

Supplementary Information

Enabling adenosine signaling to promote aged fracture healing

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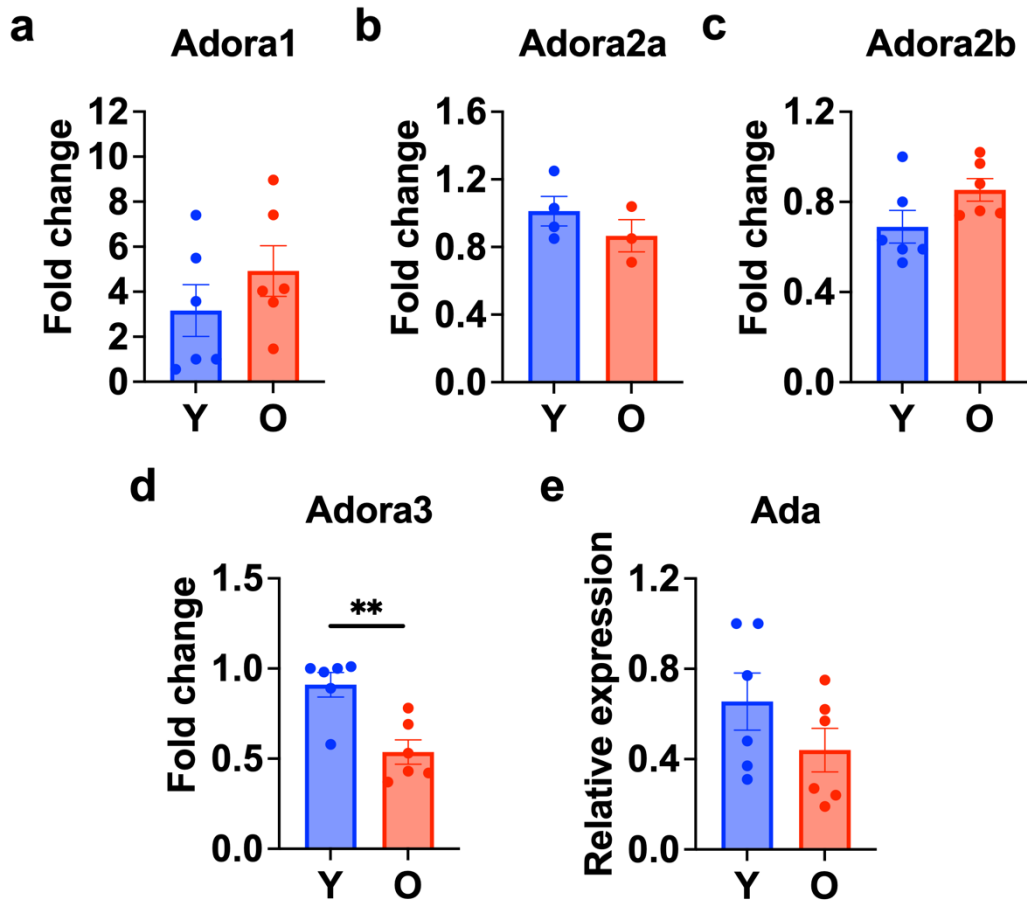
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