SUPPLEMENTAL MATERIAL

Expanded Methods

Cell culture

The Human Embryonic Kidney (HEK 293T) cell line was cultured in Dulbecco´s Modified Eagle´s Medium (DMEM; Gibco, Paisley, UK). The *Drosophila* embryonic Schneider S2 cell line was grown in *Drosophila*-Enriched Schneider´s (DES) insect medium (Sigma Aldrich, St Louis, MO, US). The primary culture of Human Umbilical Vein-derived Endothelial Cells (HUVEC) was obtained from Lonza (Walkersville, MD, US). HMEC-1 line is an immortalized Human dermal Microvascular Endothelial Cell line generated from human neonatal foreskin.[1,2] These cells retain the morphologic, phenotypic, and functional characteristics of normal human microvascular endothelial cells and have been used for wound healing experiments.[3] The HMEC-1 line was kindly provided by Dr. Edwin W. Ades (Emory University School of Medicine, Atlanta, Georgia, USA). Both HMEC-1 and HUVEC were cultured in gelatin precoated plates and grown in Endothelial Basic Medium (EBM2) supplemented with Endothelial Growth Medium (EGM2; Lonza). Human Umbilical Artery Smooth Muscle Cells (UASMCs) were obtained from Lonza and cultured in Clonetics SmGM-2 BulletKit (CC-3182, Lonza). All the media were supplemented with 2 mM L-glutamine, 10% of fetal bovine serum (FBS; Gibco) and 100 U/ml penicillin and streptomycin (Gibco). When required, cells were treated with the ALK5 inhibitor SB431542 (S4317, Sigma-Aldrich) at 5μmol/L or with endothelial cell derived conditioned media, as indicated. For endothelial denudation injury, 50- to 300-μm–wide wounds were systematically created with a sterile pipette tip throughout a confluent monolayer of HUVECs or HMEC-1 cells. Plates were washed, fresh medium was added, and cells were cultured at 37ºC. In some cases, HMEC-1 cells and HUVECs were transiently transfected with pCIneo-Sp1 and pCIneo-KLF6, prior to *in vitro* endothelial cell denudation.

Mice and mechanical injury experiments

Generation of *Klf6*$^{+/-}$ mice of the C57BL/6 strain has been previously reported[4]. For the mechanical injury experiments, *Klf6*$^{+/+}$ and *Klf6*$^{+/-}$ mice were anesthetized with Forane and underwent bilateral endoluminal injury to the common femoral artery by passing 3 times a 0.25 mm-diameter angioplasty guidewire as described previously.[5] At 28 days post-injury, mice were killed and perfused *in situ* with 5 ml of PBS followed by 10 ml of freshly prepared 4% paraformaldehyde/PBS using a peristaltic pump at approximately 1 mL/min. Hind limbs were isolated in block and the fixation continued for 24-48 hr. Specimens were decalcified for 24 hr at RT with mild shaking in Osteodec (Bio-Optica). After washes with PBS, transverse segments (approximately 2 mm-thick) were cut at the level of the injury, embedded in paraffin, and 5 μm cross-sections were obtained throughout the injured fragment. Alk1 was stained as described above, followed by hematoxylin-eosin staining. For the study, fifteen *Klf6*$^{+/+}$ and seven *Klf6*$^{+/-}$ mice underwent bilateral femoral artery injury, whereas four *Klf6*$^{+/+}$ and two *Klf6*$^{+/-}$ animals were uninjured controls.

Plasmids and DNA transfection assays

The cloning of the -1,035/+210 sequence and four 5´-deleted fragments (-898/+59, -587/+59, -422/+59 and -284/+59) of *ALK1* promoter (p*ALK1*) in the pGL2-*luc* reporter plasmid was previously described.[6] For KLF6 expression in *Drosophila* Schneider S2 cells, pAc-KLF6[3] was used. For expression in mammalian cells (HEK293T and HMEC-1), the plasmids used were pCIneo-Sp1 and pCIneo-KLF6.[7,8] The pGL3-IL6-Luc reporter construct driven by a 651-bp promoter fragment of human IL-6 has been described[9] and was kindly provided by Dr. Manuel Fresno (Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Cantoblanco, Madrid, Spain). DNA transfections were performed using the Superfect Reagent (Qiagen, Hilden, Germany) following the manufacturer´s guide. For luciferase assays, HEK293T cells were co-transfected with 500 ng/well of p*ALK1* in pGL2-*luc* reporter-construct (pGL2-p*ALK1*) and with different amounts of pCIneo-KLF6 and pCIneo-Sp1 for each condition, as indicated in the text. The pCIneo empty vector was used to equalize the amount of DNA in each well. Schneider S2 cells were transfected with 1 μg/well of pGL2-p*ALK1* and with different amounts of pAc-KLF6 for each condition, as indicated. The pAc empty vector was used to

complete the amount of DNA in each well. pRL-TK plasmid (Promega, Madison, WI, US) was co-transfected in both cases as a control for transfection efficiency. Forty eight hours after transfection, cell lysates were analyzed using dual-luciferase reporter assay system (Promega) in a Dynex luminometer (Dynex Technologies, Chantilly, VA, US). Transfection efficiency was normalized to *Renilla* luciferase activity. For overexpression of Sp1 and KLF6, HMEC-1 cells were transfected with Superfect, whereas HUVECs were nucleofected with Amaxa HUVEC nucleofector kit (VPB-1002, Lonza), using pmax-GFP vector as a control of efficiency. Both cell types were transfected with 1 µg/plate of pCIneo-Sp1 and/or pCIneo-KLF6, completing with pCIneo-empty vector. To assess the effectivity of SB431542 inhibitor, the sequence -1585/+88 of the ID1 human promoter inserted in pGL2 was used.[10]

Knock down assays with siRNA

HUVECs were transfected with siRNA specific for KLF6 (S3374, Ambion), Sp1 (S13319, Ambion) or a negative control scrambled siRNA (Ambion) with lipofectamine 2000. Each siRNA was added to a final concentration of 20 nmol/L using OptiMEM (Gibco). For nucleofection experiments, cells were nucleofected with Amaxa HUVEC nucleofector kit (VPB-1002, Lonza). KLF6 was overexpressed with pCiNeo-KLF6 or suppressed with pSuperKLF6, using pmax-GFP vector as a control of efficiency.

