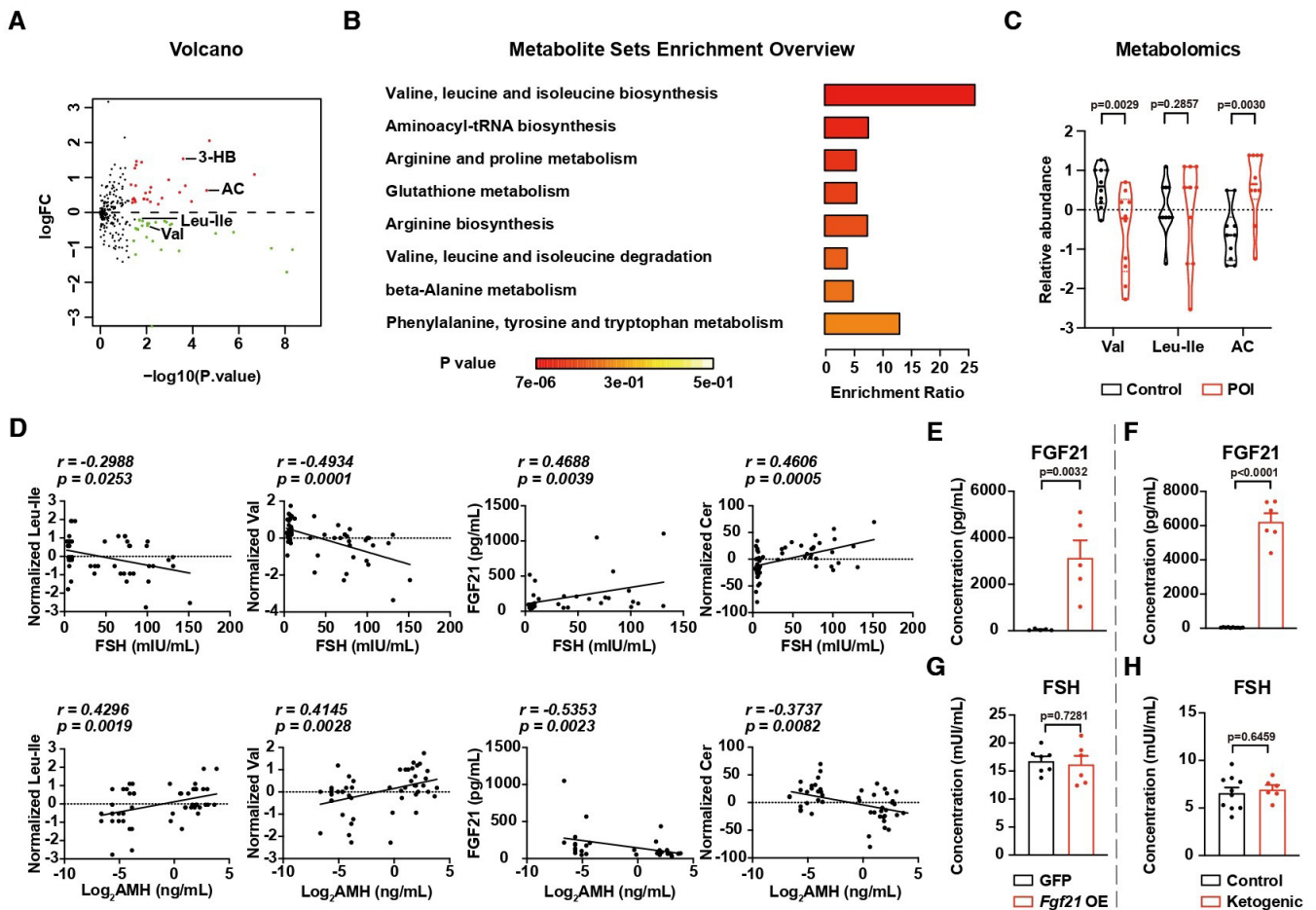# Expanded View Figures



**Figure EV1.  Metabolic details of POI patients.**

A       Volcano plot showing the fold-change and *P*-value of metabolites. *N* = 18.
B       Enrichment score and *P*-value of metabolites downregulated in POI patients. *N* = 18.
C       Relative abundance of valine (Val), acetyl-carnitine (AC) but not leucine-isoleucine (Leu-Ile) in the serum of the Shandong Cohort. *N* = 10; Truncated violin plot, central band stands for median, and dotted lines stand for the upper quartile or the lower quartile of the data.
