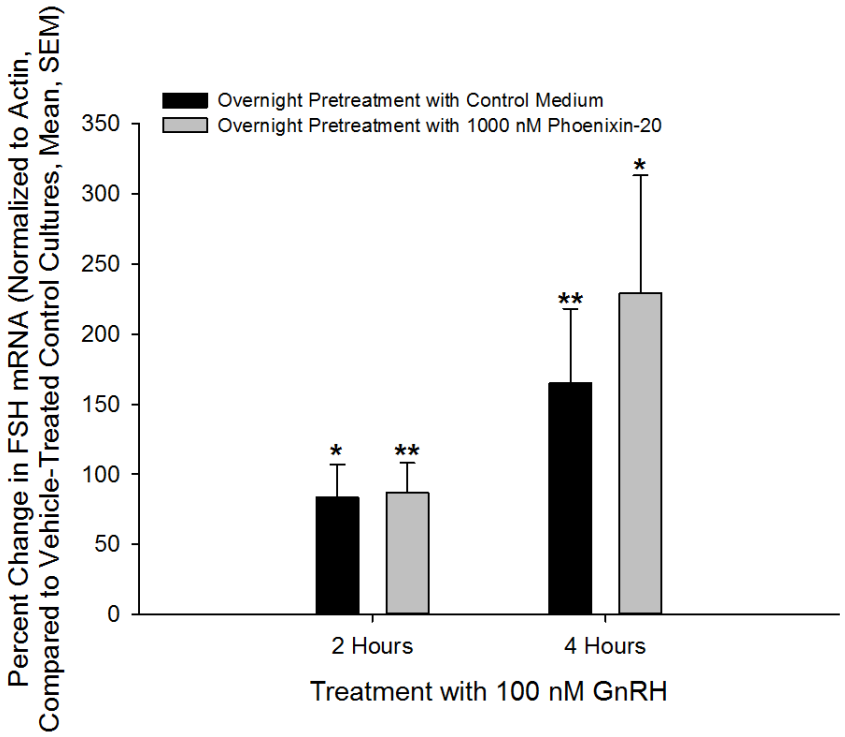
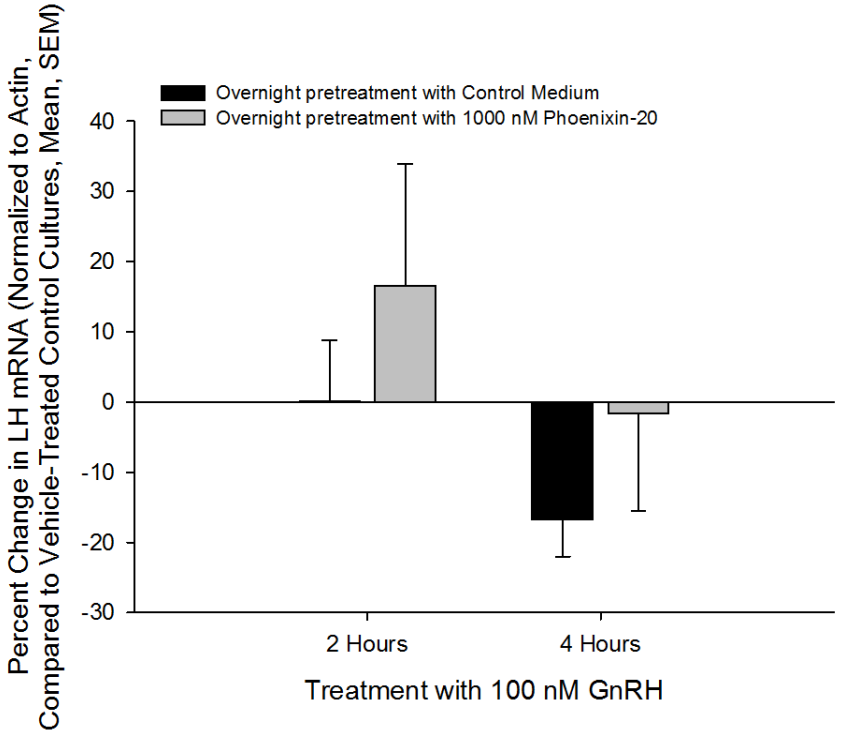
Supplemental Figure 1

A.

