Supplemental Tables

Table S1. List of primary antibodies used for immunohistochemistry, FACS, and immunofluorescence.

Name of antibodies	Manufacturer	Catalog Number
Rabbit anti-PDYN	Bioss USA	bs-13041R
Rabbit anti-KOR-1	Assay Biotech	G491
Mouse anti-CD105	AbCam	ab11414
Rabbit anti-CD146	AbCam	ab75769
Mouse anti-Collagen I	AbCam	ab90395
Mouse anti-Collagen II	AbCam	ab3092
Rabbit anti-MMP3	Abgent	AP13536a
Rabbit anti-MMP13	Abgent	AP13706c
Rabbit anti-ADAMTS4	Abgent	AP7439b
Rabbit anti-ADAMTS5	Abgent	AP7447c
Goat anti-Prg4	Everest	EB08496
Rabbit anti-CREBpS133	Abgent	AP3077a
Mouse IgG Isotype Control	Tonbo Biosciences	70-4714
Rabbit IgG Isotype control	AbCam	ab199376
Mouse anti-CD31	Biolegend	303116
Mouse anti-CD45	BD Biosciences	555485
Mouse anti-CD34	BD Biosciences	555822
Mouse anti-CD146	BD Biosciences	562135

Table S2. List of primer sequences used for quantitative RT-PCR. Bp=base pairs.

Gene Name	Primer Sequence	Product size (bp)	GenBank Accession
β-2 microglobulin (B2M)	forward: 5' gaggctatccagcgtactcca 3' reverse: 5'cggcaggcatactcatctttt3'	248	NM_004048
Ribosomal protein L7 (RPL7)	forward: 5' caaggcttcgattaacatgctga 3' reverse: 5'gccataaccacgcttgtagatt 3'	103	NM_000971
TATA Box Binding Protein (TBP)	forward: 5' tgcacaggagccaagagtgaa 3' reverse: 5' cacatcacagctccccacca 3'		NM_003194
Prodynorphin (PDYN)	forward: 5' tgtgctgtaaagacccaggat3' reverse: 5' tctctcccattcctcagaggg 3'	99	NM_001190892
Proteoglycan 4 (PRG4)	forward: 5' aggccccatgtgttcatgc 3' reverse: 5' gcgcaaagtagtcagtccatct 3'	147	NM_001127710
Matrix metallopeptidase 3 (MMP3)	forward: 5' agtcttccaatcctactgttgct 3' reverse: 5' tccccgtcacctccaatcc 3'	226	NM_002422
Matrix metallopeptidase 13 (MMP13)	forward: 5' actgagaggctccgagaaatg 3' reverse: 5' gaaccccgcatcttggctt 3'	103	NM_002427
ADAM metallopeptidase with thrombospondin type 1 motif, 4 (ADAMTS4)	forward: 5' gaggaggagatcgtgtttcca 3' reverse: 5' ccagctctagtagcagcgtc 3'	118	NM_005099
ADAM metallopeptidase with thrombospondin type 1 motif, 5 (ADAMTS5)	forward: 5' gaacatcgaccaactctactccg 3' reverse: 5' caatgcccaccgaaccatct 3'	107	NM_007038
Xylosyltransferase I (XYLT1)	forward: 5' gaaagtgcgaacagacagcaa3' reverse: 5' gagtcctgggtgcgaagttg 3'	86	NM_022166
Beta 1,4-galactosyltransferase, polypeptide 7 (B4GALT7)	forward: 5'atccggcaccacatctacg 3' reverse: 5' caacgtcgtgcatggcaat 3'	124	NM_001497

