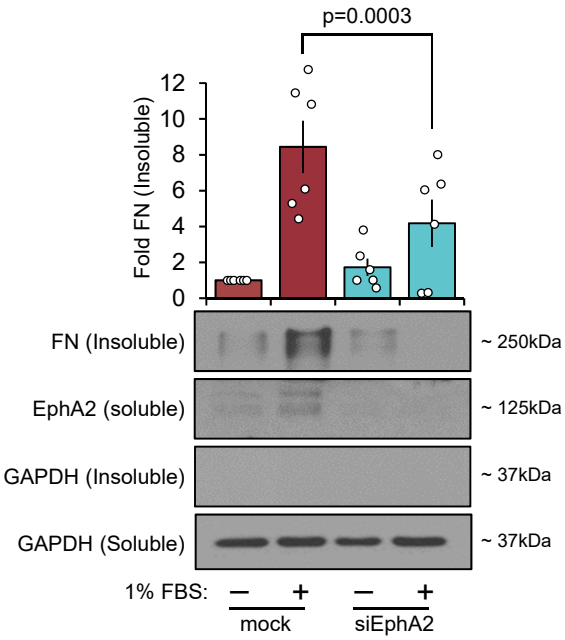
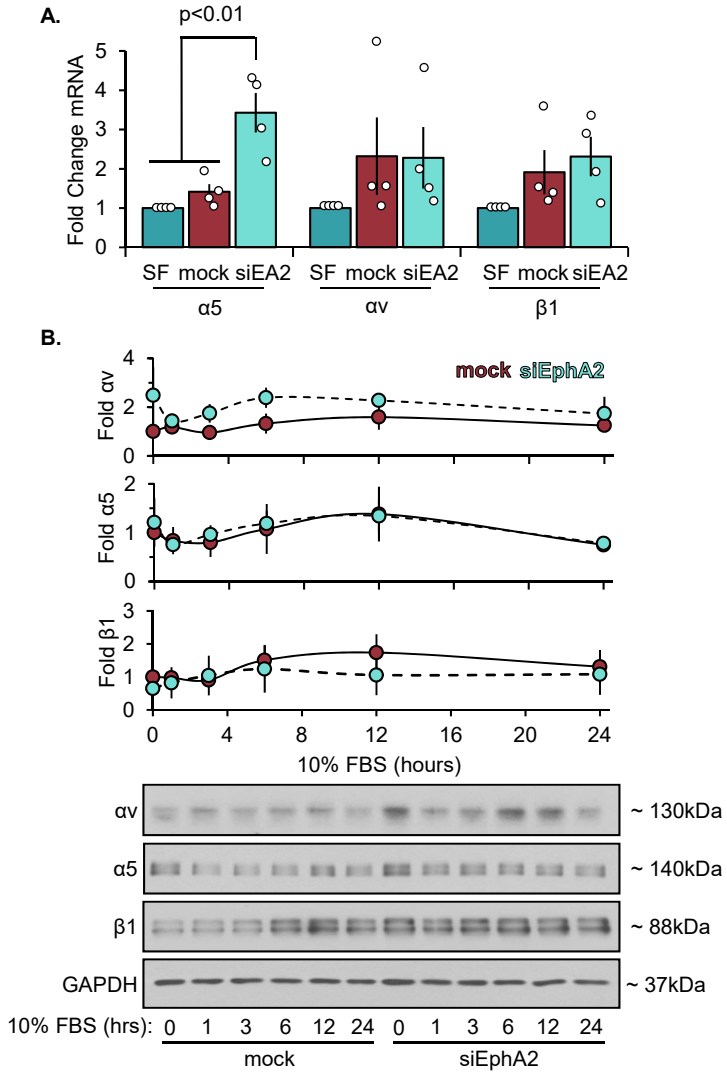
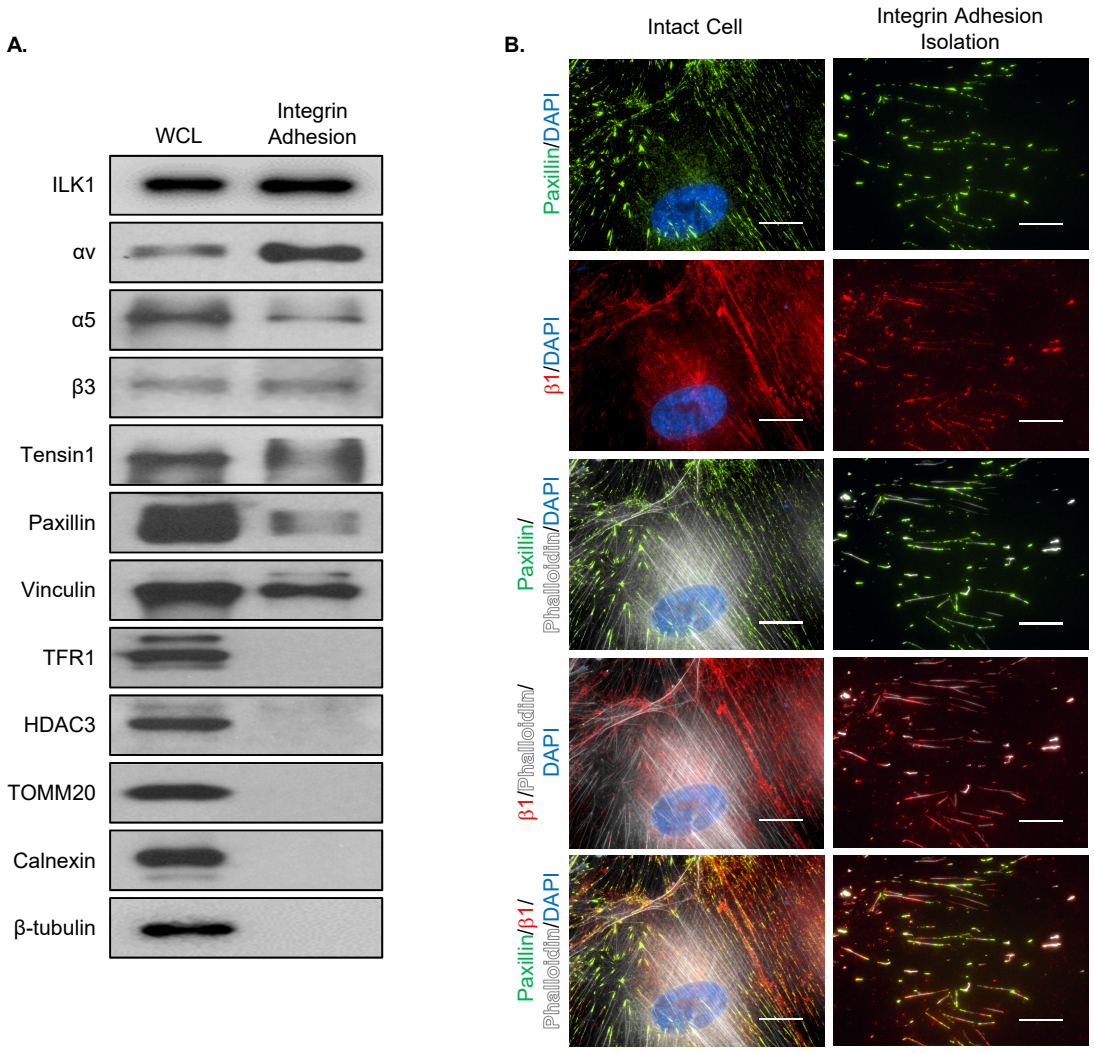


