Biophysical Journal, Volume *122*

Supplemental information

A biochemical necroptosis model explains cell-type-specific responses to cell death cues

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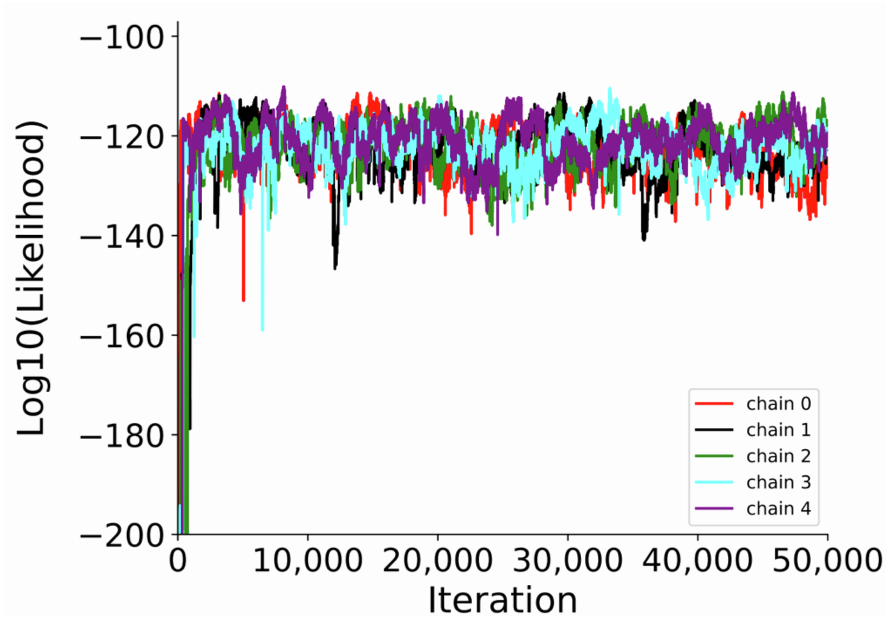

Figure S1. Log-likelihood vs. iteration for all five Markov chains used in the Bayesian parameter calibration. For each chain, the first 25,000 iterations were discarded (considered burn-in), leaving a total of 125,000 parameter sets, of which 10,628 are unique.

