Supporting Information

Covalent Modification of Synthetic Hydrogels with Bioactive Proteins via Sortase-Mediated Ligation

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Primary endometrial epithelial cell isolation and purification

Tissues were dissociated and cells purified as described by Osteen and coworkers[1] with some modifications. Biopsy specimens were collected using a pipelle and immediately placed in an ice-cold 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's F-12 (Gibco) supplemented with 1% penicillin/streptomycin (DMEM/F12). The tissue was washed twice by centrifugation at 400X g in DMEM/F12 and dissected into small pieces (1-2 mm^3). Tissue pieces were incubated for 1h at 37°C in DMEM-F12 supplemented with 0.5% collagenase Type IV (Worthington Biochemical Corporation LS004188), 0.02% DNAase (Sigma-Aldrich DN25) and 2% chicken serum (Sigma-Aldrich C5405) and vortexed every 15 min. As a result of this first dissociation, stromal cells are present as single cells while epithelial cells remain aggregated. The cell suspension was then filtered twice through a 70 μm membrane filter (Falcon 352350) in order to separate the stromal cells from the epithelial cell clumps. The latter were collected on the surface of the filters and washed by centrifugation with sterile PBS. Further dissociation of the epithelial aggregates was achieved by incubation with an enzyme mixture supplemented with 0.5% collagenase, 0.1% hyaluronidase (Sigma-Aldrich H3506), 0.1% pronase (Sigma-Aldrich P5147), 0.02% DNAase and 2% chicken serum in PBS for 15-20 min at 37°C in a water bath. The cell preparation was filtered through a 70 μm membrane filter in order to get rid of the remaining stromal cells that were released during this digestion and epithelial cell clumps were collected again and further digested with fresh enzyme mixture for 30-45 min at 37°C. This final digestion resulted in small epithelial cell clumps of 50-100 cells that were purified by differential sedimentation at unit gravity as follows. Cells were centrifuged and resuspended in 2 mL DMEM/F12 containing 10% *v/v*

dextran/charcoal treated fetal bovine serum (Atlanta Biologicals) (DMEM/F12/FBS). Cells were layered slowly over 10 mL of DMEM/F12/FBS in a sterile 15 mL conical tube and the tube was incubated in an upright position at 37°C, 95% air, 5% CO_2 for 30 min. The top 10 mL of sedimentation medium were discarded and the sedimentation step was repeated with the bottom 2 mL. The top 10 mL of sedimentation medium were discarded again and 5 mL of DMEM/F12/FBS were added. Final purification was achieved by selective attachment of any remaining stromal cells to plastic substrate as follows. Cells were seeded in a 75 cm^2 tissue culture flask and incubated at 37°C, 95% air, 5% CO_2 for 1h. Non-attached epithelial cells were collected and cultured in DMEM/F12/FBS at a density of 3 X 10^5 cells/mL for 2-3 days. After this plating period, medium was changed every other day for 2-3 days until further use.

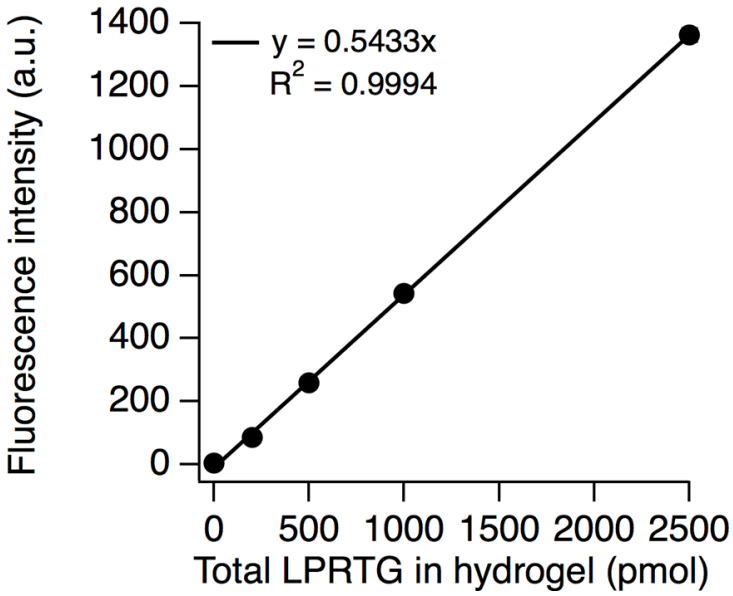

Figure S1. Linear standard curve for conversion of fluorescence arbitrary units into amount of *LPRTG* in pmol. The standard curve was established by measuring the fluorescence of hydrogels containing 0, 20, 50, 100 or 250 μM of total *LPRTG* peptide. Error bars represent standard error of the mean (n = 8). a.u. = arbitrary units.

