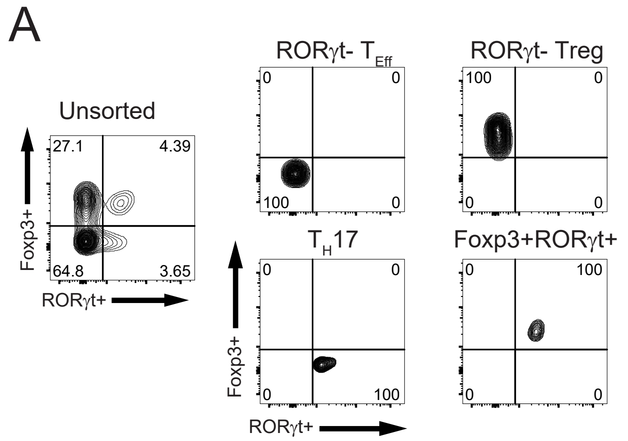
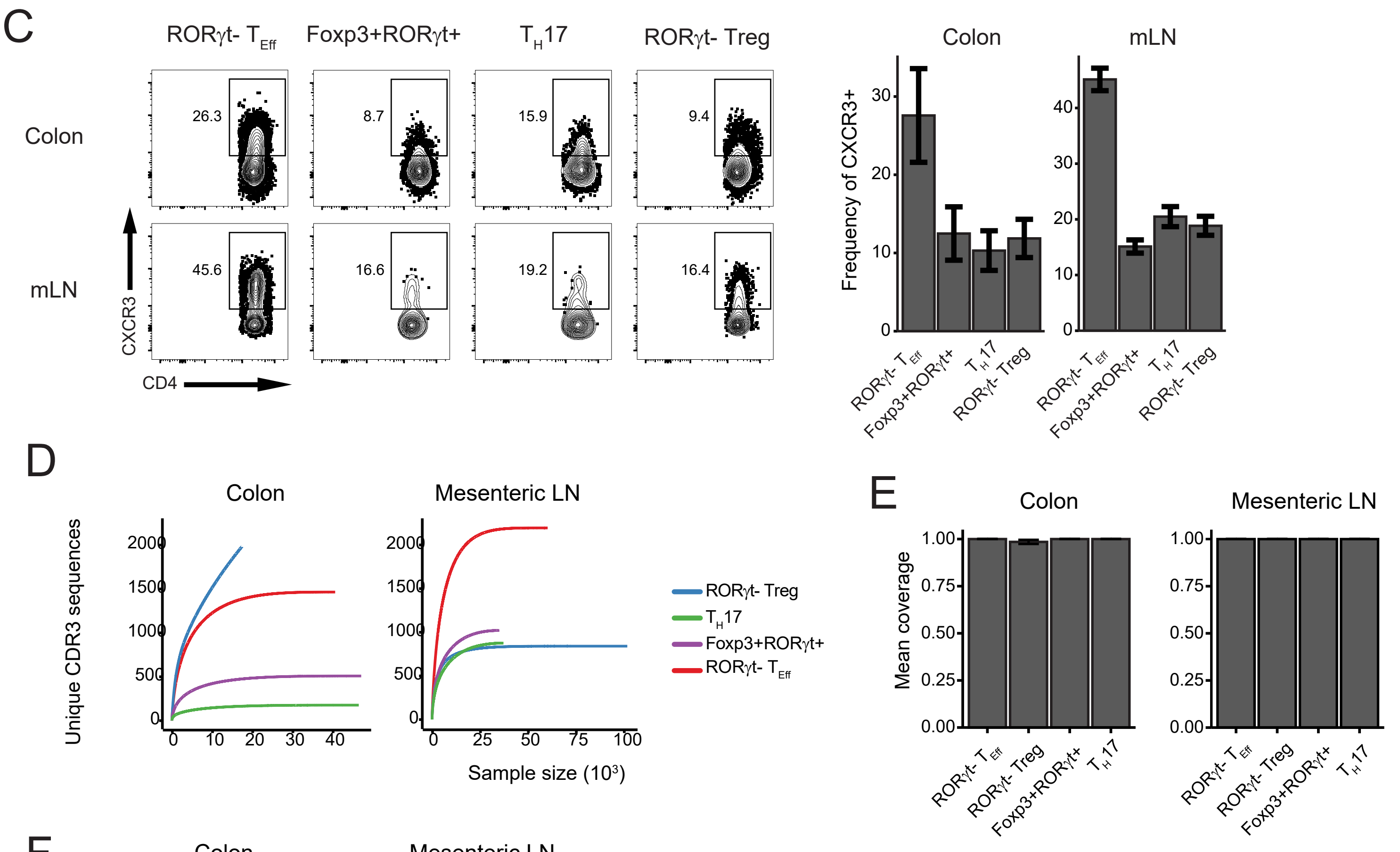
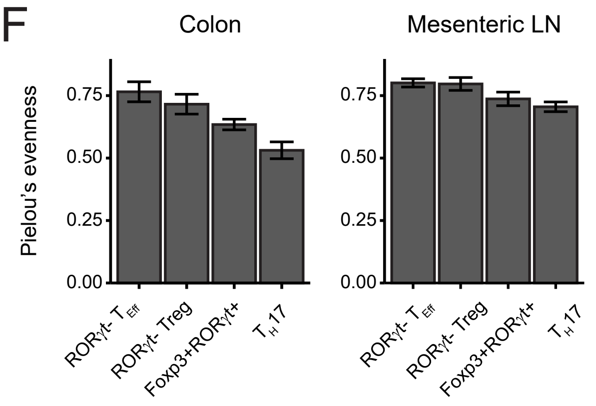


