Supplemental Data

PAM Transcripts in PAM$^{+/-}$ Mice. The neomycin resistance gene replaced Exons 2 and 3 in the targeting vector used to generate PAM$^{+/-}$ mice (1) (Supplementary Figure 2d). Offspring of PAM$^{+/-}$ matings were genotyped using multiplex PCR with Neo and Ex3 sense primers and a common antisense primer in the intron following Exon 3 (primer RI) (Supplementary Figure 2a). When analyzed using quantitative PCR, levels of PAM mRNA in PAM$^{+/-}$ and WT mice were indistinguishable (Figure 3b), despite a two-fold decline in PAM activity and PAM protein (Figure 1). RT-PCR analysis using a sense primer in Exon 3, which has been replaced by the Neomycin cassette in the knockout allele, revealed the expected decrease in transcript level (Supplementary Figure 2b). To determine whether RNA that included the Neomycin cassette might be stable enough to contribute to the QPCR signal, RT-PCR was carried out using a sense primer in the Neomycin gene and an antisense primer in Exon 6; cDNA containing the Neomycin cassette was readily detected in PAM$^{+/-}$ mouse pituitary (Supplementary Figure 2c). Sequence analysis confirmed the identity of this product (data not shown). The transcript generated from the PAM^{KO} allele does not undergo extensive nonsense mediated decay and contributes to the QPCR signal observed using primers directed to a sequence near the 3'-end of the PAM transcript.