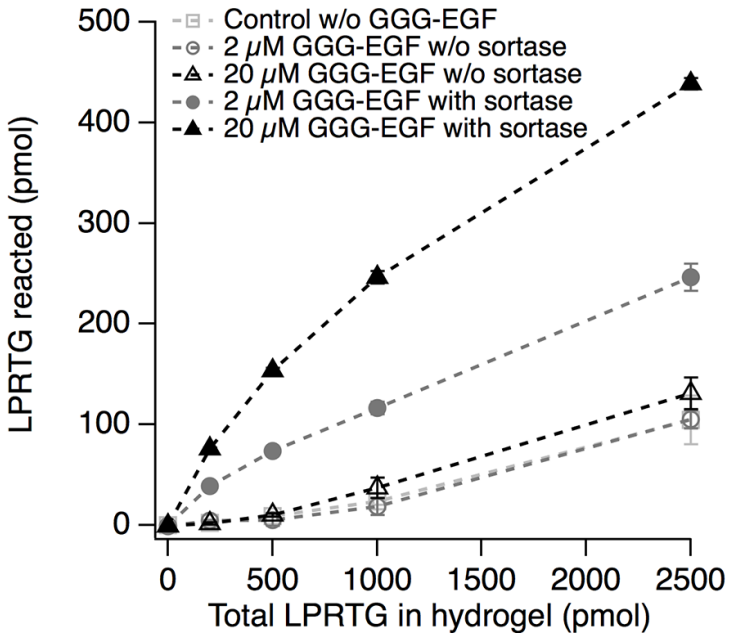

Figure S2. Amount of supposedly reacted *LPRTG* as a function of total initial *LPRTG* amount in hydrogel. Fluorescence intensities were converted to pmol *LPRTG* in hydrogel and the amount of reacted *LPRTG* was calculated as the difference between the values before and after sortase-mediated ligation. Controls in the absence of GGG-*EGF* or sortase allowed estimating photobleaching. Error bars represent standard error of the mean (n = 2).

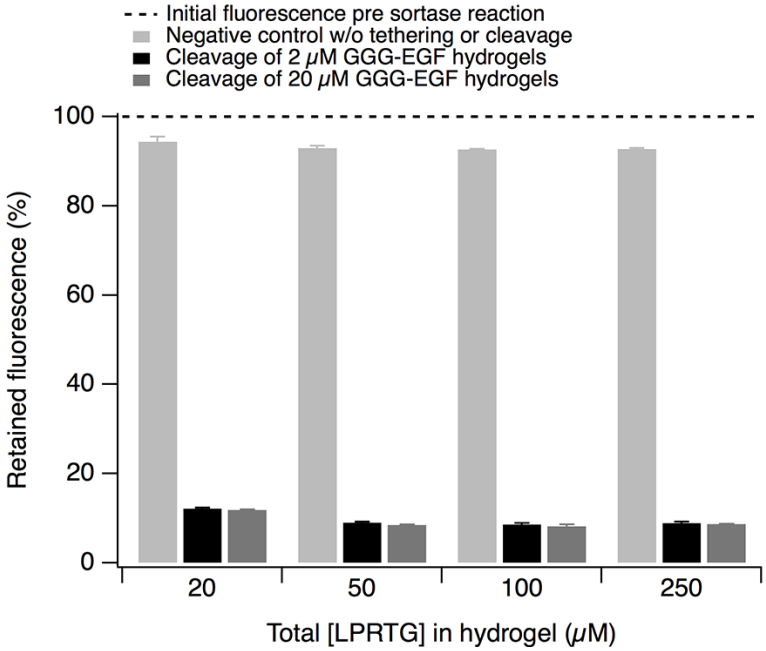

Figure S3. Percentage of retained hydrogel fluorescence as a function of total *LPRTG* in the hydrogel. Hydrogel fluorescence is measured before sortase-mediated ligation of GGG-*EGF* at 2 or 20 μM and after sortase-mediated cleavage of hydrogels. Error bars represent standard error of the mean (n = 2).

