Biochem. J. (2012) **445**, 93–100 (Printed in Great Britain) doi:10.1042/BJ20120304

online data

SUPPLEMENTARY ONLINE DATA

Embryonic poly(A)-binding protein (ePAB) phosphorylation is required for *Xenopus* oocyte maturation

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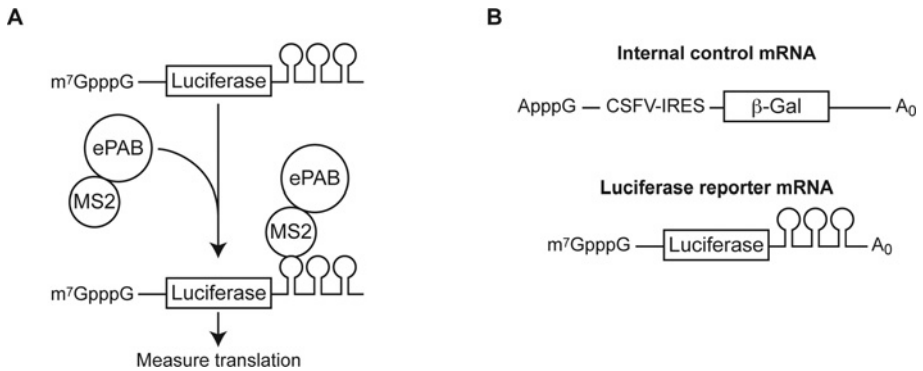

Figure S1 Tethered-function analysis of the role of ePAB phosphorylation in translational stimulation

(**A**) Oocytes were injected with *in vitro*-transcribed mRNAs encoding MS2 protein alone (negative control) or MS2-fusions with ePAB or 4×Ala-ePAB and incubated to allow for expression. Subsequently, *in vitro*-transcribed reporter and control mRNAs (depicted in **B**) were co-injected directly into the cytoplasm. Interaction of the MS2 protein with MS2-binding sites (stem-loops) in the 3′ UTR (untranslated region) of the reporter mRNA recruits or 'tethers' ePAB to the mRNA. Oocytes were incubated in the presence or absence of progesterone to induce maturation, homogenized and assayed to detect reporter protein activity. (**B**) The control mRNA has a non-functional (ApppG) cap, but is initiated via a Classical Swine Fever Virus internal ribosome entry site (CSFV-IRES) within its 5′ UTR, and contains neither MS2-binding sites nor a poly(A) tail. Since its translation is insensitive to PABP function [1], it can be used to normalize for small differences in injection efficiency. The luciferase reporter mRNA containing the MS2 sites has a functional m^7GpppG cap and is non-adenylated.

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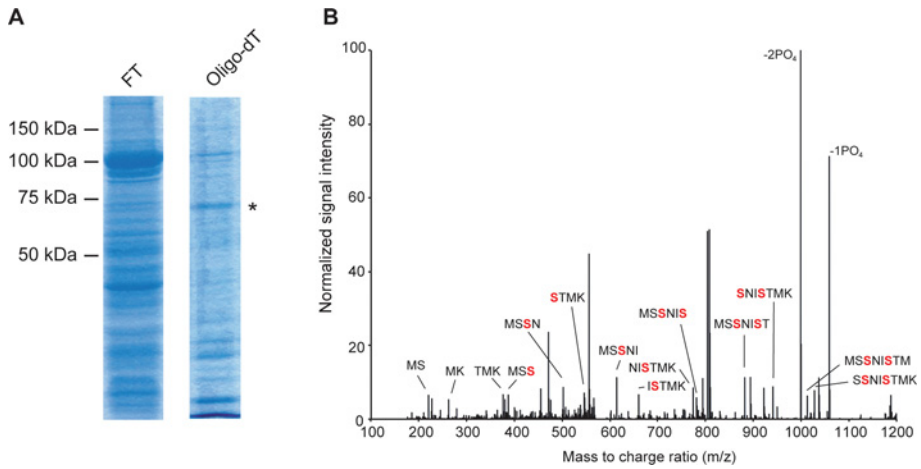

Figure S2 Identification of *in vivo* ePAB phosphorylations by mass spectrometry

(**A**) Endogenous hyperphosphorylated ePAB was enriched from immature oocyte lysate by oligo(dT) cellulose affinity selection (Oligo-dT). Proteins in the the flow-through (FT) and eluate were subjected to SDS/PAGE and stained with Coomassie Blue. Hyperphosphorylated ePAB is indicated (*). (**B**) The oligo(dT)-enriched ePAB band from (**A**) was excised and subjected to collision-induced dissociation MS spectrometry. The spectrum for ePAB peptides exhibiting phosphorylation at Ser^{461} or Ser^{464} is shown. Major $-2PO_4$ and $-1PO_4$ peaks result from neutral loss of phosphate during peptide dissociation. Phosphorylated residues are shown in red.

REFERENCE

1 Smith, R. W., Anderson, R. C., Smith, J. W., Brook, M., Richardson, W. A. and Gray, N. K. (2011) DAZAP1, an RNA-binding protein required for development and spermatogenesis, can regulate mRNA translation. RNA **17**, 1282–1295

Received 16 February 2012/9 April 2012; accepted 13 April 2012
Published as BJ Immediate Publication 13 April 2012, doi:10.1042/BJ20120304

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