Real time PCR

For quantitative analysis of the amount of ALK1, Endoglin, GAPDH (Glyceraldehyde-3-phosphate dehydrogenase), KLF6, ALK5 or Pai1 mRNA transcripts, total RNA was isolated from HUVECs or UASMCs using the RNeasy kit (Qiagen) and was reverse-transcripted using Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase (iScript cDNA Synthesis kit; BioRad, Hercules, CA, US). The resultant cDNA was used as a template for real time PCR performed using the LightCycler 480 PCR Master SYBR Green (Roche Applied Biosciences, Indianapolis, IN, US) with the following forward (Fwd) and reverse (Rev) primers: hALK1 Fwd 5´-ATCTGAGCAGGGCGACAGC-3´ and Rev 5´-ACTCCCTGTGGTGCAGTCA-3´; hENG Fwd 5´-GCCCCGAGAGGTGCTTCT-3´ and Rev 5´-TGCAGGAAGACACTGCTGTTTAC-3´; hGAPDH Fwd 5´-AGCCACATCGCTCAGACAC-3´ and Rev 5´-GCCCAATACGACCAAATCC-3´; hKLF6 Fwd 5´-CGGACGCACACAGGAGAAAA-3´ and Rev 5´-CGGTGTGCTTTCGGAAGTG-3´; hALK5 Fwd 5´-CATTAGATCGCCCTTTTATTTC-3´ and Rev 5´- CACAATAGTTCTCGCAATTGTT -3´; hPAI1 Fwd 5´-CACCCTCAGCATGTTCATTG-3´ and Rev 5´-GGTCATGTTGCCTTTCCAGT-3´. As an internal control, mRNA levels of 18S were measured using primers Fwd 5´-CTCAACACGGGAAACCTCAC-3´ and Rev 5´-CGCTCCACCAACTAAGAACG-3´. For quantitative RT-PCR measure of murine mRNA transcripts from *Klf6*$^{+/-}$ mice vs. wild type, the following primers were used: mAlk1 Fwd 5´-TGACCTCAAGAGTCGCAATG-3´ and Rev 5´-CTCGGGTGCCATGTATCTTT-3´; mEng Fwd 5´-CTTCCAAGGACAGCCAAGAG-3´ and Rev 5´-TTCTGGCAAGCACAAGAATG-3´; and mKlf6 Fwd 5´-GAGGCTGGCAGCGGAGCTTTG-3´ and Rev 5´-GTCGGGTTGGAAAAGACAGTCC-3´. As an internal control, mRNA levels of mGapdh were measured using primers mGapdh Fwd 5´-CAACGACCCCTTCATTGACC-3´ and Rev 5´-GATCTCGCTCCTGGAAGACG -3´. Amplicons were detected using a LightCycler 480 System II-384 (Roche Applied Biosciences). Triplicates of each experiment were performed.

Immunofluorescence Microscopy

In order to monitor the KLF6 translocation to the nucleus, HUVECs or UASMCs were grown to confluence onto 12 mm-diameter coverslips previously coated with 0.2% (v/v) gelatin (Sigma-Aldrich) in PBS. Then, monolayers were disrupted several times with micropipette tips, so that ~75% of the surface was denuded. At different time points, cells were fixed with 3.5% (v/v) formaldehyde in PBS. Afterwards, cells were permeabilized with 100 µg/mL L-α-lysophosphatidylcholine (Sigma-Aldrich) prior to the incubation with the primary antibody against human KLF6 (sc-7158; Santa Cruz Biotechnology, Santa Cruz, CA, US). After blocking, samples were incubated with Alexa 488 goat anti-rabbit IgG (Molecular Probes, Invitrogen; Eugene, OR, US). For nuclear counterstaining, the slides were incubated for 5 minutes with 4',6-diamidino-2-phenylindole (DAPI) at 10% in PBS and mounted with coverslips using Prolong mounting reagent (Molecular Probes, Invitrogen). Results

were observed with a spectral confocal microscope (Leica Microsystems; Bannockburn, IL, US). For the semiquantification of fluorescence intensity, the Image J™ software tool was used.

Flow cytometry

For the analysis of ALK1 expression levels at the endothelial cell surface, HUVECs collected at different time-points after the endothelial injury were fixed with 3.5% paraformaldehyde/PBS during 5 minutes and incubated with a monoclonal antibody against human ALK1 (MAB370, R&D Systems) or a polyclonal antibody against human ALK5 (SC-398, V-22 epitope, Santa Cruz Biotechnology). As a negative control, a primary irrelevant antibody from the same isotype of anti-ALK1 (IgG_{2A}) or a pool of rabbit IgGs were used, in each case. After washing, cells were incubated with Alexa 488 goat anti-mouse or goat anti rabbit IgG (Molecular Probes, Invitrogen). The fluorescence intensity was estimated with a EPICS XL flow cytometer (Coulter, Hialeah, FL, US). A minimum of 10,000 cells were counted for each experimental point. When necessary, the percentage of positive cells and the mean fluorescence intensity are indicated in each histogram (Figs. 5B, 6A, 7A, 7C and 8A). As a control to assess that ALK1 detection in HUVEC and UASMC corresponds to surface expression and not to intracellular ALK1, cells were incubated with 100 μg/mL L-α-lysophosphatidylcholine (Sigma-Aldrich) to permeabilize the cell membrane, prior to the incubation with the primary antibody (Supplementary Fig. V).

Immunohistochemistry

Paraffin embedded sections of livers or femoral arteries were prewarmed at 60ºC and deparaffinized with xylene prior to hydration with a series of ethanol graded dilutions followed by distilled water. Then, slides were subjected to epitope retrieval with citrate buffer pH 6.0 for 45 min at 95ºC in a water bath. The endogenous peroxidase activity of the tissue as well as unspecific epitopes were blocked. All the reagents used belong to the Novolink Polymer Detection System kit for IHC (Novocastra, Millipore; Billerica, MA, US). Alk1 staining was detected with rat anti-mouse monoclonal anti-Alk1 antibody (CMA106; Cell Sciences. Canton, MA, US) and with biotin goat anti-rat IgG (H+L) (Molecular Probes), followed by incubation with streptavidin-HRP (Cat #21126; Pierce. Rockford, IL, US). IL-6 staining was detected with a rabbit polyclonal antibody anti-IL-6 (ab6672; Abcam, Cambridge, MA, US). For development of the peroxidase activity, 3,3'-diaminobenzidine (DAB) chromogen was used. Nuclei were counterstained with Mayer´s haematoxylin 0.02% followed by immersion in ammonia water. Slides were dehydrated in a graded ethanol series and immersed in xylene previous to mounting in HiMo (Bio-Optica, Milano, Italy).

