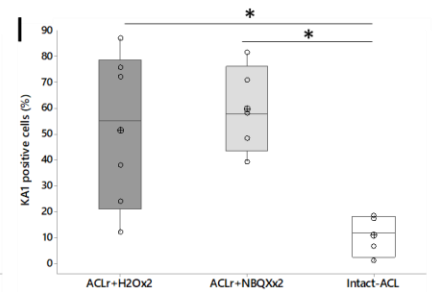
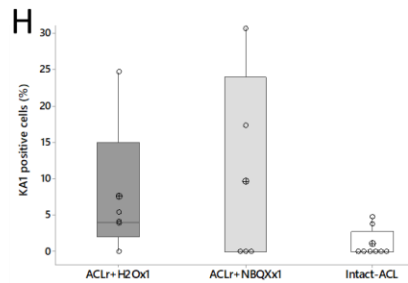
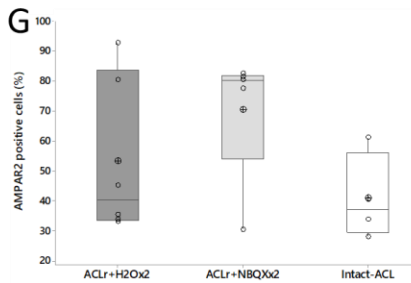
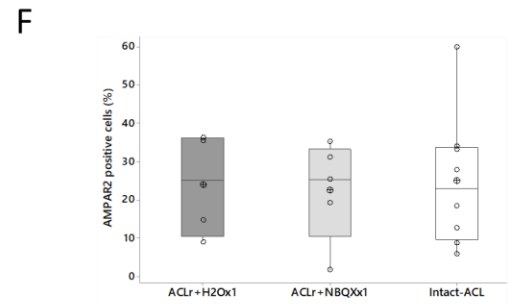
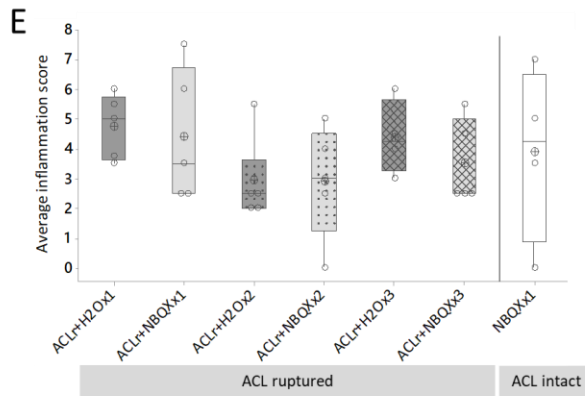
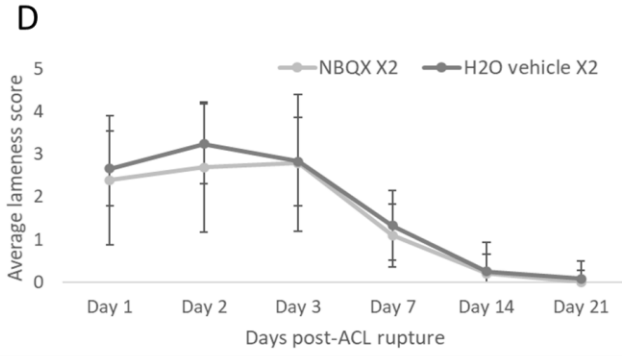
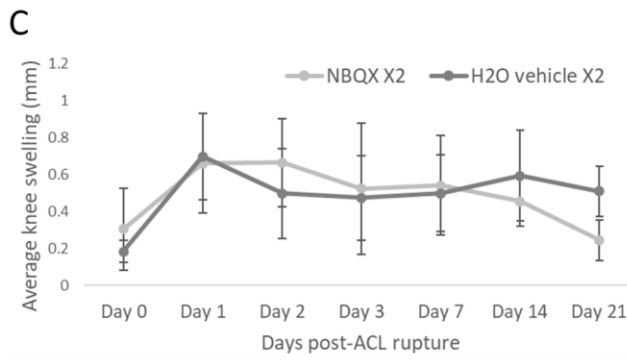
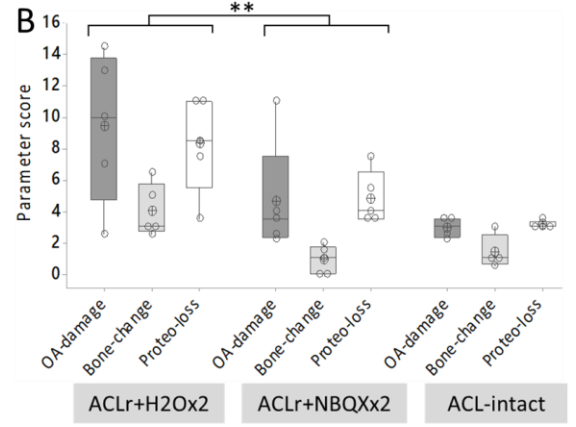
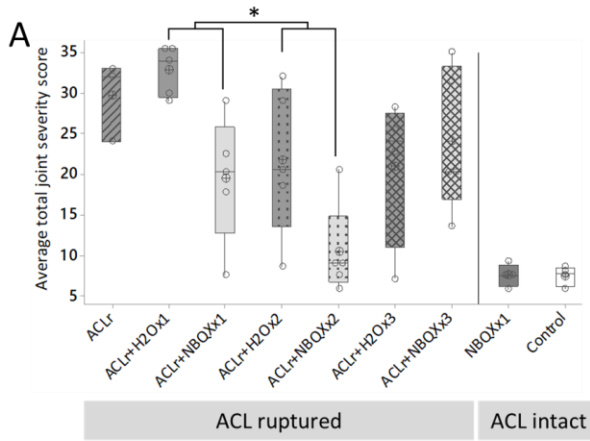


