Supplement

Mouse Genotyping

Genotyping was performed on ear tissue samples obtained at weaning. Ear tissue was lysed with 0.3 mg/mL proteinase K (Sigma-Aldrich, St. Louis, MO). Normalized to weight, pre-cleared ear lysate solution was added directly to the PCR. A hot-start mix (KAPA2G Robust HotStartReadyMix; KapaBiosystems, Shanghai, China) was used in PCRs, run for 35 cycles at annealing temperatures of 65°C for primers directed against the sense and antisense strands (Table S1). PCR products were resolved by gel electrophoresis (2% agarose) in the presence of DNA gel stain (SYBR Safe; Invitrogen, Shanghai, China). Genotyping results of the DKO mice are shown in Figure S1.

Table -S1 PCR primers

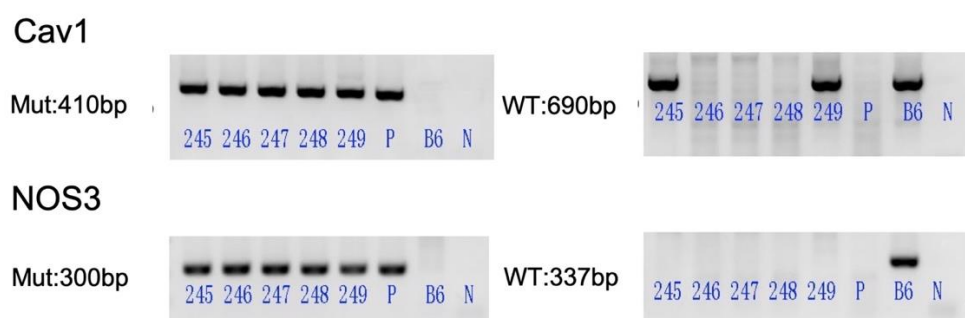| Gene	Primer sequence	Note
Cav1	CTAGTGAGACGTGCTACTTCC	Mut:410bp
	CTTGAGTTCTGTTAGCCCAG	
	GTGTATGACGCGCACACCAAG	Wt:690bp
	CTTGAGTTCTGTTAGCCCAG	
NOS3	CTTGTCCCCTAGGCACCTCT	Mut:300bp
	AATTCGCCAATGACAAGACG	
	AGGGGAACAAGCCCAGTAGT	Wt:337bp
	CTTGTCCCCTAGGCACCTCT	

Figure S1 Genotyping of a typical litter of pups in NOS3 and Cav1 double knockout mice by PCR analyzed using electrophoresis of a stained agarose gel. In this case, three animals were found to have the deletion of the wild type Cav1 and NOS3 gene and expressed the mutant genes (246, 247, 248). Positive control, negative controls and

blank control for the PCR are indicated by P, N and B6 above the three lanes. WT, wild type allele; Mut, mutant allele.

