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Andrographolide Ameliorates Abdominal Aortic Aneurysm Progression by Inhibiting Inflammatory Cell Infiltration through Downregulation of Cytokine and Integrin Expression

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Running title: Andrographolide Ameliorates Abdominal Aortic Aneurysm

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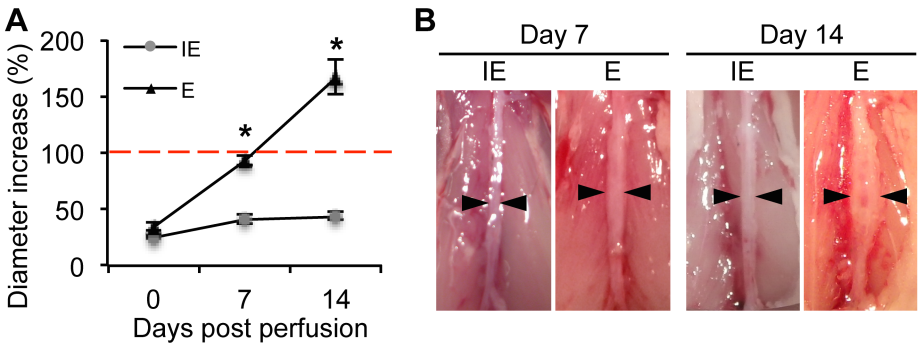
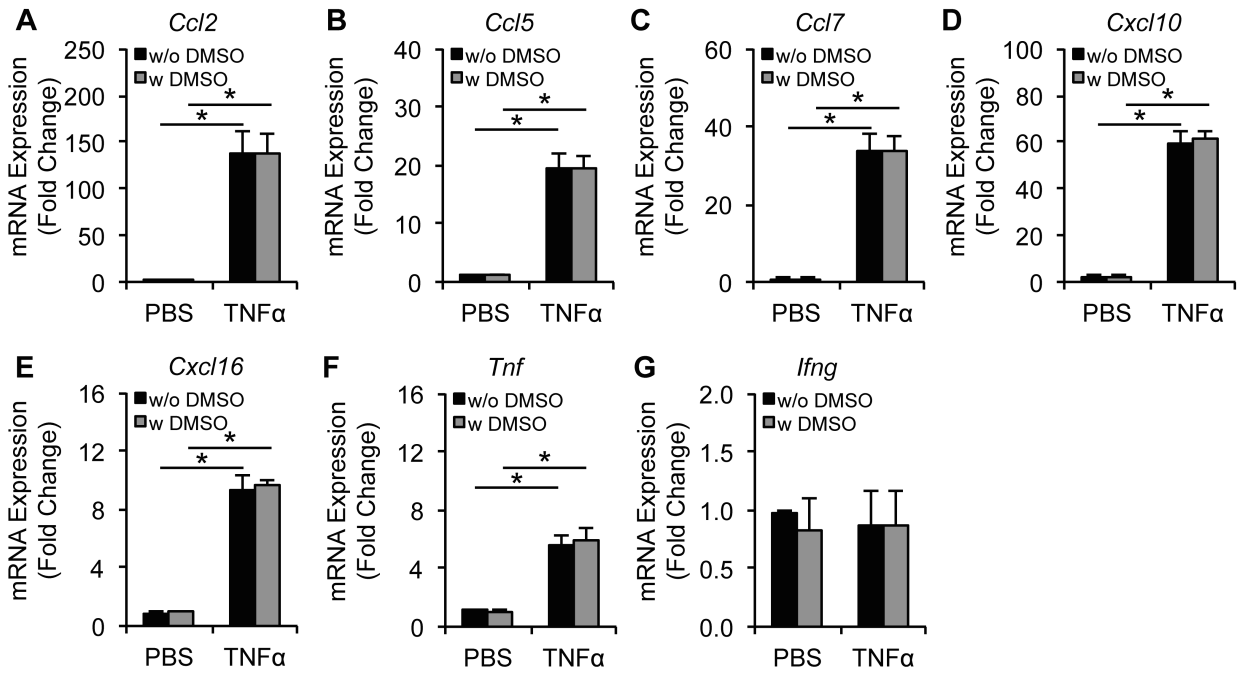
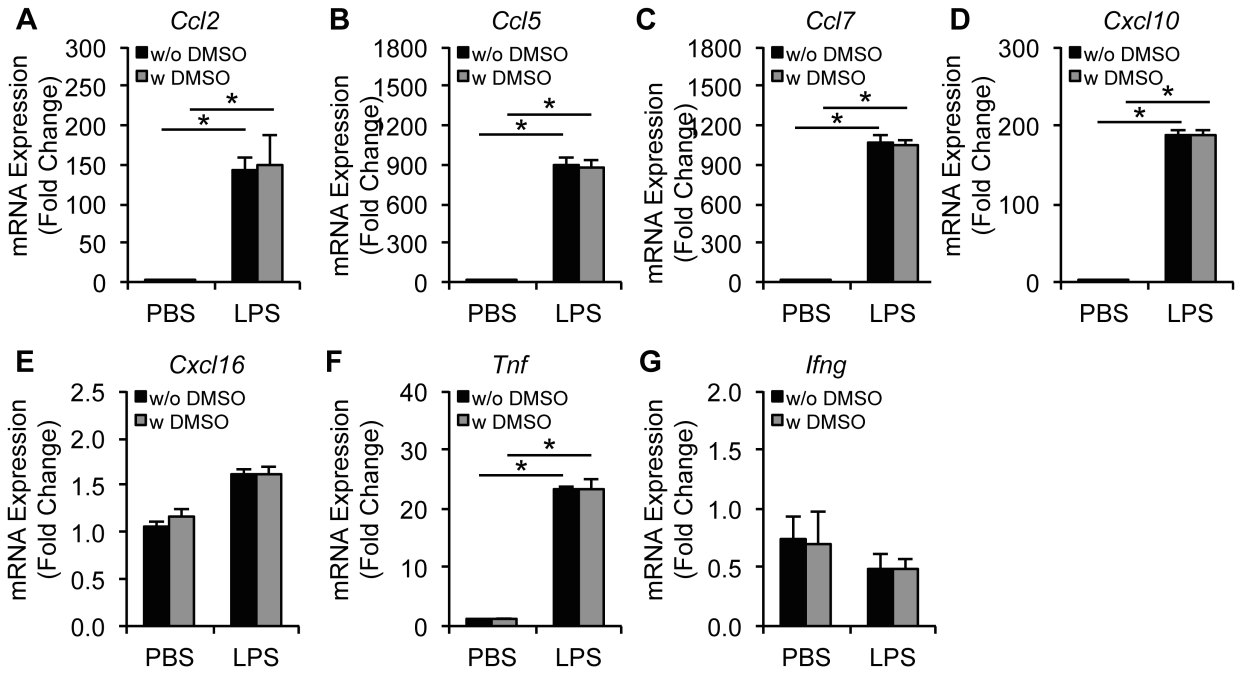
Supplemental Figures



