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Supplemental Information

Extensive Homeostatic T Cell Phenotypic Variation within the Collaborative Cross

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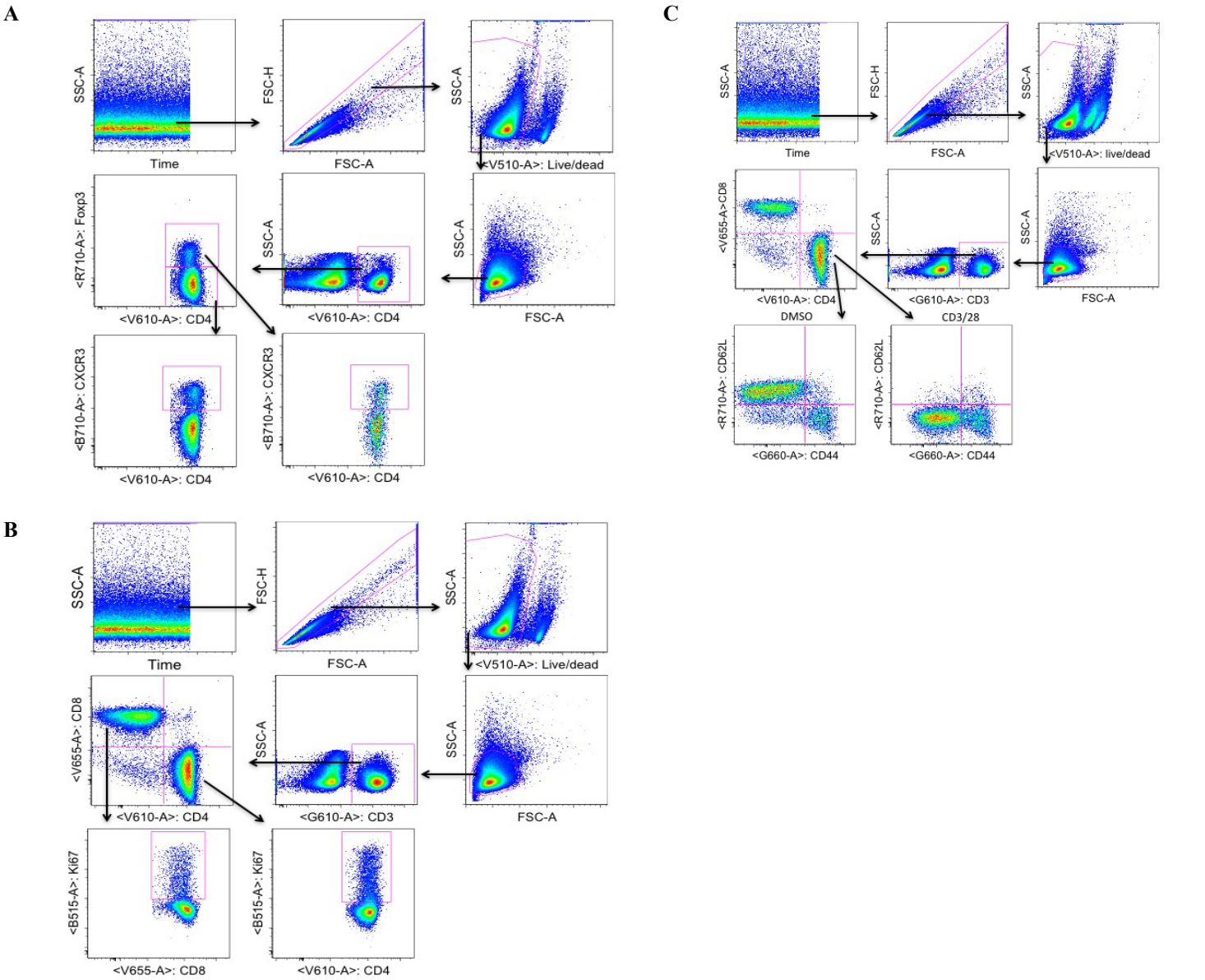
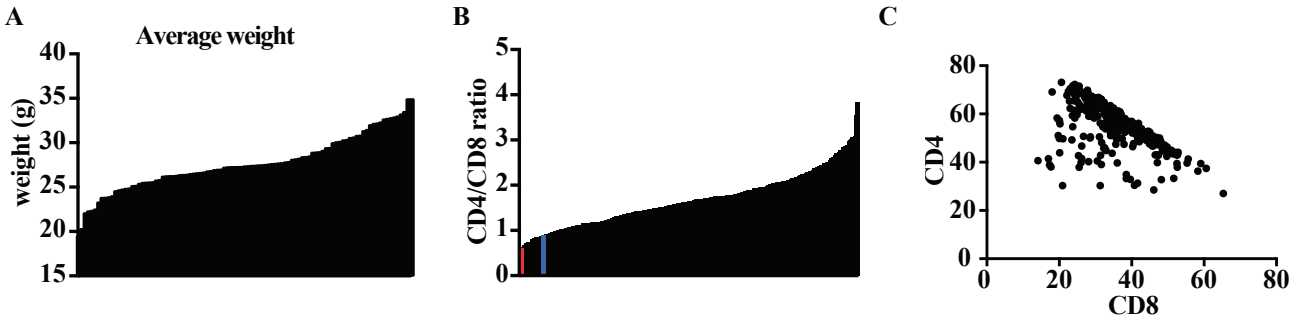
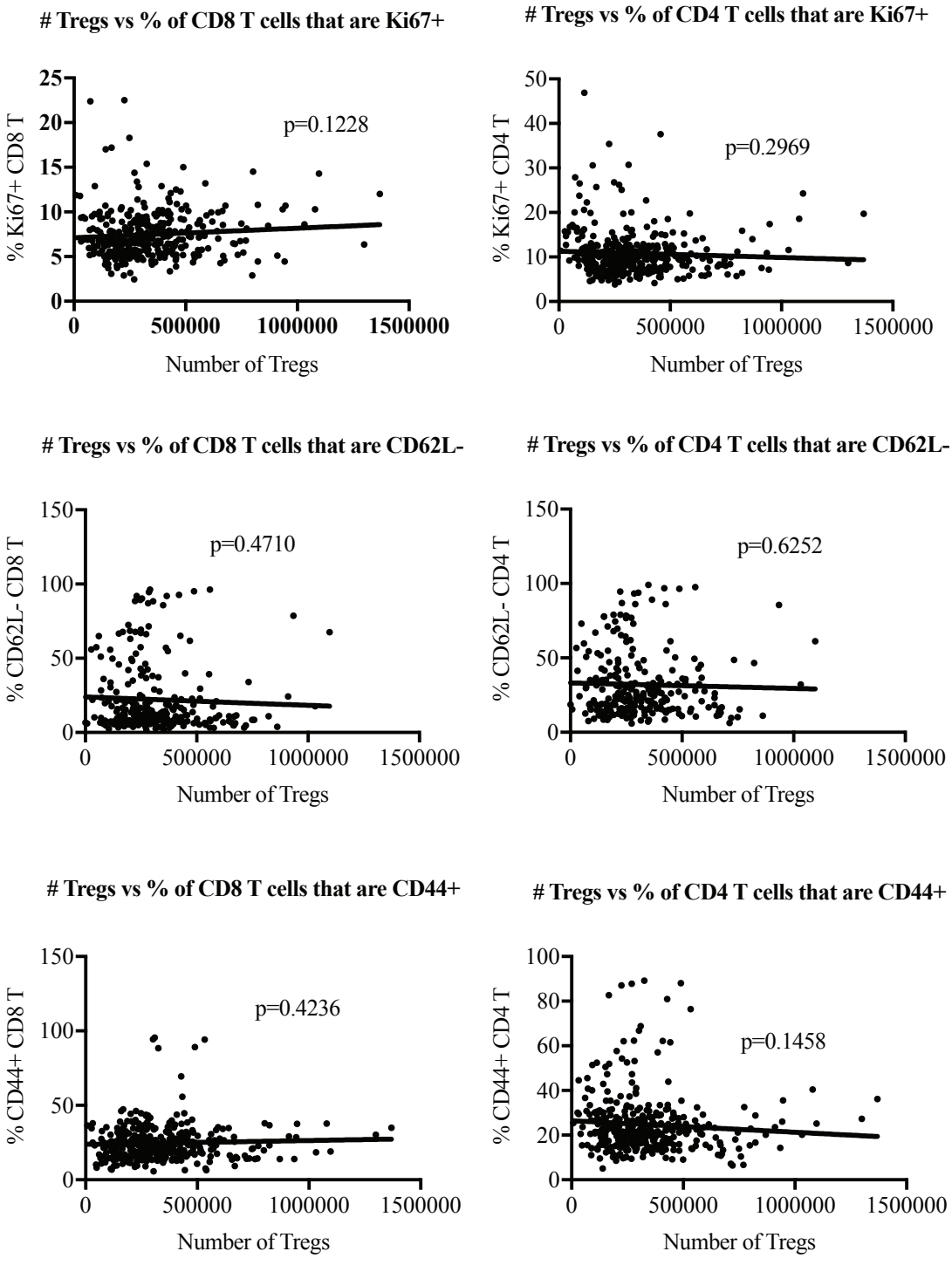


