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Supplemental Information

Ablation of Immunoproteasome β5i Subunit Suppresses Hypertensive Retinopathy by Blocking ATRAP Degradation in Mice

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Supplemental data

1. Figures

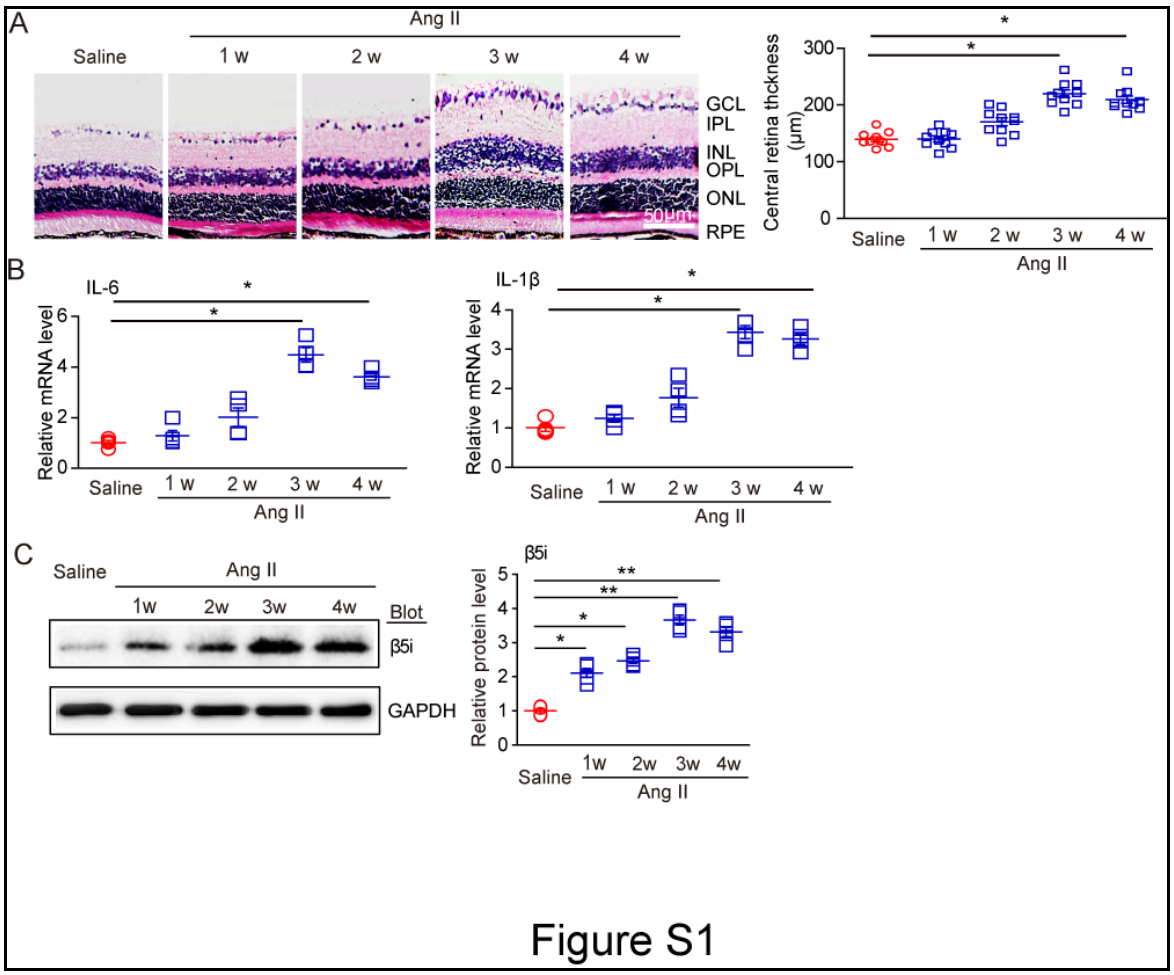

Figure S1

Figure S1. Ang II induces central retinal thickness and proinflammatory cytokine expression at different time points. (**A**) Male WT and β5i KO mice at 10–12-week-old were infused with saline or Ang II at a dose of 3000 ng/kg/minute using ALZET 1004 micro-osmotic pumps for 1-4 weeks. H&E staining of central retinal sections (left) and quantification of retinal thickness (right; n = 5 per group). Scale bar: 50 μm. (**B**) PCR analysis of *IL-6* and *IL-1β* messenger ribonucleic acid (mRNA) levels in the retinas (n = 4 per group). (**C**) Immunoblotting analysis of β5i protein levels in the retinas (n = 4 per group). Data are presented as mean ± SEM. **P* < 0.05, ***P* < 0.01 versus saline control.

