# Supplementary Materials for

# Elucidating cellular population dynamics by molecular density function perturbations

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## Supplementary Material S1. Probability Distance Metrics

***Signed Engineering Metric:***

$$\Delta_E(f^A_{X_i}(t,x_i)||f^B_{X_i}(t,x_i)) = sign(\Delta\mu_{X_i}) \int_{-\infty}^{\infty} (x_i f^A_{X_i}(t,x_i) - x_i f^B_{X_i}(t,x_i))^2 dx_i \quad \text{(S. 1)}$$

***Signed Jeffrey Divergence:***

$$\Delta_{JD}(f^A_{X_i}(t,x_i)||f^B_{X_i}(t,x_i)) = sgn(\Delta\mu_{X_i}) \int_{-\infty}^{\infty} (f^A_{X_i}(t,x_i) - f^B_{X_i}(t,x_i)) ln\left(\frac{f^A_{X_i}(t,x_i)}{f^B_{X_i}(t,x_i)}\right) dx_i \quad \text{(S. 2)}$$

***Signed Kullback-Leibler Distance:***

$$\Delta_{KLD}(f^A_{X_i}(t,x_i)||f^B_{X_i}(t,x_i)) = sgn(\Delta\mu_{X_i}) \int_{-\infty}^{\infty} f^B_{X_i}(t,x_i) ln\left(\frac{f^B_{X_i}(t,x_i)}{f^A_{X_i}(t,x_i)}\right) dx_i \quad \text{(S. 3)}$$

***Signed Jensen-Shannon Divergence:***

$$\Delta_{JSD} f_{X_i}(t,x_i) = sgn(\Delta\mu_{X_i}) \frac{f^+_{X_i}(t,x_i) + f^-_{X_i}(t,x_i)}{2} \quad \text{(S. 4)}$$

***Signed Kolmogorov-Smirnov Metric:***

$$\Delta_{KS}(f^A_{X_i}(t,x_i)||f^B_{X_i}(t,x_i)) = sgn(\Delta\mu_{X_i}) sup|F^A_{X_i}(t,x_i) - F^B_{X_i}(t,x_i)| \quad \text{(S. 5)}$$

**Supplementary Material S2. TRAIL induced programmed cell death model of Hela cells** [1, 2]

| State Variable | Definition |
|---|---|
| L | Death ligand, such as TRAIL and TNF |
| R | Inactive receptor complex |
| Rs | Active receptor complex |
| flip | FLICE inhibitory proteins inhibiting active receptors |
| C8 | Procaspase 8 and 10, inactive form of caspase 8 and 10 |
| C8s | Cleaved caspase 8 and 10, active form of caspase 8 and 10 |
| Bar | Bifunctional apoptosis regulator, binds to active caspase 8 and 10 acting as an inhibitor |
| C3 | Procaspase 3 and 7, inactive form of caspase 3 and 7 |
| C3s | Cleaved caspase 3 and 7, active form of caspase 3 and 7 |
| C6 | Procaspase 6, inactive form of caspase 6 |
| C6s | Cleaved caspase 6, active form of caspase 6 |
| XIAP | X-linked inhibitor of apoptosis protein |
| PARP | Poly ADP ribose polymerase, a DNA damage repair enzyme representing all substrates of cleaved caspase 3 |
| cPARP | Cleaved poly ADP ribose polymerase measuring single cell death |

| | |
|---|---|
| Bid | BH3 interacting domain, substrate of cleaved caspase 8, inactive form |
| tBid | Truncated BH3 interacting domain, active form of Bid |
| Bcl2c | Cleaved B-cell lymphoma 2 represents the family of apoptotic proteins in the cellular compartment (CC), it binds to tBid and acts as inhibitor |
| Bax | Bcl2 associated X protein, substrate of tBid, inactive form |
| Baxs | Substrate of Bcl2 associated X protein, active form |
| Baxms | Active Baxs in mitochondrial compartment |
| Bcl2 | All antiapoptotic proteins in the mitochondrial compartment |
| Bax2 | Complex of Baxms and Baxms in mitochondria |
| Bax4 | Complex of Bax2 and Bax2 |
| M | Number of unoccupied Bax4 binding sites on outer membrane of the mitochondria in mitochondrial compartment |
| Ms | Number of pores Bax4 created on the outer membrane of the mitochondria |
| CyCm | Cytochrome C inside the mitochondria |
| CyCr | Cytochrome C released from the mitochondria, but remaining in the mitochondrial membrane |
| Smacm | Smac/Diablo homolog inside mitochondria |

| | |
|---|---|
| Smacr | Smac/Diablo released from the mitochondria, but remaining in the mitochondrial membrane |
| CyCc | Cytochrome C in the cellular compartment |
| Apaf | Apoptosis activating factor (Apaf-1), substrate of cytochrome C, inactive form |
| Apafs | Active form of Apaf-1 |
| C9 | Procaspaes 9, inactive form |
| Apop | Apafs-C9 complex apoptosome |
| Smacc | Smac/Diablo in the cellular compartment |
| C3s:Ub | Caspase 3 ubiquitinated and targeted for degradation, inactive form |
| Pseudo:Variable | Pseudo variable with random initial condition and zero dynamics |

