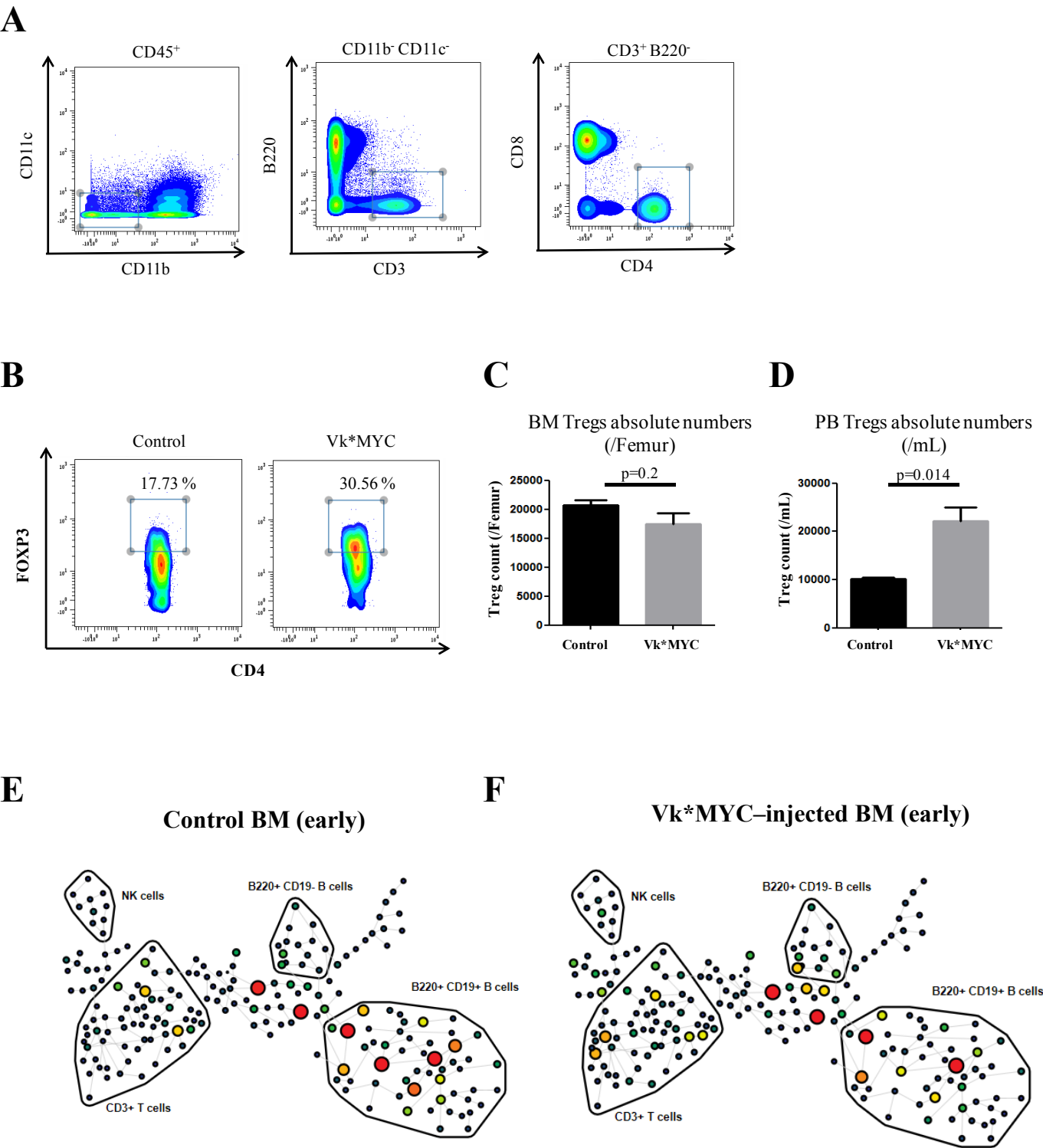


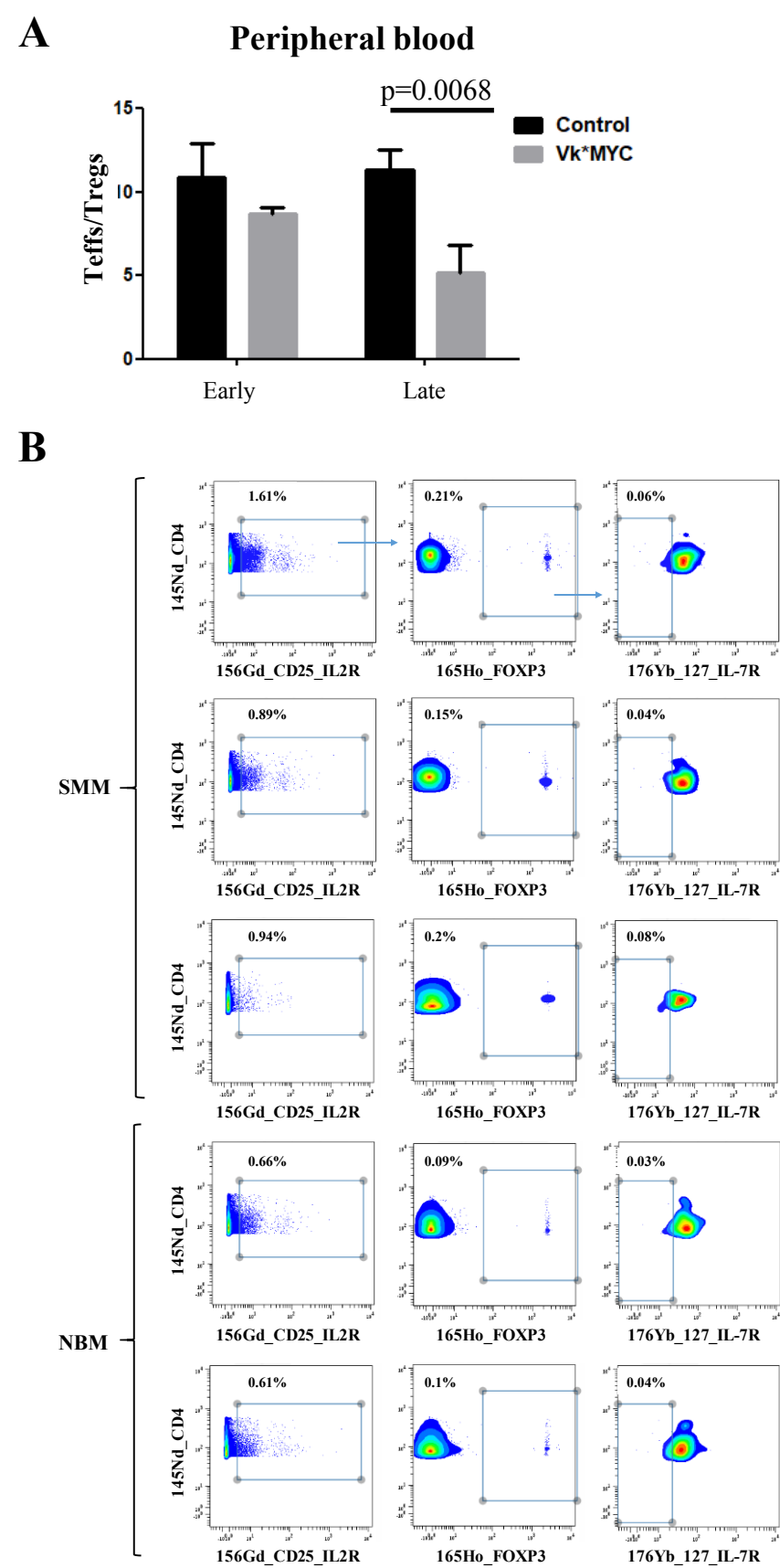
Supplementary Figure 1



Supplementary Figure 1. Gating strategy for Treg analysis by CyTOF.

(A) Gating strategy of CD4⁺ T cells by CyTOF. BM and PB CD4⁺ T cells were determined by first excluding non-viable cells. After initial gating on CD45⁺ cells, CD11b⁻ CD11c⁻ non myeloid cell population was gated, followed by selecting CD3⁺ B220⁻ T cell population and finally gated on CD4⁺ CD8⁻ population. (B) Representative Treg plots by CyTOF. FOXP3⁺ cells were determined within CD4⁺ T cells. Control and Vk*MYC injected BM Tregs of late time points are shown. (C) Absolute numbers of Treg cells in BM of Vk*MYC-injected mice at late time point. (D) Number of Treg per 1 ml PB in Vk*MYC-injected mice at late time point. SPADE tree was conducted on viable BM cells of control (E) and Vk*MYC-injected (F) mice at the early time point. The nodes of the tree represent clusters of cells that are similar in marker expression. The color indicates level of marker expression. Size of the nodes represents number of cells. Increased numbers of CD3-expressing cells were observed in the BM of Vk*MYC-injected mice compared to control BM cells.