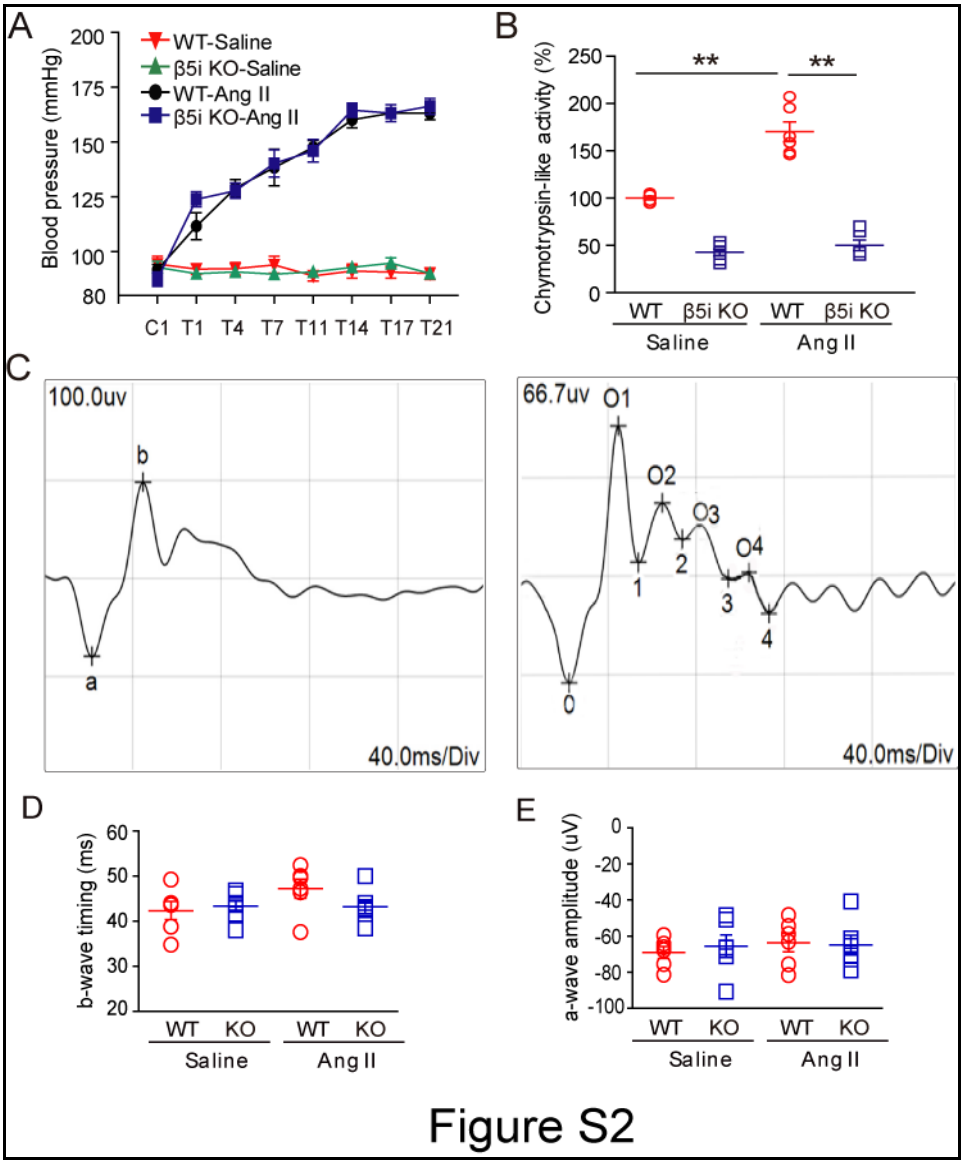

Figure S2. Effect of β5i knockout on average blood pressure and chymotrypsin-like activity. **(A)** WT and β5i KO mice were infused with Ang II at a dose of 3000 ng/kg/minute or saline for 3 weeks. Measurement of average systolic blood pressure (SBP) in each group before (C) and after saline or Ang II treatment (T) period. C1: day 1 before saline or Ang II treatment, T1: day 1 after saline or Ang II treatment etc (n=10 per group). **(B)** The chymotrypsin-like activity of retinas in each group after saline or Ang II treatment (n=6 per group). (**C**) A representative ERG waveform (left) and OPs (right) wave from original data. (**D**) Quantification of b-wave timing (n = 6 per group). (**E**). Quantification of b-wave amplitude (n = 6 per group). Results are the mean ± SEM. ***P* < 0.01 versus WT mice with saline or Ang II infusion.

