

## SUPPLEMENTAL MATERIAL

Hoerter et al., <http://www.jem.org/cgi/content/full/jem.20122528/DC1>**Table S1.** Information on the peptides tested in RMA-S cell assays

Sequence	Len	Organism	Protein	Position	ID used in manuscript
YSLPNAGDVI	10	Vaccinia	K3L (VACWR034) [A034]	6	
YAPVSPIVI	9	Vaccinia	A42R (VACWR167) [A167]	88	
YSQVNKRYI	9	Vaccinia	B2R (VACWR184) [A184]	54	
SMYCSKTFI	9	Vaccinia	D1R (VACWR106) [A106]	578	
NLIRNRDYI	9	Vaccinia	A51R (VACWR177) [A177]	310	
CTLLNAGAL	9	Vaccinia	K1L (VACWR032) [A032]	47	
IGMFNLTFI	9	Vaccinia	A23R (VACWR143) [A143]	297	
STILNSIDI	9	Vaccinia	A23R (VACWR143) [A143]	360	
YMLENIQVM	9	Vaccinia	G6R (VACWR084) [A084]	77	
VVVNWIQI	9	Vaccinia	–/– (VACWR003/VACWR216) [A003/A216]		
RMNSNQVCI	9	Vaccinia	E9L (VACWR065) [A065]	858	
YSPSNHHIL	9	Vaccinia	A3L (VACWR122) [A122]	191	
FAIKNTDDV	9	Vaccinia	E11L (VACWR067) [A067]	17	
SQTPNYDRL	9	Vaccinia	B12R (VACWR194) [A194]	268	
VAVCNLASV	9	Vaccinia	I4L (VACWR073) [A073]	441	
TMMINPFMI	9	Vaccinia	A47L (VACWR173) [A173]	93	
SAPMNVNDL	9	Vaccinia	F5L (VACWR044) [A044]	279	
FSNINDFNI	9	Vaccinia	– (VACWR016) [A016]	17	
IMGSNNISI	9	Vaccinia	A10L (VACWR129) [A129]	540	
YAVINRNVL	9	Vaccinia	J4R (VACWR096) [A096]	24	
AAYVNDLLL	9	Vaccinia	A ORF M (Moss226) [B057]	55	
ISVANKIYM	9	Vaccinia	B16R (VACWR197) [A197]	275	
FLMMNKDEL	9	Vaccinia	N2L (VACWR029) [A029]	60	
FVLLNRRWI	9	Vaccinia	A20R (VACWR141) [A141]	129	
FTIQNNTKL	9	Vaccinia	F13L (VACWR052) [A052]	307	
ASPYNFFKRI	10	Vaccinia	C2L (VACWR026) [A026]	22	
YAHINALEYI	10	Vaccinia	A47L (VACWR173) [A173]	171	
SAPMNVNDLI	10	Vaccinia	F5L (VACWR044) [A044]	279	
FSYNFTSGI	10	Vaccinia	A21L (VACWR140) [A140]	51	
LSISNDLNSI	10	Vaccinia	F14L (VACWR053) [A053]	10	
YSLPNAGDVI	10	Vaccinia	K3L (VACWR034) [A034]	6	
NSNINIIHI	9	Vaccinia	H1L (VACWR099) [A099]	67	
STITNEFCV	9	Vaccinia	B13R (VACWR195) [A195]	309	
HAITMYDKI	9	Vaccinia	E6R (VACWR062) [A062]	251	
YAPVSPIVI	9	Vaccinia	A42R (VACWR167) [A167]	88	
FAISYCRAFI	10	Vaccinia	F12L (VACWR051) [A051]	587	
KAVYNFATM	9	LCMV	GP	33	
WMHHNMDLI	9	Mouse	UTY	246	UTY
ASNENMDAM	9	Influenza	NP	366	NP68
ASNENMETM	9	Influenza	NP	366	NP34
ASNENIDTM	9	Influenza	NP	366	NP3711
SSLENFRAYV	10	Influenza	PA	224	PA224
SIINFEKL	9	Chicken	OVA	257	OVA
SIIRFEKL	9	Chicken	OVA APL	257	R4
SSYSYSSL	8	NA	Engineered null peptide		Poly-serine
RGVYVQGL	9	VSV	NP	52	VSV
VNTIFTVV	8	Mouse	Glu3	314	Glu3
ATLVFHNL	8	Mouse	Stat3	53	Statp
AAYSFYNV	8	Mouse	Nmt1	419	Mytr