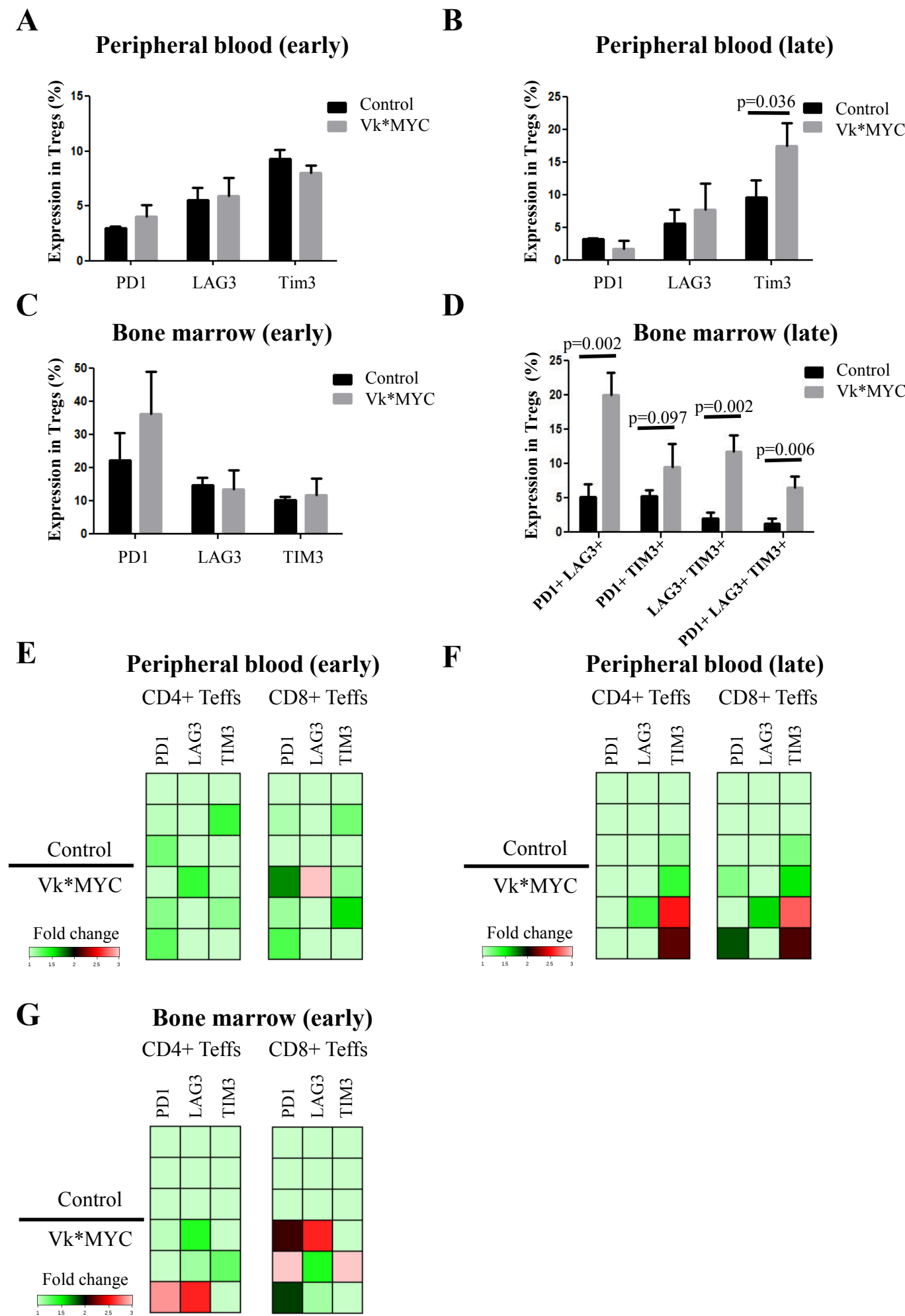
Supplementary Figure 2



Supplementary Figure 2. Decrease of Teff/ Tregs ratio in PB of Vk*MYC-injected mice and increased number of Tregs in BM of SMM patients.

(A) Significant decrease of Teffs (CD4⁺ CD44⁺⁺ CD62L^{low} and CD8⁺ CD44⁺⁺ CD62L^{low})/Tregs ratio was observed in PB of Vk*MYC injected mice at the late time point as compared to BM of control mice. (B) Representative CyTOF profiles showing proportion of CD4⁺CD25⁺, CD4CD25FOXP3⁺ and CD4CD25FOXP3CD127⁻/low cells in CD45-expressing bone marrow fraction of 3 SMM patients compared to healthy donors. (t-test 2-tailed, error bars indicate SD)

