Appendix

Appendix Figures legend

Appendix Fig. S1. Subcellular localization of FATE1 wild-type and mutant proteins in transfected HeLa cells.

A. Subcellular localization of transfected N-Flag tagged FATE1 (red) and fluorescent markers for Golgi, ER and mitochondria, respectively (green). Scale bars, 10 µm.
B. Subcellular localization of wild-type and mutant EGFP-FATE1 fusion proteins. The mitochondrial marker TOM20 is stained in red. Scale bars, 10 µm.
C. Subcellular localization of EGFP-FATE1 fusion proteins mutated in C-terminal domain basic residues. The mitochondrial marker TOM20 is stained in red. Scale bars, 10 µm.

Appendix Fig. S2. Predictions of coiled-coil structures in the FATE1 protein.

A. Hydrophobic cluster analysis (HCA) plot (Callebaut *et al*., 1997) of the FATE1 protein sequence. Amino acid residues are indicated with their one-letter code, except that proline is indicated with a star, threonine with a square, serine with a dotted square and glycine with a diamond. Clusters of hydrophobic residues in the protein sequence are evidenced in the bidimensional diagram. The position of L151 is highlighted with a red circle.
B. Prediction of coiled-coil structures in the wild-type FATE1 protein by the COILS software.
C. Prediction of coiled-coil structures in the L151D FATE1 mutant by the COILS software. The mutant greatly decreases the probability of coiled-coil formation in the C-terminal portion of the FATE1 protein.

Appendix Fig. S3. Blue native PAGE for detection of FATE-1 containing complexes in crude mitochondrial extracts from H295R/TR N-Flag FATE1 cells.
Extracts were prepared from cells cultured in basal conditions or after Dox stimulation using either digitonin (Dig.) or *n*-dodecyl-β-D-maltoside (DDM), run on native PAGE and transferred to a PVDF membrane. Immunoblotting was performed with an anti-Flag antibody.

Appendix Fig. S4. Colocalization of FATE1 with ER and mitochondria in Dox-treated H295R/TR SF-1 cells.
Endogenous FATE1 (green), ER labelled by anti-calreticulin antibody (red) and mitochondria labelled by HSP60 staining (blue). Scale bar, 10 μm. A custom-made ImageJ macro software was used to draw colocalization profiles in two different positions of the same image shown in Fig. 2G. Pixel size=81 nm.

