Supporting information

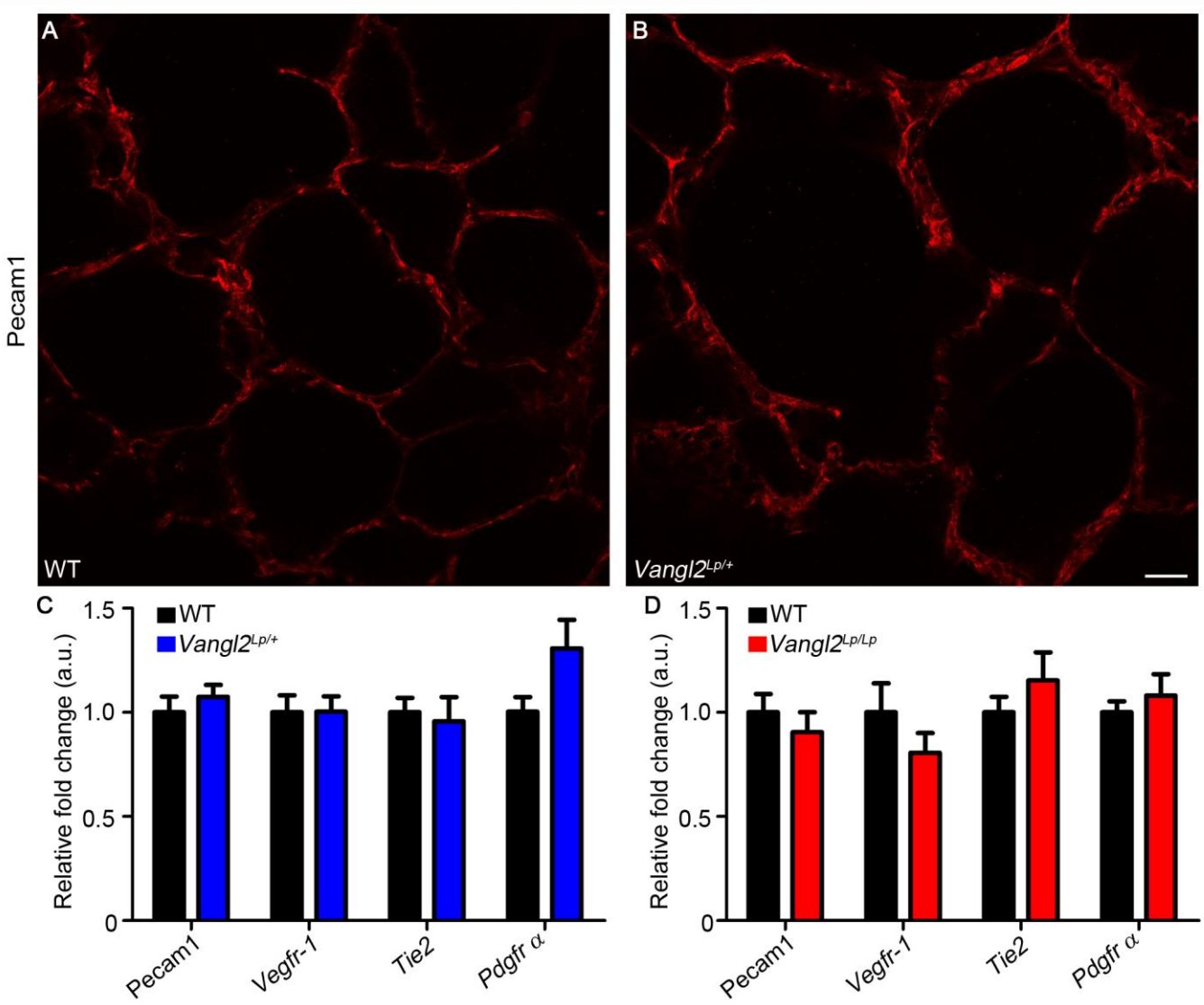

Fig. S1: Alveolar vasculature is not affected by the *Vangl2*$^{Lp/+}$ mutation.

