Supplementary Information: Nature Protocols

Directed differentiation of human pluripotent stem cells into diverse organ-specific mesenchyme of the digestive and respiratory systems.

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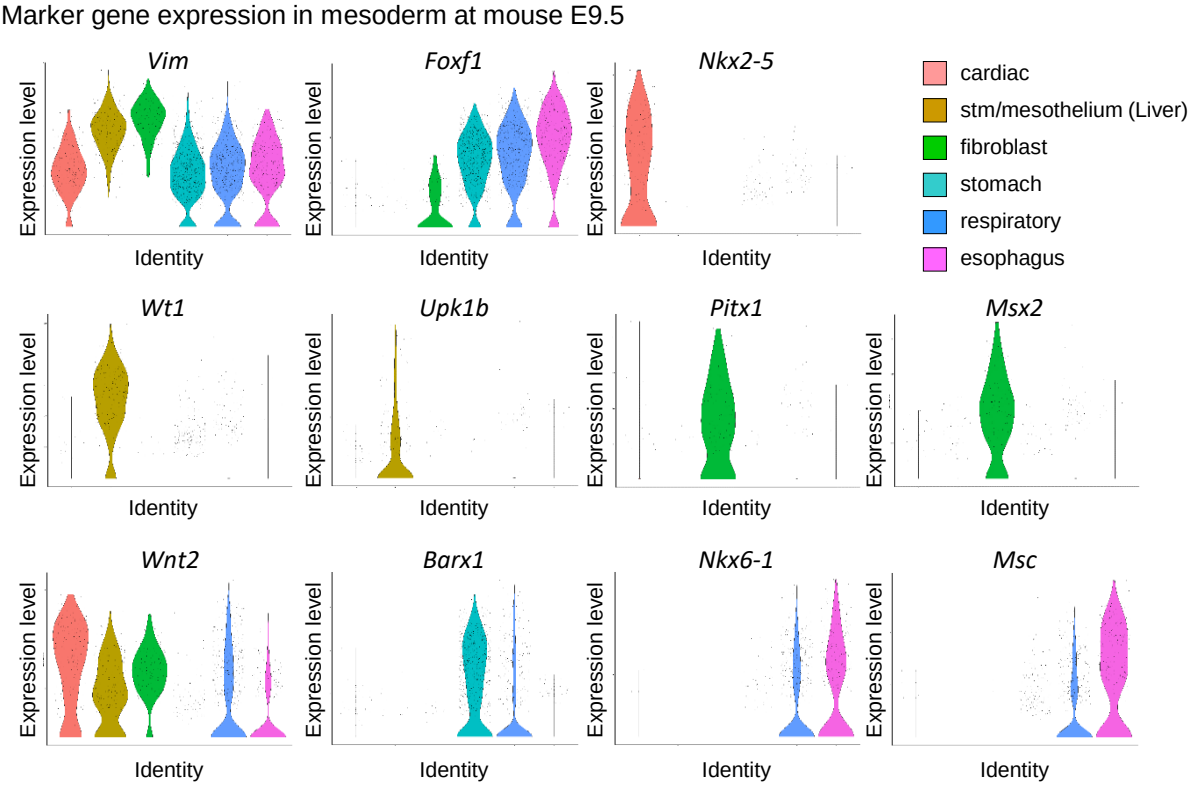
Supplementary Figure 1

