Supplementary Material

Table S1: List of human joints used in this study

Joint #	Gender	Age	Joint type	Modified Collins Grade	Use in Figures
1.	Female	64	Ankle	1	5A & 5B & 6
2.	Female	66	Ankle	1	5A & 5B & 6B
3.			Ankle	1	5A & 5B & 6B
4.	Male	69	Ankle	1	5A & 5B
5.	Male	70	Ankle	1	5A & 5B
6.			Ankle	1	5A & 5B
7.	Male	74	Ankle	1	5A & 5B
8.			Ankle	1	5A & 5B
9.	Male	19	Knee (tibia plateau)	0	5C & 5D
10.	Male	52	Knee (tibia plateau)	1	5C & 5D
11.	Female	66	Knee (femur)	2	5C & 5D &6B
12.	Male	67	Ankle	1	S1A&B
13.			Ankle	1	S1A&B
14.	Male	71	Ankle	1	S1A&B
15.			Ankle	1	S1A&B
16.	Female	76	Ankle	1	S1A&B
17.			Ankle	1	S1A&B
18.	Male	59	Knee (femur)	1	S1C&D
19.	Male	34	Knee (femur)	0	S1C&D
20.	Female	63	Knee (femur)	1	S1C&D

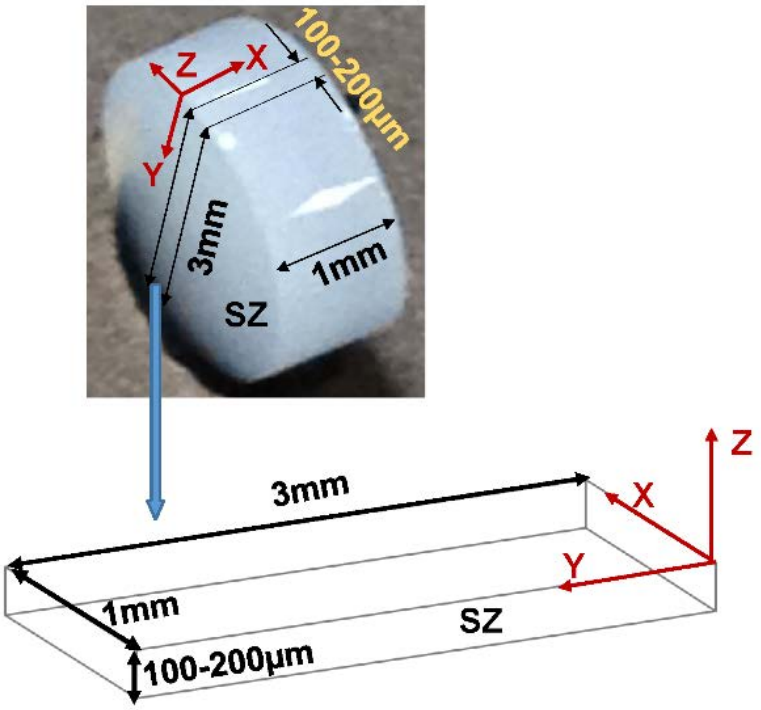

Figure S1. Slicing of explants for live/dead fluorescence imaging of human and bovine cartilage disks. A cartilage slice (100-200µm thick) was cut from the center of the cartilage disk (3 mm diameter, 1 mm thick), stained with propidium iodide (stains dead cells) and fluorescein diacetate (stains live cells), and the entire 1mm X 3mm area was imaged using fluorescence microscopy at 4X magnification in the X-Y plane of the entire slice. SZ indicates superficial zone. (Based on Bajpayee, Biomaterials, 2014 with modifications[27]).

