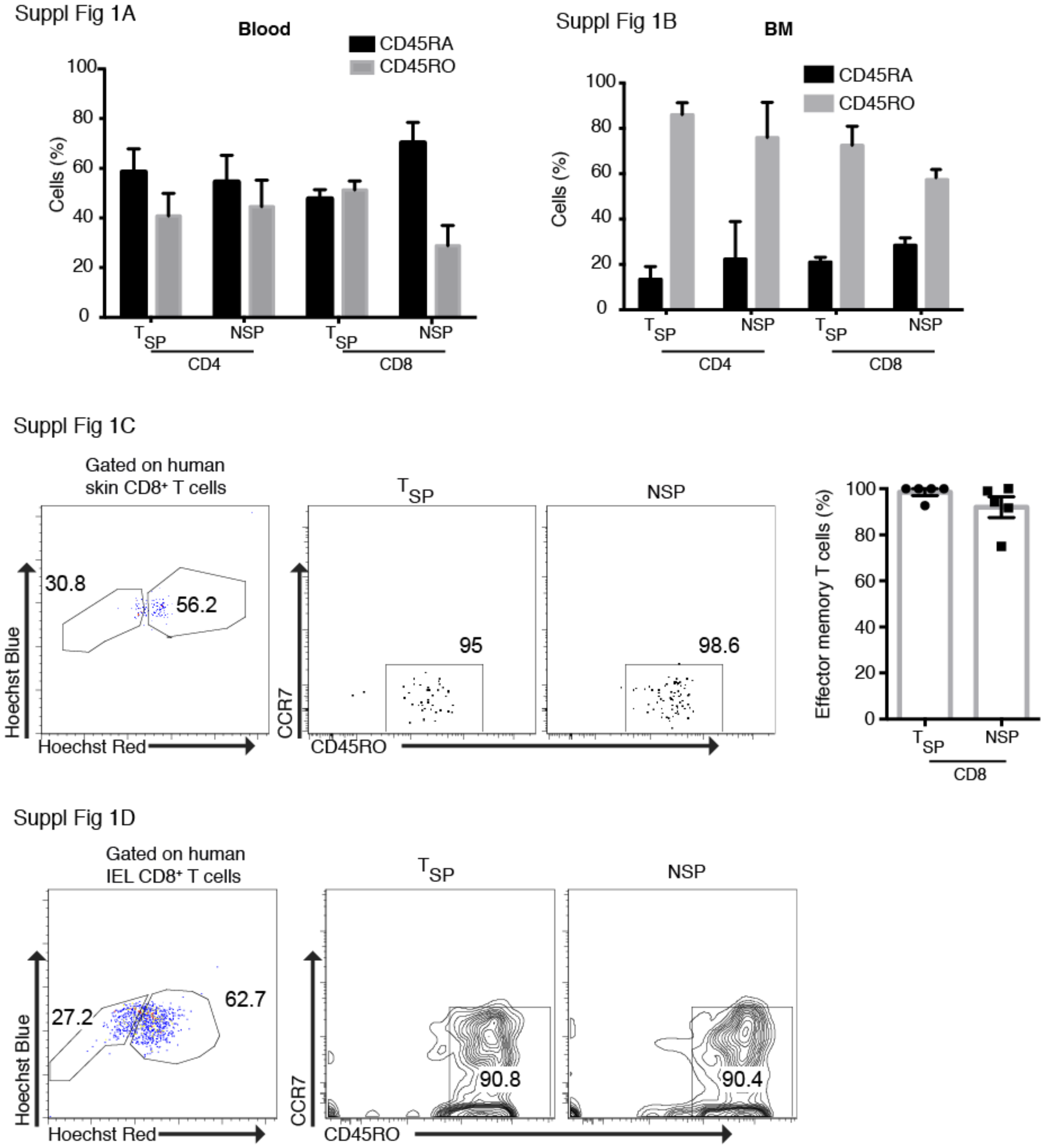
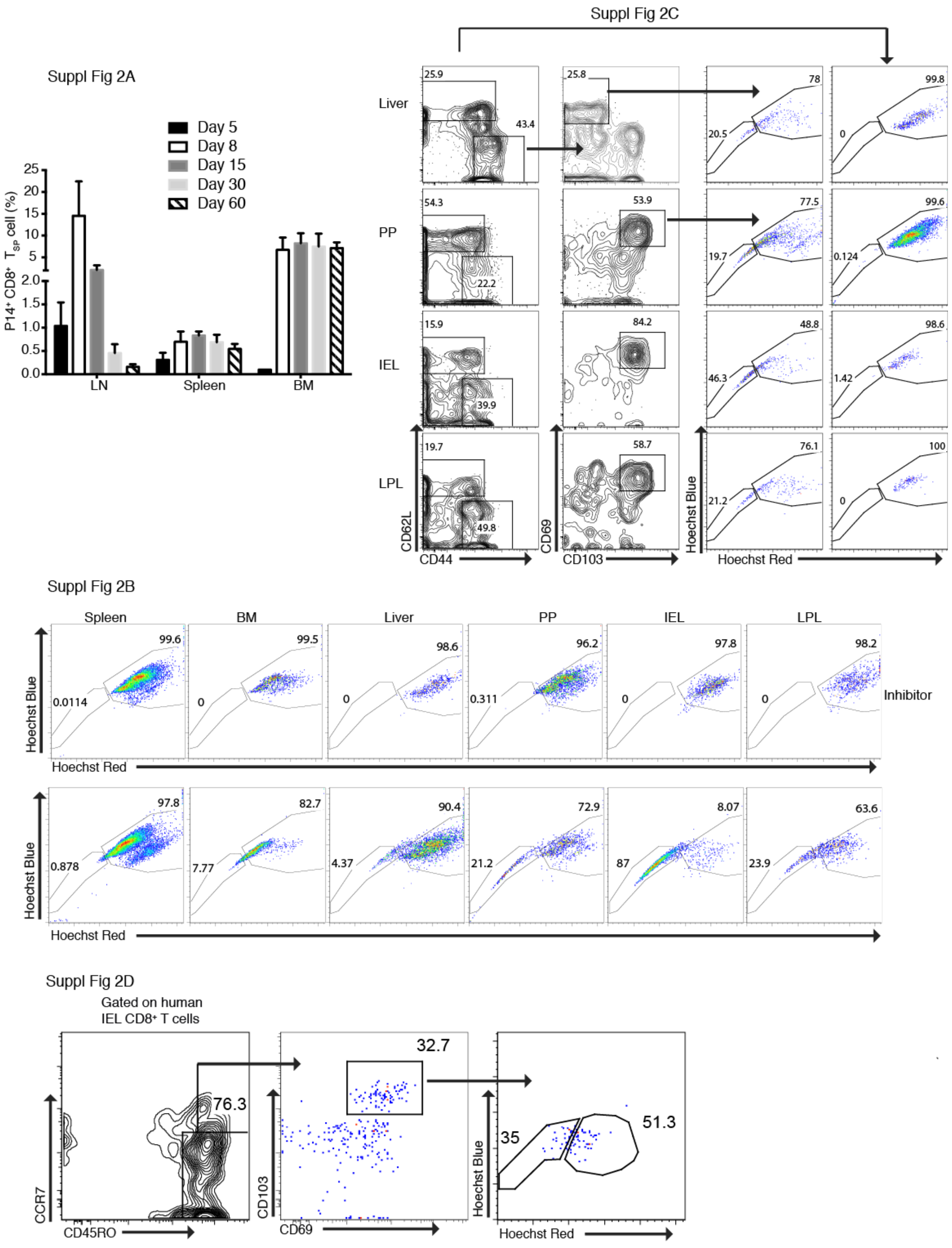


