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Supplemental information

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SUPPLEMENTAL INFORMATION

β-Resorcylic acid released by *Limosilactobacillus reuteri* protects against cisplatin-induced ovarian toxicity and infertility

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Supplementary Information (including figures and tables):

Supplementary figures (Figure S1-S6)

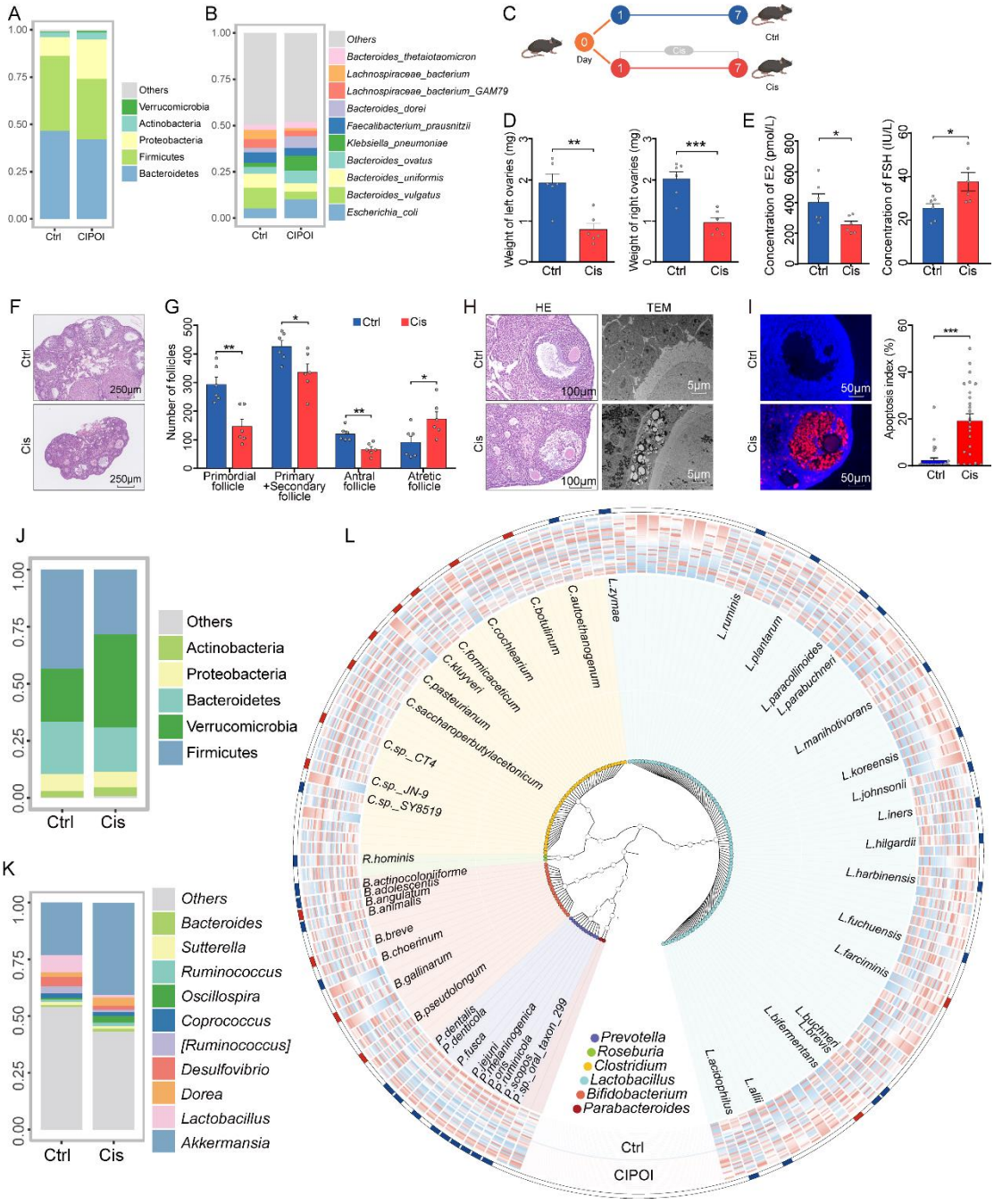

Fig. S1 Microbial composition of patients and mice with CIPOI, related to Figure 1.