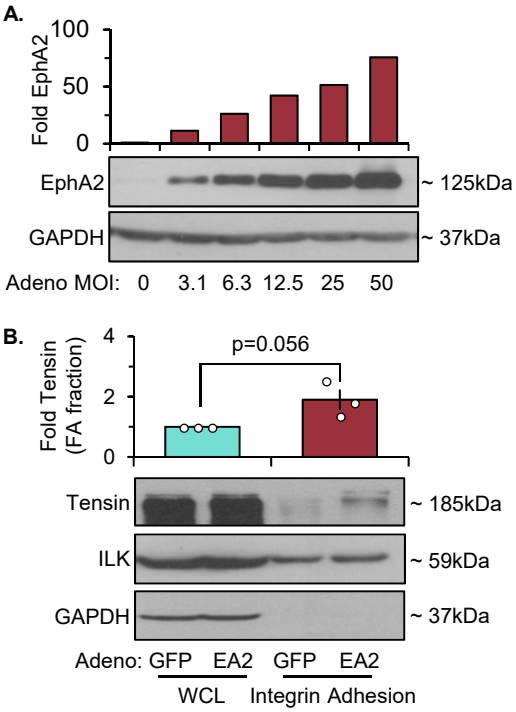
Supplemental Figure I: EphA2 depletion impairs fibronectin deposition in human dermal fibroblasts. Human dermal fibroblasts (HDFs) were transfected with mock or siRNA targeted against EphA2 for 24 hours. Cells were then plated onto Matrigel overnight in serum-free or 1% serum. A) Fibronectin deposition was measured by deoxycholate extraction, and protein was analyzed by immunoblot. Deoxycholate insoluble (deposited) fibronectin was normalized to deoxycholate soluble GAPDH. Deoxycholate insoluble GAPDH shown for isolation purity. n=3-4. Data are expressed as data ±SEM. Statistical comparisons were made using 2-way ANOVA with Bonferroni post-test. A p-value less than 0.05 is considered significant.



