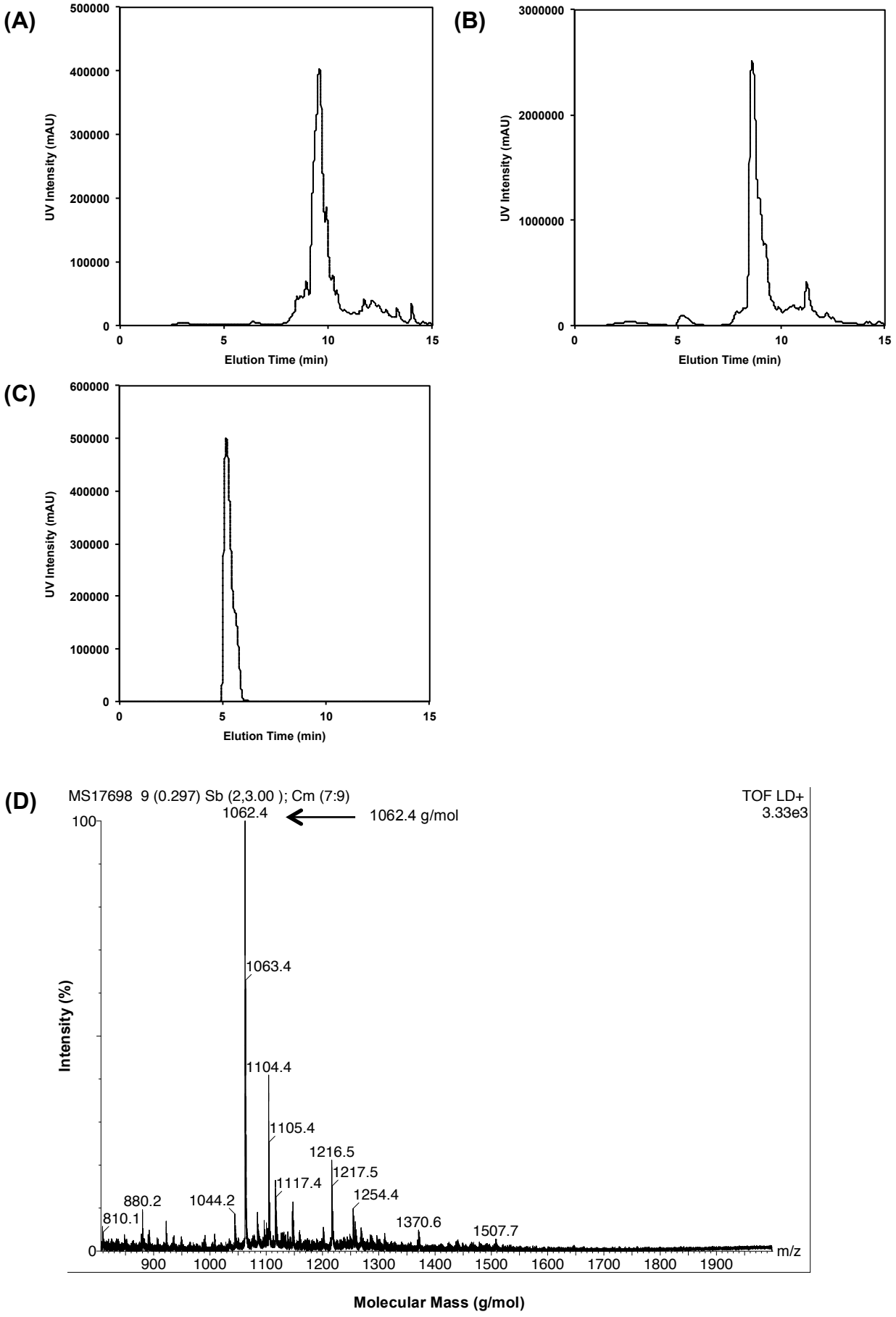
(A)
UV Intensity (mAU)
Elution Time (min)
(B)
UV Intensity (mAU)
Elution Time (min)
(C)
UV Intensity (mAU)
Elution Time (min)
(D)
MS17698 9 (0.297) Sb (2,3.00); Cm (7:9)
TOF LD+
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1062.4 g/mol
1063.4
1104.4
1105.4
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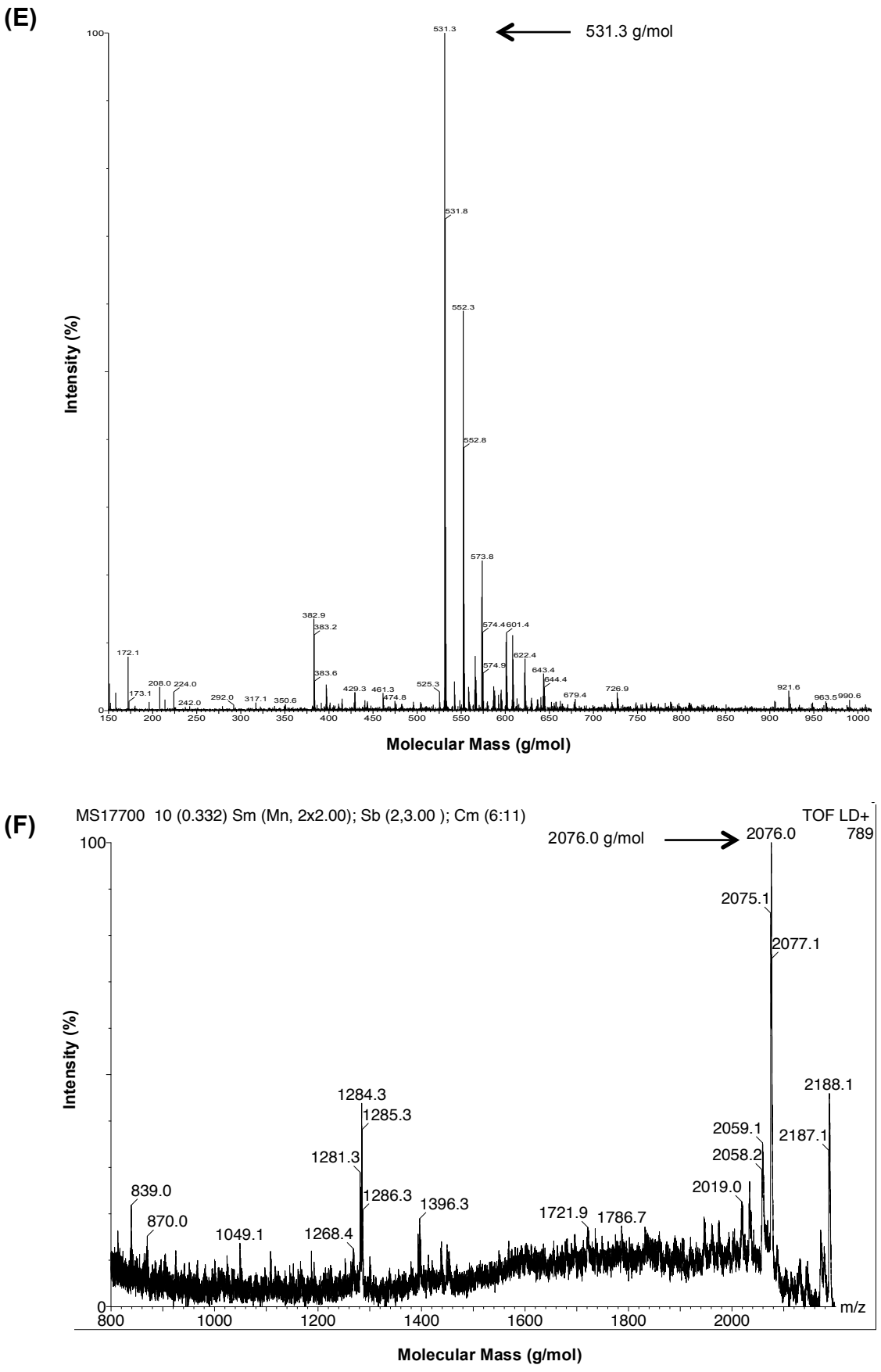
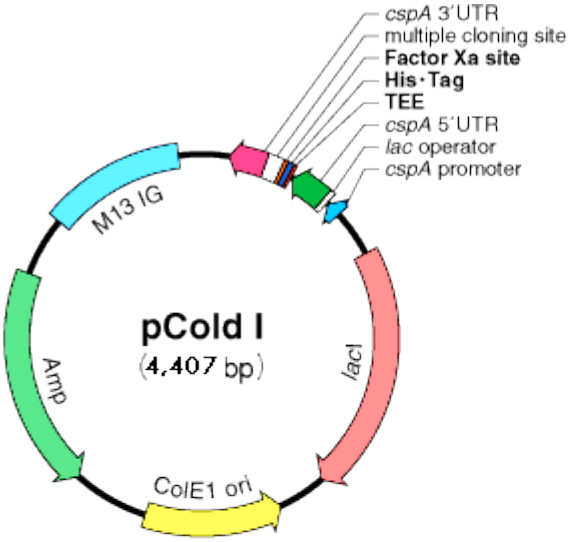


