SUPPLEMENTARY INFORMATION

Figure S1 shows that $N1_{1-13}$ decoy blocks activation of Notch1 by DLL1 but not JAG2 whereas $N1_{10-24}$ decoy blocks activation of Notch1 by JAG2 but not DLL1. **Figure S2** shows that purified $N1_{1-13}$ decoy preferentially inhibited DLL4-mediated Notch1 activation, while purified $N1_{1-24}$ decoy inhibited both DLL4 and JAG1-mediated Notch1 activation. **Figure S3** shows using co-immunoprecipitation of N1 decoys and soluble versions of DLL4 and JAG1 lacking transmembrane domains that similar ligand specificity of the decoys was observed as when using membrane bound DLL4 or JAG1. **Figure S4** shows serum levels of Notch1 decoys in P5 neonatal mice. **Figure S5** shows N1 decoys that block JAG disrupted vascular smooth muscle cell association in the actively remodeling neonatal retinal vasculature, but not the mature retinal vasculature. **Figure S6** shows that $N1_{1-13}$, $N1_{10-24}$, and $N1_{1-24}$ decoys block soft agar growth of Mm5MT-FGF4 tumor cells but do not affect growth of KP1-VEGF, LLC or B16-F10 tumor lines. **Figure S7** shows N1 decoys detected in mouse serum after adenovirus infection and quantification of these levels using ELISA. **Figure S8** shows that JAG-inhibiting decoys disrupt vascular smooth muscle coverage in tumors. **Figure S9** shows that N1 decoys expressed in mice cause a modest increase of intestinal goblet cells but no significant change in murine weight. **Figure S10** shows N1 decoys had no effect on serum markers of liver damage. **Figure S11** shows N1 decoys did not affect liver and kidney histopathology.