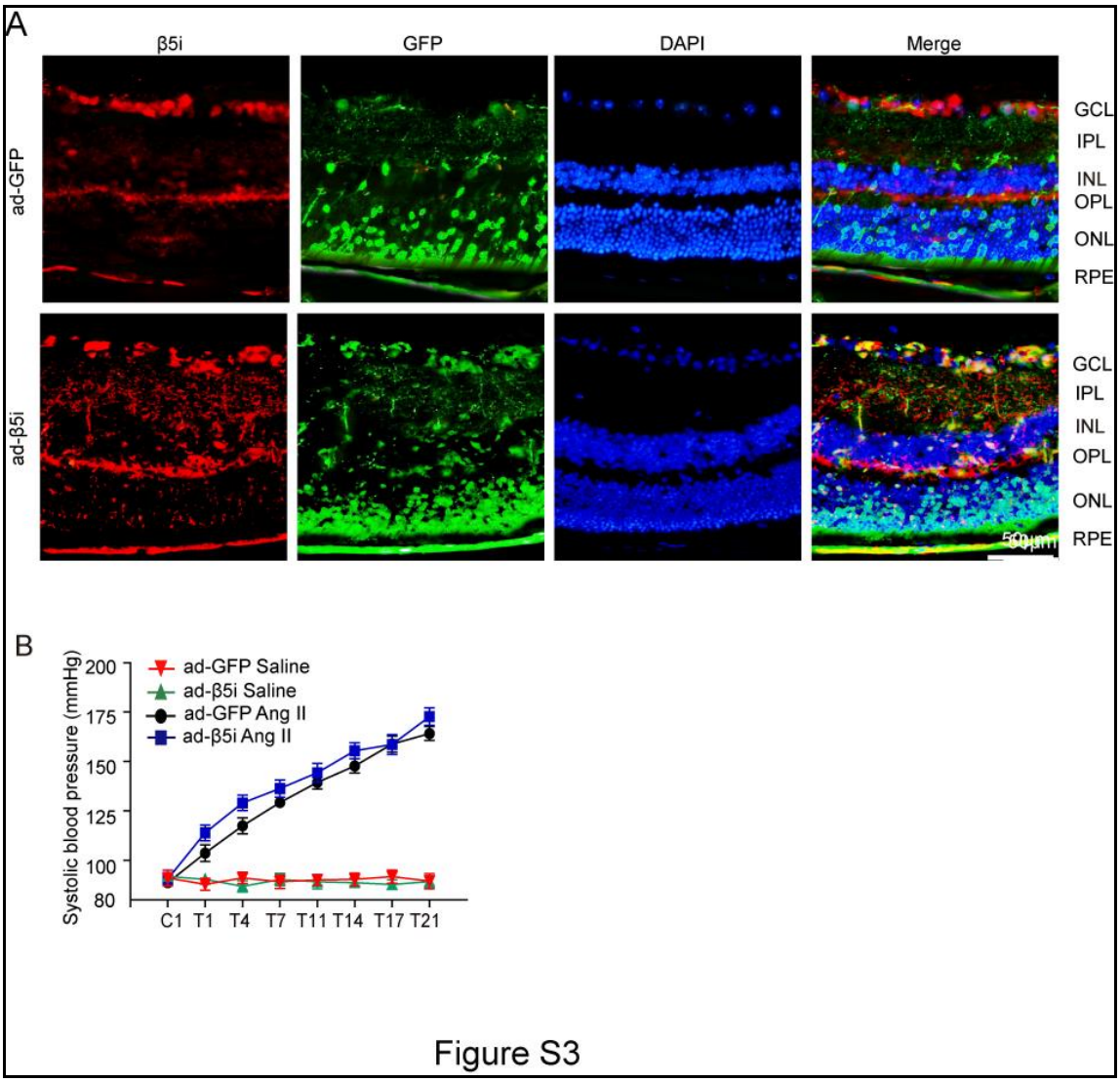

Figure 3. Effect of β5i overexpression on systolic blood pressure in mice. (**A**) WT mice were locally injected with Ad-β5i or Ad-GFP at a dose of 1.2×10^{12} pfu/ml and then infused with Ang II (3000 ng/kg/minute) for 3 weeks. Evaluation of GFP fluorescence and immunostaining of β5i expression were performed 3 days after second injection. Nuclei were counterstained with DAPI (blue). Scale bar, 50 μm. **(B)** Average systolic blood pressure (SBP) in Ad-GFP-injected mice and Ad-β5i-injected mice before (C) and after saline or Ang II treatment (T) period. C1: day 1 before saline or Ang II treatment, T1: day 1 after saline or Ang II treatment etc (n=9 per group). Data are the mean ± SEM.

