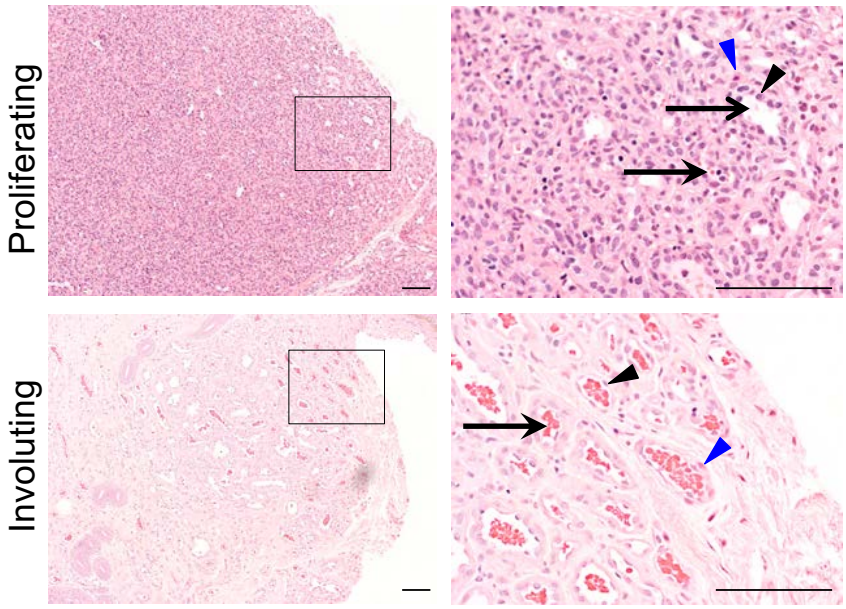
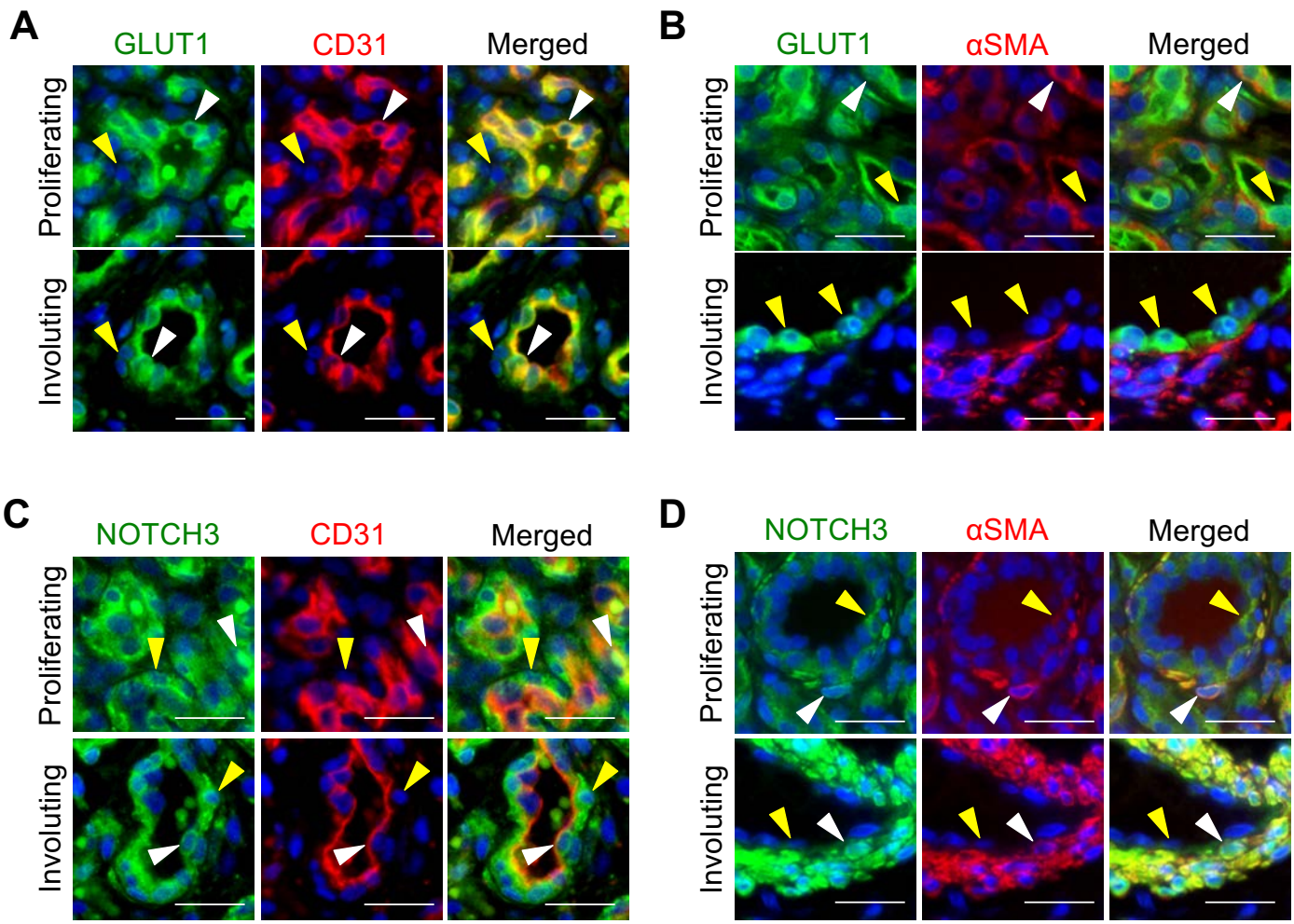
Supplemental Figure 1, Edwards et al.

