

3 **Local and Systemic IKK ϵ and NF- κ B-signaling Associated with Sjögren's**

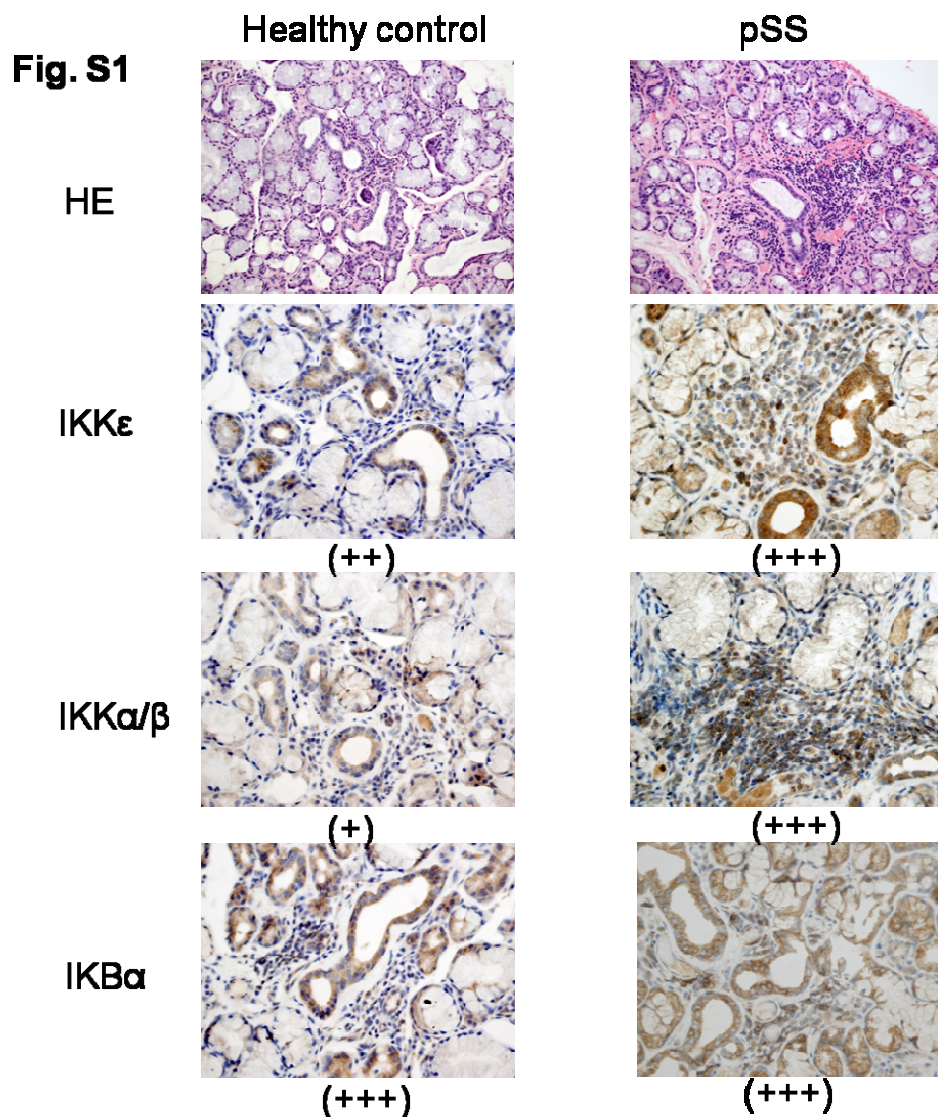
4 **Syndrome Immunopathogenesis**

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7 **Supplemental Information**



