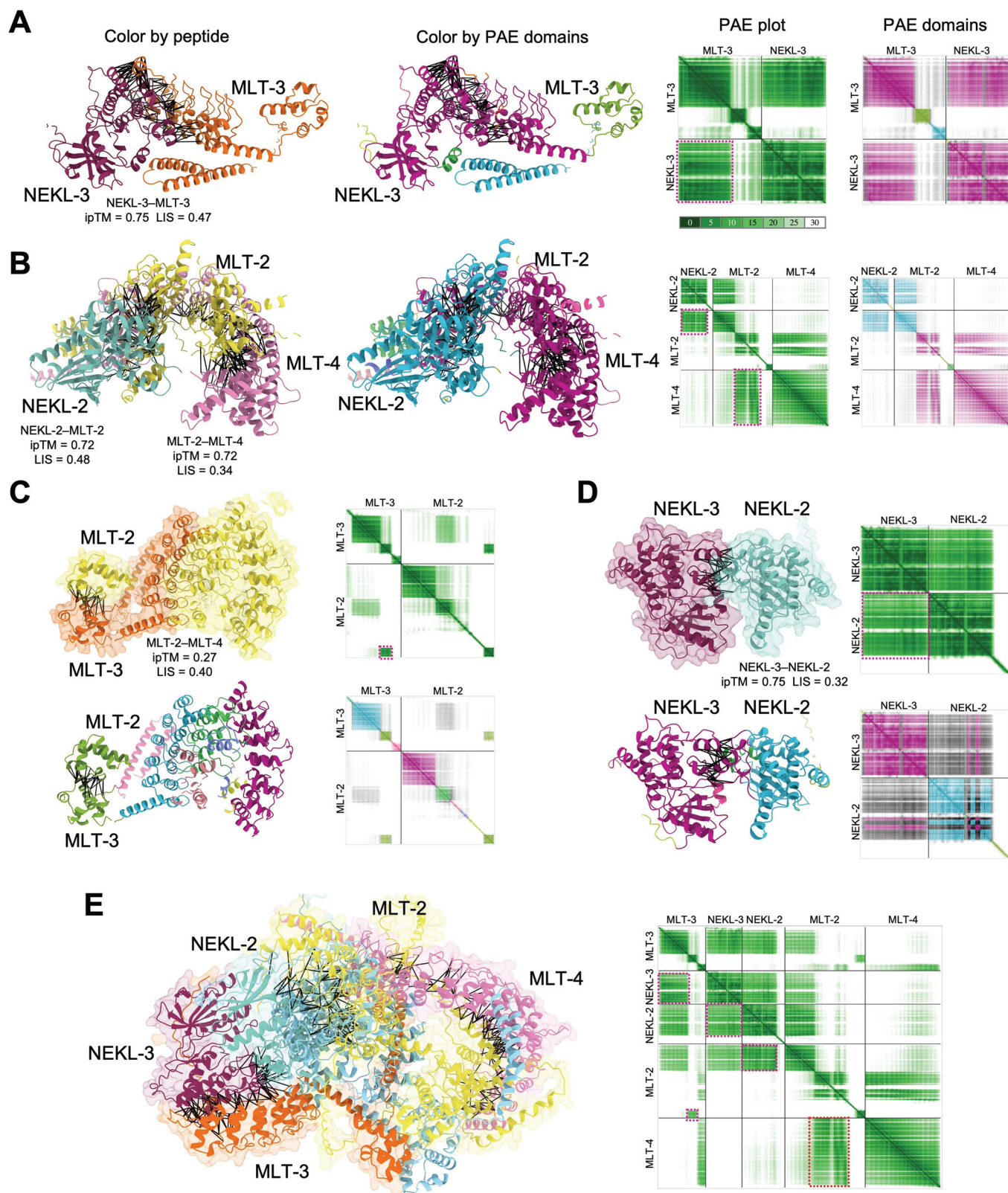
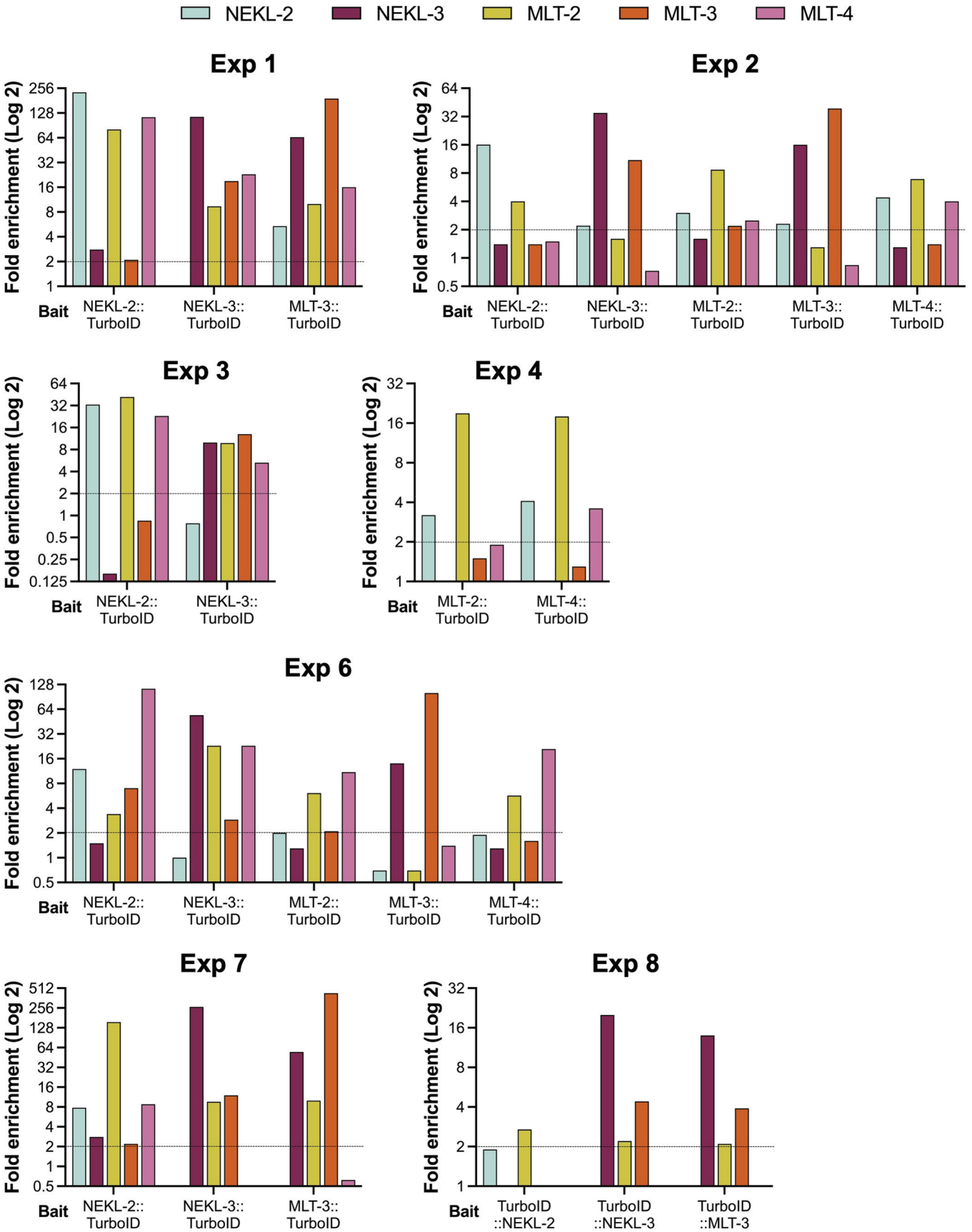


