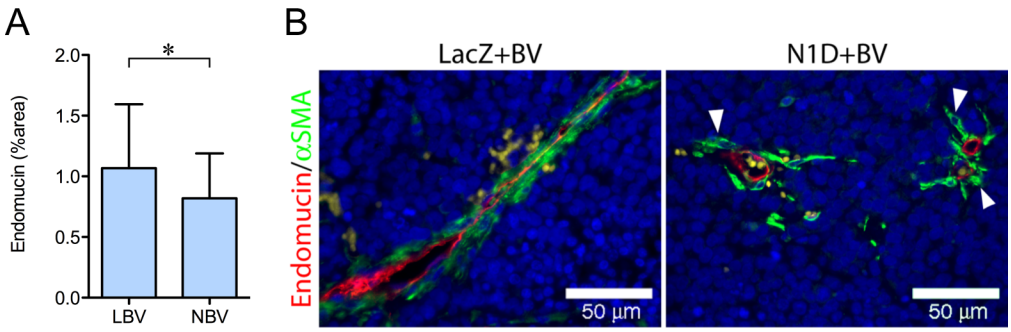
Supplementary Figure S1

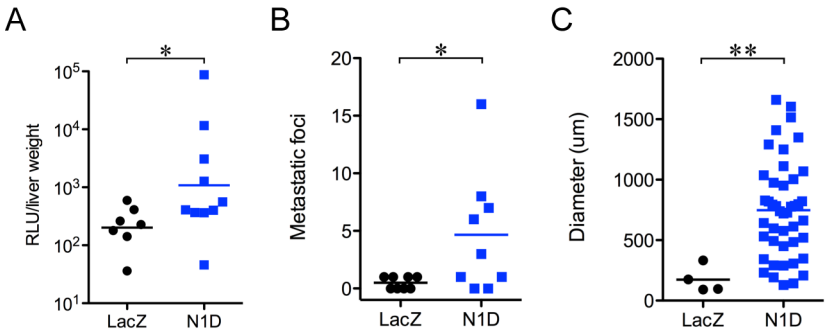


Combined Inhibition of Notch and VEGF disrupts interaction of pericytes with endothelial cells in primary tumors

(A) The endothelial cell marker endomucin was used to quantify the vasculature of NGP-LacZ+BV (LBV) and NGP-N1D+BV (NBV) primary tumors. Combined inhibition of Notch and VEGF caused a 23% decrease in endomucin (%area). **P*<0.05

(B) Double IHC for endothelial cells with endomucin (red), and for pericytes with αSMA (green), and nuclear staining with DAPI in primary tumors. Arrowheads indicate αSMA(+) cells that appear dissociated from the endomucin(+) vessel. Red blood cells (RBCs) autofluoresce yellow (merge of green and red). Bar = 50 µm.

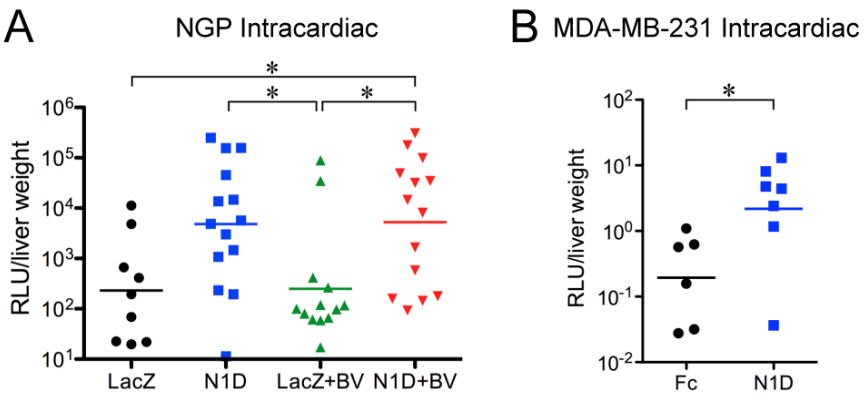
Supplementary Figure S2



Inhibition of Notch increases liver metastases.
After intrarenal implantation of NGP-LacZ (n=8) or NGP-N1D (n=10), tumors were monitored by bioluminescence, and mice sacrificed at day 39.