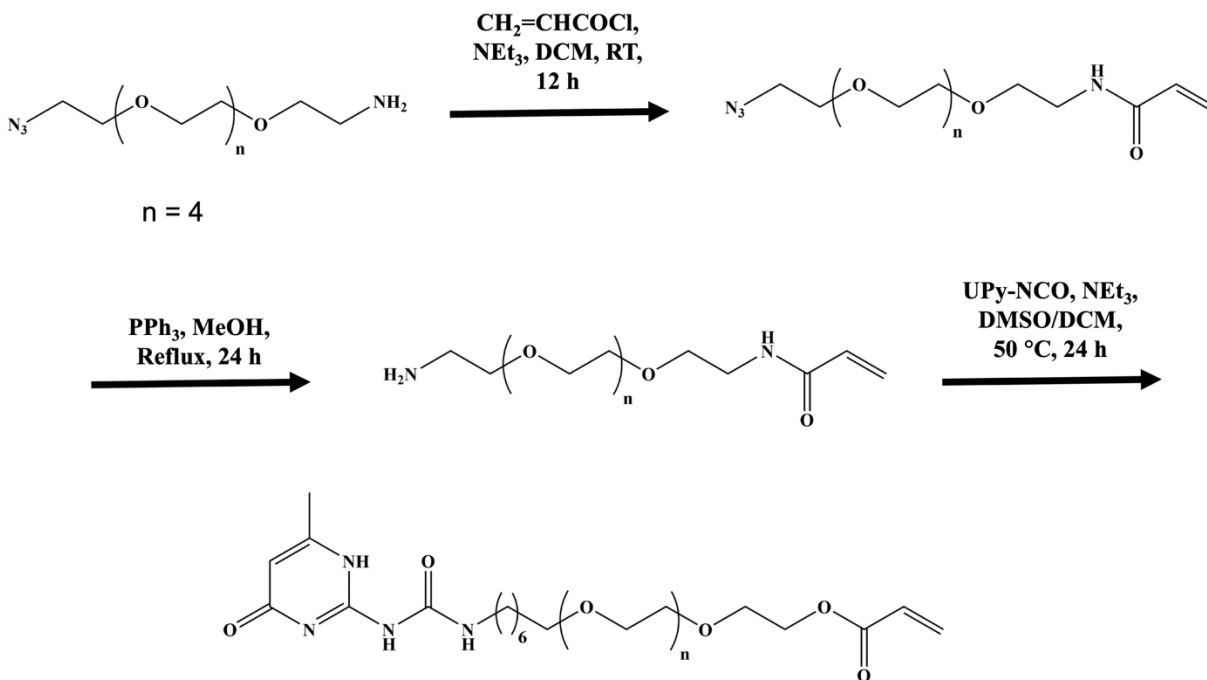
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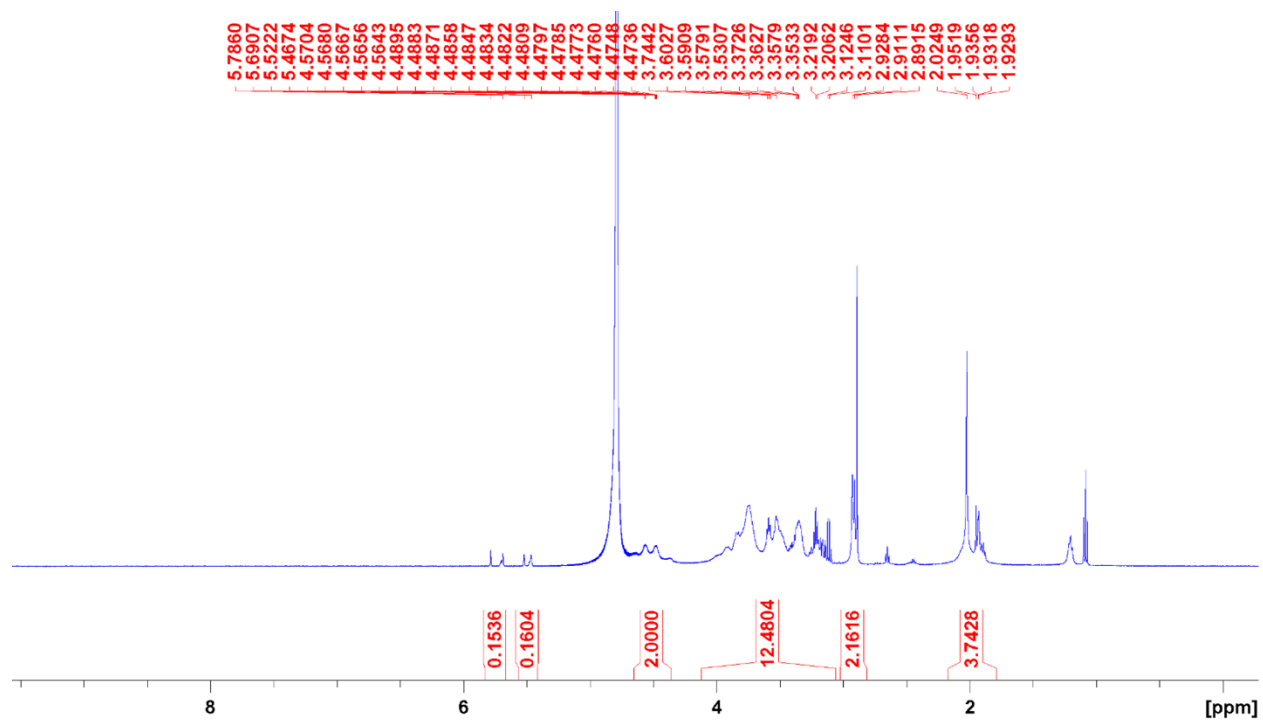
Keywords: adenosine, fracture repair, age, injectable biomaterial, drug delivery, scRNA seq



