995 **Supplemental Materials:**

996 **Figs. S1 to S9**:

997 Supplemental Figure 1. UVB-driven IFN responses are mtDNA dependent and UV-induced Z-

998 DNA derives from mtDNA.

999 Supplemental Figure 2. UVB induces oxidative DNA damage in the cytosol and mitochondrial

1000 compartment.

1001 Supplemental Figure 3. Cytosolic Z-DNA accumulation is associated with mitochondrial

1002 fragmentation.

1003 Supplemental Figure 4. IFNα does not increase mitochondrial or total cellular ROS in N/TERTs.

1004 Supplemental Figure 5. ZBP1 expression does not correlate with systemic autoantibodies or

1005 patient age.

1006 Supplemental Figure 6. mitoTEMPO rescues UVB-induced IFN expression in lupus KCs.

1007 Supplemental Figure 7. UVB leads to cytosolic shift of cGAS in N/TERTs.

1008 Supplemental Figure 8. ISGs are significantly increased after Z-DNA transfection in N/TERTs

1009 and primary KCs.

1010 Supplemental Figure 9. Overexpression of ZBP1 results in cytosolic expression.

1011 **Tables S1 to S4:**

1012 Supplemental Table 1. Demographics and characteristics of patients and controls for primary
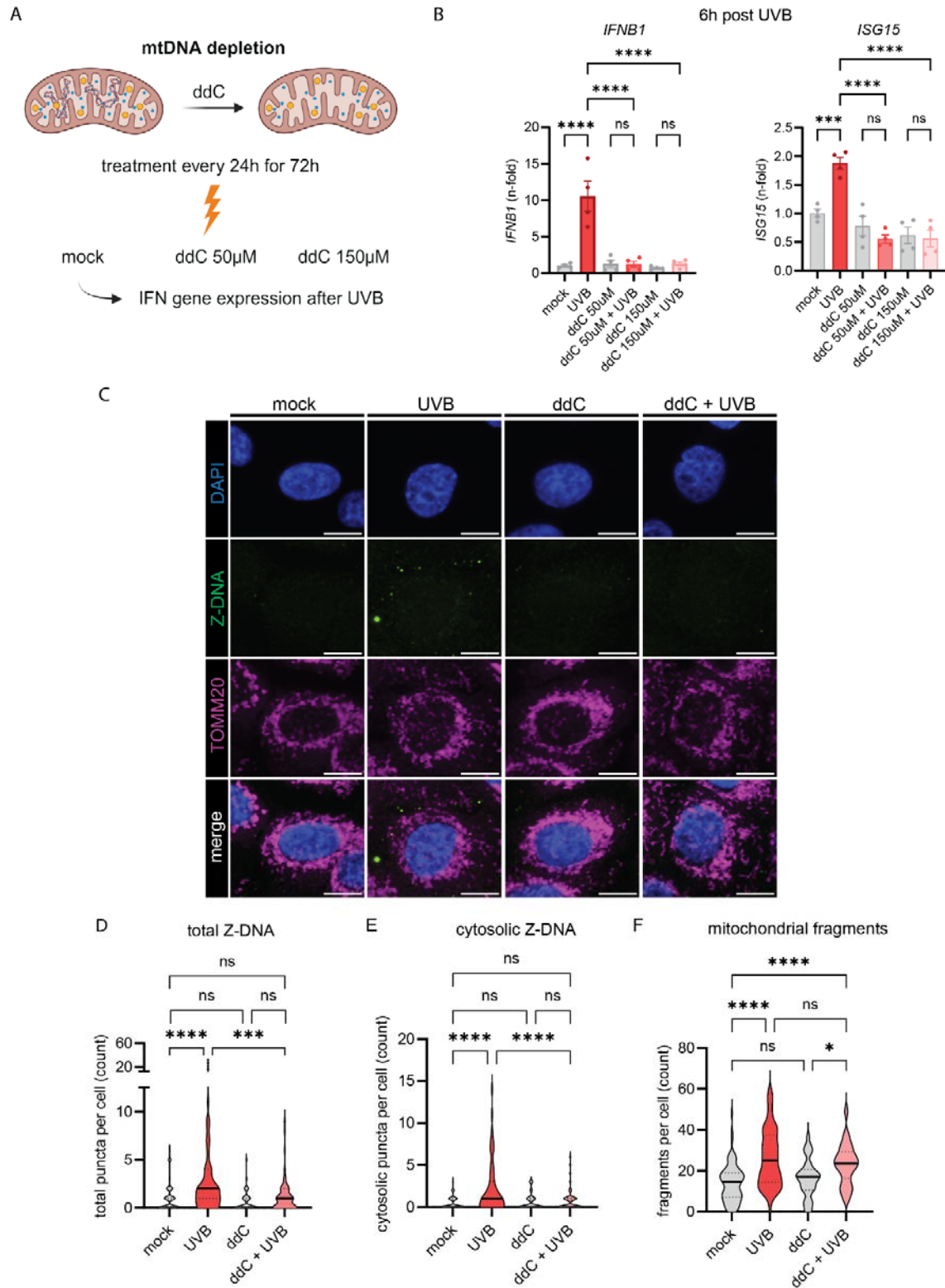
1013 keratinocyte cell culture

