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Supplementary Materials for

Synthetic matrix enhances transplanted satellite cell engraftment in dystrophic and aged skeletal muscle with comorbid trauma

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/4/8/eaar4008/DC1)

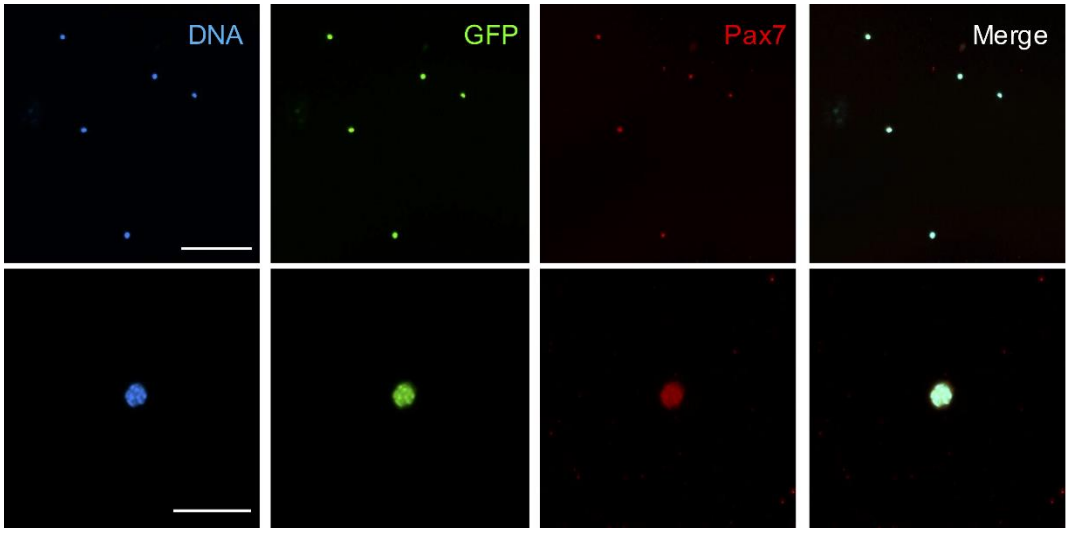

Fig. S1. Isolated primary MuSCs are Pax7^{+}. Freshly isolated cells were encapsulated in PEG-4MAL hydrogel and immediately fixed and stained. Top row scale bar: 100 μm. Bottom row scale bar: 10 μm.

