Supplemental Figures

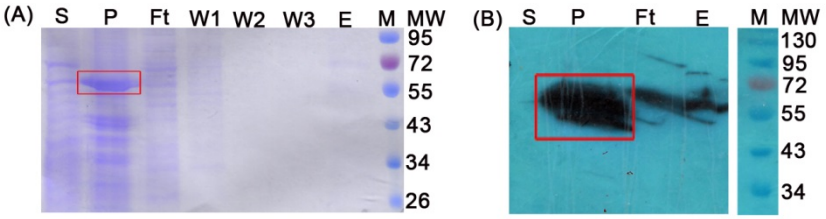

Figure S1. (A) SDS-PAGE showing MICU2-I accumulation in the precipitant. (B) Western blot showing MICU2-I in the precipitant. (Lane S and P: the supernatant and precipitant samples of MICU2-I after sonication; Lane Ft: samples flow through the resin; Lane W: after loading the supernatant on the MBP resin, samples from buffer wash; Lane E: the eluted samples form the resin; Lane M: molecular weight marker) The white vertical line refers to the fact that MW markers were loaded on the same SDS-PAGE, but not in the adjacent lanes.

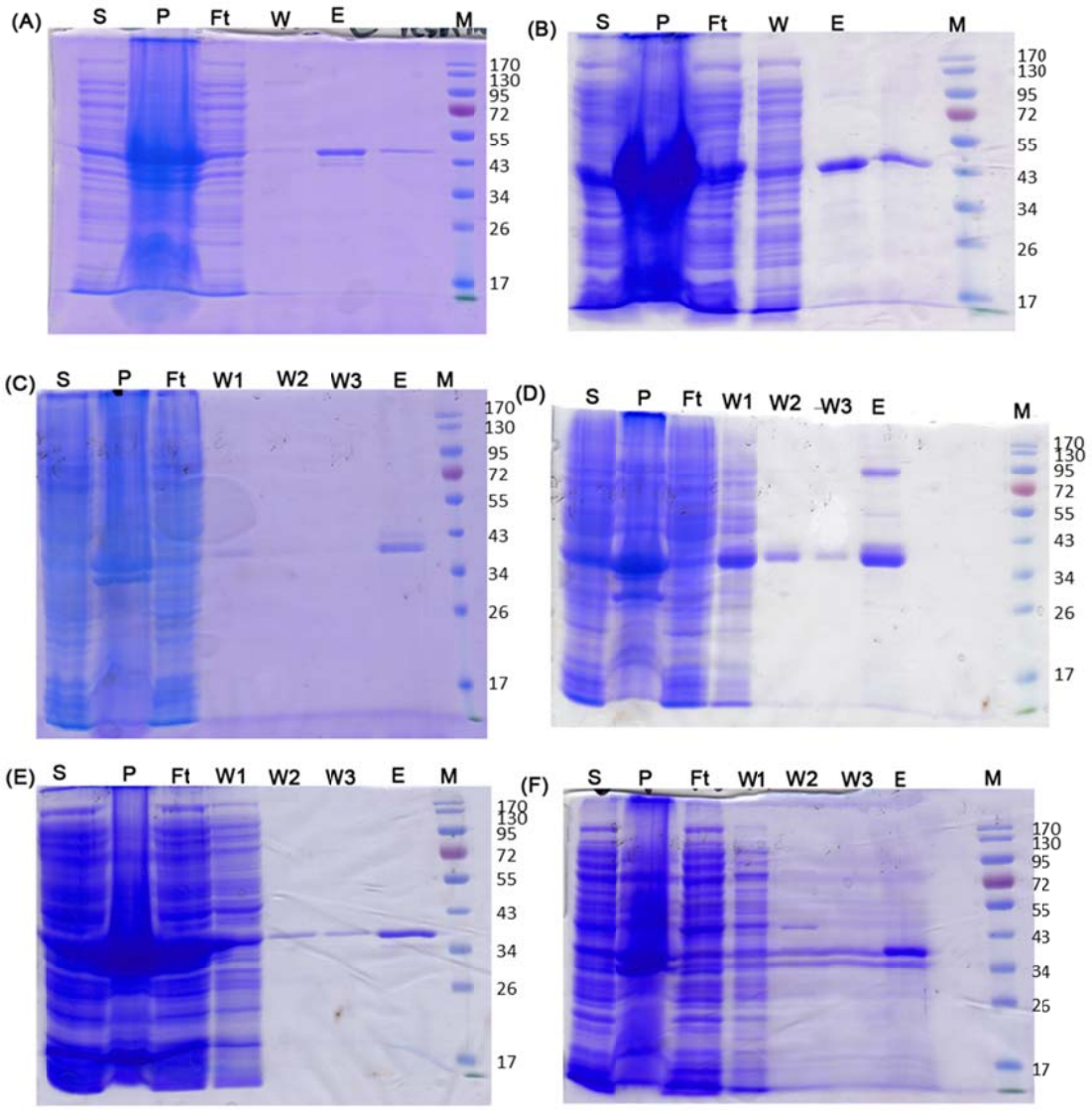

Figure S2. SDS-PAGE analysis in protein purification by His-tag based affinity chromatography. SDS-PAGE analysis of MICU2-NΔ66 (A), MICU2-NΔ84 (B), MICU2-NΔ66-CΔ28 (C), MICU2-NΔ66-CΔ36 (D), MICU2-NΔ84-CΔ28 (E), MICU2-NΔ84-CΔ36 (F). (Lane S and P: the supernatant and precipitant samples after sonication; Lane Ft: samples flow through the resin; Lane W: after loading the supernatant on the MBP resin, samples from buffer wash; Lane E: the eluted samples form the resin; Lane M: molecular weight marker)