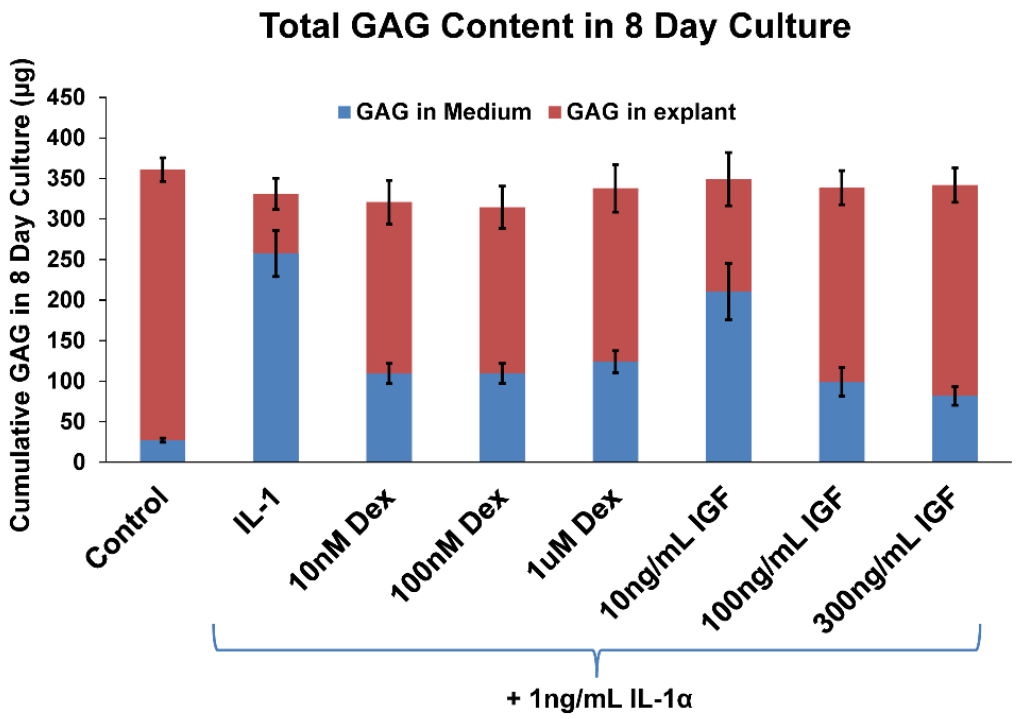

Figure S2. Total sGAG content and the corresponding distribution of sGAG in medium and explants for the immature bovine cartilage samples shown in Figure 1A. Explant disks were subjected to different doses of Dex or IGF-1 in the presence of IL-1± (1 ng/ml) for 8 days; N=23-24 explants per treatment condition from 4 animals (4 independent experiments). Total values are the mean sGAG content per 3mm x 1mm disk plus sGAG content lost to the corresponding medium for that disk. Shown separately are the medium contents (blue) and explant contents (red). Error bars are +/- 95% confidence intervals. The percentages of sGAG lost to the medium shown in Fig. 1A are equal to the medium values shown here divided by the corresponding total values for each treatment condition.

