

## **Supplementary data**

### **Detailed Materials and Methods:**

#### **Animals and hyperoxia exposure**

Newborn C57BL/6 murine pups from several littermates were mixed together at the same day. Neonates at postnatal day 4 (P4) with their nursing mothers were randomly placed into room air (RA) or hyperoxia (80% oxygen) for the studies. The dams exposed to hyperoxia and room air were rotated every 24h to avoid high oxygen toxicity. YCP-160D chamber (Changsha Huaxi Electronics Technetronic Co, Ltd) was used for hyperoxia experiments. For systemic pharmacologic inhibition of Dll4 or Notch1 signaling at P5 or P6 pups, anti-Dll4 antibody (3mg/kg; Bio X Cell; HMD4-2, BE0127) or  $\gamma$ -Secretase inhibitor DAPT (50 mg/kg; Millipore, 565770) were injected twice subcutaneously, once at 3h and once right before RA or short-time (6-12h) hyperoxia exposure. In total, the period of treatment was 9h (3+6) or 15h (3+12). Control mice were injected with IgG or vehicle respectively. All animal experiments were approved by the Animal Care and Use Committees of Shanghai Tenth People's Hospital and the NIH guidelines (Guide for the Care and Use of Laboratory Animals).

#### **Retinal whole-mount staining**

The details of whole-mount retinal immunostaining were described in our previous publication[1]. Briefly, retinas were fixed overnight in 4% PFA, followed by incubation with the blocking buffer (1% BSA and 0.3% triton-100 dissolved in PBS) for 1hr. The retinas for double or triple staining were incubated overnight at 4 °C in the blocking buffer with biotinylated isolectin B4 (IB4) (1:200; Vector Labs, B-1205) and the primary antibodies: rabbit anti GFAP (1:500; Abcam, ab7260), goat anti Dll4

(1:200; R&D Systems, AF1389), VEGF-A (1:100; R&D Systems, AF493). Alexa Fluor streptavidin conjugated (1:200; Invitrogen, S32351) and suitable Alexa Fluor-coupled secondary antibodies (1:500; Jackson Immuno Research) were incubated in the blocking buffer at room temperature for 2 hr. After brief wash with PBS, the retinas were flat mounted and analyzed by Olympus IX83 microscope or confocal laser scanning microscopy (Zeiss LSM 710 confocal microscope). NICD (1:500; CST; Val 1744, #2421) immunostaining amplified by TSA-Cy3 (Perkin Elmer) was performed according to the protocol published by Ralf Adams' group[2]. The immunofluorescence intensity was measured with the ImageJ software.

### **RNA isolation and RT-qPCR analysis**

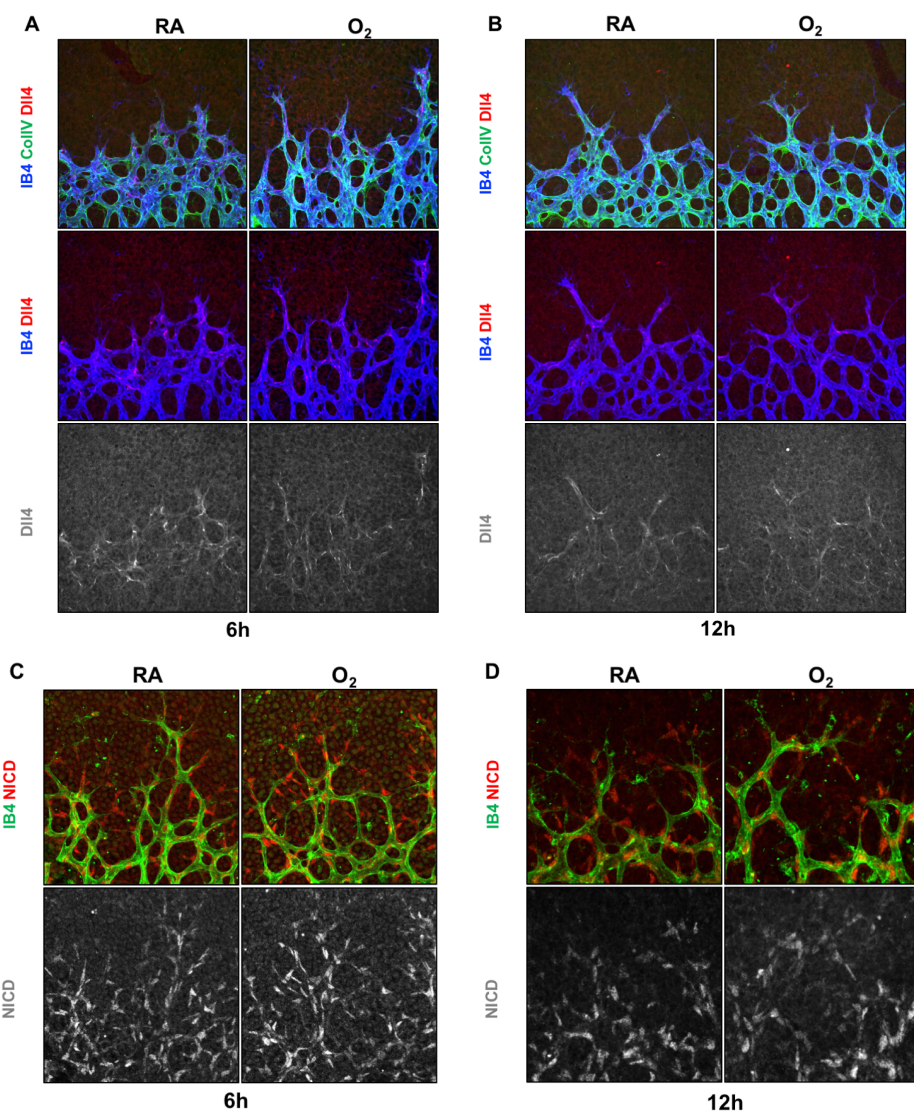
RNA of whole fresh isolated retinas was extracted by Trizol Reagent (Invitrogen) and 1 $\mu$ g/ reaction was conducted for generating cDNA by a PrimeScript RT Reagent Kit (Takara), followed by performing the quantitative PCR with the SYBR Premix Ex Taq Kit (Takara). The primers designed for analysis of mRNAs were documented in Supplementary Table S1. Relative gene expression changes were normalized to the interference gene HPRT.

