

Supplemental Data for:

Rorc restrains the potency of ST2⁺ regulatory T cells in ameliorating intestinal Graft-versus-host disease

Authors: Jinfeng Yang^{1#}, Abdulraouf Ramadan^{1#}, Dawn K. Reichenbach^{2#}, Michael Loschi^{2#}, Jilu Zhang¹, Brad Griesenauer¹, Hong Liu¹, Keli L. Hippen², Bruce R Blazar^{2*}, Sophie Paczesny^{1*}

Affiliations: ¹ Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, USA; ² Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA

These four first authors contributed equally to the study

* These two last authors contributed equally and are corresponding authors

This PDF file includes:

Supplemental Table 1. Transcriptome analysis of sorted Treg cells from WT and ST2^{-/-} mouse.

Supplemental Table 2. Transcriptional signature and gene ontology process for upregulated genes in sorted Tregs of ST2^{-/-} mice than WT mice (not shown in Figure 1).

Supplemental Table 3. Antibodies used for flow cytometry analyses.

Supplemental Figure 1. ST2 deficiency reduces donor tTreg expansion, activation, and function.

Supplemental Figure 2. Treg_{IL-33} displayed better suppressive capability than Tregs cultured without IL-33 or with IL-23+IL-17 in a ST2 dependent manner.

Supplemental Figure 3. Adoptive transfer of IL-33 cultured donor polyclonal Tregs lead to superior protection from aGVHD compared to those cultured without IL-33.

Sup. Table 1. Transcriptome analysis of sorted Treg cells from WT and ST2^{-/-} mouse

Sample ID			WT Treg	WT Treg	ST2 KO Treg	ST2 KO Treg
FOV Count			555	555	555	555
FOV Counted			554	552	555	553
Binding Density			0.19	0.22	0.17	0.19
Messages						
Positive	POS_A	ERCC_00117.1	105027	102348	107597	103634
Positive	POS_B	ERCC_00112.1	19916	20014	20956	20067
Positive	POS_C	ERCC_00002.1	5498	5458	5853	5644
Positive	POS_D	ERCC_00092.1	1621	1634	1741	1715
Positive	POS_E	ERCC_00035.1	359	379	360	398
Positive	POS_F	ERCC_00034.1	103	101	94	104
Negative	NEG_A	ERCC_00096.1	15	16	10	16
Negative	NEG_B	ERCC_00041.1	13	6	11	6
Negative	NEG_C	ERCC_00019.1	4	2	7	6
Negative	NEG_D	ERCC_00076.1	7	3	3	1
Negative	NEG_E	ERCC_00098.1	8	11	18	9
Negative	NEG_F	ERCC_00126.1	8	9	10	6
Negative	NEG_G	ERCC_00144.1	6	1	5	3
Negative	NEG_H	ERCC_00154.1	1	1	6	5
Endogenous	Abcb10	NM_019552.2	21	14	27	16
Endogenous	Abcb1a	NM_011076.1	140	149	171	160
Endogenous	Abcf1	NM_013854.1	143	136	144	136
Endogenous	Abl1	NM_009594.3	10	15	18	11
Endogenous	Adal	NM_029475.1	1	5	6	5
Endogenous	Ahr	NM_013464.4	122	103	38	53
Endogenous	Aicda	NM_009645.2	2	1	7	11
Endogenous	Aire	NM_009646.1	6	2	4	5
Endogenous	App	NM_007471.2	545	532	565	573
Endogenous	Arhgdib	NM_007486.4	462	475	99	106
Endogenous	Atg16l1	NM_029846.3	51	54	46	58
Endogenous	Atm	NM_007499.1	13	16	16	12
Endogenous	B2m	NM_009735.3	5125	4962	1689	1758
Endogenous	Batf	NM_016767.2	54	55	25	15
Endogenous	Batf3	NM_030060.2	12	11	8	5

Endogenous	Bax	NM_007527.3	28	24	16	23
Endogenous	Bcap31	NM_012060.4	217	216	383	351
Endogenous	Bcl2	NM_009741.3	30	34	38	33
Endogenous	Bcl3	NM_033601.3	77	72	55	64
Endogenous	Bcl6	NM_009744.3	15	16	29	23
Endogenous	Bid	NM_007544.3	21	22	20	30
Endogenous	Blnk	NM_008528.4	61	68	154	149
Endogenous	Bst1	NM_009763.3	22	30	8	7
Endogenous	Bst2	NM_198095.2	111	104	92	97
Endogenous	Btk	NM_013482.2	14	12	14	13
Endogenous	Btla	NM_177584.3	18	15	4	3
Endogenous	Btl1	NM_001111094.1	75	68	75	76
Endogenous	Btl2	NM_079835.2	44	49	37	31
Endogenous	C1qa	NM_007572.2	136	137	69	83
Endogenous	C1qb	NM_009777.2	178	193	55	59
Endogenous	C1qbp	NM_007573.2	220	186	352	332
Endogenous	C1ra	NM_023143.3	12	10	4	7
Endogenous	C1s	NM_144938.2	4	3	6	9
Endogenous	C2	NM_013484.2	10	11	4	6
Endogenous	C3	NM_009778.2	89	83	16	14
Endogenous	C4a	NM_011413.2	12	11	7	3
Endogenous	C4bp	NM_007576.3	1	2	6	5
Endogenous	C6	NM_016704.2	13	11	8	12
Endogenous	C7	XM_356827.6	2	1	2	3
Endogenous	C8a	NM_146148.1	1	1	2	2
Endogenous	C8b	NM_133882.2	3	1	4	1
Endogenous	C8g	NM_027062.1	1	1	1	2
Endogenous	C9	NM_013485.1	2	3	3	2
Endogenous	Camp	NM_009921.2	20	7	1	1
Endogenous	Card9	NM_001037747.1	21	19	10	12
Endogenous	Casp1	NM_009807.2	215	188	241	231
Endogenous	Casp2	NM_007610.1	32	35	57	50
Endogenous	Casp3	NM_009810.2	273	316	107	103
Endogenous	Casp8	NM_009812.2	249	251	191	200
Endogenous	Ccbp2	NM_021609.3	4	1	3	2
Endogenous	Ccl11	NM_011330.3	4	4	8	5
Endogenous	Ccl12	NM_011331.2	3	3	4	4
Endogenous	Ccl19	NM_011888.2	4	2	1	3
Endogenous	Ccl2	NM_011333.3	60	50	33	25
Endogenous	Ccl20	NM_016960.1	14	14	26	30
Endogenous	Ccl22	NM_009137.2	89	130	33	26

