SI Appendix

Supplementary Methods

Validation of surface blocking

In assays where tropoelastin was added as a soluble media supplement, wells were pre-incubated with full-serum media for 5 hrs to enable surface blocking by serum proteins and prevent protein adhesion. To confirm surface blocking, tropoelastin was added to pre-incubated or bare well surfaces for 1 hr at room temperature. Excess protein was removed with three PBS washes. Levels of bound tropoelastin were detected via an enzyme-linked immunosorbent assay, using 1:2000 mouse anti-elastin BA4 primary antibody (Sigma) for 1 hr at room temperature, 1:5000 goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody (Sigma) for 1 hr at room temperature, and visualized with 40 mM 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Sigma) solution in 0.1 mM sodium acetate, 0.05 mM NaH_2PO_4, pH 5 containing 0.01% (v/v) H_2O_2 for 1 hr at room temperature and absorbances were read at 405 nm.

Flow cytometry

Mesenchymal stem cells (MSCs) cultured for 5 or 7 days in various media formulations and on bare or protein-coated tissue culture wells were trypsinized and pelleted. The cell pellets were washed with 0.22 μm filtered FACS buffer (5% v/v FBS in PBS) and re-centrifuged at 270 g for 5 min. The cells were resuspended in FACS buffer to a concentration of 100,000 cells in 100 uL total volume, and probed for MSC marker expression using the Human Mesenchymal Stem Cell Verification Flow Kit (R&D Systems). Isotype and

unstained control samples were prepared using MSCs cultured in standard growth media on tissue culture plastic. Cells were analyzed using a BD Biosciences LSR II Flow Cytometer System. Singlet cells were determined by their forward scatter-to-side scatter and scatter height-to-width ratios, while viable cells were identified by exclusion of 1:20 propidium iodide. Only singlet, viable cells were analyzed for marker expression.

Cell differentiation

MSCs were grown in various media formulations and on bare or protein-coated tissue culture wells for 7 days. The expanded cells were harvested, re-seeded on TCP, and differentiated into the adipogenic, osteogenic and chondrogenic lineages using the hMSC Adipogenic BulletKit, hMSC Osteogenic BulletKit, and hMSC Chondrogenic BulletKit (Lonza), respectively, following the manufacturer's instructions.

To confirm adipogenesis, cells that had been induced for 25 days were washed with PBS, fixed with 10% (v/v) formalin for 30 min, then washed with water. Cells were incubated with 60% (v/v) isopropanol for 5 min, and stained for intracellular lipid droplets with 1.8 mg/mL Oil Red O in isopropanol for 20 min. Excess stain was removed with 5 washes of water.

To confirm osteogenesis, cells that had been induced for 14 days were fixed and stained for mineralized calcium deposits with Alizarin Red S, as previously described(1). Cells from the adipogenic and osteogenic experiments were imaged with a Zeiss Axio Vert.A1 microscope using an AxioCam 105 colour camera.

To confirm chondrogenesis, cell pellets that had been induced for 14 days were washed with PBS, embedded in 1.5% (w/v) agar containing 0.85% (w/v) NaCl, and fixed with 10% (v/v) formalin overnight. The samples were dehydrated in 70% (v/v) ethanol for 1

day, then paraffin-embedded, sectioned, mounted onto slides, stained with Alcian Blue (pH 2.5) for 1 hr and counterstained with Nuclear Fast Red. Samples were imaged with an Olympus VS120 Slide Scanner.