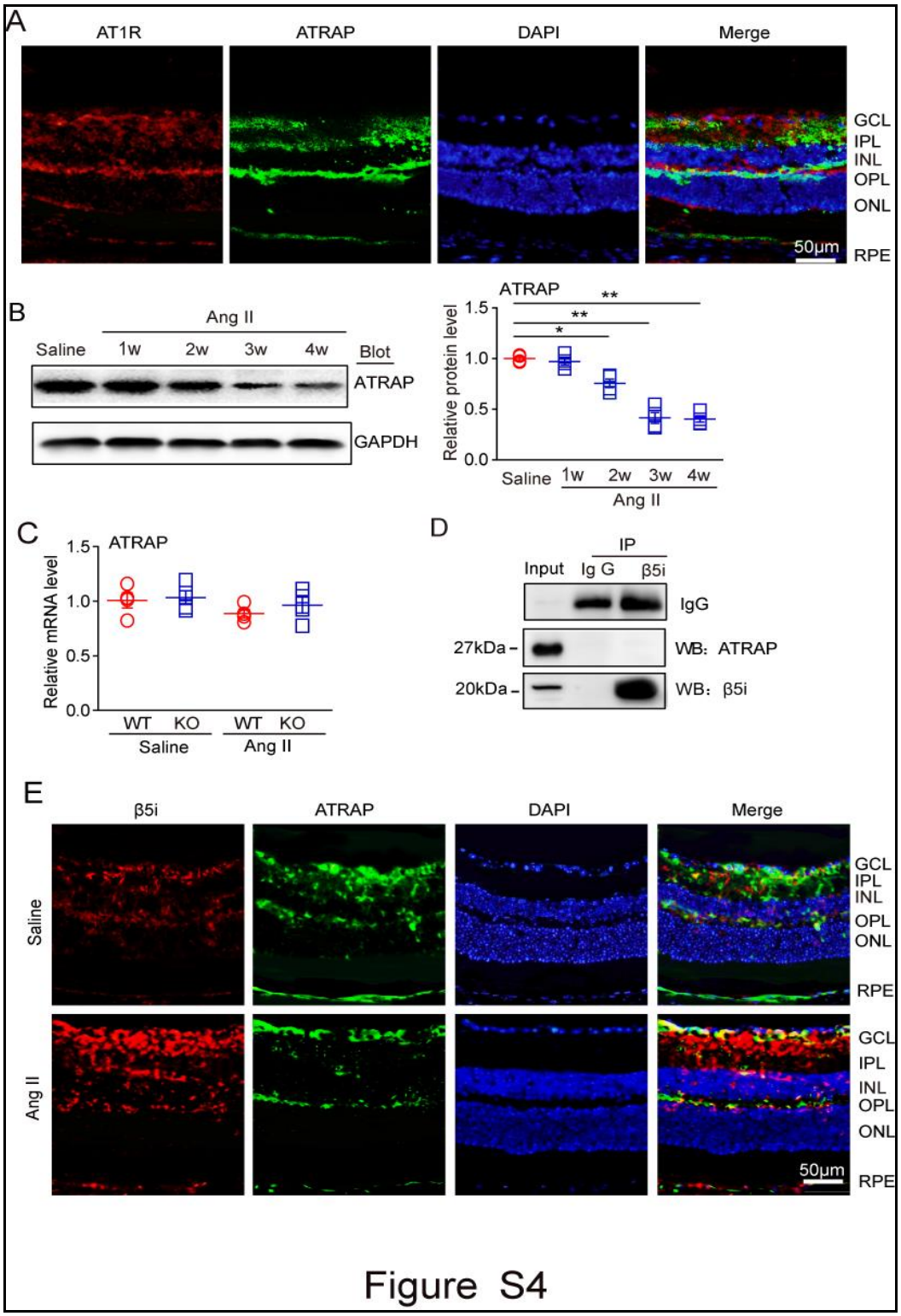

Figure 4. Expression patterns of AT1R, ATRAP and β5i in the retinal sections. (**A**) Immunostaining of the expression of endogenous AT1R and ATRAP proteins in the central retinal sections with antibody against anti-AT1R and anti-ATRAP. Scale bar: 50 μm. (**B**) PCR analysis of *ATRAP* mRNA levels in the retinas from WT and β5i KO mice after saline or Ang II infusion (n=4 per group). (**C**) Immunoprecipitation (IP)

was performed in retinal lysates with IgG control or anti-β5i antibody, and analyzed by western blot (WB) with antibody to detect endogenous ATRAP or β5i. (**D**) Immunostaining of the expression of endogenous β5i and ATRAP proteins in the central retinal sections with antibody against anti-β5i and anti-ATRAP. Scale bar: 50 μm. *$P < 0.05$, **$P < 0.01$, vs. saline control mice.

