

1

2 **SUPPLEMENTARY MATERIALS**

3

4 **CircPDE4B prevents articular cartilage degeneration and promotes repair by acting**
5 **as a scaffold for RIC8A and MID1**6 Shuying Shen#, Yute Yang#, Panyang Shen#, Jun Ma, Bin Fang, Qingxin Wang, Kefan
7 Wang, Peihua Shi, Shunwu Fan*&Xiangqian Fang*

8

9 **SUPPLEMENTARY MATERIALS AND METHODS**

10

11 **Human cartilage**

12 The collection and classification of human cartilage samples was approved by the Ethics
13 Committee of Sir Run Run Shaw Hospital (Zhejiang, China). Informed consent was
14 obtained from all participants. Control cartilage was collected from ten amputees without
15 a history of OA (n = 10), and damaged cartilage was obtained from individuals with a
16 history of OA after total knee replacement surgery (n = 10). The cartilage was divided
17 into three groups: normal medial, OA lateral and OA medial. The tibial plateau cartilage
18 without the protection of a meniscus cover comprising an area of approximately 1 cm
19 (width) × 2 cm (length) along the midline of the medial or lateral tibial plateau was
20 defined as the region of interest (ROI). The Kellgren-Lawrence grade (upon preoperative
21 imaging), Outerbridge grade (by visual observation in isolated specimens), and OARSI
22 grade (after histomorphological staining) was exploited to evaluate specimen OA
23 severity. Among them, Outerbridge and OARSI grading were used to evaluate the
24 cartilage ROI.

25

26 **Plasmids**

1 Human/mouse circPDE4B linear sequence (351/350 bp) was synthesized and subcloned
2 into pHBAAd-circRNA (HanBio., Shanghai, China). For RNA binding protein
3 immunoprecipitation (RIP) assay, linear circPDE4B truncated mutants and wild type
4 (WT) were cloned into the pcDNA3.1 vector. For RIP and immunoprecipitation (IP)
5 assay, human RIC8A cDNA (1,614 bp) was synthesized and its truncations were
6 subcloned into pcDNA3.1 vector. Mutants of RIC8A were prepared with GeneTailor™
7 Site-Directed Mutagenesis System (Invitrogen) and primers (table S3). Specially, RIC8A
8 K415R was synthesized and subcloned to pcDNA3.1 vector. For HEK-293T co-IP assay,
9 RIC8A cDNA (1614) /N/C terminal and MID1 cDNA (2,004 bp) were synthesized and
10 then subcloned into pLVX-IRES-puro-Flag or pLVX-IRES-blasticidin-Myc (Addgene).
11 For human chondrocytes (HCs) IP/co-IP assay, Myc-MID1 and Flag-RIC8A lentivirus
12 were then packaged. mmu_RIC8A was also synthesized and then subcloned into
13 pLVX-IRES-puro-Flag, further packaged. CircPDE4B-s and circPDE4B-s-del were also
14 synthesized (circPDE4B-s comprises presumed FUS-binding sites on both flanking
15 introns preserved; circPDE4B-s-del resembles circPDE4B-s, but with FUS sites deleted
16 from the surrounding introns) and subcloned into pLVX-puro vector (Addgene), then
17 packaged lentivirus. RIC8A K415R was also subcloned into
18 pAdEasy-EF1-MCS-CMV-GFP vector and packaged adenovirus (Hanbio Co. Ltd,
19 Shanghai, China). For recombinant adenovirus sh hsa/mmu circPDE4B and hsa/mmu
20 universal shRIC8A construction, oligonucleotides with gene targeting sequences (table
21 S3) were used for the cloning of small hairpin RNA (shRNA)-encoding sequences into
22 pAdEasy-U6-CMV-EGFP vector (Hanbio Co. Ltd, Shanghai, China). For lentivirus
23 shFUS construction, oligonucleotides with gene targeting sequences (table S3) were used
24 for the cloning of small hairpin RNA (shRNA)-encoding sequences into pKLO.1 vector
25 (addgene) and then packaged. HA-ubiquitin (Ubc) lentivirus and control virus were
26 purchased from Hanbio Co. Ltd, Shanghai, China.

1

2 Chondrocyte culture

3 Human chondrocytes (HCs) were extracted from knee cartilage of amputees or subjects
4 after total knee replacement, and mouse chondrocytes (MCs) were obtained from knee
5 cartilage of 3-day-old C57BL/6. Chondrocytes were cultured in Dulbecco's modified
6 eagle medium (DMEM) supplemented with 10% FBS (Thermo Fisher Scientific,
7 Waltham, MA, USA) within 24 h at 37 °C. Before culture or mRNA isolation, cells were
8 leached using the 0.075 mm filter and rinsed with sterile phosphate-buffered saline (PBS).
9 Primary chondrocytes with 70–80% fusion were used in subsequent experiments. While
10 culturing, cells were cultured in an environment with 5% CO₂ at 37 °C

