

**Web Supplement for “Activation or Tolerance of Natural Killer Cells is Modulated by Ligand Quality in a Non-Monotonic Manner”**

**Table SI: Reactions and rate constants used for the results in Fig. 2**

Reaction	$k_{on}$ ( $\mu\text{M}^{-1}\text{s}^{-1}$ )	$k_{off}$ ( $\text{s}^{-1}$ )	$K_D = k_{off}/k_{on}$ ( $\mu\text{M}$ )	$k_{cat}$ ( $\text{s}^{-1}$ )
1. $NKp46 + \text{Heparin} \leftrightarrow NKp46 - \text{Heparin}$ stimulatory ligand (mouse)	0.0072 (1)	0.0029 (1)	0.401	---
2. $Ly49A + H - 2D^d \leftrightarrow Ly49A - H - 2D^d$ inhibitory ligand (mouse)	0.0039	0.025 (2)	7.6 (2)	---
3. $\text{Lck} \rightarrow \text{Lck}^{pY394}$  $\text{Lck}$ – denotes basally activated Lck $\text{Lck}^{pY394}$ – denotes fully activated Lck (phosphorylated at Y394)	---	---	---	0.1 * (* implies estimated)
4. $\text{Lck} \leftarrow \text{Lck}^{pY394}$	---	---	---	0.01 *
5. $-ITAM^{0,P,PP} + \text{Lck}^{0pY394} \leftrightarrow ITAM^{0,P,PP} - \text{Lck}^{0pY394}$  $-ITAM^{0,P,PP}$ – denotes de-activated ( $ITAM^0$ ), partially phosphorylated ( $ITAM^P$ ) or fully phosphorylated ( $ITAM^{PP}$ ) states of a stimulatory receptor associated with a ligand. $\text{Lck}^{0pY394}$ – denotes states of Lck, Lck or $\text{Lck}^{pY394}$	$> 0.869 \times 10^{-3}$ calculated from $k_{cat}/K_M$ (3), $K_M = 2.3 \text{ mM}$ , $k_{cat} = 2.0 \text{ s}^{-1}$ ; lower bound of $k_{on}$ . Used the value, 0.869 $\times 10^{-2}$ *	0.1 *	< 2300 calculated from $K_M$ , (3) using, $K_D \leq K_M$ ; used the value 230*	---
6. $-ITAM - \text{Lck} \leftrightarrow ITAM^P - \text{Lck}$				0.01 *
7. $-ITAM^P - \text{Lck} \leftrightarrow ITAM^{PP} - \text{Lck}$				0.01 *
8. $ITAM - \text{Lck}^{pY394} \longrightarrow ITAM^P - \text{Lck}^{pY394}$	---	---	---	2.0 (3, 4)
9. $ITAM^P - \text{Lck}^{pY394} \longrightarrow ITAM^{PP} - \text{Lck}^{pY394}$	---	---	---	2.0 (3)
10. $ITAM^{PP} \rightarrow ITAM^P \rightarrow ITAM$	---	---	---	2.5 *
11. $ITIM^{0,P} + \text{Lck}^{pY394} \leftrightarrow ITIM^{0,P} - \text{Lck}^{pY394}$	$0.869 \times 10^{-2}$ *	0.1 *		---
12. $ITIM - \text{Lck} \rightarrow ITIM^P - \text{Lck}$				0.01 *
13. $ITIM - \text{Lck}^{pY394} \rightarrow ITIM^P - \text{Lck}^{pY394}$	---	---	---	2.0

					(3)
14. $ITIM^P \rightarrow ITIM$					0.1 *
15. $-ITAM^P + SHP1 \leftrightarrow -ITAM^P - SHP1$ $-ITAM$ – denotes ITAMs associated to a complex formed by receptor, ligand or any other signaling molecule	8.69 *	0.05(5 ) *	0.0057 *	---	
16. $-ITAM^{PP} + SHP1 \leftrightarrow -ITAM^{PP} - SHP1$	8.69 *	0.05(5 ) *	0.0057 *		
17. $-ITIM^P + SHP1 \leftrightarrow -ITIM^P - SHP1$ $-ITIM$ – denotes ITIMs associated to a complex formed by receptor, ligand or any other signaling molecule	8.69 *	0.05 *	0.0057 *		
18. $Syk^P \rightarrow Syk$	---	---	---	---	0.5 *
19. $-ITAM^{PP} + Syk^{0,P} \leftrightarrow -ITAM^{PP} - Syk^{0,P}$	12.0 (5)	0.11 (5)	0.009	---	
20. $-ITAM^{PP} - Syk - Lck^{pY394} \rightarrow -ITAM^{PP} - Syk^P - Lck^{pY394}$	---	---	---	---	2.0 *
21. $-ITAM^{PP} - Syk - Lck \rightarrow -ITAM^{PP} - Syk^P - Lck$	---	---	---	---	0.01 *
22. $-ITAM^P - SHP1 \rightarrow -ITAM^P - SHP1^P$					5.4*
23. $-ITIM^P - SHP1 \rightarrow -ITIM^P - SHP1^P$	---	---	---	---	5.4 *
24. $-ITAM^P - SHP1^P \rightarrow -ITAM^P - SHP1$					0.07*
25. $-ITIM^P - SHP1^P \rightarrow -ITIM^P - SHP1$	---	---	---	---	0.07 *
26. $-Syk^P + Vav \leftrightarrow -Syk^P - Vav \rightarrow -Syk^P + Vav^P$ (6, 7) $-Syk$ – denotes Zap70/Syk associated with a complex	5.0 *	0.1 *	0.02*	---	1.0 *
27. $-SHP1 + Vav^P \leftrightarrow -SHP1 - Vav^P \rightarrow -SHP1 + Vav$ (8, 9) $-SHP1$ – denotes SHP1 associated with a complex	8.69 *	0.05 *	0.0057 *	0.07(10)	
28. $-SHP1^P + Vav^P \leftrightarrow -SHP1^P - Vav^P \rightarrow -SHP1^P + Vav$ (8, 9)	0.88 *	0.05 *	0.0057 *	0.18 (10)	
29. $Vav^P \rightarrow Vav$					0.01 *
30. $Erk + Vav^P \leftrightarrow Erk - Vav^P \rightarrow -Erk^P + Vav^P$ (11, 12)	0.5 *	1.0 *	2.0	0.5 *	
31. $Erk^P + W \leftrightarrow Erk^P - W \rightarrow -Erk + W$	50 *	0.1 *	0.002 *	0.25 *	

We enforce a strict version of the kinetic proof-reading scheme, i.e., whenever, the ligand comes off the ligand receptor complex, the entire complex dissociates with the off-rate of the ligand dissociation. Reactions related to that are not shown in the above table but can

be easily seen from the SSC reaction files available on the website  
<http://planetx.nationwidechildrens.org/~jayajit/>.

**Table SII: Species Concentrations used for the results in Fig. 2**

Species	Concentration
NKp46	125 molecules/ $(\mu\text{m})^2$ *
Heparin	0 – 250 molecules/ $(\mu\text{m})^2$ *
Ly49A	125 molecules/ $(\mu\text{m})^2$ *
H-2D <sup>d</sup>	0 – 500 molecules/ $(\mu\text{m})^2$ *
Lck/Fyn	1250 molecules/ $(\mu\text{m})^2$ *
Zap70/Syk	72000 molecules/ $(\mu\text{m})^3$ *
SHP1	48000 molecules/ $(\mu\text{m})^3$ *
Vav	25000 molecules/ $(\mu\text{m})^3$ *
Erk	6250 molecules/ $(\mu\text{m})^3$ *
Erk phosphatase	2500 molecules/ $(\mu\text{m})^3$ *

#### **Implementation of Stochastic Simulation:**

The size of the simulation box is taken as,  $V=2 \times 2 \times 0.02 (\mu\text{m})^3$ , where the surface area (A) of the box is of  $4 (\mu\text{m})^2$ . The typical diffusion constants of molecules in the plasma membrane and the cytosol are  $\sim 0.01 (\mu\text{m})^2/\text{s}$  (13) and  $\sim 1.0 (\mu\text{m})^2/\text{s}$  (14) respectively, therefore, this box size ensures that the molecules are homogeneously mixed at much shorter time scales compared to the time scale of interest (minutes) in the simulation. The receptors, ligands, and kinases Lck/Fyn reside in the plasma membrane, these molecules are confined to the surface of the simulation box in our model. Cytosolic molecules, such as, Zap70/Syk, SHP-1, Vav and Erk are homogeneously distributed throughout the simulation box. When receptor ligand complexes are formed involving the cytosolic molecules, they also become confined to the surface of the simulation box. For the reactions occurring in the surface of the simulation box, we convert the binding rate ( $(k_{on})_{3D}$ ) reported in 3 dimensions into a two dimensional rate by dividing  $(k_{on})_{3D}$  by a small length scale,  $d$ .  $d$  denotes the length scale in which two molecules react, we take  $d=0.002 \mu\text{m}$ , which is of the order of the radius of gyration of a small protein molecule (15). These parameters were used to convert the above rate constants into unit of  $\text{second}^{-1}$  when we performed the Gillespie simulation in the simulation box. The files with the reactions and the input parameters used for Fig.2 are available from the website <http://planetx.nationwidechildrens.org/~jayajit/>.

#### **Unit Conversion Table:**

$$1 \mu\text{M} = 600 \text{ molecules} / (\mu\text{m}^3)$$

$$(k_{on})_{3D} = 1 (\mu\text{M})^{-1} \text{ s}^{-1} = 0.16 \times 10^{-2} (\mu\text{m})^3/\text{molecules s}^{-1}$$

**Table SIII: Reactions and rate constants used for the results in Fig. 3**

Reaction	$k_{on}$ ( $\mu M$ ) $^{-1}s^{-1}$	$k_{off}$ ( $s^{-1}$ )	$K_D = k_{off}/k_{on}$ ( $\mu M$ )	$k_{cat}$ ( $s^{-1}$ )
1. $CD16 + IgG \leftrightarrow CD16 - IgG$ stimulatory ligand (mouse)	0.0065 (13)	0.0047 (13)	0.72	---
2. $Ly49D + H - 2D^d \longleftrightarrow Ly49D - H - 2D^d$ weaker stimulatory ligand (mouse)	0.0046 *	0.8 *	173.97 *	

Reaction #1 in table SI is replaced by the above reaction #1. The rest parameters for the reactions are the same as in table SI.

**Table SIV: Species Concentrations used for the results in Fig. 3**

Species	Concentration
CD16	125 molecules/ $(\mu M)^2$ *
Ly49A	12.5 molecules/ $(\mu M)^2$ *
Ly49D	125 molecules/ $(\mu M)^2$ *
IgG	37.5 molecules/ $(\mu M)^2$ *
H-2D <sup>d</sup>	0 – 250 molecules/ $(\mu M)^2$ *

NKp46 in table SII is replaced by CD16, and rest of the parameters used are the same as in table SII. The SSC files showing the reactions and input parameters are available on the website <http://planetx.nationwidechildrens.org/~jayajit/>.

**Table SV: Reactions and rate constants used for the results in Fig. 4**

Reaction	$k_{on}$ ( $\mu M$ ) $^{-1}s^{-1}$	$k_{off}$ ( $s^{-1}$ )	$K_D = k_{off}/k_{on}$ ( $\mu M$ )	$k_{cat}$ ( $s^{-1}$ )
1. stim. receptor+ligand $\longleftrightarrow$ stim. receptor-ligand	0.0065 (13)	0.4	61.5	---
5. $-ITAM^{0,P,PP} + Lck^{0pY394} \leftrightarrow ITAM^{0,P,PP} - Lck^{0pY394}$  $-ITAM^{0,P,PP}$ – denotes de-activated ( $ITAM^0$ ), partially phosphorylated ( $ITAM^P$ ) or fully phosphorylated ( $ITAM^{PP}$ ) states of a stimulatory receptor associated with a ligand. $Lck^{0pY394}$ – denotes states of Lck, Lck or $Lck^{pY394}$	$14.98 \times 10^{-2}*$  †	0.1*  (same as in table SI)	3965*  ---	
27. $-SHP1 + Vav^P \leftrightarrow -SHP1 - Vav^P \rightarrow -SHP1 + Vav$ (8, 9)  $-SHP1$ – denotes SHP1 associated with a complex	8.69 *  (same as in table SI)	0.05 *  (same as in table SI)	0.0057 *  0.1*  †	

28. $-SHP1^P + Vav^P \leftrightarrow -SHP1^P - Vav^P \rightarrow -SHP1^P + Vav$ (8, 9)	0.88 * (same as in table SI)	0.05 * (same as in table SI)	0.0057 *	1.0*
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† Note the no change is qualitative features upon 10 times increase or decrease in the sensitivity analysis.

Reaction #1 in table SI is replaced by the above reaction #1. The rest parameters for the reactions are the same as in table SI.

**Table SVI: Species Concentrations used for the results in Fig. 4**

Species	Concentration
Weak affinity stim. ligand	0-125 molecules/ $\mu\text{m}^2$ *
Stimulatory receptor	125 molecules/ $\mu\text{m}^2$ *
SHP1	1250 molecules/ $\mu\text{m}^3$ *

Heparin and NKp46 in table SII are replaced by the weak affinity stimulatory ligand and receptor, and the concentration of SHP-1 is reduced significantly. The rest of the parameters used are the same as in table SII. The SSC files showing the reactions and input parameters are available on the website

<http://planetx.nationwidechildrens.org/~jayajit/>.

**Table SVII: Rate constants used for Fig. S14**

Rate constants	Values ( $\text{s}^{-1}$ )
$k_p$	0.5
$k_d$	0.01
$k_{on}$	0.00144
$k_{off}$	0.1
$k_{oni}$	0.00066
$k_{offi}$	0.025
$k_{ons}$	0.176
$k_{offs}$	0.05
$k_{ions}$	0.176
$k_{ioffs}$	0.05
$k_{onz}$	0.24
$k_{offz}$	0.1

**Table SVIII: Species concentrations used for Fig. S14**

Species concentrations	Values (# of molecules) (red line, Fig. S14)	Values (# of molecules) (blue line, Fig. S14)
T <sub>0</sub>	500	500
R <sub>T0</sub>	500	500
Z <sub>0</sub>	5760	5760
S <sub>0</sub>	300	3000
I <sub>0</sub>	500	500
R <sub>I0</sub>	0 - 500	0 - 500

*Numerical solution of ODEs:* The Eqs 1-11 are solved numerically for the data shown in Fig. S14. The ODEs are solved following a standard Euler discretization (16) scheme, which replaces, a derivative  $dc / dt$  by,  $(c(t + \Delta t) - c(t)) / \Delta t$  and the resulting algebraic equations are solved to obtain the solution at the time  $t + \Delta t$  from the known concentrations at time  $t$ . We used a  $\Delta t = 0.001$  for the data in Fig. S14.

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We show the sensitivity of the results in Fig. 2A in the tables below. Most of the concentrations are varied 2 times and the rate constants are varied 10 times. The relatively parameters are indicated by a variation that is less than the above quoted fold changes.

<b>Table SIX: Sensitivity analysis of the results shown in Fig. 2A for concentration variations</b>		
<b>Parameter</b>	<b>Variation</b>	<b>Result of variation</b>
Lck	Increased 2 times	Hardly any change (Fig. S1A) in the activation profile of Fig. 2a.
	Decreased 2 times	Hardly any change (Fig. S1A) in the activation profile of Fig. 2a, the threshold moves slightly to the right.
SHP-1	Increased 2 times	Large decrease in activation (Fig. S1B). When the number of inhibitory ligands is decreased 20 times, it increases the activation, however, the threshold shifts to the right and the sharpness of the response is also decreased.
	Decreased 2 times	Increases activation, the threshold in Fig. 2a shifts to the left (Fig. S1B).
Zap70/Syk	Increased 2 times	Increases activation, the threshold in Fig. 2a shifts to the left (Fig. S1C).
	Decreased 2 times	Large decrease in activation. When the number of inhibitory ligands is decreased 20 times, it increases the activation, however, the threshold shifts to the right and the sharpness of the response is also decreased (Fig. S1C).
	Increased 2 times	Increases activation, almost by 2 times at large stimulatory concentrations, the threshold in Fig. 2a

Vav	Increased 2 times	shifts to the right (Fig. S1D).
	Decreased 2 times	Decreases activation, more than 4 times at large stimulatory concentrations, the threshold in Fig. 2a shifts to the left (Fig. S1D).
Erk	Increased 2 times	Increases Erk activation, almost by 3 times at large stimulatory concentrations, the threshold in Fig. 2a shifts to the right (Fig. S1E).
	Decreased 2 times	Large decrease in activation. Threshold shifts to the right (Fig. S1E).
Erk phosphatase	Increased 2 times	Large decrease in activation. Threshold shifts to the right (Fig. S1F).
	Decreased 2 times	Increases Erk activation by more than 1.5 times at large stimulatory concentrations, the threshold in Fig. 2a shifts to the right (Fig. S1F).