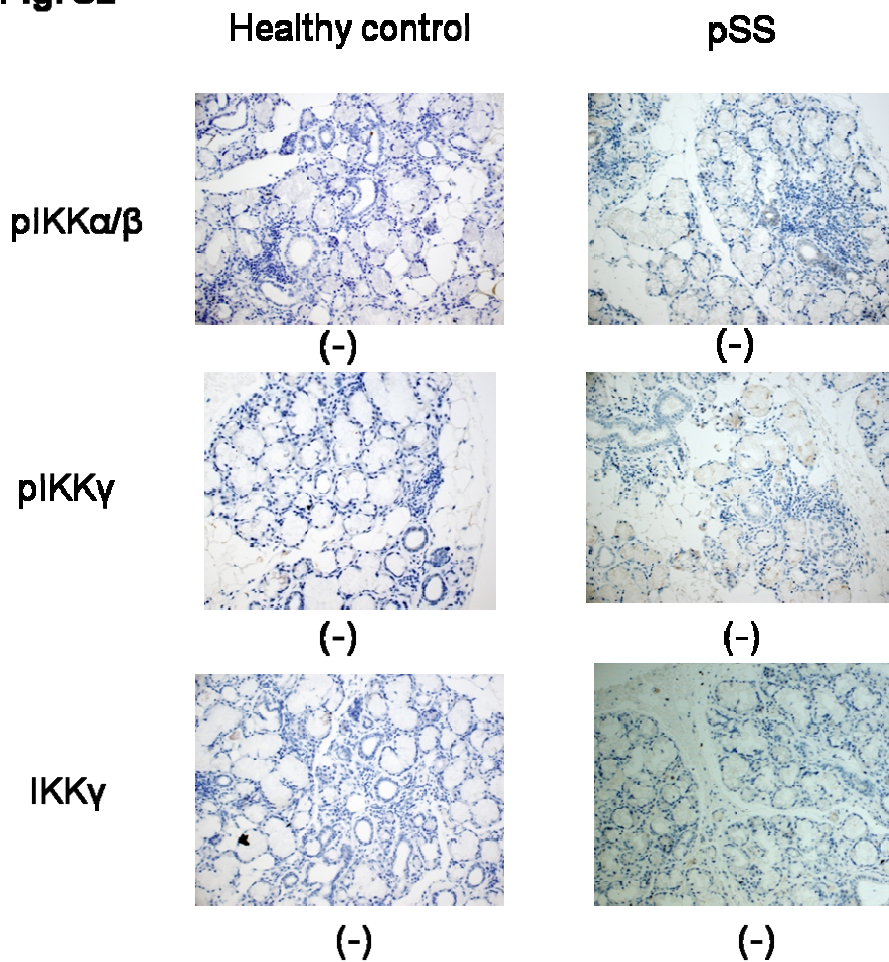
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11 **Figure S1.** Expression of total IKK ϵ , total IKK α/β and total IKB α protein in minor
12 salivary glands (MSG) from patients with primary Sjögren's syndrome (pSS) and
13 healthy controls. Sections of MSG tissue were stained with HE (top row) to observe

18 typical morphology of the ductal epithelium, acinar epithelium and infiltrating cells in
19 MSG tissues. These sections were also stained for expression of total IKK ϵ (second
20 row), total IKK α/β (third row) and total IKB α (bottom row), and observed by
21 immunohistochemical (IHC) analysis (brown), combined with counterstaining with
22 Mayer's hematoxylin (blue). Representative examples are shown in patients with pSS
23 and normal control. Original magnification: $\times 200$ for HE staining, $\times 400$ for total
24 IKK ϵ , IKK α/β and IKB α IHC.