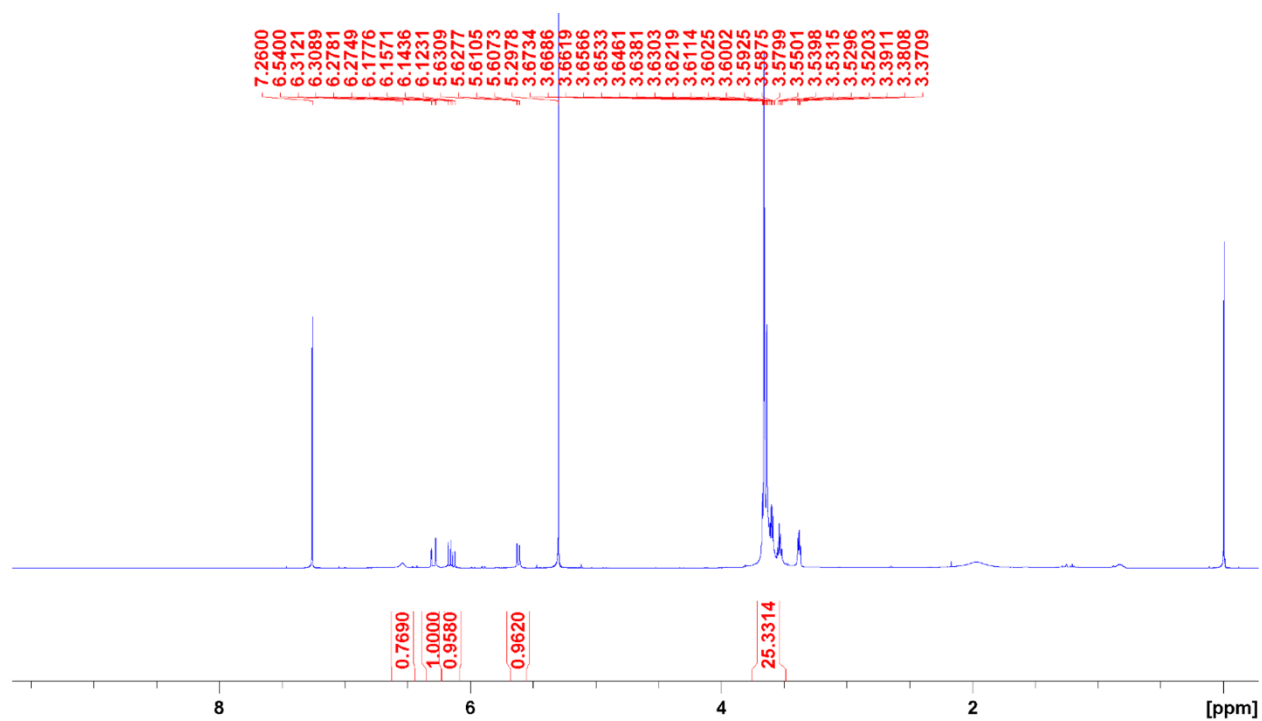
Supplementary Figure 1. Whole bone and marrow of young (3 mo.) and old (20 mo.) mice were harvested and analyzed for gene expressions of (a) *Adora1*, (b) *Adora2a*, (c) *Adora2b*, (d) *Adora3*, and (e) *Ada*. Mean $\pm$ SEM, $n=6$ mice per group. Statistical analyses were performed by two-tailed unpaired t test. ** $P < 0.01$. Unless indicated in the figure, no statistical significance between the groups was observed.