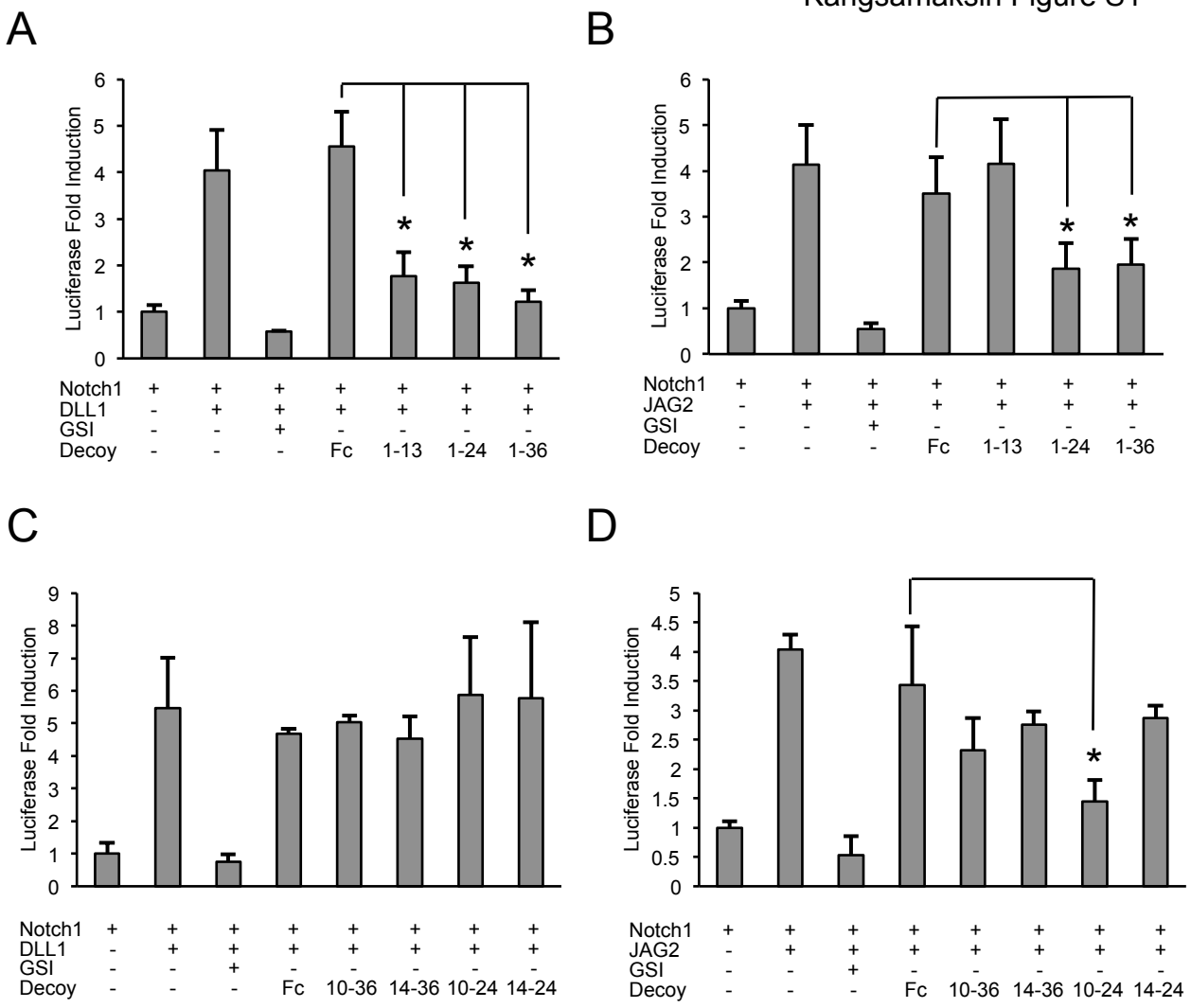


Figure S1. DLL1 and JAG2 ligand specificity of N1 decoys. Notch reporter assays were performed by co-culturing HeLa cells expressing either DLL1 or JAG2, and individual N1 decoy variants or Fc with HeLa cells expressing full-length rat Notch1 and a CSL-Luciferase reporter. GSI was used as a control for Notch1 signal inhibition. (**A** and **B**) $N1_{1-13}$ decoy blocked DLL1/Notch1 signaling, but had no affect on JAG2/Notch1 signaling. $N1_{1-24}$, and $N1_{1-36}$ decoys blocked both DLL1/Notch1 and JAG2/ Notch1 signaling. (**C** and **D**) $N1_{10-24}$ decoy blocked JAG2/Notch1 signaling, but had no effect on DLL1/Notch1 signaling. $N1_{10-36}$, $N1_{10-24}$, and $N1_{14-36}$ decoys did not function as Notch inhibitors. Mean luciferase fold induction ± S.D. * P value < 0.005. Co-culture assays were performed in triplicate and repeated three times.

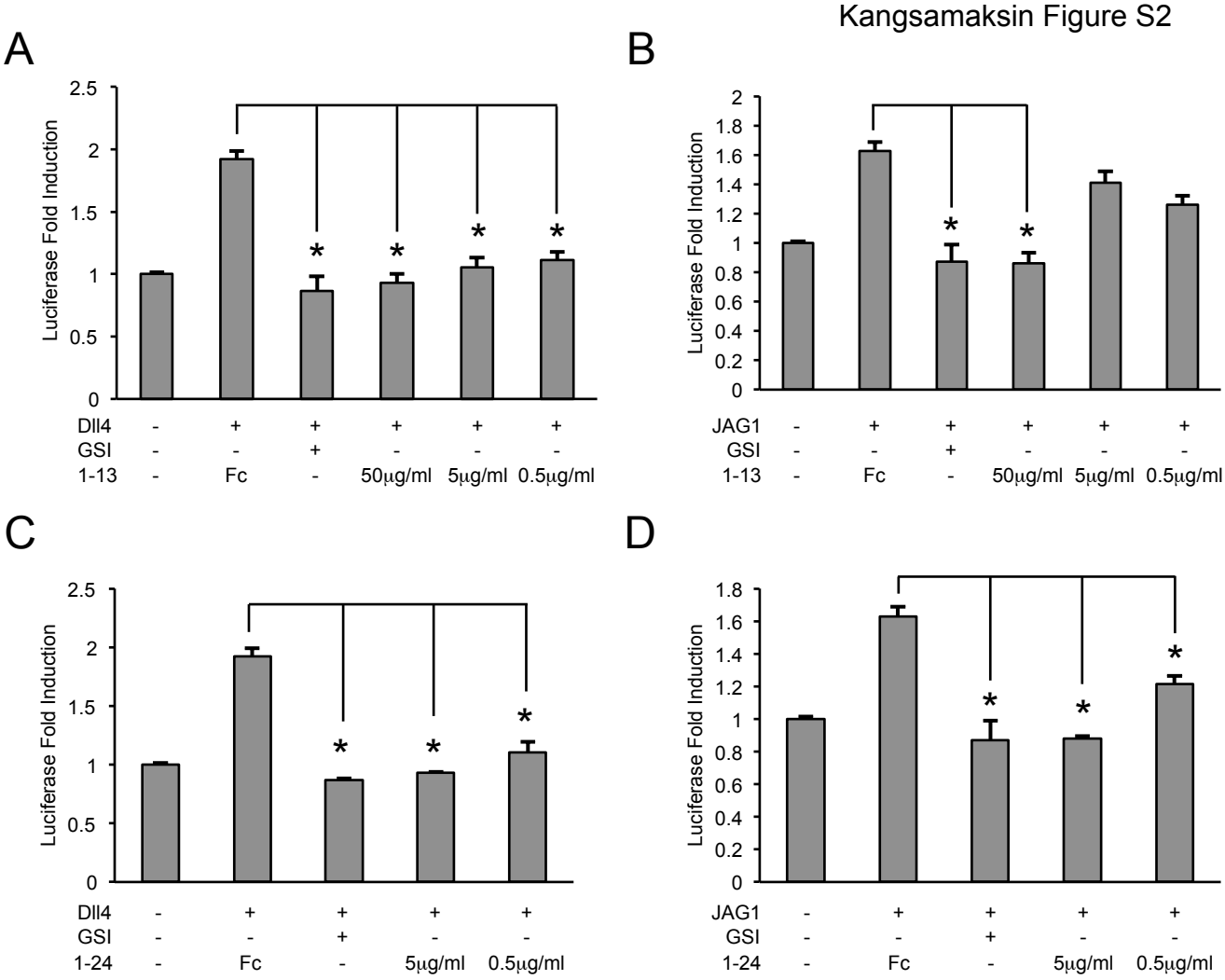


Figure S2. N1 decoys exhibit differential dose-dependent effects on ligand-induced Notch1 signaling. Notch reporter assays were performed using N1 decoy variants or Fc purified from CHO cells in co-cultures with HeLa cells expressing either DLL4 or JAG1, and HeLa cells expressing full-length rat Notch1 and 11CSL-Luciferase reporter. GSI was used as a control for Notch1 signal inhibition. (**A**) 0.5 mg/ml $N1_{1-13}$ decoy was sufficient to block DLL4/Notch1 signaling. (**B**) A high dose of 50 mg/ml $N1_{1-13}$ decoy was necessary to block JAG1/Notch1 signaling, while lower doses did not inhibit JAG1/Notch1 signaling. (**C** and **D**) 0.5 mg/ml $N1_{1-24}$ decoy was sufficient to block DLL4/Notch1 and JAG1/Notch1 signaling. Co-culture assays were performed in triplicate. Mean luciferase fold induction ± S.D. * P value < 0.002.

