Supplemental Figure 1

- ■ *Control*
- □ *Wnt5a*flox/flox;*CYP19*-Cre
- ▦ *Wnt11*flox/flox;*CYP19*-Cre
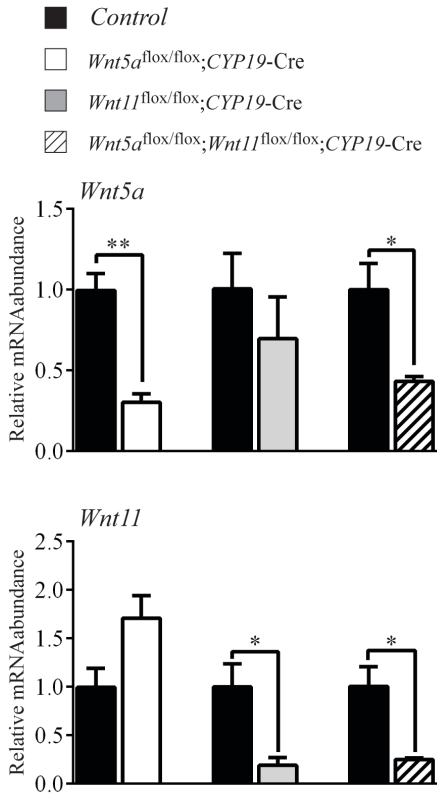- ▨ *Wnt5a*flox/flox;*Wnt11*flox/flox;*CYP19*-Cre

Figure S1 (left): *Wnt5a* and *Wnt11* knockdown efficiency in the conditional knockout models. Expression of *Wnt5a* and *Wnt11* was determined by RT-qPCR in granulosa cells isolated from immature, eCG treated mice of the indicated genotypes. Expression of each transcript was normalized to the housekeeping gene *Rpl19*. Control = corresponding genotype lacking the *CYP19*-cre transgene. Values for each experimental genotype are expressed relative to their corresponding control. n=4 animals per genotype, columns = means, error bars = SEM, * = values are statistically different (P<0.05), ** = values are statistically different (P<0.01).
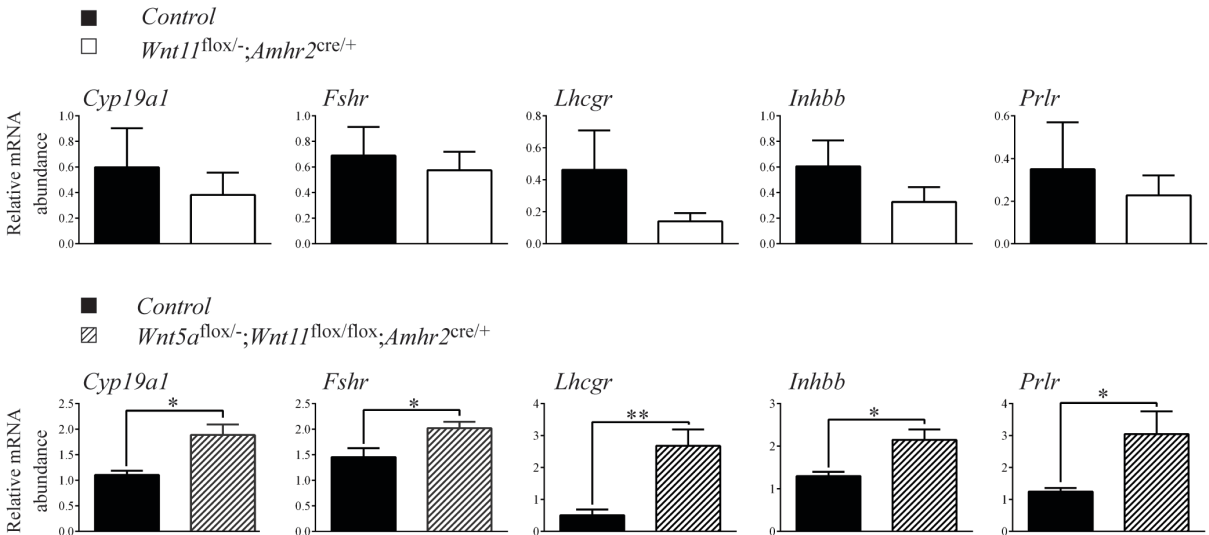
Supplemental Figure 2

