SI Appendix

Notation for Species and Reactions

As there are too many to mention individually, we describe basic types of chemical species present in our models by breaking them down into *complexes* of *elementary species*, described below.

Chemical modifications to the "elementary" species described in SI Tables 1-11 are indicated in parentheses, e.g. Species(*Modification*), while binding of other molecular species is indicated by brackets, e.g. Species[*Substrate*]. To completely specify exactly what species is being referred to, both the modification state (if different modifications exist for the species type in question) and the substrate binding state (if any substrates can bind to the species type in question) must be indicated. For example, T(p_)[Z(*)] indicates the species of partially-phosphorylated TCRs bound to activated ZAP70, while T[Z] indicates TCR bound to ZAP70, but does not indicate the phosphorylation state of the TCR or the activation state of the ZAP70.

Beyond the types of elementary species described in SI Tables 1-11, the models we discuss include many species consisting of complexes incorporating TCR, pMHC, and various other species. Complexes generally consist of an ordered set of separate *positions*, each of which contains an representative of an elementary species; our notation for complexes consists of writing the descriptions of these elementary species sequentially in positional order (without spaces). For example, T(p_)[Z(_)]AntC(p__)[Shp(*)_] would indicate a (length 3) complex consisting of a partially-phosphorylated, unactivated ZAP70-bound TCR bound to an antagonist pMHC and further complexed with unactivated CD4/Lck phosphorylated at Y394 but not S59 and bound to activated SHP (but not Unc). The types of complexes can be broken down into five major classes as follows:

Length 1 Complexes = Elementary Species

One position, which may contain any type of elementary species described above.

Length 2 Complexes (TM)

Two positions: Position 1 must contain one of the elementary TCR species, while Position 2 must contain one of the above pMHC species.

Length 3 Complexes (TMC)

Three positions: Positions 1-2 as above, Position 3 must contain one of the elementary
CD4/Lck species.

Length 4 Complexes (TMCM)

Four positions: Positions 1-3 as above, Position 4 must contain one of the elementary
pMHC species.

Length 5 Complexes (TMCMT)

Five positions: Positions 1-4 as above, Position 5 must contain one of the elementary
TCR species.

For the models used to generate the results shown in SI Figs. 18-21, the CD4/Lck
elementary species types are replaced with separate CD4 and Lck elementary species
types, as described in SI Tables 1-11 (otherwise all elementary species unchanged). In
these models, position 3 of complexes of length 3 or greater may contain either a CD4
species, an Lck species, or both. If both CD4 and Lck are simultaneously present in
Position 3, the complex is written with position 3 as in the Lck species above, but with
the letter "L" replaced by "B."

Finally, we offer an important note regarding the interpretation of reaction data tables. If
a reaction does not specify modification (binding) state of elementary species in one of
complex positions, the reaction can occur involving complexes containing the elementary
species type in question with any modification (binding) state. Similarly, if "?" is
mentioned as the required modification (binding) state somewhere, the reaction in
question may occur involving any complex meeting all of the other listed criteria.
Finally, the appearance of pointy brackets <> around a length 5 complex indicates that
the reaction may in question can occur involving either complexes as written within the
pointy brackets or "reversed" complexes. By "reversed" complexes, we mean complexes
in which Position 1 matches the requirements listed for Position 5, Position 2 matches the
requirements listed for Position 4, and the requirements for Position 3 remain unchanged.

Further Results for Bare Model

The chemical reaction network making up the bare model is pictorially displayed
in Fig. 1 of the main text, while the exact details of the species and reaction parameters

for the bare model are described in SI Tables 12 and 13. TCR binds pMHC (SI Table 13, reactions 13.1 and 13.2), and TCR-pMHC complexes bind CD4/Lck (reactions 13.15 and 13.16), which may then phosphorylate the TCR (reactions 13.31 and 13.32), in a manner similar to that in Li, et. al. (16). ZAP70 binds to (fully) phosphorylated TCR (reaction 13.22), and ZAP70 in turn is activated by Lck bound to the same TCR (reaction 13.33), similar to Lee, et. al. (20), as discussed in the main text. SHP binds to Lck in TCR-pMHC-CD4/Lck complexes (reaction 13.26); Lck may then activate SHP (reaction 13.34), and active SHP may dephosphorylate the Y394 site on Lck (reaction 13.28), as well as deactivating ZAP70 (reactions 13.29 and 13.30) (12,20).

Starting from the idea that antagonist pMHC-TCR interactions are too short-lived to promote full ITAM phosphorylation of TCRs, one might postulate that the rate of TCR phosphorylation by Lck is lower than the antagonist pMHC-TCR dissociation rate. However, SI Fig. 5a demonstrates that if this TCR phosphorylation rate is indeed this slow, the population of endogenous pMHC is not able to synergistically increase ERK signaling resulting from agonist-nucleated complexes, even in the absence of antagonist pMHC. That this must be so can also be understood from consideration of the inequality $\tau_{p|\text{Lck present}} > \tau_{\text{ant}} \gg \tau_e$, which implies that endogenous pMHC-TCR interactions are too short-lived to stimulate TCR phosphorylation even if Lck is already present in complex containing endogenous pMHC.

Thus, in order for the bare model to allow for cooperativity of endogenous pMHC with agonist pMHC in stimulation of T-cell signaling, we must have $\tau_{\text{ant}} \gg \tau_e > \tau_{p|\text{Lck}}$ (or at least $\tau_e \sim \tau_{p|\text{Lck present}}$). SI Fig. 5$b$ demonstrates that the bare model is indeed capable of displaying synergistic ERK signaling with such a choice of parameters. However, as is discussed in the main text, this parameter regime leads to problems with incorporation of the phenomenon of antagonism into the bare model; Fig. 2 of the main text illustrates these problems.

SI Figs. 6-9 display the results of simulations varying the endogenous pMHC-TCR dissociation rate (reactions 13.3-13.6), the rate at which ERK is activated by ZAP70 (reaction 13.24), the on-rate for SHP binding to Lck (reaction 13.26), and the number of CD4/Lck complexes, respectively. Each of these figures displays both the full:partial TCR phosphorylation ratio (SI Figs. 6a-9a) and the amount of ERK activation (SI Figs.

6b-9b) observed as these parameters are varied. Note that the phenomenon of full:partial TCR phosphorylation ratio increasing with the addition of antagonist molecules is robust to the variation of these parameters in the bare model; however, we see that, for values of the endogenous pMHC-TCR dissociation rate ~30 s^{-1}, there is still a slight inhibition of ERK signaling resulting from the addition of 500 antagonist pMHC molecules.

SI Fig. 10 shows more detailed data regarding the response of the bare model with this value (30 s^{-1}) for the endogenous pMHC off-rate to varying numbers of antagonists. At this parameter value, the bare model does exhibit some (very slight) antagonism of ERK activation at moderate antagonist numbers (~50-500), but this is followed by highly *stimulatory* behavior for antagonist numbers above a certain threshold (here ~1,000) . That this slight antagonistic effect appears at moderate antagonist numbers, only to be reversed at larger antagonist numbers, can be attributed to the phenomenon of SHP more effectively suppressing endogenous-derived ERK activation than that stimulated by agonists. This is because ZAP70 molecules bound to TCRs which remain localized near Lck for a comparatively long time (i.e., TCRs bound to agonist or antagonist pMHC in TCR-pMHC-CD4/Lck complexes) may be reactivated by Lck very quickly upon being dephosphorylated by SHP. At moderate antagonist numbers, antagonist-derived ERK activation is offset by SHP suppression of endogenous-derived ERK activation, while at higher antagonist numbers, antagonist-derived ERK activation dominates. In light of this, it is not surprising that the curve representing the bare model with neither agonist nor endogenous pMHC present shows strictly increasing ERK activation with increasing antagonist number.

For the results discussed above, the dynamics of ERK activation are relatively simple (SI Fig. 11*a*), generally showing a fairly rapid relaxation to a pseudo-steady-state. To study phenomena at larger time scales than of interest here, additional features need to be incorporated, e.g., the reactivation of Lck, TCR downregulation, etc. However, if the number of ZAP70 molecules is sufficiently small, saturation effects can lead to different dynamical behavior, as demonstrated in SI Fig. 11*b*. In this case, the fact that there are many more TCR molecules than ZAP70 molecules can lead to a situation in which almost all of the ZAP70 molecules are bound to TCR (it should be emphasized here that

ZAP70 is only allowed to bind phosphorylated TCRs, though ZAP70 may bind to TCRs which are no longer associated with pMHC if these TCRs remain phosphorylated). Since TCR is present in large excess over ZAP70, most TCR will still not be bound to ZAP70, and since there is very little free ZAP70 remaining, these ZAP70-free TCR will not be able to recruit ZAP70 upon binding pMHC and CD4/Lck either. Thus, the ZAP70-free TCR can act as competitive inhibitors of ZAP70 phosphorylation by Lck. The odd dynamical behavior can be understood by noting that there will be a transient period before most ZAP70 is bound to TCR in which the large free ZAP70 population allows recruitment to signaling complexes, and hence leads to activation of ERK, followed by a period over which ERK activation is drastically reduced once the free ZAP70 population is depleted. It should be noted here in passing that this phenomenon of complex ERK dynamics for insufficiently large ZAP70 population occurs at a much different threshold for the bare model than it would for the unified model discussed below (for which it would require much lower ZAP70 populations to be observed) because of greater kinetic proof-reading in the latter model.

Further Results for Unified Model

Fig. 4 of the main text is a pictorial representation of the unified model, while the details of the species and reaction parameters used for simulation are described in SI Tables 14 and 15. Note that some of the phosphatase parameters differ from the bare model in the unified model; the reason for these changes is that the added proofreading the unified model reduces the total amount of TCR phosphorylation and ERK signaling which would occur at the same parameter values greatly (since increased proofreading means more failures to phosphorylate). Thus, to compare qualitatively similar regions, a shift in parameter space is required. If such a parameter shift were not made, the qualitative results of the unified model would be similar to those shown, though the degree to which, for instance, antagonists would suppress ERK activation, would be greatly reduced. Note in particular that the dynamics of ERK deactivation (SI Table 15, reaction 15.42 for unified model vs. SI Table 13, reactions 13.39-13.41 for bare model) are chosen to be of a simpler form, requiring fewer input parameters, in the unified model (simple 1st order decay of activated ERK to deactivated ERK, appropriate when

phosphatases for ERK are not saturated) than in the bare model. Saturation effects in ERK deactivation are not necessary to exhibit a large synergistic effect of the endogenous pMHC population with a small agonist pMHC population in the unified model, in contrast to the bare model, which exhibits cooperativity more readily when phosphatases are limiting. This is demonstrated by SI Fig. 12c, which demonstrates that the bare model, when modified to have the same parameters (including 1st-order ERK deactivation; see SI Table 17, reaction 17.39) as the unified model, fails to exhibit any synergistic response to mixed agonist and endogenous populations. In contrast, the unified model may exhibit cooperativity with either type of ERK deactivation kinetics (see SI Table 16, reactions 16.42-16.45 for parameters of unified model with explicit ERK phosphatases included), as shown in SI Fig. 12a,b.

SI Fig. 12b demonstrates that the unified model exhibits synergistic ERK activation by the endogenous pMHC population with small numbers of agonist pMHC. As discussed in the main text, Fig. 3 shows that the unified model does not exhibit the pathologies of the bare model with respect to the behavior of antagonist pMHC.

Again, parameter sensitivity has been considered for the endogenous pMHC-TCR dissociation rate (SI Table 15, reactions 15.3-15.6), the rate at which ERK is activated by ZAP70 (reaction 15.24), the on-rate for SHP binding to Lck (reaction 15.26), and the number of CD4/Lck complexes; the results of these simulations are presented in SI Figs. 13-16, respectively. As for the bare model, parts a of these figures show the full:partial TCR phosphorylation ratios, while parts b of these figures display the ERK activation rates. The phenomena of both full:partial TCR phosphorylation ratio and ERK activation decreasing with the addition of antagonists is robust in the unified model with variation of all of these parameters.

In SI Fig. 16, while the curves for both full:partial TCR phosphorylation ratio and ERK activation are both basically independent of variation of number CD4/Lck complexes from 2,000 to 50,000 when no antagonists are present, both curves decline with CD4/Lck number when antagonists are present. This simply indicates that, given the long lifetime of agonist-nucleated complexes, it takes relatively small numbers of CD4/Lck in order for the model to be in a regime in which agonist pMHC are essentially always complexed with CD4/Lck, while given the shorter lifetime of antagonist-

nucleated complexes, larger numbers of CD4/Lck are required before the effect of antagonists is manifested.

Removal of SHP Feedback from Models

SI Fig. 17 displays the results of simulations of the bare model (SI Fig. 17*a*) and unified model (SI Fig. 17*b*) when Lck feedback regulation is removed by performing simulations without any SHP molecules present. In both cases, it is observed that antagonists are unable to inhibit ERK activation at any concentration (and, in fact, result in increased ERK activation), though in the unified model, there is still a decrease in the full:partial TCR phosphorylation ratio observed as antagonist number increases.

