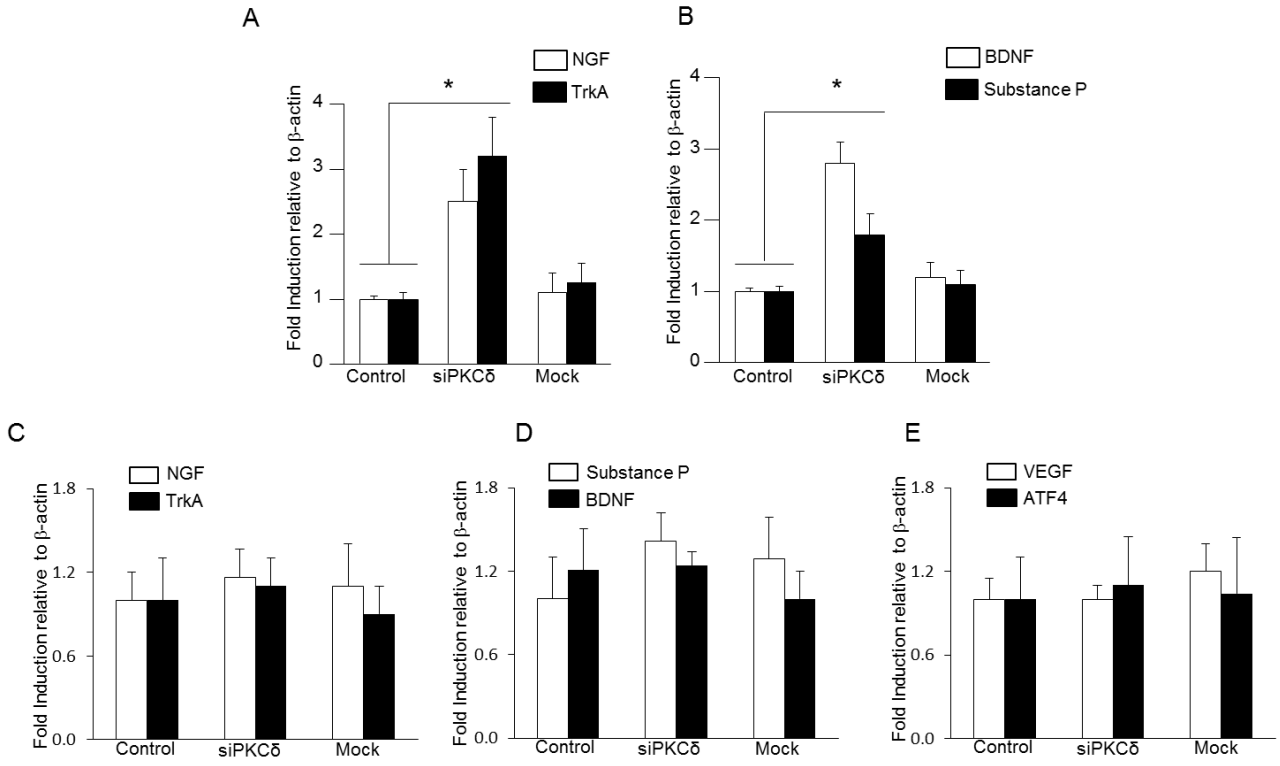
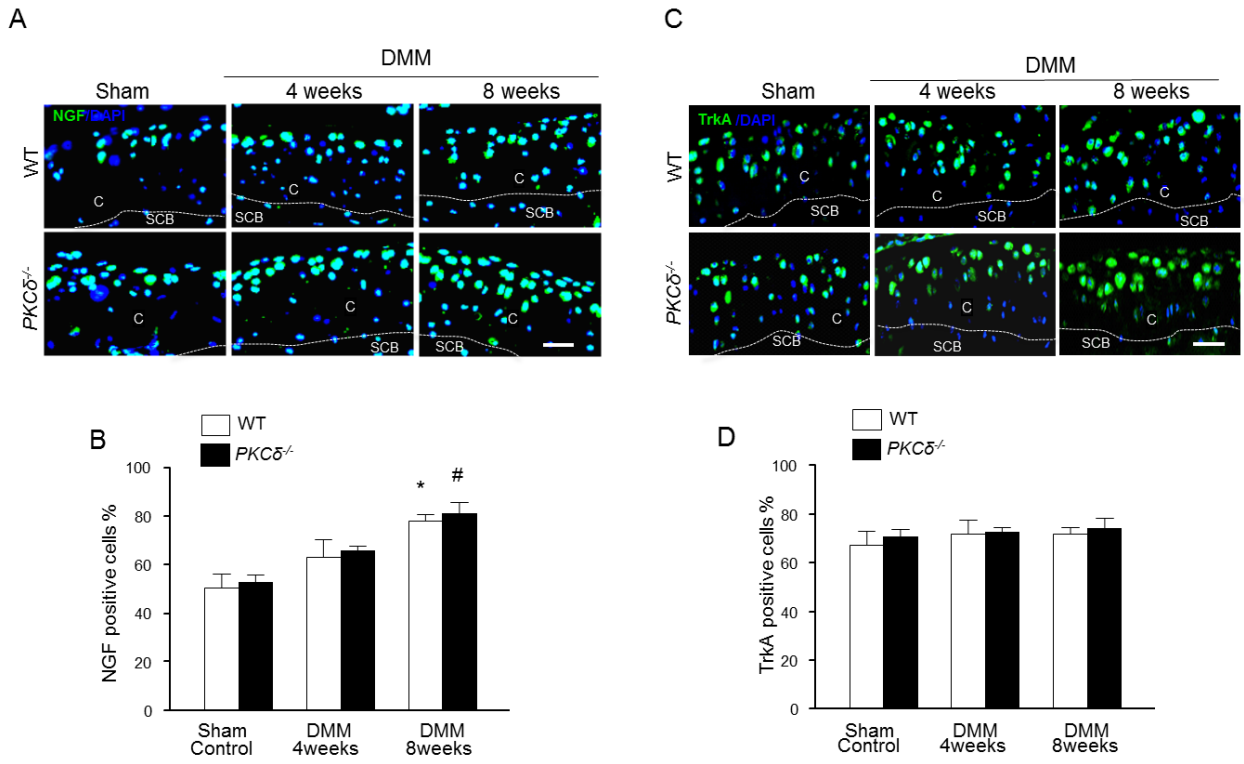
SUPPLEMENTARY FIGURES AND TABLES

