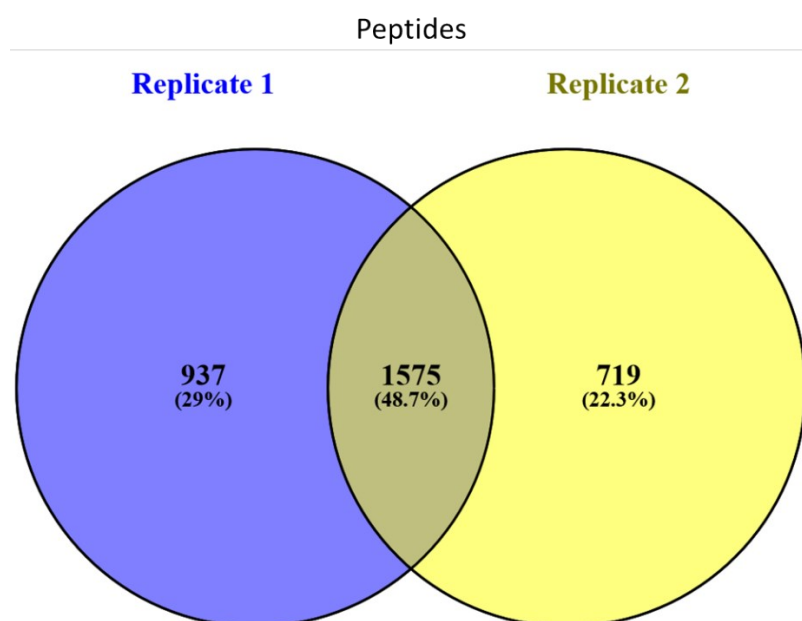


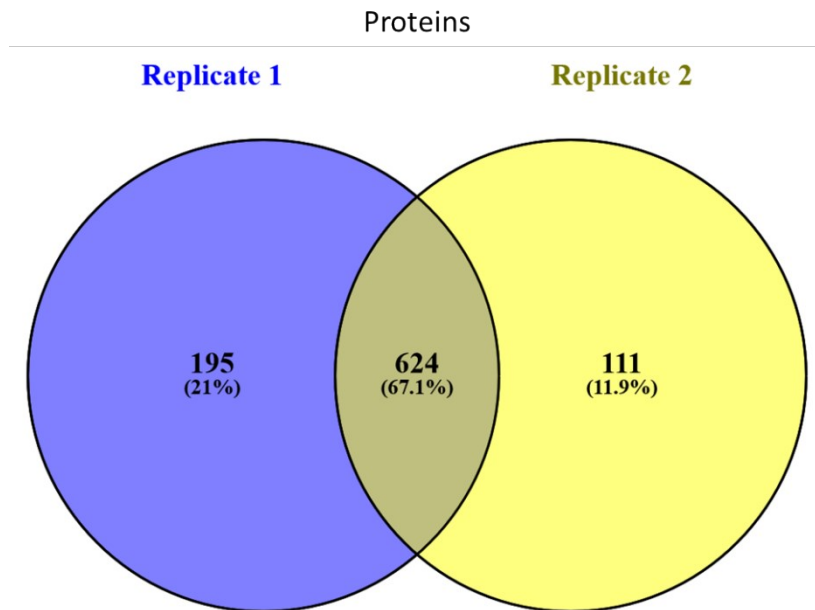
SUPPLEMENTARY INFORMATION

Supplementary material 1. Complete information on HeLa lysates crosslinked with amide BDP-NHP. Table comprises the crosslinking information for the whole dataset, replicates 1 and 2 separately, and specifically for RNA-binding proteins. pep A,B: cross-linked peptides; position A,B: corresponding number of the cross-linked residue in the protein sequence; accession A,B: protein accession number; Uniprot A,B: protein entry from Uniprot; modpos A,B: position of the cross-linked peptide in the protein sequence; PDB A,B: PDB structure identifier; PDB type: indicates if the PDB structure was modelled (e.g., Phyre, Modeller) or resulted from structural experiments; XL distance: distance in Å based on PDB structures; type: type of cross-linking found, if intra or inter-link. Information includes 2 technical replicates. The number of identifications, confidence (*p-value*), scan number of the identification, and ion charge are informed.