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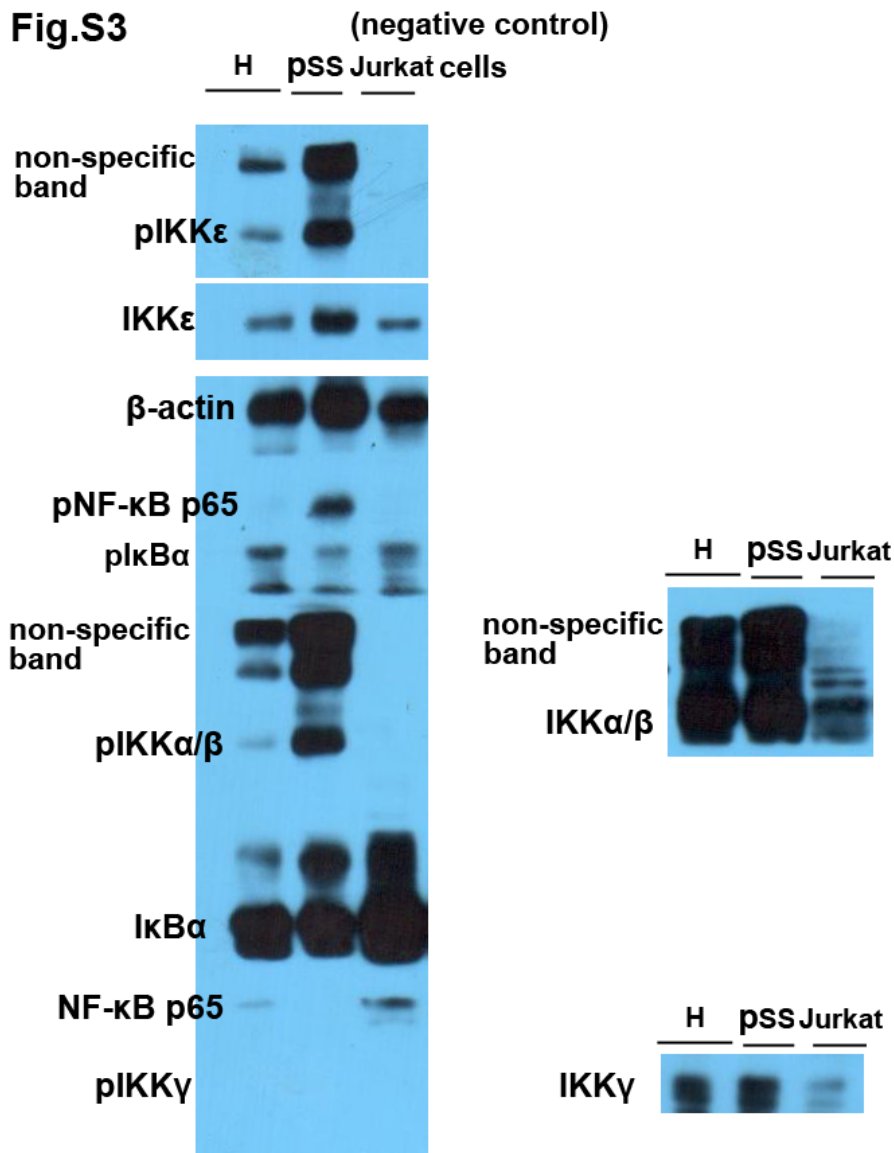
Fig. S2



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23 **Figure S2.** Expression of pIKK α/β , pIKK γ and total IKK γ was not seen in MSG
24 tissues by IHC analysis. Staining was performed as in Figure S1, using antibodies
25 against phosphorylated IKK α/β (top row), phosphorylated IKK γ (second row), and

23 total IKK γ (bottom row), combined with counterstaining with Mayer's hematoxylin
 24 (blue). Representative examples are shown from patients with pSS and healthy
 25 controls. Original magnification: $\times 200$ for pIKK α/β , pIKK γ and total IKK γ IHC.
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 28 **Figure S3.** Protein expression of pIKK ϵ , total IKK ϵ and pNF- κ B p65 was
 29 significantly increased in PBMC from patients with pSS. Peripheral blood
 30 mononuclear cells (PBMC) from patients with primary Sjögren's syndrome (pSS) and
 31 healthy individuals (H) were lysed and assayed for phosphorylated and total protein

32 expression of IKK ϵ , IKK α/β , IKK γ , I κ B α and NF- κ B p65 using western blot.

