Supporting Information

Topology-Resolved Imaging of Mitochondrial Nucleoids Uncovers Dynamic G-Quadruplex Remodeling

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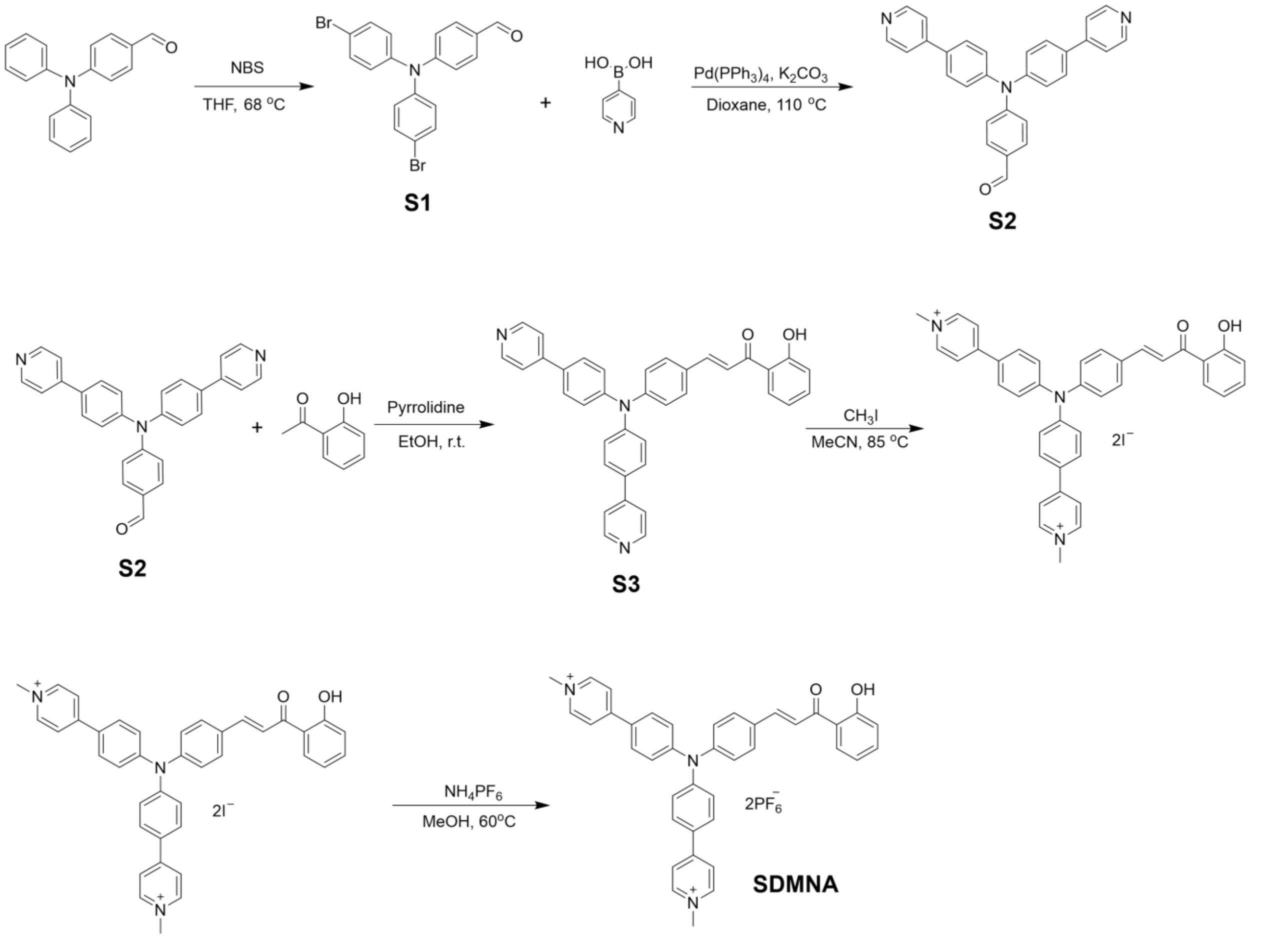
Experimental procedures



Scheme S1. Synthetic route to SDMNA.

Supplementary Figures.

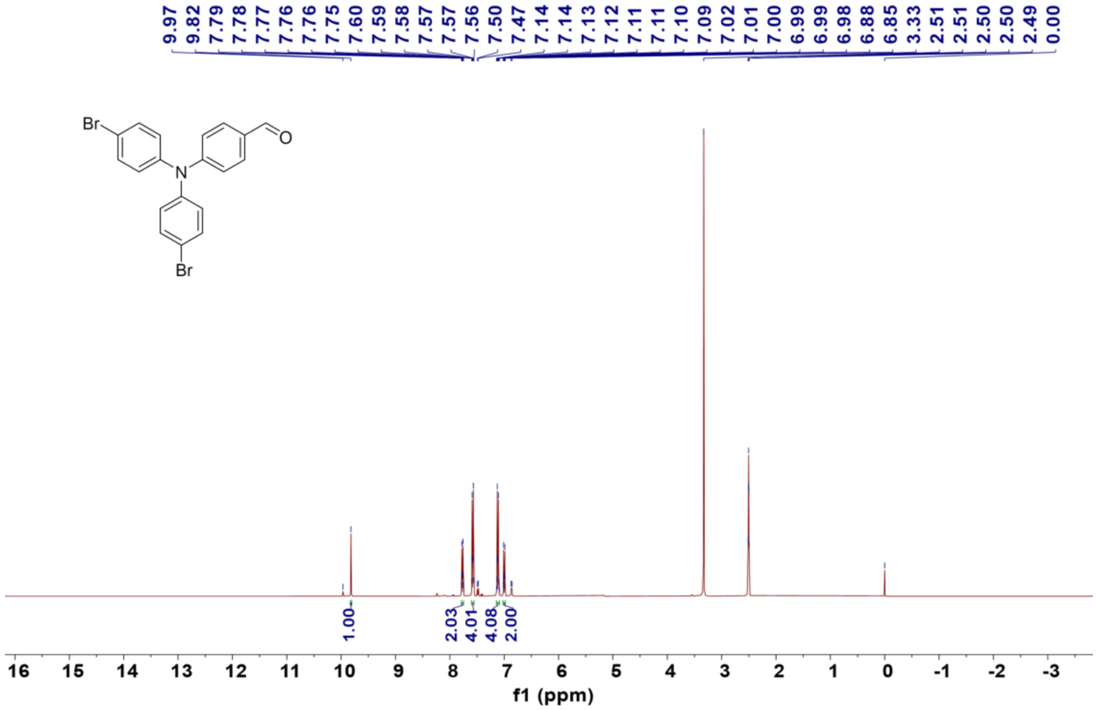


Fig. S1. ^{1}H NMR spectra of S1 in DMSO-d_6.

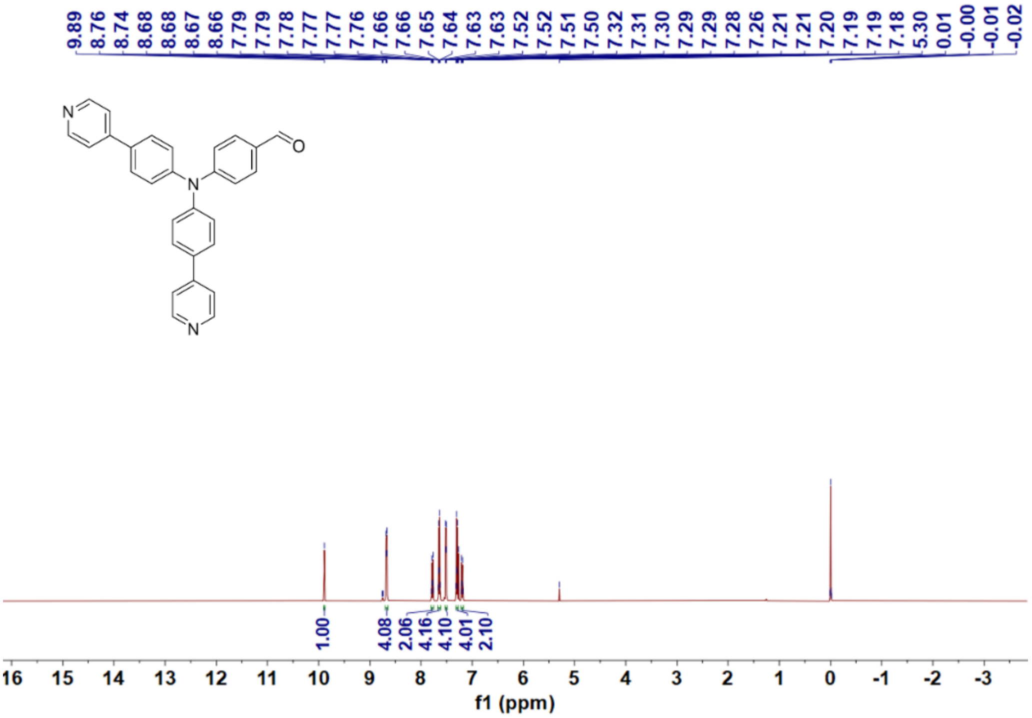


Fig. S2. ^{1}H NMR spectra of S2 in Chloroform-*d*.