(A-B) Microbial composition at the phylum (A) and species (B) level of patients with CIPOI and healthy control. (C)Schematic representation of Cis-POI modelling. (D) Ovarian weight of Ctrl and

Cis-POI mice (n = 6). (E) Serum levels of E2 and FSH in Ctrl and Cis-POI group. (n = 6). (F) H&E staining of ovaries from Ctrl and Cis-POI group. (G) Quantification of primordial, primary + secondary, antral, and atretic follicles (n =6). (H) Representative images of antral follicles of recipient mouse ovaries by H&E staining and TEM. (I) TUNEL-based quantification of the apoptotic index in the antral follicles (n =6). (J, K) Microbial composition of Cis-POI and control mice. (J) Microbial composition at the phylum, and (K) at the genus level. (L) The distribution of identified differential genera in human metagenome data. Each bar of the internal circle refers to one species, and the colour refers to its genus. The middle cycle refers to the relative abundance of species. The external cycle refers to the statistics between CIPOI and Ctrl women, whereas red and blue represent enrichment in CIPOI and Ctrl women, respectively. Data are presented as mean ± SEM; *$p < 0.05$, ***$p < 0.001$, two-tailed unpaired *t*-test.

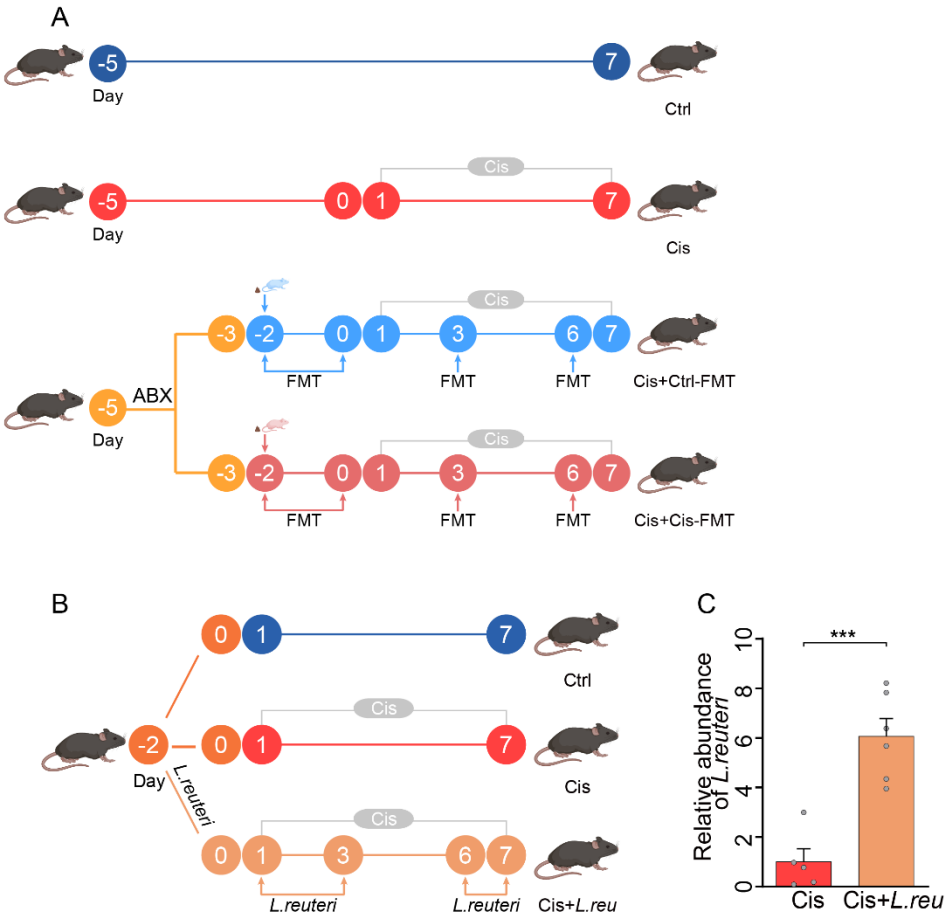

Fig.S2 The gut microbiota regulated ovarian toxicity induced by cisplatin, related to Figure 2.

