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Supplemental Information

IFNα Impairs Autophagic Degradation of mtDNA Promoting Autoreactivity of SLE Monocytes in a STING-Dependent Fashion

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Supplemental Figures

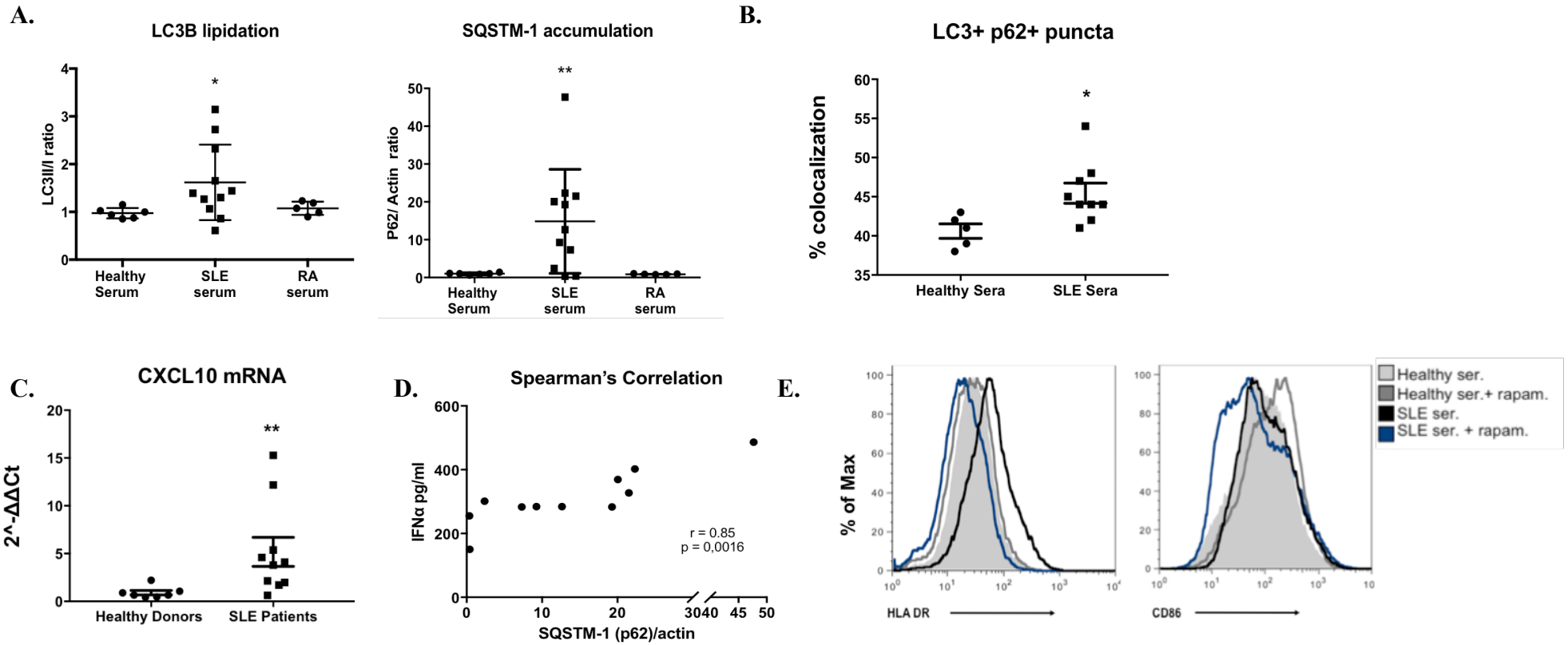


Figure S1: SLE serum impairs autophagic degradation of healthy monocytes in an [IFNα]-correlative way that enhances their immunogenicity. Related to Figures 1B, 2, 3A.

