Supplementary Figure legends

Figure S1. $S1P_2$ receptor function is dispensable during normal retina vascular development. (A) $S1P_2$ mRNA expression normalized to cyclophilin A mRNA and expressed as fold induction over the relative gene expression value measured at P5, determined by quantitative RT-PCR analysis ($n = 3$). $S1P_2$ receptor expression is decreased during normal vascular development ($^{*}P < 0.03$). (B) Frozen retinal cross sections stained with GS-lectin and DAPI during normal development (normoxia). Developing vessels of *S1p2*$^{-/-}$ retinas (P6) finely spread in NFL, towards the periphery of the retina and are able to form deeper capillary networks (P12 and P20) in IPL and OPL similarly to their WT littermates. (C) At P6, whole mount *S1p2*$^{-/-}$ retinas stained for GS-lectin and GFAP show similar vascular and astrocytic network to WT littermates. Scale bar, 100 μm. (D) *S1p2*$^{-/-}$ retinas display proper tip cell filopodia formation while at P20 ensheathment of vessels with smooth muscle cells (SMA staining) occurs as well as in *S1p2*$^{+/+}$ retinas (E). Scale bar: 50μm (B, C), 20μm (D) and 200μm (E).

Figure S2. Immunoblot analysis of cell extracts with polyclonal rabbit $S1P_2$ antibody; first and second lanes: VSMCs and MEFs expressing endogenous $S1P_2$ receptor. Third and fourth lane: HEK-293 transfected with control (pcDNA) and $S1P_2$ receptor expressing plasmid. The antibody specifically recognizes overexpressing as well as endogenous levels of $S1P_2$ receptor (*, ~ 40kDa).

Figure S3. (A, B) At P17, both *S1p2*$^{+/+}$ and *S1p2*$^{-/-}$ flat mount retinas optically sectioned at the GCL, show increased number of proliferative cells (BrdU staining) colocalizing with ECs (arrowhead), small number of mitogenic cells co-localize with astrocytes (GFAP, arrows). Scale bar: 20μm. (C) During normal development (P17, normoxia), WT

animals display normal ensheathment of vessels (GS-lectin) with pericytes (NG2) while (D) astrocytes (GFAP) are in close association with vessels (GS-lectin) as well. Scale bar: 50μm.

Figure S4. Western blot analysis of extracts from HUVECs transduced with AdGFP or Ad$S1P_2$-V5 (50 MOI). HUVECs transduced with Ad$S1P_2$-V5 show decreased eNOS protein expression that was blocked upon treatment with a specific Rho-associated protein kinase (ROCK) inhibitor (Y-27632, 10μM; $^{*}P < 0.05$, results from one representative experiment, $n = 2$).