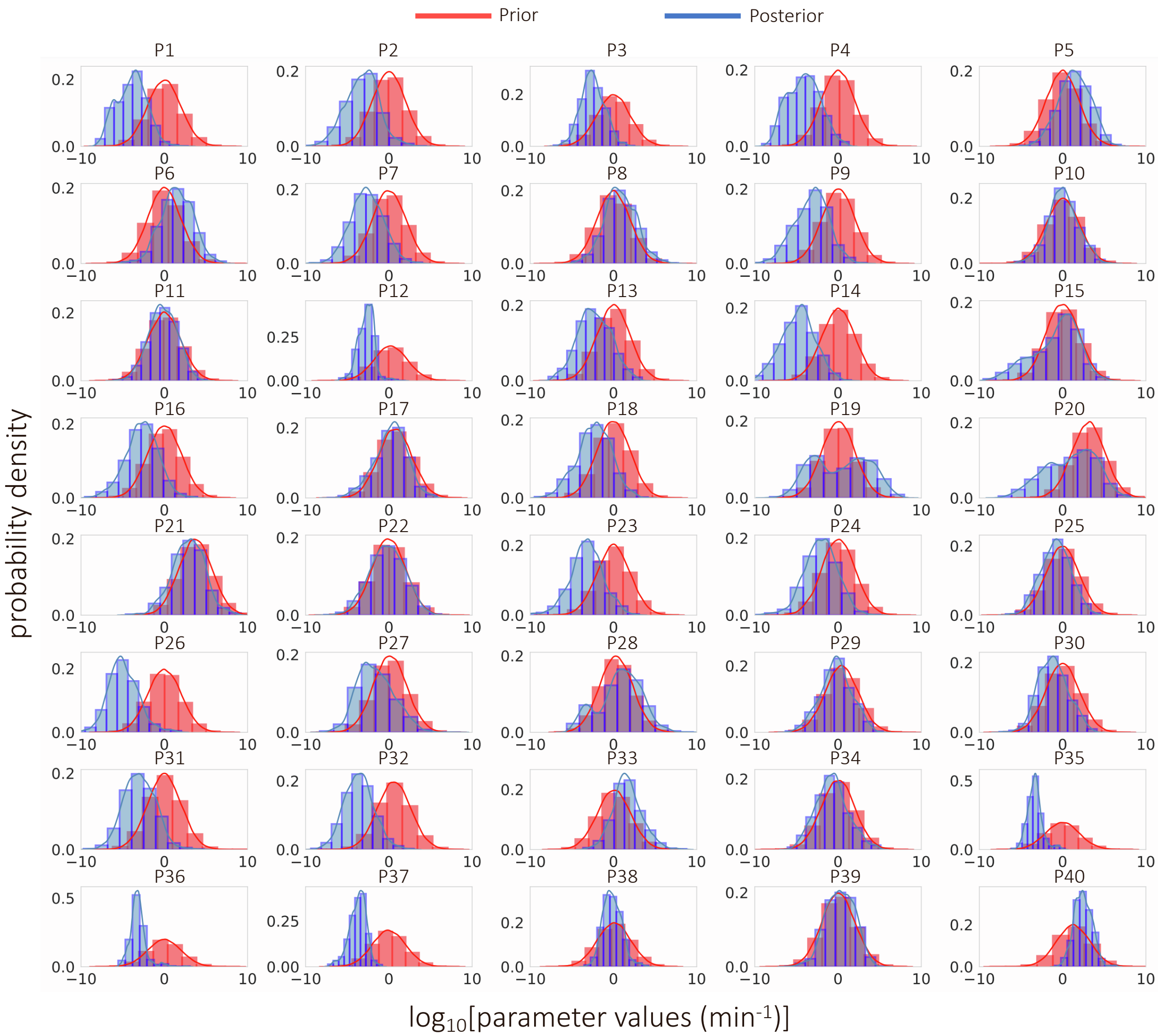

Figure S2. Distributions of parameter values from Bayesian model calibration. Both prior (red) and posterior (blue) distributions are shown.

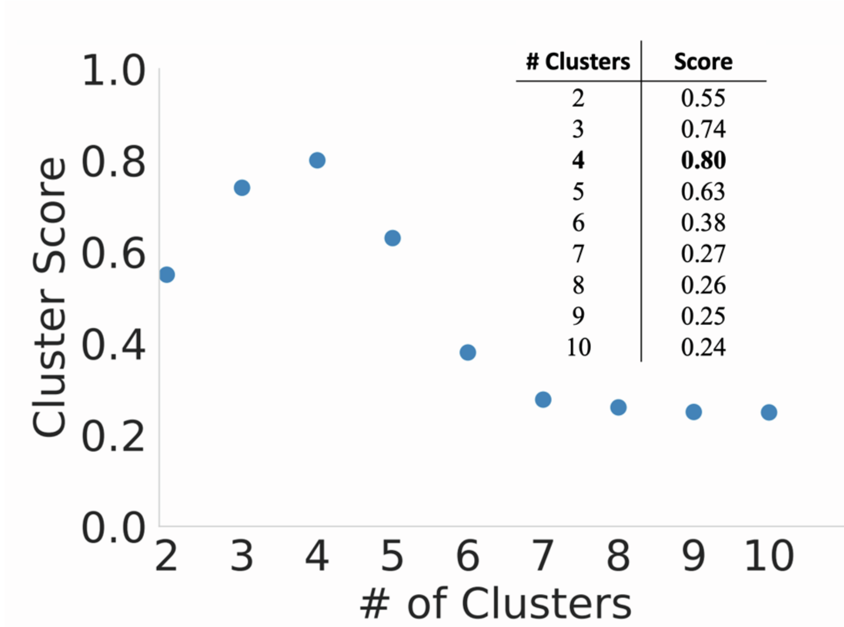

Figure S3. Silhouette clustering scores for determining the number of modes of necroptosis execution. The maximum value is for four clusters. Values were also calculated for 11-20 clusters and were all <0.3 (data not shown).

