

Figure S1. *Fgf10^{Cre}* knock-in line labels a subset of *Fgf10*-expressing cells in the embryonic lung without evidence of ectopic expression. Pregnant mice carrying *Fgf10^{Cre/lacZ}; tomato^{flax/+}* embryos received a single IP injection of tamoxifen at E11.5 and lungs were harvested at E13.5. (A-C) Single-channel fluorescent images showing RFP (red) and LacZ (green) double staining on cryosections. (D) Merged image of A-C showing that all RFP⁺ cells, labeled at E11.5, express *lacZ* at E13.5. Note the presence of RFP⁻ LacZ⁺ cells and the absence of RFP⁺ LacZ⁻ cells. (E) Quantification of RFP⁺ LacZ⁺, RFP⁻ LacZ⁺ and RFP⁺ LacZ⁻ cell populations relative to total LacZ⁺ cells. *n*=3. Data are shown as average values ± s.e.m. Scale bar: 25 μm.

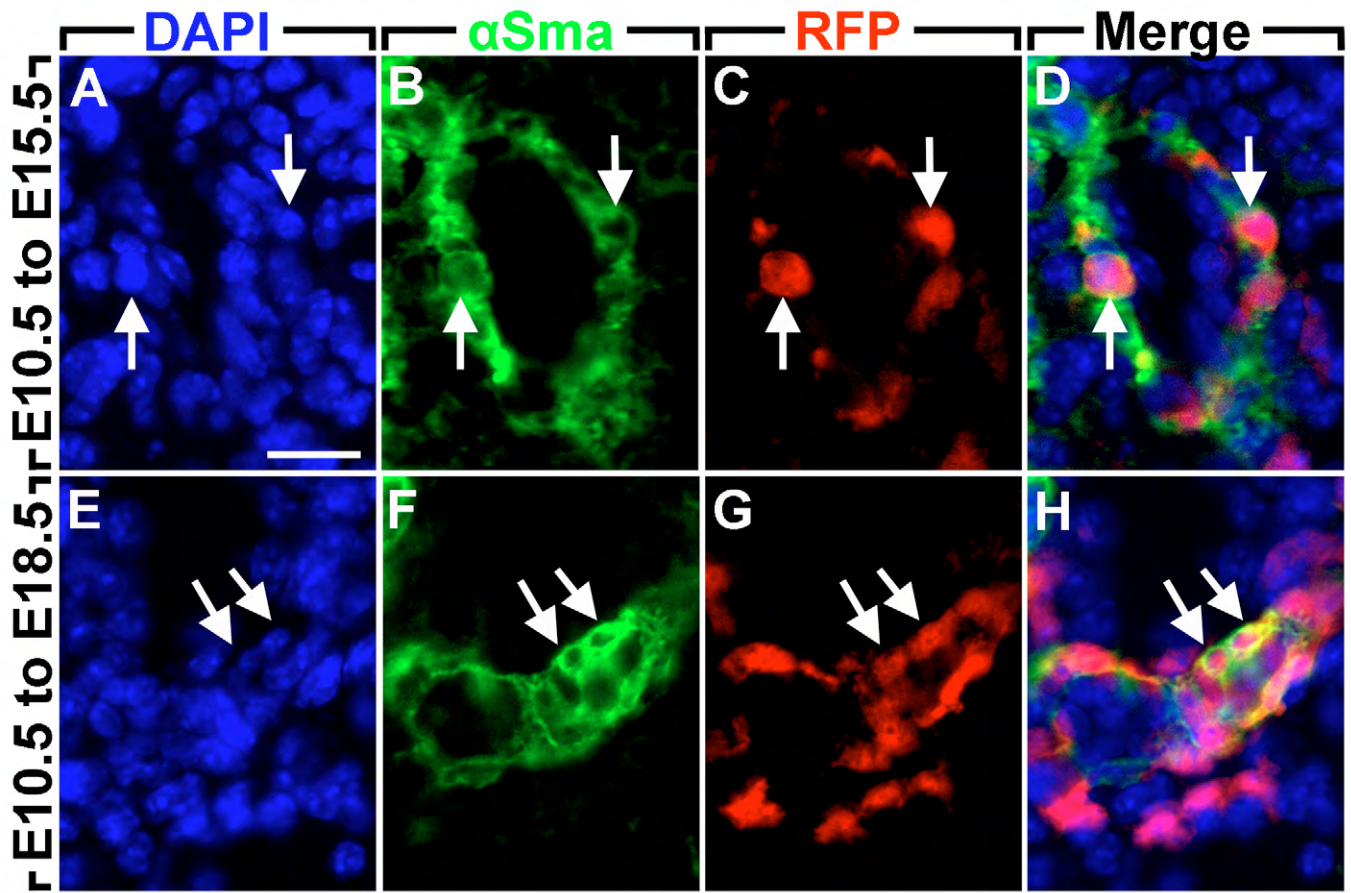


