Supplementary Tables

Table S4. Differentially expressed genes between E8I$^{WT(Trac)}$ and E8I$^{\Delta(Trac)}$ CD4^{+}CD8αα^{+} IELs. Related to Figure 5.

Overexpressed in E8I$^{WT(Trac)}$			Overexpressed in E8I$^{\Delta(Trac)}$		
gene	**ave_logFC**	**p_val_adj**	**gene**	**ave_logFC**	**p_val_adj**
Cd83	8.577585	2.93E-10	*Gm21762*	-10.5303	1.67E-20
Klrb1a	6.074966	8.77E-10	*Klk1*	-9.84137	1.74E-13
Klrb1c	8.030817	3.8E-09	*Btla*	-7.32358	5.65E-08
Klre1	4.849967	4.02E-09	*Bcl11a*	-6.9866	3.84E-06
Spry2	5.75853	4.02E-09	*Cox6a2*	-9.01962	5.51E-06
Lag3	2.264442	7.29E-09	*Fcrla*	-7.98591	8.32E-06
Gm19590	7.492748	2.45E-06	*Gm17021*	-7.08457	3.41E-05
Gm16299	7.230879	3.84E-06	*Cct6a*	-1.04941	3.97E-05
Bcl2a1b	1.348215	4.8E-06	*Dnase1l3*	-5.57644	0.000122
Zdhhc15	3.332176	5.51E-06	*Alkbh3os1*	-6.74636	0.000178
Ky	4.523866	1.38E-05	*Zfp607b*	-3.9259	0.000315
Gm4956	4.323781	2.86E-05	*Gm43375*	-6.5652	0.00034
Cnrip1	6.095595	5.15E-05	*Gm38214*	-6.29071	0.000786
Gins1	5.599377	8.68E-05	*Gm43196*	-3.80262	0.001025
Kazald1	6.476025	9.52E-05	*Olfr99*	-5.29606	0.001253
Cd200r1	1.51688	0.000134	*Sell*	-6.53934	0.001317
Spin2c	6.760562	0.000135	*Gm49179*	-7.03964	0.001445
Gm1821	1.658691	0.000147	*Dntt*	-8.32337	0.001759
Zfp932	2.325318	0.000204	*Olfr528-ps1*	-5.79965	0.001884
Nrp2	3.161478	0.000387	*Tlr6*	-4.83628	0.002789
Gm17928	3.056256	0.000687	*Gm37321*	-5.22711	0.002818
Cyp2j9	6.412093	0.000786	*Lyst*	-2.68811	0.003276
Bcl2a1d	1.583238	0.001253	*Gm50221*	-5.98038	0.003785
Tspan4	5.606894	0.001253	*Fbxl13*	-6.66278	0.003851
Gm15996	3.612176	0.001445	*Gm26575*	-5.36307	0.004354
Gm42611	2.160161	0.001884	*1500002F19Rik*	-5.90029	0.004702
Hist1h2ap	2.174963	0.002789	*Trav9-2*	-2.84198	0.005862
Tnfrsf9	4.01641	0.002789	*Siglech*	-5.70372	0.006438
A930001A20Rik	6.932589	0.003114	*Flt3*	-3.48482	0.007187
Tmem30a	1.048406	0.00345	*Gm33882*	-5.21009	0.007605
Rorc	5.216056	0.00345	*Gm44958*	-6.21954	0.008696
Pla2g4b	5.985127	0.00345	*Wdr13*	-2.58232	0.009802
Golph3	1.529466	0.003785	*Mynn*	-1.66741	0.01207
Gm14435	3.132717	0.003795	*Cfap97*	-1.59325	0.013068
Zfp160	2.240651	0.003997	*Gm37357*	-1.68319	0.013597
Egr3	5.691184	0.004354	*Ppfia4*	-7.76848	0.016735
Zdhhc3	1.780378	0.004517	*Mtfp1*	-4.89168	0.017021
Gm11802	5.439506	0.004591	*Fam220a*	-1.48648	0.017486
Trav7d-5	6.150892	0.004702	*Gm48939*	-4.25497	0.021422
Trav7n-5	6.150892	0.004702	*Gm45495*	-3.3197	0.023629
Nfkbib	1.538276	0.005151	*Plaur*	-4.72063	0.032266
Stmn1	2.374753	0.006036	*Gm28373*	-4.53106	0.034206
Setmar	4.208802	0.006036	*Cd300c*	-7.77426	0.034914
Tsga10	4.828529	0.006226	*Plag1*	-5.82179	0.034914
Il3ra	4.934256	0.006226	*Appbp2*	-1.16342	0.034914
Fam174b	5.608441	0.006704	*9030407P20Rik*	-3.58214	0.03579
Cks2	1.710192	0.00711	*Plce1*	-2.54427	0.036514
Plek	3.135804	0.008186	*Gm16060*	-3.98283	0.037372
Gm47015	1.492324	0.008696	*Adcy3*	-2.10636	0.039316
Enkd1	3.061886	0.008868	*Lrp8*	-3.96959	0.039567
Gm16572	5.696436	0.009159	*Rlf*	-1.53071	0.040456
Creb3l1	5.436078	0.00985	*Rbm33*	-1.44965	0.040456
Osgin1	1.476867	0.010278	*Ltb4r1*	-4.5426	0.043707
Cenpf	3.138301	0.010639	*Trav13-1*	-2.70602	0.045393
Zfp458	2.6881	0.010772	*Trav10*	-2.52101	0.045947
Pnpo	1.792949	0.012193			
Clec16a	2.552224	0.012193			
Ska1	5.192346	0.012193			
Bcap29	1.224712	0.01458			
Rpl36-ps2	4.058048	0.01458			

Gem	5.760524	0.014852
Gm11423	2.545579	0.015383
Vps54	1.181797	0.017021
Itih5	3.820922	0.017109
Mlkl	4.21895	0.017486
Rictor	1.43574	0.018067
6330403K07Rik	3.216177	0.0202
Rida	1.775842	0.021422
Aoah	4.281722	0.021745
Mbnl3	1.335676	0.022484
Ephx1	2.189419	0.024468
Txnl4b	1.785039	0.027541
Gm25363	4.259935	0.029876
Ttc30a1	6.915919	0.03179
Glp1r	1.449691	0.032266
Ciart	4.260166	0.032266
As3mt	2.00779	0.032315
Gm36262	4.862997	0.032958
Igflr1	1.165425	0.033625
Gm47830	4.834154	0.034206
Gm26510	1.513678	0.036514
Nat14	4.424568	0.037372
Zfp212	1.779624	0.038153
Nup133	2.660052	0.039147
Ucp2	1.135401	0.040002
Kat6a	1.507153	0.041213
Gm48735	1.834047	0.041332
Penk	3.417682	0.041658
Retreg3	1.891107	0.042317
Pgm1	1.312999	0.043707
Tacc2	1.768766	0.043707
Cog7	1.98717	0.044089
Ylpm1	1.194531	0.044847
Lrrc20	1.862899	0.044847
Gm45110	2.411711	0.045947
Egr1	5.215026	0.04765

List of significantly expressed genes relatively overexpressed in $E8I^{WT(Trac)}$ $CD4^+CD8\alpha\alpha^+$ intraepithelial lymphocytes ($CD4^+CD8\alpha\alpha^+$IELs; left) or in $E8I^{\Delta(Trac)}$ $CD4^+CD8\alpha\alpha^+$ IELs (right) as assessed by the wald-test. Average log fold change of expression (ave_logFC) and adjusted p value (p_val_adj) as indicated. N=3 per genotype, $p_{sig}<0.05$. See also Figure 5.

Table S5. Differentially expressed genes between E8I$^{WT(Trac)}$ and E8I$^{\Delta(Trac)}$ CD8αβ^{+}CD8αα^{+} IELs. Related to Figure 5.