Expanded View Figures

Figure EV1. AlphaFold modeling of NEKL-MLT interactions.

(A-E) AlphaFold3 modeling and ChimeraX rendering of predicted (A) NEKL-3-MLT-3, (B) MLT-2-MLT-4-NEKL-2, (C) MLT-2-MLT-3, (D) NEKL-2-NEKL-3, and (E) MLT-3-NEKL-3-NEKL-2-MLT-2-MLT-4 protein complexes. Black lines between proteins indicate high-confidence Predicted Aligned Error (PAE) pseudobonds ($\text{PAE} \leq 6 \text{ \AA}$; interatomic-distance $\leq 5 \text{ \AA}$). For clarity, highly unstructured portions of the peptides ($\text{B-factor} \leq 30$) were hidden. (A-E) Show protein-backbone ribbon diagrams (colored by peptide) along with single-color (green) PAE diagrams indicating regions of high structural confidence (low PAE) within and between the indicated proteins. Dashed-line cyan boxes correspond to regions where the two proteins are predicted for form a coherent structural unit. (A-D) Additionally show protein ribbon diagrams colored by PAE domain along with corresponding color-matched PAE diagrams. Ribbon diagrams in (C-E) also show partially transparent surface renderings. Inter-chain predicted Template Modeling (ipTM) values, and Local Interaction Scores (LIS) are shown for the indicated proteins modeled as heterodimers by AlphaFold3. Note that the five-protein complex (E) predicts very similar protein-protein interfaces as the subcomplex models, consistent with the hypothesis that the two subcomplexes can physically interact.





**Figure EV2. Fold changes of NEKL-MLT bait and prey proteins.**

Bar graphs of fold changes shown in Fig. 2B and Datasets EV1-8 for Exp 1-4 and 6-8.

