Figure S1. Alignment of the WT, expected and actual sequences encoding Vim (NM_003380) and the knock-in region. The expected KI and actual sequences matched perfectly while they differ to the WT in the expected regions. Two reads amplified using each of the following primers: 170F, 324R, 324F and 170R had been shown. Recoded bases corresponding to gRNA targeting site were coloured in red.

`5'HA`; `VIM missing part`; `T2A`; `mCardinal`; `3xFLAG`; `3'HA`; `VIM gRNA target site`

```
expected  TCGAACCCAAGTACACTCTTGCATTCTATGCTTTAAGTTAAATGCAAACTCCTTTTTCCT 60

genomic   ------------------------------------TAAATGCAAACTCCTTTTTCCT 60

170FI     ------------------------------------------------------------ 60

170FII    ------------------------------------------------------------ 60

324RI     ------------------------------------------------------------ 60

324RII    ------------------------------------------------------------ 60

324FI     ------------------------------------------------------------ 60

324FII    ------------------------------------------------------------ 60

170RI     ------------------------------------------------------------ 60

170RII    ------------------------------------------------------------ 60


expected  TCTTCCTGCTGCAAGTACTATCTCATCCTGATGCTCAAGAGTGTCAGGGCCTGGGTTTCC 120

genomic   TCTTCCTGCTGCAAGTACTATCTCATCCTGATGCTCAAGAGTGTCAGGGCCTGGGTTTCC 120

170FI     --------------------TCTCATCCTGATGCTCAAGAGTGTCAGGGCCTGGGTTTCC 120

170FII    --------------------CTCATCCTGATGCTCAAGAGTGTCAGGGCCTGGGTTTCC 120

324RI     ------------------------------------------------------------ 120

324RII    ------------------------------------------------------------ 120

324FI     ------------------------------------------------------------ 120

324FII    ------------------------------------------------------------ 120

170RI     ------------------------------------------------------------ 120

170RII    ------------------------------------------------------------ 120


expected  AAACAGAGACTACCCTAAAATTATTTGGCGAGTAGTACTTTACACAATTGCCTCTCCCCC 180

genomic   AAACAGAGACTACCCTAAAATTATTTGGCGAGTAGTACTTTACACAATTGCCTCTCCCCC 180

170FI     AAACAGAGACTACCCTAAAATTATTTGGGGAGTAGTACTTTACACAATTGCCTCTCCCCC 180

170FII    AAACAGAGACTACCCTAAAATTATTTGGGGAGTAGTACTTTACACAATTGCCTCTCCCCC 180

324RI     ------------------------------------------------------------ 180

324RII    ------------------------------------------------------------ 180

324FI     ------------------------------------------------------------ 180

324FII    ------------------------------------------------------------ 180

170RI     ------------------------------------------------------------ 180

170RII    ------------------------------------------------------------ 180


expected  ACAAATCATAATTGTTTCAGTAAAATGGTTACTTGGTTTTTCCAAGAAAAAACTCGTTTT 240

genomic   ACAAATCATAATTGTTTCAGTAAAATGGTTACTTGGTTTTTCCAAGAAAAAACTCGTTTT 240

170FI     ACAAATCATAATTGTTTCAGTAAAATGGTTACTTGGTTTTTCCAAGAAAAAACTCGTTTT 240

170FII    ACAAATCATAATTGTTTCAGTAAAATGGTTACTTGGTTTTTCCAAGAAAAAACTCGTTTT 240

324RI     ------------------------------------------------------------ 240

324RII    ------------------------------------------------------------ 240

324FI     ------------------------------------------------------------ 240

324FII    ------------------------------------------------------------ 240
```

```
170RI     ------------------------------------------------------------ 240

170RII    ------------------------------------------------------------ 240


expected  TACTCATTTTTGGCCTGTTTGTTTATTTAGAAACTAATCTGGATTCACTCCCTCTGGTTG 300

genomic   TACTCATTTTTGGCCTGTTTGTTTATTTAGAAACTAATCTGGATTCACTCCCTCTGGTTG 300

170FI     TACTCATTTTTGGCCTGTTTGTTTATTTAGAAACTAATCTGGATTCACTCCCTCTGGTTG 300

170FII    TACTCATTTTTGGCCTGTTTGTTTATTTAGAAACTAATCTGGATTCACTCCCTCTGGTTG 300

324RI     ------------------------------------------------------------ 300

324RII    ------------------------------------------------------------ 300

324FI     ------------------------------------------------------------ 300

324FII    ------------------------------------------------------------ 300

170RI     ------------------------------------------------------------ 300

170RII    ------------------------------------------------------------ 300


expected  ATACCCACTCAAAAAGGACACTTCTGATTAAGACGGTTGAAACTAGAGATGGACAGGTTG 360

genomic   ATACCCACTCAAAAAGGACACTTCTGATTAAGACGGTTGAAACTAGAGATGGACAGGTTG 360

170FI     ATACCCACTCAAAAAGGACACTTCTGATTAAGACGGTTGAAACTAGAGATGGACAGGTTG 360

170FII    ATACCCACTCAAAAAGGACACTTCTGATTAAGACGGTTGAAACTAGAGATGGACAGGTTG 360

324RI     ------------------------------------------------------------ 360

324RII    ------------------------------------------------------------ 360

324FI     ------------------------------------------------------------ 360

324FII    ------------------------------------------------------------ 360

170RI     ------------------------------------------------------------ 360

170RII    ------------------------------------------------------------ 360


expected  GTATCTTTTAAGGAAAAAATAGGGTAATCTCAGACAGGAGTTGATATATTTTAAAATCAG 420

genomic   GTATCTTTTAAGGAAAAAATAGGGTAATCTCAGACAGGAGTTGATATATTTTAAAATCAG 420

170FI     GTATCTTTTAAGGAAAAAATAGGGTAATCTCAGACAGGAGTTGATATATTTTAAAATCAG 420

170FII    GTATCTTTTAAGGAAAAAATAGGGTAATCTCAGACAGGAGTTGATATATTTTAAAATCAG 420

324RI     ----------------------------------------------------AAAATCAG 420

324RII    -----------------------------------------------------AAAATCAG 420

324FI     ------------------------------------------------------------ 420

324FII    ------------------------------------------------------------ 420

170RI     ------------------------------------------------------------ 420

170RII    ------------------------------------------------------------ 420


expected  TGAATCTGAATCTCAGATACAGCTGGCTAATTTGAGAGGTTCAGGTTTCATTCATGCCTA 480

genomic   TGAATCTGAATCTCAGATACAGCTGGCTAATTTGAGAGGTTCAGGTTTCATTCATGCCTA 480

170FI     TGAATCTGAATCTCAGATACAGCTGGCTAATTTGAGAGGTTCAGGTTTCATTCATGCCTA 480

170FII    TGAATCTGAATCTCAGATACAGCTGGCTAATTTGAGAGGTTCAGGTTTCATTCATGCCTA 480

324RI     TGAATCTGAATCTCAGATACAGCTGGCTAATTTGAGAGGTTCAGGTTTCATTCATGCCTA 480

324RII    TGAATCTGAATTTCAGATACAGCTGGCTAATTTGAGAGGTTCAGGTTTCATTCATGCCTA 480

324FI     ------------------------------------------------------------ 480

324FII    ------------------------------------------------------------ 480

170RI     ------------------------------------------------------------ 480
```

```
170RII     ------------------------------------------------------------ 480


expected   CTAAAAAAAGAATAGGC-TTCTTCTTCCAGCAGTACACACAGCCAACTAATTATTTGGCT 540

genomic    CTAAAAAAAGAATAGGC-TTCTTCTTCCAGCAGTACACACAGCCAACTAATTATTTGGCT 540

170FI      CTAAAAAAAGAATAGGC-TTCTTCTTCCAGCAGTACACACAGCCAACTAATTATTTGGCT 540