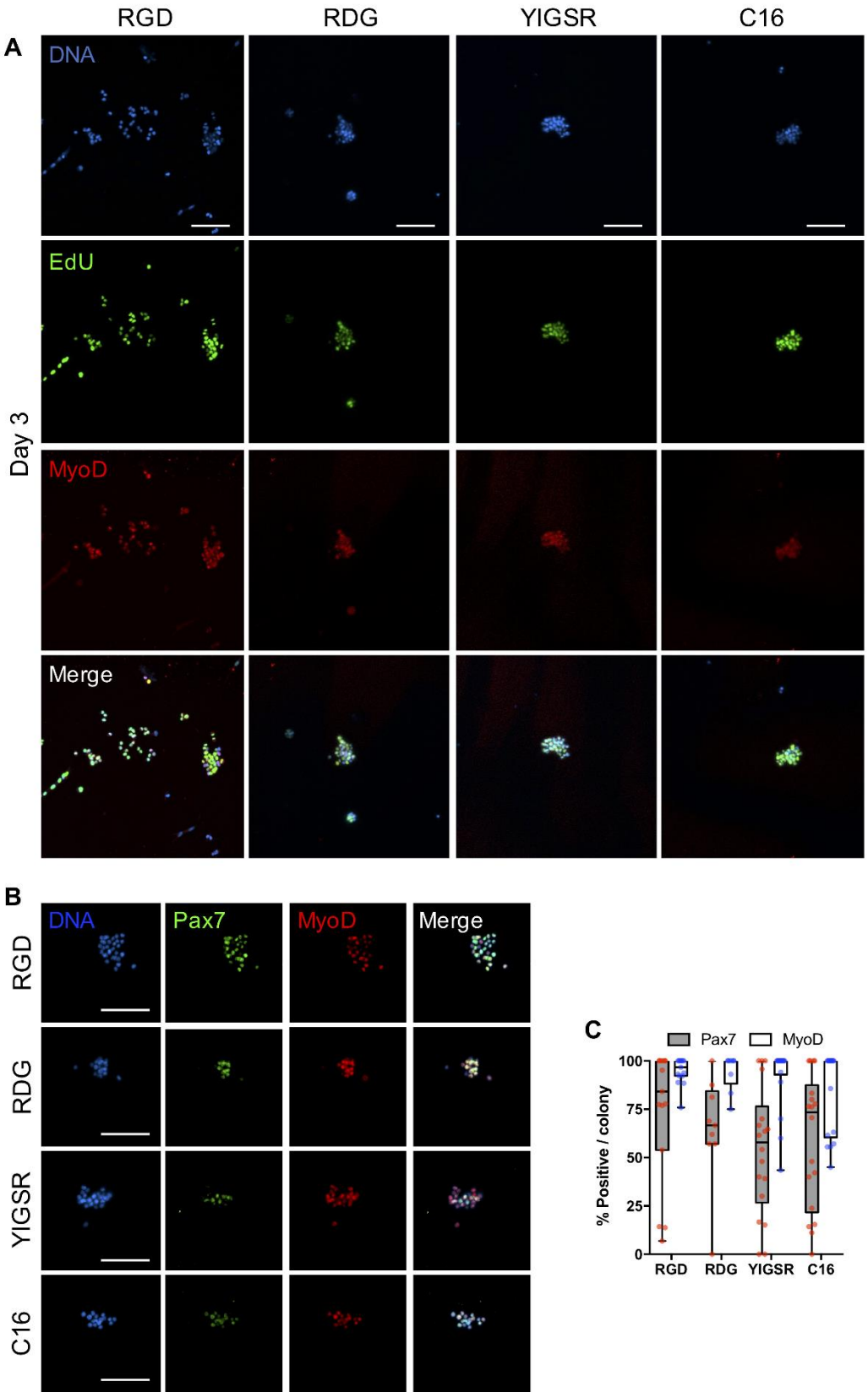

Fig. S2. RGD-presenting hydrogels promote MuSC activation and proliferation. (**A**) Representative z-projections myogenic colonies formed in hydrogels presenting synthetic cell adhesive peptides. Day 3. Scale bars: 100 μm. (**B**) MuSCs encapsulated and cultured in PEG-4MAL hydrogels become activated ($Pax7^+$/$MyoD^+$) by 72 hours post-encapsulation in culture. Scale bars: 50 μm. (**C**) Quantification of $Pax7^+$ and $MyoD^+$ cells. n=9-18. *p*=0.28 (Pax7) and *p*=0.69 (MyoD) Kruskal-Wallis test.

