SUPPLEMENTARY MATERIAL

ISCA1 MUTATION IN A PATIENT WITH INFANTILE-ONSET LEUKODYSTROPHY CAUSES DEFECTS IN MITOCHONDRIAL [4Fe-4S] PROTEINS

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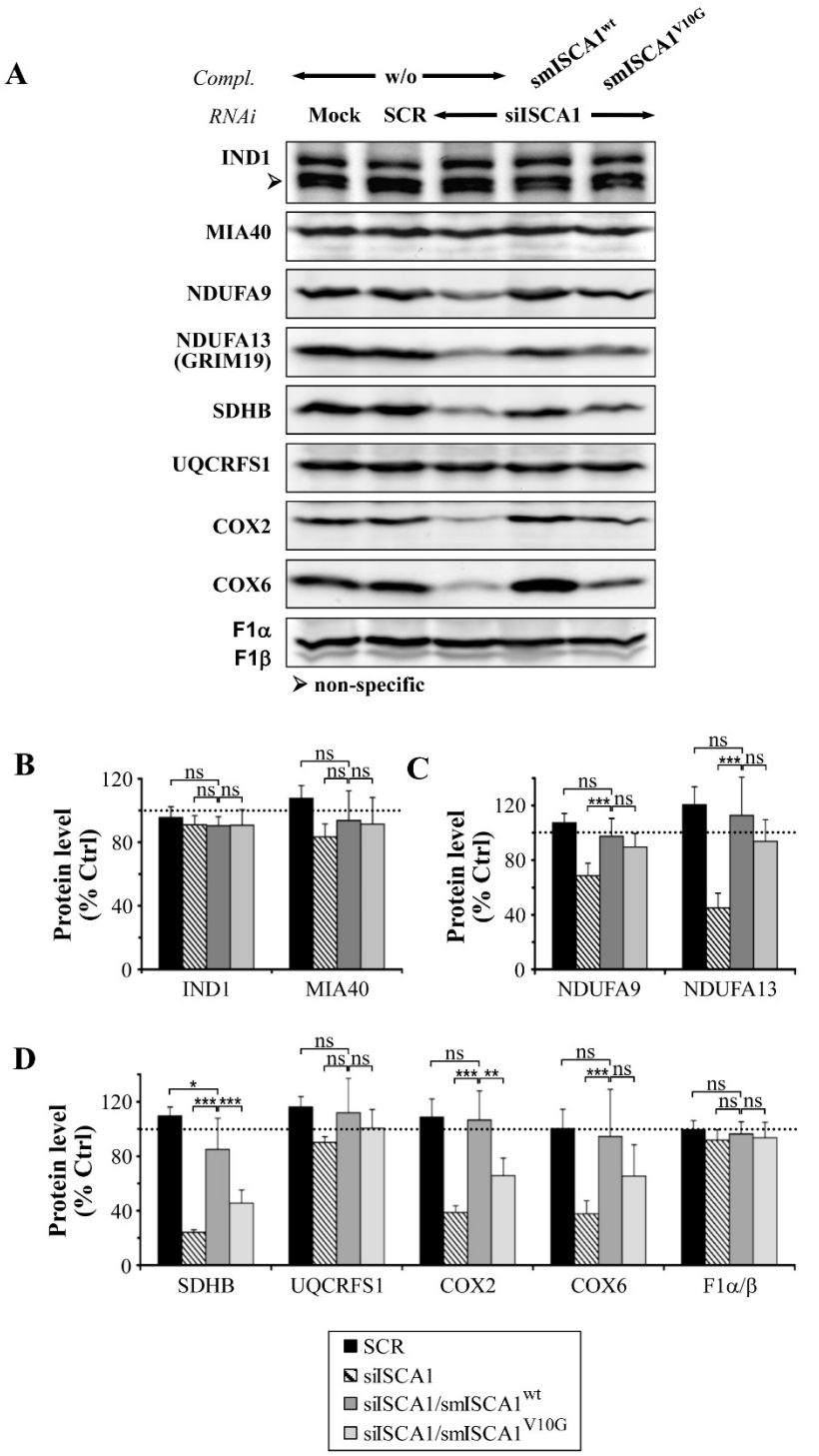
