Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Controlling placental spheroid growth and phenotype using engineered synthetic hydrogel matrices

*Emily M. Slaby, Seema B. Plaisier, Sarah R. Brady, Shivani C. Hiremath, Jessica D. Weaver**

Table S1. Crosslinker and adhesion ligand sequences purchased from GenScript.

Peptide	Sequence	Description
RGD	GRGDSPC	Adhesive, fibronectin
RDG	GRDGSPC	Adhesive, scrambled fibronectin
GFOGER	GGYGGGPP(GPP)5GFOGER(GPP)5GPC	Adhesive, collagen
YIGSR	CGGEGYGEGYIGSR	Adhesive, laminin
IKVAV	CGQAASIKVAVSADR	Adhesive, laminin
VPM	GCRDVPMSMRGGDRCG	Crosslinker, degradable (MMP1, MMP2, MMP3, MMP7, MMP9, MT1-MMP/MMP14) [72–74]
GDQ	GCRDGDQGIAGFDRCG	Crosslinker, degradable (MMP1) [73,75]
GPQ-W	GCRDQGWIGQPGDRCG	Crosslinker, degradable (MMP1, MMP2, MMP3, MMP8, MMP9, MMP12) [73,76,77]

Table S2. Table of Proteomics Normalized Abundances – available in Supporting Information

Table S3. Table of Functional Annotation – available in Supporting Information

Table S4. Table of Proteins Identified in Proteomics Analysis – available in Supporting Information

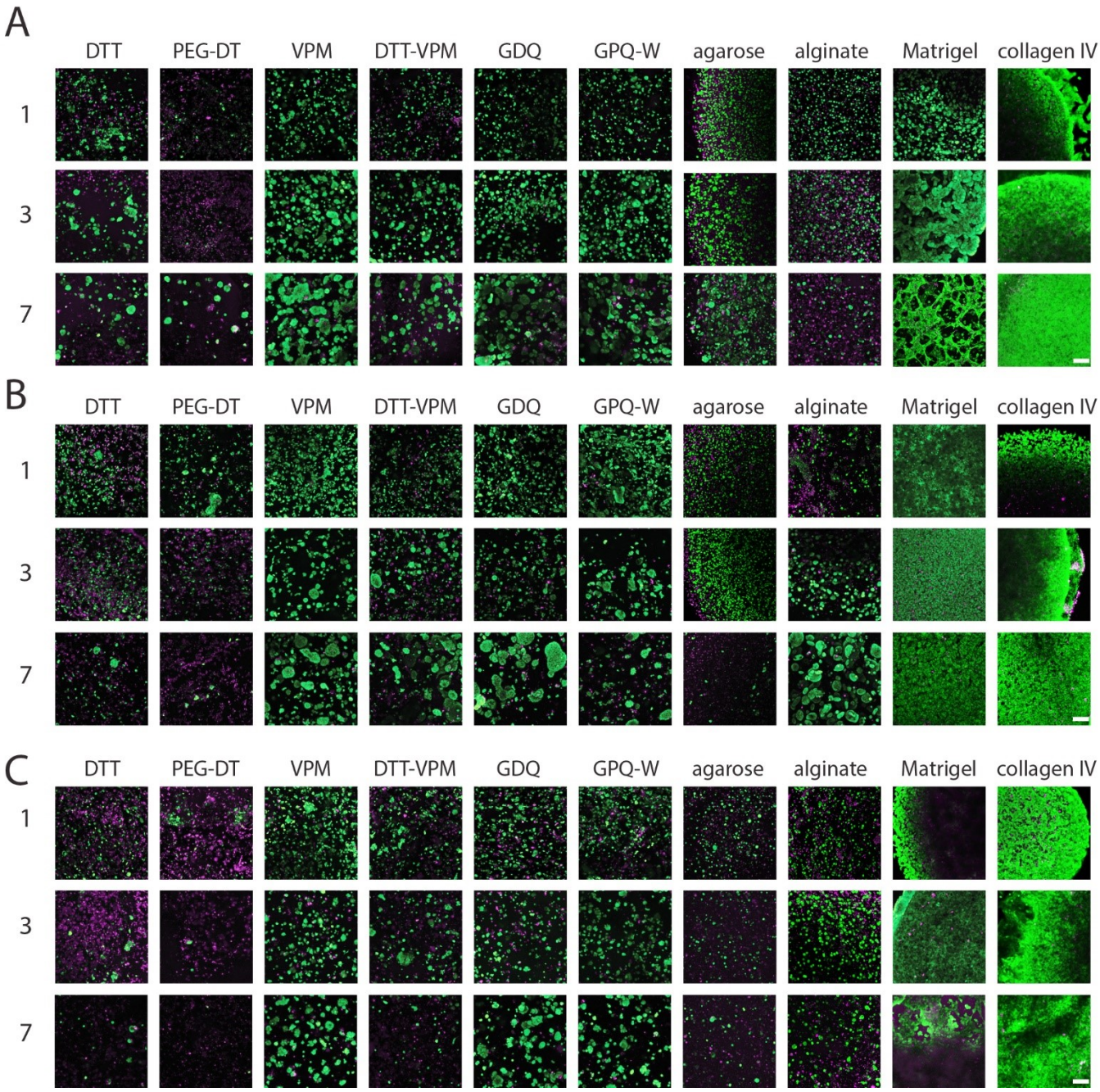

Figure S1. Cell viability and morphology through z-maximum projected confocal microscopy images (green – live, magenta – dead) of (A) JEG, (B) JAR, (C) BeWo viability over time on days 1, 3, and 7 cultured in PEG-RGD with varying crosslinkers or natural hydrogel controls. Scale bar = 200 μm.

