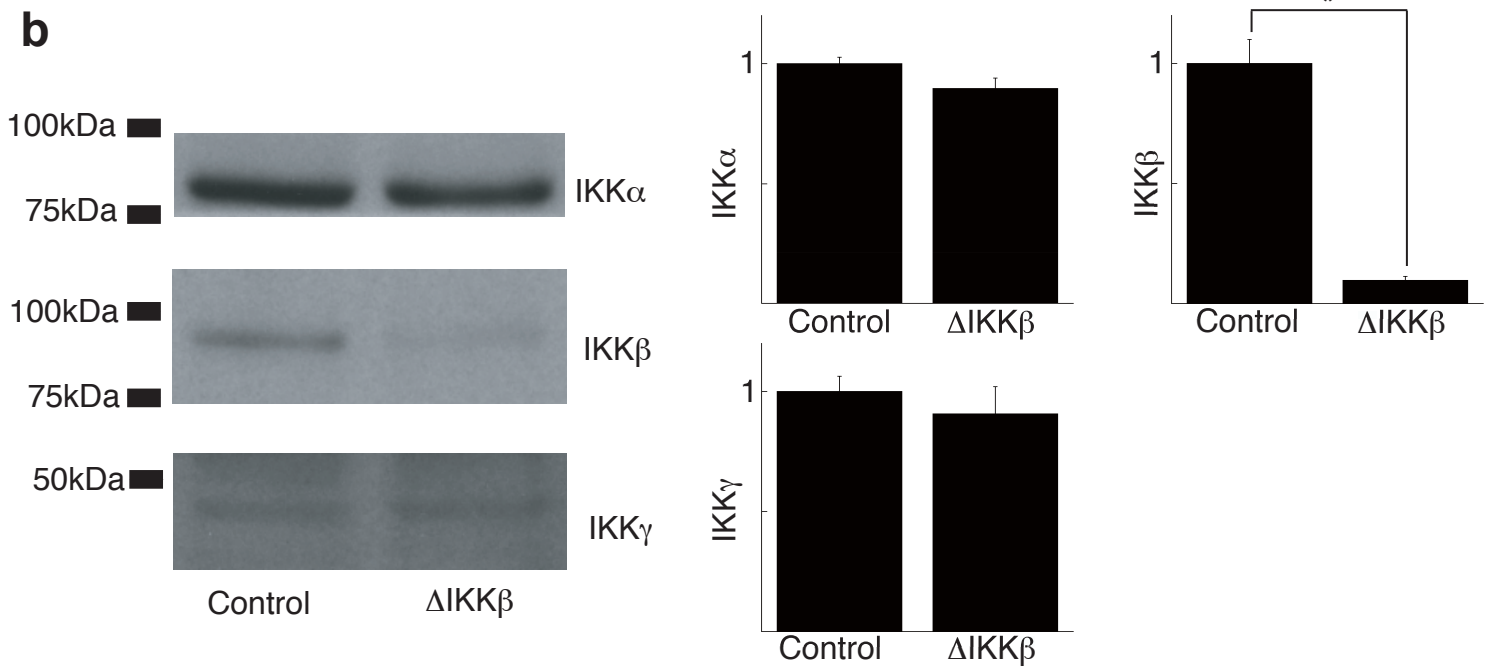
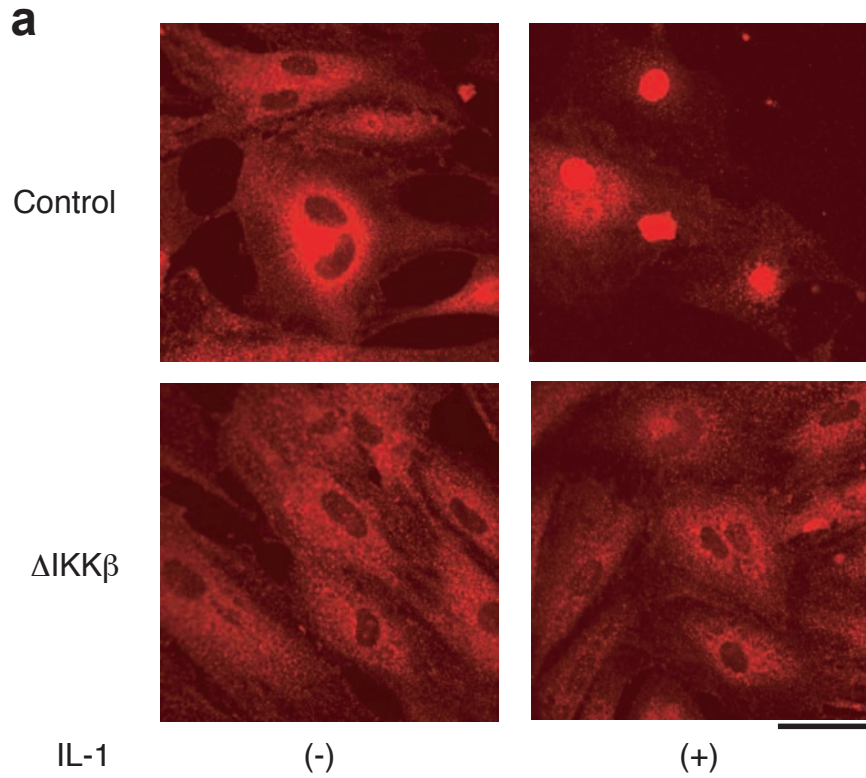