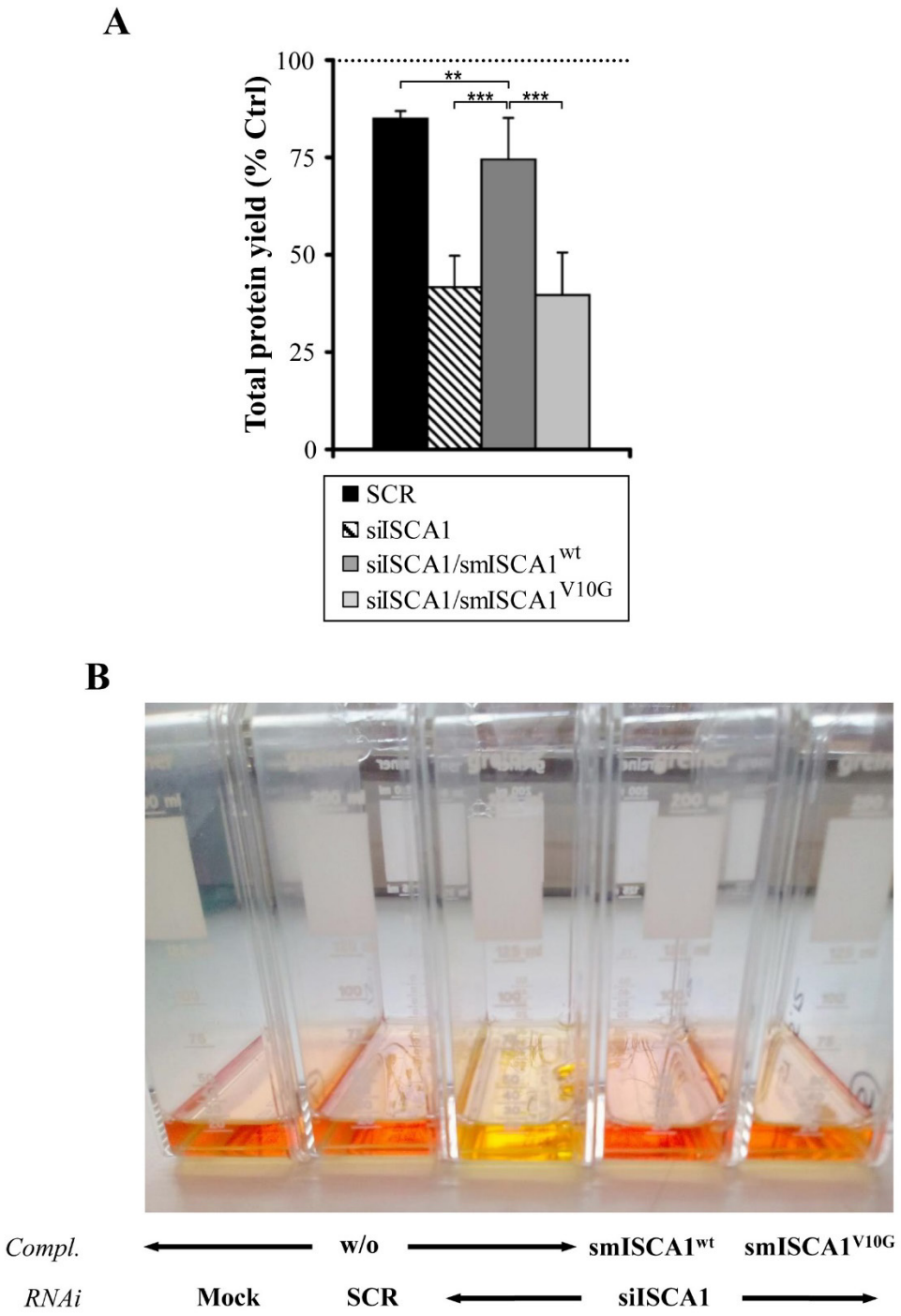
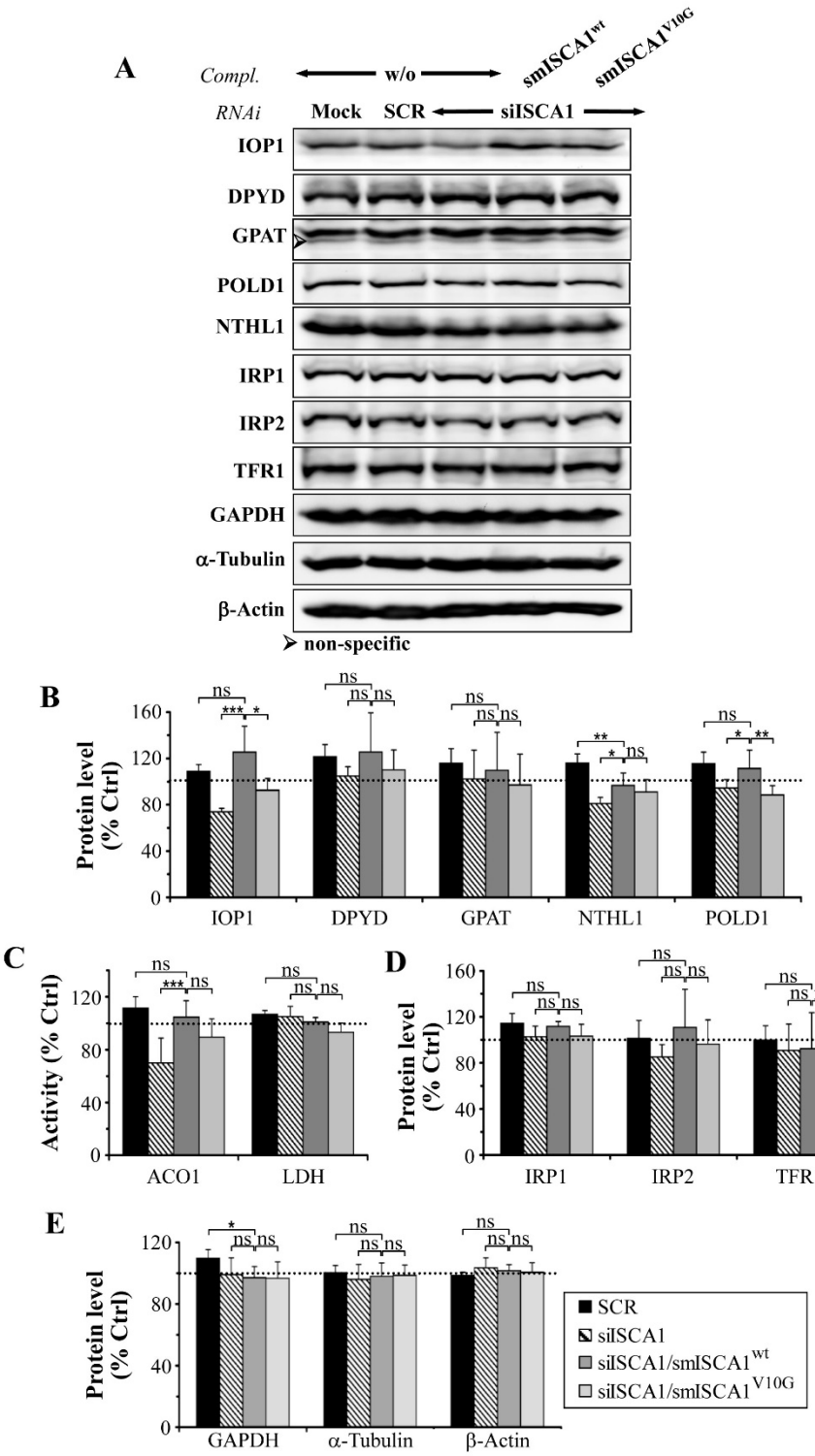
Supplementary Figures