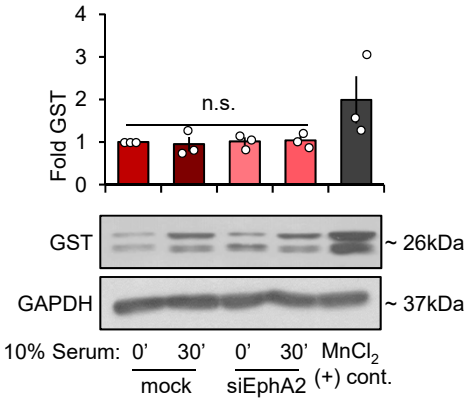
Supplemental Figure II: EphA2 depletion does not alter integrin expression or activation. Human VSMCs were transfected with mock or siRNA against EphA2 for 24 hours. Cells were plated onto tissue culture dishes overnight in serum-free or 1% serum. A) Gene expression was analyzed with RT-qPCR and normalized to Rpl13a housekeeping gene. B) Following transfection, cells were plated overnight and serum-starved for four hours prior to treatment with 1% serum at the indicated timepoints. Protein expression was measured by immunoblot and normalized to GAPDH. n=4. Data are expressed as mean ±SEM. Statistical comparisons were made using One-way ANOVA with Bonferroni post-test (A) or 2way ANOVA with Bonferroni post-test (B). A p-value less than 0.05 is considered significant.



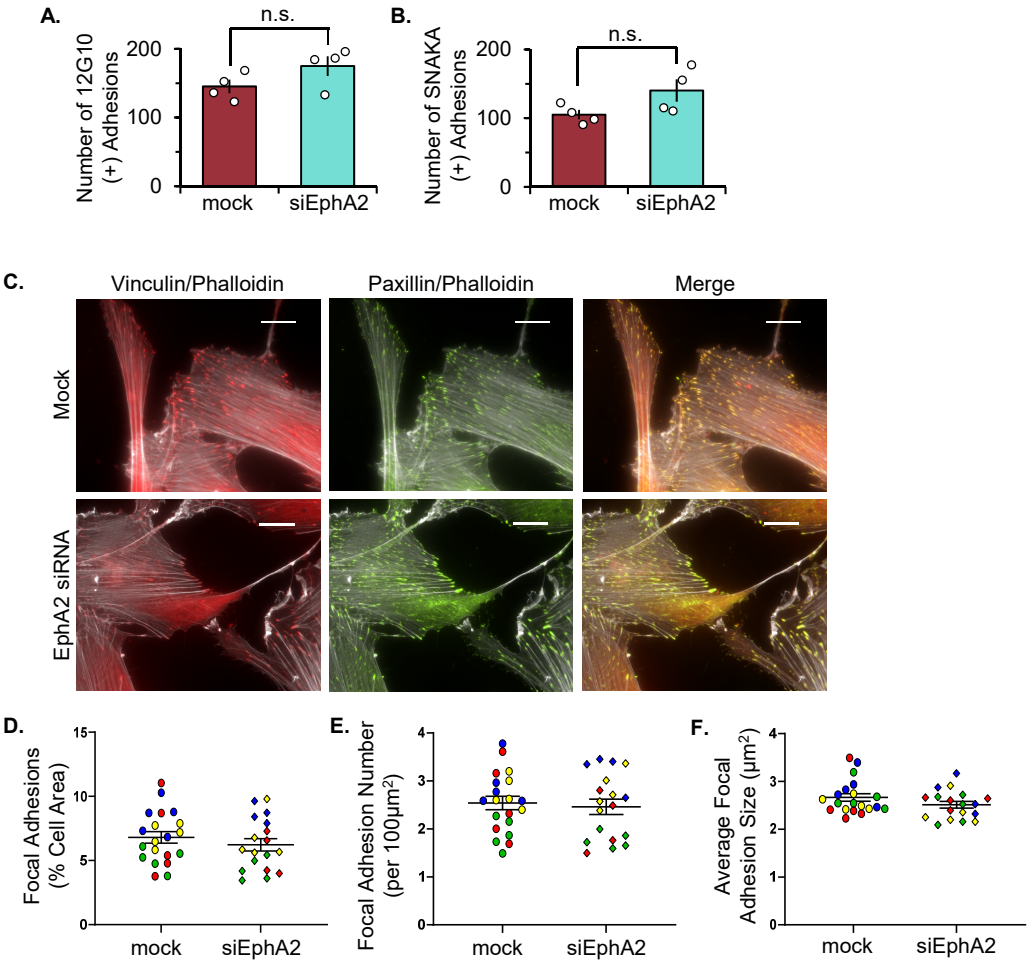
Supplemental Figure III: Integrin adhesion isolation purity. Human VSMCs were plated onto Matrigel-coated glass slides overnight and integrin adhesion isolation was performed and observed by (A) immunoblot or (B) immunofluorescence. A) Integrin-associated proteins ILK, αv integrin, α5 integrin, β3 integrin, tensin, paxillin, and vinculin are present in both whole cell lysate (WCL) and integrin adhesion fractions. Proteins associated with the plasma membrane (TFR-1), nucleus (HDAC3), mitochondria (TOMM20), endoplasmic reticulum (Calnexin), and cytoskeleton (β-tubulin) represent negative controls and are absent in the integrin adhesion fractions. B) Integrin-associated proteins paxillin (green), β1 integrin (red), and phalloidin (white) were visualized with DAPI (blue). Scale bar = 25μm. Intact cells and integrin adhesion isolation fractions are shown. n=3.