**Table SX: Sensitivity analysis of the results shown in Fig. 2A for rate constant variations**

Parameter	Variation	Result of variation
Stimulatory ligand binding rate	10 times increase	Hardly in change in the activation profile shown in Fig. 2A.
	10 times decrease	Activation threshold shifts slightly to the right.
Stimulatory ligand unbinding rate	10 times increase	Activation threshold shifts to the right at a stimulatory ligand concentration 5 times larger than that shown in Fig. 2a. The results are shown in Fig. S2A.
	10 times decrease	Activation threshold shifts

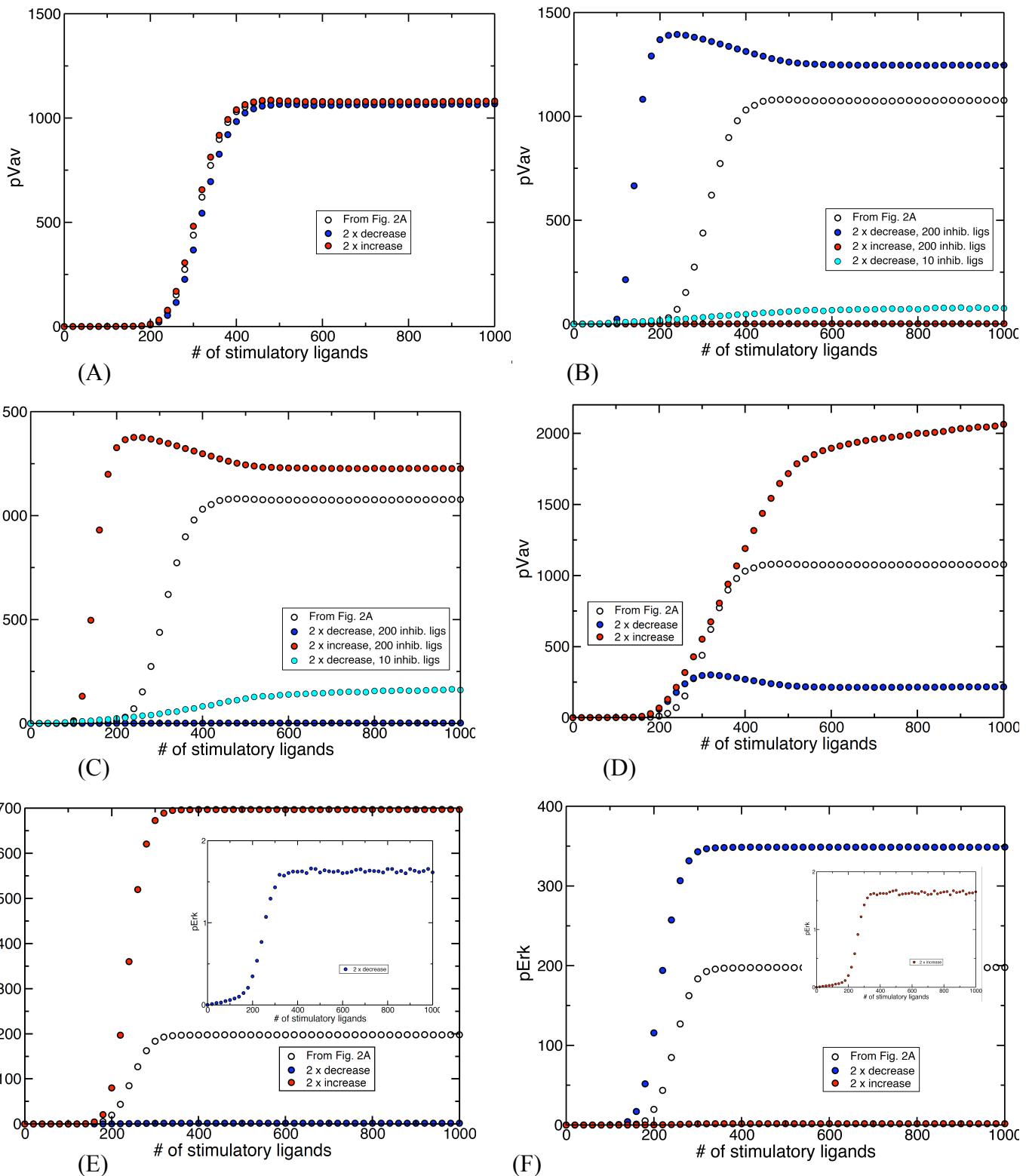
		slightly to the left. The results are shown in Fig. S2A.
Lck binding rate	10 times increase	Hardly in change in the activation profile shown in Fig. 2a
	10 times decrease	Activation threshold shifts slightly to the right. Activation decreases at large stimulatory ligand concentrations.
Lck unbinding rate	10 times increase	Activation threshold shifts slightly to the right (Fig. S2B). Activation decreases at large stimulatory ligand concentrations.
	10 times decrease	Hardly in change in the activation profile shown in Fig. 2a (Fig. S2B).
ITAM activation rate by basally activated Lck	10 times increase	Hardly in change in the activation profile shown in Fig. 2a (Fig. S2C).
	10 times decrease	Hardly in change in the activation profile shown in Fig. 2a (Fig. S2C).
ITAM activation rate by activated Lck	2 times increase	Activation threshold shifts to the left. Activation increases at large stimulatory ligand concentrations (Fig. S2D).
	2 times decrease	Activation threshold shifts to the right. Activation decreases by a large amount (more than 10 times) at large stimulatory ligand concentrations (Fig. S2D).
ITAM de-activation rate	2 times increase	Activation threshold shifts to the right. Overall large decrease in activation.
	2 times decrease	Activation threshold shifts to the left. Overall increase in activation
SHP1 binding rate	5 times increase	Activation decreases by a large amount. By lowering the inhibitory concentration

		20 times we get appreciable Vav activation with a threshold shifted at higher stimulatory concentrations (Fig. S2E).
	10 times decrease	Activation threshold shifts to the left. Overall increase in activation (Fig. S2E).
SHP1 unbinding rate	10 times increase	Activation threshold shifts to the left. Overall increase in activation (Fig. S2F).
	2 times decrease	Activation decreases by a large amount. By lowering the inhibitory concentration 20 times we get appreciable Vav activation with a threshold shifted at higher stimulatory concentrations (Fig. S2F). The transition to the activated state also becomes less sharp.
SHP-1 activation rate	10 times increase	Activation threshold shifts to the right. Slight decrease in overall activation (Fig. S2G).
	10 times decrease	Activation threshold shifts to the left. Overall increase in activation (Fig. S2G).
SHP-1 de-activation rate	10 times increase	Hardly any change.
	10 times decrease	Activation threshold shifts to the left. Overall increase in activation.
de-activation rate by non-activated SHP-1	10 times increase	Activation threshold shifts to the right. Slight decrease in overall activation (Fig. S2H).
	10 times decrease	Hardly any change in activation (Fig. S2H).
de-activation rate by activated SHP-1	2 times increase	Activation decreases by a large amount. By lowering the inhibitory concentration 20 times we get appreciable Vav activation with a threshold shifted at higher stimulatory concentrations (Fig. S2I). The transition to

		the activated state also becomes less sharper.
	2 times decrease	Activation threshold shifts to the left. Overall increase in activation (Fig. S2I).
Zap70/Syk binding rate	10 times increase	Activation threshold shifts to the left. Overall increase in activation.
	2 times decrease	Large decrease in activation. Threshold shifts to the right.
Zap70/Syk unbinding rate	2 times increase	Large decrease in activation. Activation threshold around the stimulatory ligand concentration as in Fig. 2A occurs at a 10 times lower inhibitory ligand concentration (Fig. S2J).
	10 times decrease	Activation threshold shifts to the left. Overall increase in activation (Fig. S2J).
Activation rate of Zap70/Syk mediated by basally activated Lck	10 times increase	Hardly any change in activation.
	10 times decrease	Hardly any change in activation.
Activation rate of Zap70/Syk mediated by activated Lck	2 times increase	Activation threshold shifts to the left. Overall increase in activation (Fig. S2K).
	2 times decrease	Activation threshold shifts to the left. Overall decrease in activation (Fig. S2K).
Zap70/Syk de-activation rate	2 times increase	Activation threshold shifts to the left. Overall increase in activation (Fig. S2L).
	2 times decrease	Activation threshold shifts to the left. Overall decrease in activation (Fig. S2L).
Binding rate of Zap70/Syk to Vav	10 times increase	Activation threshold shifts slightly to the left. Overall small increase in activation (Fig. S2M).
	10 times decrease	Activation threshold shifts slightly to the right. Overall decrease in activation (Fig. S2M).

Unbinding rate of Zap70/Syk to Vav	10 times increase	Hardly any change in activation threshold. Small decrease in activation at large stimulatory ligand concentrations.
	10 times decrease	Hardly any change in activation threshold. Small increase in activation at large stimulatory ligand concentrations.
Vav activation rate	2 times increase	Activation threshold shifts slightly to the left. Overall small increase in activation (Fig. S2N).
	2 times decrease	Large decrease in activation. The threshold shifts to about 400 stimulatory ligands as the concentration of the inhibitory ligands is reduced 20 times (Fig. S2N).
Erk binding rate	10 times increase	Small change in activation threshold as it shifts to the left. Small increase in activation at large stimulatory ligand concentrations (Fig. S2O).
	10 times decrease	Activation threshold shifts slightly to the right. Overall small decrease in activation (Fig. S2O).
Erk unbinding rate	10 times increase	Small change in activation threshold as it shifts to the right. Small decrease in activation at large stimulatory ligand concentrations.
	10 times decrease	Small change in activation threshold. Small decrease in activation at large stimulatory ligand concentrations.
Erk activation rate	2 times increase	Activation increases, threshold shifts to the left by a small amount (Fig.

		S2P).
	2 times decrease	Activation decreases, threshold shifts to the right by a small amount (Fig. S2P).
Inhibitory ligand binding rate	10 times increase	Small change in activation threshold as it shifts to the right (Fig. S2Q).
	10 times decrease	Small change in activation threshold as it shifts to the left. Small increase in activation at large stimulatory ligand concentrations (Fig. S2Q).
Inhibitory ligand unbinding rate	10 times increase	Small change in activation threshold as it shifts to the left. Small increase in activation at large stimulatory ligand concentrations (Fig. S2R).
	10 times decrease	Small change in activation threshold as it shifts to the right (Fig. S2R).
Erk phosphatase binding rate	10 times increase	Hardly any change in Erk activation profile.
	10 times decrease	Hardly any change in Erk activation threshold. Small increase in activation at large stimulatory ligand concentrations.
Erk phosphatase unbinding rate	10 times increase	Hardly any change in Erk activation profile.
	10 times decrease	Hardly any change in Erk activation profile.
Erk de-activation rate	2 times increase	Activation threshold shifts to the right. Overall decrease in activation.
	10 times decrease	Activation threshold shifts to the left. Overall increase in activation.



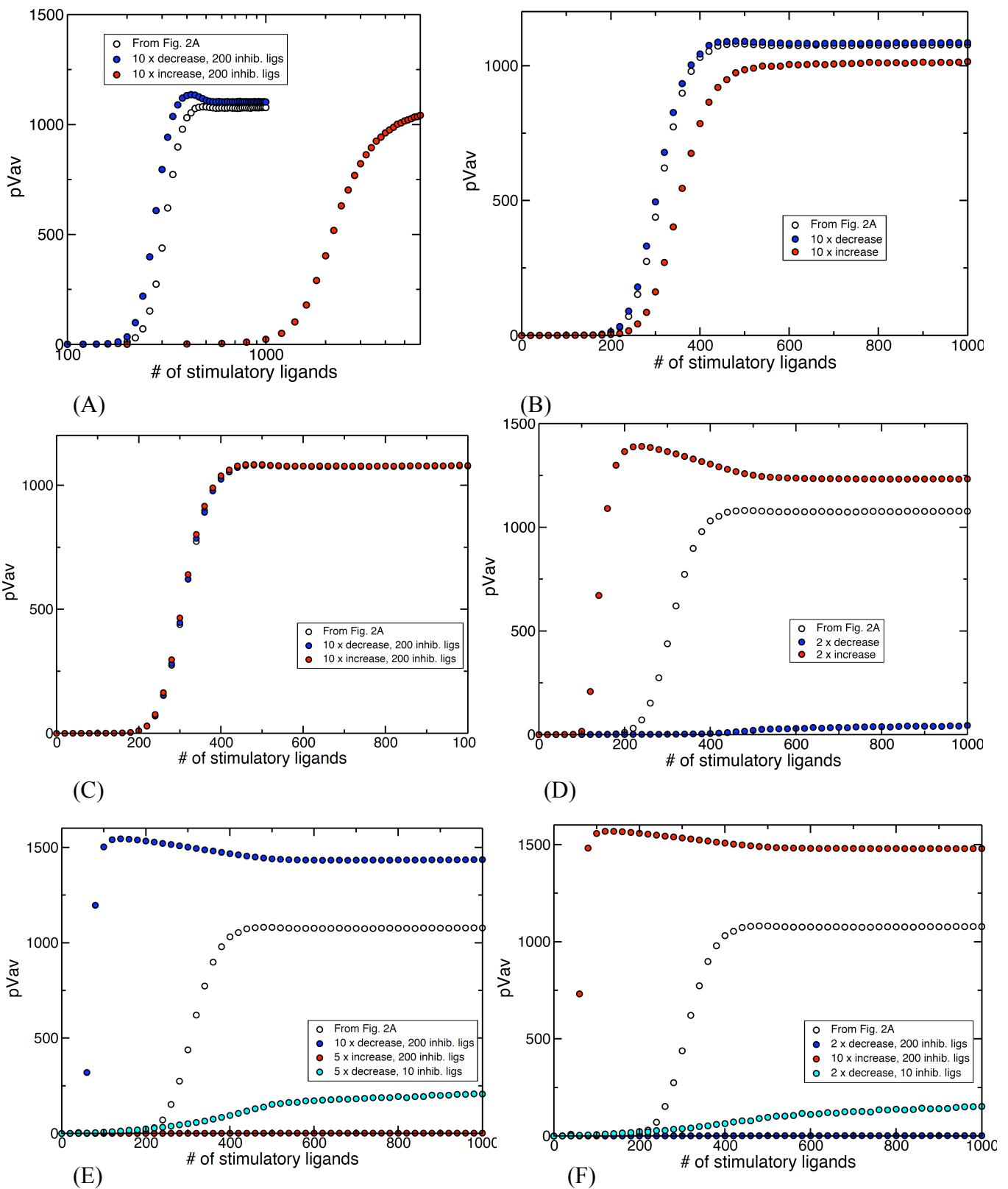
**Fig. S1 Effect of concentration variations on NK cell activation.** Parameters are varied from the values (Table SII) used for Fig. 2A. Fig. 2A is shown on all the graphs (black circles) for reference. (A) **Lck/Fyn variation.** Variations of Lck concentration do not show any significant change. (B) **SHP-1 variation.** Decreasing SHP-1 concentration by 2

