

Supporting Information

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Table S1. Genes differentially expressed by Helios KO and WT Tregs

Gene	Log ₂ -fold change	Adjusted <i>P</i> value	Gene	Log ₂ -fold change	Adjusted <i>P</i> value
Spleen (Helios KO vs. WT)					
<i>Lgals7</i>	2.658067543	2.68447E-09	<i>Cenpk</i>	-1.58585413	0.002388457
<i>Scin</i>	2.646040334	4.95706E-10	<i>Cxcr4</i>	-1.606547252	1.07696E-05
<i>Ifitm3</i>	2.57950702	1.35082E-05	<i>Cacna1s</i>	-1.622634173	0.000344232
<i>Xcr1</i>	2.56641222	1.30049E-06	<i>Susd2</i>	-1.63454853	2.37677E-14
<i>Cdkn2b</i>	2.369197852	5.27007E-05	<i>1810006J02Rik</i>	-1.645727504	6.1686E-06
<i>Epas1</i>	2.148578533	4.95706E-10	<i>Ankrd6</i>	-1.757201789	0.000722667
<i>Nmur1</i>	2.112438075	0.002740879	<i>Ston1</i>	-1.771411532	0.003611358
<i>Pydc3</i>	2.081854816	0.007220163	<i>Fam164a</i>	-1.827083637	0.008741077
<i>Fam69c</i>	2.027060833	0.002791488	<i>Lima1</i>	-1.8880349	0.005211432
<i>Igfbp7</i>	2.022091538	0.002389996	<i>Optn</i>	-2.016705042	0.000118596
<i>Ccl1</i>	2.015471591	0.008350112	<i>Ctla2b</i>	-2.040417953	9.09252E-07
<i>Cxcr6</i>	1.924874372	2.48162E-10	<i>Itga7</i>	-2.083249223	9.37614E-06
<i>G530011O06Rik</i>	1.924669607	0.000344232	<i>6720401G13Rik</i>	-2.143348671	1.9745E-06
<i>Sico4a1</i>	1.891511954	0.00338922	<i>Ncam1</i>	-2.243420584	0.009098171
<i>5830416I19Rik</i>	1.734463988	0.003912622	<i>Gatm</i>	-2.318714341	0.003450343
<i>Havcr2</i>	1.671731332	0.003153528	<i>Spns3</i>	-2.341298729	0.006970052
<i>Rln3</i>	1.658814917	2.30231E-17	<i>Gprc5a</i>	-2.394962548	0.001382246
<i>Rell1</i>	1.535072895	1.21901E-05	<i>Ccdc46</i>	-2.511417308	4.85426E-05
<i>4930412O13Rik</i>	1.50060528	0.002027753	<i>F5</i>	-2.551207716	0.003611358
<i>Gem</i>	-1.522927512	0.004468241	<i>B3galt5</i>	-2.698244164	7.75252E-06
<i>Edaradd</i>	-1.529142153	0.002820085	<i>Trim10</i>	-2.72454881	0.00165481
<i>Serinc3</i>	-1.549741145	1.31232E-13			
Tumor (Helios KO vs. WT)					
<i>Il4</i>	3.658177124	1.45E-11	<i>Vash1</i>	1.840240595	0.001120079
<i>Scin</i>	3.435824949	7.18E-19	<i>Il1r2</i>	1.821296123	9.68E-07
<i>Pydc3</i>	3.256324595	1.77E-07	<i>Ifitm2</i>	1.814620313	1.30E-14
