

## Monocytic differentiation of human acute myeloid leukemia cells: a proteomic and phosphoproteomic comparison of FAB-M4/M5 patients with and without nucleophosmin-1 mutations

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**Table S1:** Clinical and biological characteristics of the 25 patients included in the study; **Table S2:** Proteins showing significantly increased levels in *NPM1-Ins* patients compared with patients without *NPM1-Ins*; **Table S3:** The proteomic comparison of FAB-M4/M5 AML cells with and without *NPM1-Ins*; a summary of proteins forming interacting networks and showing increased levels in patients with *NPM1-Ins*; **Table S4:** Proteins showing significantly increased levels in patients without *NPM1-Ins* compared with patients with *NPM1-Ins*; **Table S5:** The proteomic comparison of FAB-M4/M5 AML cells with and without *NPM1-Ins*; a summary of proteins forming interacting networks and showing increased levels in patients without *NPM1-Ins*; **Table S6:** Protein phosphorylation sites showing significantly increased levels in patients with *NPM1-Ins* compared with patients without *NPM1-Ins*; **Table S7:** The phosphoproteomic comparison of FAB-M4/M5 AML cells with and without *NPM1-Ins*; a summary of proteins forming interacting networks and showing increased phosphorylation levels in patients with (spliceosome, proteasome) and without (ribosome biogenesis, NuRD complex) *NPM1-Ins*; **Table S8:** Protein phosphorylation sites showing significantly increased levels in patients without *NPM1-Ins* compared with patients with *NPM1-Ins*; **Figure S1:** Molecular genetic analyses of primary AML cells with and without *NPM1-Ins*; **Figure S2:** Hierarchical clustering analysis including all our FAB-M4/M5 patients and based on ribosomal proteins included in identified protein-protein interaction (PPI) networks; **Figure S3:** Principal component (a) and unsupervised hierarchical clustering (b) analysis of FAB-M4/M5 patients with and without (referred to as *NPM1-wt* in the figure) *NPM1-Ins*; a comparison with normal CD34<sup>+</sup> bone marrow cells (referred to as healthy controls in the figure); **Figure S4:** Principal component (a) and unsupervised hierarchical clustering (b) analysis of FAB-M4/M5 patients with and without (referred to as *NPM1-wt* in the figure) *NPM1-Ins*; a comparison with normal CD34<sup>+</sup> bone marrow cells (referred to as healthy controls in the figure).

**Table S1.** Clinical and biological characteristics of 25 patients included in the study. The table presents the patient identity, gender (M, male; F, female), patients with secondary AML (MDS, myelodysplastic syndrome), bone marrow blasts (percent of nucleated cells), peripheral blood blast count ( $\times 10^9/L$ ) and genetic abnormalities. (wt, wildtype) and time to/course after relapse.

| Study ID | Gender | Age (years) | Secondary    | BM blasts | Blood blast count | Karyotype                                     | Flt3 | NPM1 | Months from diagnosis to relapse | Clinical course after relapse                |
|----------|--------|-------------|--------------|-----------|-------------------|-----------------------------------------------|------|------|----------------------------------|----------------------------------------------|
| P2       | M      | 29          |              | 90        | 18.6              | Normal                                        | ITD  | Ins  | 12                               | Death from relapse                           |
| P13      | F      | 45          | Chemotherapy | 75        | 22.1              | Normal                                        | wt   | Ins  |                                  |                                              |
| P14      | F      | 42          |              | 86        | 40.4              | inv(16)(q13q22)                               | wt   | Ins  |                                  |                                              |
| P18      | F      | 59          | MDS          | 83        | 57.8              | Normal                                        | ITD  | Ins  | 8                                | Death from relapse                           |
| P23      | M      | 46          |              | 97        | 170.0             | Normal                                        | ITD  | Ins  | 11                               | Death from relapse                           |
| P24      | M      | 53          |              | 50        | 31.4              | del(9)(q13q33)                                | wt   | Ins  |                                  |                                              |
| P34      | M      | 48          |              | 76        | 14.3              | Normal                                        | ITD  | Ins  | 6                                | Death from relapse                           |
| P44      | M      | 65          |              | 77        | 101.0             | Normal                                        | wt   | Ins  |                                  |                                              |
| P46      | F      | 60          |              | 83        | 36.9              | Normal                                        | ITD  | Ins  |                                  |                                              |
| P47      | F      | 41          |              | 55        | 8.6               | Normal                                        | wt   | Ins  |                                  |                                              |
| P49      | M      | 54          | Chemotherapy | 80        | 29.1              | Normal                                        | wt   | Ins  |                                  |                                              |
| P51      | F      | 32          |              | 95        | 112.0             | t(9;11)(p21;q23)                              | wt   | Ins  | 18                               | Long-term survival after allotransplantation |
| P53      | M      | 48          |              | 50        | 65.3              | Normal                                        | wt   | Ins  |                                  |                                              |
| P10      | M      | 57          |              | 89        | 10.8              | 46,XY,inv(16)(p13q22)                         | wt   | wt   |                                  |                                              |
| P11      | F      | 18          |              | 40        | 33.8              | inv(16)(13;q22)/46,idem,t(X;6)(p22;p12)/46,XX | wt   | wt   | 11                               | Long-term survival after allotransplantation |
| P17      | M      | 60          |              | 53        | 14.2              | inv(16)                                       | ITD  | wt   | 6                                | Death from relapse                           |
| P22      | M      | 41          |              | 30        | 3.8               | inv(16)(p13q22)                               | wt   | wt   | 26                               | Death from relapse                           |
| P29      | M      | 58          |              | 83        | 70.7              | Multiple                                      | wt   | wt   |                                  |                                              |
| P31      | F      | 36          | Chemotherapy | 86        | 37.6              | Normal                                        | wt   | wt   |                                  |                                              |
| P36      | M      | 36          |              | 32        | 33.1              | Normal                                        | wt   | wt   |                                  |                                              |
| P37      | F      | 57          |              | 47        | 65.4              | Normal                                        | wt   | wt   | 12                               | Death from relapse                           |
| P42      | M      | 43          |              | 90        | 351.0             | Normal                                        | wt   | wt   |                                  |                                              |
| P43      | M      | 48          |              | 52        | 50.1              | del(5)(q31q34)[5]/46,XX[15]                   | wt   | wt   |                                  |                                              |
| P45      | F      | 61          | MDS          | 95        | 24.1              | +8                                            | ITD  | wt   | 12                               | Long-term survival after allotransplantation |
| P52      | M      | 62          |              | 25        | 33.3              | Normal                                        | wt   | wt   | 8                                | Death from relapse                           |

**Table S2.** Proteins showing significantly increased levels in *NPM1-Ins* patients compared with patients without *NPM1-Ins*. A total of 217 proteins were identified, and the table presents the gene name, the protein name and the p-value from ANOVA analysis. The proteins are ranged according to their p-value. Proteins also identified in protein-protein interaction (PPI) analyses are marked with grey shadow.

| Gene name       | Protein name                                                         | ANOVA p-value |
|-----------------|----------------------------------------------------------------------|---------------|
| AK2             | Adenylate kinase 2, mitochondrial                                    | 1.25E-07      |
| LNPEP           | Leucyl-cystinyl aminopeptidase                                       | 6.28E-07      |
| PIGK            | GPI-anchor transamidase                                              | 8.66E-07      |
| NCEH1           | Neutral cholesterol ester hydrolase 1                                | 1.19E-06      |
| DMXL2           | DmX-like protein 2                                                   | 3.60E-06      |
| COMMD5          | COMM domain-containing protein 5                                     | 4.60E-06      |
| C17orf59        | Uncharacterized protein C17orf59                                     | 6.33E-06      |
| HSD17B4         | Peroxisomal multifunctional enzyme type 2                            | 7.28E-06      |
| FDXR            | NADPH:adrenodoxin oxidoreductase, mitochondrial                      | 1.21E-05      |
| EMD             | Emerin                                                               | 160E-05       |
| OSBPL8          | Oxysterol-binding protein-related protein 8                          | 2.63E-05      |
| PIGS            | GPI transamidase component PIG-S                                     | 2.67E-05      |
| PIGT            | GPI transamidase component PIG-T                                     | 3.03E-05      |
| RAB3D           | Ras-related protein Rab-3D                                           | 3.92E-05      |
| LPCAT3          | Lysophospholipid acyltransferase 5                                   | 3.93E-05      |
| RAB4B           | Ras-related protein Rab-4B                                           | 4.36E-05      |
| EVI2B           | Protein EVI2B                                                        | 4.53E-05      |
| EPB41L3         | Band 4.1-like protein 3                                              | 5.86E-05      |
| C10orf54        | Platelet receptor Gi24                                               | 6.15E-05      |
| PARP4           | Poly [ADP-ribose] polymerase 4                                       | 6.85E-05      |
| MAN1B1          | Endoplasmic reticulum mannosyl-oligosaccharide 1,2-alpha-mannosidase | 8.02E-05      |
| VPS18           | Vacuolar protein sorting-associated protein 18 homolog               | 9.05E-05      |
| COMMD9          | COMM domain-containing protein 9                                     | 9.11E-05      |
| ACSL1           | Long-chain-fatty-acid--CoA ligase 1                                  | 9.57E-05      |
| TMEM65          | Transmembrane protein 65                                             | 0.0001        |
| PIGU            | Phosphatidylinositol glycan anchor biosynthesis class U protein      | 0.0001        |
| COMMD10         | COMM domain-containing protein 10                                    | 0.0001        |
| GPAA1           | Glycosylphosphatidylinositol anchor attachment 1 protein             | 0.0001        |
| SGPL1           | Sphingosine-1-phosphate lyase 1                                      | 0.0001        |
| CFD             | Complement factor D                                                  | 0.0001        |
| ADPGK           | ADP-dependent glucokinase                                            | 0.0002        |
| METTL7B         | Methyltransferase-like protein 7B                                    | 0.0002        |
| HSDL2           | Hydroxysteroid dehydrogenase-like protein 2                          | 0.0002        |
| TMEM192         | Transmembrane protein 192                                            | 0.0002        |
| GGT1;GGT3P;GGT2 | Gamma-glutamyltranspeptidase 1                                       | 0.0002        |
| FCGR1A          | High affinity immunoglobulin gamma Fc receptor I                     | 0.0002        |
| CCDC22          | Coiled-coil domain-containing protein 22                             | 0.0002        |
| CD97            | CD97 antigen subunit alpha/ beta                                     | 0.0002        |
| ATP11A          | Probable phospholipid-transporting ATPase 1H                         | 0.0003        |
| FAM101B         | Protein FAM101B                                                      | 0.0003        |
| NUFIP2          | Nuclear fragile X mental retardation-interacting protein 2           | 0.0003        |

|               |                                                                     |        |
|---------------|---------------------------------------------------------------------|--------|
| B3GAT3        | Galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 3 | 0.0003 |
| DNASE1L1      | Deoxyribonuclease-1-like 1                                          | 0.0004 |
| IGF2BP2       | Insulin-like growth factor 2 mRNA-binding protein 2                 | 0.0004 |
| VAPA          | Vesicle-associated membrane protein-associated protein A            | 0.0004 |
| ARMCX3        | Armadillo repeat-containing X-linked protein 3                      | 0.0004 |
| HOMER3        | Homer protein homolog 3                                             | 0.0005 |
| ERLIN1        | Erlin-1                                                             | 0.0005 |
| LMAN2         | Vesicular integral-membrane protein VIP36                           | 0.0005 |
| IGF2R         | Cation-independent mannose-6-phosphate receptor                     | 0.0005 |
| UGGT1         | UDP-glucose:glycoprotein glucosyltransferase 1                      | 0.0005 |
| IGF2BP3       | Insulin-like growth factor 2 mRNA-binding protein 3                 | 0.0005 |
| RAB24         | Ras-related protein Rab-24                                          | 0.0005 |
| ARL6IP5       | PRA1 family protein 3                                               | 0.0005 |
| AGPAT3        | 1-acyl-sn-glycerol-3-phosphate acyltransferase gamma                | 0.0005 |
| SLC38A10      | Putative sodium-coupled neutral amino acid transporter 10           | 0.0005 |
| MTCH1         | Mitochondrial carrier homolog 1                                     | 0.0005 |
| LACTB         | Serine beta-lactamase-like protein LACTB, mitochondrial             | 0.0006 |
| TM9SF4        | Transmembrane 9 superfamily member 4                                | 0.0006 |
| ETFA          | Electron transfer flavoprotein subunit alpha, mitochondrial         | 0.0006 |
| FAM45A;FAM45B | Protein FAM45A;Protein FAM45B                                       | 0.0006 |
| KPNA4         | Importin subunit alpha-3                                            | 0.0007 |
| C16orf62      | UPF0505 protein C16orf62                                            | 0.0007 |
| GHDC          | GH3 domain-containing protein                                       | 0.0007 |
| ETFB          | Electron transfer flavoprotein subunit beta                         | 0.0008 |
| MTDH          | Protein LYRIC                                                       | 0.0008 |
| PLBD2         | Putative phospholipase B-like 2                                     | 0.0008 |
| ABHD16A       | Alpha/beta hydrolase domain-containing protein 16A                  | 0.0009 |
| FAM134C       | Protein FAM134C                                                     | 0.0010 |
| ASCC2         | Activating signal cointegrator 1 complex subunit 2                  | 0.0010 |
| CPNE8         | Copine-8                                                            | 0.0010 |
| HSF1          | Heat shock factor protein 1                                         | 0.0010 |
| STX8          | Syntaxin-8                                                          | 0.0011 |
| LAIR1         | Leukocyte-associated immunoglobulin-like receptor 1                 | 0.0011 |
| ADCY7         | Adenylate cyclase type 7                                            | 0.0011 |
| RNASE2        | Non-secretory ribonuclease                                          | 0.0012 |
| POFUT1        | GDP-fucose protein O-fucosyltransferase 1                           | 0.0012 |
| UBE2J1        | Ubiquitin-conjugating enzyme E2 J1                                  | 0.0012 |
| BCL2L13       | Bcl-2-like protein 13                                               | 0.0013 |
| CTSA          | Lysosomal protective protein                                        | 0.0014 |
| FNDC3B        | Fibronectin type III domain-containing protein 3B                   | 0.0014 |
| CLINT1        | Clathrin interactor 1                                               | 0.0014 |
| NFKB2         | Nuclear factor NF-kappa-B p100 subunit                              | 0.0015 |
| PLAUR         | Urokinase plasminogen activator surface receptor                    | 0.0015 |
| ABHD11        | Alpha/beta hydrolase domain-containing protein 11                   | 0.0016 |
| WDFY1         | WD repeat and FYVE domain-containing protein 1                      | 0.0016 |
| ACADVL        | Very long-chain specific acyl-CoA dehydrogenase, mitochondrial      | 0.0017 |