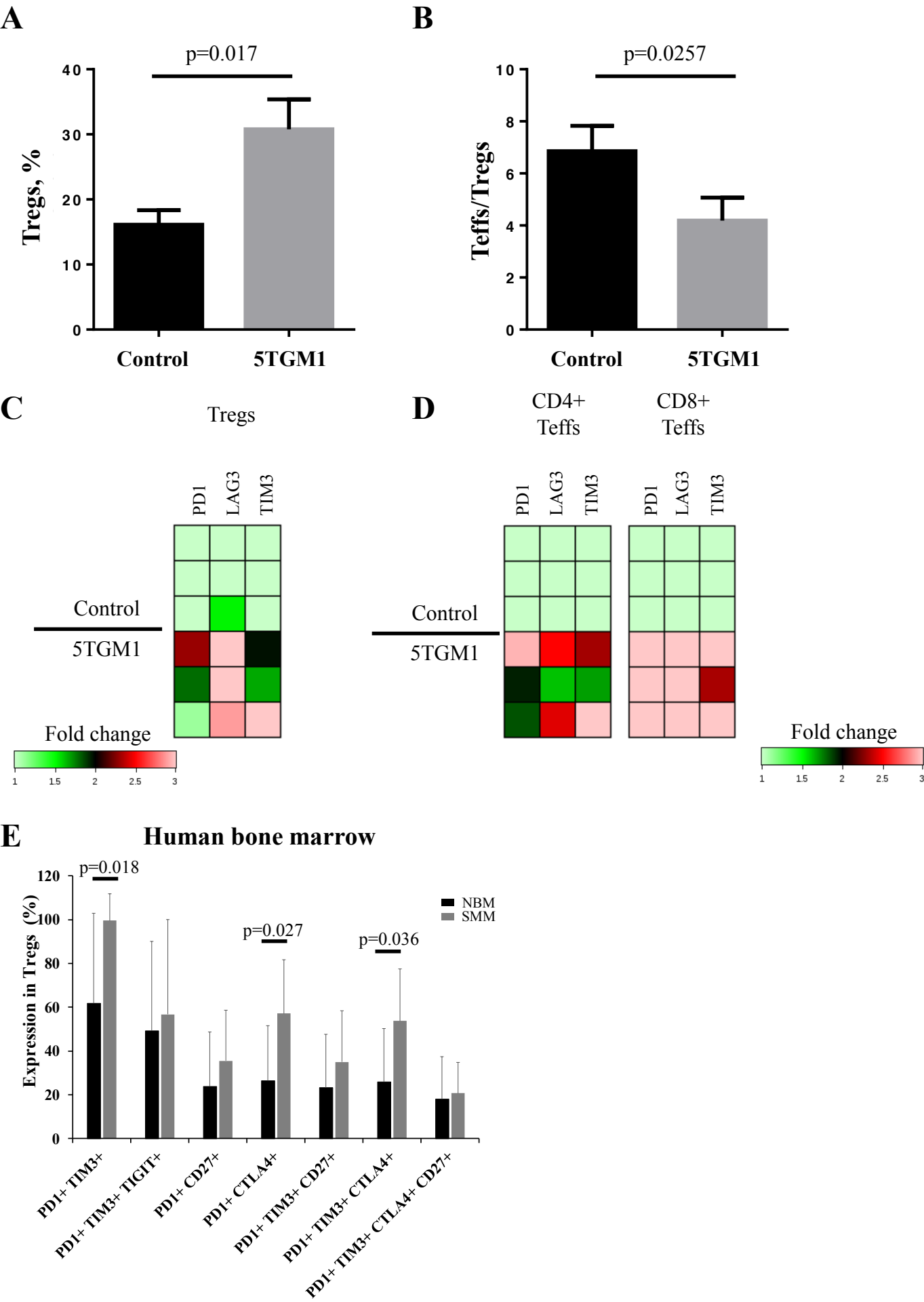
Supplementary Figure 3



Supplementary Figure 3. Decrease of Teff/ Tregs ratio in PB of Vk*MYC-injected mice and expression of immune checkpoint molecules in Tregs in the BM and PB at different time points post injection.

Expression of immune checkpoint receptor (PD1, LAG3 and TIM3) on Tregs in PB of Vk*MYC-injected mice (n=3) compared to control Tregs (n=3) at early (A), late (B) time point. (C) Expression of immune checkpoint receptor (PD1, LAG3 and TIM3) on Tregs in BM of Vk*MYC-injected mice compared to control Tregs at early time point. (D) Co-expression of checkpoint receptor (PD1, LAG3 and TIM3) on Tregs in BM of Vk*MYC-injected