

Chronic Binge Alcohol Impairs Myoblast Differentiation: Role of MicroRNA-206

Introduction

- People living with HIV (PLWH) experience aging related comorbidities earlier in life than the general population
- Skeletal muscle (SKM) myopathy occurs in 40-60% of people with alcohol use disorder
- At-risk alcohol use is twice as likely in PLWH
- Impaired SKM myoblast differentiation contributes to reduced functional SKM
- Chronic binge alcohol (CBA) impairs myoblast differentiation and decreases microRNA-206 in SIV infected macaques

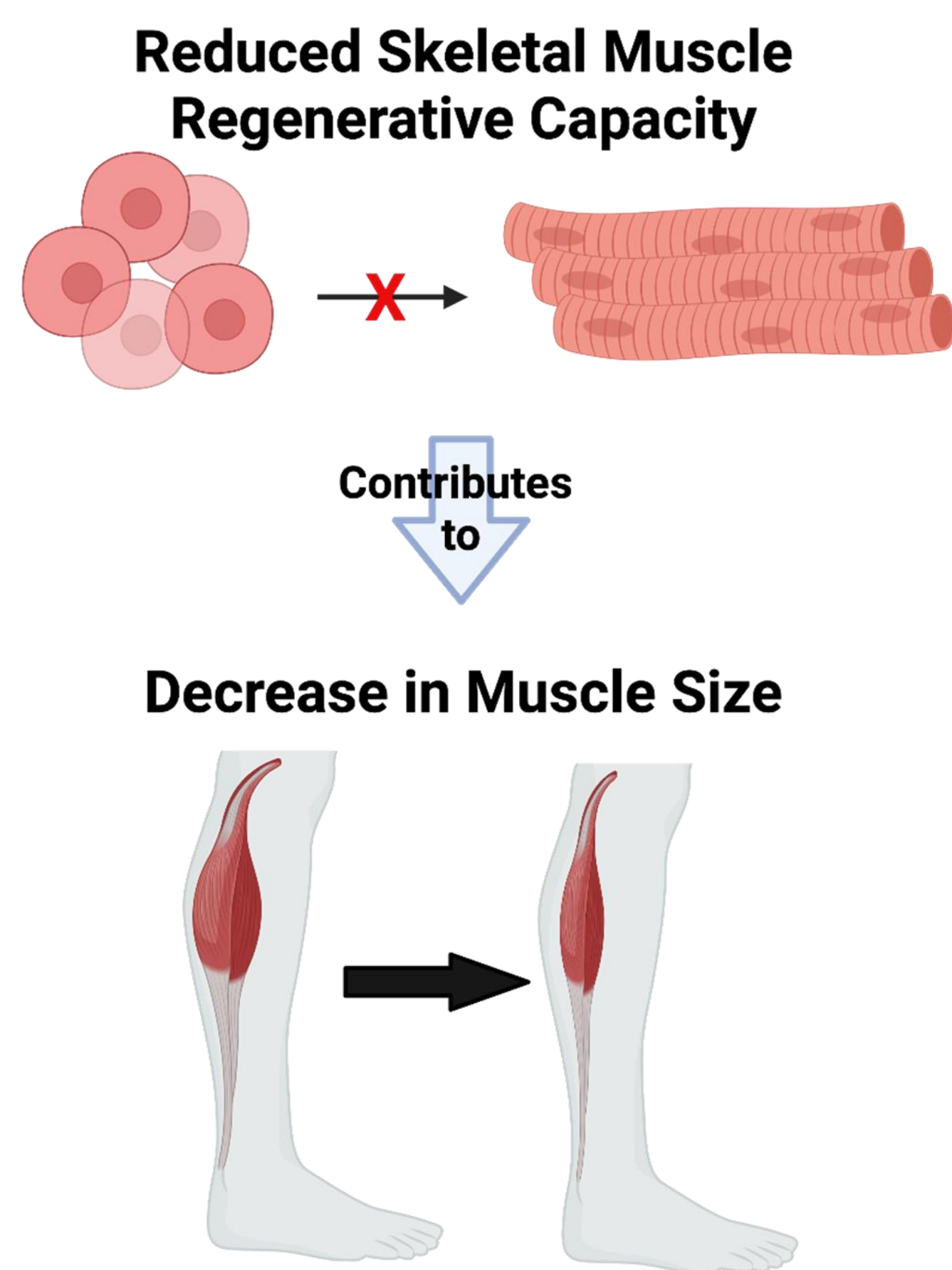
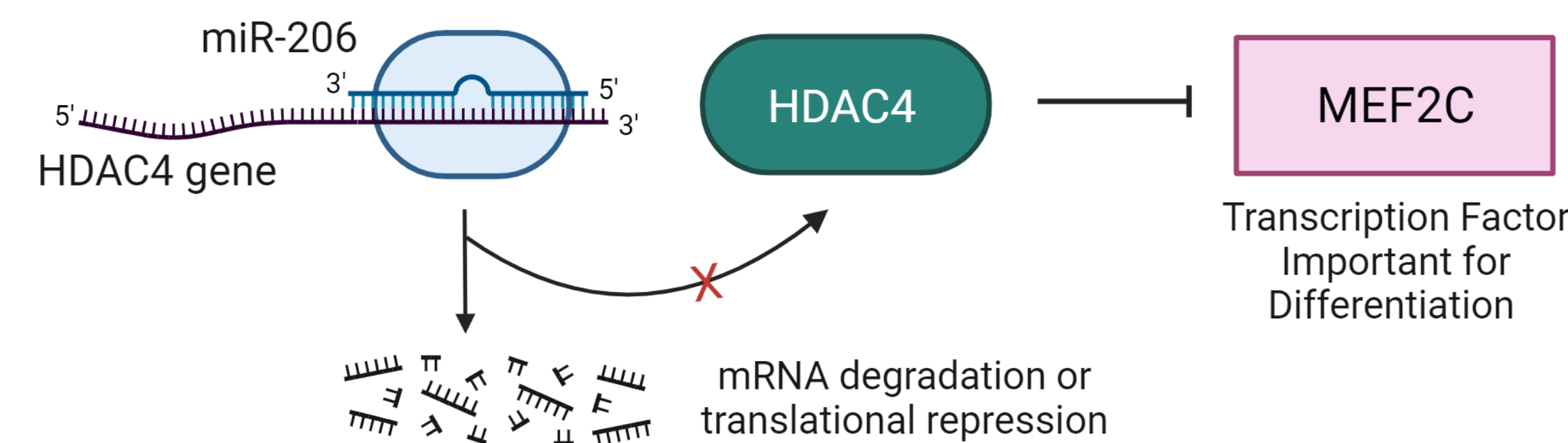
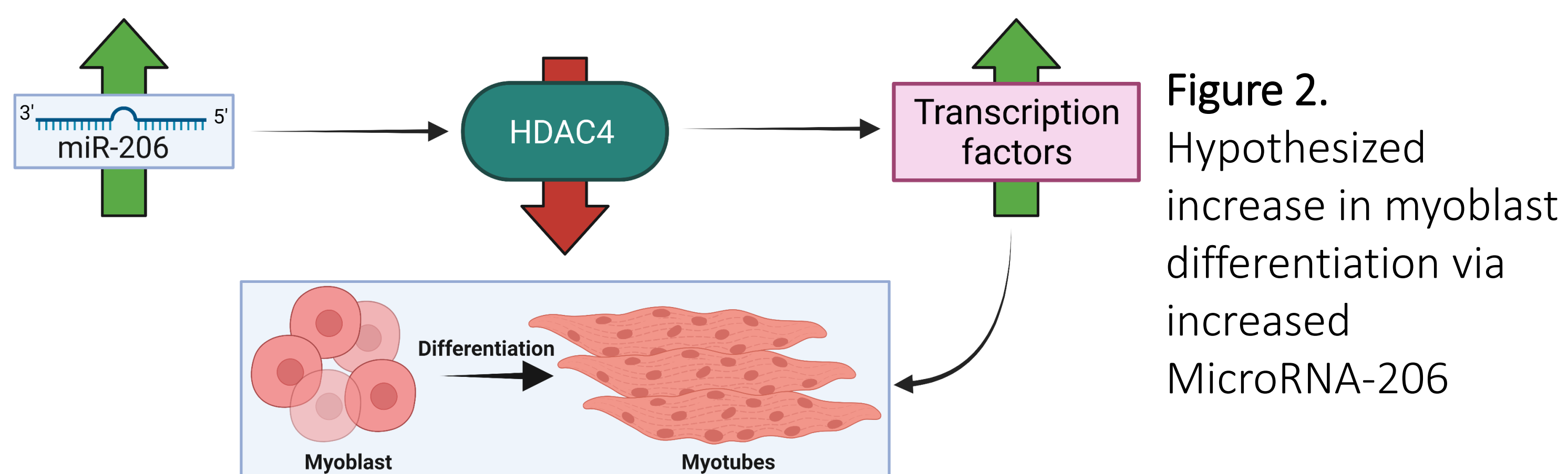