(A) Schematic representation and timeline for the recipient mice transplanted with Ctrl, Cis, Cis-POI or Ctrl mice faecal microbiota. (B) Schematic representation and timeline for the Cis-POI mice subjected to *L. reuteri* administration. (C) Relative abundance of *L. reuteri* in faeces from cisplatin-exposed mice treated with *L. reuteri* (10^9 CFU per day), gavaged to mice from 3 days prior to cisplatin-exposure, and throughout Cis-POI modelling (n = 5 for Cis-POI, and n = 6 for Cis-POI+*L. reuteri*). Data are all presented as the mean ± SEM. *p < 0.05, ***p < 0.001; two-tailed Student's *t* test.

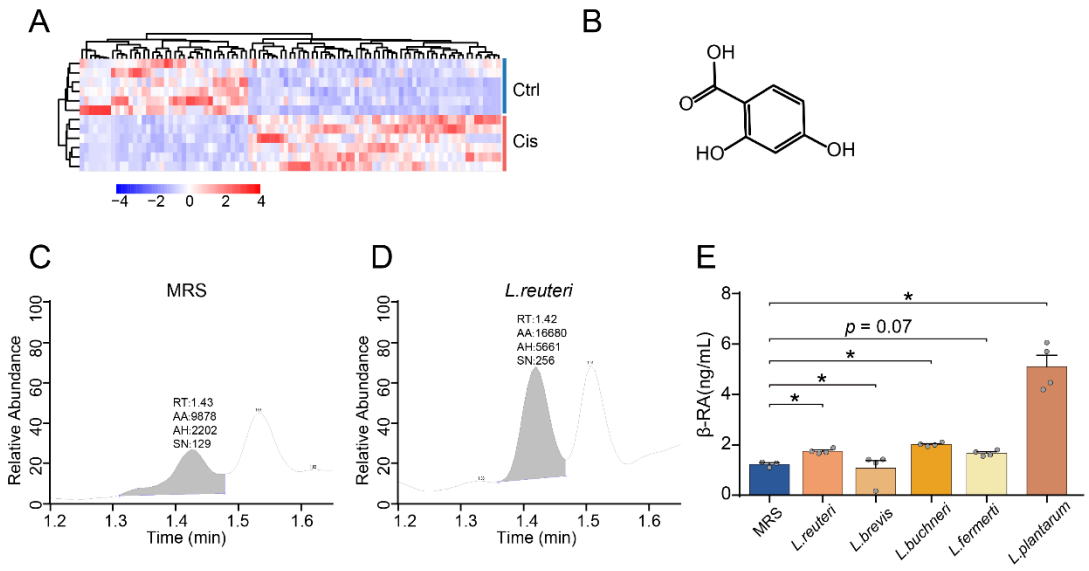

Fig.S3 The gut metabolite of Cis-POI mice and chromatograms of β-RA, related to Figure 3.

(A) Heatmap showing the differential metabolites between Cis-POI mice and control mice. (B) The chemical structure of β-RA. (C, D) Representative chromatograms of β-RA in the MRS medium *or* *L. reuteri*. (E) β-RA levels in MRS medium and several strains of *Lactobacillus* solution. (n=3 for MRS, n=4 for *Lactobacillus* solution). The data are all presented as the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001; Welch ANOVA with Bonferroni adjustment for multiple comparisons.

