14-3-3ζ : A Novel Suppressor of Inflammatory Arthritis

Supplementary Figures

Fig S1: Loss of specific antibodies and IA susceptibility in 14-3-3ζ KO animals. (A) The DNA gel showing a 58-bp deletion in the ear biopsy from WT, heterozygous, and 14-3-3ζ KO rat, generated by CRISPR-Cas9, is shown. (B) The 14-3-3ζ antibodies in the plasma of WT and 14-3-3ζ KO rats were measured at different dilutions to standardize in-house ELISA. (C) The 14-3-3eta antibodies were compared in WT and 14-3-3ζ KO rat plasma using in-house ELISA. (D) The typical PIA model is shown. (E) The 14-3-3ζ KO rats were compared with WT for IA-susceptibility in a collagen-induced arthritis (CIA) model by injecting 0.2 ml of soluble type II collagen (1 mg/kg) intradermally. At days 1 and 8 post-collagen, animals received 150µl of IFA plus 14-3-3ζ (1mg/ml) or IFA alone. Animals were scored twice weekly, and IA scored were plotted against time.

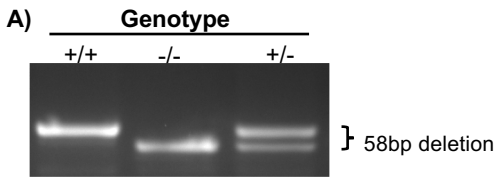
Fig S2: Negative correlation between IA scores and 14-3-3ζ antibodies. (A) The 14-3-3ζ antibodies in the plasma were measured over the course of PIA induction in WT Lewis rats. The IA scores in the same animals were plotted against time to observe the negative correlation between the IA and 14-3-3ζ antibody. The 2-way ANOVA test was used to determine the significance ($p<0.0001$). (B-C) Infusion with either complete or 14-3-3ζ antibody depleted plasma was performed in WT (B) and KO (C) animals at two time points, as indicated by the arrow. IA scores and body weights were measured.

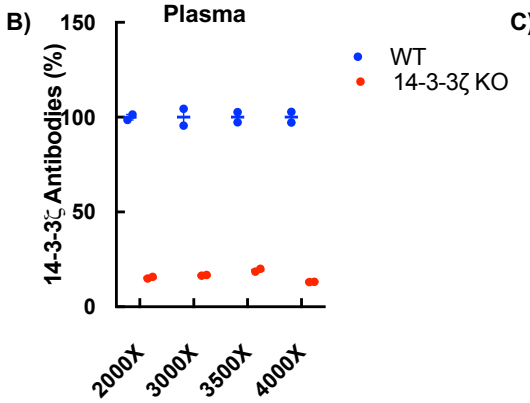
Fig S3: 14-3-3ζ immunization does not cause IA. (A) The 14-3-3ζ knockout animals were subjected to the collagen-induced arthritis model using 0.2 ml of soluble type II collagen (1 mg/kg) injected intradermally**.** At days 1 and 8, animals received 150µl of IFA plus 14-3-3ζ (1mg/ml) or IFA alone. Animals were scored twice weekly, and the scores were plotted against time. The 2-way ANOVA was used to determine the significance of the difference between the two treatments. (B) The effect of 14-3-3ζ immunization on the 14-3-3eta antibody level was examined in the WT animals in the PIA model. (C) WT Lewis rats were treated with two doses of 150µl of IFA plus 14-3-3ζ (1mg/ml) or IFA alone and observed for IA scores in the absence of pristane treatment. (D) Wistar rats were subjected to PIA followed by 150µl of IFA plus 14-3-3ζ (1mg/ml) or IFA alone. IA score and soluble levels of 14-3-3ζ and IL-17A at the end of 32d are shown.

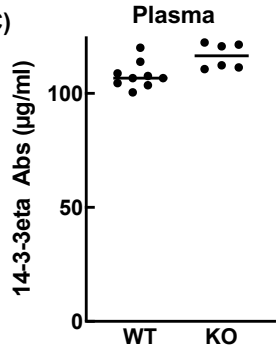
Fig S4: Exposure to recombinant 14-3-3ζ does not alter MSC growth. (A) Different amounts of recombinant His-tagged 14-3-3ζ protein purified from over-expressing HEK-293T cells were immunoblotted. (B) Rat mesenchymal cells were incubated with 0-1000ng of recombinant 14-3-3ζ for 7d followed by MTT assay, as described previously in Ref 32.

