



Smart sensor promoters drive state-specific gene circuits to convert immunosuppressive macrophages into an anti-tumor phenotype



GENEFAB



BlueRock
THERAPEUTICS

Frances D. Liu,^{1,2} Michelle Hung,^{1,2} Assen Roguev,^{1,2} Yin Yin Chong,^{1,2} Cesar Juarez,^{1,2} Russell M. Gordley,^{1,2} Mark Tomishima,³ Jessica M Haverkamp,⁴ Panos Douvaras,³ Alex Danza,¹ Monika Avina,¹ Timothy K. Lu,¹ and Philip Lee^{1,2}

¹Senti Biosciences, South San Francisco, CA,

²GeneFab, Alameda, CA,

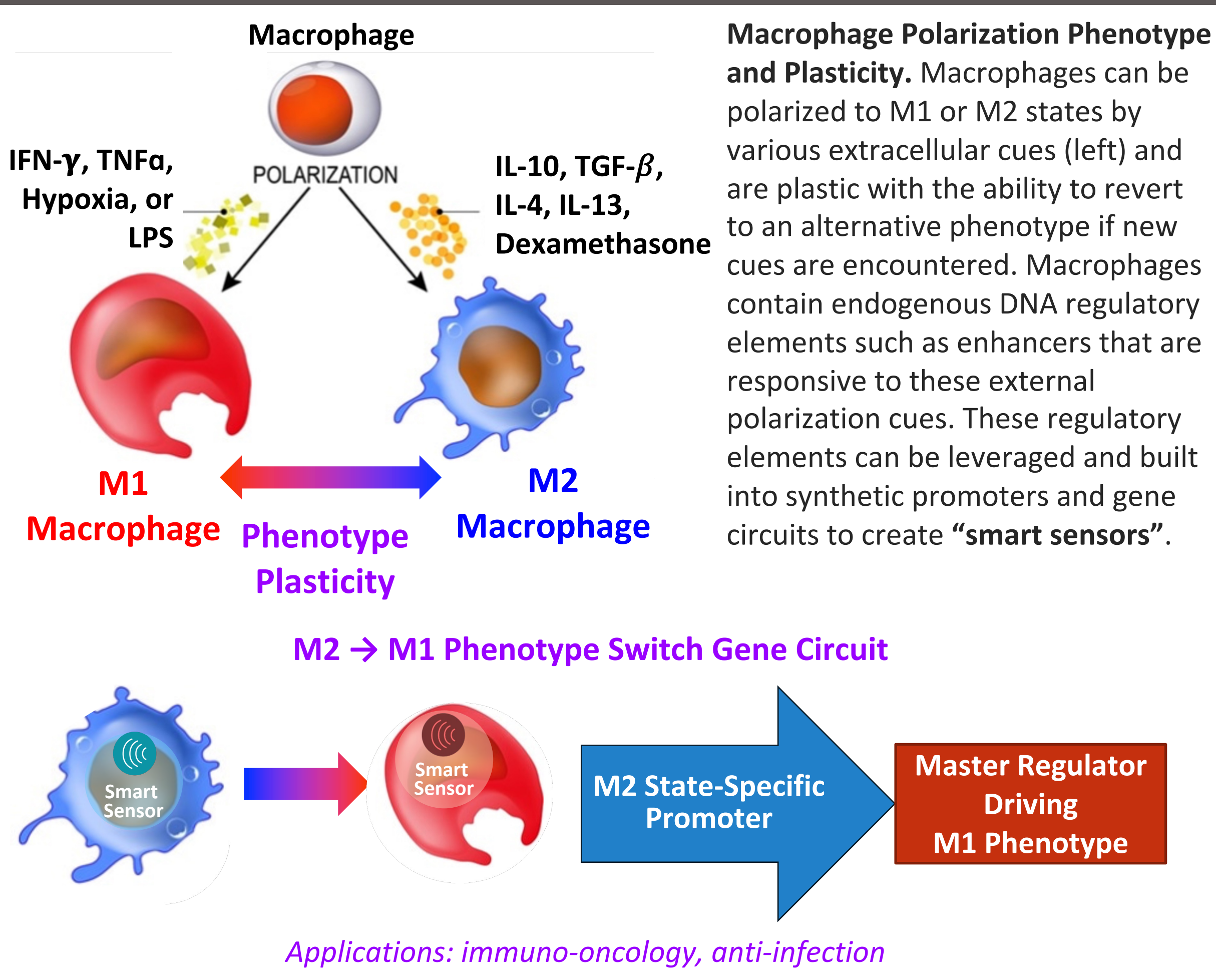
³BlueRock Therapeutics, New York, NY, and ⁴Cambridge, MA