Dose-response IOP data and statistical analysis

IOP data of four strains of mice in response to a NO donor and two NOS inhibitors are in Figure S2-S4 (each data point represents mean IOP of three measurements of an individual mouse). IOP data, percentage of IOP change and statistical analysis is summarised in Table S2.
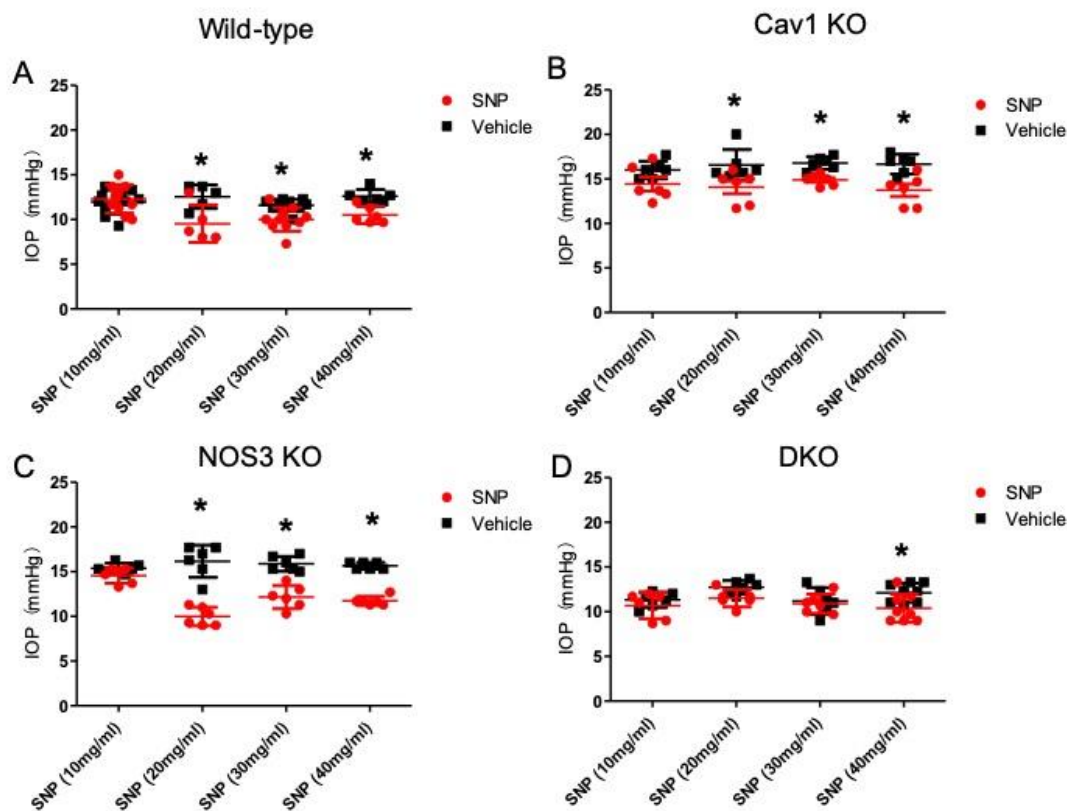

Figure S2: IOP measurements for SNP-treated wild-type mice, Cav1 KO, NOS3 KO and DKO mice. A) Wild-type animals: 20 mg/mL (n=5), 30 mg/mL (n=10), and 40 mg/mL (n=6) SNP significantly decreases IOP, whereas 10 (n=10) mg/mL does not. B) Cav1 KO: SNP significantly reduces IOP in Cav1 KO mice at concentrations of 20 mg/mL (n=6), 30 mg/mL (n=6), and 40mg/mL (n=6); 10 mg/mL (n=6) has no significant effect on IOP. C) NOS3 KO: SNP significantly reduces IOP in NOS3 KO mice at concentrations of 20 mg/mL (n=6), 30 mg/mL (n=6), and 40 mg/mL (n=6). 10 mg/mL (n=6) has no significant effect on IOP. D) DKO: 10 mg/mL (n=6), 20 mg/mL (n=6), and 30 mg/mL (n=6) SNP