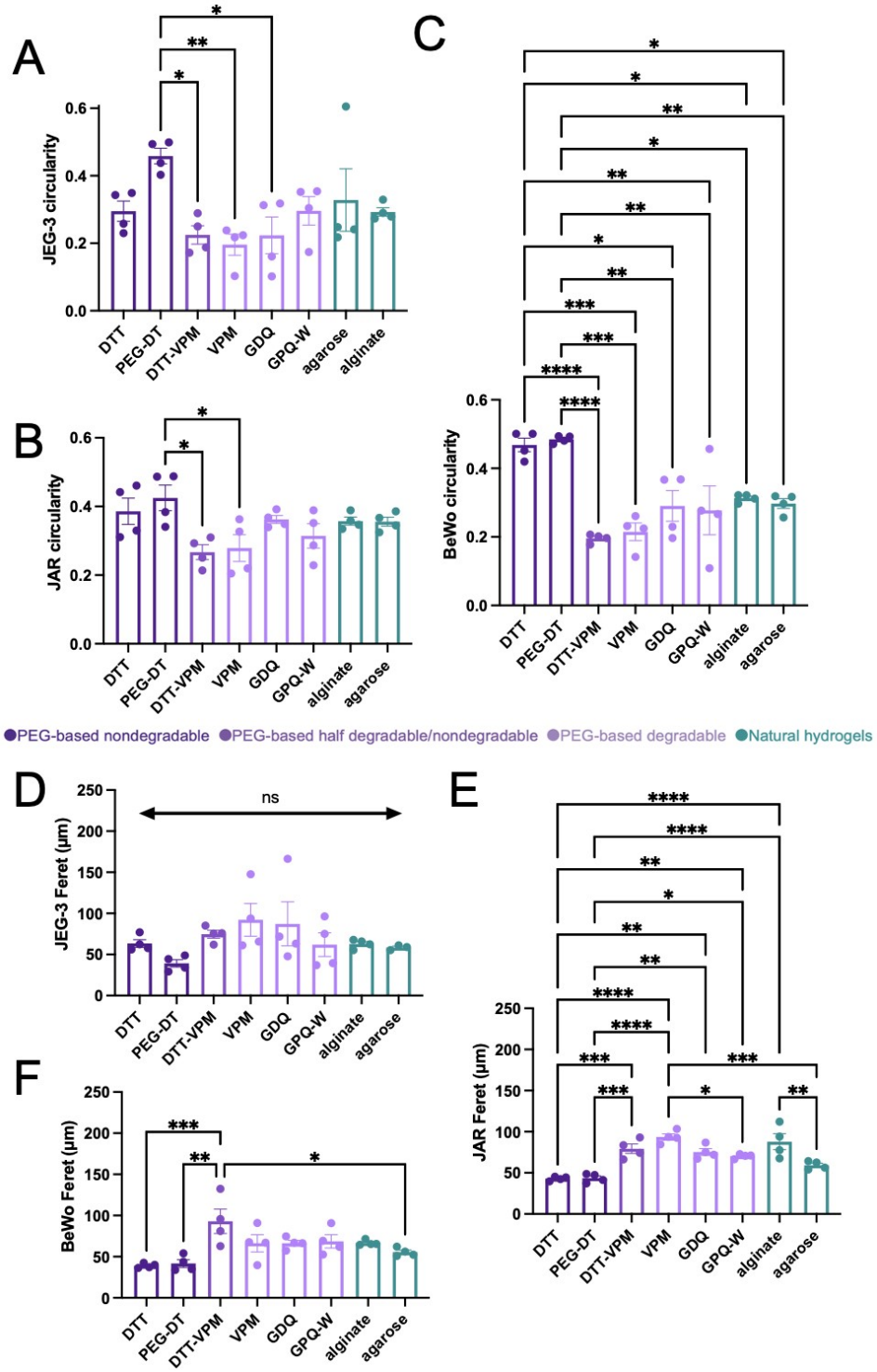

Figure S2. Cell morphology analysis of circularity (A-C) and feret diameter (D-F) on live z-maximum projected confocal microscopy images of (A, D) JEG-3, (B, E) JAR, and (C, F) BeWo cells on day 7 cultured in PEG-RGD with varying crosslinkers or natural hydrogel controls. Data is shown as mean ± SEM. Analyzed by ordinary one-way ANOVA with Tukey's multiple comparisons test; ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

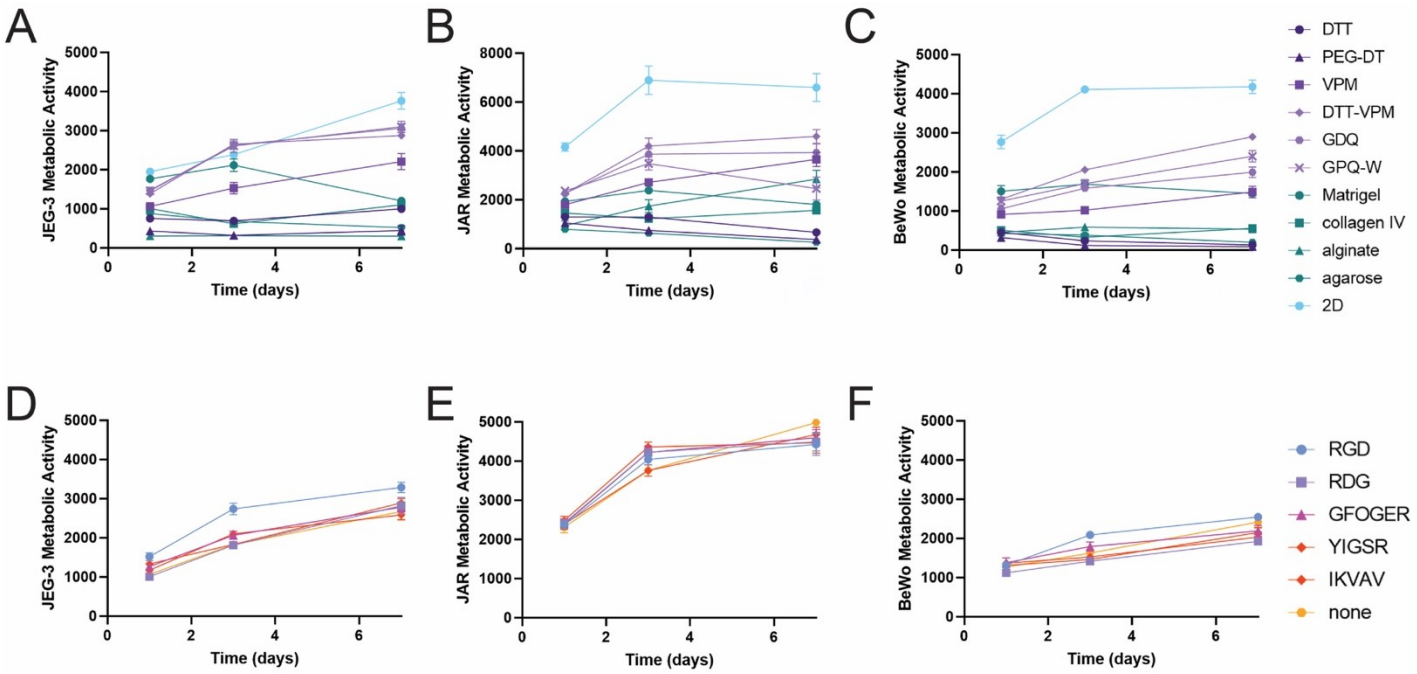

Figure S3. AlamarBlue metabolic activity over time in hydrogel controls and varying crosslinkers (A-C) or adhesion ligands (D-F) in JEG-3 (A, D), JAR (B, E), and BeWo (C, F) cell lines. Data is shown as mean ± SEM.

