Supplementary material

Targeting the PD-1/PD-L1 pathway potentiates immunoediting to counterbalance neutral evolution in a mouse model of colorectal cancer

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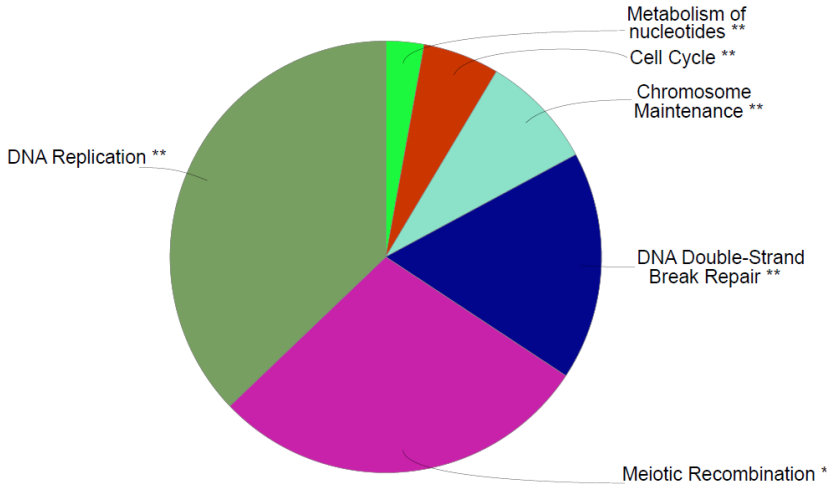


Figure S1. Enriched pathways of the significantly upregulated genes in the MC38 cell line vs normal skin samples. The pie chart is created using ClueGO and shows the enriched groups (the name of the group is given by the most significant term). The sizes of the sections correlate with the number of terms included in a group.

