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# Proximity proteomics of C9orf72 dipeptide repeat proteins identifies molecular chaperones as modifiers of poly-GA aggregation

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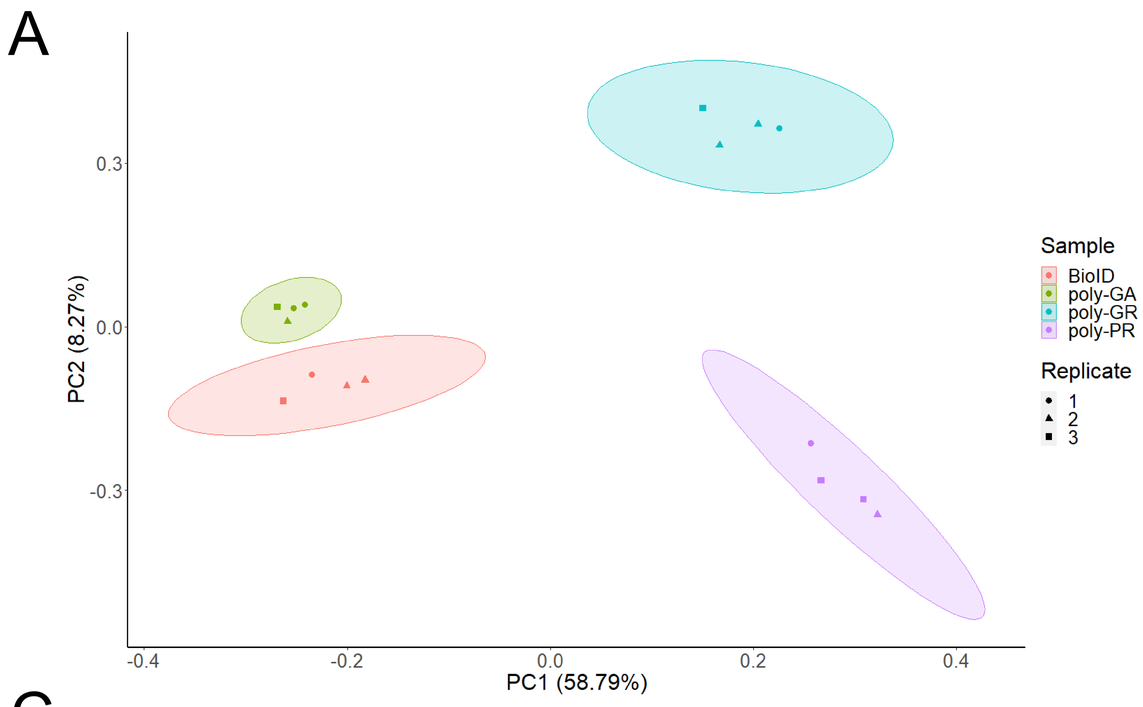
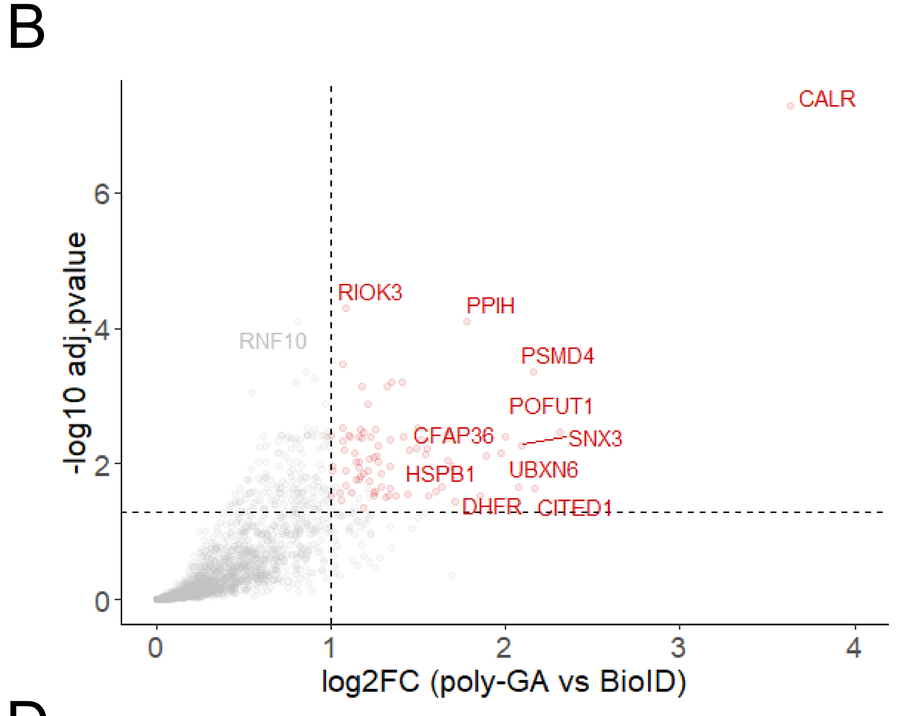
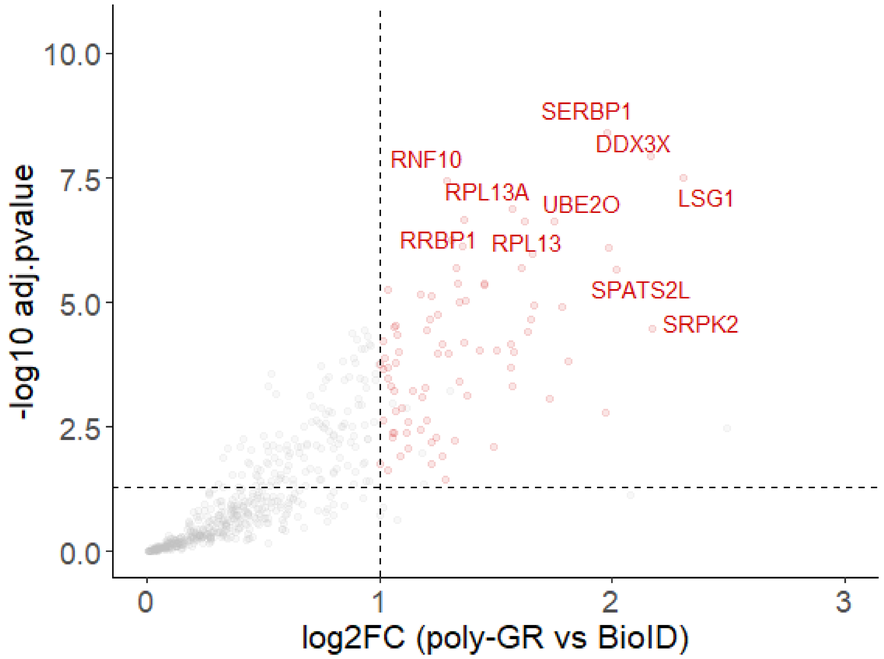
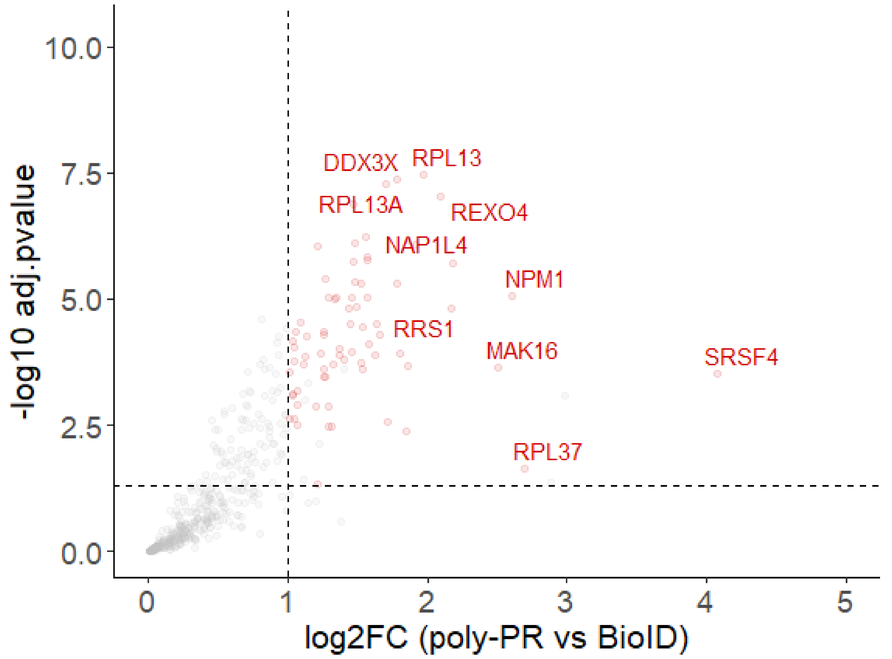
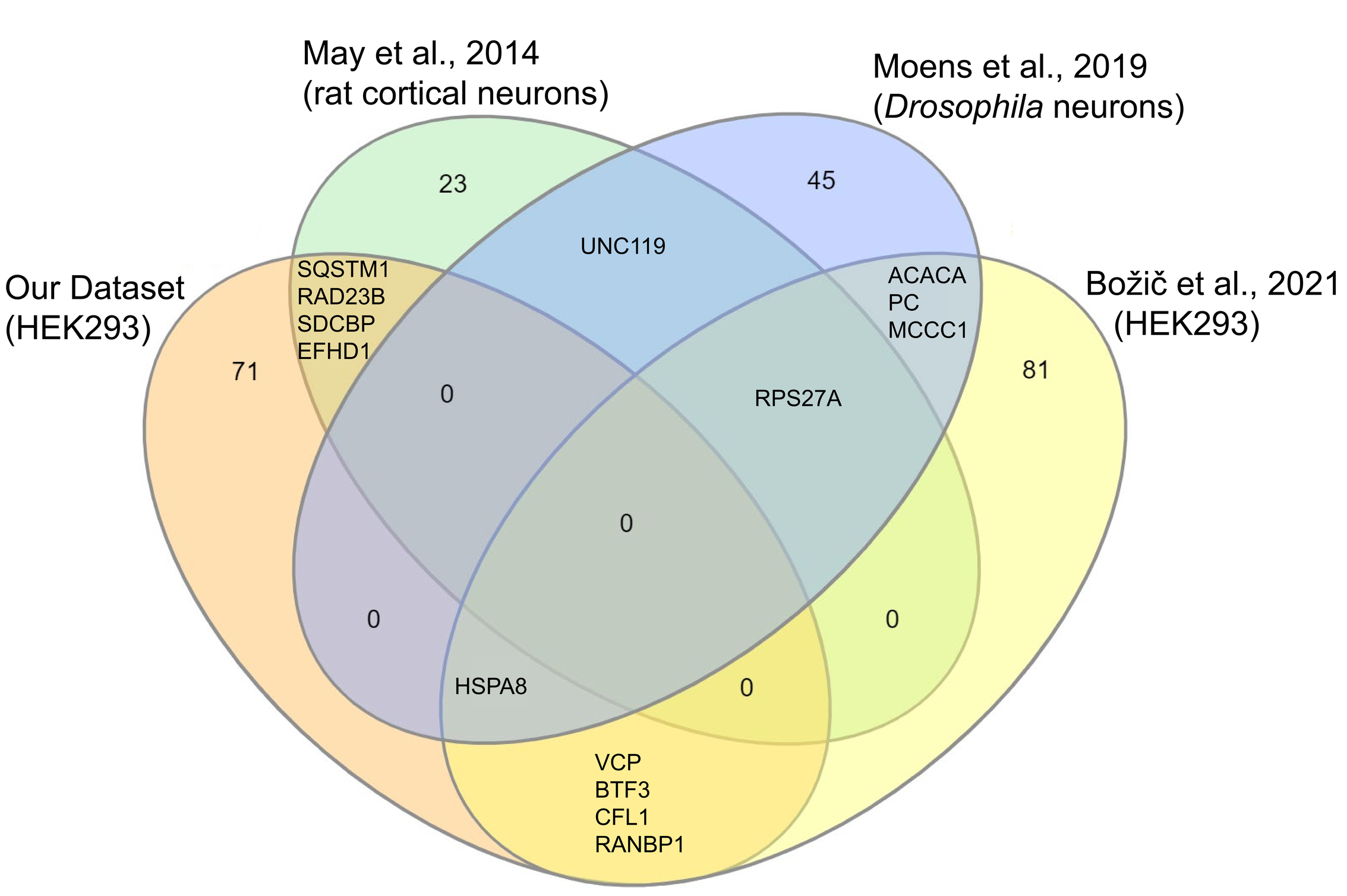


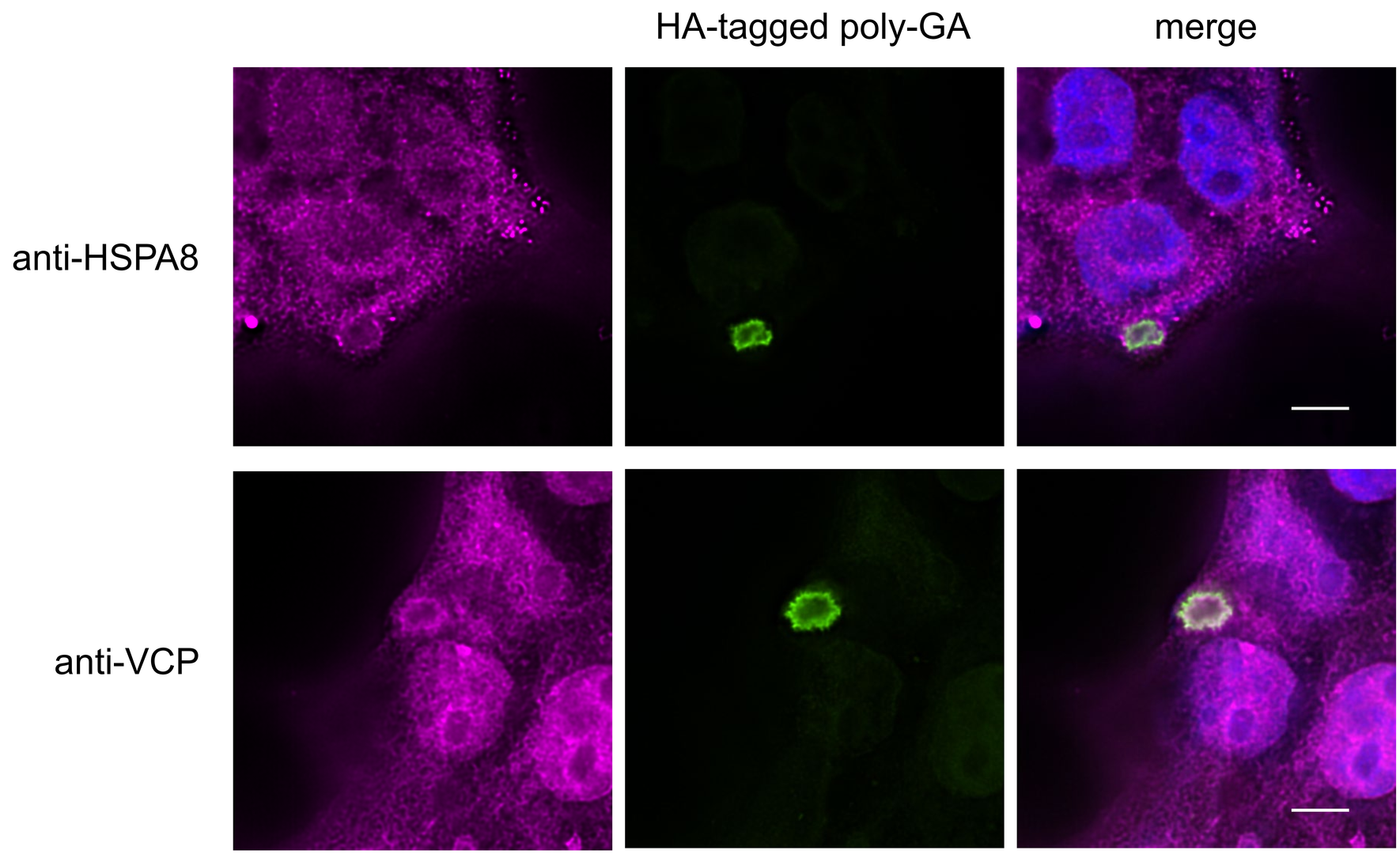



