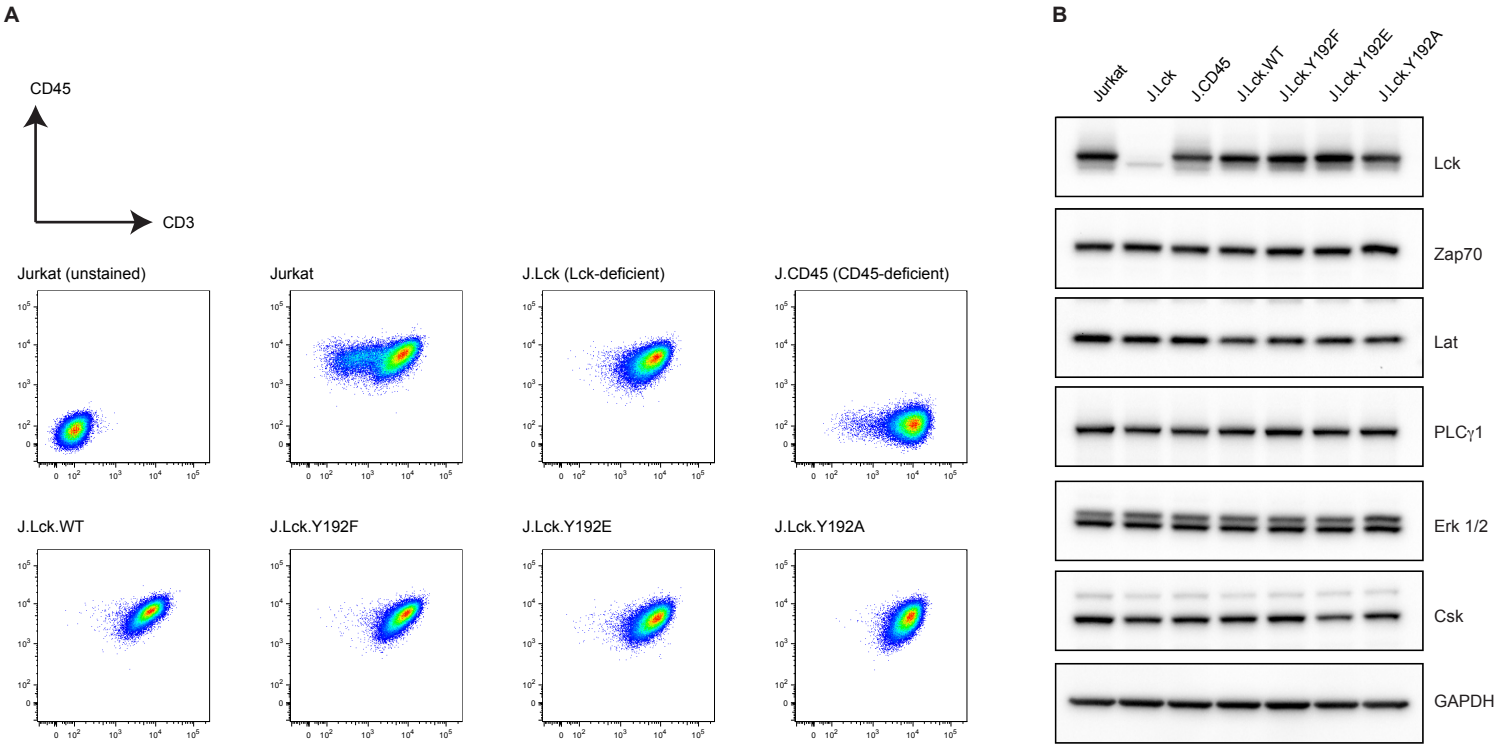
SUPPLEMENTAL FIGURES



C

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WT       GGGGGAGCAGATCTTGGGGGAGCCCCTTCAGCCCCCTCTTCCATTCCCTCAGGGACCATG
J.Lck 1  GGGGGAGCAGATCTTGGGGGAGCCCCTTCAGCCCCCTCTTCCATTCCCTC----ACCATG
J.Lck 2  GGGGGAGCAGATCTTGGGGGAGCCCCTTCAGCCCCCTCTTCCATTC--------------
         *********************************************