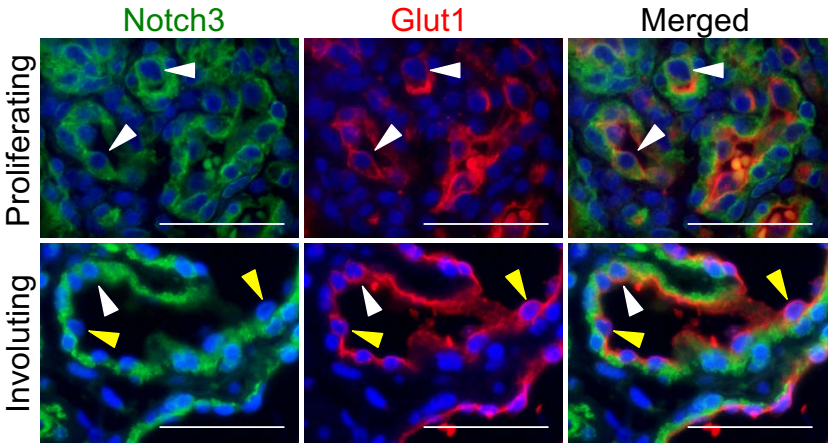


Supplemental Figure 1. Proliferating and involuting IH morphology. Representative H&E staining of proliferating (n=3) and involuting IHs (n=3). Boxed area enlarged to right. Black arrowheads mark endothelial cells. Blue arrowheads mark perivascular cells. Black arrows mark red blood cells. Scale bars - 100μm. IH, infantile hemangioma.

