Supplementary Materials of "Dual stem cell therapy from adipose tissue facilitates arteriogenesis and limb preservation in experimental critical limb ischemia via direct vascular integration"

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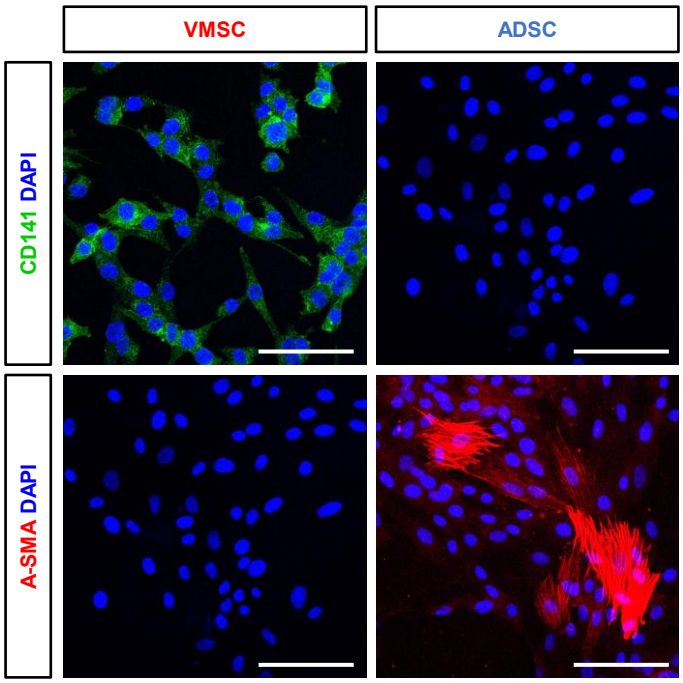
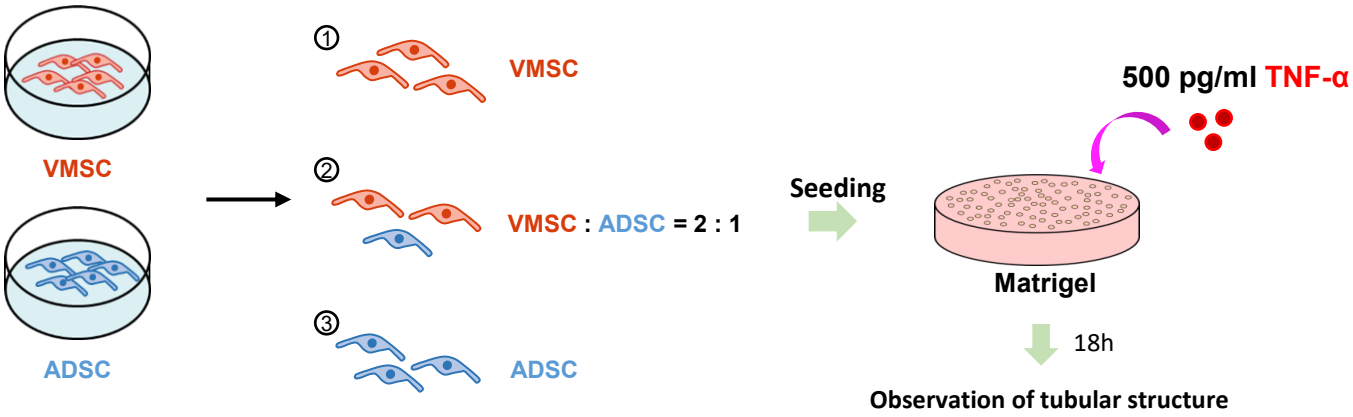
Supplementary Fig 1



Supplementary Fig 1. Immunofluorescence staining for CD141 and α-SMA in VMSCs and ADSCs
VMSC and ADSC was coverslip on 24 well plate and fixed with 3.7% formalin, followed by anti-CD141 or α-SMA staining. anti Scale bar:100 μm.

