

Supplementary Figure legends

Suppl. Figure 1: A) Chord plot showing pathways associated with the top 10 proteins (sorted by intensity score) associating with $\alpha 5$ integrin. B-D) STRING interaction networks identifying associations between $\alpha 5$ integrin and RabGTPase proteins (B), myosin motor proteins (C) and Endocytic trafficking-associated proteins (D).

Suppl. Figure 2: A) Densitometric analysis of total expression levels of $\alpha 5$ integrin, αV integrin and paxillin by Western blotting as shown in Figure 2G, N = 3.

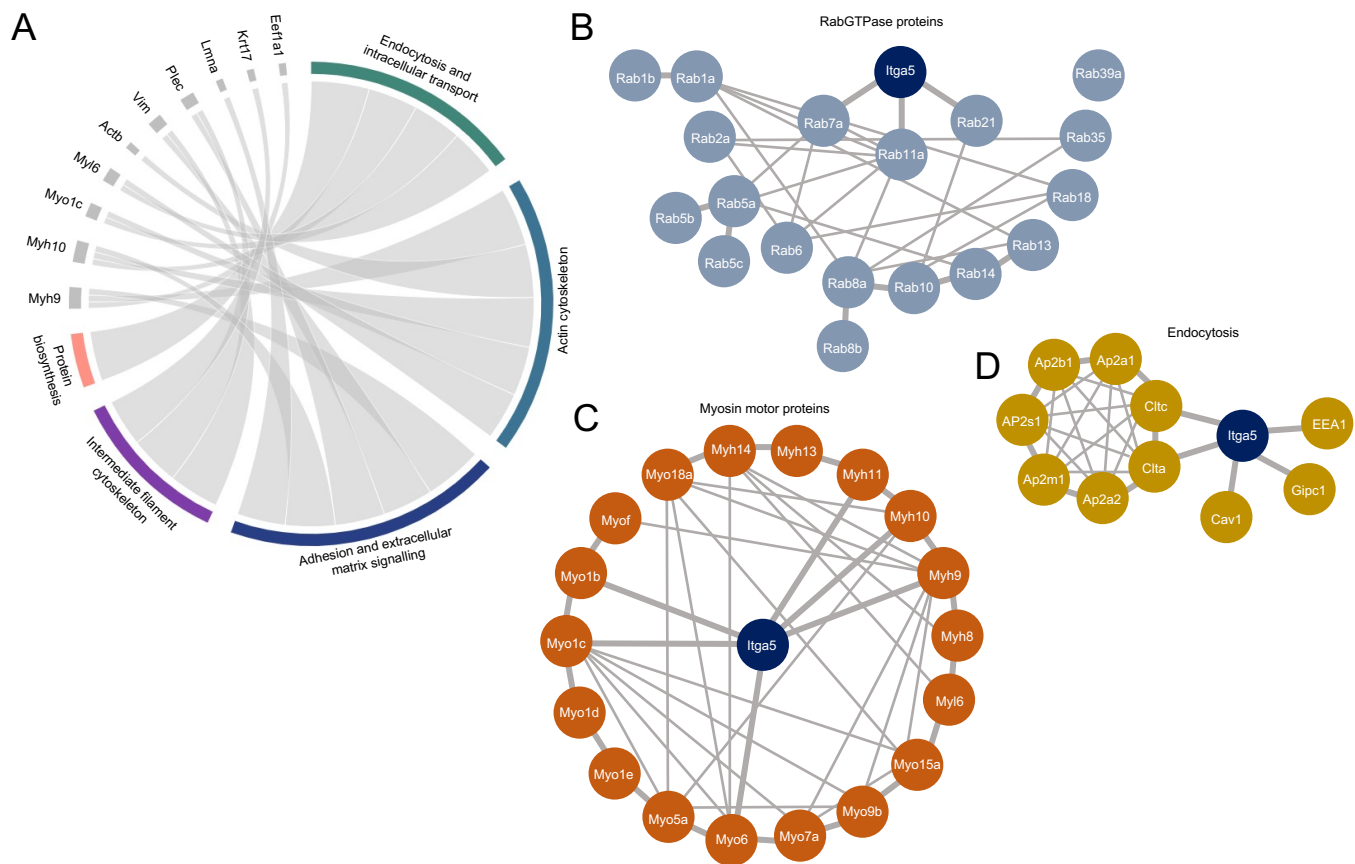
Suppl. Figure 3: A) Total cell lysate input showing expression levels of tensin-1 by Western blotting in siRNA-depleted ECs. B) Supporting densitometric analysis of A), N = 3.

