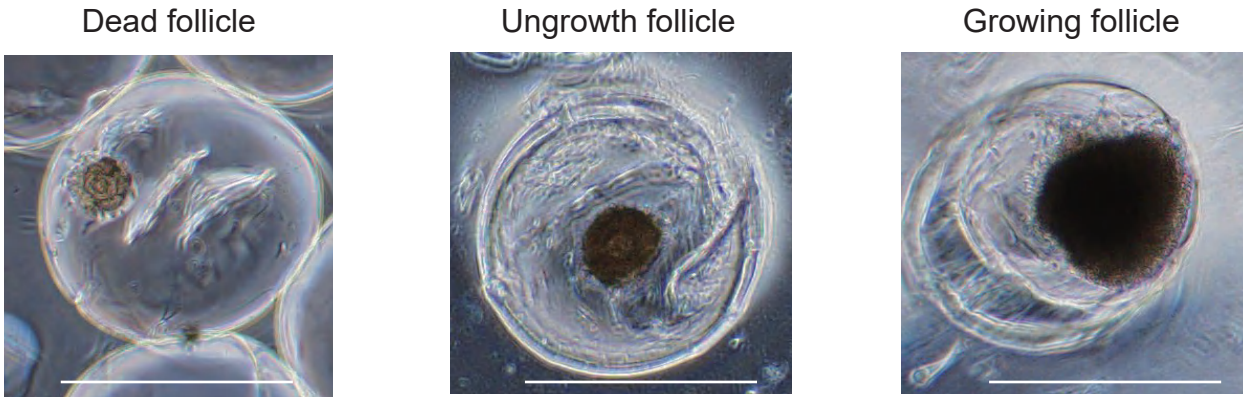


Figure S1. The death/live ratio was measured according to the intact morphology of follicles and oocytes, the scale bar was 500 μm.

S2

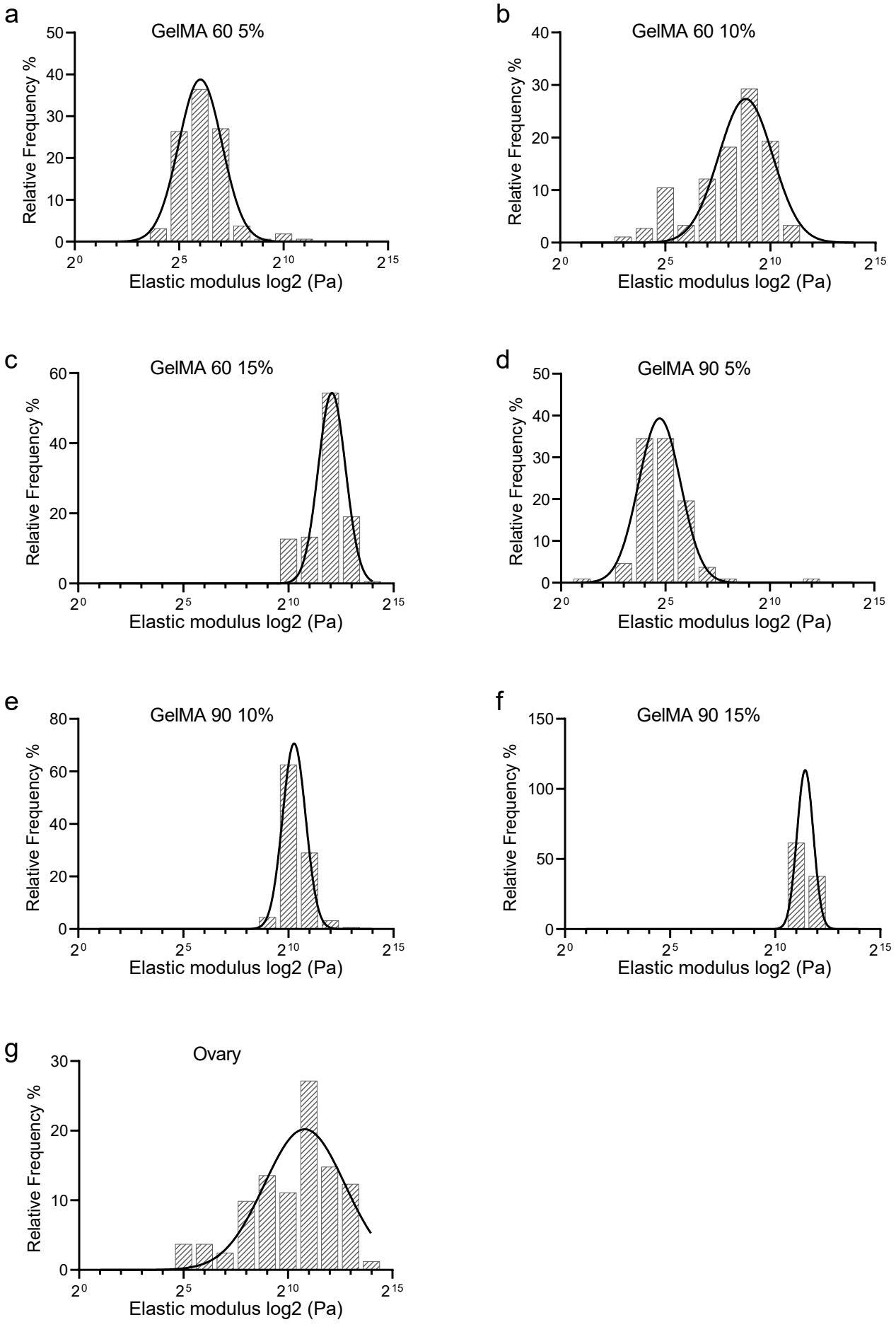


Figure S2. The elastic modulus distribution frequency of 6 GelMA conditions and ovary.The lognormal fitting curve was performed for an individual sample.

