

RESEARCH ARTICLE

A visible light-crosslinkable decellularized kidney matrix bioink for 3D bioprinting of organoids and drug testing

Supplementary file

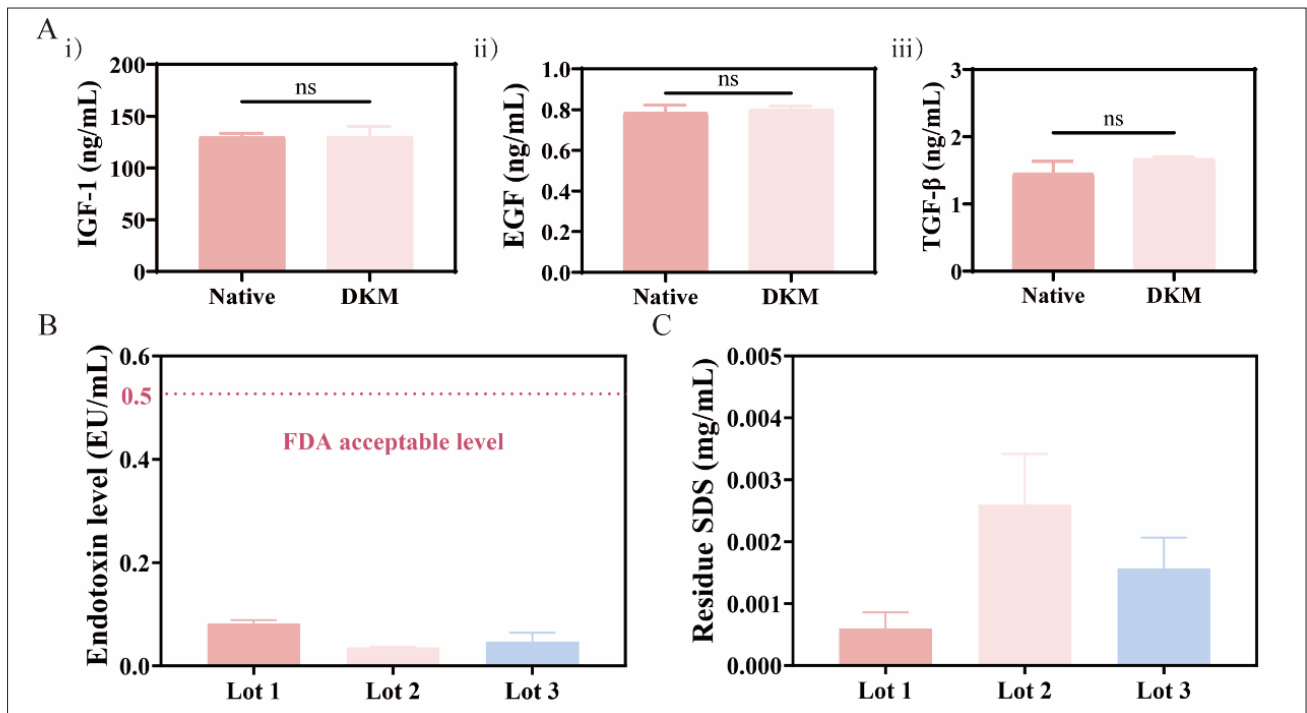


Figure S1. Characterization of growth factor content and process residuals in DKM bioink(A) Concentrations of (i) IGF-1, (ii) EGF, and (iii) TGF-β in the DKM bioink. Analysis of (B) endotoxin levels and (C) residual SDS in different lots of DKM bioink. Abbreviations: DKM: Decellularized kidney extracellular matrix; EGF: Epidermal growth factor; IGF-1: Insulin-like growth factor 1; Ru/SPS: Tris(2,2'-bipyridyl) ruthenium (II) chloride hexahydrate/sodium persulfate; SDS: Sodium dodecyl sulfate; TGF-β: Transforming growth factor-beta.