Figure 1. Mechanism of microRNA-206 in myoblast differentiation

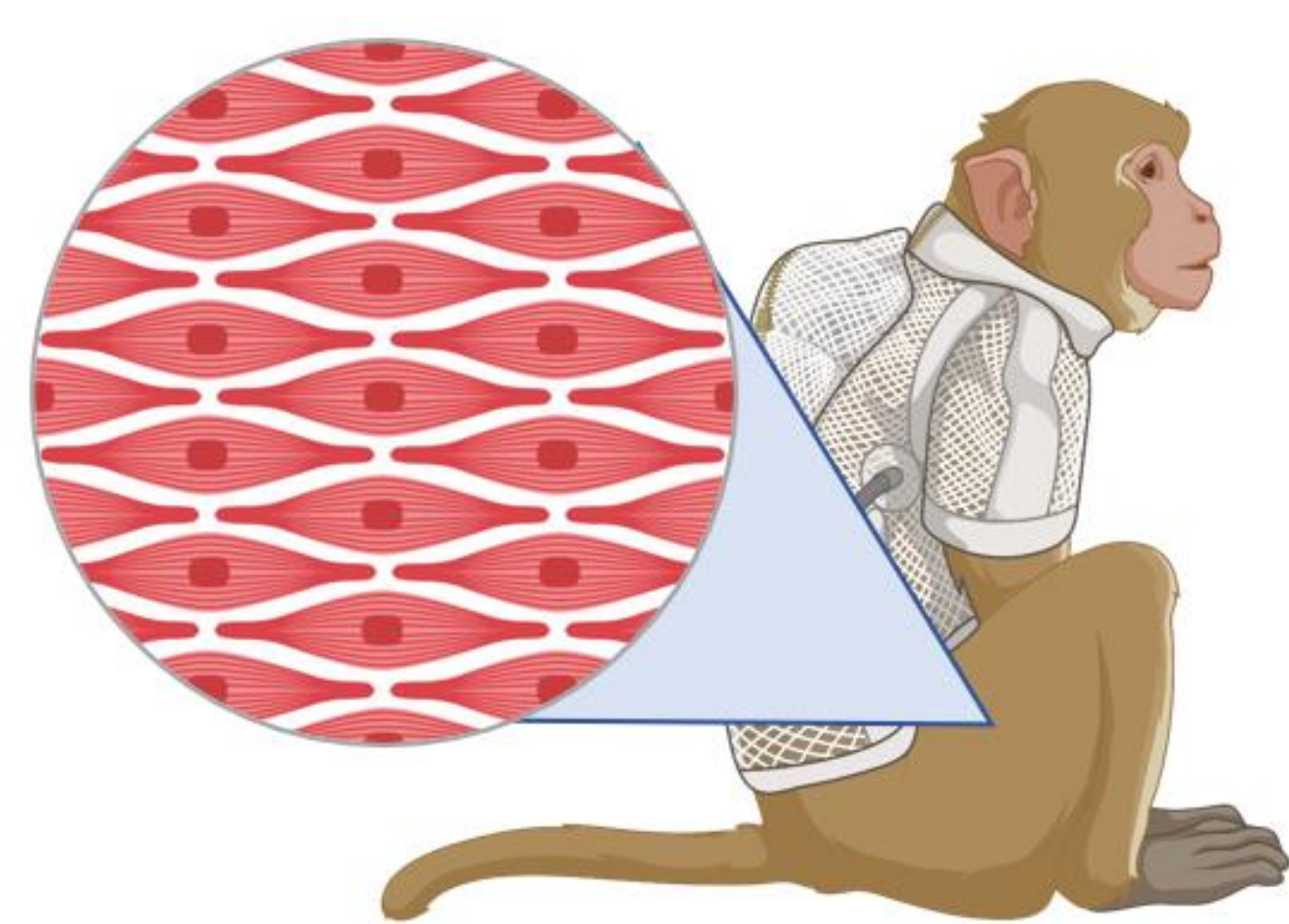


Hypothesis

- Increasing MicroRNA-206 expression in CBA primary macaque myoblasts will increase differentiation



