

Supplemental Information

Differential Expression of Ly6C and T-bet

Distinguish Effector and Memory Th1 CD4⁺

Cell Properties during Viral Infection

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Inventory of Supplemental Information

Figure S1. Comparison of Smarta TCR transgenic and GP₆₆₋₇₇ tetramer⁺ CD4 T cells. Associated with Figure 1.

Figure S2. Phenotypes and locations of LCMV-specific CD4 T cell effector and memory subsets. Associated with Figure 3.

Table S1. Top 100 signature genes from each subset relative to naïve. Associated with Figure 6.

Table S2. Differentially expressed transcription factors, chemokines, chemokine receptors, cytokines, and cytokine receptors. Associated with Table 1 and supplied as an Excel spreadsheet. Available online.

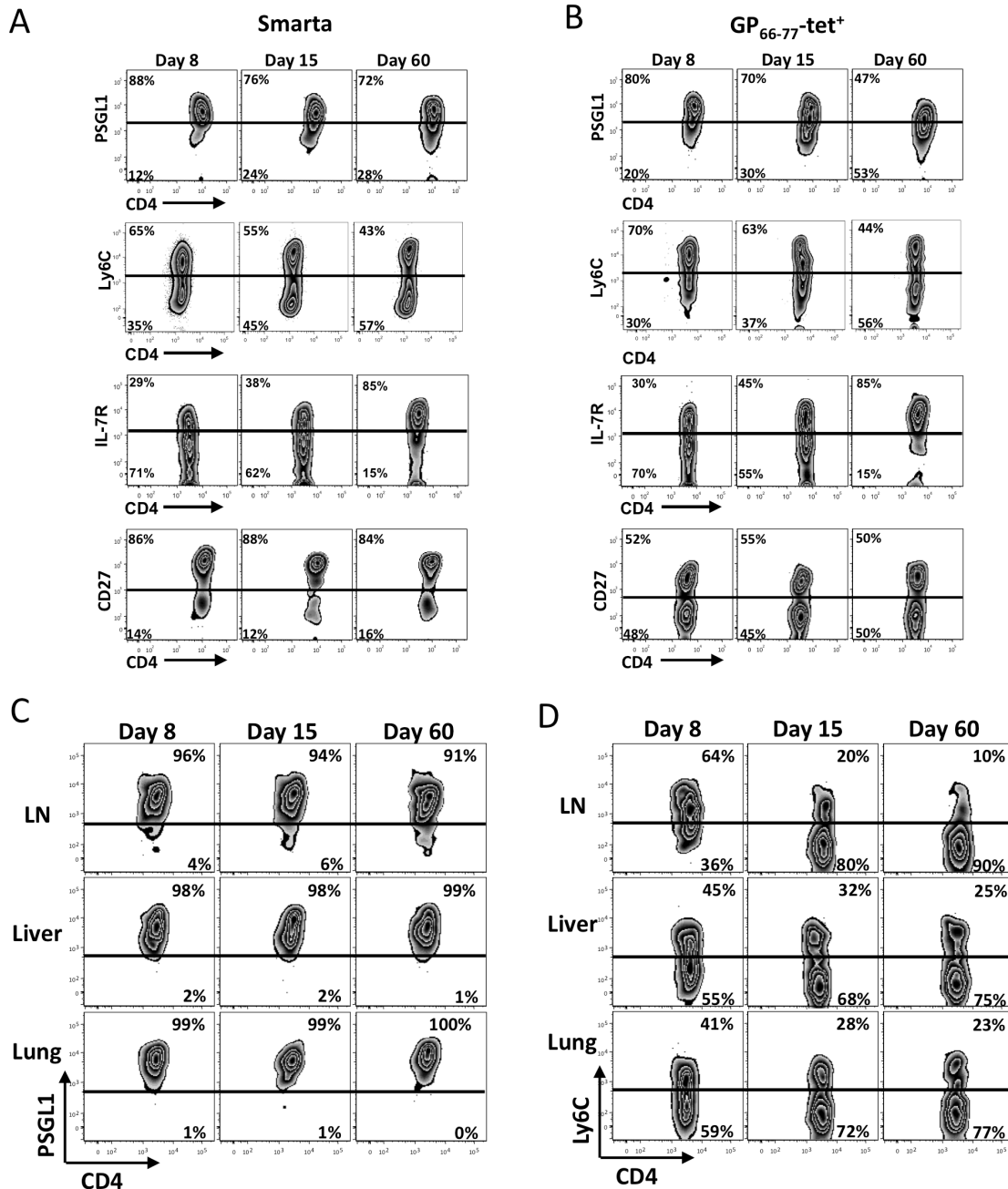
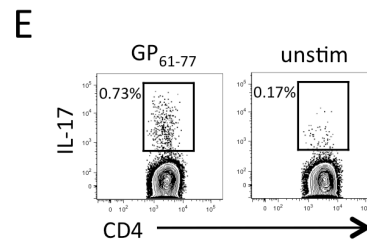
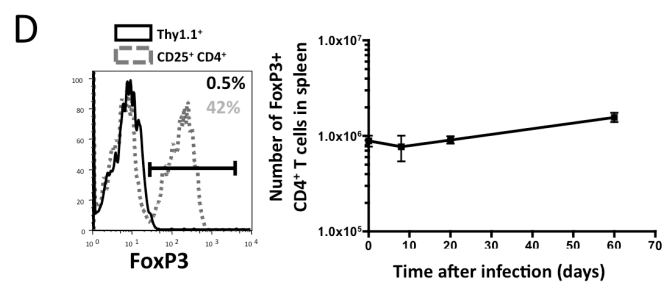
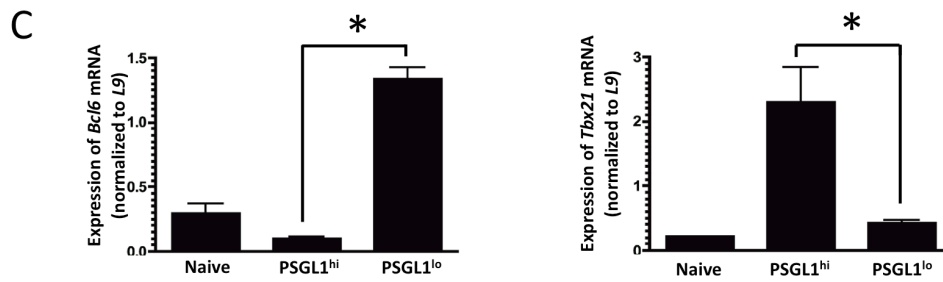
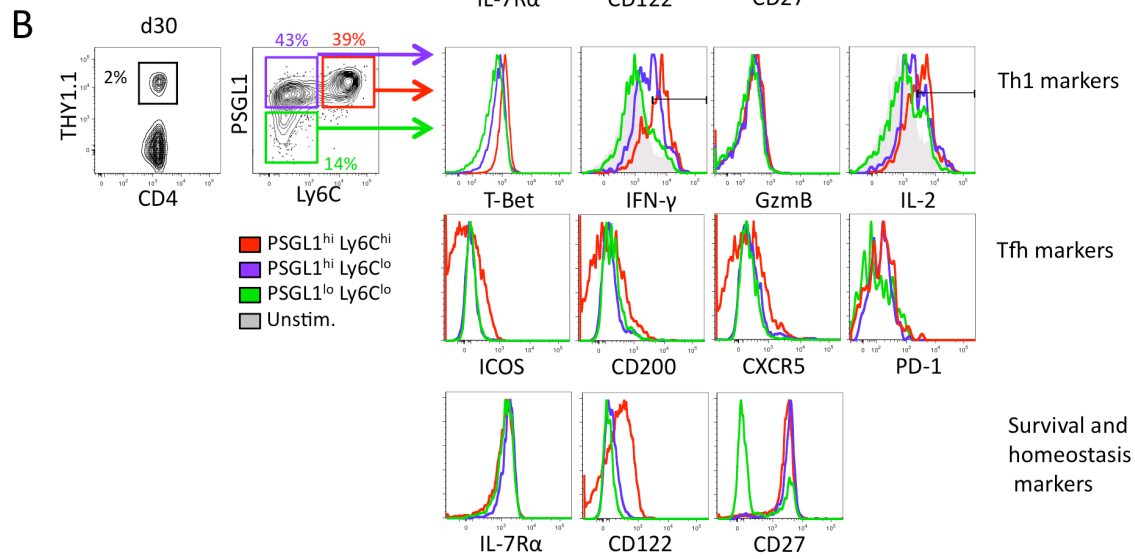
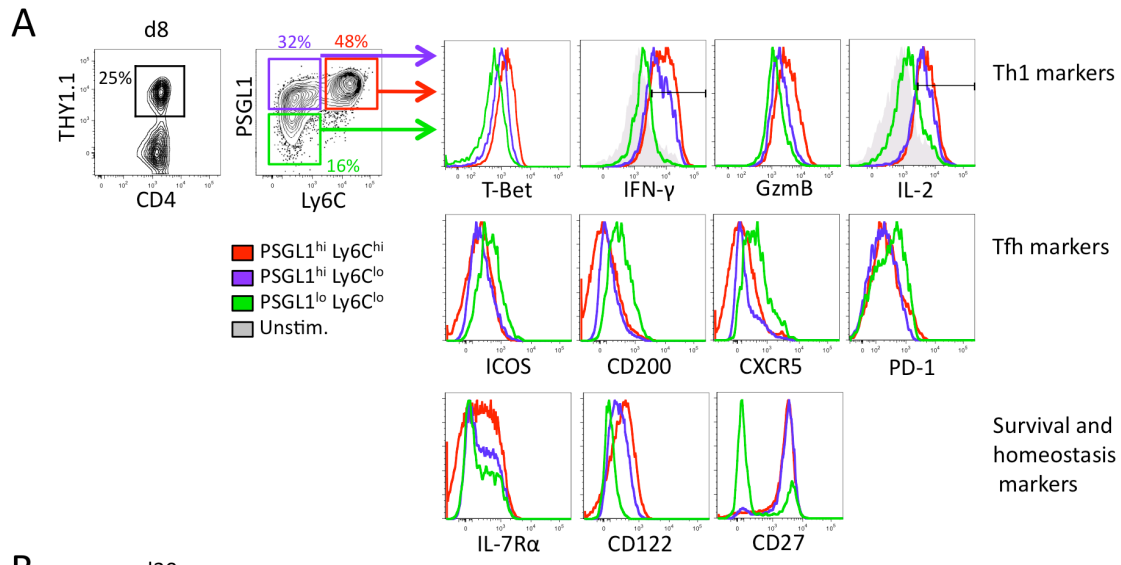


