

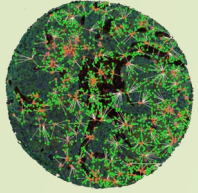
High multiplex spatial phenotyping analysis of ischemic muscle response to autologous bone marrow concentrate (cBMA) treatment

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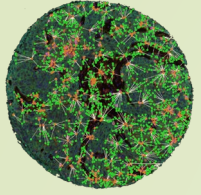
R.L. Roudebush VA Medical Center

Indianapolis, Indiana

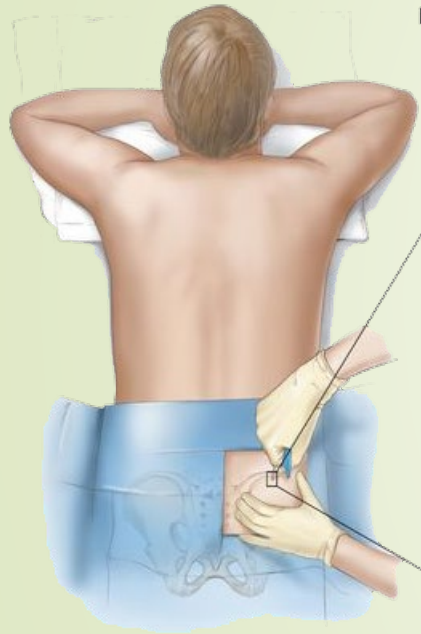


Rationale

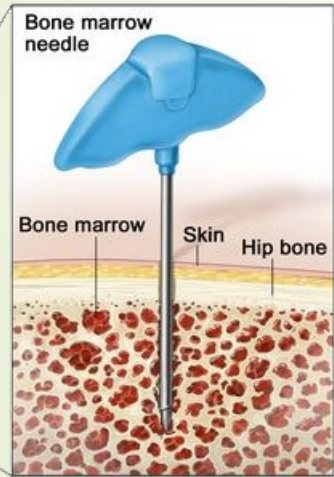
- For 1/3 of CLTI patients, the traditional surgical methods of revascularization are not technically possible
- For these patients, we looked at **cell therapy** with autologous bone marrow cells, as a means to promote limb preservation and to improve quality of life in patients with below knee amputation (BKA)
- The **Marrow CHAMP** Phase I clinical trial was thus designed, with a double purpose:
 - ❖ **Clinical**: Test the safety and efficacy of concentrated bone marrow aspirate (**cBMA**) in preventing wound complications in BKA:
 - Promotion of wound healing
 - Prevention of BKA conversion to above knee amputation (AKA)
 - ❖ **Scientific**: Characterize the host tissue response to, paracrine activity of, and fate of transplanted cBMA
- cBMA was also used in the multi-center **MOBILE** trial, to assess the efficacy of intramuscular injections of cBMA in promoting amputation-free survival in patients with poor-option CLTI



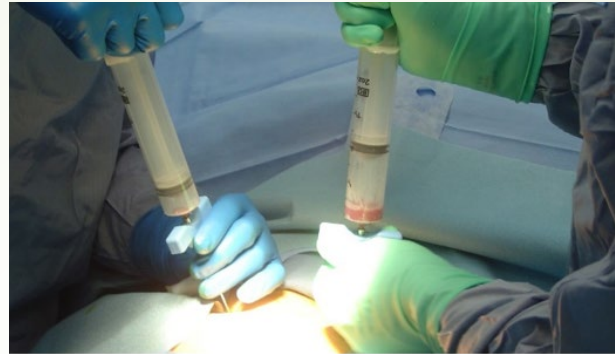
cBMA collection



Bone Marrow Aspiration and Biopsy

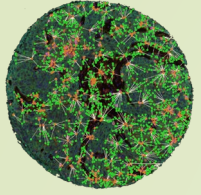


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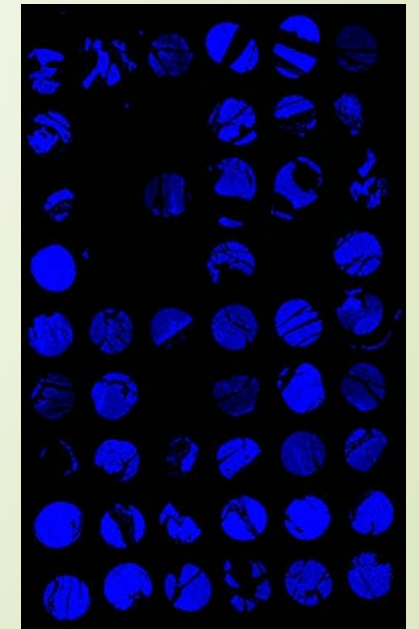
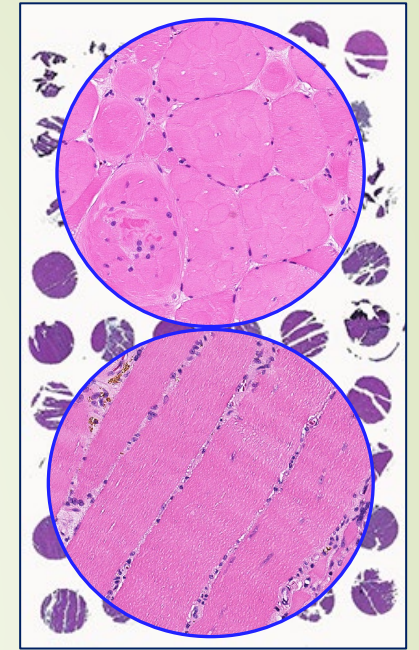
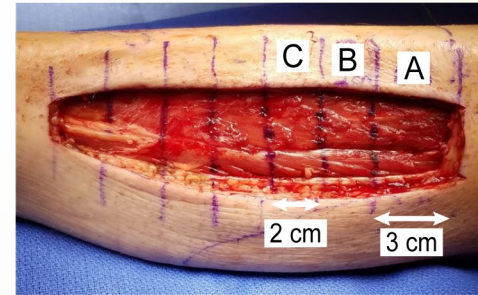
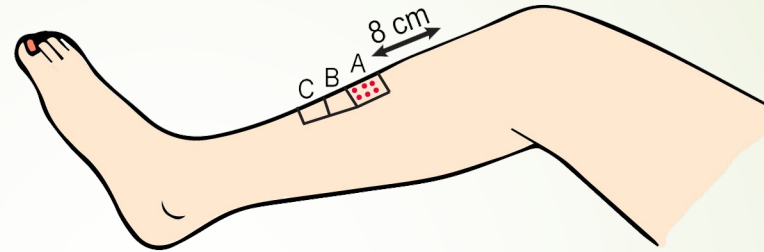
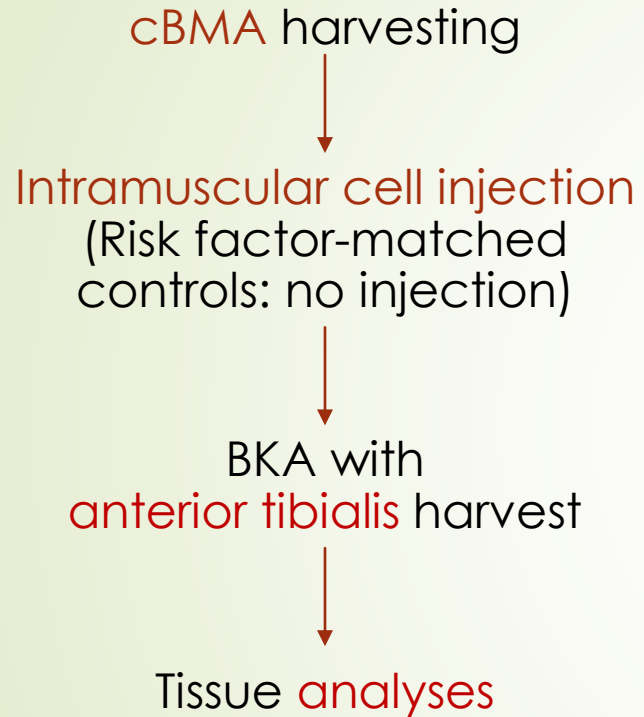


