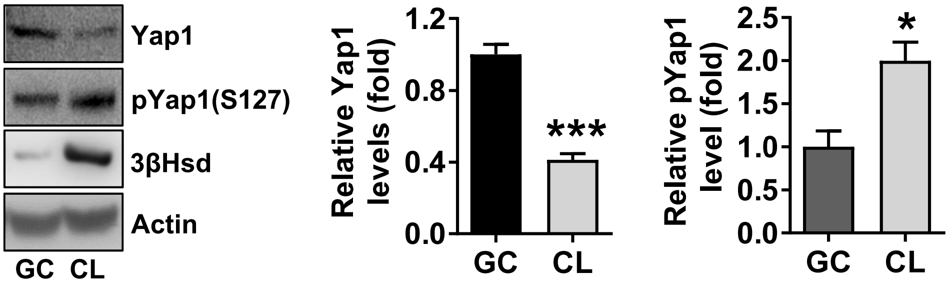
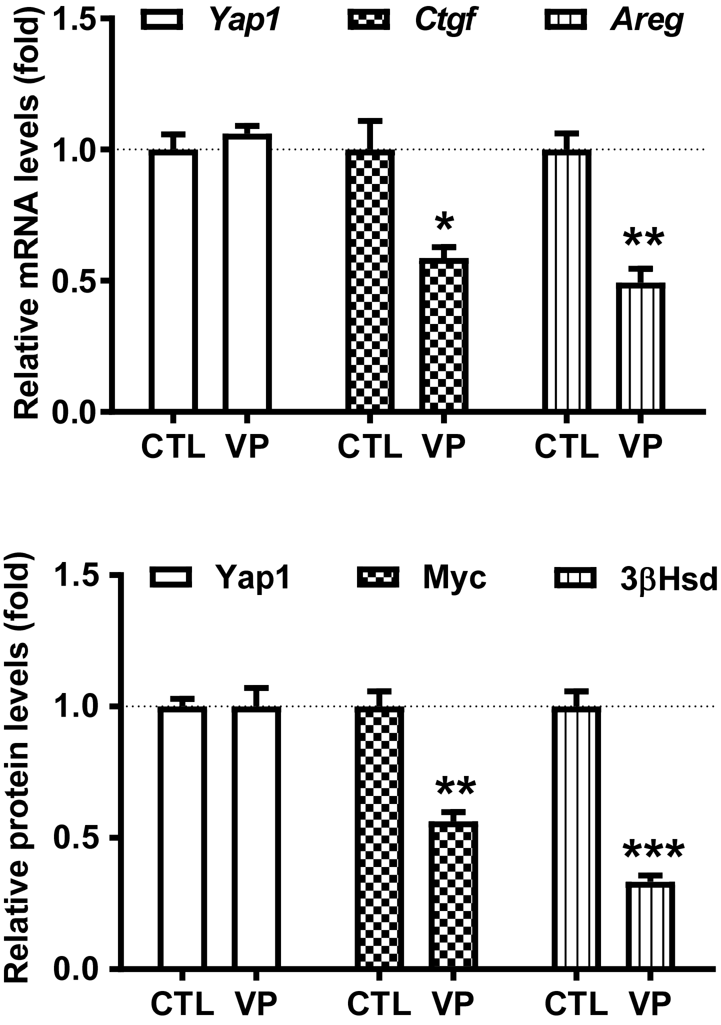
Supplemental information