**Rate Equations:**

r1 = kf1*L*R - kr1*L:R
r2 = kf2*L:R
r3 = kf3*C8*Rs - kr3*Rs:C8
r4 = kf4*Rs:C8
r5 = kf5*C3*C8s - kr5*C8s:C3
r6 = kf6*C8s:C3
r7 = kf7*C3s*PARP - kr7*C3s:PARP
r8 = kf8*C3s:PARP
r9 = kf9*Bid*C8s - kr9*C8s:Bid
r10 = kf10*C8s:Bid
r11 = kf11*Bax*tBid - kr11*tBid:Bax
r12 = kf12*tBid:Bax
r13 = kf13*Baxs - kr13*Baxms
r14 = (kf14*(Baxms*Baxms))/v - kr14*Bax2
r15 = (kf15*(Bax2*Bax2))/v - kr15*Bax4
r16 = (kf16*Bax4*M)/v - kr16*Bax4:M
r17 = kf17*Bax4:M
r18 = (kf18*CyCm*Ms)/v - kr18*Ms:CyCm
r19 = kf19*Ms:CyCm
r20 = kf20*CyCr - kr20*CyCc
r21 = (kf21*Ms*Smacm)/v - kr21*Ms:Smacm
r22 = kf22*Ms:Smacm
r23 = kf23*Smacr - kr23*Smacc
r24 = kf24*Apaf*CyCc - kr24*CyCc:Apaf
r25 = kf25*CyCc:Apaf
r26 = kf26*Apafs*C9 - kr26*Apop
r27 = kf27*Apop*C3 - kr27*Apop:C3
r28 = kf28*Apop:C3

r29 = kf29*C3s*C6 - kr29*C3s:C6
r30 = kf30*C3s:C6
r31 = kf31*C6s*C8 - kr31*C6s:C8
r32 = kf32*C6s:C8
r33 = kf33*Rs*flip - kr33*Rs:flip
r34 = kf34*Bar*C8s - kr34*C8s:Bar
r35 = kf35*Bcl2c*tBid - kr35*tBid:Bcl2c
r36 = (kf36*Baxms*Bcl2)/v - kr36*Baxms:Bcl2
r37 = (kf37*Bax2*Bcl2)/v - kr37*Bax2:Bcl2
r38 = (kf38*Bax4*Bcl2)/v - kr38*Bax4:Bcl2
r39 = kf39*Smacc*XIAP - kr39*Smacc:XIAP
r40 = kf40*Apop*XIAP - kr40*Apop:XIAP
r41 = kf41*C3s*XIAP - kr41*C3s:XIAP
r42 = kf42*C3s:XIAP

**Ordinary differential equations**

d[L]/dt = -r1
d[R]/dt = -r1
d[L:R]/dt = r1 -r2
d[Rs]/dt = r2 -r3 +r4 -r33
d[flip]/dt = -r33
d[Rs:flip]/dt = r33
d[C8]/dt = -r3 -r31
d[Rs:C8]/dt = r3 -r4
d[C8s]/dt = r4 -r5 +r6 -r9 +r10 +r32 -r34
d[Bar]/dt = -r34
d[C8s:Bar]/dt = r34
d[C3]/dt = -r5 -r27
d[C8s:C3]/dt = r5 -r6
d[C3s]/dt = r6 -r7 +r8 +r28 -r29 +r30 -r41
d[C6]/dt = -r29
d[C3s:C6]/dt = r29 -r30
d[C6s]/dt = r30 -r31 +r32
d[C6s:C8]/dt = r31 -r32
d[XIAP]/dt = -r39 -r40 -r41 +r42
d[C3s:XIAP]/dt = r41 -r42
d[PARP]/dt = -r7
d[C3s:PARP]/dt = r7 -r8
d[cPARP]/dt = r8
d[Bid]/dt = -r9
d[C8s:Bid]/dt = r9 -r10
d[tBid]/dt = r10 -r11 +r12 -r35
d[Bcl2c]/dt = -r35
d[tBid:Bcl2c]/dt = r35