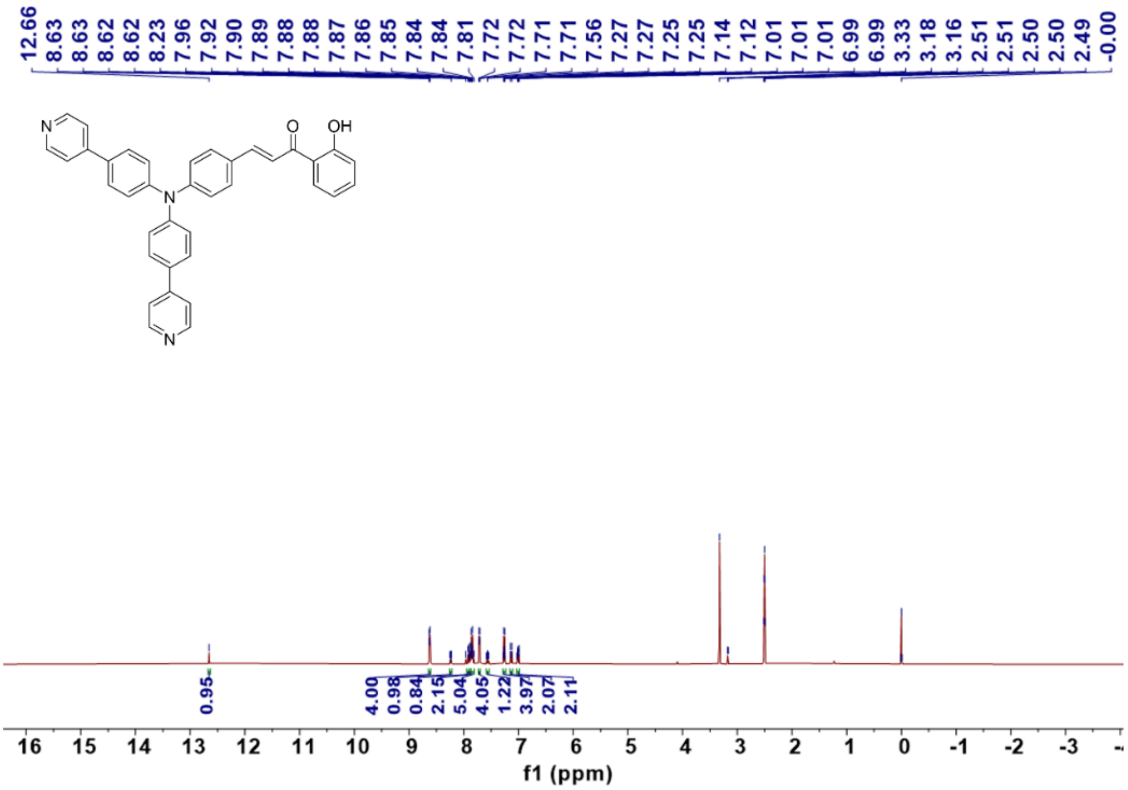


Fig. S3. ^{1}H NMR spectra of S3 in DMSO-d_6.

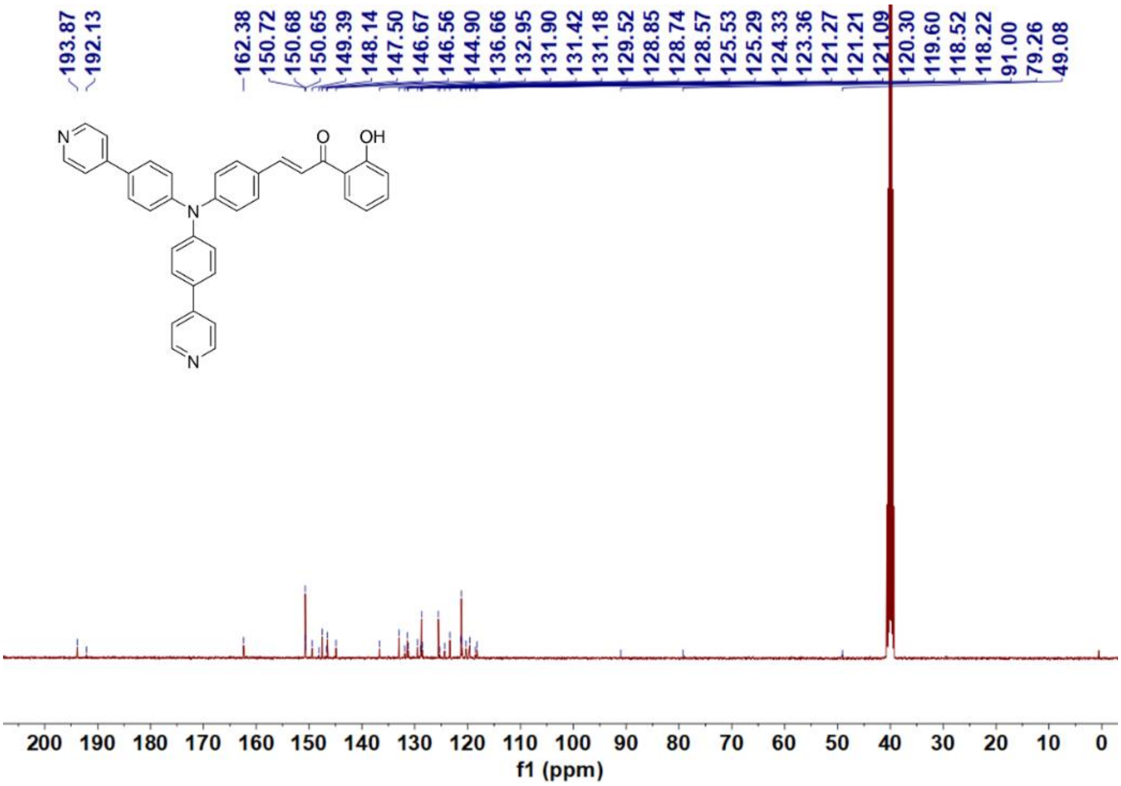


Fig. S4. ^{13}C NMR spectra of S3 in DMSO-d_6.

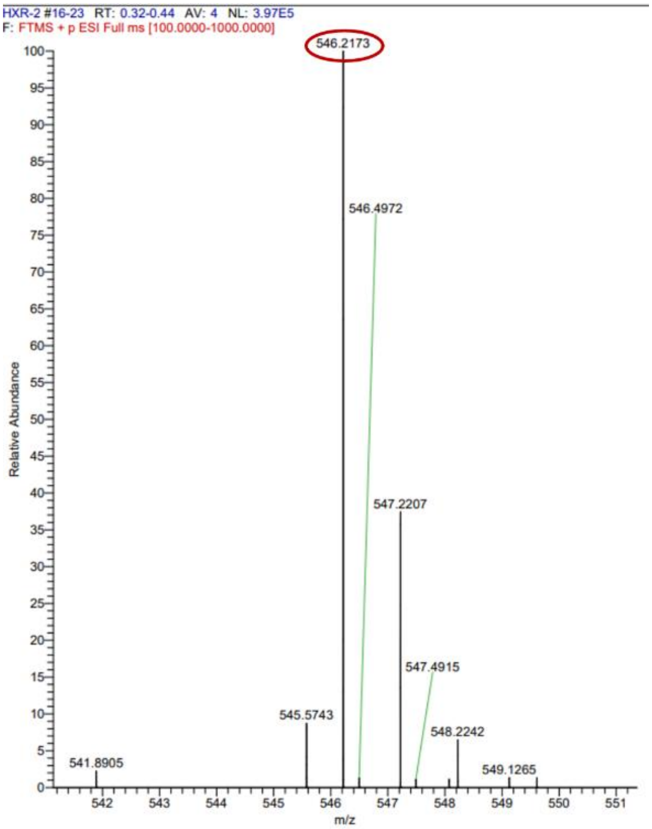


Fig. S5. HRMS of S3 in CH_2Cl_2.

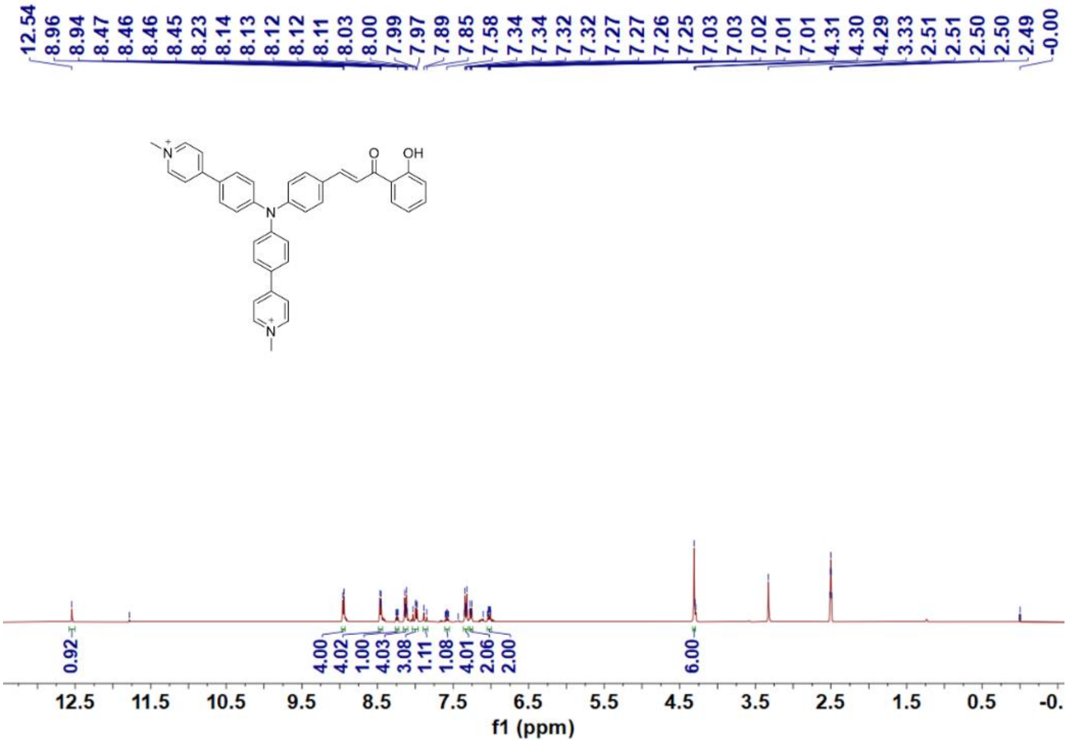


Fig. S6. ^{1}H NMR spectra of SDMNA in DMSO-d_6.