Models in which Lck is Not Associated with CD4

SI Tables 18-21 describe modifications to the bare and unified models described above in which it is assumed that CD4 and Lck are not bound together. In these models, it is assumed that CD4 can still bridge together two TCR-pMHC complexes in a "pseudodimer" structure just as CD4/Lck complexes do in the models described above (this simply represents a mechanism for association of endogenous ligands to complexes nucleated by agonists). Lck may bind to pMHC-TCR complexes even if CD4 has not yet bound them, but cannot itself bridge together two different pMHC-TCR complexes. However, in this model, it is allowed for Lck and CD4 to both bind the same pMHC-TCR complex, and, in this case, it is still possible for the CD4 to bring in a second pMHC and TCR, in which case, the Lck may phosphorylate both TCR molecules involved in the resulting "pseudodimer" complex.

SI Figs. 18 and 19 displays the results of simulations varying the number of antagonist pMHC molecules in these models. The results are qualitatively similar in behavior for these versions of the bare and unified models as for the versions with CD4/Lck assumed to be always complexed discussed above.

To this point, all models have allowed the ERK and SHP feedback pathways to act only on Lck molecules which are bound to TCR. In SI Figs. 20 and 21, we consider the case in which uncomplexed Lck may also be modified by ERK and SHP; again, the

results are qualitatively unchanged, though the threshold for effective antagonist inhibition of agonist-signaling in the unified model is shifted somewhat.

Phenomenological Model Encapsulating the Key Molecular Events in the Unified Molecular Model

The structure of the phenomenological model is identical to Fig. 4. However, some results emerging from stochastic simulation of the model shown in Fig. 4 are put in as ansatz. These are: (i) TCR bound to both endogenous and agonist peptides in dimeric signaling complexes get fully phosphorylated, (ii) TCR bound to antagonists get partially phosphorylated upon recruitment of Lck, and (iii) Lck, upon activation by Erk, is protected from dephosphorylation by SHP.

We use Ordinary Differential Equations (ODEs) to describe the reactions shown in Fig. 4 in a layer of dimensions $25\mu m \times 25\mu m \times 0.01\mu m$. A typical reaction, such as, $TCR + p_{ag}MHC \xleftrightarrow{koff,kon} TCR - p_{ag}MHC$ is described by the following ODEs:

$$\frac{d[T]}{dt} = -k_{on}[T][M] + k_{off}[TM]$$

$$\frac{d[M]}{dt} = -k_{on}[T][M] + k_{off}[TM]$$

$$\frac{d[TM]}{dt} = k_{on}[T][M] - k_{off}[TM]$$

where [T], [M] and [TM] denote the concentrations of TCR, pMHC, and TCR-pMHC complexes respectively. The TCR, pMHC, and Lck molecules are confined to the top of the layer with uniform density. The ZAP70, Erk, and SHP-1 molecules are homogenously distributed throughout the volume.

In SI Tables 22 and 23 we show the values of the parameters used to study the model. The results are shown in SI Fig. 22.

Table 1. Elementary TCR species (T)
Two modification sites:
T(__) – unphosphorylated TCR
T(p_) or T(_p) – partially phosphorylated TCR
T(pp) – fully phosphorylated TCR
One ZAP70 binding site:
T[_] – TCR which has not bound ZAP70
T[Z] – TCR which has bound one of the ZAP70 species described below

Table 2. Elementary pMHC species (M)
Three types of MHC species:
Ag – agonist pMHC
Ant – antagonist pMHC
E – endogenous pMHC

Table 3. Elementary CD4/Lck species (C) (bare model)
Two modification sites:
C(_?) – Y394 not phosphorylated
C(p?) – Y394 phosphorylated
C(?_) – S59 not phosphorylated
C(?p) – S59 phosphorylated
One SHP binding site:
C[_] – CD4/Lck without Shp bound
C[Shp] – CD4/Lck with Shp bound

Table 4. Elementary CD4/Lck species (C) (unified model)
Three modification sites:
C(_??) – Y394 not phosphorylated
C(p??) – Y394 phosphorylated
C(?_?) – S59 not phosphorylated
C(?p?) – S59 phosphorylated
C(??_) – Lck not activated
C(??*) – Lck activated
One SHP binding site, one Unc binding site:
C[_?] – CD4/Lck without Shp bound
C[Shp?] – CD4/Lck with Shp bound
C[?_] – CD4/Lck without Unc bound
C[?U] – CD4/Lck with Unc bound

Table 5. Elementary SHP species (Shp)
One modification site:
Shp(_) – unactivated SHP
Shp(*) – activated SHP

Table 6. Elementary ZAP70 species (Z)
One modification site:
Z(_) – unactivated ZAP70
Z(*) – activated ZAP70

Table 7. Elementary ERK species (Erk)
One modification site:
Erk(_) – unactivated ERK
Erk(*) – activated ERK

Table 8. Elementary ERK phosphatase species (Pase) (bare model)
One ERK-binding site:
Pase[_] – Pase without bound ERK
Pase[Erk] – Pase with bound ERK

Table 9. Elementary Unc species (U) (unified model only)
U – only one type, no modifications

Table 10. Elementary CD4 species (C) for models w/separate CD4, Lck
C – CD4 (now with no associated Lck)

Table 11. Elementary Lck species (L) for models w/separate CD4, Lck
Three modification sites (only first two for bare models)*:*
L(_??) – Y394 not phosphorylated
L(p??) – Y394 phosphorylated
L(?_?) – S59 not phosphorylated
L(?p?) – S59 phosphorylated
L(??_) – Lck not activated
L(??*) – Lck activated
One SHP binding site, one Unc binding site (only SHP site for bare models)*:*
L[_?] – Lck without Shp bound
L[Shp?] – Lck with Shp bound
L[?_] – Lck without Unc bound
L[?U] – Lck with Unc bound

Table 12. Bare model initial conditions

Species	Initial Number
T(__)	100,000
E	5,000
Ag	100
C(p_)	10,000*
Shp(_)	10,000
Z(_)	100,000
Erk(_)	10,000
Pase	1,000
All others	0

Unless explicitly mentioned elsewhere, the initial numbers of the various species in bare model simulations are as described in this table. *Note that for the simulations with free CD4 and Lck described in section 5, the initial numbers of both the species C and the species L(p_) are 10,000.

Table 13. Bare model reaction parameters

Reaction	Rate Coefficient (s^{-1})
(TCR binds to and unbinds from pMHC)	
1. M+T → TM	2.5e-5
2. TMCM+T → TMCMT	2.5e-5
3. TE → T+E	100
4. TEC → T+E+C	100
5. TECM → T+E+C+M	100
6. <TMCET> → TMCE+T	100
7. TAnt → T+Ant	1
8. TAntC → T+Ant+C	1
9. TAntCM → T+Ant+C+M	1
10. <TMCAntT> → TMCAnt+T	1
11. TAg → T+Ag	0.019
12. TAgC → T+Ag+C	0.019
13. TAgCM → T+Ag+C+M	0.019
14. <TMCAgT> → TMCAg+T	0.019
(CD4/Lck binds to and unbinds from TCR-pMHC complex)	
15. TM+C → TMC	2.0e-5
16. TMC+TM → TMCMT	2.0e-5
17. TMC → TM+C	0.02
18. TMCM → TM+C+M	0.02
19. <TMCMT> → TMC+TM	0.02

(TCR-pMHC-CD4/Lck binds to and unbinds from second pMHC)
20. TMC+M → TMCM   2.0e-5
21. TMCM → TMC+M   0.02

(ZAP70 binds to and unbinds from fully-phosphorylated TCR)
22. T(pp)[_]...+Z → T(pp)[Z]...   0.0025
23. T[Z]... → T[_]...+Z   0.02
(Activated ZAP70 activates ERK; activated ERK phosphorylates Lck)
24. Erk(_)+Z(*)... → Erk(*)+Z(*)...   0.001
25. TMC(?_)...+Erk(*) → TMC(?p)...+Erk(*)   5.0e-4

(SHP binding to and unbinding from TCR-pMHC-CD4/Lck)
26. TMC[_]...+Shp → TMC[Shp]...   0.0025
27. C[Shp]... → C[_]...+Shp   1000
(SHP dephosphorylates Lck and ZAP70)
28. C(p_)[Shp(*)]... → C(__)[Shp(*)]...   25
29. Z(*)...+Shp(*) → Z(_)...+Shp(*)   2.0e-4
30. Z(*)...Shp(*)... → Z(_)...Shp(*)...   0.2

(Lck phosphorylates TCR, activates ZAP70 and SHP)
31. T(_?)MC(p?)... → T(p?)MC(p?)...   1000
32. T(p_)MC(p?)... → T(pp)MC(p?)...   1000
33. T[Z(_)]MC(p?)... → T[Z(*)]MC(p?)...   100
34. TMC(p?)[Shp(_)]... → TMC(p?)[Shp(*)]...   200

(Phosphatases dephosphorylate Lck, TCR, and deactivate SHP, ERK and ZAP70)
35. C(?p)... → C(?_)...   0.025
36. T(p?)... → T(_?)...   0.1
37. T(?p)... → T(?_)...   0.1
38. Shp(*)... → Shp(_)...   0.25
39. Erk(*)+Pase → Erk(*)Pase   0.01
40. Erk(*)Pase → Erk(_)Pase   100
41. ErkPase → Erk+Pase   10
42. Z(*)... → Z(_)...   0.05

Table 14. Unified model initial conditions

Species	Initial Number
T(__)	100,000
E	5,000
Ag	100
C(p__)	10,000*
Shp(_)	10,000
Z(_)	100,000
Erk(_)	10,000
U	10,000
All others	0

Unless explicitly mentioned elsewhere, the initial numbers of the various species in unified model simulations are as described in this table. *Note that for the simulations with free CD4 and Lck described in section 5, the initial numbers of both the species C and the species L(p__) are 10,000.

Table 15. Unified model reaction parameters

Reaction	Rate Coefficient (s^{-1})
(TCR binds to and unbinds from pMHC)	
1. M+T → TM	2.5e-5
2. TMCM+T → TMCMT	2.5e-5
3. TE → T+E	100
4. TEC → T+E+C	100
5. TECM → T+E+C+M	100
6. <TMCET> → TMCE+T	100
7. TAnt → T+Ant	1
8. TAntC → T+Ant+C	1
9. TAntCM → T+Ant+C+M	1
10. <TMCAntT> → TMCAnt+T	1
11. TAg → T+Ag	0.019
12. TAgC → T+Ag+C	0.019
13. TAgCM → T+Ag+C+M	0.019
14. <TMCAgT> → TMCAg+T	0.019
(CD4/Lck binds to and unbinds from TCR-pMHC complex)	
15. TM+C → TMC	2.0e-5
16. TMC+TM → TMCMT	2.0e-5
17. TMC → TM+C	0.02
18. TMCM → TM+C+M	0.02
19. <TMCMT> → TMC+TM	0.02

(TCR-pMHC-CD4/Lck binds to and unbinds from second pMHC)
20. TMC+M → TMCM   2.0e-5
21. TMCM → TMC+M   0.02

(ZAP70 binds to and unbinds from fully-phosphorylated TCR)
22. T(pp)[_]...+Z → T(pp)[Z]...   0.0025
23. T[Z]... → T[_]...+Z   0.02
(Activated ZAP70 activates ERK; activated ERK phosphorylates Lck)
24. Erk(_)+Z(*)... → Erk(*)+Z(*)...   0.001
25. TMC(?_?)...+Erk(*) → TMC(?p?)...+Erk(*)   5.0e-4

(SHP binding to and unbinding from TCR-pMHC-CD4/Lck)
26. TMC[_?]...+Shp → TMC[Shp?]...   0.0025
27. C[Shp?]... → C[_?]...+Shp   1000
(SHP dephosphorylates Lck and ZAP70)
28. C(p_?)[Shp(*)?]... → C(___)[Shp(*)?]...   25
29. Z(*)...+Shp(*) → Z(_)...+Shp(*)   2.0e-4
30. Z(*)...Shp(*)... → Z(_)...Shp(*)...   0.2

(Lck phosphorylates TCR, activates ZAP70 and SHP)
31. T(_?)MC(p?_)... → T(p?)MC(p?_)...   0.1
32. T(_?)MC(p?*)... → T(p?)MC(p?*)...   1000
33. T(p_)MC(p?_)... → T(pp)MC(p?_)...   0.1
34. T(p_)MC(p?*)... → T(pp)MC(p?*)...   1000
35. T[Z(_)]MC(p?_)... → T[Z(*)]MC(p?_)...   0.1
36. T[Z(_)]MC(p?*)... → T[Z(*)]MC(p?*)...   100
37. TMC(p??)[Shp(_)?]... → TMC(p??)[Shp(*)?]... 200

(Phosphatases dephosphorylate Lck, TCR, and deactivate SHP and ERK)
38. C(?p?)... → C(?_?)...   0.025
39. T(p?)... → T(_?)...   0.01
40. T(?p)... → T(?_)...   0.01
41. Shp(*)... → Shp(_)...   0.025
42. Erk(*) → Erk(_)   0.5