(a)

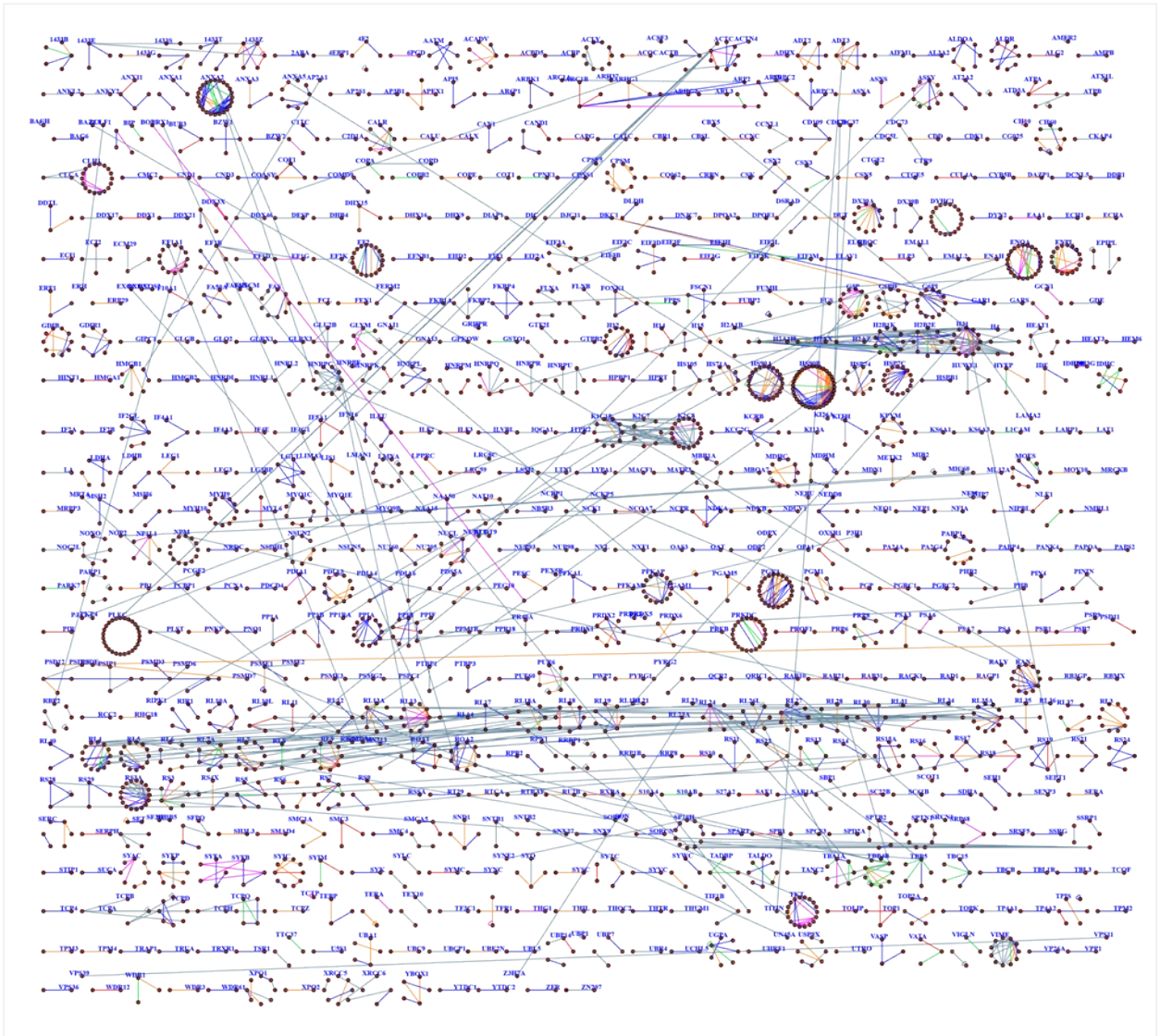


(b)