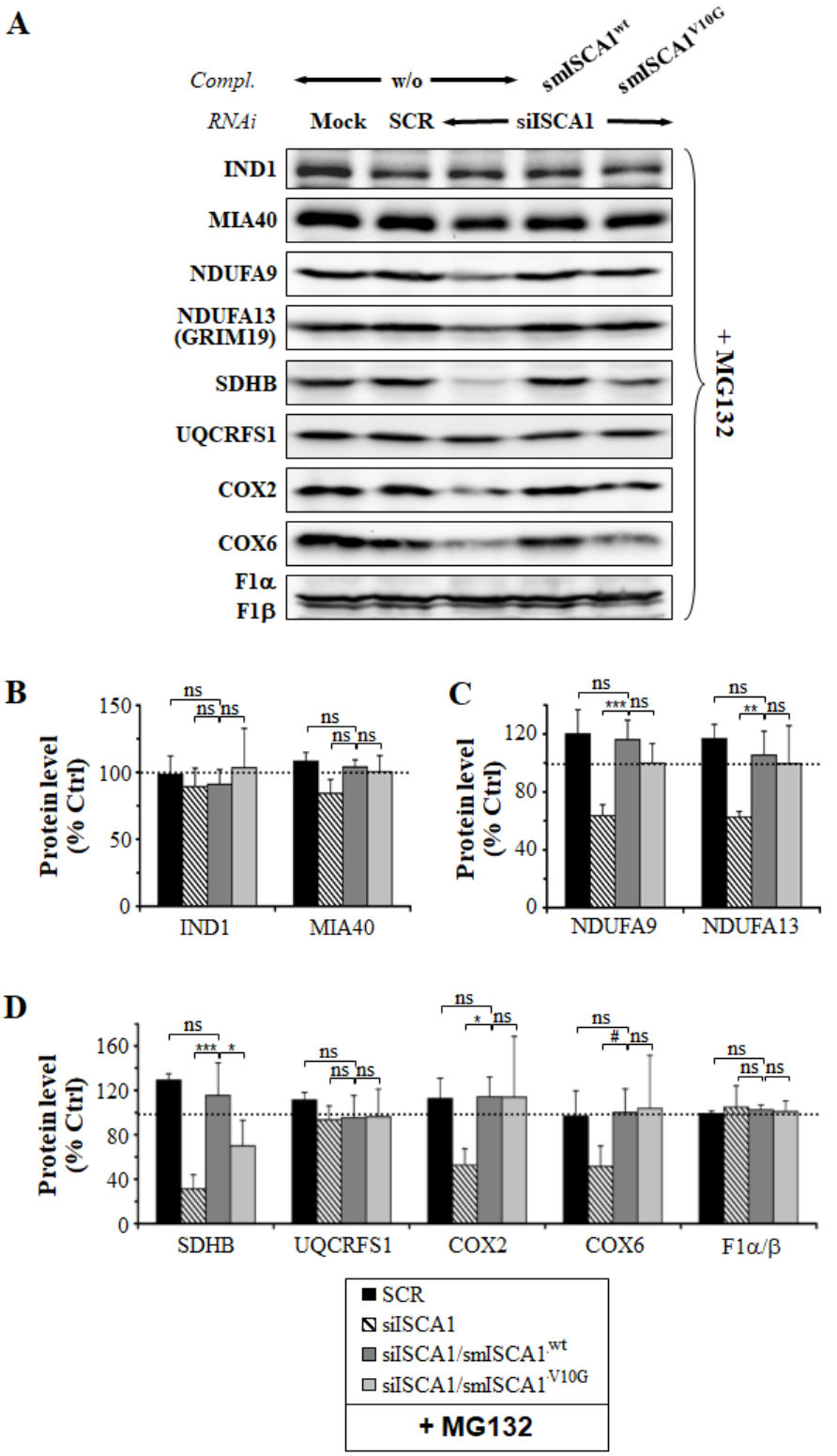
Supplementary Figure S1. The ISCA1 p.V10G mutation leads to decreased steady-state protein levels of RCC subunits. HeLa cells were transfected with siRNAs and ISCA1-encoding plasmids as in Fig. 4. Analyses were performed after the second round of transfection, i. e. after a total of 6 days of ISCA1 depletion. **(A)** Total cell lysates were subjected to immunoblotting and analyzed for the steady-state protein levels of the indicated proteins. The asterisk indicates a non-specific cross-reactivity of the anti-IND1 antiserum (Ref. 1). Representative blots are shown. **(B-D)** Immunoblot signals were quantified and the protein per β-actin ratios (Fig. 4A) were normalized to mock-transfected cells. All values are given as the mean ± SD (n = 4 to 5); *P<0.05, **P< 0.01, ***P<0.001; ns, not significant; dashed lines, 100%-value of mock-transfected control cells.



