

## SUPPLEMENTAL MATERIAL

Mapping Proximity Associations of Core Spindle Assembly Checkpoint Proteins

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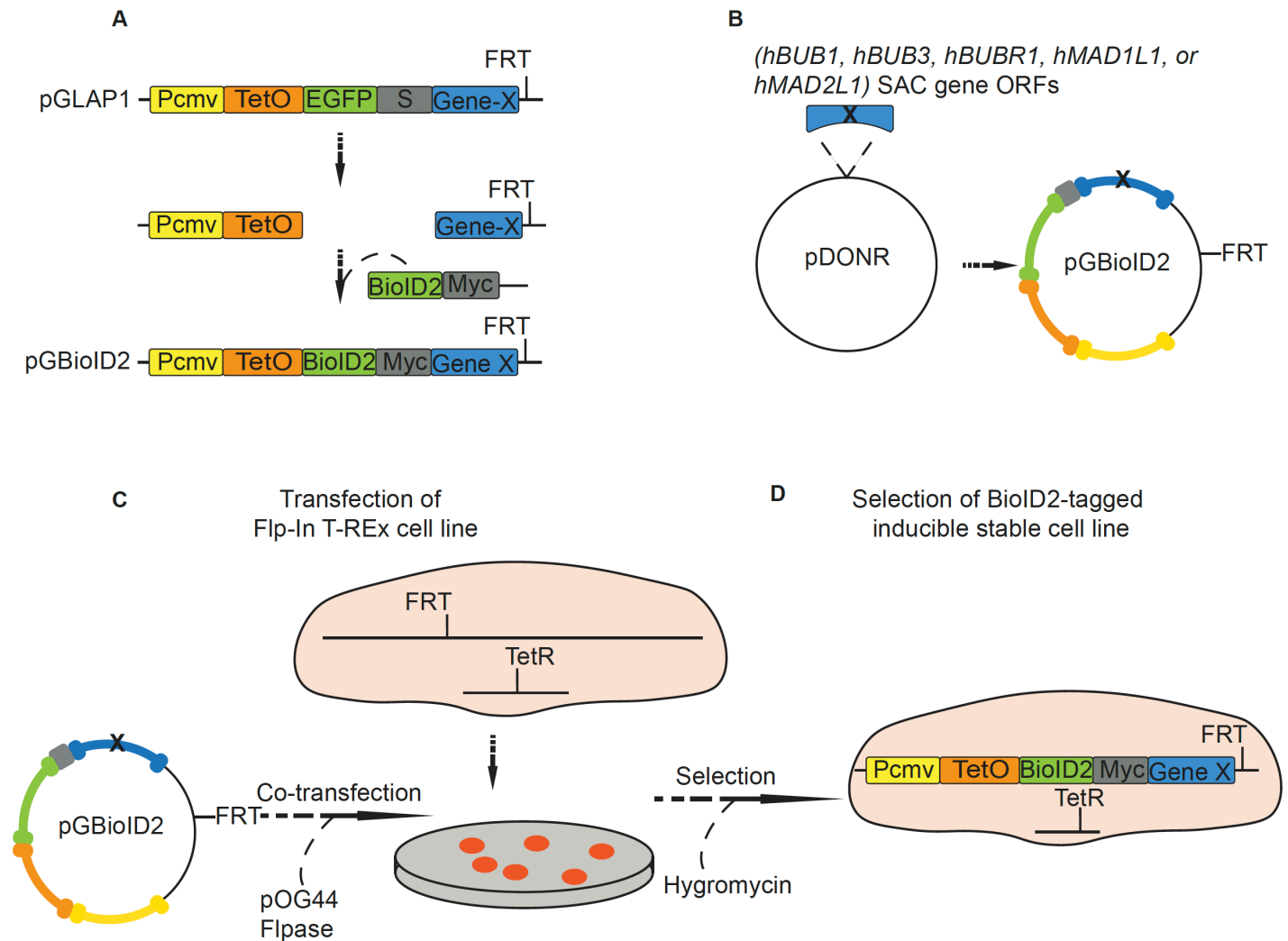
**Table S5.** Summary of all identified proteins from all BioID2 purifications. (.xlsx)

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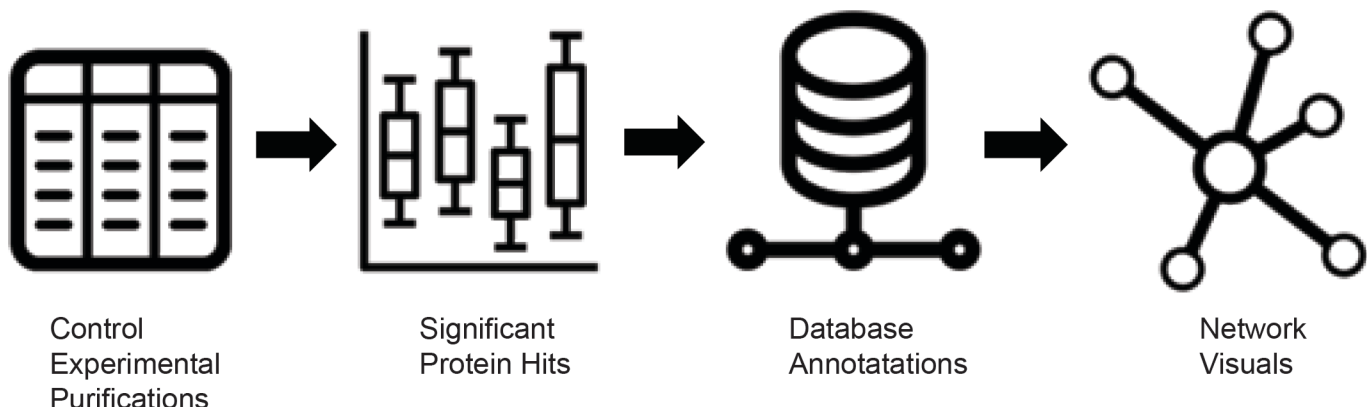
**Table S7.** Summary of significant SAC protein proximity associated proteins. (.xlsx)

**Table S8.** List of Gene Ontology (GO) annotations used in the core SAC protein proximity association network analyses. (.xlsx)