Overexpressed in E8I$^{WT(Trac)}$			Overexpressed in E8I$^{\Delta(Trac)}$		
gene	**ave_logFC**	**p_val_adj**	**gene**	**ave_logFC**	**p_val_adj**
Il2rb	1.838553	6.58E-18	*Plac8*	-1.89668	1.78E-26
Calcb	8.540629	3.06E-15	*Iglc3*	-8.77103	5.07E-17
Twist2	7.857668	7.89E-11	*Ly6d*	-9.98308	1.9E-14
Trim16	7.818193	1.3E-10	*Rnase6*	-7.78589	1.99E-13
Tigit	1.669783	2.94E-09	*Btnl7-ps*	-8.08394	3.67E-12
Tsga10	2.336488	9.2E-09	*Cd74*	-3.47087	5.08E-12
Gjb2	6.788909	2E-08	*Sdc4*	-4.4874	1.3E-11
Rgs16	7.370943	3.09E-08	*Ppp1r14d*	-8.84606	7.01E-10
Itih5	2.927409	4.68E-08	*Mgst1*	-8.59324	1.35E-09
Grk3	2.914981	2.08E-07	*Tcf7l2*	-5.52242	2.29E-09
Mtus2	6.363309	4.5E-07	*Dlg3*	-3.61153	1.75E-08
Sh2d2a	1.038083	4.79E-07	*Eya2*	-3.81918	4.91E-07
Rpl36-ps2	5.473854	1.93E-06	*Heatr5a*	-1.45863	2.77E-06
Spry2	1.78517	2.08E-06	*Slc51a*	-4.2348	2.89E-06
Phtf1os	6.594028	3.61E-06	*Gzmk*	-1.25234	3.41E-06
1700047G07Rik	2.376492	6.52E-06	*Clca4b*	-9.82448	3.61E-06
Nr4a1	5.926869	7.91E-06	*Smim22*	-7.36771	5.48E-06
Nusap1	5.378206	7.91E-06	*Cds1*	-6.52016	6.2E-06
Gm45716	2.275984	1.27E-05	*Cdc42ep4*	-6.15667	6.2E-06
Cdkn3	6.381035	1.32E-05	*Gprin3*	-6.52167	2.09E-05
Wnt10a	6.549708	1.35E-05	*Thy1*	-1.40797	2.09E-05
Plek	1.972456	1.35E-05	*Atp1b1*	-3.07396	2.18E-05
Ephx1	2.602742	2.09E-05	*Glipr2*	-3.34186	2.3E-05
Ckap2l	5.762481	2.09E-05	*Kcnj8*	-6.59678	2.79E-05
Serp2	4.215937	2.63E-05	*Ptprf*	-6.41495	2.9E-05
Dram1	6.451004	2.9E-05	*Ifit1bl1*	-1.94358	4.31E-05
Crem	2.716409	3.27E-05	*Prss30*	-6.19576	7.83E-05
2900026A02Rik	1.467805	3.27E-05	*Gpa33*	-6.5909	9.16E-05
Birc5	3.068787	3.44E-05	*4930447K03Rik*	-6.24899	9.16E-05
6720464F23Rik	1.830093	4.56E-05	*Gm39091*	-5.62813	0.00011
Klrb1a	4.024957	5.03E-05	*Pak6*	-6.75568	0.000113
Nrn1	5.842732	5.29E-05	*Rnf186*	-7.1091	0.000119
Ptpn6	1.302889	0.000113	*Btbd16*	-2.15688	0.000146
Cdc6	5.69186	0.000114	*Trim31*	-6.1468	0.000181
9530050K03Rik	4.68472	0.000119	*Crb3*	-2.5452	0.000243
Ska1	5.539225	0.000128	*Dkkl1*	-1.8539	0.000244
Cpa2	6.142104	0.000151	*Gtf3c4*	-2.53712	0.00033
Tox	1.322971	0.000202	*Il7r*	-2.17182	0.000428
Gm43636	3.863844	0.000225	*Soat2*	-1.02958	0.000455
Iqsec1	1.396754	0.000232	*Lypd8*	-10.6959	0.000476
Gm156	3.508176	0.000239	*Klf3*	-2.15986	0.000542
Gm48182	5.344042	0.000274	*H2-Ab1*	-3.23751	0.000642
Igflr1	1.194761	0.00033	*Phf11b*	-2.10252	0.000652
Gm38190	1.094031	0.000365	*Pou4f1*	-3.14446	0.000669
Gm30790	5.7876	0.000388	*Gdpd1*	-5.81388	0.000727
Mnd1	5.976298	0.000415	*Cdr2*	-2.52604	0.000759
Pdzd2	2.764092	0.000423	*Lta*	-2.9727	0.000775
Bcl2l11	1.152136	0.000444	*Gm36043*	-2.6426	0.001065
Tnfsf4	5.938302	0.00049	*Tm4sf5*	-3.21862	0.001082
Hist1h2ap	1.655867	0.000535	*Slc5a1*	-9.46691	0.001115
Nepro	2.364155	0.000554	*F2rl2*	-2.12807	0.001198
Zfp935	6.530183	0.000572	*Lgr4*	-4.04175	0.00123
Gm44901	1.768357	0.000577	*Ptafr*	-3.26735	0.00123
Slc35g2	5.537121	0.000642	*Cd24a*	-5.67781	0.001467
S100a1	1.906517	0.00071	*Mthfr*	-5.57881	0.001816
Zfp318	2.623999	0.000727	*S100g*	-4.58647	0.001886
Rnf19a	2.466756	0.000731	*App*	-2.26836	0.001928
Cd101	1.255209	0.000804	*Gm2237*	-2.79401	0.001977
4833445I07Rik	5.727192	0.000969	*Nfatc2*	-1.41669	0.00229
Dusp5	1.972161	0.001065	*Slc4a10*	-3.39131	0.0036
M1ap	3.148858	0.001065	*Mansc4*	-5.44433	0.004484
Unc13a	4.269183	0.00123	*Src*	-5.01571	0.00449
Bcl2a1b	1.37234	0.001232	*Ski*	-1.5631	0.004634
Gm12036	2.784046	0.001346	*Tmem86a*	-4.87196	0.005005

Gm20689	6.455044	0.00143
Cnih3	5.229141	0.00163
Lag3	2.059385	0.00173
Cib2	3.854989	0.001848
Trav9d-3	2.29694	0.001856
Trav9n-3	2.29694	0.001856
Cep85l	2.013048	0.001856
Gm14377	2.262636	0.001856
Ceacam-ps1	5.748506	0.001886
Kif11	2.632826	0.001886
Tnfrsf9	3.015079	0.001886
Endod1	2.086059	0.001886
Gm17928	2.721317	0.001886
Gm44860	4.017727	0.001966
Pabpc4	1.348914	0.00198
Zfp36l1	1.330951	0.002061
Ypel2	2.061505	0.002138
Dars2	2.221242	0.002253
Gmpr	4.610124	0.002282
C1qbp	1.010959	0.002475
Nrcam	4.278282	0.0036
Gm19590	2.897056	0.003952
Trav4d-4	6.622715	0.004201
Trav4n-4	6.622715	0.004201
Bcl2l15	2.359205	0.00449
Gm15287	3.360704	0.004735
Il6st	1.170593	0.004781
Stat4	1.089641	0.004801
Sulf2	2.2495	0.004921
Gm44752	3.64351	0.005005
Ccdc102a	1.601084	0.00514
Gm16638	2.277832	0.005644
Galnt4	2.330559	0.005662
Helz	1.22547	0.005887
Tctex1d1	4.153338	0.005903
Gm816	4.302373	0.005903
A530041M06Rik	1.451263	0.005993
Senp8	5.329353	0.006425
Gm16575	4.086798	0.006452
Ighv6-4	5.320397	0.007003
Fam83d	5.76987	0.007041
Cd200r2	2.3063	0.007041
Gm48904	2.976209	0.007674
BB557941	5.118324	0.007736
Wnk3	4.884693	0.007752
Cd200r1	1.846769	0.00815
Trbj1-1	2.12548	0.00858
Got2-ps1	5.089218	0.008827
Trav16d-dv11	5.299982	0.008938
Gm14021	5.048681	0.009171
Xlr3b	4.612688	0.009539
Ptrhd1	1.903929	0.009642
Ehmt1	1.514238	0.010158
Gm45406	4.974128	0.012009
Ltf	4.987067	0.0123
Tnfsf8	2.271699	0.013892
Arap3	1.686096	0.014451
Bcl2a1d	1.772999	0.01465
Lilr4b	1.425025	0.014869
E230029C05Rik	2.421245	0.015039
Gm16741	3.995124	0.016117
Glp1r	1.029124	0.016762
Capn3	1.017118	0.018631
Cela1	2.111948	0.018868
Pecam1	1.200806	0.018949
Birc2	1.054818	0.018978
Egr2	4.890629	0.019792
C330011M18Rik	3.062187	0.0205
Abhd14a	3.228564	0.021022
Trav14n-3	4.776638	0.021022

Taf4	-2.27375	0.006452
Cish	-1.14121	0.006919
Trpv2	-1.31028	0.007024
Myo7b	-5.99949	0.007253
Mirt1	-1.73149	0.007375
Tnfaip1	-2.0164	0.007449
Cox6a2	-5.60526	0.007597
Arhgef11	-6.1055	0.007849
Rbm47	-2.11455	0.007981
Tmem97	-1.09369	0.00858
Rogdi	-2.46868	0.008938
Ces2e	-8.64236	0.009581
Kdelr1	-1.6114	0.010074
Ap1m2	-3.36303	0.011595
5430431A17Rik	-1.14449	0.012706
Lactb2	-1.4014	0.014797
Trav13d-3	-5.12508	0.014838
Cmbl	-6.38465	0.015846
Cdkn2b	-4.55511	0.016762
Mttp	-3.84505	0.016762
Aoc1	-7.88392	0.017424
Tagln2	-1.48519	0.01779
Cxcr3	-1.52725	0.018036
Pde11a	-2.13368	0.018923

Ggct	1.201207	0.021022
Egr1	2.031614	0.021416
Mcoln2	2.041385	0.022436
9130019O22Rik	2.691536	0.022549
Gm17173	5.158085	0.022879
Klrd1	1.319259	0.023979
Rnf144a	3.170648	0.023994
Slc2a9	1.637185	0.023994
Sik1	2.309241	0.025315
Casp3	1.116233	0.02536
Zfp7	1.486469	0.025889
Gm11775	2.615296	0.026245
Gm49004	1.893869	0.026245
Fes	1.35603	0.026759
Gas2l3	2.790885	0.027359
Gm16675	3.018773	0.027845
Ubtd2	3.271827	0.028088
Zfp948	3.78703	0.028876
Cyth3	1.06176	0.030383
Ntf5	3.50029	0.030383
Sft2d2	1.095261	0.030458
Lig4	2.710782	0.030458
Cenpa	2.588078	0.030459
Gm44641	3.09145	0.030722
AW549877	1.320012	0.031428
Acoxl	1.092486	0.031582
Gm43042	1.125295	0.032614
Gm17056	4.447523	0.033808
Pdp1	1.637505	0.036477
4930505O20Rik	4.560369	0.036717
Rasgef1c	2.089432	0.036717
Rps26-ps1	1.741871	0.036976
Tyrobp	1.087746	0.037073
Fam174b	1.677018	0.037073
Gm11767	1.972068	0.037971
Cblb	1.202986	0.038716
Spo11	2.715193	0.040457
2700062C07Rik	1.304598	0.040457
Gm47571	2.421928	0.040457
Itgav	1.232708	0.040559
Gm48430	1.95179	0.041389
Trbv31	4.709295	0.041487
Matk	1.887288	0.042519
Ccdc112	2.424079	0.0427
Gm49359	1.577839	0.043574
Nr4a2	2.071877	0.043789
Ckm	2.559892	0.044383
Rasgef1a	1.9702	0.04661
Rtn4rl1	1.482473	0.046831
Cd200	1.696852	0.049049
Xaf1	1.549737	0.049049
Rbm11	1.773925	0.049794

List of significantly expressed genes relatively overexpressed in E8I$^{WT(Trac)}$ CD8β^+CD8$\alpha\alpha^+$ intraepithelial lymphocytes (CD8$\alpha\beta^+$CD8$\alpha\alpha^+$; left) or in E8I$^{\Delta(Trac)}$ CD8$\alpha\beta^+$CD8$\alpha\alpha^+$ (right) as assessed by the wald-test. Average log fold change of expression (ave_logFC) and adjusted p value (p_val_adj) as indicated. N=3 per genotype, p_{sig}<0.05. See also Figure 5.

