

Supplementary Materials for  
**MRE11-dependent instability in mitochondrial DNA fork protection activates  
a cGAS immune signaling pathway**

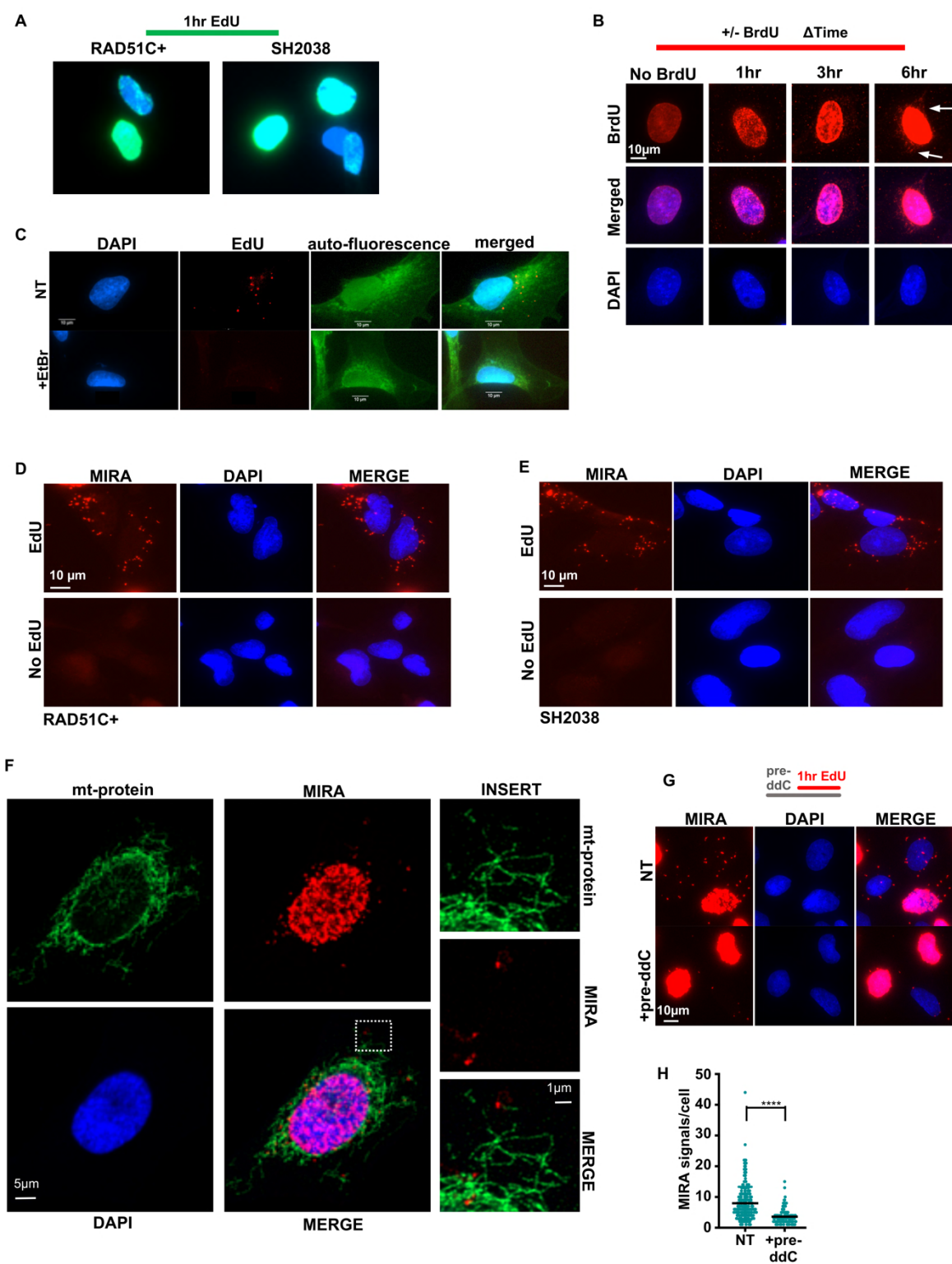
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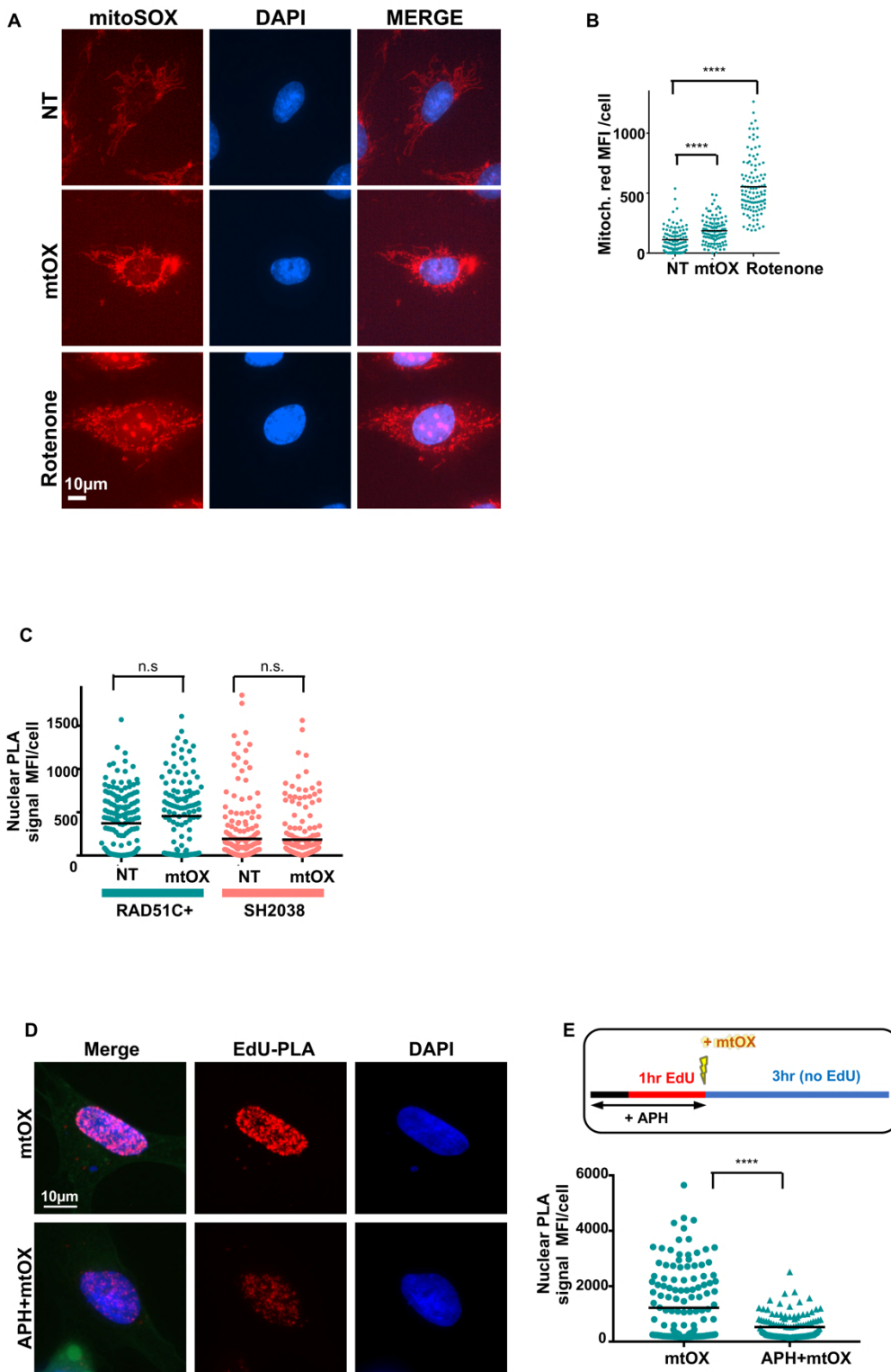