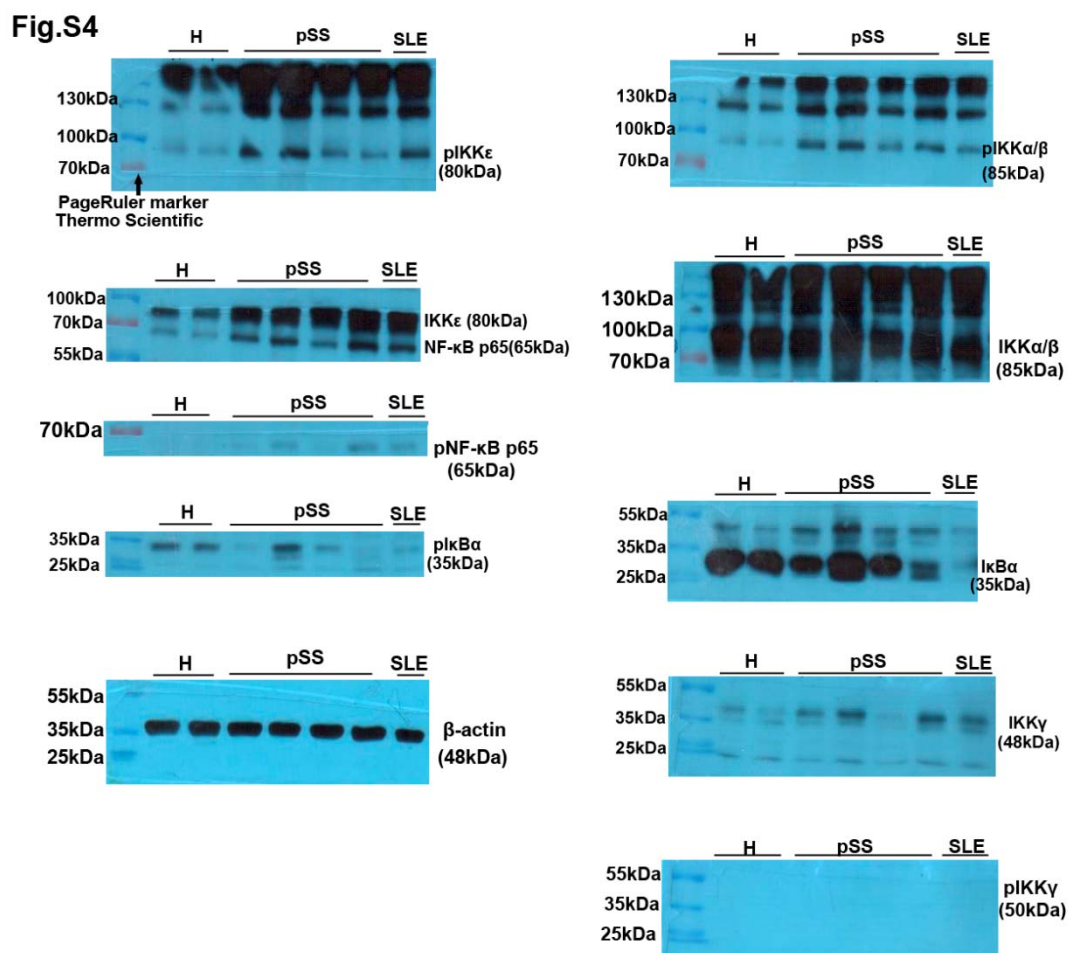
33 Untreated Jurkat T leukemia cells, obtained from the Cell Bank of the Chinese

34 Academy of Sciences (Shanghai, China) served as a negative control. β -actin served

35 as loading control for normalization. All data are representative of three repeated

36 experiments.

