

Supplemental Material

Protein phosphatase PPM1A inhibition attenuates osteoarthritis *via* regulating TGF- β /Smad2 signaling in chondrocytes

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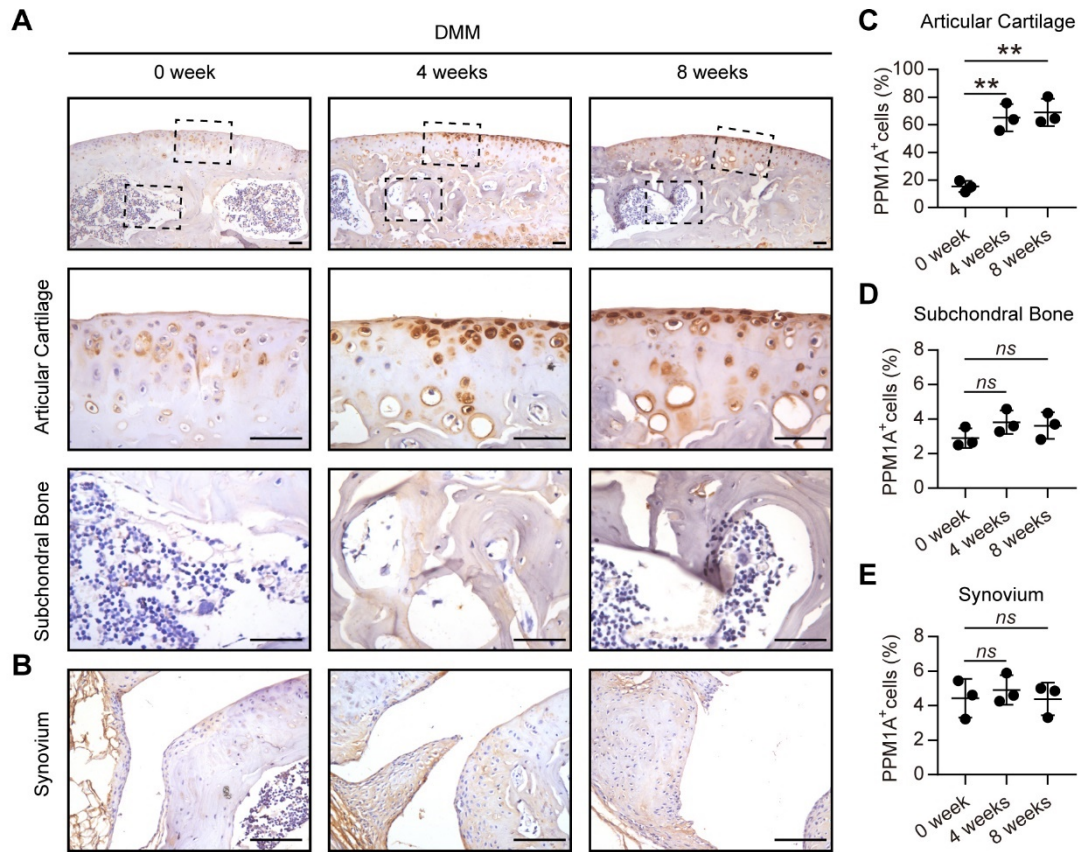
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25 **Supplementary Figure 1. DMM operation induce PPM1A expression in articular cartilage**

26 **rather than subchondral bone or synovium. (A)** Representative immunohistochemical

27 staining for PPM1A in articular cartilage and subchondral bone area at 0 week, 4 weeks, and 8

28 weeks post DMM initiation. Scale bar = 50 μ m. **(B)** Representative immunohistochemical

29 staining for PPM1A in synovium tissue at 0 week, 4 weeks, and 8 weeks after DMM surgery.

