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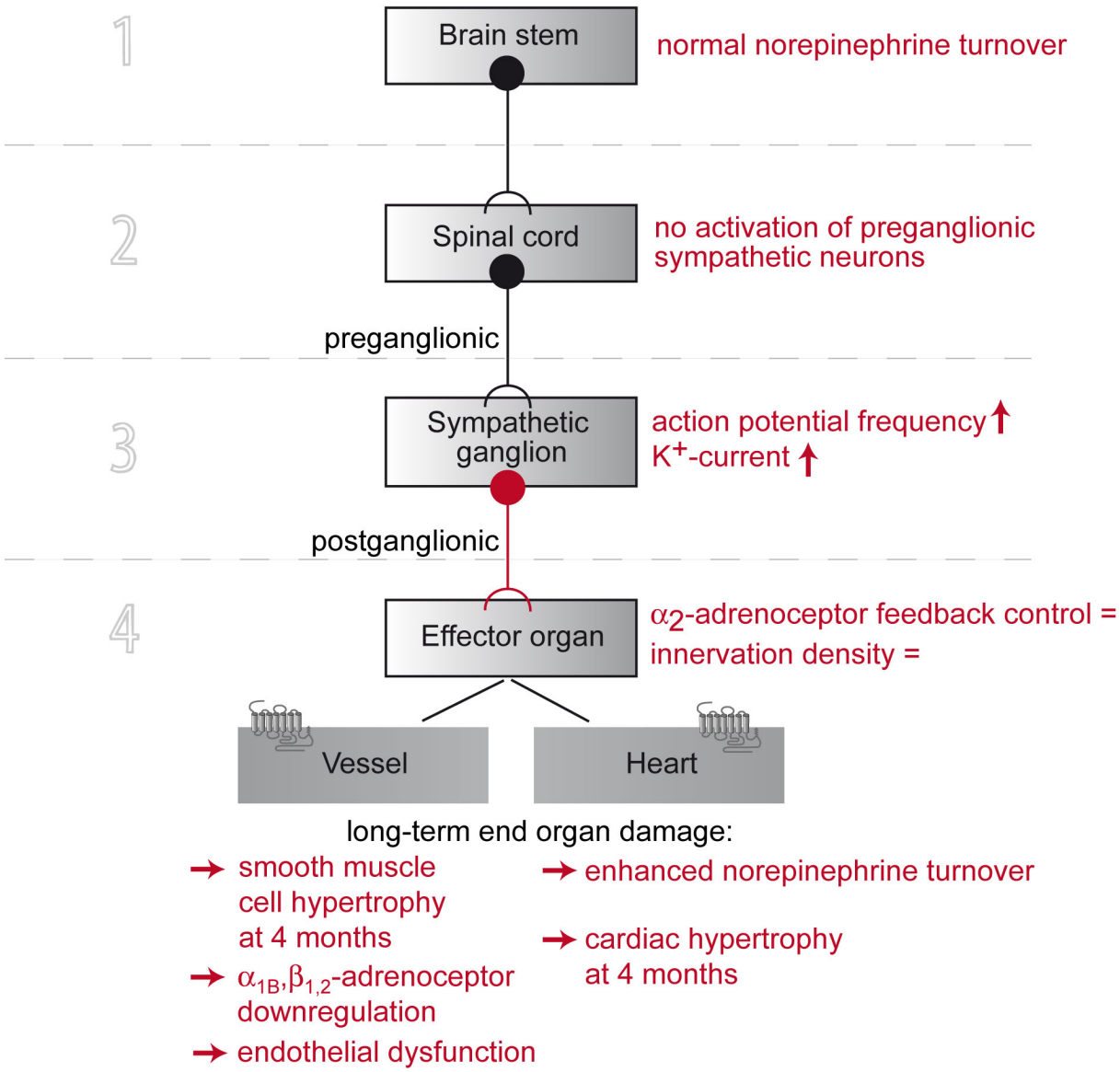
Supplemental Data

Phosducin Influences Sympathetic Activity and Prevents Stress-Induced Hypertension in Humans and Mice

