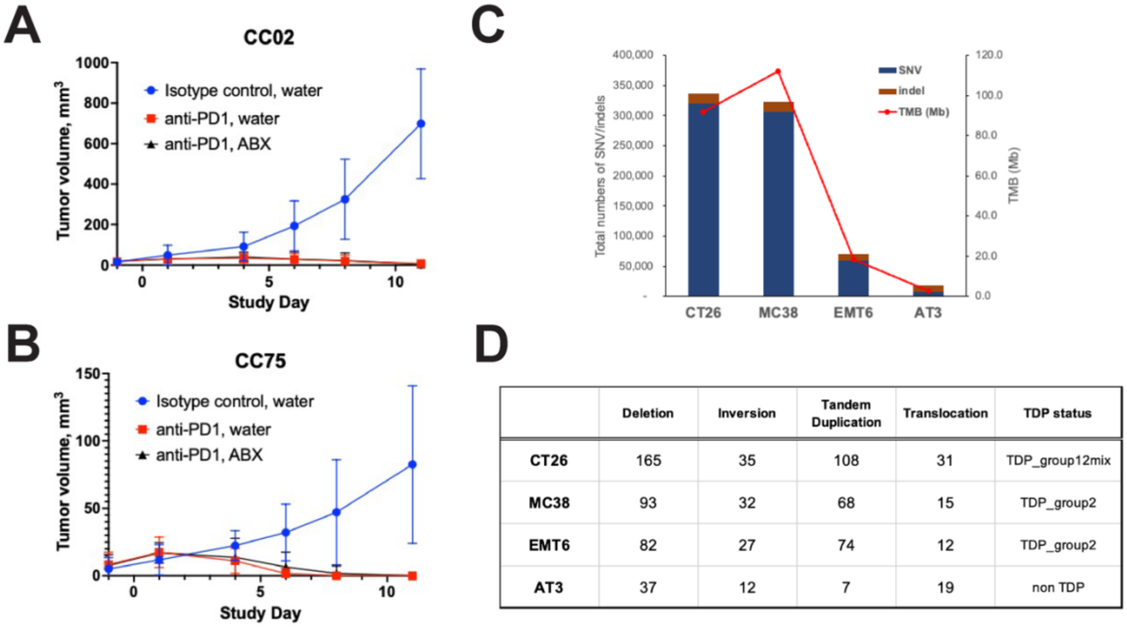
Mapping the genetic landscape establishing a tumor immune microenvironment favorable for anti-PD-1 response in mice and humans.

Supplementary Figures and Tables



	Deletion	Inversion	Tandem Duplication	Translocation	TDP status
CT26	165	35	108	31	TDP_group12mix
MC38	93	32	68	15	TDP_group2
EMT6	82	27	74	12	TDP_group2
AT3	37	12	7	19	non TDP

Figure S1. (A-B) Responder strain (CC02 and CC075 F1) mice given broad spectrum antibiotics (vancomycin, streptomycin, ampicillin, and colistin) *ad libitum* in drinking water display no loss in response to PD1 blockade. (C) SNP/indel count and tumor mutation burden (per megabase) in each tumor model. (D) Summary of single base and structural variations, and TDP assessment, quantified in each tumor model.