Supplementary Figures

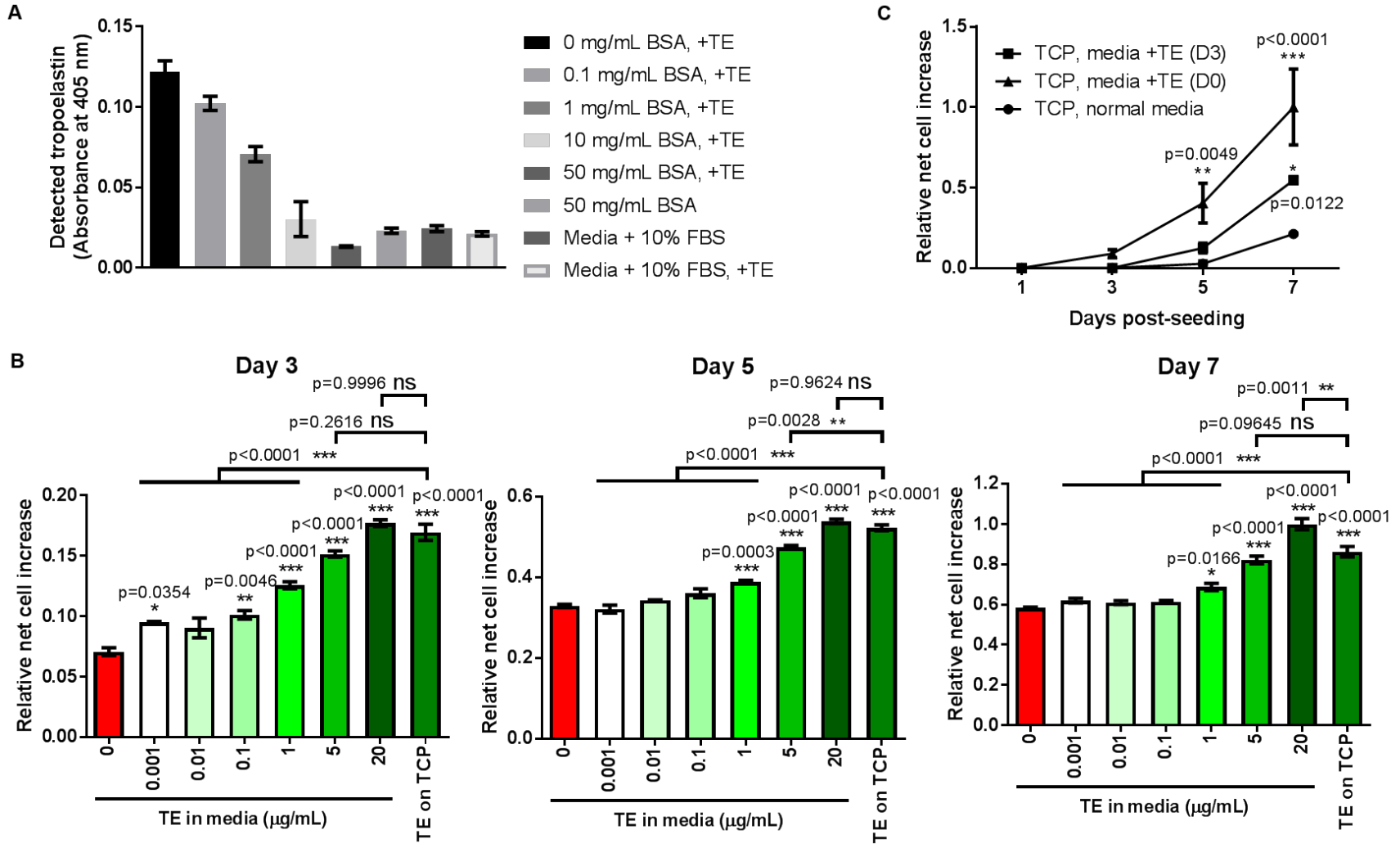