360 cc bone marrow



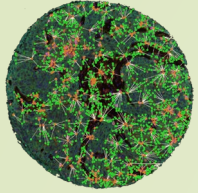


Study Design



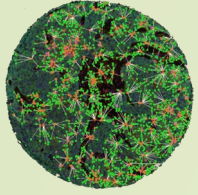
- **Genomics**
 - ❖ mRNA sequencing
 - ❖ Real-time PCR

- **Histology**
 - ❖ Capillary density
 - ❖ Muscle morpho-pathology
 - ❖ **Multiplex spatial analysis**



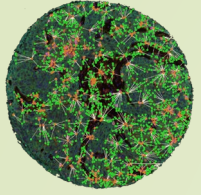
Markers of interest

- Markers of fibers **regeneration**:
 - ❖ frequency **PAX7** as marker of satellite cells (muscle progenitor cells)
 - ❖ frequency of **MYOD** (a primitive form of myosin) as marker of newly formed myofibers
 - ❖ **CD31** as marker of endothelial cells, thus blood vessels (of all calibers)
 - ❖ **KI67**, an antigen expressed in proliferating cells
- markers of muscle **degeneration**:
 - ❖ apoptosis, as detected by **BrdU** TUNEL assay
 - ❖ **PDGFRA**, as marker of fibroadipose infiltration
- **mononuclear cells infiltration**:
 - ❖ **CD3** (T cells)
 - ❖ **CD14** (monocytes/macrophages)
 - ❖ **CD68** (M1 macrophages; pro-inflammatory)
 - ❖ arginase (**ARG**; M2 macrophages; anti-inflammatory, promoting inflammation resolution)



