Thioredoxin-1 improves the immuno-metabolic phenotype of anti-tumor T cells

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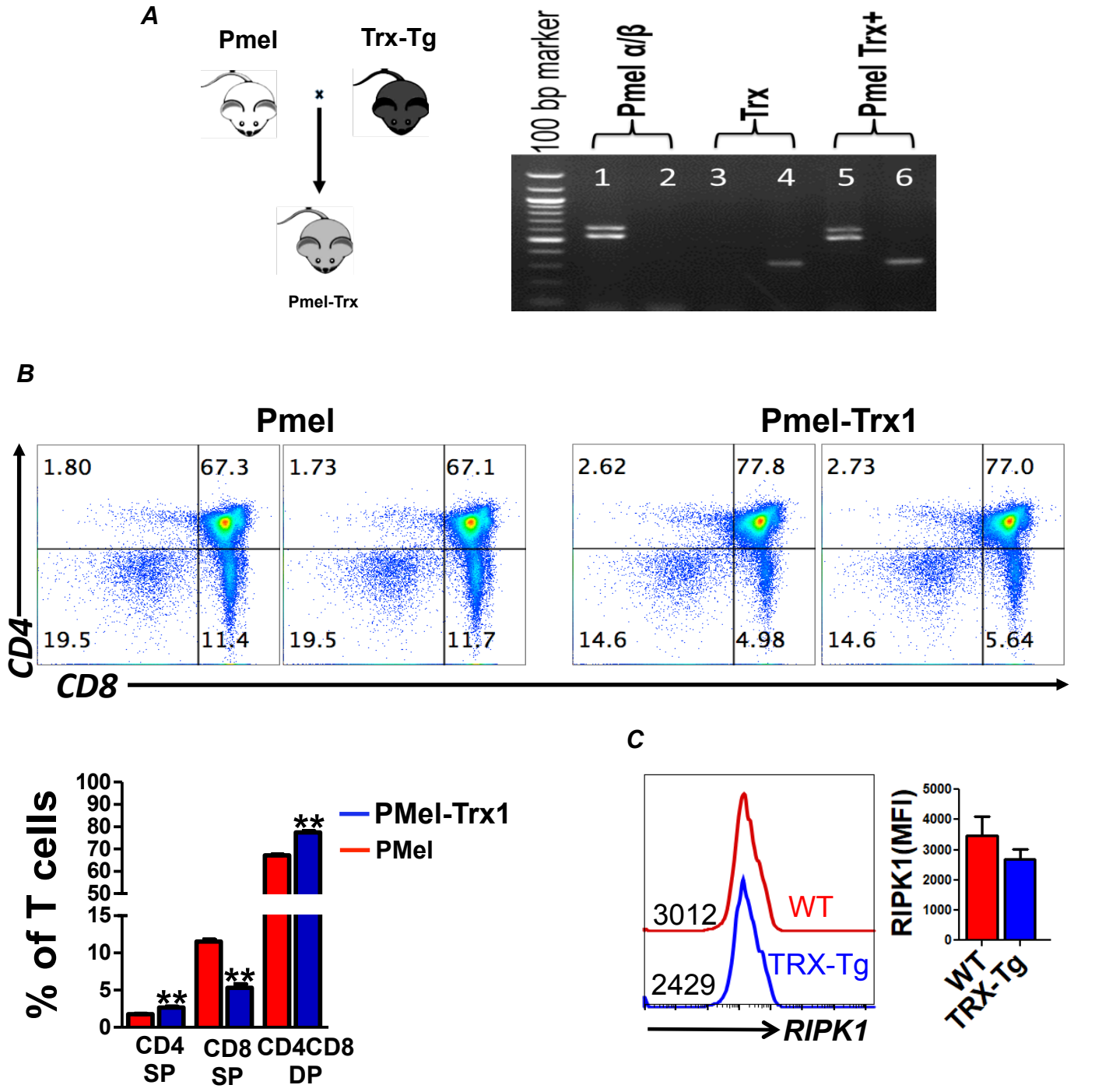
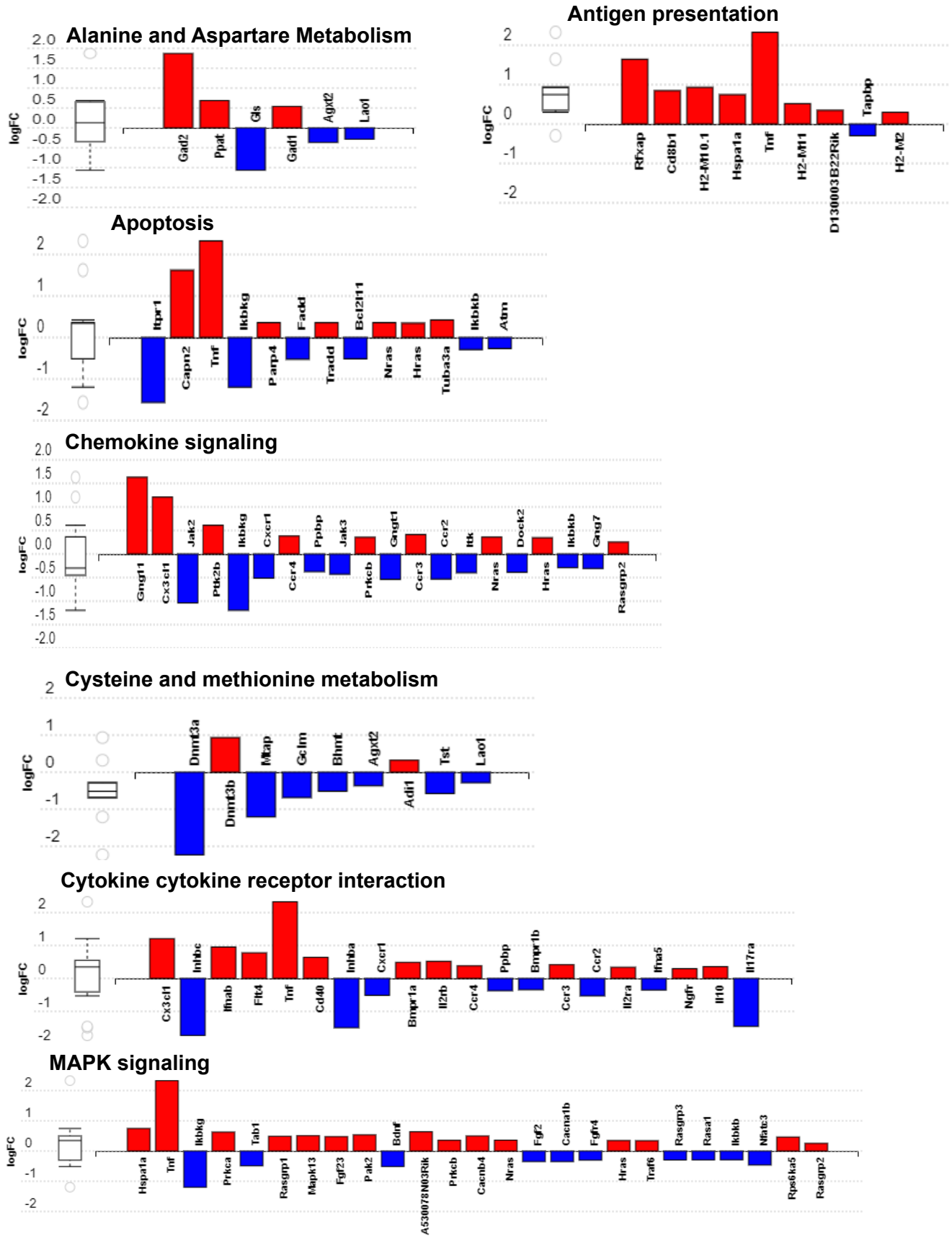


Figure S1: **Characterization of Pmel-Trx T cells.** ***A).*** The gel picture shows PCR based genotyping for Pmel and Pmel-Trx mice. ***B)*** Thymocytes were prepared from the six-week old Pmel and Pmel-Trx mice and stained for flurochrome conjugated CD4 and CD8 antibody. The dot-plot represents distribution of CD4 and CD8 single positives (SP) and double positive (DP) thymocytes obtained from two different mice. *Lower panel* shows percent of each fraction (CD4 SP, CD8 SP, CD4CD8 DP) represented in bar diagram using the data from *upper panel* dot plots. ***p* value <0.01. ***C)*** Three day TCR activated T cells from Pmel and Pmel-Trx mice were restimulated with cognate antigen for 4 hr. and stained intracellularly using flurochrome conjuagetd anti-RIPK1 antibody.

