SUPPLEMENTARY MATERIAL

Supplementary Methods:

Antibody details and immunohistochemistry technique.

Anti-iGluR2 recognises the phosphorylation site of serine 880 in the GluR2 protein (I-E-S-V-K). This amino acid sequence is present in both human and rat GluR2 protein. Anti-KA1 recognises a sequence within the last 50 amino acids of the human KA1 protein (exact sequence is unknown as it is proprietary knowledge). The manufactures state that this antibody also reacts with rat KA1, which shares 98% homology with the last 50 amino acids of the human KA1 amino acid sequence.

Anti-iGluR2 was used at 10µg/ml and anti-KA1 at 2.5µg/ml in human and rat immunohistochemistry. For human samples, antigen retrieval was carried out using 1mg/ml trypsin for 20 minutes at 37ºC followed by 1000U/ml hyaluronidase for 30 minutes at 37ºC. For rat samples, 1mg/ml trypsin for 20 minutes at 37ºC was used.

For all samples the following method was used:
Sections were deparaffinised and rehydrated prior to antigen retrieval (as stated above). Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide (Sigma) for 30 minutes followed by 3 washes in 1XTBS/0.1% tween for 15 minutes each. Sections were subsequently treated with 10% normal blocking serum (Sigma) for 1 hour prior to overnight incubation at 4ºC with primary antibody, followed by 3 washes in 1XTBS/0.1% tween. Immunostaining was detected using the rabbit VECTASTAIN ELITE ABC horseradish peroxidase kit (Vector Laboratories). Biotinylated secondary antibody was applied and incubated for 30 minutes followed by three 5 minute 1XTBS washes. Avidin-biotin complex (ABC) was added for 30

minutes followed by three 5 minute 1XTBS washes. Sections were developed using nickel enhanced diaminobenzidine (DAB) (Vector Laboratories), counterstained in Mayer's haematoxylin (Fisher Scientific), and blued in tap water. Sections were finally dehydrated, cleared in xylene and mounted. Slides were viewed on a Leica DMRB microscope. All incubations were at room temperature unless otherwise stated.

TRAP staining.

Samples were TRAP stained as previously described (33). Sections were deparaffinised, rehydrated and incubated in pre-warmed acetate-tartrate buffer (0.1M sodium tartrate (Sigma) in 0.2M acetate buffer (Sigma) (pH5.2)) at 37ºC for 5 min, followed by a 30 minute incubation at 37ºC in 20 mg/ml naphthol AS-BI phosphate (Sigma)/dimethylformamide (Fisher Scientific) in acetate-tartrate buffer. The sections were then incubated for 15 minutes at 37ºC in acetate-tartrate buffer hexazotised pararosaniline solution, rinsed twice in water, and counterstained in Mayer's haematoxylin. Sections were finally dehydrated, cleared in xylene and mounted. Slides were viewed on a Leica DMRB microscope.

Osteoblast assays.

Human primary osteoblast (HOB) cultures were grown from bone explants obtained during TKR for OA. HOBs were maintained in basal medium [minimal essential medium alpha (α-MEM) without ribonucleosides and deoxyribonucleosides, 10% foetal calf serum, penicillin (50 U/ml) and streptomycin (50 µg/ml) (Life Technologies Ltd). Assays were done between passage 2 and 3.

To determine NBQX effect on cell number, HOBs were seeded in 96 well plates ($1.5x10^2$) for 24 hours before incubation with basal medium +/- NBQX (200 µM) for 2 and 5 days (media replenished after 2 days). Cells were incubated in fresh basal medium containing MTS (CellTiter 96®, Promega) for 2 hours, before measuring absorbance at 490 nm. Assays were repeated in cells from 3 patients, 12 replicates per patient.

To determine NBQX effect on mineralisation, HOBS were seeded in 24 well plates (20×10^3 cells per well) and after 24 hours, ascorbate-2-phosphate (50 µg/ml), β-gycerolphosphate (2mM) and dexamethasone (10^{-7}M) added +/- NBQX (200 µM). Treatments were replenished twice weekly. After 20–23 days, mineralised matrix was stained with Alizarin Red S (37). Assays were repeated in cells from 3 patients, 4 replicates per patient.