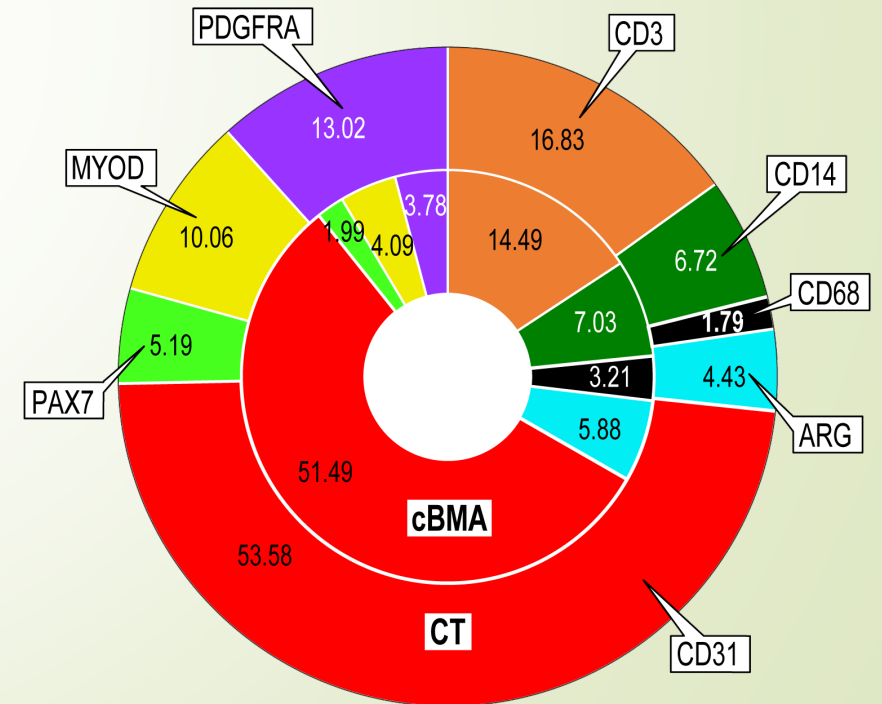
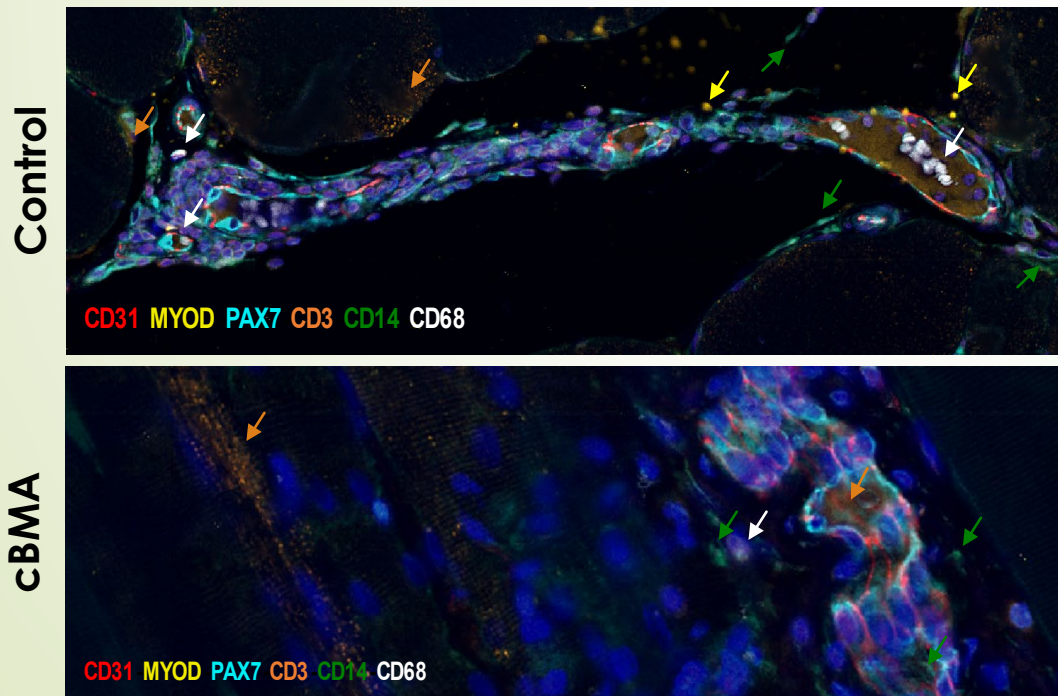
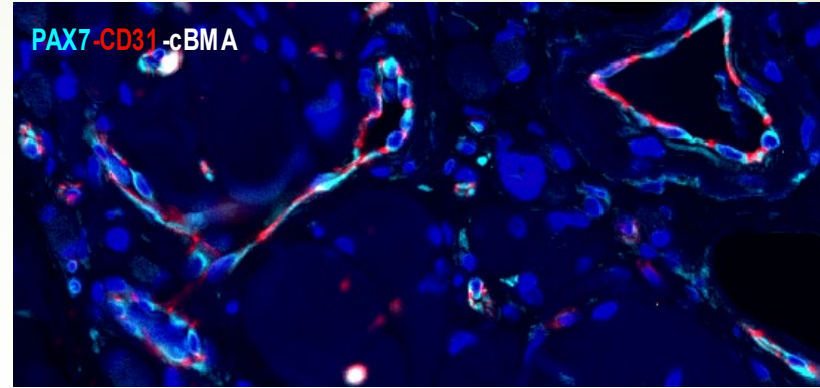
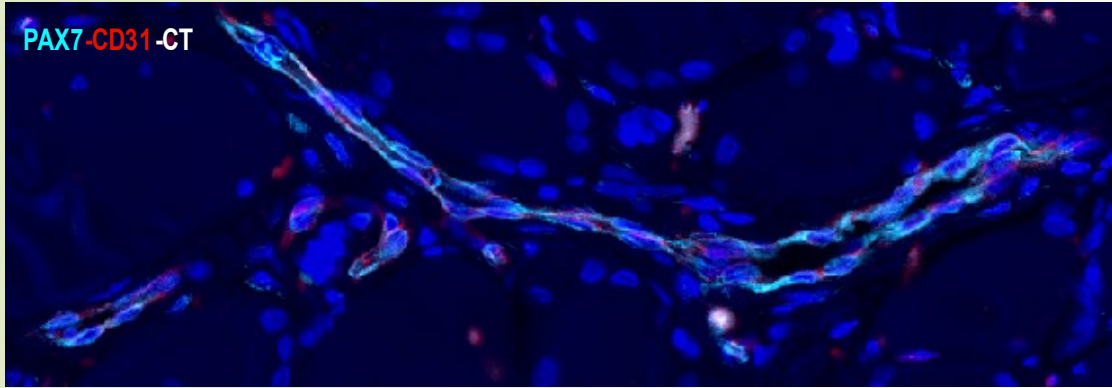
Relative proportions of the phenotypes analysed

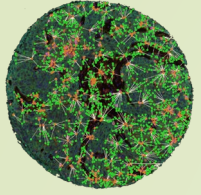
CD3: T cells
CD14: monocytes
CD68: M1 (pro-inflam.)
ARG: M2 (anti-inflam.)
CD31: blood vessels
KI67: proliferation
BrdU: apoptosis
PAX7: muscle progen.
MYOD: new fibers
PDGFRA: fibroadip.



Blood vessels microenvironment

- CD3:** T cells
- CD14:** monocytes
- CD68:** M1 (pro-inflam.)
- ARG:** M2 (anti-inflam.)
- CD31:** blood vessels
- KI67:** proliferation
- BrdU:** apoptosis
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- MYOD:** new fibers
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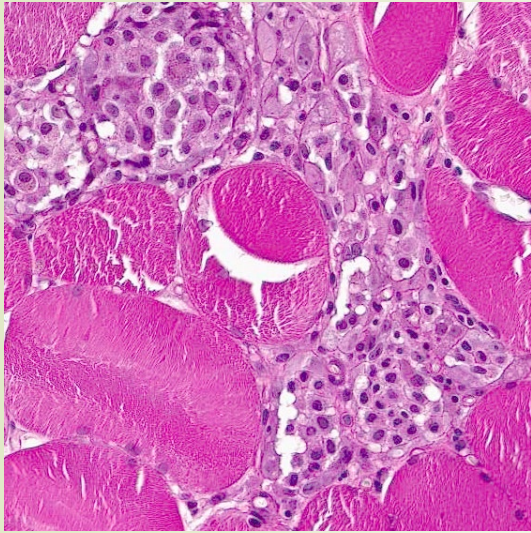




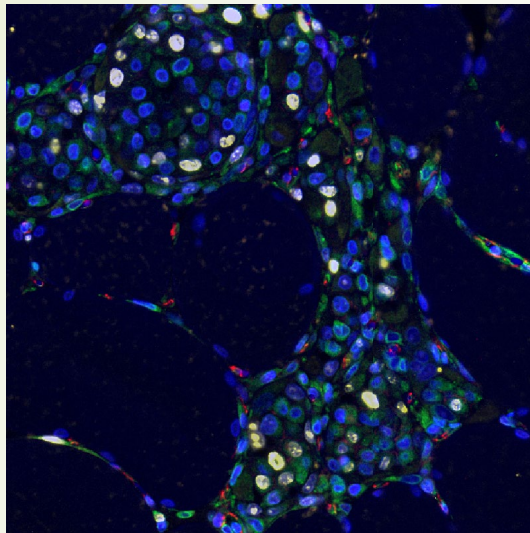
Cells invading damaged fibers

- CD3**: T cells
- CD14**: monocytes
- CD68**: M1 (pro-inflam.)
- ARG**: M2 (anti-inflam.)
- CD31**: blood vessels
- KI67**: proliferation
- BrdU**: apoptosis
- PAX7**: muscle progen.
- MYOD**: new fibers
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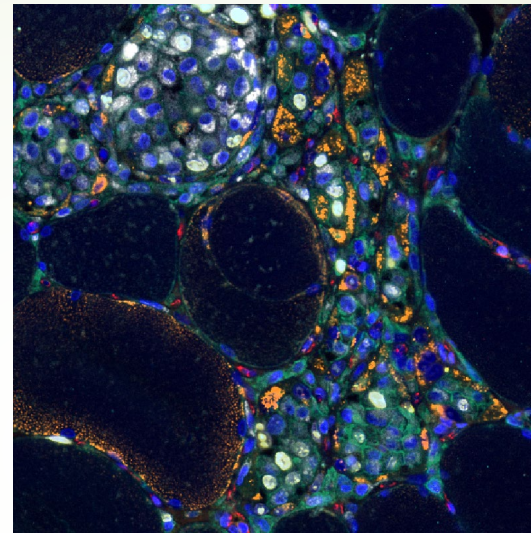
Control



