Supplemental Material

Online Supplement Methods

Induction of polymicrobial sepsis. Polymicrobial sepsis was induced by CLP using a 21-gauge needle.[1,2] Briefly, mice were anesthetized with inhaled isofluorane (2.5% mixed with room air) and subject to either CLP involved two 20-gauge punctures or laparotomy surgery (sham control). When the mouse failed to respond to paw pinch, buprenex (0.1 mg/kg) was administered subcutaneously prior to proper sterilization of the skin with providone iodine, and then a midline abdominal incision was made. The cecum was exposed and ligated with a 3-0 silk tie 1 cm from the tip and the cecal wall was perforated with a 20-gauge needle. Control mice underwent anesthesia, laparotomy, and wound closure but no cecal ligation and puncture. Immediately following the procedure, 500ul of warmed normal saline was administered subcutaneously. Within 5min following surgery, the mice were able to wake from anesthesia. The mice received a second dose of buprenex at 6-8h post-surgery subcutaneously.

Myeloperoxidase assay. Lung tissues were homogenized in 5 mM phosphate buffer and then centrifuged at 15,000 ×g for 20 minutes at 4°C. The pellets were resuspended in phosphate buffer containing 0.5% hexadecyl trimethylammonium bromide and subjected to a cycle of freezing and thawing. Subsequently the pellets were homogenized and the homogenates were centrifuged again. The supernatants were assayed for MPO activity using kinetics readings for 3 minutes and absorbance was measured at 460 nm. The results were presented as ΔOD_{460}/min/g lung tissue.

Lung transvascular albumin flux assessment. Evans blue-albumin (EBA) flux assay was performed as described.[3-5] EBA at a dose of 20 mg/kg BW was retroorbitally injected into mice 40min before tissue collection. Lungs were perfused free of blood with PBS, blotted dry, weighed and snap frozen in liquid nitrogen. The right lung was homogenized in 1 ml PBS and incubated with 2 volumes of formamide at 60°C for 18h. Then the homogenate was centrifuged

at 5,000 × g for 30min. The optical density of the supernatant was determined at 620 nm and 740 nm. Extravasated EBA in lung homogenates was expressed as micrograms of Evans blue dye per g lung tissue.

Scanning electron microscopy. Preparation of lung tissues for scanning electron microscopy was following the procedure described previously.[6] Briefly, mice were anesthetized and the pulmonary artery was cannulated with an 18-gauge catheter. Then 2 mL of partially polymerized methyl methacrylate (Mercox; Ladd Research Industries) mixed with benzoyl peroxide (accelerator) was injected into the lung over 2 min using a Harvard Pump. The methacrylate resin was allowed to harden overnight. The tissue was macerated in sodium hydroxide solution for at least 2 weeks with daily changes of the bath. The casts were cut and mounted on aluminum studs, sputter-coated with palladium-gold, and imaged with a Hitachi S-3000N scanning electron microscope at 10kV.

Intravital microscopy. Intravital microscopy of the mouse cremaster muscle circulation was performed as previously described.[7] At 48h post-LPS challenged (7.5 mg/kg BW, i.p.), the mice were anesthetized, and FITC-conjugated dextran (MW= 20kD, 1 mg/kg BW, Sigma) was injected through jugular cannulus. The scrotum was incised at 30 min after FITC-dextran administration, the surrounding cremaster muscle were exteriorized onto an intravital microscopy tray. Then the cremaster muscle was superfused with thermo-controlled (37°C) and aerated (95% N_2 and 5% CO_2) biocarbonate-buffered saline. For the permeability measurements, images were recorded using an Olympus BX61W microscope with 60 x/0.9 NA water immersion objective lens and a high speed camera (Hamamatsu C9300) through an intensifier (Video Scope International). Data were analyzed using Slidebook v5.0 (Intelligent Imaging Innovations). Vascular permeability was expressed as the ratio of extravascular fluorescence signal over the

intravascular fluorescence signal.

