SUPPLEMENTARY DATA

Supplementary Table 1. Engraftment of human MSCs in vivo. Tissue distribution of Scr siRNA or TSG-6 siRNA transfected hMSCs ($1X10^6$ cells) at 24 h after intravenous administration by human GAPDH real time RT-PCR. Most of infused cells were trapped in the lung. A small number of human cells homed in the spleen after IV infusion at day 1, however these cells disappeared after 2 days. There was no difference in cell survival of siRNA transfected hMSCs in vivo following injection.

	Scr siRNA transfected hMSCs (n=3)	TSG-6 siRNA transfected hMSCs (n=3)
Lung	82,616 ±7,078	76431 ± 13153.3[†]
Spleen	165.4 ± 39.4	101.0 ± 61[†]
Pancreas	N.D.[‡]	N.D.
PLNs	N.D.	N.D.

[†] Values are mean +/- S.D. Data not significant by T-Test.
[‡]N.D.-not detected.

Supplementary Table 2. TSG-6 inhibits the expression of co-stimulatory molecules on CD11c+ APCs. (a) Control-BM-APCs or (b) TSG-6-BM-APCs were generated in vitro and stimulated with LPS (50ng/ml) in the presence of different concentrations of TSG-6. FACS analysis of APCs generated utilizing antibodies against mouse CD11c, CD80 and CD86. FACS analysis of co-stimulatory molecules expressed in BM-APCs 18 h after LPS activation.

a.

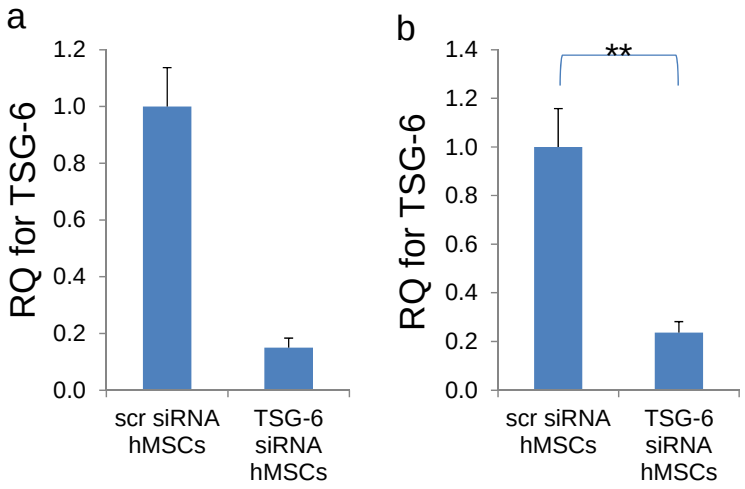
	CD86+ (%)	CD80+ (%)
Control	79.45	85.67
200 ng/ml TSG-6	74.54	84.76
400 ng/ml TSG-6	72.29	81.61

b.

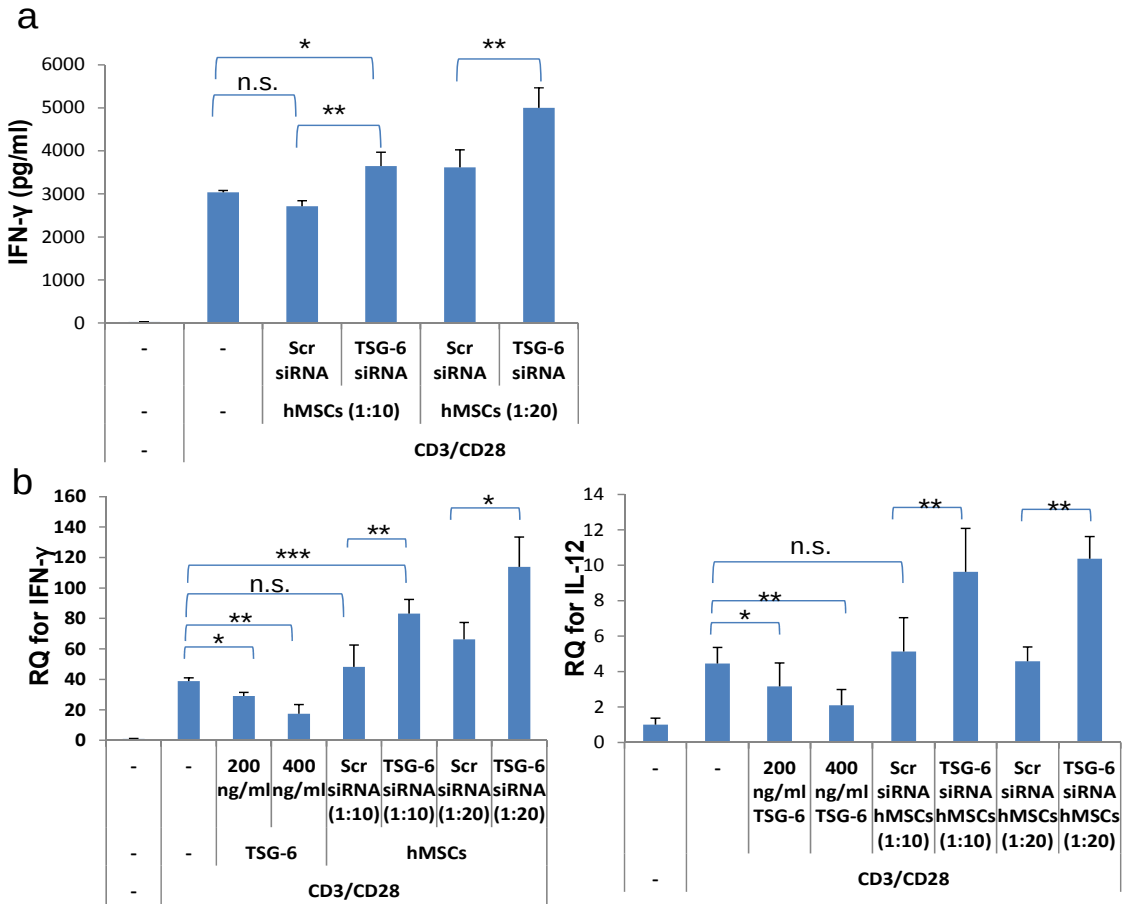
	CD86+ (%)	CD80+ (%)
Control-BM-APCs	84.12	84.23
TSG-6-BM-APCs (40 ng/ml TSG-6)	71.58	80.91
TSG-6-BM-APCs (400 ng/ml TSG-6)	67.12	80.21

