



SUPPLEMENTARY ONLINE DATA

Identification of autophosphorylation sites in eukaryotic elongation factor-2 kinase

Sébastien PYR DIT RUYS^{*1}, Xuemin WANG^{†‡1}, Ewan M. SMITH^{†2}, Gaëtan HERINCKX^{*}, Nusrat HUSSAIN^{*}, Mark H. RIDER^{*}, Didier VERTOMMEN^{*} and Christopher G. PROUD^{†‡3}

^{*}Université catholique de Louvain and de Duve Institute, 75 Avenue Hippocrate, B-1200 Brussels, Belgium, [†]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada, V6T 1Z3, and [‡]Centre for Biological Sciences, Life Sciences Building, University of Southampton, Southampton SO17 1BJ, U.K.

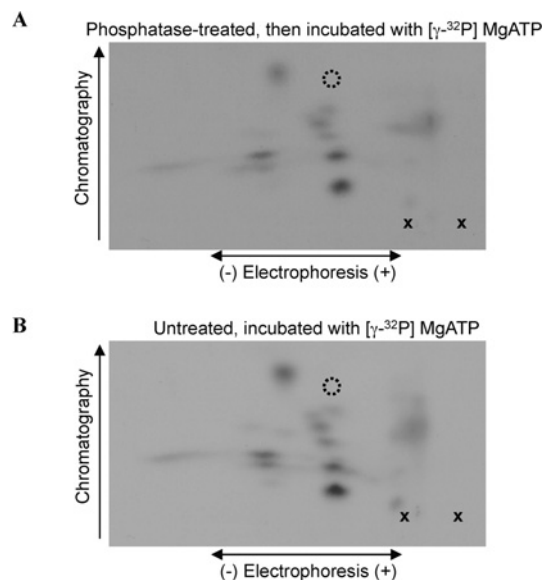


Figure S1 2D autophosphorylation peptide maps of alkaline-phosphatase-treated compared with untreated wild-type eEF2K

(A) Wild-type eEF2K was first dephosphorylated with alkaline phosphatase and subsequently allowed to undergo autophosphorylation in the presence of Ca^{2+} /CaM as described in the Experimental section of the main text. (B) Wild-type eEF2K phosphorylated in the presence of Ca^{2+} /CaM without pre-treatment. After tryptic digestion, phosphopeptides were resolved by 2D electrophoresis and chromatography (polarity and directions are indicated). The positions where the sample (larger 'X') and the DNP-lysine (smaller 'x') were applied, and the final migration position of the DNP-lysine marker (broken open circle) are also shown.

Received 15 September 2011/21 December 2011; accepted 4 January 2012
Published as BJ Immediate Publication 4 January 2012, doi:10.1042/BJ20111530

¹ These authors contributed equally to this work.

² Present address: MRC Toxicology Unit, Hodgkin Building, Lancaster Road, Leicester, LE1 9HN, U.K.

³ To whom correspondence should be addressed (email c.g.proud@soton.ac.uk).