WT       GGCTGTGGCTGCAGCTCACACCCGGAAGATGACTGGATGGAAAACATCGATGTGTGTGAG
J.Lck 1  GGCTGTGGCTGCAGCTCACACCCGGAAGATGACTGGATGGAAAACATCGATGTGTGTGAG
J.Lck 2  ----------------------CCGGAAGATGACTGGATGGAAAACATCGATGTGTGTGAG
                              ************************************

WT       AACTGCCATTATCCCATAGTCCCACTGGATGGCAAGGGCACGGTA----------AGAGG
J.Lck 1  AACTGCCATTATCCCATAGTCCCACTGGATGGCAAGGGCACGGTAGGGGTTGCCAAGAGG
J.Lck 2  AACTGCCATTATCCCATAGTCCCACTGGATGGCAAGGGCACGGCCT--------------
         ******************************************** .

WT       CGAGACAGGGGCCTTGGTGAGGGAGTTGGGTAGAGAA
J.Lck 1  CGAGACAGGGGCCTTGGTGAGGGAGTTGGGTAGAGAA
J.Lck 2  -AAGACAGGGGCCTTGGTGAGGGAGTTGGGTAGAGAA
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WT       CTTAGGGACACGGCTGACTTCCAGATATGACCATGTATTTGTGGCTTAAACTCTTGGCA
J.CD45 1 CTTAGGGACACGGCTGACTTCCAGATATGACCATGTATTTGTGGCTTAAACTCTTGGCG
J.CD45 2 CTTAGGGACACGGCTGACTTCCAGATATGACCATGTATTTGTGGCTTAAACTCTTG---
         ************************************************************

WT       TTTGGCTTTGCCTTTCTGGACACAGAAGTATTTGTGACAG
J.CD45 1 G-----CTTGCCTTTCTGGACACAGAAGTATTTGTGACAG
J.CD45 2 ----GCTTTGCCTTTCTGGACACAGAAGTATTTGTGACAG
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Figure S1. Surface staining and protein expression of Jurkat T cell variants described in Figure 1. (A) CD3 and CD45 expression was assessed by flow cytometry. (B) Protein levels of components of the TCR signaling pathway were assessed by immunoblot. (C) Sequences of CRISPR/Cas9 targeted mutations within Lck (J.Lck) and CD45 (J.CD45).