Laser microdissection (LMD) and qPCR analysis

Ten μm paraffin-embedded sections from hind limbs containing the mouse femoral artery were mounted on PET-slides (Leica Microsystems) and stained with Mayer's hematoxylin following a standard protocol. Tissue structures were visualized and then LMD was performed using a fully motorized LMD6000 system (Leica) under a 20× objective. Microdissected tissues were collected in triplicates in the lid of 0.5 ml microtubes, pelleted and resuspended in 250 μL TRIzol reagent (Invitrogen) for RNA isolation. Total RNA was quantified in a NanoVue Spectrophotometer and equal RNA amounts retrotranscribed to cDNA with the iScript cDNA Synthesis Kit (Bio-Rad). Then, qPCR was set up to detect murine Alk1 using the primers pair indicated above in an iQ5 PCR thermal cycler (Bio-Rad). The 18S gene was used as an internal housekeeping gene control. Data were analyzed using the CFX Manager software (Bio-Rad).

Chromatin immunoprecipitation (ChIP)

ChIP was performed with ChIP-IT Express kit (Active Motif, Rixensart, Belgium), following the manufacturer´s instructions. Briefly, HUVEC were grown to confluence and subsequently fixed with 1% formaldehyde in Opti-MEM medium (Gibco). Cells were scrapped in the presence of PMSF (phenylmethylsulphonyl fluoride) and lysed. Nuclei were separated using a dounce homogenizer and digested with enzymatic shearing cocktail for 15 min. One aliquot of this sheared chromatin was used as "input chromatin" and the rest was incubated with protein G magnetic beads and 10 μg of rabbit polyclonal antibody anti-human KLF6 (R-173; sc-7158, Santa Cruz Biotechnology), anti-histone H3 rabbit antiserum (07-690, Upstate Biotechnology, Inc. Charlottesville, VA, US) or control IgG (sc-

2027, Santa Cruz Biotechnology). Protein G magnetic beads bound to the immune complexes were pelleted, washed and eluted. Then, the crosslinking was reversed and the samples were incubated with proteinase K. Primers used for PCR were selected by mapping the two main KLF6-sites-rich regions in the promoter sequence. The first region encompasses from -864 to -662 (202 bp) and the second from -200 to +40 (240 bp). Sequences of both couples of primers are the following: First region Fwd 5´-GTCAGCAGAGTTCCAGGGAG-3´ and Rev 5´-TTAGCCCTGAGGATGGTTTG-3´; and second region Fwd 5´-CCCACGGCCTGAGTCCAAGG-3´ and Rev 5´-GGCCCAGCTCCTCCACTCC-3´. For negative and positive control PCRs, primers from ChIP-IT control human kit (Cat#53010, Active motif) were used (data not shown). The experiment was performed in control situation and after 3 hr of monolayer disruption.

ELISA of angiogenic factors

HUVECs monolayers were grown in EBM/EGM2 media until confluence and scratched with a pipet tip. At 0h, 2h, 6h, 8h, 12h and 24h after wounding, culture supernatants were collected and subjected to ELISA using a commercial kit (Human Angiogenesis ELISA Strip I for Profiling 8 Cytokines; #EA-1101, Signosis, Sunnyvale, CA, US), which allows profiling and measuring of angiogenic proteins, including TNF-α, IGF1, VEGF, IL6, FGFb, TGF-β, EGF and leptin.

Western blot analysis

Cells were lysed in Laemmli buffer and protein concentrations were determined (Bradford, BioRad). Protein samples were separated by 10% SDS-PAGE and electrotransferred to nitrocellulose membranes using an iBlot gel transfer system (Invitrogen). Immunodetection was carried out with the rabbit polyclonal antibody anti-KLF6 (R-173; sc-7158, Santa Cruz Biotechnology) or the mouse monoclonal antibody anti-β-actin (A-2103, Sigma), followed by incubation with the appropriate secondary antibody, anti-rabbit or anti-mouse IgG, both coupled to HRP (Dako, Glostrup, Denmark). Protein bands were revealed using the SuperSignal chemiluminescent substrate (Pierce, Rockford, IL, USA) according to the manufacturer's instructions.

In silico analysis of promoter sequences

For the *in silico* analysis of GC-boxes containing putative KLF6 and Sp1 motifs in the IL-6 proximal promoter, we retrieved the upstream sequence of the human (ID: 3569) and mouse (ID: 16193) genes. These sequences were submitted to the Genomatix MatInspector software tool: http://www.genomatix.de/products/MatInspector.

Statistics

Data were subjected to statistical analysis and results are shown as mean ±SD. Differences in mean values were analyzed using Student´s t-test. In the figures, the statistically significant values are marked with asterisks (*$p<0.05$; **$p<0.01$; ***$p<0.005$; ns= not significant).

References

1. Ades EW, Candal FJ, Swerlick RA, George VG, Summers S, Bosse DC, Lawley TJ. HMEC-1: establishment of an immortalized human microvascular endothelial cell line. *J. Invest. Dermatol.* 1992;99:683-690.