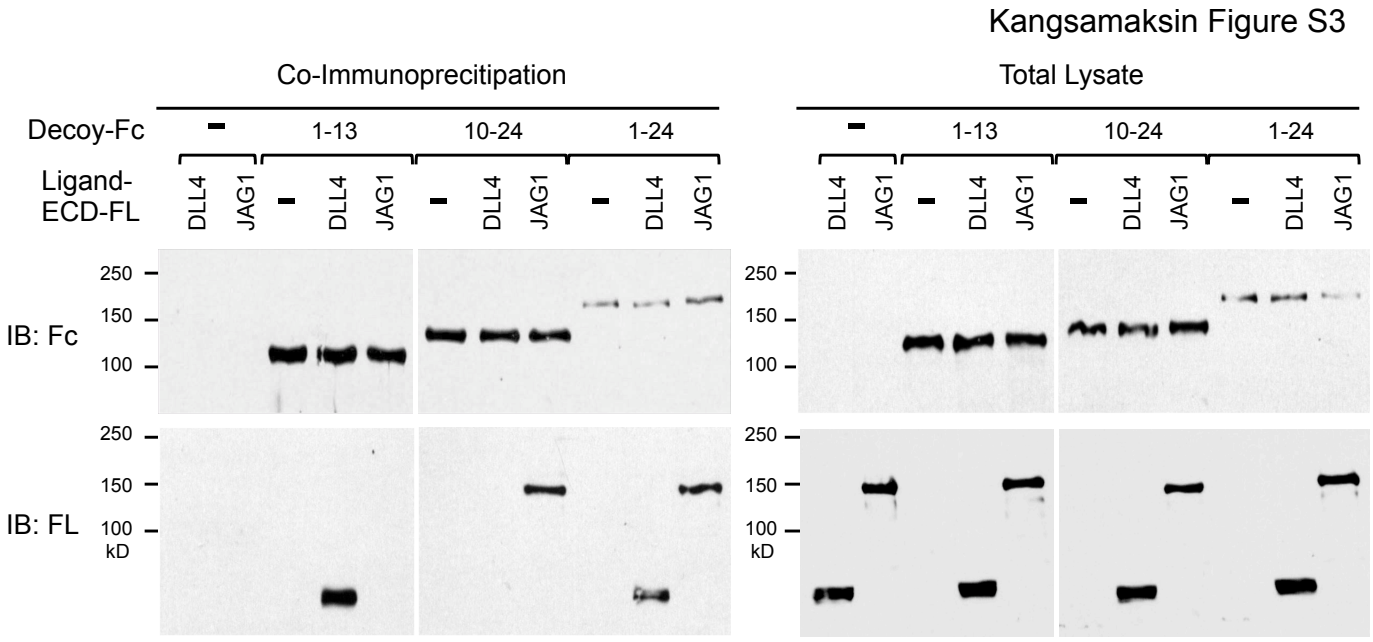


Figure S3. Co-immunoprecipitation of N1 decoys and soluble DLL4 and JAG1. $N1_{1\text{-}13}$ decoy interacted with DLL4, $N1_{10\text{-}24}$ decoy interacts with JAG1, and $N1_{1\text{-}24}$ decoy interacted with both DLL4 and JAG1. 293T cells were co-transfected with N1 decoys ($N1_{1\text{-}13}$, $N1_{10\text{-}24,}$ $N1_{1\text{-}24}$) and either FLAG-tagged soluble DLL4 or JAG1 and proteins cross-linked with DSG. Lysates were incubated with Protein A/G agarose to pull down Fc portion of N1 decoys and immunoblotted (IB) with an anti-Fc or anti-FLAG antibody. Assays were repeated twice.

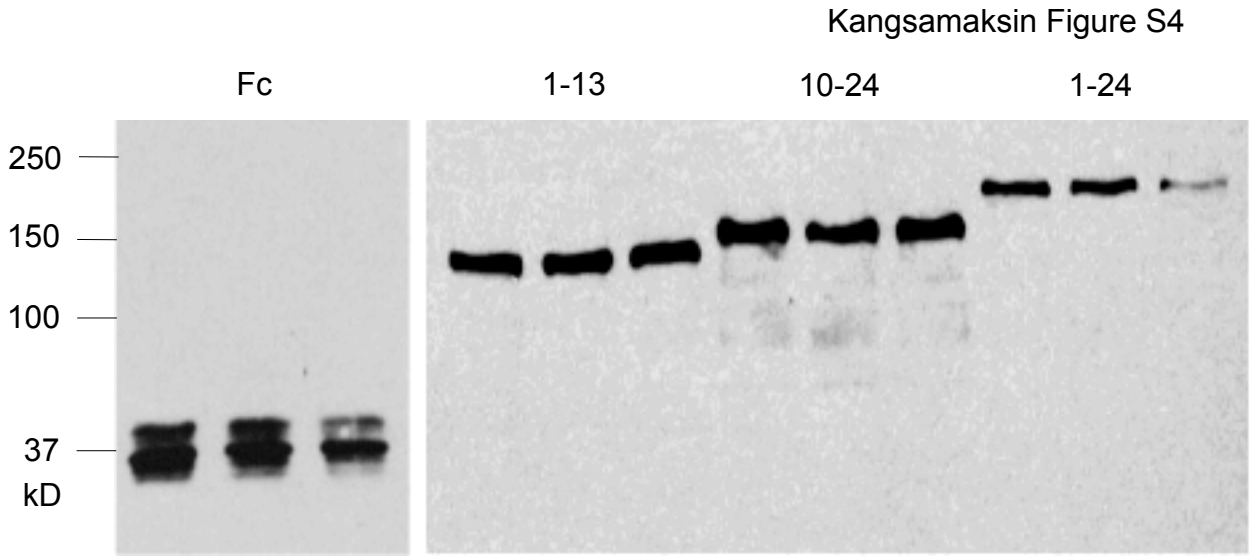


Figure S4. Serum levels of N1 decoys in murine neonates. $N1_{1-13}$, $N1_{10-24}$, and $N1_{1-24}$ decoys and Fc are secreted into the mouse serum. Immunoblot using an anti-Fc antibody of serum from 3 mice collected at P5.