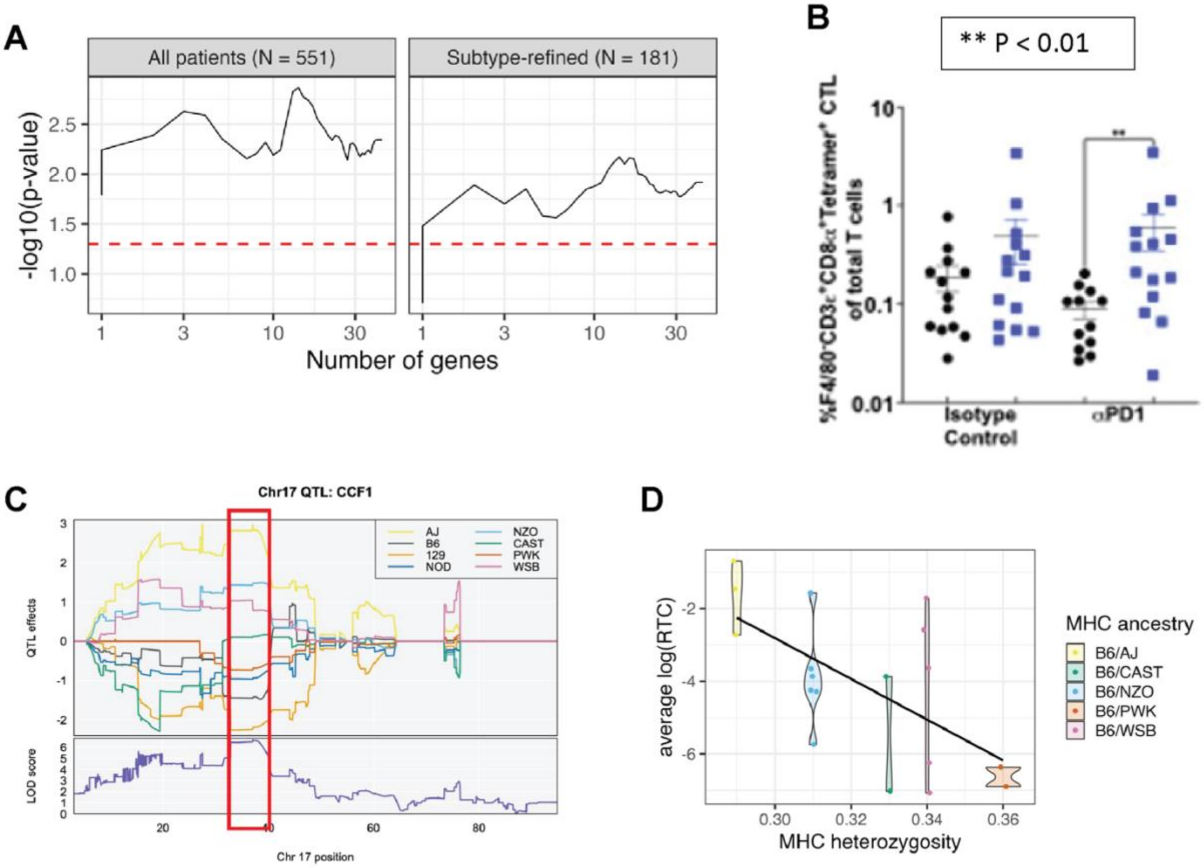


Figure S2. (A) Statistical significance levels of different numbers of genes in ICI response prognostic gene set for all patients (left) or subtype 4-restricted patients (right). (B) FACS data showing MC38-specific CTL as a percentage of total T cells. black circles indicate measurements taken from non-responder strain mice (CC36 F1, CC79 F1, and CC80 F1), blue boxes indicate measurements taken from responder strain mice (CC01 F1, CC02 F1, and CC075 F1). (C) Top panel shows QTL effects plots showing the effect on RTC of carrying a haplotype derived from each of the eight founder parental lines of the CC. Lower number indicates a lower value of RTC, which is associated with better response. Bottom panel shows LOD score across the locus from QTL mapping, as in Figure 2. Red box indicates approximate boundaries of the MHC locus. (D) Plot of ICI response as measured by RTC, stratified by each CCF1 line's ancestry at the MHC locus, versus MHC heterozygosity. As in (C), a lower RTC number is associated with better response.

