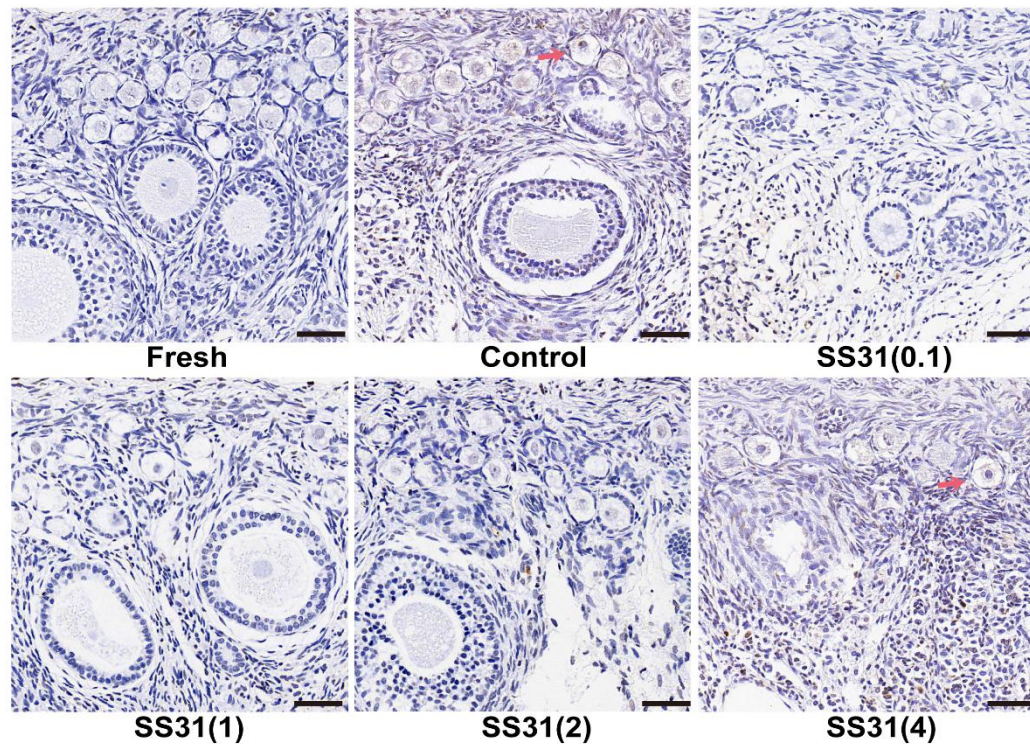


Supplementary Figures

A



B

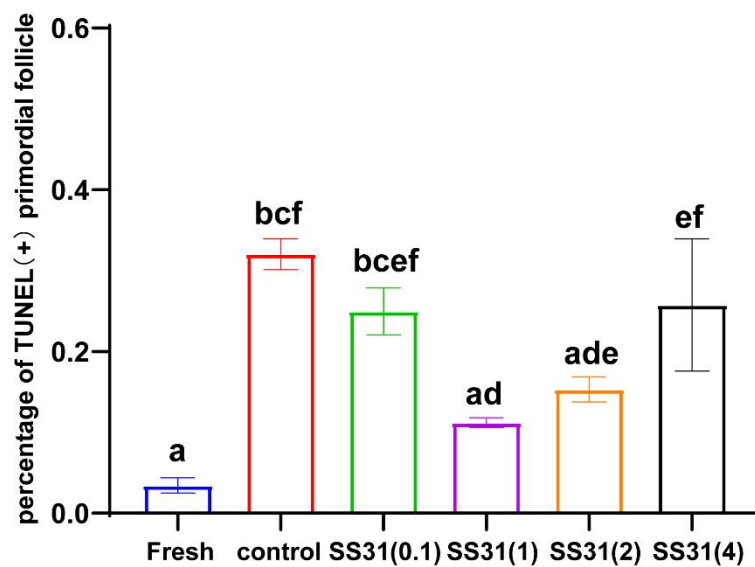


Figure S1. SS31 reduced apoptosis of primordial follicles caused by ovarian freezing and thawing.

A. TUNEL immunohistochemical staining was performed to assess oocyte apoptosis in ovarian tissue across different groups. Scale bars: 50 μ m; red arrows indicate TUNEL-positive oocytes. B. Statistical analysis of oocyte apoptosis was conducted among groups. $n = 5$; Statistical analysis using one-way ANOVA (Multiple comparisons) was performed, different letters indicate statistically significant differences between the two groups ($p < 0.05$). The error bars represent s.e.m.

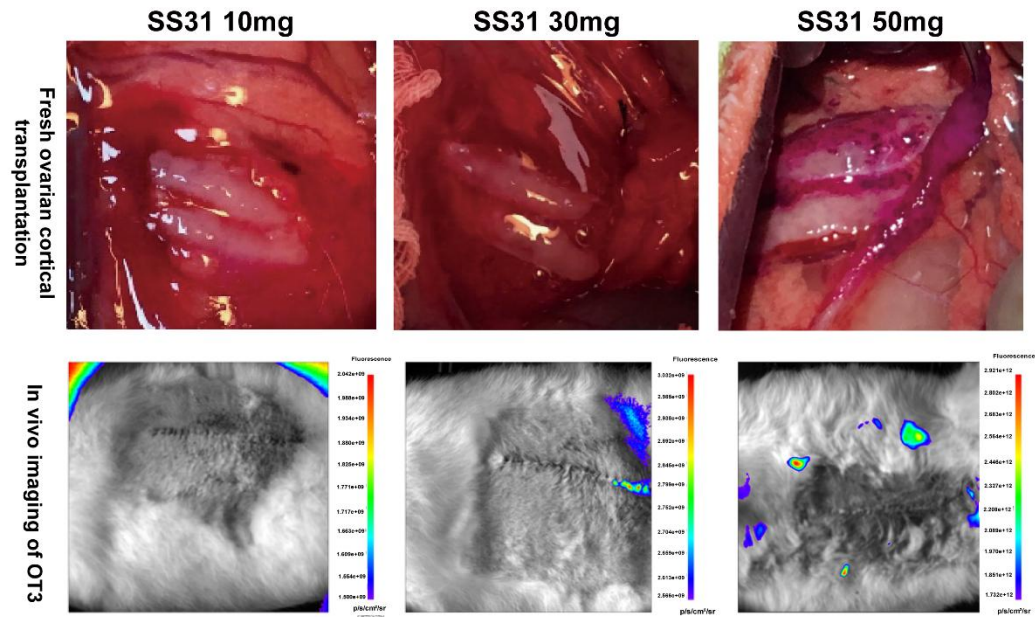


Figure S2. *In vivo* imaging of transplanted ovarian tissues three days post-transplantation. Upper Panel: Fresh ovarian cortical fragments were co-transplanted with GM-CSF gel loaded with 10 mg, 30 mg, or 50 mg of rhodamine-labeled SS31. Lower Panel: *In vivo* imaging was performed three days post-transplantation to detect fluorescence signals corresponding to the doses of rhodamine-SS31 used in the upper panel. When 10 mg of SS31 was loaded, fluorescence signals were nearly undetectable after three days. A weak fluorescence signal was observed with 30 mg of SS31, whereas a strong fluorescence signal was evident with 50 mg of SS31.

