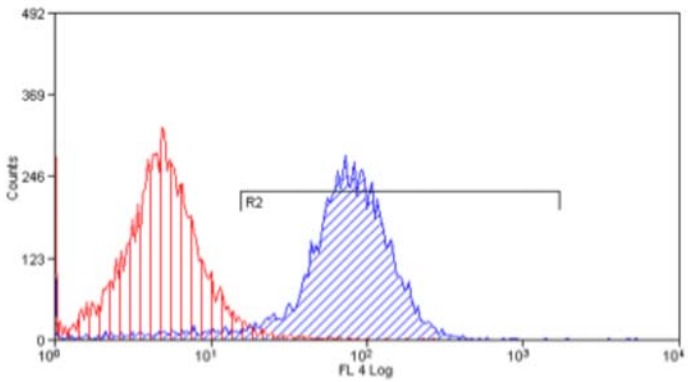
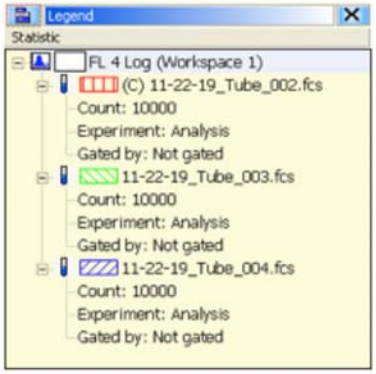
SUPPLEMENTARY DATA

Supplementary Figure 1. Identification of human retinal microvascular endothelial cells

A. Flow cytometry with an antibody against VE-Cadherin



IgG anti-VE-Cadherin



Region	Count	% Hist	% All	Bounds	Mode Count	Mode	Mean	Median
Total	10000	100.00	100.00	(1.00,10...	365	1.00	5.88	4.90
R2	323	3.23	3.23	(15.57,1...	29	15.57	27.03	19.33
Total	10000	100.00	100.00	(1.00,10...	328	21.54	68.93	20.78
R2	7362	73.62	73.62	(15.57,1...	328	21.54	33.04	24.01
Total	10000	100.00	100.00	(1.00,10...	278	73.56	88.37	76.27
R2	9227	92.27	92.27	(15.57,1...	278	73.56	93.04	81.98

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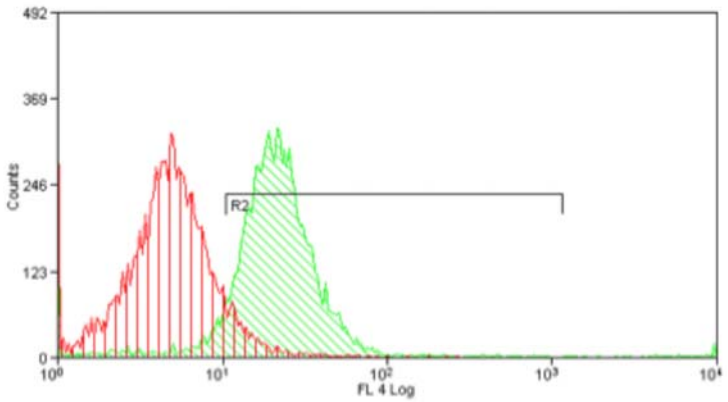
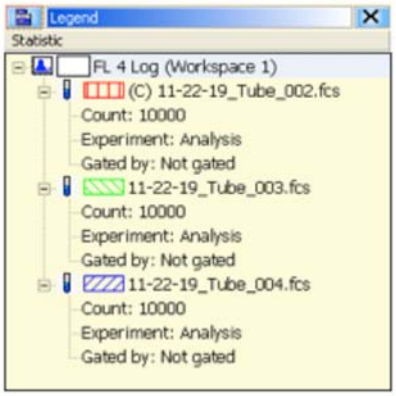
B. Flow cytometry with an antibody against CD31