Fig. S1. Representative reverse phase HPLC chromatograms and MALDI/ESI spectra of (A, D) MMP7 (MW: 1062.4 g/mol), (B, E) ScrMMP7 (MW/2: 531.3 g/mol, MW: 1062.6 g/mol), and (C, F) ACAN (MW: 2076.0 g/mol) peptides, respectively.

(A)



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           ------+
             NheI
           -+-----
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                       AbsI
                     --+------
                KpnI                                  PstI
              -----+                                 ------+
          SacI        PspXI                    AccI
        ------+     --+------                --+---
       Eco53kI       XhoI         EcoRI       SalI
       ---+---      -+-----      -+----      -+----
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                                                 ClaI
                                               --+---
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         BsmI    EcoO109I                                                NspI
        ------   --+----                                               -----+
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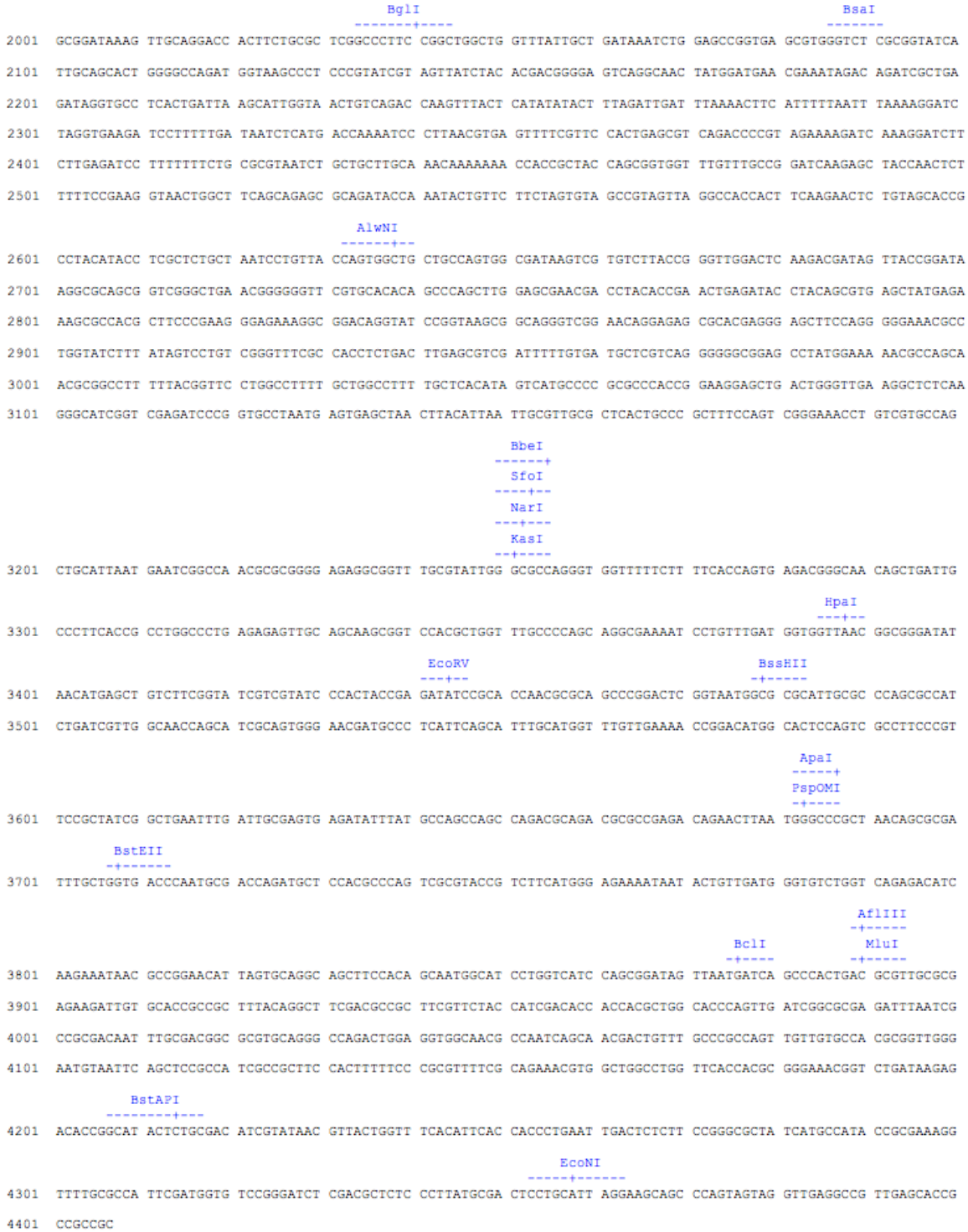
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(B)