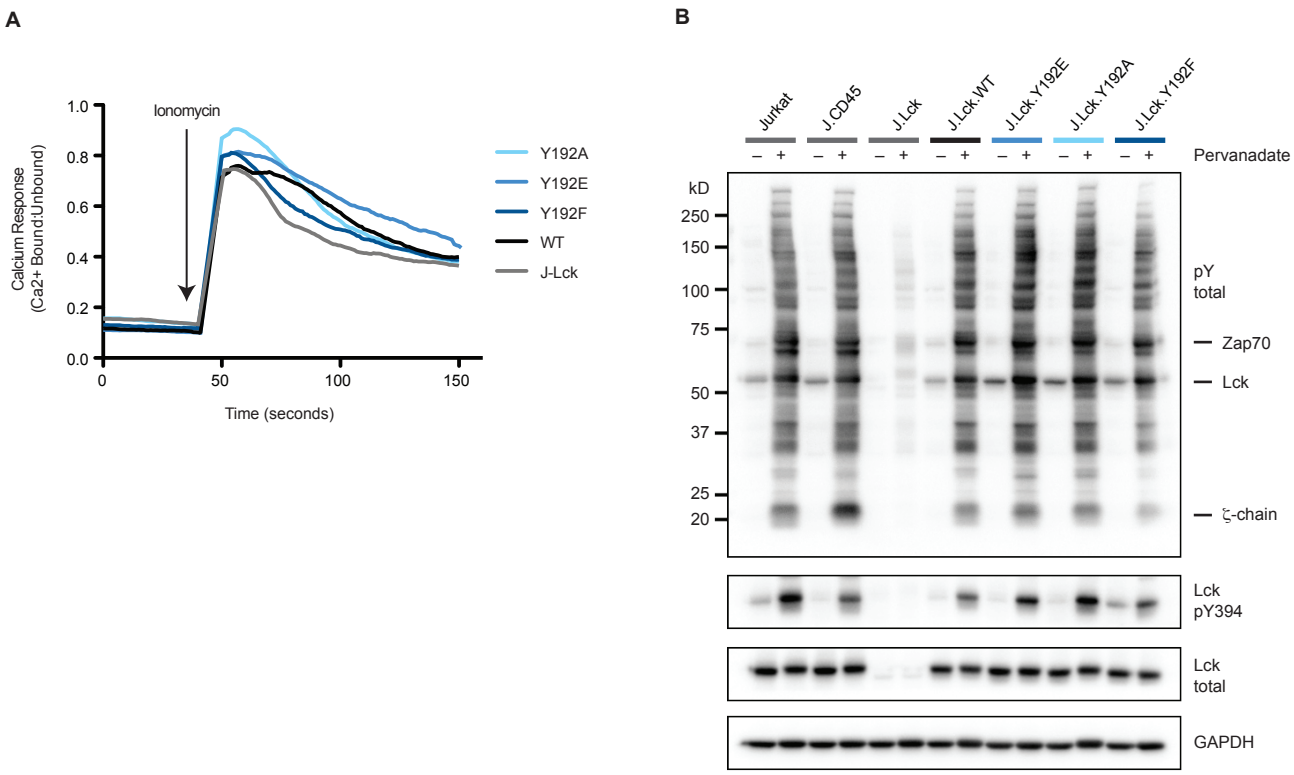
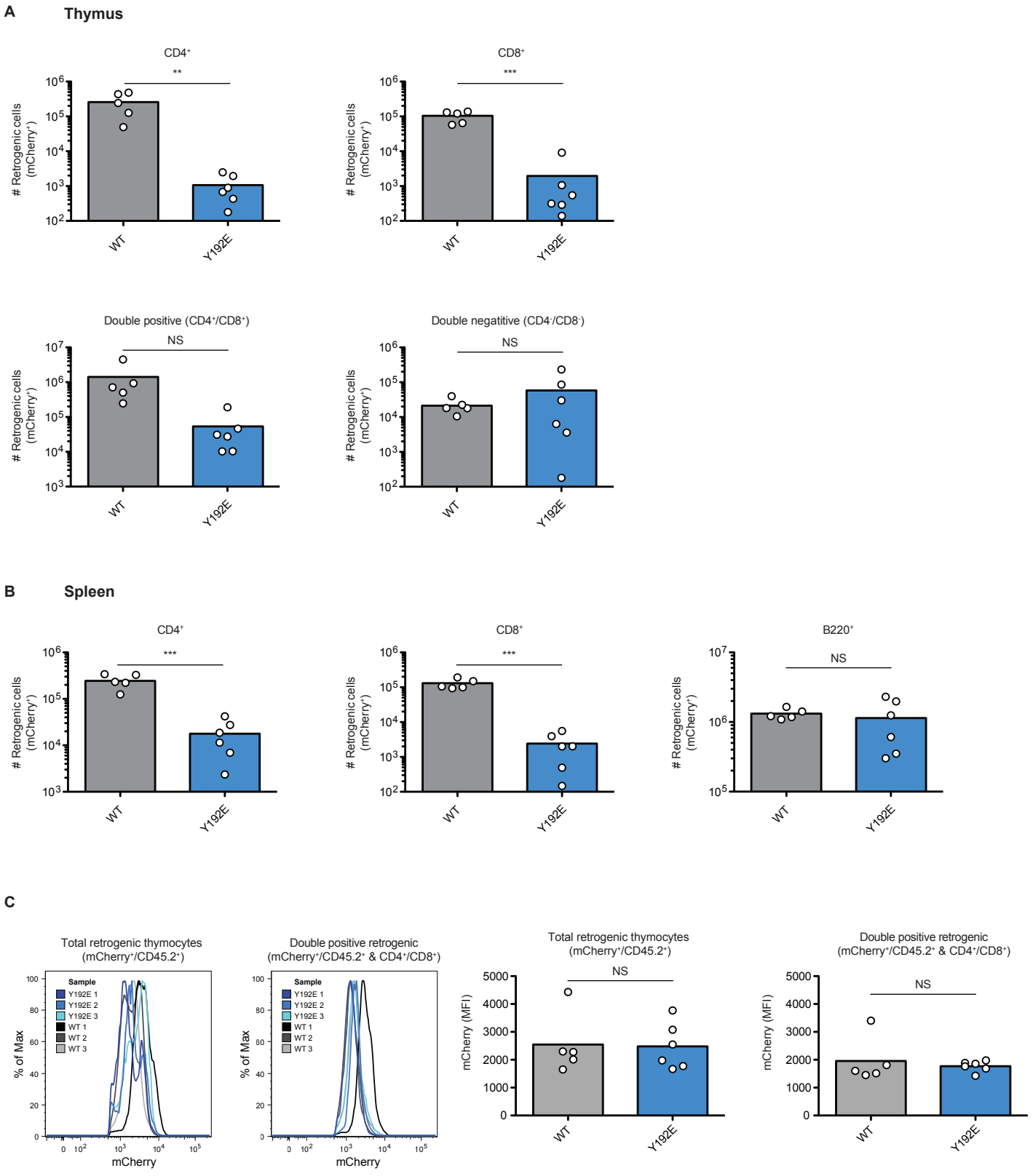


