CRISPR activation-mediated human *LAMA1* upregulation rescues aberrant cellular migration in *LAMA2*-deficient congenital muscular dystrophy (MDC1A)

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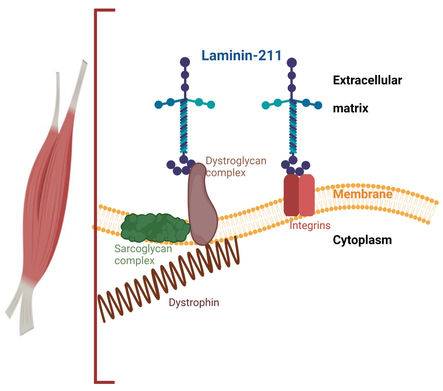
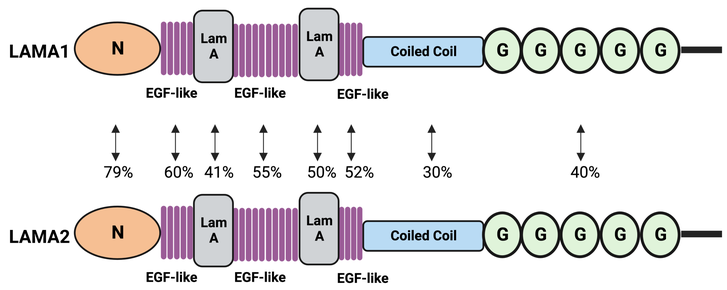
LAMA2 mutation causes congenital muscular dystrophy type 1 A (MDC1A)



- Patients exhibit **muscle weakness**, skeletal deformations and ambulatory difficulties.
- There is no cure for MDC1A.
- **Heterogeneity of mutations** makes therapy challenging.

LAMA1 as a compensatory gene for *LAMA2*



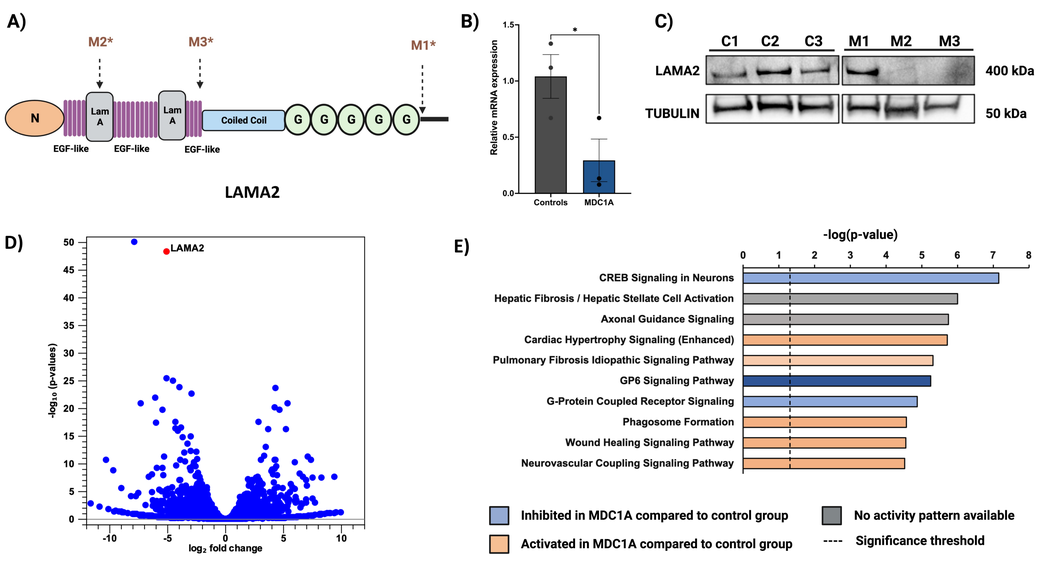
- Aid in basement membrane stability and **myogenesis** and possess **similar binding sites to cell surface receptors.**
- **Previous research:** Postnatal upregulation of *Lama1* using CRISPR activation **improved the disease phenotypes** of the MDC1A mice *(Kemaladewi*, Bassi*, et al, 2019)*.
- It remains unknown whether human *LAMA1* can compensate for lack of *LAMA2* in MDC1A patients.