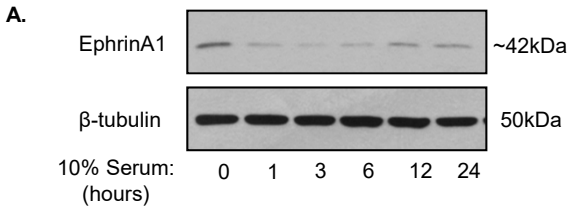
Supplemental Figure IV: Overexpression of EphA2 is sufficient to promote tensin localization to integrin adhesions. A) Human VSMCs were transduced with EphA2 adenovirus at the indicated MOI treatments for 24 hours, then serum-starved for three days. EphA2 was analyzed by immunoblot and normalized to GAPDH. B) Human VSMCs were treated with either GFP (control) or EphA2 adenovirus (MOI = 3) for 24 hours, then plated onto Matrigel-coated glass slides. Integrin adhesion isolation was performed, and integrin adhesion-associated Tensin was analyzed by immunoblot and normalized to ILK. GAPDH from whole cell lysate (WCL) shown for integrin adhesion isolation purity. (A) n = 1 (B) n=3. Data are expressed as mean ±SEM. Statistical comparisons were made using Student's T-test. A p-value less than 0.05 is considered significant.

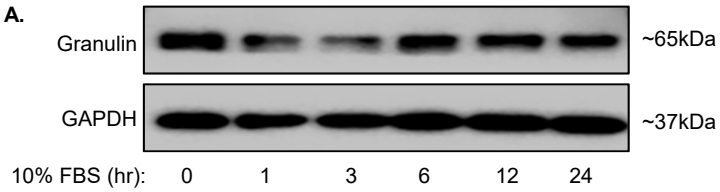


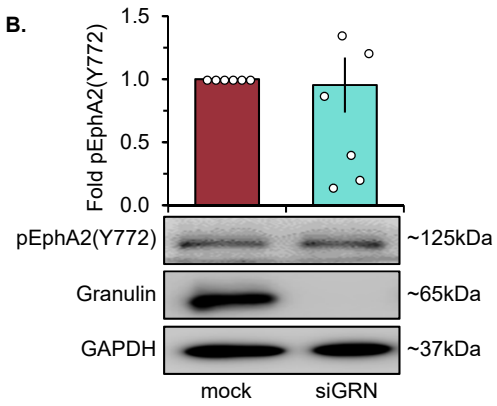
Supplemental Figure V: Integrin activation following depletion of EphA2 in VSMCs. Human VSMCs were transfected with either mock or siRNA directed against EphA2 for 24 hours. After plating, cells were serum-starved for 4 hours, then treated with 10% serum at the indicated time points. The fibronectin mimetic GST-$FNIII_{9-10}$ (20ug/mL) was added to each well for 30 minutes. 0.5mM $MnCl_2$ was added for a positive control. Bound GST-FN was analyzed by immunoblot and normalized to GAPDH. n=3. Data are expressed as mean ±SEM. Statistical comparisons were made using One-way ANOVA with Bonferroni post-test. A p-value less than 0.05 is considered significant.



