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Supplementary Materials for

MNK2 Inhibits eIF4G Activation Through a Pathway Involving Serine-Arginine–Rich Protein Kinase in Skeletal Muscle

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
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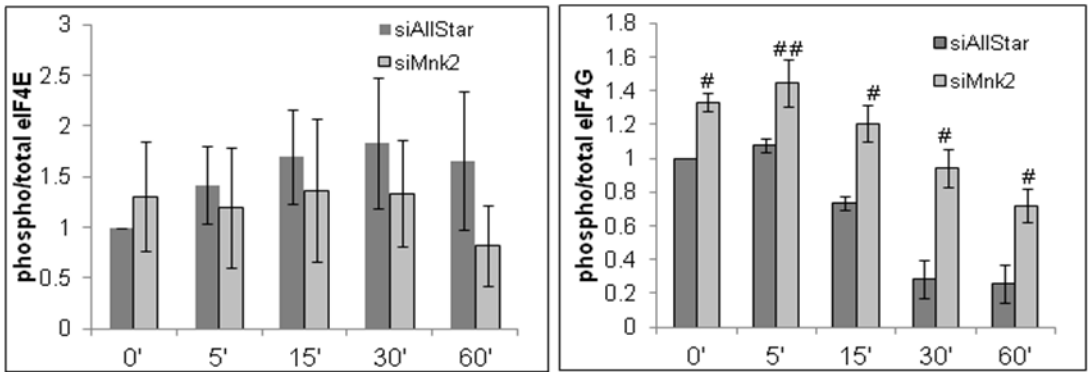

Fig. S1. Densitometry quantification of the ratio of phospho/total eIF4G and eIF4E from Fig. 1A. The left panel shows the ratio of phosphorylated to total (phospho/total) eIF4E from three independent experiments. The right panel shows the ratio of phospho/total eIF4G from three independent experiments (#p<0.05; ##p<0.07 vs the respective siAllStar time points). Error bars indicate the SD. P values were determined by unpaired t-test. See Figure 1 legend for conditions.

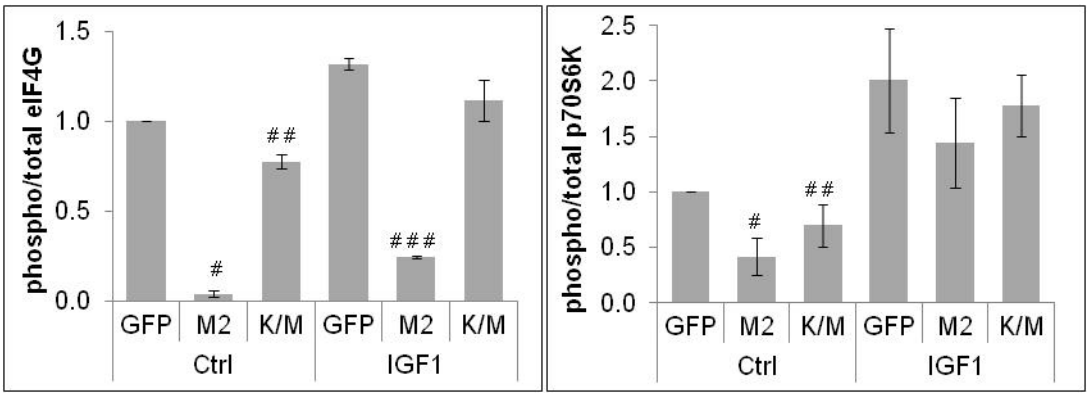

Fig. S2. Densitometry quantification of the ratio of phospho/total eIF4G and p70S6K from Fig. 3A. The left panel shows the ratio of phospho/total eIF4G from three independent experiments (#p<0.0001 vs Ctrl GFP; ##p<0.001 vs Ctrl GFP; ###p<0.0001 vs IGF1 GFP). The right panel shows the ratio of phospho/total p70S6K from three independent experiments (#p<0.01 vs Ctrl GFP; ##p=0.53 vs Ctrl GFP). Error bars indicate the SD. P values were determined by unpaired t-test. Ctrl, control; GFP, cell expressing green fluorescent protein; M2, cells overexpression MNK2; K/M, cells overexpressing a catalytically inactive mutant of MNK2. See Figure 3 legend for conditions.

