Supplementary Figure 1. CD155 expression on tumor cell lines

A) RNA was extracted from CT26, LLC, B16F10 and MC38 cell lines (Qiagen) and cDNA prepared (BioRad iScript). Real-time PCR was performed using Taqman probes and the 7500 Fast Real-time PCR system (Applied Biosystems). All samples were normalized to β-actin expression. **B)** CT26, LLC, B16F10, and MC38 cell lines were stained with antibody against CD155 (blue histogram). Fluorescence minus one, FMO (red histogram).

Supplementary Figure 2. TIGIT is enriched on tumor-infiltrating lymphocytes

Spleen, tumor draining lymph node (DLN), and tumor-infiltrating lymphocytes (TILs) were harvested from WT Foxp3-GFP knock-in mice (n=7) bearing CT26 colon carcinoma and stained with antibodies against CD4, CD8, and TIGIT. **A)** Frequency +/- SEM of TIGIT$^+$ cells within CD4$^+$ and CD8$^+$ T cells in the spleen, DLN, and TILs as indicated. **B)** Left panels, representative flow cytometry data showing TIGIT expression in CD4$^+$ Foxp3$^-$ (GFP$^-$) and CD4$^+$ Foxp3$^+$ (GFP$^+$) TILs. Right panel, frequency +/- SEM of TIGIT$^+$ Foxp3$^-$ and Foxp3$^+$ cells as indicated. Data are representative of 2 experiments. **** $p<0.0001$ (one-way ANOVA and Tukey's multiple comparisons test). TIGIT$^+$ Foxp3$^+$ cells among tissues are compared for (B).

Supplementary Figure 3. TIGIT$^{-/-}$ CD8$^+$ TILs produce more cytokines

WT or TIGIT$^{-/-}$ mice (n=3-6) were implanted s.c. with B16F10 (A) or MC38-OVA^{dim} (B). On days 10-12, TILs were isolated and stimulated with 10 μg/ml gp100 peptide for 12 hrs (A) or 10 μg/ml OVA$_{257-264}$ peptide for 4 hrs (B) and stained with antibodies against surface proteins and IFN-γ (A, B) and IL-2, TNF-α. **A and B)** Representative contour plots and bar graphs showing the frequency +/- SEM of cytokine$^+$ cells within CD8$^+$ TILs.

Supplementary Figure 4. Efficacy of anti-Tim-3 in TIGIT$^{-/-}$ mice requires CD8$^+$ T cells. WT or TIGIT$^{-/-}$ mice were implanted s.c. with B16F10 melanoma cells and treated with 250 μg of isotype control or anti-Tim-3 (RMT3-23) antibodies on day 3, 6, 9, 12. In some groups, mice were treated i.p. with anti-CD8β (53.5.8, 100 μg) or anti-asGM1 (50 μg) on days 2, 3, 10 and 14 to deplete CD8$^+$ T cells or NK cells, respectively.

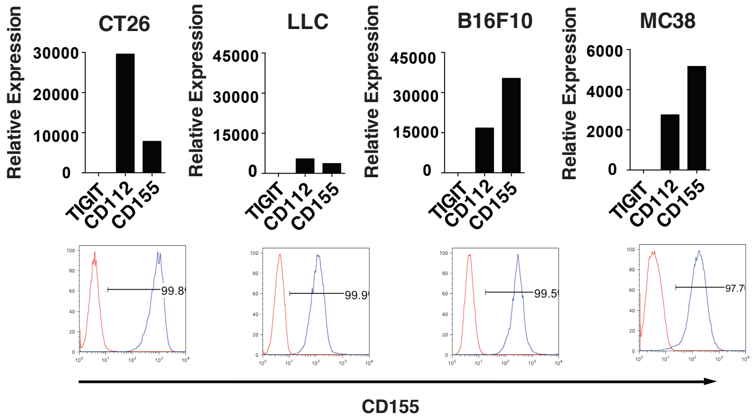
Supplementary Table 1. List of genes that are differentially regulated in TIGIT$^+$ Treg compared to their TIGIT$^-$ Treg counterparts in the tumor tissue