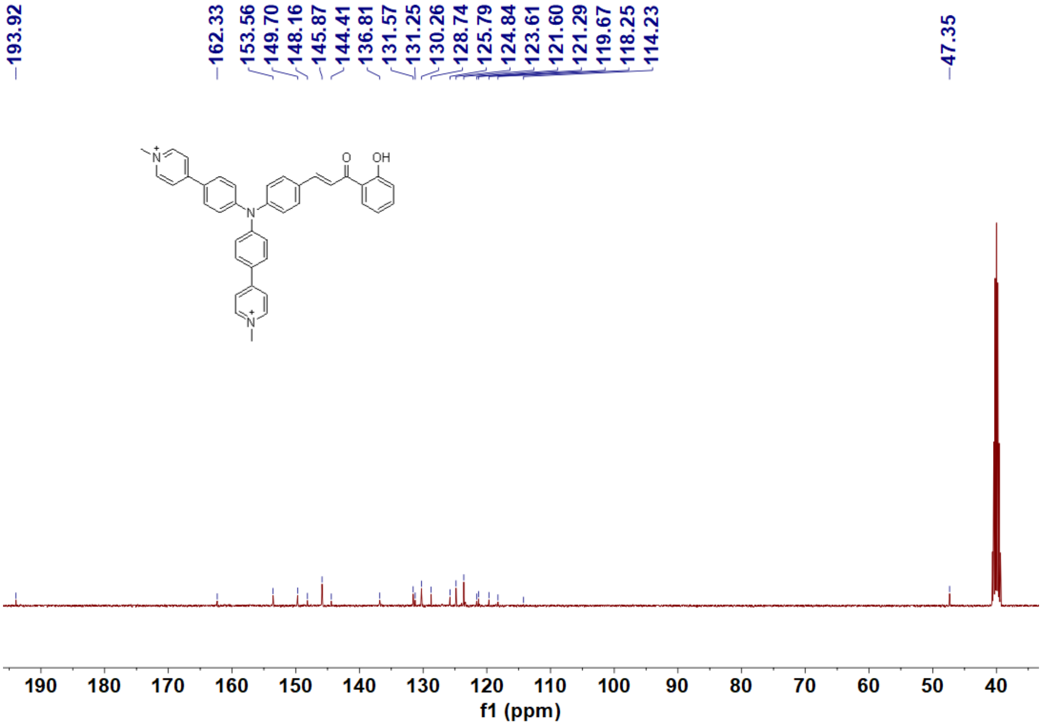


Fig. S7. ^{13}C NMR spectra of SDMNA in DMSO-d_6.

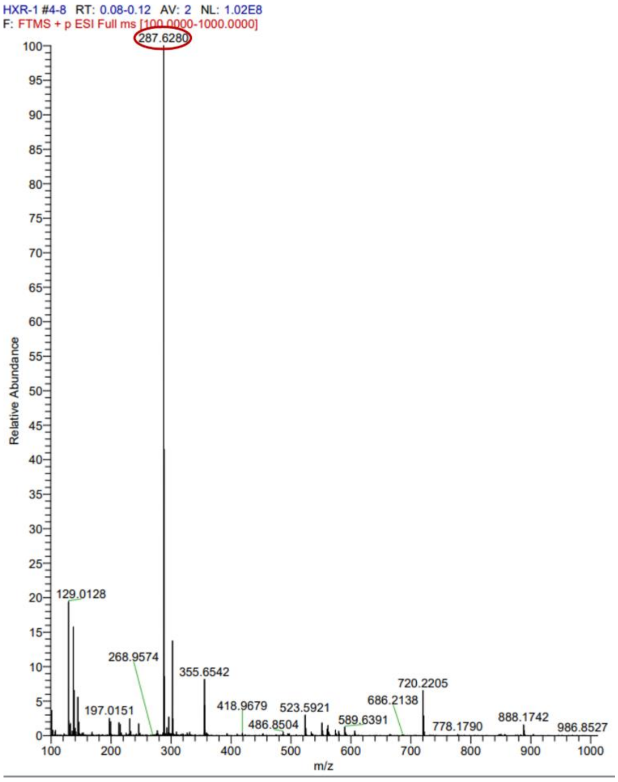


Fig. S8. HRMS of SDMNA in CH_2Cl_2.

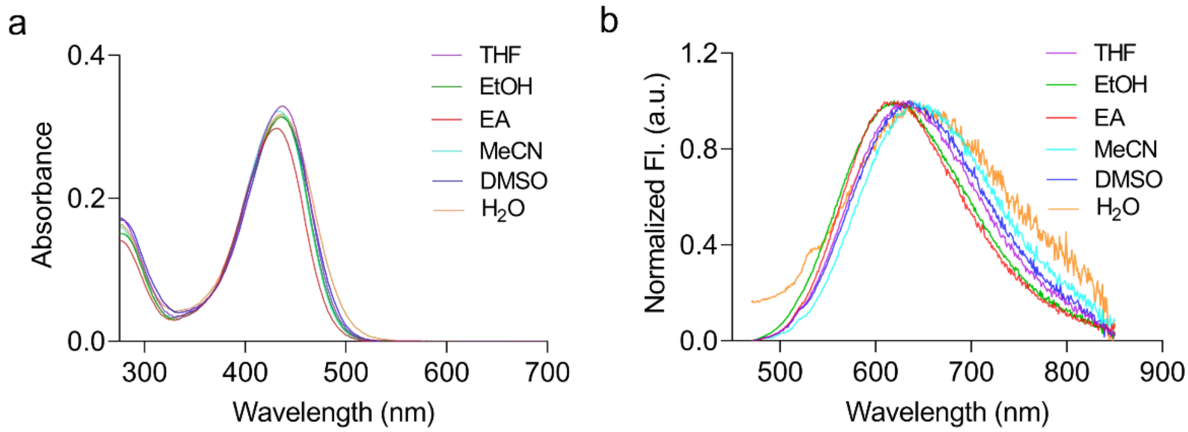


Fig. S9. Absorption (a) and fluorescence emission (b) spectra of SDMNA in different solvents: tetrahydrofuran (THF), ethanol (EtOH), ethyl acetate (EA), acetonitrile (MeCN), dimethyl sulfoxide (DMSO), and H_2O. λ_{ex} = 450 nm.

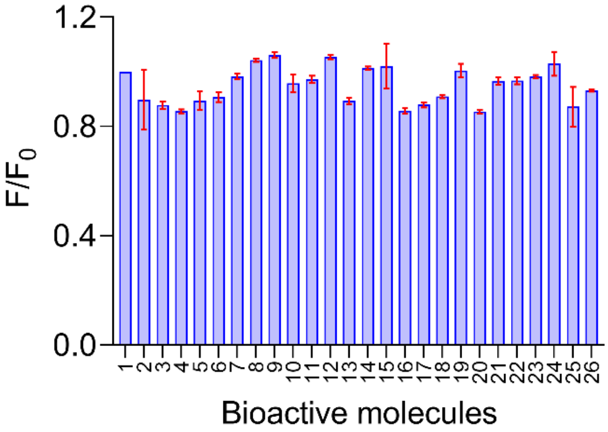


Fig. S10. Fluorescence intensity changes of SDMNA in ultrapure water in the presence of various inorganic ions and biological molecules (1: Control (H_2O), 2: CH_3COONa, 3: $CuCl_2$, 4: CuCN, 5: K_2CO_3, 6: KCl, 7: $MgSO_4$, 8: Na_2HPO_4, 9: Na_2SO_4, 10: Na_3PO_4, 11: NaBr, 12: NaCl, 13: NaF, 14: NaH_2PO_4, 15: $NaHCO_3$, 16: NaHS, 17: $NaHSO_3$, 18: NaI, 19: NaOH, 20: NH_4Cl, 21: Gln, 22: Asp, 23: His, 24: Cys, 25: GSH, 26: Glucose).

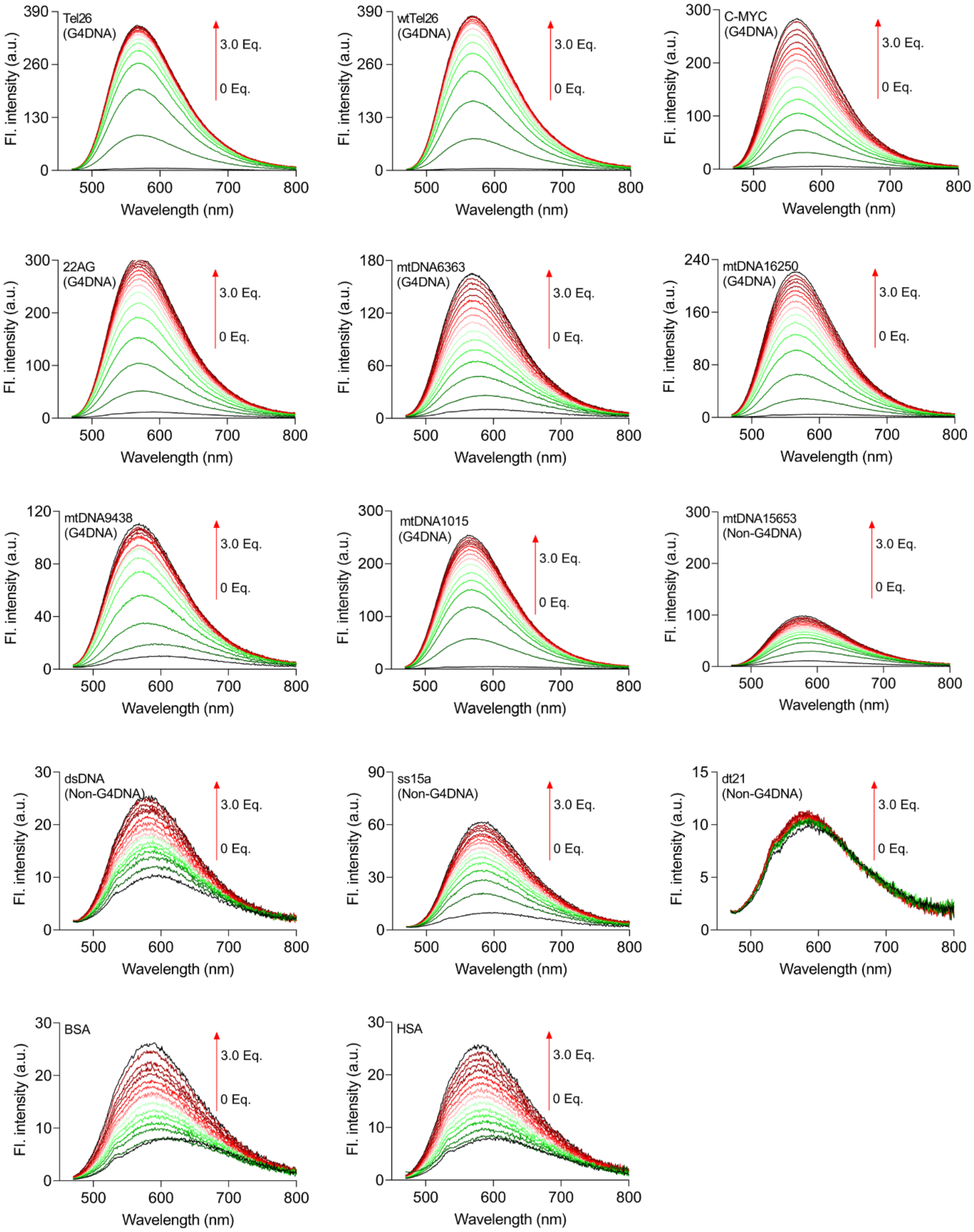


Fig. S11. Fluorescence emission spectra of SDMNA (1 μM) upon titration with different DNA topologies in 10 mM Tris-HCl buffer (pH 7.4, 100 mM K^+) at λ_{ex} = 450 nm.

