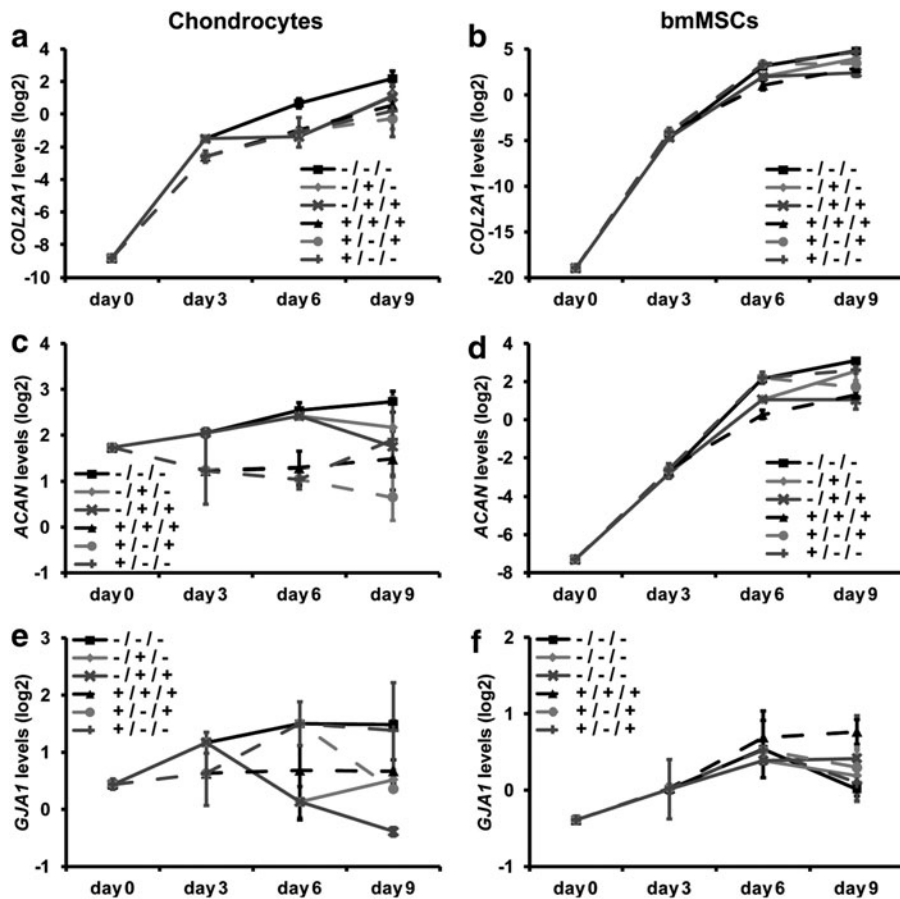


## Supplementary Data

### Effect of Intermittent Blocking of Gap Junctional Communication on Chondrogenic Differentiation

To determine whether the importance of gap junction communication changes with time in pellet differentiation cultures, chondrocyte and bone marrow-derived mesenchymal stromal cell (BM-MSC) pellets were alternately exposed to the gap junction blocker 18- $\alpha$  glycyrrhetic acid (18 $\alpha$ GCA) at various 3 day intervals during a 9 day culture period. Chondrocyte pellets receiving 18 $\alpha$ GCA over the entire 9 days of culture (+/+ /+) had lower transcript levels for *COL2A1* ( $p=0.01$ ) and *ACAN* ( $p=0.01$ ) than untreated pellets (-/- /-; Supplementary Fig. S1a, b). This was also observed with BM-MSCs (Supplementary Fig. S1d, e; *COL2A1*:  $p=0.003$ ; *ACAN*:  $p<0.001$ ). However, these two treatment groups were not significantly different with regard to *GJA1* expression (Supplementary Fig. S1c, f; chondrocytes: 0.09; MSC: 0.35). There appeared to be cell-specific differences when gap junction blocking had the greatest effect on pellet differentiation. Chondrocyte pellets

with gap junction communication blocked in the first 3 days tended to show reduced expression of these markers at day 6 even when 18 $\alpha$ GCA was removed from the culture media between day 3 and 6 (e.g.: -/- versus +/- at day 6; *ACAN*:  $p=0.01$ ; Supplementary Fig. S1b). In contrast, MSC pellets that only received gap junction blockers in the first 3 days of culture but not thereafter recovered quickly and increased expression of chondrogenic markers compared with pellets treated with 18 $\alpha$ GCA between days 3 and 6 (e.g.: +/- versus -/+ at day 6; *COL2A1*:  $p=0.03$ ; *ACAN*:  $p=0.02$ ; Supplementary Fig. S1d, e). In general, chondrogenic differentiation of BM-MSCs in pellets was more affected by disruptions in gap junctional communication between day 3 and 9 as shown by reduced *ACAN* ( $p=0.005$ ) and *COL2A1* ( $p=0.01$ ) mRNA levels compared with untreated controls at day 9 of culture (-/- /- versus -/+ /+; Supplementary Fig. S1d, e). There was no such effect on chondrocyte pellets, and mRNA levels were comparable at day 9 irrespective of when the intermittent gap junction blocking occurred (Supplementary Fig. S1a, b).



**SUPPLEMENTARY FIG. S1.** Gene expression levels of (a, d) collagen II (*COL2A1*), (b, e) aggrecan (*ACAN*), and (c, f) connexin 43 (*GJA1*) in (a-c) human articular chondrocytes or (d-f) BM-MSCs after intermittent treatment with the gap junction blocker 18 $\alpha$ GCA. Cells were differentiated in pellet cultures over 9 days, and media was changed every 3 days supplemented either with (+) or without (-) 18 $\alpha$ GCA. The different groups received 18 $\alpha$ GCA treatments in various intervals as indicated in the graphs. Control pellets were cultured in the complete absence (-/- /-) or in the presence (+/+ /+) of 18 $\alpha$ GCA for the entire period (mean  $\pm$  standard error,  $n=2$  per time point and condition, one donor). 18 $\alpha$ GCA, 18- $\alpha$  glycyrrhetic acid; BM-MSC, bone marrow-derived mesenchymal stromal cell.