

Self-organized emergence of hyaline cartilage in hiPSC-derived multi-tissue organoids

Author Information

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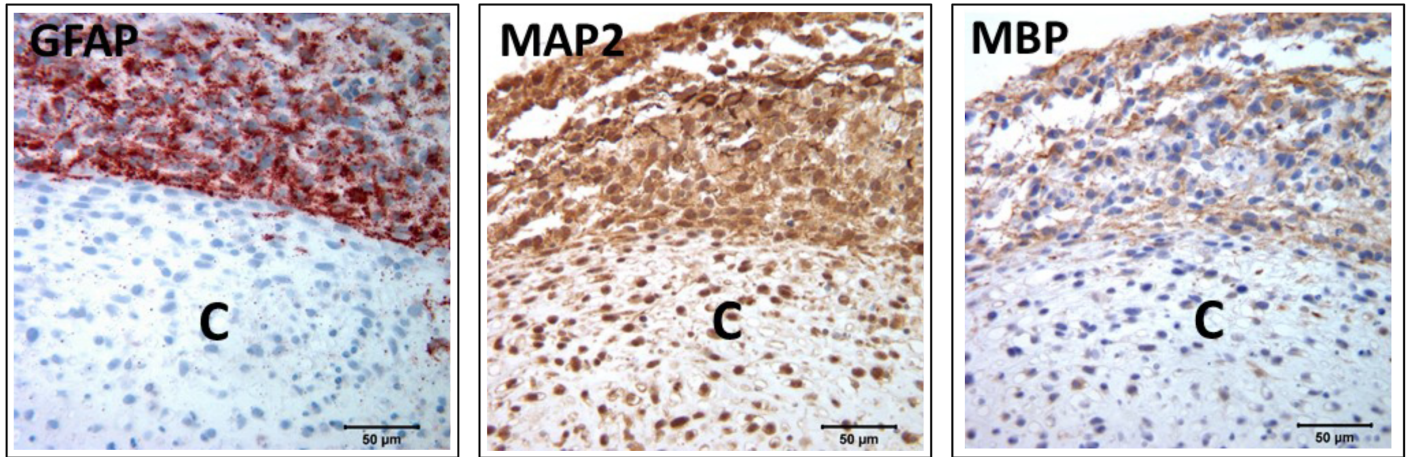
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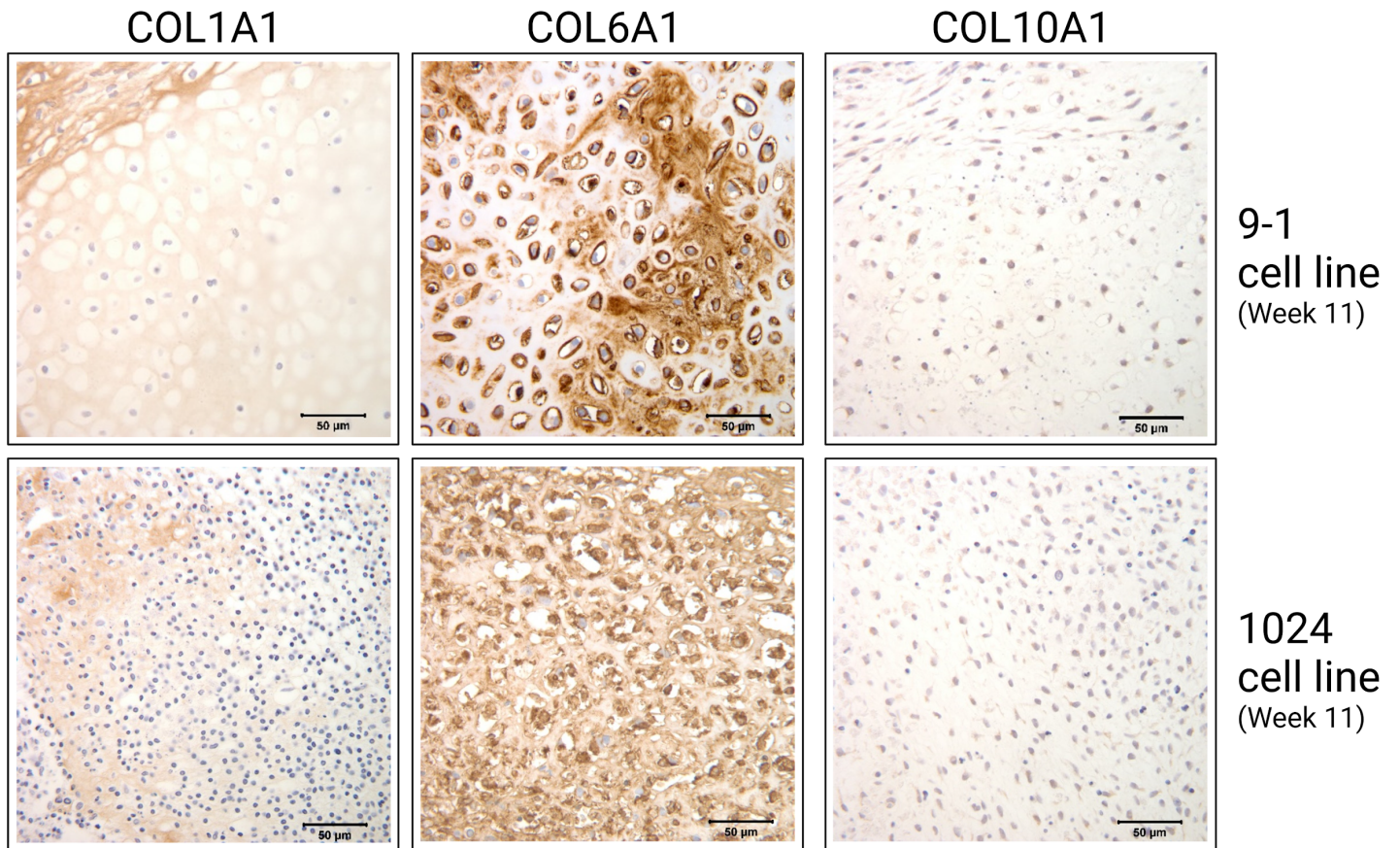
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Fig. S1