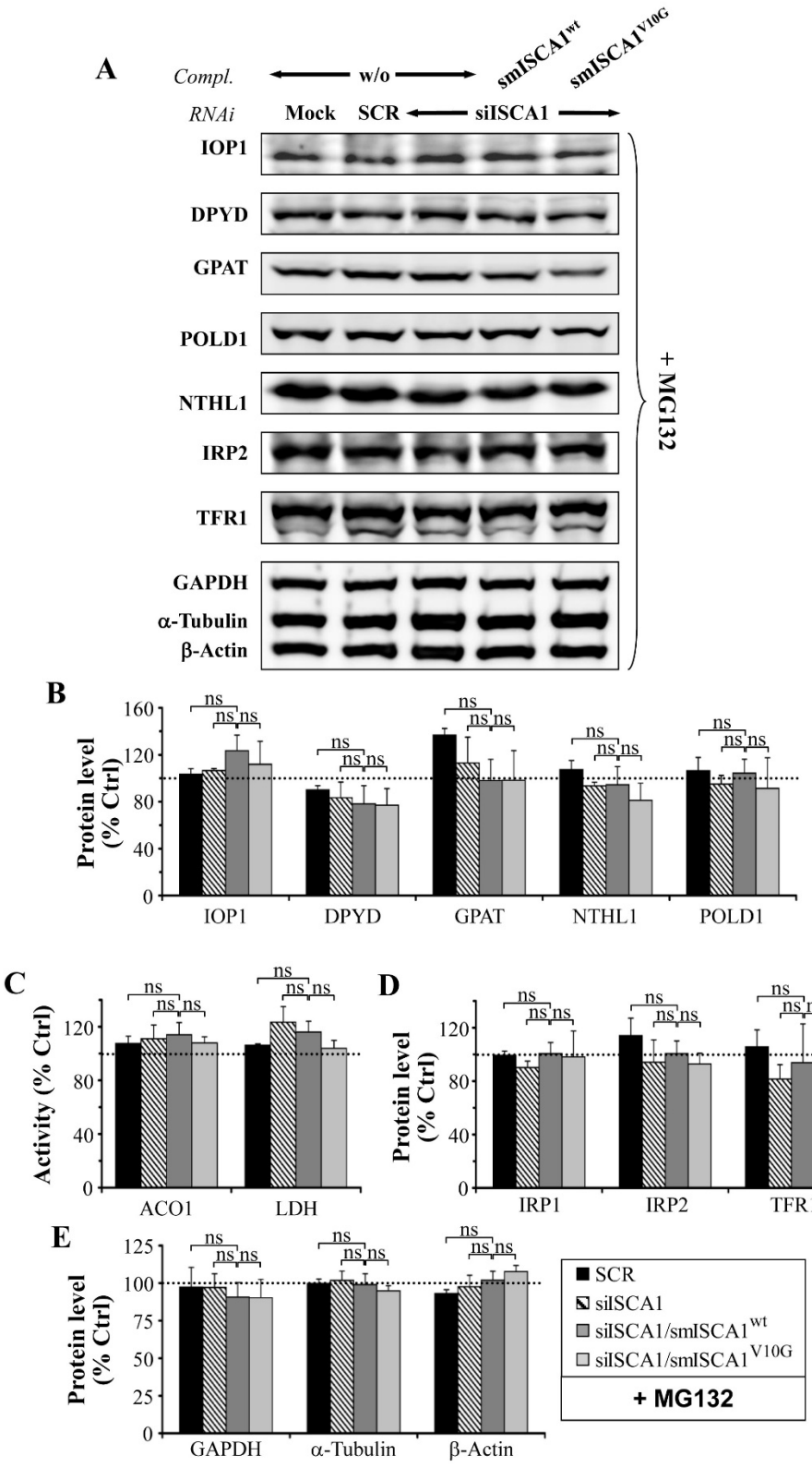
Supplementary Figure S2. The ISCA1^{V10G} protein does not restore growth of ISCA1-depleted cells. HeLa cells were transfected with siRNAs and ISCA1-encoding plasmids as in Fig. 4. **(A)** At each harvest (3 and 6 days after the initial transfections) total protein yield of the cultures was determined. The entire protein production during the entire 6-days culturing time was calculated and normalized to mock-transfected cells. Values are given as the mean ± SD (n = 6); *P<0.05, **P< 0.01, ***P<0.001; dashed line, 100%-value of mock-transfected control cells. **(B)** Culture media of cells from (A) contain the pH indicator phenol red. A color change from red to yellow indicates a drop in pH.



