Functional validation of macrophage subtypes and co-culture models to identify targets in the inflammatory muscle microenvironment

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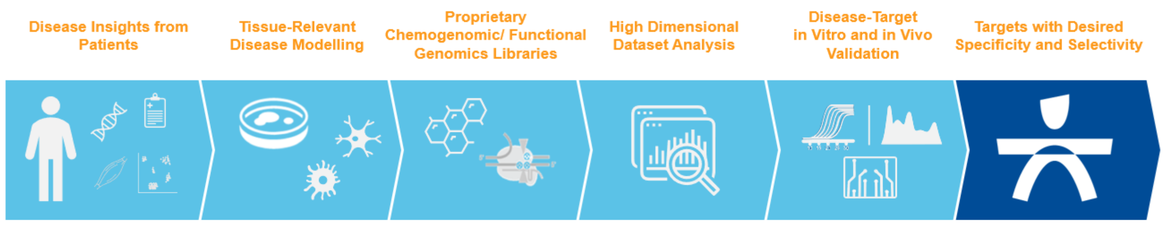
Abstract

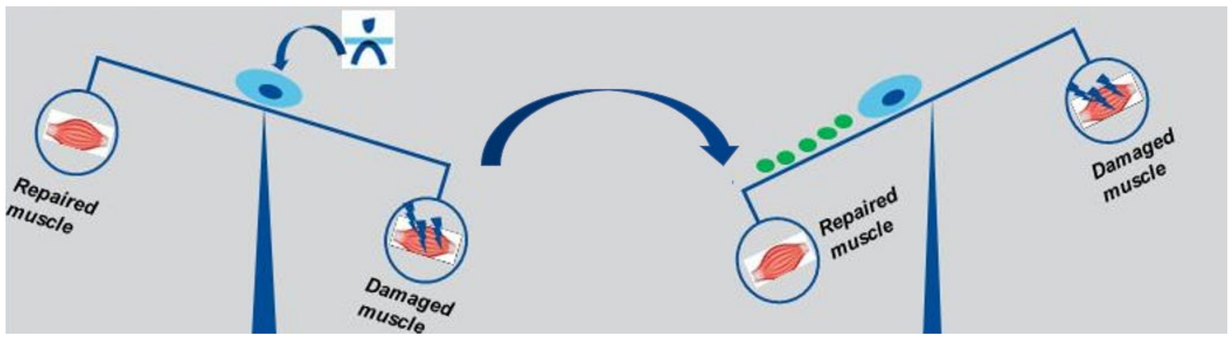
Macrophages are highly plastic, and functionally diverse cells that play key roles during the process of tissue repair. While required for proper muscle regeneration following acute injury, the enduring presence of macrophages in chronic muscle injury represents a hallmark of dystrophic muscle that likely plays a key role in driving fibrofatty replacement. In order to better understand the interactions of macrophages that drive this pathogenic process, we developed an iPSC-derived model of macrophage polarization. M0 macrophages were stimulated towards either classically "pro-inflammatory" M1 macrophages or "anti-inflammatory" M2 macrophages, which can be further divided into their own subsets of M2a, M2b, M2c, and M2d macrophages. These discrete polarization states were validated based on the expression of markers for these various macrophage subtypes using gene expression and protein markers. Coculture models were also developed to functionally evaluate the role of these discrete M2 macrophage subtypes in driving pathogenic differentiation of fibro-adipogenic progenitor (FAPs) cells. The development of these co-culture models and functional identification of these discrete macrophage subtypes allows us to elucidate how the interaction between macrophages and FAPs drive muscle replacement. This will facilitate our target identification efforts to prevent pathogenic macrophage persistence and differentiation to re-balance muscle repair and replacement as a means to treat patients living with devastating muscle diseases.

