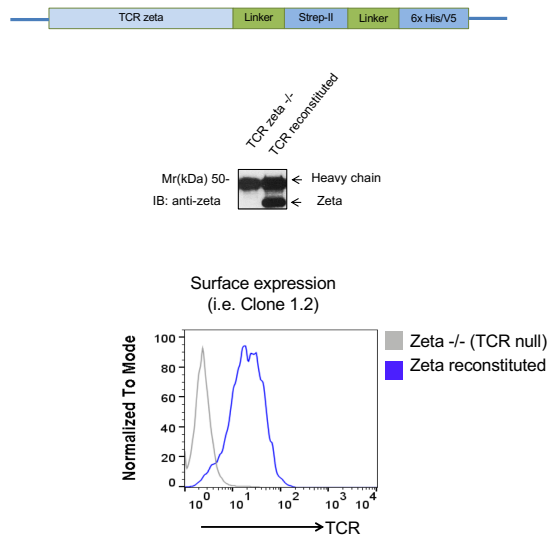


## Supplementary Figures

Thaker et al (2019)

SFigure 1



**SFigure 1: Tandem affinity purification (TAP) from reconstituted TCR-null zeta<sup>-/-</sup> 3A9 cells.** Upper panel: structure of TAP zeta chain construct with tandem Step-II and His/V5 tags for purification. Middle panel: presence of the TAP zeta chain in constituted TCR-null zeta<sup>-/-</sup> 3A9 cells. Lower panel: flow cytometry showing the surface expression of the TCR complex on TCR-null zeta<sup>-/-</sup> 3A9 cells transfected to express TAP zeta complex.