Representative Immunofluoresent staining of Pecam1 highlighting vascular architecture in lung slices of 10 week-old (**A**) WT and (**B**) *Vangl2*$^{Lp/+}$ mice (n=3 per genotype). Although some difference in the pattern of Pecam distribution is seen in *Vangl2*$^{Lp/+}$ slices, these are consistent with the overall changes in lung histology observed in these mutants (see Fig1). (**C**) Relative fold change in *Pecam1, Vegfr-1, Tie2* and *Pdgfr-α* levels normalised to housekeeping genes *Hprt* and *B2m* expression in 10-14 week old adult lungs (mean ±SEM, WT n=8 and *Vangl2*$^{Lp/+}$ n=6, student's T-test $p>0.05$), and (**D**) embryonic E18.5 lungs (mean ±SEM, WT n=4 and *Vangl2*$^{Lp/Lp}$ n=4, student's T-test $p>0.05$) showed no significant change in expression levels of these genes. Scale bars; (**A,B**) 18 µm.

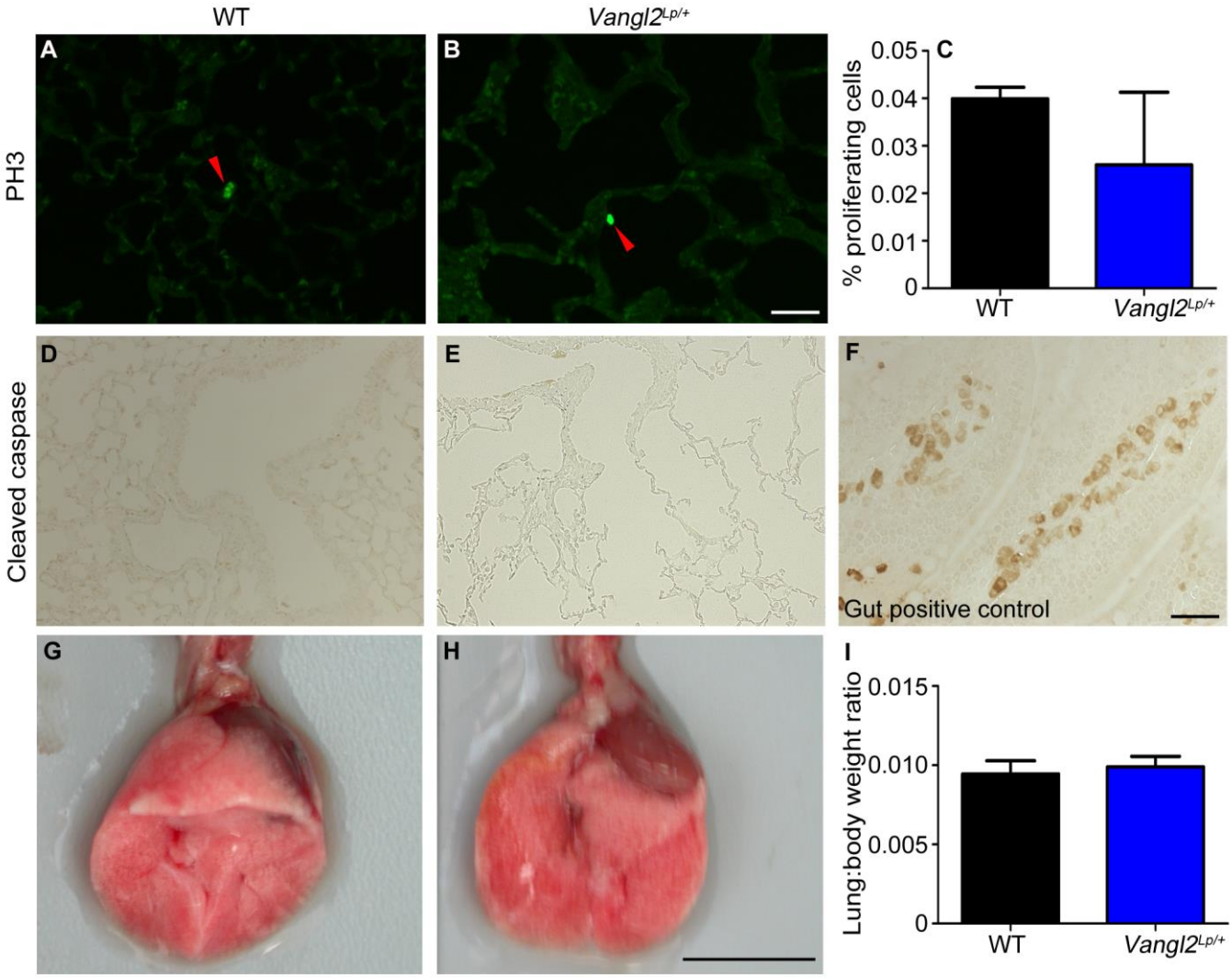

Fig. S2: Proliferation, apoptosis and lung size are not altered in *Vangl2*$^{Lp/+}$ mice. (**A-C**) Immunostaining of 10 week old WT and *Vangl2*$^{Lp/+}$ lungs for PH3, to mark proliferating cells, showed no significant difference in the percentage of proliferating cells between WT and *Vangl2*$^{Lp/+}$ lungs (mean ±SEM, WT n=3 and *Vangl2*$^{Lp/+}$ n=4, student's T-test P=0.48). (**D,E**) No apoptotic cells were observed when stained with cleaved caspase 3. (**F**) Gut sections from WT littermates were used as a positive control for cleaved caspase 3. (**G,H**) No differences were seen in macroscopic size between 10 week old WT and *Vangl2*$^{Lp/+}$ lungs. (**I**) Quantification of lung to body weight ratio did not show a significant difference between WT and *Vangl2*$^{Lp/+}$ littermates (mean ±SEM, n=3 per genotype, student's T-test). (**A,B,D-F**) 65 µm, (**G,H**) 1 cm.