Endogenous	Ccl24	NM_019577.4	48	37	5	6
Endogenous	Ccl25	NM_009138.3	300	313	819	830
Endogenous	Ccl26	NM_001013412.2	1	1	3	1
Endogenous	Ccl3	NM_011337.1	574	615	125	109
Endogenous	Ccl4	NM_013652.1	306	325	133	137
Endogenous	Ccl5	NM_013653.1	409	388	103	99
Endogenous	Ccl6	NM_009139.2	272	222	90	91
Endogenous	Ccl7	NM_013654.2	25	28	23	23
Endogenous	Ccl8	NM_021443.2	16	19	6	5
Endogenous	Ccl9	NM_011338.2	70	85	97	92
Endogenous	Ccr10	NM_007721.4	6	6	10	4
Endogenous	Ccr2	NM_009915.2	81	97	10	11
Endogenous	Ccr3	NM_009914.4	32	24	6	6
Endogenous	Ccr4	NM_009916.2	7	7	3	3
Endogenous	Ccr5	NM_009917.5	77	83	17	23
Endogenous	Ccr6	NM_001190333.1	3	2	9	10
Endogenous	Ccr7	NM_007719.2	100	146	94	111
Endogenous	Ccr8	NM_007720.2	58	54	3	2
Endogenous	Ccr9	NM_009913.6	160	158	116	132
Endogenous	Ccr11	NM_145700.2	3	6	5	4
Endogenous	Ccr12	NM_017466.4	580	589	108	118
Endogenous	Cd109	NM_153098.3	1	2	4	4
Endogenous	Cd14	NM_009841.3	144	154	53	43
Endogenous	Cd160	NM_001163496.1	1	3	4	3
Endogenous	Cd163	NM_053094.2	5	3	7	5
Endogenous	Cd164	NM_016898.2	259	247	318	310
Endogenous	Cd19	NM_009844.2	3	1	50	40
Endogenous	Cd1d1	NM_007639.3	30	27	12	14
Endogenous	Cd2	NM_013486.2	450	448	18	19
Endogenous	Cd209g	NM_027343.3	1	2	1	1
Endogenous	Cd22	NM_001043317.2	14	8	30	35
Endogenous	Cd226	NM_001039149.1	49	37	7	5
Endogenous	Cd244	NM_018729.2	25	17	4	3
Endogenous	Cd247	NM_001113391.2	126	116	18	11
Endogenous	Cd24a	NM_009846.2	124	124	252	241
Endogenous	Cd27	NM_001042564.1	87	65	8	1
Endogenous	Cd274	NM_021893.2	316	287	54	59
Endogenous	Cd28	NM_007642.4	66	50	15	20
Endogenous	Cd34	NM_001111059.1	7	8	20	20
Endogenous	Cd36	NM_007643.3	11	12	13	13
Endogenous	Cd3d	NM_013487.2	265	243	24	14

Endogenous	Cd3e	NM_007648.4	281	273	33	33
Endogenous	Cd3eap	NM_145822.2	8	8	6	14
Endogenous	Cd4	NM_013488.2	219	233	34	33
Endogenous	Cd40	NM_011611.2	38	39	16	16
Endogenous	Cd40lg	NM_011616.2	16	12	4	5
Endogenous	Cd44	NM_009851.2	173	158	66	83
Endogenous	Cd46	NM_010778.3	1	3	4	2
Endogenous	Cd48	NM_007649.4	86	84	23	23
Endogenous	Cd5	NM_007650.3	160	198	10	10
Endogenous	Cd53	NM_007651.3	41	36	15	14
Endogenous	Cd55	NM_010016.2	32	32	44	42
Endogenous	Cd59b	NM_181858.1	3	2	4	1
Endogenous	Cd6	NM_001037801.2	156	134	6	9
Endogenous	Cd69	NM_001033122.3	99	99	52	49
Endogenous	Cd7	NM_009854.1	47	44	58	64
Endogenous	Cd74	NM_001042605.1	13339	14266	6739	7074
Endogenous	Cd79a	NM_007655.3	7	6	6	9
Endogenous	Cd79b	NM_008339.2	7	4	43	47
Endogenous	Cd80	NM_009855.2	124	107	37	26
Endogenous	Cd81	NM_133655.2	462	482	644	634
Endogenous	Cd82	NM_001271430.1	270	281	200	201
Endogenous	Cd83	NM_009856.2	176	181	168	190
Endogenous	Cd86	NM_019388.3	77	93	44	48
Endogenous	Cd8a	NM_001081110.2	48	49	18	25
Endogenous	Cd8b1	NM_009858.2	11	5	3	7
Endogenous	Cd9	NM_007657.3	422	409	297	337
Endogenous	Cd96	NM_032465.2	7	5	1	2
Endogenous	Cd97	NM_011925.1	285	255	170	196
Endogenous	Cd99	NM_025584.2	306	293	194	226
Endogenous	Cdh5	NM_009868.3	3	2	4	3
Endogenous	Cdkn1a	NM_007669.4	328	323	349	323
Endogenous	Ceacam1	NM_001039185.1	254	267	216	210
Endogenous	Cebpb	NM_009883.3	419	428	292	306
Endogenous	Cfb	NM_008198.2	60	81	50	57
Endogenous	Cfd	NM_013459.1	1	2	1	2
Endogenous	Cfh	NM_009888.3	3	4	6	2
Endogenous	Cfi	NM_007686.2	3	4	4	4
Endogenous	Cfp	NM_008823.3	47	51	30	29
Endogenous	Chuk	NM_001162410.1	81	85	93	91
Endogenous	Ciita	NM_007575.2	54	66	34	35
Endogenous	Cish	NM_009895.3	48	33	5	10

Endogenous	Clec4a4	NM_001005860.2	9	2	6	2
Endogenous	Clec4e	NM_019948.2	105	117	8	13
Endogenous	Clec5a	NM_001038604.1	22	25	2	5
Endogenous	Clu	NM_013492.2	15	37	37	23
Endogenous	Cmklr1	NM_008153.3	36	39	16	15
Endogenous	Cr2	NM_007758.2	4	2	8	7
Endogenous	Cradd	NM_009950.2	15	7	12	17
Endogenous	Crlf2	NM_001164735.1	139	141	48	42
Endogenous	Csf1	NM_001113530.1	58	49	11	8
Endogenous	Csf1r	NM_001037859.1	177	161	47	53
Endogenous	Csf2	NM_009969.4	27	19	6	5
Endogenous	Csf2rb	NM_007780.4	149	168	85	76
Endogenous	Csf3r	NM_001252651.1	34	27	6	7
Endogenous	Ctla4	NM_009843.3	807	759	30	30
Endogenous	Ctnnb1	NM_007614.2	218	234	268	280
Endogenous	Ctsc	NM_009982.2	138	138	46	46
Endogenous	Ctsg	NM_007800.1	4	4	3	4
Endogenous	Ctss	NM_021281.2	597	570	195	197
Endogenous	Cul9	NM_001081335.2	5	3	5	5
Endogenous	Cx3cl1	NM_009142.3	14	15	20	20
Endogenous	Cx3cr1	NM_009987.3	11	10	20	13
Endogenous	Cxcl1	NM_008176.1	64	65	51	56
Endogenous	Cxcl10	NM_021274.1	171	168	30	44
Endogenous	Cxcl11	NM_019494.1	131	135	8	5
Endogenous	Cxcl12	NM_021704.3	3	3	8	11
Endogenous	Cxcl13	NM_018866.2	1	1	1	3
Endogenous	Cxcl15	NM_011339.2	4	3	1	2
Endogenous	Cxcl3	NM_203320.2	108	108	13	9
Endogenous	Cxcl9	NM_008599.2	265	261	16	12
Endogenous	Cxcr1	NM_178241.4	5	4	1	1
Endogenous	Cxcr2	NM_009909.3	9	4	1	1
Endogenous	Cxcr3	NM_009910.2	78	70	12	12
Endogenous	Cxcr4	NM_009911.3	364	315	119	132
Endogenous	Cxcr5	NM_007551.2	2	3	10	16
Endogenous	Cxcr6	NM_030712.4	103	79	11	8
Endogenous	Cybb	NM_007807.2	323	318	147	149
Endogenous	Ddx58	NM_172689.3	62	66	38	44
Endogenous	Defb1	NM_007843.3	5	2	6	5
Endogenous	Defb14	NM_183026.2	3	3	1	3
Endogenous	Dpp4	NM_001159543.1	46	52	78	82
Endogenous	Ebi3	NM_015766.2	29	32	7	6