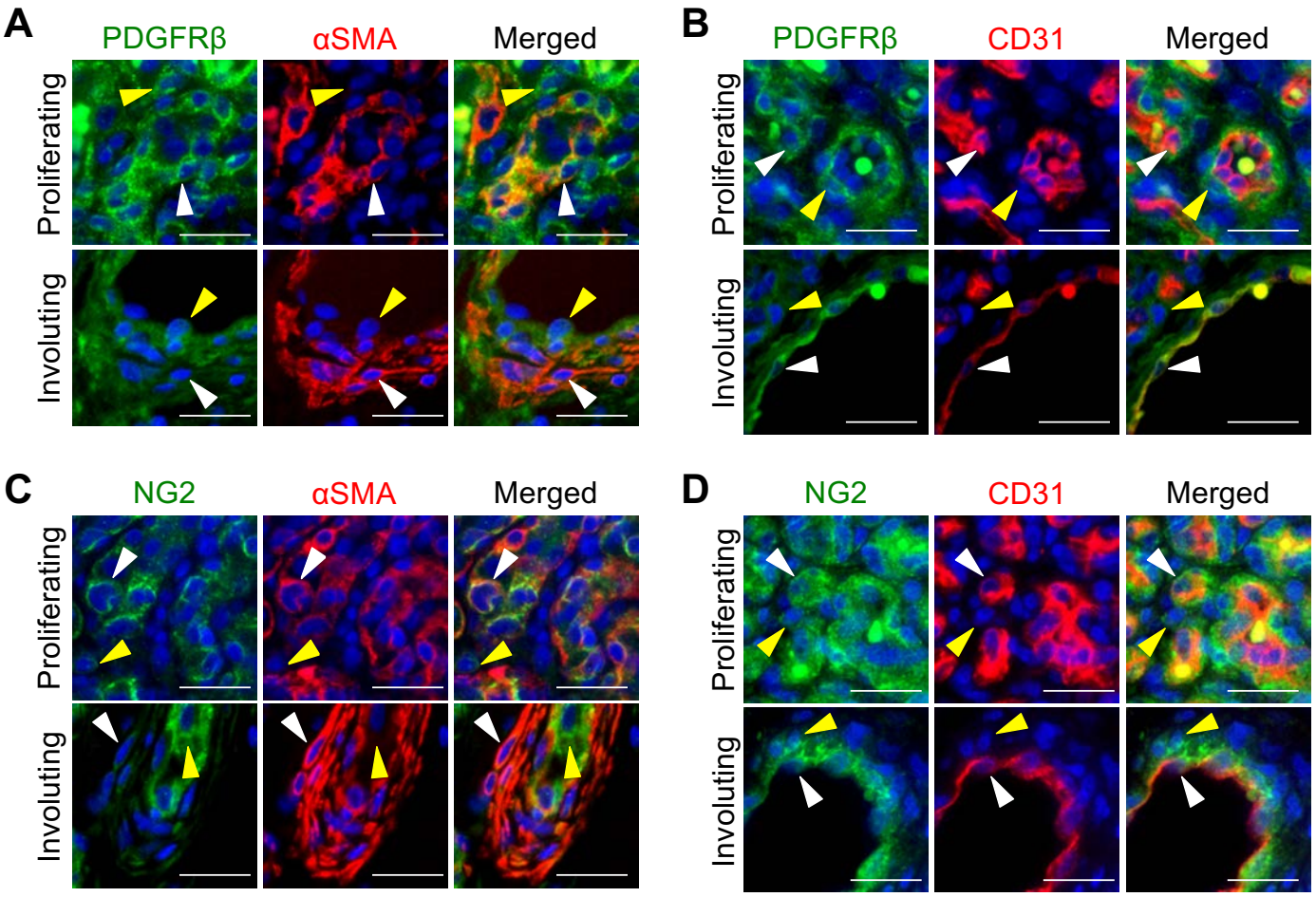


Supplemental Figure 2. High magnification images of proliferating and involuting IHs presented in Figure 1. A) GLUT1 and CD31 co-staining. White arrowheads mark GLUT1+/CD31+ cells. Yellow arrowheads mark GLUT1+/CD31- cells. 3, Proliferating IH n=2, involuting IH n=3. **B**) GLUT1 and αSMA co-staining. Yellow arrowheads mark αSMA+/GLUT1- perivascular cells. Proliferating IH n=2, involuting IH n=7 **C**) NOTCH3 and CD31 co-staining. White arrowheads mark NOTCH3+/CD31+ cells. Yellow arrowheads mark NOTCH3+/CD31- cells. Proliferating IH n=4, involuting IH n=5. **D**) NOTCH3 and αSMA co-staining. White arrowheads mark NOTCH3+/αSMA+ perivascular cells. Yellow arrowheads mark NOTCH3+/αSMA- lumenal cells. Proliferating IH n=5, involuting IH n=8. Scale bars - 25μm. Total number of IH specimens assessed for each antigen presented in Supplemental Table 3. αSMA, alpha smooth muscle actin; GLUT1, glucose transporter 1; IH, infantile hemangioma.

