Supporting Information

Synthetic Chemoselective Rewiring of Cell Surfaces: Generation of Three-Dimensional Tissue Structures

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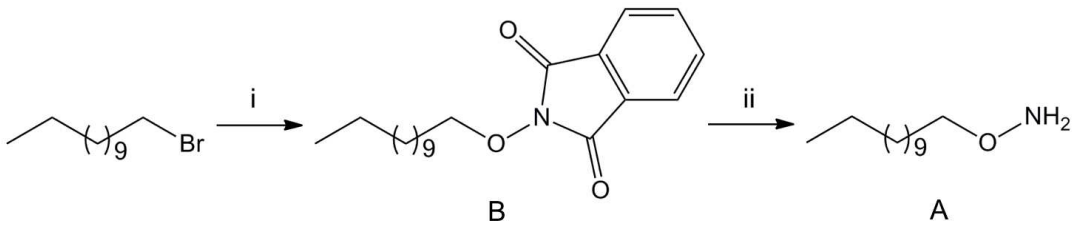
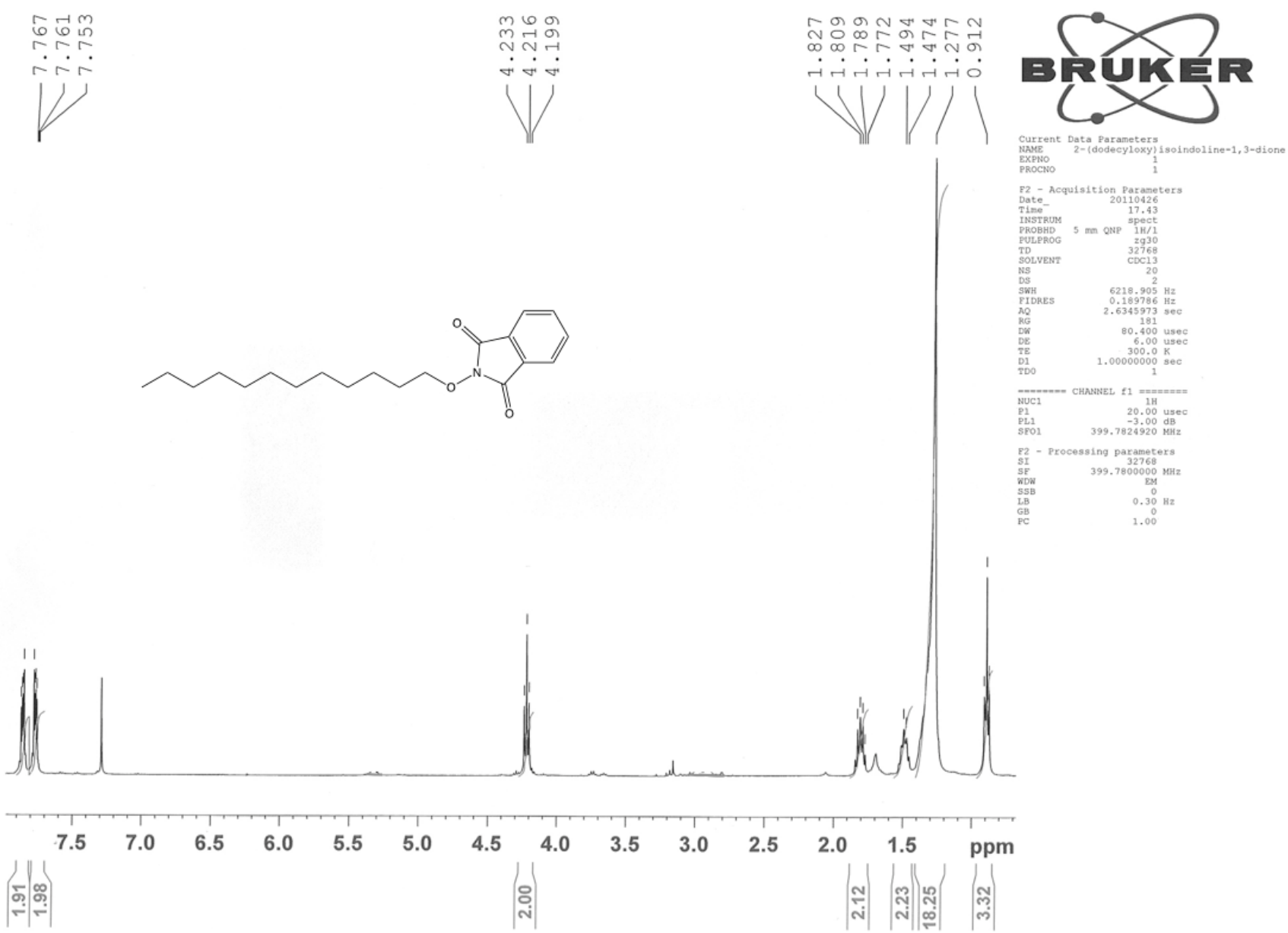
Scheme *S*1. Synthesis of *O*-dodecyloxyamine[a] (**A**)



