Biochemical and structural characterization of human core Elongator and its subassemblies

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Table S1: Plasmids used in this study

| Plasmid	Protein(s)/Insert	Sequence(s)	Modifications/Tags	Source
pLIB-ELP1	ELP1	1-1332	C-term 3xFLAG	This study
pLIB-ELP2	ELP2	1-826	N-term 10xHis	This study
pBIG1a-ELP1/3	ELP1, ELP3	1-1332 (ELP1), 1-547 (ELP3)	C-term 3xFLAG (ELP1)	This study
pBIG1a-ELP1/2/3	ELP1, ELP2, ELP3	1-1332 (ELP1), 1-826 (ELP2), 1-547 (ELP3)	C-term 3xFLAG (ELP1), N-term 10xHis (ELP2)	This study
pQLinkH-ELP4/5/6	ELP4, ELP5, ELP6	1-535 (ELP4), 1-317 (ELP5), 1-266 (ELP6)	N-term 6xHis (ELP4), N-term TwinStrepII (ELP6)	This study
pQLinkH-yElp4/5/6	yElp4, yElp5. yElp6	1-456 (yElp4), 1-309 (yElp5), 1-273 (yElp6)	N-term 6xHis (yElp6)	Setiaputra et al., 2017 [22]
pMA-T-tRNA$_{Glu}$^{UUC}	Human tRNA$_{Glu}$^{UUC}	1-107 bp	-	This study
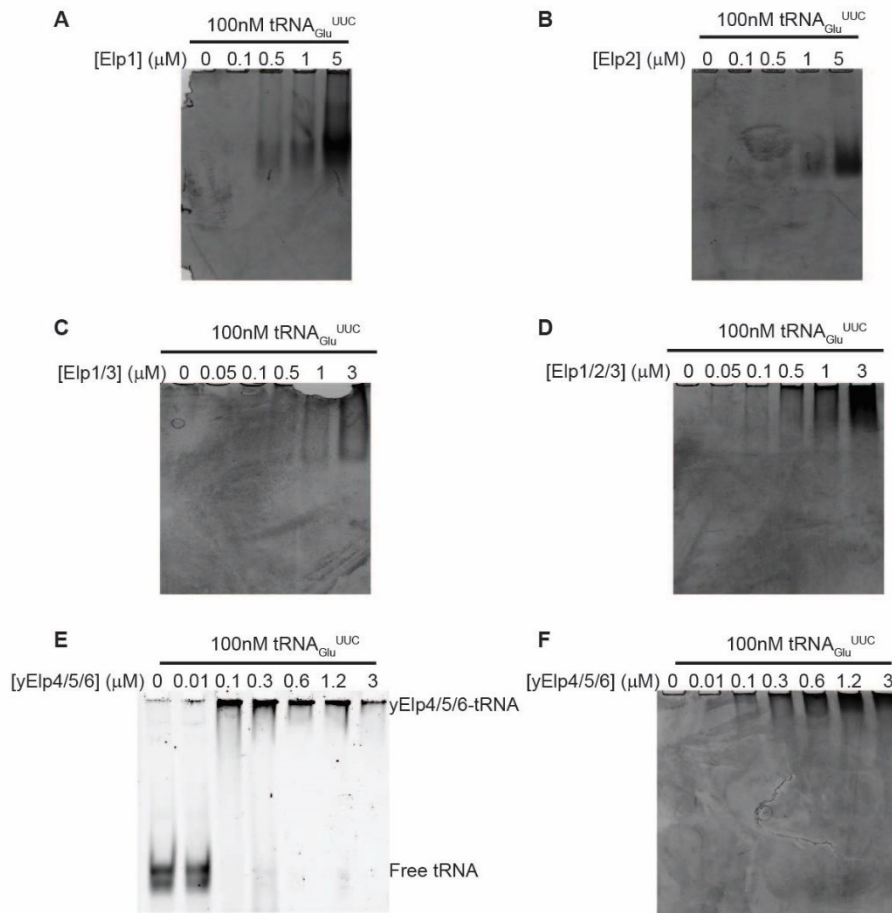
Figure S1: Interactions of human Elongator subassemblies with tRNA.