S3

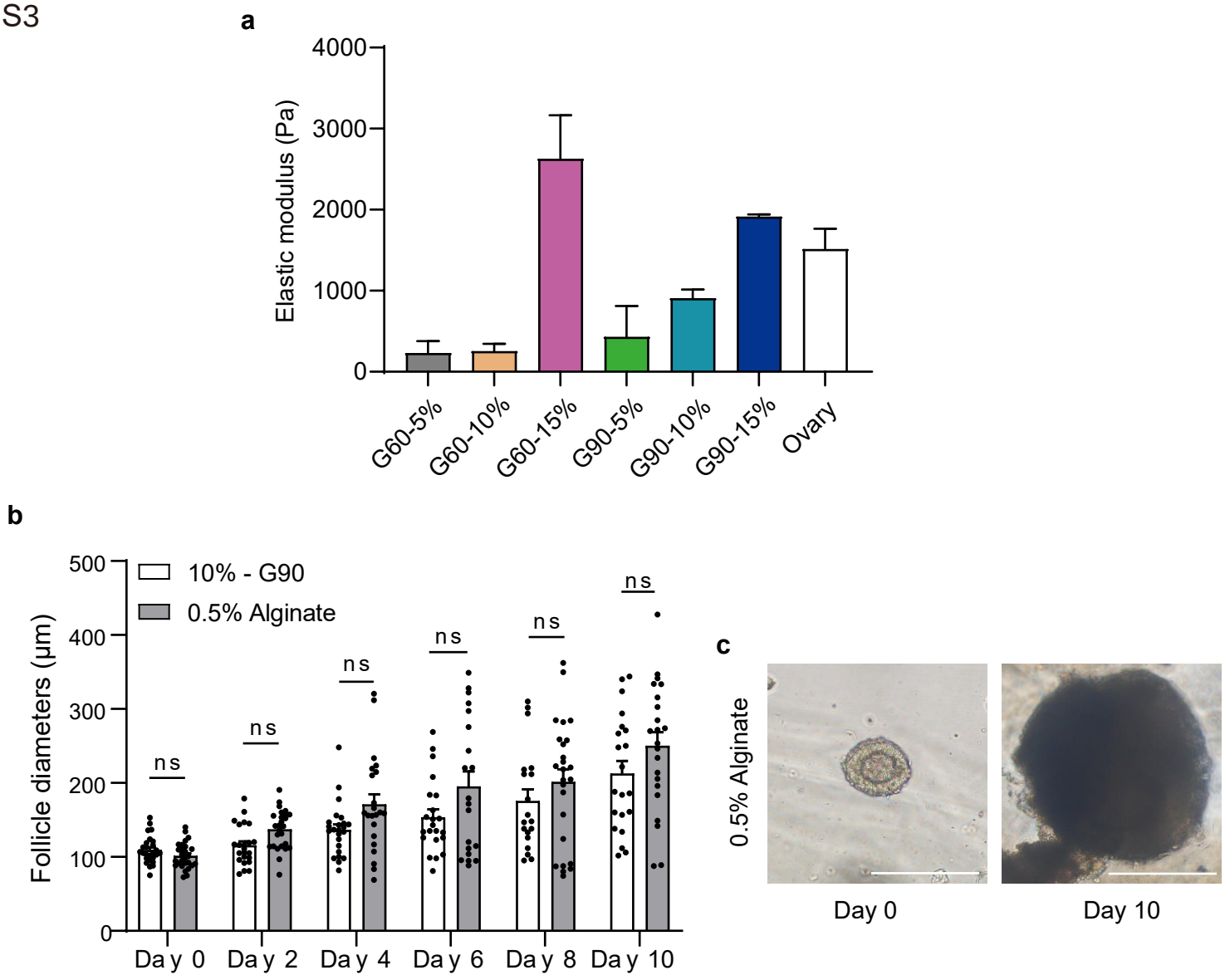


Figure S3. a. The elastic modulus test of G60 - 5%, G60 - 10%, G60 - 15%, G90 - 5%, G90 - 10%, G90 - 15%, and ovary. The error bar stands for SEM, n=2, the data passed one-way ANOVA with p value < 0.01. **b**. Evaluation of follicular growth between 10% - G90 and 0.5% alginate, $p > 0.05$ passed two-way ANOVA test. **c**. The follicular morphology in 0.5% alginate condition.

S4

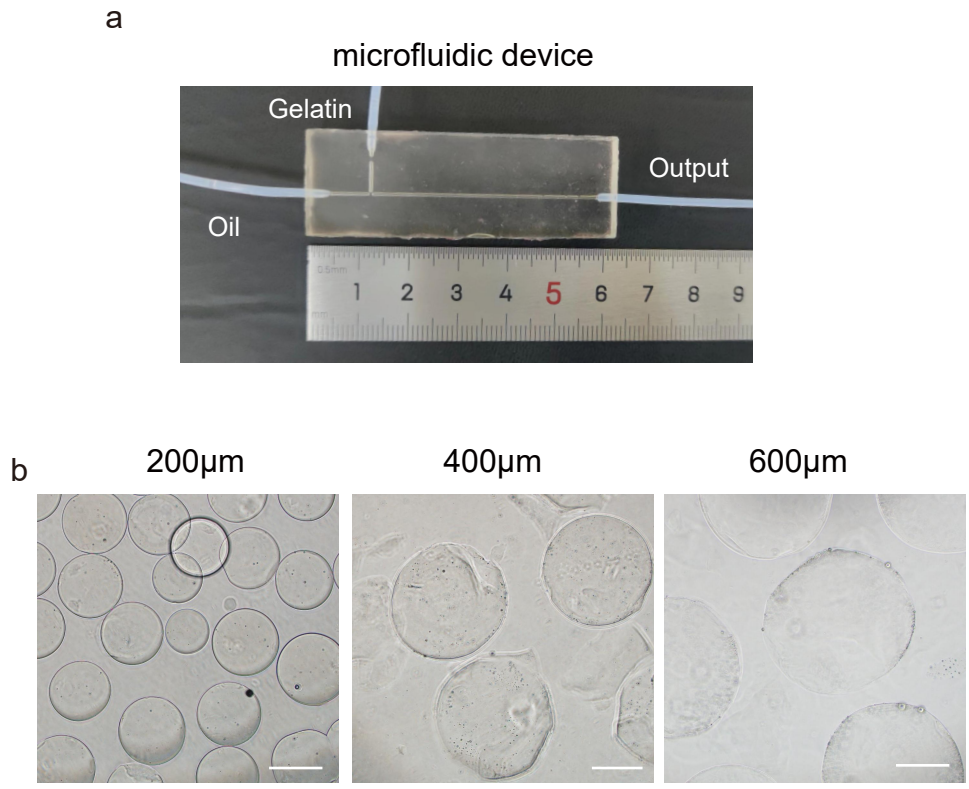


Figure S **4**. The microfluidic device (a) and gelatin microspheres in different diameters (b), the bar was 200 μm.

