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Supplemental Information

Suppression of Choroidal Neovascularization and Fibrosis by a Novel RNAi Therapeutic Agent against (Pro)renin Receptor

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Supplemental information

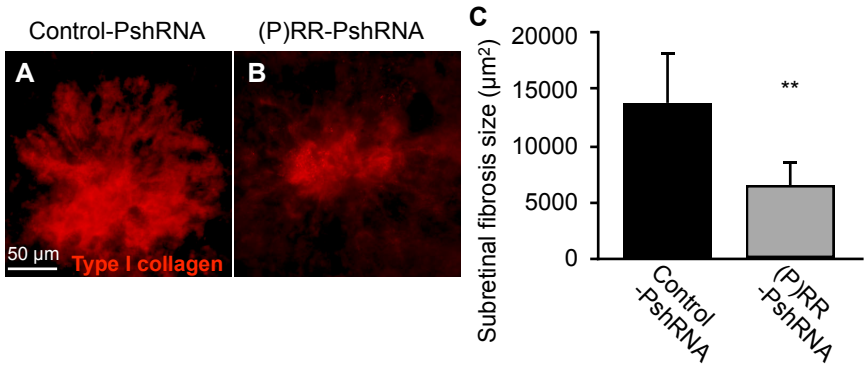

Figure S1. Suppression of subretinal fibrosis at 21 days after laser photocoagulation by (P)RR-PshRNA

(A, B) Representative micrographs of subretinal fibrosis lesions (type I collagen, *red*) in the RPE-choroid flat mounts at post-laser day 21 from mice treated with 100 pmol control-PshRNA (A) or (P)RR-PshRNA (B). Scale bar, 50 μm. (C) Quantification analysis of the size of subretinal fibrosis [control-PshRNA = 13,695 ± 4,566 μm², (P)RR-PshRNA = 6,381 ± 2,236 μm²]. $^{**}p < 0.01$ (n = 5).

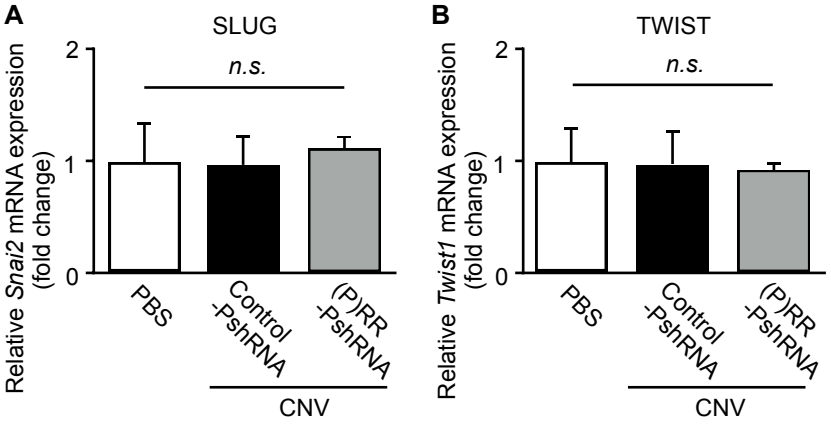

Figure S2. Neither SNAI2 nor TWIST1 is associated with CNV induction in mice

(A, B) Relative mRNA expression levels of *Snai2* (A) and *Twist1* (B) in the RPE-choroid complex from untreated normal mice (control) and CNV mice treated with 100 pmol control-PshRNA or (P)RR-PshRNA. n.s., not significant (n = 6).