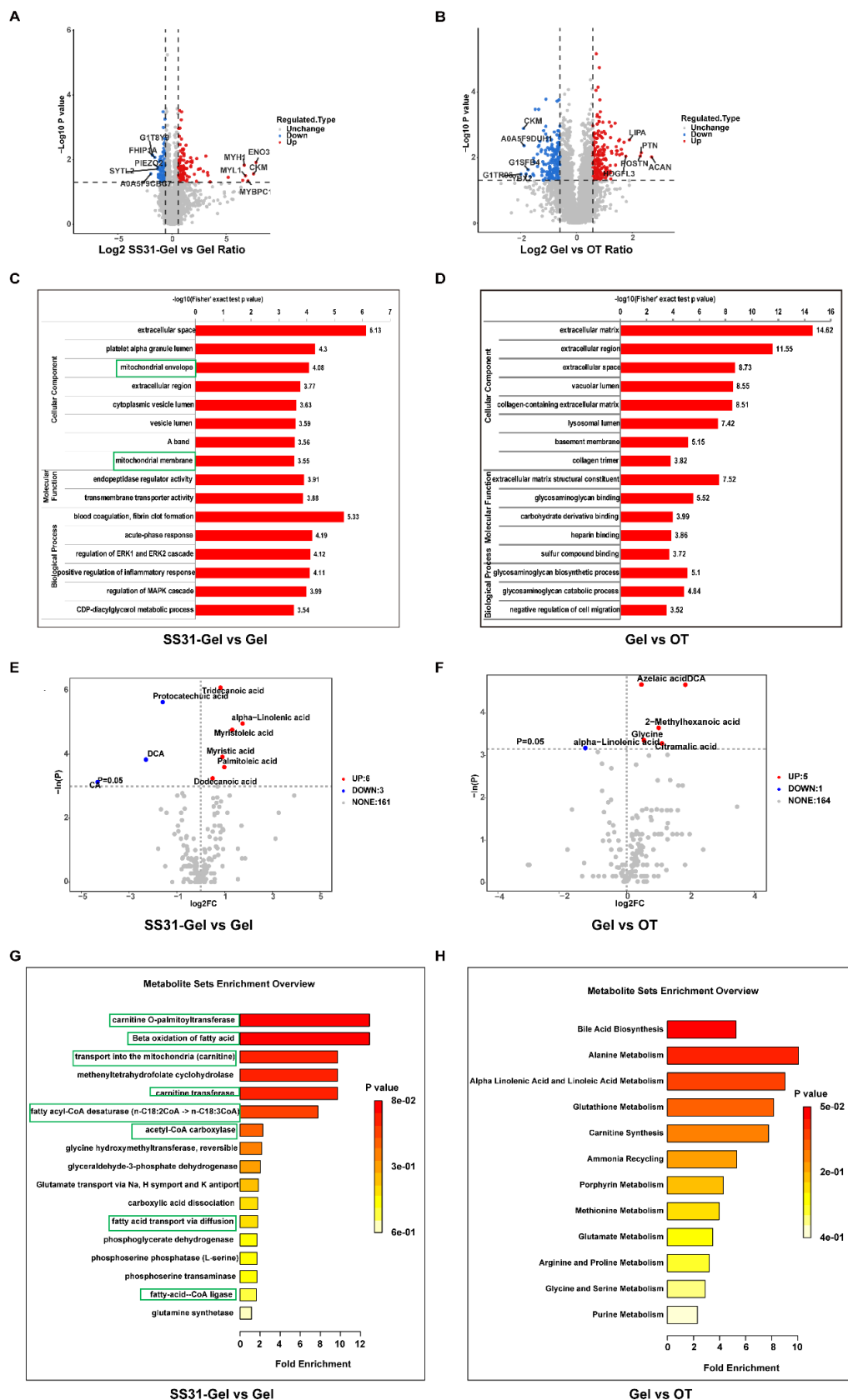


Figure S3. GO Enrichment of differentially expressed proteins of SS31/GM-CSF vs GM-CSF and GM-CSF vs OT. A, B. Volcano plots of differential proteins for SS31-Gel vs. Gel and Gel vs. OT

comparisons. The horizontal axis represents the log₂-transformed fold-change (Ratio) values, while the vertical axis represents the -log₁₀-transformed P values from the T-test. Red dots indicate significantly upregulated proteins, blue dots indicate significantly downregulated proteins, and gray dots represent non-significant differences. The selection criteria were as follows: significantly upregulated (fold-change ≥ 1.5 and $p < 0.05$) and significantly downregulated (fold-change $\leq 1/1.5$ and $p < 0.05$). C. GO terms significantly upregulated in the SS31-Gel group compared to the Gel group. GO terms related to mitochondrial structure are highlighted with a green box. D. GO terms significantly upregulated in the Gel group compared to the OT group. The vertical axis represents the descriptions of GO functions, while the horizontal axis represents the -log₁₀-transformed P values for enrichment significance, with larger values indicating stronger enrichment. E, F. Volcano plots of differential metabolites for SS31-Gel vs. Gel and Gel vs. OT comparisons. Red dots in the upper-right quadrant represent metabolites elevated in the SS31/GM-CSF group, while blue dots in the upper-left quadrant represent metabolites decreased in the SS31/GM-CSF group. G, H. SMPDB pathway enrichment bar plots for SS31-Gel vs. Gel and Gel vs. OT comparisons. The vertical axis lists the corresponding SMPDB pathway descriptions, and the horizontal axis represents fold enrichment, with larger values indicating higher enrichment levels. Pathways related to fatty acid oxidation are highlighted with a green box.