Supplementary Fig.1. Phenotype of T_{sp} and NSP cells from human blood, BM and skin

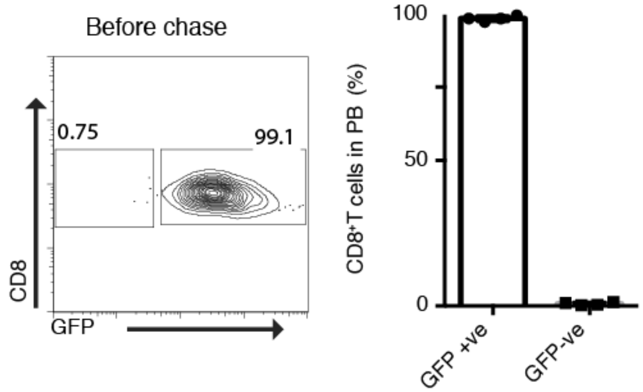
Graph shows proportion of CD45RA^{+} and CD45RO^{+} cells in T_{SP} and NSP fractions of CD4 or CD8 T cells in **(A)** human blood, **(B)** bone marrow T cells. Graphs represent compiled data from 4 to 5 human blood and BM samples. **(C)** FACS analysis on skin CD8 T_{SP} and NSP cells, bar graph showing enrichment of effector memory phenotype (CD45RO^{+}CCR7^{-} CD62L^{-}) in CD8 T_{SP} and NSP fractions, bar graph represents the compiled data (n=5). **(D)** Representative Flow analysis on IEL CD8 T_{SP} and NSP cells for effector phenotype. Experiments were repeated for 3-4 independent human IEL samples.



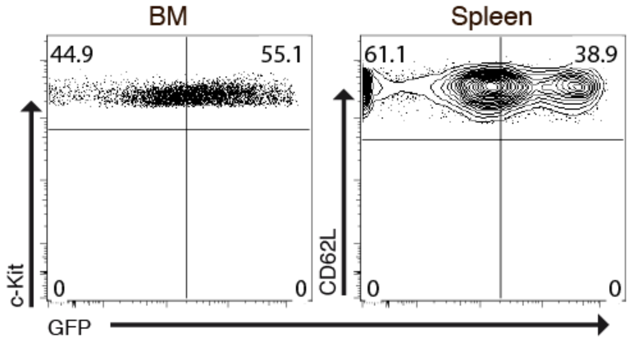
Supplementary Fig.2. Kinetics of CD8 T_{SP} in mice

P14 chimeras were infected with LCMV-arm and analyzed sequentially. **(A)** Bar graph documents kinetics of P14 CD8 T_{SP} at day 5, 8, 15, 30 and 60 (p.i) in LN, spleen and BM of infected mice. **(B)** FACS analysis detecting P14⁺ CD8⁺ T_{SP} in different organs at day 30 (p.i) (bottom panel), complete absence of P14 CD8 T_{SP} when treated with inhibitor (top panel). (C) SP analysis on P14⁺ CD8⁺ T_{RM} and non T_{RM} cells from liver, PP, IEL and LPL. Experiment was performed with n=3 mice for each group. (D) FACS plot indicates detection of SP phenotype on human IEL CD8 T_{RM} cells. Experiment repeated 6 times, representative FACS analysis is documented over here.