H & E

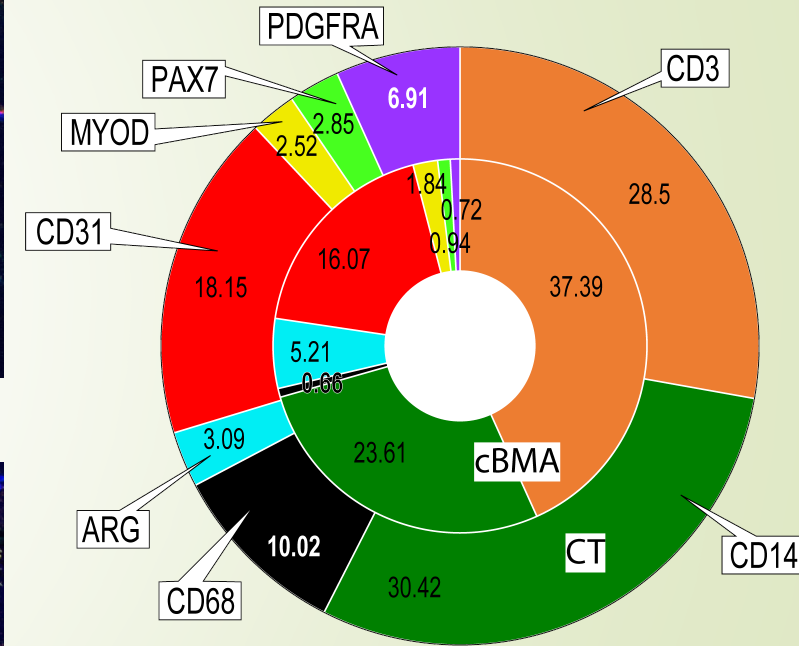
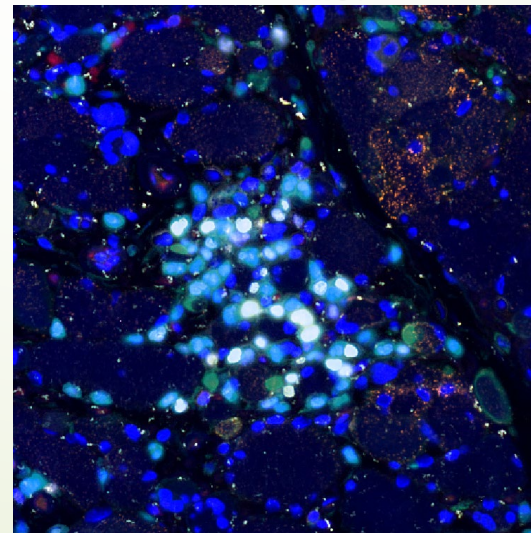
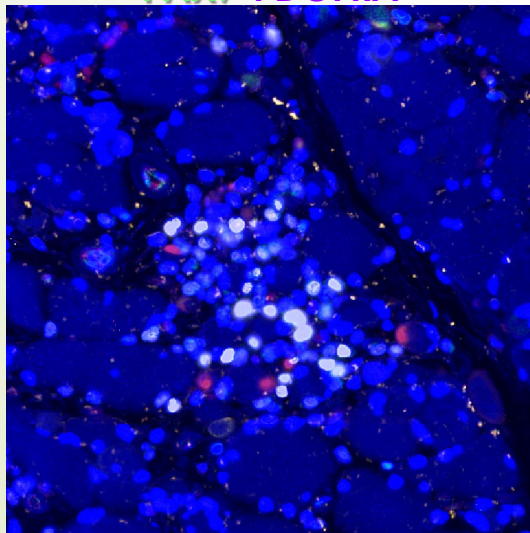
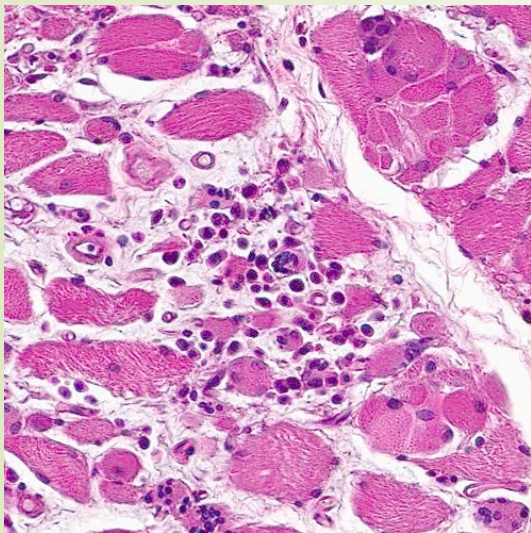


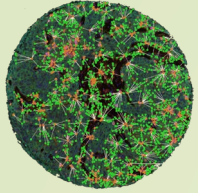
BrdU-CD31-KI67-MYOD-
PAX7-PDGFRA



ARG-CD14-CD3-CD68-
CD31-KI67

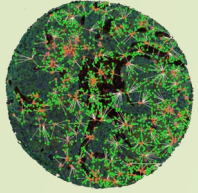
cBMA





Conclusions

- cBMA injection into muscles from CLTI patients induces pro-angiogenic, anti-inflammatory and pro-reparatory processes
- Multiplexed spatial analysis uncovered interesting inter-cellular interactions, leading to a better understanding of this particular cellular therapy
- Among the new observations are:
 - ❖ Association of PAX7 with the endothelial “niche”
 - ❖ Decreased presence of primitive markers (PAX7 and MYOD) in cBMA-treated patients, conceivably due to the respective cells differentiation
 - ❖ Decrease of the fibro-adipocyte marker PDGFRA (reduced fibrotic processes)
 - ❖ A high proportion of T cells (CD3⁺) among the incoming mononuclear infiltrate, as opposed to the lower proportion of monocytes/macrophages
 - ❖ A reversal of the M1:M2 macrophages (CD68⁺:ARG⁺) in cBMA-treated patients, suggesting an anti-inflammatory effect of the injected cells