**fig. S1. Nascent mtDNA Replication Assay (MIRA) Development**

(A) Representative images of EdU labeled cells (1hr) clicked to Alexa Fluor488 in RAD51C+ cells. Note the absence of cytoplasmic signals with one hour labeling only.

(B) Visualization of DNA by BrdU incorporation for extended times in RAD51C+ cells. Arrows indicate mtDNA. Note that cytoplasmic signals increase with time of labeling and are minute with one hour labeling insufficient for efficient and accurate quantitation.

- (C)** Representative images of intra-cellular and extra-nuclear EdU-PLA signals. Auto-fluorescence of the cell displays the cytoplasm. The cytoplasmatic signals are inhibited in the presence of Ethidium bromide (EtBr), inhibiting mtDNA replication.
- (D, E)** Representative images of MIRA assays in RAD51C<sup>+</sup> and SH2038 cells, with and without EdU.
- (F)** Airyscan super-resolution image of RAD51C<sup>+</sup> cells with immunofluorescent stain against mitochondrial protein (mt-protein) and MIRA signals.
- (G)** Representative images of MIRA assay in RAD51C<sup>+</sup> cells in which cells were incubated with or without dideoxycytidine (20 $\mu$ M, ddC) for thirty minutes prior to and during EdU incubation to suppress EdU incorporation into mtDNA.
- (H)** Scatter dot plot quantification of (G) MIRA signals. Top, sketch of experimental scheme.



**fig. S2. Validation of mtOX and Aphidicolin**

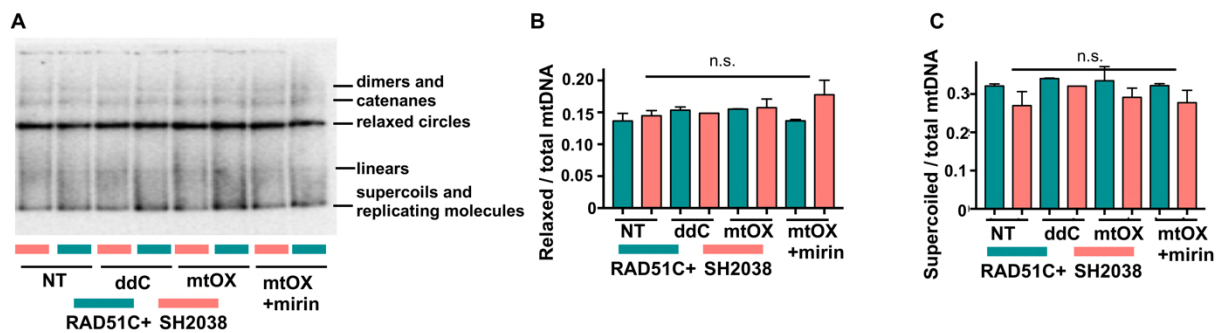
(A) Representative images and (B) scatter dot blot analysis of red fluorescent mitoSOX signals in RAD51C+ cells treated with mtOX (4  $\mu$ M) or Rotenone (5  $\mu$ M, strong positive control for oxidative damage producing mitochondrial agent). The mitoSOX dye shows the statistically significant induction of mitochondrial superoxide with mtOX and the stronger Rotenone.

(C) Scatter dot plot quantification of nuclear EdU-PLA signals with and without mtOX (4  $\mu$ M).

(D) Representative images and (E) scatter dot blot quantification of MIRA signals in RAD51C+ cells treated with mtOX (4  $\mu$ M) or additionally aphidicolin (APH, 7 $\mu$ M), which represses nuclear EdU incorporation

MFI, mean fluorescence intensity. Bars represent the mean of compiled data and the  $p$ -values are derived from the Mann-Whitney.