Figure S2


Alanine and Aspartare Metabolism
logFC
Gad2
Ppat
Gls
Gad1
Agxt2
Lao1
Antigen presentation
logFC
Rfxap
Cd8b1
H2-M10.1
Hspa1a
Tnf
H2-M11
D130003B22Rik
Tapbp
H2-M2
Apoptosis
logFC
Itpr1
Capn2
Tnf
Ikbkg
Parp4
Fadd
Tradd
Bcl2l11
Nras
Hras
Tuba3a
Ikbkb
Atm
Chemokine signaling
logFC
Gngt1
Cx3cl1
Jak2
Ptk2b
Ikbkg
Cxcr1
Ccr4
Ppbp
Jak3
Prkcb
Gngt1
Ccr3
Ccr2
Itk
Nras
Dock2
Hras
Ikbkb
Gng7
Rasgrp2
Cysteine and methionine metabolism
logFC
Dnmt3a
Dnmt3b
Map
Gclm
Bhmt
Agxt2
Adi1
Tst
Lao1
Cytokine cytokine receptor interaction
logFC
Cx3cl1
Inhbc
Ifnab
Flt4
Tnf
Cd40
Inhba
Cxcr1
Bmpr1a
Il2rb
Ccr4
Ppbp
Bmpr1b
Ccr3
Ccr2
Il2ra
Ifna5
Ngfr
Il10
Il17ra
MAPK signaling
logFC
Hspa1a
Tnf
Ikbkg
Prkca
Tab1
Rasgrp1
Mapk13
Fgf23
Pak2
Bdnf
A530078N03Rik
Prkcb
Cacnb4
Nras
Fgf2
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Hras
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Ikbkb
Nfatc3
Rps6ka5
Rasgrp2

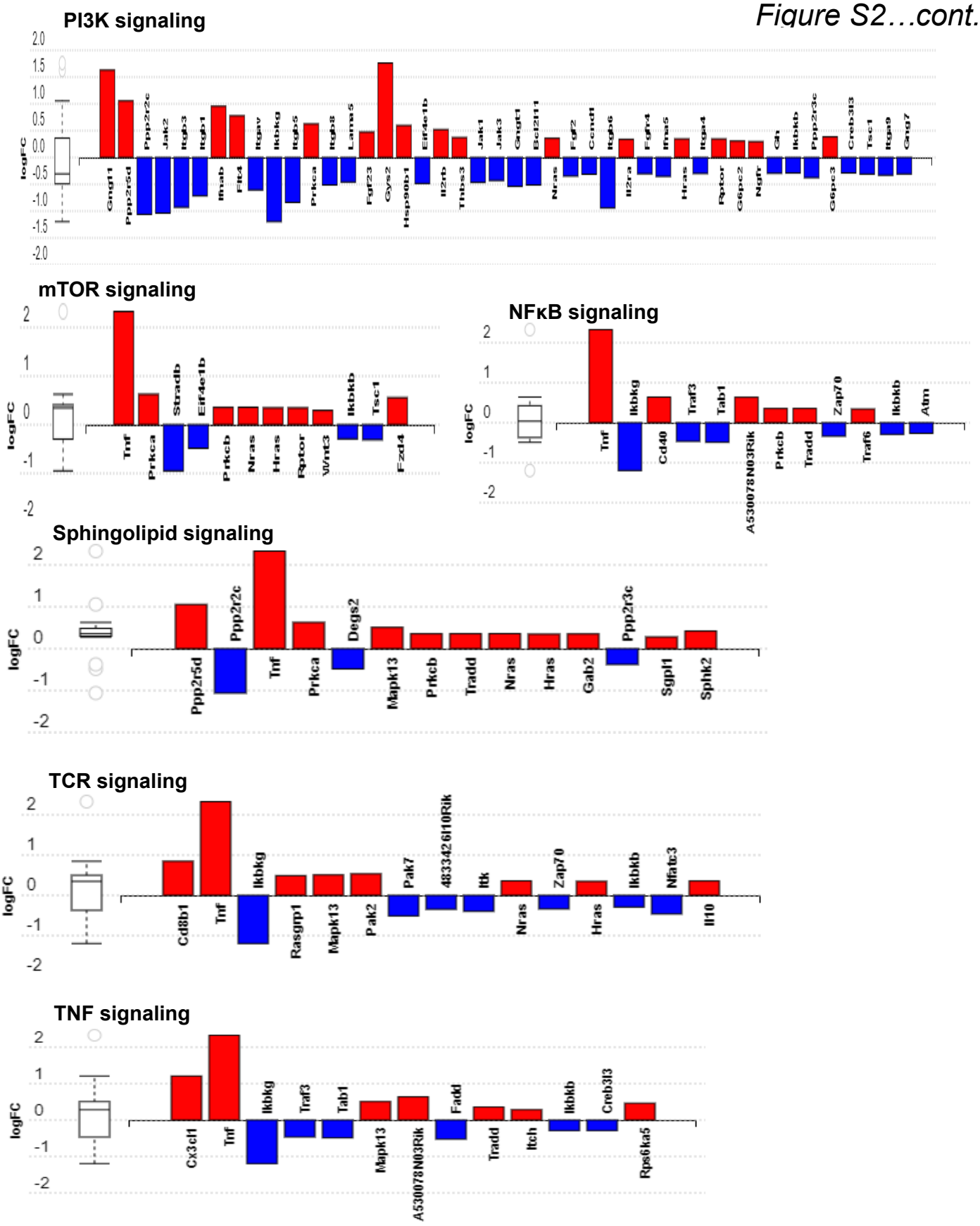

Figure S2: **Gene perturbation bar plot.** All the genes in different pathways are ranked based on their absolute perturbation values computed by propagating the measured expression changes across the pathway topology. For each gene, the signed