Table S6. Differentially expressed genes between E8I$^{WT(Trac)}$ and E8I$^{\Delta(Trac)}$ nIELs. Related to Figure 5.

Overexpressed in E8I$^{WT(Trac)}$			Overexpressed in E8I$^{\Delta(Trac)}$		
gene	**ave_logFC**	**p_val_adj**	**gene**	**ave_logFC**	**p_val_adj**
Trac	1.651493	2.71E-26	*Trdc*	-4.53865	1.26254E-46
Spry2	2.094503	1.23E-21	*Cish*	-1.27063	7.03338E-10
Fcrl1	8.550116	2.84E-16	*A130014A01Rik*	-4.93386	1.30022E-09
Gm1821	1.164367	6.83E-11	*Bbs10*	-7.48837	2.01595E-09
Tigit	1.679362	7.03E-10	*Plac8*	-1.16391	3.38171E-08
Cd200r2	1.165216	4.22E-09	*Thy1*	-2.31287	4.3433E-08
Klhdc9	7.42437	4.22E-09	*Syk*	-6.91316	1.66875E-07
Rpl36-ps2	6.328376	4.21E-08	*Lat*	-1.18016	1.93934E-07
Cela1	1.21494	4.54E-08	*Krt18*	-8.56492	3.91255E-07
Smim10l2a	4.245129	5.05E-08	*Scin*	-1.94765	9.48968E-07
Klrb1a	1.827496	1.67E-07	*Gm47322*	-6.45336	2.60109E-06
Gm28112	1.008876	1.93E-07	*Kbtbd13*	-6.45716	2.99673E-06
Bcl2a1d	1.643027	1.96E-07	*5031439G07Rik*	-4.1797	3.08547E-06
Ikzf2	1.072478	2.2E-07	*Cd63*	-3.00503	3.08547E-06
Nr4a2	2.143791	2.72E-07	*Gm42872*	-5.24863	5.84823E-06
Cntln	2.19592	3.91E-07	*Gm39091*	-6.42539	1.77748E-05
Cnih3	4.192692	3.91E-07	*Thnsl2*	-4.97354	3.23055E-05
Sh2d2a	1.183584	8.47E-07	*B9d1*	-6.32686	3.26977E-05
Arhgap11a	4.707478	1.62E-06	*Cdc14b*	-3.06993	3.67793E-05
Apol10a	6.531363	1.88E-06	*Gzmk*	-1.00806	8.87897E-05
Gm156	2.721936	2.24E-06	*Susd2*	-5.59168	0.000233757
Bcl2a1b	1.163319	2.37E-06	*Gm26353*	-6.01318	0.000274643
Bcl2l15	1.619873	3E-06	*Pdzk1*	-7.3396	0.000320842
Gjb2	4.760034	3.73E-06	*Rnf146*	-1.10054	0.000595956
Gm17928	1.431437	3.73E-06	*Tpcn1*	-2.57531	0.000658268
Sft2d2	1.474215	4.37E-06	*Gm30934*	-5.83734	0.000711777
Pik3ap1	1.316334	5.85E-06	*Ifi208*	-1.46736	0.000723897
Tex2	1.209198	5.98E-06	*Zscan30*	-5.80015	0.000810335
Trav6-1	8.050649	1.4E-05	*Atp8a2*	-1.8044	0.001098701
Klhdc2	1.038815	1.76E-05	*Osm*	-6.16468	0.001165619
Ccrl2	1.647218	1.98E-05	*Gm45378*	-5.7467	0.001165619
Hal	3.449997	3.68E-05	*Fnbp1l*	-3.07833	0.001165619
Il2rb	1.278441	7.47E-05	*Gm45867*	-2.1641	0.001168729
Gm43042	1.033033	8.97E-05	*Galnt10*	-2.4545	0.001353819
Map4k5	2.318255	9.73E-05	*Cd6*	-3.89704	0.001390277
Ptpn6	1.312568	0.000137	*E430021H15Rik*	-1.39668	0.001390277
Pdgfb	5.47043	0.000145	*Ctrl*	-5.69373	0.001429243
Igip	2.471296	0.000256	*Gm8000*	-5.66916	0.001445218
Trio	1.65572	0.00026	*Acp2*	-1.75448	0.001881934
Arhgef3	1.033652	0.000338	*Gm10509*	-5.3901	0.002097053
Gm44937	3.799383	0.000372	*Cd226*	-1.89377	0.002118614
Klra3	4.05604	0.000385	*Ptafr*	-2.42438	0.002436268
Dclre1a	2.138831	0.000454	*Gm44745*	-4.37293	0.002618644
Gm12474	4.680125	0.000472	*Mgat4a*	-1.69174	0.002840022
Crem	2.195776	0.000486	*Bcl11b*	-1.54578	0.002858117
Gm38304	5.693536	0.000506	*Acss2*	-1.16265	0.003634139
Usp46	1.656611	0.000541	*Vezf1*	-1.04997	0.004021531
Nlrp1b	5.537333	0.00055	*Trav15d-1-dv6d-1*	-6.06744	0.004101732
Wls	1.250482	0.000674	*Pdzd9*	-5.42141	0.004181892
Mdfic	1.145723	0.000728	*Cdo1*	-3.67976	0.005921496
Arap3	1.213769	0.000821	*Trav15d-2-dv6d-2*	-4.01615	0.006417876
Gm44175	1.071119	0.000992	*Trav15n-2*	-4.01615	0.006417876
Gm37313	5.728253	0.001166	*Cxcr3*	-1.01362	0.006417876
Gsap	1.132776	0.001184	*Gm11869*	-5.30471	0.00755292
Gm47571	2.698057	0.001462	*AA914427*	-5.07344	0.00855734
Gm4032	2.935027	0.001462	*Gm19331*	-4.02333	0.009319369
Arhgef25	2.190686	0.001809	*Fbxl21*	-3.59406	0.009494792
A930002I21Rik	6.382722	0.001971	*Gm43566*	-5.2179	0.011211491
Ttc30a1	6.701831	0.002094	*Gbp11*	-5.19167	0.011374094
Trav9d-3	2.018748	0.002097	*Hist1h4n*	-4.66668	0.01169224
Trav9n-3	2.018748	0.002097	*Eepd1*	-5.03142	0.011742266
Pde7b	1.861599	0.002375	*Fbxo8*	-1.10453	0.011742266
Bend4	2.325691	0.002433	*Lncbate6*	-1.84667	0.012738966
Tsga10	1.196677	0.002619	*Gm12713*	-5.14117	0.013551604

Cables1	1.073842	0.002781	*Klf3*	-2.28268	0.013568717
Itih5	1.691067	0.002781	*Rgs4*	-2.68301	0.014253307
Ckm	1.563373	0.002835	*Cox7a1*	-2.99604	0.014653697
Nr4a1	2.516035	0.003536	*BE692007*	-2.24481	0.015664207
Gm19557	1.169353	0.003572	*Gm48565*	-4.69275	0.01658852
Kctd15	4.331551	0.00382	*Gm37352*	-3.38135	0.016781735
Zmym6	1.595695	0.004102	*Cdkl4*	-5.81185	0.020335977
Cd200	2.313767	0.006078	*Hist3h2ba*	-2.16839	0.02065085
Ace2	5.363982	0.006418	*Deptor*	-2.11977	0.021642335
Gm45716	1.398436	0.006627	*Mbtps2*	-1.19896	0.022154154
Slc16a11	2.017222	0.008392	*Cd4*	-1.9196	0.023487106
Gm10801	1.056987	0.008683	*A530041M06Rik*	-1.52878	0.024581149
Gm45640	3.758381	0.009084	*Gm49368*	-1.62591	0.025536346
Cers6	1.427203	0.009356	*Gm42161*	-3.40853	0.026337231
Naa16	1.142078	0.011211	*Gm10392*	-1.77524	0.026872804
Zfx	1.599695	0.011223	*Gmeb2*	-2.02454	0.030630494
4930431P03Rik	2.610789	0.011223	*Emp3*	-1.06952	0.030630494
Gm37124	3.526872	0.011541	*Inpp4b*	-1.75433	0.031222696
A530083M17Rik	1.692958	0.011692	*Gm28791*	-1.02655	0.031222696
Pidd1	2.467629	0.011742	*Mirt1*	-1.09175	0.031408479
Gm45235	2.023999	0.012155	*Ifit1bl1*	-1.10279	0.032532433
Gm17300	1.782229	0.01246	*Sit1*	-3.39506	0.034254991
Asah2	3.111448	0.012703	*Uchl1os*	-2.19954	0.034405101
Plek	1.905637	0.013253	*Zfp574*	-1.55884	0.041200011
Gm43137	5.171314	0.013253	*Clec12a*	-2.16814	0.042411778
Lag3	1.051722	0.014253	*Ifitm10*	-1.27995	0.042678342
Cyth3	1.065344	0.014253	*1600012H06Rik*	-1.32861	0.042843538
Tspan4	2.155092	0.014253	*Muc1*	-4.83637	0.04396801
Med14	1.352428	0.014598	*Trbj1-1*	-1.7668	0.046394178
Trav16n	2.107524	0.015334	*Gm42780*	-4.53285	0.048510052
Crim1	2.693165	0.015334	*B4galnt2*	-1.38957	0.048510052
Zfp595	1.562694	0.015742	*Fam53b*	-1.65382	0.048924305
Mettl9	1.161021	0.016583	*Trdc*	-4.53865	1.26254E-46
Cdkn2b	4.452195	0.016758	*Cish*	-1.27063	7.03338E-10
Pom121	1.440646	0.017047	*A130014A01Rik*	-4.93386	1.30022E-09
Gm29112	3.285148	0.018774	*Bbs10*	-7.48837	2.01595E-09
Gm43413	1.11705	0.018933	*Plac8*	-1.16391	3.38171E-08
Pabpc4	1.906865	0.020651	*Thy1*	-2.31287	4.3433E-08
Egr1	1.990024	0.020902	*Syk*	-6.91316	1.66875E-07
H60b	1.952307	0.020941	*Lat*	-1.18016	1.93934E-07
Klrb1	1.701475	0.023204	*Krt18*	-8.56492	3.91255E-07
Filip1	6.355295	0.023276	*Scin*	-1.94765	9.48968E-07
Kit	1.196299	0.023361	*Gm47322*	-6.45336	2.60109E-06
Gm11168	1.025009	0.023487	*Kbtbd13*	-6.45716	2.99673E-06
6030400A10Rik	5.006698	0.023729	*5031439G07Rik*	-4.1797	3.08547E-06
Pcdhb7	4.754488	0.025397	*Cd63*	-3.00503	3.08547E-06
Ints14	1.582965	0.02603	*Gm42872*	-5.24863	5.84823E-06
Itgav	1.039502	0.02789	*Gm39091*	-6.42539	1.77748E-05
Ccdc102a	1.119039	0.029585	*Thnsl2*	-4.97354	3.23055E-05
Usp38	1.29133	0.029926	*B9d1*	-6.32686	3.26977E-05
Pheta2	1.103337	0.030221	*Cdc14b*	-3.06993	3.67793E-05
Cd40lg	4.582232	0.032083	*Gzmk*	-1.00806	8.87897E-05
Gm33104	1.142824	0.033322	*Susd2*	-5.59168	0.000233757
Klra1	1.382559	0.033322	*Gm26353*	-6.01318	0.000274643
Hpf1	1.157305	0.034255	*Pdzk1*	-7.3396	0.000320842
Spred1	2.414797	0.034741	*Rnf146*	-1.10054	0.000595956
Gm3650	3.878987	0.035173	*Tpcn1*	-2.57531	0.000658268
Fam92a	1.220107	0.037203	*Gm30934*	-5.83734	0.000711777
Kbtbd7	1.920862	0.037684	*Ifi208*	-1.46736	0.000723897
Naprt	1.655793	0.0412	*Zscan30*	-5.80015	0.000810335
Gm44037	2.876672	0.041263	*Atp8a2*	-1.8044	0.001098701
Gm45894	3.898248	0.041696	*Osm*	-6.16468	0.001165619
Adh1	1.836137	0.041785	*Gm45378*	-5.7467	0.001165619
Dclk2	2.886998	0.043968	*Fnbp1l*	-3.07833	0.001165619
Zfp65	1.799708	0.044198	*Gm45867*	-2.1641	0.001168729
Gm20707	1.776769	0.044671	*Galnt10*	-2.4545	0.001353819
Gm10717	1.016993	0.044708	*Cd6*	-3.89704	0.001390277
Crybg1	1.237992	0.044708	*E430021H15Rik*	-1.39668	0.001390277
Tmem186	1.184771	0.046619	*Ctrl*	-5.69373	0.001429243
1700037H04Rik	1.390203	0.046891	*Gm8000*	-5.66916	0.001445218