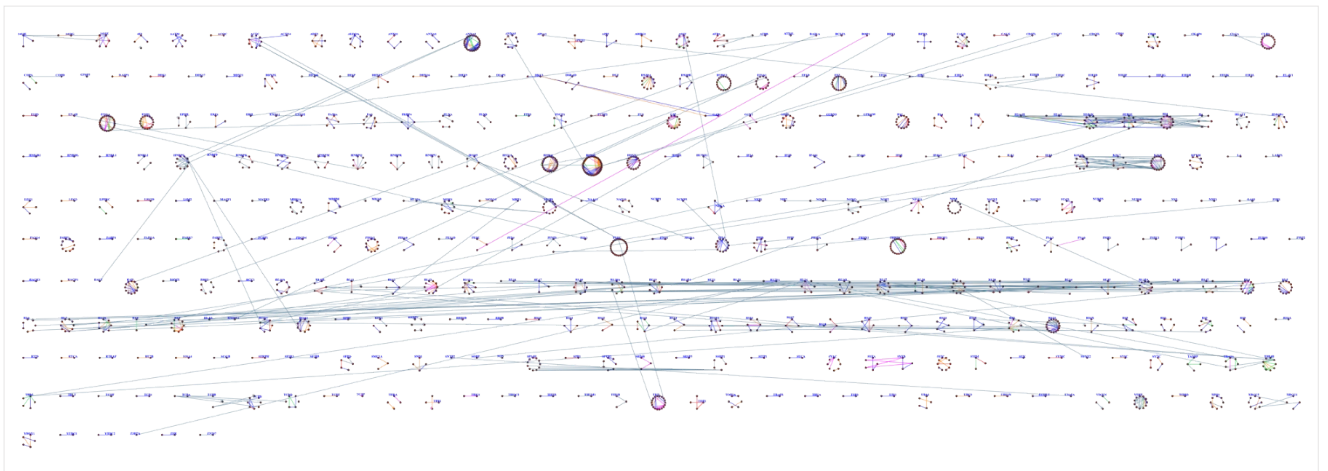


Supplementary material 2. Venn diagram of crosslinked peptides identified with the two replicates. (a) A total of 3,231 crosslinks were identified with 1% FDR before upload to XLinkDB. From those, 937 were identified uniquely in the first replicate, 719 uniquely in the second, and 1,575 were common to both replicates. (b) A total of 930 non-redundant proteins were identified. From those, 195 were unique to replicate 1, 111 to replicate 2, and 624 proteins were common to the two replicates. A total of 2,207 non-redundant crosslinks were uploaded to XLinkDB with estimated 1% FDR and probability>0.5, corresponding to 795 protein pairs (Supplementary material 1). XLinkDB applies statistical filters that could lead to a lower number of crosslinks uploaded to the database⁷⁷.

(a)