Figure S1. Comparison of Smarta TCR transgenic and GP₆₆₋₇₇ tetramer+ CD4⁺ T cells. (A) Smarta chimeric mice or (B) C57BL/6 mice were infected with LCMV and the Smarta CD4⁺ T cells (A) and GP₆₆₋₇₇ tetramer+ CD4⁺ T cells (B) were analyzed for expression of PSGL1, Ly6C, IL-7R, and CD27 at days 8, 15, and 60 p.i. (C) Smarta chimeric mice were infected with LCMV and at days 8, 15, and 60 p.i., the expression of PSGL1 (C) and Ly6C (D) was assessed on Smarta CD4⁺ T cells in the inguinal lymph nodes, liver, and lung. Related to Figure 1.



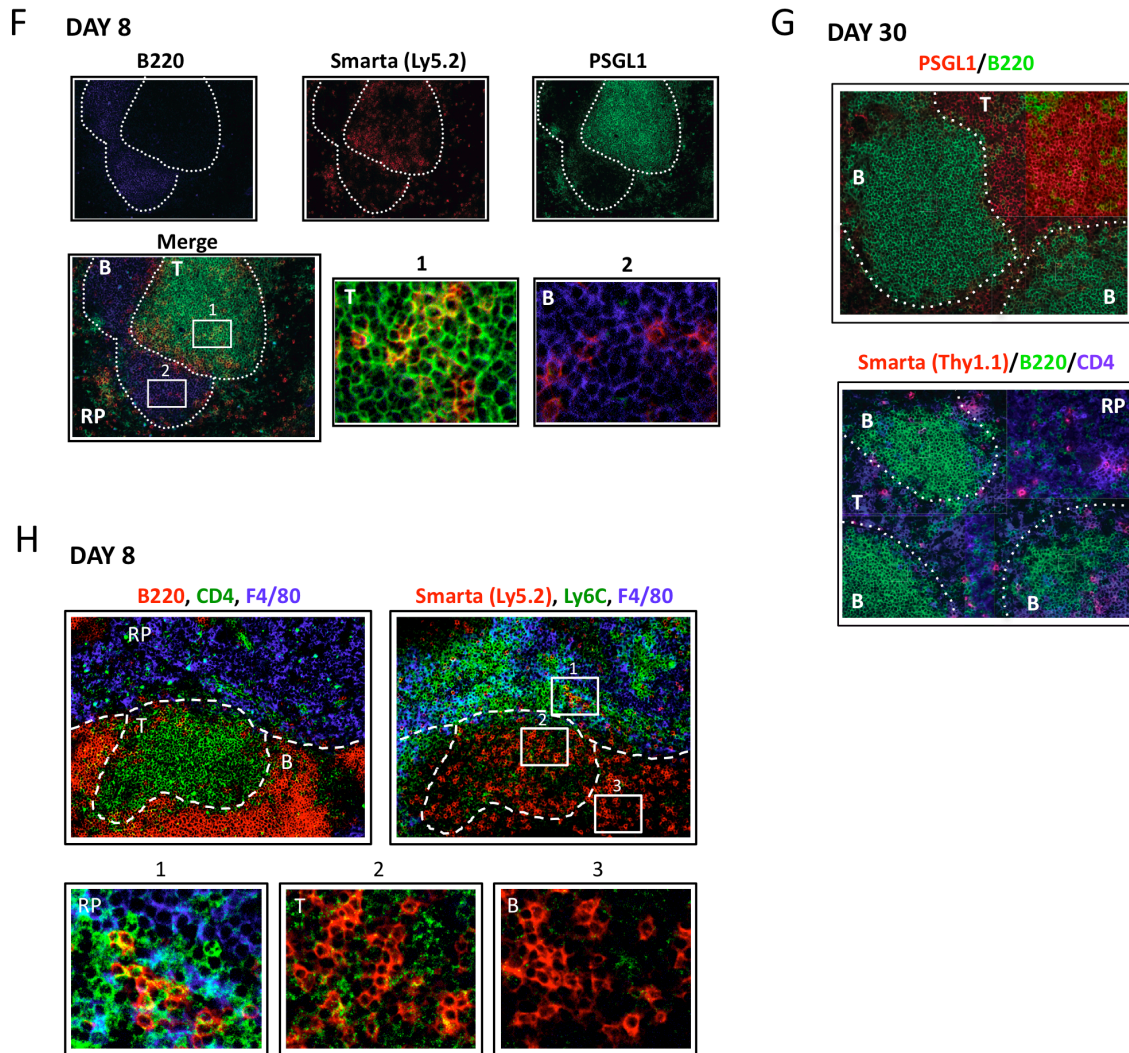


Figure S2. Phenotypes and locations of LCMV-specific $CD4^+$ T cell effector and memory subsets. (A-B) Smarta chimeric mice were infected with LCMV and 8 days (A) or 30 days (B) p.i. splenocytes were stained with antibodies to Thy1.1, PSGL1 and Ly6C and analyzed by flow cytometry for the expression of the indicated proteins. Overlapping histograms show the expression of the indicated proteins on $PSGL1^{hi}$ $Ly6C^{hi}$ (red histograms), $PSGL1^{hi}$ $Ly6C^{lo}$ (blue histograms) and $PSGL1^{lo}$ $Ly6C^{lo}$ cells (green histograms). (C) RT-PCR of *Bcl6* (left) and *Tbx21* (right) mRNA from naïve or $PSGL1^{hi}$, and $PSGL1^{lo}$ Smarta cells at day 8 p.i. (D) FoxP3 staining in Smarta cells ($Thy1.1^+$) or total $CD25^+$ $CD4^+$ T cells is shown in FACS plots (left). Line graph shows the number of FoxP3⁺ $CD4^+$ T cells throughout LCMV infection (right). (E) Smarta $CD4^+$ T cells were stimulated with GP₆₁₋₇₇ peptide for 5 hours and intracellular accumulation of IL-17 was assessed. * $p < 0.05$. (F) Ly5.1⁺ mice containing small numbers of Ly5.2⁺ Smarta $CD4^+$ T cells were infected with LCMV and 8 days later the locations of $PSGL1^{hi}$ and $PSGL1^{lo}$ effector $CD4^+$ T cells were examined in the spleen. Frozen splenic sections were stained for Ly5.2⁺ Smarta cells (red), B220 (blue), and PSGL1 (green). Dotted line delineates the B and T cell zones and magnified views of boxed areas #1 and #2 are shown. (G) At day 30, frozen sections were stained for PSGL1 (red) and