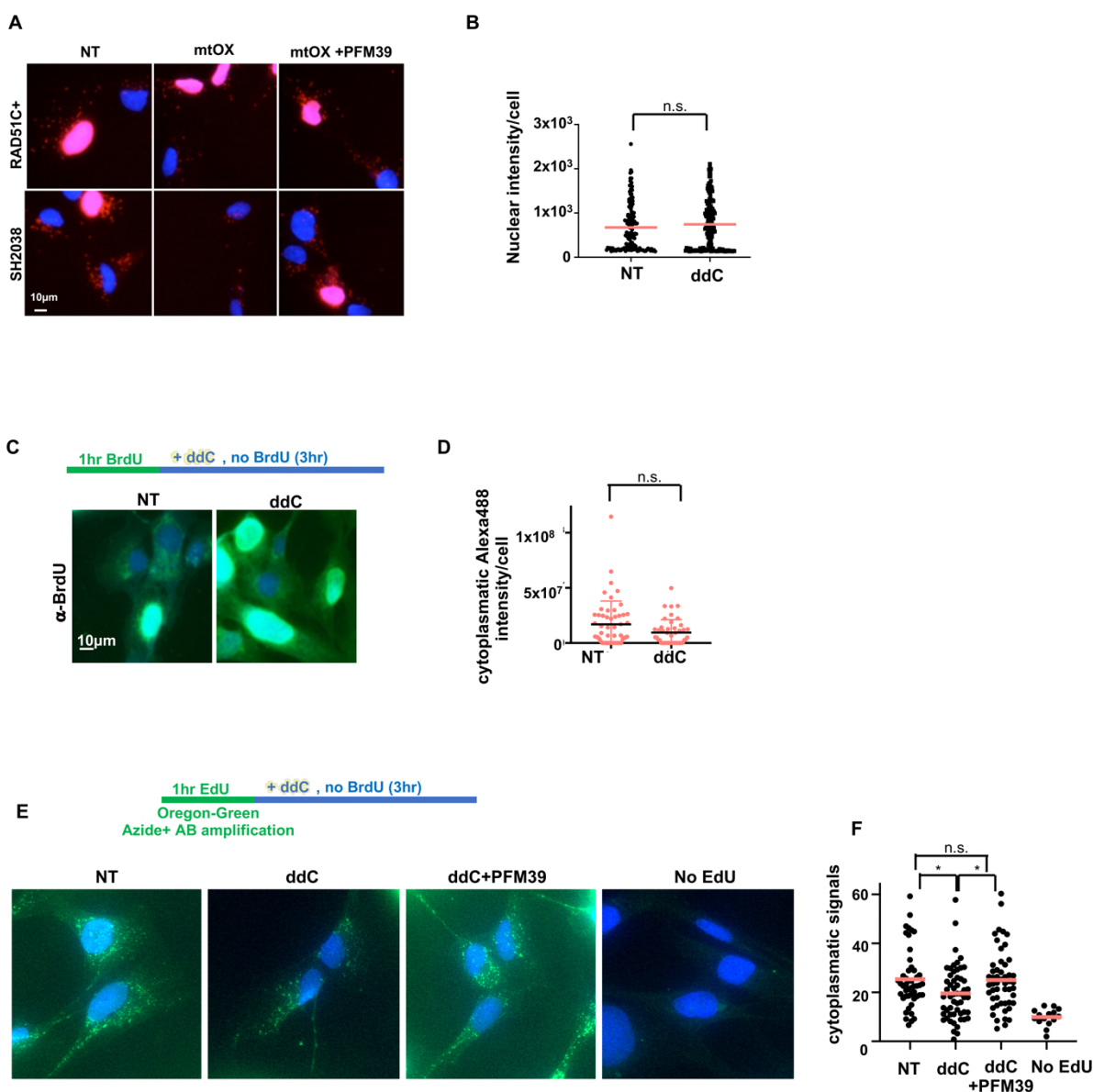
\*\*\*\* $p < 0.0001$ , n.s. not significant



**fig. S3. mtOX Does Not Cause mtDNA Linearization.**

(A) Southern blot for assessment of mtDNA topology.

(B, C) Bar graph quantification of (A). There was no significant difference between any of the conditions (ANOVA/Tukey).



