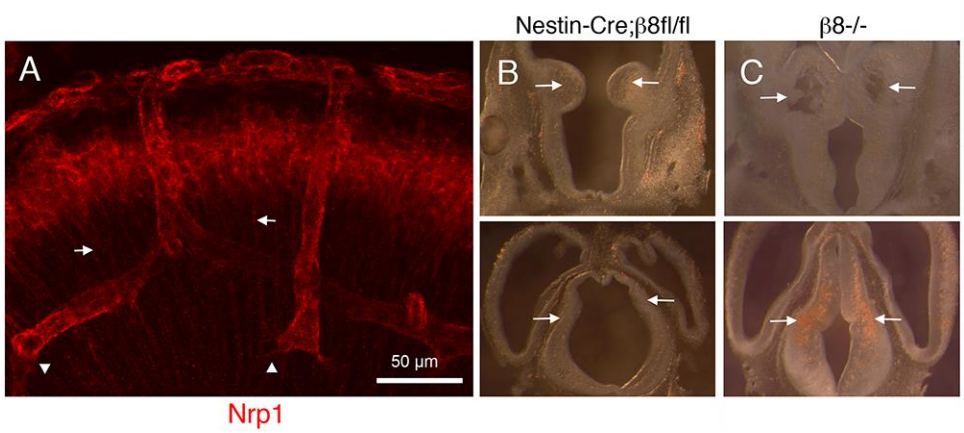


Fig. S1. Selective ablation of β8 integrin in neuroepithelial cells leads to brain vascular pathologies. (A) A horizontal section through the E13.5 mouse brain cortex was stained with an anti-Nrp1 antibody. Note that Nrp1 protein is expressed predominantly in cerebral blood vessels (arrow heads), and at lower levels in neuroepithelial cell processes (arrows). **(B-C)** Horizontal sections (100-200 μm) through the brains of *Nestin-Cre/+;β8*$^{fl/fl}$ (B, E12.5), or *β8*$^{-/-}$ (C, E13.5) embryos were cut with a vibratome. Note the cavitations and punctate microhemorrhage within the ganglionic eminences (arrows, upper panels) and thalamus (arrows, lower panels).

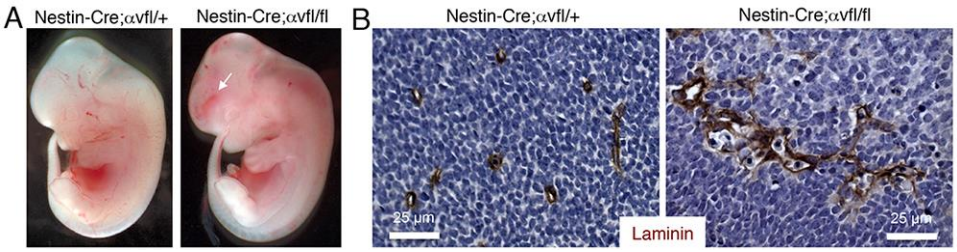


Fig. S2. Selective ablation of αv integrin in neuroepithelial cells leads to defective brain angiogenesis. (A) Images of representative E12.5 control (left) and *Nestin-Cre/+;*$\alpha v^{fl/fl}$ mutant embryos (right). Note the focal hemorrhage in the mutant brain (arrow). **(B)** Horizontal sections thorough E12.5 control (left) and *Nestin-Cre/+;*$\alpha v^{fl/fl}$ (right) mutant brains were immunolabeled with anti-laminin antibodies. Note the abnormal morphologies of cerebral blood vessels in αv integrin conditional knockouts.