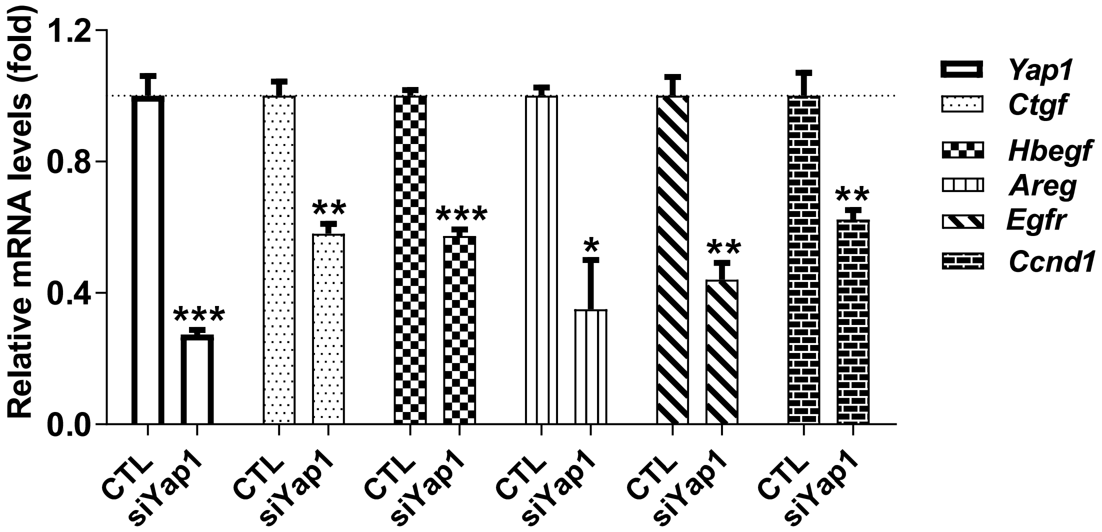
Supplementary Figure S1. YAP1 is differentially expressed in granulosa cells and luteal cells in the corpus luteum. Western blot showing the expression of YAP1 and serine(127) phosphorylated YAP1 [YAP1(S127)] in isolated granulosa cells (GC) and corpora lutea (CL) of C57BL/6 mice. Left two bar graphs show the quantitative immunosignal intensity of Yap1 and phosphorylated Yap1 in GC and CL detected by the Western blot. *: $P < 0.05$, compared to GC. ***: $P < 0.001$, compared to GC.