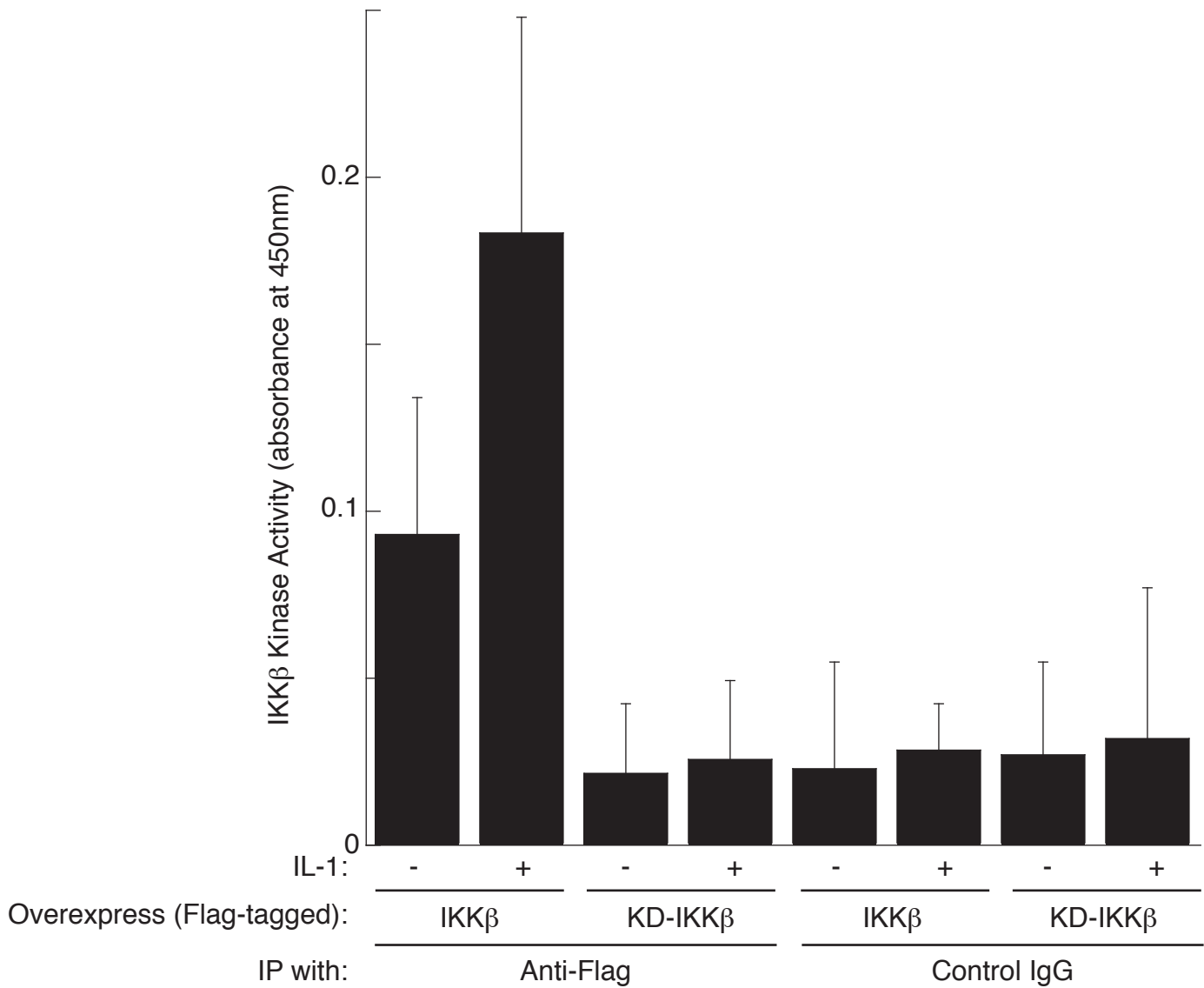