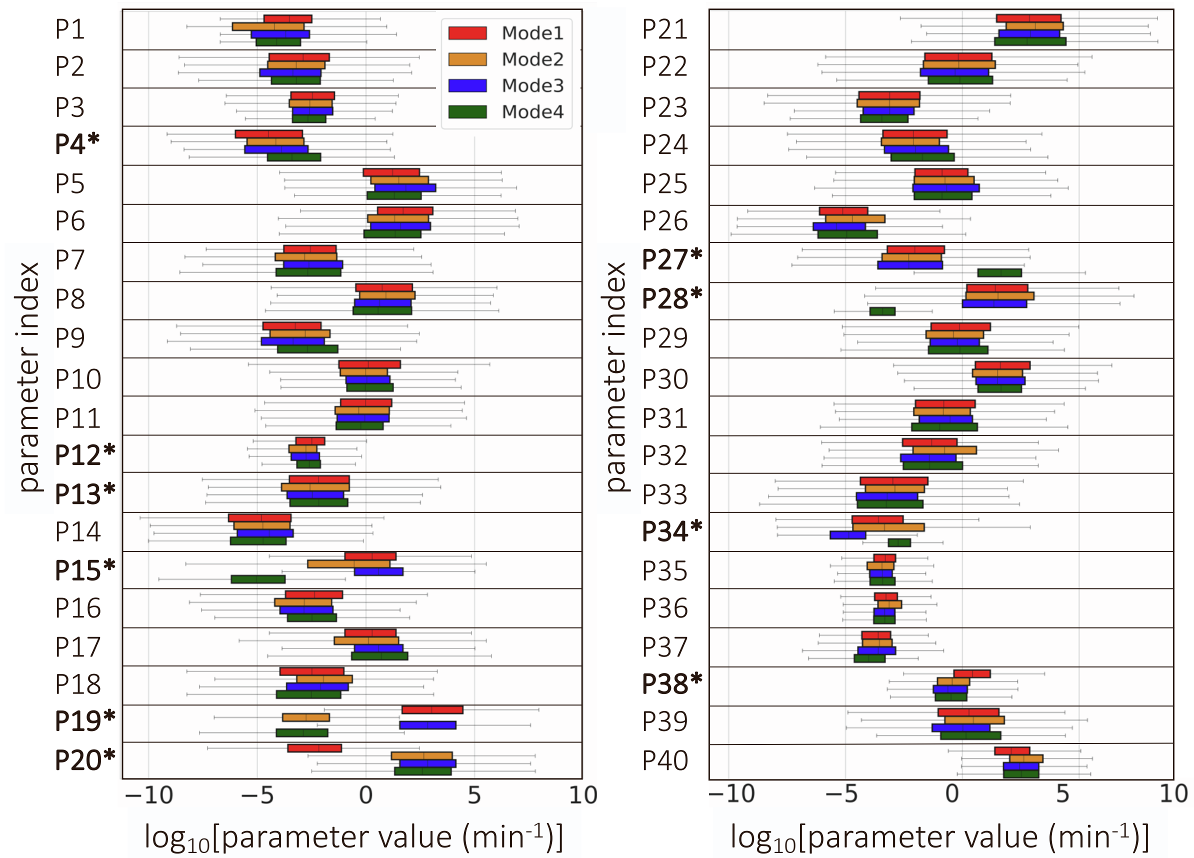

Figure S4. Rate constant distributions for all four modes of execution. Parameters with asterisks (*) are included in Figure 3C of the main text. Parameter indices (P*N*) match reaction indices (R*N*) in Figure 1 and Table 2 of the main text.