- **(A)** Liver metastatic burden was quantified by measuring bioluminescence of liver homogenates [RLU (relative luciferase units)/liver weight]. There was a 40-fold increase in liver metastatic burden in NGP-N1D (■), compared to the NGP-LacZ (●). **P*<0.05. Geometric mean is plotted.
- **(B)** Quantification of liver metastatic foci showed a significant increase in NGP-N1D (■, n=9) as compared to NGP-LacZ (●, n=8), (mean 4.6 *vs* 0.5 metastatic foci, respectively, *P*<0.05). Mean is plotted.
- **(C)** There was a marked increase in the mean diameter of the metastases in NGP-N1D (■) compared with NGP-LacZ (●), (747 *vs* 174μm, respectively, ***P*<0.01). Mean is plotted.

Supplementary Figure S3

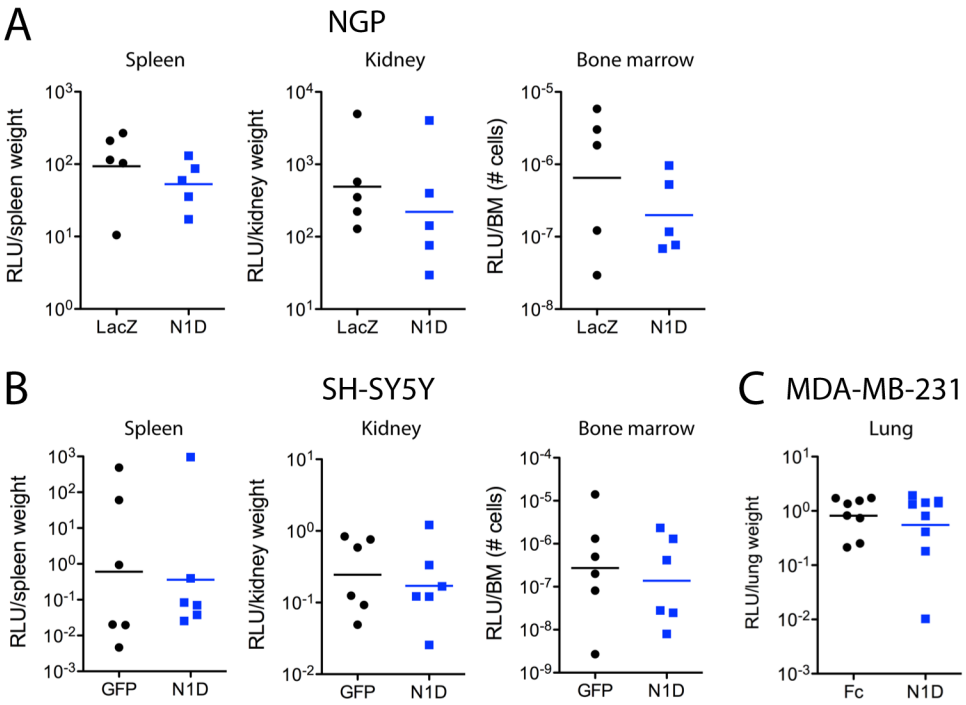


Inhibition of Notch increases liver metastases as measured by bioluminescence in liver homogenates.

(A) Quantification of liver metastases by bioluminescence of liver homogenates (RLU/liver weight) after intracardiac injection of NGP-LacZ (●), NGP-N1D (■), NGP-LacZ+BV (▲), and NGP-N1D+BV (▼). There was significantly increased liver metastasis in N1D and N1D+BV in comparison to LacZ+BV, and for N1D+BV compared to LacZ. **P*<0.05. Geometric mean is plotted.