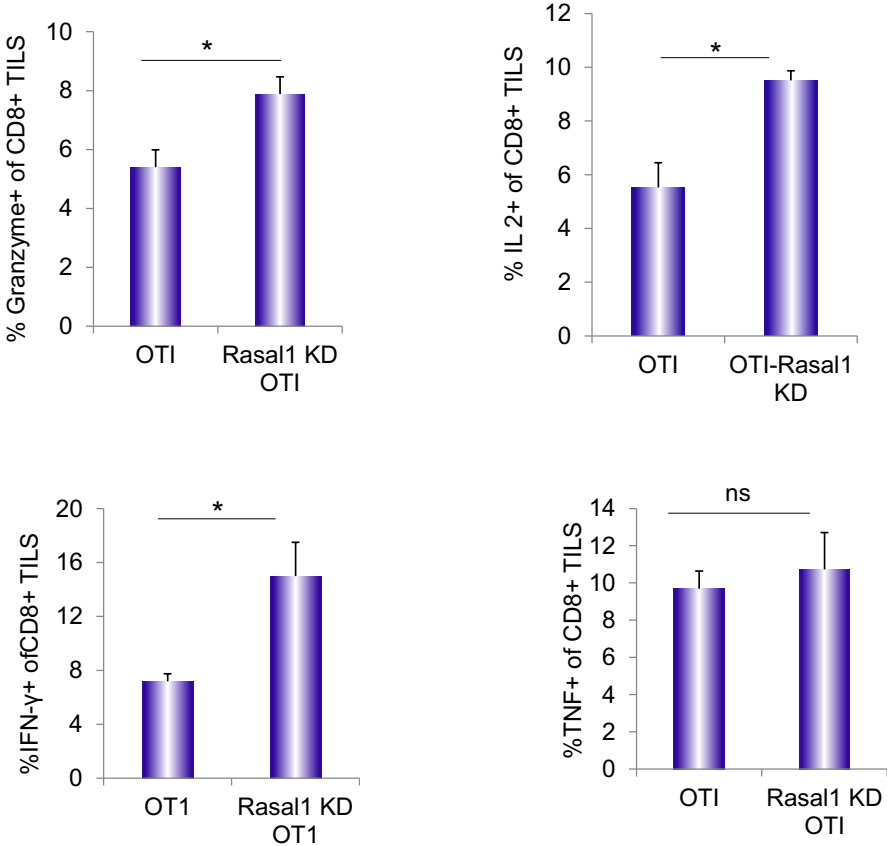
SFigure 2

EXPERIMENT 1									
Protein Accession	Protein Description	Protein Masscot Score	Protein Expect	Protein Mass	Protein Matches	Protein Rank	Peptide Rank	Peptide sequence	Peptide sequence
sp P24161-1 CD32_MOUSE	Isolform CD-3 zeta of T-cell surface glycoprotein CD3 zeta chain OS=Mus musculus GN=CD327	675	1.00E-63	18780	45	1	1	EDVDLTK	EDVDLTK
sp P24161-1 CD92_MOUSE	Isolform CD-3 zeta of T-cell surface glycoprotein CD3 zeta chain OS=Mus musculus GN=CD247	508	8.00E-47	18780	23	1	1	REVDLTK	REVDLTK
tr E9Q357 E9Q357_MOUSE	Uncharacterized protein OS=Mus musculus GN=Hd3p	489	7.00E-45	35358	33	1	1	AQIDNLR	AQIDNLR
tr E9Q320 E9Q320_MOUSE	Uncharacterized protein OS=Mus musculus GN=4732456N10R[K] [RIKEN cDNA 4732456N10 gene]	446	1.30E-40	58587	33	1	1	FAFDIK	FAFDIK
tr E9Q386 E9Q386_MOUSE	Uncharacterized protein OS=Mus musculus GN=CD3g	336	1.30E-29	20725	16	1	1	NLVGVDSR	NLVGVDSR
sp P04231 C030_MOUSE	T-cell surface glycoprotein CD3 delta chain OS=Mus musculus GN=CD3d	220	5.40E-18	19305	13	1	1	LGDNWPR	LGDNWPR
tr A0N813 A0N813_MOUSE	T-cell receptor chain (Fragment) OS=Mus musculus GN=Trbc2	131	4.30E-09	32088	11	1	1	VSLEFSK	VSLEFSK
sp P22461 CODE_MOUSE	T-cell surface glycoprotein CD3 epsilon chain OS=Mus musculus GN=CD3e	131	4.60E-09	21779	13	1	1	DVSGLNDR	DVSGLNDR
tr A2NTU7 A2NTU7_MOUSE	Cytotoxic T-cell receptor OS=Mus musculus GN=Trac	124	2.10E-08	30063	8	1	1	TMSEGFIDK	TMSEGFIDK
tr A2NTV6 A2NTV6_MOUSE	Beta-chain (Fragment) OS=Mus musculus	107	1.00E-06	15916	8	1	1	GDIPGGK	GDIPGGK
tr E9Q400 E9Q400_MOUSE	Uncharacterized protein OS=Mus musculus GN=281062205R[K]	71	0.0046	22277	5	1	1	TTTENVSQTSNVK	TTTENVSQTSNVK
tr B2R0C3 B2R0C3_MOUSE	Tyrosine-protein kinase receptor OS=Mus musculus GN=Harr	63	0.03	147291	5	1	1	SEVTEEA	SEVTEEA
sp Q92288 HSA1_MOUSE	<b>RasGAP-activating-like protein 1 OS=Mus musculus GN=Raal1</b>	61	0.03	90285	4	1	1	EAQGTSPAR	EAQGTSPAR
sp A2A400 DHX8_MOUSE	ATP-dependent RNA helicase DHX8 OS=Mus musculus GN=Dhx8	57	0.11	143337	3	1	1	VAAASVAK	VAAASVAK
tr A2M266 A2M266_MOUSE	CD3 zeta OS=Mus musculus GN=CD247	56	0.14	21549	2	1	1	NPGEGVYVALOK	NPGEGVYVALOK
tr D324W5 D324W5_MOUSE	Uncharacterized protein OS=Mus musculus GN=I200074P13R[K]	49	0.75	20422	6	1	1	NDLMLK	NDLMLK
tr D324W5 D324W5_MOUSE	Uncharacterized protein OS=Mus musculus GN=I200074P13R[K]	47	1.1	20422	6	1	1	NDLMLK	NDLMLK
tr E9QL28 E9QL28_MOUSE	Uncharacterized protein OS=Mus musculus GN=Igf1n1	47	1.1	140949	3	1	1	LTVIEDLR	LTVIEDLR
tr D9CFN9 D9CFN9_MOUSE	RIKEN cDNA 2210010C04, Isolform CRA, b OS=Mus musculus GN=2210010C04R[K]	46	1.4	27089	1	1	1	VYVNWICQTIAAN	VYVNWICQTIAAN
tr D3YX85 D3YX85_MOUSE	Uncharacterized protein OS=Mus musculus GN=6539001G17R[K]	43	2.6	107593	3	1	1	EISEVQR	EISEVQR