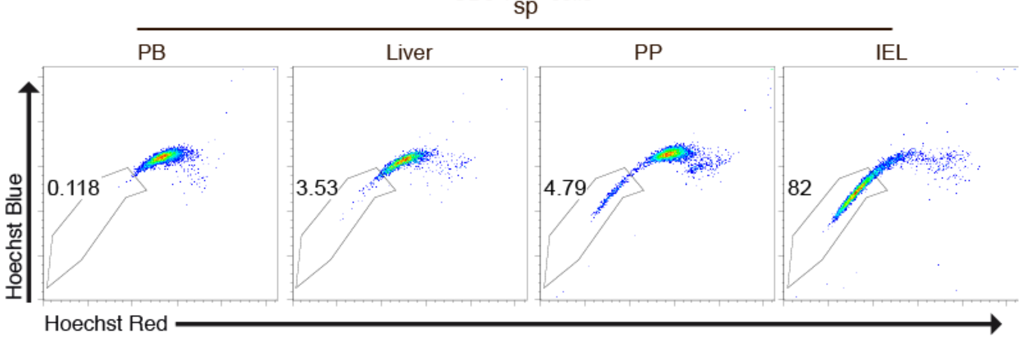
Suppl Fig 3A



Suppl Fig 3B



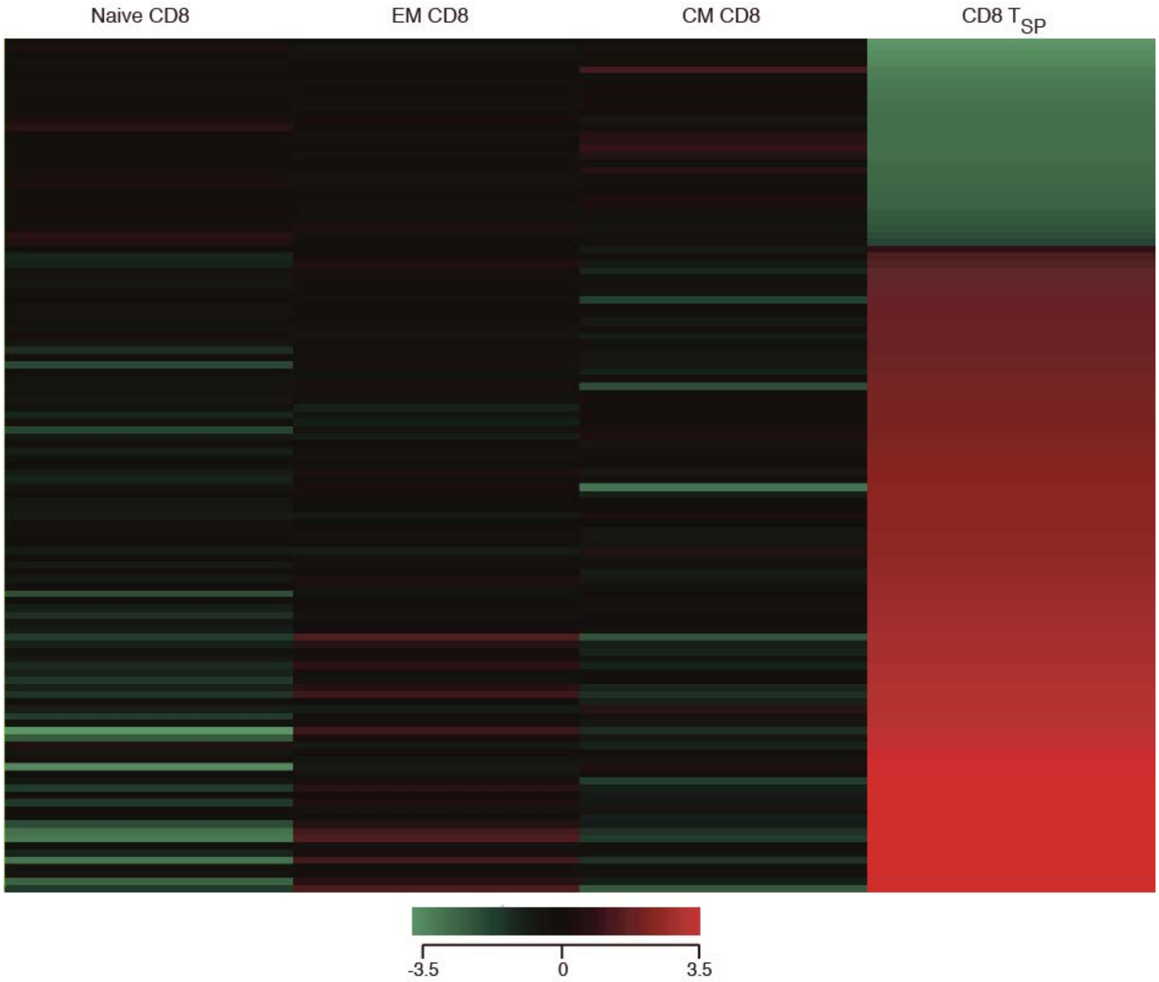
Suppl Fig 3C



Supplementary Fig.3. H2B GFP label retention analysis

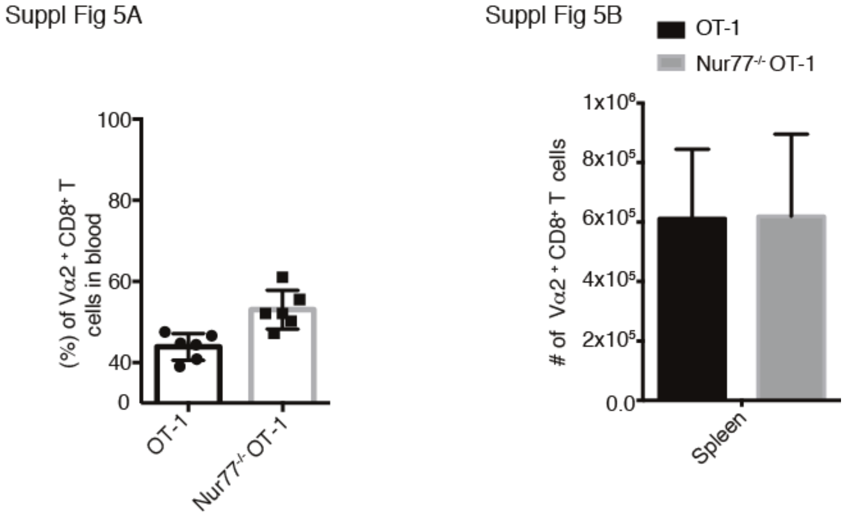
(A) FACS plot shows GFP label on blood CD8 T cells before chase, the same is documented as bar graph. **(B)** GFP label retention in the bone marrow c-Kit$^+$ cells (left) and spleen naïve CD44$^-$ CD62L$^+$ cells (right). (C) SP analysis of CD8 T cells from Blood, liver, PP and IEL. Experiments were repeated twice with n=4, data presented here from one of the two experiments.

Suppl Fig 4



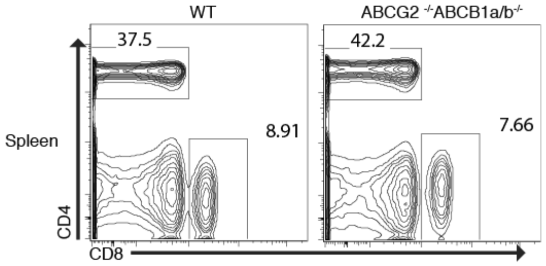
Supplementary Fig.4. Heat map comparing gene expression profile of T_{SP} versus other T cell subsets.