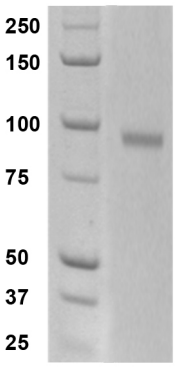

Fig. S2. (A) A fully annotated plasmid map of the pColdI vector system used to sub–clone the final DNA sequence for expression in *E. coli*. (B) A representative SDS–PAGE gel of the HIHA-Scl2 protein.

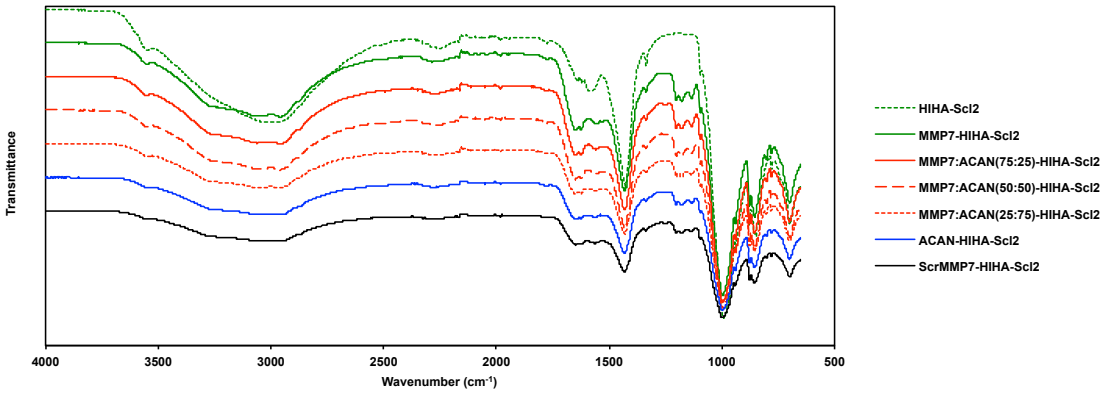

Fig. S3. Representative FTIR spectra of functionalized Scl2 proteins confirming the conjugation of Scl2 with acrylate–functionalized MMP7 and ACAN peptides.