|          |                                                                |        |
|----------|----------------------------------------------------------------|--------|
| LNP      | Protein lunapark                                               | 0.0017 |
| SP110    | Sp110 nuclear body protein                                     | 0.0018 |
| KIAA1033 | WASH complex subunit 7                                         | 0.0019 |
| AFTPH    | Aftiphilin                                                     | 0.0019 |
| CRELD1   | Cysteine-rich with EGF-like domain protein 1                   | 0.0019 |
| HEATR3   | HEAT repeat-containing protein 3                               | 0.0020 |
| JMJD6    | Bifunctional arginine demethylase and lysyl-hydroxylase JMJD6  | 0.0020 |
| DNAJC1   | DnaJ homolog subfamily C member 1                              | 0.0020 |
| DCXR     | L-xylulose reductase                                           | 0.0022 |
| SLC39A11 | Zinc transporter ZIP11                                         | 0.0022 |
| CCDC93   | Coiled-coil domain-containing protein 93                       | 0.0023 |
| OSBPL5   | Oxysterol-binding protein-related protein 5                    | 0.0024 |
| PLA2G4A  | Cytosolic phospholipase A2                                     | 0.0024 |
| PNPLA6   | Neuropathy target esterase                                     | 0.0024 |
| ASCC3    | Activating signal cointegrator 1 complex subunit 3             | 0.0025 |
| CLTB     | Clathrin light chain B                                         | 0.0025 |
| SEC22B   | Vesicle-trafficking protein SEC22b                             | 0.0025 |
| MYOF     | Myoferlin                                                      | 0.0027 |
| COMMD3   | COMM domain-containing protein 3                               | 0.0027 |
| UFSP2    | Ufm1-specific protease 2                                       | 0.0027 |
| STAB1    | Stabilin-1                                                     | 0.0028 |
| ADAM17   | Disintegrin and metalloproteinase domain-containing protein 17 | 0.0028 |
| PLEC     | Plectin                                                        | 0.0029 |
| DYNC1LI2 | Cytoplasmic dynein 1 light intermediate chain 2                | 0.0030 |
| SPCS1    | Signal peptidase complex subunit 1                             | 0.0031 |
| TRIP12   | E3 ubiquitin-protein ligase TRIP12                             | 0.0031 |
| DHRS9    | Dehydrogenase/reductase SDR family member 9                    | 0.0031 |
| DHRS7B   | Dehydrogenase/reductase SDR family member 7B                   | 0.0033 |
| CAT      | Catalase                                                       | 0.0033 |
| DNAJC10  | DnaJ homolog subfamily C member 10                             | 0.0037 |
| SFXN3    | Sideroflexin-3                                                 | 0.0034 |
| STX12    | Syntaxin-12                                                    | 0.0034 |
| VAMP7    | Vesicle-associated membrane protein 7                          | 0.0034 |
| TPP1     | Tripeptidyl-peptidase 1                                        | 0.0035 |
| CISD3    | CDGSH iron-sulfur domain-containing protein 3, mitochondrial   | 0.0036 |
| ABCD3    | ATP-binding cassette sub-family D member 3                     | 0.0036 |
| STAU1    | Double-stranded RNA-binding protein Staufen homolog 1          | 0.0037 |
| RRBP1    | Ribosome-binding protein 1                                     | 0.0037 |
| TMEM205  | Transmembrane protein 205                                      | 0.0038 |
| LAMTOR4  | Regulator complex protein LAMTOR4                              | 0.0038 |
| LMNA     | Prelamin-A/C; Lamin-A/C                                        | 0.0039 |
| CCDC6    | Coiled-coil domain-containing protein 6                        | 0.0039 |
| PTPLAD2  | Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 4           | 0.0039 |
| RETSAT   | All-trans-retinol 13,14-reductase                              | 0.0042 |
| FAM21A   | WASH complex subunit FAM21A                                    | 0.0043 |
| TLR2     | Toll-like receptor 2                                           | 0.0044 |

|                |                                                                           |        |
|----------------|---------------------------------------------------------------------------|--------|
| TOLLIP         | Toll-interacting protein                                                  | 0.0048 |
| LAMTOR2        | Ragulator complex protein LAMTOR2                                         | 0.0048 |
| STIM1          | Stromal interaction molecule 1                                            | 0.0049 |
| GLB1           | Beta-galactosidase                                                        | 0.0049 |
| CTSD           | Cathepsin D light/heavy chain                                             | 0.0050 |
| HK1            | Hexokinase-1                                                              | 0.0051 |
| BASP1          | Brain acid soluble protein 1                                              | 0.0052 |
| VPS8           | Vacuolar protein sorting-associated protein 8 homolog                     | 0.0056 |
| SPR            | Sepiapterin reductase                                                     | 0.0057 |
| PRCP           | Lysosomal Pro-X carboxypeptidase                                          | 0.0057 |
| VIM            | Vimentin                                                                  | 0.0058 |
| ERLIN2         | Erlin-2                                                                   | 0.0058 |
|                | Uncharacterized protein FLJ45252                                          | 0.0059 |
| MFSD10         | Major facilitator superfamily domain-containing protein 10                | 0.0059 |
| TBL2           | Transducin beta-like protein 2                                            | 0.0061 |
| COMMD4         | COMM domain-containing protein 4                                          | 0.0062 |
| SOD2           | Superoxide dismutase [Mn], mitochondrial                                  | 0.0063 |
| RFT1           | Protein RFT1 homolog                                                      | 0.0070 |
| BST2           | Bone marrow stromal antigen 2                                             | 0.0071 |
| RPS6KA5        | Ribosomal protein S6 kinase alpha-5                                       | 0.0071 |
| SLC35A1        | CMP-sialic acid transporter                                               | 0.0071 |
| RNF170         | E3 ubiquitin-protein ligase RNF170                                        | 0.0071 |
| CARD9          | Caspase recruitment domain-containing protein 9                           | 0.0072 |
| VAMP8          | Vesicle-associated membrane protein 8                                     | 0.0077 |
| MGST2          | Microsomal glutathione S-transferase 2                                    | 0.0079 |
| GLA            | Alpha-galactosidase A                                                     | 0.0079 |
| STK11IP        | Serine/threonine-protein kinase 11-interacting protein                    | 0.0079 |
| TMED7 (TICAM2) | Transmembrane emp24 domain-containing protein 7                           | 0.0079 |
| DSCR3          | Down syndrome critical region protein 3                                   | 0.0080 |
| CPVL           | Probable serine carboxypeptidase CPVL                                     | 0.0086 |
| GPD1L          | Glycerol-3-phosphate dehydrogenase 1-like protein                         | 0.0089 |
| OTUD4          | OTU domain-containing protein 4                                           | 0.0092 |
| DNAJC3         | DnaJ homolog subfamily C member 3                                         | 0.0096 |
| COMMD8         | COMM domain-containing protein 8                                          | 0.0096 |
| PHKA2          | Phosphorylase b kinase regulatory subunit alpha, liver isoform            | 0.0101 |
| SELENOF        | 15 kDa selenoprotein                                                      | 0.0103 |
| BLOC1S2        | Biogenesis of lysosome-related organelles complex 1 subunit 2             | 0.0105 |
| BCKDK          | [3-methyl-2-oxobutanoate dehydrogenase [lipoamide]] kinase, mitochondrial | 0.0105 |
| SLC44A1        | Choline transporter-like protein 1                                        | 0.0107 |
| MVP            | Major vault protein                                                       | 0.0108 |
| VPS16          | Vacuolar protein sorting-associated protein 16 homolog                    | 0.0110 |
| DTYMK          | Thymidylate kinase                                                        | 0.0115 |
| CC2D1B         | Coiled-coil and C2 domain-containing protein 1B                           | 0.0116 |
| WDR11          | WD repeat-containing protein 11                                           | 0.0122 |
| SCARB2         | Lysosome membrane protein 2                                               | 0.0123 |
| PRKCH          | Protein kinase C eta type                                                 | 0.0129 |

|          |                                                            |        |
|----------|------------------------------------------------------------|--------|
| EIF2AK4  | Eukaryotic translation initiation factor 2-alpha kinase 4  | 0.0132 |
| SRGN     | Serglycin                                                  | 0.0136 |
| ALDH3B1  | Aldehyde dehydrogenase family 3 member B1                  | 0.0136 |
| DPP7     | Dipeptidyl peptidase 2                                     | 0.0140 |
| ZBTB7B   | Zinc finger and BTB domain-containing protein 7B           | 0.0142 |
| TOR1AIP1 | Torsin-1A-interacting protein 1                            | 0.0145 |
| AGTRAP   | Type-1 angiotensin II receptor-associated protein          | 0.0147 |
| USP32    | Ubiquitin carboxyl-terminal hydrolase 32                   | 0.0148 |
| STX16    | Syntaxin-16                                                | 0.0148 |
| ERLEC1   | Endoplasmic reticulum lectin 1                             | 0.0148 |
| PTPN9    | Tyrosine-protein phosphatase non-receptor type 9           | 0.0155 |
| VPS26B   | Vacuolar protein sorting-associated protein 26B            | 0.0158 |
| CMAS     | N-acetylneuraminyl transferase                             | 0.0159 |
| TOM1     | Target of Myb protein 1                                    | 0.0167 |
| TTYH3    | Protein tteety homolog 3                                   | 0.0171 |
| VAPB     | Vesicle-associated membrane protein-associated protein B/C | 0.0172 |
| ERO1L    | ERO1-like protein alpha                                    | 0.0175 |
| AP3M1    | AP-3 complex subunit mu-1                                  | 0.0176 |
| VPS11    | Vacuolar protein sorting-associated protein 11 homolog     | 0.0192 |
| CLCN7    | H(+)/Cl(-) exchange transporter 7                          | 0.0194 |
| PSTPIP2  | Proline-serine-threonine phosphatase-interacting protein 2 | 0.0202 |
| TOR4A    | Torsin-4A                                                  | 0.0202 |
| SCYL2    | SCY1-like protein 2                                        | 0.0209 |
| TCIRG1   | V-type proton ATPase 116 kDa subunit a isoform 3           | 0.0210 |
| LEMD2    | LEM domain-containing protein 2                            | 0.0238 |
| EHHADH   | Peroxisomal bifunctional enzyme;Enoyl-CoA hydratase        | 0.0240 |
| SEC16A   | Protein transport protein Sec16A                           | 0.0248 |
| SCAMP1   | Secretory carrier-associated membrane protein 1            | 0.0248 |
| TMEM175  | Transmembrane protein 175                                  | 0.0257 |
| AGA      | N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase            | 0.0270 |
| SDF2L1   | Stromal cell-derived factor 2-like protein 1               | 0.0276 |
| CPM      | Carboxypeptidase M                                         | 0.0276 |
| RAB44    | Ras-related protein Rab-44                                 | 0.0278 |
| TOR1A    | Torsin-1A                                                  | 0.0296 |
| TRIM4    | Tripartite motif-containing protein 4                      | 0.0297 |
| TACC1    | Transforming acidic coiled-coil-containing protein 1       | 0.0301 |
| AP3B1    | AP-3 complex subunit beta-1                                | 0.0326 |
| AKAP13   | A-kinase anchor protein 13                                 | 0.0391 |

**Table S3.** The proteomic comparison of FAB-M4/M5 AML cells with and without *NPM1-Ins*; a summary of proteins forming interacting networks (**Figure 1** of the main text) and showing increased levels in patients with *NPM1-Ins*. The table presents the gene name, protein names and key words with regard to protein function. The table is based on information from the Gene database and selected references from the PubMed database (accessed 29<sup>th</sup> of October 2023) [37,38,51,52,57, 60-64].