**Code, parameters, and chemical species described in the kinetic model****SSC CODE FOR COMPUTER SIMULATIONS OF THE KINETIC MODEL****-REACTIONS: Formation of complexes****-MHC/TCR bond**

```
rxn m:MHC(t#, c#, p="ag") t:TCR(m#, c#) at konAg -> m.t # t.m
rxn m:MHC(t#, c#1, p="ag") CD8(m#1, t#) t:TCR(m#, c#) at konAg -> m.t # t.m
rxn m:MHC(t#, c#, p="ag") t:TCR(m#, c#1) CD8(t#1, m#) at konAg -> m.t # t.m
rxn m:MHC(c#2, t#, p="ag") t:TCR(m#, c#1) CD8(t#1, m#2) at konAg -> m.t # t.m
```

```
rxn m:MHC(t#, c#, p="en") t:TCR(m#, c#) at konEn -> m.t # t.m
rxn m:MHC(t#, c#1, p="en") CD8(m#1, t#) t:TCR(m#, c#) at konEn -> m.t # t.m
rxn m:MHC(t#, c#, p="en") t:TCR(m#, c#1) CD8(t#1, m#) at konEn -> m.t # t.m
rxn m:MHC(c#2, t#, p="en") t:TCR(m#, c#1) CD8(t#1, m#2) at konEn -> m.t # t.m
```

```
rxn TCR(m#1) MHC(t#1, p="ag") at koffAg -> break 1
rxn TCR(m#1) MHC(t#1, p="en") at koffEn -> break 1
```

**-TCR/CD8 bond**

```
rxn t:TCR(c#, m#) c:CD8(t#, m#) at konLck1 -> t.c # c.t
rxn t:TCR(c#, m#1) MHC(t#1, c#) c:CD8(t#, m#) at konLck1 -> t.c # c.t
rxn t:TCR(c#, m#) c:CD8(t#, m#1) MHC(c#1, t#) at konLck1 -> t.c # c.t
rxn t:TCR(m#2, c#) c:CD8(t#, m#1) MHC(c#1, t#2) at konLck2 -> t.c # c.t
```

```
rxn TCR(c#1, m#2) MHC(t#2) CD8(t#1) at koffLck -> break 1
rxn TCR(c#1, m#) CD8(t#1) at koffLck2 -> break 1
```

**-MHC/CD8 bond**

```
rxn m:MHC(c#, t#) c:CD8(m#, t#) at konCD -> m.c # c.m
rxn m:MHC(c#, t#1) TCR(m#1, c#) c:CD8(m#, t#) at konCD -> m.c # c.m
rxn m:MHC(c#, t#) c:CD8(m#, t#1) TCR(c#1, m#) at konCD -> m.c # c.m
rxn m:MHC(t#2, c#) c:CD8(m#, t#1) TCR(c#1, m#2) at konCD -> m.c # c.m
```

```
rxn MHC(c#1, p="en") CD8(m#1) at koffCD_en -> break 1
rxn MHC(c#1, p="ag") CD8(m#1) at koffCD_ag -> break 1
```

