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**Table S1** List of mecciRNAs from RNA-seq data

**Table S2** Mass spectrometry results of mecciND1 and mecciND1 pull-down proteins

**Table S3** Oligos used in this study

**FIGURE LEGENDS**

**Figure S1** Features of mecciRNAs, Related to Figure 1. **A**, Maps of mecciRNA junction positions on mitochondrial genome; backsplicing sites are connected by lines. Mitochondria encoded genes are shown on the circle; the outer ring, heavy strand genes; the inner ring, light strand genes. Maps constructed using Circos (Krzywinski et al., 2009). **B**, Junction motif of human, mouse and zebrafish g-circRNAs. **C**, Distance between flanking sites of mecciRNAs.

**Figure S2** Experimental verification of mecciRNAs, Related to Figure 1. **A**, Northern blots of mecciND1 and mecciCYB (sense probe, as a negative control). **B**,divergent and convergent primers design was shown. **C**, PCR with divergent and convergent primers for the verification of mecciRNAs. GAPDH mRNA, negative control; g+mtDNA, nuclear and mitochondrial DNA; RT, reverse transcription; for several gel images, a small open triangle is used to indicate the specific band from mecciRNA. **D**, Real-time qPCR and regular PCR showing the resistance of human mecciRNAs to RNase R digestion in HeLa cell. ciRS-7, positive controls; GAPDH mRNA, negative control. **E**, Real-time qPCR and regular PCR showing resistance of mouse mecciRNAs to RNase R digestion in HeLa cell and N2a cell. GAPDH mRNA, negative control; R+, R-, with or without RNase R digestion. In (**D**, **E**), error bars, s.e.m.; n=3 independent experiments.

**Figure S3** Analyses of related NGS data, Related to Figure 1. **A**, No DNA read matching junction sequences of human mecciRNAs in re-sequencing reads of genomic DNA (Nuc-DNA) and mitochondrial DNA (Mt-DNA) from HeLa cells. **B**,In RNA-seq data of cells without mitochondria (Rho0 MEF cells), no mecciRNA was identified, whereas mecciRNAs were found in wildtype MEF cells (Shimada et al., 2018). **C**,Nascent circRNAs in HeLa cells identified from RNA-seq data (Bao et al., 2018). **D**, The 4nt (allowing 1nt mismatch) upstream of the 5’ junction site was repeated at the upstream of the 3’ junction site in about 40-50% of mecciRNAs, and the 4nt (allowing 1nt mismatch) downstream of the 3’ junction site was repeated at the downstream of the 5’ junction site in about 40-50% of mecciRNAs. 300 g-circRNAs randomly picked were analyzed for comparison.

**Figure S4** Mitochondrial and cytosolic distributions of mecciRNAs, Related to Figure 1. **A**, g-circRNAs and mecciRNAs from sequencing data of cytoplasmic (cyto) and mitochondrial (mito) RNAs (HeLa cells). **B**,mecciRNAs have both mitochondrial and cytosolic distributions. ATPase6 is a mitochondrial encoded mRNA. The mitochondrial 16S rRNA is used as endogenous control in qRT-PCR. **C**, IF of TOM20 together with FISH signals of ND1 mRNA and mecciND1. Boxed areas are enlarged. Colocalization between ND1 FISH signals (G, green) and TOM20 (R, red) is shown (n=20 randomly selected areas). R/G, The proportion of red signal to green signal colocalization; G/R, The proportion of green signal to red signal colocalization. In (**C**), scale bars, 5 µm and 500 nm (enlarged areas); in (**B**), error bars, s.e.m.; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001 by Student’s *t*-test.