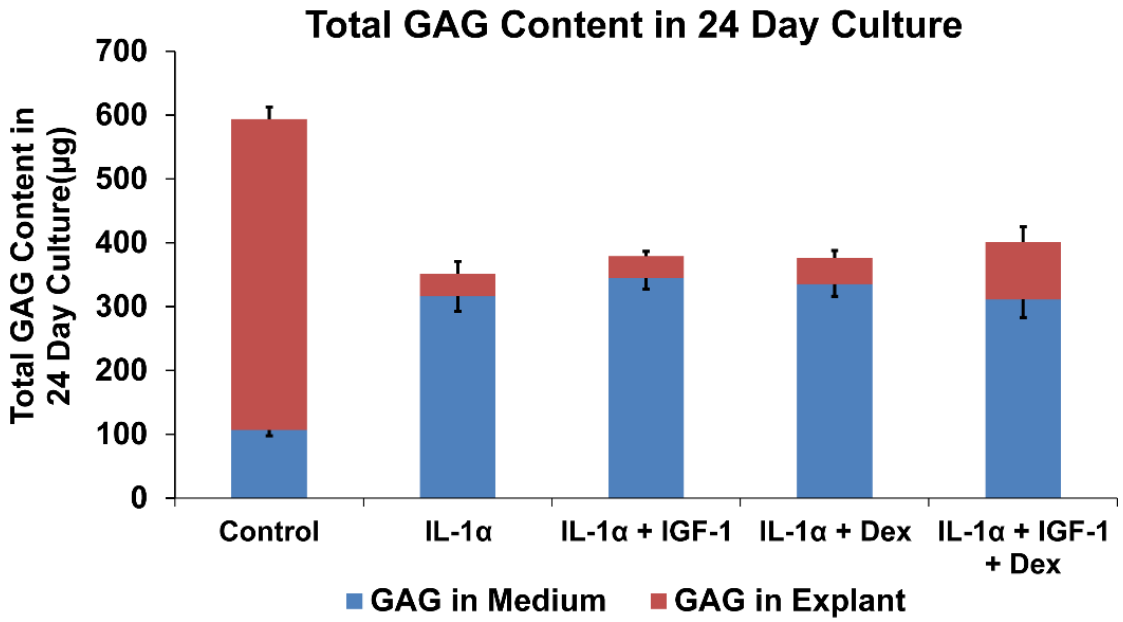

Figure S3. Total sGAG content of immature bovine cartilage of the same samples as Figure 2B. These explants were subjected to five treatments: untreated control, IL-1±, [IL-1± + IGF-1], [IL-1± + Dex], or [IL-1± + IGF-1 + Dex]. IL-1± (1 ng/ml) was added on Day 0 while IGF-1 (100 ng/ml) and Dex (100 nM) were introduced on Day 12 after the majority of sGAG was already depleted; N=18-20 disks/condition from n=3 independent experiments. Total values are the mean sGAG content per 3mm x 1mm disk plus sGAG content lost to the corresponding medium for that disk. Shown separately are the medium contents (blue) and explant contents (red). Error bars are +/- 95% confidence intervals.

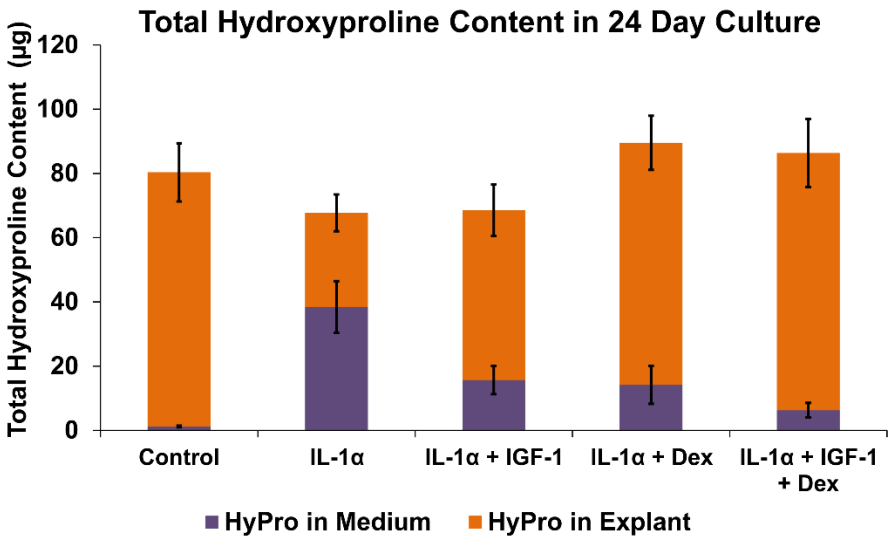

Figure S4. Total hydroxyproline content (remaining in explants after 24 days of culture + lost to the medium from day 12-24) for the same immature bovine cartilage samples shown in Figure 2B. These explants were subjected to five treatments: untreated control, IL-1±, [IL-1± + IGF-1], [IL-1± + Dex], or [IL-1± + IGF-1 + Dex]. IL-1± (1 ng/ml) was added on Day 0 while IGF-1 (100 ng/ml) and Dex (100 nM) were introduced on Day 12 after the majority of sGAG was already depleted; N=18-20 disks/condition from n=3 independent experiments. Total values are the mean hydroxyproline content per 3mm x 1mm disk plus the hydroxyproline content lost to the corresponding medium from day 12-24. Shown separately are the medium contents (blue) and explant contents (red). Error bars are +/- 95% confidence interval.

