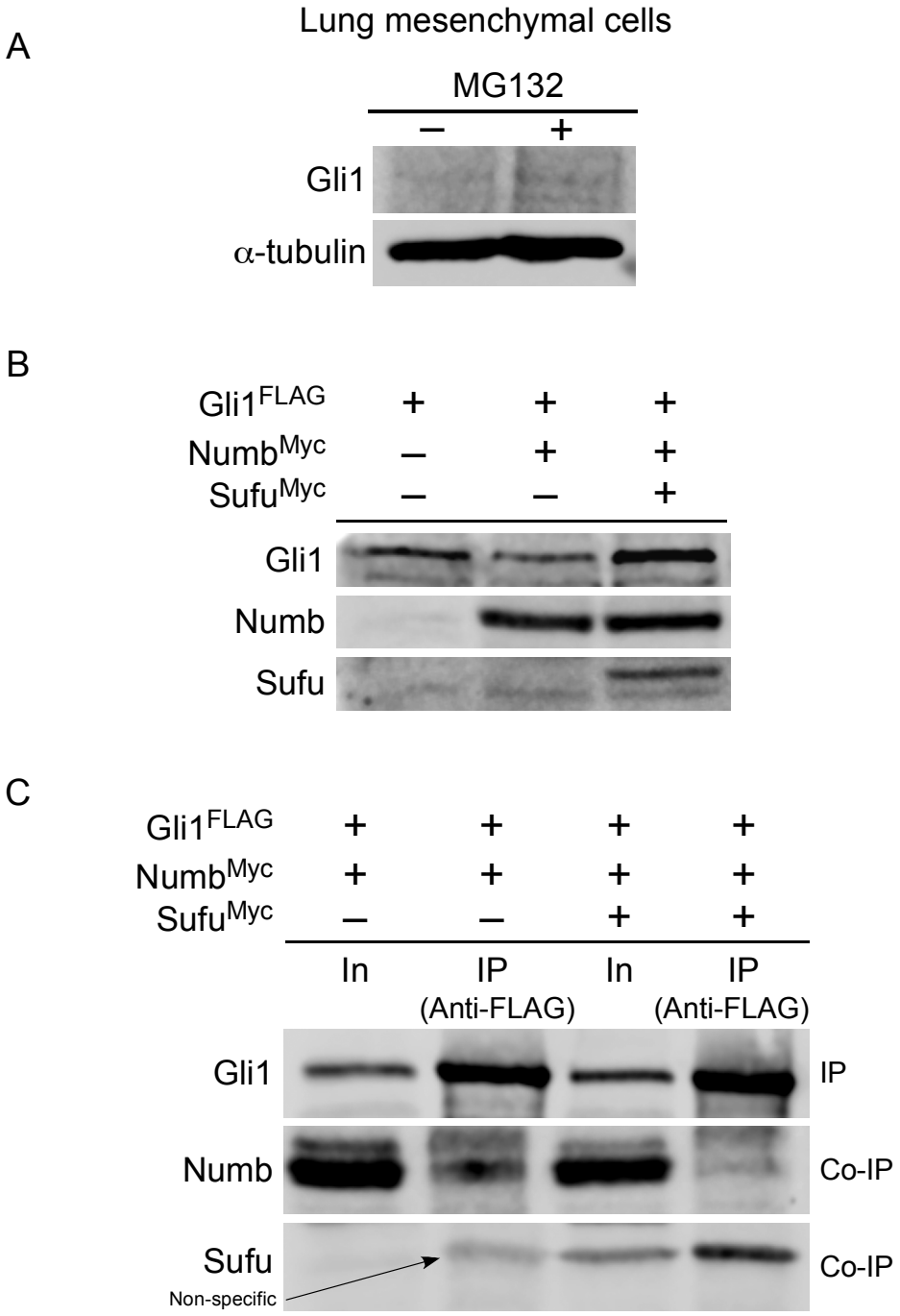

Fig. S1. Control of Gli1 protein levels by Sufu in lung mesenchymal cells
(A) Western blot analysis of lysates from wild-type mouse lung mesenchymal cells treated with MG132 to block proteasome-mediated degradation. Endogenous Gli1 protein levels did not show obvious difference in the presence of MG132. (B) Western blot analysis of lysates from lung mesenchymal cells expressing various combinations of Gli1, Numb and Sufu. Numb expression resulted in reduction in Gli1 protein levels and this effect could be reversed when Sufu was co-expressed with Numb. (C) Western blot analysis of immunoprecipitated $Gli1^{FLAG}$ from lysates of lung mesenchymal cells to test the competition between Sufu and Numb in binding to Gli1. Co-immunoprecipitated $Numb^{Myc}$ by Gli1 was significantly reduced when $Sufu^{Myc}$ was also pulled down by Gli1. In, input; IP, immunoprecipitation.

