

	Ratio of Morisita-Horn indices for all TCRs	Ratio of Morisita-Horn indices for T <sub>reg</sub> restricted TCRs	p-value
CD25 <sup>+</sup> T <sub>reg</sub> P to T <sub>reg</sub>	0.31±0.29	0.44±0.37	p>0.2
Foxp3 <sup>lo</sup> T <sub>reg</sub> P to T <sub>reg</sub>	0.35±0.35	0.41±0.36	p>0.2

**Supplementary Table 1.** Shown are the ratios of the Morisita –Horn indices for CD25<sup>+</sup> T<sub>reg</sub>P cells to mature T<sub>reg</sub> cells and Foxp3<sup>lo</sup> T<sub>reg</sub>P to mature T<sub>reg</sub> cells when analysis is based on all sequenced TCR sequences or when restricting analysis to only TCRs that were found in mature CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells. p-values calculated using two-sided t-test.

REAGENT or RESOURCE	SOURCE	Catalog number
Antibodies		
anti-mouse CD4 (GK1.5)- BV786	BD Biosciences	563331
anti-mouse CD4 (RM4-5)- BV510	BD Biosciences	563106
anti-mouse CD4 (RM4-5)- PE	eBioscience	12-0042-83
anti-mouse CD4 (GK1.5)- eF450	eBioscience	48-0041-82
anti-mouse CD4 (GK1.5)- APC	eBioscience	17-0041-83
Anti-mouse CD4 (RM4-5)- FITC	Tonbo Biosciences	35-0042-U500
Anti-mouse CD4 (GK1.5)- PE-Cy7	Biolegend	100422
Anti-mouse CD4 (RM4-5)- BV605	Biolegend	100548
Anti-mouse CD8 (53-6.7)- BV786	BD Biosciences	563332
Anti-mouse CD8 (53-6.7)- APC-eF780	eBioscience	47-0081-82
Anti-mouse CD8 (53-6.7)- Biotin	Tonbo Biosciences	30-0081-U500
Anti-mouse CD8 (53-6.7)- PerCP-Cy5.5	Biolegend	100734
Anti-mouse CD3 (145-2C11)- PE-Cy7	eBioscience	25-0031-82
Anti-mouse CD25 (PC61.5)- PerCP-Cy5.5	Tonbo Biosciences	65-0251-U100
Anti-mouse CD25 (PC61.5)- BV421	Biolegend	102043
Anti-mouse CD25 (PC61.5)- PE-Cy7	eBioscience	25-0251-82
Anti-mouse CD25 (PC61.5)- APC	eBioscience	17-0251-82
Anti-mouse FOXP3 (FJK-16s)- PE	eBioscience	12-5773-82
Anti-mouse FOXP3 (FJK-16s)- APC	eBioscience	17-5773-82
Anti-mouse FOXP3 (FJK-16s)- FITC	Biolegend	320112
Anti-mouse FOXP3 (FJK-16s)- AF700	eBioscience	56-5773-82
Anti-mouse CD45.1 (A20)- BV650	BD Biosciences	563754
Anti-mouse CD45.2 (104)- APC	eBioscience	17-0454-82
Anti-mouse CD90.1 (HIS51)- eF450	eBioscience	57-0900-82
Anti-mouse CD90.2 (53-2.1)- APC-eF780	eBioscience	47-0902-82
Anti-mouse CD73 (eBioTY/11.8)- eF450	eBioscience	48-0731-82
Anti-mouse CD73 (TY/11.8)- PE-Cy7	Biolegend	127224

Anti-mouse CD73 (TY/11.8)- BV605	Biolegend	127215
Anti-mouse CD73 (TY/11.8)- PE	Biolegend	127206
Anti-mouse TCRbeta (H57-597)- APC	eBioscience	17-5961-83
Anti-mouse TCRbeta (H57-597)- APC-eF780	eBioscience	47-5961-82
Anti-mouse TCRbeta (H57-597)- Pacific Blue	Biolegend	109226
Anti-mouse TCRbeta (H57-597)- BV421	Biolegend	109230
Anti-mouse Ter-119 (Ter-119)- Biotin	BD Biosciences	553672
Anti-mouse Ter-119 (Ter119)- APC-eF780	eBioscience	47-5921-82
Anti-mouse CD44 (IM7)- PE	eBioscience	12-0441-83
Anti-mouse CD44 (IM7)- BV786	BD Biosciences	563736
Anti-mouse CD45RB (C363.16A)- PE	eBioscience	12-0455-81
Anti-mouse CD11c (N418)- APC-eF780	eBioscience	47-0114-82
Anti-mouse CD11b (M1/70)- APC-eF780	eBioscience	47-0112-82
Anti-mouse NK1.1 (PK136)- APC-eF780	eBioscience	47-5941-82
Anti-mouse F4/80 (BM8)- APC-eF780	eBioscience	47-4801-82
Anti-mouse B220 (RA3-6B22)- APC-eF780	eBioscience	47-0452-82
Anti-cleaved-caspase 3 Asp175 (D3E9)- Alexa 647	Cell Signaling Technology	9602S
Anti-cleaved-caspase 3 Asp175 (D3E9)- PE	Cell Signaling Technology	12768S
Anti-GFP-FITC	Rockland	600-402-215
Anti-mouse Qa2 (69H1-9-9)- FITC	eBioscience	11-5996-82
Anti-mouse HSA (M1/69)- PE	eBioscience	12-0242-83
Anti-mouse CD69 (H1.2F3)- PE	BD Biosciences	553237
Anti-mouse CD69 (H1.2F3)- APC	Biolegend	104514
Anti-mouse ROR $\gamma$ T (Q31-378)- PE-CF594	BD Biosciences	562684
Anti-mouse MHC-I H2Kb (AF6-88.5.5.3)- APC	eBioscience	17-5958-82
Chemicals, Peptides, and Recombinant Proteins		
Human recombinant IL2	R&D Systems	202-IL
Mouse recombinant IL4	Tonbo Biosciences	21-8041

Annexin V- APC	BD Biosciences	550475
Dasatinib	Axon Medchem	BMS-354825
MOG <sub>35-55</sub> Peptide (MEVGWYRSPFSRVVHLYRNGK)	GenScript	RP10245
Complete Freund's Adjuvant	Sigma Life Science	F5881
M. Tuberculosis Des. H37 RA	Difco Laboratories	231141
Pertussis Toxin #180	List Biological Laboratories	#180
Streptavidin APC-eF780	eBioscience	47-4317-82
Live/Dead Fixable Viability Dye eF780	eBioscience	65-0865-14
Live/Dead Fixable Blue Dead Cell Stain	ThermoFisher	L-23105
Critical Commercial Assays		
Transcription Factor Staining Kit	eBioscience	00-5523-00
RNeasy Kit	Qiagen	74004
Annexin V Staining Buffer	eBioscience	00-0055-43