Supplemental Materials

Molecular Biology of the Cell

Barbosa et al.

Supplementary Figures

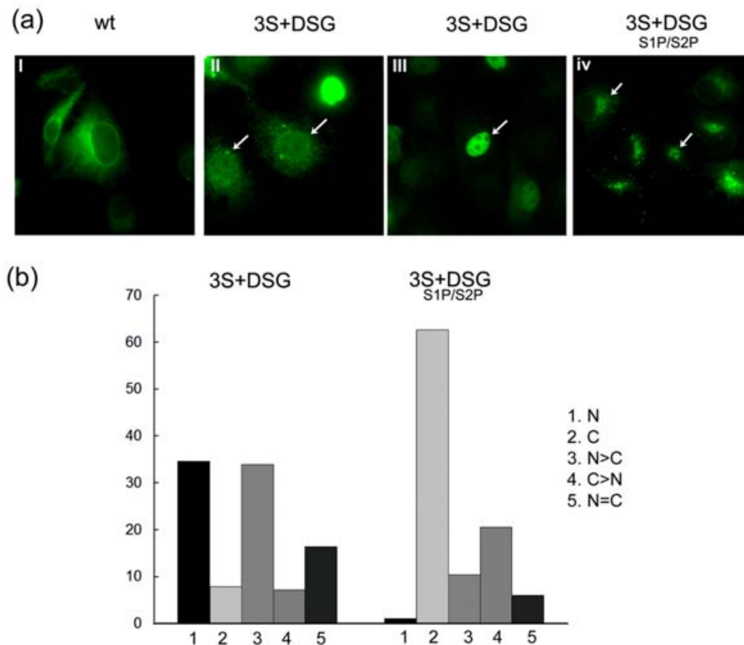

Figure S1. Quantitation of distinct subcellular patterns the CREB-H 3S+DSG variant in the context of normal (S1P+/S2P+) or mutated (S1P-/S2P-) cleavage sites.

(a) Typical patterns of localisation for classification and quantitation. COS-1 cells were transfected with CREB-H w/t or the 3S+DSG variant containing either intact (S1P+/S2P+) or mutated (S1P-/S2P-) cleavage sites. Cells were fixed (48 hrs) in 4% PFA and stained using the primary anti-SV5 epitope antibody and the appropriate secondary Alexa488 antibody. Panel I illustrates the ER pattern obtained for the w/t protein. Panel II and III illustrate patterns obtained for the 3S+DSG phosphorylation variant with intact S1P and S2P cleavage site. Panel II shows the nuclear plus ER mixed pattern indicated by the arrow, while panel III shows an example of an exclusively nuclear pattern. Panel IV illustrates the pattern obtained for the 3S+DSG variant with the mutated S1P and S2P sites (S1P-/S2P-). The pattern is typical for Golgi localization. (b) Quantitation of these different patterns for the 3S+DSG phosphorylation variant with either intact or mutated S1P and S2P cleavage site (S1P+/S2P+ and S1P-/S2P-, respectively). The different patterns are classified as follows: 1, nuclear only; 2, cytoplasmic only; 3, nuclear greater than cytoplasmic; 4 cytoplasmic greater than nuclear; 5 approximately equal nuclear and cytoplasmic localisation. A clear and complete exclusion of the protein from the nuclei was observed for the S1P-/S2P- mutant when compared to the intact variant where a large proportion of the protein is found in the nuclei in patterns 1 or 3.

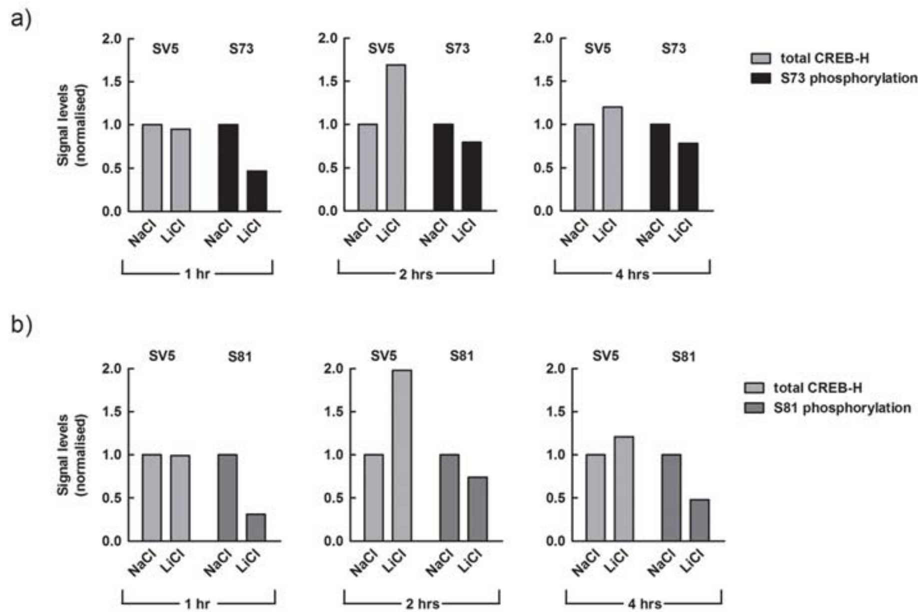

Figure S2. Quantitative analysis of the effects of LiCl on CREB-H abundance and phosphorylation.

Quantitative analysis of blots shown in Figure 5. Cos-1 cells transfected with wt CREB-H under the control of the TK promoter were treated for 1, 2 or 4 hrs with 20 mM of NaCl or LiCl. Total abundance was detected using the anti-SV5 epitope tag antibody in one channel and the amount of phosphorylation at serine 73 or serine 81 was detected using the phosphospecific pS73 or pS81 antibodies in the second channel. Western blots were scanned by laser scanning densitometry using a Licor Odyssey Imaging system and intensities for each band quantified using Image Studio software (Licor Biosciences). At each time point the signals intensities for either SV5 versus p73 or SV5 versus p81 were compared. The value obtained for the NaCl treatment were normalised to 1 and the signal for LiCl treatment then quantified by comparison. Note that the absolute signal for SV5 and those for the phosphospecific antibodies were not the same, though for ease of reference, they have been normalised to 1 on the same plot. Thus a value of 1 for anti-S73 is not comparable to a value of 1 for anti-SV5 in absolute terms. However the value for LiCl is directly comparable to that for NaCl for all antibodies. (a) Plots of normalised values for total CREB-H (SV5) and S73 phosphorylation (S73). (b) Plots normalised values for total CREB-H (SV5) and S81 phosphorylation (S81). Thus for example, at 2 hrs (panel a), while the total signal for the protein (SV5 signal) is elevated for LiCl over NaCl, the signal for anti-S73 is reduced. Therefore taking into account the increased protein levels, the proportion of phosphorylated species is lower for LiCl treatment, as discussed in the text.