Supplementary Table 1.

Human synovial inflammation scoring system (30).

Grade	Inflammation	Tissue appearance
0	Normal tissue displaying no signs of inflammation.	-Synovial lining is less than 4 cells thick. -Sparse cellular distribution. -Few or no inflammatory cells present.
1	Mild inflammation.	-Synovial lining is 4 or 5 cells thick. -Increased cellularity. -Some inflammatory cells present.
2	Moderate inflammation.	-Synovial lining is 6 or 7 cells thick. -Dense cellularity. -Inflammatory cells present. -No lymphoid aggregates.
3	Severe inflammation.	-Synovial lining is greater than 7 cells thick. -Dense cellularity. -Dense inflammatory cell infiltration. -May contain perivascular lymphoid aggregates.

Supplementary Table 2.

Rat synovial inflammation scoring system (31).

Component	Sub-component	Grade
Sub-synovial inflammation	Normal (adipose tissue appears normal, no infiltrate).	0
	Focal inflammatory infiltrates, adiposity hardly affected (10% inflammatory cells, 90% adipose tissue).	1
	Random inflammatory infiltrate equals adiposity (50% inflammatory cells, 50% adipose tissue).	2
	Inflammatory infiltrate dominating tissue (70% inflammatory cells, 30% adipose tissue).	3
	Substantial infiltrate with severe loss of adiposity (90% inflammatory cells, 10% adipose tissue).	4
	Ablation of adiposity due to infiltrate (100% inflammatory cells, 0% adipose tissue).	5
Synovial exudate	Normal	0
	Evidence of inflammatory cells in joint space.	1
	Moderate numbers of inflammatory cells in joint space, possibly with evidence of fibrin deposits.	2
	Substantial numbers of inflammatory cells with large fibrin deposits.	3
Synovial hyperplasia and pannus formation	Normal, 1-3 layers thick.	0
	Over three layers thick.	1
	Over three layers thick with some	2

	overgrowth over joint surfaces.	
	Over three layers thick with overgrowth over joint surfaces and evidence of cartilage loss.	3
Total score		0-14

Supplementary Table 3.

Human OA joint degradation scoring system based on the Mankin score (32, 62).

Component	Sub-component	Grade
Cartilage surface integrity	Normal	0
	Surface irregularities	1
	Pannus and surface irregularities	2
	≥2 clefts to transitional zone	3
	≥2 clefts to radial zone	4
	≥2 clefts to calcified zone	5
	Complete disorganisation	6
Chondrocyte appearance	Normal	0
	Diffuse hypercellularity	1
	Cloning*	2
	Hypocellularity	3
Proteoglycan loss	Normal	0
	Slight reduction	1
	Moderate reduction	2
	Severe reduction	3
	No dye noted	4
Tidemark integrity	Intact	0
	Crossed by blood vessels	1
Total score		0-14

* A clone is accepted if there are ≥5 nuclei present in one chondron. A sample must contain a minimum of 5 clones to be accepted as a grade 2 for chondrocyte appearance (cloning).

Supplementary Table 4.

Joint degradation scoring system. A literature search revealed no suitable single scoring system to accurately score joint degradation observed in our AIA rat model. Bone changes were often ignored. We therefore incorporated a simple subchondral bone scoring paradigm (63) into a previously used modified Mankin score (32).

Component	Sub-component	Grade
Cartilage surface integrity	Normal	0
	Surface irregularities	1
	Pannus and surface irregularities	2
	Clefts to middle zone	3
	Clefts to deep zone	4
	Clefts to calcified zone	5
	Complete disorganisation	6
Chondrocyte appearance	Normal	0
	Irregular cell organisation	1
	Cellular organisation highly varied. -e.g. highly cellular/acellular	2
Proteoglycan loss	Normal	0
	Slight reduction	1
	Moderate reduction	2

	Severe reduction	3
	No dye noted	4
Tidemark integrity	Intact	0
	Tip of bone or vessel touching	1
	Significant contact/breach	2
Bone changes	None	0
	Mild	1
	Moderate	2
	Severe	3
TOTAL		0-17

Supplementary Table 5. GluR, IL6 and bone marker primers and cycling conditions for QRT-PCR.