St7l	1.267517	0.047474	*Acp2*	-1.75448	0.001881934
Tssk6	3.311044	0.047474			
Zfp850	2.04788	0.04851			
Edem3	1.512383	0.049055			
Gm10720	1.092274	0.049493			
Slc35f5	1.934453	0.049793			

List of significantly expressed genes relatively overexpressed in $E8I^{WT(Trac)}$ $CD4^{-}CD8\beta^{-}CD8\alpha\alpha^{+}$ intraepithelial lymphocytes (nIELs; left) or in $E8I^{\Delta(Trac)}$ nIELs (right) as assessed by the wald-test. Average log fold change of expression (ave_logFC) and adjusted p value (p_val_adj) as indicated. N=3 per genotype, $p_{sig}<0.05$. See also Figure 5.

Supplementary Figures and Legends

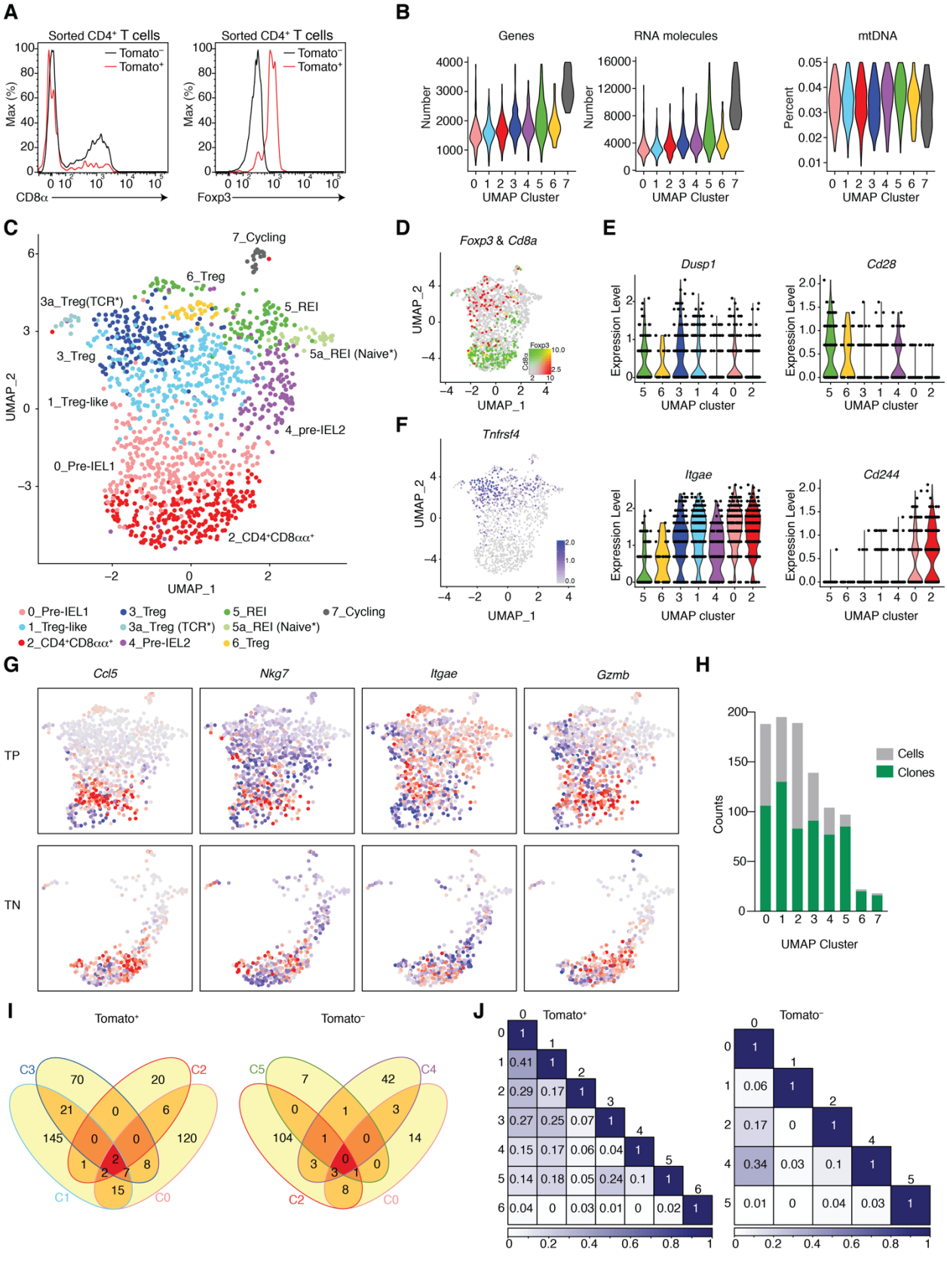
A
Sorted CD4+ T cells
Tomato−
Tomato+
Max (%)
CD8α
Foxp3
B
Genes
RNA molecules
mtDNA
Number
Percent
UMAP Cluster
C
7_Cycling
6_Treg
3a_Treg(TCR*)
5_REI
5a_REI (Naive*)
3_Treg
1_Treg-like
4_pre-IEL2
0_Pre-IEL1
2_CD4+CD8αα+
UMAP_1
UMAP_2
D
Foxp3 & Cd8a
E
Dusp1
Cd28
Expression Level
UMAP cluster
F
Tnfrsf4
Itgae
Cd244
G
Ccl5
Nkg7
Itgae
Gzmb
TP
TN
H
Cells
Clones
Counts
I
Tomato+
Tomato−
C0
C1
C2
C3
C4
C5
J
Tomato+
Tomato−

Figure S1 (related to Figure 1; Clonal distribution of intraepithelial CD4$^+$ T cells follows the single-cell trajectories). (**A-J**) A iFoxp3^{Tom} mouse was treated with tamoxifen for 10 weeks, and Tomato$^+$ (library 1) and Tomato$^-$ (library 2) CD4$^+$ T cells from the intestinal epithelium (IE) were sorted for scRNAseq using 10X Genomics platform. (**A**) Surface CD8α (left) and intranuclear Foxp3 (right) expression of sequenced CD4$^+$ Tomato$^+$ (red) and Tomato$^-$ (black) cells. (**B**) Number of sequenced genes (left) and RNA molecules (middle) per cluster and percent of mitochondrial DNA (right) per UMAP cluster. (**C**) UMAP clustering of 8 (0-7) distinct populations of single cells, including sub-clusters (3a, 5a). Sub-cluster 3a_Treg (TCR*) indicates Treg cells expressing TCR-stimulated genes. Sub-clusters 5a_REI-Treg (Naïve*) indicates *Sell*-expressing Treg cells. Cluster names correspond to cell colors throughout the figure as indicated. (**D**) Expression of *Foxp3* (red), *Cd8a* (green), or both (yellow) by all analyzed cells. (**E**) Expression of genes related to TCR signaling (top) or IEL program (bottom) in each UMAP cluster ordered by pseudotime trajectories. (**F**) Expression of *Tnfrsf4* (encoding Ox40) by analyzed cells. (**G**) UMAP projection color-coded by the residual expression of the indicated genes; red indicating levels of unspliced (upregulation) and blue indicating higher levels of spliced (downregulation) RNA molecules of each gene. (**H**) Total number of cells with paired $\alpha\beta$TCR sequences (grey) and total number of clones (green) within each UMAP cluster. (**I**) Venn diagram showing number of clones overlapping between indicated scRNAseq clusters. (**J**) Normalized Morisita-Horn index of paired $\alpha\beta$TCR per UMAP cluster among Tomato$^+$ (left) and Tomato$^-$ (right) cells. N=1 mouse, 1,294 sequenced cells.