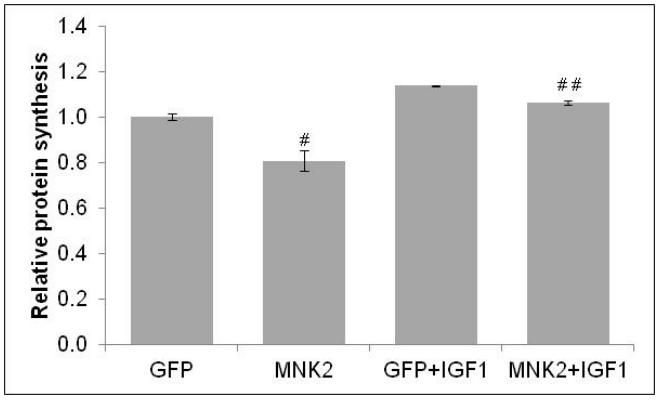

Fig. S3. MNK2 overexpression partially inhibits global protein synthesis. C2C12 cells were transduced with the indicated adenovirus on day 1 of differentiation. On day 3 of differentiation, myotubes were labeled with 10 μCi S^{35}-methionine for 4 hours in the presence or absence of 100 ng/mL IGF1. Protein synthesis was assessed by taking the ratio of S^{35}-incorporation to total number of nuclei. All values were then normalized to GFP, as depicted above (each treatment was in triplicates; #p<0.05 vs GFP; ##p<0.05 vs GFP+IGF1). This experiment has been repeated twice.

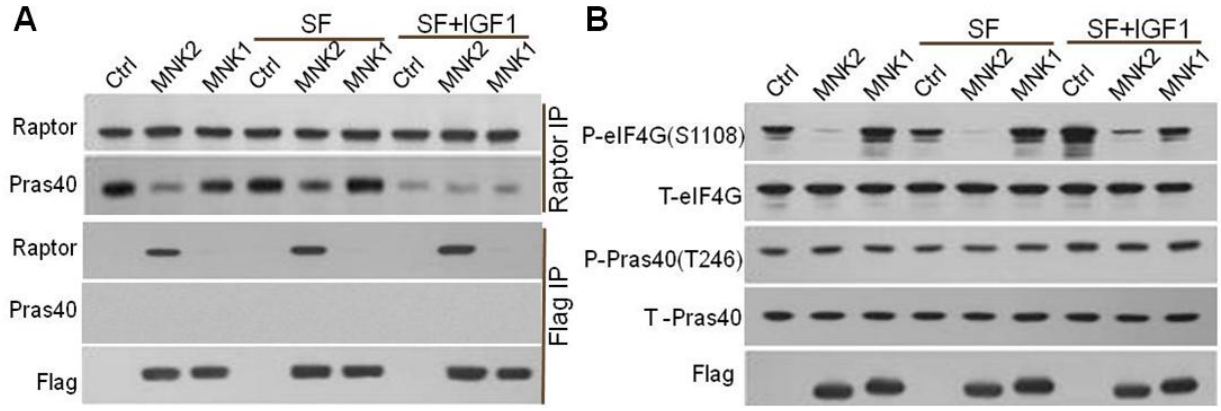

Fig. S4. MNK2 and Pras40 compete for binding to Raptor. (**A**) C2C12 cells were transduced with the indicated MNK-expressing adenovirus at day 1 of differentiation. At day 3 of differentiation, fresh differentiation medium or serum free medium (SF) was added for an addition 4 hrs. Half of the SF wells were treated with IGF1 (10 nM) for 30 min before cell lysates were prepared. Immunoprecipitation was then performed with antibodies recognizing Flag or Raptor. (**B**) The lysates used in panel A were analyzed for eIF4G and Pras40 phosphorylation by Western blotting. MNK1: adeno-MNK1; MNK2: adeno-MNK2. This experiment has been repeated twice.