Supplementary Figure S1



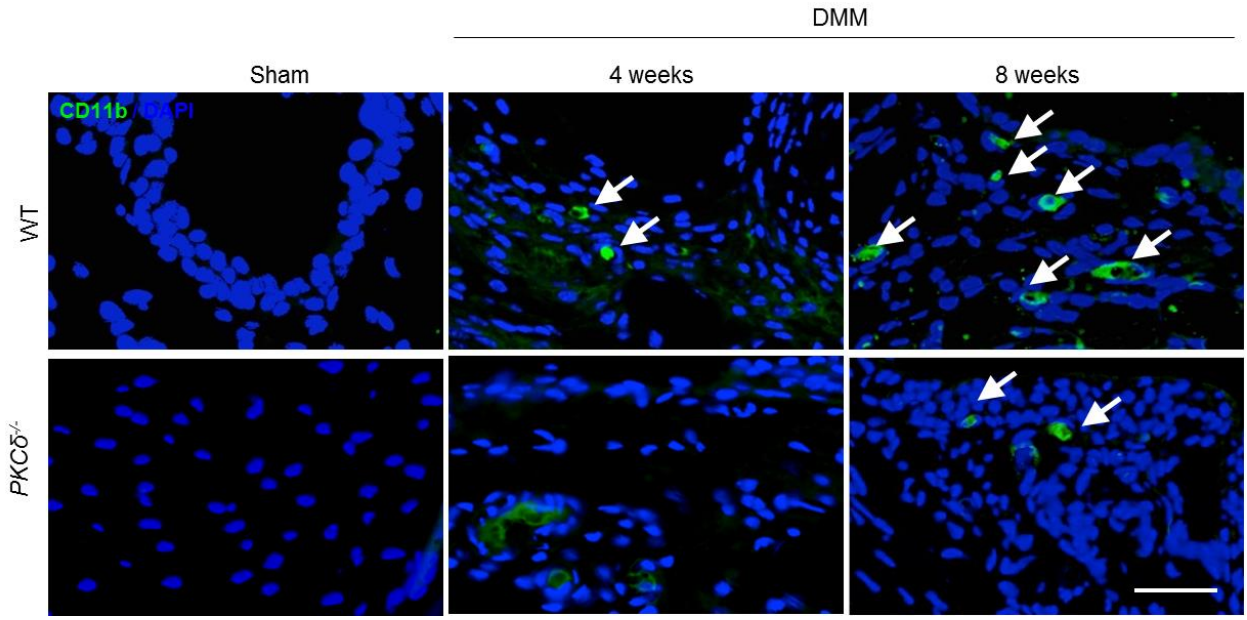
Supplementary figure S1. qPCR analyses in human fibroblast-like synovial cells and chondrocytes. (**A,B**) mRNA levels were quantified by qPCR in human fibroblast-like synovial cells after transfection with siRNA that targets PKCδ. The knockdown of PKCδ upregulates mRNA levels of NGF/TrkA and BDNF/substance P (P <0.05). Values are presented as mean±SD. (**C-E**) Human chondrocytes were transfected with siRNA that targets PKCδ. There were no changes in mRNA levels of NGF/TrkA (**C**), Substance P/BDNF (**D**) and VEGF/ATF4 (**E**) after knockdown of PKCδ in human chondrocytes.