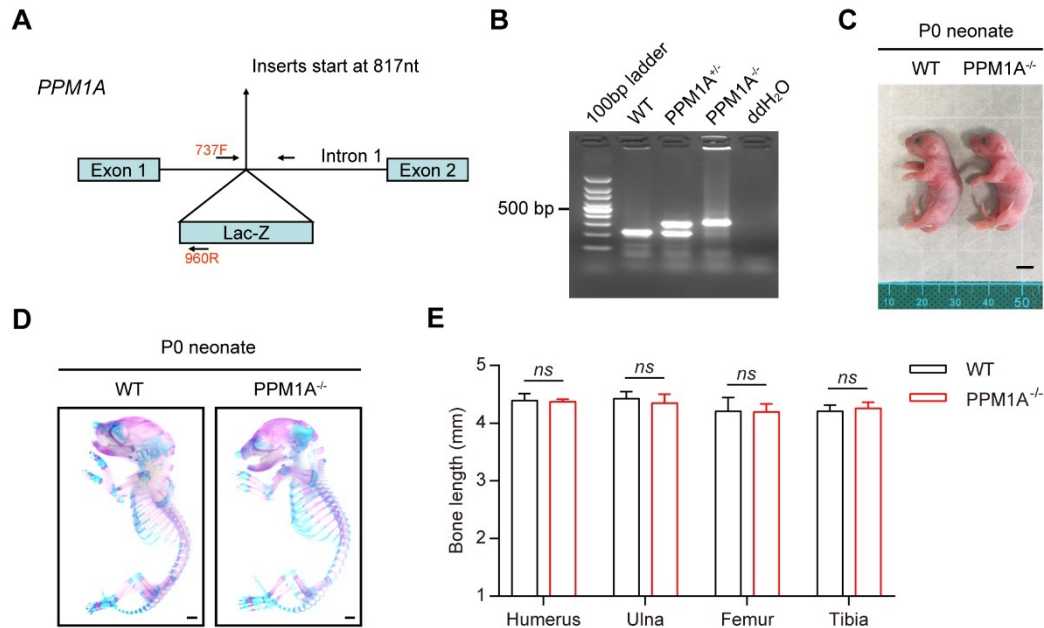
30 Scale bar = 100 μ m. **(C-E)** Quantitative data of PPM1A positive cells in articular cartilage (C),

31 subchondral bone (D), and synovium (E) at different timepoints. Data were presented as means

32 \pm S.D., n = 5 mice at per timepoint, ** = $P < 0.01$ and ns = no significance by one-way ANOVA

33 with Dunnett's *t*-test.

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36 **Supplementary Figure 2. Comparison of skeletal development between WT and PPM1A-**

37 **KO postnatal mice. (A)** The schematic diagram of generation of PPM1A-KO mouse. **(B)**

38 Genotyping for WT, PPM1A^{+/-}, and PPM1A^{-/-} mice. KO band = 300~400 bp, WT band = 200~300

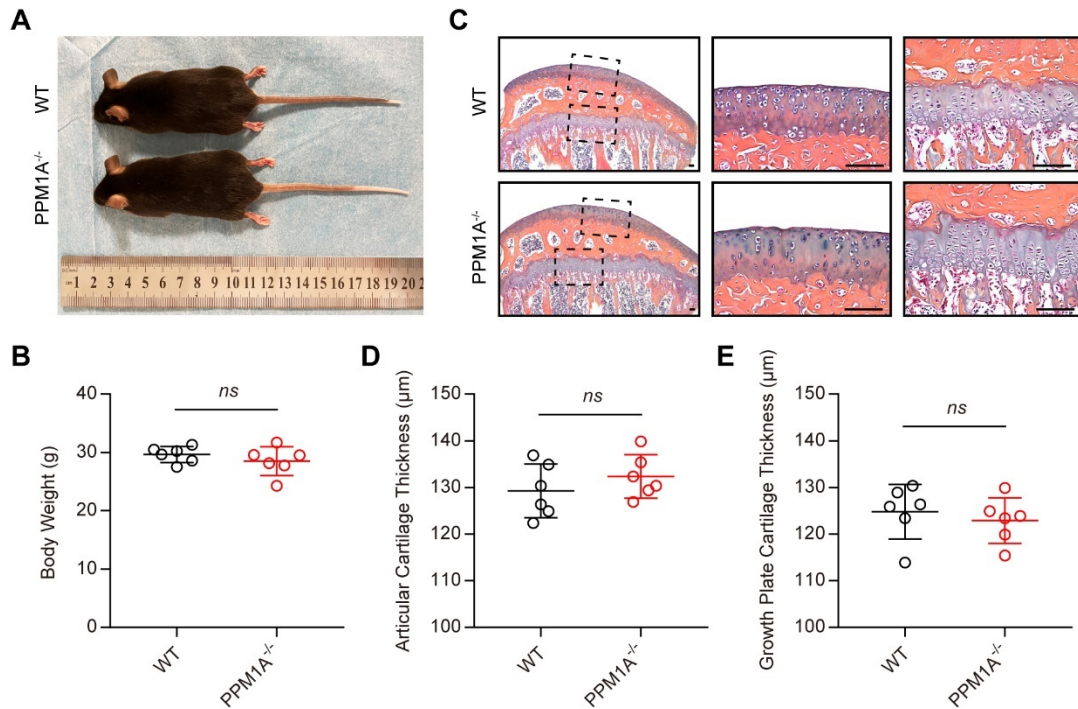
39 bp. **(C)** Gross appearance of WT and PPM1A-KO postnatal mice at day 0 (P0). Scale bar = 5