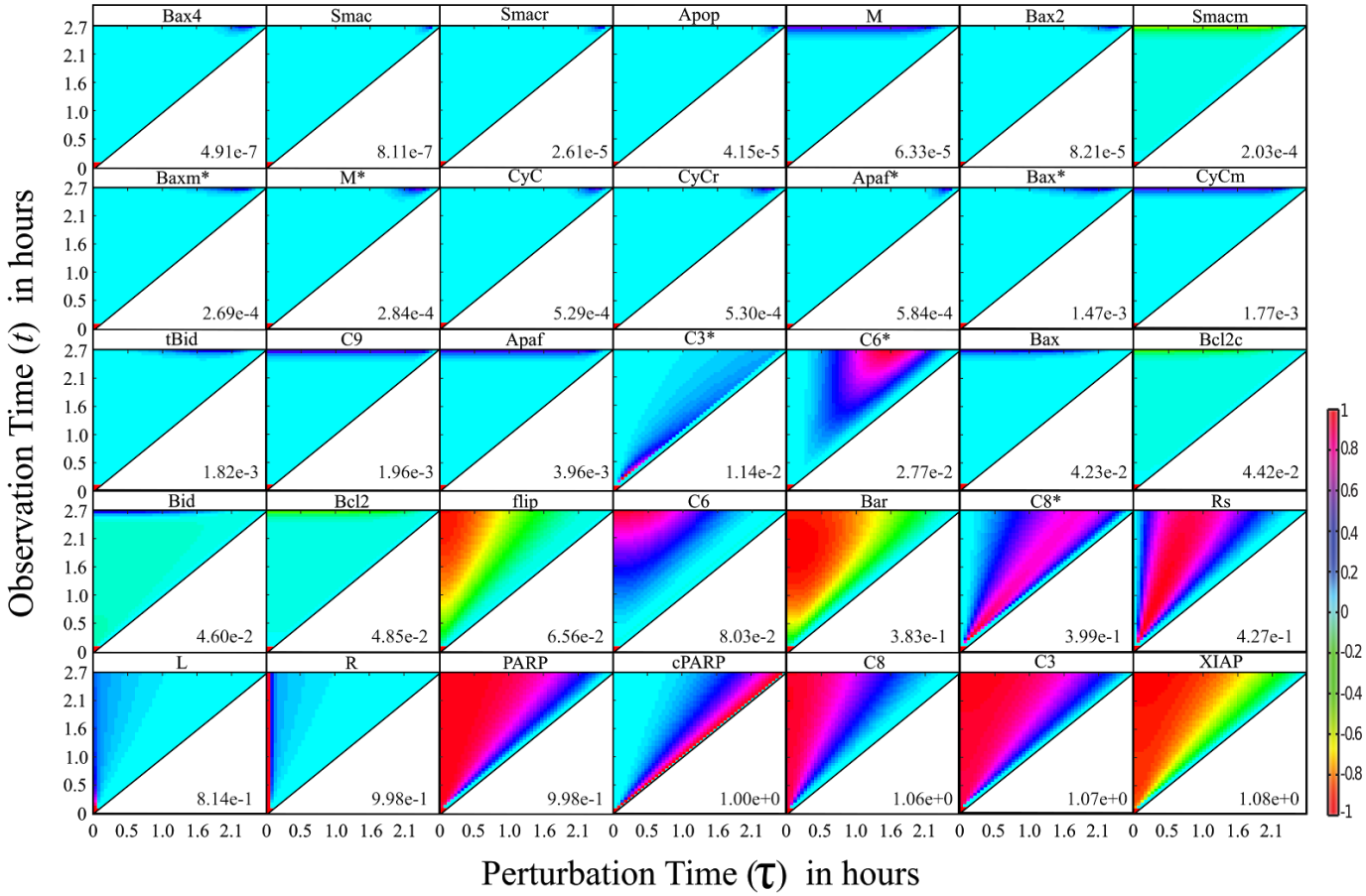
d[Bax]/dt = -r11
d[tBid:Bax]/dt = r11 -r12
d[Baxs]/dt = r12 -r13
d[Baxms]/dt = r13 -2*r14 -r36
d[Bcl2]/dt = -r36 -r37 -r38
d[Baxms:Bcl2]/dt = r36
d[Bax2]/dt = r14 -2*r15 -r37
d[Bax2:Bcl2]/dt = r37
d[Bax4]/dt = r15 -r16 -r38
d[Bax4:Bcl2]/dt = r38
d[M]/dt = -r16
d[Bax4:M]/dt = r16 -r17
d[Ms]/dt = r17 -r18 +r19 -r21 +r22
d[CyCm]/dt = -r18
d[Ms:CyCm]/dt = r18 -r19
d[CyCr]/dt = r19 -r20
d[Smacm]/dt = -r21
d[Ms:Smacm]/dt = r21 -r22
d[Smacr]/dt = r22 -r23
d[CyCc]/dt = r20 -r24 +r25
d[Apaf]/dt = -r24
d[CyCc:Apaf]/dt = r24 -r25
d[Apafs]/dt = r25 -r26
d[C9]/dt = -r26
d[Apop]/dt = r26 -r27 +r28 -r40
d[Apop:C3]/dt = r27 -r28
d[Smacc]/dt = r23 -r39
d[Apop:XIAP]/dt = r40
d[Smacc:XIAP]/dt = r39
d[C3s:Ub]/dt = r42
d[Pseudo:Variable]/dt = 0

