

Appendix Figure and Tables

Dauden et al.

Architecture of the yeast Elongator complex

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Sample preparation and crosslinking validation

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Appendix Figure S3

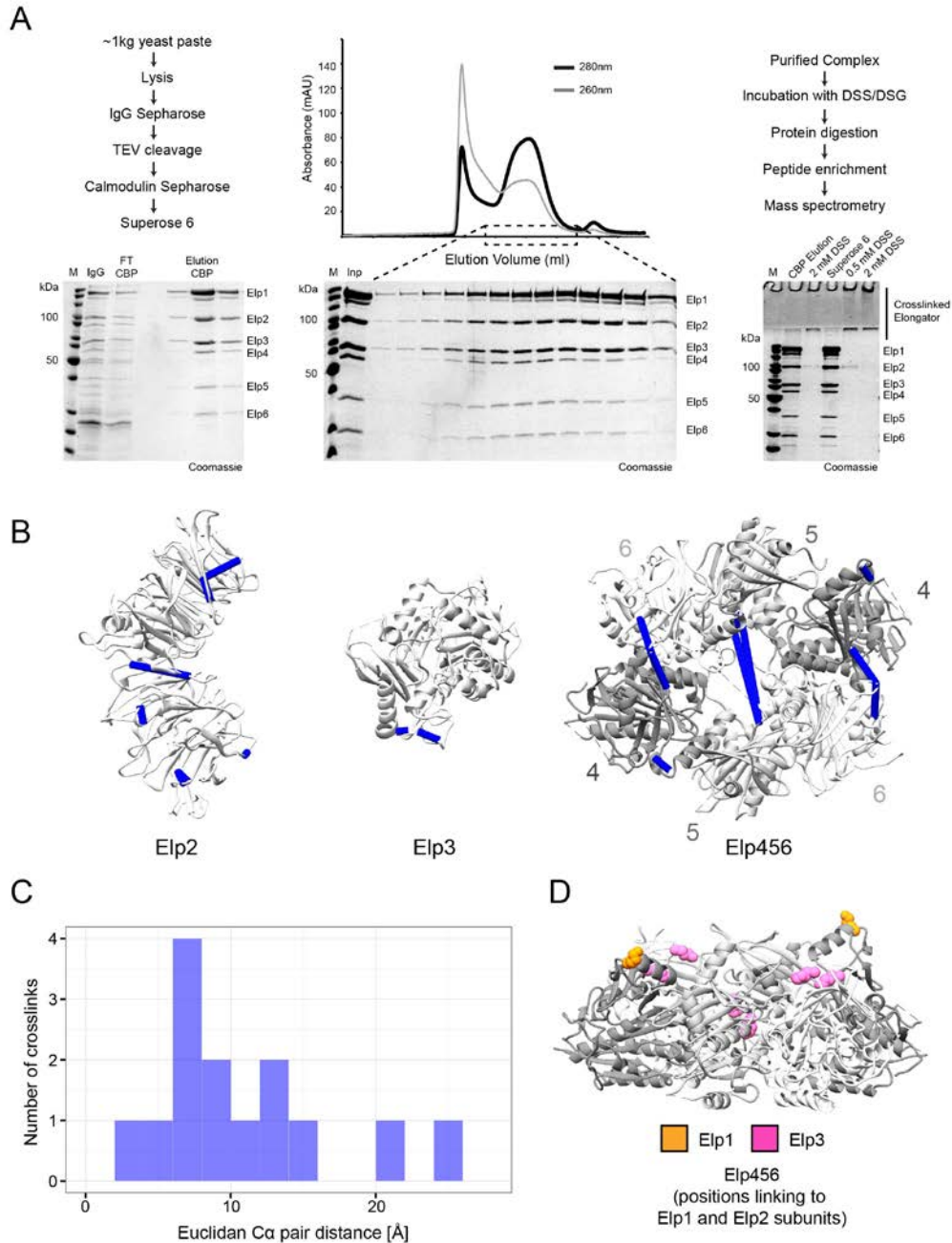
Conservation and charge distribution of holoElongator

