Supplemental figure S1. Immunohistochemistry of all cartilage donors used in this study.

Supplemental figure S1. Protein expression of DKK1, FRZB and β-catenin was detected by IHC in all donors. Images were taken using the Nanozoomer (scale bar 100μm). D1-D10=Donor 1-10.
Supplemental figure S2. The effects of IL1β on DKK1 and FRZB and Cartilage and WNT related genes. A. DKK1 and B. FRZB are illustrated in red and nuclei are in blue (DAPI). C, D. IL1β decreased mRNA expression of cartilage markers ACAN and COL2A1 while increased hypertrophic and apoptotic markers, WNT receptor FRZD10 and transcription factors TCF4 and LEF1 were induced by IL1β. WNT inhibitor WIF2, WNT4 expression was decreased upon IL1β stimulation. E,F. qPCR was used to measure the expression of IL1β target gene IL16, IL1β and MMP3; G. Expression of WNT antagonists DKK1 and FRZB mRNA at indicated time and dose point after IL1β treatment.
**Supplemental figure S3.** The effects of IL1β and iNOS inhibitor on DKK1 and FRZB and β-catenin expression visualized by immunofluorescence. A. DKK1 and B. FRZB and C. β-catenin in red and nuclei are in blue.

**Supplemental table S1. Primer sequences.** PCR Reactions were carried out using the Bio-Rad CFX96 (Bio-Rad, Hercules, CA) under the following conditions: cDNA was denatured for 5 min at 95°C, followed by 39 cycles consisting of 15 s at 95°C, 15 s at 60°C and 30 s at 72°C. For each reaction, a melting curve was generated to test primer dimer formation and non-specific priming. Gene expression was normalized using GAPDH as housekeeping gene.

| Gene Name | Primer Sequence                        | Product Size | Annealing Temperature |
|-----------|----------------------------------------|--------------|-----------------------|
| GAPDH     | Forward primer: 5′ CGCTCTCTGCTCTCTCTCTGTT 3′<br>Reverse primer: 5′ CCAATTGTTGAAAGCACAT3′ | 81           | 60                    |
| IL6       | Forward primer: 5′GGCACTGCCAGAAACAGACC 3′<br>Reverse primer: 5′GCAAGTCTCTCATTGAATCC 3′ | 85           | 60                    |
| TCF4      | Forward primer: 5′GCACTGCGCGACTACATAGG 3′<br>Reverse primer: 5′GACCTCGGATCTACACAGTGG 3′ | 98           | 60                    |
| MMP3      | Forward primer: 5′TGGAATTCTCCCTCTATGGA 3′<br>Reverse primer: 5′AGGCAACAGGACTACAGTGG 3′ | 116          | 60                    |
| MMP13     | Forward primer: 5′AGGAGCAGATGCGACTTGCT 3′<br>Reverse primer: 5′TGCCGGAGGAAAGCAGC 3′ | 72           | 60                    |
| IL1β      | Forward primer: 5′TCCCCAGCCCTCTTTGTAAG 3′<br>Reverse primer: 5′TTCAGAACCAGTGCGGCTG3′ | 91           | 60                    |
| INOS      | Forward primer: 5′CCTACTCCGGTCAGTGGGT 3′<br>Reverse primer: 5′AGGCAACAGGACTACAGTGG 3′ | 168          | 60                    |
| DKK1      | Forward primer: 5′AGTTACTGCCTCTCCACTAC 3′<br>Reverse primer: 5′GCCGCGACTACCTCTCTGGA 3′ | 172          | 60                    |
| FRZB      | Forward primer: 5′ACGGAGCAGATGCAACCTCTT 3′<br>Reverse primer: 5′CGAGACTGATCCTCTACCTTAA 3′ | 155          | 60                    |
| FASL      | Forward primer: 5′CTTTGAGACCTACAGGAAAAGG 3′<br>Reverse primer: 5′ATGGCGAGTGTCAGTACAGG 3′ | 107          | 60                    |
| AIXN2     | Forward primer: 5′AGTGCGAGTTCCACCGAAAC 3′<br>Reverse primer: 5′CTGGTGCAAGACATAGCCAGAA 3′ | 103          | 60                    |
| BMP2      | Forward primer: 5′GCTAGACCTGTAACGAGGCA 3′<br>Reverse primer: 5′TTTCCCCACCTGTTCTCTG 3′ | 74           | 60                    |
| FZD10     | Forward primer: 5′AAAGTGTCTGAGGAGCAATGC 3′<br>Reverse primer: 5′AGAAACCCAGTCGTCCTACA 3′ | 205          | 60                    |
| LEF1      | Forward primer: 5′CAGAGGGCAAGGAGGATTTAG 3′<br>Reverse primer: 5′CTGAGAGGTTGGCTGCTG 3′ | 109          | 60                    |
| WIF1      | Forward primer: 5′TCAGAAAAGCGCAACAGAGA 3′<br>Reverse primer: 5′TGATGCCCTTTATCAGAGGAG 3′ | 132          | 60                    |
| WNT4      | Forward primer: 5′CTCTCTCCTTGGGCTGCT 3′<br>Reverse primer: 5′AGTGGCTGACGCTGTTCTC 3′ | 101          | 60                    |
| ACAN      | Forward primer: 5′TTCCCATCGTGCTTTTCA 3′ | 121          | 60                    |
Building an ANIMO model for investigating signaling cross-talk

