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Supplemental Information

PTPN2 Deficiency Enhances Programmed T Cell Expansion and Survival Capacity of Activated T Cells

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Fig. S1

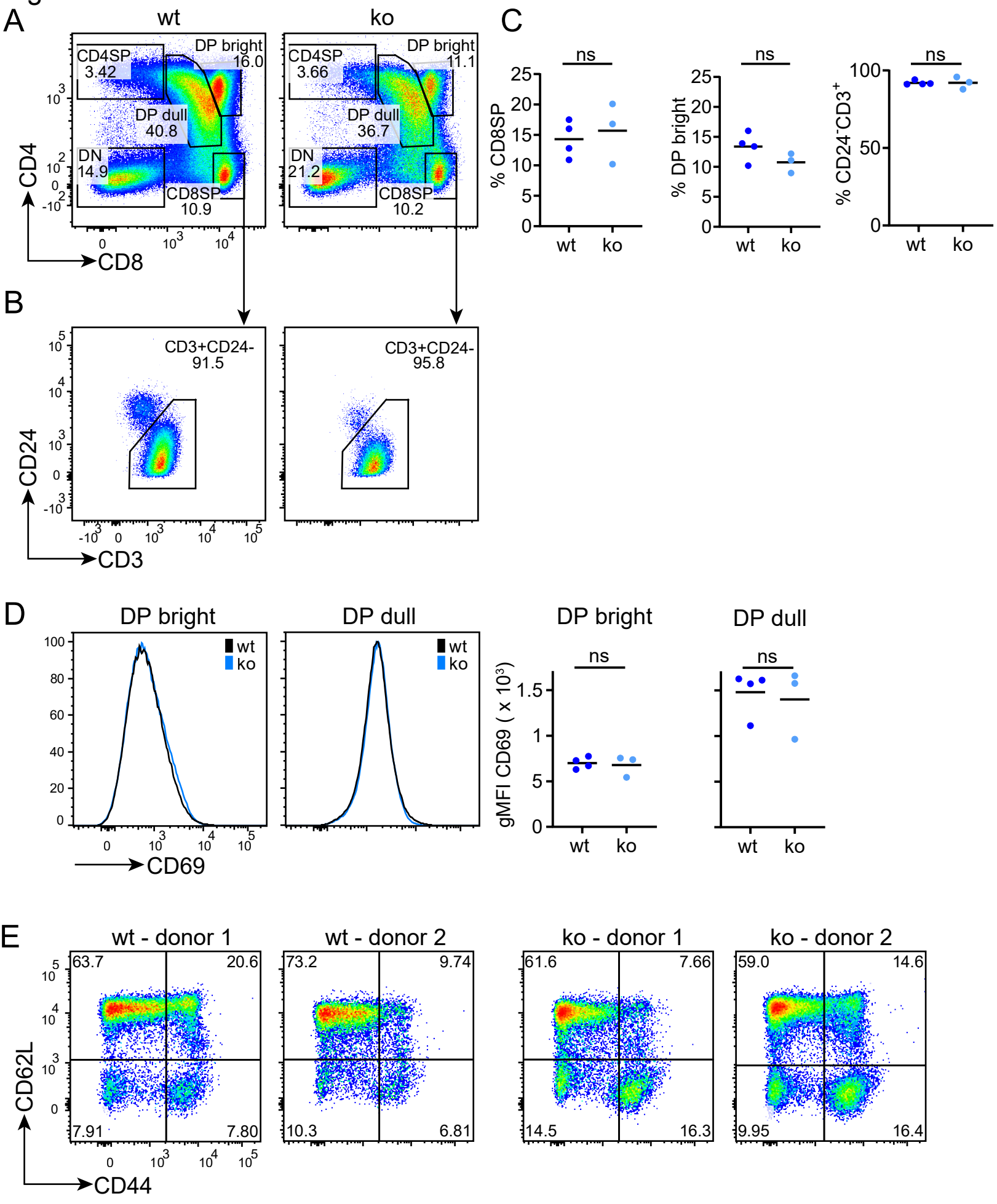
A
wt
ko
CD4SP
3.42
DP bright
16.0
DP dull
40.8
DN
14.9
CD8SP
10.9
CD4SP
3.66
DP bright
11.1
DP dull
36.7
DN
21.2
CD8SP
10.2
CD4
CD8
B
CD3+CD24-
91.5
CD3+CD24-
95.8
CD24
CD3
C
ns
% CD8SP
% DP bright
% CD24-CD3+
wt
ko
D
DP bright
DP dull
wt
ko
CD69
gMFI CD69 (x 10³)
E
wt - donor 1
wt - donor 2
ko - donor 1
ko - donor 2
63.7
20.6
7.91
7.80
73.2
9.74
10.3
6.81
61.6
7.66
14.5
16.3
59.0
14.6
9.95
16.4
CD62L
CD44