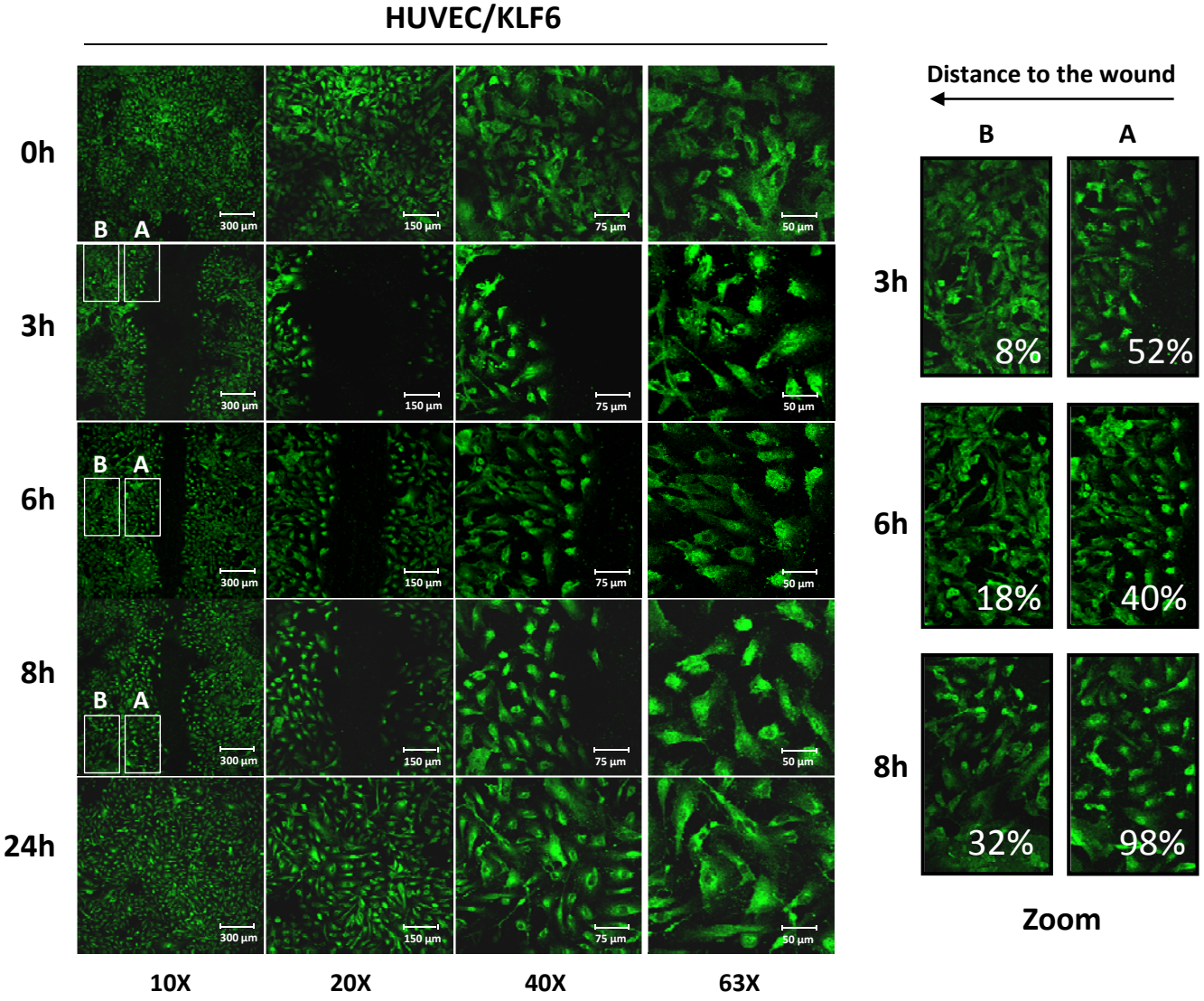
2. Xu Y, Swerlick RA, Sepp N, Bosse D, Ades EW, Lawley TJ. Characterization of expression and modulation of cell adhesion molecules on an immortalized human dermal microvascular endothelial cell line (HMEC-1). *J. Invest. Dermatol*. 1994;102:833-837.

3. Botella LM, Sanchez-Elsner T, Sanz-Rodriguez F, Kojima S, Shimada J, Guerrero-Esteo M, Cooreman MP, Ratziu V, Langa C, Vary CP, Ramirez JR, Friedman S, Bernabeu C. Transcriptional activation of endoglin and transforming growth factor-beta signaling components by cooperative interaction between Sp1 and KLF6: their potential role in the response to vascular injury. *Blood*. 2002;100:4001-4010.

4. Matsumoto N, Kubo A, Liu H, Akita K, Laub F, Ramirez F, Keller G, Friedman SL. Developmental regulation of yolk sac hematopoiesis by Kruppel-like factor 6. *Blood*. 2006;107:1357-1365.

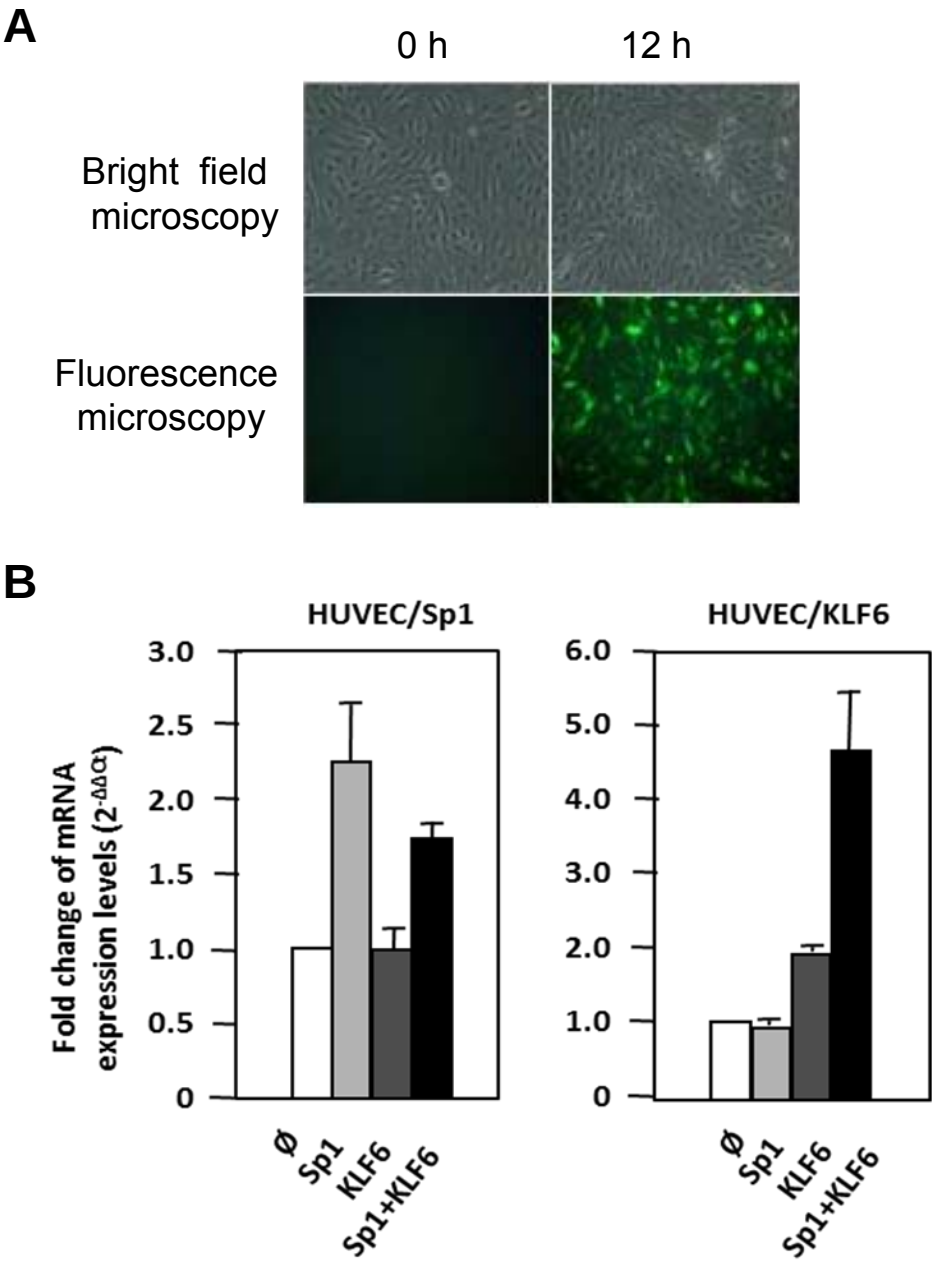
5. Roque M, Fallon JT, Badimon JJ, Zhang WX, Taubman MB, Reis ED. Mouse model of femoral artery denudation injury associated with the rapid accumulation of adhesion molecules on the luminal surface and recruitment of neutrophils. *Arterioscler. Thromb. Vasc. Biol.* 2000;20:335-342.
6. Garrido-Martin EM, Blanco FJ, Fernandez LA, Langa C, Vary CP, Lee UE, Friedman SL, Botella LM, Bernabeu C. Characterization of the human Activin-A receptor type II-like kinase 1 (ACVRL1) promoter and its regulation by Sp1. *BMC Mol. Biol*. 2010;11:51.
7. Ratziu V, Lalazar A, Wong L, Dang Q, Collins C, Shaulian E, Jensen S, Friedman SL. Zf9, a Kruppel-like transcription factor up-regulated in vivo during early hepatic fibrosis. *Proc. Natl. Acad. Sci. USA*. 1998;95:9500-9505.
8. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J. Biol. Chem.* 2000;275:2247-2250.
9. Eickelberg O, Pansky A, Mussmann R, Bihl M, Tamm M, Hildebrand P, Perruchoud AP, Roth M. Transforming growth factor-beta1 induces interleukin-6 expression via activating protein-1 consisting of JunD homodimers in primary human lung fibroblasts. *J. Biol. Chem*. 1999;274:12933-12938.
10. Tournay O, Benezra R. Transcription of the dominant-negative helix-loop-helix protein Id1 is regulated by a protein complex containing the immediate-early response gene Egr-1. *Mol. Cell. Biol*. 1996;16:2418-2430.