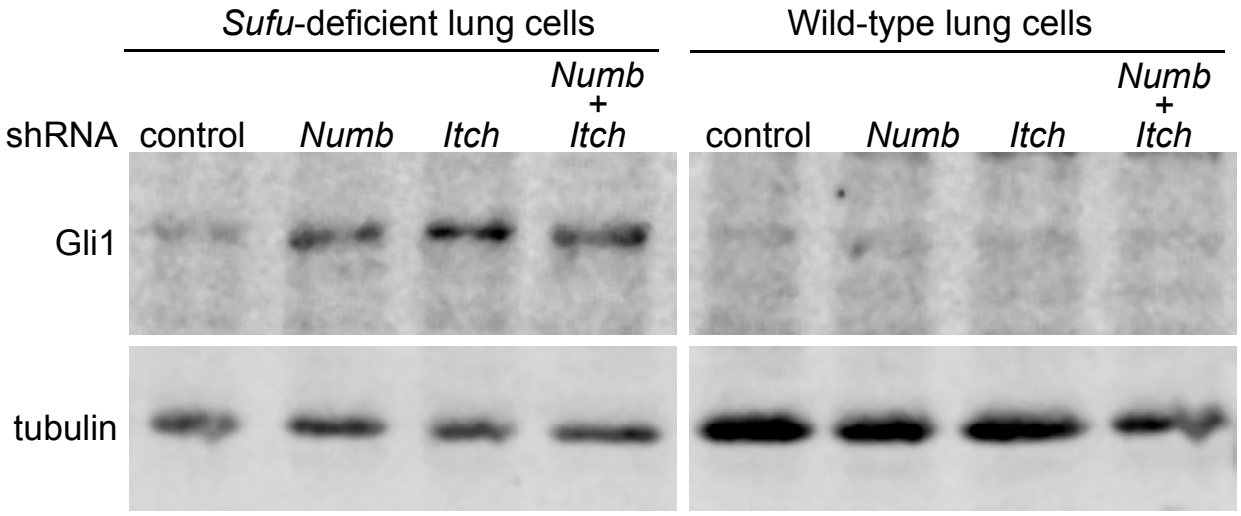

Fig. S2. shRNA-mediated knockdown of *Numb* or *Itch* increases endogenous Gli1 protein levels in *Sufu*-deficient lung cells

Western blot analysis of lysates from wild-type or *Sufu*-deficient mouse lung cells in which *GFP* (control), *Numb*, *Itch* or *Numb/Itch* were knocked down by shRNA. Immortalized lung mesenchymal cells were derived from wild-type or $Sufu^{f/-}$; $Dermo1^{Cre/+}$ (*Sufu*-deficient) embryos. Note that the baseline Gli1 protein levels in *Sufu*-deficient lung cells did not recapitulate those of intact lung tissues. While Gli1 protein levels were reduced in *Sufu*-deficient lungs compared with wild-type lungs, Gli1 protein levels were not reduced in immortalized *Sufu*-deficient lung cells compared with immortalized wild-type lung cells. This was likely due to immortalization and culturing, which altered the transcription of *Gli1*.