**fig. S4. MIRA Detects Nascent mtDNA Other Than 7S.**

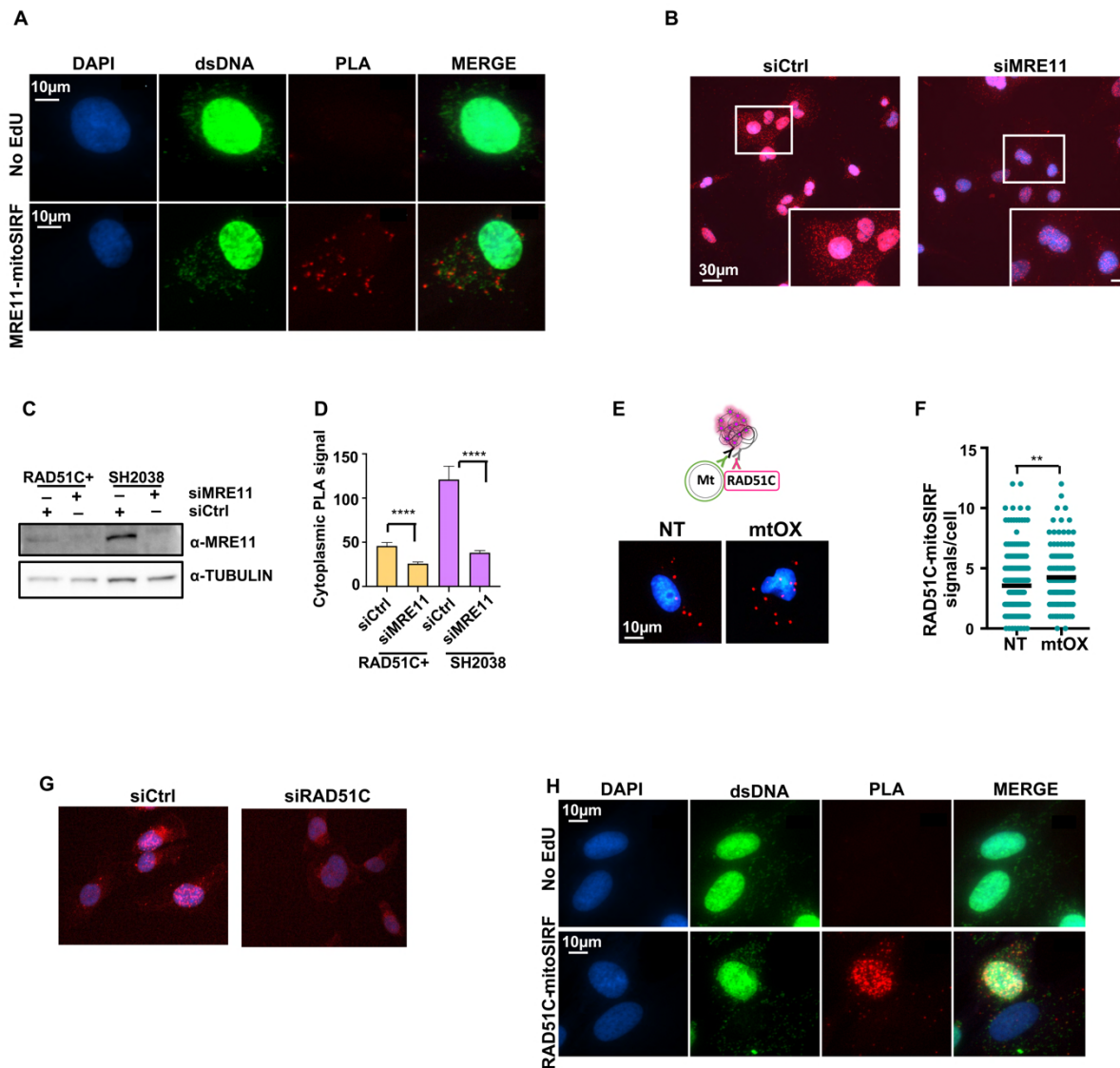
(A) Representative images corresponding to data in Fig. 3C.

**(B)** Nuclear intensity of PLA signals in SH2038 cells with and without ddC.

**(C, D)** Representative images and scatter dot plot quantification of SH2038 cells labeled for one hour with BrdU and released into media with or without ddC (20  $\mu$ M) as indicated in the experimental sketch on top.

**(E, F)** Representative images and scatter dot plot quantification of SH2038 cells labeled for one hour with EdU and released into media with or without ddC (20  $\mu$ M) as indicated in the experimental sketch on top. EdU was detected by click-it conjugation to Oregon-green and subsequent anti-Oregongreen antibody detection for signal amplification.

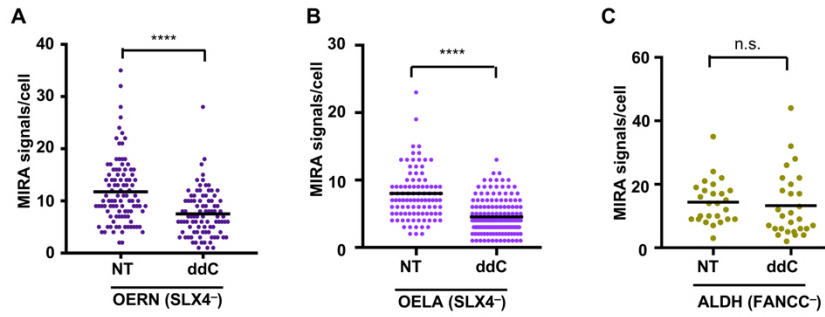
Bars in scatter dot blots represent the mean of compiled data and the  $p$ -values are derived from the Mann-Whitney.  $p$ -values of the bar graphs for 7S DNA and mtDNA topology were derived using the ANOVA/Tukey analysis. \*\*\* $p$ <0.001, \*\* $p$ <0.01, \* $p$ <0.05 n.s. not significant



**fig. S5. Validation of mitoSIRF**

(A) Representative images of MRE11-mitoSIRF in BJ fibroblasts with and without EdU.  
 (B) Representative images of PLA against MRE11 in RAD51C+ cells transfected with siRNA against MRE11 or scramble siRNA (siCtrl)  
 (C) Western blot against MRE11 in SH2038 and RAD51C+ cells transfected with siRNA against MRE11 or scramble siRNA (siCtrl)  
 (D) Quantification of cytoplasmic MRE11-PLA signals of (C) in SH2038 and RAD51C+ cells transfected with siRNA against MRE11 or scramble siRNA (siCtrl)  
 (E, F) Representative images and scatter dot blot of analysis of RAD51C-mitoSIRF signals in RAD51C+ cells with and without mtOX (4  $\mu$ M, 3 hours).  
 (G) Representative images of PLA against RAD51C in RAD51C+ cells transfected with siRNA against RAD51C or scramble siRNA (siCtrl)  
 (H) Representative images of RAD51C-mitoSIRF in BJ fibroblasts with and without EdU.  
 Bars in scatter dot blots represent the mean of compiled data and the *p*-values are derived from the Mann-Whitney. \*\*\**p*<0.001, \*\**p*<0.01





