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SUPPLEMENTARY FIGURES



Supplementary Figure 1. mRNA expression levels of ACPM, ISCU, LIAS and ISCA1 after transfection of HEK293T cells with their respective targeting siRNA as determined by ddPCR. Data are shown as mean \pm SD (n=3). TBP was used as housekeeping gene and expressions were compared to cells transfected with non-targeting siRNA (siNT).



Supplementary Figure 2. Isolation and directed mutagenesis screen of the ACPM-ISD11 complex. A) Analysis of the ACPM-ISD11 at different steps of the purification by SDS PAGE and coomassie blue staining. GF: fraction after gel filtration; + TEV: fraction after TEV treatment to remove the His tag on ISD11; Ni rebind: fraction after nickel rebind. B) Representation of the ACPM-ISD11 complex using the *E. coli* Acp-ISD11 structure from pdb file 5usr. ISD11 is shown in green and ACPM in blue. Residues that showed an effect in the alanine screen are indicated. C) Western blot of input and elution fractions of the ACPM mutant screen. D) Western blot of input and elution fractions of the ISD11 mutant screen.



Supplementary Figure 3. Deconvoluted mass spectra for ACPM-ISD11 sample. **A)** Retention time 2.94 min. Intact ISD11 was detected as 10829 Da but with relative low abundance. The major species 9058 Da was assigned as the truncated version $ISD11_{tr}$, and other minor truncated species were also detected and assigned. **B)** Retention time 4.8-5.07 min. Masses higher than the expected 10169.5Da for apo-ACPM were detected, and assigned as the holo-ACPM with 4'-PP-acyl-chains.



Supplementary Figure 4. LC-UV-MS profiles for ACPM-ISD11 sample with major mass labeled for each of the abundant peaks.