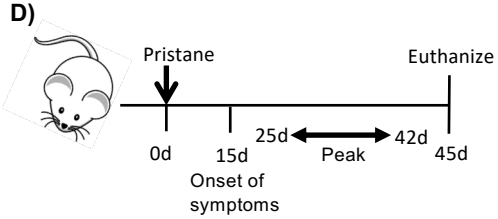
Fig S5: IA-induced immunological changes are suppressed by 14-3-3ζ. RT-pCR measured expression of Cxcl1 (A), Ifng (B), and Tnfa (C) in the circulating PBMCs of WT and 14-3-3ζ KO animals at the end of the PIA model where animals received either IFA alone or IFA+14-3-3ζ immunization.

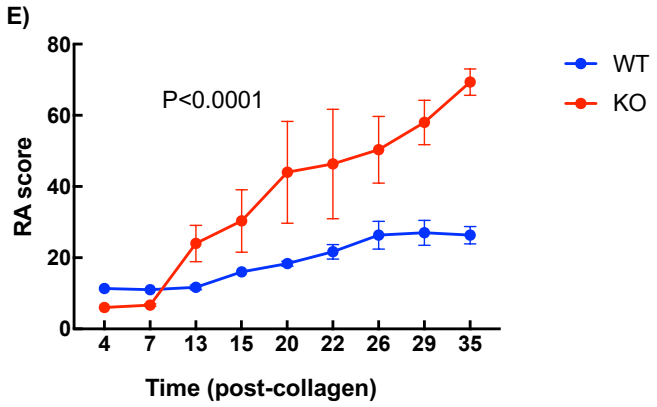
Fig S1


A)
Genotype
+/+
-/-
+/-
58bp deletion


B)
Plasma
14-3-3ζ Antibodies (%)
150
100
50
0
2000X
3000X
3500X
4000X
WT
14-3-3ζ KO


C)
Plasma
14-3-3eta Abs (µg/ml)
100
50
0
WT
KO


D)
Pristane
Euthanize
0d
15d
Onset of symptoms
25d
Peak
42d
45d


E)
P<0.0001
RA score
80
60
40
20
0
4
7
13
15
20
22
26
29
35
Time (post-collagen)
WT
KO