Electrophoretic mobility shift assay (EMSA) demonstrating the interaction between recombinant tRNA$_{Glu}^{UUC}$ and Elongator subassemblies. 100nM of tRNA was incubated with the indicated amount of hELP1 (**A**), hELP2 (**B**), hELP1/3 (**C**), hELP1/2/3 (**D**), or yeast Elp4/5/6 (**E**, **F**) and run on a 5% native polyacrylamide gel. Proteins within the gel were then stained using PAGE Blue (**A-D**, **F**) or nucleic acids were stained using SYBR Gold (**E**) and visualized.
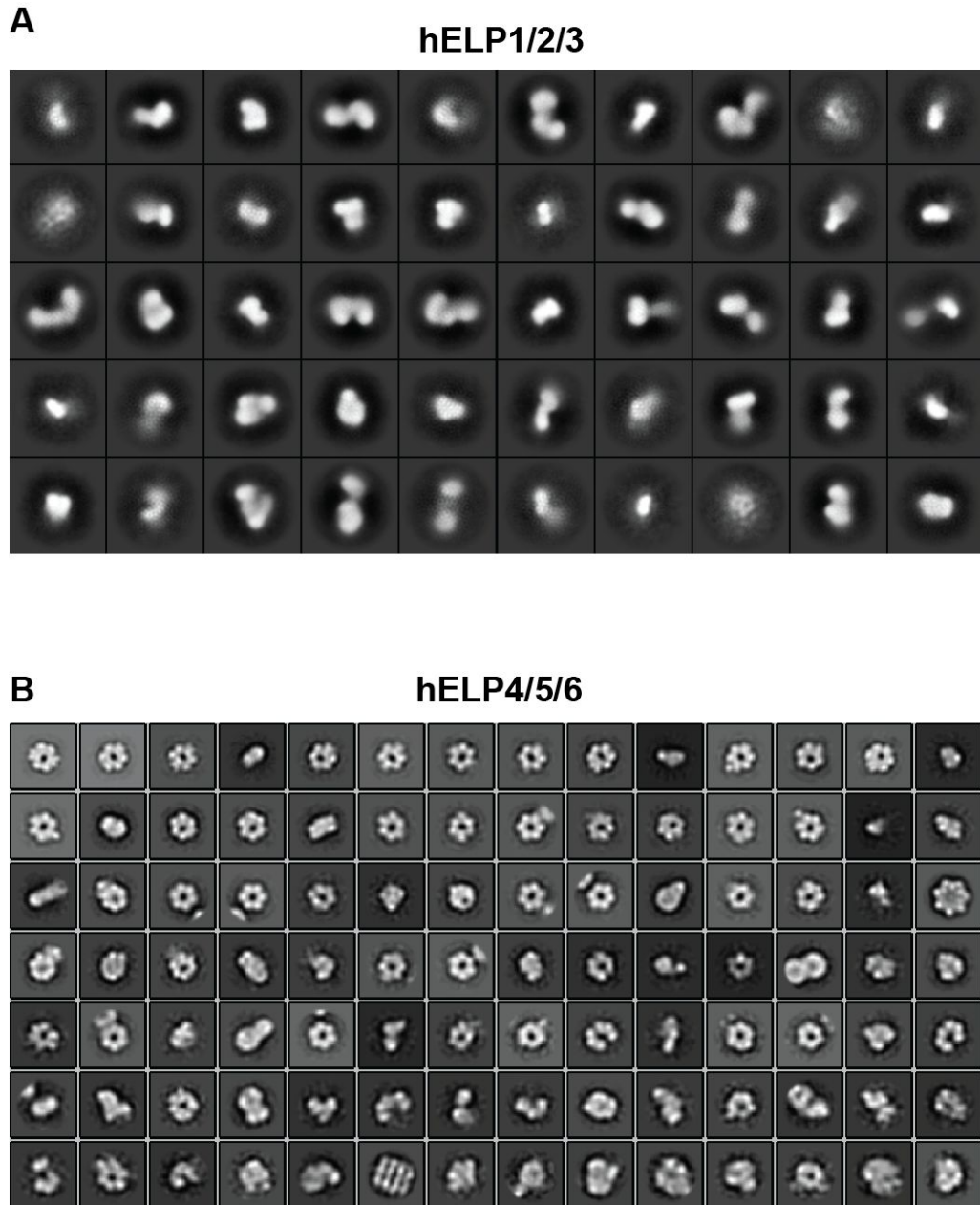
Figure S2. Negative stain EM class averages human Elongator subassemblies. A. 38,529 particles of recombinant hELP1/2/3 were classified and aligned into 50 classes. Class averages are sorted randomly. Box edge length is 460 Å. **B.** 35,272 particles of purified hELP4/5/6 were classified and aligned into 100 classes. Class averages are sorted by particle distribution, with averages containing the most particles starting from the top left. Box edge length is 280 Å.
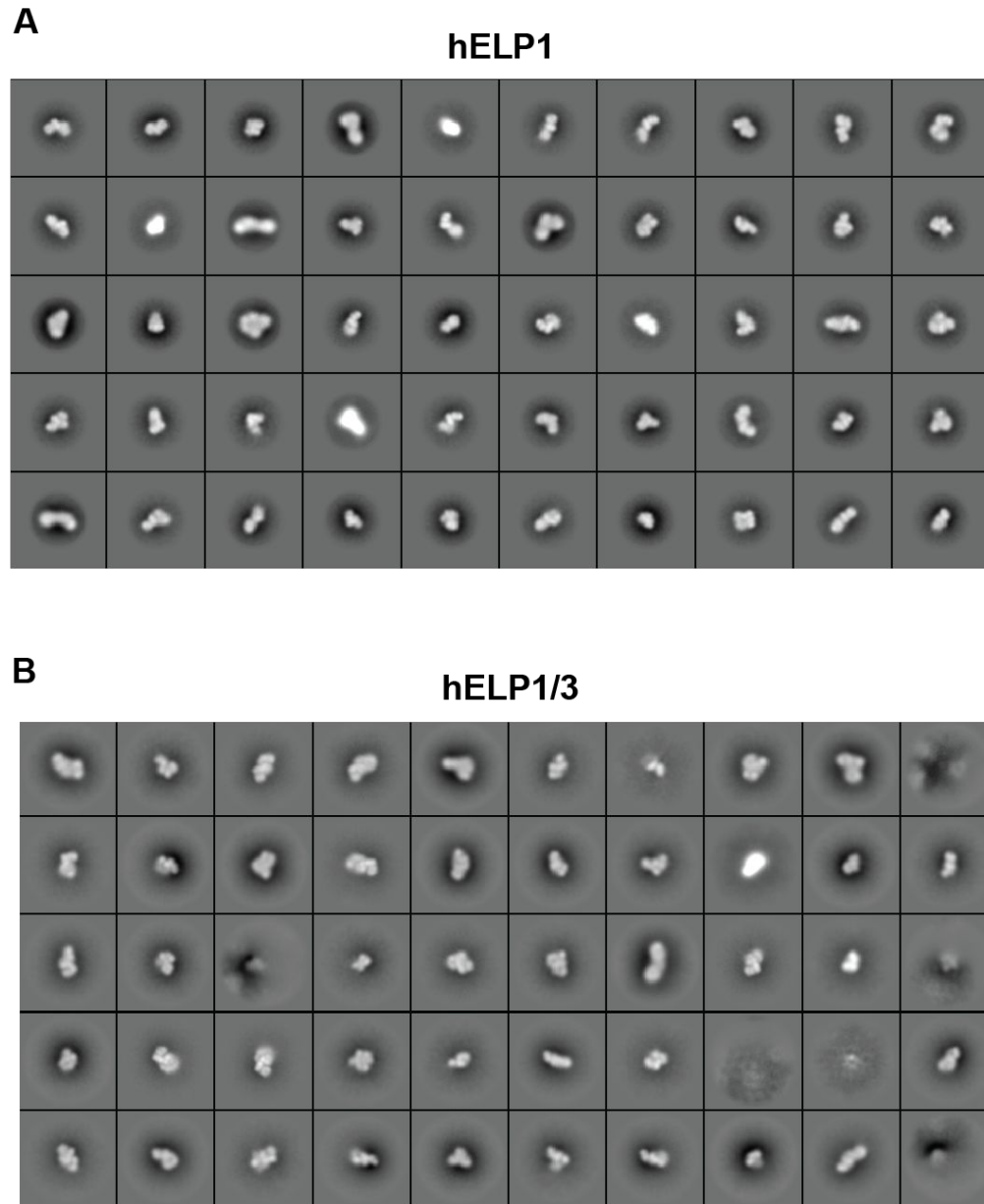
A