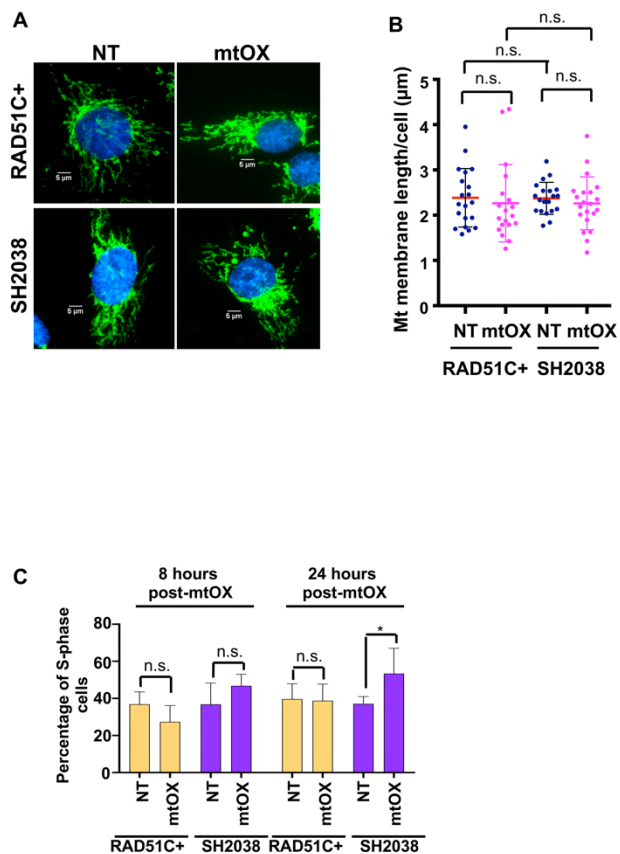
**fig. S6. SLX4 But Not FANCC Core Complex Proteins are Required for mtDNA Fork Protection Defects**

(A, B) Scatter dot plot quantification of MIRA assay signals with and without ddC in OERN, OELA, (SLX4 defective) patient fibroblasts.

(C) Scatter dot plot quantification of MIRA assay signals with and without ddC in ALDH (FANCC defective) patient fibroblasts.

Bars represent the mean of compiled data. *p*-values are derived using the Mann-Whitney test.

\*\*\*\**p*<0.0001, n.s. not significant



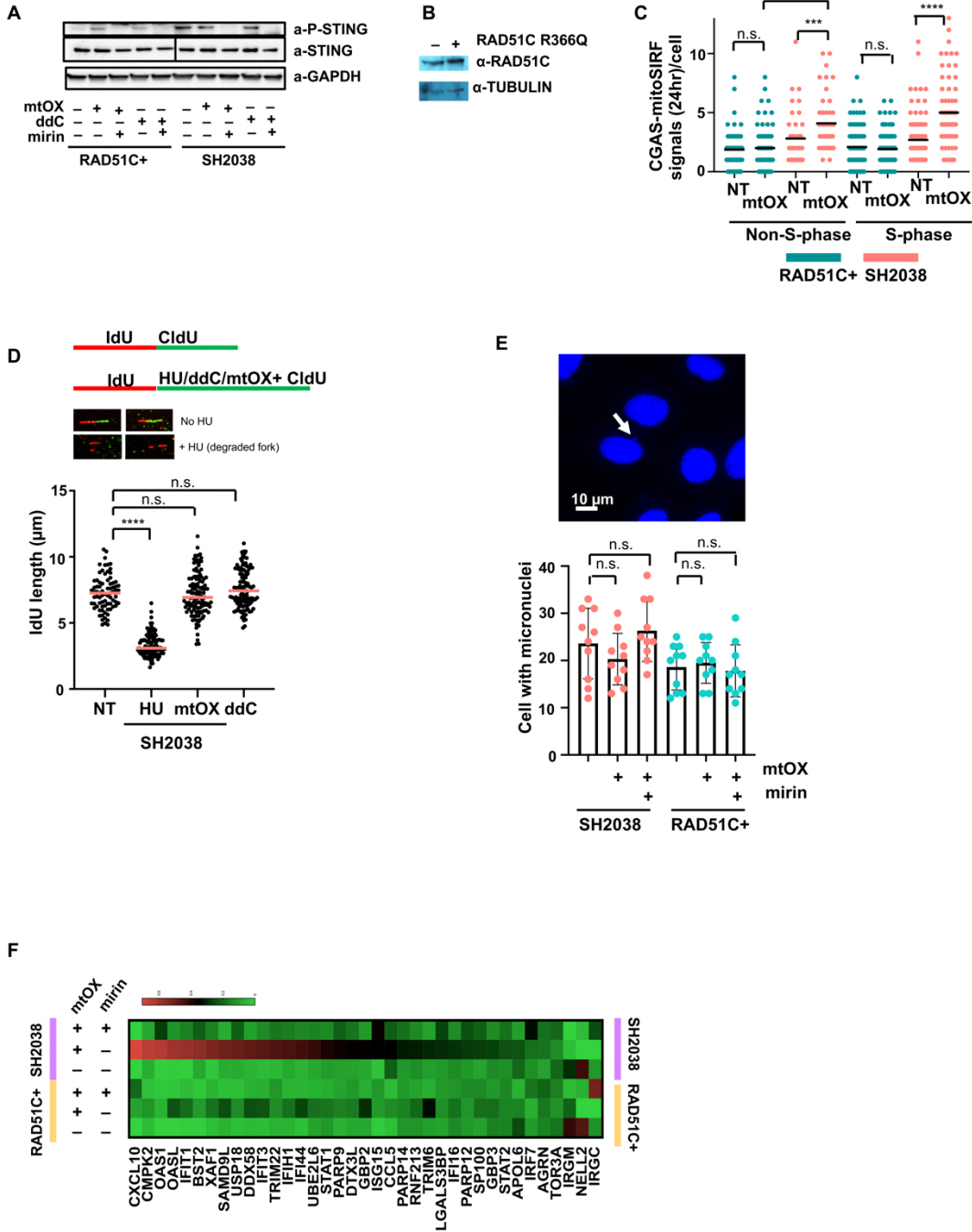
**fig. S7. Effects of mtOX on Mitochondrial Physiology**

(A) Representative immunofluorescence images of cells treated with mtOX (4 μM), or left untreated as indicated and stained for mitochondrial protein (mt-protein, depicted in green; DAPI depicted in blue).

