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Supplementary Materials for

Monoclonal antibodies against GARP/TGF-β1 complexes inhibit the immunosuppressive activity of human regulatory T cells in vivo

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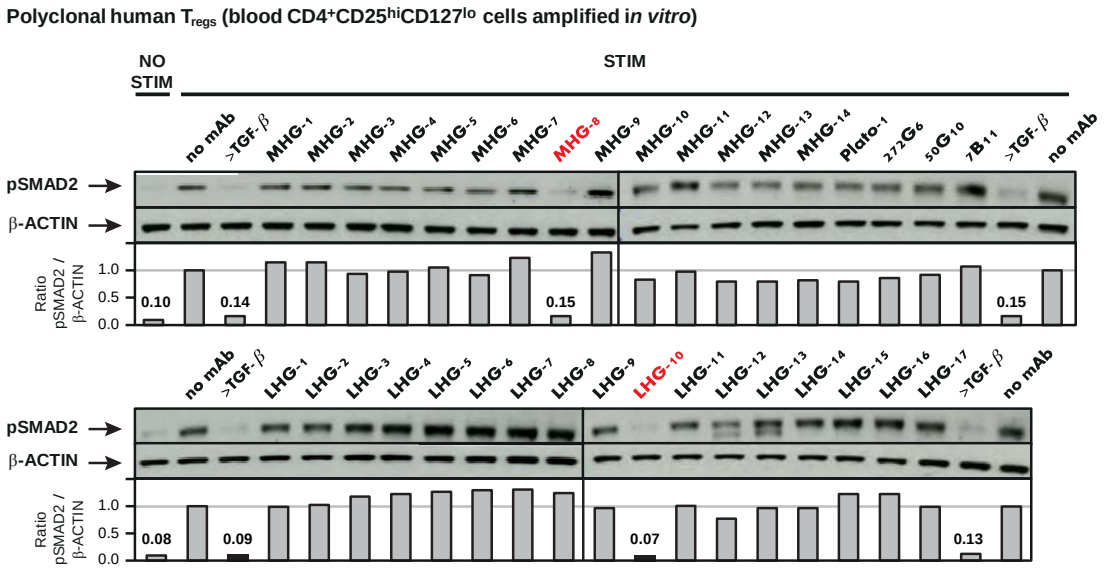
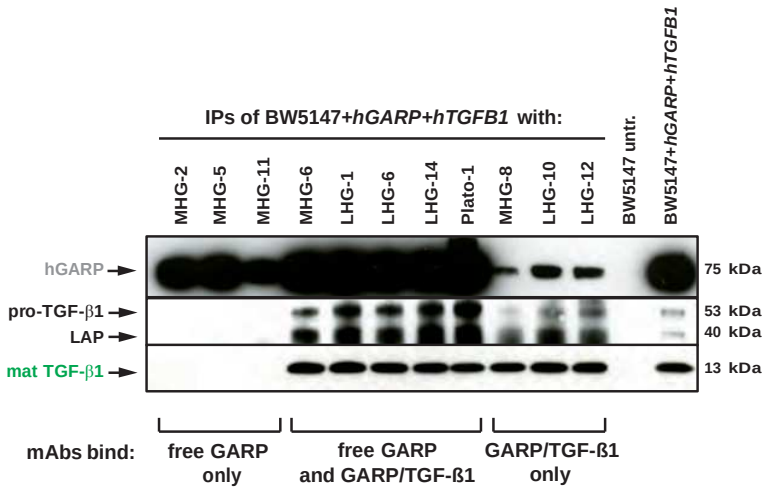
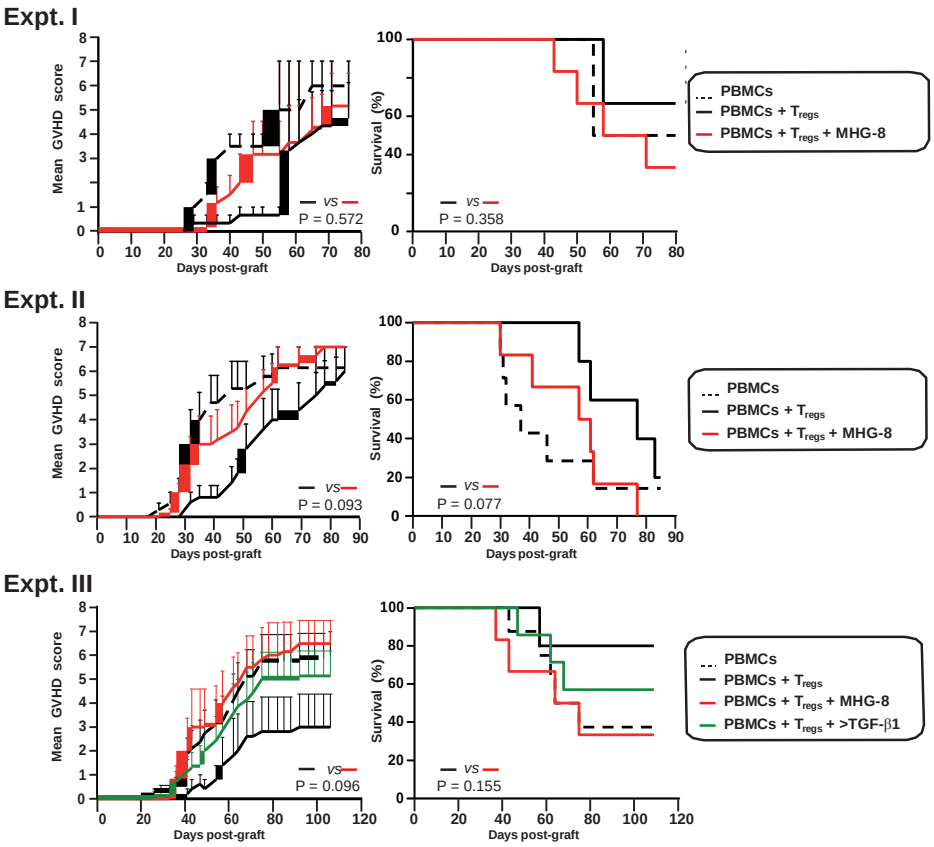
Table S1. Purity of polyclonal human T_{regs}.