Study Design



- Female rhesus macaques given CBA or VEH prior to SIV infection
- All animals received ART therapy
- At study endpoint, primary cells from the vastus lateralis muscle were isolated and SKM stem cells proliferated from fresh tissue

9 Months

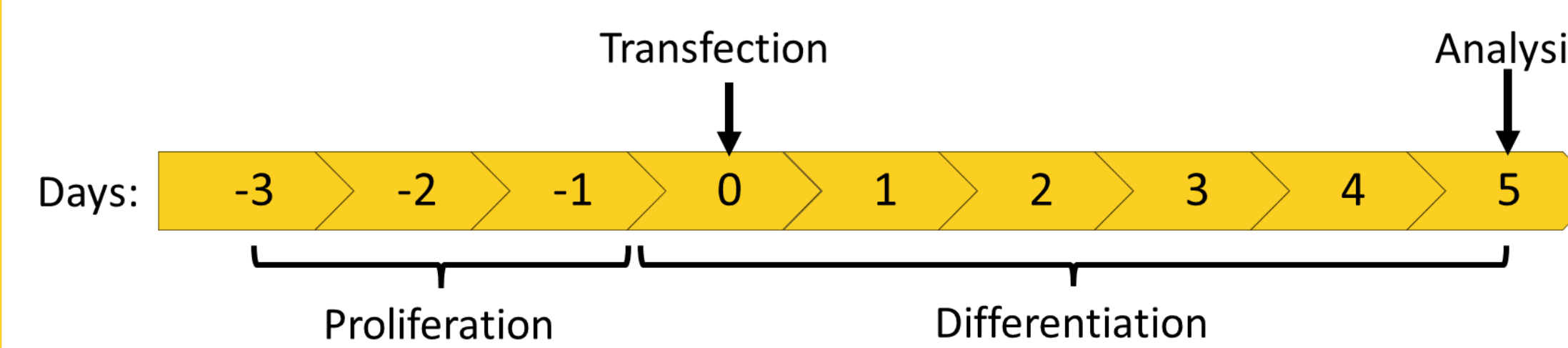
2.5 Months Antiretrovirals (Tenofovir & Emtricitabine)

3 Months SIV_{mac251} Infection

Chronic Binge Alcohol (CBA) or Vehicle (VEH) – 13-14 g/kg/week

14.5 Months

Methods



- Cell plating with cryopreserved VEH or CBA myoblasts at passage 4
- Myoblasts were proliferated for 3 days
- Transfection occurred on day 0 of differentiation using Lipofectamine 2000
- Myoblasts were allowed to differentiate for 5 days
- At day 5 of differentiation, HEMA 3 staining was used to determine the fusion index. RNA was isolated and cDNA synthesized for qRT-PCR