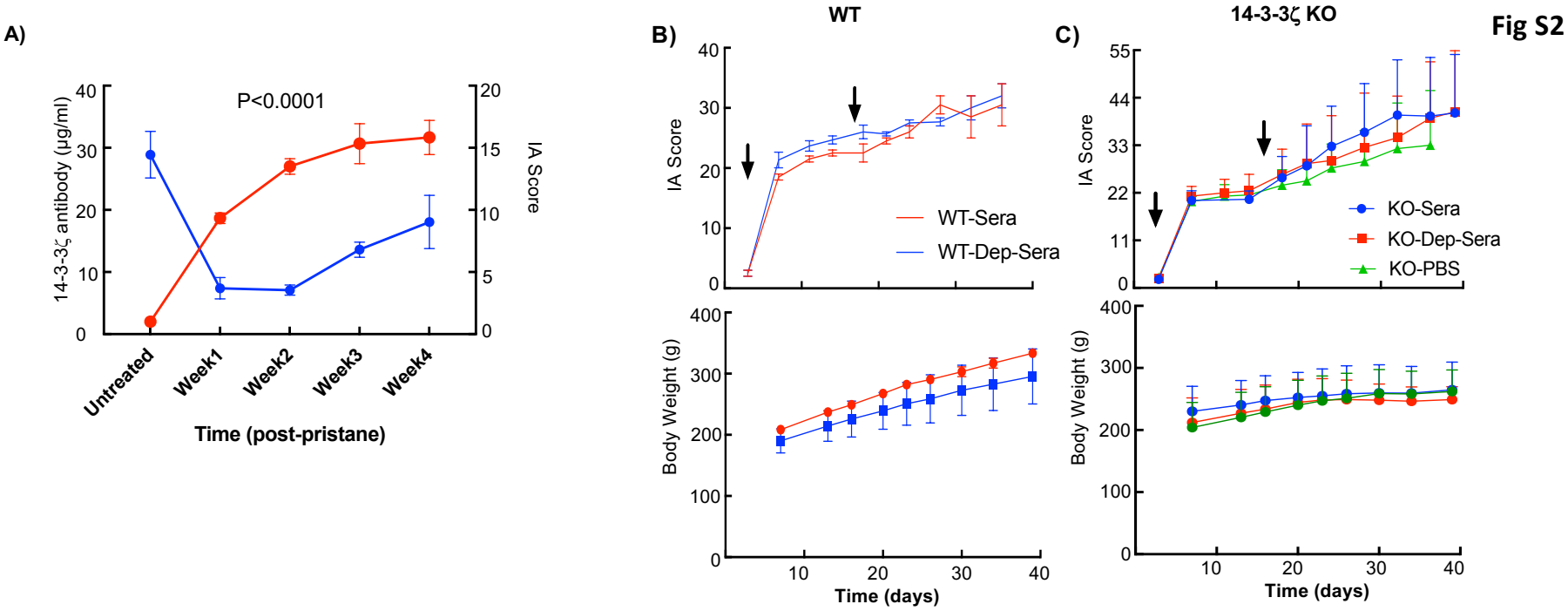

A)
P<0.0001
14-3-3ζ antibody (µg/ml)
IA Score
Untreated
Week1
Week2
Week3
Week4
Time (post-pristane)
B)
WT
IA Score
WT-Sera
WT-Dep-Sera
Body Weight (g)
Time (days)
C)
14-3-3ζ KO
IA Score
KO-Sera
KO-Dep-Sera
KO-PBS
Body Weight (g)
Time (days)