ACKNOWLEDGMENTS

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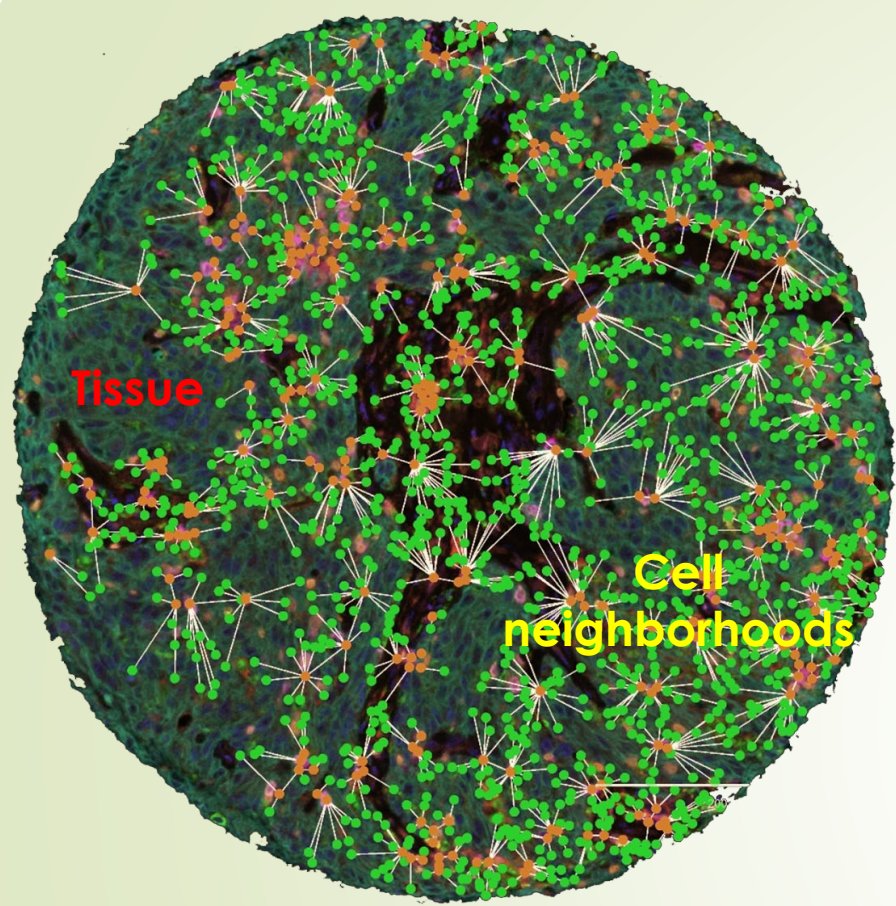
Other contributors