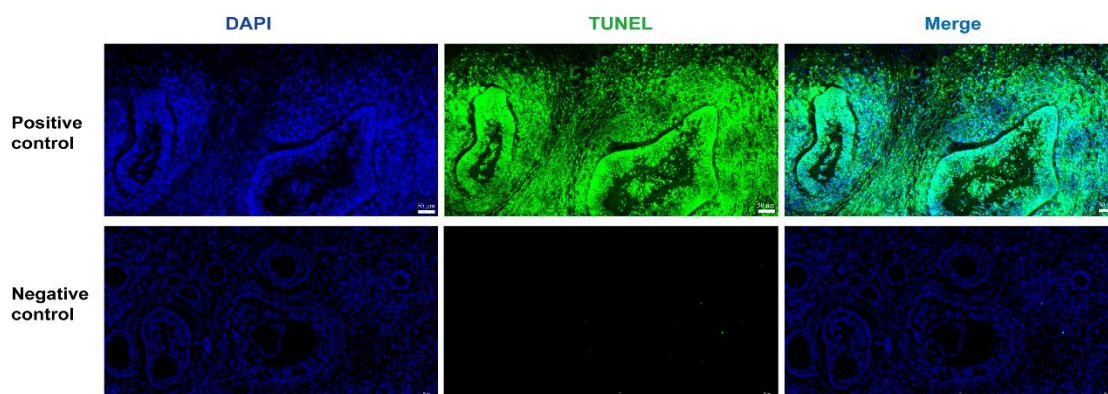


Figure S4. TUNEL Immunohistochemistry Staining of Ovarian Tissue. The blue color represents DAPI staining (for cell nuclei), and the green color represents TUNEL positive. The scale bar indicates 50 μm . and the positive control group was treated with DNase I working solution (usually at a final concentration of 0.1 $\mu\text{g/mL}$) dropwise for 20 minutes, the negative control, use only the fluorescein-conjugated dUTP solution (50 μL).

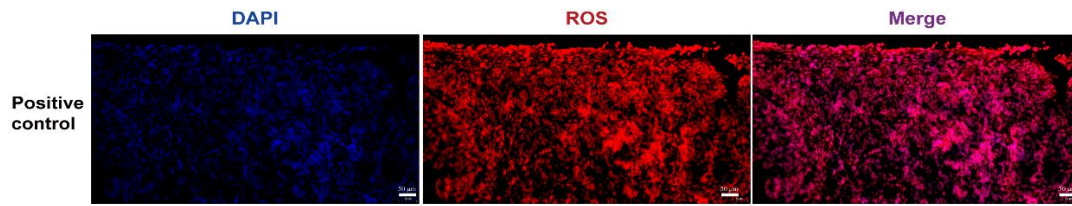


Figure S5. ROS Immunofluorescence Staining: ROS levels in ovarian tissue of various groups are shown using immunofluorescence staining. The blue color represents DAPI staining (for cell nuclei), and the red color represents ROS. The scale bar indicates 50 μ m. and the positive control group was treated with 100 μ M H_2O_2 for 30 minutes during the incubation process of ovarian tissue sections.

Supplementary Tables

Table S1. Levels of E₂, FSH, and AMH before and after bilateral ovariectomy of rabbits

Groups	Resection	E ₂ (pg/ml)	FSH (mIU/ml)	AMH (pg/ml)
F-T-OT	Before	26.79±2.56 ^a	2.70±1.73 ^c	2179.07±527.07 ^e
	After	5.71±1.19 ^b	7.76±1.19 ^d	1121.49±301.09 ^f
GM-CSF+OT	Before	26.97±4.10 ^a	2.49±1.79 ^c	2252.02±393.36 ^e
	After	6.25±1.08 ^b	7.98±0.94 ^d	1297.96±216.05 ^f
SS31/ GM-CSF+OT	Before	26.12±4.49 ^a	2.72±2.03 ^c	2190.12±511.54 ^e
	After	4.64±0.91 ^b	8.57±2.92 ^d	926.47±146.81 ^f

Note: Under the same ovarian treatment, different groups in the same column with the same letter indicated no significant difference ($p > 0.05$), and different letters indicated significant difference ($p < 0.05$). OT: frozen-thawed ovarian transplantation control group, GM-CSF+OT: GM-CSF gel and frozen-thawed ovarian transplantation group, SS31/GM-CSF+OT: GM-CSF gel with SS31 and frozen-thawed ovarian transplantation group.

Table S2. Recovery of E₂, FSH, and AMH at 3 and 7 days after frozen-thawed ovarian tissue transplantation

Groups	Days of transplantation	E ₂ recovery (pg/ml)	FSH recovery (mIU/ml)	AMH recovery (pg/ml)
OT	3	1.99±1.05 ^a	7.27±0.96 ^c	410.33±501.35 ^e
	7	8.37±6.20 ^b	3.38±2.81 ^d	231.00±273.65 ^f
GM-CSF+OT	3	11.82±3.49 ^a	4.10±2.38 ^c	502.76±331.04 ^e
	7	7.60±5.50 ^b	3.22±3.06 ^d	559.97±107.39 ^f
SS31/ GM-CSF+OT	3	6.99±7.13 ^a	4.96±3.20 ^c	1193.17±410.83 ^e
	7	17.36±6.86 ^b	2.78±2.42 ^d	1278.13±522.85 ^g

Note: At the same transplantation day, the same letter in the same column in different groups showed no significant difference ($p > 0.05$), and the different letter showed significant difference ($p < 0.05$).

E₂ recovery: post-transplant E₂- pre-transplant E₂; FSH recovery: post-transplant FSH- pre-transplant FSH; AMH recovery: AMH after transplantation -AMH before transplantation. OT: frozen-thawed ovarian transplantation control group, GM-CSF+OT: GM-CSF gel and frozen-thawed ovarian transplantation group, SS31/GM-CSF+OT: GM-CSF gel with SS31 and frozen-thawed ovarian transplantation group.

Raw WB data

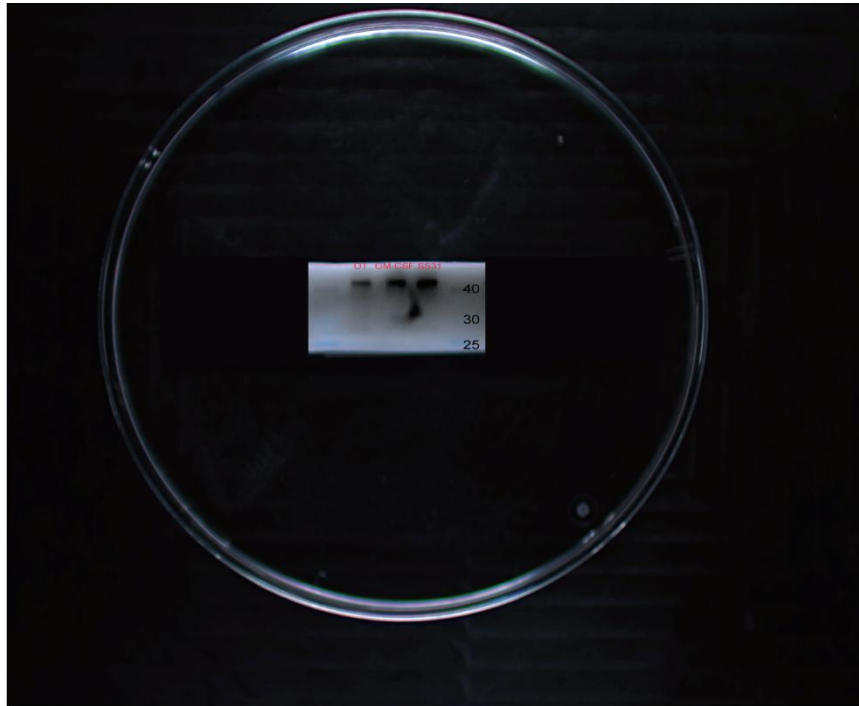
Figure 9G. Western Blot Analysis of Protein Expression of SDHA, SDHC, ACADM, ACADS, and CPT1 in Ovarian Tissue at 7 Days Post-Transplantation