Gene (Protein)	Primers (5'-3')	Annealing temp. (ºC)	Amplicon size	Primer conc. (µm)	$MgCl_2$ conc. (mM)
Gria1	F-CGAGTTCTGCTACAAATCCCG	61	91bp	0.2	2.5
(AMPAR1)	R-TGTCCGTATGGCTTCATTGATG				
Gria2	F-GGAAGTAAGGAAAAGACCAGTGCCCTC	60	85bp	0.2	3.5
(AMPAR2)	R-TTGCCAAACCAAGGCCCCCG				
Gria3	F-GGCAGGAAAAGCGATACTTG	60	116bp	0.2	2.5
(AMPAR3)	R-CCAGGTTAGCGAGCATGTAG				
Grik1	F-TGAGCAGTGTCTCTCTTTCAATGCC	62	145bp	0.1	2.5
(GluR5)	R-TCTCTGAGTTCGTCTCTGGTGACAA				
Grik2	F-AAACCCTGGCGCTTCGGGAC	62	180bp	0.1	3.5
(GluR6)	R-GCCACTGGCTGGATCCCACG				
Grik3	F-CGCTTCGGTGGCCGCTTCAT	64	151bp	0.1	2.5
(GluR7)	R-CCCCGACCTTCTCGAGGCCA				
Grik4	F-GAACTTGGGATGGTGTCAGC	64	135bp	0.1	3.5
(KA1)	R-AGAAAGCATGGGATTGGTTG				
Grik5	F-GCCCTCCGTCCCACCAGGAT	62	137bp	0.1	2.5
(KA2)	R-GACAGCACCTGGCAGCTGGG				
Grin1	F-CCGGGTCATCATCCTTTCT	59	180bp	0.2	3.5
(NMDAR1)	R-TTCTTGCCATTGATGAGCTG				
Il6	F-CCGGAGAGGAGACTTCACAG	61	161bp	0.1	3.5
(IL-6)	R-ACAGTGCATCATCGCTGTTC				
Tnfrsf11b	F-GAGTGTGCGAATGTGAGGAA	60	150bp	0.1	2.5
(OPG)	R-CACCTGAGAAGAACCCATCC				
Tnfsf11	F-CGACTCTGGAGAGCGAAGAC	62	125bp	0.1	3.5
(RANKL)	R-AACTCCTGAGAAGCGCTGTG				

Ctsk (Cathepsin K)	F-CAGAGTGGGAAGGCAGAGTC R-CCAACAGGAACCACACTGG	64	95bp	0.1	2.5
Col1a1 (Type 1 collagen)	F- ACTGCCCTCCTGACGCATGG R-TCGCACACAGCCGTGCCATT	64	140bp	0.1	3.5

