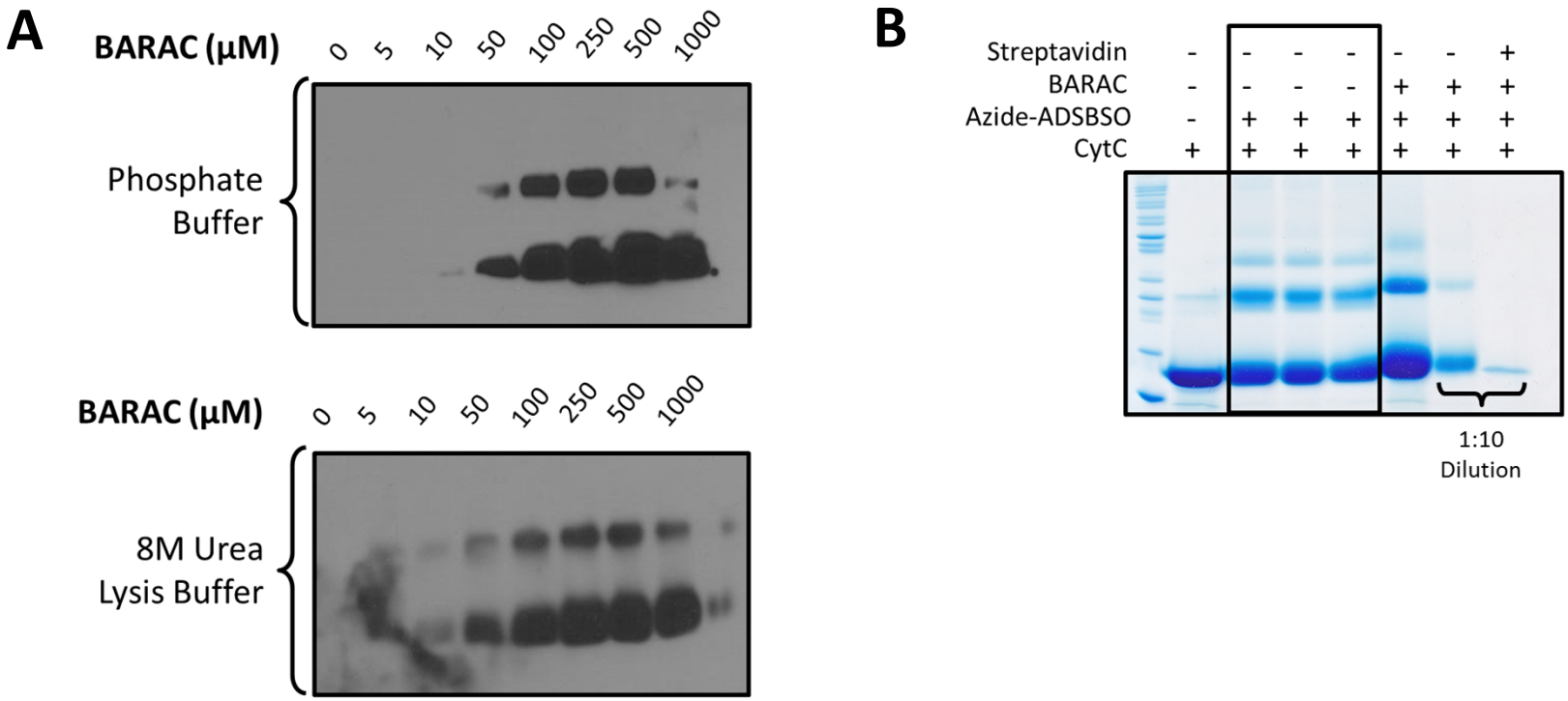
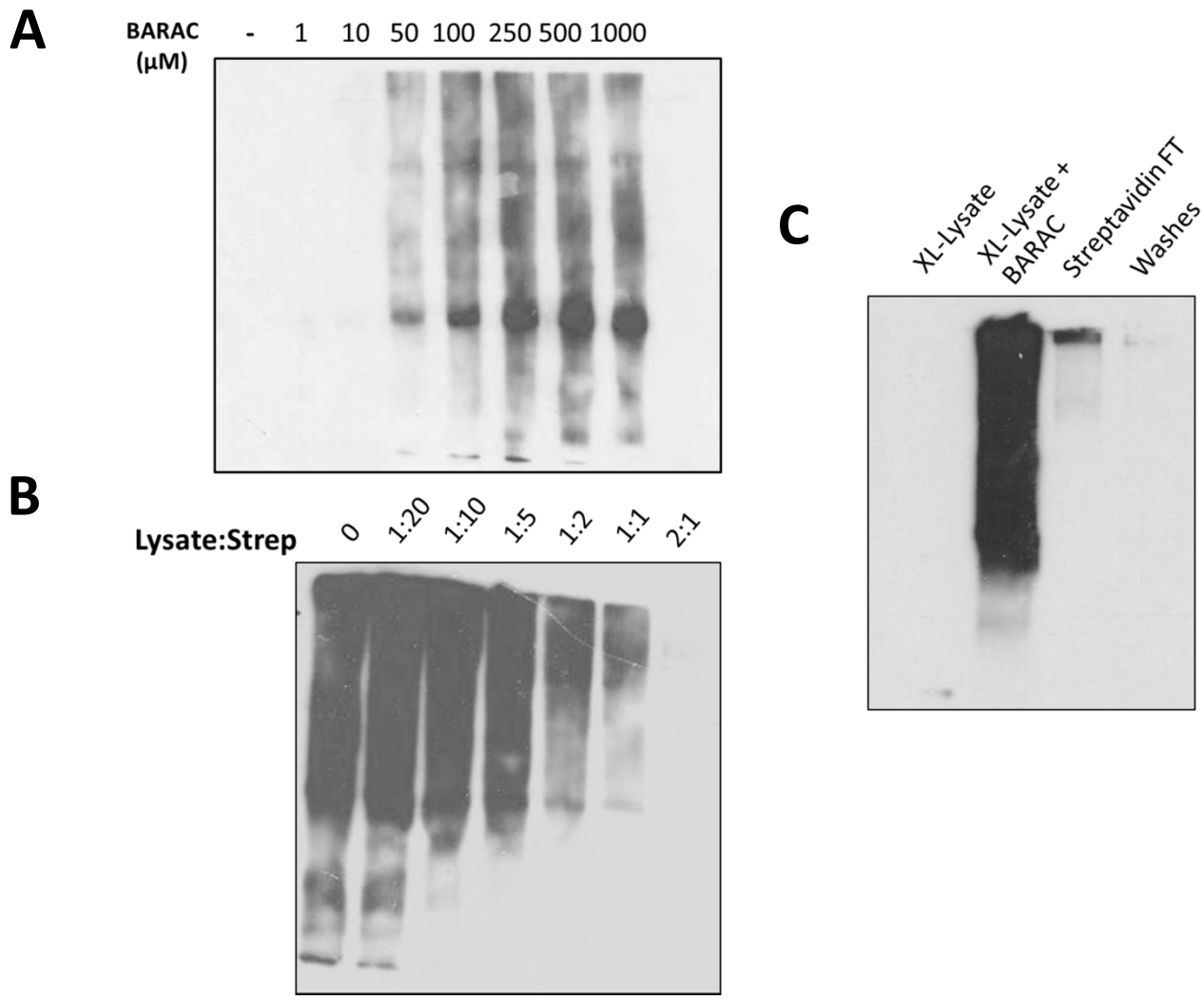
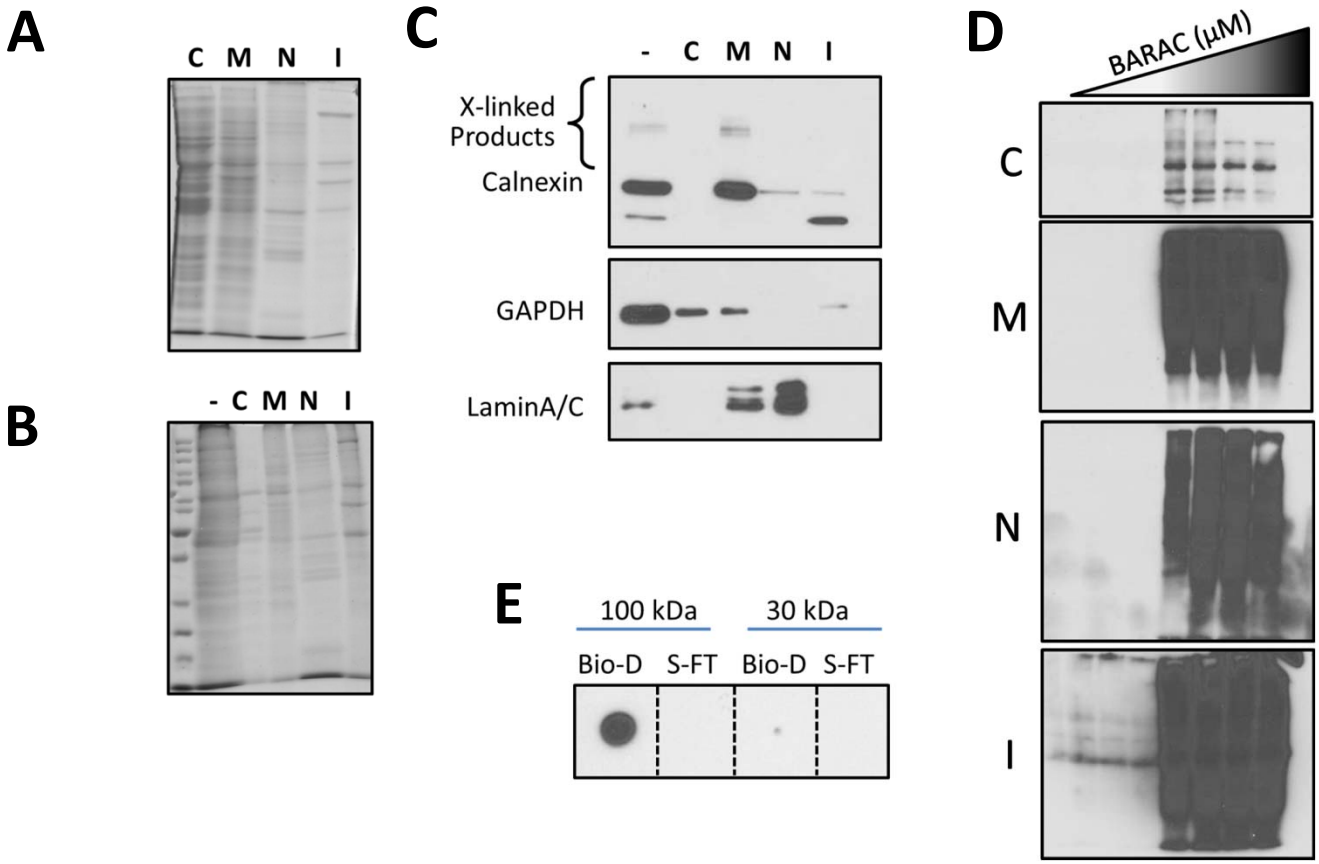


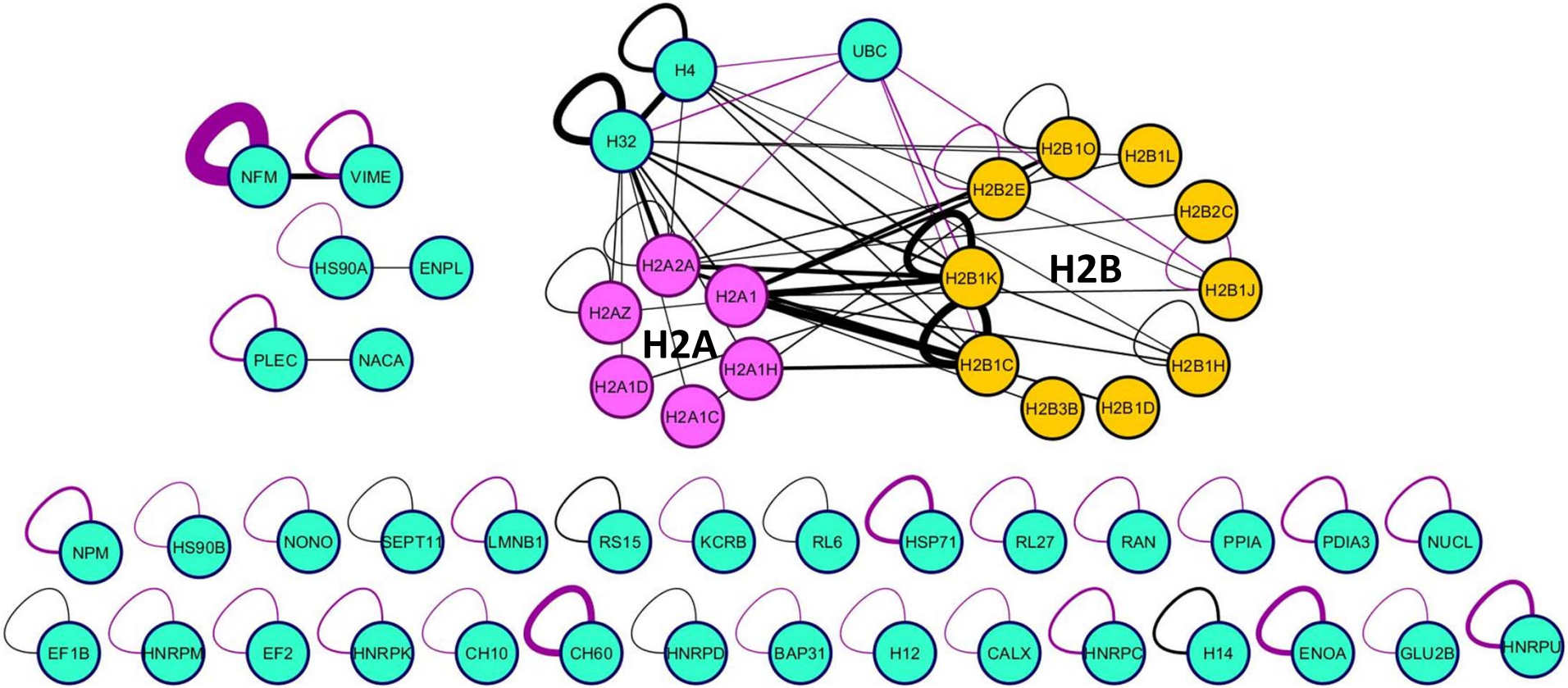
Supplemental Figure 1. Optimization of Biotin-Conjugation and Subsequent Affinity Purification Using Azide-A-DSBSO Cross-linked CytC. A) Western blot analysis of biotin-conjugated products of Azide-A-DSBSO cross-linked CytC after reacting with increasing amounts of BARAC. The effectiveness of biotin conjugation of Azide-A-DSBSO cross-linked cytochrome C and subsequent purification by binding to streptavidin resins was monitored by SDS-PAGE and immunoblotting. Top: reaction in phosphate buffer conditions; bottom: reaction in 8M urea lysis buffer conditions. Western blots are probed with streptavidin-conjugated to HRP. **B)** Assessment of CytC cross-linking, biotin-conjugation, and