Heat map of differentially expressed genes between $CD8^+$ T_{SP} and naïve, EM and CM (Fold change > 4). The list of T_{SP} signature genes is noted in supplementary table 1.

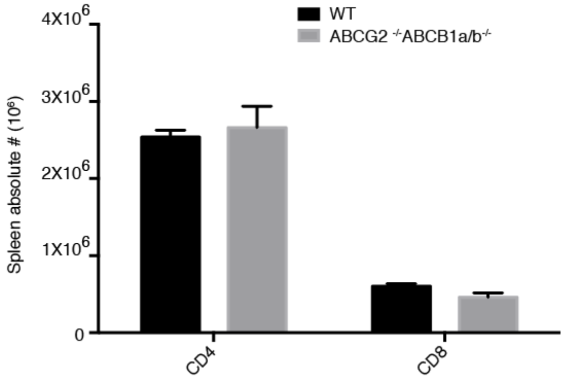


Supplementary Fig. 5. Analysis of Flu-ova infected mice
(A) Bar graph represents Vα2 $CD8^+$ T cell chimerism in blood at day 8 after Flu ova infection. **(B)** Graph documents the same in spleen at Day 14 post infection. Experiment was repeated twice with n=3 mice for each group, compiled data from two experiments was documented above.

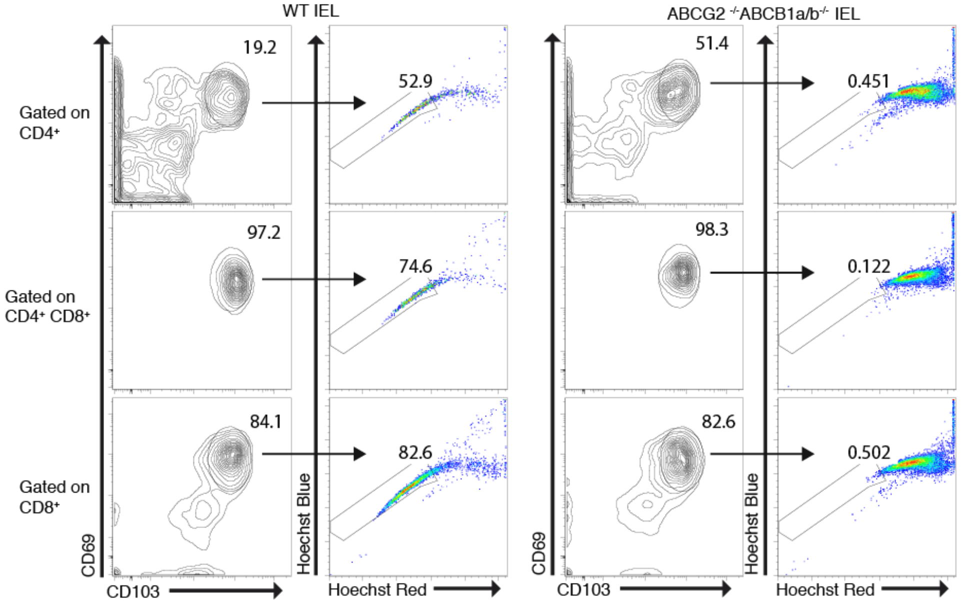
Suppl Fig 6A



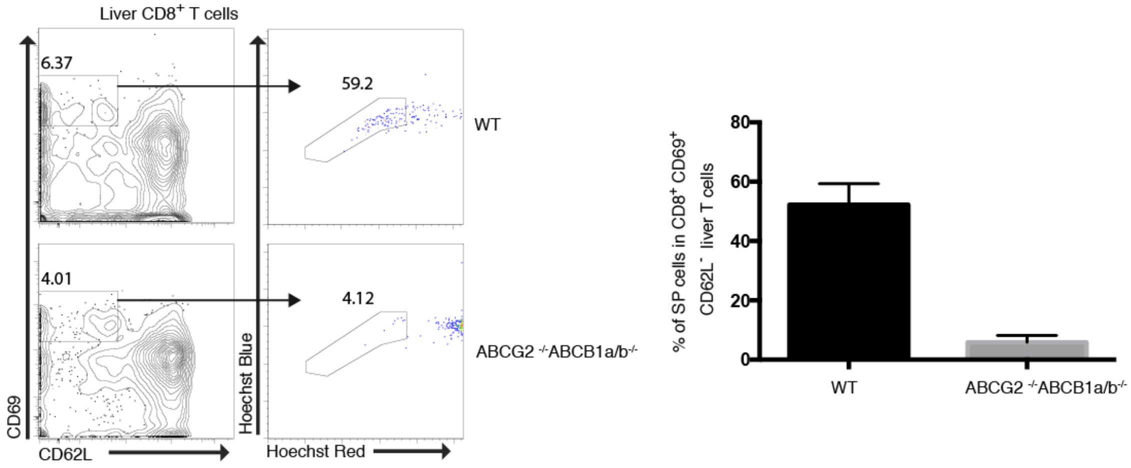
Suppl Fig 6B



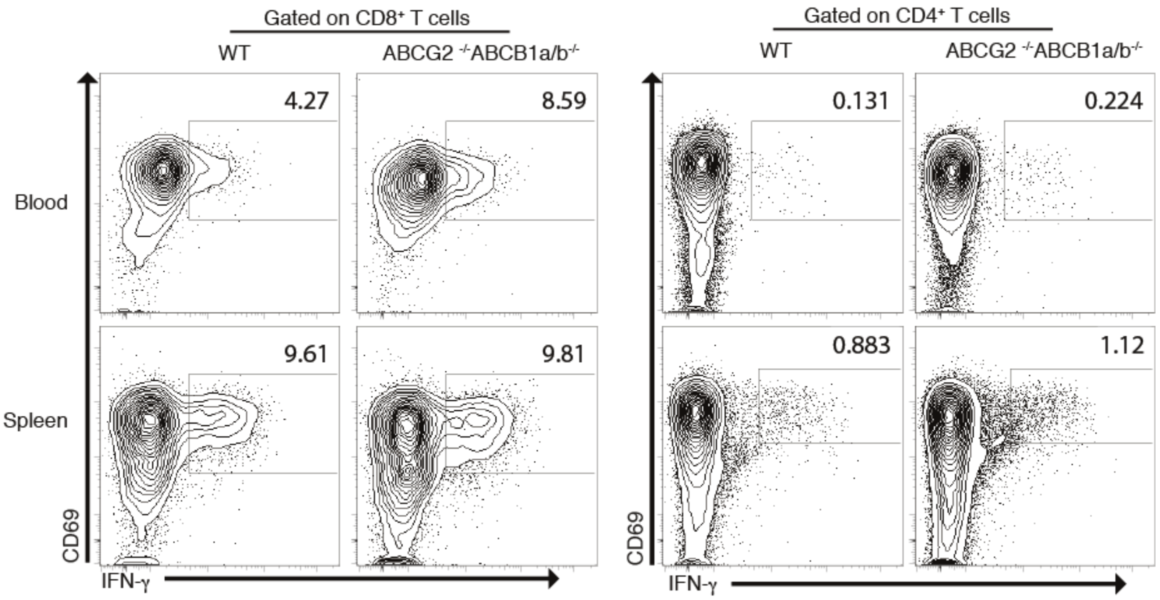
Suppl Fig 6C



Suppl Fig 6D

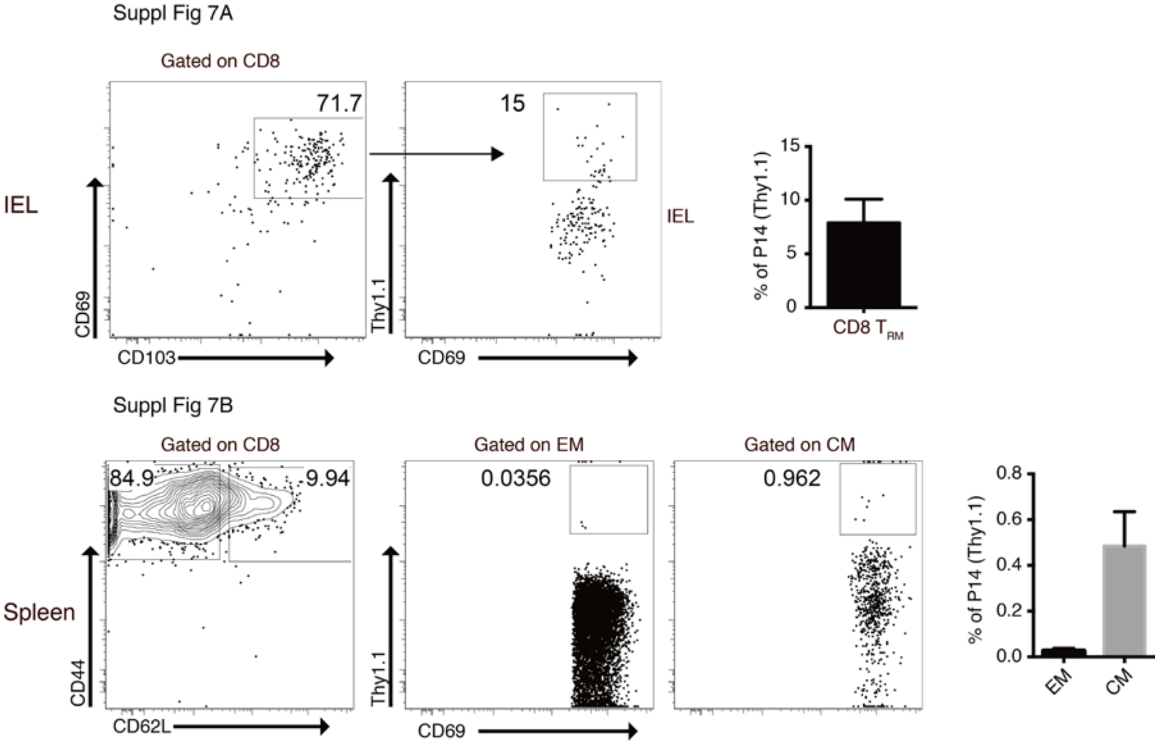


Suppl Fig 6E



Supplementary Fig.6. SP analysis in WT and ABCG2$^{-/-}$ ABCB1a/b$^{-/-}$ mice