| Initial Conditions: | #/cell | Coefficient of variation |
|---|---|---|
| L | 15000 | 0 |
| R | 200 | 0.25 |
| L:R | 0 | 0 |
| Rs | 0 | 0 |
| flip | 100 | 0.25 |
| Rs:flip | 0 | 0 |
| C8 | 20000 | 0.25 |
| Rs:C8 | 0 | 0 |
| C8s | 0 | 0 |
| Bar | 1000 | 0.25 |
| C8s:Bar | 0 | 0 |
| C3 | 10000 | 0.282 |
| C8s:C3 | 0 | 0 |
| C3s | 0 | 0 |
| C6 | 10000 | 0.25 |
| C3s:C6 | 0 | 0 |
| C6s | 0 | 0 |
| C6s:C8 | 0 | 0 |
| XIAP | 100000 | 0.288 |
| C3s:XIAP | 0 | 0 |
| PARP | 1000000 | 0.25 |
| C3s:PARP | 0 | 0 |
| cPARP | 0 | 0 |
| Bid | 40000 | 0.288 |
| C8s:Bid | 0 | 0 |
| tBid | 0 | 0 |
| Bcl2c | 20000 | 0.25 |
| Bid:Bcl2c | 0 | 0 |
| Bax | 100000 | 0.271 |

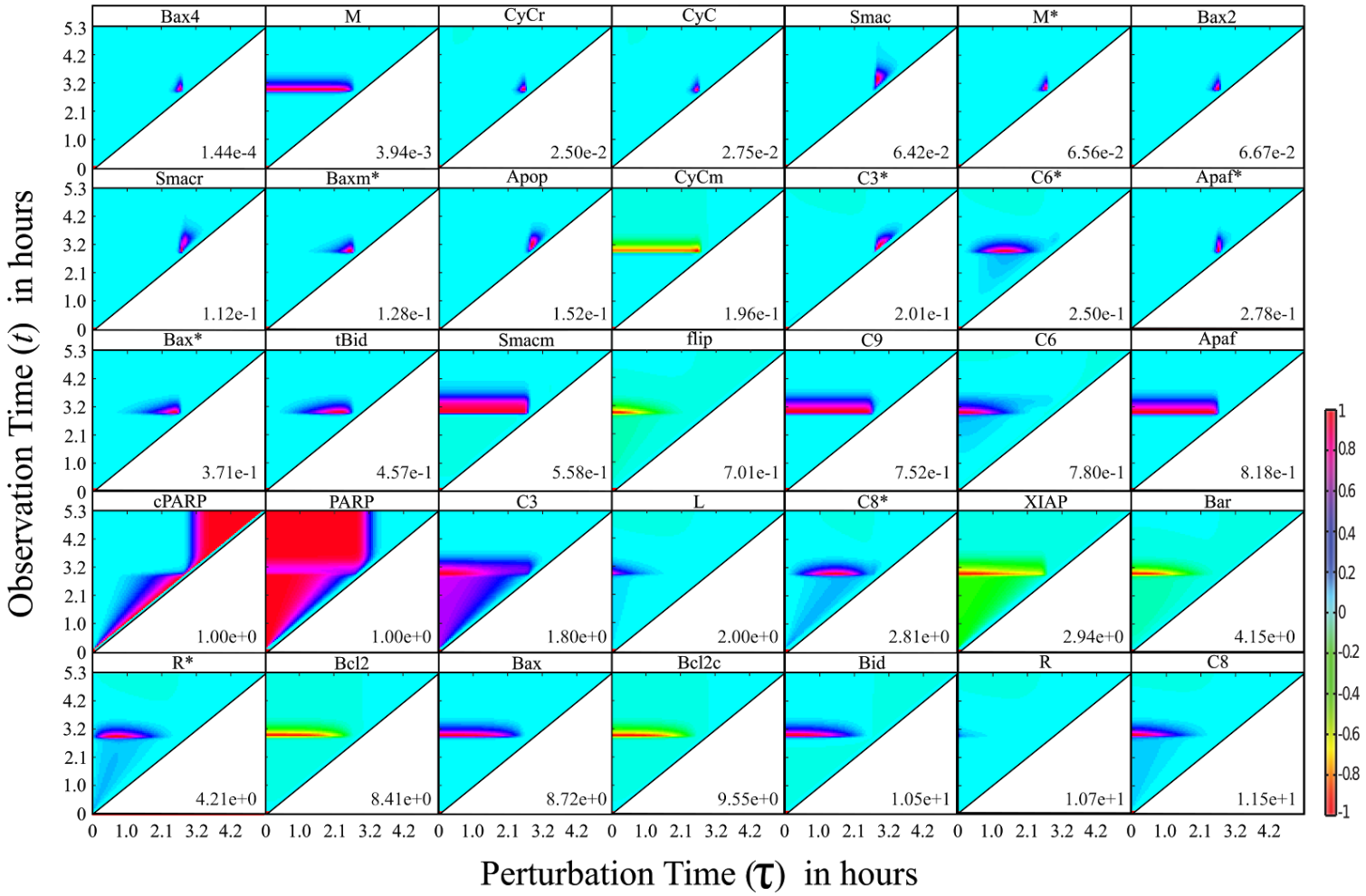
| | | |
|---|---|---|
| tBid:Bax | 0 | 0 |
| Baxs | 0 | 0 |
| Baxms | 0 | 0 |
| Bcl2 | 20000 | 0.294 |
| Baxms:Bcl2 | 0 | 0 |
| Bax2 | 0 | 0 |
| Bax2:Bcl2 | 0 | 0 |
| Bax4 | 0 | 0 |
| Bax4:Bcl2 | 0 | 0 |
| M | 500000 | 0.25 |
| Bax4:M | 0 | 0 |
| Ms | 0 | 0 |
| CyCm | 500000 | 0.25 |
| Ms:CyCm | 0 | 0 |
| CyCr | 0 | 0 |
| Smacm | 100000 | 0.25 |
| Ms:Smacm | 0 | 0 |
| Smacr | 0 | 0 |
| CyC | 0 | 0 |
| Apaf | 100000 | 0.25 |
| CyC:Apaf | 0 | 0 |
| Apafs | 0 | 0 |
| C9 | 100000 | 0.25 |
| Apop | 0 | 0 |
| Apop:C3 | 0 | 0 |
| Smac | 0 | 0 |
| Apop:XIAP | 0 | 0 |
| Smac:XIAP | 0 | 0 |
| C3s:Ub | 0 | 0 |
| Pseudo | 1 | 0 |