S5

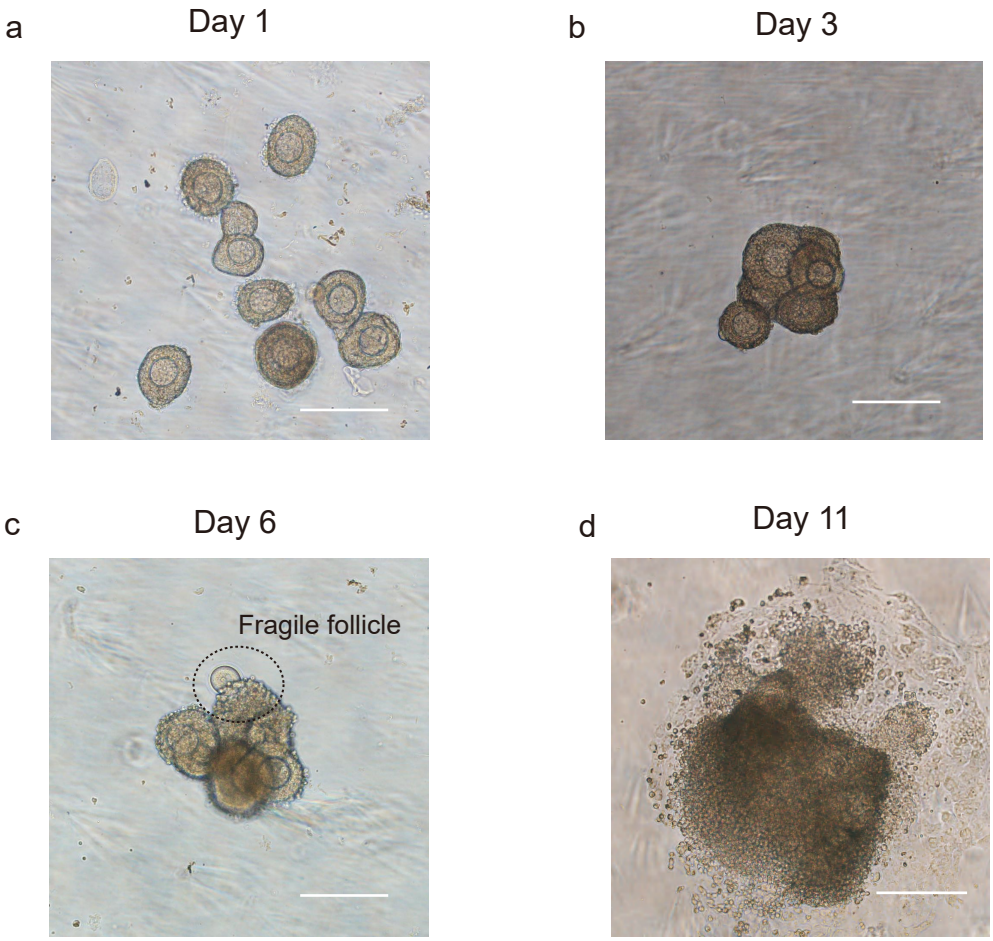


Figure S5. Follicles under G90 - 10% condition without microspheres. **a**. Follicles were cultured for 1 day, and none of the follicles were attached to the GelMA surface. **b**. Follicles formed a cluster instead of attaching to the GelMA surface. **c**. After culturing for 6 days, some follicles became fragile and released the oocyte. **d**. After culturing for 11 days, granulosa cells dissociated from the follicles. The bar was 200 μm.

S6

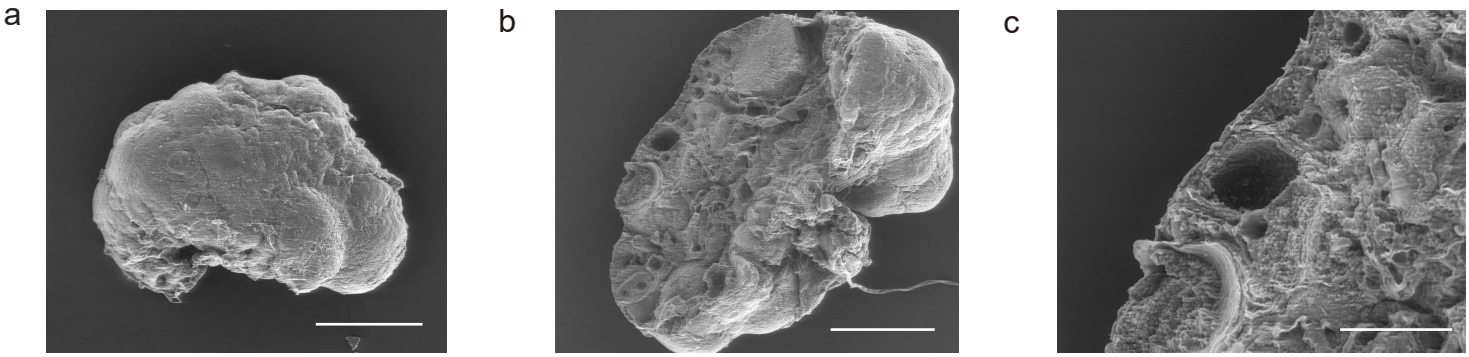


Figure S6. Follicles grew under the surface epithelium. **a.** SEM imaged the intact ovary, the scale bar was 400 μm. **b.** SEM visualized the follicle cavity under the surface epithelium, the scale bar was 400 μm. **c.** The amplified image of the follicle cavity, the scale bar was 100 μm.

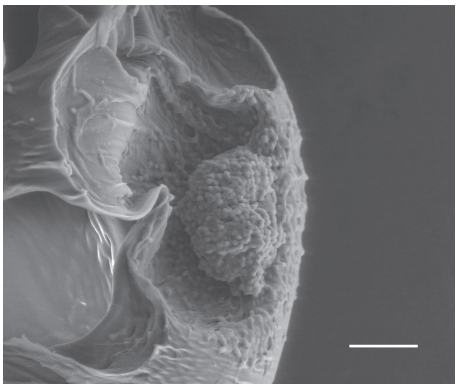
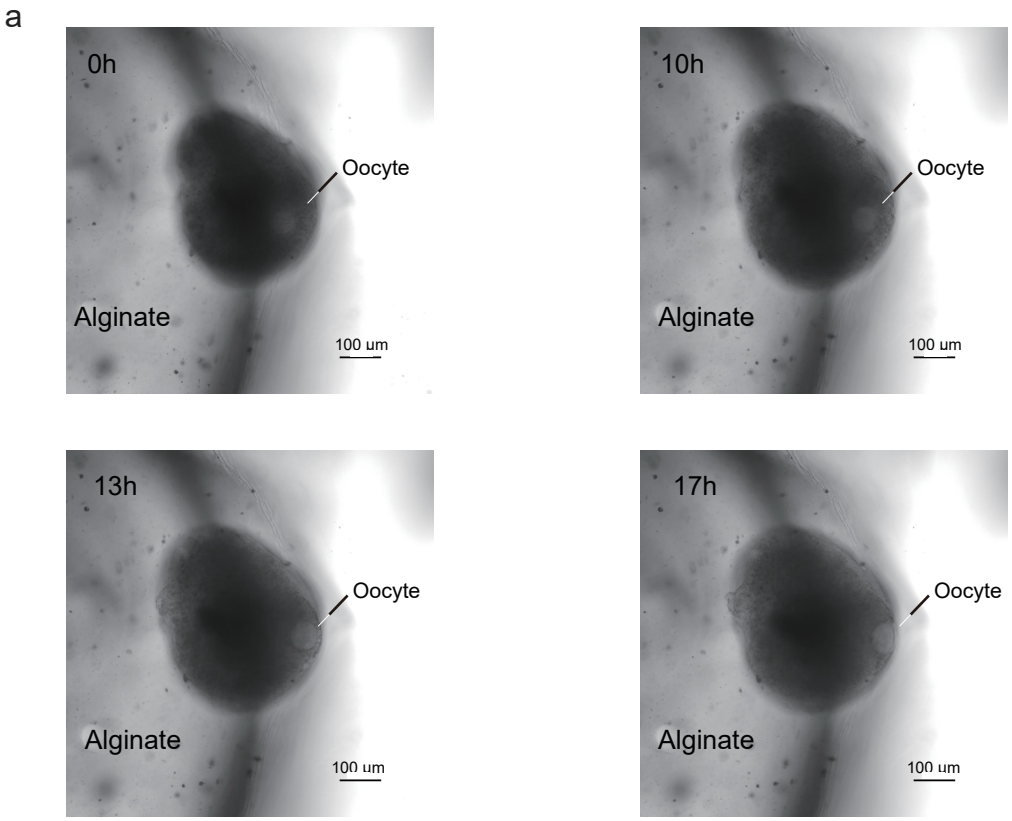


Figure S7. SEM visualized the follicle to confirm its growing location within the cavities. The scale bar was 50 μm.