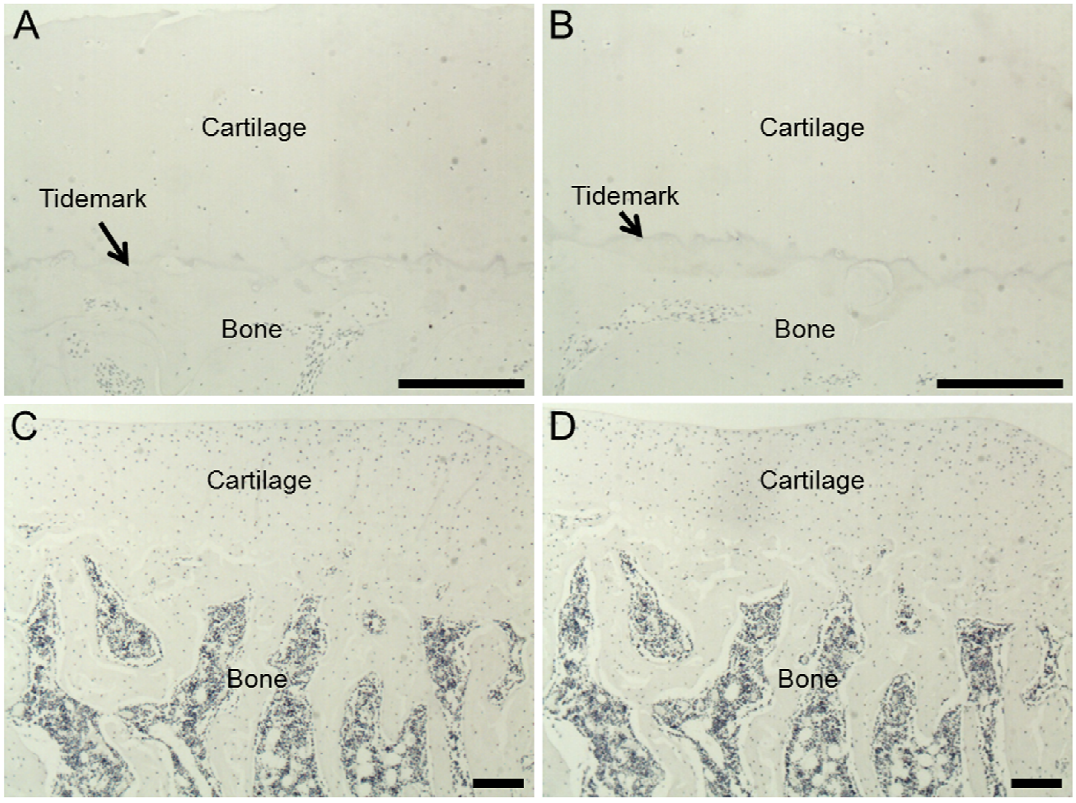
Supplementary Table 6. Individual severity score component values for AIA, AIA+NBQX and naïve rats.

	CSI	CA	PL	TI	BC
Naïve	1.83±0.44	3.33±0.58	3.17±0.6	2.5±0.76	0.83±0.46
AIA vs Naïve	10.89±1.66 *P*<0.001	6.28±0.63 *P*<0.001	7.44±1.05 *P*<0.001	7.06±0.43 *P*<0.001	7.61±1.1 *P*<0.001
AIA+NBQX vs Naïve vs AIA	7.67±0.66 *P*<0.001 30%↓ *P*>0.05	5.39±0.39 *P*<0.001 14%↓ *P*<0.05	5.31±0.36 *P*<0.01 28%↓ *P*<0.01	6.06±0.45 *P*<0.001 14%↓ *P*<0.01	3.69±0.67 *P*<0.001 51%↓ *P*<0.001

CSI, cartilage surface integrity; CA, chondrocyte appearance; PL, proteoglycan loss; TI, tidemark integrity; BC, bone changes. Percentage reductions of AIA+NBQX scores compared to AIA scores are shown.