Figure S2. Jurkat Y192 variants respond to stimuli that bypass the TCR, related to Figure 1. (A) Calcium responses upon ionomycin treatment were similar across J.Lck Y192 variants. (B) Jurkat T cells, J.Lck, J.CD45 and Y192 variants were treated with the general protein tyrosine phosphatase (PTP) inhibitor pervanadate. Robust tyrosine phosphorylation is induced in cell lines expressing Lck, including Y192 variants. Data are representative of three independent experiments.



Figures S3. (A) Quantification of retrogenic cell numbers isolated from thymus and spleen described in Figure 4. (B) Lck expression levels were assessed by comparing mCherry intensity in total thymocytes and double positive thymocytes described in Figure 4. * $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$, and NS $P > 0.05$. P values were calculated using the unpaired Student's t test (N=5 or 6 mice per group).

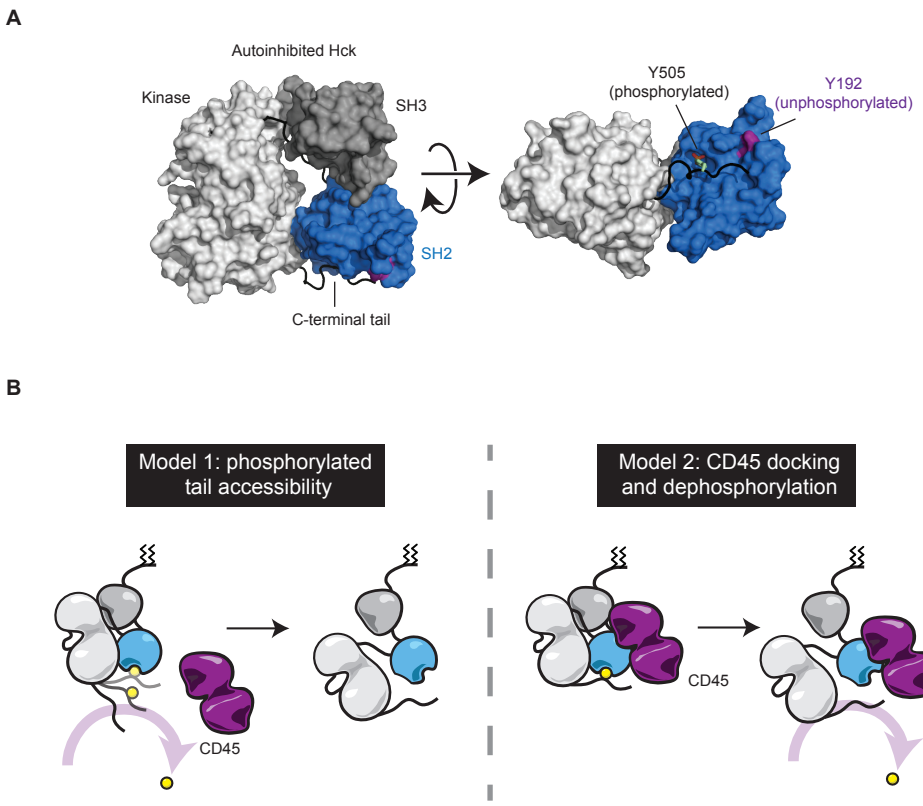
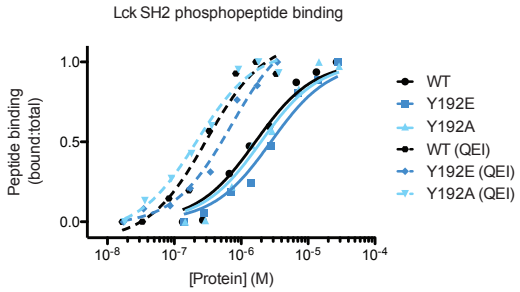


