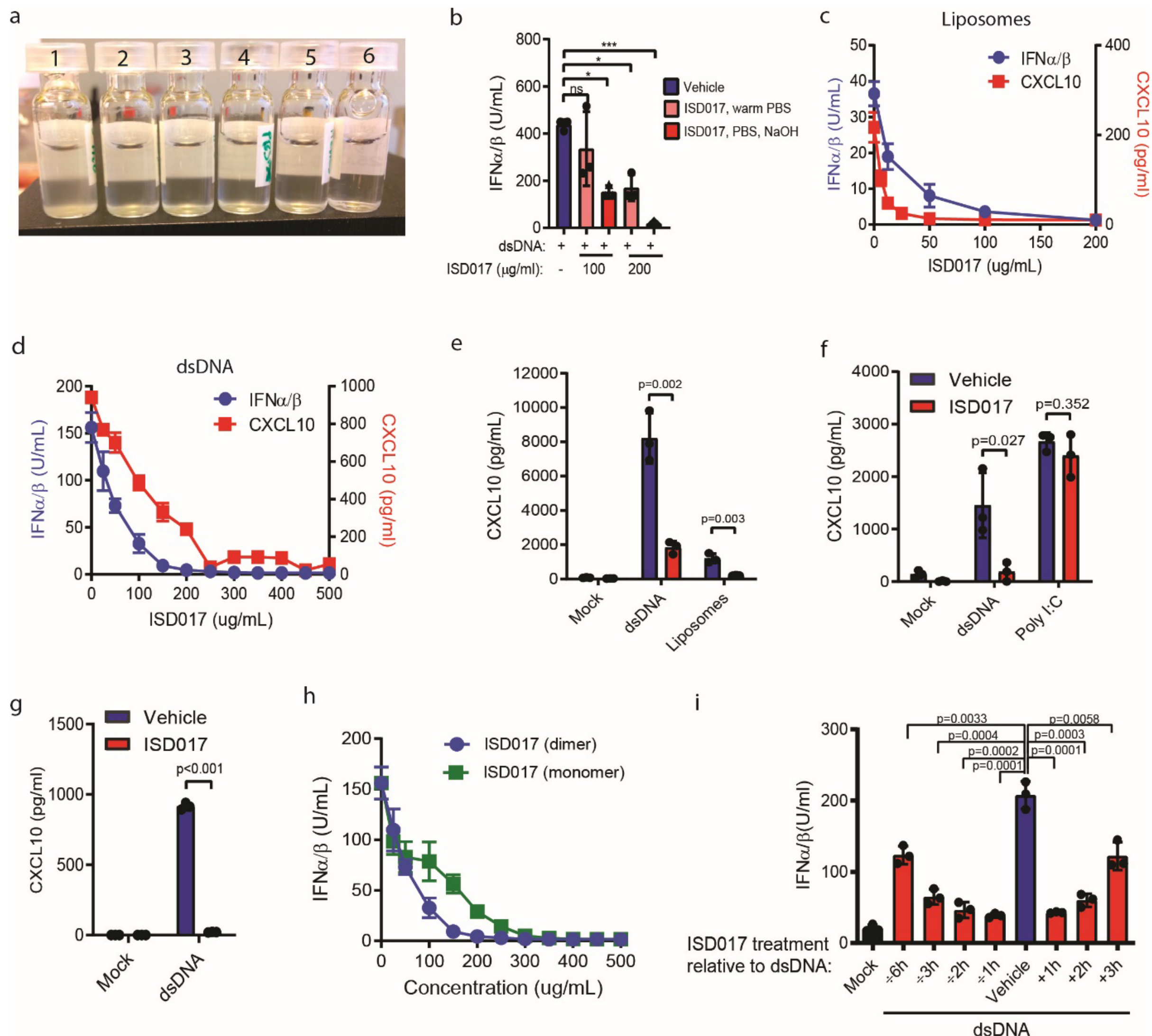
Supplemental Information

A STING antagonist modulating the interaction with STIM1 blocks ER-to-Golgi trafficking and inhibits lupus pathology