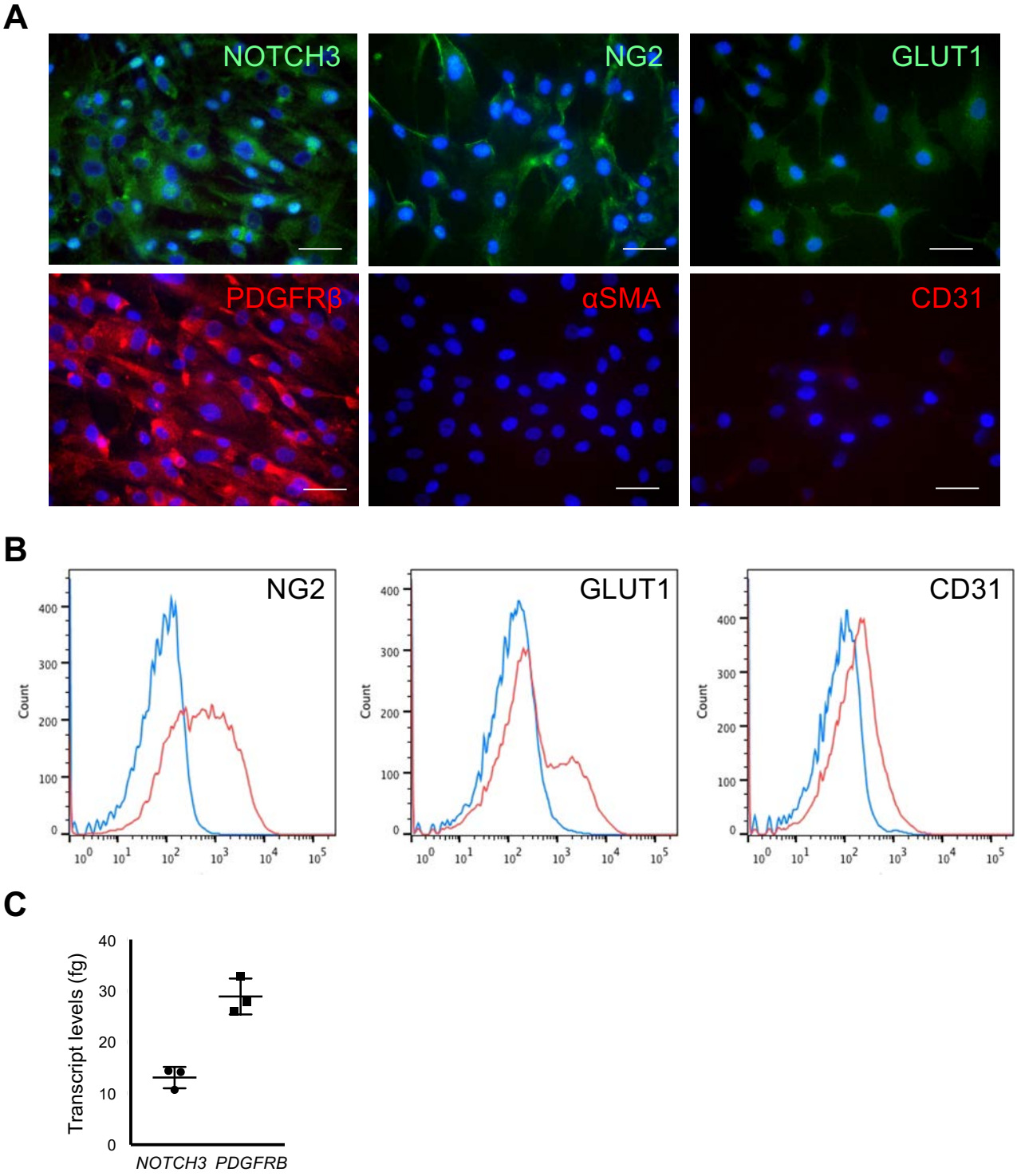
Supplemental Figure 3, Edwards et al.



Supplemental Figure 3. NOTCH3 is expressed in a subset of Glut1+ IH cells. Proliferating and involuting IH specimens stained for NOTCH3 and GLUT1. White arrowheads mark NOTCH3+/GLUT1+ cells. Yellow arrowheads mark GLUT1+/NOTCH3- lumenal cells. Proliferating IH n=4, involuting IH n=2 Scale bars - 50μm. GLUT1, glucose transporter 1; IH, infantile hemangioma.

