Supplementary Data

Supplementary ImageJ Macro

```
title = getTitle;
dotIndex = indexOf(title, ".");
Name = substring(title, 0, dotIndex);
run("Stack to Images");
selectWindow(Name+"–0001");
setOption("BlackBackground", false);
run("Make Binary", "thresholded remaining black");
run("Analyze Particles…", "size = 50–1000 clear summarize");
selectWindow(Name+"–0002");
setOption("BlackBackground", false);
run("Make Binary", "thresholded remaining black");
run("Analyze Particles…", "size = 50–1000 clear summarize");
selectWindow(Name+"–0003");
setOption("BlackBackground", false);
run("Make Binary", "thresholded remaining black");
run("Analyze Particles…", "size = 50–1000 clear summarize");
selectWindow(Name+"–0004");
setOption("BlackBackground", false);
run("Make Binary", "thresholded remaining black");
run("Analyze Particles…", "size = 50–1000 clear summarize");
selectWindow(Name+"–0005");
setOption("BlackBackground", false);
run("Make Binary", "thresholded remaining black");
run("Analyze Particles…", "size = 50–1000 clear summarize");
selectWindow(Name+"–0006");
setOption("BlackBackground", false);
run("Make Binary", "thresholded remaining black");
run("Analyze Particles…", "size = 50–1000 clear summarize");
close("*")
```

Supplementary Table S1. Sequence of Primers Used for Digital Droplet Polymerase Chain Reaction Amplification

Gene	*Forward sequence*	*Reverse sequence*
Actin NM_007393.3[42–44]	AAATCGTGCGTGACATCAAAGA	GCCATCTCCTGCTCGAAGTC
HPRT NM_013556.2[42–44]	GACCTCTCGAAGTGTTGGATAC	TCAACAGGACTCGTATTTG
RPL13-a NM_009438.4[45]	ATCCCTCCACCCTATGACAA	GCCCCAGGTAAGCAAACTT
Col1 NM_007742.4[46]	GCAACAGTCGCTTCACCTAC	GTGGGAGGGAACCAGATTG
Col2 NM_031163[47]	CGAGTGGAAGAGCGGAGACTAC	CCAGTTTTTCCGAGGGACAGT
ColX NM_009925[46]	TTCTGCTGCTAATGTTCTTGACC	GGGATGAAGTATTGTGTCTTGGG
PTHrP NM_008970	GAAACGCAGAGAACAGGAGAA	AATTTCAATGCGTCCTTAAGCTG
IHH NM_010544[48]	CCCCAACTACAATCCCGACA	TCATGAGGCGGTCGG

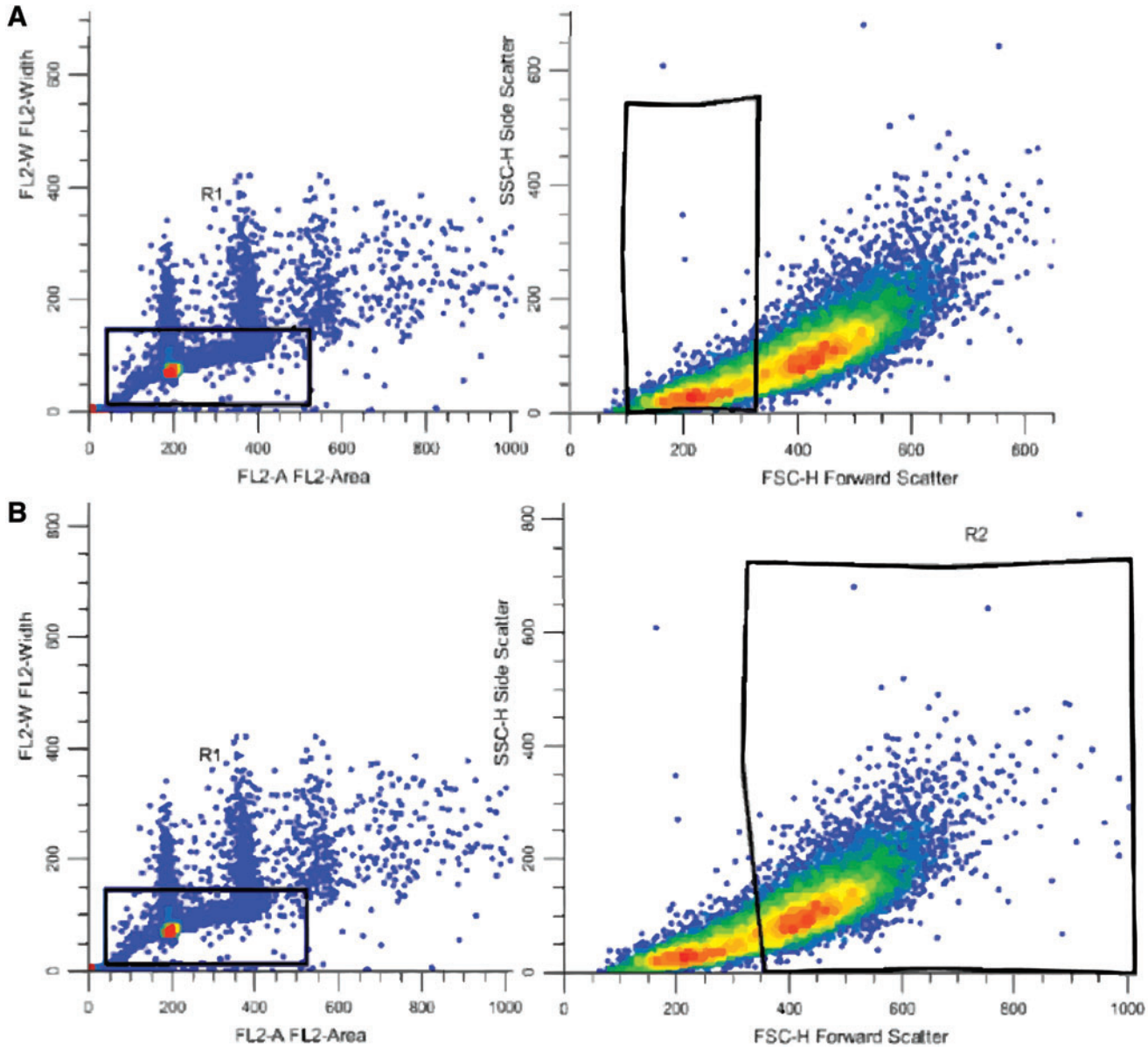
Primers used for ddPCR gene expression analysis. The table lists provided earlier give the name of all genes analyzed by ddPCR in the current study, the gene accession numbers that were used to generate each set of primers, and the publications that guided the selection of primers, where applicable (left column). Full sequences for each primer used in the current study (forward and reverse) are shown to the right.