(Unc119 binds to and unbinds from Lck)
43. TMC[?_]...+U → TMC[?U]...   1.0e-5
44. TMC[?U]... → TMC[?_]...+U   0.02
45. C[?U] → C[?_]+U   100
(Unc 119 activates Lck)
46. TMC(p?_)[?U]... → TMC(p?*)[?U]...   0.1
(Lck deactivated immediately upon unbinding from TCR)
47. C(??*) → C(??_)   ∞

Table 16. Unified model w/explicit ERK phosphatase reaction parameters

Reaction	Rate Coefficient (s^{-1})
(TCR binds to and unbinds from pMHC)	
1. M+T → TM	2.5e-5
2. TMCM+T → TMCMT	2.5e-5
3. TE → T+E	100
4. TEC → T+E+C	100
5. TECM → T+E+C+M	100
6. <TMCET> → TMCE+T	100
7. TAnt → T+Ant	1
8. TAntC → T+Ant+C	1
9. TAntCM → T+Ant+C+M	1
10. <TMCAntT> → TMCAnt+T	1
11. TAg → T+Ag	0.019
12. TAgC → T+Ag+C	0.019
13. TAgCM → T+Ag+C+M	0.019
14. <TMCAgT> → TMCAg+T	0.019
(CD4/Lck binds to and unbinds from TCR-pMHC complex)	
15. TM+C → TMC	2.0e-5
16. TMC+TM → TMCMT	2.0e-5
17. TMC → TM+C	0.02
18. TMCM → TM+C+M	0.02
19. <TMCMT> → TMC+TM	0.02
(TCR-pMHC-CD4/Lck binds to and unbinds from second pMHC)	
20. TMC+M → TMCM	2.0e-5
21. TMCM → TMC+M	0.02
(ZAP70 binds to and unbinds from fully-phosphorylated TCR)	
22. T(pp)[_]...+Z → T(pp)[Z]...	0.0025
23. T[Z]... → T[_]...+Z	0.02
(Activated ZAP70 activates ERK; activated ERK phosphorylates Lck)	
24. Erk(_)+Z(*)... → Erk(*)+Z(*)...	0.001
25. TMC(?_?)...+Erk(*) → TMC(?p?)...+Erk(*)	5.0e-4
(SHP binding to and unbinding from TCR-pMHC-CD4/Lck)	
26. TMC[_?]...+Shp → TMC[Shp?]...	0.0025
27. C[Shp?]... → C[_?]...+Shp	1000
(SHP dephosphorylates Lck and ZAP70)	
28. C(p_?)[Shp(*)?]... → C(___)[Shp(*)?]...	25
29. Z(*)...+Shp(*) → Z(_)...+Shp(*)	2.0e-4
30. Z(*)...Shp(*)... → Z(_)...Shp(*)...	0.2

(Lck phosphorylates TCR, activates ZAP70 and SHP)
31. T(_?)MC(p?_)... → T(p?)MC(p?_)...   0.1
32. T(_?)MC(p?*)... → T(p?)MC(p?*)...   1000
33. T(p_)MC(p?_)... → T(pp)MC(p?_)...   0.1
34. T(p_)MC(p?*)... → T(pp)MC(p?*)...   1000
35. T[Z(_)]MC(p?_)... → T[Z(*)]MC(p?_)...   0.1
36. T[Z(_)]MC(p?*)... → T[Z(*)]MC(p?*)...   100
37. TMC(p??)[Shp(_)?]... → TMC(p??)[Shp(*)?]...   200

(Phosphatases dephosphorylate Lck, TCR, and deactivate SHP and ERK)
38. C(?p?)... → C(?_?)...   0.025
39. T(p?)... → T(_?)...   0.01
40. T(?p)... → T(?_)...   0.01
41. Shp(*)... → Shp(_)...   0.025
42. Erk(*) → Erk(_)   0.5
43. Erk(*)+Pase → Erk(*)Pase   0.0005
44. Erk(*)Pase → Erk(_)Pase   100
45. ErkPase → Erk+Pase   10

(Unc119 binds to and unbinds from Lck)
46. TMC[?_]...+U → TMC[?U]...   1.0e-5
47. TMC[?U]... → TMC[?_]...+U   0.02
48. C[?U] → C[?_]+U   100
(Unc 119 activates Lck)
49. TMC(p?_)[?U]... → TMC(p?*)[?U]... 0.1
(Lck deactivated immediately upon unbinding from TCR)
50. C(??*) → C(??_)   ∞

Table 17. Bare model w/1ˢᵗ-order ERK deactivation reaction parameters

Reaction	Rate Coefficient (s⁻¹)
(TCR binds to and unbinds from pMHC)	
1. M+T → TM	2.5e-5
2. TMCM+T → TMCMT	2.5e-5
3. TE → T+E	100
4. TEC → T+E+C	100
5. TECM → T+E+C+M	100
6. <TMCET> → TMCE+T	100
7. TAnt → T+Ant	1
8. TAntC → T+Ant+C	1

9. TAntCM → T+Ant+C+M   1
10. \<TMCAntT\> → TMCAnt+T   1
11. TAg → T+Ag   0.019
12. TAgC → T+Ag+C   0.019
13. TAgCM → T+Ag+C+M   0.019
14. \<TMCAgT\> → TMCAg+T   0.019

(CD4/Lck binds to and unbinds from TCR-pMHC complex)
15. TM+C → TMC   2.0e-5
16. TMC+TM → TMCMT   2.0e-5
17. TMC → TM+C   0.02
18. TMCM → TM+C+M   0.02
19. \<TMCMT\> → TMC+TM   0.02

(TCR-pMHC-CD4/Lck binds to and unbinds from second pMHC)
20. TMC+M → TMCM   2.0e-5
21. TMCM → TMC+M   0.02

(ZAP70 binds to and unbinds from fully-phosphorylated TCR)
22. T(pp)[_]...+Z → T(pp)[Z]...   0.0025
23. T[Z]... → T[_]...+Z   0.02
(Activated ZAP70 activates ERK; activated ERK phosphorylates Lck)
24. Erk(_)+Z(*)... → Erk(*)+Z(*)...   0.001
25. TMC(?_)...+Erk(*) → TMC(?p)...+Erk(*)   5.0e-4

(SHP binding to and unbinding from TCR-pMHC-CD4/Lck)
26. TMC[_]...+Shp → TMC[Shp]...   0.0025
27. C[Shp]... → C[_]...+Shp   1000
(SHP dephosphorylates Lck and ZAP70)
28. C(p_)[Shp(*)]... → C(__)[Shp(*)]...   25
29. Z(*)...+Shp(*) → Z(_)...+Shp(*)   2.0e-4
30. Z(*)...Shp(*)... → Z(_)...Shp(*)...   0.2

(Lck phosphorylates TCR, activates ZAP70 and SHP)
31. T(_?)MC(p?)... → T(p?)MC(p?)...   1000
32. T(p_)MC(p?)... → T(pp)MC(p?)...   1000
33. T[Z(_)]MC(p?)... → T[Z(*)]MC(p?)...   100
34. TMC(p?)[Shp(_)]... → TMC(p?)[Shp(*)]...   200

(Phosphatases dephosphorylate Lck, TCR, and deactivate SHP and ERK)
35. C(?p)... → C(?_)...   0.025
36. T(p?)... → T(_?)...   0.01
37. T(?p)... → T(?_)...   0.01
38. Shp(*)... → Shp(_)...   0.025
39. Erk(*) → Erk(_)   0.5

Table 18. Bare model reaction parameters (free CD4, Lck)

Reaction	Rate Coefficient (s^{-1})
(TCR binds to and unbinds from pMHC)	
1. M+T → TM	2.5e-5
2. TMCM+T → TMCMT	2.5e-5
3. TMBM+T → TMBMT	2.5e-5
4. TE → T+E	100
5. TEC → T+E+C	100
6. TEL → T+E+L	100
7. TEB → T+E+C+L	100
8. TECM → T+E+C+M	100
9. TEBM → T+E+C+L+M	100
10. <TMCET> → TMCE+T	100
11. <TMBET> → TMBE+T	100
12. TAnt → T+Ant	1
13. TAntC → T+Ant+C	1
14. TAntL → T+Ant+L	1
15. TAntB → T+Ant+C+L	1
16. TAntCM → T+Ant+C+M	1
17. TAntBM → T+Ant+C+L+M	1
18. <TMCAntT> → TMCAnt+T	1
19. <TMBAntT> → TMBAnt+T	1
20. TAg → T+Ag	0.019
21. TAgC → T+Ag+C	0.019
22. TAgL → T+Ag+L	0.019
23. TAgB → T+Ag+C+L	0.019
24. TAgCM → T+Ag+C+M	0.019
25. TAgBM → T+Ag+C+L+M	0.019
26. <TMCAgT> → TMCAg+T	0.019
27. <TMBAgT> → TMBAg+T	0.019
(CD4 binds to and unbinds from TCR-pMHC complex)	
28. TM+C → TMC	2.0e-5
29. TMC+TM → TMCMT	2.0e-5
30. TML+C → TMB	2.0e-5
31. TMB+TM → TMBMT	2.0e-5
32. TMC → TM+C	0.02
33. TMCM → TM+C+M	0.02
34. <TMCMT> → TMC+TM	0.02
35. TMB → TML+C	0.02
36. TMBM → TML+C+M	0.02
37. <TMBMT> → TMB+TM	0.02
(TCR-pMHC-CD4 binds to and unbinds from second pMHC)	
38. TMC+M → TMCM	2.0e-5

39. TMB+M → TMBM   2.0e-5
40. TMCM → TMC+M   0.02
41. TMBM → TMB+M   0.02

(Lck binds to and unbinds from TCR-pMHC complex)
42. TM+L → TML   2.0e-5
43. TMC... → TMB...   2.0e-5
44. TML → TM+L   0.02
45. TMB... → TMC...+L   0.02

 (ZAP70 binds to and unbinds from fully-phosphorylated TCR)
46. T(pp)[_]...+Z → T(pp)[Z]...   0.0025
47. T[Z]... → T[_]...+Z   0.02
(Activated ZAP70 activates ERK; activated ERK phosphorylates Lck)
48. Erk(_)+Z(*)... → Erk(*)+Z(*)...   0.001
49. TML(?_)+Erk(*) → TML(?p)+Erk(*)   5.0e-4
50. TMB(?_)...+Erk(*) → TMB(?p)...+Erk(*)   5.0e-4

(SHP binding to and unbinding from TCR-pMHC-Lck)
51. TML[_]+Shp → TML[Shp]   0.0025
52. TMB[_]...+Shp → TMB[Shp]...   0.0025
53. L[Shp]... → L[_]...+Shp   1000
54. TMB[Shp]... → TMB[_]...+Shp   1000
(SHP dephosphorylates Lck and ZAP70)
55. L(p_)[Shp(*)]... → L(__)[Shp(*)]...   25
56. TMB(p_)[Shp(*)]... → TMB(__)[Shp(*)]...   25
57. Z(*)...+Shp(*) → Z(_)...+Shp(*)   2.0e-4
58. Z(*)...Shp(*)... → Z(_)...Shp(*)...   0.2

(Lck phosphorylates TCR, activates ZAP70 and SHP)
59. T(_?)ML(p?) → T(p?)ML(p?)   1000
60. T(_?)MB(p?)... → T(p?)MB(p?)...   1000
61. T(p_)ML(p?) → T(pp)ML(p?)   1000
62. T(p_)MB(p?)... → T(pp)MB(p?)...   1000
63. T[Z(_)]ML(p?) → T[Z(*)]ML(p?)   100
64. T[Z(_)]MB(p?)... → T[Z(*)]MB(p?)...   100
65. TML(p?)[Shp(_)] → TML(p?)[Shp(*)]   200
66. TMB(p?)[Shp(_)]... → TMB(p?)[Shp(*)]...   200

(Phosphatases dephosphorylate Lck, TCR, and deactivate SHP, ERK and ZAP70)
67. L(?p)... → L(?_)...   0.025
68. TMB(?p)... → TMB(?_)...   0.025
69. T(p?)... → T(_?)...   0.1
70. T(?p)... → T(?_)...   0.1
71. Shp(*)... → Shp(_)...   0.25
72. Erk(*)+Pase → Erk(*)Pase   0.005

73. Erk(*)Pase → Erk(_)Pase   100
74. ErkPase → Erk+Pase   10
75. Z(*)... → Z(_)...   0.05

Table 19. Unified model reaction parameters (free CD4, Lck)

Reaction	Rate Coefficient (s^{-1})
(TCR binds to and unbinds from pMHC)	
1. M+T → TM	2.5e-5
2. TMCM+T → TMCMT	2.5e-5
3. TMBM+T → TMBMT	2.5e-5
4. TE → T+E	100
5. TEC → T+E+C	100
6. TEL → T+E+L	100
7. TEB → T+E+C+L	100
8. TECM → T+E+C+M	100
9. TEBM → T+E+C+L+M	100
10. <TMCET> → TMCE+T	100
11. <TMBET> → TMBE+T	100
12. TAnt → T+Ant	1
13. TAntC → T+Ant+C	1
14. TAntL → T+Ant+L	1
15. TAntB → T+Ant+C+L	1
16. TAntCM → T+Ant+C+M	1
17. TAntBM → T+Ant+C+L+M	1
18. <TMCAntT> → TMCAnt+T	1
19. <TMBAntT> → TMBAnt+T	1
20. TAg → T+Ag	0.019
21. TAgC → T+Ag+C	0.019
22. TAgL → T+Ag+L	0.019
23. TAgB → T+Ag+C+L	0.019
24. TAgCM → T+Ag+C+M	0.019
25. TAgBM → T+Ag+C+L+M	0.019
26. <TMCAgT> → TMCAg+T	0.019
27. <TMBAgT> → TMBAg+T	0.019
(CD4 binds to and unbinds from TCR-pMHC complex)	
28. TM+C → TMC	2.0e-5
29. TMC+TM → TMCMT	2.0e-5
30. TML+C → TMB	2.0e-5
31. TMB+TM → TMBMT	2.0e-5