**Figure S5** mecciND1 interacts with RPA70 and RPA32, Related to Figure 2. **A**, The position of antisense oligo and mecciND1 pulldown efficiency are shown; Actin mRNA served as negative control. **B**,Pulldown of mecciND1 with biotin labeled antisense oligo (AS oligo) in HeLa mitochondrial lysate. RPA70 and RPA32 co-pulled down with mecciND1 were verified by Western blots; NDUFB8, negative control. **C**, mecciRNAs from RIP against RPA32 (α-RPA32) with whole-cell HeLa cell lysates were quantified by real-time qPCR (supplement for Figure 2D). **D,** Confocal images in single z-section of immunofluorescence (IF) for RPA70 (upper) and RPA32 (lower) together with TOM20 as well as FISH of mecciND1. Boxed areas are enlarged. Colocalization between RPA70 or RPA32 (G, green), TOM20 (R, red), and mecciND1 (M, magenta) is shown (n=20 randomly selected areas). **E**, Representative structured illumination microscopy (N-SIM) image in z-stacks and single z-section of immunofluorescence (IF) for RPA32 together with TOM40 as well as FISH of mecciND1 in fixed HeLa cells. Boxed areas are enlarged. In (**D**), scale bars, 5 µm and 500 nm (enlarged areas); in (**E**), scale bars, 2 µm and 200 nm (enlarged areas); in (**A**-**C**), error bars, s.e.m.; n=3 independent experiments; \*\*P < 0.01; \*\*\*P < 0.001, Student’s *t*-test.

**Figure S6** mecciND5 interacts with hnRNPA proteins, Related to Figure 2. **A**, Upper panel, the position of antisense oligo and mecciND5 pulldown efficiency are shown; Actin mRNA served as negative control. Bottom panel, mecciRNAs from RIP against hnRNPA1 (α-hnRNPA1) with whole-cell HeLa cell lysates were quantified by real-time qPCR (supplement for Figure 2H). **B**, Homology of hnRNPA1, hnRNPA2B1, and hnRNPA3 proteins and alignment of their RNA recognition motif (RRM). **C**,Confocal images in single z-section ofimmunofluorescence (IF) for hnRNPA1 (upper), hnRNPA2B1 (middle), and hnRNPA3 (lower) together with TOM20 as well as FISH of mecciND5. Boxed areas are enlarged. Colocalization between hnRNPA1, hnRNPA2B1, or hnRNPA3 (G, green), TOM20 (R, red), and mecciND5 (M, magenta) is shown (n=20 randomly selected areas). **D**, Representative structured illumination microscopy (N-SIM) image in z-stacks and single z-section of immunofluorescence (IF) for hnRNPA1 together with TOM40 as well as FISH of mecciND5 in fixed HeLa cells. Boxed areas are enlarged. In (**C**), scale bars, 5 µm and 500nm (enlarged areas). In(**D**), scale bars, 2 µm and 200 nm (enlarged areas); in (**A**), error bars, s.e.m.; n=3 independent experiments; \*\*\*P < 0.001, Student’s *t*-test.

**Figure S7** Correlations between mecciND1 and mitochondrial RPA levels, Related to Figure 3. **A**, Knockdown efficiency of mecciND1 in 293T for Figure 3A; si-NC, siRNA with scrambled sequences. **B**,Changes in mitochondrial RPA protein levels upon the transfection of antisense morpholino oligos (AMO) against mecciND1 (mecciND1-AMO). Quantification of RPA proteins is shown (normalized to TIM23, a mitochondrial inner membrane protein, and then with scra-AMO control group as 1.0; scra-AMO, AMO with scrambled sequences). Whole-cell levels and cytosolic levels of mecciND1 are shown with bar figure. **C**, Diagram of mecciND1 overexpression plasmid. MecciND1 RNA levels increased in both whole cells (total level) and mitochondria (mito level) through the transfected overexpression plasmid (OE-mecciND1). **D**, RPA70 and RPA32 mRNA levels were unchanged after the knockdown or overexpression of mecciND1. **E**, Knockdown efficiency of mecciND1 in RPE-1 for Figure 3C, 3D and Figure S7F; si-NC, siRNA with scrambled sequences. **F**, Representative structured illumination microscopy images in z-stacks (3D N-SIM) of immunofluorescence (IF) for PRA32 together with TOM40 as well as FISH of mecciND1 in fixed RPE-1 cells transfected with siRNA (si-NC or si-mecciND1). Single z-section images of boxed areas are enlarged. Scale bars, 2 µm and 200 nm (enlarged areas).**G,** Representative structured illumination microscopy images in z-stacks (3D N-SIM) of immunofluorescence (IF) for PRA32 in the nucleus of fixed RPE-1 cells transfected with siRNA (si-NC or si-mecciND1). Scale bars, 2 µm. Quantification of immunofluorescence intensity of PRA32 is shown in the bar figure. In (**A-E**), relative RNA levels were normalized to 18S rRNA (for total level and cytosol level) and 16S rRNA (for mito level), error bars; s.e.m.; n=3 independent experiments; ns, not significant; \*P < 0.05; \*\*\*P < 0.001, Student’s *t*-test.