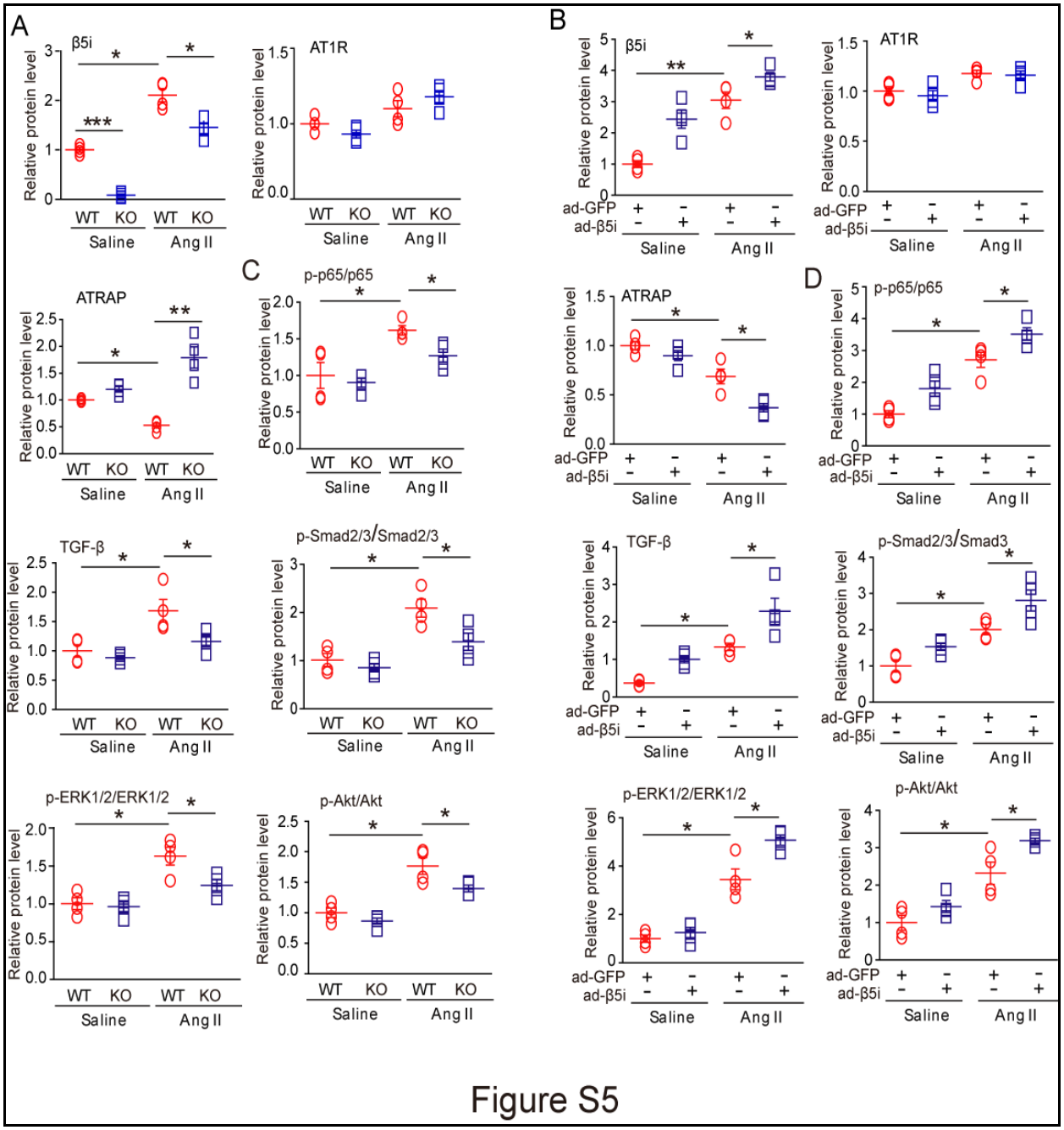

Figure S5. Quantification of corresponding protein bands. (A) Quantification of β5i, AT1R, ATRAP, p-p65, p65, TGF-β, Smad2/3, p-Smad2/3, ERK1/2, p-ERK1/2, Akt and p-Akt protein levels in the retinas from WT and β5i KO mice infused with

saline or Ang II (3000 ng/kg/minute) or saline for 3 weeks (n = 4 per group). **(B)** Quantification of β5i, AT1R, ATRAP, p-p65, p65, TGF-β, Smad3, p-Smad2/3, ERK1/2, p-ERK1/2, Akt, and p-Akt protein levels in the retinas from WT mice locally injected with Ad-β5i or Ad-GFP infused with saline or Ang II (3000 ng/kg/minute) or saline for 3 weeks (n = 4 per group). Data are presented as the mean ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs. saline control or Ang II-infused WT or ad-GFP-injected WT mice.