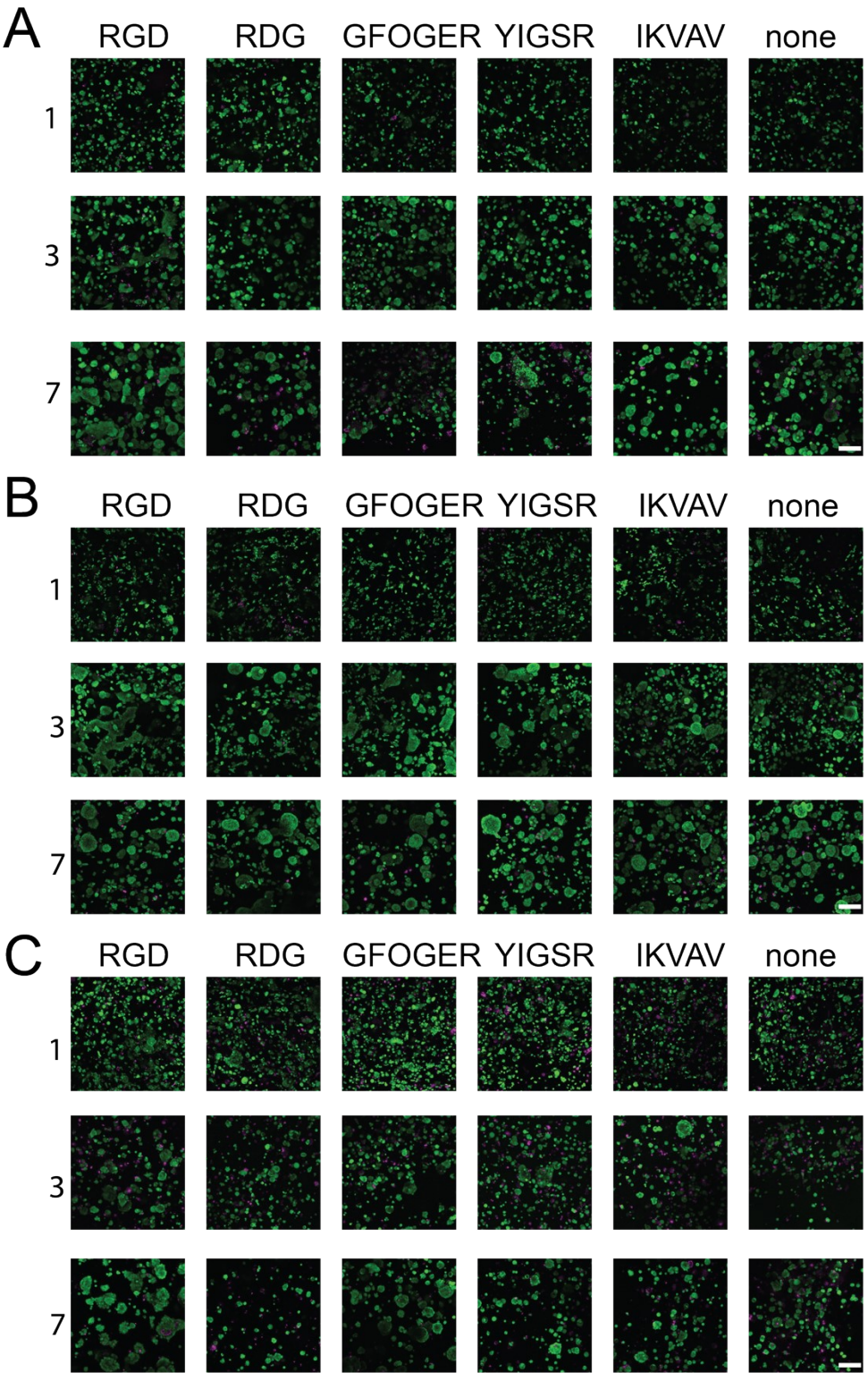

Figure S4. Cell viability and morphology through z-maximum projected confocal microscopy images (green – live, magenta – dead) of (A) JEG, (B) JAR, (C) BeWo viability over time on days 1, 3 and 7 with varying adhesion ligands. Scale bar = 200 μm.

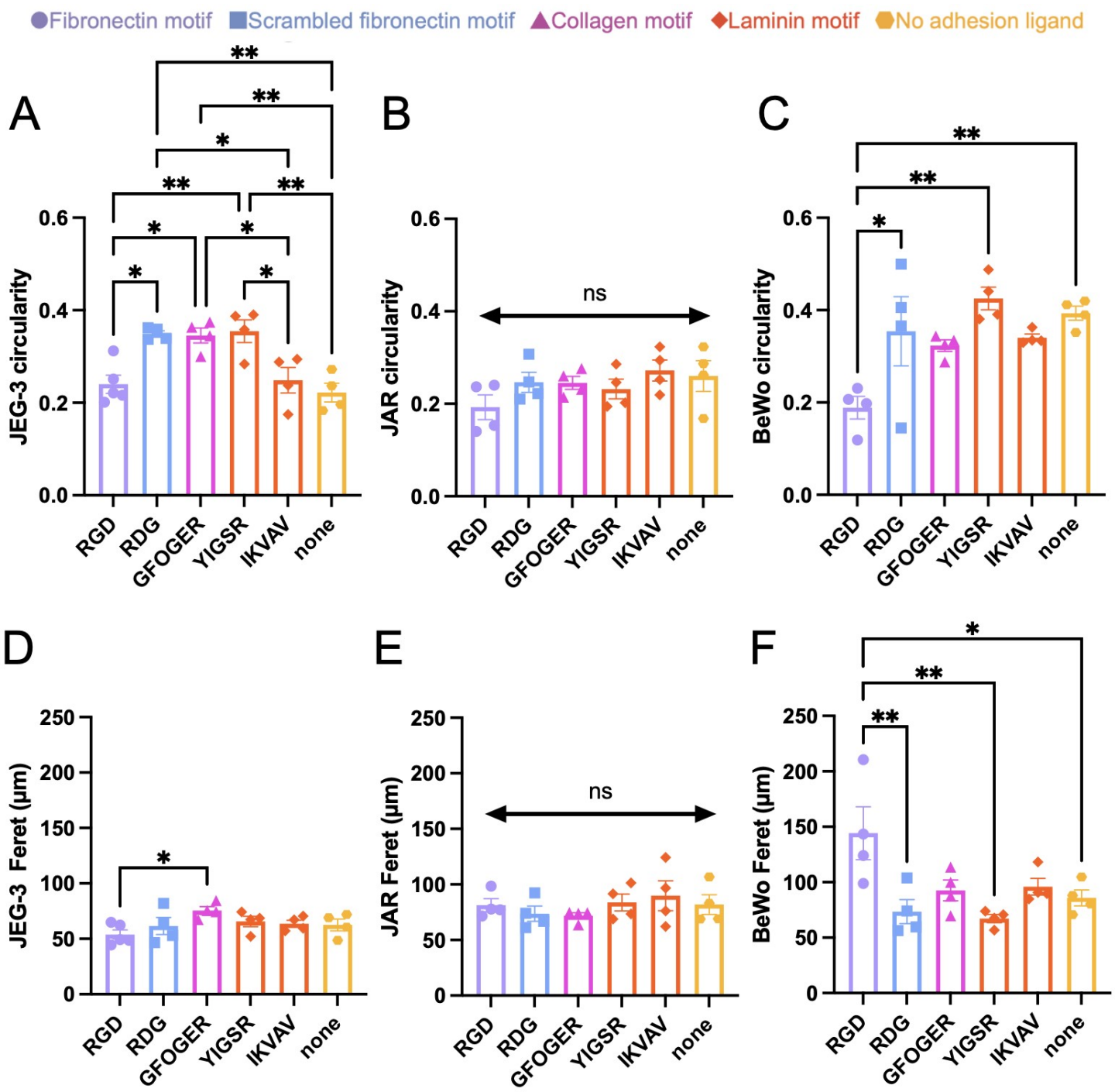

Figure S5. Cell morphology analysis of circularity (A-C) and feret diameter(D-F) on live z-maximum projected confocal microscopy images of (A, D) JEG, (B, E) JAR, and (C, F) BeWo cells on day 7 cultured in PEG-VPM with varying adhesive ligands or no adhesion ligand ("none"). Data is shown as mean ± SEM. Analyzed by ordinary one-way ANOVA with Tukey's multiple comparisons test; ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