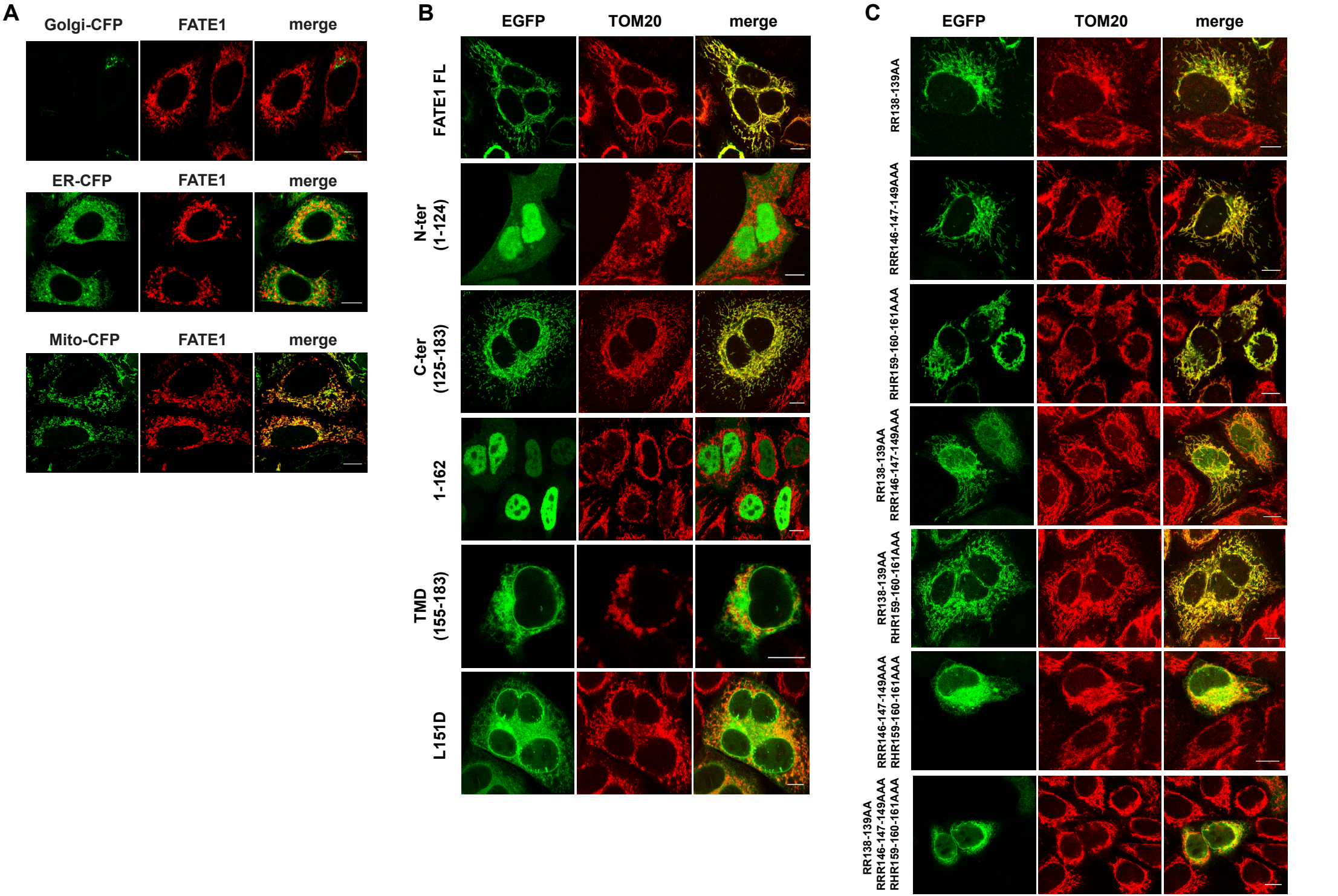
Appendix Fig. S5. Colocalization of FATE1 with ER and mitochondria in Dox-treated H295R/TR N-Flag FATE1 cells.
Endogenous FATE1 (green), ER labelled by anti-calreticulin antibody (red) and mitochondria labelled by MitoTracker staining (blue). Two areas (a and b) showing close apposition of red, green and blue staining (white signals) are shown at higher magnification. Scale bar, 10 μm. A custom-made ImageJ macro software was used to draw colocalization profiles in three different positions of the image. Pixel size=60 nm.

Appendix Fig. S6. BIK expression in H295R cell lines used in this study. HeLa cells were used as positive control, as shown in ref. 53.

Additional reference

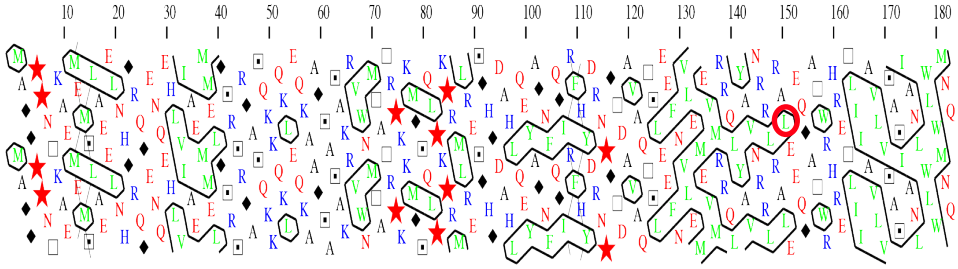
Callebaut I, Labesse G, Durand P, Poupon A, Canard L, Chomillier J, Henrissat B, Mornon JP (1997) Deciphering protein sequence information though hydrophobic cluster analysis. Current status and perspectives. *Cell Mol Life Sci* **53**: 621-645