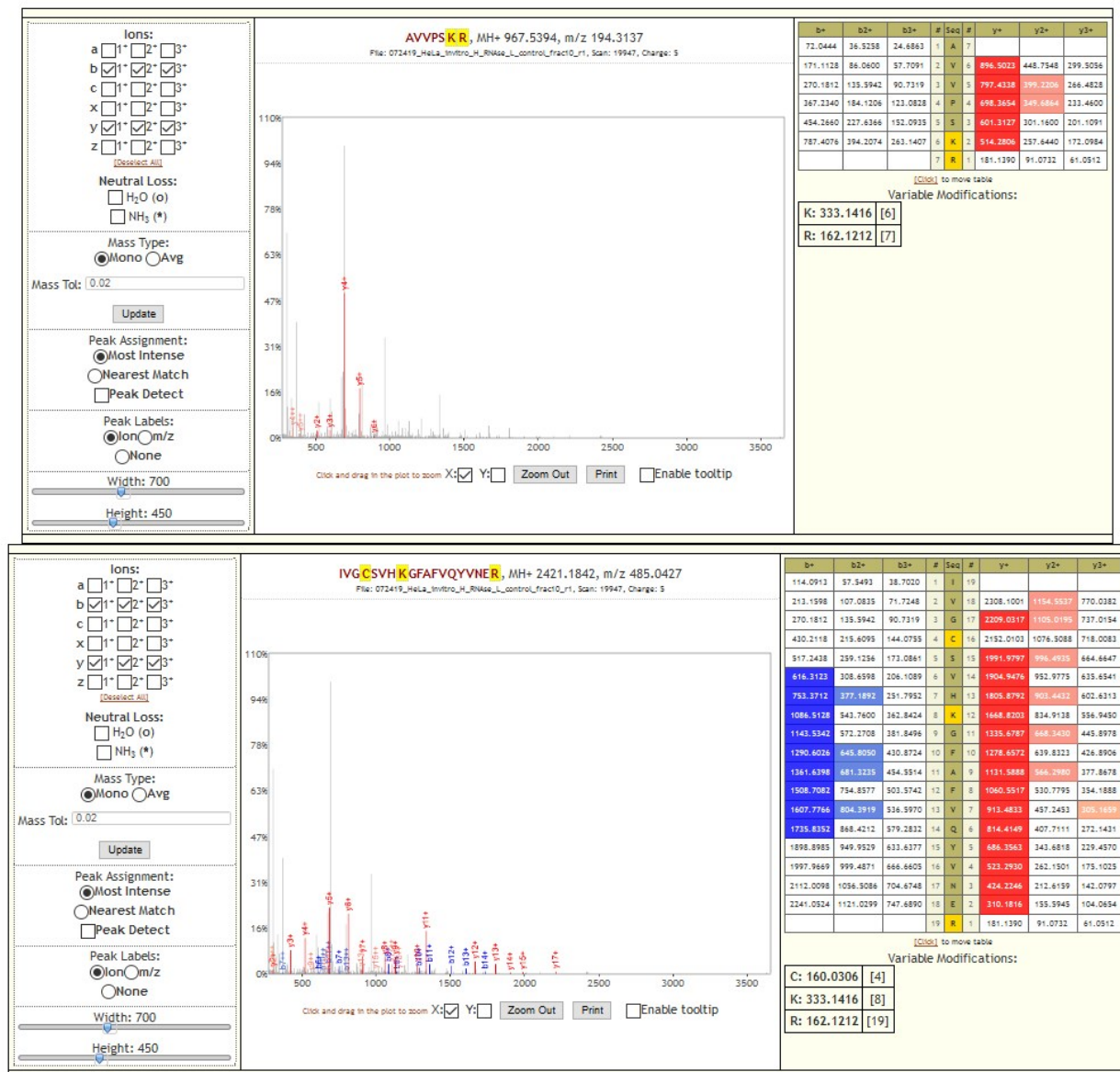


(b)

