# Supplemental Materials

***Molecular Biology of the Cell***

Barbosa et al.

**Figure S1.**

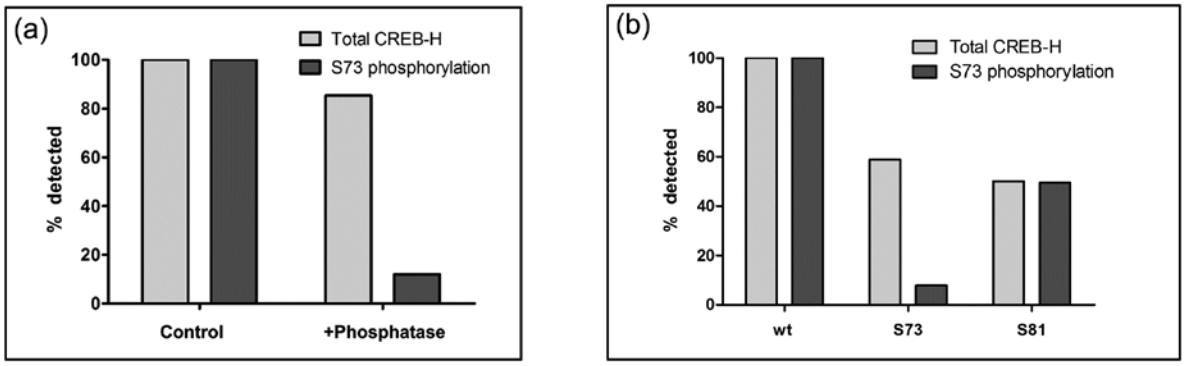


**Figure S1. Quantitative analysis of phosphorylation of CREB-HΔTMC using anti-phosphopeptide antibodies**

Quantitative analysis of blots shown in Figure 4. CREB-H was simultaneously detected in separate channels using a Licor Odyssey fluorescence detection scanner system, after probing with antibody to the SV5 epitope tag (to detect total levels, green channel) or anti-pS73 or pS81 antibodies to detect phosphorylated forms (red channel). (a) The differential effect of phosphatase treatment. For each antibody, levels for the control (phosphatase treatment in the presence of inhibitors, Figure 4a, lane 4) are normalised to 100. Total values in the same area in the presence of phosphatase but without inhibitor (lanes 3) were quantified and expressed relative to 100. The differential loss of pS73 is evident. (b) Quantitative analysis of the effect of mutation in S73. As before samples shown in Figure 4c, were subject to quantitative analysis to detect the total levels of the wt and S73 or S81 mutant proteins. For each antibody, levels for the wt are normalised to 100 (Figure 4c, lane 2). Total values for each antibody in the same area for S73A mutant (lane 3) and S81A mutant (lane 4) were evaluated and expressed relative to 100. The differential loss of detection by anti-pS73 in the S733A mutant was readily apparent, though residual reactivity remained (<10%). Results are discussed in the text.