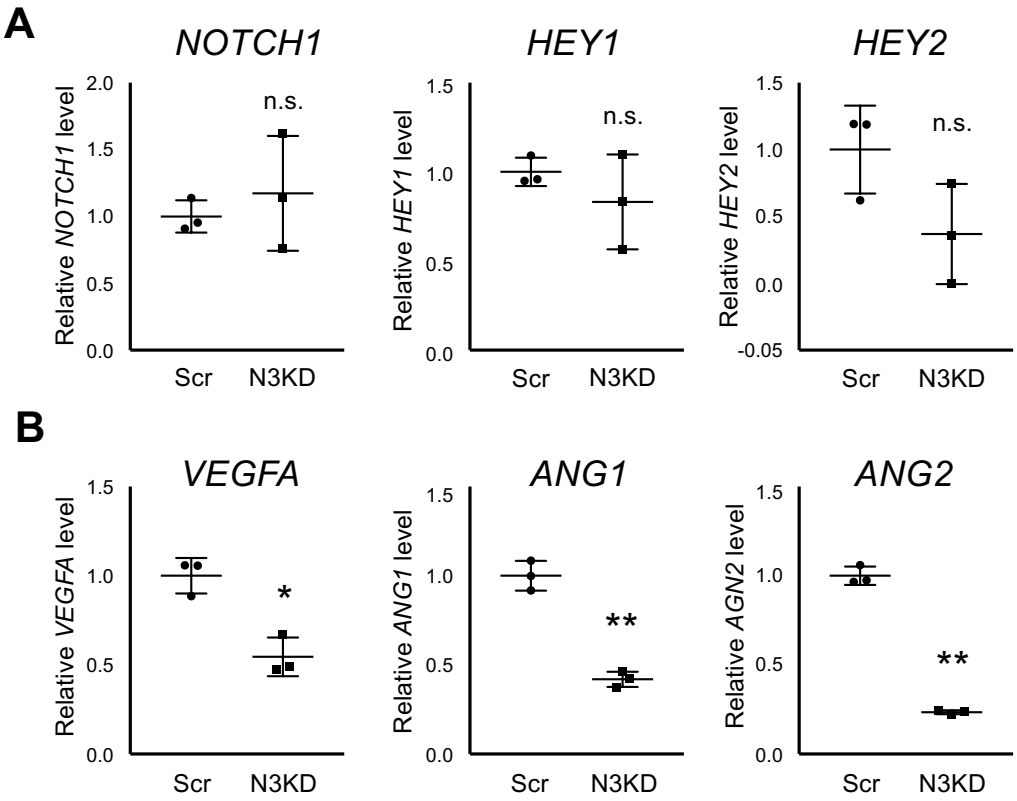


Supplemental Figure 4. High magnification images of proliferating and involuting IHs presented in Figure 2. A) PDGFRβ and αSMA co-staining. White arrowheads mark PDGFRβ+/αSMA+ perivascular cells. Yellow arrowheads mark PDGFRβ+/αSMA- cells. Proliferating IH n=2, involuting IH n=3. **B**) PDGFRβ and CD31 co-staining. White arrowheads mark PDGFRβ+/CD31+ cells. Yellow arrowheads mark PDGFRβ+/CD31- perivascular cells. Proliferating IH n=3, involuting IH n=2. **C**) NG2 and αSMA co-staining. White arrowheads mark NG2+/αSMA+ cells, and yellow arrowheads mark NG2+/αSMA- cells. Proliferating IH n=7, involuting IH n=7. **D)** NG2 and CD31 co-staining. White arrowheads mark NG2+/CD31+ cells, and yellow arrowheads mark NG2+/CD31- cells. Proliferating IH n=7, involuting IH n=3. Scale bars - 25μm. Total number of IH specimens assessed for each antigen presented in Supplemental Table 3. αSMA, alpha smooth muscle actin; GLUT1, glucose transporter 1; IH, infantile hemangioma; NG2, neuron-glial antigen 2.



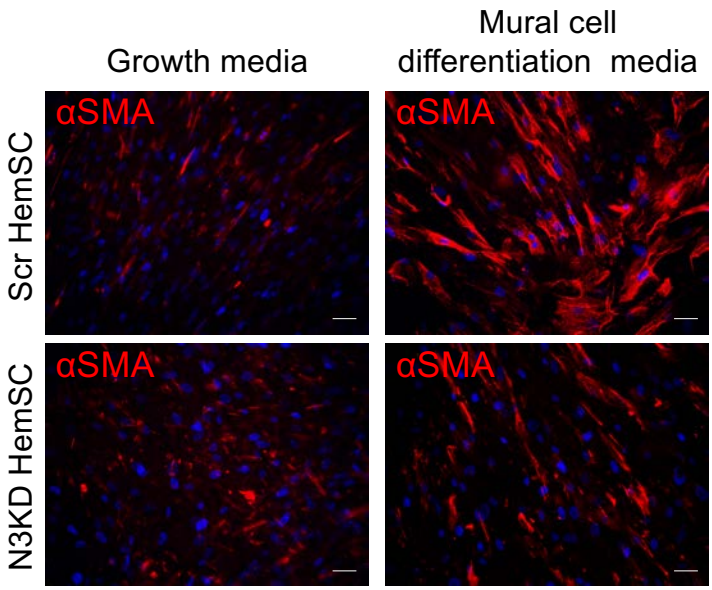
Supplemental Figure 5. Expression of endothelial and perivascular markers in isolated HemSCs. A) CD133+ HemSCs (n=3, done in duplicate) stained for NOTCH3, NG2, GLUT1, PDGFRβ, αSMA, and CD31. Scale bars - 50μm. **B**) NG2, GLUT1 and CD31 (red line) and control IgG (blue line) FACS of HemSCs (n=3, done in duplicate). **C**) Representative data of HemSC *NOTCH3* and *PDGFRB* transcript levels determined by qRT-PCR and normalized to *BACTIN*. (n=3, done in duplicate) Error bars represent ± S.D. αSMA, alpha smooth muscle actin; GLUT1, glucose transporter 1; IH, infantile hemangioma; HemSC, hemangioma stem cell; NG2, neuron-glial antigen 2.

Supplemental Figure 6, Edwards et al.



