



**Figure S1.** PI3K/AKT and ERK1/2 pathways alone are not involved in the LH-dependent activation of the Hippo pathway in granulosa cells

Primary cultured GCs were pre-treated with or without inhibitors against PKA (H-89, 50 $\mu$ M for 30 mins), AKT1/2/3 (MK-2206, 10 $\mu$ M for 60 mins) or MEK1/2 (UO126, 10 $\mu$ M for 60 mins) followed by treatment with LH (50 ng/ml) or vehicle control for 30 mins. Representative immunoblots show 1 replicate per condition, whereas quantification was done using 4 replicates per condition. CREB, AKT and ERK1/2 are well established substrates of PKA, AKT (by auto-phosphorylation) and MEK1/2 in GCs and confirmed the inhibition by H-89, MK-2206 and UO126, respectively.  $\beta$ -Actin (ACTB) was used as the loading control. Data are normalized by the sum of all data points for each replicate and are represented as means  $\pm$  SEM. Different letters above histograms indicate significant differences between treatment conditions.  $P \leq 0.05$ ; two-way ANOVA, followed by Tukey's post hoc test.