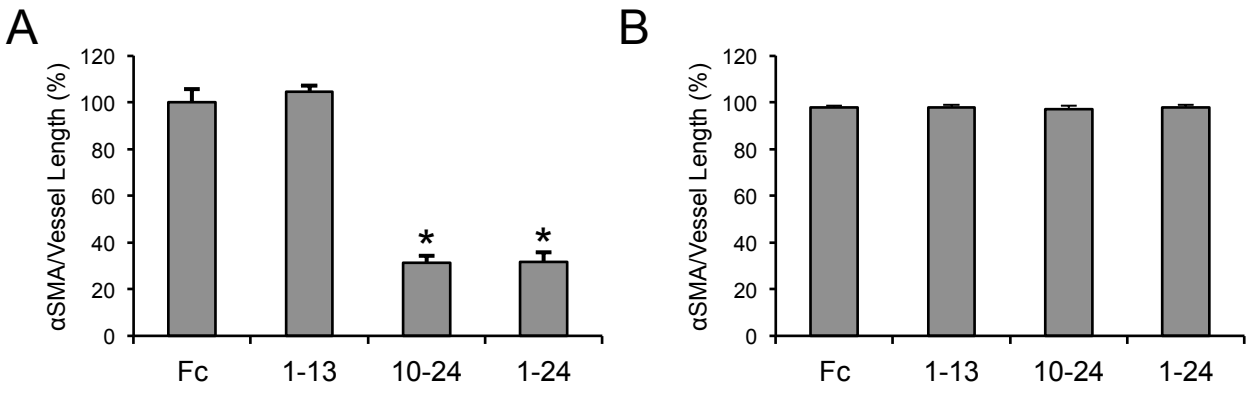
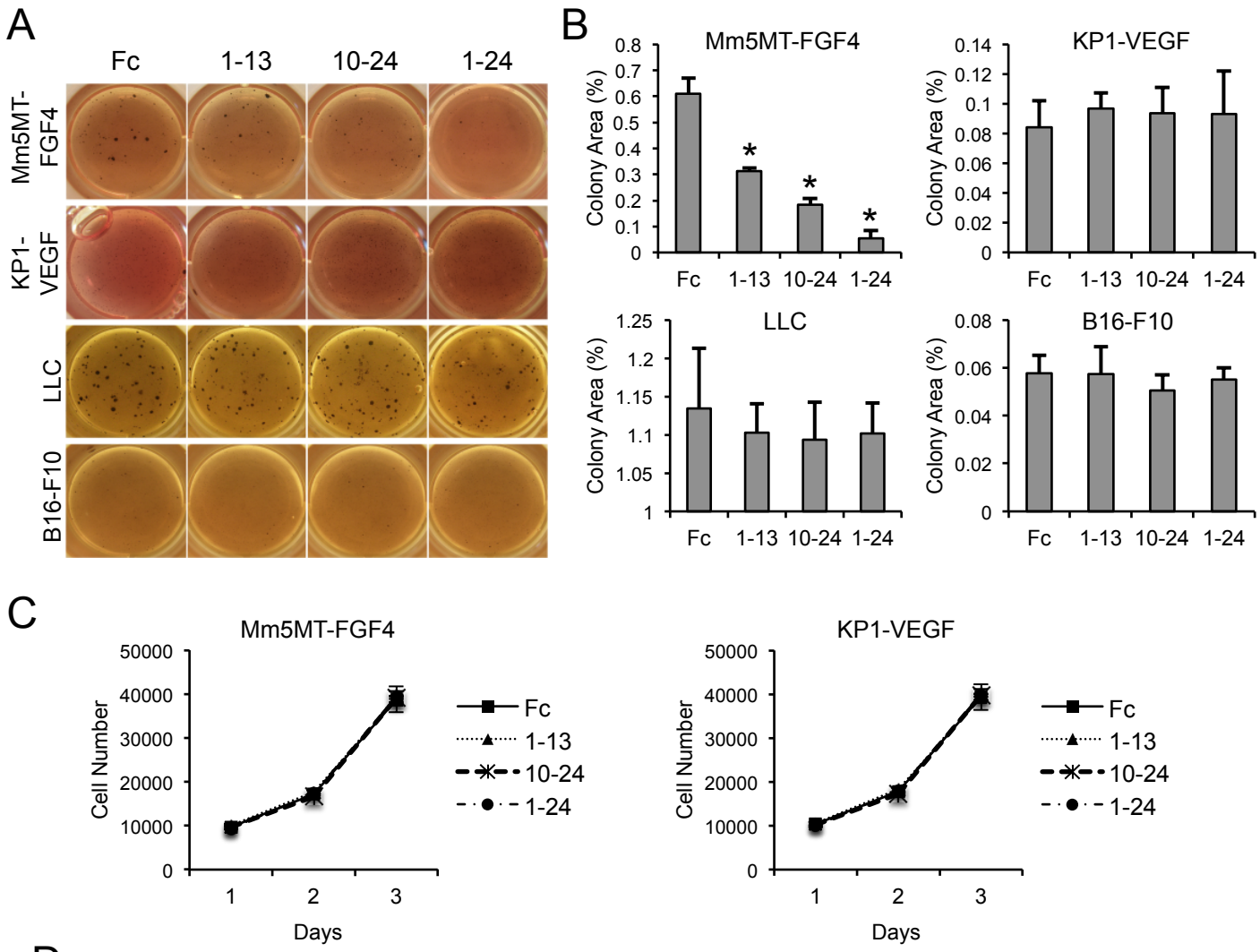