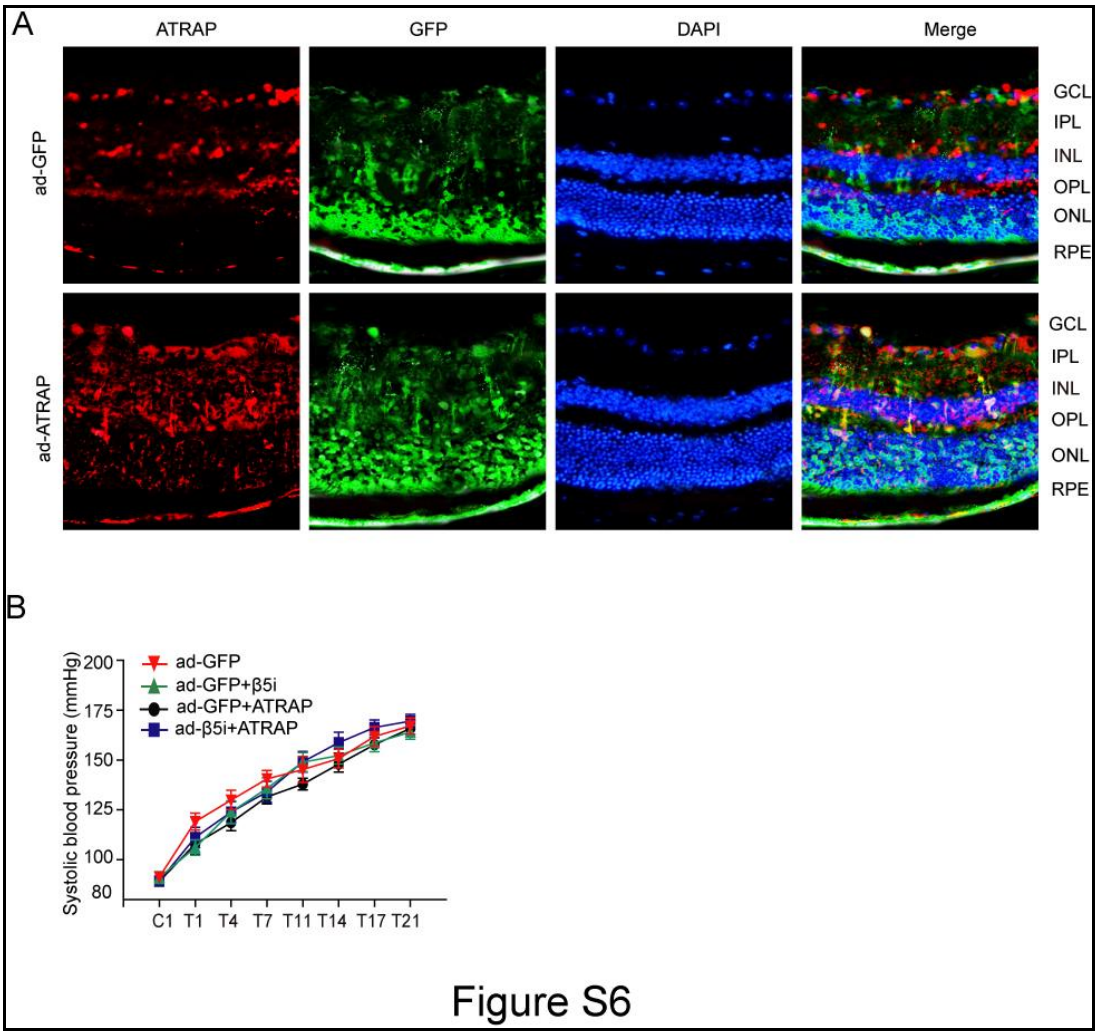

Figure S6. Effect of β5i and ATRAP overexpression on systolic blood pressure in mice. (A) WT mice were locally injected with Ad-GFP, Ad-β5i, or Ad-ATRAP at a dose of 1.2×10^{12} pfu/ml and then infused with Ang II (3000 ng/kg/minute) for 3 weeks. Evaluation of GFP fluorescence and immunostaining of ATRAP expression were performed 3 days after second injection. Nuclei were counterstained with DAPI

(blue). Scale bar, 50 μm. **(B)** Average systolic blood pressure (SBP) in WT mice injected with Ad-GFP, Ad-GFP+β5i, Ad-GFP+ATRAP and Ad-β5i+ATRAP before (C) and after saline or Ang II treatment (T) period. C1: day 1 before saline or Ang II treatment, T1: day 1 after saline or Ang II treatment etc (n=10 per group). Data are the mean ± SEM.