(B) Quantification of liver metastases by bioluminescence of liver homogenates (relative luciferase units/liver weight) after intracardiac injection of MDA-MB-231-Fc (●), and MDA-MB-231-N1D (■). There was a significantly increased liver metastasis in N1D in comparison to Fc. **P*<0.05. Geometric mean is plotted.

Supplementary Figure S4



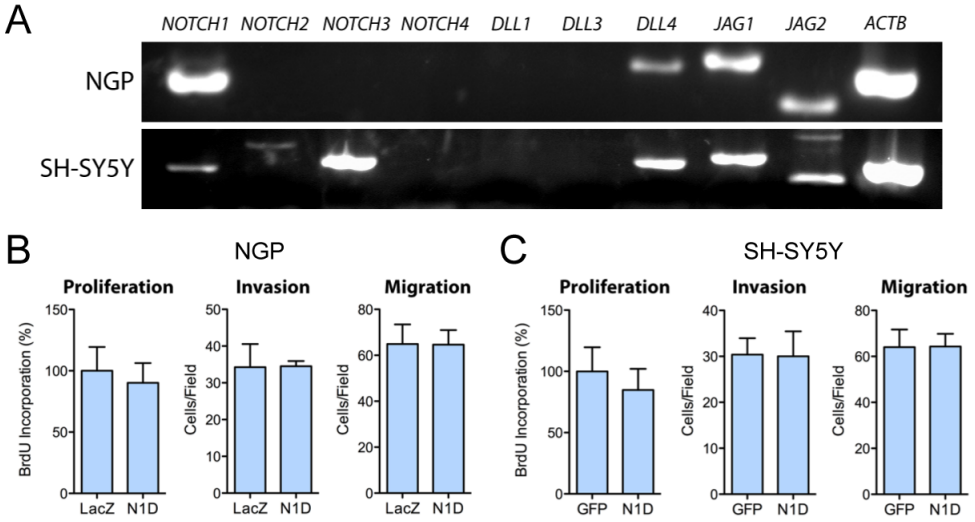
Notch blockade does not increase metastases to other organs.

(A) NGP metastases. Quantification of spleen, kidney, and bone marrow metastases by bioluminescence of organ homogenates (RLU/organ weight) or from bone marrow/# cells, after intracardiac injection of NGP-LacZ (●), NGP-N1D (■). There was no significant difference in metastasis to spleen, kidney, or bone marrow in NGP-LacZ compared to NGP-N1D. Geometric mean is plotted.

(B) SH-SY5Y metastases. Quantification of spleen, kidney, and bone marrow metastases by bioluminescence of organ homogenates (RLU/organ weight) or from bone marrow/# cells, after intracardiac injection of SH-SY5Y-GFP (●), SH-SY5Y-N1D (■). There was no significant difference in metastasis to spleen, kidney, or bone marrow in SH-SY5Y-GFP compared to SH-SY5Y-N1D. Geometric mean is plotted.

(C) MDA-MB-231-N1D metastases. Quantification of lung metastases by bioluminescence of lung homogenates (RLU/lung weight) after intracardiac injection of MDA-MB-231-Fc (●), and MDA-MB-231-N1D (■). There was no significant difference in lung metastases in MDA-MB-231-Fc compared to MDA-MB-231-N1D. Geometric mean is plotted.