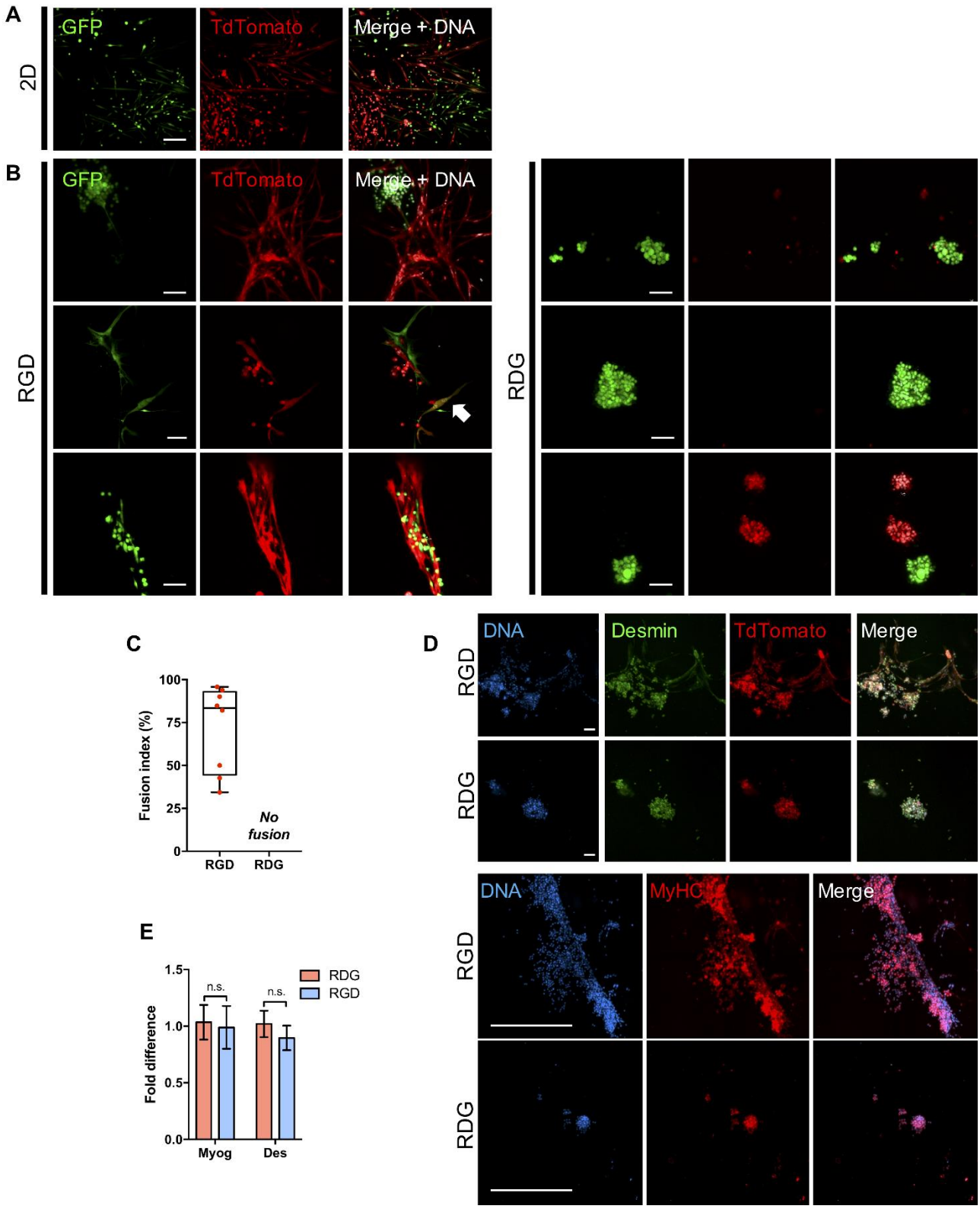

Fig. S3. RGD-presenting hydrogels promote MuSC differentiation. (**A**) Freshly isolated GFP$^+$ and TdTomato$^+$ MuSCs were seeded (1:1) collagen/laminin-coated tissue culture plastic to demonstrate fusion through co-expression of GFP and TdTomato. Scale bar: 200 μm. (**B**) Freshly isolated GFP$^+$ and TdTomato$^+$ MuSCs were seeded (1:1) in PEG-4MAL hydrogels functionalized with RGD or RDG. Cells cultured in RGD-functionalized hydrogels fuse (arrow) and become more elongated compared to the cells cultured in RDG-functionalized hydrogels. Scale bar: 100 μm. (**C**) Quantification of fusion index (%). n=8. p<0.0001 via unpaired two-tailed t-test. (**D**) Cells cultured in both RGD- and RDG-functionalized hydrogels stain positive for desmin and myosin heavy chain (MyHC). Cells were cultured in growth media for 6 days, then in differentiation media for 4 days. Scale bars: 100 μm (Desmin), 500 μm (MyHC). (**E**) Quantification of myogenin and desmin gene expression. n=4-5. n.s. p>0.05. Mean ± SEM.