B

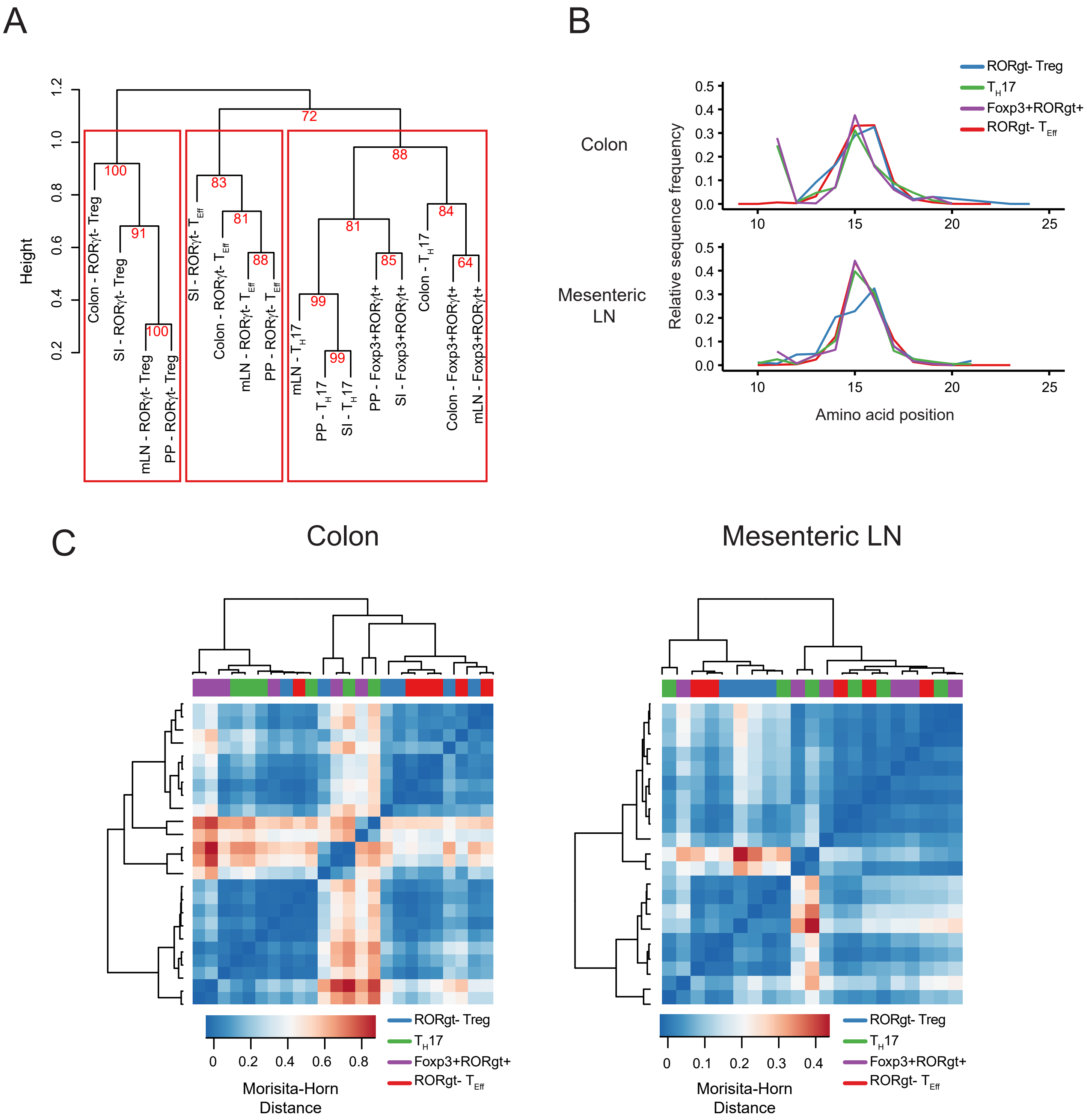
	Colon LP	mLN	PP	SI LP
RORγt- T_{Eff}	4680	6863	6206	1594
RORγt- Treg	3623	3488	3278	1031
Foxp3+RORγt+	1471	4552	907	632
T_H17	753	2918	1632	1100