perturbation is represented with negative values in blue and positive values in red. The box and whisker plot on the left summarizes the distribution of all gene perturbations in this pathway. The box represents the 1st quartile, the median and the 3rd quartile, while circles represent the outliers.

Figure S3

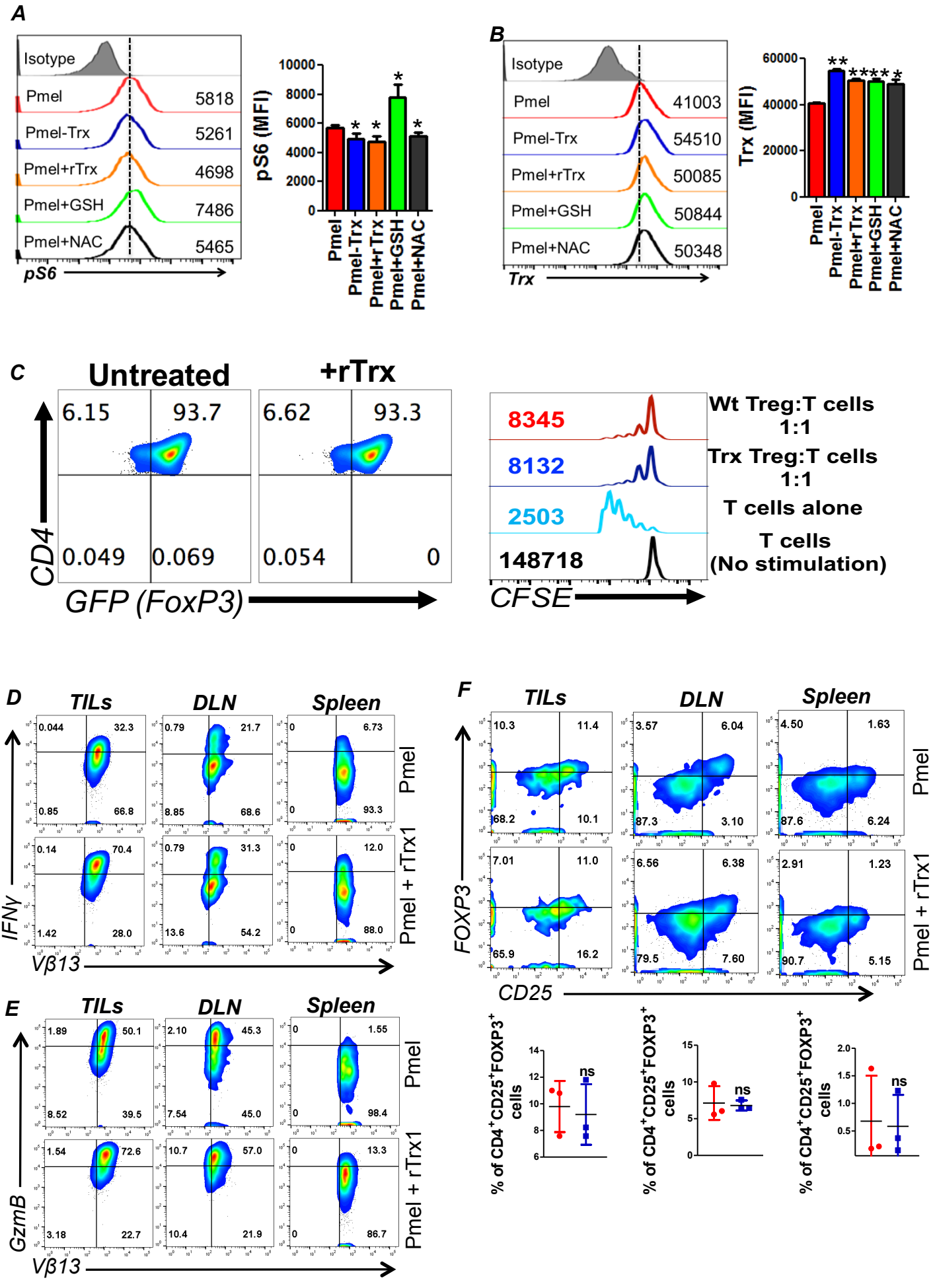
A
Isotype
Pmel
5818
Pmel-Trx
5261
Pmel+rTrx
4698
Pmel+GSH
7486
Pmel+NAC
5465
pS6
pS6 (MFI)
Pmel
Pmel-Trx
Pmel+rTrx
Pmel+GSH
Pmel+NAC
B
Isotype
Pmel
41003
Pmel-Trx
54510
Pmel+rTrx
50085
Pmel+GSH
50844
Pmel+NAC
50348
Trx
Trx (MFI)
C
Untreated
+rTrx
6.15
93.7
0.049
0.069
6.62
93.3
0.054
0
CD4
GFP (FoxP3)
8345
Wt Treg:T cells 1:1
8132
Trx Treg:T cells 1:1
2503
T cells alone
148718
T cells (No stimulation)
CFSE
D
TILs
DLN
Spleen
0.044
32.3
0.85
66.8
0.79
21.7
8.85
68.6
0
6.73
93.3
Pmel
0.14
70.4
1.42
28.0
0.79
31.3
13.6
54.2
0
12.0
88.0
Pmel + rTrx1
IFNγ
Vβ13
E
TILs
DLN
Spleen
1.89
50.1
8.52
39.5
2.10
45.3
7.54
45.0
0
1.55
98.4
Pmel
1.54
72.6
3.18
22.7
10.7
57.0
10.4
21.9
0
13.3
86.7
Pmel + rTrx1
GzmB
Vβ13
F
TILs
DLN
Spleen
10.3
11.4
68.2
10.1
3.57
6.04
87.3
3.10
4.50
1.63
87.6
6.24
Pmel
7.01
11.0
65.9
16.2
6.56
6.38
79.5
7.60
2.91
1.23