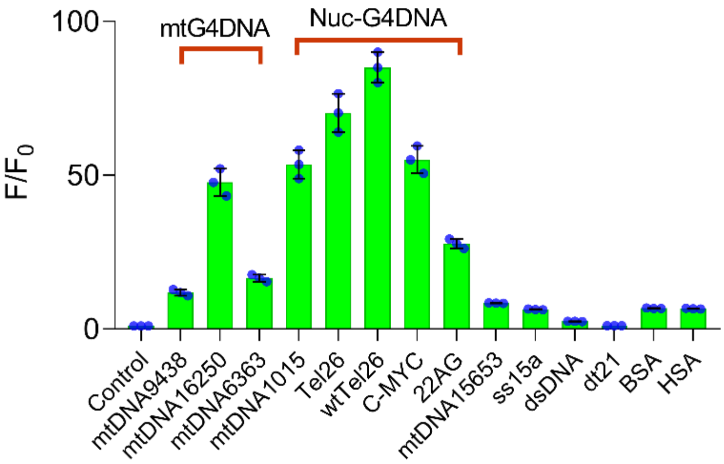


Fig. S12. Relative fluorescence intensity ratios of SDMNA interacting with different DNA topologies at an SDMNA: DNA molar ratio of 3:1 in 10 mM Tris-HCl buffer (pH 7.2, 100 mM K^+), λ_{ex} = 450 nm.

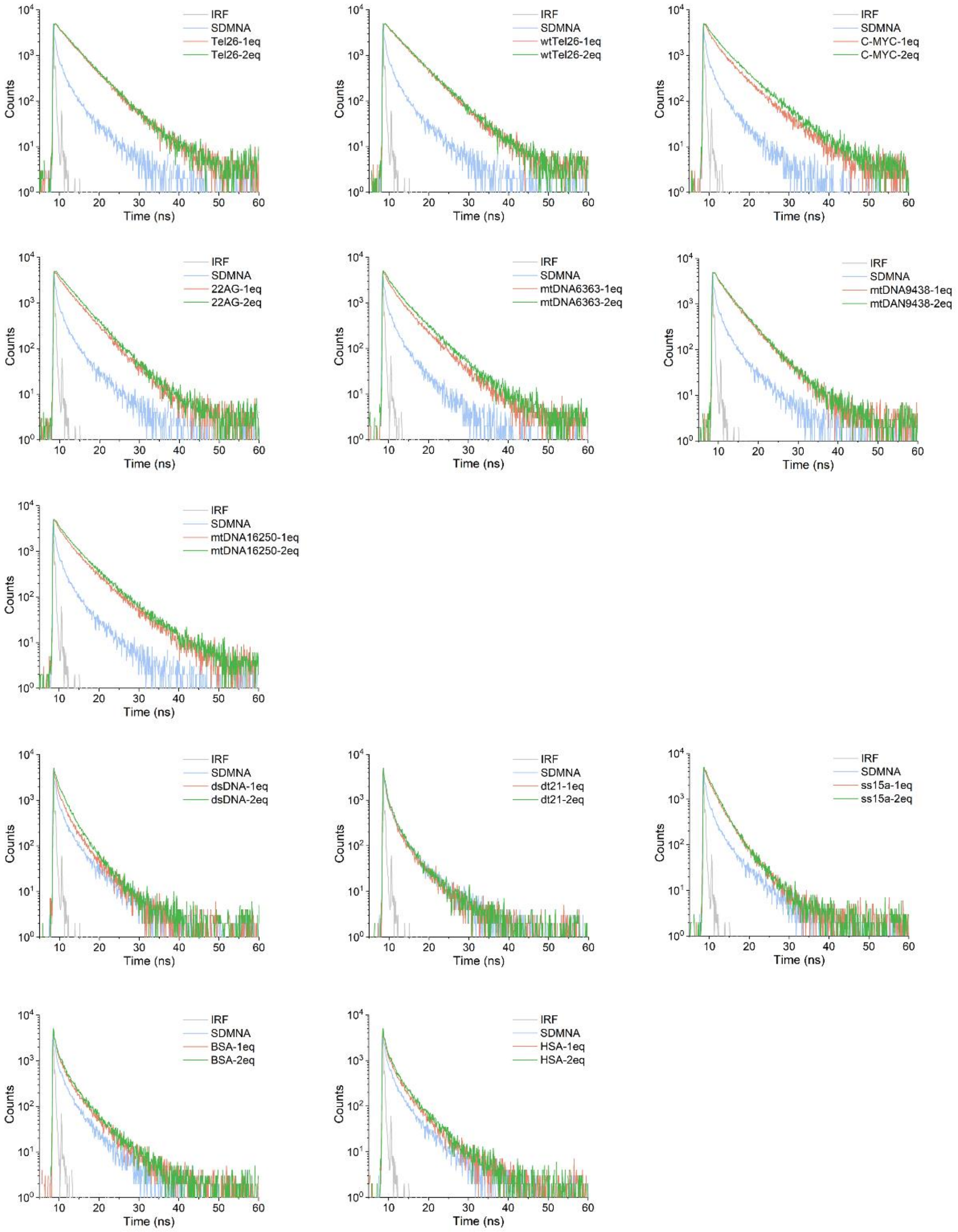


Fig. S13. Fluorescence lifetime decay curves of SDMNA (1 μM) upon titration with different DNA topologies in 10 mM Tris-HCl buffer (pH 7.4, 100 mM K^+). λ_{ex} = 445 nm, λ_{em} = 570 nm.

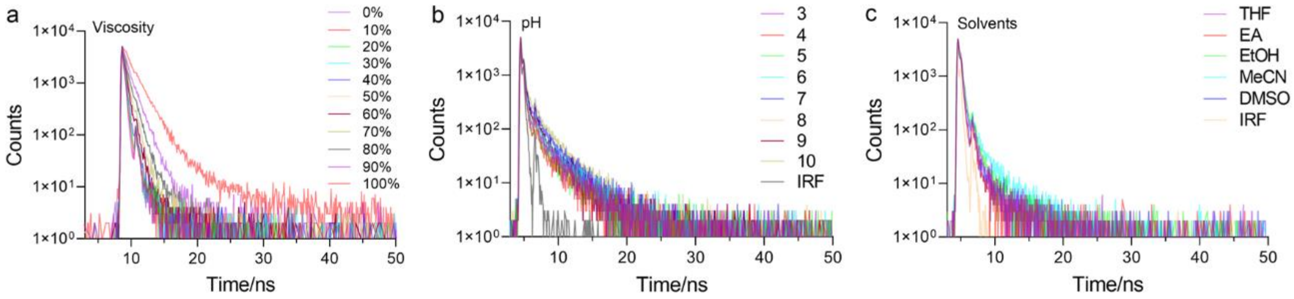


Fig. S14. Fluorescence lifetime decay curves of SDMNA measured under different viscosity, pH, and solvent environments. λ_{ex} = 445 nm, λ_{em} = 570 nm.

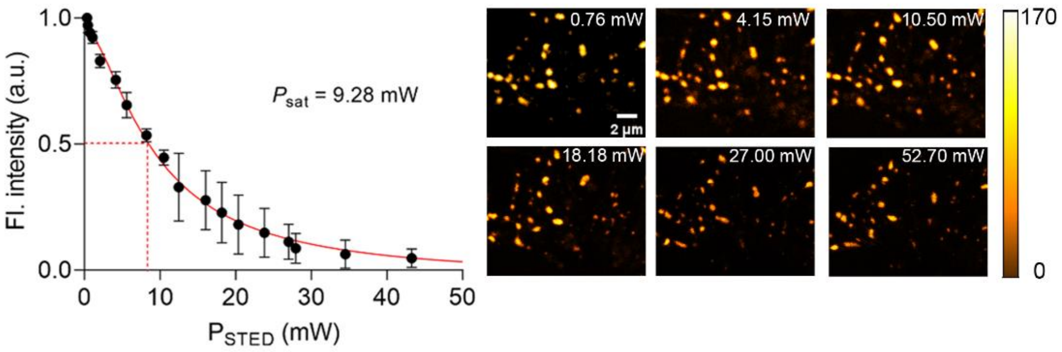


Fig. S15. STED images of SDMNA (2 μM) in COS-7 cells acquired under different depletion laser powers using a 775 nm depletion beam. Right: plot of normalized fluorescence intensity of SDMNA (2 μM) in COS-7 cells as a function of STED depletion laser power. λ_{ex} = 488 nm; λ_{em} = 575-625 nm, λ_{STED} = 775 nm.

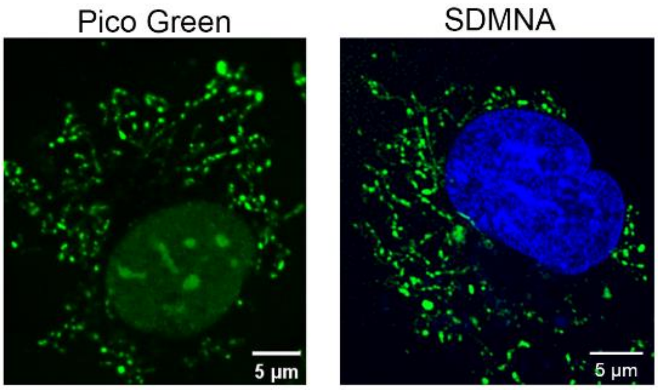


Fig. S16. Comparison of PicoGreen staining (left) and SDMNA staining (right, co-labeled with Hoechst 33342) in COS-7 cells. PicoGreen, λ_{ex} = 488 nm, λ_{em} = 575-625 nm; SDMNA, λ_{ex} = 488 nm, λ_{em} = 575-625 nm; Hoechst33342, λ_{ex} = 405 nm, λ_{em} = 420-475 nm.