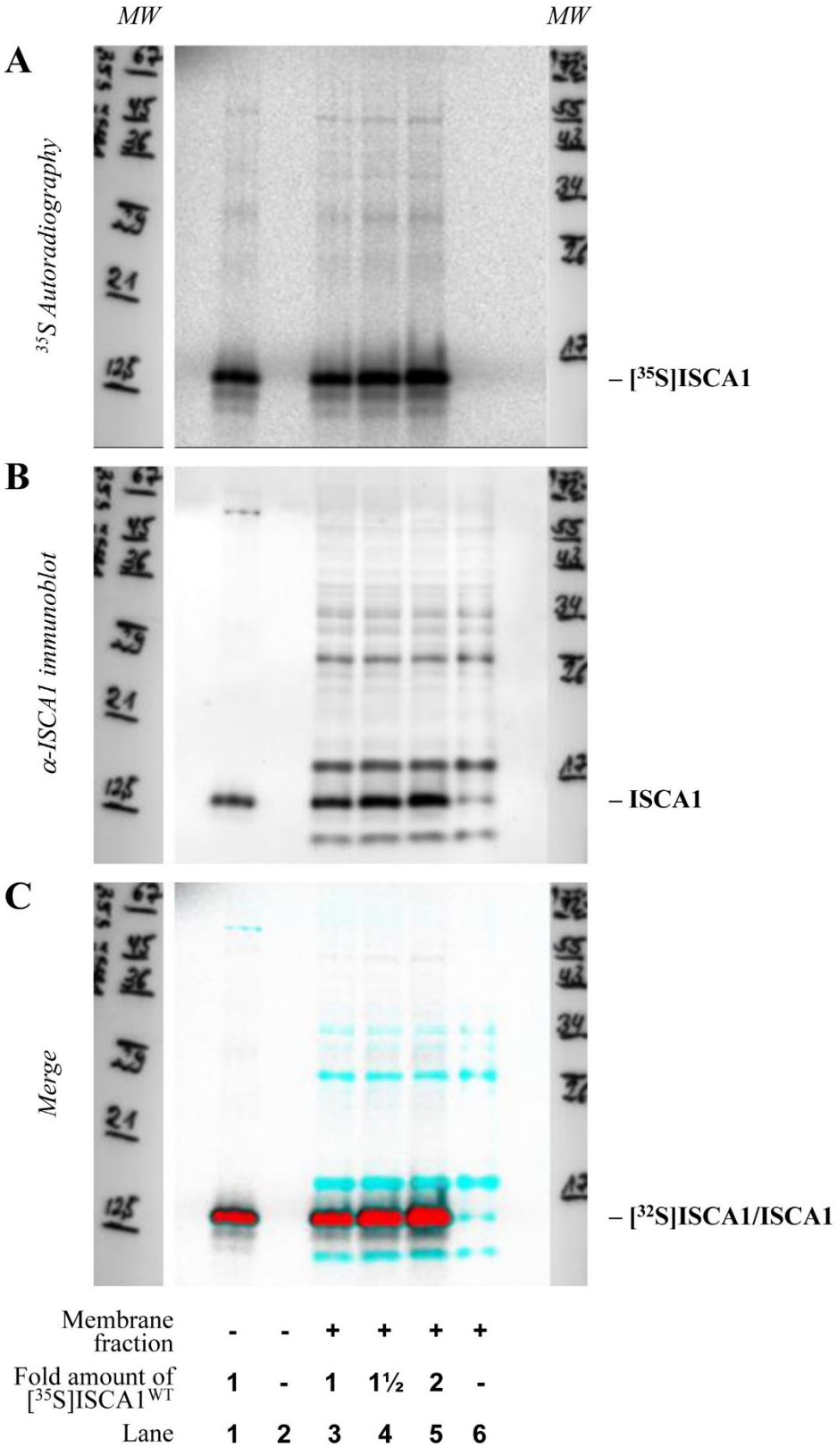
Supplementary Figure S3. The ISCA1 p.V10G mutation hardly affects extra-mitochondrial Fe-S proteins. HeLa cells were transfected with siRNAs and ISCA1-encoding plasmids and treated as in Fig. 4. **(A)** Total cell lysates were subjected to immunoblotting and analyzed for the steady-state protein levels of the indicated proteins. Representative blots are shown. **(B, D, E)** Immunoblot signals were quantified, and the protein per β-actin ratios were normalized to mock-transfected cells. The levels of β-actin itself were directly normalized to its levels in mock-treated cells. **(C)** The specific activity of cytosolic aconitase (ACO1/IRP1) was determined in the cytosolic fractions prepared by digitonin-based cell fractionation and normalized to the values deduced from mock-transfected control (Ctrl) cells. As a reference, the specific activity of lactate dehydrogenase (LDH) was determined. All values are given as the mean ± SD (n = 4 to 5); *P<0.05, **P< 0.01, ***P<0.001; ns, not significant; dashed lines, 100%-value of mock-transfected control cells.