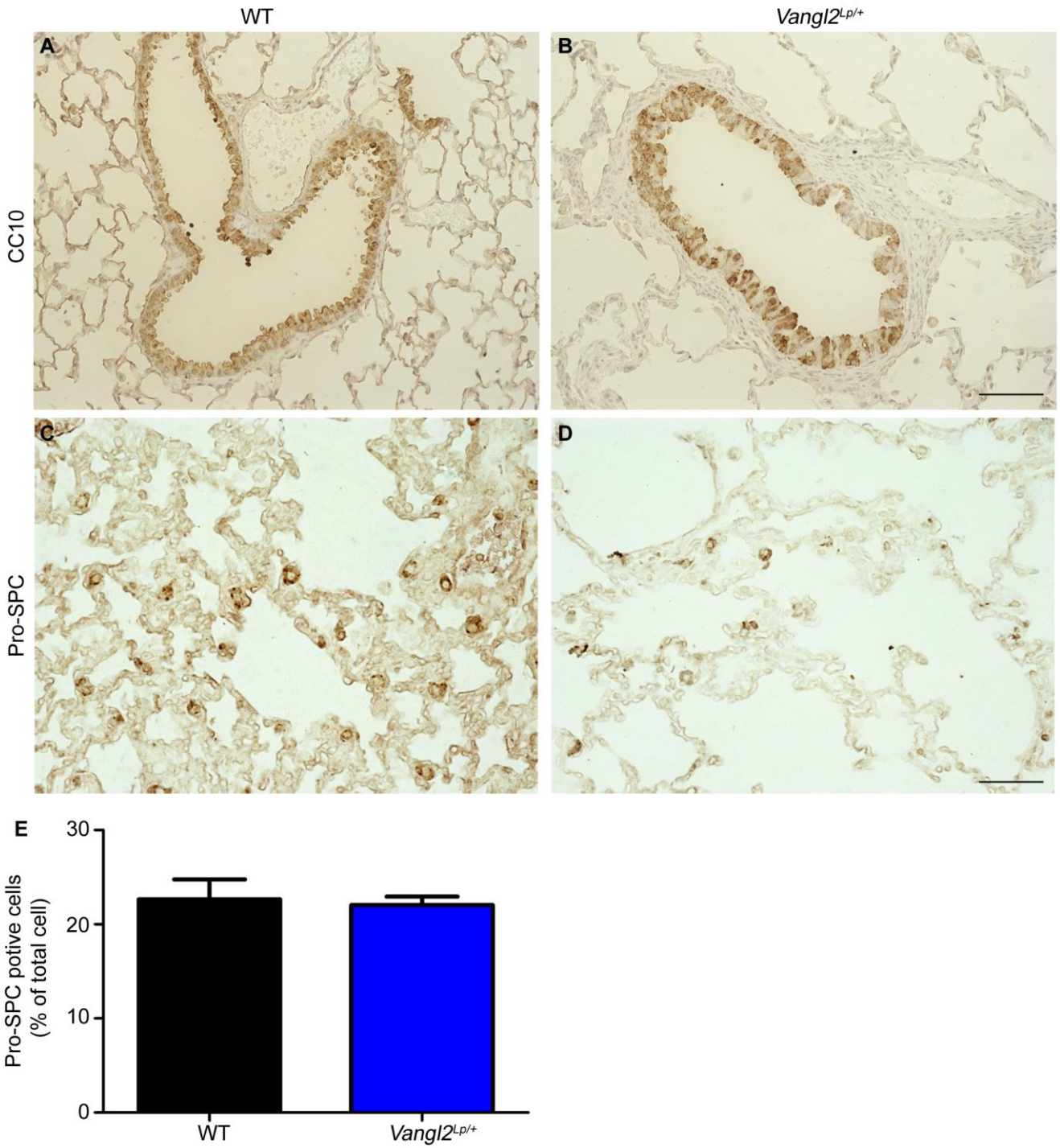

Fig. S3: The *Vangl2*$^{Lp/+}$ mutation does not affect cell differentiation in adult lungs.

Analysis of cell differentiation in 10 week-old (**A,C**) WT and (**B,D**) *Vangl2*$^{Lp/+}$ mice by immunohistochemistry showed no differences in (**A,B**) club cell 10, CC10 (a proximal airway marker), or (**C,D**) pro surfactant protein-C, Pro-SPC (an alveolar type II cell marker) between WT and *Vangl2*$^{Lp/+}$ mice**.** (**E**) Quantification of Pro-SPC positive cells revealed no significant difference in the percentage ATII cells between WT and *Vang2*$^{Lp/+}$ lungs (mean ±SEM, WT n=3 per genotype, student's T-test P>0.05). Scale bars; (**A,B**) 62 µm, (**C,D**) 28 µm.