32. TMC → TM+C	0.02
33. TMCM → TM+C+M	0.02
34. <TMCMT> → TMC+TM	0.02
35. TMB → TML+C	0.02
36. TMBM → TML+C+M	0.02
37. <TMBMT> → TMB+TM	0.02

(TCR-pMHC-CD4 binds to and unbinds from second pMHC)

38. TMC+M → TMCM	2.0e-5
39. TMB+M → TMBM	2.0e-5
40. TMCM → TMC+M	0.02
41. TMBM → TMB+M	0.02

(Lck binds to and unbinds from TCR-pMHC complex)

42. TM+L → TML	2.0e-5
43. TMC... → TMB...	2.0e-5
44. TML → TM+L	0.02
45. TMB... → TMC...+L	0.02

 (ZAP70 binds to and unbinds from fully-phosphorylated TCR)

46. T(pp)[_]...+Z → T(pp)[Z]...	0.0025
47. T[Z]... → T[_]...+Z	0.02

(Activated ZAP70 activates ERK; activated ERK phosphorylates Lck)

48. Erk(_)+Z(*)... → Erk(*)+Z(*)...	0.001
49. TML(?_?)+Erk(*) → TML(?p?)+Erk(*)	5.0e-4
50. TMB(?_?)...+Erk(*) → TMB(?p?)...+Erk(*)	5.0e-4

(SHP binding to and unbinding from TCR-pMHC-Lck)

51. TML[_?]+Shp → TML[Shp?]	0.0025
52. TMB[_?]...+Shp → TMB[Shp?]...	0.0025
53. L[Shp?]... → L[_?]...+Shp	1000
54. TMB[Shp?]... → TMB[_?]...+Shp	1000

(SHP dephosphorylates Lck and ZAP70)

55. L(p_?)[Shp(*)?]... → L(___)[Shp(*)?]...	25
56. TMB(p_?)[Shp(*)?]... → TMB(___)[Shp(*)?]...	25
57. Z(*)...+Shp(*) → Z(_)...+Shp(*)	2.0e-4
58. Z(*)...Shp(*)... → Z(_)...Shp(*)...	0.2

(Lck phosphorylates TCR, activates ZAP70 and SHP)

59. T(_?)ML(p?_) → T(p?)ML(p?_)	0.1
60. T(_?)MB(p?_)... → T(p?)MB(p?_)...	0.1
61. T(_?)ML(p?*) → T(p?)ML(p?*)	1000
62. T(_?)MB(p?*)... → T(p?)MB(p?*)...	1000
63. T(p_)ML(p?_) → T(pp)ML(p?_)	0.1
64. T(p_)MB(p?_)... → T(pp)MB(p?_)...	0.1
65. T(p_)ML(p?*) → T(pp)ML(p?*)	1000

66. T(p_)MB(p?*)... → T(pp)MB(p?*)...   1000
67. T[Z(_)]ML(p?_) → T[Z(*)]ML(p?_)   0.1
68. T[Z(_)]MB(p?_)... → T[Z(*)]MB(p?_)...   0.1
69. T[Z(_)]ML(p?*) → T[Z(*)]ML(p?*)   100
70. T[Z(_)]MB(p?*)... → T[Z(*)]MB(p?*)...   100
71. TML(p??)[Shp(_)?] → TML(p??)[Shp(*)?]   200
72. TMB(p??)[Shp(_)?]... → TMB(p??)[Shp(*)?]...   200

(Phosphatases dephosphorylate Lck, TCR, and deactivate SHP and ERK)
73. L(?p?)... → L(?_?)...   0.025
74. TMB(?p?)... → TMB(?_?)...   0.025
75. T(p?)... → T(_?)...   0.01
76. T(?p)... → T(?_)...   0.01
77. Shp(*)... → Shp(_)...   0.025
78. Erk(*) → Erk(_)   0.5

(Unc119 binds to and unbinds from Lck)
79. TML[?_]+U → TML[?U]   1.0e-5
80. TMB[?_]...+U → TMB[?U]...   1.0e-5
81. TML[?U] → TML[?_]+U   0.02
82. TMB[?U]... → TMB[?_]...+U   0.02
83. L[?U] → L[?_]+U   100
(Unc 119 activates Lck)
84. TML(p?_)[?U] → TML(p?*)[?U]   0.1
85. TMB(p?_)[?U]... → TMB(p?*)[?U]...   0.1
(Lck is deactivated immediately upon unbinding from TCR)
86. L(??*) → L(??_)   ∞

Table 20. Bare model reaction parameters (free CD4, Lck; ERK/SHP act on free Lck)

Reaction	Rate Coefficient (s^{-1})
(TCR binds to and unbinds from pMHC)	
1. M+T → TM	2.5e-5
2. TMCM+T → TMCMT	2.5e-5
3. TMBM+T → TMBMT	2.5e-5
4. TE → T+E	100
5. TEC → T+E+C	100
6. TEL → T+E+L	100
7. TEB → T+E+C+L	100
8. TECM → T+E+C+M	100
9. TEBM → T+E+C+L+M	100

10. <TMCET> → TMCE+T   100
11. <TMBET> → TMBE+T   100
12. TAnt → T+Ant   1
13. TAntC → T+Ant+C   1
14. TAntL → T+Ant+L   1
15. TAntB → T+Ant+C+L   1
16. TAntCM → T+Ant+C+M   1
17. TAntBM → T+Ant+C+L+M   1
18. <TMCAntT> → TMCAnt+T   1
19. <TMBAntT> → TMBAnt+T   1
20. TAg → T+Ag   0.019
21. TAgC → T+Ag+C   0.019
22. TAgL → T+Ag+L   0.019
23. TAgB → T+Ag+C+L   0.019
24. TAgCM → T+Ag+C+M   0.019
25. TAgBM → T+Ag+C+L+M   0.019
26. <TMCAgT> → TMCAg+T   0.019
27. <TMBAgT> → TMBAg+T   0.019

(CD4 binds to and unbinds from TCR-pMHC complex)
28. TM+C → TMC   2.0e-5
29. TMC+TM → TMCMT   2.0e-5
30. TML+C → TMB   2.0e-5
31. TMB+TM → TMBMT   2.0e-5
32. TMC → TM+C   0.02
33. TMCM → TM+C+M   0.02
34. <TMCMT> → TMC+TM   0.02
35. TMB → TML+C   0.02
36. TMBM → TML+C+M   0.02
37. <TMBMT> → TMB+TM   0.02

(TCR-pMHC-CD4 binds to and unbinds from second pMHC)
38. TMC+M → TMCM   2.0e-5
39. TMB+M → TMBM   2.0e-5
40. TMCM → TMC+M   0.02
41. TMBM → TMB+M   0.02

(Lck binds to and unbinds from TCR-pMHC complex)
42. TM+L → TML   2.0e-5
43. TMC... → TMB...   2.0e-5
44. TML → TM+L   0.02
45. TMB... → TMC...+L   0.02

(ZAP70 binds to and unbinds from fully-phosphorylated TCR)
46. T(pp)[_]...+Z → T(pp)[Z]...   0.0025
47. T[Z]... → T[_]...+Z   0.02

(Activated ZAP70 activates ERK; activated ERK phosphorylates Lck)
48. Erk(_)+Z(*)... → Erk(*)+Z(*)...   0.001
49. L(?_)...+Erk(*) → L(?p)...+Erk(*)   5.0e-4
50. TMB(?_)...+Erk(*) → TMB(?p)...+Erk(*)   5.0e-4

(SHP binding to and unbinding from TCR-pMHC-Lck)
51. TML[_]+Shp → TML[Shp]   0.0025
52. TMB[_]...+Shp → TMB[Shp]...   0.0025
53. L[Shp]... → L[_]...+Shp   1000
54. TMB[Shp]... → TMB[_]...+Shp   1000
(SHP dephosphorylates Lck and ZAP70)
55. L(p_)[Shp(*)]... → L(__)[Shp(*)]...   25
56. TMB(p_)[Shp(*)]... → TMB(__)[Shp(*)]...   25
57. L(p_)+Shp(*) → L(__)+Shp(*)   6.25e-5
58. Z(*)...+Shp(*) → Z(_)...+Shp(*)   2.0e-4
59. Z(*)...Shp(*)... → Z(_)...Shp(*)...   0.2

(Lck phosphorylates TCR, activates ZAP70 and SHP)
60. T(_?)ML(p?) → T(p?)ML(p?)   1000
61. T(_?)MB(p?)... → T(p?)MB(p?)...   1000
62. T(p_)ML(p?) → T(pp)ML(p?)   1000
63. T(p_)MB(p?)... → T(pp)MB(p?)...   1000
64. T[Z(_)]ML(p?) → T[Z(*)]ML(p?)   100
65. T[Z(_)]MB(p?)... → T[Z(*)]MB(p?)...   100
66. TML(p?)[Shp(_)] → TML(p?)[Shp(*)]   200
67. TMB(p?)[Shp(_)]... → TMB(p?)[Shp(*)]...   200

(Phosphatases dephosphorylate Lck, TCR, and deactivate SHP, ERK and ZAP70)
68. L(?p)... → L(?_)...   0.025
69. TMB(?p)... → TMB(?_)...   0.025
70. T(p?)... → T(_?)...   0.1
71. T(?p)... → T(?_)...   0.1
72. Shp(*)... → Shp(_)...   0.25
73. Erk(*)+Pase → Erk(*)Pase   0.005
74. Erk(*)Pase → Erk(_)Pase   100
75. ErkPase → Erk+Pase   10
76. Z(*)... → Z(_)...   0.05

Table 21. Unified model reaction parameters (free CD4, Lck; ERK/SHP act on free Lck)

Reaction	Rate Coefficient (s^{-1})
(TCR binds to and unbinds from pMHC)	
1. M+T $\rightarrow$ TM	2.5e-5
2. TMCM+T $\rightarrow$ TMCMT	2.5e-5
3. TMBM+T $\rightarrow$ TMBMT	2.5e-5
4. TE $\rightarrow$ T+E	100
5. TEC $\rightarrow$ T+E+C	100
6. TEL $\rightarrow$ T+E+L	100
7. TEB $\rightarrow$ T+E+C+L	100
8. TECM $\rightarrow$ T+E+C+M	100
9. TEBM $\rightarrow$ T+E+C+L+M	100
10. <TMCET> $\rightarrow$ TMCE+T	100
11. <TMBET> $\rightarrow$ TMBE+T	100
12. TAnt $\rightarrow$ T+Ant	1
13. TAntC $\rightarrow$ T+Ant+C	1
14. TAntL $\rightarrow$ T+Ant+L	1
15. TAntB $\rightarrow$ T+Ant+C+L	1
16. TAntCM $\rightarrow$ T+Ant+C+M	1
17. TAntBM $\rightarrow$ T+Ant+C+L+M	1
18. <TMCAntT> $\rightarrow$ TMCAnt+T	1
19. <TMBAntT> $\rightarrow$ TMBAnt+T	1
20. TAg $\rightarrow$ T+Ag	0.019
21. TAgC $\rightarrow$ T+Ag+C	0.019
22. TAgL $\rightarrow$ T+Ag+L	0.019
23. TAgB $\rightarrow$ T+Ag+C+L	0.019
24. TAgCM $\rightarrow$ T+Ag+C+M	0.019
25. TAgBM $\rightarrow$ T+Ag+C+L+M	0.019
26. <TMCAgT> $\rightarrow$ TMCAg+T	0.019
27. <TMBAgT> $\rightarrow$ TMBAg+T	0.019
(CD4 binds to and unbinds from TCR-pMHC complex)	
28. TM+C $\rightarrow$ TMC	2.0e-5
29. TMC+TM $\rightarrow$ TMCMT	2.0e-5
30. TML+C $\rightarrow$ TMB	2.0e-5
31. TMB+TM $\rightarrow$ TMBMT	2.0e-5
32. TMC $\rightarrow$ TM+C	0.02
33. TMCM $\rightarrow$ TM+C+M	0.02
34. <TMCMT> $\rightarrow$ TMC+TM	0.02
35. TMB $\rightarrow$ TML+C	0.02
36. TMBM $\rightarrow$ TML+C+M	0.02
37. <TMBMT> $\rightarrow$ TMB+TM	0.02

(TCR-pMHC-CD4 binds to and unbinds from second pMHC)