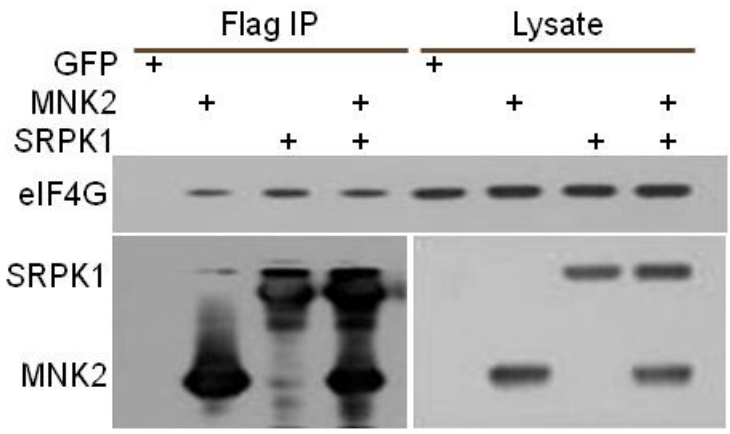

Fig S5. MNK2 and SRPK1 interact with eIF4G. C2C12 cells were transduced with the adenovirus for the indicated Flag-tagged protein (MNK2 or SRPK1) or GFP at day 1 of differentiation. Cell lysates were prepared at day 3 of differentiation. Immunoprecipitation was performed with anti-Flag antibody. The lines indicate immunoprecipitation part and protein loading control part of the blot respectively. This experiment has been repeated three times.

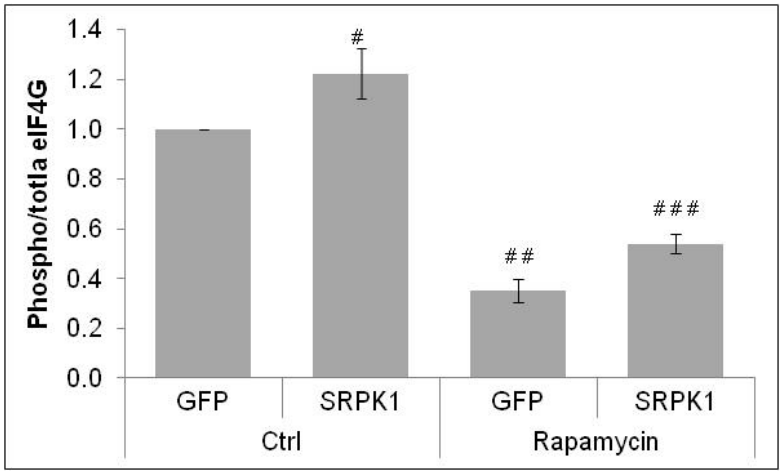

Fig S6. Densitometry quantification of the ratio of phospho/total eIF4G in Fig. 5. The bar graph shows the ratio of phospho/total eIF4G from three independent experiments. Error bars indicate the SD. P values were determined by unpaired t-test. #p<0.05; ##p<0.0001 vs Ctrl GFP; ###p<0.01 vs rapamycin GFP. See Figure 5 legend for conditions.