mice compared to healthy controls at late time point. Heatmap of immune checkpoint receptor (PD1, LAG3 and TIM3) expression on PB CD4⁺ and CD8⁺ Teffs (CD44⁺⁺ CD62L^{low}) at early (E) and late (F) time points. The color indicates the mean expression fold change of each molecule. (G) Heatmap of PD1, LAG3 and TIM3 expression on BM CD4⁺ and CD8⁺ Teffs (CD44⁺⁺ CD62L^{low}) at early time point. (t-test 2-tailed, error bars indicate SD)

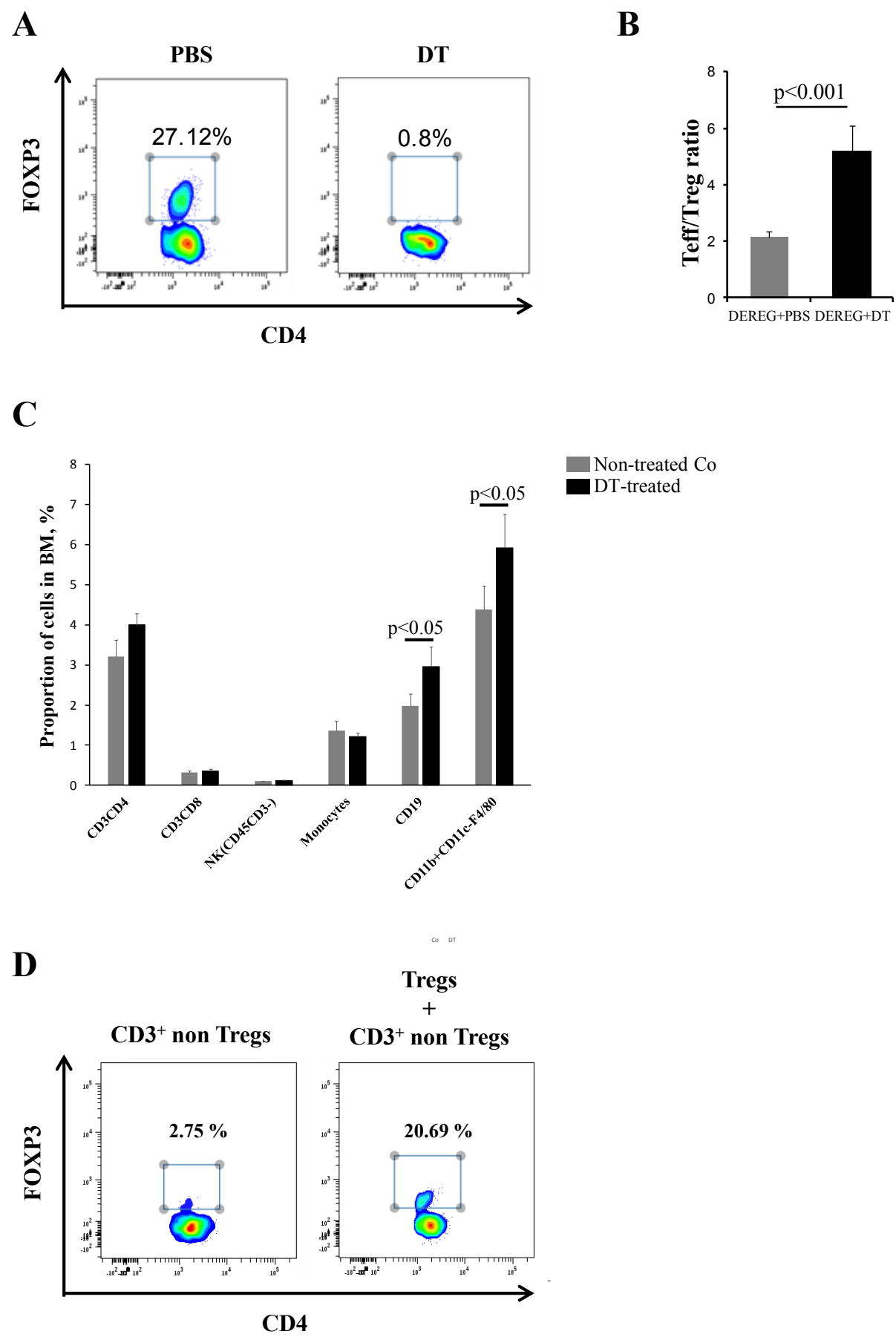
Supplementary Figure 4



Supplementary Figure 4. Treg increased and activated in BM of 5TGM1-injected C57BL/KalwRij mice and co-expression of immune checkpoint molecules in BM of SMM patients.

