

Polarization state specific synthetic promoters for control of macrophage cell therapies

Control of macrophage cell therapies
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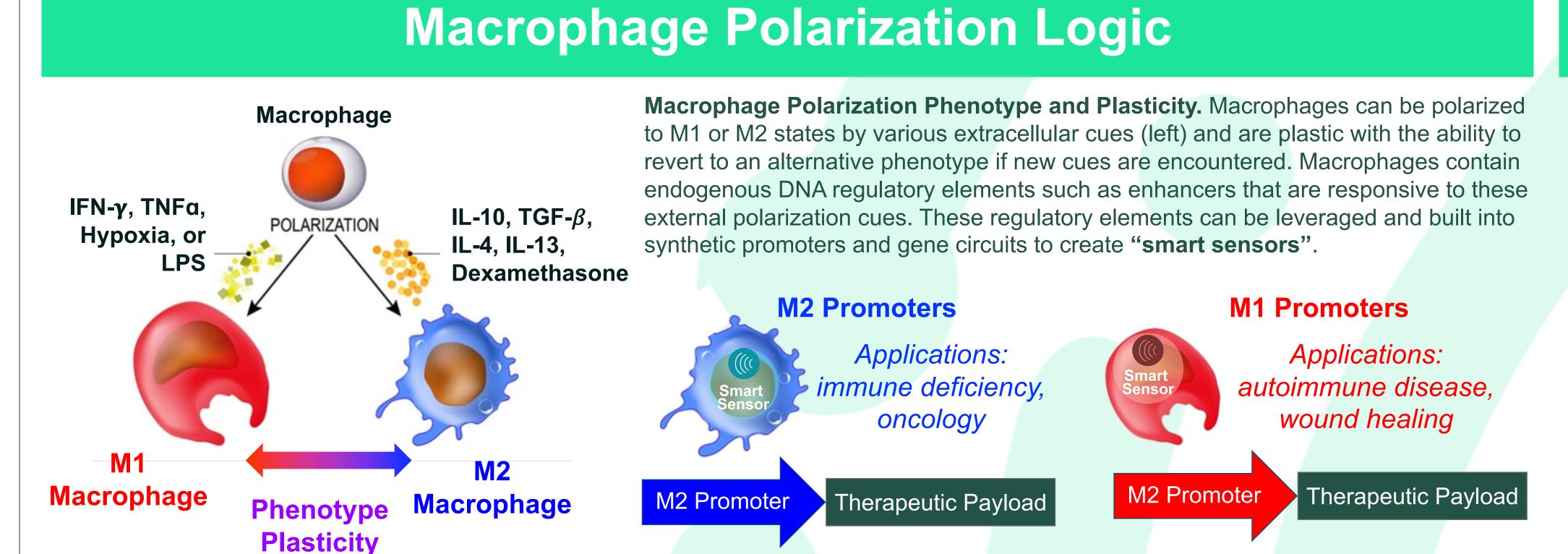
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BlueRock

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## Macrophage Polarization State Specific Promoters



