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Supplemental Information

HIF-1α Dependent Wound Healing Angiogenesis In Vivo Can Be Controlled by Site-Specific Lentiviral Magnetic Targeting of SHP-2

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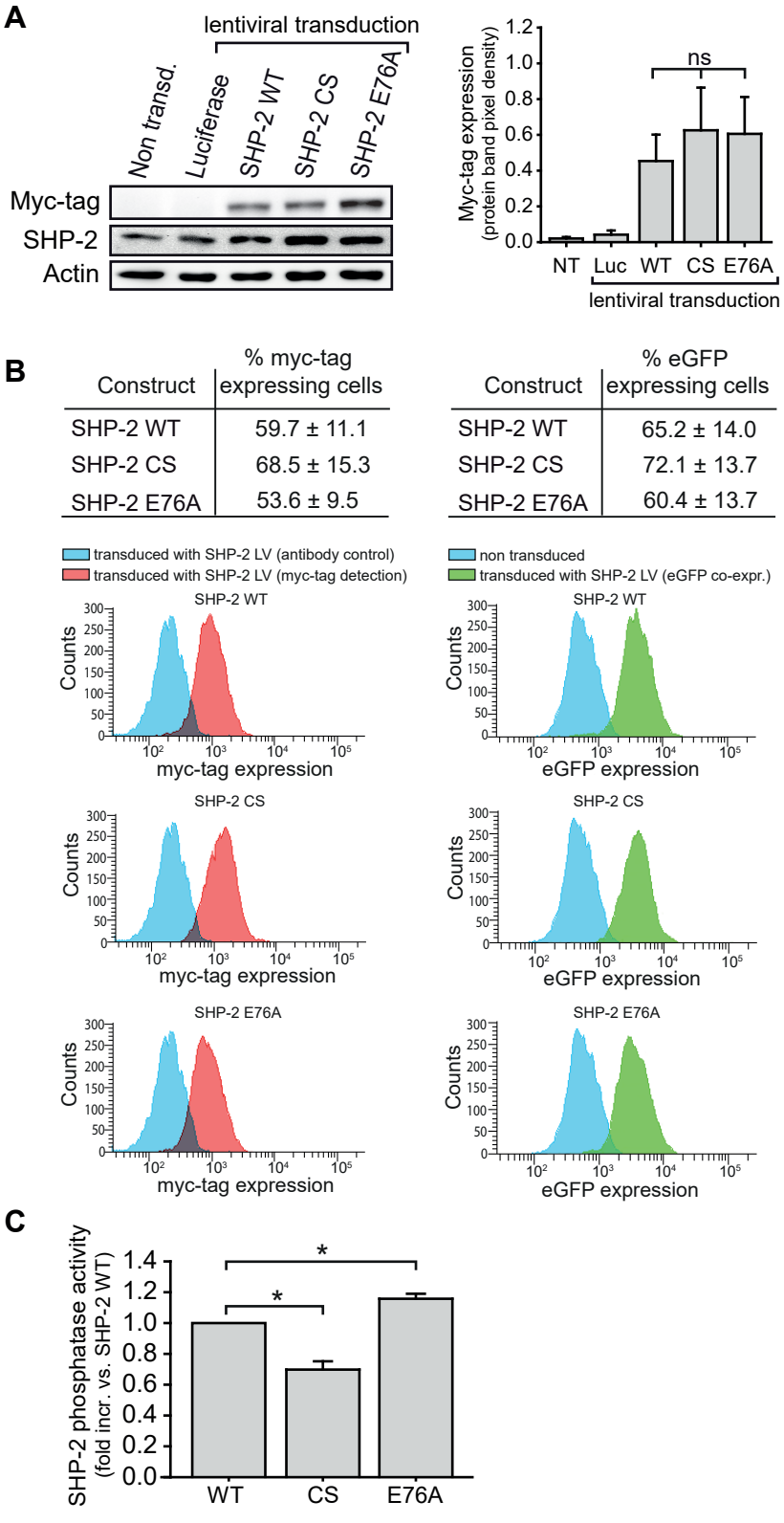
Figure S1



Construct	% myc-tag expressing cells
SHP-2 WT	59.7 ± 11.1
SHP-2 CS	68.5 ± 15.3
SHP-2 E76A	53.6 ± 9.5

Construct	% eGFP expressing cells
SHP-2 WT	65.2 ± 14.0
SHP-2 CS	72.1 ± 13.7
SHP-2 E76A	60.4 ± 13.7

Figure S1. Expression of SHP-2 constructs in human endothelial cells

A) Lentiviral transduction of SHP-2 WT, CS or E76A constructs of HMEC resulted in expression of these constructs as shown by myc-tag detection by western blotting (n=6). A luciferase expressing lentivirus was used as control. Graph next to blot shows the SHP-2-myc-tag expression as measured from pixel densities of the protein bands normalized to actin. ns: not significant. B) Myc-tag expression was also detected by flow cytometry (n=3). Expression of eGFP (expressed together with SHP-2-myc-tag through an IRES sequence) in these cells was also detected by flow cytometry (n=3). Representative histograms of myc-tag expressing cells (red) and antibody control (blue) as well as eGFP expressing cells (green) and non-transduced cells (blue) are shown below the respective table. C) Phosphatase activity of SHP-2 WT, CS and E76A in HMEC was assessed by dephosphorylation of pNPP of myc-tag precipitates 96h after transduction (*$p<0.05$, n=3). Quantitative data are represented as mean±SEM.

