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Supplementary Materials for

Sleep deprivation impairs memory by attenuating mTORC1-dependent protein synthesis

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The PDF file includes:

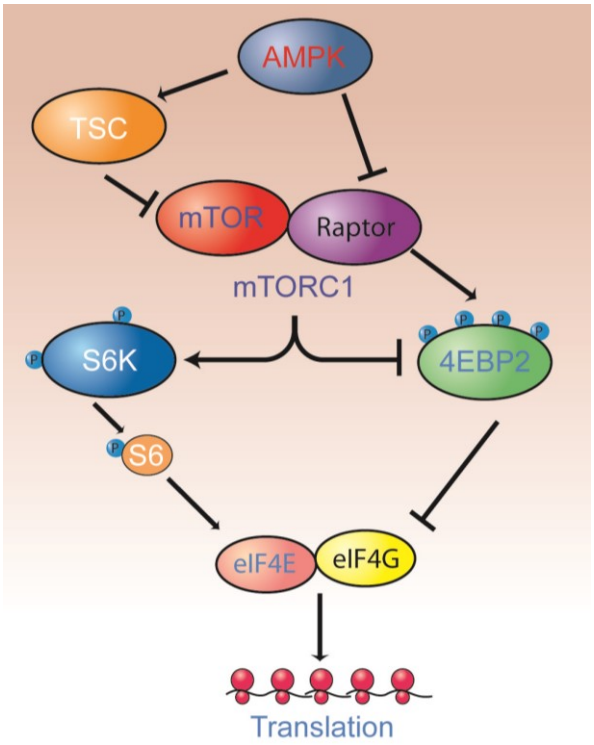

Fig. S1. Schematic showing the mechanism of attenuated translation in the hippocampus caused by sleep deprivation. Blue lettering indicates reduced abundance in the mouse hippocampus after 5 hours of sleep deprivation, red lettering indicates increased signaling, and white lettering indicates no change. Our findings indicate that five hours of sleep deprivation increases AMPK activity, which inhibits mTORC1. Because mTORC1 inhibits the translation repressor 4EBP2 through phosphorylation, reduced mTORC1 activity in the hippocampus enables the activity of 4EBP2. This subsequently leads to reduced interactions between eIF4E and eIF4G, thus leading to lower levels of translation.

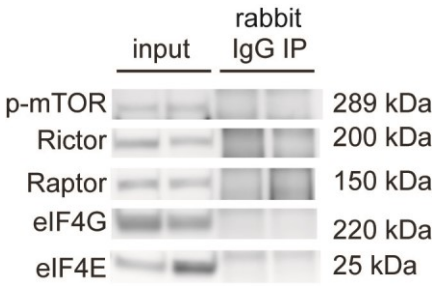

Fig. S2. Control immunoblots of hippocampal extracts treated with rabbit IgG. Representative Western blots of hippocampus extracts from 6 mice that were treated with rabbit IgG. These IgG control blots are applicable to all immunoprecipitations in this study (Fig. 3B, 3E, and 4F) as all immunoprecipitation experiments were conducted with antibodies raised in rabbit.

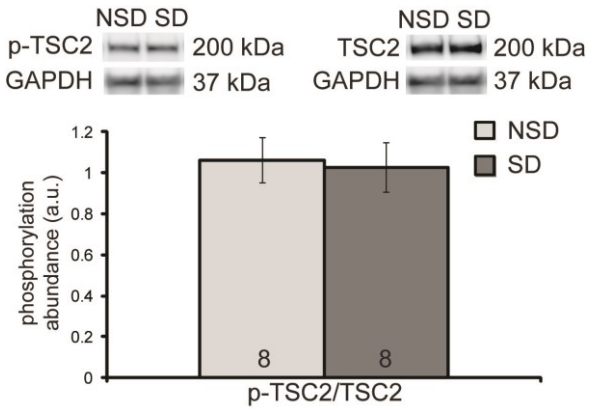

Fig. S3. The abundance of phosphorylated TSC2 does not change after 5 hours of sleep deprivation. Representative Western blots and quantitation of phosphorylated TSC2 from hippocampus extracts from mice that were not sleep-deprived (NSD) and those that were sleep-deprived (SD) for 5 hours. GAPDH served as a loading control. Abundance of phosphorylated TSC2 was corrected against total TSC2 protein in each condition. Data are means ± SEM from 8 mice in each condition, a.u., arbitrary units.