Figure S5. N1 decoys that block JAG disrupt vascular smooth muscle cell association in the actively remodeling neonatal retinal vasculature. Quantification of mean percent αSMA+ vascular smooth muscle cell coverage of arteries normalized by vessel length. (**A**) $N1_{10\text{-}24}$ and $N1_{1\text{-}24}$ decoys disrupted vascular smooth muscle cell coverage in P5 neonates (n = 3). (**B**) N1 decoys had no affect on vascular smooth muscle coverage of mature retinal arteries assessed after 21 days. Data presented ± S.D. * P value < 0.05.



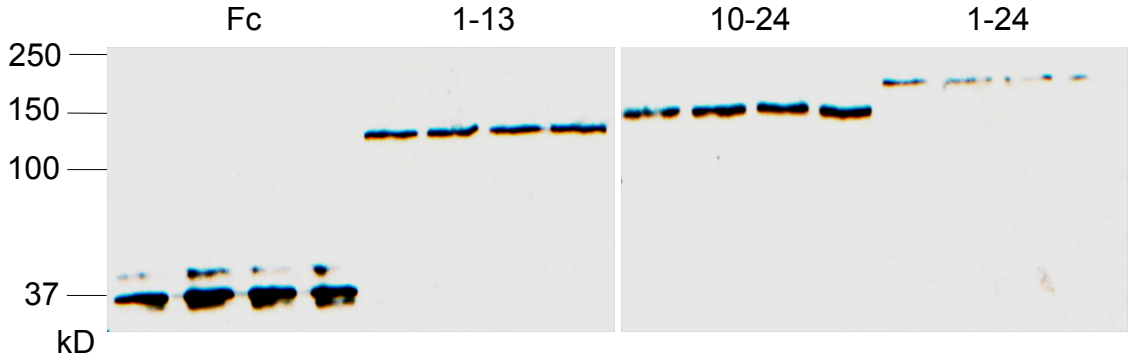
D

Quantitation of apoptotic cells

	Mm5MT-FGF4	KP1-VEGF
Fc	4.98±0.84%	0.11±0.25%
1-13	5.46±0.75%	0.19±0.14%
10-24	5.12±0.44%	0.16±0.30%
1-24	4.89±0.65%	0.23±0.28%

Figure S6. Effects of N1 decoys on tumor cells in vitro. (**A** and **B**) $N1_{1-13}$, $N1_{10-24}$, and $N1_{1-24}$ decoys reduce colony formation in Mm5MT-FGF4. (**A**) Soft agar colony formation assay of Mm5MT-FGF4, KP1-VEGF, LLC, and B16-F10 tumor cells expressing $N1_{1-13}$, $N1_{10-24}$, and $N1_{1-24}$ decoys or Fc. 3 x 10^3 cells/ well (24 well plate) were cultured for 3 weeks and 3 mg/ml MTT added to visualize colonies. (**B**) Quantification of the colony area. Data presented as average percentage of colony area ± S.D. * P value < 0.001. (**C**) $N1_{1-13}$, $N1_{10-24}$, and $N1_{1-24}$ decoys did not inhibit proliferation. Proliferation assay of Mm5MT-FGF4 and KP1-VEGF transductants determined by WST-8 assay on days 1 and 4. Experiments performed in triplicate and presented as average cell number ± S.D. (**D**) $N1_{1-13}$, $N1_{10-24}$, and $N1_{1-24}$ decoys did not induce apoptosis. Apoptosis of Mm5MT-FGF4 and KP1-VEGF transductants were determined using Annexin V-FITC kit (Biovision). Average percentage of apoptotic cells was determined ± S.D.

A



B

Serum Levels of adenoviral-expressed transgenes

	Day 5 (ug/ml)	Day 7 (ug/ml)	Day 14 (ug/ml)	Day 21 (ug/ml)
Fc	29 ± 2.86	30 ± 1.34	32 ± 1.22	36 ± 6.37
1-13	18 ± 3.97	18 ± 1.34	20 ± 0.88	21 ± 3.27
10-24	14 ± 1.46	14 ± 0.75	11 ± 1.05	16 ± 1.81
1-24	6 ± 0.35	4 ± 1.17	5 ± 1.34	3 ± 0.53
1-36	0.23 ± 0.18	0.48 ± 0.41	0.11 ± 0.23	0.24 ± 0.18

Figure S7. Serum levels of N1 decoys in tumor-bearing mice. $N1_{1-13}$, $N1_{10-24}$, and $N1_{1-24}$ decoys and Fc are secreted into the mouse serum of Mm5MT-FGF4 implanted mice. (**A**) Immunoblot using an anti-Fc antibody of serum from 4 tumor-bearing mice at day 21. (**B**) Human Fc ELISA assay of mouse serum collected at day 5, 7, 14, and 21 post-adenoviral injection.