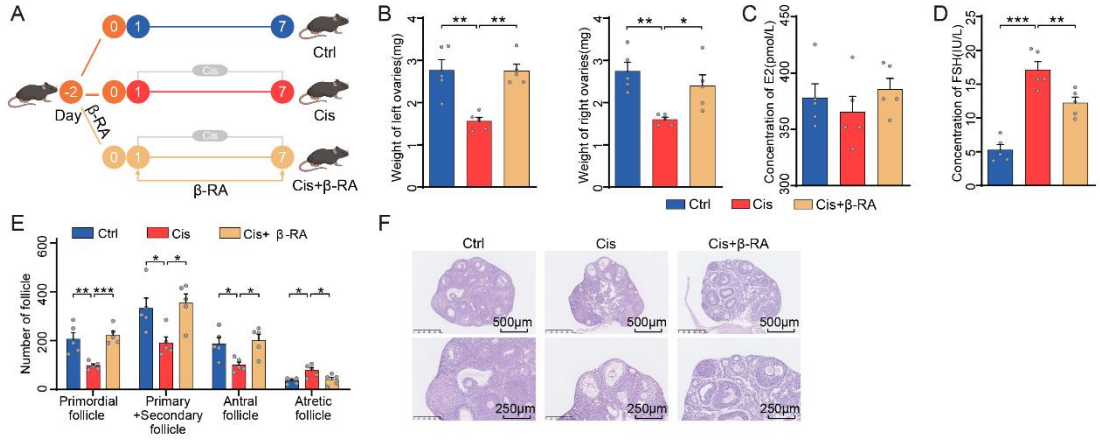

Fig.S4 β-RA prevents cisplatin-induced ovarian damage in germ-free condition, related to Figure 4.

(A-F) β-RA prevents cisplatin-induced ovarian damage in germ-free condition (n=5 in each group). (A) Schematic representation and timeline for the Cis-POI mice subjected to β-RA administration. (B) Ovarian weight. (C) Serum levels of E2. (D)Serum levels of FSH. (E)Quantification of the primordial, primary + secondary, antral and atretic follicles. (F) Representative images of ovaries and antral follicles in recipient mice by H&E. The data are all presented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; one-way ANOVA following the Dunnett's multiple comparisons test.