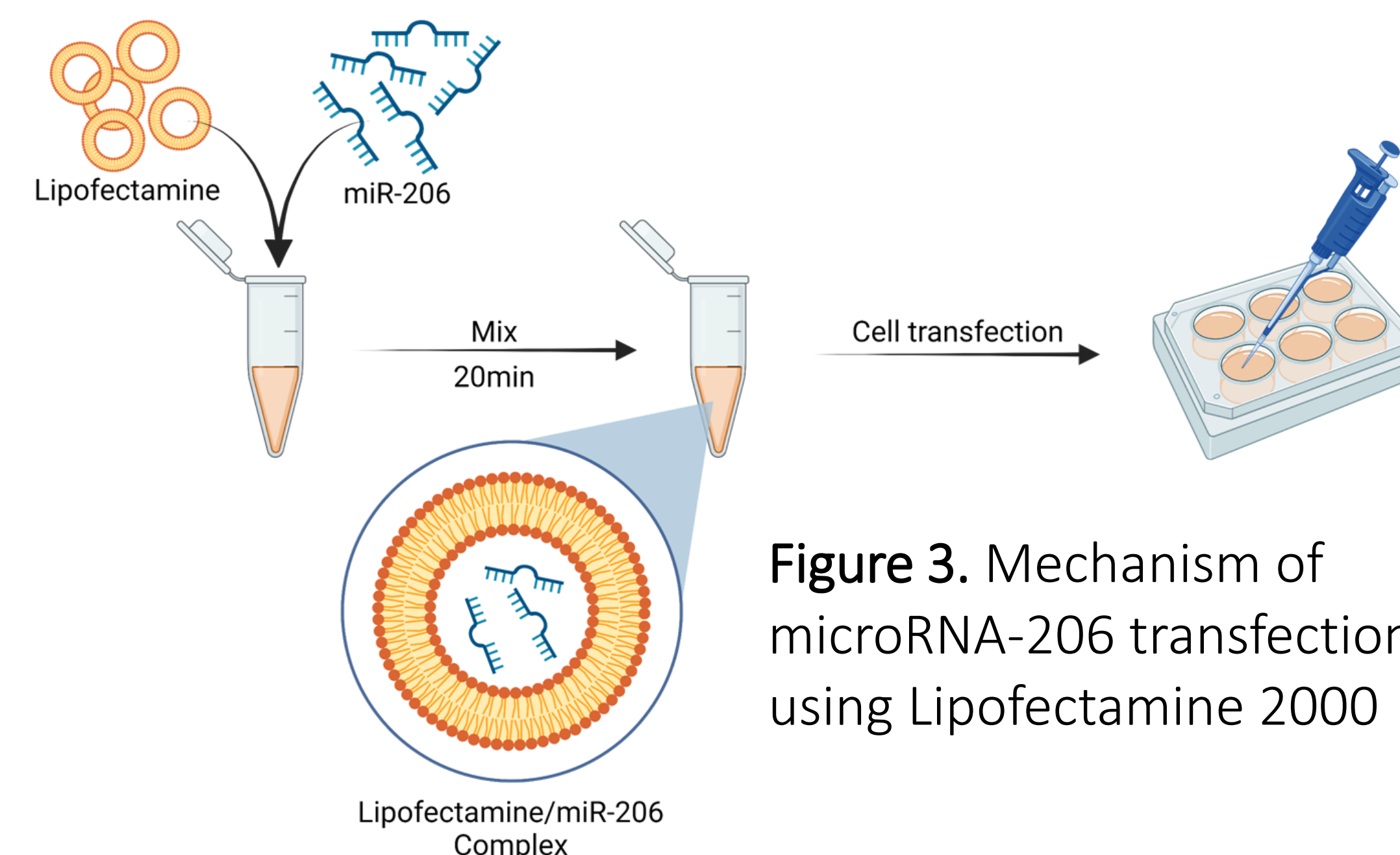


Figure 3. Mechanism of microRNA-206 transfection using Lipofectamine 2000

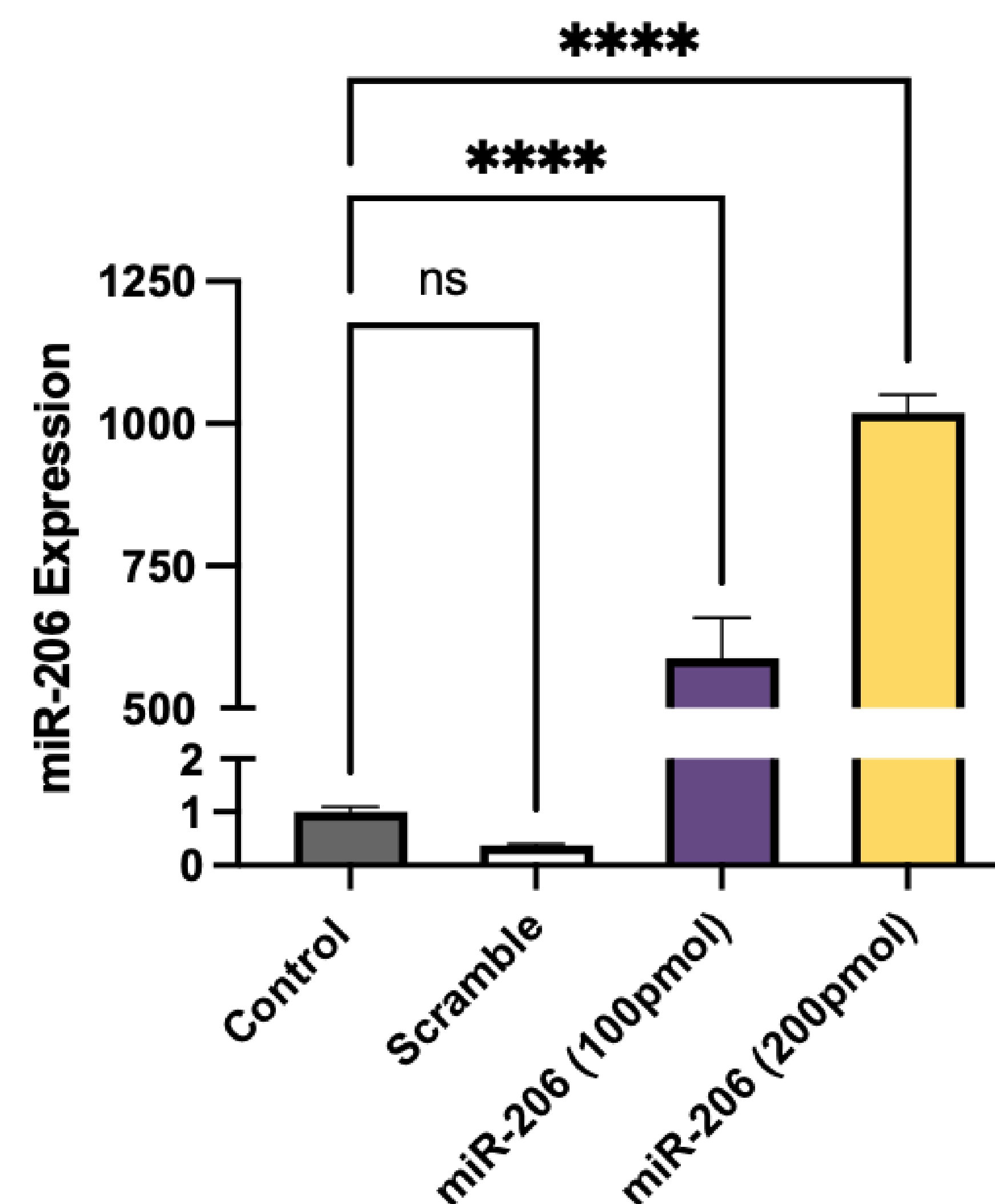


Figure 4. microRNA-206 transfection optimization