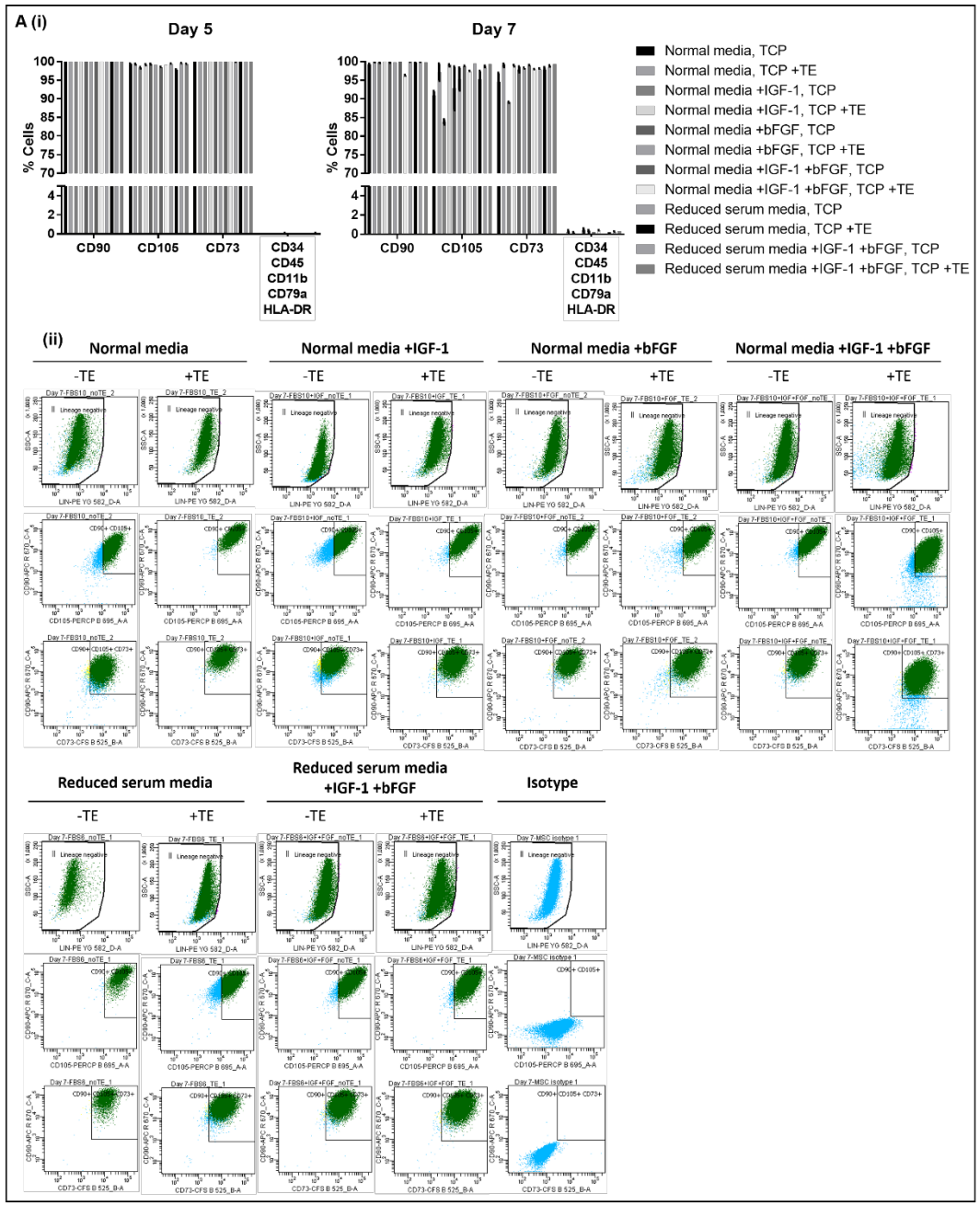