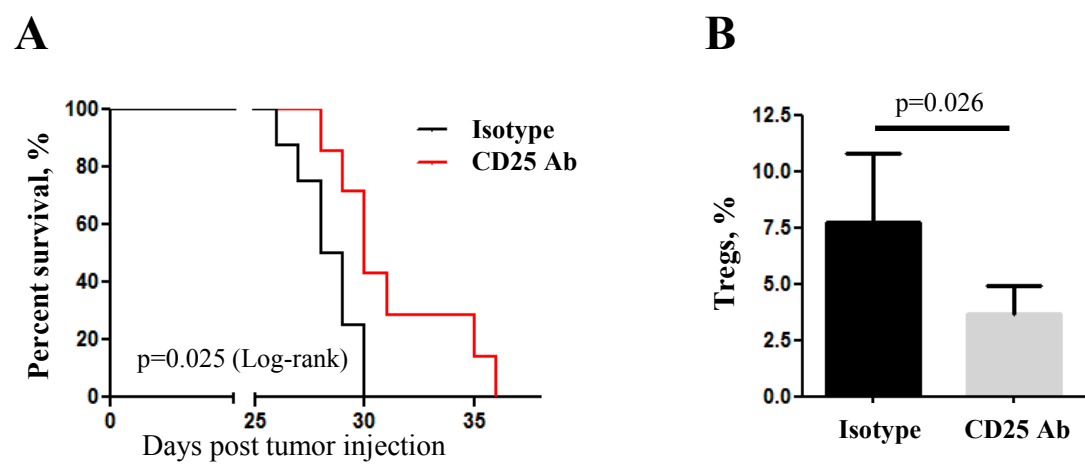
(A) Significant increase of Tregs frequency within CD4⁺ T cells in the BM of 5TGM1 injected mice (n=3) compared to control mice (n=3) at late time point (day 28). (B) Significant decrease of Teffs / Tregs ratio was observed at the late time point of 5TGM1 injected mice compared to control mice. (C) Heatmap of immune checkpoint receptor (PD1, LAG3 and TIM3) expression of BM Tregs at late time point. The color indicates the mean expression fold change of each molecule. BM Tregs of 5TGM1 injected mice had higher expression of immune checkpoint receptors compared to control BM Tregs. (D) Heatmap of PD1, LAG3 and TIM3 expression of BM CD4⁺ and CD8⁺ Teffs at late time point. Higher expression of PD1, LAG3 and TIM3 on 5TGM1 injected BM Teffs. (E) Increased proportion of Tregs co-expressing PD1, TIM3 and CTLA4 in BM aspirates of SMM patients (n=17) compared to healthy donors (n=11). Data combined from 3 independent experiments. (t-test 2-tailed, error bars indicate SD)

Supplementary Figure 5



Supplementary Figure 5. Proportion of Tregs after depletion by DT or adoptive transfer. (A) Effective depletion of BM Tregs in Vk*MYC-injected DEREG mice by DT injection compared to PBS injection at day 20 post Vk*MYC injection. (B) Flow cytometry data of BM cells at day 3 after last DT treatment in Vk*MYC-injected mice demonstrate a significantly increased Teff/Treg ratio compared to PBS treated mice (4 weeks post Vk*MYC injection). (C) Phenotypic distribution of bone marrow cells in Vk*MYC-injected mice 4 weeks post Vk*MYC injection and DT treatment compared to Vk*MYC-injected, non-treated DEREG mice (n=5 per group). (D) Representative FACS analysis of BM Tregs of mice injected with Tregs and CD3⁺ non Tregs (Tregs + CD3⁺ non Tregs) and mice injected only with CD3⁺ non Tregs (CD3⁺ non Tregs). (t-test 2-tailed, error bars indicate SD)