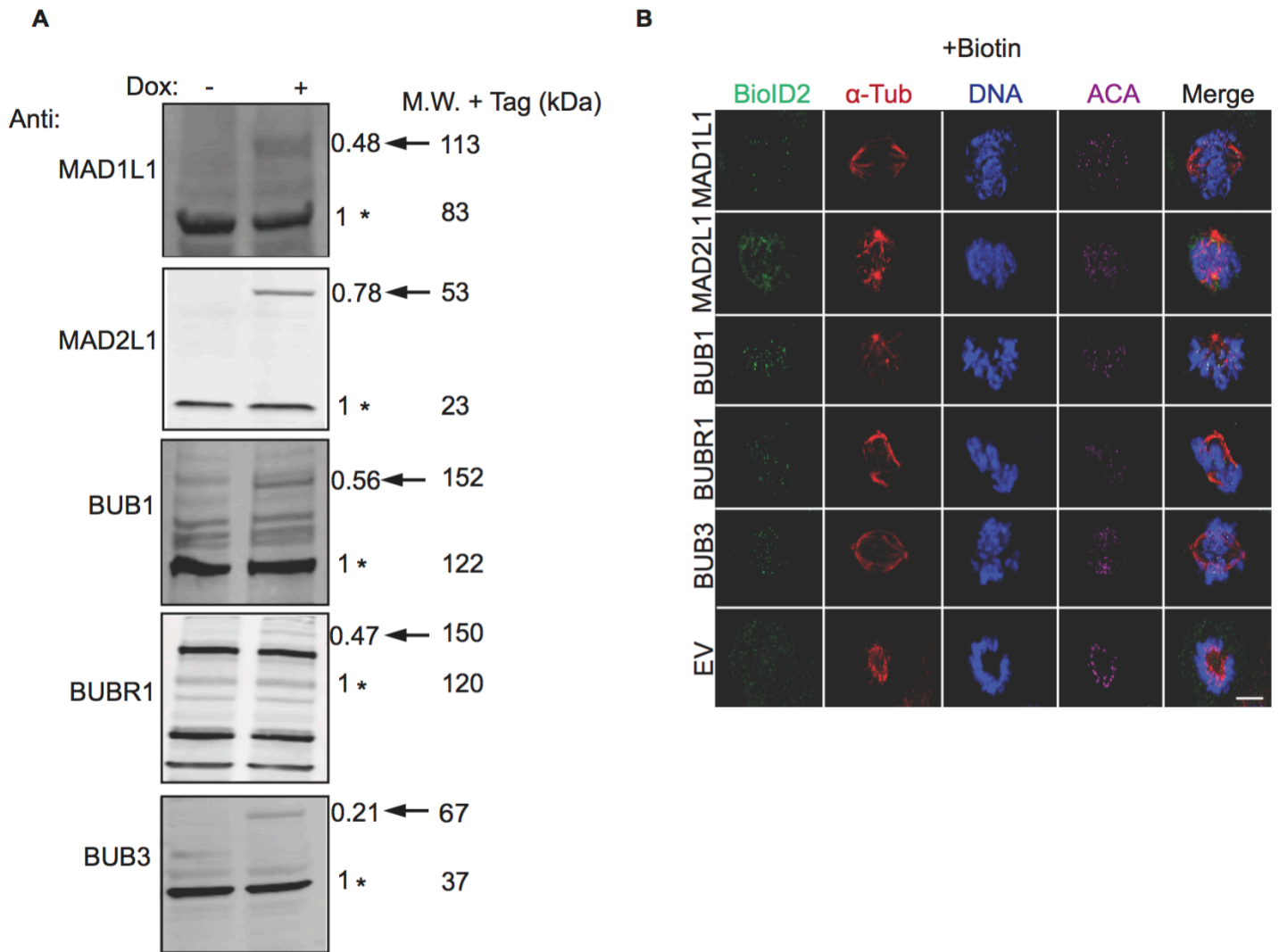
## Supplemental Figures



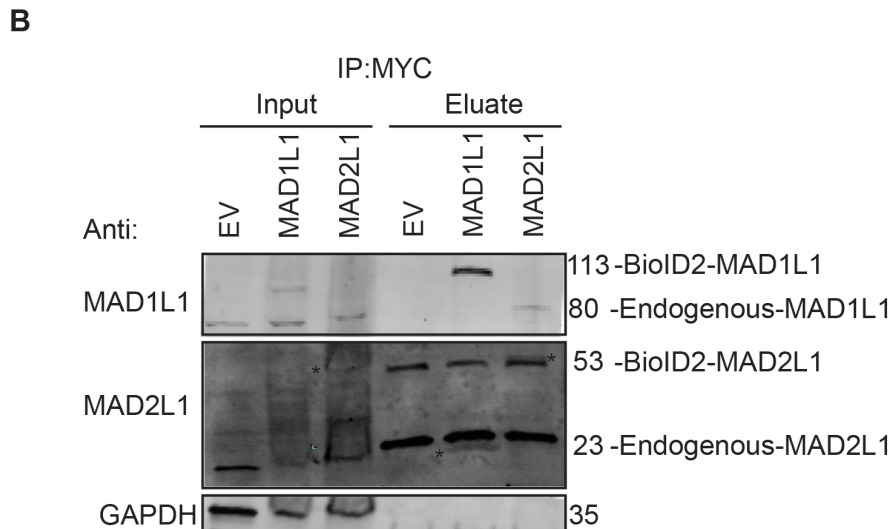
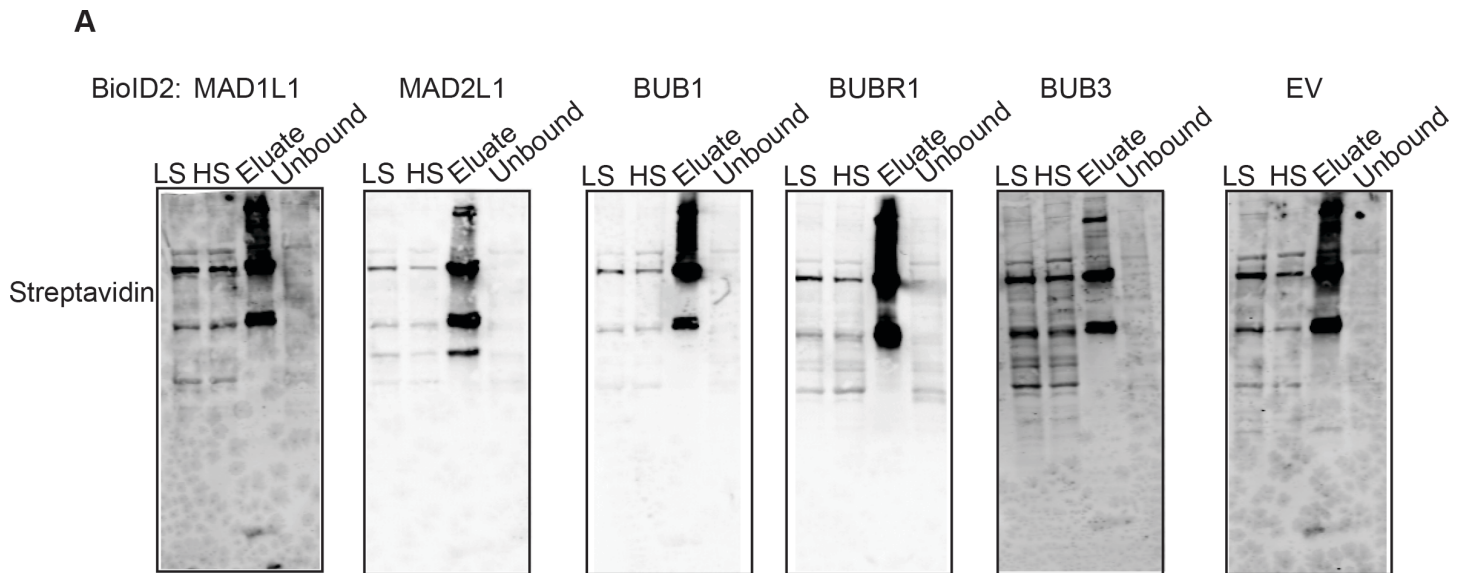
**Figure S1.** Generation of BioID2-tagged SAC protein inducible stable cell lines. (A) Generation of the Gateway compatible pGBioID2 vectors (pGBioID2-27 and pGBioID2-47) using the pGLAP1 vector backbone. S denotes S-tag and M denotes Myc-tag. (B) Transfer of core human SAC gene ORFs from the pDONR221 vector to the pGBioID2 vector. (C-D) pGBioID2-SAC gene vectors were co-transfected with the Flpase containing vector pOG44 into HeLa Flp-In-TREx cells and stable integrants were selected with Hygromycin. See Tables S2 and S3 for primer amplification sequences and information on the vectors generated.



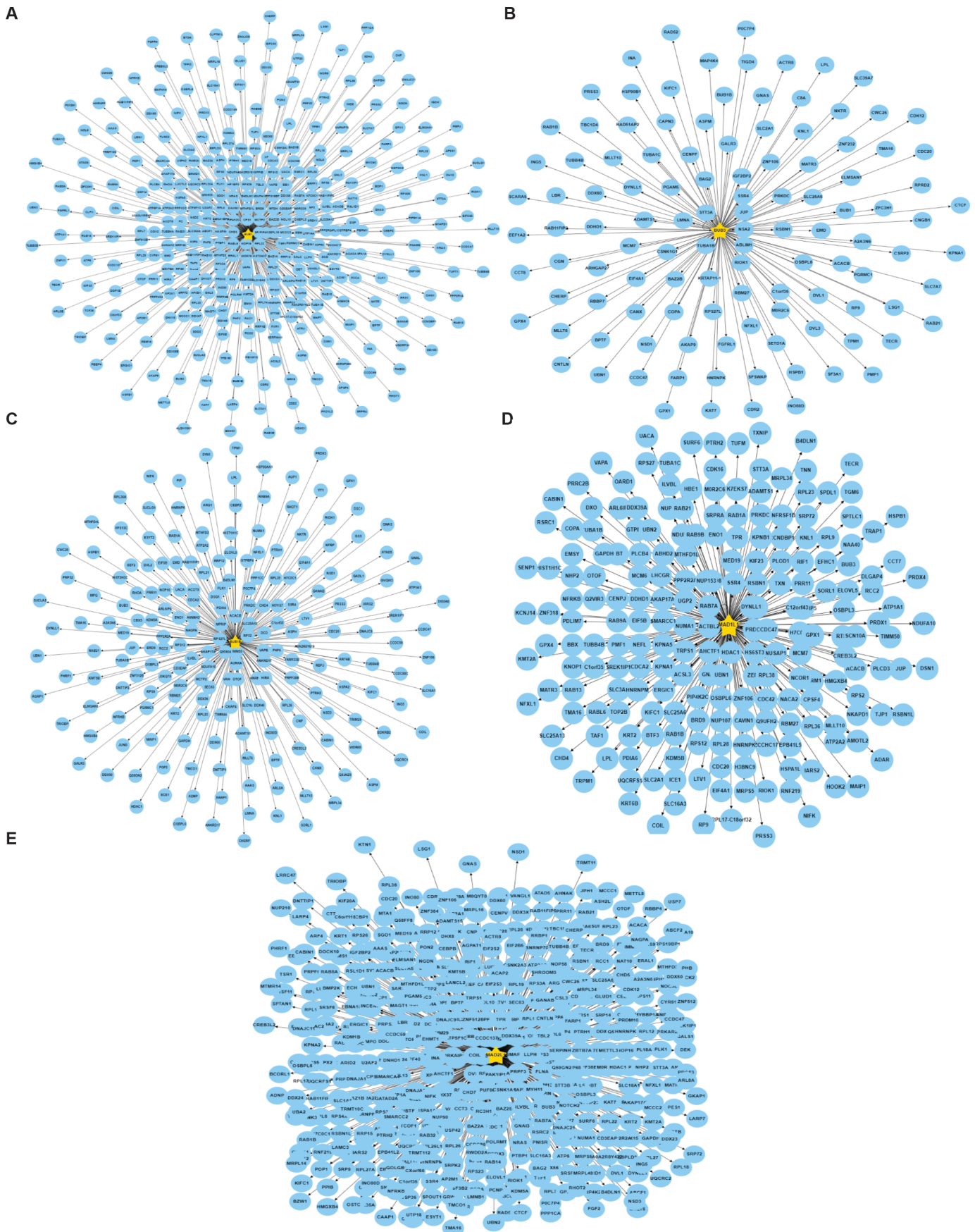
**Figure S2.** Workflow of mass spectrometry data acquisition and analysis. Control and experimental SAC core protein BioID2 purifications were analyzed by mass spectrometry and the mass spectrometry data was analyzed with a Mascot search that yielded 8,403 unique peptides, from which 903 unique proteins were identified. The experimental purifications, considering all 5 baits and replicates, identified 870 proteins while the control purifications identified 480 proteins. Due to the overlap between proteins identified in the control and experimental purifications, we determined hits by identifying proteins that showed a significant difference when compared to the control. Briefly, proteins shared between control and experimental purifications were compared by calculating the mean difference and then simulating the distribution of the mean difference (10,000 simulations). Once the distribution was simulated the 95% confidence interval (CI) were calculated and proteins which lied outside the 95% CI and also showed a higher spectral count in the experimental compared to the control were considered hits. A total of 378 unique proteins were considered hits across all experimental purifications. Protein hits were then queried in several databases (Geneontology; BioGRID; Reactome) and Cytoscape protein association networks were used to visualize the results.