Figure S1. (A) Detection of surface-bound tropoelastin with an enzyme-linked immunosorbent assay. Tropoelastin (TE) was added to bare TCP, TCP pre-incubated with increasing concentrations of bovine serum albumin (BSA), or TCP pre-incubated with normal serum-containing media. Samples incubated with BSA or media, but without added tropoelastin, were used as negative controls. **(B)** MSC proliferation on TCP in media containing increasing amounts of tropoelastin in solution, or on tropoelastin-coated TCP in normal media over 7 days. Panels show relative net cell increase at 3, 5 and 7 days post-seeding. Asterisks directly above data columns indicate statistical differences from the no-tropoelastin controls. **(C)** MSC proliferation on TCP in normal media with and without soluble tropoelastin over 7 days. Tropoelastin was added at 20 μg/mL in solution, either on the day of seeding (D0), or at 3 days post-seeding (D3). Cell abundance in tropoelastin-supplemented media increases above that in normal media, corresponding to the time of tropoelastin addition.


A (i)
Day 5
Day 7
% Cells
CD90
CD105
CD73
CD34
CD45
CD11b
CD79a
HLA-DR
Normal media, TCP
Normal media, TCP +TE
Normal media +IGF-1, TCP
Normal media +IGF-1, TCP +TE
Normal media +bFGF, TCP
Normal media +bFGF, TCP +TE
Normal media +IGF-1 +bFGF, TCP
Normal media +IGF-1 +bFGF, TCP +TE
Reduced serum media, TCP
Reduced serum media, TCP +TE
Reduced serum media +IGF-1 +bFGF, TCP
Reduced serum media +IGF-1 +bFGF, TCP +TE
(ii)
Normal media
Normal media +IGF-1
Normal media +bFGF
Normal media +IGF-1 +bFGF
-TE
+TE
Reduced serum media
Reduced serum media +IGF-1 +bFGF
Isotype

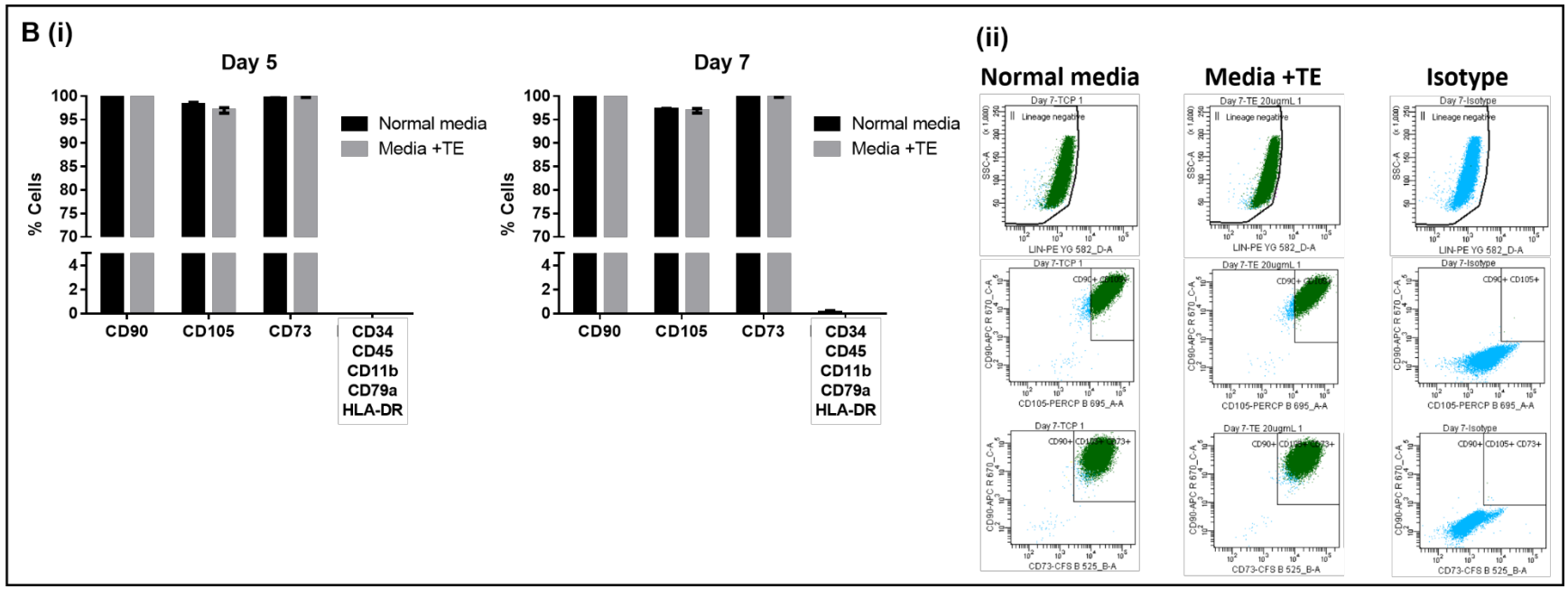


Figure S2. Surface marker expression of MSCs expanded with tropoelastin. Cells were cultured **(A)** on bare or tropoelastin-coated TCP in normal (10% (v/v) FBS) or reduced serum (6% (v/v) FBS) media, with or without IGF-1 and/or bFGF growth factors; or **(B)** on TCP in normal media or in media containing 20 μg/mL soluble tropoelastin (TE). (i) Percentage of the cell population expressing the positive MSC markers CD90, CD105, CD73, and the cocktail of negative markers CD34 CD45, CD11b, CD79a and HLA-DR after 5 or 7 days culture. Marker expression was quantified as the percentage of positive events detected from gated singlet viable cells. (ii) Representative flow cytometry dot plots of cells grown in various culture conditions at 7 days post-seeding. The first row depicts the selection gating for cells that do not express the negative markers. The second row shows the population of lineage-negative cells which express both

