Title; Sirt6 regulates postnatal growth plate differentiation and proliferation via Ihh signaling.

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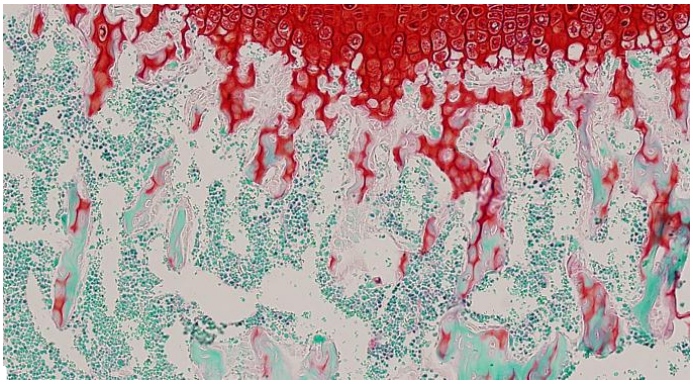
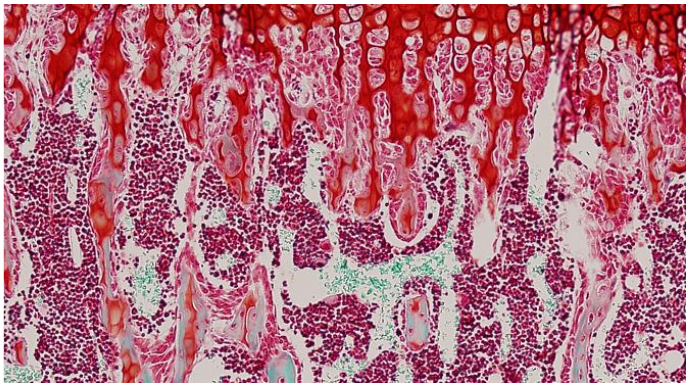
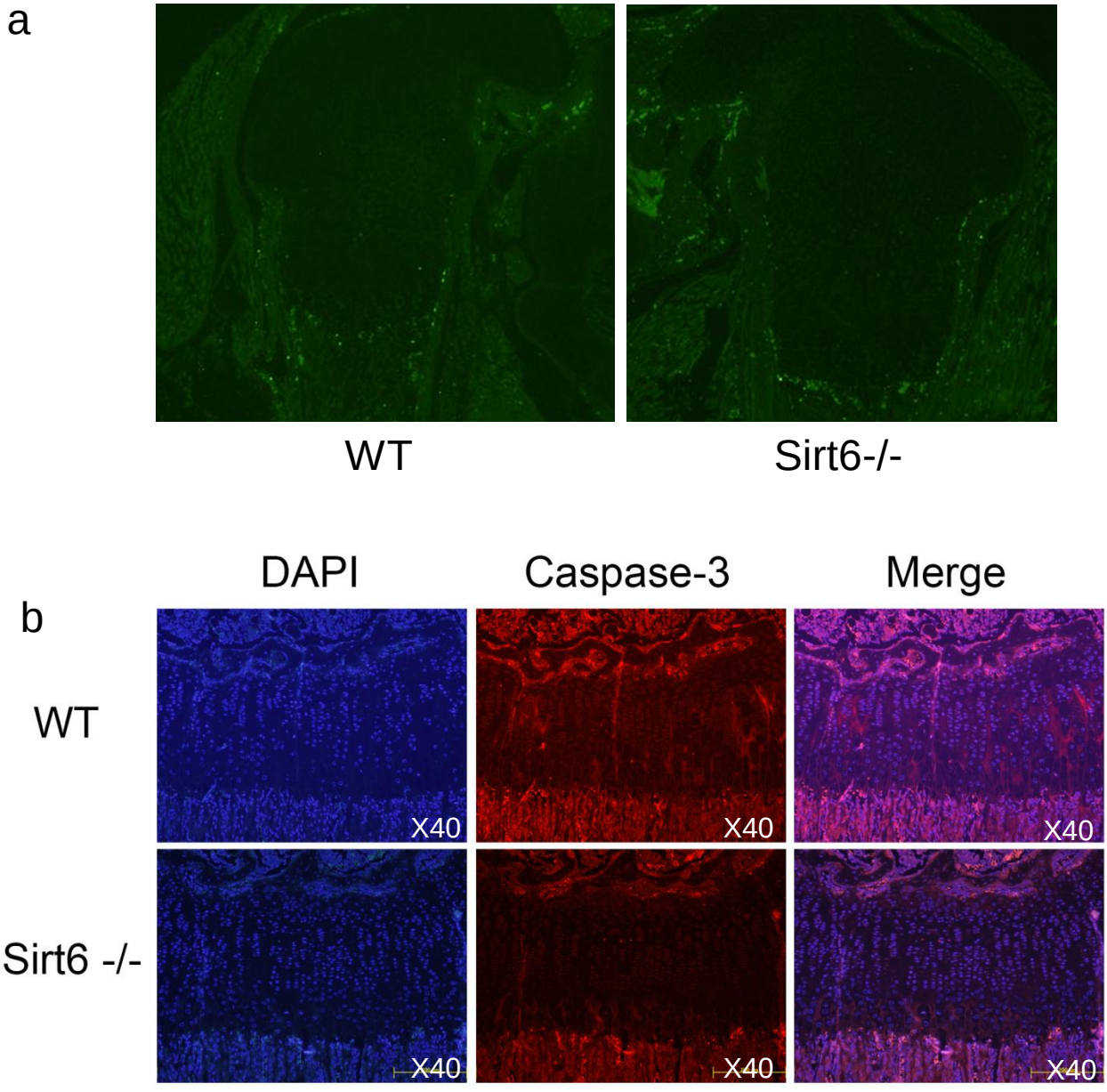
WT