Supplementary materials.

Supplementary figure 1. (A) Two injections significantly reduce joint severity scores compared to a single injection following ACLr (n=5 per treatment, *p<0.05, GLM). (B) When broken down into parameters, 2 doses of NBQX significantly reduced scores compared to 2 doses of sH₂O vehicle (n=5 per treatment**p<0.01, GLM). (C) Knee swelling and (D) lameness score over time, and (E) day 21 histological inflammation, were not affected by NBQX dosing regimen in ACL ruptured mice (n=5 per treatment). (F&G) Numbers of AMPAR2 positive cells (presented as a percentage of total cells counted in the ACL) were similar for ruptured ACL and intact ACL, however, numbers of KA1 positive cells (H&I) were lower in intact-ACL compared to ruptured ACL, significantly lower when compared to 2 doses of vehicle or NBQX (I, *p<0.05, one-way ANOVA, tukey post-hoc). Data presented as mean ±SD in C & D and boxplots representing interquartile range, median and all data points (including mean, indicated by crossed circle) in A, B & E-I.



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 37°C followed by 1000U/ml hyaluronidase for 30 minutes at 37°C. For rat samples, 1mg/ml trypsin for 20 minutes at 37°C 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 4°C 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.

Supplementary table 1. Patient details.

ID	Use in study	Glutamate conc (umol/L)	Weeks post injury	Weight (kg)	Height (cm)	BMI	Pathology	Age at surgery	Sex
483	Glutamate conc.	71.094	17				ACL reconstruction	29	male
445	Glutamate conc.	19.924	275				ACL reconstruction	55	female
518	Glutamate conc.	54.74	37	92	180	28	ACL reconstruction	33	male
649	Glutamate conc.	96.186	10	83	171	29	ACL reconstruction	20	male
266	Glutamate conc.	52.496	134	77	180	25	ACL reconstruction	33	male
952	Glutamate conc.	34.714	10	71			ACL reconstruction	24	female
1170	Glutamate conc.	81.464					ACL reconstruction		male
1789	Glutamate conc.	66.3	361	103	185	30	ACL reconstruction	32	male
1808	Glutamate conc.	61.71	14	78	173	26	ACL reconstruction	38	male
1817	Glutamate conc.	56.78	14	86	168	29	ACL reconstruction	32	female
1842	Glutamate conc.	45.458	14	81	183	25	ACL reconstruction	18	male
1928	Glutamate conc.	61.268	40	87	177	27.8	ACL reconstruction	19	male
2011	Glutamate conc.	43.248	13	75	168	27	ACL reconstruction	33	female
874	Glutamate conc.	45.73	47				ACL reconstruction	30	male
895	Glutamate conc.	129.098	74	60	160	23	ACL reconstruction	22	male
1596	Glutamate conc.	49.538	161				ACL reconstruction	22	female
1708	Glutamate conc.	37.74	14				ACL reconstruction	17	male
1768	Glutamate conc.	19.618	524				ACL reconstruction	26	male
1841	Glutamate conc.	44.336	7	68	173	23	ACL reconstruction	21	male
1874	Glutamate conc.	21.08	383				ACL reconstruction	37	male