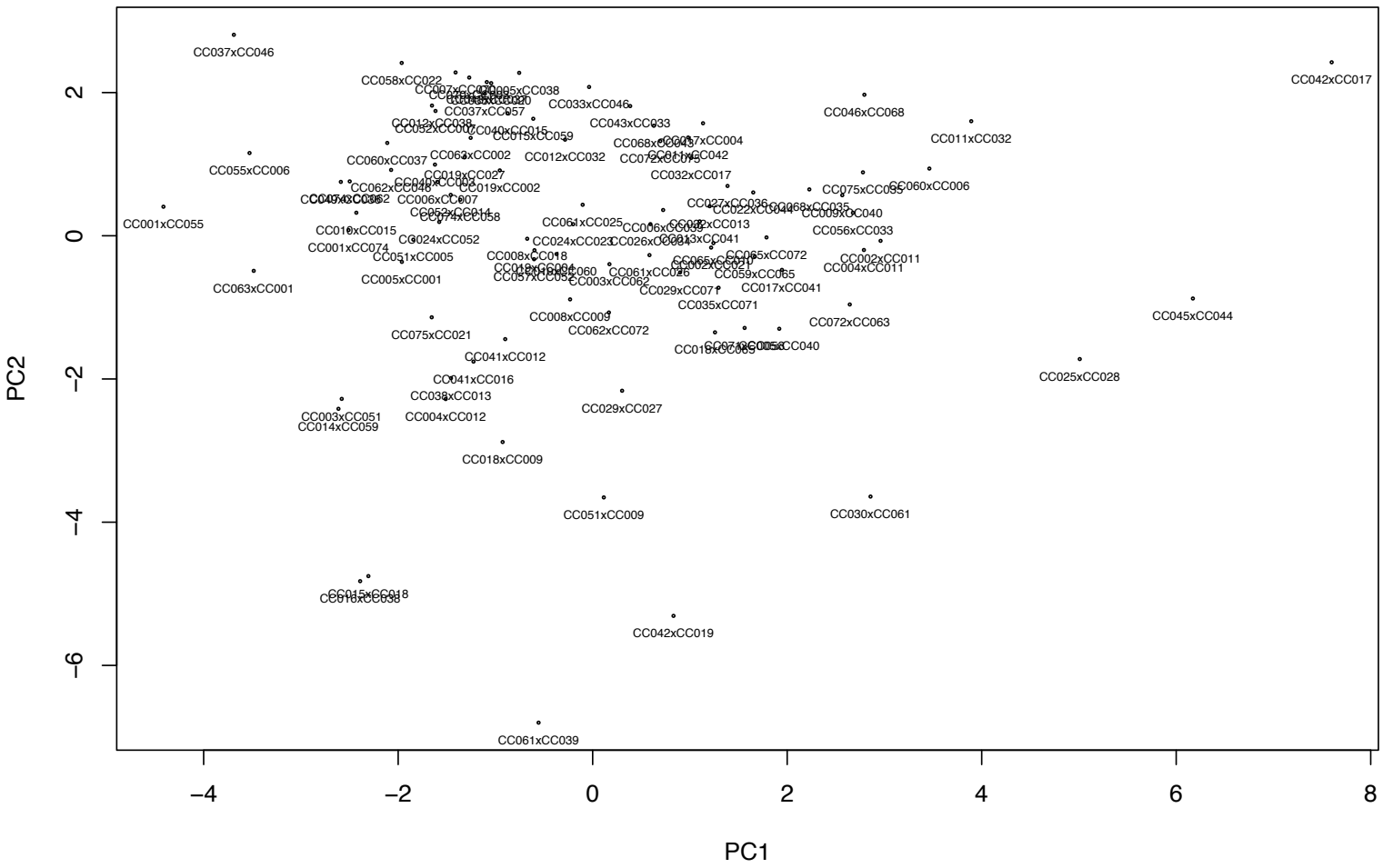
Supplemental Figure 1. Gating scheme for flow cytometry panels, related to Figs. 1-3. (a) Regulatory T Cell panel. The panel is gated in the following order: time, singlets, live, lymphocytes, CD4+, CD4+ Foxp3- and CD4+ Foxp3+, CD4+ Foxp3- CXCR3+, and CD4+ Foxp3+ CXCR3+. (b) T cell panel. The panel is gated in the following order: time, singlets, live, lymphocytes, CD3+, CD4+ and CD8+, CD8+ Ki67+, and CD4+ Ki67+. (c) Intracellular cytokine panel. The panel is gated in the following order: time, singlets, live, lymphocytes, CD3+, CD4+, CD62L versus CD44 (DMSO stimulation), and CD62L versus CD44 (anti-CD3/28 stimulation)