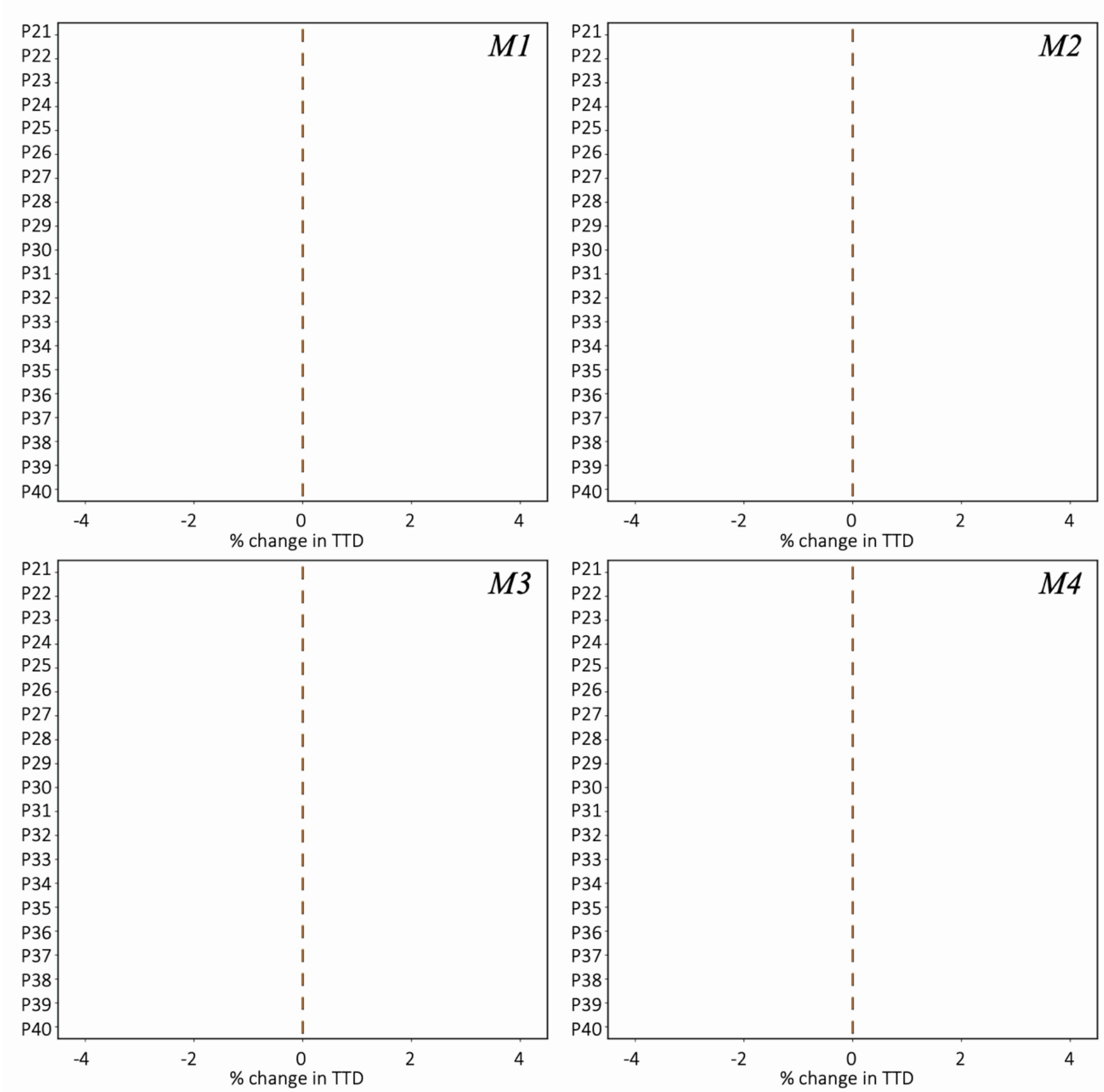

Figure S5. Rate constant sensitivity analyses show no sensitivity to rate constants P21-P40 in any mode. Values were varied in a range ±20% around the reference parameter set for each mode. Parameter indices (P*N*) match reaction indices (R*N*) in Figure 1 and Table 2 of the main text.