Hypothesis of the study

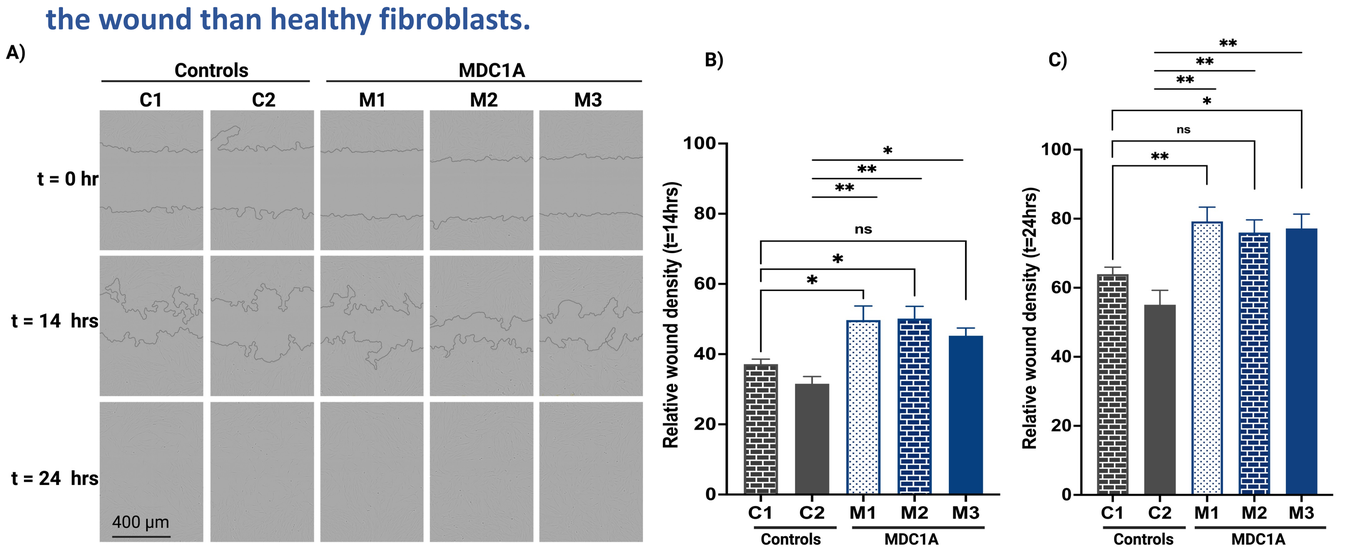
We hypothesize that CRISPR activation mediated upregulation of human *LAMA1* can compensate for the lack of *LAMA2* and rescue cellular abnormalities in MDC1A fibroblasts.

LAMA2-deficiency in MDC1A patient-derived fibroblasts manifests as overactive wound healing/migration

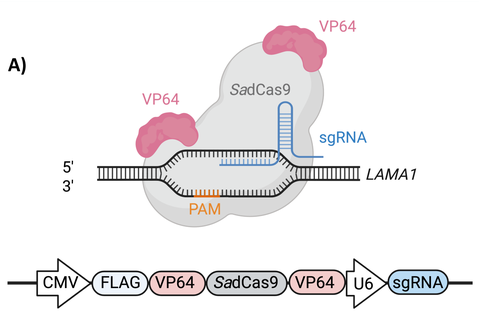
- MDC1A fibroblasts (M1, M2, and M3) with different *LAMA2* mutations exhibit **activated wound healing pathway.**

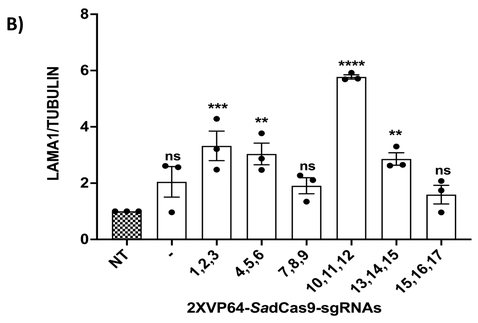


- We employed migration assay using the Incucyte instrument, which couples an automated wound creation and live cell imaging. We observed that MDC1A **patient fibroblasts migrated faster to close the wound than healthy fibroblasts.**