| Rate Constants: | $(\#/CC)^{-1}.sec^{-1}$ | Rate Constants: | $sec^{-1}$ | Rate Constants: | $sec^{-1}$ |
|---|---|---|---|---|---|
| Kf1 | 4.00E-07 | kr1 | 1.00E-03 | kf2 | 1.00E-05 |
| Kf3 | 1.00E-06 | kr3 | 1.00E-03 | kf4 | 1.00E+00 |
| Kf5 | 1.00E-07 | kr5 | 1.00E-03 | kf6 | 1.00E+00 |
| Kf7 | 1.00E-06 | kr7 | 1.00E-02 | kf8 | 1.00E+00 |
| Kf9 | 1.00E-07 | kr9 | 1.00E-03 | kf10 | 1.00E+00 |
| Kf11 | 1.00E-07 | kr11 | 1.00E-03 | kf12 | 1.00E+00 |
| Kf13 | 1.00E-02 | kr13 | 1.00E-02 | kf17 | 1.00E+00 |
| Kf14 | 1.00E-06 | kr14 | 1.00E-03 | kf19 | 1.00E+01 |
| Kf15 | 1.00E-06 | kr15 | 1.00E-03 | kf22 | 1.00E+01 |
| Kf16 | 1.00E-06 | kr16 | 1.00E-03 | kf25 | 1.00E+00 |
| Kf18 | 2.00E-06 | kr18 | 1.00E-03 | kf28 | 1.00E+00 |
| Kf20 | 1.00E-02 | kr20 | 1.00E-02 | kf30 | 1.00E+00 |
| Kf21 | 2.00E-06 | kr21 | 1.00E-03 | kf32 | 1.00E+00 |
| Kf23 | 1.00E-02 | kr23 | 1.00E-02 | kf42 | 1.00E-01 |
| Kf24 | 5.00E-07 | kr24 | 1.00E-03 | | |
| Kf26 | 5.00E-08 | kr26 | 1.00E-03 | | |
| Kf27 | 5.00E-09 | kr27 | 1.00E-03 | | |
| Kf29 | 1.00E-06 | kr29 | 1.00E-03 | | |
| Kf31 | 3.00E-08 | kr31 | 1.00E-03 | | |
| Kf33 | 1.00E-06 | kr33 | 1.00E-03 | | |
| Kf34 | 1.00E-06 | kr34 | 1.00E-03 | | |
| Kf35 | 1.00E-06 | kr35 | 1.00E-03 | | |
| Kf36 | 1.00E-06 | kr36 | 1.00E-03 | | |
| Kf37 | 1.00E-06 | kr37 | 1.00E-03 | | |
| Kf38 | 1.00E-06 | kr38 | 1.00E-03 | | |
| Kf39 | 7.00E-06 | kr39 | 1.00E-03 | | |
| Kf40 | 2.00E-06 | kr40 | 1.00E-03 | | |
| Kf41 | 2.00E-06 | kr41 | 1.00E-03 | | |
| Ratio of mitochondrial volume to cell volume (v) is taken as 0.07 | | | | | |