Supplementary Figure S2



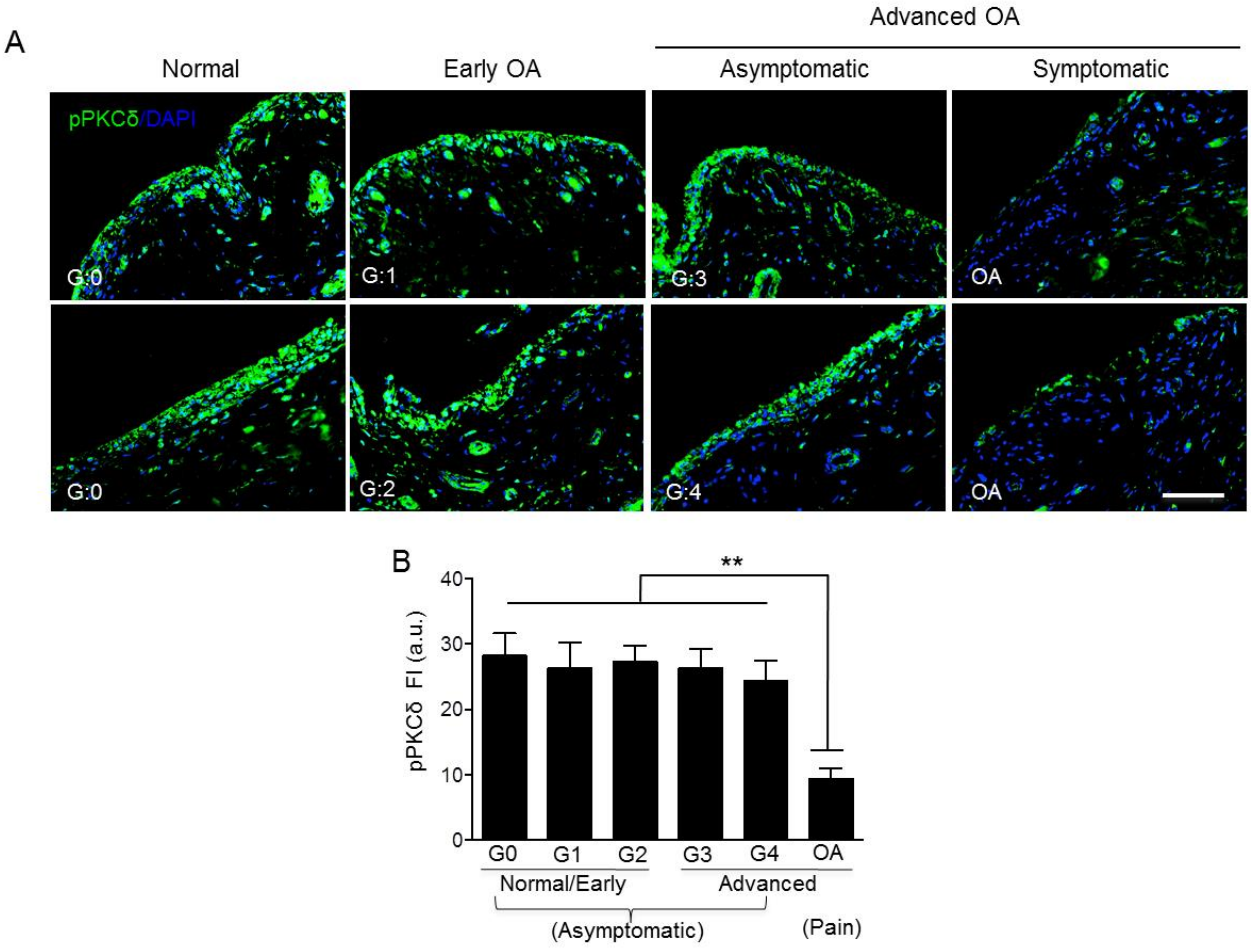
Supplementary figure S2. NGF and TrkA expression in articular cartilage after DMM. Representative immunofluorescence images of NGF (green) (**a**) and TrkA (**b**) expression in the articular cartilage of *PKCδ*$^{-/-}$ and WT mice. DAPI stains nuclei (blue). C = Cartilage; SCB = Subchondral bone. Scale bars, 50 μm, n=5 each group. (**c,d**) Quantitative analyses of NGF and TrkA expression in cartilage. Values are presented as mean ± SD (Compared between WT sham and DMM *, *P* <0.05; Compared between *PKCδ*$^{-/-}$ mice sham and DMM #, *P* <0.05)