hELP1

B

hELP1/3

Figure S3. Negative stain EM class averages of hELP1 and hELP1/3 assemblies.
A. 61,968 particles of hELP1 were classified and aligned into 50 classes. Class averages are sorted randomly. Box edge length is 320 Å. **B.** 75,859 particles of purified hELP1/3 were classified and aligned into 50 classes. Class averages are sorted randomly. Box edge length is 320 Å.
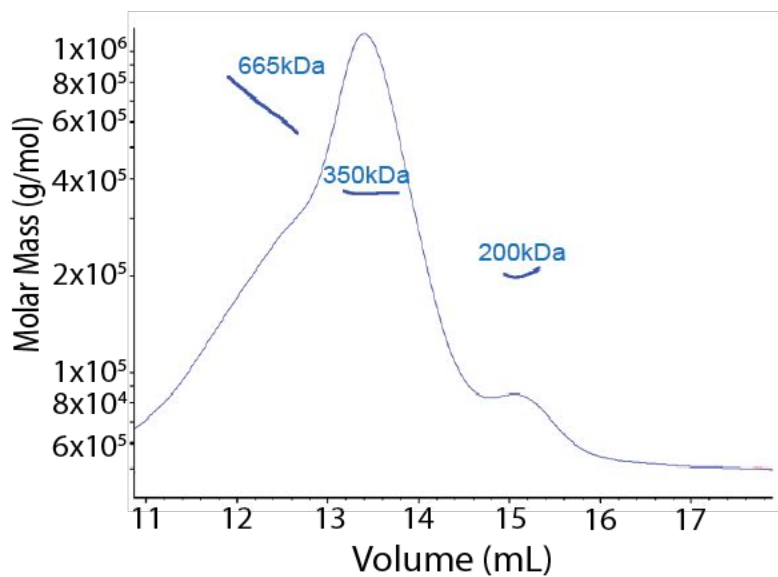
Figure S4. Size-exclusion chromatography coupled with multi angle light scattering (SEC-MALS) elution profile of purified hELP1. The average molecular mass of molecules in each SEC peak was calculated from MALS and is denoted above the peak.