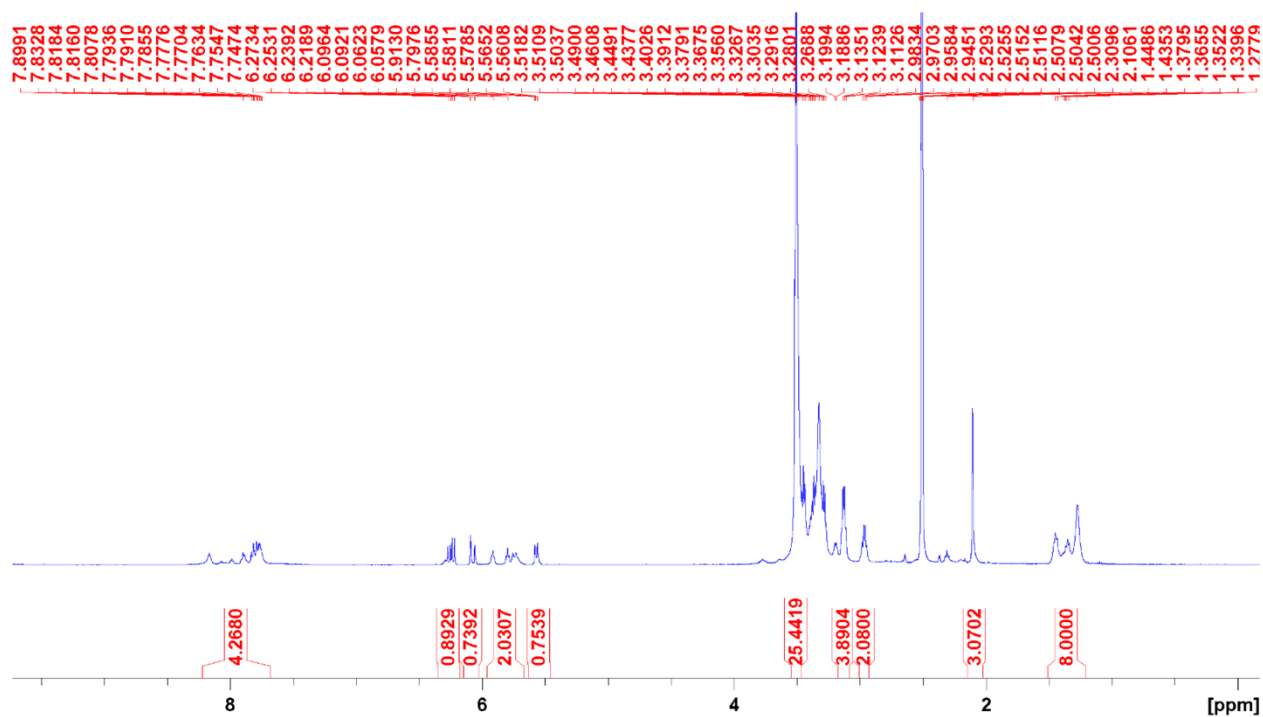
Supplementary Figure 2. Reaction scheme for the synthesis of UPy linker.