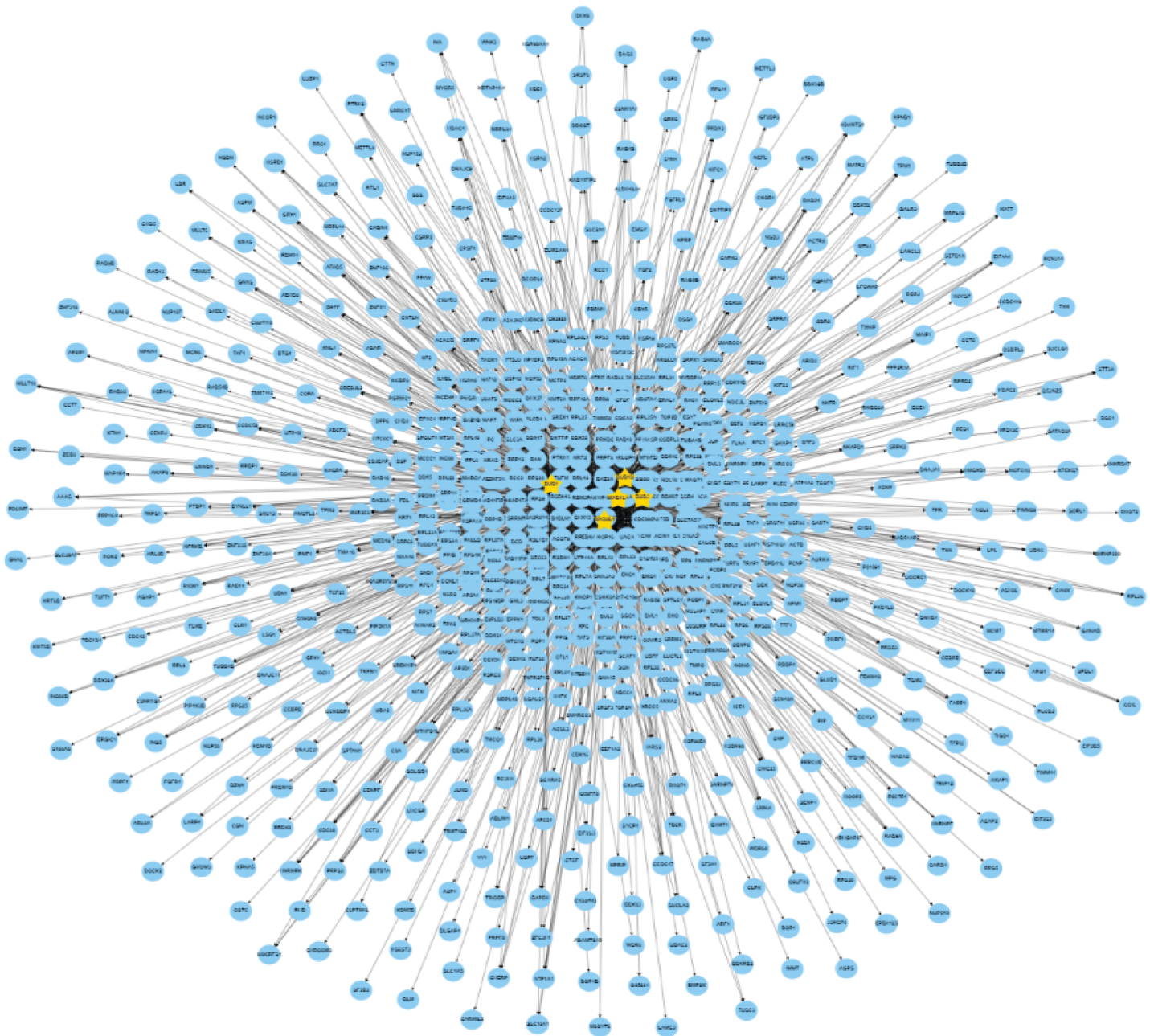
**Figure S3.** Establishment of inducible BioID2-tagged SAC protein (BUB1, BUB3, BUBR1, MAD1L1 and MAD2L1) stable cell lines. (A) Immunoblot analysis of extracts from doxycycline (Dox)-inducible BioID2-tag alone or BioID2-tagged SAC protein (BUB1; BUB3; BUBR1; MAD1L1; MAD2L1) expression cell lines in the absence (-) or presence (+) of Dox for 16 hours. For each cell line, blots were probed with the indicated antibodies that recognize the endogenous protein (labeled with a 1\* to indicate 1x endogenous protein levels) and the corresponding overexpressed BioID2-tagged protein (labeled with .21-.78 and an arrow to indicate that their level of expression is below endogenous levels). M.W. indicates molecular weight. Note that BioID2-tagged SAC proteins are only expressed in the presence of Dox. (B) Fixed-cell immunofluorescence microscopy of the BioID2-tag alone (empty vector, EV) or the indicated BioID2-tagged SAC proteins during prometaphase, a time when the SAC is active. HeLa BioID2-tagged protein expression cell lines were induced with Dox and treated with 50  $\mu$ M Biotin for 16 hours, fixed and stained with Hoechst 33342 DNA dye and anti-BioID2, anti- $\alpha$ -Tubulin and anti-centromere antibodies (ACA). Bar indicates 5  $\mu$ m. Note that all BioID2-tagged SAC proteins localize to the kinetochore region, overlapping with the ACA signal, whereas the BioID2-tag alone (EV) was diffused throughout the cell.



**Figure S4.** BioID2 biochemical purifications. (A) HeLa BioID2-tag alone (empty vector, EV) or BioID2-tagged SAC protein (BUB1; BUB3; BUBR1; MAD1L1; MAD2L1) stable cell lines were induced to express the BioID2-tagged proteins for 16 hours in the presence of 100 nM Taxol and 50  $\mu$ M of Biotin. BioID2 biochemical purifications were then performed with streptavidin beads to capture biotinylated proteins. Diagnostic samples of each purification (low speed supernatant (LS), high speed supernatant (HS), eluate, and unbound) were resolved by western blotting and the blots were probed with streptavidin to visualize the biotinylated eluates of each BioID2-tagged SAC protein purification. (B) BioID2-Myc (empty vector, EV), BioID2-Myc-MAD1L1, or BioID2-Myc-MAD2L1 inducible HeLa stable cell lines were induced with Dox and treated with 100 nM Taxol to arrest cells in mitosis. Mitotic cell lysates were then used for reciprocal Myc immunoprecipitations and subjected to immunoblot analysis with the indicated antibodies. Note that endogenous MAD1L1 immunoprecipitates with BioID2-Myc-MAD2L1 and endogenous MAD2L1 immunoprecipitates with BioID2-Myc-MAD1L1. Asterisks denote endogenous MAD2L1 and BioID2-Myc-MAD2L1.