SITC 2023

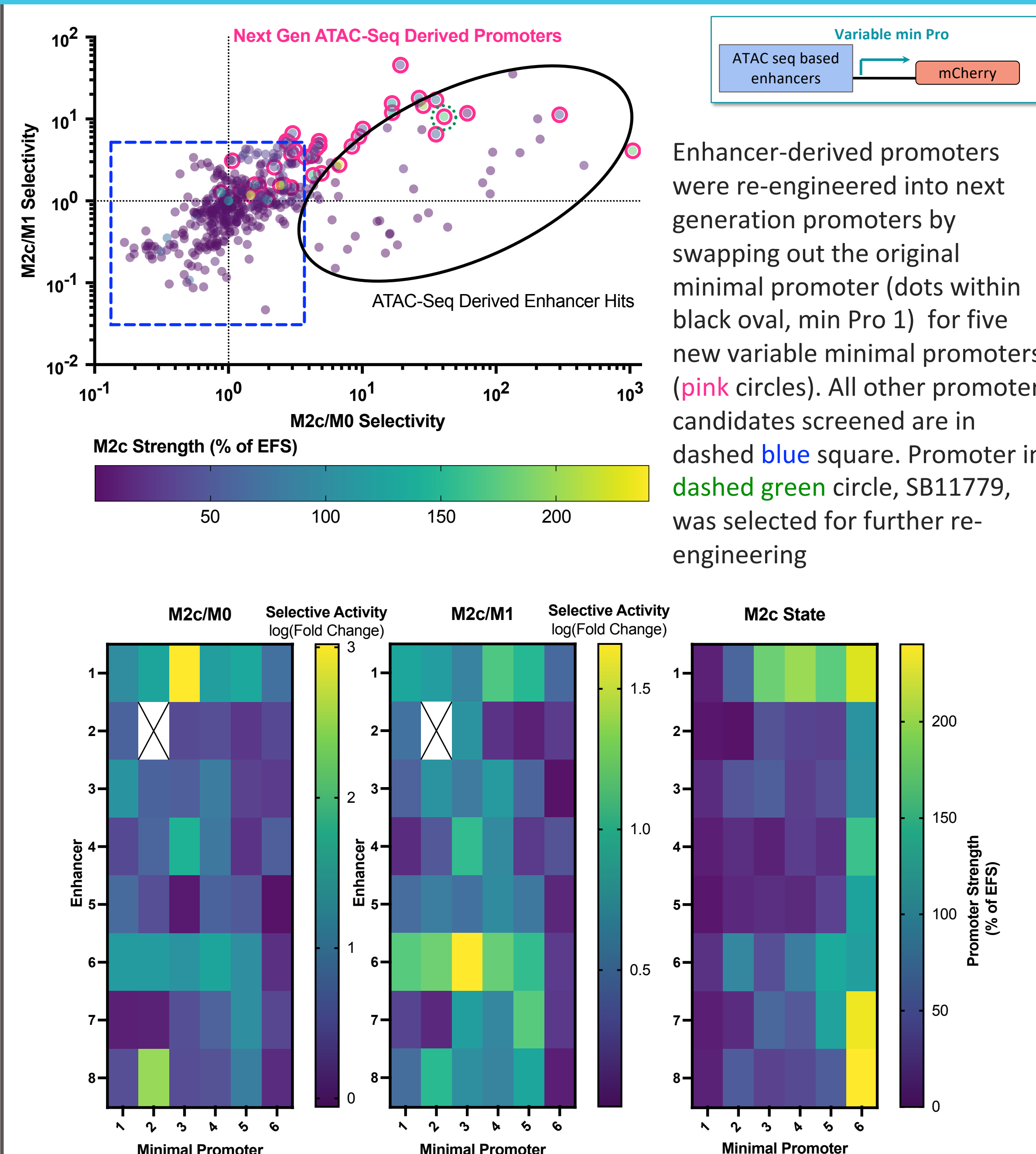
San Diego, CA

Abstract # 430