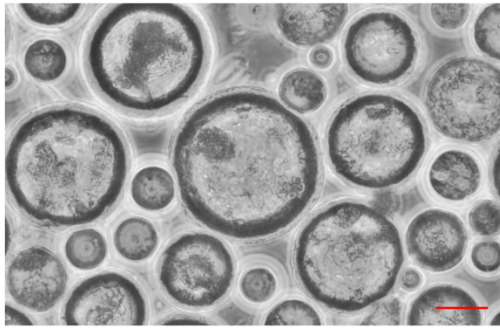
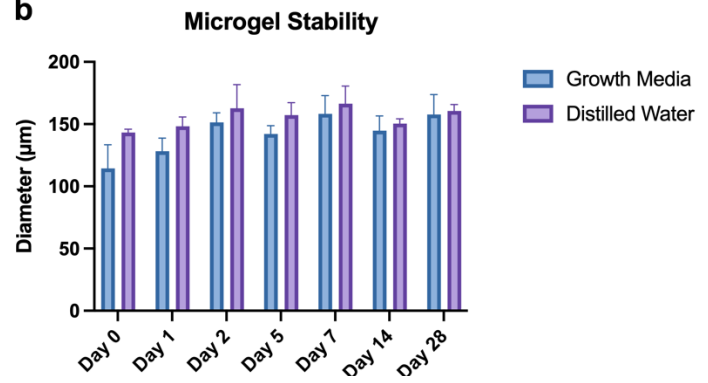
Supplementary Figure 3. ¹H NMR spectrum of HA-AEMA recorded at 500 MHz in D₂O.



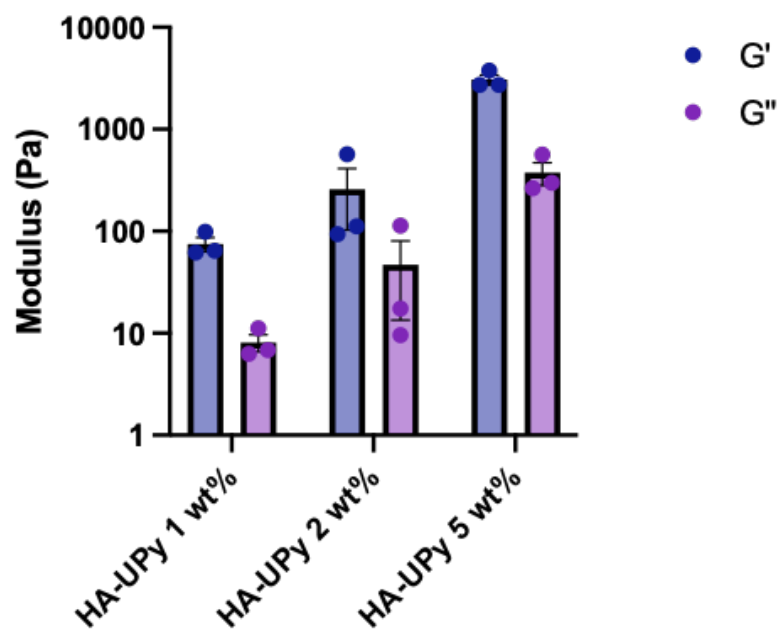
Supplementary Figure 4. ^1H NMR spectrum of N-(17-azido-3,6,9,12,15-pentaoxaheptadecyl)acrylamide recorded at 500 MHz in CDCl_3 .



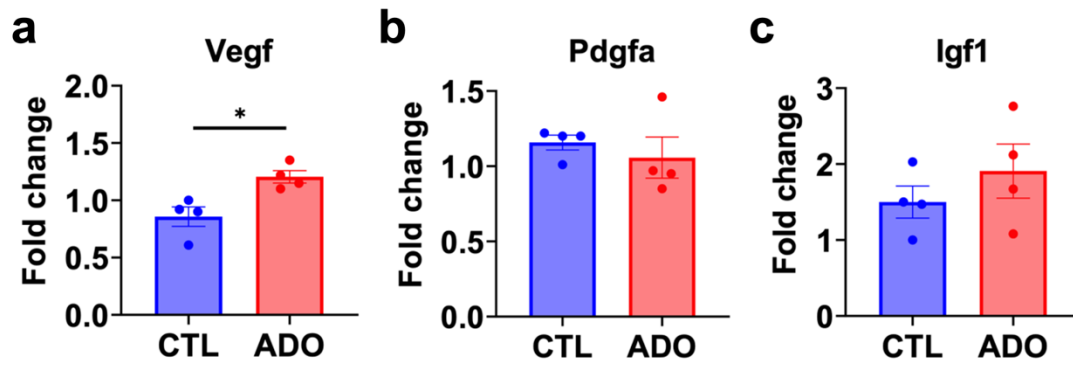
Supplementary Figure 5. ¹H NMR spectrum of UPy linker recorded at 500 MHz in DMSO-d₆.