EXPERIMENT 2									
Protein Accession	Protein Description	Protein Masscot Score	Protein Expect	Protein Mass	Protein Matches	Protein Rank	Peptide Rank	Peptide sequence	Peptide sequence
sp A2AFR3-1 FRPD4_MOUSE	Isolform 1 of FERM and PDZ domain-containing protein 4 OS=Mus musculus GN=Frmpd4	44	2.3	146084	3	1	1	KADESPR	KADESPR
sp P01739 TV42_MOUSE	T-cell receptor alpha chain V region 2B4 OS=Mus musculus	146	1.30E-10	14773	9	1	1	ALMSFVNGEK	ALMSFVNGEK
sp P04231 C030_MOUSE	T-cell surface glycoprotein CD3 delta chain OS=Mus musculus GN=CD3d	288	8.60E-25	19305	25	1	1	LGDNWPR	LGDNWPR
sp P10126 EFT1A1_MOUSE	Elongation factor 1-alpha 1 OS=Mus musculus GN=Eft1a1	88	0.0081	50424	5	1	1	LRLDVYK	LRLDVYK
sp P17897 LVZ1_MOUSE	Lysozyme C-1 OS=Mus musculus GN=Lvz1	53	0.29	17240	1	1	1	STDVGFQISR	STDVGFQISR
sp P20203 GPR78_MOUSE	78 kDa glucose-regulated protein OS=Mus musculus GN=Hsp95	508	8.30E-47	72492	22	2	2	VLESDLK	VLESDLK
sp P22461 CODE_MOUSE	T-cell surface glycoprotein CD3 epsilon chain OS=Mus musculus GN=CD3e	460	6.10E-42	21779	67	1	1	GTFGASPPR	GTFGASPPR
sp P24161-1 CD92_MOUSE	Isolform CD-3 zeta of T-cell surface glycoprotein CD3 zeta chain OS=Mus musculus GN=CD247	652	3.00E-61	18780	110	1	1	ADDPENGGK	ADDPENGGK
sp P62697 CYC_MOUSE	Cyclothrome c, somatic OS=Mus musculus GN=Cy3	47	1.1	11712	3	1	1	ADLAATK	ADLAATK
sp P63017 HSP70_MOUSE	Heat shock cognate 71 kDa protein OS=Mus musculus GN=Hsp98	500	5.10E-46	71055	23	1	1	NTITPTK	NTITPTK
sp Q5F2E7-1 NUFP2_MOUSE	Isolform 1 of Nuclear fragile X mental retardation-interacting protein 2 OS=Mus musculus GN=N715	41	1.60E-67	75668	27	1	1	SEGTDAVDK	SEGTDAVDK
sp Q61171 PBRX2_MOUSE	Peroviradoin-2 OS=Mus musculus GN=Pbrx2	187	4	21936	3	1	1	QITVDLPIGR	QITVDLPIGR
sp Q91V65 DDX1_MOUSE	ATP-dependent RNA helicase DDX1 OS=Mus musculus GN=DDX1	62	1.10E-14	83474	12	1	1	VPVDFEDGK	VPVDFEDGK
sp Q91F41 RTCA_MOUSE	RNA-splicing ligase RtcB homolog OS=Mus musculus GN=D10W54.52e	44	0.032	55727	3	1	1	TALDESVDVPAK	TALDESVDVPAK
sp Q9G048 NUDC2_MOUSE	Nucl domain-containing protein 2 OS=Mus musculus GN=Nuclcd2	103	2.1	17820	2	1	1	MSAPFERE	MSAPFERE
sp Q9G042 F195A_MOUSE	Protein FAM195A OS=Mus musculus GN=Fam195a	103	2.90E-06	17974	5	1	1	TGPTPOODFSR	TGPTPOODFSR
sp Q92288 HSA1_MOUSE	<b>RasGAP-activating-like protein 1 OS=Mus musculus GN=Raal1</b>	40	0.03	90285	4	1	1	BDLAAPRDSIGTS	BDLAAPRDSIGTS
tr A0N813 A0N813_MOUSE	T-cell receptor chain (Fragment) OS=Mus musculus GN=Trbc2	55	0.19	32088	3	1	1	EYHSYGTDPQATYK	EYHSYGTDPQATYK
tr A2A420 A2A420_MOUSE	Dystropin, muscular dystrophy OS=Mus musculus GN=Dmd	52	0.35	427676	3	1	1	ERIKK	ERIKK
tr A2A163 A2A163_MOUSE	Hephaestin OS=Mus musculus GN=Hsth	49	2.8	130956	3	1	1	GLTVQGGSR	GLTVQGGSR