MicroRNA-206 Expression

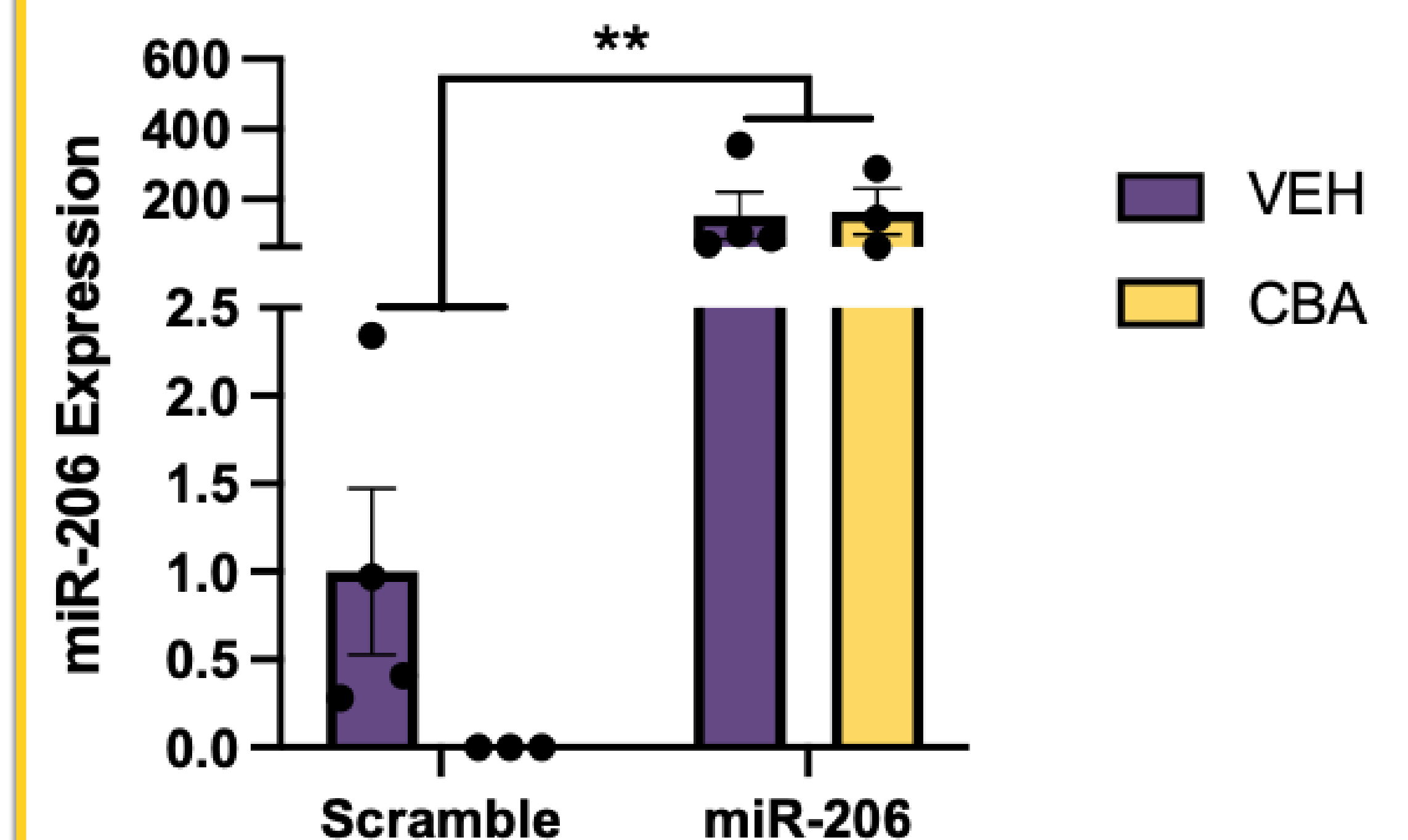


Figure 5. MicroRNA-206 expression was increased in VEH and CBA myoblasts that were transfected.

HEMA 3 Staining for Fusion Index