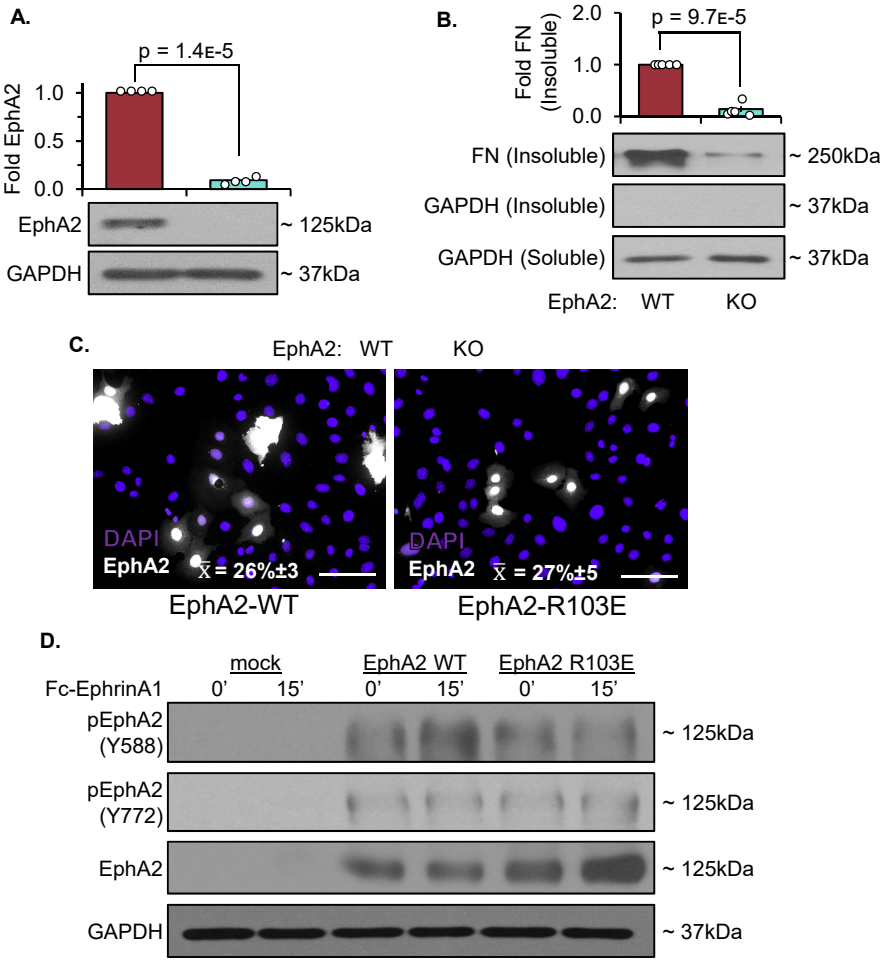
Supplemental Figure VI: Focal adhesions in Vascular Smooth Muscle Cells. Human VSMCs were treated with either mock or siEphA2 for 24 hours and plated onto Matrigel overnight. A) Cells were stained for active β1 integrin (12G10), and adhesion number was quantified. B) Cells were stained for active α5 integrin (SNAKA51), and adhesion number was quantified. C) Vinculin (red), Paxillin (green) and phalloidin (white) were visualized by immunofluorescence. Vinculin and Paxillin-positive focal points are considered focal adhesions, which were quantified for each high-powered field by (D) percent of cell area, (E) focal adhesion number per 100μm2, and (F) average focal adhesion size in μm2. Scale bar = 25μm. n=4. At least 4 high power fields were assessed for each condition. (D-E) are color coded by individual high power field to allow visualization of the data. Data were not significant using Student's T-tests.



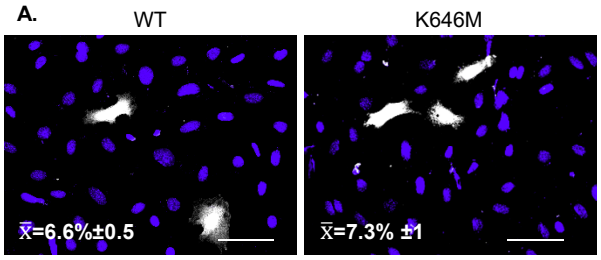


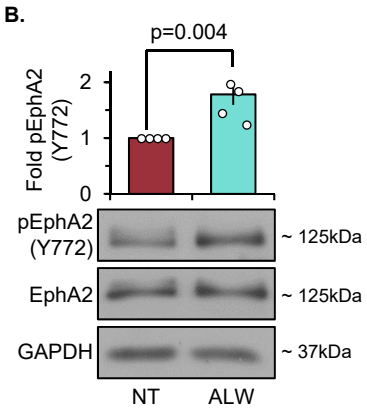


Supplemental Figure VII: EphA2 ligand expression and function. A/B) Expression of ephrinA1 and progranulin in primary hVSMCs were measured by immunoblot in cells following a 10% serum treatment for the indicated timepoints. B) A) Primary hVSMCs were serum-starved for 72 hours, followed by treatment with 10% serum for indicated time points. Levels of Granulin and GAPDH were assessed by immunoblotting. B) Primary hVSMCs were transfected with siGRN overnight. Cells were then plated onto FN-coated plates overnight in 1% serum. EphA2 Y772 phosphorylation, Granulin, and GAPDH levels were assessed by immunoblotting. n=6. Statistical analysis was performed using Student's T-test. Data are expressed as mean ±SEM. Statistical comparisons were made using Student's T-test. A p-value less than 0.05 is considered significant.