Histology and imaging. Lung tissues were fixed and processed for H & E staining and immunofluorescent staining as described previously.[11] Briefly, lung tissues were fixed by 5 min instillation of 10% PBS-buffered formalin through trachea catheterization at a transpulmonary pressure of 15 cm H_2O, and then overnight at 4^oC with agitation. After paraffin processing, the tissues were cut into semi-thin 4 to 5 μm thick, and stained with H & E for histological analysis. Cryosections (3-5 μm thick) of mouse lung tissues were fixed with 4% paraformaldehyde and then immunostained with anti-p110γ antibody (1:100, Cell Signaling Technology) and anti-VE-cadherin antibody (1:400, Santa Cruz Biotechnology).

The sections of human lung tissues were stained with anti-p110γ antibody (1:100, Cell Signaling Technology) and donkey anti-rabbit Cy5-conjugated secondary antibody (1:100, Jackson ImmunoResearch). Lung ECs were stained with anti-CD31 (1:40, Abcam). Sections were imaged with a confocal microscope system (LSM510; Carl Zeiss, Inc) equipped with a 63×1.2 NA objective lens (Carl Zeiss, Inc.).

Cell proliferation and apoptosis assay. BrdU (75 mg/kg BW, Sigma) was intraperitoneally injected into mice 4h prior to tissue collection. Mouse lung cryosections (3-5 μm thick) were stained with FITC-conjugated anti-BrdU antibody according to the manufacturer's instructions (In Situ Cell Proliferation kit; Roche Diagnostics), and nuclei were counterstained with DAPI. Anti-vWF (1:250, Sigma) and anti-CD31 antibodies (1:40, Abcam) were used to identify endothelial cells. *In situ* apoptosis detection in mouse lungs was carried out with the *in situ* cell death detection kit (Roche Applied Science). Anti-vWF anti-CD31 antibodes was used to stain ECs.

Primary cultures of human lung microvascular ECS. Human lung microvascular ECs were purchased from Lonza and cultured in EBM2 complete medium (Lonza). ECs with up to 7 passages were used for the studies. Subconfluent ECs were starved overnight before treated with either recombinant human SDF-1α (R & D Systems) or various reagents including AS605240 (Cayman Chemical Co.), TGX-221 (Cayman Chemical Co.), AS1842856 (Merck Millipore), and psammaphysene A (a gift from Dr. Robert Kalb).

Molecular analysis. Total RNA was isolated using an RNeasy Mini kit including DNase I digestion (Qiagen). Then QRT-PCR analysis was performed with a sequence detection system (ABI Prism 7000; Applied Biosystems/Invitrogen) with a SYBR Green 1-step kit (Invitrogen). Total RNA was isolated using an RNeasy Mini kit including DNase I digestion (Qiagen). Then QRT-PCR analysis was performed with a sequence detection system (ABI Prism 7000; Applied Biosystems/Invitrogen) with a SYBR Green 1-step kit (Invitrogen). The following primer sets were used for analyses: mouse FoxM1 primers, 5’-CACTTGGATTGAGGACCACTT-3’ and 5’-GTCGTTTCTGCTGTGATTCC-3’; and mouse Cyclophilin primers, 5’-CTTGTCCATGGCAAATGCTG-3’ and 5’-TGATCTTCTTGCTGGTCTTGC-3’; Primers for mouse ICAM-1, TNF-α, IL-6, iNOS, Cdc25C, cyclin B1, and cyclin A2 were purchased from Qiagen. Human FOXM1 primers, 5'-GGAGGAAATGCCACACTTAGCG-3' and 5'-TAGGACTTCTTGGGTCTTGGGGTG-3'; Human 18S rRNA primers, 5'-TTCCGACCATAAACGATGCCGA-3' and 5'- GACTTTGGTTTCCCGGAAGCTG-3'. The mouse gene expression was normalized to mouse Cyclophilin. The human gene expression was normalized to 18S rRNA.

Western blot analysis was performed using anti-p110γ (1:1000, Cell Signaling Biotechnology), and anti-FoxM1 (1:500, Santa Cruz Biotechnology), respectively. The same

blots were re-probed with an anti-β-actin antibody (1:3000, BD Biosciences) as loading control.

Liposome-mediated transduction of cDNA into mouse lung vascular endothelial cells.

