Cell Reports

Supplemental Information

MITRAC7 Acts as a COX1-Specific Chaperone and Reveals a Checkpoint during Cytochrome *c* Oxidase Assembly

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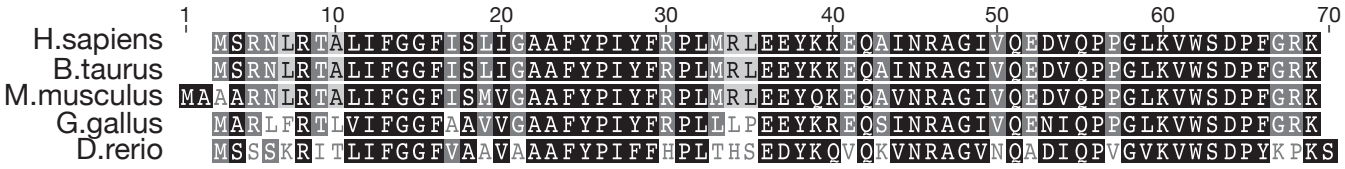

Figure S1. MITRAC7 is conserved in metazoa. Related to Figure 1.
Alignment of MITRAC7 homologous proteins of selected metazoa species using ClustalW2. Black boxes, 100% similar amino acids; dark grey, 80–100% similarity; light gray, 60– 80% similarity between species.