**Figure S2.**

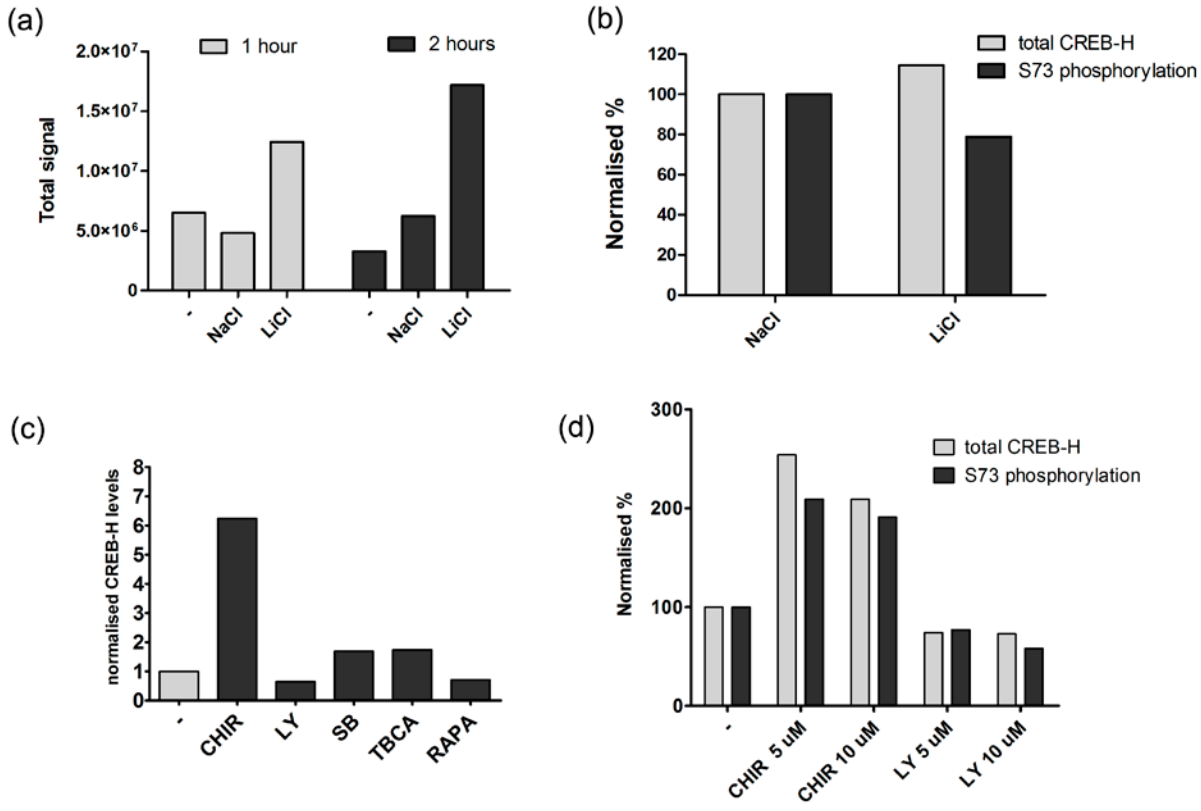


**Figure S2. Quantitative analysis of the effects of inhibitors on CREB-HΔTMC abundance and phosphorylation**

Quantitative analysis of blots shown in Figure 8. (a) Cells transfected with the vector for wt CREB-HΔTMC under the control of the TK promoter were untreated or treated for 1 or 2 hrs with 20 mM of NaCl or LiCl. Total abundance was detected using the anti-SV5 epitope tag and expressed as raw absolute numbers. Treatment with LiCl resulted in a specific 2.5-3-fold increase in abundance. (b) Quantitative analysis of blots shown in Figure 8b. Levels for each antibody were normalised to 100 and the relative levels after LiCl treatment showed a modest increase in total abundance but a decrease in phosphorylation at S73. (c) Quantitative analysis of blots shown in Figure 8c, lanes 1-6. Cells transfected with the wt protein were treated with each of the inhibitors as described in the legend to Figure 8, and total abundance was detected using the anti-SV5 epitope tag and normalised to the untreated control, set as a value of 1. The quantitative analysis revealed an over 6-fold increase after treatment with CHIR99021 (CHIR) as discussed in the text. (d) Quantitative analysis of blots shown in Figure 8d. Levels of the wt protein detected by each antibody in untreated cells were normalised to 100 and the relative levels detected by each antibody after inhibitor treatment expressed relative to 100. The results show an increase in total abundance of approximately 2.5-fold in the presence of CHIR but a modest shift in ratio with less phosphorylation detected by the anti-pS73 antibody. Clearly, despite the increase in abundance, CREB-H retains phosphorylation at pS73 in the presence of CHIR with possible reasons discussed in the text. In contrast no significant effect was observed with LY294002 (Ly).