Liposomes were prepared as described.[15,35] Briefly, the mixture comprised of dimethyldioctadecylammonium bromide and cholesterol (1:1 molar ratio) was dried using the Rotavaporator (Brinkmann), and dissolved in 5% glucose followed by 20 min sonication. The complex consisting of plasmid DNA expressing human p110γ or FOXM1 under the control of human *VE-cadherin* promoter or empty vector and liposomes was combined at the ratio of 1 μg of DNA to 8 nmol of liposomes. The DNA/liposome complex (50 μg of DNA/mouse) was injected into the retro-orbital venous plexus.

Online Supplement Figures and Figure Legends

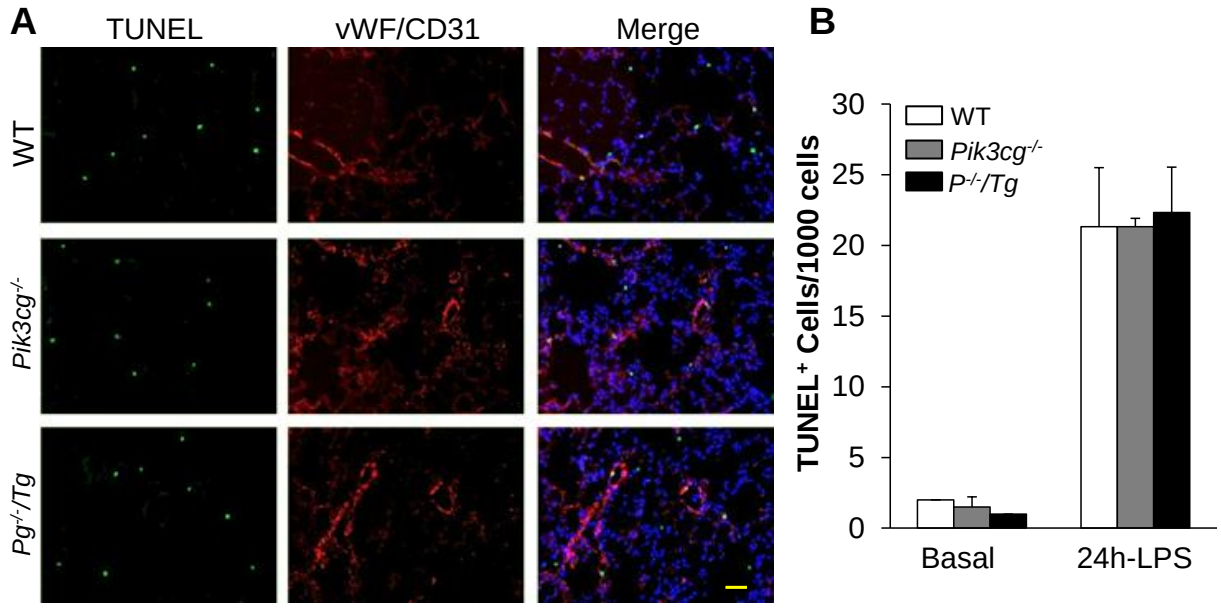

Figure 1. Quantification of cell apoptosis in mouse lungs. (**A**) Representative micrographs of TUNEL staining of mouse lungs. At 24h post-LPS challenge (7.5 mg/kg, i.p.), mouse lungs were collected for cryosectioning and TUNEL staining (green). ECs were immuniostained with anti-vWF and anti-CD31 antibodies (red). Nuclei were counterstained with DAPI (blue). (**B**) Quantfication of apoptosis. Data are expressed as mean ± SD (n=4-5mice/group). All mice of the 3 genotypes exhbited similar rate of apoptosis at 24h post-LPS challenge.

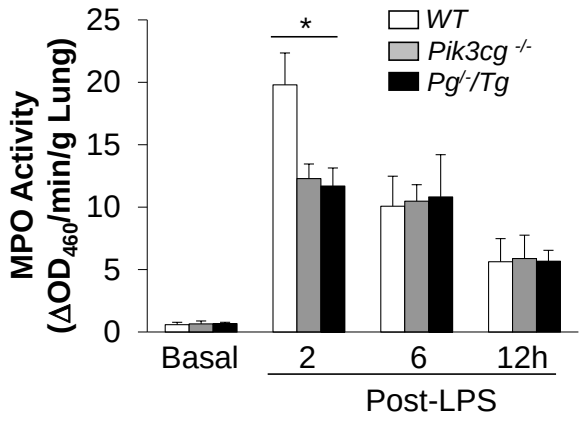

Figure 2. Decreased lung MPO activity in *Pik3cg*$^{-/-}$ mice at 2h post-LPS challenge. At 2h post-LPS (7.5 mg/kg, i.p.), lungs were perfuse free of blood with PBS and collected for MPO activity assay. Data are expressed as mean ± SD (n = 5 mice/group). *, P = 0.003 (ANOVA).