| ENDOCYTIC RECYCLING (11 proteins) |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                      |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| COMMD3                            | <i>COMM domain containing 3</i> . The encoded protein is predicted to be involved in sodium ion transport, and it is predicted to be located in the extracellular region and ficolin-1-rich granule lumen.                                                                                                                                                                                                         | Membrane protein<br>Sodium transport                                 |
| COMMD4                            | <i>COMM domain containing 4</i> . The encoded protein is located in the cytosol, intracellular membrane-bounded organelles and the plasma membrane.                                                                                                                                                                                                                                                                | Cytosole<br>Plasma membrane                                          |
| COMMD5                            | <i>COMM domain containing 4</i> . The protein I located in cytosol, intracellular membrane-bounded organelles and the plasma membrane.                                                                                                                                                                                                                                                                             | Cytosole<br>Plasma membrane                                          |
| COMMD8                            | <i>COMM domain containing 8</i> . The protein binds coiled-coil domain-containing protein 22 (CCDC22), and this complex can regulate the turnover of I-kappa-B and the activation of NF-kappa-B.                                                                                                                                                                                                                   | NFκB<br>CCDC22 (see below)                                           |
| COMMD9                            | <i>COMM domain containing 9</i> . The protein is predicted to be involved in sodium ion transport and to act upstream of or within cholesterol homeostasis. It is located in Golgi apparatus, cytosol and nucleoplasm.                                                                                                                                                                                             | Membrane protein<br>Sodium transport<br>Golgi, cytosole, nucleoplasm |
| COMMD10                           | <i>COMM domain containing 10</i> . Located in nucleoplasm and involved in endosomal recycling.                                                                                                                                                                                                                                                                                                                     | Nucleoplasm                                                          |
| CCDC22                            | <i>Coiled-coil domain containing 22</i> . This gene encodes a protein containing a coiled-coil domain. The encoded protein functions in the regulation of NFκB by <u>interacting with COMMD</u> (copper metabolism Murr1 domain-containing) proteins. The mouse orthologous protein has been shown to bind copines, which are calcium-dependent, membrane-binding proteins that may function in calcium signaling. | NFκB<br>COMMD protein interactions                                   |
| CCDC93                            | <i>Coiled-coil domain containing 22</i> . The encoded protein is involved in Golgi to plasma membrane transport and endocytic recycling. It is located in intracellular membrane-bounded organelle.                                                                                                                                                                                                                | Golgi                                                                |
| VPS26C                            | <i>VPS26 endosomal protein sorting factor C</i> . The protein is important for endosomal transport [37,60,61].                                                                                                                                                                                                                                                                                                     | Endosome                                                             |
| VPS35L                            | <i>VPS35 endosomal protein sorting factor like</i> . The encoded protein is involved in Golgi to plasma membrane transport and endocytic recycling. It is located in endosome.                                                                                                                                                                                                                                     | Golgi to plasma membrane<br>Endosome                                 |
| DENND10                           | <i>DENN domain containing 10</i> . The protein enables guanyl-nucleotide exchange factor activity and <u>small GTPase binding</u> activity. It is involved in <u>endosome transport</u> via the <u>multivesicular body sorting pathway</u> , protein transport and regulation of early endosome to late endosome transport. The protein is thus located in late endosomes.                                         | Endosome<br>Protein transport                                        |
| VESICLE FUSION (10 proteins)      |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                      |
| SEC22B                            | <i>SEC22 homolog B, vesicle trafficking protein b</i> . The encoded protein is one of the SNARE proteins, and it functions in membrane fusion of vesicle trafficking between the endoplasmic reticulum and the Golgi apparatus and in secretory autophagy [38].                                                                                                                                                    | SNARE<br>Endoplasmic reticulum<br>Golgi<br>Autophagy                 |
| STX8                              | <i>Syntaxin 8</i> . The encoded protein is a member of the syntaxin family and is involved in protein trafficking from early to late endosomes via <u>vesicle fusion</u> and <u>exocytosis</u> . It interacts with the SNARE complex [62-64].                                                                                                                                                                      | Endosomes<br>Protein trafficking<br>SNARE                            |
| STX12                             | <i>Syntaxin 12</i> . The protein is predicted to enable SNAP receptor activity and <u>SNARE binding activity</u> and is involved in autophagosome assembly and protein stabilization. It is located in several cellular components, including membrane rafts, phagocytic vesicles, and phagophore assembly site.                                                                                                   | Autophagosome<br>Protein stabilization<br>SNARE                      |
| STX16                             | <i>Syntaxin 16</i> . The encoded protein is a member of the syntaxin or t-SNARE (target-SNAP receptor) family. These proteins are found on cell membranes and serve as the targets for V-SNARES ( <u>vesicle-SNAP receptors</u> ) permitting specific vesicle docking and fusion.                                                                                                                                  | Vesicle docking and fusion<br>SNARE                                  |

|       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                       |
|-------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|
| VAMP7 | <i>Vesicle associated membrane protein 7.</i> The encoded <u>transmembrane protein</u> that is a member of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family. It localizes to late endosomes and lysosomes and is involved in the <u>fusion of transport vesicles</u> to their target membranes.                                                                                                                                                                                                                                                | Vesicle fusion<br>SNARE               |
| VAMP8 | <i>Vesicle associated membrane protein 8.</i> The encoded protein is an integral membrane protein that belongs to the <u>synaptobrevin/vesicle-associated membrane protein subfamily</u> of soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs). The encoded protein is involved in the fusion of vesicles with the membrane.                                                                                                                                                                                                                              | Vesicle fusion<br>SNARE               |
| VPS8  | <i>VPS8 subunit of CORVET complex.</i> The encoded protein is predicted to enable metal ion binding activity. It is involved in <u>endosomal vesicle fusion</u> and located in early endosomes.                                                                                                                                                                                                                                                                                                                                                                                        | Endosome                              |
| VPS11 | <i>VPS11 core subunit of CORVET and HOPS complexes.</i> Vesicle mediated protein sorting plays an important role in segregation of intracellular molecules into distinct organelles. Genetic studies in yeast have identified more than 40 vacuolar protein sorting (VPS) genes involved in vesicle transport to vacuoles. This gene encodes the human homolog of yeast class C Vps11 protein. The mammalian class C Vps proteins are predominantly associated with <u>late endosomes/lysosomes</u> and may mediate <u>vesicle trafficking steps</u> in the endosome/lysosome pathway. | Late endosomes<br>Vesicle trafficking |
| VPS16 | <i>VPS16 core subunit of CORVET and HOPS complexes.</i> This gene encodes the human homolog of yeast class C Vps16 protein. The mammalian class C Vps proteins are predominantly associated with <u>late endosomes/lysosomes</u> and may mediate vesicle trafficking steps in the endosome/lysosome pathway.                                                                                                                                                                                                                                                                           | Late endosome<br>Vesicle trafficking  |
| VPS18 | <i>VPS18 core subunit of CORVET and HOPS complexes.</i> This gene encodes the human homolog of yeast class C Vps18 protein. The mammalian class C Vps proteins are predominantly associated with <u>late endosomes/lysosomes</u> and mediate vesicle trafficking steps in the endosome/lysosome pathway.                                                                                                                                                                                                                                                                               | Late endosome<br>Vesicle trafficking  |

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**THE GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) TRANSAMIDASE COMPLEX; ATTACHMENT OF THE GPI ANCHOR TO PROTEIN (5 proteins)**

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|       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                              |
|-------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
| GPAA1 | <i>Glycosylphosphatidylinositol anchor attachment 1.</i> Posttranslational glycosylphosphatidylinositol (GPI) anchor attachment serves as a general mechanism for linking proteins to the cell surface membrane. The protein encoded by this gene presumably functions in GPI anchoring at the <u>GPI transfer step</u> . The anchor attachment protein 1 contains an N-terminal signal sequence, 1 cAMP- and cGMP-dependent protein kinase phosphorylation site, 1 leucine zipper pattern, 2 potential N-glycosylation sites, and 8 putative transmembrane domains. These five proteins form the GPI transamidase complex that mediates binding of GPI to target proteins [51,52]. GPI molecules as membrane anchors. GPI-transamidase is a membrane-bound protein complex found in the endoplasmic reticulum; after attachment of GPI to target proteins the lipid reconstitution occurs upon transport to the Golgi [52] . However, GPI anchored proteins are almost exclusively localized to the cell surface where they are associated to the plasma membrane through the lipid portion of the anchor. | GPI anchoring                                                |
| PIGK  | <i>Phosphatidylinositol glycan anchor biosynthesis class K.</i> This member of the cysteine protease family C13 is involved in glycosylphosphatidylinositol (GPI)-anchor biosynthesis. The GPI-anchor is a glycolipid that serves to anchor proteins to the cell surface. This protein is a member of the multisubunit enzyme, GPI transamidase and is thought to be its <u>enzymatic component</u> . GPI transamidase mediates GPI anchoring in the <u>endoplasmic reticulum</u> , by catalyzing the transfer of fully assembled GPI units to proteins.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Membrane anchor<br>GPI transamidase<br>Endoplasmic reticulum |
| PIGS  | <i>Phosphatidylinositol glycan anchor biosynthesis class S.</i> The encoded protein is involved in GPI-anchor biosynthesis. The glycosylphosphatidylinositol (GPI) anchor is a <u>glycolipid</u> serves to anchor proteins to the cell surface. This gene encodes an essential component of the multisubunit enzyme GPI transamidase that mediates GPI anchoring in the endoplasmic reticulum by catalyzing the transfer of fully assembled GPI units to proteins.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Membrane anchor<br>GPI transamidase<br>Endoplasmic reticulum |
| PIGT  | <i>Phosphatidylinositol glycan anchor biosynthesis class T.</i> This gene encodes a protein involved in glycosylphosphatidylinositol (GPI)-anchor biosynthesis. This protein is an essential component of the multisubunit enzyme, GPI transamidase that mediates GPI anchoring in the endoplasmic reticulum, by catalyzing the transfer of fully assembled GPI units to proteins.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Membrane anchor<br>GPI transamidase<br>Endoplasmic reticulum |
| PIGU  | <i>Phosphatidylinositol glycan anchor biosynthesis class U.</i> The encoded protein is a predicted integral membrane protein that may function in cell division control. It is the fifth subunit of GPI transamidase that attaches GPI-anchors to proteins.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Membrane anchor<br>GPI transamidase<br>Endoplasmic reticulum |

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| GALACTOSIDASE ACTIVITY (4 proteins) |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                             |
|-------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|
| HK1                                 | <i>Hexokinase 1</i> . Hexokinases phosphorylate glucose to produce glucose-6-phosphate, the first step in most <u>glucose metabolism</u> pathways. This gene encodes a ubiquitous form of hexokinase which localizes to the <u>outer membrane of mitochondria</u> .                                                                                                                                                                                                                                                                                             | Mitochondria<br>Glucose metabolism                          |
| GLB1                                | <i>Galactosidase beta 1</i> . This gene encodes a member of the glycosyl hydrolase 35 family of proteins. Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein that is proteolytically processed to generate the mature <u>lysosomal enzyme</u> . This enzyme catalyzes the hydrolysis of a terminal beta-linked galactose residue from ganglioside substrates and other glycoconjugates.                                                                                                                | Lysosomal enzyme                                            |
| GLA                                 | <i>Galactosidase alpha</i> . This gene encodes a homodimeric glycoprotein that hydrolyses the terminal alpha-galactosyl moieties from glycolipids and glycoproteins. This enzyme predominantly hydrolyzes ceramide trihexoside, and it can catalyze the hydrolysis of melibiose into galactose and glucose. A variety of mutations in this gene affect the synthesis, processing, and stability of this enzyme, which causes Fabry disease, a rare lysosomal storage disorder that results from a failure to catabolize alpha-D-galactosyl glycolipid moieties. | Lysosomal enzyme                                            |
| CTSA                                | <i>Cathepsin A</i> . This gene encodes a member of the <u>peptidase S10 family</u> of serine carboxypeptidases. Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein that is proteolytically processed to generate two chains that comprise the heterodimeric active enzyme. This enzyme possesses deamidase, esterase and carboxypeptidase activities and acts as a scaffold in the lysosomal multienzyme complex.                                                                                      | Lysosome                                                    |
| CLATHRIN BINDING (3 proteins)       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                             |
| CLINT1                              | <i>Clatrin interactor 1</i> . The encoded protein has similarity to the epsin family of endocytic adapter proteins. It interacts with clathrin, the adapter protein AP-1 and phosphoinositides. This protein may be involved in the formation of <u>clathrin coated vesicles</u> and trafficking between the <u>trans-Golgi network and endosomes</u> .                                                                                                                                                                                                         | Clathrin-coated vesicles<br>Endosome<br>Trans-Golgi network |
| CLTB                                | <i>Clathrin light chain beta</i> . Clathrin is a large, soluble protein composed of heavy and light chains. It functions as the main structural component of the lattice-type cytoplasmic face of coated pits and vesicles which entrap specific macromolecules during <u>receptor-mediated endocytosis</u> . This gene encodes one of two clathrin light chain proteins which are believed to function as regulatory elements.                                                                                                                                 | Clathrin-mediated and receptor-mediated endocytosis         |
| AFTPH                               | <i>Aftiphilin</i> . The encoded protein enables clathrin binding activity and is predicted to be involved in intracellular transport. It is located in the Golgi apparatus, cytosol and nucleoplasm, and it is a part of AP-1 adaptor protein complex. It can also bind to the clathrin adaptor protein AP-2 that is regarded as a key component in clathrin-mediated endocytosis [57].                                                                                                                                                                         | Intracellular transport<br>Golgi, cytosol, nucleoplasm      |

**Table S4.** Proteins showing significantly increased levels in patients without *NPM1-Ins* compared with patients with *NPM1-Ins*. A total of 132 proteins were identified, and the table presents the gene name, the protein name and the p-value from ANOVA analysis. The proteins are ranged according to their p-value. Proteins also identified in PPI analyses are marked with grey shadow.