streptavidin enrichment under optimal conditions by SDS-PAGE analysis and Coomassie blue staining. We tested different membranes capabilities of handling cross-linked samples, the boxed region shows the same sample that was filtered with 3 different filter membranes; last two lanes were loaded at a 1:10 dilution in order to compare equivalent amounts of biotin-conjugated CytC before (original load) and after (flow thru) binding to streptavidin beads.



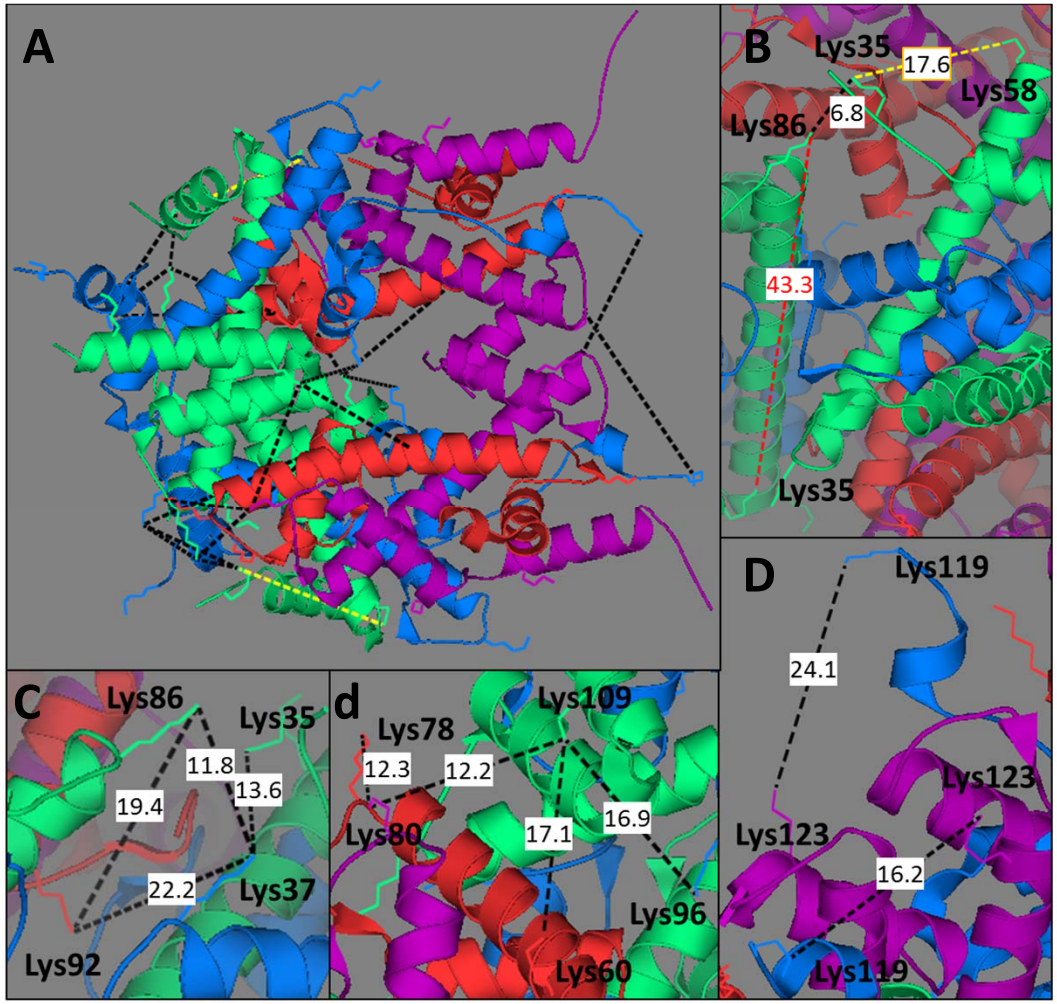
Supplemental Figure 2. Optimization of BARAC Conjugation and Affinity Purification on Lysates of *In Vivo* Azide-A-DSBSO Cross-linked Human 293 Cells. A) Western blot analysis of click chemistry reaction with Azide-A-DSBSO cross-linked 293 cell lysate and increasing amounts of BARAC (1-1000μM). **b)** Western blot analysis of streptavidin