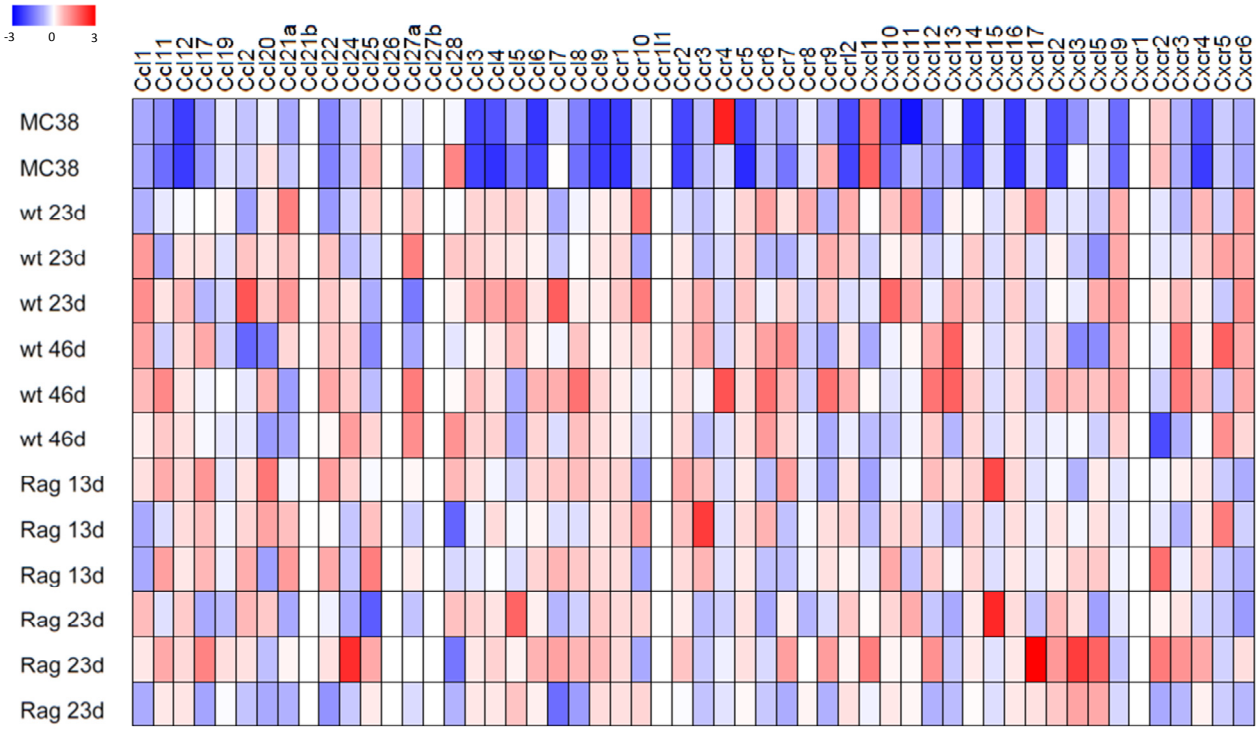


Figure S2. z-score heatmaps of the log2 transformed normalized counts of chemokines and chemokine receptors in MC38, wild type and RAG1$^{-/-}$ samples.

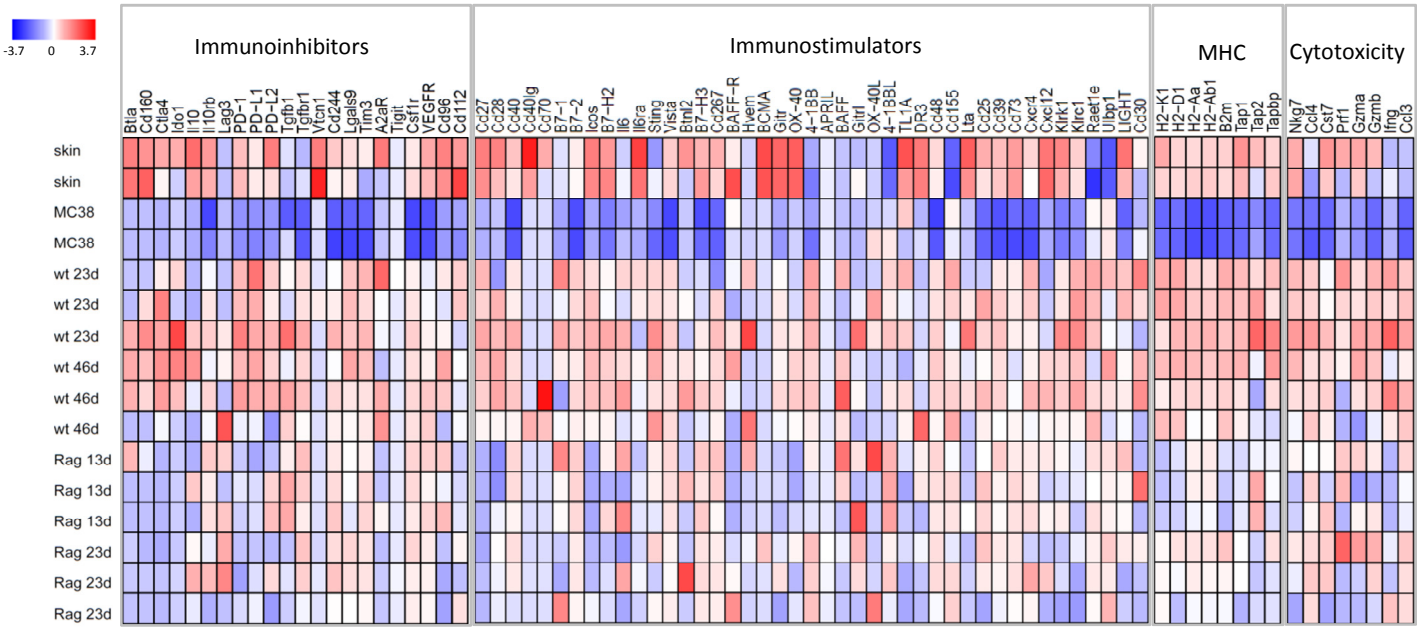
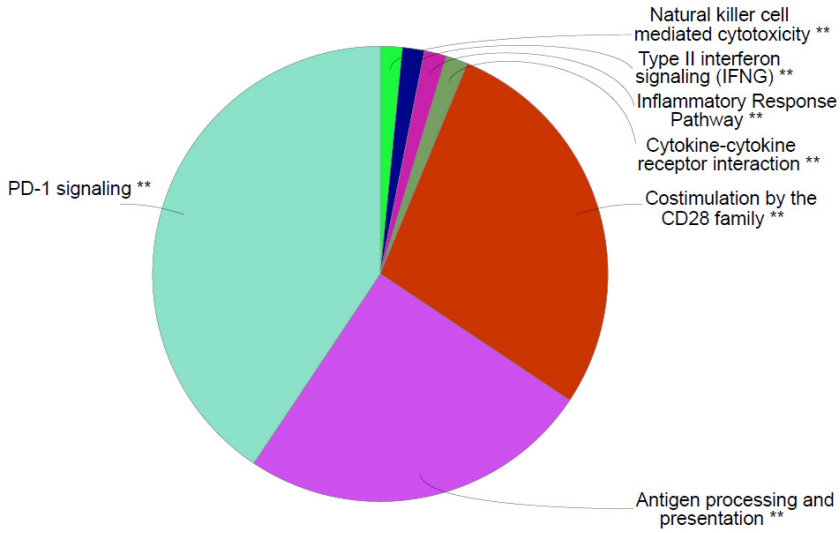