Nadine Beetz, Michael D. Harrison, Marc Brede, Xiangang Zong, Michal J. Urbanski, Anika Sietmann, Jennifer Kaufling, Michel Barrot, Mathias Seeliger, Maria Augusta Vieira-Coelho, Pavel Hamet, Daniel Gaudet, Ondrej Seda, Johanne Tremblay, Theodore A. Kotchen, Mary Kaldunski, Rolf Nüsing, Bela Szabo, Howard J. Jacob, Allen W. Cowley Jr., Martin Biel, Monika Stoll, Martin J. Lohse, Ulrich Broeckel, Lutz Hein

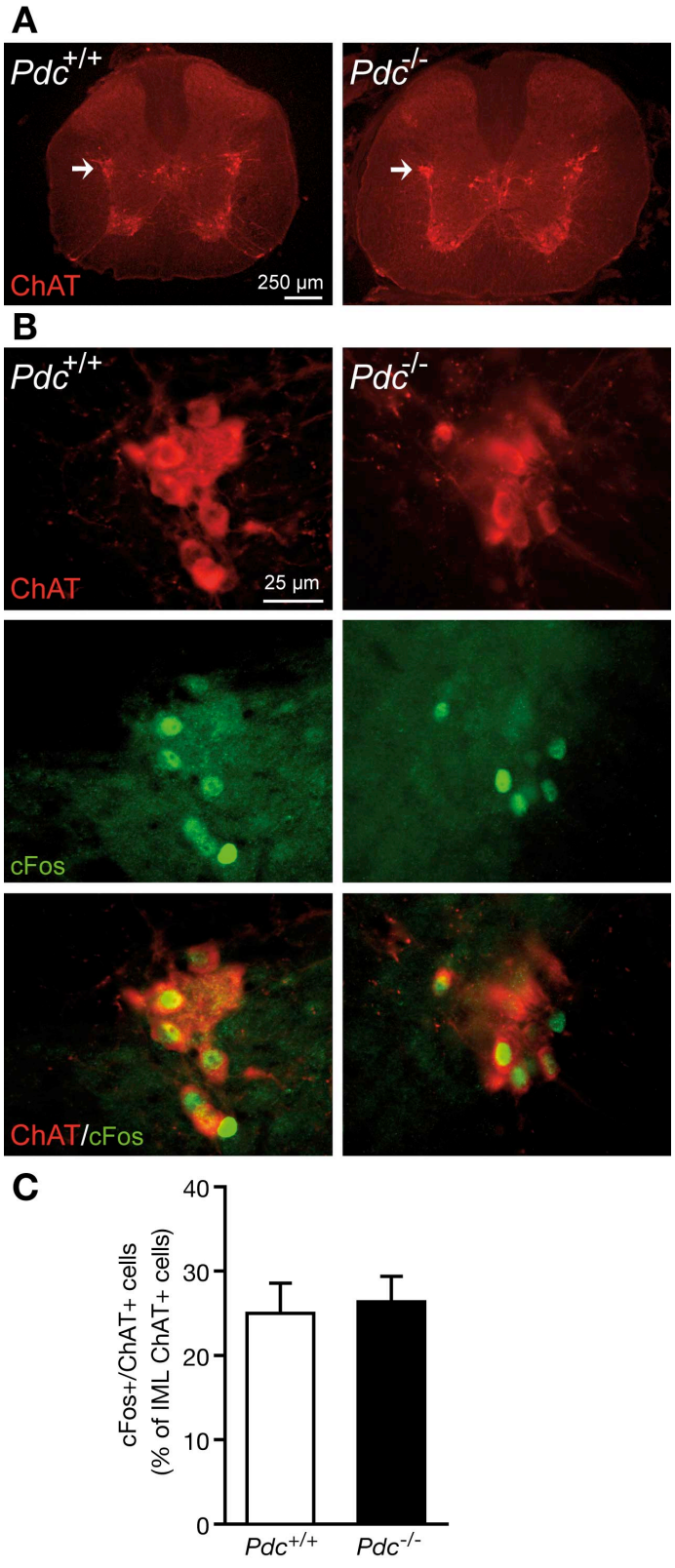
Supplemental Figure 1 | Overview over the phenotypic characterization of regulatory circuits in the sympathetic nervous system in phosducin-deficient mice.