11

12 Animal models

13 Adult male C57BL/6 mice (n = 40), eight weeks of age, were used for *in vivo*
14 experiments. According to a previous study^{1,2}, medial meniscus destabilization (DMM)
15 surgery was performed to induce post-traumatic OA to form the positive control. We
16 randomly divided mice into four groups with ten mice per group: SHAM + vector, DMM
17 + vector, DMM + circPde4b, and DMM + circPde4b + RIC8A groups. Briefly, mice (n =
18 10 per group) were anesthetized, and their medial joint capsules were incised to expose
19 the medial meniscotibial ligament (MMTL). Subsequently, the MMTL was transected
20 with microsurgical scissors to release the ligament linked to the tibial plateau,
21 consequently destabilizing the medial meniscus. The joints were closed after being rinsed
22 with sterile saline. At the same time, the medial knee joint capsule was incised for sham
23 operation. HanBio (Shanghai, China) created and packaged adeno-associated virus (AAV)
24 vectors for circPde4b and RIC8A. One week after operation, a total of 10 µL
25 (approximately 1×10^{12} vg/mL) of vector AAV, circPde4b AAV, or a mixture including
26 5 µL circPde4b AAV and 5 µL RIC8A AAV was delivered intra-articularly into the knee

1 joints. Eight weeks later, knee pain was evaluated using a series of assessments³,
2 including a hot plate test, knee extension test, and electric shock stimulated treadmill test.
3 Briefly, in the 55 °C hot plate test, latency was recorded when the hind paw of the mouse
4 was lifting and/or flicking/licking. In the knee extension test, the number of vocalizations
5 that occurred during five extensions was recorded. For the electric shock stimulated
6 treadmill test, the number of electric shocks stimulating the mouse was recorded within 2
7 min at a speed of 50 m/min. Following the assessments, mice were sacrificed, and both
8 knee articular cartilages were harvested for histological analysis or gene expression
9 analyses. We conducted all animal experiments following the approval of the Institute of
10 Health Sciences Institutional Animal Care and Use Committee (Zhejiang, China).

11

12 **Immunofluorescence**

13 Human osteoarthritis chondrocytes were fixed in 4% paraformaldehyde for 30 min,
14 covered with PBS containing 0.25% Triton X-100 for 15 min, and sealed with 5% BSA
15 containing 0.25% Triton X-100 at RT for 30 min. Cells were then incubated with the
16 following primary antibodies: anti-MMP13 (18165-1-AP, proteintech, 1:50), anti-MMP3
17 (ab52915, abcam, 1:100), anti-COL2 (ab34712, abcam, 1:100), and anti-aggrecan
18 (13880-1-AP, proteintech, 1:50). After overnight incubation at 4 °C, the cells were
19 washed three times with PBS and incubated with goat anti-rabbit IgG (H&L) at room
20 temperature for 1 h, rinsed and the nuclei were restained with DAPI for 5 min. Target
21 proteins were visualized by fluorescence microscopy (Carl Zeiss, USA).

22

23 **Micro-CT analysis**

24 After fixation in 70% ethanol, all samples were scanned in a 16 mm scanning tube with
25 10.5 mm³ volume at 55,000 V, 180 mA, with a 115 min acquisition time. To avoid

1 dehydration, joints were wrapped in napkins dampened with PBS while scanning. The
2 data were analyzed using Skyscan software.

3

4 ***In vitro* transcription**

5 Biotin-labeled oligonucleotide probes targeting junction sites of circPDE4B were
6 synthesized (RiboBio, Guangzhou, China). Linear circPDE4B was in vitro transcribed
7 using Biotin RNA Labeling Mix (Roche) and T7 RNA polymerase, circularized using T4
8 RNA ligase I, treated with RNase R, and purified with RNeasy Mini kit (Qiagen Inc.).

9

10 **Histological analysis, scoring system and immunohistochemistry**

11 After fixing in 4% paraformaldehyde, paraffin was used to embed cartilage specimens.

12 All samples were cut into 5 µm sections, and each one-tenth was stained with 0.1%

13 Safranin O solution as well as 0.001% Fast Green solution (Sigma-Aldrich, St. Louis,

14 MO, USA). OARSI grade^{4,5} was used to grade the severity of cartilage degeneration by

15 two observers blinded to group-identifying information. For human cartilage, the ROI

16 was evaluated using OARSI, while the OA severity of mice cartilage was recorded with

17 the maximal score of observed cartilage.

18 For immunohistochemistry, the sections were incubated with primary antibodies at

19 4 °C overnight. Subsequently, the sections were incubated with secondary antibodies for

20 1 h (Beyotime Institute of Biotechnology, Inc., Jiangsu, China) at 25 °C. All positively

21 stained cells along the joint surface of each sample were counted in the femoral ankle and

22 tibial plateau region, and the proportion of positive cells was evaluated using Image-Pro

23 Plus 6.0⁶.

24

25 **RNA extraction and RT-qPCR analysis**

1 Total RNA was extracted using RNAEX reagent (Accurate Biotechnology, Hunan) from
2 cultured or isolated chondrocytes. Reverse transcription of mRNAs to cDNA was
3 performed using total RNA, with kits from Accurate Biotechnology (Hunan, China).
4 Specific circRNAs or mRNAs were quantified with SYBR® Green Premix Pro Taq HS
5 qPCR kit (Accurate Biotechnology, Hunan, China). The ABI 7500 Sequencing Detection
6 System (Applied Biosystems, Foster City, CA, USA) was employed for amplification. To
7 obtain circRNA, using 3 U/μg RNase R (Epicenter, San Diego, CA, USA) or not covered
8 total RNA at 37 °C within 20 min. The RNeasy MinElute cleanup kit (Qiagen) was then
9 used to purify RNA. Specific primers were designed to amplify circPDE4B. Agarose
10 gel electrophoresis and sequencing were used to detect amplification products. All
11 reactions were repeated three times. Housekeeping β-actin gene were used for
12 standardization. All primers are listed in table S3.