Endogenous	Emr1	NM_010130.1	112	102	39	33
Endogenous	Entpd1	NM_009848.3	102	104	34	18
Endogenous	Eomes	NM_010136.2	10	2	3	4
Endogenous	Ets1	NM_001038642.1	527	502	99	104
Endogenous	Fadd	NM_010175.5	11	8	15	15
Endogenous	Fas	NM_007987.2	19	25	8	13
Endogenous	Fasl	NM_010177.3	21	11	3	4
Endogenous	Fcamr	NM_001170632.1	11	4	11	7
Endogenous	Fcer1a	NM_010184.1	10	6	21	29
Endogenous	Fcer1g	NM_010185.4	65	53	12	13
Endogenous	Fcgr1	NM_010186.5	26	26	8	10
Endogenous	Fcgr2b	NM_001077189.1	108	127	68	58
Endogenous	Fcgr3	NM_010188.5	37	44	6	8
Endogenous	Fcgr4	NM_144559.1	58	63	11	9
Endogenous	Fcgrt	NM_010189.3	35	29	46	42
Endogenous	Fkbp5	NM_010220.3	87	64	37	41
Endogenous	<td>NM_010233.1</td> <td>15</td> <td>16</td> <td>12</td> <td>12</td>	NM_010233.1	15	16	12	12
Endogenous	Folr4	NM_022888.2	99	98	4	2
Endogenous	Foxp3	NM_054039.1	276	285	7	2
Endogenous	Frmpd4	NM_001033330.2	1	2	1	3
Endogenous	Fyn	NM_008054.2	125	120	47	54
Endogenous	Gata3	NM_008091.3	82	86	6	5
Endogenous	Gfi1	NM_010278.2	11	13	8	9
Endogenous	Gm10499	XM_003086920.1	13	10	18	22
Endogenous	Gp1bb	NM_010327.2	41	41	49	44
Endogenous	Gpi1	NM_008155.3	2	4	8	5
Endogenous	Gpr183	NM_183031.2	44	41	52	57
Endogenous	Gpr44	NM_009962.2	7	4	2	4
Endogenous	Gzma	NM_010370.2	223	247	84	79
Endogenous	Gzmb	NM_013542.2	827	770	37	37
Endogenous	H2-Aa	NM_010378.2	2377	2554	1313	1532
Endogenous	H2-Ab1	NM_207105.2	2762	3067	987	1050
Endogenous	H2-DMa	NM_010386.3	169	177	87	103
Endogenous	H2-DMb2	NM_010388.4	35	36	70	65
Endogenous	H2-Ea-ps	NM_010381.2	561	611	1400	1457
Endogenous	H2-Eb1	NM_010382.2	1956	2135	1110	1160
Endogenous	H2-K1	NM_001001892.2	3844	3873	515	564
Endogenous	H2-Ob	NM_010389.3	2	6	23	18
Endogenous	H2-Q10	NM_010391.4	44	55	15	16
Endogenous	H60a	NM_010400.2	1	2	6	5
Endogenous	Hamp	NM_032541.1	1	1	1	1

Endogenous	Hc	NM_010406.1	2	1	1	1
Endogenous	Hcst	NM_011827.3	95	74	20	8
Endogenous	Hfe	NM_010424.4	13	18	16	11
Endogenous	Hif1a	NM_010431.1	126	113	32	34
Endogenous	Hlx	NM_008250.2	15	16	10	5
Endogenous	Icam1	NM_010493.2	88	85	20	31
Endogenous	Icam2	NM_010494.1	15	13	10	12
Endogenous	Icam4	NM_023892.2	10	5	4	2
Endogenous	Icam5	NM_008319.2	1	2	4	2
Endogenous	Icos	NM_017480.1	271	254	13	7
Endogenous	Icosl	NM_015790.3	28	22	25	25
Endogenous	Ifi204	NM_008329.2	25	27	3	4
Endogenous	Ifi35	NM_027320.4	86	98	70	74
Endogenous	Ifih1	NM_027835.2	112	111	66	60
Endogenous	Ifit2	NM_008332.2	114	104	11	8
Endogenous	Ifitm1	NM_001112715.1	141	133	94	99
Endogenous	Ifna1	NM_010502.2	5	3	1	4
Endogenous	Ifna2	NM_010503.2	1	2	3	1
Endogenous	Ifnar1	NM_010508.1	300	283	197	204
Endogenous	Ifnar2	NM_001110498.1	46	52	24	43
Endogenous	Ifnb1	NM_010510.1	4	3	4	2
Endogenous	Ifng	NM_008337.1	83	91	8	4
Endogenous	Ifngr1	NM_010511.2	462	421	156	182
Endogenous	Ifngr2	NM_008338.3	131	136	187	198
Endogenous	Igf2r	NM_010515.1	25	31	33	38
Endogenous	Ikbkap	NM_026079.3	86	96	92	86
Endogenous	Ikbkb	NM_010546.2	33	40	38	36
Endogenous	Ikbke	NM_019777.3	94	90	86	82
Endogenous	Ikbkg	NM_178590.2	48	41	50	46
Endogenous	Ikzf1	NM_001025597.1	15	22	11	16
Endogenous	Ikzf2	NM_011770.4	216	198	13	15
Endogenous	Ikzf3	NM_011771.1	85	76	25	31
Endogenous	Ikzf4	NM_011772.2	27	27	7	2
Endogenous	Il10	NM_010548.1	74	88	5	7
Endogenous	Il10ra	NM_008348.2	117	130	37	33
Endogenous	Il10rb	NM_008349.5	85	94	70	97
Endogenous	Il11ra1	NM_010549.3	7	4	6	4
Endogenous	Il12a	NM_008351.1	4	4	2	5
Endogenous	Il12b	NM_008352.1	26	13	8	8
Endogenous	Il12rb1	NM_008353.2	52	45	4	4
Endogenous	Il12rb2	NM_008354.3	19	14	4	4