Wild-type and *Sufu*-deficient lung cells were transduced with lentiviruses that carry shRNA for the respective gene. Knockdown of *Numb*, *Itch* or *Numb/Itch* led to increased levels of endogenous Gli1 protein in *Sufu*-deficient but not wild-type lung cells. We speculate that Sufu in wild-type lung cells protects Gli1 from Numb-mediated degradation. As such, knockdown of *Numb/Itch* in wild-type lung cells has minimal effects on Gli1. Together, these results suggest that Numb/Itch mediate Gli1 protein degradation and supports a model in which Sufu stabilizes Gli1 by antagonizing Numb/Itch action.

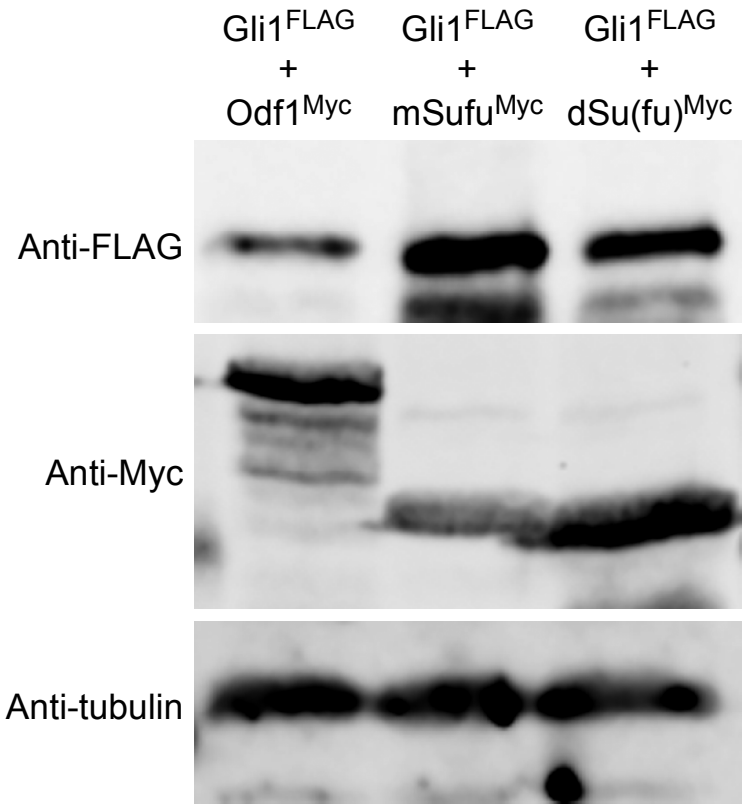

Fig. S3. Drosophila Su(fu) stabilizes Gli1 protein
Western blot analysis of lysates from HEK293T cells expressing various combinations of epitope-tagged mouse Gli1, mouse Sufu, Drosophila Su(fu) and Odf1 (control). Both mouse and fly Sufu stabilized Gli1 protein. Tubulin was used as the loading control.

