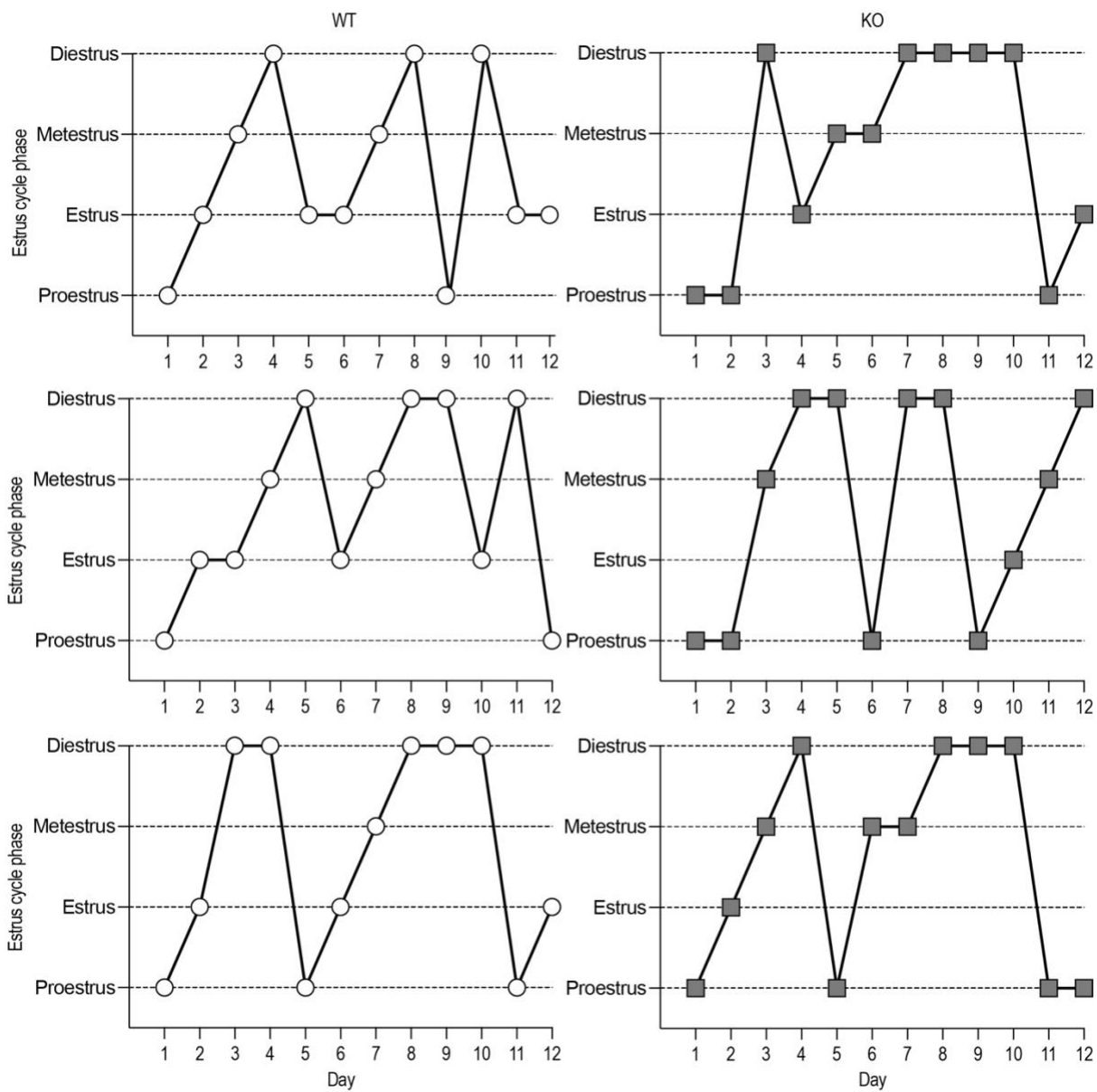


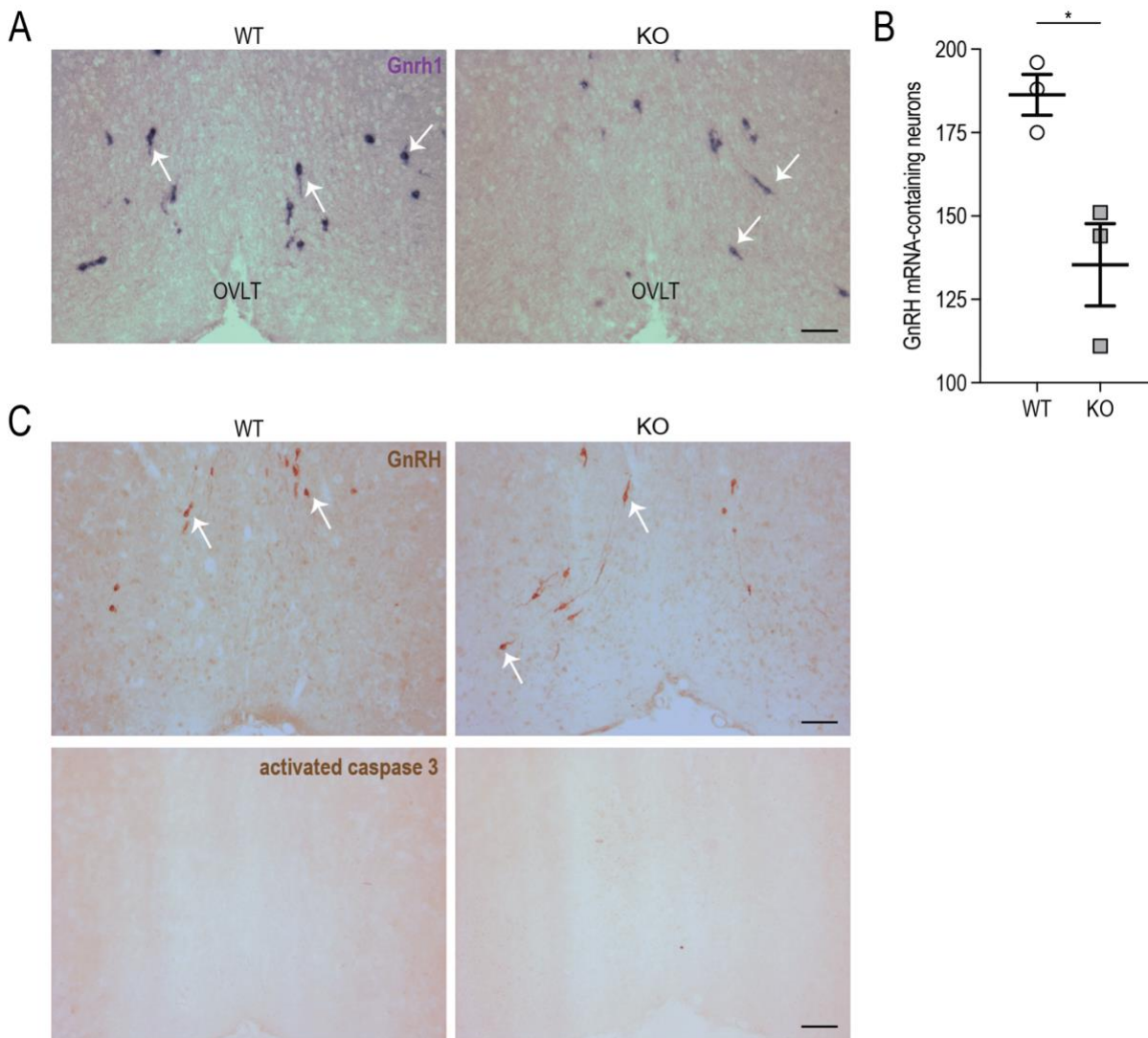
Supplementary Figure 1. Mouse anti-p140Cap antibody specificity.

Maximum intensity projections of z-stack images (5 serial image planes; z step size = 1 μm) of p140Cap in the cortex of *p140Cap* KO and WT P120 mice. Scale bar: 100 μm .