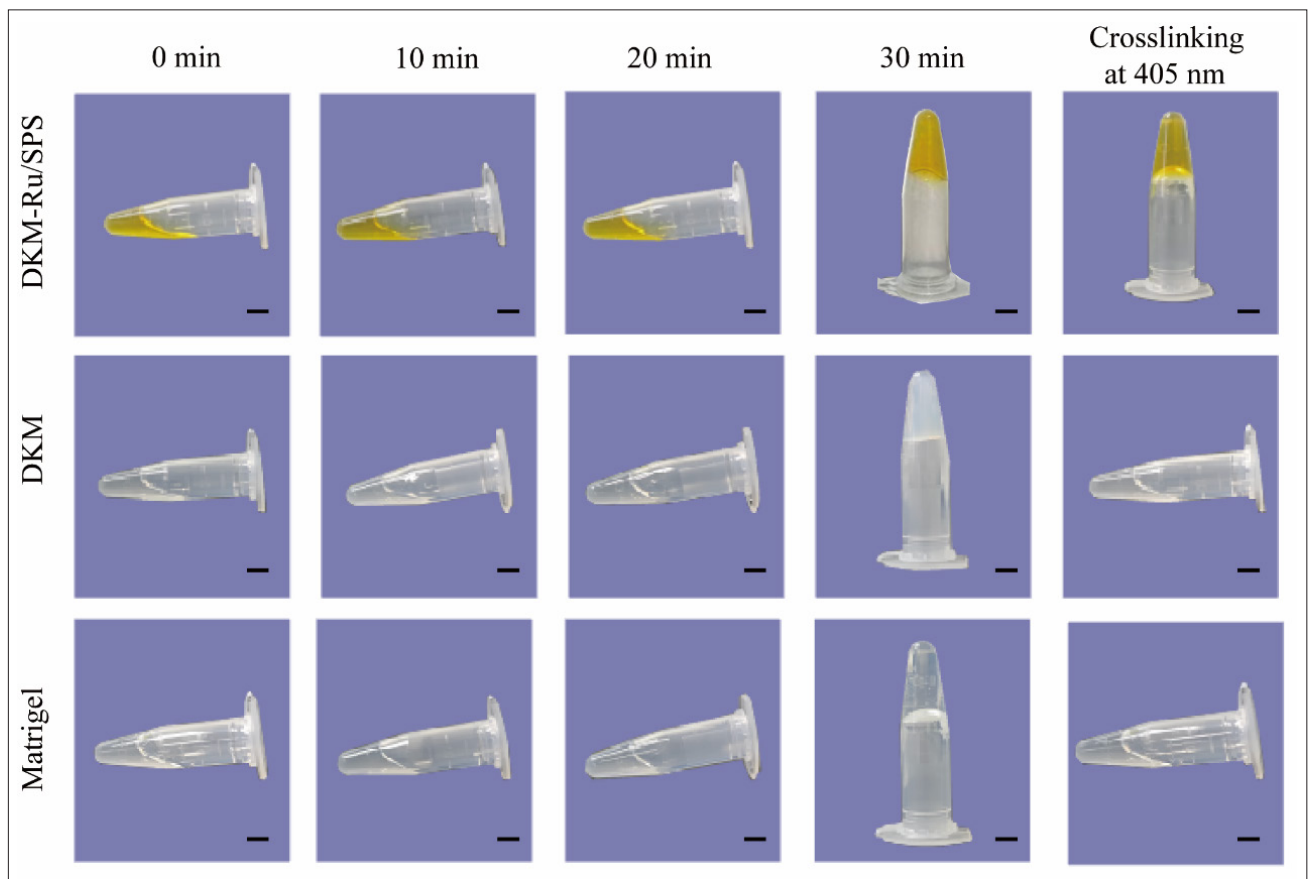


Figure S2. Physical and chemical characterization of DKM–Ru/SPS bioinks. Temperature-responsive characteristics of DKM–Ru/SPS bioink, DKM, Matrigel, and composite bioinks at 37°C, along with the photoinitiated crosslinking capabilities of these materials. Scale bar = 10 mm. Abbreviations: DKM: Decellularized kidney extracellular matrix; Ru/SPS: Tris(2,2'-bipyridyl) ruthenium (II) chloride hexahydrate/sodium persulfate.

