Supplementary information

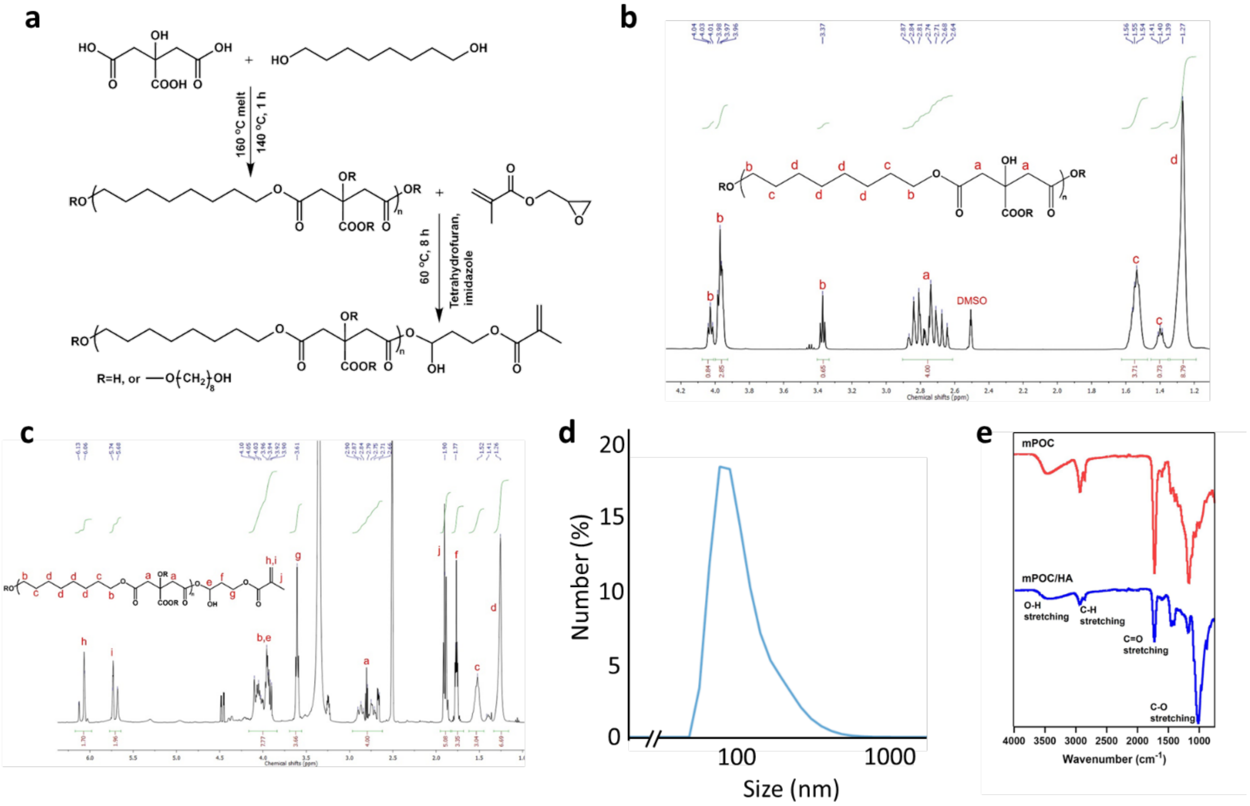
Microtopography-induced changes in cell nucleus morphology enhance bone regeneration by modulating the cellular secretome

Supplementary Table S1. List of Proteins Differentially Secreted by MSCS on Micropillars compared to Flat Surface (Secretome).

