Supplementary Table 1

A. Parameter values used in simulation				
Species concentration	**Values used in Simulation**	**Source**	**Remarks**	
Ligand (Anti-CD16 antibody)	60 molecules/μm^3	Estimated	We assumed the antibody molecules in a volume of 1.0 μm × 25 μm^2 (area of the plasma membrane in the simulation box) bind to the CD16 molecules in the simulation.	
CD16 Receptor	80 molecules /μm^2	Fig. 1B in (1)	Ref. (1) estimates 70000-110000 CD16 receptor per NK cells obtained from PBMCs. Assuming the diameter of a primary human NK cell to be ~7 μm, the concentration of CD16 in the plasma membrane is ~ 450 molecules /μm^2. For KHYG1 cell line we have assumed a smaller concentration of CD16 than the primary NK cells in the PBMCs.	
CD3ζ	80 molecules/μm^2	Assumption	Surface expressions of human CD16 is stabilized by CD3ζ adaptors (Fig. S3 in (2)). We assumed the concentrations of CD16 are CD3ζ are similar.	
LCK	390 molecules /μm^2	Table S1 in (3)	Taken from the values measured for Jurkat T cells.	
SHP-1	0.3 μM =180 molecules/μm^3	Table S1 in (3)	Value used using estimation of SHP-2 in Jurkat T cells, maxQB database (4)	
Cbl	141 molecules/μm^3	See Table S2	Estimated by fitting our model to the Ca^{++} kinetics data.	
Kinetic reaction rates	**Values used in Simulation**	**Region of reaction**	**Source**	**Remarks**

CD16 binding with anti-CD16 antibody (k_{ON})	1.7×10^{-5} μm^3 s^{-1} molecule $^{-1}$	Extracellular volume (V_e) V_e = 5μm × 5μm × 0.002μm = 0.05 μm^3		CD16 are crosslinked by anti-CD16 antibody (2). We considered here, K_D to be ~ 1 μM.
CD16 unbinding from anti-CD16 antibody (k_{OFF})	9.95×10^{-3} s^{-1}≅ 0.01 s^{-1}	Extracellular volume (V_e)	Table 1 in (5)	Value corresponds to IgG1 Fc (WT) unbinding from human CD16A – 158V in ref. (5).
CD3ζ adaptor (homodimer) binding to the CD16 receptor	2×10^{-3} μm^2 s^{-1} molecule^{-1}	Membrane (A) A= 5μm × 5μm = 25 μm^2	Assumption	
CD3ζ adaptor (homodimer) unbinding from CD16 receptor	0.01 s^{-1}	Membrane (A)	Assumption	
Phosphorylation of CD3ζ ITAMs by LCK	1.5×10^{-2} μm^2 s^{-1}	Membrane (A)	Table 1 in (6)	Values of k_{cat}/k_M for Lck pY394-pY505 were calculated by fitting Michaelis Menten model in ref. (6).
Dephosphorylation of ITAMs on CD3ζ	0.001 s^{-1}	Membrane (A)		Assumption
ZAP70 binding to fully phosphorylated ITAM	5 x 10^6 M^{-1}s^{-1} =5 (μM s)$^{-1}$=8.3 × 10^{-3} μm^3 s^{-1} molecule^{-1}	Cytosolic volume (V_c) V_c = 5 μm× 5μm×1 μm = 25 μm^3	(7)	Measured values reported in ref. (7) for T cells. K_a (association rate)

				= 5 x 10^6 $M^{-1}s^{-1}$
ZAP70 unbinding from fully phosphorylated ITAM	0.125 s^{-1}	Cytosolic volume (V_c)	(7)	Measured values reported in ref. (7) for T cells. K_a (association rate)= 5 x 10^6 $M^{-1}s^{-1}$, and, K_D=25 nM We approximated the unbinding rate as $K_a \times K_D$ using the values reported in ref. (7) .
ZAP70 phosphorylation by LCK	3×10^{-5} $(nMs)^{-1}$=0.03 $(\mu M\ s)^{-1}$ = 4.98×10^{-5} $\mu m^3\ s^{-1}$ molecule $^{-1}$	Cytosolic volume (V_c)	Table 2 in (8)	Value used for phosphorylation of ZAP70 Y493 by Lck.
ZAP70 (free or bound) dephosphorylation by SHP-1	0.034 $(\mu M\ s)^{-1}$ = 5.64×10^{-5} $\mu m^3\ s^{-1}$ molecule $^{-1}$	Cytosolic volume (V_c)	Table 2 in (9)	Catalytic rate of SHP-1(tethered) for substrate PEG12 calculated in (9).
SYK binding to fully phosphorylated ITAM	5 $(\mu M\ s)^{-1}$=8.3×10^{-3} $\mu m^3\ s^{-1}$ molecule $^{-1}$	Cytosolic volume (V_c)	(7)	SYK and ZAP70 affinities to fully phosphorylated ITAMs correspond to K_a (association rate)= 5 x 10^6 $M^{-1}s^{-1}$ reported in (7).
SYK binding to partially phosphorylated ITAM	8.3×10^{-4} $\mu m^3\ s^{-1}$ molecule	Cytosolic volume (V_c)	Assumption	We assumed SYK binds to the partially phosphorylated

