

Supplemental Information

**Pathological polyQ expansion does not alter
the conformation of the Huntingtin-HAP40
complex**

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Supplemental Figures

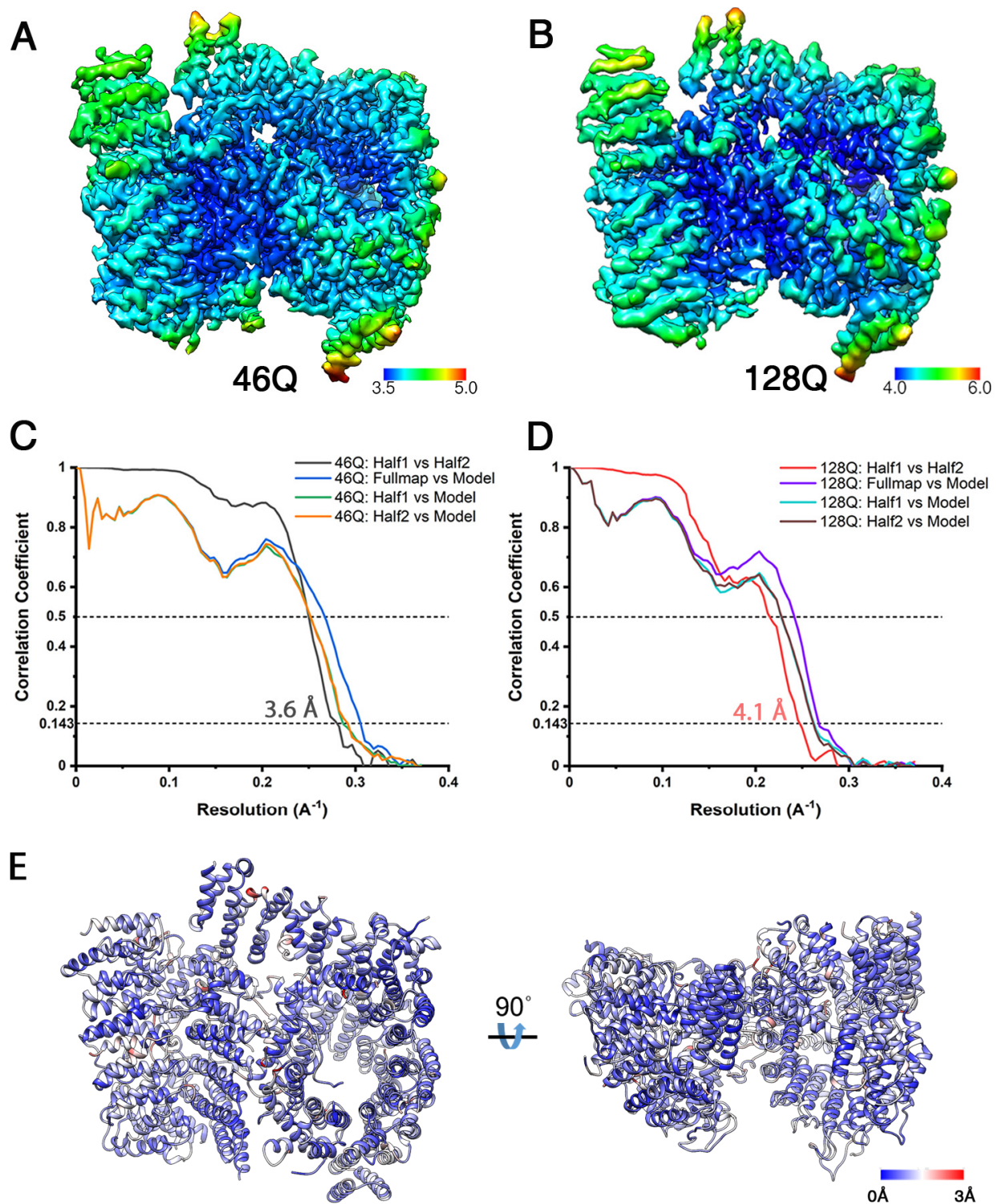


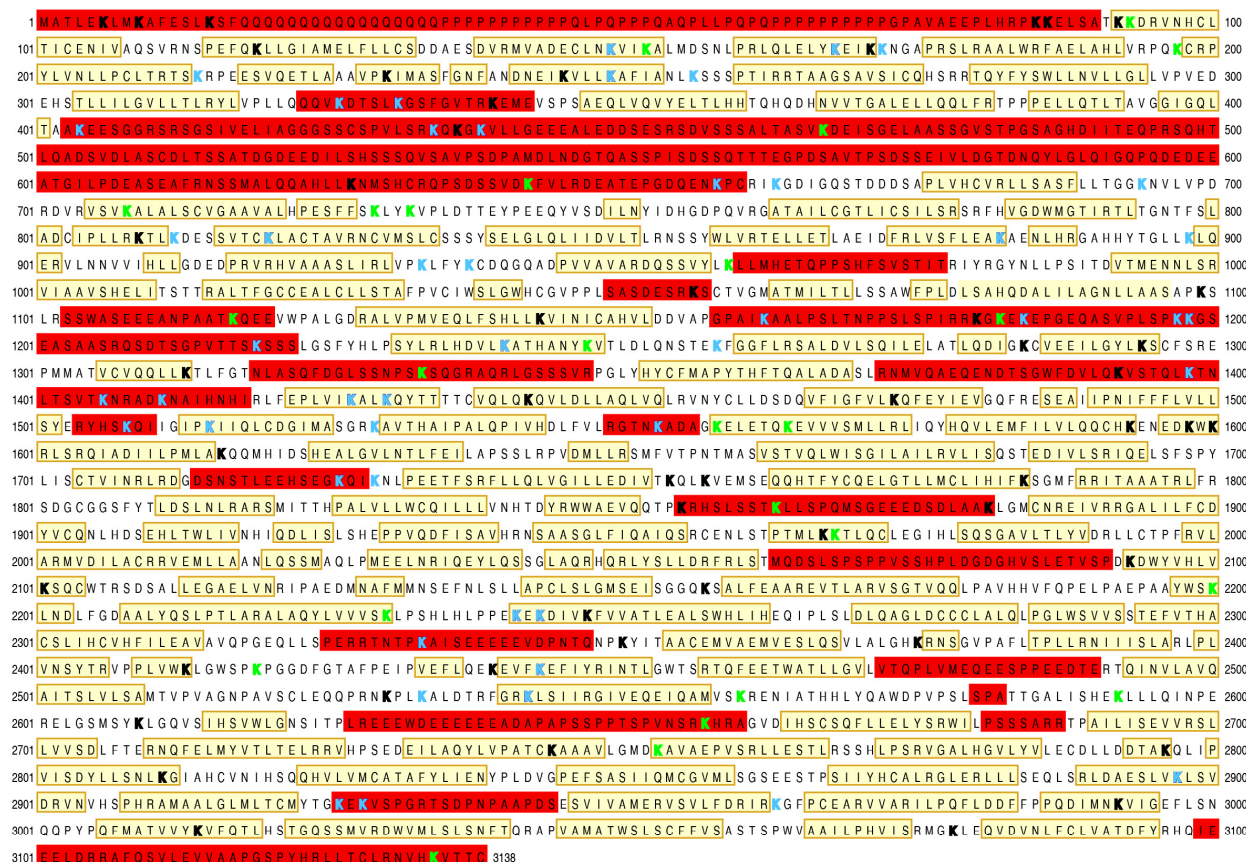
Figure S 1: Local resolution of cryo-EM structures. Related to Figure 2.

(A, B) Density maps of 46QHTT-HAP40 and 128QHTT-HAP40 complexes colored according to local resolution. The maps were filtered according to the local resolution.

(C, D) FSC plots of the two structures. FSC curves comparing maps obtained with two independent half sets of particles (Half1 vs Half2), the full map against the model, as well as maps obtained from each half set of particles against the model are shown. The FSC = 0.143 criterion was used for the estimation of the global resolution.

(E) Atomic model for 128QHTT-HAP40 shown in ribbon representation, colored according to its RMSD with 46QHTT-HAP40.