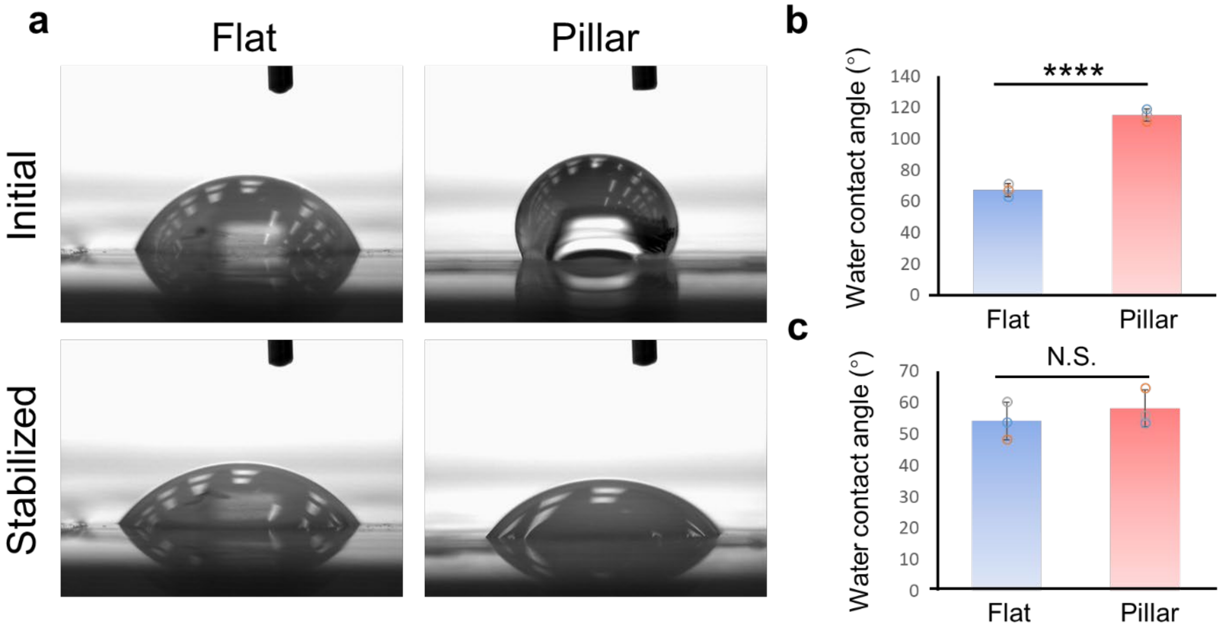
Supplementary Table S2. List of Proteins Differentially Expressed by MSCS on Micropillars compared to Flat Surface (Proteomic).

Supplementary Table S3. Gene Ontology Analysis on Genes Differentially Expressed in Tissues Regenerated with Flat and Micropillar Implants.

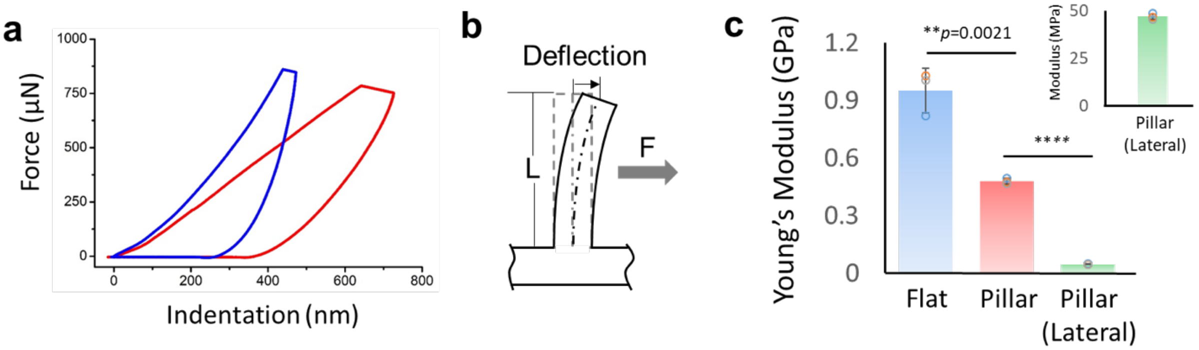
Supplementary Table S4. Top 20 Ontology Gene Sets Enriched in LMPs compared to other cell lineages.



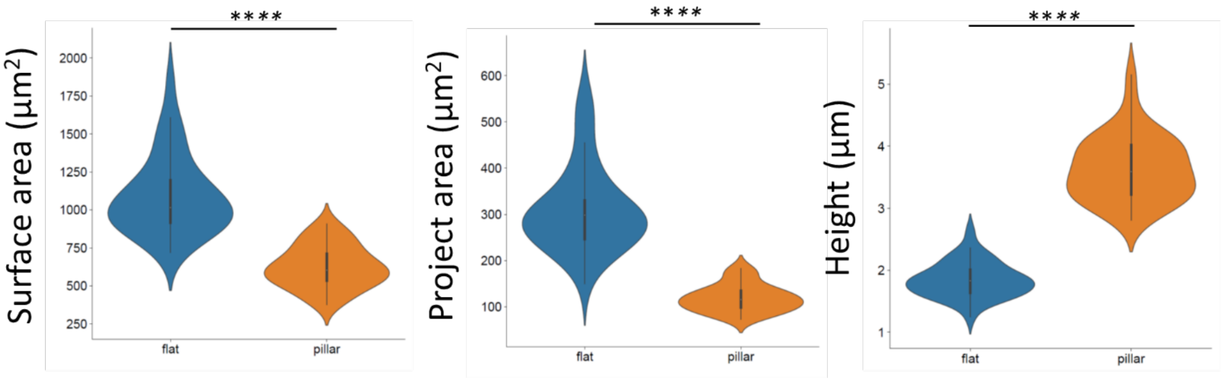
Supplementary Figure S1. Characterization of mPOC ploymers. a. Synthesis scheme of mPOC prepolymer. ^{1}HNMR spectrum of **b.** POC prepolymer and **c.** mPOC prepolymer. d. Analysis of size distribution of HA nanoparticles using DLS. **e.** FTIR spectrum of mPOC and mPOC/HA films.