Supplementary Figure S5



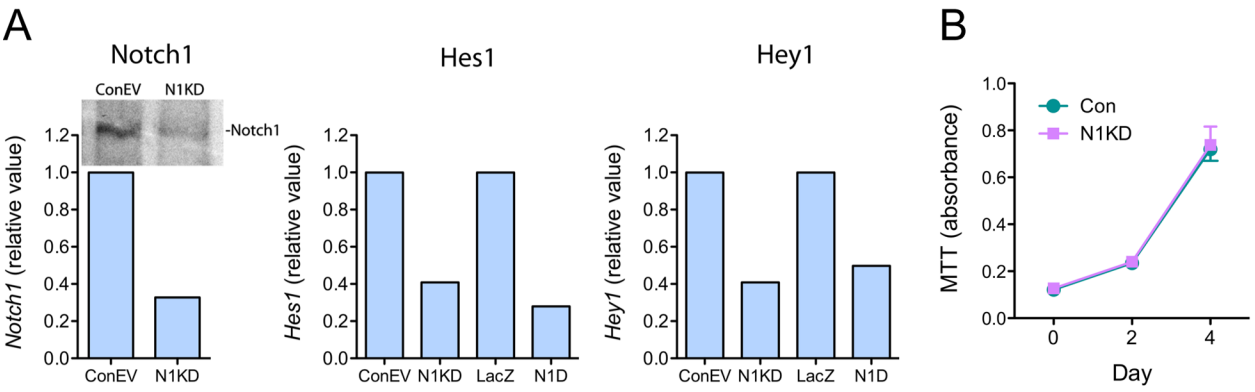
N1D does not affect proliferation, invasion or migration of neuroblastoma cell lines

(A) RNA from neuroblastoma cell lines NGP and SH-SY5Y was isolated, reverse transcribed, and PCR performed for Notch receptors and ligands, as described in Supplemental Methods.

(B) Proliferation, invasion, and migration was determined for NGP as described in Supplemental Methods. There was no significant difference in proliferation (BrdU Incorporation % control, mean $\pm$ std dev), invasion (cells/field mean $\pm$ std dev), or migration (cells/field, mean $\pm$ std dev). and;

(C) Proliferation, invasion, and migration was determined for SH-SY5Y. There was no significant difference in proliferation (BrdU Incorporation % control, mean $\pm$ std dev), invasion (cells/field mean $\pm$ std dev), or migration (cells/field, mean $\pm$ std dev).