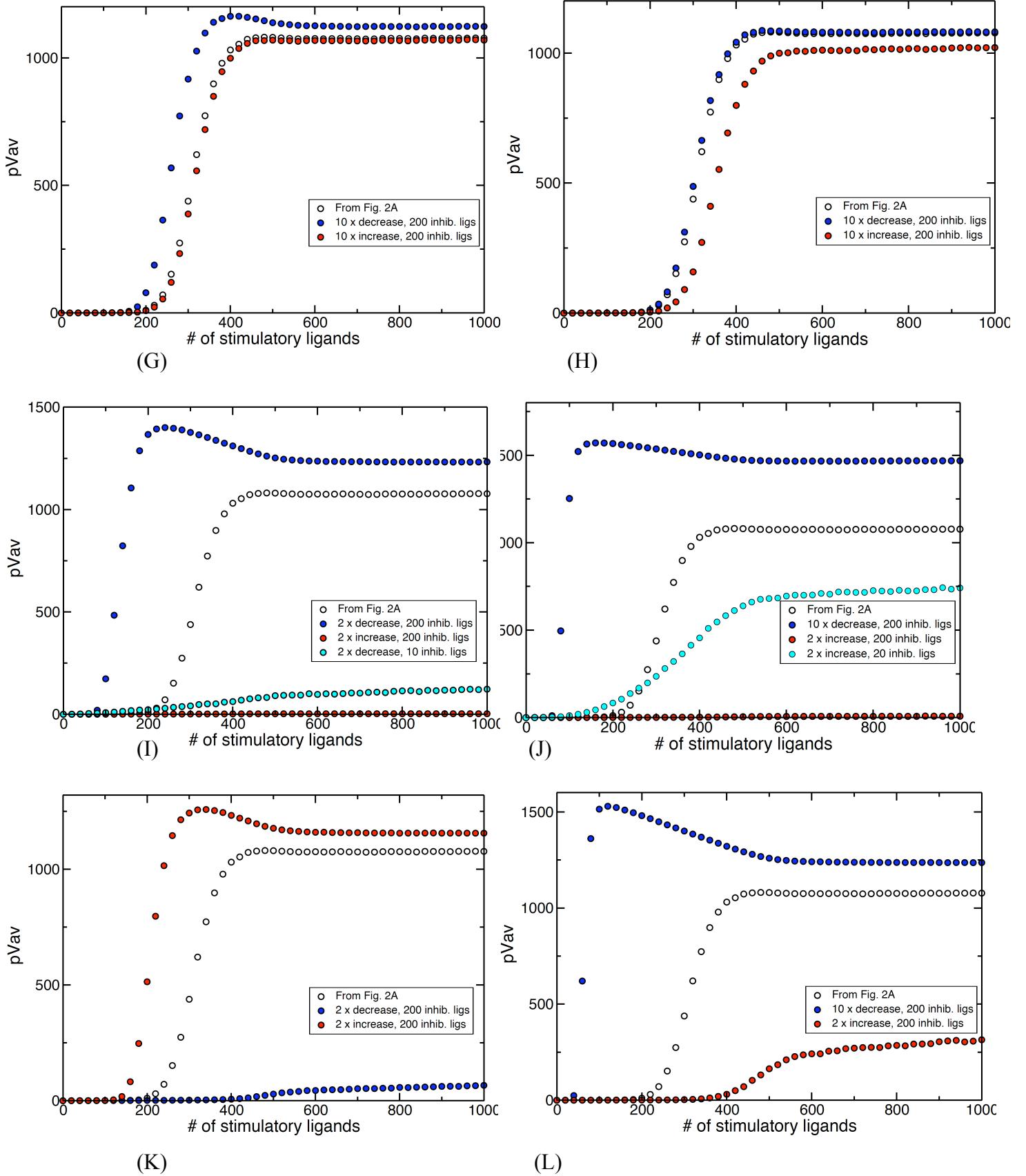
times decreases the threshold. Increasing the SHP-1 concentration by 2 times significantly decreases the activation. When the number of inhibitory ligands is decreased 20 times, it increases the activation, however, the threshold appears at a larger stimulatory concentration and the sharpness of the response is also decreased. (C)

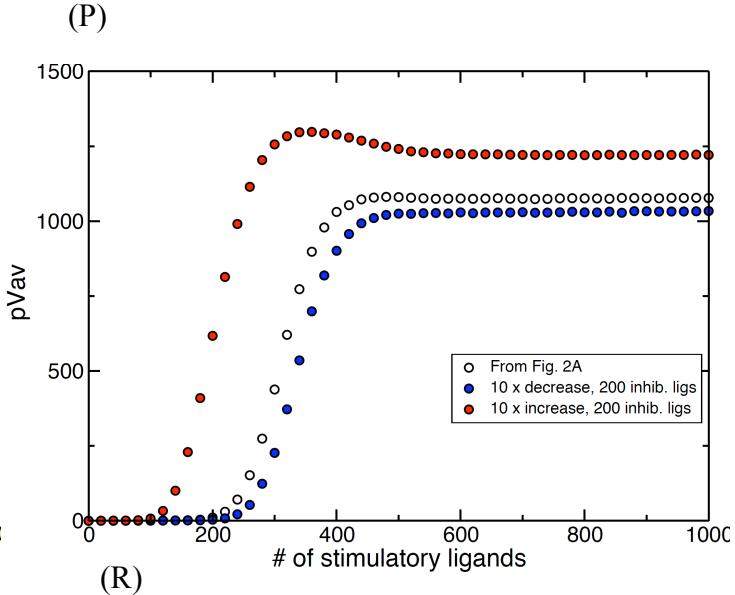
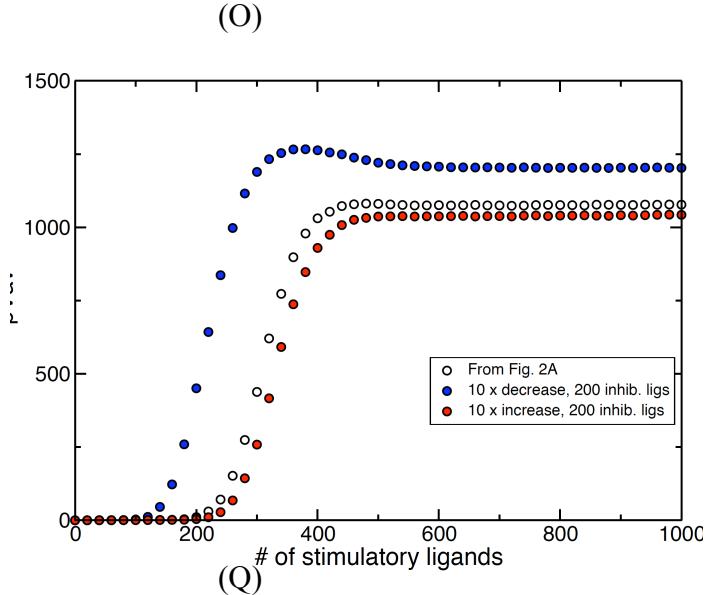
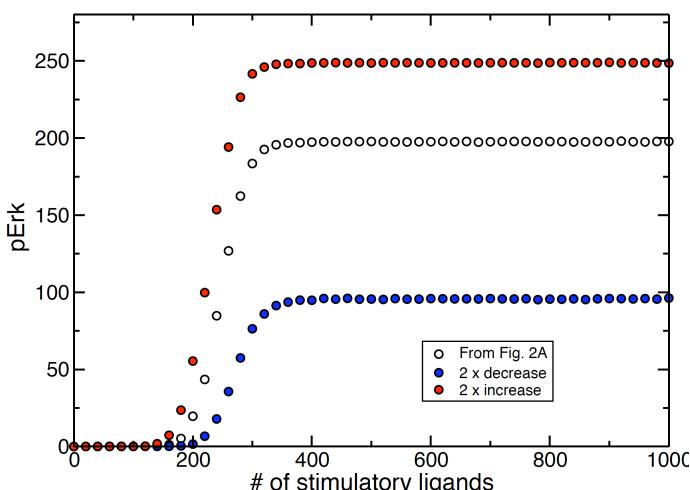
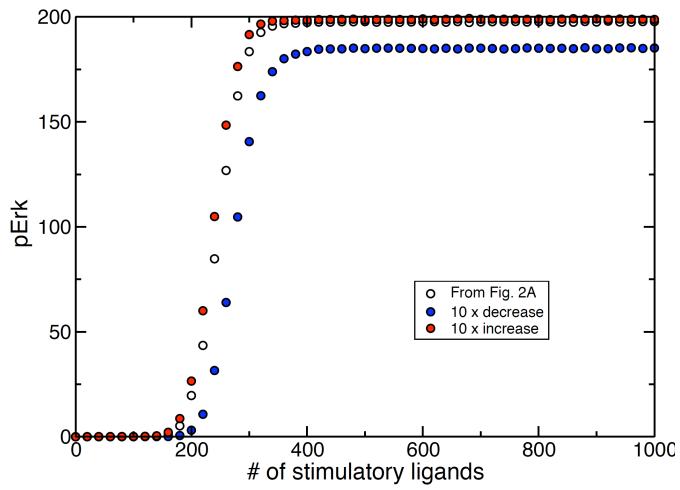
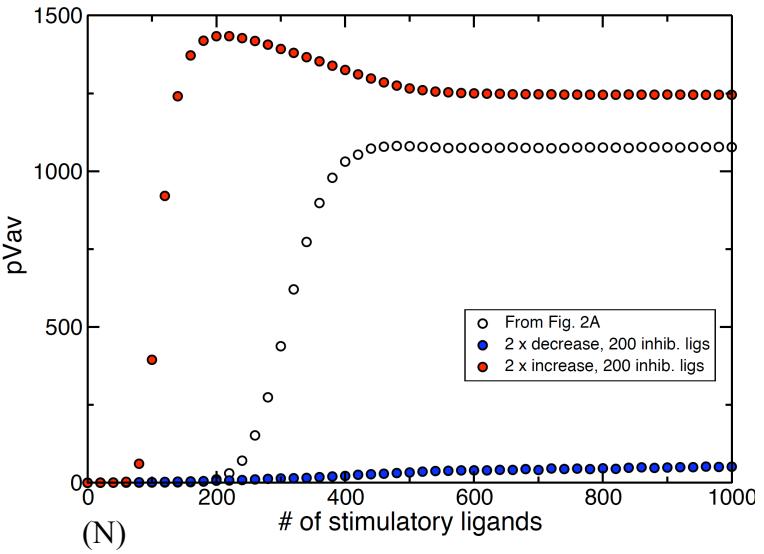
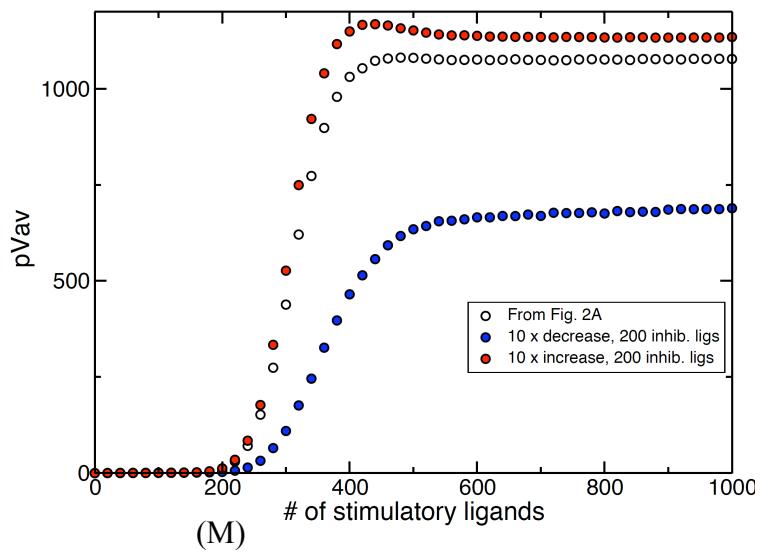
**Zap70/Syk variation.** Increasing Zap70/Syk concentration (2 times) helps activation and the threshold is shifted at a lower stimulatory ligand concentration. Two-fold decrease in Zap70/Syk concentration significantly decreases activation. The activation is partially recovered as the inhibitory ligand concentration is reduced 20 times in this case,

however, the threshold of activation increases and the sharpness of the response is also reduced. (D) **Vav variation.** Increase and decrease in Vav concentrations result in increase and decrease in Vav activation, respectively. However, the threshold does not change appreciably. (E) **Erk variation.** Increase and decrease in Erk concentrations

result in increase and decrease in Erk activation. Erk activation decreases significantly as the concentration is decreased 2 times (shown in the inset), though the qualitative nature of the activation profile and the position of the threshold does not change significantly. (F) **Erk phosphatase variation.** Increase and decrease in Erk phosphatase concentrations result in decrease and increase in Erk activation. Erk activation decreases significantly as the concentration is increased 2 times (shown in the inset), though the qualitative nature of the activation profile and the position of the threshold does not change significantly.

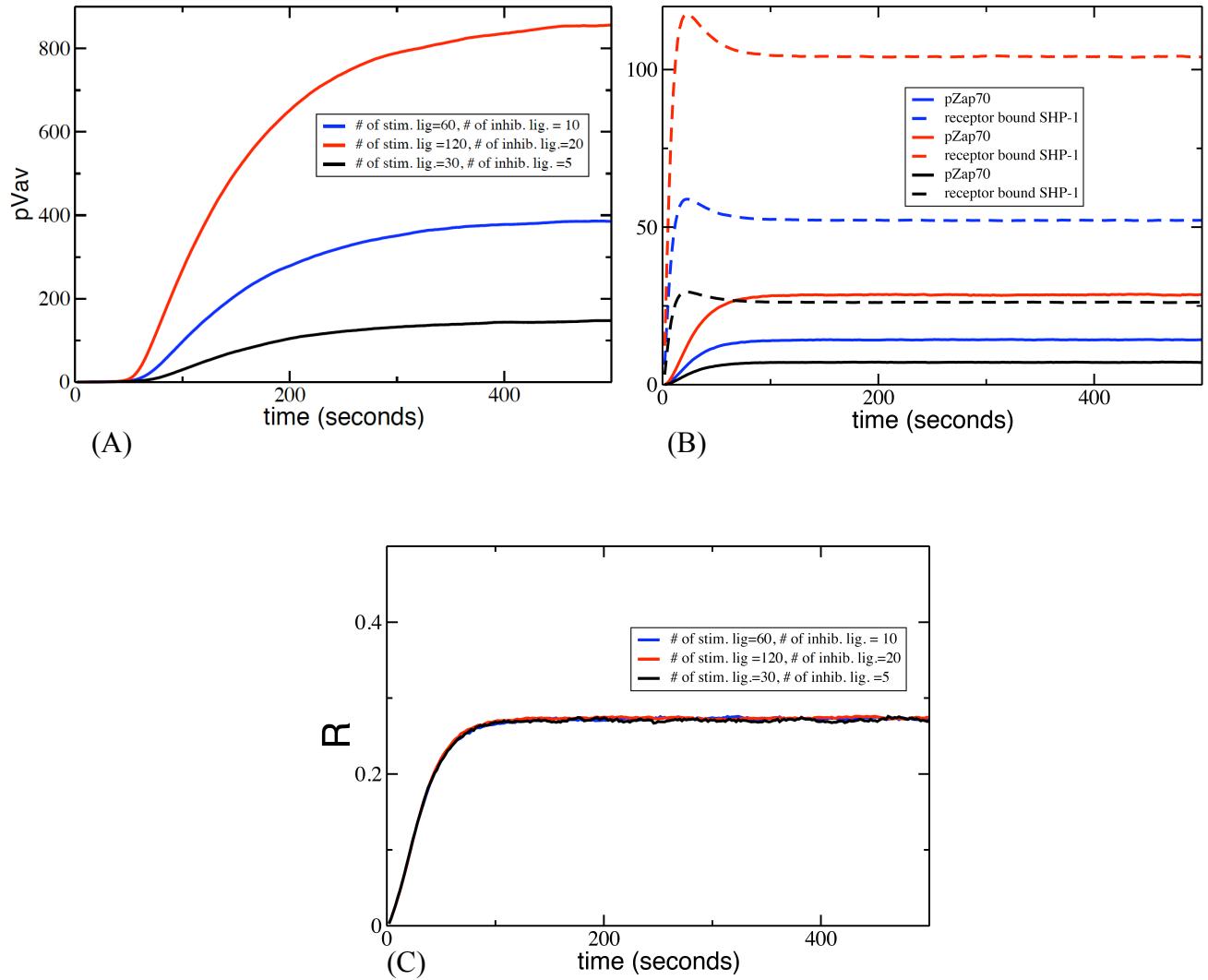






**Fig. S2 Sensitivity of Fig. 2A with variations of rate constants.** Parameters are varied from the values (Table SII) used for Fig. 2A. Fig. 2A is shown on all the graphs (black circles) for reference. **(A) Stimulatory ligand unbinding rate.** Increasing and decreasing the rate constant by 10 times move the threshold of activation to lower and higher concentrations of stimulatory ligands, respectively. **(B) Lck/Fyn unbinding rate.** Ten fold increase in the value of the rate reduces activation by a small amount and the threshold moves to a higher ligand concentration. Decreasing the rate constant by 10 times does not result in a significant change. **(C) Catalytic rate of basally activated Lck/Fyn.** 10 times increase or decrease does not lead to any significant change. **(D) Catalytic rate of fully activated Lck/Fyn.** 2 times decrease reduces the activation significantly and the threshold moves to a higher ligand concentration. 2 times increase results in a higher activation while the threshold moves to a lower ligand concentration. **(E) SHP-1 binding rate.** 5 times increase in the rate constant decreases the activation appreciably. However, decreasing the inhibitory ligand concentration 20 times results in a partial recovery of the initial activation, and the threshold shift to a higher stimulatory ligand concentration. Decreasing the rate 10 times increases activation and moves the threshold to a lower ligand concentration. **(F) SHP-1 unbinding rate.** Decreasing the rate 2 times decreases the activation significantly. Reducing the inhibitory ligand concentration 20 times results in a partial recovery of the initial activation, and the threshold shift to a higher stimulatory ligand concentration. Increasing the rate 10 times increases activation and moves the threshold to a lower ligand concentration. **(G) SHP-1 activation rate.** 10 times increase does not change the activation profile significantly and 10 times decrease increases activation while shifts the threshold to a lower ligand concentration. **(H) Catalytic rate of non-activated SHP-1.** 10 times increase decreases activation while shifts the threshold to a lower ligand concentration. 10 times decrease does not change the activation profile significantly. **(I) Catalytic rate of activated SHP-1.** 2 times increase decreases the activation appreciably, the system recovers partially as the inhibitory ligand concentration is decreased 20 times while shifts the threshold to a higher ligand concentration. Decreasing the rate 2 times increases activation and the threshold move to a lower ligand concentration. **(J) Zap70/Syk unbinding rate.** 2 times increase results in a large de-activation, however, the reducing the inhibitory ligand concentration 20 times brings back the activation and the threshold moves to a higher stimulatory ligand concentration. Decreasing the rate 10 times increases activation and the threshold shifts to a lower ligand concentration. **(K) Zap70/Syk activation rate mediated by activated Lck/Fyn.** 2 times increase results in an increased activation and the threshold moves to a lower ligand concentration. 2 times decrease reduces activation while the threshold moves to a higher ligand concentration. **(L) Zap70/Syk de-activation rate.** 2 times increase in the rate reduces activation and moves the threshold to a higher ligand concentration. 10 times decrease increases activation and the threshold shifts to a lower ligand concentration. **(M) Rate of Zap70/Syk binding to Vav.** 10 times increase does not change the activation profile significantly, whereas, 10 times decrease in the rate lowers activation and moves the threshold to a higher ligand concentration. **(N) Vav activation rate.** 2 times increase in the rate increases activation and moves the threshold to a lower ligand concentration. 2 times decrease decreases activation and the threshold shifts to a higher ligand concentration. **(O) Rate of Vav binding to Erk.** 10 times