ddPCR, digital droplet polymerase chain reaction; IHH, Indian hedgehog.

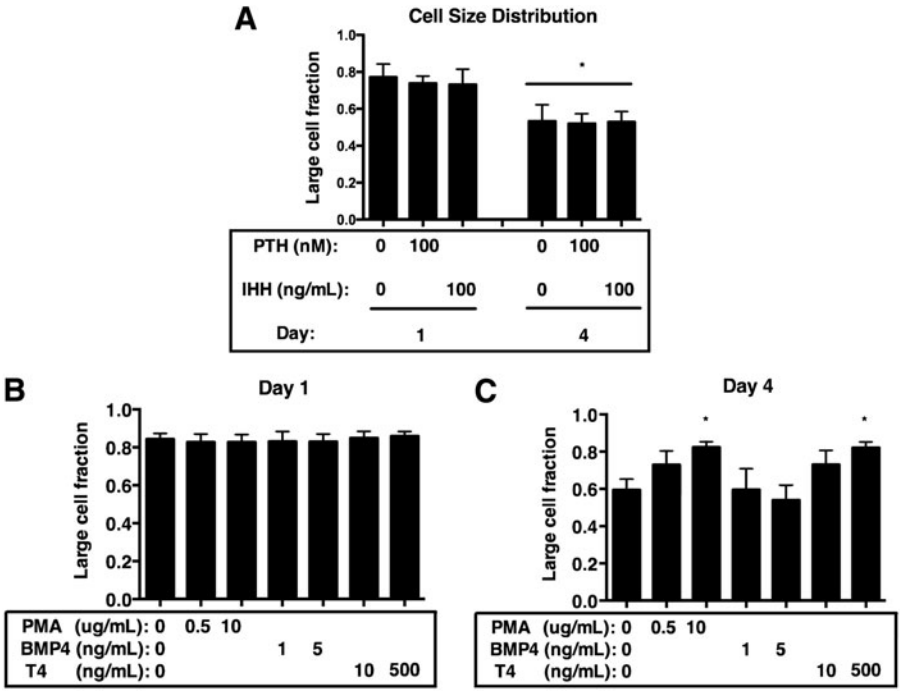
SUPPLEMENTARY TABLE S2. QUANTIFICATION OF THE LIVE-DEAD STAINS FROM FIGURE 1A

Time point	*Live count*	*Dead count*	*Total cells*	*Live/total*	*Percent viability*
Day 0	100	9	109	0.92	92
Day 1	143	11	154	0.93	93
Day 4	314	10	324	0.97	97
Day 7	258	13	271	0.95	95

Fluorescence images resulting from our viability assay were input to our automated ImageJ object-counting macro. Results from all time points analyzed are shown in each row. Counts in the green channel (live cells) and red channel (dead cells) were summed to calculate total cells. The ratio of living and total cells was multiplied by 100 to calculate percent viability (rightmost column).



SUPPLEMENTARY FIG. S1. Two distinct populations of chondrocytes isolated from growth plate cartilage and cultured alginate beads. **(A, B)** Examples of scatterplots generated in ModFit LTE software showing distributions of cell area with respect to cell width (*left*) and forward scatter with respect to side scatter (*right*) of propidium iodide-stained chondrocytes isolated from alginate bead culture. Rectangles show the gating strategy used to separate FACS data pertaining to smaller **(A)** versus larger **(B)** chondrocytes. $*p < 0.05$.



SUPPLEMENTARY FIG. S2. Cell size distribution modulated by exogenously activating IHH signaling. For all panels, counts of large, small, and total cell populations derived from propidium iodide S-phase analysis and used to calculate the fraction of total cells that were in the larger cell population. **(A)** Large cell fraction of chondrocytes cultured for 1 (*left*) and 4 *(right)* days in the presence or absence of PTH1–34 or IHH ($n = 3$). **(B, C)** Large cell fraction of chondrocytes cultured in the presence or absence of IHH-stimulating factors for 1 day **(B)** and 4 days **(C)** in culture. BMP4, bone morphogenetic protein-4; IHH, Indian hedgehog; PMA, purmorphamine; PTH1-34, parathyroid hormone 1–34. For all graphs, $*p < 0.05$.