Appendix Table S1

Crystallographic data collection, phasing and refinement statistics

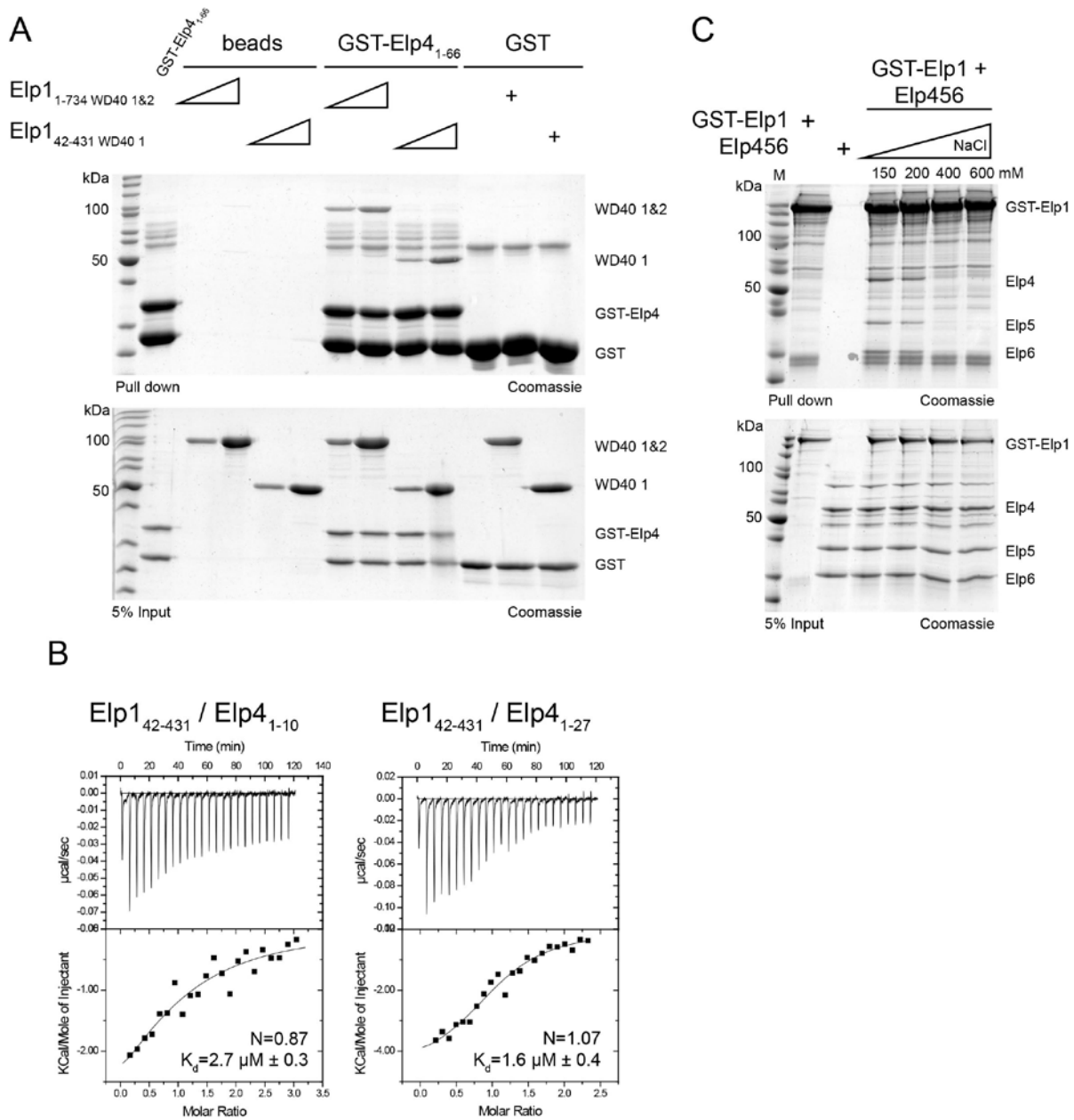
Appendix Table S2

List of highly confident crosslinks identified in this work



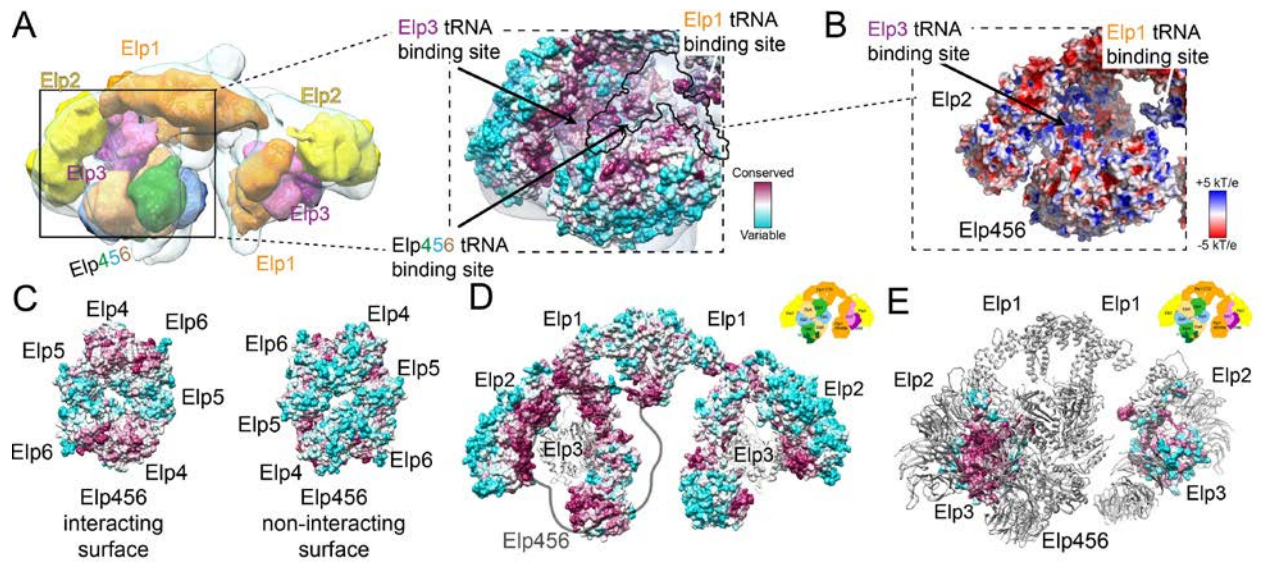
Appendix Figure S1 | Sample preparation and crosslinking validation. **A.** Schematic overview of the Elp1-TAP purification protocol and SDS-PAGE analyses of the initial purification steps (left panel). Samples were analyzed by SDS-PAGE and stained by Coomassie. Identities of respective proteins are indicated on the right. Gel filtration of purified Elp1-TAP tagged Elongator complex shows UV absorption profiles at 260nm and 280nm (middle panel). The respective fractions were analyzed by SDS-PAGE and stained by Coomassie. Identities of respective proteins are indicated. Schematic overview of the used XL-MS protocol and SDS-PAGE analyses of the samples before and after

incubation with indicated amounts of crosslinking reagent (right panel). Gel was stained by Coomassie and identities of respective proteins are indicated on the right. **B.** Crosslinks (blue lines) identified in this work mapped to the subunit structures. Only high confidence crosslinks (LD score greater than or equal to 30) are displayed. **C.** Histogram of crosslink distances. All crosslinks satisfy the C α -C α distance threshold of 30 Å. **D.** Residues of the Elp456 sub-complex subunits crosslinking to Elp1 and Elp3 subunits (no residue crosslinks to Elp2). All crosslinking positions exclusively locate to one side of the hexamer, suggesting its orientation relatively to the Elp123 sub-complex.