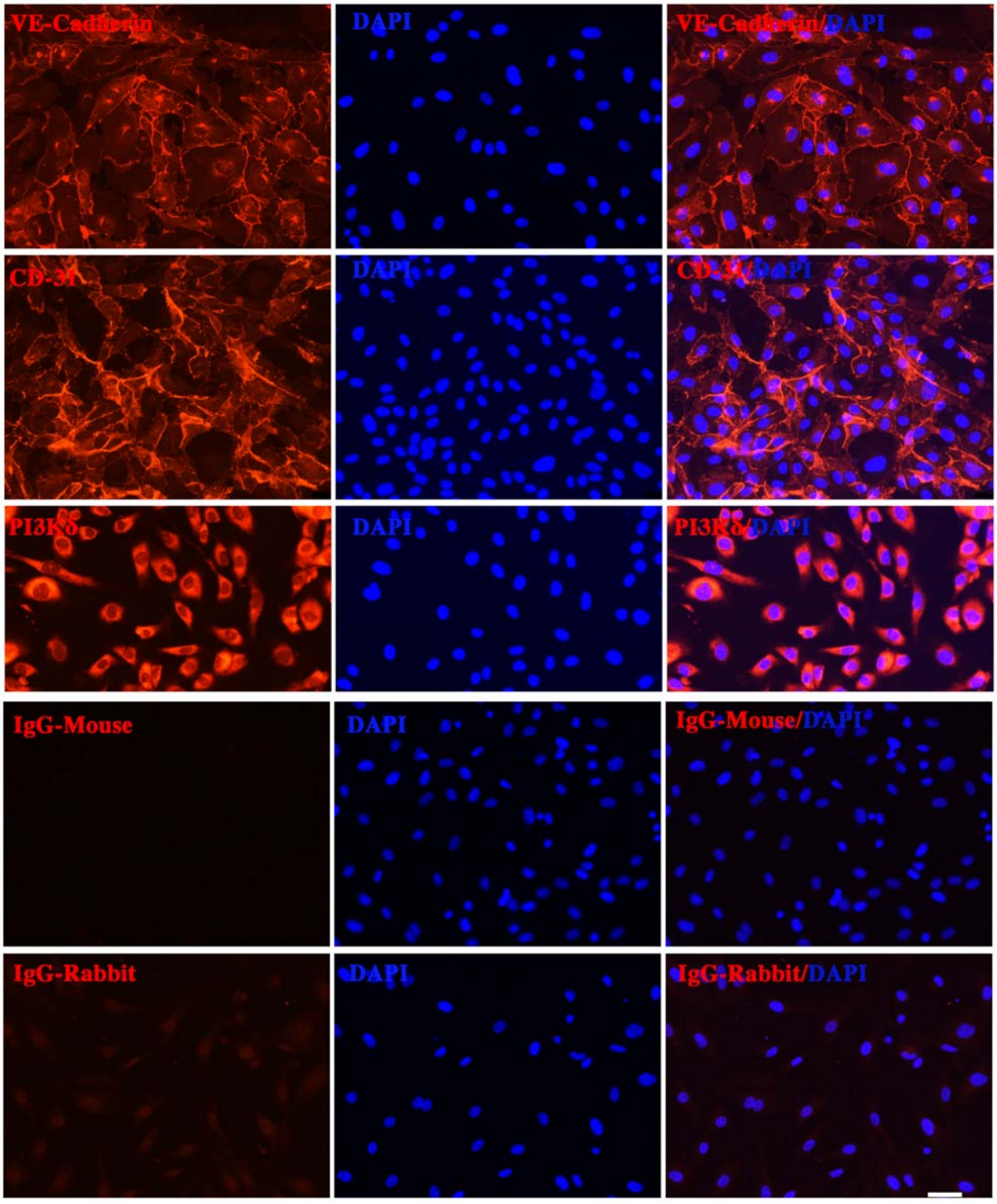
IgG anti-CD31 antibody



Region	Count	% Hist	% All	Bounds	Mode Count	Mode	Mean	Median
Total	10000	100.00	100.00	(1.00,10...	365	1.00	5.88	4.90
R2	910	9.10	9.10	(10.46,1...	81	11.66	17.48	13.47
Total	10000	100.00	100.00	(1.00,10...	328	21.54	68.93	20.78
R2	8919	89.19	89.19	(10.46,1...	328	21.54	28.02	22.34
Total	10000	100.00	100.00	(1.00,10...	278	73.56	88.37	76.27
R2	9377	93.77	93.77	(10.46,1...	278	73.56	91.60	79.08

