

Additional file 2. Validation of PCOS-like stimuli in human granulosa cells.

(A) Relative mRNA expression of AR following DHEA-induced HA. (B) Relative mRNA expression of IRS1 after palmitic-acid-induced IR. (C) Relative mRNA expression of IL6 and (D) secreted IL-6 protein levels after LPS-induced CLI. (E-H) Same read-outs under combined HA + IR + CLI treatment (100 μ M DHEA + 100 μ M PA + 0.5 μ g/mL LPS, 12 h). (I) Cell viability assessed by CCK8 assay across increasing relative combined doses (0.25 $\times$, 0.5 $\times$, 1 $\times$, 2 $\times$). (J-M) Dose-response profiles of (J) AR, (K) IRS1, (L) IL6 mRNA expression, and (M) IL-6 secretion under combined stimulation for 12 h. (N-Q) Time-course profiles at the selected dose (1 $\times$): (N) AR, (O) IRS1, (P) IL6 mRNA, and (Q) IL-6 protein levels at 0, 3, 6, 12, and 24 h. Bars and curves represent mean $\pm$ SD (n = 3), with individual data points shown where applicable. *p < 0.05 vs. vehicle controls (Student's t-test or ANOVA where appropriate). These results validate the successful induction of HA, IR, and CLI phenotypes in hGCs, and confirm the selected dose (1 $\times$) and time (12 h) as biologically active yet non-toxic.

