



| Histogram # | Filename | Parameter | Low bound | High bound | # of Events | % of gated cells | Median | Geometric Mean | CV |
|----------------|-----------------------------------|-----------|--------------|------------|----------------|------------------------|----------|-------------------|-------|
| 1 | SCs-2 nd antibody only | FITC-A | 267.95 | 254300.03 | 0 | 0.00 | n/a | n/a | n/a |
| 2 | Unstained SCs | FITC-A | 267.95 | 254300.03 | 2 | 0.02 | 283.388 | 283.53 | 7.02 |
| 3 | SCs-desmin (A488) | FITC-A | 267.95 | 254300.03 | 9180 | 99.43 | 10410.50 | 8389.41 | 80.03 |
| 4 | SCs-MYH (A647) | FITC-A | 267.95 | 254300.03 | 7 | 0.08 | 312.08 | 344.72 | 26.87 |



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|-----------|-----------------------------------|-----------|--------|------------|--------|-------|--------|-----------|--------|
| # | | | bound | | Events | gated | | Mean | |
| | | | | | | cells | | | |
| 1 | SCs-2 nd antibody only | APC-A | 542.12 | 262144.00 | 8 | 0.09 | 773.55 | 936.94 | 78.47 |
| 2 | Unstained SCs | APC-A | 542.12 | 262144.00 | 2 | 0.02 | 648 | 645.89 | 11.39 |
| 3 | SCs-desmin (A488) | APC-A | 542.12 | 262144.00 | 12 | 0.13 | 847.8 | 1011.04 | 79.5 |
| 4 | SCs-MYH (A647) | APC-A | 542.12 | 262144.00 | 7922 | 85.18 | 2907.9 | 2683.90 | 115.68 |

Figure S1. Flow cytometry analysis of desmin- and myosin-positive mouse SCs. (A) Representative confocal images of SCs, 7 days post-seeding. Desmin-positive cells are stained in green and nuclei are stained in blue. (B) Approximately 99% of the SCs isolated from the tibialis were desmin-positive. (C) Approximately 85% of the cells within the cell culture were myosin-positive.

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Figure S2. Representative confocal images of SCs, 7 days post-seeding. Desmin-positive cells are stained in green, Pax 7 positive cells are stained in magenta and nuclei are stained in blue; scale bar = $100 \,\mu$ m.



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|----------------|-----------------------------------|-----------|--------|------------|--------|-------|--------|-----------|--------|
| # | | | bouna | | Events | cells | | wean | |
| 1 | Unstained ECs | FITC-A | 356.74 | 254300.77 | 0 | 0.00 | n/a | n/a | n/a |
| 2 | ECs-CD31 (A488) | FITC-A | 356.74 | 254300.77 | 7265 | 84.09 | 915.56 | 945.31 | 68.07 |
| 3 | ECs-2 nd antibody only | FITC-A | 356.74 | 254300.77 | 7 | 0.08 | 391.98 | 839.68 | 193.35 |
| 4 | SCs-CD31 (A488) | FITC-A | 356.74 | 254300.77 | 5 | 0.06 | 382.11 | 623.12 | 177.31 |

Figure S3. Flow cytometry analysis of CD31-positive mouse ECs.Approximately 84% of the mouse ECs were CD31-positive.



Figure S4. Representative confocal images of whole-mount immunofluorescent scaffold populated with ECs and SCs, 14 days post-seeding. ECs are stained in green, desmin-positive cells are stained in magenta and nuclei are stained in blue; scale bar = $100 \mu m$.



Figure S5. (**A**) Representative intravital images, obtained through the AIW, of an ECand SC-embedded graft, 18 days post-transplantation. Green: host vasculature; red: TRITC-conjugated dextran. Scale bar = 100 μ m. (**B**) Representative intravital images, obtained through the AIW, of an EC- and SC-containing graft, 14 days posttransplantation. Green: Flk-1 GFP vessels; blue: mouse CD31. Scale bar = 100 μ m.



Figure S6. Image of a mouse stabilized in a custom-made SID for intravital confocal imaging. (A) lateral view of an anesthetized mouse in the SID. (B) Representative image of a mouse with an abdominal imaging window (AIW) 14 days post-surgery.