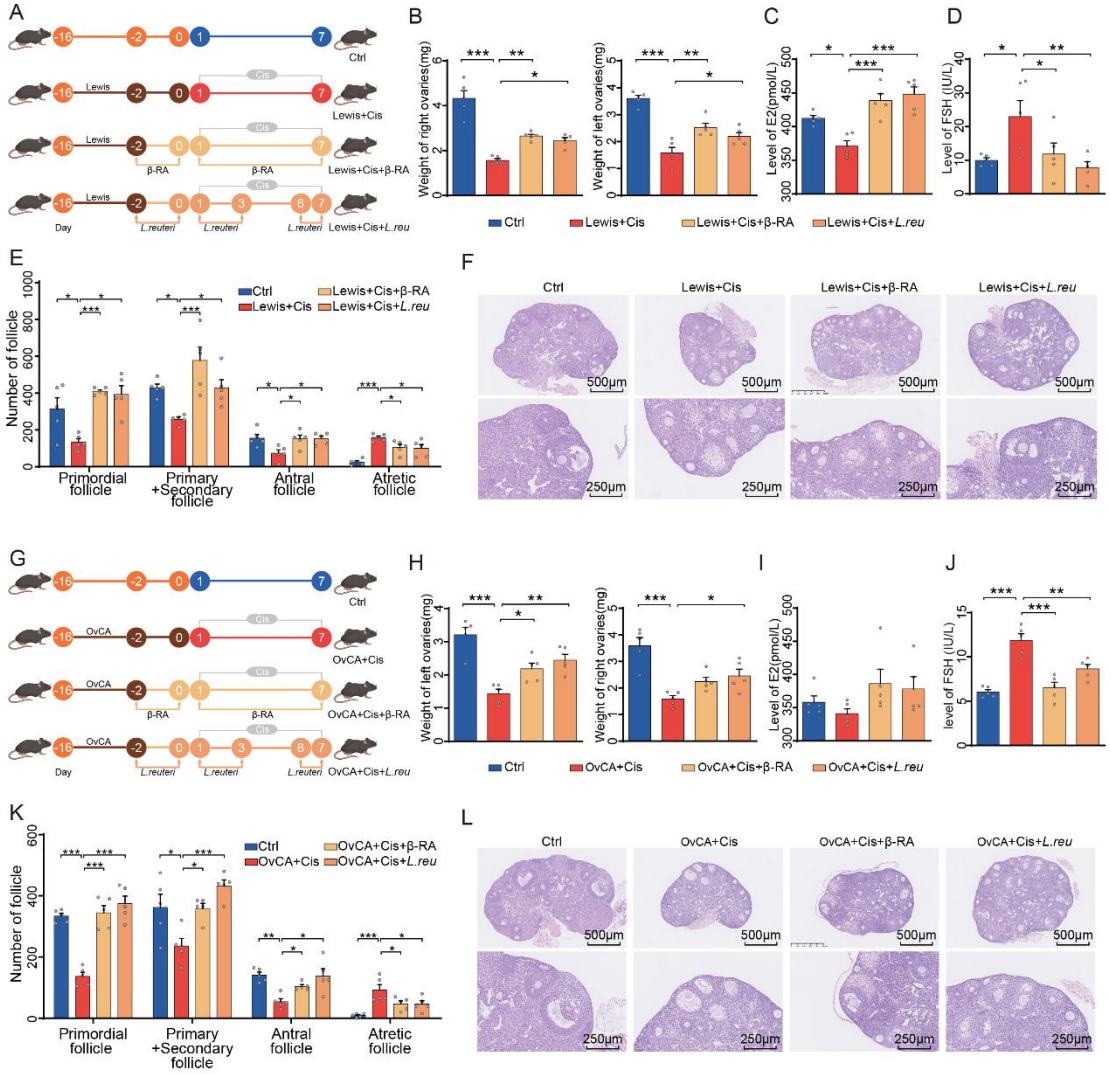

Fig.S5 β-RA prevents cisplatin-induced ovarian damage in two tumour-bearing mice, related to Figure 4.

(A-F) β-RA prevents cisplatin-induced ovarian damage in Lewis-bearing mice (n=5 in each group). (A) Schematic representation and timeline for β-RA and *L.reuteri* administration in Lewis-bearing plus cisplatin mice. (B) Ovarian weight. (C) Serum levels of E2. (D)Serum levels of FSH. (E) Quantification of the primordial, primary + secondary, antral and atretic follicles. (F) Representative images of ovaries and antral follicles in recipient mice by H&E. (G-L) β-RA prevents cisplatin-induced ovarian damage in OvCa-bearing mice (n=5 in each group). (G) Schematic representation and timeline for β-RA and *L.reuteri* administration in OvCa-bearing

plus cisplatin mice. (H) Ovarian weight. (I) Serum levels of E2. (J)Serum levels of FSH. (K)Quantification of the primordial, primary + secondary, antral and atretic follicles. (L) Representative images of ovaries and antral follicles in recipient mice by H&E. The data are all presented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; one-way ANOVA following the Dunnett's multiple comparisons test.