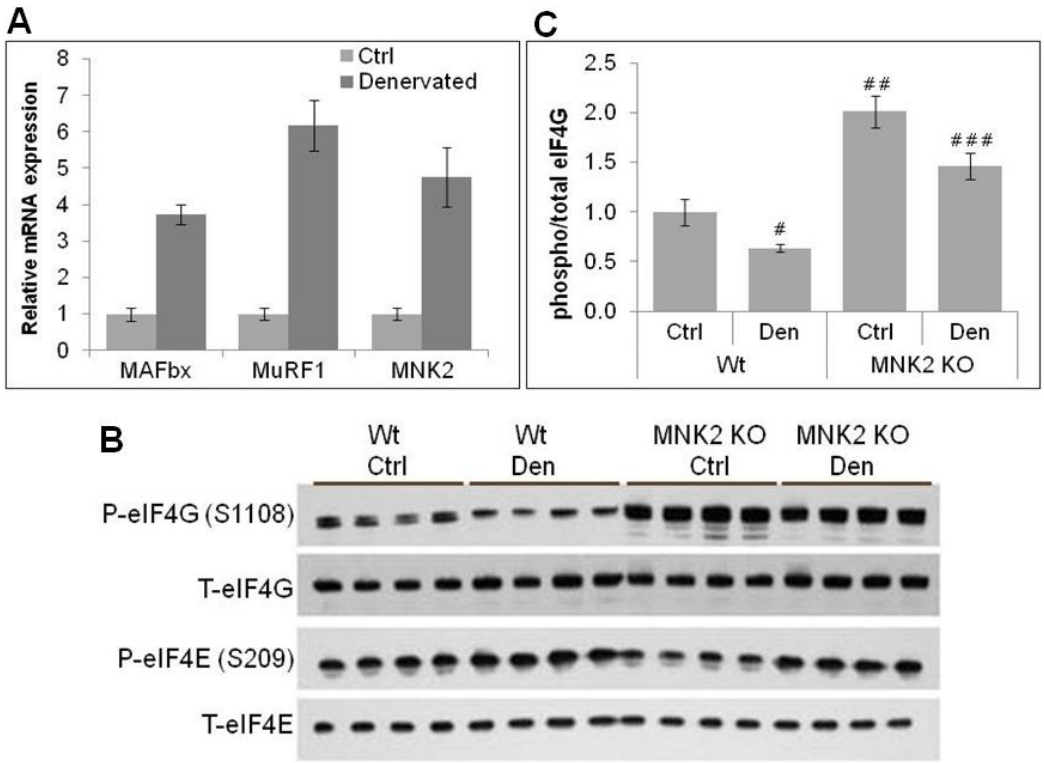

Fig. S7. Expression of atrophy-associated genes and eIF4G phosphorylation status in gastrocnemius in a denervation-induced atrophy model. (**A**) *MNK2*, *MAFbx*, and *MuRF1* expression in denervated and contralateral innvervated gastrocnemius at day 4 after denervation (n = 5 mice). (**B**) The phosphorylation status of eIF4G in denervated and contralateral innervated gastrocnemius of wild-type (Wt) and MNK2- knockout (KO) mice at day 14 after denervation (n = 4 mice). (**C**) Bar graph presentation of p-eIF4G to total eIF4G ratio of samples in B. Error bars indicate the SD. P values were calculated by unpaired t-test. #p<0.01 vs Wt Ctrl; ##p<0.0001 vs Wt Ctrl; ###p<0.0001 vs Wt Den.