<i>Lgals7</i>	3.179605436	1.34E-20	<i>Nckap5</i>	1.809936707	0.000152949
<i>Il21</i>	2.937181996	3.76E-05	<i>4930412O13Rik</i>	1.768880177	8.77E-06
<i>Ermn</i>	2.82523349	5.41E-05	<i>Xcr1</i>	1.767769129	0.000504895
<i>Tnfsf8</i>	2.811147709	1.94E-72	<i>Mid1</i>	1.765606414	3.70E-05
<i>Tnc</i>	2.767405532	1.50E-07	<i>Lgmn</i>	1.751292508	1.27E-14
<i>G530011O06Rik</i>	2.726413992	3.65E-10	<i>Cd40lg</i>	1.707430232	1.71E-15
<i>Tnfsf11</i>	2.647264577	4.54E-21	<i>Sema7a</i>	1.640529976	0.00931739
<i>Csf2</i>	2.626345581	2.63E-06	<i>Palld</i>	1.635001673	0.000268784
<i>5830416I19Rik</i>	2.580757562	1.77E-07	<i>Gm14446</i>	1.629863198	2.94E-05
<i>Gzmc</i>	2.482794504	6.55E-09	<i>Cldn25</i>	1.611467866	8.20E-47
<i>1300014I06Rik</i>	2.4004248	0.001851397	<i>Il1rl1</i>	1.563280988	1.21E-09
<i>Ccr3</i>	2.37421554	4.08E-17	<i>Arl4c</i>	1.560941305	4.98E-26
<i>Ramp3</i>	2.364773339	6.65E-21	<i>Mmp25</i>	1.543150071	0.008611886
<i>Ipmk</i>	2.353821768	1.58E-19	<i>Rps4y2</i>	1.537731201	0.000381017
<i>Ifitm1</i>	2.301511362	0.001838493	<i>Lancl3</i>	1.533832523	0.00817211
<i>Fam69c</i>	2.299333984	8.08E-05	<i>Bmyc</i>	-1.506848564	2.36E-27
<i>Nmur1</i>	2.228184638	9.92E-05	<i>Irak1bp1</i>	-1.512953124	0.003342283
<i>Fam20a</i>	2.210932465	2.24E-09	<i>Fam73a</i>	-1.528608297	0.000156832
<i>6430562O15Rik</i>	2.130053409	0.006970673	<i>Ablim2</i>	-1.540172337	0.002187868
<i>Gpr15</i>	2.128342831	1.49E-12	<i>Pgcp</i>	-1.541979997	0.000140215
<i>A930001A20Rik</i>	2.106485353	0.004051292	<i>Zfp365</i>	-1.546820849	0.001186914
<i>Slc17a6</i>	2.092939968	0.005317657	<i>Ehbp1</i>	-1.562837802	0.004152742
<i>Mmp9</i>	2.085351066	6.38E-11	<i>Dse</i>	-1.571899587	0.007814486
<i>Ffar2</i>	1.999312782	0.007291635	<i>L2hgdh</i>	-1.572353601	0.004019477
<i>Pqlc1</i>	1.997829885	4.99E-05	<i>Ctla2b</i>	-1.578041354	3.50E-08
<i>Cebpa</i>	1.988728501	0.004802616	<i>Gucy1a3</i>	-1.588923283	0.00579425
<i>Cxcl10</i>	1.97550785	3.39E-06	<i>Btbd16</i>	-1.590587224	0.006531401
<i>Furin</i>	1.945924012	6.00E-19	<i>Smyd2</i>	-1.592413828	6.38E-08
<i>Ifi204</i>	1.937065553	0.000656685	<i>Zfp3612</i>	-1.596172541	2.25E-20
<i>Ccl1</i>	1.928155309	0.003948586	<i>Camk1</i>	-1.596457274	0.005468302

Table S1. Cont.