Gene name	Fold expression TIGIT+/TIGIT- TIL Treg	SEM
Lag3	13.57369442	0.348895137
TIGIT	8.569699312	3.460576538
CCR8	7.62873218	3.58964762
IL-10	6.578844942	2.355730978
Arnt2	6.066391762	0.927354329
RUNX2	5.538881806	0.556077067
Il2ra	5.25078646	1.667739759
IL1r2	5.159677665	1.009931752
Pdcd1	5.091941289	0.181209093
ITGA3	4.849276943	0.445533627
IL12RB2	4.794796421	2.544775772
Klrg1	4.613976658	1.794179219
CXCR6	4.58486956	1.929836322
IKZF3	4.42299837	1.800529894
CTLA4	4.256684205	1.995032999
Rgs16	4.12312849	0.351629116
ICOS	4.064769166	2.345165865
FoxP3	4.016600576	2.490899939
CCL5	4.009624818	0.120641355
TBX21	3.799587462	1.198606633
IL27R	3.751491521	1.615061559
IL12RB1	3.481480436	1.828983408
prdm1	3.450755365	1.690409181
IL2RB	3.436597248	1.289463645
NFATc1	3.284659262	0.845606911
BATF	3.194940439	0.440481462
Havcr2	2.841018212	0.729312527
CD39	2.740638883	0.29257825
Il15ra	2.634837605	0.615221021
CXCR3	2.621173972	1.354337885
CCL4	2.507625622	0.185196491

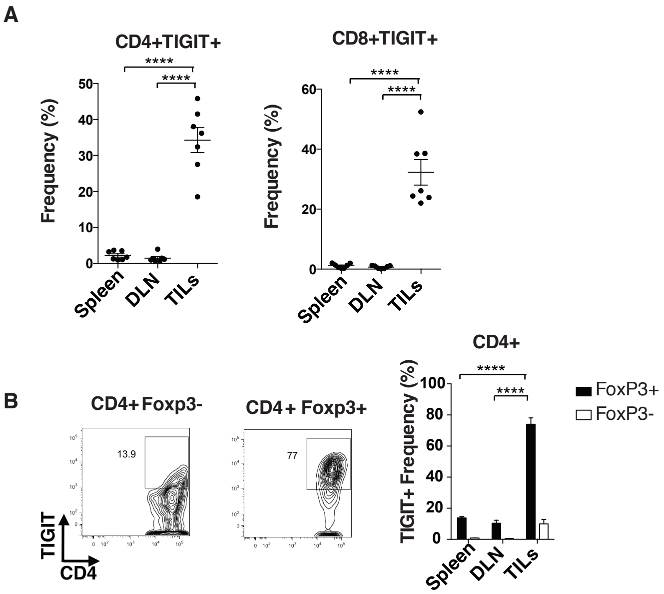
CMTM6	2.484592365	0.152596646
EBi3	2.363257325	0.496560937
CCR5	2.328110797	0.269163629
RORA	2.321802166	0.535809949
IL10RA	2.27833987	0.181799242
ID2	2.143822143	0.022666183
CCR2	2.125290693	0.436892902
STAT1	2.092107006	0.577375443
Lef1	2.025888507	0.41005116
TGFB1	2.017581607	0.14908506
PRF1	2.00468882	0.197182837
CASP6	1.949569049	0.143528865
RUNX1	1.943855276	0.214004255
NFIL3	1.918059155	0.292151627
IRF8	1.910407709	0.105236063
STAT3	1.809905639	0.139969333
Pdcd1l	1.755902118	0.055761873
L1CAM	1.708409463	0.034906045
Tob	-2.263776955	0.113320179
ACVR2A	-2.228971361	0.086179776
BATF3	-2.375177754	0.064317176
PROCR	-2.584209713	0.141885713
IFNGR2	-2.675907683	0.082911768
KLF7	-2.708204363	0.133016108
JUN	-2.792687699	0.032070834
BATF2	-2.858338676	0.077493974
Bcl6	-3.015987778	0.015749056
Bcl2	-3.057464012	0.009271697
CCL12	-3.275708924	0.052316091
Grail	-3.599756216	0.277413015
Tcf7	-3.851438535	0.11926965
NFE2L2	-3.906298901	0.071287882
Gpr56	-4.322811077	0.054410515
IL1rn	-4.414808494	0.011458391
KLF3	-5.82088016	0.082153866
DDR1	-6.404698133	0.099294648
Serpine1	-12.58766961	0.066357279