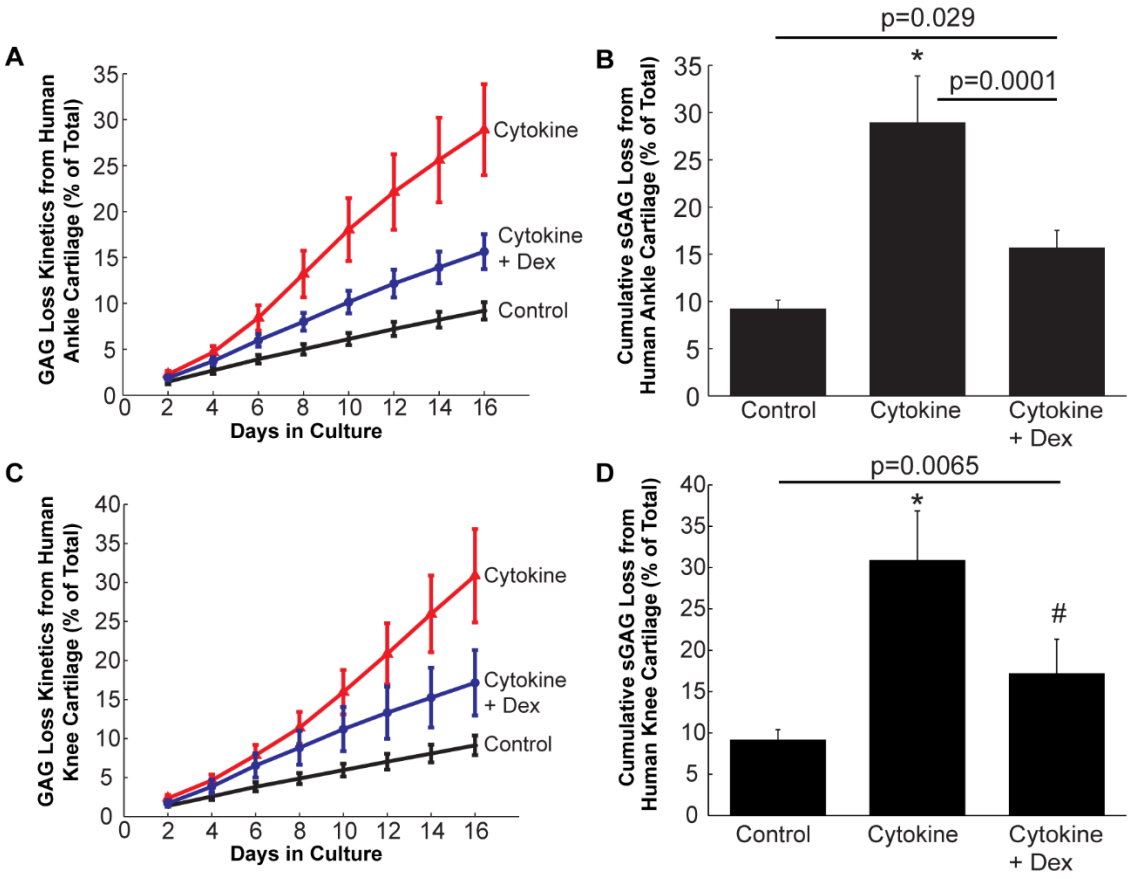

Figure S5. sGAG loss from adult human cartilage explants in response to three treatments using low glucose DMEM (1g/L) as the culture medium: control (basal medium: 1g/mL low glucose DMEM medium supplemented according to Methods section), cytokine (25 ng/mL TNF±, 50 ng/mL IL-6, 250 ng/mL sIL6Ra), and cytokine+dex (cytokine as above plus 100nM Dexamethasone). These conditions were chosen to enable direct comparison with previous experiments using exactly the same cytokine conditions but in high glucose medium[22]. **A,** Kinetics of sGAG loss from adult human ankle cartilage in response to 16-day treatments, N = 51 explants per treatment from 3 donors (see Table S1 for list of associated donor joints; 3independent experiments). **B,** Corresponding cumulative sGAG loss by day 16 for the same treatments and explants of **A**. **C,** Kinetics of sGAG loss from adult human knee cartilage in response to 16-day

treatments, N = 17 explants per treatment from 3 donors (3 independent experiments). **D,** Corresponding cumulative sGAG loss by day 16 for the same treatments and explants of **C**. Values are mean +/- 95% confidence interval. * vs. untreated control ($p<0.0001$); # vs. cytokine alone ($p<0.0001$). We first note that cumulative GAG loss by day 16 (low glucose, TNF± + IL-6/sIL-6R) was ~30% of total for both knee and ankle specimens, while in the present study by day 17, cumulative GAG loss was also about 30% for both knee and ankle (Fig. 5, high glucose, IL-1). Addition of 100nM Dex reduced GAG loss by almost 50% in both ankle and knee cartilage regardless of the glucose concentration or cytokine used to trigger the catabolic responses. Comparing Figure S5 to the data of Sui (2009, high glucose, identical cytokines)[22], TNF+IL-6/sIL-6R treatment by Sui et al. resulted in ~30% GAG loss by day 8 from knee cartilage and ~20% GAG loss from ankle cartilage by day 8; TNF alone resulted in ~25% GAG loss from knee cartilage and 15% GAG loss from ankle cartilage by day 8. Here, in Figure S1, TNF+IL-6/sIL-6R resulted in ~10-15% GAG loss from both knee and ankle cartilage by day 8. Thus, with these normal (non-OA) donor tissues, the catabolic effects associated with GAG loss were on the same order for both low and high glucose conditions. Thus, we found that glucose concentration does not change our conclusion regarding the ability of Dex to suppress sGAG loss in adult human donor tissues under cytokine challenge, whether by IL-1 (Fig. 5) or TNF±, IL-6, and sIL-6R.