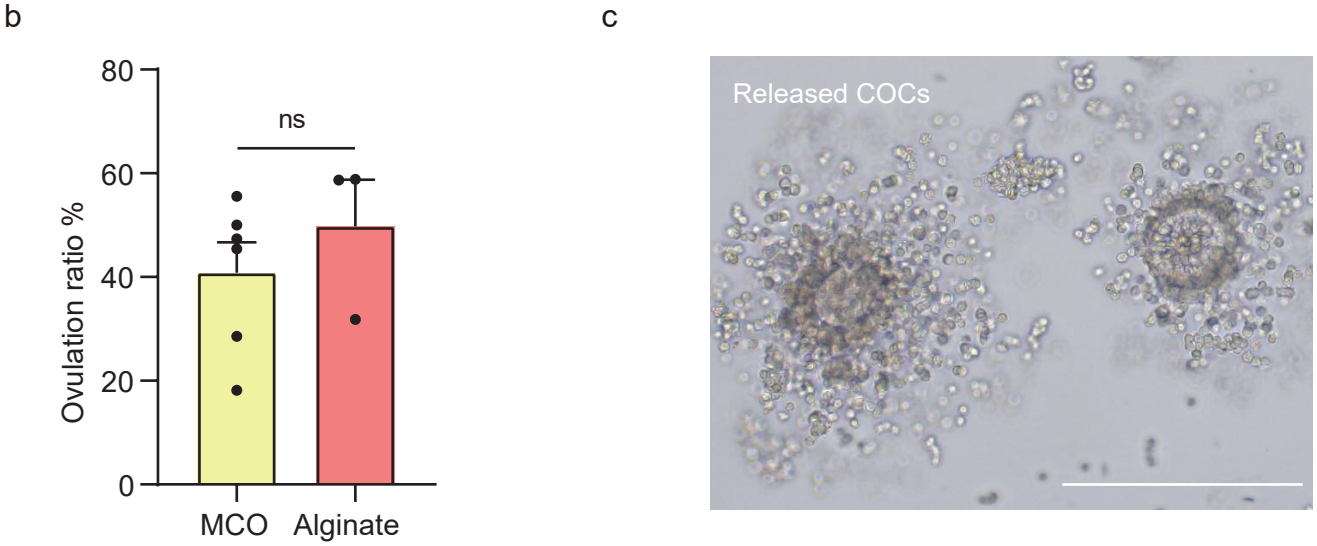


Figure S8. **a**. Time-lapse of follicles cultured in alginate hydrogel. The follicle received the simulus of hCG to ovulate the oocyte, but the process was impeded by the alginate barrier, the scale bar was 100 μm. **b**. The ovulation ratio bewteen MCO and alginate condition showed no obvious different, $p > 0.05$ through T test. **c**. COCs were released out of the MCO into the medium, the scale bar was 200 μm.

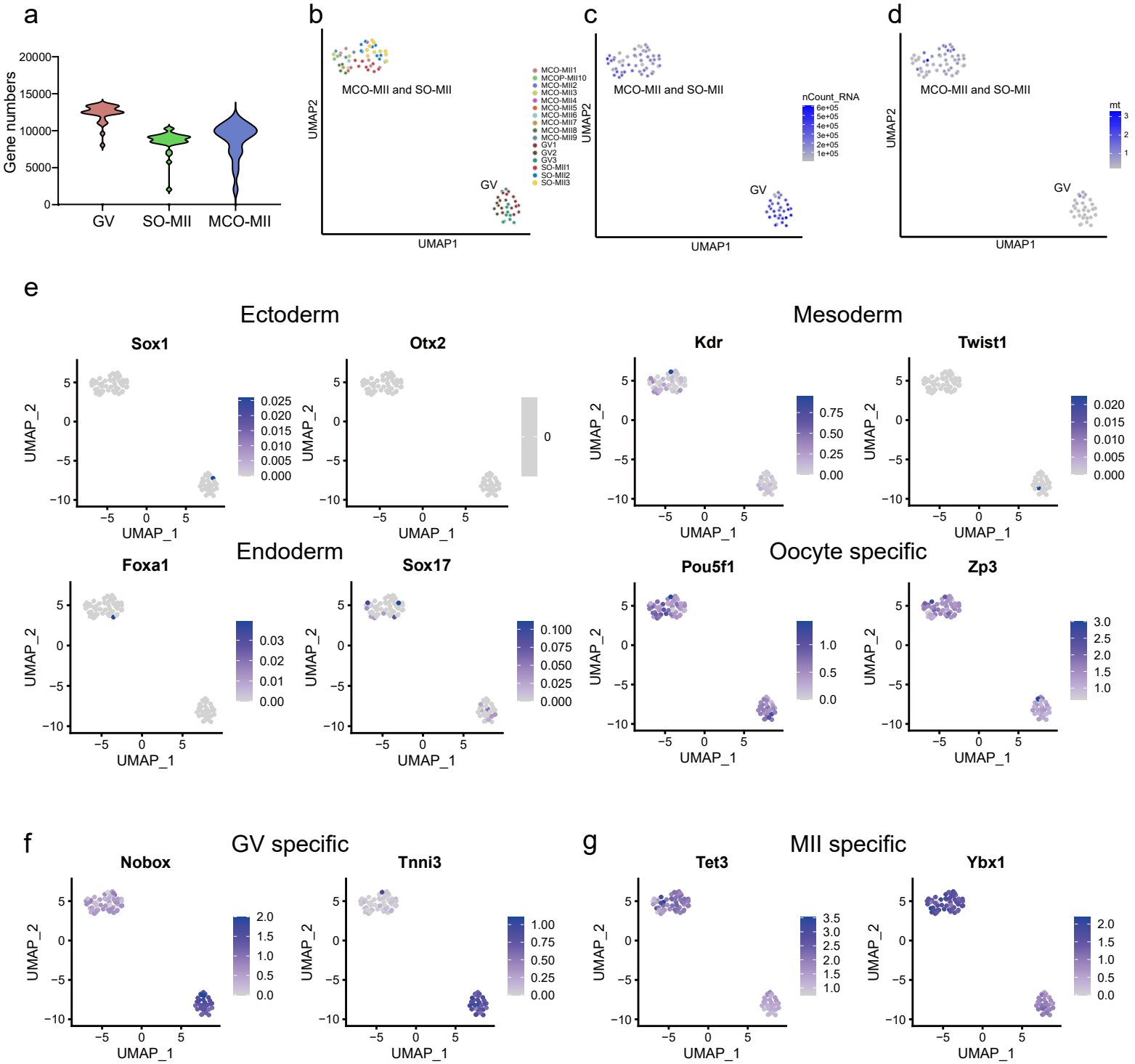


Figure S9. Quality control of GV, MII, and OLM oocytes. **a**. The average number of expressed genes in GV, MII, and OLM oocytes. **b**. The batch effect analysis by UMAP plot. **c**. The UMI counts analysis by UMAP plot. **d**. The mitochondrial contamination test by UMAP plot. **e**. Feature plots of ectoderm, mesoderm, endoderm, and oocyte markers. **f**. GV oocytes specific genes by feature plot. **g**. MII oocytes specific genes by feature plot.