Endogenous	ll13	NM_008355.2	15	12	13	13
Endogenous	ll13ra1	NM_133990.4	94	101	90	93
Endogenous	ll15	NM_008357.2	8	3	6	5
Endogenous	ll15ra	NM_008358.2	7	18	10	12
Endogenous	ll16	NM_010551.3	86	65	21	22
Endogenous	ll17a	NM_010552.3	3	2	2	5
Endogenous	ll17b	NM_019508.1	1	2	1	1
Endogenous	ll17f	NM_145856.2	1	3	6	2
Endogenous	ll17ra	NM_008359.1	70	61	37	39
Endogenous	ll17rb	NM_019583.3	13	10	32	24
Endogenous	ll17re	NM_001034029.1	4	2	6	4
Endogenous	ll18	NM_008360.1	15	15	54	57
Endogenous	ll18r1	NM_001161842.1	114	86	15	8
Endogenous	ll18rap	NM_010553.2	60	53	2	2
Endogenous	ll19	NM_001009940.1	4	2	1	1
Endogenous	ll1a	NM_010554.4	209	232	33	33
Endogenous	ll1b	NM_008361.3	413	471	188	208
Endogenous	ll1r1	NM_001123382.1	6	4	13	12
Endogenous	ll1r2	NM_010555.4	176	200	39	49
Endogenous	ll1rap	NM_134103.2	13	12	11	8
Endogenous	ll1rl1	NM_001025602.2	203	205	10	5
Endogenous	ll1rl2	NM_133193.3	8	5	6	6
Endogenous	ll1rn	NM_031167.5	263	246	39	40
Endogenous	ll2	NM_008366.2	10	3	1	2
Endogenous	ll20	NM_021380.1	4	1	1	1
Endogenous	ll21	NM_021782.2	6	8	4	1
Endogenous	ll21r	NM_021887.1	22	27	2	3
Endogenous	ll22	NM_016971.1	1	1	7	3
Endogenous	ll22ra2	NM_178258.5	2	3	70	82
Endogenous	ll23a	NM_031252.1	21	15	8	2
Endogenous	ll23r	NM_144548.1	3	3	6	3
Endogenous	ll25	NM_080729.2	3	3	4	2
Endogenous	ll27	NM_145636.1	15	13	2	6
Endogenous	ll27ra	NM_016671.3	56	48	1	3
Endogenous	ll28a	NM_001024673.2	1	4	5	3
Endogenous	ll2ra	NM_008367.2	301	280	8	5
Endogenous	ll2rb	NM_008368.3	586	537	38	32
Endogenous	ll2rg	NM_013563.3	206	209	37	30
Endogenous	ll3	NM_010556.4	3	1	3	4
Endogenous	ll33	NM_133775.1	5	7	8	5
Endogenous	ll4	NM_021283.1	10	10	1	3

Endogenous	Il4ra	NM_001008700.3	318	369	121	106
Endogenous	Il5	NM_010558.1	5	2	3	2
Endogenous	Il6	NM_031168.1	21	33	17	11
Endogenous	Il6ra	NM_010559.2	9	13	10	8
Endogenous	Il6st	NM_010560.2	41	36	31	28
Endogenous	Il7	NM_008371.2	3	3	4	3
Endogenous	Il7r	NM_008372.3	117	125	31	26
Endogenous	Il9	NM_008373.1	4	2	3	3
Endogenous	Ilf3	NM_010561.2	24	33	36	36
Endogenous	Irak1	NM_008363.2	50	57	55	76
Endogenous	Irak2	NM_001113553.1	231	267	142	152
Endogenous	Irak3	NM_028679.3	43	46	13	12
Endogenous	Irak4	NM_029926.5	108	87	128	133
Endogenous	Irf1	NM_008390.1	585	637	264	302
Endogenous	Irf3	NM_016849.3	8	7	13	16
Endogenous	Irf4	NM_013674.1	63	81	58	72
Endogenous	Irf5	NM_012057.3	59	65	44	32
Endogenous	Irf7	NM_016850.2	106	103	96	81
Endogenous	Irf8	NM_008320.3	112	101	97	94
Endogenous	Irgm1	NM_008326.1	390	371	111	104
Endogenous	Itga2b	NM_010575.2	3	2	6	5
Endogenous	Itga4	NM_010576.3	75	89	20	21
Endogenous	Itga5	NM_010577.3	16	10	7	15
Endogenous	Itga6	NM_008397.3	150	143	223	217
Endogenous	Itgal	NM_008400.2	144	140	30	32
Endogenous	Itgam	NM_001082960.1	105	112	34	35
Endogenous	Itgax	NM_021334.2	105	124	63	68
Endogenous	Itgb1	NM_010578.1	148	149	166	154
Endogenous	Itgb2	NM_008404.4	169	159	35	39
Endogenous	Itln1	NM_010584.3	41	58	196	201
Endogenous	Jak1	NM_146145.2	235	242	144	134
Endogenous	Jak2	NM_001048177.1	364	401	137	118
Endogenous	Jak3	NM_010589.5	150	128	47	42
Endogenous	Kir3dl1	NM_177749.3	1	3	2	2
Endogenous	Kir3dl2	NM_177748.2	3	3	4	2
Endogenous	Kit	NM_001122733.1	7	6	10	9
Endogenous	Klra1	NM_016659.3	2	3	3	1
Endogenous	Klra21	NM_053151.1	2	1	2	1
Endogenous	Klra4	NM_010649.3	1	1	1	1
Endogenous	Klra5	NM_008463.2	4	3	4	2
Endogenous	Klra6	NM_008464.2	3	1	1	1

Endogenous	Klra7	NM_001110323.1	1	2	1	3
Endogenous	Klra8	NM_010650.3	2	1	1	2
Endogenous	Klrb1	NM_001099918.1	1	1	1	1
Endogenous	Klrc1	NM_001136068.1	50	41	4	3
Endogenous	Klrc2	NM_001098669.1	4	2	5	5
Endogenous	Klrc3	NM_021378.1	1	1	1	1
Endogenous	Klrd1	NM_010654.2	21	12	5	8
Endogenous	Klrk1	NM_001083322.1	15	22	12	9
Endogenous	Lair1	NM_001113474.1	13	12	13	15
Endogenous	Lck	NM_010693.2	181	183	22	24
Endogenous	Lcp2	NM_010696.3	20	20	7	5
Endogenous	Lef1	NM_010703.3	26	16	6	3
Endogenous	Lif	NM_008501.2	24	20	21	19
Endogenous	Lilra5	NM_001081239.2	9	11	8	7
Endogenous	Lilra6	NM_011090.2	7	8	4	2
Endogenous	Lilrb3	NM_011095.2	35	28	6	10
Endogenous	Lilrb4	NM_013532.2	590	522	74	75
Endogenous	Litaf	NM_019980.1	207	208	61	86
Endogenous	Lta	NM_010735.1	8	7	4	5
Endogenous	Ltb	NM_008518.2	150	138	46	34
Endogenous	Ltb4r1	NM_008519.2	41	36	9	10
Endogenous	Ltb4r2	NM_020490.2	8	7	6	9
Endogenous	Ltbr	NM_010736.3	74	78	87	88
Endogenous	Ltf	NM_008522.3	2	2	1	1
Endogenous	Ly86	NM_010745.2	37	45	43	40
Endogenous	Ly96	NM_016923.1	5	5	6	6
Endogenous	Maf	NM_001025577.2	52	51	25	31
Endogenous	Map4k1	NM_008279.2	33	27	16	23
Endogenous	Map4k2	NM_009006.2	60	44	34	33
Endogenous	Map4k4	NM_008696.2	40	57	44	49
Endogenous	Mapk1	NM_011949.3	138	127	139	156
Endogenous	Mapk11	NM_011161.5	4	3	7	5
Endogenous	Mapk14	NM_011951.2	49	46	61	54
Endogenous	Mapkapk2	NM_008551.1	289	280	110	123
Endogenous	Marco	NM_010766.2	2	1	2	2
Endogenous	Masp1	NM_008555.2	7	2	4	3
Endogenous	Masp2	NM_010767.3	1	2	8	9
Endogenous	Mbl2	NM_010776.1	1	3	1	1
Endogenous	Mbp	NM_010777.3	13	11	13	15
Endogenous	Mif	NM_010798.2	678	663	566	686
Endogenous	Mme	NM_008604.3	15	18	32	32