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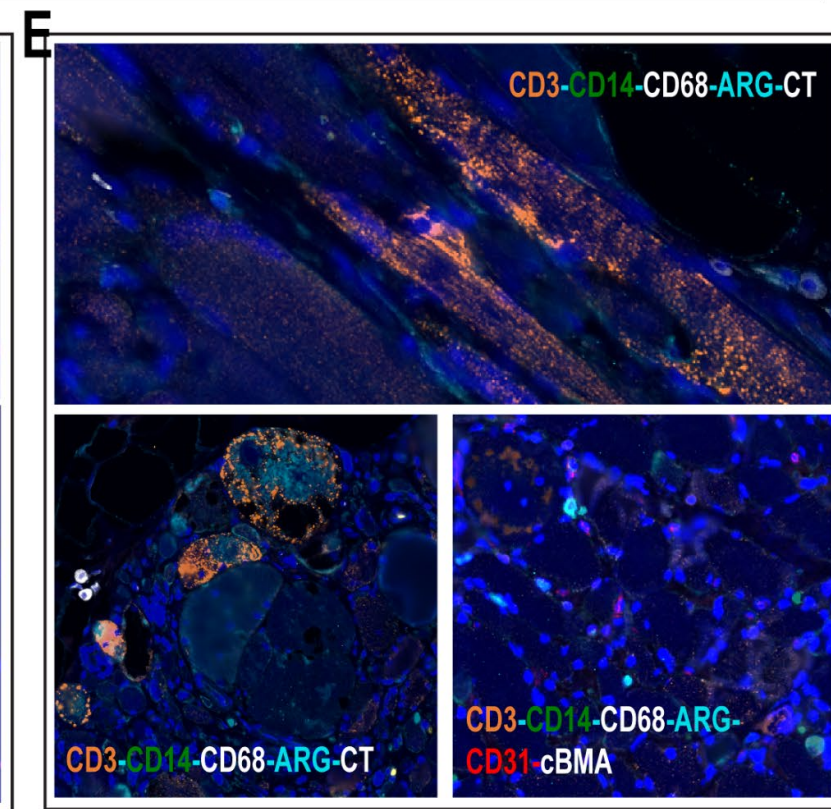
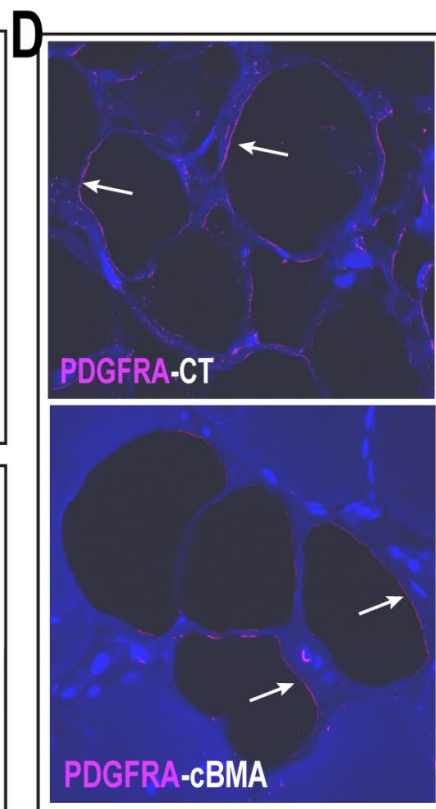
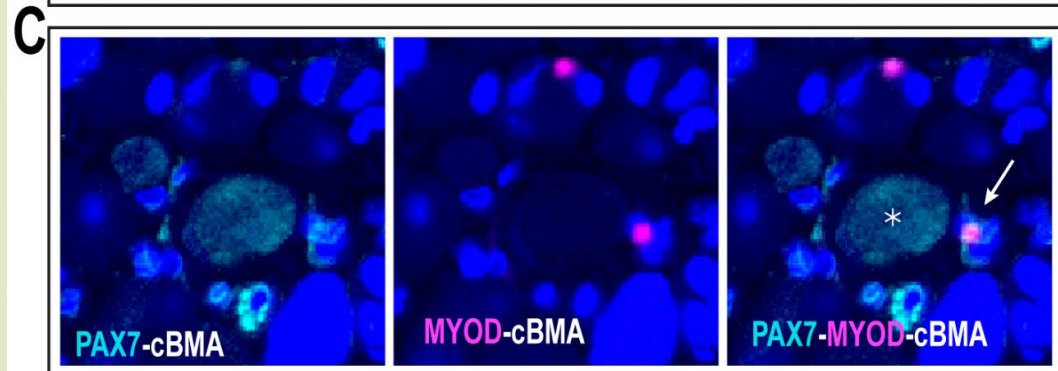
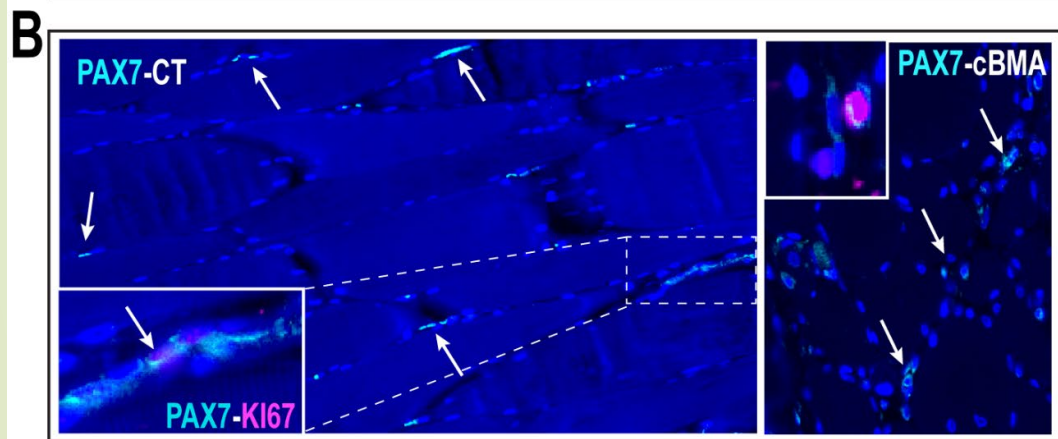
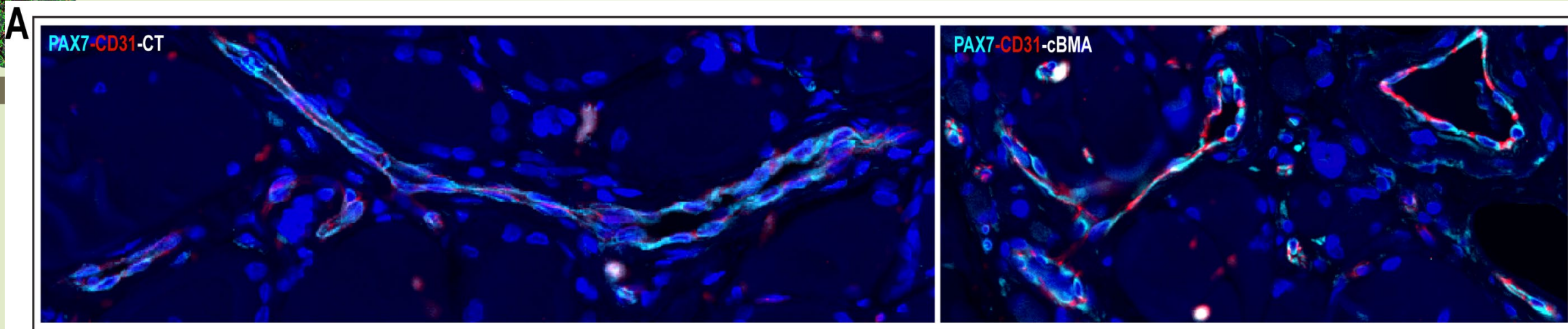
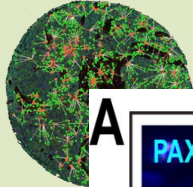