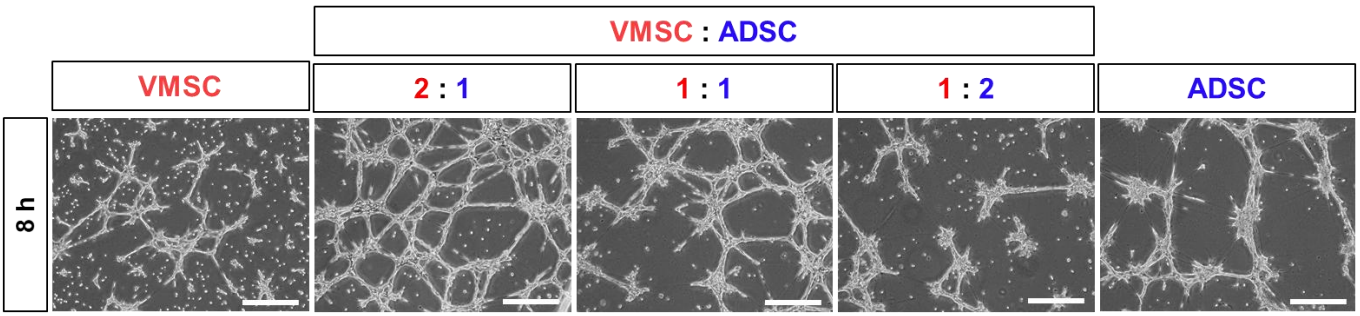
Supplementary Fig 2



Supplementary Fig 2. Experimental scheme of tube formation assay for assessing angiogenic capacity of VMSCs and ADSCs under the inflammatory stress

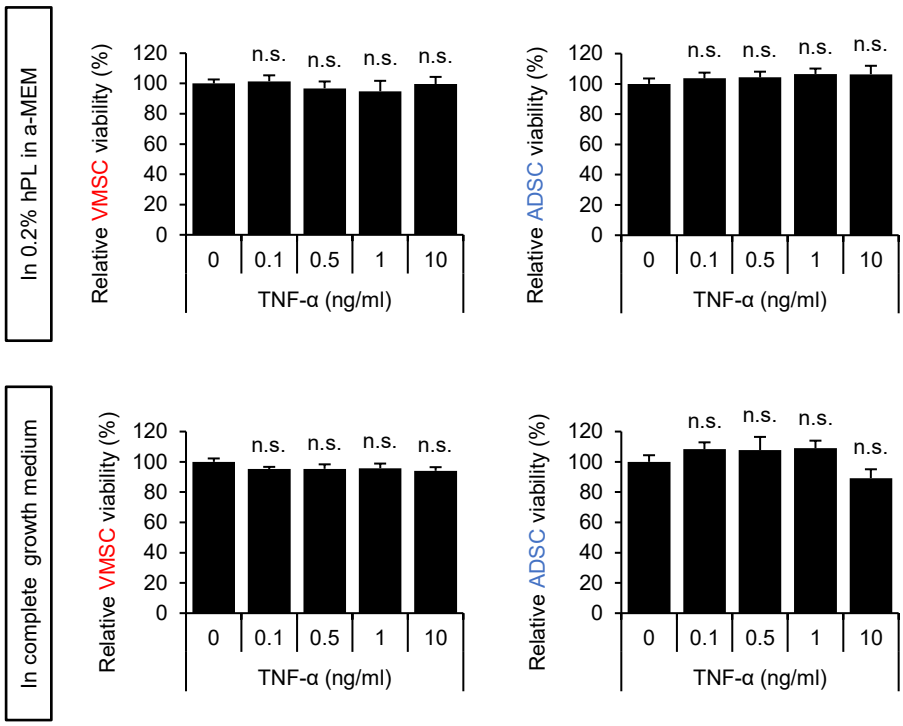
VMSCs, ADSCs, and combination of VMSCs and ADSCs were seeded onto Matrigel in the presence or absence of 500 pg/ml TNF-α. 18h later, tubular structure was comparatively observed

Supplementary Fig 3



Supplementary Fig 3. Optimization of combination ratio for VMSC and ADSC
Representative images showing In vitro Matrigel tube formation assay on μ-slide of VMSCs, ADSCs, and mixture of VMSCs and ADSCs. Tube formation assay was allowed for 24 h. (Total cells: 9,000 cells/well) (Scale bar: 500 μm).(F) Tube formation of PKH-red-labeled ADSC and PKH-greed-labeled VMSC on Matrigel (Scale bar: 100 μm).