Supplemental Figure S6

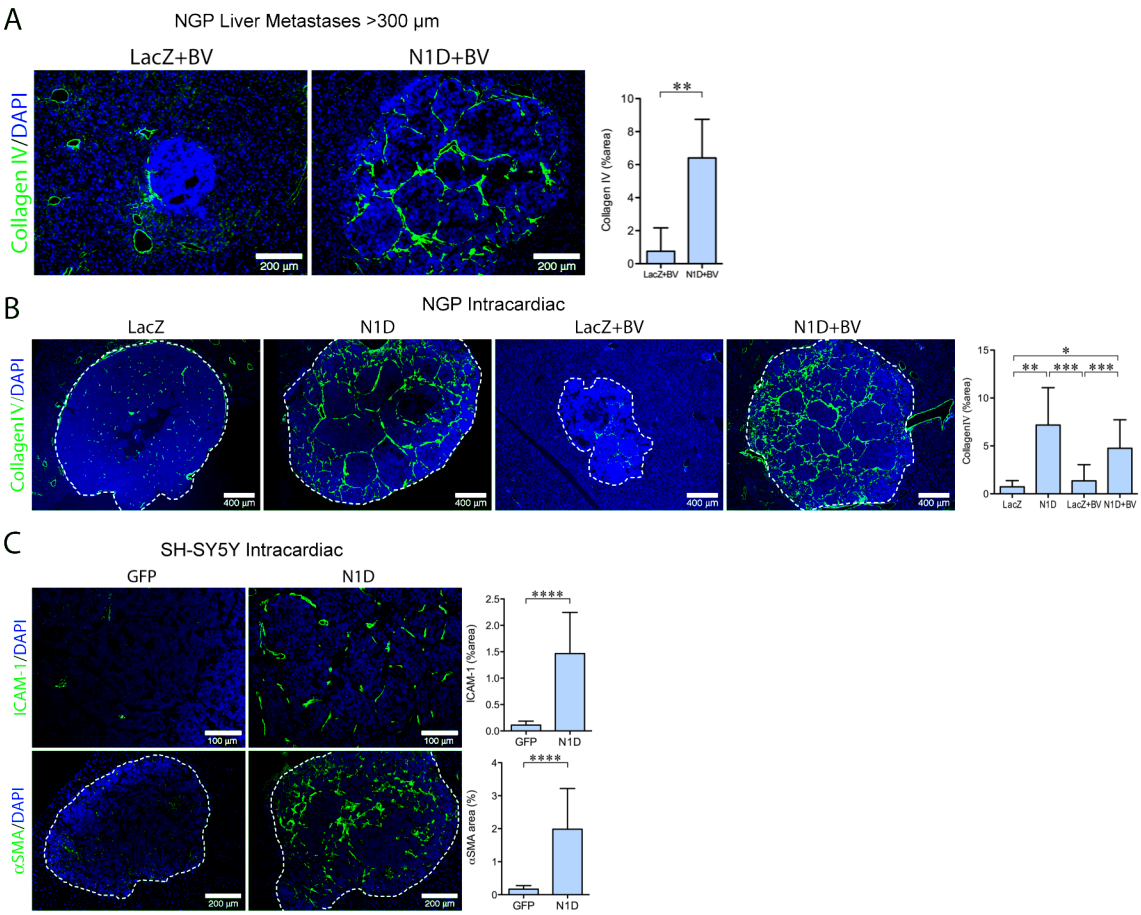


N1KD decreases Hes1 and Hey1 but does not affect proliferation of NGP cells

(A) N1KD decreased *Notch1* expression by 63% compared to empty vector control (ConEV), as measured by Taqman Real Time PCR. Inset shows marked decrease of Notch1 protein by Western analysis. N1KD induced a decrease in the Notch responsive genes *Hes1* and *Hey1*, similar to that seen with NGP cells expressing the N1D as measured by Taqman Real Time PCR.

(B) Proliferation of NGP-ConEV (●), or NGP-N1KD (■) or cells was measured at 0, 2, 4 days by MTT assay. There was no difference in proliferation (mean ± std dev). *P*=n.s.

Supplementary Figure S7



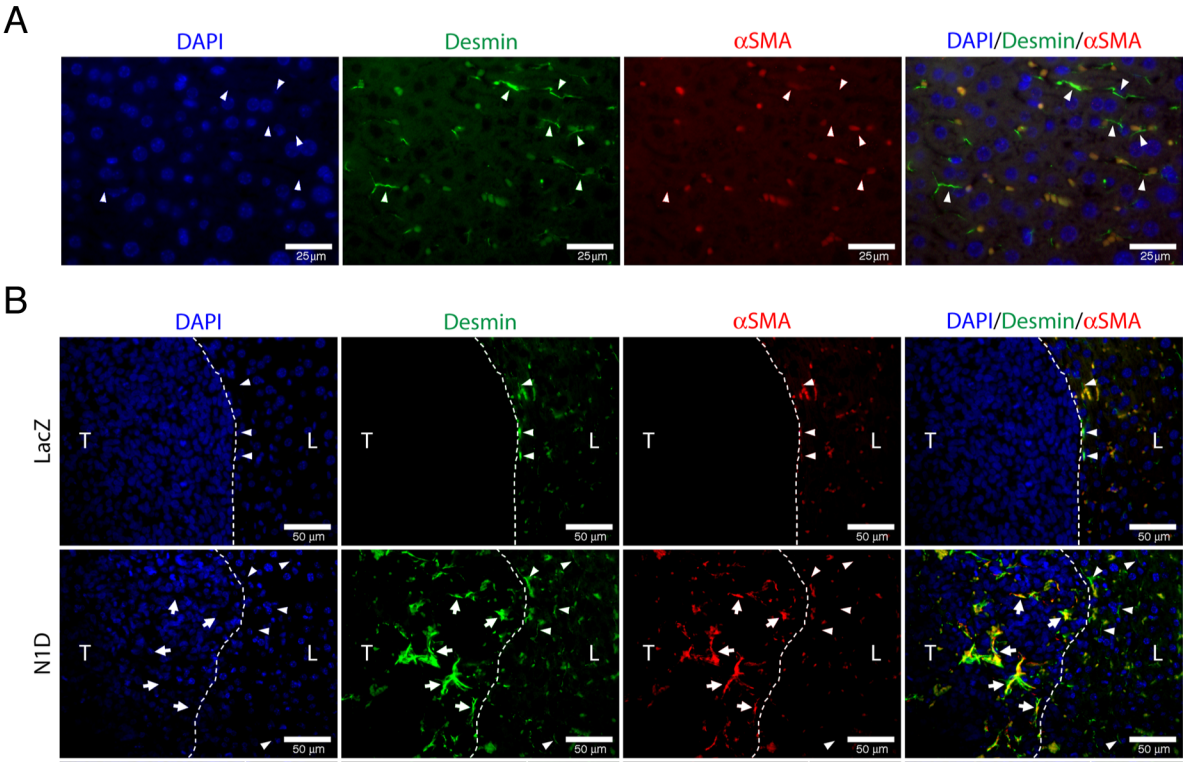
Increased vasculature of liver metastases with Notch blockade