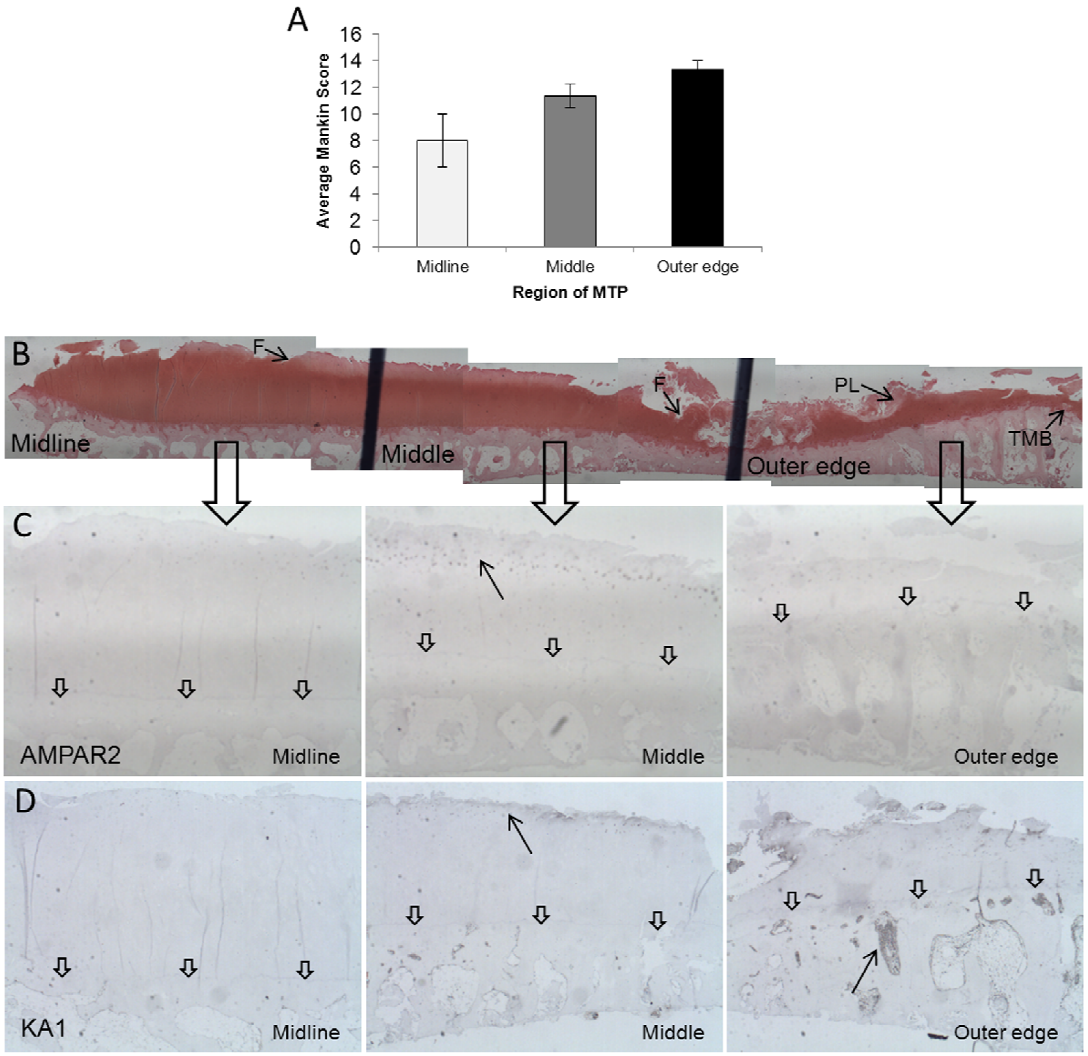
Supplementary Figure 1.

Human and rat tibial plateaux immunohistochemistry negative controls. (A) Human TBS control with primary antibody omitted. (B) Human rabbit IgG control. (C) Rat TBS control with primary antibody omitted. (D) Rat rabbit IgG control. Scale bars: A&B, 500μm; C&D, 100μm.