Supplementary Figure S1. Endothelial cells in $\Delta IKK\beta$ mice (a) Lungs from control and $\Delta IKK\beta$ mice were stained with anti-von Willebrand Factor antibody. (b) Electron microscope image of endothelial cells. x44,000. RBC: red blood cells. (c) Electron microscope image of endothelial cells. x94,800. Arrows point at caveolae. (d) Electron microscope image of endothelial cells. x56,800. Arrow points tight junction. (e) Electron microscope image of endothelial cells. x111,300. Arrow points Weibel-Palade body. Scale bars: (a)100 μ m (b - e) 100nm

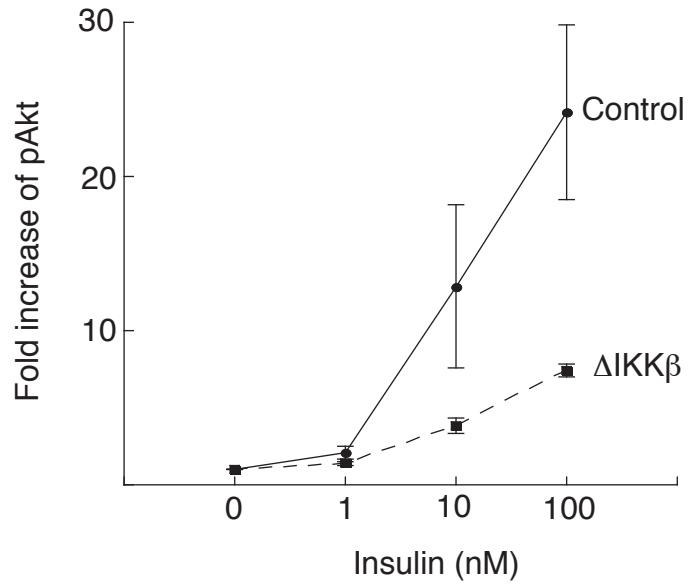


Supplementary Figure S2. Translocation of p65 to nucleus and the expression of IKK $\alpha/\beta/\gamma$ in IKK β -deleted endothelial cells. (a) Primary cultured endothelial cells from lungs of IKK β flox/flox mice with/without infection of Ad-Cre were stimulated with 2.5ng/ml IL-1, and stained with anti-p65 antibody (n=3). Scale bar: 10 μ m (b) Representative blots for IKK α , IKK β and IKK γ in IKK β -deleted endothelial cells. Graphs indicate fold increase of cumulative quantitative densitometry to control from six independent experiments in each group (p<0.0001 in two-side Student's t-test for IKK β (*), p>0.05 in two-side Student's t-test for both IKK α and IKK γ). Error bars: \pm SE

