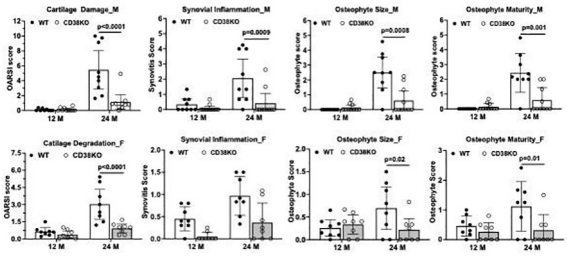
Figure 1. Both male and female mice deficient in CD38 were protected from age-related spontaneous OA development.



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DEFINING THE HIERARCHY OF FIBROBLASTS AND THEIR STEM CELLS IN THE ADULT SYNOVIAL JOINT AT SINGLE CELL RESOLUTION

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Purpose: The synovium, a specialised connective tissue, encapsulates synovial joints providing a barrier between the joint space and surrounding tissues. The healthy synovium consists of two layers, the sub-lining, composed of fibroblasts and other cell types, and the synovial lining that contains a specialised tissue-resident fibroblast known as the fibroblast-like synoviocyte (FLS). The FLS and sub-lining fibroblasts play a critical role in joint health and osteoarthritis. In the healthy joint, FLS produce essential joint lubricants such as hyaluronic acid and lubricin. In contrast, in osteoarthritis, synovial fibroblasts exhibit enhanced expression of inflammatory cytokines, chemokines, matrix metalloproteinases and other catabolic enzymes leading to cartilage breakdown. In the adult knee, FLS and sub-lining fibroblasts are derived, in part, from embryonic *Gdf5*-expressing joint progenitor cells, a subset of Pdgfrα-expressing fibroblasts, present in the joint interzone. These cells give rise to synovial joints during development forming joint tissues such as the synovium, cartilage, menisci and ligaments. Despite their critical roles, understanding of synovial fibroblast ontogeny, phenotypic diversity, molecular regulation of fate determination and renewal from adult stem/progenitor cells is limited.
Methods: Synovial fibroblasts were isolated from adult transgenic *Gdf5-Cre*;*Tom;Pdgfrα-H2BGFP* mouse knees, either healthy or 6 days after injury to the articular cartilage. Cells were sorted, by fluorescence-activated cell sorting, based on their ontogeny, and processed for single-cell RNA-sequencing using the 10x Genomics Chromium controller. Transcriptomic analysis was performed using the Seurat R package. RNA velocity was analysed using scVelo to infer differentiation pathways, and gene regulatory networks (regulons) were analysed to determine molecular regulation of fate determination using SCENIC. Cell cycle analysis was performed computationally to identify proliferating synovial fibroblast populations and confirmed by Ki67 immunofluorescence staining.
Results: In healthy joints, sub-lining fibroblasts were found to be of mixed ontogeny with partly overlapping phenotypes, while FLS descended from the *Gdf5*-expressing cells of the embryonic joint interzone. After cartilage injury, we identified an actively cycling, facultative *Gdf5*-lineage stem cell population that supplied new chondrocytes, immunoregulatory fibroblasts and FLS, the latter via transit-amplifying progenitors. Furthermore, we detected the appearance of injury-induced FLS and chondrocyte sub-populations that are not of *Gdf5*-lineage, demonstrating lineage plasticity during repair. Finally, we reveal the molecular regulation of the synovial fibroblast phenotype, with both mouse and human FLS exhibiting *Sox5* and *Creb5* regulon activity regardless of ontogeny or injury.
Conclusions: Our findings elucidate the functional hierarchies and differentiation trajectories of ontogenetically defined stromal cell populations in the knee, from adult stem cells to FLS and immunoregulatory fibroblasts, and provide novel insight into the molecular regulation that governs cell fate in the adult joint. These data advance our knowledge of the cell populations that maintain and repair the synovial joint in adult life.