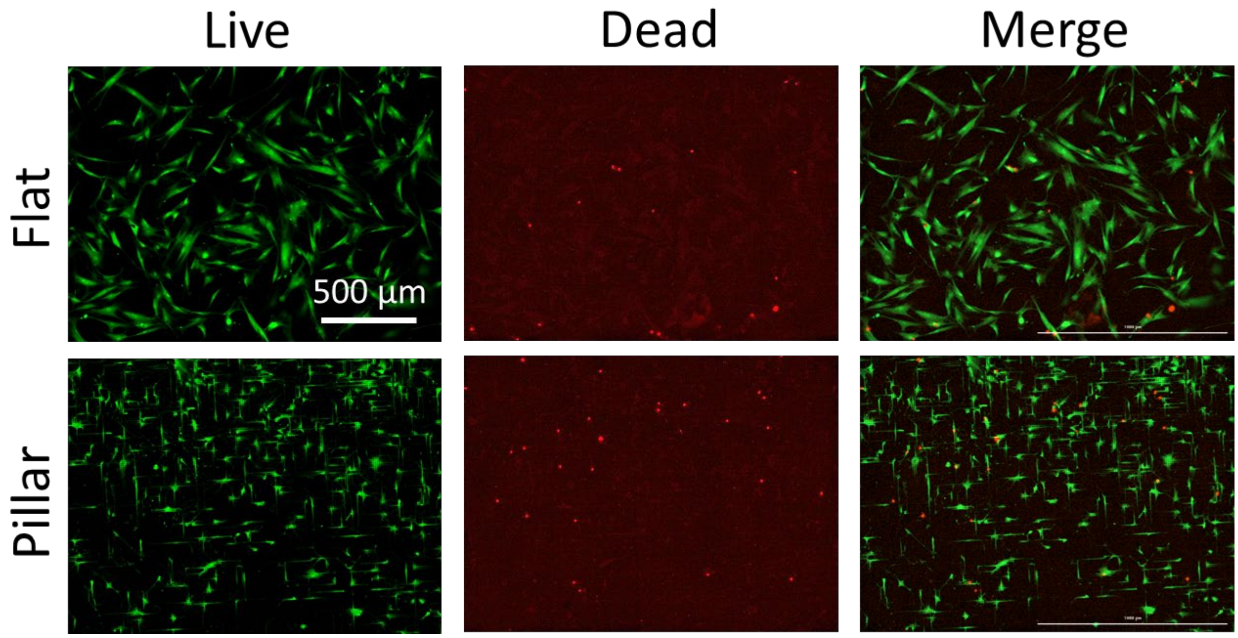
Supplementary Figure S2. Characterization of hydrophilicity of the mPOC/HA implants. a. Representative images show a water droplet on a flat or micropillar surface captured at the initial state or after stabilization (5 min). Analysis of water contact angle at **b.** initial and **c.** stabilized state. N.S., no significant difference, *****p*< 0.0001, n = 3.