Indiana Center for Biological
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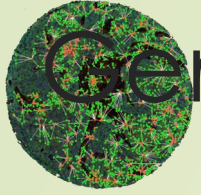
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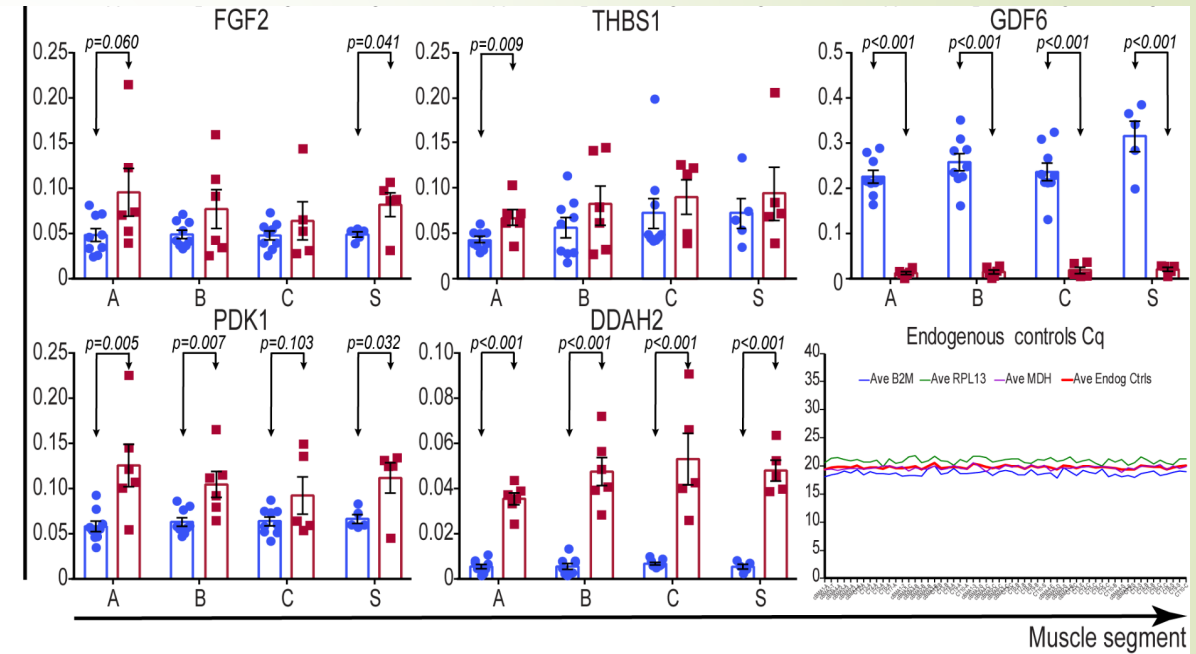
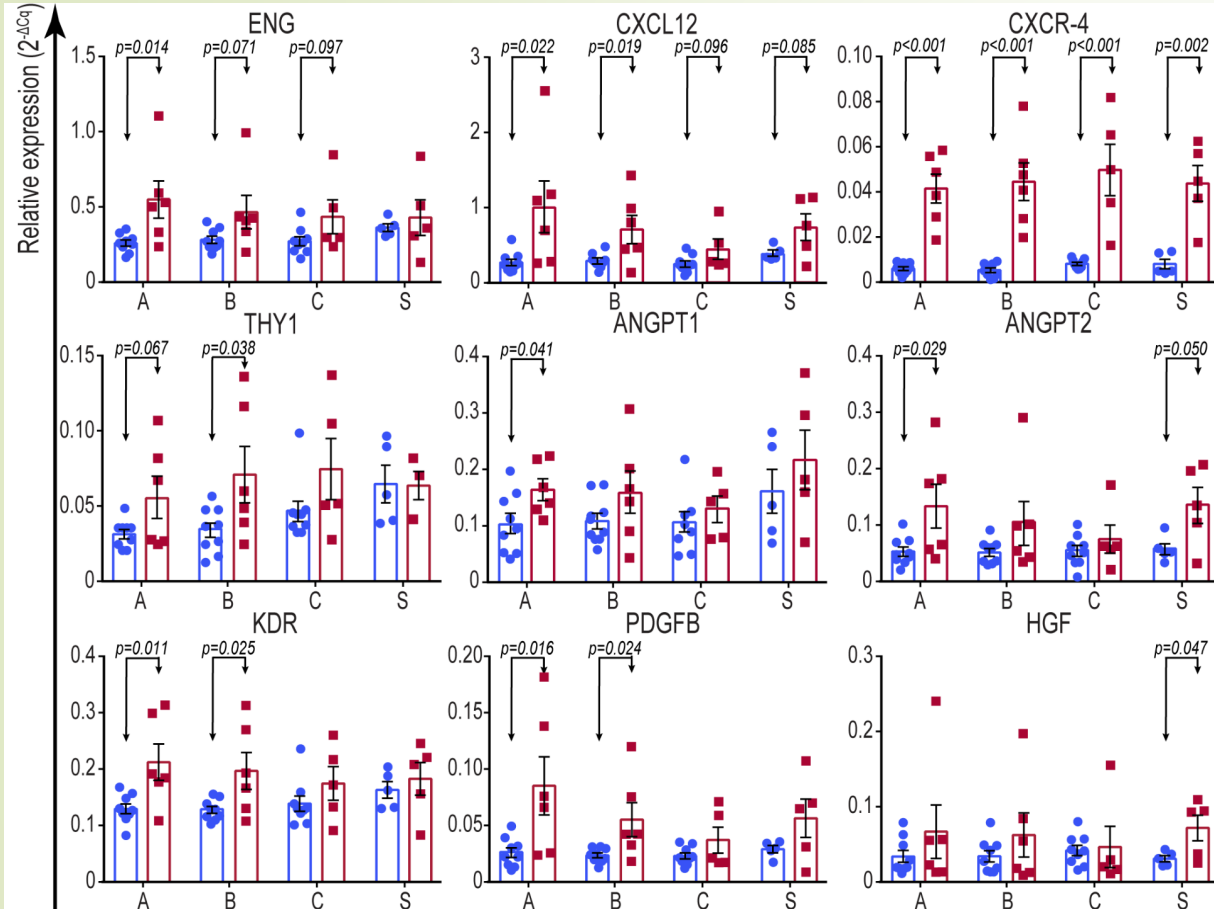