Kangsamaksin Figure S8

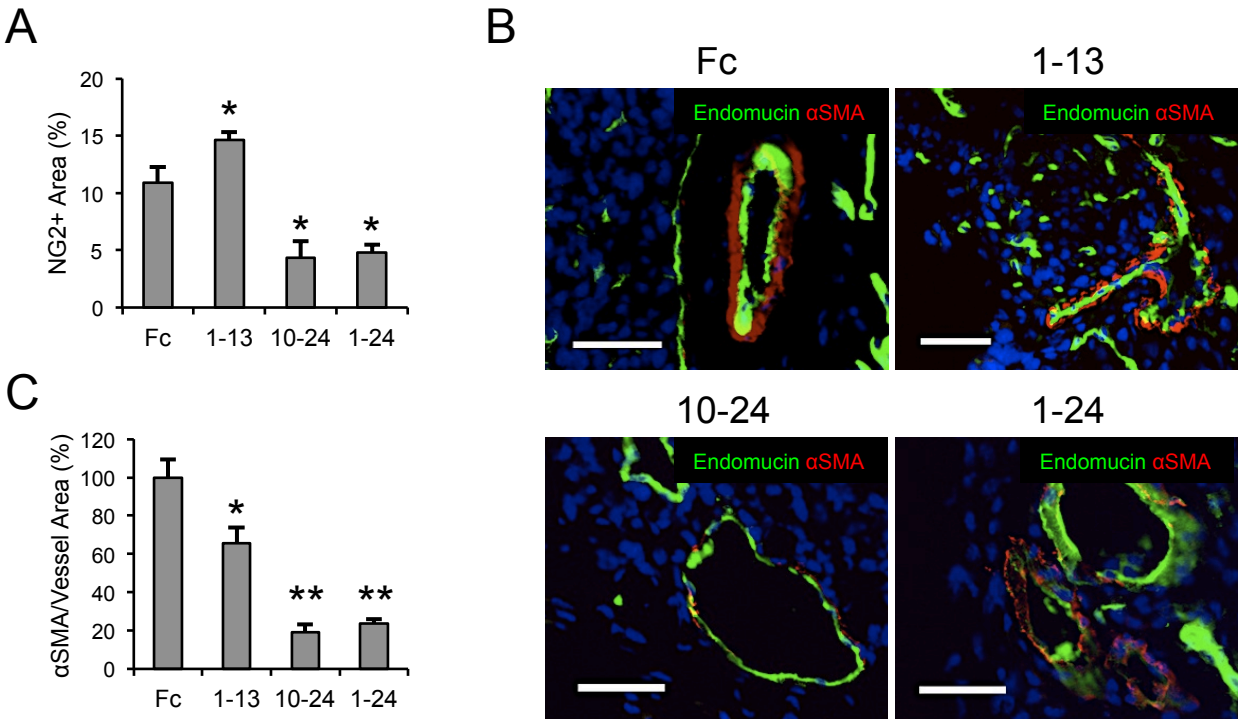


Figure S8. N1 decoys that block JAG disrupt pericyte and vascular smooth muscle cell coverage in tumors. Tumor sections were immunostained with NG2 for pericytes and αSMA for vascular smooth muscle cells. $N1_{1\text{-}13}$ which blocks only DLL, did not affect vascular smooth muscle cells but $N1_{10\text{-}24}$, and $N1_{1\text{-}24}$ decoys significantly disrupt vascular smooth muscle cell coverage and morphology. (**A**) Quantification of mean percent NG2 area, ± S.D. P value < 0.02 (n = 4-5). (**B**) Endomucin and αSMA staining of large vessels with vascular smooth muscle cell coverage located at the tumor periphery. Scale bars: 10 μm. (**C**) Quantification of mean percent αSMA+ vascular smooth muscle cell coverage of arteries normalized by vessel length. Data presented ± S.D. * P value < 0.02 (n = 4-5).