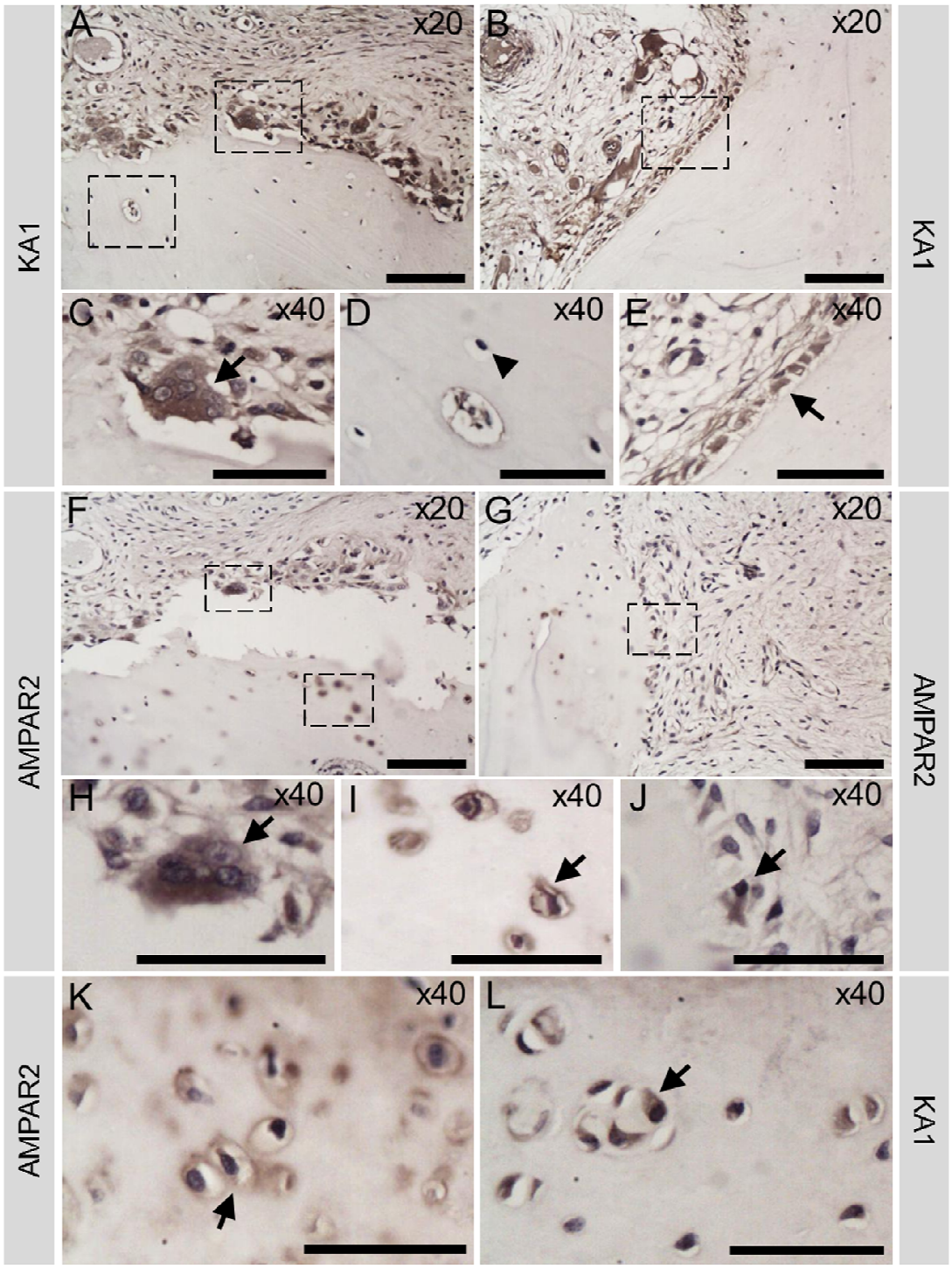
Supplementary Figure 2.

Histology and AMPAR2 and KA1 staining patterns across the MTP in a representative randomly selected OA patient. (A) Cartilage degradation severity (indicated by increasing Mankin score) appears to become more severe towards the outer edge of the MTP (n=3, *P*=0.07). (B) Histologically, more fibrillation (F), proteoglycan loss (PL) and breaching of the tidemark (TMB) can be seen towards the outer edge of the MTP. (C&D) AMPAR2 and KA1 localisation is more abundant in chondrocytes and bone cells towards the middle and outer edge (line arrows), however, severe loss of cartilage near the outer edge results in less chondrocytes and subsequently less AMPAR2 and KA1 staining in the cartilage. Block arrows indicate the location of the tidemark in C & D.