Thaneas Prabakaran, Anne Troldborg, Sarinya Kumpunya, Isara Alee, Emilija Marinković, Samuel J Windross, Ramya Nandakumar, Ryo Narita, Bao-cun Zhang, Mikkel Carstensen, Pichpisith Vejvisithsakul, Mikkel H S Marqvorsen, Marie B Iversen, Christian K Holm, Lars J Østergaard, Finn Skou Pedersen, Trairak Pisitkun, Rayk Behrendt, Prapaporn Pisitkun, Søren R Paludan

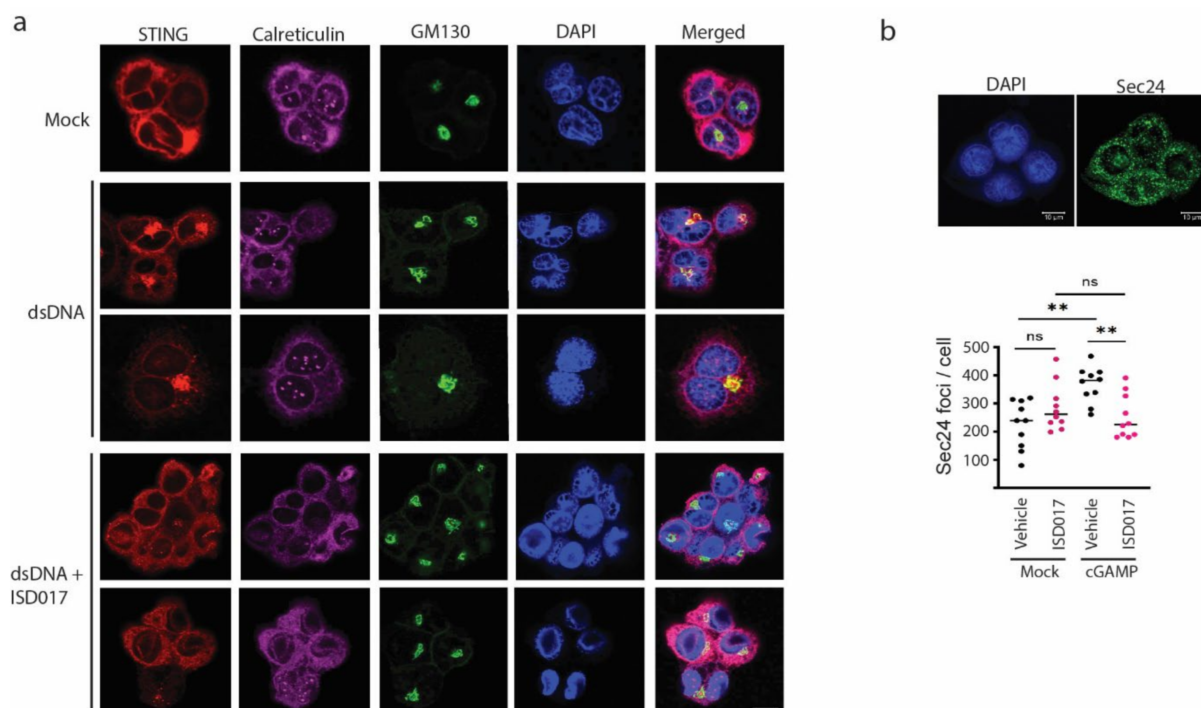
The file includes:

Supplementary Fig 1.	ISD017 is a STING antagonist in human cells.
Supplementary Fig 2.	ISD017 inhibits STING ER exit and trafficking to the Golgi.
Supplementary Fig 3.	ISD017 blocks STING activation *in vivo*.
Supplementary Fig 4.	Inhibitory activity of reported cGAS-STING antagonists and effect of ISD017 on mouse growth.
Supplementary Fig5.	ISD017 treatment improves disease outcome in a mouse model for STING-dependent lupus.
Supplementary Fig6.	Characterization of serum from lupus patients.
Supplementary Table 1.	Patient demographics.