Beta 1,3-galactosyltransferase polypeptide 6 (B3GALT6)	forward: 5' cgcgcttcgacaccgaata 3' reverse: 5' cgtacacgtaggacaggcg n3'	159	NM_080605
Beta-1,3-glucuronyltransferase 3 (B3GAT3)	forward: 5' aaggagtcgtctactttgctga 3' reverse: 5' gggcattgggcttatctaacag 3'	249	NM_012200
UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1 (GALNT1)	forward: 5' cacctccttgatttgggtactc3' reverse: 5'aggaatgacgactggtttccc 3'	169	NM_020474
protein kinase cAMP-activated catalytic subunit alpha (PRKACA)	Forward: 5' accctgaatgaaaagcgcatc 3' Reverse: 5' cgtaggtgtgagaacatctccc 3'		
protein kinase cAMP-activated catalytic subunit beta (PRKACB)	Forward: 5' ccatgcacggttctatgcag 3' Reverse: 5' gtctgtgacctggatatagcctt 3'		

Supplemental Figures

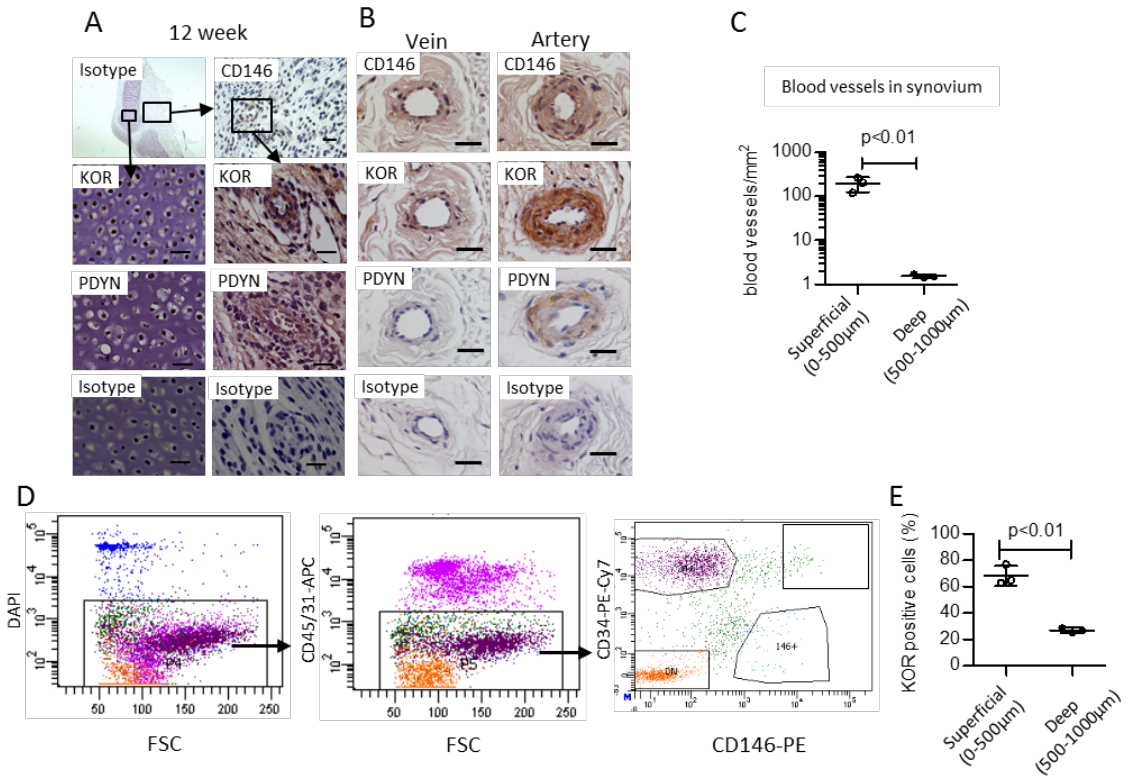

Figure S1. Expression pattern of PDYN and KOR in fetal and adult human cartilage and synovial tissue. (A) Expression of PDYN and KOR in human limbs at 12 weeks of development. Bar = 20μm. Boxes in upper images indicate relative positions of lower 6 images in a developing femur. (B) PDYN staining in adult synovial membrane of knee joint. Bar = 20μm. (C) Quantification of blood vessels in the superficial (0-500μm from synovial surface) and the deep layer (500-1000 μm from synovial surface) of synovial membrane. Mean ± S.D. (n=3). (D) FACS isolation of four distinct mesenchymal cell populations from synovial tissues. (E) Quantification of KOR positive chondrocyes in adult cartilage. Mean ± S.D. (n=3). P values were calculated by Student's t-test. Images in panel A, B and D were representative data from 3 independent experiments (three different tissue donors).