Supplementary Figure I



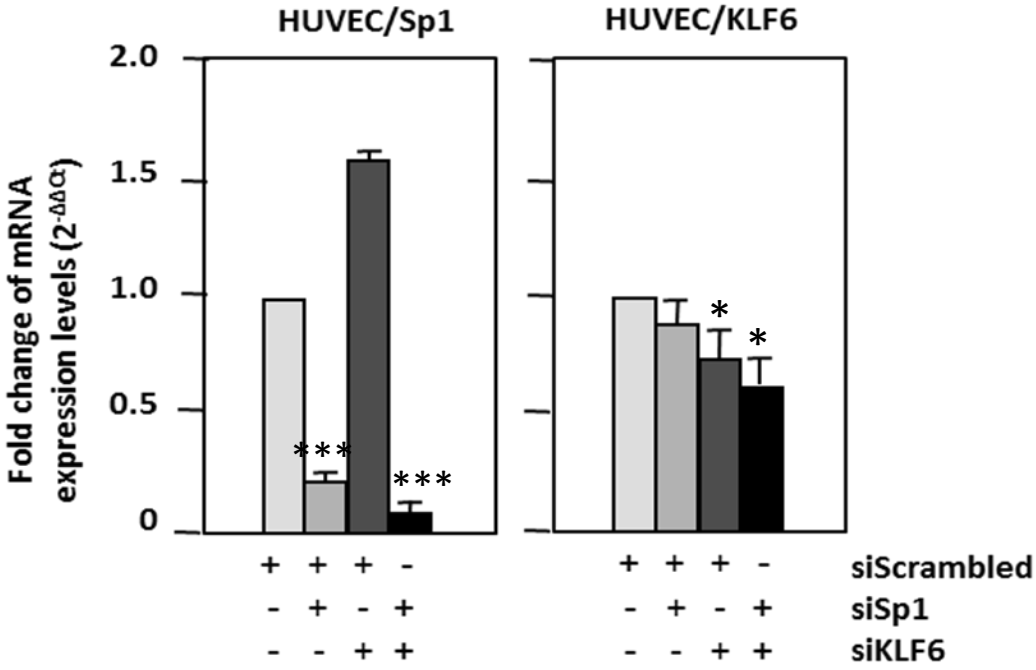
Supplementary Figure I. KLF6 translocation to the nucleus is dependent on the distance to the wound. HUVEC monolayers were wounded *in vitro* and at different time points (0h, 3h, 6h, 8h and 24h) KLF6 was detected by immunofluorescence microscopy. Magnification of two different areas, **A** (close to the wound) and **B** (distant to the wound) is shown on the right. The number of cells with nuclear or cytoplasmic KLF6 was quantified and the percentage of cells with nuclear KLF6 are indicated. The closer to the wound, the higher the percentage of cells with nuclear KLF6.

Supplementary Figure II



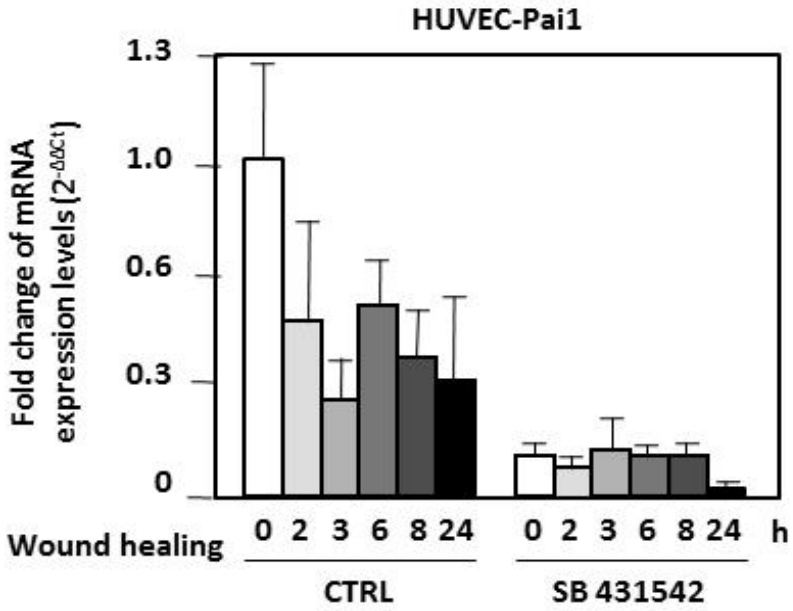
Supplementary Figure II. Nucleofection efficiency of Sp1 and KLF6 expression vectors in HUVECs. A. Cells nucleofected with pmax-GFP as a positive control showing over 90% efficiency of nucleofection. **B**. Real time PCR of Sp1 and KLF6 mRNA levels after nucleofection. Nucleofected transcription factors are indicated. mRNA levels of Sp1 and KLF6 are shown on the left and right histogram, respectively.