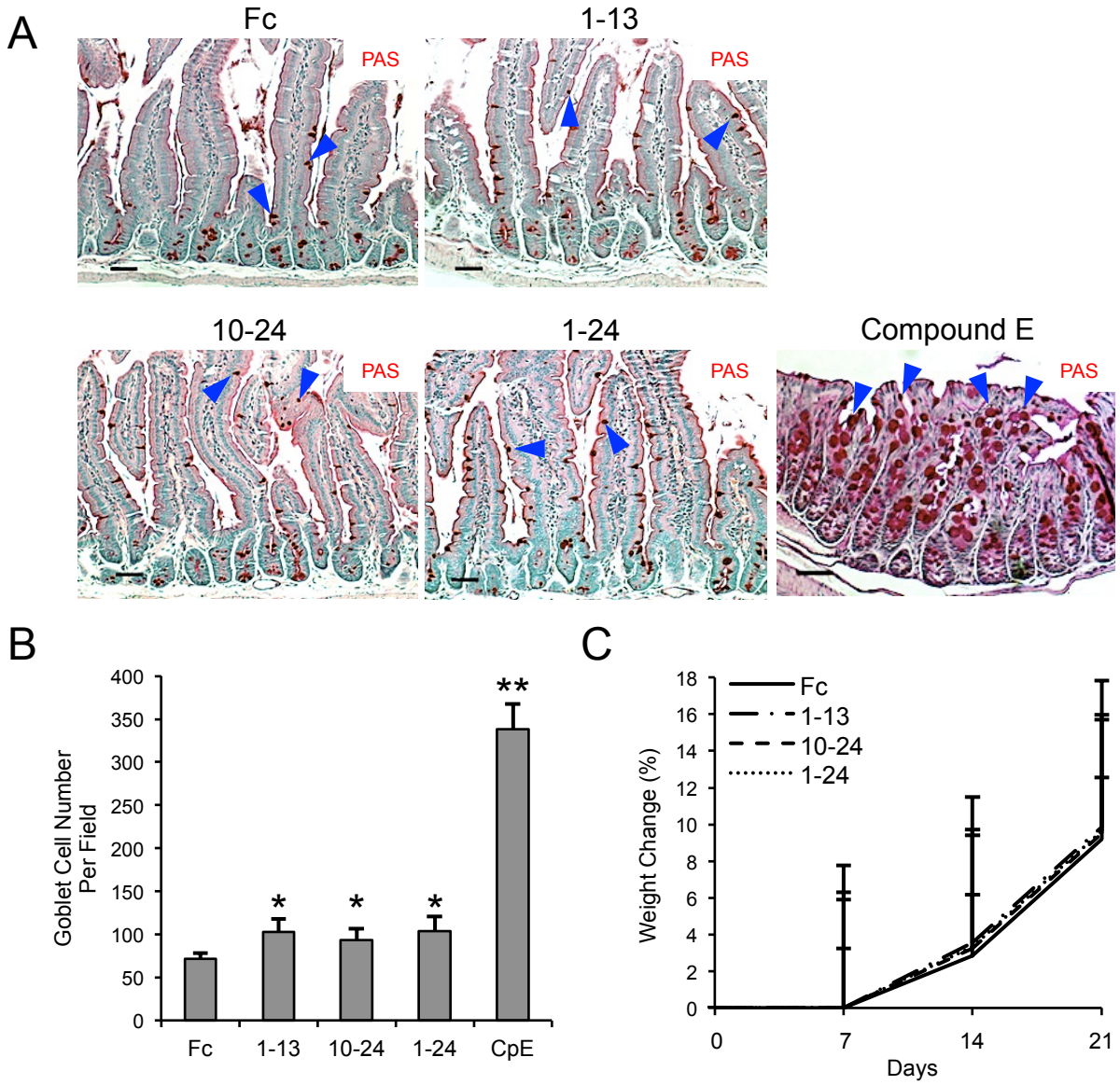


Figure S9. N1 decoys are minimally toxic to tumor-bearing mice. (**A**) Periodic acid-Schiff (PAS) staining of globlet cells (arrows) in the duodena. Intestines from N1 decoy treated tumor-bearing mice collected at Day 21. Compound E (CpE) treated mice received 100 mmol/kg CpE daily and intestines collected at Day 5. Scale bars: 10 μm. (**B**) Quantification of PAS+ goblet cell number represented as mean number of goblet cells per field ± S.D. * P value < 0.02, ** P value < 0.0002 (n = 5). (**C**) No significant difference in weight change was observed between the N1 decoy groups and control. Tumor bearing mice were weighed weekly after adenovirus injection. Data presented as percent mean weight change ± S.D. (n = 5).