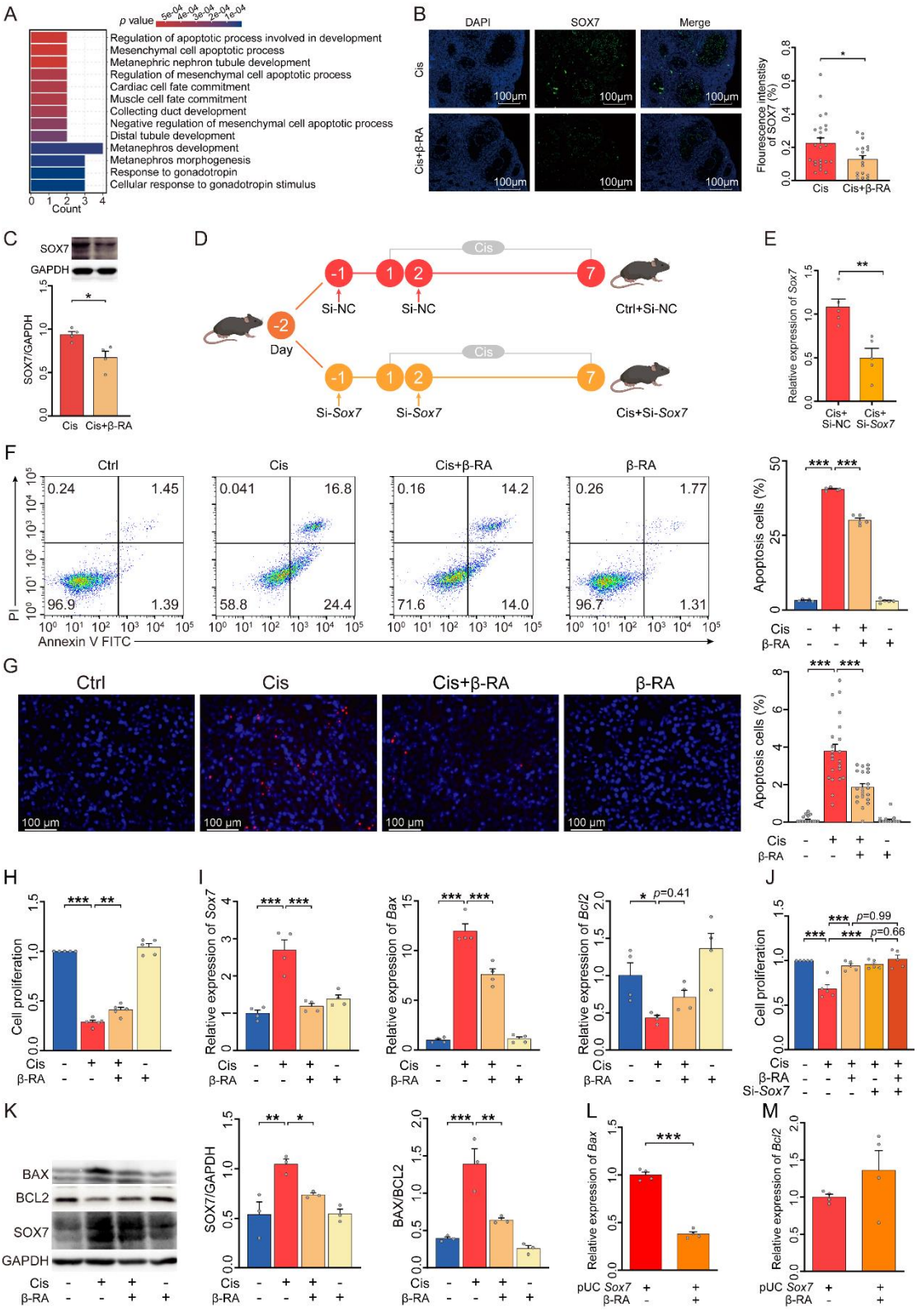

Fig.S6 β-RA suppresses apoptosis in granulosa cells by inhibiting SOX7 transcription, related to Figure 5.

(A) Gene ontology analysis of the top 100 downregulated genes in β-RA-pretreated granulosa cells.