binding efficiency using increasing ratios of lysate to streptavidin beads. **c)** Evaluation of biotin-conjugation and streptavidin binding efficiency of cross-linked lysate by immunoblotting. Samples were loaded in the following order: cross-linked lysate, biotin-conjugated cross-linked lysate, flow thru of biotin-conjugated cross-linked lysate after binding to streptavidin, and washes of streptavidin beads. XL = Cross-linked; FT = Flow Thru.



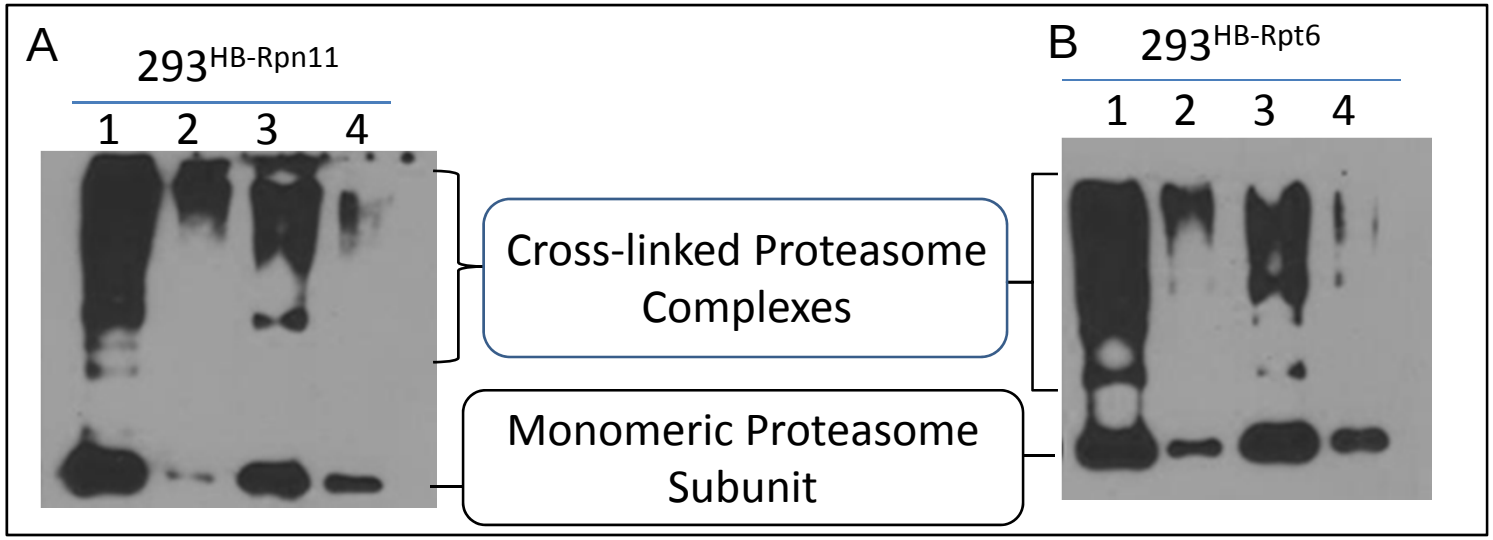
Supplemental Figure 3. Optimization of Biotin-Conjugation and Affinity Purification on Cell Fractionated and Size Fractionated *In Vivo* Azide-A-DSBSO Cross-linked 293 Cell Lysates. **A)** SDS-PAGE analysis of non-cross-linked 293 cell fractions [C=Cytosolic; M=Membrane; N=Nuclear; I=Insoluble]. **B)** SDS-PAGE analysis of Azide-A-DSBSO cross-linked 