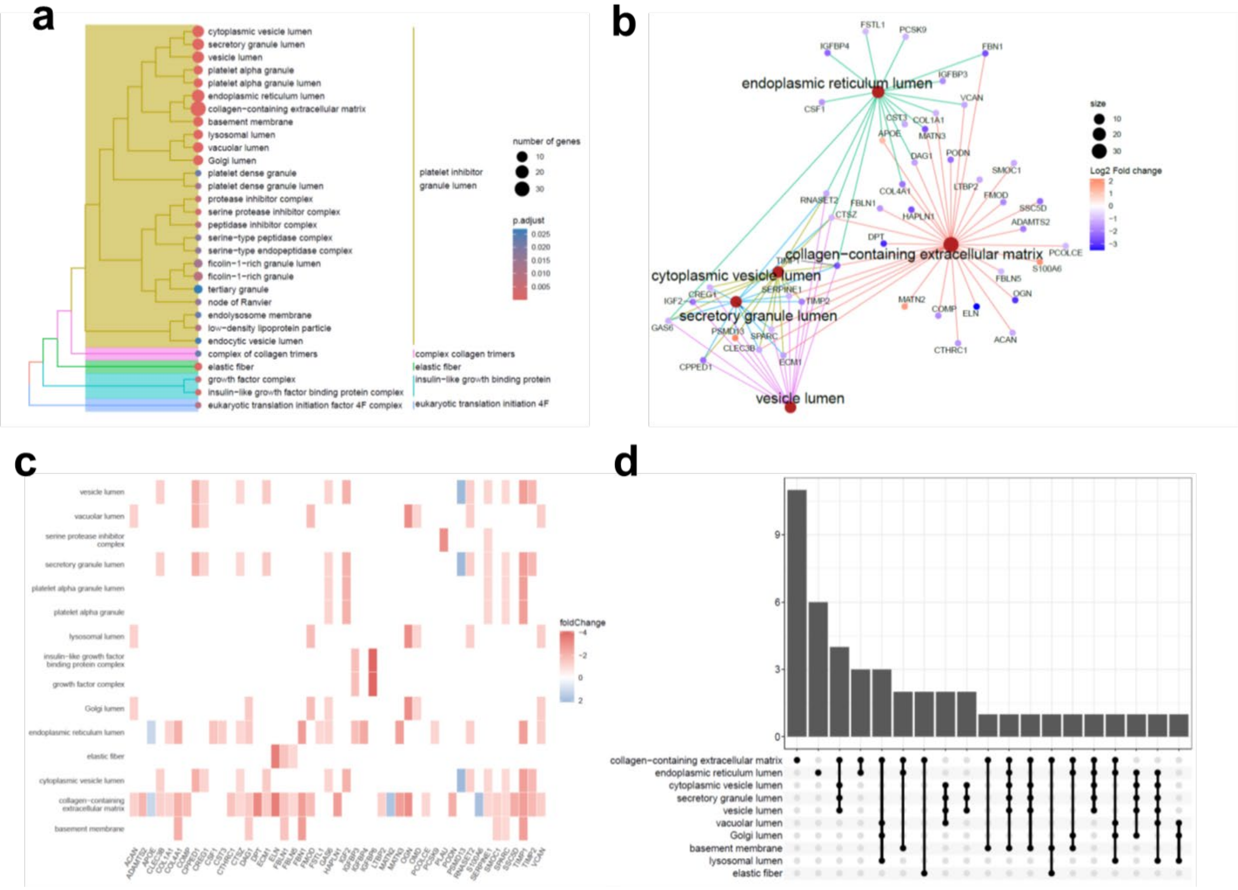
Supplementary Figure S3. Mechanical test of flat and micropillar mPOC/HA implants. a. Representative indentation-force curves collected from flat and micropillar implants. **b.** illustration of lateral deflection. c. Young's Modulus of flat and micropillar implants, as well as the calculated lateral modulus of micropillars. Insert: enlarged plot of later modulus of micropillars, *****p* < 0.0001, n = 3.



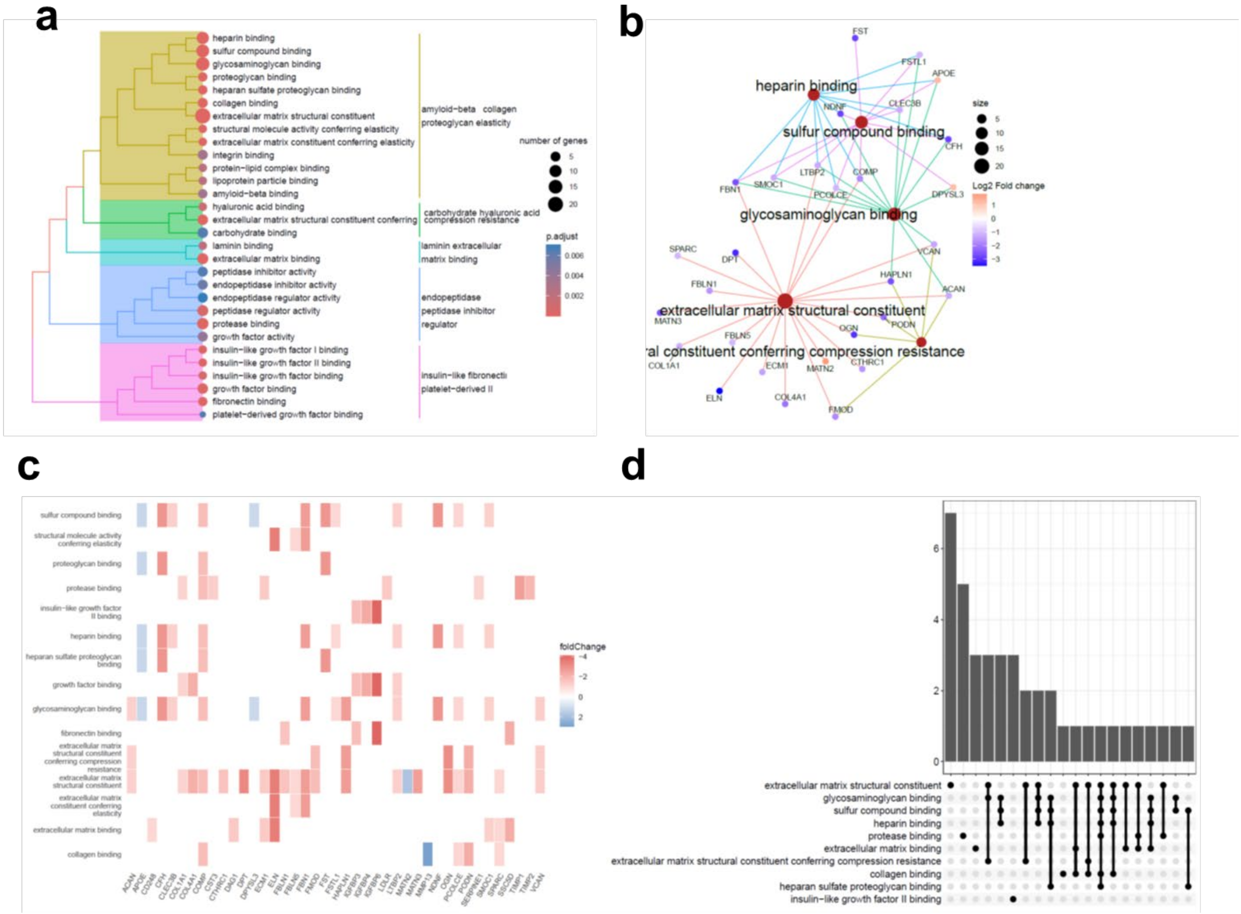
Supplementary Figure S4. 3D morphological features on cell nuclei. a. Surface area, **b.** project area, and **c.** height of cell nuclei on flat and micropillar mPOC/HA surfaces. n=35, **** p<0.0001.