\* protein hits from control samples (TCR deficient 3A9 T cells) and common contaminants have been excluded.  
 \* a representative peptide from each hit is shown

SFigure 2: Rasal1 peptides are detected by TCR complex by tandem affinity purification (TAP) and MS/MS analysis. Peptides detected by MS/MS from 2 separate experiments.

SFigure 3



**SFigure 3:** MFI expression values for granzyme B and cytokines on scrambled siRNA or Rasal1 KD siRNA T-cells. Histogram showing the % of CD8 TILs expressing granzyme, Interleukin 2, IFN- $\gamma$ 1 and TNF $\alpha$ . T-cells were transfected with scrambled siRNA or Rasal1 KD siRNA T-cells followed by removal of TILs from EL4 tumors as described in the text.

Fig. 1B

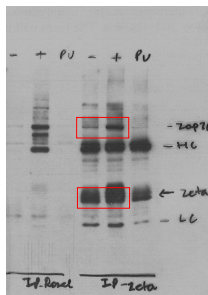


Fig. 1D

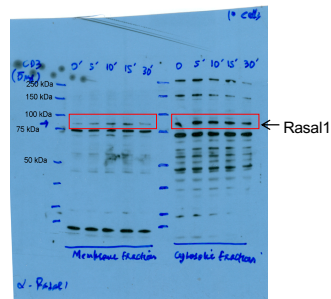


Fig. 2B

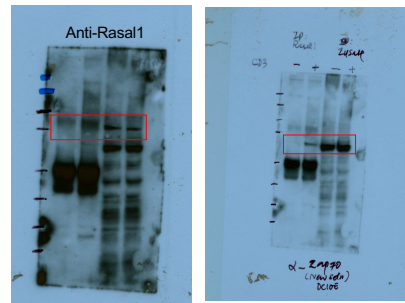
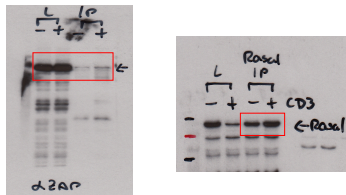


Fig. 2C



**Figure 4:** Uncropped versions of Figures 1-2





Fig. 4A (top)

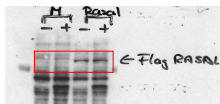
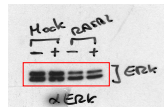
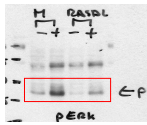


Fig. 4A (bottom)

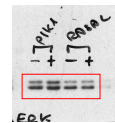
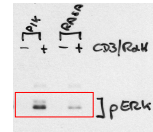
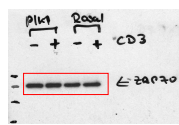


Fig. 4A inset



Fig. 4C

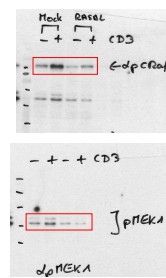


Fig. 5A uncropped

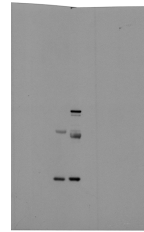
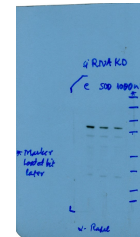


Fig. 5D uncropped



**SFigure 4:** Uncropped versions of figures 4-5