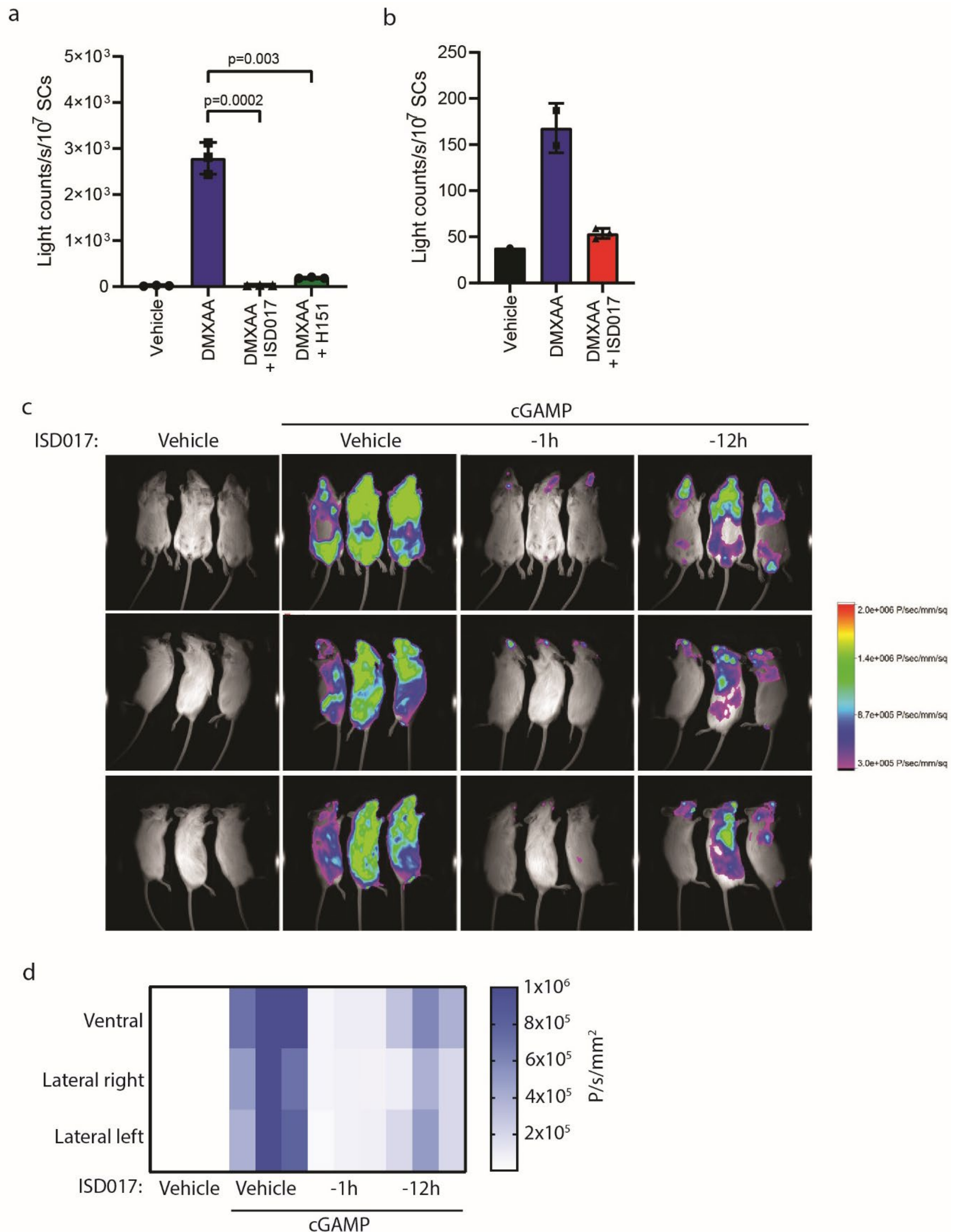


Supplementary Fig. 1. ISD017 is a STING antagonist in human cells. (a) Comparison of ISD017 solubility on the following six solvents: 1, H_2O; 2, 0.9% NaCl; 3, TBS (pH 7.3); 4, PBS (pH 7.4); 5, HEPES; 6, PBS (pH7.4) + 1M NaOH. For dissolving of peptide with solvent no 6, 194 uL of PBS mixed with 6 uL 1 M NaOH was added to 1 mg ISD017. (b) Human PBMCs were treated with ISD017 (200 µg/ml) solubilized in either warm PBS or PBS supplemented with NaOH as specified in the methods section. One h later, the cells were stimulated with ISD017 in the indicated concentrations. Supernatants were harvested 16 h post-stimulation, and IFN-I activity was determined (n=2). (c, d) PBMCs were treated with ISD017 at the indicated doses. One h later, the cells were treated with (c) liposomes (8 µg/ml) or (d) transfected with dsDNA (2 µg/ml). Supernatants were harvested 16 h post-stimulation, and levels of IFN-I activity (blue) and CXCL10 (red) were determined (n=3). (e) Murine peritoneal cells were treated with ISD017 (200 µg/ml). One h later, the cells were treated with liposomes (8 µg/ml) or transfected with dsDNA (2 µg/ml). Supernatants were harvested 16 h post-stimulation, and levels of CXCL10 were determined by ELISA (n=3). (f) PBMCs were treated with ISD017 (200 µg/ml). One h later, the cells were transfected with either dsDNA or polyIC (both 2 µg/ml). Supernatants were harvested 16 h post-stimulation, and levels of CXCL10 were determined by ELISA (n=3). (g) PMA-differentiated THP1 cells were treated with ISD017 (200 µg/ml). One

h later, the cells were transfected with either dsDNA or polyIC (both 2 µg/ml). Supernatants were harvested 16 h post-stimulation, and levels of CXCL10 were determined by ELISA (n=5). (h) PBMCs were treated with monomeric (green) or dimeric (blue) ISD017 at the indicated doses. One h later, the cells were transfected with dsDNA (2 µg/ml). Supernatants were harvested 16 h post-stimulation, and levels of IFN-I activity were determined (n=3). (i) PMA-differentiated THP1 cells were treated with ISD017 (200µg/ml) at the indicated time points relative to stimulation with dsDNA (2 µg/ml). Supernatants were isolated 16 h post-stimulation and analyzed for IFN-I bioactivity. The data in panel b-i are presented as means of biological triplicates +/- st.dev. [Statistical analysis of the data in b, e-g, i were performed using two-tailed one-way ANOVA test].