1014 Supplemental Table 2. Demographics and characteristics of lupus and dermatomyositis patients

1015 from which skin biopsies were used for tissue immunofluorescence

1016

1017   **Supplemental Data**

1018

1019   **Supplemental Figure 1**

1020
1021

1022 **Supplemental Figure 1.UVB-driven IFN responses are mtDNA dependent and UV-induced Z-DNA**

1023 **derives from mtDNA.**

1024 **A.** Experimental approach for mtDNA depletion in N/TERTs using nucleoside 2',3' dideoxycytidine (ddC).

1025 Treatment with ddC was performed for 72h. After irradiation, medium was changed to ddC-free medium

1026 until gene expression analysis. **B.** Quantitative gene expression 6h after UVB exposure. n=2 for each

1027 experiment. **C.** Representative confocal images of N/TERTs treated with +/- ddC +/- UVB 3h after UVB

1028 exposure stained for Z-DNA, TOMM20 and DAPI. Scale bar 10μm. **D.** Quantification of Z-DNA puncta

1029 and mitochondrial fragments using CellProfiler open-source software from conditions in (**C.**), n=3.

1030 Comparisons were done via ordinary one-way ANOVA followed by Sidak's multiple comparison test.

1031 Mean and SEM. *P<0.05, **P<0.01, ***P<0,001, ****P<0.0001.

1032
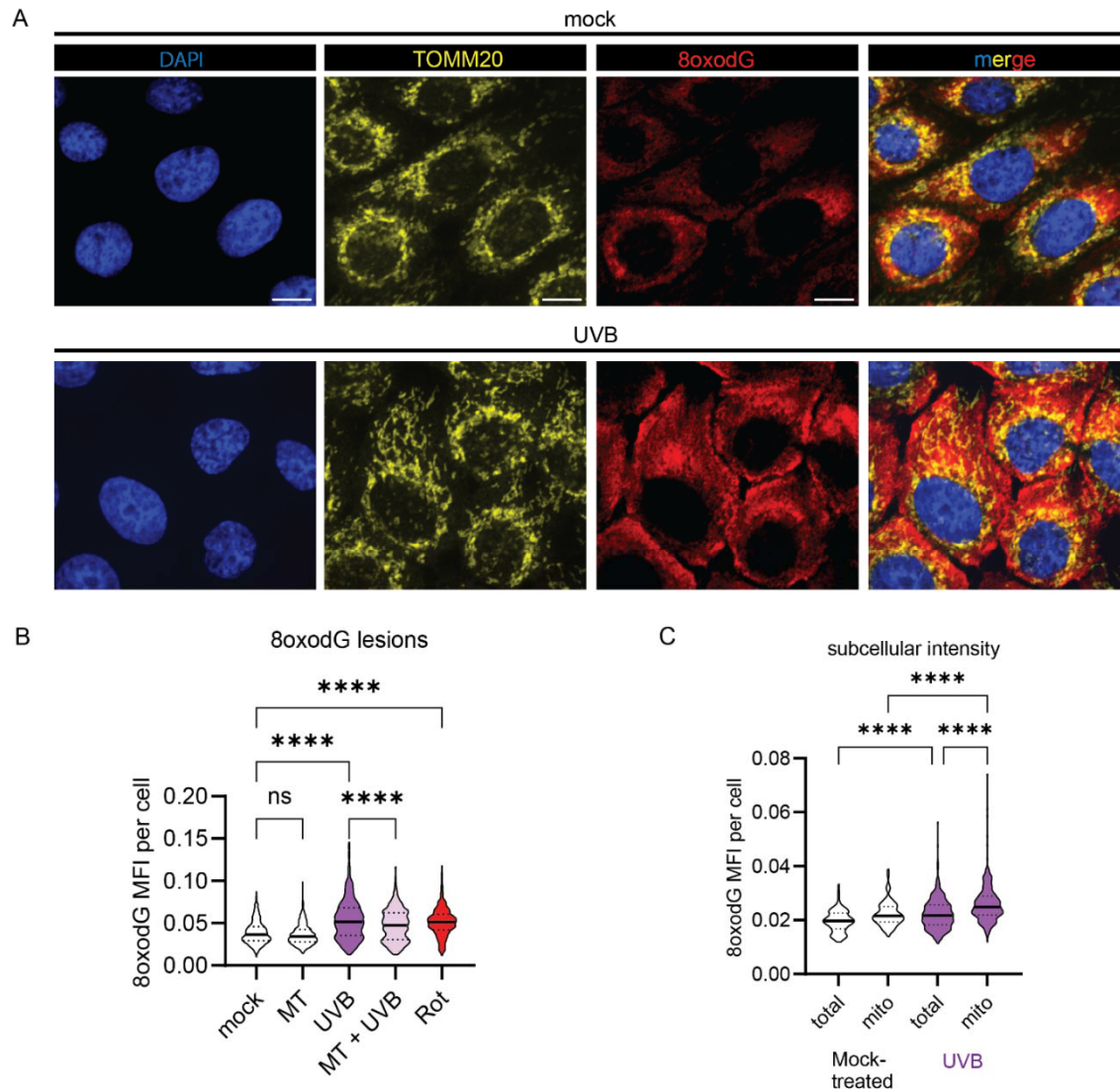
Supplemental Figure 2.