[a] Reagents and conditions. (i) N-hydroxyphthalimide (1.5 eq), $NaHCO_3$ (1.5 eq), DMF, reflux, 80 °C, 12 h; 87 % and (ii) hydrazine (6 eq), dry DCM, N_2, 12 h; 74 %.

2-(dodecyloxy)isoindoline-1,3-dione (B). To a solution of N-hydroxyphthalimide (1.96 g, 12.04 mmol, 1.5 eq) and sodium bicarbonate (10.11 g, 12.04 mmol, 1.5 eq) in DMF (20 mL) at 80°C was added 1-bromododecane (1.93 mL, 8.02 mmol). The mixture was refluxed and stirred for 12 h. The reaction was diluted with DCM and washed with H_2O (6 x 50 mL), 1 M $NaHCO_3$ (3 x 50 mL), and H_2O (2 x 50 mL), dried over $MgSO_4$, and concentrated to afford a white solid, **B** (2.66 g, 87 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (m, 3H), 1.28 (bm, 16H), 1.47-1.49 (m, J = 9.2 Hz, 2H), 1.77-1.83 (m, J = 22.0 Hz, 2H), 4.20-4.23 (t, J = 13.6 Hz, 2H), 7.28-7.30, 7.75-7.77 (dm, J = 4.8, Hz, J = 5.6 Hz, 2H, 2H). (ESI) (*m/z*) [M + H^+]: 332.28.

O-dodecyloxyamine (A). To a solution of ***B*** (2.65 g, 8.00 mmol) in dry DCM (30 mL) under inert atmosphere (Ar) was slowly added hydrazine (1.53 mL, 48.00 mmol, 6 eq). Upon addition, a white precipitate immediately formed. The mixture was stirred for 12 h. The reaction was diluted with DCM and washed with H_2O (6 x 50 mL), dried over $MgSO_4$, and concentrated to afford a pale yellow oil, **A** (1.18 g, 74 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88-0.91 (t, J = 13.6 Hz, 3H), 1.28 (s, 18H), 1.57-1.60 (m, J = 14.0 Hz, 2H), 3.65-3.69 (t, J = 13.2 Hz, 2H). (ESI) (*m/z*) [M + H^+]: 201.22.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BRUKER
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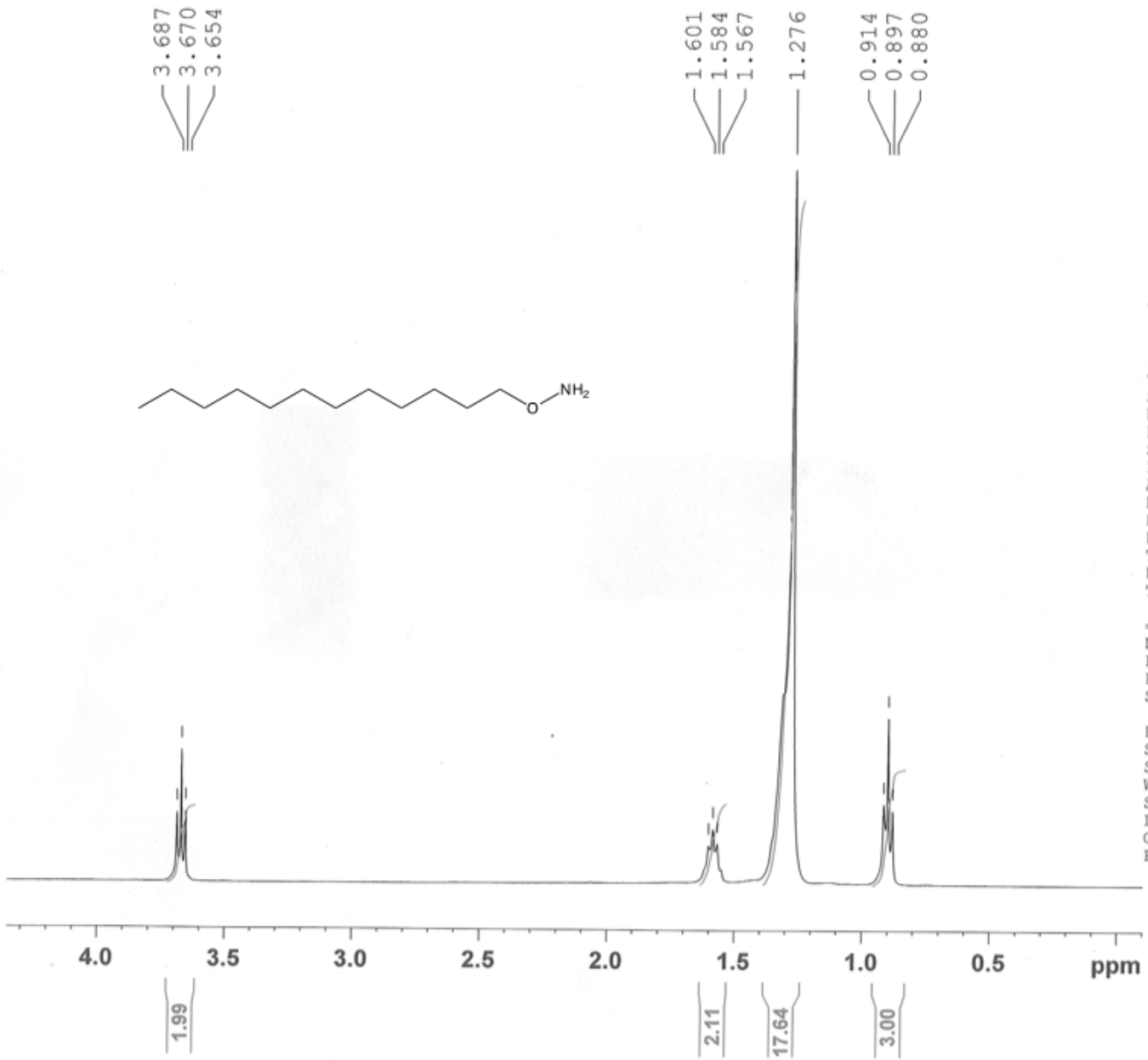

