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SUPPLEMENTARY ONLINE DATA

Neuropilin-1 regulates platelet-derived growth factor receptor signalling in mesenchymal stem cells

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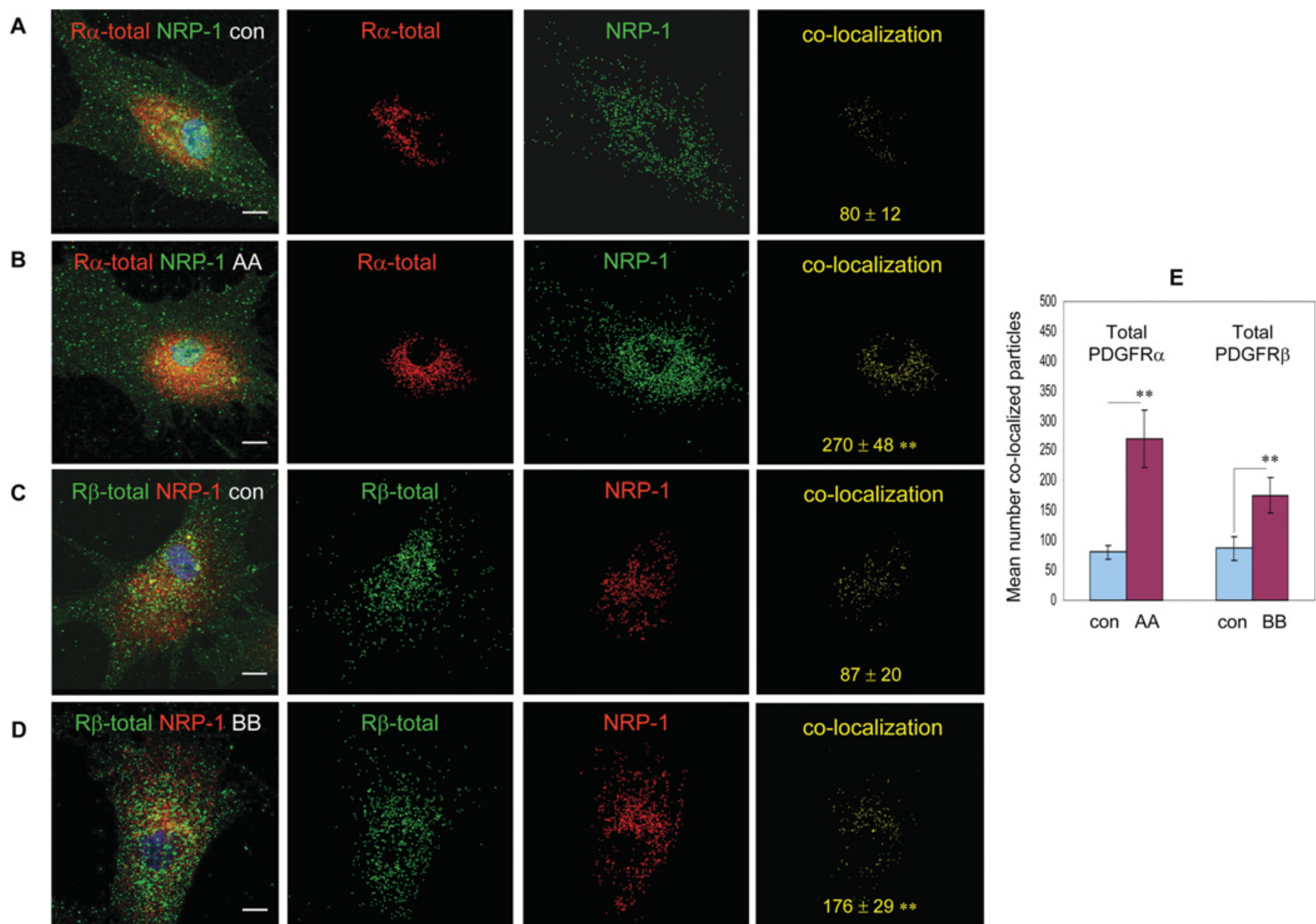

Figure S1 Co-localization of NRP-1 with total PDGFRs

The cellular distribution of NRP-1 and total PDGFRs was examined using pan-PDGFR antibodies. MSCs grown on 0.1% gelatin were cultured for 24 h in serum-free conditions, exposed to PDGF ligands, then co-localization of NRP-1 with either total PDGFRα or PDGFRβ was examined by immunofluorescence microscopy. (**A**) Control unstimulated and (**B**) exposed to 20 ng/ml PDGF-AA for 10 min, showing total PDGFRα (red) and NRP-1 (green). (**C**) Control unstimulated and (**D**) exposed to 20 ng/ml PDGF-BB for 10 min, showing total PDGFRβ (green) and NRP-1 (red). For each image, the corresponding red and green channels having similar threshold values and the same particle size range are shown, together with their co-localization represented by the image in yellow. The mean number of co-localized particles$\pm$S.D. derived from four different single-cell images is denoted in yellow. Nuclei are counter-stained with DAPI (blue). Representative images of at least six independent experiments are shown. Scale bars = 20 μm. (**E**) Histogram showing the ligand-induced increase in co-localization between NRP-1 and total PDGFRα or PDGFRβ, as determined by immunofluorescence analysis. Values are the mean number of co-localized particles$\pm$S.D. derived from six different single-cell images. $^{**}P<0.001$, compared with the corresponding unstimulated control.