Supplemental Figure 1



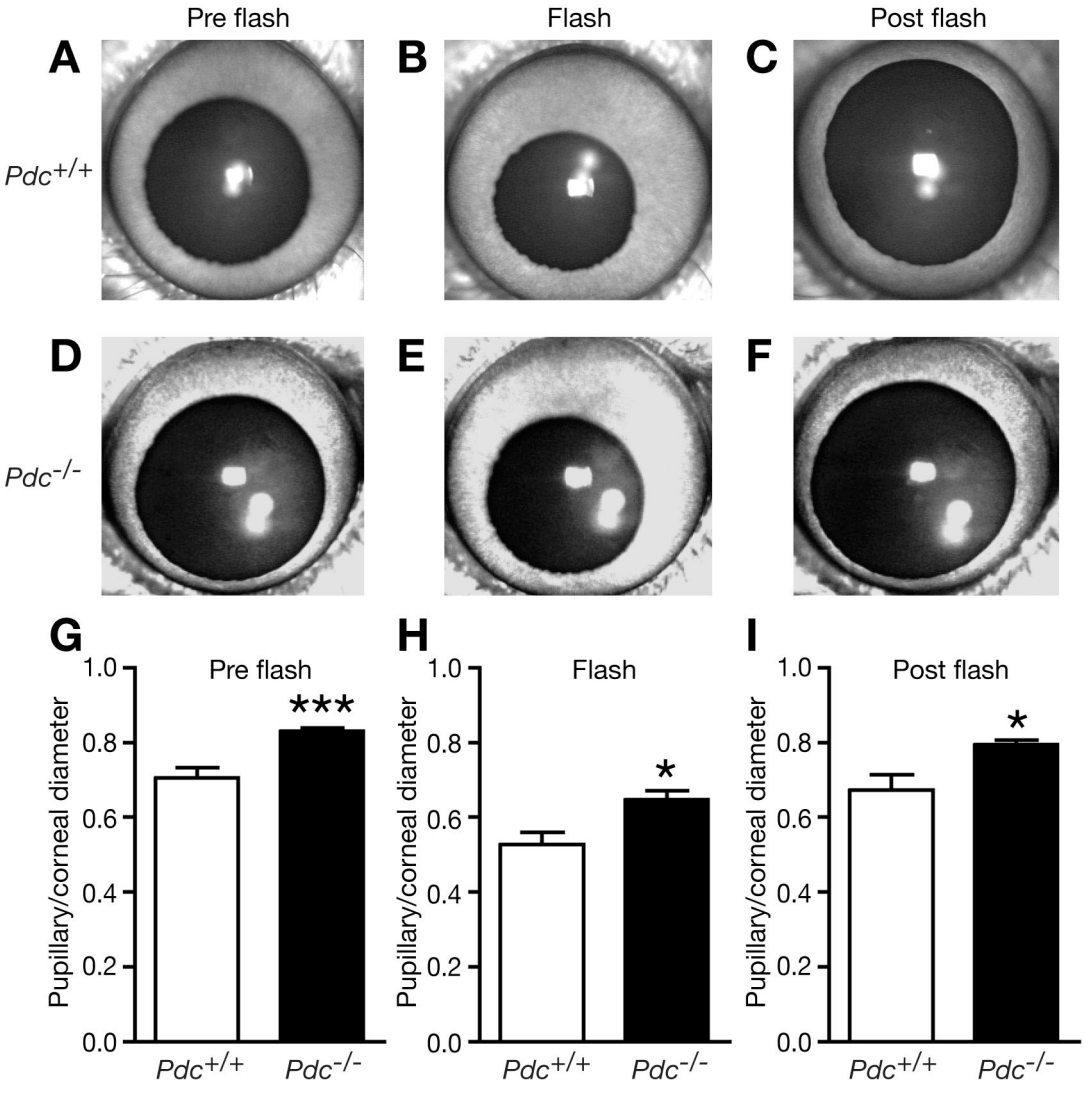
Supplemental Figure 2 | cFos immunostaining to assess activation of preganglionic sympathetic neurons in the thoracic spinal cord. (**A**) Choline acetyltransferase (ChAT) immunostaining in *Pdc*$^{+/+}$ and *Pdc*$^{-/-}$ mice. The deletion of *Pdc* did not affect the organization of the spinal cholinergic system. The white arrow indicates the intermediolateral column (IML) location. (**B**) cFos, marker of activity, was detected by immunofluorescence in the IML cholinergic neurons of both *Pdc*$^{+/+}$ and *Pdc*$^{-/-}$ mice. (**C**) The proportion of IML cholinergic neurons expressing cFos did not differ between *Pdc*$^{+/+}$ and *Pdc*$^{-/-}$ mice (n=6 per genotype, P=0.78).