Supplemental Methods

Table S1. Sequence of the primers used in the quantitative real-time PCR assay

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
β1i	CTGGAGCTACACGGGTTGGA	ATATACCTGTCCCCCCTCACATT
β2i	CAGCCGTCTGCCCTTTACTG	AGAGCCCAGGTCACTCAGGAT
β5i	CTTGGCACCATGTCTGGTTGT	CCGGTACTGCAGCATCATGT
β1	CCAATCGAGTGACTGACAAGCT	GGACTAGTGGAGGCTCGTTCA
β2	AGGCCAGATATGGAGGAGGAA	GGGCACTGAGAATGGACGAA
β5	TGCTCGCTAACATGGTGTATCAGTA	AGCCAGAGCCCACTGAGAAG
NOX1	CAGTTATTCATATCATTGCACACCTATTT	CAGAAGCGAGAGATCCATCCA
NOX4	GCACGCTGTTGATTTTTATGG	GCGAGGCAGGAGAGTCAGTA
ph22phox	CTCCTCTTCACCCTCACTCG	GTGGACTCCCATTGAGCCTA
IL-1β	CTTCCCCAGGGCATGTTAAG	ACCCTGAGCGACCTGTCTTG
IL-6	TTCCATCCAGTTGCCTTCTTG	TTGGGAGTGGTATCCTCTGTGA
ATRAP	CGTTGGAACTGGCGCAAC	ACCAGGAGAATAACCTGAGCG
GAPDH	GTGTTTCCTCGTCCCGTAGA	AATCTCCACTTTGCCACTGC