(A) Larger metastases (>300 μm) had increased vascularity as seen by collagen IV (green), with collagen IV quantification (***P*<0.01, mean ± std dev). Nuclei shown by DAPI (blue).

(B) Intracardiac injection of NGP-N1D and NGP-N1D+BV showed significantly higher vasculature by collagen IV in comparison to NGP-LacZ and NGP-LacZ+BV. DAPI nuclear counterstain. CollagenIV (%area), mean ± std dev. **P*<0.05, ***P*<0.01, ****P*<0.001. Bar = 400 μm.

(C) Intracardiac injection of SH-SY5Y-N1D showed markedly increased vascularity as demonstrated by ICAM-1 (**top panel** %area, mean ± std dev, 13-fold, P<0.0001, Bar = 100 μm) and αSMA (**bottom panel,** %area, mean ± std dev, 12-fold, *****P*<0.0001, Bar = 200 μm). DAPI nuclear counterstain.

Supplementary Figure S8

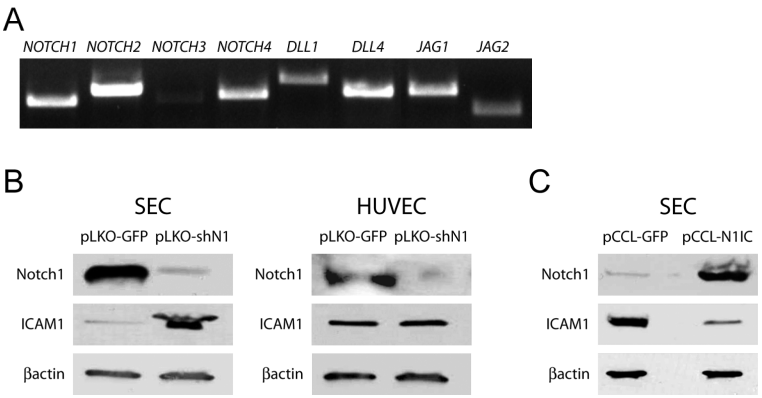


Activation of HSCs in liver metastases with Notch blockade

(A) Double IHC for desmin (green), αSMA (red), and nuclear staining with DAPI in normal mouse liver. Arrowheads indicate desmin(+)/αSMA(-) cells indicating that the HSC are not activated in the normal liver. Red blood cells (RBCs) are also present in the liver and autofluoresce yellow (merge of green and red). Bar = 25 μm.

(B) Double IHC for desmin (green), αSMA (red), and nuclear staining with DAPI, of liver metastases after intracardiac injection of NGP-LacZ and NGP-N1D. Dashed line indicates border between, T=tumor. L=liver. Arrows indicate desmin(+)/αSMA(+) located within tumor metastases in which Notch is blocked (N1D, N1D+BV), suggesting that HSCs have been activated. Similar increase in desmin(+)/αSMA(+) cells was seen with NGP-N1D+BV, but not NGP-LacZ+BV (data not shown). Arrowheads indicate desmin(+)/αSMA(-) HSC within the liver. Bar = 50 μm.