40 mm. **(D)** Representative images of whole skeleton staining with Alizarin red and Alcian blue of

41 WT and PPM1A^{-/-} mice at P0. Scale bar = 1 mm. **(E)** Quantitative data of long bone length of

42 WT and PPM1A-KO mice at P0. All the data were presented as means ± S.D., n = 3 pups per

43 group, *ns* = no significance by unpaired parametric Student's *t* test.



44

45 **Supplementary Figure 3. PPM1A-KO mice presented normal cartilage homeostasis in**

46 **adulthood. (A)** Gross appearance of male WT and PPM1A-KO mice at 3-month-old. Scale bar

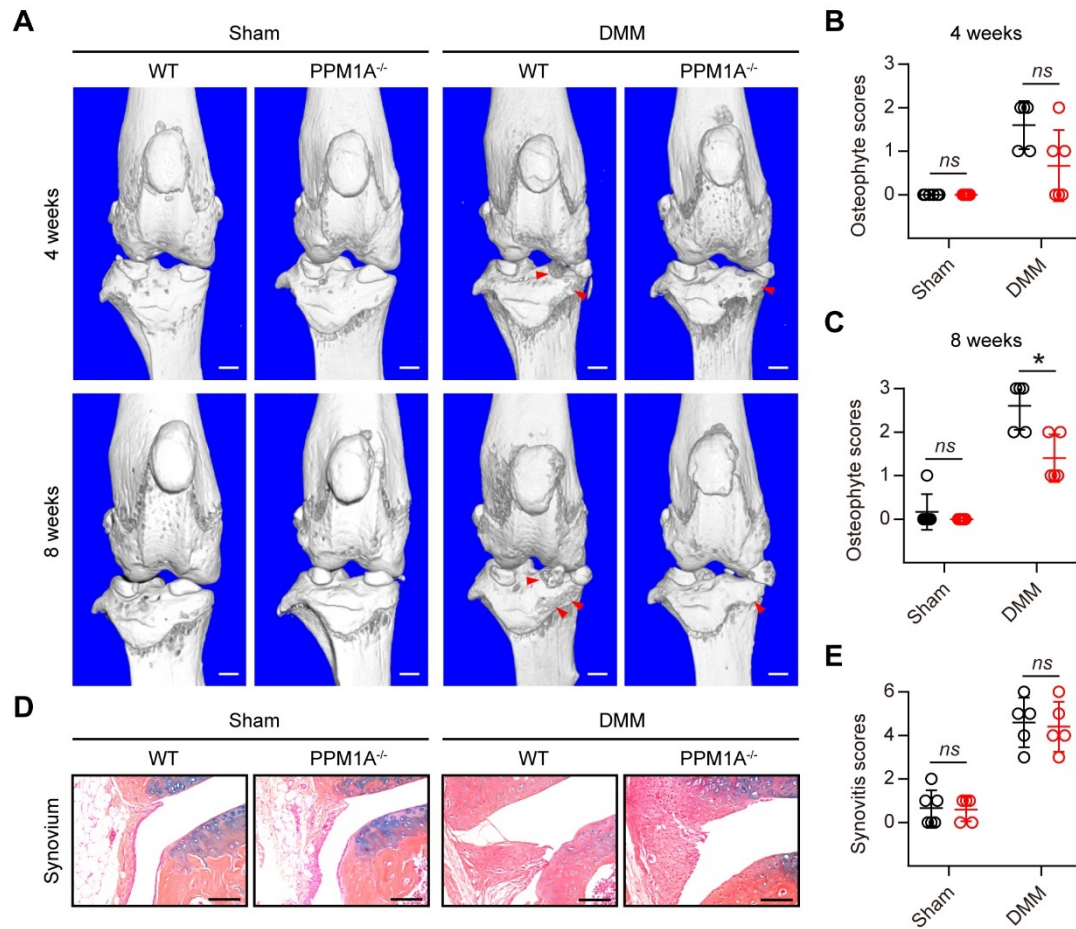
47 = 5 mm. **(B)** Quantification of body weight for male WT and PPM1A-KO mice at 3-month-old.

