**Supplementary Materials and Methods**

**Animals.** Wild type and Ang-2-deficient mice backcrossed in the C57Bl/6 background were housed and bred from heterozygous animals. Animals of both sexes were used at 6-12 weeks of age. All experiments were performed in accordance with the German Legislation on the Protections of Animals and the Guide for the Care and Use of Laboratory Animals. All animal experiments were approved by the respective local regulatory committee (Freiburg, Karlsruhe, Homburg/Saar).

**Materials.** Recombinant myc-tagged Ang-1, myc-tagged Ang-2, and sTie2-Fc were produced and purified as described previously[1,2]. In brief, proteins were expressed in baculovirus infected Sf9 cells and purified from the supernatants. Myc-tagged Ang-1 and myc-Ang-2 were purified using a Tie2-Fc-ProteinA-sepharose column as described previously. The protein containing fractions were pooled and dialyzed against 50 mM Tris-HCl pH 7.5, 150 mM NaCl and 0.05% CHAPS. Purified samples were stored frozen and tested for activity prior to use in a 3D sprouting angiogenesis assay[3]. Soluble Tie2-Fc was purified from the supernatant of sTie2-Fc expressing Sf9 cells as described previously[1]. TNF$\alpha$ was purchased from R&D Systems (R&D Systems, www.rndsystems.com). Predesigned annealed siRNAs were purchased from Ambion (Ambion, www.ambion.com).

**Cell culture.** Human umbilical vein endothelial cells (HUVEC), endothelial cell growth medium (EGM) and endothelial cell basal medium (EBM), and corresponding supplements were purchased from Promocell (PromoCell, www.promocell.com). Cells were cultured at 37°C, 5% $CO_2$ in the appropriate growth medium containing 10% fetal calf serum (Invitrogen, www.invitrogen.com). HUVEC were used between passage 2 and 5.

**SiRNA transfection of HUVEC.** HUVEC were seeded in 6-well dishes at a density of 120000 cells per well 24 h prior to the experiment. Cells were transfected with pre-designed annealed siRNAs (ID# 121284, ID# 121287) directed to human Ang-2 (100 nM each) and a negative control siRNA (ID# 4611) (Ambion, www.ambion.com) using Oligofectamine (Invitrogen, www.invitrogen.com) according to the manufacturer's instructions. The transfection was carried out in 1 ml OptiMEM (Invitrogen, www.invitrogen.com). Five hours after transfection, cells were supplemented with additional 3 ml endothelial cell growth medium and cultured for another 2 days. Downregulation of Ang-2 mRNA was analyzed by RT-PCR as described previously[4] and downregulation of Ang-2 protein was verified by Western blotting. To this end, conditioned medium of transfected HUVEC (48 h after transfection) was precipitated with sTie2-Fc and ProteinA-Sepharose. Samples were

resolved on a 10% SDS-PAGE and blotted. Ang-2 was detected using an Ang-2 specific antibody (Regeneron Pharmaceuticals, www.regeneron.com).

**Flow cytometry of mouse peripheral blood leukocytes.** Identification of mouse peritoneal neutrophils and analysis of leukocyte populations in the peripheral blood was assessed using directly conjugated, subset-specific rat-anti mouse antibodies. These included Gr-1 (APC, clone RB6-8C5), CD19 (PE, clone 1D3) and CD4 (Cychrome, clone RM4-$5_a$), and corresponding isotype controls (all purchased from BD Pharmingen, www.pharmingen.com). Rat anti-mouse F4/80 antigen (FITC, clone MCA497F) was from Serotec (www.serotec.com). For flow cytometry, neutrophils were harvested from the peritoneal cavity as described (see peritonitis experiments). To obtain peripheral blood leukocytes, mouse blood was collected and sedimentation of erythrocytes was achieved using PBS-EDTA buffer (PBS, 5mM EDTA, 2% Dextran) for 30 min at 37°C. Further depletion of red blood cells was performed with red blood cell lysis buffer (Roche, www.roche-diagnostics.com). After blocking with 10% rat IgG, cells were stained with 1 µg Gr-1-APC or a combination of F4/80-FITC, CD19-PE, CD4-Cychrome, and Gr-1-APC for 30 min on ice. Cells were washed, and analyzed using CELLQuestTM software (Becton Dickinson, www.bd.com).