(B) Immunofluorescence staining of ovarian sections with SOX7 antibody. Green represents SOX7 and blue represents DAPI. (C) Western blotting was performed to quantify SOX7 in ovarian tissues (n = 4). (D) Schematic representation and timeline for the Cis-POI mice subjected to (NC-)siRNA or *Sox7*-siRNA administration. (E)The relative mRNA level of SOX7 in ovarian tissue. Mice were injected with siRNA targeting mouse *Sox7* or control siRNA (2 mg/kg) before cisplatin exposure. (F) The proportion of apoptotic KGN cells was determined by Annexin V-FITC/PI staining and flow cytometry (n = 5). (G) Quantification of apoptosis index in KGN cells based on TUNEL staining. (H) Cell Counting Kit-8 was performed to measure the relative cell viability (n = 5). (I) KGN cells were pre-stimulated with or without β-RA for 6 h and subjected to cisplatin or PBS. Relative mRNA levels of *Sox7*, *Bax,* and *Bcl2* (n = 4). (J) After transfection with or without siRNA targeting Sox7 or control siRNA for 36h, cisplatin-primed KGN cells were co-incubated with or without β-RA for 6 h. Relative viability of KGN cells (n=5). (K) Representative western blot analysis for SOX7, BAX, and BCL2 in KGN cells, and quantifications (n = 3). (L and M) The relative mRNA levels of *Bax* and *Bcl2*. After transfected with Puc-SOX7 for 6 h, KGN cells were treated with or without β-RA (n = 4). Data are all presented as the mean ± SEM. *$p < 0.05$, ***$p < 0.001$; two-tailed Student's *t* test in B, C, E, L, M; one-way ANOVA following the Dunnett's multiple comparisons test in (F-K).

Supplementary tables (Table S3 & S4)

Table S3 Clinical characteristics of the participants (Mean±SD), related to STAR Methods

	Ctrl (n=18)	CIPOI (n=21)	*p*-value
Demographics			
Age (yrs)	31.00±5.82	33.19±5.91	0.26
Height (cm)	157.41±4.03	157.93±5.17	0.74
Weight (kg)	52.40±5.99	52.78±5.90	0.85
Serum hormone levels			
FSH (IU/L)	5.07±1.87	50.12±31.03	<0.001
E2 (ng/L)	83.17±36.34	32.58±39.49	<0.001
AMH (ng/ml)	2.94±1.75	0.40±1.54	<0.001
Chemotherapy regiments			
Cisplatin/Etoposide	/	21	
Cancer stage			
limited stage	/	3	
extensive stage	/	18	

Differences in characteristics between CIPOI and Control group were evaluated, using two-tailed unpaired Student's t-test. Abbreviations: Ctrl, healthy controls.

POF, premature ovarian failure; n, sample size; SD, standard deviation; FSH, follicle stimulating hormone; E2, oestradiol; AMH, anti-Müllerian hormone.

Table S4 Primers used in qPCR analysis, related to STAR Methods.

	Forward	Reverse
16sRNA	GTGSTGCAYGGYTGTCGTCA	ACGTCRTCCMCACCTTCCTC
L reuteri	GGCGGCTGTCTGGTCTGCAA	GCTTGCGACTCGTTGTACCGTC
L.brevis	CTTCTGGATGATCCCGCGGCG	ACCGCCTGCGCTCGCTTTAC
L.plantarum	ATTCATAGTCTAGTTGGAGGT	CCTGAACTGAGAGAATTTGA
L.fermenti	GCACCTGATTGATTTTGGTCG	GTCCATTGTGGAAGATTCCC
L.buchneri	GAAACAGGTGCTAATACCGTATAACA	CGCCTTGGTAGGCCGTTACCTTACCAA
	ACCA	CA
L.delteri	TACTGTTAAGGTTGGCGACAGC	TGTAGACTTGGCCCTTGAAAGT
Bcl-2	CCCGAGAGGTCTTTTTCCGAG	CCAGCCCATGATGGTTCTGAT
BAX	CCCGAGAGGTCTTTTTCCGAG	CCAGCCCATGATGGTTCTGAT
SOX7(mouse)	ATGCTGGGAAAGTCATGGAAG	CGTGTTCTGGTCACGAGAGA
SOX7(human)	TCGACGCCCTGGATCAACT	CTGGGAGACCGGAACATGC
Gapdh	GACAGTCAGCCGCATCTTCT	TTAAAAGCAGCCCTGGTGAC