Healthy $CD14^+$ monocytes were treated with 10% human sera for 24hours as indicated. **(A)** Quantification of W.B. protein band intensity ratios N_{HEAL}= 6, N_{SLE} = 10, N_{RA}= 5 (numbers of sera), **(B)** Analysis of % colocalization of $LC3^{555}$ with $P62^{488}$ puncta. N_{HEAL}= 5, N_{SLE} = 9, n= 3 (number of different healthy donors). **(C)** Relative CXCL10 mRNA levels in $CD14^+$ PBMCs of the SLE patients whose sera were assayed in (A) and (B). **(D)** Spearman's correlation analysis between SLE sera IFNα concentration as measured by ELISA (pg/ml) and SQSTM-1 (P62)/actin ratio of immunoblot band intensities of the cell lysates. **(E)** Histograms of a representative flow cytometric analysis of HLADR and CD86 expression on $CD14^+$ monocytes from a healthy donor treated with allogeneic sera +/- rapamycin (24hrs). Results are expressed as mean +SEM. $*P < 0.05$, $**P < 0.005$. All data sets were analyzed using non parametric Mann Whitney U test.

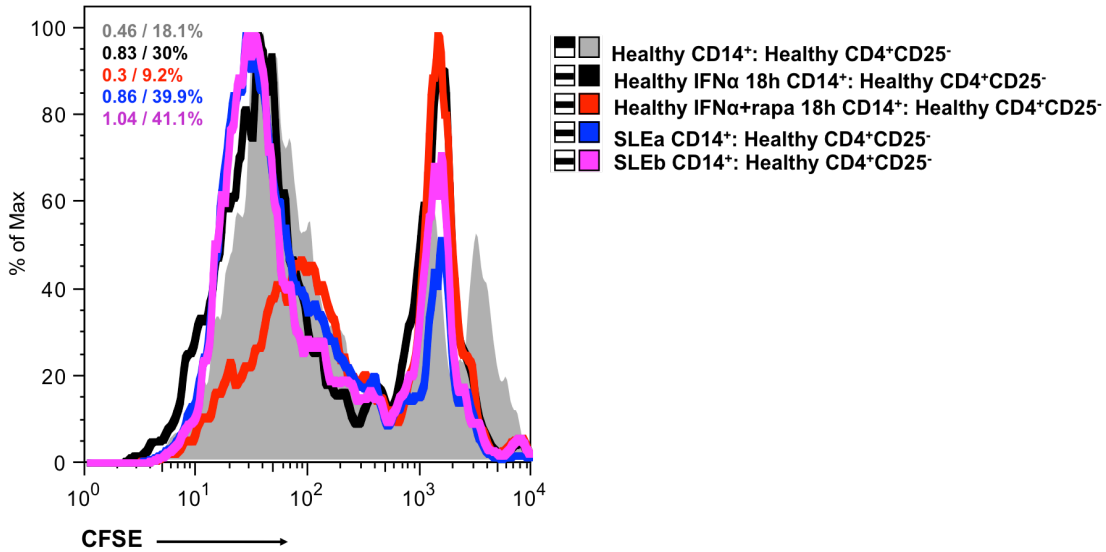


Figure S2: SLE monocytes exhibit increased ability to induce CD4 T cell proliferation compared to healthy controls. Related to Figure 3E.
SLE or healthy $CD14^+$ monocytes were isolated and co-cultured with allogeneic naïve CFSE-labeled $CD4^+$ T cells (isolated from cord blood as described in Experimental procedures) for 6 days. Proliferation index analysis demonstrated an increased proliferation of T cells in the presence of SLE monocytes compared to healthy controls. Proliferation Index / % Divided cells are listed on the upper left part of the representative result of CFSE dilution presented.