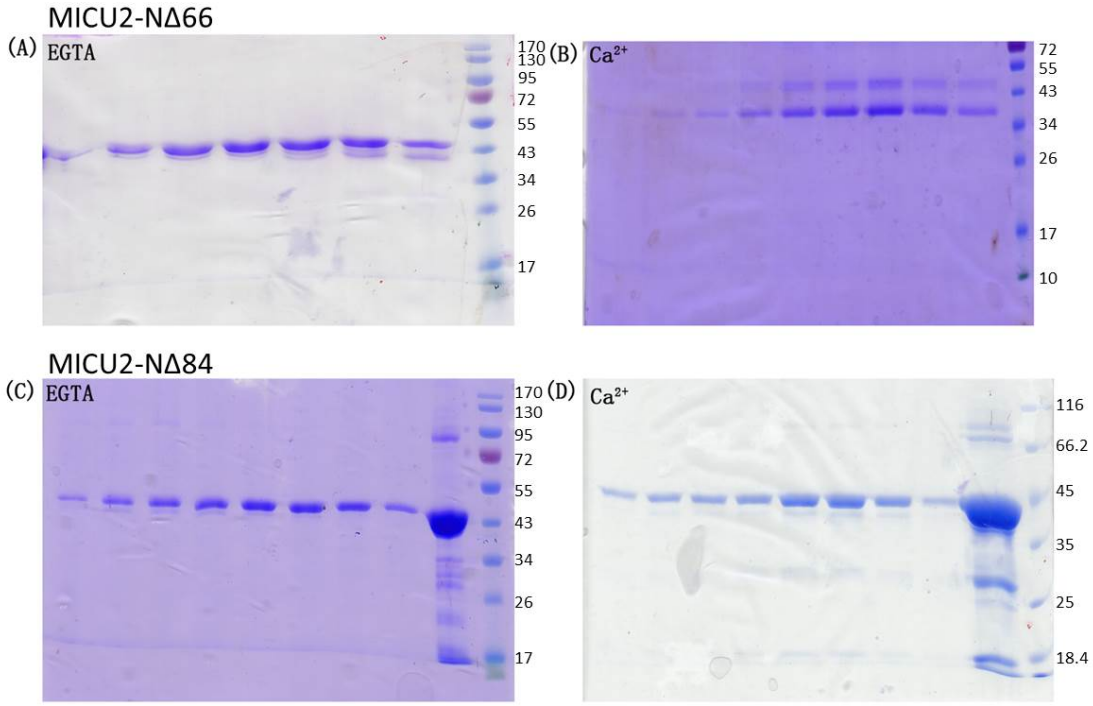

Figure S3. SDS-PAGE analysis of the main peak fractions of MICU2 proteins purified by size exclusion chromatography with or without Ca^{2+}. (A,B) Fraction analysis of MICU2-NΔ66 with EGTA or Ca^{2+}. (C,D) Fraction analysis of MICU2-NΔ84 in the conditions of EGTA or Ca^{2+}.