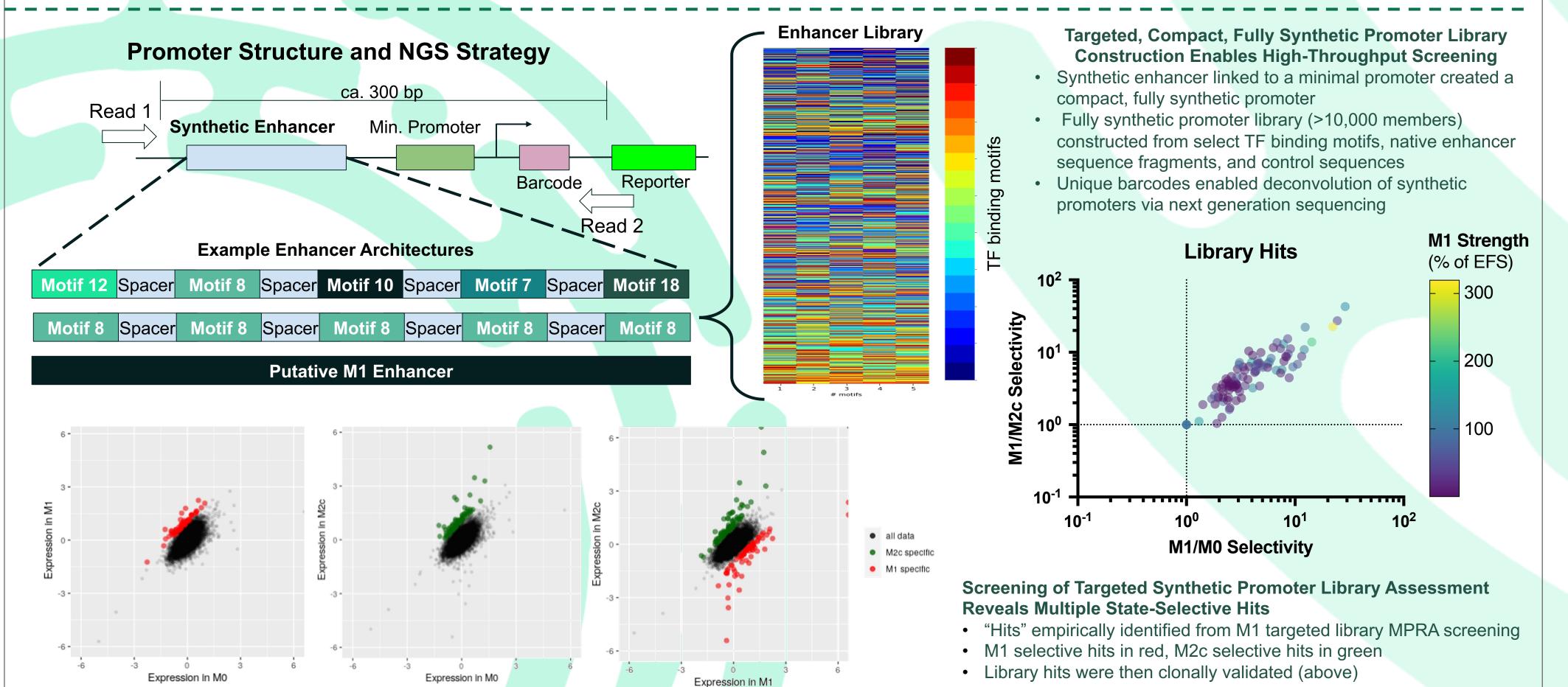
Polarization state specific promoters sense pathological states and locally control payload expression

## **Promoter Discovery Strategies Re-engineered Native Compact Fully Synthetic Enhancer-based Synthetic Promoter Screening (<200bp) Promoters (500-2000bp) Promoters (500-2000bp)** Pooled screening of fully synthetic Native promoters mined Putative enhancers mined from RNA-Seq promoter libraries from ATAC-Seq Bioinformatic selection of Functional mapping via Selective Enhancer **Core Promoter MPRA Hits** mutational variants Engineering Identification Clonal Re-design into 3<sup>rd</sup> generation variants **Outcome: Outcome: Outcome:** Strong & Specific M2 Promoters Strong & Specific Strong & Specific M1 Promoters M1 Promoters

Successful discovery of state-specific "sensors" from three parallel promoter discovery strategies