Supplementary Figure S3



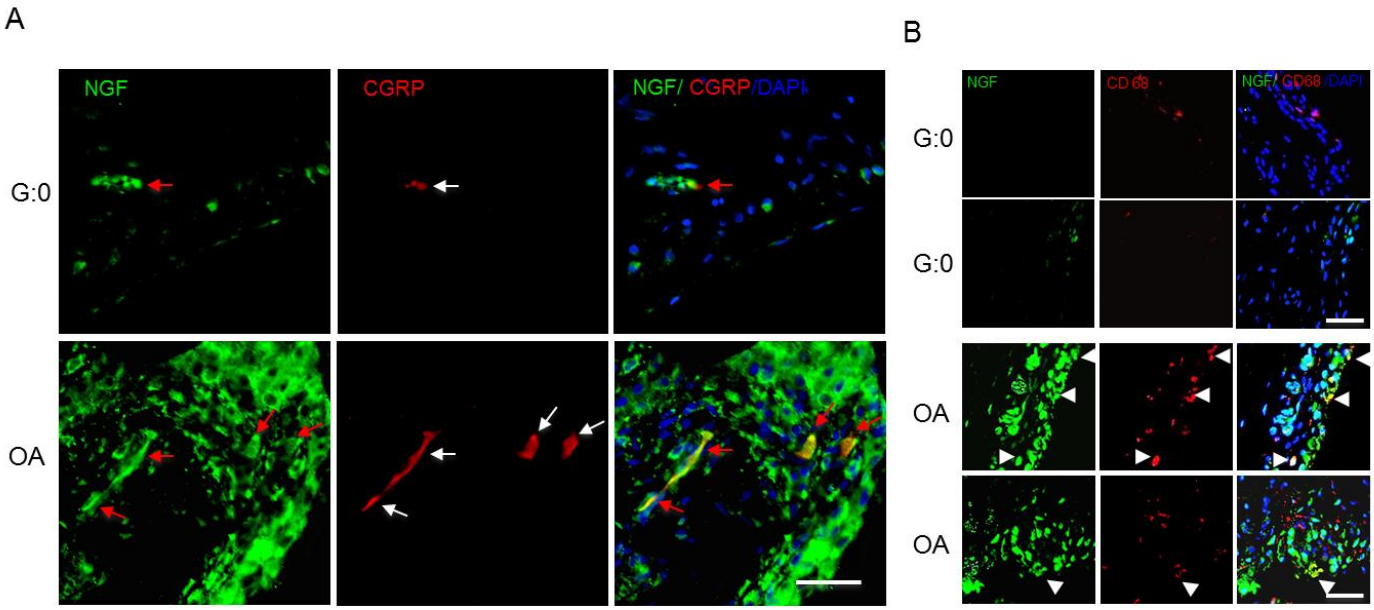
Supplementary figure S3. Decreased macrophage infiltration in knee joint synovium of *PKCδ*$^{-/-}$ mice after DMM. The ipsilateral knee joints of the sham and the DMM surgery groups were immunostained for CD11b, a marker for macrophage activation, at 4 and 8 wks post-DMM. Representative fluorescence images indicate progressively increased macrophage activation (green) in WT after DMM, compared to *PKCδ*$^{-/-}$ mice. Arrows indicate CD11b^{+} cells. DAPI stains nuclei (blue). Scale bars, 50 μm, n=5 per group.