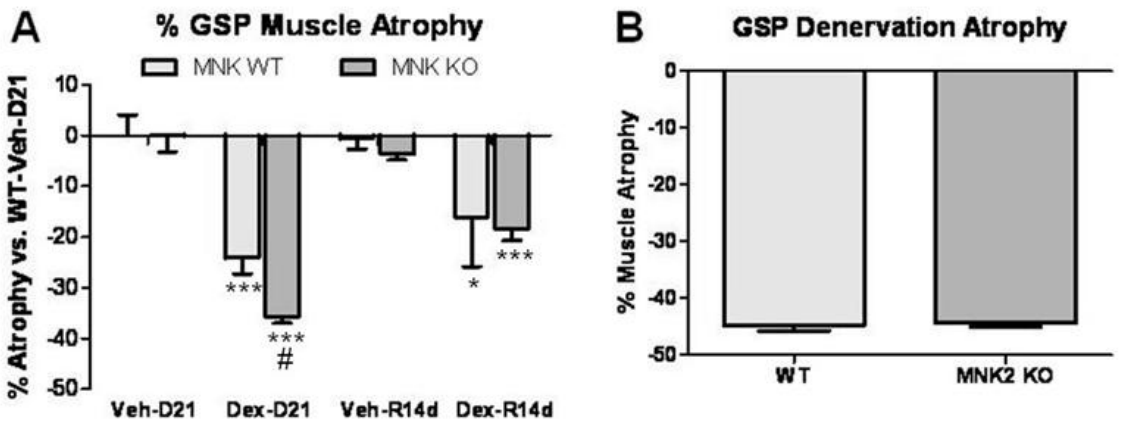

Fig. S8. Muscle mass in Dexamethasone-induced and denervation-induced atrophy models. (**A**) Percent atrophy of the gastrocnemius, soleus, and plantaris (GSP) muscles after 21 days of vehicle (Veh-D21) or dexamethasone (Dex-D21) treatment and at recovery day 14 (Veh-R14d and Dex-R14d) after cessation of dexamethasone (n = 8-10 mice). * $p<0.05$; ***$p<0.001$ vs corresponding Veh-D21; #$p<0.05$ vs corresponding wildtype. P values were obtained by Tow-Way ANOVA followed by Bonferroni post-hoc test using GraphPad Prism 5. Treatment with dexamethasone for 21 days caused less atrophy of the gastrocnemius-soleus-plantaris muscle complex in wild-type compared to MNK2 KO mice (23% vs. 36%, $p < 0.01$); however, by day 14 after cessation of dexamethasone treatment the degree of muscle atrophy was comparable between wild-type and MNK2 KO mice (20% vs. 18%, respectively). (**B**) The percent atrophy of the GSP muscles at day 14 after unilateral sciatic neurectomy (n = 10 mice). Muscle atrophy was comparable in wild-type and MNK2 KO mice at day 14 of denervation (45% vs. 44%, respectively).

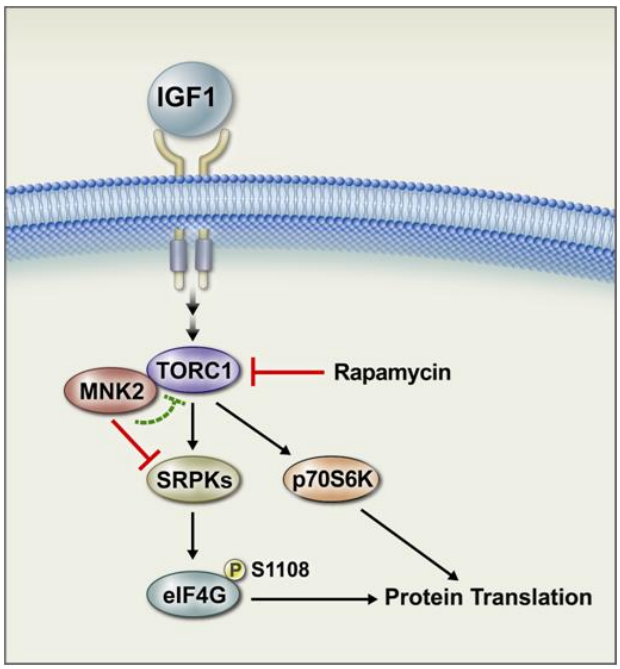

Fig. S9. Proposed negative role of MNK2 on protein translation through SRPK and TORC1. Our results suggest that SRPKs are the kinases responsible for eIF4G phosphorylation at Ser^{1108}. The activities of these kinases are regulated positively by TORC1 and negatively by MNK2. In addition to suppressing SRPK activity, MNK2 through its physical interaction with TORC1 also affects TORC1's activity towards p70S6K. By the combination of these two actions, MNK2 inhibits pathways controlling protein translation. Red solid inhibitory lines represent inhibiton of TORC1 by rapamycin and negative regulation of SRPKs by MNK2; green dotted inhibitory line represents suppression of TORC1 activity by MNK2-TORC1 interaction.

Table S1. Summary of primary mouse kinase panel siRNA screen (Excel file). Four siRNAs (Qiagen) against each gene were screened in duplicates. Phospho-eIF4G signal intensity was normalized with signal of negative control siRNA (Silencer Negative Control 2, Ambion) and signal of positive control eIF4G siRNA (SI00992278, Qiagen) by linear scaling to 0 as negative control and -100 as positive control. Two out of 4 siRNAs that resulted in less than -30 of normalized phospho-eIF4G intensity were selected for further confirmation study.