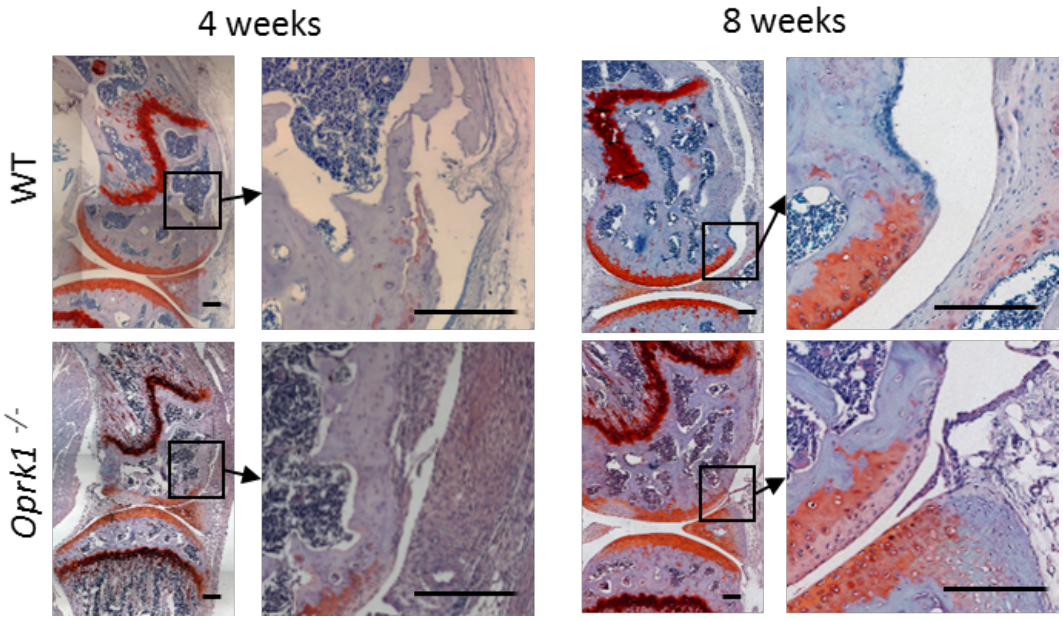

Figure S2. Defects on the trochlear groove are shown on sagittal section of a mouse knee joint, 4 weeks or 8 weeks post-injury. Bar = 200μm. Six mice were examined. Representative images of Safranin O were shown.

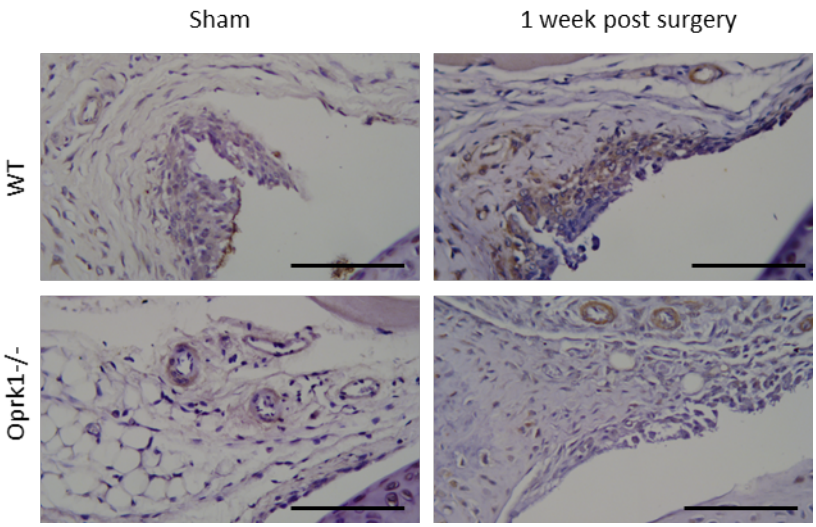

Figure S3. Immunohistochemical staining for PDYN was performed on sagittal section of mouse knee joint 1 week post-injury. Bar=100μm. Six mice were examined. Representative images were shown.