Protein expression levels of ACADM in ovarian tissues from each group 7 days after transplantation



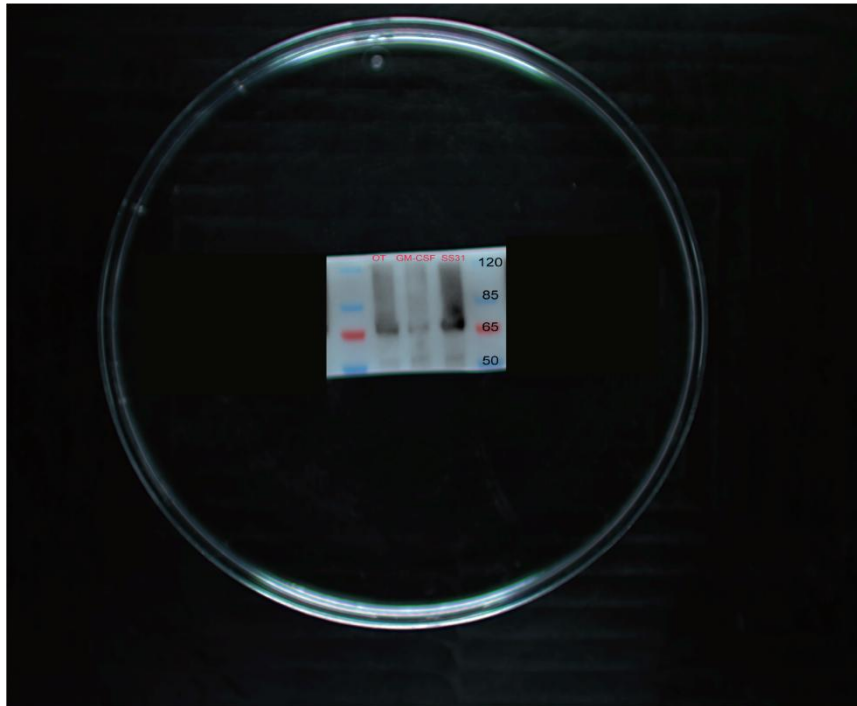
Protein expression levels of SDHC in ovarian tissues from each group 7 days after transplantation



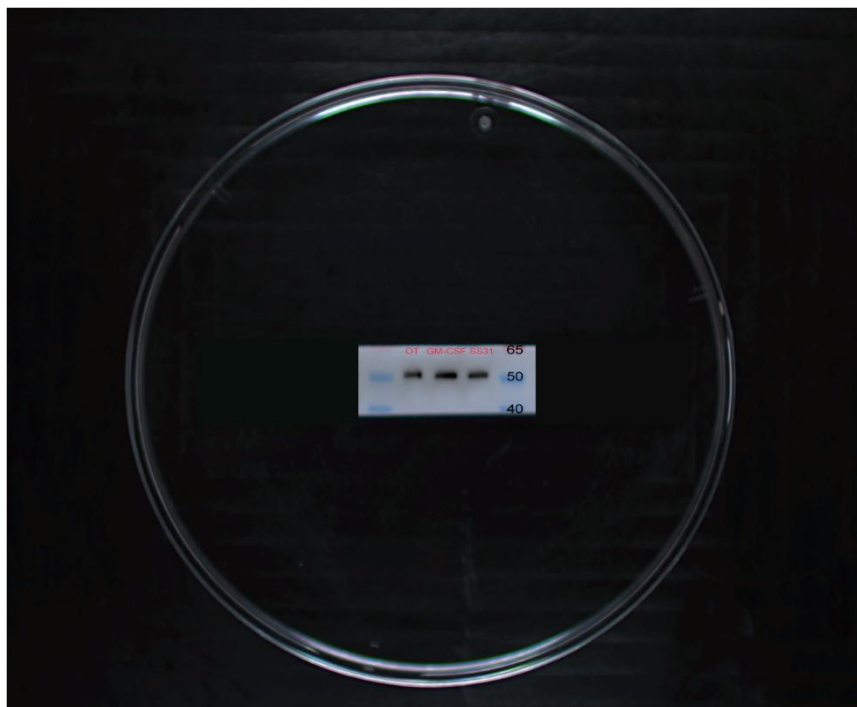
Protein expression levels of ACADS in ovarian tissues from each group 7 days after transplantation



Protein expression levels of CPT1 in ovarian tissues from each group 7 days after transplantation

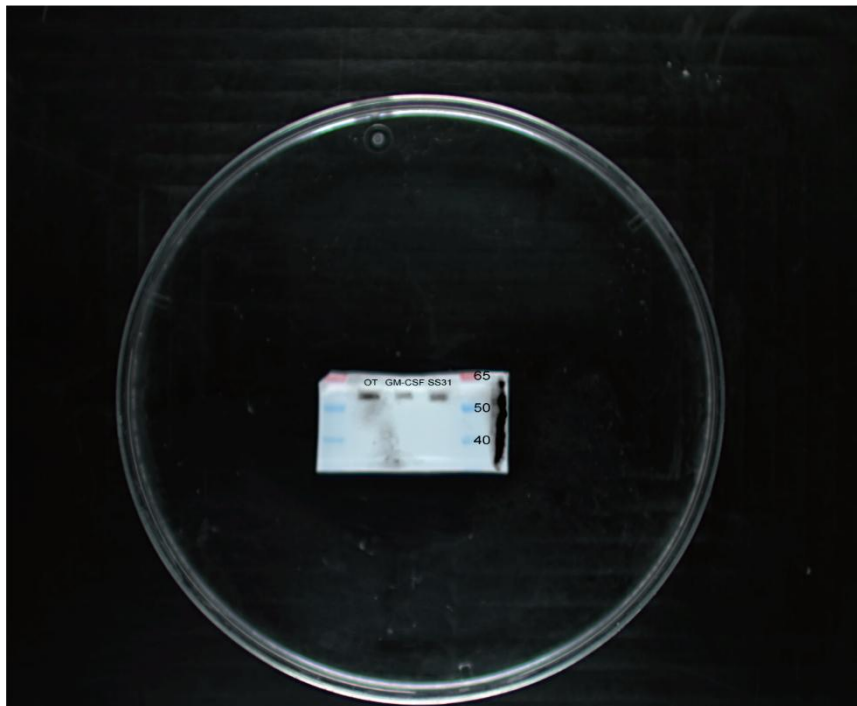


Protein expression levels of SDHA in ovarian tissues from each group 7 days after transplantation