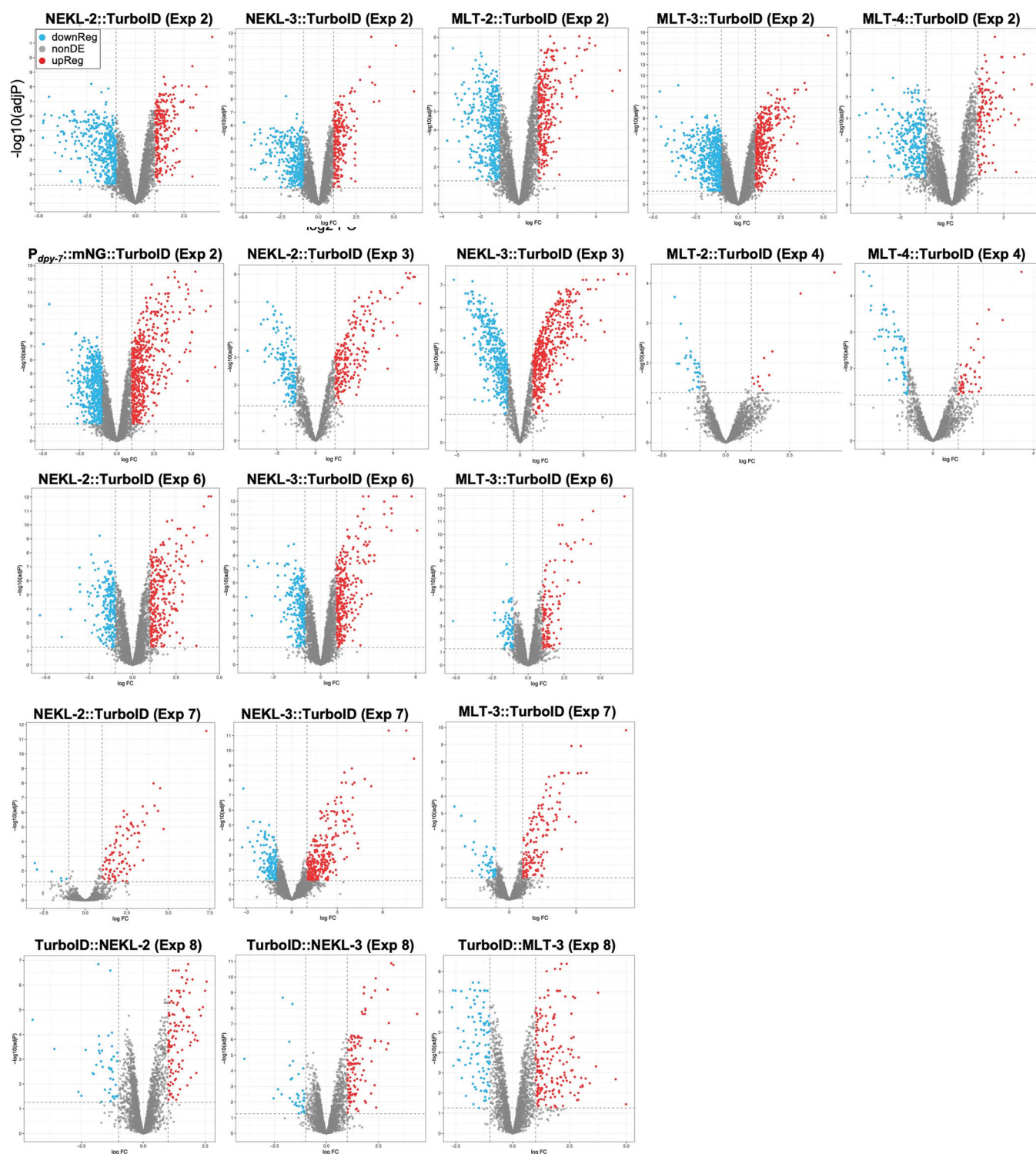


Figure EV3. Volcano plots.

Volcano plots of bait-TurboID strains versus N2 control showing the $\log_2$ fold change (FC) versus $-\log_{10}$ adjusted P value. Red and blue dots indicate proteins that were up and down, respectively, in the bait strain relative to the N2 strain; select identified bait and prey proteins are indicated by arrows. NonDE, not differentially expressed. Red and blue dots indicate proteins that were up in the bait and N2 strains, respectively. Additional volcano plots are shown in Figs. 3A, 6B, EV5, and EV7. Data corresponding to volcano plots are available in Datasets EV1-8.