Endogenous	Mr1	NM_008209.4	7	3	3	2
Endogenous	Ms4a1	NM_007641.5	3	6	90	87
Endogenous	Msr1	NM_001113326.1	22	28	6	8
Endogenous	Muc1	NM_013605.1	6	5	13	7
Endogenous	Mx1	NM_010846.1	26	24	17	13
Endogenous	Myd88	NM_010851.2	51	56	58	63
Endogenous	Ncam1	NM_001113204.1	1	1	1	3
Endogenous	Ncf4	NM_008677.2	89	101	31	29
Endogenous	Nfatc1	NM_016791.4	31	44	15	23
Endogenous	Nfatc2	NM_001037177.1	51	57	21	18
Endogenous	Nfatc3	NM_010901.2	72	78	60	71
Endogenous	Nfil3	NM_017373.3	289	293	198	218
Endogenous	Nfkb1	NM_008689.2	92	103	54	47
Endogenous	Nfkb2	NM_019408.2	495	485	388	396
Endogenous	Nfkbia	NM_010907.2	2277	2305	1072	1101
Endogenous	Nfkbiz	NM_030612.1	607	620	276	313
Endogenous	Nod2	NM_145857.2	13	11	6	6
Endogenous	Nos2	NM_010927.3	110	72	90	64
Endogenous	Notch1	NM_008714.2	143	129	119	145
Endogenous	Notch2	NM_010928.1	65	79	50	44
Endogenous	Nox1	NM_172203.1	18	18	23	32
Endogenous	Nox3	NM_198958.2	1	2	2	2
Endogenous	Nox4	NM_015760.4	2	1	1	1
Endogenous	Npc1	NM_008720.2	59	63	63	69
Endogenous	Nt5e	NM_011851.3	29	37	16	11
Endogenous	Pax5	NM_008782.2	3	2	20	23
Endogenous	Pdcd1	NM_008798.1	69	54	4	6
Endogenous	Pdcd1lg2	NM_021396.2	36	28	6	4
Endogenous	Pdcd2	NM_008799.2	8	8	24	11
Endogenous	Pdgfb	NM_011057.3	18	12	13	12
Endogenous	Pdgfrb	NM_008809.1	7	3	12	9
Endogenous	Pecam1	NM_008816.2	58	66	36	24
Endogenous	Phlpp1	NM_133821.3	51	51	30	37
Endogenous	Phlpp2	NM_001122594.2	33	38	43	34
Endogenous	Pigr	NM_011082.3	1125	1172	4020	3970
Endogenous	Pla2g2a	NM_001082531.1	395	425	784	801
Endogenous	Pla2g2e	NM_012044.2	5	1	4	5
Endogenous	Plau	NM_008873.2	69	70	24	30
Endogenous	Plaur	NM_011113.3	323	309	152	148
Endogenous	Pml	NM_008884.2	38	43	53	45
Endogenous	Pou2f2	NM_001163554.1	24	24	15	16

Endogenous	Pparg	NM_011146.1	21	32	26	25
Endogenous	Ppbp	NM_023785.2	2	2	4	2
Endogenous	Prdm1	NM_007548.3	52	59	22	19
Endogenous	Prf1	NM_011073.2	19	15	4	3
Endogenous	Prim1	NM_008921.2	24	24	40	26
Endogenous	Prkcd	NM_011103.2	265	282	304	286
Endogenous	Psemb10	NM_013640.3	308	314	195	193
Endogenous	Psemb11	NM_175204.4	3	1	2	4
Endogenous	Psemb5	NM_011186.1	90	83	125	138
Endogenous	Psemb7	NM_011187.1	80	65	94	75
Endogenous	Psemb9	NM_013585.2	537	555	197	216
Endogenous	Psmc2	NM_011188.3	190	182	222	215
Endogenous	Psmc7	NM_010817.2	77	78	121	110
Endogenous	Ptafr	NM_001081211.1	156	148	41	43
Endogenous	Ptger4	NM_008965.1	75	74	61	61
Endogenous	Ptgs2	NM_011198.3	249	263	39	52
Endogenous	Ptk2	NM_007982.2	55	50	68	60
Endogenous	Ptpn2	NM_001127177.1	53	52	47	46
Endogenous	Ptpn22	NM_008979.1	101	81	27	19
Endogenous	Ptpn6	NM_013545.2	74	81	70	80
Endogenous	Ptprc	NM_011210.3	372	362	109	133
Endogenous	Rae1	NM_175112.5	58	56	68	71
Endogenous	Rag1	NM_009019.2	1	1	1	2
Endogenous	Rag2	NM_009020.3	2	1	1	3
Endogenous	Rela	NM_009045.4	151	149	104	112
Endogenous	Relb	NM_009046.2	153	157	109	117
Endogenous	Rorc	NM_011281.2	41	35	168	199
Endogenous	Runx1	NM_001111021.1	34	40	20	9
Endogenous	Runx3	NM_019732.2	35	29	20	18
Endogenous	S100a8	NM_013650.2	561	342	9	12
Endogenous	S100a9	NM_009114.2	347	240	11	9
Endogenous	Sele	NM_011345.2	4	1	3	2
Endogenous	Sell	NM_001164059.1	38	48	38	36
Endogenous	Selplg	NM_009151.3	1	2	1	3
Endogenous	Serping1	NM_009776.3	10	9	13	7
Endogenous	Sh2d1a	NM_011364.3	24	31	2	4
Endogenous	Sigirr	NM_023059.3	36	38	39	47
Endogenous	Ski	NM_011385.2	187	154	176	158
Endogenous	Slamf1	NM_013730.4	34	32	8	7
Endogenous	Slamf7	NM_144539.5	60	62	39	43
Endogenous	Smad3	NM_016769.3	58	64	44	61