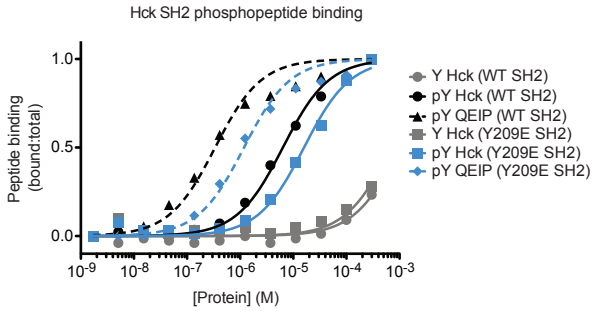
Figure S4. Model for Y192-mediated regulation of Lck activity in T cells, related to Figures 5 and 6. (A) Structure of autoinhibited Hck (PDB: 1AD5). Kinase and regulatory domains are shown as surface renderings with linker regions depicted as cartoons. Denoted are: the regulatory C-terminal tail phosphosite Y522, equivalent to Y505 in Lck; and Hck Y209, which is equivalent to Lck Y192. Lck Y192/Hck 209 is located distal to the interface of the SH2 and kinase domains in the autoinhibited structure of Hck. (B) Models for Y192 regulation of CD45-mediated activation of Lck. *Model 1*: CD45-mediated dephosphorylation of Lck is governed by accessibility of the phosphorylated C-terminal tail. *Model 2*: CD45 interacts with Lck prior to accessing the phosphorylated C-terminal tail.

A



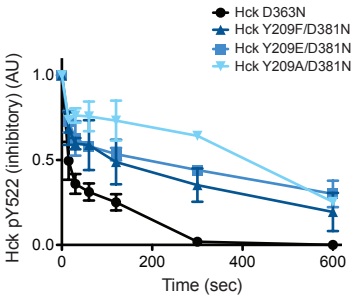
		K_D (μM)		
Peptide	Sequence	WT Lck SH2	Y192E Lck SH2	Y192A Lck SH2
pY Lck	F*-TEGQ[pY]QPQP	1.7 ± 0.3	3.4 ± 0.3	1.9 ± 0.2
pY QEIP	F*-TEGQ[pY]QEIP	0.24 ± 0.05	0.66 ± 0.02	0.21 ± 0.01

B



		K_D (μM)	
Peptide	Sequence	WT Hck SH2	Y209E Hck SH2
Y Hck	F*-Ahx-TESQYQQQP	>1000	>1000
pY Hck	F*-Ahx-TESQ[pY]QQQP	7.2 ± 0.9	19.5 ± 1.5
pY Lck	F*-TEGQ[pY]QPQP	3.3 ± 0.3	10.0 ± 1.3
pY QEIP	F*-TEGQ[pY]QEIP	0.3 ± 0.02	1.0 ± 0.1

C



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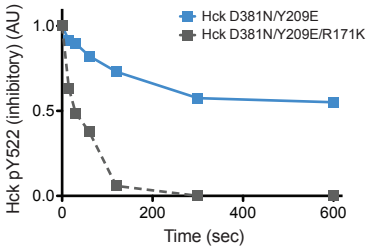

Figure S5. Mutation of Y192 within the SH2 domain does not substantially alter binding affinity for C-terminal tail phosphopeptides, related to Figures 5 and 6. (A) Binding affinity of the SH2 domain of Lck was assessed by a fluorescence polarization assay using a phosphopeptide derived from the C-terminal tail (FITC-TEGQ[pY]QPQP) and a higher affinity variant (FITC-TEGQ[pY]QEIP. Binding affinities were calculated (right panel). (B) Hck SH2 domains were purified and assessed for phosphopeptide binding by a fluorescence polarization assay. Additional C-terminal phosphopeptides, including a higher affinity variant (QEIP), were also assessed and binding affinities calculated (right panel). (C) Dephosphorylation of additional Hck D381N/Y209 variants by CD45. (D) Dephosphorylation by CD45 of Hck D381N/Y209E ± an additional mutation that disrupts SH2 binding (R171K). Data are representative of three independent experiments.