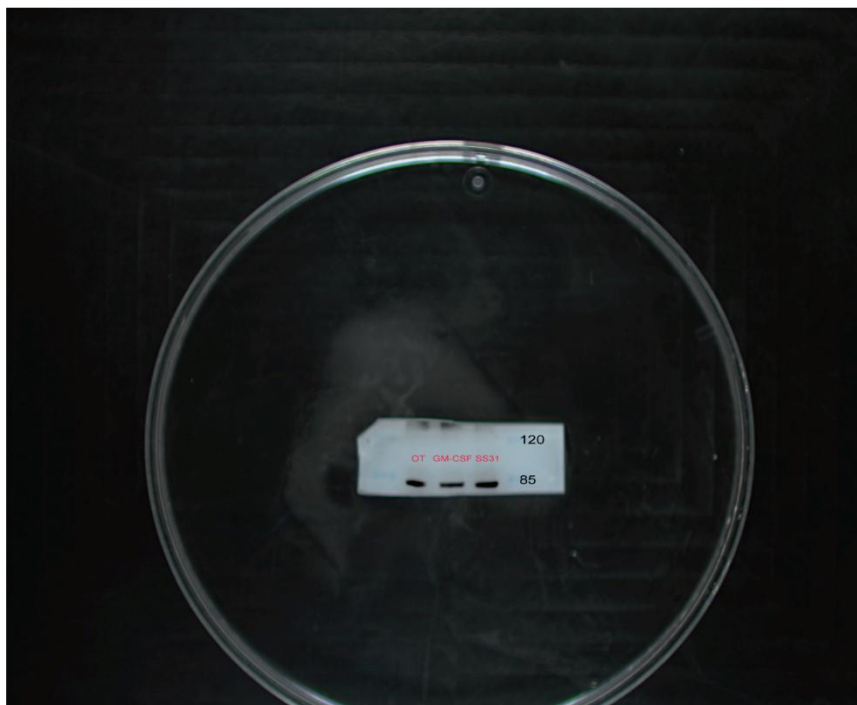


Protein expression levels of TUBULIN in ovarian tissues from each group 7 days after transplantation

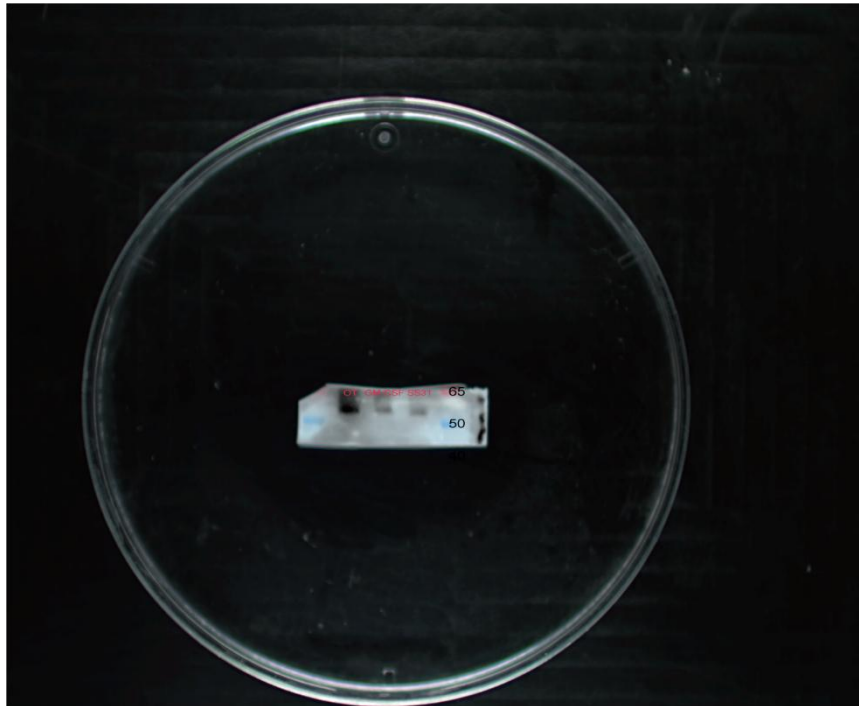
Figure 11D. Western blot analysis of mTOR, p-mTOR, Akt, p-Akt, P85, and p-P85 expression levels at 3 days post-transplantation



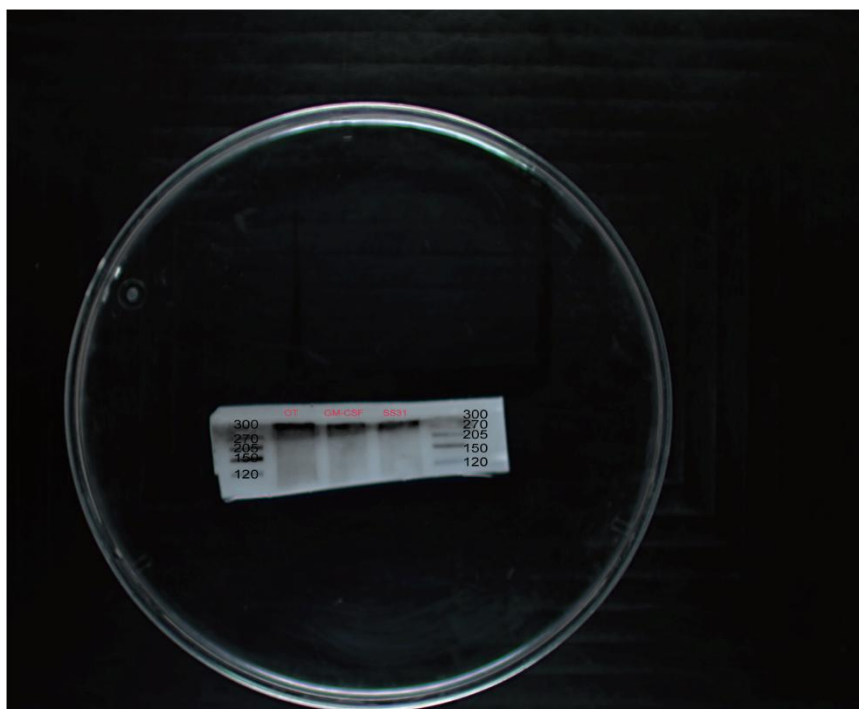
Protein expression levels of AKT in ovarian tissues from each group 3 days after transplantation