Figure S2

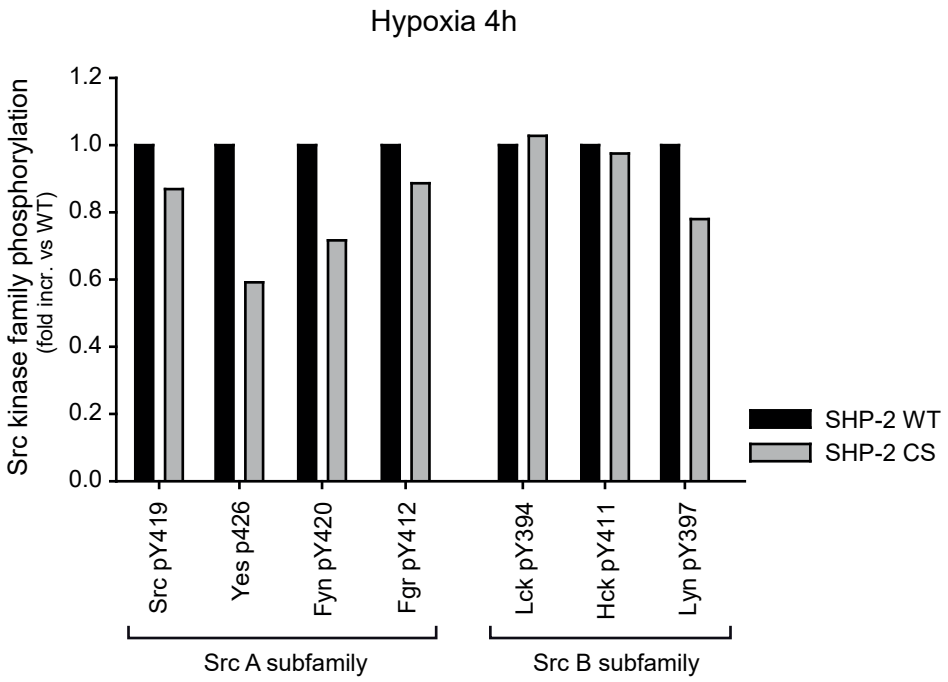


Figure S2. SHP-2 CS reduces phosphorylation of Src kinase family members

A phospho-kinase array was performed with lysates from hypoxic (4h) cells expressing SHP-2 WT or CS to screen for activating phosphorylation of Src kinase family members (n=2).

Figure S3

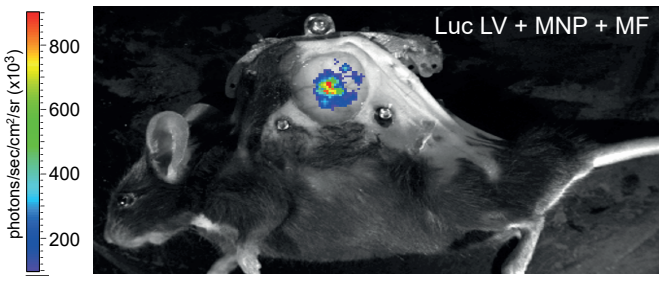


Figure S3. Magnetic targeting of MNP and lentivirus complexes is site specific
Magnetic targeting of MNP associated to luciferase expressing lentivirus to the dorsal skin of mice shows transgene expression at the target site only. Bioluminescence images were taken 6 days post transduction.

Supplemental materials and methods

Detection of expression of SHP-2 constructs with flow cytometry

96h after lentiviral transduction, HMEC were detached with Accutase (PAA Laboratories, Cölbe, Germany), pelleted, and washed with phosphate buffered saline supplemented with calcium, fixated with 1% formalin for 10 minutes, pelleted and permeabilized with 0.1% Triton-X for 2 minutes. After rinsing, the cell pellet was incubated with anti-myc-tag antibody (1:200) for 1h at room temperature, rinsed and incubated with AF-546 labeled secondary antibody (#A11035 from Invitrogen, Molecular Probes) for 30 minutes at room temperature in the dark. After rinsing the cells were analysed with a BD FACS Canto II (BD Biosciences). To control for background staining, cells were stained with secondary antibody only.

Phosphokinase array (Src)

The phosphorylation profile of Src kinases in lysates of hypoxic HMEC overexpressing the SHP-2 constructs (WT, CS or E76A) was screened using the human phospho-kinase array form R&D Systems (#ARY003B) following the manufacturer's instructions. Pixel densities of the duplicate array spots were measured using the Hokawo software (Hamamatsu).