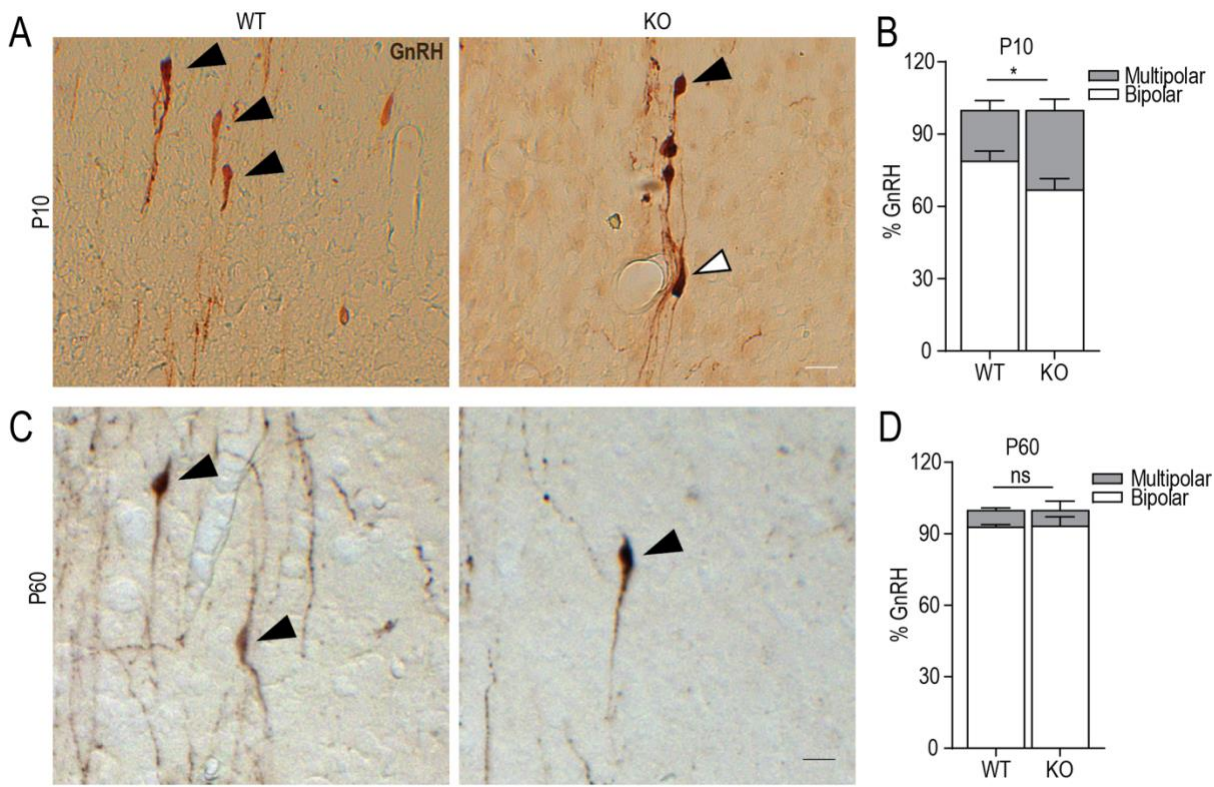
Supplementary Figure 2. *p140Cap* KO females display abnormal estrous cyclicity.

Representative estrous cycle transitions in *p140Cap* KO and WT adult mice. Each graph shows the transitions observed in a single animal over 12 days. The phase of the estrus cycle was determined as described in the material and methods section.