90.7
5.15
Pmel + rTrx1
FOXP3
CD25
% of CD4+CD25+FOXP3+ cells
ns
% of CD4+CD25+FOXP3+ cells
ns
% of CD4+CD25+FOXP3+ cells
ns

Figure S3*: Effect of rTrx on pS6 and iTreg generation.** Splenic T cells obtained from Pmel transgenic mice were stimulated with cognate antigen gp100 for three days either alone or in presence of rTrx, GSH, or L-NAC before evaluating for the expression levels of: ***A) pS6, and ***B)*** Trx. Adjacent bar represents cumulative data from three repeat experiments. *p* value *< 0.05, **<0.01. ***C)*** Purified $CD4^+$ T cells from EGFP-FoxP3-knockin mouse splenocytes were used for *in vitro* differentiation to induced regulatory T cells (iTreg's) by activating with plate-bound anti-CD3 (5 µg/ml), anti-CD28 (5 µg/ml) in presence TGFβ (4ng/ml), IL2 (100 unit/ml) for three days either in absence or presence of recombinant thioredoxin (rTrx). *Left panel* demonstrates expression of CD4 and FoxP3-GFP using BD FACS Accuri. *Right panel* represents iTreg's function in a T cell suppression assay that was performed using C57BL/6 mouse derived splenic $CD4^+CD25^{neg}$ T cells pre-labeled with CFSE dye (0.5µM) and co-cultured with Treg's at 1:1 ratio (100K each), and