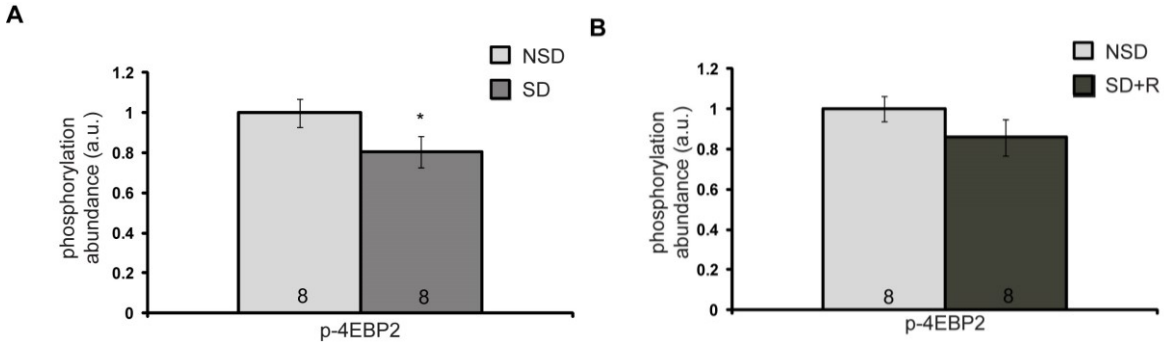

Fig. S4. Five hours of sleep deprivation reduces the abundance of phosphorylated 4EBP2, which rebounds after 2.5 hours of recovery sleep. (**A**) Abundance of phosphorylated 4EBP2 (p-4EBP2) was measured by ELISA from the hippocampus of mice that were not sleep-deprived (NSD) and those that were sleep-deprived (SD) for 5 hours. Data are means ± SEM from 8 mice in each condition; * $p = 0.042$ by a *t* test; a.u., arbitrary units. (**B**) Abundance of phosphorylated 4EBP2 was measured by ELISA from the hippocampus of NSD mice and mice given 2.5 hours of recovery sleep after 5 hours of sleep deprivation (SD+R). Abundance of phosphorylated 4EBP2 was corrected against total protein loaded into the ELISA. Data are means ± SEM from 8 mice in each condition; a.u., arbitrary units; $p = 0.221$ by a *t* test.

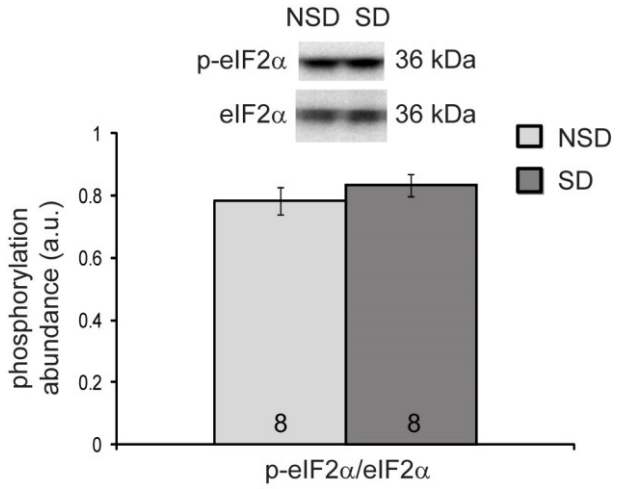

Fig. S5. Abundance of phosphorylated eIF2α does not change after 5 hours of sleep deprivation. Representative Western blots and quantitation of phosphorylated eIF2α from hippocampus extracts from mice that were not sleep-deprived (NSD) and those that were sleep-deprived (SD) for 5 hours. Abundance of phosphorylated eIF2α was corrected against total eIF2α protein in each condition. Data are means ± SEM from 8 mice in each condition, a.u., arbitrary units.