Eom et al., http://www.jcb.org/cgi/content/full/jcb.201401005/DC1

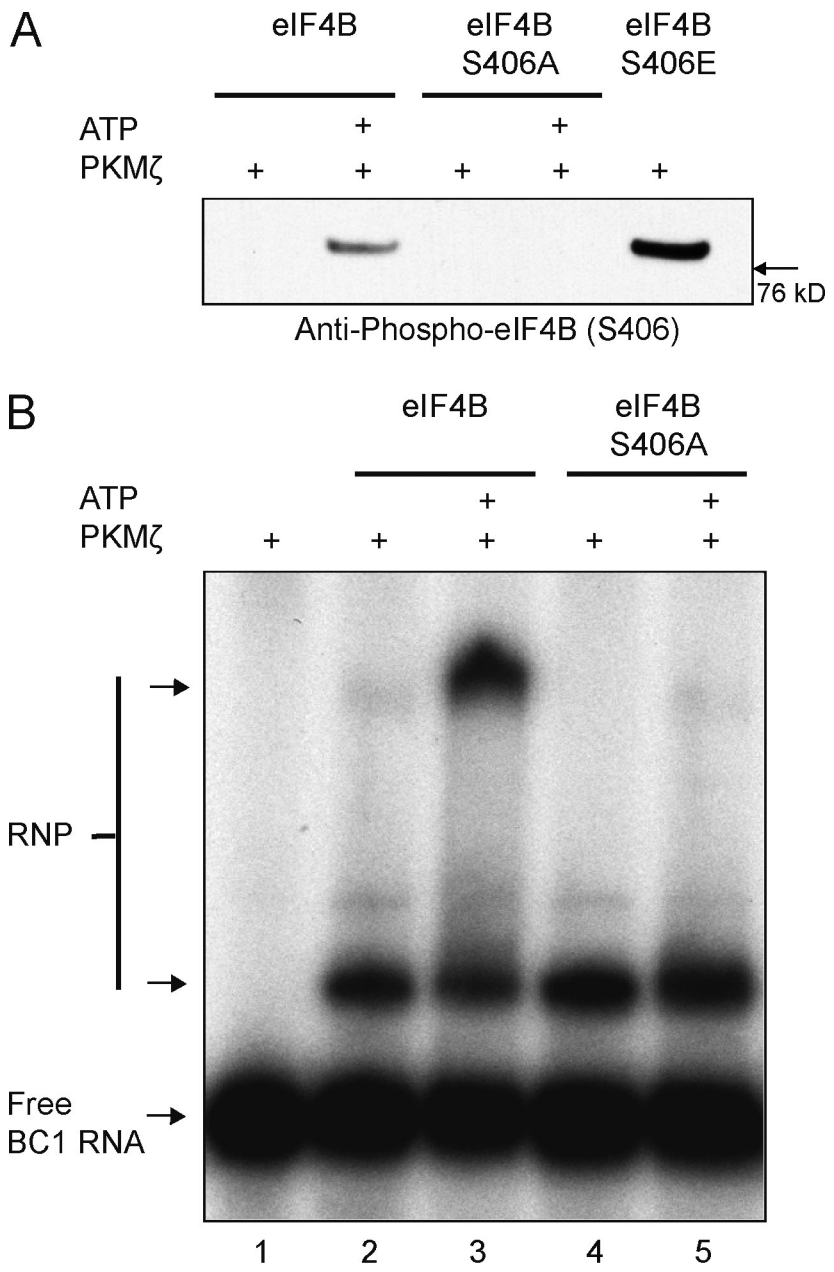

Figure S1. **Phosphorylation of eIF4B S406 increases binding of BC1 RNA.** (A) Unmodified eIF4B and eIF4B S406A were incubated with recombinant PKMζ either with or without ATP. Western blot analysis was performed with an antibody specific for phospho-S406 eIF4B. The results obtained indicate that unmodified eIF4B was S406-phosphorylated by PKMζ in the presence (lane 2), but not in the absence (lane 1), of ATP. In contrast, eIF4B S406A was phosphorylated neither in the presence nor in the absence of ATP (lanes 3 and 4). Phosphomimetic eIF4B S406E was used as a positive control (lane 5). (B) EMSA analysis was performed with ^{32}P-labeled BC1 RNA and eIF4B proteins (unmodified eIF4B and eIF4B S406A) that were or were not