Supplementary Figure S9



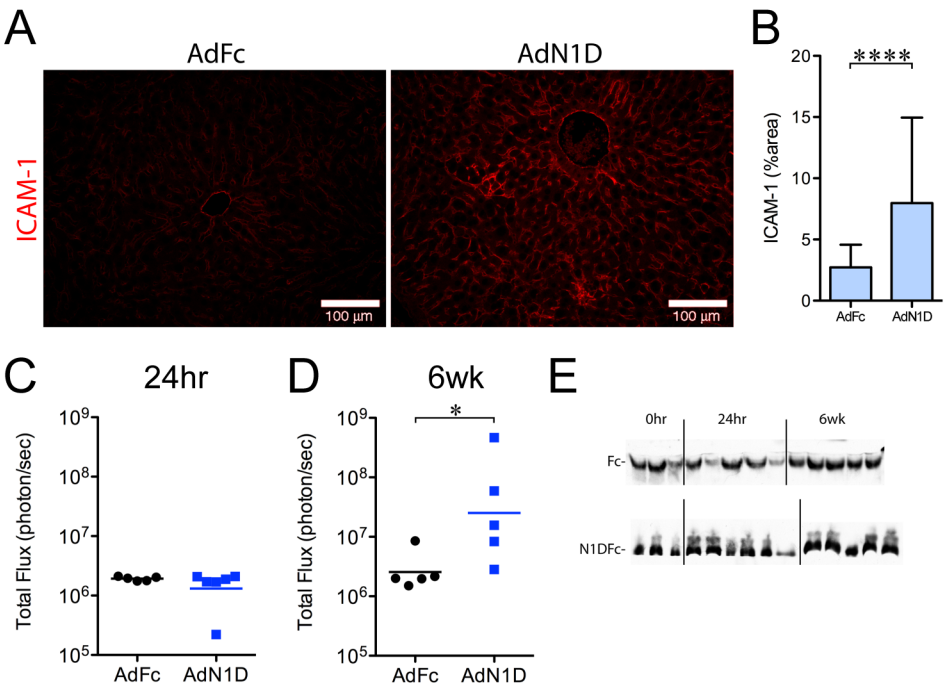
SEC expression of ICAM-1 is regulated by Notch

(A) The expression of Notch receptors and ligands in mSECs was examined by RT-PCR. mSECs expressed Notch1, 2, 4, and Dll1, Dll4, Jag1, and Jag2.

(B) Knockdown of Notch1 (shN1) in mSEC, but not HUVEC, showed increased expression of ICAM-1 as shown by Western blot analysis. βactin was used as a loading control.

(C) Expression of N1IC in SEC resulted in a decrease in ICAM-1 expression. βactin was used as a loading control

Supplementary Figure S10



AdN1D increases expression of liver ICAM-1 but does not increase retention of tumor cells in the liver.

(A) Mice were injected with AdFc (n=2) or AdN1D (n=3). After 3 days, mice were sacrificed and ICAM-1 IHC was performed. AdN1D demonstrated increased ICAM-1 expression. Bar = 100 µm

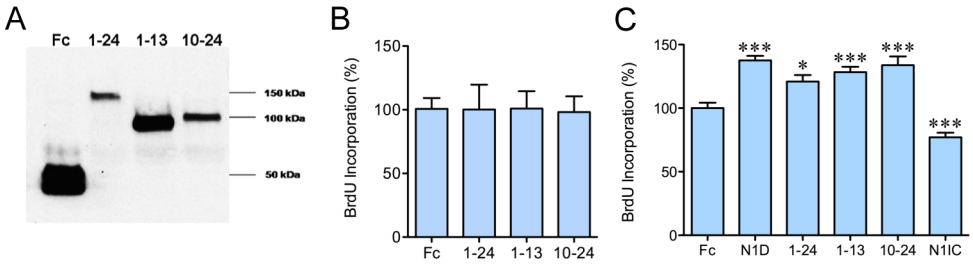
(B) ICAM-1 was 2.9 fold higher in AdN1D compared to AdFc, mean ± std dev, $P<0.0001$.