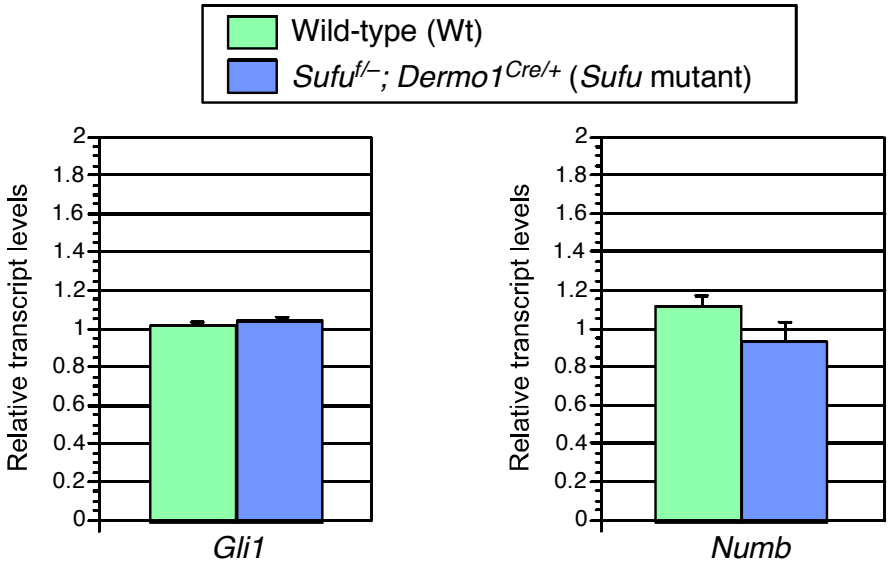

Fig. S4. *Gli1* and *Numb* mRNA levels are not altered in the absence of Sufu
qPCR analysis of *Gli1* and *Numb* mRNA in wt and *Sufu* mutant lungs. Neither *Gli1* nor *Numb* transcript levels were altered due to loss of Sufu. This suggests that regulation of Gli1 by Numb and Sufu occurs at post-transcriptional steps.

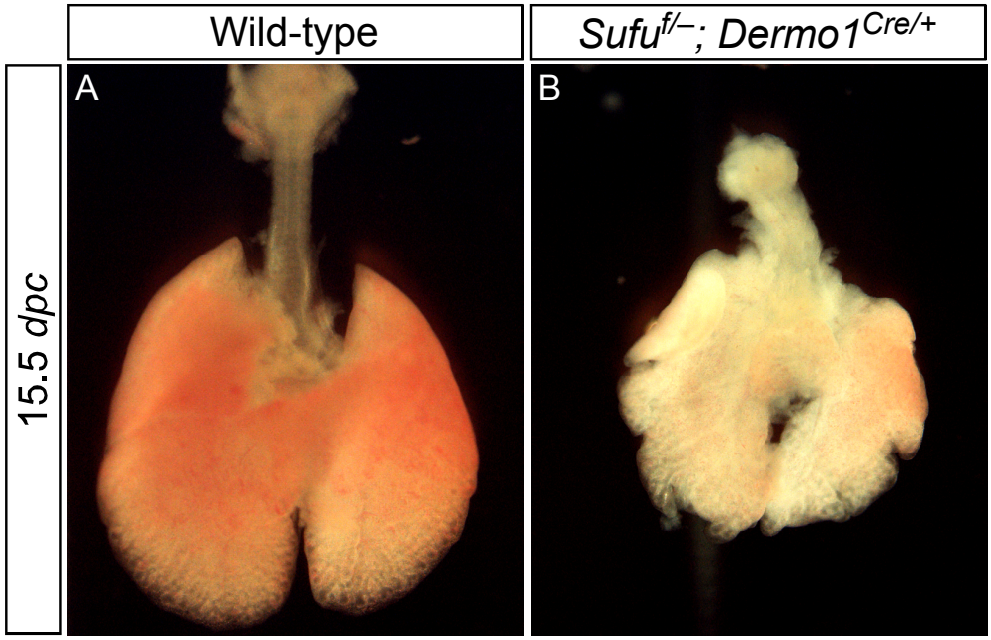

Fig. S5. Reduced epithelial branching in the absence of *Sufu*
External morphology of dissected lungs from wild-type (A) and *Sufu*-deficient (B) embryos at 15.5 *dpc*. *Sufu* mutant lungs were smaller in size compared to wild-type. The contour of the more terminal branches could be discerned and a reduced number of branches was detected in the absence of *Sufu*.