**-REACTIONS: Chemical modifications****-Phosphorylation/dephosphorylation**

```
rxn t:TCR(p="p0", c#1, m#2) MHC(t#2) CD8(lck="basal", t#1) at kp1 -> t.p = "p1"
rxn t:TCR(p="p1", c#1, m#2) MHC(t#2) CD8(lck="basal", t#1) at kp2 -> t.p = "p2"

rxn t:TCR(p="p0", c#1, m#2) MHC(t#2, c#) CD8(lck="active", t#1, m#) at kpact1 -> t.p = "p1"
rxn t:TCR(p="p0", c#, m#2) MHC(t#2, c#1) CD8(lck="active", t#, m#1) at kpact1 -> t.p = "p1"
rxn t:TCR(p="p0", c#1, m#2) MHC(t#2, c#3) CD8(lck="active", t#1, m#3) at kpact1 -> t.p = "p1"

rxn t:TCR(p="p1", c#1, m#2) MHC(t#2, c#) CD8(lck="active", t#1, m#) at kpact2 -> t.p = "p2"
rxn t:TCR(p="p1", c#, m#2) MHC(t#2, c#1) CD8(lck="active", t#, m#1) at kpact2 -> t.p = "p2"
rxn t:TCR(p="p1", c#1, m#2) MHC(t#2, c#3) CD8(lck="active", t#1, m#3) at kpact2 -> t.p = "p2"

rxn t:TCR(p="p0", m#1, c#) MHC(t#1) CD8(lck="active") at kpact1cross -> t.p = "p1"
rxn t:TCR(p="p1", m#1, c#) MHC(t#1) CD8(lck="active") at kpact2cross -> t.p = "p2"

rxn t:TCR(p="p1", m#, c#) at kdp1 -> t.p = "p0"
rxn t:TCR(p="p2", m#, c#) at kdp2 -> t.p = "p1"

rxn t:TCR(p="p1", m#, c#1) CD8(t#1, m#) at kdp1 -> t.p = "p0"
rxn t:TCR(p="p2", m#, c#1) CD8(t#1, m#) at kdp2 -> t.p = "p1"
```

-Lck activation/deactivation

rxn c:CD8(lck="basal", t#1, m#) TCR(c#1, p="p2", m#2) MHC(t#2, c#) at kact -> c.lck = "active"

rxn c:CD8(lck="basal", t#, m#1) TCR(p="p2", c#, m#2) MHC(c#1, t#2) at kact -> c.lck = "active"

rxn c:CD8(lck="basal", t#1, m#3) TCR(c#1, p="p2", m#2) MHC(t#2, c#3) at kact -> c.lck = "active"

rxn c:CD8(lck="active", t#, m#) at kdeact -> c.lck = "basal"

## -DEFINITION OF MEMBRANE LATTICE

region World

box width 100 height 100 depth 1

subvolume edge 1

## -DEFINITION OF INTIAL CONDITIONS AND DIFFUSION RATES

new MHC(p="ag") at count\_ag

new MHC(p="en") at count\_en

new TCR(p="p0") at 300

new CD8(lck="basal") at 100

diffusion at 0

diffusion MHC(t#, c#) at kdiff

diffusion TCR(m#, c#) at kdiff

diffusion CD8(m#, t#) at kdiffCD8

## -RECORD THE NUMBER OF FULLY PHOSPHORYLATED TCR

record TCR(p="p2")

## RATE PARAMETERS USED IN SIMULATIONS

(Experimentally derived parameters are underlined)

OT-I	F5	Reaction described
<u>52</u>	<u>1212</u>	$k_{on, TCR-agonist\ MHC}$ , TCR-agonist pMHC on rate
<u>52</u>	<u>52</u>	$k_{on, TCR-nonstimulatory\ MHC}$ , TCR-nonstimulatory pMHC on rate
<u>0.02</u>	<u>0.8</u>	$k_{off, TCR-agonist\ MHC}$ , TCR-MHC off rate for agonist peptide
<u>1000</u>	<u>1000</u>	$k_{on, CD8-MHC}$ , MHC-coreceptor (CD8) on rate
<u>varied</u>	<u>varied</u>	$k_{off, CD8-agonist\ MHC}$ , Agonist MHC-coreceptor (CD8) off rate
<u>20</u>	<u>20</u>	$k_{off, CD8-nonstimulatory\ MHC}$ , Nonstimulatory MHC-coreceptor (CD8) off rate
<u>600</u>	<u>600</u>	$k_{diff}$ , Rate of diffusion of membrane surface proteins ( $D = 0.01 \mu m^2/s$ )
<u>varied</u>	<u>varied</u>	$k_{off, TCR-nonstimulatory\ MHC}$ , TCR-MHC off rate for nonstimulatory peptide
0.1, 0.05	0.1, 0.05	$k_p$ , Rate of phosphorylation of TCR by basal Lck (rate with no sites phosphorylated, rate with one site phosphorylated)
0.1, 0.05	0.1, 0.05	$k_{dp}$ , Rate of dephosphorylation of TCR (rate with one site phosphorylated, rate with two sites phosphorylated)
1	1	$k_{on, Lck-TCR}$ , Rate of Lck engagement with TCR
1	1	$k_{off, Lck-TCR}$ , Rate of Lck disengagement with TCR
300	300	$k_{p, active}$ , Rate of phosphorylation by fully active Lck
1	1	$k_{act}$ , Rate of Lck activation
0.15	0.15	$k_{deact}$ , Rate of Lck deactivation when away from TCR-pMHC complex