Fig S2

Fig S3

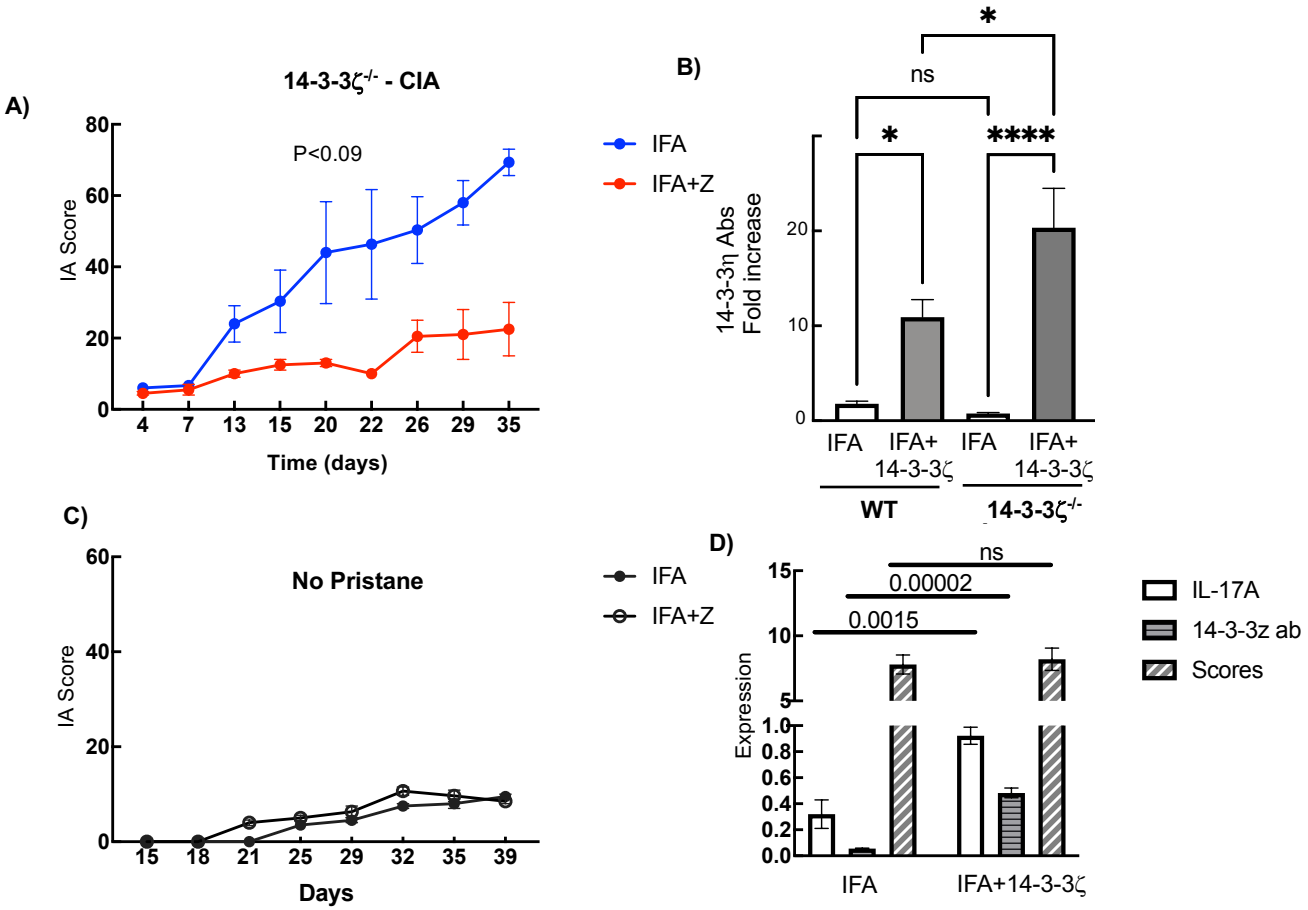

A)
14-3-3ζ-/- - CIA
P<0.09
IA Score
Time (days)
B)
IFA
IFA+Z
14-3-3η Abs
Fold increase
ns
*
****
IFA
IFA+ 14-3-3ζ
WT
14-3-3ζ-/-
C)
No Pristane
IA Score
Days
D)
Expression
0.00002
0.0015
IL-17A
14-3-3z ab
Scores
IFA
IFA+14-3-3ζ

Fig S4

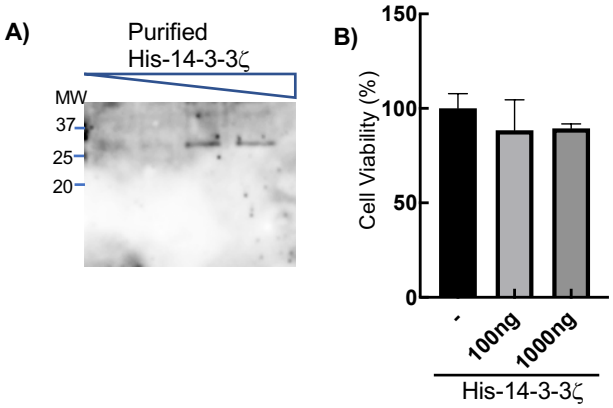
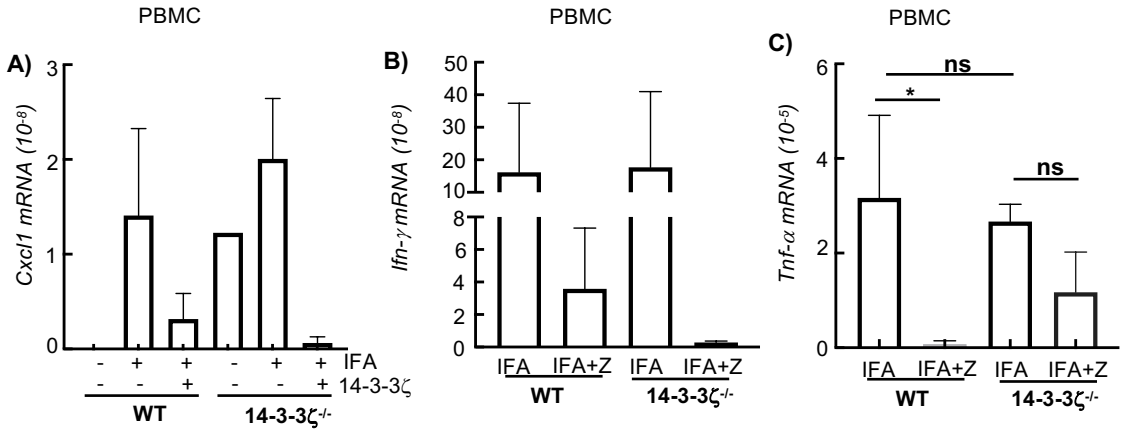

A)
Purified
His-14-3-3ζ
MW
37
25
20
B)
150
100
50
0
Cell Viability (%)
-
100ng
1000ng
His-14-3-3ζ

Fig S5


PBMC
A)
Cxcl1 mRNA (10-8)
3
2
1
0
- + + - + + IFA
- - + - - + 14-3-3ζ
WT
14-3-3ζ-/-
PBMC
B)
Ifn-γ mRNA (10-8)
50
40
30
20
10
8
6
4
2
0
IFA IFA+Z IFA IFA+Z
WT
14-3-3ζ-/-
PBMC
C)
Tnf-α mRNA (10-5)
6
4
2
0
ns
*
ns
IFA IFA+Z IFA IFA+Z
WT
14-3-3ζ-/-