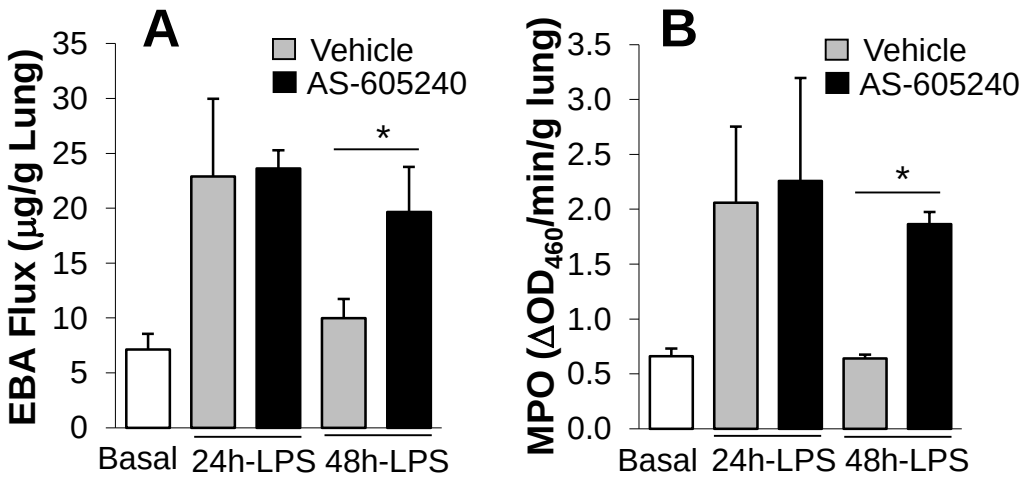

Figure 3. p110γ inhibition at 12h post-LPS challenge impairs vascular repair. (**A**) EBA extravasation assay demonstrating impaired vascular repair in mice treated with p110γ inhibitor. To exclude potential cofounding effect of p110γ inhibition on leukocytes recruitment in the initial responses, p110γ inhibitor, AS-605240 (30mg/kg BW, *per os,* every 12h) was administered into WT mice at 12h post-LPS challenge (7.5 mg/kg, i.p). 0.5% carboxymethycellulose/0.25% Tween-20 was used as vehicle control. At 24h or 48h post-LPS challenge, lungs were perfused free of blood with PBS and collected for EBA assay. Data are expressed as mean ± SD (n = 5). *, $P < 0.01$ (t test). (**B**) MPO activity assay revealing sustained elevation of lung inflammation in mice treated with p110γ inhibitor. *, $P < 0.01$ (t test).

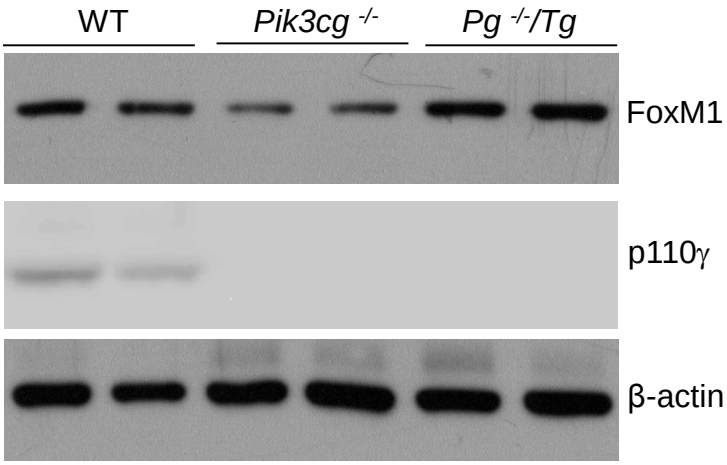

Figure 4. Western blotting demonstrating marked expression of FoxM1 in *Pg*$^{-/-}$*/Tg* mouse lungs. Representative Western blots showing prominent FoxM1 expression in *Pg*$^{-/-}$*/Tg* lungs in contrast to *Pik3cg*$^{-/-}$ lungs at 72h post-LPS challenge (7.5 mg/kg BW, i.p.). The experiments were performed 3 times with similar data.