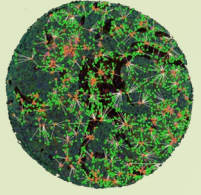
Thank you!





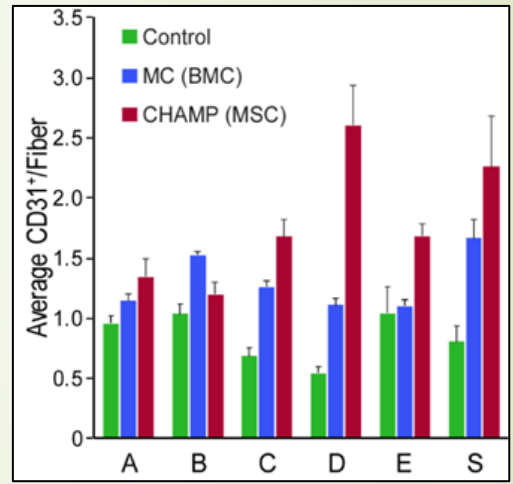
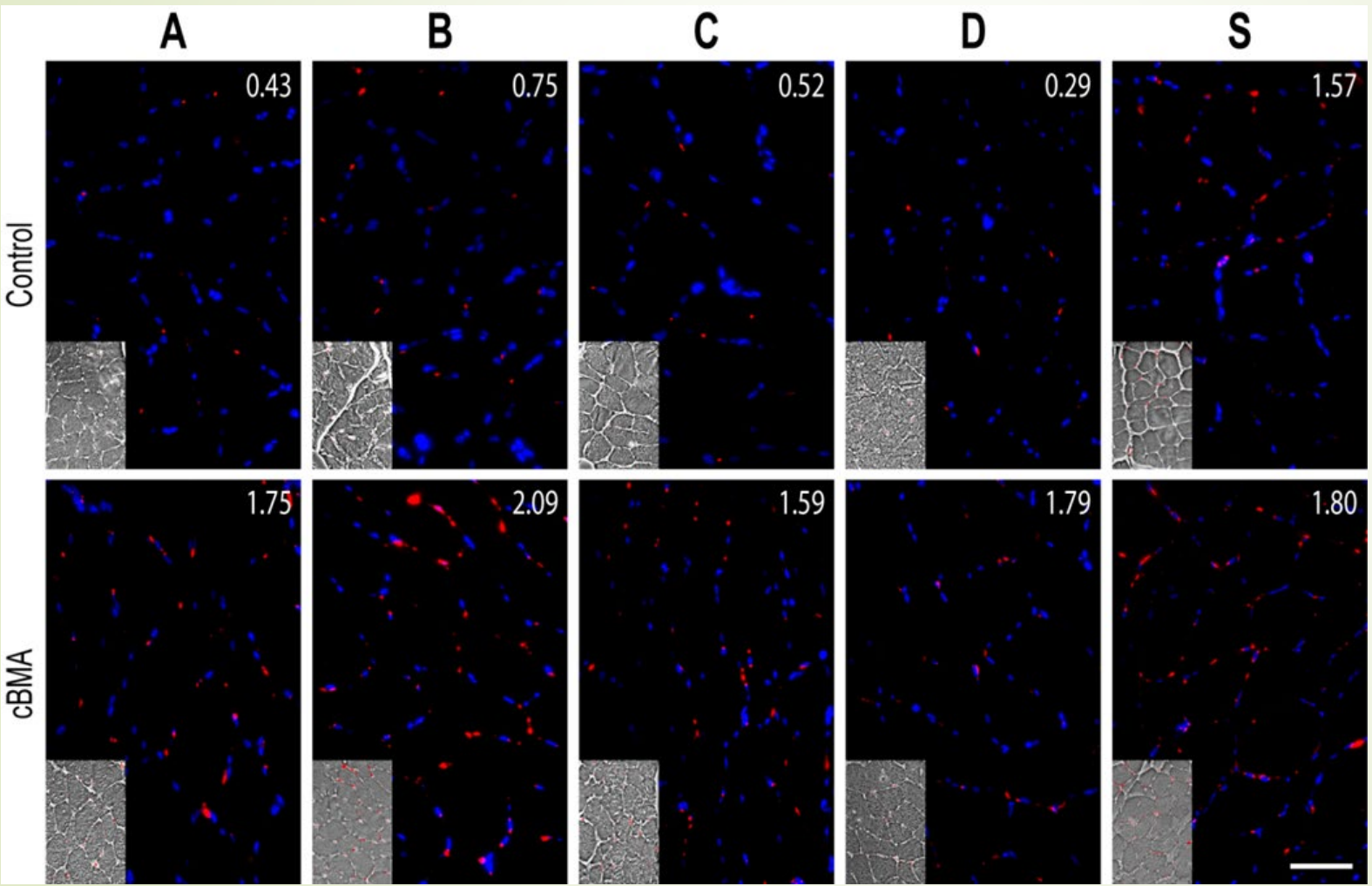
Genomics: real-time PCR [BMC only analyzed so far]

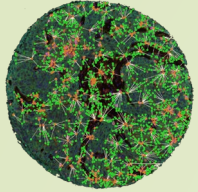




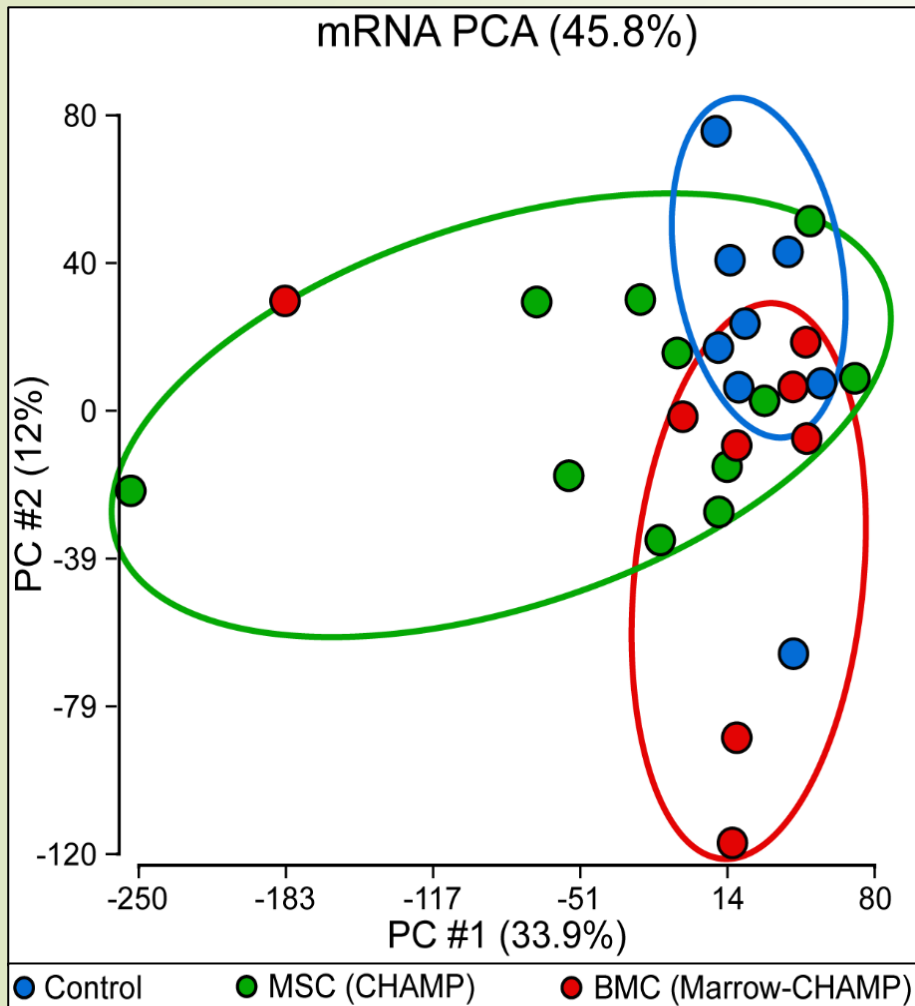
Previous findings: 1. Increased capillary density

Nuclei
CD31
(capillaries)





2. Genomics: differential gene expression



Common in MSC and BMC vs. control

MSC vs. control only

BMC vs. control only

- Blood vessels (development, angiogenesis, functioning)
- Immune system signaling
- Interleukins and cytokine signaling
- Neutrophil degranulation
- Extracellular matrix organization
- Degradation of the extracellular matrix
- Integrin cell surface interactions

- Mitochondria and energy metabolism
- IL10 signaling
- Haemostasis, platelet signaling
- Toll-like receptors signaling
- Plasma lipoprotein assembly, remodeling, and clearance

- Cell cycle, mitosis
- Integrins, intercellular adhesion

- Negative regulation of proliferation

- Hemoglobins
- Granulocyte differentiation and function

- Transcriptional repression
- Chondrogenic differentiation
- Cell proliferation and migration