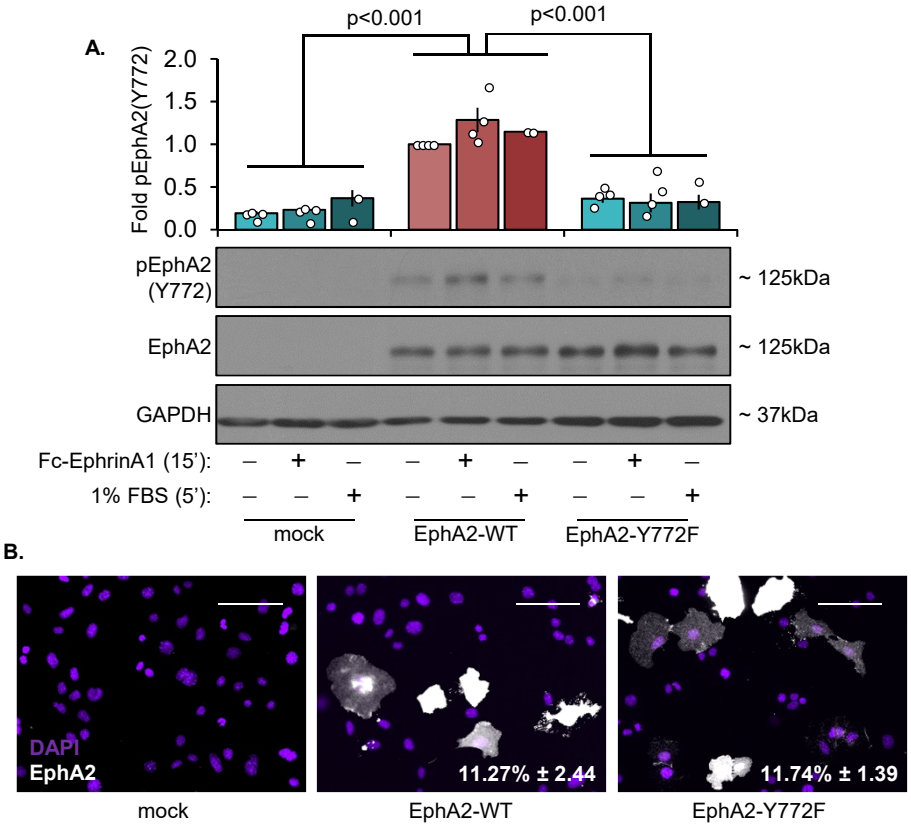


Supplemental Figure VIII: EphA2 KO SMCs and R103E Validation. EphA2 WT or EphA2 KO VSMCs were isolated. A) EphA2 expression was analyzed by immunoblot and normalized to GAPDH. B) Fibronectin deposition was measured by deoxycholate extraction, and protein was analyzed by immunoblot. Deoxycholate insoluble (deposited) fibronectin was normalized to deoxycholate soluble GAPDH. Deoxycholate insoluble GAPDH shown for isolation purity. C) EphA2 KO VSMCs were transfected with wildtype EphA2 or EphA2 R103E (ligation-deficient). Cells were stained for EphA2 (white) and DAPI (purple), and percent positive EphA2 was analyzed per total DAPI per high powered field. D) Cells were serum-starved for 4 hours, then treated with 1µg/mL fc-EphrinA1 for 15 minutes, followed by immunoblot analysis of Y588- and Y772-phospho EphA2, total EphA2, and GAPDH. n=4-5. Data are expressed as mean ±SEM. Statistical comparisons were made using Student's T-test. A p-value less than 0.05 is considered significant.