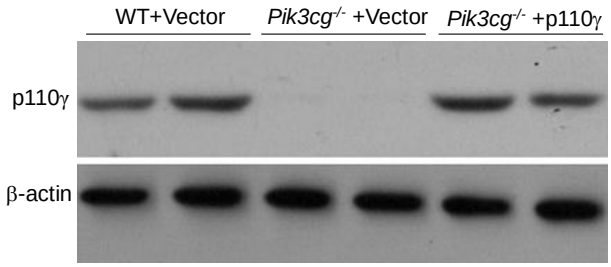

Figure 5. Liposome-mediated restoration of p110γ expression in *Pik3cg*$^{-/-}$ lungs. Plasmid DNA expressing human p110γ under the control of human *CDH5* promoter (p110γ) were transduced to *Pik3cg*$^{-/-}$ mice. As control, empty vector was transduced to *Pik3cg*$^{-/-}$ mice as well as WT mice. At 30h post-transduction, lung tissues were collected for Western blotting with an anti-p110γ antibody. Anti-β-actin was used as loading control. The experiment was repeated twice with similar results.

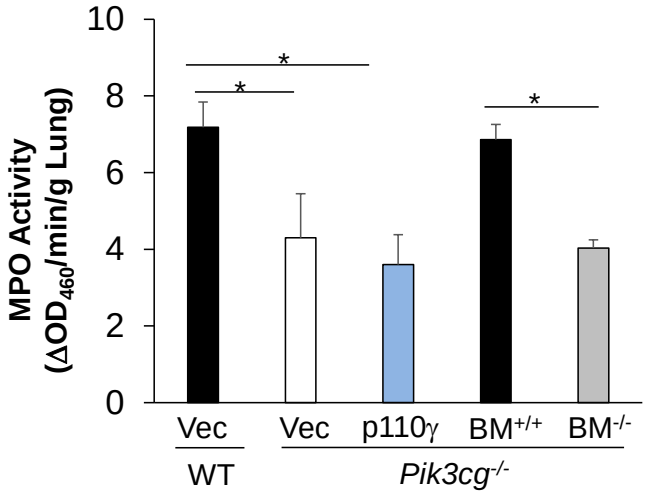

Figure 6. Impaired neutrophil recruitment to *Pik3cg*$^{-/-}$ mouse lungs in the initial response to LPS challenge was ascribed to p110γ deficiency in neutrophils not in ECs. WT and *Pik3cg*$^{-/-}$ mice were transduced with either empty vector DNA (Vec) or p110γ plasmid DNA (p110γ) expressing p110γ under the control of *CDH5* promoter. At 30h post-liposome transduction, the mice were challenged with LPS (7.5 mg/kg, i.p.) and lungs were collected at 2h post-LPS for MPO assay. In a separate study, *Pik3cg*$^{-/-}$ mice subjected to lethal irradiation were transplanted with bone marrow cells isolated from either WT (BM$^{+/+}$) or *Pik3cg*$^{-/-}$ (BM$^{-/-}$) mice. These chimeric mice were then challenged with LPS and lung tissues were collected at 2h post-LPS for MPO assay. Data are expressed as mean ± SD. n=4-5 mice/group. *, $P < 0.05$ (t test). Restored expression of p110γ in lung ECs of *Pik3cg*$^{-/-}$ mice by liposome-mediated transduction failed to rescue the defective neutrophil infiltration in *Pik3cg*$^{-/-}$ mouse lungs at 2h post-LPS whereas WT but not *Pik3cg*$^{-/-}$ bone marrow cell transplantation resulted in normalization of neutrophil recruitment and migration to *Pik3cg*$^{-/-}$ mouse lungs at 2h post-LPS challenge. These data demonstrate that p110γ deficiency in neutrophils not in ECs is responsible for the defective infiltration of neutrophils to mouse lungs at 2h post-LPS challenge seen in *Pik3cg*$^{-/-}$ mice.