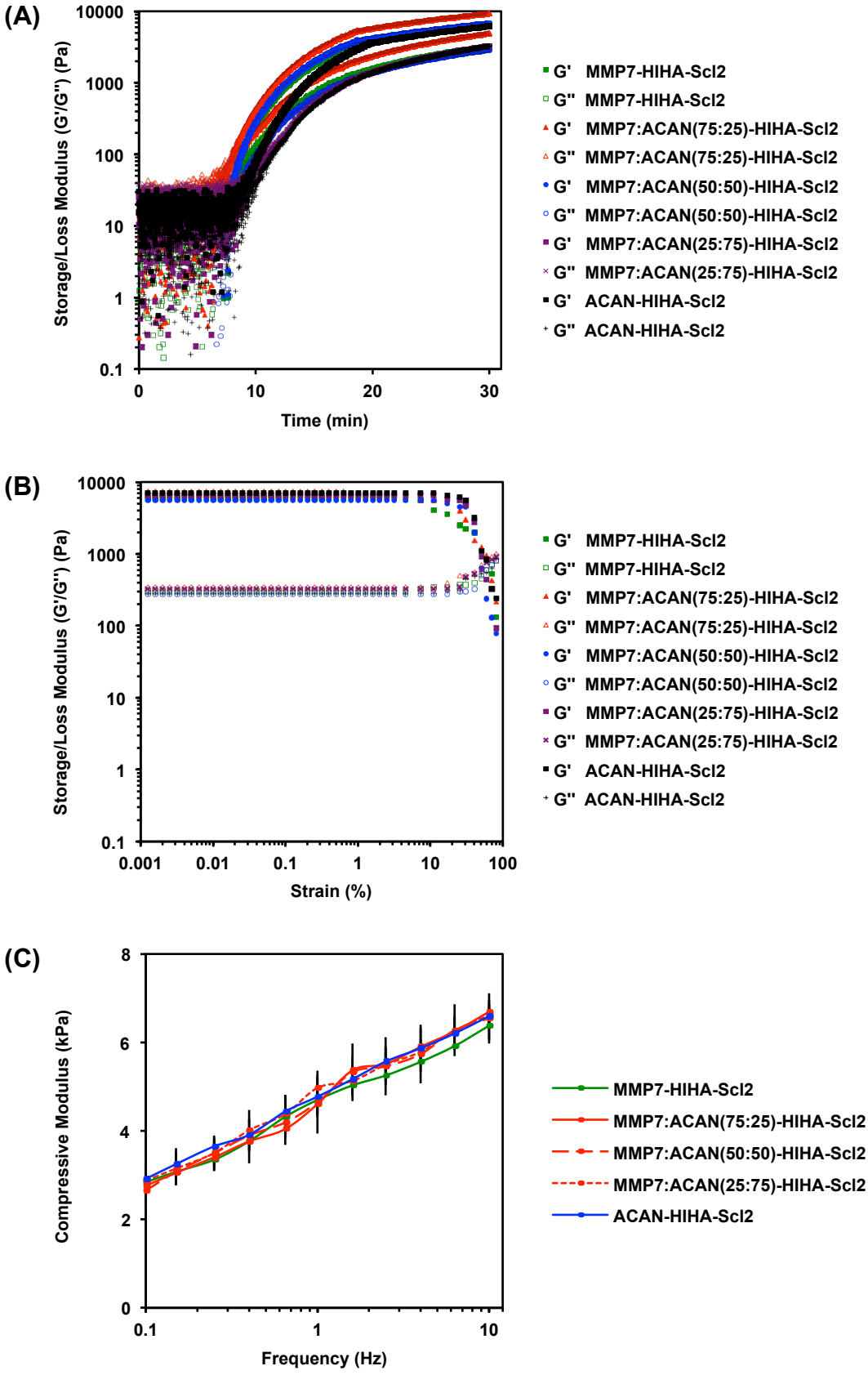

Fig. S4. Rheological properties of acellular functionalized Scl2 hydrogels. (A) Time to gelation determined at a temperature of 37 °C, angular frequency of 6.28 rad/s, and strain of 0.5% shown as G' and G''. (B) Strain sweep at a temperature of 37 °C and an angular frequency of 6.28 rad/s shown as G' and G''. (C) Dynamic mechanical analysis (DMA) used to determine the elastic modulus in unconfined compression of hydrogels compressed to 10% strain at 0.5% strain/min from 0.1 to 10 Hz. Values represent means ± SD (n = 3).

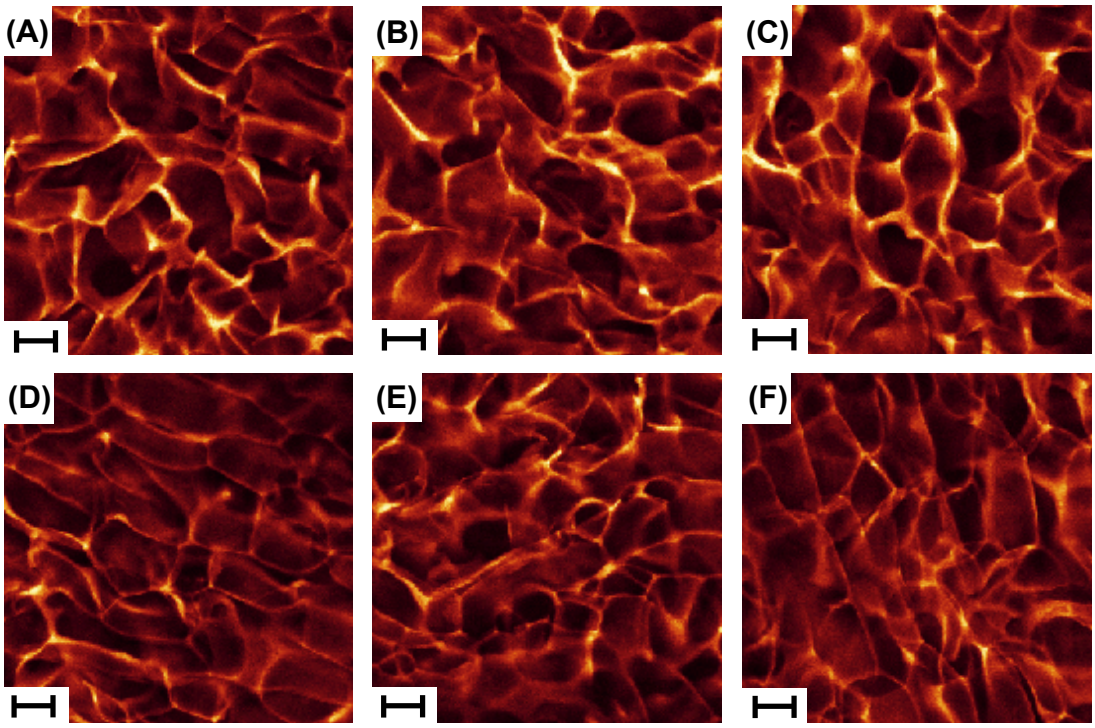

Fig. S5. Representative multi–photon second harmonic generation (MP–SHG) images of acellular (A) ScrMMP7-HIHA-Scl2, (B) MMP7-HIHA-Scl2, (C) MMP7:ACAN(75:25)-HIHA-Scl2, (D) MMP7:ACAN(50:50)-HIHA-Scl2, (E) MMP7:ACAN(25:75)-HIHA-Scl2, and (F) ACAN-HIHA-Scl2 hydrogels (scale bars are 10 μm).