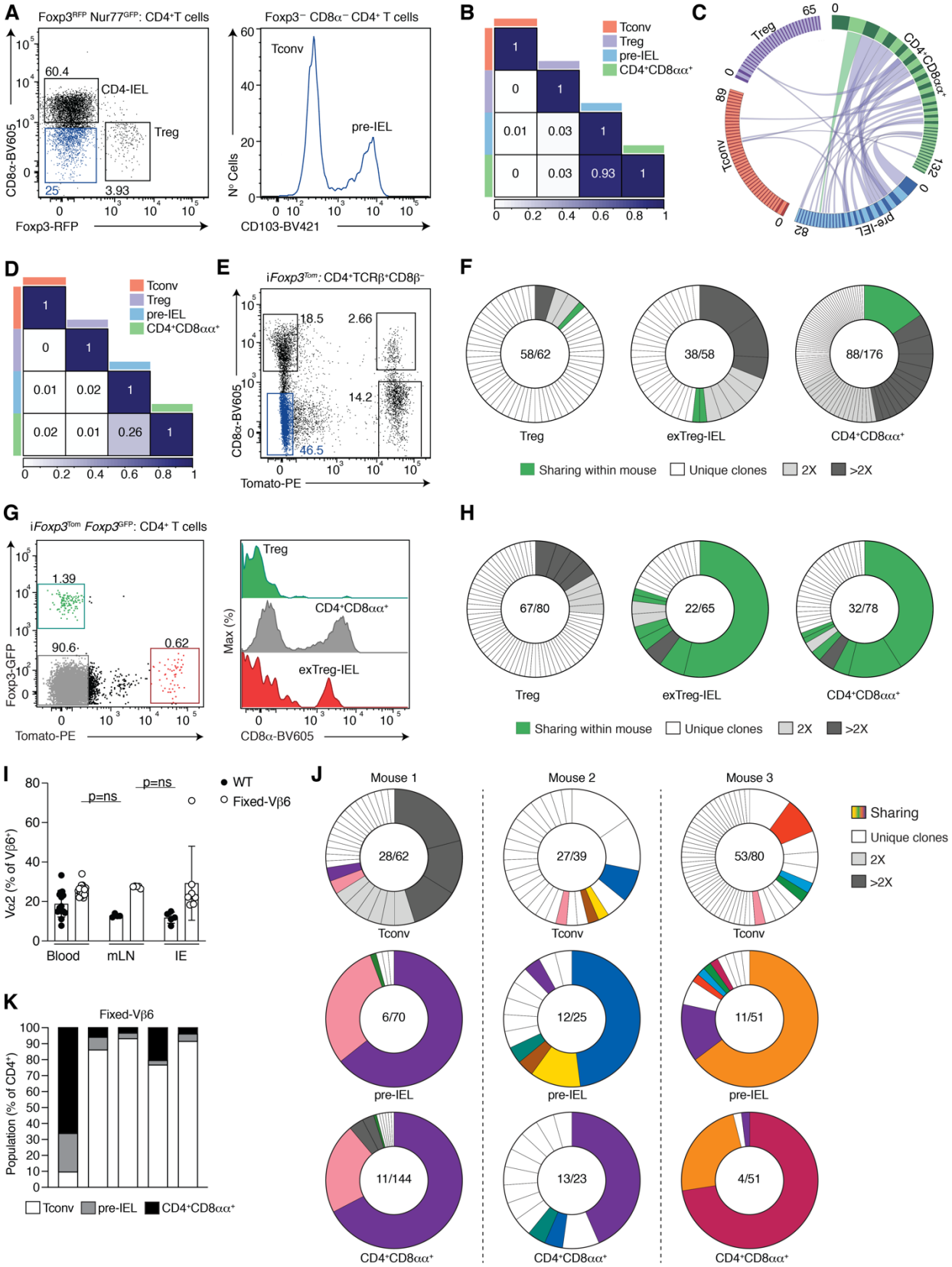
A
Foxp3RFP Nur77GFP: CD4+T cells
60.4
CD4-IEL
Treg
25
3.93
CD8α-BV605
Foxp3-RFP
Foxp3− CD8α− CD4+ T cells
Tconv
pre-IEL
№ Cells
CD103-BV421
B
Tconv
Treg
pre-IEL
CD4+CD8αα+
C
Treg
Tconv
pre-IEL
CD4+CD8αα+
D
E
iFoxp3Tom: CD4+TCRβ+CD8β−
18.5
2.66
14.2
46.5
Tomato-PE
F
58/62
38/58
88/176
Treg
exTreg-IEL
CD4+CD8αα+
Sharing within mouse
Unique clones
2X
>2X
G
iFoxp3Tom Foxp3GFP: CD4+ T cells
1.39
90.6
0.62
Foxp3-GFP
Tomato-PE
Treg
CD4+CD8αα+
exTreg-IEL
Max (%)
CD8α-BV605
H
67/80
22/65
32/78
Treg
exTreg-IEL
CD4+CD8αα+
Sharing within mouse
Unique clones
2X
>2X
I
p=ns
p=ns
WT
Fixed-Vβ6
Vα2 (% of Vβ6+)
Blood
mLN
IE
J
Mouse 1
Mouse 2
Mouse 3
28/62
27/39
53/80
Tconv
6/70
12/25
11/51
pre-IEL
11/144
13/23
4/51
CD4+CD8αα+
Sharing
Unique clones
2X
>2X
K
Fixed-Vβ6
Population (% of CD4+)
Tconv
pre-IEL
CD4+CD8αα+

Figure S2 (related to Figure 2; CD4$^+$CD8αα$^+$ IELs are clonally expanded with decreased TCR diversity). (**A-C**) CD4$^+$ T cells from the intestinal epithelium (IE) of two *Foxp3*^{RFP}*Nur77*^{GFP} mice were sorted as follows: CD4$^+$ conventional (Tconv; RFP$^-$CD103$^-$CD8α$^-$), regulatory T cells (Treg; RFP$^+$), pre-IEL (RFP$^-$CD103$^+$CD8α$^-$) and CD4$^+$CD8αα$^+$ IEL (RFP$^-$CD103$^+$CD8α$^+$). TCRβ and TCRα were sequenced via the MiSeq platform. (**A**) Dot plot of sorted cells as indicated. Blue histogram (right) of Foxp3$^-$CD8α$^-$ cells (blue in right graph). (**B**) Normalized Morisita-Horn index of paired αβTCR per population of mouse 1 of 2 sequenced mice. (**C**) Circos plot shows the distribution of paired αβTCR CDR3 sequences, with clones ordered clockwise from largest to smallest. Links indicate clonal sharing between populations within the mouse; purple links indicate clonal expansions lower than 10 cells, green links 10 or more cells. (**D**) Normalized Morisita-Horn index of paired αβTCR per population of 2 of 2 sequenced mice. (**E-F**) CD4$^+$ T cells from the IE of *Foxp3*$^{eGFP\text{-}cre\text{-}ERT2}$ x *Rosa26*$^{lsl\text{-}tdTomato}$ (iFoxp3^{Tom}) mice following 10 weeks of tamoxifen treatment were sorted as follows: CD4$^+$ conventional (Tconv; Tomato$^-$CD103$^-$CD8α$^-$), regulatory T cells mixed with Treg cell-derived pre-IELs (Treg cells and pre-IEL; Tomato$^+$CD8α$^-$), Tconv-derived pre-IELs (pre-IEL; Tomato$^-$CD103$^+$ CD8α$^-$), Treg cell-derived CD4$^+$CD8αα$^+$ IELs (exTreg-IEL; Tomato$^+$CD103$^+$CD8α$^+$) and Tconv derived CD4$^+$CD8αα$^+$ IELs (CD4$^+$CD8αα$^+$; Tomato$^-$CD103$^+$CD8α$^+$). TCRβ and TCRα were sequenced via the MiSeq platform. (**E**) Dot plot depicting CD8α and Tomato expression by IE CD4$^+$ T cells. Blue cells indicate population from which pre-IELs were derived, as in (**A**). (**F**) αβTCR clonal diversity of indicated populations of mouse 2 of 2 sequenced mice. Each slice represents a distinct αβTCR CDR3. Green clones represent sharing within each mouse. White slices represent unique clones and grey-scale slices represent expanded clones at indicated populations. The numbers enclosed in each graph indicate number of clones (numerator) and total number of cells (denominator) sequenced per indicated population. (**G-H**) CD4$^+$ T cells from the IE of *Foxp3*$^{eGFP\text{-}cre\text{-}ERT2}$ x *Rosa26*$^{lsl\text{-}tdTomato}$ x *Foxp3*$^{IRES\text{-}GFP}$ (iFoxp3^{Tom} *Foxp3*^{GFP}) mice after a 10 week tamoxifen treatment were sorted as follows: regulatory T cells (Treg; GFP$^+$Tomato$^-$CD8α$^-$), Treg cell-derived CD4$^+$CD8αα$^+$ IELs (exTreg-IEL; Tomato$^+$CD8α$^+$) and Tconv cell derived CD4$^+$CD8αα$^+$ IELs **(**CD4$^+$CD8αα$^+$; Tomato$^-$CD8α$^+$). TCRβ (and TCRα of expanded TCRβ clones) were sequenced via the MiSeq platform. (**G**) Dot plot of GFP$^+$ (green), Tomato$^+$ (red) and double-negative (grey) CD4$^+$ T cells in the IE (left) and their corresponding CD8α expression (histogram, right) with sorted populations as indicated. (**H**) αβTCR clonal diversity of indicated populations of mouse 1 of 2 sequenced mice. Label scheme as in (F). (**I-K**) Total Vα2$^+$ Vβ6$^+$ CD4$^+$ T cells from three fixed-Vβ6 x *Foxp3*^{GFP} mice were single-cell sorted as follows: Tconv cell

(CD103^{-}CD8α^{-}), pre-IEL (CD103^{+}CD8α^{-}) and CD4^{+}CD8$\alpha\alpha^{+}$ IEL (CD103^{+}CD8α^{-}). TCRα were sequenced via the MiSeq platform. (**I**) TCR Vα2 frequencies among TCR Vβ6 CD4^{+} T cells in blood, mesenteric lymph nodes (mLN) and intestinal epithelium (IE) of WT and fixed-Vβ6 mice. (**J**) Clonal diversity of indicated populations of three sequenced mice. Each slice represents a distinct TCRα CDR3. Colored clones represent sharing between populations and mice; pink clone is the same as the transnuclear (TN) monoclonal strain, purple is 1 amino acid different from the TN clone. White slices represent unique clones and grey-scale slices represent expanded clones at indicated numbers. (**K**) Relative frequencies of indicated IE populations among CD4^{+} T cells, each bar represents 1 mouse.

