Materials and Methods:

Cell lines, reagents, antibodies and plasmids. HEK293 cells were obtained from ATCC and maintained in DMEM (Invitrogen Life Technologies, San Diego, CA) supplemented with 10% FBS, 100U /ml penicillin, 200ug/ml streptomycin, and 0.25ug/ml amphotericin B. Synoviolin expression plasmids and anti-Synoviolin antibodies were used as described (Gao et al., 2006). IRE1-expression plasmid was purchased from Addgene (Cambridge, MA) and subcloned to pCMV expression plasmid (Sigma, St. Louis, MO). Anti-IRE1 antibody was purchased from ABCAM (Cambridge, MA). Anti-Myc polyclonal antibody was obtained from Santa Cruz (Santa Cruz, CA). Tunicamycin is from Calbiochem (La Jolla, CA).

Collagen-induced arthritis (CIA) DBA1 mice (Jackson Laboratories) were bred and maintained in the accordance with the guidelines of the IACUC and all the experimental procedures were approved by the IACUC of the University of Missouri.
Native bovine collagen II (Worthington Biochemical Co. Lakewood, NJ) was emulsified with an equal volume of complete Freund's Adjuvant (CFA). Disease was induced by intradermal injection of DBA1 mice with 50 μl of emulsion containing 100 μg collagen in CFA. On day 21, the mice were boosted by intradermal injection with 100 μg collagen in incomplete Freund's Adjuvant (IFA). Clinical arthritis was assessed by the following scoring system: grade 0, no swelling; grade 1, mild, but definite redness and swelling of the ankle or wrist or digits; grade 2, moderate redness and swelling of ankle and wrist; grade 3, severe redness and swelling of entire paw including digits; grade 4, maximally inflamed limb with involvement of multiple joints. Each limb was graded, giving a maximum possible score of 16/mouse. About 80% of DBA1 mice developed arthritis 40 days after first injection with collagen, most of these mice developed severe arthritis with an average score of 12.

Isolation of synovial fibroblasts. CIA induction was performed as we previously reported (Gao et al., 2006). Synovial tissues were obtained from DBA1 mice as described (Gouze et al., 2004). These joint tissues were minced and incubated with 1 mg/ml of

collagenase(Worthington Biochemical Co. Lakewood, NJ) in serum-free Dulbecco's modified Eagle's medium (DMEM) for 3 hours at 37°C, filtered through a nylon mesh, extensively washed, and cultured in DMEM supplemented with 10% fetal calf serum (FCS) (Fisher Scientific INC), 100 U penicillin, 100 μg/ml streptomycin, and 50 mg/ml L-glutamine in a humidified atmosphere containing 5% CO_2. After overnight culture, we removed the nonadherent cells, and trypsinized the adherent cells split at a ratio of 1:3, and cultured them in medium. Synoviocytes were used from passages 3 through 9 in these experiments, during which they consisted of a homogeneous population of synovial fibroblasts monitored by flow cytometery with less than 1% of CD11b, phagocytic, and Fcϒ receptor II positive cells.

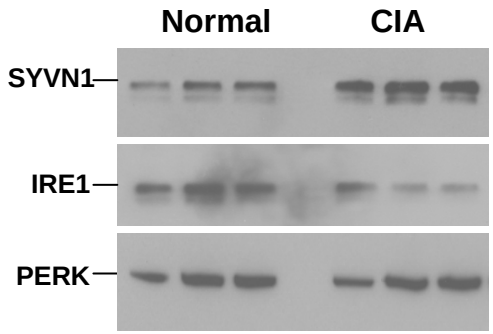
Co-immunoprecipitation, SDS-polyacrylamide gel electrophoresis (PAGE) and Western blotting: Transient transfected HEK293 cells or mouse synovial fibroblasts were collected from culture dishes and lysed with Nonidet P-40 (NP-40) lysis buffer (20 mM Tris-HCl with pH 7.5, 150 mM NaCl, 1% NP-40, and protease inhibitor cocktail was added freshly). For co-immunoprecipitation, the whole cell lysates were incubated with antibodies (1 μg/ml) on ice for 2 hours followed by adding 25 μl of protein G sepharose beads and incubated for additional 2 hours. The beads were washed with ice-cold NP-40 lysis buffer for three times and boiled in 20 μl of Laemmli's buffer (50 mM Tris-HCl, pH 6.8, 30% glycerol, 4% SDS and 1% β-mercaptoethanol). Samples were subjected to 8% or 10% analysis by SDS-PAGE and electrotransferred onto polyvinylidene difluoride membranes (PVDF; Millipore). Membranes were probed with the indicated primary antibodies (usually 1 μg/ml), followed by horseradish peroxidase (HRP)-conjugated secondary antibodies. Membranes were then washed and visualized with an enhanced chemiluminescence detection system (ECL; Amersham Pharmacia Biotech). When necessary, membranes were stripped by incubation in stripping buffer (62.5 mM Tris-HCl, pH 5.7, 100 mM 2-mercaptoethanol, and 2% SDS) for 30 min at 70°C with constant agitation, washed, and then reprobed with other antibodies as indicated.