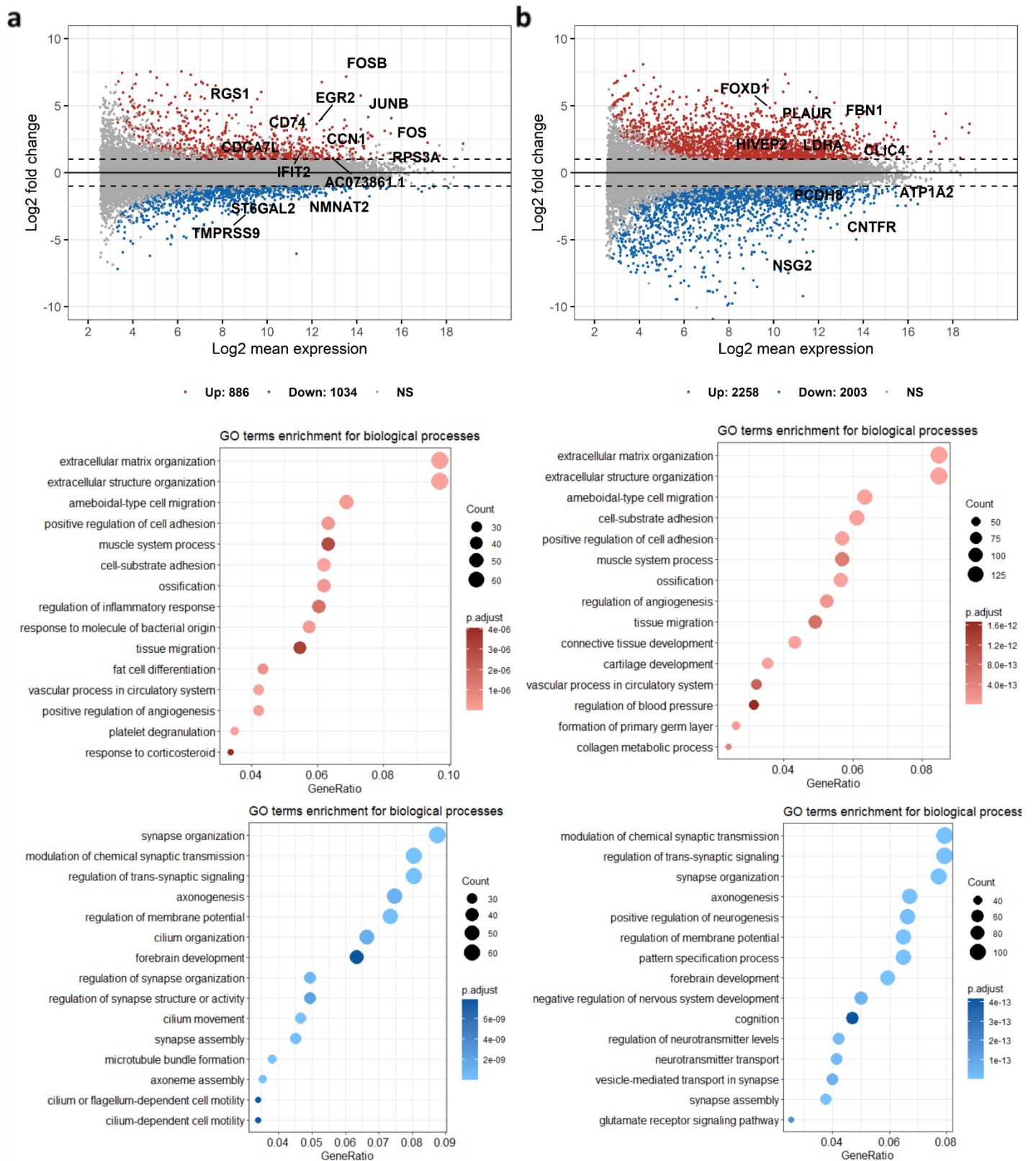
Immunohistochemical staining for brain cell lineages in 1024 MTOs at 8 weeks demonstrates strong labeling for glial fibrillary acidic protein (GFAP, astrocytes), microtubule-associated protein 2 (MAP2, neurons), and myelin basic protein (MBP, oligodendrocytes) in surface regions of the MTO adjacent to the chondrogenic region (C).