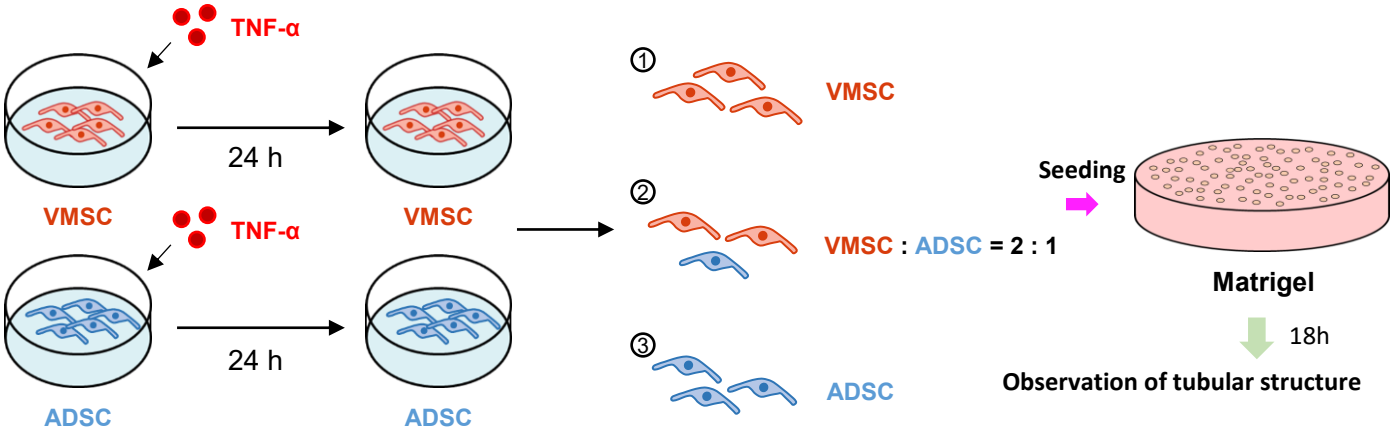
Supplementary Fig 4



Supplementary Fig 4. Effect of diverse concentrations of TNF-α on the viability of VMSCs and ADSCs

VMSCs or ADSCs were treated with the indicated concentrations of TNF-α, and cell viability was measured 24 h later using the WST-1 assay. Viability is expressed as a percentage relative to the untreated control (0 ng/mL).Values are mean ± SD, One-way ANOVA test followed by Tukey's multiple comparison test, n=5; n.s.: not significant)