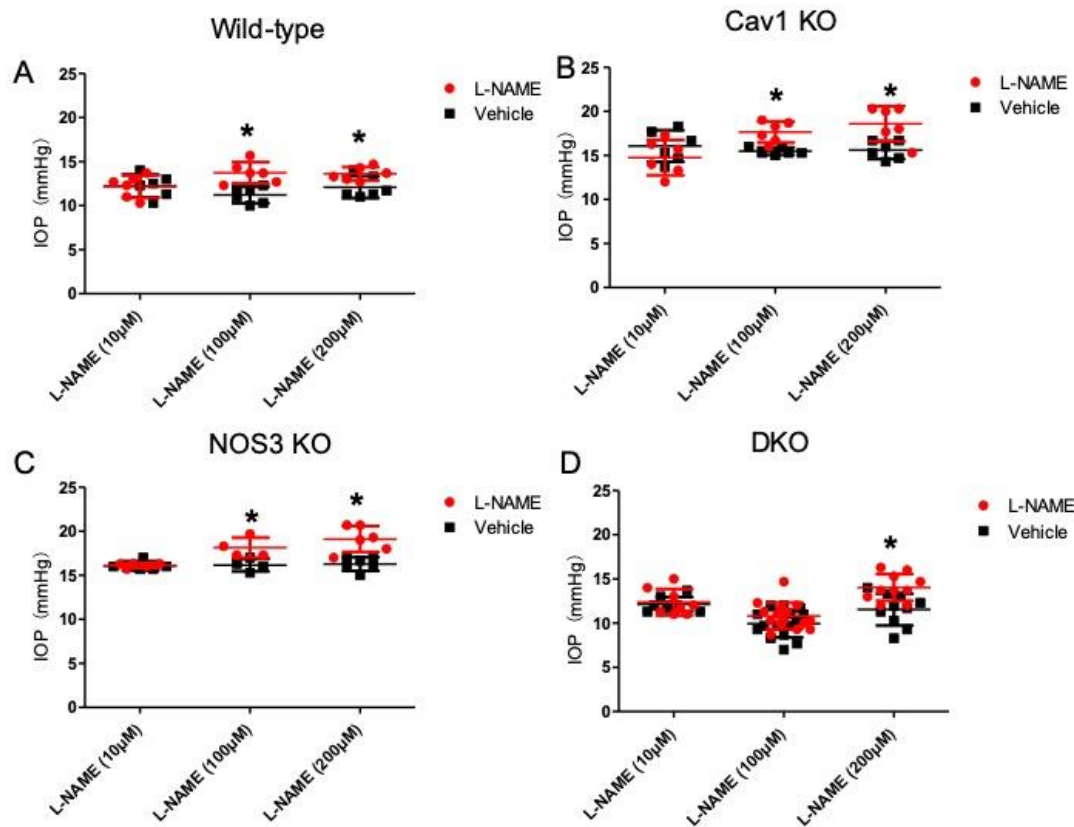does not cause a significant change of IOP in DKO mice. Error bar shows standard error of the mean. *p<0.05.