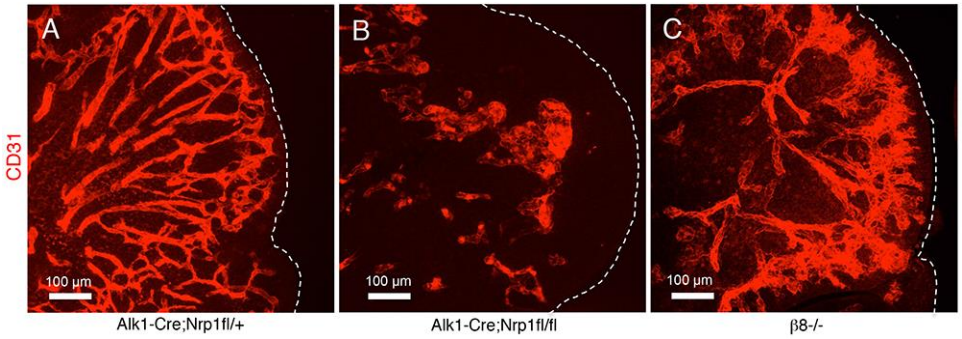


Fig. S3. Genetic ablation of Nrp1 in endothelial cells leads to defective cerebral blood vessel sprouting. (A-C) Horizontal sections thorough the ganglionic eminences of E13.5 control (A), *Alk1-Cre/+;Nrp1*$^{fl/fl}$ (B), and *β8*$^{-/-}$ (C) brains were immunolabeled with anti-CD31 mAb. Note the abnormal vascular patterning in the Nrp1 and β8 integrin mutants. Blood vessels in Nrp1 mutants form glomeruloid-like tufts prior to reaching subventricular regions, whereas blood vessels in β8-/- brains reach subventricular regions and form glomeruloid-like tufts. The dashed lines indicate boundaries between brain tissue and ventricles.

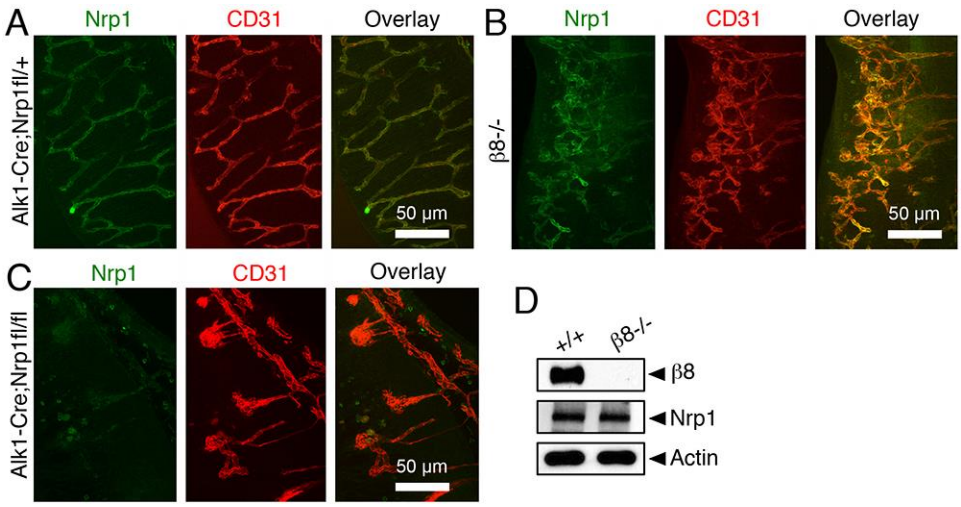


Fig. S4. Analysis of Nrp1 and β8 integrin levels in knockout mouse models reveals lack of reciprocal regulation of protein expression. (A-C) Brain sections from control (A); *β8*$^{-/-}$ (B) and *Alk1-Cre/+;Nrp1*$^{fl/fl}$ embryos (C) were fluorescently labeled with anti-Nrp1 (green) and anti-CD31 (red) antibodies. Note that Nrp1 protein expression in endothelial cells is significantly diminished in *Alk1-Cre/+;Nrp1*$^{fl/fl}$ samples, but is not impacted in *β8*$^{-/-}$ samples. **(D)** Brain lysates from control and *β8*$^{-/-}$ embryos were immunoblotted for Nrp1 and β8 integrin antibodies. Ablation of β8 integrin in the neuroepithelium does not alter Nrp1 protein expression.