**Flow cytometry of human endothelial cells.** Confluent siRNA transfected HUVEC monolayers were stimulated with 0.01 ng/ml TNF$\alpha$ in the presence or absence of 2 µg/ml Ang-2 in 1 ml/well EBM containing 10% FCS at 37°C. After 4 h, cells were washed with PBS, detached from the cell culture dish with accutase (PAA Laboratories, www.paa.at), and suspended in FACS-buffer (HBSS, 25mM Hepes, 10% FCS). Cells were centrifuged at 200 x g for 5 min at 4°C. The supernatant was removed and cells were stained for ICAM-1 on ice. Unspecific binding sites were blocked with 50µl of 10% goat serum in PBS for 20 min. Cells where then incubated with biotinylated anti-human ICAM-1 antibody (R&D systems, www.rndsystems.com) (final concentration: 1 µg/ml) for 20 min. Cells were washed with 1 ml FACS-buffer followed by centrifugation at 200 x g for 5 min at 4°C. Antibody binding was detected with Cy5-conjugated streptavidin (BD Pharmingen, www.pharmingen.com) for 15 min in the dark. Cells were washed again with FACS-buffer and fixed in 1% PFA / PBS at 4°C. Cells (10.000 per experimental group) were analyzed for ICAM-1 expression by flow cytometry (FACSCalibur, BD Biosciences) applying the Cell Quest software (BD Biosciences). Intensity of ICAM-1 expression was quantitatively plotted as mean fluorescence intensity (mean fluorescence channel of experimental group minus mean fluorescence channel of corresponding control group).

**Flow cytometry of murine neutrophils.** Murine neutrophils were collected by peritoneal lavage following thioglycollate-induced peritonitis. Collected cells were washed with FACS-buffer (HBSS, 25mM HEPES, 10% FCS) and stained with anti-mouse Tie-2 IgG (clone 4G8HE, www.chemicon.com). Phycoerythrin labelled $F(ab`)_2$ fragment (www.jacksonimmuno.com) was used as secondary antibody. Cells were analyzed for Tie-2 expression by flow cytometry (FACSCalibur, BD Biosciences) applying the Cell Quest software.

**Western blot analysis**. HUVE cells were grown to confluence in endothelial cell growth medium. Cells were stimulated with 0.01 ng/ml TNF$\alpha$ in the absence and presence of Ang-2 in endothelial cell basal medium containing 10% FCS for 4 h. Whole cell lysates were resolved on a 10 % SDS PAGE, blotted and probed with an anti-ICAM-1 antibody (H-276) and anti-VCAM-1 antibody (H-108) (Santa Cruz, www.scbt.com). The stripped blots were then re-probed with an anti-actin antibody (I-19) (Santa Cruz, www.scbt.com) to monitor equal loading.

**Immuncytochemical analysis.** HUVEC were grown to confluence in endothelial cell growth medium. Cells were stimulated with 0.01 ng/ml TNF in the absence and presence of Ang-2 in endothelial cell basal medium containing 10% FCS for 4 h. Cells were washed twice with PBS and fixed with cold methanol. Fixed cells were washed twice with PBS and blocked with 250 µl buffer (PBS containing 5 % donkey serum) for 30 min. After blocking, an anti-human ICAM-1 antibody (BBA 17; R&DSystems, www.rndsystems.com) or anti-human VCAM-1 antibody (BBA 19; R&DSystems, www.rndsystems.com) 1:200 diluted in blocking buffer was added for 1 h at room temperature. The cells were washed three times for 10 min with PBS before the addition of the Cy3 coupled donkey-anti-goat antibody for 30 min at room temperature. Cells were washed three times and incubated with Hoechst dye for an additional 5 min.

**Ang-2/vWF staining of human tissues.** Immunhistochemistry and immunofluorescence stainings were performed on unfixed frozen samples on a human tissue array (BioChain, www.biochain.com). Sections were air-dried for 1 h at room temperature and fixed with 4% paraformaldehyde for 30 min. Nonspecific binding was blocked by incubation in 10% goat serum in PBS for 20 min. Slides were incubated with the mouse-anti human Ang-2

monoclonal antibody 4B10 (Regeneron, www.regeneron.com) and vWF (Dako, www.dako.com) over night at 4°C. The primary antibodies were detected with a Cy3-coupled goat anti-rabbit IgG antibody and a goat-anti-mouse biotinylated antibody. The biotinylated antibody was detected using Streptavidin-Alexa 488.

***Streptococcus pneumoniae* infection experiments.** Infection experiments in homozygous Ang-2-deficient mice and wild type littermates were performed by intranasal application of 20 µl of a bacterial suspension of *Streptococcus pneumoniae* type 3 (NCTC 7978) ($5 \times 10^6$ cfu) into anesthetized mice (10 weeks old, 19-22 g of weight). Mice were sacrificed 36 h after infection and a bronchoalveolar lavage was performed. A total of 1 ml lavage fluid was retrieved per mouse and total cell numbers were determined from each sample by hemocytometer count. The BAL fluid differential cell count was determined by FACS analyses.

## Reference


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