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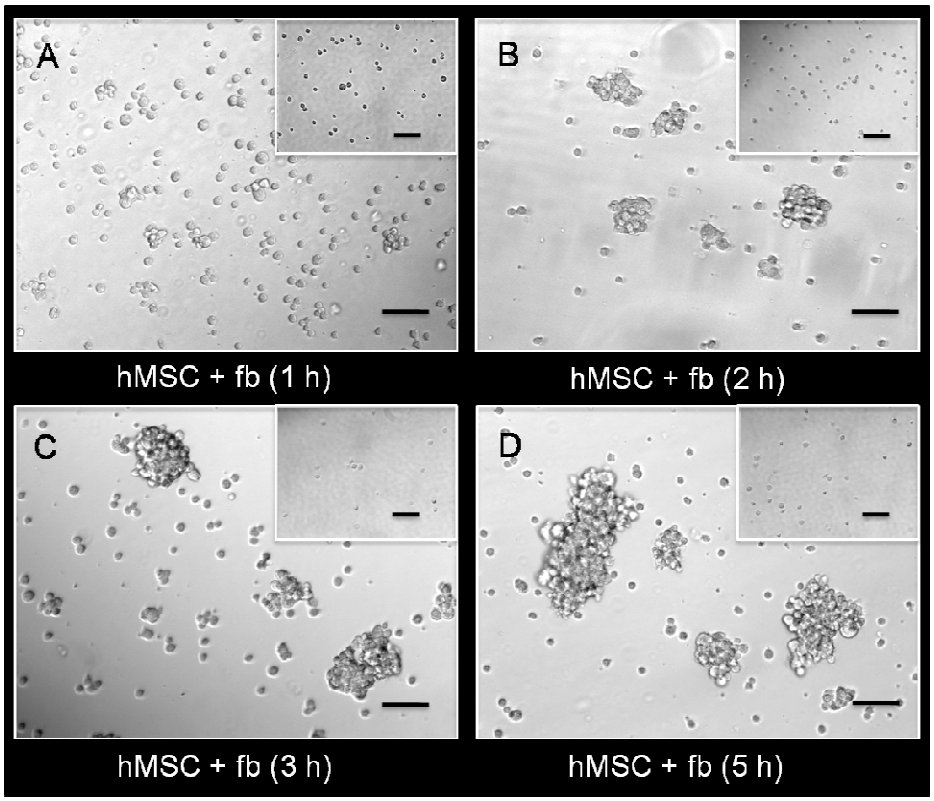