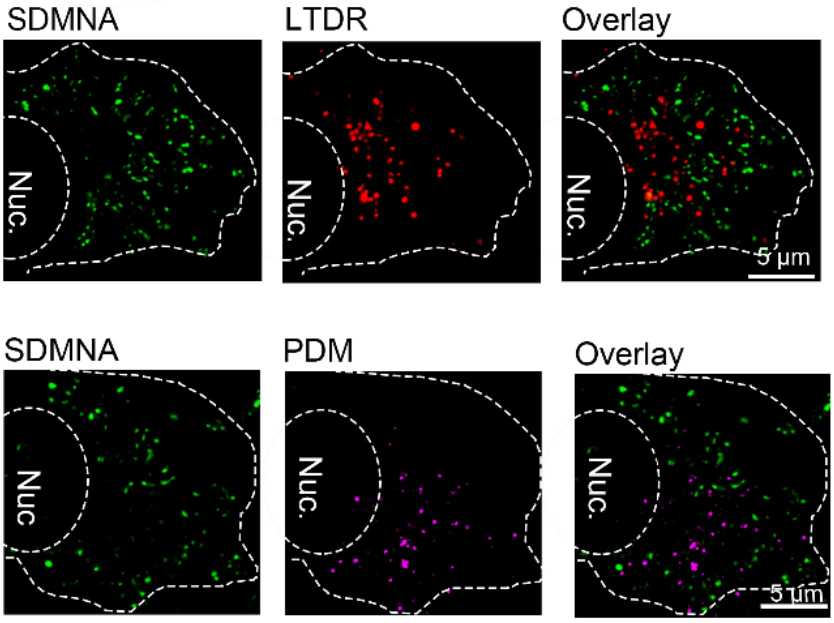


Fig. S17. Colocalization experiment of SDMNA (2 µM, 2 h) with LysoTracker Deep Red (LTDR, 200 nM) and PDM (1 µM, 30 min) in COS-7 cells, respectively.

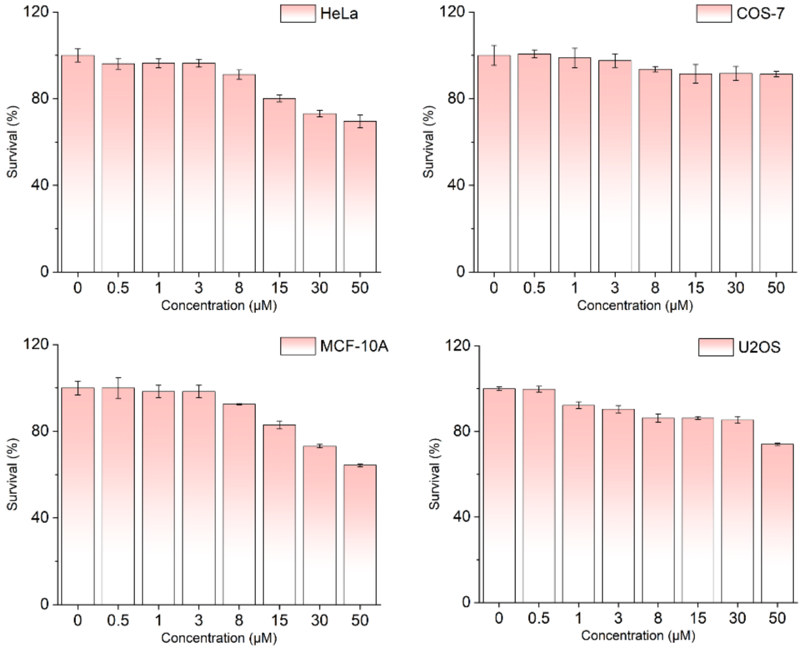


Fig. S18. Cytotoxicity of SDMNA at various concentrations in HeLa, COS-7, MCF-10A, and U2OS cells after 24 h of incubation.

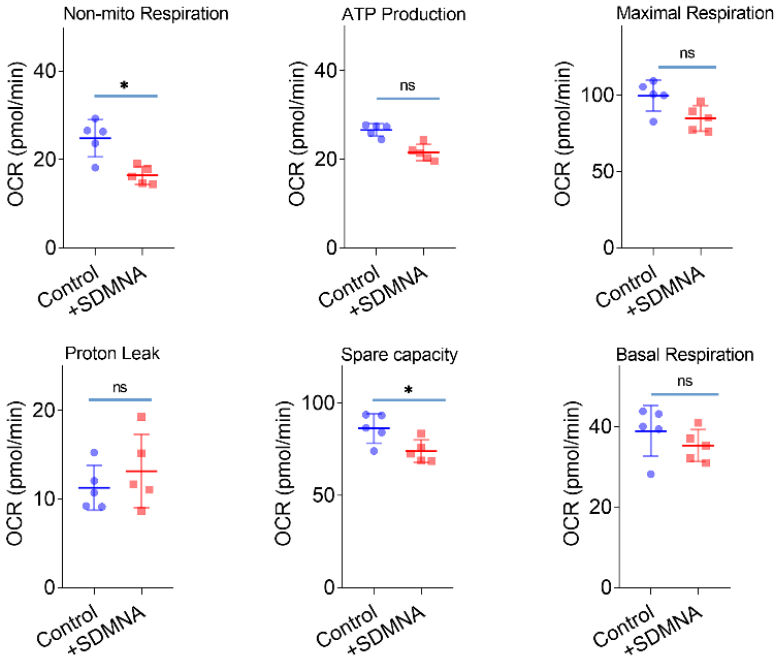


Fig. S19. OCR traces and corresponding bar graphs indicate comparable basal respiration, ATP production, maximal respiration, proton leak, non-mitochondrial respiration, and spare respiratory capacity, as shown in Fig. 2h.

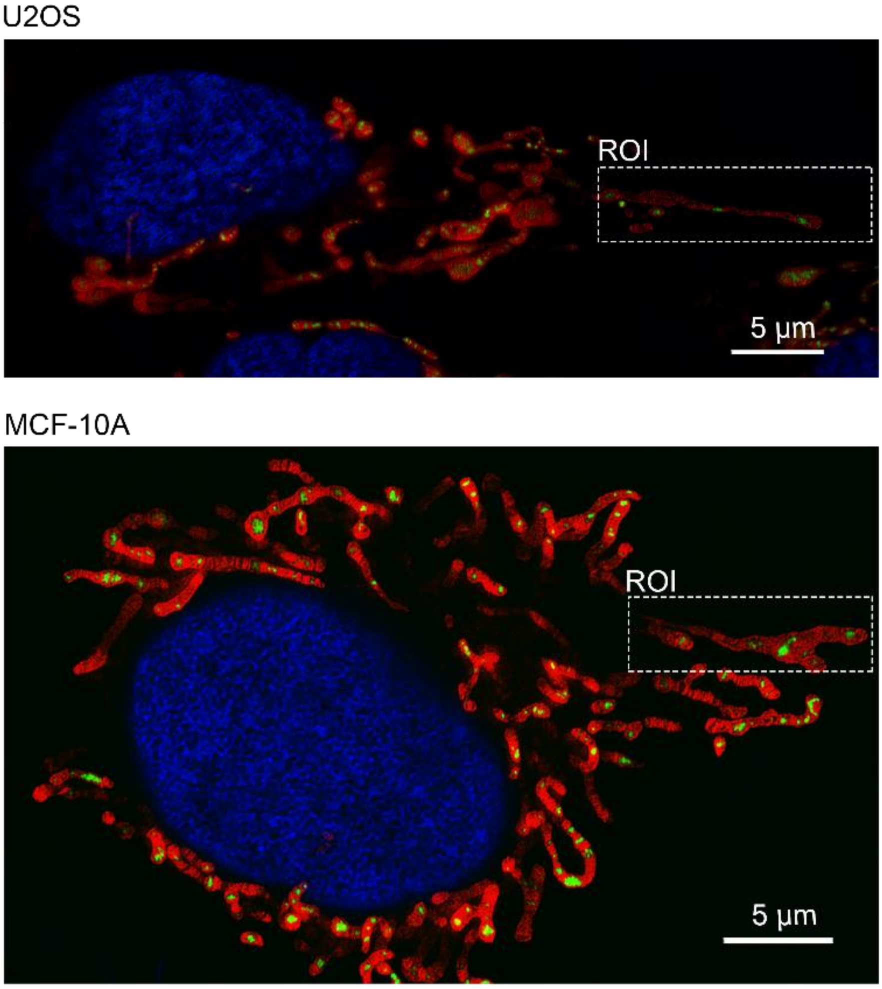


Fig. S20. STED imaging of mitochondrial inner membrane and mtDNA was performed using PKMO and SDMNA in different cell types (U2OS and MCF-10A). The ROI magnification areas are shown in Figs. 2i-2j.

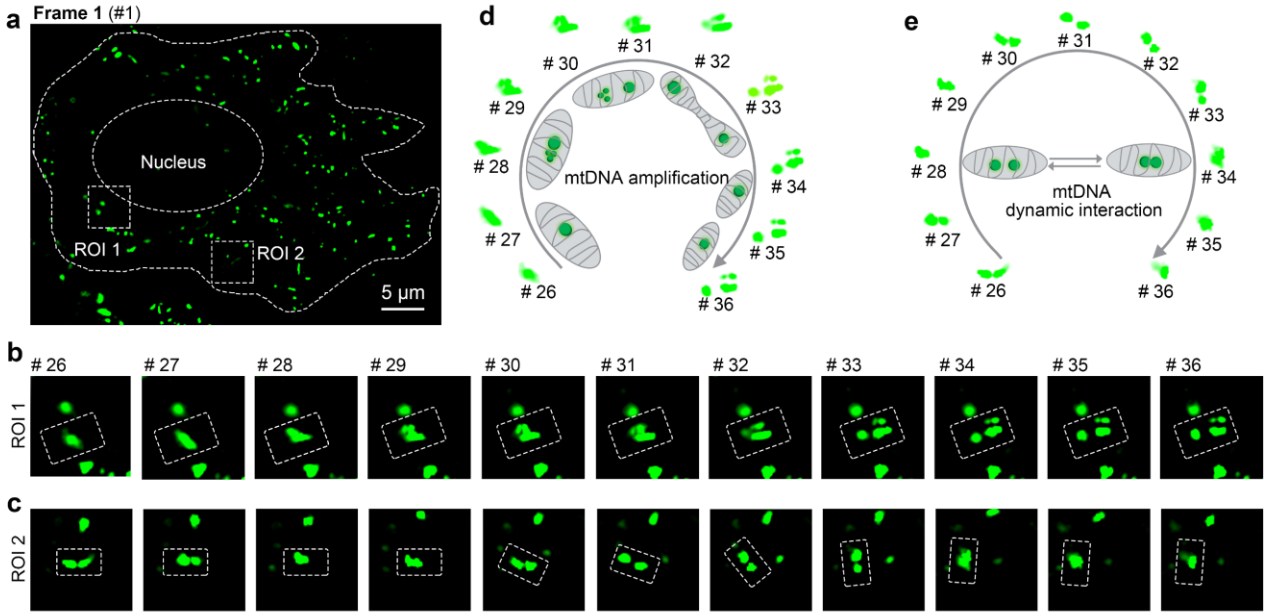


Fig. S21. Long-term dynamic imaging of mtDNA using SDMNA. (a) Time-lapse ultrafast structured illumination microscopy (SIM) imaging of fluorescently labeled mtDNA in living cells stained with SDMNA. (b, c) Frame-by-frame analysis of mtDNA dynamics within ROI 1 (b) and ROI 2 (c) from (a). (d, e) Schematic illustrations depicting mtDNA replication events (d) and transient dynamic interactions between adjacent mtDNA copies (e).