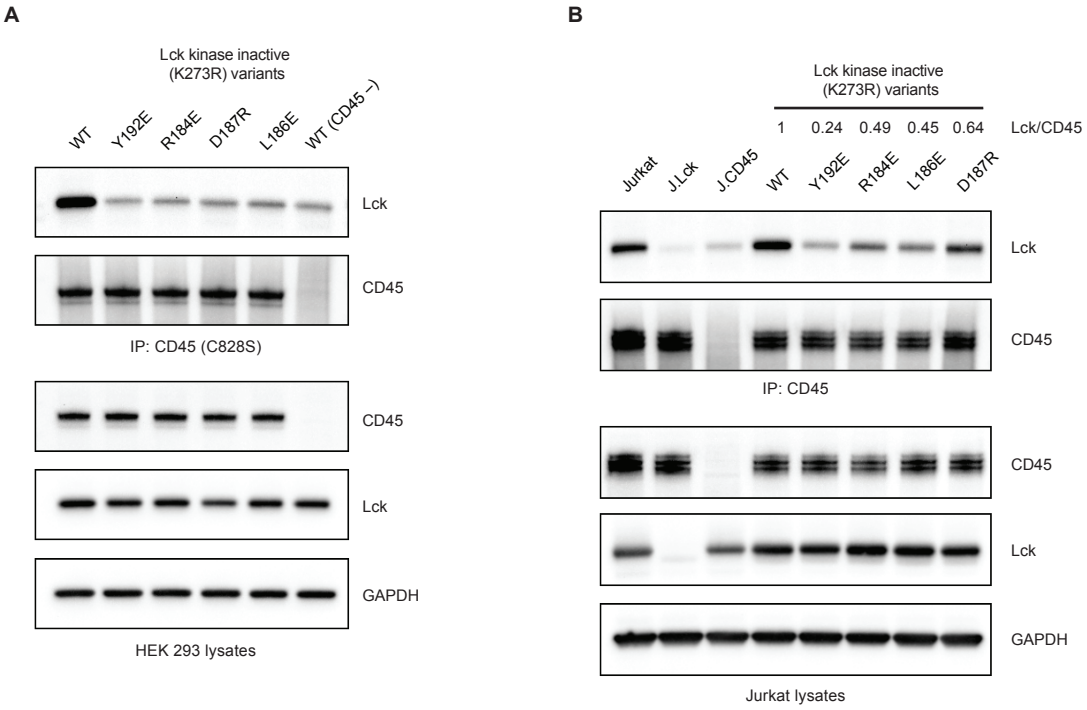

Figure S6. Lck SH2 domain variants assessed for association with CD45, related to Figure 7. (A) HEK 293 cells were transfected with inactive CD45 (C828S) and inactive Lck (K273R) variants. Cells that did not express CD45 were used as a control. CD45 was immunoprecipitated and associated Lck assessed by immunoblot. (B) Lck-deficient J.Lck cells were transiently transfected with inactive Lck (K273R) variants. Jurkat, J.CD45 and untransfected J.Lck cells were included as controls. CD45 was immunoprecipitated and associated Lck assessed by immunoblot. Quantification of Lck to CD45 ratio from IP is denoted. Data are representative of two independent experiments.