D       The correlations between metabolites/FGF21 and clinical parameters. The *P* and *r* were calculated by the nonparametric Spearman test. *N* = 56.
E, F    The concentration of FGF21 in mouse serum. (E) *N* = 5; (F) Control *N* = 10; Ketogenic diet, *N* = 6.
G, H    The concentration of FSH in mouse serum. (G) Control, *N* = 7; *Fgf21* OE, *N* = 6; and (H) control, *N* = 10; Ketogenic diet, *N* = 6.

Data information: Error bars stand for SEM. The *P*-value was calculated by a two-tailed *t*-test with 2-way ANOVA correction.
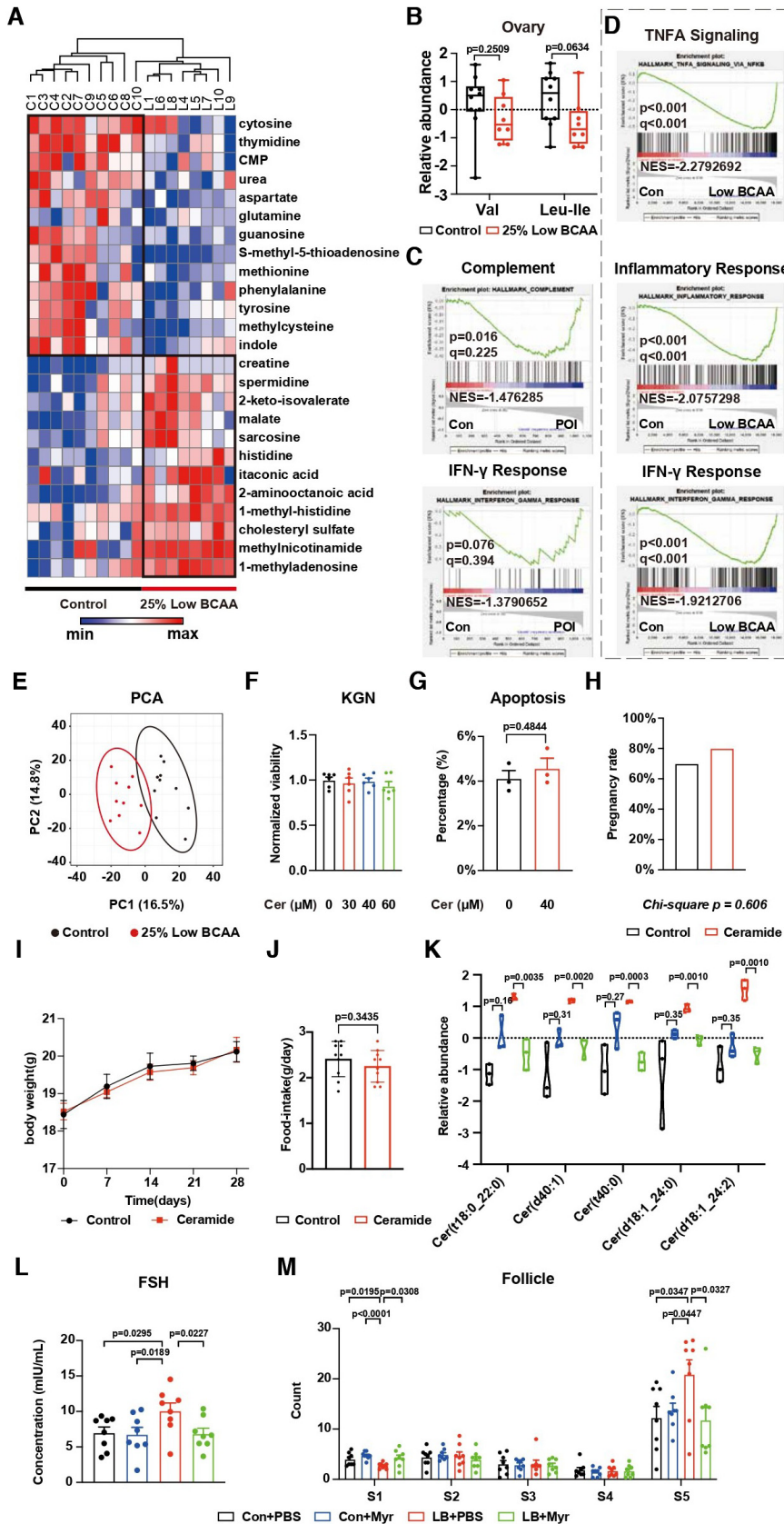Source data are available online for this figure.