Figure S2. Contribution of *Fgf10*-positive cells labeled at E10.5 to the vascular smooth muscle lineage *in vivo*. Single-channel fluorescent images showing α Sma staining (green) on E15.5 (A-D) and E18.5 (E-H) lungs from *Fgf10^{iCre/+}; tomato^{flax/+}* embryos that were exposed to tamoxifen at E10.5. RFP⁺ cells within the vascular smooth muscle compartment are marked by white arrows. Scale bar: 12.5 μ m.

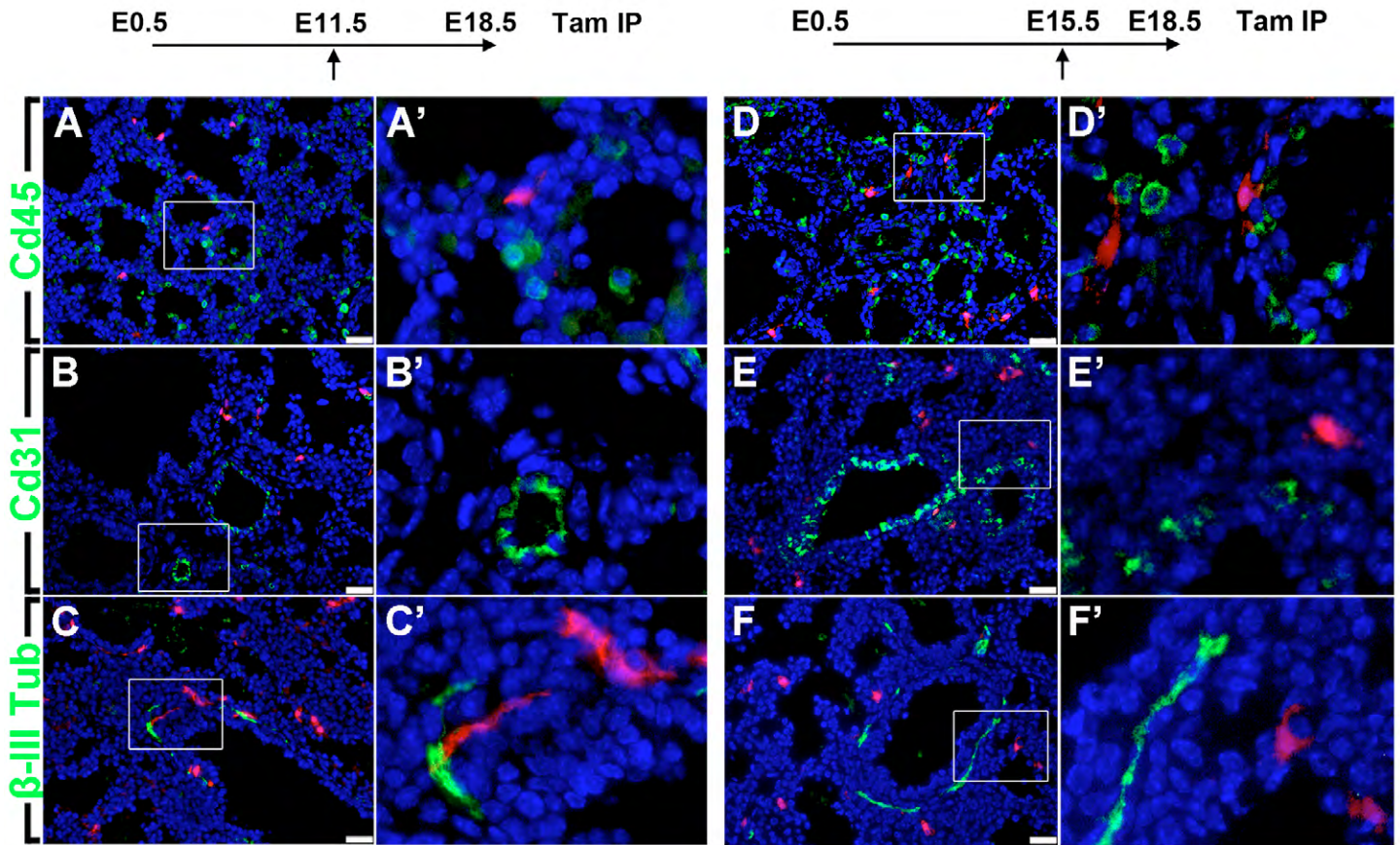


Figure S3. *Fgf10*-expressing cells from E11.5 or E15.5 do not express hematopoietic, endothelial or neuronal markers at E18.5. Immunofluorescence staining for Cd45, Cd31 and β -III Tubulin of E18.5 *Fgf10*^{Cre/+}; *tomato*^{lox/+} lungs that were exposed to tamoxifen at E11.5 (A-C) or E15.5 (D-F). High magnification images for the areas in the boxes are shown in (A'-C') and (D'-F'), respectively. Scale bars: 25 μ m.