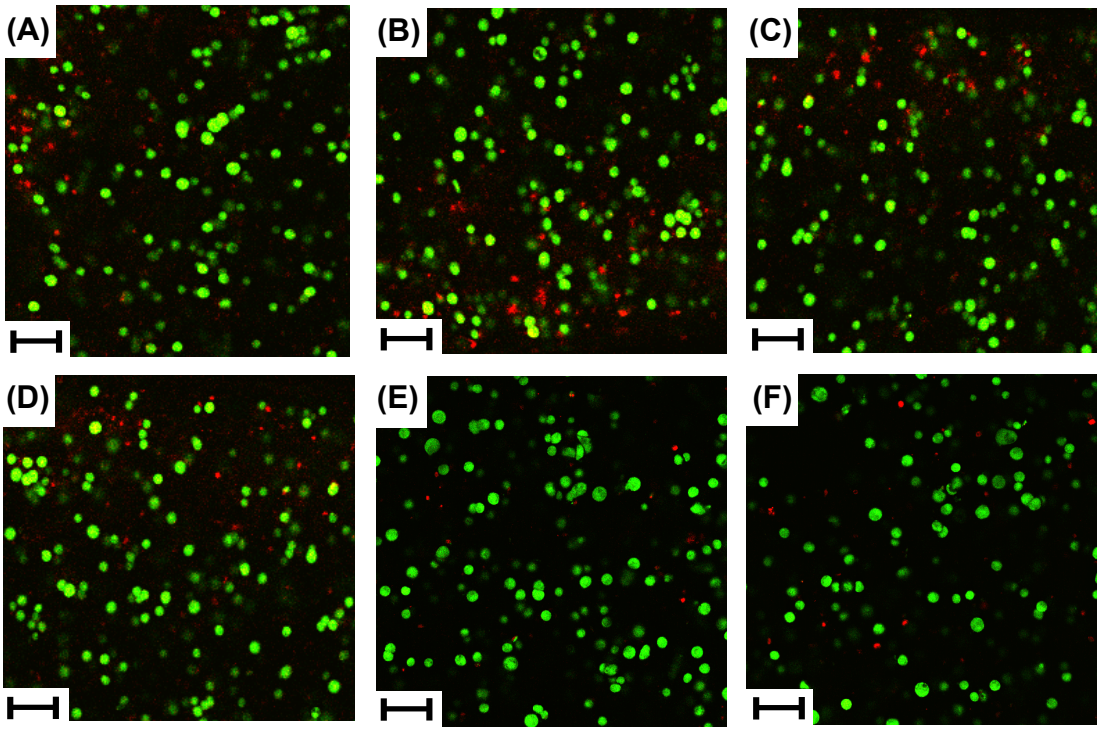

Fig. S6. hMSC viability in Scl2 hydrogels. LIVE/DEAD® Viability/Cytotoxicity assay on hMSCs cultured for 6 weeks in hydrogels. Representative confocal images of cells in (A) ScrMMP7-HIHA-Scl2, (B) MMP7-HIHA-Scl2, (C) MMP7:ACAN(75:25)-HIHA-Scl2, (D) MMP7:ACAN(50:50)-HIHA-Scl2, (E) MMP7:ACAN(25:75)-HIHA-Scl2, and (F) ACAN-HIHA-Scl2 hydrogels (scale bars are 50 μm).

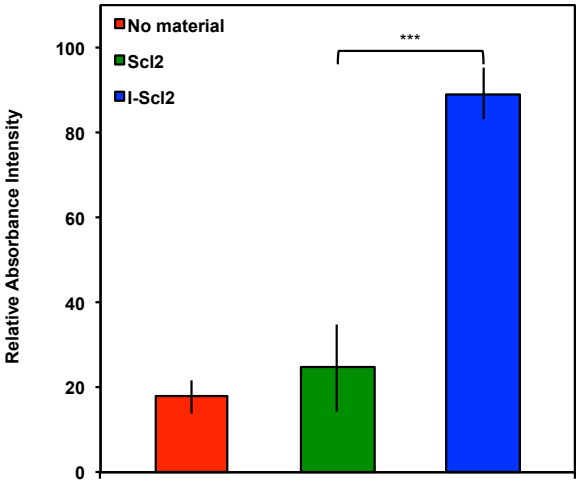

Fig. S7. Integrin (α1β1 and α2β1) binding of hMSCs on Scl2 proteins. Empty wells were used as a negative control denoted 'no material'. Values represent means ± SD. ***$p < 0.001$ (n = 3 for each donor; 3 different bone marrow–derived hMSC donors).