Supplemental Figure 2. UVB induces oxidative DNA damage in the cytosol and mitochondrial compartment.

A. Representative confocal microscopy images of N/TERTs 3h after UVB exposure stained for TOMM20, 8oxodG lesions and DAPI. Scale bar 20μm. **B.** Quantification of 8oxodG intensity per cell using open-source software, CellProfiler, in N/TERTs treated +/- mitoTEMPO (50μM), +/-UVB or Rotenone (0.5μM) as a positive control, n=3. **C.** Quantification of subcellular intensity of 8oxodG intensity per cell (total) or mitochondrial (mito) assessed by TOMM20$^+$ merged area. Comparisons were done via ordinary one-way ANOVA followed by Sidak's multiple comparison test. Mean and SEM. *P<0.05, **P<0.01, ***P<0,001, ****P<0.0001.
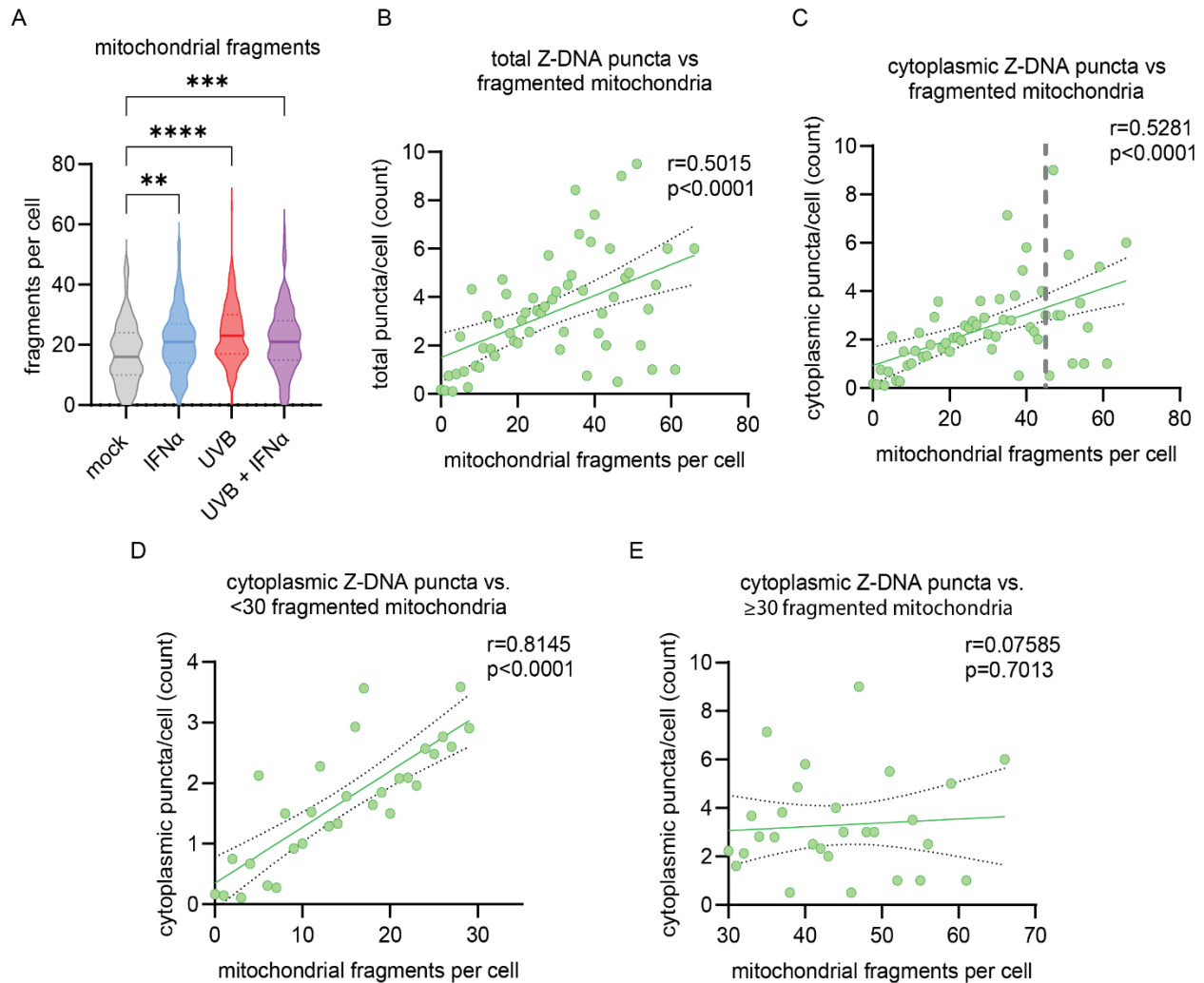
Supplemental Figure 3.