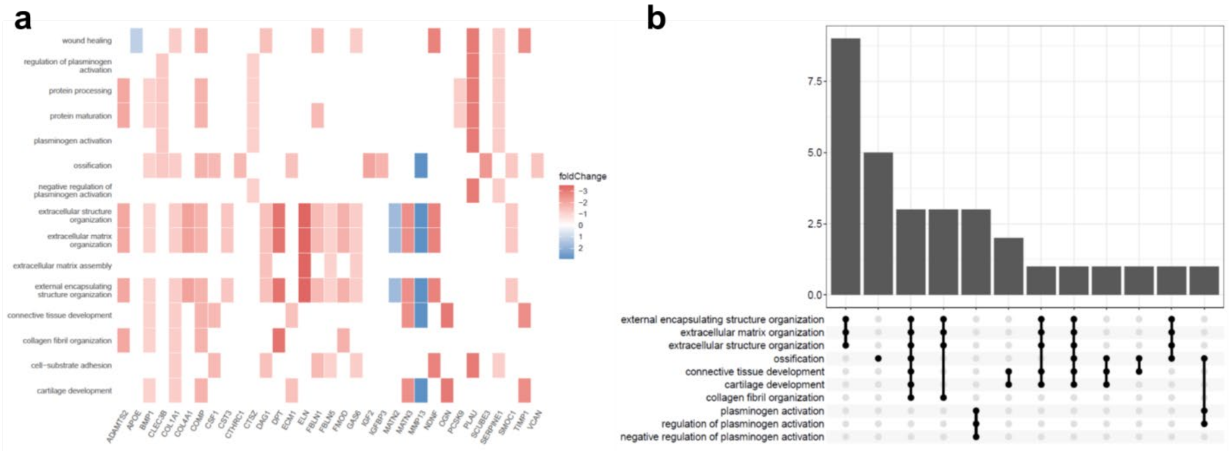
Supplementary Figure S5. Live/dead staining of hMSCs on flat and micropillar mPOC/HA surfaces after 24 h culture.