SUPPLEMENTARY DATA

C. Immunofluorescence with antibodies against VE-Cadherin, CD31, p110δ or non-immune IgG



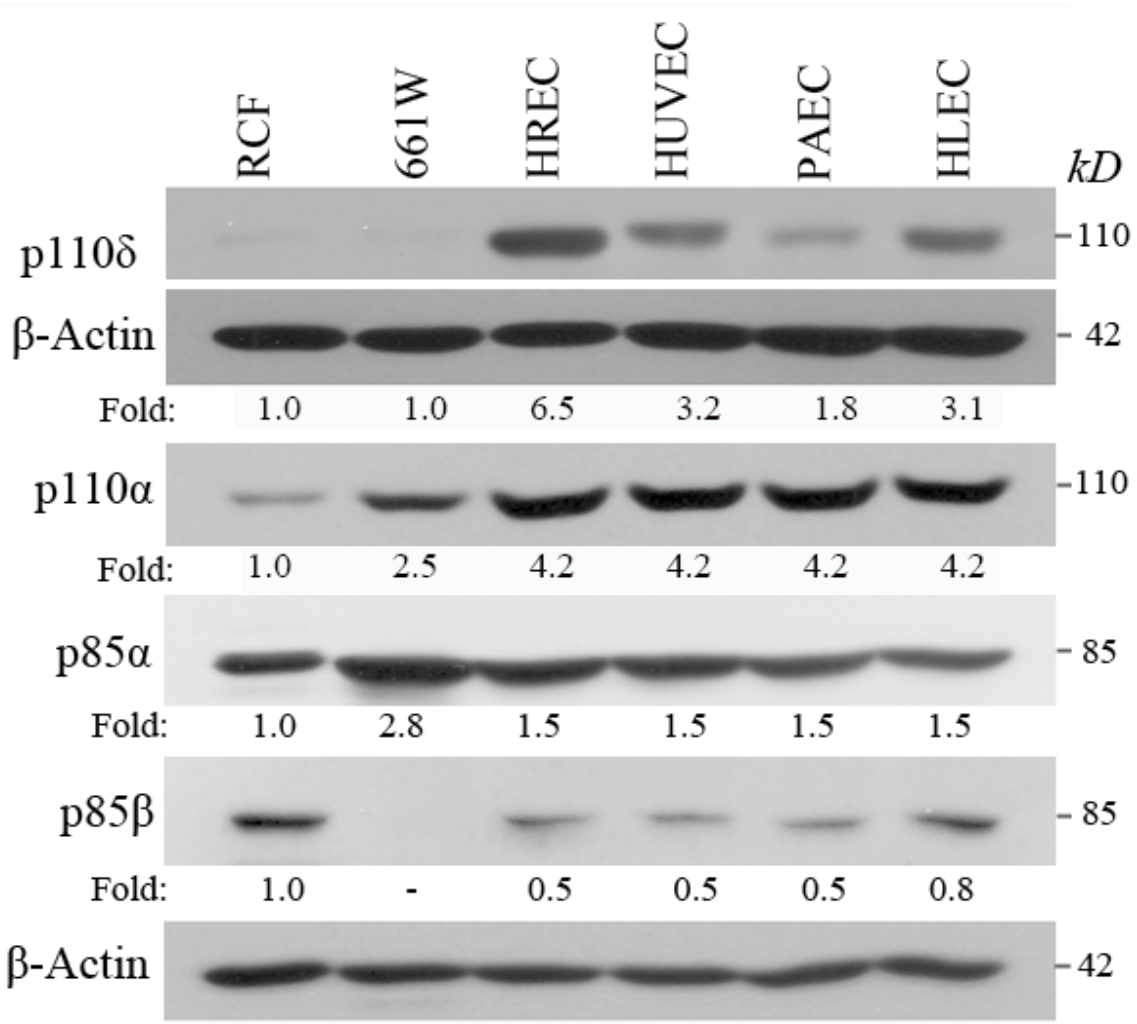
SUPPLEMENTARY DATA

A-B. Human retinal microvascular endothelial cells (HRECs) were cultured to 80% confluence and harvested for staining with antibodies against VE-cadherin (A), CD-31 (B) or non-immune IgG (IgG) and then fluorescence-labeled secondary antibodies (Dylight 549). These stained cells were analyzed by fluorescence-activated cell sorting (FACS). This is representative of three independent experiments.

C. HRECs were cultured in a 4-well chamber to 80% confluence. After fixature with 3.7% formaldehyde, the cells were incubated with primary antibodies against VE-Cadherin, CD3, p110d (PI3Kd) or non-immune IgG from rabbits or mouse, and then stained with fluorescence-label secondary rabbit or mouse antibodies. This is representative of three independent experiments. Scale bar: 40 μm.

