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3 cells before (A, B) or after 7 (C, D) and 14 (E, F) days of chondrogenic differentiaton
4 in the absence (Veh, A, C, E) or the presence of 20 nM rapamycin (Rapa, B, D,
5 F).Scale bar: 1 mm. (G) qPCR analyses of *Acan*, *Col2a1*, and *Sox9* expression in
6 ATDC5 cells before **chondrogenic induction (-IM)** or after 7 days of culture in
7 chondrogenic media (IM) supplemented without (**IM+DMSO**) or with 20 nM
8 rapamycin (IM+Rapa). n=3, *: $P<0.05$.
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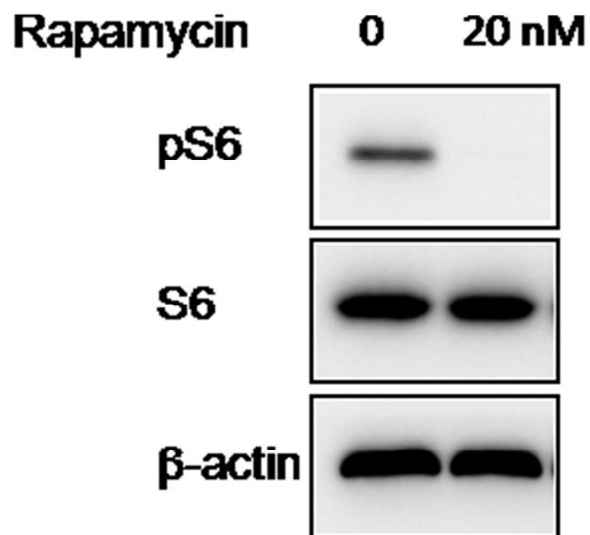
18 **Supplemental Figure 1.** Western blot analysis of protein samples isolated from
19 DMSO- or 20 nM rapamycin-treated ATDC5 cells. ATDC5 cells were subjected to
20 DMSO or rapamycin treatment for 18 hours prior to protein isolation.
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24 **Supplemental Figure 2.** Enlarged images of H&E staining on paraffin sections of
25 humerus from E12.5 wild-type (Ctrl) and RapCKO (F) embryos. Scale bar: 1 μm .
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29 **Supplemental Figure 3.** Lower magnification images of Alcian blue staining of
30 ATDC5 cells before (A, B) or after 7 (C, D) and 14 (E, F) days of chondrogenic
31 differentiaton in the absence (Veh, A, C, E) or the presence of 20 nM rapamycin
32 (Rapa, B, D, F).Scale bar: 4 mm.
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40 **Supplemental Figure 4.** Effects of rapamycin treatment on cell proliferation. ATDC5
41 cells were seeded in 6-well plates at 2×10^5 cells/well. After overnight culture, cells
42 were treated with either DMSO (Veh) or 20 nM rapamycin. Cell numbers were
43 counted after 1 day and 2 days of rapamycin treatment using a hemacytometer.
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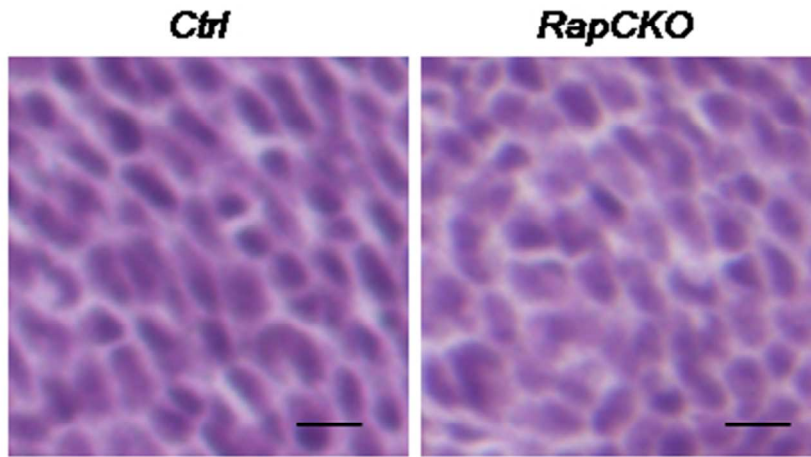
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Supplemental Figure 1
92x95mm (96 x 96 DPI)