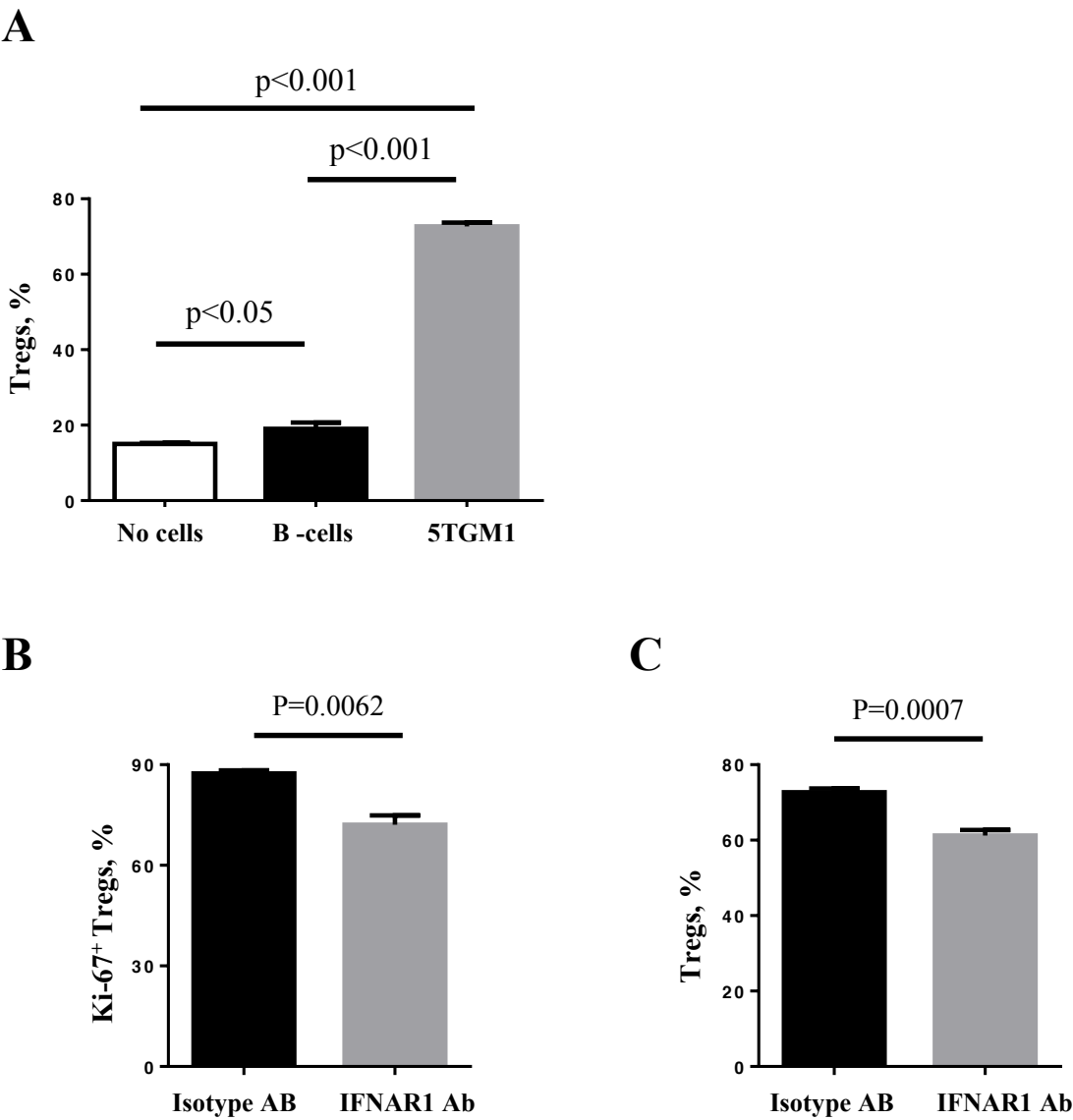
Supplementary Figure 6



Supplementary Figure 6. Blocking of CD25 receptor using CD25 antibody prolongs survival of Vk*MYC injected animals.

(A) Treatment with anti-CD25 antibody leads to better survival of injected mice. Mice were treated with anti-CD25 antibody on day -4 and day 10 post Vk*MYC cell injection (n=6). Isotype antibody treatment was used in the control group (n=6). (B) Significantly lower numbers of Tregs were detected in PB of anti-CD25 antibody treated mice as compared to isotype controls. PB analysis was done on day 11 post Vk*MYC injection. (t-test 2-tailed, error bars indicate SD)

Supplementary Figure 7



Supplementary Figure 7. Type-1 IFN pathway plays a role in Treg expansion by 5TGM1 cells.
(A) 5TGM1 cells are capable of expanding Tregs in vitro. CD4⁺ CD25⁺ Tregs were co-cultured with C57BL/KalwRij B cells or 5TGM1 cells for 72 hrs using trans-well. FOXP3⁺ cells within the CD4⁺ cells are shown. Significant Treg expansion was observed in the 5TGM1 co-culture group under trans-well compared to “No cells” or “B cells” groups. Average of experiments performed in triplicate is shown. The experiment was repeated 3 times. (B) Significant decrease of ki-67 expression of 5TGM1 expanded Tregs co-cultured with IFNAR1 Ab was observed compared to Isotype Ab. Average of experiments performed in triplicate is shown. The experiment was repeated 3 times. (C) Significant decrease of Treg expansion by 5TGM1 cells co-cultured with IFNAR1 Ab compared to Isotype Ab. Average of experiments performed in triplicate is shown. The experiment was repeated 3 times. (t-test 2-tailed, error bars indicate SD)

SUPPLEMENTARY TABLE 1

gene_name	log2FoldChange	pvalue
CR2	-1.754105105	1.14E-07
GM38257	-1.663325205	5.07E-07
GM11175	-1.625350548	1.78E-08
H2-DMB2	-1.580643499	9.61E-07
NTRK3	-1.538300689	3.19E-06
RGS2	-1.535722917	2.48E-07
PPP1R15A	-1.493961489	3.26E-06
DNAJB9	-1.489792391	2.98E-13
BAMBI	-1.453324869	3.85E-06
TNFAIP3	-1.413637676	6.27E-06
SLC30A4	-1.363309794	5.53E-06
GM10698	-1.360612906	6.90E-07
FOS	-1.312912328	5.00E-05
ALAS2	-1.311925596	2.46E-10
HBA-A1	-1.308836618	2.61E-16
CD22	-1.304986205	6.08E-06
SCD1	-1.299237228	6.01E-05
PTPRF	-1.293199031	5.00E-05
ZFP354C	-1.278876062	0.000119035
PAX5	-1.269783046	9.99E-05
RP23-4F16.12	-1.246683443	5.08E-06
BLK	-1.232624802	0.000211979
JUN	-1.229247208	0.000153806
ATP1B1	-1.198212543	1.09E-08
ZSCAN10	-1.196748707	0.000137553
CACNA2D2	-1.181106971	0.000334108
E430014B02RIK	-1.180031188	0.000216646
VANGL2	-1.173394111	0.000122724
RASL11B	-1.169355359	9.85E-05
MAFF	-1.16763885	0.000336557
RYR2	-1.16547011	0.000458805
PIM3	-1.155030723	3.51E-08
C1QL3	-1.153808501	0.000493352
NR4A2	-1.147471441	5.88E-05
C230085N15RIK	-1.145522682	0.000538342