Gene	Log ₂ -fold change	Adjusted P value	Gene	Log ₂ -fold change	Adjusted P value
<i>Trim2</i>	-1.616557997	0.001053006	<i>Gm5134</i>	-2.125418632	0.002656836
<i>Cul9</i>	-1.619370034	7.54E-05	<i>Stau2</i>	-2.149372521	0.006492957
<i>Ankrd13d</i>	-1.636165617	0.001838493	<i>Dctd</i>	-2.153496299	2.38E-05
<i>Phlda3</i>	-1.646101035	0.00219099	<i>Sema5b</i>	-2.155932759	0.008606469
<i>Rasl11b</i>	-1.651355467	0.001368078	<i>Klhl13</i>	-2.157845084	0.005547559
<i>Eno2</i>	-1.658863183	0.000352835	<i>Car6</i>	-2.16304197	0.008238871
<i>Gtf2ird1</i>	-1.65931663	4.11E-07	<i>Tnxb</i>	-2.167863078	0.007933612
<i>Man1c1</i>	-1.669647101	0.006129299	<i>Lhfpl1</i>	-2.175027677	0.004323686
<i>Tfpi</i>	-1.675191724	6.81E-06	<i>Tbc1d24</i>	-2.19966545	0.004957513
<i>Fam69b</i>	-1.677423558	0.000376046	<i>Msrp2</i>	-2.202615877	1.66E-11
<i>Gm14085</i>	-1.682320302	0.000772184	<i>Magi1</i>	-2.218479485	9.17E-06
<i>Nap1l3</i>	-1.695063004	0.005841561	<i>Lhfpl2</i>	-2.225813928	0.006398797
<i>Tctex1d2</i>	-1.695473232	0.001040002	<i>Zfp947</i>	-2.228526809	0.001300151
<i>Gga2</i>	-1.707784224	0.000157496	<i>Procr</i>	-2.236432734	0.005636025
<i>Cfp</i>	-1.714870515	0.009143508	<i>4921507P07Rik</i>	-2.239212824	0.001772505
<i>Hpgds</i>	-1.717314029	0.000184871	<i>Flt4</i>	-2.250065315	0.003425783
<i>Slc7a10</i>	-1.719650044	0.000618918	<i>Tpst1</i>	-2.266869666	2.72E-05
<i>Dab2ip</i>	-1.742441474	0.001042275	<i>9530059O14Rik</i>	-2.267321814	0.004890418
<i>Plxna1</i>	-1.750195458	0.000925722	<i>Fam149a</i>	-2.270602296	0.00490624
<i>Capn3</i>	-1.756321546	7.02E-05	<i>Kif21a</i>	-2.277002999	0.003802187
<i>Adam12</i>	-1.759520847	0.005663936	<i>Gylt11b</i>	-2.288285558	0.003568323
<i>Azi1</i>	-1.760651572	8.77E-05	<i>Sh3tc1</i>	-2.292027706	6.25E-05
<i>Sorbs3</i>	-1.771797834	0.007061593	<i>Slmo1</i>	-2.297802715	0.004128109
<i>Ube2c</i>	-1.783279211	0.00302029	<i>Bicd1</i>	-2.307696846	3.49E-20
<i>Rhod</i>	-1.786634035	2.80E-06	<i>Tph2</i>	-2.308876951	0.004334488
<i>Ttc16</i>	-1.789396645	0.003149327	<i>Psrc1</i>	-2.312963135	6.76E-05
<i>Sgsm2</i>	-1.810265098	5.95E-05	<i>Slpi</i>	-2.324083689	0.003568323
<i>Clic3</i>	-1.816438786	0.009657127	<i>D630045J12Rik</i>	-2.33708223	0.002939655
<i>Sec16b</i>	-1.822416125	0.000864339	<i>Slc35d3</i>	-2.376340449	0.001891695
<i>Gabrr2</i>	-1.824576074	0.000398807	<i>Kndc1</i>	-2.379933068	0.001144623
<i>Aldh2</i>	-1.849117783	8.54E-05	<i>Myo7a</i>	-2.381248071	9.07E-06
<i>Tet1</i>	-1.871961731	9.57E-08	<i>Txnrd3</i>	-2.389391091	0.000102358