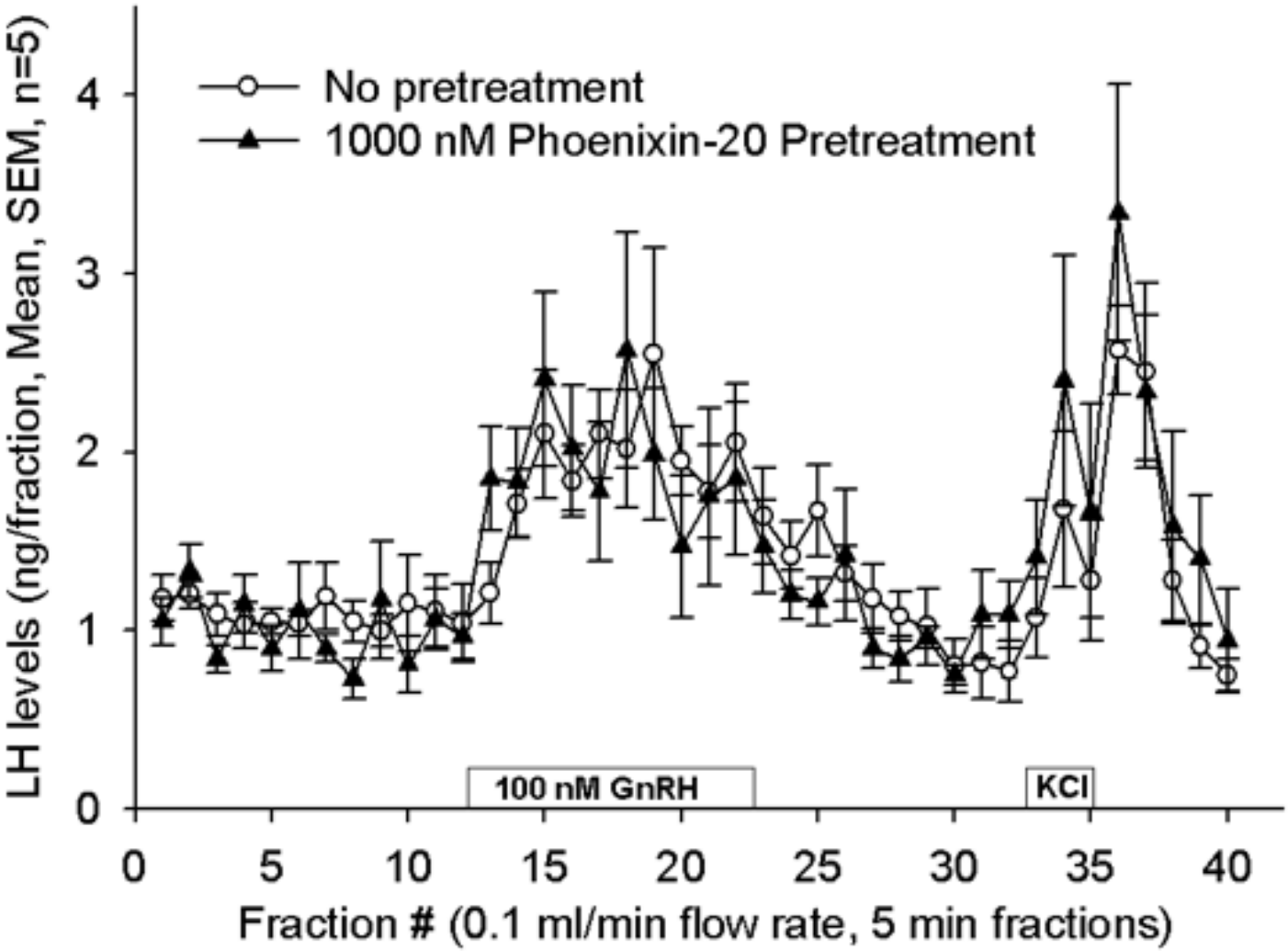


B.