## CHEMICAL SPECIES PRESENT IN THE KINETIC MODEL

MHC(p="ag")

MHC(p="en")

TCR(p="p0")

CD8(lck="basal")

MHC(p="ag", t#1) TCR(p="p0", m#1)

MHC(p="en", t#1) TCR(p="p0", m#1)

TCR(p="p0", c#1) CD8(lck="basal", t#1)  
 MHC(p="en", c#1) CD8(lck="basal", m#1)  
 MHC(p="ag", c#1) CD8(lck="basal", m#1)  
 TCR(p="p0", c#1, m#2) CD8(lck="basal", t#1) MHC(p="ag", t#2)  
 MHC(p="ag", c#1, t#2) CD8(lck="basal", m#1) TCR(p="p0", m#2)  
 TCR(p="p0", c#1, m#2) CD8(lck="basal", t#1) MHC(p="en", t#2)  
 MHC(p="en", c#1, t#2) CD8(lck="basal", m#1) TCR(p="p0", m#2)  
 MHC(p="en", c#1) CD8(lck="basal", m#1, t#2) TCR(p="p0", c#2)  
 MHC(p="ag", c#1) CD8(lck="basal", m#1, t#2) TCR(p="p0", c#2)  
 MHC(p="ag", c#1, t#2) CD8(lck="basal", m#1, t#3)  
     TCR(p="p0", m#2, c#3)  
 TCR(p="p1", m#1, c#2) MHC(p="ag", t#1) CD8(lck="basal", t#2)  
 MHC(p="en", c#1, t#2) CD8(lck="basal", m#1, t#3)  
     TCR(p="p0", m#2, c#3)  
 TCR(p="p1", m#1, c#2) MHC(p="en", t#1) CD8(lck="basal", t#2)  
 TCR(p="p1", m#1, c#2) MHC(p="ag", t#1, c#3)  
     CD8(lck="basal", t#2, m#3)  
 TCR(p="p1", c#1) CD8(lck="basal", t#1)  
 TCR(p="p1", m#1) MHC(p="ag", t#1)  
 TCR(p="p2", m#1, c#2) MHC(p="ag", t#1) CD8(lck="basal", t#2)  
 TCR(p="p1", m#1, c#2) MHC(p="en", t#1, c#3)  
     CD8(lck="basal", t#2, m#3)  
 TCR(p="p1", m#1) MHC(p="en", t#1)  
 TCR(p="p2", m#1, c#2) MHC(p="en", t#1) CD8(lck="basal", t#2)  
 TCR(p="p1", c#1) CD8(lck="basal", t#1, m#2) MHC(p="ag", c#2)  
 TCR(p="p1", m#1) MHC(p="ag", t#1, c#2) CD8(lck="basal", m#2)  
 TCR(p="p2", m#1, c#2) MHC(p="ag", t#1, c#3)  
     CD8(lck="basal", t#2, m#3)  
 TCR(p="p1")  
 MHC(p="en", c#1) CD8(lck="basal", m#1, t#2) TCR(p="p1", c#2)  
 TCR(p="p2", c#1) CD8(lck="basal", t#1)  
 TCR(p="p2", m#1) MHC(p="ag", t#1)  
 CD8(lck="active", t#1) TCR(p="p2", c#1, m#2) MHC(p="ag", t#2)  
 TCR(p="p1", m#1) MHC(p="en", t#1, c#2) CD8(lck="basal", m#2)  
 TCR(p="p2", m#1, c#2) MHC(p="en", t#1, c#3)  
     CD8(lck="basal", t#2, m#3)  
 TCR(p="p2", m#1) MHC(p="en", t#1)  
 CD8(lck="active", t#1) TCR(p="p2", c#1, m#2) MHC(p="en", t#2)  
 TCR(p="p2", c#1) CD8(lck="basal", t#1, m#2) MHC(p="ag", c#2)  
 TCR(p="p2", m#1) MHC(p="ag", t#1, c#2) CD8(lck="basal", m#2)  
 CD8(lck="active", t#1, m#2) TCR(p="p2", c#1, m#3)  
     MHC(p="ag", c#2, t#3)  
 TCR(p="p2")  
 MHC(p="en", c#1) CD8(lck="basal", m#1, t#2) TCR(p="p2", c#2)  
 TCR(p="p2", c#1) CD8(lck="active", t#1)  
 CD8(lck="active")  
 TCR(p="p2", m#1) MHC(p="en", t#1, c#2) CD8(lck="basal", m#2)  
 CD8(lck="active", t#1, m#2) TCR(p="p2", c#1, m#3)  
     MHC(p="en", c#2, t#3)  
 CD8(lck="active", m#1) MHC(p="ag", c#1, t#2) TCR(p="p2", m#2)  
 TCR(p="p2", c#1) CD8(lck="active", t#1, m#2) MHC(p="ag", c#2)  
 MHC(p="en", c#1) CD8(lck="active", m#1, t#2) TCR(p="p2", c#2)  
 TCR(p="p1", c#1) CD8(lck="active", t#1)  
 TCR(p="p0", c#1) CD8(lck="active", t#1)  
 TCR(p="p1", c#1, m#2) CD8(lck="active", t#1) MHC(p="en", t#2)  
 TCR(p="p1", c#1, m#2) CD8(lck="active", t#1) MHC(p="ag", t#2)