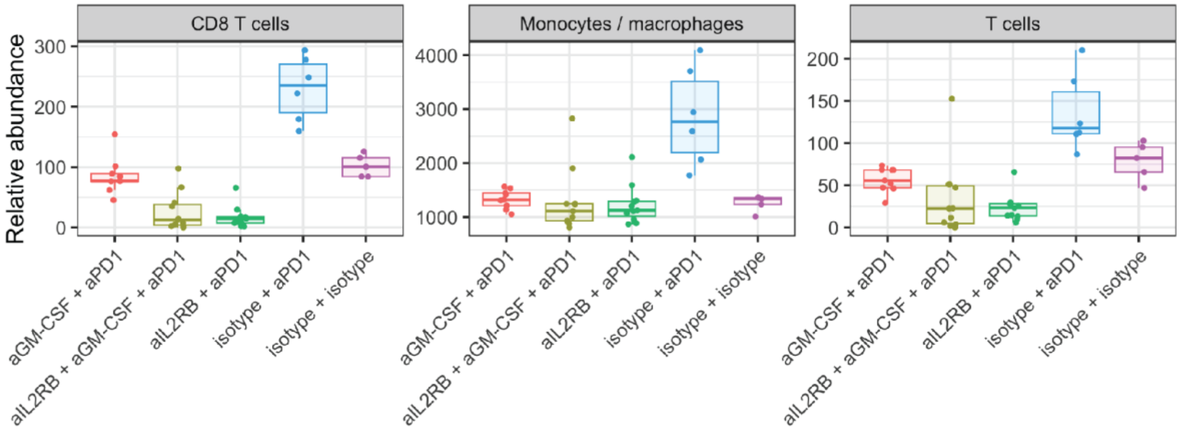
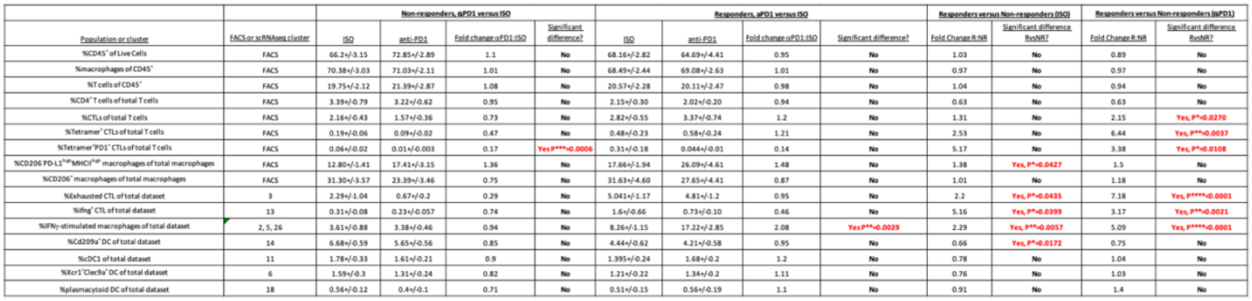


Figure S3. Bulk RNA-Seq deconvolution using mMCP-Counter on samples of MC38 tumors from CC75F1 mice treated with blocking antibodies to PD1, GM-CSF, and/or IL2RB.

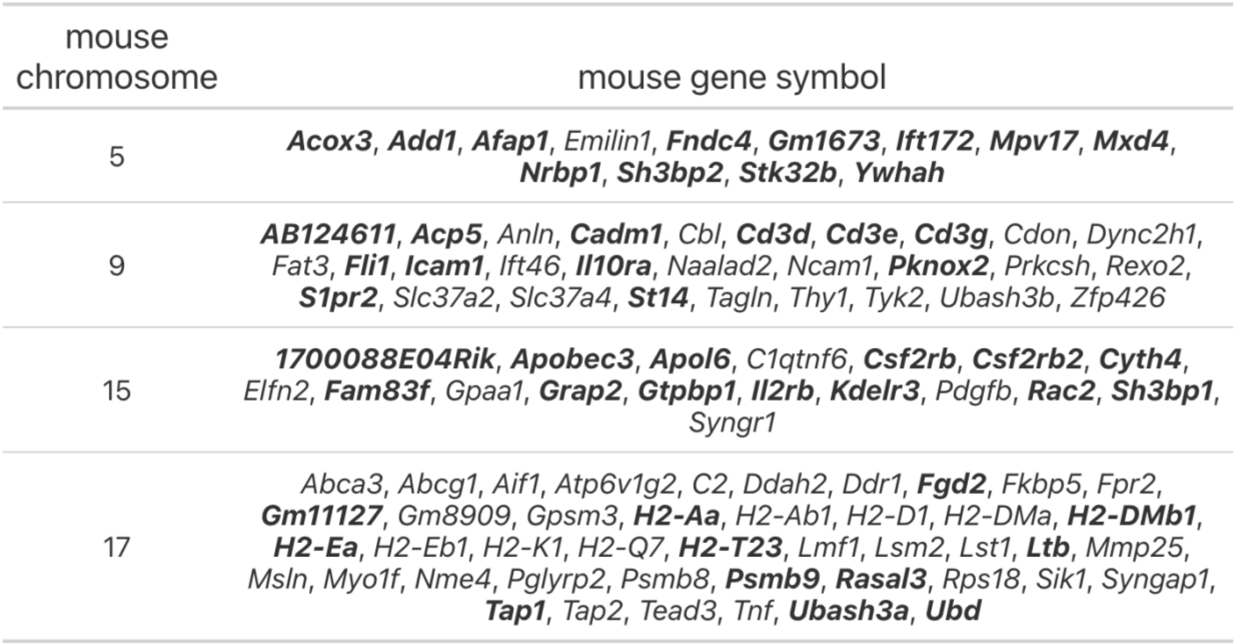
JAX strain number	Strain name
664	C57BL/6J
651	BALB/cJ
21238	CC001
21236	CC002
21237	CC003
20944	CC004
20945	CC005
29625	CC007
26971	CC008
18856	CC009
21889	CC010
18854	CC011
28409	CC012
18859	CC015
24684	CC016
22870	CC017
25131	CC023
21891	CC024
18857	CC025
25130	CC027
20946	CC032
25910	CC033
25127	CC036
26426	CC044
24683	CC057
27298	CC058
26427	CC060
23830	CC065
25908	CC068
18855	CC074
27293	CC075
25989	CC078
25990	IL6411(CC079)
25988	IL6573(CC080)