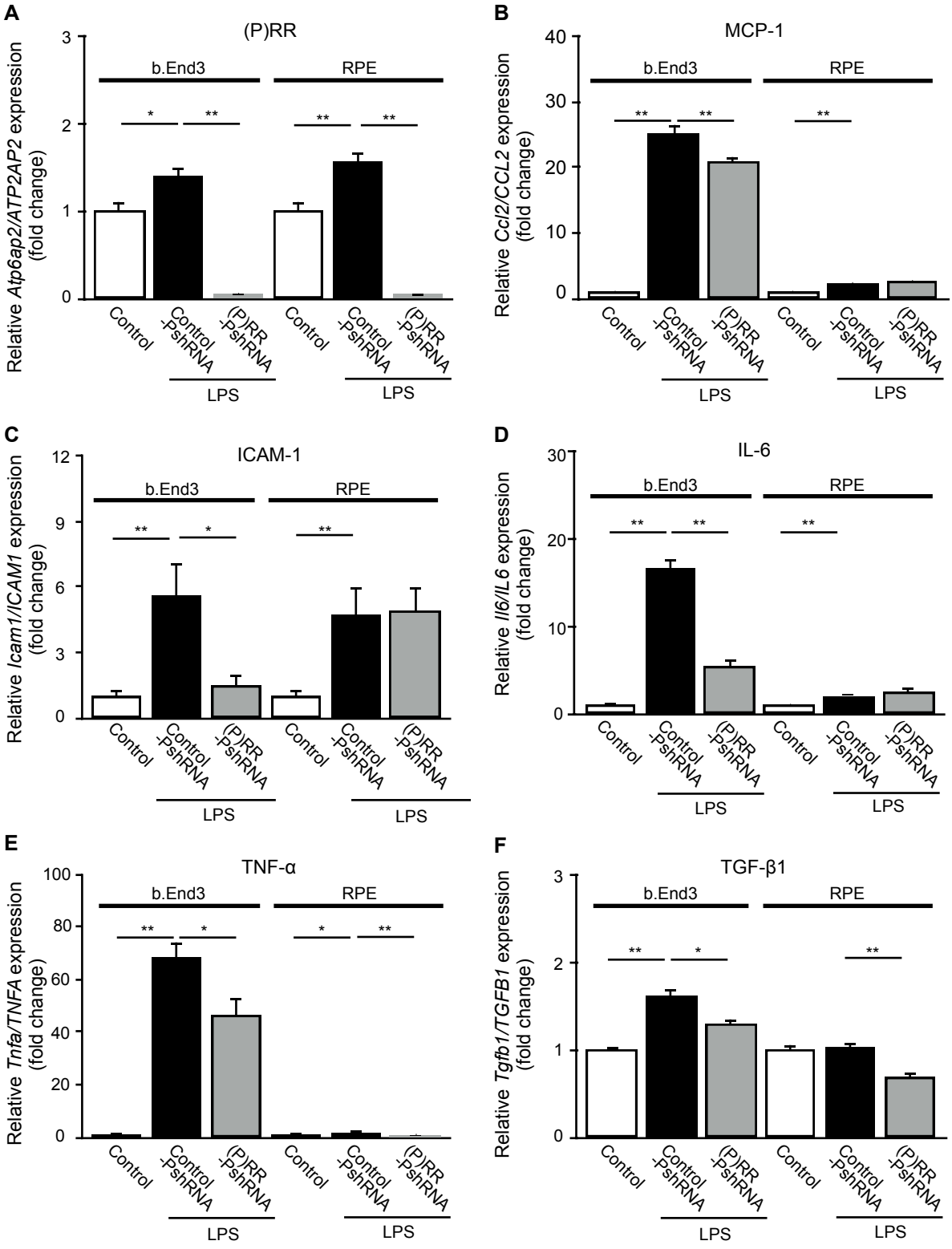

Figure S3. Blockade of inflammatory responses by (P)RR-PshRNA in LPS-stimulated endothelial cells and RPE cells

(A-E) Gene expression levels of inflammatory molecules *Atp6ap2/ATP6AP2* (A), *Ccl2/CCL2* (B), *Icam1/ICAM1* (C), *Il6/IL6* (D), *Tnfa/TNFA* (E) and profibrotic cytokine *Tgfb1/TGFB1* (F) in 1 nM control-PshRNA or (P)RR-PshRNA transfected mouse microvascular endothelial cells stimulated with 10 ng/ml LPS for 8 hours and 1 nM control-PshRNA or (P)RR-PshRNA transfected human RPE cells stimulated with 10 ng/ml LPS for 12 hours. $*p < 0.05$, $**p < 0.01$ (n = 6).

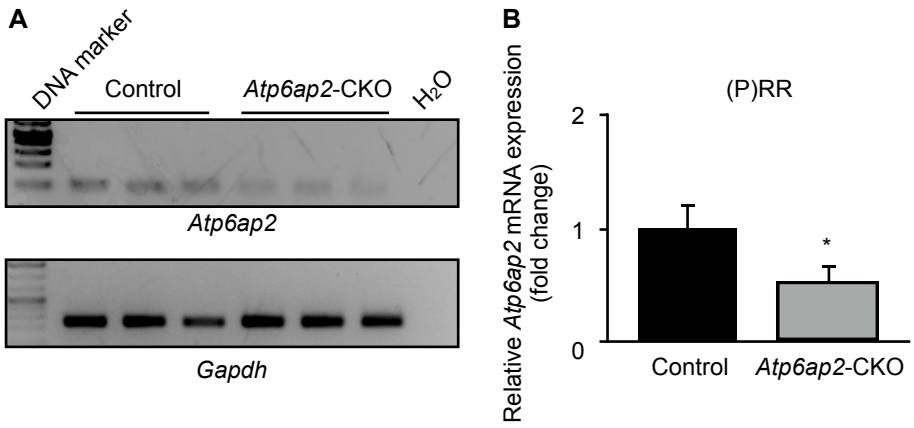

Figure S4. Gene expression of *(P)RR/Atp6ap2* in the RPE-choroid complex of *(P)RR*/*Atp6ap2*-CKO mice