a**b**

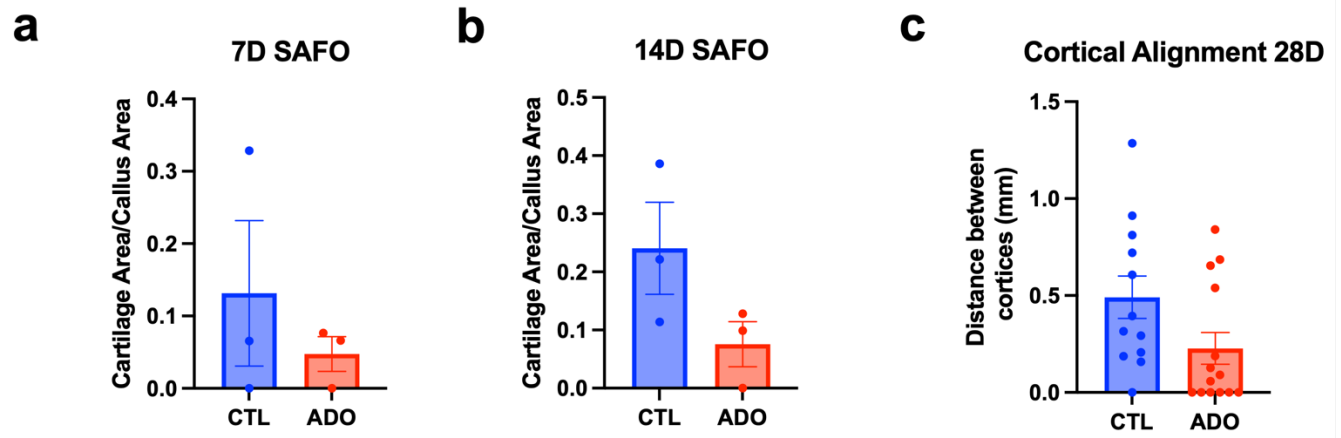
Supplementary Figure 6. (a) Image of microgels in distilled water and growth media over 28 days (scale bar: 100 μm), and (b) quantification of microgel diameter.



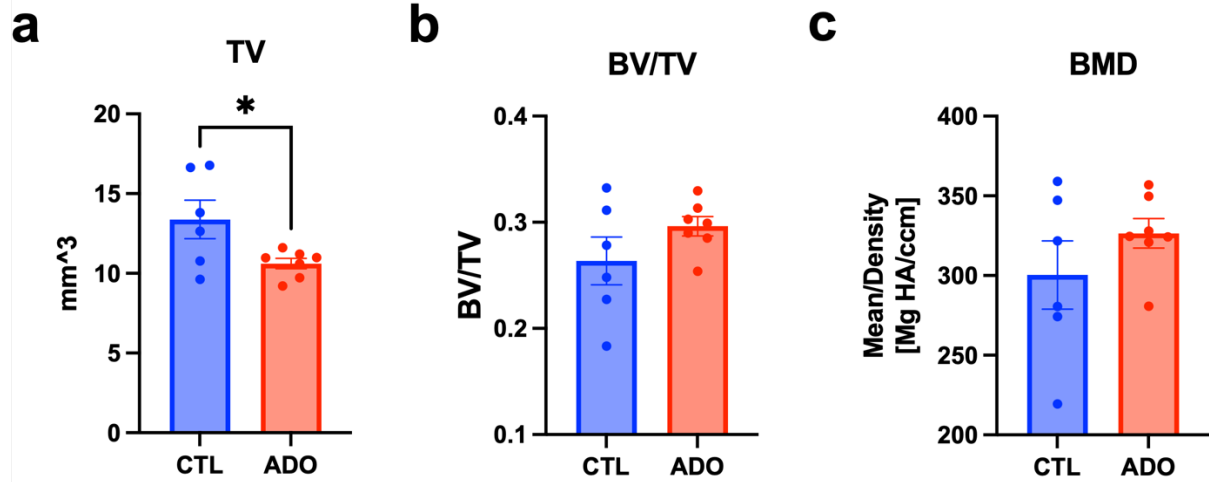
Supplementary Figure 7. Storage moduli of 1 wt%, 2 wt%, and 5 wt% microgel scaffolds UPy at a frequency of 1 Hz and 0.5% strain.



