Supplementary Figure 1: Mammary epithelial cell preparation. In order to determine receptor expression specifically in MECs without contamination of fat and stromal cells, primary MECs were isolated from post-pubertal virgin females, or age-matched pregnant females at early, mid, or late pregnancy using Percoll gradient purification. (A, B) Sample phase contrast photomicrographs of epithelial (A) and stromal (B) fractions suspended in 1X PBS following Percoll gradient were captured at 10X on a Zeiss Observer.Z1 microscope with an AxioCamMR3 camera and Axiovision 4.6.3 software (Carl Zeiss MicroImaging, Inc. Thornwood, NY).. Purified epithelial cells (A) are present as 3-dimensional (3D) organoid structures rather than single cells. (C) To further confirm that the primary cell preparations contained purified MECs, we analyzed the relative expression of epithelial (CK18 and E-cadherin) and mesenchymal (N-cadherin) markers normalized to β-actin by Q-PCR. RNA from primary mouse mammary fibroblasts was used as a positive control for N-cad expression and as a representative of mammary gland stromal cells. Any MEC sample that was +1 standard deviation outside of the mean for N-cad expression was removed from further analysis to minimize any interference from small amounts of contaminating stromal cells. (D) In addition to visualization of conceptus prior to MEC isolation, we analyzed β-casein expression in primary samples by Q-PCR to confirm the pregnancy status of mice {Robinson, 1995 #39}. β-casein expression was low, yet detectable in virgin samples, increased 8-fold by P5 and increased further through P12 and P18. Numbers at the top of columns indicate relative quantity (R.Q.) of β-casein after normalization to β-actin. L.P., late puberty, 10-week virgin; P5, pregnancy day 5; P12, pregnancy day 12; P18, pregnancy day 18. a, $P<0.04$ vs P5; b, $P<0.01$ vs P18, *, N-cad R.Q.$\leq 0.074 \pm 0.016$; c, $P<0.02$ vs LP and P5; d, $P<0.05$ vs P12. Data are expressed as mean ± SEM. n=5 for L.P. and P5, n=4 for P12 and P18.

Supplementary Figure 2: Ligand-dependent activation of IR and Akt in primary MECs. Primary MECs from virgin animals were treated with 0 nM (Control), 10 nM, 50 nM or 100 nM of insulin (A, B), IGF-I (C) or IGF-II (D) for 15 min immediately followed by protein isolation. (A) Quantification of IR activation by ELISA in primary MEC samples. Data indicate mean ± SEM units of phosphorylated (pYpY1162/1163) IRβ subunit per nanogram total IRβ subunit protein. (B-D) Graphs depict densitometric analysis of Akt western immunoblot images (percent of control). Bars indicate mean ± SEM of phosphorylated Akt (Ser473)/total Akt expression. a, $P\leq 0.05$ vs control; b, $P\leq 0.02$ vs control and 10 nM; c, $P\leq 0.04$ vs control, 10 nM and 50 nM. n=3 samples per treatment.

Supplementary Figure 3: Schematic representation of hybridR, IGF-1R and IR expression, ligand affinity, signaling and downstream biological effects in MECs during puberty. < and > symbols indicate relative levels of receptors (top) or Akt phosphorylation (bottom) based on our data. Solid lines indicate reported activation of signaling molecules or biological outcomes. Dashed lines of signaling molecules indicate low or unknown levels of activation and dashed lines of biological outcomes indicate hypothesized functions. Model is based on data from these experiments and from studies in text references 15,23,28,35.