Sirt6-/-



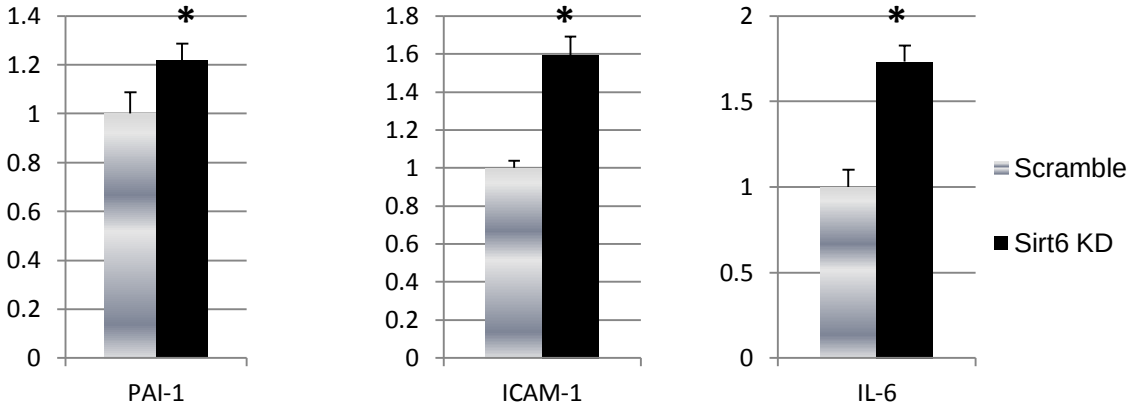
Supplementary Figure S1
Primary spongiosa from 3.5-week-old mice were stained with safranin O (X100).
Note the remarkable delay of ossification in Sirt6-/- mice.



Supplementary Figure S2
(a)TUNEL staining of P1 WT and Sirt6-/- mouse femur sections.
The number of apoptotic cells was similar between Sirt6-/- and WT.
(b) Immunohistological findings for activated caspase-3 in P14.
Caspase-3 was not activated in the proliferating zone of both WT and Sirt6-/- mice.