**Supplemental Figure 3. Cytosolic Z-DNA accumulation is associated with mitochondrial fragmentation.**

A. Violin plots represent quantification of mitochondrial fragments (defined as TOMM20$^+$ objects smaller than 1μm) in N/TERTs after 16h of IFNα treatment followed by UVB (50mJ/cm^2) exposure. Comparisons were done via ordinary one-way ANOVA followed by Sidak's multiple comparison test. **P<0.01, ***P<0,001, ****P<0.0001. **B and C.** Correlation of total or cytoplasmic Z-DNA puncta and fragmented mitochondria with simple linear regression. **D and E.** Correlations of data in C divided by # of mitochondrial fragments per cell. Pearson correlation coefficient (r) and p-values for indicated correlations are shown in the upper right.
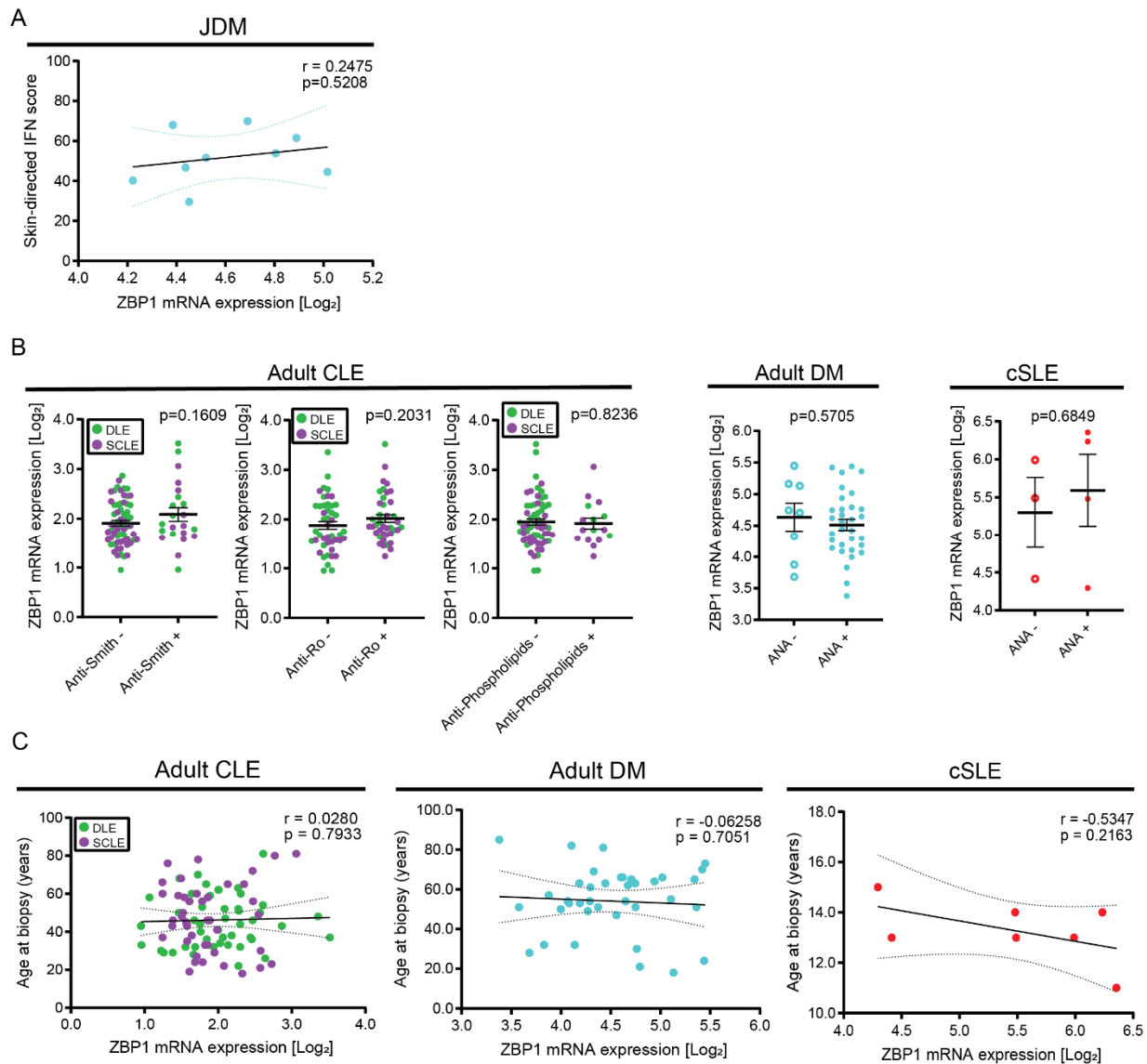
1056 **Supplemental Figure 4.**