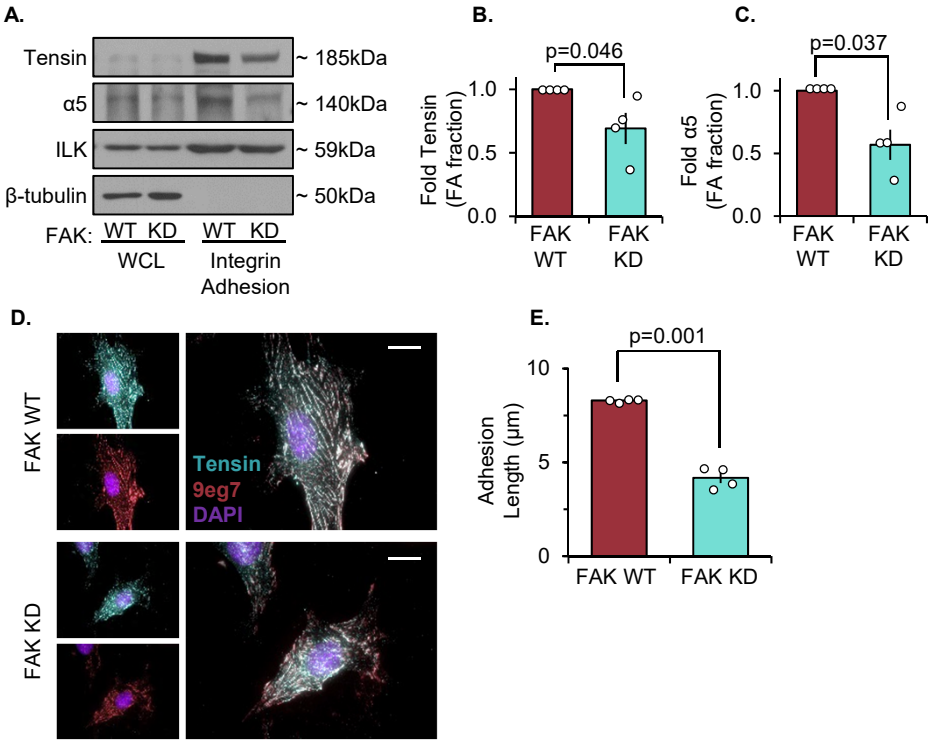




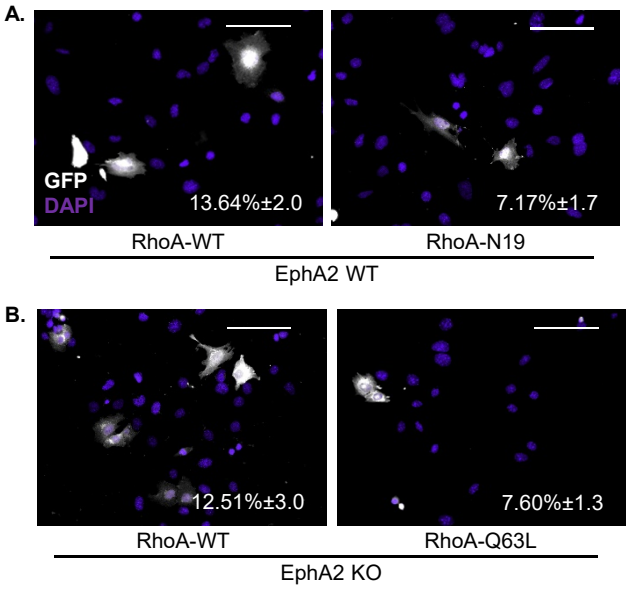
Supplemental Figure IX: EphA2 kinase activity and Y772 phosphorylation. A) EphA2 KO VSMCs were transfected with wildtype EphA2 or EphA2 K646M (kinase dead). Cells were stained for EphA2 (white) and DAPI (purple), and percent positive EphA2 was analyzed per total DAPI per high powered field. Scale bar = 100μm. n=4. B) EphA2 WT mouse aortic VSMCs cells were serum-starved for 4 hours, then treated with ALW (0.5μM) for 30 minutes. Phospho-EphA2 Y772 was quantified by immunoblot and normalized to total EphA2. n=4-5. Data are expressed as mean ±SEM. Statistical comparisons were made using Student's T-test. A p-value less than 0.05 is considered significant.