Figure S3. z-score heatmaps of the log2 transformed normalized counts of selected genes in MC38, skin, wild type and RAG1$^{-/-}$ samples.

A



B

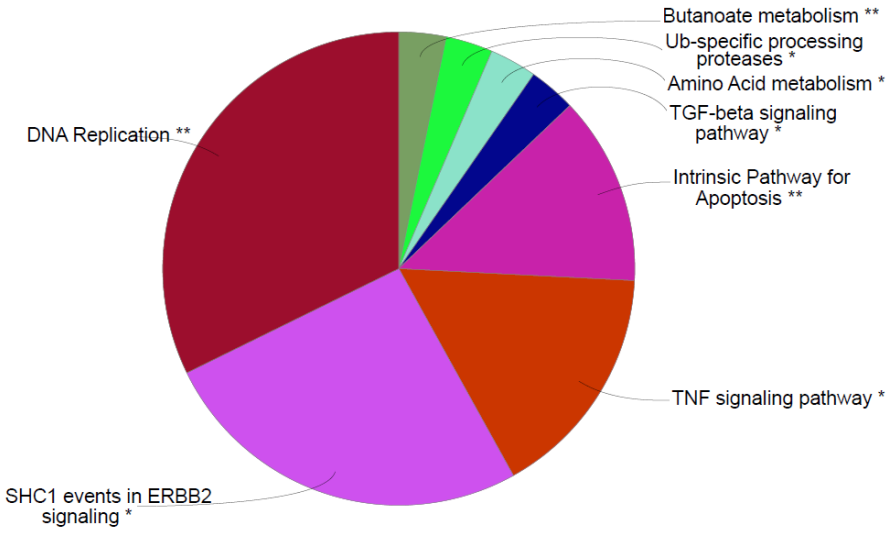
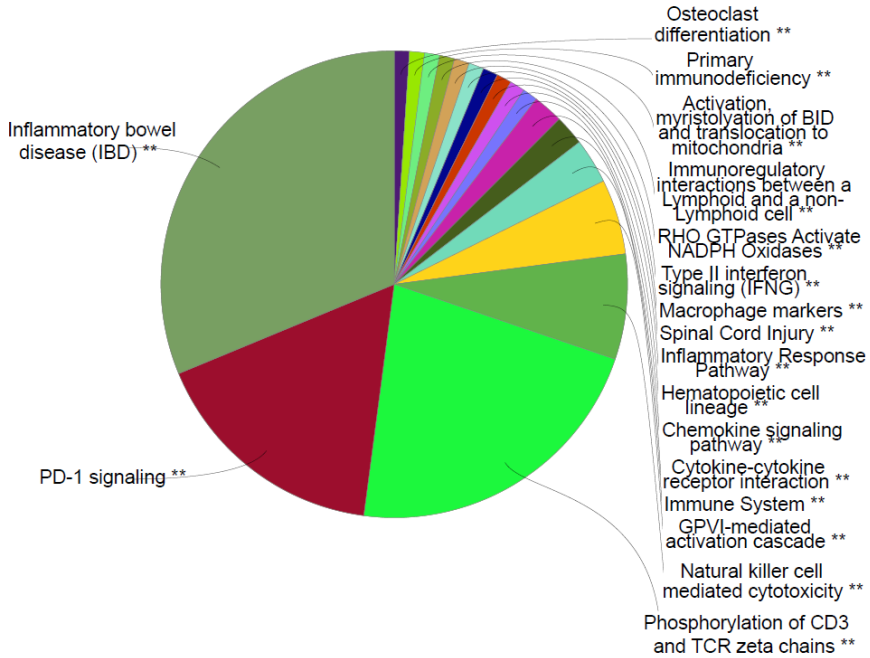