Figure S1: Absence of PTPN2 does not impair thymic development. Related to Figure 1. The thymi of OT-I;*Lck*-Cre;*Ptpn2*$^{fl/fl}$ (ko) and OT-I;*Ptpn2*$^{fl/fl}$ (wt) mice were taken from donors at the age of 6-10 weeks. **A**) Frequencies of developing T cells among total thymocytes were determined by flow cytometry and FACS plots of the major developmental stages of two mice are shown. Data are representative of seven donor mice. SP: single positive, DP: double positive, DN: double negative **B**) The FACS plots show the percentage of $CD24^{-}CD3^{+}$ cells of CD8-single positive cells. **C**) The dot plots show the summary of all 7 animals and each dot represents one individual mouse, horizontal lines the mean. **D**) The histograms show a representative overlay of the CD69 expression levels of the $CD8^{+}CD4^{+}$ bright and dull populations and the dot plots show the geometric mean fluorescent intensity (gMFI) of all seven animals. **E**) The FACS plots show the pre-activation status of isolated OT-I CD8 T cells by means of CD44 and CD62L expression of 4 individual donor mice that were used in our experiments. Statistical analysis: unpaired t-test. ns (not significant) $p \geq 0.05$.

Fig. S2

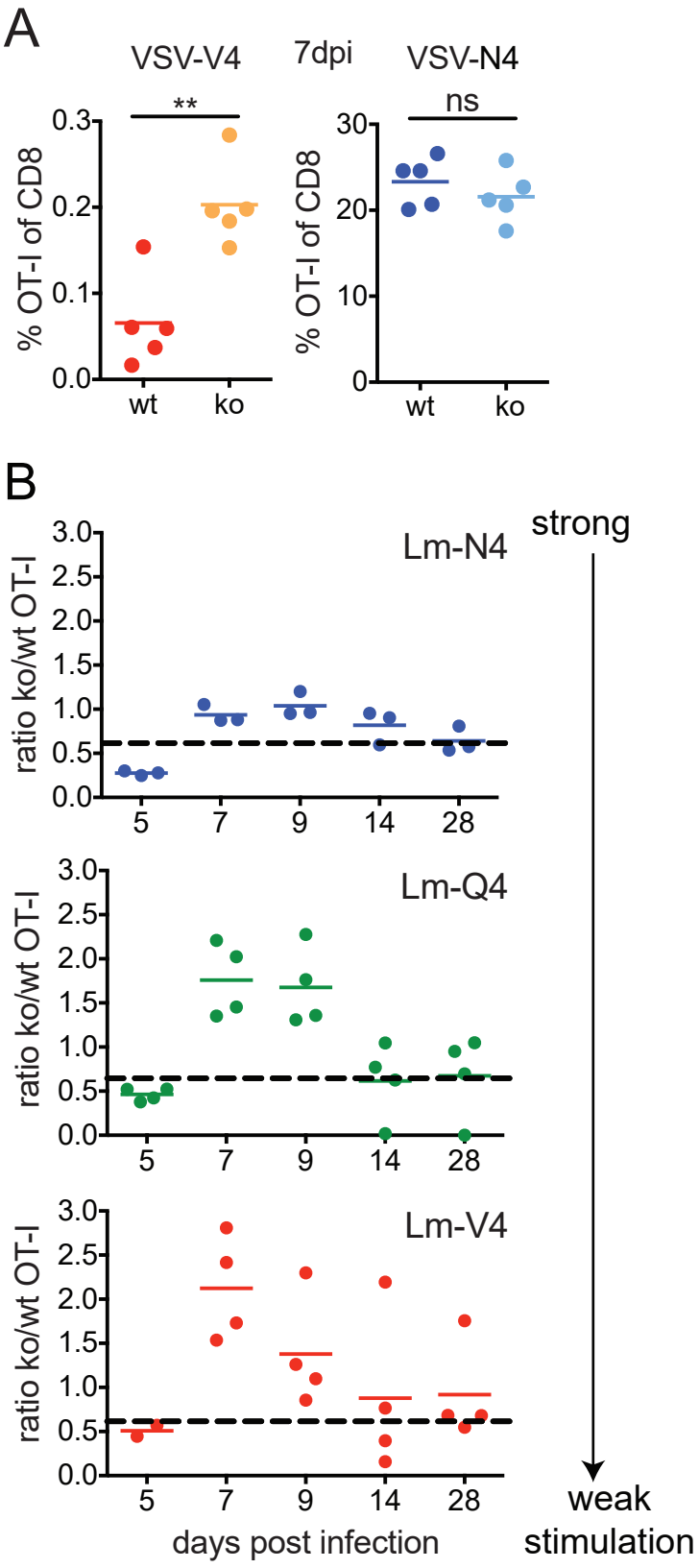
A
VSV-V4
7dpi
VSV-N4
**
ns
% OT-I of CD8
0.3
0.2
0.1
0.0
30
20
10
0
wt
ko
B
strong
Lm-N4
Lm-Q4
Lm-V4
ratio ko/wt OT-I
3.0
2.5
2.0
1.5
1.0
0.5
0.0
5
7
9
14
28
days post infection
weak
stimulation

