

Supplemental Material

CTLA-4 Protects against Angiotensin II-Induced Abdominal Aortic Aneurysm Formation in Mice

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Supplemental Table 1

Parameters	Control	CTLA-4-Tg
Body weight, g	27.9 ± 1.6 (n=15)	28.7 ± 1.5 (n=15)
SBP (before), mmHg	103.5 ± 6.6 (n=15)	105.4 ± 4.7 (n=15)
SBP (after), mmHg	134.0 ± 14.1* (n=15)	128.7 ± 25.3* (n=15)
Total-cholesterol, mg/dl	826.3 ± 284.3 (n=19)	815.4 ± 163.9 (n=20)
Triglycerides, mg/dl	45.0 ± 23.6 (n=19)	50.2 ± 26.2 (n=20)

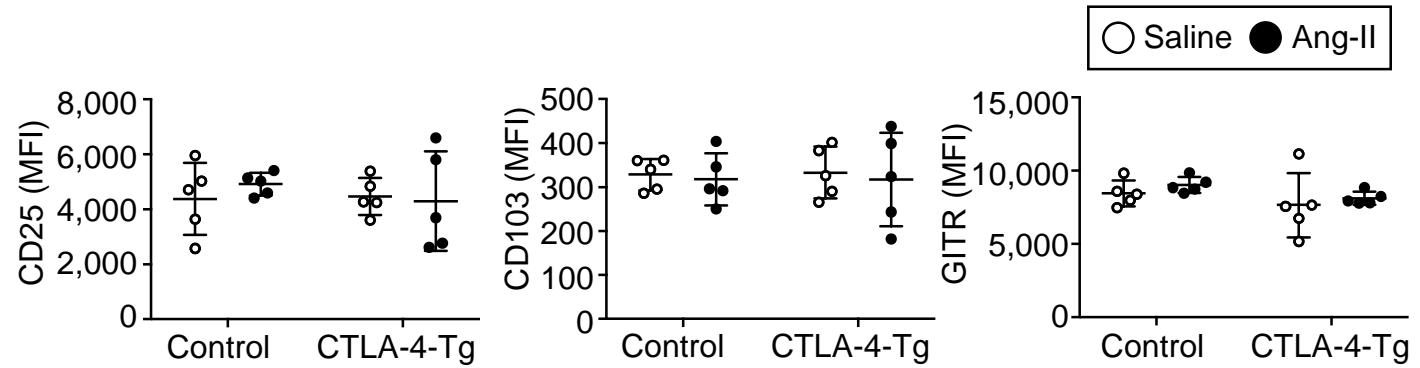
Body weight, SBP, and plasma lipid profile, in 16-week-old control apolipoprotein E-deficient (*ApoE*^{-/-}) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4)-Tg/*ApoE*^{-/-} mice. Data are expressed as mean ± SD. SBP indicates systolic blood pressure; SBP (before), SBP before angiotensin II infusion; SBP (after), SBP after angiotensin II infusion for 4 weeks. **P*<0.05 vs SBP (before) in the same group; paired *t*-test. There are no statistically significant differences in all parameters between angiotensin II-infused *ApoE*^{-/-} and CTLA-4-Tg/*ApoE*^{-/-} mice.

Supplemental Table 2

Genes	Forward primers	Reverse primers
<i>Ifng</i>	CGGCACAGTCATTGAAAGCCTA	GTTGCTGATGGCCTGATTGCT
<i>Ccl2</i>	GCATCCACGTGTTGGCTCA	CTCCAGCCTACTCATTGGGATCA
<i>Il1b</i>	TCCAGGATGAGGACATGAGCAC	GAACGTACACACCAGCAGGTTA
<i>Il6</i>	CCACTTCACAAGTCGGAGGCTTTA	GCAAGTGCATCATCGTTGTTTCATAC
<i>Icam1</i>	CAATTCACACTGAATGCCAGCTC	CAAGCAAGTCCGTCTCGTCCA
<i>Vcam1</i>	TGCCGGCATATACGAGTGTGA	CCCGATGGCAGGTATTACCAAG
<i>Mmp2</i>	GATAACCTGGATGCCGTCGTG	CTTCACGCTCTTGAGACTTTGGTTC
<i>Mmp9</i>	GCCCTGGAACCTCACACGACA	TTGGAAACTCACACGCCAGAAG
<i>Gapdh</i>	TGTGTCCGTCGTGGATCTGA	TTGCTGTTGAAGTCGCAGGAG