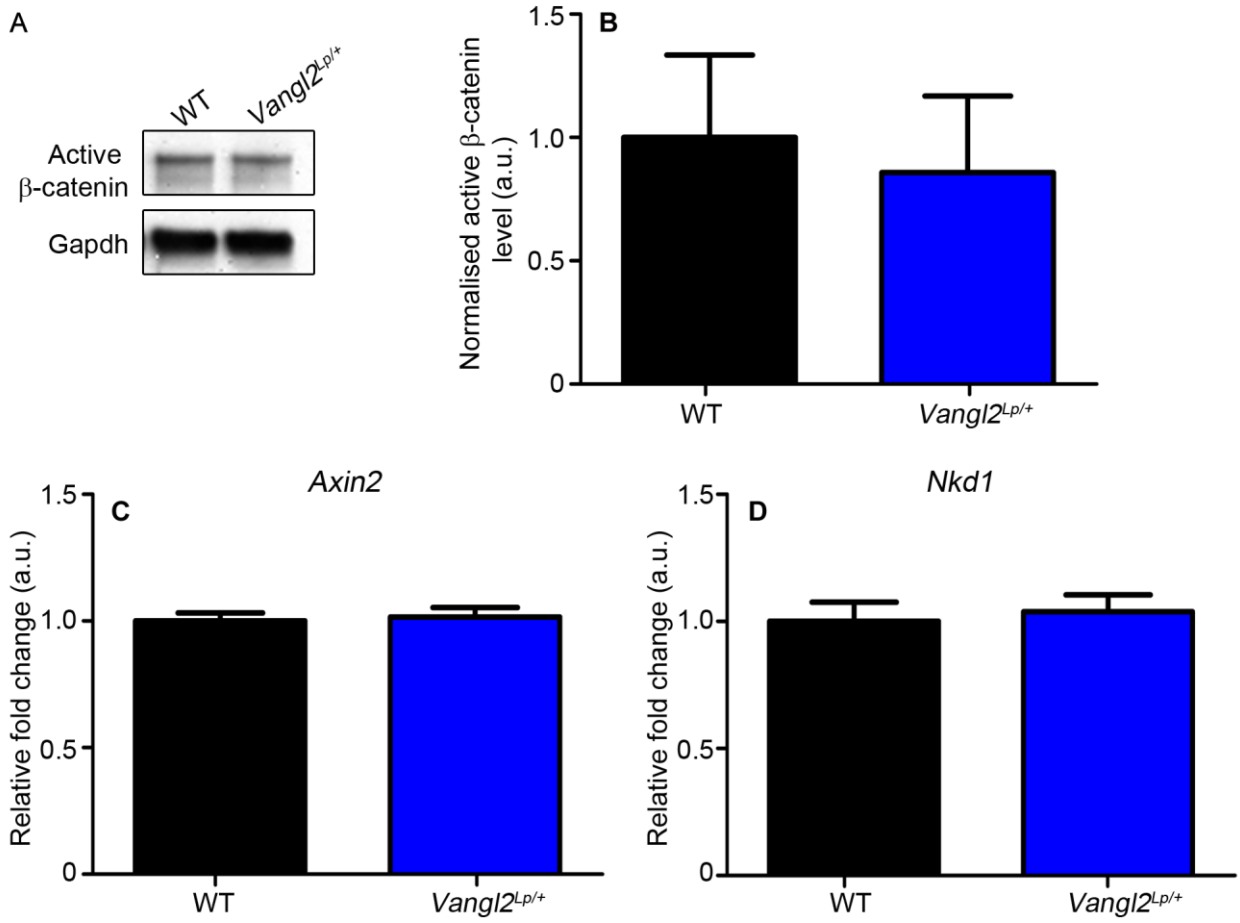

Fig. S4: Canonical Wnt/β-catenin signalling is not altered in adult *Vangl2*$^{Lp/+}$ lungs.

(**A**) Representative western blot of active β-catenin (92 kDa) and Gapdh (37 kDa) loading control in the adult lung. (**B**) Quantification of active β-catenin showed no significant difference between WT and *Vangl2*$^{Lp/+}$ lungs (mean ±SEM, n=4 per genotype, with all samples run on a single gel, student's T-test P>0.05). qRT-PCR of canonical Wnt signalling target genes (**C**) *Axin2* and (**D**) *Nkd1* showed no significant difference in these transcripts between WT and *Vangl2*$^{Lp/+}$ (mean ±SEM, n=3 per genotype, student's T-test P>0.05).