Figure *S*1. Phase contrast images representing control of spheroid size and composition. Human Mesencymal stem cells (hMSCs) were cultured with ketone-containing liposomes (16 h), resulting in fusion and display of ketone groups from the cell surface. Similarly, Swiss 3T3 albino mouse fbs were cultured with oxyamine-functionalized liposomes. The two populations were mixed together (10^4 cells/mL, 1:1), allowed to react for (A) 1, (B) 2, (C) 3, and (D) 5 h, pipeted onto glass substrates, and were imaged by phase contrast microscopy. As shown, spheroids continue to grow in size as mixing duration increases. Scale bars represent 60 µm. The insets represent control experiments where ketone-functionalized hMSCs were co-cultured with fibroblasts (not displaying oxyamine groups) for each of the corresponding time points (A) 1, (B) 2, (C) 3, and (D) 5 h, and no spheroid assembly occurs. Scale bars for all inset images represent 300 µm.

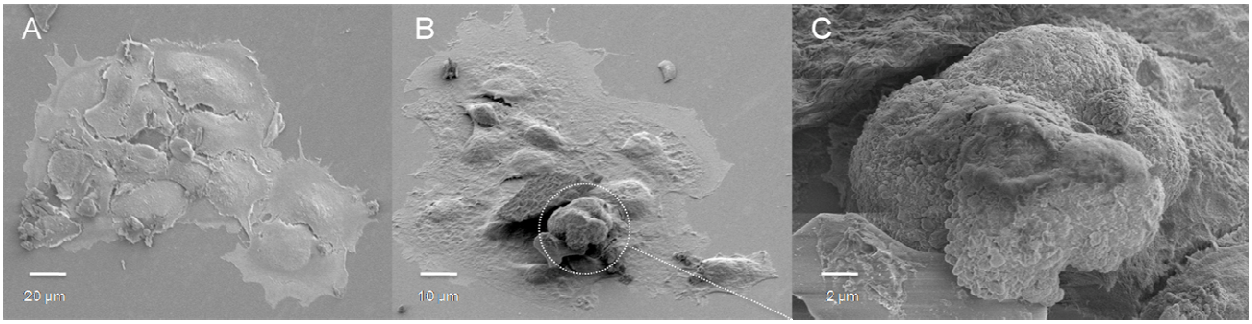

Figure *S*2. The following SEM images representing the formation of 3D spheroid assemblies in solution via chemoselective cell-surface engineering are shown. Two fibroblast populations were cultured separately with ketone- or oxyamine-containing liposomes overnight and were then mixed together for 3 h before imaging. (A and B) Low-resolution micrographs are displayed with a (C) high-resolution image of B.