TRP53INP2	-1.141599881	0.000274122
BTG2	-1.137583817	2.61E-15
LY6D	-1.132680814	0.000252716
SPTY2D1	-1.116065657	2.57E-08
DAPK1	-1.103332552	4.56E-05
SIK1	-1.087969101	0.000940377
GFOD2	-1.07715986	0.000948759
TRIB1	-1.073847646	2.72E-09
CD55	-1.070912389	1.32E-13
GEM	-1.068014009	0.000236276
CECR2	-1.067194084	0.001331875
GM37811	-1.065655387	0.00122256
ZFP36	-1.065106335	1.05E-12
DUSP1	-1.05100995	6.25E-11
GM30292	-1.047008312	0.001008288
IRS2	-1.043354899	3.16E-07
HBA-A2	-1.042529375	8.39E-05
SLC25A23	-1.041967417	7.54E-06
HBB-BS	-1.036951957	0.000248255
TET1	-1.035944757	0.000285789
HBB-BT	-1.026969464	9.51E-05
PRRT2	-1.011071105	0.000669147
CERS6	-1.009686382	3.13E-05
TOB1	-1.008933656	4.46E-07
IGHD	-1.004497214	9.82E-05
GM20518	-1.002087894	0.002512408
SCAND1	1.001369748	0.002539504
EAF2	1.003642029	0.002477922
SH3BP2	1.008766975	5.94E-06
GM20559	1.013158536	4.92E-17
RGS12	1.018507433	0.000596453
MPP4	1.018949259	0.001831878
ICA1	1.024838143	0.001353931
HIP1	1.029539163	6.89E-07
BCL2A1A	1.034434774	0.000710139
GM3164	1.035517446	0.001872974
GM6637	1.038669623	0.000156508
ADGRE1	1.039700291	0.001723903
CP	1.041958608	0.001698374

TMEM158	1.04279845	8.42E-05
CHIL3	1.044581948	0.000156244
BC147527	1.047536484	2.42E-05
SOCS1	1.048840236	0.000789071
CASP4	1.049297949	6.17E-06
BASP1	1.05746133	0.001501014
NES	1.05757639	0.000452303
FCNA	1.068582796	0.001082541
EBI3	1.073064945	5.91E-07
SLC52A3	1.074613694	1.65E-06
GM6548	1.079941637	3.09E-07
CYP51	1.082781175	5.66E-08
IGKC	1.083198381	0.000733206
SH3PXD2B	1.087953986	0.001080636
MT-ND3	1.088637219	0.000786208
TCTEX1D2	1.08926198	0.000927467
BSPRY	1.091614041	8.79E-06
HMGN3	1.093532681	2.51E-05
STAT1	1.094043084	1.70E-20
LPCAT2	1.095535467	0.000645603
CD200R4	1.096444242	1.37E-05
5430427O19RIK	1.098073883	0.000707204
GM4841	1.098776209	0.000827399
FKBP11	1.10287262	0.000760344
H2-T24	1.105629551	1.11E-09
4930512H18RIK	1.108047937	0.000724009
BCL2A1B	1.110126289	5.95E-08
GM5970	1.110754461	0.000788536
GM5431	1.110866239	0.000777824
TOR3A	1.113582731	1.13E-13
GNGT2	1.113997466	0.000119205
GM18752	1.114592703	0.000795976
MPO	1.114824206	9.52E-05
RASA4	1.121610185	0.000575146
PHF11C	1.12277566	8.08E-12
CAMP	1.123186311	7.88E-05
GPR55	1.125583986	2.16E-08
RBMS3	1.128988564	0.00025565
TREX1	1.129992747	3.52E-10

ABCB1A	1.131277826	1.36E-16
CD63	1.133631118	0.000665654
CASP1	1.135809142	2.54E-07
TRIM30A	1.136887548	3.24E-20
CCR3	1.137533447	2.79E-06
PTAFR	1.139419711	5.59E-05
TMEM67	1.152764987	1.50E-05
PHF11D	1.152877254	4.99E-05
KLRG1	1.156370238	3.05E-13
GRN	1.156994471	0.000241286
GM15135	1.157079313	0.000211861
LDLR	1.159771627	1.07E-07
MAFB	1.167243015	0.000458463
APOBR	1.168616595	4.42E-05
IL1R2	1.173275516	0.00014506
TMCC3	1.174761286	9.20E-05
PON3	1.176099496	2.43E-05
CCRL2	1.187297139	1.95E-08
GM10175	1.187953348	2.99E-09
F830016B08RIK	1.190081222	0.000105571
MS4A7	1.195775093	0.000289061
GM3468	1.196550035	1.03E-05
CLIC4	1.197818519	1.91E-11
HERC6	1.199291097	4.61E-16
ERDR1	1.199785325	0.00030423
GZMA	1.199934318	0.00022304
SEMA7A	1.209890061	0.000208051
PYDC3	1.212671092	2.88E-14
MS4A4C	1.216484615	0.000223092
IGKV1-117	1.221325905	0.00021762
NR4A3	1.227888915	4.29E-10
IGTP	1.234096576	1.14E-31
CXCL11	1.248418621	4.89E-05
HOXB4	1.252410074	3.69E-06
NAMPT	1.252749973	1.59E-22
CD300A	1.26064519	0.000153922
CCL6	1.261792632	9.56E-05
GM26917	1.26385414	0.000108552
AW112010	1.266039911	3.26E-14