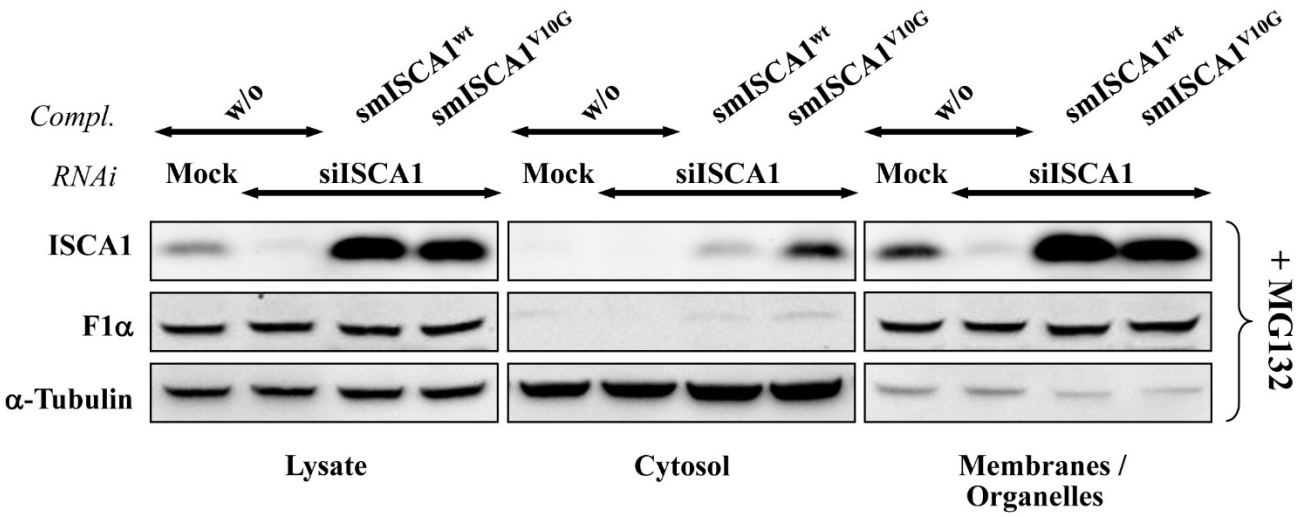
Supplementary Figure S4. MG132 treatment increases mitochondrial ISCA1^{V10G} levels without raising the low amounts of RCC subunits in ISCA1-depleted HeLa cells. HeLa cells were transfected and treated as in Fig. 4 and further incubated with MG132 protease inhibitor as in Fig. 5. **(A)** Total cell lysates were subjected to immunoblotting and analyzed for the steady-state protein levels of the indicated proteins. Representative blots are shown. **(B - D)** Immunoblot signals were quantified and the protein per β-actin ratios were normalized to mock-transfected cells. Compared to the results shown in Suppl. Fig. S1 (no MG132 treatment) the increased amounts of ISCA1^{V10G} upon MG132 treatment did not significantly alleviate the analyzed RCC subunit levels. All values are given as the mean ± SD (n = 3 to 4); *P<0.05, **P< 0.01, ***P<0.001; #, p < 0.1; ns, not significant; dashed lines, 100%-value of mock-transfected control cells.



