

iScience, Volume 27

Supplemental information

eIF2B localization and its regulation

during the integrated stress

response is cell-type specific

Filipe M. Hanson, Madalena I. Ribeiro de Oliveira, Alison K. Cross, K. Elizabeth Allen, and Susan G. Campbell

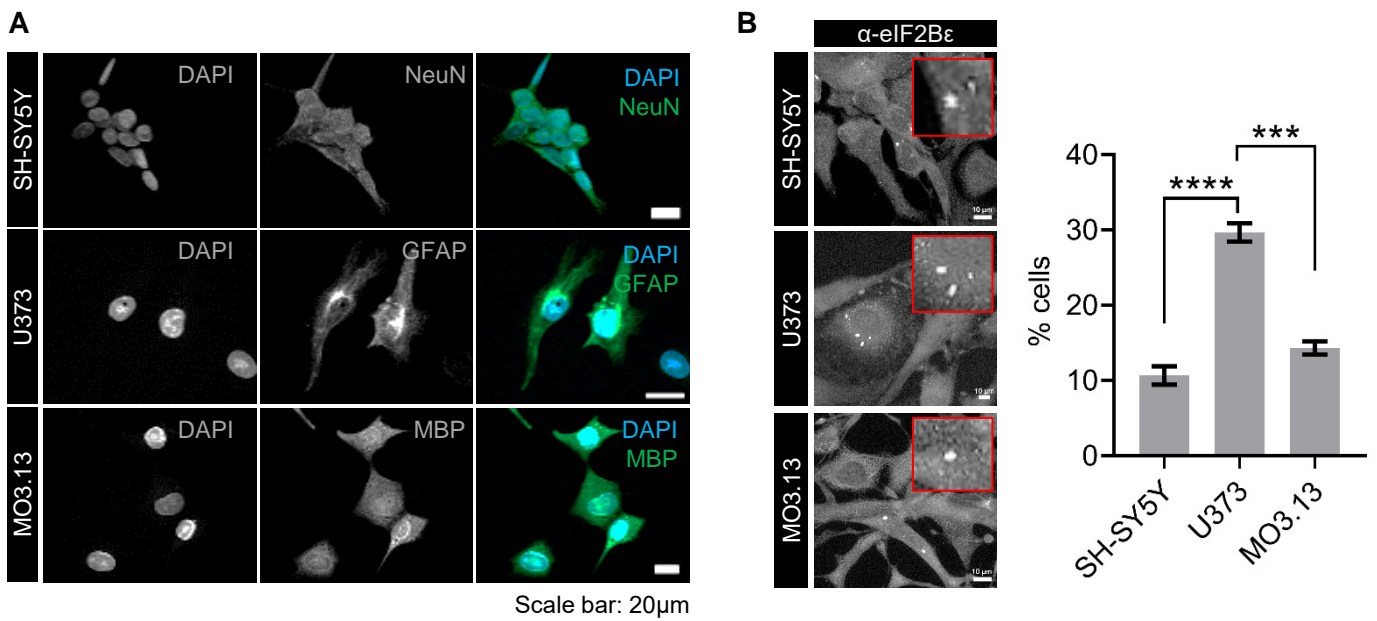


Figure S1. Endogenous eIF2B bodies are expressed in SH-SY5Y, U373 and MO3.13 cells, related to Figure 1. (A) Confocal images of SH-SY5Y, U373 and MO3.13 cells immunostained for neural marker *NeuN*, astrocytic marker *GFAP* and oligodendrocytic marker *MBP*, respectively. **(B)** Confocal images of SH-SY5Y, U373 and MO3.13 cells immunostained with α -eIF2B ϵ (*left panel*). Mean percentage of cells displaying at least one eIF2B ϵ -containing body in a population of 100 cells (mean \pm SEM; $N=3$; **** $p\leq 0.0001$, *** $p\leq 0.001$ according to one-way ANOVA) (*right panel*). Scale bar: 10 μ m.