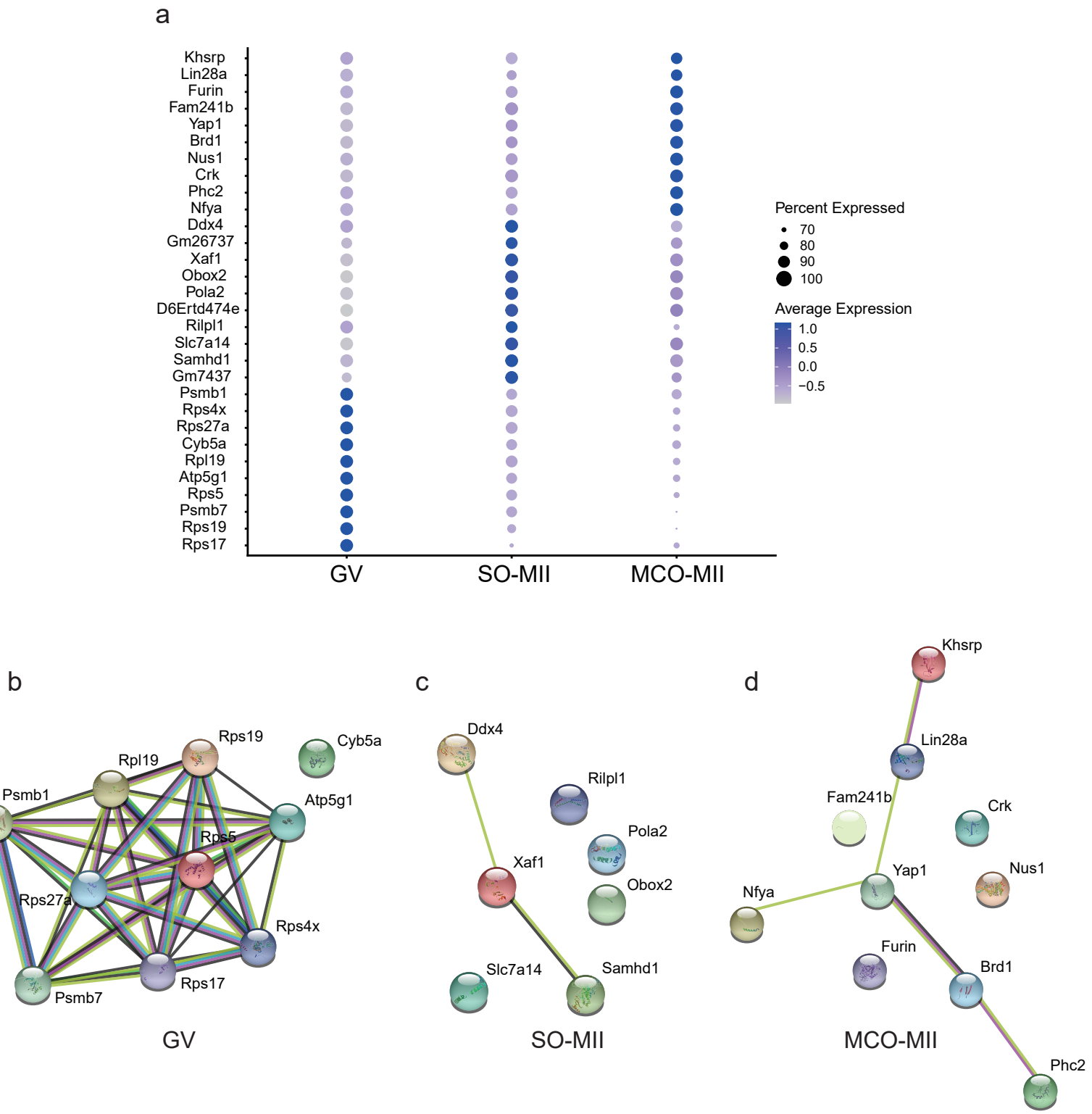


Figure S10. DEGs might affect the oocyte status. **a**. Top 10 DEGs of GV, SO-MII, and MCO-MII oocytes. **b**. Protein network analysis of top 10 DEGs in GV oocytes. **c**. Protein network analysis of top 10 DEGs in SO-MII oocytes. **d**. Protein network analysis of top 10 DEGs in MCO-MII oocytes.