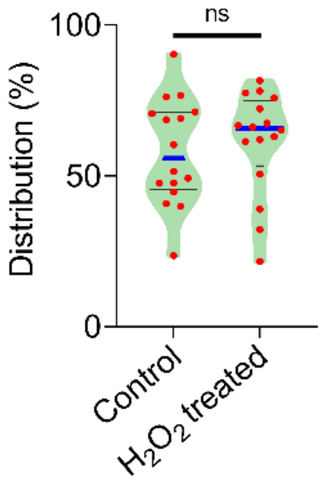


Fig. S22. Comparison of the 2.0-3.0 ns fluorescence lifetime distributions in Figs. 4b, 4d.

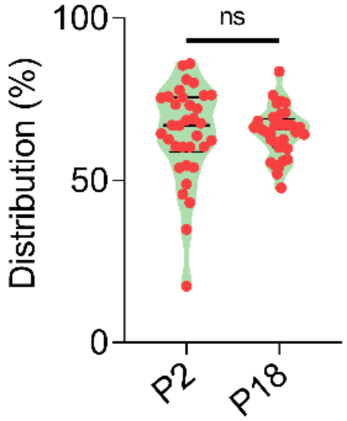


Fig. S23. Comparison of the 2.0-3.0 ns fluorescence lifetime distributions in Figs. 4f, 4h.

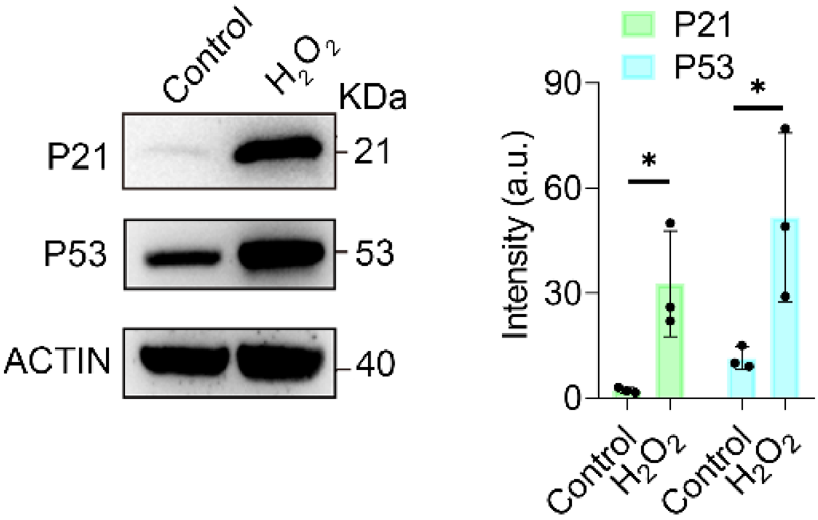


Fig. S24. Western blot analysis of the senescence markers P21 and P53 and corresponding densitometric quantification in the oxidative stress-induced senescence model, n = 3.

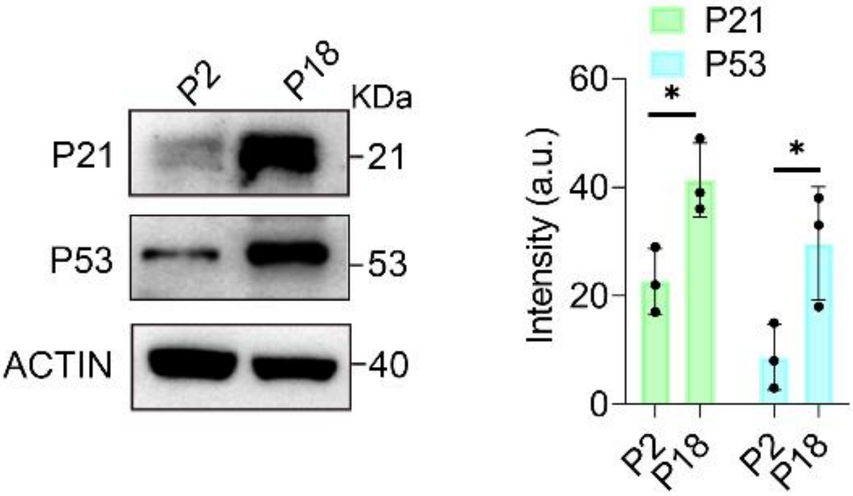


Fig. S25. Western blot analysis of the senescence markers P21 and P53 and corresponding densitometric quantification in the replicative senescence model, n = 3.

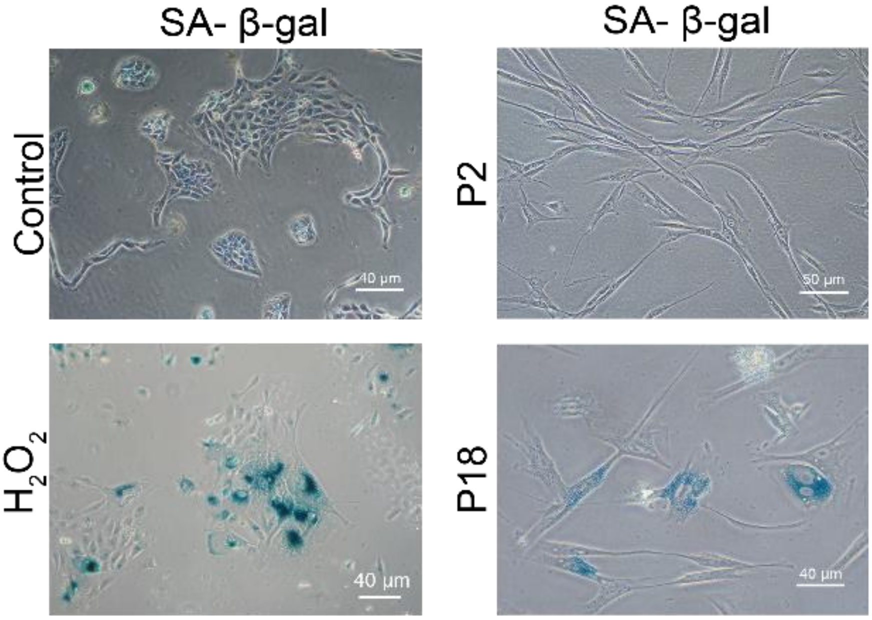


Fig. S26. Senescence *β*-galactosidase staining in H_2O_2-treated and replicative senescent models.

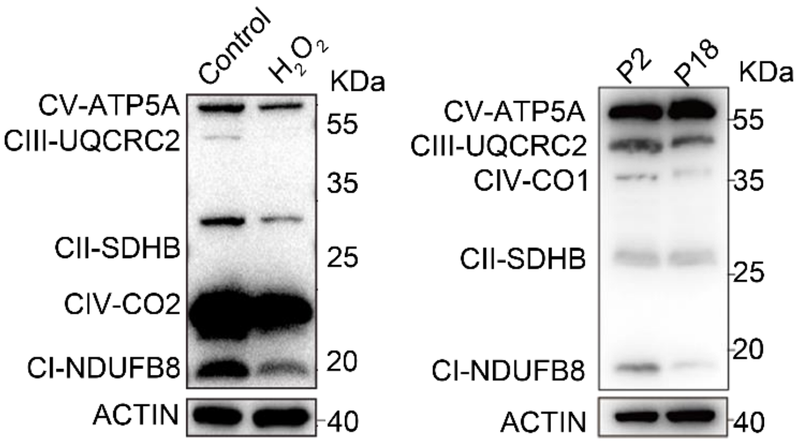


Fig. S27. Western blot analysis of oxidative phosphorylation (OXPHOS)-related proteins in H_2O_2-treated and replicative senescent cells.