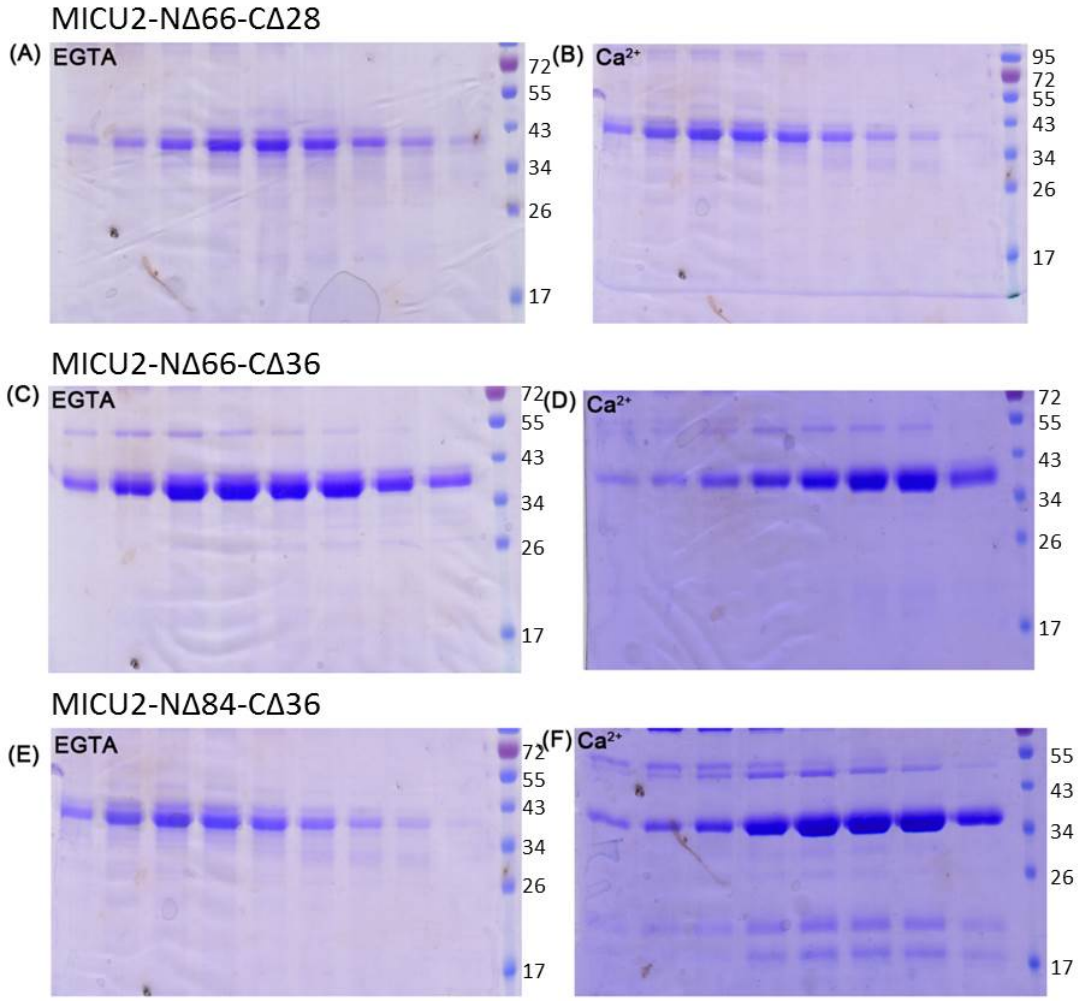

Figure S4. SDS-PAGE analysis of the main peak fractions for MICU2 proteins purified by size exclusion chromatography in the conditions with or without Ca^{2+}. (A,B) Fraction analysis of MICU2-NΔ66-CΔ28 in EGTA or Ca^{2+}conditions. (C,D) Fraction analysis of MICU2-NΔ66-CΔ36 in EGTA or Ca^{2+}conditions. (E,F) Fraction analysis of MICU2-NΔ84-CΔ28 in EGTA or Ca^{2+}conditions. (G,H) Fraction analysis of MICU2-NΔ84-CΔ36 in EGTA or Ca^{2+}conditions.

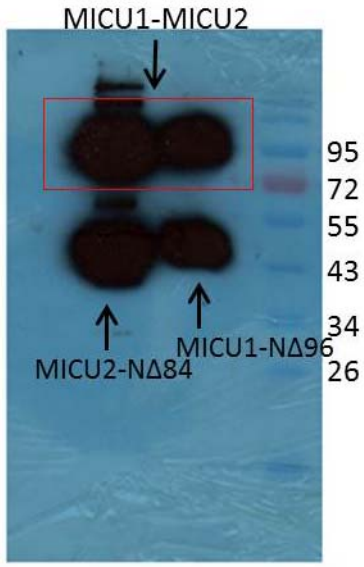

Figure S5. Pull-down experiment of MICU1-Δ96 and MICU2-Δ84 through coexpression in *E. coli* and purified by Ni-NTA resin. Eluted samples were analysed by western blot using non-reducing SDS-PAGE. Bands at ~95 kDa indicate that MICU1 and MICU2 could form heterodimer through disulfide bond.

Supplemental Tables

Table S1. Constructs for MICU1 and MICU2 truncations

Clone	Vector	Restriction site		
MICU1-NΔ54	pET-28a(+)		*Nco*I	*Xho*I
MICU1-NΔ96	pET-28a(+)		*Nco*I	*Xho*I