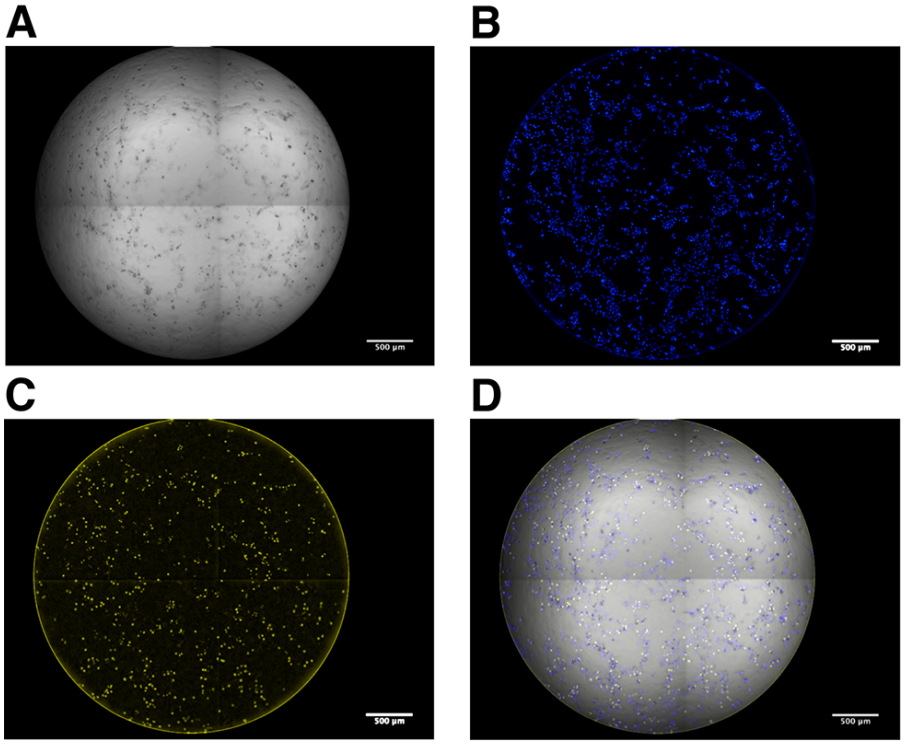

Figure S4. Cryopreserved human hepatocytes seeded on a hydrogel containing 500 µM *synKRGD* and 250 µM *LPRTG* and exposed to tethered EGF. A) bright field B) nuclei staining with Hoechst C) positive nuclei staining with FITC D) overlay.

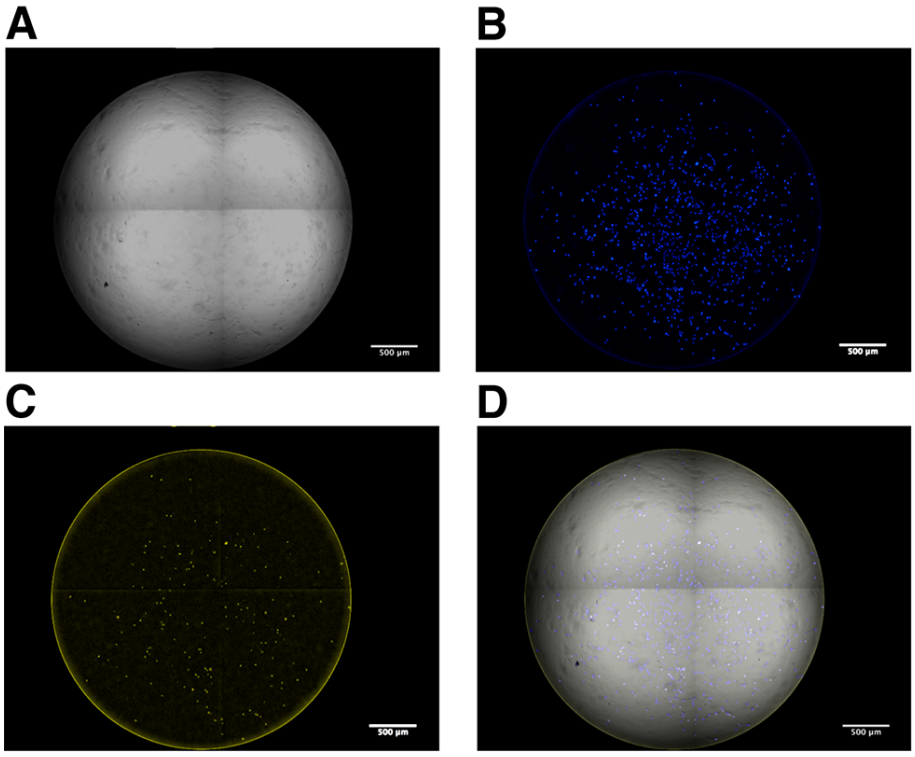

Figure S5. Primary human endometrial epithelial cells seeded on a hydrogel containing 500 µM synKRGD and 250 µM LPRTG and exposed to tethered EGF. A) bright field B) nuclei staining with Hoechst C) positive nuclei staining with FITC D) overlay.

REFERENCES

(1) Osteen, K. G.; Hill, G. A.; Hargrove, J. T.; Gorstein, F. *Fertil. Steril.* **1989**, *52*, 965–972.

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