Appendix Figure S2 | Validation of the Elp1-Elp3-Elp4 interaction network. **A.** GST-pull down assays of purified GST-tagged N-terminus of Elp4 with the two predicted untagged WD40 domains of Elp1 (1st WD40 aa 42-431 and both WD40s aa 1-734). GSH resin and untagged proteins were used as input controls. Lower panel shows 5% of the input and upper panel shows bound fractions. All samples were analyzed by SDS-PAGE and stained by Coomassie. Identities of respective proteins are indicated on the right. **B.**

ITC measurement using the purified first WD40 domain of Elp1 (10 μ M; cell) and synthetic peptides of the N-terminus of Elp4, aa1-10 and aa1-27 respectively (both 100 μ M; syringe). Calculated K_d and stoichiometry (N) are indicated. **C.** Same as **A**, but using GST-tagged full length Elp1 in combination with untagged full length Elp456 in presence of increasing concentrations of NaCl.



Appendix Figure S3 | Analysis of surface properties of the holoElongator model. **A.** Sequence conservation of the tentative tRNA binding cleft mapped onto the molecular surface of the model. The full model is shown in a low-resolution representation within the negative stain envelope as viewed from the top (left). Sequence conservation is mapped onto the surface of the cleft as a color gradient (right). The black outline depicts a possible tRNA binding mode. **B.** Electrostatic potential mapped onto the surface of the cleft. Residues 74-94 and 374-405 of Elp3 (depicted as gray ribbons) were not used for calculating the charge distribution because their exact atomic structure is uncertain.

Note also that the depicted electrostatic potential is only approximate due to low resolution of the model and missing regions, such as Arg-rich region of Elp1. **C.** Sequence conservation mapped onto the surface of Elp456 hexamer that interacts with Elp123 sub-complex (left) and the opposite, non-interacting surface (right). **D.** Sequence conservation mapped onto the surface of Elp1 and Elp2. Elp3 is shown in ribbon representation. For clarity, the Elp456 hexamer is depicted as an outline. The cartoon (top right) indicates the viewing angle. **E.** Sequence conservation mapped onto the surface of Elp3. All other subunits are shown in ribbon representation.

Appendix Table S1. Crystallographic data collection, phasing and refinement statistics

| | Elp2 full length SeMet PDB ID 5M2N |
|---|--|
| Data collection | |
| Space group | P6 ₁ 22 |
| Cell dimensions | |
| <i>a</i> , <i>b</i> , <i>c</i> (Å) | 79.69 79.69 532.15 |
| α , β , γ (°) | 90, 90, 120 |
| Wavelength | 0.97898 |
| Resolution (Å) [†] | 50-2.8 (2.89-2.8) |
| <i>R</i> _{meas} | 14.3 (81.9) |
| <i>I</i> / σ (<i>I</i>) | 14.40 (2.58) |
| <i>CC</i> _{1/2} | 0.99 (0.68) |
| Completeness (%) | 99.6 (95.7) |
| Redundancy | 9.3 (7.2) |
| Refinement | |
| Resolution (Å) | 50-2.8 |
| No. reflections | 45798 |
| <i>R</i> _{work} / <i>R</i> _{free} | 19.9/23.5 |
| No. atoms | |
| Protein | 5763 |
| Ligand/ion | 0 |
| Water | 23 |
| <i>B</i> factors | |
| Protein | 45.3 |
| Ligand/ion | |
| Water | 23.8 |
| r.m.s deviations | |
| Bond lengths (Å) | 0.002 |
| Bond angles (°) | 0.561 |

^a Values in parentheses are for highest-resolution shell.

[†] Resolution limits according to *I*/ σ of 2 is are 2.7 Å for Elp2

Appendix Table S2. List of highly confident crosslinks identified in this work (ld-score at least 30)

Please see source data 1 for a complete list and additional specifications of the detected cross links