<i>B230216N24Rik</i>	-1.873381334	0.002489468	<i>Plekha4</i>	-2.424496994	0.001724651
<i>Nme4</i>	-1.879373077	0.008238871	<i>Igf2bp1</i>	-2.436390458	0.001922516
<i>Mctp1</i>	-1.884777842	9.64E-07	<i>Entpd2</i>	-2.441019403	0.002112437
<i>Ston1</i>	-1.88718766	0.0006993	<i>Aldh3b1</i>	-2.461497906	0.001628802
<i>B3galt5</i>	-1.889273207	0.00088104	<i>Zfhx3</i>	-2.48104622	0.001163186
<i>Rapgef4</i>	-1.899663593	0.000141919	<i>Mmp11</i>	-2.495047381	1.02E-05
<i>Gucy1b3</i>	-1.906505161	0.001447867	<i>Bfsp2</i>	-2.498560224	0.000691909
<i>Ephx1</i>	-1.916783765	4.67E-43	<i>Cdcp1</i>	-2.510828416	2.36E-05
<i>Kifc3</i>	-1.93782179	0.007028312	<i>Rassf4</i>	-2.518626301	0.000824271
<i>6720401G13Rik</i>	-1.957367198	2.65E-06	<i>Tmem144</i>	-2.536243046	0.00047794
<i>Ankrd6</i>	-1.993802609	1.98E-05	<i>Pls1</i>	-2.547385146	0.000757595
<i>Mcc</i>	-2.001992203	0.00951619	<i>Phactr1</i>	-2.606909076	0.000896616
<i>Bend6</i>	-2.002947714	0.000100668	<i>Wbscr27</i>	-2.63251965	2.48E-08
<i>Extl1</i>	-2.021282719	8.84E-05	<i>Alas2</i>	-2.659077977	3.27E-06
<i>Rragd</i>	-2.028301528	3.82E-11	<i>Lcn13</i>	-2.687851266	0.000413576
<i>Tmcc3</i>	-2.037881069	0.000570731	<i>Ptpn14</i>	-2.72916564	0.00020935
<i>Nckap5l</i>	-2.040244556	0.000209139	<i>Adamts14</i>	-2.73440504	4.12E-06
<i>Enpp2</i>	-2.059185153	0.005914905	<i>Crtam</i>	-2.734757366	7.99E-05
<i>Maoa</i>	-2.067895621	0.00476702	<i>1810006J02Rik</i>	-2.765147644	2.55E-11
<i>Tiam2</i>	-2.08048923	0.001266127	<i>A530050N04Rik</i>	-2.766781473	0.000341778
<i>Fras1</i>	-2.083339494	0.009102222	<i>Muc4</i>	-2.782517055	6.98E-06
<i>Pilra</i>	-2.084839799	0.008160749	<i>Ncf1</i>	-2.790503745	2.41E-09
<i>Cav2</i>	-2.097957721	0.007209038	<i>Nos2</i>	-2.796626074	0.000143762
<i>nr2</i>	-2.102304013	0.000130369	<i>Siglec1</i>	-2.890510475	0.000114157
<i>Sifn4</i>	-2.107904161	0.006390156	<i>Cd14</i>	-2.910917127	0.000118145
<i>1700123M08Rik</i>	-2.110891405	0.003217499	<i>Gsdmc</i>	-3.005662839	5.43E-05
<i>BC051142</i>	-2.119664502	0.00931739	<i>F13a1</i>	-3.032965187	2.53E-06
<i>Tmem146</i>	-2.123484509	0.001632094	<i>Ltbp4</i>	-3.375969027	6.47E-08

FoxP3-Cre (WT) and Helios^{fl/fl}.FoxP3-Cre (KO) mice were inoculated s.c. with B16/F10 before small numbers (~400) of splenic and tumor Tregs (YFP⁺) were sorted for gene expression profiling. Genes that display differential expression between intrasplenic (spleen) and intratumoral (tumor) Tregs from FoxP3-Cre (WT) and Helios^{fl/fl}.FoxP3-Cre (KO) mice are presented according to a Log₂-fold change ≥ 1.5 , and P value < 0.01 .