Figure S4. Enriched pathways of the significantly A) upregulated and B) downregulated genes in wild type day 23 vs RAG1$^{-/-}$ day 23 tumors. The pie chart is created using ClueGO and shows the enriched groups (the name of the group is given by the most significant term). The sizes of the sections correlate with the number of terms included in a group.

A



B

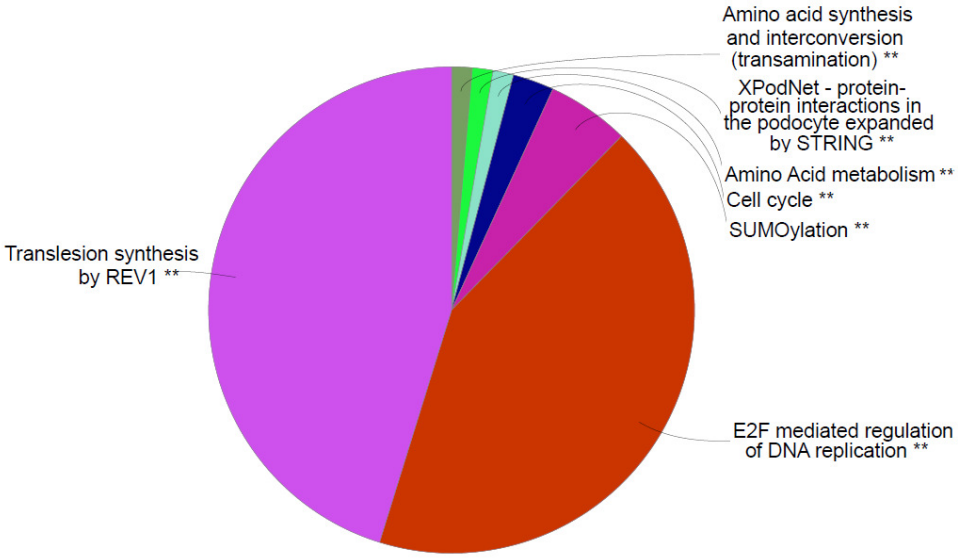


Figure S5. Enriched pathways of the significantly A) upregulated and B) downregulated genes in the antiPD-L1 vs the IgG2b tumors. The pie chart is created using ClueGO and shows the enriched groups (the name of the group is given by the most significant term). The sizes of the sections correlate with the number of terms included in a group.