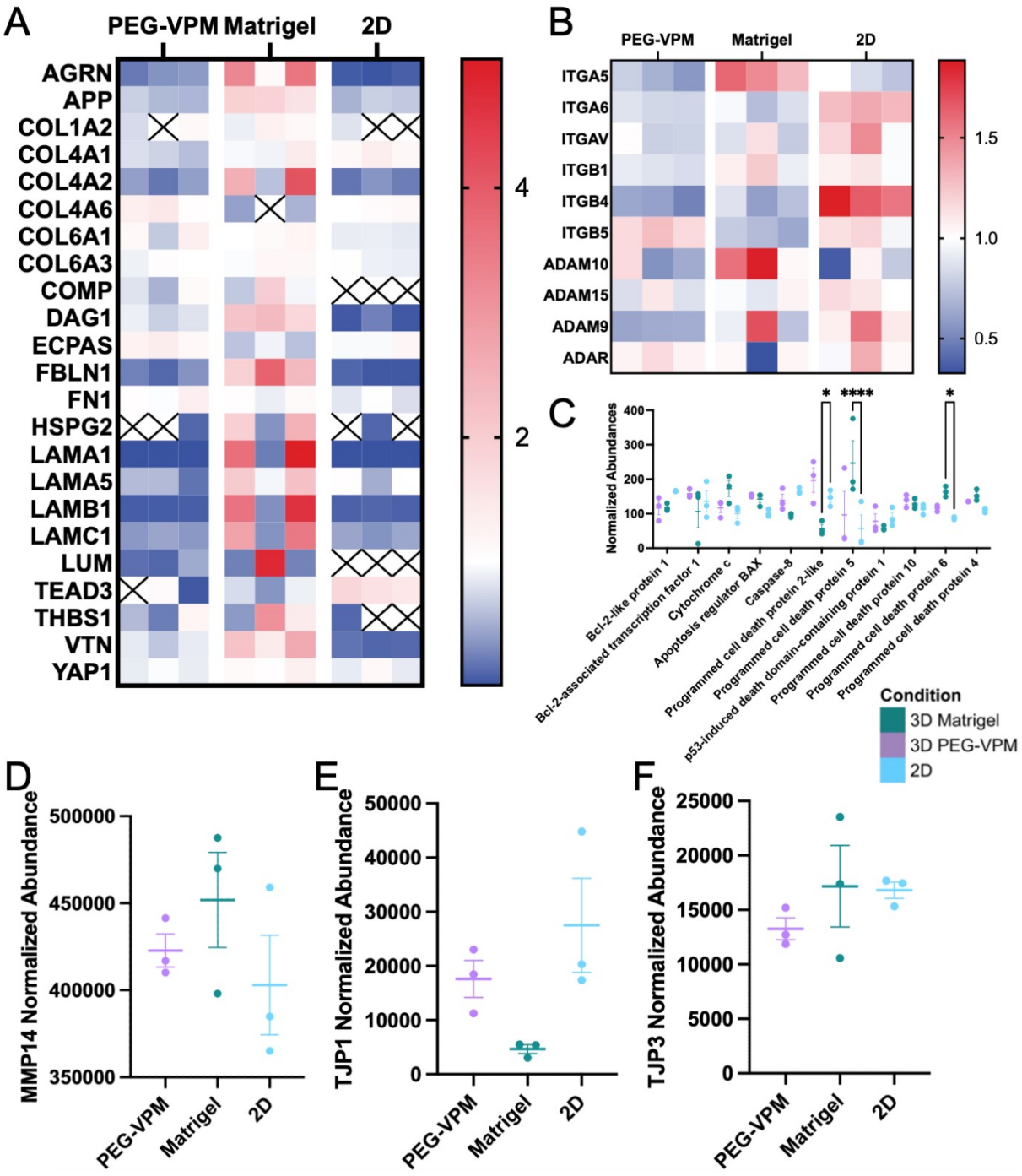

Figure S6. Normalized protein abundances of JAR cells cultured in PEG-VPM-RGD, Matrigel, or 2D from proteomic analysis. (A) ECM and structural integrity proteins, (B) integrins and disintegrin and metalloproteinase domain-containing proteins, (C) apoptosis-associated proteins, (D) MMP14, (E) Tight junction protein 1 (TJP1), and (F) Tight junction protein 3 (TJP3) normalized abundances. Data shown as mean ± SEM. (C) Analyzed by two-way with Dunnett's multiple comparisons test to 2D or (D-F) one-way ANOVA with Tukey's multiple comparison test; * $p < 0.05$, **** $p < 0.0001$.

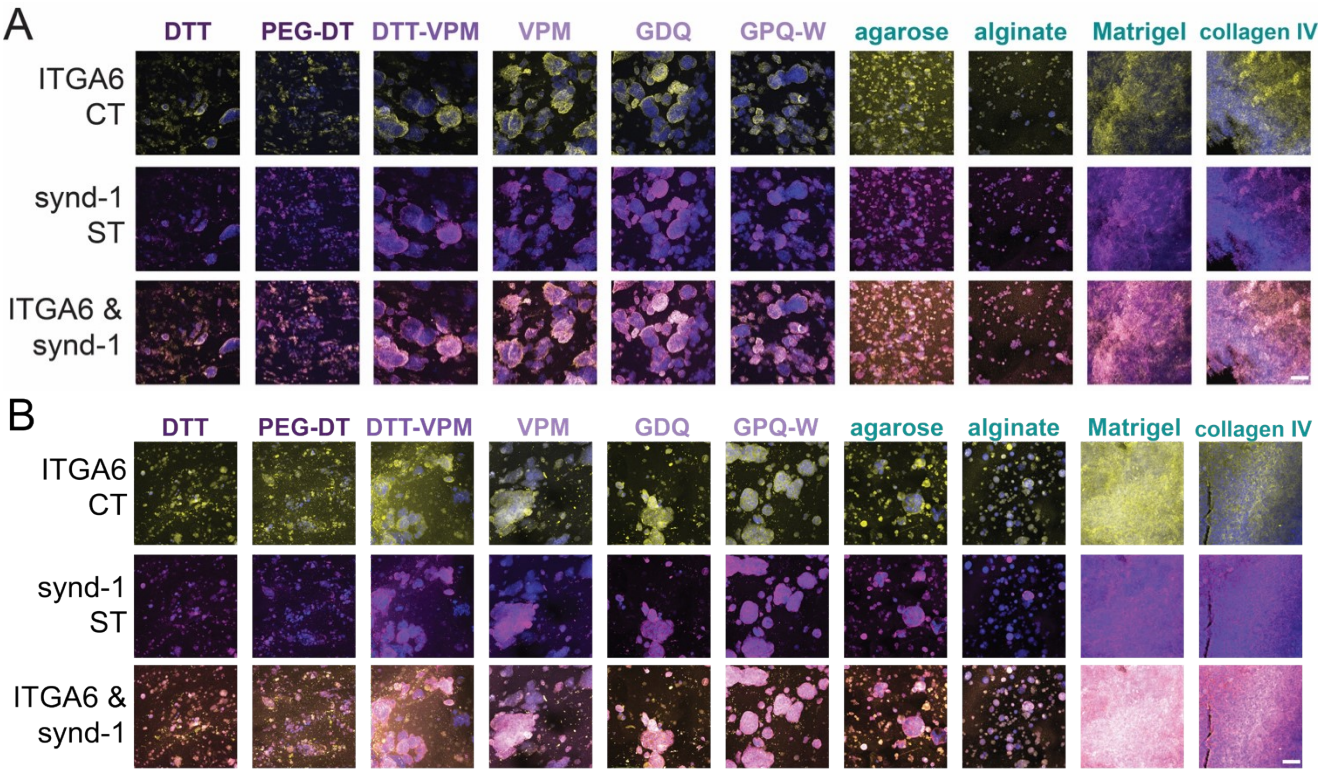

Figure S7. Impact of hydrogel degradability on trophoblast phenotype by surface markers. (A) JEG-3 and (B) BeWo stained with ITGA6 (yellow), synd-1 (magenta), and DAPI (blue) after culture in PEG-RGD hydrogels with varying crosslinkers or hydrogel controls for 7 days. Scale bar = 100 μm.