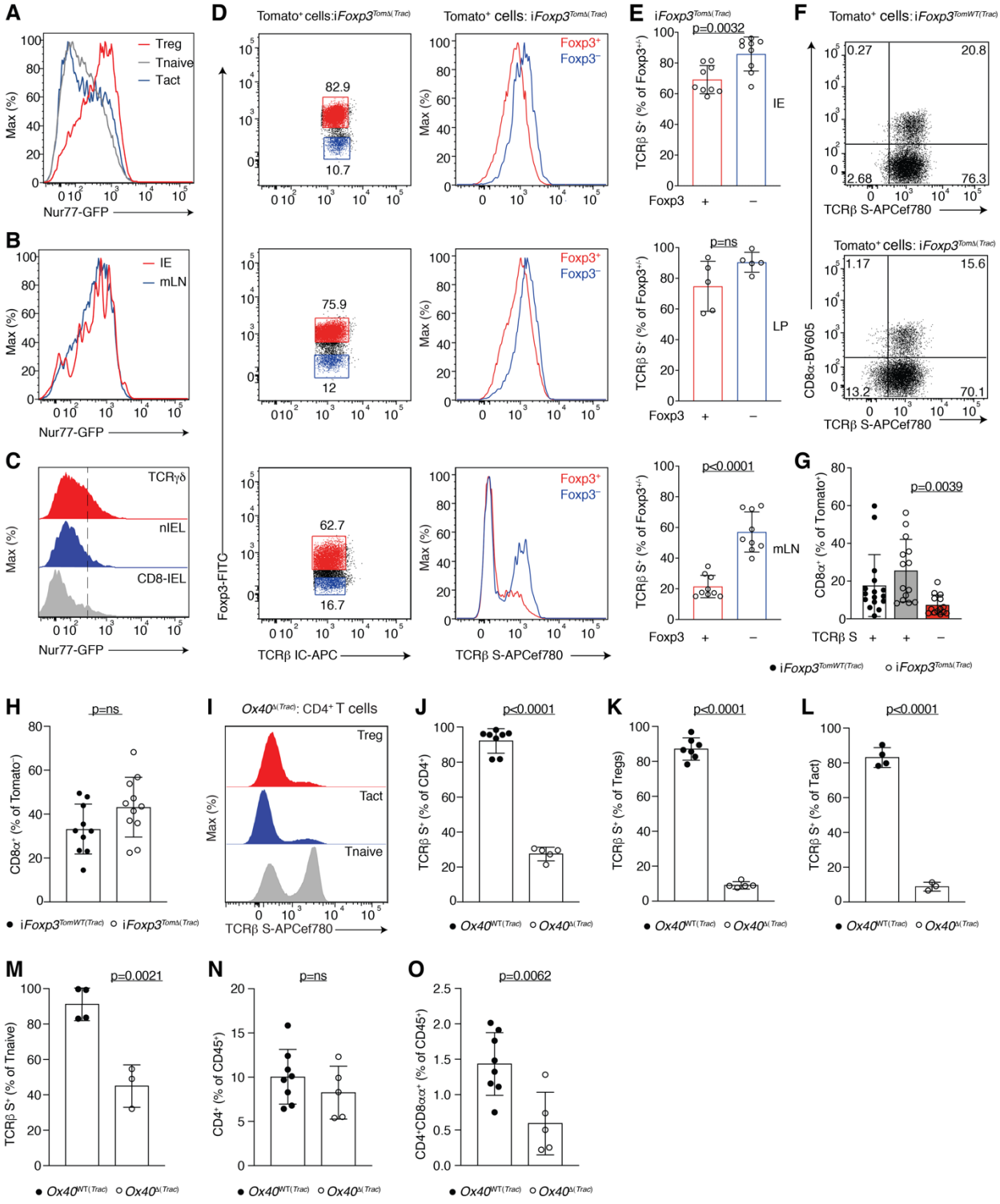
A
Treg
Tnaive
Tact
Max (%)
Nur77-GFP
B
IE
mLN
C
TCRγδ
nIEL
CD8-IEL
D
Tomato⁺ cells:iFoxp3Tomδ(Trac)
82.9
10.7
75.9
12
62.7
16.7
Foxp3-FITC
TCRβ IC-APC
Tomato⁺ cells: iFoxp3Tomδ(Trac)
Foxp3⁺
Foxp3⁻
TCRβ S-APCef780
E
iFoxp3Tomδ(Trac)
p=0.0032
IE
p=ns
LP
p<0.0001
mLN
TCRβ S⁺ (% of Foxp3+/-)
Foxp3 + −
F
Tomato⁺ cells: iFoxp3TomWT(Trac)
0.27
20.8
2.68
76.3
1.17
15.6
13.2
70.1
CD8α-BV605
G
p=0.0039
CD8α⁺ (% of Tomato⁺)
TCRβ S + + −
iFoxp3TomWT(Trac)
iFoxp3Tomδ(Trac)
H
p=ns
CD8α⁺ (% of Tomato⁻)
I
Ox40Δ(Trac): CD4⁺ T cells
Treg
Tact
Tnaive
J
p<0.0001
TCRβ S⁺ (% of CD4⁺)
K
p<0.0001
TCRβ S⁺ (% of Tregs)
L
p<0.0001
TCRβ S⁺ (% of Tact)
M
p=0.0021
TCRβ S⁺ (% of Tnaive)
N
p=ns
CD4⁺ (% of CD45⁺)
O
p=0.0062
CD4⁺CD8αα⁺ (% of CD45⁺)
Ox40NT(Trac)
Ox40Δ(Trac)

Figure S3 (related to Figure 3; TCR signaling is required for CD4$^+$CD8αα$^+$ IEL differentiation).

(**A-C**) *Nur77*-GFP expression by indicated cell types from *Nur77*^{GFP} *Foxp3*^{RFP} double-reporter mice. (**A**) *Nur77*^{GFP} expression among CD4$^+$ Foxp3$^+$ regulatory T cells (Treg, red), CD62L^{high}CD44^{low} naïve T cells (Tnaïve, grey), and CD62L^{low}CD44^{high} Foxp3$^-$ activated T cells (Tact, blue) in the mesenteric lymph nodes (mLN). (**B**) *Nur77*^{GFP} expression among Foxp3$^+$ Treg cells in the intestinal epithelium (IE, red) and mLN (blue). (**C**) *Nur77*^{GFP} expression among TCRγδ-IELs (red), CD8αα$^+$CD8β$^-$CD4$^-$TCRαβ$^+$ natural IELs (nIEL, blue) and CD8αα$^+$CD8β$^+$TCRαβ$^+$ IELs (CD8αβ$^+$CD8αα$^+$, grey) in the small intestine epithelium. (**D-H**) Flow cytometry analysis of iFoxp3$^{Tom\,WT(Trac)}$ (*Trac*$^{+/+}$ iFoxp3^{Tom}) or iFoxp3$^{Tom\,\Delta(Trac)}$ (*Trac*$^{f/f}$ iFoxp3^{Tom}) mice 8-12 weeks after tamoxifen administration. (**D**) Dot plots (left) for intracellular TCRβ and Foxp3, or histograms (right) of surface TCRβ expression among tomato$^+$ Foxp3$^+$ Treg cells (red) and tomato$^+$ Foxp3$^-$ CD4$^+$ T cells (blue) in the intestinal epithelium (IE, top), lamina propria (LP, middle) and mesenteric lymph nodes (mLN, bottom). (**E**) Frequencies of surface TCRβ-expressing cells among Foxp3$^+$ or Foxp3$^-$ among tomato$^+$ CD4$^+$ T cells from iFoxp3$^{Tom\,\Delta(Trac)}$ IE (top), LP (middle) or mLN (bottom). (**F**) Dot plots of surface CD8α and TCRβ of Tomato$^+$ CD4$^+$ T cells in iFoxp3$^{Tom\,WT(Trac)}$ (top) and iFoxp3$^{Tom\,\Delta(Trac)}$ (bottom) mice. (**F**) Frequencies of CD8α$^+$ cells among Tomato$^+$ CD4$^+$ T cells within TCR-sufficient cells from iFoxp3$^{Tom\,WT(Trac)}$ (white bar) or iFoxp3$^{Tom\,\Delta(Trac)}$ (grey bar) mice, or TCR-deficient cells from iFoxp3$^{Tom\,\Delta(Trac)}$ (red bar) mice. (**H**) Frequency of CD8α-expressing CD4$^+$ (CD4$^+$CD8αα$^+$ IELs) cells among tomato$^-$ cells in the IE of iFoxp3$^{Tom\,WT(Trac)}$ or iFoxp3$^{Tom\,\Delta(Trac)}$ mice. (**I-O**) Flow cytometry analysis of cells isolated from the IE and mLN of 9-12 week-old *Ox40*$^{WT(Trac)}$ (*Trac*$^{+/+}$ *Ox40*$^{cre+/-}$ or *Trac*$^{f/f}$ *Ox40*$^{cre-/-}$) or *Ox40*$^{\Delta(Trac)}$ (*Trac*$^{f/f}$ *Ox40*$^{cre+/-}$) mice. (**I**) Histogram of surface TCRβ expression among Treg cells (red), Tact cells (blue) and Tnaïve cells (grey) CD4$^+$ T cells in the mLN. (**J-M**) Frequencies of surface TCRβ-expressing cells among CD4$^+$ T cells (**J**), Tregs (**K**), CD4$^+$ activated cells (Tact, Foxp3$^-$CD44^{high}CD62L^{low}) (**L**) and CD4$^+$ naïve cells (Tnaïve, Foxp3$^-$CD44^{low}CD62L^{high}) (**M**) cells in the mLN. (**N, O**) Frequencies of total CD4$^+$ T cells (**N**) and CD4$^+$CD8αα$^+$IELs (CD8α$^+$TL-Tetramer$^+$) (**O**) among total CD45$^+$ cells in the IE. Data are expressed as mean +/- SEM of individual mice (n=5-16, 2-6 experiments). Significant p values as indicated [student's t test (**E,H-L, N,O**) or one-way ANOVA and Bonferroni (**G**)].