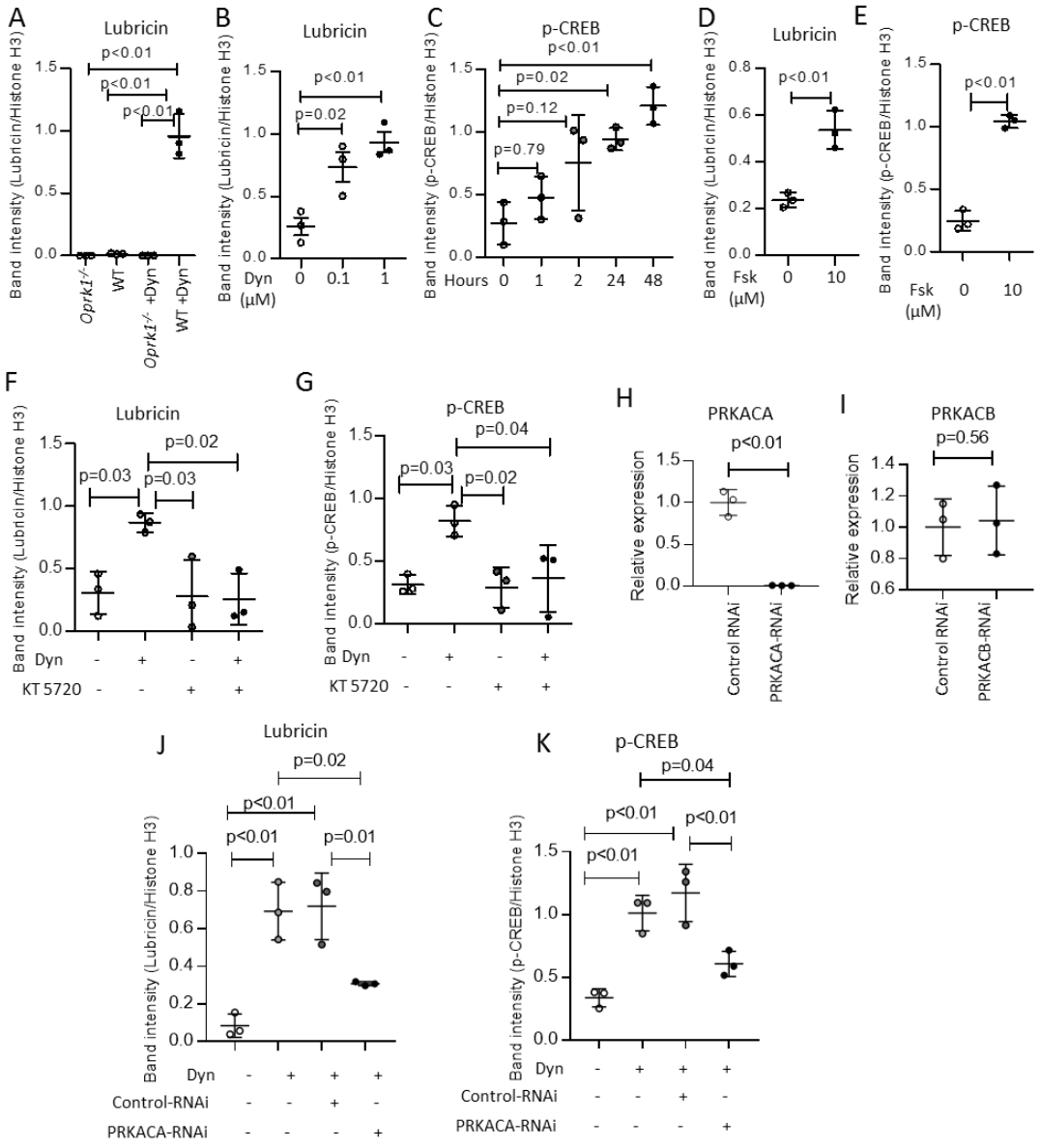

Figure S4. Quantification of lubricin and phospho-CREB expression by Western Blot. (A) Rib chondrocytes from *Oprk1*$^{-/-}$ show no upregulation of lubricin expression in response to dynorphin A; while WT chondrocytes highly upregulate the levels of lubricin after the exposure to dynorphin A. Mean ± S.D. (n=3). (B) Fetal human chondrocytes treated with dynorphin A for 24 hours. The expression levels of lubricin were normalized to the levels of Histone H3 (house keeping gene). Mean ± S.D. (n=3). (C) Adult chondrocytes treated with 1μM of dynorphin A for 0, 1, 2, 24 and 48 hours. Mean ± S.D. (n=3). (D-E) Chondrocytes were treated with forskolin for 24 hours. The expression level of lubricin and phosphorylation of CREB were quantified. Mean ± S.D. (n=3). (F-G) Chondrodytes were treated with Dynorphin and PKA inhibitor KT 5720 for 24 hours. The expression level of lubricin and phosphorylation of CREB were quantified. Mean ±

S.D. (n=3). Each dot represents one sample from one tissue donor. (H) Quantitative PCR confirmed significant reduction of the expression of PKA catalytic subunit A (*PRKACA*) by small interference RNA (RNAi). (I) Expression of PKA catalytic subunit B (*PRKACB*) was not repressed by small interference RNA. (J-K) Chondrocytes were transfected with small interference RNA for 72 hours, then treated with Dynorphin (1μM) for 24 hours. The expression levels of lubricin and phosphorylation of CREB were quantified using Image J software. Mean ± S.D. (n=3). Each dot represents one sample from one tissue donor. P values in panel A, B, C, F, G, J and K were calculated with one way ANOVA followed by Tukey HSD Post-hoc Test, while p values in panel D, E, H and I were calculated with student's t-test.