(C) Mice were injected with AdFc (n=5) or AdN1D (n=6). After 3 days, 10^5 NGP cells were injected into the heart and 24 hr later mice were sacrificed. Quantification of total flux (photon/sec) by *ex vivo* liver bioluminescence showed no difference between AdFc (●) or AdN1D (■). Geometric mean is plotted.

(D) Mice were injected with AdFc (n=5) or AdN1D (n=5). After 3 days 10^5 NGP cells were injected intracardically and 6 weeks later mice were sacrificed. Quantification of total flux (photon/sec) by *ex vivo* liver bioluminescence showed a 34-fold higher liver flux for AdN1D (■) compared to AdFc (●), $*P<0.05$. Geometric mean is plotted.

(E) Western analysis of blood collected at 3 days, prior to injection of tumor cells, detected circulating Fc or N1D-Fc in all mice.

Supplementary Figure S11



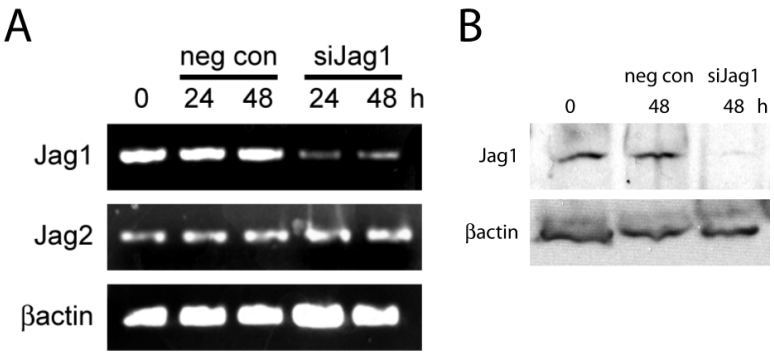
Notch1 decoy variants increase proliferation of mSEC but not NGP cells.

(A) *Notch1 decoy variants are secreted.* The secretion of decoy proteins were confirmed by immunoprecipitating conditioned media with a goat anti-human IgG(Fc) antibody (Thermo Scientific, #31413) and agarose A/G beads (Santa Cruz Biotechnology) followed by immunoblot analysis.

(B) *Notch1 decoy variants do not affect proliferation of NGP cells.* The proliferation of NGP expressing Fc, $N1_{1\text{-}24}$ decoy $N1_{1\text{-}24}$ decoy, $N1_{1\text{-}13}$ decoy or $N1_{10\text{-}24}$ decoy was determined by BrdU assay after 24hrs. Percent of Fc control, mean $\pm$ std dev. *P*=n.s.

(C) *Notch Decoy Variants increase proliferation of mSEC.* The proliferation of mSEC expressing Fc, N1D, N1 decoy variants: $N1_{1\text{-}24}$ decoy, $N1_{1\text{-}13}$ decoy or $N1_{10\text{-}24}$ decoy, and Notch1 intracellular domain (N1IC) was determined by BrdU assay after 24hrs. Blockade of Notch with N1D and the N1 decoy variants significantly increased proliferation in mSECs, while activation of Notch signaling with N1IC significantly inhibited proliferation. Percent Fc control, mean $\pm$ std dev. $^{*}P<0.05$, $^{***}P<0.001$.

Supplementary Figure S12



Jag1 siRNA decreases expression of Jag1 in NGP cells.
NGP cells were transfected with ON-TARGET*plus* pooled siJag1 or negative control ON-TARGET*plus* Non-targeting Control Pool siRNA.

(A) RT-PCR was performed for *Jag1*, *Jag2*, and *βactin*. siJag1 decreased expression of *Jag1* but not *Jag2* at 24h and 48h after transfection.

(B) Western blot analysis demonstrated that siJag1 decreased protein expression of Jag1.