Supplementary Figure S4



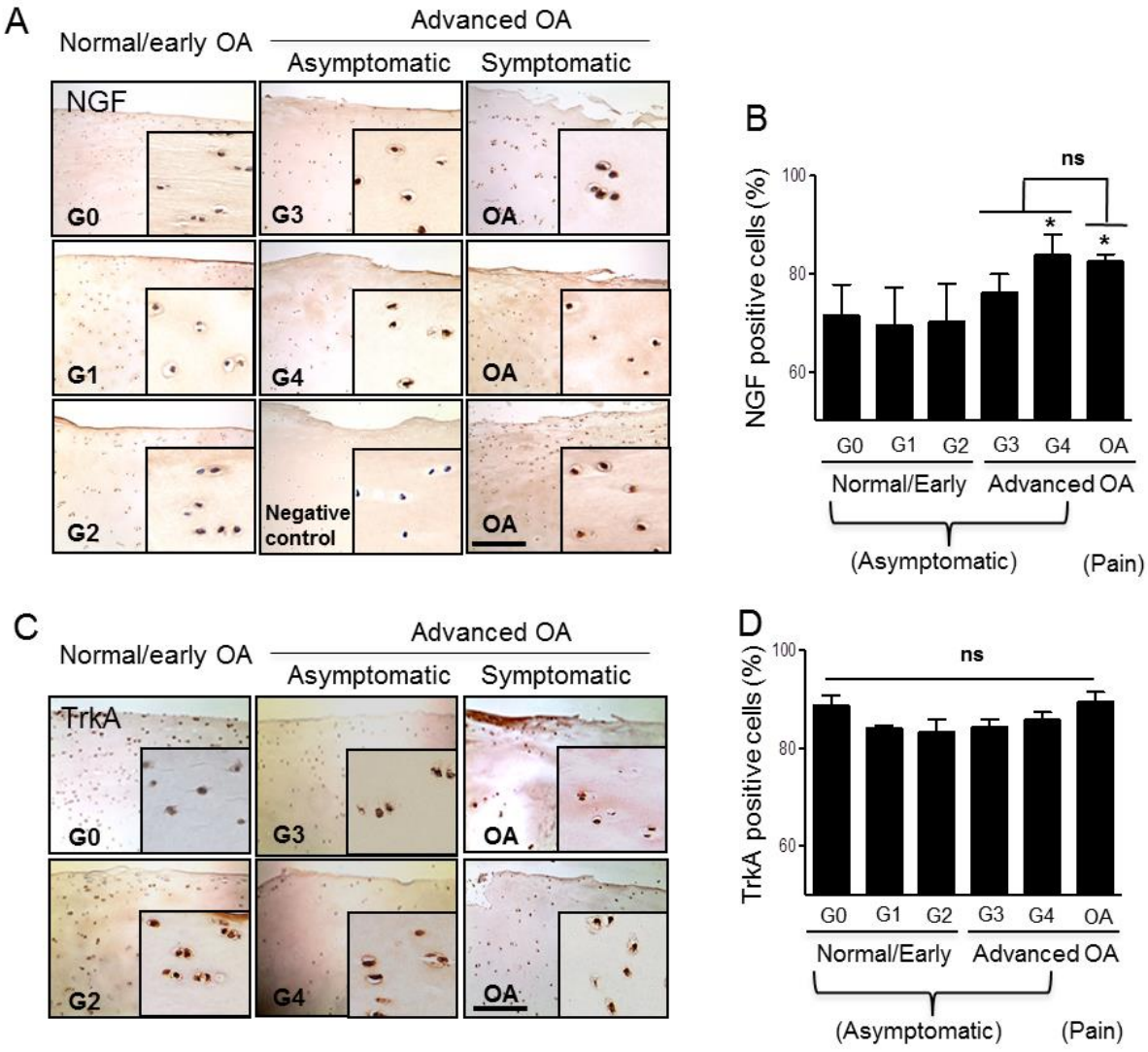
Supplementary figure S4. Activity of PKCδ in human knee joint synovium. (**A**) Representative images of phospho-PKCδ (Ser645) expression in human joint synovium. (**B**) Quantitative analysis of phospho-PKCδ (Ser645) expression in human synovium. Values are presented as mean±SD (Compared between asymptomatic and symptomatic group: **, *P* <0.01. DAPI stains nuclei blue. G= OA Grade. Scale bars, 50μm. FI=fluorescence intensity; a.u.=arbitrary unit.

