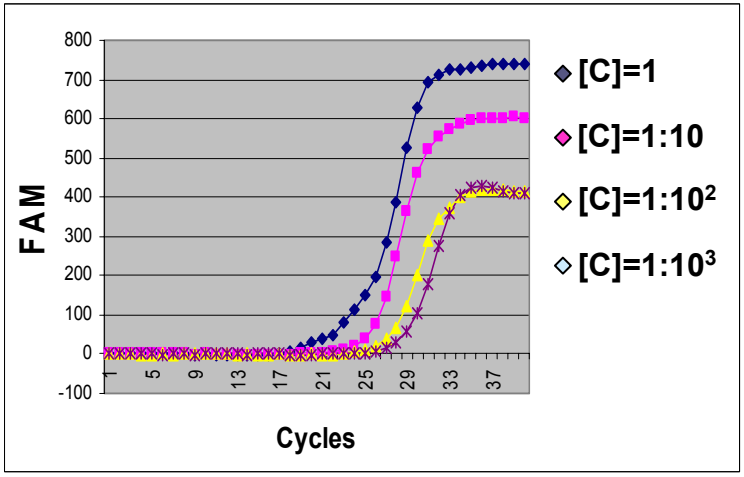
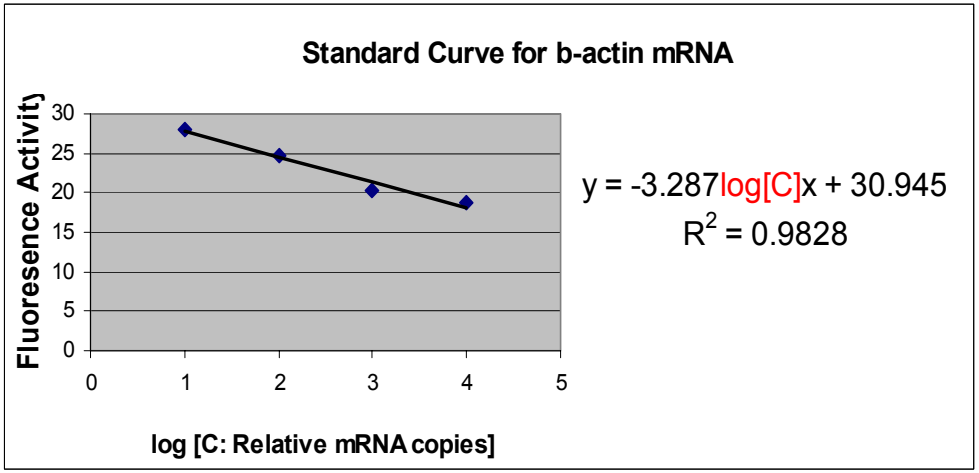
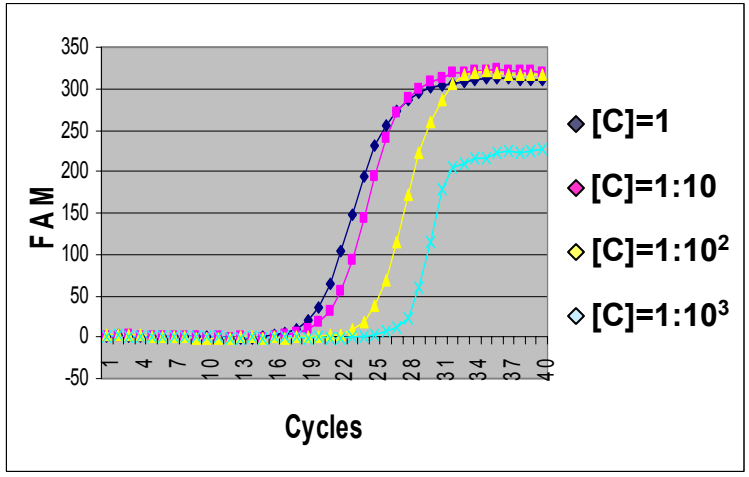
Quantification of PP2A mRNA levels using Real Time - RT - PCR