13

14 **Co-immunoprecipitation (co-IP)**

15 Co-IP was performed as previously described (Jiao et al, 2018; Li et al, 2018b), with
16 antibodies (1:100 dilution) specific for Myc (Abcam Inc), HA (Cell Signaling
17 Technology), FLAG (Abcam Inc), anti-Ubiquitin antibody (Abcam Inc), RIC8A (Abcam
18 Inc), or MID1 (Abcam Inc). Bead-bound proteins were released and analyzed by western
19 blot.

20

21 **RNA interference**

22 siRNA-mediated knockdown was used to inhibit circRNA expression. Three different
23 siRNAs (RiboBio, Guangzhou, China) were designed and examined for circPDE4B
24 knockdown efficiency. SiRNA sequences are listed table S3. A specific analog (RiboBio,
25 Guangzhou, China) was used to induce miRNA expression. Plasmid transfection was
26 performed using Lipofectamine 3000 transfection reagent (ThermoFisher). The

1 Lipofectamine RNAiMAX transfection reagent (ThermoFisher) was applied for siRNA
2 delivery.

3

4 **RNA binding protein immunoprecipitation**

5 RIP assay was performed using Magna RIPTM RNA Binding Protein
6 Immunoprecipitation kit (Millipore), with antibodies (1:100 dilution) specific for FUS
7 (Abcam Inc), Flag (Abcam Inc) or RIC8A (Abcam Inc). Co-precipitated RNAs were then
8 extracted with TRIzol, and the amount of circPDE4B in the eluate was determined by
9 RT-qPCR (table S3).

10

11 **RNA pull-down assay and Mass Spectrometry (MS)/ qRT-PCR**

12 The RNA pull-down kit (BersinBio) was used for the RNA pull-down assay. The assay is
13 based on circRNA hybridization with the target-specific biotinylated probe, followed by
14 pull-down, reverse transcription to cDNA for amplification through quantitative real-time
15 PCR (RT-qPCR), and sequencing. After washing with PBS, a total of 10^7 HCs were
16 exposed to ultraviolet irradiation at 254 nm, followed by lysis with 1 mL lysis buffer and
17 homogenized with a 0.4 mm injector. A biotinylated antisense probe (0.2 nmol), which
18 targets the adapter sequence, or Lac Z probes (control probes) were then added to the
19 circPDE4B-RAP mixture. Incubation at 65 °C for 10 mins was carried out, followed by
20 hybridization for 2 h at room temperature. Thereafter, 200 μ L of streptavidin-coated
21 magnetic beads was added. Washing followed to remove non-specific binding. Protein
22 digestion yielded peptide fragments, which were then dissolved and vortexed. We used a
23 loading tube to load the supernatants for MS identification. Probes are displayed in table
24 S3.

25

26 ***In vitro* binding assay**

1 For RIC8A GST pull-down assays, GST fusion proteins from *E. coli* BL21 cells were
2 purified overnight at 4 °C and fixed on glutathione Sepharose 4B columns. HEK-293T
3 cells transfected with Flag-MID1 were treated as indicated. Cells were then lysed in the
4 NETN buffer with the protease inhibitor and cultured with Sepharose, and immobilized
5 with indicated RIC8A GST protein and circPDE4B at 4 °C for 8 h. The
6 RIC8A/MID1-circPDE4B complex were pulled down using GST beads (Sigma). Protein
7 was detected by SDS-PAGE and western blot.

8

9 **Western blot**

10 SDS -PAGE separated total cellular protein, which was then transferred to an
11 Immobilon-P membrane (0.2 µm pore size, Millipore, Billerica, MA, USA). The
12 membranes were blocked and then incubated with primary antibodies (BD Biosciences,
13 San Jose, CA, USA) overnight at 4 °C, and subsequently incubated with secondary
14 antibodies for 1 h. β-actin served as the internal standard and Image J was used to
15 quantify blots intensities. The antibodies used were as follows: anti-MMP13 antibody
16 (1:1000, abcam, ab51072), anti-MMP3 antibody (1:1000, abcam, ab52915),
17 anti-ADAMTS4 antibody (1:1000, abcam, ab185722), anti-COL2 antibody (1:1000,
18 abcam, ab34712), anti-SOX9 antibody (1:1000, abcam, ab185966), anti-aggrecan
19 antibody (1:1000, abcam, ab3778), anti-RIC8A antibody (1:1000, abcam, ab194941),
20 anti-MID1 antibody (1:1000, abcam, ab70770), anti-HA antibody (1:2000, Cell Signaling
21 Technology, 4970s), anti-FLAG antibody (1:1000, abcam, ab205606), anti-Ubiquitin
22 antibody (1:1000, abcam, ab140601), anti-Myc antibody (1:1000, abcam, ab32) and
23 anti-β-actin antibody (1:2000, Cell Signaling Technology, 4970s).

24

25 **RNA fluorescent *in situ* hybridization (FISH)**

1 The cells were seeded in 12-well plates with sterile glass covers and allowed to culture
2 overnight. Cells were then fixed with PBS containing 37% formaldehyde for 15 min at
3 25 °C and dehydrated with 70% ethanol at 4 °C for 1 h. Slides were hybridized for 14–16
4 h at 37 °C. Cy3-labeled circPDE4B probes were constructed by RiboBio (Guangzhou,
5 China). Hybridization buffer for RNA FISH dissolved the probes at a concentration of 20
6 nM. Following overnight hybridization, the slides were washed with 10% formamide/2 ×
7 SSC at 37 °C on an oscillator for 30 min, and subsequently washed with PBS and 0.1%
8 (v/v) Tween 20 three times. Cells were then stained with Alexa Fluor 546-conjugated
9 streptavidin for 1 h at the same temperature as for IF. The cover glass was restained with
10 DAPI, fixed with ProLong Gold anti-fading reagents, and observed under a Nikon A1Si
11 Laser Scanning Confocal Microscope (Nikon Instruments Inc., Japan). For FISH of
12 samples from in vivo experiments, ahead of hybridization, we performed the biopsy at 37°
13 C under 0.8% pepsin processing. Slices were then deparaffinized, rehydrated, and
14 permeated for 30 min. The primers and probe sequences are shown in table S3.

