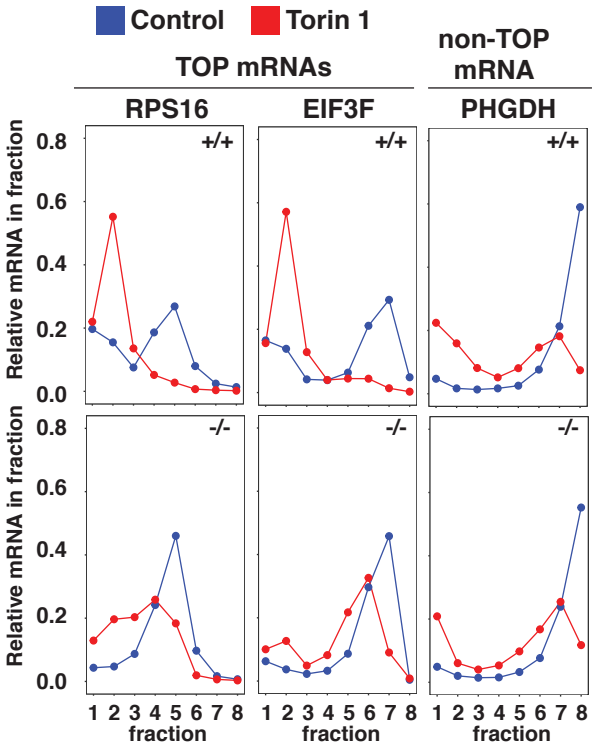

Figure S1. Polysome analysis of TOP and non-TOP mRNAs
Changes in the distribution of additional TOP (RPS16, EIF3F) and non-TOP mRNAs (PHGDH) in sucrose gradient fractions from wild-type and LARP1-null HEK-293T cells treated with vehicle (DMSO) or 250 nM Torin 1 for 2 h. Abundance of the indicated mRNAs was measured by qPCR and calculated as a proportion of the total in all fractions.

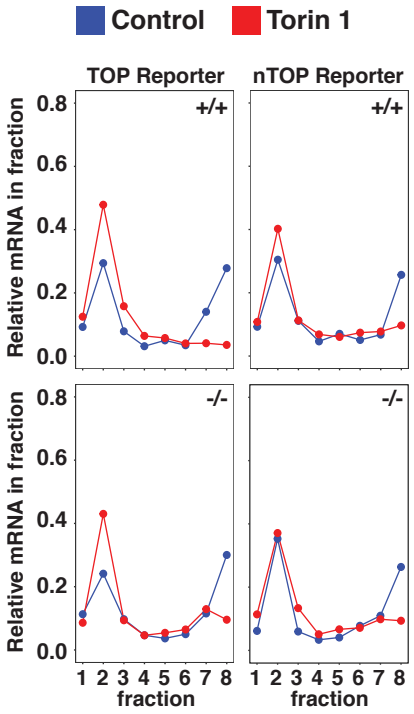

Figure S2. Polysome analysis of TOP and non-TOP reporter mRNAs
Extracts from wild-type or LARP1-null HEK-293T cells stably expressing TOP or non-TOP Renilla luciferase reporters were analyzed by sucrose gradient fractionation. Abundance of the reporter mRNA was measured by QPCR and calculated as a proportion of the total in all fractions.