Supplementary Table 1. List of genes that are differentially regulated in TIGIT$^+$ Treg compared to their TIGIT$^-$ Treg counterparts in the tumor tissue

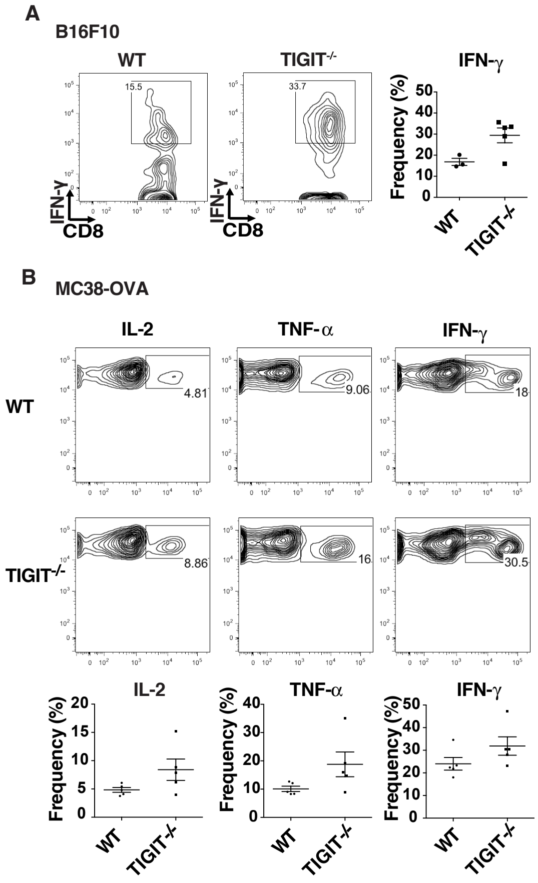
TIGIT$^+$ and TIGIT$^-$ Treg cells were sorted from B16F10 tumors of Foxp3 GFP KI mice. Gene expression was analyzed using a custom nanostring codeset. Fold expression +/- SEM of differentially regulated genes in the TIGIT$^+$ TIL Treg compared to TIGIT$^-$ counterparts are shown. Data are pooled from two independent experiments.



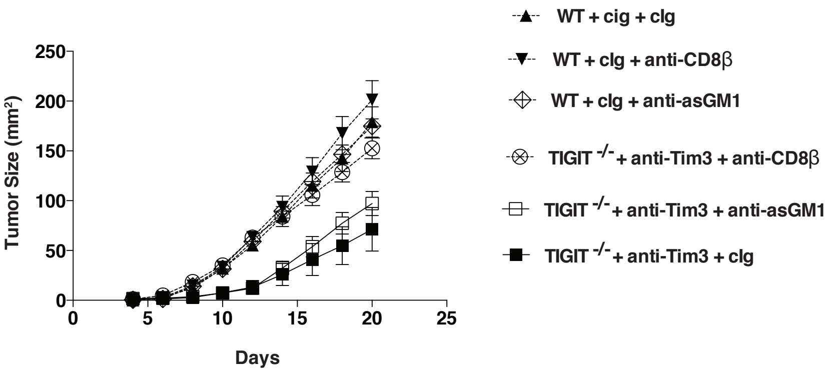
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4