38. TMC+M → TMCM   2.0e-5
39. TMB+M → TMBM   2.0e-5
40. TMCM → TMC+M   0.02
41. TMBM → TMB+M   0.02

(Lck binds to and unbinds from TCR-pMHC complex)
42. TM+L → TML   2.0e-5
43. TMC... → TMB...   2.0e-5
44. TML → TM+L   0.02
45. TMB... → TMC...+L   0.02

 (ZAP70 binds to and unbinds from fully-phosphorylated TCR)
46. T(pp)[_]...+Z → T(pp)[Z]...   0.0025
47. T[Z]... → T[_]...+Z   0.02
(Activated ZAP70 activates ERK; activated ERK phosphorylates Lck)
48. Erk(_)+Z(*)... → Erk(*)+Z(*)...   0.001
49. L(?_?)...+Erk(*) → L(?p?)...+Erk(*)   5.0e-4
50. TMB(?_?)...+Erk(*) → TMB(?p?)...+Erk(*)   5.0e-4

(SHP binding to and unbinding from TCR-pMHC-Lck)
51. TML[_?]+Shp → TML[Shp?]   0.0025
52. TMB[_?]...+Shp → TMB[Shp?]...   0.0025
53. L[Shp?]... → L[_?]...+Shp   1000
54. TMB[Shp?]... → TMB[_?]...+Shp   1000
(SHP dephosphorylates Lck and ZAP70)
55. L(p_?)[Shp(*)?]... → L(___)[Shp(*)?]...   25
56. TMB(p_?)[Shp(*)?]... → TMB(___)[Shp(*)?]...   25
57. L(p__)+Shp(*) → L(___)+Shp(*)   6.25e-5
58. Z(*)...+Shp(*) → Z(_)...+Shp(*)   2.0e-4
59. Z(*)...Shp(*)... → Z(_)...Shp(*)...   0.2

(Lck phosphorylates TCR, activates ZAP70 and SHP)
60. T(_?)ML(p?_) → T(p?)ML(p?_)   0.1
61. T(_?)MB(p?_)... → T(p?)MB(p?_)...   0.1
62. T(_?)ML(p?*) → T(p?)ML(p?*)   1000
63. T(_?)MB(p?*)... → T(p?)MB(p?*)...   1000
64. T(p_)ML(p?_) → T(pp)ML(p?_)   0.1
65. T(p_)MB(p?_)... → T(pp)MB(p?_)...   0.1
66. T(p_)ML(p?*) → T(pp)ML(p?*)   1000
67. T(p_)MB(p?*)... → T(pp)MB(p?*)...   1000
68. T[Z(_)]ML(p?_) → T[Z(*)]ML(p?_)   0.1
69. T[Z(_)]MB(p?_)... → T[Z(*)]MB(p?_)...   0.1
70. T[Z(_)]ML(p?*) → T[Z(*)]ML(p?*)   100
71. T[Z(_)]MB(p?*)... → T[Z(*)]MB(p?*)...   100
72. TML(p??)[Shp(_)?] → TML(p??)[Shp(*)?]   200
73. TMB(p??)[Shp(_)?]... → TMB(p??)[Shp(*)?]...   200

(Phosphatases dephosphorylate Lck, TCR, and deactivate SHP and ERK)

74. L(?p?)... → L(?_?)...   0.025
75. TMB(?p?)... → TMB(?_?)...   0.025
76. T(p?)... → T(_?)...   0.01
77. T(?p)... → T(?_)...   0.01
78. Shp(*)... → Shp(_)...   0.025
79. Erk(*) → Erk(_)   0.5

(Unc119 binds to and unbinds from Lck)

80. TML[?_]+U → TML[?U]   1.0e-5
81. TMB[?_]...+U → TMB[?U]...   1.0e-5
82. TML[?U] → TML[?_]+U   0.02
83. TMB[?U]... → TMB[?_]...+U   0.02
84. L[?U] → L[?_]+U   100

(Unc 119 activates Lck)

85. TML(p?_)[?U] → TML(p?*)[?U]   0.1
86. TMB(p?_)[?U]... → TMB(p?*)[?U]...   0.1

(Lck is deactivated immediately upon unbinding from TCR)

87. L(??*) → L(??_)   ∞

Table 22. Concentration values used in phenomenological model

Species	Concentration
TCR	200 molecules/$(\mu m)^2$
Agonist pMHC	10 molecules/$(\mu m)^2$
Antagonist pMHC	Varied from 1.0e-8 to 1000 molecules/$(\mu m)^2$
Endogenous pMHC	Varied from 1.0e-8 to 1.0e+4 molecules/$(\mu m)^2$
Lck	10 molecules/$(\mu m)^2$
SHP	2.0e+4 molecules/$(\mu m)^3$
ZAP70	2.0e+4 molecules/$(\mu m)^3$
ERK	2.0e+4 molecules/$(\mu m)^3$