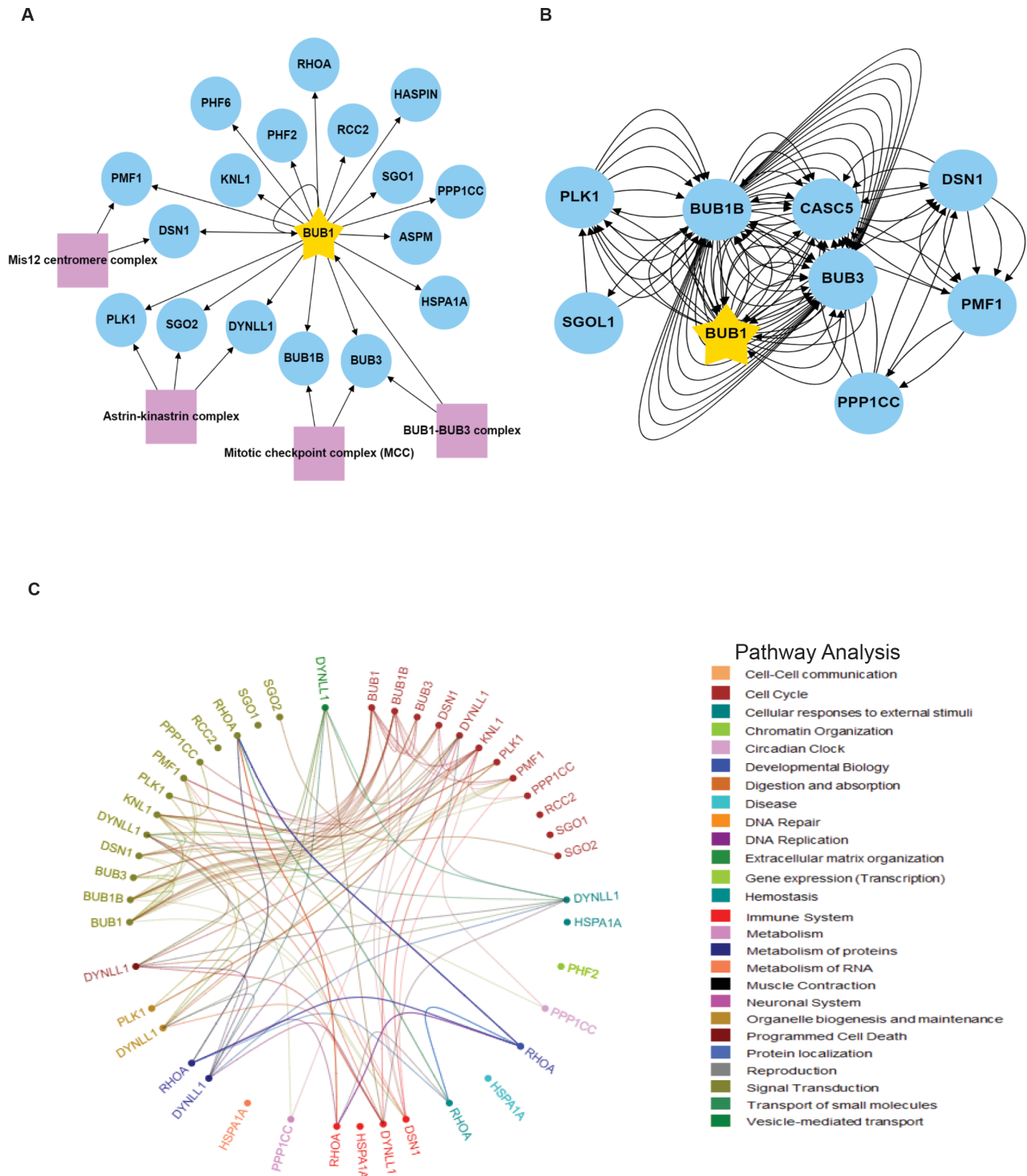


**Figure S5.** Core SAC protein proximity association maps. (A-E) Protein proximity association maps for each of the core SAC proteins BUB1 (A), BUB3 (B), BUBR1 (C), MAD1L1 (D), and MAD2L1 (E). See Table S4 for a list of proteins in each map. The maps were visualized using RCytoscapeJS.

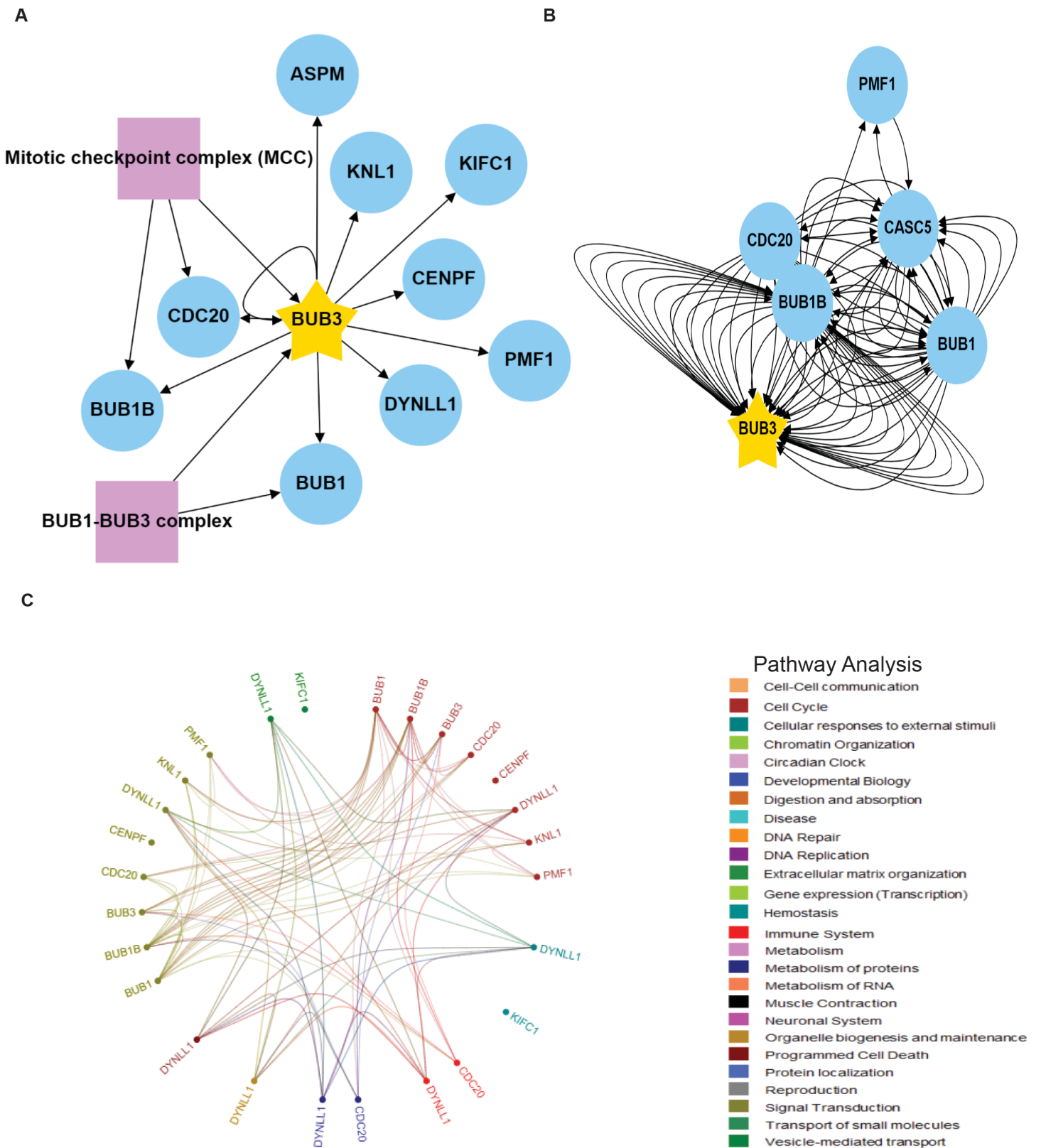


**Figure S6.** Core SAC protein proximity network. The five core SAC protein (BUB1; BUB3; BUBR1; MAD1L1; MAD2L1) proximity association maps in Figure S5 were combined to generate the core SAC protein proximity association network. The network was visualized using RCytoscapeJS.