Supplementary Figure S5



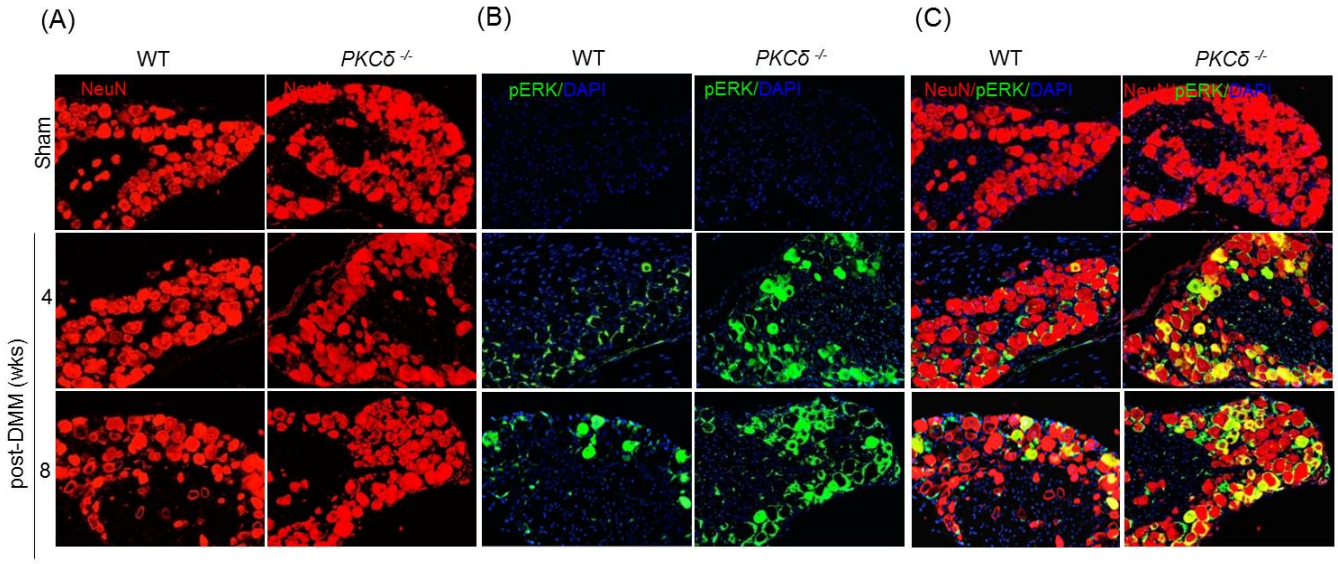
Supplementary figure S5. Expression of NGF in human knee joint synovium. (**A**) Double immunofluorescence staining of NGF (green) and CGRP (red) in human knee joint synovium. Arrows indicate co-localization of NGF with CGRP+ peptidergic sensory nerve fibers. (**B**) Double immunofluorescence staining of NGF (green) and CD68 (red) in human knee joint synovium. Arrowheads indicate few macrophages expressing NGF. Scale bars, 50 μm, n=6 per group. DAPI stains nuclei (blue).