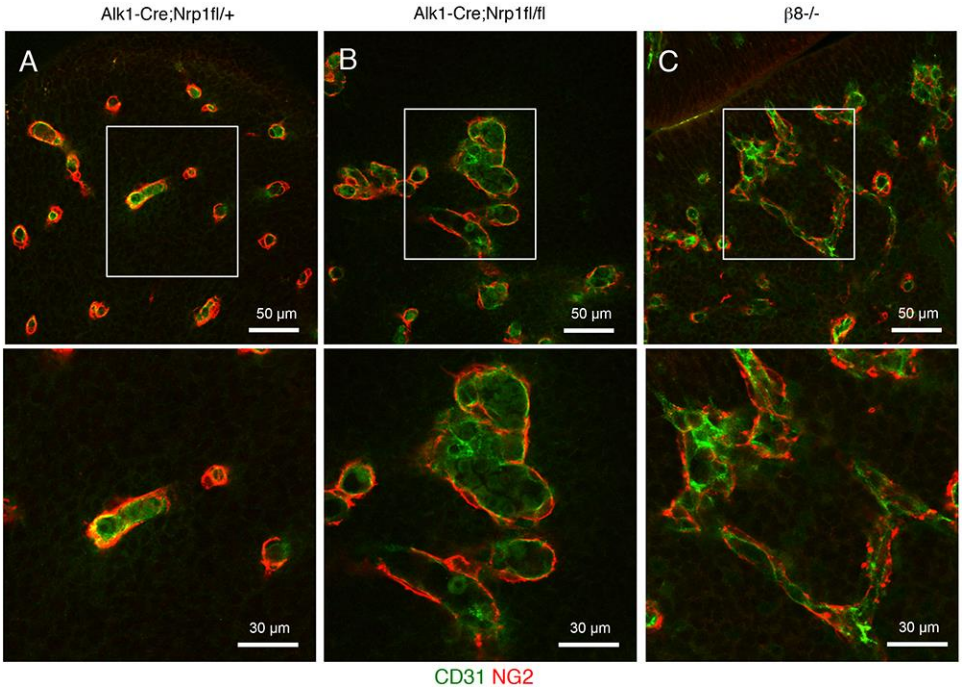


Fig. S5. Analysis of endothelial-pericyte interactions in Nrp1 and β8 integrin mutant mouse models. (A-C) Horizontal sections through the brain ganglionic eminences of control (A), *Alk1-Cre/+;Nrp1*$^{fl/fl}$ mutant (B) and *β8*$^{-/-}$ embryos (C) labeled with CD31 (green) and NG2 (red) to reveal endothelial cells and pericytes. Note that vascular pericytes are associated with endothelial cells in both the control and mutant samples.

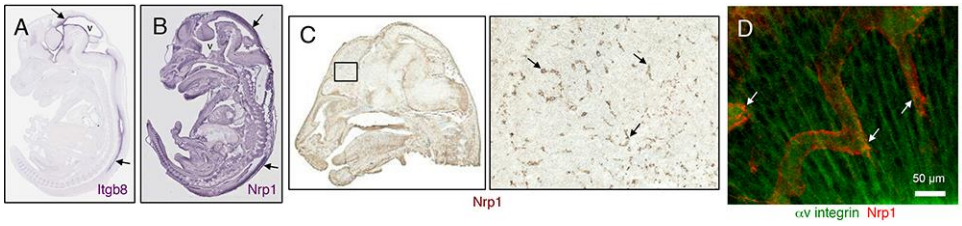


Fig. S6. In vivo gene expression patterns of mouse β8 integrin and Nrp1. (A, B) Genepaint.org was queried for *itgb8* (A) or *nrp1* (B) expression patterns in E14.5 mouse embryos. Note that *Itgb8* mRNA is expressed mainly in neuroepithelial cells adjacent to the brain ventricles (arrows, A), whereas *nrp1* (B) is expressed within and outside the CNS (B). **(C)** Nrp1 mRNA expression patterns from analysis in the gensat.org, revealing robust gene expression in cerebral blood vessels (arrows). **(D)** Horizontal sections through the E13.5 mouse brain were stained with antibodies recognizing αv integrin (green) and Nrp1 (red). Note that αv integrin protein is expressed in neuroepithelial cells that closely juxtapose endothelial cells expressing Nrp1 protein (arrows).