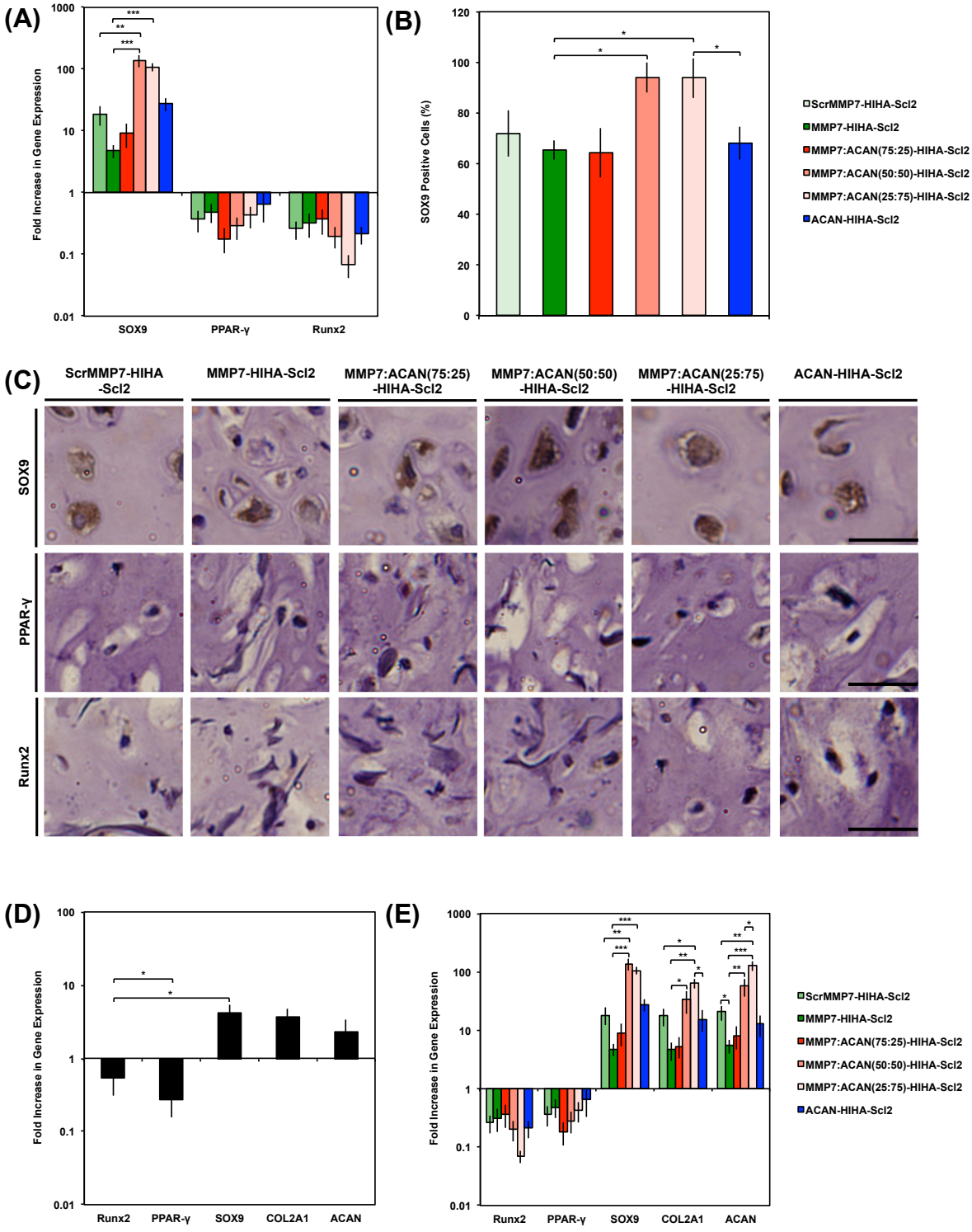

Fig. S8. (A) hMSC gene expression in Scl2 hydrogels. SOX9, Runx2, and PPAR-γ gene expression of hMSCs encapsulated in hydrogels after 2 weeks in culture, as analyzed using the ΔΔCt method. Data presented as a fold difference relative to undifferentiated hMSCs (calibrator) prior to encapsulation and normalized to GAPDH (housekeeping gene). (B) Percentage positive SOX9 cells determined from immunohistochemical stained sections for SOX9 and normalized to total number of cells. (C) High magnification images showing representative immunohistochemical examination of hMSC–seeded hydrogels after 14 days in culture. Hydrogels are stained for SOX9, PPAR-γ, and Runx2, respectively, from top to bottom. Scale bars are 100 μm. (D) hMSC gene expression in pellet cultures. Runx2, PPAR-γ, SOX9, COL2A1, and ACAN gene expression of hMSCs after 2 weeks in culture, as analyzed using the ΔΔCt method. Data presented as a fold difference relative to undifferentiated hMSCs (calibrator) and normalized to GAPDH (housekeeping gene). (E) hMSC gene expression in Scl2 hydrogels. Runx2, PPAR-γ, SOX9, COL2A1, and ACAN gene expression of hMSCs encapsulated in hydrogels after 2 weeks in culture, as analyzed using the ΔΔCt method. Data presented as a fold difference relative to undifferentiated hMSCs (calibrator) prior to encapsulation and normalized to GAPDH (housekeeping gene). The data in Fig. S8 is reproduced from Fig. 6. Values represent means ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$ (n = 3 for each donor; 3 different bone marrow–derived hMSC donors).

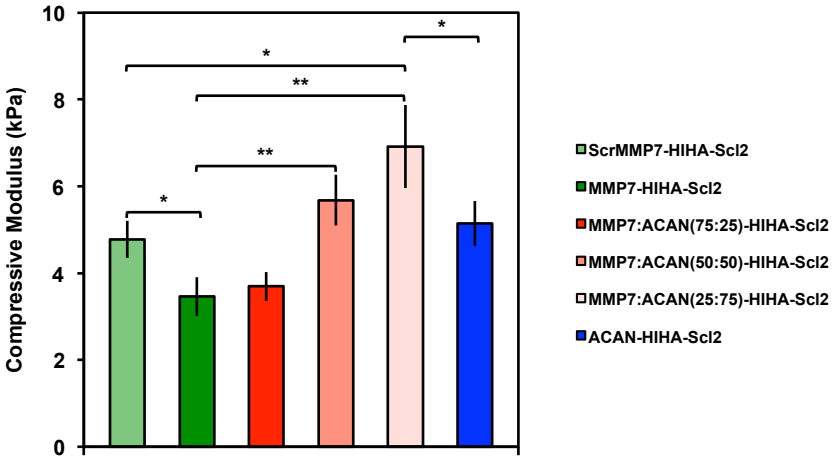

Fig. S9. Dynamic mechanical analysis (DMA). Elastic compression moduli of cell–seeded Scl2 hydrogels compressed to 10% strain at 0.5% strain/min and 1 Hz after 6 weeks of culture. Values represent means ± SD. (n = 3 for each donor; 3 different bone marrow–derived hMSC donors).