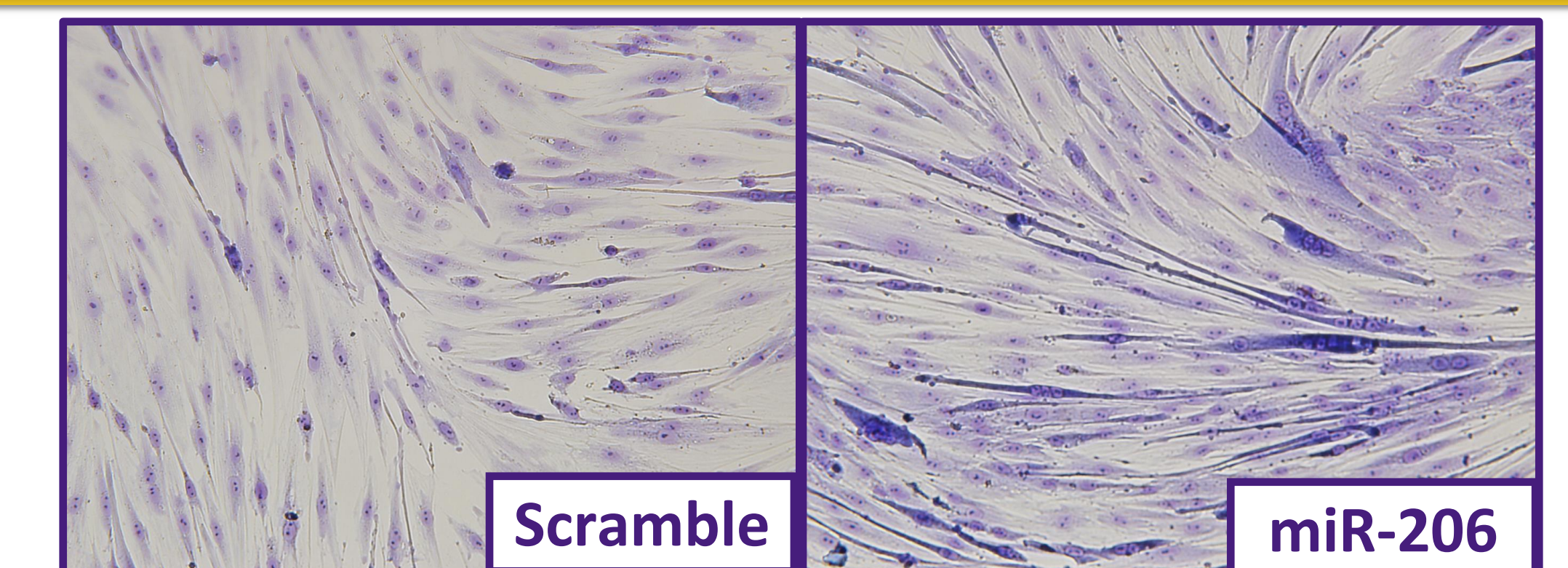


Figure 6. Myoblast differentiation determined using fusion index after HEMA 3 staining. Increased fusion index occurred in myoblasts transfected with MicroRNA-206