(B) Scatter dot plot of mitochondrial length measurements. Error bars represent the standard deviation and the *p*-values are derived from the Mann Whitney-test.

(C) Bar graph of EdU positive cells 8 hours or 24 hours after treatment with mtOX and labeled with EdU thirty minutes prior to fixation. Error bars represent the standard deviation and the *p*-values are derived from the Student T-test.

Figure S8.



**fig. S8. cGAS after mtOX Treatment is Activated by Mitochondrial and Not Nuclear DNA**

(A) Western blot of STING and P-STING twenty-four hours after mtOX treatment with and without MRE11 inhibition by mirin.

(B) Western blot against RAD51C in SH2038 cells with and without expressing RAD51C R366Q from stably transfected expression plasmid.

(C) Scatter dot plot quantification of cGAS-mitoSIRF signals in S-phase cells and non-S phase cells following the experimental schemes of main Fig. 6D.

(D) Single-molecule DNA fiber analysis of SH2038 cells exposed to HU, mtOX or ddC as delineated in sketch above.

(E) Analysis of micronuclei twenty-four hours after mtOX treatment with and without MRE11 inhibition by mirin.

(F) Heatmap of RNAseq data of SH2038 and RAD51C+ cells with and without mtOX (4  $\mu$ M) and mirin (50  $\mu$ M) of previously reported mtDNA-cGAS-activated inflammation genes in TFAM heterozygous cells.

Bars of the scatter graphs represent the mean of compiled data and  $p$ -values are derived using the Mann-Whitney test. \*\*\*\* $p$ <0.001, \*\*\* $p$ <0.001, n.s. not significant

Gene ID	Gene name	fpkm (RAD51C+, no treatment)	fpkm (RAD51C+, mtOX)	fpkm (RAD51C+, mtOX+mirin)	fpkm (SH2038, no treatment)	fpkm (SH2038, mtOX)	fpkm (SH2038, mtOX+mirin)
ENSG00000134321	RSAD2	0.289907472	4.691412128	0.272464236	0.767713348	26.94902321	3.241440588
ENSG00000183486	MX2	0.37108727	6.5048623	1.498821472	1.277381313	35.91227627	10.33842858
ENSG00000089127	OAS1	0.267472421	3.814568561	1.417087861	0.638860957	40.67265218	17.13831598
ENSG00000162654	GBP4	0.007239406	0.190606306	0	0.008645703	0.404970821	0.037421084
ENSG00000157601	MX1	4.410018988	26.55722604	5.006118109	11.08294883	130.6270565	33.74761984
ENSG00000135114	OASL	0.574392214	27.39483418	2.948494273	1.549786801	69.30856698	14.10313172
ENSG00000111335	OAS2	0.412263857	8.421203212	3.609892132	1.163732781	47.17217145	19.49404116
ENSG00000185745	IFIT1	1.249460371	32.60947612	8.086254409	1.989566986	96.66952721	25.99985584
ENSG00000130303	BST2	0.696460736	6.56385268	1.439060372	5.042497975	35.16127556	13.44398358
ENSG00000132530	XAF1	1.224284934	5.553072453	1.836933876	0.818781224	16.0257785	4.746321767
ENSG00000184979	USP18	2.000075596	7.380376437	2.013343054	4.155171868	27.29498386	11.71164371
ENSG00000119917	IFIT3	14.14553331	81.71000621	24.04234899	17.14805872	163.2300765	48.73096985
ENSG00000117228	GBP1	2.645274336	17.05329812	2.750317739	1.710470398	24.26480546	4.06054213
ENSG00000132274	TRIM22	1.231694541	2.681720403	2.226567893	1.063343346	8.898985166	3.41348771
ENSG00000172183	ISG20	1.086458352	2.082274089	0.930992014	1.363324822	5.311315228	0.885129196
ENSG00000185885	IFITM1	0.466903612	4.221873943	4.155796612	2.973879073	37.49453841	33.05103476
ENSG00000165949	IFI27	1.697569306	3.342316872	3.179758548	4.141860901	27.30402933	21.74847971
ENSG00000156587	UBE2L6	12.85840081	25.95135754	8.892068674	11.61819214	57.66988388	21.35370063
ENSG00000111331	OAS3	10.22580757	27.59665273	19.32326581	13.52096428	103.2090323	73.80162548
ENSG00000068079	IFI35	22.98211696	31.31134789	19.17875977	15.1532139	76.81697333	31.13094982
ENSG00000115415	STAT1	37.02838136	57.76410338	23.16198874	34.32747407	127.1715774	46.30797103
ENSG00000126709	IFI6	92.61457857	196.8710434	123.3961691	387.4971505	994.3317131	823.1279084
ENSG00000106785	TRIM14	3.943626827	21.86997668	14.23298358	3.865283301	41.55853478	25.84150196
ENSG00000162645	GBP2	0.156394348	0.547107821	0.137673141	0.158040258	0.686433779	0.202103796
ENSG00000187608	ISG15	28.57239967	210.9168689	221.9514361	63.77527591	694.9611298	717.8270286
ENSG00000101347	SAMHD1	20.48119932	28.92759471	10.90511907	24.50408133	61.38827049	25.58061402
ENSG00000142089	IFITM3	83.93574775	178.8295255	179.1819909	135.1184993	480.8355182	484.8768107
ENSG00000179583	CIITA	0.103393574	0.281224074	0.286840925	0.061739185	0.394350957	0.157905636
ENSG00000121236	TRIM6	0.886334803	2.823443424	0.613224679	0.85614815	2.551982593	0.808506554
ENSG00000055332	EIF2AK2	9.165343014	17.03208371	11.92927761	9.911850553	25.27708735	15.47439067
ENSG00000185201	IFITM2	31.76928372	74.91348446	83.01683455	39.38753913	136.2996587	137.6021821
ENSG00000067066	SP100	13.42558364	26.04965122	19.41023427	12.41749642	36.16421351	28.85182469
ENSG00000163462	TRIM46	0.721885332	2.687790187	2.379750923	0.830185501	3.350219367	3.144121082
ENSG00000026508	CD44	64.66345407	134.2076486	79.77621024	50.15565488	137.1422591	75.09398828
ENSG00000140464	PML	16.44118866	26.87167206	40.89085458	15.1657907	46.89219985	47.1163422
ENSG00000182179	UBA7	3.522652594	8.318991711	12.24082127	2.520957471	11.4021959	12.47516749
ENSG00000102882	MAPK3	8.881536183	22.8093079	21.04841948	9.96711443	25.28619504	24.71872173
ENSG00000120738	EGR1	208.2236856	11.84152305	46.04749783	360.4913255	14.96028287	98.25459308
ENSG00000058404	CAMK2B	1.705610339	0.119370039	0.126827292	2.072670666	0.078114655	0.296459221