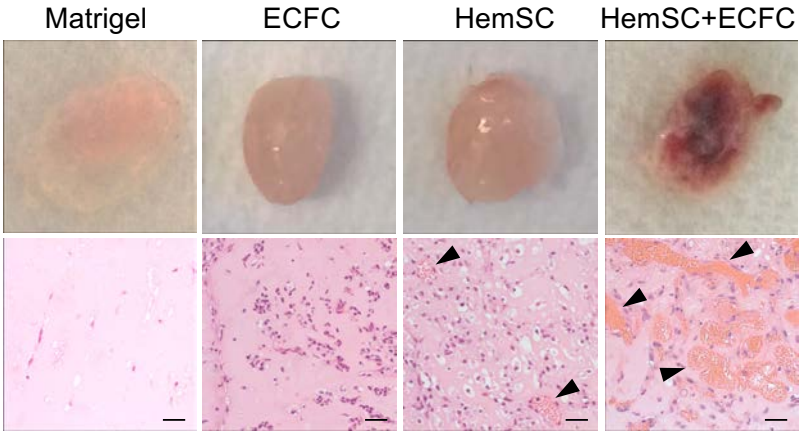
Supplemental Figure 6. Characterization of *NOTCH3* knockdown HemSCs. A) Relative *NOTCH1*, *HEY1* and *HEY2* transcript levels. Representative data from 4 independent N3KD HemSC populations and matching Scr HemSC populations determined by qRT-PCR and normalized to *BACTIN*. Error bars represent ± S.D. n.s. - not significant. Student T-Test. **B**) Relative *VEGFA*, *ANG1*, and *ANG2* transcript levels. Representative data from 2 independent N3KD HemSC populations and matching Scr HemSC populations determined by qRT-PCR and normalized to *BACTIN*. Error bars represent ± S.D. *p<0.01, **p<0.005. Student T-Test. ANG1, angiopoietin 1; ANG2, angiopoietin 2; HemSC, hemangioma stem cell; N3KD, Notch3 knockdown,

Supplemental Figure 7, Edwards et al.

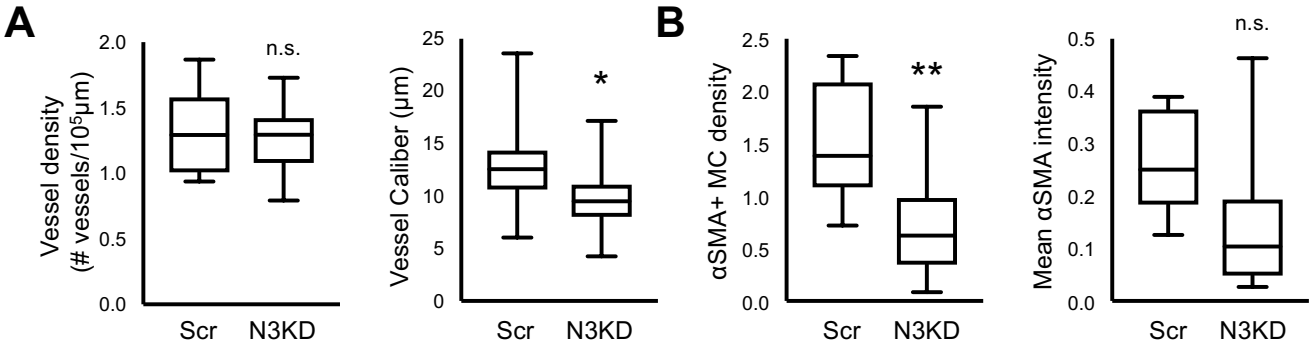


Supplemental Figure 7. *NOTCH3* knockdown blocks HemSC mural cell differentiation. Scrambled HemSCs (Scr HemSC) or *NOTCH3* knockdown HemSCs (N3KD HemSC) were grown in growth or mural cell differentiation media for 2 weeks and stained for αSMA. Scale bars - 50μm. (n=3 HemSC populations done in duplicate) αSMA, alpha smooth muscle actin; HemSC, hemangioma stem cell.

Supplemental Figure 8, Edwards et al.