Fig. S2



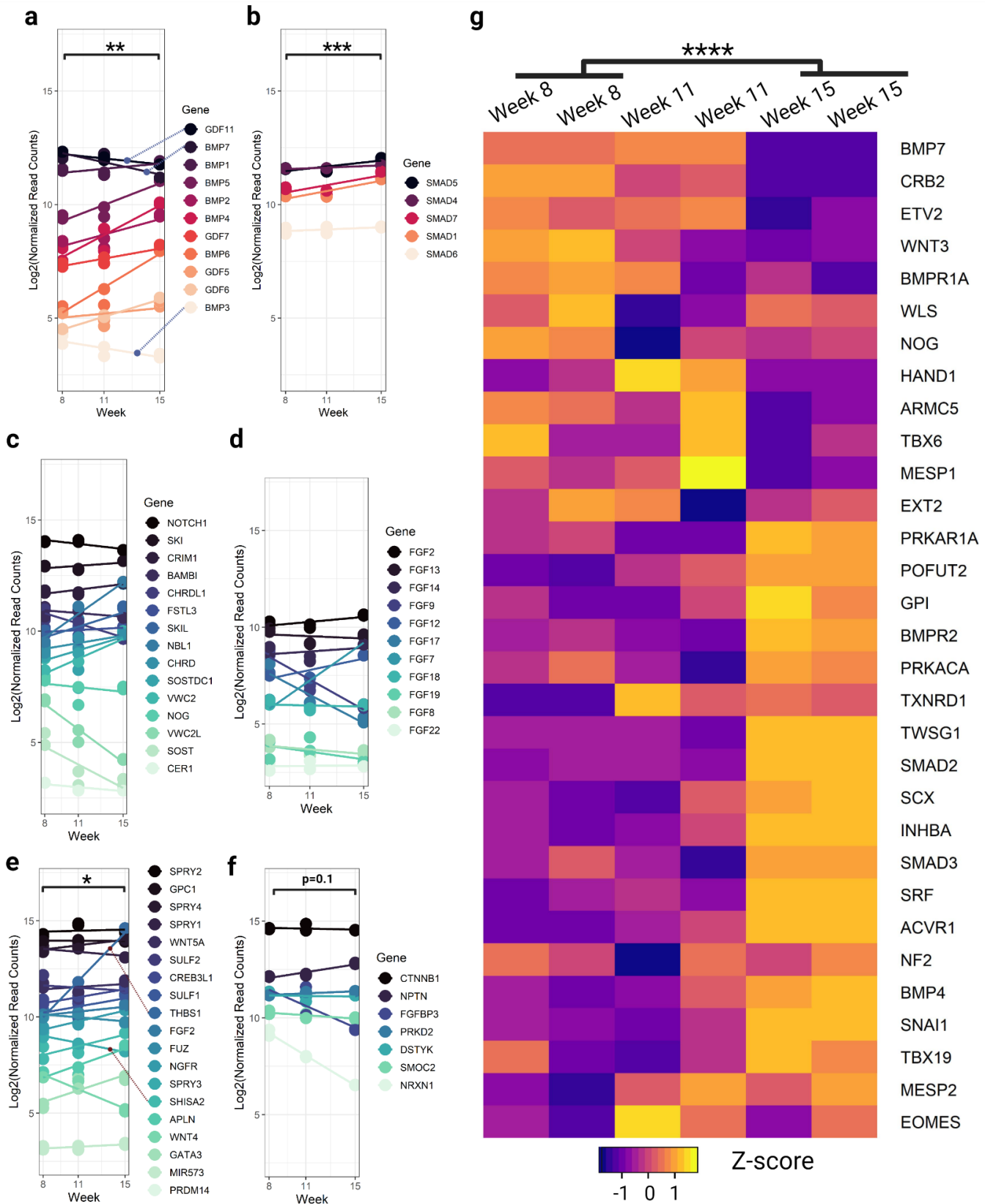
11-week MTOs from 9-1¹ and 1024 cell lines showing some peripheral weak to moderate staining for type I collagen (COL1A1), pericellular and some patchy matrix staining for type VI collagen (COL6A1), and negative staining for type X collagen (COL10A1).