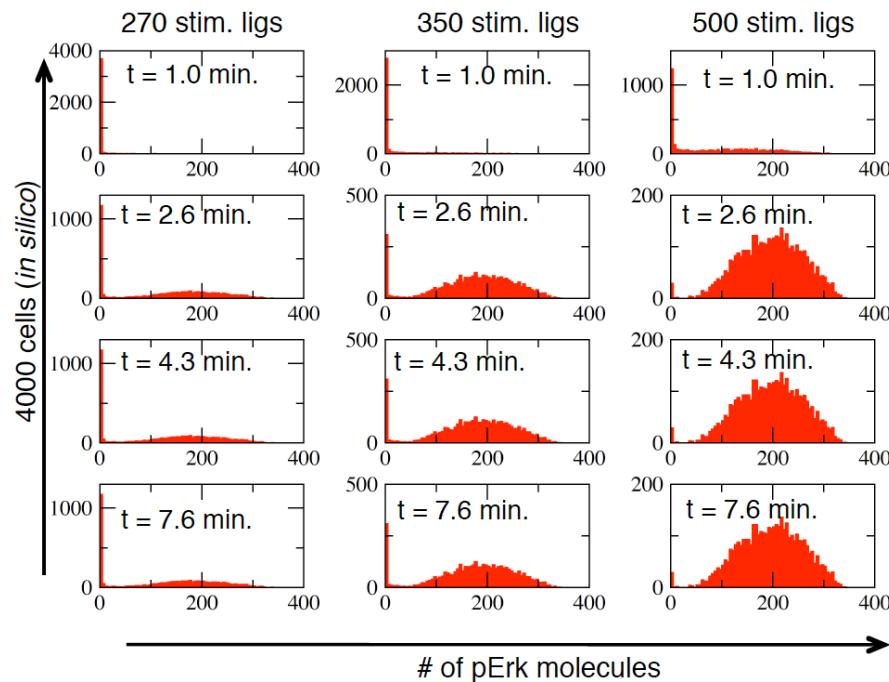
decrease decreases activation while shifts the threshold to a higher ligand concentration. 10 times increase does not change the activation profile significantly. **(P) Erk activation rate.** 2 times increase in the rate increases activation and moves the threshold to a lower ligand concentration. 2 times decrease decreases activation and the threshold shifts to a higher ligand concentration. **(Q) Rate of inhibitory ligand binding.** 10 times increase in the rate decreases activation and moves the threshold to a higher ligand concentration. 10 times decrease increases activation and the threshold shifts to a lower ligand concentration. **(R) Rate of inhibitory ligand unbinding.** 10 times decrease in the rate decreases activation and moves the threshold to a higher ligand concentration. 10 times increase increases activation and the threshold shifts to a lower ligand concentration.



**Fig. S3 Effect of concentrations of activated Zap70/Syk and receptor bound SHP-1 molecules on Vav activation kinetics.** (A) Shows kinetics of Vav activation for three different combinations of stimulatory and inhibitory ligand concentrations. All the three cases produce very similar values for the ratio  $R$  as shown in part (C) of the figure. The time scales for reaching maximal activation decreases as the concentrations of Zap70/Syk and receptor bound SHP-1 molecules (shown in part B of the figure) decrease. (B) Shows kinetics of activated Zap70/Syk and receptor bound SHP-1 concentrations. (C)  $R$  vs time for all the three case. The parameters (except for ligand concentrations) of the simulations are same as in Fig. 2.

### Section SA: Effect of protein level variations due to extrinsic noise fluctuations on distribution of Erk activation in a cell population

We introduce variability in protein level expressions in the model to probe the effect of extrinsic noise fluctuations on the bimodal nature of distribution of Erk activation in a cell population. The variations in the protein concentrations are set to 35% of the respective protein concentrations. Such variations may arise from extrinsic noise fluctuations at the single cell level. The value of the variation is estimated from the variance of extrinsic noise fluctuations in gene expression in yeast(1). We use a uniform random distribution to generate the variations in the protein concentrations. The distributions shown in the figure below demonstrates that the bimodal nature of pErk distribution is retained even in the presence of extrinsic noise fluctuations.



**Fig. S4 Effect of extrinsic noise fluctuations on Erk distributions.** The pErk distributions show a bimodal response for the same times and concentrations used for the results in Fig. 2D. We concentrations are varied as described above and the rate constants are the same as in Fig. 2D

1. Volfson, D., J. Marciniak, W. J. Blake, N. Ostroff, L. S. Tsimring, and J. Hasty. 2006. Origins of extrinsic variability in eukaryotic gene expression. *Nature* 439:861-864.

We show the sensitivity of the results in Fig. 4B in the tables below. Most of the concentrations are varied 2 times and the rate constants are varied 10 times. The relatively parameters are indicated by a variation that is less than the above quoted fold changes.

**Table SXI: Effect of variation of concentrations on Fig. 4B**

Parameter	Variation	Result of variation
Stimulatory receptor	Increased 2 times	The region where inhibitory ligands aid stimulation moves to the right at weaker ligand affinities (Fig. S5A).
	Decreased 2 times	The region where inhibitory ligands aid stimulation moves to the left at stronger ligand affinities (Fig. S5A).
Stimulatory ligand	Increased 2 times	The region where inhibitory ligands aid stimulation moves to the right at weaker ligand affinities (Fig. S5B).
	Decreased 2 times	The region where inhibitory ligands aid stimulation moves to the left at stronger ligand affinities (Fig. S5B).
Lck	Increased 2 times	Hardly any change in the behavior shown in Fig. 4B. Results shown in Fig. S5C.
	Decreased 2 times	Hardly any change in the behavior shown in Fig. 4B. Results shown in Fig. S5C.
SHP-1	Increased 1.5 times	The region where inhibitory ligands aid stimulation moves to the left at stronger ligand affinities, and the range over which this phenomenon is observed is widened. Results shown in Fig. S5D.
	Decreased 2 times	The region where inhibitory ligands aid stimulation moves to the right at weaker ligand affinities, and the range over which this phenomenon is observed is shrunk. Results shown in Fig. S5D.

Zap70/Syk	Increased 1.4 times	Hardly any change in the behavior shown in Fig. 4B. Results shown in Fig. S5E.
	Decreased 2 times	Hardly any change in the behavior shown in Fig. 4B. Results shown in Fig. S5E.
Vav	Increased 2 times	Activation increases, no in the affinity range where inhibitory ligands aid stimulation (Fig. S5F).
	Decreased 2 times	Activation decreases, no in the affinity range where inhibitory ligands aid stimulation (Fig. S5F).

**Table SXII: Effect of variation of rate constants for the results shown in Fig. 4B**

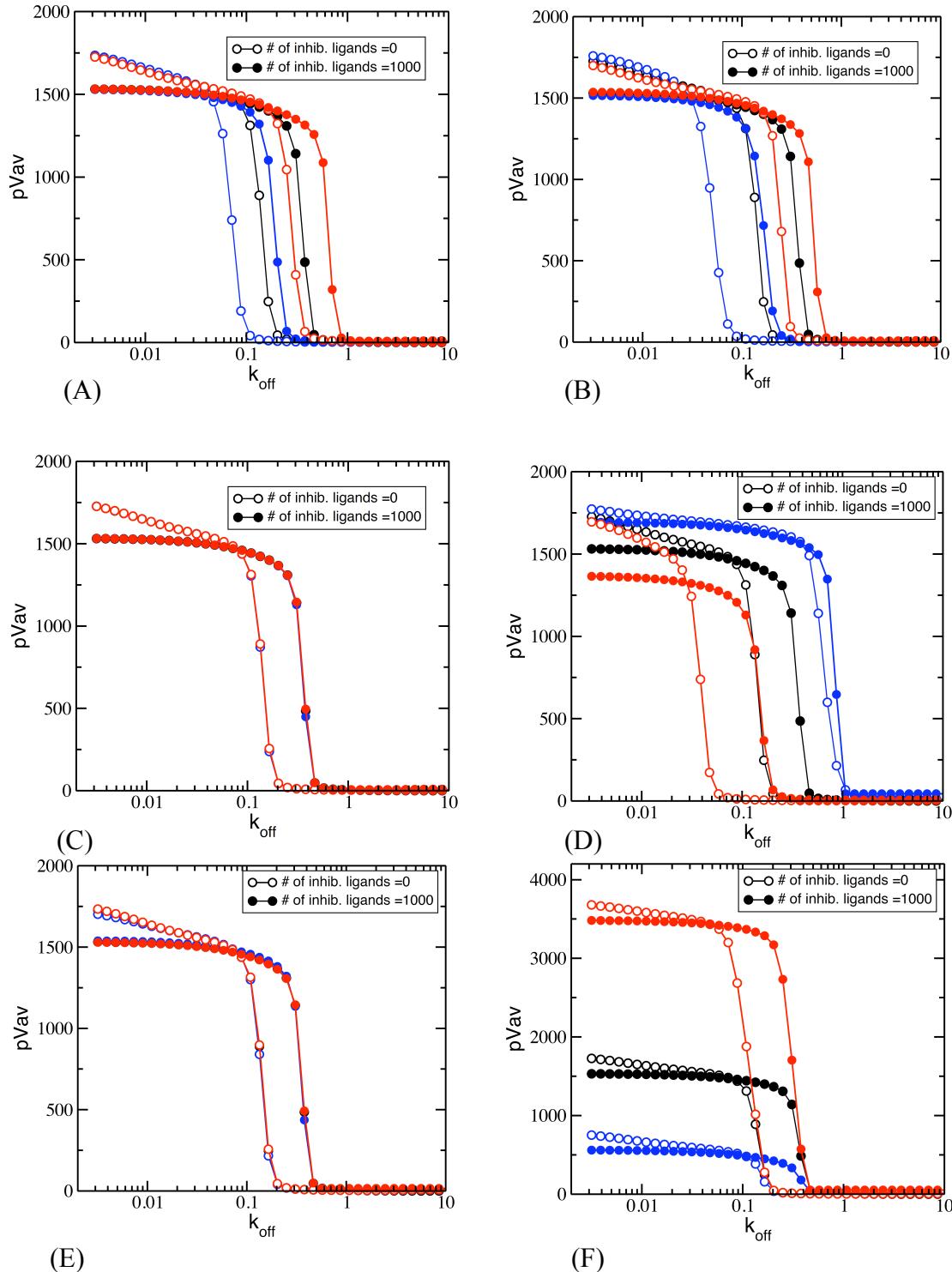
Parameter	Variation	
Stimulatory ligand binding rate	5 times increase	The region where inhibitory ligands aid stimulation moves to the right at weaker ligand affinities, and the range over which this phenomenon is observed is shrunk (Fig. S6A).
	10 times decrease	The region where inhibitory ligands aid stimulation moves to the left at stronger ligand affinities, and the range over which this phenomenon is observed is shrunk (Fig. S6A).
Lck binding rate	10 times increase	Hardly any change in the $k_{off}$ range.
	10 times decrease	Hardly any change in the $k_{off}$ range.
Lck unbinding rate	10 times increase	Hardly any change in the $k_{off}$ range.
	10 times decrease	Hardly any change in the $k_{off}$ range.
ITAM activation rate by basally activated Lck.	10 times increase	Hardly any change in the in Fig. 4B.
	10 times decrease	Hardly any change in the in Fig. 4B.

ITAM activation rate by activated Lck.	2 times increase	The region where inhibitory ligands aid stimulation moves to the right at weaker ligand affinities, and the range over which this phenomenon is observed is widened (Fig. S6B).
	10 times decrease	The region where inhibitory ligands aid stimulation moves to the left at stronger ligand affinities, and the range over which this phenomenon is observed is reduced (Fig. S6B).
ITAM de-activation rate	10 times increase	The region where inhibitory ligands aid stimulation moves slightly to the left at stronger ligand affinities, and the range over which this phenomenon is observed is reduced (Fig. S6C).
	10 times decrease	The region where inhibitory ligands aid stimulation moves slightly to the right at weaker ligand affinities, and the range over which this phenomenon is observed is widened (Fig. S6C).
SHP1 binding rate	10 times increase	The region where inhibitory ligands aid stimulation moves to the left at stronger ligand affinities, and the range over which this phenomenon is observed is widened (Fig. S6D).
	2 times decrease	The region where inhibitory ligands aid stimulation moves to the right at weaker ligand affinities, and the range over which this phenomenon is observed is shrunk (Fig. S6D).
SHP1 unbinding rate	2 times increase	The region where inhibitory ligands aid stimulation

		moves to the right at weaker ligand affinities, and the range over which this phenomenon is observed is shrunk (Fig. S6E).
	10 times decrease	The region where inhibitory ligands aid stimulation moves to the left at stronger ligand affinities, and the range over which this phenomenon is observed is widened (Fig. S6E).
SHP1 activation rate	10 times increase	Hardly any change (Fig. S6F).
	10 times decrease	The region where inhibitory ligands aid stimulation moves to slightly the right at weaker ligand affinities (Fig. S6F).
SHP1 de-activation rate	10 times increase	The region where inhibitory ligands aid stimulation moves to slightly the right at weaker ligand affinities (Fig. S6G).
	10 times decrease	Hardly any change (Fig. S6G).
De-activation by non-activated SHP-1	10 times increase	Hardly any change.
	10 times decrease	Hardly any change.
De-activation by activated SHP-1	2 times increase	The region where inhibitory ligands aid stimulation moves slightly to the left at stronger ligand affinities, and the range over which this phenomenon is observed is reduced (Fig. S6H).
	2 times decrease	The region where inhibitory ligands aid stimulation moves to slightly the right at weaker ligand affinities (Fig. S6H).
Zap70/Syk binding rate	10 times increase	Hardly any change in the $k_{off}$ range shown in Fig. S6I
	10 times decrease	Hardly any change in the $k_{off}$ range shown in Fig. S6I
Zap70/Syk unbinding rate	10 times increase	The region where inhibitory

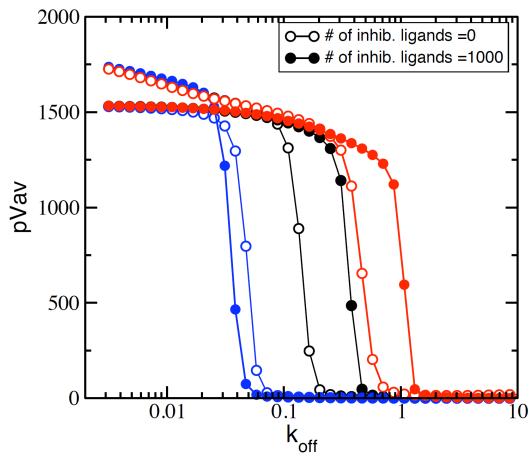
		ligands aid stimulation moves slightly to the right at weaker ligand affinities, and the range over which this phenomenon is observed is reduced (Fig. S6J).
	10 times decrease	Hardly any change in the $k_{off}$ range shown in Fig. 4B (Fig. S6J).
Activation rate of Zap70/Syk mediated by Lck	10 times increase	Hardly any change in the $k_{off}$ range shown in Fig. 4B.
	10 times decrease	Hardly any change in the $k_{off}$ range shown in Fig. 4B.
Activation rate of Zap70/Syk mediated by activated Lck	10 times increase	The region where inhibitory ligands aid stimulation moves slightly to the right at weaker ligand affinities.
	2 times decrease	The region where inhibitory ligands aid stimulation moves to the left at stronger ligand affinities.
Binding rate of Zap70/Syk to Vav	10 times increase	Hardly any change in the $k_{off}$ range.
	10 times decrease	The region where inhibitory ligands aid stimulation moves slightly to the left at stronger ligand affinities, and the range over which this phenomenon is observed is reduced.
Unbinding rate of Zap70/Syk to Vav	10 times increase	Slight increase in activation in the range where the phenomenon is observed (Fig. S6K).
	10 times decrease	Decrease in activation, the size of range where the phenomenon is observed is much reduced (Fig. S6K).
Vav activation rate	2 times increase	The region where inhibitory ligands aid stimulation moves slightly to the right at weaker ligand affinities (Fig. S6L).
	2 times decrease	The region where inhibitory ligands aid stimulation

		moves slightly to the left at stronger ligand affinities (Fig. S6L).
Inhibitory ligand binding rate	10 times increase	Hardly any change (Fig. S6M).
	10 times decrease	Hardly any change (Fig. S6M).
Inhibitory ligand unbinding rate	10 times increase	Hardly any change (Fig. S6N).
	10 times decrease	Hardly any change (Fig. S6N).