48 **(C)** ABH/OG staining for both tibial articular cartilage and growth plate from WT and PPM1A^{-/-}

49 mice at 3-month-old. **(D and E)** Quantification of articular cartilage thickness (D) and growth

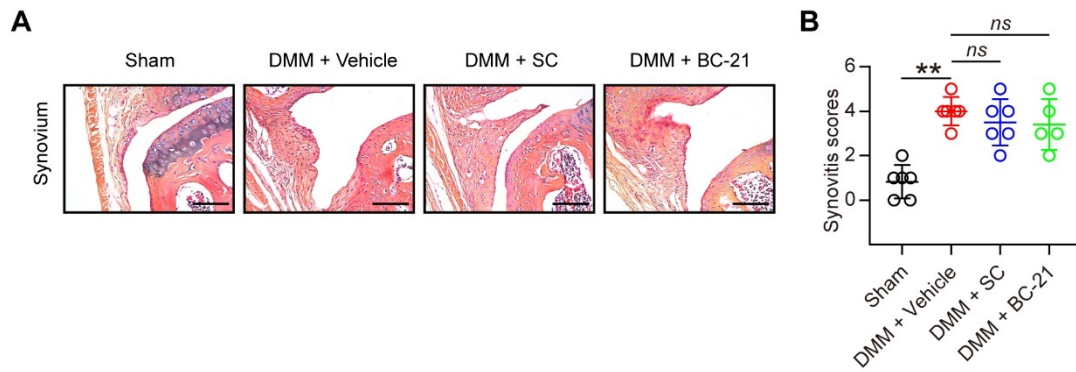
50 plate cartilage thickness (E). Data were presented as means ± S.D., n = 6 mice per group. *ns*

51 = no significance by unpaired parametric Student's *t* test.



52

53 **Supplementary Figure 4. PPM1A-KO mice exhibited less osteophyte formation but no**
 54 **effect on synovitis in response to DMM surgery. (A)** Representative images of 3D
 55 reconstruction for knee joint after sham or DMM surgery at 4 weeks and 8 weeks. Red arrows
 56 indicated osteophyte. Scale bar = 1 mm. **(B and C)** Quantification of osteophyte scores for
 57 gross knee joint at 4 weeks (B) and 8 weeks (C) after operation. **(D)** Representative ABH/OG-
 58 stained sections of anterior synovium from WT and PPM1A^{-/-} mice at 8 weeks after sham or
 59 DMM surgery. Scale bar = 100 μ m. **(E)** Synovitis scores for assessment of the severity of both
 60 synovial hyperplasia and synovial inflammation at 8 weeks after surgery. Data were presented
 61 as means \pm S.D., $n \geq 5$ mice per group. * = $P < 0.05$ and *ns* = no significance by two-way
 62 ANOVA with Sidak's test.



63

64 **Supplementary Figure 5. Intra-articular injection of PPM1A inhibitor displayed no**

65 **therapeutic effect on OA synovitis. (A)** Representative ABH/OG staining images of anterior

66 synovium from mice treated with vehicle or PPM1A inhibitor at 8 weeks after sham or DMM

67 surgery. Scale bar = 100 μ m. **(B)** Synovitis scores for assessment of the severity of both

68 synovial hyperplasia and synovial inflammation at 8 weeks after surgery. Data were presented

69 as means \pm S.D., ** = $P < 0.01$ and ns = no significance by one-way ANOVA with Dunnett's *t*-

70 test.

71

72 **Table S1.** Primers sequence for RT-qPCR.

Gene	Primer sequences	Origin
<i>PPM1A</i>	Forward: 5'-GGACAAGTACCTGGAGAGCAGA-3'	Mus
	Reverse: 5'-GGGATGTTCTCACTGGCTAACG-3'	Mus
<i>β-actin</i>	Forward: 5'-GGAGATTACTGCCCTGGCTCCTA-3'	Mus
	Reverse: 5'-GACTCATCGTACTCCTGCTTGCTG-3'	Mus

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74 **Table S2.** Primers sequence for *PPM1A* genotyping.

	Primer sequences
Primer 1	5'-GGCTGAAGCCTGAGAAGGTTC-3'
Primer 2	5'-TCCTCCAGGCAAGTTACAAGC-3'
Primer 3	5'-ATCCTCTGCATGGTCAGGTC-3'
Primer 4	5'-CGTGGCCTGATTCATTCC-3'

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