| Gene name | Protein name                                                                     | ANOVA p-value |
|-----------|----------------------------------------------------------------------------------|---------------|
| SRP14     | Signal recognition particle 14 kDa protein                                       | 4.10E-08      |
| SRP9      | Signal recognition particle 9 kDa protein                                        | 1.05E-07      |
| GNG2      | Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2                | 9.67E-06      |
| NPM1      | Nucleophosmin                                                                    | 1.16E-05      |
| NLE1      | Notchless protein homolog 1                                                      | 4.22E-05      |
| RRM1      | Ribonucleoside-diphosphate reductase large subunit                               | 4.70E-05      |
| NO66      | Bifunctional lysine-specific demethylase and histidyl-hydroxylase NO66           | 4.76E-05      |
| RPL15     | 60S ribosomal protein L15                                                        | 5.28E-05      |
| OGT       | UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase 110 kDa subunit | 8.07E-05      |
| MEF2D     | Myocyte-specific enhancer factor 2D                                              | 8.43E-05      |
| PAICS     | Multifunctional protein ADE2                                                     | 0.0002        |
| YBX1      | Nuclease-sensitive element-binding protein 1                                     | 0.0002        |
| RPL12     | 60S ribosomal protein L12                                                        | 0.0004        |
| RAD23B    | UV excision repair protein RAD23 homolog B                                       | 0.0004        |
| PA2G4     | Proliferation-associated protein 2G4                                             | 0.0004        |
| RPL29     | 60S ribosomal protein L29                                                        | 0.0004        |
| NACA      | Nascent polypeptide-associated complex subunit alpha                             | 0.0004        |
| RPL19     | 60S ribosomal protein L19                                                        | 0.0005        |
| PLD4      | Phospholipase D4                                                                 | 0.0006        |
| NPM3      | Nucleoplasmin-3                                                                  | 0.0007        |
| RPL34     | 60S ribosomal protein L34                                                        | 0.0008        |
| RPS9      | 40S ribosomal protein S9                                                         | 0.0008        |
| SERPINB9  | Serpin B9                                                                        | 0.0008        |
| EIF5B     | Eukaryotic translation initiation factor 5B                                      | 0.0009        |
| CLIC4     | Chloride intracellular channel protein 4                                         | 0.0011        |
| RPL13     | 60S ribosomal protein L13                                                        | 0.0011        |
| MAD2L1    | Mitotic spindle assembly checkpoint protein MAD2A                                | 0.0011        |
| UBA5      | Ubiquitin-like modifier-activating enzyme 5                                      | 0.0013        |
| PM20D2    | Peptidase M20 domain-containing protein 2                                        | 0.0013        |
| EEF2      | Elongation factor 2                                                              | 0.0014        |
| NAP1L4    | Nucleosome assembly protein 1-like 4                                             | 0.0015        |
| RPL32     | 60S ribosomal protein L32                                                        | 0.0016        |
| ENY2      | Transcription and mRNA export factor ENY2                                        | 0.0017        |
| FTSJ3     | pre-rRNA processing protein FTSJ3                                                | 0.0017        |
| SERBP1    | Plasminogen activator inhibitor 1 RNA-binding protein                            | 0.0017        |
| UBQLN2    | Ubiquilin-2                                                                      | 0.0019        |
| RPL4      | 60S ribosomal protein L4                                                         | 0.0019        |
| TXNL1     | Thioredoxin-like protein 1                                                       | 0.0021        |
| RPL13A    | 60S ribosomal protein L13a                                                       | 0.0022        |
| MTHFD1    | C-1-tetrahydrofolate synthase, cytoplasmic                                       | 0.0022        |
| CBX5      | Chromobox protein homolog 5                                                      | 0.0023        |

|         |                                                                        |        |
|---------|------------------------------------------------------------------------|--------|
| IPO5    | Importin-5                                                             | 0.0026 |
| UTP3    | Something about silencing protein 10                                   | 0.0027 |
| METAP2  | Methionine aminopeptidase 2                                            | 0.0029 |
| RPL14   | 60S ribosomal protein L14                                              | 0.0029 |
| PFAS    | Phosphoribosylformylglycinamide synthase                               | 0.0029 |
| RPL36   | 60S ribosomal protein L36                                              | 0.0031 |
| HAX1    | HCLS1-associated protein X-1                                           | 0.0032 |
| RPL18A  | 60S ribosomal protein L18a                                             | 0.0032 |
| MPO     | Myeloperoxidase                                                        | 0.0032 |
| RPS28   | 40S ribosomal protein S28                                              | 0.0034 |
| RPL7    | 60S ribosomal protein L7                                               | 0.0034 |
| RPS5    | 40S ribosomal protein S5                                               | 0.0035 |
| FAF1    | FAS-associated factor 1                                                | 0.0035 |
| DDX54   | ATP-dependent RNA helicase DDX54                                       | 0.0037 |
| SLK     | STE20-like serine/threonine-protein kinase                             | 0.0037 |
| CCT2    | T-complex protein 1 subunit beta                                       | 0.0040 |
| RPL18   | 60S ribosomal protein L18                                              | 0.0043 |
| SAMD9   | Sterile alpha motif domain-containing protein 9                        | 0.0045 |
| SGTA    | Small glutamine-rich tetratricopeptide repeat-containing protein alpha | 0.0046 |
| BMS1    | Ribosome biogenesis protein BMS1 homolog                               | 0.0049 |
| KRR1    | KRR1 small subunit processome component homolog                        | 0.0051 |
| ASF1A   | Histone chaperone ASF1A                                                | 0.0054 |
| RPL27A  | 60S ribosomal protein L27a                                             | 0.0055 |
| IPO4    | Importin-4                                                             | 0.0056 |
| CDK1    | Cyclin-dependent kinase 1                                              | 0.0056 |
| NOC3L   | Nucleolar complex protein 3 homolog                                    | 0.0056 |
| TCP1    | T-complex protein 1 subunit alpha                                      | 0.0057 |
| NLRX1   | NLR family member X1                                                   | 0.0058 |
| RCL1    | RNA 3-terminal phosphate cyclase-like protein                          | 0.0059 |
| NUDT5   | ADP-sugar pyrophosphatase                                              | 0.0059 |
| ZCCHC11 | Terminal uridylyltransferase 4                                         | 0.0061 |
| CCT8    | T-complex protein 1 subunit theta                                      | 0.0062 |
| RRS1    | Ribosome biogenesis regulatory protein homolog                         | 0.0062 |
| RPL26   | 60S ribosomal protein L26                                              | 0.0066 |
| RPL21   | 60S ribosomal protein L21                                              | 0.0067 |
| UBFD1   | Ubiquitin domain-containing protein UBFD1                              | 0.0068 |
| RPL23A  | 60S ribosomal protein L23a                                             | 0.0070 |
| IRGQ    | Immunity-related GTPase family Q protein                               | 0.0072 |
| GID8    | Glucose-induced degradation protein 8 homolog                          | 0.0074 |
| GALK2   | N-acetylgalactosamine kinase                                           | 0.0075 |
| RPL28   | 60S ribosomal protein L28                                              | 0.0079 |
| STMN1   | Stathmin                                                               | 0.0082 |
| PFDN2   | Prefoldin subunit 2                                                    | 0.0084 |
| DCAF8   | DDB1- and CUL4-associated factor 8                                     | 0.0087 |
| RPL31   | 60S ribosomal protein L31                                              | 0.0089 |
| GNL3    | Guanine nucleotide-binding protein-like 3                              | 0.0090 |

|          |                                                         |        |
|----------|---------------------------------------------------------|--------|
| SERPINH1 | Serpin H1                                               | 0.0091 |
| UBE2A    | Ubiquitin-conjugating enzyme E2 A                       | 0.0094 |
| RPL11    | 60S ribosomal protein L11                               | 0.0095 |
| EBNA1BP2 | Probable rRNA-processing protein EBP2                   | 0.0098 |
| CMPK2    | UMP-CMP kinase 2, mitochondrial                         | 0.0098 |
| SAAL1    | Protein SAAL1                                           | 0.0099 |
| NAA15    | N-alpha-acetyltransferase 15, NatA auxiliary subunit    | 0.0101 |
| RPL7A    | 60S ribosomal protein L7a                               | 0.0106 |
| DNMT3A   | DNA (cytosine-5)-methyltransferase 3A                   | 0.0109 |
| RBM3     | Putative RNA-binding protein 3                          | 0.0110 |
| PDCD5    | Programmed cell death protein 5                         | 0.0113 |
| FSCN1    | Fascin                                                  | 0.0113 |
| RPIA     | Ribose-5-phosphate isomerase                            | 0.0113 |
| MYO18A   | Unconventional myosin-XVIIIa                            | 0.0113 |
| RPS20    | 40S ribosomal protein S20                               | 0.0117 |
| RPL3     | 60S ribosomal protein L3                                | 0.0119 |
| FKBP4    | Peptidyl-prolyl cis-trans isomerase FKBP4;              | 0.0122 |
| RPS17    | 40S ribosomal protein S17                               | 0.0123 |
| PATL1    | Protein PAT1 homolog 1                                  | 0.0123 |
| RPL24    | 60S ribosomal protein L24                               | 0.0125 |
| BCAT1    | Branched-chain-amino-acid aminotransferase, cytosolic   | 0.0127 |
| CTPS1    | CTP synthase 1                                          | 0.0130 |
| BTF3     | Transcription factor BTF3                               | 0.0138 |
| RPS24    | 40S ribosomal protein S24                               | 0.0139 |
| RPS29    | 40S ribosomal protein S29                               | 0.0143 |
| RPL9     | 60S ribosomal protein L9                                | 0.0147 |
| RPS18    | 40S ribosomal protein S18                               | 0.0158 |
| RPLP1    | 60S acidic ribosomal protein P1                         | 0.0160 |
| RPL36AL  | 60S ribosomal protein L36a-like                         | 0.0165 |
| PEF1     | Peflin                                                  | 0.0175 |
| RPLP2    | 60S acidic ribosomal protein P2                         | 0.0180 |
| CCS      | Copper chaperone for superoxide dismutase               | 0.0184 |
| RPS8     | 40S ribosomal protein S8                                | 0.0194 |
| RPS3     | 40S ribosomal protein S3                                | 0.0196 |
| DCTD     | Deoxycytidylate deaminase                               | 0.0225 |
| UBE2E1   | Ubiquitin-conjugating enzyme E2 E1                      | 0.0225 |
| ZC3HAV1L | Zinc finger CCCH-type antiviral protein 1-like          | 0.0229 |
| HN1L     | Hematological and neurological expressed 1-like protein | 0.0229 |
| DDX3X    | ATP-dependent RNA helicase DDX3X                        | 0.0229 |
| RPS23    | 40S ribosomal protein S23                               | 0.0237 |
| FAU      | 40S ribosomal protein S30                               | 0.0277 |
| PCBP2    | Poly(rC)-binding protein 2                              | 0.0284 |
| EIF4A1   | Eukaryotic initiation factor 4A-I                       | 0.0285 |
| ISYNA1   | Inositol-3-phosphate synthase 1                         | 0.0297 |
| LGALS9   | Galectin-9                                              | 0.0321 |

**Table S5.** The proteomic comparison of FAB-M4/M5 AML cells with and without *NPM1-Ins*; a summary of proteins forming interacting networks (**Figure 1** of the main text) and showing increased levels in patients without *NPM1-Ins*. The table presents the gene name, protein names and key words with regard to protein function. The table is based on information from the Gene database and selected references from the PubMed database (accessed 29<sup>th</sup> of October 2023) [65-76].

| CYTOPLASMIC TRANSLATION (61 proteins) |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                         |
|---------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|
| BMS1                                  | <i>BMS1 ribosome biogenesis factor</i> . The gene encodes a ribosome assembly protein. A similar protein in yeast functions in 35S-rRNA processing, which includes a series of cleavage steps critical for formation of 40S ribosomes.                                                                                                                                                                                                                                                                                                                       | Ribosome assembly<br>Ribosome 40S                       |
| BTF3                                  | <i>Basic transcription factor 3</i> . This gene encodes the basic transcription factor 3. This protein forms a stable complex with RNA polymerase IIB and is required for transcriptional initiation.                                                                                                                                                                                                                                                                                                                                                        | Transcription initiation<br>RNA polymerase              |
| EBNA1BP2                              | <i>EBNA1 binding protein 2</i> . The encoded protein enables RNA binding activity. It is predicted to be involved in rRNA processing and ribosomal large subunit biogenesis. The protein is located in chromosome and nucleolus.                                                                                                                                                                                                                                                                                                                             | RNA binding and processing<br>Nucleolus<br>Ribosome 40S |
| EEF2                                  | <i>Eukaryotic translation elongation factor 2</i> . The encoded member of the GTP-binding translation elongation factor family is essential for protein synthesis. It promotes the GTP-dependent translocation of the nascent protein chain from the A-site to the P-site of the ribosome. The protein is completely inactivated by EF-2 kinase phosphorylation.                                                                                                                                                                                             | Translation<br>Elongation<br>Ribosome                   |
| EIF4A1                                | <i>Eukaryotic translation initiation factor 4A1</i> . The protein enables double-stranded RNA binding activity; it is predicted to be involved in cytoplasmic translational initiation.                                                                                                                                                                                                                                                                                                                                                                      | RNA binding<br>Translation initiation                   |
| EIF5B                                 | <i>Eukaryotic translation initiation factor 5B</i> . Accurate initiation of translation in eukaryotes is complex and requires many factors, some of which are composed of multiple subunits. The encoded protein eIF5B interacts with eIF1A on the ribosome along with other initiation factors and GTP to position the initiation methionine tRNA on the start codon of the mRNA so that translation initiates accurately.                                                                                                                                  | Ribosome<br>Translation initiation                      |
| FAU                                   | <i>FAU ubiquitin like and ribosomal protein S30 fusion</i> . This gene encodes a fusion protein consisting of the ubiquitin-like protein fubi at the N terminus and ribosomal protein S30 at the C terminus. It has been proposed that the fusion protein is post-translationally processed to generate free fubi and free ribosomal protein S30. Fubi is a member of the ubiquitin family, and ribosomal protein S30 belongs to the S30E family of ribosomal proteins. Ribosomal protein S30 is a component of the 40S subunit of the cytoplasmic ribosome. | Ribosome S40<br>Cytoplasm                               |
| FTSJ3                                 | <i>FtsJ RNA 2'-O-methyltransferase 3</i> . The gene encodes an RNA methyltransferase; the protein is localized to the nucleolus where it colocalizes with NIP7 and contributes in the formation of preribosomal complexes [70-72].                                                                                                                                                                                                                                                                                                                           | RNA<br>Nucleolus                                        |
| GNL3                                  | <i>G protein nucleolar 3</i> . The encoded protein may interact with p53, may be involved in carcinogenesis and protein also appears to be important for stem cell proliferation. This protein is found in both the nucleus and nucleolus. GNL3 seems to be involved ribosomal biogenesis as well as in regulation of cell proliferation, resistance to apoptosis, cellular differentiation and aging (for references see [73]. It may also be involved in myeloid leukemogenesis [65].                                                                      | Ribosome<br>Nucleus, nucleolus<br>Carcinogenesis        |
| KRR1                                  | <i>KRR1 small subunit processome component homolog</i> . The protein has RNA binding activity and is predicted to be involved in rRNA processing. It is localized in the nucleus.                                                                                                                                                                                                                                                                                                                                                                            | RNA binding<br>tRNA processing                          |
| NACA                                  | <i>Nascent polypeptide associated complex subunit alpha</i> . This encoded protein associates with basic transcription factor 3 (BTF3) to form the nascent polypeptide-associated complex (NAC). This complex binds to nascent proteins that lack a signal peptide motif as they emerge from the ribosome, blocking interaction with the signal recognition particle (SRP) and preventing mistranslocation to the endoplasmic reticulum.                                                                                                                     | Ribosome-RNA<br>Protein synthesis                       |
| NCC3/CCL15                            | <i>C-C motif chemokine ligand 15</i> . This secreted protein is chemotactic for T cells and monocytes, and acts through C-C chemokine receptor type 1 (CCR1). The proprotein is further processed into numerous smaller functional peptides. Naturally-occurring readthrough transcripts occur from this gene into the downstream gene CCL14.                                                                                                                                                                                                                | Chemokine CCL15                                         |
| NLE1                                  | <i>Notchless homolog 1</i> . Predicted to be involved in Notch signaling pathway and ribosomal large subunit assembly. Predicted to act in hematopoietic stem cell homeostasis and regulation of signal transduction. Located in nucleolus and nucleoplasm.                                                                                                                                                                                                                                                                                                  | Notch signaling<br>Ribosome S60 assembly                |