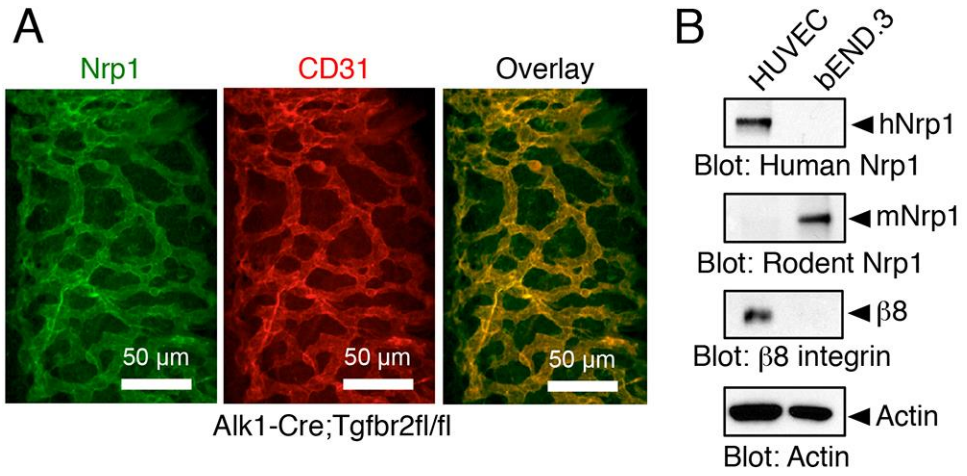


Fig. S7. Genetic deletion of Tgfbr2 in endothelial cells does not impact Nrp1 protein expression. (A) Horizontal brain sections from *Alk1-Cre/+;Tgfbr2*$^{fl/fl}$ embryos were labeled with anti-Nrp1 (green) and anti-CD31 (red) antibodies. Note that Nrp1 protein is expressed in endothelial cells. **(B)** Detergent-soluble lysates from HUVECs or bEND.3 cells were then immunoblotted with antibodies recognizing human or rodent Nrp1 protein or pan-species antibody recognizing β8 integrin.

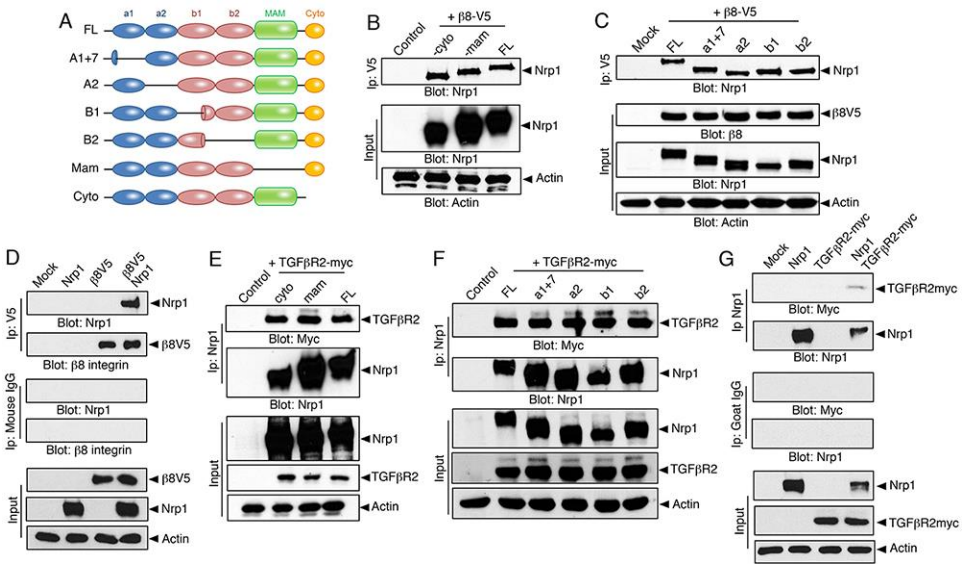


Fig. S8. Nrp1 forms protein complexes with β8 integrin and TGFβR2. (A) Schematic diagram showing domain composition of full-length, wild type rat Nrp1 (FL) and various constructs containing deletions in the extracellular region or cytoplasmic domain. **(B, C)** HEK-293 cells were transfected with a pcDNA3.1A plasmid expressing V5-tagged human β8 integrin in combination with pcDNA3.1 expression plasmids expressing full-length Nrp1 or cDNA constructs containing deletions in the cytoplasmic tail or Mam domain (B). Alternatively, cells were transfected with plasmids expressing V5-tagged human β8 integrin with the full-length Nrp1 cDNA or cDNAs encoding various extracellular domain deletions in the pMT21 expression plasmid (C). Detergent-soluble lysates were then immunoprecipitated with anti-V5 antibodies and then immunoblotted with anti-Nrp1 antibodies recognizing the full-length control or mutated proteins. **(D)** HEK-293 cells were transfected with plasmids expressing V5-tagged human β8 integrin alone, rat Nrp1 alone, or both plasmids in combination. Lysates were immunoprecipitated with mouse IgGs or anti-V5 mAb and then immunoblotted with anti-Nrp1 antibodies. Note that immunoprecipitation with anti-V5, but not negative control IgGs, reveal a