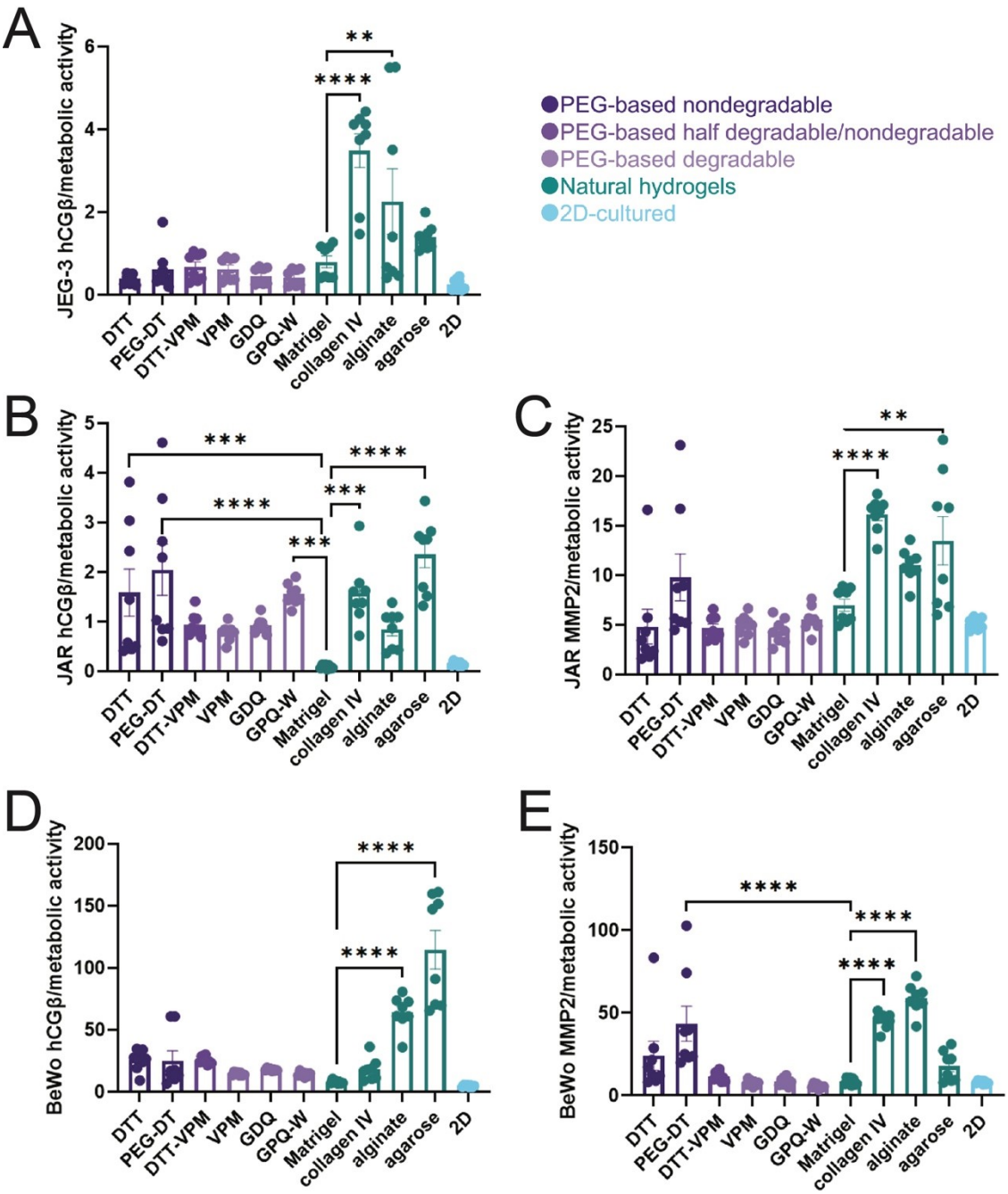

Figure S8. Cell line secretion on day 7 in various crosslinked PEG-RGD and control hydrogels normalized to metabolic activity. Secretion of (A, B, D) hCGβ and (C, E) MMP2 from (A) JEG-3, (B-C) JAR, and (D-E) BeWo cells normalized to the average metabolic activity of the cells in each condition on day 7. Data shown as mean ± SEM. Analyzed by ordinary one-way ANOVA with Dunnett's multiple comparisons test compared to Matrigel; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

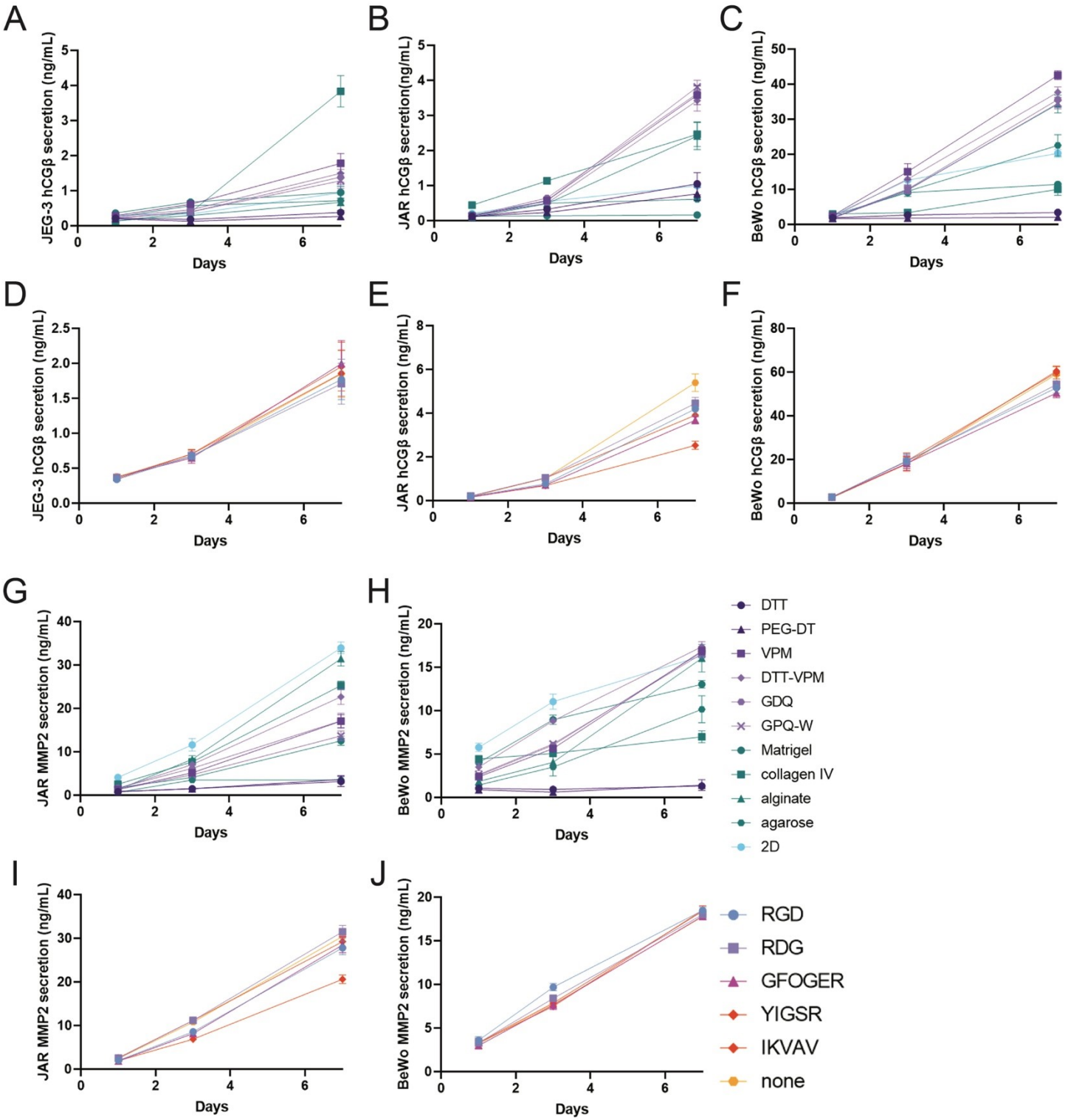

Figure S9. Secretion of (A-F) hCGβ and (G-J) MMP2 from JEG-3 (A, D), JAR (B, E, G, I), and BeWo (C, F, H, J) cell lines over time in hydrogels with varying crosslinked PEG-RGD hydrogels or controls (A-C, G-H) or degradable PEG-VPM hydrogels with varying adhesion ligands (D-F, I-J). Data shown as mean ± SEM.