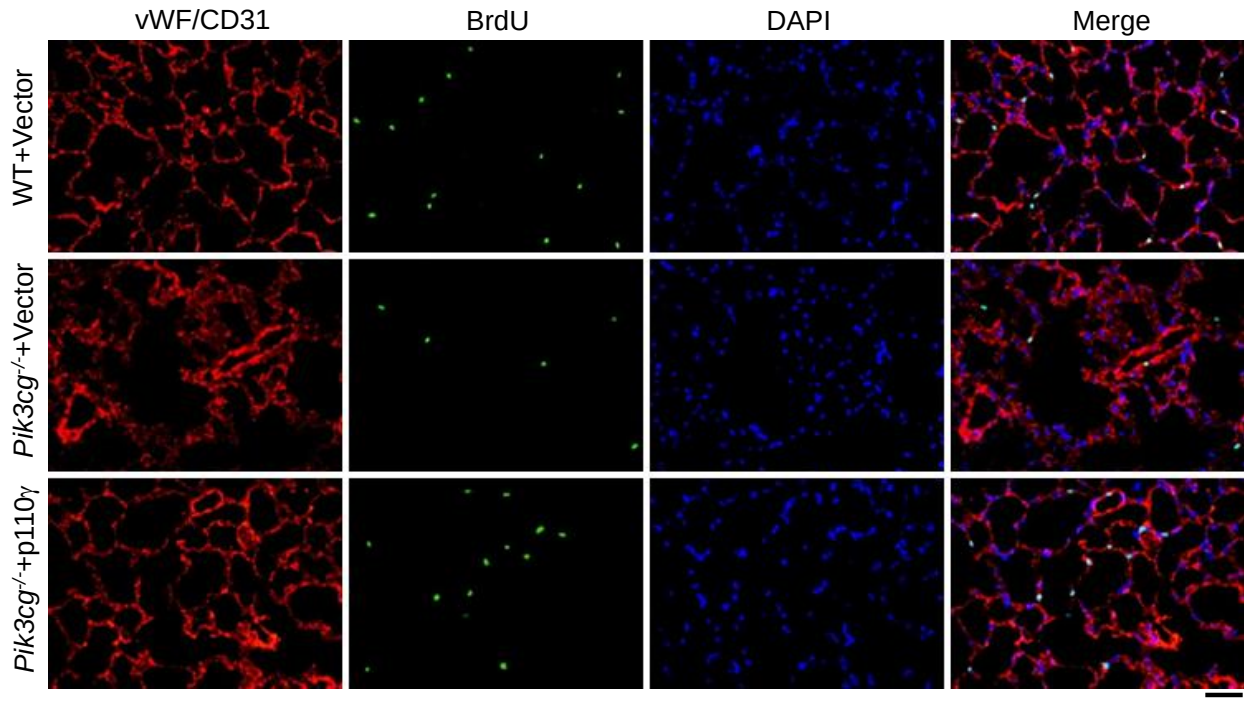

Figure 7. Representative micrographs showing normalized lung EC proliferation in *Pik3cg*$^{-/-}$ mice transduced with p110γ plasmid. At 30h post-liposome-mediated Plasmid DNA transduction [empty vector (Vector) or plasmid DNA expressing p110γ under control of the *CDH5* promoter (p110γ)], mice were challenged with LPS (7.5 mg/kg, i.p.). At 60h post-LPS challenge, lung tissues were collected for cryosectioning and immunostaining. BrdU was administered i.p. at 4h prior to tissue collection. Scale bar, 50μm.

Online Supplement References:

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5. Kolosova IA, Mirzapoiazova T, Moreno-Vinasco L, Sammani S, Garcia JG, Verin AD. Protective effect of purinergic agonist ATPgammaS against acute lung injury. *Am J Physiol Lung Cell Mol Physiol.* 2008; 294: L319-324.
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7. Barthel SR, Wiese GK, Cho J, Opperman MJ, Hays DL, Siddiqui J, Pienta KJ, Furie B, Dimitroff CJ. Alpha 1,3 fucosyltransferases are master regulators of prostate cancer cell trafficking. *Proc. Natl. Acad. Sci. U S A.* 2009;106:19491-19496.