Neuropilin-1 promotes Hedgehog signaling through a novel cytoplasmic motif

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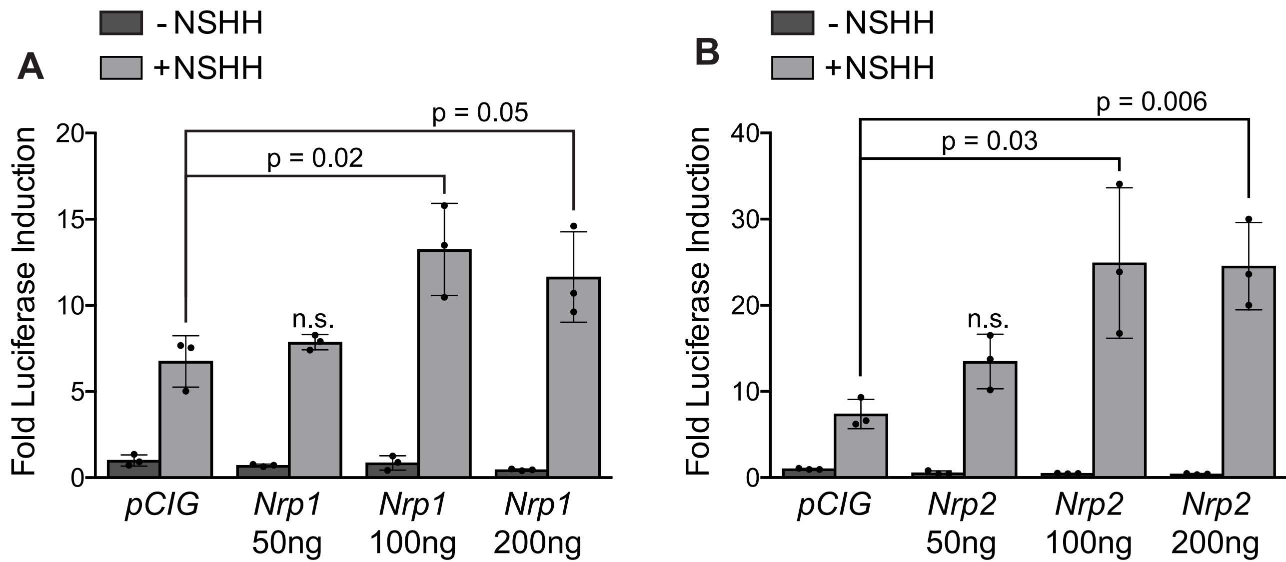
Supplemental Material to follow:

Figure S1
Figure S2
Figure S3
Figure S4
Figure S5



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assay	NRP1 fold change	NRP1 p-value	NRP2 fold change	NRP2 p-value
1	2.63	0.008	1.34	0.095
2	2.74	0.005	1.29	0.284
3	2.40	0.027	1.52	0.055
4	1.55	0.115	1.39	0.025
5	2.11	0.007	1.82	0.027
6	1.40	0.007	0.83	0.197
7	1.87	0.004	1.53	0.012
8	1.66	0.008	1.16	0.103
avg.	2.04	0.022	1.36	0.100

FIGURE S1. Optimization of NIH/3T3 transfection conditions. NIH/3T3 cells were transfected with 50, 100, and 200ng of *Nrp1* (A) or *Nrp2* (B), respectively. HH-dependent luciferase reporter activity was measured in ligand stimulated (+NSHH) and unstimulated (-NSHH) cells. Data reported as mean fold induction ± SD, with p-values calculated using a two-tailed Student's t test. 100 ng transfection amount was chosen for subsequent assays. (C) Summary of luciferase assay data in which *Nrp1* and *Nrp2* were directly compared in eight independent assays. Fold change reported between ligand-stimulated vector only (*pCIG*) triplicate wells and ligand-stimulated *Nrp1*- or *Nrp2*- transfected triplicate wells. Yellow highlight denotes significance ($p<0.05$).

