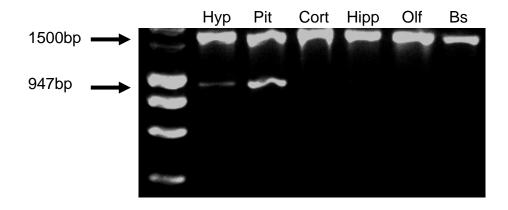
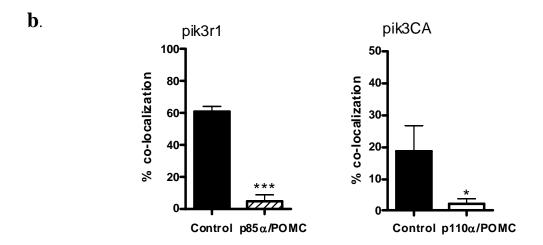
Supplemental Figure 1

a.





Supplemental Figure 1. Deletion of exon 1 of *pik3CA* in genomic DNA from POMC expressing regions of *pik3CA POMCKO* mice. (a) The 1500bp region between primer binding sites becomes 947bp in length when exon 1 is excised by cre recombinase. (b) Gels showing amplified genomic DNA from a *pik3r1 POMCKO* mouse. Hyp = DNA from whole hypothalamus, Pit = pituitary DNA with evidence of p110alpha deletion from POMC expressing corticotrophs, Cort = frontal cortex, Hipp = hippocampus, Olf = olfactory bulb DNA, Bs = Brainstem. Note that any POMC

expression in brainstem was not high enough to elicit evidence of pik3CA exon 1 deletion. (b) Immunohistochemistry for β -endorphin was performed following ISHH for $p85\alpha$ or $p110\alpha$ in brain slices from targeted knockouts and littermate controls and the number of dual positive neurons were quantified throughout the rostral-caudal extent of the hypothalamus. Very stringent criteria were used to assess coexpression to minimize false positives; coexpression was considered present if silver grains clustered around the outline of the cell beneath. * p<0.05, ** p<0.01