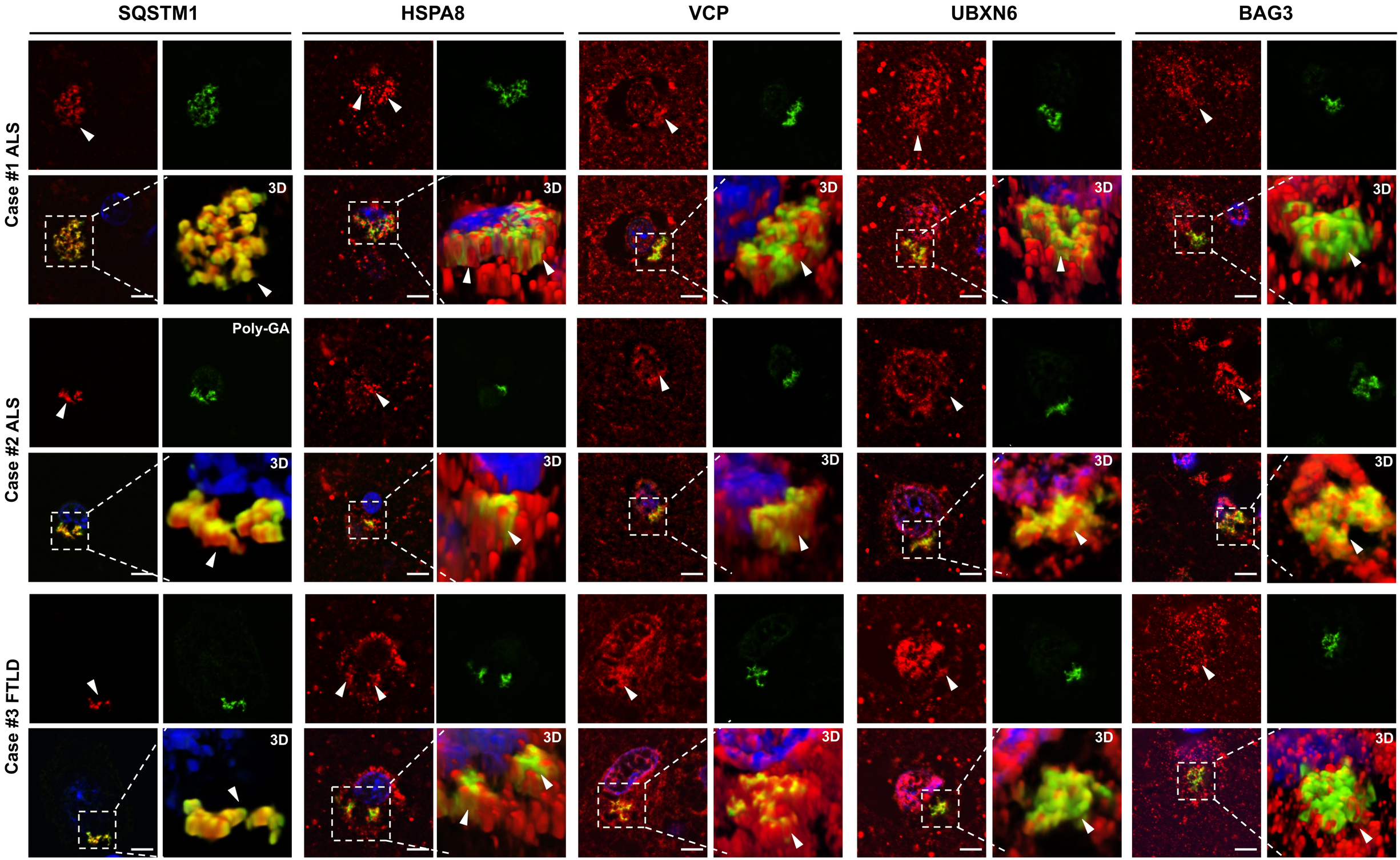

**Supplementary Fig. 1. A)** Principal component analysis plot for interactomes of samples used in this study (n=4); **B-D**) Volcano plots for the comparisons of protein abundances in poly-GA (**B**), poly-GR (**C**) and poly-PR (**D**) samples with the myc-BioID specificity control samples.



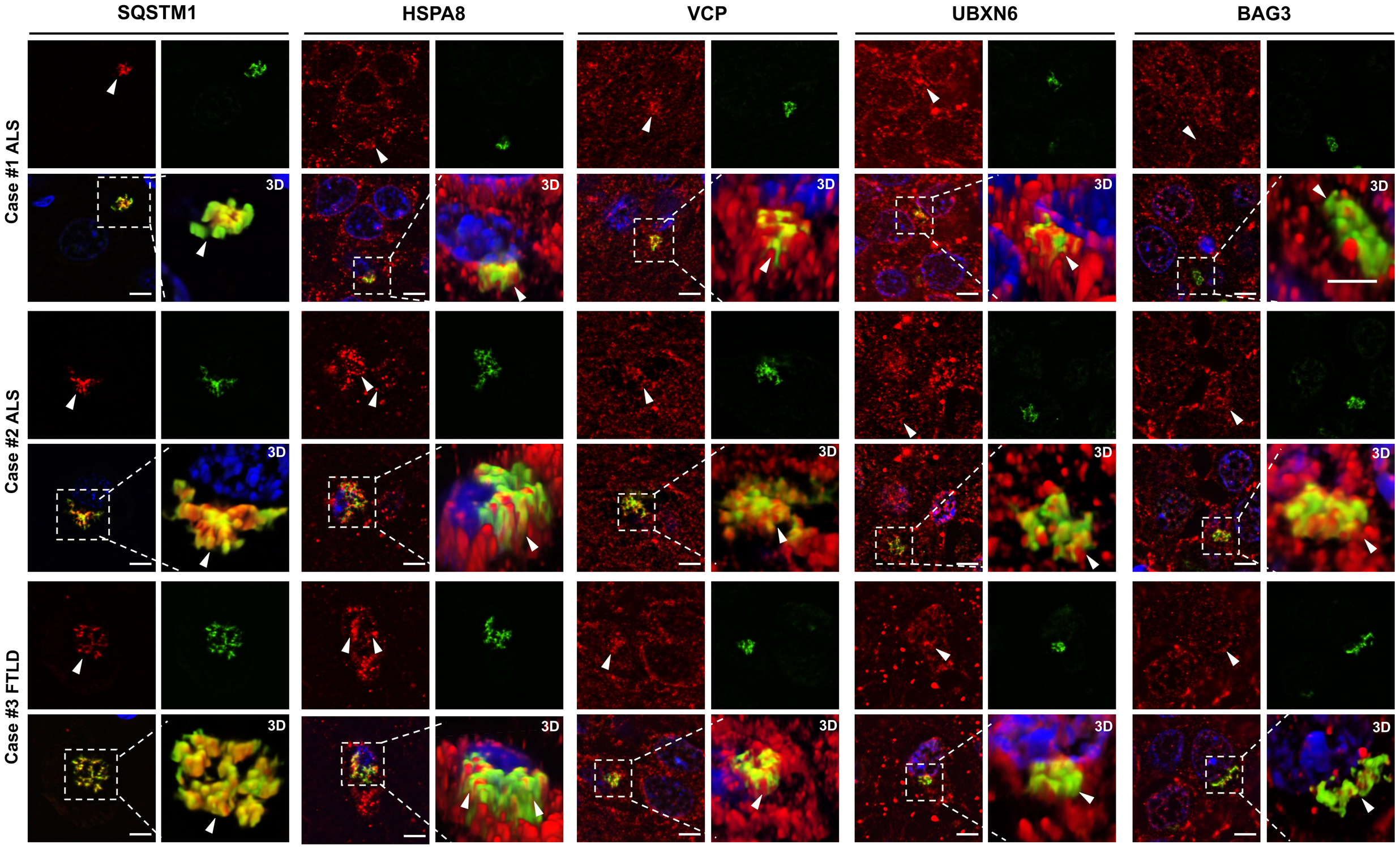

**Supplementary Fig. 2.