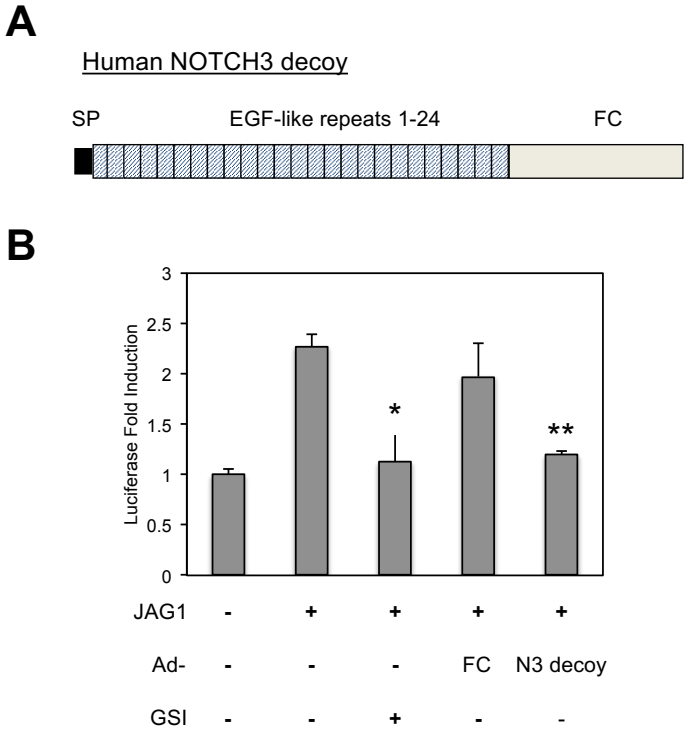


Supplemental Figure 8. **HemSC/ECFC xenografts robustly develop an IH like phenotype.** HemSCs, ECFCs or both ECFCs and HemSCs in 1:1 ratio were resuspended in Matrigel, and subcutaneously implanted into the flanks of immunocompromised mice. Matrigel without cells served as a control. Xenografts were evaluated at Day 14 post-implantation. Two independent experiments were done and representative data presented. **Top**) Gross appearance of explanted Matrigel. **Bottom**) H&E staining of representative xenograft sections. Arrowheads mark large caliber red-blood cell containing IH-like vessel. Scale bar - 50μm. IH, infantile hemangioma; HemSCs, hemangioma stem cells; ECFCs, endothelial colony forming cells.



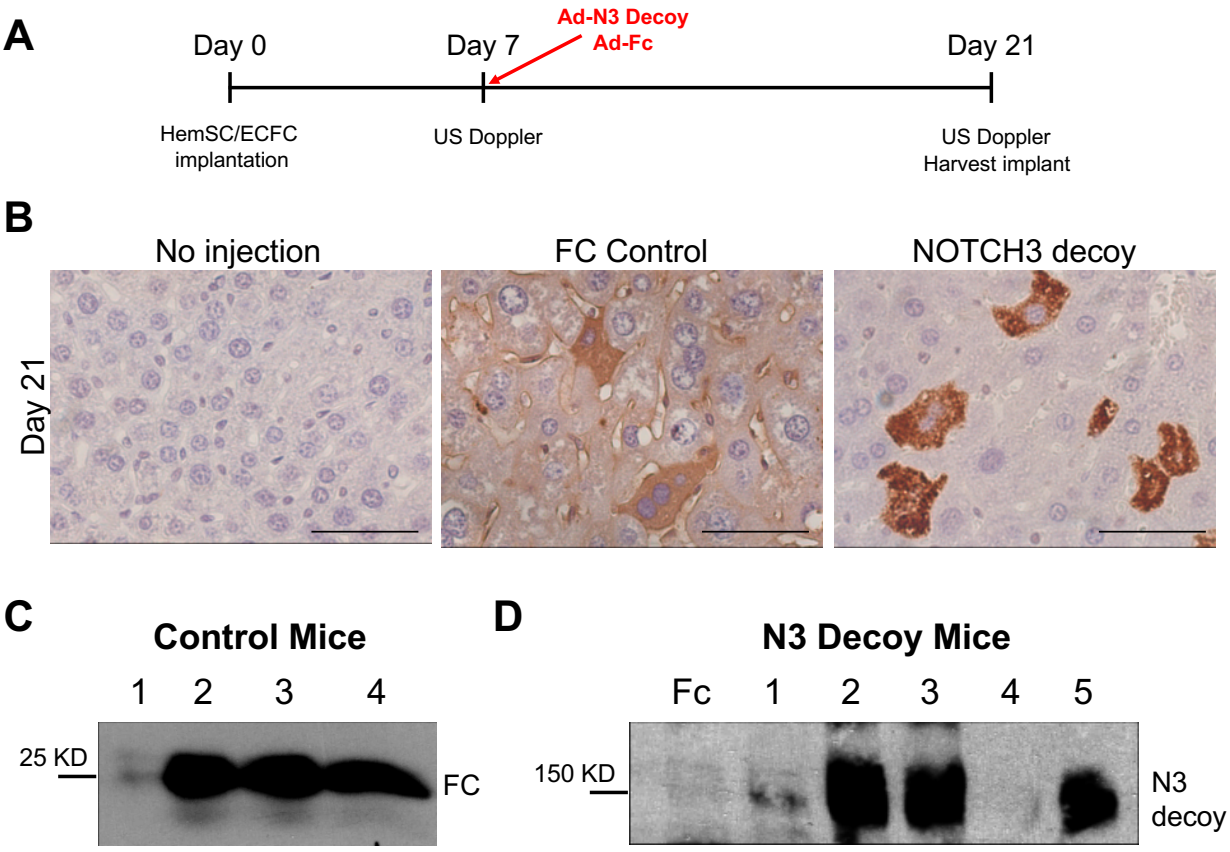
Supplemental Figure 9. *NOTCH3* knockdown in HemSCs inhibits IH development in a HemSC-only xenograft mouse model. A) Quantification of vessel density and caliber. Results representative of n = 3 HemSC populations (2 implants each). Error bars represent ± S.D. n.s., not significant, *p<0.0001. Student T-Test. **B**) αSMA+ mural cell density determined as mean mural cell αSMA signal intensity normalized to IH endothelial GLUT1 signal intensity. Average mural cell αSMA expression determined as mean αSMA signal intensity normalized DAPI+/αSMA+ cell number. Results representative of n = 3 HemSC populations (2 implants each). Error bars represent ± S.D. n.s., not significant, **p<0.01. Student T-Test. αSMA, alpha smooth muscle actin; GLUT1, glucose transporter 1; HemSC, hemangioma stem cell; MC, mural cell.