|        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                         |
|--------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PA2G4  | <i>Proliferation associated 2G4</i> . This RNA-binding protein is present in pre-ribosomal ribonucleoprotein complexes and may be involved in ribosome assembly and the regulation of intermediate and late steps of rRNA processing. This protein can interact with the cytoplasmic domain of the ErbB3 receptor and may contribute to transducing regulatory signals. This protein is also a transcriptional co-repressor of cell cycle regulatory genes through its interactions with histone deacetylases. This protein has been implicated in differentiation of human cancer cells. The protein seems to support leukemogenesis in human AML; Ebp1 is highly expressed in AML cells and regulates the level of ribosomal RNA synthesis by binding to RNA Polymerase I and enhancing the formation of the Pol I initiation complex [66,67]. The protein is also a substrate for MDM2 [66]. | Nucleoplasm and nucleolus<br>AML<br>Transcriptional corepressor<br>Histone deacetylation<br>RNA binding<br>Ribosomal assembly<br>Signal transduction<br>Differentiation |
| RCL1   | <i>RNA terminal phosphate cyclase like 1</i> . The protein is predicted to enable endoribonuclease activity and to be involved in endonucleolytic cleavage of tricistronic rRNA transcript. Predicted to be located in nucleoplasm and to be active in nucleolus.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Nucleoplasm, nucleolus<br>RNA-endoribonuclease                                                                                                                          |
| RPL2   | <i>Ribosomal protein L2</i> .                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Ribosome?                                                                                                                                                               |
| RPL3   | <i>Ribosomal protein L3</i> . This gene encodes a ribosomal protein that is a component of the 60S subunit. The protein belongs to the <u>L3P family</u> of ribosomal proteins and it is located in the cytoplasm. This gene is co-transcribed with several small nucleolar RNA genes.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Ribosome 60S<br>Cytoplasm                                                                                                                                               |
| RPL4   | <i>Ribosomal protein L4</i> . This ribosomal protein is a component of the 60S subunit and belongs to the <u>L4E family</u> of ribosomal proteins. It is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Ribosome 60S<br>Cytoplasm                                                                                                                                               |
| RPL7   | <i>Ribosomal protein L7</i> . The encoded ribosomal protein is a component of the 60S subunit and belongs to the <u>L30P family</u> of ribosomal proteins. It contains an N-terminal basic region-leucine zipper (BZIP)-like domain and the RNP consensus submotif RNP2. In vitro the BZIP-like domain mediates homodimerization and stable binding to DNA and RNA, with a preference for 28S rRNA and mRNA. The protein can inhibit cell-free translation of mRNAs, suggesting that it plays a regulatory role in the translation apparatus. It is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                   | Ribosome 60S<br>Cytoplasm<br>RNA binding<br>DNA binding<br>Inhibition of translation?                                                                                   |
| RPL7A  | <i>Ribosomal protein L7A</i> . This gene encodes a ribosomal protein that is a component of the 60S subunit. The protein belongs to the <u>L7AE family</u> of ribosomal proteins. It can interact with a subclass of nuclear hormone receptors and inhibit their ability to transactivate by preventing their binding to their DNA response elements. The gene is co-transcribed with the U24, U36a, U36b, and U36c small nucleolar RNA genes. This gene may be involved in carcinogenesis; it rearranges with the trk proto-oncogene to form the chimeric oncogene trk-2h, which encodes an <u>oncoprotein</u> consisting of the N terminus of ribosomal protein L7a fused to the receptor tyrosine kinase domain of trk [68,69].                                                                                                                                                              | Ribosome 60S<br>Nuclear receptor inhibition<br>Chimeric oncoprotein                                                                                                     |
| RPL9   | <i>Ribosomal protein P1</i> . This gene encodes a ribosomal protein that is a component of the 60S subunit. The protein belongs to the <u>L6P family</u> of ribosomal proteins. It is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Ribosome 60S<br>Cytoplasm                                                                                                                                               |
| RPL11  | <i>Ribosomal protein L11</i> . Ribosomes consist of a small 40S subunit and a large 60S subunit. Together these subunits are composed of 4 RNA species and approximately 80 structurally distinct proteins. This gene encodes a ribosomal protein that is a component of the 60S subunit. The protein belongs to the <u>L5P family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Ribosome 60S<br>Cytoplasm                                                                                                                                               |
| RPL12  | <i>Ribosomal protein L12</i> . This gene encodes a component of the 60S ribosomal subunit. It belongs to the <u>L11P family</u> of ribosomal proteins and is located in the cytoplasm. The protein binds directly to the 26S rRNA. This gene is co-transcribed with the U65 snoRNA.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Ribosome 60S<br>Cytoplasm                                                                                                                                               |
| RPL13  | <i>Ribosomal protein L13</i> . The encoded ribosomal protein is a component of the 60S subunit. It belongs to the <u>L13E family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Ribosome 60S<br>Cytoplasm                                                                                                                                               |
| RPL14  | <i>Ribosomal protein L14</i> . This ribosomal protein is a component of the 60S subunit. It belongs to the <u>L14E family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Ribosome 60S<br>Cytoplasm                                                                                                                                               |
| RPL15  | <i>Ribosomal protein L15</i> . This protein is a member of the <u>L15E family</u> of ribosomal proteins and a component of the 60S subunit.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Ribosome 60S                                                                                                                                                            |
| RPL18  | <i>Ribosomal protein L18</i> . This protein is a member of the <u>L18E family</u> of ribosomal proteins and is a component of the 60S subunit.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Ribosome 60S                                                                                                                                                            |
| RPL18A | <i>Ribosomal protein L18A</i> . This gene encodes a member of the <u>L18AE family</u> of ribosomal proteins and is a component of the 60S subunit. This gene is co-transcribed with the U68 snoRNA, located within the third intron.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Ribosome 60S                                                                                                                                                            |
| RPL19  | <i>Ribosomal protein L19</i> . This gene encodes a ribosomal protein that is a component of the 60S subunit. The protein belongs to the <u>L19E family</u> of ribosomal proteins. It is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Ribosome 60S<br>Cytoplasm                                                                                                                                               |

|         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                          |
|---------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| RPL21   | <i>Ribosomal protein L21.</i> This gene encodes a ribosomal protein that is a component of the 60S subunit. It belongs to the <u>L21E family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                           | Ribosome 60S<br>Cytoplasm                                                                |
| RPL23A  | <i>Ribosomal protein L23A.</i> This gene encodes a ribosomal protein that is a component of the 60S subunit. It belongs to the <u>L23P family</u> of ribosomal proteins and is located in the cytoplasm. This gene is co-transcribed with the U42A, U42B, U101A, and U101B small nucleolar RNA genes, which are located in its third, first, second, and fourth introns, respectively.                                                                                                                                                                            | Ribosome 60S<br>Cytoplasm                                                                |
| RPL24   | <i>Ribosomal protein L24.</i> The encoded ribosomal protein is a component of the 60S subunit. It belongs to the <u>L24E family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                                        | Ribosome 60S<br>Cytoplasm                                                                |
| RPL26   | <i>Ribosomal protein L26.</i> This encoded ribosomal protein is a component of the 60S subunit. The protein belongs to the <u>L24P family</u> of ribosomal proteins. It is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                              | Ribosome 60S<br>Cytoplasm                                                                |
| RPL27A  | <i>Ribosomal protein L27A.</i> This gene encodes a ribosomal protein that is a component of the 60S subunit. It belongs to the <u>L15P family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                          | Ribosome 60S<br>Cytoplasm                                                                |
| RPL28   | <i>Ribosomal protein L28.</i> This gene encodes a ribosomal protein that is a component of the 60S subunit. The protein belongs to the L28E family of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                         | Ribosome 60S<br>Cytoplasm                                                                |
| RPL29   | <i>Ribosomal protein L29.</i> The gene encodes a cytoplasmic ribosomal protein that is a component of the 60S subunit. The protein belongs to the <u>L29E family</u> of ribosomal proteins. The protein is also a peripheral membrane protein expressed on the cell surface.                                                                                                                                                                                                                                                                                      | Ribosome 60S<br>Cytoplasm<br>Cell surface                                                |
| RPL31   | <i>Ribosomal protein L31.</i> This gene encodes a ribosomal protein that is a component of the 60S subunit. The protein belongs to the <u>L31E family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                  | Ribosome 60S<br>Cytoplasm                                                                |
| RPL32   | <i>Ribosomal protein L32.</i> This gene encodes a ribosomal protein that is a component of the 60S subunit. It belongs to the <u>L32E family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                           | Ribosome 60S<br>Cytoplasm                                                                |
| RPL34   | <i>Ribosomal protein L34.</i> The encoded ribosomal protein is a component of the 60S subunit and belongs to the <u>L34E family</u> of ribosomal proteins. It is located in the cytoplasm. Overexpression of this gene has been observed in some cancer cells.                                                                                                                                                                                                                                                                                                    | Ribosome 60S<br>Cytoplasm<br>Cancer                                                      |
| RPL36   | <i>Ribosomal protein L36.</i> The encoded ribosomal protein is a component of the 60S subunit and protein belongs to the <u>L36E family</u> of ribosomal proteins. It is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                                | Ribosome 60S<br>Cytoplasm                                                                |
| RPL36AL | <i>Ribosomal protein L36A like.</i> This gene encodes a ribosomal protein that is a component of the 60S subunit and belongs to the <u>L44E (L36AE) family</u> of ribosomal proteins.                                                                                                                                                                                                                                                                                                                                                                             | Ribosome 60S                                                                             |
| RPLP1   | <i>Ribosomal protein lateral stalk subunit P1.</i> This encoded ribosomal phosphoprotein is a component of the 60S subunit, belongs to the <u>L12P family</u> of ribosomal proteins and has an important role in the elongation step of protein synthesis. The protein is located in the cytoplasm.                                                                                                                                                                                                                                                               | Ribosome 60S<br>Protein elongation<br>Cytoplasm                                          |
| RPLP2   | <i>Ribosomal protein lateral stalk subunit P2.</i> The encoded ribosomal phosphoprotein is a component of the 60S subunit, it belongs to the L12P family of ribosomal proteins and has a role in the elongation step of protein synthesis. The protein is located in the cytoplasm.                                                                                                                                                                                                                                                                               | Ribosome 60S<br>Protein elongation<br>Cytoplasm                                          |
| RPS1    | <i>Ribosomal protein S1.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Ribosome                                                                                 |
| RPS3    | <i>Ribosomal protein S3.</i> This ribosomal protein that is a component of the 40S subunit, where it forms part of the domain where translation is initiated. It belongs to the <u>S3P family</u> of ribosomal proteins. Studies of the mouse and rat proteins have demonstrated that the protein has an <u>extraribosomal role as an endonuclease</u> involved in DNA damage. The protein appears to be located in both the cytoplasm and nucleus but not in the nucleolus. This gene is co-transcribed with the small <u>nucleolar RNA genes U15A and U15B.</u> | Ribosome 40S<br>Translation initiation<br>Cytoplasm, nucleus<br>Endonuclease, DNA repair |
| RPS5    | <i>Ribosomal protein S5.</i> This gene encodes a ribosomal protein that is a component of the 40S subunit. It belongs to the <u>S7P family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                                                                                                                                                                                                                             | Ribosome 40S<br>Cytoplasm                                                                |
| RPS8    | <i>Ribosomal protein S8.</i> The protein belongs to the <u>S8E family</u> of ribosomal proteins and is located in the cytoplasm. This gene is co-transcribed with the small nucleolar RNA genes U38A, U38B, U39, and U40 located in its fourth, fifth, first, and second introns, respectively.                                                                                                                                                                                                                                                                   | Ribosome<br>Cytoplasm                                                                    |

|        |                                                                                                                                                                                                                                                                                                                                                                         |                                           |
|--------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| RPS9   | <i>Ribosomal protein S9</i> . The encoded ribosomal protein is a component of the 40S subunit and belongs to the <u>S4P family</u> of ribosomal proteins. It is located in the cytoplasm.                                                                                                                                                                               | Ribosome 40S<br>Cytoplasm                 |
| RPS17  | <i>Ribosomal protein S17</i> . This gene encodes a ribosomal protein that is a component of the 40S subunit. The protein belongs to the <u>S17E family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                       | Ribosome 40S<br>Cytoplasm                 |
| RPS18  | <i>Ribosomal protein S18</i> . This gene encodes a ribosomal protein that is a component of the 40S subunit. The protein belongs to the <u>S13P family</u> of ribosomal proteins and is located in the cytoplasm. The gene product of the E. coli ortholog (ribosomal protein S13) is involved in the binding of fMet-tRNA, and thus, in the initiation of translation. | Ribosome 40S<br>Cytoplasm<br>Translation? |
| RPS20  | <i>Ribosomal protein S20</i> . The encoded ribosomal protein is a component of the 40S subunit. It belongs to the <u>S10P family</u> of ribosomal proteins and is located in the cytoplasm. This gene is co-transcribed with the small nucleolar RNA gene U54, which is located in its second intron.                                                                   | Ribosome S40<br>Cytoplasm                 |
| RPS23  | <i>Ribosomal protein S23</i> . This ribosomal protein is a component of the 40S subunit and belongs to the <u>S12P family</u> of ribosomal proteins. It is located in the cytoplasm.                                                                                                                                                                                    | Ribosome 40S<br>Cytoplasm                 |
| RPS24  | <i>Ribosomal protein S24</i> . This gene encodes a ribosomal protein that is a component of the 40S subunit. The protein belongs to the <u>S24E family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                       | Ribosome 40S<br>Cytoplasm                 |
| RPS28  | <i>Ribosomal protein S28</i> . This gene encodes a ribosomal protein that is a component of the 40S subunit. It belongs to the <u>S28E family</u> of ribosomal proteins and is located in the cytoplasm.                                                                                                                                                                | Ribosome 40S<br>Cytoplasm                 |
| RPS29  | <i>Ribosomal protein S29</i> . This gene encodes a ribosomal protein that is a component of the 40S subunit and a member of the <u>S14P family</u> of ribosomal proteins. It can enhance the tumor suppressor activity of Ras-related protein 1A (KREVI) and is located in the cytoplasm.                                                                               | Ribosome 40S<br>Cytoplasm                 |
| SERBP1 | <i>SERPINE1 mRNA binding protein 1</i> . The encoded protein enables SUMO binding activity; mRNA 3'-UTR binding activity; and ribosome binding activity. It is involved in PML body organization. The protein is located in cytosol and nucleus.                                                                                                                        | Ribosome binding<br>Cytosol and nucleus   |
| SPL13A | <i>Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein</i> .                                                                                                                                                                                                                                                                        |                                           |
| SRP14  | <i>Signal recognition particle 14</i> . The protein has RNA binding activity and is involved in protein targeting to endoplasmic reticulum. It is also located in nucleus.                                                                                                                                                                                              | RNA binding                               |
| SRP9   | <i>Signal recognition particle 9</i> . The protein is predicted to have RNA binding activity and signal recognition particle binding activity. It is also predicted to be involved in SRP-dependent cotranslational protein targeting to membrane. Predicted to be located in cytosol.                                                                                  | RNA binding                               |
| UTP3   | <i>UTP3 small subunit processome component</i> . The protein has RNA binding activity. Predicted to be involved. It is located in the nucleolus.                                                                                                                                                                                                                        | RNA binding                               |