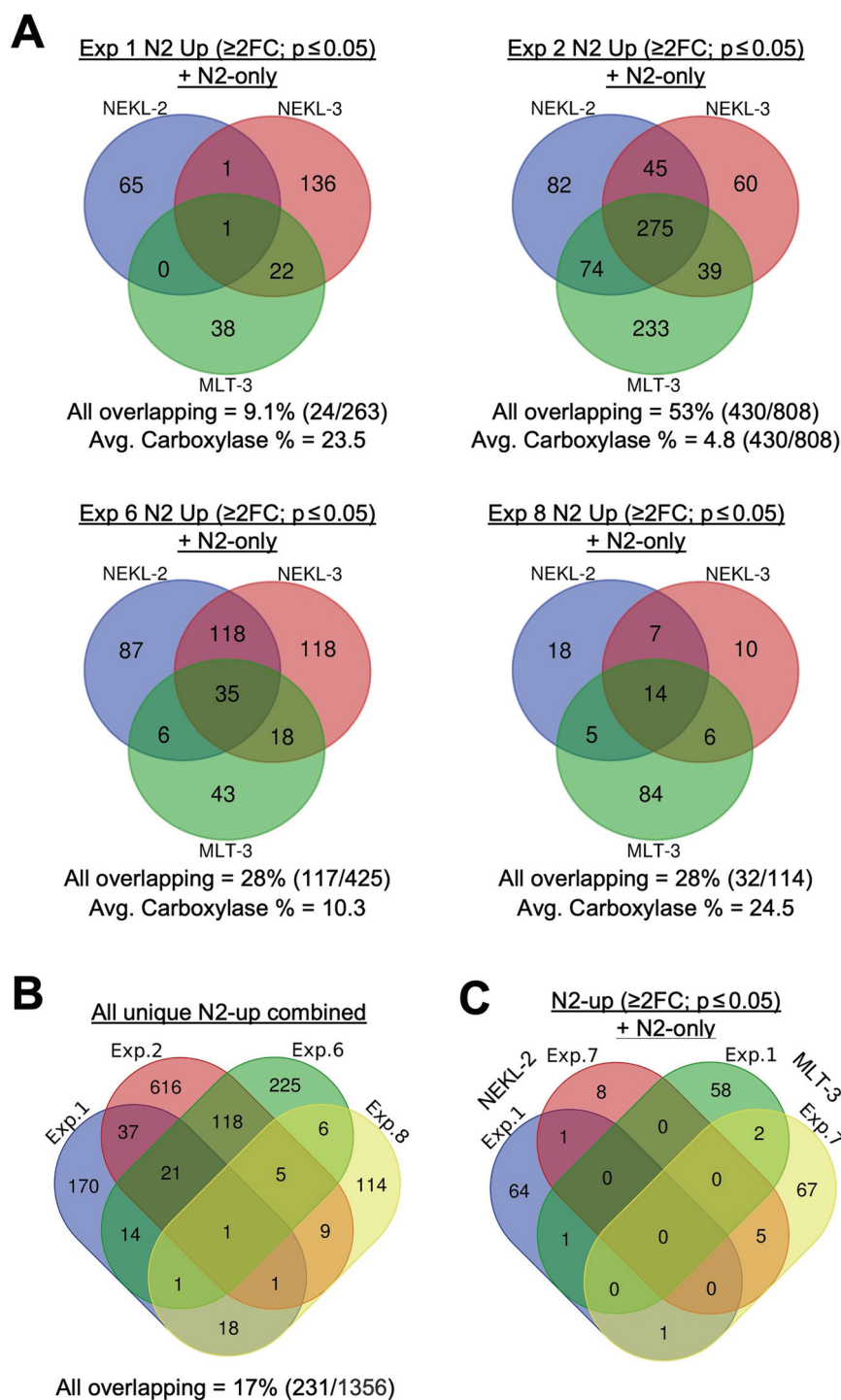


Figure EV4. Analysis of N2-enriched proteins.

(A) Venn diagrams showing overlap of proteins enriched (≥ 2 -fold (FC); adjusted $P \leq 0.05$) in N2 samples (N2 Up) versus NEKL-2::TurboID, NEKL-3::TurboID, and MLT-3::TurboID bait samples from Exp 1, Exp 2, Exp 6, and Exp 8. N2-only indicates proteins that were present in all technical replicates of the N2 sample but were absent in all technical replicates of the corresponding bait-TurboID; Also see Fig. 2B. Note variability in the extent of overlap among experiments. (B) Venn diagram comparing overlaps among the combined N2 Up proteins from Exp 1, Exp 2, Exp 6, and Exp 8. Note the relative lack of consistently overlapping proteins among experiments. (C) Venn diagram showing overlaps of N2-enriched proteins from NEKL-2::TurboID and MLT-3::TurboID (Exp 1 and Exp 7). Little overlap was observed between comparable biological replicates of NEKL-2::TurboID and MLT-3::TurboID as well as between the two bait proteins within the same biological replicate.

