Supporting information

Generation and characterization of human U-2 OS cell lines with the CRISPR/Cas9-edited protoporphyrinogen oxidase IX gene

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Table S1

Nucleotide sequences of designed guide RNAs specific for the *PPOX* gene.

CRISPR/Cas9 target site	Designed guide RNA sequence
Arginin 38	5´-GTGGAGAGCAGTGAGCGTCT-3´
Tryptophan 227	5´-GACCTCCACGAAGTGACCAC-3´
Cystein 459	5´-GTTAATGACTGTATAGAGAG-3´

Table S2

List of primers used for heteroduplex identification and genotyping of *PPOX* alleles.

CRISPR/Cas9 target site	Primer name-orientation	Sequence
Arginin 38	R38-forward	5'-AAGGTGAGTGCTCCACTTGTGCCAG-3'
	R38-reverse	5'-GCTCAGAAACCTGCTCTCCACATGC-3'
Tryptophan 227	W227-forward	5'-TTGTAGAGATGCAGTTTCACTATGTTGGCC-3'
	W227-reverse	5'-CTAGGATTCTGGGGTAGCCCATGTC-3'
Cystein 459	C459-forward	5'-GGAAAACAGCTGGGCTGAGGAGG-3'
	C459-reverse	5'-CTGGGCATTTTCTGCCTATGCTGGAATA-3'

Table S3 (an Excel file)

MS-LFQ analysis of protein expression levels in PPO-KO cells. Data of all proteins identified are shown in the Table S3 (separate file). The difference between the control groups (average of U-2 OS and N2) and the PPO-KO groups (average of R38/1 and W227/1) is expressed as log2(PPO-KO)-log2(control). An imputation method was employed for proteins with the MS signal below the detection limit of the method in one of analyzed samples.

Fig. S1. Analysis of the heme content in cell extracts. The example of an HPLC chromatogram of the U-2 OS cell lysate. Heme was detected by the absorbance at 400 nm in 4.7 min.

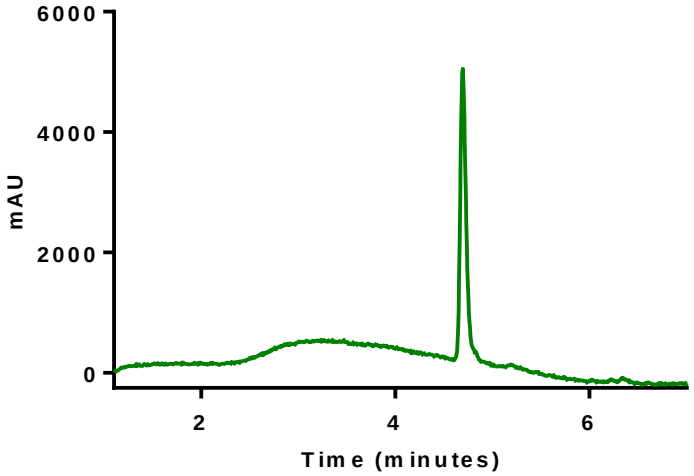

Fig. S2. Analysis of PP-IX content in cell extracts. The example of HPLC chromatogram of the W227/1 cell lysate. PP-IX was detected by fluorescence (λex/λem = 400/ 615 nm) in 6.5 min.

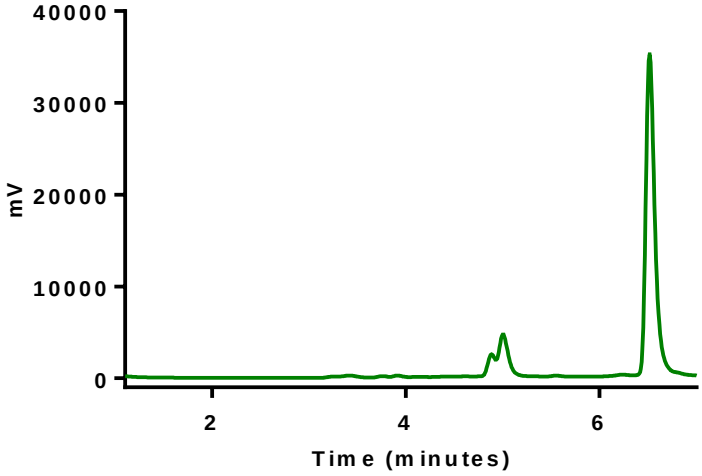

Fig. S3. Uncropped western blot images shown in Fig. 4 of the main text. Individual OXPHOS complexes were separated by BN-PAGE and visualized with antibodies specific for NDUFB8, SDHA, UQCRC2, MT-CO1 and ATP5F1B that were used for detection of the complex CI, CII, CIII, CIV and CV, respectively.