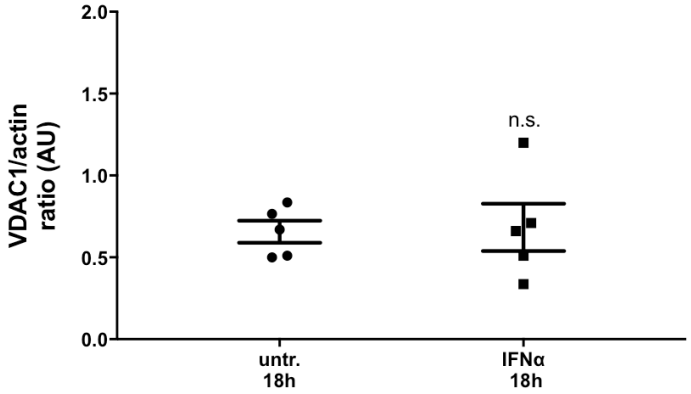
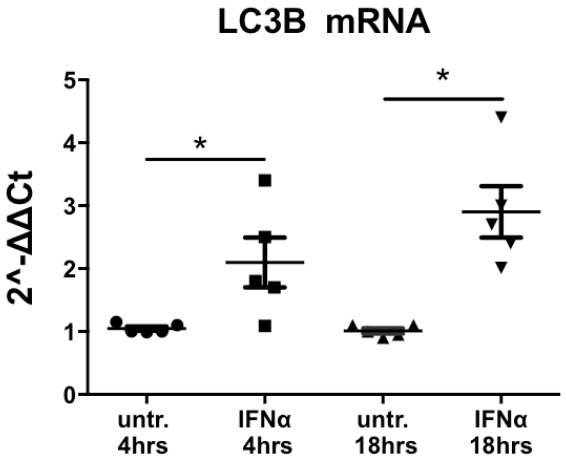


Figure S3: VDAC1 protein levels remain unchanged upon IFNα mediated effects in lysosomal degradation. Related to Figure 4E-G.
Quantification of Western Blot protein bands intensity ratios of VDAC1 to actin, from 5 independent experiments ($N_{HEAL.}$ = 5). Data are expressed as mean +SEM. *n.s.* indicates absence of statistical significance.

A.



B.

Figure S4: IFNα upregulates LC3B transcription. Related to Figure 2D.
(A) Relative mRNA levels of LC3B compared to GAPDH upon IFNα(400ng/m) treatment of healthy monocytes for 4 and 18hours (*n =5*). **(B)** *in silico* analysis (http://tfbind.hgc.jp/) of LC3B promoter indicated the presence of stat and irf binding sites. Results are expressed as mean +SEM. *P < 0.05. Results were analyzed using paired, student's t test.