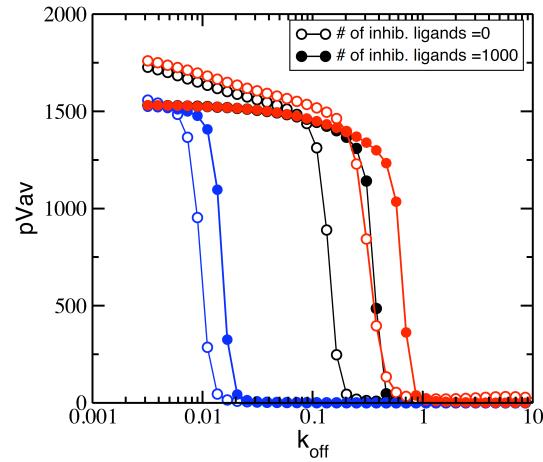


**Fig. S5. Effect of variation of concentrations on the results shown in Fig. 4B.** The concentrations are increased (red circles) and decreased (blue circles) from the values (shown in Table S VI) used for Fig. 4B. The cases with zero and 1000 inhibitory ligands are shown with empty and filled circles. The results in Fig. 4B are reproduced (black circles) in the figures for comparison. (A) Variation in stimulatory receptor

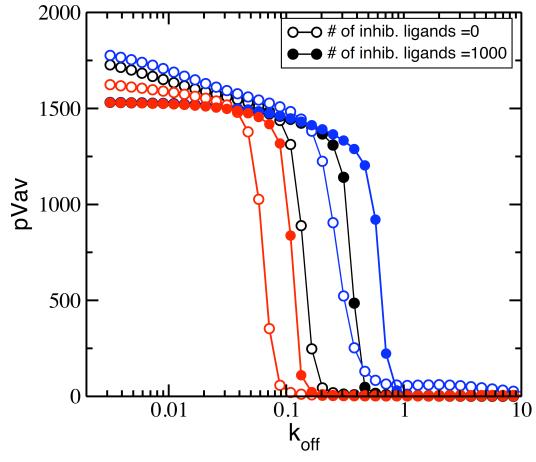
concentrations. (B) Variation in stimulatory ligand concentrations. (C) Variation in Lck/Fyn concentrations. (D) Variation in SHP-1 concentrations. (E) Variation in Zap70/Syk concentrations. (F) Variation in Vav concentrations.



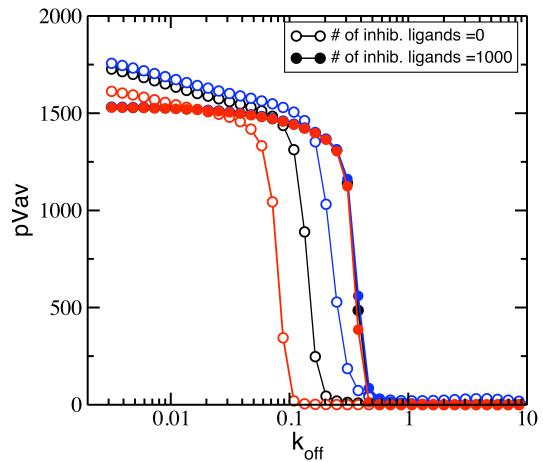
(A)



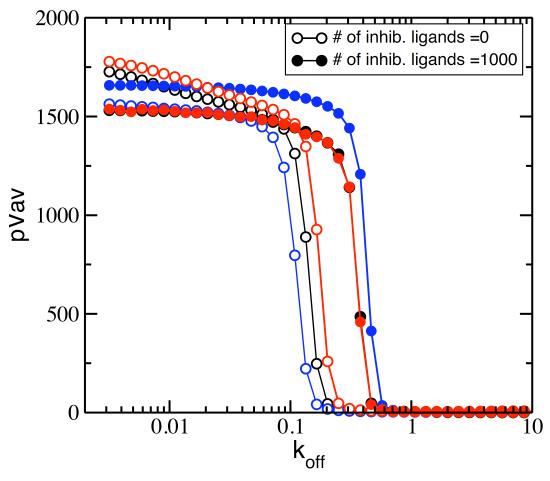
(B)



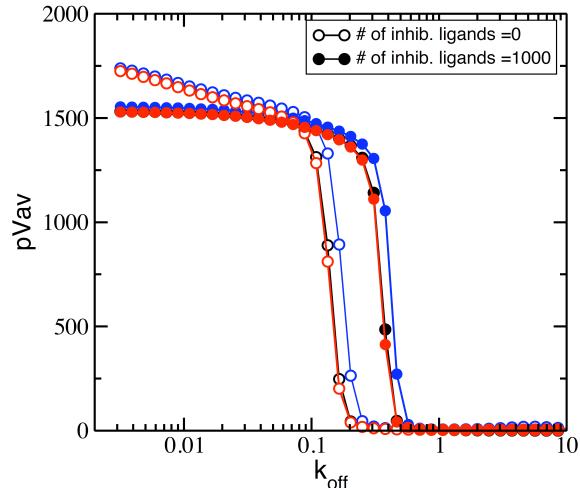
(C)



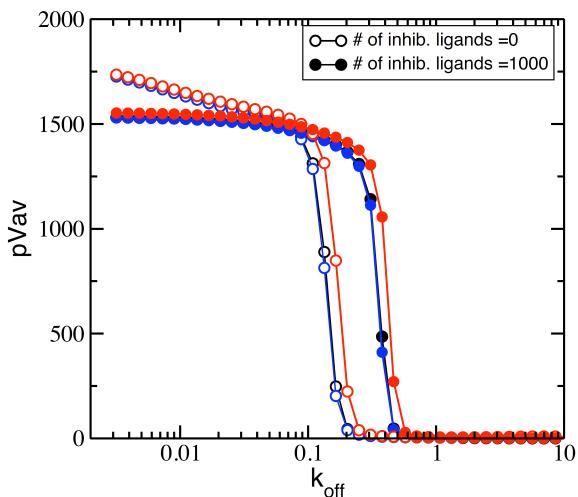
(D)



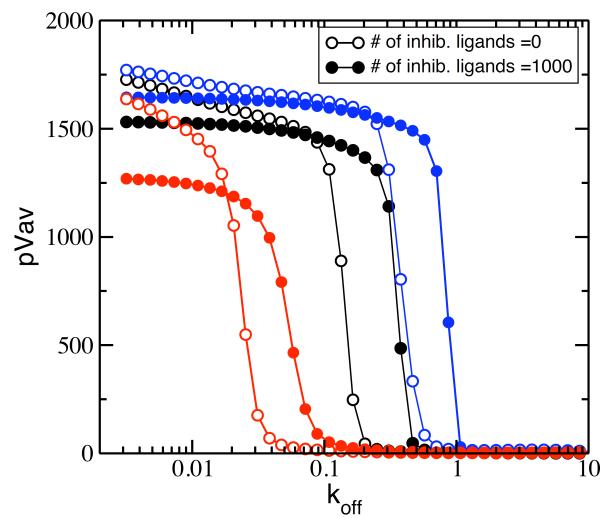
(E)



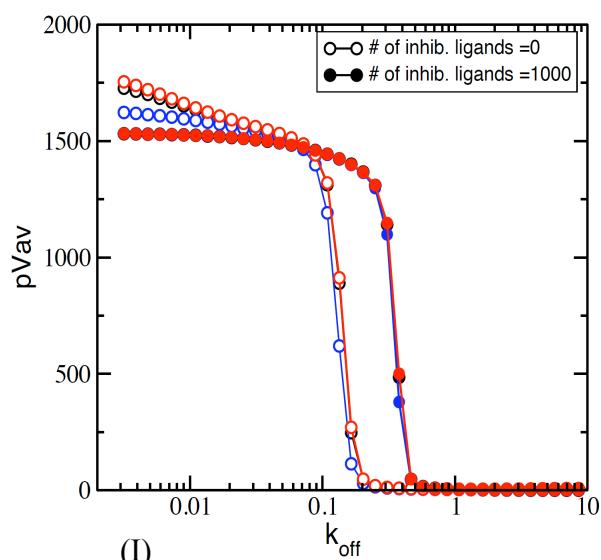
(F)



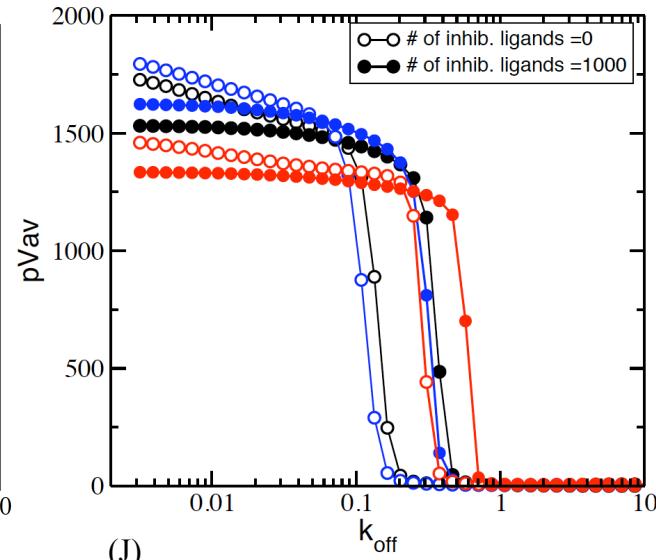
(G)



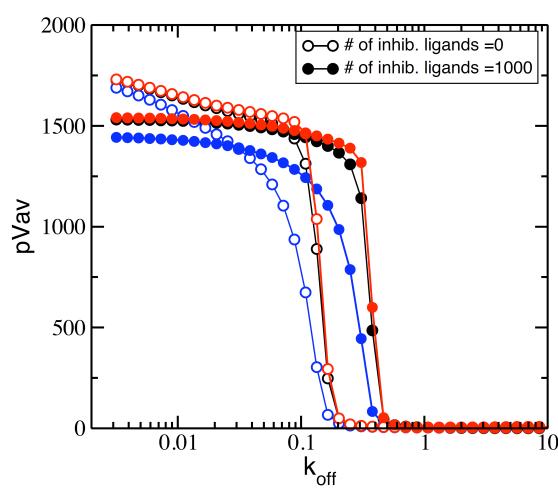
(H)



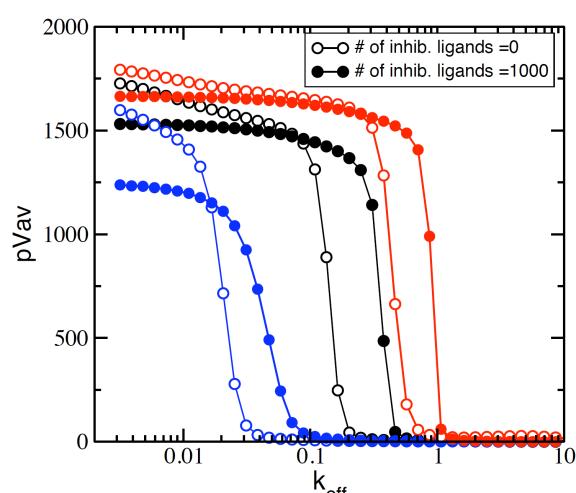
(I)



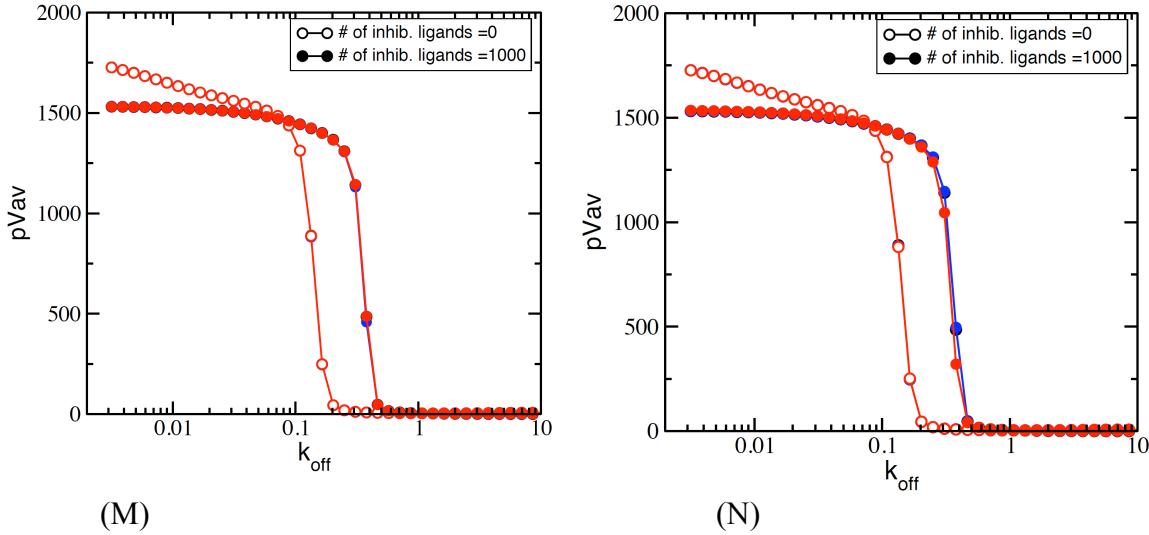
(J)



(K)



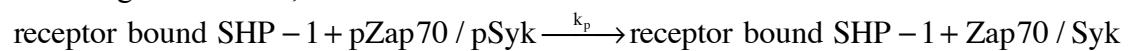
(L)



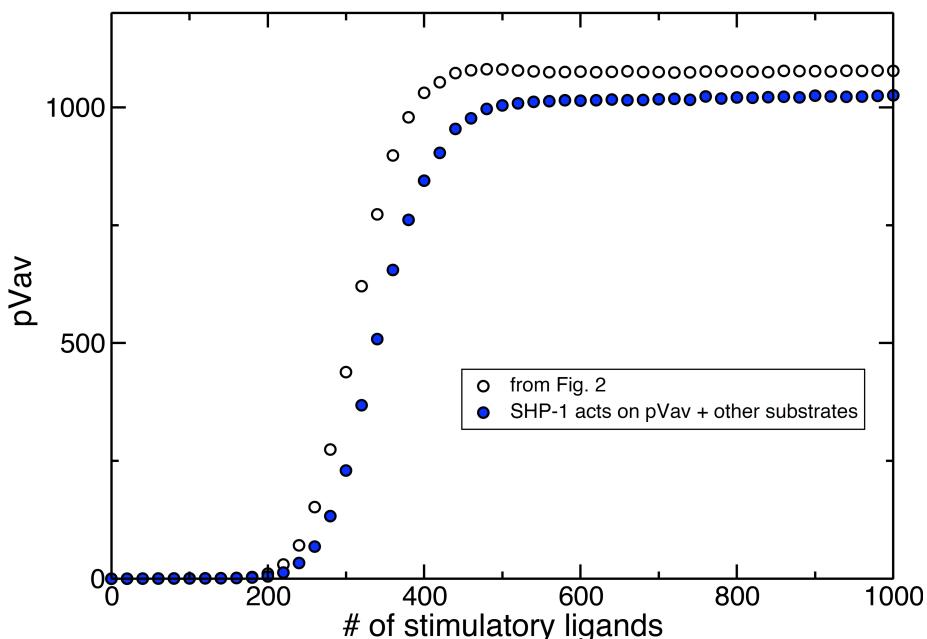
**Fig. S6. Effect of variation of rate constants on the results shown in Fig. 4B.** The concentrations are increased (red circles) and decreased (blue circles) from the values (shown in Table SV) used for Fig. 4B. The cases with zero and 1000 inhibitory ligands are shown with empty and filled circles. The results in Fig. 4B are reproduced (black circles) in the figures for comparison. (A) Variation in rate of stimulatory ligand binding. (B) Variation in catalytic rate of fully activated Lck/Fyn. (C) Variation in rate of de-activation of activated stimulatory receptors. (D) Variation in SHP-1 binding rate. (E) Variation in SHP-1 unbinding rate. (F) Variation in SHP-1 activation rate. (G) Variation in SHP-1 de-activation rate. (H) Variation in de-activation rate by activated SHP-1. (I) Variation in rate of Zap70/Syk binding to fully activated stimulatory receptors. (J) Variation in rate of Zap70/Syk unbinding from fully activated stimulatory receptors. (K) Variation in rate of Zap70/Syk binding to Vav. (L) Variation in rate of Vav activation. (M) Variation in binding rate of inhibitory ligands. (N) Variation in unbinding rate of inhibitory ligands.