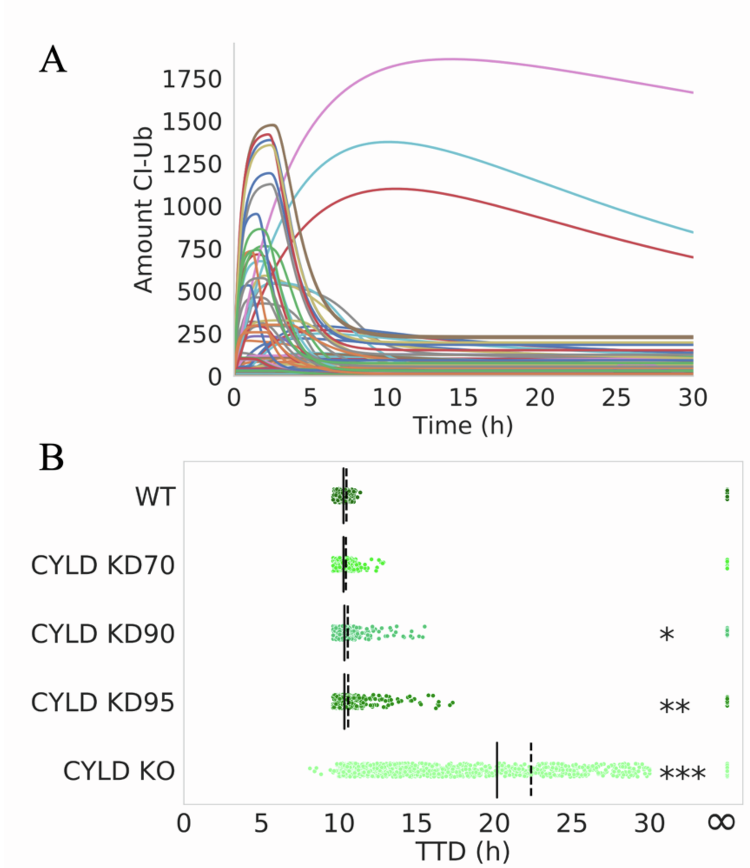

Figure S6. Dynamics in necroptosis execution mode 4. (A) Time courses for ubiquitinated complex I (CI-Ub) for all parameter sets in mode 4 show that CYLD (8,868 molecules; Table 1 of the main text) is always in great excess. (B) TTD distributions over all parameter sets in mode 4 for CYLD knockdowns (KDs; 70%-95%) and knock out (KO), compared to wild-type (WT). Solid black lines = medians, dashed black lines = means; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Mood's median test).

Table S1. List of molecular species in the necroptosis model. `C8i`, `C8a`: inactive and active caspase-8; `MLKL-u`, `MLKL-p`: unphosphorylated and phosphorylated MLKL; `RIP1-u`, `RIP1-Ub`, `RIP1-p`, `RIP1-trunc`: unmodified, ubiquitinated, phosphorylated, and truncated (degraded) RIP1; `RIP3-u`, `RIP3-p`: unphosphorylated and phosphorylated RIP3. Percent sign (`%`) signifies that proteins are within the same complex (not necessarily bound to each other).

Variable	Species
x_0	TNF
x_1	TNFR
x_2	TRADD
x_3	RIP1-u
x_4	TRAF
x_5	cIAP
x_6	MLKL-u
x_7	A20
x_8	CYLD
x_9	FADD
x_{10}	RIP3-u
x_{11}	FLIP
x_{12}	LUBAC
x_{13}	C8i
x_{14}	TNF % TNFR
x_{15}	TNF % TNFR % TRADD
x_{16}	RIP1-u % TNF % TNFR % TRADD
x_{17}	RIP1-u % TNF % TNFR % TRADD % TRAF
x_{18}	RIP1-u % TNF % TNFR % TRADD % TRAF % cIAP
x_{19}	RIP1-Ub % TNF % TNFR % TRADD % TRAF % cIAP
x_{20}	LUBAC % RIP1-Ub % TNF % TNFR % TRADD % TRAF % cIAP
x_{21}	A20 % LUBAC % RIP1-Ub % TNF % TNFR % TRADD % TRAF % cIAP
x_{22}	CYLD % LUBAC % RIP1-Ub % TNF % TNFR % TRADD % TRAF % cIAP
x_{23}	RIP1-u % TRADD
x_{24}	FADD % RIP1-u % TRADD
x_{25}	C8i % FADD % RIP1-u % TRADD
x_{26}	FADD % RIP1-u % RIP3-u % TRADD
x_{27}	C8i % FADD % FLIP % RIP1-u % TRADD
x_{28}	RIP1-u % RIP3-u
x_{29}	C8a % FADD % FLIP % RIP1-u % TRADD
x_{30}	RIP1-u % RIP3-p
x_{31}	RIP1-trunc
x_{32}	C8a % FLIP
x_{33}	RIP1-p % RIP3-p
x_{34}	C8a % FADD % FLIP % RIP1-u % RIP3-u % TRADD
x_{35}	MLKL-u % RIP1-p % RIP3-p
x_{36}	MLKL-p

