SUPPLEMENTAL MATERIAL

Balanced single-vector co-delivery of VEGF / PDGF-BB improves functional collateralization in chronic cerebral ischemia

Aiki Marushima, MD[1]; Melina Nieminen, MSc[1]; Irina Kremenetskaia, MSc[1]; Roberto Gianni-Barrera, PhD[2]; Johannes Woitzik, MD[1]; Georges von Degenfeld, MD[3]; Andrea Banfi MD[2]; *Peter Vajkoczy, MD[1] and *Nils Hecht, MD[1]

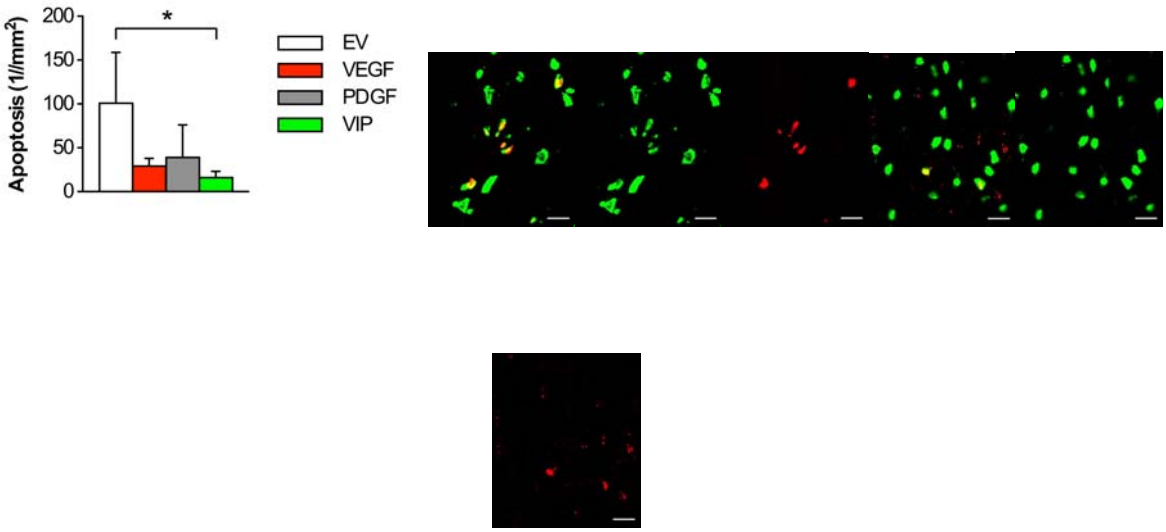
**equal contribution*

[1]Department of Neurosurgery and Center for Stroke research Berlin (CSB), Charité - Universitätsmedizin Berlin, Berlin, Germany.

[2]Department of Biomedicine, University Hospital Basel and University of Basel, Switzerland

[3]Global Medical Affairs, Bayer Pharma AG, Wuppertal, Germany.

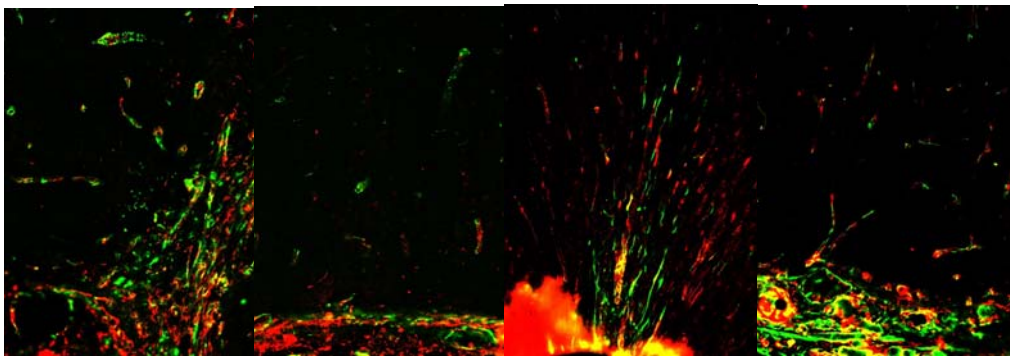
SUPPLEMENTAL FIGURE I



Neuronal apoptosis

(A) Photomicrographs of TUNEL / NeuN co-localization in the cortical region below the EMS on day 42 illustrate decreased neuronal apoptosis in animals that received VIP myoblasts (lower panels) compared to treatment with EV myoblasts (upper panels). Bar = 25μm. **(B)** Bar graph of neuronal apoptosis (TUNEL / NeuN co-localization / mm^2) in the cortical region below the EMS. *$p<0.05$.