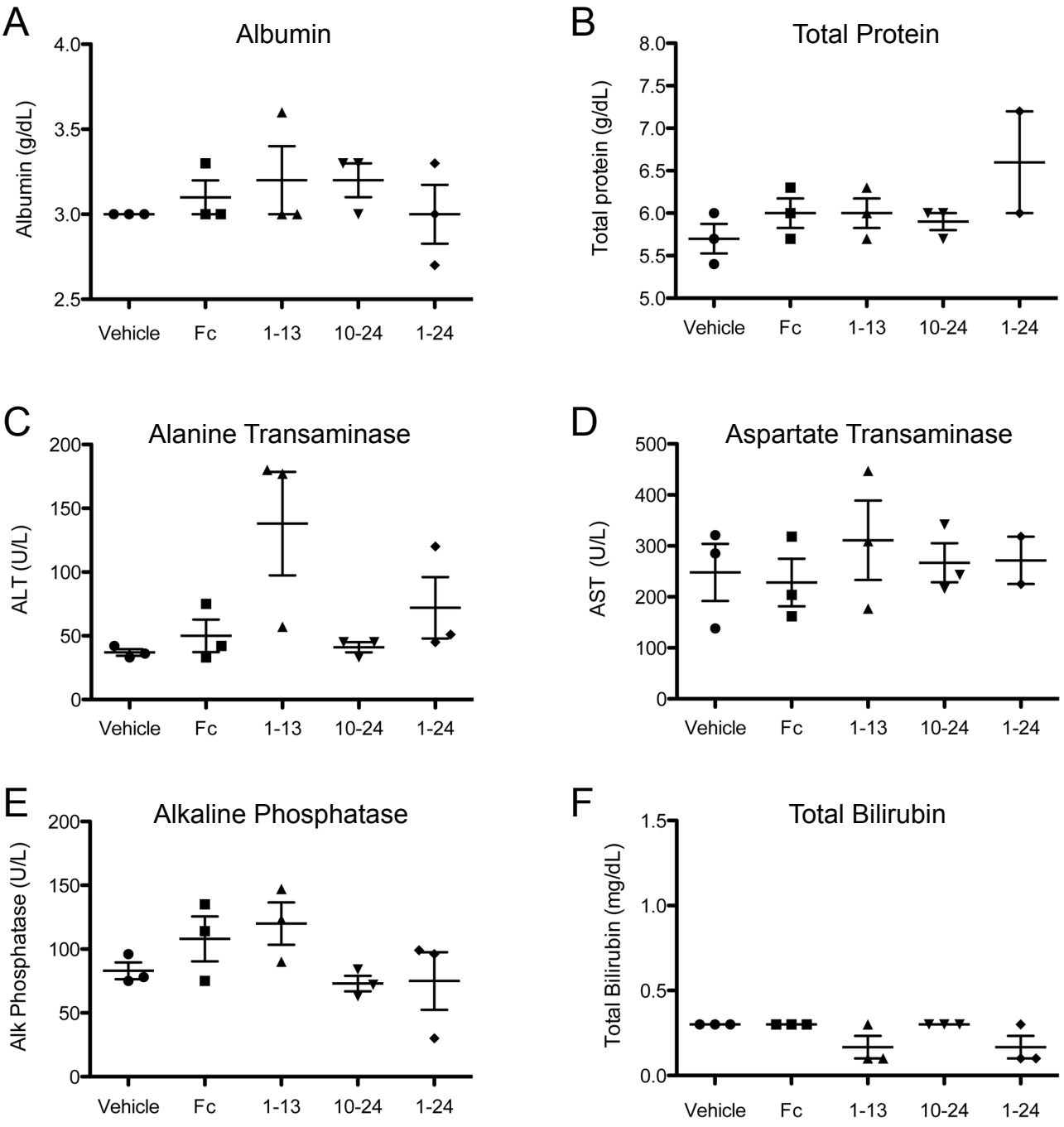


Figure S10. N1 decoys had no effect on serum markers of liver damage. Sera were collected from vehicle or N1 decoy treated mice at day 21 (n=3). (**A**) Albumin, (**B**) total protein, (**C**) alanine transaminase, (**D**) aspartate transaminase, (**E**) alkaline phosphatase and (**F**) total bilirubin levels were determined by ANTECH Diagnostics. Data plotted, and mean and ± S.E.M. indicated. No significant differences were observed for any of the markers using one-way ANOVA.

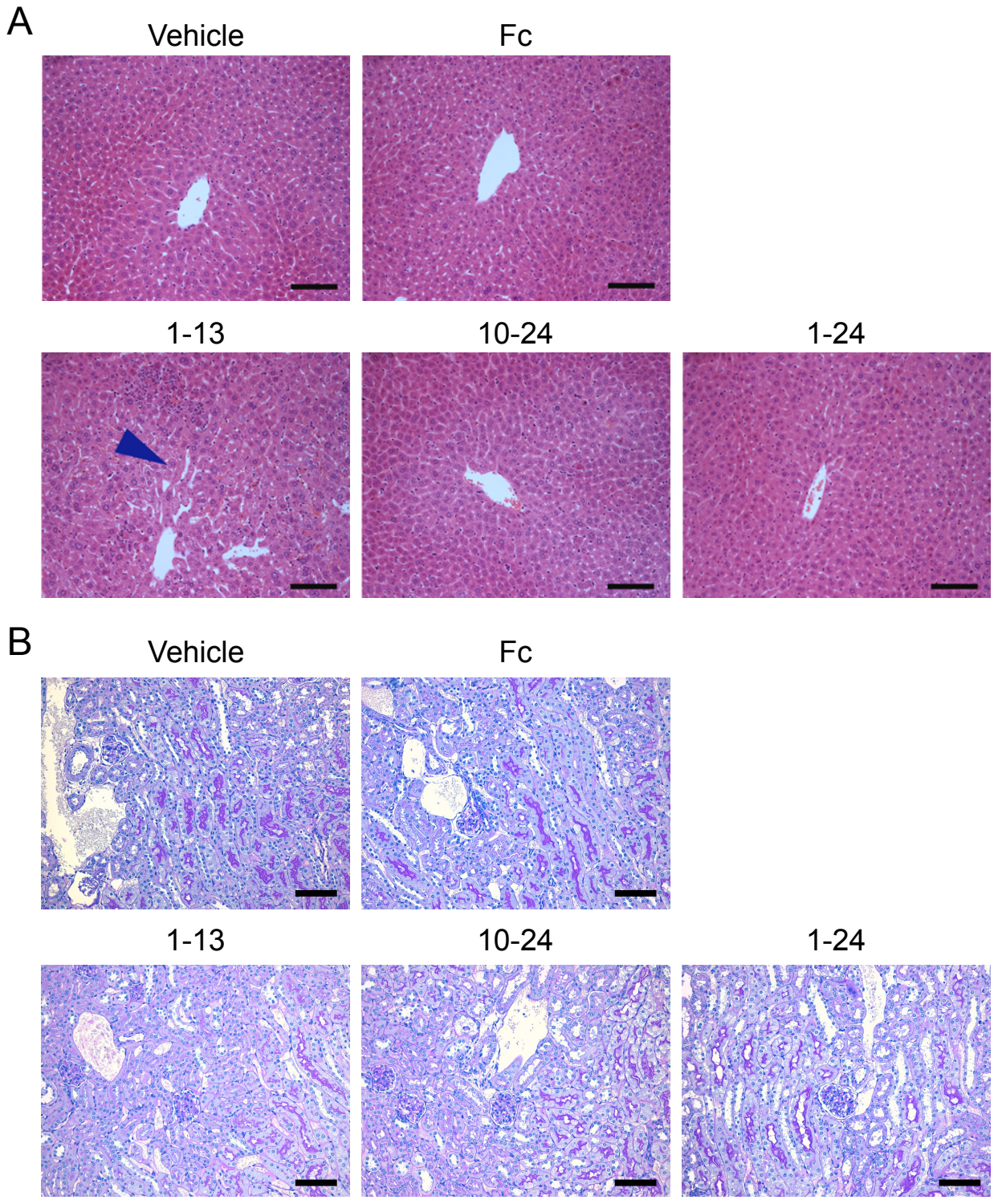


Figure S11. Liver and kidney histopathology of N1 decoy treated mice. Livers and kidneys were collected from vehicle or N1 decoy treated mice at day 21 (n=3). (**A**) H&E staining of liver sections. Blue arrow marks mild sinusoid dilation. (**B**) Periodic acid-Schiff staining of kidney sections. No significant differences were observed. Scale bars: 100 μm.