Table S2. Set of coupled differential equations for the necroptosis model. Species corresponding to each variable, x_i, are given in Table S1. Square brackets indicate concentration. Median values of the rate constants (p_i) for each mode are given in Table 2 of the main text.

$\boldsymbol{d[x_0]/dt}$	=	$\boldsymbol{p_{19}[x_{21}] + p_2[x_{14}] + p_{20}[x_{22}] - p_3[x_0] - p_1[x_0][x_1]}$
$\boldsymbol{d[x_1]/dt}$	=	$p_{19}[x_{21}] + p_2[x_{14}] + p_{20}[x_{22}] - p_1[x_0][x_1]$
$\boldsymbol{d[x_2]/dt}$	=	$p_{29}[x_{29}] + p_{34}[x_{34}] + p_{35}[x_{26}] + p_5[x_{15}] - p_4[x_{14}x_2]$
$\boldsymbol{d[x_3]/dt}$	=	$p_7[x_{16}] - p_6[x_{15}][x_3]$
$\boldsymbol{d[x_4]/dt}$	=	$p_{19}[x_{21}] + p_{20}[x_{22}] + p_9[x_{17}] - p_8[x_{16}][x_4]$
$\boldsymbol{d[x_5]/dt}$	=	$p_{11}[x_{18}] + p_{19}[x_{21}] + p_{20}[x_{22}] - p_{10}[x_{17}][x_5]$
$\boldsymbol{d[x_6]/dt}$	=	$p_{39}[x_{35}] - p_{38}[x_{33}][x_6]$
$\boldsymbol{d[x_7]/dt}$	=	$p_{16}[x_{21}] + p_{19}[x_{21}] - p_{15}[x_{20}[x_7]$
$\boldsymbol{d[x_8]/dt}$	=	$p_{18}[x_{22}] + p_{20}[x_{22}] - p_{17}[x_{20}][x_8]$
$\boldsymbol{d[x_9]/dt}$	=	$p_{22}[x_{24}] + p_{29}[x_{29}] + p_{34}[x_{34}] + p_{35}[x_{26}] - p_{21}[x_{23}][x_9]$
$\boldsymbol{d[x_{10}]/dt}$	=	$p_{31}[x_{26}] + p_{34}[x_{34}] - p_{30}[x_{10}][x_{24}]$
$\boldsymbol{d[x_{11}]/dt}$	=	$p_{26}[x_{27}] - p_{25}[x_{11}][x_{25}]$
$\boldsymbol{d[x_{12}]/dt}$	=	$p_{14}[x_{20}] + p_{19}[x_{21}] + p_{20}[x_{22}] - p_{13}[x_{12}][x_{19}]$
$\boldsymbol{d[x_{13}]/dt}$	=	$p_{24}[x_{25}] - p_{23}[x_{13}][x_{24}]$
$\boldsymbol{d[x_{14}]/dt}$	=	$-p_2[x_{14}] + p_5[x_{15}] + p_1[x_0][x_1] - p_4[x_{14}][x_2]$
$\boldsymbol{d[x_{15}]/dt}$	=	$-p_5[x_{15}] + p_7[x_{16}] + p_4[x_{14}][x_2] - p_6[x_{15}][x_3]$
$\boldsymbol{d[x_{16}]/dt}$	=	$-p_7[x_{16}] + p_9[x_{17}] + p_6[x_{15}][x_3] - p_8[x_{16}][x_4]$