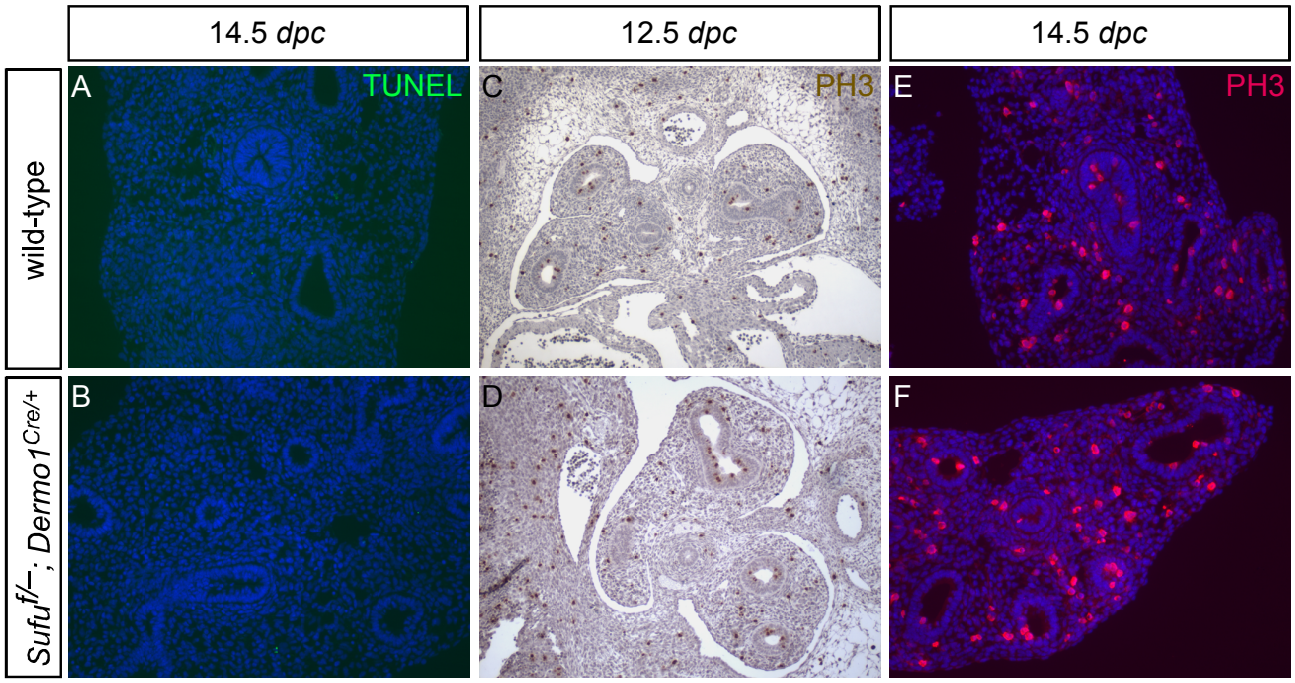

Fig. S6. The rate of cell proliferation and cell death in the lungs is not significantly altered in the absence of *Sufu* TUNEL analysis (A, B), immunohistochemistry (C, D) and immunofluorescence (E, F) analysis of wild-type and *Sufu*-deficient lungs at the indicated stages. No apparent difference in cell proliferation rate (PH3) or cell death (TUNEL) was detected between wild-type and *Sufu* mutant lungs.