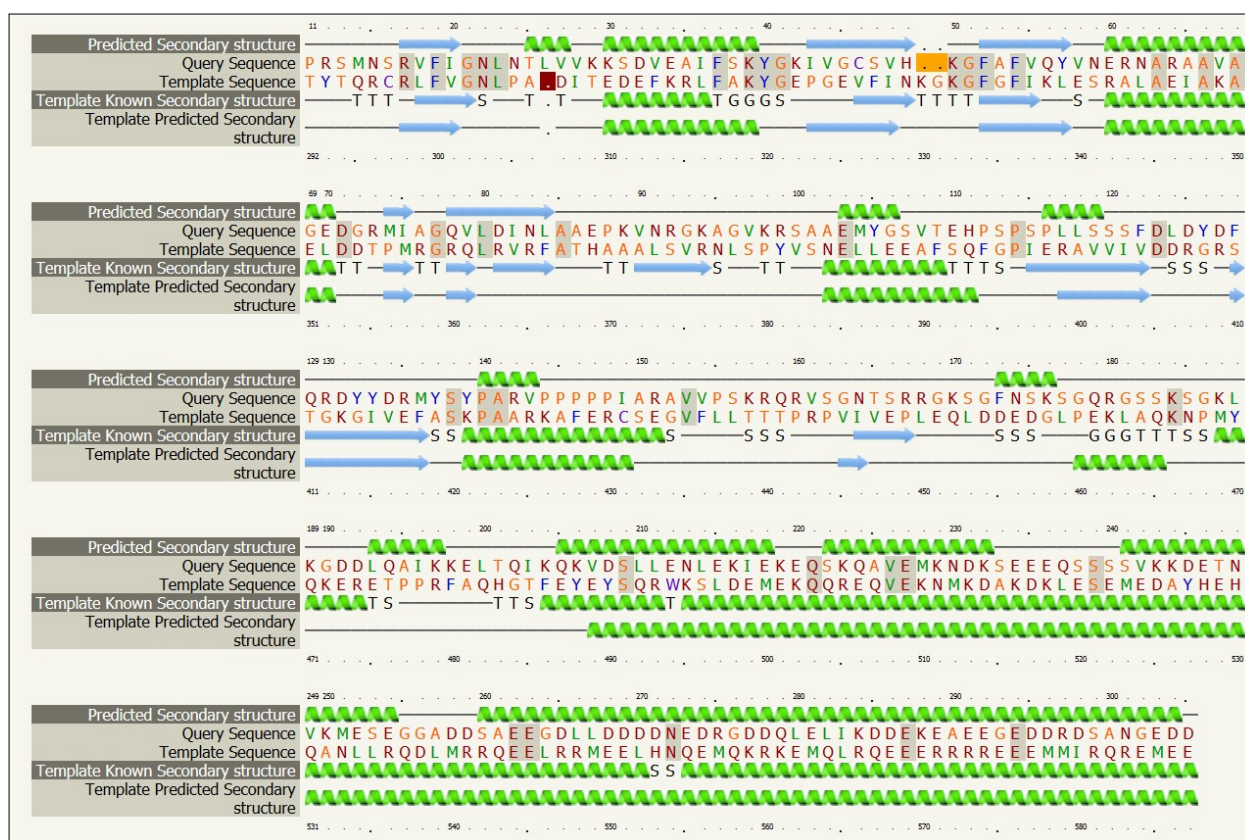


Supplementary material 3. Crosslinking interaction network. (a) Crosslinking interaction network of the whole dataset and (b) for crosslinks happening in RNA-binding proteins.

XLinkDB uses Cytoscape⁷⁸ to generate protein interaction networks for visualization and analysis of protein interactors. Distance in PDB Context (Å): in red, distances under 10, in blue, distances from 10 to 20, in orange, distances from 20 to 30, in green, 30 to 40, and in magenta, distances of 40 or greater.



Supplementary material 4. Tandem mass spectra of crosslinked peptides. MS2 spectra view of peptides AVVPSK¹⁵⁷R and IVGCSVHK⁵⁰GFAFVQYVNER of hnRNPC, with b and y fragment ions of crosslinked peptides shown on the right panel, in blue and red, respectively. XLinkDB uses Lorikeet (<https://uwpr.github.io/Lorikeet/>) to generate the spectrum view and integrates it to the interaction table⁷⁷.



Supplementary material 5. Phyre2 alignment of hnRNPC and SFPQ protein sequences.
 hnRNPC: query sequence; SFPQ: template sequence.

Supplementary material 6. Crosslinking information on EnigmRBPs. pep A,B: cross-linked peptides; startPosA,B: corresponding number of the cross-linked residue in the protein sequence; accession A,B: protein accession number; Uniprot A,B: protein entry from Uniprot; modpos A,B: position of the cross-linked peptide in the protein sequence; PDB A,B: PDB structure identifier; PDB type: indicates if the PDB structure was modelled (e.g., Phyre, Modeller) or resulted from structural experiments; XL distance: distance in Å based on PDB structures; type: type of cross-linking found, if intra or inter-link. Information includes 2 technical replicates. The number of identifications, confidence (*p-value*), scan number of the identification, ion charge, and gene ontology analysis are informed.