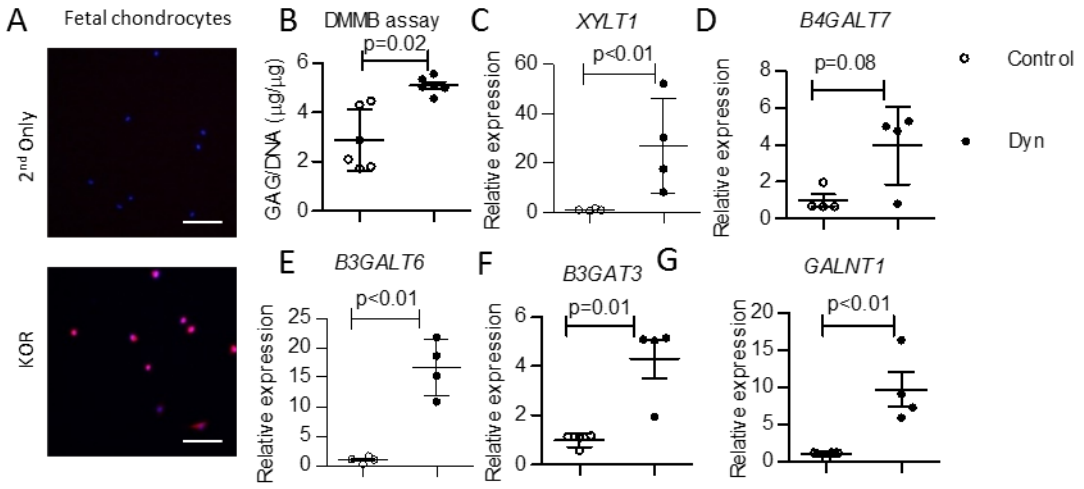

Figure S5. Dynorphin A increases the expression levels of GAG-synthesizing enzymes in chondrocytes. (A) Immunofluorescent staining confirms the expression of KOR on fetal human chondrocytes at passage 1 ex vivo. Representative images were from a total of 4 donors. Bar = 20μm. (B) GAG levels were measured in the medium conditioned with chondrocytes in the presence or absence of 1μM of dynorphin A. Mean ± S.D. Four donors were tested. Data six samples from one representative donor was shown. Each dot represents the average of three