B220 (green) on the left or Thy1,1+ Smarta cells (red), B220 (green), and CD4 (blue) on the right. (H) Day 8 frozen sections were stained for B220 (red), CD4 (green), and F4/80 (blue) on the left or Ly5.2+ Smarta (red), Ly6C (green), and F4/80 (blue) on the right. Magnified views of boxed areas are shown. Each experiment is a representative of three independent animals. Related to Figure 3.

Supplemental Table 1. Top 100 signature genes for each effector and memory subset.

	day 8 PSGL1 ^{lo} Ly6C ^{lo} Naive		day 8 PSGL1 ^{hi} Ly6C ^{hi} Naive		day 8 PSGL1 ^{hi} Ly6C ^{lo} Naive		day 60 PSGL1 ^{hi} Memory Naive	
	Gene	Fold change	Gene	Fold change	Gene	Fold change	Gene	Fold change
Lag3	70.38	Lgals3	148.58	Erdr1	82.17	Erdr1	72.44	
Erdr1	67.19	Nkg7	143.36	Cxcr3	59.23	Cxcr3	56.40	
Sostdc1	63.86	Erdr1	122.71	Ccl5	50.52	Ccl5	51.48	
Cxcr5	38.14	Ccl4	116.57	Nkg7	45.57	Serpina3g	36.06	
Cxcr5	35.87	Ccl5	97.93	Lgals3	34.34	Nkg7	35.12	
1810009J06Rik	33.78	Ifng	95.63	Serpina3g	34.24	Myo1f	31.31	
Il1r2	33.39	LOC381140	84.49	Myo1f	33.16	Lyz	29.36	
Rgs16	32.64	AA467197	76.97	Ccl4	31.97	Casp1	27.53	
LOC381140	32.45	Cxcr3	76.23	Casp1	31.83	Igk-C	26.74	
Bhlhb2	30.84	Ifng	70.91	Lag3	30.93	LOC100047628	25.67	
Spp1	28.58	Bhlhb2	69.17	Zbtb32	28.79	LOC100047788	25.38	
Casp1	24.65	Plac8	62.62	LOC381140	27.28	Lag3	22.23	
Smtn	24.33	Myo1f	59.16	Bhlhb2	27.00	LOC100047419	21.68	
0610007P22Rik	23.78	Tbx21	58.36	Ifng	24.03	Zbtb32	19.37	
Cxcr5	22.97	Ifitm1	52.13	LOC100047419	23.42	S100a6	19.18	
Il21	22.95	Mt1	49.97	S100a6	23.32	Ccr6	18.84	
Sccpdh	22.94	Emp1	43.17	Ccr6	20.55	Asb2	18.56	
0610007P22Rik	22.92	LOC100047788	41.00	Il1r2	19.98	Tbx21	18.05	
Il4	22.64	Pik3ap1	38.31	Asb2	19.87	Lgals3	17.34	
Slc24a3	22.62	Osbpl3	37.29	Osbpl3	19.42	Bhlhb2	17.18	
Cxcr3	22.59	Casp1	37.18	Casp4	19.38	Igh-VJ558	16.99	
Serpina3g	22.11	Smpdl3b	36.93	Tbx21	18.62	Itgae	16.41	
Stx11	21.99	Gldc	36.33	Stx11	18.27	Itgae	16.24	
Asb2	21.33	Kcnk5	32.64	Emp1	17.21	Casp4	15.39	
Nrbp2	21.18	Ahnak	32.60	Itgae	16.87	LOC381140	14.96	
LOC100047419	21.11	S100a4	31.75	Ifng	16.28	Tnfsf14	14.72	
Bzrap1	20.18	Gp49a	31.58	0610007P22Rik	16.12	Serpina3f	14.35	
Ryk	19.55	Cxcr6	31.47	Itgae	15.96	Emp1	13.57	
Synpo	19.28	Ccl3	31.40	Anxa2	15.79	Osbpl3	13.28	
LOC100047611	19.08	Rbm47	31.21	Ahnak	14.60	Ifng	13.24	
BC029169	18.45	S100a6	29.29	0610007P22Rik	14.12	Bzrap1	13.21	
Casp4	18.19	Zbtb32	29.08	Esm1	13.91	0610007P22Rik	13.17	
Zbtb32	17.84	Ccl4	28.60	LOC100047611	13.81	0610007P22Rik	13.12	
Cd83	17.77	A230057G18Rik	28.06	Tnfsf14	13.74	Ahnak	12.52	
Npas4	16.41	Ahnak	27.61	Plac8	13.71	Al324046	12.41	
Ifng	16.18	LOC100047934	25.05	Cxcr5	13.59	Xcl1	11.91	
Smtn	15.89	Casp4	24.15	Rgs16	13.48	Chst2	11.86	
Igk-C	15.81	Garnl4	23.59	Serpina3f	13.12	Cxcr5	11.81	
Esm1	15.76	Podnl1	23.58	Bzrap1	12.99	Stx11	11.44	
Angptl2	15.67	Havcr2	23.33	Ccr6	12.01	Nrn1	11.23	
Tiam1	15.55	Il18rap	23.31	Smpdl3b	12.00	Nrp1	11.16	
LOC100047628	15.03	LOC100047628	23.17	Cxcr5	11.98	Ccl4	11.16	
Nrn1	14.68	Lrrk1	23.14	Synpo	11.78	Ahnak	10.94	
Ecel1	14.54	Anxa2	23.13	Ryk	11.63	Nrp1	10.69	
Tmem2	14.29	Esm1	23.10	Chst2	11.50	Anxa2	10.60	
Gna14	14.06	Cx3cr1	22.95	Zcchc18	11.49	Cxcr6	10.49	
Nrp1	13.89	Igk-C	22.43	S100a4	11.39	Ccr6	10.27	
Nrp1	13.87	LOC100047419	20.60	Ahnak	11.32	Tiam1	10.12	
Chst2	13.82	Klrg1	20.32	Nrp1	11.15	Zcchc18	10.06	
Muc16	13.59	Popdc2	20.20	Nrp1	10.95	Plac8	9.85	
LOC100046232	13.25	0610007P22Rik	19.59	Kcnk5	10.80	Cxcr5	9.48	