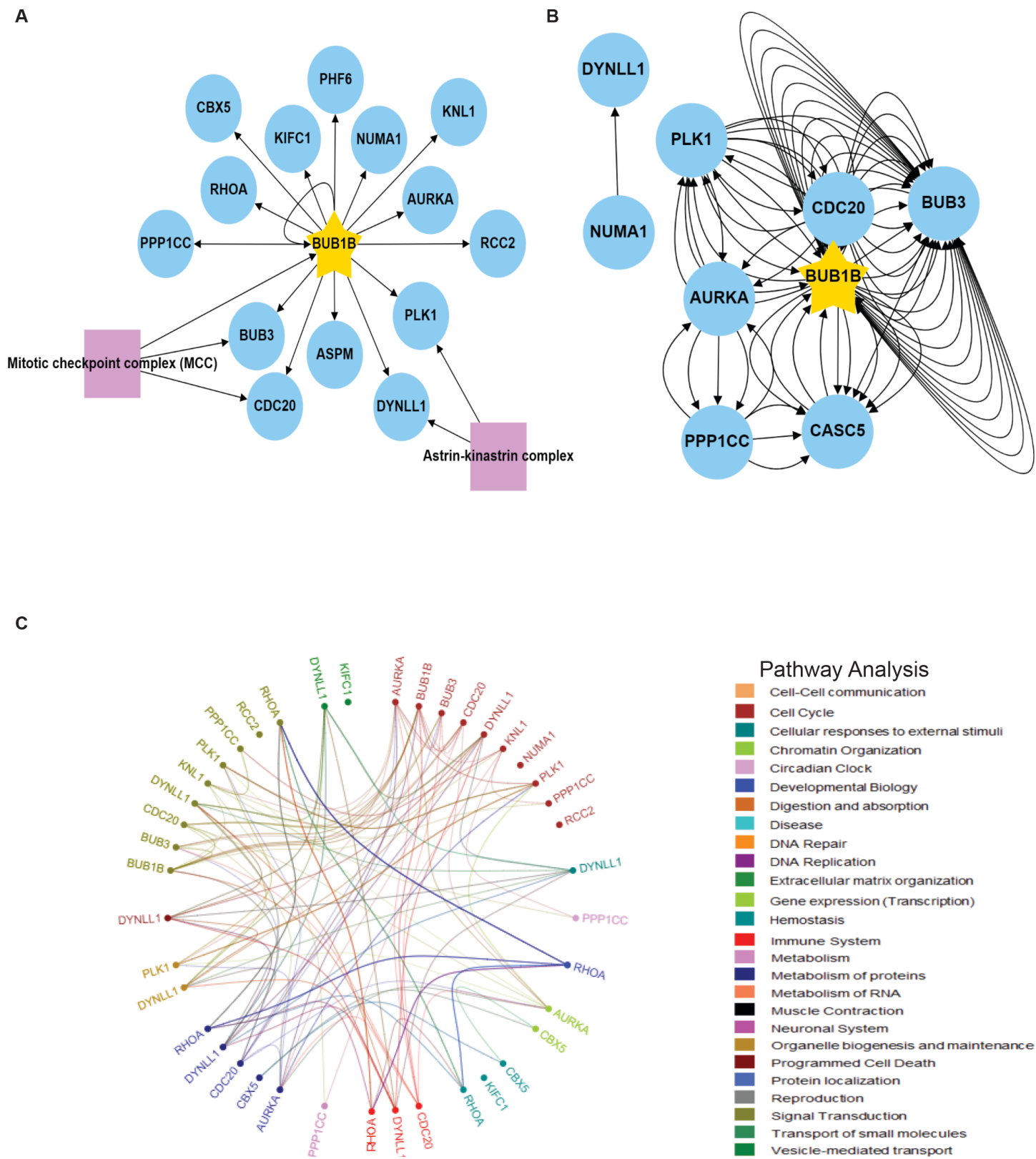




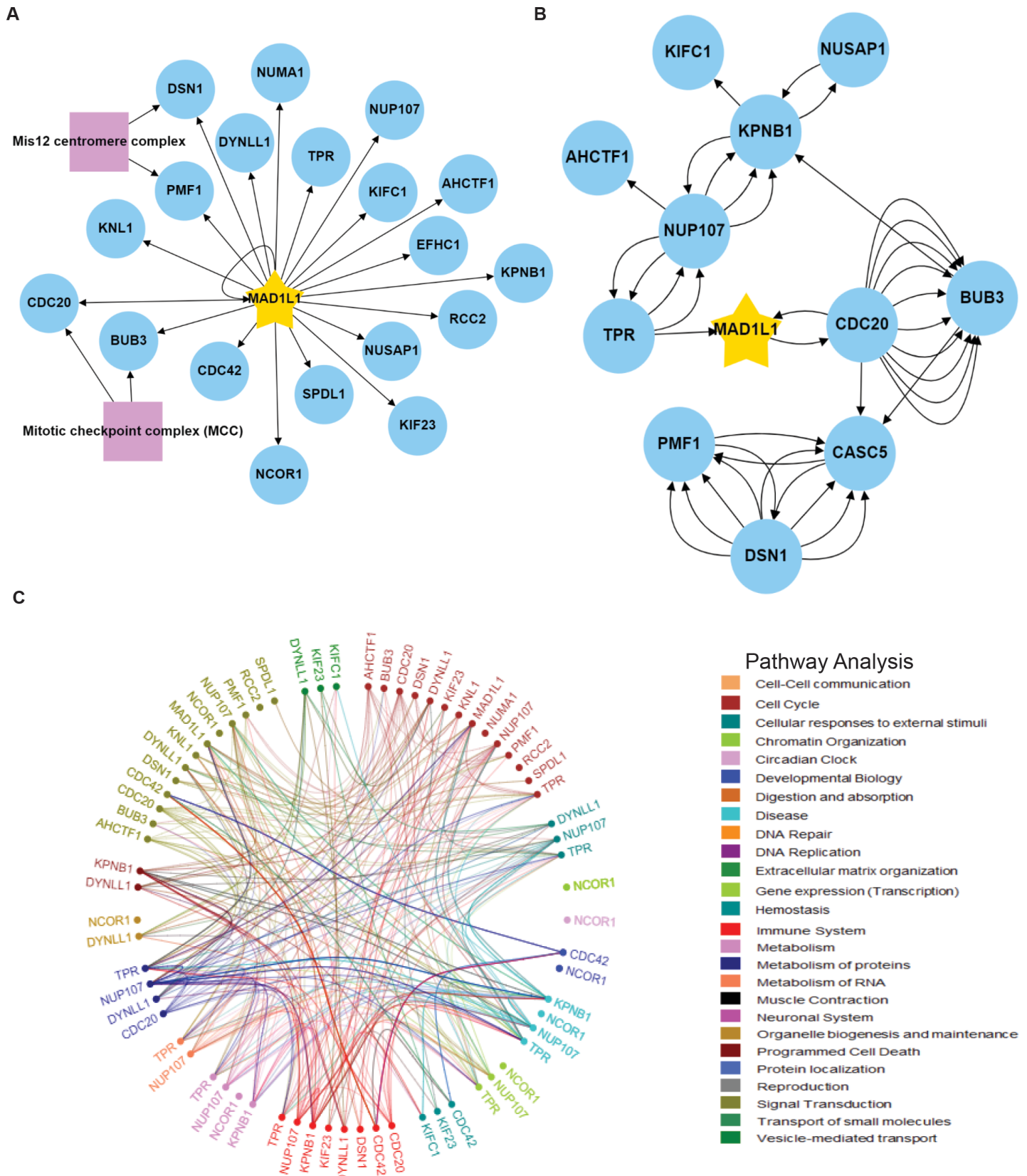
**Figure S7.** The BioID2-BUB1 proximity protein association map. (A) Generation of the BUB1 protein proximity association map using kinetochore related Gene Ontology annotations and CORUM complex annotation analyses. The map was visualized using RCytoscapeJS. Purple boxes highlight protein complexes known to assemble with BUB1 as annotated by the CORUM database. Arrows indicate the direction of the detected interactions. (B) The BUB1 proximity association map was analyzed with BioGRID to reveal previously verified protein associations. Each arrow indicates an experimentally annotated interaction curated in the BioGRID database. Direction of arrows indicate an annotated interaction from a bait protein to the prey. (C) Reactome pathway analysis of the BUB1 proximity association map. The Reactome circular interaction plot depicts the associations between the identified proteins within the BUB1 proximity association map and the corresponding pathways in which they function. Legend presents color-coded pathways that correspond to the circular interaction plot.



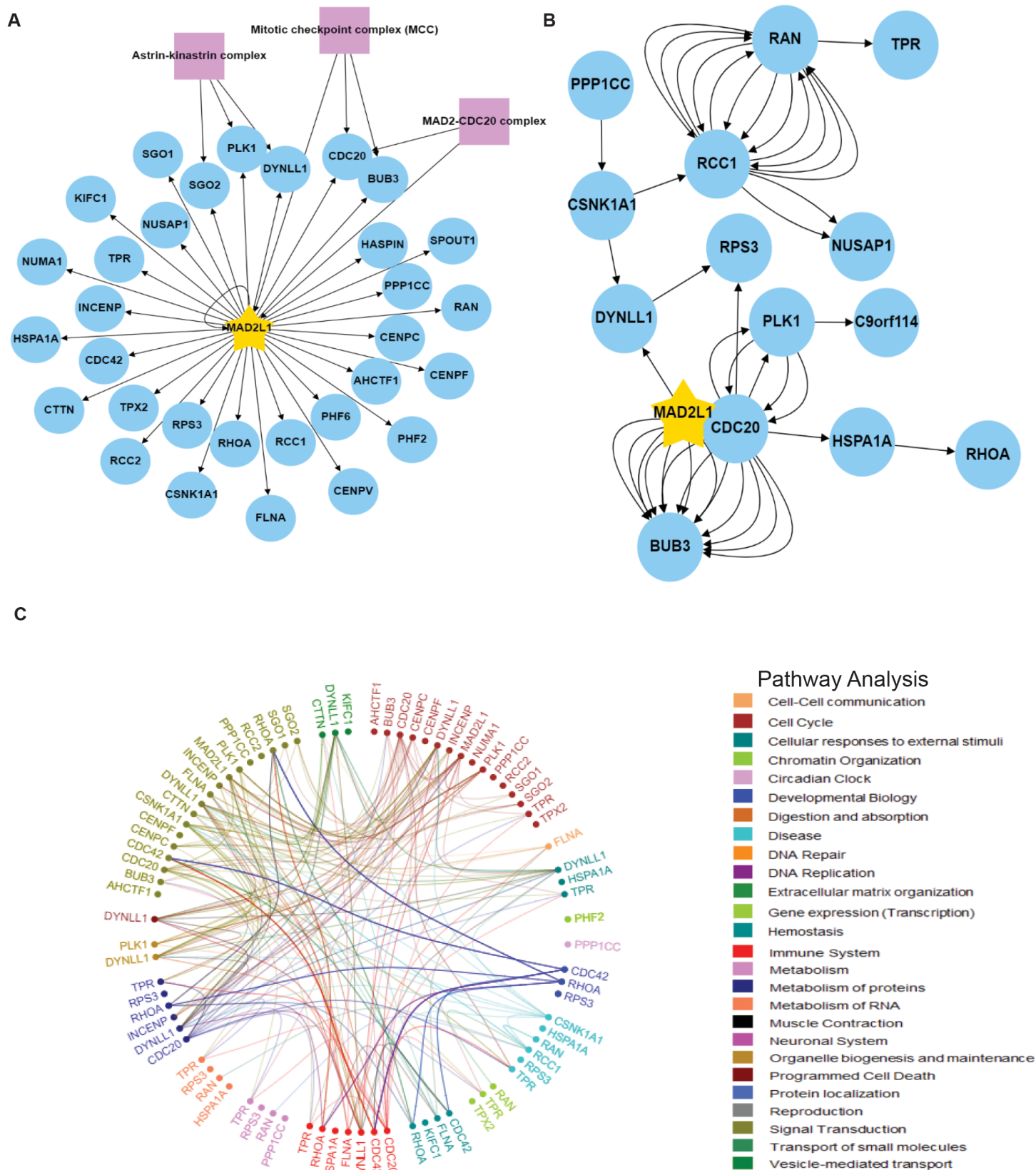
**Figure S8.** The BioID2-BUB3 proximity protein association map. A, B, and C are as described in Figure S7.