The Fulcrum Therapeutics Product Engine



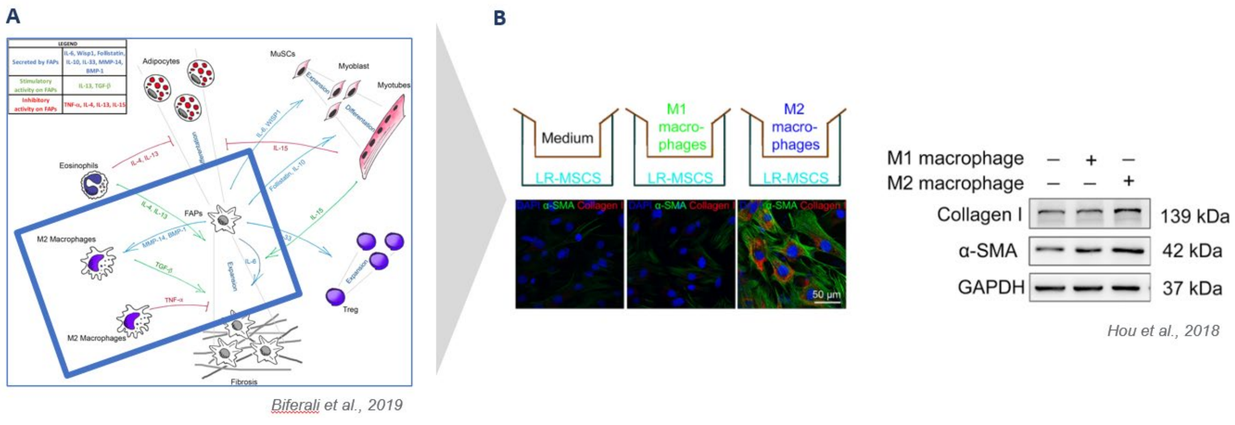
Fulcrum's patient-centric approach *is built over best-in-class cell modelling, chemogenomic tools, high dimensional phenotypic screening and systematic target validation using tissue/disease relevant in vitro and in vivo tools*

Fulcrum's Approach to Re-balancing Muscle Repair and Replacement



By understanding the Hallmarks of Muscular Dystrophy, we can systematically target discrete pathomechanisms to re-balance muscle repair and replacement.

Background

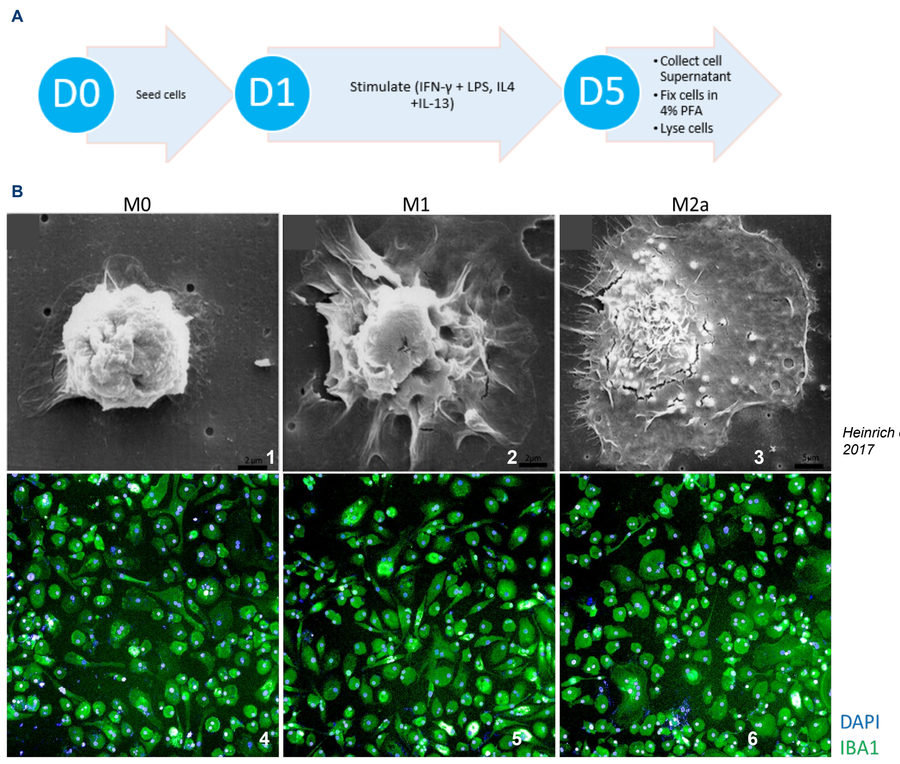


Biferali et al., 2019

Hou et al., 2018

(A) Macrophage:FAP interactions have been characterized to drive fibrofatty replacement **(B)** M2 Macrophages in co-culture with MSCs recapitulate increased fibrotic differentiation within the dystrophic microenvironment