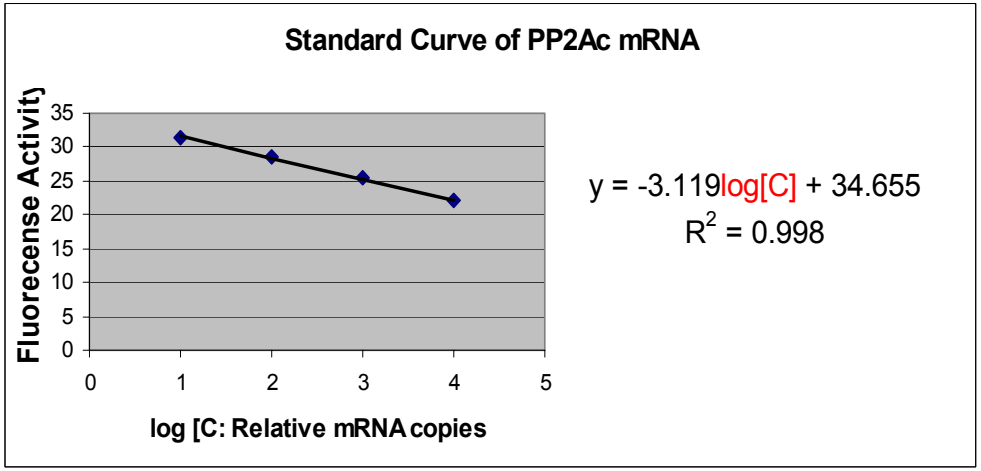


PP2Ac





β-actin



Supplemental Figure Legend

RNA quantitation with real time PCR is based on the study of Higuchi *et al*. (Biotechnology, 11,1026, 1993) which demonstrated that a plot of the log of initial mRNA copy number for a set of standards versus their respective threshold cycles (Ct) as determined by real time PCR is a straight line. Consequently, quantitation of the amount of target RNA in unknown samples is accomplished by measuring the Ct and using the standard curve to determine the starting copy number. There are two approaches to construct the standard curve (Bustin, SA, J. Mol Endocrinol. 25, 169, 2000):

1)Absolute method: the standard curve is created using RNA with known concentration

2) Relative method: in this method, one of the experimental samples (the calibrator, or 1x sample) is used as the basis for comparative analysis:

- Serial 10 fold dilutions of the calibrator sample are made: initial mRNA copies = C_1, $C_2=C_1/10$, $C_3=C1/100$, $C_4=C1/1000$ (usually 6 dilutions are made).

- An arbitrary value is given to describe the log concentration of a sample. For example $\log C_4=1$. That means that $\log C_3=2$, $\log C_2=3$ and $\log C_1=4$.

- Threshold cycle numbers for each sample are plotted against the logC values (out of the 6 dilutions the results from the middle 4 are used).

- Least square fit is used as the standard curve. The equation that describes the standard curve ($y= \beta x + z$) is used to determine the "relative mRNA copies" of the target RNA, where y is the Ct number, x the logC and β the slope of the curve, which reflects the efficiency of the reaction. Samples that differ by a factor of 2 in the original concentration of cDNA (derived from mRNA) would be expected to be 1 cycle apart. Thus ideally (PCR has 100% efficiency) samples that differ by a factor of 10 (as in our dilution series) would be ~3.3 cycles apart. In addition, the

correlation coefficient R2 would ideally be 1. Standard curves are accepted when $3.1 < \beta < 3.6$ and $R2 > 0.9$

To measure PP2Ac mRNA in our samples we employed the relative quantitation method described above. β-actin, a well described house-keeping gene, was used to correct for errors made during the collection and processing of the samples. Real time RT-PCR results for PP2Ac and β-actin mRNA derived from the calibrator samples and the respective standard curves are shown in the figure. Moreover, as shown in the figure the slope and the R2 of each standard curve meet the requirements of this methodology. From each RNA sample we run simultaneously real time PCR for PP2Ac and β-actin in different tubes. PP2Ac and β-actin relative mRNA copies were calculated applying the equations shown in the figure and subsequently we calculated their ratio. Figure 3B in our manuscript presents the evaluation of the amount of PP2Ac mRNA in our samples as PP2Ac / β-actin mRNA relative copies ratio.