Suppl. Figure 4: A) Recycling assay schematic. Surface proteins were biotin labelled prior to incubation at 37 °C to stimulate receptor internalisation. Following MESNA stripping, internalised proteins were allowed to recycle for the indicated timepoints at 37 °C alongside a MESNA stripped control. EC lysates were immunoprecipitated against biotin to detect the recycled fraction of total $\alpha 5$ integrin by SDS-PAGE and Western blotting. B-C) Quantification of siRNA-depletion of p120RasGAP and Rab21 from total cell lysates, N = 3, N = 2 respectively.

Suppl. Figure 5: A) Densitometric quantification of FAK phosphorylation at Try³⁹⁷ and Tyr⁴⁰⁷ residues relative to total FAK or β -actin expression in Ctrl and siRNA-depleted ECs, N = 3. B) Quantification of siRNA-depletion of Rab11 from total cell lysates, N = 4. C) Representative confocal microscopy images showing colocalisation between $\alpha 5$ integrin and EEA1 in Ctrl and siRab11 ECs fixed at 180 minutes. D) Representative confocal microscopy images showing colocalisation between $\alpha 5$ integrin and Rab7 in Ctrl and siRab11 ECs fixed at 180 minutes. E) Representative confocal microscopy images showing GM130⁺ Golgi-body positioning and F-actin protrusions in Ctrl and siRab11 ECs at the scratch-wound edge. Arrows indicate correctly positioned Golgi-bodies. Asterisks indicate statistical significance.

Suppl. Figure 6: A) Representative confocal microscopy images showing ERG1/2⁺ ECs in Pdgfb-iCreER^{T2}-negative, NRP1^{fl/fl}.EC^{KO}, NRP2^{fl/fl}.EC^{KO} and NRP1^{fl/fl}NRP2^{fl/fl}.EC^{KO} retinas harvested at P6. B) Quantification of ERG1/2⁺ EC density shown as relative percentages of respective littermate control animals, N ≥ 2 (n ≥ 4). C) Representative confocal microscopy images showing BS-1 lectin⁺ collagen IV⁺ regressed vessels in Pdgfb-iCreER^{T2} negative and NRP1^{fl/fl}NRP2^{fl/fl}.EC^{KO} animals. D) Quantification of raw vessel regression, N = 3 (n ≥ 8). E) Right panel: quantification of filopodial length (shown as relative percentages of respective littermate control animals), and filopodial tortuosity (shown as ratios) in Pdgfb-iCreER^{T2}-negative, NRP1^{fl/fl}.EC^{KO}, NRP2^{fl/fl}.EC^{KO} and NRP1^{fl/fl}NRP2^{fl/fl}.EC^{KO} animals, (n = 5). F) Individual polar plots showing Golgi-body orientation in Pdgfb-iCreER^{T2} negative and NRP1^{fl/fl}NRP2^{fl/fl}.EC^{KO} animals (n = 50 ECs). Asterisks indicate statistical significance.

(Suppl. Figure 1)



A

Figure A displays three bar graphs showing the relative expression of $\alpha 5$ integrin, αV integrin, and total paxillin in Ctrl, sNRP1, sNRP2, and sNRP1/2 groups. The y-axis for all graphs represents relative expression, ranging from 0.0 to 1.5. The x-axis labels are Ctrl, sNRP1, sNRP2, and sNRP1/2. The Ctrl group is represented by a grey bar, sNRP1 by a light blue bar, sNRP2 by a medium blue bar, and sNRP1/2 by a dark blue bar. Error bars represent standard deviation. Individual data points are overlaid on each bar. A horizontal line with 'ns' (not significant) is drawn above the bars for each graph, indicating no significant difference between the groups.

Protein	Ctrl	sNRP1	sNRP2	sNRP1/2
Relative $\alpha 5$ integrin expression	1.0	~0.8	~0.75	~0.9
Relative αV integrin expression	1.0	~0.9	~1.1	~0.95
Relative total paxillin expression	1.0	~0.95	~0.7	~0.75

A

TCL input

kDa

185

42

Tensin-1

β -actin

Ctrl

siNRP1

siNRP2

siNRP1/2

B

Relative tensin-1 expression

ns

Ctrl

siNRP1

siNRP2

siNRP1/2

Detailed description: Panel A shows a Western blot of Tensin-1 (185 kDa) and β -actin (42 kDa) in total cell lysate (TCL input) from cells treated with Ctrl, siNRP1, siNRP2, or siNRP1/2. Panel B is a bar graph showing the relative Tensin-1 expression normalized to β -actin for the same four conditions. The expression levels are approximately 1.0 for Ctrl, 0.85 for siNRP1, 0.8 for siNRP2, and 0.75 for siNRP1/2. A horizontal line with 'ns' indicates no significant difference between the groups.