soluble anti-CD3 (2 µg/ml). After three days in co-culture the proliferation of CFSE labeled T cells was determined using FACS. Data was analyzed using FloJo software. Similar data was obtained in two independent experiments. ***D-F)*** C57BL/6 mice (n=3 mice/group) were inoculated (*s.c*) with 0.25×10^6 B16-F10 melanoma cells for 14 days, after which mice were adoptively transferred with 1×10^6 three day gp100 activated Pmel T cells, Pmel cells treated with rTrx (10 µg/ml). After 12 days of T cells transfer, lymphocytes were retrieved from the excised tumor and the indicated lymphoid organs. FACS plot is shown for the retrieved lymphocytes that were stimulated overnight with αCD3 (2 µg/ml) and αCD28 antibody (5 µg/ml) before staining with flurochrome-conjugated antibodies to determine intracellular IFNγ level **(D)**, and Granzyme B (GzmB) **(E)** level. *N*=3. *p<0.05, **p<0.005. ***F)*** *Upper panel* shows the FACS plot obtained for determining the regulatory T cells by intracellular FoxP3 staining on $CD4^+CD25^+$ fraction using the retrieved lymphocytes (TILs, DLN, spleen). *Lower panel* represent the cumulative data from different recipient mice. *N*=3. *p<0.05, **p<0.005.