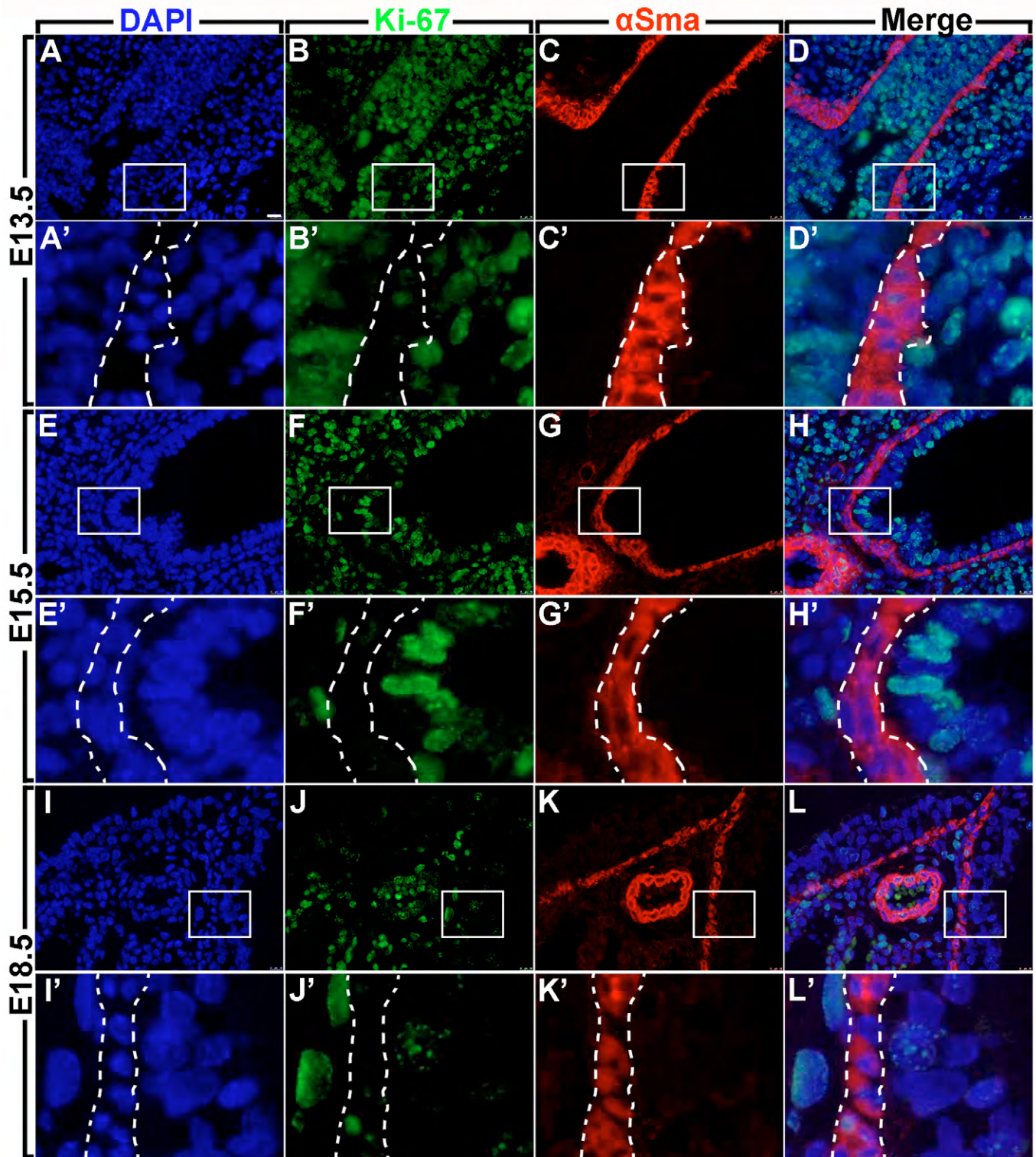
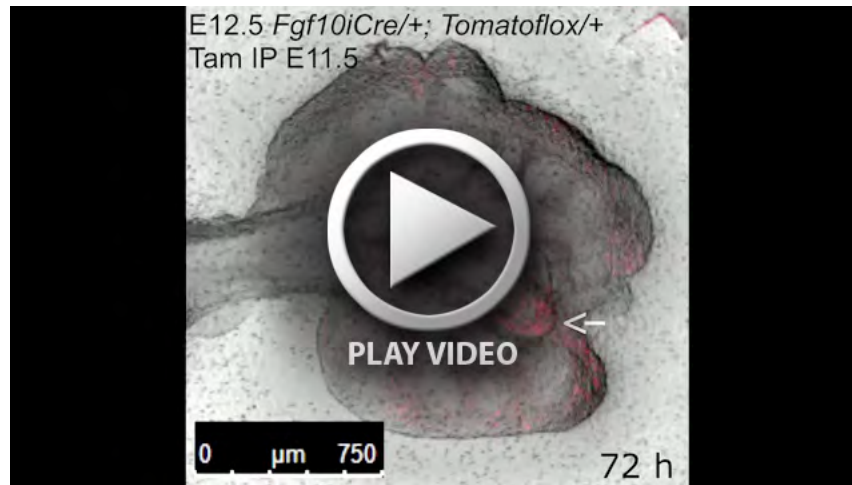