Table 23. Rate constants used in phenomenological model

Reaction	**Rate constant**
Agonist pMHC-TCR binding	0.003 $(\mu m)^2$ molecule^{-1} s^{-1}
Agonist pMHC-TCR unbinding	0.01 s^{-1}
Endogenous pMHC-TCR binding	$3.0e\text{-}4$ $(\mu m)^2$ molecule^{-1} s^{-1}
Endoegnous pMHC-TCR unbinding	20 s^{-1}
Antagonist pMHC-TCR binding	0.001 $(\mu m)^2$ molecule^{-1} s^{-1}
Antagonist pMHC-TCR unbinding	0.1 s^{-1}
Lck binding to pMHC-TCR	0.01 $(\mu m)^2$ molecule^{-1} s^{-1}
Lck unbinding from pMHC-TCR	1.0 s^{-1}
Full phosphorylation of TCR in TCR-p_{ag}MHC complex	20 s^{-1}
Partial phosphorylation of TCR in TCR-p_{ant}MHC complex	0.1 s^{-1}
TCR dephosphorylation	0.1 s^{-1}
ZAP70 binding to phosphorylated TCR	0.02 $(\mu m)^2$ molecule^{-1} s^{-1}
ZAP70 unbinding from TCR	0.1 s^{-1}
Phosphorylation of ZAP70 in TCR[ZAP]-pMHC-Lck complex	1.1 s^{-1}
ZAP70 dephosphorylation	0.5 s^{-1}
ERK binding to Lck in TCR-pMHC-Lck complex	0.02 $(\mu m)^2$ molecule^{-1} s^{-1}
ERK unbinding from Lck	0.1 s^{-1}
Activation of ERK bound to Lck	2.0 s^{-1}
ERK deactivation	0.01 s^{-1}
SHP binding to Lck in TCR-pMHC-Lck complex	0.02 $(\mu m)^2$ molecule^{-1} s^{-1}
SHP unbinding from Lck	0.1 s^{-1}
Activation of SHP bound to Lck	2.0 s^{-1}
SHP deactivation	0.01 s^{-1}
Lck protection by activated ERK	0.02 $(\mu m)^2$ molecule^{-1} s^{-1}
Lck deactivation by activated SHP	0.02 $(\mu m)^2$ molecule^{-1} s^{-1}
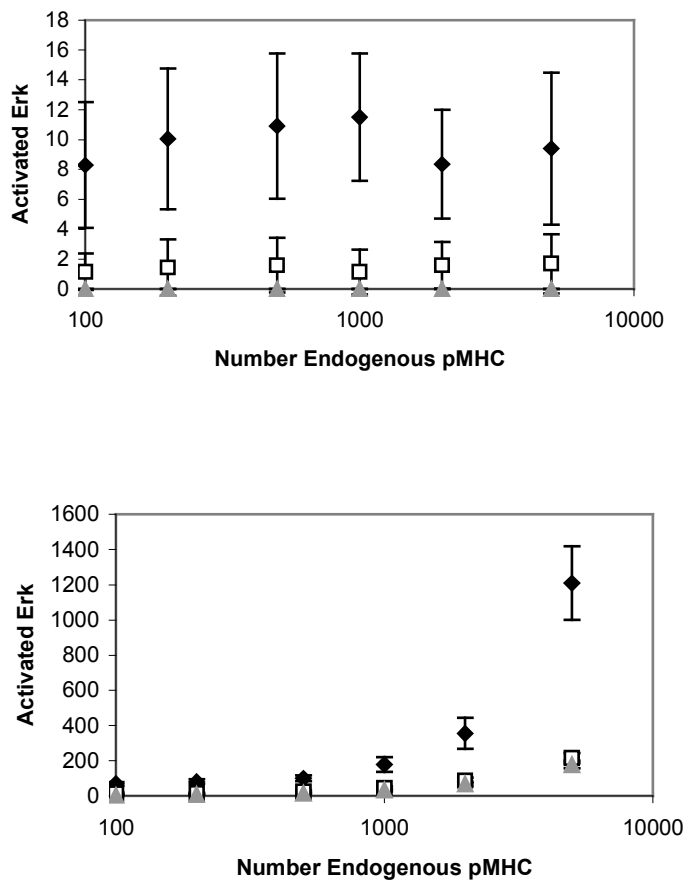
Fig. 5. Solid black diamonds indicate 100 agonist, open squares 10 agonist, and gray triangles 0 agonist pMHC. (*a*) Bare model with TCR phosphorylation rate set to 0.1 s^{-1}, slower than the dissociation rate for antagonist pMHC-TCR (1 s^{-1}), does not exhibit synergistic ERK activation increase from increasing endogenous pMHC population when small numbers of agonist pMHC are present. (*b*) Bare model with TCR phosphorylation rate set to 1000 s^{-1}, faster than dissociation rate for *endogenous* pMHC-TCR (100 s^{-1}), *does* exhibit amplification of agonist-induced ERK signaling resulting from increasing endogenous pMHC population. In particular, the amount of ERK activation resulting from inclusion of 100 agonist and 5000 endogenous pMHC is significantly greater than the sum of the ERK activation resulting from 100 agonist and few endogenous pMHC and the ERK activation resulting from 0 agonist and 5000 endogenous pMHC.
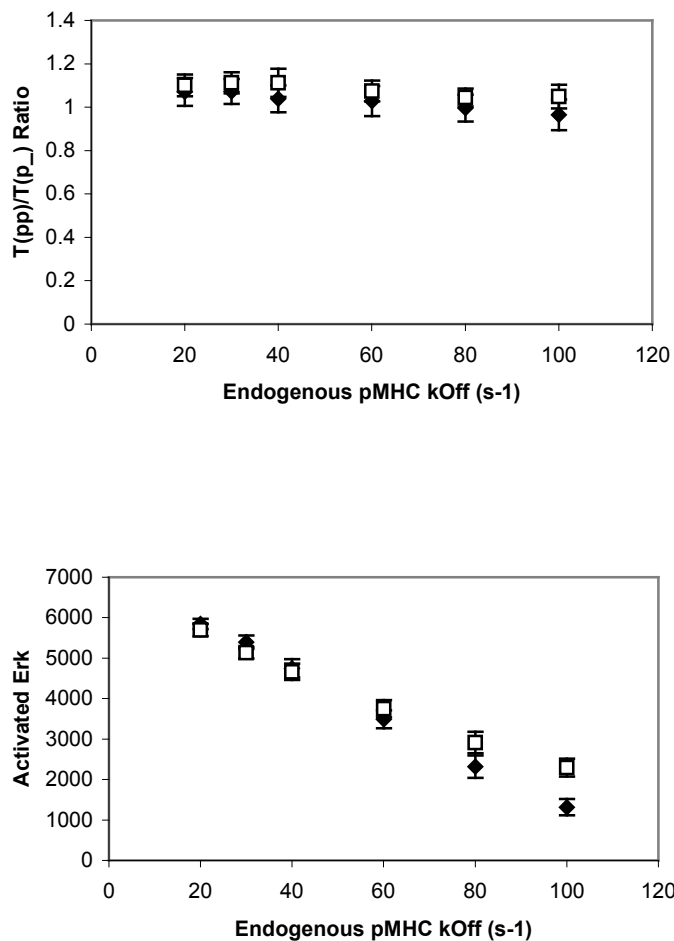
Fig. 6. Solid black diamonds indicate 0 antagonist, open squares 500 antagonist pMHC. (**a**) Slight increase in ratio of fully- to partially-phosphorylated TCRs resulting from inclusion of 500 antagonist pMHC in the bare model is robust to decrease in endogenous pMHC-TCR dissociation rate. (**b**) Comparison of simulations with 0 vs. 500 antagonist pMHC as endogenous pMHC-TCR dissociation rate is reduced. Note that for endogenous dissociation rates in the range 20-40 s⁻¹, there is a very slight decrease in ERK activation in the simulations with 500 antagonist pMHC compared with those with no antagonists (but see also SI Fig. 10).
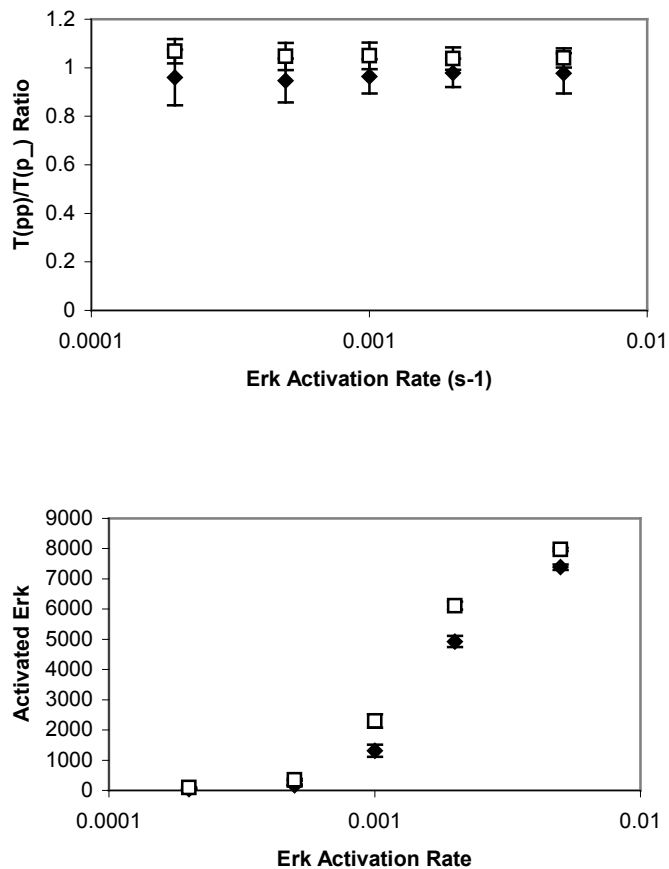
Fig. 7. Solid black diamonds indicate 0 antagonist, open squares 500 antagonist pMHC. (*a*) Variation of ERK activation rate in bare model has small effect on resulting ratio of fully- to partially-phosphorylated TCRs. (*b*) ERK activation increases sharply with increase in ERK activation rate. Simulations with 500 antagonists lead to more ERK activation than those with 0 antagonists at all values of ERK activation rate.
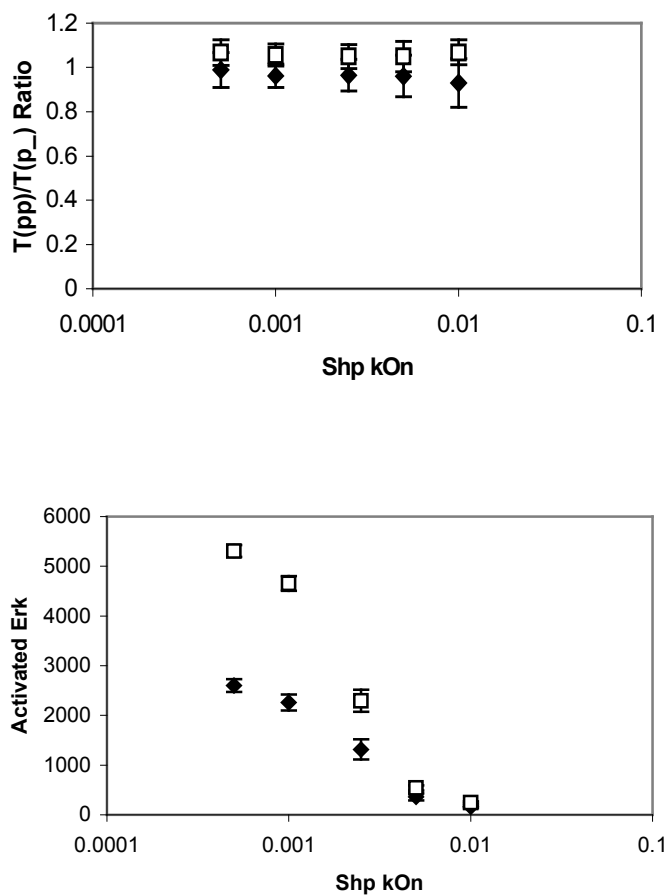
Fig. 8. Solid black diamonds indicate 0 antagonist, open squares 500 antagonist pMHC. (***a***) Variation of rate coefficient for SHP binding to Lck in bare model has small effect on resulting ratio of fully- to partially-phosphorylated TCRs. (***b***) ERK activation decreases sharply with increase in rate coefficient for SHP binding to Lck. Simulations with 500 antagonists lead to more ERK activation than those with 0 antagonists at all values of SHP-Lck binding rate coefficient.
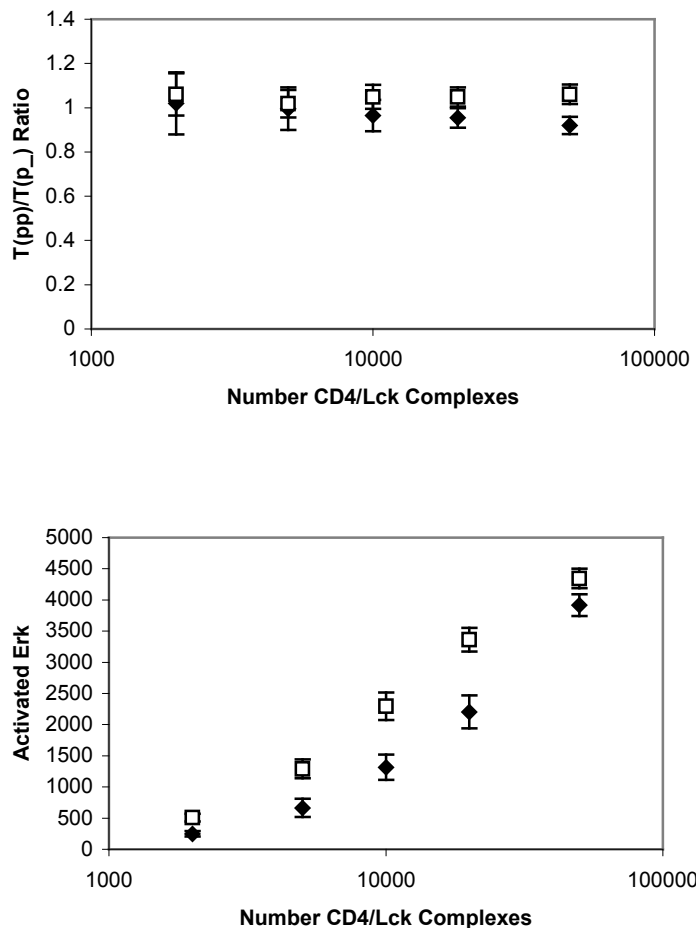
Fig. 9. Solid black diamonds indicate 0 antagonist, open squares 500 antagonist pMHC. (***a***) Variation of number of CD4/Lck complexes in bare model has small effect on resulting ratio of fully- to partially-phosphorylated TCRs. (***b***) ERK activation increases sharply with increase in CD4/Lck number in bare model. Simulations with 500 antagonists lead to more ERK activation than those with 0 antagonists at all values of CD4/Lck number.
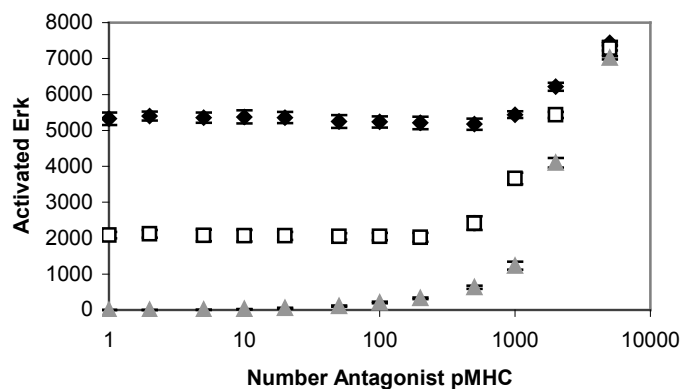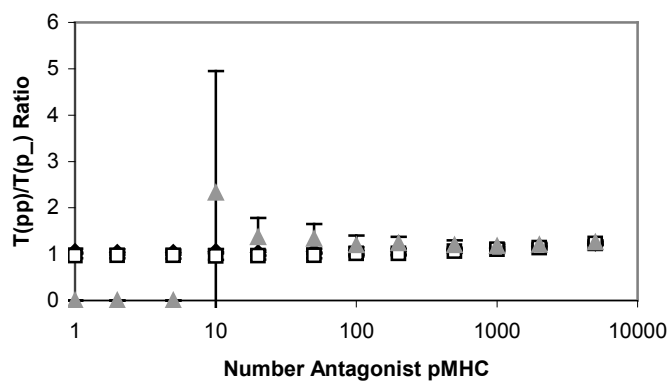
Fig. 10. Solid black diamonds indicate 100 agonist pMHC, open squares 0 agonist pMHC, and gray triangles 0 agonist and 0 endogenous pMHC. (***a***) In the bare model with endogenous pMHC-TCR dissociation rate taken to be 30 s^{-1}, ratio of fully- to partially-phosphorylated TCRs increases slightly with increasing numbers of antagonist pMHC present. (***b***) While intermediate numbers of antagonists (~500) very slightly suppress ERK activation (relative to case with 0 antagonists) in the bare model with endogenous pMHC-TCR dissociation rate 30 s^{-1}, further increase in antagonist number leads to marked increase in ERK activation.
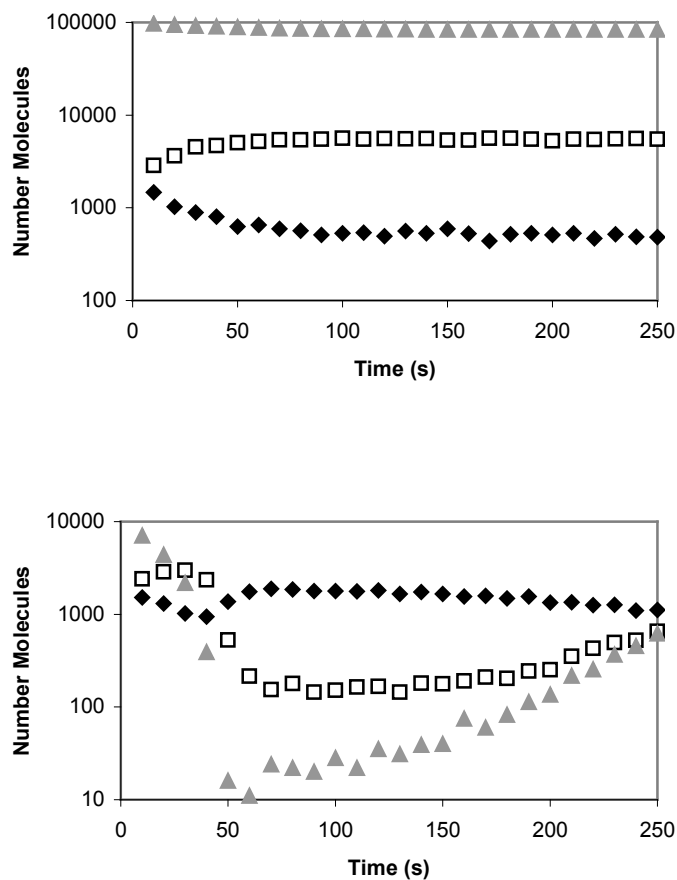
Fig. 11. Solid black diamonds indicate SHP activation, open squares ERK activation, and gray triangles unbound ZAP70. (***a***) The bare model (with 0 antagonist pMHC) with excess ZAP70 (here, 100,000 molecules) rapidly approaches a pseudo-steady state with regard to ERK and SHP activation (representative single trajectory shown). (***b***) When available ZAP70 is reduced sufficiently (here, to 10,000 molecules), ERK and SHP dynamics becomes more complicated. At short times (t < 50 s), trajectory resembles that of excess ZAP70 plot above, but as number of unbound ZAP70 falls to very low levels, ERK activation also declines rapidly.
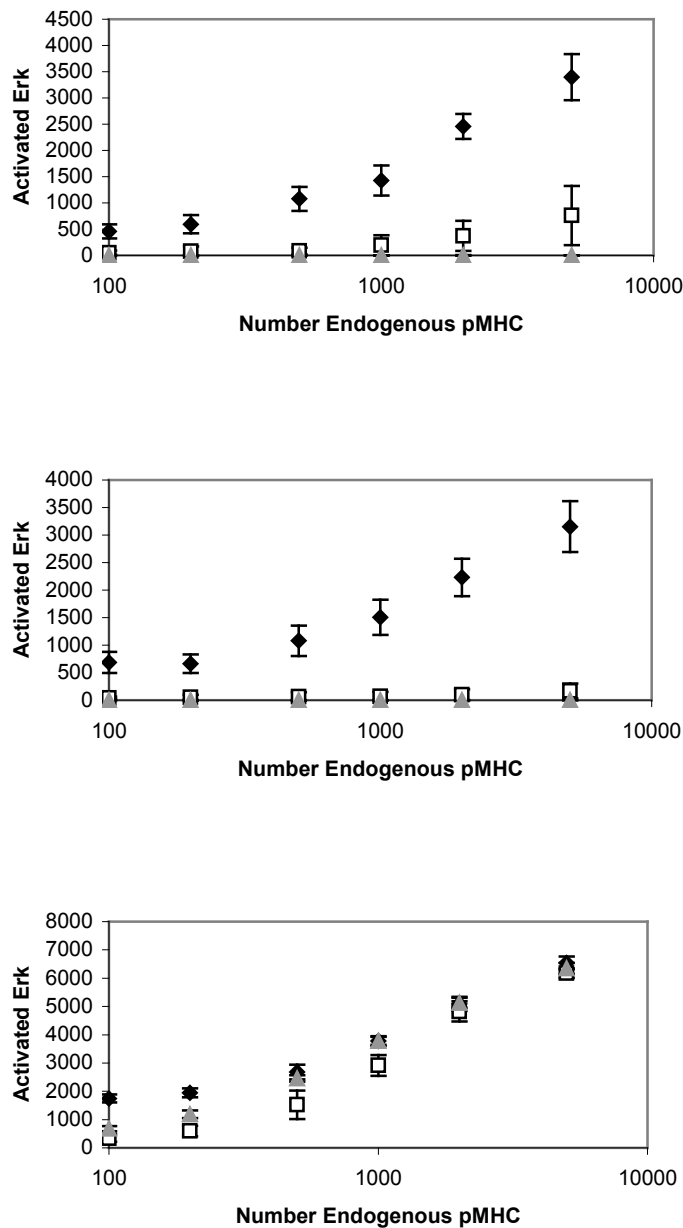
Fig. 12. Solid black diamonds indicate 100 agonist, open squares 10 agonist, and gray triangles 0 agonist pMHC. (*a*) Unified model exhibits synergistic ERK activation increase resulting from increasing endogenous pMHC population when small numbers of agonist pMHC are present. In particular, ERK activation in simulations with both 100 agonist pMHC and 5000 endogenous pMHC is significantly greater than sum of ERK activation resulting from 100 agonist and few endogenous pMHC and ERK activation resulting from 0 agonist and 5000 endogenous pMHC. (*b*) Unified model modified to include ERK phosphatase saturation (as described in SI Table 16) also exhibits endogenous pMHC-driven amplification of agonist-induced ERK signaling. (*c*) In contrast, the bare model, modified by removal of ERK phosphatase saturation (as described in SI Table 17), does not exhibit this synergistic signal amplification.
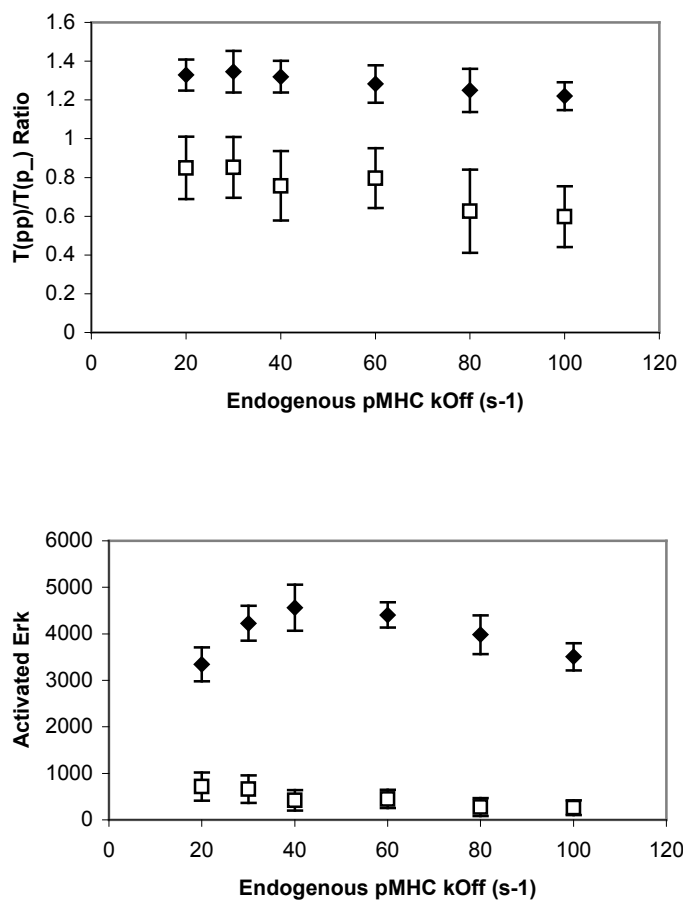
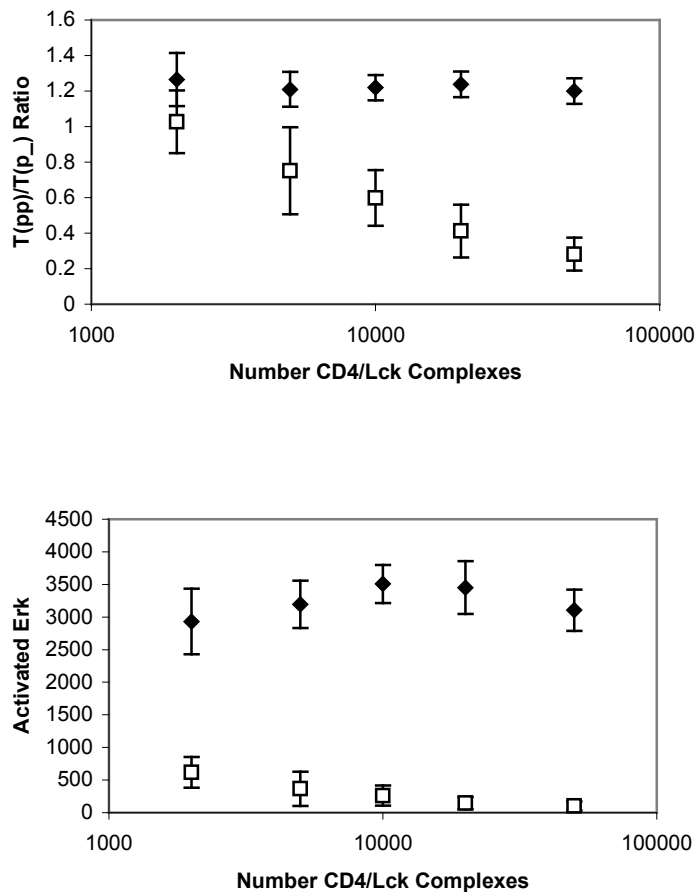**Fig. 13.** Solid black diamonds indicate 0 antagonist, open squares 500 antagonist pMHC. (**a**) Decrease in ratio of fully- to partially-phosphorylated TCRs resulting from inclusion of 500 antagonist pMHC in the unified model is robust to decrease in endogenous pMHC-TCR dissociation rate. (**b**) Comparison of simulations with 0 vs. 500 antagonist pMHC as endogenous pMHC-TCR dissociation rate is reduced. Phenomenon of antagonist suppression of ERK activation is qualitatively robust. Note appearance of maximum in ERK signaling (for 0 antagonist simulations) when endogenous pMHC-TCR dissociation rate is set to intermediate value ($\sim$40 s^{-1}).
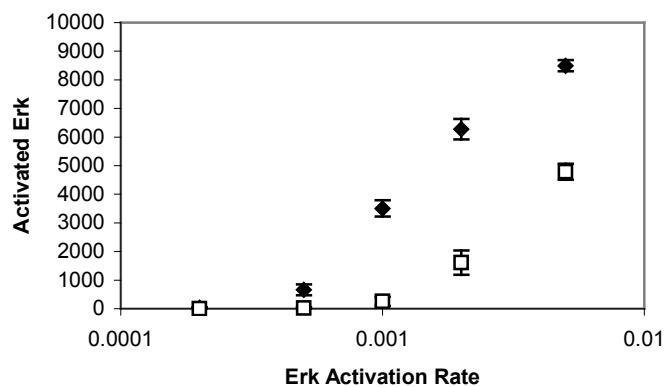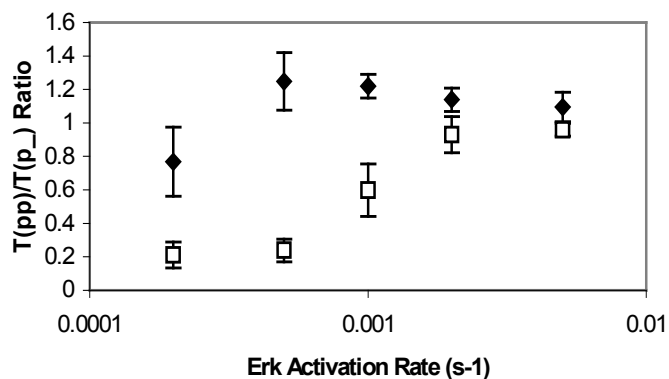
Fig. 14. Solid black diamonds indicate 0 antagonist, open squares 500 antagonist pMHC. (**a**) Effect on ratio of fully- to partially-phosphorylated TCRs of variation of ERK activation rate in unified model. Note that difference in this ratio between simulations with 0 and 500 antagonist pMHC is largest for intermediate values of ERK activation rate. (**b**) ERK activation increases sharply with increase in ERK activation rate. Simulations with 0 antagonists lead to more ERK activation than those with 500 antagonists at all values of ERK activation rate.
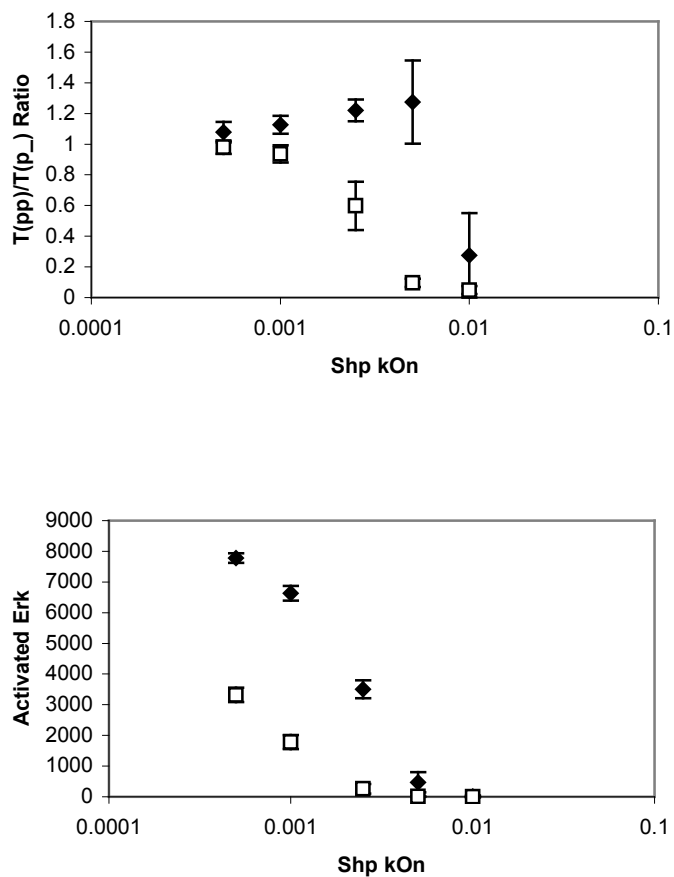
Fig. 15. Solid black diamonds indicate 0 antagonist, open squares 500 antagonist pMHC. (*a*) Effect on ratio of fully- to partially-phosphorylated TCRs of variation in the rate coefficient for SHP binding to Lck in unified model. Note that difference in this ratio between simulations with 0 and 500 antagonist pMHC is largest for intermediate values of SHP-Lck binding rate coefficient. (*b*) ERK activation decreases sharply with increase in SHP-Lck binding rate coefficient. Simulations with 0 antagonists lead to ore ERK activation than those with 500 antagonists at all values of SHP-Lck binding rate coefficient.