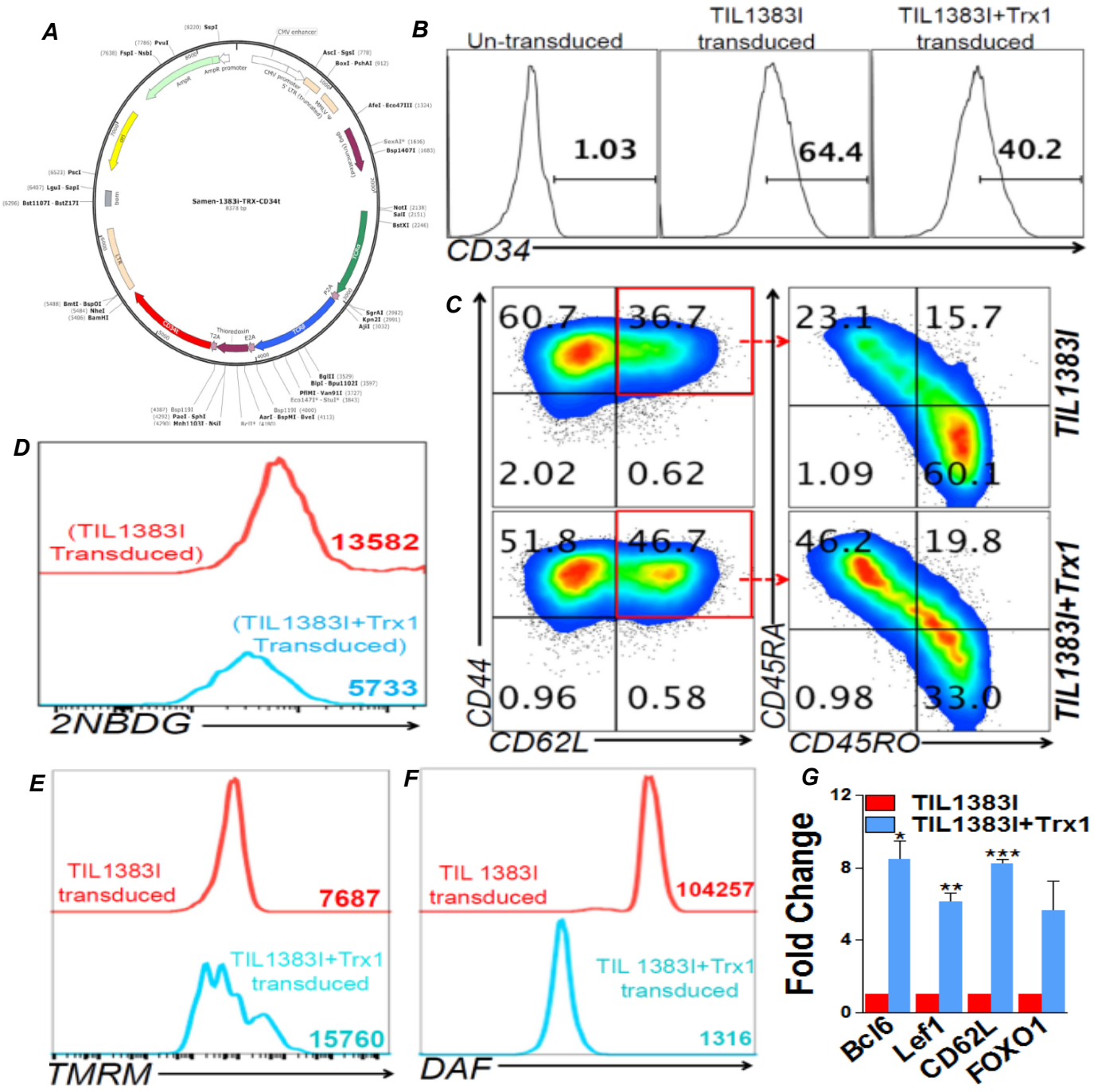

Figure S4: **Characterization of the human T cells transduced with tyrosinase reactive TIL1383I TCR construct with Trx gene insert.** Human T cells from normal healthy donor peripheral blood were retrovirally transduced with TIL1383I+Trx vector ***(A)*** or TIL1383I vector, and characterized for: ***B)*** transduction efficiency based on CD34 expression, ***C)*** CD62L, CD44, CD45RA, CD45RO expression, and ***D)*** Glucose uptake using 2NBDG assay. Cells were restimulated overnight with cognate hTyr peptide antigen using T2 cells for determining: ***E)*** Mitochondrial membrane potential using TMRM, and *F)* NO secretion using DAF. ***G)*** RNA from TCR transduced cells after REP was used to determine expression of 'stemness' genes using qPCR. *N=2. *p* value < *0.05; **p* value < 0.01, ****p* value < 0.005.