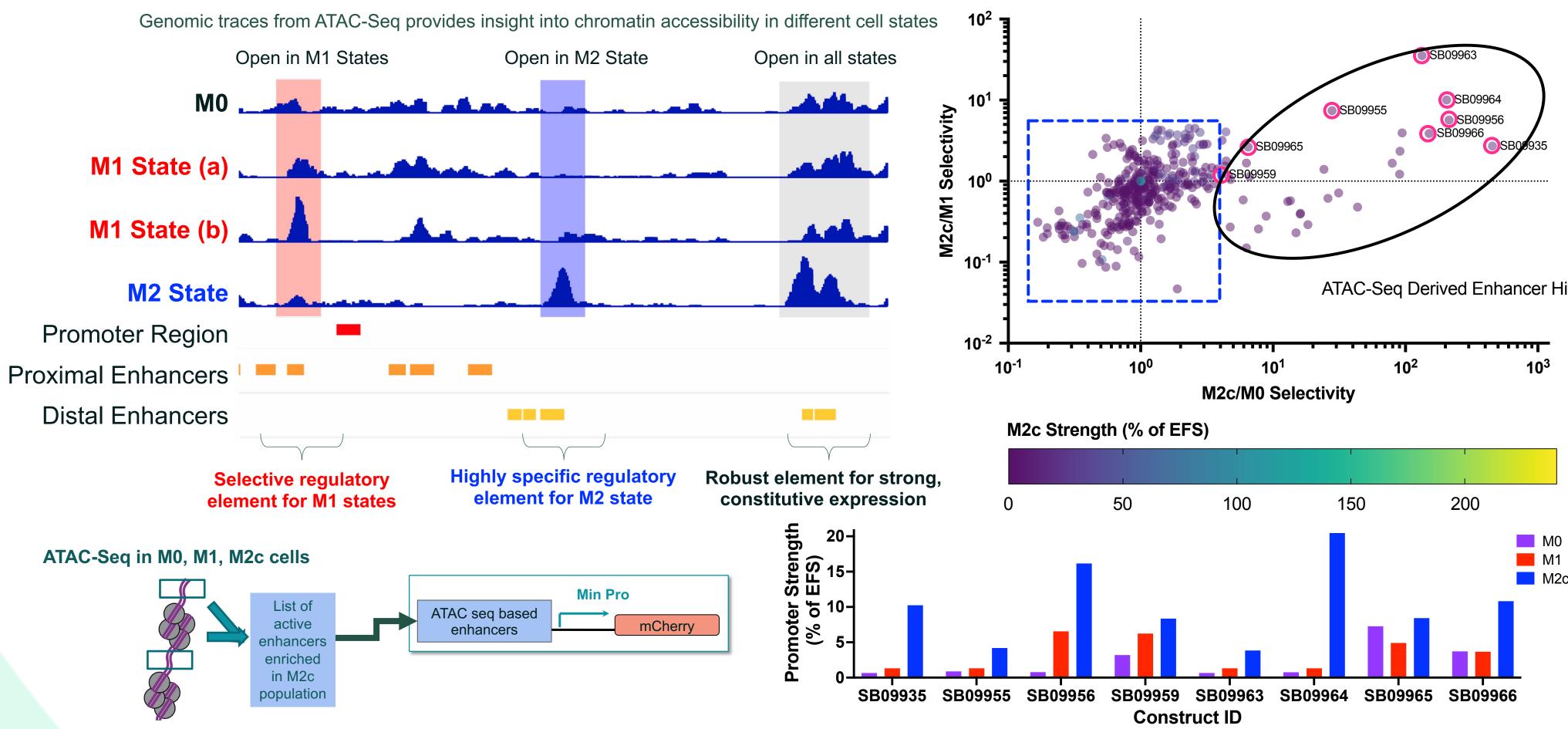
## M1 Promoter Discovery Native Macrophage Promoter Screening Identifies M1 State-Selective Promoters (left) >20 native promoter-reporter constructs mined from RNA-Seg data tested for state-selective activity (% of EFS) SB09406/SB05132 • All promoter activity normalized to a constitutive control, EFS, to assess promoter strength SB09406/SB05132 (blue rectangle) selected as a highly M1-state selective native promoter for engineering CHOSAOT LEOSAOS CHOSATO CHOSAT M1/M0 Selectivity 2nd Generation Promoter Variant Screening Reveal Regions of Functional Regulatory Elements (above) 3rd Gen Pro Native Pro M1 Strength (% of EFS) SB09413 SB12093 109.73 SB12094 3rd Generation Promoter Variants Improve Specificity by an Order of Magnitude