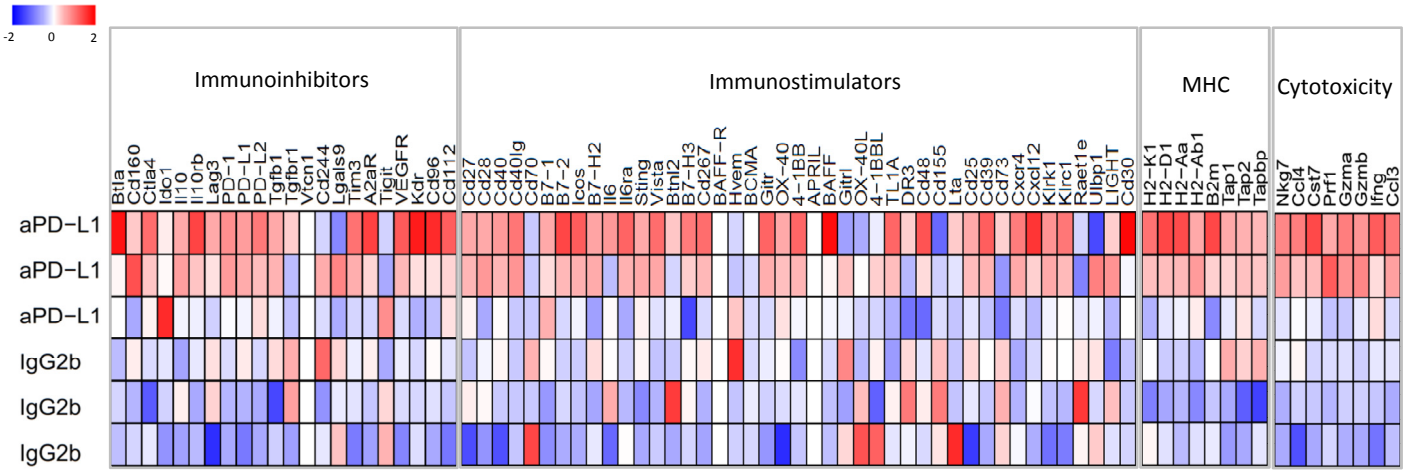


Figure S6. z-score heatmaps of the log2 transformed normalized counts of selected genes in the anti-PD-L1 and the IgG2b control samples.

Table S1. Number of mutations that changed their variant allele frequencies (VAF) and cancer cell fractions (CCF) between time points in the three wt and $RAG1^{-/-}$ samples out of the total 3394 (shared between wt23 and wt46) and 3443 (shared between $RAG1^{-/-}$13 and $RAG1^{-/-}$23) mutations.

wt23 vs wt46 diff in VAF > 0.2	wt23 vs wt46 diff in CCF > 0.3	wt23 vs wt46 not shared mutations	$RAG1^{-/-}$13 vs $RAG1^{-/-}$23 diff in VAF > 0.2	$RAG1^{-/-}$ vs $RAG1^{-/-}$23 diff in CCF > 0.3	$RAG1^{-/-}$ vs $RAG1^{-/-}$23 not shared mutations
138	496	1057	113	446	1087
177	542	1031	105	394	1130
144	614	1015	125	675	1004

Table S2. List of expressed predicted neoantigens present in the MC38 cell line and the RAG1$^{-/-}$ samples from day 23, and absent from the wild type samples from day 23

Peptide_mut	Chr	Start	Ref	Alt	Gene	Protein	Amino_acids	Db_nM	Db_Rank	Kb_nM	Kb_Rank
YLVANDGEI	chr15	6658562	A	C	Fyb	ENSMUSP00000087947	D/A	285.48	0.125	NA	NA
ASFHSGNI	chr3	145314878	A	G	Col24a1	ENSMUSP00000029848	T/A	NA	NA	145.5	0.5
LTLLAGLL	chr7	43468715	G	A	Vsig10l	ENSMUSP00000103611	A/T	NA	NA	482.14	1.3
LGLTLLAGL	chr7	43468715	G	A	Vsig10l	ENSMUSP00000103611	A/T	NA	NA	310.16	0.9
KGTLYYYTL	chr10	80322726	G	T	Pcsk4	ENSMUSP00000020340	T/K	NA	NA	39.498	0.125
IIPEYVSAF	chr11	82987611	T	C	Slfn9	ENSMUSP00000044435	I/V	NA	NA	190.61	0.6
IGDLRLATL	chr11	101263944	G	A	Wnk4	ENSMUSP00000099397	G/R	NA	NA	301	0.9
KGYYFNKGTL	chr10	80322726	G	T	Pcsk4	ENSMUSP00000020340	T/K	NA	NA	150.84	0.5
KGTLYYYTLL	chr10	80322726	G	T	Pcsk4	ENSMUSP00000020340	T/K	NA	NA	50.742	0.175
LSFLCRYVAV	chr17	56769541	C	G	Dus3l	ENSMUSP00000007747	P/A	NA	NA	109.83	0.4
NKGYYFNKGTL	chr10	80322726	G	T	Pcsk4	ENSMUSP00000020340	T/K	NA	NA	482.24	1.3
FNKGTLYYYTL	chr10	80322726	G	T	Pcsk4	ENSMUSP00000020340	T/K	NA	NA	353.97	1
NKGTLYYYTLL	chr10	80322726	G	T	Pcsk4	ENSMUSP00000020340	T/K	NA	NA	134.84	0.5
KGTLYYYTLLL	chr10	80322726	G	T	Pcsk4	ENSMUSP00000020340	T/K	NA	NA	115.6	0.4
IMRKCFPQNRL	chr13	59512429	T	G	Agtpbp1	ENSMUSP00000022040	K/Q	NA	NA	361.08	1
WLSFLCRYVAV	chr17	56769541	C	G	Dus3l	ENSMUSP00000007747	P/A	NA	NA	326.53	0.9
SSLLAGPRL	chr9	108448377	T	A	Klhdc8b	ENSMUSP00000142325	Q/L	NA	NA	378.61	1.1