(A) FACS plot comparing CD4 and CD8 frequency in WT and ABCG2$^{-/-}$ ABCB1a/b$^{-/-}$ mice, the same is plotted in graph **(B)**. **(C)** FACS plot shows WT IEL CD4 and CD8 T$_{RM}$ cells with SP phenotype that is completely absent in IELs from ABCG2$^{-/-}$ABCB1a/b$^{-/-}$ mice. **(D)** Representative FACS analysis and bar graph documents the same in liver. **(E)** IFN-γ production from blood and spleen CD4^{+}, CD8^{+} T cells was compared between WT and ABCG2$^{-/-}$ ABCB1a/b$^{-/-}$ mice. Experiments are repeated twice with 3 to 4 mice each, graphs represent data from one experiment (n=4 mice)



Supplementary Fig.7. Generation of gut tissue resident memory (T_{RM}) cells by adoptive transfer of SP CD8 T cells

(A) FACS plot represent the percent of Thy1.1$^+$ CD8 T cells in IEL CD69$^+$ CD103$^+$ T_{RM} cells, summary of data is presented as bar graph (n=5). **(B)** FACS analysis displays the percent Thy1.1$^+$ CD8 T cells in splenic T_{EM} and T_{CM} compartment. Combined analysis is shown in bar graph (n=5).