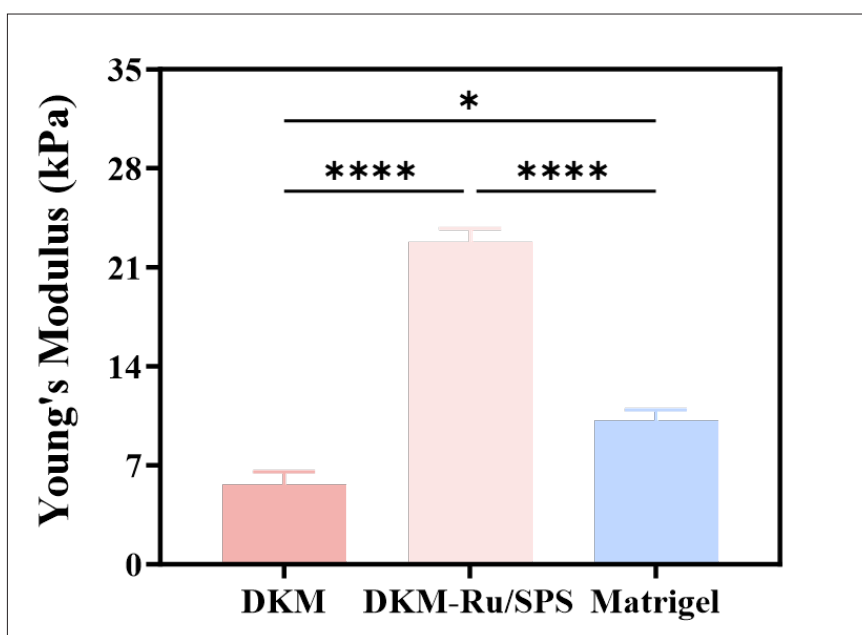


Figure S3. Young's modulus of DKM, DKM-Ru/SPS hydrogels, and Matrigel ($n = 3$; mean \pm SEM; $*p < 0.05$, $****p < 0.0001$). Abbreviations: DKM: Decellularized kidney extracellular matrix; Ru/SPS: Tris(2,2'-bipyridyl) ruthenium (II) chloride hexahydrate/sodium persulfate.

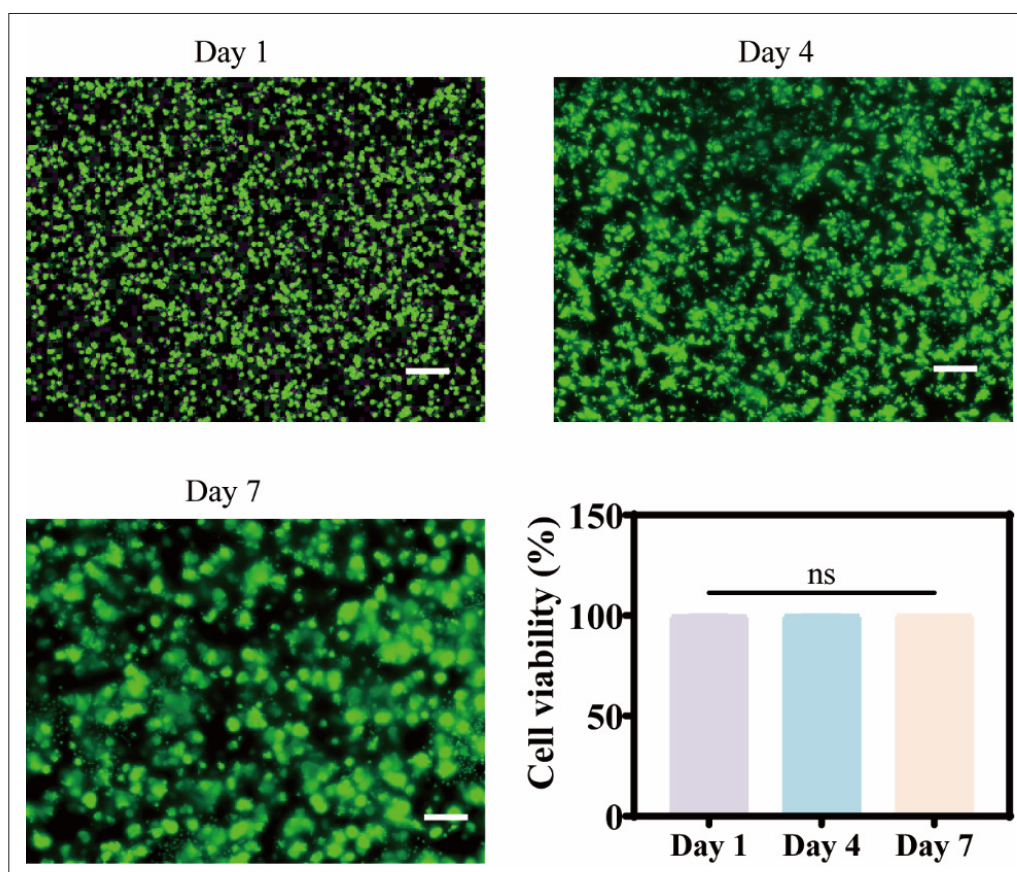


Figure S4. Representative fluorescence images and quantitative analysis of live/dead cell viability from day 1 to day 7. Scale bar = 300 μ m; magnification: 4 \times . Notes: $n = 3$; mean \pm standard error of the mean; ns = not statistically significant.