Supplemental information



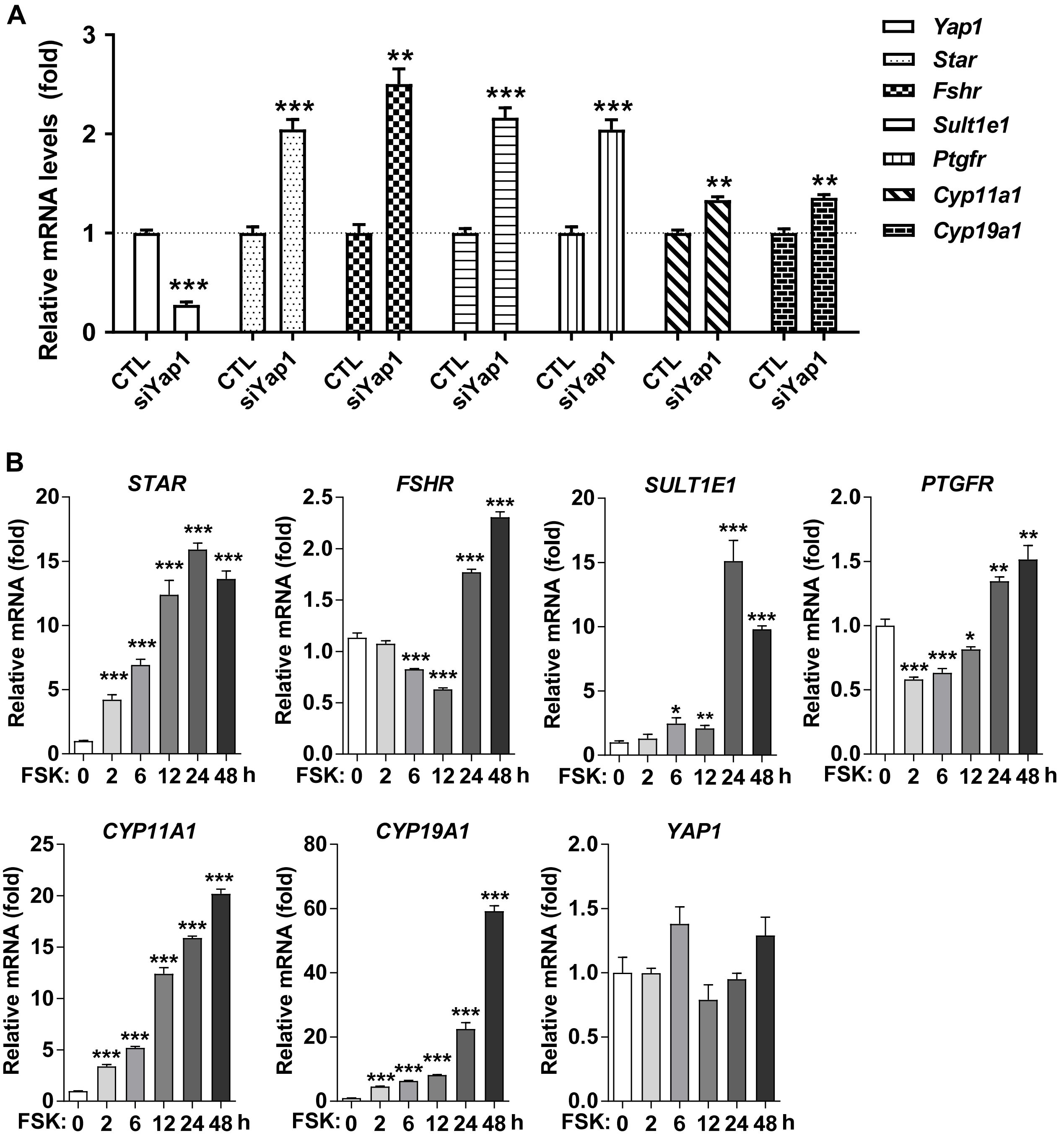
Supplementary Figure S2. Pharmacological inhibition of YAP1 in granulosa cells with Verteporfin. Quantitative data of Figure 3E showing relative mRNA (*Yap1, Ctgf, Areg*) and protein (Yap1, Myc, 3βHsd) expression in mouse ovarian tissues treated with vehicle (CTL) or Verteporfin (VP). mRNA and protein were extracted from vehicle-treated control (CTL) or Verteporfin-treated (VP) mouse ovaries. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$, compared with CTL.

Supplemental information



Supplementary Figure S3. YAP1 regulates HBEGF expression in granulosa cells. Quantitative data of Figure 6A showing relative mRNA levels of *Yap1, Ctgf, Hbegf, Areg, Egfr, and Ccnd1* in mouse granulosa cells with or without knockdown of Yap1 using Yap1 siRNA. RNA was extracted from mouse granulosa cells treated with non-targeting control siRNA (CTL) or Yap1 targeting siRNA (siYap1). Gene expression was determined by semi-quantitative PCR. Gapdh mRNA was used as a internal control to normalize gene expression data. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Supplemental information



Supplementary Figure S4. YAP1 suppresses granulosa cell differentiation.

A) Quantitative data of Figure 7D showing relative mRNA levels of *Yap1, Star, Fshr, Sult1e1, Ptgfr, Cyp11a1, and Cyp19a1* in mouse granulosa cells with or without knockdown of Yap1 using *Yap1* siRNA (siYap1). Total RNA was extracted from cultured primary mouse granulosa cells treated with non-targeting control siRNA (CTL) or Yap1-targeting siRNA (siYap1). **: $P < 0.01$; ***: $P < 0.001$, compared with CTL. **B)** Quantitative PCR results showing expression of granulosa cell differentiation-associated genes in KGN cells treated with forskolin (FSK, 10μM) for 0-48 hours. *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$, compared with control (0h).