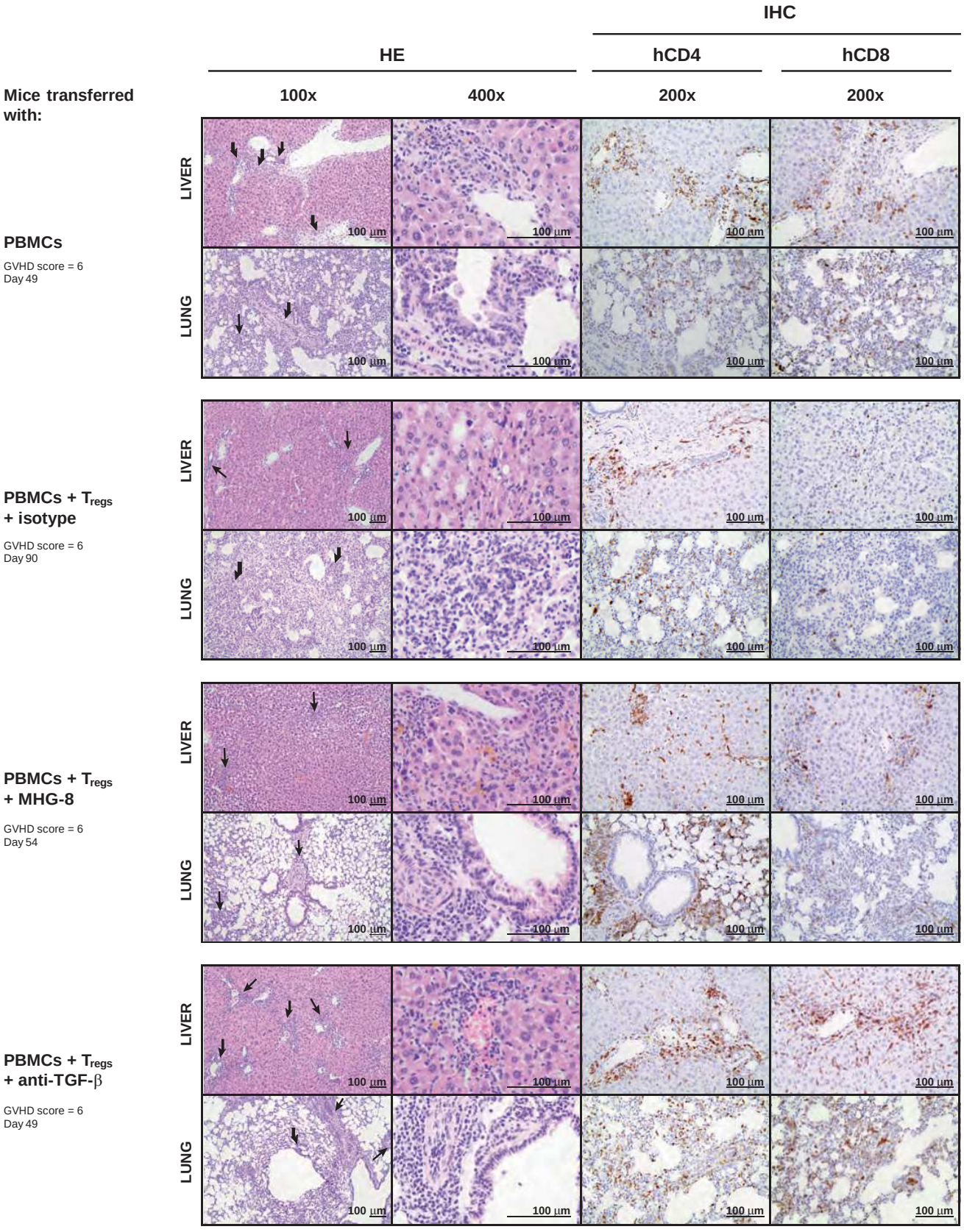
Supplementary Figure S1. MHG-8 and LHG-10 inhibit production of active TGF-β1 by human polyclonal T_{regs}.
After a short in vitro amplification, human $CD4^{+}CD25^{hi}CD127^{lo}$ cells (T_{regs}) were re-stimulated with anti-CD3/CD28 coated beads during 24 hours, in the presence or absence of the indicated mAbs (10 µg/ml). Plato-1, 272G6, 50G10 and 7B11 are commercially available anti-hGARP mAbs. Cells lysates were analyzed by Western Blot with antibodies against phosphorylated SMAD2 (pSMAD2) as a read-out for active TGF-β1 production, or β-ACTIN as loading control. Bar graphs show quantification of ECL signals, represented as ratios of pSMAD2 to β-ACTIN, normalized to the ratio in cells stimulated in the absence of anti-hGARP mAb (no mAb) measured on the same gel.