S11

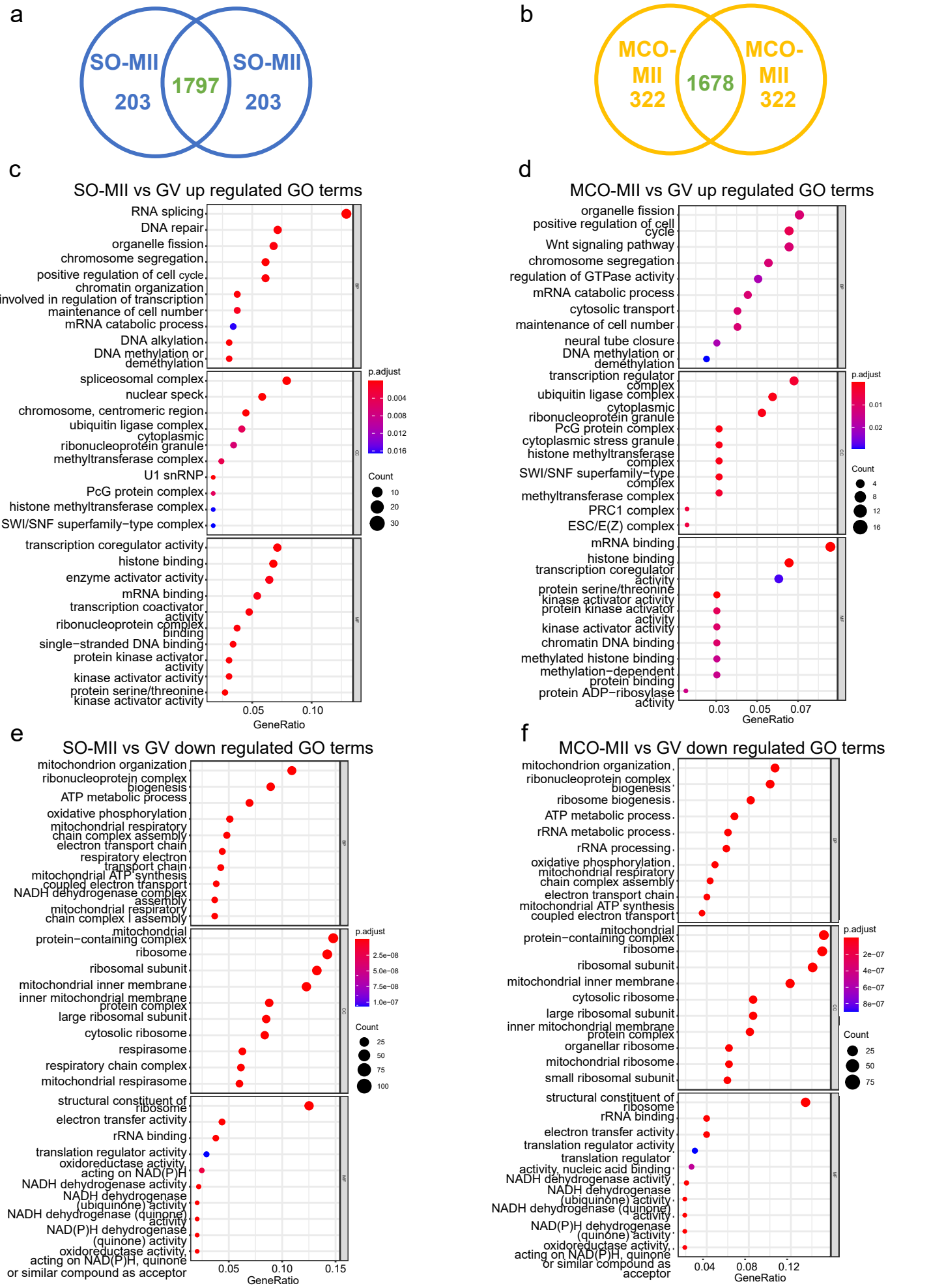


Figure S11. Characterization of DEGs. **a**. The Resampling method revealed DEGs among SO-MII oocytes and visualized by a VENN map. **b**. The Resampling method revealed DEGs among MCO-MII oocytes and visualized by a VENN map. **c**. GO analysis of upregulated DEGs between SO-MII oocytes and GV oocytes. **d**. GO analysis of upregulated DEGs between MCO-MII oocytes and GV oocytes. **e**. GO analysis of downregulated DEGs between SO-MII oocytes and GV oocytes. **f**. GO analysis of downregulated DEGs between MCO-MII oocytes and GV oocytes.

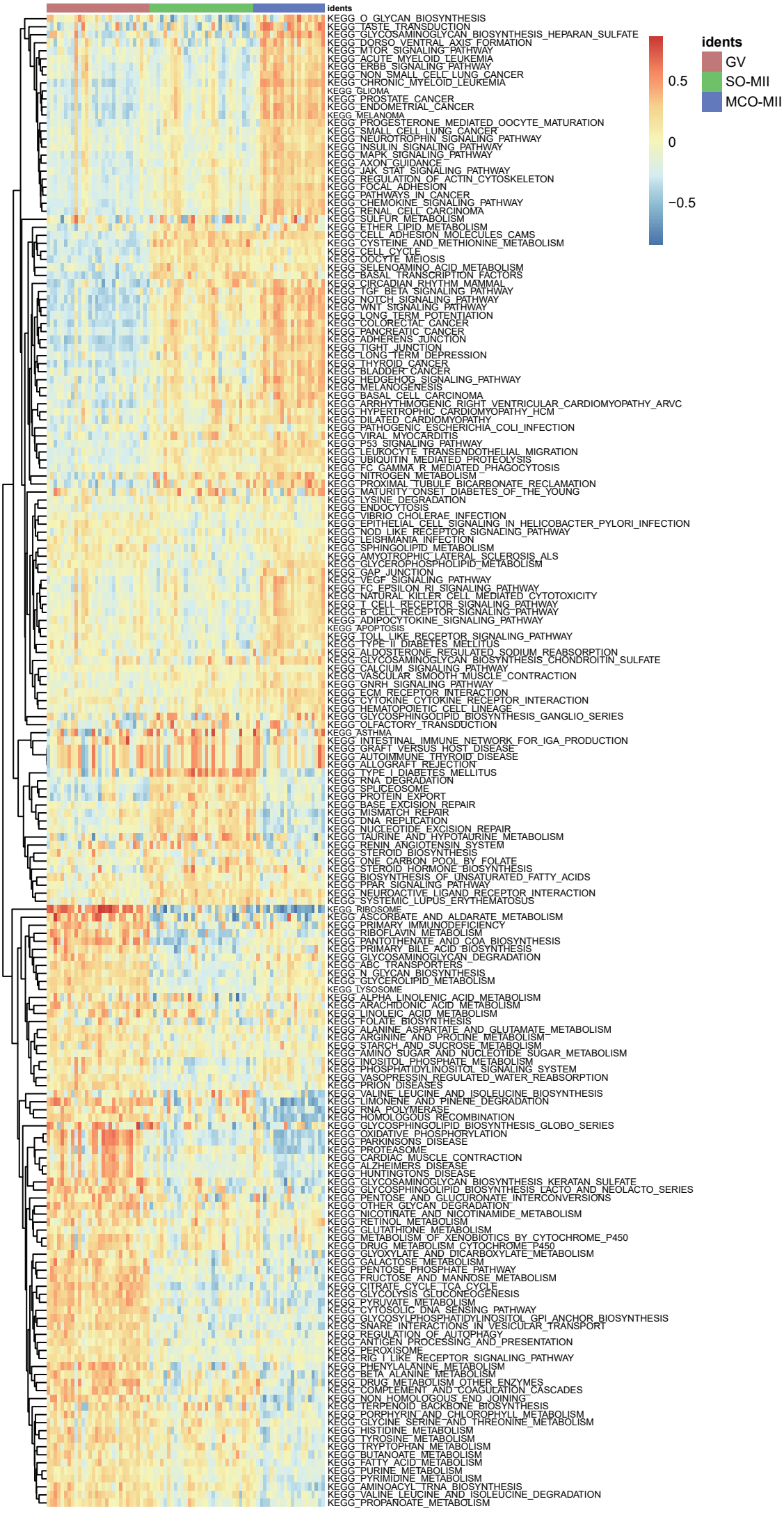


Figure S12. The KEGG pathway analysis.