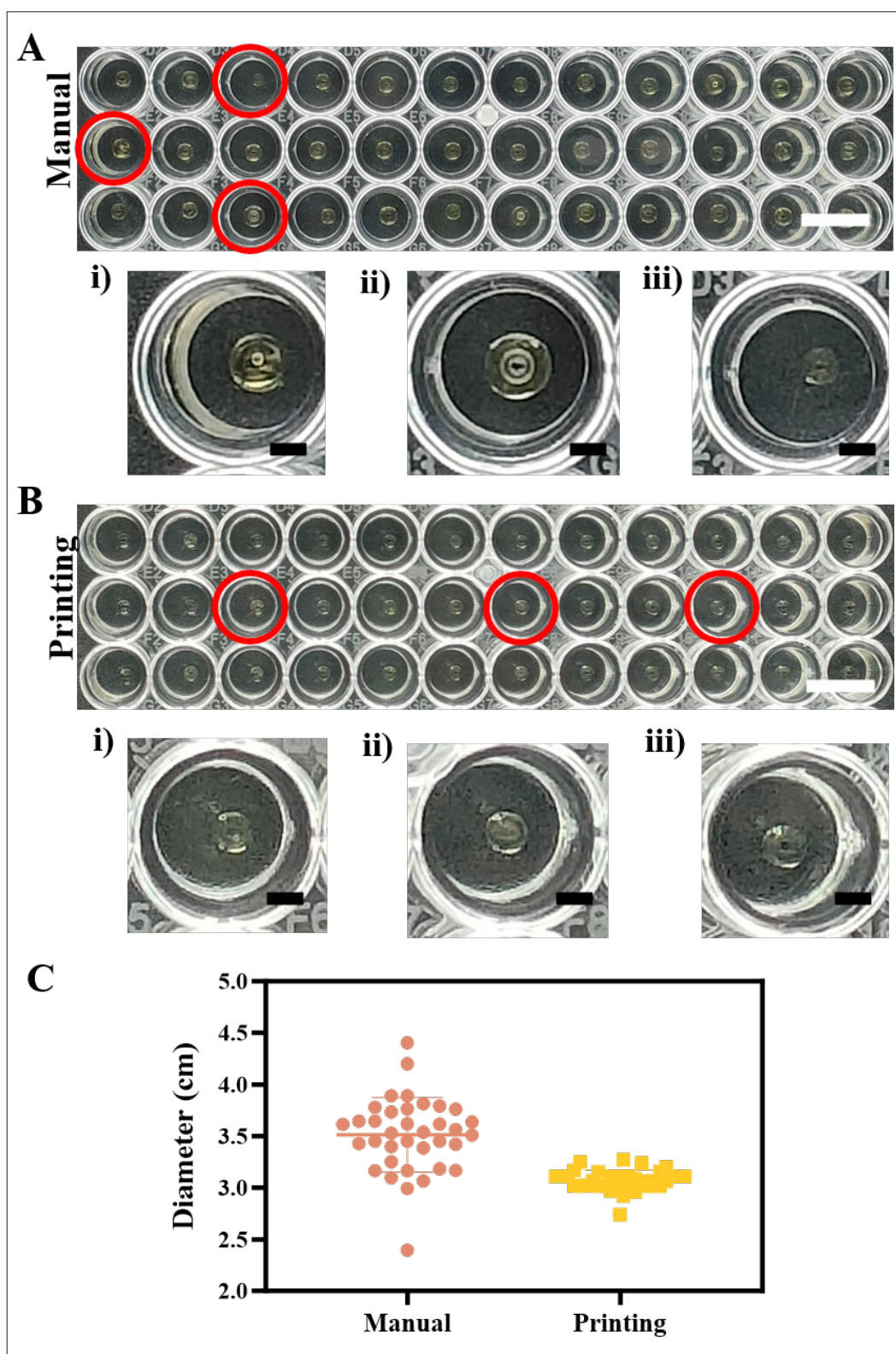


Figure S5. Uniformity analysis of hydrogels between manual and bioprinting methods. Representative magnifying camera enlarged images (i–iii) of 15 mg/mL DKM–Ru/SPS hydrogels formed by (A) manual dispensing or (B) bioprinting at 3 kpa. Scale bars: 5 mm, 1 mm; (C) Diameter analysis of light-crosslinked DKM hydrogels in panels A and B. Abbreviations: DKM: Decellularized kidney extracellular matrix; Ru/SPS: Tris(2,2'-bipyridyl) ruthenium (II) chloride hexahydrate/sodium persulfate.