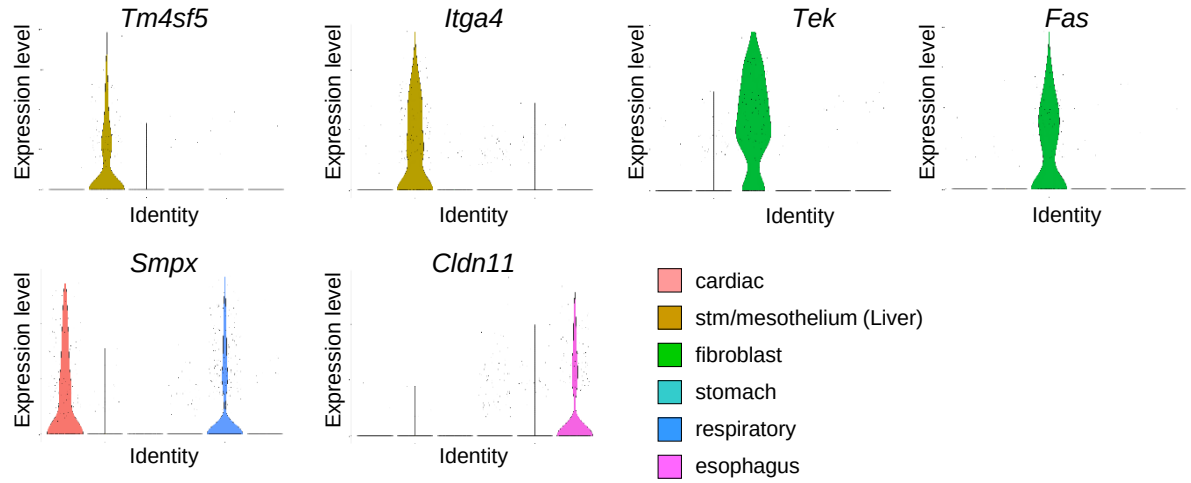
a



b

Organ-specific mesoderm	Marker genes
Liver STM/Mesothelium	*Wt1, Upk1b, Gata4, Tbx18*
Liver fibroblasts	*Pitx1, Krt19, Msx1, Msx2*
Gastric mesoderm	*Barx1, Nkx3-2, Foxf1*
Respiratory mesoderm	*Nkx6-1, Tbx5, Wnt2, Foxf1*
Esophageal mesoderm	*Nkx6-1, Msc, Wnt4, Foxf1*