Endogenous	Smad5	NM_008541.2	18	21	53	37
Endogenous	Socs1	NM_009896.2	74	65	27	35
Endogenous	Socs3	NM_007707.2	94	122	73	72
Endogenous	Spn	NM_001037810.1	133	126	16	21
Endogenous	Src	NM_001025395.2	166	185	162	166
Endogenous	Stat1	NM_009283.3	529	557	106	103
Endogenous	Stat2	NM_019963.1	24	28	6	5
Endogenous	Stat3	NM_213659.2	469	474	211	203
Endogenous	Stat4	NM_011487.4	30	35	6	3
Endogenous	Stat5a	NM_011488.2	97	87	34	38
Endogenous	Stat5b	NM_011489.3	101	82	43	42
Endogenous	Stat6	NM_009284.2	106	101	96	107
Endogenous	Syk	NM_011518.2	123	116	73	78
Endogenous	Tagap	NM_145968.2	73	77	39	41
Endogenous	Tal1	NM_011527.2	7	8	4	10
Endogenous	Tap1	NM_001161730.1	625	701	110	127
Endogenous	Tapbp	NM_009318.2	125	147	70	52
Endogenous	Tbk1	NM_019786.4	59	63	40	54
Endogenous	Tbx21	NM_019507.1	46	37	9	5
Endogenous	Tcf4	NM_013685.1	6	16	75	60
Endogenous	Tcf7	NM_009331.3	12	11	21	15
Endogenous	Tfrc	NM_011638.3	254	270	496	512
Endogenous	Tgfb1	NM_011577.1	259	281	92	82
Endogenous	Tgfb2	NM_009367.1	4	4	3	4
Endogenous	Tgfb3	NM_009368.2	6	7	4	5
Endogenous	Tgfb1	NM_009369.4	216	191	85	91
Endogenous	Tgfb1	NM_009370.2	56	51	44	57
Endogenous	Tgfb2	NM_009371.2	167	160	103	105
Endogenous	Thy1	NM_009382.3	77	88	7	5
Endogenous	Tigit	NM_001146325.1	206	193	10	7
Endogenous	Tirap	NM_001177847.1	12	8	12	12
Endogenous	Tlr1	NM_030682.1	52	41	29	36
Endogenous	Tlr2	NM_011905.2	16	27	12	9
Endogenous	Tlr3	NM_126166.2	22	18	23	19
Endogenous	Tlr4	NM_021297.2	28	17	10	5
Endogenous	Tlr5	NM_016928.2	4	5	4	4
Endogenous	Tlr8	NM_133212.2	15	14	4	5
Endogenous	Tlr9	NM_031178.2	18	14	7	8
Endogenous	Tmem173	NM_028261.1	43	38	9	7
Endogenous	Tnf	NM_013693.1	173	173	56	58
Endogenous	Tnfai3	NM_009397.2	774	748	149	165

Endogenous	Tnfaip6	NM_009398.2	2	4	11	7
Endogenous	Tnfrsf11a	NM_009399.3	55	61	79	81
Endogenous	Tnfrsf13b	NM_021349.1	15	11	18	22
Endogenous	Tnfrsf13c	NM_028075.2	6	3	14	14
Endogenous	Tnfrsf14	NM_178931.2	119	152	124	115
Endogenous	Tnfrsf17	NM_011608.1	2	4	15	10
Endogenous	Tnfrsf1b	NM_011610.3	305	296	43	35
Endogenous	Tnfrsf4	NM_011659.2	501	487	6	7
Endogenous	Tnfrsf8	NM_009401.2	15	9	4	2
Endogenous	Tnfrsf9	NM_001077508.1	55	42	1	1
Endogenous	Tnfsf10	NM_009425.2	60	70	43	43
Endogenous	Tnfsf11	NM_011613.3	31	15	4	3
Endogenous	Tnfsf12	NM_011614.3	14	7	15	10
Endogenous	Tnfsf13b	NM_033622.1	21	20	8	10
Endogenous	Tnfsf14	NM_019418.2	15	9	4	5
Endogenous	Tnfsf15	NM_177371.3	4	5	5	9
Endogenous	Tnfsf18	NM_183391.3	4	3	1	2
Endogenous	Tnfsf8	NM_009403.2	2	3	2	3
Endogenous	Tollip	NM_023764.3	178	201	142	159
Endogenous	Traf1	NM_009421.3	113	111	25	26
Endogenous	Traf2	NM_009422.2	13	15	10	12
Endogenous	Traf3	NM_001048206.1	53	48	21	21
Endogenous	Traf4	NM_009423.4	26	14	37	37
Endogenous	Traf5	NM_011633.1	24	28	13	16
Endogenous	Traf6	NM_009424.2	67	56	58	47
Endogenous	Trem1	NM_021406.3	39	44	6	5
Endogenous	Trem2	NM_031254.2	6	4	1	2
Endogenous	Trp53	NM_011640.1	119	126	156	156
Endogenous	Tslp	NM_021367.1	3	2	4	3
Endogenous	Tyk2	NM_018793.2	33	34	36	41
Endogenous	Tyrobp	NM_011662.2	481	507	196	210
Endogenous	Ube2l3	NM_009456.2	61	64	87	94
Endogenous	Vcam1	NM_011693.2	20	27	6	7
Endogenous	Vtn	NM_011707.2	1	1	1	2
Endogenous	Xbp1	NM_013842.2	221	210	462	428
Endogenous	Xcl1	NM_008510.1	93	86	16	13
Endogenous	Xcr1	NM_011798.4	2	2	3	2
Endogenous	Zap70	NM_009539.2	66	83	6	5
Endogenous	Zbtb7b	NM_009565.4	84	99	128	123
Endogenous	Zeb1	NM_011546.2	23	33	10	8
Housekeeping	Alas1	NM_020559.2	338	311	319	301

Housekeeping	Eef1g	NM_026007.4	205	245	319	294
Housekeeping	G6pdx	NM_008062.2	107	130	115	107
Housekeeping	Gapdh	NM_001001303.1	6037	6019	4256	4365
Housekeeping	Gusb	NM_010368.1	58	60	38	42
Housekeeping	Hprt	NM_013556.2	180	158	228	197
Housekeeping	Oaz1	NM_008753.4	985	1050	1172	1267
Housekeeping	Polr1b	NM_009086.2	8	6	9	8
Housekeeping	Polr2a	NM_009089.2	178	175	233	257
Housekeeping	Ppia	NM_008907.1	1863	1951	2685	2745
Housekeeping	Rpl19	NM_009078.2	4393	4572	5199	5235
Housekeeping	Sdha	NM_023281.1	150	166	223	220
Housekeeping	Tbp	NM_013684.3	25	27	32	29
Housekeeping	Tubb5	NM_011655.4	298	261	56	74

Sup. Table 2. Transcriptional signature and gene ontology process for upregulated genes in sorted Tregs of ST2^{-/-} mice than WT mice (not shown in Figure 1)

