Supplemental Figure Legends

Suppl. Figure I. (A) In the matrigel assay, preincubation of CAC with neutralizing antibodies against leptin (anti-Ob) or the leptin receptor (anti-ObR), or transient downregulation of ObR mRNA expression using small interfering RNA (ObR siRNA), abolished the increase in CAC adhesion to the HUVEC network following stimulation with leptin (10 ng/mL; CAC+L10). (B) Summary of 3-6 independent experiments. $^{**}P<0.01$ for leptin-treated vs. control-treated CAC in the presence of nonspecific IgG or control siRNA, respectively; $\#P<0.05$ for the presence vs. absence of anti-Ob or anti-ObR antibody, or ObR siRNA, in leptin-stimulated cells. Bars represent 100 µm.

Suppl. Figure II. The angiogenic effect of leptin on CAC could significantly be reduced by RGD peptides (Gly-Arg-Gly-Asp-Ser) or neutralizing antibodies against αvβ5 integrins, *in vitro* using the matrigel assay (A) and *in vivo* using the CAM assay (B). $^{*}P<0.05$, and $^{**}P<0.01$ for leptin vs. control-treated cells; $\#P<0.05$, and $\#\#\#P<0.001$ for the presence vs. absence of RGD peptide, or anti-αvβ5 monoclonal antibody, in leptin-stimulated CAC.

Suppl. Figure III. (A) Stimulation of CAC with leptin resulted in time-dependent phosphorylation of the β5, but not β3, integrin chain; immunoprecipitation (IP) of phosphotyrosine residues (PY20) followed by Western blot (WB) analysis. (B) Summary of 6 independent experiments. $^{**}P<0.01$ vs. unstimulated cells defined as 100%. (C) The leptin-induced integrin αvβ5 phosphorylation was absent in CAC pretreated with leptin receptor-neutralizing antibodies (anti-ObR) as opposed to control IgG (n=1). (D, E) IPs followed by WB analysis suggested complex formation of ObR and β5 integrin in response to leptin.

Graphs show quantitative summary of n=3-5 independent experiments. *P<0.05 vs. unstimulated cells defined as 100%.

Suppl. Figure IV. (A) Functional analysis using the *in vitro* spheroid assay confirmed that the effects of leptin (10 ng/mL) on the number of CAC adherent to HUVEC sprouts could be reduced by preincubation of CAC with 10 µM of the JAK inhibitor AG490, or the Src kinase inhibitors PP2, but not with the nonspecific homologue PP3. *P<0.05 for the comparison of leptin- vs. DMSO-treated cells defined as 100%, ##P<0.01 for the comparison of DMSO and AG490-treated, leptin stimulated cells, and #P<0.05 vs. PP2-treated cells. (B and C) Similar findings were obtained in the *in vivo* hindlimb ischemia model using athymic mice, after preincubation of CAC with 10 µM of the Src kinase inhibitor SU6656. *P<0.05 for leptin vs. control-treated cells; #P<0.05, and ##P<0.01 for the presence vs. absence of each inhibitor in leptin-stimulated CAC, §P<0.001 vs. no CAC. Representative immunofluorescence images showing CD31-positive capillaries are displayed. Bars represent 50 µm.

Suppl. Figure V. (A, B) The increase in Src kinase phosphorylation at tyroine (Y) 416 following stimulation of CAC with leptin (10 ng/mL) requires the presence of ObR, as it was abolished in ObR siRNA-transfected CAC. **P<0.01 for leptin-treated vs. control-treated CAC defined as 100% in the presence of control siRNA; and #P<0.05 for the presence vs. absence of ObR siRNA in leptin-stimulated cells. (C, D) Src phosphorylation at Y416 also was abolished after preincubation with the JAK inhibitor AG490, and the PLCγ inhibitor U73122. ***P<0.001 for leptin vs. control-treated CAC defined as 100%; ##P<0.01 for the presence vs. absence of AG490 in leptin-stimulated cells.

Suppl. Figure VI. Incubation of endothelial cells (HUVEC) with leptin (10 ng/mL) induced phosphorylation of αvβ5 integrins (A), and resulted in a time-dependent increase in Src

kinase phosphorylation at Y416 and dephosporylation at Y527 (B), while FAK became phosphorylated at Y397, 861, and 925 (C). The amount of total Src and FAK protein is shown as loading control.