Table S1: Mouse strains used in this study including links for sourcing mice.



		Non-responders, αPD1 versus ISO				Responders, aPD1 versus ISO				Responders versus Non-responders (ISO)		Responders versus Non-responders (αPD1)	
Population or cluster	FACS or scRNAseq cluster	ISO	anti-PD1	Fold change αPD1:ISO	Significant difference?	ISO	anti-PD1	Fold change αPD1:ISO	Significant difference?	Fold Change R:NR	Significant difference RvsNR?	Fold Change R:NR	Significant difference RvsNR?
%CD45^{+} of Live Cells	FACS	66.2+/-3.15	72.85+/-2.89	1.1	**No**	68.16+/-2.82	64.69+/-4.41	0.95	**No**	1.03	**No**	0.89	**No**
%macrophages of CD45^{+}	FACS	70.38+/-3.03	71.03+/-2.11	1.01	**No**	68.49+/-2.44	69.08+/-2.63	1.01	**No**	0.97	**No**	0.97	**No**
%T cells of CD45^{+}	FACS	19.75+/-2.12	21.39+/-2.87	1.08	**No**	20.57+/-2.28	20.11+/-2.47	0.98	**No**	1.04	**No**	0.94	**No**
%CD4^{+} T cells of total T cells	FACS	3.39+/-0.79	3.22+/-0.62	0.95	**No**	2.15+/-0.30	2.02+/-0.20	0.94	**No**	0.63	**No**	0.63	**No**
%CTLs of total T cells	FACS	2.16+/-0.43	1.57+/-0.36	0.73	**No**	2.82+/-0.55	3.37+/-0.74	1.2	**No**	1.31	**No**	2.15	**Yes, P*=0.0270**
%Tetramer^{+} CTLs of total T cells	FACS	0.19+/-0.06	0.09+/-0.02	0.47	**No**	0.48+/-0.23	0.58+/-0.24	1.21	**No**	2.53	**No**	6.44	**Yes, P**=0.0037**
%Tetramer^{+}PD1^{+} CTLs of total T cells	FACS	0.06+/-0.02	0.01+/-0.003	0.17	**Yes P***=0.0006**	0.31+/-0.18	0.044+/-0.01	0.14	**No**	5.17	**No**	3.38	**Yes, P*=0.0108**
%CD206^{-}PD-L1^{high}MHCII^{high} macrophages of total macrophages	FACS	12.80+/-1.41	17.41+/-3.15	1.36	**No**	17.66+/-1.94	26.09+/-4.61	1.48	**No**	1.38	**Yes, P*=0.0427**	1.5	**No**
%CD206^{+} macrophages of total macrophages	FACS	31.30+/-3.57	23.39+/-3.46	0.75	**No**	31.63+/-4.60	27.65+/-4.41	0.87	**No**	1.01	**No**	1.18	**No**
%Exhausted CTL of total dataset	3	2.29+/-1.04	0.67+/-0.2	0.29	**No**	5.041+/-1.17	4.81+/-1.2	0.95	**No**	2.2	**Yes, P*=0.0435**	7.18	**Yes, P****<0.0001**
%Ifng^{+} CTL of total dataset	13	0.31+/-0.08	0.23+/-0.057	0.74	**No**	1.6+/-0.66	0.73+/-0.10	0.46	**No**	5.16	**Yes, P*=0.0399**	3.17	**Yes, P**=0.0021**
%IFNγ-stimulated macrophages of total dataset	2, 5, 26	3.61+/-0.88	3.38+/-0.46	0.94	**No**	8.26+/-1.15	17.22+/-2.85	2.08	**Yes P**=0.0029**	2.29	**Yes, P**=0.0057**	5.09	**Yes, P****<0.0001**
%Cd209a^{+} DC of total dataset	14	6.68+/-0.59	5.65+/-0.56	0.85	**No**	4.44+/-0.62	4.21+/-0.58	0.95	**No**	0.66	**Yes, P*=0.0172**	0.75	**No**
%cDC1 of total dataset	11	1.78+/-0.33	1.61+/-0.21	0.9	**No**	1.395+/-0.24	1.68+/-0.2	1.2	**No**	0.78	**No**	1.04	**No**
%Xcr1^{+}Clec9a^{+} DC of total dataset	6	1.59+/-0.3	1.31+/-0.24	0.82	**No**	1.21+/-0.22	1.34+/-0.2	1.11	**No**	0.76	**No**	1.03	**No**
%plasmacytoid DC of total dataset	18	0.56+/-0.12	0.4+/-0.1	0.71	**No**	0.51+/-0.15	0.56+/-0.19	1.1	**No**	0.91	**No**	1.4	**No**

