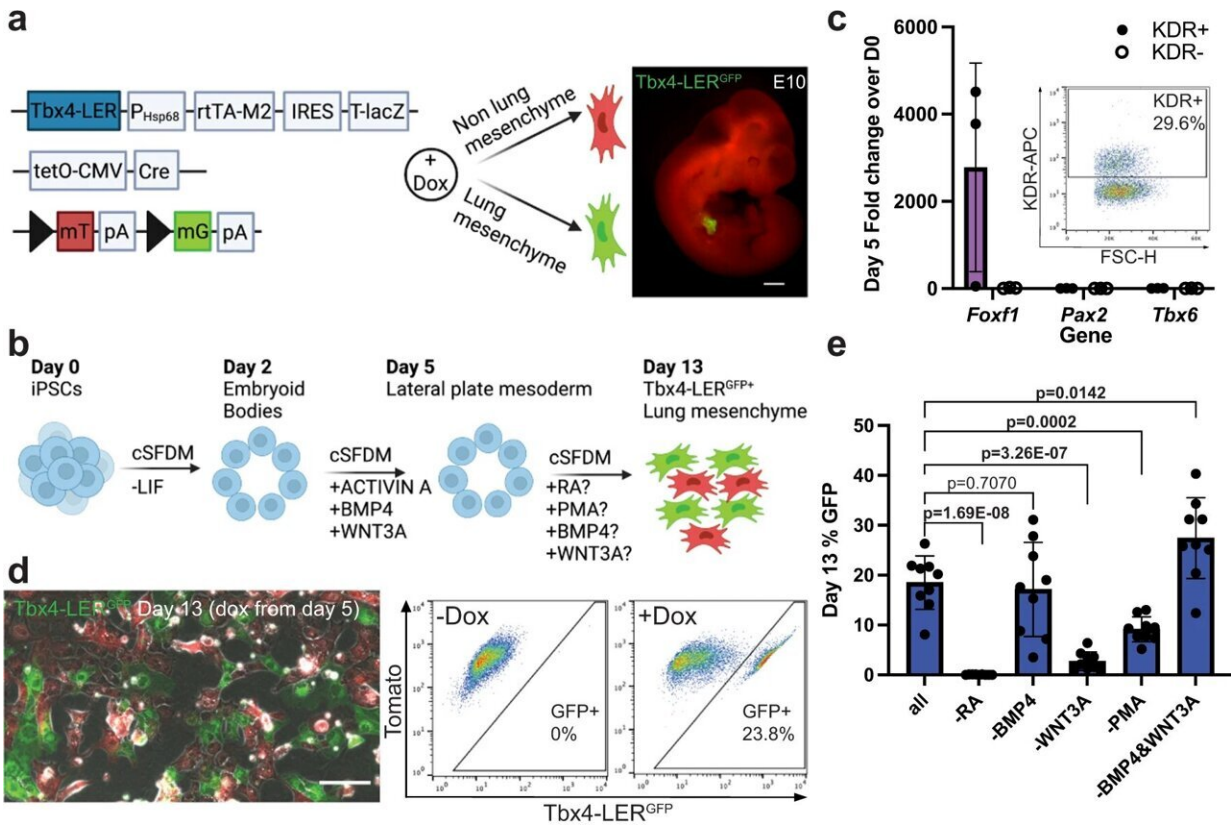


Researchers create new model of lung mesenchymal cells

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In vitro differentiation of iPSCs toward the lung mesenchyme lineage through a mesodermal progenitor. **a** Schematic of the *Tbx4* lung enhancer reporter/tracer (LER) line, and image of an E10 embryo (dox from E6.5 to E10). Scale bar = 0.5 mm. Created with BioRender.com. **b** Directed differentiation of iPSCs into lung mesenchyme through a mesodermal progenitor. cSFDM = complete serum-free differentiation medium, LIF = Leukemia inhibitory factor. Created with BioRender.com. **c** Expression relative to day 0 iPSCs of *Foxf1* (lateral plate mesoderm), *Pax2* (intermediate mesoderm) and *Tbx6* (paraxial mesoderm) in

KDR \pm cells on day 5. *N* = 3. **d** Image and flow cytometry plot showing expression of GFP (green) in the Tbx4-LER iPSC line on day 13. Red = Tomato in non-recombined cells. Scale bar = 100 μ m. **e** GFP percentage on day 13 using all 4 medium factors (RA, PMA, BMP4, WNT3A), or one or two factors removed at a time. Dox from day 5 on. *N* = 9. **f** Day 13 GFP percentage in RA&PMA medium, with either the XAV or recombinant mouse WNT3A. Dox from day 5 on. *N* = 3. **g** GFP percentage on day 13 in RA&PMA medium, in RA or PMA only medium, or upon addition of Cyclopamine. Dox from day 5 on. *N* = 4 for “RA&PMA” and “RA”, *N* = 3 for “PMA” and “RA&Cyclo”. **h** GFP percentage over time from day 5 to 13 of differentiation. Dox from day 5 on. *N* = 4 for day 8-12, *N* = 3 for day 13. **i** GFP percentage on day 13 in RA&PMA medium after sorting for KDR \pm on day 5 of differentiation. *N* = 3. Created with BioRender.com. **j** Representative image showing reporter activation on day 13 of lung epithelial differentiation and GFP percentage in the mesenchymal versus epithelial (i.e., co-development, cLM) differentiation protocol. Dox from day 5 on in the mesenchymal differentiation protocol, and from day 6 (anterior foregut endoderm stage) on in the epithelial (co-development) protocol. Scale bar = 100 μ m. *N* = 4 for “Mesenchymal”, *N* = 5 for “Co-development”. All bars show mean \pm sd. *p* values were determined by unpaired, two-tailed Student’s *t* test. Significant (*p*

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