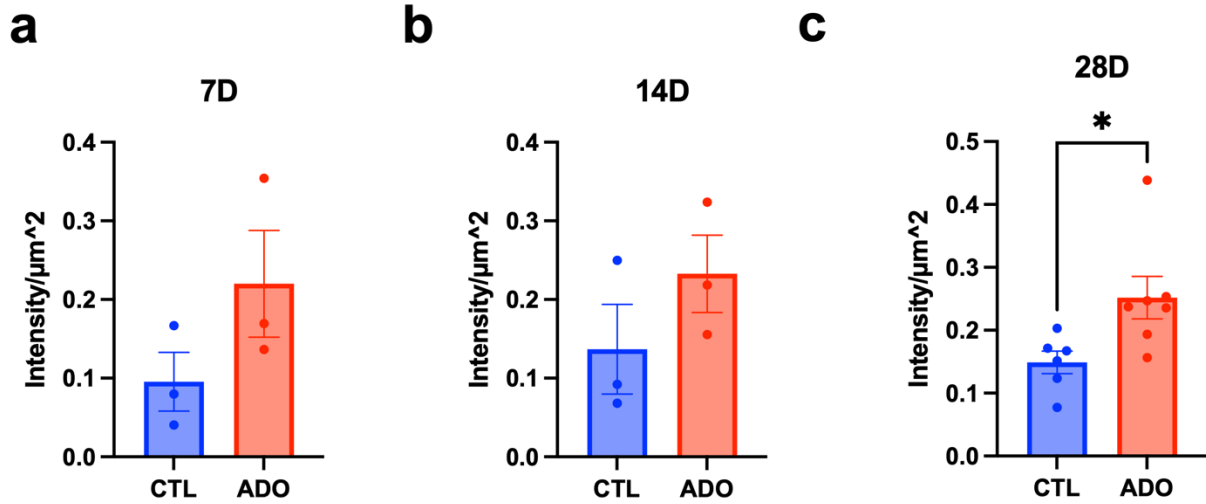
Supplementary Figure 8. Gene expression of (a) Vegf, (b) Pdgfa, and (c) Igf1 in the fractured callus assessed at 3 days post-fracture (mean $\pm$ SEM, $n=3$ mice per group). Statistical analyses were performed by two-tailed unpaired t test. * $p<0.05$. Unless indicated in the figure, no statistical significance between the groups was observed.