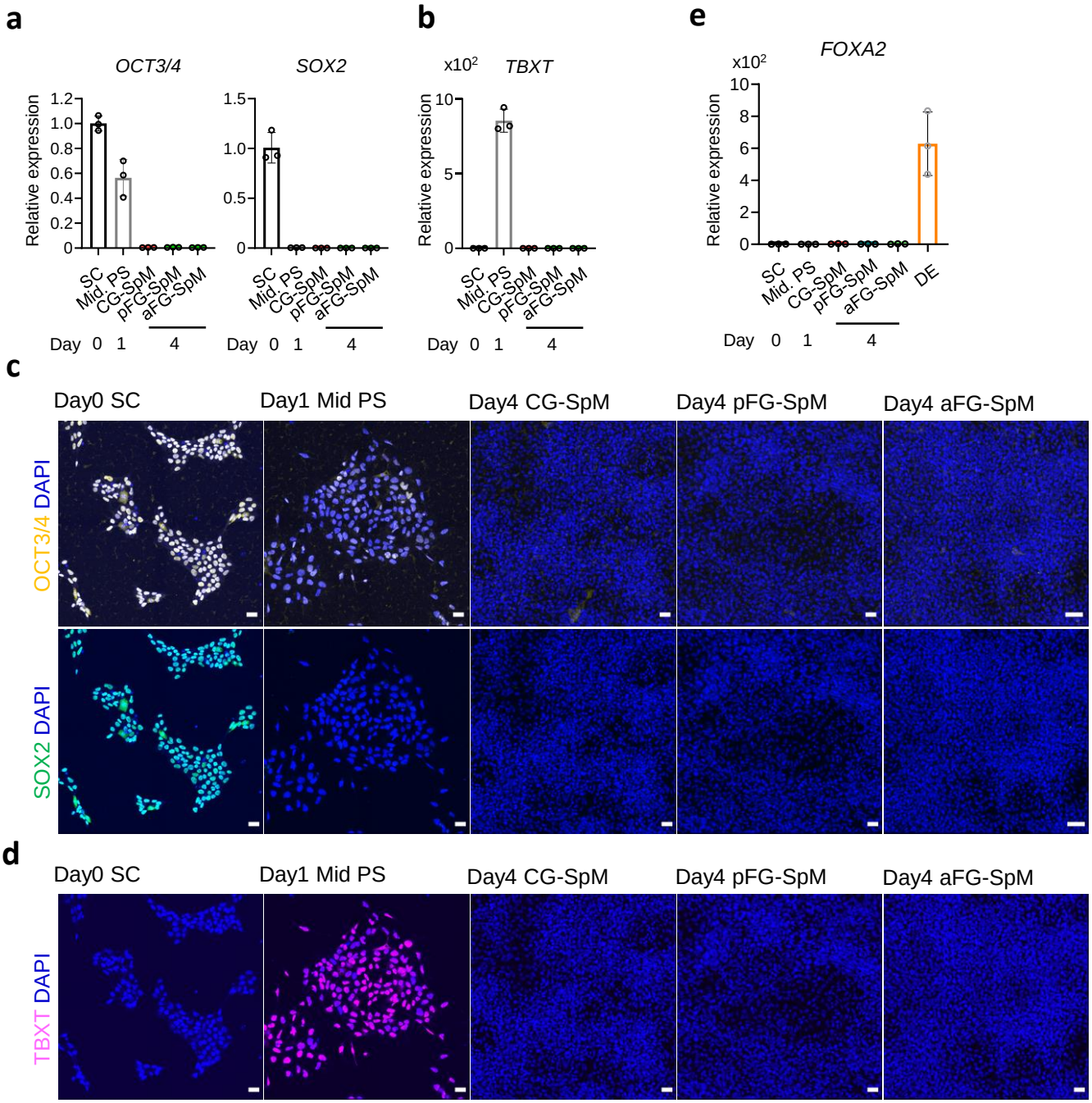
c



Supplementary Fig. 1

a, The violin plots display the sc-RNA-seq expression of organ-specific mesenchyme markers in different mouse embryonic mesoderm cell populations at E9.5. **b,** Summary of combinatorial marker gene expression from the mouse foregut that were used to define the different hPSC-derived cell types. **c,** The violin plots display the expression of organ-specific mesenchymal surface markers in mouse embryonic mesoderm at E9.5. The plasma membrane genes (1,917 genes) were extracted from the human protein atlas (https://www.proteinatlas.org/). Among these genes, 390 genes were overlapped with differentially expressed genes in mesoderm at E9.5. Representative organ-specific mesoderm markers are shown.