readings from each sample. (C-G) Dynorphin A induces mRNA expression of the genes involved in chondroitin sulfate biosynthesis. Mean ± S.D. (n=4). Each dot represents the average of six samples from one tissue donor. P values are calculated with Student's t-test.

Uncropped immunoblots used in original figures

Fig. 5C

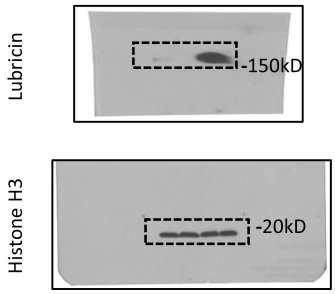

Fig. 5D

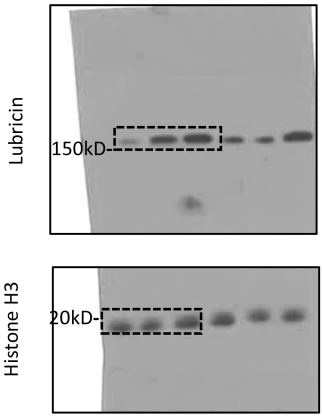

Fig. 5F

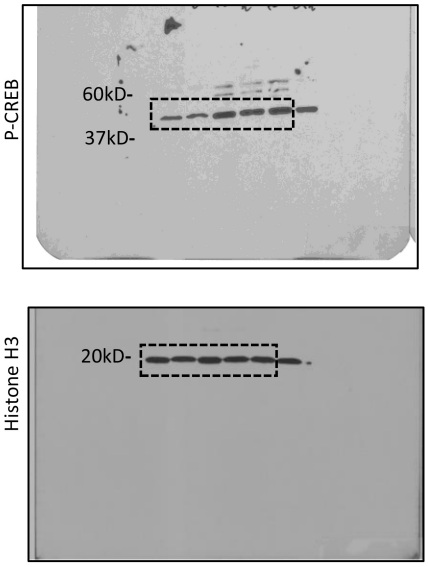

Fig. 5H

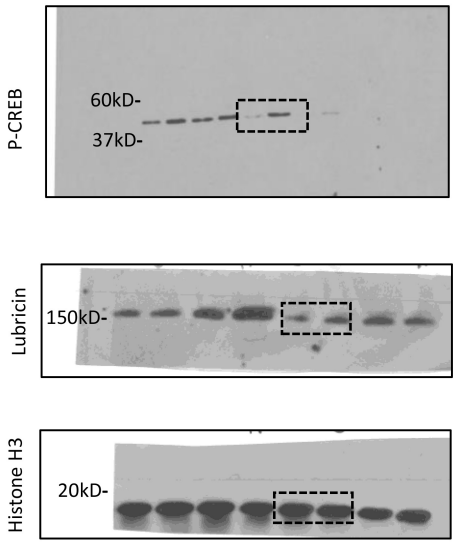

Fig. 5I

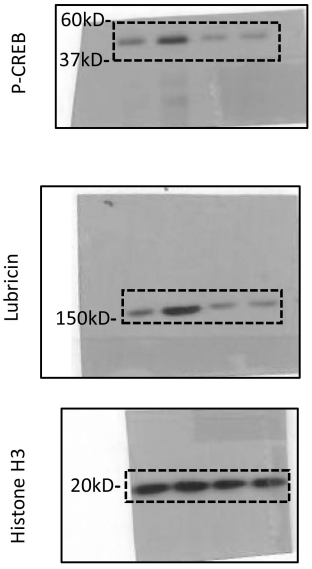

Fig. 5J