- ■ *Control*
- □ *Wnt11*flox/-;*Amhr2*cre/+

- ■ *Control*
- ▨ *Wnt5a*flox/-;*Wnt11*flox/flox;*Amhr2*cre/+

Figure S2 (above). Expression of WNT5a target genes in *Wnt11*flox/-;*Amhr2*cre/+ and *Wnt5a*flox/-;*Wnt11*flox/-;*Amhr2*cre/+ mice. RT-qPCR analysis of the indicated genes was done on granulosa cell samples collected from immature mice 48h after eCG treatment (n=4 animals/ genotype). All data were normalized to the housekeeping gene *Rpl19*. Control = corresponding genotype lacking the *Amhr2*cre allele. Columns = means, error bars = SEM, asterisks indicate a statistically significant (*: P<0.05, **: P<0.01) difference vs control.
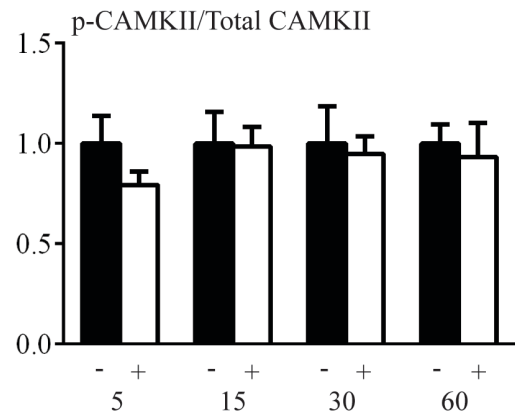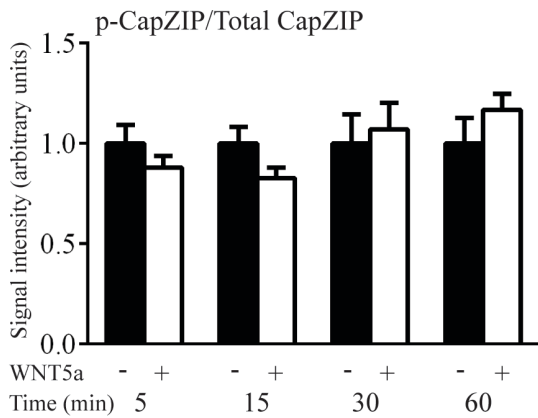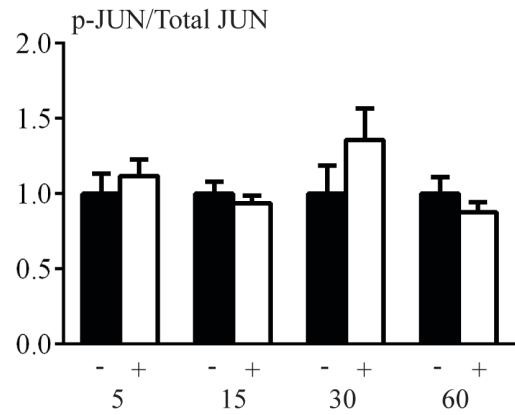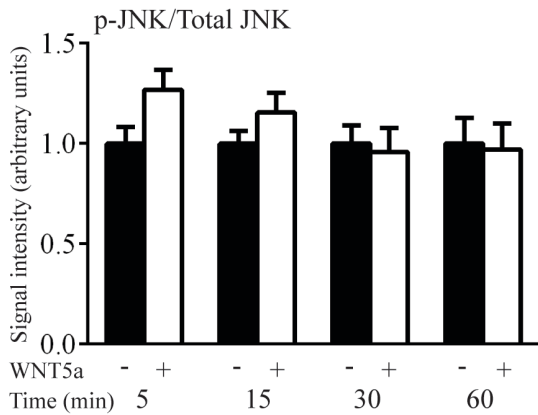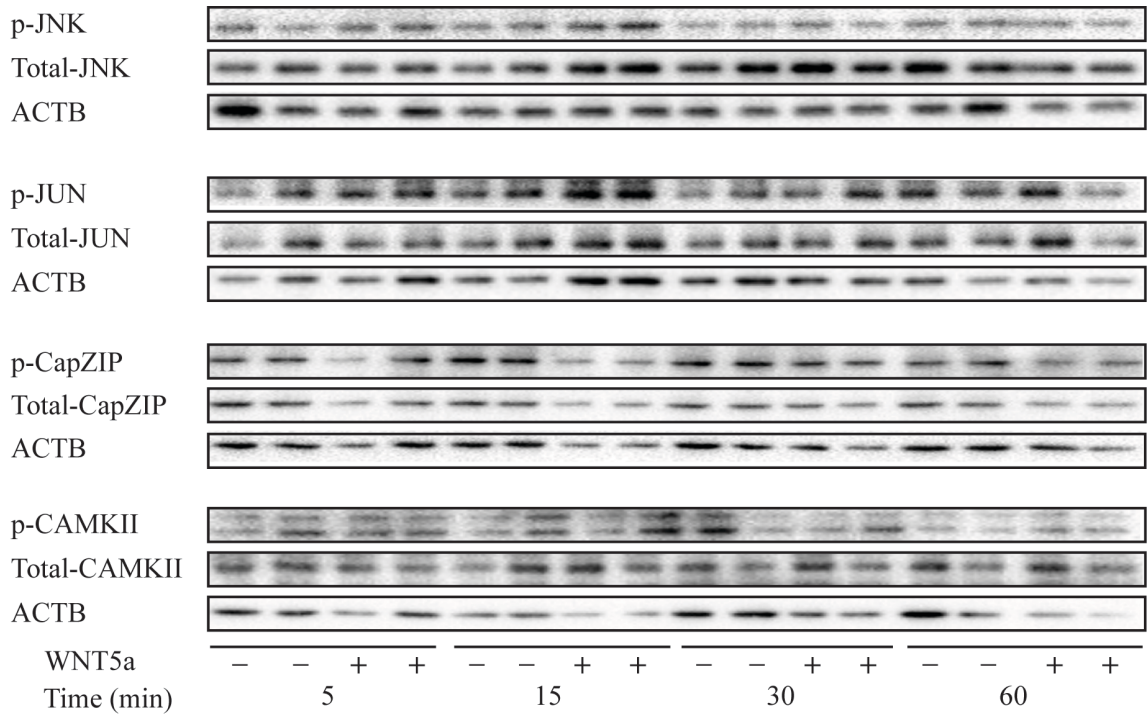
Supplemental Figure 3