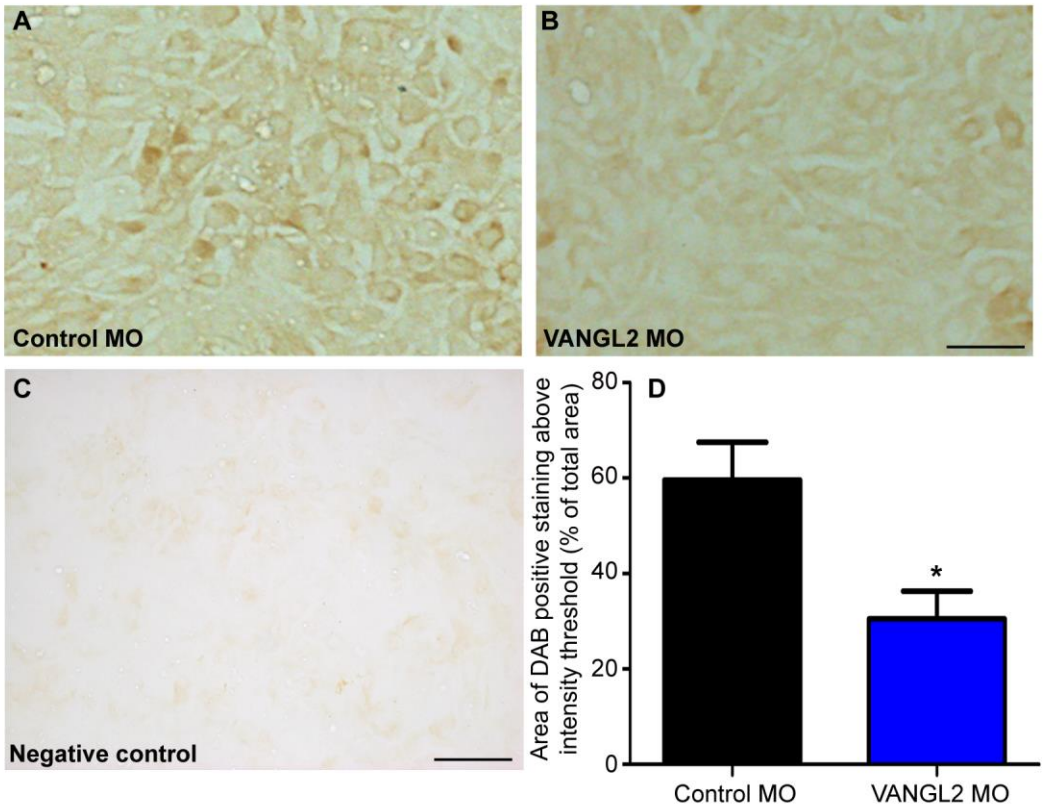

Fig S5: Quantification of VANGL2 knockdown in A549 cells following morpholino treatment.

(**A-B**) Immunohistochemical staining for VANGL2 in A549 cells after treatment with (**A**) control or (**B**) VANGL2 MO for 72 hr. (**C**) Negative control with primary antibody omitted (image obtained from the same 8 well chamber slide as A and B immunostained at the same time but captured in a different imaging session) (**D**) Quantification of VANGL2 staining determined by DAB intensity showed a 48.8% reduction following VANGL2 MO treatment compared to control MO treatment (mean ±SEM, n=3 independent experiments, each containing 3 technical replicates per treatment, student's T-test *p<0.05). Scale bar; (**A, B)** 13 µm **C**) 25 µm.