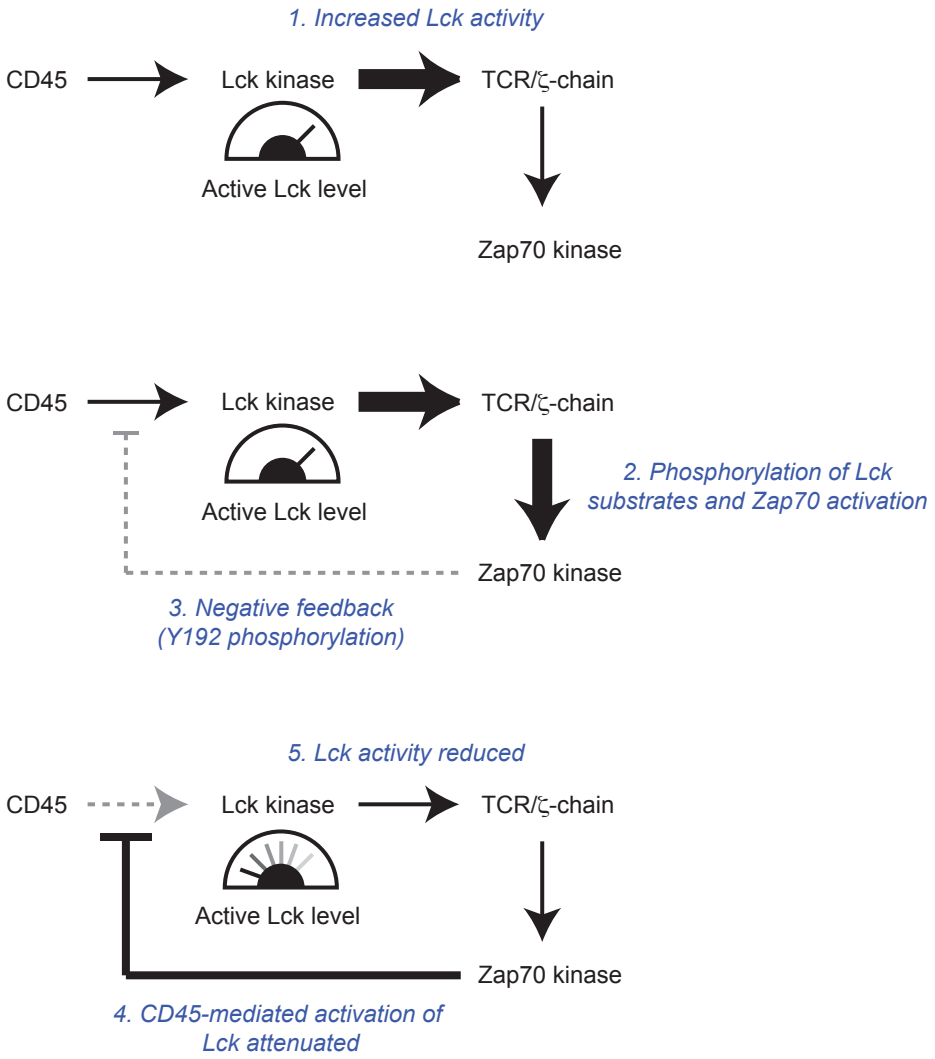

Figure S7. A negative feedback loop to regulate Lck activation by CD45 is proposed, related to Figure 7. (1) An increase in Lck activity occurs which results in phosphorylation of Lck substrates, including the TCR ζ-chain and Zap70. (2) Increased Zap70 activity results in signaling events in resting or stimulated cells. (3) These signaling events are proposed to activate negative feedback loops, including the phosphorylation of Lck Y192. (4) Phosphorylation of Y192 disrupts the ability of CD45 to activate Lck thereby reducing the amount of active Lck (5). Reducing the amount of active Lck will reduce tonic TCR signaling and the sensitivity of the TCR to stimulation.

SUPPLEMENTAL TABLES

Table S1. List of Lck phosphorylation sites identified in vitro, related to Figure 6.

Phosphorylation by Lck		
Protein	Residue	Location
Lck	Y192	SH2
Lck	Y263	Kinase
Lck	Y394	Kinase (activation loop)
Lck	Y414	Kinase
Lck	Y489	Kinase
Lck	Y505	Tail
CD45	Y640	Cytoplasmic
CD45	Y687	P1
CD45	Y705	P1
CD45	Y710	P1
CD45	Y720	P1
CD45	Y880	P1
CD45	Y978	P2
CD45	Y980	P2
CD45	Y1081	P2
CD45	Y1116	P2
CD45	Y1216	P2