A

B
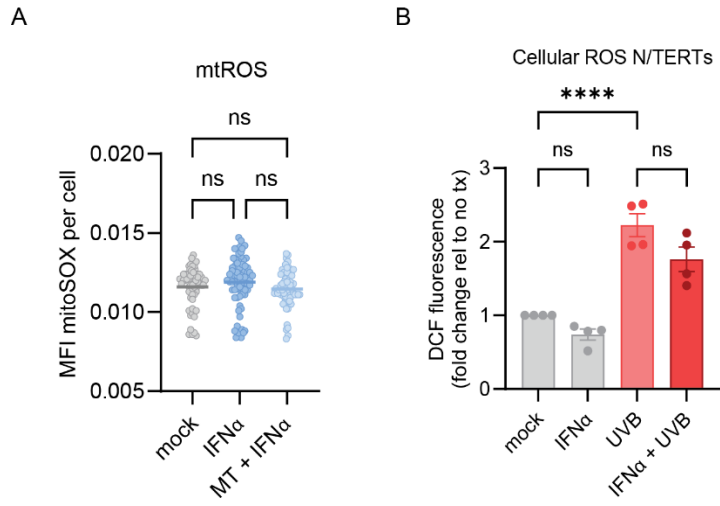
1057

1058 **Supplemental Figure 4. IFNα does not increase mitochondrial or total cellular ROS in N/TERTs.**

1059 **A.** Violin plots represent quantification of mitoSOX staining intensity per cell in N/TERTs stimulated with

1060 IFNα (1000U/ml) for 16h compared to mock. **B.** Fold change of fluorescence of

1061 Dichlorodihydrofluorescein (DFC) after treatment with IFNα for 16h +/- UVB exposure in N/TERTs 5min

1062 after UVB exposure, n=4. Comparisons were done via ordinary one-way ANOVA followed by Sidak's

1063 multiple comparison test. Mean and SEM. ****$P<0.0001$.

1064

Supplemental Figure 5.

Supplemental Figure 5. ZBP1 expression does not correlate with systemic autoantibodies or patient age.

A. Correlation of cutaneous *ZBP1* expression in juvenile dermatomyositis (n=9) with skin-directed IFN score showing no significant correlation. **B.** Comparison of cutaneous *ZBP1* expression with autoantibodies in adult CLE, DM and childhood onset SLE (cSLE) showing independence of *ZBP1* expression with autoantibody status. **C.** Correlation of cutaneous *ZBP1* expression with age in adult CLE, adult DM and childhood SLE (cSLE) showing no significant correlation with age.

