**Supplementary Methods and Results**

*Optimization of RNA isolation from intact cartilage tissue*

Frozen cartilage pieces were placed in either a pre-cooled stainless-steel canister with a 25mm stainless steel grinding ball (large ball) or in an Eppendorf Biopur RNase/DNase free tube with a 5mm stainless steel grinding ball (small ball). For large ball homogenization, the canister was closed and immersed in liquid nitrogen for 2 minutes. The tissue was then disrupted using an oscillating milling machine (Retsch Mixer Mill MM 400; RETSCH; Haan, Germany) for 2-3 minutes at 30Hz. For small ball homogenization, individual Eppendorf tubes were put into a milling block, which was able to hold a maximum of 8 tubes. Tubes were loaded onto the block and then immersed into liquid nitrogen for 2 minutes. The tissue was then disrupted, following the same procedure as above. Following homogenization, total RNA was isolated from disrupted native (undigested) cartilage tissue using the mirVana total RNA isolation kit (Life Technologies) or miRNeasy isolation kit (Qiagen), following the protocols as outlined by each manufacturer for total RNA isolation. Homogenized cartilage tissue was subjected to either an incubation period with lysis buffer or no incubation prior to extracting RNA using either kit. Isolated RNA was purified using the RNA Clean and Concentrator Kit (Zymo Research; Irvine, CA, USA) and stored at -80°C. RNA quality was measured on an Agilent 2100 BioAnalyzer to determine the RNA Integrity Number (RIN) (Agilent, Santa Clara, CA, USA).

Homogenization of native equine articular cartilage with a large ball (25mm) resulted in increased yield of RNA content as compared to small ball (5mm) homogenization (51.1 ng/g versus 11.4 ng/g respectively, $p<0.001$). RNA quality did not differ significantly between homogenization methods (RIN values 2.21 and 1.93 respectively, $p=0.16$). No differences in quantity or quality were observed between RNA kits used, or whether samples were incubated

for 24 hours in lysis buffer or immediately extracted. However, quantity of RNA isolated using the large ball method with the mirVana kit without a lysis buffer incubation produced was lower than the amount yielded by digested chondrocytes.

*Reference gene selection*

NormFinder, an excel-based software, evaluates candidate reference genes across various samples and assigns genes with a stability value. Lower stability values represent more stable reference genes. NormFinder can differentiate between samples and assign them under different treatment groups (intact versus digested cartilage) to allow the algorithm to take into account treatment group variance. NormFinder assigned stability values to each candidate reference gene. The most stable reference genes were *18S* (stability value = 0.321), *B2M* (0.497), *RPLP0* (0.600), *SOX9* (0.655), *GAPDH* (0.854), and *GUSB* (0.863). The software also produced a combination of reference genes that was most stable across both treatment groups. The combination of *B2M + RPLP0* resulted in a stability value of 0.220. The most unstable genes were *COL1A2* and *ACTB*, with stability values of 2.991 and 4.125, respectively. Thus, *B2M* and *RPLP0* were both used as reference genes in the analysis of gene expression in intact and digested cartilage.