Supplemental Figure 1, related to Figure 1. Sorting and TCR sequencing of mucosal CD4+ T cell subsets.

(A) Representative post-sort purity of sequencing populations. **(B)** Number of unique TCRα (TRAV_CDR3) sequences for each of the sorted T cell populations in indicated tissues. (LP) lamina propria, (mLN) mesenteric LN, (PP) Peyer's patch, (SI) small intestine. **(C)** Frequency of CXCR3+ cells within inidicated populations of CD4+ CD44-hi CD62L-lo cells. **(D)** Representative rarefaction curves for indicated sequencing populations. **(E)** Mean of calculated population coverage across all sequencing experiments for indicated populations and tissues. **(F)** Polyclonality indicated by average Pielou's evenness (evenness at order = 1) for each population in indicated tissues. n=6, each of 2 pooled mice. Error bars/ribbon = ± 1 s.e.m.



Supplemental Figure 2, related to Figure 2. Clustering of mucosal TCR repertoire based on CDR3 similarity

(A) Hierarchical clustering of all sequenced populations and tissues based on Euclidean distance of combined set of Morisita-Horn values from each mouse for a given population and tissue. Node value indicates reproducibility of branch point in bootstrapped trees. **(B)** Spectratype analysis of TCRs from sorted populations. Lines represent mean frequency of indicated sequence length for each population. **(C)** Hierarchical clustering of TCR perturbation calculated from MH distance (inverse of MH similarity with 0=identical, 1=complete dissimilarity) of spectratype profiles (set of relative frequency for each sequence length) between individual sequencing samples of indicated populations and tissues. n=6, each of 2 pooled mice.

