

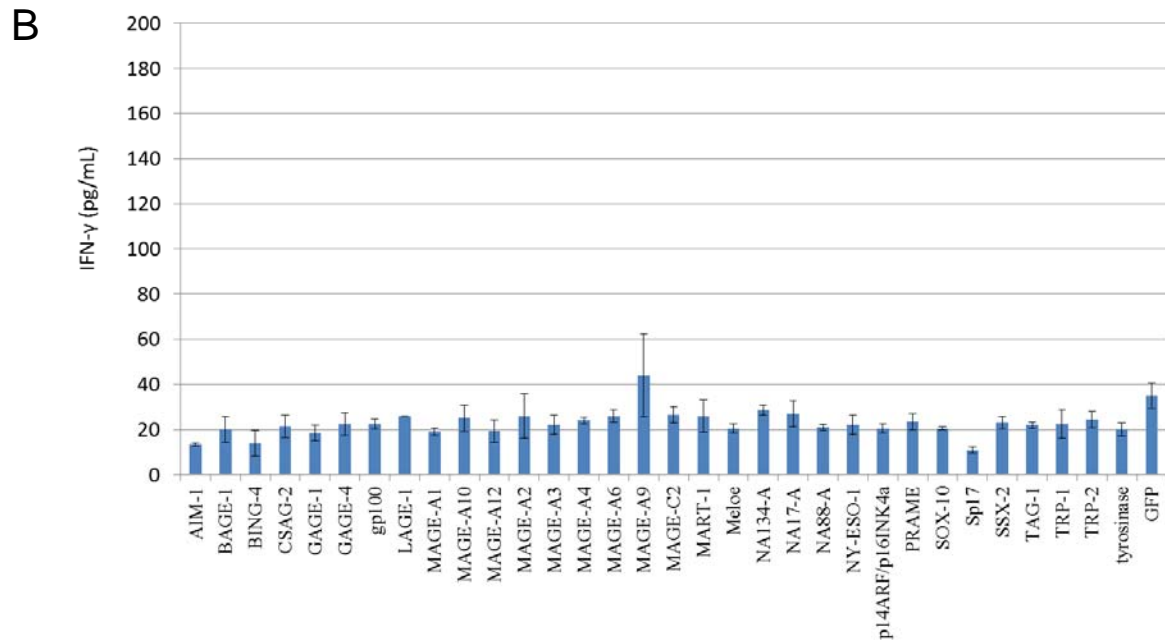
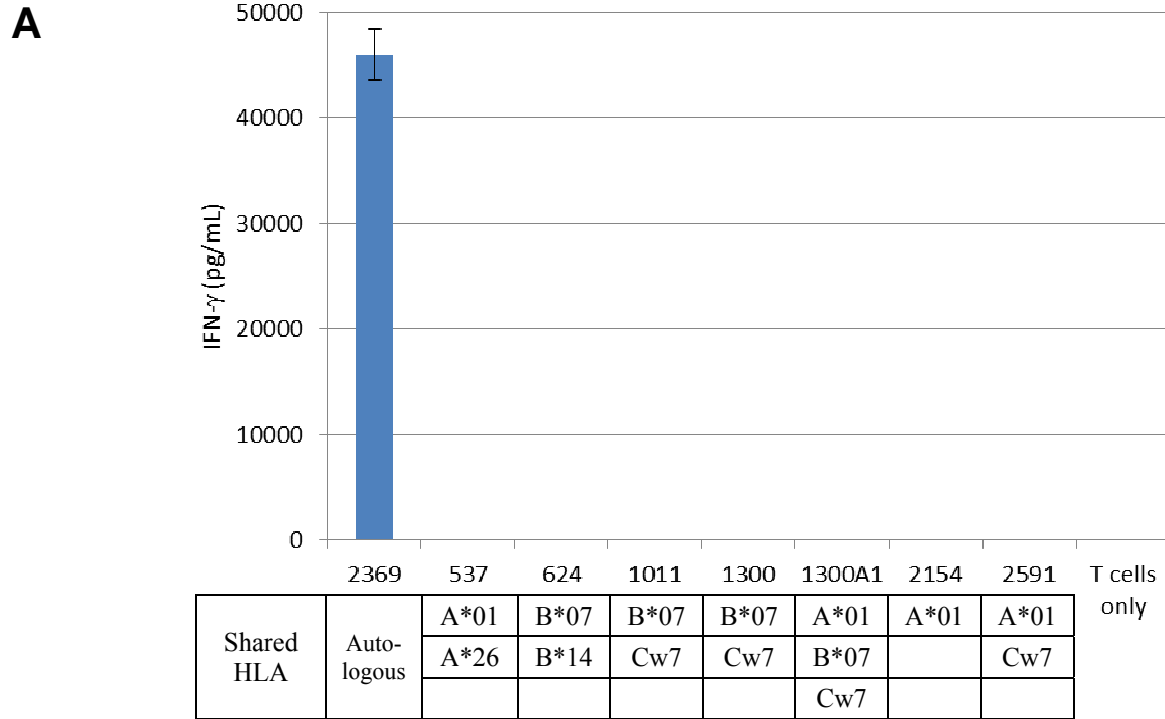
## Supplementary Material

### **Mutated PPP1R3B is recognized by T cells used to treat a melanoma patient who experienced a durable complete tumor regression**

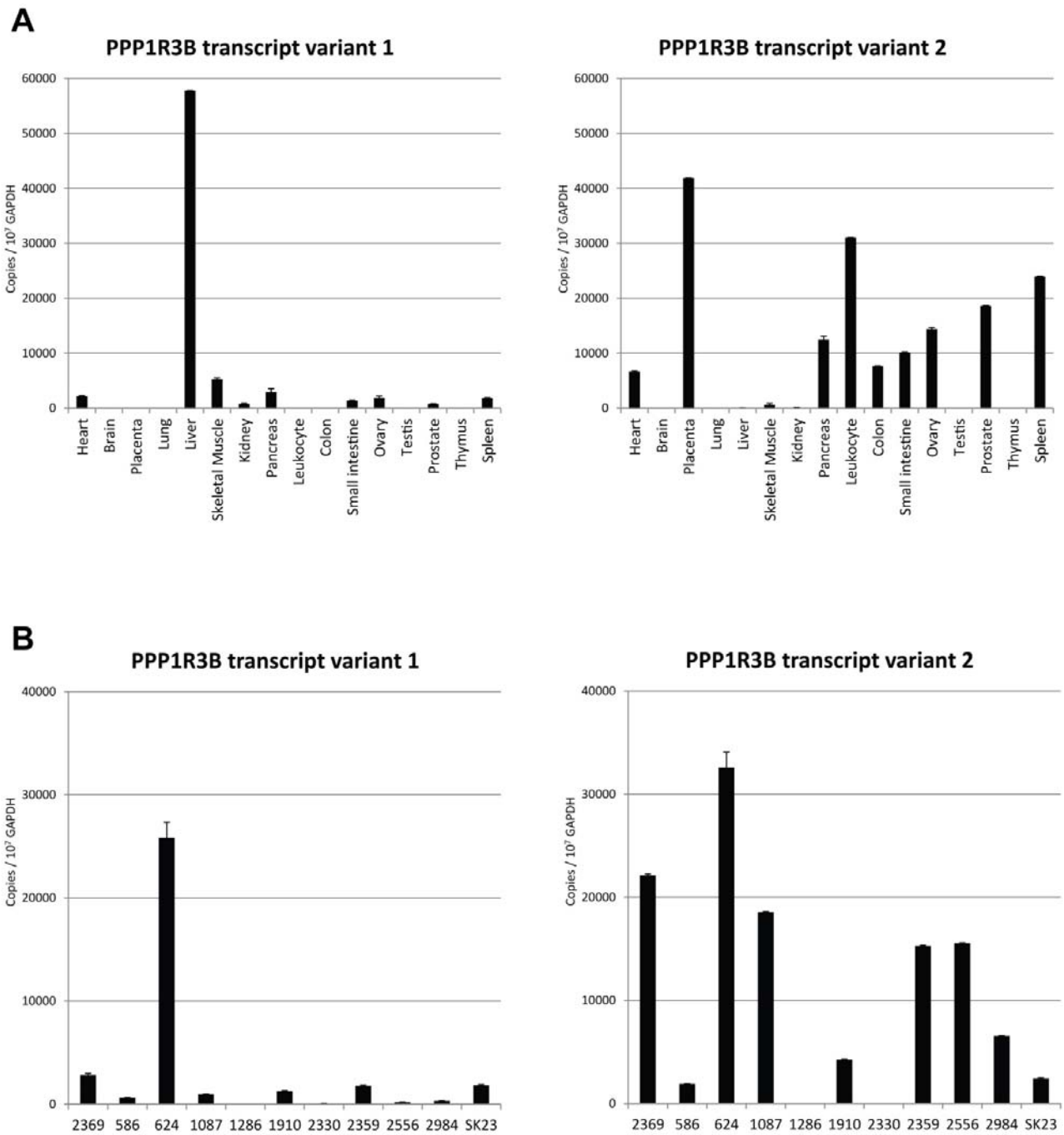
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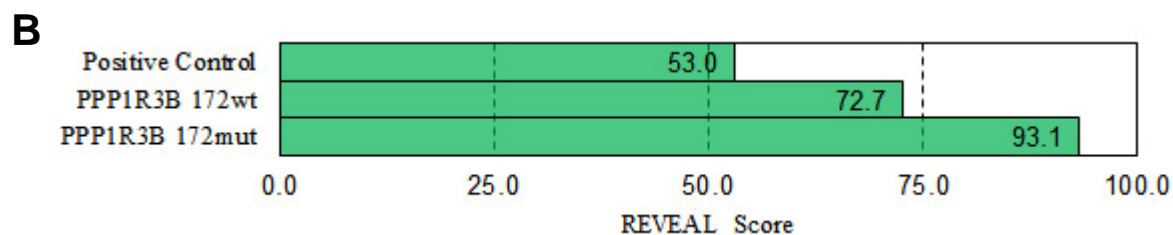
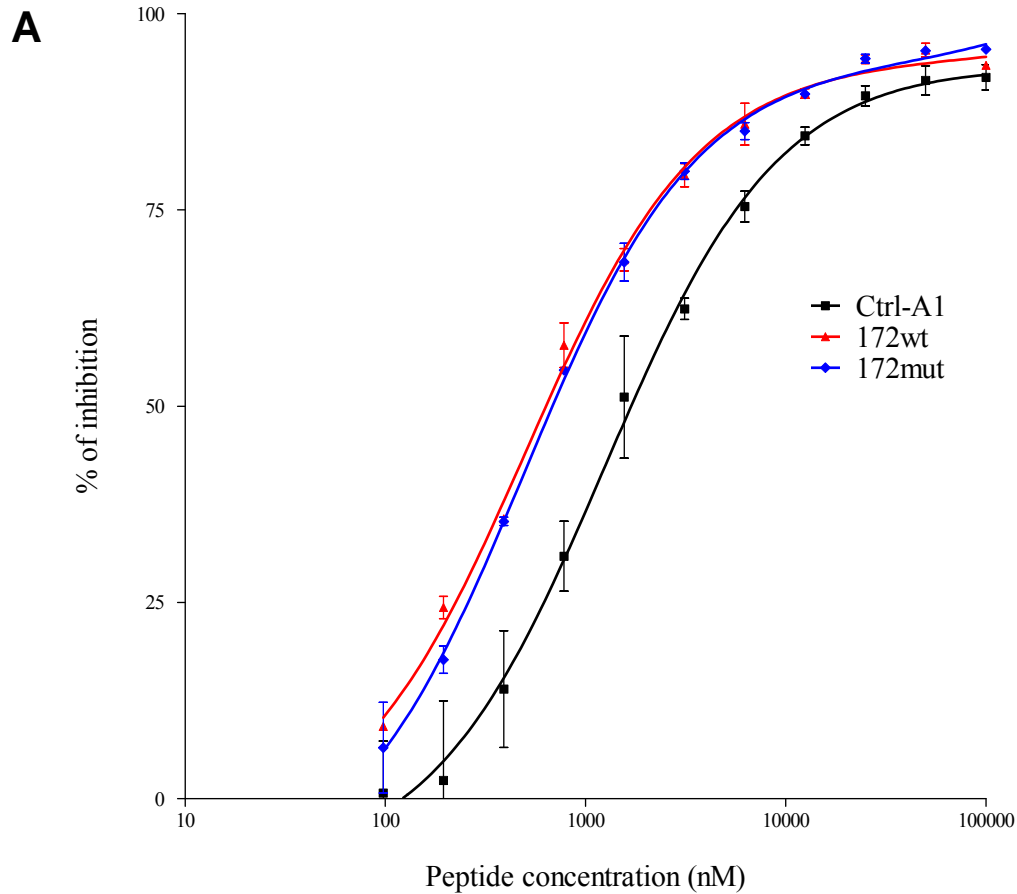
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**Supplementary Figure 1.