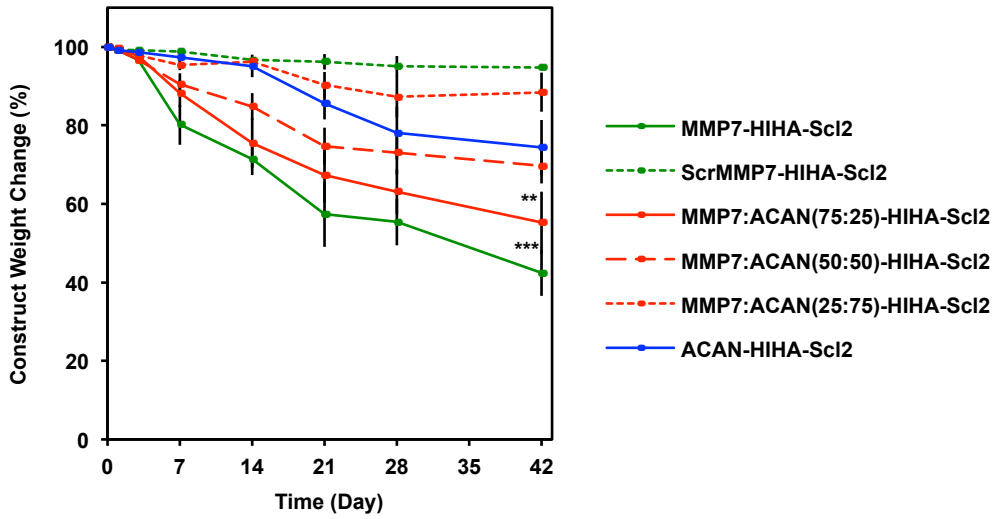

Fig. S10. Scl2 hydrogel dry weight change over time in culture with hMSCs. Weight change was normalized to dry weight at day 0. Values represent means ± SD. $^{**}p < 0.01$ versus MMP7:ACAN(25:75)-HIHA-Scl2, $^{***}p < 0.001$ versus MMP7:ACAN(25:75)-HIHA-Scl2 (n = 3 for each donor; 3 different bone marrow–derived hMSC donors).

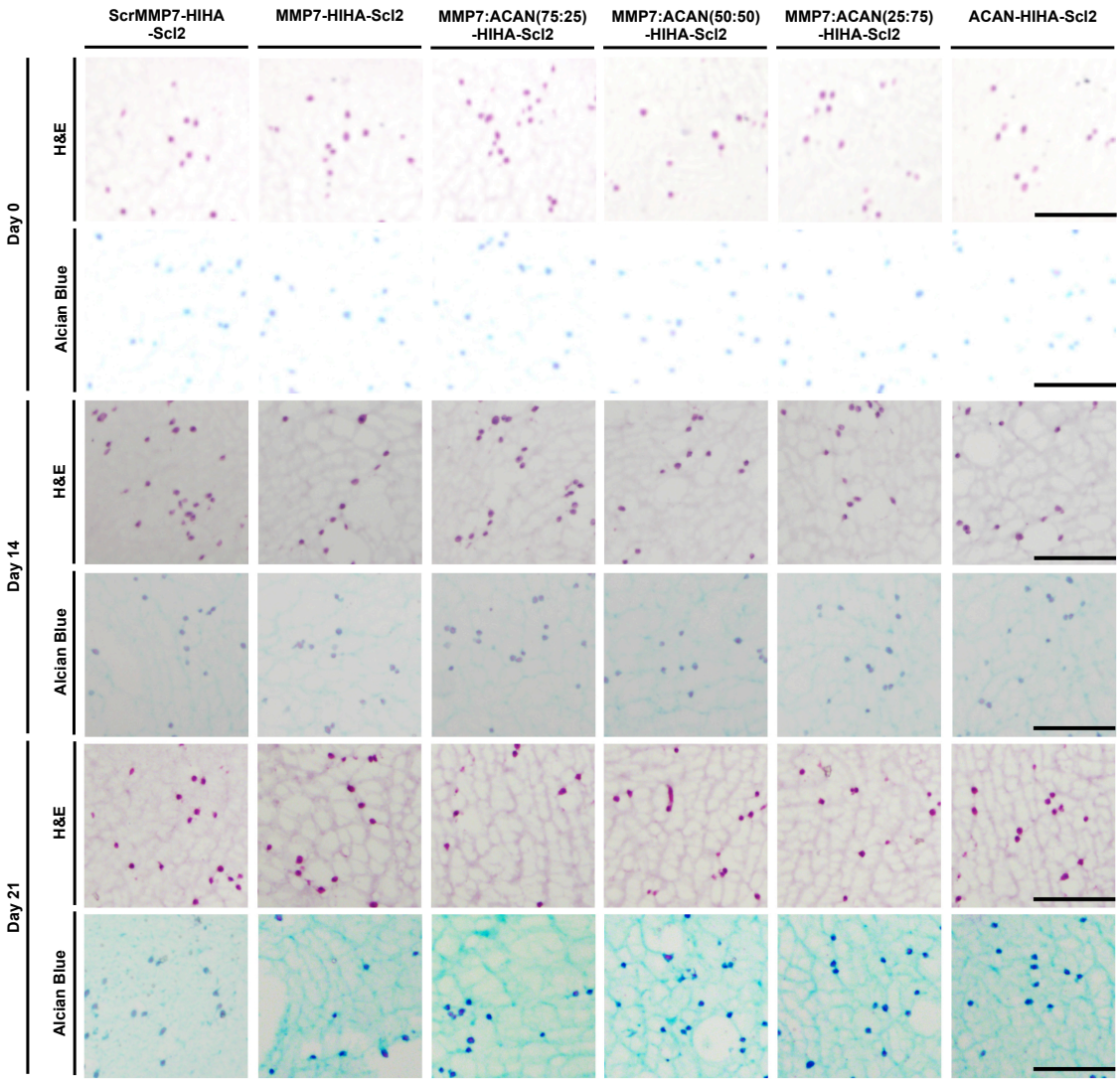

Fig. S11. ECM accumulation of hMSC–seeded Scl2 hydrogels. Representative histological examination of hMSC–seeded hydrogels after 0, 14, and 21 days in culture. Hydrogels are stained with haematoxylin and eosin (H&E) and alcian blue for sGAG. Scale bars are 50 μm.