### **Statistical analysis**

Data was generated by 3-6 room air or hyperoxia treated pups for each experiment and analyzed by SPSS version 20.0 (SPSS Inc). Values are presented as mean  $\pm$  SE unless stated otherwise. For statistical analysis, unpaired Student's t-test was used and  $p < 0.05$  was considered significant.

Figures and legends:

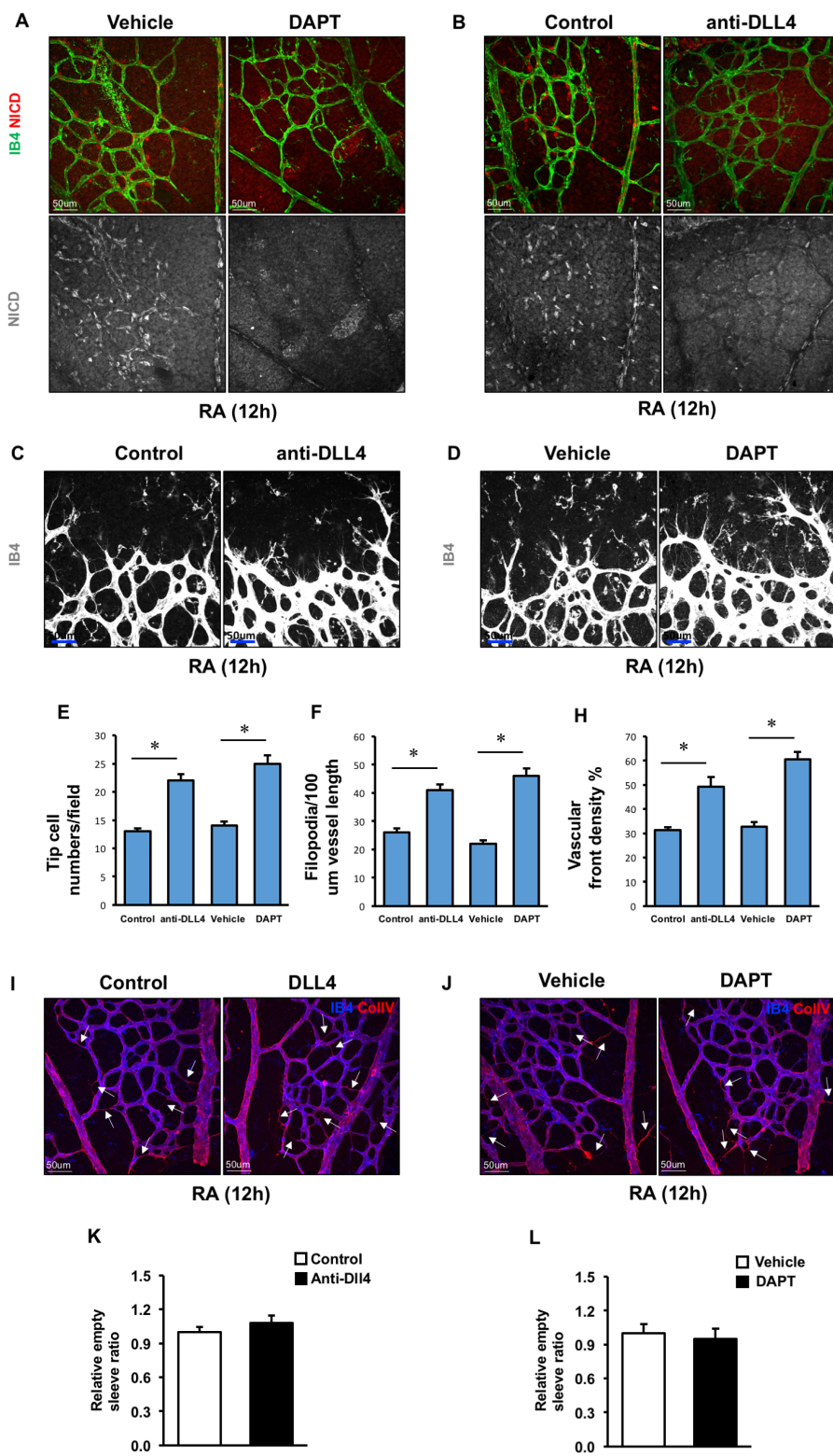
Fig. S1



**Fig. S1 Comparable Dll4-Notch1 signaling in the retinal angiogenic front after short-time hyperoxia exposure.**

**A-B.** Whole-mount staining with Dll4 (red), IB4 (blue) and Collagen IV (green) in the retinas treated by room air (RA) and hyperoxia (O<sub>2</sub>) for 6h or 12h, showing comparable Dll4 expression in angiogenic front. **C-D.** Confocal images of IB4 (green) and NICD (red) co-staining in retinal angiogenic front. NICD immunosignal in the angiogenic region was similar at 6h and 12h after RA or O<sub>2</sub> exposure.

**Fig. S2**



**Fig. S2 The role of short-time pharmacologic Dll4-Notch1 signaling inhibition on Notch1 activation and sprouting angiogenesis.**

**A-B.** Retinal double immunofluorescence with IB4 (green) and NICD (red) in the central plexus. P6 pups in room air (RA) were subcutaneously injected with DAPT or anti-Dll4 antibody twice and retinas were harvested 15hrs after first injection. **C-D.** IB4 staining of DAPT or anti-Dll4 antibody treated retinas at P6. **E-F.** Quantification of indicated parameters in the DAPT or anti-Dll4 antibody treated retinas.  $*p < 0.05$ , data are presented as mean  $\pm$  SEM (n=4). **I-J.** IB4 (blue) and Collagen IV(red) co-staining at P6 retinas treated with DAPT or anti-Dll4 antibody. White arrows indicate Collagen-positive and IB4-negative staining. **K-L.** Empty sleeves (Collagen IV+ IB4-) were quantified.  $*p < 0.05$  (mean  $\pm$  SEM; n = 4).

**Table 1S. qPCR primer sequences**

<b>Gene name</b>	<b>Species</b>	<b>Sequences of primers</b>