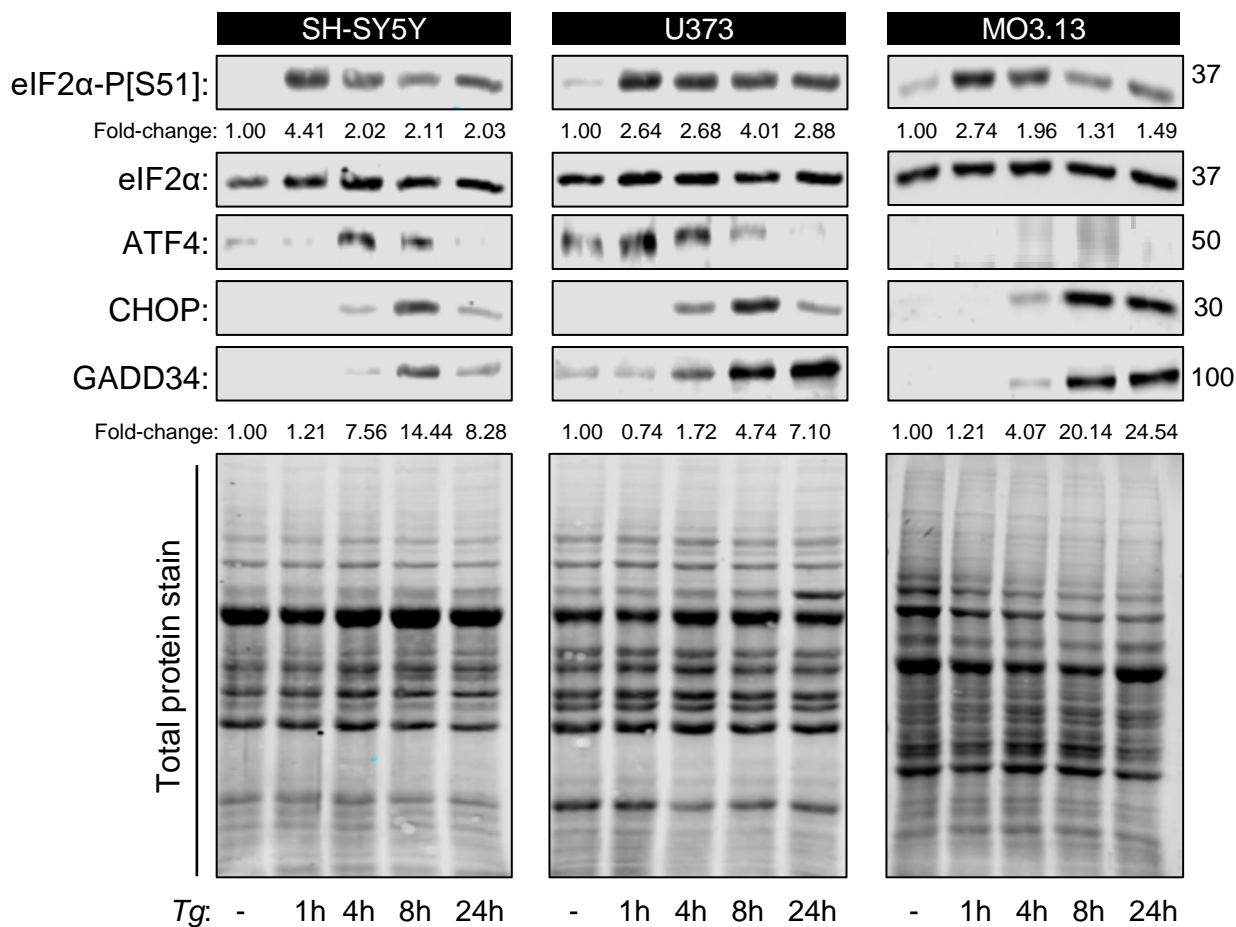


Figure S2. ATF4 expression peaks before the 24h time point of Tg exposure, related to Figure 3. Representative western blot of the ISR expression profile (eIF2α-P[S51], pan-eIF2α, ATF4, CHOP and GADD34) and total protein staining (loading control) in SH-SY5Y, U373 and MO3.13 cells treated with vehicle (DMSO) for 24h or thapsigargin for 1, 4, 8 and 24 hours.