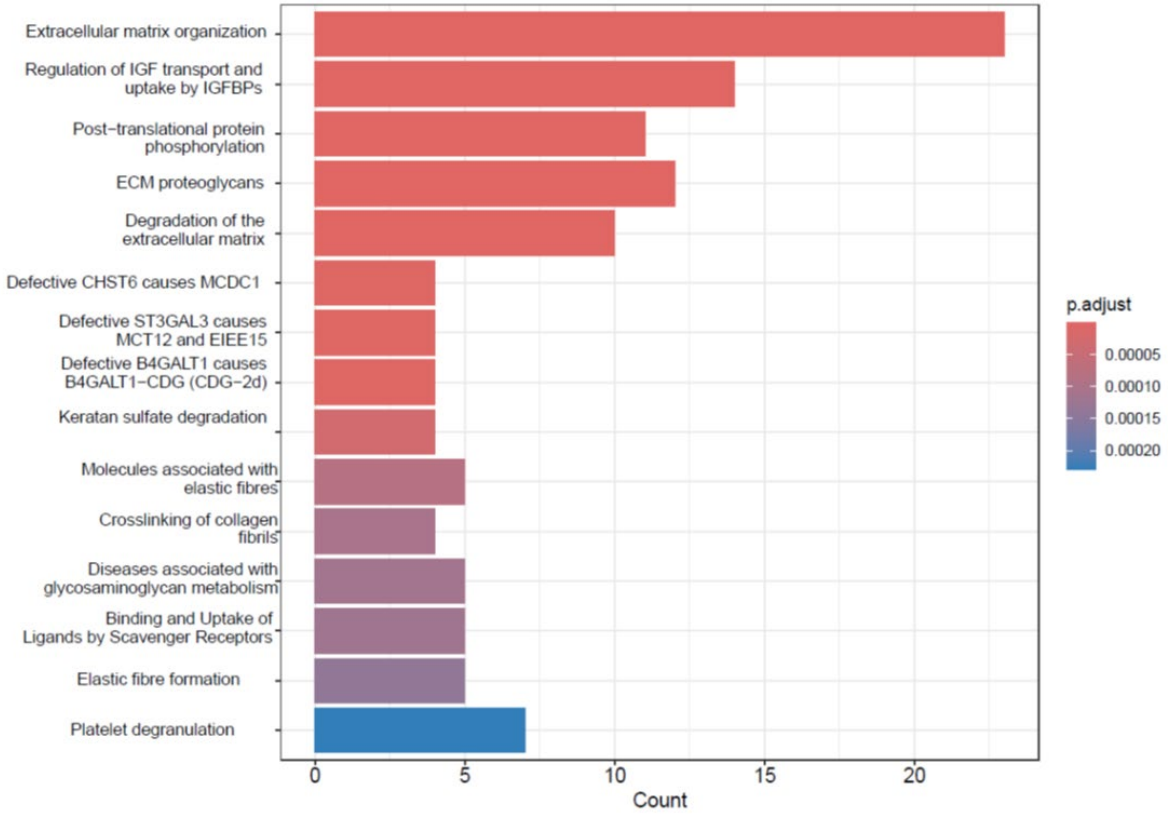
Supplementary Figure S6. GO analysis shows the biological domain with respect to the cellular component. a. Hierarchical clustering of enriched terms. It relies on the pairwise similarities of the enriched terms. The agglomeration method was average. **b**. The linkages of proteins and GO terms as a network. **c.** The heat-plot display the relationships between proteins and enriched terms. **d.** the protein overlapping among different GO gene sets.



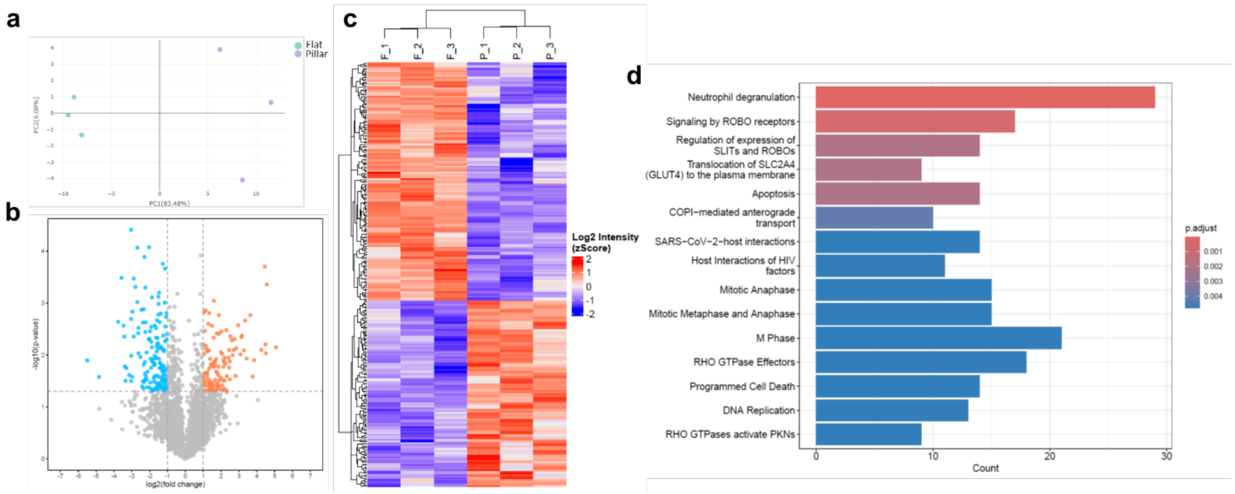
Supplementary Figure S7. GO analysis shows the biological domain with respect to the molecular function. a. Hierarchical clustering of enriched terms. **b**. The linkages of proteins and GO terms as a network. **c.** The heat-plot display the relationships between proteins and enriched terms. **d.** the protein overlapping among different GO gene sets.