Supplemental Figure 2



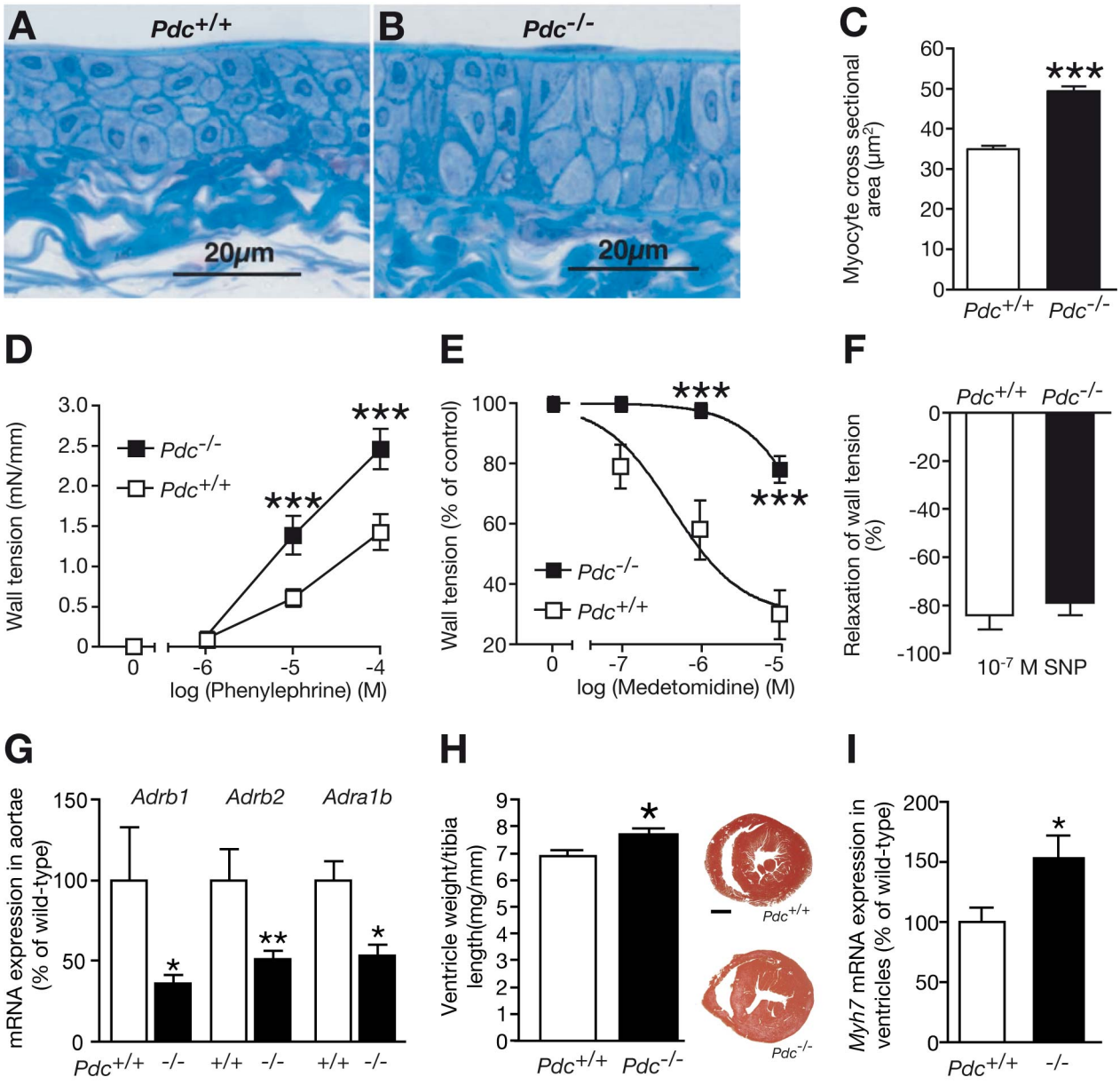
Supplemental Figure 3 | Pupil diameter in wild-type and *Pdc*-deficient mice. Pupil size was determined in awake *Pdc*$^{+/+}$ (**A-C**) and *Pdc*$^{-/-}$ (**D-F**) mice by infrared confocal scanning-laser ophthalmoscopy after over-night dark adaptation (”pre flash"), immediately after a brief white light flash ("flash") and 30 s after the light flash ("post flash"). (**G-I**) Mean values of pupillary / corneal diameter in 8 *Pdc*$^{+/+}$ and 8 *Pdc*$^{-/-}$ mice (* $P<0.05$, *** $P<0.001$).