Fusion Index is Increased after Transfection

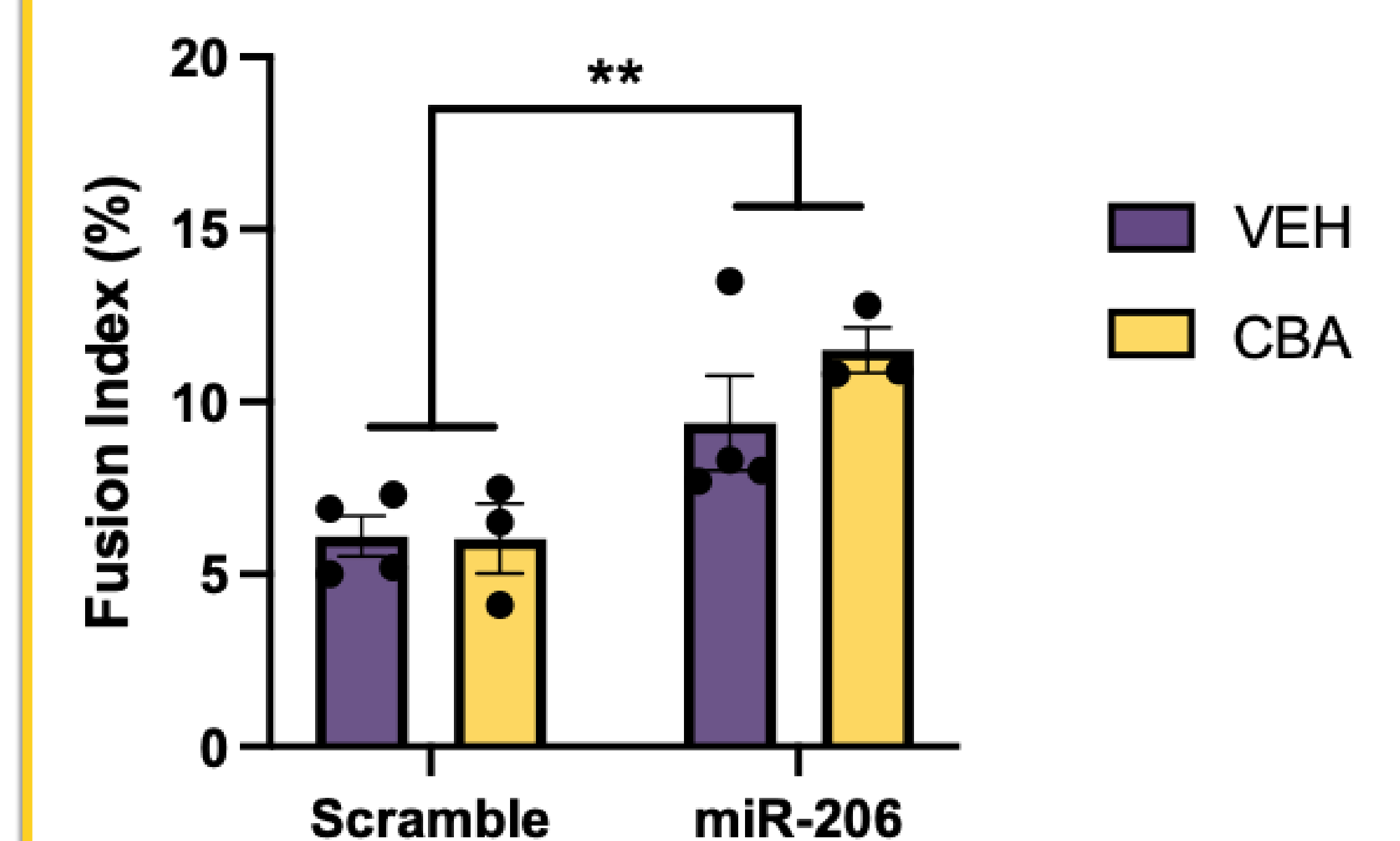


Figure 7. Fusion index for VEH and CBA myoblasts was higher in cells that were transfected with MicroRNA-206. Two-way ANOVA performed for figures 5 and 7.

Summary and Future Direction

Summary

- Transfection of VEH and CBA myoblasts with miR-206 increases the expression of miR-206 and increases differentiation

Future Direction

- Future studies will measure HDAC4 and MEF2C expression levels and determine if our hypothesis can be applied to humans

Thank You!

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COMPREHENSIVE ALCOHOL-
HIV/AIDS RESEARCH CENTER

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