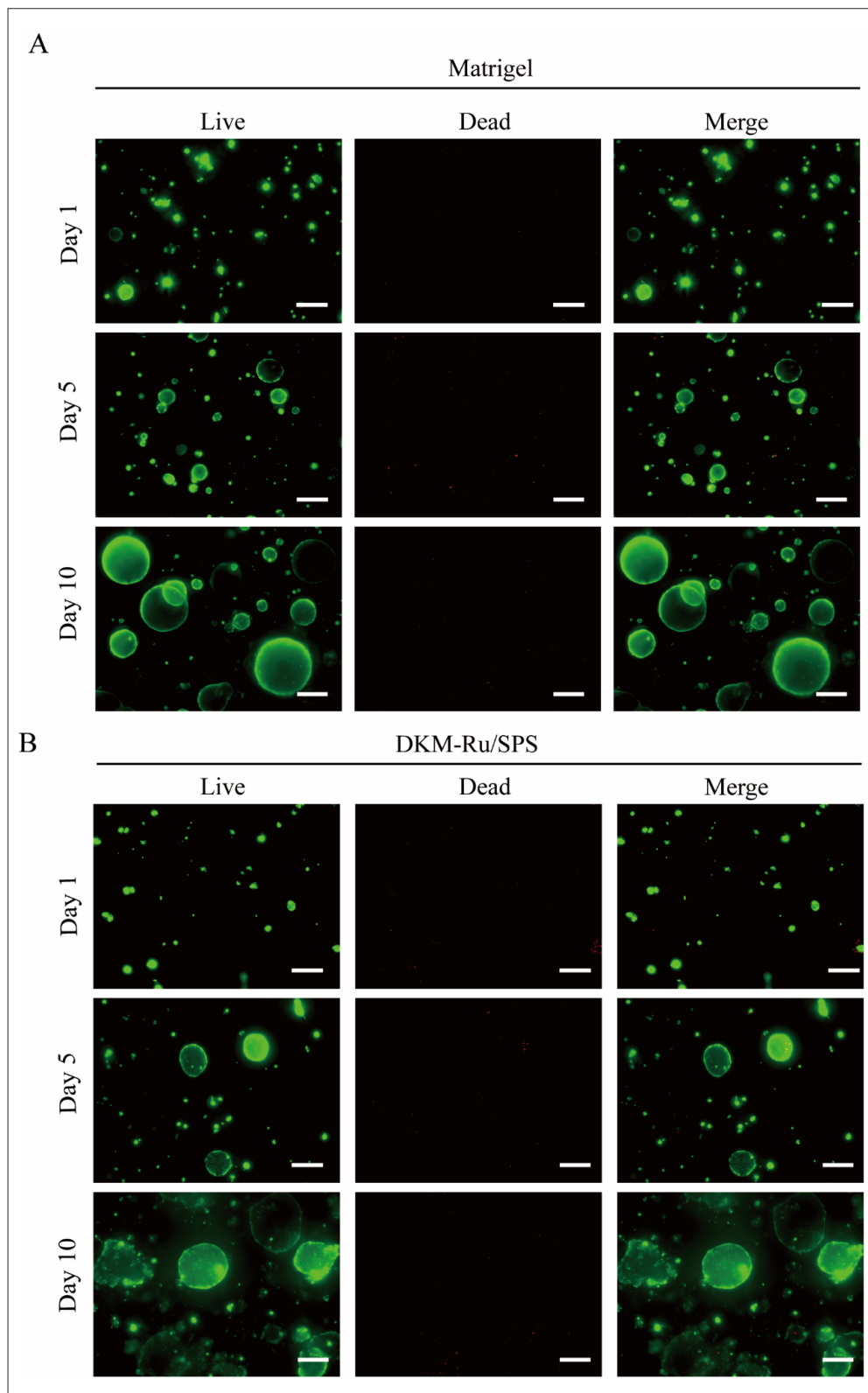


Figure S6. Representative live/dead fluorescence images of organoids in DKM-Ru/SPS bioinks or Matrigel after 10 days of culture. Scale bar = 300 μ m; magnification: 4 \times . Abbreviations: DKM: Decellularized kidney extracellular matrix; Ru/SPS: Tris(2,2'-bipyridyl) ruthenium (II) chloride hexahydrate/sodium persulfate.

