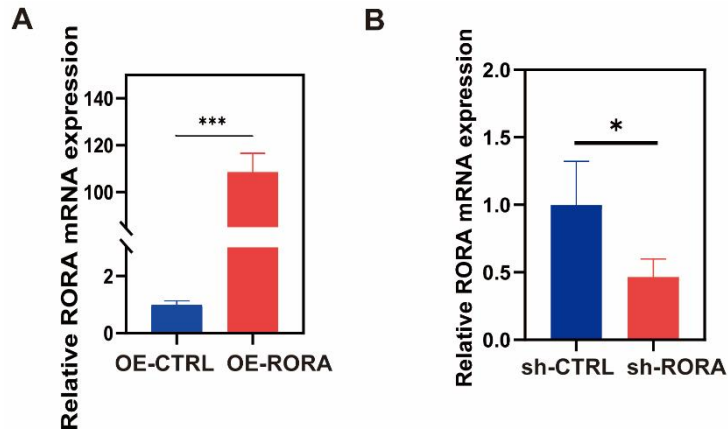
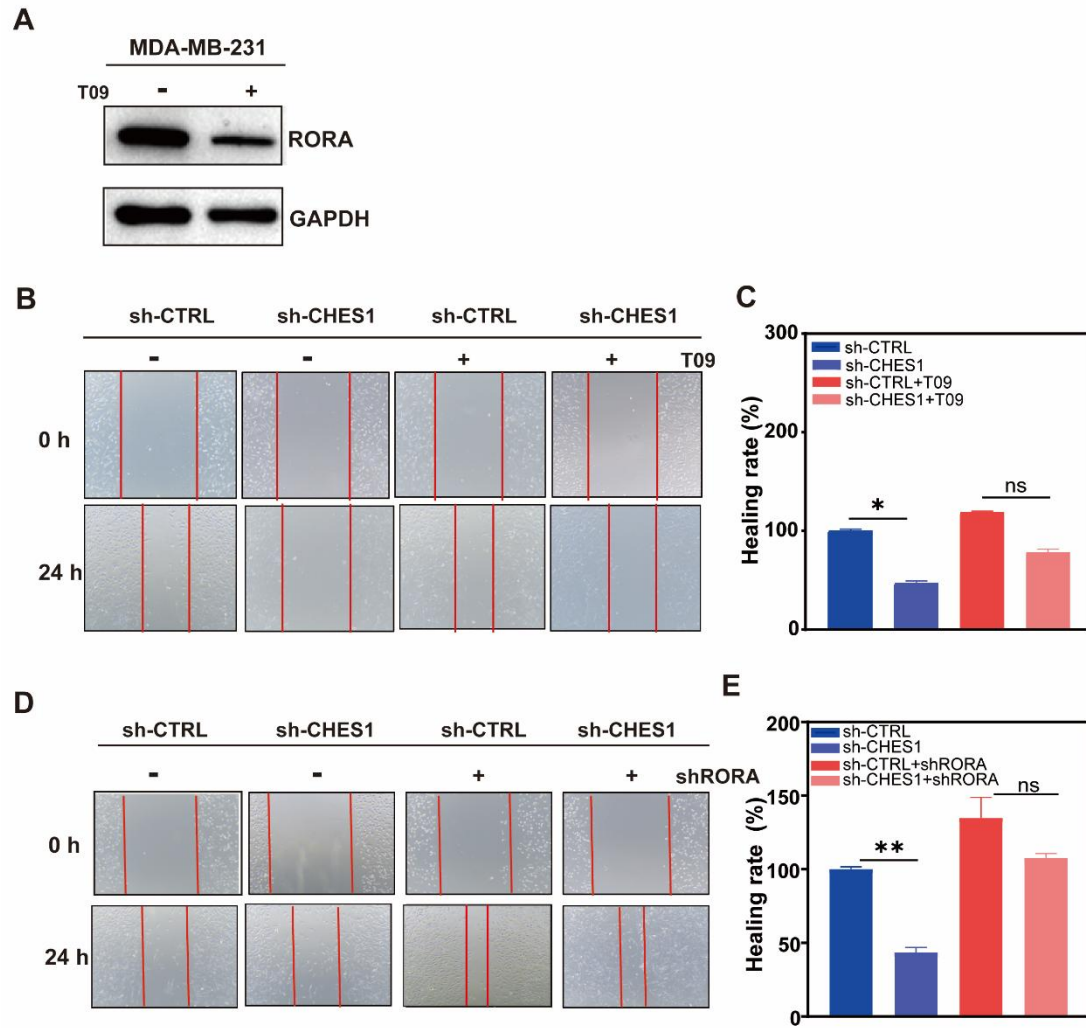