S13

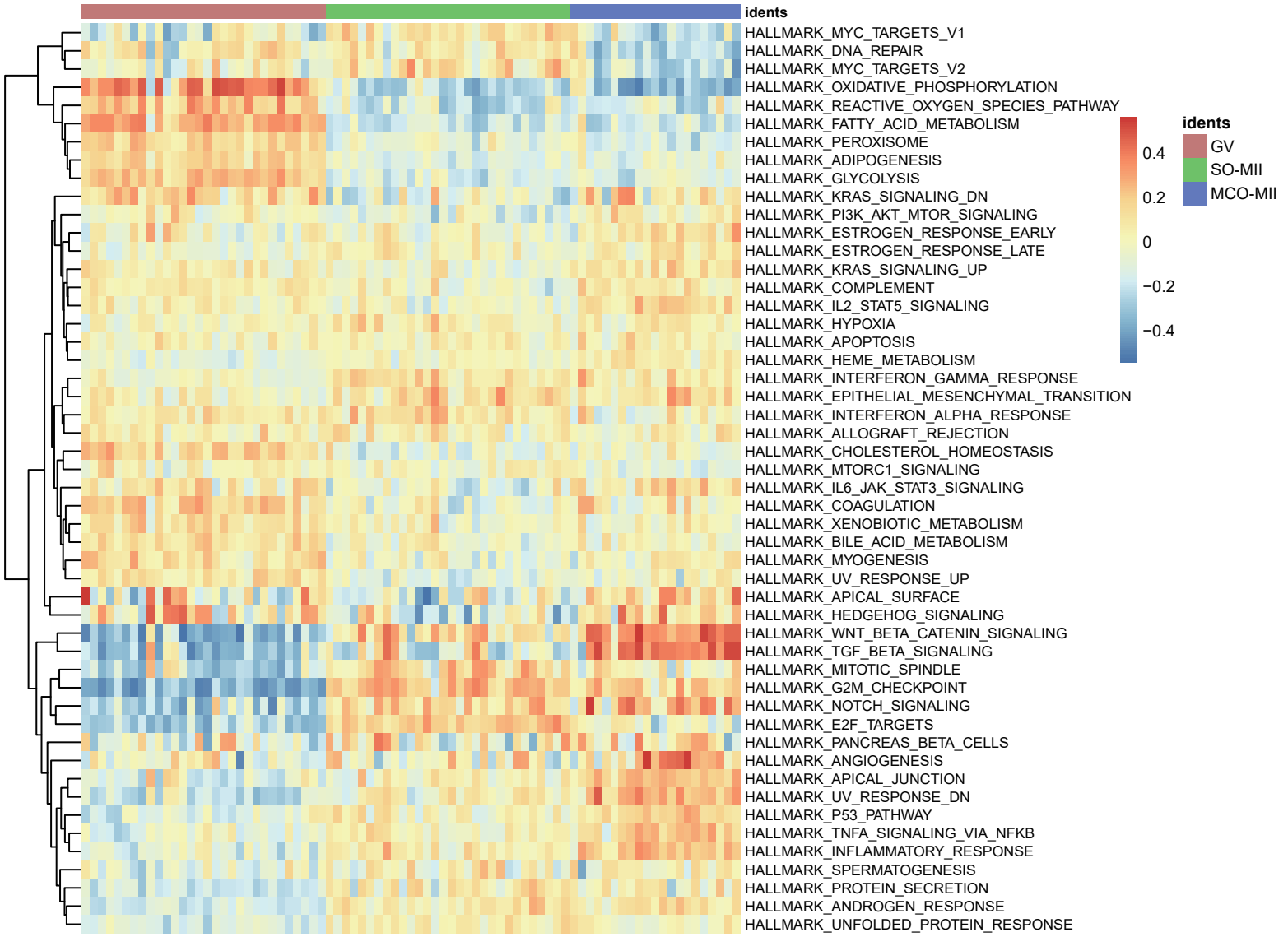


Figure S13. The Hallmarks pathway analysis.

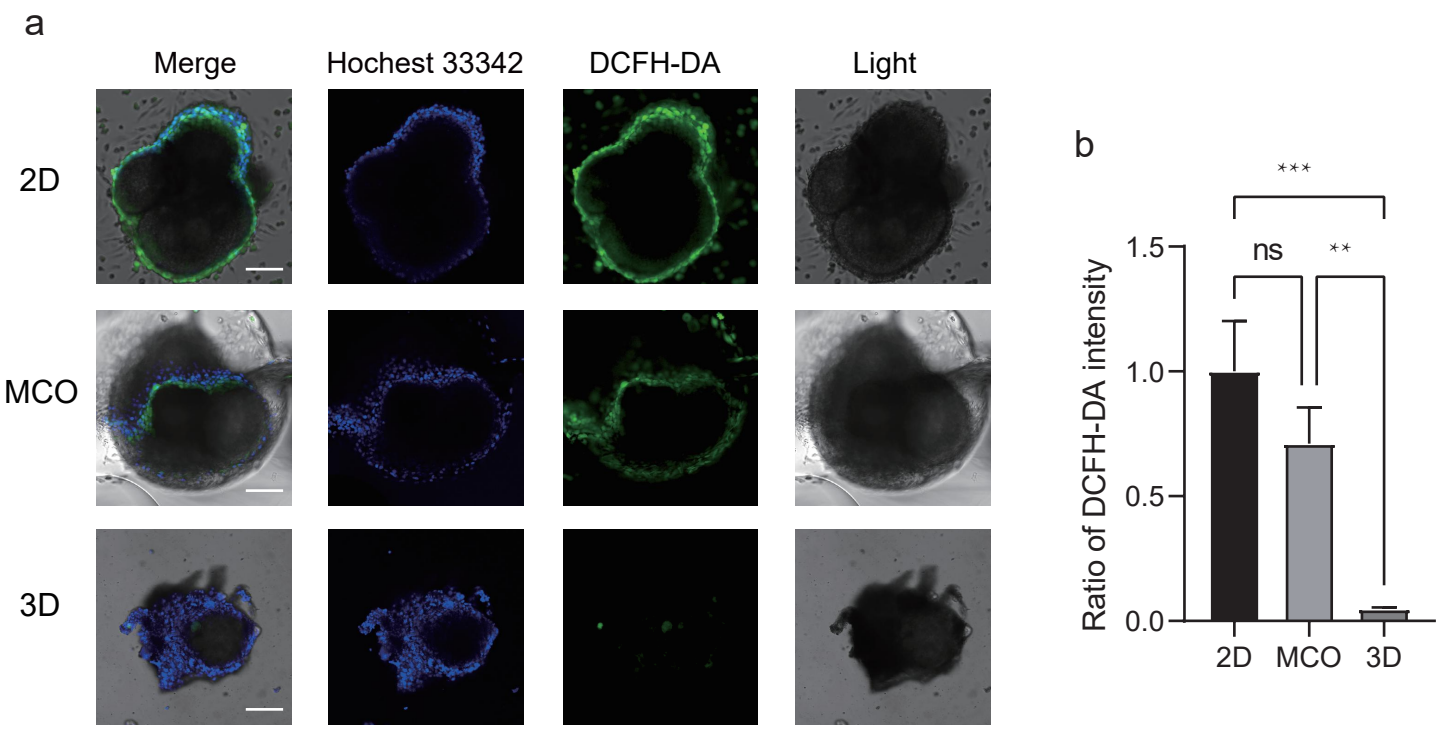


Figure S14. The ROS level in 2D, MCO, and 3D culture system after 24h of H_2O_2 treatment.