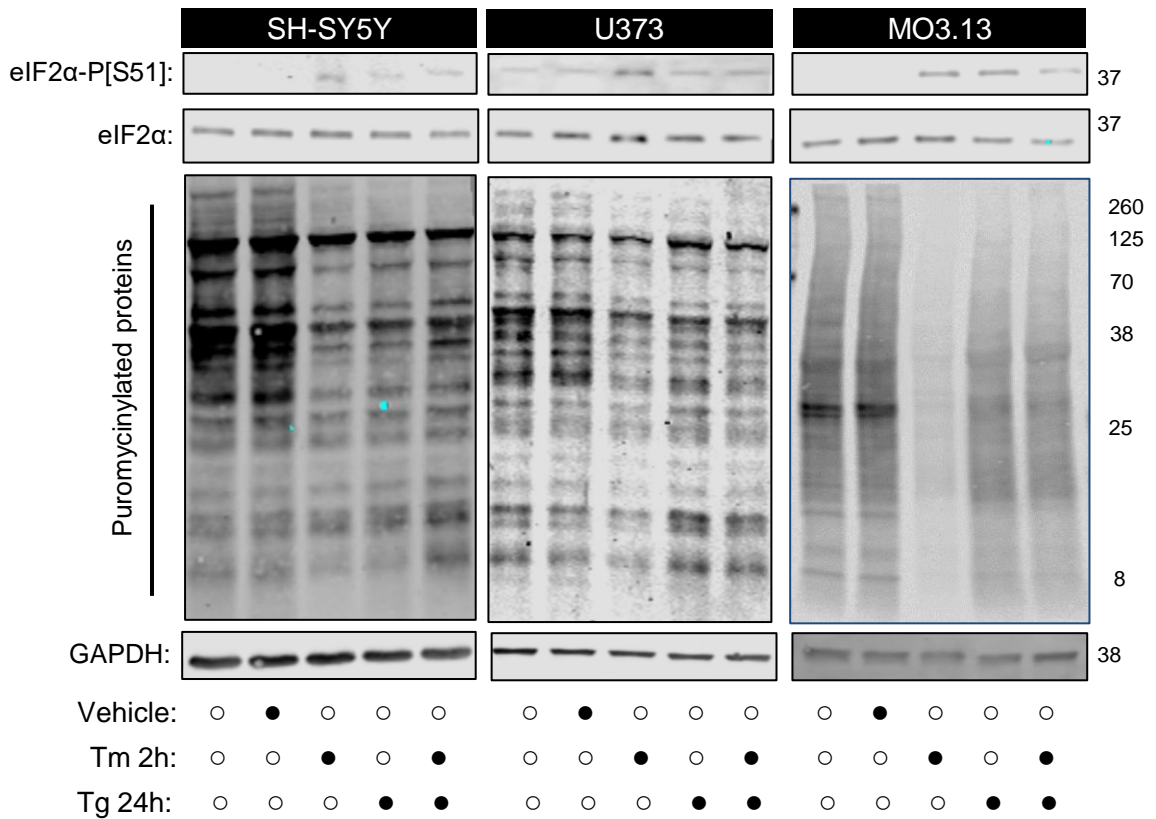


Figure S3. Tunicamycin treatment prior to Tg preconditioning for 24h does not further impact on eIF2α-P[S51] levels and protein synthesis, related to Figure 3. Western blot of the ISR expression profile (eIF2α-P[S51], pan-eIF2α) and global newly synthesized proteins (puromycin incorporation assay) in SH-SY5Y, U373 and MO3.13 cells treated with vehicle (DMSO), acute ER stress inducer (Tm 3 μg/mL for 2h), chronic ER stress (Tg 300 nM for 24h) and chronic ER stress further challenged with acute ER stress inducer (Tg 300 nM for 24h + Tm 3 μg/mL on the last 2h).

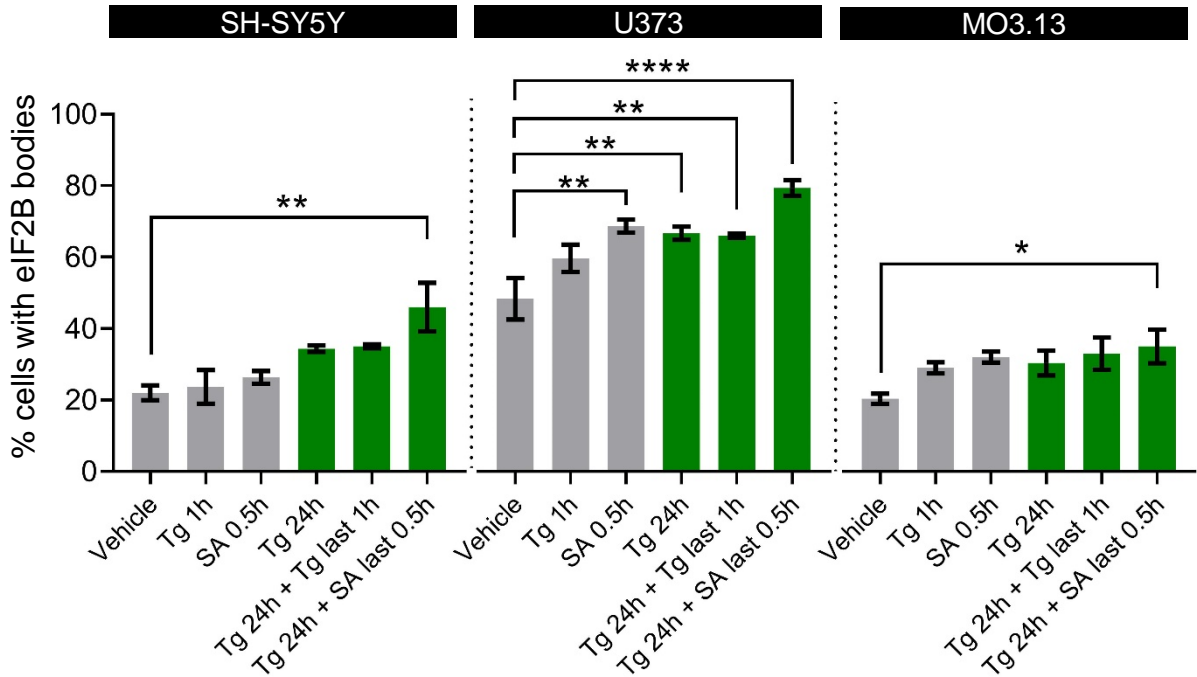


Figure S4. eIF2B localisation increases with cellular stress, related to Figure 4. SH-SY5Y, U373 and MO3.13 cells were subjected to acute stress inducers (Tg 1 μ M for 1h and SA 125 μ M for 30 min); chronic ER stress (Tg 300nM for 24h) or subsequently challenged with previously described acute stress treatments (Tg 24h + Tg last 1h; Tg 24h + SA last 0.5h). Mean percentage of cells displaying localised eIF2B (at least one eIF2B body present) in a population of 100 transfected cells (mean \pm SEM; $N=3$).