Fig. S1

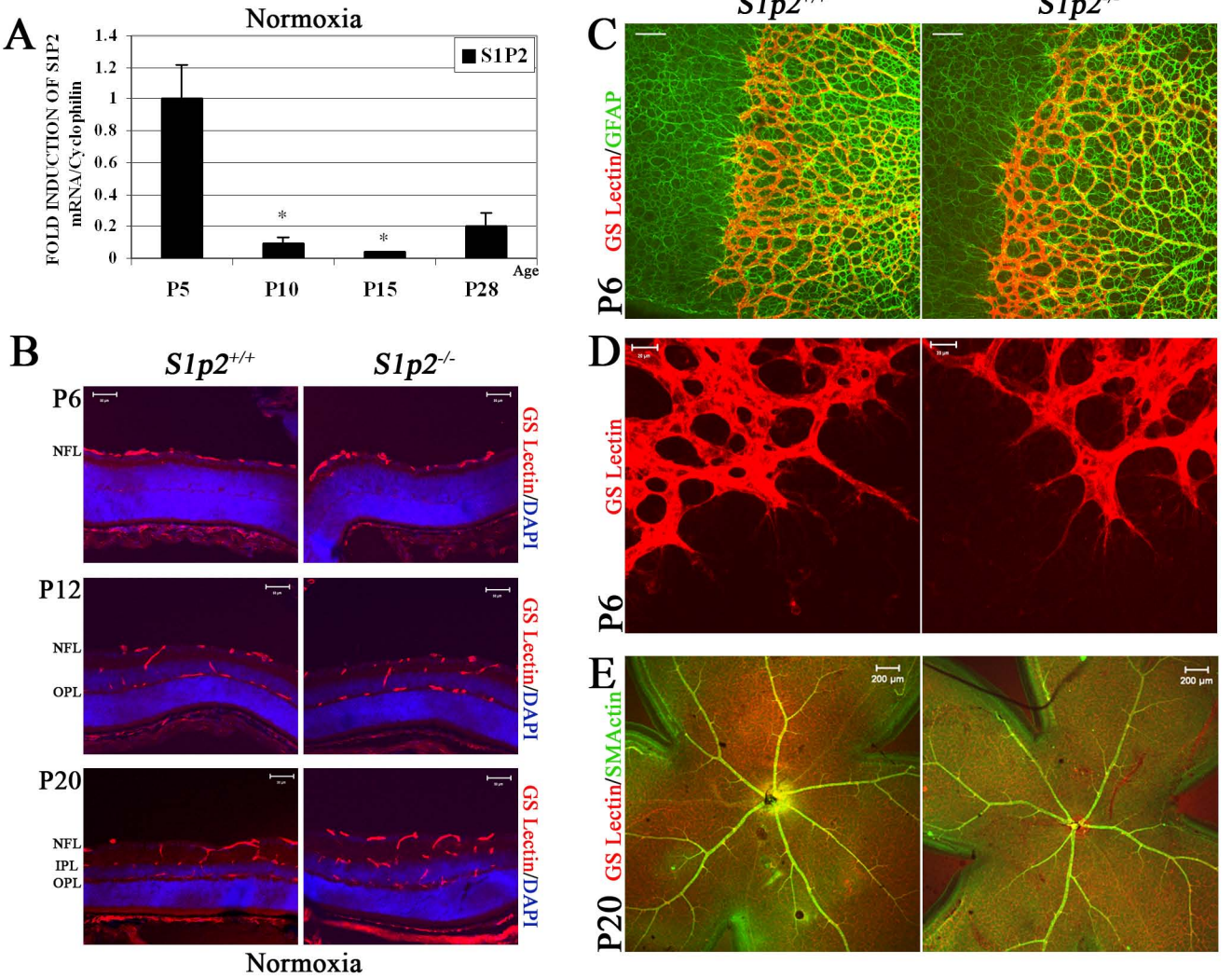

A
Normoxia
S1P2
FOLD INDUCTION OF SIP2 mRNA/Cyclophilin
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
Age
P5
P10
P15
P28
B
S1p2+/+
S1p2-/-
P6
NFL
GS Lectin/DAPI
P12
NFL
OPL
GS Lectin/DAPI
P20
NFL
IPL
OPL
GS Lectin/DAPI
Normoxia
C
S1p2+/+
S1p2-/-
GS Lectin/GFAP
P6
D
GS Lectin
P6
E
GS Lectin/SMActin
200 µm
200 µm
P20