Supplemental Figure 2. Additional phenotypic variation in CC-RIX mice, related to Figs. 1-6. (A) Weight variability of CC-RIX mice at steady state at similar ages (8-10 weeks old). (B) CD4/CD8 ratio and (C) CD4 vs CD8 scatter plot for all CC-RIX lines in the screen.



Supplemental Figure 3, related to Fig. 3. Correlations between the number of Tregs in the spleen and the frequency of Ki67+ (proliferated) CD8 or CD4 T cells, CD62L- (activated) CD8 or CD4 T cells, or CD44+ (memory phenotype) CD8 or CD4 T cells.



Supplemental Figure 4, related to Figs. 1-3 and Supp. Table 4. Principal Component Analysis was performed to demonstrate the overall immune system phenotypic diversity among CC-RIX lines. The first two principal components (PCs) explained 19.8% and 14.2% of the total phenotypic variance, respectively. The top 3 phenotypes contributing to PC1 are frequency of Ki67+ CD4+ T cells (contributing 11.9%), frequency of CD4+ cells (11.2%), and frequency of CD3+ T cells (9.8%). The top 3 phenotypes contributing to PC2 are frequency of CD4+ T cells (ICS panel, CD3CD28 stimulation) (16.8%), frequency of TNFA- IFNG+ CD4+ T cells (ICS panel, CD3CD28 stimulation) (10.9%), and frequency of TNFA- IFNG+ CD8+ T cells (ICS panel, CD3CD28 stimulation) (10.6%).

Supplementary Table 1. T cell phenotypes identified in RIX screen, related to Figs. 1-3	
PHENOTYPE	**MARKERS**
Total T cells	CD3+
CD4 T	CD3+CD4+
CD8 T	CD3+CD8+
Regulatory T	CD3+CD4+Foxp3+
Tissue-migrating T	CD4+, CD8+, or Foxp3+ CCR5+, CXCR3+, or CD29+
Central memory T	CD4+ or CD8+ CD44+CCR7+CD62L+
Effector memory T	CD4+ or CD8+ CD44+CCR7-CD62L-
Memory/Ag experienced T	CD4+, CD8+, or Foxp3+ CD44-hi
Naïve T	CD4+ or CD8+ CD44-CD62L+
Tissue-resident memory T	CD4+ or CD8+ CD44-hi CD69+ and/or CD103+
Activated T	CD4+ or CD8+ combo of CD25+, ICOS+, CTLA-4+, Ki67+,CD62L-
Activated Treg	CD73+, CTLA-4+, or ICOS+ CD4+Foxp3+ cells
Th17	CD4+ IL-17+
Proliferating T	CD4+ or CD8+ Ki67+
Activated Th1	CD4+Ly6C+PSGL-1+
Follicular helper T	CD4+Ly6C-PSGL-1-
Th1	CD4+Tbet+ and/or IFNg+
Cytokine producing T	CD4+ or CD8+ TNFa+ and/or IFNg+
Short-lived effector T	CD8+CD44+KLRG-1+CD127-
Memory precursor T	CD8+CD44+KLRG-1-CD127+