** TIL 2369 T cells predominantly recognize unique, not shared antigens. **(A)** TIL 2369 T cells were co-cultured with a panel of melanoma cell lines that shared HLA gene expressions with autologous cells. **(B)** 293-A1 cells were transfected with a panel of previously described non-mutated melanoma antigens, and these transfected cells were then co-cultured with TIL 2369 T cells overnight. The concentration of IFN- $\gamma$  was determined by ELISA. A positive control of GFP was included to demonstrate the effectiveness of the transfection.



**Supplementary Figure 2.** PPP1R3B mRNA expression in normal tissue and melanoma cell lines. The copy numbers of PPP1R3B transcript variant 1 and variant 2 from normal tissue (**A**) and melanoma samples (**B**) were determined by quantitative PCR.



**Supplementary Figure 3.** Both PPP1R3B 172wt and 172mut bind HLA-A\*01. **(A)** Acid-treated autologous EBV-transformed B cells were incubated 24 hr with the fluorescent reference peptide Fl-A1, together with test peptides at various concentrations. The binding of fluorescent peptide was determined by flow cytometry, and the percentage of inhibition of fluorescent peptide was calculated. The  $IC_{50}$  of Ctrl-A1 (A\*01 consensus sequence) is 200 nM **(B)** Graphical representation of ProImmune REVEAL MHC-peptide binding assay for HLA A\*0101. Peptides and the positive control in DMSO (1 mM) were used in this assay. Peptides with scores > 45% of the positive control (REVEAL Score > 24) are considered to be good binders.