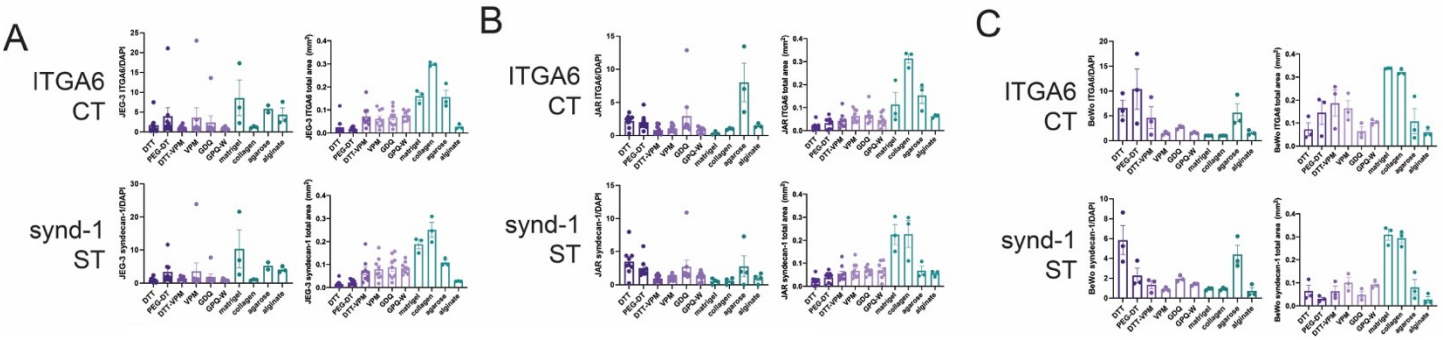

Figure S10. Image analysis of protein area expression data from Figure 5 and S5 of JEG-3 (A), JAR (B), and BeWo. (C) Total expression (right) of ITGA6 and syndecan-1 and normalized (left) to DAPI in PEG-RGD with various crosslinkers and natural hydrogel controls. Data shown as mean ± SEM.

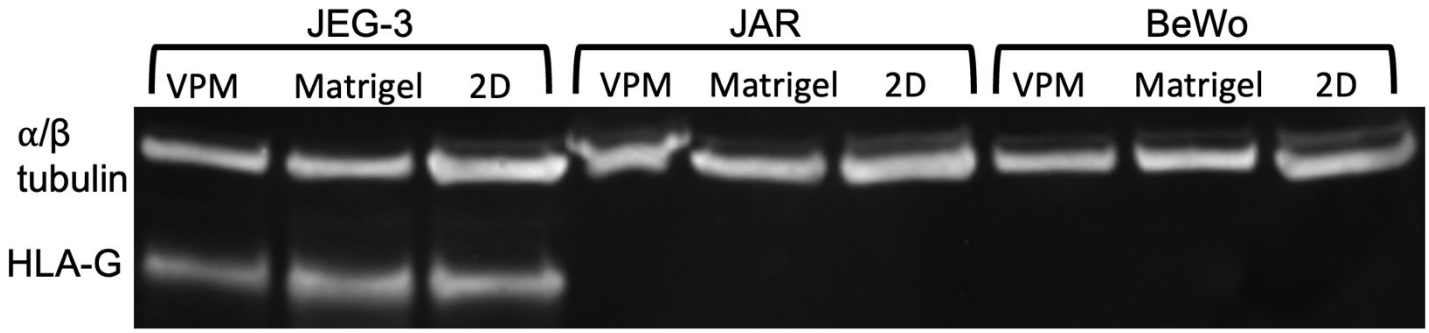

Figure S11. Western blot analysis of JEG-3, JAR, and BeWo cell lysates after culture in PEG-RGD-VPM ("VPM"), Matrigel, or 2D for 7 days and probed for HLA-G expression relating to the EVT subtype and α/β tubulin housekeeping gene.

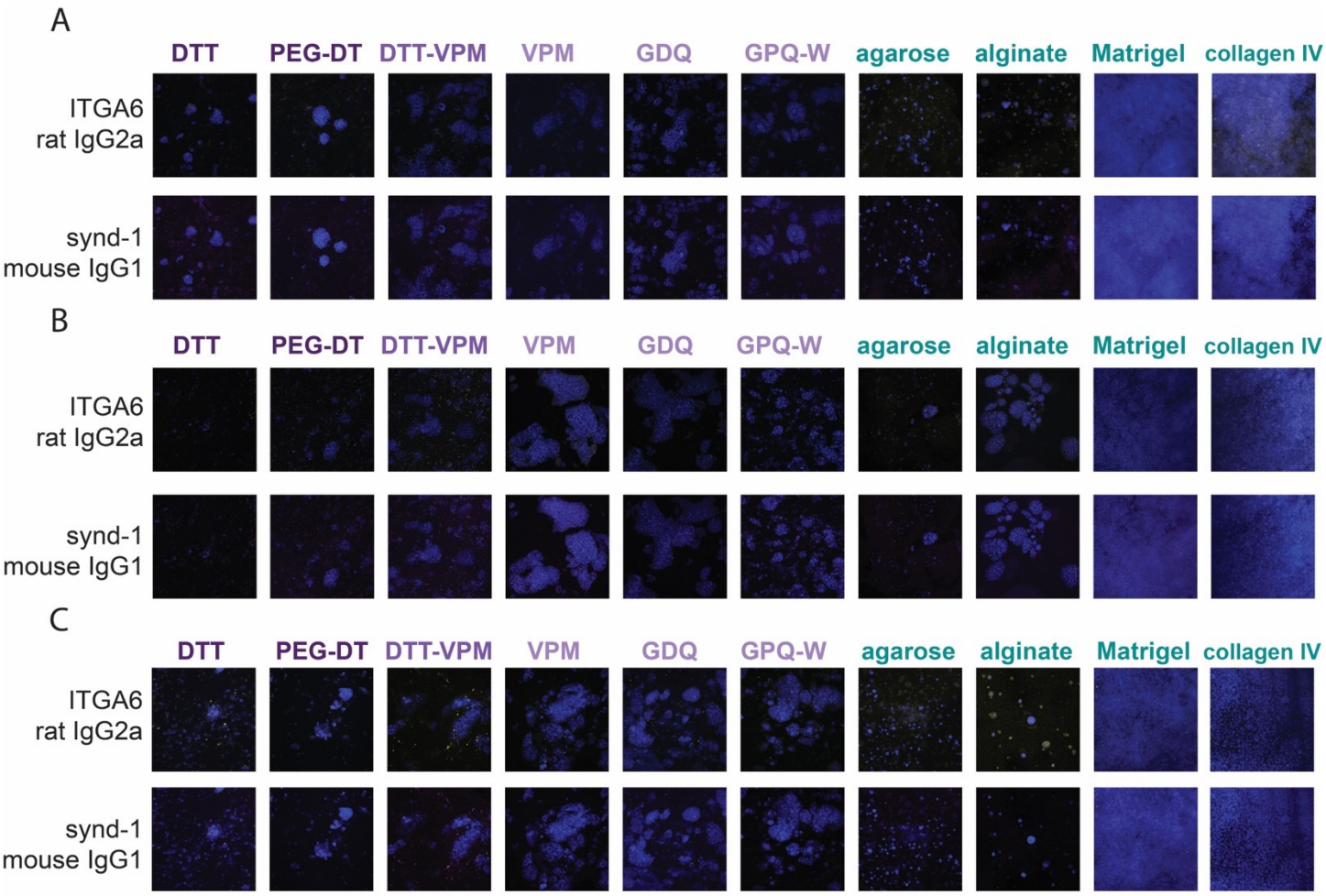

Figure S12. Isotype controls for data shown in Figure 5 and S5. (A) JEG-3, (B) JAR, and (C) BeWo stained with DAPI (blue) and isotype controls for ITGA6 (rat IgG2a - yellow) and syndecan-1 (mouse IgG1 - magenta) after culture in PEG-RGD hydrogels with varying crosslinkers or hydrogel controls for 7 days.

