**Supplementary Information**

**Functional proximity mapping of RNA binding proteins uncovers a mitochondrial mRNA anchor that promotes stress recovery**

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This file includes supplementary figure 1-7 and supplementary dataset 1-6

**Supplementary Figures**

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**Supplementary Figure 1. Evaluation of APEX-PS, related to Figure 1.** **a,** Enrichment of subcellular RBPs. RBPs are enriched after phase separation. Biotinylated RBPs are enriched after the second enrichment using streptavidin beads. **b,** Schematic of five subcellular regions investigated in this study. **c-f,** Evaluation of APEX-PS in the nucleolus (**c**), cytosol (**d**), OMM (**e**) and ERM (**f**). **g,** UV crosslinking-based APEX-PS enables the enrichment of nuclear RBPs. After 1 minute of labeling with APEX-NLS, UV was applied to crosslink proteins to RNA at 254 nm with 400 mJ/cm2. Streptavidin blotting was performed after each step of the tandem enrichment. **h,** Comparison of FA- and UV-based APEX-PS. Silver staining of nuclear proteins was performed after streptavidin enrichment. **i,** After UV-based APEX-PS, we detected the four protein markers in Figure 1D using western blot. Samples blotted were whole cell lysate (left), material after phase separation (middle), and material after both phase separation and streptavidin bead enrichment (right). **j,** Capture of cytosolic RBPs by TurboID-PS. Cytosolic proteins were biotinylated by TurboID-NES and RNA-protein interactions were crosslinked by FA treatment. The samples were processed by phase separation and streptavidin enrichment, following by streptavidin blotting.



**Supplementary Figure 2. Additional analysis of nuclear APEX-PS dataset, related to Figure 2.**  **a,** Correlation of biological replicates. **b,** Receiver operating characteristic (ROC) curves of TMT ratios used for assignment of nuclear RBPs. Proteins were ranked in descending order based on the TMT ratio. True positive denotes known nuclear RBPs collected by overlapping known human RBPs and nuclear proteins annotated by GOCC (Table S1). False positives include cytosolic proteins that were not previously identified as RBPs. **c,** Sample histogram showing how the cutoff for 128N/126C ratio was applied. **d,** The number of tRNA ligases identified by APEX-PS and fractionation-based methods. The identified tRNA ligases were shown. (**E**) GO biological process analysis of nuclear RBP orphans identified by APEX-PS.



**Supplementary Figure 3. Additional analysis of nucleolar APEX-PS dataset, related to Figure 3.** **a,** Receiver operating characteristic (ROC) curve of TMT ratios used for assignment of nucleolar RBPs. True positives are known nuclear RBPs collected by overlapping known human RBPs and nucleolar proteins annotated by GOCC (Table S1). False positives include cytosolic proteins that were not previously identified as RBPs. **b,** GO biological process analysis of nucleolar RBP orphans. **c,** Subclassification of RBPs in nucleolar APEX-PS dataset. Many RBPs we enriched bind to poly(A)[6](#_ENREF_6), [7](#_ENREF_7), [12](#_ENREF_12), [32](#_ENREF_32), [34](#_ENREF_34), [35](#_ENREF_63). Of the remainder, 19 have been experimentally shown to bind to the 6 RNA classes shown at right. The information of RNA types is shown in Supplementary Dataset 2.



**Supplementary Figure 4. RNA binding domain (RBD) analysis for nucleolar APEX-PS dataset, related to Figure 4.** **a,** Percentages of nucleolar RBPs with classical versus non-classical RNA binding domains (RBDs). **b,** The counts of RBPs with each type of classical and non-classical RNA binding domain. The RBDs of each RBP are listed in Table S2. **c,** Comparison of formaldehyde-dependent enrichment between classical (green) versus non-classical (yellow)-RBD-containing proteins in the nucleolar APEX-PS dataset. The relative biotinylation extent of +FA versus –FA is shown with the mean value of log2 (130C/130N), log2 (131N/130N) and log2 (131C/130N).



**Supplementary Figure 5. Additional analysis of mitochondrial APEX-PS dataset, related to Figure 5.** **a,** Correlation between biological replicates for APEX2-PS-OMM profiling. **b,** Receiver operating characteristic (ROC) curve of TMT ratios used for assignment of OMM-localized RBPs. Proteins were ranked in descending order based on the TMT ratio. True positive denotes known OMM proteins annotated by GOCC. False positive includes cytosolic proteins that were not previously identified as RBPs. False positive proteins are mitochondrial matrix proteins identified by APEX profiling. **c,** Characterization of OMM-localized RBPs involved in mitochondrion-ER contact sites. The annotations of mitochondrial-ER contact are based on split-TurboID profiling54 and shown in Supplementary Table 4.



**Supplementary Figure 6. SYNJ2BP binds and promotes the OMM localization of its clients, related Figure 6.**  **a,** Validation of mitochondria-related SYNJ2BP clients in the absence or presence of protein translation inhibitor PUR by CLIP and qRT-PCR. **b,** Confocl imaging of SYNJ2BP localization at OMM under PUR condition. Anti-TOM20 stains OMM and DAPI stains nuclei. Scale bars, 10 μm. **c,** Validation of non-targeted guide control cells and SYNJ2BP knock-out cells stably expressing APEX2-OMM and APEX2-NES respectively. **d,** Evaluation of OMM localization of SYNJ2BP clients in SYNJ2BP knock-out cells by APEX-mediated proximity labeling of RNA.

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**Supplementary Figure 7. SYNJ2BP promotes the cellular recovery from stresses, related Figure 7.**  **a,** Evaluation of the cell viability of SYNJ2BP KO cells. **b,** Evaluation of cell viability of SYNJ2BP KO cells pre-treated with cycloheximide (CHX). **c,** Detection of ATP level in SYNJ2BP KO cells pre-treated with CHX and PUR. **d,** Evaluation of OMM localization of SYNJ2BP-regulated clients in SYNJ2BP knock-out cells by APEX-mediated proximity labeling of RNA. The OMM localization was determined by comparing APEX2-OMM with APEX2-NES labeling. IARS2 is an OMM-localized mRNA not identified to bind with SYNJ2BP. **e,** Evaluation of complex III activity in SYNJ2BP knock-out cells under sodium arsenite stress. **f-g,** Evaluation of complex IV activity in SYNJ2BP knock-out cells under heat (**f**) and sodium arsenite (**g**) stress. **h,** Evaluation of cell proliferation in SYNJ2BP knock-out cells during the recovery from sodium arsenite stress.

**Supplementary Datasets**

**Supplementary Dataset 1. The 11-plex TMT proteomics results for assigning nuclear and nucleolar RBPs.**

**Supplementary Dataset 2. The list of nuclear RBPs.**

**Supplementary Dataset 3. The list of nucleolar RBPs.**

**Supplementary Dataset 4. The 11-plex TMT proteomics results for assigning OMM-localized RBPs.**

**Supplementary Dataset 5. The lists of OMM-localized RBPs under basal and PUR conditions.**

**Supplementary Dataset 6. The list of SYNJ2BP mRNA clients.**

The supplementary datasets are provided in separated files.