Table S3. List of expressed predicted neoantigens present in the MC38 cell line and the IgG2b control samples, and absent from the anti-PD-L1 samples

Peptide_mut	Chr	Start	Ref	Alt	Gene	Protein	Amino_acid	Db_nM	Db_Rank	Kb_nM	Kb_Rank
AQLANDVVL	chr10	18056095	C	G	Reps1	ENSMUSP00000123238	P/A	39.083	0.015	NA	NA
ISFVNAIPW	chr11	75489864	A	C	Prpf8	ENSMUSP00000018449	E/A	407.2091	0.15	206.0801	0.6
YFLDNLDTL	chr11	109392383	A	C	Gna13	ENSMUSP00000020930	K/T	186.9848	0.08	NA	NA
YLVANDGEI	chr15	6658562	A	C	Fyb	ENSMUSP00000087947	D/A	285.4786	0.125	NA	NA
YSLLLDTYI	chr15	37945070	T	G	Rrm2b	ENSMUSP00000022901	I/L	51.0251	0.02	NA	NA
AAQLANDVVL	chr10	18056095	C	G	Reps1	ENSMUSP00000123238	P/A	102.2957	0.05	NA	NA
APSLLNWTRV	chr10	82699172	G	C	Hcfc2	ENSMUSP00000020478	R/T	465.7171	0.175	NA	NA
ISFVNAIPWV	chr11	75489864	A	C	Prpf8	ENSMUSP00000018449	E/A	119.3035	0.05	NA	NA
KALLNGDGAI	chr14	24164486	C	A	Dlg5	ENSMUSP00000087879	E/D	34.5384	0.015	NA	NA
RAAQLANDVVL	chr10	18056095	C	G	Reps1	ENSMUSP00000123238	P/A	241.6992	0.09	NA	NA
AQLANDVVLQI	chr10	18056095	C	G	Reps1	ENSMUSP00000123238	P/A	496.5346	0.175	NA	NA
AAPSLLNWTRV	chr10	82699172	G	C	Hcfc2	ENSMUSP00000020478	R/T	439.9904	0.15	NA	NA
ISFVNAIPWVI	chr11	75489864	A	C	Prpf8	ENSMUSP00000018449	E/A	111.3205	0.05	NA	NA
IKALLNGDGAI	chr14	24164486	C	A	Dlg5	ENSMUSP00000087879	E/D	434.6625	0.15	NA	NA
IATVQAFL	chr2	12410009	G	T	Fam188a	ENSMUSP00000028105	P/T	NA	NA	440.4284	1.2
LSYLRATL	chr2	120030296	G	C	Jmjd7	ENSMUSP00000041220	V/L	NA	NA	9.8735	0.02
ICYGSAPL	chr3	110250130	C	A	Prmt6	ENSMUSP00000140836	S/I	NA	NA	109.7087	0.4
YAFAFSQV	chr6	28885053	T	G	Snd1	ENSMUSP00000001460	I/S	NA	NA	11.6314	0.025
KARKYFEL	chr7	28185387	G	T	Dyrk1b	ENSMUSP00000083064	R/L	NA	NA	138.8444	0.5
LTLLAGLL	chr7	43468715	G	A	Vsig10l	ENSMUSP00000103611	A/T	NA	NA	482.1449	1.3
KILTFDRL	chr7	45720370	A	G	Rpl18	ENSMUSP00000103365	Q/R	NA	NA	102.6694	0.4
LTFDRLAL	chr7	45720370	A	G	Rpl18	ENSMUSP00000103365	Q/R	NA	NA	71.8819	0.25
AMFLGANV	chr9	57546426	T	C	Mpi	ENSMUSP00000034856	D/G	NA	NA	126.6132	0.4
TSSGYPTL	chr9	102885994	G	C	Ryk	ENSMUSP00000135858	S/T	NA	NA	290.6021	0.9
FVMDYIPV	chr10	7705809	G	T	Lats1	ENSMUSP00000132078	G/V	NA	NA	205.7593	0.6
SSYAHGGF	chr11	55487518	G	T	G3bp1	ENSMUSP00000018727	L/F	NA	NA	46.7578	0.15
VSRHHRAL	chr11	72796528	G	C	Zzef1	ENSMUSP00000068790	G/R	NA	NA	285.9487	0.8
VIATVQAFL	chr2	12410009	G	T	Fam188a	ENSMUSP00000028105	P/T	NA	NA	468.1571	1.3
SVYGEVSRL	chr2	129066190	C	G	Ttl	ENSMUSP00000046883	A/G	NA	NA	96.1962	0.3
KSFRQKPNL	chr6	48619619	C	G	Zfp775	ENSMUSP00000056290	S/R	NA	NA	93.9273	0.3
AGLRRLLLM	chr7	25203598	C	A	Dedd2	ENSMUSP00000049763	R/L	NA	NA	430.6135	1.2
KARKYFELL	chr7	28185387	G	T	Dyrk1b	ENSMUSP00000083064	R/L	NA	NA	77.7964	0.25
LGLTLLAGL	chr7	43468715	G	A	Vsig10l	ENSMUSP00000103611	A/T	NA	NA	310.1638	0.9
ISPTVRATL	chr9	21981403	C	A	Rgl3	ENSMUSP00000035726	V/L	NA	NA	162.2901	0.5
LNWTRVSSF	chr10	82699172	G	C	Hcfc2	ENSMUSP00000020478	R/T	NA	NA	290.533	0.9
SAWVPFGGL	chr10	128802236	C	A	Tmem198b	ENSMUSP00000050451	V/F	NA	NA	42.9606	0.15
VSRHHRALL	chr11	72796528	G	C	Zzef1	ENSMUSP00000068790	G/R	NA	NA	149.4984	0.5
IIPEYVSAF	chr11	82987611	T	C	Slfn9	ENSMUSP00000044435	I/V	NA	NA	190.6065	0.6