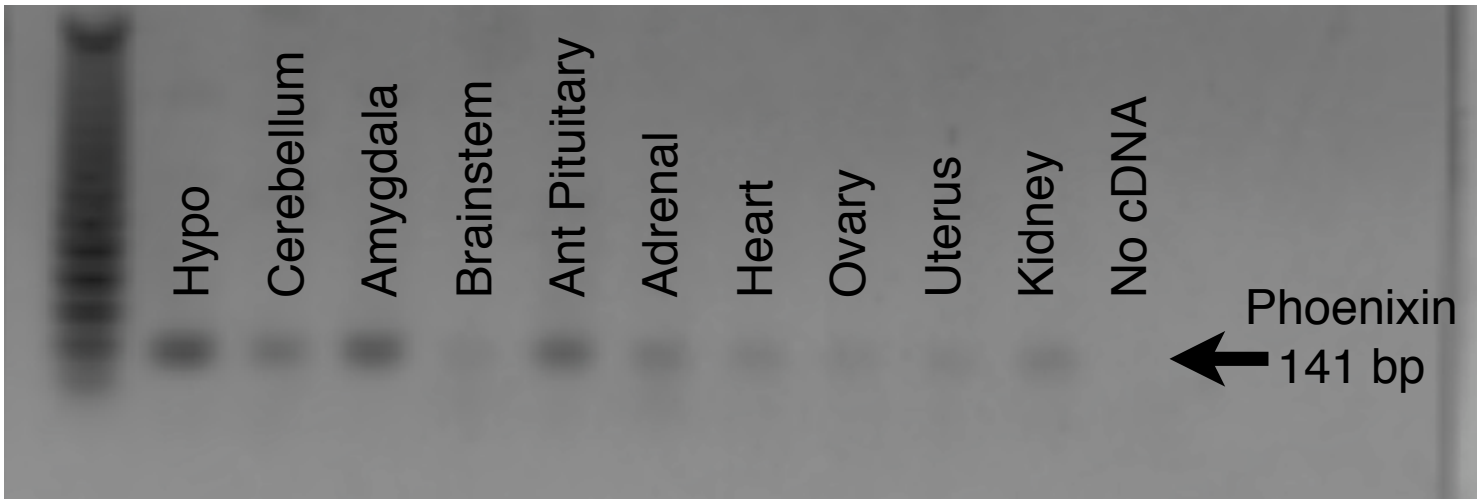
Supplemental Figure 1: Pretreatment with phoenixin-20 enhances GnRH-stimulated expression of FSH. Primary pituitary cultures obtained from male rat donors were pretreated overnight with either control media or 100 nM phoenixin-20. The following day, cells were exposed to either control media alone, or media containing 100 nM GnRH for 2 or 4 hours. Cells were lysed, total RNA was collected, and cDNA was produced. Pretreatment with phoenixin-20 potentiated GnRH-stimulated expression of FSH (A), following 4 hours of exposure to GnRH. Phoenixin-20 appeared to enhance GnRH-stimulated expression of LH (B), although this effect failed to attain significance. *$p<0.05$, **$p<0.01$, vs. vehicle-treated cultures, as determined by t test.