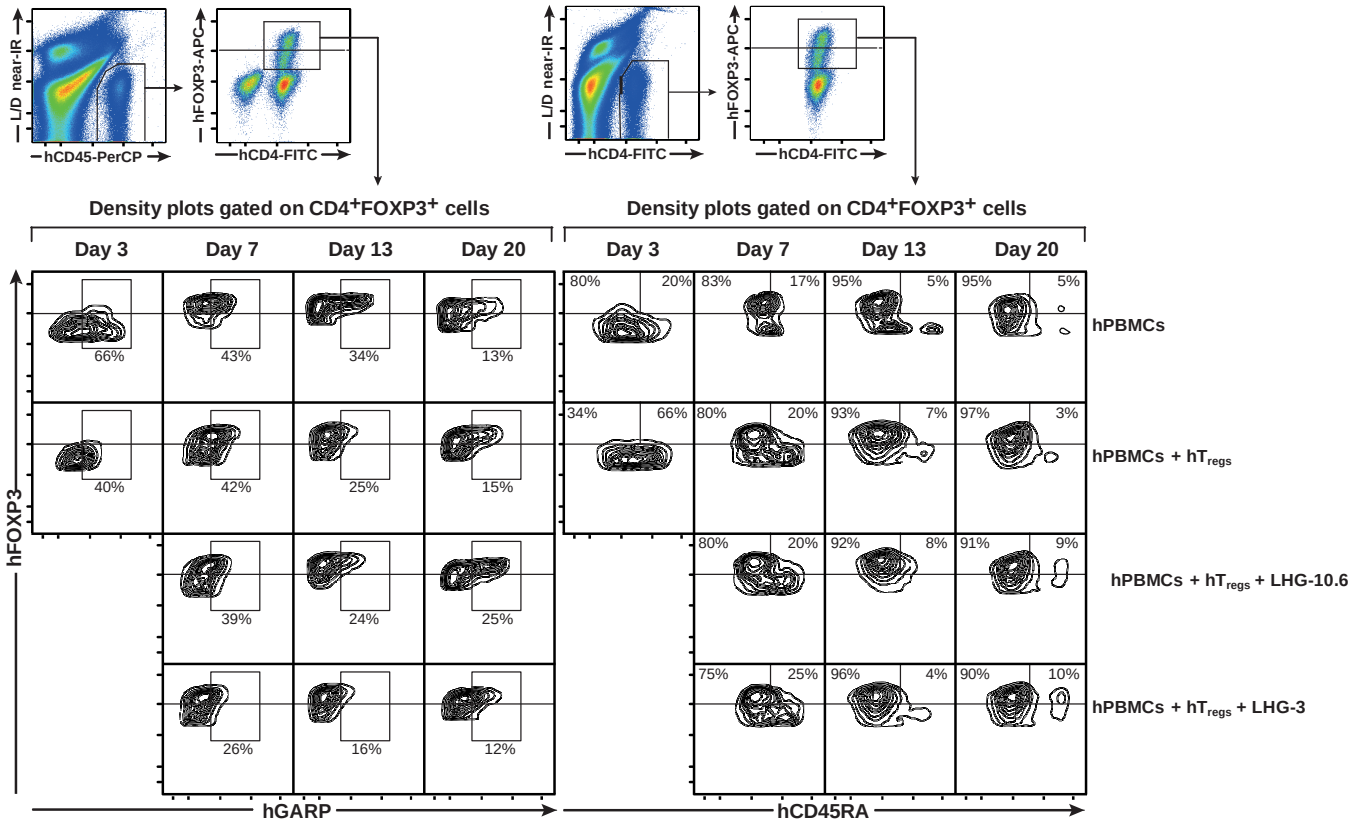
Supplementary Figure S2. Some anti-hGARP mAbs bind free GARP only.
Cell lysates of BW5147 cells transfected with *hGARP* and *hTGFB1* were immunoprecipitated with the indicated anti-hGARP mAbs. Total lysates (BW5147+*hGARP*+*hTGFB1* or untransfected controls; 30% of input in IP) and IP products were analysed by Western Blot with antibodies against hGARP (clone Plato-1), LAP or the mature TGF-β1 peptide. IPs with mAbs representative of each category are shown, but all 31 new anti-hGARP were tested in this assay.