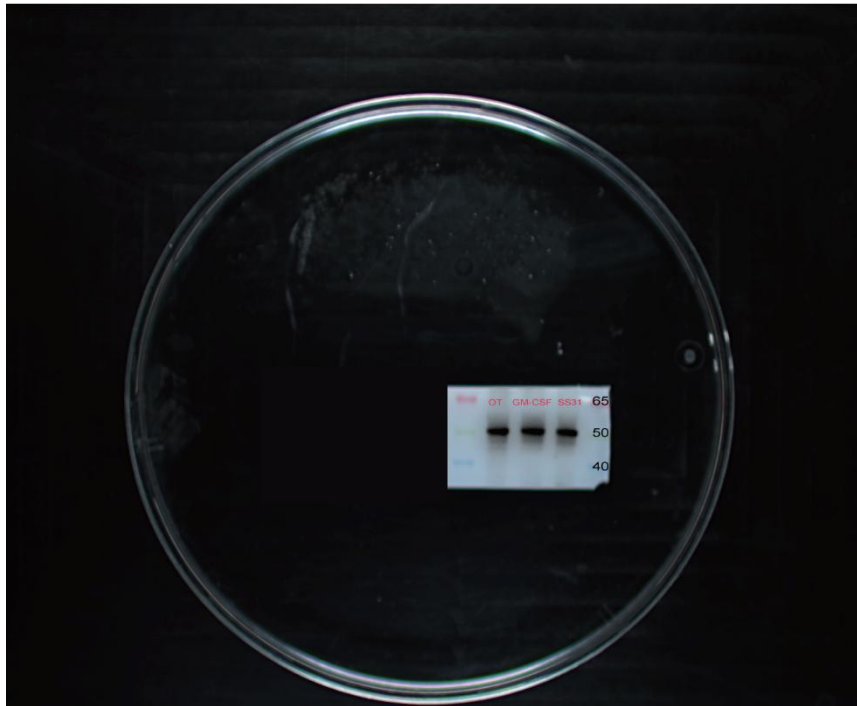
Protein expression levels of P85 in ovarian tissues from each group 3 days after transplantation



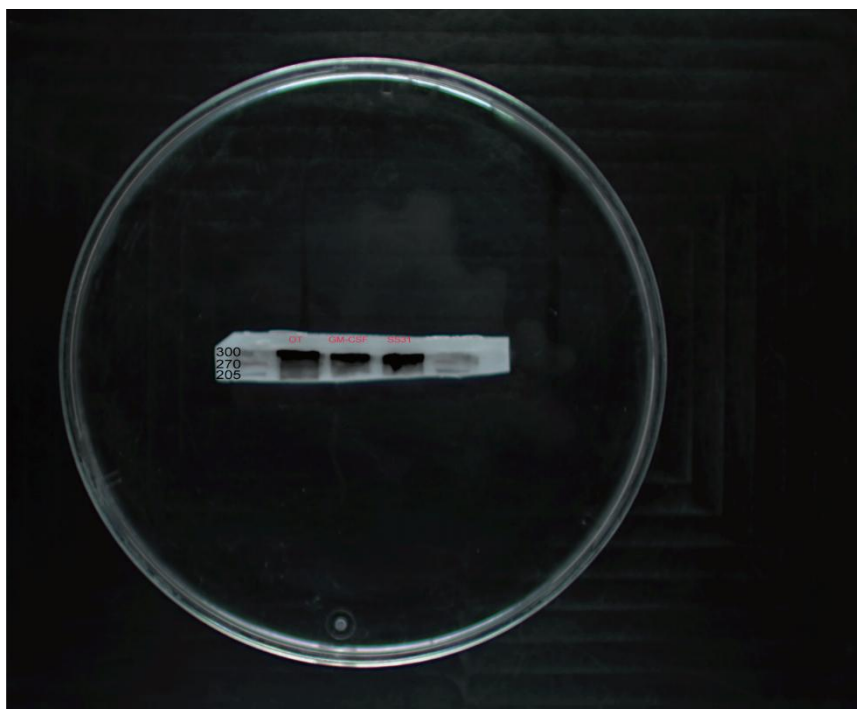
Protein expression levels of p-AKT in ovarian tissues from each group 3 days after transplantation



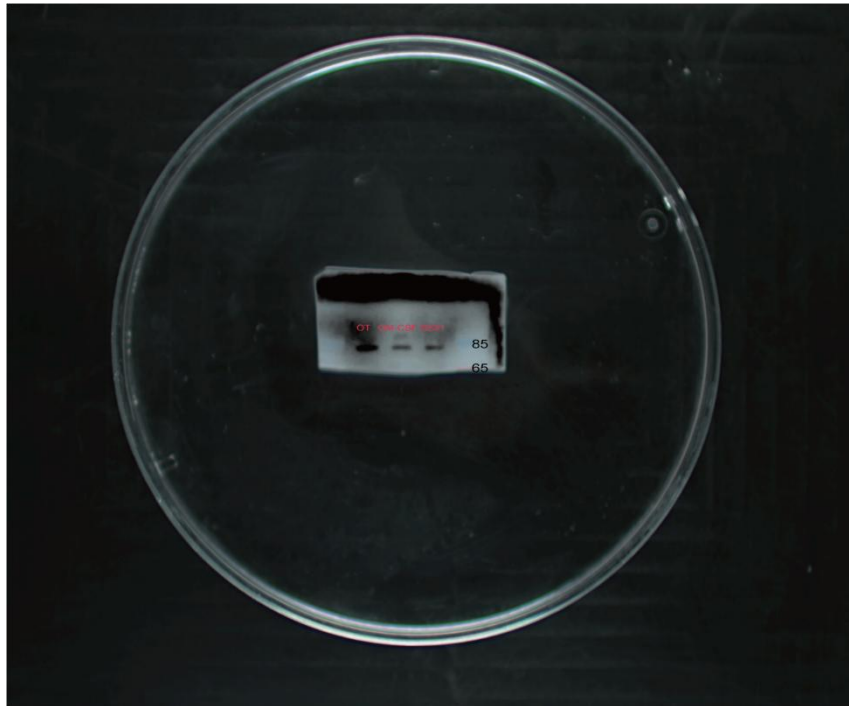
Protein expression levels of p-mTOR in ovarian tissues from each group 3 days after transplantation



Protein expression levels of TUBULIN in ovarian tissues from each group 3 days after transplantation

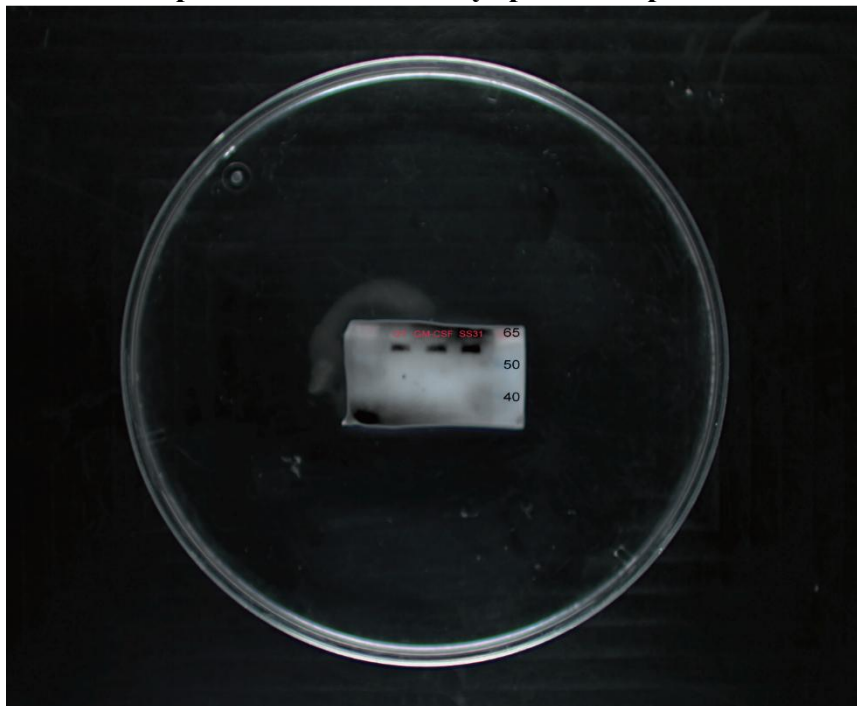


Protein expression levels of mTOR in ovarian tissues from each group 3 days after transplantation