293 cell lysate and cell fractions. ["-" = No fractionation]. **C)** Western blot analysis of 293 cell lysate and cell fractions. Top: probed with calnexin antibody (membrane protein); Middle: probed with GAPDH antibody (cytosolic protein); Bottom: probed with Lamin A/C antibody (nuclear protein). **D**) 293 cell lysates reacted with increasing amounts of BARAC (0, 1, 10, 50, 100, 250. 500, 1000 μM), separated by SDS-PAGE and analyzed by Western blot probing for Streptavidin-HRP. **E**) Dot blot of 100 and 30 kDa filtered lysates; [Bio-D=biotin-conjugated digest; S-FT=streptavidin flow thru].



Supplemental Figure 4. Azide-A-DSBSO Cross-link Derived Protein-Protein Interaction Network from 293 Cells. Proteins are represented by a single node and interactions between proteins as a single edge. Edge thickness represents the number of inter-links (redundant and unique) captured and identified for a given interactions. Purple edges represent interactions also found in the IDB (Interaction Database) network. Nodes with shared/homologous peptides are grouped (H2A and H2B).



Supplemental Figure 5. Identified Intra- and Inter-Subunit Inter-Linked Peptides, Mapped onto 2.5Å Nucleosome Crystal Structure Containing Histone H2A, H2B, H3.2, and H4. **A)** Nucleosome crystal structure downloaded from the Protein Data Bank (PDB); PDB ID=3AV1. Nucleosome complex consists of two copies each of Histone H2A (Blue), H2B (Green), H3.2 (Purple), and H4 (Red). Azide-A-DSBSO intra-subunit (yellow) and inter-subunit (black) inter-linked peptides are represented by dashed lines. **B-E)** Zoomed in views of identified *intra-subunit* inter-links between **B)** H2BLys35-Lys58 and H2BLys35-Lys86 (black=dimer; red=monomer); and *inter-subunit* inter-links between **C)** H4Lys78-H2BLys86, H4Lys92-H2ALys37, H2ALys37-H2BLys35, and H2ALys37-H2BLys86; **D)** H3.2Lys80-H4Lys78, H3.2Lys80-H2BLys109, H2BLys109-H4Lys60, and H2B109-H2ALys96; **E)** H2ALys119-H3.2Lys123. Visualization and distance measurements (in Å) were done with Pymol.



Supplemental Figure 6. Evaluation of HB-tag Based Tandem Affinity Purification of *In Vivo* Azide-A-DSBSO Cross-linked Proteasome Complexes from A) $293^{HB\text{-}Rpn11}$ and B) $293^{HB\text{-}Rpt6}$ cells. Lanes: 1- cell lysate; 2-Flow through after the first step purification by binding to Ni-sepharose beads; 3-Elution from Ni-sepharose resins; 4-Flow through after second step purification by binding to streptavidin beads. Streptavidin-HRP was used to probe HB tagged proteasome subunits.