170FII     CTAAAAAAAGAATAGGC-TTCTTCTTCCAGCAGTACACACAGCCAACTAATTATTTGGCT 540

324RI      CTAAAAAAAGAATAGGCTTTTTTTTTCCAGCAGTACACACAGCCAACTAATTATTTGGCT 540

324RII     CTAAAAAAAGAATAGGC-TTCTTTTTCCAGCAGTACACACAGCCAACTAATTATTTGGCT 540

324FI      ------------------------------------------------------------ 540

324FII     ------------------------------------------------------------ 540

170RI      ------------------------------------------------------------ 540

170RII     ------------------------------------------------------------ 540


expected   CCTGGATGTGAAGTTGAGATAGCAGTCTTCCTGTGCTCCAGAATTAGTGATTTGCTTTGG 600

genomic    CCTGGATGTGAAGTTGAGATAGCAGTCTTCCTGTGCTCCAGAATTAGTGATTTGCTTTGG 600

170FI      CCTGGATGTGAAGTTGAGATAGCAGTCTTCCTGTGCTCCAGAATTAGTGATTTGCTTTGG 600

170FII     CCTGGATGTGAAGTTGAGATAGCAGTCTTCCTGTGCTCCAGAATTAGTGATTTGCTTTGG 600

324RI      CCTGGATGTGAAGTTGAGATAGCAGTCTTCCTGTGCTCCAGAATTAGTGATTTGCTTTGG 600

324RII     CCTGGATGTGAAGTTGAGATAGCAGTCTTCCTGTGCTCCAGAATTAGTGATTTGCTTTGG 600

324FI      ------------------------------------------------------------ 600

324FII     ------------------------------------------------------------ 600

170RI      ------------------------------------------------------------ 600

170RII     ------------------------------------------------------------ 600


expected   TGCTTAATTTGAAGTGGGAGTAAGCTTCCTTAAACCACTTCCTAAAGCAGCTACATGAAA 660

genomic    TGCTTAATTTGAAGTGGGAGTAAGCTTCCTTAAACCACTTCCTAAAGCAGCTACATGAAA 660

170FI      TGCTTAATTTGAAGTGGGAGTAAGCTTCCTTAAACCACTTCCTAAAGCAGCTACATGAAA 660

170FII     TGCTTAATTTGAAGTGGGAGTAAGCTTCCTTAAACCACTTCCTAAAGCAGCTACATGAAA 660

324RI      TGCTTAATTTGAAGTGGGAGTAAGCTTCCTTAAACCACTTCCTAAAGCAGCTACATGAAA 660

324RII     TGCTTAATTTGAAGTGGGAGTAAGCTTCCTTAAACCACTTCCTAAAGCAGCTACATGAAA 660

324FI      ------------------------------------------------------------ 660

324FII     ------------------------------------------------------------ 660

170RI      ------------------------------------------------------------ 660

170RII     ------------------------------------------------------------ 660


expected   CAGCTTCACTAGACTACCTCAATATGAGGAATGTTTTGATCCTGGACATATGGTGTCTTC 720

genomic    CAGCTTCACTAGACTACCTCAATATGAGGAATGTTTTGATCCTGGACATATGGTGTCTTC 720

170FI      CAGCTTCACTAGACTACCTCAATATGAGGAATGTTTTGATCCTGGACATATGGTGTCTTC 720

170FII     CAGCTTCACTAGACTACCTCAATATGAGGAATGTTTTGATCCTGGACATATGGTGTCTTC 720

324RI      CAGCTTCACTAGACTACCTCAATATGAGGAATGTTTTGATCCTGGACATATGGTGTCTTC 720

324RII     CAGCTTCACTAGACTACCTCAATATGAGGAATGTTTTGATCCTGGACATATGGTGTCTTC 720

324FI      ------------------------------------------------------------ 720

324FII     ------------------------------------------------------------ 720

170RI      ------------------------------------------------------------ 720

170RII     ------------------------------------------------------------ 720
```

```
expected   CTACCTCCATACTTTATAGATTCCTAAACCCATCTATATAATACAAGCATGTGCCATACG 780

genomic    CTACCTCCATACTTTATAGATTCCTAAACCCATCTATATAATACAAGCATGTGCCATACG 780

170FI      CTACCTCCATACTTTATAGATTCCTAAACCCATCTATATAATACAAGCATGTGCCATACG 780

170FII     CTACCTCCATACTTTATAGATTCCTAAACCCATCTATATAATACAAGCATGTGCCATACG 780

324RI      CTACCTCCATACTTTATAGATTCCTAAACCCATCTATATAATACAAGCATGTGCCATACG 780

324RII     CTACCTCCATACTTTATAGATTCCTAAACCCATCTATATAATACAAGCATGTGCCATACG 780

324FI      ------------------------------------------------------------ 780

324FII     ------------------------------------------------------------ 780

170RI      ------------------------------------------------------------ 780

170RII     ------------------------------------------------------------ 780


expected   ATCATTTAGTTTCTTATTACCTCCCTATGCCAGGAAAGAAATAGTTGCAATTTATTGTAG 840

genomic    ATCATTTAGTTTCTTATTACCTCCCTATGCCAGGAAAGAAATAGTTGCAATTTATTGTAG 840

170FI      ATCATTTAGTTTCTTATTACCTCCCTATGCCAGGAAAGAAATAGTTGCAATTTATTGTAG 840

170FII     ATCATTTAGTTTCTTATTACCTCCCTATGCCAGGAAAGAAATAGTTGCAATTTATTGTAG 840

324RI      ATCATTTAGTTTCTTATTACCTCCCTATGCCAGGAAAGAAATAGTTGCAATTTATTGTAG 840

324RII     ATCATTTAGTTTCTTATTACCTCCCTATGCCAGGAAAGAAATAGTTGCAATTTATTGTAG 840

324FI      ------------------------------------------------------------ 840

324FII     ------------------------------------------------------------ 840

170RI      ------------------------------------------------------------ 840

170RII     ------------------------------------------------------------ 840


expected   TCATCATGAAATCTTCCCTTGCACATAAATTTAAAATGTACCTGCTGCACATTTTAATAT 900

genomic    TCATCATGAAATCTTCCCTTGCACATAAATTTAAAATGTACCTGCTGCACATTTTAATAT 900

170FI      TCATCATGAAATCTTCCCTTGCACATAAATTTAAAATGTACCTGCTGCACATTTTAATAT 900

170FII     TCATCATGAAATCTTCCCTTGCACATAAATTTAAAATGTACCTGCTGCACATTTTAATAT 900

324RI      TCATCATGAAATCTTCCCTTGCACATAAATTTAAAATGTACCTGCTGCACATTTTAATAT 900

324RII     TCATCATGAAATCTTCCCTTGCACATAAATTTAAAATGTACCTGCTGCACATTTTAATAT 900

324FI      ------------------------------------------------------------ 900

324FII     ------------------------------------------------------------ 900

170RI      ------------------------------------------------------------ 900

170RII     ------------------------------------------------------------ 900


expected   GTCTTAATTGCTTTTAAACTTGGCTGTATTGTGTACAACTATTATACCATCTTTTATAAA 960

genomic    GTCTTAATTGCTTTTAAACTTGGCTGTATTGTGTACAACTATTATACCATCTTTTATAAA 960

170FI      GTCTTAATTGCTTTTAAACTTGGCTGTATTGTGTACAACTATTATACCATCTTTTATAAA 960

170FII     GTCTTAATTGCTTTTAAACTTGGCTGTATTGTGTACAACTATTATACCATCTTTTATAAA 960

324RI      GTTTTAATTGCTTTTAAACTTGGCTGTATTGTGTACAACTATTATACCATCTTTTATAAA 960

324RII     GTTTTAATTGCTTTTAAACTTGGCTGTATTGTGTACAACTATTATACCATCTTTTATAAA 960

324FI      ------------------------------------------------------------ 960

324FII     ------------------------------------------------------------ 960

170RI      ------------------------------------------------------------ 960

170RII     ------------------------------------------------------------ 960
```

```
expected  CACAGTTTTTTAAGAAATTTCTTTTTGTAAGTTACAACATTCCACTGGAtccttatAGGA 1020
genomic   CACAGTTTTTTAAGAAATTTCTTTTTGTAAGTTACAACATTCCACTGGATCCTTATATTG 1020
170FI     CACAGTTTTTTAAGAAATTTCTTTTTGTAAGTTACAACATTCCACTGGATCCTTATAGGA 1020
170FII    CACAGTTTTTTAAGAAATTTCTTTTTGTAAGTTACAACATTCCACTGGATCCTTATAGGA 1020
324RI     CMCAGTTTTTTWAGAAATTTCTTTTTGTAAGTTACAACMTTYCACTGGATCCTTATWGGA 1020
324RII    CACAGTTTTTTWAGAAATTTCTTTTTGTAAGTTACAACMTTYCACYGGATCCTTATWGGA 1020
324FI     ------------------------------------------------------------ 1020
324FII    ------------------------------------------------------------ 1020
170RI     ------------------------------------------------------------ 1020
170RII    ------------------------------------------------------------ 1020


expected  CATATAATACAAGAGGGTCTTGTGTGTCTGCCCCTTCTAGTTTTCACTCATGCAGAAGCA 1080