Differentially expressed genes between CD8 TSP vs other substes (Naïve, TEM and TCM) with fold change > 4

Probe Set ID	Entrez Gene	Gene Symbol	Gene Title	Chromosomal Location	CD8 SP vs CD8Naive (Fold change >= 4.0)	CD8 SP vs CD8 Naive (Fold change >= 4.0)	CD8 SP vs CD8 EM (Fold change >= 4.0)	CD8 SP vs CD8 EM (Fold change >= 4.0)	CD8SP vs CD8 CM (Fold change >= 4.0)
201694_s_at	1958	EGR1	early growth response 1	chr5q31.1	355.5871	9.474059	51.15658	51.15658	124.87742
206115_at	1960	EGR3	early growth response 3	chr8p23-p21	289.78473	9.178838	38.253395	38.253395	504.18884
205476_at	6364	CCL20	chemokine (C-C motif) ligand 20	chr2q36.3	270.70932	9.080601	30.37064	30.37064	70.05293
204103_at	6351	CCL4	chemokine (C-C motif) ligand 4	chr17q12	196.91464	8.6214266	4.272974	4.272974	20.440428
207113_s_at	7124	TNF	tumor necrosis factor	chr6p21.3	126.245674	7.98009	14.664831	14.664831	48.19655
205249_at	1959	EGR2	early growth response 2	chr10q21.1	107.77646	7.7518983	6.0749693	6.0749693	60.476353
204141_at	7280	TUBB2A	tubulin, beta 2A class IIa	chr6p25	65.34731	7.030056	10.428625	10.428625	9.634062
204622_x_at	4929	NR4A2	nuclear receptor subfamily 4, group A, member 2	chr2q22-q23	54.601883	6.770879	15.674133	15.674133	46.64736
216248_s_at	4929	NR4A2	nuclear receptor subfamily 4, group A, member 2	chr2q22-q23	53.58953	6.7438793	15.962476	15.962476	46.797432
204621_s_at	4929	NR4A2	nuclear receptor subfamily 4, group A, member 2	chr2q22-q23	51.97353	6.699705	18.666348	18.666348	45.962627
201693_s_at	1958	EGR1	early growth response 1	chr5q31.1	51.154873	6.6768	39.128742	39.128742	52.97839
209959_at	8013	NR4A3	nuclear receptor subfamily 4, group A, member 3	chr9q22	46.40343	6.5361595	20.578075	20.578075	54.392975
227404_s_at	1958	EGR1	early growth response 1	chr5q31.1	44.67448	6.481379	39.543037	39.543037	40.120064
209301_at	760	CA2	carbonic anhydrase II	chr8q22	32.94041	6.0417867	7.0097632	7.0097632	32.982986
214696_at	84981///407004	MIR22///MIR22HG	microRNA 22///MIR22 host gene (non-protein coding)	chr17p13.3	30.229372	5.917879	6.1357107	6.1357107	12.3511715
201631_s_at	8870	IER3	immediate early response 3	chr6p21.3	27.202187	5.7656507	9.576074	9.576074	18.72205
204170_s_at	1164	CKS2	CDC28 protein kinase regulatory subunit 2	chr9q22	24.957727	5.6414146	18.127895	18.127895	23.489655
1555847_a_at	284454	LOC284454	uncharacterized LOC284454	chr19p13.13	23.94513	5.5816603	6.1539874	6.1539874	9.336185
211538_s_at	3306	HSPA2	heat shock 70kDa protein 2	chr14q24.1	21.163752	5.4035234	21.78429	21.78429	19.529013
202768_at	2354	FOSB	FBJ murine osteosarcoma viral oncogene homolog B	chr19q13.32	20.526165	5.359392	16.849552	16.849552	10.563432
201324_at	2012	EMP1	epithelial membrane protein 1	chr12p12.3	20.480429	5.356174	9.242455	9.242455	30.256481

203725_at	1647	GADD45A	growth arrest and DNA-damage-inducible, alpha	chr1p31.2	19.194828	5.2626457	5.743819	5.743819	5.754538
226777_at	8038///100967225	ADAM12///ADAM12-OT1	ADAM metallopeptidase domain 12///ADAM12 overlapping transcript 1 (non-protein coding)	chr10q26.2///chr10q26.3	18.11175	5.178854	5.7755084	5.7755084	15.916521
202672_s_at	467	ATF3	activating transcription factor 3	chr1q32.3	14.561832	4.86412	8.323889	8.323889	23.341032
206683_at	7718	ZNF165	zinc finger protein 165	chr6p21.3	14.351564	4.843136	16.481129	16.481129	7.969202
205239_at	374///727738	AREG///AREGB	amphiregulin///amphiregulin B	chr4q13.3	14.213691	4.8292093	13.109322	13.109322	12.483153
207978_s_at	8013	NR4A3	nuclear receptor subfamily 4, group A, member 3	chr9q22	13.687983	4.774838	10.182067	10.182067	11.195989
224657_at	54206	ERRFI1	ERBB receptor feedback inhibitor 1	chr1p36	13.554325	4.7606814	5.3092318	5.3092318	5.3449774
212558_at	10252	SPRY1	sprouty homolog 1, antagonist of FGF signaling (Drosophila)	chr4q28.1	13.262747	4.7293077	8.5359125	8.5359125	8.022035
213975_s_at	4069	LYZ	lysozyme	chr12q15	13.113254	4.7129538	5.4884505	5.4884505	7.1658773
238542_at	80328	ULBP2	UL16 binding protein 2	chr6q25	12.617626	4.6573687	6.9703155	6.9703155	10.107688
230966_at	259307	IL4I1	interleukin 4 induced 1	chr19q13.3-q13.4	11.728041	4.5518901	5.5644503	5.5644503	9.15149
241824_at					11.376574	4.5079942	5.3485584	5.3485584	10.498419
202284_s_at	1026	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	chr6p21.2	11.2014885	4.4856186	6.066156	6.066156	10.663784
1559249_at	6310	ATXN1	ataxin 1	chr6p23	10.998722	4.459264	4.4999056	4.4999056	4.06728
211583_x_at	259197	NCR3	natural cytotoxicity triggering receptor 3	chr6p21.3	10.955801	4.453623	4.1315193	4.1315193	5.778989
230052_s_at	84807	NFKBID	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta	chr19q13.12	10.664459	4.414739	7.9631886	7.9631886	9.268031
223394_at	29950	SERTAD1	SERTA domain containing 1	chr19q13.1-q13.2	10.461591	4.3870304	8.963221	8.963221	13.052696
201939_at	10769	PLK2	polo-like kinase 2	chr5q12.1-q13.2	10.437913	4.3837614	11.050534	11.050534	12.048437
204472_at	2669	GEM	GTP binding protein overexpressed in skeletal muscle	chr8q13-q21	10.275794	4.361178	13.535637	13.535637	11.746499
210763_x_at	259197	NCR3	natural cytotoxicity triggering receptor 3	chr6p21.3	10.244114	4.3567233	4.3087697	4.3087697	5.2594666
223484_at	84419	C15orf48	chromosome 15 open reading frame 48	chr15q21.1	10.171895	4.3465166	4.6200757	4.6200757	15.595555