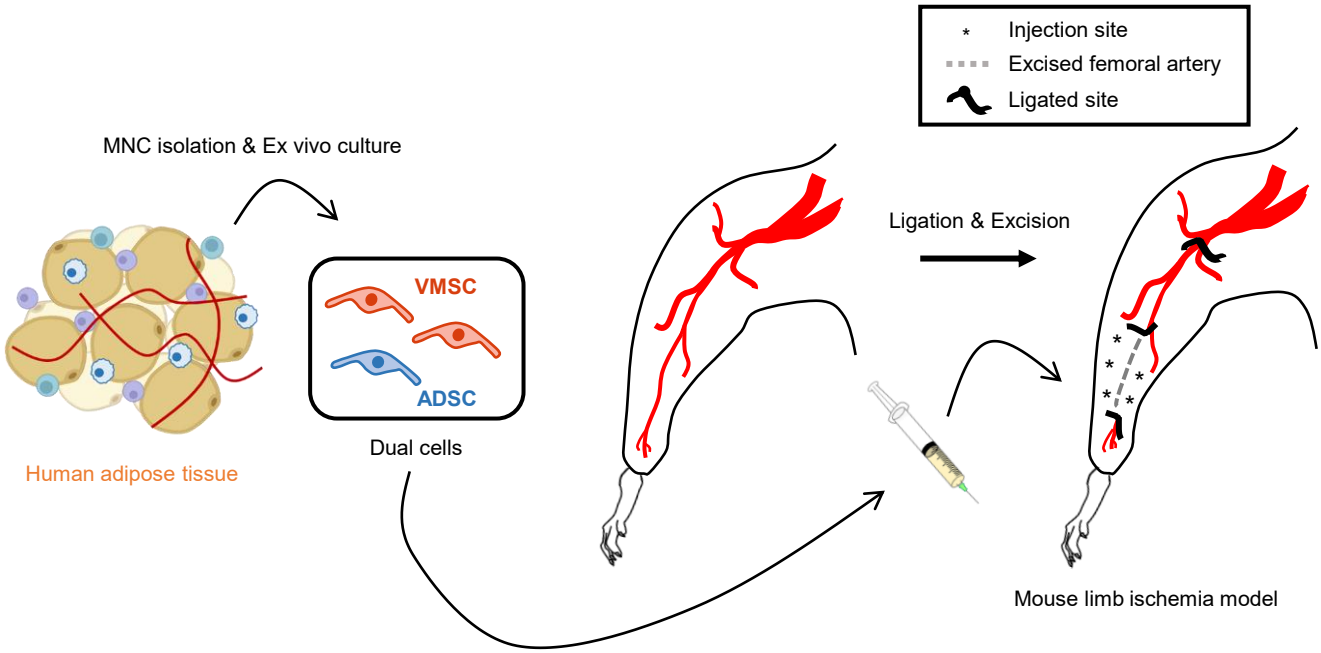
Supplementary Fig 5



Supplementary Fig 5. Experimental scheme of tube formation assay for evaluating angiogenic capacity of TNF-α primed VMSCs and ADSCs

VMSCs and ADSCs were pretreated with 10 ng/mL TNF-α for 24 h, followed by seeding of VMSCs, ADSCs, or VMSC–ADSC co-cultures (2:1 ratio) onto Matrigel.

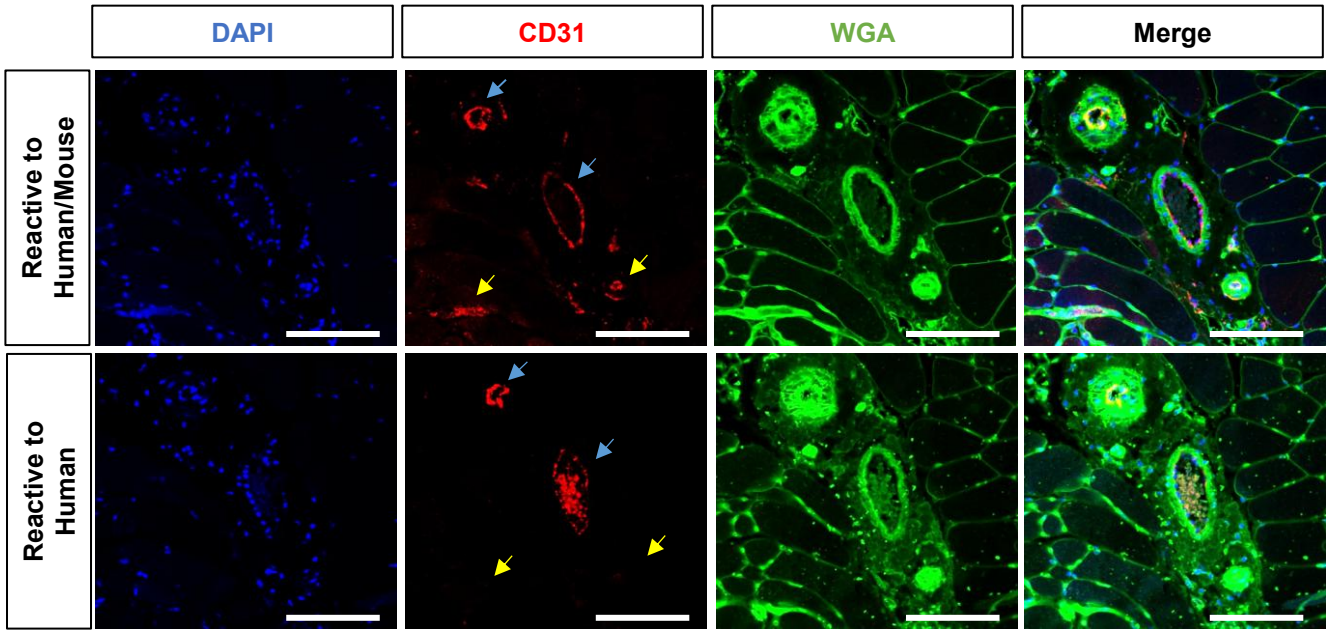
Supplementary Fig 6



Supplementary Fig 6. Co-transplantation of VMSCs and ADSCs at 2:1 ratio into mouse limb ischemia model

VMSCs and ADSCs were isolated from human adipose tissue and expanded in culture. A total 2.4 × 10^5 cells (VMSCs: 1.6 × 10^5 cells and ADSCs: 0.8 × 10^5 cells) were injected into each 5 sites of legs intramuscularly.