**Table S1.** fpkm values related to Fig. 7B

Gene ID	Gene name	fpkm (RAD51C+, no treatment)	fpkm (RAD51C+, mtOX)	fpkm (RAD51C+, mtOX+mirin)	fpkm (SH2038, no treatment)	fpkm (SH2038, mtOX)	fpkm (SH2038, mtOX+mirin)
ENSG00000137959	IFI44L	0.068875533	1.009747265	0.054736119	0.061691251	6.713647454	0.945686801
ENSG00000183486	MX2	0.37108727	6.5048623	1.498821472	1.277381313	35.91227627	10.33842858
ENSG00000135114	OASL	0.574392214	27.39483418	2.948494273	1.549786801	69.30856698	14.10313172
ENSG00000111335	OAS2	0.412263857	8.421203212	3.609892132	1.163732781	47.17217145	19.49404116
ENSG00000185745	IFIT1	1.249460371	32.60947612	8.086254409	1.989566986	96.66952721	25.99985584
ENSG00000130303	BST2	0.696460736	6.56385268	1.439060372	5.042497975	35.16127556	13.44398358
ENSG00000168062	BATF2	0.258987017	0.805866248	0.263449265	0.577353907	4.133817289	1.450285513
ENSG00000133321	RARRES3	0.425589113	3.112592847	0.12025607	0.734156581	4.973093487	0.54997624
ENSG00000136514	RTP4	0	0.375754452	0	0	0.60674219	0.258197054
ENSG00000107201	DDX58	2.10212717	10.23538539	2.733958386	2.533404207	20.55453106	4.192615042
ENSG00000119917	IFIT3	14.14553331	81.71000621	24.04234899	17.14805872	163.2300765	48.73096985
ENSG00000185885	IFITM1	0.466903612	4.221873943	4.155796612	2.973879073	37.49453841	33.05103476
ENSG00000115267	IFIH1	2.048141854	6.20173007	1.961060392	2.711234677	14.85302749	5.389172213
ENSG00000165949	IFI27	1.697569306	3.342316872	3.179758548	4.141860901	27.30402933	21.74847971
ENSG00000107201	DDX58	2.10212717	10.23538539	2.733958386	2.533404207	20.55453106	4.192615042
ENSG00000111331	OAS3	10.22580757	27.59665273	19.32326581	13.52096428	103.2090323	73.80162548
ENSG00000138642	HERC6	3.038392853	12.48123464	3.952929297	4.301103371	25.86911402	12.6888726
ENSG00000068079	IFI35	22.98211696	31.31134789	19.17875977	15.1532139	76.81697333	31.13094982
ENSG00000187608	ISG15	28.57239967	210.9168689	221.9514361	63.77527591	694.9611298	717.8270286
ENSG00000188313	PLSCR1	28.08739911	26.22100017	15.55864741	30.03261611	64.71489573	33.2418589
ENSG00000138646	HERC5	9.671669757	10.51704068	3.756795522	11.38457923	19.01378235	8.239815448

**Table S2.** fpkm values related to Fig. 7J

Gene ID	Gene name	fpkm (RAD51C+, no treatment)	fpkm (RAD51C+, mtOX)	fpkm (RAD51C+, mtOX+mirin)	fpkm (SH2038, no treatment)	fpkm (SH2038, mtOX)	fpkm (SH2038, mtOX+mirin)
ENSG00000137959	IFI44L	0.068875533	1.009747265	0.054736119	0.061691251	6.713647454	0.945686801
ENSG00000183486	MX2	0.37108727	6.5048623	1.498821472	1.277381313	35.91227627	10.33842858
ENSG00000135114	OASL	0.574392214	27.39483418	2.948494273	1.549786801	69.30856698	14.10313172
ENSG00000111335	OAS2	0.412263857	8.421203212	3.609892132	1.163732781	47.17217145	19.49404116
ENSG00000185745	IFIT1	1.249460371	32.60947612	8.086254409	1.989566986	96.66952721	25.99985584
ENSG00000130303	BST2	0.696460736	6.56385268	1.439060372	5.042497975	35.16127556	13.44398358
ENSG00000168062	BATF2	0.258987017	0.805866248	0.263449265	0.577353907	4.133817289	1.450285513
ENSG00000133321	RARRES3	0.425589113	3.112592847	0.12025607	0.734156581	4.973093487	0.54997624
ENSG00000136514	RTP4	0	0.375754452	0	0	0.60674219	0.258197054
ENSG00000107201	DDX58	2.10212717	10.23538539	2.733958386	2.533404207	20.55453106	4.192615042
ENSG00000119917	IFIT3	14.14553331	81.71000621	24.04234899	17.14805872	163.2300765	48.73096985
ENSG00000185885	IFITM1	0.466903612	4.221873943	4.155796612	2.973879073	37.49453841	33.05103476
ENSG00000115267	IFIH1	2.048141854	6.20173007	1.961060392	2.711234677	14.85302749	5.389172213
ENSG00000165949	IFI27	1.697569306	3.342316872	3.179758548	4.141860901	27.30402933	21.74847971
ENSG00000107201	DDX58	2.10212717	10.23538539	2.733958386	2.533404207	20.55453106	4.192615042
ENSG00000111331	OAS3	10.22580757	27.59665273	19.32326581	13.52096428	103.2090323	73.80162548
ENSG00000138642	HERC6	3.038392853	12.48123464	3.952929297	4.301103371	25.86911402	12.6888726
ENSG00000068079	IFI35	22.98211696	31.31134789	19.17875977	15.1532139	76.81697333	31.13094982
ENSG00000187608	ISG15	28.57239967	210.9168689	221.9514361	63.77527591	694.9611298	717.8270286
ENSG00000188313	PLSCR1	28.08739911	26.22100017	15.55864741	30.03261611	64.71489573	33.2418589
ENSG00000138646	HERC5	9.671669757	10.51704068	3.756795522	11.38457923	19.01378235	8.239815448