15

16 **Patient and public involvement statement**

17 Patients or the public WERE NOT involved in the design, or conduct, or reporting, or
18 dissemination plans of our research

19

20 **Statistical analysis**

21 All statistical analyses were performed using SPSS v22.0. Data distribution was assessed
22 using the Shapiro-Wilk test. The Levene test was used to assess the equality of variances.
23 Statistical analysis was performed by unpaired two-tailed Student's t-test (normal
24 distribution and equal variances, using 95% confidence intervals for between-group
25 differences), Welch t-test (unequal variances), or Mann–Whitney U test (non-normal
26 distribution). Multiple group comparisons were performed by one-way analysis of

1 variance (normal distribution) or Kruskal–Wallis (non-normal distribution) test followed
 2 by Bonferroni or Dunn post hoc test, respectively. A P value < 0.05 was considered
 3 statistically significant.

4

5 **REFERENCES**

- 6 1. Glasson SS, Blanchet TJ, Morris EA. The surgical destabilization of the medial
 7 meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. *Osteoarthritis*
 8 *Cartilage* 2007;15(9):1061-9. doi: 10.1016/j.joca.2007.03.006
- 9 2. Chen D, Shen J, Zhao W, et al. Osteoarthritis: toward a comprehensive understanding
 10 of pathological mechanism. *Bone Res* 2017;5:16044. doi:
 11 10.1038/boneres.2016.44 [published Online First: 2017/02/06]
- 12 3. Piel MJ, Kroin JS, van Wijnen AJ, et al. Pain assessment in animal models of
 13 osteoarthritis. *Gene* 2014;537(2):184-8. doi: 10.1016/j.gene.2013.11.091
- 14 4. Glasson SS, Chambers MG, Van Den Berg WB, et al. The OARSI histopathology
 15 initiative - recommendations for histological assessments of osteoarthritis in the
 16 mouse. *Osteoarthritis Cartilage* 2010;18 Suppl 3:S17-23. doi:
 17 10.1016/j.joca.2010.05.025 [published Online First: 2010/10/01]
- 18 5. Pritzker KP, Gay S, Jimenez SA, et al. Osteoarthritis cartilage histopathology: grading
 19 and staging. *Osteoarthritis Cartilage* 2006;14(1):13-29. doi:
 20 10.1016/j.joca.2005.07.014
- 21 6. Chen P, Xia C, Mei S, et al. Intra-articular delivery of sinomenium encapsulated by
 22 chitosan microspheres and photo-crosslinked GelMA hydrogel ameliorates
 23 osteoarthritis by effectively regulating autophagy. *Biomaterials* 2016;81:1-13. doi:
 24 10.1016/j.biomaterials.2015.12.006 [published Online First: 2015/12/30]

25

26

27 **SUPPLEMENTARY TABLES**

28

29 **Table S1.** Top 50 differentially expressed circRNAs in control and OA tissues ranked by
 30 expression level.

id	log2FC(Con-trol/OA)	pval	regulate	significant	mean_Con	mean_OA
1:65913245 65918835	2.298017962	0.013420134	up	yes	71.57389833	14.47433303

10

2:223998115 224001922	2.017322781	0.037510887	up	yes	57.89743315	14.2263021
6:159682474 159688242	3.277353459	0.000604761	up	yes	38.51538065	3.882709433
7:84129123 8 4194668	4.416423377	0.000404841	up	yes	23.89613671	1.023738907
4:102304317 102315830	3.801482647	0.003360458	up	yes	21.73943548	1.4663222
2:223991803 224001922	2.334035326	0.013161838	up	yes	18.03811377	3.497306791
13:42953948 42970670	2.907556748	0.009637056	up	yes	16.17980657	2.069638833
5:65170617 6 5197151	4.154129743	0.015296915	up	yes	15.86773823	0.796862253
17:27765535 27766588	7.209593338	2.94E-10	up	yes	14.70143599	0
1:102979382 102989571	7.01468859	9.33E-11	up	yes	12.83098702	0
1:32948222 3 2949774	1.899306098	0.048974712	up	yes	7.811225125	2.020780085
8:17558295 1 7562110	5.830035008	7.60E-07	up	yes	7.605289257	0.035448292
19:45025329 45025737	2.667894244	0.014344495	up	yes	6.992268417	1.016012274
7:80761616 8 0789528	2.166891001	0.025288244	up	yes	6.740826549	1.423384001
19:6702127 6 702579	5.725610351	3.17E-08	up	yes	5.191520209	0
8:17543318 1 7543715	4.576294017	1.42E-05	up	yes	4.946360357	0.111532203
5:59180595 5 9215968	5.27607838	1.18E-07	up	yes	3.774876381	0
10:7243558 7 285954	1.954291245	0.049795018	up	yes	3.444247889	0.814584431
17:27768977 27769584	5.136722549	0.000186202	up	yes	3.418095033	0
20:54157169 54171670	4.243602481	8.27E-05	up	yes	3.089979272	0.068397727
13:42917541	3.094148388	0.011943706	up	yes	2.836359073	0.243856855

