

Supplemental Table 1: Antibodies used for staining of stimulated PBMCs. All antibodies were obtained from Miltenyi Biotec (Bergisch Gladbach, Germany), the fixable viability dye eFluor520 was obtained from Thermo Fisher (Waltham, USA).

Antibody Specificity	Antibody Conjugate	Clone
CD8	FITC	REA734
CD56	FITC	REA196
CD19	FITC	REA675
CD14	FITC	REA599
Viability	eFluor520	
CD25	APC	REA570
CD45RA	PerCP-Vio700	REA562
CD154	PE-Vio770	5C8
CD137	PE	4B4-1
CD127	APC-Vio770	REA614
CD197	VioBlue	REA546
CD4	VioGreen	REA623

Supplemental Table 2: Responsiveness of CD4⁺ non-Tregs (A) and Tregs (B) based on expression of CD154 and CD137. The observed rates of reacting samples are presented descriptively with counts, percentages and its two-sided 95% Clopper-Pearson confidence intervals (CIs). Higher expression of markers in stimulated samples in comparison to the unstimulated control is presented as “1”, a lower expression as “0”. PBMCs of all analysed Buffy Coats responded to the anti-CD3 stimulus as positive control.

A)

CD154-positive non-Tregs

	1 IU/ml rFVIII	1 IU/100 µl rFVIII	1 IU/ml rFVIIIFc	1 IU/100 µl rFVIII Fc	740 pM MOG	7.4 nM MOG	anti-CD3 (5 µg/ml)
Sample 1	1	0	1	0	0	0	1
Sample 2	1	1	1	0	0	1	1
Sample 3	1	0	0	0	0	1	1
Sample 4	1	0	1	0	1	1	1
Sample 5	0	1	0	1	0	1	1
Sample 6	0	1	0	1	0	1	1
Sample 7	1	1	1	1	0	0	1
Sample 8	0	1	0	0	0	0	1
Sample 9	0	1	0	1	1	0	1
Sample 10	1	1	0	0	1	0	1
Sample 11	0	1	0	1	0	0	1
Sample 12	0	0	0	0	1	0	1
Sample 13	0	0	0	1	0	1	1
Sample 14	0	1	0	0	1	1	1
Sample 15	0	1	1	1	1	1	1
Sample 16	1	1	1	1	1	0	1
Sample 17	0	1	1	1	0	0	1
Sample 18	0	0	0	0	0	1	1
Sample 19	0	0	0	0	0	0	1
Total	7/19	12/19	7/19	9/19	7/19	9/19	19/19
Rate (%)	36.8	63.2	36.8	47.4	36.8	47.4	
95% CI	(16.3-61.6)	(38.4-83.7)	(16.3-61.6)	(24.5-71.1)	(16.3-61.6)	(24.5-71.1)	

B)

CD137-positive Tregs

	1 IU/ml rFVIII	1 IU/100 µl rFVIII	1 IU/ml rFVIII Fc	1 IU/100 µl rFVIII Fc	740 pM MOG	7.4 nM MOG	anti-CD3 (5 µg/ml)
Sample 1	1	1	1	0	0	0	1
Sample 2	0	0	0	0	0	0	1
Sample 3	0	1	0	0	0	0	1
Sample 4	1	1	1	1	1	0	1
Sample 5	0	0	0	1	0	0	1
Sample 6	1	1	1	1	1	1	1
Sample 7	1	1	1	1	1	0	1
Sample 8	0	1	0	0	0	0	1
Sample 9	1	1	0	1	1	1	1
Sample 10	0	1	0	1	0	0	1
Sample 11	1	1	1	1	1	1	1
Sample 12	0	1	0	1	1	0	1
Sample 13	1	1	1	1	1	0	1
Sample 14	1	1	1	1	0	1	1
Sample 15	1	1	0	1	0	0	1
Sample 16	1	1	1	1	1	1	1
Sample 17	1	1	0	1	0	0	1
Sample 18	1	1	0	0	0	0	1
Sample 19	0	0	0	0	0	0	1
Total	12/19	16/19	8/19	13/19	8/19	5/19	19/19
Rate (%)	63.2	84.2	42.1	68.4	42.1	26.3	
95% CI	(38.4-83.7)	(60.4-96.6)	(20.3-66.5)	(43.5-87.4)	(20.3-66.5)	(9.1-51.2)	

Supplemental Table 3: Summary of medians, quartiles, and ranges for the intensity of responsiveness towards mentioned stimuli.

	1 IU/100 μl rFVIII	1 IU/100 μl rFVIII-Fc	7.4 nM MOG
Median	1.39	1.11	0.88
1. Qu - 3. Qu	1.05 - 1.55	0.95 - 1.33	0.67 - 0.99
Min - Max	0.72 - 3.69	0.50 - 2.52	0.56 - 1.27

Supplemental Table 4: Summary of medians, quartiles, and ranges for proportion of antigen-specific CD197⁺CD45RA⁺ naïve non-Tregs reacting towards the mentioned stimuli.

	Unstimulated	1IU/100 µl rFVIII	µl rFVIIIc	7.4 nM MOG	anti-CD3
Median	57.4	78.20	68.40	65.40	53.20
1. Qu - 3. Qu	42.60 - 67.65	58.70 - 85.65	57.10 - 79.75	53.00 - 74.90	42.00 - 61.40
Min - Max	22.2 - 86.8	44.10 - 89.90	27.20 - 91.90	27.70 - 87.10	28.70 - 79.30