1899	Glutamate conc.	74.086	14				ACL reconstruction	26	male
1934	Glutamate conc.	71.604	19	80	180	25	ACL reconstruction	24	female
1944	Glutamate conc.	75.82	20	84.5	185	24	ACL reconstruction	22	male
2001	Glutamate conc.	99.076	27	92	175	30	ACL reconstruction	22	male
2031	Glutamate conc.	65.314	35	73	175	24	ACL reconstruction	17	male
2355	Immuno & glutamate conc.	66.266	48	77	175	25	ACL reconstruction	32	male
2357	Immuno & glutamate conc.	15.198	156	61	178	19	ACL reconstruction	19	male
2358	Immuno & glutamate conc.	43.622	61	57	150	25	ACL reconstruction	22	female
970	Glutamate conc.	88.4		109	183	33	ACL reconstruction	38	male
2362	Immuno & glutamate conc.	25.908		98	180	30	OA TKR	73	male
2361	Immuno & glutamate conc.	24.208	30	76	175	25	OA TKR	81	male
2360	Immuno & glutamate conc.	30.6	130	87	165	31	OA TKR	64	female
1882	Glutamate conc.	75.344					OA TKR		male
1680	Glutamate conc.	39.746					OA TKR		Female
2364	Immuno & glutamate conc.	28.22	15	65	165	24	Men. Arthroscopy	51	female
2365	Immuno & glutamate conc.	99.518	56				Men. Arthroscopy	66	female
1795	Glutamate conc.	22.984					Men. Arthroscopy	63	male
1911	Glutamate conc.	24.65		105	178	33	Men. Arthroscopy	39	male
1972	Glutamate conc.	40.29		56	160	22	Men. Arthroscopy	42	female

1678	Glutamate conc.	79.322					RA TKR		male
1617	Glutamate conc.	54.026					RA TKR		female

Supplementary table 2.

Mouse synovial inflammation scoring system (61).

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 overgrowth over joint surfaces.	2
	Over three layers thick with overgrowth over joint surfaces and evidence of cartilage loss.	3
Total score		0-14

Supplementary table 3.

Mouse knee degeneration scoring system (62).

Parameter	Sub-parameter	Grade
Osteoarthritic Damage	Normal	0
	Loss of toluidine blue	0.5
	Small fibrillations without loss of cartilage	1
	Vertical clefts down to the layer immediately below the superficial layer and some loss of surface lamina	2
	Vertical clefts/ erosion to the calcified cartilage extending to <25% of the articular surface	3
	Vertical clefts/ erosion to the calcified cartilage extending to 25-50% of the articular surface	4
	Vertical clefts/ erosion to the calcified cartilage extending to 50-75% of the articular surface	5
	Vertical clefts/ erosion to the calcified cartilage extending to >75% of the articular surface	6
Subchondral Bone Changes	Normal	0
	Mild	1

	Moderate	2
	Severe	3
Loss of Proteoglycan	Normal staining of non-calcified cartilage	0
	Decreased but not complete loss of Toluidine blue staining over 1-100% of the articular cartilage	1
	Complete loss of Toluidine blue staining in the non-calcified cartilage extending to <25% of the articular surface	2
	Complete loss of Toluidine blue staining in the non-calcified cartilage extending to 25-50% of the articular surface	3
	Complete loss of Toluidine blue staining in the non-calcified cartilage extending to 50-75% of the articular surface	4
	Complete loss of Toluidine blue staining in the non-calcified cartilage extending to >75% of the articular surface	5