### Effect of SHP-1 de-activating Lck, activated stimulatory receptors and activated Zap70/Syk in addition to activatedVav.

SHP-1 has been found to de-activate substrates such as, phosphorylated ITAMs, phosphorylated Zap70/Syk and phosphorylated tyrosine residues in Lck in biochemical studies. However, the only substrate for SHP-1 in NK cells has been found to be Vav1. We studied the effect of having activated stimulatory receptors, pZap70/Syk and Lck as additional substrates of receptor bound SHP-1 in our model. To keep the number of additional reactions because inclusion of the above effects we use a first order decay of the substrates. For example, a receptor bound SHP-1 molecule de-activates pZap70/pSyk following the reaction,

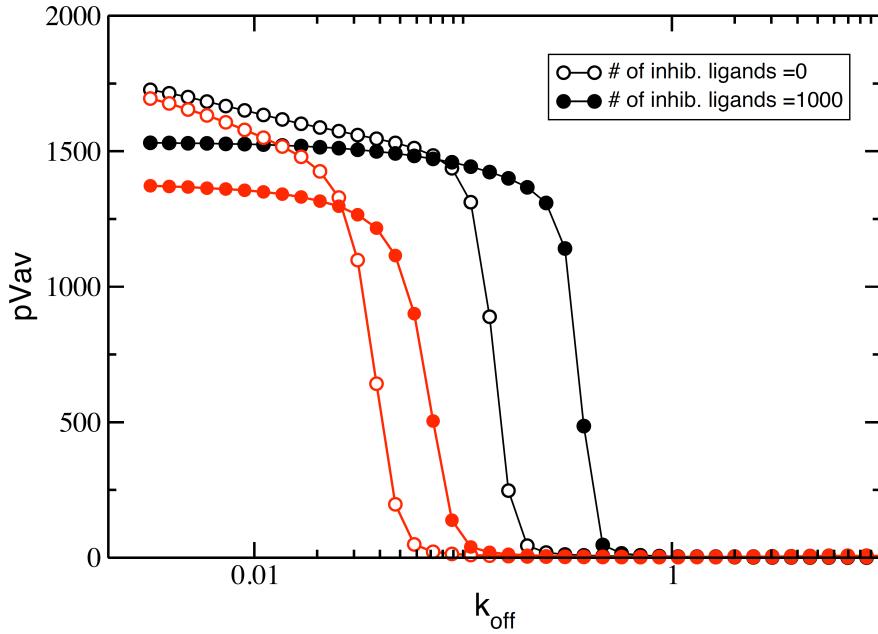


The fully and basally activated forms of Lck are de-activated to basally and a de-activated form respectively by SHP-1. Similarly, activated states of stimulatory receptors, when not bound to SHP-1/Zap70/Syk get de-activated by receptor bound SHP-1. The results of including these effects to Fig. 2A and 4B are shown below.



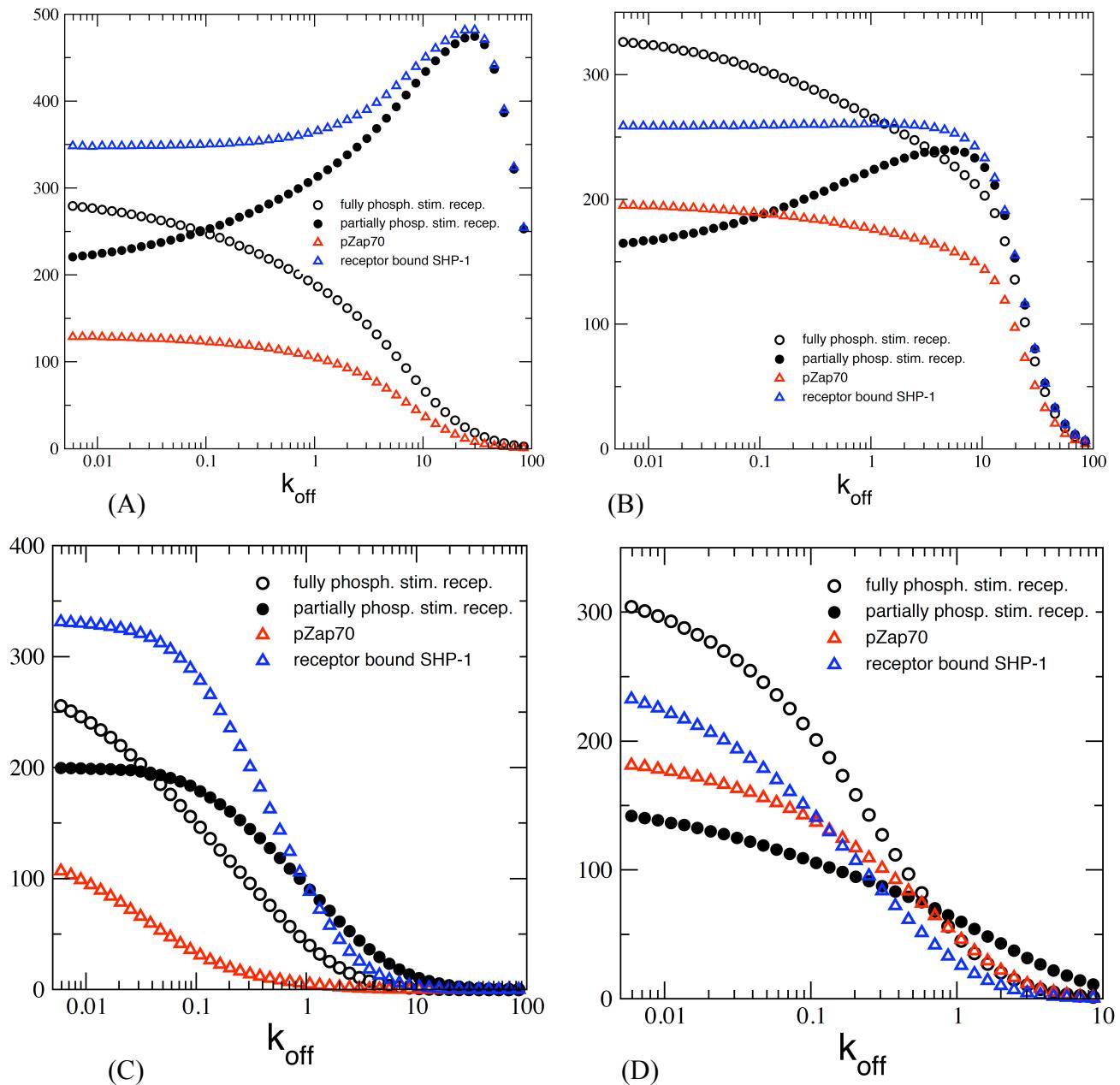
**Fig. S7 Effect on Vav activation as the concentration of stimulatory ligands is increased.** Inclusion of the above reactions reduce Vav activation and the threshold of activation moves to higher stimulatory ligand concentrations. The values of the rate constants for the additional reactions are the following: pZap70/pSyk de-activation ( $k_p=10^{-7}\text{s}^{-1}$ ,  $k_{p1}=10^{-5}\text{s}^{-1}$ ,  $k_p$  and  $k_{p1}$  refer to de-activation by non-activated SHP-1 and activated SHP-1 respectively), Lck de-activation ( $k_p=10^{-7}\text{s}^{-1}$ ,  $k_{p1}=10^{-5}\text{s}^{-1}$ ), activated stimulatory receptor de-activation ( $k_p=10^{-7}\text{s}^{-1}$ ,  $k_{p1}=10^{-5}\text{s}^{-1}$ ). When rate constants were

higher than the values shown here, Vav activation decreases substantially. The rest of the parameters are the same as in Fig. 2.



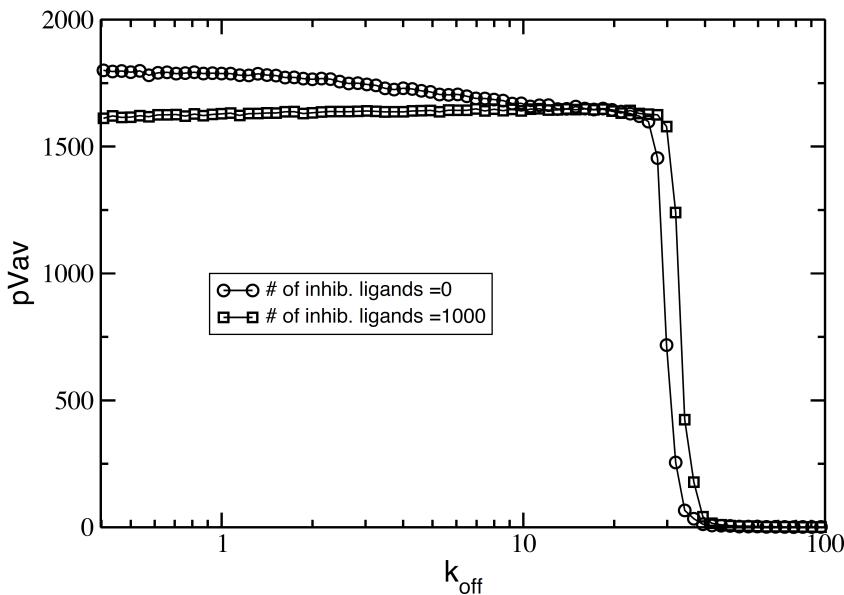
**Fig. S8 Effect on Vav activation when inhibitory receptors help mediate activation.** When SHP-1 acts on other substrates (pZap, Lck, activated stimulatory receptors) in addition to pVav, our model shows (red circles) that inhibitory receptors can help mediate activation under the same conditions detailed in the main text. The empty and filled red circles refer to the cases when zero and 1000 inhibitory ligands are present in the system, respectively. The results from Fig. 4B are shown in black circles for comparison. The values of the rate constants for the additional reactions are the following: pZap70/pSyk de-activation ( $k_p=10^{-3} s^{-1}$ ,  $k_{p1}=10^{-2} s^{-1}$ ,  $k_p$  and  $k_{p1}$  refer to de-activation by non-activated SHP-1 and activated SHP-1, respectively), Lck de-activation ( $k_p=10^{-6} s^{-1}$ ,  $k_{p1}=10^{-4} s^{-1}$ ), activated stimulatory receptor de-activation ( $k_p=10^{-3} s^{-1}$ ,  $k_{p1}=10^{-2} s^{-1}$ ). When rate constants were higher than the values shown here, Vav activation decreases substantially. The rest of the parameters are the same as in Fig. 4B.

### Comparison of different kinetic proof-reading schemes:



**Fig. S9 Comparison between weak and strict kinetic proof reading schemes.** A weaker version of a kinetic proof reading scheme is implemented where only receptor-ligand and receptor-ligand-Lck/Fyn complexes are dissociated immediately upon ligand unbinding. For any other complexes, the rest of a complex remains intact as the ligand unbinds. In the strict version of the kinetic proof reading scheme, any complex associated with a receptor ligand complex dissociates immediately upon ligand unbinding. The results are compared when there are 500 stimulatory ligands and no inhibitory ligands in

the system. The rest of the parameters are the same as in tables SI and SII. The SSC code for this version of the kinetic proof reading is available at <http://planetx.nationwidechildrens.org/~jayajit/>. (A) Concentrations of pZap70/pSyk, receptor bound SHP-1, partially and fully activated stimulatory receptors decrease slowly as the off-rate of ligand unbinding from the receptor is increased. The system shows appreciable unrealistic activation even for a very weak affinity peptide possessing an off-rate of  $5\text{ s}^{-1}$ . The slower de-activation arises from the effect of protection of the activated states of the stimulatory receptors bound to Zap-70/Syk and SHP-1 molecules, which are not affected appreciably by the unbinding of stimulatory ligands in this scheme, from the action of the phosphatases. (B) Variations of the concentrations of pZap70/pSyk, receptor bound SHP-1, partially and fully activated stimulatory receptors when the binding affinity of SHP-1 to partially phosphorylated stimulatory receptors is reduced 1000 times. The system shows a much slower decrease compared to (A). The rest of the parameters are same as in (A). (C) Variation of the activation markers (pZap70/pSyk, receptor bound SHP-1, partially and fully activated stimulatory receptors) as the off-rate of ligand unbinding increases for the model with the strict kinetic proof reading scheme shows a more realistic faster decay of activation. For a weak affinity ligand of an off-rate  $1.0\text{ s}^{-1}$  the system shows significant decrease in activation. The parameters used are the same as in (A). (D) Results for the model with the strict kinetic proof reading scheme when SHP-1 binds to the partially phosphorylated stimulatory receptors with a 1000 fold less affinity. The system shows a slower deactivation, however, the activation decreases substantially for an off-rate of  $5\text{ s}^{-1}$ .



**Fig. S10 Activation mediated by inhibitory receptors for a model with the weaker form of kinetic proof reading scheme.** The model with the weaker form of kinetic proof reading scheme shows activation mediated by inhibitory receptors when SHP-1 is limiting in the system. The range of affinities for the stimulatory ligand receptor binding

that shows this behavior is shifted to a much weaker range of affinities ( $>k_{\text{off}}=30\text{s}^{-1}$ ) compared to the model with the strict kinetic proof reading scheme (Fig. 4B). The parameter values used for the simulation is the same as in the parameters used for Fig. 4B.

## Section SB: Enzymatic activation and de-activation of pVav

Consider a substrate W (Vav in our model) getting activated and de-activated by two enzymes,  $E_1$  (pZap70/pSyk in the model) and  $E_2$  (receptor bound SHP-1 in the model), respectively, following the reaction scheme below. The same reactions were used by Goldbeter and Koshland (1) to show presence of zero order ultra-sensitivity in the system.



When Michaelis Menten approximations hold

(when,  $[E_1](t=0)/([W](t=0)+(k_{-1}+k_{1f})/k_1) \ll 1$ , and,  $[E_2](t=0)/([W^*](t=0)+(k_{-2}+k_{2f})/k_2) \ll 1$ ) (2) we can write down the kinetics of activation of W as,

$$\frac{d[W^*]}{dt} = \frac{k_{1f}E_{1T}(W_T - [W^*])}{K_{1M} + (W_T - [W^*])} - \frac{k_{2f}E_{2T}[W^*]}{K_{2M} + [W^*]} \quad (1)$$

where,  $E_{1T} = [E_1] + [WE_1]$ ,  $E_{2T} = [E_2] + [W^*E_2]$ ,  $W_T = [W] + [W^*] + [WE_1] + [W^*E_2]$ ,

$K_{1M} = (k_{1f} + k_{-1})/k_1$ , and  $K_{2M} = (k_{2f} + k_{-2})/k_2$ . At the steady state,  $[W^*]$  can be calculated from the quadratic equation given by,

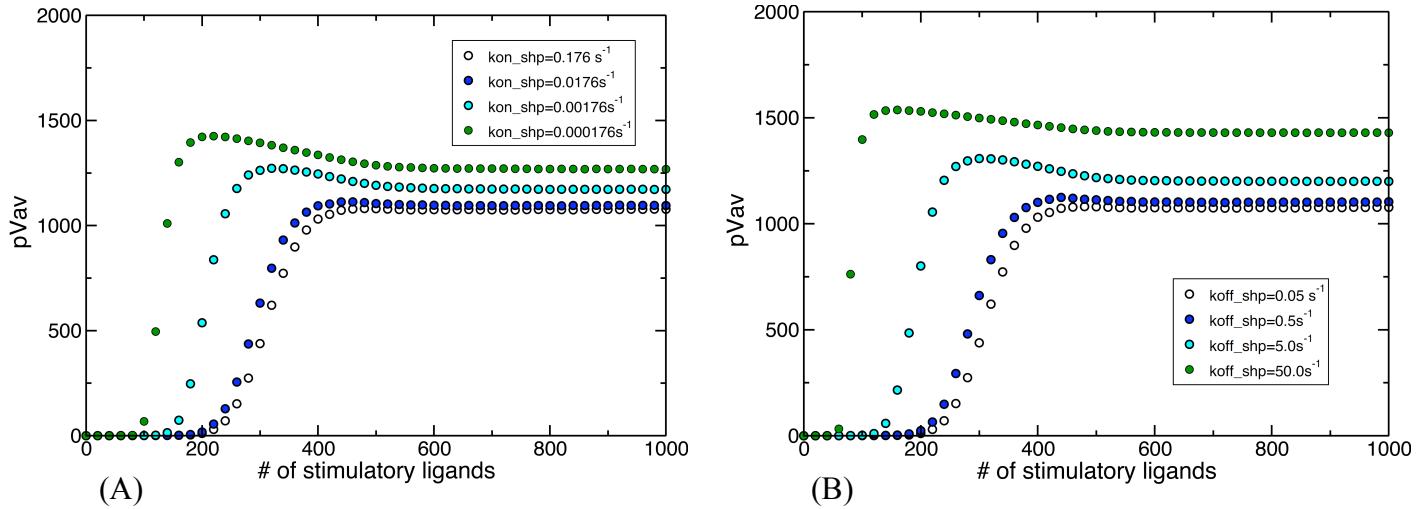
$$k_{1f}R(W_T - [W^*])(K_{2M} + [W^*]) - k_{2f}[W^*](K_{1M} + (W_T - [W^*])) = 0, \quad (2)$$

where,  $R = E_{1T}/E_{2T}$ . The above equation shows that the steady state concentration does not depend on the enzyme concentrations separately, but depends on the ratio, R. Thus we will find a scaling relation,  $[W^*] = f(E_{1T}, E_{2T}) = g(R)$ . In addition, Eq.(2) shows that the time scales in the kinetics depend on,  $1/E_{1T}$  and  $1/E_{2T}$ . Therefore, the larger the concentrations of the enzymes,  $E_{1T}$  and  $E_{2T}$ , the faster is the kinetics of activation.