specific interaction between Nrp1 and β8 integrin. **(E, F)** HEK-293 cells were transfected with a pcDNA3.1A plasmid expressing myc-tagged human TGFβR2 in combination with pcDNA3.1 expression plasmids expressing full-length Nrp1 or cDNA constructs containing deletions in the cytoplasmic tail or Mam domain (E). Alternatively, cells were transfected with plasmids expressing myc-tagged human TGFβR2 with the full-length Nrp1 cDNA or cDNAs encoding various extracellular domain deletions in the pMT21 expression plasmid (F). Detergent-soluble lysates were then immunoprecipitated with anti-myc antibodies and then immunoblotted with anti-Nrp1 antibodies recognizing the full-length control or mutated proteins. **(G)** Cells were transfected with plasmids expressing rat Nrp1 alone, myc-tagged human TGFβR2 alone, or Nrp1 and TGFβR2-myc in combination. Lysates were then immunoprecipitated with goat IgGs or anti-Nrp1 antibodies and then immunoblotted with anti-myc antibodies. Note that Nrp1 and TGFβR2 interactions are detected only when specific anti-Nrp1 antibodies are used for immunoprecipitation, but not with negative control goat IgGs.

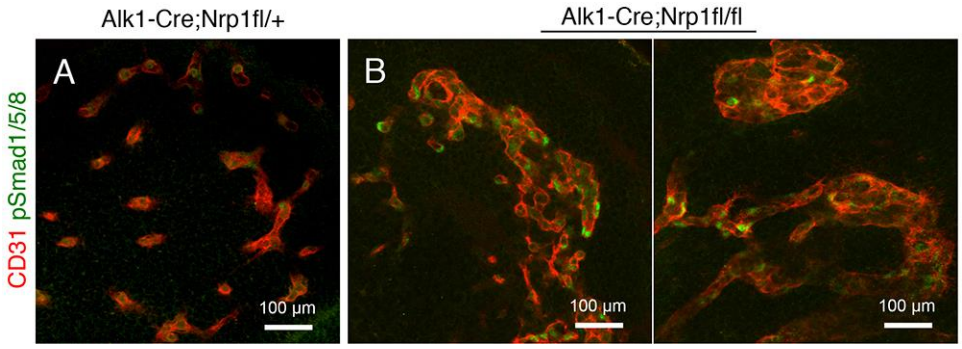


Fig. S9. Genetic deletion of Nrp1 in endothelial cells in vivo leads to enhanced phosphorylation of Smad1/5/8. (A, B) Horizontal sections through the cerebral cortices of E13.5 *Alk1-Cre/+;Nrp1*$^{fl/+}$ (A) and *Alk1-Cre/+;Nrp1*$^{fl/fl}$ (B) embryonic brains were immunostained with anti-pSmad1/5/8 and anti-CD31 antibodies. Note the increased levels of pSmad1/5/8 within *Nrp1*$^{-/-}$ endothelial cell nuclei.