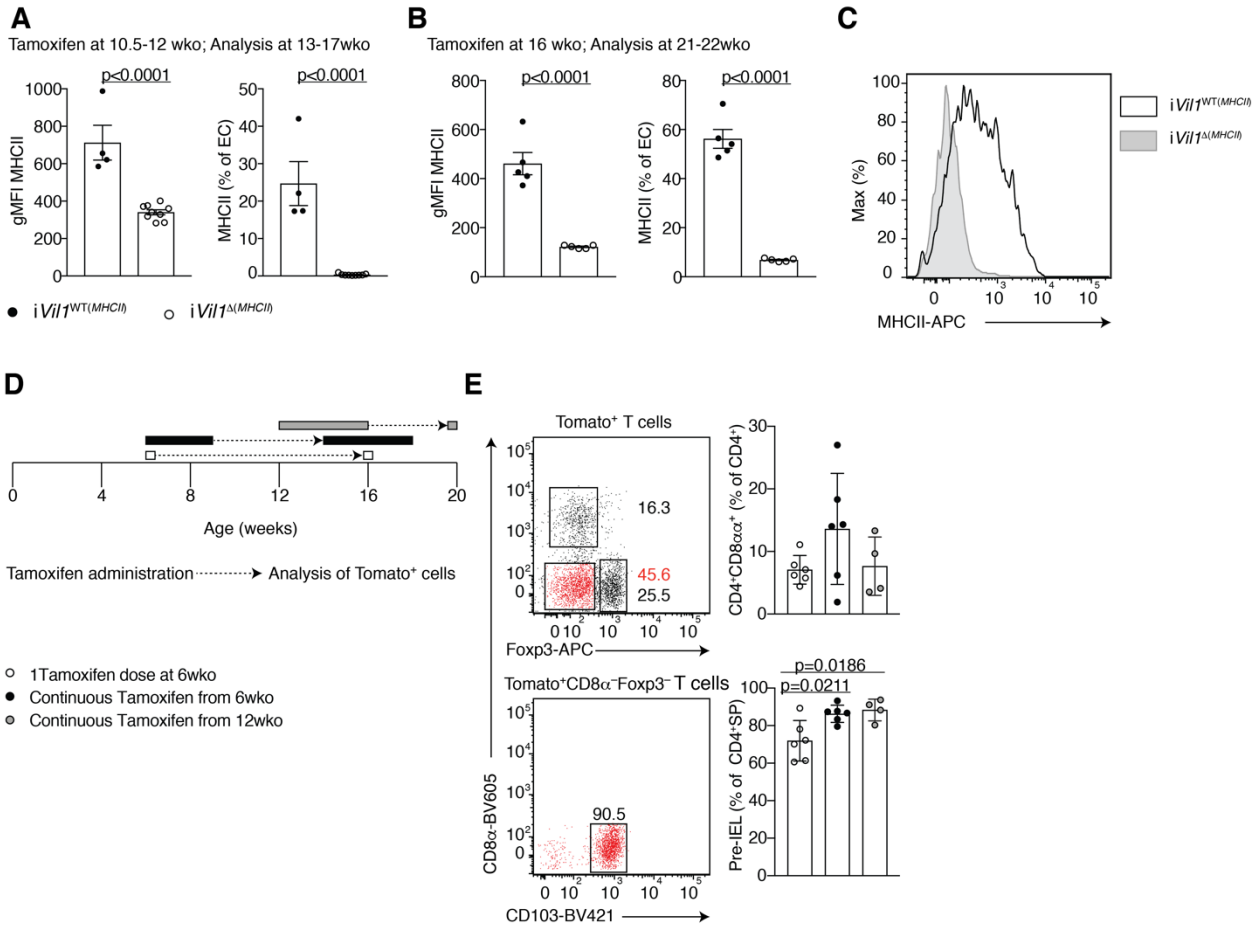

Figure S4 (related to Figure 4; MHCII expression by epithelial cells is required for CD4$^+$CD8αα$^+$ IEL differentiation). (**A-C**) Flow cytometry analysis of the intestinal epithelium (IE) of i*Vil1*$^{WT(MHCII)}$ (*H2-Ab1*$^{+/+}$ *Vil1*$^{creERT2+/-}$ or *H2-Ab1*$^{f/f}$ *Vil1*$^{creERT2-/-}$) or i*Vil1*$^{Δ(MHCII)}$ (*H2-Ab1*$^{f/f}$ Vil1$^{creERT2+/+}$) mice after tamoxifen administration to 5-7 week-old mice, analyzed at 10-12.5 weeks (**A**) and to 16 week-old mice, analyzed at 21-22 weeks of age (**B, C**). (**A, B**) Geometric mean fluorescence intensity (gMFI) (left) and frequency (right) of MHCII expression by epithelial cells 3-5 weeks after tamoxifen administration. (**C**) Histogram of MHCII expression by EpCAM$^+$ epithelial cells in 21 week-old i*Vil1*$^{WT(MHCII)}$ (white) or i*Vil1*$^{Δ(MHCII)}$ (grey) mice 4 weeks after tamoxifen administration. (**D, E**) iFoxp3^{Tomato} mice were treated with either 1 dose or continuous doses of Tamoxifen, and Tomato$^+$ CD4$^+$ T cells were analyzed from the intestinal epithelium. (**D**) Experimental lay out of tamoxifen administration connected to analysis by dashed line. (**E**) Dot plots of surface CD8α expression and intracellular Foxp3 expression among Tomato$^+$ cells (top left) and surface CD103 expression among CD4$^+$SP (Tomato$^+$CD4$^+$CD8α$^-$) cells (bottom left). Graphs represent CD4$^+$CD8αα$^+$ IEL frequencies among Tomato$^+$ CD4$^+$ T cells (top right) and

pre-IELs frequencies among Tomato^{+} CD4^{+}SP cells (bottom right) of indicated tamoxifen administration schemes. Data are expressed as mean +/- SEM of individual mice (n=4-9). Significant p values as indicated [student's t test (**A, B**) or one-way ANOVA and Bonferroni (**E**)].

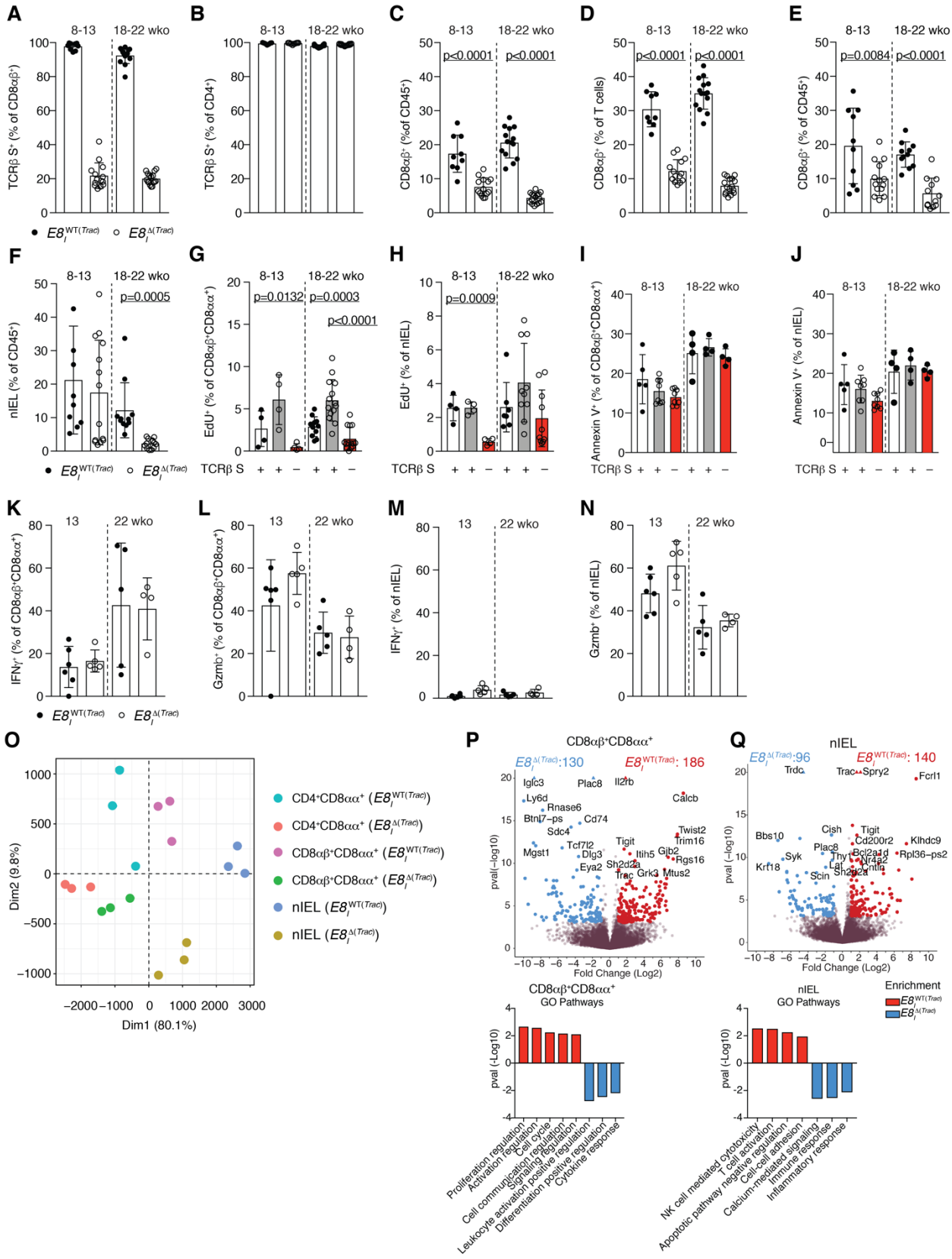
A
8-13
18-22 wko
TCRβ S+ (% of CD8αβ+)
E8I WT(Trac)
E8I Δ(Trac)
B
TCRβ S+ (% of CD4+)
C
CD8αβ+ (%of CD45+)
p<0.0001
p<0.0001
D
CD8αβ+ (% of T cells)
p<0.0001
p<0.0001
E
CD8αβ+ (% of CD45+)
p=0.0084
p<0.0001
F
nIEL (% of CD45+)
p=0.0005
G
EdU+ (% of CD8αβ+CD8αα+)
p=0.0132
p=0.0003
p<0.0001
H
EdU+ (% of nIEL)
p=0.0009
I
Annexin V+ (% of CD8αβ+CD8αα+)
J
Annexin V+ (% of nIEL)
TCRβ S + + − + + −
K
13
22 wko
IFNγ+ (% of CD8αβ+CD8αα+)
L
Gzmb+ (% of CD8αβ+CD8αα+)
M
IFNγ+ (% of nIEL)
N
Gzmb+ (% of nIEL)
O
Dim2 (9.8%)
Dim1 (80.1%)
CD4+CD8αα+ (E8I WT(Trac))
CD4+CD8αα+ (E8I Δ(Trac))
CD8αβ+CD8αα+ (E8I WT(Trac))
CD8αβ+CD8αα+ (E8I Δ(Trac))
nIEL (E8I WT(Trac))
nIEL (E8I Δ(Trac))
P
CD8αβ+CD8αα+
E8I Δ(Trac):130
E8I WT(Trac): 186
pval(−log10)
Fold Change (Log2)
Igfc3
Plac8
Il2rb
Ly6d
Calcb
Rnase6
Btnl7−ps
Cd74
Sdc4
Twist2
Trim16
Tcf7l2
Dlg3
Tigit
Mgst1
Itih5
Gib2
Rgs16
Eya2
Sh2d2a
Trac
Grk3
Mtus2
CD8αβ+CD8αα+
GO Pathways
pval (-Log10)
Proliferation regulation
Activation regulation
Cell cycle
Cell communication regulation
Signaling regulation
Leukocyte activation positive regulation
Differentiation positive regulation
Cytokine response
Q
nIEL
E8I Δ(Trac):96
E8I WT(Trac): 140
Trdc
Trac
Spry2
Fcrl1
Bbs10
Cish
Tigit
Cd200r2
Klhdc9
Plac8
Bcl2a1d
Rpl36−ps2
Syk
Thy1
Nr4a2
Krt18
Lgl1
Ntn1
Scin
Sh2d2a
Enrichment
E8I WT(Trac)
E8I Δ(Trac)
nIEL
GO Pathways
NK cell mediated cytotoxicity
T cell activation
Apoptotic pathway negative regulation
Cell-cell adhesion
Cell-cell adhesion signaling
Calcium-mediated signaling
Immune response
Inflammatory response