1075   **Supplemental Figure 6.**
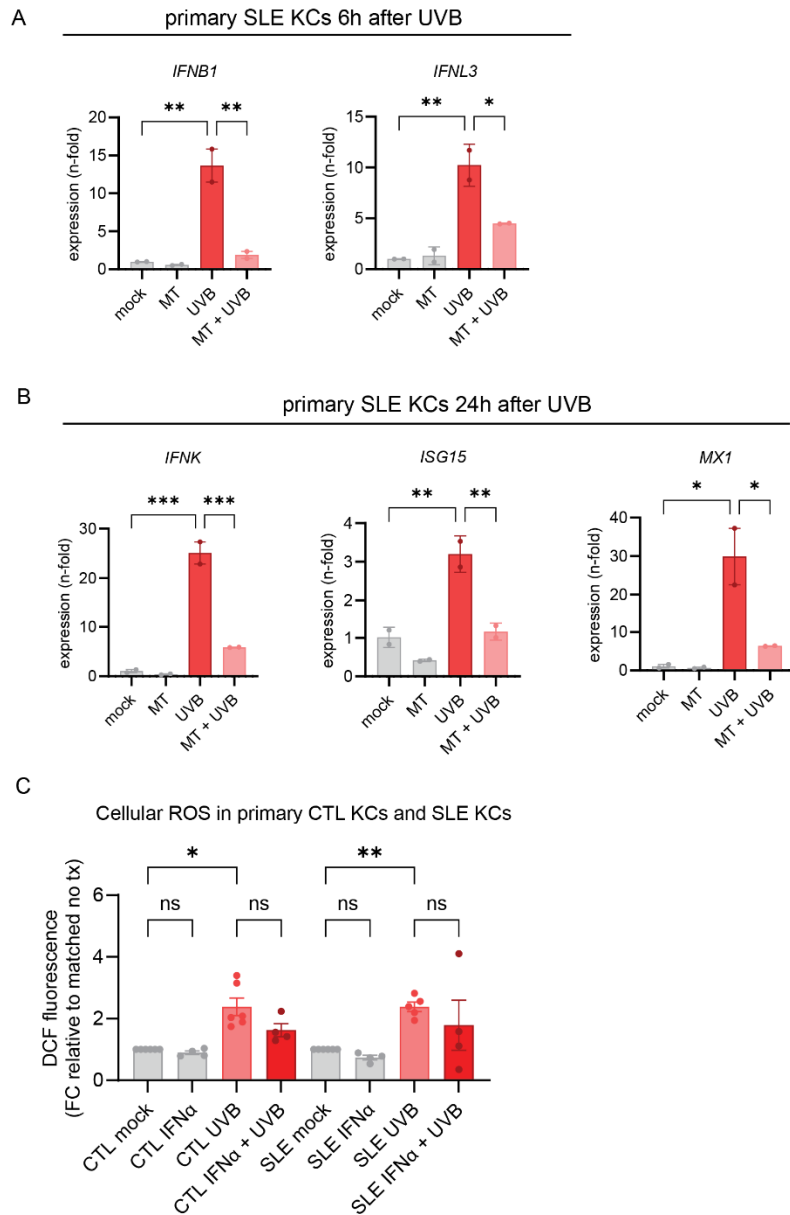

1076

1077   **Supplemental Figure 6. mitoTEMPO rescues UVB-induced IFN expression in lupus KCs.**

1078   **A.** Nonlesional SLE KCs (n=2) were treated +/- mitoTEMPO (50µM) and irradiated with UVB. Gene

1079   expression was analyzed 6h after UVB exposure. **B.** Gene expression analysis 24h after UVB exposure

1080   was normalized to β-Actin. n=2. Mean and SEM. **C.** Measurement of cellular ROS in primary HC KCs

1081   (n=4) and SLE KCs (n=4) at baseline and after IFNα treatment +/- UVB exposure. Comparisons were

1082   done via ordinary one-way ANOVA followed by Sidak's multiple comparison test. Mean and SEM.

1083   *P<0.05, **P<0.01, ***P<0,001, ****P<0.0001.

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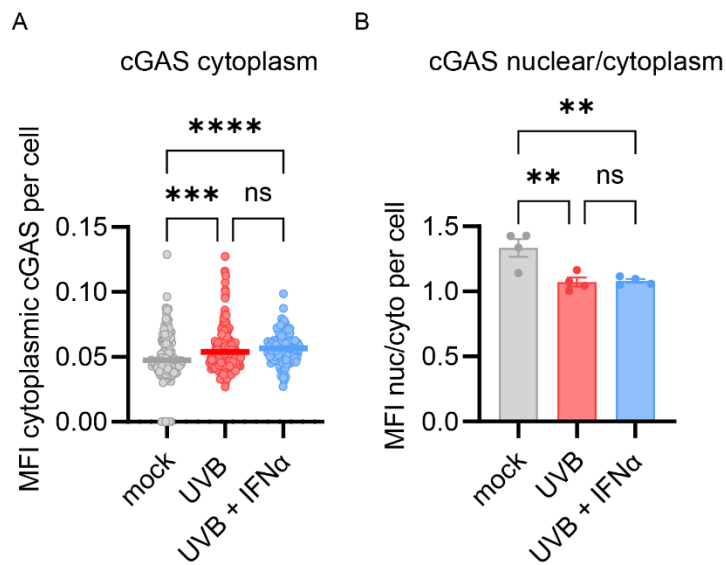
Supplemental Figure 7.

A

B

cGAS cytoplasm

cGAS nuclear/cytoplasm

Supplemental Figure 7. UVB leads to cytosolic shift of cGAS in N/TERTs.

A. Quantification of cytosolic mean fluorescence intensity (MFI) of cytoplasmic cGAS defined by the DAPI-negative area in N/TERTs using open-source software CellProfiler. **B.** Ratio of nuclear and cytoplasmic MFI per cell and shown is the mean ratio per cell of each experiment (n=4). Comparisons were done via ordinary one-way ANOVA followed by Sidak's multiple comparison test. Mean and SEM. **P<0.01, ***P<0,001, ****P<0.0001.