** Comparison of the poly-GA associated protein dataset from this study with previously published datasets.




**Supplementary Fig. 3. Molecular chaperones are sequestered by cytoplasmic poly-GA aggregates lacking lysine residues**. Endogenous HSPA8 and VCP proteins are sequestered around HA-tagged poly-GA aggregates. Size bar = 5μm.




**Supplementary Fig. 4. Molecular chaperones and co-chaperone association with insoluble poly-GA DPR aggregates in temporal cortex post-mortem tissue.** Immunofluorescence co-staining for SQSTM1, HSPA8, VCP, UBXD1 and BAG3 (red) with poly-GA (green) and Hoechst (blue) in the temporal cortex from neuropathologically diagnosed ALS and FTLD cases. Each staining is shown as a panel of single optical sections for red, green, and merged channels and an inset of the volume rendered z-stacks (3D). Scale bar: 5 μm. Abbreviations: ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; DPR, dipeptide repeat protein; SQSTM1, sequestosome 1; HSPA8, heat shock protein family A member 8; VCP, valosin-containing protein; UBXN6, UBX-domain containing protein 6; BAG3, Bcl2-associated athanogene 3.




**Supplementary Fig. 5. Molecular chaperones and co-chaperone association with insoluble poly-GA DPR aggregates in hippocampal human post-mortem tissue.** Immunofluorescence co-staining for SQSTM1, HSPA8, VCP, UBXD1 and BAG3 (red) with poly-GA (green) and Hoechst (blue) in the hippocampus from neuropathologically diagnosed ALS and FTLD cases. Each staining is shown as a panel of single optical sections for red, green, and merged channels and an inset of the volume rendered z-stacks (3D). Scale bar: 5 µm. Abbreviations: ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; DPR, dipeptide repeat protein; SQSTM1, sequestosome 1; HSPA8, heat shock protein family A member 8; VCP, valosin-containing protein; UBXN6, UBX-domain containing protein 6; BAG3, Bcl2-associated athanogene 3.