(A) Gene expression analysis of *(P)RR/Atp6ap2* in the RPE-choroid complex of control and *(P)RR*/*Atp6ap2*-CKO mice. *Gapdh* was used as an internal control. (B) Quantification analysis of the relative expression of *(P)RR*/*Atp6ap2*. **p* < 0.05 (n = 3).

Table S1. Primer sequences used in quantitative RT-PCR

Target gene	Sequence
Mouse	
(P)RR/Atp6ap2	forward 5'- CCTCATTAGGAAGACAAGGACTATCC -3'
	reverse 5'- GGGTTCTTCGCTTGTTTTGC -3'
Ccl2	forward 5'- TTGGCTCAGCCAGATGCA -3'
	reverse 5'- CCTACTCATTGGGATCATCTTGC -3'
Icam1	forward 5'- CCTGTTTCCTGGCTCTGAAG -3'
	reverse 5'- GTCTGCTGAGACCCCTCTTG -3'
Il6	forward 5'- CACAGAGGATACCACTCCCAACA -3'
	reverse 5'- TCCACGATTTCCCAGAGAAACA -3'
Tnfa	forward 5'- GGTGCCTATGTCTCAGCCTCTT -3'
	reverse 5'- CGATCACCCCGAAGTTCAGTA -3'
Emr1	forward 5'- CTTTGGCTATGGGCTTCCAGTC -3'
	reverse 5'- GCAAGGAGGACAGAGTTTATCGTG -3'
Tgfb1	forward 5'- CAGTGGCTGAACCAAGGAGAC -3'
	reverse 5'- ATCCCGTTGATTTCCACGTG -3'
Acta2	forward 5'- TCTGTAAGGCCGGCTTTGC -3'
	reverse 5'- TGTCCCATTCCCACCATCA -3'
Col1a1	forward 5'- TGACTGGAAGAGCGGAGAGT -3'
	reverse 5'- GACGGCTGAGTAGGGAACAC -3'
Fn1	forward 5'- GTCAGTGTCTCCAGTGTCTAC -3'
	reverse 5'- TGGCTTGCTGGCCAATCAGT -3'
Snai1	forward 5'- CACACGCTGCCTTGTGTCT -3'
	reverse 5'- GGTCAGCAAAAGCACGGTT -3'
Snai2	forward 5'- CAGCGAACTGGACACACACA -3'
	reverse 5'- ATAGGGCTGTATGCTCCCGAG -3'
Twist1	forward 5'- GGACAAGCTGAGCAAGATTCA -3'
	reverse 5'- CGGAGAAGGCGTAGCTGAG -3'
Gapdh	forward 5'- AGGTCGGTGTGAACGGATTTG -3'
	reverse 5'- TGTAGACCATGTAGTTGAGGTCA -3'
Human	
(P)RR/ATP6AP2	forward 5'- AGGCAGTGTCATTTCGTACC -3'
	reverse 5'- GCCTTCCCTACCATATACACTC -3'
CCL2	forward 5'- CGCCTCCAGCATGAAAGTCT -3'
	reverse 5'- ATGAAGGTGGCTGCTATG -3'
ICAM1	forward 5'- GCAAGCTCCCAGTGAAATGCAAAC -3'
	reverse 5'- TGTCTACTGACCCCAACCCTTGATG -3'
IL6	forward 5'- CCACTCACCTCTTCAGAACG -3'
	reverse 5'- CATCTTTGGAAGGTTCAGGTTG -3'
TNFA	forward 5'- ACTTTGGAGTGATCGGCC -3'
	reverse 5'- GCTTGAGGGTTTGCTACAAC -3'
TGFB1	forward 5'- GCCCTGGACACCAACTATTG -3'
	reverse 5'- CGTGTCCAGGCTCCAAATG -3'
GAPDH	forward 5'- CCTGGCCAAGGTCATCCATG -3'
	reverse 5'- GGAAGGCCATGCCAGTGAGC -3'