				state of the ITAM (state U) with a 10 × lower rate (=250 nM)
SYK unbinding from fully or partially phosphorylated ITAM	0.125 s^{-1}	Cytosolic volume (V_c)	(7)	SYK and ZAP70 affinities to the ITAM correspond to K_a (association rate)= 5 x 10^6 $M^{-1}s^{-1}$ and K_D=25 nM reported in ref. (7).
Catalytically active SYK phosphorylating ITAM	1.21 s^{-1}	Cytosolic volume (V_c)	Table S1 in (10)	Assumed following BCR signaling where phospho-ITAM bound catalytically active SYK fully phosphorylates the ITAM it is attached to (11).
Basally active SYK phosphorylating ITAM	8.4 $\mu M^{-1}s^{-1}$ =0.014 μm^3 s^{-1} molecule $^{-1}$	Cytosolic volume (V_c)	Table S1 in (10)	Assumed following BCR signaling where phospho-ITAM bound basally active SYK fully phosphorylates other ITAMs (12).
Catalytically active SYK phosphorylating ITAM	15.1 $\mu M^{-1}s^{-1}$=0.025 μm^3 s^{-1} molecule $^{-1}$	Cytosolic volume (V_c)	Table S1 in (10)	Assumed following BCR signaling where phospho-ITAM bound catalytically active SYK fully phosphorylates other ITAMs.

SYK or ZAP70 (bound or free) dephosphorylation rate by phosphatase SHP-1	5.64×10^{-5} μm^3 s^{-1} molecule $^{-1}$	Cytosolic volume (V_c)	(13)	Assumed similar dephosphorylation rates for pSYK and pZAP70.
SHP-1 binding rate to fully phosphorylated ITAM	0.25 $(\mu M\ s)^{-1}$ $= 4.15 \times 10^{-4}$ μm^3 s^{-1} $molecule^{-1}$	Cytosolic volume (V_c)	Table 2 in (9) SI in (14)	Value measured for PEG12 peptides in SPR experiment. SHP-1 injected over immobilized phosphorylated PEG-PD1 peptides.
SHP-1 unbinding rate from fully phosphorylated ITAM	1.7 s^{-1}		Table 2 in (9)	Value measured for PEG12 peptides.
SHP-1 binding to partially phosphorylated ITAM	0.25 $(\mu M\ s)^{-1}$ $= 4.15 \times 10^{-4}$ μm^3 s^{-1} $molecule^{-1}$	Cytosolic volume (V_c)	Table 2 in (9) SI in (14)	We considered the same rates for binding of SHP-1 to partially or fully phosphorylated ITAMs.
SHP-1 unbinding from partially phosphorylated ITAM	1.7 s^{-1}		Table 2 in (9)	
Cbl binds to ITAM bound ZAP70 or SYK	0.4 $(\mu M\ s)^{-1}$ $= 6.7 \times 10^{-4}$ μm^3 s^{-1} $molecule^{-1}$		Assumption	
Cbl unbinds from ITAM bound ZAP70 or SYK	0.004 s^{-1}		Assumption	
ITAM bound ZAP70 or SYK degradation rate by Cbl	0.07 s^{-1}		Assumption	Cbl binds to the phospho-ITAM bound ZAP70 and

				SYK and degrades the ITAM bound molecule (ZAP70 or SYK) and whole CD16 (15).
Free pZAP70 or pSYK dephosphorylation	0.01 s^{-1}		Assumption	Represents dephosphorylation of ZAP70 and SYK by other phosphatases than SHP-1.
Parameter k_1 in Ca^{++} ODE	0.7 μM		(16, 17)	Determined by fitting data for Xenopus oocyte in ref. (17)
parameter k_2 in Ca^{++} ODE	0.7 μM		(16, 17)	Determined by fitting data for Xenopus oocyte in ref.(17)
Parameter b in Ca^{++} ODE	0.111		(16, 17)	Determined by fitting data for Xenopus oocyte in ref. (17)
Rate for the kinetic proof-reading reaction step	0.01 s^{-1}		Assumption	

B: Estimated parameters from *in silico* model training for limited ITAMs (concentrations of CD16 and CD3ζ homodimers = 80 molecules/μm^2)

	Parameters	**Estimated values**
CD16 signaling model	initial ZAP70 concentration	1131 molecules/um^3
CD16 signaling model	initial SYK concentration	754 molecules/um^3
	SYK auto-phosphorylation rate	0.0026 s^{-1}
	SYK trans-phosphorylation rate	0.0022 μm^3 molecule^{-1} s^{-1}
	Cbl concentration	141 molecules/μm^3
	Parameters for ΔSYK	**Parameters for ΔZAP70**

Ca^{++} ODE model	**Parameters**	**Estimated Values**	**Parameters**	**Estimated Values**
	C_{1Z}	0.785 s^{-1}	C_{1S}	0.058 s^{-1}
	C_{2Z}	0.161 μM^{-1} s^{-1}	C_{2S}	0.0001 μM^{-1} s^{-1}
	γ_Z	0.012 s^{-1}	γ_S	0.00103 s^{-1}

C. Estimated parameters from *in silico* model training for higher ITAMs (concentrations of CD16 and CD3ζ homodimers = 200 per μm^2)

CD16 signaling model	**Parameters**		**Estimated Values**	
	initial ZAP70 concentration		896 molecules/μm^3	
	initial SYK concentration		598 molecules/μm^3	
	SYK auto-phosphorylation rate		0.014959 s^{-1}	
	SYK trans-phosphorylation rate		2.823×10^{-5} μm^3 $molecule^{-1}$ s^{-1}	
Ca^{++} ODE model	**Parameters for ZAP70**		**Parameters for SYK**	
	Parameters	**Estimated Values**	**Parameters**	**Estimated Values**
	C_{1Z}	0.2243 s^{-1}	C_{1S}	0.0545 s^{-1}
	C_{2Z}	0.538418 μM^{-1} s^{-1}	C_{2S}	0.00107 μM^{-1} s^{-1}
	γ_Z	0.00559 s^{-1}	γ_S	0.000264 s^{-1}

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