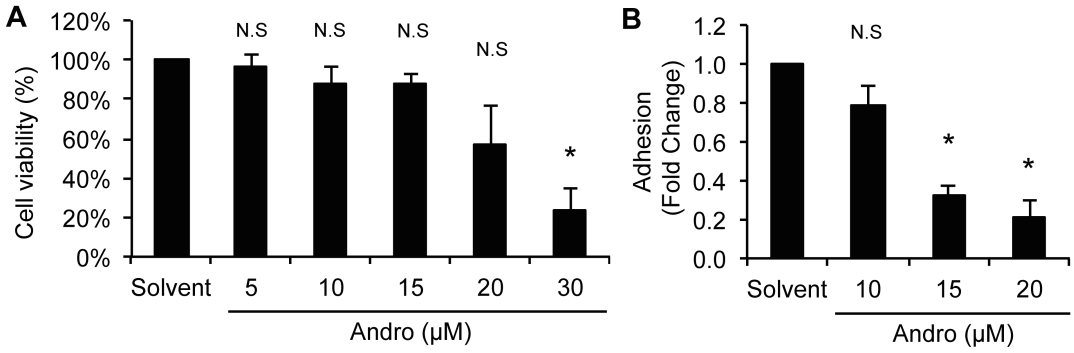
Supplement Figure 1. Elastase-induced mouse abdominal aortic aneurysms (AAAs). **(A)** Diameter increase of elastase (E) or inactivated elastase (IE) perfused mice abdominal aortas. An AAA is defined as a percentage increase in aortic diameter that is equal or greater than 100% (red dashed line). All values represent mean±SEM. n=5-7; * $p<0.05$, two-tailed Student's *t* test. **(B)** Representative photos and diameter increase of abdominal aortas of E- or IE- mice, taken 7 days and 14 days after surgery.