Supplementary Figure S5. The ISCA1 p.V10G mutation hardly affects extra-mitochondrial Fe-S proteins in MG132-treated cells. HeLa cells were transfected and treated with MG132 as in Fig. 5. **(A)** Total cell lysates were subjected to immunoblotting and analyzed for the steady-state protein levels of the indicated proteins. Representative blots are shown. **(B, D, E).** Immunoblot signals were quantified and the protein per β-actin ratios were normalized to mock-transfected cells. The levels of β-actin itself were directly normalized to its levels in mock-treated cells. **(C)** The specific activities of cytosolic aconitase (ACO1/IRP1) and LDH were determined in the cytosolic fractions prepared by digitonin-based cell fractionation. All values are given as the mean ± SD (n = 3 to 4); *P<0.05, **P< 0.01, ***P<0.001; #, p < 0.1; ns, not significant dashed lines, 100%-value of mock-transfected control cells.



Supplementary Figure S6. ISCA1 is not processed. HeLa organellar material and *in vitro* translated [^{35}S] radiolabeled ISCA1^{wt} were mixed (lanes 3 to 5) or remained unmixed (lanes 1 and 6), and were subjected to 16% tricine-SDS gel electrophoresis and blotting according to Fig. 6C. **(A)** Autoradiograph and **(B)** anti-ISCA1 immunostaining were **(C)** merged by superimposition. Immunoblot signals are shown in cyan, matching signals are highlighted in red. MW, molecular mass marker.



Supplementary Figure S7. Overexpression of ISCA1^{V10G} leads to partial mis-localization in the cytosol. HeLa cells were transfected with siRNAs and ISCA1-encoding plasmids prior to MG132 treatment as in Fig. 5. Total cell lysates, cytosolic and membrane samples obtained by digitonin-based cell fractionation were subjected to immunoblotting and analyzed for the indicated proteins. Overexpression of smISCA1^{wt} or smISCA1^{V10G} results in partial cytosolic mis-localization.

Supplementary Tables

Supplementary Table 1. Respiratory chain enzymatic activities in muscle and brain tissues obtained from autopsy samples

	CI/CS	CII/CS	SDH/CS	CIII/CS	CIV/CS	Citrate synthase*
Patient-Muscle	0.53	**0.06 (52%)**	**0.02 (35%)**	0.61	**1.05 (46%)**	118
Control range	0.214-0.226	0.116-0.124	0.057-0.063	0.3-0.4	2.3-3.1	150-230
Patient-Brain	0.31 (89%)	**0.12 (54%)**	**0.08 (44%)**	**0.21 (68%)**	**0.65 (73%)**	180
Control range	0.35-0.37	0.22-0.30	0.18-0.27	0.31-0.38	0.89-1.37	133-164

*Citrate synthase (CS) expressed as: nmoles/min/mg protein. Values below the control range are given in bold. In brackets the percentage of the enzyme activities relative to the lowest value of controls (n=3) is provided (mean ± one value of the standard deviation).

Supplementary Table 2. ATP synthase enzymatic activities in mitochondria from patient fibroblasts

	CV (ATP synthesis)*		
	Succ	Mal	Mal+Pyr
Patient	11.92 (33%)	10.84 (69%)	8.83 (74%)
Control range	35.97-37.57	15.61-17.87	11.95-13.79

*CV (ATP synthase) activity was measured in the direction of ATP synthesis using succinate (Succ), malate (Mal) or malate plus pyruvate (Mal+Pyr) as substrates. The values are expressed as nmoles/min/mg protein. In brackets the percentage of the enzyme activities relative to the lowest value of controls (n=53) is provided (mean ± one value of the standard deviation).

Supplementary Reference

1. Sheftel, A.D., Stehling, O., Pierik, A.J, Netz, D.J., Kerscher, S., Elsässer, H.P., Wittig, I., Balk, J., Brandt, U., Lill, R. (2009) Human ind1, an iron-sulfur cluster assembly factor for respiratory complex I. *Mol. Cell. Biol.*, **29**, 6059-6073.