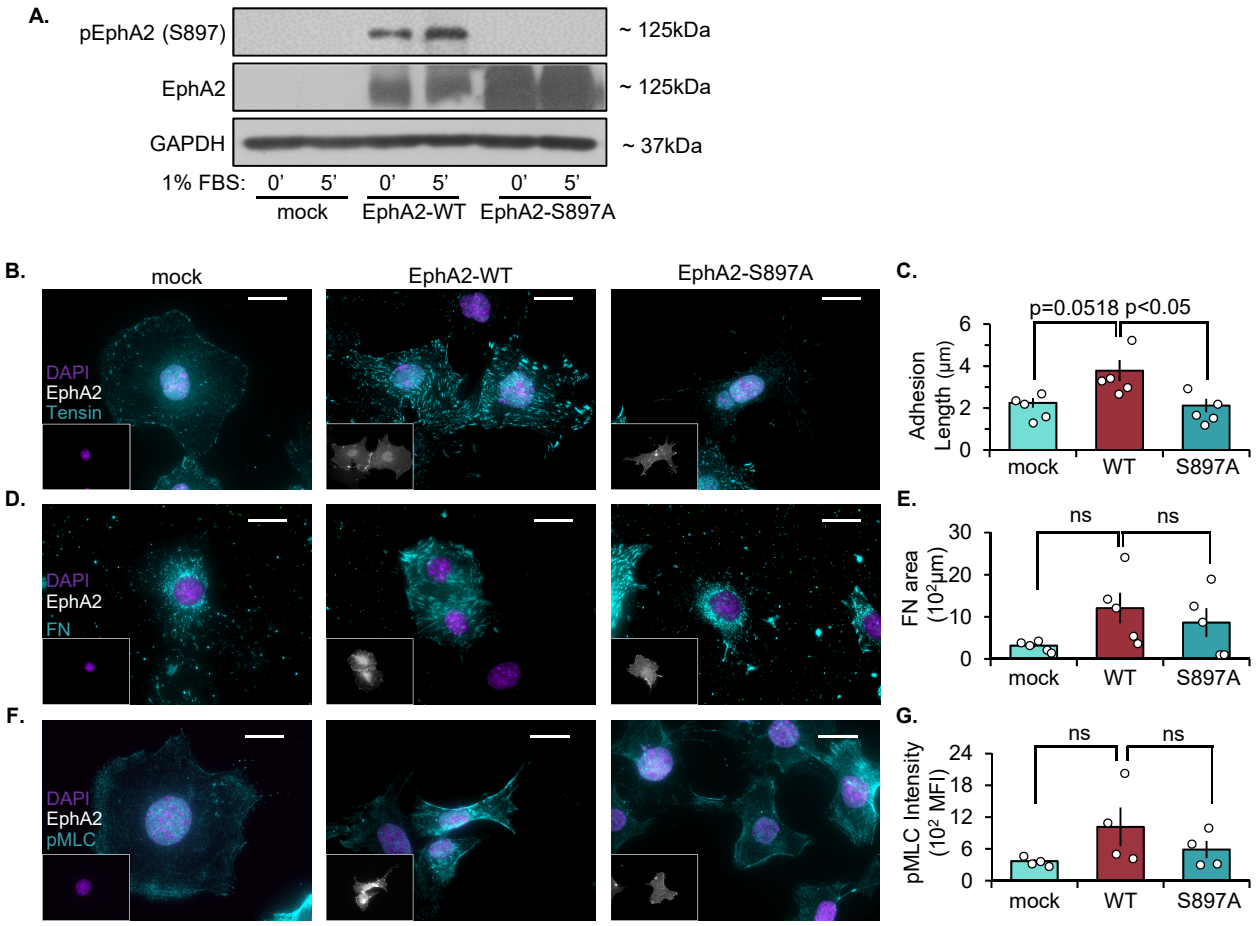
Supplemental Figure X: Validation of EphA2-WT and EphA2-Y772F point mutation constructs. EphA2 KO VSMC were transfected with mock, EphA2-WT, or EphA2-Y772F constructs for 24 hours. Cells were plated onto Matrigel overnight in 1% serum. A) Cells were serum-starved for four hours, then treated with either 1% serum or 1µg/mL fc-EphrinA1 at the indicated timepoints. Phospho-EphA2 was analyzed by immunoblot and normalized to GAPDH. B) Cells were stained for EphA2 (white) and DAPI (nuclei), and percent-positive EphA2 was analyzed per total DAPI per high powered field. Scale bar = 100µm. n=4. Data are expressed as mean ±SEM. Statistical comparisons were made using 2-way ANOVA with Bonferroni post-test. A p-value less than 0.05 is considered significant.



Supplemental Figure XI: FAK kinase-dead VSMCs have reduced fibrillar adhesion formation. A-E) FAK WT or FAK KD mouse VSMCs were plated onto Matrigel overnight. A-C) Cells were plated onto glass slides, and integrin adhesion isolation was performed. Protein expression was measured by immunoblot and normalized to integrin-linked kinase (ILK). β-tubulin from the whole cell lysate (WCL) fraction is shown for integrin adhesion isolation purity. B,C) Tensin and α5 integrin expression from the integrin adhesion fraction were quantified. D,E) Cells were stained for tensin (teal) and active β1 integrin (9eg7, pink) and DAPI (purple), and adhesion length was measured in microns. Scale bar = 25μm. n=4. Data are expressed as mean ±SEM. Statistical comparisons were made using Student's T-test (B,C,E). A p-value less than 0.05 is considered significant.



Supplemental Figure XII: Transfection efficiency of Rho constructs in EphA2-WT and EphA2-KO VSMCs. A-B) EphA2 WT or EphA2 KO VSMCs were transfected with either RhoA-WT, (A) RhoA-N19, or (B) RhoA-Q63L constructs for 24 hours. Cells were plated onto Matrigel-coated coverslips in 1% serum overnight, and percent-positive GFP cells (white) were normalized to total DAPI (purple) per high powered field. Scale bar = 100μm. n=4. Data are expressed as mean ±SEM. Statistical comparisons were made using Student's T-test. A p-value less than 0.05 is considered significant.



Supplemental Figure XIII: EphA2 S897 is required for fibrillar adhesion formation. EphA2 KO VSMCs were transfected with mock, EphA2-WT, or EphA2-S897A constructs for 24 hours. Cells were plated onto Matrigel overnight in 1% serum. A) Cells were serum-starved for four hours, then treated with 1% serum for 5 minutes. S897-phospho EphA2 was examined by immunoblot and the shown blot represents at least 4 independent experiments. B-G) Cells were stained for EphA2 (white), DAPI (purple) B,C) for tensin (teal) and adhesion length was measured in microns, D,E) fibronectin (teal) was quantified as fibronectin-positive area in microns, and F,G) phospho-MLC (pink) intensity was measured and quantified as mean fluorescence intensity (MFI). Scale bar = 100μm. n=4-5. Data are expressed as mean ±SEM. Statistical comparisons were made using one-way ANOVA with Bonferroni post-test.