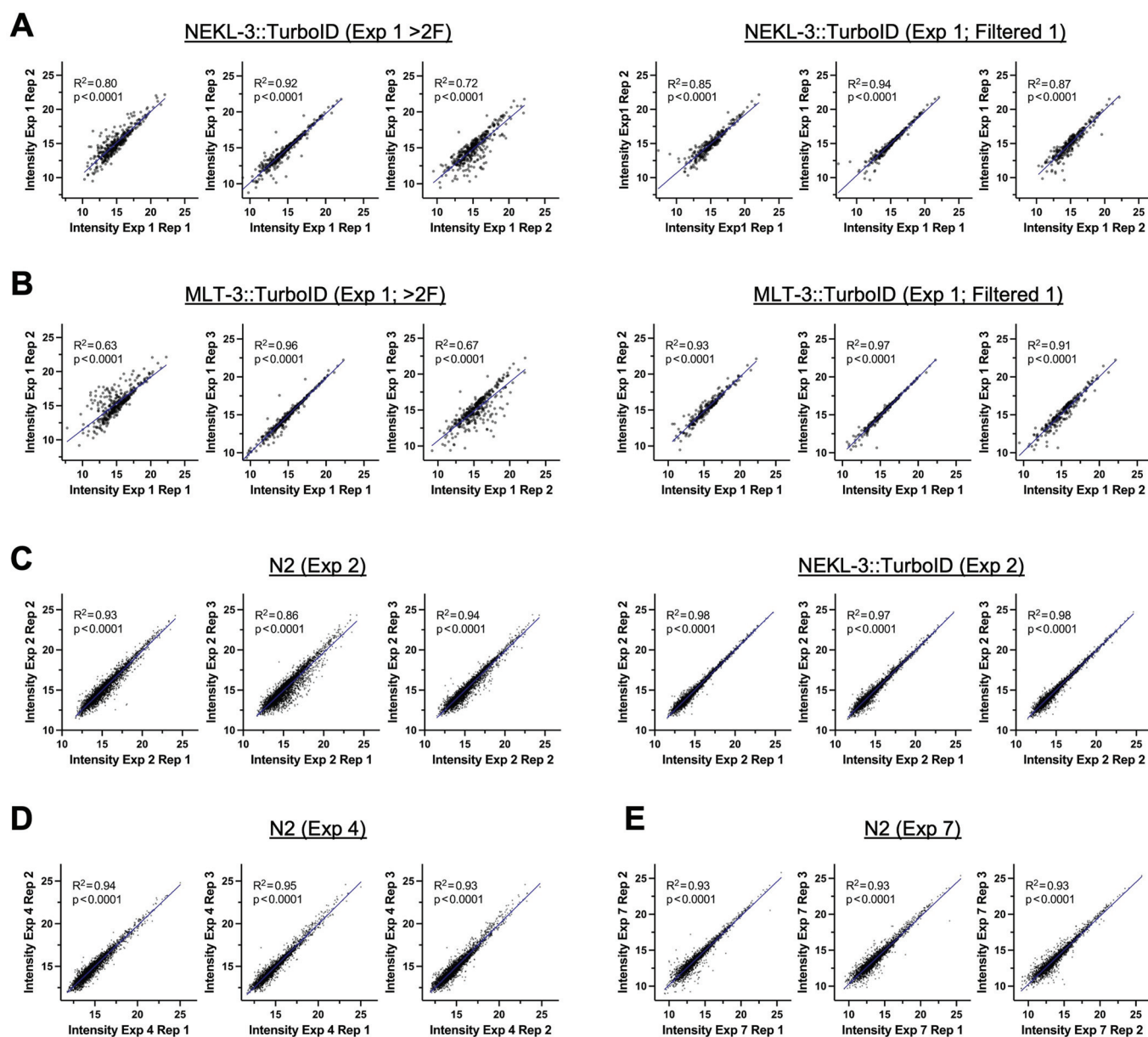


Figure EV5. Additional analysis of technical replicates.

(A–E) Scatter plot diagrams showing correlations of individual protein intensities (abundance) between the indicated replicate pairs. R^2 values were derived using simple linear regression; raw data are available in Dataset EV10. Also see Fig. 5.