**Figure S8** Correlations between mecciND5 and mitochondrial hnRNPA levels, Related to Figure 4. **A**, Knockdown efficiency of mecciND5 for Figure 4A,si-NC, siRNA with scrambled sequences; hnRNPA1, hnRNPA2B1, and hnRNPA3 mRNA levels were examined under mecciND5 knockdown. **B,** Changes in mitochondrial hnRNPA protein levels upon the transfection of antisense morpholino oligos (AMO) against mecciND5 (mecciND5-AMO). Quantification of hnRNPA proteins is shown (normalized to TIM23, a mitochondrial inner membrane protein, and then with scra-AMO control group as 1.0; scra-AMO, AMO with scrambled sequences). Whole cell levels and cytosolic levels of mecciND5 are shown with bar figure. **C**, Diagram of mecciND5 overexpression plasmid. MecciND5 levels increased in both whole cells (total level) and mitochondria (mito level) through plasmid overexpression (OE). **D**, hnRNPA1, hnRNPA2B2, and hnRNPA3 mRNA levels upon mecciND5 overexpression. **E**, Knockdown efficiency of mecciND5 for Figure 4C, 4D and Figure S8F. **F**, Representative structured illumination microscopy images in z-stacks (3D N-SIM) of immunofluorescence (IF) for hnRNPA1 together with TOM40 as well as FISH of mecciND5 in fixed RPE-1 cells transfected with siRNA (si-NC or si-mecciND5). Single z-section images of boxed areas are enlarged. Scale bars, 2 µm and 200 nm (enlarged areas). **G,** Representative structured illumination microscopy images in z-stacks (3D N-SIM) of immunofluorescence (IF) for hnRNPA1 in the nucleus of fixed RPE-1 cells transfected with siRNA (si-NC or si-mecciND5). Scale bars, 2 µm. Quantification of immunofluorescence intensity of hnRNPA1 is shown in the bar figure. In (**A-E**), relative RNA levels were normalized to 18S rRNA (total level and cytosol level) and 16S rRNA (mito level); error bars, s.e.m.; n=3 independent experiments; ns, not significant; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001, Student’s *t*-test.

**Figure S9** mecciND1 and mecciND5 copy number per cell and *in vitro* assays, Related to Figure 5. **A**, mecciND1 and mecciND5 copy number per cell in 293T, HeLa, RPE-1, and HepG2 cell lines. **B**, Schematic diagram showing procedures of linear RNA generation and *in vitro* circularization (Wesselhoeft et al., 2018). E1, 14 bp of Td gene exon1; E2, 15 bp of Td gene exon2. **C**, 5% Urea PAGE gel of purified circular RNAs, mecciND1, mecciND5, and circSRSF. **D**, Semi-quantitative RT-PCR of RNA import results for Figure 5B. **e**, Semi-quantitative RT-PCR of RNA import results for Figure 5C. **F**, mecciND1 or mecciND5 added with RPA32 mRNA in the rabbit reticulocyte translational system (co-translational for 30 mins) and the mitochondrial importation of the in vitro-translated RPA32-FLAG protein. Upper, translation products of RPA32-FLAG protein (Input); lower, RPA32-FLAG protein imported into mitochondria. G, mecciND5 or mecciND1 added together with hnRNPA1 mRNA in the rabbit reticulocyte translational system (co-translational for 30 mins) and the mitochondrial importation of in vitro-translated hnRNPA1-FLAG protein. Upper, translation products of hnRNPA1-FLAG protein (Input); lower, hnRNPA1-FLAG protein imported into mitochondria. **H**, Semi-quantitative RT-PCR showed that mecciND1 and mecciND5 imported into mitochondria could be exported out in the *in vitro* assays. Super, supernatant; Pellet, mitochondria. In (**F** and **G**), data is mean±s.e.m., n=3 independent experiments; ns, not significant; \*P < 0.05; \*\*P < 0.01; Student’s *t*-test.