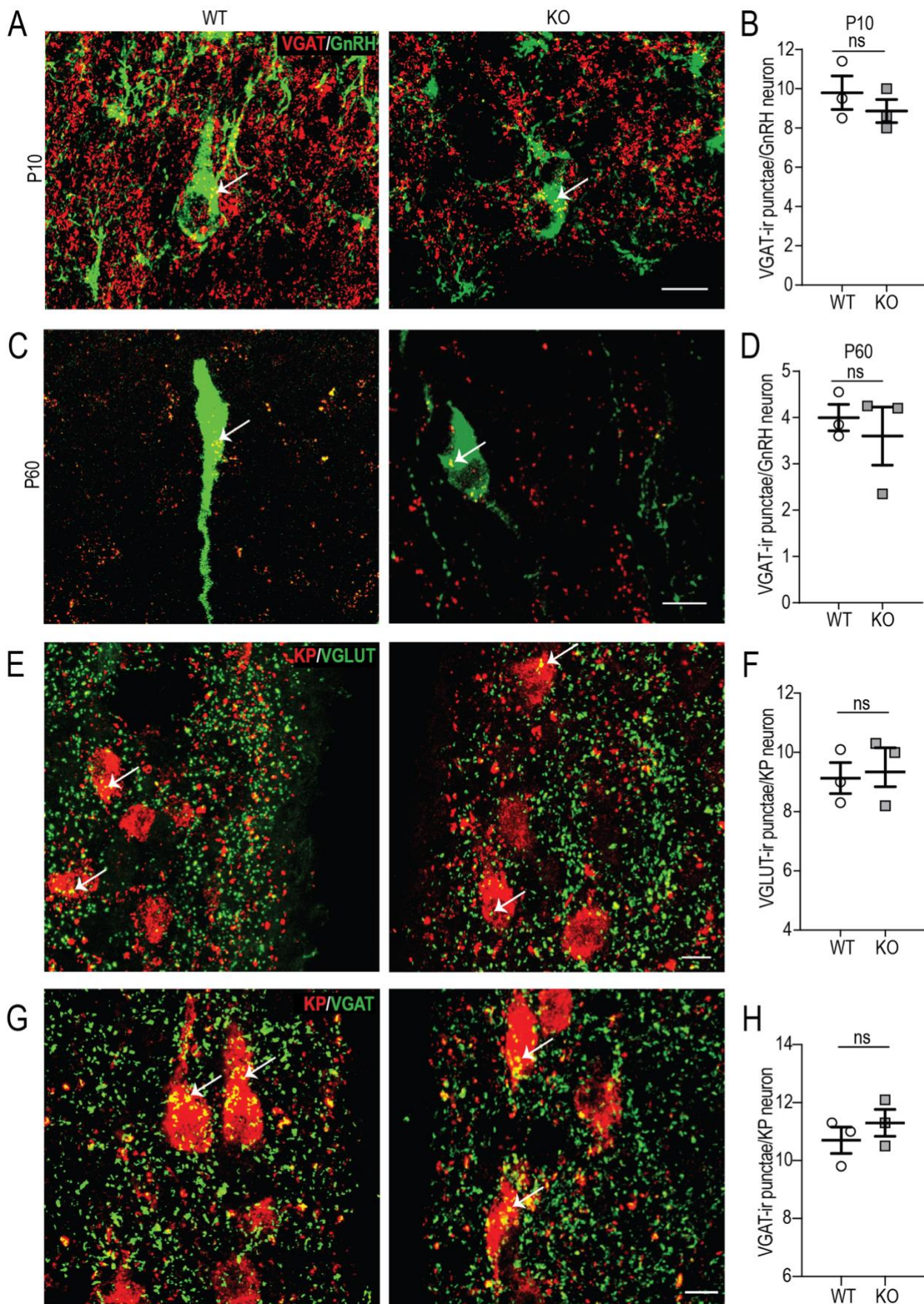
Supplementary Figure 3. *p140Cap* KO female mice show a reduced number of *Gnrh1* mRNA-containing neurons at P60 and unaltered cell death in the MPOA at P30.

(A) *In situ* hybridization for the *Gnrh1* transcript in the MPOA of *p140Cap* KO and WT P60 female mice. Arrows indicate *Gnrh1*-expressing neurons. Scale bar: 250 μ m. (B) Number of *Gnrh1*-expressing neurons in *p140Cap* KO and WT P60 female mice. $n = 3$ WT mice; 3 *p140Cap* KO mice. Unpaired, two-tailed t test, $P = 0.02$. (C) Immunostaining of GnRH neurons (upper panels) and apoptotic cells (lower panels) in adjacent sections of the MPOA of *p140Cap* KO and WT P30 female mice. Arrows indicate *GnRH*-ir neurons. Scale bar: 250 μ m. OVLT = organum vasculosum of lamina terminalis. Data are represented as means \pm SEM. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant.