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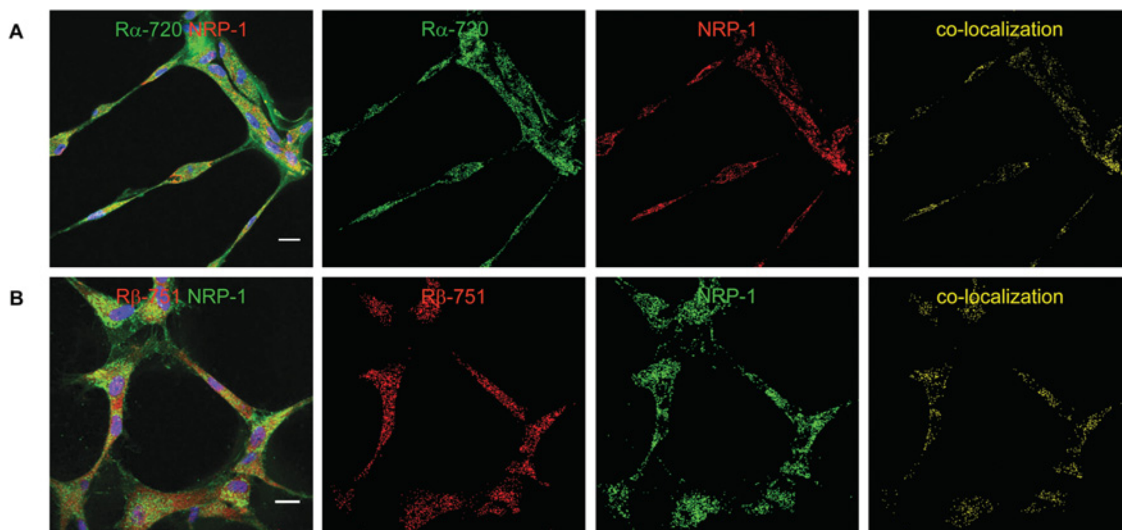

Figure S2 Co-localization of NRP-1 with additional phosphorylated PDGFR sites

The co-localization of NRP-1 with two additional PDGFR phosphorylation sites was examined during the assembly of MSC networks. MSCs were seeded on to Matrigel™ and cultured for 24 h, then co-localization of NRP-1 with either PDGFRα at site Tyr^{720}, or PDGFRβ at site Tyr^{751} was examined by immunofluorescence microscopy. (**A**) MSCs showing PDGFRα-Tyr^{720} (green) and NRP-1 (red). (**B**) MSCs showing PDGFRβ-Tyr^{751} (red) and NRP-1 (green). For each image, the corresponding red and green channels which have similar threshold values and the same particle size range are shown, together with their co-localization represented by the image in yellow. Nuclei are counterstained with DAPI (blue). Representative images of at least four independent experiments are shown. Scale bars = 20 μm.