Huntingtin



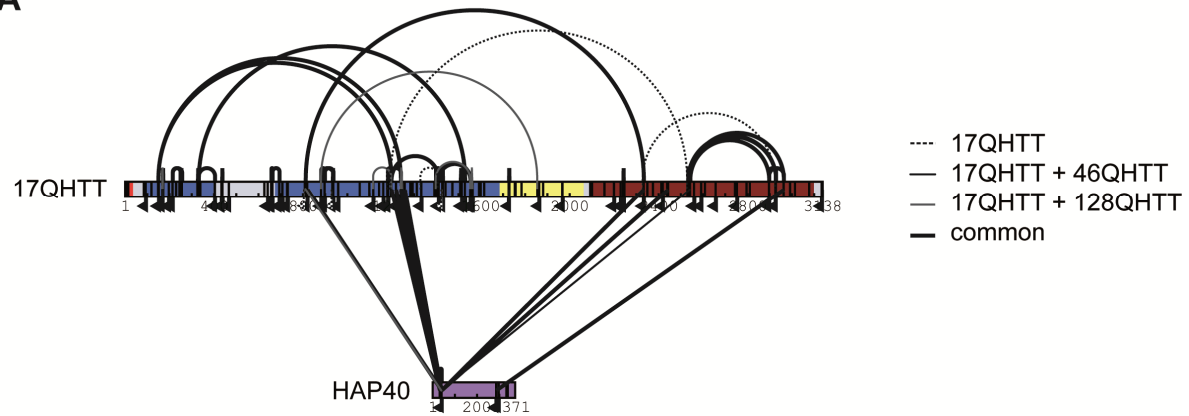
HAP40



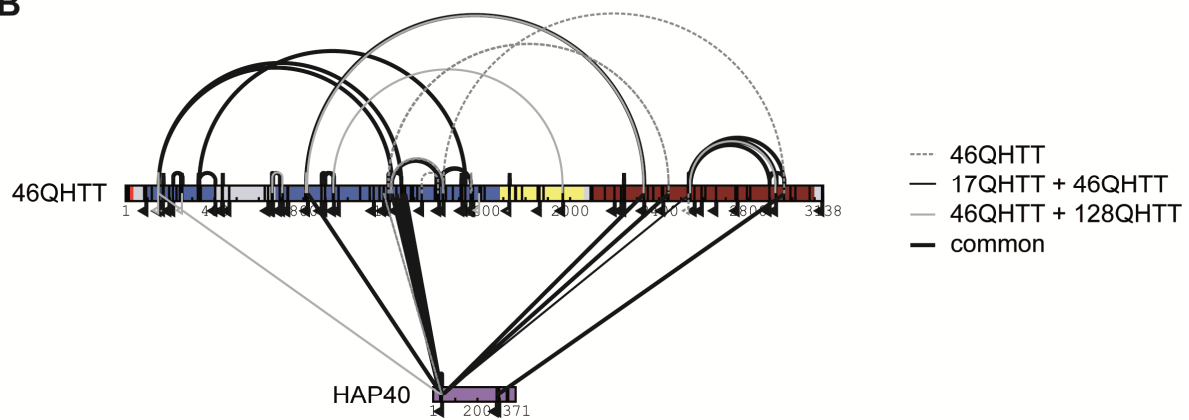
Figure S 2: Mapping of monolinks and crosslinks in the sequence of HTT and HAP40. Related to Figure 2 and Figure 3.

Structural elements of 17QHTT are indicated as follows: invisible in the model (red boxes) and α -helices (yellow boxes). Lysine residues (K) with monolinks (green) or crosslinks (blue) detected in all three HTT-HAP40 complexes (17QHTT-HAP40, 46QHTT-HAP40 and 128QHTT-HAP40) are marked. Only high-confidence crosslinks that were reliably identified in at least two out of three biological replicates are shown (see methods for details). The remaining lysines are marked in black bold.