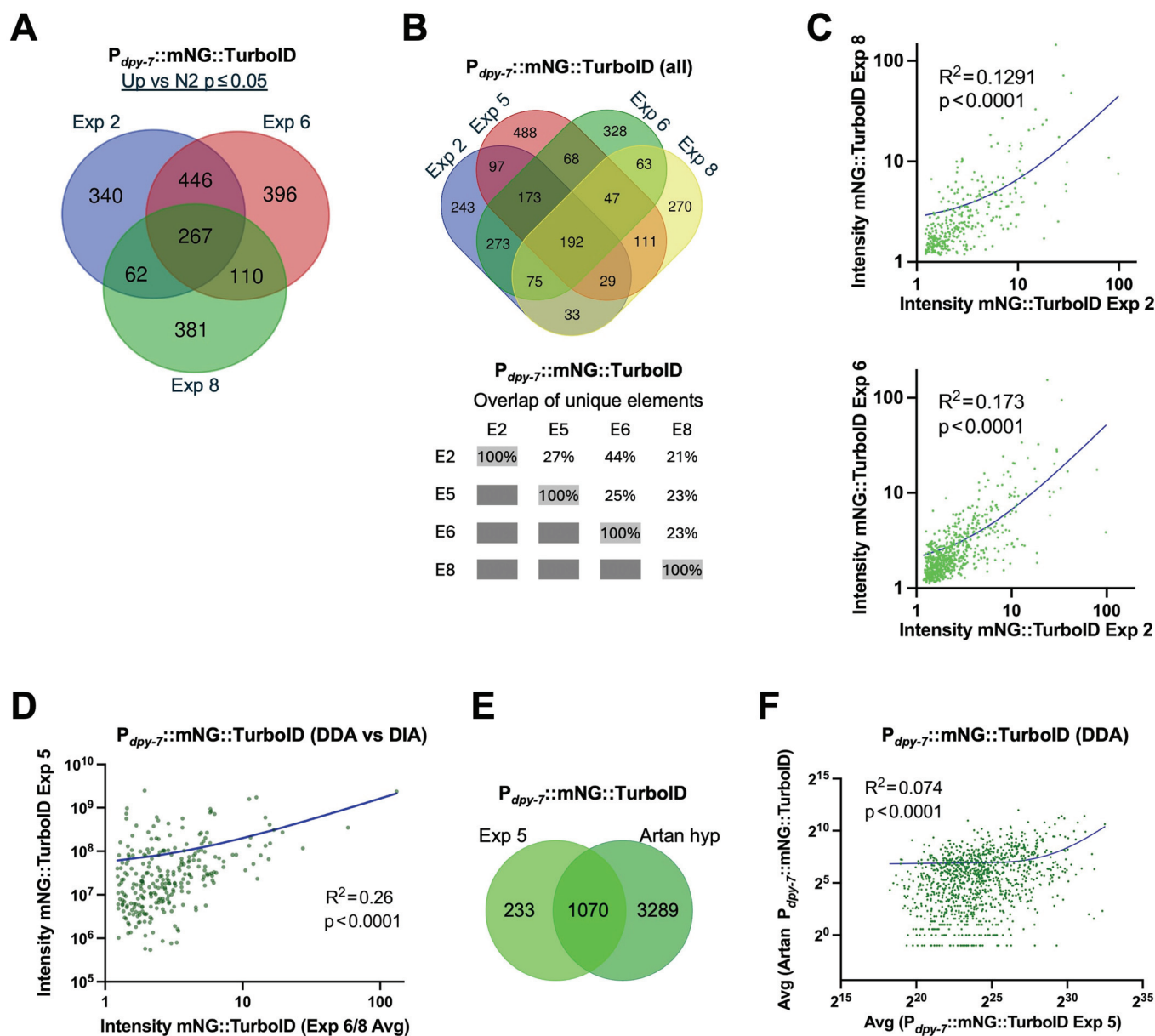


Figure EV6. Additional analysis of $P_{dpy-7::mNG::TurboID}$ datasets.

(A) Venn diagram overlap of proteins significantly enriched (based on adjusted P values) in the three DIA-MS $P_{dpy-7::mNG::TurboID}$ experiments. (B) Venn diagram and quantification of pairwise overlaps for the four $P_{dpy-7::mNG::TurboID}$ experiments. Proteins included were detected in all technical replicates. Note that although Exp 2 and Exp 6 showed the greatest overlap in detected proteins, the correlation in protein levels was strongest between Exp 6 and Exp 8 (also see Fig. 6C). (C) Scatter plots showing correlations of protein intensities (abundance) for the mNG::TurboID control strain from indicated experimental pairs. R^2 values were derived using simple linear regression; raw data are available in Dataset EV11. The curved regression lines are due to the conversion of axes to a log scale. The markedly lower R^2 values, relative to Exp 6 vs Exp 8, are due in large part to discrepancies in intensity values at the high end of the scale. (D) Scatter plot showing weak correlation between Exp 5 (DDA-MS) and averaged data from Exp 6 and Exp 8 (DIA-MS). (E) Overlap and (F) correlation between Exp 5 and DDA-MS experiment from Artan and colleagues (PMID: 35665632). Note that although the proteins detected in Exp 5 overlapped extensively with a subset of those previously reported, the correlation in their expression levels was weak.