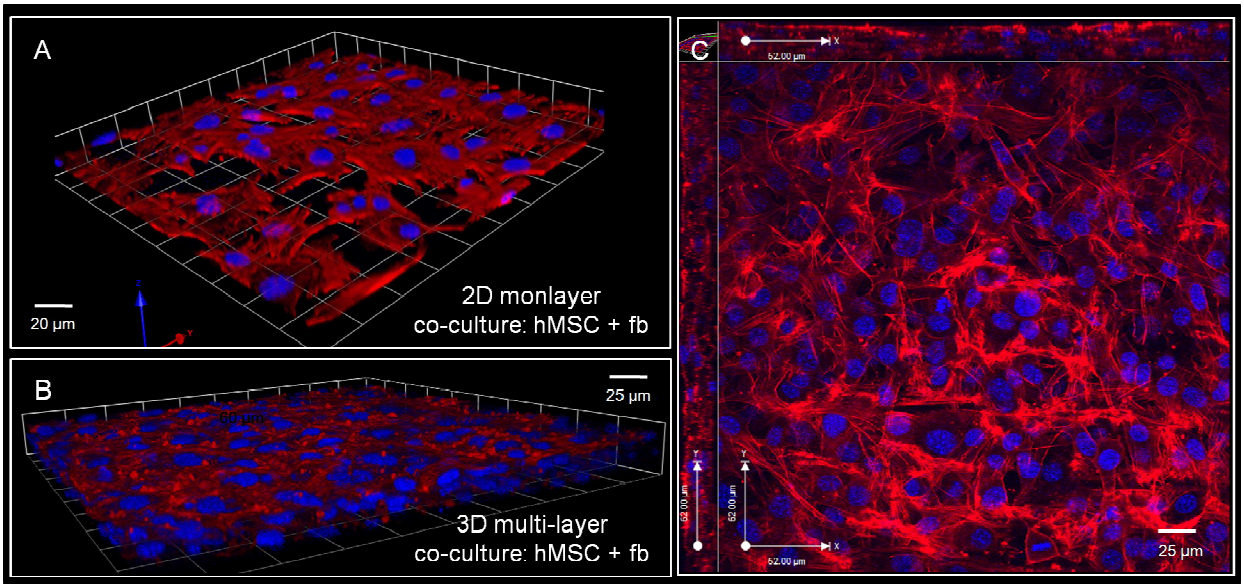

Figure *S*3. Confocal images representing images of oxime-mediated, 3D tissue-like structure formation with hMSC/fb co-cultures. Separate hMSC and fb populations were functionalized with ketone- and oxyamine-containing liposomes, respectively, resulting in membrane fusion and subsequent presentation of the ketone and oxyamine groups from cell surfaces. By culturing these cells on substrates, alternating cell population seeding layer-by-layer, gave rise to multi-layered, tissue-like cell sheets through stable oxime chemistry. (A) A 2D reconstruction image showing only a monolayer of cells after ketone-tethered hMSCs were co-cultured with non-functionalized fb. (B) A 3D reconstruction and (C) confocal micrograph displaying 3D multiple cell layers after oxyamine-presenting fb were added to hMSCs presenting ketone groups. Cells were stained with DAPI (blue, nucleus) and phalloidin (red, actin).

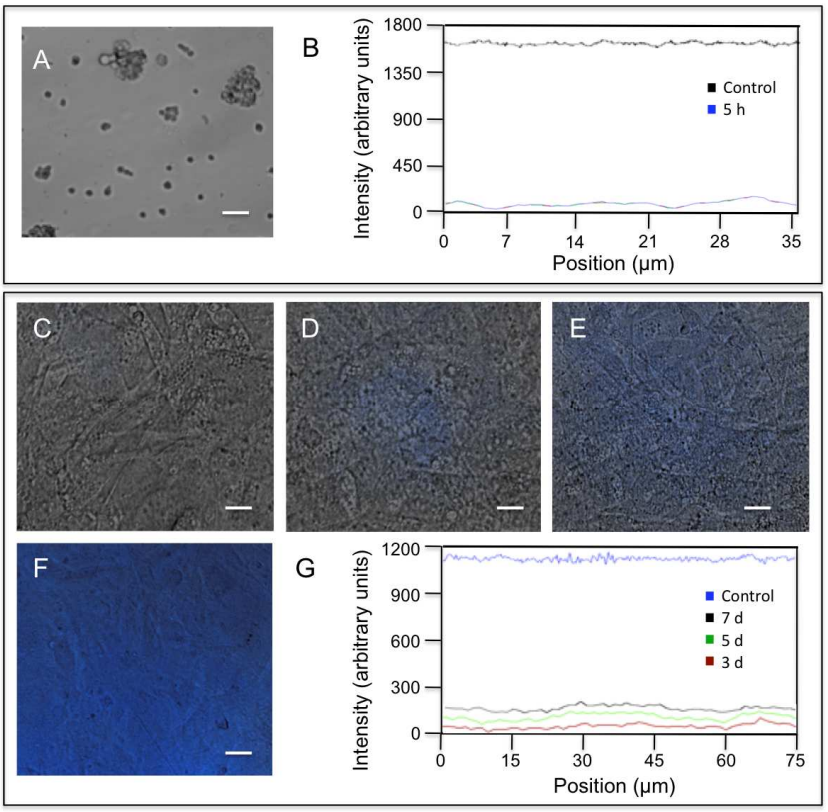

Figure *S*4. 3D spheroid and multi-layer structure cell viability, assayed with trypan blue. (A) Ketone- and oxyamine-tethered fb were mixed together in solution for 1, 2, 3, and 5 h, resulting in 3D spheroid formation and were then tested for viability using trypan blue dye (0.4 % in PBS, 2 min). (B) Trypan blue linescans (fluorescence false-colored for enhanced visualization) of the spheroids generated after 5 h of culture were compared to a control population in which spheroids were generated for 5 h, fixed with formaldehyde, and stained with trypan blue under the same conditions. Greater than 99 % of cells were determined to be viable. Similarly, 3D multi-layered tissue-like structures were generated and cultured for (C) 3 (D) 5, and (E) 7 days and tested for viability using trypan blue dye. (F) Again as a control, cells were grown in a multilayer for 7 days, then fixed with formaldehyde, and stained with trypan blue, and linescans were constructed for all samples and compared to the control. Cell viability decreased with time and number of cell layers from 3 to 5 to 7 days of culture with approximated viabilities of (C) 98, (D) 91, and (E) 84 %, respectively. The scale bars represent 60 (A) and 30 μm (C-F).