positive markers CD90 and CD105. The third row shows the population of CD90+ and CD105+ cells which also express the MSC marker CD73. Cells stained with isotype antibody controls for all markers are also shown.

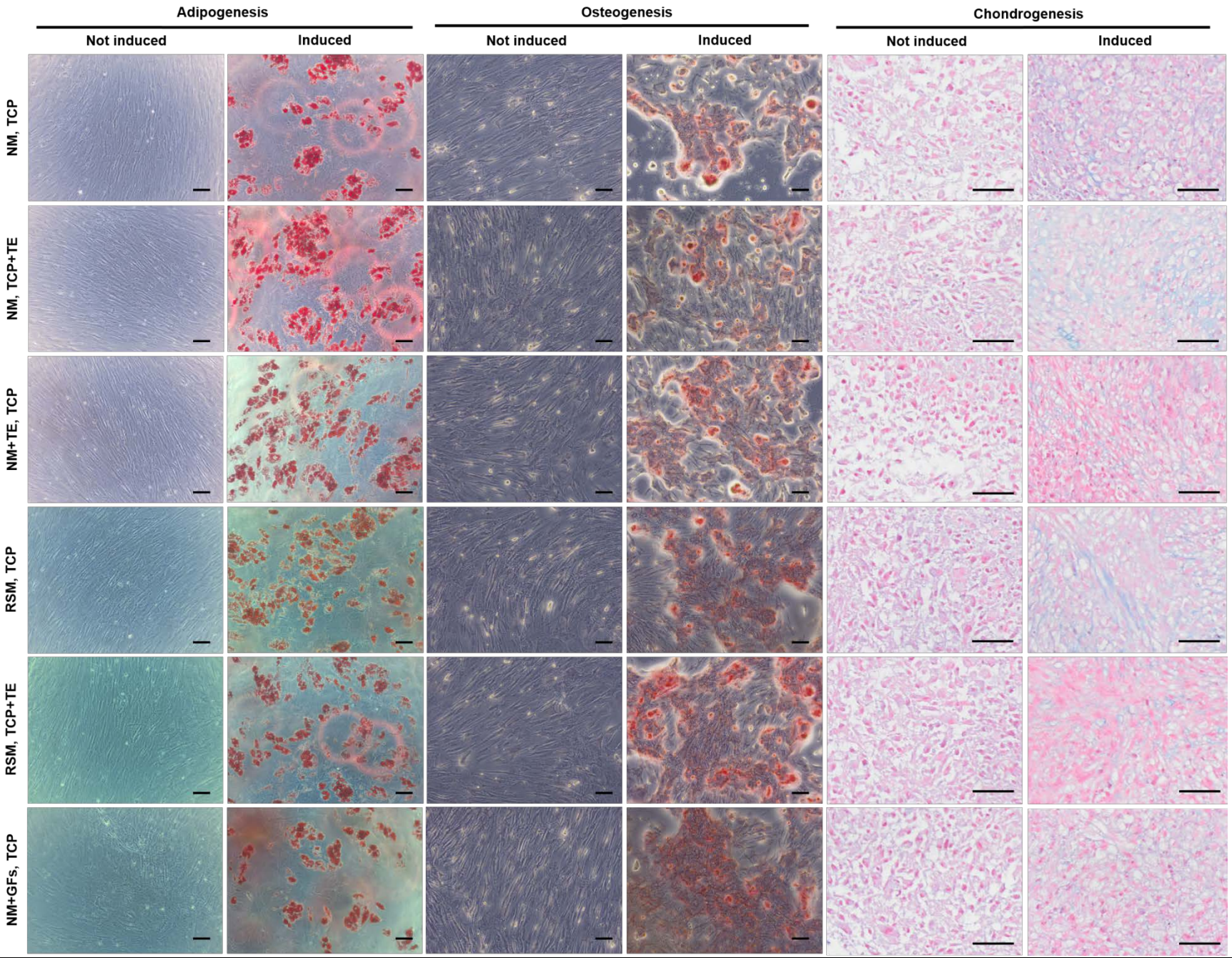

Adipogenesis
Osteogenesis
Chondrogenesis
Not induced
Induced
Not induced
Induced
Not induced
Induced
NM, TCP
NM, TCP+TE
NM+TE, TCP
RSM, TCP
RSM, TCP+TE
NM+GFs, TCP

Figure S3. Tri-lineage differentiation of MSCs expanded with tropoelastin. Cells were cultured on TCP or tropoelastin (TE)-coated TCP, in normal media (NM) or reduced serum media (RSM) supplemented with tropoelastin or IGF-1 and bFGF growth factors for 7 days, then harvested and differentiated into adipogenic, osteogenic, and chondrogenic lineages. Induced and non-induced cells were stained for intracellular lipid droplets with Oil Red O, mineralized calcium nodules with Alizarin Red, and glycosaminoglycans with Alcian Blue. Scale bar: 50 μm.