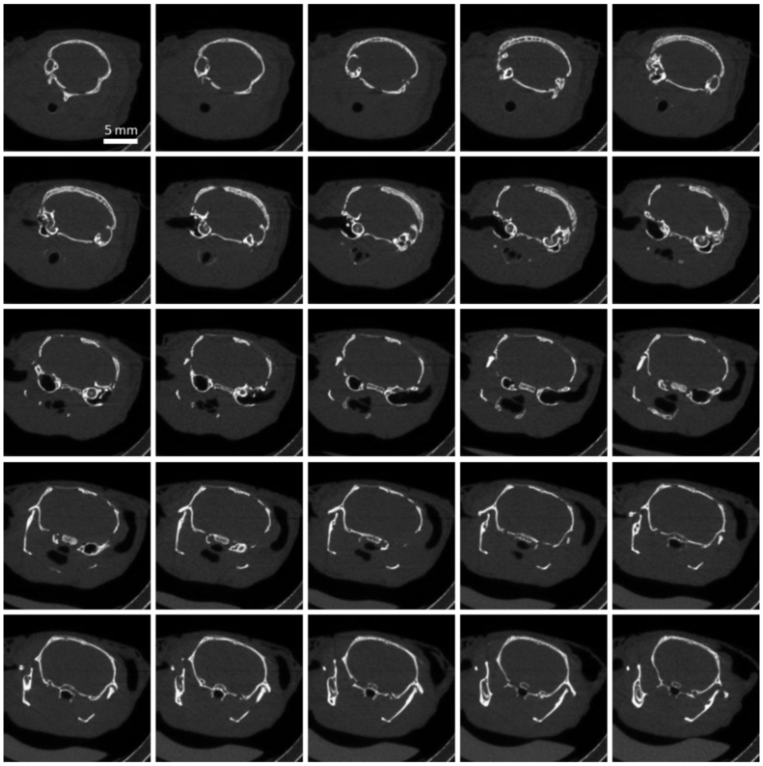
Supplementary Figure S8. GO analysis shows the biological domain with respect to the biological process. a. The heat-plot display the relationships between proteins and enriched terms. **b.** the protein overlapping among different GO gene sets.



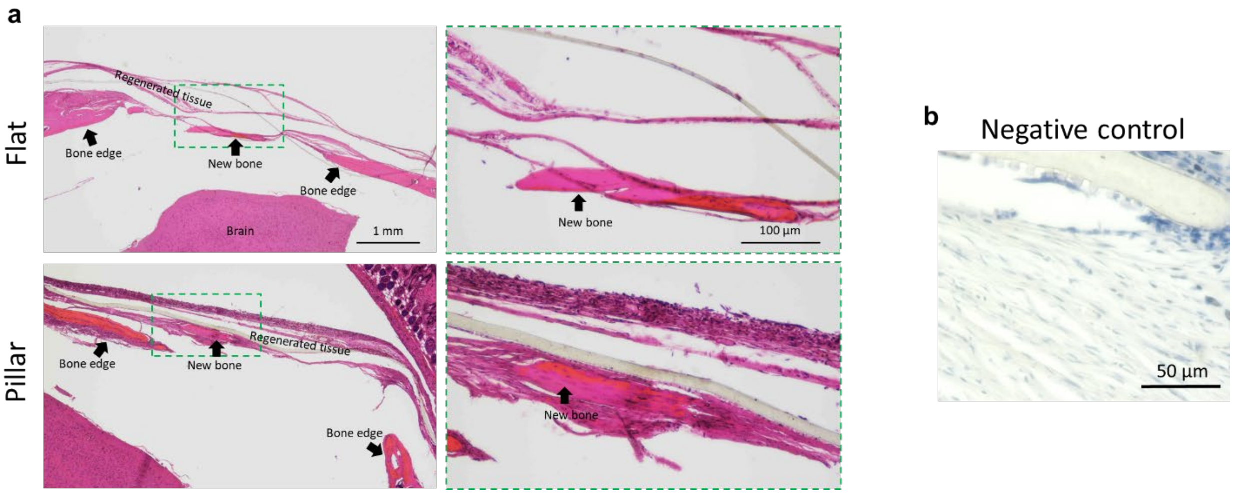
Supplementary Figure S9. Reactome pathway analysis. Top 15 most significant enriched pathways.