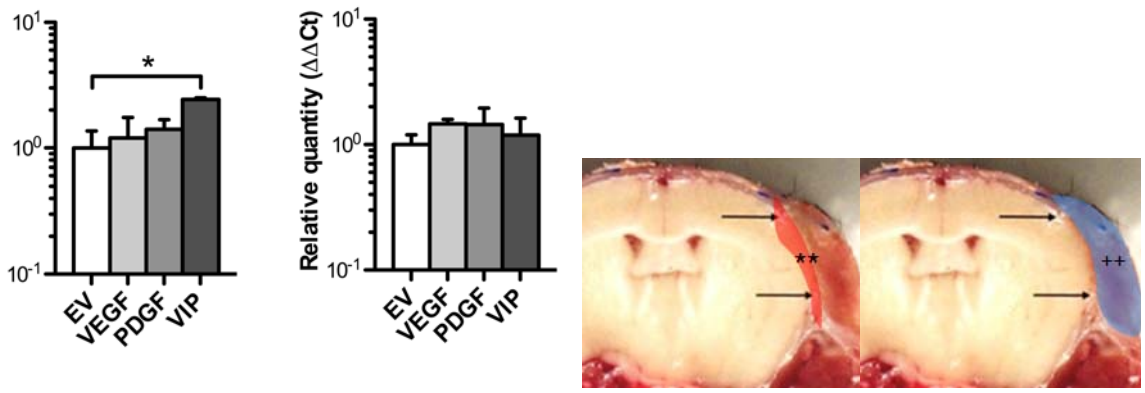
SUPPLEMENTAL FIGURE II



PDGF receptor beta (PDGF-Rβ) expression

Confocal laser scanning photomicrographs of 20μm sections of the cortical region below the EMS show marked PDGF-Rβ expression in the area of transpial collateralization at the muscle / brain EMS interface (dashed line) following implantation of VIP and PDGF myoblasts. Bar = 50μm.

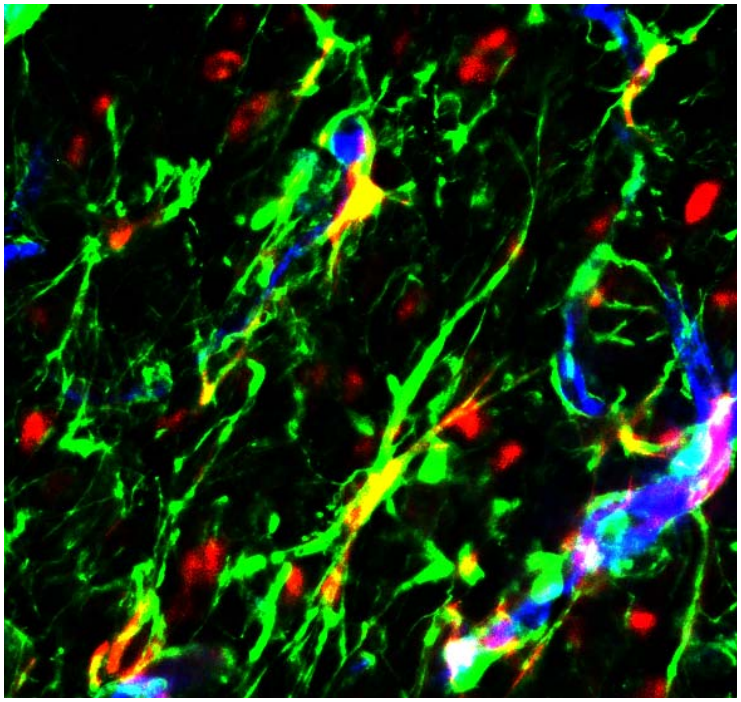
SUPPLEMENTAL FIGURE III



Endogenous $VEGF_{164}$ expression on day 42

To distinguish between endogenous and exogenous $VEGF_{164}$ transcripts and determine whether the increased VEGF protein levels in animals that received hPDGF-BB alone could be due to endogenous upregulation, we designed specific primer and probe sequences with Primer Express software 3.0 (Applied Biosystems, Forster City, California, USA). Briefly, to detect the endogenous $mVEGF_{164}$, rt-PCR primers and probes are spanning the 5'UTR untranslated region and samples from n=4 animals per group that were used for rtPCR analysis shown in Figures 1 and 6 were analyzed.

SUPPLEMENTAL FIGURE IV



VEGF localization on day 42

High-power confocal laser scanning photomicrograph of a 20μm section of the cortical region below the EMS of an animal treated with VIP myoblasts shows signs of VEGF-positive immunofluorescence in areas without GFAP or CD31 co-localization (arrows). Bar =

50μm.