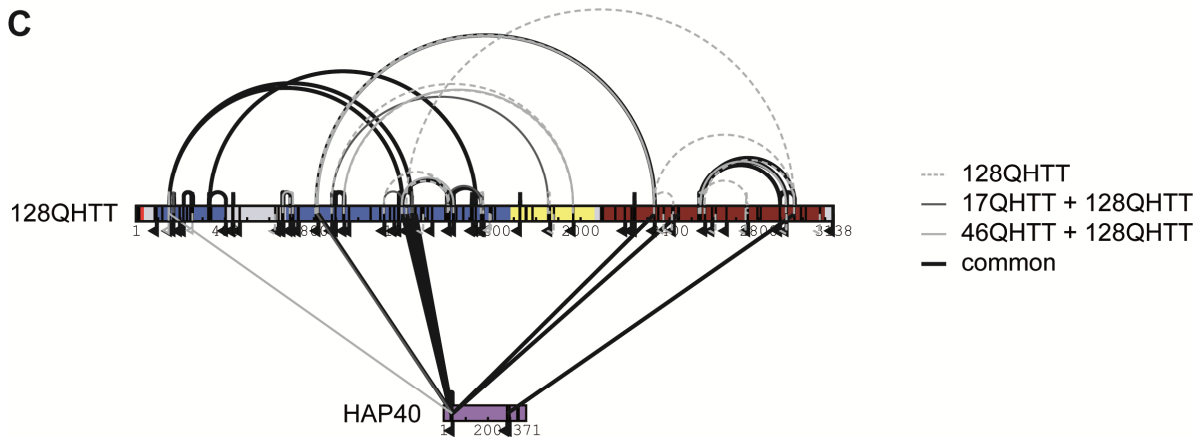
A



B



C



D

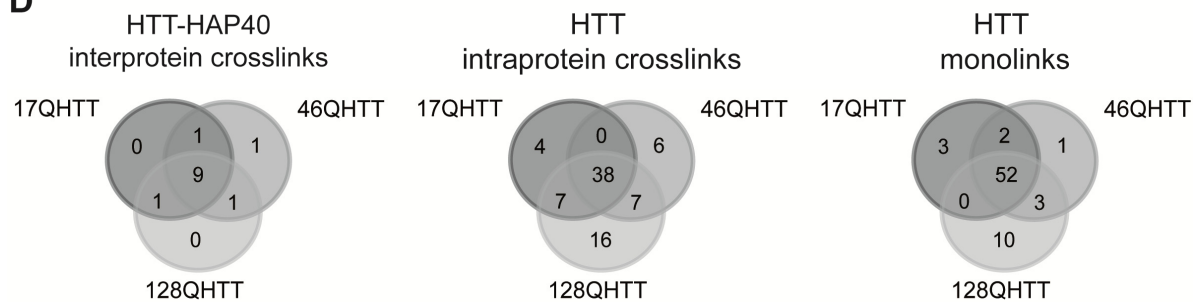


Figure S 3: Effects of polyQ expansion on overall crosslink patterns of HTT-HAP40 complexes.

Related to Figure 3.

(A, B, C) Comparative representation of all crosslinks identified in three different polyQ expanded HTT-HAP40 complexes, namely 17QHTT-HAP40 (A), 46QHTT-HAP40 (B) and 128QHTT-HAP40 (C). A uniform numbering scheme based on the 17QHTT sequence was used. Crosslinks common to all three complexes are represented by thick, black solid lines. Crosslinks identified in only two of the complexes are represented by thin solid lines (17QHTT and 46QHTT, black; 17QHTT and 128QHTT dark grey; 46QHTT and 128QHTT, light grey). Crosslinks identified in only one of the complexes are depicted by dotted lines (17QHTT, black; 46QHTT, dark grey; 128QHTT light grey). Only high-confidence crosslinks that were reliably identified in at least two out of three biological replicates are shown (see Methods for details). Interlinks are shown as straight line, intralinks as curved line and monolinks as a flag. The HTT N-HEAT domain, bridge domain and C-HEAT domains are colored in blue, yellow and maroon, respectively. HAP40 is colored in purple.

(D) Venn diagrams showing the overlap of crosslinks identified in the three different HTT-HAP40 complexes (A, B, C).

Supplemental Table

Table S 1: Cryo-EM data collection, refinement and validation statistics. Related to Figure 2.

	46Q-Huntingtin- HAP40 complex (EMD-30911) (PDB 7DXJ)	128Q-Huntingtin- HAP40 complex (EMD-30912) (PDB 7DXK)
Data collection and processing		
Magnification	105,000X	105,000X
Voltage (kV)	300	300
Electron exposure (e-/Å ²)	32	32
Defocus range (µm)	1.4-3.0	1.4-3.0
Pixel size (Å)	1.35	1.35
Symmetry imposed	C1	C1
Initial particle images (no.)	581,397	463,213
Final particle images (no.)	192,473	122,992
Map resolution (Å)	3.6	4.1
FSC threshold	0.143	0.143
Map resolution range (Å)	3.5-5.0	4.0-6.0
Refinement		
Map sharpening <i>B</i> factor (Å ²)	-140	-187
Model composition		
Non-hydrogen atoms	20,491	20,491
Protein residues	2,621	2,621
R.m.s. deviations		
Bond lengths (Å)	0.0076	0.0074
Bond angles (°)	1.38	1.30
Validation		
MolProbity score	1.66	1.70
Clashscore	4.80	5.93
Poor rotamers (%)	0.97	0.48
Ramachandran plot		
Favored (%)	93.86	94.60
Allowed (%)	5.90	5.24
Disallowed (%)	0.23	0.16