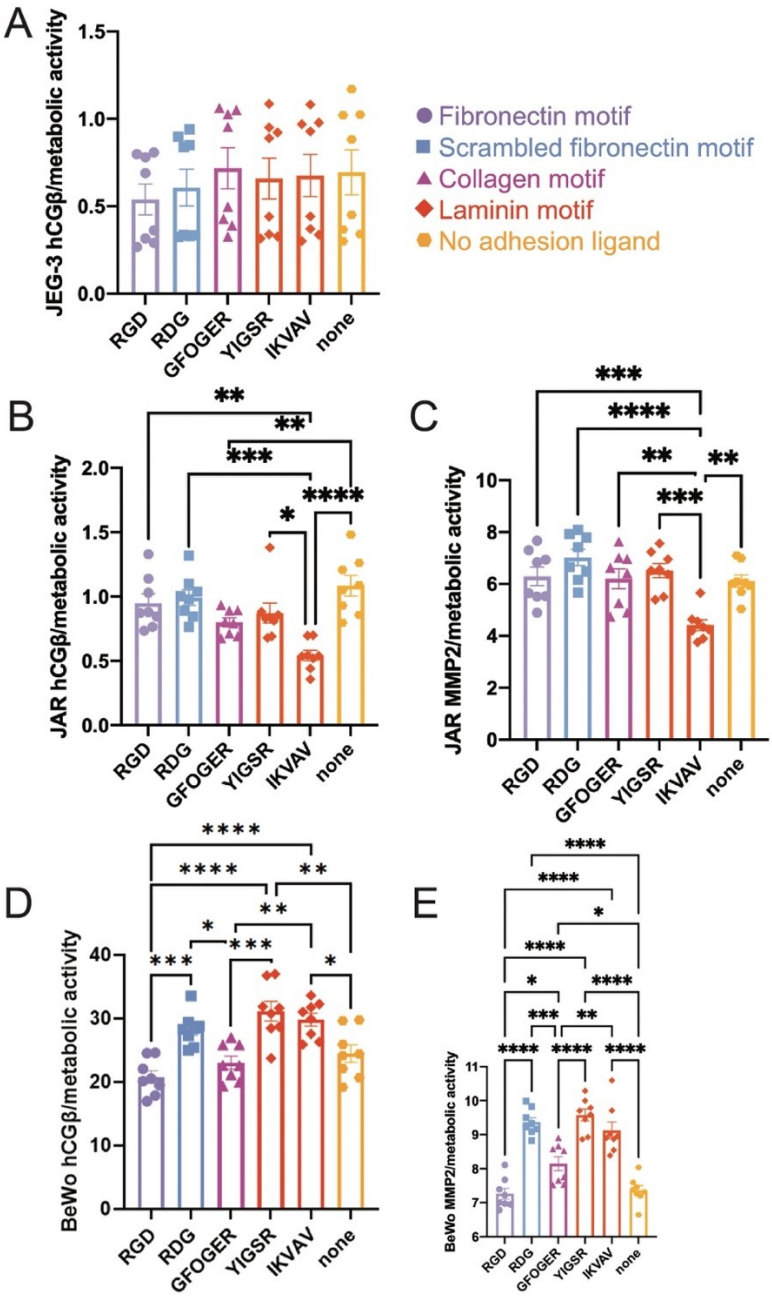

Figure S13. Cell line secretion on day 7 in degradable PEG-VPM hydrogels with varying adhesion ligands normalized to metabolic activity. Secretion of (A, B, D) hCGβ and (C, E) MMP2 from (A) JEG-3, (B-C) JAR, and (D-E) BeWo cells normalized to the average metabolic activity of the cells in each condition on day 7. Data shown as mean ± SEM. Analyzed by ordinary one-way ANOVA with Tukey's multiple comparisons test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