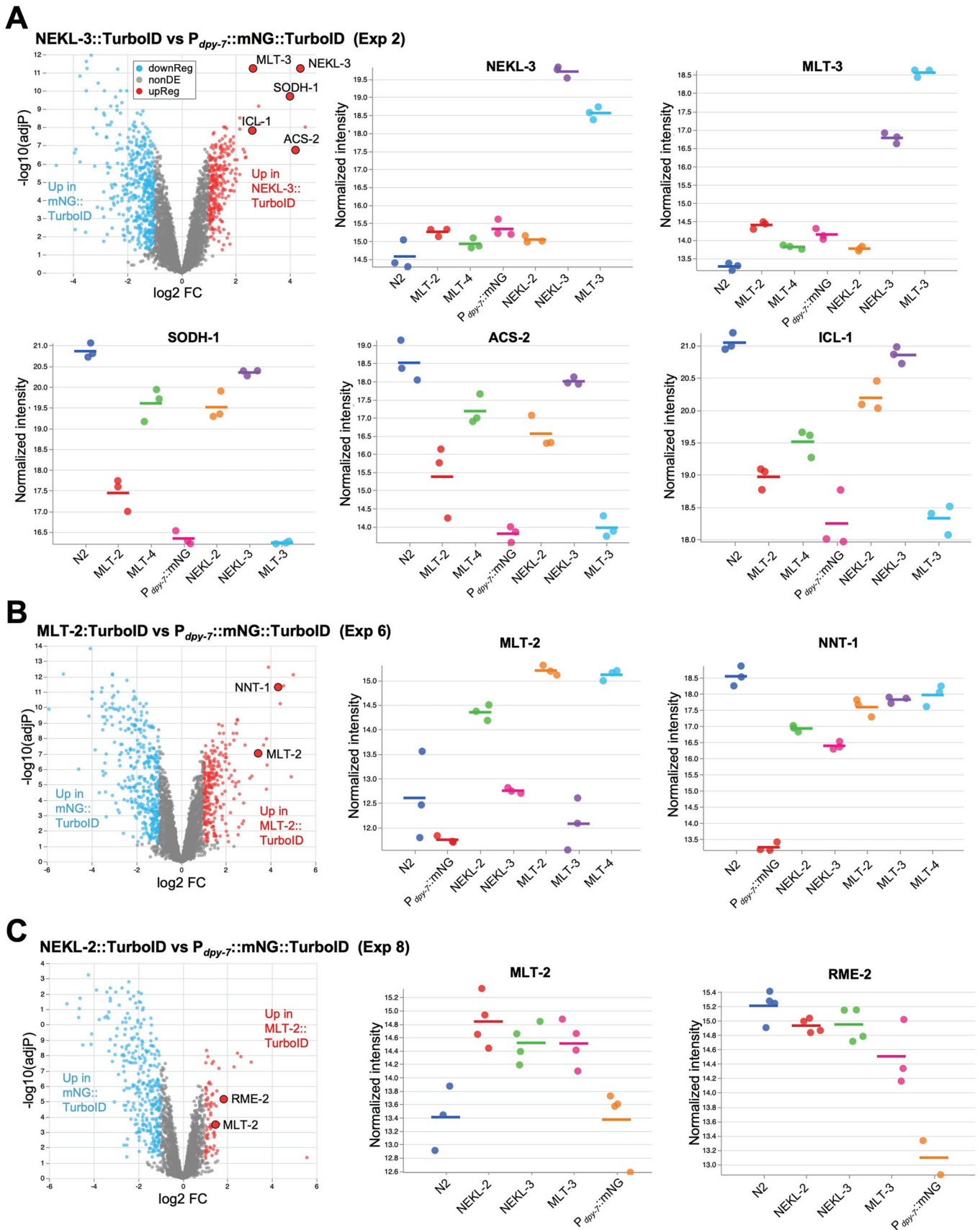


Figure EV7. $P_{dpy-7::mNG::TurboID}$ down proteins.

(A–C) Volcano plots of $P_{dpy-7::mNG::TurboID}$ versus NEKL-3::TurboID (A), MLT-2::TurboID (B), and NEKL-2 TurboID (C) indicating the $\log_2$ fold change (FC) versus $-\log_{10}$ adjusted P values. Enlarged red dots indicate proteins shown in the accompanying intensity plots. Intensity plots ($\log_2$ scale) showing the abundance of the indicated bait and prey proteins; individual dots represent values from the three technical replicates. For SODH-1, ACS-2, ICL-1, NNT-1, and RME-2, levels in $P_{dpy-7::mNG::TurboID}$ were markedly lower than in the N2 or most bait-TurboID strains.

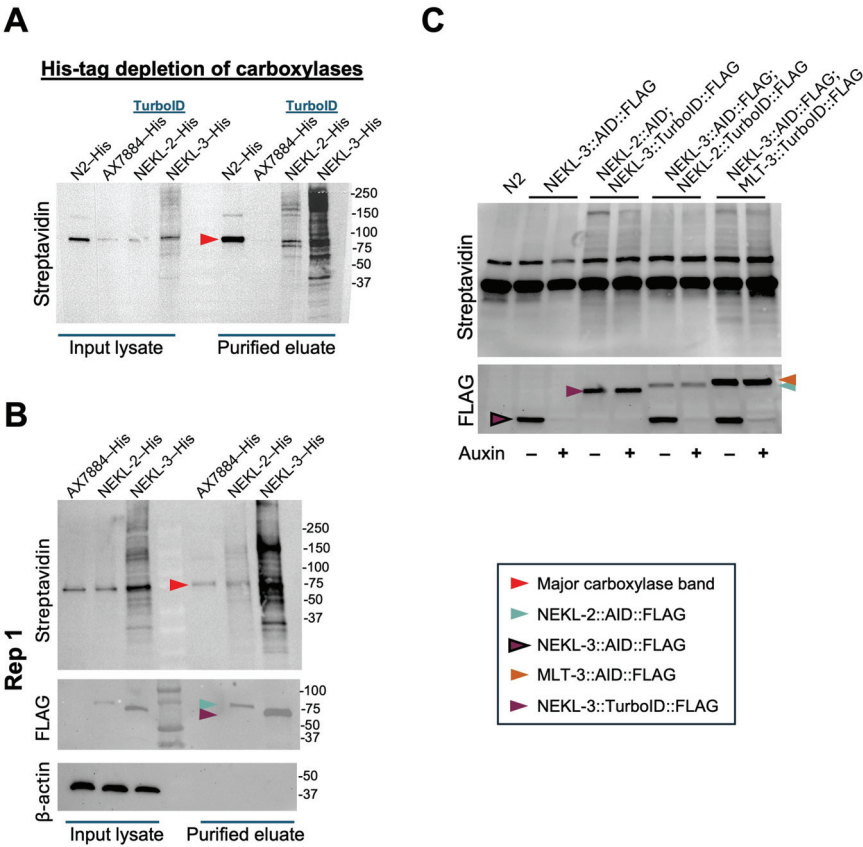


Figure EV8. Methodological variations.