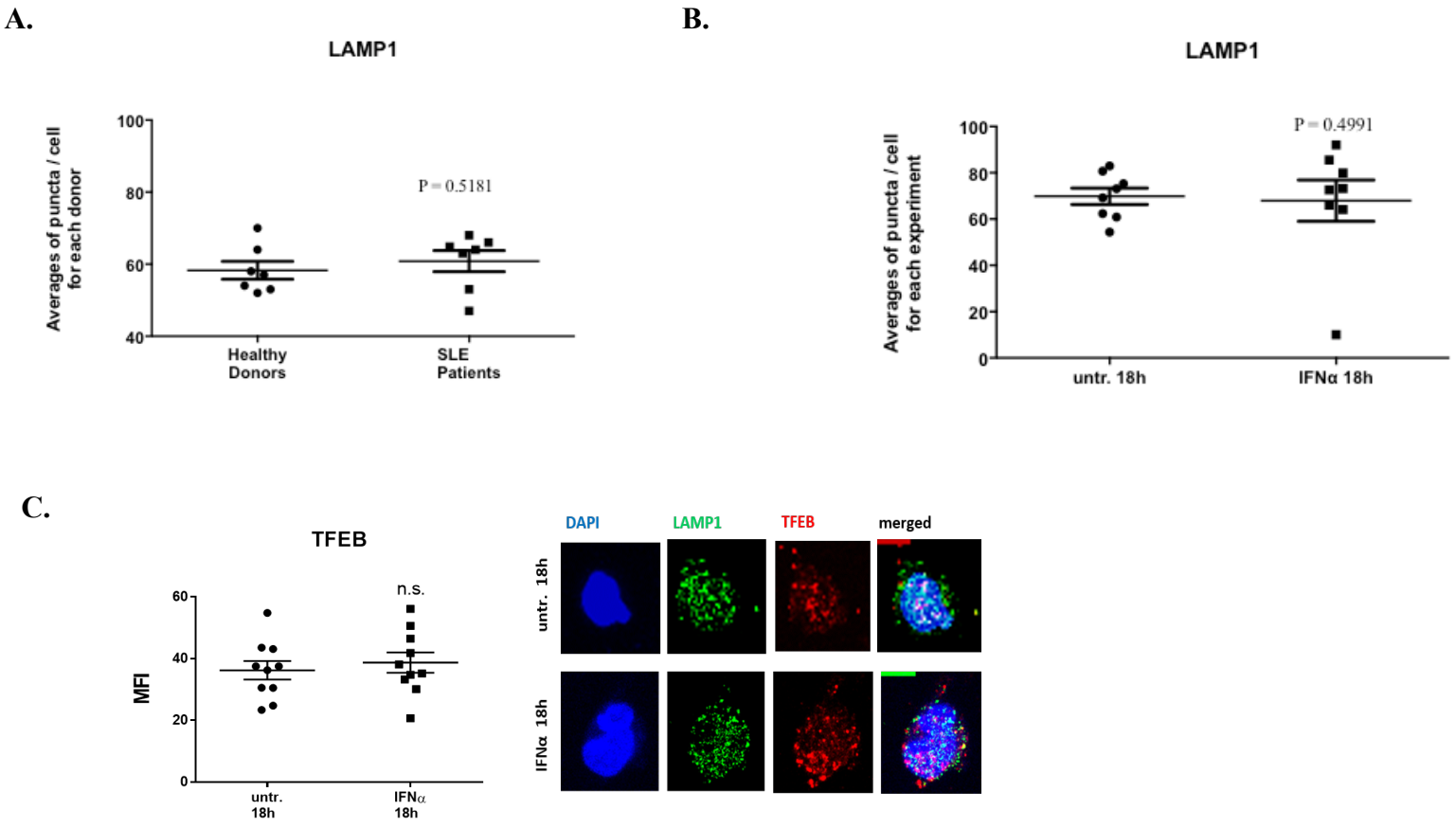
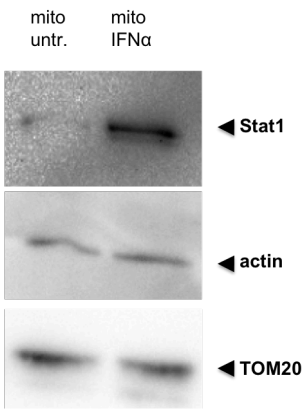


Figure S5: Lysosomal biogenesis is not affected in SLE and IFNα-treated monocytes. Related to Figures 1D, 2E.
(A) Averages of 15 measurements of LAMP1+ puncta/cell were calculated for each donor ($n_{healthy}$=7, n_{SLE} =7) and plotted. **(B)** same as in (A) for healthy $CD14^+$ monocytes ($n_{healthy}$=8) +/- IFNα (400ng/ml) for 18hrs. **(C)** TFEB expression and localization in healthy $CD14^+$ monocytes ($n_{healthy}$=8) +/- IFNα (400ng/ml) for 18hrs. P values were calculated by non parametric Mann Whitney U test for (A) and paired student's t test for (B). Scale bars 5μm.

A.



B.