genomic   CCT--------------------------------------------------------- 1080
170FI     CATATAATACAAGAGGGTCTTGTGTGTCTGCCCCTTCTAGTTTTCACTCATGCAGAAGCA 1080
170FII    CATATAATACAAGAGGGTCTTGTGTGTCTGCCCCTTCTAGTTTTCACTCATGCAGAAGCA 1080
324RI     CATATAATACMAGAGGGTCTTGKGKGTCTGCCCCTTCTAGTTTTCAMTCATGCAGAAGCA 1080
324RII    CATATAATACMAGAGGGTCTTGKGKGTCTGCCCCTTCTAGTTTTCAMTCATGCAGAAGCA 1080
324FI     ------------------------------------------------------------ 1080
324FII    ------------------------------------------------------------ 1080
170RI     ------------------------------------------------------------ 1080
170RII    ------------------------------------------------------------ 1080



expected  ACATAACCTTCTGATTTGCACAATAAATTACATATATTTAGCAGGATTTTTATTTGCCGT 1140
genomic   ------------------------------------------------------------ 1140
170FI     ACATAACCTTCTGATTTGC----------------------------------------- 1140
170FII    ACATAACCTTCTGATTT------------------------------------------- 1140
324RI     ACATAACCTTYTGATTTGCACAATAAATTACATATATTTAGCMGGATTTTTWTTTGCCST 1140
324RII    ACATAACCTTYTGATTTGCACAATAAATTACAWATATTTAGCMGGATTTTTATTTGCCST 1140
324FI     ------------------------------------------------------------ 1140
324FII    ------------------------------------------------------------ 1140
170RI     ------------------------------------------------------------ 1140
170RII    ------------------------------------------------------------ 1140


expected  GATATATAGGATAATTTAGTCTTTGGCATGTGGCATTATATTTATTTTGGTTTTTTTTTT 1200
genomic   ------------------------------------------------------------ 1200
170FI     ------------------------------------------------------------ 1200
170FII    ------------------------------------------------------------ 1200
324RI     GATATATAGGATAAWTTAGTYTTTGGCMTGKGGCMTTATATTTATTTTGGTTTTTTTTTT 1200
324RII    GATATATAGGATAAWTTAGTYTTTGGCMTGTGGCMTTATATTTATTTTGGTTTTTTTTTT 1200
324FI     ------------------------------------------------------------ 1200
324FII    ------------------------------------------------------------ 1200
170RI     ------------------------------------------------------------ 1200
170RII    ------------------------------------------------------------ 1200
```

```
expected   TAAACAGGTTATCAACGAAACTTCTCAGCATCACGATGACCTTGAAggaagcggagaggg 1260
genomic    ------------------------------------------------------------ 1260
170FI      ------------------------------------------------------------ 1260
170FII     ------------------------------------------------------------ 1260
324RI      TAAACAGGTTATCAACGAAACTTCTYAGCATCACGATGACCTTGAAGGAAGCGGAGAGGG 1260
324RII     TAAACAGGTTATCAACGAAACTTCTYAGCATCACGATGACCYTGAAGGAAGCGGAGAGGG 1260
324FI      ------------------------------------------------------------ 1260
324FII     ------------------------------------------------------------ 1260
170RI      ------------------------------------------------------------ 1260
170RII     ------------------------------------------------------------ 1260


expected   cagaggaagtctgctaacatgcggtgacgtcgaggagaatcctggacctATGGTGAGCAA 1320
genomic    ------------------------------------------------------------ 1320
170FI      ------------------------------------------------------------ 1320
170FII     ------------------------------------------------------------ 1320
324RI      CAGRGGAAGTCTGSTAACMTGCGGTGACGTCGAGGAGAATCCTGGACCTATGGTGAGCAA 1320
324RII     CAGRGGAAGTCTGSTAACMTGCGGTGACGTCGAGGAGAATCCTGGACCTATGGTGAGCAA 1320
324FI      ------------------------------------------------------------ 1320
324FII     ------------------------------------------------------------ 1320
170RI      ------------------------------------------------------------ 1320
170RII     ------------------------------------------------------------ 1320


expected   GGGCGAGGAGCTGATCAAGGAGAACATGCACATGAAGCTGTACATGGAAGGCACCGTGAA 1380
genomic    ------------------------------------------------------------ 1380
170FI      ------------------------------------------------------------ 1380
170FII     ------------------------------------------------------------ 1380
324RI      GGGCGAGGAGCTGATCAAGGAGAACATGCACATGAAGCTGTACATGGAAGGC-------- 1380
324RII     GGGCGAGGAGCTGATCAAGGAGAACATGCACATGAAGCTGTACATGGAAGGCACC----- 1380
324FI      ------------------------------------------------------------ 1380
324FII     ------------------------------------------------------------ 1380
170RI      ------------------------------------------------------------ 1380
170RII     ------------------------------------------------------------ 1380


expected   CAACCACCACTTCAAGTGCACCACCGAAGGGGAGGGCAAGCCCTACGAGGGCACCCAGAC 1440
genomic    ------------------------------------------------------------ 1440
170FI      ------------------------------------------------------------ 1440
170FII     ------------------------------------------------------------ 1440
324RI      ------------------------------------------------------------ 1440
324RII     ------------------------------------------------------------ 1440
324FI      ---------------------------------GAGGGCAAGCCCTACGAGGGCACCCAGAC 1440
324FII     ---------------------------------GGGCAAGCCCTACGAGGGCACCCAGAC 1440
170RI      ------------------------------------------------------------ 1440
170RII     ------------------------------------------------------------ 1440


expected   CCAGAGGATTAAGGTGGTGGAGGGAGGCCCCCTGCCGTTCGCATTCGACATCCTGGCCAC 1500
```

```
genomic    ------------------------------------------------------------ 1500

170FI      ------------------------------------------------------------ 1500

170FII     ------------------------------------------------------------ 1500

324RI      ------------------------------------------------------------ 1500

324RII     ------------------------------------------------------------ 1500

324FI      CCAGAGGATTAAGGTGGTGGAGGGAGGCCCCCTGCCGTTCGCATTCGACATCCTGGCCAC 1500

324FII     CCAGAGGATTAAGGTGGTGGAGGGAGGCCCCCTGCCGTTCGCATTCGACATCCTGGCCAC 1500

170RI      ------------------------------------------------------------ 1500

170RII     ------------------------------------------------------------ 1500


expected   CTGCTTTATGTACGGGAGCAAGACCTTCATCAACCACACCCAGGGCATCCCCGATTTCTT 1560

genomic    ------------------------------------------------------------ 1560

170FI      ------------------------------------------------------------ 1560

170FII     ------------------------------------------------------------ 1560

324RI      ------------------------------------------------------------ 1560

324RII     ------------------------------------------------------------ 1560

324FI      CTGCTTTATGTACGGGAGCAAGACCTTCATCAACCACACCCAGGGCATCCCCGATTTCTT 1560

324FII     CTGCTTTATGTACGGGAGCAAGACCTTCATCAACCACACCCAGGGCATCCCCGATTTCTT 1560