42970670						
7:84110470 8 4194668	4.361466685	0.000348323	up	yes	2.810081997	0.041570553
6:107503657 107533610	2.581974853	0.045526372	up	yes	2.492951616	0.333054478
20:18297985 18306393	4.687371106	0.002144755	up	yes	2.476554324	0
4:88475841 8 8479507	4.543128739	4.12E-06	up	yes	2.231406616	0
7:84046324 8 4060558	4.025668711	0.012672298	up	yes	2.160629655	0.038797737
7:90747681 9 0790652	4.475075842	3.16E-06	up	yes	2.123986063	0
1:27668226 2 7669346	3.850469968	0.001064668	up	yes	2.109467326	0.053172438
17:66513336 66514466	4.390178942	0.001302459	up	yes	1.996889514	0
18:6301903 6 312056	2.876917257	0.039095578	up	yes	1.93391171	0.176881352
1:186893011 186911389	3.106673582	0.00897813	up	yes	1.899981712	0.132179616
18:6263947 6 312056	3.497797506	0.00378226	up	yes	1.830524648	0.070896584
X:148661908 148662768	2.136810258	0.026904799	up	yes	1.829022055	0.338624828
3:112109543 112113145	3.347239351	0.003786519	up	yes	1.827445988	0.089392497
4:150467673 150491035	4.255549433	1.27E-05	up	yes	1.810064475	0
17:27778690 27779056	4.225978288	1.16E-05	up	yes	1.771312099	0
22:33761371 33765953	4.194653636	0.001595289	up	yes	1.731118987	0
17:80289659 80290728	2.289891436	0.029722666	up	yes	1.684744047	0.26496392
8:105419144 105561481	2.399627165	0.025199407	up	yes	1.584695203	0.219272552
19:45022053 45028992	3.28409336	0.006756638	up	yes	1.569980322	0.071435494

2:223912253 223918010	4.053146681	0.000134317	up	yes	1.560040669	0
13:42926336 42970670	2.181505031	0.034446179	up	yes	1.511971175	0.255352047
15:45476079 45476552	3.959830453	0.001227535	up	yes	1.456065035	0
1:46077515 4 6080750	3.950560938	8.63E-05	up	yes	1.446099152	0
5:65170617 6 5215487	3.94001855	6.71E-05	up	yes	1.434842326	0
5:65242104 6 5273447	2.83649554	0.031810662	up	yes	1.431521832	0.114413908
14:10035368 7 100361921	2.884414792	0.048709863	up	yes	1.424099749	0.106403957
17:80325030 80328477	3.013454971	0.006302126	up	yes	1.419825328	0.088214614
7:84110470 8 4134951	3.482653818	0.002962863	up	yes	1.414107802	0.035448292
1:102939035 102946956	2.892345557	0.03182733	up	yes	1.300464786	0.088620731

1

2 **Table S2.** Top 50 circPDE4B-binding proteins identified by mass spectrometry (ranked

3 by pep_score).

num	prot_acc	prot_desc	pep_s core
1	P07237	Protein disulfide-isomerase OS=Homo sapiens OX=9606 GN=P4HB PE=1 SV=3	118.07
2	Q9NPQ8	Synembryn-A OS=Homo sapiens OX=9606 GN=RIC8A PE=1 SV=3	96.92
3	P06733	Alpha-enolase OS=Homo sapiens OX=9606 GN=ENO1 PE=1 SV=2	95.8
4	Q16891	MICOS complex subunit MIC60 OS=Homo sapiens OX=9606 GN=IMMT PE=1 SV=1	95.59
5	P04259	Keratin, type II cytoskeletal 6B OS=Homo sapiens OX=9606 GN=KRT6B PE=1 SV=5	92.43

13

6	P67936	Tropomyosin alpha-4 chain OS=Homo sapiens OX=9606 GN=TPM4 PE=1 SV=3	90.97
7	P08133	Annexin A6 OS=Homo sapiens OX=9606 GN=ANXA6 PE=1 SV=3	87.9
8	Q6WCQ1	Myosin phosphatase Rho-interacting protein OS=Homo sapiens OX=9606 GN=MPRIIP PE=1 SV=3	84.9
9	P27348	14-3-3 protein theta OS=Homo sapiens OX=9606 GN=YWHAQ PE=1 SV=1	83.69
10	O00458	Interferon-related developmental regulator 1 OS=Homo sapiens OX=9606 GN=IFRD1 PE=1 SV=4	82.38
11	P04181	Ornithine aminotransferase, mitochondrial OS=Homo sapiens OX=9606 GN=OAT PE=1 SV=1	81.54
12	Q9UNL2	Translocon-associated protein subunit gamma OS=Homo sapiens OX=9606 GN=SSR3 PE=1 SV=1	80.32
13	P10809	60 kDa heat shock protein, mitochondrial OS=Homo sapiens OX=9606 GN=HSPD1 PE=1 SV=2	77.24
14	Q15084	Protein disulfide-isomerase A6 OS=Homo sapiens OX=9606 GN=PDIA6 PE=1 SV=1	72.89
15	Q7Z406	Myosin-14 OS=Homo sapiens OX=9606 GN=MYH14 PE=1 SV=2	71.8
16	E9PRG8	Uncharacterized protein C11orf98 OS=Homo sapiens OX=9606 GN=C11orf98 PE=4 SV=1	71.22
17	P68871	Hemoglobin subunit beta OS=Homo sapiens OX=9606 GN=HBB PE=1 SV=2	67.41
18	P83731	60S ribosomal protein L24 OS=Homo sapiens OX=9606 GN=RPL24 PE=1 SV=1	66.31
19	P21333	Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4	66.06
20	P21127	Cyclin-dependent kinase 11B OS=Homo sapiens OX=9606	65.88