**Figure EV2. Lipid metabolism features of POI.**

A   Heatmap showing the top 25 differentially abundant metabolites in the ovaries of mice on a low BCAA diet. Control, $N = 10$; low BCAA, $N = 8$.

B   The relative abundance of BCAAs in the ovaries of mice on a low BCAA diet. Control, $N = 10$; low BCAA, $N = 8$; Boxplot, central band stands for median, boxes stand for 50% of the data, and whiskers stand for min or max of the data.

C   GSEA of the proteomics data in the serum of the Fudan Cohort. $N = 11$.

D   GSEA of RNA-seq data from the liver in the mice on a low BCAA diet or control diet. $N = 6$.

E   The PCA of serum from mice on a low BCAA diet or control diet. $N = 10$.

F   The relative cell viability of KGN cells with ceramide treatment. $N = 6$.

G   The percentage of Annexin V positive cells of KGN cells with ceramide treatment. $N = 3$.

H–J   The pregnancy rate, body weight, and food intake from mice with ceramide treatment. $N = 10$.

K   The relative abundance of ceramide in the serum of mice with myriocin treatment. $N = 3$; Truncated violin plot, central band stands for median, and dotted lines stand for the upper quartile or the lower quartile of the data.

L   The serum concentration of FSH in mice with ceramide treatment. $N = 8$.

M   The changes in follicle count from mice with ceramide treatment. $N = 8$.