Supplementary Figure S3. Detailed results of experiments I, II, and III summarized in Fig. 5B.
Graphs show progression of disease score (mean + sem) and survival. Numbers of mice per group are indicated in Fig. 5B. Statistical significance of differences were calculated using 2-way ANOVA followed by a Bonferroni *post hoc* test for disease scores, and Log-rank (Mantel-Cox) test for survival.

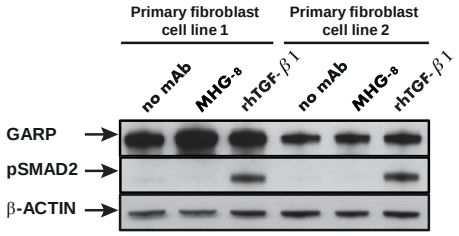


Supplementary Figure S4. Analyses of tissues from NSG mice grafted with human PBMCs.
Sections from lung and liver of NSG mice grafted with human PBMCs +/- T_{regs}, and treated or not with MHG-8, anti-TGF-β or isotype control antibodies as indicated in Fig. 5A, were stained with Hematoxilin/Eosin (HE) or with antibodies against human CD4 (hCD4) or CD8 (hCD8). Organs were collected from mice with a GVHD score of 6 at the indicated day post graft. Arrows in liver sections indicate areas of periportal inflammation, interface hepatitis or endothelitis. Arrows in lung sections show endothelitis or interstitial inflammation.



Supplementary Figure S5. FACS analysis of splenocytes collected from NSG mice at various time points after transfer of human PBMCs ± T_{regs}.

NSG mice injected as in Fig. 5A with human cells and antibodies indicated on the right were sacrificed at various time points after cell transfer. Permeabilized splenocytes were labeled with a Live/Dead cell marker, anti-hCD45, anti-hCD4, anti-hFOXP3 and MHG-6 antibodies (plots on the left), or with a Live/Dead cell marker, anti-hCD4, anti-hCD45RA, and anti-hFOXP3 (plots on the right). One representative mouse per group is represented (n = 2 mice per group). The gating strategy is indicated on top of the density plots. Dotted horizontal lines indicate the delineation between $FOXP3^{lo}$ and $FOXP3^{hi}$ cells. In the density plots on the left, the rectangle and the percentage below indicate $GARP^+$ cells in $CD4^+FOXP3^+$ cells. In the density plots on the right, the 2 rectangles and the inset percentages indicate $CD45RA^-$ and $CD45RA^+$ cells in $CD4^+FOXP3^{hi}$ cells, respectively.



Supplementary Figure S6. Human fibroblasts express GARP but do not produce active TGF-β1.
Human primary fibroblast cell lines were derived from abdominal plasty or tumor fragments. Cells were plated in complete medium. After 24 hrs, medium was changed to X-VIVO10, and cells were incubated for an additional 24 hours. In conditions indicated on the figure, MHG-8 was added at a final concentration of 10 µg/ml during the last 24 hours of culture, or recombinant hTGF-β1 was added during the last 30 minutes of culture. Cell lysates were analyzed by Western blot with anti-GARP, anti-pSMAD2 or anti-β Actin antibodies.