Fig. 16. Solid black diamonds indicate 0 antagonist, open squares 500 antagonist pMHC. (***a***) Ratio of fully- to partially- phosphorylated TCRs in unified model with 0 antagonists is robust to variation of number of CD4/Lck complexes, but this ratio declines in simulations with 500 antagonists as CD4/Lck number increases. (***b***) Similarly, ERK activation is only weakly affected by change in CD4/Lck number for 0 antagonist simulations, while for 500 antagonist simulations, ERK activation decreases several-fold as CD4/Lck number increases from 2,000 to 50,000.
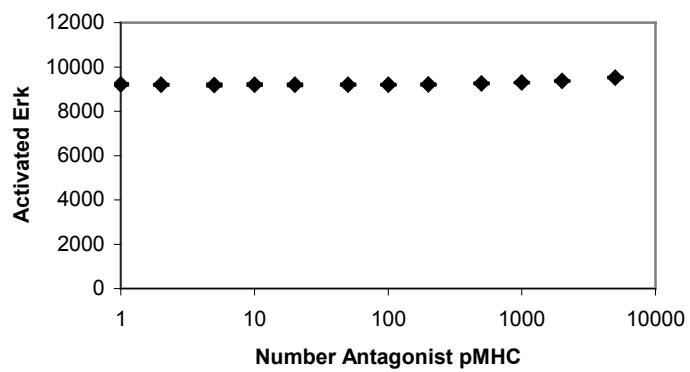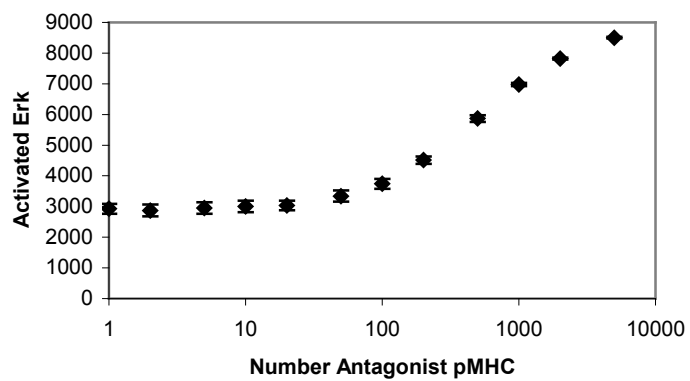
Fig. 17. (*a*) Bare model and (*b*) unified model both show rise in ERK activation with increasing numbers of antagonist pMHC when SHP is excluded from simulations.
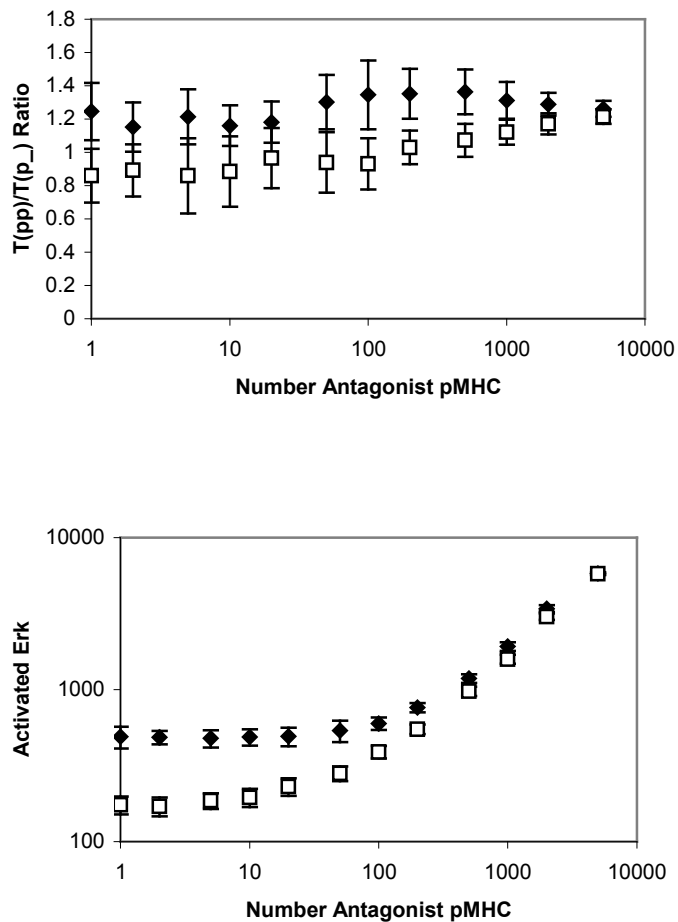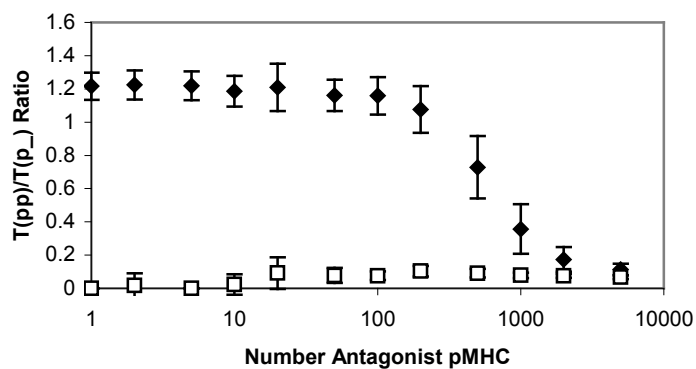
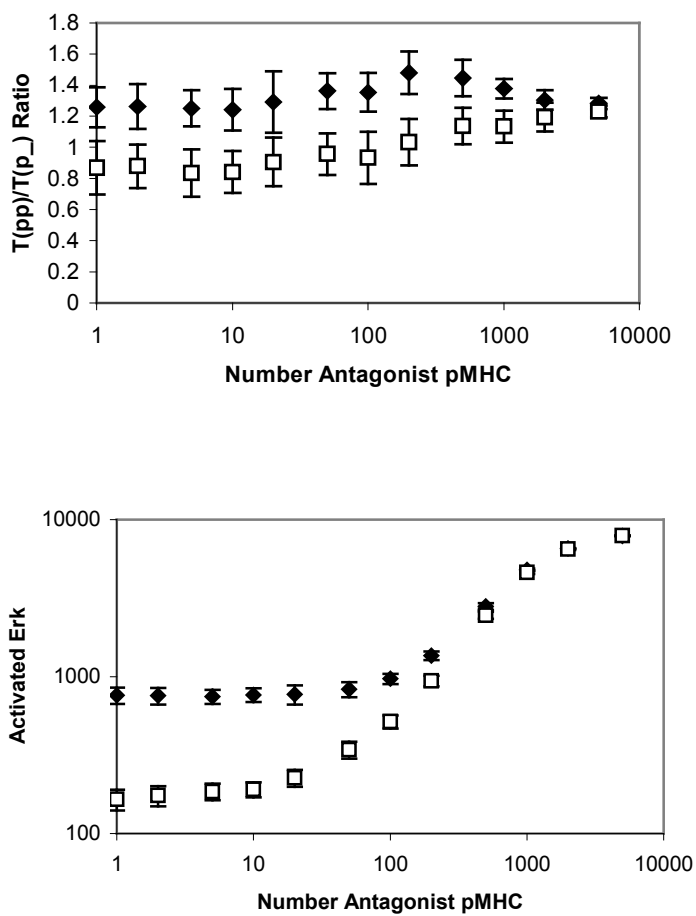**Fig. 18.** Solid black diamonds indicate 100 agonist, open squares 0 agonist pMHC. (***a***) Bare model in which CD4 and Lck are not complexed (described in SI Table 18) shows small increase in ratio of fully- to partially-phosphorylated TCRs resulting from increase of antagonist pMHC number. (***b***) Similarly, in this model, ERK activation increases with number of antagonists.
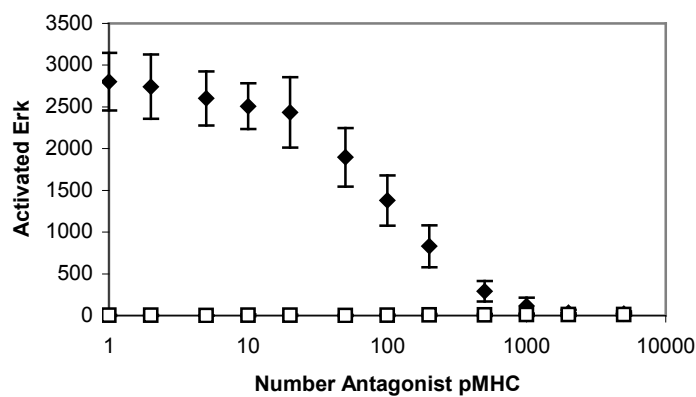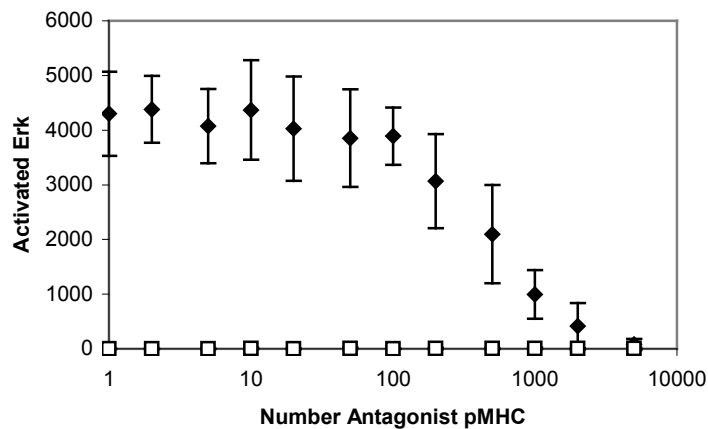
Fig. 19. Solid black diamonds indicate 100 agonist, open squares 0 agonist pMHC. (***a***) Unified model in which CD4 and Lck are not complexed (described in SI Table 19) shows decrease in ratio of fully- to partially-phosphorylated TCRs resulting from increase of antagonist pMHC number. (***b***) This version of unified model also exhibits antagonist suppression of ERK activation.