A
Average Mankin Score
16
14
12
10
8
6
4
2
0
Midline
Middle
Outer edge
Region of MTP
B
F
F
PL
TMB
Midline
Middle
Outer edge
C
AMPAR2
Midline
Middle
Outer edge
D
KA1
Midline
Middle
Outer edge

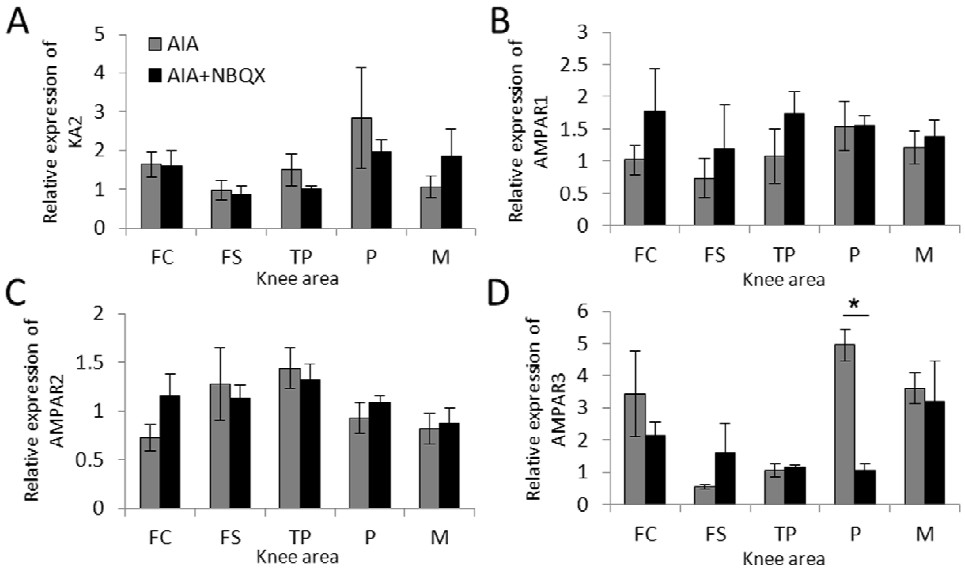
Supplementary Figure 3.

Kainate 1 (KA1) and AMPA receptor 2 (AMPAR2) immunohistochemistry in bone and cartilage from a medial tibial plateau sample from a rheumatoid arthritis (RA) patient. (A&B) KA1 staining was found in osteoclasts (C, arrow) and osteoblasts (E, arrow), but not osteocytes (D, arrow head). (F&G) Osteoclasts (H, arrow) and osteocytes (I, arrow) stained strongly for AMPAR2. Osteoblasts (J, arrow) were also positive for AMPAR2, but staining was not as intense and not in every cell. Chondrocytes stained positive for AMPAR2 (K, arrow) and KA1 (L, arrow). Dashed boxes indicate where x40 images were taken. Scale bars: A, B, F, G, 100μm; C, D, E, H, I, J-L, 50μm.


KA1
A
x20
B
x20
KA1
C
x40
D
x40
E
x40
AMPAR2
F
x20
G
x20
AMPAR2
H
x40
I
x40
J
x40
AMPAR2
K
x40
L
x40
KA1

Supplementary Figure 4.

QRT-PCR mRNA expression analysis of KA2 (A), AMPAR1 (B), AMPAR2 (C) and AMPAR3 (D) in AIA and AIA+NBQX rats. Data is presented as the Pfaffl ratio of the right leg divided by that of the contralateral left leg. AMPAR3 mRNA expression increased 5 fold in the patella of AIA rats, but was reduced to normal by NBQX treatment ($P<0.05$). FC, femoral condyle; FS, femoral shaft; TP, tibial plateaux; P, patella; M, meniscus. *$P<0.05$.



Supplementary Figure 5.

Mineralisation (A) and cell number (B) assays of human primary osteoblasts with and without NBQX treatment (200μM). (A) NBQX treatment prevents mineralisation. (B) Osteoblast cell number was significantly reduced (*P*<0.001) following incubation with NBQX. Values shown are mean ±SEM. ****P*<0.001.