Major mismatch HCT model		
Gene	ST2 ^{-/-} vs. WT intestinal Tregs ratio	Gene ontology process
<i>Rorc</i> *	4.79	T helper 17 differentiation
<i>Itln1</i>	4.04	Positive regulation of protein phosphorylation
<i>Pigr</i>	3.48	Immunoglobulin transcytosis in epithelial cells mediated by polymeric immunoglobulin receptor
<i>Ccl25</i> *	2.69	Cell chemotaxis and cellular response to interferon-gamma
<i>H2-Ea-ps</i> *	2.44	Encode immune response antigens that function in the T-cell-dependent immune response
<i>Blnk</i>	2.35	Intracellular signal transduction and transmembrane receptor protein tyrosine kinase signaling pathway
<i>Xbp1</i>	2.06	Phosphatidylinositol 3-kinase regulatory subunit binding
<i>Cd24a</i>	1.99	Protein tyrosine kinase activator activity
<i>Pla2g2a</i>	1.93	Calcium-dependent phospholipase A2 activity
<i>Tfrc</i>	1.92	Transferrin transmembrane transporter activity
<i>H2-DMb2</i> *	1.89	Antigen processing and presentation of exogenous peptide antigen via MHC class II
<i>Bcap31</i> *	1.70	Immune response and calcium-mediated signaling using intracellular calcium source
<i>C1qbp</i> *	1.68	Innate and adaptive immune system process
<i>Dpp4</i> *	1.63	T cell activation and regulation of T cell mediated immunity
<i>Casp2</i>	1.62	Apoptotic signaling pathway
<i>Psmb5</i>	1.52	Proteasome-mediated ubiquitin-dependent protein catabolic process
<i>Itga6</i>	1.50	Integrin-mediated signaling pathway
Minor mismatch HCT model		
Gene	ST2 ^{-/-} vs. WT intestinal Tregs ratio	Gene ontology process
<i>Ifnar1</i> *	6.17	T cell activation and positive regulation of interferon-gamma production
<i>Rorc</i> *	3.73	T helper 17 differentiation
<i>Cd4</i> *	2.22	T cell activation and helper T cell enhancement of adaptive immune response

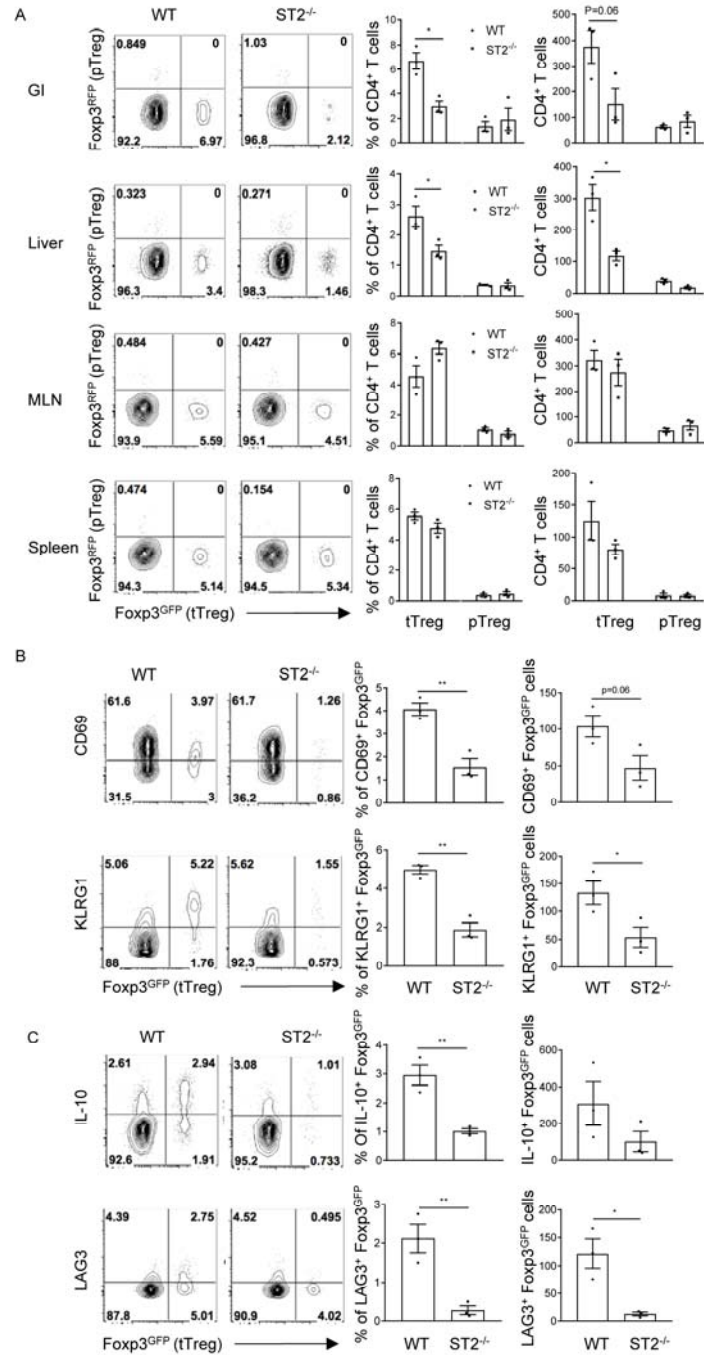
<i>Pecam1</i>	2.18	Leukocyte cell-cell adhesion and Rho protein signal transduction
<i>Stat1</i>*	2.03	Interferon-gamma-mediated signaling pathway
<i>Relb</i>*	1.85	T-helper 1 type immune response
<i>Lck</i>	1.84	Cell surface receptor signaling pathway
<i>Tubb5</i>	1.82	Cellular process and regulation of synapse organization
<i>Ccl9</i>*	1.82	Cellular response to interferon-gamma and chemokine-mediated signaling pathway
<i>Tnfrsf1b</i>*	1.70	Inflammatory response and regulation of T cell cytokine production
<i>Masp1</i>	1.65	Immune system process and complement activation, lectin pathway
<i>Gzma</i>	1.63	Apoptotic process
<i>Nfkbiz</i>*	1.57	Positive regulation of T-helper 17 cell differentiation

* *Genes associated with inflammatory phenotype*

Sup. Table 3. Antibodies used for flow cytometry analyses

Antibody	Company	Clone	Fluorochrome
CD4	eBioscience	GK1.5	PE/ PerCP-eFluor® 710
CD8	eBioscience	53-6.7	FITC/ PE-CY7
IFN γ	eBioscience	XMG1.2	PE/ APC/ PerCP-Cy5.5
ROR γ t	BD biosciences	Q31-378	BV650
T-bet	eBioscience	4B10	PE-CY7
KLRG1	eBioscience	2F1	APC
CD69	eBioscience	H1.2F3	PE-CY7
AREG	R&D	206220	Unconjugated
Foxp3	eBioscience	FJK-16s	PE-CY7
Helios	eBioscience	22F6	PercCP-eF710
ST2	mdbioproduct	Dj8	PE
TIGIT	eBioscience	GIGD7	eF660
IL-17	eBioscience	17B7	APC
IL-4	eBioscience	11B11	PE
IL-10	BD biosciences	JES5-16E3	BV711
LAG3	eBioscience	C9B7W	PE-CY7
Ki67	eBioscience	SolA15	PercCP-eF710