Supplemental Figure 3



Supplemental Figure 4 | Cardiovascular function in 4-6 month old phosducin-deficient mice. (**A**-**G**) Hypertrophy and altered function in segments of the femoral and iliac artery isolated from 4 months old phosducin-deficient mice. (**A**-**C**) Longitudinal sections through the femoral artery of wild-type (**A**) and *Pdc*$^{-/-}$ mice (**B**) revealed an increased media thickness as well as increased smooth muscle cells cross sectional areas (**C**, n=6, *** *P*<0.0001). (**D**) The vasoconstrictory response to α_1-adrenergic receptor activation by phenylephrine was significantly enhanced in *Pdc*$^{-/-}$ iliac artery segments (n=6-10, *** *P*<0.001). (**E**) Endothelium-dependent relaxation induced by the α_2-adrenoceptor agonist medetomidine was diminished in *Pdc*$^{-/-}$ as compared with *Pdc*$^{+/+}$ vessels (n=8-10, *** *P*<0.001). (**F**) Vasorelaxation of precontracted vessels by sodium nitroprusside was unaltered in *Pdc*$^{-/-}$ vessels (n=6, *P*>0.05). (**G**) In aortae of mice aged 4 months, α- and β-adrenoceptors were downregulated as detected by quantitative real-time PCR (n=7-8, * *P*<0.05, ** *P*<0.01). Abbreviations: *Adrb1*, β_1-adrenoceptor; *Adrb2*, β_2-adrenoceptor; *Adra1b*, α_{1B}-adrenoceptor. (**H** and **I**) Cardiac hypertrophy and β-MHC regulation. (**H**) In animals aged 4 months, analysis of cardiac morphology revealed significant cardiac hypertrophy as expressed by increased heart weight to tibia length ratio (n=7-8; * *P*<0.05). (**I**) The β-isoform of myosin heavy chain was found to be upregulated in quantitative real-time PCR experiments of whole ventricles (* *P*<0.05; n=7-8). Abbreviation: *Myh7*, β-myosin heavy chain. Bar 1 mm.

Supplemental Figure 4



Supplemental Table 1 | Blood pressure after implantation of telemetric pressure transducers during isoflurane anesthesia. Maximal arterial blood pressure was determined during the first 12 hours after implantation of a telemetric blood pressure transducer into the left carotid artery (n=6-8 per genotype).