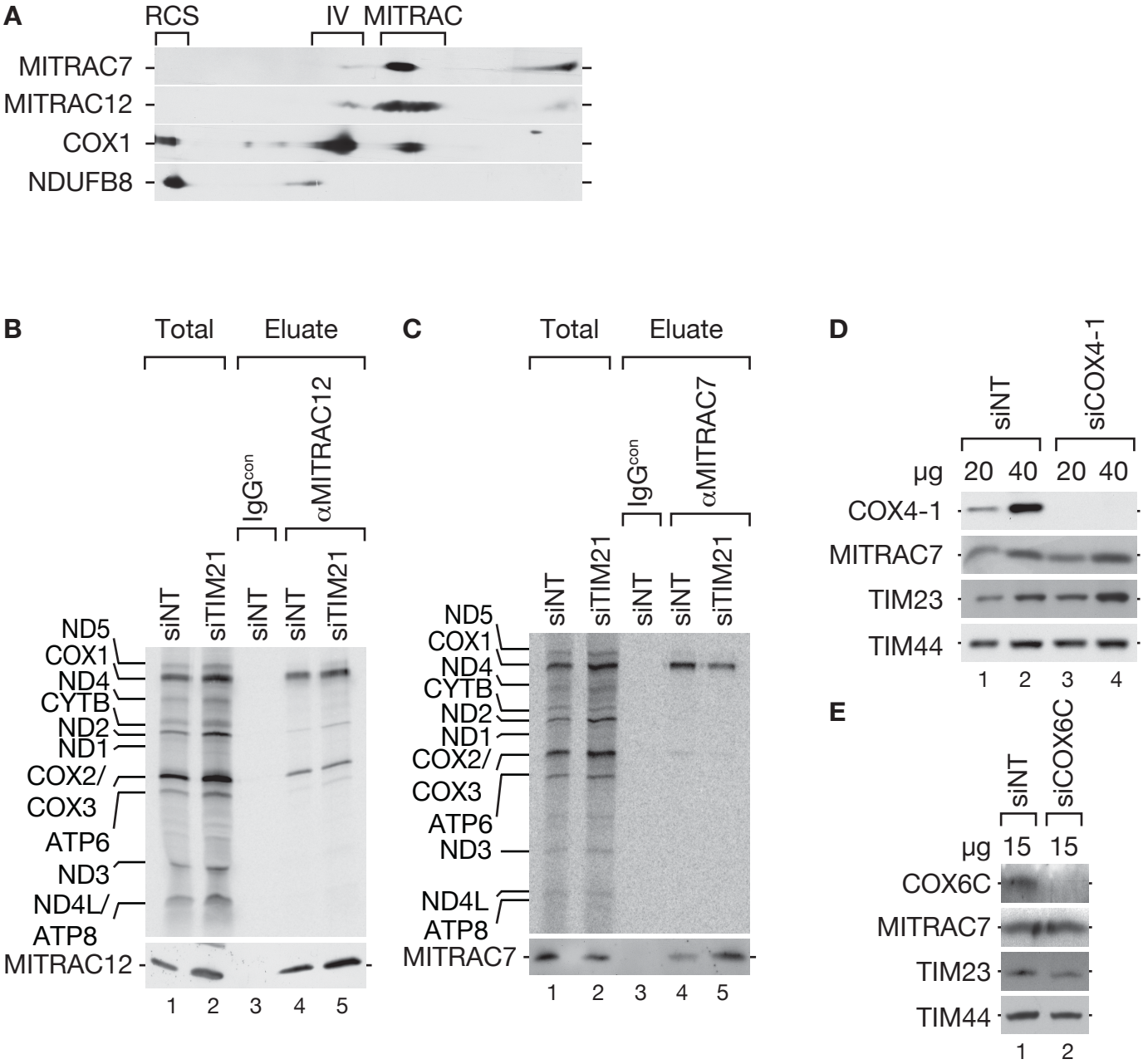

Figure S2. MITRAC7 comigrates with a slower MITRAC fraction and the association of MITRAC7 with COX1 depends on TIM21, COX4-1 and COX6C function.
Related to Figure 3.
(A) HEK293T mitochondria were analyzed by 2D-BN-/SDS-PAGE and Western-blotting. Membranes were probed with anti-MITRAC7, anti-MITRAC12, anti-COX1 and anti-NDUFB8 antibodies. RCS, respiratory-chain supercomplexes; IV, complex IV.
(B) and (C) HEK293T cells were treated with TIM21-specific or non-targeting control siRNA for 72 hr and mitochondrial translation products were labeled with [^{35}S]methionine. Whole cell lysates were subjected to immunoisolation with control (IgG), anti-MITRAC12 (B) or anti-MITRAC7 (C) antisera (Total, 5%; Eluate, 100%).
COX4-1 (D) or COX6C (E) were depleted with siRNA-applications, whole cell lysates analysed by Western-blotting and membranes probed with indictaed antibodies.

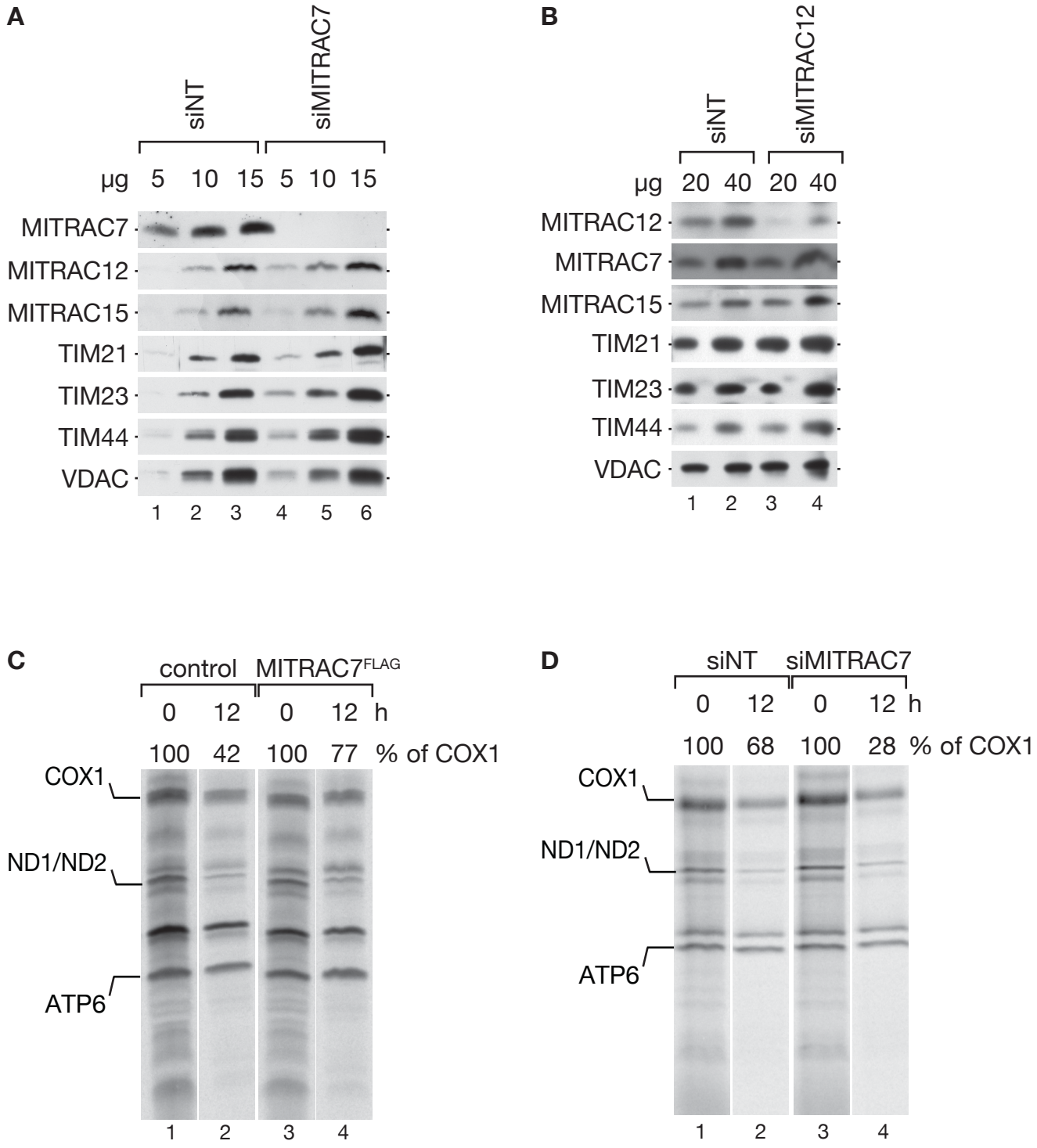

Figure S3. MITRAC7 and MITRAC12 stability do not depend on each other. Related to Figure 4 and 6.

After depletion of MITRAC7 or MITRAC12 in HEK293T cells, mitochondria (A) or cell extracts (B) were analyzed by Western-blotting. Membranes were probed with indicated antibodies.

(C) and (D) Mitochondrial translation products were pulse-labeled with [35S]methionine for 1hr either after MITRAC7 over expression (C) or depletion (D) and further cultured in the absence of [35S]methionine (chase) for 12 hr. Whole cell lysates (50 µg) were subjected to digital autoradiography.