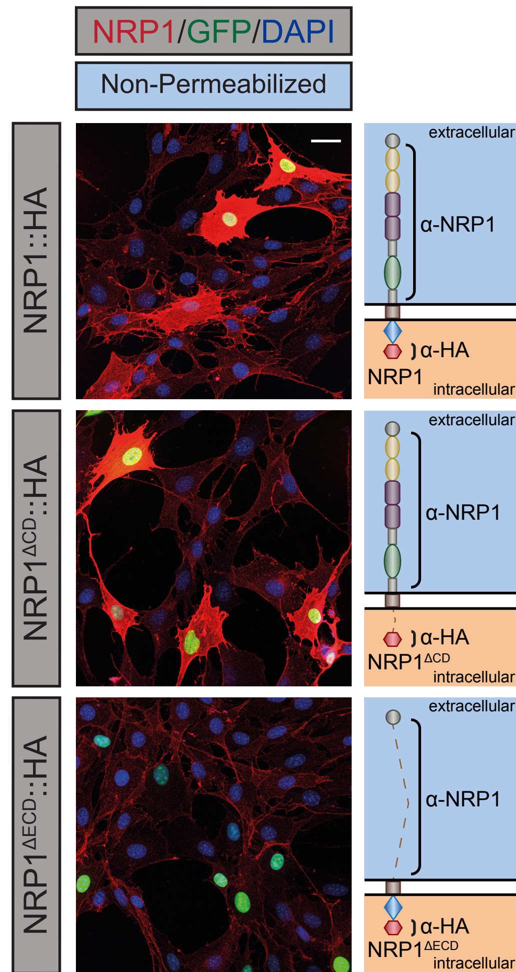

FIGURE S2. NRP1 antibody detects endogenous as well as overexpressed NRP1 protein. Antibody detection of endogenous NRP1 (red) in NIH/3T3 cells transfected with NRP1, NRP1$^{\Delta CD}$, and NRP1$^{\Delta ECD}$ as indicated. Exposure adjusted for endogenous rather than overexpressed protein (cf. Figure 2). DAPI staining indicates nuclei (blue). GFP expression identifies transfected cells (green). Diagrams of each construct to right indicate antibody-binding sites. Scale bar, 10µm.

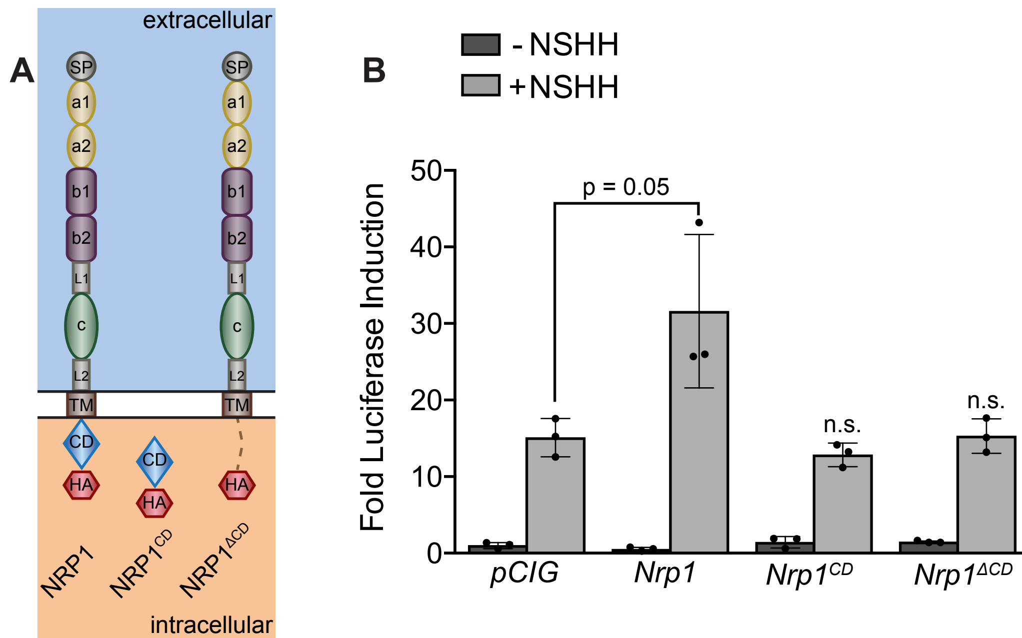

FIGURE S3. Cytosolic NRP1 cytoplasmic domain does not promote HH signaling. (A) Diagram of full-length NRP1, a cytosolic version lacking the extracellular and transmembrane domains ($NRP1^{CD}$), and a version lacking the cytoplasmic domain ($NRP1^{\Delta CD}$). (B) HH-dependent luciferase reporter activity measured in NIH/3T3 cells. Data reported as mean fold induction ± SD, with p-values calculated using a two-tailed Student's t test.