SUPPLEMENTARY DATA

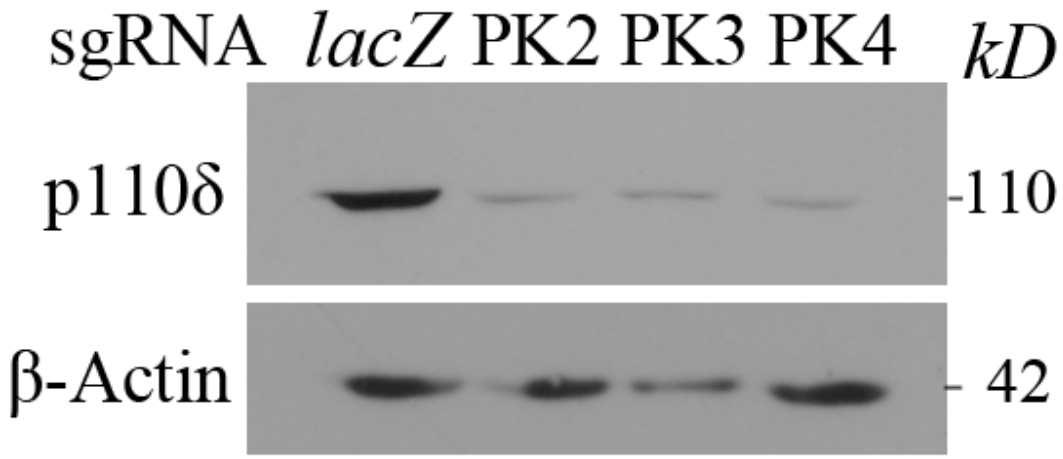
Supplementary Figure 2. Expression of PI3Kd in vascular endothelial cells



Lysates of rabbit conjunctival fibroblasts (RCFs), mouse cone photoreceptor cells (661W), human retinal microvascular endothelial cells (HRECs), human umbilical vein endothelial cells (HUVECs), porcine aortic endothelial cells (PAECs), and human lymphatic endothelial cells (HLECs) were subjected to western blot analysis using indicated antibodies. The intensity of the p110δ and p110α, and p85α and -β bands was firstly normalized to that of the corresponding β-Actin bands, and then calculated to establish the ratio of the control in the first lane shown as "Fold" at the bottom of the panels. This is representative of three independent experiments.