| Protein 1 | Protein 2 | Residue 1 | Residue 2 | ld-Score |
|-----------|-----------|-----------|-----------|----------|
| Elp6 | Elp4 | 230 | 218 | 47.01 |
| Elp3 | Elp1 | 521 | 759 | 42.93 |
| Elp1 | Elp4 | 1223 | 218 | 42.93 |
| Elp1 | Elp3 | 510 | 473 | 43.22 |
| Elp1 | Elp3 | 1217 | 401 | 38.38 |
| Elp1 | Elp3 | 1223 | 401 | 38 |
| Elp1 | Elp4 | 1270 | 451 | 38 |
| Elp4 | Elp1 | 177 | 1217 | 36.66 |
| Elp4 | Elp3 | 399 | 61 | 36.96 |
| Elp6 | Elp3 | 225 | 401 | 36.92 |
| Elp4 | Elp3 | 399 | 14 | 38.22 |
| Elp1 | Elp3 | 220 | 473 | 40.6 |
| Elp4 | Elp3 | 399 | 6 | 33.17 |
| Elp1 | Elp4 | 190 | 218 | 32.95 |
| Elp5 | Elp3 | 140 | 6 | 32.62 |
| Elp1 | Elp3 | 1270 | 401 | 32.41 |
| Elp1 | Elp3 | 1223 | 61 | 32.18 |
| Elp5 | Elp3 | 140 | 14 | 35.3 |
| Elp1 | Elp4 | 198 | 218 | 37.14 |
| Elp1 | Elp4 | 1223 | 213 | 34.29 |
| Elp2 | Elp1 | 460 | 199 | 33.01 |
| Elp4 | Elp3 | 399 | 9 | 32.69 |
| Elp1 | Elp4 | 199 | 218 | 34.34 |
| Elp1 | Elp4 | 1217 | 213 | 32.96 |
| Elp3 | Elp4 | 342 | 370 | 30.46 |
| Elp1 | Elp3 | 1270 | 6 | 34.14 |
| Elp4 | Elp1 | 213 | 199 | 37.5 |
| Elp2 | Elp1 | 249 | 967 | 43.34 |
| Elp1 | Elp4 | 1223 | 177 | 39.77 |
| Elp4 | Elp3 | 143 | 401 | 33.98 |
| Elp1 | Elp3 | 1270 | 14 | 38.89 |
| Elp1 | Elp3 | 1223 | 14 | 31.29 |
| Elp1 | Elp4 | 1223 | 143 | 30.06 |
| Elp1 | Elp3 | 1217 | 19 | 32.56 |
| Elp4 | Elp1 | 186 | 199 | 32.36 |
| Elp4 | Elp1 | 176 | 756 | 30.34 |
| Elp1 | Elp1 | 1223 | 1270 | 45.86 |
| Elp2 | Elp2 | 249 | 257 | 44.11 |
| Elp4 | Elp4 | 235 | 143 | 43.75 |

| Protein 1 | Protein 2 | Residue 1 | Residue 2 | Id-Score |
|-----------|-----------|-----------|-----------|----------|
| Elp1 | Elp1 | 1223 | 818 | 44.79 |
| Elp4 | Elp4 | 143 | 177 | 42.47 |
| Elp1 | Elp1 | 1308 | 1223 | 48.58 |
| Elp2 | Elp2 | 260 | 250 | 41.37 |
| Elp4 | Elp4 | 143 | 218 | 41.35 |
| Elp3 | Elp3 | 528 | 473 | 40.16 |
| Elp1 | Elp1 | 190 | 199 | 41.03 |
| Elp3 | Elp3 | 14 | 61 | 39.4 |
| Elp4 | Elp4 | 143 | 55 | 40.81 |
| Elp4 | Elp4 | 207 | 213 | 43.56 |
| Elp1 | Elp1 | 1119 | 1223 | 40.63 |
| Elp1 | Elp1 | 1246 | 1223 | 38.74 |
| Elp1 | Elp1 | 1246 | 1217 | 40.07 |
| Elp3 | Elp3 | 86 | 79 | 37.45 |
| Elp3 | Elp3 | 401 | 61 | 36.91 |
| Elp1 | Elp1 | 220 | 199 | 37.73 |
| Elp1 | Elp1 | 818 | 1217 | 41.38 |
| Elp4 | Elp4 | 143 | 5 | 42.95 |
| Elp3 | Elp3 | 19 | 61 | 36.28 |
| Elp1 | Elp1 | 1270 | 1217 | 42.12 |
| Elp3 | Elp3 | 325 | 401 | 35.69 |
| Elp4 | Elp4 | 143 | 63 | 39.78 |
| Elp1 | Elp1 | 1308 | 1217 | 40.02 |
| Elp1 | Elp1 | 1308 | 846 | 37.25 |
| Elp3 | Elp3 | 6 | 61 | 34.93 |
| Elp4 | Elp4 | 143 | 213 | 34.95 |
| Elp3 | Elp3 | 453 | 401 | 34.37 |
| Elp3 | Elp3 | 423 | 401 | 34.28 |
| Elp1 | Elp1 | 1223 | 756 | 33.78 |
| Elp2 | Elp2 | 650 | 680 | 35.49 |
| Elp1 | Elp1 | 1119 | 1217 | 35.11 |
| Elp4 | Elp4 | 177 | 218 | 33.99 |
| Elp1 | Elp1 | 818 | 846 | 38.2 |
| Elp1 | Elp1 | 1217 | 1217 | 33.3 |
| Elp4 | Elp4 | 177 | 211 | 32.83 |
| Elp4 | Elp4 | 211 | 218 | 33 |
| Elp4 | Elp4 | 177 | 207 | 32.8 |
| Elp1 | Elp1 | 1062 | 1223 | 36.13 |
| Elp4 | Elp4 | 207 | 218 | 35.86 |
| Elp1 | Elp1 | 220 | 198 | 32.12 |
| Elp3 | Elp3 | 401 | 14 | 32.09 |
| Elp4 | Elp4 | 186 | 218 | 31.78 |

