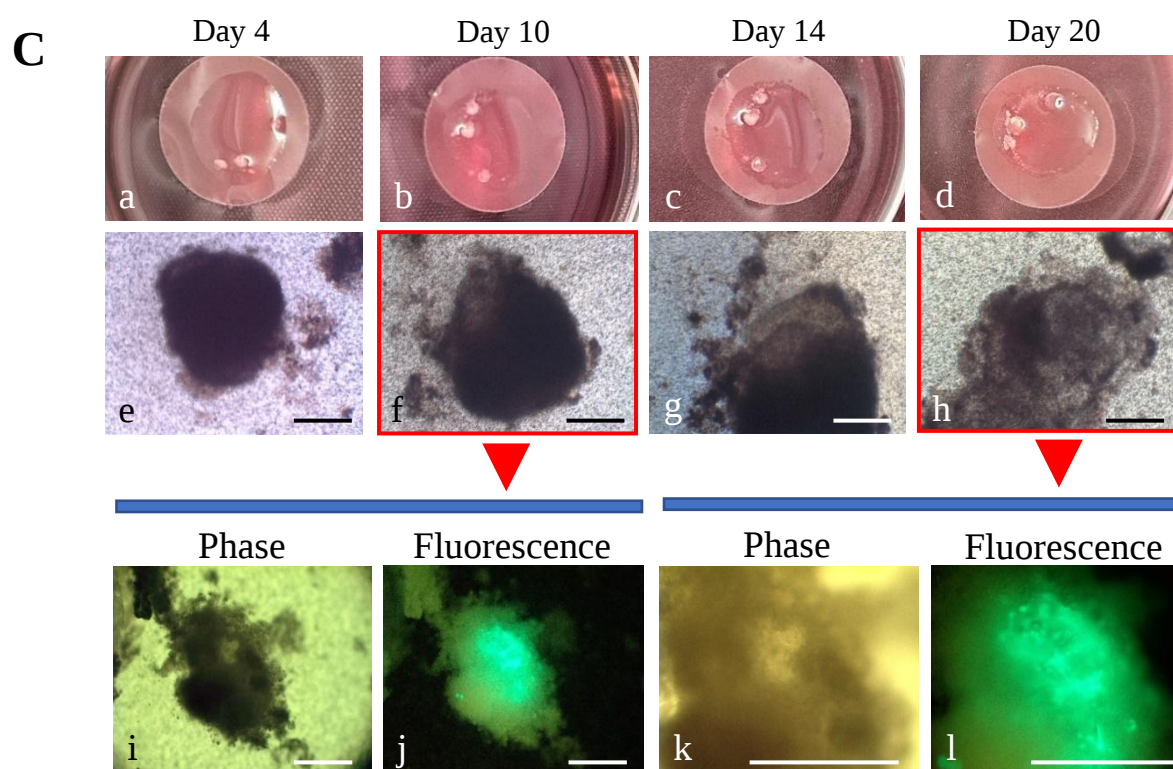
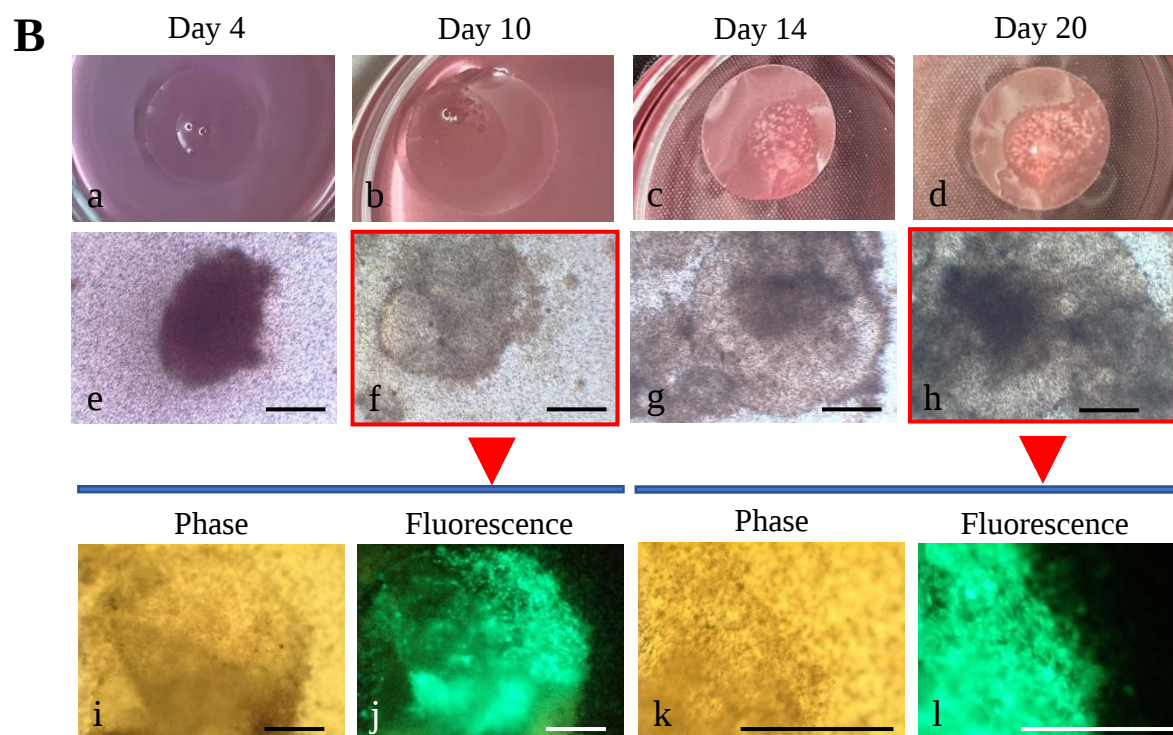
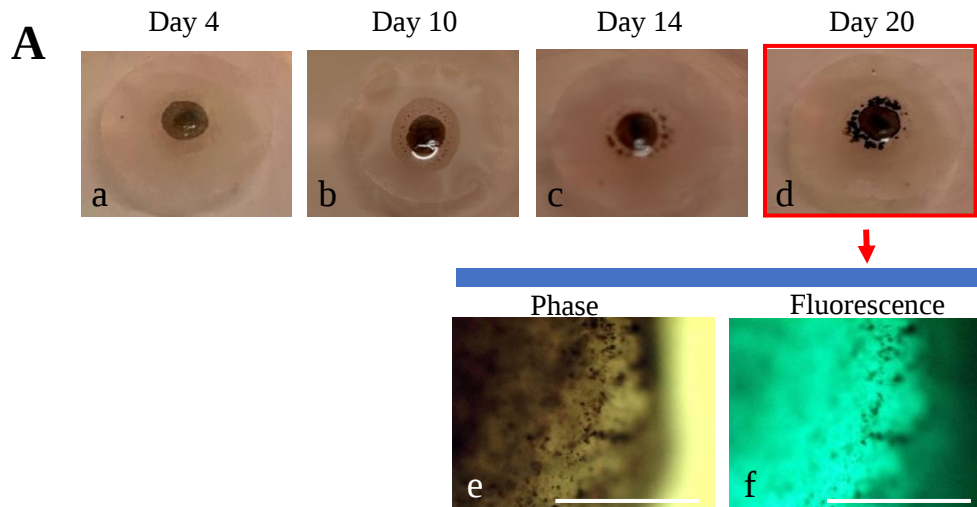




Supplementary Figure S1.

Time-course observation of cell growth and aggregation within the Matrigel-based 3D culture system.

(A) EGFP-B16 cells embedded in Matrigel. Cells were initially throughout the scaffold but progressively accumulated toward the central region by day 4. Continued proliferation resulted in expansion of melanin-containing cell aggregates, and by day 20, pigmented cells occupied most of the scaffold. Persistent EGFP-derived green fluorescence was observed throughout the culture period, indicating the presence of viable EGFP-B16 cells.

(B) Naïve EGFP-iPSCs embedded in Matrigel. During culture, the cells proliferated and gradually formed central cell aggregates within the scaffold. EGFP-derived green fluorescence remained detectable throughout the culture period, indicating the persistence of viable EGFP-iPSCs. (C) Naïve EGFP-iPSC-derived embryoid bodies (EBs) embedded in Matrigel. During culture, the EBs increased in size and became concentrated within the scaffold. By day 20, the EBs had formed a single cohesive multicellular structure with a whitish appearance. EGFP-derived green fluorescence remained detectable throughout the culture period, indicating the persistence of viable EB-derived cells.

EGFP, enhanced green fluorescent protein; iPSCs, induced pluripotent stem cells; EBs, embryoid bodies. Scale bar = 50 μ m.