Figure S3 (previous page). WNT5a does not regulate non-canonical signaling effectors in granulosa cells. Primary cultured granulosa cells were treated or not with WNT5a (3.5 µg/ml) for the indicated times, and the expression of the indicated proteins and phosphoproteins was studied by immunoblotting. Representative immunoblots showing 2 samples/treatment and time are shown in the upper panels, quantitative analyses of 4 samples/treatment and time are shown in the graphs. Columns = means, error bars = SEM, values for the WNT5a treated groups are expressed relative to their corresponding controls at each time point.
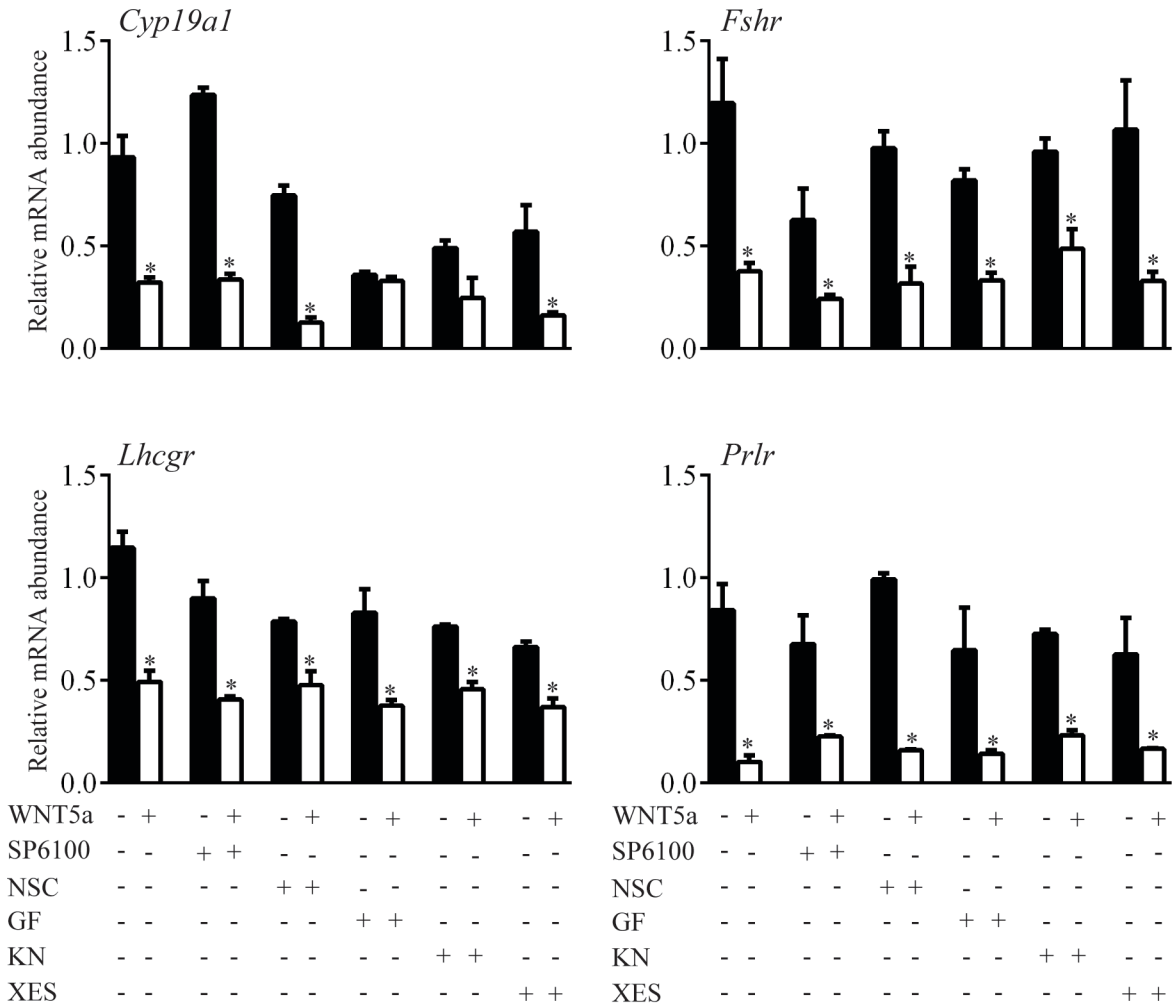
Supplemental Figure 4

Figure S4. Inhibition of noncanonical signaling does not prevent WNT5a-mediated suppression of its target genes. Primary cultured granulosa cells were treated with WNT5a (3.5 µg/ml) for 15 minutes, with or without pretreatment with the inhibitors SP600125 (SP6), NSC23766 (NSC), GF109203X (GF), KN-93 (KN) or Xestrospongin C (Xes), and the mRNA levels of the indicated WNT5a target genes were studied by RT-qPCR. Expression of each transcript was normalized to the housekeeping gene *Rpl19*, n = 4 samples/treatment. Columns = means, error bars = SEM, asterisks (*) indicate a statistically significant difference vs control, $P < 0.05$.