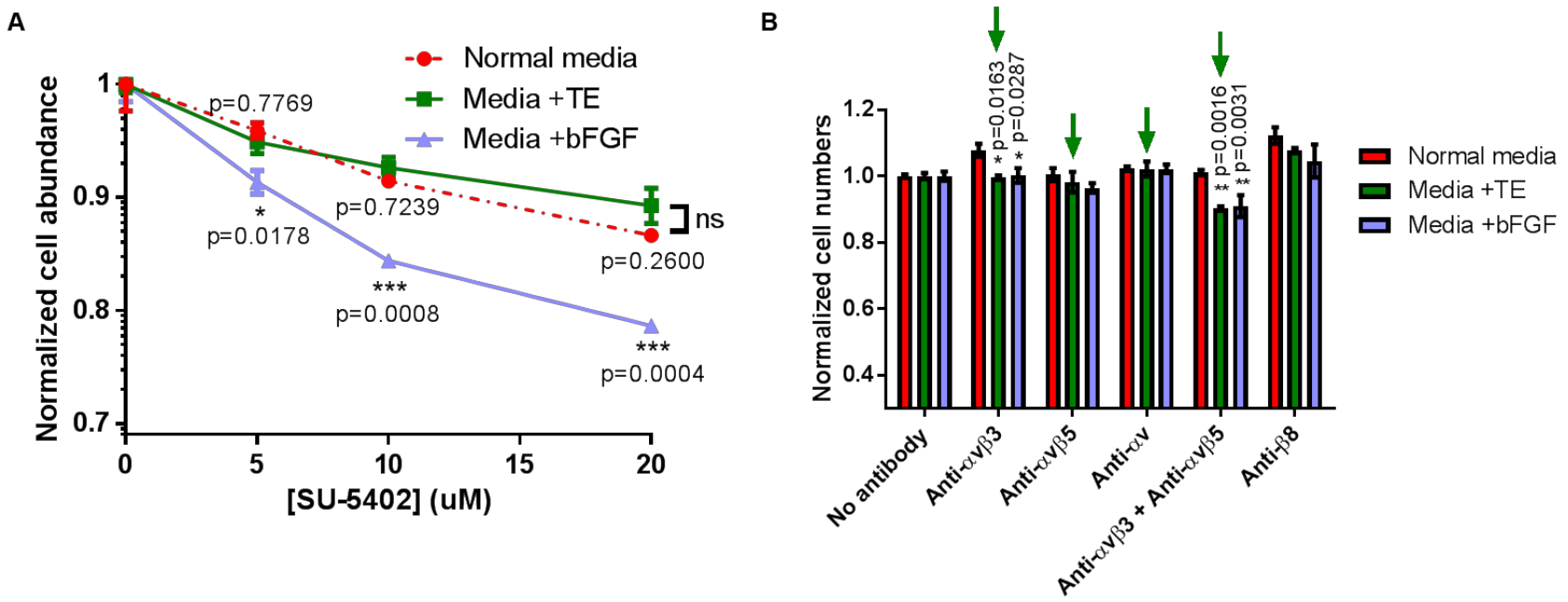


Figure S4. MSC abundance in the presence of (A) fibroblast growth factor receptor (FGFR) and (B) integrin inhibitors, one day post-seeding. Cells were grown on TCP in normal media, in media with 20 μg/mL tropoelastin (TE), or in bFGF-supplemented media. **(I)** Increasing doses of the FGFR inhibitor, SU-5402, were added to the media. Cell numbers were normalized against samples without SU-5402. Cell numbers in media containing tropoelastin or bFGF were compared with those in normal media at each inhibitor concentration to account for the non-specific toxicity of SU-5402. **(B)** Optimal inhibitory concentrations of anti-αvβ3, anti-αvβ5, anti-αvβ5 and anti-αvβ3, or anti-αv, were added to the media. Controls without antibodies or with an antibody against a non-expressed integrin (anti-β8)

were included. Green arrows indicate cells grown in the presence of tropoelastin and αv integrin subunit antibodies. Asterisks above individual columns denote significant differences from cells in normal media at each antibody condition.