Supplementary Figure III



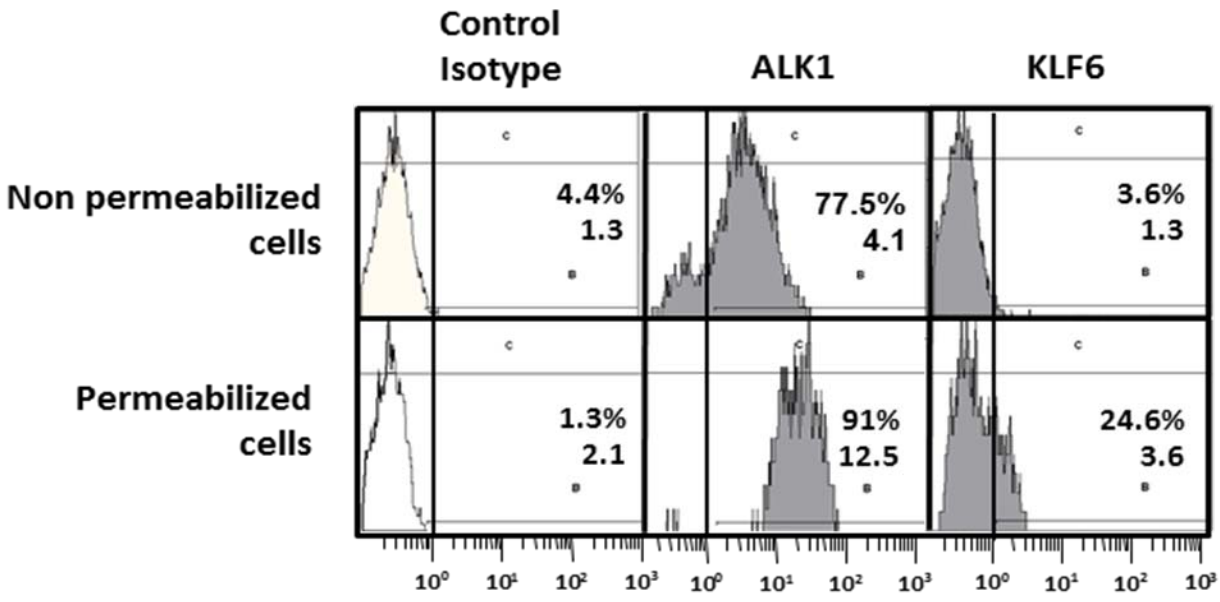
Supplementary Figure III. Efficiency of Sp1 and KLF6 knock-down in HUVECs by siRNA (si) transfection. Real time RT-PCR analysis shows mRNA levels of Sp1 (left) and KLF6 (right) in HUVECs upon transfection with different combinations of siSp1, siKLF6 and SiScrambled.

Supplementary Figure IV



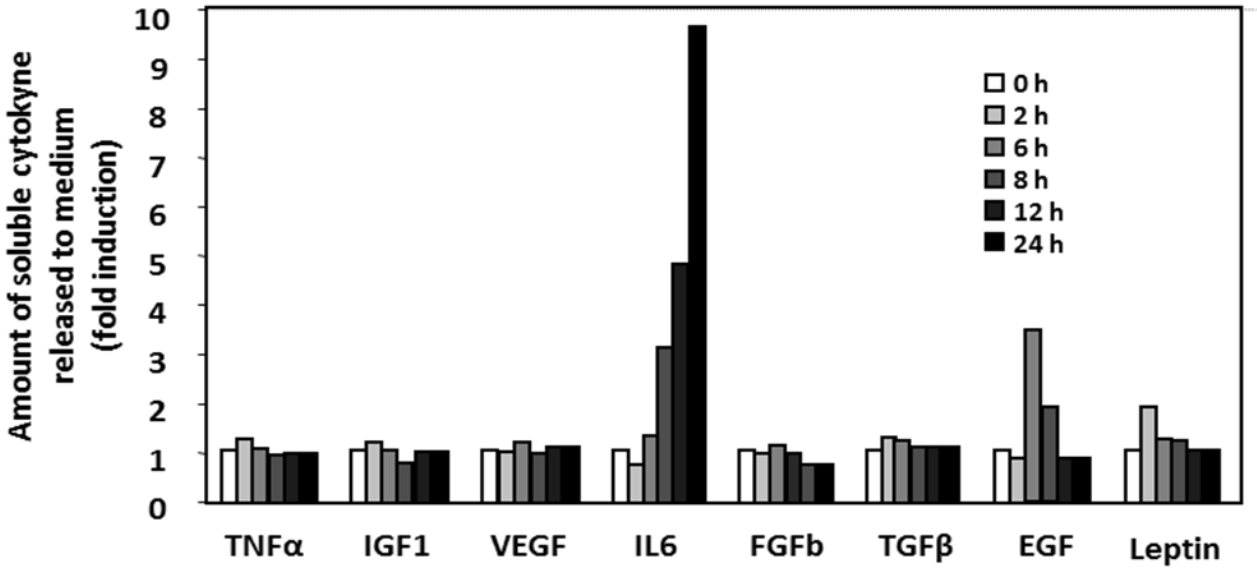
Supplementary Figure IV. Effect of SB431542 treatment on ALK5 activity. HUVECs were wounded *in vitro* and incubated in the absence (CTRL) or in the presence of the ALK5 inhibitor SB431542. At the rimes indicated, the levels of PAI-1 mRNA were analyzed by Real time RT-PCR. Upon treatment with SB431542, mRNA levels of *PAI1* (a downstream target of ALK5) were decreased respect to controls, demonstrating the efficiency of the SB431542 treatment.