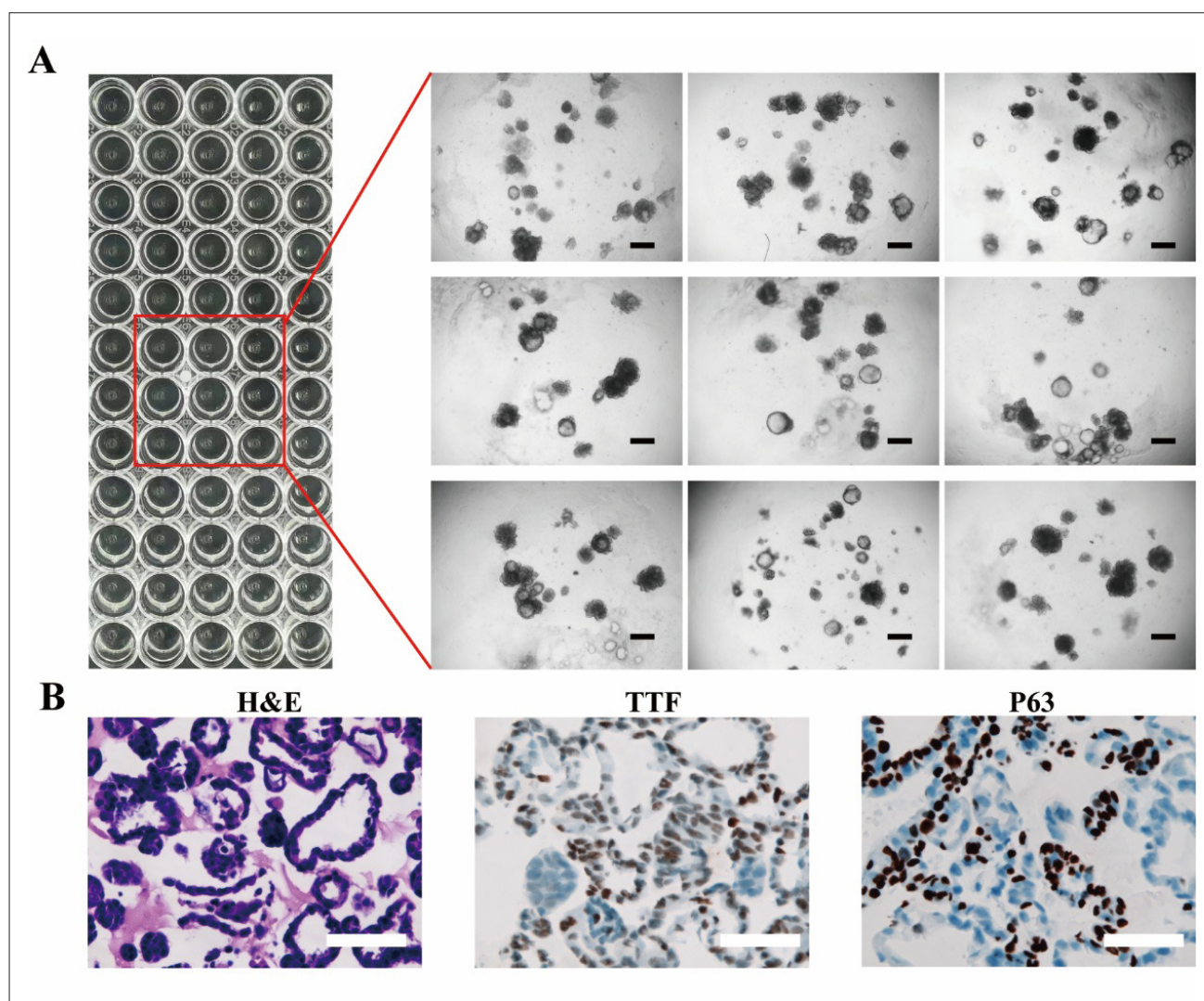


Figure S7. Culture and characterization of lung organoids in DKM–Ru/SPS bioink under bioprinting (A) Lung organoids mixed with DKM–Ru/SPS bioink and cultured in a 96-well plate (left column) with corresponding magnified views (right column). Scale bar: 300 μm ; magnification: 4 \times . (B) Representative images of hematoxylin and eosin (H&E) staining and immunohistochemical staining for thyroid transcription factor 1 (TTF) and P63 staining in lung organoids. Scale bar: 50 μm ; magnification: 40 \times . Abbreviations: DKM: Decellularized kidney extracellular matrix; Ru/SPS: Tris(2,2'-bipyridyl) ruthenium (II) chloride hexahydrate/sodium persulfate.