(A–C) Western blots with specific proteins indicated by the key. (A) Streptavidin-probed western blot showing a reduction in the major carboxylase band in strains that underwent carboxylase depletion (AX7884, NEKL-2, and NEKL-3). –His, lysates were incubated with nickel-coated (His-binding) beads to remove the endogenous His-tagged versions of PYC-1, PCCA-1, MCCC-1, and POD-2. N2 does not encode His-tagged carboxylases, accounting for the stronger band in this strain. Increased protein concentration in the eluate lanes facilitates the visualization of the bait-induced biotinylated bands. (B) Western blots from Exp 3, replicate 1 Exp showing biotinylated products (streptavidin), bait proteins (FLAG), and loading control (β -actin). (C) Western blot of lysates from the indicated strains after incubation in the presence or absence of auxin, which leads to the degradation of the AID-tagged proteins. Note that the NEKL-2::AID strain contained a defective FLAG tag and was thus not detectable.

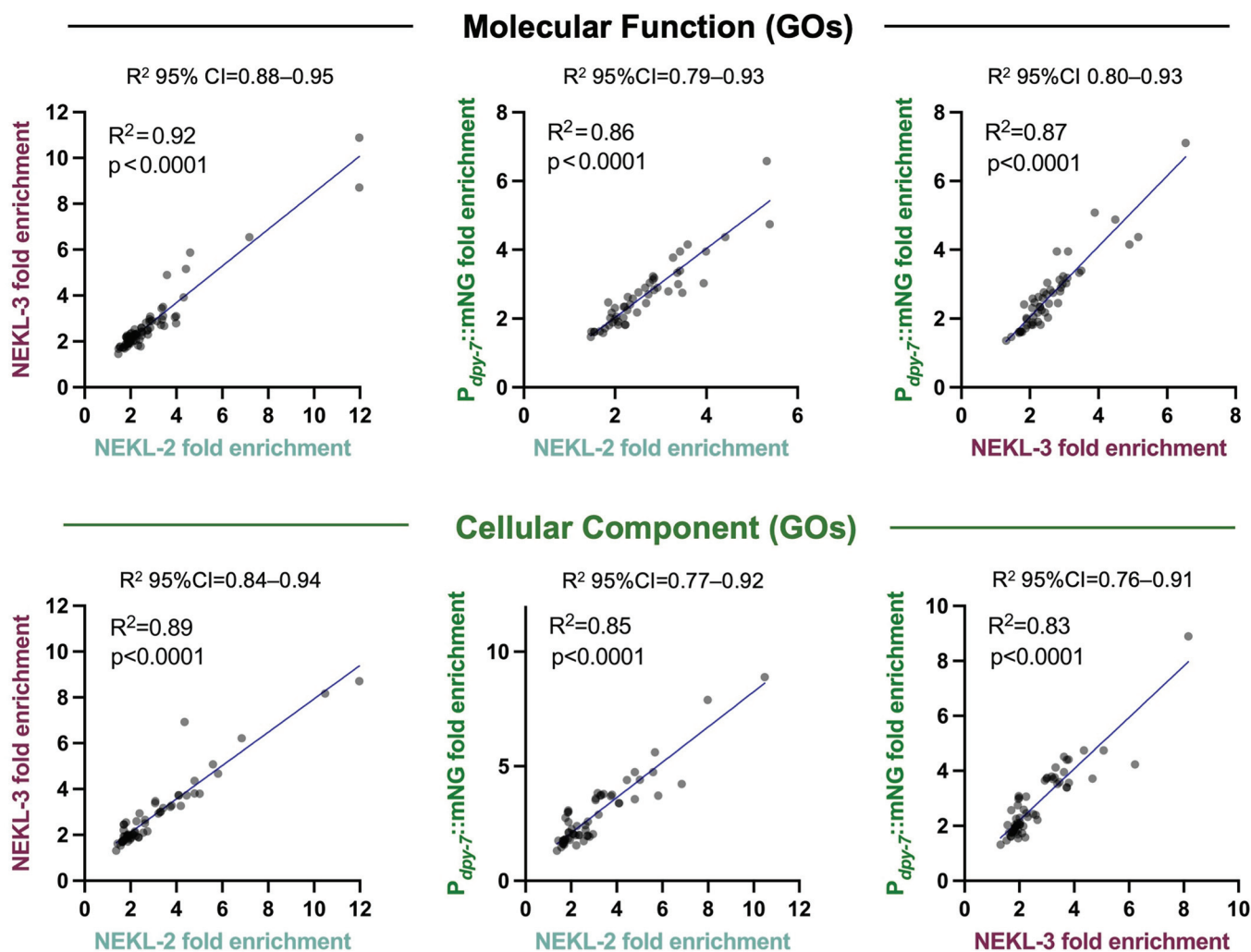


Figure EV9. Correlations of GO terms.

Scatterplots showing correlations in fold enrichments of Molecular Function and Cellular Component GOs for the combined NEKL-2::TurboID, NEKL-3::TurboID, and $P_{dpy-7}::mNG::TurboID$ datasets. 95%CI, 95% confidence interval. Although correlation was greatest between NEKL-2–NEKL-3, this was not statistically significant based on CIs.