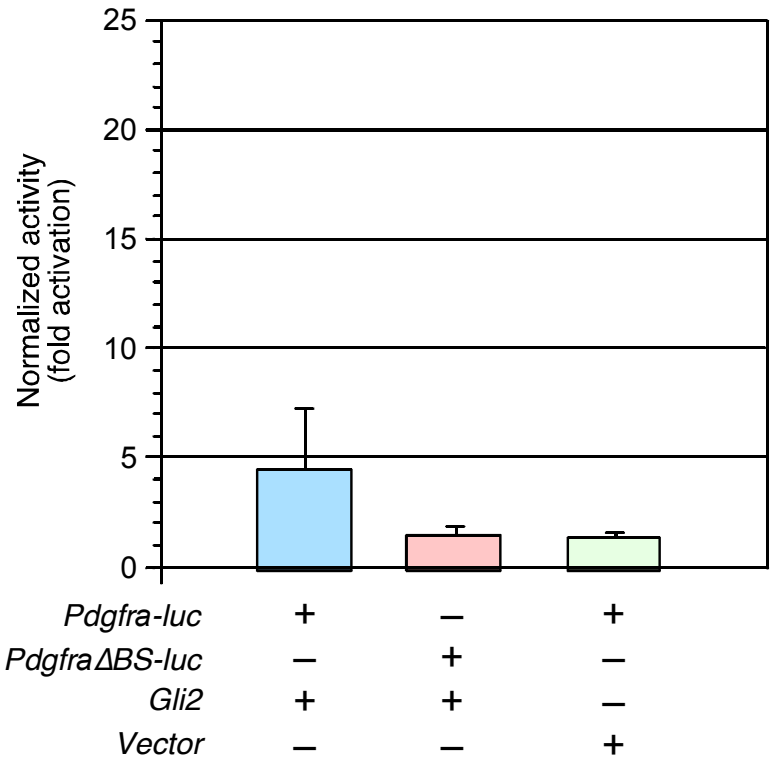

Fig. S7. Gli2 has low activity in activating the *Pdgfra* promoter fragment *in vitro*
A canonical Gli-binding site (GliBS) is present in the mouse *Pdgfra* promoter and is mutated in the control construct *Pdgfra*Δ*BS.* The *Pdgfra* promoter fragments are placed upstream of *firefly luciferase* (*luc*). Unlike Gli1, addition of Gli2 had low activity in inducing expression of *luc* from the *Pdgfra* promoter.

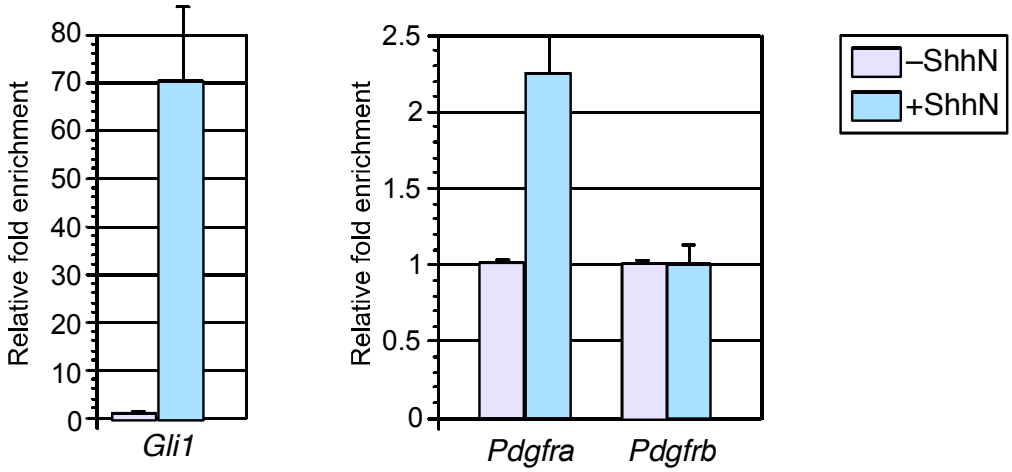

Fig. S8. Hedgehog (Hh) stimulation induces *Pdgfra* but not *Pdgfrb* expression
qPCR analysis of gene activation in response to Hh stimulation in wild-type mouse lung mesenchymal cells. Expression of *Gli1* is vastly upregulated upon Hh pathway activation as previously reported. *Pdgfra* was induced upon ShhN addition while *Pdgfrb* levels were unaltered.