
Supplementary information

NAC controls nascent chain fate through tunnel sensing and chaperone action

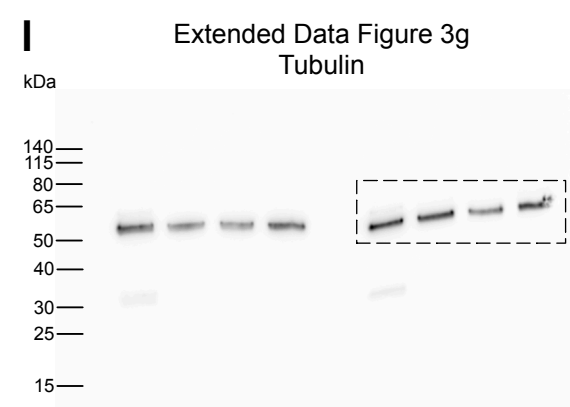
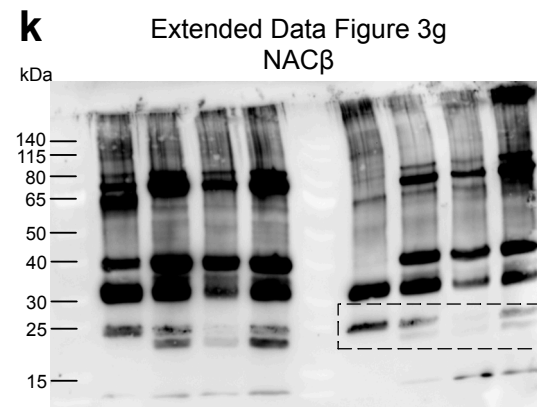
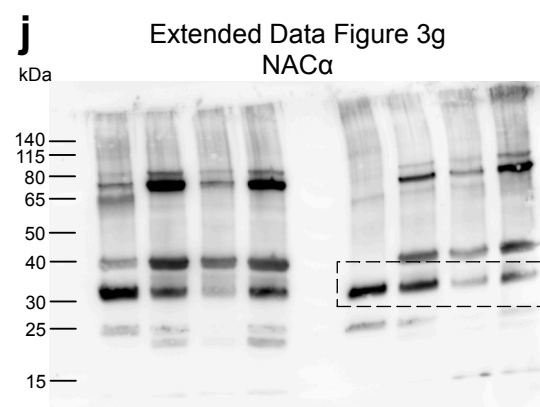
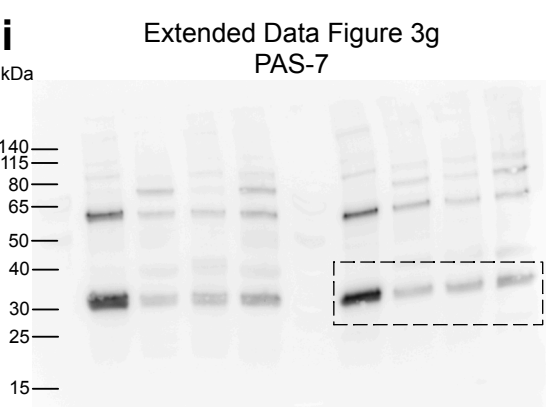
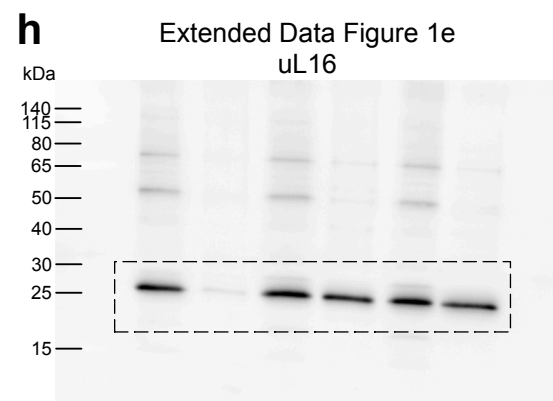
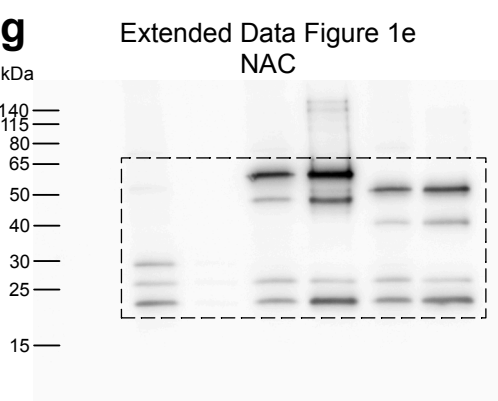
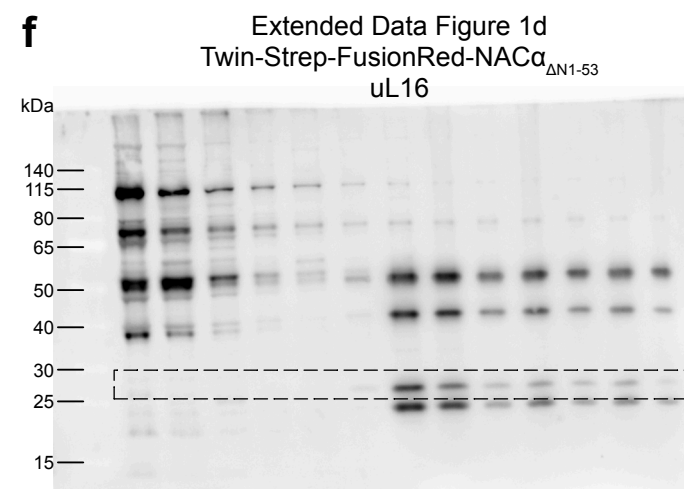
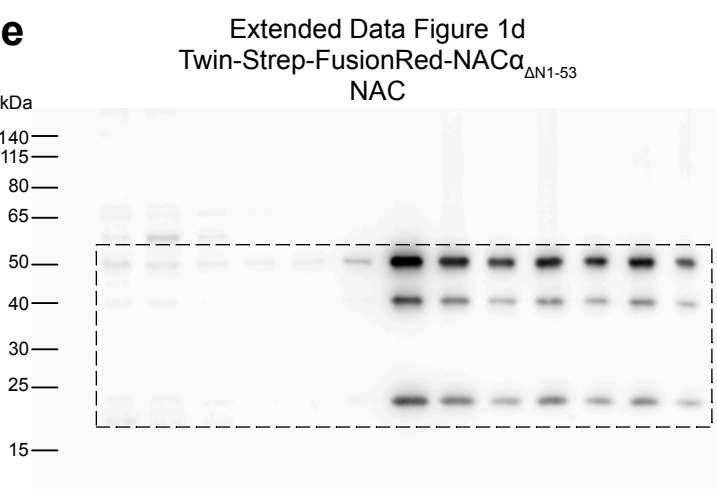
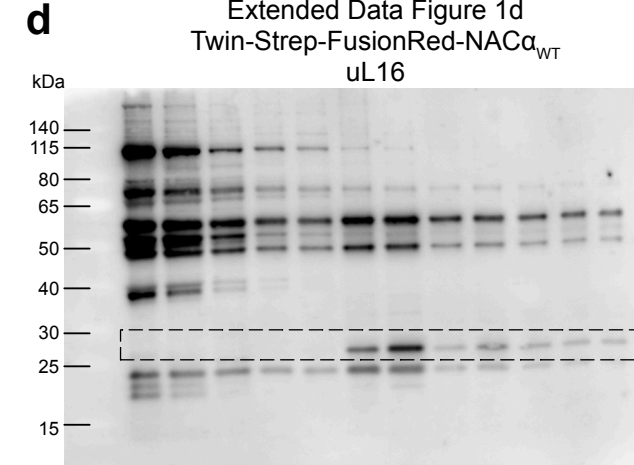
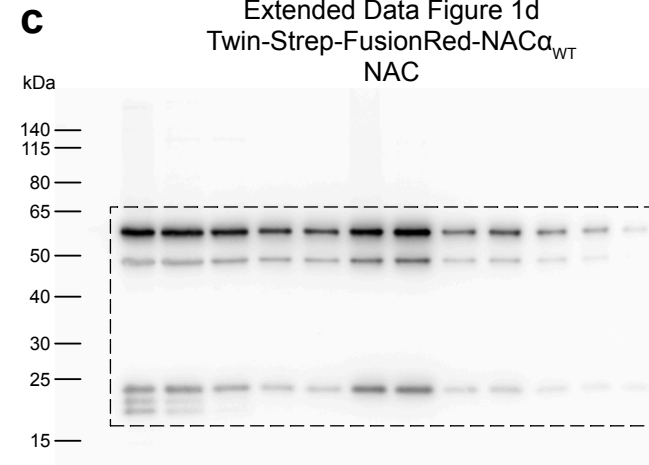
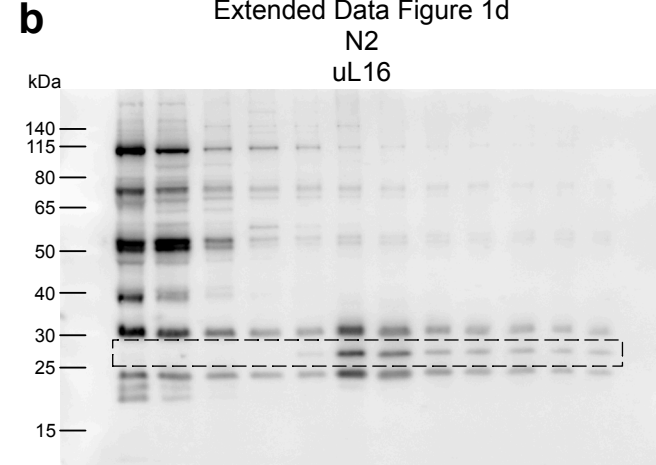
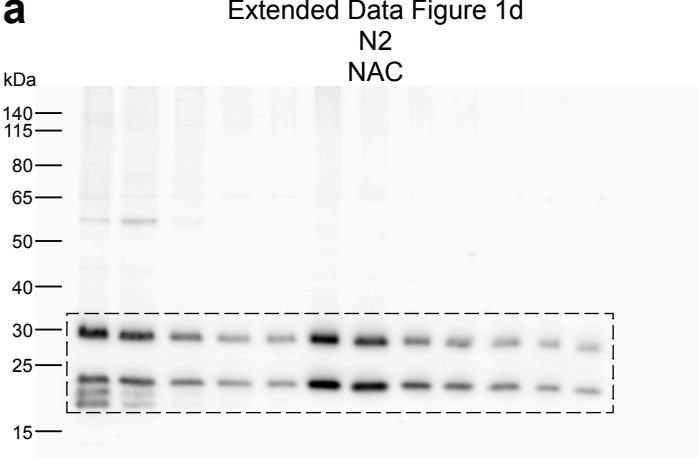
In the format provided by the
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SI Figure 1. Uncropped immunoblots from Extended Data Figures. a-f, Immunoblots for Extended Data Fig. 1d showing migration of wildtype NAC (**a**), Twin-Strep-NAC (**c**), Twin-Strep-NAC Δ N1-53 (**e**), and ribosomal proteins (**b**, **d**, and **f**) on sucrose gradient. **g-h**, Immunoblots for Extended Data Fig. 1e showing the effective IP against NAC (**g**) to isolate NAC-bound ribosomes (**h**). **i-l**, Immunoblots of Extended Data Fig. 3g to verify the knockdown efficiency of PAS-7 (**i**), NAC α (**j**), NAC β (**k**), and loading control Tubulin (**l**).