Nodes in an ANIMO network can represent both proteins directly involved in signal transduction (e.g., kinases) and other related entities, such as cytokines, genes and mRNA. An activity level is associated to each node, to represent for example the relative amount of phosphorylated kinase or the concentration of mRNA. The activity level of a node can be altered by interactions with other nodes. ANIMO networks can include activations (→) and inhibitions (←), which will increase (resp. decrease) the activity level of the target node if the source node is active. For example, A → B will increase the activity level of B if A is active. The speed at which an interaction occurs is defined by its $k$ parameter, which can be estimated qualitatively by choosing among a pre-defined set of options (very slow, slow, medium, fast, very fast) or by directly inputting a numerical value. Using the indicated qualitative choices already leads to useful models: e.g. a slow interaction to represent the production of a protein, and a fast one for a post-translational modification such as phosphorylation is sufficient to provide a realistic behavior in a network with the proper node topology [30-32].

**Step 1: Building the IL1 pathway**

When building our model in ANIMO we aimed to make it as simple as possible, using the minimal amount of proteins and interactions necessary to describe a process. The canonical IL1β/NFκB pathway is important for inflammation. We therefore drew nodes to represent IL1β, IL1R, NFκB and its inhibitor IKβα, and iNOS, see Figure S4B,C. In our models, we assume that there are 2 types of reactions: fast reactions for post-translational modifications, such as phosphorylation, and slow reactions where gene transcription occurs. We therefore added reactions between nodes using these 2 types of reaction speed with a “scenario 1” setting. We also add auto-inhibition to indicate inhibition as described in the literature for e.g. receptor internalization, phosphatase activity and, in the case of NFκB, nuclear export as regulated by IκB.
Supplemental figure S4. IL1 and WNT signaling in ANIMO models. A. Legend. Shape of the nodes defines the type of node, activity is on a scale of red = inactive, to green = active. B. IL1β activates iNOS via NFκB after addition of IL1β. Green is active and red inactive. C. the heatmap indicates protein activity in time (relative time units); D, E. Model of an inactive WNT pathway where the destruction complex, consisting of GSK3β, AXIN2 and APC, is active resulting in degradation of β-catenin. F, G. When WNT is added to the network GSK3β is inactivated thereby alleviating the downregulation of β-catenin. β-catenin then accumulates and can bind to TCF/LEF, activating this transcriptional complex. H, I. Simplifying the WNT signaling pathway results in WNT activating β-
catenin, and thereby TCF/LEF, at similar rates; J, K. Simple diagram of the active IL1 and WNT pathways.