GM4955	1.271731042	3.13E-20
STAB1	1.281071179	2.40E-06
IFI47	1.282309228	1.86E-29
GM14005	1.286809507	4.65E-05
HAVCR2	1.287290048	6.65E-06
CITED4	1.295169111	8.08E-05
AW011738	1.306109907	5.61E-07
PPA1	1.320178061	2.15E-12
OAS1G	1.328872033	6.64E-05
GM43197	1.336949545	4.50E-11
SLC15A3	1.339568654	2.08E-06
GM12250	1.344169383	6.05E-14
OAS1B	1.347048356	2.30E-14
GBP6	1.353023467	3.13E-37
STAT2	1.354924947	1.24E-20
DLL1	1.378800133	2.31E-05
GM12185	1.38074827	7.18E-09
JCHAIN	1.380878605	1.39E-05
HOXB3	1.383021836	3.24E-05
PYDC4	1.385414901	2.56E-24
TGTP2	1.394913467	3.60E-35
PHF11B	1.40665229	2.04E-28
TRAFFD1	1.410275673	1.17E-20
CLEC7A	1.413461174	2.18E-05
GM7609	1.420988286	5.86E-07
IFITM3	1.421733729	1.97E-05
CYBB	1.43884076	1.79E-15
TRIM30D	1.443810351	9.50E-27
A230005M16RIK	1.444853517	1.44E-05
GJB2	1.450925577	5.91E-06
PGAM2	1.459750164	1.03E-05
IGKV1-110	1.460227786	3.78E-06
TNFRSF8	1.464298932	2.29E-07
2310031A07RIK	1.478324191	1.81E-06
PARP9	1.483784389	2.75E-25
CTLA2B	1.4875031	6.37E-06
GM16464	1.489977611	8.97E-07
FGL2	1.491789641	2.09E-38
TDRD7	1.493025722	4.06E-12

GM43126	1.494357031	3.34E-06
IRGM1	1.496371348	3.41E-29
GBP6	1.511196014	1.70E-06
CAPN11	1.519456885	2.36E-06
DAXX	1.52137526	2.01E-21
PHF11A	1.532932568	1.68E-21
ASB2	1.536593957	2.46E-06
IFIH1	1.543091248	1.59E-22
TGTP1	1.547998408	9.61E-32
NBEA	1.555243044	3.01E-06
AI607873	1.569320377	2.31E-06
CLEC4A3	1.572916155	2.26E-06
ALOX15	1.598276628	1.10E-06
TRIM30C	1.60348943	1.48E-06
CCR5	1.610138097	1.91E-11
MLKL	1.629821429	1.30E-08
XAF1	1.645732679	7.43E-36
TBX21	1.648506268	2.38E-11
DDX60	1.654399355	1.55E-29
IFIT1BL1	1.669001668	9.40E-33
BC094916	1.684393885	5.76E-26
PLAC8	1.684564995	2.22E-11
LGALS3BP	1.694255968	3.50E-26
RNF213	1.711070519	2.19E-31
GM12338	1.722961279	1.23E-07
H2AFJ	1.730319213	2.54E-16
SERPINA3F	1.738391129	5.76E-10
NKG7	1.740084454	2.05E-11
DHX58	1.756183428	2.45E-28
CD86	1.778766006	3.41E-21
ZBP1	1.783705644	5.92E-37
FCGR4	1.88251529	1.60E-08
GBP10	1.905632856	2.57E-45
ISG20	1.935859627	1.97E-41
SDC3	1.949803332	8.09E-16
APOL9B	1.953308856	9.34E-11
USP18	2.008189107	2.41E-30
CCL5	2.01812883	1.89E-30
LY6A	2.023697966	3.07E-52

PARP12	2.030801403	2.54E-32
IIGP1	2.039347672	1.67E-38
CCL4	2.049569457	5.49E-10
IFI27L2A	2.050202143	1.16E-76
OAS1A	2.160719625	1.66E-33
CMPK2	2.19332456	5.27E-33
RTP4	2.193588902	4.17E-40
GM4951	2.212083177	1.50E-16
RSAD2	2.230319355	3.12E-36
BST2	2.274620521	3.44E-69
IRF7	2.33301622	7.37E-45
CXCL10	2.369495039	1.16E-13
OAS3	2.4292666	3.95E-39
OASL1	2.586914015	2.26E-29
IFIT1	2.645642235	4.44E-46
OASL2	2.68690895	4.73E-20
IFIT3	2.693997567	3.02E-20
IFIT3B	2.697812916	5.60E-19
GZMB	2.710077584	9.17E-71
MX2	2.735753401	1.50E-31
MX1	2.7380499	3.01E-22
MNDA	2.884359591	1.81E-25
IFI204	3.051636274	2.89E-27
OAS2	3.275260084	2.34E-53
ISG15	3.282058987	1.43E-83
IFI44	3.437057621	1.78E-43

Supplementary Table 1. Genes up-regulated and down-regulated in Vk*MYC injected BM Tregs compared to control BM Tregs.

A list of the genes significantly up-regulated and down-regulated in BM Tregs obtained from Vk*MYC injected mice (n=3) compared to BM Tregs from control mice (n=3).