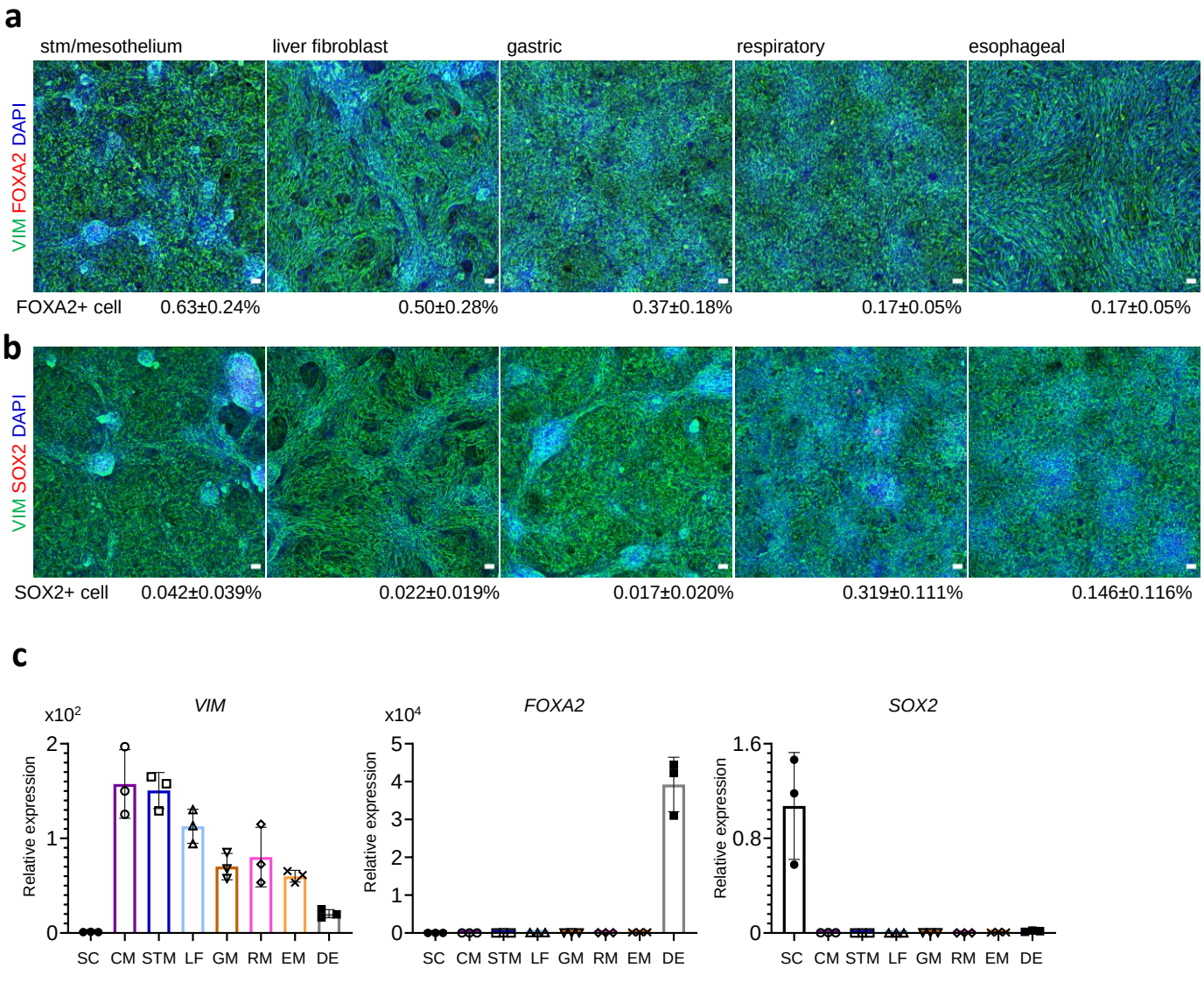
Supplementary Figure 2



Supplementary Fig. 2

a, Relative mRNA expression of pluripotent marker genes, *OCT3/4* and *SOX2* by quantitative RT-PCR from day0 to day4. **b,** Relative mRNA expression of *TBXT* as an early mesoderm marker by quantitative RT-PCR from day0 to day4. **c,** Immunostaining for OCT3/4 (*yellow*) and SOX2 (*green*). **d,** Immunostaining for TBXT (*red*). The images in c and d are maximum intensity projection of confocal stacks. **e,** Relative mRNA expression of *FOXA2* as an endoderm marker by quantitative RT-PCR from day0 to day4. Each bar indicates the average from the 3 independent wells with standard deviation .Scale bar; 50μm

Supplementary Figure 3



Supplementary Fig. 3

a, Immunostaining for VIM (*green*), FOXA2 (*red*), and DAPI (*blue*) at day7 mesenchyme. **b,** Immunostaining for VIM (*green*), SOX2 (*red*), and DAPI (*blue*) at day7 mesenchyme. The images in a and b are maximum intensity projection of confocal stacks. **c,** Relative mRNA expression of pan-mesoderm marker, *VIM*, endoderm marker, *FOXA2*, and ectoderm marker, *SOX2*, by quantitative RT-PCR. Each bar indicates the average from the 3 independent wells with standard deviation. Scale bar; 50μm (**a, b**).