		GN=CDK11B PE=1 SV=4	
21	P48444	Coatomer subunit delta OS=Homo sapiens OX=9606 GN=ARCN1 PE=1 SV=1	64.61
22	Q12965	Unconventional myosin-Ie OS=Homo sapiens OX=9606 GN=MYO1E PE=1 SV=2	62.35
23	P42766	60S ribosomal protein L35 OS=Homo sapiens OX=9606 GN=RPL35 PE=1 SV=2	61.42
24	P05783	Keratin, type I cytoskeletal 18 OS=Homo sapiens OX=9606 GN=KRT18 PE=1 SV=2	60.52
25	P05783	Keratin, type I cytoskeletal 18 OS=Homo sapiens OX=9606 GN=KRT18 PE=1 SV=2	60.52
26	P10412	Histone H1.4 OS=Homo sapiens OX=9606 GN=HIST1H1E PE=1 SV=2	58.75
27	P43246	DNA mismatch repair protein Msh2 OS=Homo sapiens OX=9606 GN=MSH2 PE=1 SV=1	57.6
28	O43707	Alpha-actinin-4 OS=Homo sapiens OX=9606 GN=ACTN4 PE=1 SV=2	57.2
29	P08865	40S ribosomal protein SA OS=Homo sapiens OX=9606 GN=RPSA PE=1 SV=4	56.12
30	Q9NX58	Cell growth-regulating nucleolar protein OS=Homo sapiens OX=9606 GN=LYAR PE=1 SV=2	55.3
31	P06396	Gelsolin OS=Homo sapiens OX=9606 GN=GSN PE=1 SV=1	55.09
32	Q9Y4I1	Unconventional myosin-Va OS=Homo sapiens OX=9606 GN=MYO5A PE=1 SV=2	54.65
33	P62937	Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens OX=9606 GN=PPIA PE=1 SV=2	54
34	Q15233	Non-POU domain-containing octamer-binding protein OS=Homo sapiens OX=9606 GN=NONO PE=1 SV=4	51.87

35	Q7KZF4	Staphylococcal nuclease domain-containing protein 1 OS=Homo sapiens OX=9606 GN=SND1 PE=1 SV=1	51.86
36	Q5BJD5	Transmembrane protein 41B OS=Homo sapiens OX=9606 GN=TMEM41B PE=1 SV=1	50.71
37	P13796	Plastin-2 OS=Homo sapiens OX=9606 GN=LCP1 PE=1 SV=6	48.92
38	Q02809	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1 OS=Homo sapiens OX=9606 GN=PLOD1 PE=1 SV=2	48.83
39	P47755	F-actin-capping protein subunit alpha-2 OS=Homo sapiens OX=9606 GN=CAPZA2 PE=1 SV=3	47.71
40	P62701	40S ribosomal protein S4, X isoform OS=Homo sapiens OX=9606 GN=RPS4X PE=1 SV=2	46.2
41	Q96D15	Reticulocalbin-3 OS=Homo sapiens OX=9606 GN=RCN3 PE=1 SV=1	46.01
42	P62241	40S ribosomal protein S8 OS=Homo sapiens OX=9606 GN=RPS8 PE=1 SV=2	45.16
43	P27797	Calreticulin OS=Homo sapiens OX=9606 GN=CALR PE=1 SV=1	44.29
44	P31946	14-3-3 protein beta/alpha OS=Homo sapiens OX=9606 GN=YWHAB PE=1 SV=3	42.45
45	P04844	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 OS=Homo sapiens OX=9606 GN=RPN2 PE=1 SV=3	42.04
46	P46777	60S ribosomal protein L5 OS=Homo sapiens OX=9606 GN=RPL5 PE=1 SV=3	41.71
47	P14618	Pyruvate kinase PKM OS=Homo sapiens OX=9606 GN=PKM PE=1 SV=4	41.29
48	P60866	40S ribosomal protein S20 OS=Homo sapiens OX=9606 GN=RPS20 PE=1 SV=1	40.91
49	Q9Y281	Cofilin-2 OS=Homo sapiens OX=9606 GN=CFL2 PE=1 SV=1	40.89

50	Q9UN86	Ras GTPase-activating protein-binding protein 2 OS=Homo sapiens OX=9606 GN=G3BP2 PE=1 SV=2	40.52
----	--------	---	-------

1

2 **Table S3.** Primers and sequences used in this study.