**Step 2: Modeling the WNT pathway**

In this model, we only consider WNT signaling via β-catenin, since DKK1 is a WNT antagonist that functions by binding to LRP5/6, which are co-factors for the WNT receptors FRIZZLED, that activate β-catenin by inhibition of the β-catenin destruction complex.

In order to represent the canonical WNT/β-catenin signaling pathway we have to consider that when no WNT ligand is present the destruction complex is active. Its function is to destroy the ubiquitously expressed β-catenin (Figure S4D,E). As an effect, β-catenin is inactive.

However, when WNT is present, GSK3β is inactivated, AXIN and APC are recruited to the receptor complex, indicated by the inhibiting edges (Figure S4F,G). As an effect, β-catenin is not degraded and accumulates in the cytoplasm and translocates to the nucleus to form a transcriptional complex with the TCF/LEF transcription factors.

Although we can model this quite well, it would be easier to simplify the exact protein interactions so that in absence of WNT, β-catenin is inactive and in presence of WNT β-catenin is active. We therefore chose to model the WNT signaling pathway as shown in Figure S4H, I. As can be seen in the activity plots on the right, the timing and intensity of β-catenin and TCF/LEF is similar to Figure S4G.

**Step 3: Combining the IL and the WNT pathways**

We added the WNT network representation as in Figure S4H to the IL1β model in Figure S4B, creating the model in Figure S4J. As can be seen from the network diagram, the only interaction between these networks is that β-catenin downregulates NFκB. It is known that the WNT signaling pathway influences the IL1β pathway by β–catenin inhibiting NFκB [26]. We therefore added an edge from β-catenin to NFκB (Figure S4J). This addition slows down, but does not completely inhibit NFκB activation.

**Step 4: Adding the WNT antagonists DKK1 and FRZB**

To test our hypothesis that IL1β activates WNT signaling through DKK1 and FRZB repression (see main manuscript), we added nodes to the network for ‘DDK1 mRNA’ and ‘FRZB mRNA’. The upstream signals activating the transcription of these genes is not completely clear. However, we do know that in healthy articular cartilage these factors are expressed, whereas in OA the expression of these genes is greatly reduced [12,13]. We therefore added a node “anabolic regulator” or ‘ANAR’ to our model that induces the transcription of DKK1 and FRZB. We then added the nodes DKK1 and FRZB protein, that both function to antagonize WNT signaling. If there is no cross-talk from the IL1β pathway to the WNT pathway, WNT and β-catenin become inactive due to the presence of DKK1 and FRZB (Figure S5).
Supplemental Figure S5. ANIMO models used to test the hypothesis that IL1β can activate WNT signaling by downregulating DKK1 and FRZB via iNOS. A, B. Model 1, in which iNOS downregulates both DKK1 and FRZB resulting in upregulation of WNT activity; C, D. Model 2, the WNT and IL pathways including the WNT antagonists DKK1 and FRZB. When only DKK1 is inhibited by iNOS, as is described [27] there is no WNT activity; E,F. Model 3, 1400W is added to inhibit iNOS, thereby restoring DKK1 and FRZB expression resulting in inhibition of WNT activity; G,H. Model 4, iNOS regulates MMP expression. If 1400W is added the expression of MMP is also decreased.

Step 5: Adaptation of the model to fit the biological data
In our ANIMO models of the WNT and IL1β pathways in chondrocytes we used very simple reaction kinetics, in which activation via post-translational modification was considered a fast reaction, and activation via gene expression was considered a slow reaction. This simplification was
sufficient to describe the trends of the activation, but not the in a realistic time line. We have therefore changed the time scale of the model to match the timing of events as reported by the experimental data. This was done achieved by comparing our experimental data with timing information (Figures 3E, 3F, and Figure 4D) to the time scale of the model. As experimental data showed events that are about 4 times slower than the model interactions, we lowered the \( k \) parameter of each interaction by 4-fold for all interactions in the ANIMO model. In addition, we observed in our experimental data that FRZB was inhibited at a slower rate than DKK1. In our model, we lowered the value of the \( k \) parameter for the iNOS \( \rightarrow \) FRZB mRNA interaction to 0.001 and the FRZB mRNA to FRZB protein to 0.001 to match this better.