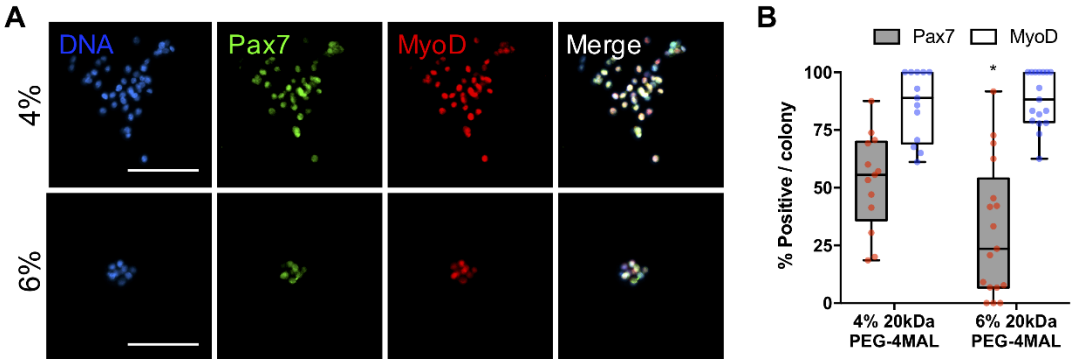

Fig. S4. Pax7/MyoD expression of MuSCs in 4 and 6% 20-kDa PEG-4MAL hydrogels. (**A**) MuSCs encapsulated and cultured in PEG-4MAL hydrogels become activated (Pax7$^+$/MyoD$^+$) by 76 hours post-encapsulation in culture. Scale bars: 50 μm. (**B**) Quantification of Pax7$^+$ and MyoD$^+$ cells. n=13-17. * p<0.05 via unpaired t-test.