	Genotype	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)
C57BL6/J	*Pdc*$^{+/+}$	133.8 ± 4.2	100.5 ± 3.0
	Pdc$^{-/-}$	188.6 ± 7.8	142.8 ± 3.6
	t-test	***P*<0.001**	***P*<0.001**
C3H/HeN	*Pdc*$^{+/+}$	123.4 ± 3.3	89.0 ± 4.6
	Pdc$^{-/-}$	165.4 ± 4.1	124.1 ± 3.0
	t-test	***P*<0.001**	***P*<0.001**
„long day“ light 20h/dark 4h	*Pdc*$^{+/+}$	133.3 ± 7.6	98.3 ± 5.1
	Pdc$^{-/-}$	170.4 ± 11.9	127.2 ± 8.7
	t-test	***P*<0.05**	***P*<0.05**
„long night“ light 4h/dark 20h	*Pdc*$^{+/+}$	136.0 ± 7.2	103.4 ± 6.2
	Pdc$^{-/-}$	176.2 ± 6.4	138.3 ± 4.5
	t-test	***P*<0.01**	***P*<0.01**
MT_{1a}/MT_{1b} KO	*MT1a*$^{+/+}$ *MT1b*$^{+/+}$ *Pdc*$^{+/+}$	124.6 ± 13.3	97.7 ± 5.2
	MT1a$^{-/-}$ *MT1b*$^{-/-}$ *Pdc*$^{+/+}$	117.2 ± 5.2	87.9 ± 2.7
	t-test	*P*=0.623	*P*=0.144

Supplemental Table 2 | Brain and brain stem levels of norepinephrine and its metabolites in *Pdc*$^{+/+}$ and *Pdc*$^{-/-}$ mice. Norepinephrine, dihydroxyphenylglycol (DHPG) and normetanephrine (NMN) did not differ between genotypes (P>0.05, n=7-12 per genotype).

	Catecholamine	**Genotype**	
	(pmol/g tissue)	*Pdc*$^{+/+}$	*Pdc*$^{-/-}$
Whole brain	Norepinephrine	643 ± 23	631 ± 19
	DHPG	41.2 ± 1.9	42.2 ± 1.8
	Normetanephrine	83.0 ± 12.1	79.3 ± 8.1
Brain stem	Norepinephrine	4868 ± 206	5131 ± 118
	Normetanephrine	331 ± 51	348 ± 44

Supplemental Table 3 | Map of variation found in the human *PDC* gene. The table displays SNPs found in *PDC* gene from direct sequencing. Underlined SNPs are previously known SNPs and non-underlined SNPs were found through sequencing. SNPs marked by an asterisk were genotyped in the full FC and AA populations.

SNP	Position	Population	MAF	PDC Region
ss79332289	184676382	FC/AA	FC:0.12,AA:0.06	
ss79332318	184676521	AA	0.03	
ss79332290	184677635	FC	0.08	
ss79332320	184678535	AA	0.04	
rs1929095*	184679344	FC/AA	FC:0.13,AA:0.44	3' UTR
ss79332321	184679672	AA	0.19	3' UTR
rs11812050	184680337	FC/AA	FC:0.1,AA:0.04	Intronic
ss79332323	184680698	AA	0.32	Intronic
ss79332292	184681408	FC	0.02	Intronic
ss79332324	184681457	AA	0.01	Intronic
ss79332293	184682446	FC	0.08	Intronic
ss79332295	184683662	FC/AA	FC:0.5,AA:0.44	Intronic
rs6659683	184684073	FC/AA	FC:0.27,AA:0.49	Intronic
rs6672638	184684107	FC/AA	FC:0.08,AA:0.27	Intronic
rs6672836*	184684320	FC/AA	FC:0.26,AA:0.49	Intronic
ss79332327	184684858	AA	0.04	Intronic
ss79332328	184685012	AA	0.26	Intronic
rs12407957	184685223	FC/AA	FC:0.13,AA:0.45	5' UTR
rs10911863	184688299	FC/AA	FC:0.2,AA:0.39	Intronic
ss79332330	184688304	AA	0.03	Intronic
rs12402521*	184689144	FC/AA	FC:0.26,AA:0.49	Intronic
rs12239322	184689437	FC/AA	FC:0.01,AA:0.2	Intronic
ss79332298	184689927	FC	0.12	Intronic
ss79332331	184690705	AA	0.14	Intronic
rs10911864	184692078	FC/AA	FC:0.06,AA:0.1	Intronic