Supplemental Figure Legends:

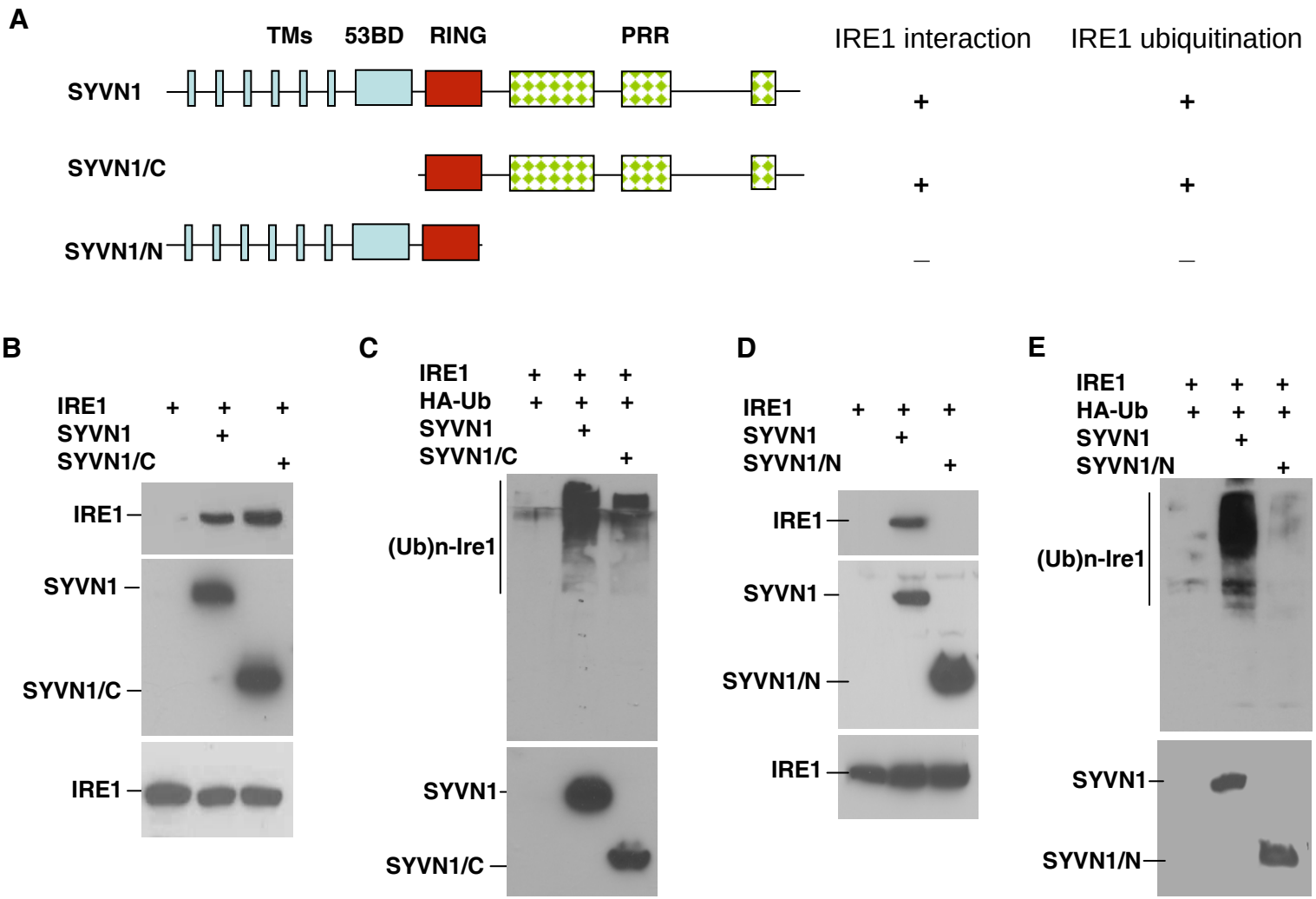
sFig. 1. Decreased IRE1 protein expression in synovial fibroblasts from CIA mice. The protein expression of SYVN1 (top panel), IRE1 (middle panel) and PERK (bottom panel) in the lysates of synovial fibroblasts from normal and CIA mice were determined by western blotting.

sFig. 2. The C-terminus of SYVN1 binds to and mediates IRE1 ubiquitination. (A) The schematic representation of SYVN1 and its N-terminus deletion mutant: TMs, transmembrane domains; RING, RING finger; 53BD, p53-binding domain; PR, proline-rich sequence. **(B)** IRE1 was cotransfected with SYVN1 or its N-terminus deletion (SYVN/C). The proteins of SYVN1 or its mutant were immunoprecipitated with anti-Flag antibody. The interacting IRE1 was detected with anti-IRE1 antibody (top panel). The same membrane was reblotted with anti-SYVN1 antibody (middle panel). The expression levels of IRE1 in the whole cell lysates were examined with anti-IRE1 antibody (bottom). **(C)** IRE1 and HA-Ub expression plasmids were co-transfected with SYVN1 or SYVN1/C truncated mutant into HEK293 cells. IRE1 ubiquitination was examined as described in Figure 3A. **(D & E)** The effects of deletion of SYVN1 C-terminus on its interaction with IRE1 (D) and on IRE1 ubiquitination (E) were analyzed as describe in (B & C).

Supplemental Fig. 1



sFig. 2


A
TMs
53BD
RING
PRR
IRE1 interaction
IRE1 ubiquitination
SYVN1
SYVN1/C
SYVN1/N
+
+
+
+
–
–
B
IRE1 + + +
SYVN1 +
SYVN1/C +
IRE1
SYVN1
SYVN1/C
IRE1
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IRE1 + + +
HA-Ub + + +
SYVN1 +
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