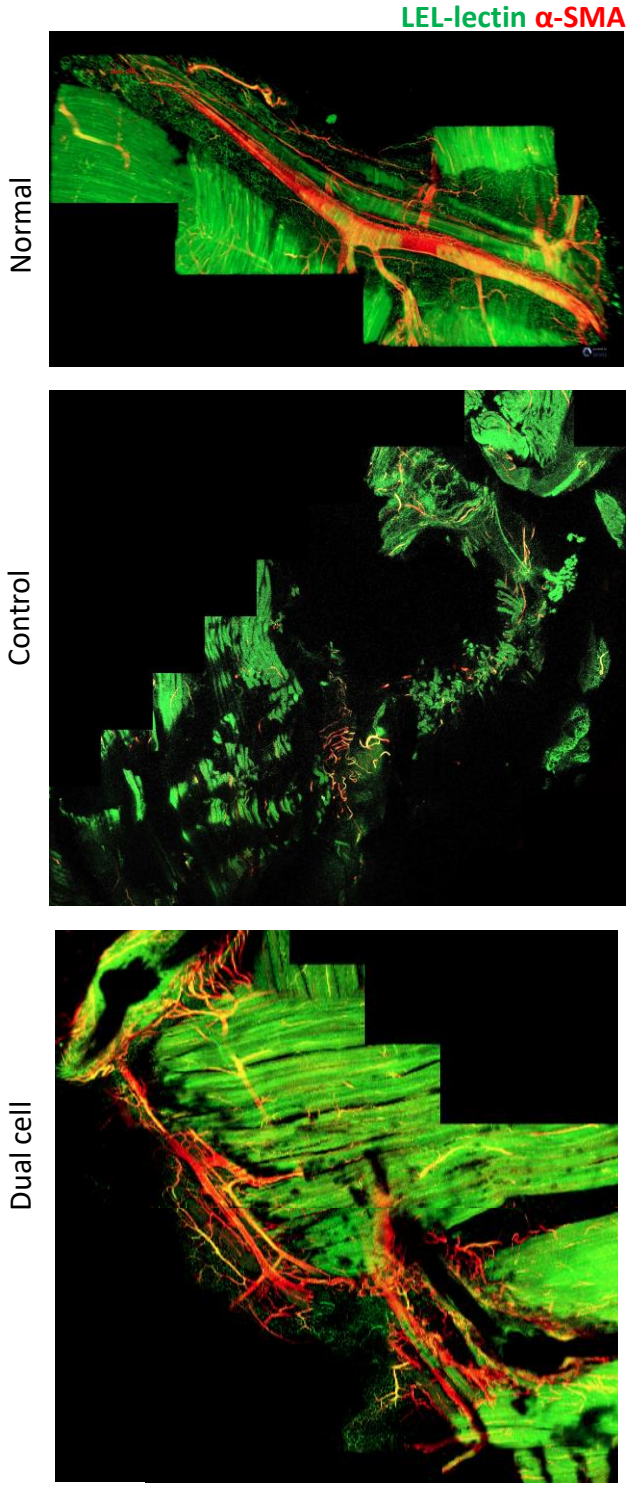
Supplementary Fig 7



Supplementary Fig. 7. Immunofluorescence staining with anti-human CD31 and anti-human/mouse CD31 antibodies

Dual cells were transplanted into CLI mice, and 28 days later, tissues were isolated and fixed. Paraffin-embedded serial sections were stained to distinguish vessels derived from transplanted human cells (human $CD31^+$) from host mouse vessels (human/mouse $CD31^+$). Blue arrows indicate human $CD31^+$ vessels; yellow arrows indicate mouse vessels