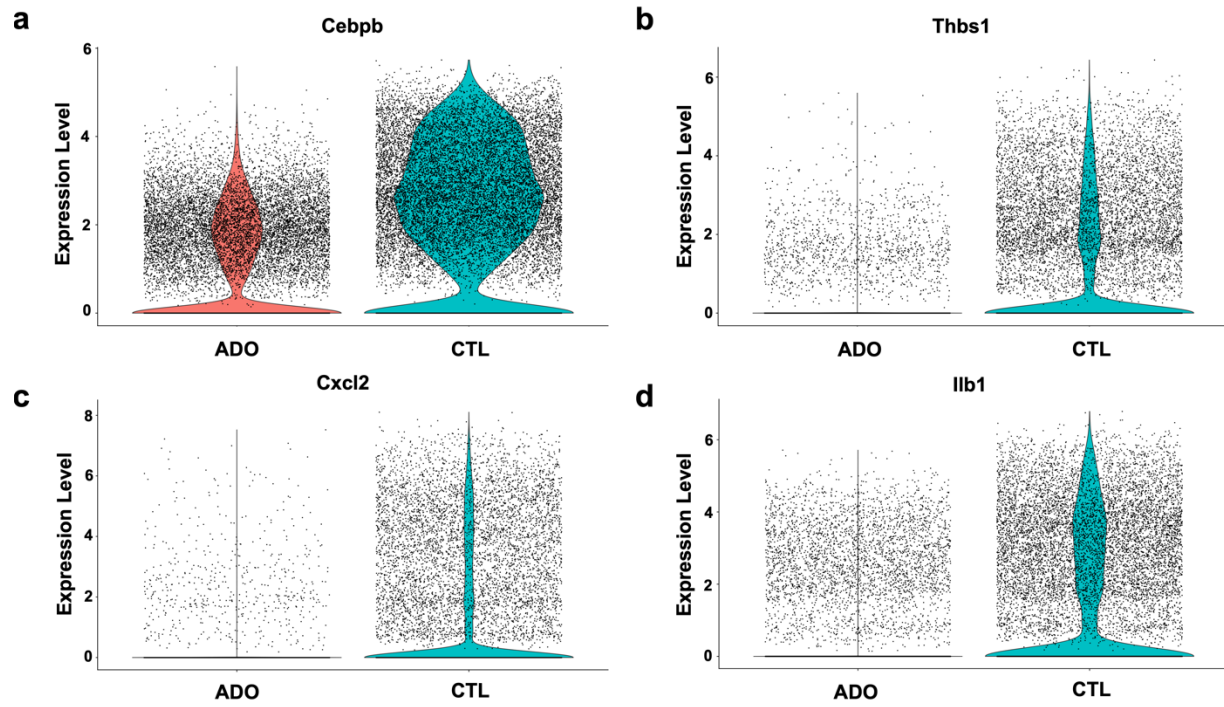
Supplementary Figure 9. Fractured callus evaluation of Safarin-O positive staining at (a) 7 days and (b) 14 days post-fracture, and (c) Cortical alignment assessed at 28 days post-fracture (mean $\pm$ SEM, $n=3$ mice per group for SAFO and $n=12$ cortices per group for cortical alignment). Statistical analyses were performed by two-tailed unpaired t test. Unless indicated in the figure, no statistical significance between the groups was observed.



Supplementary Figure 10. Quantification of the micro-CT images of fractured tibiae of old (20 mo.) mice treated with control (microgel without adenosine) or adenosine-loaded microgels. (a) Callus Volume (total volume (TV)); (b) Ration of bone volume (BV/TV); (c) bone mineral density (BMD); n = 6-7 per group. Statistical analyses were performed by two-tailed unpaired *t* test. *p<0.05; Unless indicated in the figure, no statistical significance between the groups was observed.



Supplementary Figure 11. Quantification of the endomucin staining of (a) 7 day, (b) 14 day, and (c) 28 day fractured calluses of old (20 mo.) mice at treated with control (microgel without adenosine) or adenosine-loaded microgels; $n = 3$ per group for 7D and 14D, $n = 6-7$ per group for 28D. Statistical analyses were performed by two-tailed unpaired t test. $*p < 0.05$. Unless indicated in the figure, no statistical significance between the groups was observed.



Supplementary Figure 12. Violin plots showing expression of (a) *Cebpb*, (b) *Thbs1*, (c) *Cxcl2*, and (d) *Ilb1* between aged mice treated with control or adenosine-containing microgel scaffolds.