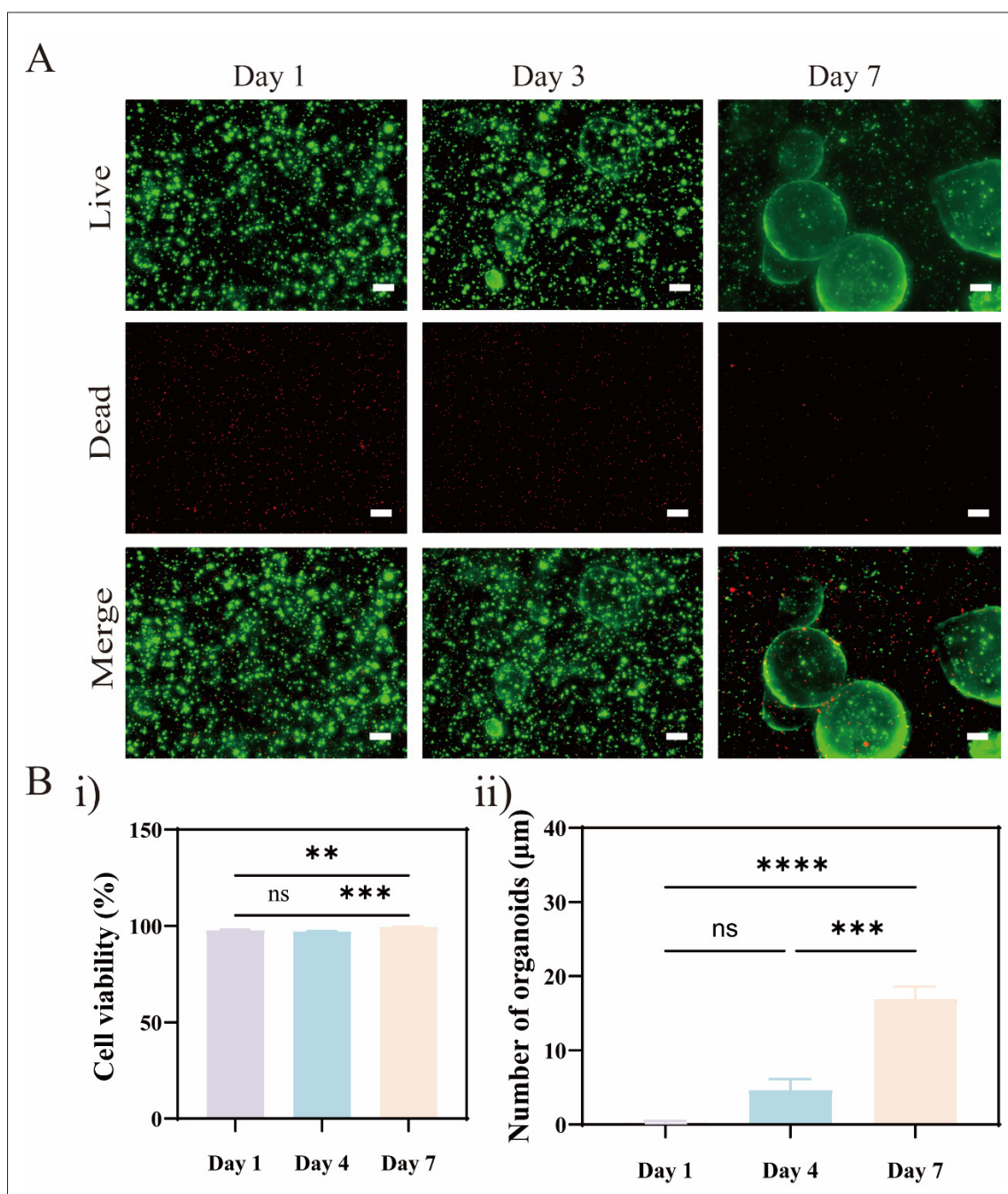


Figure S8. Live/dead staining and quantitative analysis of liver organoids. (A) Representative fluorescence images of live/dead of liver organoids throughout the culture period. Scale bar = 300 µm; magnification: 4×. (B) (i) Live/dead analysis and (ii) number of liver organoids. Notes: $n = 3$; mean \pm standard error of the mean; one way analysis of variance; **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$; ns, not statistically significant.