CRISPR activation system to upregulate human *LAMA1*

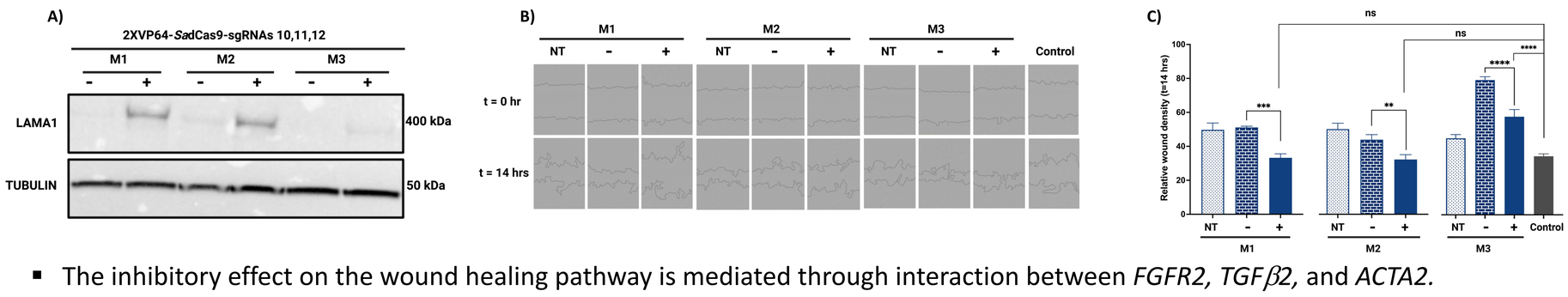




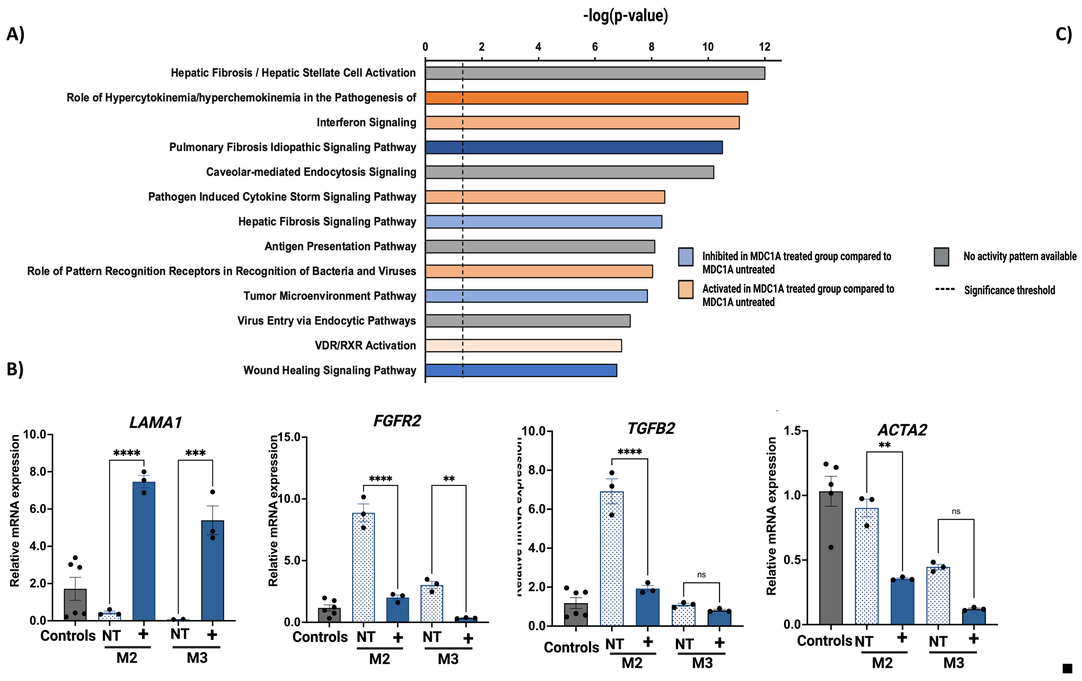
CRISPR activation system: We designed **17 sgRNAs** targeting the proximal promoter region of the **human *LAMA1* gene.** Each sgRNA was inserted into a plasmid consisting of 2X VP64 transcriptional activators flanking the dCas9 derived from *S. aureus.* Screening in 293T cells shows that **sgRNAs 10,11,12** resulted in the highest upregulation of *LAMA1*.

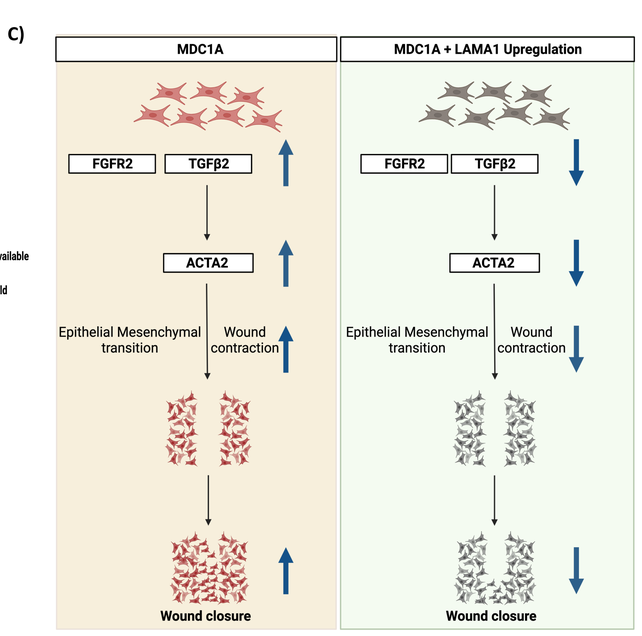
CRISPR activation-mediated *LAMA1* upregulation compensates for the lack of *LAMA2* and rescues aberrant migration in MDC1A fibroblasts

- CRISPR activation induces ***LAMA1* upregulation** in MDC1A patient fibroblasts and reduces the overactive migration.



- The inhibitory effect on the wound healing pathway is mediated through interaction between *FGFR2, TGFβ2,* and *ACTA2.*





- The MDC1A fibroblasts express high *FGFR2, TGFβ2,* and *ACTA2,* facilitating increased migration and wound contraction.
- The signaling mechanisms are downregulated in response to the upregulation of *LAMA1*, resulting in reduced migration.

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CRISPRa-induced upregulation of human *LAMA1* compensates for *LAMA2*-deficiency in Merosin-deficient congenital muscular dystrophy

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I am graduating in April 2023 with a Ph.D. in Human Genetics and looking for job opportunities in biotech and/or nonprofits.

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