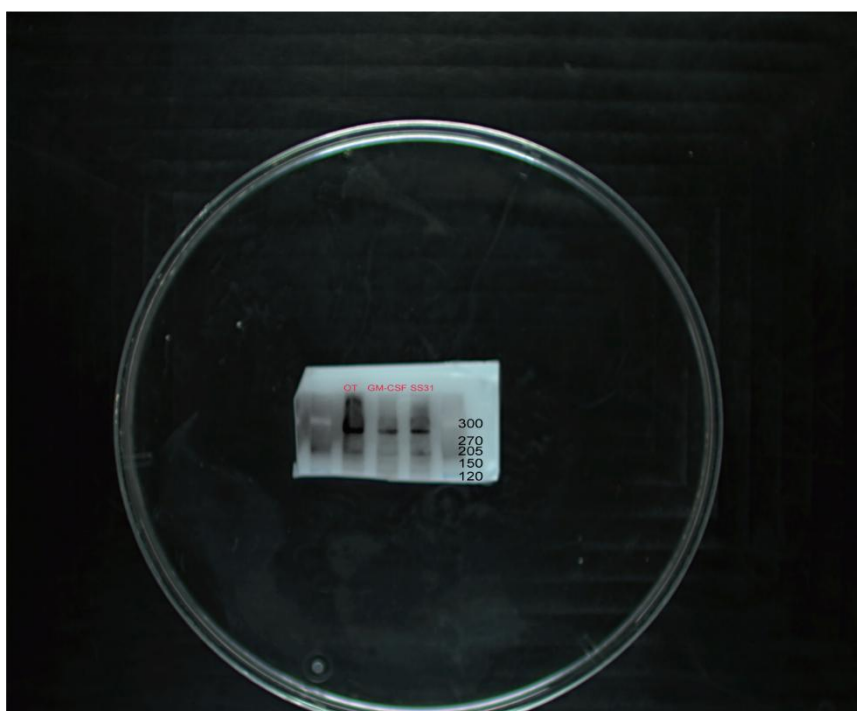


Protein expression levels of p-P85 in ovarian tissues from each group 3 days after transplantation

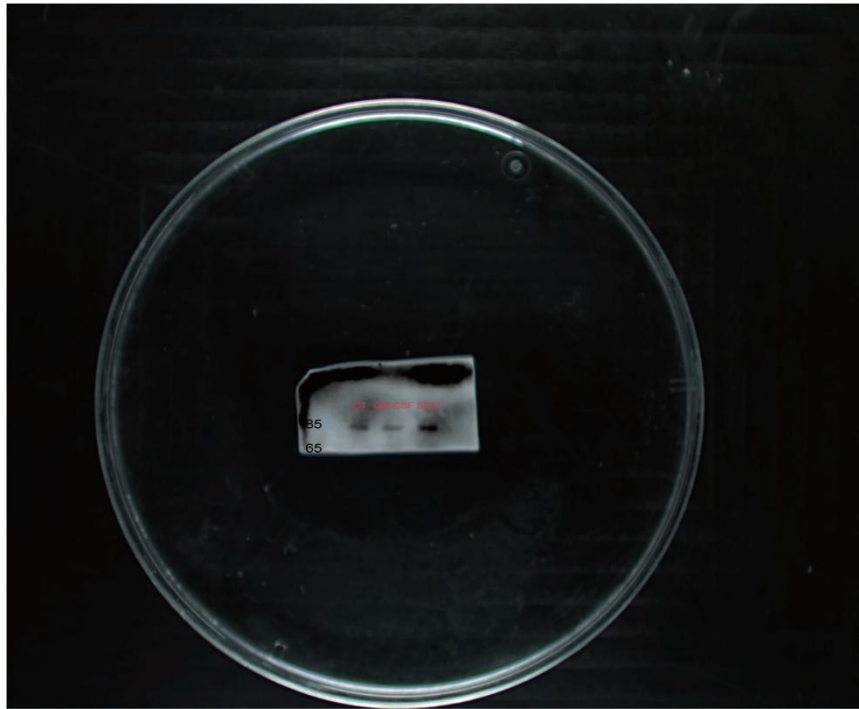
Figure 11H. Western blot analysis of mTOR, p-mTOR, Akt, p-Akt, P85, and p-P85 expression levels at 7 days post-transplantation



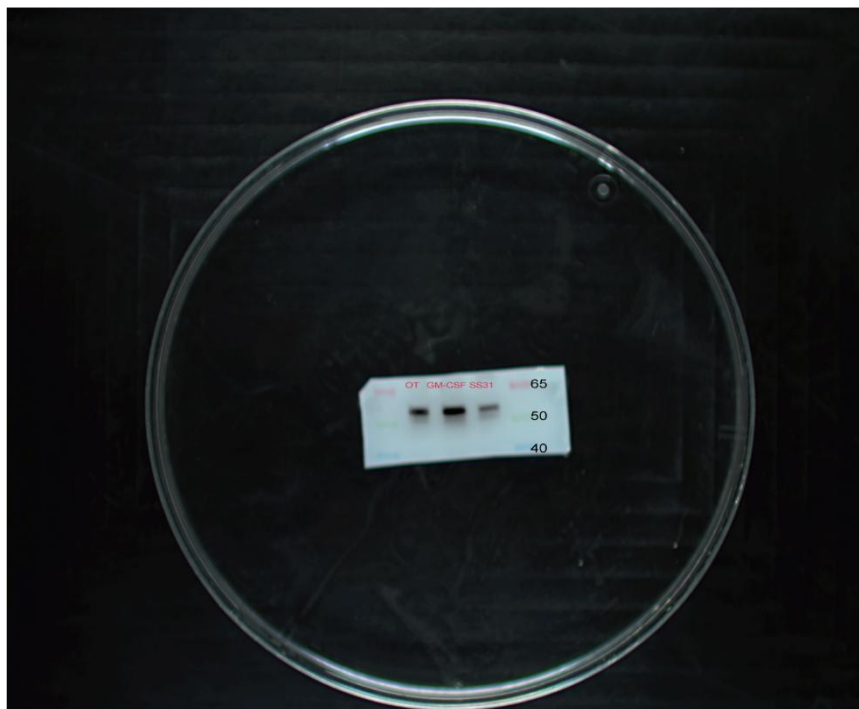
Protein expression levels of AKT in ovarian tissues from each group 7 days after transplantation