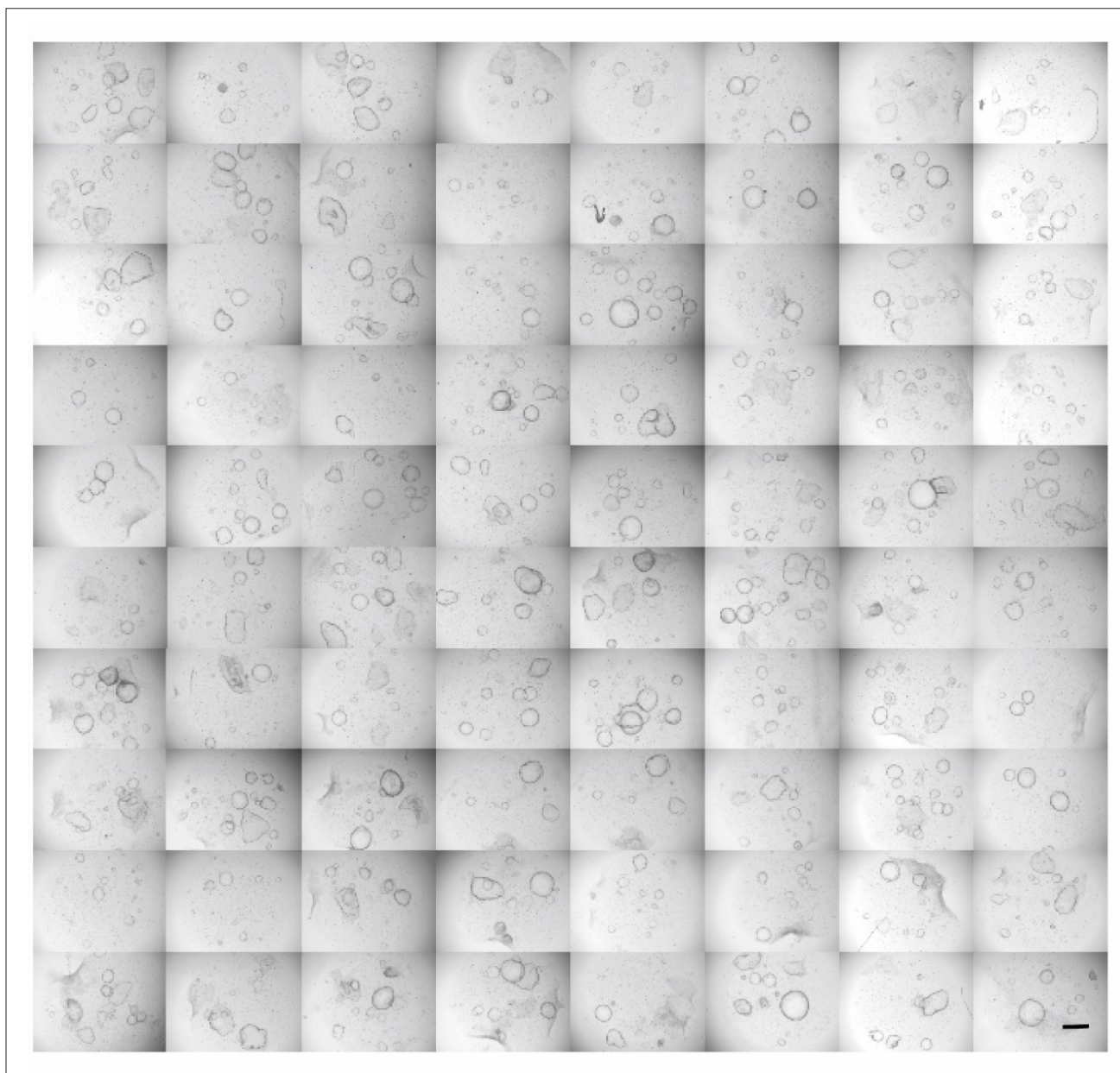


Figure S9. Representative bright-field images of organoids in DKM–Ru/SPS bioinks after seven days of culture. Scale bar = 300 μm ; magnification: 4 \times . Abbreviations: DKM: Decellularized kidney extracellular matrix; Ru/SPS: Tris(2,2'-bipyridyl) ruthenium (II) chloride hexahydrate/sodium persulfate.