SUPPLEMENTARY DATA

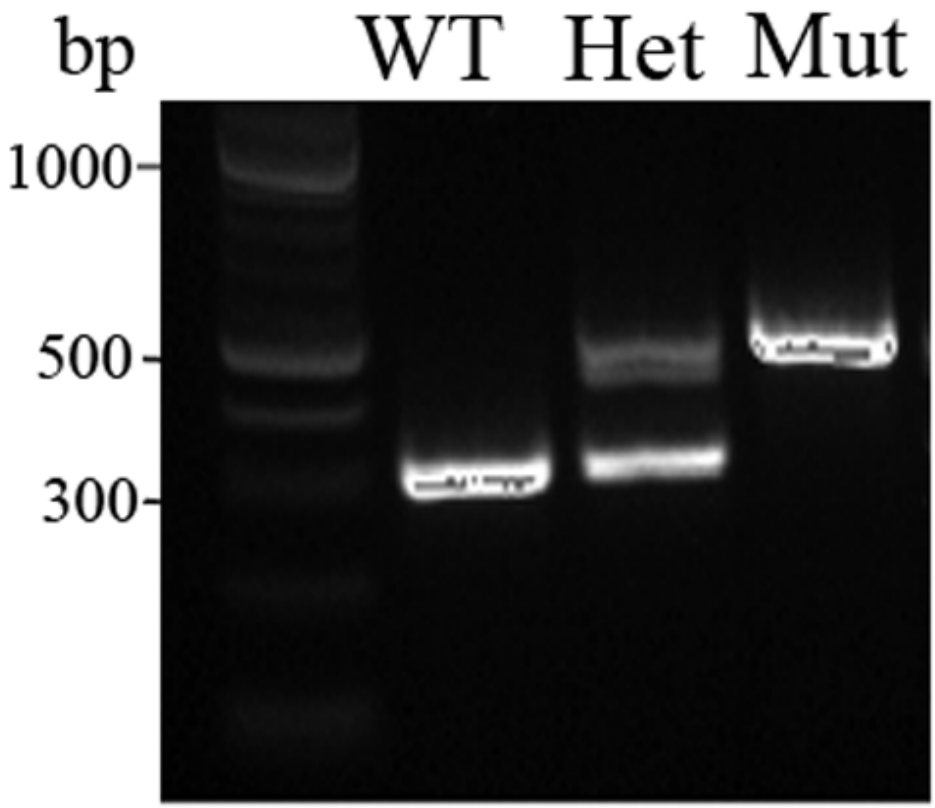
Supplementary Figure 3. Depletion of p110δ in HRECs using CRISPR/Cas9



HRECs infected by lentiviruses containing SpCas9 and sgRNA-PK2, PK3 and PK4 (targeting human *PI3KCD*) or lacZ (targeting bacterial *lacZ)* were selected with puromycin, and their lysates were subjected to western blot using indicated antibodies. This is representative of three independent experiments.