Figure S5 (related to Figure 5; TCR signaling is not essential for CD4$^+$CD8αα$^+$IEL maintenance).

(**A-N)** Flow cytometry analysis of 8-22 week-old E8I$^{WT(Trac)}$ (*Trac*$^{+/+}$ E8I^{cre+} or *Trac*$^{+/+}$ E8I^{cre-} or *Trac*$^{f/f}$ E8I^{cre-}) or E8I$^{\Delta(Trac)}$ (*Trac*$^{f/f}$ E8I^{cre+}) mice, grouped by age as indicated. Frequencies of surface TCRβ-expressing cells among CD8αβ$^+$ (**A**) and CD4$^+$ (**B**) T cells in the mesenteric lymph nodes (mLN). Frequency of CD8αβ$^+$ T cells among total CD45$^+$ cells (**C**) and intracellular TCRβ-expressing T cells (**D**) in the mLN. Frequencies of CD8αβ$^+$ T cells (**E**) and natural IELs (nIEL, CD4$^-$CD8αα$^+$CD8β$^-$TL-Tetramer$^+$) (**F**) among CD45$^+$ cells in the intestinal epithelium (IE). (**G, H**) Proliferation (EdU incorporation) of CD8αβ$^+$CD8αα$^+$ IELs (**G**) or nIELs (**H**) with or without surface TCRβ expression after EdU injection 16 and 4 hours prior to analysis. (**I, J**) Frequencies of Annexin V$^+$ cells among CD8αβ$^+$CD8αα$^+$ IELs (**I**) and nIELs (**J**). (**K-N**) Frequencies of IFNγ (**K, M**) and Gzmb (**L, N**) production upon PMA and ionomycin *ex-vivo* stimulation among CD8αβ$^+$CD8αα$^+$ IELs (**K, L**) and nIELs (**M, N**). (**O-Q**) Bulk RNA-sequencing was performed on TCR$^+$ CD4$^+$CD8αα$^+$ IELs, CD8αβ$^+$CD8αα$^+$ IELs and nIELs from E8I$^{WT(Trac)}$ and TCR$^-$ CD4$^+$CD8αα$^+$ IELs from E8I$^{\Delta(Trac)}$ mice. (**O**) Principal component analysis of indicated cell populations. (**P,Q**) Volcano plots of differentially expressed genes between indicated populations ($p<0.05$, in color) (top), and selected differentially-enriched gene ontology (GO) pathways between them (bottom). Data are expressed as mean +/- SEM of individual mice. (n=4-14). Significant p values as indicated [student's t test (**A-F, K-N**) or one-way ANOVA and Bonferroni (**G-J**)]. N=10-16 (**A-F**), n=4-14 (**G, H**), n=5-8 (**I, J**) and n=4-6 (**K-N**). Sequencing data is n=3 mice per group (**O-Q**).

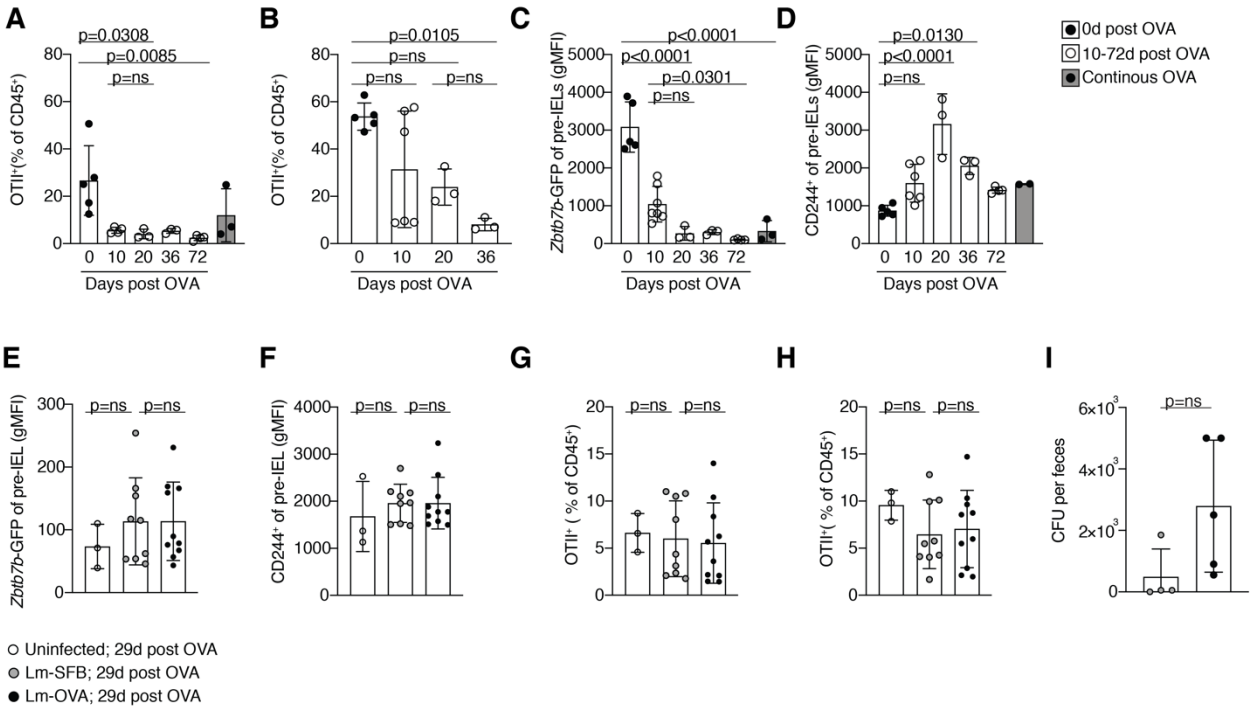

Figure S6 (related to Figure 6; Cognate ligand interaction is dispensable for the maintenance of CD4$^+$CD8$\alpha\alpha^+$IELs under steady state or after *Listeria monocytogenes* infection). (**A-D**) Total splenocytes and lymph node cells from OTII *Zbtb7b*^{GFP} *Rag1*$^{-/-}$ mice were transferred to *Rag1*$^{-/-}$ animals 1 day prior to a 10-day ovalbumin (OVA) diet treatment. Transferred cells were analyzed at different time points after OVA withdrawal as indicated. (**A**) Frequencies of OTII CD4$^+$ T cells (Vα2+) among total CD45$^+$ cells in the epithelium (left) and mesenteric lymph nodes (right). **(B, D)** geometric mean fluorescence intensity (gMFI) of *Zbtb7b*-GFP (**B**) or CD244 (2B4) (**D**) expression among pre-IELs. (**E-J**) *Rag1*$^{-/-}$ animals were treated as in (**A**) and 20 days after OVA removal, animals were orally infected with *Listeria monocytogenes* expressing full length SFB protein 3340 or OVA (Lm-SFB or Lm-OVA, respectively) or were left uninfected. All mice were treated with streptomycin 24h prior to infection. Transferred cells were analyzed 9 days after infection. (**E, F**) gMFI of *Zbtb7b*-GFP (**E**) or CD244 (**F**) expression among pre-IELs. (**G, H**) Frequencies of OTII CD4$^+$ T cells among total CD45$^+$ cells in the epithelium (**G**) and mesenteric lymph nodes (**H**). (**I**) Colony forming units (CFU) per fecal pellet 3 days post infection. (n=3-9 of two independent experiments). Significant p values as indicated [student's t test (**I**) or one-way ANOVA and Bonferroni (**A-H**)].