#### PROTEIN STABILIZATION (4 proteins)

|                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                      |
|--------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| CCT2                     | <i>Chaperonin containing TCP1 subunit 2</i> . The encoded protein is a molecular chaperone that is a member of the <u>chaperonin containing TCP1 complex (CCT)</u> , also known as the <u>TCP1 ring complex (TRiC)</u> . Unfolded polypeptides enter the central cavity of the complex and are folded in an ATP-dependent manner. The complex folds various proteins, including actin and tubulin. The complex seems to have an antiapoptotic effect through inhibition of autophagy; inhibition of the CCT/TCP1/Alt-mTOR pathway is thereby a possible strategy for targeted therapy in human AML [74]. | Molecular chaperon<br>CCT/TRiC complex<br>Actin, tubulin<br>Inhibits autophagy and apoptosis<br>AML-chemoresistance  |
| CCT8<br>(see CCT2 above) | <i>Chaperonin containing TCP1 subunit 8</i> . The encoded protein is the theta subunit of the <u>CCT chaperonin</u> , which is abundant in cytosol and may be involved in the transport and assembly of newly synthesized proteins.                                                                                                                                                                                                                                                                                                                                                                      | Molecular chaperon<br>CCT/ TRiC complex<br>Actin, tubulin<br>Inhibits autophagy and apoptosis<br>AML-chemoresistance |
| PFDN2                    | <i>Prefoldin subunit 2</i> . The encoded protein is a member of the prefoldin beta subunit family. It is one of six subunits of prefoldin, a molecular chaperone complex that binds and stabilizes newly synthesized polypeptides, thereby allowing them to fold correctly. The complex, consisting of two alpha and four beta subunits, forms a double beta barrel assembly with six protruding coiled-coils.                                                                                                                                                                                           | Prefoldin complex                                                                                                    |

|                             |                                                                                                                                                                                                  |                                                                                                                         |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| TCP1<br>(see CCT2<br>above) | <i>t-complex 1</i> . The encoded protein is a molecular chaperone that is a member of the chaperonin containing <u>TCP1 complex (CCT)</u> , also known as the TCP1 ring complex ( <u>TRiC</u> ). | Molecular chaperon<br>CCT/ TRiC complex<br>Actin, tubulin<br>Inhibits autophagy and<br>apoptosis<br>AML-chemoresistance |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|

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#### 2-DEOXYRIBONUCLEOTIDE BIOSYNTHETIC PROCESS (3 proteins)

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|       |                                                                                                                                                                                                                                                                                                                                                                                 |                                                         |
|-------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|
| CMPK2 | <i>Cytidine/uridine monophosphate kinase 2</i> . This encoded oritein is an enzymes in the nucleotide synthesis salvage pathway that may participate in monocytic differentiation. The gene encodes regulatory RNAs that may be involved in carcinogens and in addition a mitochondrial protein [75,76].                                                                        | Nucleotide synthesis<br>Mitochondria<br>Carcinogenesis? |
| DCTD  | <i>dCMP deaminase</i> . The protein catalyzes the deamination of dCMP to dUMP, the nucleotide substrate for thymidylate synthase. The encoded protein is allosterically activated by dCTP and inhibited by dTTP and is found as a homohexamer. This protein uses zinc as a cofactor.                                                                                            | Nucleotide metabolism                                   |
| RRM1  | <i>Ribonucleotide reductase catalytic subunit M1</i> . This gene encodes the large and catalytic subunit of ribonucleotide reductase, an enzyme essential for the conversion of ribonucleotides into deoxyribonucleotides. A pool of available deoxyribonucleotides is important for DNA replication during S phase of the cell cycle as well as multiple DNA repair processes. | Nucleotide metabolism<br>Cell cycle<br>DNA repair       |

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**Table S6.** Protein phosphorylation sites showing significantly increased levels in patients with *NPM1-Ins* compared with patients without *NPM1-Ins*. A total of 70 phosphosites were identified, and the table presents the gene name, the protein name, the position of the phosphorylated amino acid residue (position), the phosphorylated amino acid (AA) and the p-value from ANOVA analysis. The phosphosites are ranged according to their p-value. Phosphosites also identified in PPI analyses are marked with grey shadow.

| Gene name     | Protein name                                                            | Position | AA | ANOVA p-value |
|---------------|-------------------------------------------------------------------------|----------|----|---------------|
| TOR1AIP1      | Torsin-1A-interacting protein 1                                         | 154      | S  | 7.52E-06      |
| TOR1AIP1      | Torsin-1A-interacting protein 1                                         | 156      | S  | 7.52E-06      |
| TOR1AIP1      | Torsin-1A-interacting protein 1                                         | 157      | S  | 7.52E-06      |
| SSFA2         | Sperm-specific antigen 2                                                | 739      | S  | 3.37E-05      |
| DTNBP1        | Dysbindin                                                               | 321      | S  | 5.72E-05      |
| DNAJC1        | DnaJ homolog subfamily C member 1                                       | 479      | S  | 7.37E-05      |
| DNAJC1        | DnaJ homolog subfamily C member 1                                       | 480      | S  | 7.37E-05      |
| SRP72         | Signal recognition particle subunit SRP72                               | 625      | S  | 0.0001        |
| PSMD1         | 26S proteasome non-ATPase regulatory subunit 1                          | 315      | S  | 0.0002        |
| PSMD1         | 26S proteasome non-ATPase regulatory subunit 1                          | 311      | T  | 0,0002        |
| EEPD1         | Endonuclease/exonuclease/phosphatase family domain-containing protein 1 | 173      | S  | 0.0002        |
| TRPV2         | Transient receptor potential cation channel subfamily V member 2        | 3        | S  | 0.0002        |
| SEC61B        | Protein transport protein Sec61 subunit beta                            | 17       | S  | 0.0003        |
| MTDH          | Protein LYRIC                                                           | 568      | S  | 0.0004        |
| TMEM179B      | Transmembrane protein 179B                                              | 205      | S  | 0.0004        |
| TMEM179B      | Transmembrane protein 179B                                              | 206      | S  | 0.0004        |
| SH3BP1        | SH3 domain-binding protein 1                                            | 544      | S  | 0.0006        |
| PLEC          | Plectin                                                                 | 4613     | S  | 0.0009        |
| DTNBP1        | Dysbindin                                                               | 316      | S  | 0.0011        |
| NUCKS1        | Nuclear ubiquitous casein and cyclin-dependent kinase substrate 1       | 214      | S  | 0.0018        |
| NUCKS1        | Nuclear ubiquitous casein and cyclin-dependent kinase substrate 1       | 204      | S  | 0.0018        |
| NUCKS1        | Nuclear ubiquitous casein and cyclin-dependent kinase substrate 1       | 202      | T  | 0.0018        |
| FAM21A;FAM21C | WASH complex subunit FAM21A;WASH complex subunit FAM21C                 | 619      | S  | 0.0018        |
| FAM21A;FAM21C | WASH complex subunit FAM21A;WASH complex subunit FAM21C                 | 620      | S  | 0.0018        |
| ADAM17        | Disintegrin and metalloproteinase domain-containing protein 17          | 791      | S  | 0.0022        |
| SRRM2         | Serine/arginine repetitive matrix protein 2                             | 1103     | S  | 0.0024        |
| BRAT1         | BRCA1-associated ATM activator 1                                        | 742      | S  | 0.0027        |
| DPM1          | Dolichol-phosphate mannosyltransferase subunit 1                        | 9        | S  | 0.0031        |
| TFEB          | Transcription factor EB                                                 | 109      | S  | 0.0033        |
| TFEB          | Transcription factor EB                                                 | 114      | S  | 0.0033        |
| TFEB          | Transcription factor EB                                                 | 122      | S  | 0.0033        |
| UBE2J1        | Ubiquitin-conjugating enzyme E2 J1                                      | 266      | S  | 0.0036        |
| MEIS1         | Homeobox protein Meis1                                                  | 194      | S  | 0.0044        |
| STX12         | Syntaxin-12                                                             | 142      | S  | 0.0046        |
| IRS2          | Insulin receptor substrate 2                                            | 915      | S  | 0.0050        |
| IRS2          | Insulin receptor substrate 2                                            | 306      | S  | 0.0058        |
| ADD3          | Gamma-adducin                                                           | 649      | S  | 0.0062        |
| SLC39A7       | Zinc transporter SLC39A7                                                | 275      | S  | 0.0065        |
| SLC39A7       | Zinc transporter SLC39A7                                                | 276      | S  | 0.0065        |
| PSMD2         | 26S proteasome non-ATPase regulatory subunit 2                          | 16       | S  | 0.0074        |

|          |                                                                |      |   |        |
|----------|----------------------------------------------------------------|------|---|--------|
| ZC3H14   | Zinc finger CCCH domain-containing protein 14                  | 409  | S | 0.0080 |
| CCAR2    | Cell cycle and apoptosis regulator protein 2                   | 675  | S | 0.0104 |
| TGOLN2   | Trans-Golgi network integral membrane protein 2                | 298  | S | 0.0109 |
| CDK12    | Cyclin-dependent kinase 12                                     | 1244 | T | 0.0121 |
| PLEC     | Plectin                                                        | 4389 | S | 0.0143 |
| RCC1     | Regulator of chromosome condensation                           | 11   | S | 0.0146 |
| MTDH     | Protein LYRIC                                                  | 426  | S | 0.0154 |
| FAM21A   | WASH complex subunit FAM21A                                    | 539  | S | 0.0156 |
| CHAMP1   | Chromosome alignment-maintaining phosphoprotein 1              | 282  | S | 0.0158 |
| ITPR1    | Inositol 1,4,5-trisphosphate receptor type 1                   | 1716 | S | 0.0173 |
| CDK12    | Cyclin-dependent kinase 12                                     | 301  | S | 0.0177 |
| CDK12    | Cyclin-dependent kinase 12                                     | 303  | S | 0.0177 |
| AFF4     | AF4/FMR2 family member 4                                       | 180  | S | 0.0214 |
| VPS26B   | Vacuolar protein sorting-associated protein 26B                | 304  | S | 0.0215 |
| ZEB2     | Zinc finger E-box-binding homeobox 2                           | 760  | S | 0.0218 |
| ZEB2     | Zinc finger E-box-binding homeobox 2                           | 758  | T | 0.0218 |
| SRRM1    | Serine/arginine repetitive matrix protein 1                    | 769  | S | 0.0219 |
| BCLAF1   | Bcl-2-associated transcription factor 1                        | 339  | S | 0.0230 |
| IRS2     | Insulin receptor substrate 2                                   | 1149 | S | 0.0235 |
| SUN2     | SUN domain-containing protein 2                                | 12   | S | 0.0244 |
| ARHGAP30 | Rho GTPase-activating protein 30                               | 630  | S | 0.0248 |
| PBXIP1   | Pre-B-cell leukemia transcription factor-interacting protein 1 | 43   | S | 0.0272 |
| USP20    | Ubiquitin carboxyl-terminal hydrolase 20                       | 132  | S | 0.0287 |
| USP20    | Ubiquitin carboxyl-terminal hydrolase 20                       | 134  | S | 0.0287 |
| LNPEP    | Leucyl-cystinyl aminopeptidase                                 | 91   | S | 0.0301 |
| CHD2     | Chromodomain-helicase-DNA-binding protein 2                    | 1728 | S | 0.032  |
| STK11IP  | Serine/threonine-protein kinase 11-interacting protein         | 398  | S | 0.0333 |
| FCER1G   | High affinity immunoglobulin epsilon receptor subunit gamma    | 69   | S | 0.0340 |
| C16orf54 | Transmembrane protein C16orf54                                 | 194  | S | 0.0343 |
| CDKN2AIP | CDKN2A-interacting protein                                     | 131  | S | 0.0348 |

**Table S7.** The phosphoproteomic comparison of FAB-M4/M5 AML cells with and without *NPM1-Ins*; a summary of proteins forming interacting networks (**Figure 2** of the main text) and showing increased phosphorylation levels in patients with (spliceosome, proteasome) and without (ribosome biogenesis, NuRD complex) *NPM1-Ins*. The table presents the gene name, protein names and key words with regard to protein function. The table is based on information from the Gene database and selected references from the PubMed database (accessed 29<sup>th</sup> of October 2023) [77-85].