Supplementary Figure S6



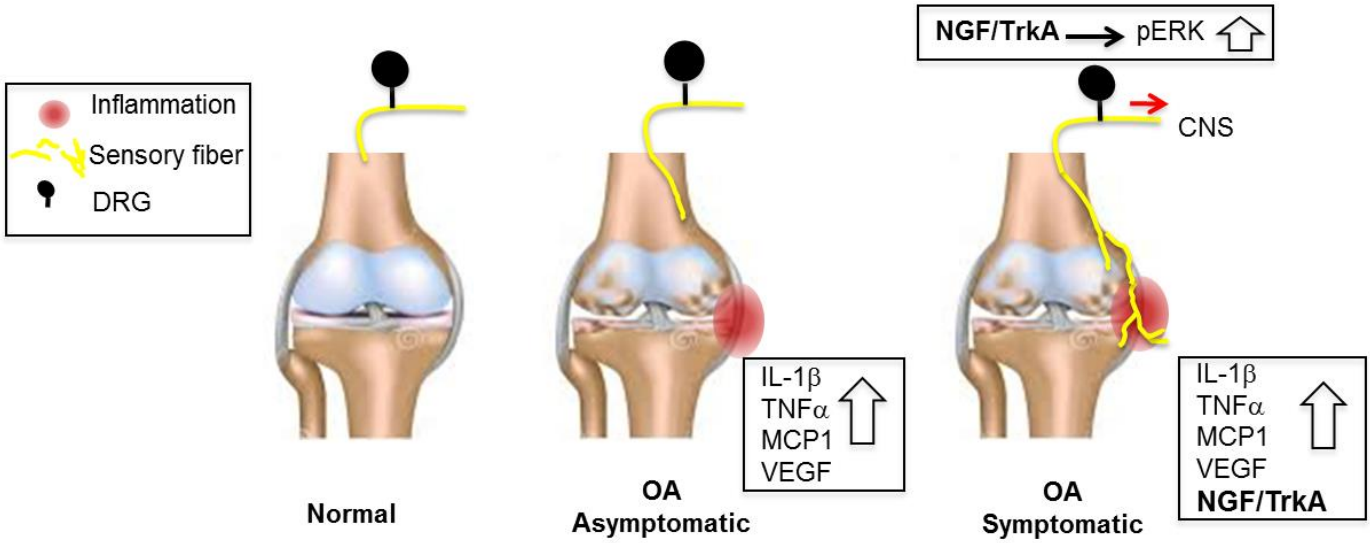
Supplementary figure S6. Expression of NGF and TrkA in human adult articular cartilage. Human knee joint cartilage tissues from asymptomatic organ donors (G:0-G:4) and age-matched symptomatic OA patients (surgically removed tissues) were immunostained for NGF and TrkA. (**A**) Representative images of NGF expression in human articular cartilage. (**C**) Expression of TrkA in human articular cartilage. Positive cells stain brown. Nuclei are counterstained with hematoxylin (blue). Scale bars, 50 μm, n=6 per group. (**B,D**) Histomorphometric analyses of NGF and TrkA expression in human articular cartilage. Values are mean ± SD (Compared with Normal *, *P* <0.05).