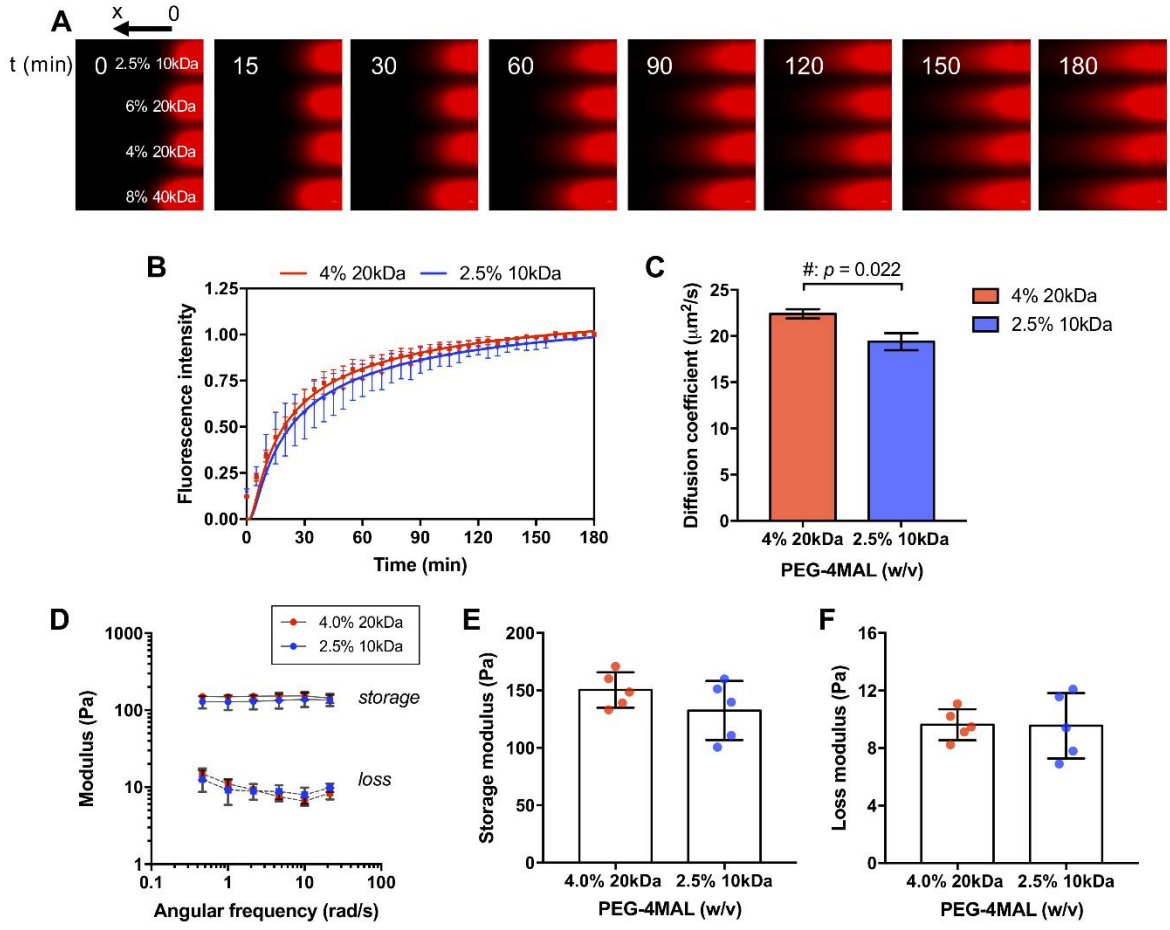

Fig. S5. 1D diffusion assay in PEG-4MAL hydrogels. (**A**) Representative micrographs of Alexa-555-labeled α-bungartoxin (8 kDa) diffusing into PEG-4MAL gels over time. (**B**) Quantification of intensity. The solution of Fick's second law was fitted through the data to determine the diffusion coefficients. n=5. Mean ± SEM. (**C**) Diffusion coefficients determined from curve fitting. n=5. Mean ± SEM. (**D-F**) Rheological assessments of 4% 20 kDa and 2.5% 10 kDa PEG-4MAL hydrogels. n=5. Mean ± SD. # p<0.05 via unpaired two-tailed t-test.