210873_x_at	200315///100913187	APOBEC3A///APOBEC3A_B	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A///APOBEC3A and APOBEC3B deletion hybrid	chr22q13 GRCh37 novel patch///chr22q13.1-q13.2	10.048314	4.3288815	7.612218	7.612218	15.065396
209324_s_at	6004	RGS16	regulator of G-protein signaling 16	chr1q25-q31	10.046491	4.3286197	4.49998	4.49998	12.079825
224185_at		OTTHUMG00000178019///RP11-199F11.2	NULL///NULL		9.966646	4.3171082	10.078484	10.078484	7.8313017
218368_s_at	51330	TNFRSF12A	tumor necrosis factor receptor superfamily, member 12A	chr16p13.3	9.679141	4.274879	5.7799697	5.7799697	6.4189095
202154_x_at	10381	TUBB3	tubulin, beta 3 class III	chr16q24.3	9.572596	4.2589102	4.803158	4.803158	7.1958427
214710_s_at	891	CCNB1	cyclin B1	chr5q12	9.534717	4.25319	7.997006	7.997006	4.5271425
230233_at					9.383252	4.230088	10.817796	10.817796	13.089007
208977_x_at	10383	TUBB4B	tubulin, beta 4B class IVb	chr9q34	9.37511	4.2288356	4.777498	4.777498	8.135268
202779_s_at	27338	UBE2S	ubiquitin-conjugating enzyme E2S	chr19q13.43	9.333766	4.2224593	5.9901953	5.9901953	6.241909
207768_at	1961	EGR4	early growth response 4	chr2p13	9.061656	4.1797748	7.19181	7.19181	6.4962726
235102_x_at	26851///780851///780852///780853///780854	SNORD3A///SNORD3B-1///SNORD3B-2///SNORD3C///SNORD3D	small nucleolar RNA, C/D box 3A///small nucleolar RNA, C/D box 3B-1///small nucleolar RNA, C/D box 3B-2///small nucleolar RNA, C/D box 3C///small nucleolar RNA, C/D box 3D	chr17p11.2	9.01752	4.1727307	8.352369	8.352369	10.413363
202340_x_at	3164	NR4A1	nuclear receptor subfamily 4, group A, member 1	chr12q13	8.794142	4.1365428	7.833911	7.833911	10.590924
213726_x_at	10383	TUBB4B	tubulin, beta 4B class IVb	chr9q34	8.675416	4.1169329	4.7794213	4.7794213	7.7755065
213476_x_at	10381	TUBB3	tubulin, beta 3 class III	chr16q24.3	8.199234	4.035489	4.691419	4.691419	6.952917
204011_at	10253	SPRY2	sprouty homolog 2 (Drosophila)	chr13q31.1	8.064626	4.0116076	7.7365823	7.7365823	6.164064
208926_at	4758	NEU1	sialidase 1 (lysosomal sialidase)	chr6p21.3	8.01454	4.0026197	4.935627	4.935627	5.2658467
36711_at	23764	MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	chr22q13.1	7.9551835	3.9918952	8.256691	8.256691	9.742798
205436_s_at	3014	H2AFX	H2A histone family, member X	chr11q23.3	7.7166157	3.9479682	5.861675	5.861675	5.9614353
201325_s_at	2012	EMP1	epithelial membrane protein 1	chr12p12.3	7.602531	3.9264798	5.0113053	5.0113053	7.8378673
240207_at					7.4801607	3.9030693	7.2756877	7.2756877	5.7175717

244447_at					7.227721	3.853541	5.531211	5.531211	22.668379
1568768_s_at	100302650	BRE-AS1	BRE antisense RNA 1	chr2p23	7.133807	3.8346722	4.065595	4.065595	6.400854
1555938_x_at	7431	VIM	vimentin	chr10p13	6.998464	3.8070383	4.099205	4.099205	4.556003
205193_at	23764	MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	chr22q13.1	6.751522	3.7552128	6.9516764	6.9516764	8.707857
228868_x_at	81620	CDT1	chromatin licensing and DNA replication factor 1	chr16q24.3	5.982863	3.580836	6.040455	6.040455	5.028311
230380_at	83591	THAP2	THAP domain containing, apoptosis associated protein 2	chr12q21.1	5.9749546	3.5789278	5.538372	5.538372	4.7617364
208078_s_at	150094	SIK1	salt-inducible kinase 1	chr21q22.3	5.887562	3.5576704	7.9521537	7.9521537	7.368583
201739_at	6446	SGK1	serum/glucocorticoid regulated kinase 1	chr6q23	5.884283	3.5568666	14.409118	14.409118	18.657473
244697_at					5.7828712	3.531786	4.6332536	4.6332536	5.898475
236898_at					5.7175794	3.5154045	4.0733852	4.0733852	4.355932
224836_at	58476	TP53INP2	tumor protein p53 inducible nuclear protein 2	chr20q11.22	5.6841	3.506932	5.172248	5.172248	4.9872746
212657_s_at	3557	IL1RN	interleukin 1 receptor antagonist	chr2q14.2	5.61198	3.48851	4.533588	4.533588	7.715889
202912_at	133	ADM	adrenomedullin	chr11p15.4	5.406382	3.4346635	4.584364	4.584364	5.7638273
237784_at	10923	SUB1	SUB1 homolog (S. cerevisiae)	chr5p13.3	5.253787	3.3933578	6.2331	6.2331	6.199801
223767_at	53831	GPR84	G protein-coupled receptor 84	chr12q13.13	5.2222047	3.384659	4.658847	4.658847	5.5034695
202871_at	9618	TRAF4	TNF receptor-associated factor 4	chr17q11-q12	5.214539	3.3825397	4.903262	4.903262	4.1313143
242125_at					5.1581297	3.366848	4.562951	4.562951	6.930153
230123_at	55707	NECAP2	NECAP endocytosis associated 2	chr1p36.13	5.0699673	3.3419764	5.238352	5.238352	4.3349404
203505_at	19	ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	chr9q31.1	5.047729	3.3356345	5.7408724	5.7408724	7.310459
203504_s_at	19	ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	chr9q31.1	4.9968443	3.3210173	5.599719	5.599719	6.5666876
1565579_at					4.977727	3.3154871	4.9052024	4.9052024	4.3654556
202393_s_at	7071	KLF10	Kruppel-like factor 10	chr8q22.2	4.9758034	3.3149295	5.0111127	5.0111127	17.111912
216979_at	8013	NR4A3	nuclear receptor subfamily 4, group A, member 3	chr9q22	4.929472	3.301433	4.1314616	4.1314616	4.420495
227943_at					4.8170514	3.2681503	4.5897217	4.5897217	4.422902
230913_at					4.689833	3.2295365	4.5253096	4.5253096	8.386161
242727_at	221079	ARL5B	ADP-ribosylation factor-like 5B	chr10p12.31	4.6144414	3.206156	4.003738	4.003738	4.363214