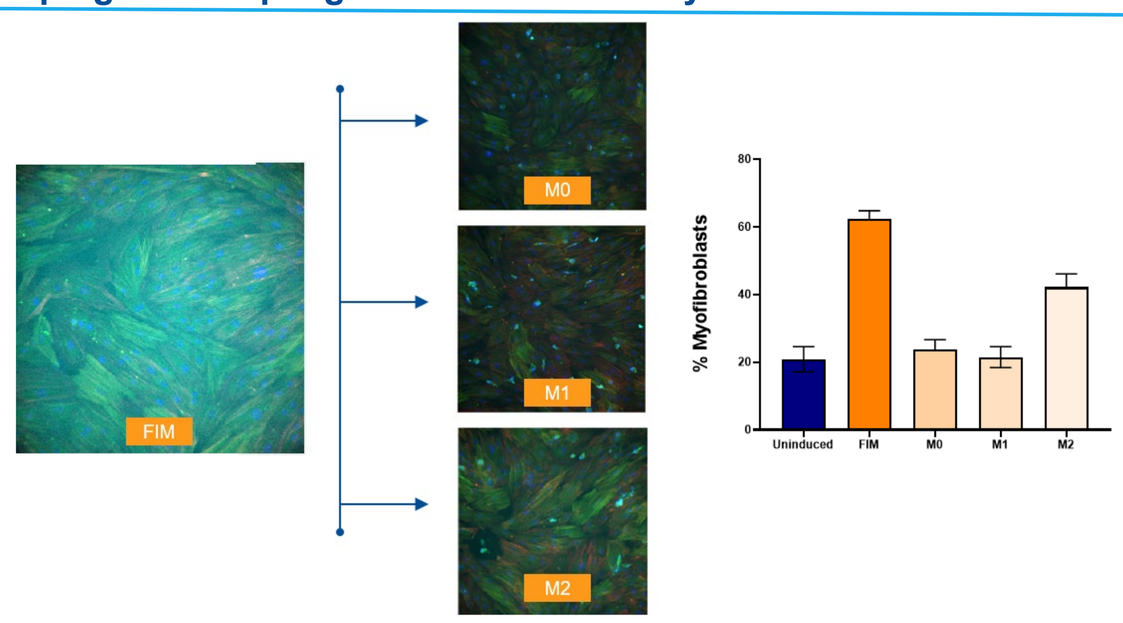
Assay Development and Validation of Macrophage Polarization States



Heinrich et al., 2017

(A) Assay Timeline **(B)** (1) M0 Macrophage reference; small, round morphology. (2) M1 reference; cytoplasmic extensions morphology (3) M2 reference; multinucleated giant cell (MNGs) morphology (4) M0 IBA1 staining to demonstrate small, round morphology (5) M1 IBA1 staining to demonstrate cytoplasmic extensions (6) M2 IBA1 staining with examples of MNGs **(C)** Validation of polarization states by RT-qPCR measuring TNF-α and MRC1 which are highly expressed in M1 and M2 macrophages, respectively

Developing a Macrophage/MSC Co-Culture System



(A) Preliminary data on a Macrophage:MSC Co-Culture utilizing iPSC-derived macrophages. This data shows differentiation to myofibroblasts when cultured with M2 macrophages but not M1 or M0, similar to induction with TGF-β.

Next Steps

- We plan to further sub-characterize into various M2 subtypes (M2b, M2c, M2d) utilizing the ICC and qPCR assays developed to assess their specific roles tied into pathogenic differentiation.
- Assess tools to perturb macrophage polarization states
- Develop co-culture model to investigate the role of macrophages in adipogenic differentiation

References

(1) Hou et al. M2 macrophages promote myofibroblast differentiation of LR-MSCs and are associated with pulmonary fibrogenesis *Cell Commun Signal 16, 89 (2018)*
(2) Biferali et al. Fibro-adipogenic progenitors cross-talk in skeletal muscle: The social network. *Front. Physiol.* 10:1074 (2019).
(3) Heinrich Fet al. (2017) Morphologic, phenotypic, and transcriptomic characterization of classically and alternatively activated canine blood-derived macrophages in vitro. *PLOS ONE* 12(8): e0183572