| <b>SPLICEOSOME (2 proteins)</b>         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                 |
|-----------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| SRRM1                                   | <i>Serine and arginine repetitive matrix 1</i> . The protein has RNA binding activity and is predicted to be involved in mRNA splicing via spliceosomes. It is located in nuclear speck.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | RNA binding<br>Spliceosome                                                                      |
| SRRM2                                   | <i>Serine and arginine repetitive matrix 2</i> . The encoded protein enables C2H2 zinc finger domain binding activity and protein N-terminus binding activity. The protein is involved in mRNA splicing, via effects on the spliceosome.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | mRNA splicing<br>Spliceosome                                                                    |
| <b>PROTEASOME (2 proteins)</b>          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                 |
| PSMD1                                   | <i>Proteasome 26S subunit, non-ATPase 1</i> . The 26S proteasome is a multicatalytic proteinase complex composed of two complexes, a 20S core and a 19S regulator. The 20S core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 alpha subunits and 2 rings are composed of 7 beta subunits. The 19S regulator is composed of a base, which contains 6 ATPase subunits and 2 non-ATPase subunits, and a lid, which contains up to 10 non-ATPase subunits. Proteasomes are distributed throughout eukaryotic cells at a high concentration and cleave peptides in an <u>ATP/ubiquitin-dependent process</u> in a non-lysosomal pathway. This gene encodes the largest <u>non-ATPase subunit</u> of the 19S regulator lid, which is responsible for <u>substrate recognition</u> and binding. A previous study suggests that proteins within the regulatory 19S subunit (including non-ATPase subunits) can be involved in leukemogenesis in human AML [77]. | 19S proteasomal subunit<br>Non-ATPase<br>AML                                                    |
| PSMD2                                   | <i>Proteasome 26S subunit ubiquitin receptor, non-ATPase 2</i> . This gene encodes one of the non-ATPase subunits of the 19S regulator lid. In addition to participation in proteasome function, this subunit may also participate in the Tumor necrosis factor (TNF) signaling pathway since it interacts with the TNF type 1 receptor. Furthermore, several molecules involved in or modulating Wnt signalling have a prognostic value in AML including PSMD2.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 19S proteasomal subunit<br>Non-ATPase<br>TNF signaling<br>Wnt signaling<br>AML chemosensitivity |
| <b>RIBOSOME BIOGENESIS (4 proteins)</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                 |
| UPT14A                                  | <i>UTP14A small subunit processome component</i> . This gene encodes a member of the uridine triphosphate 14 family. It is an essential component of a large ribonucleoprotein complex <u>bound to the U3 small nucleolar RNA</u> and is involved in <u>ribosome biogenesis</u> and 18S rRNA synthesis.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Ribosome biogenesis<br>RNA binding<br>Ribonucleoprotein                                         |
| KRR1                                    | <i>KRR1 small subunit processome component homolog</i> . The encoded protein enables RNA binding activity and is predicted to be involved in rRNA processing.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | tRNA processing                                                                                 |
| KRI1                                    | <i>KRI1 homolog</i> . This protein seems to be involved in regulation of apoptosis; it can have antiapoptotic effects possibly through modulation of MPK-1/ERK signaling. It also seems to be involved in DNA repair [78].                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Apoptosis?<br>DNA repair?                                                                       |
| AATF                                    | <i>Apoptosis antagonizing transcription factor</i> . The encoded protein was identified on the basis of its interaction with MAP3K12/DLK, a protein kinase involved in the induction of apoptosis. It contains a leucine zipper, which is a characteristic motif of transcription factors. Overexpression of this gene interfered with MAP3K12 induced apoptosis [79-82]. Due to its roles in ribosome biogenesis, checkpoint control/DNA damage and regulation of apoptosis AATF has been suggested as a possible therapeutic target in cancer treatment [83].                                                                                                                                                                                                                                                                                                                                                                                                                          | Ribosome biogenesis<br>Cell cycle regulation<br>Apoptosis<br>Transcription regulation?          |
| <b>NuRD COMPLEX (3 proteins)</b>        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                 |
| SAP30                                   | <i>Sin3A associated protein 30</i> . The encoded protein is a component of the histone deacetylase complex, which includes SIN3, SAP18, HDAC1, HDAC2, RbAp46, RbAp48 as well as other polypeptides. This complex is active in deacetylating core histone octamers, but inactive in                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Histone deacetylase                                                                             |

|         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                              |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
|         | deacetylating nucleosomal histones. Targeting of Sap30 mediated signaling is regarded as a possible strategy to eradicate leukemia-initiating cells in human AML [84].                                                                                                                                                                                                                                                                                                                                    |                                                              |
| MTA2    | <i>Metastasis associated 1 family member 2</i> . This encoded protein has been identified as a component of NuRD, a <u>nucleosome remodeling deacetylase</u> complex. Their indirect effects on transcriptional regulation may include chromatin remodeling. It has two DNA binding domains, a dimerization domain, and a domain commonly found in proteins that <u>methylate DNA</u> . The protein mediates deacetylation of p53 that is correlated with loss of growth inhibition in transformed cells. | Nuclear deacetylase<br>DNA methylation?<br>p53 deacetylation |
| GATAD2A | <i>GATA zinc finger domain containing 2A</i> . The encoded protein enables protein-macromolecule adaptor activity. It is involved in negative regulation of transcription and is located in nucleoplasm. The protein may also interact with STAT3 to suppress its activity through posttranslational modification [85].                                                                                                                                                                                   | Transcription<br>Nucleoplasm<br>STAT3                        |

**Table S8.** Protein phosphorylation sites showing significantly increased levels in patients without *NPM1-Ins* compared with patients with *NPM1-Ins*. A total of 76 phosphosites were identified, and the table presents the gene name, the protein name the position of the phosphorylated amino acid residue (position), the phosphorylated amino acid (AA) and the p-value from ANOVA analysis. The phosphosites are ranged according to their p-value. Phosphosites also identified in PPI analyses are marked with grey shadow.

| Gene name   | Protein name                                               | Position | AA | ANOVA P-value |
|-------------|------------------------------------------------------------|----------|----|---------------|
| CCDC86      | Coiled-coil domain-containing protein 86                   | 18       | S  | 3.49E-07      |
| CCDC86      | Coiled-coil domain-containing protein 86                   | 21       | S  | 3.49E-07      |
| KRI1        | Protein KRI1 homolog                                       | 639      | S  | 1.27E-06      |
| KRI1        | Protein KRI1 homolog                                       | 628      | S  | 1.39E-06      |
| SURF6       | Surfeit locus protein 6                                    | 138      | S  | 5.71E-05      |
| NPM1        | Nucleophosmin                                              | 125      | S  | 6.04E-05      |
| H1FX        | Histone H1x                                                | 31       | S  | 0.0002        |
| EZR;MSN;RDX | Ezrin;Moesin;Radixin                                       | 558      | T  | 0.0002        |
| SNIP1       | Smad nuclear-interacting protein 1                         | 54       | S  | 0,0002        |
| IQGAP2      | Ras GTPase-activating-like protein IQGAP2                  | 16       | S  | 0.0003        |
| SLC38A1     | Sodium-coupled neutral amino acid transporter 1            | 52       | S  | 0.0004        |
| TNKS1BP1    | 182 kDa tankyrase-1-binding protein                        | 1620     | S  | 0.0004        |
| TNKS1BP1    | 182 kDa tankyrase-1-binding protein                        | 1621     | S  | 0.0004        |
| DDX54       | ATP-dependent RNA helicase DDX54                           | 782      | S  | 0.0005        |
| GATAD2A     | Transcriptional repressor p66-alpha                        | 100      | S  | 0.0005        |
| GATAD2A     | Transcriptional repressor p66-alpha                        | 107      | S  | 0.0005        |
| SNIP1       | Smad nuclear-interacting protein 1                         | 52       | S  | 0.0005        |
| GATAD2A     | Transcriptional repressor p66-alpha                        | 114      | S  | 0.0006        |
| CCDC86      | Coiled-coil domain-containing protein 86                   | 66       | S  | 0.0008        |
| TNKS1BP1    | 182 kDa tankyrase-1-binding protein                        | 836      | S  | 0.0009        |
| CCDC86      | Coiled-coil domain-containing protein 86                   | 65       | T  | 0.0009        |
| DNTTIP1     | Deoxynucleotidyltransferase terminal-interacting protein 1 | 161      | S  | 0.0010        |
| MYO18A      | Unconventional myosin-XVIIIa                               | 2041     | S  | 0.0010        |
| MYO18A      | Unconventional myosin-XVIIIa                               | 2043     | S  | 0.0011        |
| NCK1        | Cytoplasmic protein NCK1                                   | 85       | S  | 0,0018        |
| BAZ1B       | Tyrosine-protein kinase BAZ1B                              | 158      | S  | 0.0019        |
| UHRF1       | E3 ubiquitin-protein ligase UHRF1                          | 287      | S  | 0.0020        |
| HIST1H1B    | Histone H1.5                                               | 18       | S  | 0.0020        |
| SKAP2       | Src kinase-associated phosphoprotein 2                     | 283      | S  | 0.0022        |
| CCDC86      | Coiled-coil domain-containing protein 86                   | 69       | S  | 0.0023        |
| FTSJ3       | pre-rRNA processing protein FTSJ3                          | 336      | S  | 0.0024        |
| CHAMP1      | Chromosome alignment-maintaining phosphoprotein 1          | 87       | S  | 0.0024        |
| HIST1H1D    | Histone H1.3                                               | 18       | T  | 0.0029        |
| FTSJ3       | pre-rRNA processing protein FTSJ3                          | 335      | S  | 0.0029        |
| NAP1L4      | Nucleosome assembly protein 1-like 4                       | 125      | S  | 0.0030        |
| SCAF11      | Protein SCAF11                                             | 481      | S  | 0.0032        |
| SCAF11      | Protein SCAF11                                             | 648      | S  | 0.0032        |
| RBM25       | RNA-binding protein 25                                     | 677      | S  | 0.0038        |
| MTA2        | Metastasis-associated protein MTA2                         | 435      | S  | 0.0042        |
| NMT1        | Glycylpeptide N-tetradecanoyltransferase 1                 | 47       | S  | 0.0051        |

|          |                                                                                                              |      |   |        |
|----------|--------------------------------------------------------------------------------------------------------------|------|---|--------|
| MYO18A   | Unconventional myosin-XVIIIa                                                                                 | 1998 | S | 0.0052 |
| MYO18A   | Unconventional myosin-XVIIIa                                                                                 | 2002 | S | 0.0052 |
| HMG1     | High mobility group protein HMG-I/HMG-Y                                                                      | 103  | S | 0.0054 |
| RBM39    | RNA-binding protein 39                                                                                       | 117  | S | 0.0057 |
| SMARCC1  | SWI/SNF complex subunit SMARCC1                                                                              | 328  | S | 0.0058 |
| SMARCC1  | SWI/SNF complex subunit SMARCC1                                                                              | 330  | S | 0.0058 |
| DDX21    | Nucleolar RNA helicase 2                                                                                     | 121  | S | 0.0060 |
| HMG1     | High mobility group protein HMG-I/HMG-Y                                                                      | 102  | S | 0.0063 |
| PPAN     | Suppressor of SWI4 1 homolog                                                                                 | 238  | S | 0.0070 |
| FNBP4    | Formin-binding protein 4                                                                                     | 499  | S | 0.0071 |
| FNBP4    | Formin-binding protein 4                                                                                     | 508  | S | 0.0071 |
| AATF     | Protein AATF                                                                                                 | 316  | S | 0.0077 |
| AATF     | Protein AATF                                                                                                 | 320  | S | 0.0077 |
| AATF     | Protein AATF                                                                                                 | 321  | S | 0.0077 |
| SNIP1    | Smad nuclear-interacting protein 1                                                                           | 58   | S | 0.0092 |
| UTP14A   | U3 small nucleolar RNA-associated protein 14 homolog A                                                       | 29   | S | 0.0093 |
| UTP14A   | U3 small nucleolar RNA-associated protein 14 homolog A                                                       | 31   | S | 0.0093 |
| SAP30    | Histone deacetylase complex subunit SAP30                                                                    | 131  | S | 0.0093 |
| SAP30    | Histone deacetylase complex subunit SAP30                                                                    | 138  | S | 0.0097 |
| TNKS1BP1 | 182 kDa tankyrase-1-binding protein                                                                          | 1666 | S | 0.0100 |
| DDX54    | ATP-dependent RNA helicase DDX54                                                                             | 39   | S | 0.0119 |
| NCOR1    | Nuclear receptor corepressor 1                                                                               | 172  | S | 0.0130 |
| DDX54    | ATP-dependent RNA helicase DDX54                                                                             | 41   | S | 0.0152 |
| ACACA    | Acetyl-CoA carboxylase 1; Biotin carboxylase                                                                 | 80   | S | 0.0155 |
| KRR1     | KRR1 small subunit processome component homolog                                                              | 3    | S | 0.0157 |
| TFAM     | Transcription factor A, mitochondrial                                                                        | 193  | S | 0.0159 |
| EIF5B    | Eukaryotic translation initiation factor 5B                                                                  | 214  | S | 0.0166 |
| TMPO     | Lamina-associated polypeptide 2, isoforms beta/gamma                                                         | 385  | S | 0.0174 |
| SMARCD1  | SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A containing DEAD/H box 1 | 212  | S | 0.0177 |
| PA2G4    | Proliferation-associated protein 2G4                                                                         | 2    | S | 0.0238 |
| TMPO     | Lamina-associated polypeptide 2, isoform alpha                                                               | 160  | T | 0.0246 |
| MKI67    | Antigen KI-67                                                                                                | 2528 | S | 0.0252 |
| DOCK2    | Dedicator of cytokinesis protein 2                                                                           | 1705 | S | 0.0293 |
| EIF5B    | Eukaryotic translation initiation factor 5B                                                                  | 135  | S | 0.0297 |
| EIF5B    | Eukaryotic translation initiation factor 5B                                                                  | 137  | S | 0.0297 |
| PDCD5    | Programmed cell death protein 5                                                                              | 119  | S | 0.0308 |

|     | <i>NPM1-Ins</i> | <i>FLT3-ITD</i> | <i>FLT3-TKD</i> | <i>NRAS</i> | <i>IKZF1</i> | <i>DNMT3A</i> | <i>TET2</i> | <i>IDH1</i> | <i>IDH2</i> | <i>RUNX1</i> | <i>CEPBA</i> | <i>KIT</i> | <i>KMT2A</i> | <i>WT1</i> | <i>RAD21</i> | <i>CDKN2A</i> | <i>ASXL1</i> | <i>BCORL1</i> | <i>PTPN11</i> | <i>CALR</i> | <i>CFR3R</i> | <i>EZH2</i> |
|-----|-----------------|-----------------|-----------------|-------------|--------------|---------------|-------------|-------------|-------------|--------------|--------------|------------|--------------|------------|--------------|---------------|--------------|---------------|---------------|-------------|--------------|-------------|
| P2  |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P13 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P14 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P18 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P23 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P24 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P34 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P44 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P46 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P47 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P49 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P51 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P53 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P10 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P11 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P17 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P22 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P29 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P31 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P36 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P37 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P42 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P43 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P45 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |
| P52 |                 |                 |                 |             |              |               |             |             |             |              |              |            |              |            |              |               |              |               |               |             |              |             |

**Figure S1.** Molecular genetic analyses of primary AML cells with (top rows) and without (bottom rows) *NPM1-Ins*. We performed a submicroscopic mutational profiling of 54 genes frequently mutated in AML by characterizing the Illuminas TruSight Myeloid Gene Panel [127]. The mutated genes are indicated at the top of the figure, the patient identity is presented in the left column and listed in the same way as in **Table S1**. The figure presents the results only for those genes that were mutated for at least one of the patients included in the present study. The four patients marked with grey were only tested for *NPM1* and *FLT3* abnormalities.