Data information: S1, Primordial; S2, Primary; S3, Secondary; S4, Antral; S5, Atretic. Error bars stand for SEM. The $P$-value was calculated by a two-tailed $t$-test with 2-way ANOVA correction.
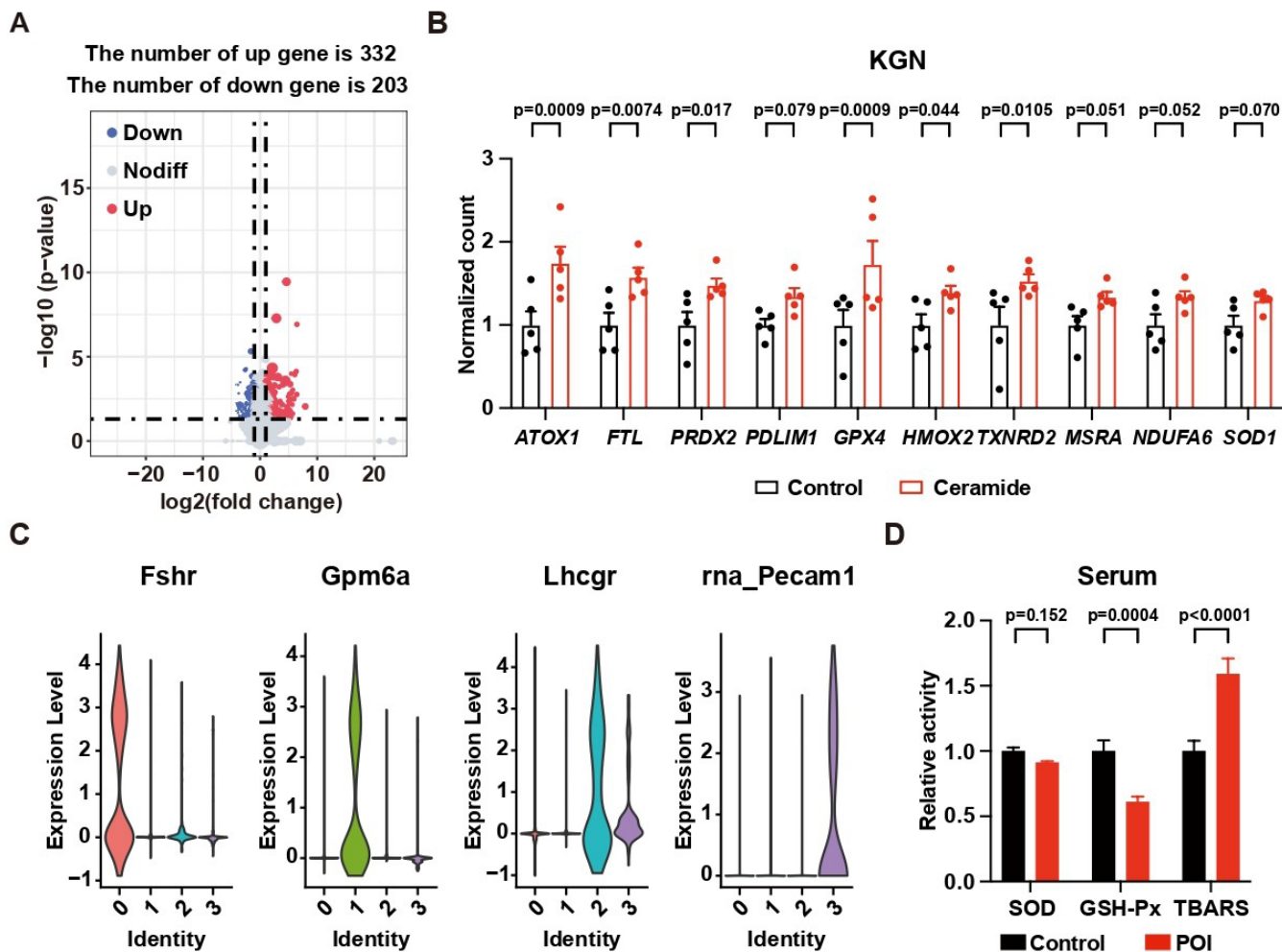
Source data are available online for this figure.

**Figure EV3. Low BCAA induces POI via elevation of ROS.**

A   Genes with two-fold changes and *P*-value < 0.05 are highlighted in the volcano plot. *N* = 5.
B   Upregulation of ROS-related genes in KGN cells treated with ceramide. *N* = 5.
C   Violin plots showing the expression of classical markers of nonimmune cells in ovaries from sNuc-seq data.
D   Relative activity of ROS-related factors in patients. Control, *N* = 30; POI, *N* = 60.