236105_at	65986	ZBTB10	zinc finger and BTB domain containing 10	chr8q13-q21.1	4.344961	3.1193433	5.197877	5.197877	7.0342
238029_s_at	151473	SLC16A14	solute carrier family 16, member 14 (monocarboxylic acid transporter 14)	chr2q36.3	4.2332196	3.0817554	4.3372245	4.3372245	5.211846
229983_at	166815	TIGD2	tigger transposable element derived 2	chr4q22.1	-4.0928354	-1.0331006	-4.661574	4.661574	-7.464864
204349_at	9443	MED7	mediator complex subunit 7	chr5q33.3	-4.265854	-1.0928345	-4.538787	4.538787	-4.721471
214820_at	54014	BRWD1	bromodomain and WD repeat domain containing 1	chr21q22.2	-4.2691836	-1.0939603	-4.120032	4.120032	-4.5338764
220391_at	79842	ZBTB3	zinc finger and BTB domain containing 3	chr11q12.3	-4.49801	-1.169287	-4.5442824	4.5442824	-5.8161073
219889_at	10023	FRAT1	frequently rearranged in advanced T-cell lymphomas	chr10q24.1	-4.533724	-1.1806965	-5.161925	5.161925	-6.3663826
209828_s_at	3603	IL16	interleukin 16	chr15q26.3	-4.680295	-1.2265995	-4.257084	4.257084	-4.2481065
206188_at	9831	ZNF623	zinc finger protein 623	chr8q24.3	-4.6892633	-1.2293613	-4.116435	4.116435	-4.800505
1553815_a_at	170082	TCEANC	transcription elongation factor A (SII) N-terminal and central domain containing	chrXp22.2	-4.6975174	-1.2318985	-4.2914343	4.2914343	-4.392349
229298_at	84078	KBTBD7	kelch repeat and BTB (POZ) domain containing 7	chr13q14.11	-4.748286	-1.2474067	-4.2015104	4.2015104	-6.6184216
219345_at	51027	BOLA1	bolA homolog 1 (E. coli)	chr1q21	-4.8257556	-1.2707548	-4.794643	4.794643	-5.8712077
219467_at	54826	GIN1	gypsy retrotransposon integrase 1	chr5q21.1	-4.8295283	-1.2718823	-4.9347363	4.9347363	-7.310824
230516_at	115416	MALSU1	mitochondrial assembly of ribosomal large subunit 1	chr7p15.3	-5.215315	-1.3827543	-4.551269	4.551269	-4.4153013
204264_at	1376	CPT2	carnitine palmitoyltransferase 2	chr1p32	-5.4366097	-1.4427073	-4.6959305	4.6959305	-6.922564
1554345_a_at	54826	GIN1	gypsy retrotransposon integrase 1	chr5q21.1	-5.4445615	-1.4448159	-5.260183	5.260183	-9.444577
206734_at	8690	JRKL	jerky homolog-like (mouse)	chr11q21	-5.606095	-1.4869962	-4.32775	4.32775	-5.287994
229970_at	84078	KBTBD7	kelch repeat and BTB (POZ) domain containing 7	chr13q14.11	-5.6139197	-1.4890084	-5.0635047	5.0635047	-6.3861938
213596_at	837	CASP4	caspase 4, apoptosis-related cysteine peptidase	chr11q22.2-q22.3	-6.2037377	-1.6331377	-7.172761	7.172761	-5.1375656
206314_at	55888	ZKSCAN7	zinc finger with KRAB and SCAN domains 7	chr3p21.32	-6.3473477	-1.666154	-5.236052	5.236052	-7.3039837

227385_at	403313	PPAPDC2	phosphatidic acid phosphatase type 2 domain containing 2	chr9p24.1	-6.385565	-1.6748142	-6.3006616	6.3006616	-6.8605313
205541_s_at	23708	GSPT2	G1 to S phase transition 2	chrXp11.22	-6.655098	-1.7344599	-4.5216727	4.5216727	-7.8936806
236153_at					-6.722345	-1.7489645	-4.393146	4.393146	-5.2560244
218689_at	2188	FANCF	Fanconi anemia, complementation group F	chr11p15	-7.0387664	-1.8153226	-5.167518	5.167518	-6.6596317
226458_at		OTTHUMG00000175805///RP1-39G22.7	NULL///NULL		-7.1893835	-1.845868	-4.684105	4.684105	-8.178004
219538_at	54554	WDR5B	WD repeat domain 5B	chr3q21.1	-7.3190475	-1.871656	-4.919258	4.919258	-8.080641
235052_at	126375	ZNF792	zinc finger protein 792	chr19q13.11	-7.3975143	-1.8870406	-6.077551	6.077551	-10.900192
219243_at	55303	GIMAP4	GTPase, IMAP family member 4	chr7q36.1	-7.8748164	-1.9772463	-6.079937	6.079937	-7.170238
232094_at	79768	KATNBL1	katanin p80 subunit B-like 1	chr15q14	-8.589153	-2.102516	-4.948485	4.948485	-5.0103774
226756_at	168455	CCDC71L	coiled-coil domain containing 71-like	chr7q22.3	-9.349459	-2.2248828	-9.173811	9.173811	-10.425746
235306_at	155038	GIMAP8	GTPase, IMAP family member 8	chr7q36.1	-11.156089	-2.4797595	-6.337486	6.337486	-8.024004