<b>Vegfa</b>	<b>mouse</b>	5'- ACATCTTCAAGCCGTCCTGT -3'
		5'- GCTTTGGTGAGGTTTGATCCG -3'
<b>Vegfa110</b>	<b>mouse</b>	5'- ATGCAGATCATGCGGATCAAA -3'
		5'- TGTCACATCTGCATTCACATC -3'
<b>Vegfa120</b>	<b>mouse</b>	5'- ATGCAGATCATG CGGATCAAA -3'
		5'- CTTGGCTTGTCACATTTTTTCT G -3'
<b>Vegfa144</b>	<b>mouse</b>	5'- AGCCAGAAAAAAAAATCAGTTCGA -3'
		5'- CTTGTCACATACGCT CCAG -3'
<b>Vegfa164</b>	<b>mouse</b>	5'- CAAAGCCAGAAAATCACTGTG A -3'
		5'- TTGTCACATCTGCAAGTACGT T -3'
<b>Vegfa188</b>	<b>mouse</b>	5'- ATCCTGGAGCGTTCCTGT -3'
		5'- TTGTCACATCTGCAAGTACGTT -3'
<b>Vegfa206</b>	<b>mouse</b>	5'- CTTTTGCCTTTTTGCAGTCACT -3'
		5'- TTG TCACATCTGCAAGTACGTT -3'
<b>HPRT</b>	<b>mouse</b>	5'- TGCTGACCTGCTGGATTACA -3'
		5'- TTTATGTCCCCCGTTGACTGA -3'

## Supplemental References

- [1] S. Majumder, G.F. Zhu, X. Xu, S. Senchanthisai, D. Jiang, H. Liu, C. Xue, X. Wang, H. Coia, Z. Cui, E.M. Smolock, R.T. Libby, B.C. Berk, J. Pang, G-Protein-Coupled Receptor-2-Interacting Protein-1 Controls Stalk Cell Fate by Inhibiting Delta-like 4-Notch1 Signaling, *Cell Rep.* 17 (2016) 2532–2541. doi:10.1016/j.celrep.2016.11.017.
- [2] M.E. Pitulescu, I. Schmidt, B.D. Giaimo, T. Antoine, F. Berkenfeld, F. Ferrante, H. Park, M. Ehling, D. Biljes, S.F. Rocha, U.H. Langen, M. Stehling, T. Nagasawa, N. Ferrara, T. Borggrefe, R.H. Adams, Dll4 and Notch signalling couples sprouting angiogenesis and artery formation, *Nat. Cell Biol.* 19 (2017) 915–927. doi:10.1038/ncb3555.