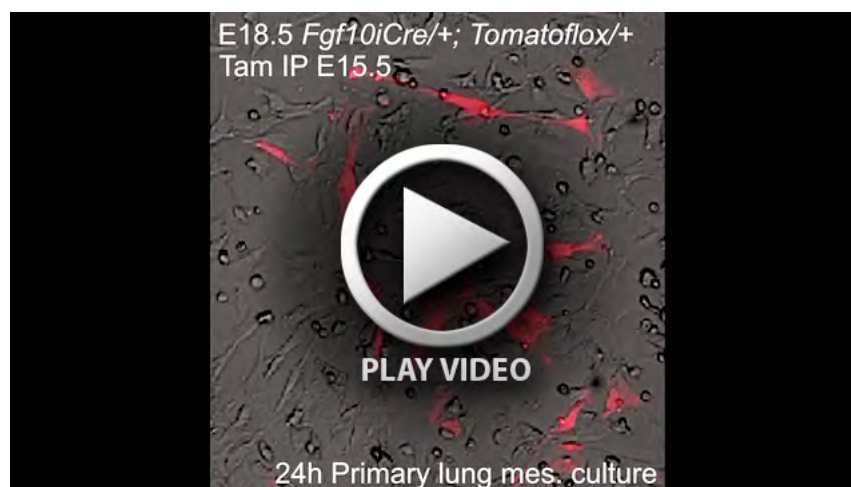
Figure S4. α Sma-positive cells rarely proliferate during lung development. Immunofluorescence staining for Ki-67 and α Sma in the lung at E13.5 (A-D), E15.5 (E-H) and E18.5 (I-L). High magnification images of the areas in the white boxes are shown in (A'-D'), (E'-H') and (I'-L'). The PBSMC layer is marked in A'-L'. Scale bars: 25 μ m.



Movie 1. Time-lapse imaging of an E12.5 *Fgf10^{iCre/+}*; *tomato^{lox/+}* lung undergoing branching morphogenesis. Cells were labeled by a single IP injection of tamoxifen at E11.5. Lungs were harvested at E12.5 and grown in an air-liquid interphase for 72 hours. An overlay of brightfield and the RFP channel is shown. The arrow shows strong tomato expression in the accessory lobe at t=0 hour.



Movie 2. Time-lapse imaging of an E18.5 *Fgf10^{iCre/+}*; *tomato^{lox/+}* lung explant that was induced with tamoxifen at E11.5. The explant was cultured on a filter in an air-liquid interphase for 24 hours. An overlay of brightfield and the RFP channel is shown. Note the presence of RFP⁺ cells with long filopodia. Dividing cells are marked with arrows and daughter cells are marked with circles.



Movie 3. Time-lapse imaging of a primary culture of lung mesenchyme from E18.5 *Fgf10^{iCre/+}*; *tomato^{lox/+}* lungs that were induced with tamoxifen at E15.5. Single cells were obtained as described in the “FACS analysis and sorting” part in the Materials and Methods. Suspensions were plated for 45 minutes for differential adhesion, washed with PBS and incubated overnight (37°C; 5% CO₂). Imaging was performed for 24 hours and an overlay of brightfield and the RFP channel is shown. Dividing cells are marked with arrows and daughter cells are marked with circles.