rs10798041	184692149	FC/AA	FC:0.17,AA:0.19	Intronic
rs10911866	184692409	FC	0.15	Intronic
ss79332299	184693528	FC/AA	FC:0.5,AA:0.42	Intronic
ss79332333	184694101	AA	0.4	Intronic
rs4007511	184694184	AA	0.34	Intronic
ss79332301	184697699	FC/AA	FC:0.05,AA:0.04	
rs1799957*	184697968	FC/AA	FC:0.26,AA:0.48	
ss79332303	184698407	FC	0.19	
ss79332304	184698744	FC	0.04	
ss79332306	184699208	FC	0.05	
ss79332334	184699677	AA	0.01	
ss79332336	184699810	AA	0.01	
ss79332307	184699852	FC	0.18	
ss79332339	184699990	AA	0.01	
ss79332309	184700051	FC/AA	FC:0.2,AA:0.07	
ss79332310	184700204	FC/AA	FC:0.08,AA:0.1	
ss79332312	184700244	FC	0.01	
ss79332314	184700282	FC	0.02	
rs3119331	184701141	FC/AA	FC:0.18,AA:0.02	
ss79332315	184701516	FC/AA	FC:0.08,AA:0.04	
rs16825403	184701847	FC/AA	FC:0.11,AA:0.17	
ss79332340	184701869	AA	0.04	
ss79332342	184701889	AA	0.17	
ss79332317	184701897	FC/AA	FC:0.03,AA:0.04	

Supplemental Table 4 | Primer sequences used for determination of gene expression by quantitative real-time PCR. Abbreviations: *Pdc*, phosducin; *Ptgs2*, cyclooxygenase 2; *Actb*, β-actin; *Srp14*, signal recognition particle 14; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *Ppib*, peptidylprolyl isomerase B; *Adrb1*, β_1-adrenoceptor; *Adrb2*, β_2-adrenoceptor; *Adra1a*,*b*,*d*, $\alpha_{1A,B,D}$-adrenoceptor; *Myh7*, β-myosin heavy chain; *Rps29*, ribosomal protein S29; s, sense primer; as, antisense primer.

Gene	**Primer [5' → 3']**	**Product size (bp)**
Pdc	s: CACCCAGCAAGAAGGAAATC as: ATGAGTTCATATTCTTGAATGCTCA	106
Pdc (nested PCR)	s: CTCAGACAAATGTCCTCTCC as: CTGCTCATTCTTTCTTTTGAG	56
Ptgs2	s: TCCCTGAAGCCGTACACATCA as: TGGACGAGGTTTTTCCACCA	132
Actb	s: TCCATCATGAAGTGTGACGT as: GAGCAATGATCTTGATCTTCAT	154
Srp14	s: TCGAGCCTGCAGAAAACAAG as: TTCTTCAGCCCGTCCATGT	142
Gapdh	s: ATGGCCTTCCGTGTTCCTA as: ATGCCTGCTTCACCACCTT	106
Ppib	s: TTCCATCGTGTCATCAAGGAC as: GAAGCGCTCACCATAGATGCT	93

Adrb1	s: CGCCATCACGTCGCCCTT as: GCAGCGGCGCGCTTCGT	154
Adrb2	s: GAGCGACTACAAACCGTCA as: TCGATGCTGGCTGTGACGCA	182
Adra1a	s: ATGTACTGTCGAGTCTACGTGGTAG as: TGGATACGGAGCGTCACTTGCT	105
Adra1b	s: GACACCGGCCACAACACATCA as: GCAGTGTGGAGTTGCTCGAG	97
Adra1d	s: AGCACCACGCGCAGCCTCGA as: GAGCGAGCTGCGCAAGGTGT	155
Myh7	s: ACTGTCAACACTAAGAGGGTCA as: TTGGATGATTTGATCTTCCAGGG	114
Rps29	s: ATGGGTCACCAGCAGCTCTA as: AGCCTATGTCCTTCGCGTACT	155