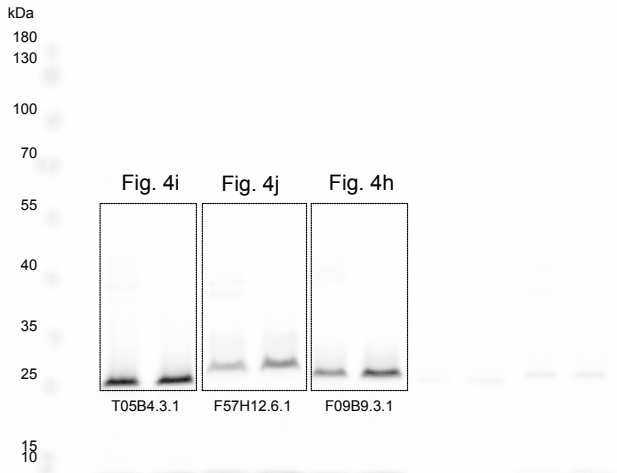
SI Figure 2. Uncropped fluorescence scan of gel from Extended Data Figures. a, Fluorescence scan of gels shown in Extended Data Fig. 7e to detect BODIPY labeled loading control as well as translation intermediates and products. **b**, Fluorescence scan of gels shown in Extended Data Fig. 7h to detect BODIPY labeled loading control as well as translation intermediates and products.

SI Figure 3. Uncropped autoradiography of gels from figures. a, Autoradiography to visualize crosslinking between nascent chain and NAC shown in Fig. 4h-j. **b**, Autoradiography to visualize crosslinking between nascent chain and NAC as shown in Extended Data Fig. 6.