PKMζ-phosphorylated, as described in A. While basal RNP complex formation was observed in the presence of any of the used eIF4B variants (lanes 2–5), substantial strengthening of BC1 RNA binding, and ensuing homo-oligomerization, was observed only with unmodified eIF4B after PKMζ-mediated S406 phosphorylation (lane 3).

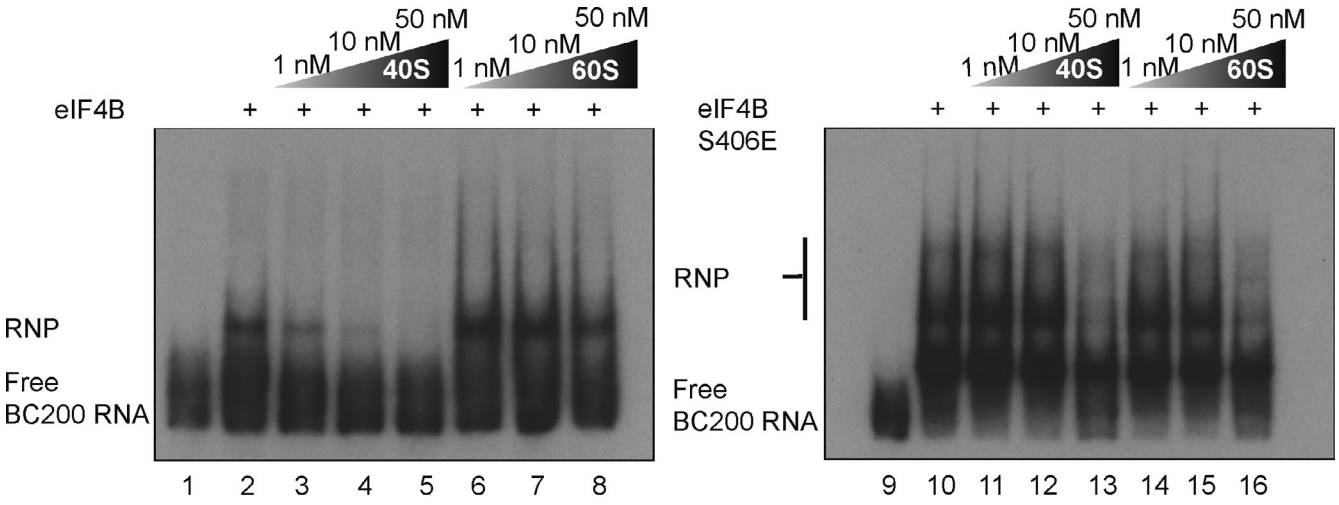

Figure S2. **Competition between human BC200 RNA and the 40S small ribosomal subunit for eIF4B is a function of S406 phosphorylation status.** (A) The 40S ribosomal subunit effectively competed human BC200 RNA off dephospho-S406 eIF4B (lanes 2–5) but not off phosphomimetic eIF4B S406E (lanes 10–13). No substantial competition was seen between the 60S ribosomal subunit and BC200 RNA for access to either the dephospho or the phosphomimetic factor (lanes 6–8 and lanes 14–16).