| Protein 1 | Protein 2 | Residue 1 | Residue 2 | Id-Score |
|-----------|-----------|-----------|-----------|----------|
| Elp4 | Elp4 | 169 | 177 | 34.62 |
| Elp1 | Elp1 | 1217 | 756 | 35.06 |
| Elp5 | Elp5 | 140 | 140 | 30.76 |
| Elp3 | Elp3 | 401 | 19 | 32.4 |
| Elp4 | Elp4 | 186 | 213 | 35.42 |
| Elp1 | Elp1 | 1119 | 1232 | 33.34 |
| Elp4 | Elp4 | 169 | 218 | 31.77 |
| Elp6 | Elp6 | 230 | 269 | 36.69 |
| Elp2 | Elp2 | 249 | 260 | 33.98 |
| Elp2 | Elp2 | 25 | 28 | 35.31 |
| Elp2 | Elp2 | 699 | 680 | 38.1 |
| Elp1 | Elp1 | 1223 | 1223 | 35.4 |
| Elp4 | Elp4 | 235 | 218 | 32.21 |
| Elp4 | Elp4 | 55 | 5 | 33.05 |
| Elp1 | Elp1 | 1062 | 1217 | 32.55 |
| Elp1 | Elp1 | 1316 | 1223 | 31.6 |
| Elp1 | Elp1 | 1217 | 759 | 31.57 |
| Elp1 | Elp1 | 1223 | 1217 | 36.7 |
| Elp4 | Elp4 | 177 | 213 | 34.82 |
| Elp4 | Elp4 | 169 | 143 | 35.43 |
| Elp2 | Elp3 | 257 | 86 | 34.49 |
| Elp4 | Elp4 | 55 | 218 | 33.58 |
| Elp1 | Elp1 | 1217 | 1232 | 32.96 |
| Elp1 | Elp1 | 1052 | 1223 | 32.4 |
| Elp1 | Elp1 | 1052 | 1217 | 32 |
| Elp3 | Elp3 | 401 | 473 | 35.71 |
| Elp1 | Elp1 | 1270 | 1232 | 30.84 |
| Elp1 | Elp1 | 1223 | 1232 | 30.69 |
| Elp1 | Elp1 | 510 | 1223 | 35.14 |
| Elp1 | Elp1 | 1223 | 759 | 38.53 |
| Elp1 | Elp1 | 1308 | 1232 | 34.04 |
| Elp1 | Elp4 | 1246 | 370 | 33.92 |
| Elp3 | Elp1 | 325 | 1217 | 33.13 |
| Elp2 | Elp3 | 249 | 79 | 31.81 |
| Elp6 | Elp4 | 230 | 399 | 30.89 |

Ld-score – linear discriminant crosslink confidence score as calculated by xQuest