Figure S2: Absence of PTPN2 delays the onset of T cell contraction. Related to Figures 1 and 2. CD45-congenic C57BL/6J host mice received 10^4 OT-I;*Lck*-Cre;*Ptpn2*$^{fl/fl}$ (ko) and OT-I;*Ptpn2*$^{fl/fl}$ (wt) CD8 T cells and were infected 24 hours later with ovalbumin-expressing pathogens. **A**) Frequencies of OT-I T cells among total CD8 T cells in the spleen were determined by flow cytometry 7 days after infection with 2 x 10^6 PFU VSV-V4 or VSV-N4. Data are representative of three independent experiments, with at least four mice per group. **B**) Plots show the ratio of ko/wt OT-I T cells at different time-points following infection with 1000 CFU Lm-N4 (SIINFEKL), Lm-Q4 (SIIQFEKL), or Lm-V4 (SIIVFEKL), which provide high, intermediate or low avidity ligands for OT-I T cells. The dashed line represents the initial engraftment ratios determined 1 day after transfer of a higher amount of wt or ko OT-I T cells of the same mixture in uninfected hosts. The data are representative of two independent experiments with 3-5 mice. Dots in all panels represent one individual mouse, horizontal lines the mean. Statistical analysis: unpaired t-test. **$p \leq 0.001$, ns (not significant) $p \geq 0.05$.

Fig. S3

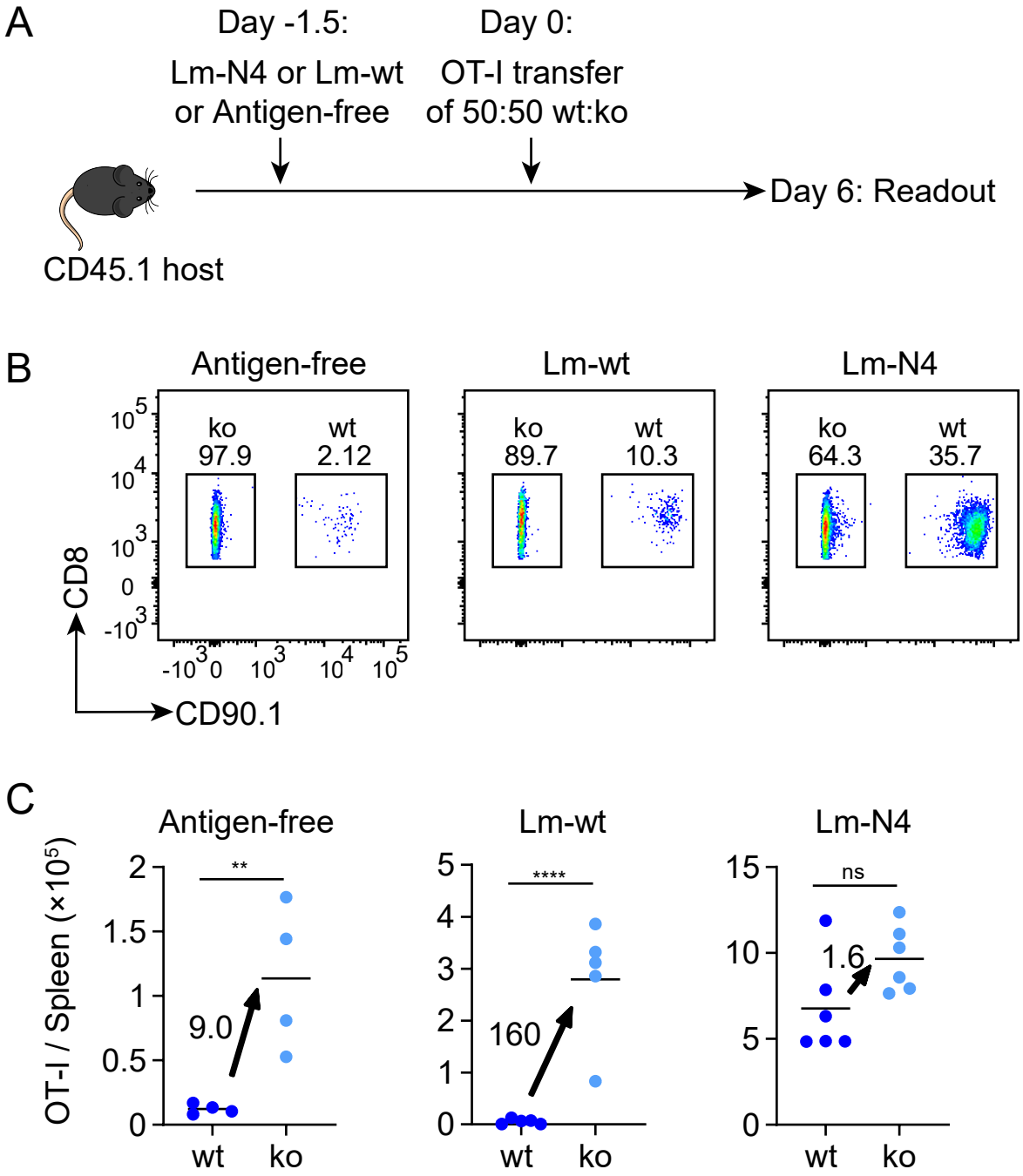
A
Day -1.5:
Lm-N4 or Lm-wt
or Antigen-free
Day 0:
OT-I transfer
of 50:50 wt:ko
Day 6: Readout
CD45.1 host
B
Antigen-free
Lm-wt
Lm-N4
ko
97.9
wt
2.12
ko
89.7
wt
10.3
ko
64.3
wt
35.7
CD8
CD90.1
C
Antigen-free
Lm-wt
Lm-N4
OT-I / Spleen (×10^5)
**
****
ns
9.0
160
1.6
wt
ko