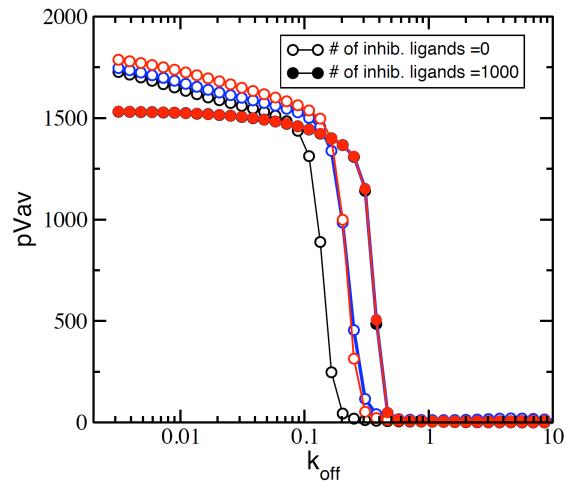
However, in the absence of the Michaelis Menten approximation, the simple nature of the kinetics is lost. In that case, the steady state of  $[W^*]$  depends on  $E_{1T}$  and  $E_{2T}$  separately, and can be obtained by solving a cubic algebraic equation (1).

1. Goldbeter, A., and D. E. Koshland, Jr. 1981. An amplified sensitivity arising from covalent modification in biological systems. Proc Natl Acad Sci U S A 78:6840-6844.
2. Murray, J. D. 1989. Mathematical biology. Springer-Verlag, Berlin ; New York.

### Effect of variation of SHP-1 affinity to partially phosphorylated stimulatory receptors



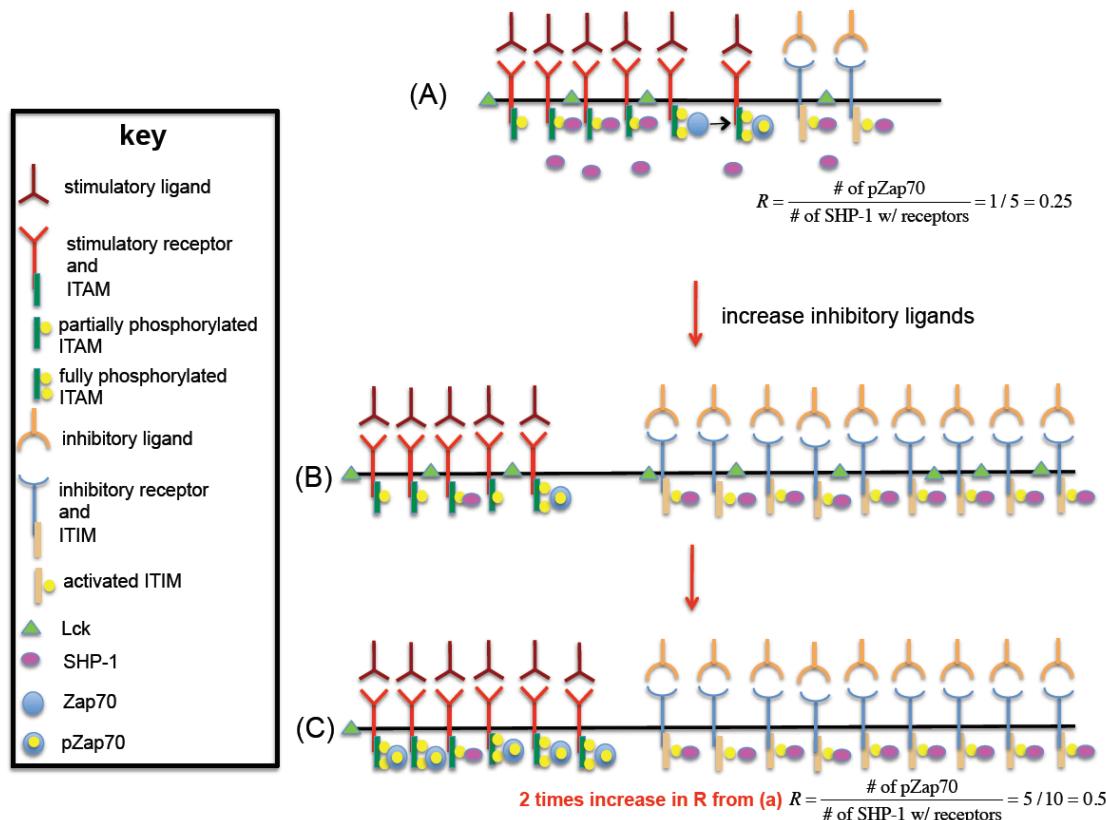
**Fig. S11 Variation in activation mediated by stimulatory ligands.** The rate of SHP-1 binding ( $\text{kon\_shp}$ ) to partially phosphorylated stimulatory receptors is decreased (A) or the rate of unbinding ( $\text{koff\_shp}$ ) from partially phosphorylated stimulatory receptors is increased (B) up to 1000 times from the value used for the results in Fig. 2A. The affinity of SHP-1 binding to fully phosphorylated stimulatory receptors and activated inhibitory receptors is kept unchanged. The rest of the parameters are the same as in Fig. 2A. The activation is increased as a result of the above variations, as well as, the threshold shifts to a lower concentration of stimulatory ligands. However, the qualitative behavior the activation profile is similar to the results in Fig. 2A (shown above in black circles).



**Fig. S12 Variation in activation mediated by inhibitory ligands.** (A) The rate of SHP-1 binding ( $\text{kon\_shp}$ ) to partially phosphorylated stimulatory receptors is decreased 2 times. The case with no inhibitory ligand and 1000 inhibitory ligands are shown in empty and filled blue circles respectively. (B) The rate of unbinding ( $\text{koff\_shp}$ ) from partially phosphorylated stimulatory receptors is increased 10 times. The results are shown in

empty red (0 inhibitory ligand) and filled red circles (1000 inhibitory ligands). The rest of the parameters are same as in Fig. 4B. The results from Fig. 4B are reproduced (black circles) for comparison. The range of the stimulatory ligand affinities where inhibitory ligands help activation decreases for both the above variations. If the affinity of SHP-1 binding to the partially activated stimulatory receptors is increased further, the system ceases to exhibit this effect.

## Steps involved in activation mediated by inhibitory ligands

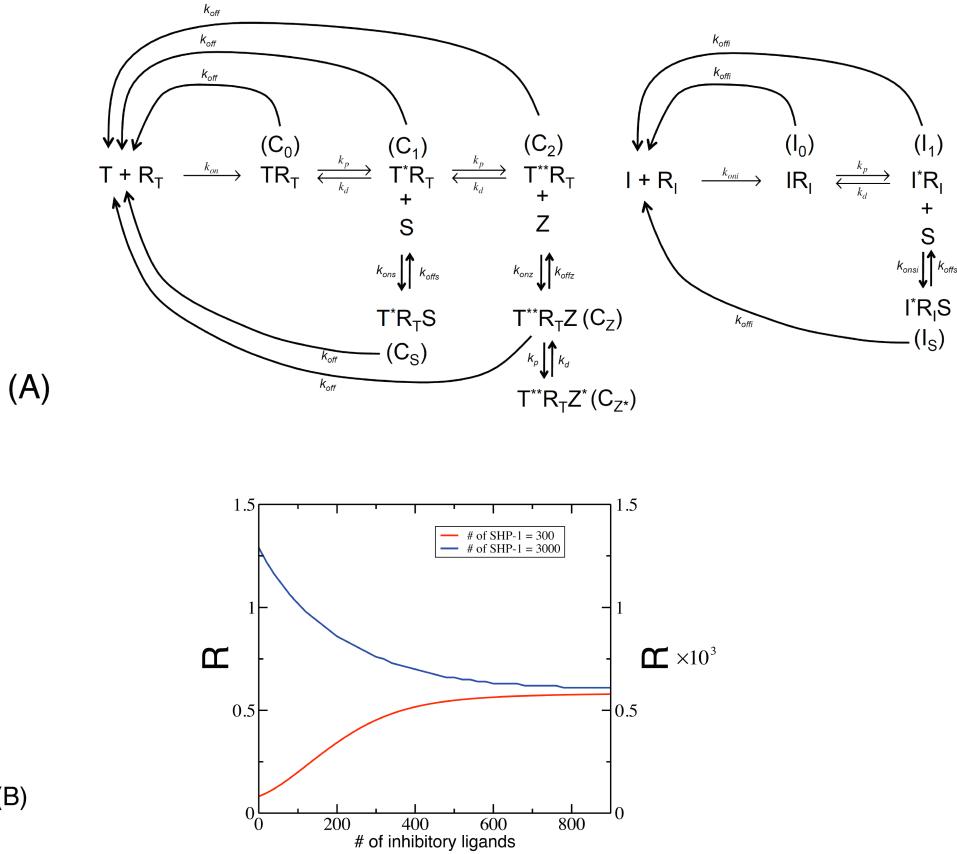


**Fig. S13 Mechanism underlying stimulation mediated by inhibitory receptors.** (A) NK cells possessing stimulatory receptors with ITAMs are presented with cognate weak affinity stimulatory ligands. Most of the ITAMs are partially phosphorylated by Lck/Fyn and they recruit SHP-1 molecules. Since the number of SHP-1 molecules is small, most of the SHP-1 molecules are recruited by the partially phosphorylated ITAMs. The few fully phosphorylated ITAMs recruit Zap70 molecules. ITAM bound Zap70 molecules get phosphorylated by Lck/Fyn and activate Vav. ITIMs associated with inhibitory receptors recruit SHP-1 upon activation by Lck/Fyn. Vav activation is determined by the ratio ( $R = \frac{\# \text{ of pZap70}}{\# \text{ of SHP-1 bound to receptors}}$ ). Since, the number of receptor bound SHP-1 molecules is much larger than the pZap70 molecules in this case, it will not result in any activation. (B) As the number of the inhibitory ligands is increased, more ITIMs are phosphorylated and become available to recruit SHP-1. When the number of activated ITIMs is much larger than the SHP-1 molecules, most of the SHP-1 molecules are recruited by the ITIMs. Hence this will lead to a situation where most of the partially phosphorylated ITAMs are not bound to any SHP-1. (C) Whenever a partially phosphorylated ITAM, not bound to a SHP-1 molecule, is converted into a fully phosphorylated ITAM it binds to a Zap70. Since Zap70 is available in excess, most of the fully phosphorylated ITAMs are always bound to Zap70 molecules, and thus escape de-activation by phosphatases. Therefore, in this situation the number of Zap70

molecules associated with the ITAMs increases. This increases the number of pZap70 molecules. If the relative increase in Zap70 molecules is larger than the relative increase in receptor bound SHP-1 molecules, then it will result in an increase in the ratio, R, favoring Vav activation. In this case, R increases 2 times from (A).

## Section SC: Minimal Model

We construct a minimal model to understand general mechanisms underlying the results in the last section. The model consists of a set of essential signaling events required to capture the competition between stimulatory and inhibitory pathways that lead to Vav activation. The kinetics of the system is described by a set of mean field kinetic rate ordinary differential equations (ODEs). This approach is justified when the participating molecules are present in large copy numbers and the effects of the stochastic number fluctuations can be ignored. Since Vav activation crucially depends on the ratio ( $R$ ) of concentrations of activated Zap70/Syk and receptor bound SHP-1 molecules, we will use the increase or decrease in  $R$  as an indicator of Vav activation or de-activation respectively.



**Fig. S14 Minimal model.** (A) The reaction network used in the minimal model. The variables,  $C_0, C_1, C_2, C_S, C_Z, C_{Z^*}, S$  and  $Z$  denote the concentrations of the stimulatory receptor ( $R_T$ ) and stimulatory ligand ( $T$ ) complexes,  $TR_T$ ,  $T^*R_T$ ,  $T^{**}R_T$ ,  $T^*R_TS$ ,  $T^{**}R_TS$ ,  $T^{**}R_TZ$ ,  $T^{**}R_TZ^*$ , and SHP-1 and Zap70/Syk molecules, respectively. The variables,  $I_0$ ,  $I_1$ , and  $I_S$  denote the concentrations of the inhibitory receptor ( $R_I$ ) and inhibitory ligand ( $I$ ) complexes,  $IR_I$ ,  $I^*R_I$ , and  $I^*R_IS$ , respectively. (B) Variation of the ratio,  $R = C_{Z^*} / (C_S + I_S)$  in the steady state.  $R$  increases (red) or decreases (blue) as the concentration of inhibitory ligands is increased for a small (<the number of inhibitory

receptors ) or large number of SHP-1 ( $>$  the number of inhibitory receptors) molecules, respectively. The values of the parameters used are shown in the web supplement (Table SVIII).

The reactions and the parameters involved in the model are shown in Fig. S14A. We analyze the steady state solution of the system to extract the conditions necessary to have inhibitory receptors mediate activation in the system. In Fig. S14B we show how the qualitative nature of variation of R with increasing concentration of inhibitory ligands is altered as the SHP-1 concentration is increased. It is difficult to get simple analytical expressions elucidating these conditions in general, however, under certain approximations, such as,  $k_d \approx 0$ , the system of coupled equations simplify. One of the important conditions obtained from the minimal model is that the SHP-1 concentration needs to be within a range for activation mediated by inhibitory receptors to occur, and, the total number of the SHP-1 molecules should be smaller than the available number of inhibitory receptor ligand complexes. Under certain simplifying approximations as detailed below, the range is given by,

$$\frac{k_{ons}k_p C_0 + (k_{off} + k_{offs})(k_{off} + k_p)}{k_{off}k_{ons}} > S_0 > \frac{(k_{off} + k_p)(k_{offs} + k_{off})}{k_{ons}k_{off}}, \text{ where } S_0 \text{ denotes the total}$$

SHP-1 concentration. The other parameters are described in Fig. S14A. The upper and lower bounds depend on the affinity ( $K_{DS} = k_{offs} / k_{ons}$ ) of SHP-1 molecules binding to the activated stimulatory receptors. For small values of the affinity (or large  $K_{DS}$ ), the range of SHP-1 concentrations becomes very small making this effect difficult to be observed in real systems. In addition, the above range is valid when Zap70/Syk molecules are present in excess compared to the receptor ligand complexes. The effect ceases to exist when Zap70/Syk molecules are present in small numbers and the affinity of Zap70/Syk to fully phosphorylated stimulatory receptors is small which is in contrast to the case for lymphocytes(48). The details of the calculation showing the above result are shown later in this section. The necessary conditions that emerge from the minimal model in order to get activation mediated through inhibitory receptors are the following: (i) weak affinity stimulatory ligand, (ii) small number of SHP-1 molecules, where the range is determined by the numbers of stimulatory and inhibitory receptor ligand complexes, (iii) high affinity binding of SHP-1 to partially activated stimulatory receptors, and (iv) excess amount of Zap70/Syk molecules. The details of the calculations are given below.

The signaling kinetics of the model shown in Fig. S14A can be described in terms of the following rate equations in Eq.1-11.

$$\frac{dC_0}{dt} = k_{on}[T][R_T] - (k_p + k_{off})C_0 + k_d C_1 \quad (1)$$

$$\frac{dC_1}{dt} = k_p C_0 - (k_d + k_p + k_{off})C_1 + k_d C_2 - k_{ons}SC_1 + k_{offs}C_S \quad (2)$$

$$\frac{dC_2}{dt} = k_p C_1 - (k_d + k_{off}) C_2 - k_{onz} Z C_2 + k_{offz} C_Z + k_d C_{Z^*} - k_p C_Z \quad (3)$$

$$\frac{dC_Z}{dt} = k_{onz} Z C_2 - (k_{offz} + k_{off}) C_Z + k_d C_{Z^*} \quad (5)$$

$$\frac{dC_{Z^*}}{dt} = k_p C_Z - (k_{off} + k_d) C_{Z^*} \quad (7)$$

$$\frac{dC_S}{dt} = k_{ons} S C_1 - (k_{offs} + k_{off}) C_S \quad (8)$$

$$\frac{dI_0}{dt} = k_{oni} [I] [R_I] - (k_p + k_{offi}) I_0 + k_d I_1 \quad (9)$$

$$\frac{dI_1}{dt} = k_p I_0 - (k_d + k_{offi}) I_1 - k_{onsi} S I_1 + k_{offsi} I_S \quad (10)$$

$$\frac{dI_S}{dt} = k_{onsi} S I_1 - (k_{offsi} + k_{offi}) I_S \quad (11)$$

The above ODEs are complemented by the conservation laws,

$T_0 = [T] + C_0 + C_1 + C_2 + C_S + C_Z + C_{Z^*}$ ,  $R_{T0} = [R_T] + C_0 + C_1 + C_2 + C_S + C_Z + C_{Z^*}$ ,  
 $Z_0 = Z + C_Z + C_{Z^*}$ ,  $S_0 = S + C_S + I_S$ ,  $I_0 = [I] + I_0 + I_1 + I_S$ , and,  $R_{I0} = [R_I] + I_0 + I_1 + I_S$ ,  
where,  $T_0$ ,  $R_{T0}$ ,  $Z_0$ ,  $S_0$ ,  $I_0$  and  $R_{I0}$  denote the total concentrations of stimulatory receptors, stimulatory ligands, Zap70, SHP-1, inhibitory receptors and inhibitory ligands respectively.