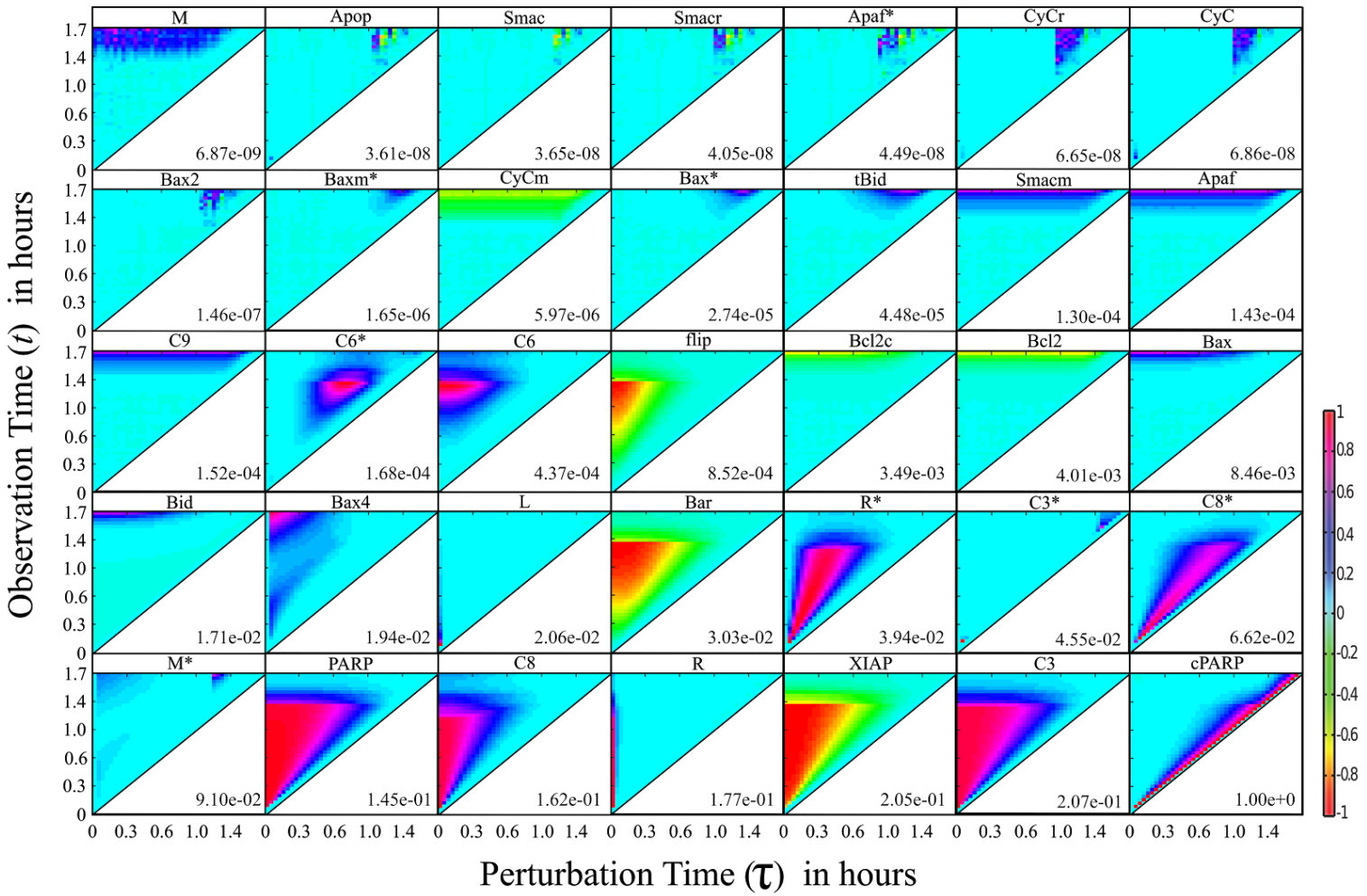
## Supplementary Figures



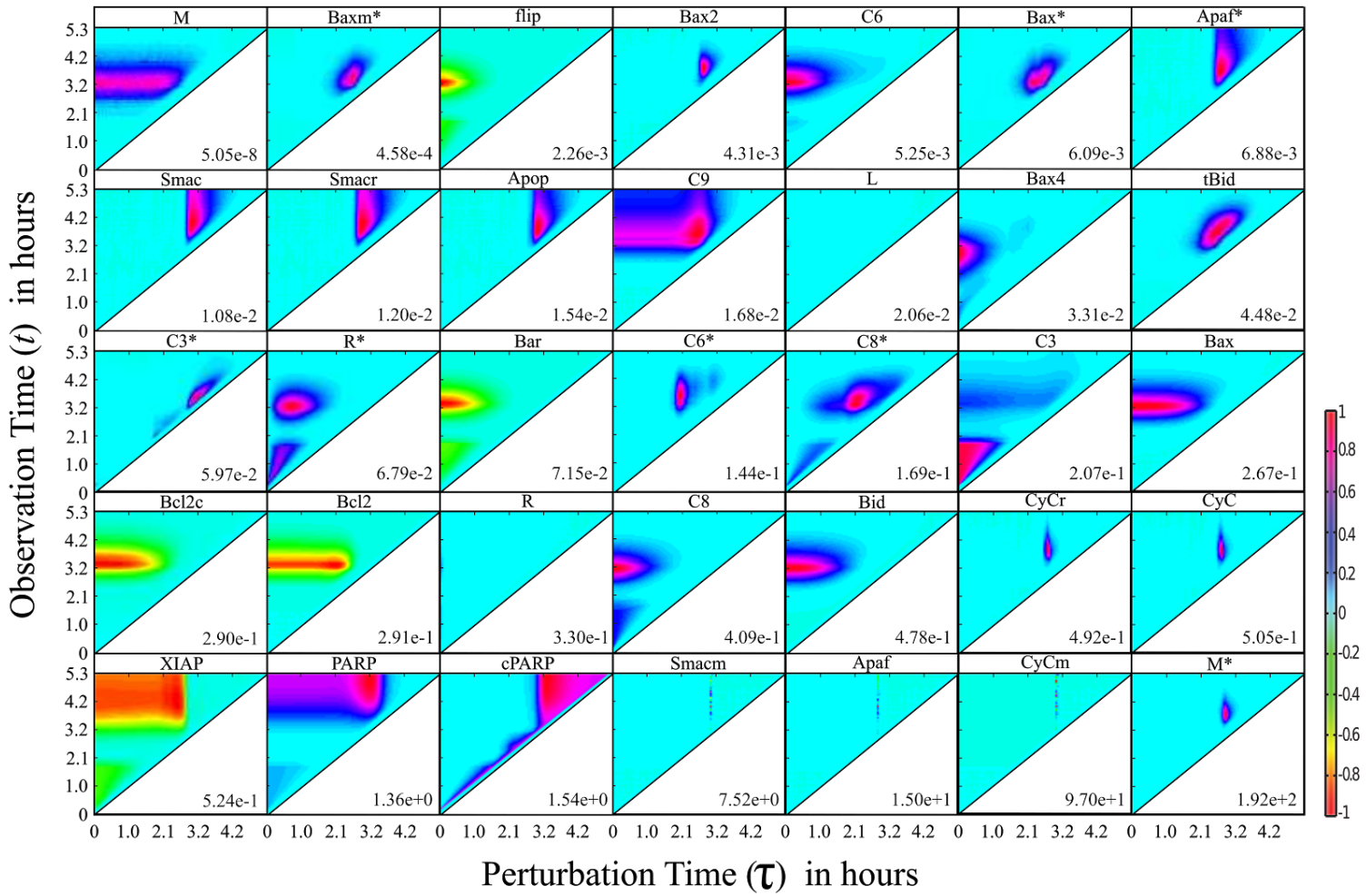

**Supplementary Figure S1.** GFM analysis of TRAIL induced apoptosis model during pre-MOMP (before 2.36 hours). The subplots give the heat maps of the GFM coefficients of cPARP levels with respect to an infinitesimal perturbations to each of the molecules. The heat maps are arranged in increasing magnitude of peak sensitivities (i.e. scaling factor shown in the bottom right corner).



