

## Supplemental Figure Legends

Supplemental Figure 1. Effect of a newly generated anti-TIGIT mAb (clone MBSA43) on T cell proliferation and IFN $\gamma$  production (A) Proliferation of human CD4<sup>+</sup> T cells labeled with CFSE and activated with anti-CD3 and anti-CD28 with plate-bound anti-TIGIT or isotype-matched control antibody. On day 5, CFSE dilution was analyzed by flow cytometry after gating on viable cells. (B) Flow cytometry of IFN $\gamma$  intracellular staining on human CD4<sup>+</sup> T cells, stimulated with PMA and ionomycin for 4 hr in the presence of GolgiStop. Cells were stained with LIVE/DEAD Fixable Dead Cell Stain Kit before fixation to allow gating on viable cells. Data are representative of three individual donors.

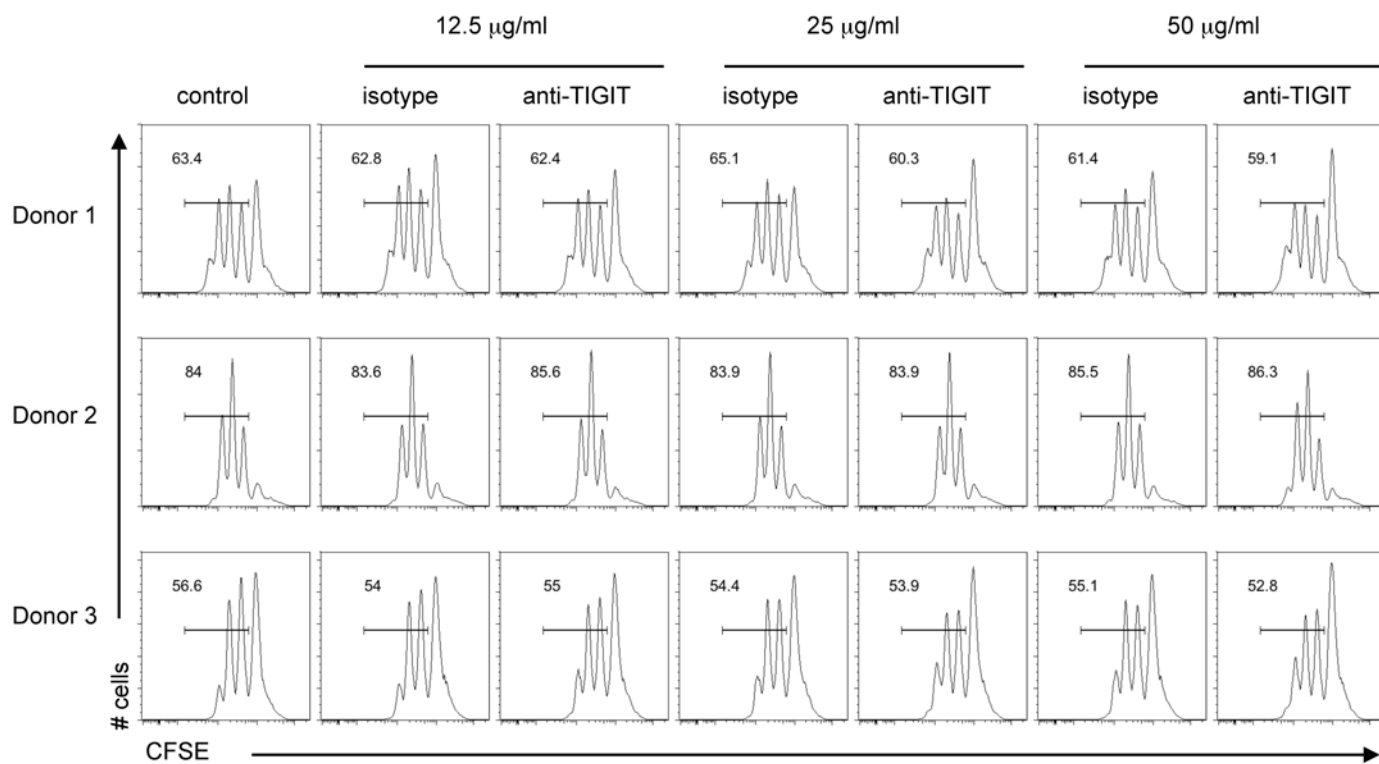
Supplemental Figure 2. TIGIT cell-intrinsic signaling does not modify T cell apoptosis. (A) Human CD4<sup>+</sup> T cells activated with anti-CD3 and anti-CD28 and incubated with plate-bound agonistic anti-TIGIT or isotype-matched control antibody. Apoptosis was analyzed by flow cytometry at 24 h. (B) and at 48 h. (C) No significant differences were observed in the frequency of early or late apoptotic cells (n=5).

Supplemental Figure 3. TIGIT expression and function in memory T cells from MS patients. (A) Relative TIGIT mRNA levels in *ex vivo* memory T cells in untreated RRMS patients and healthy age-matched controls (n=9). (B) Representative experiment showing TIGIT expression on memory T cells upon activation in MS patients. (C) Group data representing analysis of frequency of memory T cells expressing TIGIT after activation in RRMS (n=8). (D) Anti-TIGIT inhibits T cell proliferation in CD4<sup>+</sup> cells from RRMS patients. CD4<sup>+</sup> T cells were activated with anti-CD3, anti-CD28 and agonistic anti-TIGIT

or isotype-matched control. On day 4, T cell proliferation was assessed by CFSE dilution assay after gating on viable cells. (E) Group data representing analysis of T cell proliferation in RRMS patients in the presence of agonistic anti-TIGIT or isotype control (n=8). For each scattered plot, the horizontal bar represents the mean value. \*p<0.001.

Table S1. Target sequences of five different shRNAs directed against CD226 and TIGIT introduced into human CD4<sup>+</sup> T cells by lentiviral transduction.

Target gene	Name in the text	Clone name	Clone ID	Vector	Region	Target sequence
	control	pLKO.1-emptyT	TRCN0000208001	pLKO.1		
TIGIT		NM_173799.2-696s21c1	TRCN0000244766	pLKO_TRC005	CDS	AGCTGCATGACTACTTCAATG
TIGIT		NM_173799.2-323s21c1	TRCN0000244769	pLKO_TRC005	CDS	TCGCTGACCGTGAACGATACA
TIGIT	shTIGIT-1	NM_173799.2-984s21c1	TRCN0000257016	pLKO_TRC005	3UTR	TAACGTGGATCTTGATCATAA
TIGIT	shTIGIT-2	NM_173799.2-345s21c1	TRCN0000244768	pLKO_TRC005	CDS	GGGAGTACTTCTGCATCTATC
TIGIT		NM_173799.2-95s21c1	TRCN0000244767	pLKO_TRC005	CDS	GGAATGATGACAGGCACAATA
CD226		NM_006566.1-1196s1c1	TRCN0000057636	pLKO.1	CDS	CGCAGACCAAAGACTAGAGTT
CD226		NM_006566.1-781s1c1	TRCN0000057635	pLKO.1	CDS	CCCAAGACAAATAGTGAGCAA
CD226	shCD226	NM_006566.1-1125s1c1	TRCN0000057634	pLKO.1	CDS	CCGGTCAACCTACCAATCAAT
CD226		NM_006566.1-296s1c1	TRCN0000057637	pLKO.1	CDS	GCCGAGAACATGTCTCTAGAA
CD226		NM_006566.1-467s1c1	TRCN0000057633	pLKO.1	CDS	GCTTCCAATAACATGACTCTT

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