Supplementary Table 2. RIX line information, related to Figs. 1-3.
***H2b b**, Strain carries H2b from B6 or 129*
***H2b Nonb**, Strain carries H2b from A/J, NOD, NZO, CAST, PWK, or WSB*
***H2b het**, One strain was H2b b (B6 or 129) and one strain was Nonb*
H2b, One strain was segregating H2b from both b and Nonb*
***H2b#**, One strain was recombinant in the H2b region between b and Nonb*

Line	H2b status	Average weight (g)	Line	H2b status	Average weight (g)
CC001xCC055	H2b het	24.63	CC032xCC013	H2b het	26.94
CC001xCC074	H2b het	ND	CC032xCC017	H2b het	27.66
CC002xCC011	H2b het	27.78	CC033xCC046	H2b Nonb	27.43
CC002xCC021	H2b het	ND	CC033xCC068	H2b het	27.23
CC003xCC051	H2b#	26.9	CC034xCC016	H2b het	ND
CC003xCC062	H2b het	26.46	CC035xCC020	H2b het	27.42
CC004xCC011	H2bb	30.28	CC035xCC071	H2b Nonb	ND
CC004xCC012	H2bb	27.67	CC036xCC051	H2b#	29.07
CC005xCC001	H2b het	25.14	CC037xCC046	H2b het	31.31
CC005xCC038	H2b het	ND	CC037xCC057	H2b het	31.43
CC005xCC040	H2b het	27.23	CC038xCC013	H2b het	27.49
CC006xCC007	H2b*	27.27	CC039xCC020	H2b het	26.37
CC006xCC039	H2b*	27.27	CC040xCC003	H2b het	33.18
CC007xCC070	H2b*	33.45	CC040xCC015	H2b het	29.4
CC007xCC075	H2b het	32.84	CC041xCC012	H2b het	25.46
CC008xCC009	H2b Nonb	27.61	CC041xCC016	H2b het	27.18
CC008xCC010	H2b het	32.12	CC042xCC017	H2b het	26.6
CC008xCC018	H2b het	30.54	CC042xCC019	H2b het	23.78
CC009xCC040	H2b het	32.62	CC042xCC025	H2b het	ND
CC010xCC015	H2b het	30.25	CC043xCC033	H2b Nonb	28.39
CC010xCC060	H2b het	30.77	CC043xCC037	H2b het	23.27
CC011xCC032	H2b het	28.9	CC044xCC060	H2b*	25.37
CC011xCC042	H2b het	25.12	CC045xCC044	H2b*	24.72
CC012xCC032	H2b het	26.21	CC046xCC068	H2b het	28.45
CC012xCC038	H2b het	25.48	CC049xCC036	H2b Nonb	27.82
CC013xCC026	H2b Nonb	ND	CC051xCC005	H2b#	28.88
CC013xCC041	H2b het	28.07	CC051xCC009	H2b#	30.78
CC014xCC059	H2b#	25.73	CC052xCC007	H2b het	24.82
CC015xCC018	H2b het	23.78	CC052xCC014	H2b het	28.2
CC015xCC059	H2b#	26.23	CC055xCC006	H2b*	30.1