SUPPLEMENTARY DATA

Supplementary Figure 1. The efficacy of the siRNA on the expression of TSG-6 in vitro and in vivo. (a) Real-time RT-PCR assays of hMSCs transfected with Scr or TSG-6 siRNA at 24 h and (b) *in vivo* at 24 h after intravenous administration (n=3). Value are means ± S.D. **p<0.001 by two-tailed Student's t-test.

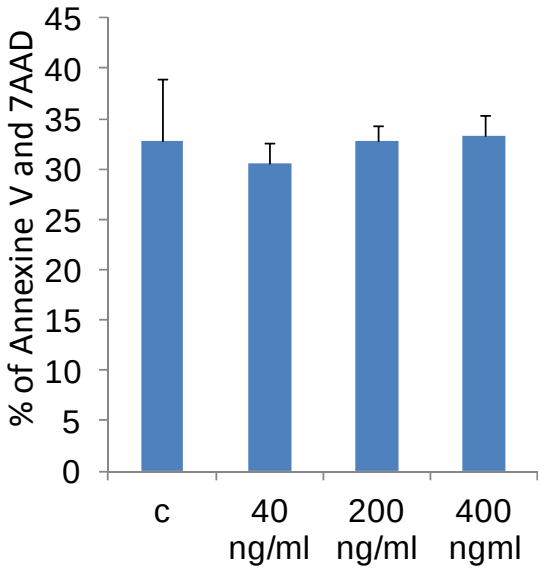


Supplementary Figure 2. hMSCs suppressed Th1 cytokine expression in part by secreting TSG-6 *in vitro*. Th1 expression after 72h (a) and 24 h (b) from splenocyte cultures ($1.0x10^6$/ml) in the presence of TSG-6 or different ratios of hMSC transfected with Scr or TSG-6 siRNA (hMSCs: splenocytes=1: 10 to 1:20).