Supplemental Figure 10, Edwards et al.



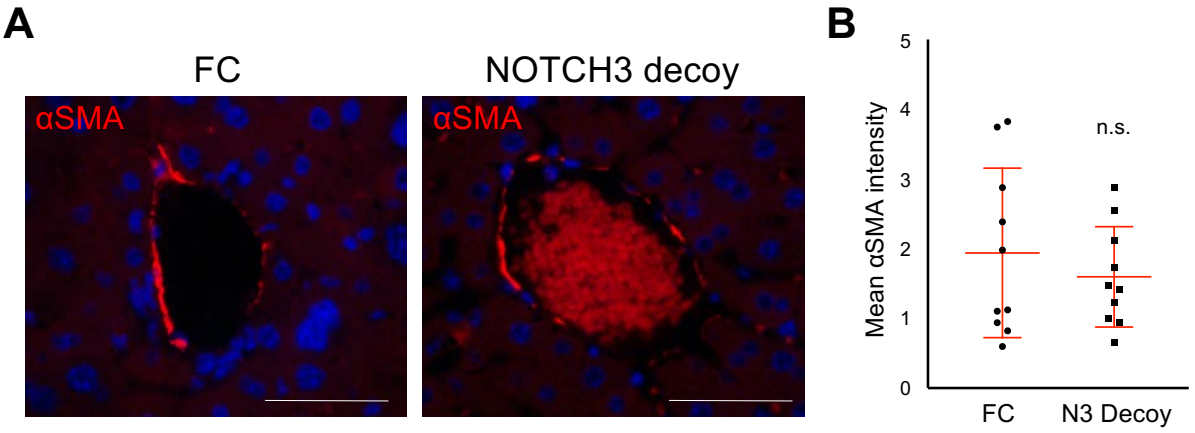
Supplemental Figure 10. The NOTCH3 inhibitor, human NOTCH3 Decoy (N3 Decoy), inhibits JAG1 activation of Notch signaling. A) N3 Decoy encodes the signal peptide (SP) and EGF-like repeats 1-24 of human NOTCH3 fused to in frame with human IgG FC. **B**) Notch/CSL signal activation measured in HeLa cells expressing full-length rat NOTCH1, N3 Decoy and a Notch luciferase reporter (11CSL-Luc) co-cultured with HeLa cells expressing the Notch ligand, JAG1. Addition of Compound E, a gamma-secretase inhibitor (GSI), was used as a control and inhibited Notch activation of the 11CSL-Luc reporter. Co-culture assays were performed in triplicate and repeated three times. Data presented as mean luciferase fold induction ± S.D. *p< 0.02, **p<0.005, Student T-Test. CSL, C promoter binding factor 1/Suppressor of Hairless/Lag-1.

Supplemental Figure 11, Edwards et al.



Supplemental Figure 11. **Design and validation of the NOTCH3 Decoy HemSC/ECFC xenograft study. A**) Schematic of experimental time-line. **B**) Staining with antibodies against human FC of livers (Day 21) from mice injected an adenovirus encoding either N3 Decoy or FC. Liver sections from un-injected mice served as a negative control. Scale bar - 50μm. **C**) Western blot with anti-FC antibody of sera (Day 21) collected from mice injected with the adenovirus encoding FC (n=4). **D**) Western blot of sera immunoprecipitated with antibodies against human FC and then probed with anti-FC antibody (n=5). N3 Decoy was not detected in the sera of mouse 4 and analysis of this xenograft was excluded from the study. ECFC, endothelial colony forming cell; HemSC, hemangioma stem cell; N3 Decoy; NOTCH3 Decoy

Supplemental Figure 12, Edwards et al.



Supplemental Figure 12. NOTCH3 Decoy treatment has no effects on αSMA+ perivasculature expression in hepatic vasculature. A) Liver sections from mice treated with FC (control) or NOTCH3 Decoy (n=2 populations; n=4 xenografts each) stained for αSMA. **B**) Mean αSMA expression determined as mean αSMA signal intensity normalized DAPI+/αSMA+ cell number. (n=2 populations; n=4 xenografts each) n.s., not significant. Student T-Test. αSMA, alpha smooth muscle actin.