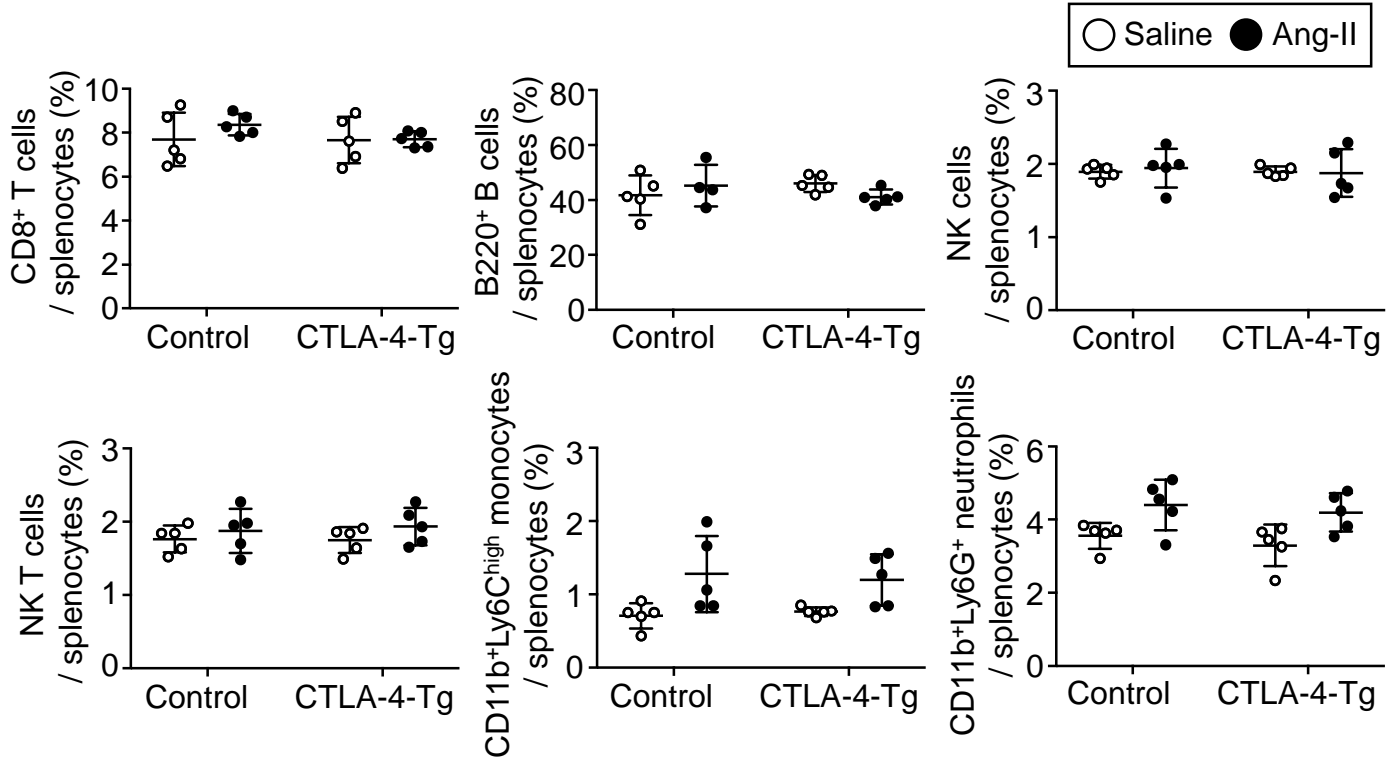
Primer sequences.

Supplemental Figure 1



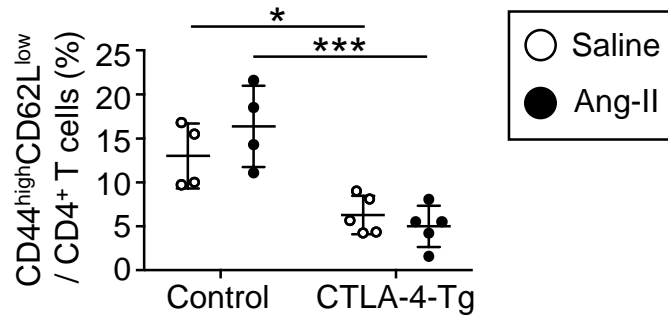
Supplemental Figure 1. The effect of cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) overexpression on Treg-associated markers. Seven days after the pump implantation, apolipoprotein E-deficient (*ApoE*^{-/-}) and CTLA-4-Tg/*ApoE*^{-/-} mice with or without angiotensin II infusion were euthanized and lymphoid cells from spleen were prepared. *ApoE*^{-/-} mice infused with angiotensin II or saline served as controls. The expression levels of Treg-associated markers were analyzed gating on splenic CD4⁺Foxp3⁺ Tregs. Histograms show mean fluorescence intensity (MFI). n=5 per group. Data points represent individual animals. Horizontal bars represent means. Error bars indicate s.d. Ang-II, angiotensin II; GITR, glucocorticoid-induced tumor necrosis factor receptor family-related gene/protein.

Supplemental Figure 2



Supplemental Figure 2. Overexpression of cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) does not affect immune cells other than CD4⁺ T cells in spleen. Seven days after the pump implantation, apolipoprotein E-deficient (*ApoE*^{-/-}) and CTLA-4-Tg/*ApoE*^{-/-} mice with angiotensin II or saline infusion were euthanized and lymphoid cells from spleen were prepared. *ApoE*^{-/-} mice infused with angiotensin II or saline served as controls. Percentages of splenic CD8⁺ T cells, B220⁺ B cells, natural killer (NK) cells, NK T cells, CD11b⁺Ly6C^{high} monocytes, CD11b⁺Ly6G⁺ neutrophils were determined by flow cytometry. n=4 to 5 per group. Data points represent individual animals. Horizontal bars represent means. Error bars indicate s.d. Ang-II, angiotensin II.

Supplemental Figure 3



Supplemental Figure 3. Overexpression of cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) suppresses Teff responses in the para-aortic lymph nodes (LNs) of angiotensin II-infused or saline-infused mice. Seven days after the pump implantation, lymphoid cells from para-aortic LNs were prepared. Apolipoprotein E-deficient (*ApoE*^{-/-}) mice infused with angiotensin II or saline served as controls. The graph represents the percentage of CD44^{high}CD62L^{low} effector T cells within the CD4⁺ population assessed by flow cytometry. n=4 to 5 per group. Data points represent individual animals. Horizontal bars represent means. Error bars indicate s.d. **P*<0.05; ****P*<0.001; one-way ANOVA followed by Tukey's post hoc test. Ang-II, angiotensin II.