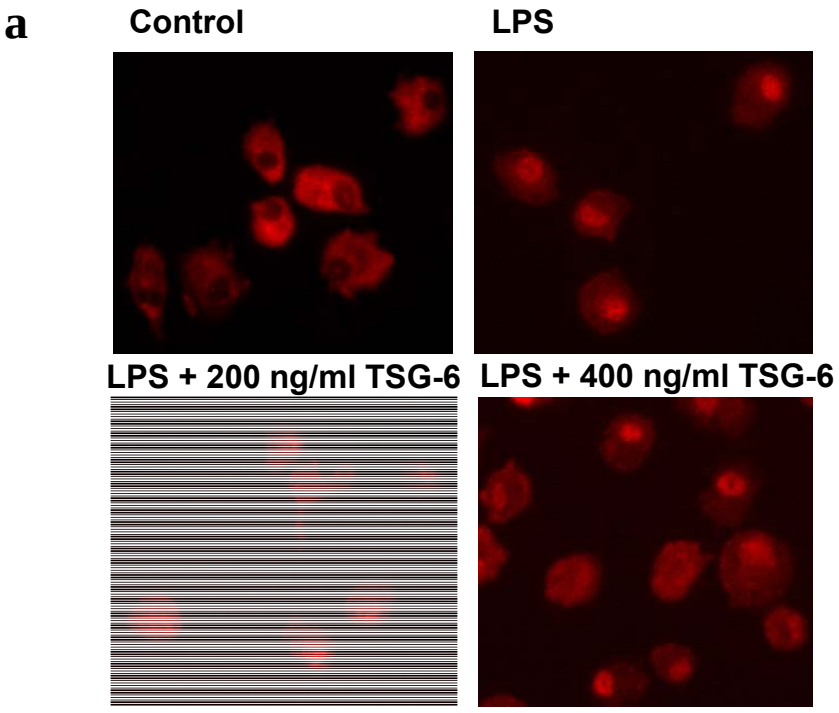


SUPPLEMENTARY DATA

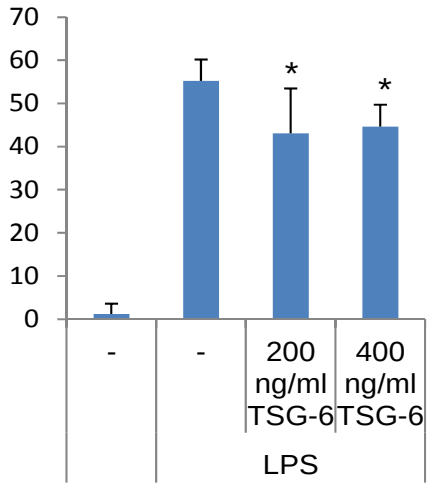
Supplementary Figure 3. TSG-6 inhibition of T cell activation and proliferation is not due to cell death. FACS analysis utilizing Annexin-V and 7-Aminoactinomycin (7-AAD) after 8 wk old female NOD splenocytes ($2.5x10^6$/ml) were activated in a 96-well plate coated with anti-CD3 in the presence of different concentrations of rhTSG-6 for 72 h. Values are means ± S.D.



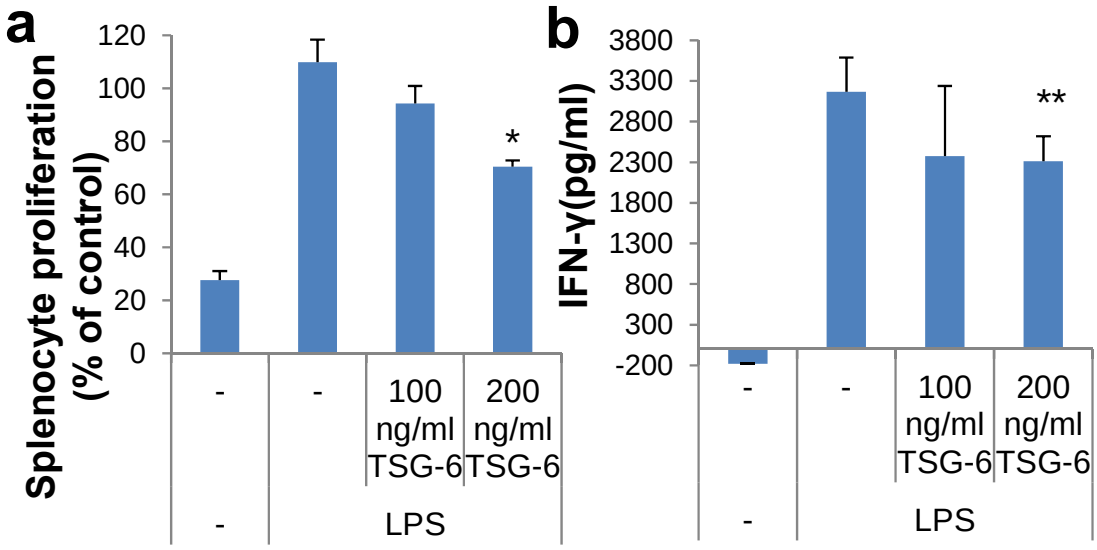
Supplementary Figure 4. TSG-6 Inhibited Nuclear Translocation of NF-κB in APCs. Murine bone marrow derived APCs were incubated with LPS (50 ng/ml) and with or without TSG-6 for 15 min. Representative micrographs of immunocytochemistry are shown for cytoplasmic and nuclear distribution on NF-κB. (b) NF-κB translocation 15 min after activation. Ten arbitrary chosen high-power fields (20x magnification) were captured at random and quantified. Values are means ± S.D. (n=10, *p<0.05 by one-way ANOVA).



b



Supplementary Figure 5. TSG-6 suppressed antigen presenting cell activation *in vitro*. Splenocytes were activated with LPS (100ng/ml) *in vitro* in the presence of TSG-6. (a) MTT assay and (b) IFN-γ expression 72 h after activation. Values are means ± S.D. n=3, *p<0.05 by two-tailed Student's t-test.



SUPPLEMENTARY DATA

Supplementary Figure 6. Schematic diagram. TSG-6, secreted by hMSCs, inhibits Th1 polarization by inhibiting both APC and T cell activation and is capable of driving the differentiation of APCs towards a tolerogenic phenotype, thereby generating regulatory T cells both *in vitro* and *in vivo*.