Table S2. Parameter settings for models in figures S4-S5. To simplify the model construction, the \( k \)-values used in the models presented in this article were mostly chosen among ANIMO's qualitative range, which has a direct correspondence with numerical values as follows: very slow = 0.001, slow = 0.002, medium = 0.004, fast = 0.008, very fast = 0.016.

| Model figure S2A | Interaction | k-values |
|------------------|------------|----------|
| activation       | IL1b \( \rightarrow \) IL1R | 0.016 |
|                  | IL1R \( \rightarrow \) NFkb | 0.016 |
|                  | NFkb \( \rightarrow \) IL1b | 0.001 |
|                  | NFkb \( \rightarrow \) iNOS | 0.002 |
|                  | iNOS \( \rightarrow \) IKb\_a | 0.001 |
| inhibition       | IL1b \( \rightarrow \) IL1b | 4.40E-04 |
|                  | IL1R \( \rightarrow \) IL1R | 4.40E-04 |
|                  | NFkb \( \rightarrow \) NFkb | 0.01 |
|                  | IKb\_a \( \rightarrow \) NFkb | 0.008 |

| Model Figure S2B | Interaction | k-values |
|------------------|------------|----------|
| activation       | bcat-mRNA \( \rightarrow \) bcat | 0.008 |
|                  | bcat \( \rightarrow \) TCF/LEF | 0.016 |
| inhibition       | Frizzled \( \rightarrow \) GSK3b | 0.016 |
|                  | LRP \( \rightarrow \) GSK3b | 0.016 |
|                  | GSK3b \( \rightarrow \) bcat | 0.016 |
|                  | GSK3b \( \rightarrow \) Axin | 0.016 |
|                  | GSK3b \( \rightarrow \) APC | 0.016 |

| Model figure S2C | Interaction | k-values |
|------------------|------------|----------|
| activation       | bcat-mRNA \( \rightarrow \) bcat | 0.008 |
|                  | WNT \( \rightarrow \) LRP | 0.016 |
|                  | WNT \( \rightarrow \) Frizzled | 0.016 |
|                  | bcat \( \rightarrow \) TCF/LEF | 0.016 |
| inhibition       | GSK3b \( \rightarrow \) APC | 0.016 |
|                  | GSK3b \( \rightarrow \) Axin | 0.016 |
|                  | GSK3b \( \rightarrow \) bcat | 0.016 |
LRP --| GSK3b 0.016
Frizzled --| GSK3b 0.016

Model figure S2D
WNT --> bcat 0.003
bcat --> TCF/LEF 0.016

Models 1-4 (figure S3A-D)

| Interaction                     | k-values |
|---------------------------------|----------|
| **Activation**                  |          |
| ANAR --> DKK1 mRNA              | 0.002    |
| ANAR --> FRZB mRNA              | 0.002    |
| B-cat --> TCF/LEF               | 0.002    |
| DKK1 mRNA --> DKK1              | 0.002    |
| FRZB mRNA --> FRZB              | 0.002    |
| IL-1R --> NFkB                  | 0.016    |
| IL-1b --> IL-1R                 | 0.016    |
| NFkB --> MMP                    | 0.001    |
| NFkB --> iNOS                   | 0.002    |
| TCF/LEF --> NFkB                | 0.001    |
| WNT --> B-cat                   | 0.003    |
| WNT source --> WNT              | 0.016    |
| iNOS --> IKB_a                  | 0.002    |
| iNOS --> MMP                    | 0.002    |
| **Inhibition**                  |          |
| 1400W --> iNOS                  | 0.016    |
| B-cat --> B-cat                 | 0.001    |
| B-cat --> NFkB                  | 0.004    |
| DKK1 mRNA --> DKK1              | 6.00E-04 |
| DKK1 --> WNT                    | 0.016    |
| DKK1 mRNA --> DKK1 mRNA         | 0.004    |
| FRZB --> FRZB                   | 6.00E-04 |
| FRZB --> WNT                    | 0.016    |
| FRZB mRNA --> FRZB mRNA         | 0.004    |
| IKB_a --> IKB_a                 | 0.001    |
| IKB_a --> NFkB                  | 0.002    |
| IL-1R --> IL-1R                 | 0.002    |
| MMP --> MMP                     | 0.003    |
| NFkB --> NFkB                   | 0.002    |
| TCF/LEF --> TCF/LEF             | 0.002    |
| WNT --> WNT                     | 0.004    |
| iNOS --> DKK1 mRNA              | 0.016    |
| iNOS --> FRZB mRNA              | 0.016    |
| iNOS --> iNOS                   | 1.00E-04 |