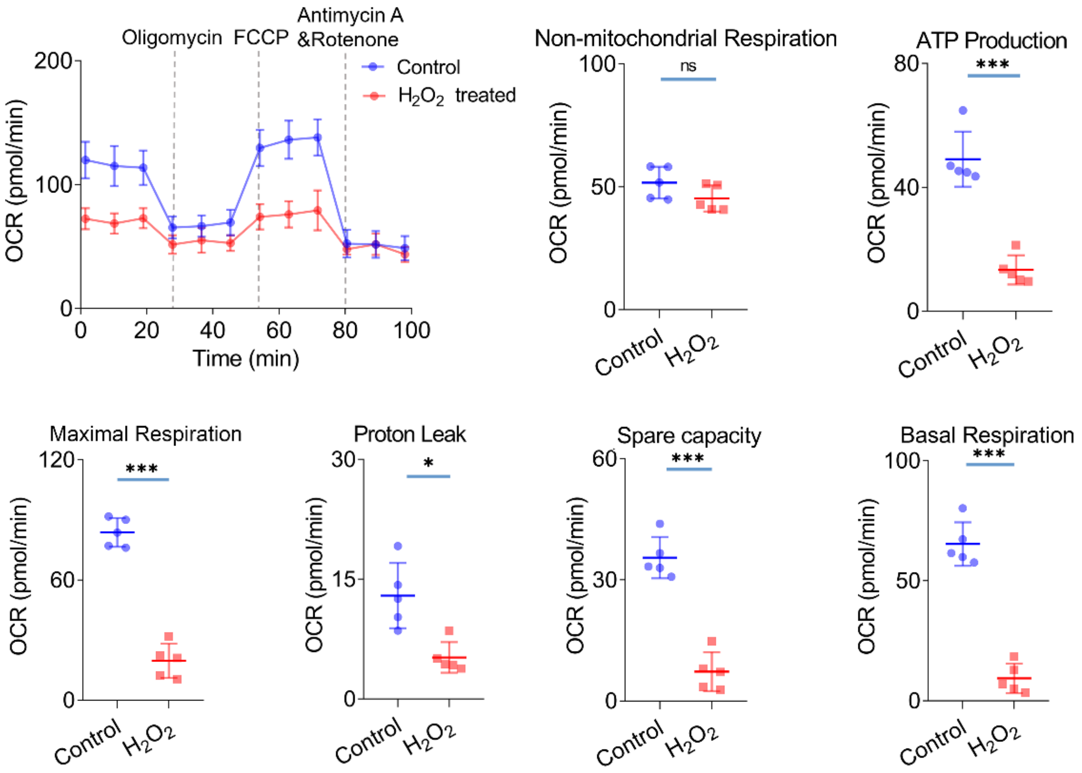


Fig. S28. Seahorse analysis reveals impaired mitochondrial respiration in H_2O_2-treated cells, including reduced basal respiration, ATP-linked respiration, maximal respiration, spare respiration capacity, and proton leak.

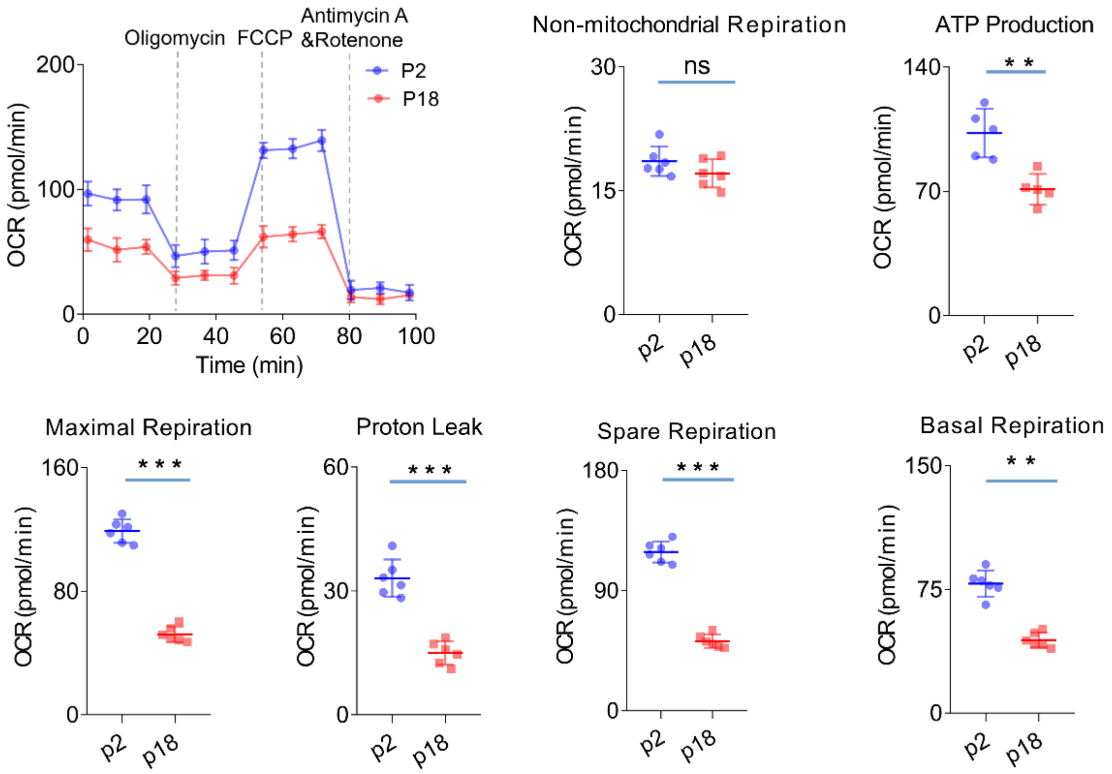


Fig. S29. Seahorse analysis reveals impaired mitochondrial respiration in replicated senescent cells, including reduced basal respiration, ATP-linked respiration, maximal respiration, spare respiration capacity, and proton leak.

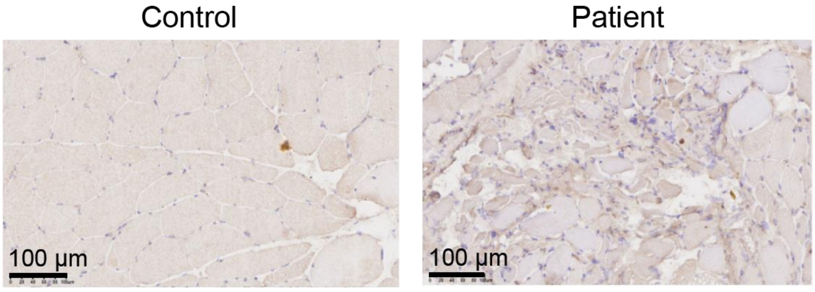


Fig. S30. Immunohistochemical staining of tissue sections from controls and patients with *FUS* gene mutations.

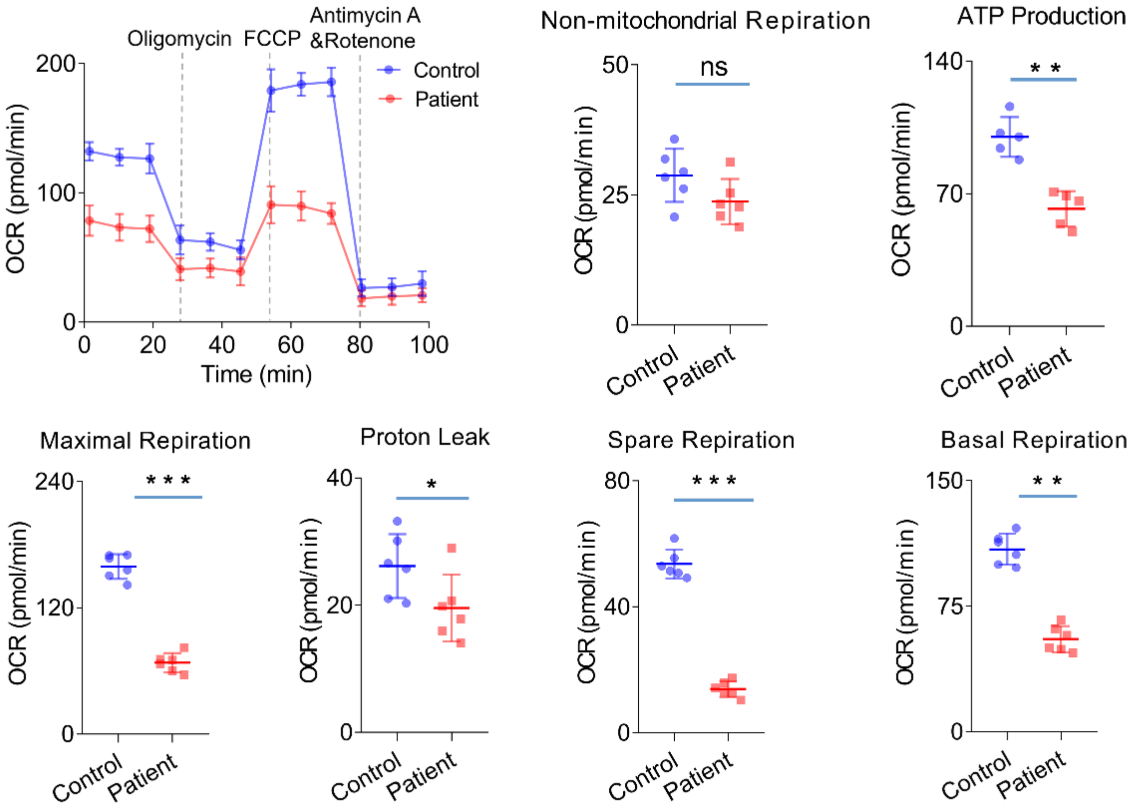


Fig. S31. Seahorse analysis reveals impaired mitochondrial respiration in ALS cells, including reduced basal respiration, ATP-linked respiration, maximal respiration, spare respiration capacity, and proton leak.

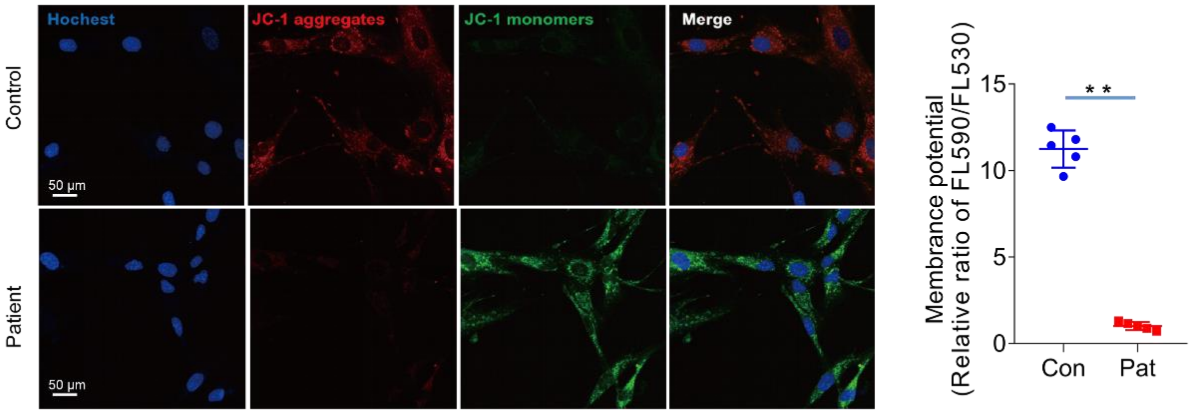


Fig. S32. JC-1 staining of normal and *FUS*-mutant patient cells.