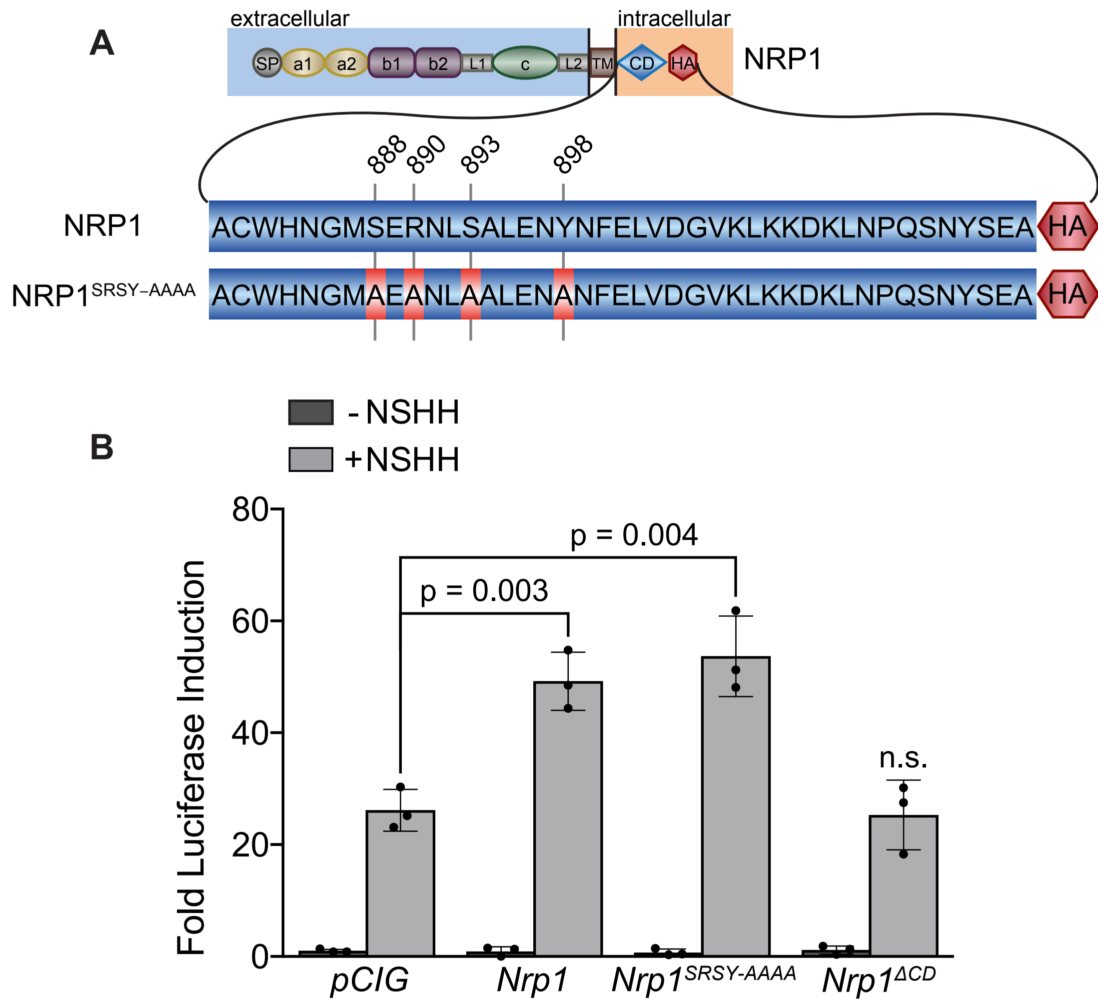

FIGURE S4. Phosphorylation of key residues is dispensable for NRP1 promotion of HH signaling. (A) Diagram of NRP1 cytopalsmic domain and $NRP1^{SRSY\text{-}AAAA}$ indicating mutations of key residues to Alanine (B) HH-dependent luciferase reporter activity measured in NIH/3T3 cells. Data reported as mean ± SD, with p-values calculated using a two-tailed Student's t test.

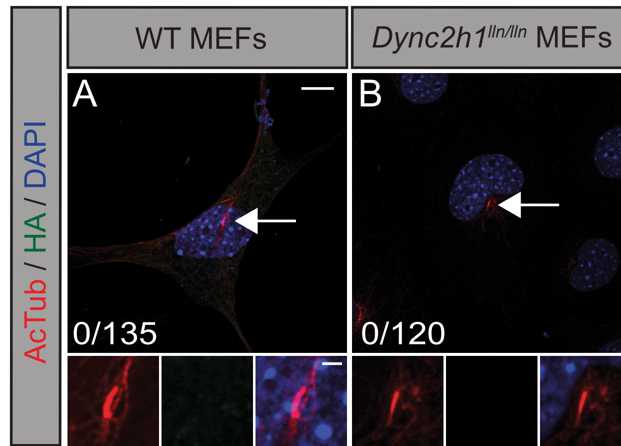

FIGURE S5. HA antibody staining in vector-transfected cells. Antibody detection of primary cilia (red, AcTub) and HA (green) in *pCIG*-transfected WT (left) and Dynein mutant (*Dync2h1*$^{lln/lln}$, right) MEFs. DAPI indicates nuclei (blue). Exposure adjusted to match Figure 5 to demonstrate the background level of HA staining.Scale bar, 10µm. Inset scale bar, 1µm.