Fig 2B
| Node name   | Initial activity |
|------------|------------------|
| ANAR       | 67               |
| B-cat      | 0                |
| DKK1       | 100              |
| DKK1 mRNA  | 26               |
| FRZB       | 100              |
| FRZB mRNA  | 28               |
| IKB_a      | 3                |
| IL-1R      | 2                |
| IL-1b      | 100              |
| MMP        | 0                |
| NFKB       | 0                |
| TCF/LEF    | 0                |
| WNT        | 0                |
| WNT source | 42               |
| iNOS       | 100              |

**Fig S4B**

| Node name | Initial activity |
|-----------|------------------|
| IKB_a     | 0                |
| IL-1R     | 0                |
| IL-1b     | 100              |
| NFKB      | 0                |
| iNOS      | 0                |

**Fig S4D**

| Node name    | Initial activity |
|--------------|------------------|
| APC          | 20               |
| Axin         | 40               |
| Frizzled     | 0                |
| GSK3b        | 100              |
| LRP          | 0                |
| TCF/LEF      | 0                |
| bcat         | 0                |
| bcat-mRNA    | 50               |
### Fig S4F

| Node name   | Initial activity |
|-------------|------------------|
| APC         | 20               |
| Axin        | 40               |
| Frizzled    | 0                |
| GSK3b       | 100              |
| LRP         | 0                |
| TCF/LEF     | 0                |
| WNT         | 100              |
| bcat        | 0                |
| bcat-mRNA   | 50               |

### Fig S4H

| Node name   | Initial activity |
|-------------|------------------|
| TCF/LEF     | 0                |
| WNT         | 100              |
| bcat        | 0                |

### Fig S4J

| Node name   | Initial activity |
|-------------|------------------|
| IKB_a       | 0                |
| IL-1R       | 0                |
| IL-1b       | 100              |
| NFkB        | 0                |
| TCF/LEF     | 0                |
| WNT         | 100              |
| bcat        | 0                |
| bcat-mRNA   | 50               |
| iNOS        | 0                |

### Fig S5A

| Node name   | Initial activity |
|-------------|------------------|
| ANAR        | 67               |
| B-cat       | 0                |
| DKK1        | 100              |
| DKK1 mRNA   | 26               |
| Node name | Initial activity |
|-----------|------------------|
| ANAR      | 67               |
| B-cat     | 0                |
| DKK1      | 100              |
| DKK1 mRNA | 26               |
| FRZB mRNA | 100              |
| FRZB      | 28               |
| IKB_a     | 3                |
| IL-1R     | 2                |
| IL-1b     | 100              |
| MMP       | 0                |
| NFkB      | 0                |
| TCF/LEF   | 0                |
| WNT       | 0                |
| WNT source| 42               |
| iNOS      | 0                |

**Fig S5C**

| Node name   | Initial activity |
|-------------|------------------|
| 1400W       | 100              |
| ANAR        | 67               |
| B-cat       | 100              |
| DKK1        | 5                |

**Fig S5E**
| Node name   | Initial activity |
|-------------|------------------|
| DKK1        | 0                |
| mRNA        |                  |
| FRZB        | 5                |
| mRNA        | 0                |
| IKB_a       | 100              |
| IL-1R       | 100              |
| IL-1b       | 100              |
| MMP         | 33               |
| NFkB        | 100              |
| TCF/LEF     | 93               |
| WNT         | 100              |
| WNT source  | 42               |
| iNOS        | 100              |

Fig S5G