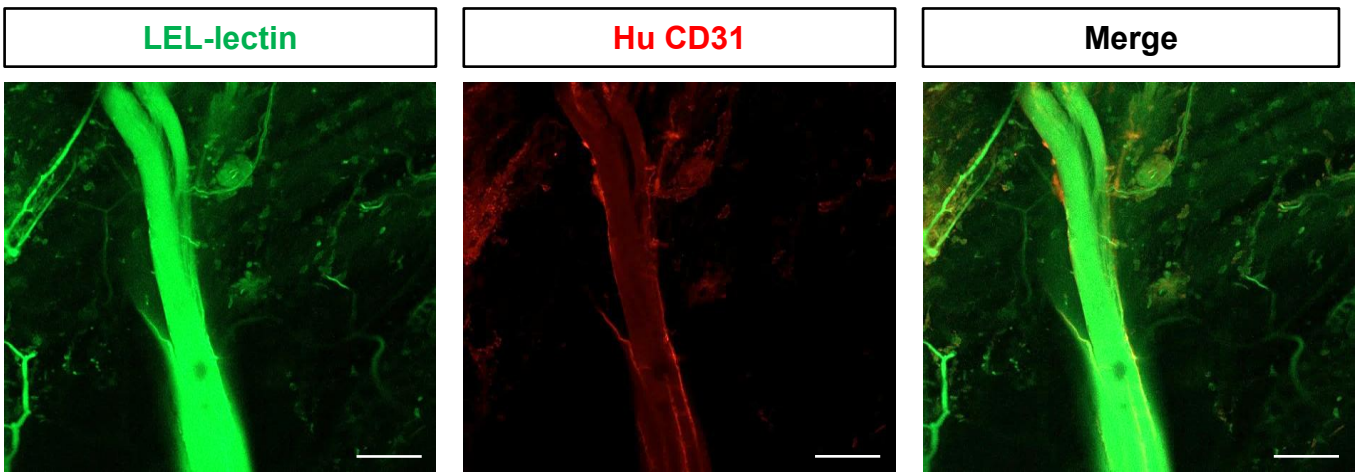
Supplementary Fig 8



Supplementary Fig 8. Low-magnification immunofluorescence image showing perfusable vasculature with perivascular cell coverage

Dual cells were transplanted into CLI mice, and 28 days later, FITC-conjugated LEL-lectin was intravenously injected immediately prior to sacrifice. Tissues were then isolated and fixed. Following a tissue-clearing procedure, samples were stained with an anti–α-SMA antibody to visualize functional vessels with perivascular coverage.