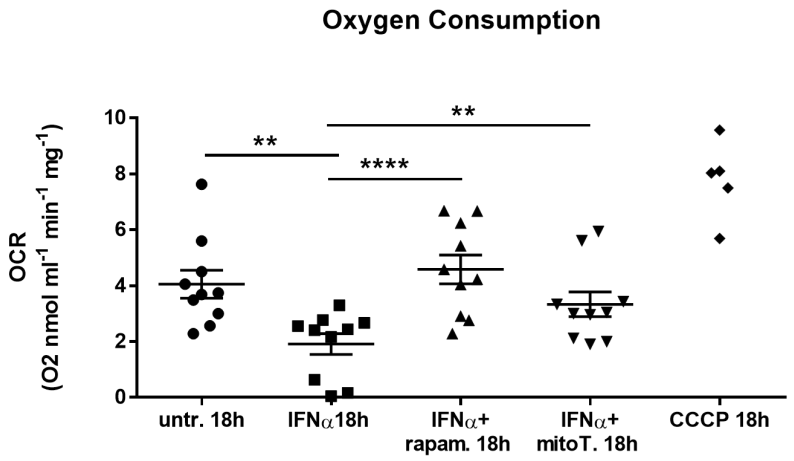


Figure S6: Stat1 is localized in mitochondria and mitochondrial respiration is deregulated upon IFNα signaling. Related to Figures 4D, 5.
(A) Mitochondrial preparations from healthy $CD14^+$ monocytes either untreated or treated with IFNα (400ng/ml) for 18hrs (ThermoScienctific Mitochondrial Isolation kit, reagent based method) were lysed with RIPA supplemented with protease and phosphatase inhibitors and 80μgs of lysates were analysed via 12% SDS-PAGE. Membranes were blotted for Stat1, actin and TOM20 as depicted. **(B)**
Averages of oxygen consumption rates of $4x10^6$ $CD14^+$ monocytes treated as indicated were normalized with the total protein content of each sample. Respiration measurements were recorded and analyzed by Oxygraph Plus Software. CCCP decoupler was used as a strong inducer of oxygen consumption. P values were calculated by paired student's t test.

Table S2. RA patients' clinical records. Related to Figures 1 and S1

Sex/gender	Age	Steroids - dose	Methotrexate (mg)	Biologics
Female	56	0	0	0
Female	46	160IM	0	adalinumab (humira)
Female	80	10	15	enbrel
Female	63	0	12,5	0
Male	48	0	0	0
Female	42	0	0	0

Table S4. MSIGBD Analysis of RNAseq data. Related to Figure 6A

Gene Set Name [# Genes (K)]	Description	# Genes in Overlap (k)	p-value	FDR q-value
REACTOME_INTERFERON_ALPHA_BETA_SIGNALING [64]	Genes involved in Interferon alpha/beta signaling	20	$1.77\ e^{-30}$	$2.35\ e^{-27}$
REACTOME_IMMUNE_SYSTEM [933]	Genes involved in Immune System	42	$1.29\ e^{-26}$	$7.06\ e^{-24}$
REACTOME_INTERFERON_SIGNALING [159]	Genes involved in Interferon Signaling	23	$2.03\ e^{-26}$	$7.06\ e^{-24}$
REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_NE_SYSTEM [270]	Genes involved in Cytokine Signaling in Immune system	27	$2.12\ e^{-26}$	$7.06\ e^{-24}$
KEGG_HEMATOPOIETIC_CELL_LINEAGE [88]	Hematopoietic cell lineage	11	$1.47\ e^{-12}$	$3.91\ e^{-10}$
NABA_MATRISOME [1028]	Ensemble of genes encoding extracellular matrix and extracellular matrix-associated proteins	27	$8.76\ e^{-12}$	$1.94\ e^{-9}$
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION [267]	Cytokine-cytokine receptor interaction	15	$1.6\ e^{-11}$	$3.04\ e^{-9}$
REACTOME_ANTIVIRAL_MECHANISM_BY_IFN_ST_STIMULATED_GENES [66]	Genes involved in Antiviral mechanism by IFN-stimulated genes	9	$7.37\ e^{-11}$	$1.22\ e^{-8}$
BIOCARTA_IL17_PATHWAY [17]	IL 17 Signaling Pathway	6	$2.3\ e^{-10}$	$3.4\ e^{-8}$
NABA_SECRETED_FACTORS [344]	Genes encoding secreted soluble factors	15	$5.49\ e^{-10}$	$7.3\ e^{-8}$