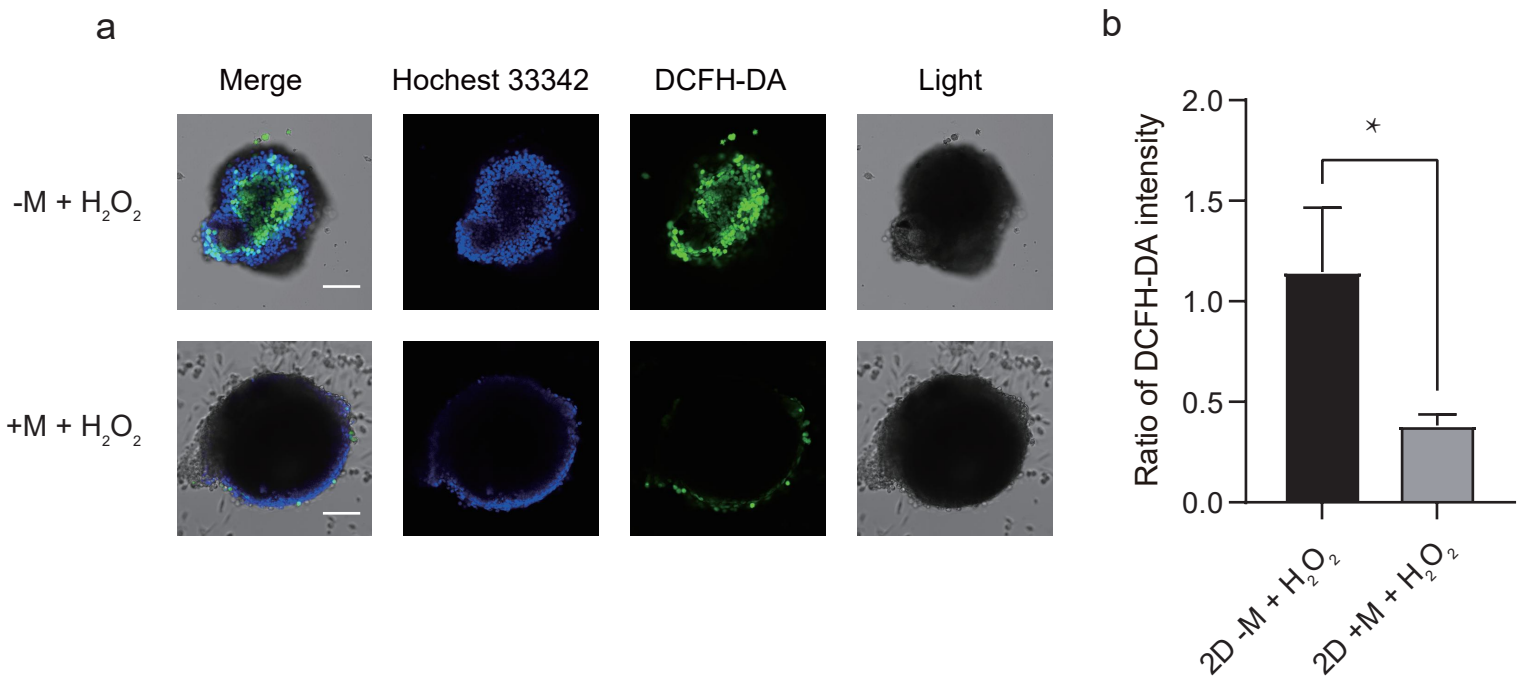


Figure S15. The ROS level in the 2D culture system with/without melatonin. a. The melatonin reduced the ROS level in 2D culture system; b. The fluorescent intensity ratio of the ROS level in the 2D culture system with/without melatonin, the *p* value passed student t test, *: $p < 0.05$.

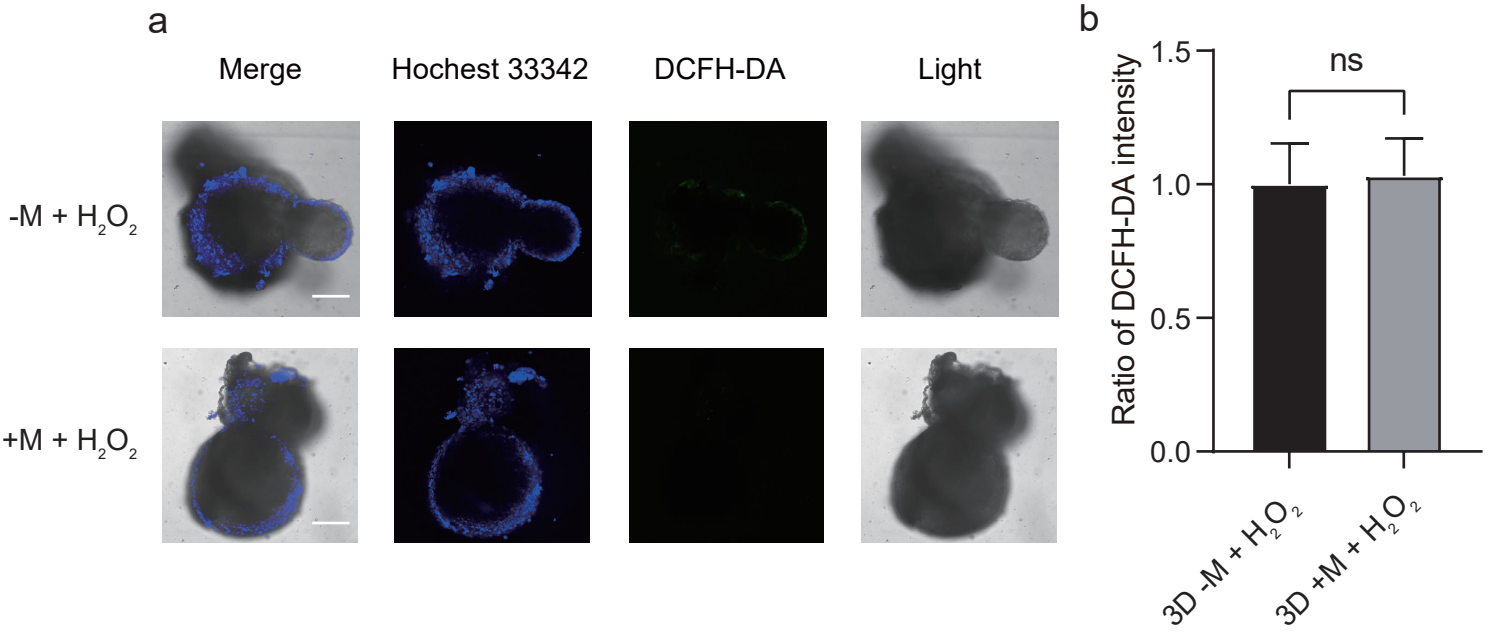


Figure S16. The ROS level in 3D culture system with/without melatonin. a. The ROS level in 3D culture system was hardly to be detected; b. The fluorescent intensity ratio of the ROS level in the 3D culture system with/without melatonin, the *p* value passed student t test, ns: $p > 0.05$.