Figure 1B

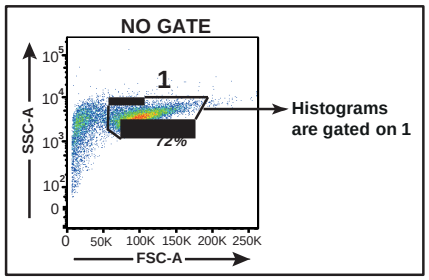


Figure 3B-D

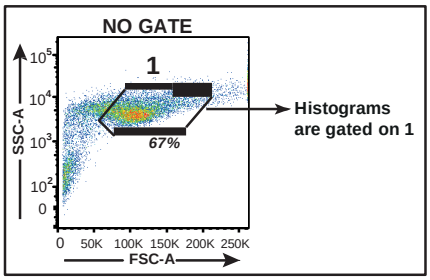


Figure 6B

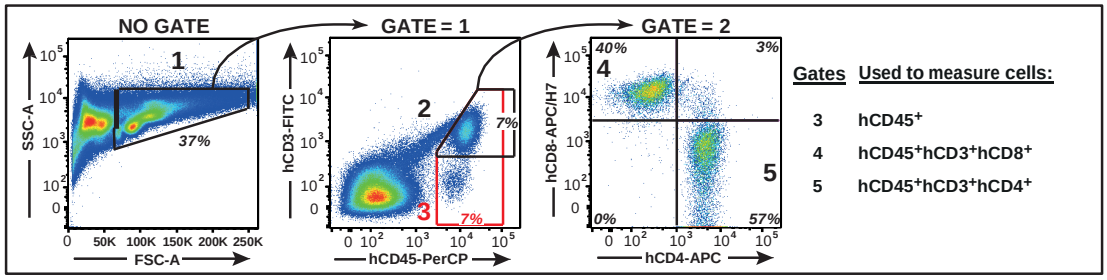