Fig. 20. Solid black diamonds indicate 100 agonist, open squares 0 agonist pMHC. (***a***) Bare model in which CD4 and Lck are not complexed, and in which SHP and ERK act on free Lck, (described in SI Table 20) shows small increase in ratio of fully- to partially-phosphorylated TCRs resulting from increase of antagonist pMHC number. (***b***) Similarly, in this model, ERK activation increases with number of antagonists.
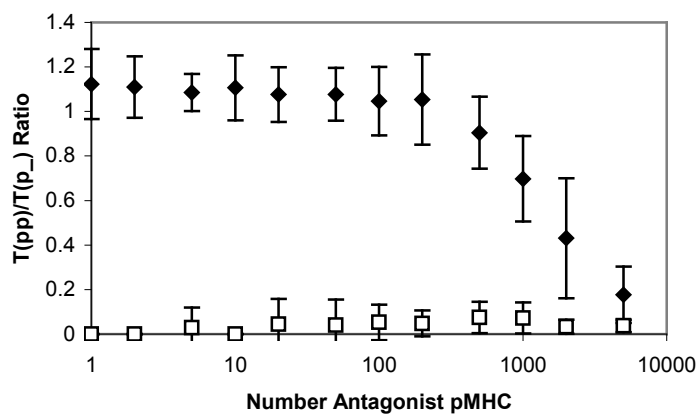
Fig. 21. Solid black diamonds indicate 100 agonist, open squares 0 agonist pMHC. (*a*) Unified model in which CD4 and Lck are not complexed, and in which SHP and ERK act on free Lck, (described in SI Table 21) shows decrease in ratio of fully- to partially-phosphorylated TCRs resulting from increase of antagonist pMHC number. (*b*) This version of unified model also exhibits antagonist suppression of ERK activation.
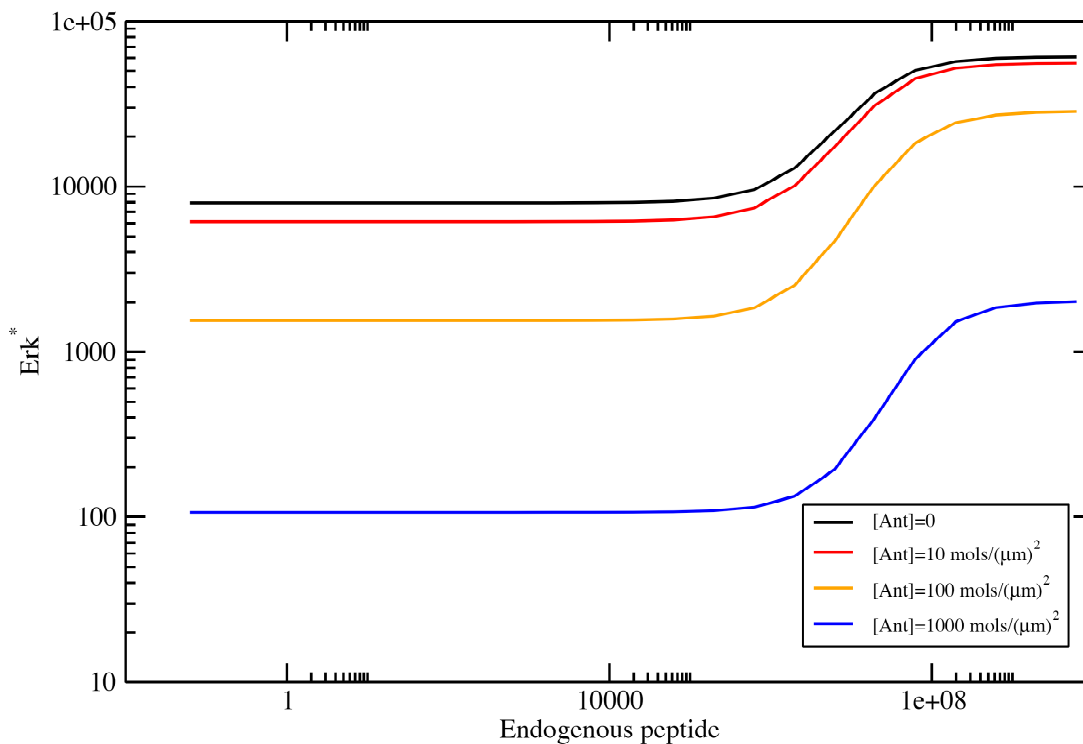
Fig. 22. Variation of Erk activation with endogenous pMHC number for different antagonist pMHC densities. The agonist density for the above plot is set at 10 molecules/$(\mu m)^2$. The values of the other parameters are shown in SI Tables 22 and 23. At very low and very large copy numbers of endogenous peptides, the change in activated ERK upon changing endogenous peptide number is very small. However, for an intermediate range of endogenous peptide copy number, which increases as the density of antagonists increases, the activated ERK level changes sharply as endogenous peptide density is varied. Also, note that the sensitivity of the saturation level of activated ERK to changes in antagonist density is much weaker at large endogenous peptide number than that at small copy number of endogenous peptides.