SUPPLEMENTARY DATA

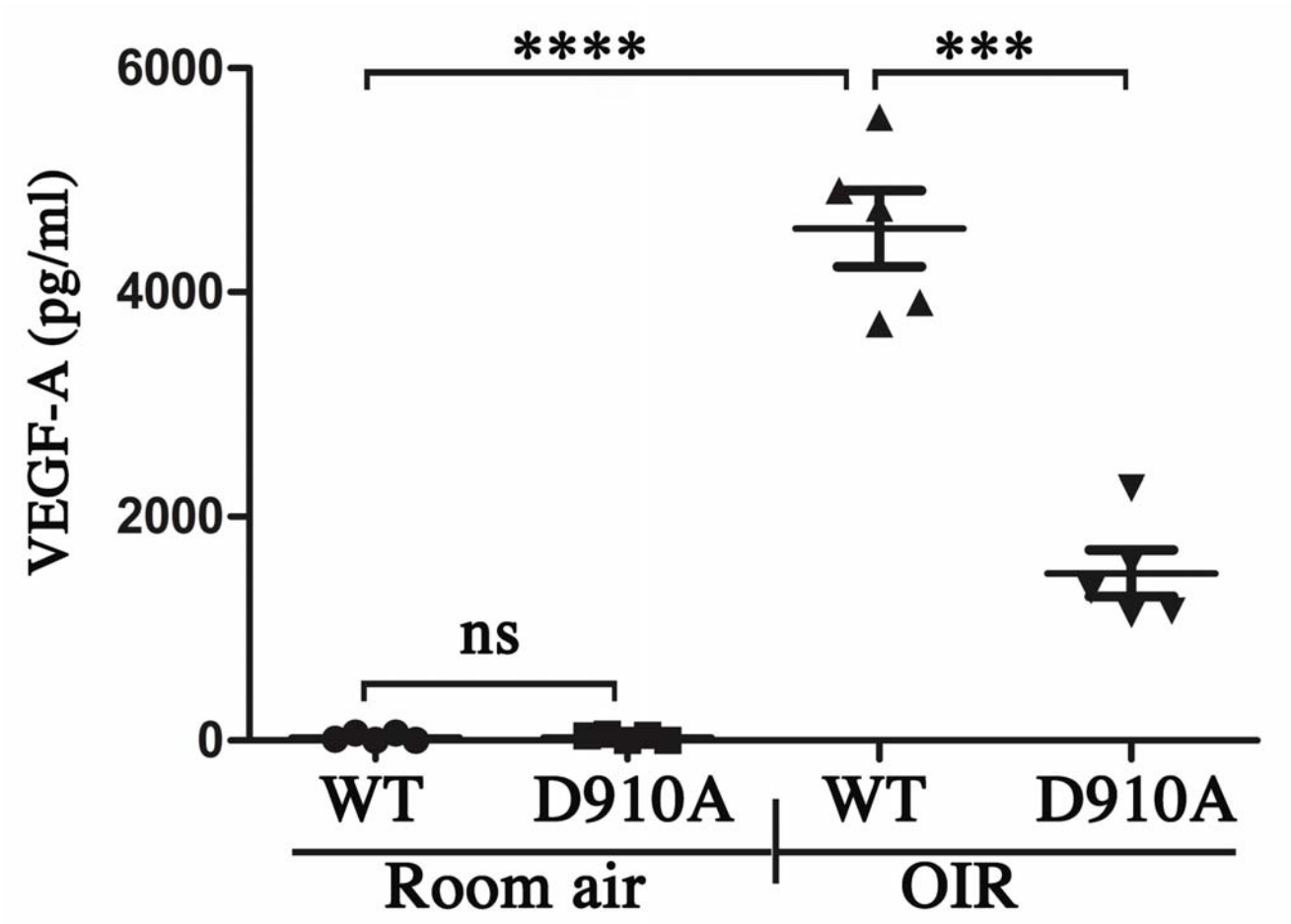
Supplementary Figure 4. Genotyping of Mut and WT mice



Genomic DNA from tails of WT, heterozygous (Het) and Mut (mutant PI3Kp110δ $^{D910A/D910A}$) mice[1] on P12 was subjected to PCR amplification using primers (forward, P28F: CCTGCACAGAAATGCACTTCC; Reverse, P28R, AACGAAGCTCTCAGAGAAAGCTG), and separated in 1.5% agarose gel. Images were taken under ultraviolet light.

SUPPLEMENTARY DATA

Supplementary Figure 5. Measurement of VEGF-A in the mouse vitreous



Clarified vitreous (5 µl) from each eye of P17 mice in room air or experiencing OIR was diluted with PBS into 50 µl, which was added into each well/a 96-well plate and subjected to a mouse VEGF 120 and 165 quantikine ELISA assay. Each group from five eyes of five mice.
WT: wild type *PIK3CD*, D910A: Mutant *PIK3CD* D910A. Room air: mice staying in room air always, OIR: mice experiencing oxygen induced retinopathy. *** and **** indicate significant difference ($p < 0.001$ and 0.0001, respectively) using one-way ANOVA followed by the Tukey post hoc-test. ns: not significant.

SUPPLEMENTARY DATA

Reference

1. Okkenhaug, K., *et al.* Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science* **297**, 1031-1034 (2002).