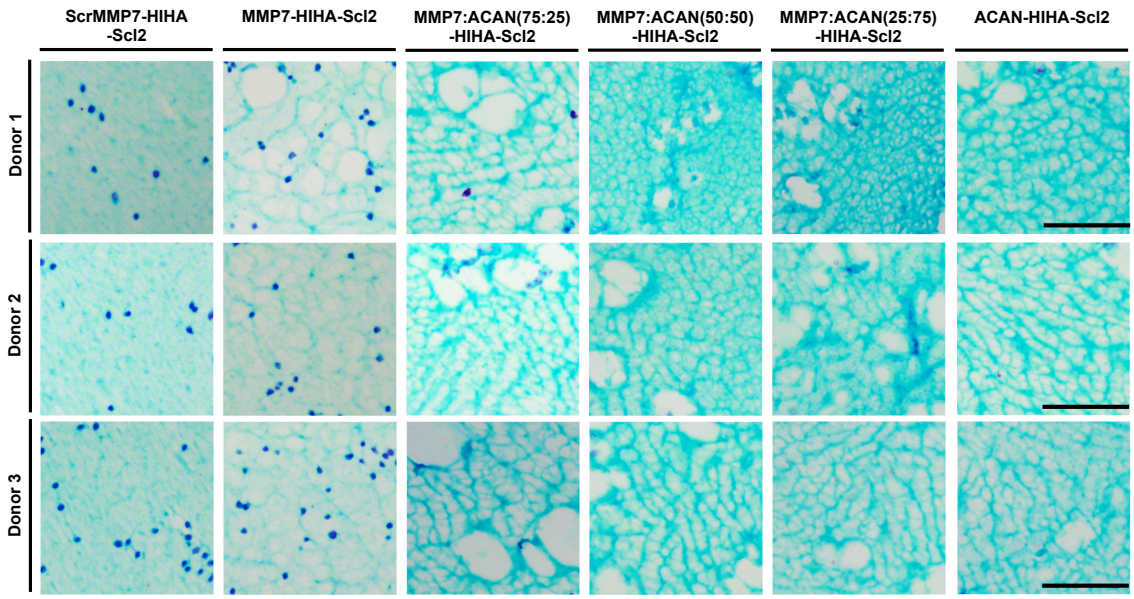

Fig. S12. ECM accumulation of hMSC–seeded Scl2 hydrogels from three different donors. Representative histological examination of hMSC–seeded hydrogels after 42 days in culture. Donor 1 images are reproduced from Fig. 7. Hydrogels are stained with alcian blue for sGAG. Scale bars are 50 μm.

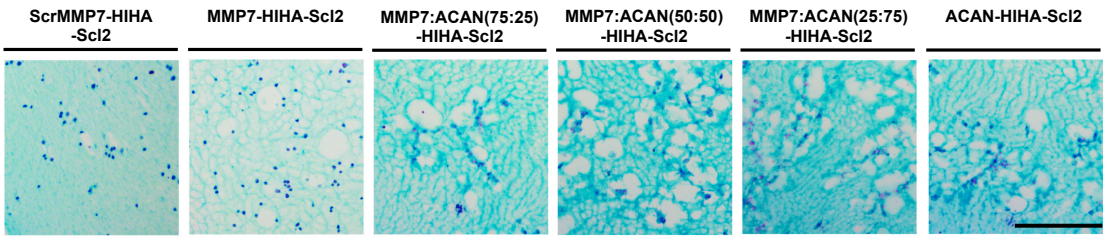

Fig. S13. Low magnification images demonstrating ECM accumulation of hMSC–seeded Scl2 hydrogels. Representative histological examination of hMSC–seeded hydrogels after 6 weeks in culture. Hydrogels are stained with alcian blue for sGAG. Scale bars are 200 μm.

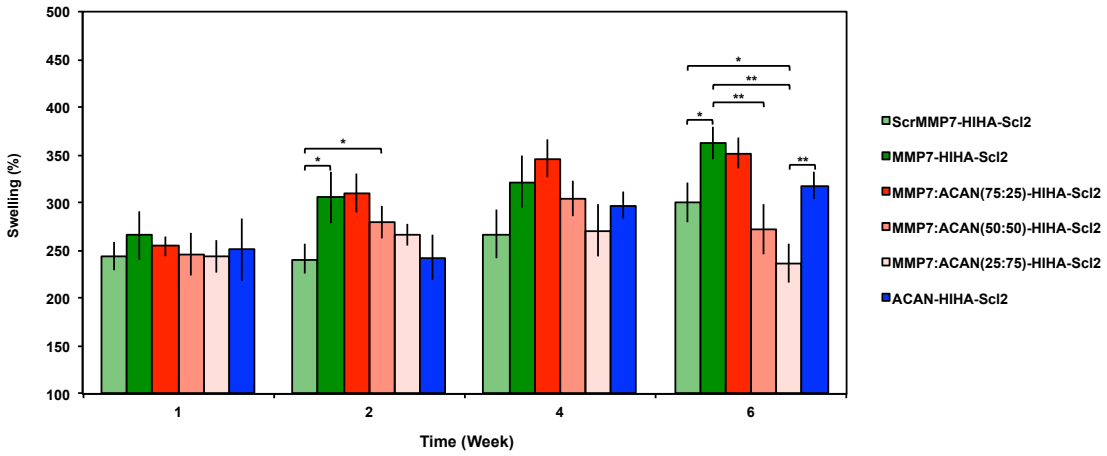

Fig. S14. Swelling behavior of cell–seeded Scl2 hydrogels over time. Values represent means ± SD. *$p < 0.05$, **$p < 0.01$ (n = 3 for each donor; 3 different bone marrow–derived hMSC donors).