Native promoter engineering into synthetic variants improves strength and specificity of M1-specific promoters

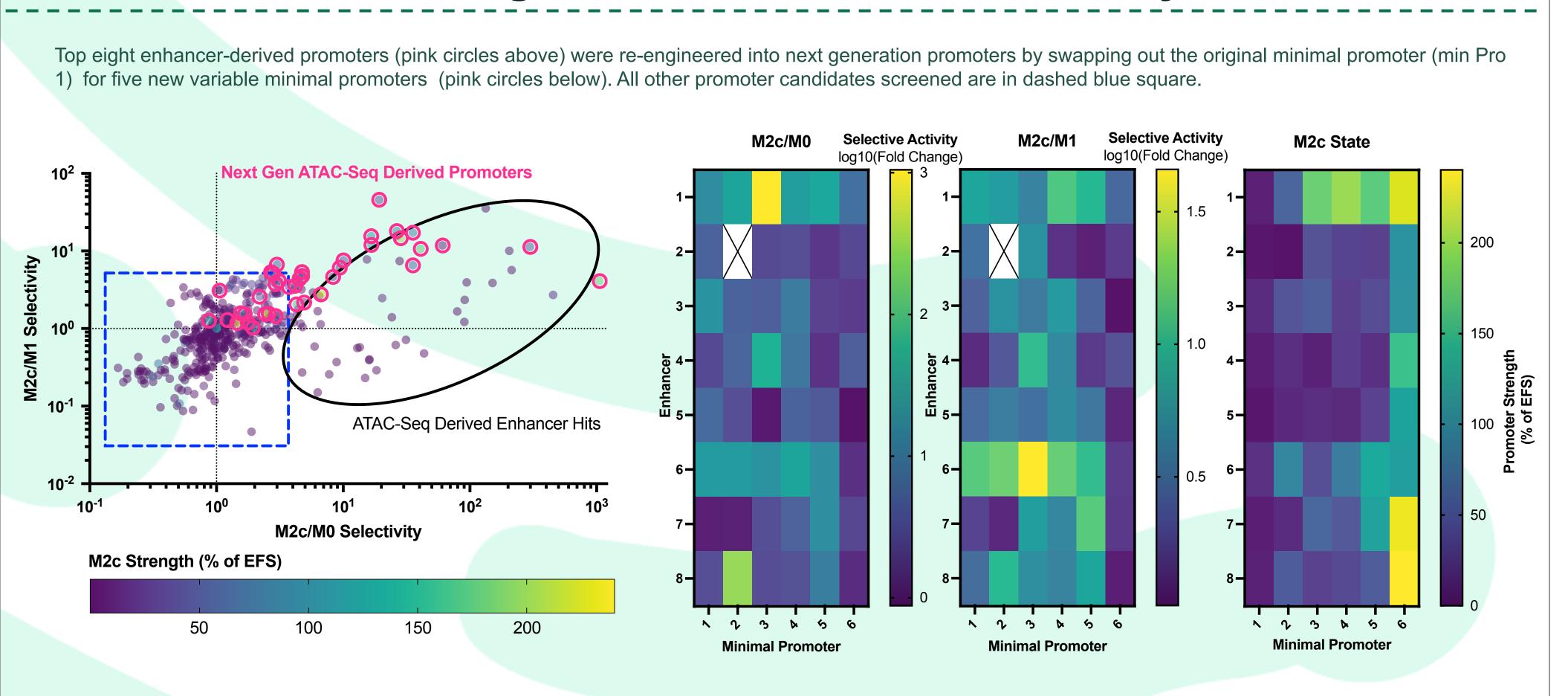


Fully synthetic promoter library hits are as strong and M1selective as native promoters

## M2 Promoter Discovery



Our bioinformatics pipeline enables potent genomic loci selection with high M2c state selective activity



Core promoter engineering increases both strength and specificity of M2c-state specific promoters