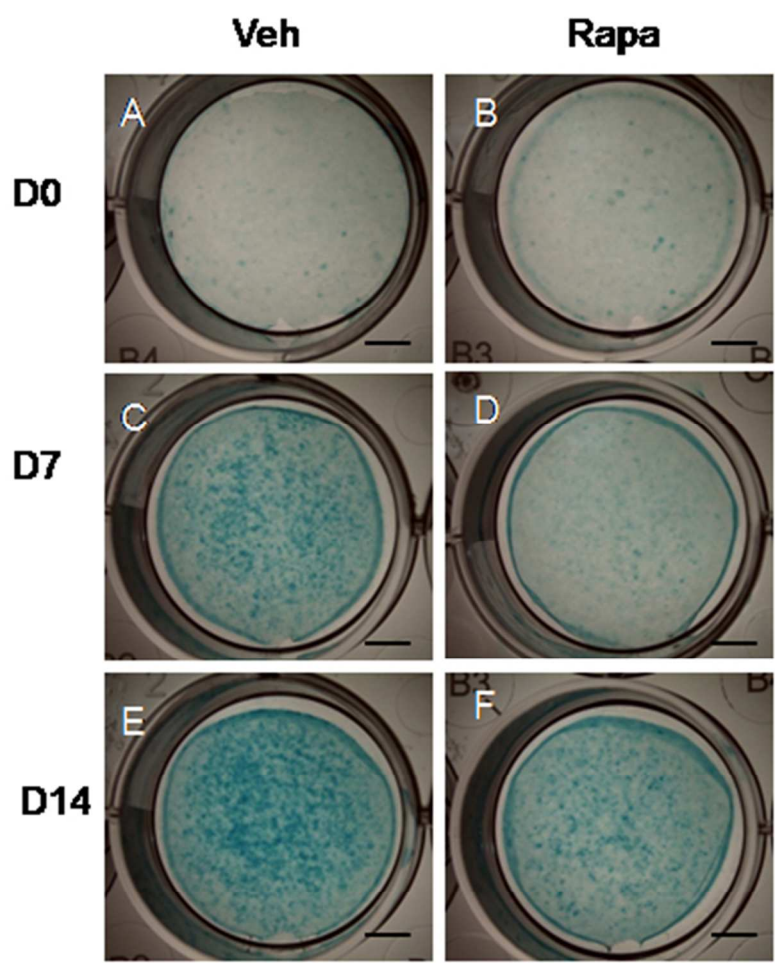
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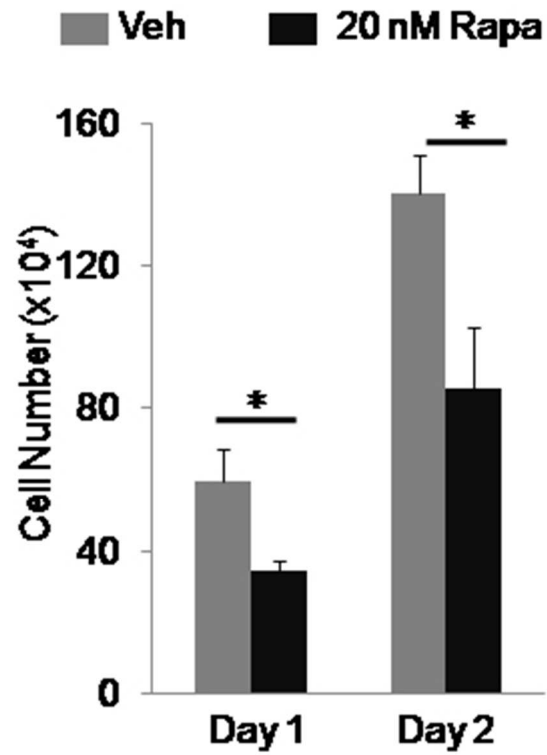


Supplemental Figure 2
127x78mm (96 x 96 DPI)

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Supplemental Figure 3
114x139mm (96 x 96 DPI)



Supplemental Figure 4
88x113mm (96 x 96 DPI)

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