Figure 6C

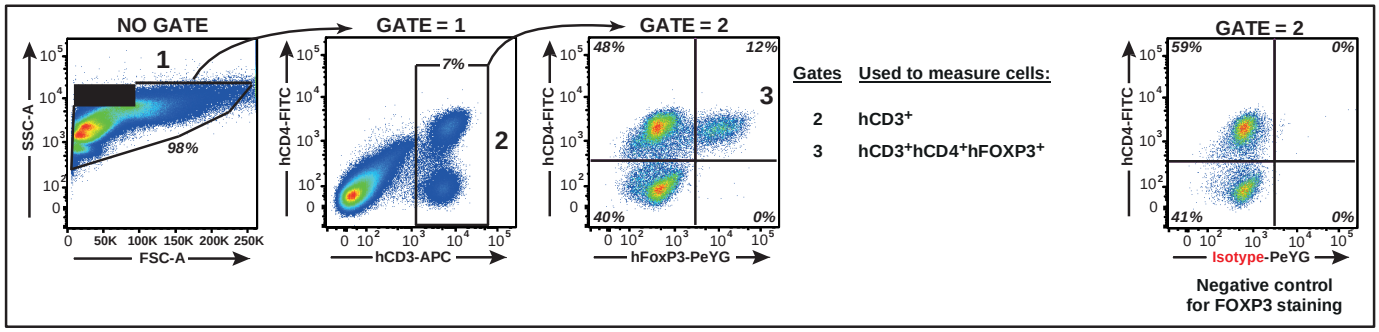
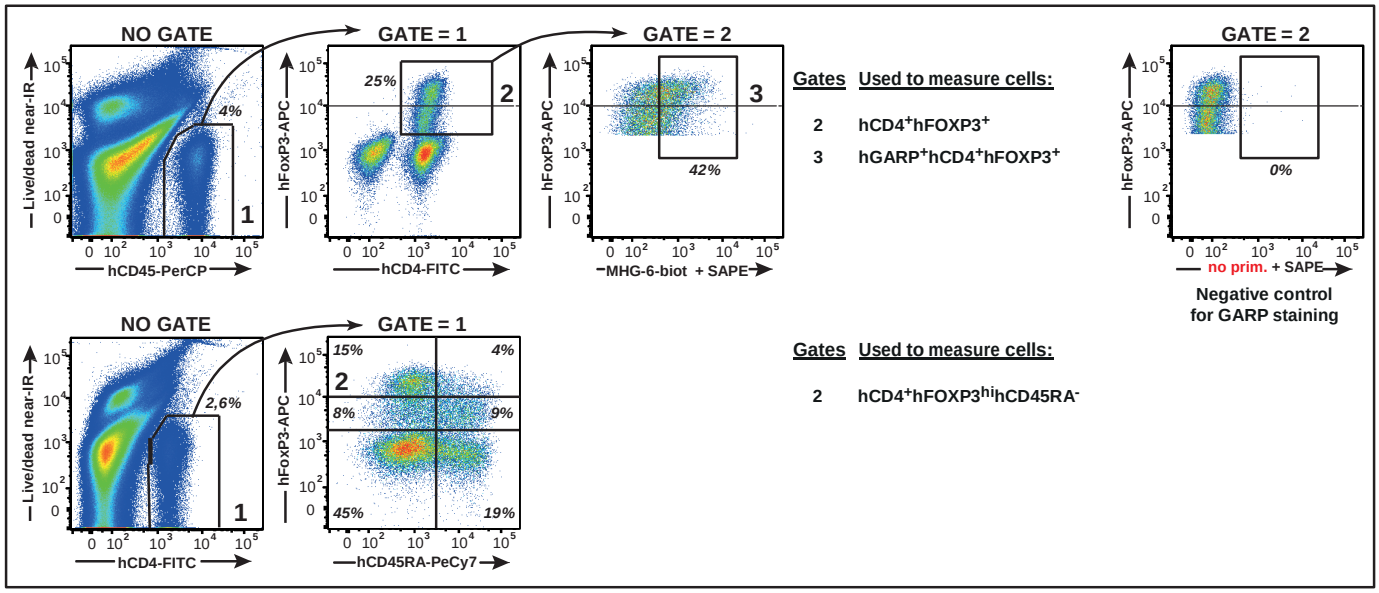
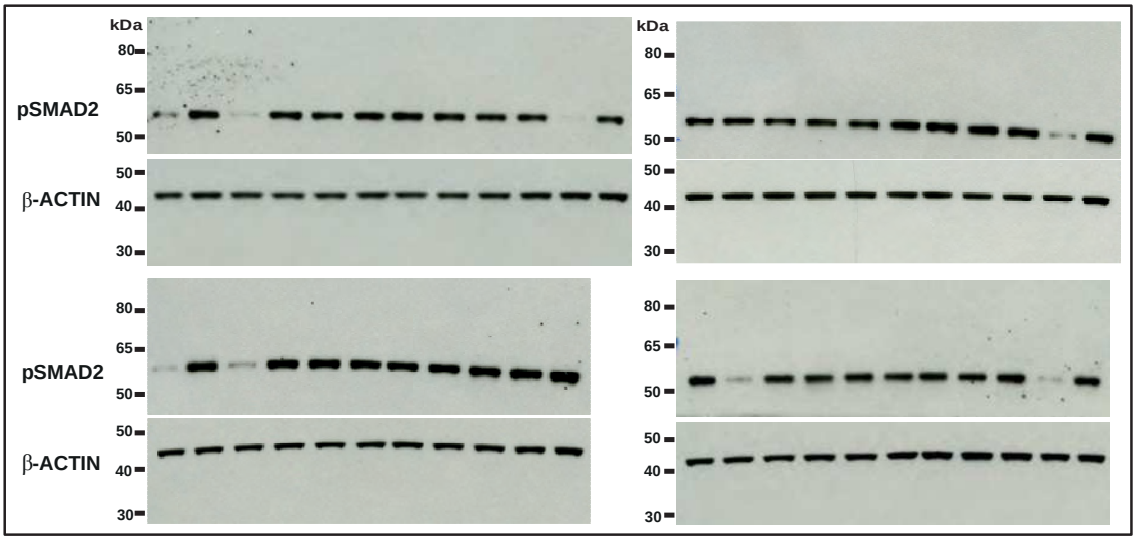