Figure S3: IOP measurements for L-NAME-treated wild-type mice, Cav1 KO, NOS3 KO and DKO mice. A) Wild-type animals: eyes treated with 100 μM (n=6) and 200 μM (n=6) L-NAME have significantly increased IOP. B) Cav1 KO animals: 10 μM (n=6) L-NAME does not change IOP in Cav1 KO mice. Eyes treated with 100 μM (n=6) or 200 μM (n=6) L-NAME have significantly increased IOP compared with vehicle treated eyes. C) NOS3 KO animals: 10 μM L-NAME(n=5) does not change IOP in NOS3 KO mice. Eyes treated with 100 μM or 200 μM L-NAME (n=4 and n=6 respectively) have significantly increased IOP compared with vehicle treated eyes. D) DKO animals: 10 μM (n=8) or 100 μM (n=15) L-NAME did not significantly change IOP in DKO mice, however 200 μM L-NAME (n=10) significantly increased IOP compared with vehicle treated eyes. Error bar shows standard error of the mean. *p<0.05.
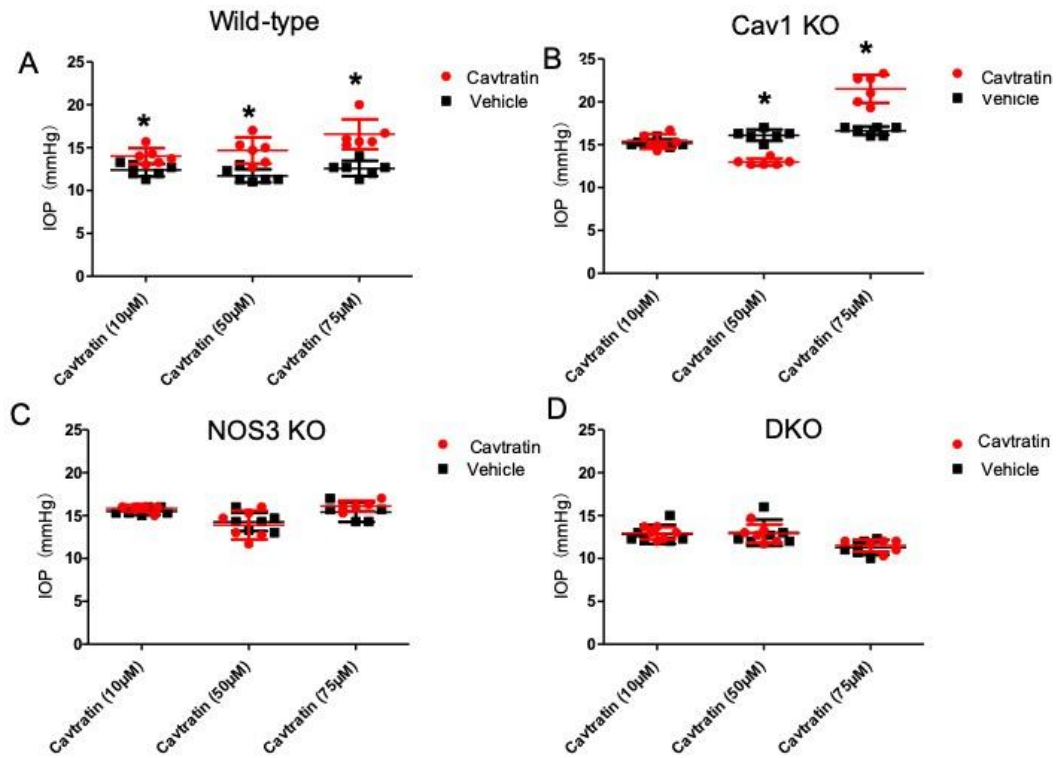
Figure S4: IOP measurements for Cavtratin-treated wild-type mice, Cav1 KO, NOS3 KO and DKO mice. A) Wild-type mice: 10 μM (n=6), 50 μM (n=6), and 75 μM (n=6) cavtratin significantly increases IOP. B) Cav1 KO mice: 75 μM (n=6) cavtratin significantly increased IOP while 50 μM (n=6) cavtratin significantly lowers IOP in Cav1 KO mice. C) NOS3 KO: 10 μM (n=6), 50μM (n=6), and 75 μM (n=5) cavtratin does not cause a significant change of IOP in NOS3 KO mice. D) DKO: 10 μM (n=6), 50 μM (n=6), and 75 μM (n=6) cavtratin does not cause a significant change of IOP in DKO mice. Error bar shows standard error of the mean. *$p < 0.05$.

Table S2 IOP of mice treated with SNP, L-NAME and cavtratin.