Figure S3: Pre-activated PTPN2-deficient T cells expand significantly in response to antigen. Related to Figure 3. A) CD45-congenic C57BL/6J host mice were infected with 1000 CFU Lm-N4 or Lm-wt or kept antigen-free for 36 hours before receiving 5×10^4 OT-I;*Lck*-Cre;$Ptpn2^{fl/fl}$ (ko) mixed with 5×10^4 OT-I;$Ptpn2^{fl/fl}$ (wt) CD8 T cells that both had been activated *ex vivo* for 36 hours. **B**) Numbers of OT-I T cells in the spleen were determined by flow cytometry 7.5 days after infection and representative FACS plots of one mouse of each condition are displayed. **C**) The dot plots show the absolute numbers of OT-I T cells per spleen and each dot represents one individual mouse, horizontal lines the mean.

Data are representative of two independent experiments for the transfer in antigen-free hosts and one experiment for the transfer in infected hosts, with at least four mice per group. Statistical analysis: unpaired t-test. ****$p \leq 0.00001$, **$p \leq 0.001$, *$p \leq 0.01$, ns (not significant) $p \geq 0.05$.

Supplementary Table 1. Differentially phosphorylated tyrosine sites of PTPN2-deficient versus wt OT-I T cells. Related to Figure 4.

Proteins	Isoforms	Positions within isoforms	Amino acid	log2 ko/wt	Localization probability	Score
Stat3	P42227-3	704	Y	2.4	1.00	115
Stat3	P42227;B7ZC18;P42227-2	705;679;705	Y	2.3	1.00	138
Stat5b	P42232	699	Y	2.2	1.00	122
Stat5a	P42230	694	Y	1.9	1.00	108
Stat4	Q3V157;P42228	693;694	Y	1.4	0.99	116
Vav1	P27870;E9PXI0;Q8VDU4	844;820;805	Y	1.2	0.90	51
Ptpn18	Q3V441;Q61152	32;381	Y	1.2	0.77	119
Fyb	O35601;O35601-2	559;559	Y	1.1	1.00	122
Ptpn6	P29351;P29351-2;P29351-3;G3UYY5	564;566;525;64	Y	1.0	1.00	108
Mapk1	P63085	185	Y	1.0	1.00	190
Pdap1	Q3UHX2	70	Y	1.0	0.93	120
Srsf1	H7BX95;Q6PDM2	202;202	Y	0.9	0.98	98
Hist1h4a	P62806	52	Y	0.9	1.00	118
Pabpc1	P29341	364	Y	0.8	1.00	90
Itsn2	E9QNG1;B2RR82;Q9Z0R6;Q9Z0R6-2;A0A1W2P775	922;949;922;922;481	Y	0.8	1.00	138
Cd28	P31041	189	Y	0.7	1.00	165
Cblb	Q3TTA7;B9EKI5;Q3TTA7-2	889;845;737	Y	0.6	1.00	70
Cfl1	P18760;F8WGL3	68;68	Y	-0.7	0.87	179

The OT-I CD8 T cells were activated for 30 hours *in vitro* with anti-CD3/anti-CD28 coupled beads and analyzed via mass spectrometry. The results were filtered for a combined MS-score of > 50 and a localization probability of > 0.75.