CC016xCC038	H2b het	27.33	CC055xCC028	H2b het	34.88
CC016xCC061	H2b het	26.38	CC056xCC033	H2b#	27.53
CC017xCC004	H2b het	25.49	CC057xCC052	H2b het	27.33
CC017xCC041	H2b het	26.55	CC058xCC022	H2b Nonb	27.38
CC018xCC009	H2b het	28.37	CC059xCC065	H2b#	32.75
CC018xCC065	H2b het	26.29	CC060xCC006	H2b*	26.76
CC019xCC002	H2b het	27.59	CC060xCC037	H2b het	25.54
CC019xCC004	H2b het	19.46	CC061xCC025	H2b het	23.85
CC019xCC027	H2b*	22.26	CC061xCC026	H2b het	23.96
CC020xCC008	H2b het	32.28	CC061xCC039	H2b het	22.32
CC021xCC023	H2b het	26.5	CC062xCC046	Non	31.96
CC022xCC024	H2b Nonb	ND	CC062xCC072	H2b het	30.06
CC022xCC044	H2b*	20.26	CC063xCC001	H2b het	26.79
CC023xCC025	H2b het	29.38	CC063xCC002	H2b het	26.64
CC024xCC023	H2b het	26.94	CC065xCC010	H2b het	32.95
CC024xCC052	H2b het	ND	CC065xCC072	H2b het	32.15
CC025xCC028	H2b het	29.95	CC068xCC035	H2b het	ND
CC026xCC034	H2b het	25.79	CC068xCC043	H2b het	27.22
CC026xCC042	H2b het	ND	CC070xCC003	H2b*	28.87
CC027xCC036	H2b*	ND	CC071xCC058	H2b Nonb	ND
CC027xCC045	H2b*	24.53	CC072xCC063	H2b het	28.12
CC028xCC024	H2b het	30.57	CC072xCC075	H2b het	32.67
CC028xCC030	H2b het	ND	CC074xCC058	H2b Nonb	27.38
CC029xCC027	H2b*	22.06	CC074xCC062	H2b Nonb	22.47
CC029xCC071	H2b Nonb	ND	CC075xCC021	H2b het	24.8
CC030xCC023	H2b het	26.35	CC075xCC035	H2b het	26.18
CC030xCC061	H2b het	26.2			

Supplementary Table 3. Metrics of phenotypic dispersion, related to Figs. 1-3.
All values represent frequency of parent population as determined by flow cytometry

Phenotype	**Min.**	**Max.**	**Median**	**mean**	**B6 mean/SD**	**Balb/c mean/SD**
Total Cell Count	$8.64x10^5$	$65x10^5$	$25x10^6$	$26.6x10^6$		
Frequency of Tregs	1.82	25.5	7.525	8.2	10.0/1.00	12.52/0.58
Frequency of CD29+ Tregs	8.84	95.6	35.55	38.8	32.22/2.45	29.90/0.89
Frequency of CD44-hi Tregs	21.1	93	56.55	56.2	39.52/3.46	44.28/1.61
Frequency of CD73+ Tregs	3.49	97.8	56.15	56.1	81.12/1.2	51.96/2.38
Frequency of CTLA-4+ Tregs	11.2	86.2	45.05	45.3	46.18/1.35	34.08/1.69
Frequency of CXCR3+ Tregs	0.325	72.2	19.7	20.1	24.02/1.35	20.12/1.70
Frequency of ICOS+ Tregs	0.0138	64.1	18.1	20.4	12.48/1.06	12.2/0.90
Frequency of CD8+ T cells	14.1	65.3	34.6	35.6	36.62/0.87	32.07/0.90
Frequency of CD44+ CD8 T cells	5.75	95.5	22.8	24.7	21.4/1.0	22.6/0.80
Frequency of CD62Lneg CD8 T cells	1.53	96.2	11.8	22.1	3.58/0.39	3.00/0.20
Frequency of Ki67+ CD8 T cells	2.45	22.5	6.92	7.5	10.83/1.05	7.24/0.53
Frequency of CD4+ T cells	27	73.1	56.7	55.2	58.96/0.66	64.50/0.82
Frequency of CD44+ CD4+ T cells	5.03	89.2	21.7	24.7	16.68/1.29	21.940/1.12
Frequency of CD62Lneg CD4+ T cells	5.84	99.1	24.2	32.0	11.5/1.3	8.75/0.61
Frequency of Ki67+ CD4+ T cells	3.87	46.9	9.88	10.8	8.924/0.591	8.554/0.233
Frequency of IL-17+ CD8+ T cells after polyclonal stimulus	0	11.3	0.2025	0.5	0.0567/0.01	0.07664/0.01
Frequency of TNFanegIFNgpos CD8+ T cells after polyclonal stimulus	0	33.1	0.1805	1.1	0.05362/0.02	0.12064/0.02
Frequency of IL-17+ CD4+ T cells after polyclonal stimulus	0	12.9	0.2865	0.8	0.02188/0.02	0.01944/0.01
Frequency of Tbet+ CD4+ T cells after polyclonal stimulus	0	35.1	1.53	3.3	2.53/0.29	2.38/0.29
Frequency of TNFanegIFNgpos CD4+ T cells after polyclonal stimulus	0	41.2	0.101	1.5	0.05138/0.01	0.06968/0.01