**Table S3.** fpkm values related to Fig. 7K

Gene ID	Gene name	fpkm (RAD51C+, no treatment)	fpkm (RAD51C+, mtOX)	fpkm (RAD51C+, mtOX+mirin)	fpkm (SH2038, no treatment)	fpkm (SH2038, mtOX)	fpkm (SH2038, mtOX+mirin)
ENSG00000169245	CXCL10	0	0.045254779	0.096163848	0	0.338449271	0
ENSG00000134326	CMPK2	0.815138588	2.22716595	0.586686167	1.322468763	11.92448377	1.55025665
ENSG00000089127	OAS1	0.267472421	3.814568561	1.417087861	0.638860957	40.67265218	17.13831598
ENSG00000135114	OASL	0.574392214	27.39483418	2.948494273	1.549786801	69.30856698	14.10313172
ENSG00000185745	IFIT1	1.249460371	32.60947612	8.086254409	1.989566986	96.66952721	25.99985584
ENSG00000130303	BST2	0.696460736	6.56385268	1.439060372	5.042497975	35.16127556	13.44398358
ENSG00000132530	XAF1	1.224284934	5.553072453	1.836933876	0.818781224	16.0257785	4.746321767
ENSG00000177409	SAMD9L	0.938846694	3.6832785	0.537595541	1.128648387	7.735231048	1.157000758
ENSG00000184979	USP18	2.000075596	7.380376437	2.013343054	4.155171868	27.29498386	11.71164371
ENSG00000107201	DDX58	2.10212717	10.23538539	2.733958386	2.533404207	20.55453106	4.192615042
ENSG00000119917	IFIT3	14.14553331	81.71000621	24.04234899	17.14805872	163.2300765	48.73096985
ENSG00000132274	TRIM22	1.231694541	2.681720403	2.226567893	1.063343346	8.898985166	3.41348771
ENSG00000115267	IFIH1	2.048141854	6.20173007	1.961060392	2.711234677	14.85302749	5.389172213
ENSG00000137965	IFI44	8.492553254	56.41929707	13.60689948	13.25090531	95.83796988	32.21050332
ENSG00000156587	UBE2L6	12.85840081	25.95135754	8.892068674	11.61819214	57.66988388	21.35370063
ENSG00000115415	STAT1	37.02838136	57.76410338	23.16198874	34.32747407	127.1715774	46.30797103
ENSG00000138496	PARP9	2.267265731	5.66991107	3.316182384	3.529394544	13.37950243	7.621180572
ENSG00000163840	DTX3L	6.583834252	13.25287139	9.159729434	7.555853608	29.19323949	14.49599572
ENSG00000162645	GBP2	0.156394348	0.547107821	0.137673141	0.158040258	0.686433779	0.202103796
ENSG00000187608	ISG15	28.57239967	210.9168689	221.9514361	63.77527591	694.9611298	717.8270286
ENSG00000161570	CCL5	0.324951232	1.127788886	0.371868771	1.785144444	2.872076795	1.805676976
ENSG00000173193	PARP14	7.812348338	9.852947588	6.845288515	7.295688135	20.18060942	11.98435839
ENSG00000173821	RNF213	7.712502578	12.89341442	5.994937868	8.39554982	20.09988414	9.141757619
ENSG00000116525	TRIM62	9.072492795	3.892095363	9.271473591	12.80765657	4.013902989	10.62341522
ENSG00000108679	LGALS3BP	49.3539444	69.46149499	60.66821231	59.07338741	156.9433247	137.4243887
ENSG00000163565	IFI16	15.99297196	20.94875234	13.86150278	15.54090775	32.51177191	15.33420945
ENSG00000059378	PARP12	7.326426148	7.984953079	9.330679883	7.507654082	17.84465089	15.83994031
ENSG00000067066	SP100	13.42558364	26.04965122	19.41023427	12.41749642	36.16421351	28.85182469
ENSG00000117226	GBP3	5.909288512	7.1586712	4.232307433	5.213687874	9.795497632	5.174049927
ENSG00000170581	STAT2	38.99741915	38.05584566	36.78566416	32.95007889	60.44939646	46.77557054
ENSG00000221963	APOL6	12.80523853	7.904634175	3.252791511	10.35625044	12.25671042	5.285594032
ENSG00000185507	IRF7	27.53046725	17.0044337	44.71309484	32.82676396	46.91521996	82.77966226
ENSG00000188157	AGRN	81.27361784	92.83694474	110.2432045	51.14210057	96.22110349	98.28031261
ENSG00000186283	TOR3A	27.12805973	18.63001708	24.22471121	30.76433686	20.53139323	30.890377
ENSG00000237693	IRGM	0.320837925	0.159987511	0.033996442	0.223511481	0.029912673	0
ENSG00000184613	NELL2	0.331563612	0	0.046843934	0.307978029	0	0.031738508
ENSG00000124449	IRGC	0.106752932	0	0.441155992	0.095617727	0	0.206930491

**Table S4.** fpkm values related to fig. S8F