Supplemental Figure 2



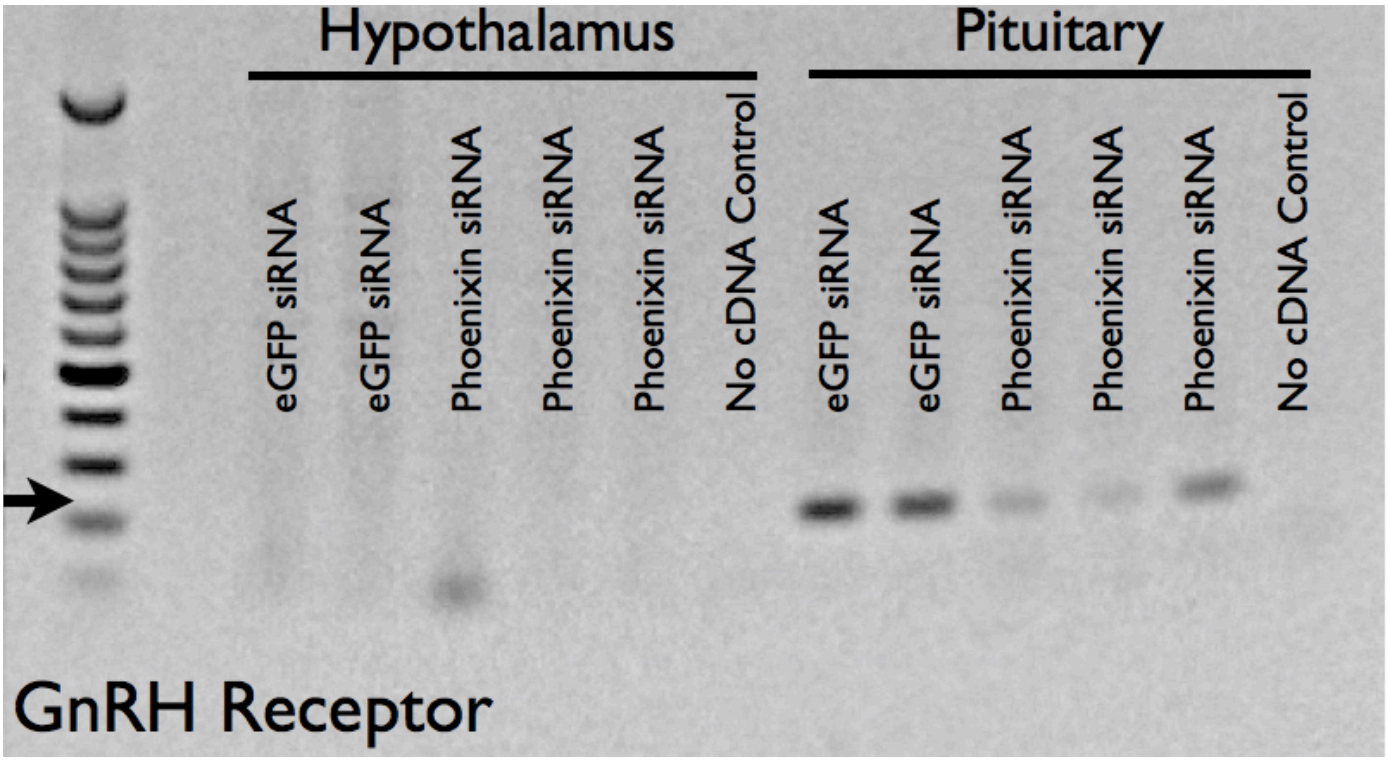
Supplemental Figure 2: Pretreatment with phoenixin-20 does not enhance GnRH-stimulated LH release from male pituitary cell cultures. Male pituitary cell cultures were pretreated with either control media or 1000 nM phoenixin-20 for 18 hours prior to exposure to 100 nM GnRH, followed by KCl. Phoenixin-20 did not alter GnRH-stimulated LH release at any time point.