Primers for qPCR		
PDE4B mRNA (hsa)	F	CACACTTGGGATCGACCTCT
	R	GGAAGCGTTGTCAAAGGCAG
circPDE4B (hsa)	F	CAGAGTGAAAGGGCAAGGACT
	R	CCGTCATCACACTCCTGCTTT
Pde4b mRNA (mmu)	F	TGCGCTTGGAACTTGAGCTT
	R	GCCGCTGTCGGATGCTTTTA
circPde4b (mmu)	F	CTGTGAGCCAGGAGTGGTCTA
	R	GGTGTAAGTGAAGAGCTGTAGGAT
Human β -actin	F	AGAGCTACGAGCTGCCTGAC
	R	AGCACTGTGTTGGCGTACAG
Mouse β -actin	F	AGCCATGTACGTAGCCATCC
	R	CTCTCAGCTGTGGTGGTGAA
Human MMP3	F	CCTACAAGGAGGCAGGCAAG
	R	CCCGTCACCTCCAATCCAAG
Human MMP13	F	TCGGCCACTCCTTAGGTCTT
	R	AAGTGGCTTTTGCCGGTGTA
Human COL2A1	F	TCCTGCCGTTTCGCTG
	R	CATTATACCTCTGCCCATCCTG
Human ADAMTS4	F	GTCCCATGTGCAACGTCAAG
	R	ATGCGGCCATCTTGTTCATCT
Human ADAMTS5	F	GGCACTGGCTACTATGTGG
	R	CGTCACAGCCAGTTCTCACA
Human Aggrecan	F	GGGACCTGCAAGGAGACAGAG
	R	TCAATCTCACACAGGTCCCCTTC
Human SOX9	F	GCTCTGGAGACTTCTGAACGA
	R	CCGTTCTTACCAGACTTCTCT
Human RIC8A	F	GCTCTGCGGTCATACAACCAG
	R	GTCTCTCCGGTCTCTCTGTT
Mouse RIC8A	F	AGATGCGGTGACAGAAGCTC
	R	CACGGTGCTTTGGTGACAAG
Mouse MMP13	F	CAAGCAGTTCCAAAGGCTACA

17

	R	TAGGGCTGGGTCACACTTCT
Mouse MMP3	F	ACTGTGTCCCAAGGAGAGGAG
	R	AAACCATCTACACAGTTCAGACAC
Mouse COL2A1	F	CACGCATGAGCCGAAGCTA
	R	GGGTTTCCACGTCTACCA
Mouse ADAMTS4	F	TTGTTCTCCCAGTCACCCTCC
	R	AGCCTGGGACTAAAGATAGGCA
Mouse ADAMTS5	F	ATGCAGCCATCCTGTTCACC
	R	AAGGCCAAGTAGATGCCCAATTT
Mouse Aggrecan	F	CACTGTCAAAGCACCATGCC
	R	TAGGCTGGCTCCCATTCAGT
Mouse SOX9	F	TAATTCCCCAGGCTCTTGAT
	R	GCAGCCGGGATTTAAGGCTC
Human MID1	F	CTGATCCGAGGCAAAGAGCC
	R	CAGGCACTCGTCACAGTAGG
Human FUS	F	TTATGGCCAGAGCCAGAACAC
	R	GCTACCGTAACTTCCCGAGG
Human DHX9	F	GTACGGCCTGGATTCTGCTT
	R	ATCACAGCATCCAAAGGGGG
Primers for Site-Directed Mutagenesis		
RIC8A K143R	F	ctctgtgagcctcaccactagggggc
	R	gcccgcctagtgtgaggtcacagag
RIC8A K187R	F	gcgactctctcagctctgaaacagctg
	R	cagctgttcaggagctgagaggagtgcgc
RIC8A K237R	F	acctccccctgatggagtccaggggtg
	R	caccctggactccatcaggggggaggt
RIC8A K285R	F	gagaacatccagacacctgaggggcaagtccc
	R	gggaactgcccctcaggtgtctgtgtctc
RIC8A K319R	F	tgtgcaaacgcctctctaggaagatgaggggg
	R	ccctctcatcttctagagagggcgttgcaca
RIC8A K328R	F	ctacactctccctcagcctgtgtcttctgtgca
	R	tgcacaagacacacaggctgagggagagtgtag
RIC8A K415R	F	cccatagcctgtgtacctgatgaatcggggcac
	R	gtgccccgattcatcaggtacacaggctatggg
RIC8A K456R	F	tttatgctggctctgctctctgtactcatctgtg
	R	cacagatgagtacaaggaagccagagccagcataaa
RIC8A K469R	F	ttagggcctctctccaccctcccg
	R	cgggagggtggaggagagggccgctaa

Primers for PCR		
Divergent- β -actin	F	CAGGGCTTACCTGTACTACTGA
	R	GCGCGGCGATATCATCATCC
β -actin	F	AGAGCTACGAGCTGCCTGAC
	R	AGCACTGTGTTGGCGTACAG
CircPDE4B	F	CAGAGTGAAAGGGCAAGGACT
	R	CCGTCATCACACTCCTGCTTT
mPDE4B	F	CACACTTGGGATCGACCTCT
	R	GGAAGCGTTGTCAAAGGCAG
Divergent- β -actin (Mouse)	F	GGCCTGTACTACTGACTTGAGA
	R	AAGGAGCTGCAAAGAAGCTGT
β -actin (Mouse)	F	AGCCATGTACGTAGCCATCC
	R	CTCTCAGCTGTGGTGGTGAA
CircPDE4B (Mouse)	F	CTGTGAGCCAGGAGTGGTCTA
	R	GGTGTAAGTGAAGAGCTGTAGGAT
PDE4B (Mouse)	F	TGCGCTTGAACTTGAGCTT
	R	GCCGCTGTCGGATGCTTTTA
ShRNA		
CircPDE4B shRNA-1	F	CCGGCCAGGAGTGGTATTA AAAACTCGAGTTTTTAAT ACCACTCCTGGTTTTTC
	R	AATTGAAAAACCAGGAGTGGTATTA AAAACTCGAGTT TTTAATACCACTCCTGG
CircPDE4B shRNA-2	F	CCGGGGAGTGGTATTA AAAAAGTGCTCGAGCACTTTTT AATACCACTCCTTTTTTC
	R	AATTGAAAAAGGAGTGGTATTA AAAAAGTGCTCGAGCA CTTTTTAATACCACTCC
RIC8A shRNA-1	F	CCGGACCGCACAGAGGAGTTCCA CTCTCGAGTGGA ACTC CTCTGTGCGGTTTTTTTC
	R	AATTGAAAAAGGAGTGGTATTA AAAAAGTGCTCGAGCA CTTTTTAATACCACTCC
RIC8A shRNA-2	F	CCGGCATGTTTGACAAGCTCTCCCTCGAGGGAGAGCT TGTC AAACATGTTTTTC
	R	AATTGAAAAACATGTTTGACAAGCTCTCCCTCGAGGG AGAGCTTGTC AAACATG
MID1 shRNA-1	F	CCGGACCGCATCCTAGTATCACACTCGAGTGTGATAC TAGGATGCGGTTTTTTTC
	R	AATTGAAAAAACCGCATCCTAGTATCACACTCGAGTG TGATACTAGGATGCGGT