1094 **Supplemental Figure 8.**

1095

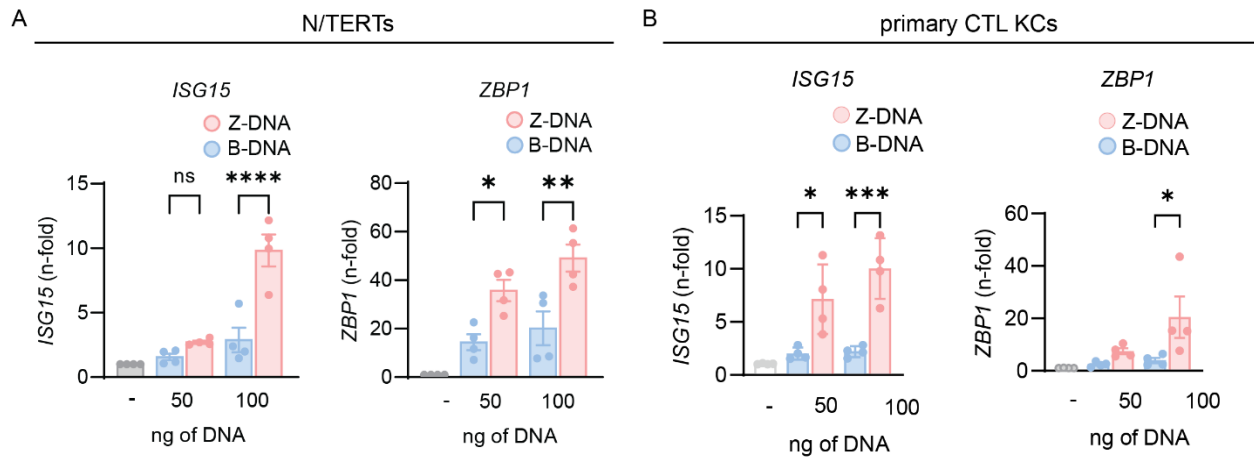1096 **Supplemental Figure 8. ISG15 and ZBP1 are significantly increased after Z-DNA transfection vs.**
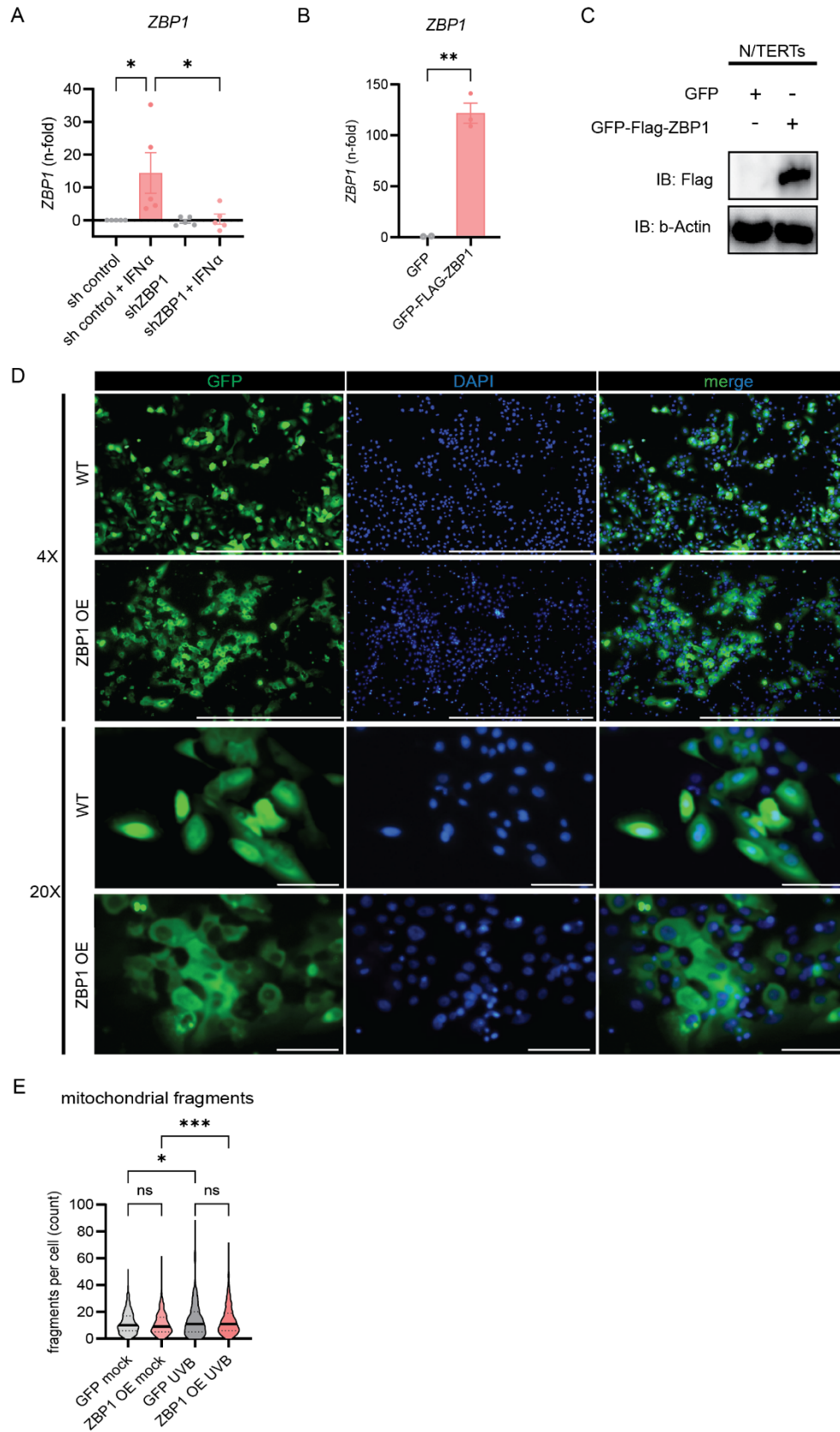
1097 **B-DNA in N/TERTs and primary KCs.**

1098 **A.** Gene expression at 24h of indicated genes from N/TERTs (n=4) and primary HC KCs (n=4) treated

1099 transfected with Z-DNA or B-DNA. Comparisons were done via ordinary one-way ANOVA followed by

1100 Sidak's multiple comparison test. Mean and SEM. **P<0.01, ***P<0,001, ****P<0.0001.

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1104 **Supplemental Figure 9.**

1106   **Supplemental Figure 9. Overexpression of ZBP1 results in cytosolic expression.**

1107   **A.** Confirmation of shRNA knockdown by qPCR compared to shcontrol after IFNα stimulation (1000U/ml)

1108   for 16h, n=5. **B.** Quantitative gene expression of ZBP1 overexpressors compared to GFP alone, n=3.