170RI      ------------------------------------------------------------ 1560

170RII     ------------------------------------------------------------ 1560


expected   TAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGACGGGGG 1620

genomic    ------------------------------------------------------------ 1620

170FI      ------------------------------------------------------------ 1620

170FII     ------------------------------------------------------------ 1620

324RI      ------------------------------------------------------------ 1620

324RII     ------------------------------------------------------------ 1620

324FI      TAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGACGGGGG 1620

324FII     TAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGACGGGGG 1620

170RI      ------------------------------------------------------------ 1620

170RII     ------------------------------------------------------------ 1620


expected   CGTGCTTACCGTTACCCAGGACACCAGCCTCCAGGACGGCTGCTTGATCTACAACGTCAA 1680

genomic    ------------------------------------------------------------ 1680

170FI      ------------------------------------------------------------ 1680

170FII     ------------------------------------------------------------ 1680

324RI      ------------------------------------------------------------ 1680

324RII     ------------------------------------------------------------ 1680

324FI      CGTGCTTACCGTTACCCAGGACACCAGCCTCCAGGACGGCTGCTTGATCTACAACGTCAA 1680

324FII     CGTGCTTACCGTTACCCAGGACACCAGCCTCCAGGACGGCTGCTTGATCTACAACGTCAA 1680

170RI      ------------------------------------------------------------ 1680

170RII     ------------------------------------------------------------ 1680


expected   GCTCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTG 1740

genomic    ------------------------------------------------------------ 1740
```

```
170FI     ------------------------------------------------------------ 1740
170FII    ------------------------------------------------------------ 1740
324RI     ------------------------------------------------------------ 1740
324RII    ------------------------------------------------------------ 1740
324FI     GCTCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTG 1740
324FII    GCTCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTG 1740
170RI     ------------------------------------------------------------ 1740
170RII    ------------------------------------------------------------ 1740


expected  GGAGGCCACCACCGAGACCCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGATGCGACAT 1800
genomic   ------------------------------------------------------------ 1800
170FI     ------------------------------------------------------------ 1800
170FII    ------------------------------------------------------------ 1800
324RI     ------------------------------------------------------------ 1800
324RII    ------------------------------------------------------------ 1800
324FI     GGAGGCCACCACCGAGACCCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGATGCGACAT 1800
324FII    GGAGGCCACCACCGAGACCCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGATGCGACAT 1800
170RI     ------------------------------------------------------------ 1800
170RII    ------------------------------------------------------------ 1800


expected  GGCCCTGAAGCTCGTGGGCGGGGGCCACCTGCACTGCAACCTGAAGACCACATACAGATC 1860
genomic   ------------------------------------------------------------ 1860
170FI     ------------------------------------------------------------ 1860
170FII    ------------------------------------------------------------ 1860
324RI     ------------------------------------------------------------ 1860
324RII    ------------------------------------------------------------ 1860
324FI     GGCCCTGAAGCTCGTGGGCGGGGGCCACCTGCACTGCAACCTGAAGACCACATACAGATC 1860
324FII    GGCCCTGAAGCTCGTGGGCGGGGGCCACCTGCACTGCAACCTGAAGACCACATACAGATC 1860
170RI     ------------------------------------------------------------ 1860
170RII    ------------------------------------------------------------ 1860


expected  CAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTTTGTGGACCGCAGACTGGA 1920
genomic   ------------------------------------------------------------ 1920
170FI     ------------------------------------------------------------ 1920
170FII    ------------------------------------------------------------ 1920
324RI     ------------------------------------------------------------ 1920
324RII    ------------------------------------------------------------ 1920
324FI     CAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTTTGTGGACCGCAGACTGGA 1920
324FII    CAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTTTGTGGACCGCAGACTGGA 1920
170RI     ------------------------------------------------------------ 1920
170RII    ------------------------------------------------------------ 1920


expected  AAGAATCAAGGAGGCCGACAATGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAG 1980
genomic   ------------------------------------------------------------ 1980
170FI     ------------------------------------------------------------ 1980
```

```
170FII    ------------------------------------------------------------ 1980

324RI     ------------------------------------------------------------ 1980

324RII    ------------------------------------------------------------ 1980

324FI     AAGAATCAAGGAGGCCGACAATGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAG 1980

324FII    AAGAATCAAGGAGGCCGACAATGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAG 1980

170RI     ------------------------------------------------------------ 1980

170RII    ------------------------------------------------------------ 1980


expected  ATACTGCGACCTCCCTAGCAAACTGGGGCACAAACTTAATGGCATGGACGAGCTGTACAA 2040

genomic   ------------------------------------------------------------ 2040

170FI     ------------------------------------------------------------ 2040

170FII    ------------------------------------------------------------ 2040

324RI     ------------------------------------------------------------ 2040

324RII    ------------------------------------------------------------ 2040

324FI     ATACTGCGACCTCCCTAGCAAACTGGGGCACAAACTTAATGGCATGGACGAGCTGTACAA 2040

324FII    ATACTGCGACCTCCCTAGCAAACTGGGGCACAAACTTAATGGCATGGACGAGCTGTACAA 2040

170RI     ------------------------------------------------------------ 2040

170RII    ------------------------------------------------------------ 2040


expected  GGACTATAAAGACCACGACGGAGACTACAAGGATCATGATATTGATTACAAAGACGATGA 2100

genomic   ------------------------------------------------------------ 2100

170FI     ------------------------------------------------------------ 2100

170FII    ------------------------------------------------------------ 2100