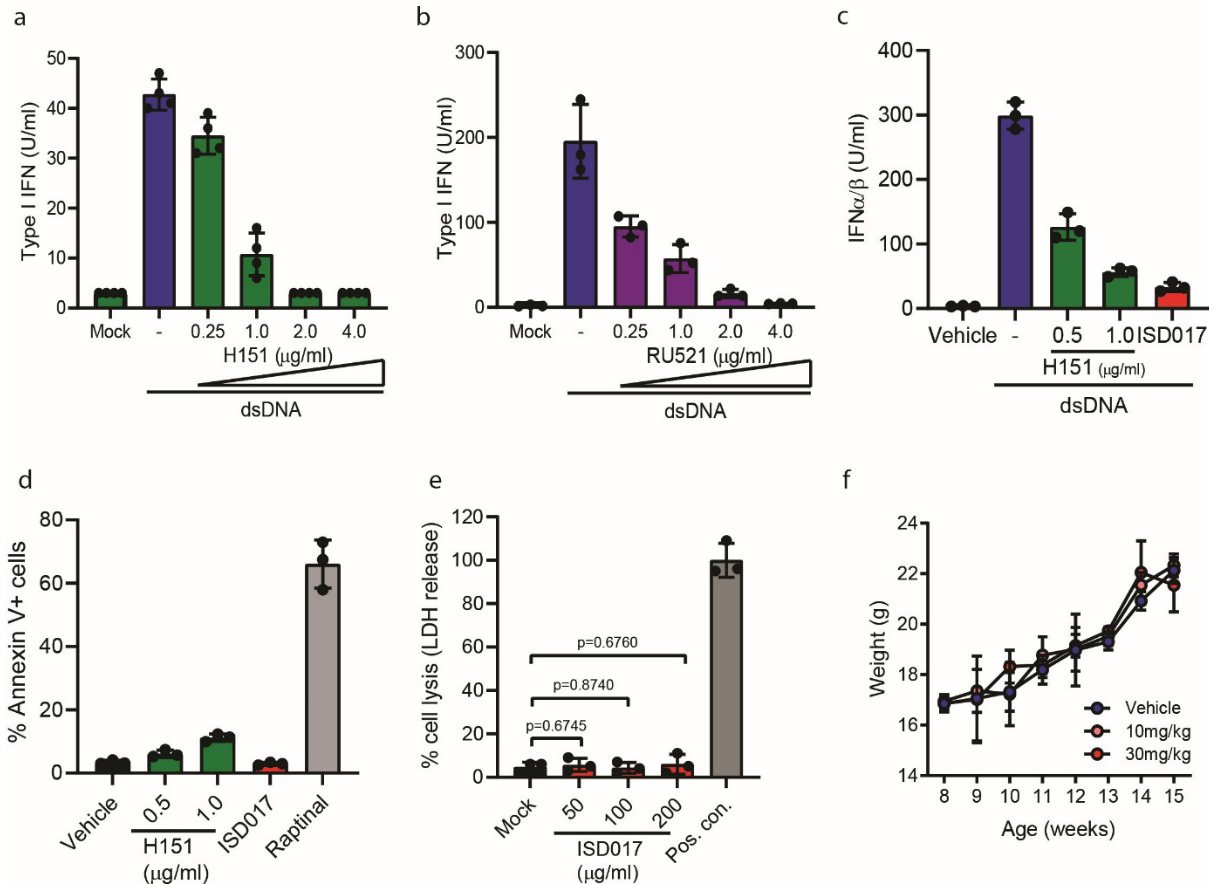


Supplementary Fig. 2. ISD017 inhibits STING ER exit and trafficking to the Golgi. (a) PMA-differentiated THP1 cells were pretreated with ISD017 (200 µg/ml) for 1 h. The cells were transfected with dsDNA (6 µg/ml) and fixed 2 h later for imaging analysis after staining with anti-STING, anti-Calreticulin (ER), anti-GM130 (Golgi), and DAPI (n=3). Scalebar, 10 µm. The cells shown are additional examples of representative cells similar to the cells shown in Fig. 2B. (b) PMA-differentiated THP1 cells were pretreated with ISD017 (200 µg/ml) for 1 h. The cells were transfected with dsDNA (6 µg/ml) and fixed 2 h later for imaging analysis after staining with anti-STING, anti-Sec24, and DAPI (n=2). A representative image of Sec24 and DAPI staining is shown. Scalebar, 10 µm. For quantification of Sec24 foci, 10 cells from each group were counted in a blinded fashion. Statistical analysis was performed using two-tailed unpaired t-test with welch's correction. Individual data points represent number of Sec24 foci in individual cells and are shown as means +/- st.dev. [Statistical analysis of the data in b were performed using two-tailed student's t test].