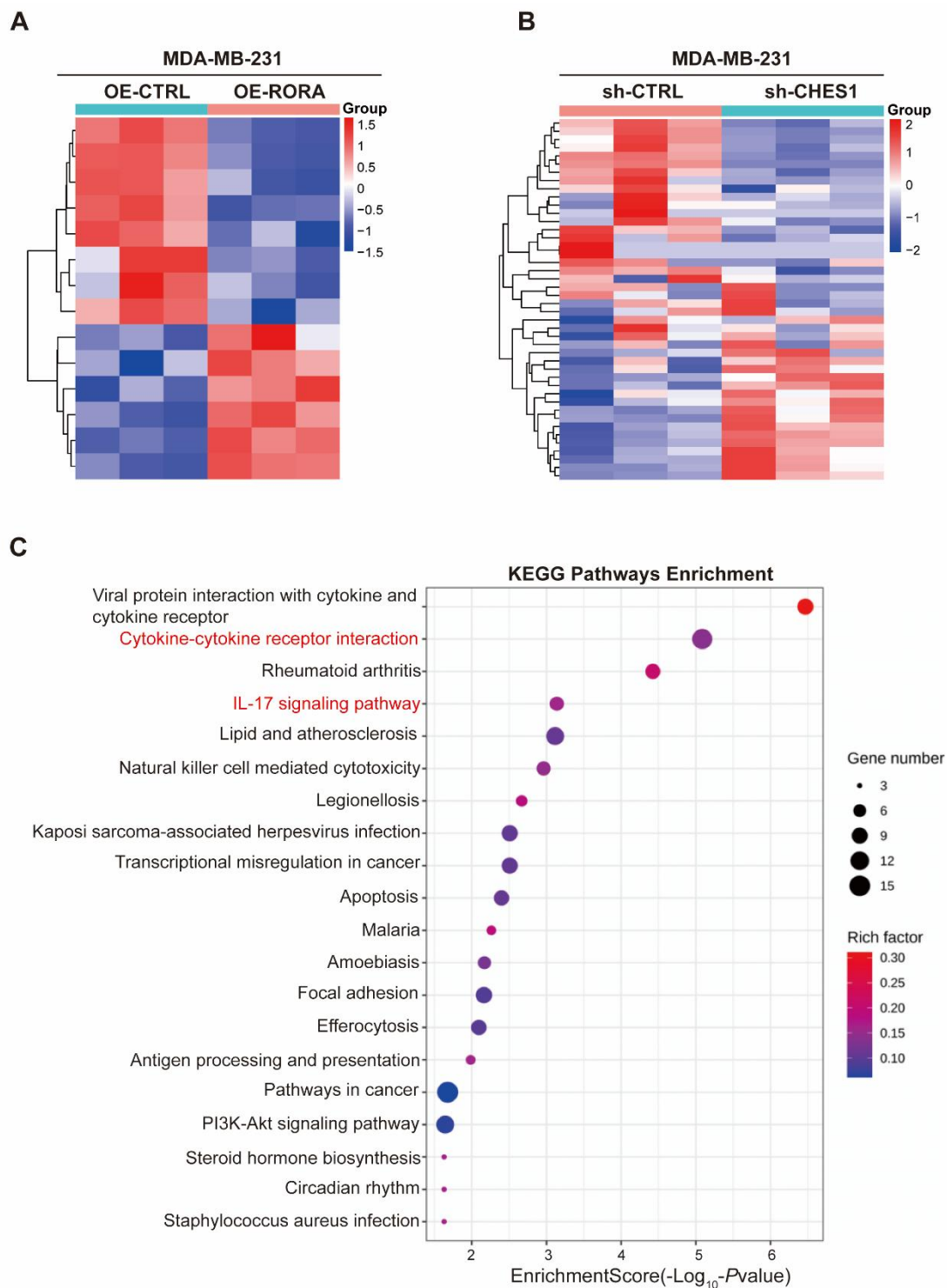
Supplementary figures



Supplementary Figure 1 (A) qRT-PCR analysis of relative RORA mRNA levels in MDA-MB-231 cells transfected with empty overexpression vector (OE-CTRL) or RORA overexpression plasmid (OE-RORA). **(B)** Relative RORA mRNA expression determined by qRT-PCR in MDA-MB-231 cells expressing control shRNA (sh-CTRL) or RORA-targeted shRNA (sh-RORA). Data are presented as mean $\pm$ SD. * $P < 0.05$, *** $P < 0.001$.