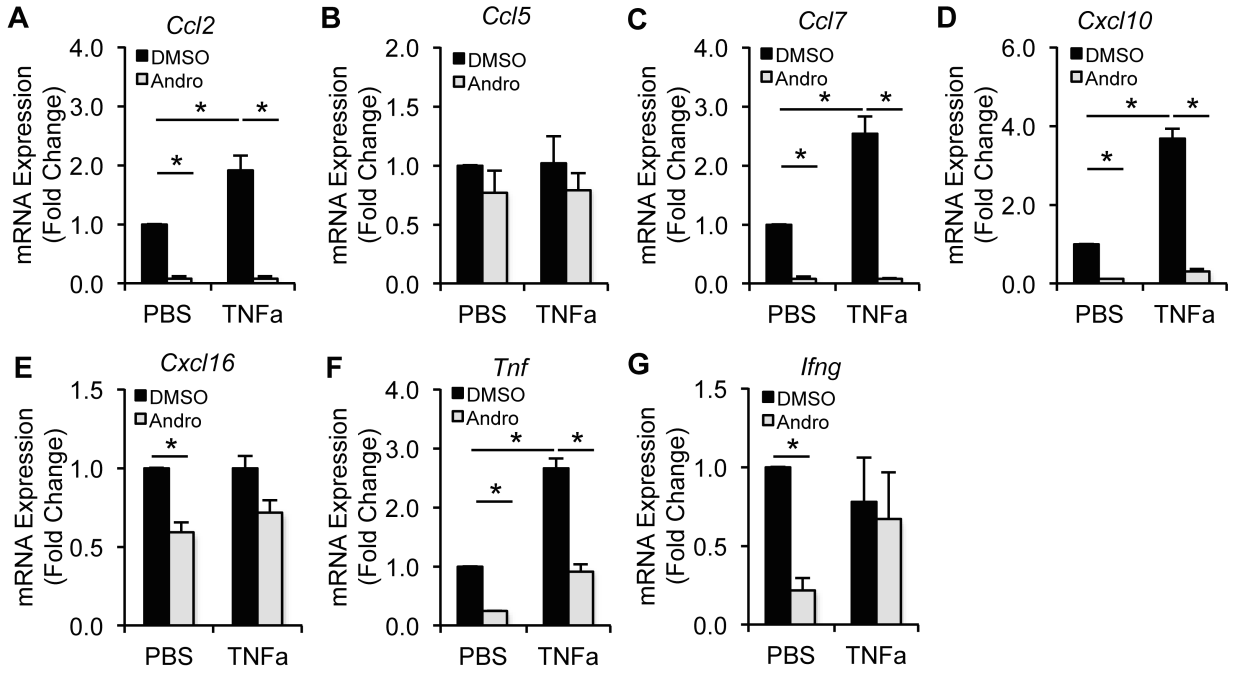
Supplement Figure 2. Effect of DMSO on cytokine production of SMCs. **(A-G)** SMCs were pretreated with or without 0.1% DMSO (the equivalent concentration of DMSO in Andro-treated culture) for 1 h before incubation with TNFα (10 ng/ml) for 6 h. mRNA expression of inflammatory chemokines and cytokines was analyzed by qPCR. All values represent mean±SEM. n=3; * $p<0.05$, one-way ANOVA.



Supplement Figure 3. Effect of DMSO on cytokine production of monocytes/macrophages. **(A-G)** RAW264.7 cells were pretreated with or without 0.1% DMSO (the equivalent concentration of DMSO in Andro-treated culture) for 1 h before incubation with LPS (100 ng/ml) for 6 h. mRNA expression of inflammatory chemokines and cytokines was analyzed by qPCR. All values represent mean±SEM. n=3; * $p<0.05$, one-way ANOVA.



Supplement Figure 4. Dose-responses of Andro in monocytes/macrophages. **(A)** RAW264.7 cells were cultured in the presence of solvent (DMSO) or increasing concentration of Andro for 48h. Cell viability was measured using the CellTiter-Glo® Cell Viability Assay. Cell viability (%) was expressed as a percentage relative to solvent-treated cells. **(B)** RAW264.7 cells were pretreated with solvent (DMSO) or 10 to 20 μM of Andro before seeding onto fibronectin-coated plates. Adherent cells were stained with crystal violet and quantified at OD550 nm. Adhesion ability was expressed as fold change to solvent-treated cells. All values represent mean±SEM. n=3; * $p<0.05$ as compared to solvent-treated cells; N.S, not significant as compared to solvent-treated cells, one-way ANOVA.



Supplement Figure 5. Andro inhibits cytokine expression in monocytes/macrophages. **(A-G)** RAW264.7 cells were pretreated with solvent (DMSO) or Andro (15μM) for 1 h before incubation with TNFα (10 ng/ml) for 6 h. mRNA expression of inflammatory chemokines and cytokines was analyzed by qPCR. All values represent mean±SEM. n=3; * $p<0.05$, one-way ANOVA (A-D&F) and two-tailed Student's *t* test (E&G).