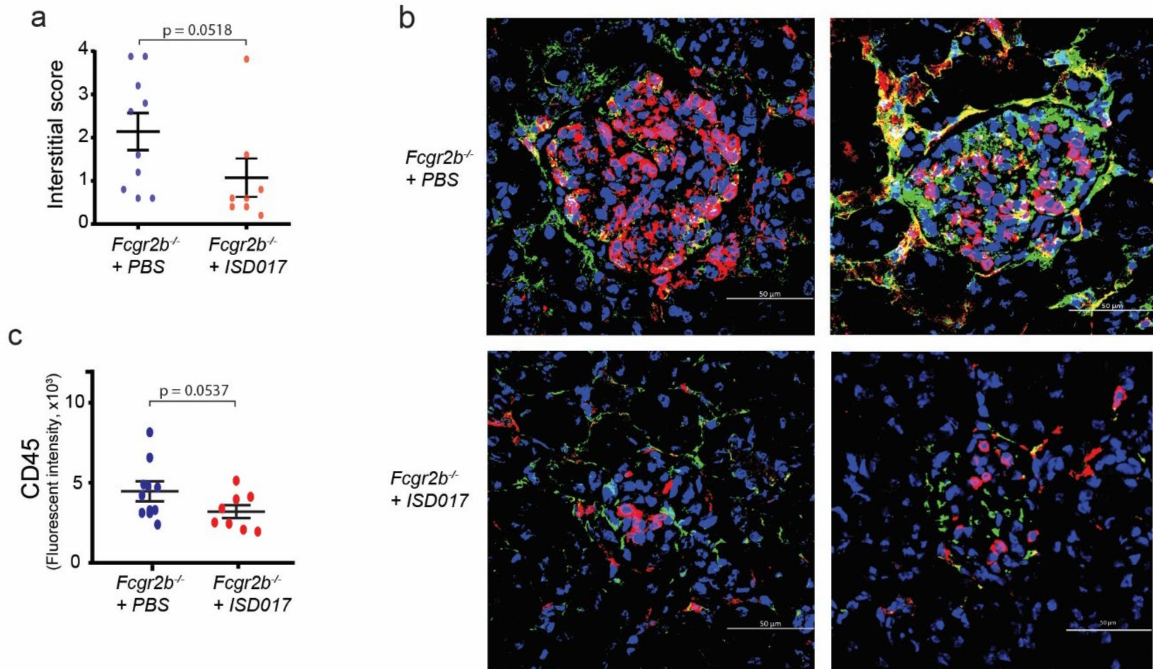


Supplementary Fig. 3. ISD017 blocks STING activation *in vivo*. (a) IFN-βΔβ-luc/Δβ-luc-reporter (ΔβLUC) mice were treated with ISD017 (30 mg/kg) or H-151 (30 mg/kg) and 1 h later with DMXAA (25 mg/kg), both i.p. The mice were sacrificed 5 h later, and luciferase activity in spleen cell homogenates was measured (n=3). (b) ΔβLUC mice were treated with ISD017 (30 mg/kg) and 6 h later with DMXAA (25 mg/kg), both i.p. The mice were sacrificed 5 h later, and luciferase activity in spleen cell homogenates were measured (n=3). (c) ΔβLUC mice were treated i.p. with ISD017 (30 mg/kg), and 1 or 12 h later injected i.v. with cGAMP

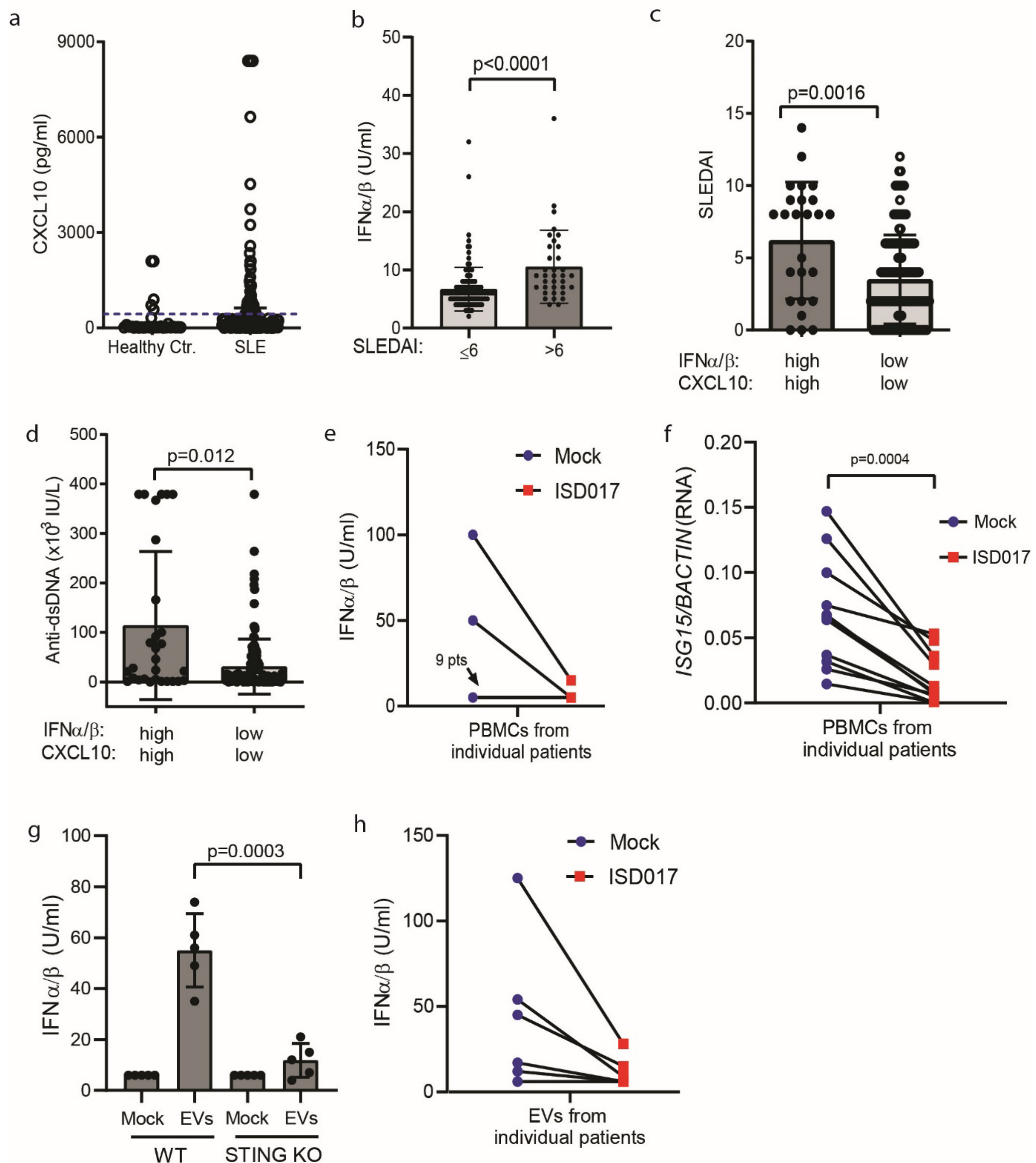
(20 mg/kg). Five h after treatment with STING agonist, whole animals were subjected to bioimaging on ventral and lateral (right and left) sides (n=3). Top row is identical to the data presented in Fig 3d. (d) Quantification of the data presented in panel c. The data are presented as means of measurements from individual mice +/- st.dev. [Statistical analysis of the data in a were performed using two-tailed one-way ANOVA test].



Supplementary Fig. 4. Inhibitory activity of reported cGAS-STING antagonists and effect of ISD017 on mouse growth. (a) PMA-differentiated THP1 cells were treated with H-151 at the indicated doses one h before stimulation with dsDNA (2 µg/ml). Supernatants were isolated 16 h post-stimulation and analyzed for IFN-I bioactivity (n=2). (b) PMA-differentiated THP1 cells were treated with RU.521 at the indicated doses one h before stimulation with dsDNA (2 µg/ml). Supernatants were isolated 16 h post-stimulation and analyzed for IFN-I bioactivity (n=3). (c) PMA-differentiated THP1 cells were treated with H151 (0.5 and 1.0 µg/ml) or ISD017 (200 µg/ml) for 24 h and apoptotic cell death was evaluated by AnnexinV (n=3). Raptonal was used as a potitive control. (d) PBMCs from 3 healhty donors were treated with ISD017 for 18 h, and cytolysis was evaluated by measurement of LDH in the supernatants. As positive control, cells were lysed with detergent lysis buffer (n=3). (e) PMA-differentiated THP1 cells were treated with H151 (0.5 and 1.0 µg/ml) or ISD017 (200 µg/ml) for 1 h followed by stimulation with dsDNA (4 µg/ml). Supernatants were isolated 20 h post-stimulation and analyzed for IFN-I bioactivity (n=3). (f) Female C57BL/6 were treated with ISD017 i.p. 3 times per week (10 mg/kg). Weights were measured once per week (n=4). The data in e are presented as means of biological triplicates +/- st.dev. [Statistical analysis of the data in e were performed using two-tailed one-way ANOVA test].



Supplementary Fig. 5. ISD017 treatment improves disease outcome in a mouse model for STING-dependent lupus. (a) Interstitial scores of kidney sections shown in Fig 5f were blindly graded (n=8–10 per group). (b) Additional images to the ones shown in Fig. 5h. Immunofluorescence staining of the kidneys from *Fcgr2b*$^{-/-}$ mice treated with PBS or ISD017 (10 mg/kg, 3 times per week). IgG (green), CD45 (red), and DAPI (blue). Data are representative of 8-10 mice per group (scale bar, 50 µm). (c) Quantification of immunofluorescence signals for CD45 (n=8-10 mice per group) from data shown in Fig 5h. Data shown as means ± st.dev.



Supplementary Fig. 6. Characterization of serum from lupus patients. (a) Serum from 164 lupus patients and 21 healthy controls was analyzed for levels of CXCL10 by ELISA. The data are presented as levels from individual donors. (b) IFN-I serum levels in patient groups with SLEDAI < 6 or SLEDAI < 6. (c, d) SLEDAI score and anti-dsDNA levels in patients with high *versus* low serum levels of both IFN-I and CXCL10. (e) PBMCs from 11 IFN-I^{high} lupus patients were cultured for 16 h in the presence or absence of ISD017 (200 μg/ml). Supernatants were isolated and analyzed for the levels of IFN-I. (f) RNA was also isolated from the same cells as in panel e, and evaluated for levels of ISG15 mRNA. The data were normalized to BACTIN, and presented as normalized ratios. (g) Wildtype and STING-deficient THP1 cells were treated with extracellular vesicles (EVs) from IFN-I^{high} patients (a mixture of EVs from seven patients).

Culture supernatants were isolated 16 h later, and levels of IFN-I were measured. (h) PBMCs from a healthy donor were treated for 16 h with EVs isolated from seven IFN-I^{high} lupus patients in the presence or absence of ISD017 (200 µg/ml). Supernatants were isolated and analyzed for the levels of IFN-I. Data in panel a-d are presented as mean levels from individual donors. Data in panel e, f, and h are presented as matched values from cultures from individual patient PBMCs in the absence (blue) or presence (red) of ISD017. [Statistical analysis of the data in b-d, f, g were performed using two-tailed one-way ANOVA test].

Supplementary Table 1. Patient demographics

Patient characteristics	
Patients included (n)	164
Age at inclusion (mean, sd[1])	45.4 (14.9)
Age at diagnosis (mean, sd)	33.5 (14.1)
Gender F (%)	90
Ethnicity: Caucasian (%)	97
Clinical data at time of inclusion:	
SLEDAI at inclusion, (mean, sd)	3.9 (3.3)
SLICC (mean, sd)	0.9 (1.2)
Treatment at time of inclusion	
Hydroxychloroquine treatment (%)	79.6
Prednisolone treatment (%)	57.4
Other immuno-suppressives (%)	39.5

[1] ds, Standard deviation