SUPPLEMENTARY DATA

Molecular insights into HSD10 disease: impact of SDR5C1 mutations on the human mitochondrial RNase P complex

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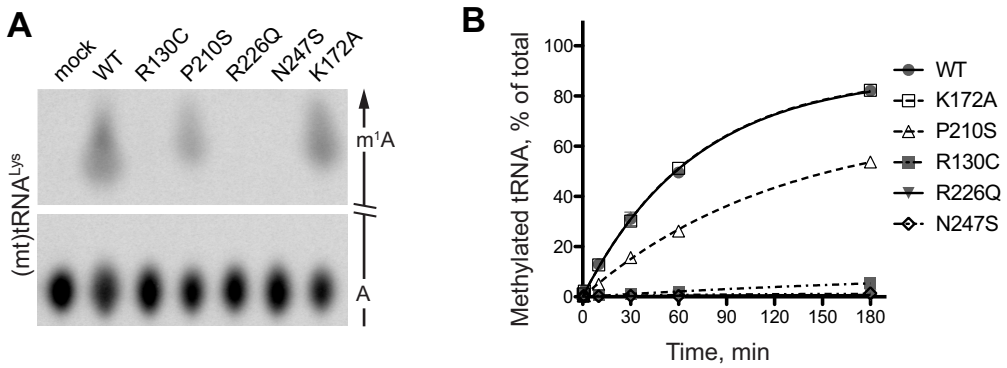
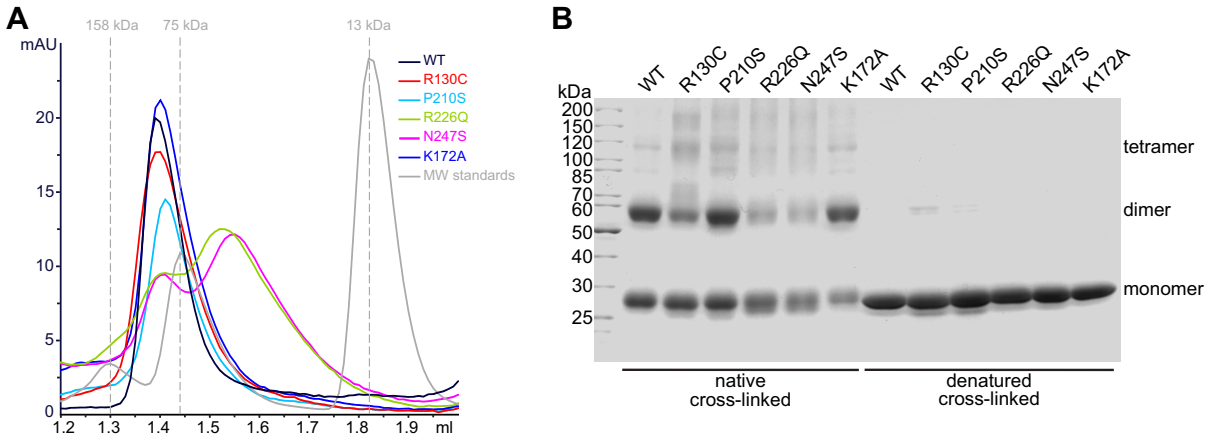


Supplementary Figure S1. Mutations in SDR5C1 affect the tRNA-methyltransferase activity of the TRMT10C-SDR5C1 complex. (**A** and **B**) The methytransferase subcomplex of mtRNase P was reconstituted from recombinant TRMT10C and wild type or mutant SDR5C1, and its activity assayed with position-9 labelled (mt)tRNA^{Lys} and SAM. Reaction aliquots were withdrawn and stopped at different time points and the tRNA hydrolysate resolved by TLC. In (**A**), the 30-minutes time point of a representative experiment is shown. The direction of migration and the positions of A and m^{1}A are indicated to the right; only the informative part of the TLC is shown. (**B**) Methylation data of two complete, independent experiments were plotted as means and SD, and fit by non-linear regression.



Supplementary Figure S2. Mutations in SDR5C1 affect its tetramerization. (**A**) Wild type and mutant, recombinant SDR5C1 were separated by size exclusion chromatography and the 280 nm-absorbance chromatograms overlaid. The elution profile of molecular weight standards is overlaid as a solid grey line, and the elution peaks are highlighted by vertical dashed lines: aldolase, 158 kDa; conalbumine, 75 kDa; cytochrome *c*, 13 kDa. Only the informative part of the chromatograms is shown. (**B**) Wild type and mutant SDR5C1 were subjected to rapid photochemical cross-linking, resolved by 6–18% gradient SDS-PAGE and stained by Coomassie brilliant blue. The molecular weight of marker proteins is indicated to the left. Cross-linked protein samples were loaded on the left part of the gel; control reactions (proteins SDS-denatured prior to cross-linking) were loaded on the right. The main bands and their interpretation with respect to oligomeric-state are specified to the right. The faint bands visible in the SDS-denatured samples R130C and P210S are minor contaminants in the protein preparation.