Condition	Relative Tensin-1 expression
Ctrl	1.0
siNRP1	~0.85
siNRP2	~0.8
siNRP1/2	~0.75

A

Diagram A illustrates a network of interactions centered on p120. The central node is p120, which is connected to five peripheral nodes: G3bp1, G3bp2, Rasal2, Iqgap1, and Rasip1. The connections are represented by lines of varying thickness, indicating the strength of the interaction. The thickest lines connect p120 to G3bp1 and G3bp2. The lines connecting p120 to Rasal2 and Iqgap1 are also relatively thick. The lines connecting p120 to Rasip1 and G3bp2 are thinner.

B siRNA-nucleofected ECs → Biotin labelling → Strip (2 min, 4 min, 10 min) → Lyse, IP: Biotin. Stripped → Lyse, IP: Biotin. Biotin-labelled surface receptor.

C Relative p120RasGAP expression: Ctrl (1.0), siP120RasGAP (~0.25).

D Relative Rab21 expression: Ctrl (1.0), siRab21 (~0.2).

A

VEGF₁₆₄ stimulation 0 mins 5 mins 30 mins

pFAK^{Tyr527} expression (Relative/total FAK expression)

Ctrl siNRP1 siNRP2 siNRP1/2 siNRP1/2+sp120RasGAP

pFAK^{Tyr527} expression (Relative/ β -actin expression)

Ctrl siNRP1 siNRP2 siNRP1/2 siNRP1/2+sp120RasGAP

pFAK^{Tyr47} expression (Relative/total FAK expression)

Ctrl siNRP1 siNRP2 siNRP1/2 siNRP1/2+sp120RasGAP

pFAK^{Tyr47} expression (Relative/ β -actin expression)

Ctrl siNRP1 siNRP2 siNRP1/2 siNRP1/2+sp120RasGAP

Panel	Condition	Time (mins)	pFAK ^{Tyr527} expression		pFAK ^{Tyr47} expression	
			Relative/total FAK	Relative/ β -actin	Relative/total FAK	Relative/ β -actin
pFAK ^{Tyr527} (Relative/total FAK)	Ctrl	0	1.0	1.0	1.0	1.0
		5	1.2	1.3	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP1	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP2	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP1/2	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.2	1.3	1.0	1.0
	siNRP1/2+sp120RasGAP	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.1	1.1	0.9	0.9
pFAK ^{Tyr527} (Relative/ β -actin)	Ctrl	0	1.0	1.0	1.0	1.0
		5	1.3	1.3	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP1	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP2	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP1/2	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.2	1.3	1.0	1.0
	siNRP1/2+sp120RasGAP	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.1	1.1	0.9	0.9
pFAK ^{Tyr47} (Relative/total FAK)	Ctrl	0	1.0	1.0	1.0	1.0
		5	1.2	1.3	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP1	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP2	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP1/2	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.2	1.3	1.0	1.0
	siNRP1/2+sp120RasGAP	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.1	1.1	0.9	0.9
pFAK ^{Tyr47} (Relative/ β -actin)	Ctrl	0	1.0	1.0	1.0	1.0
		5	1.3	1.3	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP1	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP2	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.0	1.0	0.9	0.9
	siNRP1/2	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.2	1.3	1.0	1.0
	siNRP1/2+sp120RasGAP	0	1.0	1.0	1.0	1.0
		5	1.1	1.0	0.9	0.9
		30	1.1	1.1	0.9	0.9

B

Relative Rab11 expression

Ctrl siRab11a+b

Condition	Relative Rab11 expression
Ctrl	1.0
siRab11a+b	~0.25

C

a5 integrin EEA1 DAPI

Ctrl

a5 integrin

EEA1

siRab11

a5 integrin

EEA1

D

$\alpha 5$ integrin Rab7 DAPI

Ctrl

$\alpha 5$ integrin

Rab7

s/Rab11

$\alpha 5$ integrin

Rab7

(Suppl. Figure 6)