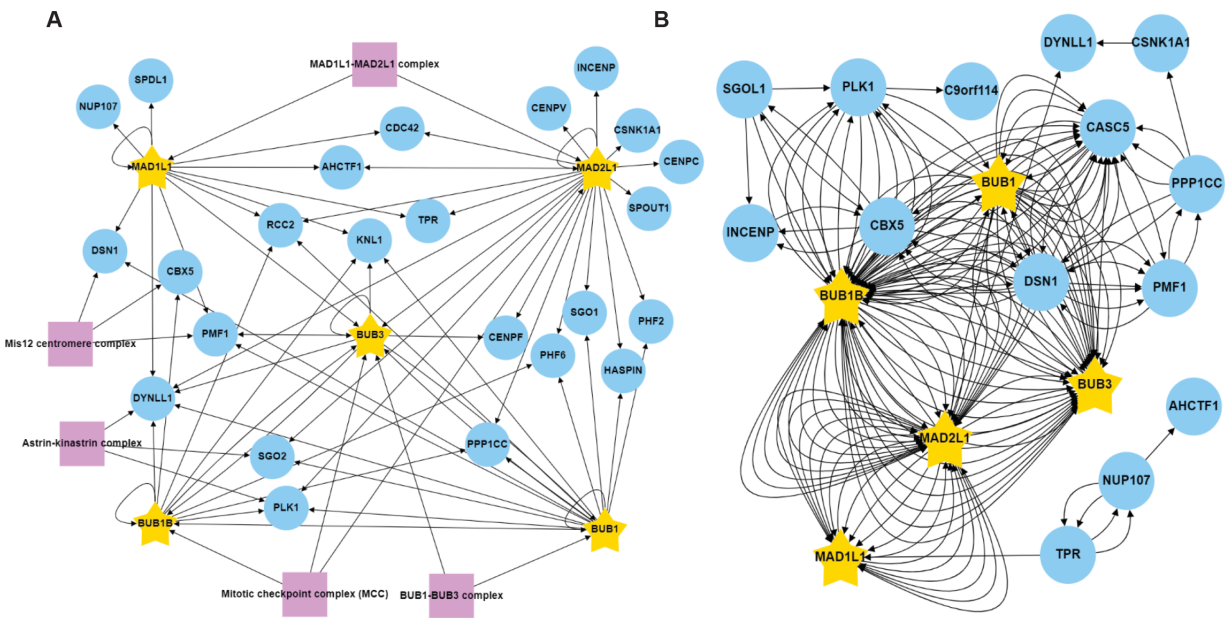
**Figure S9.** The BioID2-BUBR1/BUB1B proximity protein association map. A, B, and C are as described in Figure S7.



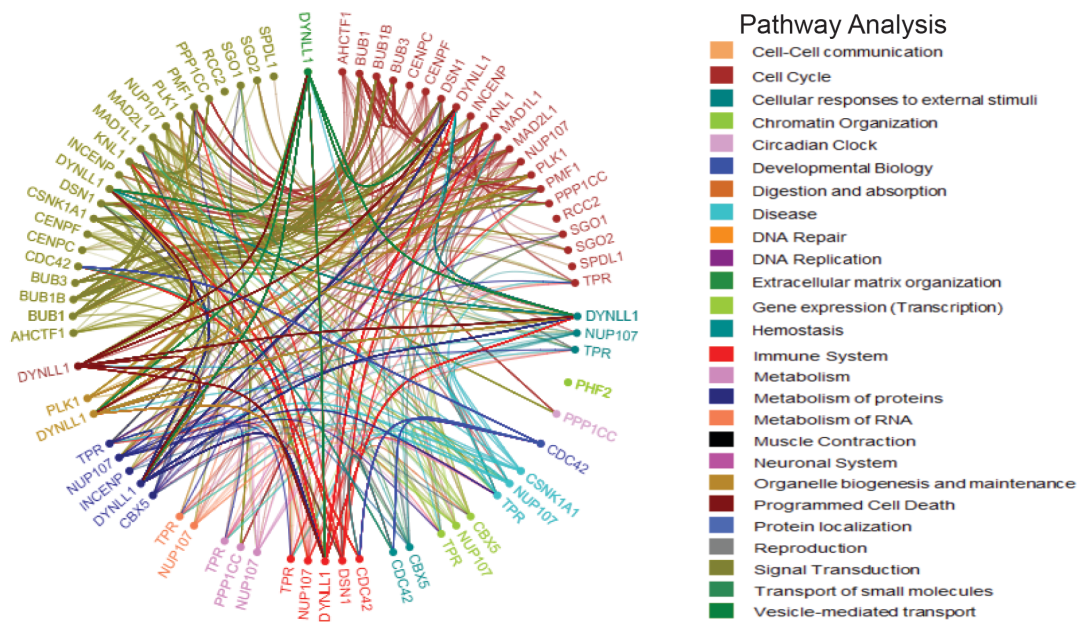
**Figure S10.** The BioID2-MAD1L1 proximity protein association map. A, B, and C are as described in Figure S7.



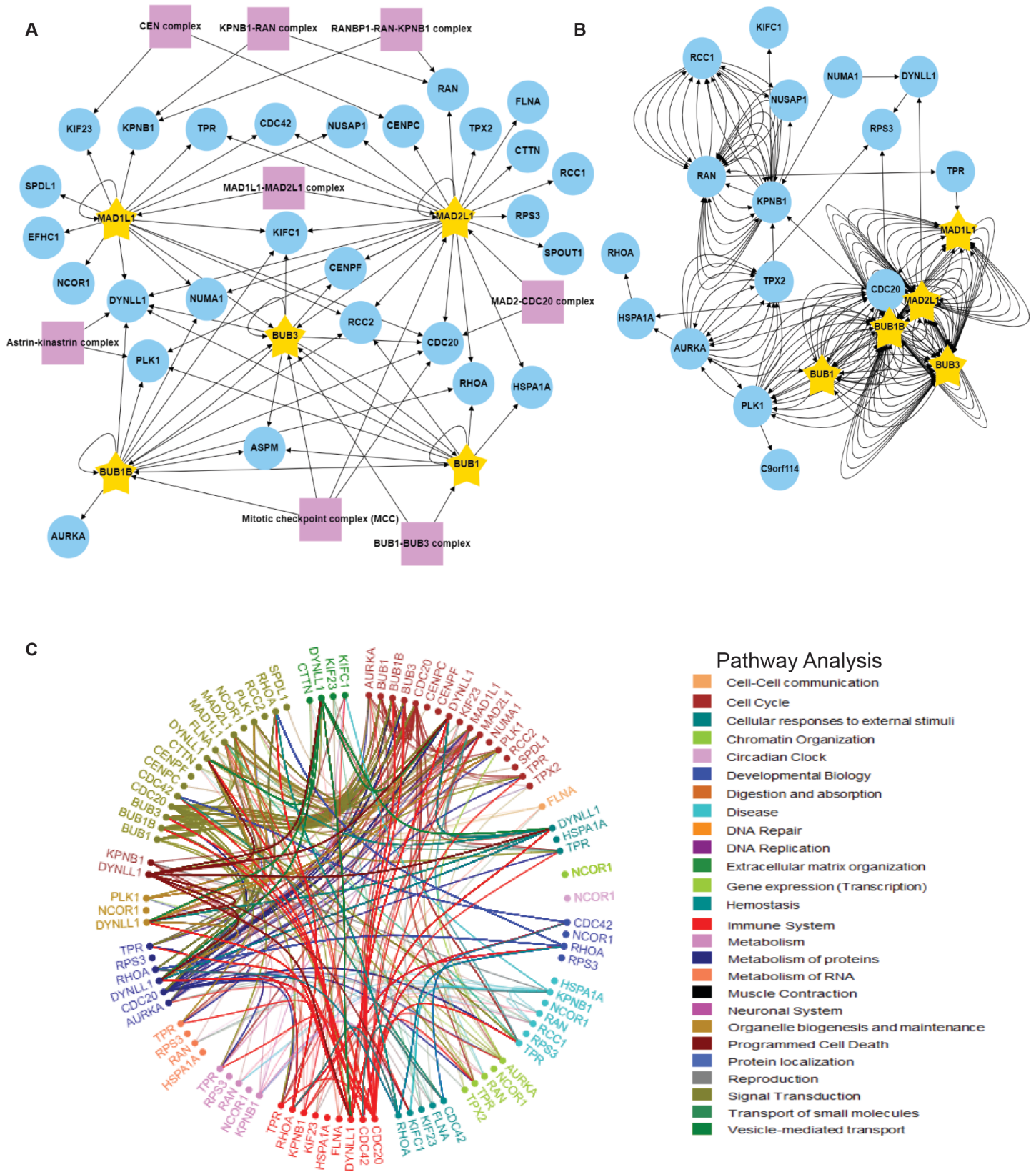
**Figure S11.** The BioID2-MAD2L1 proximity protein association map. A, B, and C are as described in Figure S7.