324RI     ------------------------------------------------------------ 2100

324RII    ------------------------------------------------------------ 2100

324FI     GGACTATAAAGACCACGACGGAGACTACAAGGATCATGATATTGATTACAAAGACGATGA 2100

324FII    GGACTATAAAGACCACGACGGAGACTACAAGGATCATGATATTGATTACAAAGACGATGA 2100

170RI     ----------GACCACGACGGAGACTACAAGGATCATGATATTGATTACAAAGACGATGA 2100

170RII    --------------------------------------------AAAGACGATGA 2100


expected  CGATAAGTAATAGTGGAAGAGGGTCTTGTGTGTCTGCCCCTTCTAGTTTTCACTCATGCA 2160

genomic   ---------GTAGTGGAAGAGGGTCTTGTGTGTCTGCCCCTTCTAGTTTTCACTCATGCA 2160

170FI     ------------------------------------------------------------ 2160

170FII    ------------------------------------------------------------ 2160

324RI     ------------------------------------------------------------ 2160

324RII    ------------------------------------------------------------ 2160

324FI     CGATAAGTAATAGTGGAAGAGGGTCTTGTGTGTCTGCCCCTTCTAGTTTTCACTCATGCA 2160

324FII    CGATAAGTAATAGTGGAAGAGGGTCTTGTGTGTCTGCCCCTTCTAGTTTTCACTCATGCA 2160

170RI     CGATAAGTAATAGTGGAAGAGGGTCTTGTGTGTCTGCCCCTTCTAGTTTTCACTCATGCA 2160

170RII    CGATAAGTTATAGTGGAAGAGGGTCTTGTGTGTCTGCCCCTTCTAGTTTTCACTCATGCA 2160


expected  GAAGCAACATAACCTTCTGATTTGCACAATAAATTACATATATTTAGCAGGATTTTTATT 2220

genomic   GAAGCAACATAACCTTCTGATTTGCACAATAAATTACATATATTTAGCAGGATTTTTATT 2220

170FI     ------------------------------------------------------------ 2220

170FII    ------------------------------------------------------------ 2220
```

```
324RI      ------------------------------------------------------------ 2220

324RII     ------------------------------------------------------------ 2220

324FI      GAAGCAACATAACCTTCTGATTTGCACAATAAATTACATATATTTAGCAGGATTTTTATT 2220

324FII     GAAGCAACATAACCTTCTGATTTGCACAATAAATTACATATATTTAGCAGGATTTTTATT 2220

170RI      GAAGCAACATAACCTTCTGATTTGCACAATAAATTACATATATTTAGCAGGATTTTTATT 2220

170RII     GAAGCAACATAACCTTCTGATTTGCACAATAAATTACATATATTTAGCAGGATTTTTATT 2220


expected   TGCCGTGATATATAGGATAATTTAGTCTTTGGCATGTGGCATTATATTTATTTTGGTTTT 2280

genomic    TGCCGTGATATATAGGATAATTTAGTCTTTGGCATGTGGCATTATATTTATTTTGGTTTT 2280

170FI      ------------------------------------------------------------ 2280

170FII     ------------------------------------------------------------ 2280

324RI      ------------------------------------------------------------ 2280

324RII     ------------------------------------------------------------ 2280

324FI      TGCCGTGATATATAGGATAATTTAGTCTTTGGCATGTGGCATTATATTTATTTTGG-TTT 2280

324FII     TGCCGTGAtATATAGGATAATTTAGTCTTTGGCATGTGGCATTATATTTATTTTGG-TTT 2280

170RI      TGCCGTGATATATAGGATAATTTAGTCTTTGGCATGTGGCATTATATTTATTTTGGTTTT 2280

170RII     TGCCGTGATATATAGGATAATTTAGTCTTTGGCATGTGGCATTATATTTATTTTGGTTTT 2280


expected   TTTTTTTAAACAGGTTATCAACGAAACTTCTCAGCATCACGATGACCTTGAATAAAAATT 2340

genomic    TTTTTTTAAACAGGTTATCAACGAAACTTCTCAGCATCACGATGACCTTGAATAAAAATT 2340

170FI      ------------------------------------------------------------ 2340

170FII     ------------------------------------------------------------ 2340

324RI      ------------------------------------------------------------ 2340

324RII     ------------------------------------------------------------ 2340

324FI      TTTTTTTAAACAGGTTATCAACGAAACTTCTCAGCATCACGATGACCTTGAATAAAAATT 2340

324FII     TTTTTTTAAACAGGTTATCAACGAAACTTCTCAGCATCACGATGACCTTGAATAAAAATT 2340

170RI      TTTTTTTAAACAGGTTATCAACGAAACTTCTCAGCATCACGATGACCTTGAATAAAAATT 2340

170RII     TTTTTTTAAACAGGTTATCAACGAAACTTCTCAGCATCACGATGACCTTGAATAAAAATT 2340


expected   GCACACACTCAGTGCAGCAATATATTACCAGCAAGAATAAAAAAGAAATCCATATCTTAA 2400

genomic    GCACACACTCAGTGCAGCAATATATTACCAGCAAGAATAAAAAAGAAATCCATATCTTAA 2400

170FI      ------------------------------------------------------------ 2400

170FII     ------------------------------------------------------------ 2400

324RI      ------------------------------------------------------------ 2400

324RII     ------------------------------------------------------------ 2400

324FI      GCAC-------------------------------------------------------- 2400

324FII     GCACACACTCAGTGCAGCAATATATTACCAGCAAGA--TAAAAAGAAATCCATATC-TAA 2400

170RI      GCACACACTCAGTGCAGCAATATATTACCAGCAAGAATAAAAAAGAAATCCATATCTTAA 2400

170RII     GCACACACTCAGTGCAGCAATATATTACCAGCAAGAATAAAAAAGAAATCCATATCTTAA 2400


expected   AGAAACAGCTTTCAAGTGCCTTTCTGCAGTTTTTCAGGAGCGCAAGATAGATTTGGAATA 2460

genomic    AGAAACAGCTTTCAAGTGCCTTTCTGCAGTTTTTCAGGAGCGCAAGATAGATTTGGAATA 2460

170FI      ------------------------------------------------------------ 2460

170FII     ------------------------------------------------------------ 2460

324RI      ------------------------------------------------------------ 2460
```

```
324RII    ------------------------------------------------------------ 2460

324FI     ------------------------------------------------------------ 2460

324FII    AGAAACAGCTTTC-AGTGCCTTTCTGCAGTTTT--------------------------- 2460

170RI     AGAAACAGCTTTCAAGTGCCTTTCTGCAGTTTTTTCAGGAGCGCAAGATAGATTTGGAATA 2460

170RII    AGAAACAGCTTTCAAGTGCCTTTCTGCAGTTTTTTCAGGAGCGCAAGATAGATTTGGAATA 2460


expected  GGAATAAGCTCTAGTTCTTAACAACCGACACTCCTACAAGATTTAGAAAAAAGTTTACAA 2520

genomic   GGAATAAGCTCTAGTTCTTAACAACCGACACTCCTACAAGATTTAGAAAAAAGTTTACAA 2520

170FI     ------------------------------------------------------------ 2520

170FII    ------------------------------------------------------------ 2520

324RI     ------------------------------------------------------------ 2520

324RII    ------------------------------------------------------------ 2520

324FI     ------------------------------------------------------------ 2520

324FII    ------------------------------------------------------------ 2520

170RI     GGAATAAGCTCTAGTTCTTAACAACCGACACTCCTACAAGATTTAGAAAAAAGTTTACAA 2520

170RII    GGAATAAGCTCTAGTTCTTAACAACCGACACTCCTACAAGATTTAGAAAAAAGTTTACAA 2520


expected  CATAATCTAGTTTACAGAAAAATCTTGTGCTAGAATACTTTTTAAAAGGTATTTTGAATA 2580

genomic   CATAATCTAGTTTACAGAAAAATCTTGTGCTAGAATACTTTTTAAAAGGTATTTTGAATA 2580

170FI     ------------------------------------------------------------ 2580

170FII    ------------------------------------------------------------ 2580

324RI     ------------------------------------------------------------ 2580

324RII    ------------------------------------------------------------ 2580

324FI     ------------------------------------------------------------ 2580

324FII    ------------------------------------------------------------ 2580

170RI     CATAATCTAGTTTACAGAAAAATCTTGTGCTAGAATACTTTTTAAAAGGTATTTTGAATA 2580

170RII    CATAATCTAGTTTACAGAAAAATCTTGTGCTAGAATACTTTTTAAAAGGTATTTTGAATA 2580


expected  CCATTAAAACTGCTTTTTTTTTTCCAGCAAGTATCCAACCAACTTGGTTCTGCTTCAATA 2640