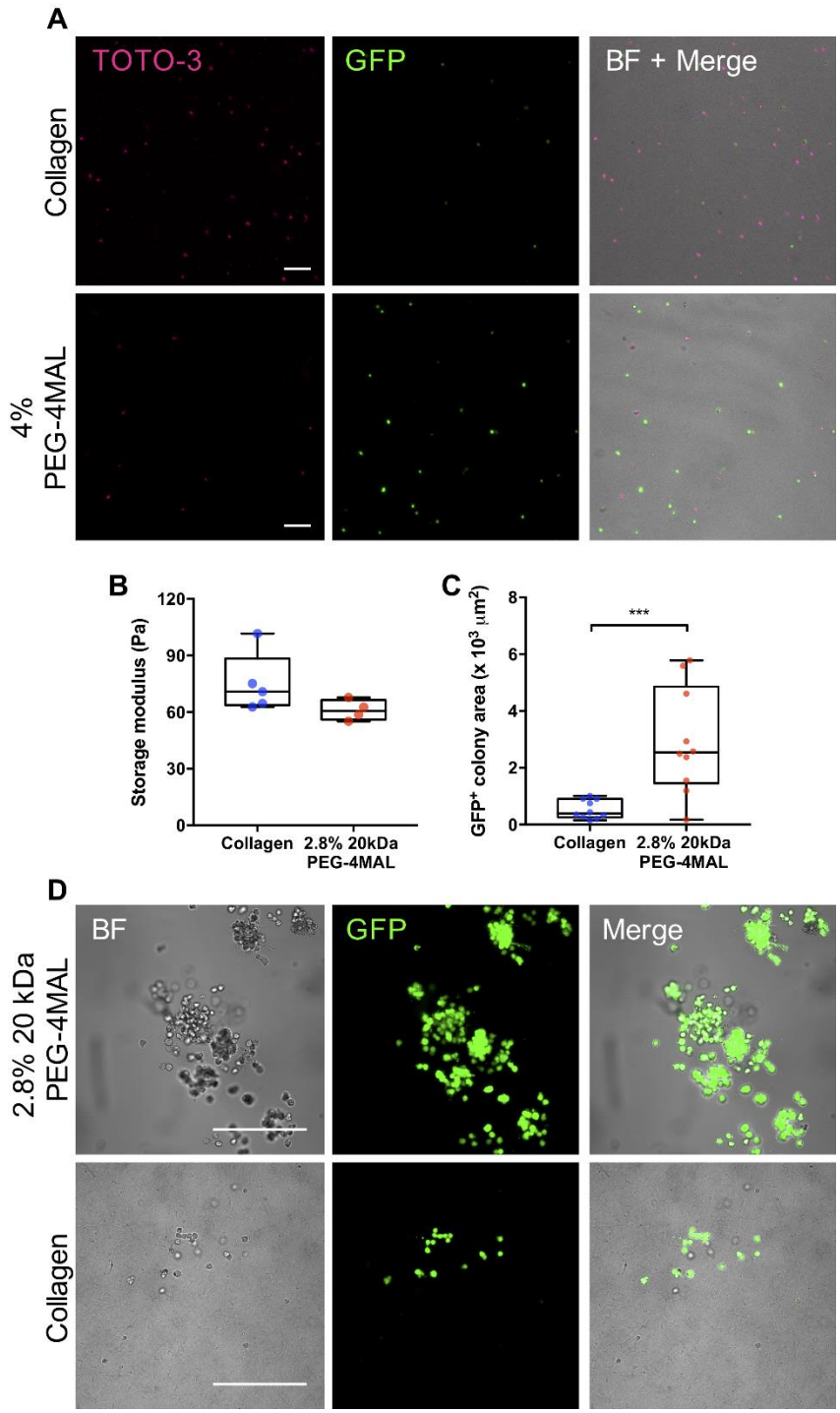

Fig. S6. Synthetic matrix supports higher MuSC proliferation potential than collagen gel. (**A**) Representative z-projections of GFP$^+$ MuSCs 1-day post-encapsulation in 4% PEG-4MAL hydrogel and 2.7 mg·ml^{-1} collagen gel. Scale bars: 100 μm. (**B**) Storage modulus of 2.8% PEG-4MAL hydrogel and 2.7 mg·ml^{-1} collagen gel. n=4-5. p=0.14 via unpaired t-test. (**C**) Quantification of GFP$^+$ myogenic colony area in 4% PEG-4MAL hydrogel and 2.7 mg·ml^{-1} collagen gel. n=10. *** p<0.001 via unpaired two-tailed t-test. (**D**) Representative z-projections of GFP$^+$ MuSCs 3-days post-encapsulation in 2.8% PEG-4MAL hydrogel and 2.7 mg·ml^{-1} collagen gel. Scale bars: 100 μm.

Table S1. List of cell-adhesive synthetic peptides and their targets.

Common Name	Sequence	Targets
RGD	GRGDSPC	$\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, $\alpha_v\beta_8$, $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_{IIb}\beta_3$
RDG (scrambled control)	GRDGSPC	N/A (inactive)
YIGSR	CGGEGYGEGYIGSR	67 kDa laminin receptor
C16	CGGKAFDITYVRLKF	Syndecan, $\alpha_v\beta_3$, $\alpha_5\beta_1$, β_1