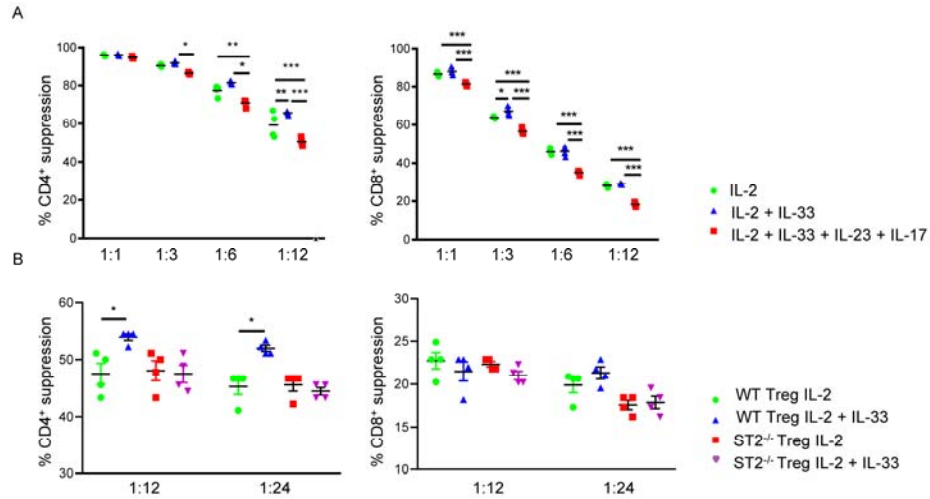
Supplementary Figures



Sup Figure 1. ST2 deficiency reduces donor tTreg expansion, activation, and function

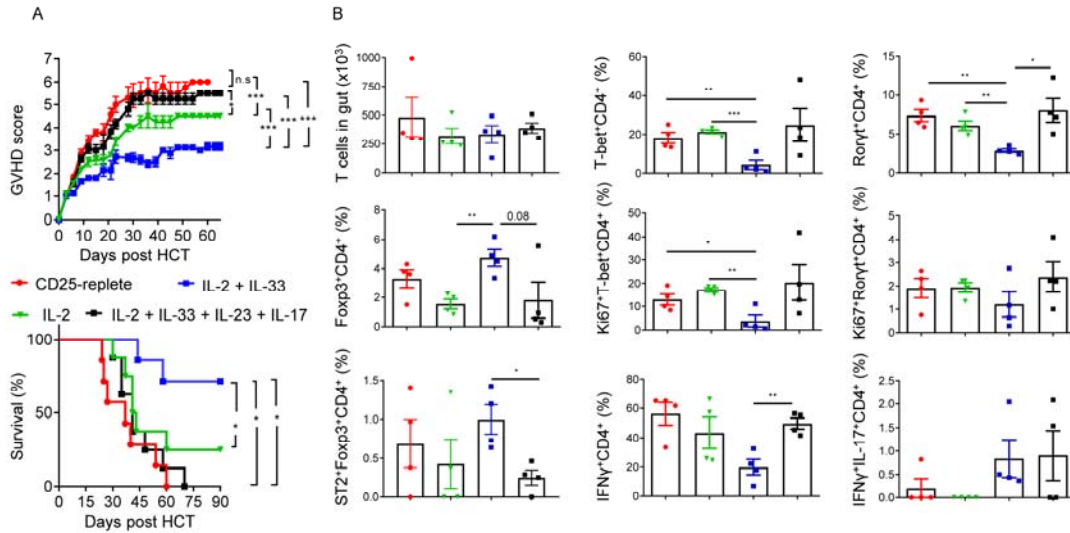
(A) Representative plots of infiltrated CD4⁺ T cells in the gut and liver of C3H.SW mice receiving allogenic B6 WT Fcpx^{RFP} CD25-depleted total T cells with either WT or ST2^{-/-} Fcpx^{GFP} sorted Tregs, ratio of 10:1, n=3, data are shown as mean ± SEM; unpaired t-test, *, P<0.05. No difference between pTregs and tTregs in lymphoid organs (spleen, MLN, not shown). **(B)** Representative plots of CD69 and KLRG1 expression on infiltrated Fcpx^{GFP}+CD4⁺ T cells in the gut of C3H.SW recipient mice at day 10 post allo-HCT, n=3, data

are shown as mean \pm SEM; unpaired t-test, *, $P < 0.05$, **, $P < 0.01$. **(C)** Representative plots of IL-10 and LAG-3 expression on infiltrated Foxp3^{GFP+}CD4⁺ T cells in the gut of C3H.SW recipient mice at day 10 post allo-HCT, n=3, data are shown as mean \pm SEM; unpaired t-test, *, $P < 0.05$, **, $P < 0.01$.



Sup Figure 2. Treg_{IL-33} displayed better suppressive capability than Tregs cultured without IL-33 or with IL-23+IL-17 in a ST2 dependent manner

(A) WT CD4⁺CD25⁺ Tregs were cultured with IL-2, IL-2+IL-33, or IL-2+IL-33+IL-23+IL-17 for 3 days. Percent suppression of *ex vivo* T cell proliferation at various ratios of Tregs:Tefts (1:1, 1:3, 1:6, 1:12) for cells cultured 3 days, (n=4, data are shown as mean ± SEM; ANOVA with Bonferroni correction for multiple comparisons, *, P<0.05, **, P<0.01, ***, P<0.001). **(B)** WT or ST2^{-/-} CD4⁺CD25⁺ Tregs were cultured with IL-2, or IL-2+IL-33 for 3 days. Percent suppression of *ex vivo* T cell proliferation at various ratios of Tregs:Tefts (1:12, 1:24) for cells cultured 3 days, (n=4, data are shown as mean ± SEM; ANOVA with Bonferroni correction for multiple comparisons, *, P<0.05, **, P<0.01, ***, P<0.001).



Sup Figure 3. Adoptive transfer of IL-33 cultured donor polyclonal Tregs lead to superior protection from aGVHD compared to those cultured without IL-33

(A) Clinical score of aGVHD and survival curve for C3H.SW mice receiving allogeneic B6 CD25-replete T cells, or CD25-depleted total T cells with *ex vivo* cultured Treg (IL-2, IL-2+IL-33, IL-2+IL-33+IL-17+IL-23), ratio of 20:1. n=8 per group, data are shown as mean \pm SEM; ANOVA with Bonferroni correction for multiple comparisons, ***, P<0.001 for clinical score, a log-rank test was used for survival analysis, *, P<0.05. **(B)** Graph bars represent total infiltrated cells in gut, and frequency of Foxp3, T-bet, ROR γ t, Ki67, ST2, IFN γ , IL-17 of infiltrated T cells isolated from the gut of C3H.SW recipient mice at day 10 after allo-HCT (n=4, data are shown as mean \pm SEM; ANOVA with Bonferroni correction for multiple comparisons, *, P<0.05, **, P<0.01, ***, P<0.001).