genomic   CCATTAAAACTGCTTTTTTTTTTCCAGCAAGTATCCAACCAACTTGGTTCTGCTTCAATA 2640

170FI     ------------------------------------------------------------ 2640

170FII    ------------------------------------------------------------ 2640

324RI     ------------------------------------------------------------ 2640

324RII    ------------------------------------------------------------ 2640

324FI     ------------------------------------------------------------ 2640

324FII    ------------------------------------------------------------ 2640

170RI     CCATTAAAACTGCTTTTTTTTTTCCAGCAAGTATCCAACCAACTTGGTTCTGCTTCAATA 2640

170RII    CCATTAAAACTGCTTTTTTTTTTCCAGCAAGTATCCAACCAACTTGGTTCTGCTTCAATA 2640


expected  AATCTTTGGAAAAACTCTTTTGTTGTGTTATTTATTGGATAATATCTAAACAATTCTCTA 2700

genomic   AATCTTTGGAAAAACTCTTTTGTTGTGTTATTTATTGGATAATATCTAAACAATTCTCTA 2700

170FI     ------------------------------------------------------------ 2700

170FII    ------------------------------------------------------------ 2700

324RI     ------------------------------------------------------------ 2700

324RII    ------------------------------------------------------------ 2700
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324FI     ------------------------------------------------------------ 2700

324FII    ------------------------------------------------------------ 2700

170RI     AATCTTTGGAAAAACTCTTTTGTTGTGTTATTTATTGGATAATATCTAAACAATTCTCTA 2700

170RII    AATCTTTGGAAAAACTCTTTTGTTGTGTTATTTATTGGATAATATCTAAACAATTCTCTA 2700


expected  CTTGGTCCTATTAGTTAATTTGTCATTACAATCATGTAAGTTGATAAATTCAGGTTATTT 2760

genomic   CTTGGTCCTATTAGTTAATTTGTCATTACAATCATGTAAGTTGATAAATTCAGGTTATTT 2760

170FI     ------------------------------------------------------------ 2760

170FII    ------------------------------------------------------------ 2760

324RI     ------------------------------------------------------------ 2760

324RII    ------------------------------------------------------------ 2760

324FI     ------------------------------------------------------------ 2760

324FII    ------------------------------------------------------------ 2760

170RI     CTTGGTCCTATTAGTTAATTTGTCATTACAATCATGTAAGTTGATAAATTCAGGTTATTT 2760

170RII    CTTGGTCCTATTAGTTAATTTGTCATTACAATCATGTAAGTTGATAAATTCAGGTTATTT 2760


expected  ATGCTTGAGATGTAGTTCTTAATTTTGTCATTTTTGATAGACCTCACTTCTTTTTATTAT 2820

genomic   ATGCTTGAGATGTAGTTCTTAATTTTGTCATTTTTGATAGACCTCACTTCTTTTTATTAT 2820

170FI     ------------------------------------------------------------ 2820

170FII    ------------------------------------------------------------ 2820

324RI     ------------------------------------------------------------ 2820

324RII    ------------------------------------------------------------ 2820

324FI     ------------------------------------------------------------ 2820

324FII    ------------------------------------------------------------ 2820

170RI     ATGCTTGAGATGTAGTTCTTAATTTTGTCATTTTTGATAGACCTCACTTCTTTTTATTAT 2820

170RII    ATGCTTGAGATGTAGTTCTTAATTTTGTCATTTTTGATAGACCTCACTTCTTTTTATTAT 2820


expected  TACTTAAAAACATTTACAAATAGGTGGTGTCGAATAAAATAACTTGTACCAAAATGAAGA 2880

genomic   TACTTAAAAACATTTACAAATAGGTGGTGTCGAATAAAATAACTTGTACCAAAATGAAGA 2880

170FI     ------------------------------------------------------------ 2880

170FII    ------------------------------------------------------------ 2880

324RI     ------------------------------------------------------------ 2880

324RII    ------------------------------------------------------------ 2880

324FI     ------------------------------------------------------------ 2880

324FII    ------------------------------------------------------------ 2880

170RI     TACTTAAAAACATTTACAAATAGGTGGTGTCGAATAAAATAACTTGTACCAAAATGAAGA 2880

170RII    TACTTAAAAACATTTACAAATAGGTGGTGTCGAATAAAATAACTTGTACCAAAATGAAGA 2880


expected  TAGGTCTCTCTAAAATGAGCTCAGGTCTGTGATTTTAATCATAACAAACAGACTTCCTAA 2940

genomic   TAGGTCTCTCTAAAATGAGCTCAGGTCTGTGATTTTAATCATAACAAACAGACTTCCTAA 2940

170FI     ------------------------------------------------------------ 2940

170FII    ------------------------------------------------------------ 2940

324RI     ------------------------------------------------------------ 2940

324RII    ------------------------------------------------------------ 2940

324FI     ------------------------------------------------------------ 2940
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324FII     ------------------------------------------------------------ 2940

170RI      TAGGTCTCTCTAAAATGAGCTCAGGTCTGTGATTTTAATCATAACAAACAGACTTCCTAA 2940

170RII     TAGGTCTCTCTAAAATGAGCTCAGGTCTGTGATTTTAATCATAACAAACAGACTTCCTAA 2940


expected   AATTAAAAAATAAAAACTTTTTTTAGTACATATAGCATATGAGTAACACAAATTCCACTT 3000

genomic    AATTAAAAAATAAAAACTTTTTTTAGTACATATAGCATATGAGTAACACAAATTCCACTT 3000

170FI      ------------------------------------------------------------ 3000

170FII     ------------------------------------------------------------ 3000

324RI      ------------------------------------------------------------ 3000

324RII     ------------------------------------------------------------ 3000

324FI      ------------------------------------------------------------ 3000

324FII     ------------------------------------------------------------ 3000

170RI      AATTAAAAAATAAAAACTTTTTTTAGTACATATAGCATATGAGTAACACA---------- 3000

170RII     AATTAAAAAATAAAAACTTTTTTTAGTACATATAGCATATGAGTAACACAAA-------- 3000


expected   TGGGAGTTAAAGCATAGGAAGTTGCCAAGATATAGGGGCTTATCTTCCGCTAGCAAGATG 3060

genomic    TGGGAGT----------------------------------------------------- 3060

170FI      ------------------------------------------------------------ 3060

170FII     ------------------------------------------------------------ 3060

324RI      ------------------------------------------------------------ 3060

324RII     ------------------------------------------------------------ 3060

324FI      ------------------------------------------------------------ 3060

324FII     ------------------------------------------------------------ 3060

170RI      ------------------------------------------------------------ 3060

170RII     ------------------------------------------------------------ 3060


expected   CAGAGAAATG 3070

genomic    ---------- 3070

170FI      ---------- 3070

170FII     ---------- 3070

324RI      ---------- 3070

324RII     ---------- 3070

324FI      ---------- 3070

324FII     ---------- 3070

170RI      ---------- 3070

170RII     ---------- 3070
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Figure S2. The DNA template for knock-in into *Vimentin* gene. Partial vKiT plasmid sequence which contains the template for the knock-in. The sequence recognized by Cas9/gRNA complex was recoded are shown in red text. Blue text are the PAM sequences.