MICU1-NΔ96-Myc	pcDNA3.1		*BamH*I	*Xho*I
MICU2- NΔ84-Flag	pcDNA3.1		*Hind*III	*Xho*I
MICU2- NΔ84-CΔ36-Flag	pcDNA3.1		*Hind*III	*Xho*I
MICU1-NΔ96-MICU2-NΔ84	pETDuet-1	MCS1: MICU1-NΔ96	*Sal*I	*Not*I
		MCS2: MICU2-NΔ84	*Nde*I	*Xho*I
MICU2-NΔ84-MICU1-NΔ96	pETDuet-1	MCS1: MICU2-NΔ84	*Sal*I	*Not*I
		MCS2: MICU1-NΔ96	*Nde*I	*Xho*I

Table S2. Retention volume of the MICU2 constructs purified by size exclusion chromatography

Construction	Retention volume (mL)	
	EGTA	Ca^{2+}
MICU2-Δ66	85.8	80.6
MICU2-Δ84	83.3	80.7
MICU2-Δ66-C-Δ28	84.7	80.2
MICU2-Δ66-C-Δ36	83.4	80.2
MICU2-Δ84-C-Δ28	84.1	79.1
MICU2-Δ84-C-Δ36	83.9	80.9

Table S3. Retention volume of the MICU2 constructs purified by size exclusion chromatography

Construction	Retention volume (mL)	
	EGTA	Ca^{2+}
MICU2-Δ84-C-Δ28	86.1	81.7
MICU2-Δ84-C-Δ28^{EF1mut}	86.4	87.1
MICU2-Δ84-C-Δ28^{EF2mut}	84.8	80.4

Table S4. PDB DOI for the Structure of MICU1

	PDB DOI
Ca^{2+}-free	10.2210/pdb4nsc/pdb
Ca^{2+}-bound	10.2210/pdb4nsd/pdb