Fig. S3.



Differential gene expression and gene ontology enrichment analyses (week 11 vs week 8 and week 15 vs week 11). **a.** Differential gene expression, gene ontology enrichment for upregulated and genes, gene ontology enrichment for downregulated genes between week 11 and week 8. **b.** Differential gene expression, gene ontology enrichment for upregulated and genes, gene ontology enrichment for downregulated genes between week 15 and week 11.

Fig S4.



Gene expression plots of gene lists of interests. **a.** Bone morphogenic proteins (BMP). **b.** SMAD proteins. **c.** BMP antagonists. **d.** Neural FGFs. **e.** Negative regulation of FGFR pathway (GO:0040037). **f.** Positive regulation of FGFR pathway (GO:0045743). **g.** Mesoderm formation (GO:0001707).

Table S9. Antibodies used for histology and immunohistochemistry.

| Antibody | Type of Antibody | Manufacturer/Cat. No. | Dilution | Antigen Retrieval |
|---|-------------------------|--|-----------------|--------------------------|
| Aggrecan (ACAN) | Goat polyclonal | R&D Systems, Cat# AF1220 | 1:200 | None |
| Type I Collagen (COL1A1) | Mouse monoclonal | Sigma c2456 | 1:100 | Proteinase K |
| Type II Collagen (COL2A1) | Mouse monoclonal | Developmental Studies Hybridoma Bank CIIC1 | 1:10 | Pepsin |
| Type VI Collagen (COL6A1) | Rabbit polyclonal | abcam ab6588 | 1:400 | Citrate |
| Type X Collagen (COL10A1) | Rabbit polyclonal | abcam ab58632 | 1:400 | Typsin |
| Glial Fibrillary Acidic Protein (GFAP) | Rabbit polyclonal | Dako Cat.#Z0334 | 1:1000 | None |
| Microtubule Associated Protein 2 (MAP2) | Chicken polyclonal | abcam ab5392 | 1:4000 | Citrate |
| Myelin Basic Protein (MBP) | Rabbit polyclonal | abcam ab40390 | 1:3000 | Citrate |

Reference:

1. Lindborg, B. A. *et al.* Rapid Induction of Cerebral Organoids From Human Induced Pluripotent Stem Cells Using a Chemically Defined Hydrogel and Defined Cell Culture Medium. 694–702 (2016).