Supplementary Figure V



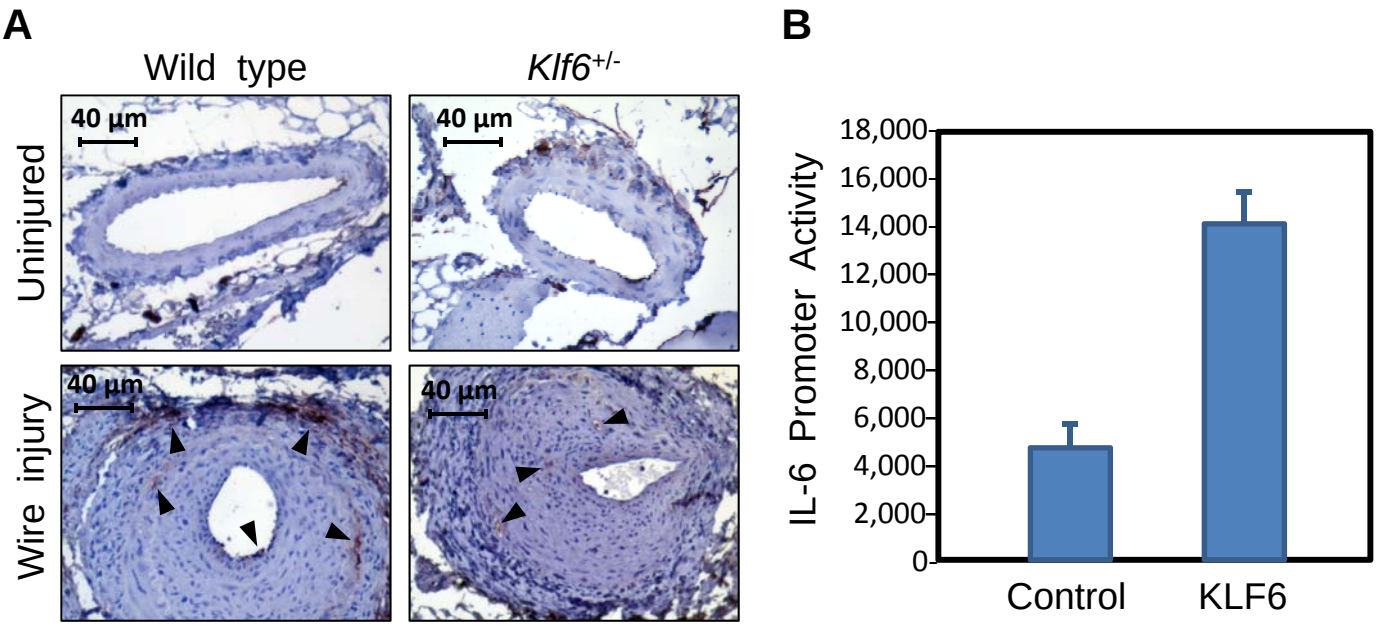
Supplementary Figure V. ALK1 and KLF6 staining of permeabilized and non-permeabilized HUVECs. Cells were treated (permeabilized) or not (non-permeabilized) with α-lysophosphatidyl-choline and analyzed by immunofluorescence flow cytometry using anti-ALK1, anti-KLF6 or an isotype matched antibody. When permeabilized, HUVECs allow the detection of intracellular KLF6, whereas ALK1 staining was increased respect to untreated cells. The percentage of positive cells and the mean fluorescence intensity are indicated.

Supplementary Figure VI



Supplementary Figure VI. ELISA of different angiogenic factors released to medium by HUVECs during wound healing. HUVECs were wounded in vitro and the culture supernatant was collected at different times**.** Levels of cytokines were measured in conditioned media from HUVECs after 2h, 6h, 8, 12h and 24h of wounding and compared to basal levels in medium from control (undamaged, 0h) HUVECs.

Supplementary Figure VII



Supplementary Figure VII. KLF6 regulates IL-6 expression. A. Effect of vascular injury on IL-6 expression. Immunohistochemistry staining of IL-6 in 4 weeks-injured femoral arteries from *Klf6*$^{+/-}$ mice in comparison with wild type littermates. In uninjured vessels from wild type and heterozygous littermates the presence of IL-6 is almost undetectable. By contrast, IL-6 staining was clearly detected upon wire injury in different layers of the femoral artery (black arrowheads). The increased signal of IL-6 in wild type mice was higher than that of *Klf6*$^{+/-}$ littermates. **B**. KLF6 transactivates the IL-6 promoter. HEK 293T were cotransfected with a luciferase reporter construct driven by a 621-bp promoter fragment of IL-6 promoter (pGL3-IL6-Luc) and the expression vector pCIneo-KLF6 (KLF6) or the corresponding empty vector (Control). Overexpression of KLF6 upregulates more than 3-fold the activity of the IL-6 promoter.

Supplementary Figure VIII

A

Human IL-6; gi|224589819|ref|NC_000007.13|:22765423-22767000 *Homo sapiens* chromosome 7, GRCh37.p5 Primary Assembly