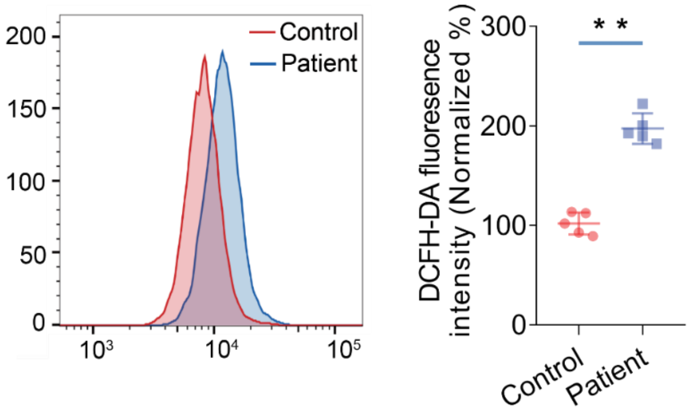


Fig. S33. Fluorescence intensity quantification of DCFH-DA measured by flow cytometry.

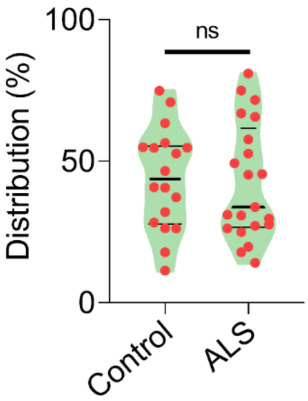


Fig. S34. Comparison of the 2.0-3.0 ns fluorescence lifetime distributions in Figs. 6g, 6i.

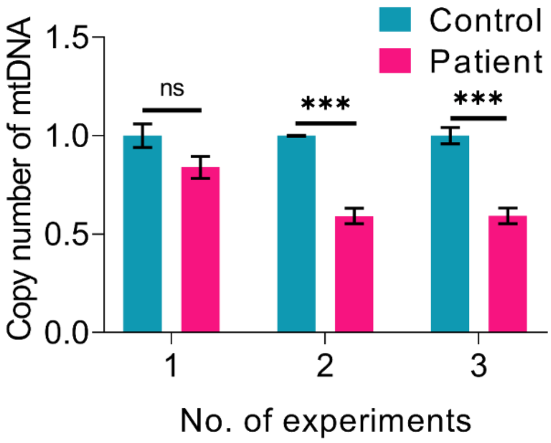


Fig. S35. qPCR analysis revealed a reduced mtDNA copy number in ALS patient–derived human skin fibroblasts.

Table S1. Fluorescence lifetime fitting parameters of SDMNA in the presence of DNA at a 1:1 DNA-to-SDMNA molar ratio.

Type	**Name**	**τ (ns)**	**τ1 (ns)/f1**	**τ2 (ns)/f2**
G-quadruplex DNA	mtDNA9438	3.6989	1.1488/18.66%	4.2839/81.34%
	mtDNA1015	4.1057	1.7249/25.18%	4.9070/74.82%
	mtDNA16250	4.1796	1.6606/30.82%	5.3018/69.18%
	mtDNA6363	3.8409	1.4464/29.69%	4.8521/70.31%
	wtTel26	4.5576	1.2526/8.78%	4.8757/91.22%
	Tel26	4.3284	1.4520/11.17%	4.6901/88.83%
	C-MYC	4.0225	1.4521/30.42%	5.1462/69.58%
	22AG	4.0729	1.3448/14.18%	4.5237/85.82%
Non G-quadruplex DNA	mt15653	2.8547	1.0922/40.07%	4.0332/59.93%
	dsDNA	0.5549	0.7674/50.79%	3.3557/49.21%
	ss15a	2.5318	1.2402/36.52%	3.2748/63.48%
	dt21	1.5268	0.6278/68.12%	3.4477/31.88%

Table S2. Fluorescence lifetime fitting parameters of SDMNA in the presence of DNA at a 1:1 DNA-to-SDMNA molar ratio.

Type	**Name**	**τ(ns)**	**τ1(ns)/f1**	**τ2(ns)/f2**
G-quadruplex DNA	mtDNA9438	3.8637	1.4559/19.50%	4.4470/80.50%
	mtDNA1015	4.3940	1.8962/21.93%	5.0957/78.07%
	mtDNA16250	4.5156	2.1513/34.02%	5.7347/65.98%
	mtDNA6363	4.2046	1.4554/23.21%	5.0356/76.79%
	wtTel26	4.6969	1.8177/8.39%	4.9606/91.61%
	Tel26	4.4526	1.9191/12.00%	4.7981/88.00%
	c-Myc	4.5663	1.7498/24.51%	5.4808/75.49%
	22AG	4.2707	2.0347/12.86%	4.6007/87.14%
Non G-quadruplex DNA	mt15653	2.9219	1.1430/37.84%	4.0049/62.16%
	dsDNA	2.3355	1.0714/43.77%	3.3195/56.23%
	ss15a	2.5793	1.7173/56.00%	3.6764/44.00%
	dt21	1.6388	0.6452/63.17%	3.3430/36.83%

Table S3. Fluorescence lifetime fitting parameters of SDMNA under different conditions.

Type		**τ(ns)**	**τ1(ns)/f1**	**τ2(ns)/f2**
pH	3	0.5687	0.0613/80.13%	2.6148/19.87%
	4	0.4057	0.0546/85.83%	2.5324/14.17%
	5	0.4716	0.0627/84.34%	2.6735/15.66%
	6	0.6627	0.0593/76.17%	2.5915/23.83%
	7	0.8150	0.0631/68.90%	2.4809/31.10%
	8	0.5589	0.0700/78.12%	2.3043/21.88%
	9	0.6153	0.0719/73.41%	2.1155/26.59%
	10	0.9268	0.0651/62.79%	2.3808/37.21%
Viscosity (Gly-MeOH)	0%	0.1945	0.1488/94.70%	1.0114/5.30%
	10%	0.2213	0.1545/91.04%	0.8999/8.96%
	20%	0.2197	0.1204/76.45%	0.5422/23.55%
	30%	0.2339	0.1192/66.89%	0.4657/33.11%
	40%	0.2788	0.1440/67.92%	0.5643/32.08%
	50%	0.3162	0.1517/63.00%	0.5962/37.00%
	60%	0.3996	0.1788/58.08%	0.7056/41.92%
	70%	0.5059	0.1690/44.23%	0.7730/55.77%
	80%	0.6802	0.1882/35.97%	0.9566/64.03%
	90%	0.9551	0.3044/30.22%	1.2369/69.78%
	100%	1.8097	1.5406/92.38%	5.0722/7.62%
Solvent	THF	0.3116	0.1762/87.63%	1.2706/12.37%
	EA	0.2070	0.1306/92.86%	1.2008/7.14%
	EtOH	0.3667	0.1713/79.44%	1.1219/20.56%
	MeCN	0.4619	0.1533/82.13%	1.8804/17.87%
	DMSO	0.2378	0.1203/88.21%	1.1173/11.79%
HSA	HSA: SDMNA=1:1	1.9142	0.7218/61.43%	3.8132/38.57%
	HSA: SDMNA=2:1	2.5195	0.8922/49.77%	4.1319/50.23%
BSA	BSA: SDMNA=1:1	2.2811	0.8611/54.90%	4.0096/45.10%
	BSA: SDMNA=2:1	2.4023	0.8820/51.46%	4.0140/48.54%

Table S4. List of DNA sequences used in this research.

Type	**Name**	**Sequence**
G-quadruplex DNA	mtDNA9438	5’-GGC GTA GGT TTG GTC TAG GG-3’
	mtDNA1015	5’-GGG CTT GAT GTG GGG AGG GGT GTT TAA GGG-3’
	mtDNA16250	5’-GAA GCG GGG GAG GGG GGG TTT GGT GGA AAT-3’
	mtDNA6363	5’-AGG GAC GCG GGC GGG GGA TAT AGG GT-3’
	wtTel26	5’-TTA GGG TTA GGG TTA GGG TTA GGG TT-3’
	Tel26	5’-AAA GGG TTA GGG TTA GGG TTA GGG AA-3’
	c-Myc	5’-TGG GGA GGG TGG GGA GGG TGG GGA AGG-3’
	22AG	5’-AGG GTT AGG GTT AGG GTT AGG G-3’
Non G-quadruplex DNA	mt15653	5’-GGG TTA ATC GTG TGA CCG CGG TGG CTG G-3’
	dsDNA	5’-AGA GGA GAG CTC T-3’
	ss15a	5’-CGC GCG TTT CGC GCG-3’
	dt21	5’-AAA AAA AAA AAA AAA AAA AAA-3’

Table S5. Primer sequences used in mtDNA copy number and mtG4DNA content.

Name	**Sequence**
COX3 (mtDNA, for copy number)	Forward: 5′-CAGCCCATGACCCCTAACAG-3′
	Reverse: 5′-TACATCGCGCCATCATTGGT-3′
GAPDH (nuclear DNA, for copy number)	Forward: 5′-CAGGTTGTCTCCTGCGACTTC-3′
	Reverse: 5′-GGGTGGTCCAGGGTTTCTTAC-3′
Homo-MT-ND6-112 (G4-enriched mtDNA region)	Forward: 5′-AATCATACAAAGCCCCCGCA-3′
	Reverse: 5′-GGGGTGGTGGTTGTGGTAAA-3′
Homo-MT-ND6-130 (G4-enriched mtDNA region)	Forward: 5′-CCAATCCTACCTCCATCGCTAA-3′
	Reverse: 5′-TTAGGGGGAATGATGGTTGTCT-3′
Homo-MT-ATP6-141 (non-G4 mtDNA control region)	Forward: 5′- ACAACTAACCTCCTCGGACTC-3′
	Reverse: 5′- GTGGGCTAGGGCATTTTTAATC-3′
Mus-MT-ND6-161 (G4-enriched mtDNA region)	Forward: 5′- TGGTTGTCTTGGGTTAGCATT-3′
	Reverse: 5′- CAAACACAACCAACATCCCCC-3′
Mus-MT-ND6-84 (G4-enriched mtDNA region)	Forward: 5′- GTTGGAAGGAGGGATTGGGG-3′
	Reverse: 5′- CCCGCAAACAAAGATCACCC-3′
Mus-MT-ATP6-95 (non-G4 mtDNA control region)	Forward: 5′- TGCCTCATTCATTACCCCAACAAT-3′
	Reverse: 5′- TTAGGCGTTTTGAGGATGGGAA-3′