VIM gRNA target site – 5'HA – VIM missing part - T2A - mCardinal –3xFLAG- 3'HA - VIM gRNA target site

GATCCTTATATTGCCTGTAGTGGAAGAAAAAACTCGTTTTTTACTCATTTTTGGCCTGTTTGTTTATTTAGAAACTAATCT
GGATTCACTCCCTCTGGTTGATACCCACTCAAAAAGGACACTTCTGATTAAGACGGTTGAAACTAGAGATGGACAGGTTG
GTATCTTTTTAAGGAAAAAATAGGGTAATCTCAGACAGGAGTTGATATATTTTTAAAATCAGTGAATCTGAATCTCAGATAC
AGCTGGCTAATTTGAGAGGTTCAGGTTTTCATTCATGCCTACTAAAAAAAGAATAGGCTTCTTCTTCCAGCAGTACACACA

GCCAACTAATTATTTGGCTCCTGGATGTGAAGTTGAGATAGCAGTCTTCCTGTGCTCCAGAATTAGTGATTTGCTTTGGT
GCTTAATTTGAAGTGGGAGTAAGCTTCCTTAAACCACTTCCTAAAGCAGCTACATGAAACAGCTTCACTAGACTACCTCA
ATATGAGGAATGTTTTGATCCTGGACATATGGTGTCTTCCTACCTCCATACTTTATAGATTCCTAAACCCATCTATATAA
TACAAGCATGTGCCATACGATCATTTAGTTTCTTATTACCTCCCTATGCCAGGAAAGAAATAGTTGCAATTTATTGTAGT
CATCATGAAATCTTCCCTTGCACATAAATTTAAAATGTACCTGCTGCACATTTTAATATGTCTTAATTGCTTTTAAACTT
GGCTGTATTGTGTACAACTATTATACCATCTTTTATAAACACAGTTTTTTAAGAAATTTCTTTTTGTAAGTTACAACATT
CCACTGGAtccttatAGGACATATAATAATACAAGAGGGTCTTGTGTGTCTGCCCCTTCTAGTTTTCACTCATGCAGAAGCAA
CATAACCTTCTGATTTGCACAATAAATTACATATATTTAGCAGGATTTTTATTTGCCGTGATATATAGGATAATTTAGTC
TTTGGCATGTGGCATTATATTTATTTTGGTTTTTTTTTTTAAACAGGTTATCAACGAAACTTCTCAGCATCACGATGACC
TTGAAggaagcggagagggcagaggaagtctgctaacatgcggtgacgtcgaggagaatcctggacctATGGTGAGCAAGGGCGAGGAGCTGATCAA
GGAGAACATGCACATGAAGCTGTACATGGAAGGCACCGTGAACAACCACCACTTCAAGTGCACCACCGAAGGGGGAGGGCAAGCC
CTACGAGGGCACCCAGACCCAGAGGATTAAGGTGGTGGAGGGAGGCCCCCTGCCGTTCGCATTCGACATCCTGGCCACCTGCTTT
ATGTACGGGAGCAAGACCTTCATCAACCACACCCAGGGCATCCCCGATTTCTTTAAGCAGTCCTTCCCTGAGGGCTTCACATGGGA
GAGAGTCACCACATACGAAGACGGGGGCGTGCTTACCGTTACCCAGGACACCAGCCTCCAGGACGGCTGCTTGATCTACAACGTC
AAGCTCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCACCACCGAGACCCTGT
ACCCCGCTGACGGCGGCCTGGAAGGCAGATGCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGCACTGCAACCTGAAGA
CCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTTTGTGGACCGCAGACTGGAAAGAATCAAGGA
GGCCGACAATGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGCAAACTGGGGCACAAACTT
AATGGCATGGACGAGCTGTACAAGGACTATAAAGACCACGACGGAGACTACAAGGATCATGATATTGATTACAAAGACG
ATGACGATAAGTAATAGTGGAAGAGGGTCTTGTGTGTCTGCCCCTTCTAGTTTTCACTCATGCAGAAGCAACATAACCTTCTGATT
TGCACAATAAATTACATATATTTAGCAGGATTTTTATTTGCCGTGATATATAGGATAATTTAGTCTTTGGCATGTGGCATTATATTTA
TTTTGGTTTTTTTTTTTAAACAGGTTATCAACGAAACTTCTCAGCATCACGATGACCTTGAATAAAAATTGCACACACTCAGTGCAGC
AATATATTACCAGCAAGAATAAAAAAGAAATCCATATCTTAAAGAAACAGCTTTCAAGTGCCTTTCTGCAGTTTTTCAGGAGCGCA
AGATAGATTTGGAATAGGAATAAGCTCTAGTTCTTAACAACCGACACTCCTACAAGATTTAGAAAAAAGTTTACAACATAATCTAG
TTTACAGAAAAATCTTGTGCTAGAATACTTTTTAAAAGGTATTTTGAATACCATTAAAACTGCTTTTTTTTTTTCCAGCAAGTATCCAAC
CAACTTGGTTCTGCTTCAATAAATCTTTGGAAAAACTCTTTTGTTGTGTTATTTATTGGATAATATCTAAACAATTCTCTACTTGGTCC
TATTAGTTAATTTGTCATTACAATCATGTAAGTTGATAAATTCAGGTTATTTATGCTTGAGATGTAGTTCTTAATTTTGTCATTTTTGA
TAGACCTCACTTCTTTTTATTATTACTTAAAAACATTTACAAATAGGTGGTGTCGAATAAAATAACTTGTACCAAAATGAAGATAGG
TCTCTCTAAAATGAGCTCAGGTCTGTCACTACAGGCAATATAAGGATC

Figure S3. Confocal imaging of HEK293 cells transfected with re-cloned *VIM-T2A-mCardinal* **(A-C)** and fusion VIM-mCardinal **(D-F).** Cellular distribution of mCardinal in *VIM-T2A-mCardinal* expressing cells shows its strong fluorescence in single dot-like area **(B)**, as well as it is weakly diffused in the cytoplasm. VIM-mCardinal fusion protein aggregated in the cytoplasm **(E)**. A) merged, B) mCardinal, C) transmission light, D) merged, E) mCardinal, F) transmission light. The scale bar is 10 μm.
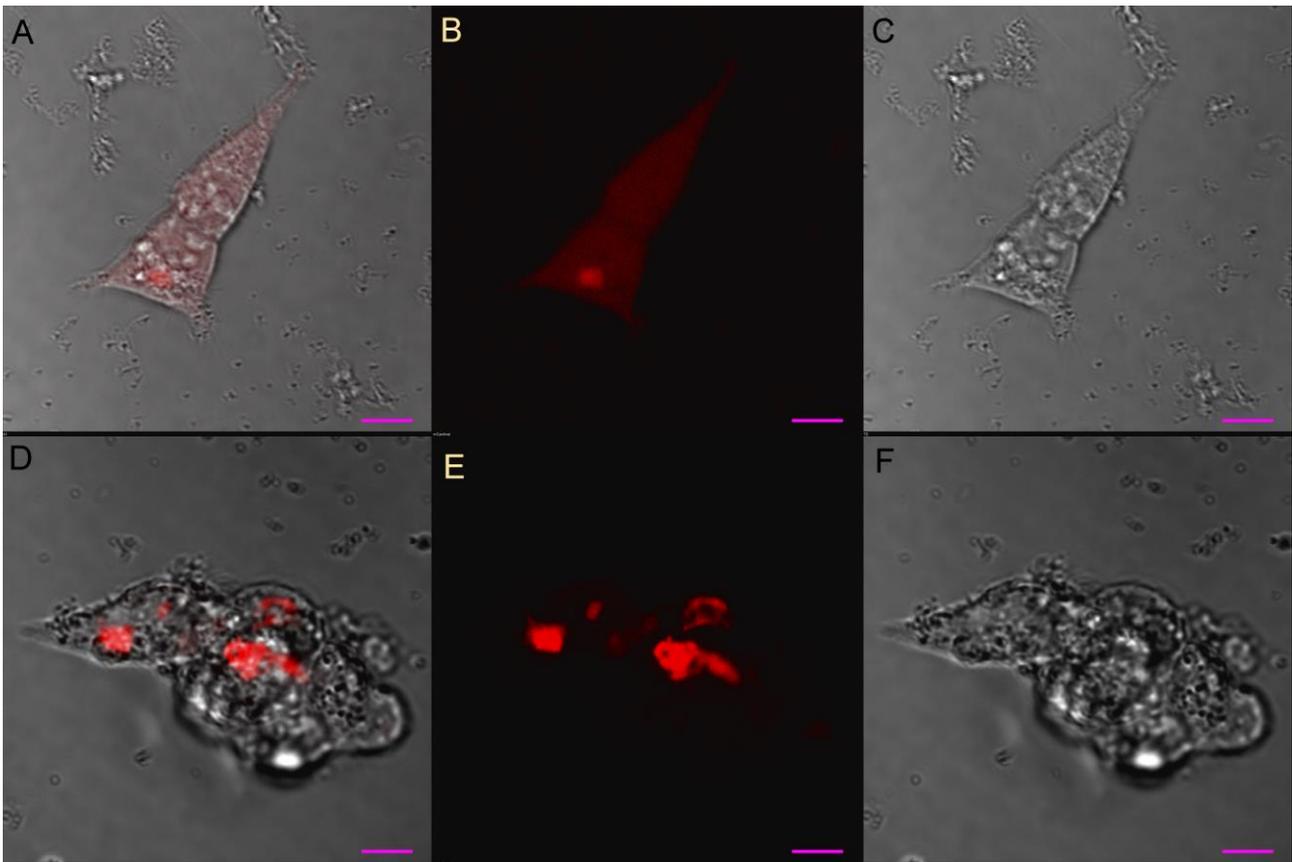
Figure S4. Sorting of VRCs by flow cytometry. **A)** P1 acquisition gate was set on FSC and SSC detectors. **B)** the cells showing red fluorescence were selected by P2 or P3 gate, then collected into the tubes and named as dim-VRCs or bright-VRCs respectively. Representative dot-plot has been shown.