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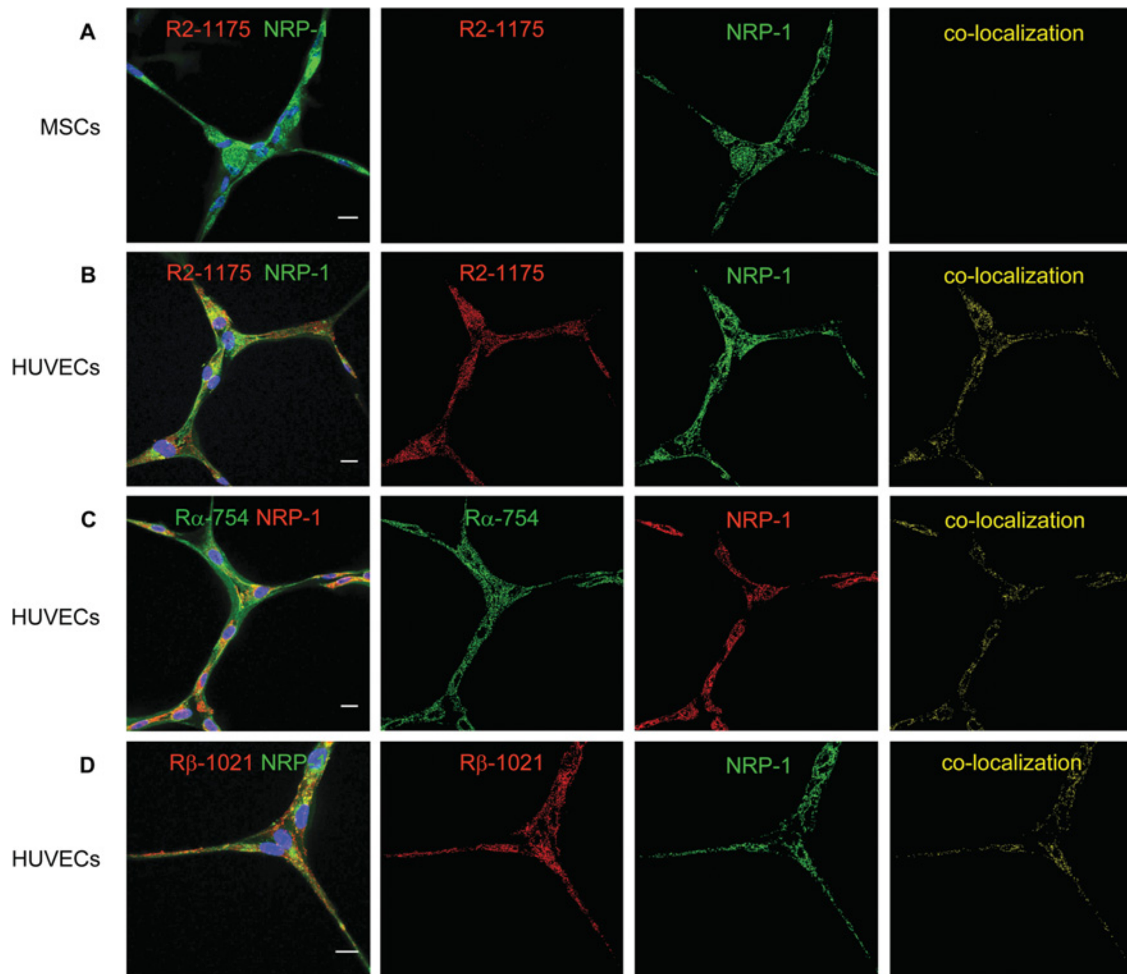

Figure S3 NRP-1 co-localization with PDGFRs in HUVEC networks

The co-localization of NRP-1 and PDGFRs was examined in HUVEC networks. HUVECs or MSCs were seeded on to Matrigel^{TM} and cultured for 24 h, then co-localization of NRP-1 with either VEGFR2 at site Tyr^{1175}, PDGFRα at site Tyr^{754} or PDGFRβ at site Tyr^{1021}, was examined by immunofluorescence microscopy. (**A**) MSCs showing VEGFR2-Tyr^{1175} (red) and NRP-1 (green). (**B**) HUVECs showing VEGFR2-Tyr^{1175} (red) and NRP-1 (green). (**C**) HUVECs showing PDGFRα-Tyr^{754} (green) and NRP-1 (red). (**D**) HUVECs showing PDGFRβ-Tyr^{1021} (red) and NRP-1 (green). For each image, the corresponding red and green channels which have similar threshold values and the same particle size range are shown, together with their co-localization represented by the image in yellow. Nuclei are counterstained with DAPI (blue). Representative images of at least three independent experiments are shown. Scale bars = 20 μm.

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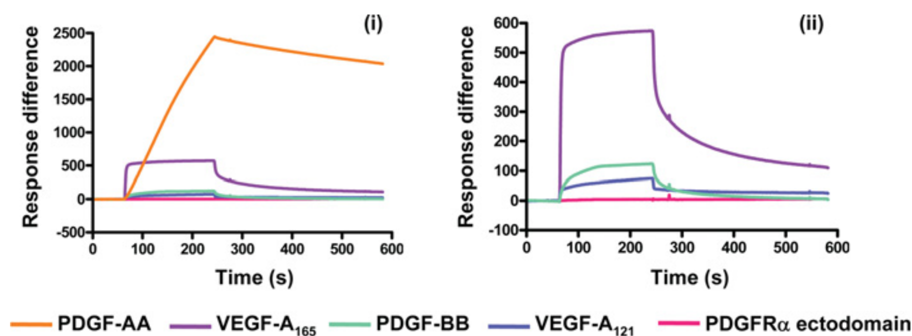

Figure S4 NRP-1 interactions with PDGF ligands and PDGFRα

BIAcore analysis was used to examine the interactions between immobilized recombinant NRP-1 (R&D Systems) and soluble ligands PDGF-AA, PDGF-BB, VEGF-A_{165}, VEGF-A_{121} and a recombinant PDGFRα ectodomain (produced by C. Bayley). Soluble ligands were injected over NRP-1 immobilized on a CM5 chip. The response difference denotes the level of interaction above respective control flow cells. (i) PDGF-AA displayed the highest binding affinity for NRP-1; however, this ligand was difficult to evaluate using BIAcore analysis, due to a high level of interaction with control flow cells [1]. (ii) Smaller response difference scale, showing VEGF-A_{165}, PDGF-BB and VEGF-A_{121} bound to NRP-1 with decreasing affinity, but the PDGFRα ectodomain exhibited no detectable binding. Data shown are representative of three independent experiments.

REFERENCE

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