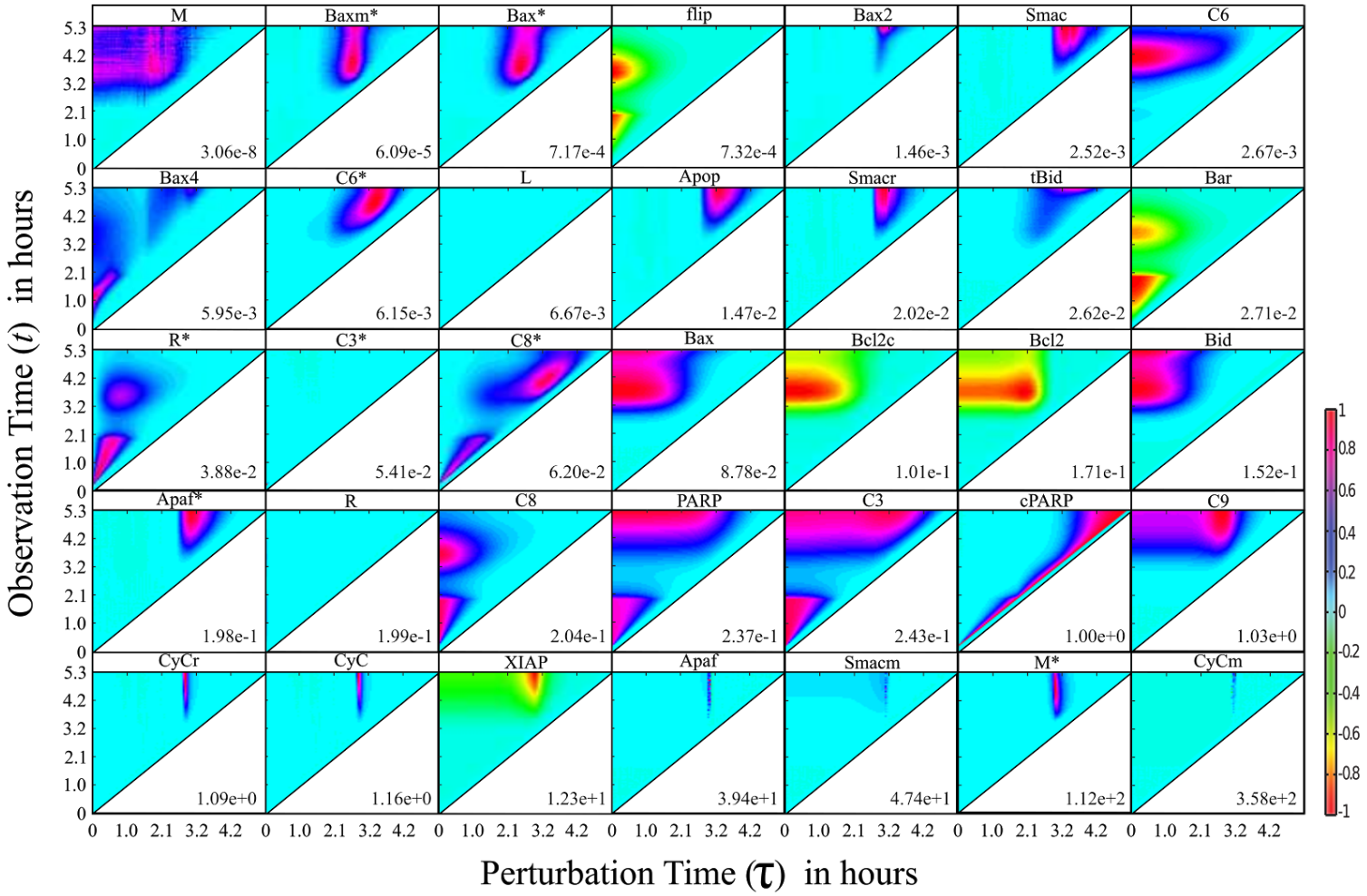

**Supplementary Figure S2.** GFM analysis of TRAIL induced apoptosis model during post-MOMP (after 2.36 hours). The subplots give the heat maps of the GFM coefficients of cPARP levels with respect to an infinitesimal perturbations to each of the molecules. The heat maps are arranged in increasing magnitude of peak sensitivities (i.e. scaling factor shown in the bottom right corner).



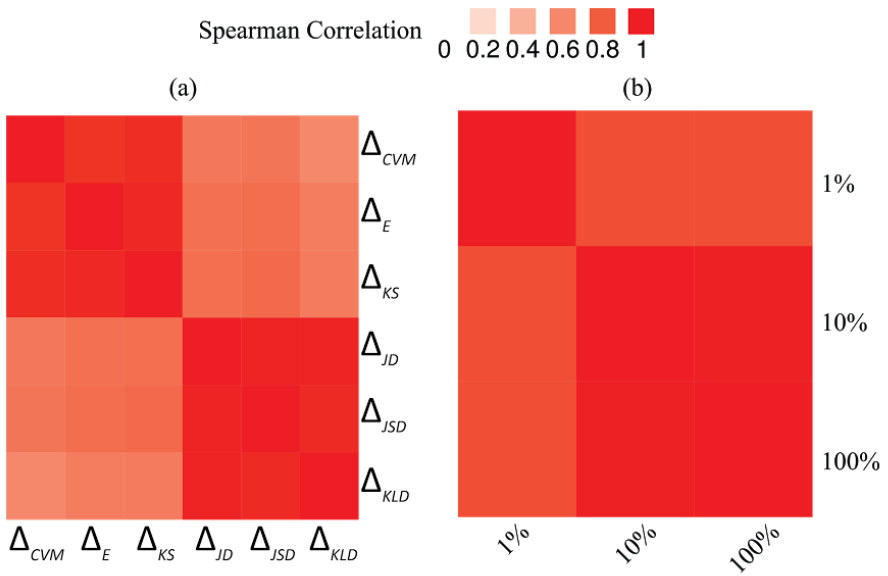

**Supplementary Figure S3.** MDFP analysis of TRAIL induced apoptosis model during pre-MOMP (before 1.76 hours). The subplots give the heat maps of the MDFP coefficients of cPARP levels with respect to a mean-shift perturbations to each of the molecules. The heat maps are arranged in increasing magnitude of peak sensitivities (i.e. scaling factor shown in the bottom right corner).




**Supplementary Figure S4.** MDFP analysis of TRAIL induced apoptosis model during post-MOMP (after 1.76 hours). The subplots give the heat maps of the MDFP coefficients of cPARP levels with respect to a mean-shift perturbations to each of the molecules. The heat maps are arranged in increasing magnitude of peak sensitivities (i.e. scaling factor shown in the bottom right corner).




**Supplementary Figure S5:** MDFP analysis of non-apoptotic Hela subpopulation. The subplots give the heat maps of the MDFP coefficients of cPARP levels with respect to a mean-shift perturbations to each of the molecules. The heat maps are arranged in increasing magnitude of peak sensitivities (i.e. scaling factor shown in the bottom right corner).




**Supplementary Figure S6:** Spearman correlations of MDFP sensitivity coefficients using different distribution distances and perturbation sizes. (a) Correlation of the MDFP sensitivity coefficients calculated using different distribution distances with a 10% mean perturbation. (b) Correlation of the MDFP sensitivity coefficients calculated using different sizes (1%, 10% and 100%) of mean perturbations with the Cramer-von Mise distance ($\Delta_{CVM}$).

## References


1. Spencer, S. L.; Gaudet, S.; Albeck, J. G.; Burke, J. M.; Sorger, P. K. Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis. *Nature* **2009**, *459*, 428–432.
2. Albeck, J. G.; Burke, J. M.; Spencer, S. L.; Lauffenburger, D. A.; Sorger, P. K. Modeling a snap-action, variable-delay switch controlling extrinsic cell death. *PLoS Biol.* **2008**, *6*, 2831–2852.