GAGTAAATGCCCAACAGAGGTCACTGTTTTATCGATCTTGAAGAGATCTCTTCTTAGCAAAGCAAAGAAACCGATTGTGA
AGGTAACACCATGTTTGGTAAATAAGTGTTTTGGTGTTGTGCAAGGGTCTGGTTTCAGCCTGAAGCCATCTCAGAGCTGT
CTGGGTCTCTGGAGACTGGAGGGACAACCTAGTCTAGAGCCCATTTGCATGAGACCAAGGATCCTCCTGCAAGAGACACC
ATCCTGAGGGAAGAGGGCTTCTGAACCAGCTTGACCCAATAAGAAATTCTTGGGTGCCGACGCGGAAGCAGATTCAGAGC
CTAGAGCCGTGCCTGCGTCCGTAGTTTCCTTCTAGCTTCTTTTGATTTCAAATCAAGACTTACAGGGAGAGGGAGCGATA
AACACAAACTCTGCAAGATGCCACAAGGTCCTccttTGACATCCCCAACAAAGAGGTGAGTAGTATTCTCCCCCTTTCTG
CCCTGAACCAAGTGGGCTTCAGTAATTTCAGGGCTCCAGGAGACCTGGGGCCCATGCAGGTGCCCCAGTGAAACAGTGGT
GAAGAGACTCAGTGGCAATGGGGAGAGCACTGGCAGCACAAGGCAAACCTCTGGCACAGAGAGCAAAGTCCTCACTGGGA
GGATTCCCAAGGGGTCACTTGGGAGAGGGCAGGGCAGCAGCCAACCTCCTCTAAGTGGGCTGAAGCAGGTGAAGAAAGTG
GCAGAAGCCACGCGGTGGCAAAAAGGAGTCacacactccACCTGGAGacgccTTGAAGTAACTGCACGAAATTTGAGGAT
GGCCAGGCAGTTCTACAACAGCCGCTCACAGGGAGAGCCAGAACACAGAAGAACTCAGATGACTGGTAGTATTACCTTCT
TCATAATCCCAGGCTTGGGGGGCTGCGATGGAGTCAGAGGAAACTCAGTTCAGAACATCTTTGGTTTTTACAAATACAAA
TTAACTGGAACGCTAAATTCTAGCCTGTTAATCTGGTCACTGAAAAAAAATTTTTTTTTTTTCAAAAAACATAGCTTTAG
CTTATTTTTTTTCTCTTTGTAAAACTTCGTGCATGACTTCAGCTTTACTCTTTGTCAAGACATGCCAAAGTGCTGAGTCA
CTAATAAAAGAAAAAAAGAAAGTAAAGGAAGAGTGGTTCTGCTTCTTAGCGCTAGCCTCAATGACGACCTAAGCTGCACT
TTTCCCCCTAGTTGTGTCTTGCCATGCTAAAGGACGTCACATTGCACAATCTTAATAAGGTTTCCAATCAGCCcacccG
CTCTGGCCcacccTCACCCTCCAACAAAGATTTATCAAATGTGGGATTTTCCCATGAGTCTCAATATTAGAGTCTCAAC
CCCCAATAAATATAGGACTGGAGATGTCTGAGGCTCATTCTGCCCTCGAGCCCACCGGGAACGAAAGAGAAGCTCTATCT
CCCCTCCAGGAGCCCAGCTATGAACTCCTTCTCCACAAGTAAGTGCAGGAAATCCTTAGCCCTGGAACTGCCAGCGGCGG
TCGAGCCCTGTGTGAGGGAGGGGTGTGTGGCCCAGGGAGGGCTGGCGGGCGGCCAGCA

B

Mouse IL-6; gi|372099105|ref|NC_000071.6|:30011733-30013310 *Mus musculus* strain C57BL/6J chromosome 5, GRCm38 C57BL/6J

TTATTTTAGTGTAATTTTTTTCTTCACATTGAATTCTAGGAGAAACTATGCTAGTGATATATAATTCTTGAACTATTAAA
CATGGGAGCATAAGAAAACAAGAATCTTAAGGCAATCTGCAGAGTGAAGAAGCTGATTGTGATCCTGAGAGTGTGTTTTG
TAAATGGTTTTGGATTTTATGTACAGAGCCTACTTTCAGCCTGGAATCATTCTGAATGCTAGCTAGATATCTGGAGACAG
GTGGACAGAAAACCAGGAACTAGTCTGAAAAAGAAACTAACCAAAGGGAAGAAGTCTGTTTAAGTTTGACCCAGCCTAGA
AGACTTGAGCATTGGAGGGGTTATTCAGAGTGAGACGTACCACCTTCAGATTCAAATCCTGTCATCCAGTAGAAGGGAGC
TTCAAACACAAGCTAGCTAAGATACAATGAGGTCCTTCTTCGATATCTTTATCTTCCATATACCATGAATCAAAGAAACT
TCAACAACATGAGGACTGCAACAGACCTTCAAGCCTCCTTGCATGACCTGGAAATGTTTTGGGGTGTCCTGGCAGCAGTG
GGATCAGCACTAACAGATAAGGGCAACTCTCACAGAGACTAAAGGTCTTAACTAAGAAGATAGCCAAGAGACCACTGGGG
AGAATGCAGAGAATAGGCTTGGACTTGGAAGCCAAGATTGCTTGACAACAGACAGAAGATATTTCTGTACTTCACCCACT
TTACCCACCTGGCAACTCCTGGAAACAACTGCACAAAATTTGGAGGTGAACAAACCATTAGAAACAACTGGTCCTGACAA
GACACAGGAAAAACAAGCAATATGCAACATTACTGTCTGTTGTCCAGGTTGGGTGCTGGGGGTGGGAGAGggagtGTGTG
TCTTTGTATGATCTGAAAAAACTCAGGTCAGAACATCTGTAGATCCTTACAGACATACAAAAGAATCCTAGCCTCTTATT
CATGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATGTGTGTCGTCTGTCATGCGCGCGTGCC
TGCGTTTAAATAACATCAGCTTTAGCTTCTCTTTCTCCTTATAAAACATTGTGAATTTCAGTTTTCTTTCCCATCAAGAC
ATGCTCAAGTGCTGAGTCACTTTTAAAGAAAAAAAAGAAGAGTGCTCATGCTTCTTAGGGCTAGCCTCAAGGATGACTTA
AGCACACTTTCCCCTTCCTAGTTGTGATTCTTTCGATGCTAAACGACGTCACATTGTGCAATCTTAATAAGGTTTCCAAT
CAGCCcacccACTCTGGCCccacccccacccTCCAACAAAGATTTTTATCAAATGTGGGATTTTCCCATGAGTCTCAAA
ATTAGAGAGTTGACTCCTAATAAATATGAGACTGGGGATGTCTGTAGCTCATTCTGCTCTGGAGCCCACCAAGAACGATA
GTCAATTCCAGAAACCGCTATGAAGTTCCTCTCTGCAAGTAAGTGAAGGCAGTTCCTTGCCCTCTGGCGGAGCTATTGAG
ACTGTGAGAGAGGAGTGTGAGGCAGAGAGCCAGCATTGTGGGTTGGCCAGCAGCCATC

Supplementary Figure VIII. ***In silico*** **analysis of putative KLF6 motifs in the IL-6 promoter.** Several GC-boxes that recruit members of the Sp/KLF transcription factors family were identified. Putative transcription factor binding motifs for KLF6 (green boxes) and Sp1 (yellow boxes) in the plus (capitals) and negative (lower case letters) strands of human (**A**) and mouse (**B**) IL-6 promoter sequences are indicated. KLF6(+); klf6(-); SP1(+); sp1(-). The translation initiation codon is in red.