Figure 6D



Supplementary Figure S7. Gating strategies, isotype controls, and representative stains for all flow experiments. One representative sample is shown for each experiment.

Figure 2



Supplementary figure S1

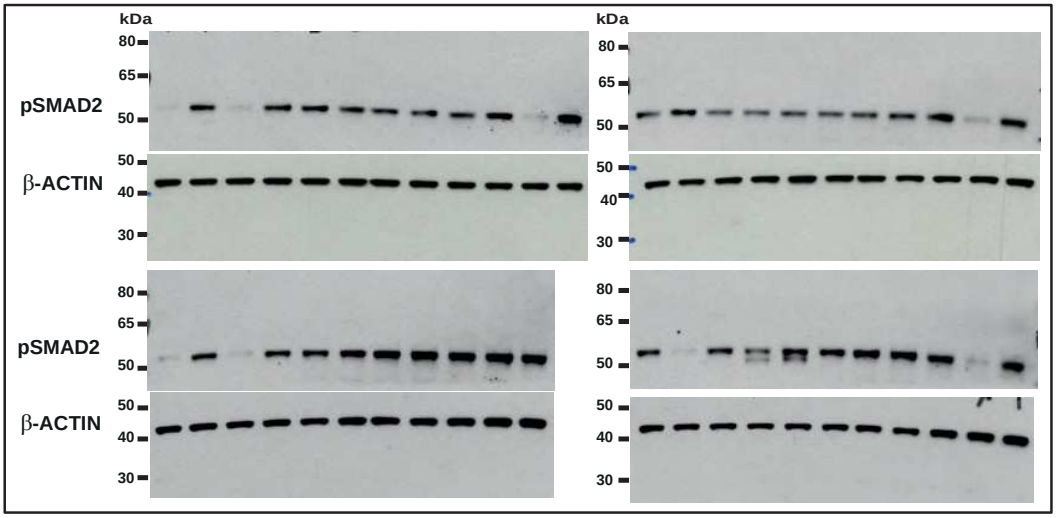


Figure S2

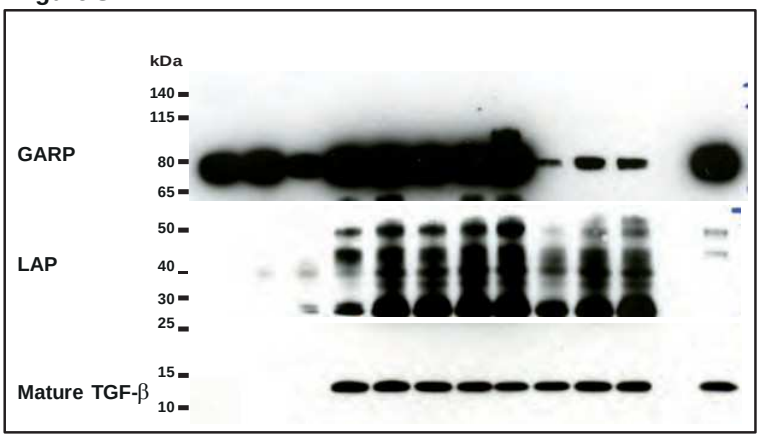
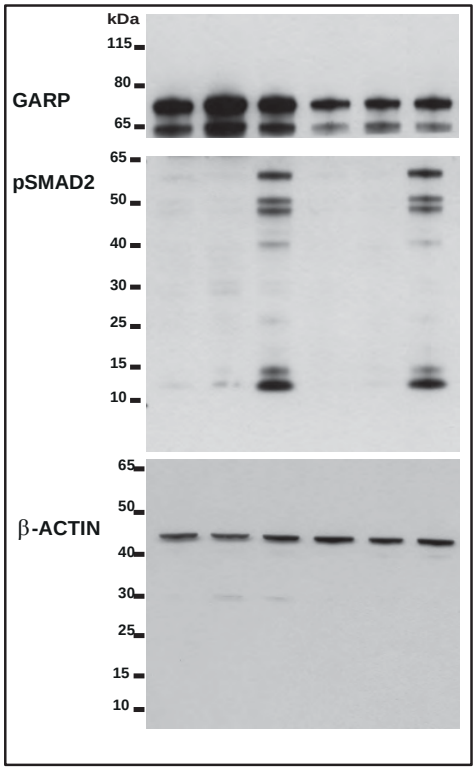


Figure S6



Supplementary Figure S8. Source data for figures showing Western blot analyses.
Uncropped Western blot images used in the figures. Some membranes were cut along horizontal lines in two or three parts before hybridization.

Supplementary Table S1. Purity of polyclonal human T_{regs}.

$CD4^+CD25^+CD127^{lo}$ cells sorted from PBMCs

Donor	% $CD4^+FOXP3^+$ cells by icFACS	% *FOXP3i1*^{demeth} cells by MS-qPCR		Used in
	on day of sorting	on day of sorting	after 12 to 14 days of culture	
A	95%	**101%**	**93%**	**Fig. 5B**
B (sort #1)	93%	**97%**	**81%**	**Fig. 5B**
B (sort #2)	99%	**80%**	**64%**	**Fig. 6A-C**
C	91%	**73%**	**55%**	**Fig. 5B,C**
D	92%	**93%**	**70%**	**Fig. 5B,D**
E	84%	**92%**	**97%**	**Supp. Fig. S1**
F	*not determined*	**84%**	**69%**	**Fig. 6D; Supp. Fig. S5**