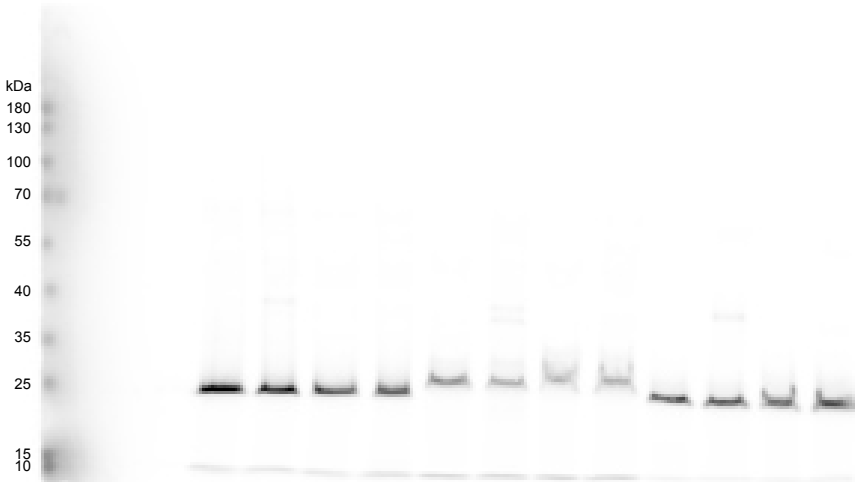


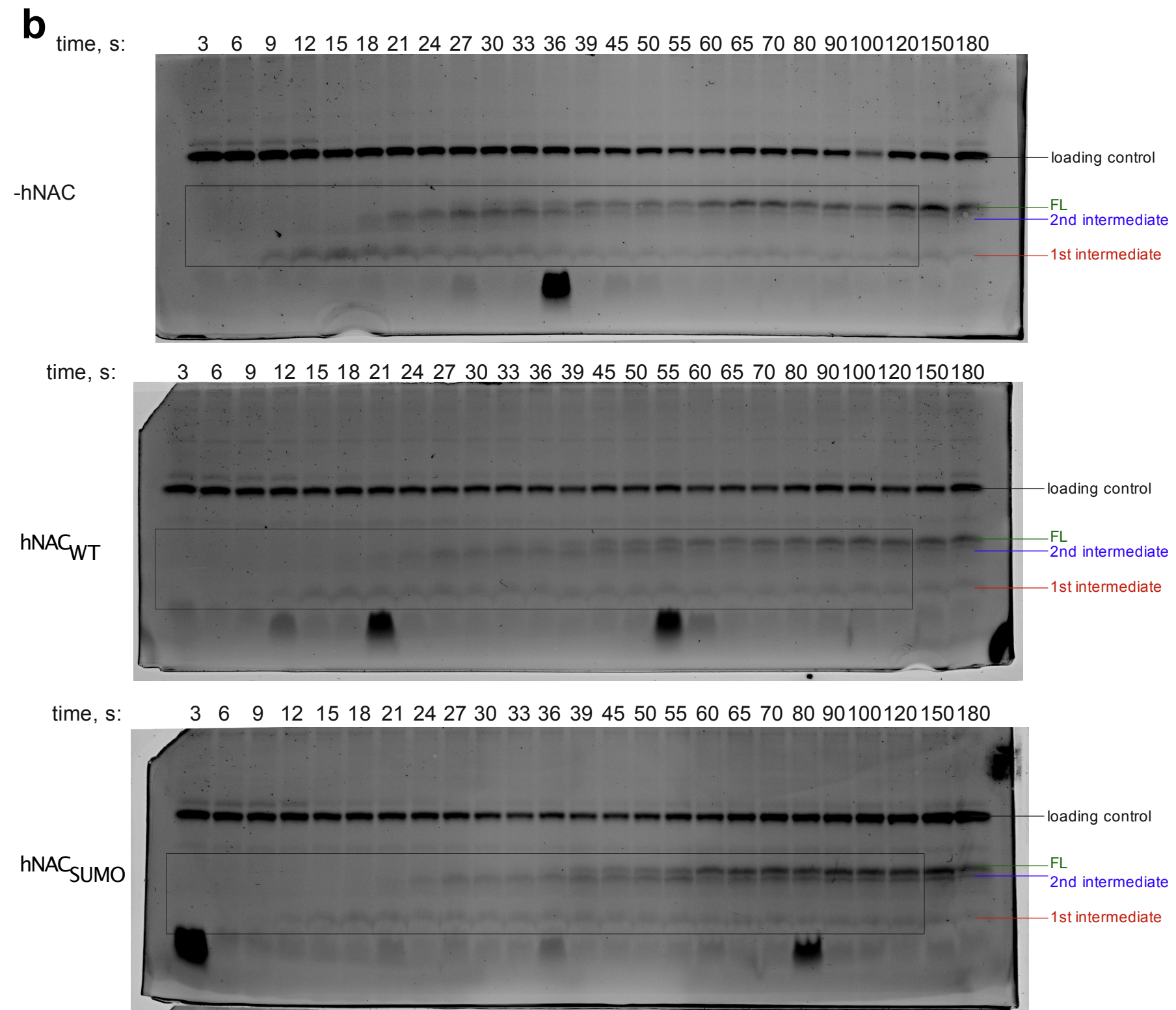
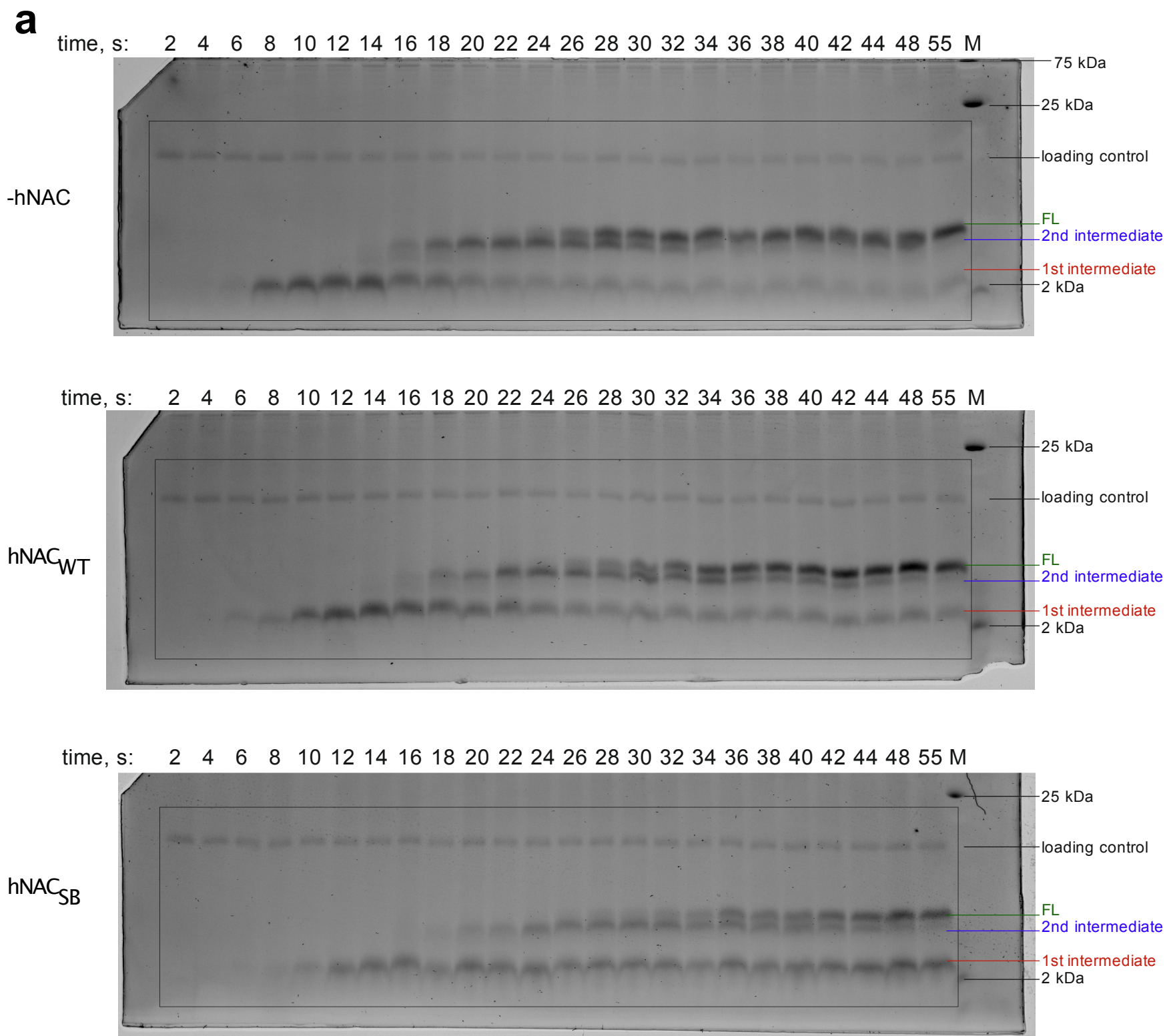
a

Fig. 4h,i,j -NAC interacts with short NCs

**b**

Ext. Fig. 6: Bpa-crosslinking of NAC and ribosome nascent chain complexes





The intensity of each *in vitro* translated peptide (1st, 2nd intermediate and full-length product (FL)) was analyzed via Image Studio software and normalized against the loading control loaded on the same gel as marked. The areas marked in the box appear in the Extended Data Figure 7.