

Supplemental Methods

Generation of the pituitary-specific c-Raf kinase deletion.

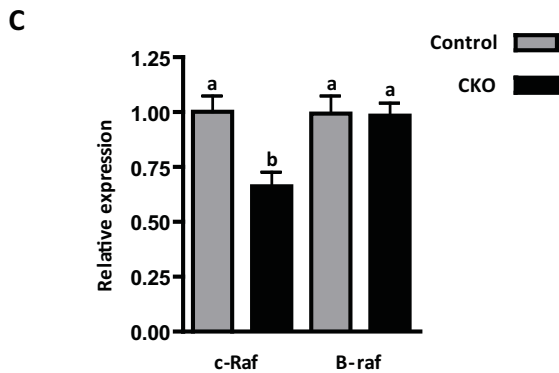
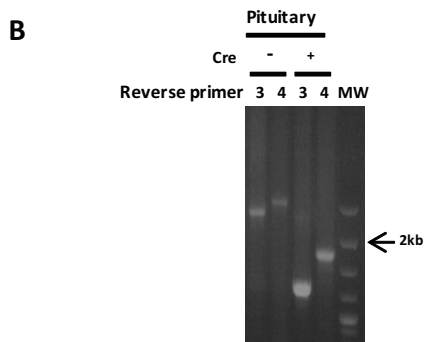
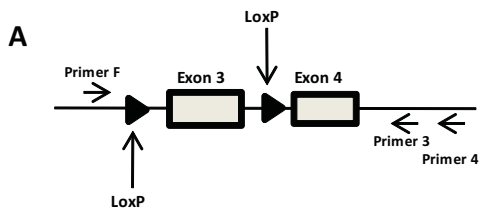
The c-Raf floxed mouse and the ERK1/2 pituitary targeted compound knockout mouse have been described previously (5, 43). α GSU-CRE mice, in which Cre recombinase is expressed under regulatory control of a 4.6 kb fragment of the mouse α GSU promoter, were purchased from Jackson Laboratories (Bar Harbor, ME). Swiss Webster mice used as wild type were purchased from Taconic Farms (Germantown, NY). Animals were maintained and treated in compliance with the specifications of the Cornell University Institutional Animal Care and Use Committee. Genotyping of the c-Raf conditional knockout animals was performed by PCR using primers 5'- AGCCGAGTCAGCAAATGCACTG, and 5'- AGTAGTCTACGGCACGTTTACT AGG. Genomic analysis of Cre-dependent recombination at the *c-Raf* locus was performed by PCR using primers "Pr. F" 5'-AGCCGAGTCAGCAAATGCACTG, "Pr. 3" 5'-TATCAC CTGCCAGGAACCAACAAGC, and "Pr. 4" 5'-AAGCCAACACTGCTCACTGTGTGGC.

Supplemental results

Figure Legend

Figure 1. Validation of the pituitary targeted c-Raf conditional knockout mouse. A. Cre-mediated recombination at the *c-Raf* locus within the pituitary was detected by PCR using genomic DNA from the specified tissues. For each sample, the forward primer designated "Primer F" was paired individually with reverse primers labeled "Primer 3" or "Primer 4" spanning the floxed genomic region as indicated in the schematic. B. PCR result using whole pituitary genomic DNA as template and Primer F in combination with Primer 3 (3) or 4 (4) in the PCR amplifications. Molecular weight marker (MW) is shown. Equivalent results were obtained from male and female mice. C. Whole pituitary transcript levels of c-Raf and B-Raf were measured by qPCR in control and c-Raf conditional knockout (CKO) animals. Bars represent mean \pm SEM from 5 animals per group. Means were compared by 2-tailed t-test. Bars with different letter designations represent mean values that are statistically significantly different at $p < 0.05$.

Figure 2. Conditional deletion of c-Raf kinase in pituitary gonadotropes does not affect growth rate or litter size in mice. A. Growth rate of c-Raf pituitary-specific conditional knockout (CKO) animals was compared to control mice at 2-6 weeks of age. Bars represent mean \pm SEM from 5 animals per group. B. Litter size was compared between Raf CKO (maternal genotype) and control mice. Litter size was determined at weaning for both genetic lines. Bars represent mean \pm SEM from 9 litters per group. In both A and B, means were compared by 2-tailed t-test and were found to be statistically non-significant.



Supplemental Figure 2 Bliss et al EN 11-0247