Table 1. Primer sequences

GENES	FORWARD PRIMER SEQUENCES	REVERSE PRIMER SEQUENCES
Mouse		
Yap1	CAATACGGAATATCAATCCCAG	GATCGGAACTATTGGTTGTC
Ctgf	CCCGCCAACCGCAAGATT	AGGCGGCTCTGCTTCTCCA
Areg	AATGAGAACTCCGCTGCTAC	CCCCTGTGGAGAGTTCACTG
Gapdh	CAATGTGTCCGTCGTGGATCT	GCCTGCTTCACCACCTTCTT
Hbegf	ATGAAGCTGCTGCCGTCGGT	TCAGTGGGAGCTAGCCACGC
Egfr	GGAGGATGTAGTTGATGCTGATGAG	GGGCTGATTGTGATAGACAGGGTTC
Ccnd1	CATCAAGTGTGACCCGGACTG	CCTCCTCCTCAGTGGCCTTG
Star	AAAGCCAGCAGGAGAACGGGGA	GCCTCCATGCGGTCCACAAGTT
Fshr	GGGCTGGAGTCCATTCAGACG	CAGTTTATAACGACTGGTCAG
Sult1e1	GCCAAAGATGTCGCCGTTTC	AACCATACGGAACTTGCCCT
Ptgfr	CTGGCCATAATGTGCGTCTC	TGTCGTTTCACAGGTCACTGG
Cyp11a1	GGGGCAACAAGCTGCCCTTCAA	TGCAGGGTCATGGAGGTCGTGT
Cyp19a1	TCGGCATGCATGAGAACGGCA	CAGGGCCCGTCAGAGCTTTCAT
Tgfβr1	TACGTCAGACATTCGGGAAGCA	AGGTAACGCCAGGAATTGTTGC
Tgfβr2	GCCTTCGCCCTCTTTACATTGA	CGGAAGCTTCGGGATTTATGGT
Smad6	CCACTGGATCTGTCCGATTC	AAGTCGAACACCTTGATGGAG
Smad7	TCCTGCTGTGCAAAGTGTTC	AGTAAGGAGGAGGGGGAGAC
Smad1	ACCCCTACCACTATAAGCGAG	TGCTGGAAAGAGTCTGGGAAC
Smad2	ATGTCGTCCATCTTGCCATTC	AACCGTCCTGTTTTCTTTAGCTT
Smad3	CACAGCCACCATGAATTACGG	TGGCGTCTCTACTCTCTGATAGT
Human		
YAP1	GCAACTCCAACCAGCAGCAACA	CGCAGCCTCTCCTTCTCCATCTG
HBEGF	GCTCCCTCCTGCATCTGCCA	GAGGCTCAGCCCATGACACCTCT
STAR	CGTGACTTTGTGAGCG	GCCACGTAAGTTTGGT
FSHR	TTCAAGAACAAGGATCCATTCC	CCTGGCCCTCAGCTTCTTAA
SULT1E1	AAGGGAATTACAGGAGACTG	TAGATTCCTTCATTTGCTGC
PTGFR	CCCATTTCTGGTTACAATGG	TAGAGATTCTTAAGGACAGCC
CYP11A1	TGGGTCGCCTATCACCAGTAT	CCACCCGGTCTTTCTTCCA
CYP19A1	TGAATATTGGAAGGATGCACAGAC	TGGAATCGTCTCAGAAGTGTAACGAG
GAPDH	CAGCCTCAAGATCATCAGCA	GTCTTCTGGGTGGCAGTGAT
Mouse genotyping		
Foxl2-Cre/Ert2	Common: AGAGAAGAGAGTGAGAGCCG	Wild Type (335 bp): GAGCGCCACGTACGAGTACG Mutant (221 bp) : GTCCAGCTCGACCAGGATGG
Cyp19-Cre	Wild Type (109 bp): AAATGAGGACAGGCACCTTG Mutant (140 bp): GAAACAGGGGCAATGGTG	Common: CGGATAAGTAATGCCCCAGA
Yap1^{flox}	Common: ACATGTAGGTCTGCATGCCAGAGGAGG	Wild Type (457 bp) & Loxp (600 bp) : AGGCTGAGACAGGAGGATCTCTGTGAG Knockout (338 Bp) : TGGTTGAGACAGCGTGCACTATGGAGC