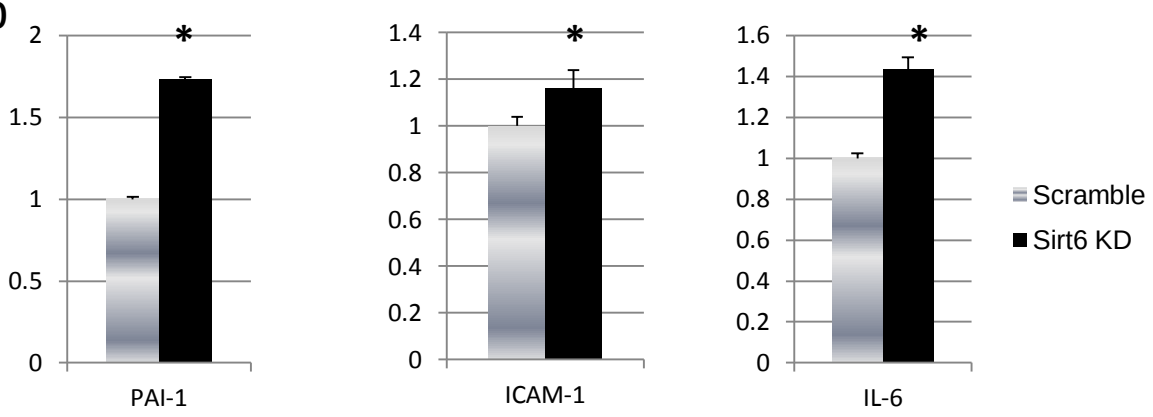
Primary chondrocyte

a

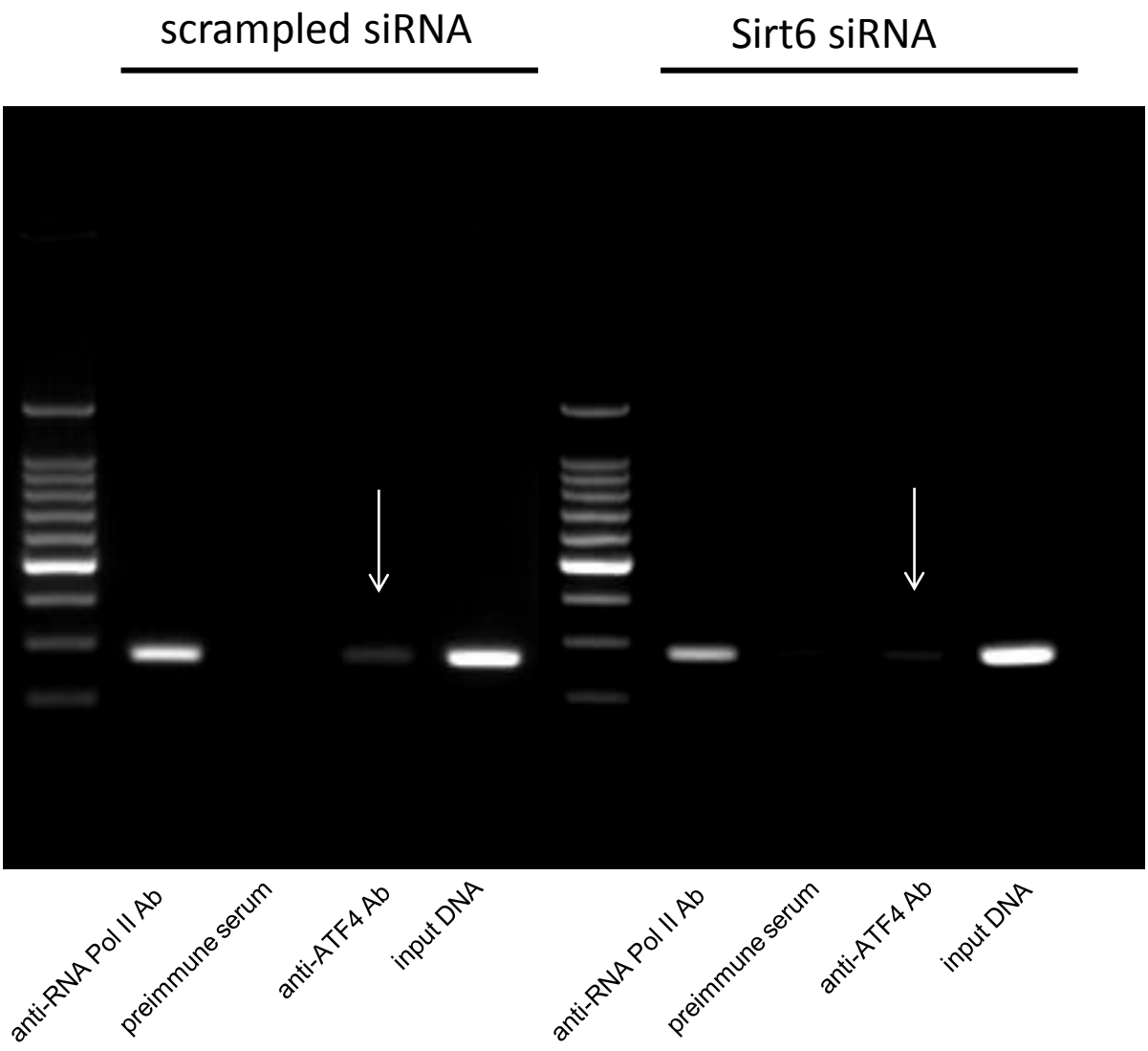


ATDC5 cells

b



Supplementary Figure S3
mRNA expression of PAI-1, ICAM-1 and IL-6 was enhanced by Sirt6 knockdown in primary chondrocyte (a) and ATDC5 cells (b).
The graph shows relative levels of gene expression.
Values represent the mean ± SD of 3 samples per group. *; $p < 0.05$.



Supplementary Figure S4
Representative ChIP of *Ihh* with anti-ATF-4 antibody (Ab) and qRT-PCR of ChIP-ed in primary chondrocytes treated with scrampled siRNA (left panal) and Sirt6 siRNA (right panel).