(A) Patient Infusion				
Rank #	TCR V $\beta$	CDR3 sequence	Counts	Freq. (%)
1	TRBV27 (V $\beta$ 14)	gccagcagcttcggacagggcgtcc ctcggcggcagctat	59752	42.58
2	TRBV25-1	gccagcagtgaaacctatggtgtctg ggccaacgtctgact	12202	8.70
3	TRBV11-3	gccagcagtgtagcagcagcggcgg ccccgtggagaccagctac	7244	5.16
4	TRBV28	gccagcagccccgcgccctcga aggaccgggagcgtgttt	5049	3.60
5	TRBV29-1	agcgttgatagtcagggcggggg gactatgctacacc	4646	3.31
6	TRBV7-3	gccagcagctatctagcgggagac ggcaatgagcagttc	2983	2.13
7	TRBV28	gccagcagttttgggactgggcagt tc	2941	2.10
8	TRBV13	gccagcagctcccgcagggggc cccaatgagcagttc	2819	2.01
9	TRBV3-1	gccagcagccaaggcgggggt gggtggagaatgagcagttc	2646	1.89
10	TRBV9	gccagcagcttcggcgggtcccgg ggagctgttt	2105	1.50
11	TRBV4-3	gccagcagccaagatctactagcg ggagaccggggagctgttt	1934	1.38
12	TRBV27	gccagcagtttatcgggacaggg ggcgtgaatggctacacc	1733	1.23
13	TRBV6-1	gccagcagagcgttgactagcgtg gagcagttc	1703	1.21
14	TRBV4-3	gccagcagccaagactccacagc gggaccagctac	1602	1.14
15	TRBV6-5	gccagcagctttggacaggggaaga accggggagcgtgttt	1595	1.14
16	TRBV12-4	gccacctcccggcccgtttgact atggctacacc	1439	1.03
17	TRBV6-2 & 6-3	gccagcagttacgtgcaaaacattc agtac	1124	0.80
18	TRBV4-1	gccagcagccaagagagagcgg ggccccggcagatagcagctat	1122	0.80
19	TRBV5-1	gccagcagcttccccggaactaat gaaaaactgttt	839	0.60
20	TRBV6-5	gccaccacctcacagggggcgc gggagatagcagctat	721	0.51

(B) After A REP				
Rank #	TCR V $\beta$	CDR3 sequence	Counts	Freq. (%)
1	TRBV27 (V $\beta$ 14)	gccagcagcttcggacagggcgtcc ctcggcggcagctat	29386	40.61
2	TRBV11-3	gccagcagtgtagcagcagcggcgg ccccgtggagaccagctac	7432	10.27
3	TRBV25-1	gccagcagtgaaacctatggtgtctg ggccaacgtctgact	1998	2.76
4	TRBV7-3	gccagcagctatctagcgggagac ggcaatgagcagttc	1426	1.97
5	TRBV13	gccagcagctcccgcagggggc cccaatgagcagttc	1256	1.74
6	TRBV28	gccagcagccccgcgccctcga aggaccgggagcgtgttt	1062	1.47
7	TRBV6-2 & 6-3	gccagcagttacgtgcaaaacatte agtac	1050	1.45
8	TRBV20-1	agtgtcagtagtagtcggggggg ggccaagagaccagctac	1002	1.38
9	TRBV29-1	agcgttgatagtcagggcggggg gactatgctacacc	941	1.30
10	TRBV30	gcctggcatgagagacaggttt aactatggctacacc	722	1.00
11	TRBV28	gccagcagttttgggactgggcagt tc	712	0.98
12	TRBV15	gccccctggggggggcggagaga aacctgaagctttc	668	0.92
13	TRBV6-5	gccagcagctttggacaggggaaga accggggagcgtgttt	489	0.68
14	TRBV25-1	gccagcagtgacgcggatcctac aatgagcagttc	435	0.60
15	TRBV12-4	gccagcagtttaagtcggggcgcac gggtccgggagaccagctac	406	0.56
16	TRBV6-5	gccagcagggcccgggtcagcaac taataaaaactgttt	403	0.56
17	TRBV4-1	gccagcagccaagagagagcgg ggccccggcagatagcagctat	388	0.54
18	TRBV6-5	gccagcagttacaccgggacaggg ggaatccattcagctac	335	0.46
19	TRBV6-5	gccaccacctcacagggggcgc gggagatagcagctat	294	0.41
20	TRBV28	gccagcagtttagatgtgtttacc aagagaccagctac	265	0.37

**Supplementary Table 1.** Representation of dominant T cell clonotypes is similar in the infused TIL 2369 (A) and *in vitro* re-expanded TILs (B). TCR beta chain variable region cDNA sequences were obtained by deep sequencing. The dominant TRBV27 (V $\beta$ 14) clonotype, as well as the majority of the additional clonotypes, is similarly represented in the infused TIL 2369 as well as in TILs that were expanded in a second REP with anti-CD3 antibody.