Appendix Figure S1



Appendix Figure S2

A



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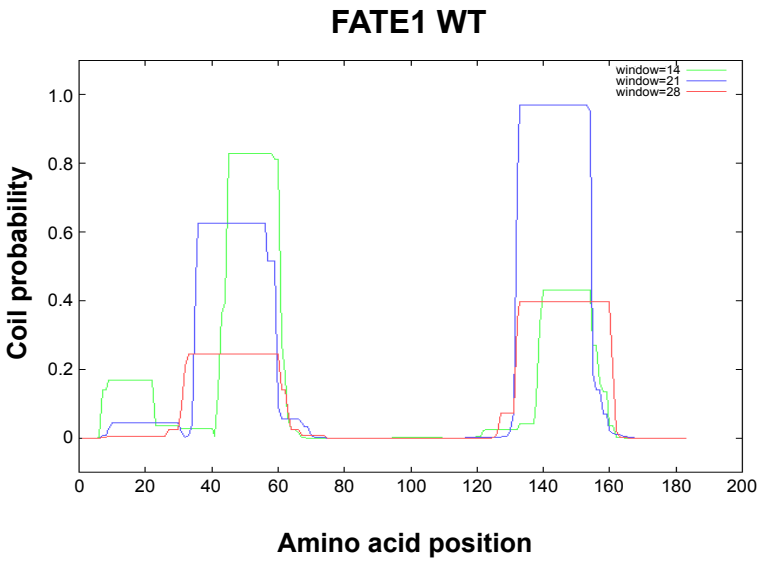