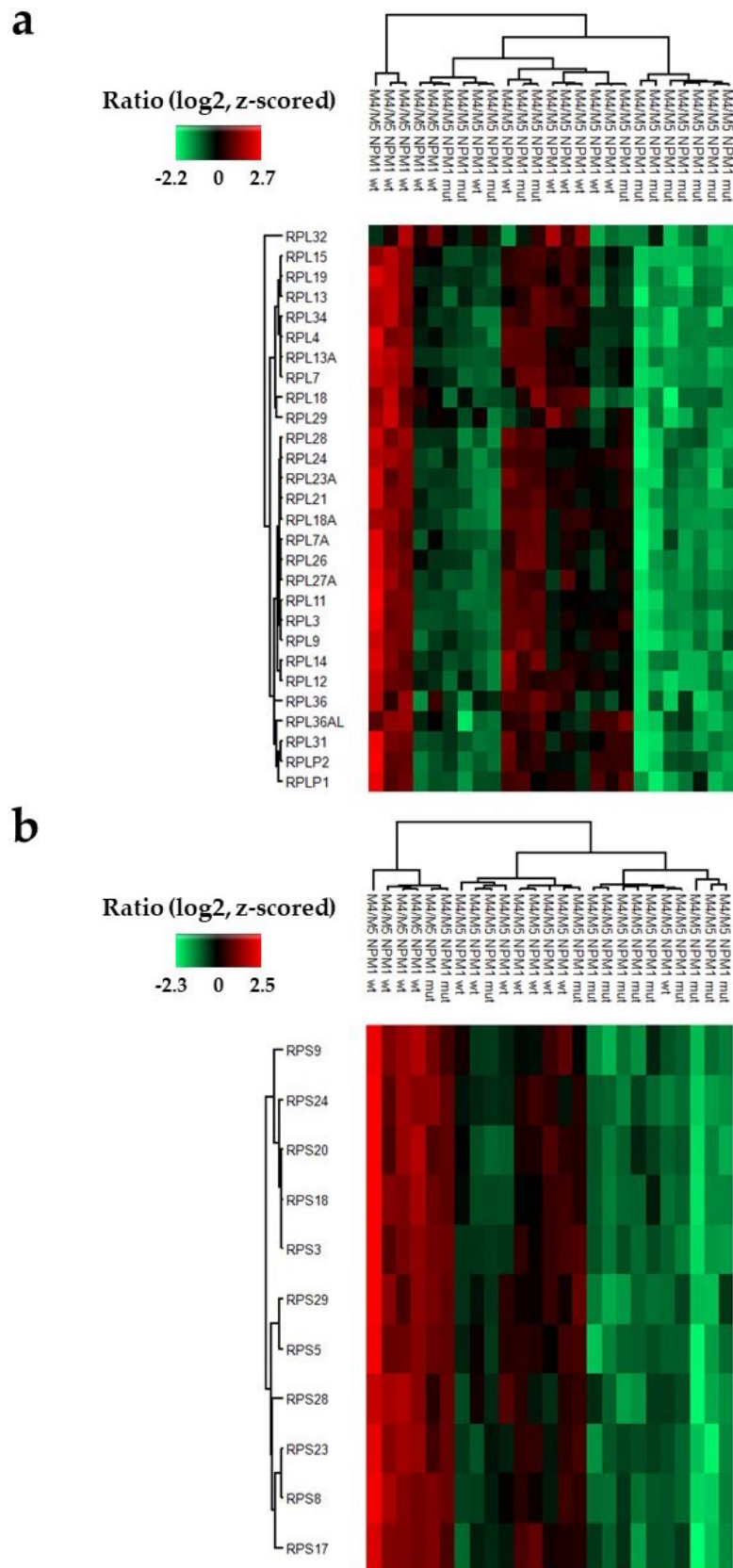
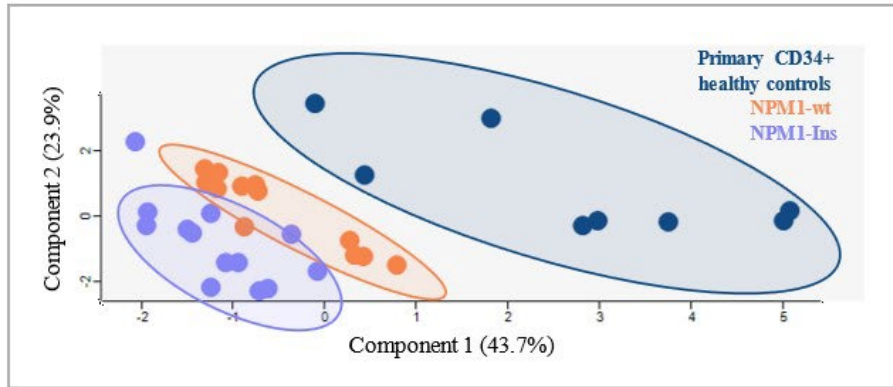


**Figure S2.** Hierarchical clustering analysis including all our FAB-M4/M5 patients and based on ribosomal proteins included in identified protein-protein interaction (PPI) networks. The two analyses were based on the differentially expressed large (**a**) and small (**b**) ribosomal subunit proteins with a significantly different regulation identified by ANOVA. Samples with and without *NPM1-Ins* are referred to as *NPM1* mut and *NPM1* wt, respectively, in this figure.

**a**



**b**

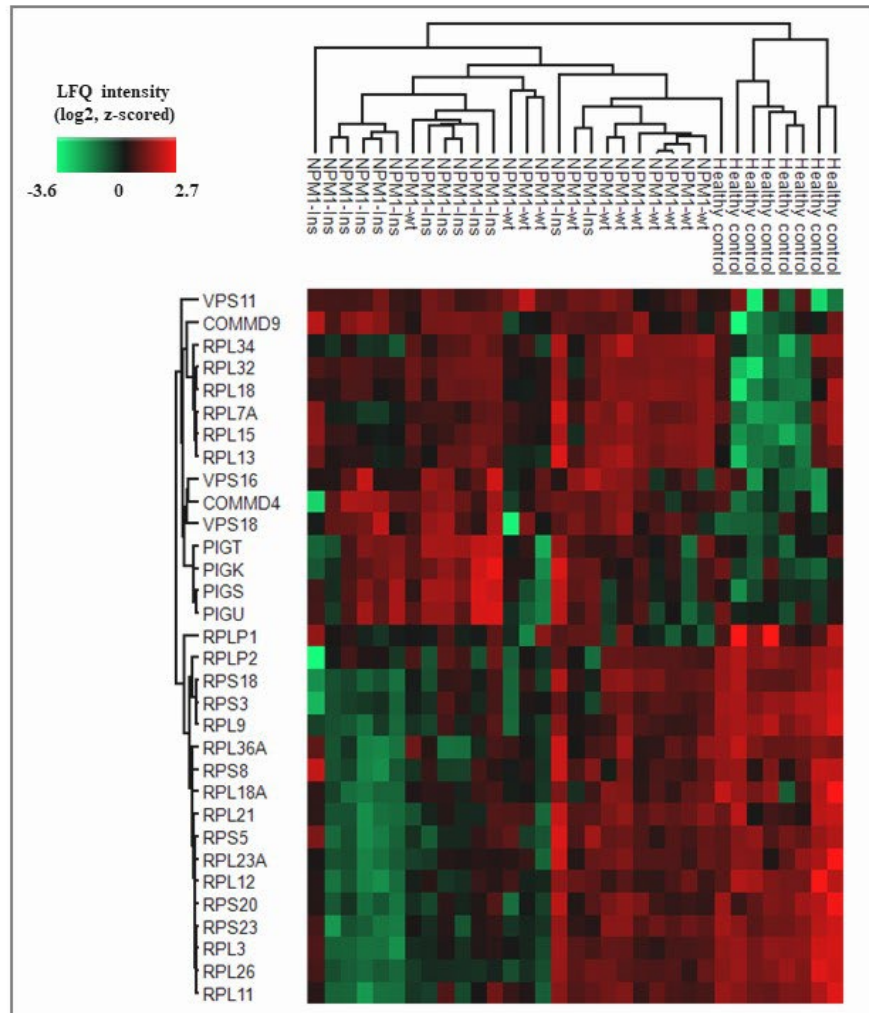


**Figure S3.** Principal component (a) and unsupervised hierarchical clustering (b) analysis of FAB-M4/M5 patients with and without (referred to as *NPM1-wt* in the figure) *NPM1-Ins*; a comparison with normal CD34<sup>+</sup> bone marrow cells (referred to as healthy controls in the figure). The label-free quantification (LFQ) analysis was based on the expression of the 32 out of 53 differentially expressed proteins that were identified by analysis of PPIs in **Figures 1b and 1d** after ANOVA test with quantitative values in all CD34<sup>+</sup> as well as in patient samples.

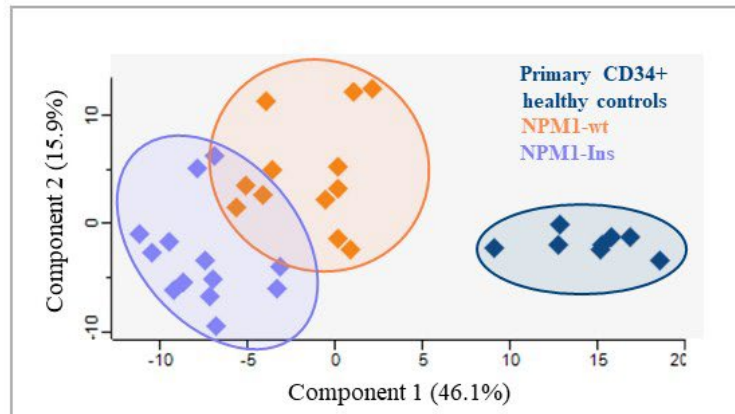
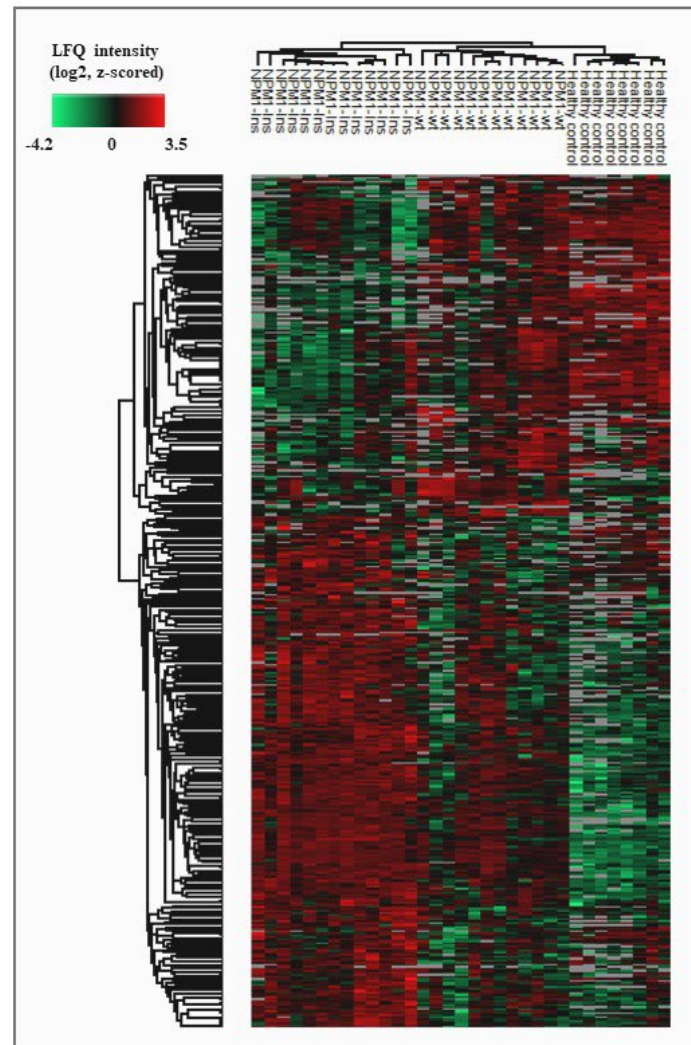
**a****b**

**Figure S4.** Principal component (a) and unsupervised hierarchical clustering (b) analysis of FAB-M4/M5 patients with and without (referred to as *NPM1-wt* in the figure) *NPM1-Ins*; a comparison with normal CD34<sup>+</sup> bone marrow cells (referred to as healthy controls in the figure). The LFQ analysis was based on the expression of 426 differentially regulated proteins found after a *t*-test performance.