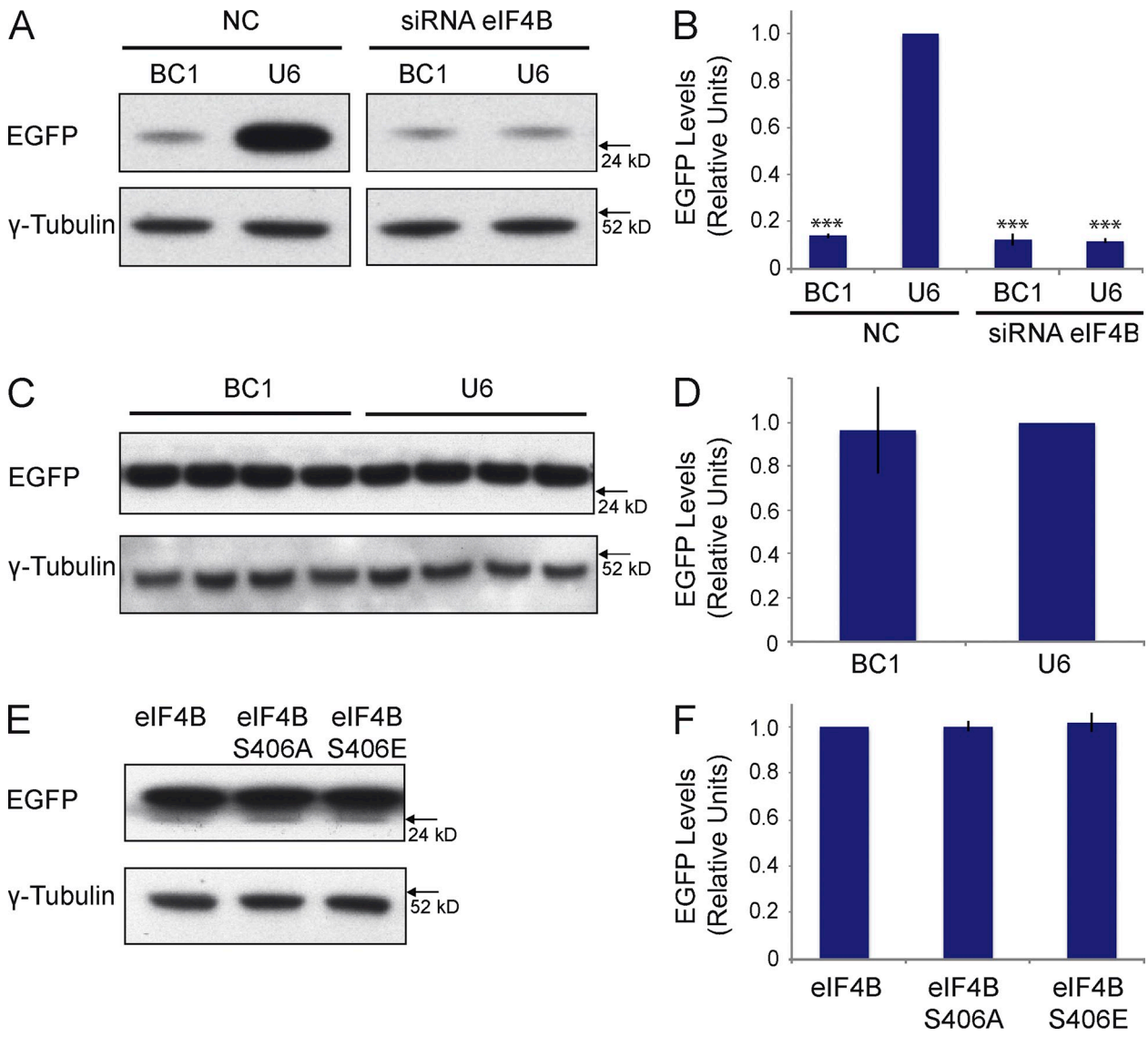

Figure S3. **eIF4B is required for BC1 RNA translational control directed at structured 5′ UTRs.** (A and B) Neuro-2A cells were transfected with siRNA directed against eIF4B or with NC siRNA, with the PKMζ reporter construct, and with BC1 RNA or U6 RNA. In the presence of eIF4B (left, NC siRNA), BC1 RNA, but not U6 RNA, significantly repressed translation of the reporter mRNA. After eIF4B knockdown (right), translational efficiency was reduced, and BC1 RNA did not repress translation of the reporter mRNA, in comparison with U6 RNA. (B) Quantitative analysis: one-way ANOVA, $P < 0.001$. Tukey post-hoc analysis, comparison with reporter level after transfection with NC siRNA and U6 RNA: $P < 0.001$ for all other transfections. $n = 4$. ***, $P < 0.001$. (C and D) In analogous transfection experiments, translation of an EGFP-C1 mRNA containing an unstructured 5′ UTR was not repressed by BC1 RNA (comparison with U6 RNA). (D) Quantitative analysis: Student's *t* test, $P = 0.892$. (E and F) To examine the relevance of the eIF4B S406 phosphorylation status on BC1 RNA repression of an mRNA with an unstructured 5′ UTR, Neuro-2A cells were transfected with siRNA directed against