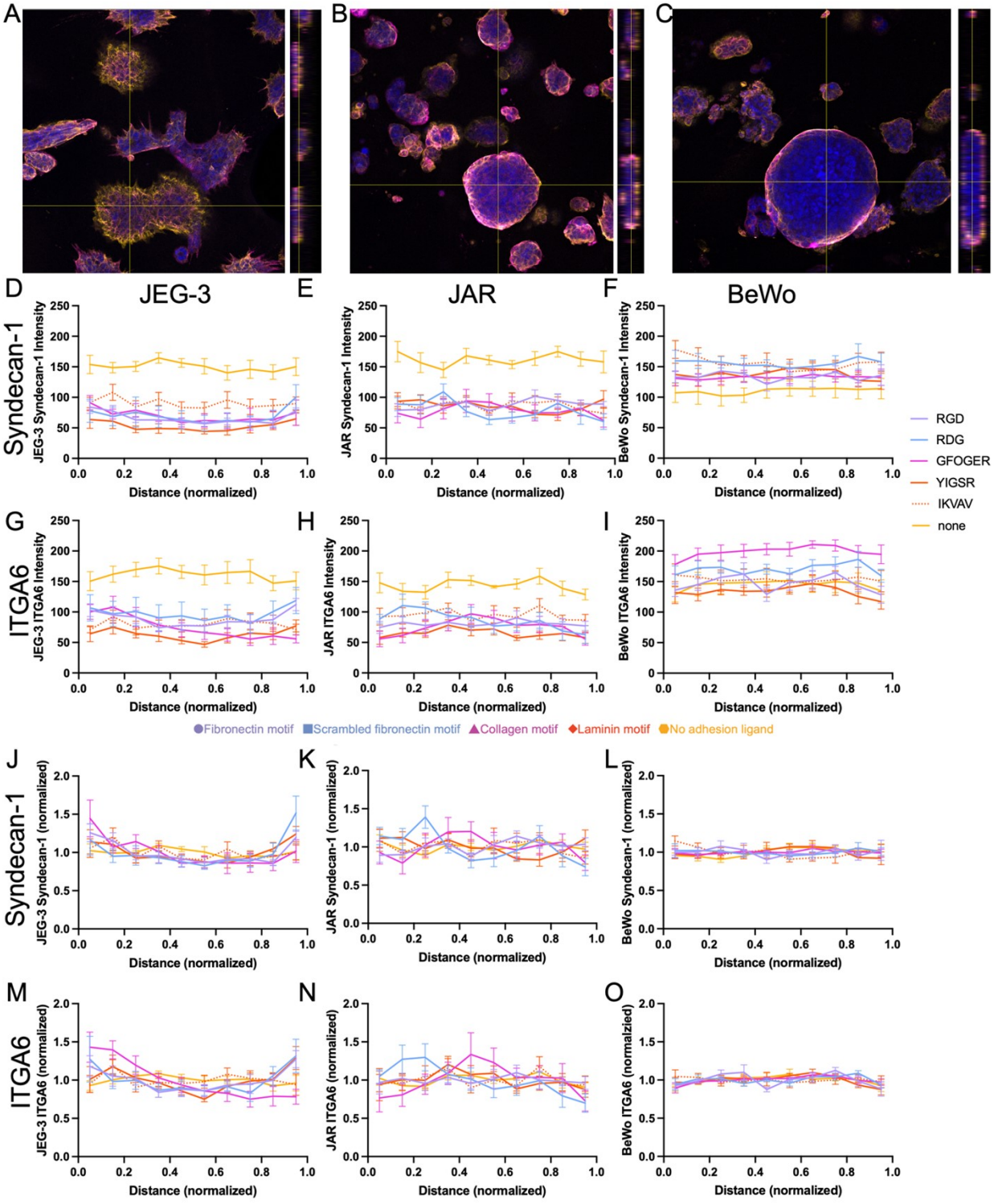

Figure S14. Distribution of select phenotypic markers across individual spheroids. (A-C) Orthogonal views of selected images of JEG-3 cultured in PEG-VPM (A) -RGD, (B) -GFOGER, and (C) -YIGSR stained with DAPI (blue), ITGA6 (yellow), and syndecan-1 (magenta). (D-O) Image analysis of sydecan-1 and ITGA6 intensity (D-I) and normalized (J-O) to the average intensity of each spheroid from n=7 spheroids within the respective images from Figure 6. Data shown as mean ± SEM.

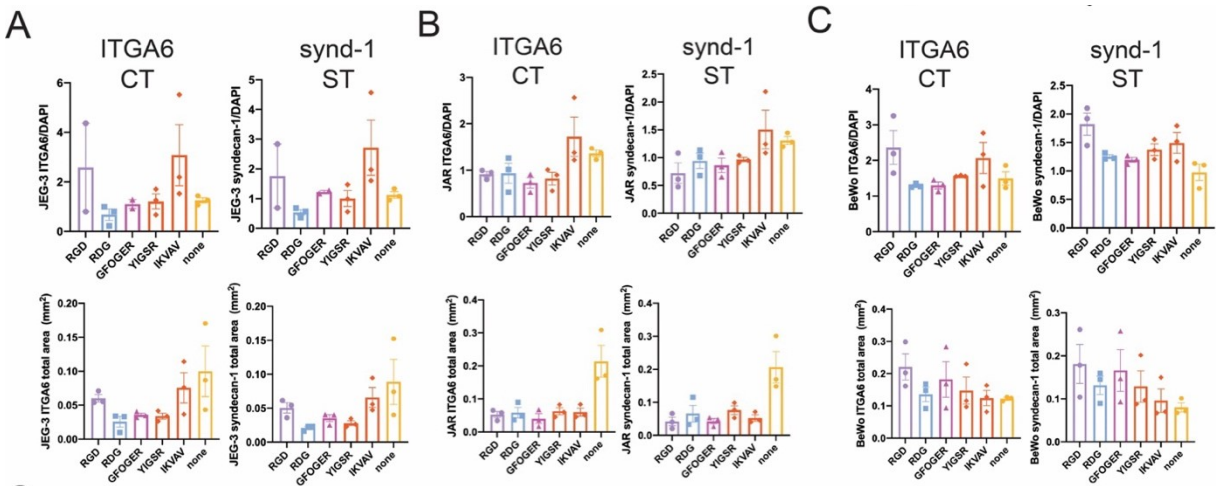

Figure S15. Image analysis of data from Figure 6 of JEG-3 (A), JAR (B), and BeWo (C) total area expression of ITGA6 and synd-1 (bottom) and normalized to DAPI (top) in PEG-VPM with various adhesive ligands. Data shown as mean ± SEM.

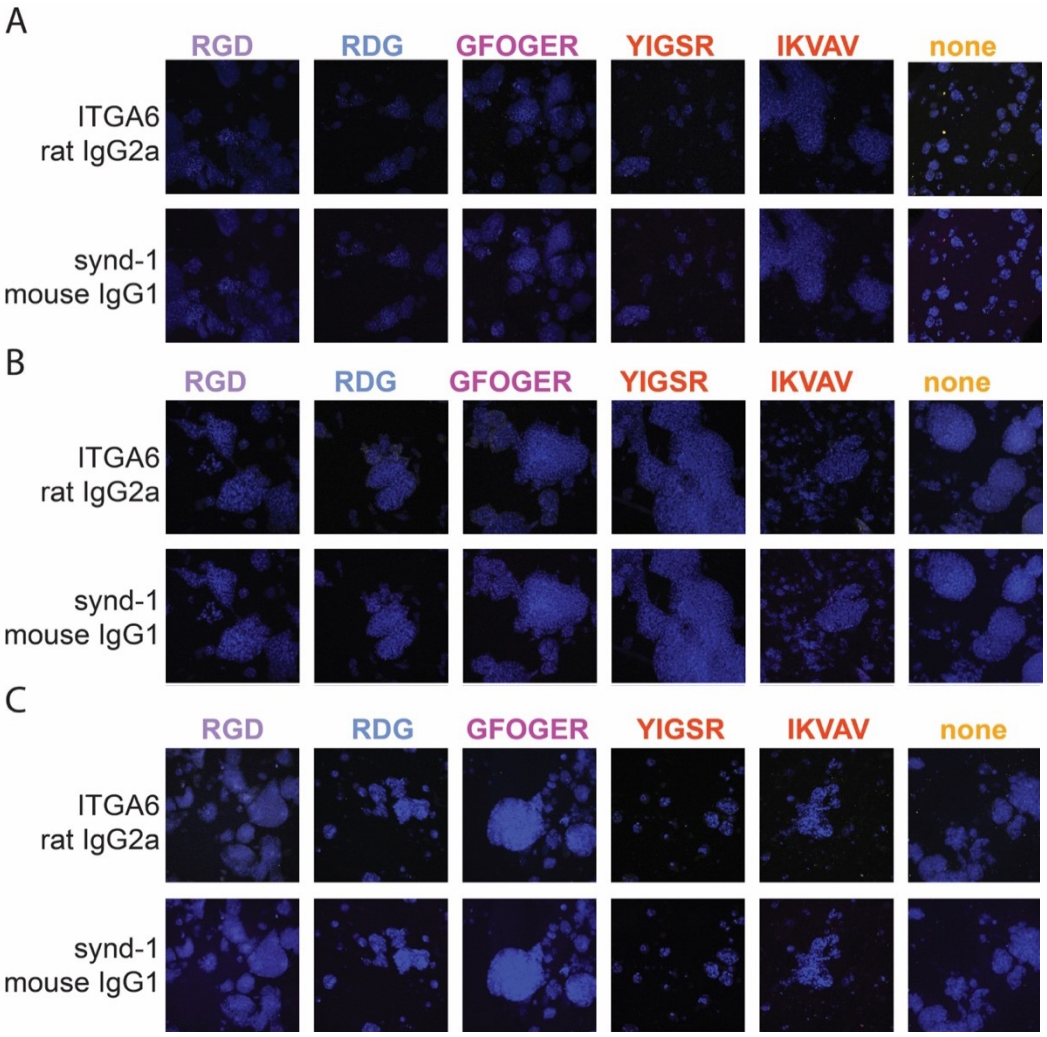

Figure S16. Isotype controls for data shown in Figure 6. (A) JEG-3, (B) JAR, and (C) BeWo stained with DAPI (blue) and isotype controls for ITGA6 (rat IgG2a - yellow) and syndecan-1 (mouse IgG1 - magenta) after culture in degradable PEG-VPM hydrogels with varying adhesion ligands for 7 days.