	Average TCR frequency				Foxp3+RORγt+ vs. RORγt- Treg		Foxp3+RORγt+ vs. T_H17	
TCR	RORγt- T_{Eff}	RORγt- Treg	Foxp3+RORγt+	T_H17	log2fold	P-adj	log2fold	P-adj
Colon								
TRAV14-3*01_FCAASAIWNTGYQNFYFG	1.2E-04	2.2E-03	3.9E-02	5.3E-04	3.186	6.5E-02	3.47	4.1E-02
TRAV14-2*02_FCAAMGYQNFYFG	3.3E-05	0	4.6E-03	0.0E+00	4.960	4.4E-04	2.58	9.9E-02
TRAV14-2*02_FCAASWASGYNKLTFG	1.9E-03	2.9E-04	4.5E-03	1.5E-04	2.798	8.8E-02	2.48	9.9E-02
TRAV14-2*02_FCAASVTGNTGKLIFG	4.0E-04	1.4E-05	3.3E-03	1.0E-04	4.897	4.0E-04	3.55	4.0E-02
TRAV14D-3/DV8*01_FCAASEYSALGRLHFG	6.6E-05	0	1.8E-03	0	4.755	4.0E-04	7.00	1.4E-06
TRAV14D-3/DV8*01_FCAASDGNYAQGLTFG	2.5E-04	1.7E-05	2.3E-04	0	1.775	8.8E-02	2.93	4.9E-02
TRAV14-2*02_FCAASWASGDNKLTFG	4.9E-05	9.5E-06	1.2E-04	0	1.928	8.8E-02	2.64	5.7E-02
TRAV14-2*02_FCAAMGDQNFYFG	0	0	1.1E-04	0	2.198	8.8E-02	2.04	9.9E-02
TRAV14-3*01_FCAASGYNQGKLIFG	0	0	1.0E-04	0	2.060	8.8E-02	3.66	1.5E-02
TRAV14-1*01_FCAASYNNAGAKLTFG	0	0	7.8E-05	0	1.874	8.8E-02	2.02	9.9E-02
Mesenteric LN								
TRAV14-3*01_FCAASAIWNTGYQNFYFG	4.9E-04	1.9E-04	9.7E-03	1.1E-03	4.00	2.3E-02	3.47	4.1E-02
TRAV14D-3/DV8*02_FCAASYNNAGAKLTFG	0	0	6.9E-03	1.2E-04	5.34	2.7E-03	4.95	3.5E-03
TRAV14-2*02_FCAAMGYQNFYFG	1.1E-04	0	1.3E-03	6.0E-04	4.09	1.8E-02	2.58	9.9E-02
TRAV14D-3/DV8*02_FCAAAGANTGKLTFG	1.3E-04	0	8.4E-04	2.1E-04	4.19	1.5E-02	3.26	5.8E-02
TRAV14D-3/DV8*03_FCAASASSGSWQLIFG	2.7E-04	0	7.4E-04	1.2E-04	3.75	2.7E-02	2.17	9.9E-02
TRAV14-1*01_FCAASWASGYNKLTFG	6.4E-05	3.8E-05	5.4E-04	1.0E-04	2.88	7.2E-02	2.01	9.9E-02
TRAV14D-3/DV8*03_FCAASEYSALGRLHFG	0	4.4E-05	5.3E-04	0.0E+00	2.66	9.7E-02	5.18	1.0E-03
TRAV14D-3/DV8*02_FCAASRGGSAKLIFG	0	0	4.6E-04	0.0E+00	4.11	1.8E-02	4.36	5.3E-03
TRAV14-2*02_FCAASDGSNYQLIWG	2.7E-05	0	3.2E-04	0.0E+00	3.21	4.6E-02	3.33	2.4E-02

Supplemental Table I, related to Figure 3. Top Foxp3+RORγt+ TCRα sequences

Top 10 Foxp3+RORγt+ TCRα sequences from indicated tissues, sorted by frequency, meeting criteria of ≥1 $\log_2$fold enrichment and ≥0.1 false discovery rate adjusted p-value for comparison with both the RORγt- Treg and T_H17 populations, calculated by DESeq2. Bold indicates CT2 sequence

Primer	Sequence
1st-PCR Forward Va2 primer	CTTCCGATCATGGACAAGATCCTGACAGC
1st and 2nd PCR Reverse Ca-adaptor primer	CAAGCAGAAGACGGCATACGAGATtcgtacgagacgacacagcaggttctgggttctg
2nd PCR Forward adaptor primer	AATGATACGGCGACCACCGAGATCTACACCTTCCGATC
Forward Va2 sequencing primer	ACACCTTCCGATCatggacaagatcctgacagc
Reverse Ca2 sequencing primer	gacgacacagcaggttctgggttctggatgt
Forward index sequencing primer	cagaacccagaacctgctgtgt

Supplemental Table II, related to TCR Sequencing Experimental Procedure. Sequence of primers used for amplification and sequencing of TRAV14 TCR library for use with the Illumina MiSeq platform.