Data information: Error bars stand for SEM. The *P*-value was calculated by a two-tailed *t*-test with 2-way ANOVA correction.
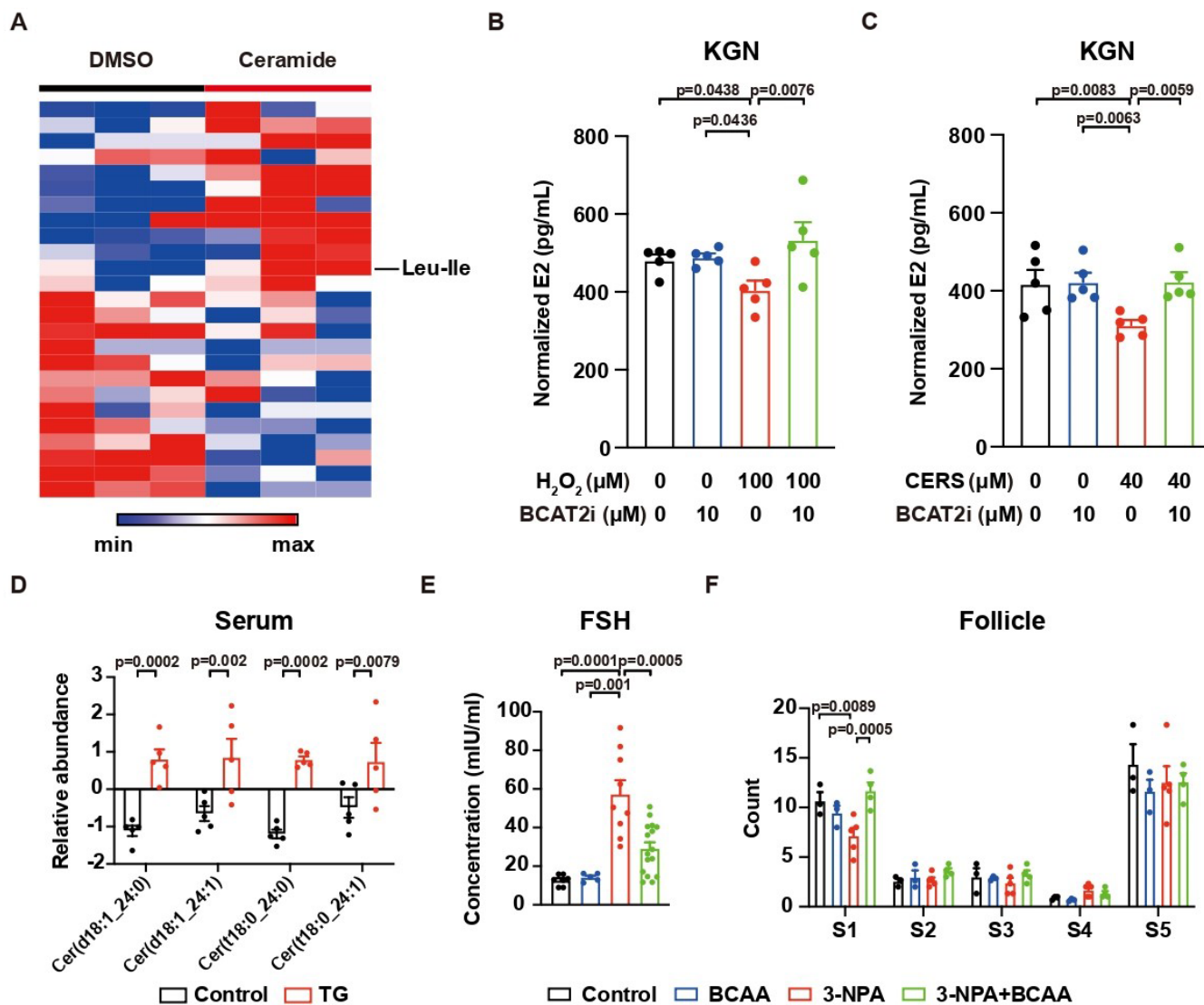Source data are available online for this figure.

**Figure EV4. BCAA supplement protected the granulosa cells from ROS inducer.**

A  Heatmap showing the relative abundance of metabolites in KGN cells treated with ceramides. $N = 3$.
B  The concentration of E2 secreted by KGN cells with $H_2O_2$ treatment w/o BCAT2 inhibitor. $N = 5$.
C  The concentration of E2 secreted by KGN cells with ceramide treatment w/o BCAT2 inhibitor. $N = 5$.
D  Elevation of ceramide in the serum of TG-treated mice. $N = 5$.
E  BCAA supplement prevented the elevation of FSH in mice with 3-NPA treatment. Control, $N = 7$; BCAA, $N = 5$; 3-NPA, $N = 9$; 3-NPA + BCAA, $N = 16$.
F  BCAA supplement rescued the decrease of primordial follicles in mice with 3-NPA treatment. Control, $N = 3$; BCAA, $N = 3$; 3-NPA, $N = 5$; 3-NPA + BCAA, $N = 4$.

Data information: S1, Primordial; S2, Primary; S3, Secondary; S4, Antral; S5, Atretic. Error bars stand for SEM. The *P*-value was calculated by a two-tailed *t*-test with 2-way ANOVA correction.
Source data are available online for this figure.