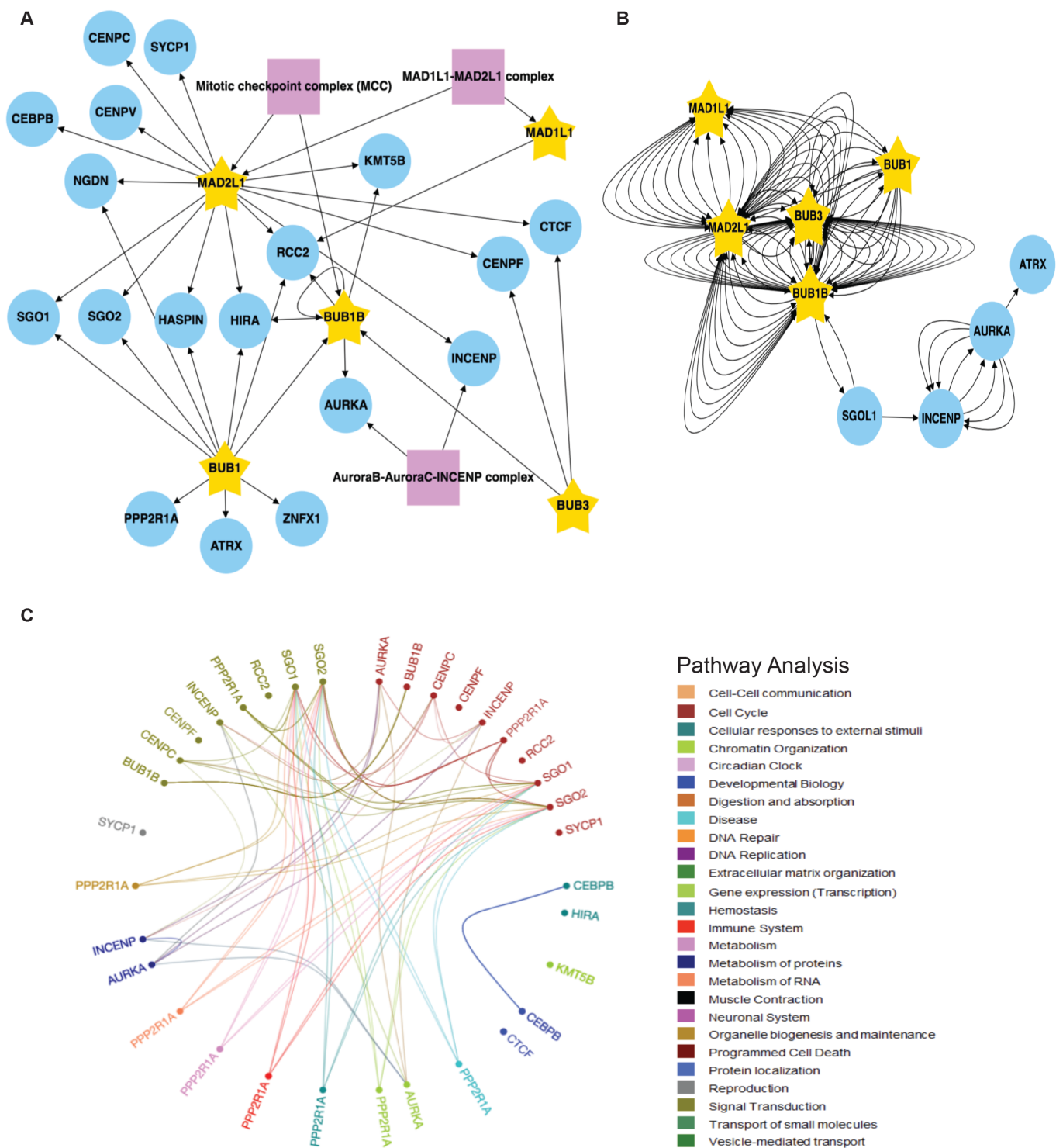
C



**Figure S12.** The core SAC protein proximity association network using kinetochore Gene Ontology annotations. (A) Generation of the core SAC protein (BUB1; BUB3; BUBR1; MAD1L1; MAD2L1) proximity association network using kinetochore related Gene Ontology annotations and CORUM complex annotation analyses. The map was visualized using RCytoscapeJS. Purple boxes highlight protein complexes known to assembly with core SAC proteins as annotated by the CORUM database. Arrows indicate the direction of the detected interactions. (B) The core SAC protein kinetochore proximity association network was analyzed with BioGRID to reveal previously verified protein associations. Each arrow indicates an experimentally annotated interaction curated in the BioGRID database. Direction of arrows indicate an annotated interaction from a bait protein to the prey. (C) Reactome pathway analysis of the core SAC protein kinetochore proximity association network. The Reactome circular interaction plot depicts the associations between the identified proteins within the core SAC protein proximity association network and the corresponding pathways in which they function. Legend presents color-coded pathways that correspond to the circular interaction plot.

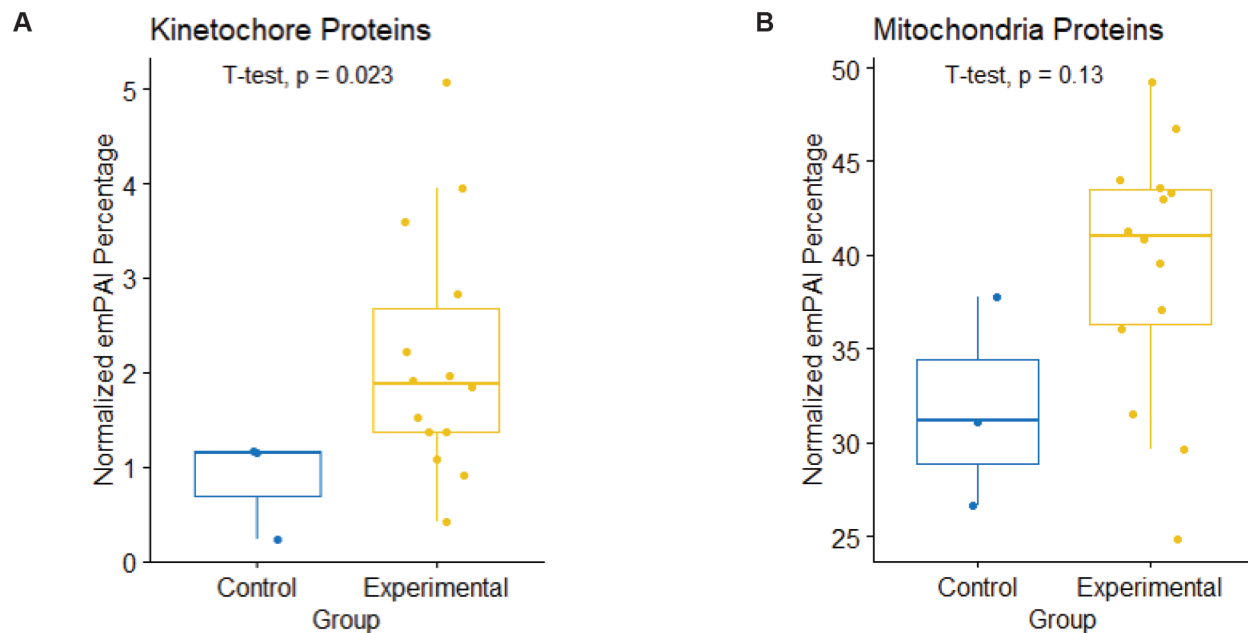


**Figure S13.** The core SAC protein proximity association network using mitotic spindle related Gene Ontology annotations. A, B, and C are as described in Figure S12, except that the mitotic spindle related Gene Ontology annotations were applied to the analysis.