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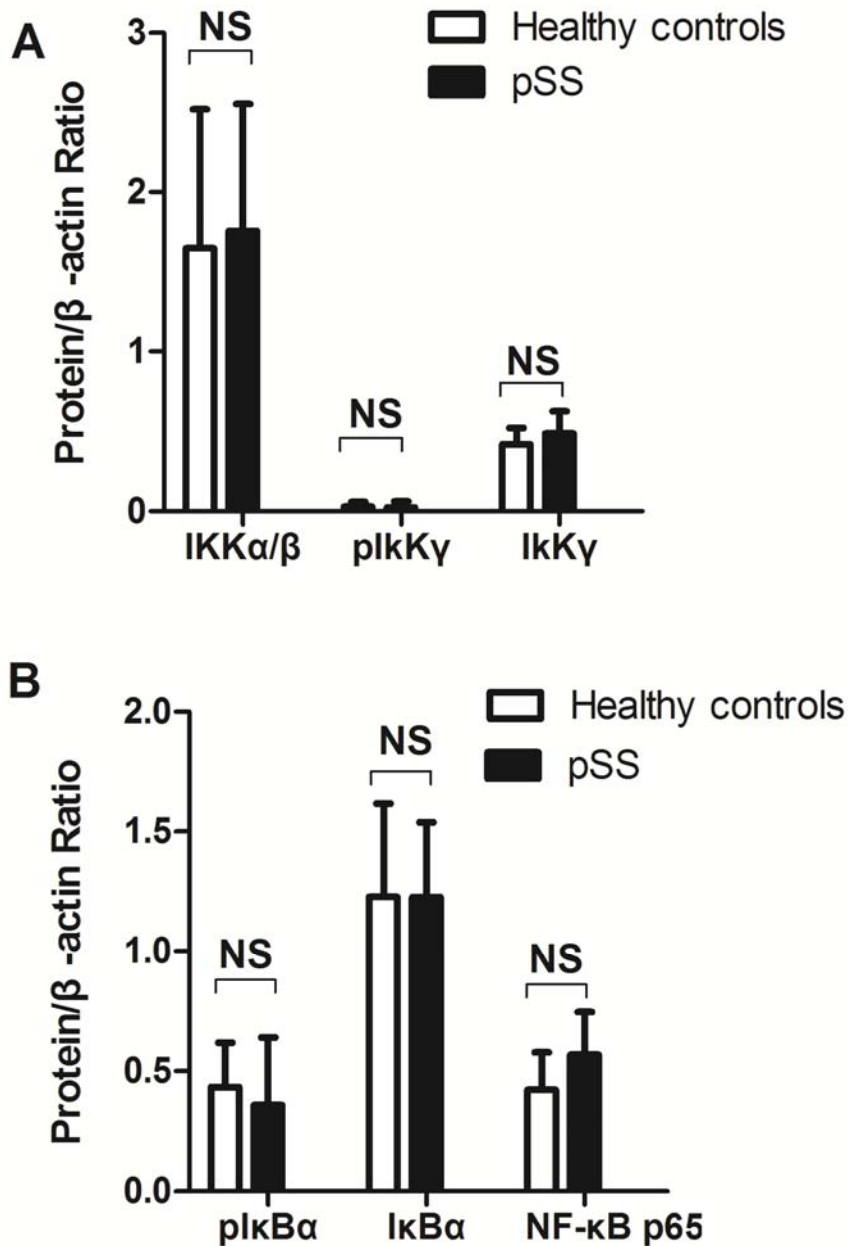
39 **Figure S4.** Full images of western blots stained for phosphorylated and total protein

40 expression of IKK ϵ , IKK α/β , IKK γ , I κ B α and NF- κ B p65 in PBMC. Peripheral blood

41 mononuclear cells (PBMC) from two healthy controls (H), four representative pSS
42 patients and one patient with systemic lupus erythematosus (SLE) were lysed and
43 analyzed for protein expression by western blot as in Figure S3. In all blots, Thermo
44 Scientific PageRuler Plus Protein Ladder served as protein size standards (red band:
45 70kDa) and β -actin served as protein control. All data are representative of three
46 repeated experiments.

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Fig.S5



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49 **Figure S5.** Relative expression of total IKK α/β , pIKK γ , total IKK γ , pIKB α , total
50 I κ B α and total NF- κ B p65 were similar in PBMC from healthy controls and patients
51 with pSS. Peripheral blood mononuclear cells (PBMC) from healthy controls (n=26)
52 and pSS patients (n=33) were lysed and analyzed for protein expression by western
53 blot as in Figure S3. For each protein, relative expression was quantified as the ratio
54 of the protein to β -actin. NS: not significant.