TCR(p="p0", c#1, m#2) CD8(lck="active", t#1) MHC(p="en", t#2)  
TCR(p="p0", c#1, m#2) CD8(lck="active", t#1) MHC(p="ag", t#2)  
MHC(p="en", c#1) CD8(lck="active", m#1)  
MHC(p="ag", c#1) CD8(lck="active", m#1)  
MHC(p="en", c#1, t#2) CD8(lck="active", m#1) TCR(p="p2", m#2)  
MHC(p="en", c#1, t#2) CD8(lck="active", m#1) TCR(p="p1", m#2)  
MHC(p="ag", c#1, t#2) CD8(lck="active", m#1) TCR(p="p1", m#2)  
MHC(p="en", c#1, t#2) CD8(lck="active", m#1) TCR(p="p0", m#2)  
MHC(p="ag", c#1, t#2) CD8(lck="active", m#1) TCR(p="p0", m#2)  
MHC(p="en", c#1) CD8(lck="active", m#1, t#2) TCR(p="p1", c#2)  
MHC(p="ag", c#1) CD8(lck="active", m#1, t#2) TCR(p="p1", c#2)  
MHC(p="en", c#1) CD8(lck="active", m#1, t#2) TCR(p="p0", c#2)  
MHC(p="ag", c#1) CD8(lck="active", m#1, t#2) TCR(p="p0", c#2)  
MHC(p="en", c#1, t#2) CD8(lck="active", m#1, t#3)  
    TCR(p="p1", m#2, c#3)  
MHC(p="ag", c#1, t#2) CD8(lck="active", m#1, t#3)  
    TCR(p="p1", m#2, c#3)  
MHC(p="en", c#1, t#2) CD8(lck="active", m#1, t#3)  
    TCR(p="p0", m#2, c#3)  
MHC(p="ag", c#1, t#2) CD8(lck="active", m#1, t#3)  
    TCR(p="p0", m#2, c#3)