Supplemental Figure 3



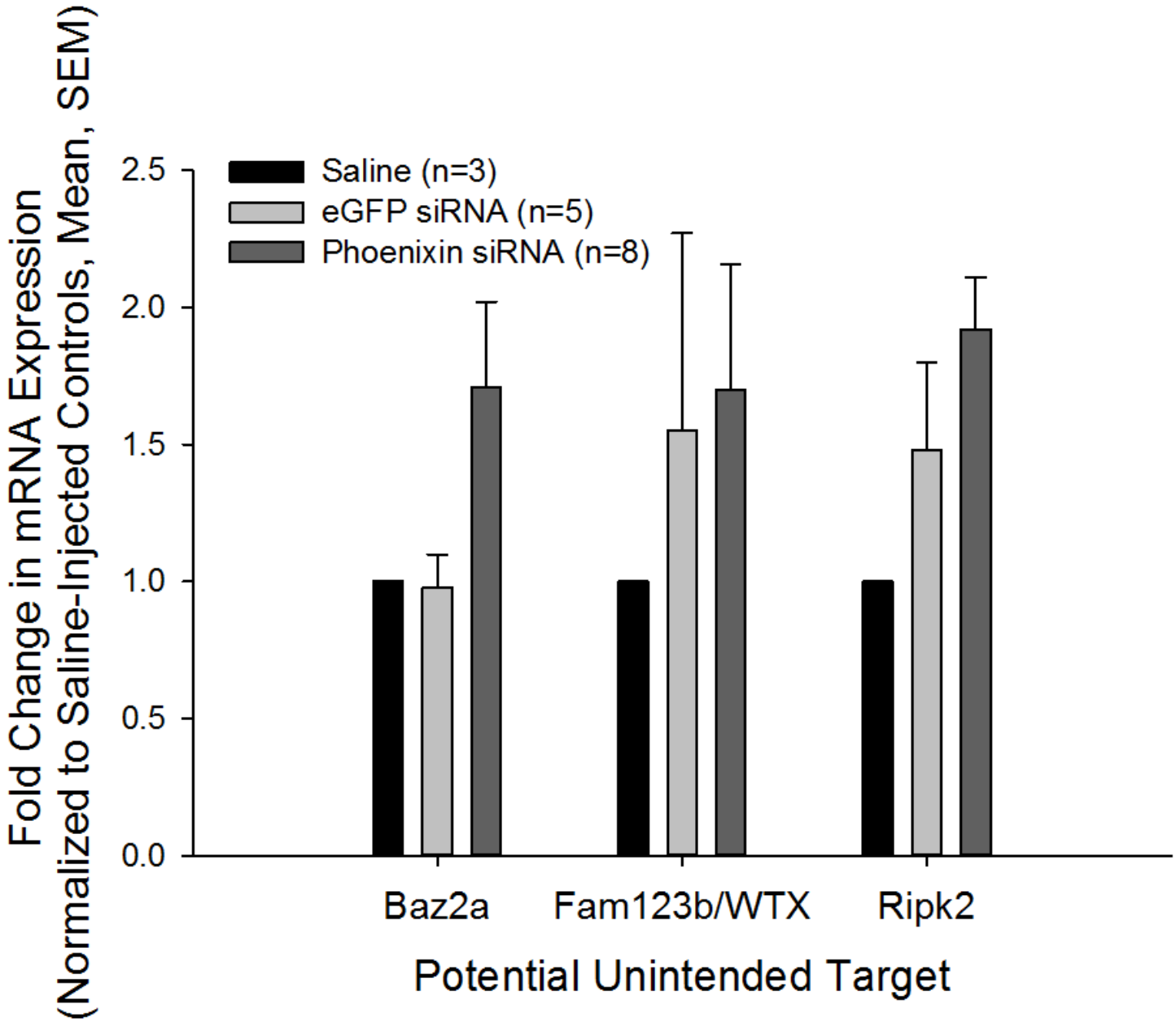
Supplemental Figure 3: Phoenixin mRNA is detected in various rat tissues. Tissues were collected from female rat donors. RNA was collected using a RNeasy Kit (Qiagen) and cDNA was produced using MML-V reverse transcriptase (Promega). PCR was performed using primers specific for phoenixin, and PCR products were separated on a 1% agarose gel. Contrast and exposure of the entire gel was adjusted using Apple iPhoto, and the gel was annotated using Apple Keynote.