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INHIBITION OF HISTONE DEMETHYLASES AS AN APPROACH TO RESTORE DEFICIENT DOT1L ACTIVITY IN OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA), the most common chronic joint disease worldwide, is characterized by progressive damage to the articular cartilage, increased joint-associated bone remodelling, and synovial inflammation. Current OA treatments are limited to pain relief, physiotherapy or replacement surgeries in severe cases, yet disease-modifying drugs are lacking. A genome-wide association study (GWAS) revealed a genetic association between polymorphisms in the *DOT1L* gene and OA. The *Disruptor of telomeric silencing 1-like (DOT1L)* gene encodes a unique histone methyltransferase that methylates Lysine 79 of Histone H3 (H3K79). We previously identified DOT1L as a key protector of cartilage homeostasis, using both human articular chondrocytes and different *Dot1l* genetic mouse models. Furthermore, we reported that DOT1L activity, indicated by the levels of H3K79 methylation (H3K79me), is reduced in OA as compared to non-OA cartilage. Therefore, maintaining H3K79me seems to be critical to preserve joint health and prevent the development or progression of OA. Here, we hypothesized that H3K79me could be restored or maintained by inhibiting demethylation at the H3K79 site, via targeting specific histone demethylases. There are two main families of histone demethylases: the lysine-specific demethylases (LSD) and Jumonji C (JmjC) demethylases. The LSD family contains two members: LSD1 and LSD2. The JmjC family is further classified into 6 subfamilies: from KDM2 to KDM7. In this study, we aimed to investigate which histone demethylases are responsible for H3K79 demethylation and whether their specific targeting can lead to protective effects in OA.
Methods: We determined the baseline mRNA expression of a panel of histone demethylases in primary human articular chondrocytes (hACs) from non-OA patients, and mapped their expression upon OA-mimicking stimuli (IL-1β and Wnt signalling activator CHIR99021), using real-time qPCR. To interrogate the role of JmjC demethylase family in H3K79me, human articular chondrocyte C28/I2 cells were treated with the JmjC pan inhibitor JIB-04. In parallel, the LSD family members LSD1 and LSD2 were pharmacologically inhibited using LSD1 inhibitor II or silenced using siRNA against LSD2, respectively. The role of the different JmjC subfamilies on H3K79me was studied using selective pharmacological inhibitors. We assessed the levels of H3K79me by Western blot and immunofluorescence analysis. Furthermore, we used a 3D micromass model of C28/I2 cells to evaluate changes in glycosaminoglycan content by Alcian blue staining upon histone demethylase targeting. siRNA silencing was used to dissect the individual role of selective JmjC histone demethylases on H3K79me in C28/I2 cells. This was followed by a translational assessment of the individual targeting in hACs from OA patients. In this setup, gene expression of healthy cartilage markers *Collagen2a1* (*COL2A1*) and *Aggrecan*, as well as of catabolic markers *MMP13* and *ADAMTS5*, was evaluated using real-time qPCR. Intra-articular injection of daminozide (inhibitor of KDM2/7 subfamily) was performed in an *in vivo* OA mouse model induced by destabilization of the medial meniscus (DMM), and histological analyses were performed.
Results: We found striking differences in the baseline expression of the different histone demethylases in hACs. Treatment with proinflammatory cytokine IL1-β resulted in an increase in *KDM6B* and *KDM7A* mRNA expression. Conversely, Wnt signalling activation by CHIR99021 led to a downregulation in most of histone demethylases' mRNA expression. Interestingly, pharmacological inhibition of the JmjC family using JIB-04 resulted in increased H3K79me levels in human articular chondrocytes. However, blockade of LSD family members did not lead to H3K79me changes. Inhibition of all JmjC demethylase subfamilies increased H3K79me levels, but only targeting of KDM2/7 and KDM6 subfamilies led to an increase in glycosaminoglycan content. Individual silencing of *KDM2B*, *KDM7A*, and *KDM6B* increased H3K79me. Interestingly, specific knockdown of *KDM7A* resulted in increased expression of chondrocyte healthy markers while reducing the expression of catabolic markers. Histological assessments after intra-articular injection of daminozide (inhibitor of the KDM2/7 subfamily) in DMM mouse model increased