Top 100 "signature genes"

Top 100 "signature genes" cont.

d8 PSGL1 ^{lo} /naive		d8 PSGL1 ^{hi} Ly6c ^{hi} /naive		d8 PSGL1 ^{hi} Ly6c ^{lo} /naive		Memory PSGL1 ^{hi} /naive	
Gene	Fold change	Gene	Fold change	Gene	Fold change	Gene	Fold change
Myo1f	13.18	LOC100047167	19.15	Igk-C	10.72	Il1r2	9.28
Ifng	13.05	Ccr5	18.70	Tiam1	10.69	Scppdh	9.27
Tmem2	12.97	Ilgad	18.35	Podnl1	10.56	Gpr68	9.17
1700019D03Rik	12.66	0610007P22Rik	18.29	LOC100047628	10.42	Ryk	9.16
S100a6	12.48	Ahnak	17.92	Tmem2	10.29	Lyzs	9.03
Vdr	11.92	Osbpl3	17.86	Ebi3	10.06	Ifng	8.98
Abhd4	11.70	Havcr2	17.82	Nrn1	9.80	LOC100047611	8.98
Osbpl3	11.55	Plek	17.61	2810439F02Rik	9.77	Rgs16	8.95
Nrp	11.45	Tnfsf14	17.51	Gpr68	9.75	S100a4	8.92
Krt17	11.44	Rora	17.37	Tspan17	9.60	Podnl1	8.90
Ttbk1	11.34	Ltb4r1	17.14	Gp49a	9.50	Esm1	8.89
Tnfsf14	11.19	Popdc2	17.13	Scppdh	9.46	2810439F02Rik	8.35
Pex11a	11.07	Stx11	16.78	Osbpl3	9.45	Kcnk5	8.22
Cd22	10.95	Tcrb-V8.3	16.69	Axl	9.25	Synpo	8.21
6330403K07Rik	10.42	Atxn1	16.66	Klrg1	9.20	Axl	8.09
Tspan17	10.42	2810439F02Rik	16.36	Cxcr6	9.15	Tspan17	8.05
Pex11a	10.32	F2rl2	16.07	Casp4	9.03	Klk1b27	7.91
B4galnt4	10.26	Lag3	16.06	Cd83	8.96	6430511F03	7.78
Axl	10.07	6430511F03	15.88	Xcl1	8.77	Al324046	7.78
Ryk	9.98	Ifitm1	15.59	Rora	8.73	Rora	7.71
Coro2b	9.86	Prc1	15.50	Sostdc1	8.63	Cd83	7.68
Trat1	-10.33	Gna15	15.14	2810439F02Rik	8.62	Slc24a3	7.68
Slc16a5	-10.59	Sytl3	14.79	Ltb4r1	8.56	Casp4	7.67
2310010M24Rik	-10.92	F730045P10Rik	14.75	6330403K07Rik	8.36	Bmp7	7.65
Rpl29	-11.32	1500009L16Rik	14.25	Slc24a3	8.28	Lyz2	7.62
Satb1	-11.58	Ifitm3	14.15	5033430115Rik	8.11	Il4	7.60
Rps6	-11.97	Dapk2	14.05	LOC100045542	8.08	Ikzf2	7.51
Sema4a	-12.30	2310010M24Rik	-13.02	Ahnak	8.04	Nrp	7.51
Prkd3	-12.58	scl0001132.1_96	-13.58	Slc15a3	8.03	Osbpl3	7.50
Actn1	-12.92	Ttc28	-14.53	LOC100046232	7.96	Actg2	7.41
LOC100041103	-13.21	Ephx1	-15.18	Rilpl2	7.85	Klrg1	7.41
Pdlim1	-13.51	Dapl1	-18.52	Endod1	7.84	Smpd13b	7.40
Klrd1	-13.69	Tmem108	-18.90	Amigo2	-7.62	2810439F02Rik	7.39
Gpr18	-13.84	LOC100041103	-20.68	Myo6	-7.65	Inpp1	7.11
Tmem108	-14.11	Ampd1	-21.52	Slc16a5	-8.39	Tmem2	7.10
Actn2	-14.15	Atp1b1	-24.90	Nme7	-8.55	Amigo2	-6.42
Dapl1	-14.59	Tmie	-25.26	Dapl1	-9.03	Dapl1	-6.75
Ampd1	-14.75	Amigo2	-26.20	A530021J07Rik	-10.22	Nme7	-6.79
Amigo2	-16.60	Nme7	-28.03	Amigo2	-10.60	Nme7	-8.26
EG666577	-17.65	Ramp1	-28.64	Nme7	-11.00	Nme7	-8.30
Myo6	-18.44	Gpr83	-30.14	Tmem108	-11.13	Amigo2	-8.39
Ampd1	-23.36	Ampd1	-32.43	Nme7	-11.60	A530021J07Rik	-9.17
Satb1	-28.09	Nme7	-33.52	Atp1b1	-11.61	Atp1b1	-9.23
A530021J07Rik	-32.38	Slc16a5	-39.92	Ampd1	-13.40	Pdlim4	-9.61
Ly6c1	-43.68	A530021J07Rik	-42.80	Pdlim4	-13.59	Tmem108	-10.36
Sell	-55.40	Sell	-67.43	Ly6c1	-15.26	Ampd1	-12.08
Igfbp4	-59.58	Pdlim4	-68.46	Ampd1	-18.18	Igfbp4	-14.50
Pdlim4	-71.26	Igfbp4	-75.30	Igfbp4	-22.86	Igfbp4	-15.19
Igfbp4	-127.92	Igfbp4	-126.34	Igfbp4	-23.46	Ampd1	-15.47

Supplemental Table 1. Top 100 signature genes from each subset relative to naive.
 The average log2 transformed relative expression intensities of day 8 PSGL1^{lo} Ly6C^{lo}, day 8 PSGL1^{hi} Ly6C^{hi}, day 8 PSGL1^{hi} Ly6C^{lo}, and day 60 PSGL1^{hi} memory Stg compared to naive Stg mRNA from Illumina microarrays.