Supplementary Figure S7



Supplementary figure S7. Increased ERK activation in DRG neurons of *PKCδ*$^{-/-}$ mice after DMM. (**A-C**) Double immunofluorescence staining of NeuN (red) **(A)**, and phospho-ERK (p-ERK, Thr202/Tyr204) (green) **(B),** in DRGs of *PKCδ*$^{-/-}$ and WT mice. (**C**) White arrows indicate p-ERK co-localized DRG neurons. Note that satellite cells (blue arrows) also express p-ERK during OA progression. (n=5 per group). All scale bars, 100μm.

Supplementary Figure S8



Supplementary figure S8. Schematic diagram of knee OA joint pain progression between asymptomatic and symptomatic conditions. Normal knee joints contain few sensory neurons. In asymptomatic OA, levels of inflammatory cytokines and chemokines and angiogenic factors, but not NGF, are significantly increased in joint synovium. In the lack of afferent sensory neuronal fibers that can sense the inflammatory signal by "nerve firing," the OA condition will be asymptomatic. Heavily innervated synovial joints are seen in symptomatic OA joint tissues. The upregulated NGF within the synovial joint can be transported retrogradely to DRG neurons through TrkA present on afferent sensory nerve terminals; this, in turn further promotes axonal outgrowth. NGF activation of ERK/MAPK may play a role in centralization of OA pain. (modified from www.depositphotos.com)

Supplementary Table S1. mRNA expressions of inflammatory cytokines/receptors and matrix degrading enzymes[#]

	WT with DMM (wk)			*PKCδ* KO with DMM (wk)		
	Sham	**4**	**8**	**Sham**	**4**	**8**
			Inflammatory cytokines/receptors			
TNFα	1	2.5**	3.7**	1	1	0.7
IL-1β	1	3**	2.5**	1	1.2	0.8
IL-6	1	2.8**	3*	1	0.8	1
NFκB	1	1.7*	2.2*	1	1	1.2
TLR-2	1	1	3.8**	1	1.2	1.3
TLR-4	1	2.2**	3.5**	1	1.3	1.4
			Matrix degrading enzymes			
MMP13	1	4.5**	2.1*	1	1	0.7
MMP3	1	14**	13**	1	8**	3.5**
MMP2	1	3.4**	1.8*	1	3.8**	1.4
MMP9	1	2.8**	2.9**	1	2.5**	1.9*
ADAMTS4	1	3.1**	2.9**	1	1	0.5
ADAMTS5	1	2.5**	1.8*	1	0.8	0.5

qPCR analysis using ipsilateral knee joint extraction of *PKCδ*$^{-/-}$ and age-, gender-matched WT mice at 4wk and 8 wk post-DMM. (Compared with sham control: *, $p<0.05$; **, $p<0.01$, each group: n=6). No significant difference between WT and *PKCδ*$^{-/-}$ was detected in the basal level of the inflammatory cytokines and enzymes.

Supplementary Table 2. Human tissues

Collin's Grade(cartilage degradation score)	Asymptomatic			OA with pain
	G:0	G:1/2	G:3/4	G:3/4
Numbers	6	11	10	17
Mean Age (min/max)	34 (26/52)	51 (36/73)	63 (47/80)	63 (52/82)
Gender (M/F)	2/4	5/6	6/4	9/8

Note: G0, perfect cartilage; G1~G2, early OA; G3~G4, advanced OA