MID1 shRNA-2	F	CCGGGCAACGTCACCCTACAGAACTCGAGTTCTGTAG GGTGACGTGCTTTTTTC
	R	AATTGAAAAAGCAACGTCACCCTACAGAACTCGAGTT CTGTAGGGTGACGTTGC
FUS shRNA-1	F	CCGGCAAGCAGATTGGTATTATTCTCGAGAATAATAC CAATCTGCTTGTTTTTTC
	R	AATTGAAAAACAAGCAGATTGGTATTATTCTCGAGAA TAATACCAATCTGCTTG
FUS shRNA-2	F	CCGGCAGAGTTACAGTGGTTATACTCGAGTATAACCA CTGTAACCTCTG TTTTTTC
	R	AATTGAAAAACAGAGTTACAGTGGTTATACTCGAGTA TAACCACTGTAACCTCTG
Mmu CircPDE4B shRNA-1	F	CCGGGGAGTGGTCTAATCTGCCACTCGAGTGGCAGAT TAGACCACTCCTTTTTTC
	R	AATTGAAAAAGGAGTGGTCTAATCTGCCACTCGAGTG GCAGATTAGACCACTCC
Mmu CircPDE4B shRNA-2	F	CCGGGCCAGGAGTGGTCTAATCTCTCGAGAGATTAGA CCACTCCTGGCTTTTTTC
	R	AATTGAAAAAGCCAGGAGTGGTCTAATCTCTCGAGAG ATTAGACCACTCCTGGC
SiRNAs		
Scramble si		UUCUCCGAACGUGUCACGUTT
Human CircPDE4B si-1		AGCCAGGAGTGGTATTA AAA
Human CircPDE4B si-2		CCAGGAGTGGTATTA AAAAA
Human CircPDE4B si-3		GGAGTGGTATTA AAAAAAGTG
Mouse CircPDE4B si-1		GGAGTGGTCTAATCTGCCA
Mouse CircPDE4B si-2		GCCAGGAGTGGTCTAATCT
Mouse CircPDE4B si-3		TGAGCCAGGAGTGGTCTAA
FUS si-1		CAAGCAGATTGGTATTATT
FUS si-2		CAGAGTTACAGTGGTTATA
DHX9 si-1		CGAACACCATTGCATGAAA
DHX9 si-2		GGACTAGTAGCAACATTGA
Human/Mouse RIC8A si-1		ACCGCACAGAGGAGTTCCA
Human/Mouse RIC8A si-2		CATGTTTGACAAGCTCTCC
Human KRT6B si		TACCATCAAGTCAACAGTTATCA
Human ENO1 si		GCATTGGAGCAGAGGTTTA

Human IMMT si	GGGTTGACTACTGGCAAAATTGC
Human P4HB si	GTCCTCTTTAAGAAGTTTGATGA
Probes for FISH	
Cy3-Hsa circPDE4B	5'-Cy3-ACACTTTTAAATACCACTCCTGGCTTACAG-3'
Cy3- Mmu circPDE4B	5'-CY3-GGCAGATTAGACCACTCCTGGCTCAC-3'
RAP and PCR sequences	
CircPDE4B pull-down probe	CATTGCTATTACAACCTGTAAGCCAGGAGTGGTATTA AAAA GTGTCAGCAAACCTGCATTGAATAACAGACATCCTAAGAGG GGATATTTTCCACCTCTATAATGAAGAAAAGCAGGAGTGT GATGACGGTGATGGCTGATGATAATGTTAAAGATTATTT GAATGTAGCTTGAGTAAATCCTACAGTTCTTCCAGTAACA CACTTGGGATCGACCTCTGGAGAGGGAGAAGGTGTTGCTC AGGAAACTTACAGTTACCACCACTGTCTCAAAGACAGAGT GAAAGGGCAAGGACTCCTGAGGGAGATGGTATTTCCAGGC CGACCACACTGCCTTTGACAACGCTTCCAAGCATTGCTAT TACAACCTGTAAGCCAGGAGTG
Lac Z pull-down probes	TGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGG CGTTACCCAACCTTAATCGCCTTGACGACATCCCCCTTC GCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCC CTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCT GATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCA CACCGCATATGGTGCCTCTCAGTACAATCTGCTCTGATG CCGCATAG

1