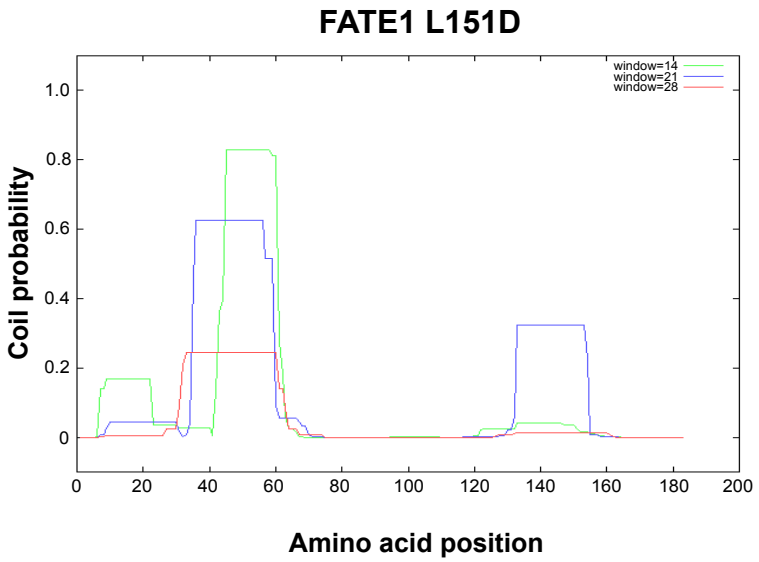
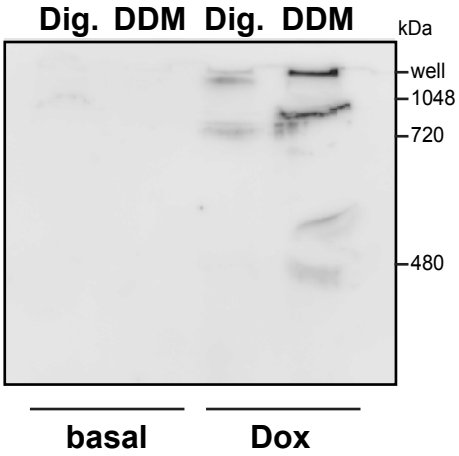
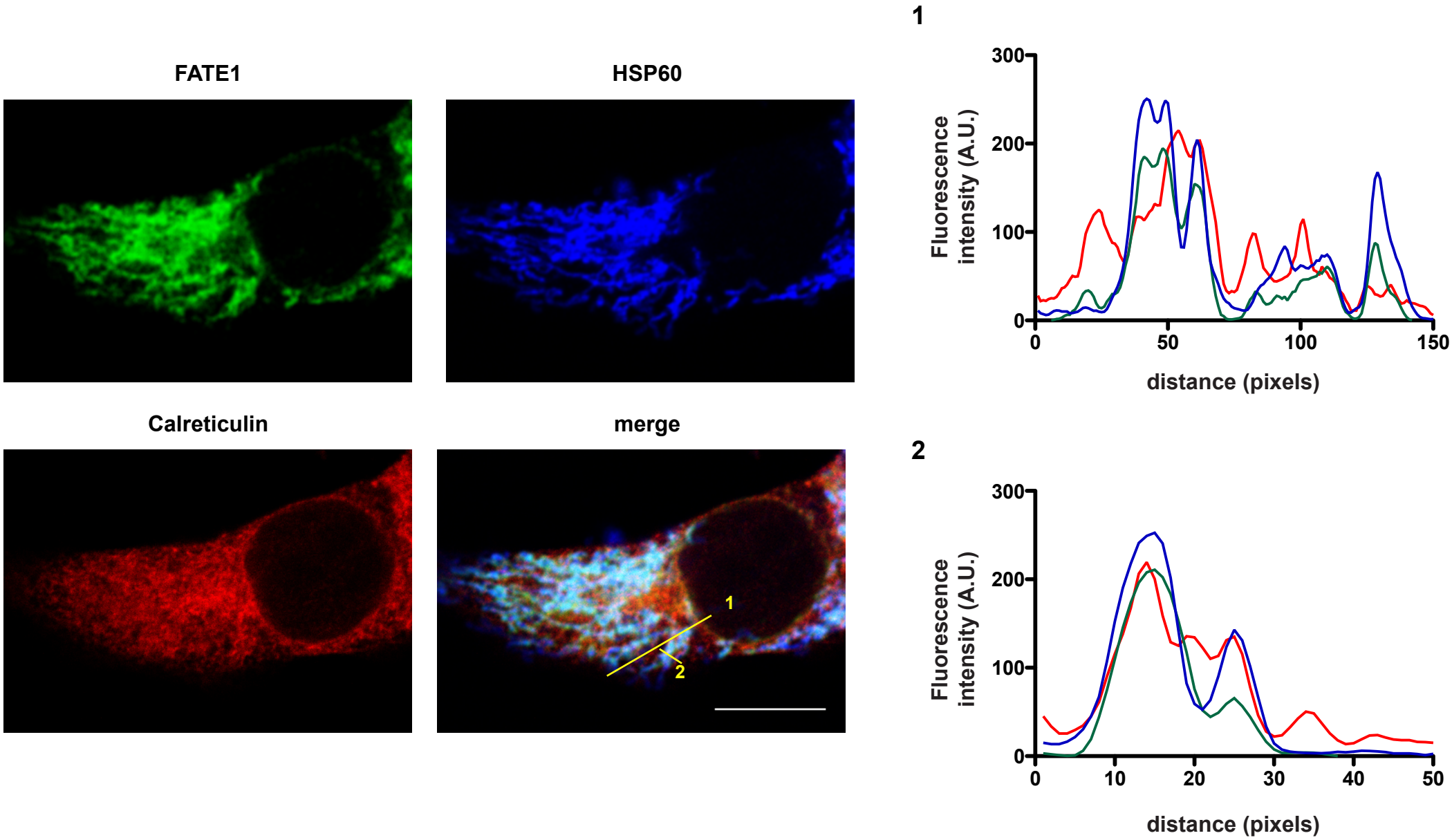