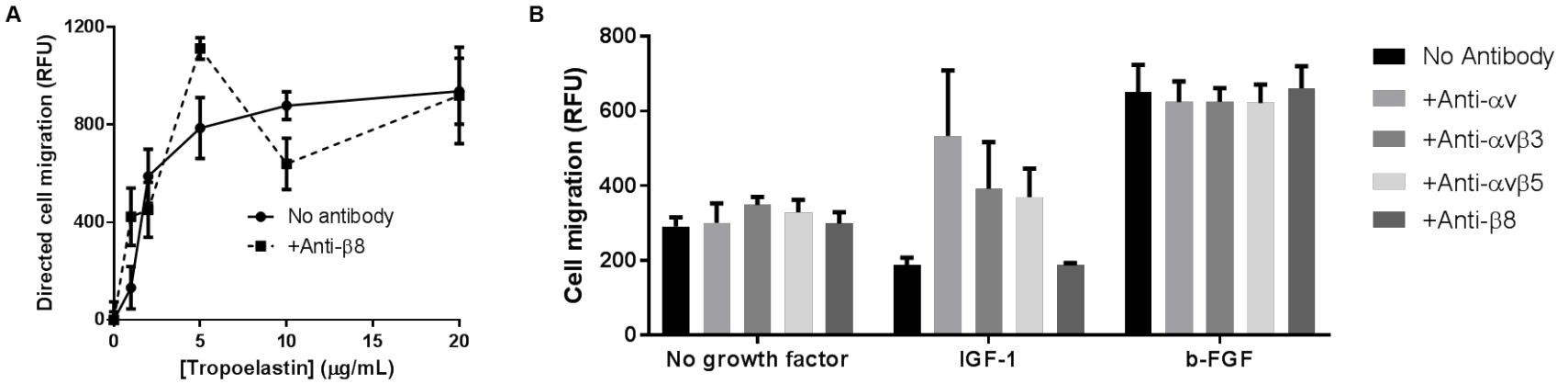


Figure S5. Chemotactic behavior of MSCs. **(A)** Cell migration towards increasing concentrations of tropoelastin as a diffusible chemoattractant in the bottom chamber of a Boyden chamber assay. Cells were incubated with or without 5 μg/mL anti-β8 integrin antibody in the top chamber. Cell migration was normalized to the level of unstimulated migration exhibited by no tropoelastin controls. **(B)** Cell chemotaxis to normal or growth factor-supplemented media in the presence of integrin-blocking antibodies. Controls were without antibodies or with an antibody against a non-expressed integrin (anti-β8).

References

1. Gregory CA, Gunn WG, Peister A, & Prockop DJ (2004) An Alizarin red-based assay of mineralization by adherent cells in culture: comparison with cetylpyridinium chloride extraction. *Analytical biochemistry* 329(1):77-84.