Fig. S2

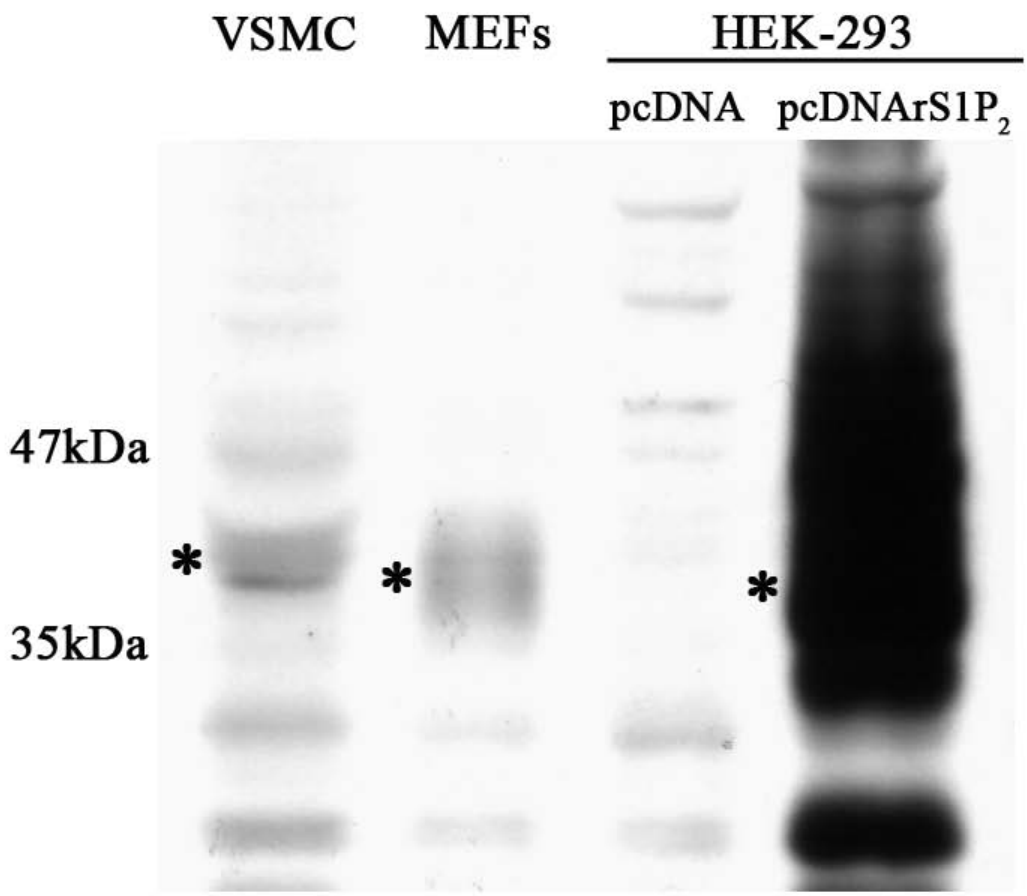


Fig.S3

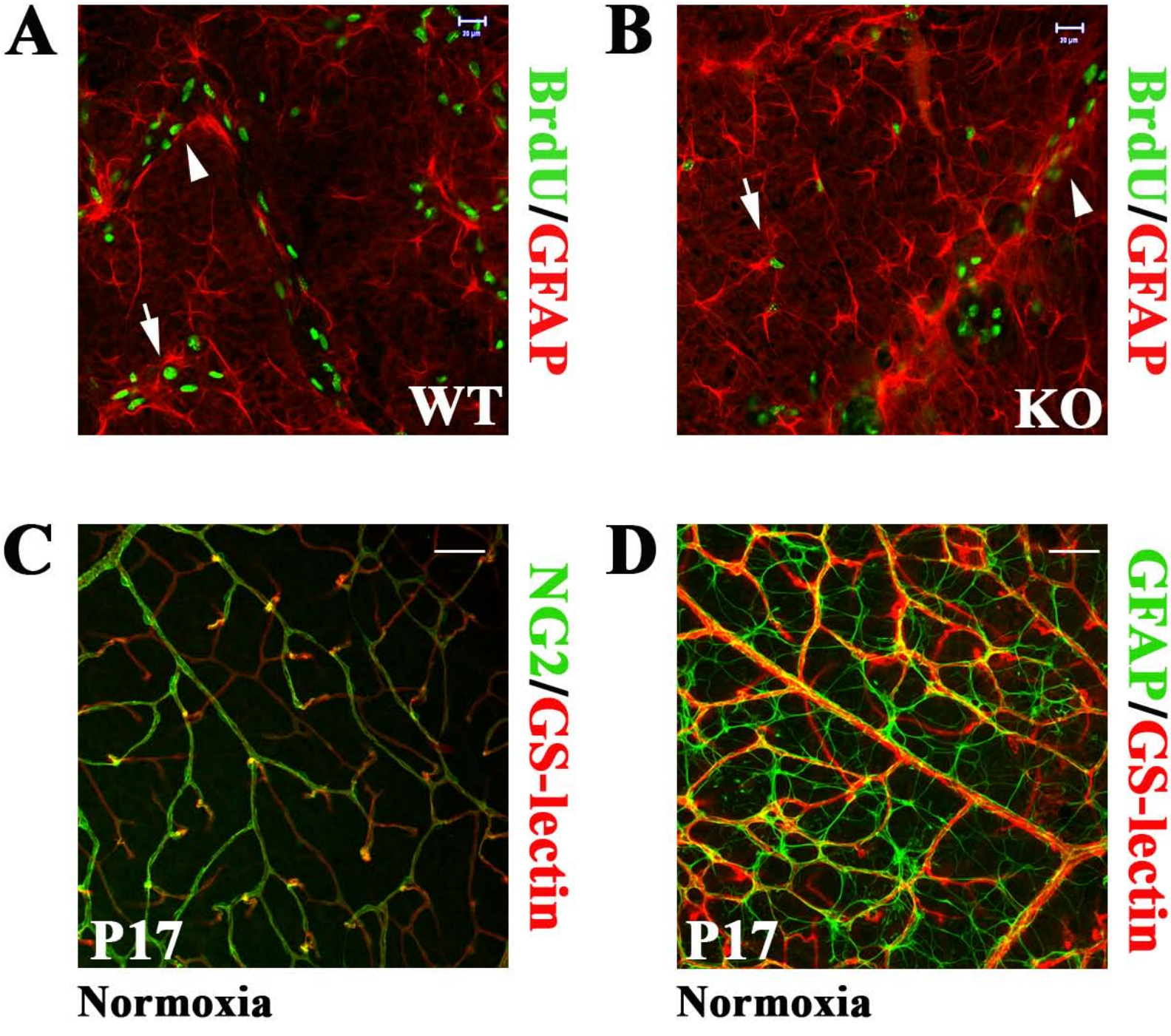
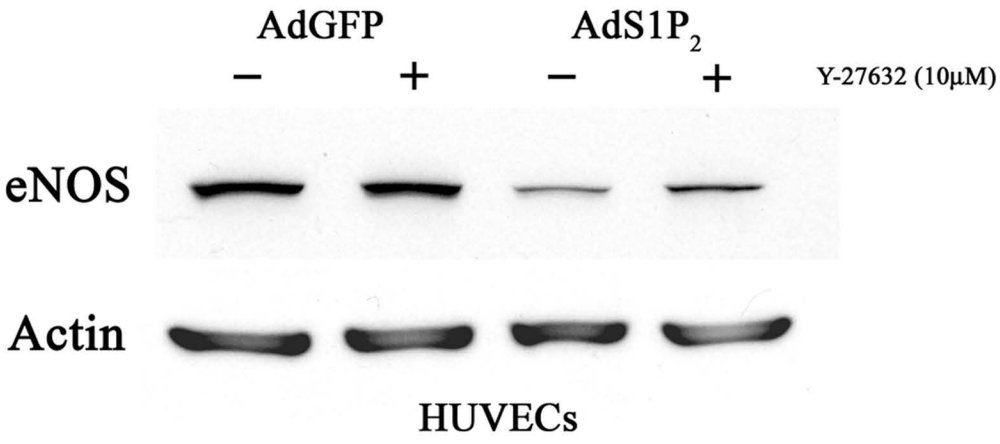