Note that the stimulatory and inhibitory signaling pathways are coupled through the variables,  $C_S$  and  $I_S$ , which denote the concentrations of SHP-1 bound complexes with stimulatory and inhibitory receptors.

#### Steady States:

The steady states are defined by,

$\frac{dC_0}{dt} = \frac{dC_1}{dt} = \frac{dC_S}{dt} = \frac{dC_2}{dt} = \frac{dC_Z}{dt} = \frac{dC_{Z^*}}{dt} = 0$  and  $\frac{dI_0}{dt} = \frac{dI_1}{dt} = \frac{dI_S}{dt} = 0$ . We get the following expressions for the steady state concentrations,

$$\begin{aligned}
C_0 &= \frac{k_{on}[T][R_T] + k_d C_1}{k_{off} + k_p}, \quad C_1 = \frac{k_p C_0 + k_d C_2 + k_{offs} C_S}{k_d + k_{off} + k_p + k_{ons} S}, \quad C_2 = \frac{k_p C_1 + k_{offz} C_Z}{k_d + k_{off} + k_{onz} Z}, \\
C_S &= \frac{k_{ons} C_1 S}{k_{offs} + k_{off}}, \quad C_Z = \frac{k_{onz} C_2 Z + k_d C_{Z^*}}{k_{offz} + k_{off} + k_p}, \quad C_{Z^*} = \frac{k_p C_Z}{k_d + k_{off}}, \quad I_0 = \frac{k_{oni}[I][R_I] + k_d I_1}{k_p + k_{offi}} \\
I_1 &= \frac{k_p I_0 + k_{offs} I_S}{k_d + k_{offi} + k_{ons} S}, \quad I_S = \frac{k_{ons} I_1 S}{k_{offs} + k_{offi}}
\end{aligned}$$

Activation of Vav is determined by the relative ratio (R) of concentrations of activated Zap70 with receptor bound SHP-1 molecules,  $R = \frac{C_{Z^*}}{C_S + I_S}$ .

**Case I<sub>0</sub> = 0 :** In absence of inhibitory receptors, i.e.,  $I_0 = 0$ ,  $R = \frac{C_{Z^*}}{C_S}$ .  $C_{Z^*}$  and  $C_S$  can be calculated from the above equations as functions of the rate constants, and the constants,  $T_0$ ,  $R_{T0}$ ,  $Z_0$ ,  $S_0$ ,  $I_0$  and  $R_{I0}$ . The resulting expressions can be quite complicated, however, in the limit,  $k_d = 0$ , they simplify. When,  $k_{on} Z \gg k_{off}$ , the concentrations,  $C_1$ ,  $C_S$ ,  $C_2$  and  $C_Z$  take the form below,

$$\begin{aligned}
C_0 &= \frac{k_{on}[T][R_T]}{k_{off} + k_p}, \quad C_1 = \frac{k_p C_0 + k_{offs} C_S}{k_{off} + k_p + k_{ons} S}, \quad C_S = \frac{k_{ons} C_1 S}{k_{offs} + k_{off}}, \quad C_2 = \frac{k_p C_1 + k_{offz} C_Z}{k_{off} + k_{onz} Z} \\
C_Z &= \frac{k_{onz} C_2 Z}{k_{offz} + k_{off} + k_p} \text{ and } C_{Z^*} = \frac{k_p C_Z}{k_{off}}. \quad C_Z \text{ can be calculated from the solution of the quadratic equation,}
\end{aligned}$$

$$y^2 - \left( \frac{K_{DZ} k_p}{(k_{off} + k_p)} + \frac{Z_0 k_{off}}{k_p} + \frac{k_p C_1}{k_{off} + k_p} \right) y + \frac{k_{off} Z_0 C_1}{(k_{off} + k_p)} = 0 \quad (12)$$

where,  $y = C_Z$  and  $K_{DZ} = (k_{offz} + k_p + k_{off}) / k_{onz}$ .

When, Zap70 is in excess, i.e.,  $Z_0 \gg C_1, K_{DZ}$ ,

$$y = C_Z \approx \frac{k_p C_1}{k_{off} + k_p} \quad (13)$$

When, Zap70 is limited,  $Z_0 \ll C_1, K_{DZ}$ ,  $y \approx \left( \frac{K_{DZ} k_p}{(k_{off} + k_p)} + \frac{k_p C_1}{k_{off} + k_p} \right)$ . Furthermore, when Zap70 binds weakly to  $C_2$ , i.e.,  $K_{DZ} \gg C_1$ ,  $C_Z$  concentration is mostly determined by  $K_{DZ}$ . In this case, increasing concentration of inhibitory ligands will decrease activation because, in this process receptor bound SHP-1 molecules will increase without affecting the activated Zap70 concentration.

In the contrary, when Zap70 is excess, i.e.,  $Z_0 \gg C_1, K_{DZ}, R$  is given by,

$$R = \frac{C_{Z^*}}{C_S} = \frac{k_p(k_{offs} + k_{off})C_Z}{k_{ons}k_{off}C_1S} \approx \frac{k_p^2(k_{offs} + k_{off})}{k_{off}(k_{off} + k_p)k_{ons}S}. \quad (14)$$

We need to calculate,  $x = S = S_0 - C_S$ , in order to write R as a function of the rate constants and total concentrations of the signaling molecules.

Using the expressions for  $C_1$  and  $C_S$  above,  $x$  is calculated from solution of the quadratic equation below,

$$\begin{aligned} & k_{ons}k_{off}x^2 + x(k'_{off}(k_{off} + k_p) - k_{off}k_{ons}S_0 + k_{ons}k_pC_0) - k'_{off}(k_{off} + k_p)S_0 = 0 \quad (15) \\ \Rightarrow & ax^2 + bx - c = 0 \\ \Rightarrow & x = \frac{-b \pm \sqrt{b^2 + 4ac}}{2a} \end{aligned}$$

where,  $a = k_{ons}k_{off}$ ,  $b = (k'_{off}(k_{off} + k_p) - k_{off}k_{ons}S_0 + k_{ons}k_pC_0)$ ,  $c = k'_{off}(k_{off} + k_p)S_0$ , and

$$k'_{off} = k_{off} + k_{offs}. \text{ The physical solution would correspond to, } x_{sol} = \frac{-b + \sqrt{b^2 + 4ac}}{2a},$$

because,  $x > 0$ . However, depending on the relative concentrations of stimulatory receptors and SHP-1 molecules,  $b > 0$  or  $b < 0$ . If,  $b > 0$ , this would imply,

$$k_{off}k_{ons}S_0 < k_{ons}k_pC_0 + k'_{off}(k_{off} + k_p), \text{ or, } S_0 < \frac{k_{ons}k_pC_0 + k'_{off}(k_{off} + k_p)}{k_{off}k_{ons}}. \text{ This situation}$$

arises when there are more stimulatory receptor ligand complexes than SHP-1 molecules. On the other hand, when there are more SHP-1 molecules than the stimulatory receptor

$$\text{ligand pairs, } b < 0 \text{ or } S_0 > \frac{k_{ons}k_pC_0 + k'_{off}(k_{off} + k_p)}{k_{off}k_{ons}}.$$

In the limit, when,  $b^2 \gg 4ac$ , the above solution is simplified,

$$x_{sol} = c/b + O(2) \approx A_1S_0/(C_1 - B_1S_0), \text{ when } b = C_1 - B_1S_0 > 0$$

$$= |b|/a + c/|b| + O(2) \approx (B_1S_0 - C_1)/k_{ons}k_{off} + A_1S_0/(B_1S_0 - C_1), \text{ when } b < 0$$

In the above expressions,  $A_1 = (k_{off} + k_{offs})(k_{off} + k_p)$ ,  $B_1 = k_{ons}k_{off}$ , and  $C_1 = k_{ons}k_pC_0 - (k_{off} + k_{offs})(k_{off} + k_p)$ .

The ratio, R, is given by,

$$R \approx \frac{k_p^2(k_{offs} + k_{off})}{k_{off}(k_{off} + k_p)k_{ons}x_{sol}} \quad (16)$$

**Case I<sub>0</sub> ≠ 0 :** When inhibitory receptors are present in large copy numbers, i.e.,  $I_0 \gg S_0$ ,

$$\text{then, } I_S = S_0 \text{ and } C_S \approx 0. \text{ Thus, } C_{Z^*} = \frac{k_p^2C_1}{k_{off}(k_{off} + k_p)} \approx \frac{k_p^3C_0}{k_{off}(k_{off} + k_p)^2}, \text{ and,}$$

$$R = R_{I_0 \neq 0} = \frac{C_{z^*}}{I_S} = \frac{k_p^2}{k_{off}} \frac{k_p C_0 (I_0 \neq 0)}{S_0 (k_{off} + k_p)^2}. \quad (17)$$

If we have more activation as we increase concentration of inhibitory receptors, it implies,  $R_{I_0 \neq 0} > R_{I_0 = 0}$ ,

$$\Rightarrow \frac{k_p^2}{k_{off}} \frac{k_p C_0 (I_0 \neq 0)}{S_0 (k_{off} + k_p)^2} > \frac{k_p^2 (k_{offs} + k_{off})}{k_{off} (k_{off} + k_p) k_{ons} x_{sol}} \quad (18)$$

1. When  $b > 0$ , the above inequality would imply,

$$\begin{aligned} \frac{k_p^2}{k_{off}} \frac{k_p C_0 (I_0 \neq 0)}{S_0 (k_{off} + k_p)^2} &> \frac{k_p^2 (k_{offs} + k_{off}) (C_1 - B_1 S_0)}{k_{off} (k_{off} + k_p) k_{ons} A_1 S_0} \\ \Rightarrow S_0 &> C_1 / B_1 - \frac{k_p C_0 (I_0 \neq 0) k_{ons} A_1}{(k_{off} + k_{offs})(k_{off} + k_p) B_1} \end{aligned} \quad (19)$$

Thus, in order to have activation as the concentration of inhibitory ligands is increased, the concentration of the SHP-1 molecules should be in the range,

$$\begin{aligned} \frac{k_{ons} k_p C_0 + (k_{off} + k_{offs})(k_{off} + k_p)}{k_{off} k_{ons}} &> S_0 > C_1 / B_1 - \frac{k_p C_0 (I_0 \neq 0) k_{ons} A_1}{(k_{off} + k_{offs})(k_{off} + k_p) B_1} \\ A_1 = (k_{off} + k_{offs})(k_{off} + k_p), \quad B_1 = k_{ons} k_{off} \quad \text{and} \quad C_1 = k_{ons} k_p C_0 + (k_{off} + k_{offs})(k_{off} + k_p). \end{aligned}$$

Therefore, the above inequality gives,

$$\frac{k_{ons} k_p C_0 + (k_{off} + k_{offs})(k_{off} + k_p)}{k_{off} k_{ons}} > S_0 > \frac{k_p (C_0 (I_0 = 0) - C_0 (I_0 \neq 0))}{k_{off}} + \frac{(k_{off} + k_p)(k_{offs} + k_{off})}{k_{ons} k_{off}}$$

When,  $Z_0 \gg C_0$ , we can expect,  $C_0 (I_0 = 0) \approx C_0 (I_0 \neq 0)$ , then the above relation is given by,

$$\frac{k_{ons} k_p C_0 + (k_{off} + k_{offs})(k_{off} + k_p)}{k_{off} k_{ons}} > S_0 > \frac{(k_{off} + k_p)(k_{offs} + k_{off})}{k_{ons} k_{off}}. \quad (20)$$

Now,  $\frac{(k_{offs} + k_{off})}{k_{ons}} = K_D$ , is the dissociation constant for the reaction,  $C_1 + S \xrightleftharpoons[k_{offs}]{k_{ons}} C_S$ .

Therefore, in order to convert half of the  $C_1$  to  $C_S$ , one needs,  $K_D + C_1 / 2$  number of  $S_0$  molecules. When,  $(k_{off} + k_p) / k_{off} \sim 1$ , the last part of the above inequality implies that one needs to have a SHP-1 concentration to produce an appreciable number of  $C_S$  molecules when there are no inhibitory ligands. This sets the stage for the significant increase in activated Zap70 molecules when the SHP-1 molecules are transferred from  $C_S$  to the inhibitory receptors in presence of large concentrations of inhibitory ligands.

2. If,  $b < 0$ , then the condition in Eq(18) gives,

$$S_0 < \frac{k_p C_0 (I_0 \neq 0) k_{ons} x_{sol}}{(k_{off} + k_{offs})(k_{off} + k_p)}$$

$$\Rightarrow S_0 < \frac{k_p C_0 (I_0 \neq 0) k_{ons}}{(k_{off} + k_{offs})(k_{off} + k_p)} [(S_0 + A_l S_0 / (B_l S_0 - C_1)) - C_1 / k_{ons} k_{off}]$$

Therefore, the range for  $S_0$  to produce activation when inhibitory ligands are added is

$$\frac{k_{ons} k_p C_0 + (k_{off} + k_{offs})(k_{off} + k_p)}{k_{off} k_{ons}} < S_0 < \frac{k_p C_0 (I_0 \neq 0) k_{ons}}{(k_{off} + k_{offs})(k_{off} + k_p)} [(S_0 + A_l S_0 / (B_l S_0 - C_1)) - C_1 / k_{ons} k_{off}]$$

(21)

In order to make the algebraic manipulations easier, we define,

$$\frac{k_{off} k_{ons}}{(k_{off} + k_{offs})(k_{off} + k_p)} = K_D,$$

$$C_1 / B_1 = (k_p / k_{off}) C_0 + (k_{off} + k_{offs})(k_{off} + k_p) / (k_{ons} k_{off}) = (k_p / k_{off}) C_0 + K_D = C'_0 + K_D$$

, where,  $C'_0 = (k_p / k_{off}) C_0$ .

Thus, Eq.(21) takes the form,

$$C'_0 + K_D < S_0 < (C'_0 (I_0 \neq 0) / K_D) [(S_0 + K_D S_0 / (S_0 - C'_0 - K_D)) - C'_0 - K_D]$$

Now,  $S_0 < (C'_0 (I_0 \neq 0) / K_D) [(S_0 + K_D S_0 / (S_0 - C'_0 - K_D)) - C'_0 - K_D]$  can be simplified to,  
 $(C'_0 (I_0 \neq 0) / K_D) [(1 + K_D / (S_0 - C'_0 - K_D)) - C'_0 / S_0 - K_D / S_0] > 1$ . Using,  
 $C'_0 + K_D < S_0 \Rightarrow C'_0 / S_0 < 1 - K_D$ , we can further simplify the relation to,

$$(C'_0 (I_0 \neq 0) / K_D) [(1 + K_D / (S_0 - C'_0 - K_D)) - K_D / S_0 - 1 + K_D / S_0] > 1$$

$$\Rightarrow C'_0 (I_0 \neq 0) / (S_0 - C'_0 - K_D) > 1$$

$$\Rightarrow (S_0 - C'_0 - K_D) < C'_0 (I_0 \neq 0)$$

$$\Rightarrow S_0 < C'_0 (I_0 \neq 0) + C'_0 + K_D$$

Thus, the range of  $S_0$  is given by,  $C'_0 + K_D < S_0 < C'_0 (I_0 \neq 0) + C'_0 + K_D$  or  
 $k_p / k_{off} C_0 + K_D < S_0 < k_p / k_{off} C_0 (I_0 \neq 0) + k_p / k_{off} C_0 + K_D$ .

Therefore, in the SHP-1 limiting case, when Zap70 is in excess, inhibitory receptors can mediate activation instead of inhibition.

In the larger model, when inhibitory receptors mediate activation, the concentrations of SHP-1 molecules are much smaller than that of the stimulatory receptors, therefore, the results there would correspond to the  $b < 0$  case in the minimal model. Furthermore, SHP-1 molecules can bind to only the partially phosphorylated receptors in the minimal model, which will be more realistic when concentration of SHP-1 molecules is smaller than that of the Zap70 molecules. Thus the minimal model represents the full molecular model when  $b < 0$ .