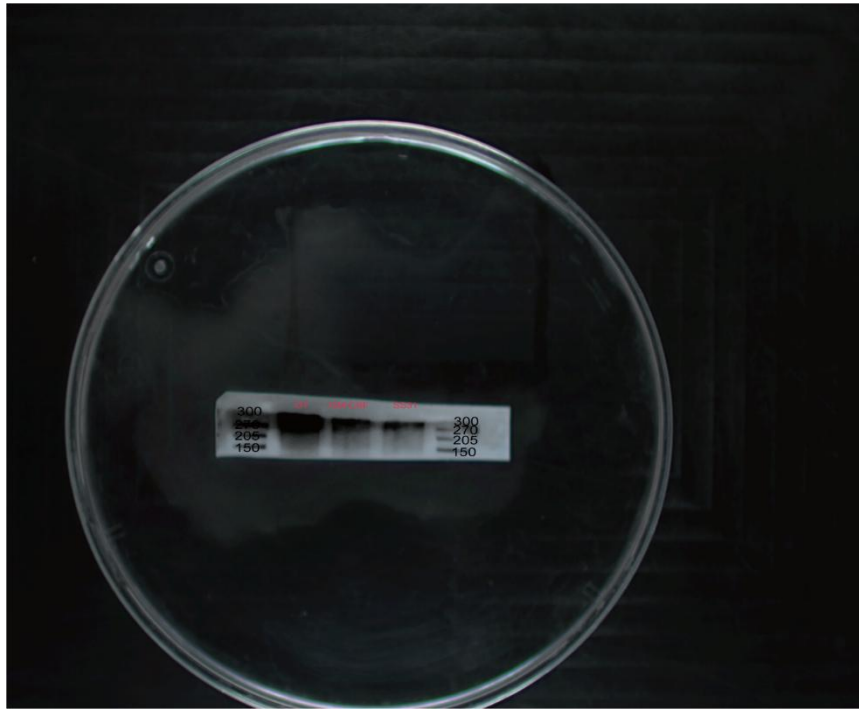
Protein expression levels of mTOR in ovarian tissues from each group 7 days after transplantation



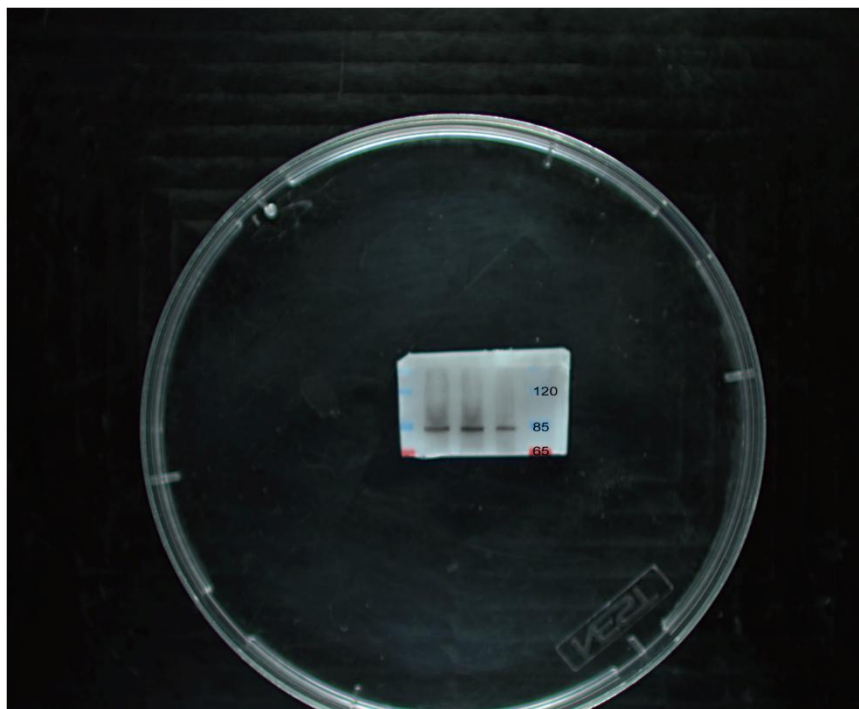
Protein expression levels of P85 in ovarian tissues from each group 7 days after transplantation



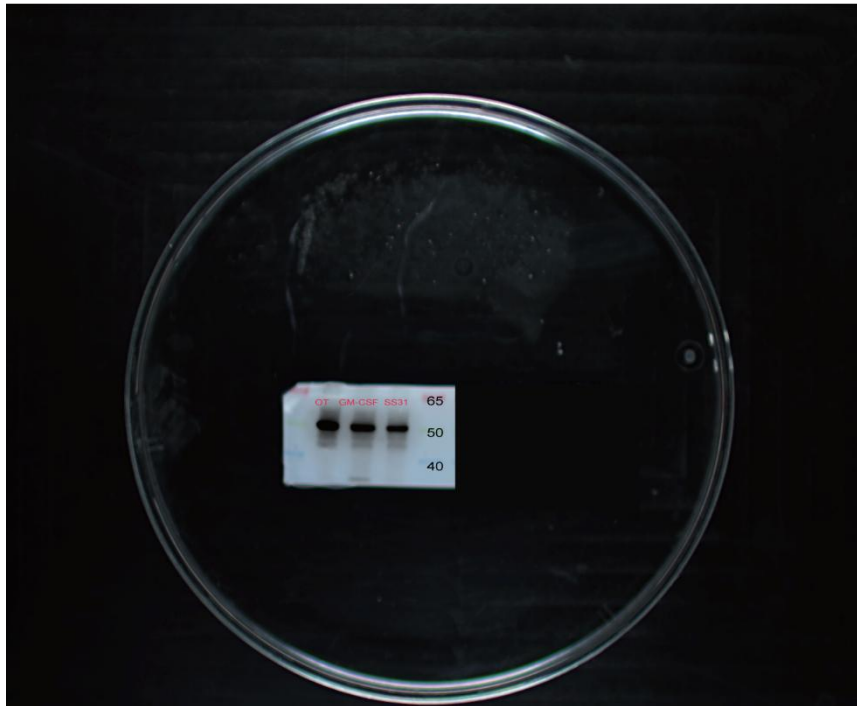
Protein expression levels of p-AKT in ovarian tissues from each group 7 days after transplantation



Protein expression levels of p-mTOR in ovarian tissues from each group 7 days after transplantation



Protein expression levels of p-P85 in ovarian tissues from each group 7 days after transplantation



Protein expression levels of TUBULIN in ovarian tissues from each group 7 days after transplantation