A
WT
BrdU/GFAP
B
KO
BrdU/GFAP
C
P17
NG2/GS-lectin
Normoxia
D
P17
GFAP/GS-lectin
Normoxia

Fig. S4


AdGFP
AdS1P2
− + − +
Y-27632 (10μM)
eNOS
Actin
HUVECs

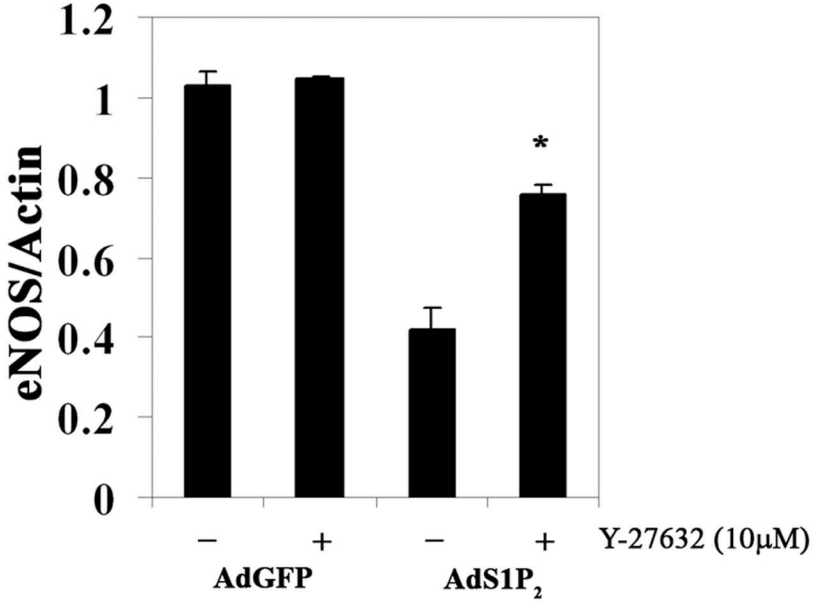

1.2
1
0.8
0.6
0.4
0.2
0
eNOS/Actin
*
− + − +
Y-27632 (10μM)
AdGFP
AdS1P2

Table S1

Primer sequences for quantitative real-time RT-PCR

Gene	Sequence of primers
mS1p1	F: ATGGTGTCCACTAGCATCCC
	R: CGATGTTCAACTTGCCTGTGTAG
mS1p2	F: ATGGGCGGCTTATACTCAGAG
	R: GCGCAGCACAAGATGATGAT
mS1p3	F: GCCTAGCGGGAGAGAAACCT
	R: CCGACTGCGGGAAGAGTGT
mAng2	F: TCAACAGCTTGCTGACCATGAT
	R: GGTTTGCTCTTCTTTACGGATAGC
mVEGF	F: CACGACAGAAGGAGAGCAGAAGT
	R: TTCGCTGGTAGACATCCATGAA
mFlt1	F: GAGGAGGATGAGGGTGTCTATAGGT
	R: GTGATCAGCTCCAGGTTTGACTT
mTNF-a	F: GGGCCACCACGCTCTTCTGTCT
	R:GCCACTCCAGCTGCTCCTCCAC
miNOS	F: TGGCCACCTTGTTCAGCTACG
	R:GCCAAGGCCAAACACAGCATAC
mCOX-2	F: GTACCCGGACTGGATTCTATGG
	R: GGGTGGGCTTCAGCAGTAATT
mCyclophilin	F: ATGGCAAATGCTGGACCAAA
	R: TGCCATCCAGCCATTCAGT
mGAPDH	F:CAACTACATGGTCTACATGTTCCAGT
	R:TGACCCGTTTGGCTCCA