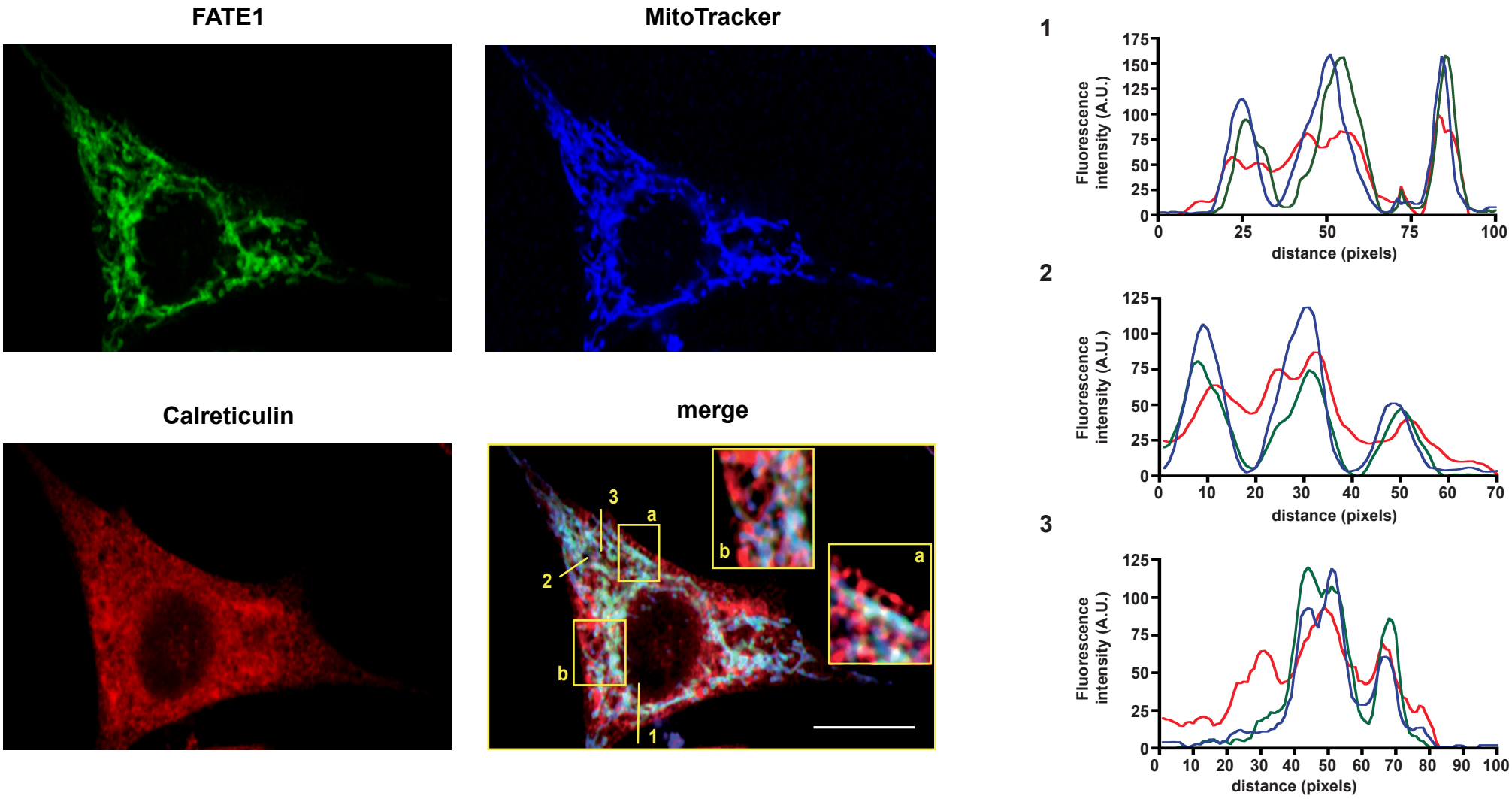
Appendix Figure S3



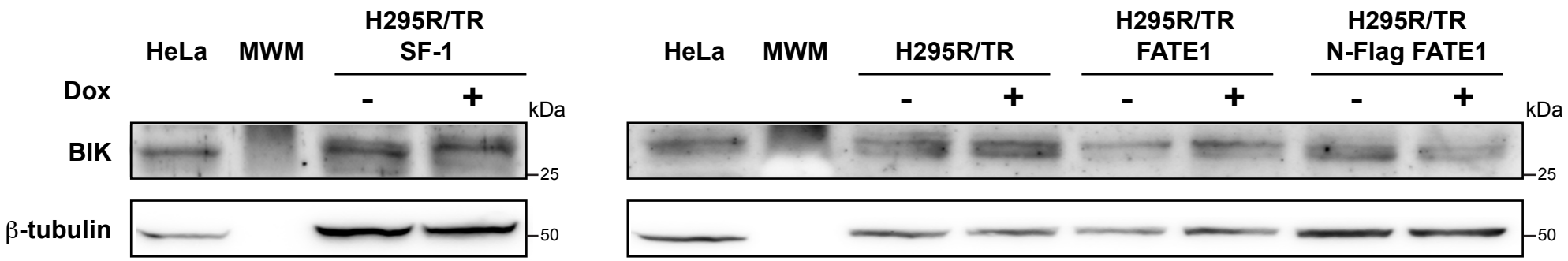
Appendix Figure S4



Appendix Figure S5



Appendix Figure S6



Appendix Table S1

Name	ID	MW	Coverage
Mitochondrial inner membrane protein	Q16891	84025 Da	27%
78 kDa glucose-regulated protein	P11021	72402 Da	18%
Heat shock cognate 71 kDa protein	P11142	71082 Da	26%
Tubulin beta-2A chain	Q13885	50274 Da	36%
Tubulin beta chain	P07437	50095 Da	26%
60S acidic ribosomal protein P0	P05388	34423 Da	25%
Emerin	P50402	29033 Da	31%
40S ribosomal protein S3a	P61247	30154 Da	32%
Oxidoreductase HTATIP2	Q9BUP3	27329 Da	31%
60S ribosomal protein L7	P18124	29264 Da	29%
40S ribosomal protein S8	Q92597	24475 Da	36%
Fetal and adult testis expressed transcript (FATE1)	Q969F0	20698 Da	78%

Proteins immunoprecipitated by the anti-Flag antibody in Dox-treated H295R/TR N-Flag FATE1 cells were identified by MS/MS. Only proteins whose Mascot score exceeded the threshold significance value ($p<0.05$) are reported in the Table.