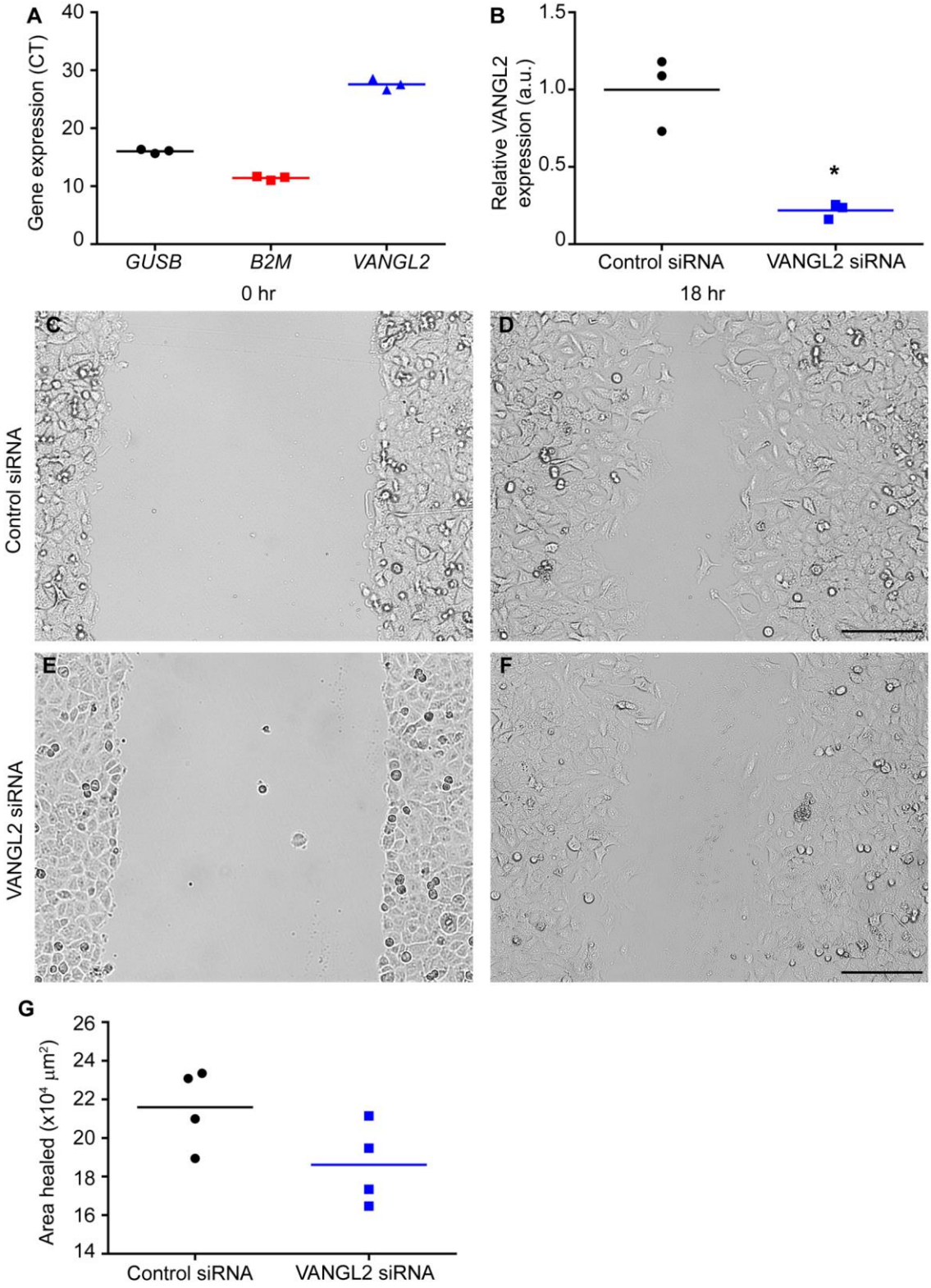

Fig S6: Knockdown of *VANGL2* using siRNA impairs wound healing in A549 cells

(**A**) qRT-PCR shows *VANGL2* expression levels in A549 cells compared to *GUSB* and *B2M* housekeeping genes (mean ±SEM, n=3). (**B**) Quantification of *VANGL2* knockdown by qRT-PCR showed an 80% reduction in *VANGL2* mRNA levels in A549 cells treated with *VANGL2* siRNA compared to scrambled control (mean ±SEM, n=3 per treatment, student's T-test *p<0.05). (**C-F**) Representative images of wound edges (red lines), in A549 cells treated with (**C,D**) control siRNA or (**E,F**) *VANGL2* siRNA, at (**A,C**) 0 hr and (**B,D**) 18 hr post scratch. (**G**) *VANGL2* knockdown led to a reduction in the area healed at 18 hr compared to controls (mean ±SEM, n=2 experiments with 2 technical replicates per treatment in each). Scale bar; (**C-F**) 115 μm.