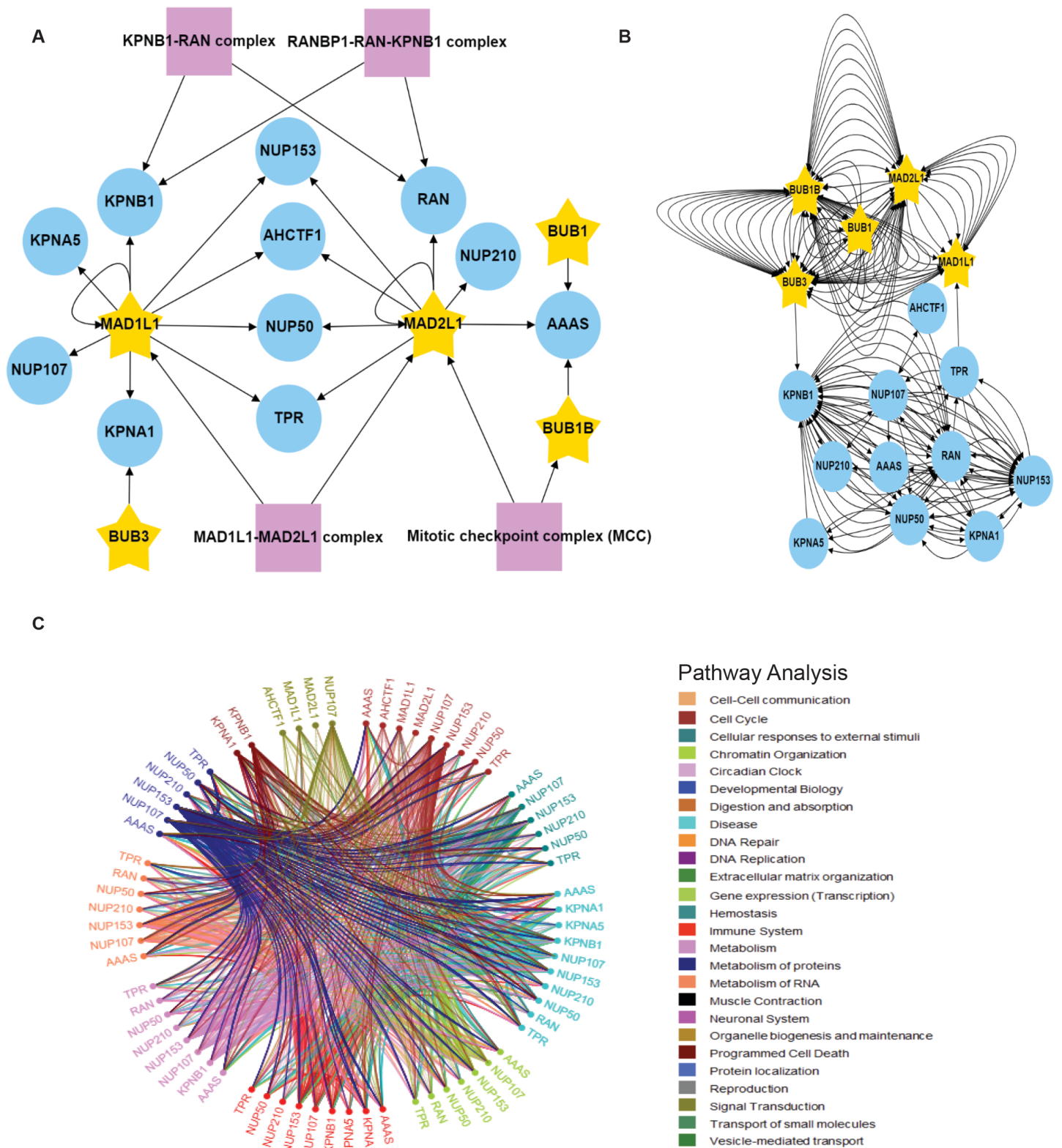


**Figure S14.** The core SAC protein proximity association network using centromere related Gene Ontology annotations. A, B, and C are as described in Figure S12, except that the centromere related Gene Ontology annotations were applied to the analysis.

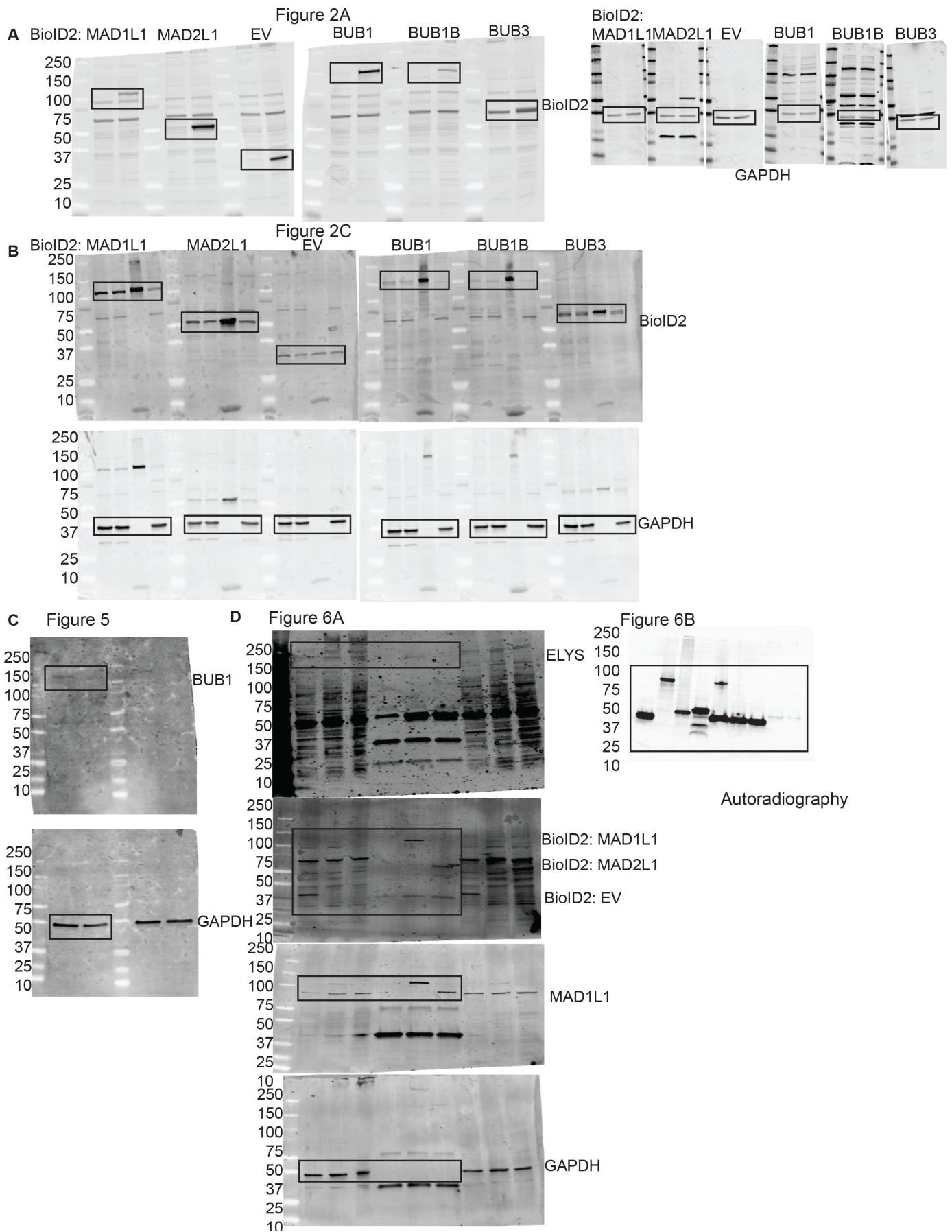




**Figure S15.** Kinetochores protein enrichment analysis. (A) Kinetochores related GO terms (see Table S8 for GO terms) were used to identify kinetochores related proteins and the normalized emPAI scores were used as a quantitative measure of the amount of each protein in each purification. Comparing the amount of kinetochores related proteins to the sum of normalized emPAI scores of all proteins in each purification resulted in a normalized emPAI percentage. This percentage was representative of the amount of kinetochores proteins compared to the total amount of proteins identified in the purification. This resulted in a range of 0.5- 5% kinetochores proteins in each experimental purification. These percentages were then compared to control purifications to determine whether there was an enrichment of kinetochores related proteins in the experimental purifications. After analyzing the control purifications as described above for the experimental, we performed a student's t-test to determine if there was a significant difference. The results indicate that there are lower amounts, 0.2-1%, of kinetochores related proteins in the control purifications, and that the difference is significant ( $p$  value = 0.023). (B) The same analysis in (A) was performed with Mitochondria related proteins. The results showed no significant difference between the percentage of mitochondria related proteins in control purifications compared to experimental purifications.

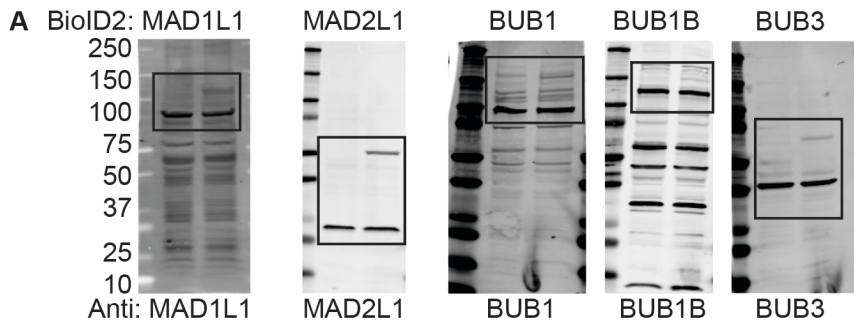


**Figure S16.** The core SAC protein proximity association network using nuclear pore related Gene Ontology annotations. A, B, and C are as described in Figure S12, except that nuclear pore related Gene Ontology annotations were applied to the analysis.

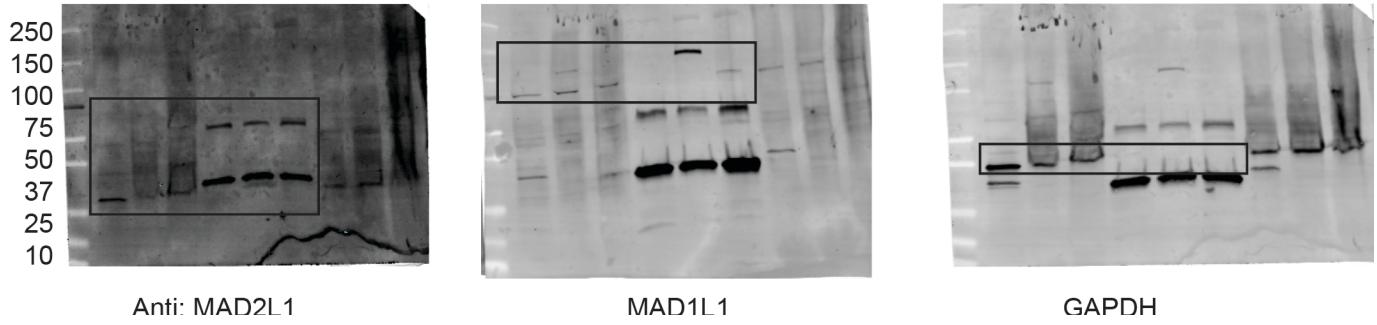


**Figure S17.** Uncropped immunoblots and autoradiography blots for all figures.

Supplemental Figure 3A



Supplemental Figure 4B



**Figure S18.** Uncropped immunoblots for all supplemental figures.