1109   **C.** Immunoblot against FLAG confirming FLAG-tag of ZBP1 overexpressor cells. **D.** Representative

1110   immunofluorescence images show efficient transfection of both GFP (first line) alone and GFP-ZBP1

1111   (second line) in 4X magnification, scale bar=1000μm. Detailed images reveal pancellular tag of GFP (third

1112   line) and cytosolic overexpression of ZBP1 (fourth line). 20X, scale bar=1000μm. 4X, scale bar=100μm.

1113   **E.** Quantification of mitochondrial fragments (TOMM20^{+} objects <1μm^2 with circularity >0.6) in GFP-tag

1114   N/TERTs and ZBP1 OE N/TERTs at baseline and after UVB exposure using CellProfiler software.

1115   Comparisons were done via ordinary one-way ANOVA followed by Sidak's multiple comparison test or t-

1116   test. *P<0.05, **P<0.01, ***P<0,001, ****P<0.0001.

1117

1118 **Supplemental Table 1. Demographics and characteristics of patients and controls for primary**
1119 **keratinocyte cell culture**

	HC (N=8)	SLE (N=8)
Median age in years (IQR)	44 (31,52)	44 (41,52)
Female sex - n (%)	4 (50%)	6 (75%)
Cutaneous lupus – n (%)	-	5 (62%)
Median SLEDAI-2k (IQR)	-	2 (0,4)
Cutaneous lupus subtype – n (%)		
ACLE	-	1 (12%)
SCLE	-	1 (12%)
DLE	-	3 (38%)
CLASI activity (IQR)		2 (0,3)
SLE treatment – n (%)		
Hydroxychloroquine	-	5 (62%)
Glucocorticoid	-	3 (38%)
Immunosuppressant	-	7 (88%)
Autoantibodies – n positive (%)	-	
ANA	-	8 (100%)
Anti-Ro/SSA	-	5 (62%)
Anti-dsDNA	-	4 (50%)
Anti-Sm/RNP	-	4 (50%)
Site of non-lesional biopsy - n (%)		
Buttock/hip	8 (100%)	7 (88%)
Arm	0	1 (12%)
HC: healthy controls; SLE: systemic lupus erythematosus; IQR: interquartile range; n: number; SLEDAI: Systemic Lupus Erythematosus Disease Activity; ACLE: acute cutaneous lupus; DLE: discoid lupus erythematosus; SCLE: subacute cutaneous lupus erythematosus; CLASI: Cutaneous Lupus Erythematosus Disease Area and Severity Index; ANA: antinuclear antibody		

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1122 **Supplemental Table 2. Demographics and characteristics of lupus and dermatomyositis**
1123 **patients from which skin biopsies were used for tissue immunofluorescence**

	CLE/SLE (N=13)		DM (N=6)	
Median age in years (IQR)	46 (41,51)		54 (35,61)	
Female sex - n (%)	11 (85%)		5 (83%)	
	Clinical manifestations CLE/SLE		**Clinical manifestations DM**	
	Cutaneous lupus only – n (%)	4 (31%)	Skin involvement	6 (100%)
	DLE	8 (62%)	Muscle involvement	4 (67%)
	SCLE	5 (38%)		
	Median CLASI activity (IQR)	4 (2,8)		
	Median SLEDAI-2k (IQR)	4 (2,8)		
Autoantibodies – n positive (%)				
ANA	12 (92%)		5 (83%)	
	Anti-Ro/SSA	6 (46%)	Anti-Mi-2	1 (17%)
	Anti-dsDNA	4 (31%)	Anti-TIF-1γ	1 (17%)
	Anti-Sm/RNP	3 (23%)	Anti-PL7	1 (17%)
Treatment – n (%)				
Hydroxychloroquine	12 (92%)		2 (33%)	
Glucocorticoid	6 (46%)		1 (17%)	
Immunosuppressant	5 (38%)		3 (50%)	
SLE: systemic lupus erythematosus; CLE: cutaneous lupus erythematosus; DM: dermatomyositis; IQR: interquartile range; n: number; DLE: discoid lupus erythematosus; SCLE: subacute cutaneous lupus erythematosus; CLASI: Cutaneous Lupus Erythematosus Disease Area and Severity Index; SLEDAI: Systemic Lupus Erythematosus Disease Activity; ANA: antinuclear antibody				

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