$\boldsymbol{d[x_{17}]/dt}$	=	$p_{11}[x_{18}] - p_9[x_{17}] - p_{10}[x_{17}][x_5] + p_8[x_{16}][x_4]$
$\boldsymbol{d[x_{18}]/dt}$	=	$-p_{11}[x_{18}] - p_{12}[x_{18}] + p_{10}[x_{17}][x_5]$
$\boldsymbol{d[x_{19}]/dt}$	=	$p_{12}[x_{18}] + p_{14}[x_{20}] - p_{13}[x_{12}][x_{19}]$
$\boldsymbol{d[x_{20}]/dt}$	=	$-p_{14}[x_{20}] + p_{16}[x_{21}] + p_{18}[x_{22}] + p_{13}[x_{12}][x_{19}] - p_{15}[x_{20}][x_7] - p_{17}[x_{20}][x_8]$
$\boldsymbol{d[x_{21}]/dt}$	=	$-p_{16}[x_{21}] - p_{19}[x_{21}] + p_{15}[x_{20}][x_7]$
$\boldsymbol{d[x_{22}]/dt}$	=	$-p_{18}[x_{22}] - p_{20}[x_{22}] + p_{17}[x_{20}][x_8]$
$\boldsymbol{d[x_{23}]/dt}$	=	$p_{19}[x_{21}] + p_{20}[x_{22}] + p_{22}[x_{24}] - p_{21}[x_{23}][x_9]$
$\boldsymbol{d[x_{24}]/dt}$	=	$-p_{22}[x_{24}] + p_{24}[x_{25}] + p_{31}[x_{26}] + p_{21}[x_{23}][x_9] - p_{23}[x_{13}][x_{24}] - p_{30}[x_{10}][x_{24}]$
$\boldsymbol{d[x_{25}]/dt}$	=	$-p_{24}[x_{25}] + p_{26}[x_{27}] + p_{23}[x_{13}][x_{24}] - p_{25}[x_{11}][x_{25}]$
$\boldsymbol{d[x_{26}]/dt}$	=	$-p_{31}[x_{26}] + p_{33}[x_{34}] - p_{35}[x_{26}] + p_{30}[x_{10}][x_{24}] - p_{32}[x_{26}][x_{32}]$
$\boldsymbol{d[x_{27}]/dt}$	=	$-p_{26}[x_{27}] - p_{27}[x_{27}] + p_{28}[x_{29}] + p_{25}[x_{11}][x_{25}]$
$\boldsymbol{d[x_{28}]/dt}$	=	$p_{35}[x_{26}] + p_{36}[x_{28}]$
$\boldsymbol{d[x_{29}]/dt}$	=	$p_{27}[x_{27}] - p_{28}[x_{29}] - p_{29}[x_{29}]$
$\boldsymbol{d[x_{30}]/dt}$	=	$p_{36}[x_{28}] - p_{37}[x_{30}]$
$\boldsymbol{d[x_{31}]/dt}$	=	$p_{29}[x_{29}] + p_{34}[x_{34}]$
$\boldsymbol{d[x_{32}]/dt}$	=	$p_{29}[x_{29}] + p_{33}[x_{34}] + p_{34}[x_{34}] - p_{32}[x_{26}][x_{32}]$
$\boldsymbol{d[x_{33}]/dt}$	=	$p_{37}[x_{30}] + p_{39}[x_{35}] + p_{40}[x_{35}] - p_{38}[x_{33}][x_6]$
$\boldsymbol{d[x_{34}]/dt}$	=	$-p_{33}[x_{34}] - p_{34}[x_{34}] + p_{32}[x_{26}][x_{32}]$
$\boldsymbol{d[x_{35}]/dt}$	=	$-p_{39}[x_{35}] - p_{40}[x_{35}] + p_{38}[x_{33}][x_6]$
$\boldsymbol{d[x_{36}]/dt}$	=	$p_{40}[x_{35}]$