murine eIF4B mRNA, with an EGFP-C1 construct containing an unstructured 5′ UTR, and with human unmodified eIF4B, eIF4B S406A, or eIF4B S406E constructs. Regardless of the eIF4B S406 phosphorylation variant used, expression levels of the EGFP reporter were not significantly different from one another. (F) Quantitative analysis: one-way ANOVA, $P = 0.8435$. Tukey post-hoc analysis, comparison with unmodified eIF4B: $P = 0.997$ for eIF4B S406A, $P = 0.854$ for eIF4B S406E. For all panels, EGFP expression levels were normalized against those of endogenous γ-tubulin. Error bars represent SEM.

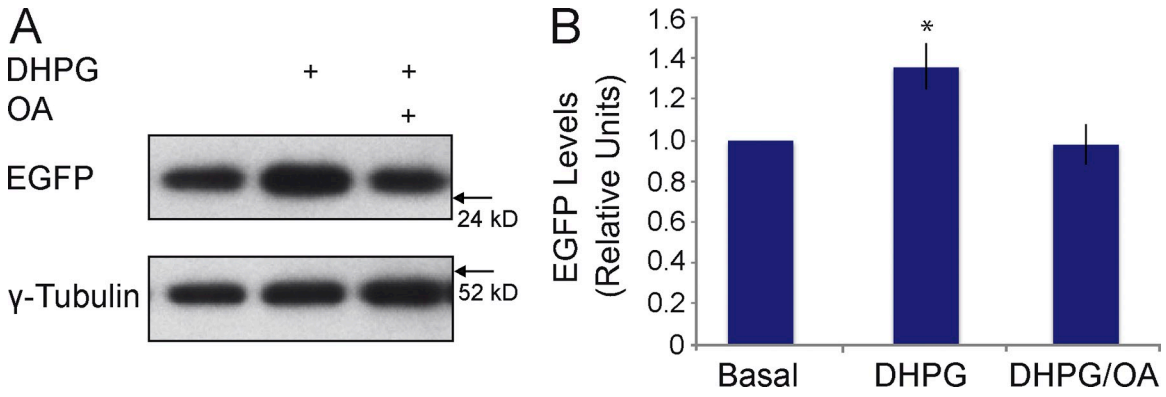

Figure S4. **PP2A is required for the induction of translation after activation of group I mGluRs.** (A and B) Stimulation of Neuro-2A cells with the group I mGluR agonist DHPG resulted in increased levels of the reporter. No such increase was observed if DHPG was applied in the presence of the PP2A inhibitor OA. (B) Quantitative analysis: one-way ANOVA, $P < 0.05$. Tukey post-hoc analysis, comparison with basal: $P < 0.05$ for DHPG, $P = 0.982$ for DHPG/OA. $n = 6$. EGFP expression levels were normalized against those of endogenous γ-tubulin. Error bars represent SEM. *, $P < 0.05$.