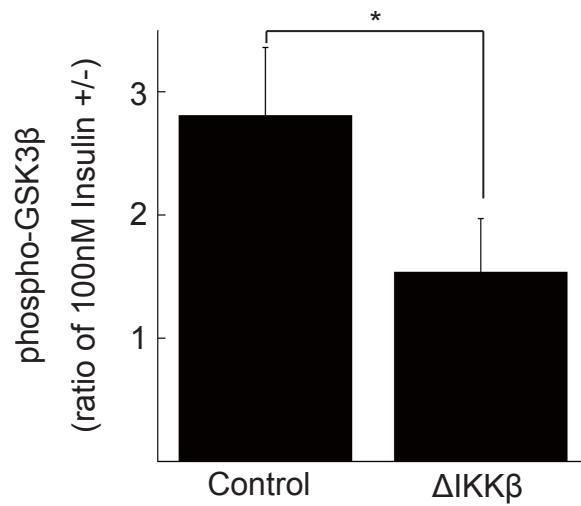
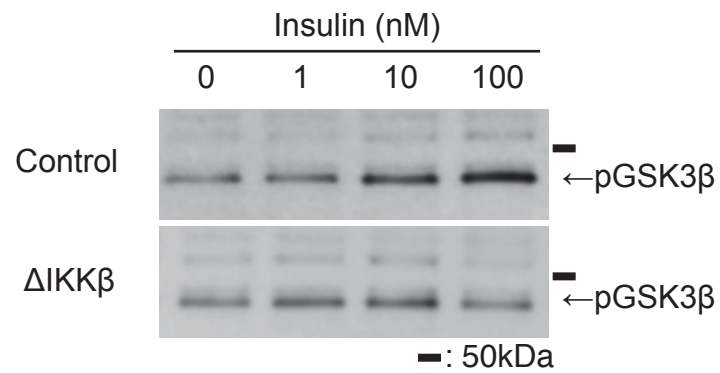


Supplementary Figure S3. IKK β kinase activity of adenovirus IKK β or KD-IKK β .

Primary cultured endothelial cells infected with Flag-tagged adenovirus of IKK β or KD-IKK β were stimulated with 2.5ng/ml for 30min. Each cell lysate was immunoprecipitated with anti-Flag antibody or control IgG, and subjected to IKK kinase assay. Cumulative data \pm SD from three independent experiments are shown.