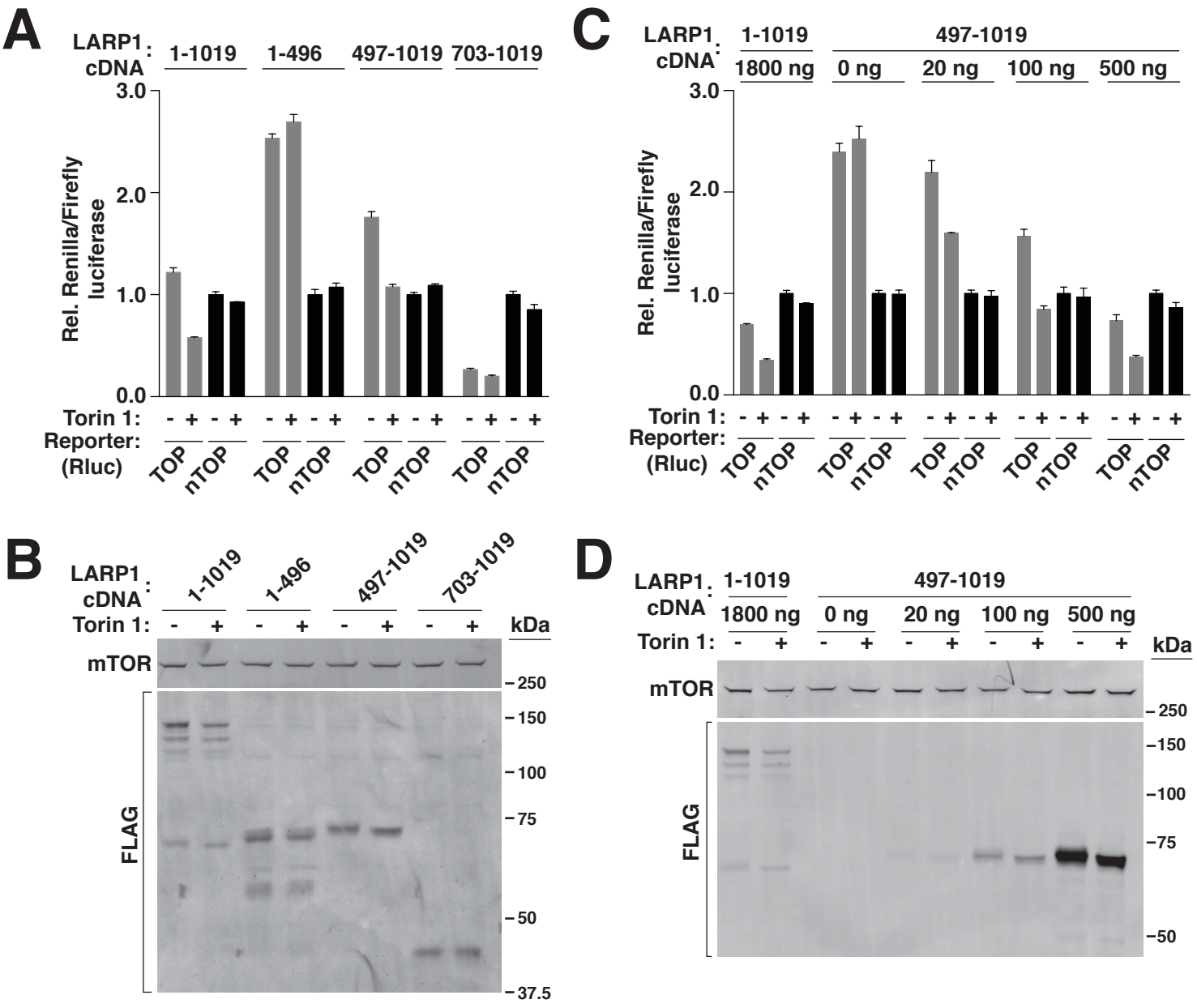

Figure S3. Effect of LARP1 expression on TOP mRNA regulation

A. Analysis of TOP mRNA regulation by LARP1 fragments expressed at equal levels. LARP1-null HEK-293T cells were transfected with varying amounts of cDNAs encoding the indicated LARP1 fragments to normalize expression levels (see (B)), Renilla luciferase with TOP or non-TOP (nTOP) 5' UTR, and a control cDNA encoding firefly luciferase. Cells were then treated with vehicle (DMSO) or 250 nM Torin 1 for 6 h and analyzed for luciferase production. Data are Renilla/firefly, normalized to vehicle-treated nTOP levels for each cDNA. **B.** LARP1 fragments are expressed at equal levels. Extracts from cells treated as in (A) expressing the indicated LARP1 fragments and the TOP mRNA reporter were analyzed by western blotting for the indicated proteins. **C.** Higher expression of LARP1$^{497\text{-}1019}$ than full-length LARP1 is required to restore TOP mRNA regulation. LARP1-null HEK-293T cells were transfected with the indicated amounts of cDNAs encoding full-length LARP1 or LARP1$^{497\text{-}1019}$ (see (D)) along with TOP and non-TOP luciferase reporters and treated as in (A), then analyzed for luciferase production. Data are Renilla/firefly, normalized to vehicle-treated nTOP levels for each cDNA. **D.** Expression of levels of LARP1 fragments from (C). Extracts from cells treated as in (C) expressing the indicated amounts of full-length LARP1 or LARP1$^{497\text{-}1019}$ and the TOP mRNA reporter were analyzed by western blotting for the indicated proteins.

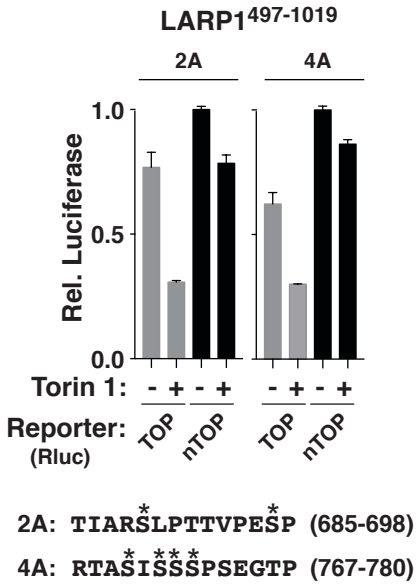

Figure S4. Function of LARP1 phosphorylation sites on TOP mRNA regulation. Alleles of LARP1$^{497-1019}$ with serine to alanine mutations at either 689 and 697 (2A) or 770, 772, 773, and 774 (4A) were transfected into LARP1-null HEK-293T cells along with TOP and non-TOP Renilla luciferase reporters, and a control firefly luciferase reporter. Cells were then treated with vehicle (DMSO) or 250 nM Torin 1 for 6 h, and analyzed by luciferase assay. Data are Renilla/firefly, normalized to vehicle-treated nTOP levels for each LARP1 allele expressed. (n=3, error bars are SD).