Drug	Concentration	WT					Cav1 KO					NOS3 KO					DKO				
		IOP(Mean ± SD, mmHg)					IOP(Mean ± SD, mmHg)					IOP(Mean ± SD, mmHg)					IOP(Mean ± SD, mmHg)				
		Vehicle	Drug	Percentage Change(%)	n	P value	Vehicle	Drug	Percentage Change(%)	n	P value	Vehicle	Drug	Percentage Change(%)	n	P value	Vehicle	Drug	Percentage Change(%)	n	P value
SNP(mg/mL)	10	12.0 ± 1.3	12.3 ± 1.6	2.5 ± 16.9	10	0.549	16.0 ± 1.0	14.4 ± 1.9	-10 ± 14.3	6	0.062	15.4 ± 0.6	14.6 ± 0.9	-5.2 ± 3.9	6	0.159	11.3 ± 0.9	10.7 ± 1.5	-5.3 ± 15.8	6	0.329
	20	12.5 ± 1.3	9.5 ± 2.1	-24 ± 13.0	5	0.001	16.6 ± 1.7	14.1 ± 1.8	-15.1 ± 9.2	6	0.004	16.2 ± 1.8	10.0 ± 1.0	-38.3 ± 6.1	6	0.000	12.7 ± 0.8	11.5 ± 1.0	-9.4 ± 8.5	6	0.082
	30	11.6 ± 0.7	10.0 ± 1.3	-13.8 ± 12.1	10	0.009	16.8 ± 0.7	14.9 ± 0.6	-11.3 ± 4.8	6	0.025	15.9 ± 0.8	12.2 ± 1.3	-23.3 ± 7.7	6	0.000	11.2 ± 1.5	10.9 ± 1.1	-2.7 ± 11.6	6	0.514
	40	12.6 ± 0.7	10.5 ± 1.0	-16.7 ± 9.8	6	0.007	16.7 ± 1.1	13.7 ± 1.7	-18 ± 13.0	6	0.001	15.7 ± 0.4	11.7 ± 0.5	-25.5 ± 2.9	6	0.000	12.1 ± 1.1	10.4 ± 1.6	-14 ± 9.9	8	0.004
L-NAME(μM)	10	12.3 ± 1.3	12.2 ± 1.3	-0.8 ± 11.3	6	0.920	16.1 ± 1.8	14.8 ± 2.0	-8.1 ± 6.5	6	0.148	16.1 ± 0.5	16.1 ± 0.3	0 ± 3.7	5	0.949	12.1 ± 0.8	12.4 ± 1.5	2.5 ± 7.5	8	0.948
	100	11.2 ± 0.9	13.7 ± 1.2	22.3 ± 16.3	6	0.001	15.5 ± 0.4	17.7 ± 1.2	14.2 ± 6.1	6	0.018	16.2 ± 0.7	18.2 ± 1.1	12.3 ± 3.0	4	0.001	9.9 ± 1.5	10.8 ± 1.5	9.1 ± 19.2	15	0.134
	200	12.1 ± 1.2	13.6 ± 0.8	12.4 ± 10.5	6	0.028	15.6 ± 1.0	18.6 ± 2.0	19.2 ± 14.0	6	0.002	16.3 ± 0.8	19.1 ± 1.5	17.2 ± 4.8	6	0.000	11.6 ± 1.8	14.0 ± 1.5	20.7 ± 29.3	10	0.001
Cavtratin(μM)	10	12.4 ± 0.7	14.0 ± 0.9	12.9 ± 13.6	6	0.024	15.2 ± 0.5	15.4 ± 0.8	1.3 ± 5.4	6	0.665	15.5 ± 0.4	15.8 ± 0.4	1.9 ± 3.4	6	0.551	13.0 ± 1.1	12.9 ± 0.7	-0.8 ± 7.9	6	0.825
	50	11.7 ± 0.8	14.7 ± 1.5	25.6 ± 8.7	6	0.000	16.1 ± 0.7	12.9 ± 0.4	-19.9 ± 2.3	6	0.000	14.3 ± 1.1	13.9 ± 1.7	-2.8 ± 15.2	6	0.532	13.0 ± 1.5	12.9 ± 1.1	-0.8 ± 7.0	6	0.868
	75	12.6 ± 0.9	16.6 ± 1.7	31.7 ± 8.3	6	0.000	16.6 ± 0.5	21.5 ± 1.6	29.5 ± 12.9	6	0.000	15.4 ± 1.1	16.1 ± 0.6	4.5 ± 4.5	5	0.266	11.3 ± 0.9	11.5 ± 0.7	1.8 ± 0.1	6	0.720

WT=wild type; Cav1 KO=Cav1 Knockout mice; NOS3 KO=NOS3 Knockout mice; DKO=Cav1$^{-/-}$ NOS3$^{-/-}$ double knockout mice; IOP=intraouclar pressure; SNP=sodium nitroprusside ; L-NAME=L-NG-nitroarginine methyl ester.