IGDLRLATL	chr11	101263944	G	A	Wnk4	ENSMUSP00000099397	G/R	NA	NA	300.9963	0.9
VSLKGLCQL	chr11	116069391	T	C	Unc13d	ENSMUSP00000074549	E/G	NA	NA	325.2974	0.9
AGFTKLAPV	chr13	89704367	G	C	Vcan	ENSMUSP00000105173	P/A	NA	NA	53.1282	0.175
SIQDFLLRM	chr14	65648256	G	C	Nuggc	ENSMUSP00000078434	M/I	NA	NA	205.8907	0.6
WSLSYLRATL	chr2	120030296	G	C	Jmjd7	ENSMUSP00000041220	V/L	NA	NA	415.0376	1.2
SAWVPFGGLM	chr10	128802236	C	A	Tmem198b	ENSMUSP00000050451	V/F	NA	NA	481.1183	1.3
VHSEMYSLLL	chr15	37945070	T	G	Rrm2b	ENSMUSP00000022901	I/L	NA	NA	377.1472	1.1
LSFLCRYVAV	chr17	56769541	C	G	Dus3l	ENSMUSP00000007747	P/A	NA	NA	109.8274	0.4
IMRKCFPQNRL	chr13	59512429	T	G	Agtpbp1	ENSMUSP00000022040	K/Q	NA	NA	361.0831	1
AGFTKLAPVPL	chr13	89704367	G	C	Vcan	ENSMUSP00000105173	P/A	NA	NA	221.6772	0.7
ISWVKEGFTFL	chr16	73978491	G	C	Robo2	ENSMUSP00000112776	L/V	NA	NA	63.4355	0.2
WLSFLCRYVAV	chr17	56769541	C	G	Dus3l	ENSMUSP00000007747	P/A	NA	NA	326.5283	0.9
ALYINLAAI	chr18	61989909	C	A	Sh3tc2	ENSMUSP00000055094	Y/*	308.6139	0.125	NA	NA
AALYINLAAI	chr18	61989909	C	A	Sh3tc2	ENSMUSP00000055094	Y/*	52.0202	0.025	NA	NA
VAALYINLAAI	chr18	61989909	C	A	Sh3tc2	ENSMUSP00000055094	Y/*	286.6858	0.125	415.9277	1.2