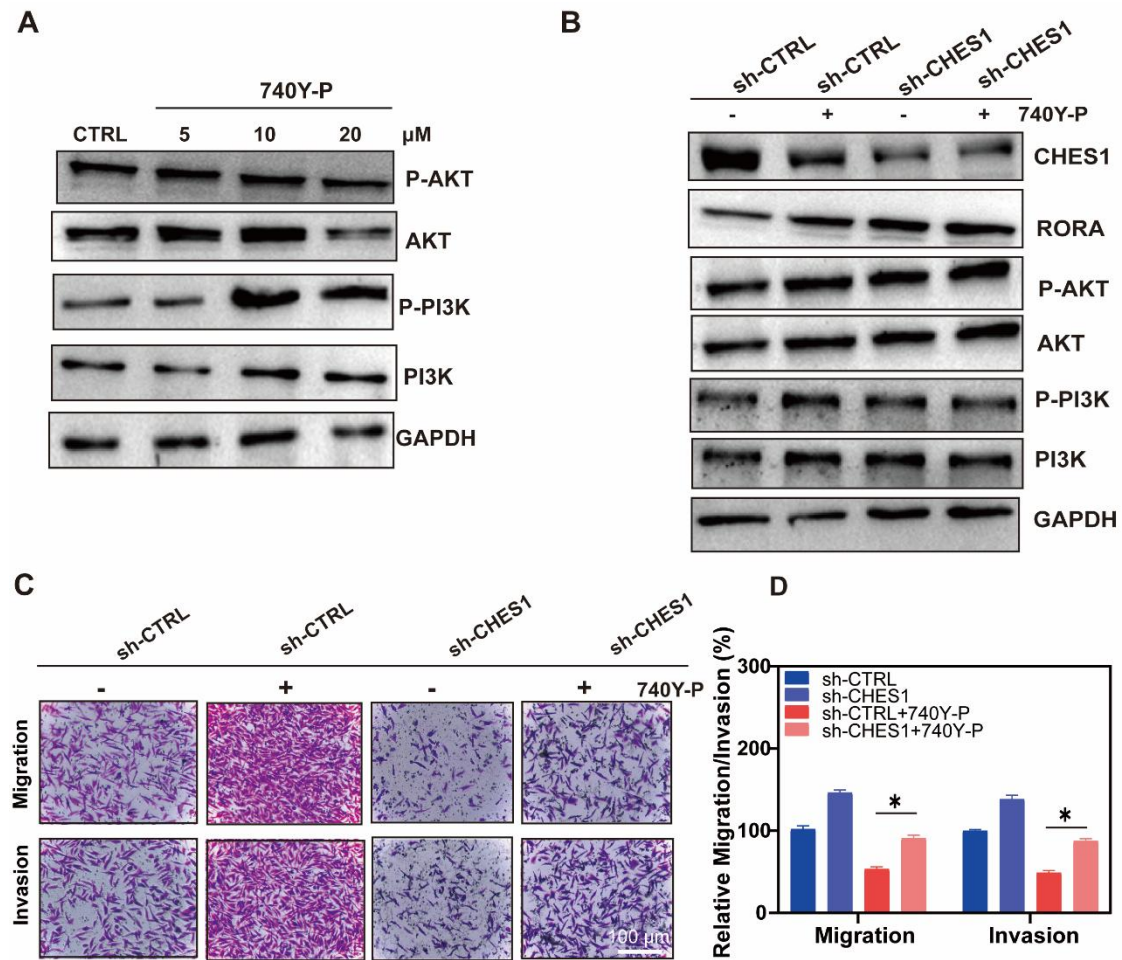


Supplementary Figure 2 (A) Western blot detection of RORA protein expression in MDA-MB-231 cells with or without T0901317 treatment, GAPDH as internal loading control. **(B-C)** Representative wound-healing images at 0 h and 24 h. **(B)** and quantitative statistics of relative migration area percentage **(C)** for four groups treated with T0901317. **(D-E)** Wound-healing bright-field micrographs at 0 h and 24 h with genetic sh-RORA intervention **(D)** and quantitative analysis of relative migration area **(E)**. Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, ns, not significant.



Supplementary Figure 3 (A) Hierarchical clustering heatmap of DEGs in OE-CTRL and OE-RORA MDA-MB-231 cells. Heatmap results showed that numerous PI3K/AKT pathway-associated DEGs were detected in OE-RORA cells. Color gradient represents normalized gene expression Z-score (red = upregulation, blue = downregulation). **(B)** Clustering heatmap of DEGs from sh-CTRL and sh-CHES1 groups. PI3K/AKT pathway-related DEGs were detected in shCHES1 MDA-MB-231 cells. Color gradient denotes normalized gene Z-score (red = upregulation, blue

= downregulation). **(C)** Bubble plot of top enriched KEGG pathways based on DEGs from OE-RORA vs OE-CTRL comparison. X-axis: enrichment score ($-\log_{10} P$ -value); Y-axis: pathway name; bubble size represents the number of enriched differential genes; bubble color gradient denotes the enrichment factor (Rich factor). PI3K-Akt signaling pathway and circadian rhythm pathways were markedly enriched.



Supplementary Figure 4 (A) Western blot analysis of total and phosphorylated PI3K/AKT (P-PI3K, P-AKT) in cells treated with gradient concentrations of PI3K agonist 740Y-P (0, 5, 10, 20 μ M). GAPDH was used as loading control. (B) Western blot detection of CHES1, RORA, PI3K, AKT and their phosphorylated proteins in sh-CTRL and sh-CHES1 cells with or without 20 μ M 740Y-P stimulation. (C) Transwell migration and invasion assays were performed to evaluate cell migratory and invasive capacities. Transmigrated cells were stained with crystal violet. (D) Quantitative histogram of relative migration and invasion rates normalized to untreated sh-CTRL group. * $P < 0.05$