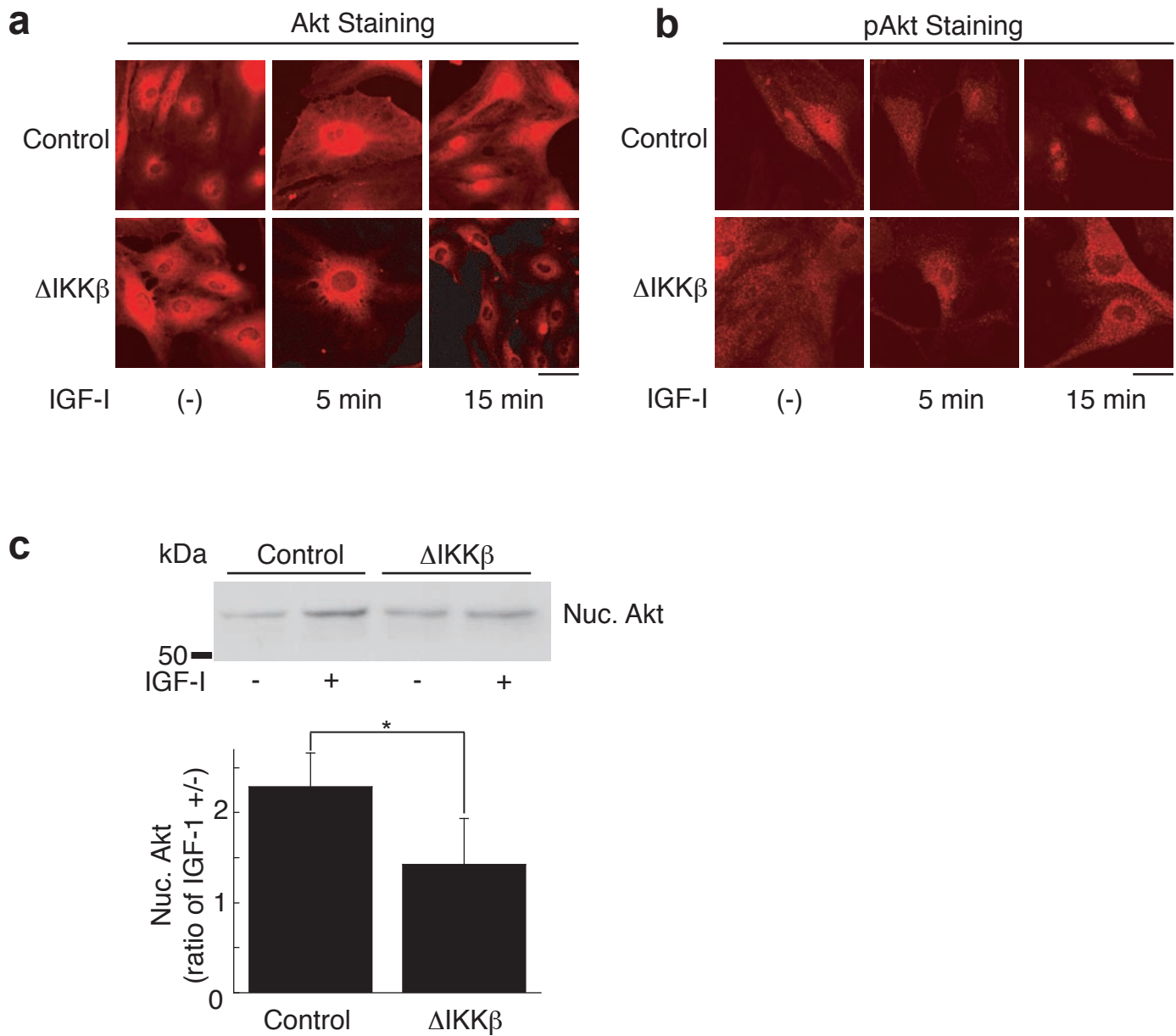


Supplementary Figure S4. Dose-dependent phosphorylation of Akt by insulin stimulation Primary cultured endothelial cells from lungs of IKK β flox/flox mice with/without infection of Ad-Cre were stimulated with insulin for 15min. Phosphorylation of Akt was quantified by accumulative densitometry of Western blots from three independent experiments in each group. Error bars: \pm SE.



Supplementary Figure S5. Change of GSK3β phosphorylation in IKKβ-deleted cells

(a) Representative blots for pGSK3β after 15min insulin stimulation at indicated concentration. (b) Cumulative quantitative densitometry data \pm SE from six independent experiments are shown. * $p < 0.05$ in two-side Student's t-test.



Supplementary Figure S6. Intracellular Akt trafficking in IKK β -deleted endothelial cells.

(a - b) Representative confocal microscopy images of intracellular trafficking pattern of Akt (a) and phospho-Akt (b) in IKK β -deleted or control endothelial cells treated with/without IGF-I. Scale bars: 10 μ m (c) Translocation of Akt to nucleus in IKK β -deleted or control endothelial cells after IGF-I stimulation. Representative immunoblots and cumulative quantitative densitometry data \pm SD from three independent experiments are shown (* p <0.05 in two-side Student's t-test).

Supplementary Methods

Electron microscopy

Tissue was fixed with a 2.5% glutaraldehyde, 2% paraformaldehyde, and 0.025% calcium chloride in 1.0M sodium cacodylate buffer (pH 7.4) for 2 hours, postfixed in 1.5% sym-collidine-buffered osmium tetroxide for 2 hours, stained en bloc with uranyl acetate, dehydrated in a series for graded alcohols, and embedded in Eponate. After polymerization at 60 degrees for 16 hours, thin sections were cut using a diamond knife on an ultramicrotome (Leica, Heidelberg, Germany). Thin sections of 85nm, were visualized with a Philips CM-10 electron microscope at 80kV³⁰.

Immunocytochemistry

Cells fixed with 4% PFA were incubated with diluted primary antibodies for Akt (1:50), pAkt (1:50) and p65 (Santa Cruz Biotechnology, 1:50) overnight at 4° C. Diluted rhodamine-conjugated donkey anti-rabbit antibody (Jackson Immunoresearch Laboratories, 1:200) was applied to the cells for 60 minutes.

IKK β kinase assay

IKK β kinase assay was performed in accordance with the manufacturer's recommendation (HTScan® IKK β Kinase Assay Kit, Cell Signaling #7549). Immunoprecipitated IKK β was incubated with I κ B- α (Ser32) Biotinylated Peptide and ATP (30min, room temperature) before transfer to 96-well streptavidin-coated plates. After incubation (60min, room temperature) and washing (PBS/Tx3), phospho-I κ B- α antibody (1:1000) was added and colorimetric ELISA detection performed.

Supplementary Reference

- ³⁰ Feng, D., Nagy, J. A., Dvorak, H. F. & Dvorak, A. M. Ultrastructural studies define soluble macromolecular, particulate, and cellular transendothelial cell pathways in venules, lymphatic vessels, and tumor-associated microvessels in man and animals. *Microsc Res Tech* **57**, 289-326 (2002).