Appendix Table S2. Primary antibodies used in this study.

Antigen	Supplier	Catalog number
Anti-Flag M2 mouse monoclonal	Sigma-Aldrich	F3165
Anti-Flag rabbit polyclonal	Sigma-Aldrich	F7425
BIK/NBK	Santa Cruz Biotechnology	sc-10770
Calreticulin	Thermo Fisher	PA3-900
Emerin	from D. Toniolo	NA
FATE1	Abcam	ab139275
FATE1	Santa Cruz Biotechnology	sc-101220
GRP75	Santa Cruz Biotechnology	sc-13967
HSP60	Santa Cruz Biotechnology	sc-1052
MCU	Sigma-Aldrich	HPA016480
MCUR1	Sigma-Aldrich	SAB2700722
Mfn1	Abcam	ab57602
Mfn2	Abcam	ab50838
Mic60/mitofilin	MitoSciences	MSM02
MICU1	Sigma-Aldrich	HPA037480
MICU2	Sigma-Aldrich	HPA045511
MICU3	Sigma-Aldrich	HPA024771
RPL7	from B. Bardoni	NA
SERCA2	Sigma-Aldrich	S1439
Sigma-1 receptor	Sigma-Aldrich	HPA018002
SOAT1	Abcam	ab39327
Tom20	Abcam	ab115746
VDAC1	MitoSciences	MSA03