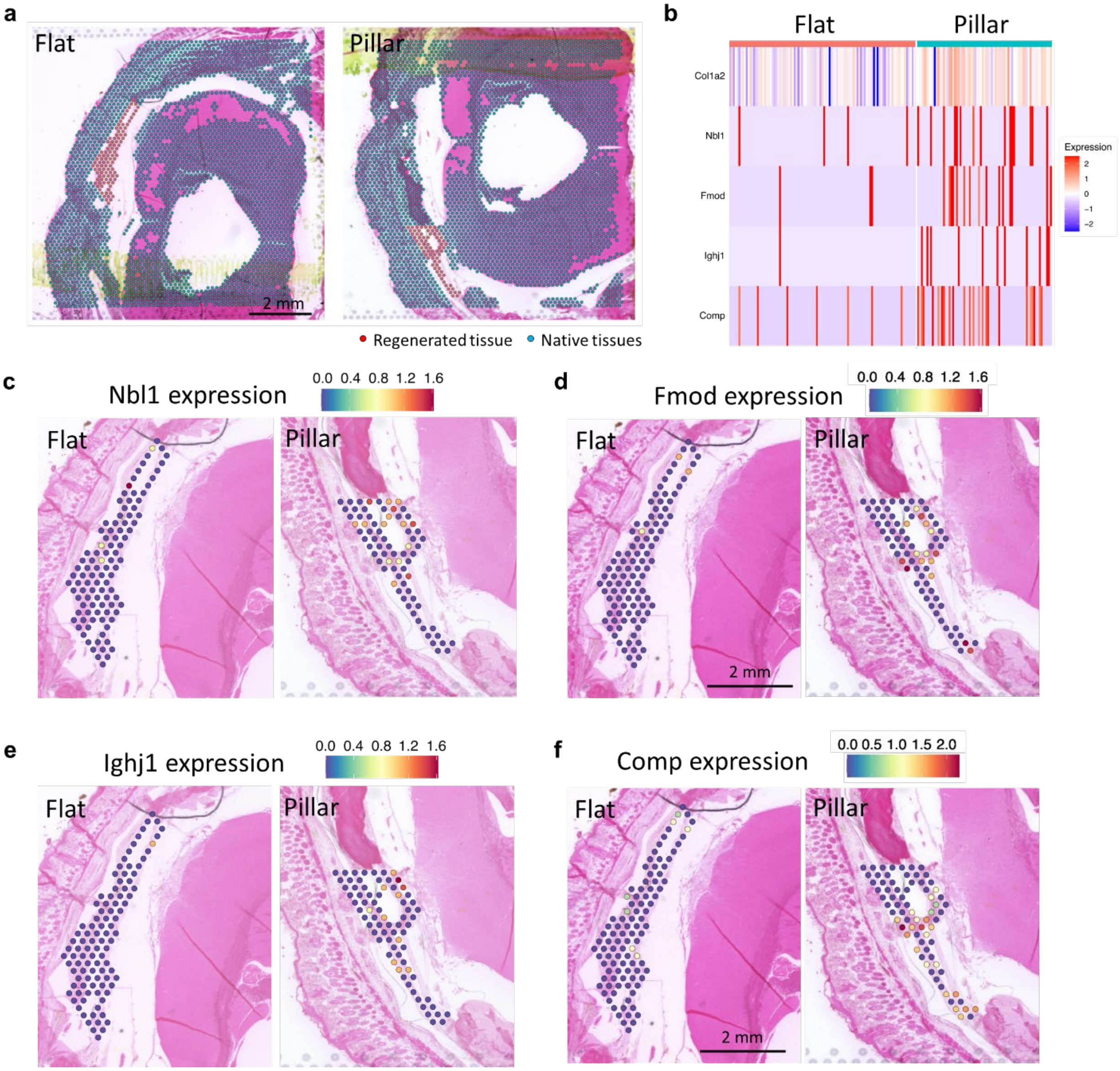
Supplementary Figure S10. Proteome analysis of cells cultured on flat and micropillar mPOC/HA scaffolds. a. PCA plot and **b.** volcano plot of the proteins collected inside cells. c. Heatmap of all significantly differently expressed proteins on flat and micropillar samples. d. Plot of the top 15 most significant enriched pathways based on reactome pathway analysis.



Supplementary Figure S11. Stack images show the regenerated tissue treated with flat (left) and micropillar (right) implants.



Supplementary Figure S12. a. H&E staining of the defect tissue treated with flat and micropillar implants. **b.** Negative control of IHC staining without primary antibody incubation.



Supplementary Figure S13. Spatial transcriptomic (ST) analysis of regenerated tissues. a. Spatial distribution and clustering of ST spots in flat and pillar group. Blue: ST spots in non-ROI (region of interest); red: ST spots in ROI. **b.** Heatmap shows the expression of the significantly differentially expressed genes. Spatial plots of **c.** Nbl1 expression profile, **d.** Fmod expression profile, **e.** Ighj1 expression profile, and **f.** Comp expression profile in tissues regenerated with flat mPOC/HA implant and micropillar mPOC/HA implant. Nbl1: neuroblastoma suppressor of tumorigenicity 1; Fmod: fibromodulin; Ighj1: Immunoglobulin Heavy Joining 1; Comp: cartilage oligomeric matrix protein.