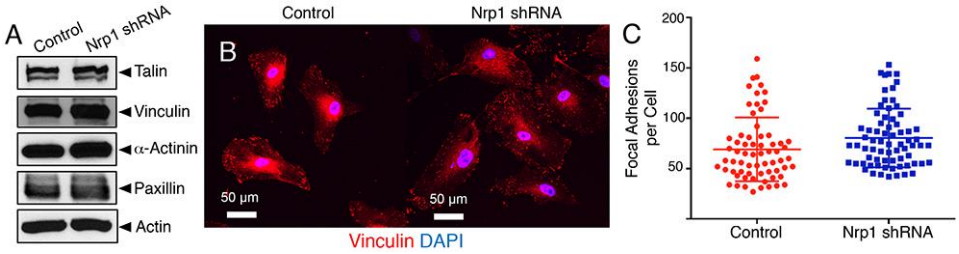
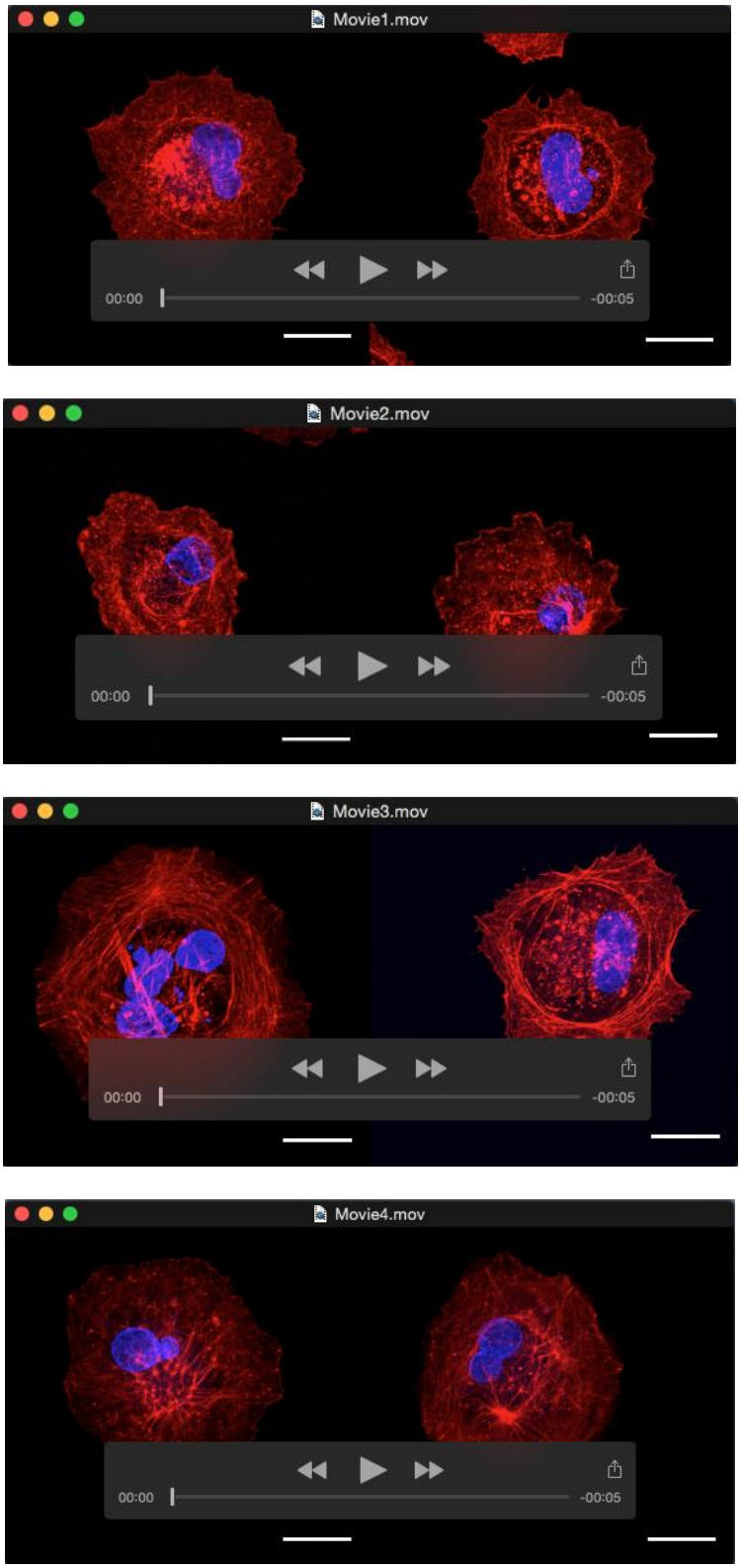


Fig. S10. Silencing Nrp1 in human endothelial cells does not impact focal adhesion formation. (A) Detergent-soluble lysates from HUVECs expressing control or Nrp1 shRNAs were immunoblotted with various antibodies recognizing focal adhesion and signaling proteins. **(B, C)** HUVECs on collagen I-coated dishes were labeled with anti-vinculin antibodies (B) and numbers of focal adhesions were quantified (C).



Movies 1-4. Nrp1 contributes to the organization of the actin cytoskeleton during cell spreading. Cells were starved for 1 h, suspended, and re-plated on fibronectin-coated surfaces for various

intervals. Following fixation, cells were permeabilized and stained with fluorescent phalloidin (red) and a nuclear dye (blue) to visualize the actin cytoskeleton and nuclei, respectively. Fiji was used for the three-dimensional rendering and animation of confocal Z-stacks consisting of 0.5μm optical sections collected at 0.25 μm Z-steps. **(1)** Control cells 10 minutes after plating, **(2)** Nrp1 shRNA cells 10 minutes after plating, **(3)** Control cells 20 minutes after plating, **(4)** Nrp1 shRNA cells 20 minutes after plating. Scale bars represent 20 μm.