Supplemental Figure 4



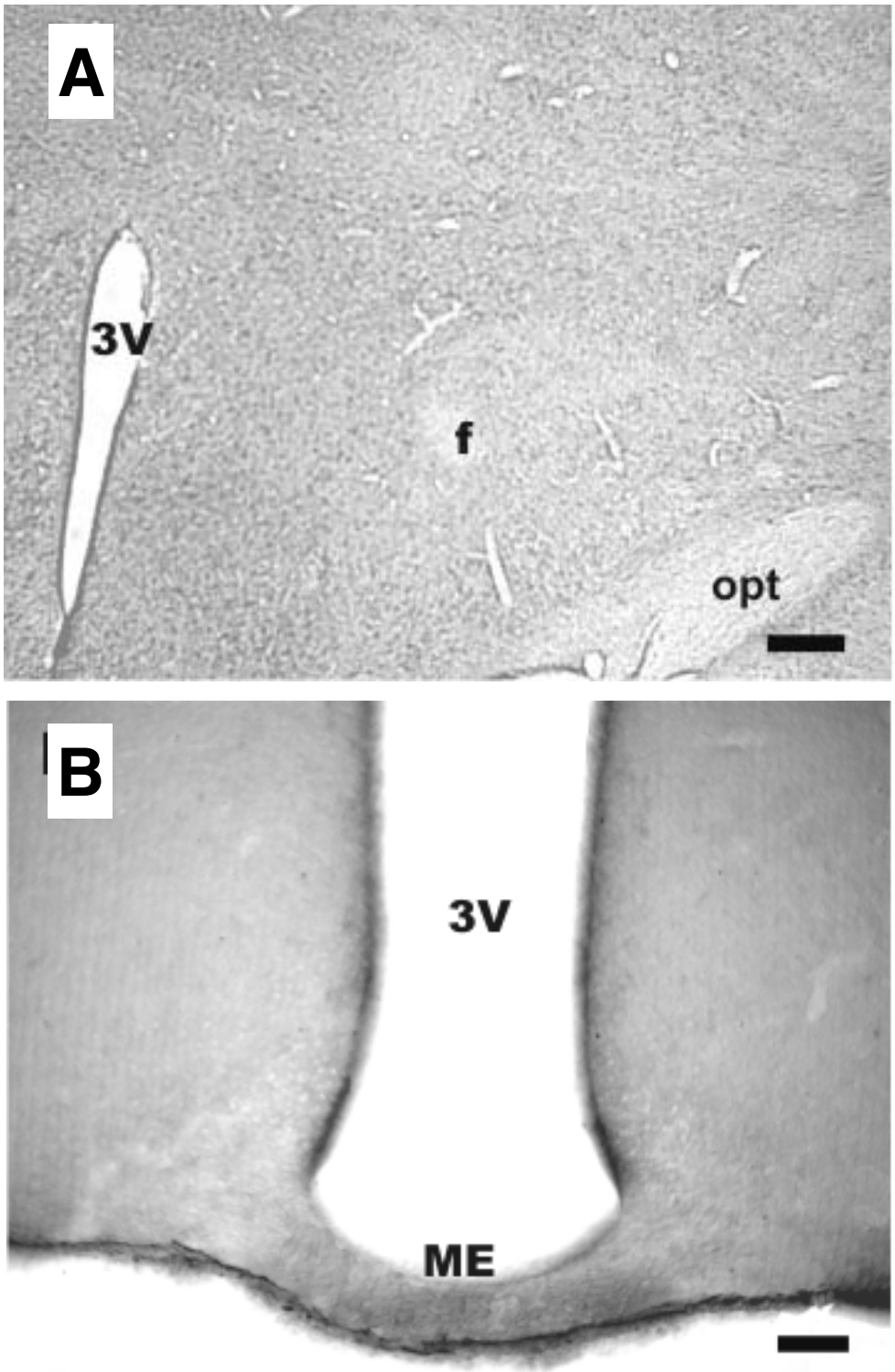
Supplemental Figure 4: In vivo compromise of phoenixin reduces GnRH receptor message in pituitary cells. Animals were treated with either control siRNA directed against eGFP or with siRNA directed against phoenixin. The following day, animals were sacrificed, and RNA was collected from anterior pituitaries. PCR was performed to assess GnRH receptor expression, and rats in which endogenous phoenixin was compromised exhibited reduced levels of GnRH receptor.

Supplemental Figure 5



Supplemental Figure 5: In vivo compromise of phoenixin does not alter the expression of potential unintended targets. Using NCBI BLAST, potential unintended targets of our phoenixin siRNA constructs were identified. We determined that the three most likely unintended targets were Baz2a (NM_001107158.1), Fam123b/WTX (NM_001109320.1), and Ripk2 (NM_001191865.1). Using RNA collected from the hypothalami of saline, phoenixin siRNA, or eGFP siRNA-treated rats, we performed RT-PCR to evaluate the expression of these potential unintended targets. Treatment with phoenixin siRNA did not compromise any of the three potential unintended targets. Data are represented as fold change compared to saline-injected control animals. Gene expression was normalized to the expression of the housekeeping gene, HPRT-1.

Supplemental Figure 6



Supplemental Figure 6: Preabsorption control for immunohistochemistry utilizing anti-phoenixin antibody. Hypothalamic sections, including the paraventricular region (A) and the median eminence (B) incubated in anti-phoenixin antiserum preabsorbed with the peptide phoenixin 15 (10 µg/ml) overnight; irPNX cells are not detected in these sections. Scale bar is 100 uM. 3V, third ventricle; opt, optic tract; f, fornix; ME, median eminence.