**Figure S10** Western blots of proteins in the RNA-IP (for Figure 7A) and knockdown and overexpression of PNPASE, Related to Figure 5. **A**, Western blots of overexpressed TOM20-FLAG, TOM40-FLAG and PNPASE-FLAG. NDUFB8, a mitochondrial marker. **B**, Western blots to show the successful IP with anti-FLAG antibodies (ɑ-FLAG). Open triangles indicate specific bands of ACTB and \* denotes antibody heavy chain. **C**, RT-qPCR and Western blots showed the knockdown efficiency of PNPASE for Figure 5G. TOM40, served as loading control. **D**, mecciND1 and mecciND5 RNA levels of 293T cells under PNPASE overexpression. RMRP RNA, a known PNPASE interacting RNA imported into mitochondria. Vec, vector control; PNP, PNPASE overexpression. **E**, Western blots to show the successful IP with anti-FLAG antibodies (ɑ-FLAG). Open triangles indicate specific bands of ACTB and \* denotes antibody heavy chain. In (**C, D**), relative RNA levels were normalized to 18S rRNA (total level) and 16S rRNA (mito level); error bars, s.e.m.; n=3 independent experiments; ns, not significant; \*\*P < 0.01; \*\*\*P < 0.001, Student’s t-test.

**Figure S11** Secondary structure of mecciND1 and mecciND5, Related to Figure 5. **A**, A predicted secondary structure of mecciND1 generated by the mfold. Boxed areas are enlarged. mecciND1 stem-loop mutation sites are shown in red (related to Figure 5I). **B,** *In vivo* secondary structure of mecciND1 sequence in published icSHAPE data (Sun et al., 2019). The underlined sequences corresponded to the boxed stem-loop structure in (**A**). **C**, A predicted secondary structure of mecciND5 generated by the mfold Web Server. Boxed areas are enlarged. mecciND5 stem-loop mutation sites are shown in red (related to Figure 5I). **D**, *In vivo* secondary structure of mecciND5 sequence in published icSHAPE data. The underlined sequences corresponded to the boxed stem-loop structure in (**D**).

**Figure S12** mecciND1 levels under stress, mecciND5 levels in HCC, mecciRNAs in *S. pombe* and *C. elegans*. Related to Figure 6. **A**, **B,** Expression of mecciND1 increased under 4 h hypoxia culture (**a**), and 2 h tunicamycin (TM) treatment (**B**). **C**, mecciND5 levels in pairs of tumor samples and adjacent tissues from 21 Hepatocellular carcinoma (HCC) patients. **D**, Identification of mecciRNAs in *S. pombe* using convergent and divergent primers. g+mtDNA, nuclear and mitochondrial DNA; no RT, reverse transcription reaction without reverse transcriptase. **E**, Identification of mecciRNAs in *C. elegans* using convergent and divergent primers. g+mtDNA, nuclear and mitochondrial DNA; no RT, reverse transcription reaction without reverse transcriptase. **F**, Images of muscle mitochondria (*myo-3p::tom20(1–49aa)::gfp*) of worms subjected to feeding RNAi against mecciRNAs; L4440, negative control. Efficient knockdown of mecciRNAs by feeding RNAi is shown. Scale bar, 10 µm. In (**A-C, F**), qRT-PCR relative RNA levels were normalized to 18S rRNA, n=3 independent experiments (**A, B, F**), error bars, s.e.m.; ns, not significant; \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, by Student’s t-test.