Supplementary Figure 4. GnRH neurons in *p140Cap* KO mice show delayed morphological maturation.

(A) Immunostaining of GnRH neurons in *p140Cap* KO and WT P10 mice. Scale bar: 20 μ m. (B) Fraction of unipolar/bipolar (1 or 2 visible extensions) and multipolar (more than 2 visible extensions) GnRH neurons in *p140Cap* KO and WT P10 mice. $n = 125$ WT neurons; 180 *p140Cap* KO neurons. Unpaired, two-tailed t test, $P = 0.05$ (C) Immunostaining of GnRH neurons in *p140Cap* KO and WT P60 mice. Scale bar: 20 μ m. (D) Fraction of bipolar and multipolar GnRH neurons in *p140Cap* KO and WT P60 mice. $n = 130$ WT neurons; 90 *p140Cap* KO neurons. Unpaired, two-tailed t test, $P = 0.29$. Black arrowheads indicate bipolar GnRH neurons, white arrowheads indicate multipolar GnRH neurons. Scale bars: 20 μ m. Data are represented as means \pm SEM. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant.



Supplementary Figure 5. *p140Cap* KO mice show normal GABAergic *punctae* on GnRH neurons and normal glutamatergic and GABAergic *punctae* on KP neurons.

(A) Maximum intensity projections of z-stack images (20 serial image planes; z step size = 0.5 μ m) of VGAT-ir *punctae* (red) and GnRH neurons (green) in *p140Cap* KO and WT P10 mice. Arrows indicate VGAT-ir *punctae* on GnRH neurons. Scale bar: 10 μ m. (B) Average number of VGAT-ir *punctae* on GnRH neurons in *p140Cap* KO and WT P10 mice. n = 3 WT mice; 3 *p140Cap* KO mice; at least 20 GnRH neurons

were analyzed for each mouse. Unpaired, two-tailed t test, $P = 0.42$. (C) Maximum intensity projections of z-stack images (20 serial image planes; z step size = $0.5 \mu\text{m}$) of VGAT-ir *punctae* (red) and GnRH neurons (green) in *p140Cap* KO and WT P60 mice. Arrows indicate VGABA-ir *punctae* on GnRH neurons. Scale bar: $10 \mu\text{m}$. (D) Average number of VGAT-ir *punctae* on GnRH neurons in *p140Cap* KO and WT P60 mice. $n = 3$ WT mice; 3 *p140Cap* KO mice; at least 20 GnRH neurons were analyzed for each mouse. Mann-Whitney test, $P > 0.99$. (E) Maximum intensity projections of z-stack images (10 serial image planes; z step size = $0.5 \mu\text{m}$) of VGLUT-ir *punctae* (green) and KP neurons (red) in P60 *p140Cap* KO and WT mice. Arrows indicate VGLUT-ir *punctae* on KP neurons. Scale bar: $10 \mu\text{m}$. (F) Average number of VGAT-ir *punctae* on KP neurons in *p140Cap* KO and WT P60 mice. $n = 3$ WT mice; 3 *p140Cap* KO mice; at least 50 GnRH neurons were analyzed for each mouse. Unpaired, two-tailed t test, $P = 0.56$. (G) Maximum intensity projections of z-stack images (10 serial image planes; z step size = $0.5 \mu\text{m}$) of VGAT-ir *punctae* (green) and KP neurons (red) in *p140Cap* KO and WT P60 mice. Arrows indicate VGAT-ir *punctae* on KP neurons. Scale bar: $10 \mu\text{m}$. (B) Average number of VGAT-ir *punctae* on KP neurons in *p140Cap* KO and WT P60 mice. $n = 3$ WT mice; 3 *p140Cap* KO mice; at least 50 GnRH neurons were analyzed for each mouse. Unpaired, two-tailed t test, $P = 0.34$. Data are represented as means \pm SEM. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant.