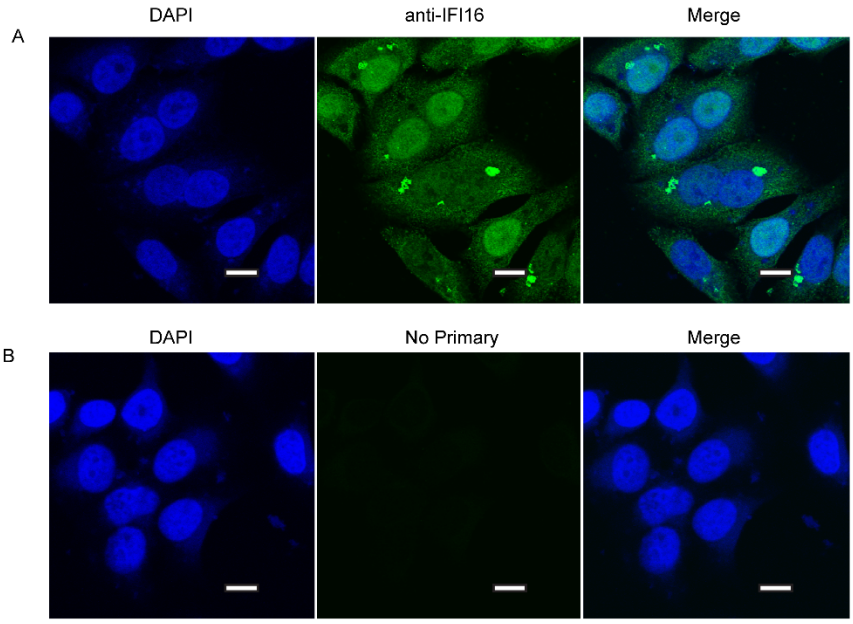
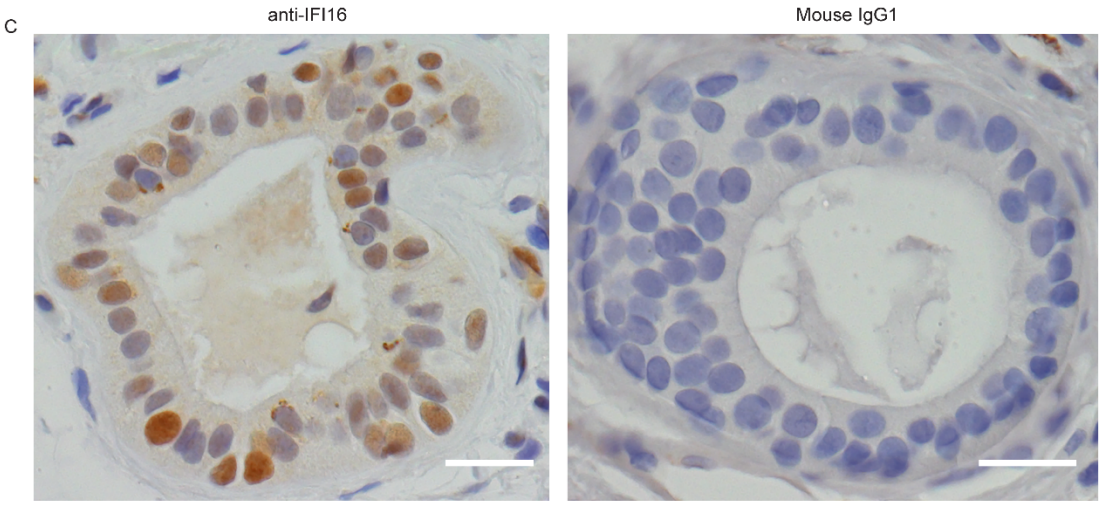
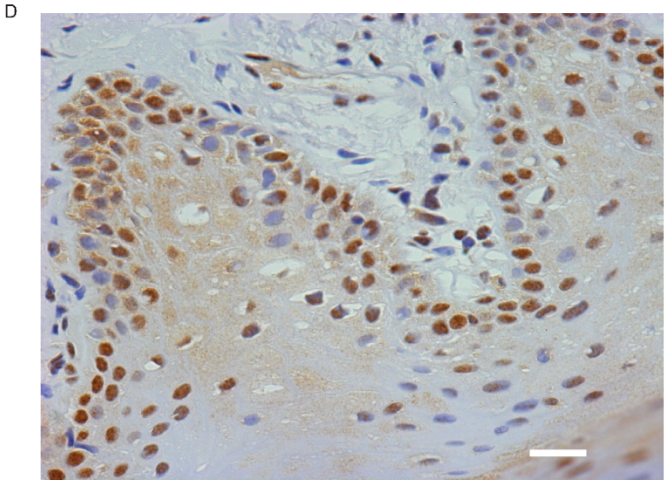
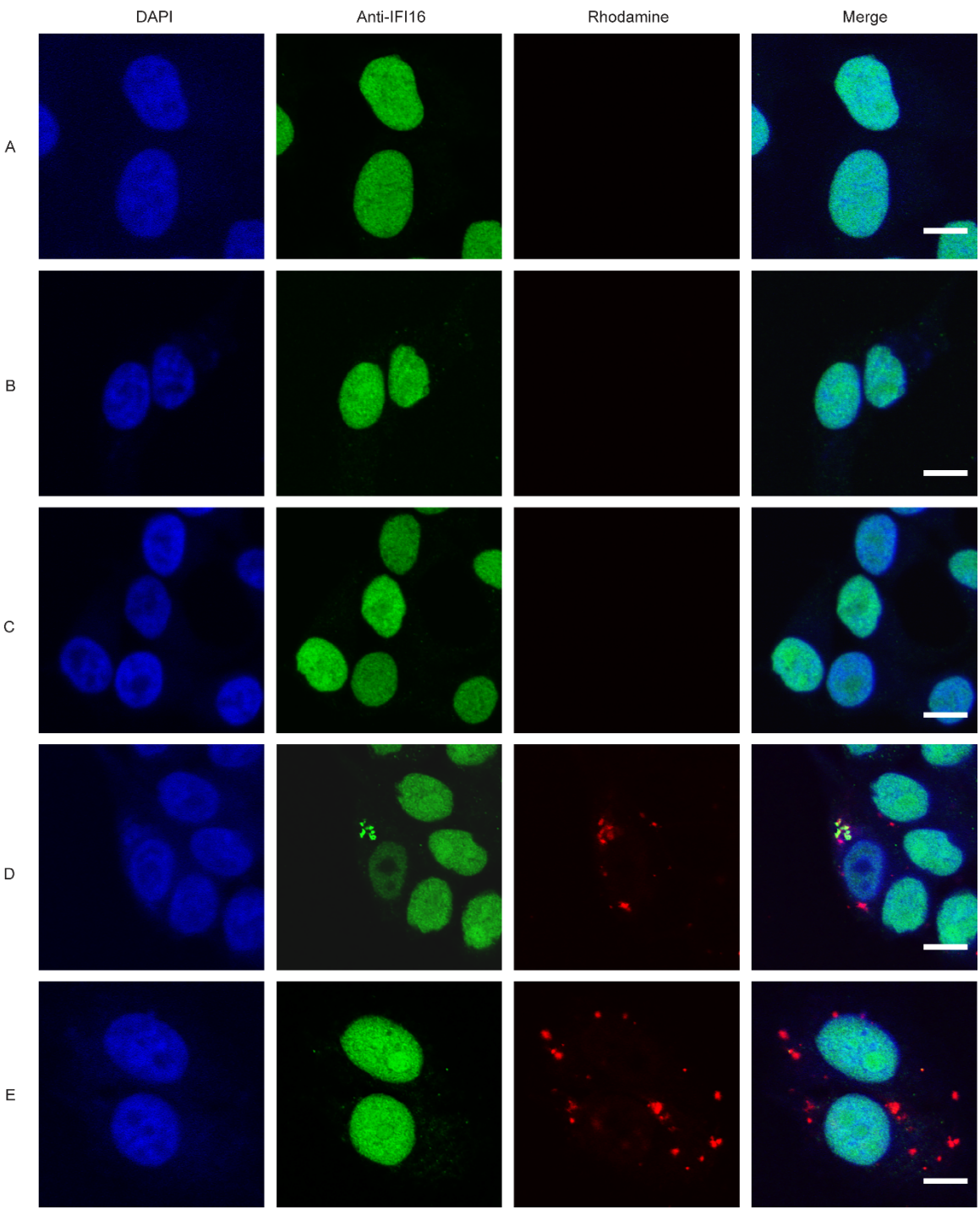

A
DAPI
anti-IFI16
Merge
B
DAPI
No Primary
Merge

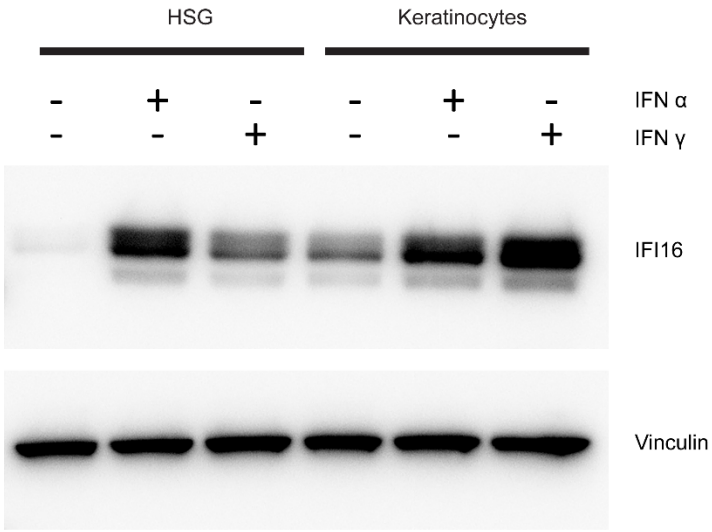

C
anti-IFI16
Mouse IgG1


D

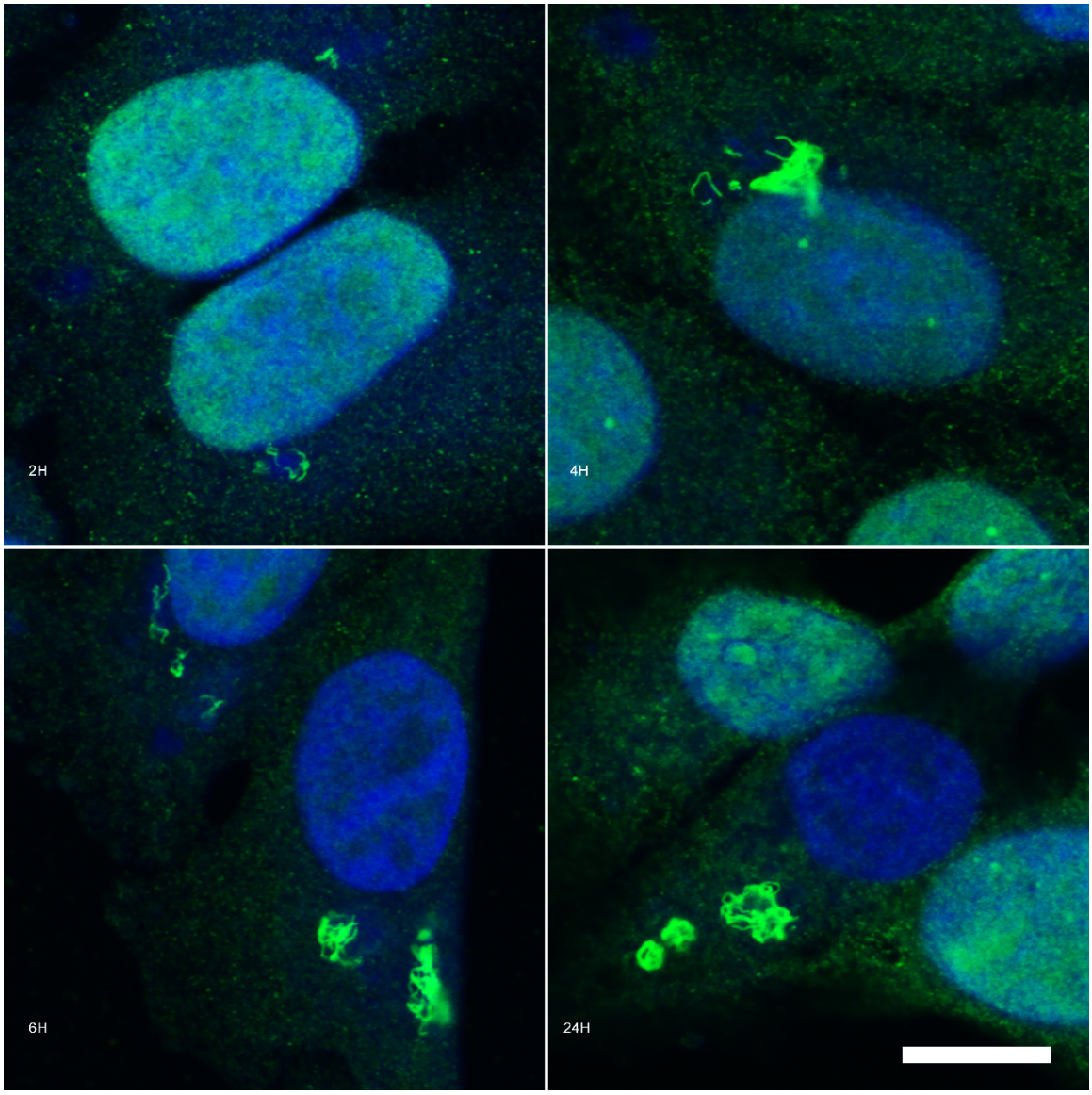
Supplemental Figure 1: IFI16 immunocytochemistry controls. (A&B): Cultured HSG cells were treated with IFNα and transfected with DNA as described in Figure 2. The fixed cells were stained with anti-IFI16 monoclonal antibody (A) or no primary antibody (B) and visualized by immunofluorescence confocal microscopy. Images were acquired using identical camera settings. Scale bars indicate 10 μM. Salivary gland paraffin sections were stained with anti-IFI16 monoclonal antibody or an equivalent final concentration of mouse IgG1 isotype control, and staining was visualized by light microscopy (C). Images were captured using identical camera settings. Image of oral mucosal epithelium stained with anti-IFI16 monoclonal antibody demonstrating nuclear localization, representative of samples examined in 6 patients (D). Scale bar indicates 20 μM.


DAPI
Anti-IFI16
Rhodamine
Merge
A
B
C
D
E

Supplemental Figure 2: Cytoplasmic IFI16 filamentation requires cytoplasmic dsDNA. HSGs were treated with IFNα (A), followed by lipofectamine transfection reagent (B), Poly(dA:dT)-Rhodamine (C), lipofectamine-Poly(dA:dT)-Rhodamine complexes (D) and lipofectamine-Poly(I:C)-Rhodamine complexes (E), then fixed and stained with DAPI (blue), anti-IFI16 antibody (green), and imaged by confocal microscopy. Despite cytoplasmic localization, transfected Poly(I:C) did not induce IFI16 filament formation. Scale bars indicate 10 μM.



Supplemental Figure 3: IFI16 expression is induced in cultured HSG cells and keratinocytes in response to IFN treatment. Cultured HSG cells and primary keratinocytes were treated with recombinant IFNα at 1000 u/mL or IFNγ at 50 ng/mL for 24 hours. Equal protein amounts of lysates generated from these cultures were immunoblotted using monoclonal antibodies against IFI16 and vinculin.


2H
4H
6H
24H

Supplemental Figure 4: Time-dependent IFI16 filament formation following dsDNA transfection in HSG cell cultures. HSG cells were treated with IFNα for 24 hours, and then transfected with plasmid DNA for the indicated times. Cells were fixed, stained with anti-IFI16 monoclonal antibody (green) and DAPI (blue), and analyzed by confocal microscopy. Small cytoplasmic filaments can be seen at 2 hours post transfection, with larger structures visible at 4 hours. At the 6 and 24 hour times, the cytoplasmic structures remained prominent but nuclear IFI16 staining was often absent. Scale bar indicates 10 μM.

Patient	Diagnosis	Age	Gender	Race	SSA	SSB	Biopsy	Focus Score	Nuclear IFI16	Cytoplasmic IFI16 Filaments	anti-IFI16 ELISA
1	SS	42	Female	White	+	+	+	6.4	+++	+	2.083
2	SS	68	Female	White	+	-	+	4.8	+++	+++	0.328
3	SS	29	Female	White	-	+	+	4	++	-	1.045
4	SS	55	Female	White	+	-	+	2.1	+++	++	0.437
5	SS	53	Female	White	+	-	+	3.9	++	+	0.144
6	SS	61	Female	White	+	-	+	3.4	++	-	0.690
7	SS	52	Female	Black	-	-	+	3.2	+++	+++	ND
8	SS	31	Female	White	+	+	+	1.2	++	-	ND
9	CT	44	Female	Hispanic	-	-	-		+	+	0.272
10	CT	25	Female	White	-	-	-		+	+	0.399
11	CT	36	Female	White	-	-	-		+	+	ND
12	CT	44	Female	White	-	-	-		++	-	0.430
13	CT	52	Female	White	-	-	-		+	-	0.559
14	CT	36	Female	White	-	-	-		+	+	ND
15	CT	35	Female	White	-	-	-		++	-	ND

Supplemental Table 1. Phenotypic characteristics and labial salivary gland IFI16 staining results in Sjögren's Syndrome (SS) and control (CT) subjects.

Focus score: Lymphocytic foci / 4 mm^2

Nuclear IFI16: Intensity of nuclear IFI16 staining; low (+), moderate (++), strong (+++)

Cytoplasmic IFI16 filaments: frequency of ductal epithelial cells containing cytoplasmic IFI16 filaments; none (-), rare (+), moderate (++), many (+++)

Anti-IFI16 ELISA OD >0.521 = positive

ND: no serum available for analysis

Antibody	IFI16 ΔCT/FL IP
Anti-NT	216%
Anti-CT	6%
209	21%
641	4%
647	9%
655	4%
677	10%
239	83%
366	78%
530	71%
547	169%
632	42%

Supplemental Table 2. C-terminal specificity of SS sera with property of IFI16-DNA binding. Commercial antibodies specific for the N-terminus (anti-NT) and C-terminus (anti-CT) of IFI16 and human sera were used to immunoprecipitate full length IFI16 (FL) or N-terminal IFI16 fragment (IFI16ΔCT) in Figure 6. Western blots were performed with anti-N terminal antibody and quantified by densitometry, and the ratio of immunoprecipitated IFI16ΔCT/IFI16 FL was calculated.

Supplemental Video 1: Cytoplasmic IFI16 structures in human salivary glands. Labial salivary gland paraffin sections were stained with anti-IFI16 antibody (green) and DAPI (blue). Z-stack images were obtained by confocal microscopy and analyzed with Imaris software. Representative cytoplasmic IFI16-containing structures in two epithelial cells are highlighted.

Supplemental Video 2: IFI16 filamentation in cultured keratinocytes following transfection with Poly(dA:dT). Primary keratinocytes were grown on coverslips and transfected with Poly(dA:dT)-Rhodamine (red) for 24 hours, then fixed in PFA, and stained with anti-IFI16 antibody (green) and DAPI (blue). Z stack images were obtained by confocal microscopy and analyzed with Imaris software. An area of cytoplasmic IFI16 filament formation in association with Poly(dA:dT) is highlighted.