Table S2: Overview of flow cytometry results.



mouse chromosome	mouse gene symbol
5	***Acox3***, ***Add1***, ***Afap1***, *Emilin1*, ***Fndc4***, ***Gm1673***, ***Ift172***, ***Mpv17***, ***Mxd4***, ***Nrbp1***, ***Sh3bp2***, ***Stk32b***, ***Ywhah***
9	***AB124611***, ***Acp5***, *Anln*, ***Cadm1***, *Cbl*, ***Cd3d***, ***Cd3e***, ***Cd3g***, *Cdon*, *Dync2h1*, *Fat3*, ***Fli1***, ***Icam1***, *Ift46*, ***Il10ra***, *Naalad2*, *Ncam1*, ***Pknox2***, *Prkcsh*, *Rexo2*, ***S1pr2***, *Slc37a2*, *Slc37a4*, ***St14***, *Tagln*, *Thy1*, *Tyk2*, *Ubash3b*, *Zfp426*
15	***1700088E04Rik***, ***Apobec3***, ***Apol6***, *C1qtnf6*, ***Csf2rb***, ***Csf2rb2***, ***Cyth4***, *Elfn2*, ***Fam83f***, *Gpaa1*, ***Grap2***, ***Gtpbp1***, ***Il2rb***, ***Kdelr3***, *Pdgfb*, ***Rac2***, ***Sh3bp1***, *Syngr1*
17	*Abca3*, *Abcg1*, *Aif1*, *Atp6v1g2*, *C2*, *Ddah2*, *Ddr1*, ***Fgd2***, *Fkbp5*, *Fpr2*, ***Gm11127***, *Gm8909*, *Gpsm3*, ***H2-Aa***, *H2-Ab1*, *H2-D1*, *H2-DMa*, ***H2-DMb1***, ***H2-Ea***, *H2-Eb1*, *H2-K1*, *H2-Q7*, ***H2-T23***, *Lmf1*, *Lsm2*, *Lst1*, ***Ltb***, *Mmp25*, *Msln*, *Myo1f*, *Nme4*, *Pglyrp2*, *Psmb8*, ***Psmb9***, ***Rasal3***, *Rps18*, *Sik1*, *Syngap1*, ***Tap1***, *Tap2*, *Tead3*, *Tnf*, ***Ubash3a***, ***Ubd***

Table S3: Mouse orthologs of the human genes prioritized using our CST algorithm predicted to have a major role establishing a tumor immune microenvironment favorable for aPD1 response in mice and humans