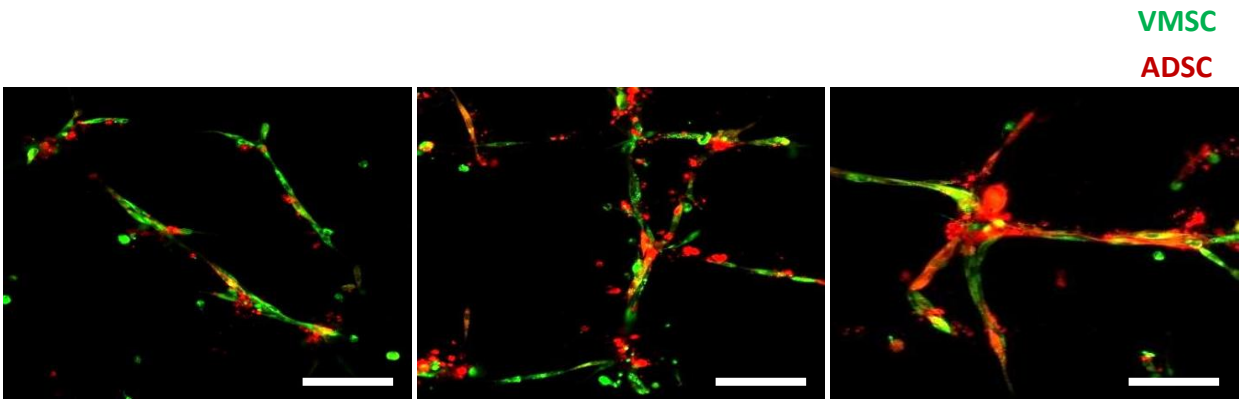
Supplementary Fig 9



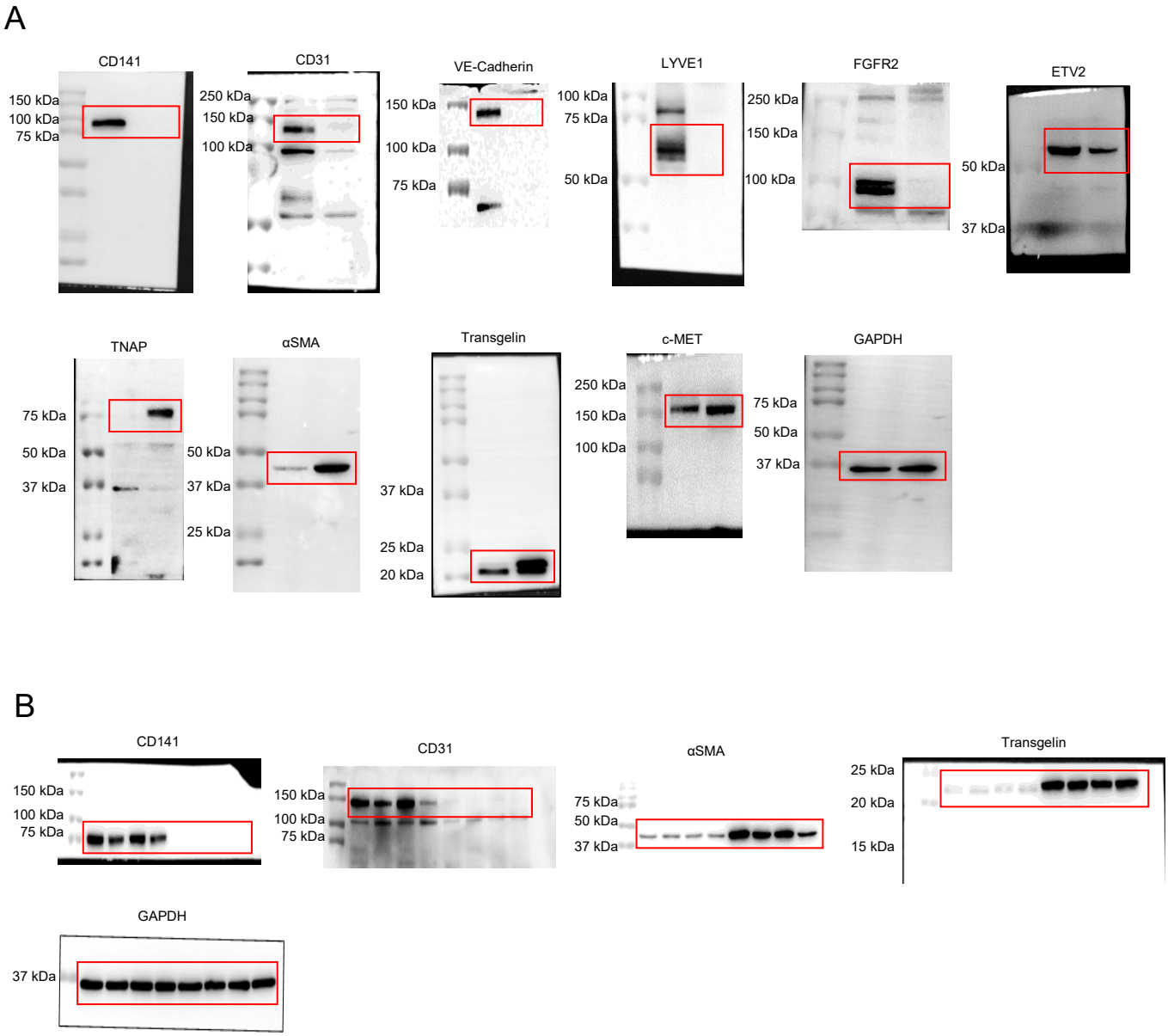
Supplementary Fig 9. Immunofluorescence detection of human antigen in perfusable vasculature of transplanted tissue

Dual-cell transplantation was performed in CLI mice, and 28 days post-transplantation, FITC-conjugated LEL-lectin was intravenously administered immediately before sacrifice. Tissues were subsequently harvested and fixed. After tissue clearing, samples were immunostained with an anti-human CD31 antibody, followed by Alexa Fluor 568–conjugated secondary antibodies to visualize human cell–integrated functional vessels with perivascular coverage.

Supplementary Fig 10



Supplementary Fig 10. Tube formation with VMSC and ADSC in vitro
PKH-red-labeled ADSC and PKH-greed-labeled VMSC was seeded on Matrigel-coated cover slip and tube formation process was observed (Scale bar: 100 μm).



Supplementary Fig 11. Un-cropped blots for Western blot data
(A) Whole blot for Figure 2A. (B) Whole blot for Figure 3A.