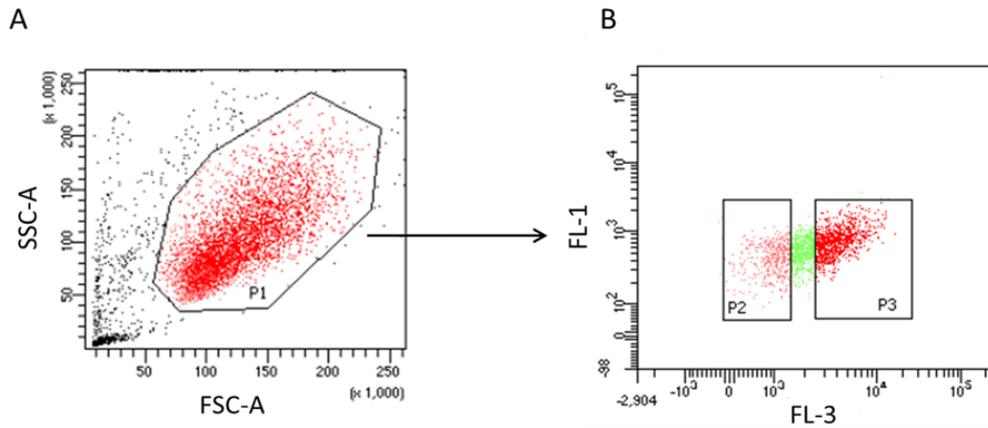

Figure S5. Expression of *VIM*, *mCardinal* and *CDH1* in dim-VRCs and bright-VRCs by qPCR. Mean $2^{(-\Delta CT)} \pm$ SEM is shown, the reference gene was *GAPDH*. The graph shows the representative result of the measurement which was done in triplicate.
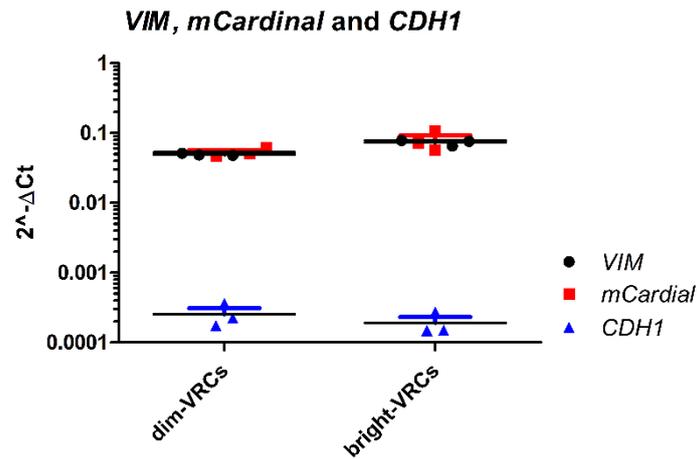
Figure S6. EMT markers in VRC and in H2170 parental cells assessed by qPCR. Relative quantification (RQ). Expression of *CDH1*, *VIM*, *SNAI1*, *ZEB1*, *ZEB2*, *TWIST1* and *TWIST2* in VRCs and in parental H2170 cell line has been shown as mean $2^{(-\Delta\Delta CT)} \pm$ SEM, with the *GAPDH* reference. The graph shows the representative result of the measurement which was done in triplicate.
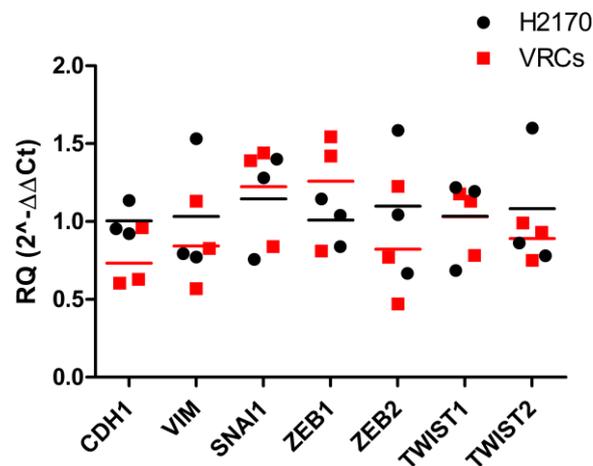
Table 1. Oligonucleotides.

	Name of the oligonucleotide	Sequence
1	gRNA-F	CACCGGATCCTTATATTGCCTGTAG
2	gRNA-R	AAACCTACAGGCAATATAAGGATCC
3	KI-F	ATGTCCACCAGGTCCGTGTC
4	KI-R	CAGACACACAAGACCCTCTTCCAC
5	BCB-F	AGGGTCTTGTGTGTCTGTCTCAGATCTCGAGCTCAAGCTTC
6	BCB-R	ACGGACCTGGTGGACATGTACGGAGACGGGATCTGACG
7	SDM-F	AGGAGAATGGCGGAGGGATGGTGAGCAAGGGCGAGG
8	SDM-R	CCCTCCGCCATTCTCCTCGACGTCACCGC
9	170	TCGAACCCAAGTACACTCTTGC
10	249	ATCCTTGTAGTCTCCGTCGTGGTC
11	GAPDH-F	CTCTGCTCCTCCTGTTCGAC
12	GAPDH-R	GCCCAATACGACCAAATCC
13	VIM-F	AGTCCACTGAGTACCGGAGAC
14	VIM-R	CATTTCACGCATCTGGCGTTC
15	mCard-F	TGATCAAGGAGAACATGCACATGAAGC
16	mCard-R	CCTTAATCCTCTGGGTCTGGGTG
17	Cdh1-F	CGAGAGCTACACGTTCACGG
18	Cdh1-R	GGGTGTCGAGGGAAAAATAGG
19	Mir145-F	AAGATCTCTACAGATGGGGCTGGATGC
20	Mir145-R	AAAGCTTCAAGAGTACGGCAGTGCTGA
21	Mir-200b-F	AAGATCTGGATTAGGACGCTCAGGTGTC
22	Mir-200b-R	AAAGCTTTGGAGTAGGAGCTCCGGATGTG
23	Mir-200c-F	AAGATCTAGGGTGGGTAAATCGGTGTG
24	Mir-200c-R	AAAGCTTACCTGAGGCGATGGATGTTG
25	Mir-205-F	AAGATCTCCTCCTTGGAGGATGTGA
26	Mir-205-R	AAAGCTTACGCACACTCCAGATGTCTC
27	ZEB1F	ACTGTGGTAGAAACAAATTCAGATTCAGATGATG
28	ZEB1R	GCCCTTCCTTTCCTGTGTCATCC
29	ZEB2F	CTCTGTAGATGGTCCAGTGAAGAATGC
30	ZEB2R	GTCACTGCGCTGAAGGTACTCC
31	SNAI1F	TCGGAAGCCTAACTACAGCGA
32	SNAI1R	AGATGAGCATTGGCAGCGAG
33	TWIST1F	GCCGGAGACCTAGATGTCATT
34	TWIST1R	TTTTAAAAGTGCGCCCCACG
35	TWIST2F	CGCCAGGGCTGTCCGTC
36	TWIST2R	GTCACTGCTGTCCCTTCTCTCG