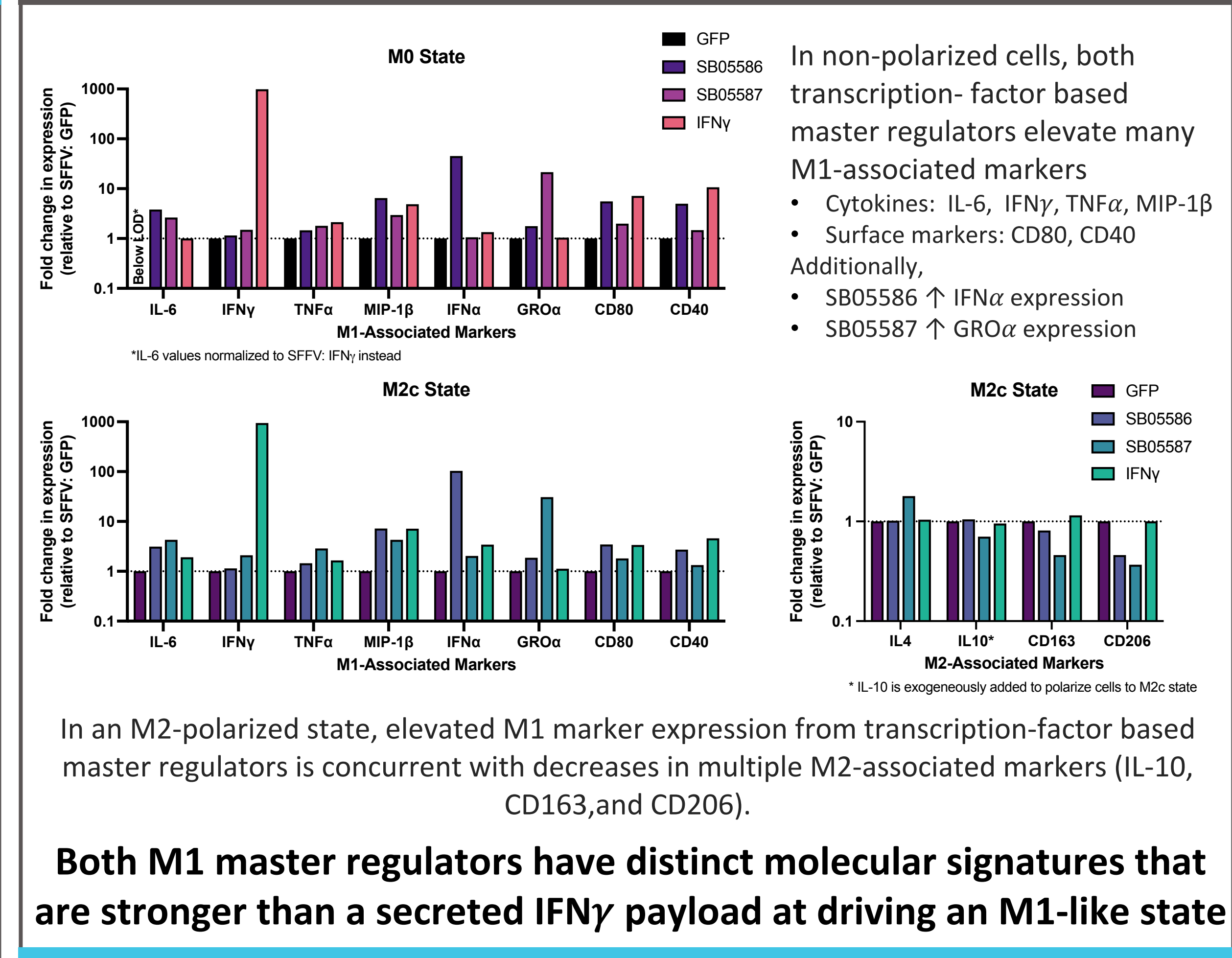
Macrophage Polarization Logic



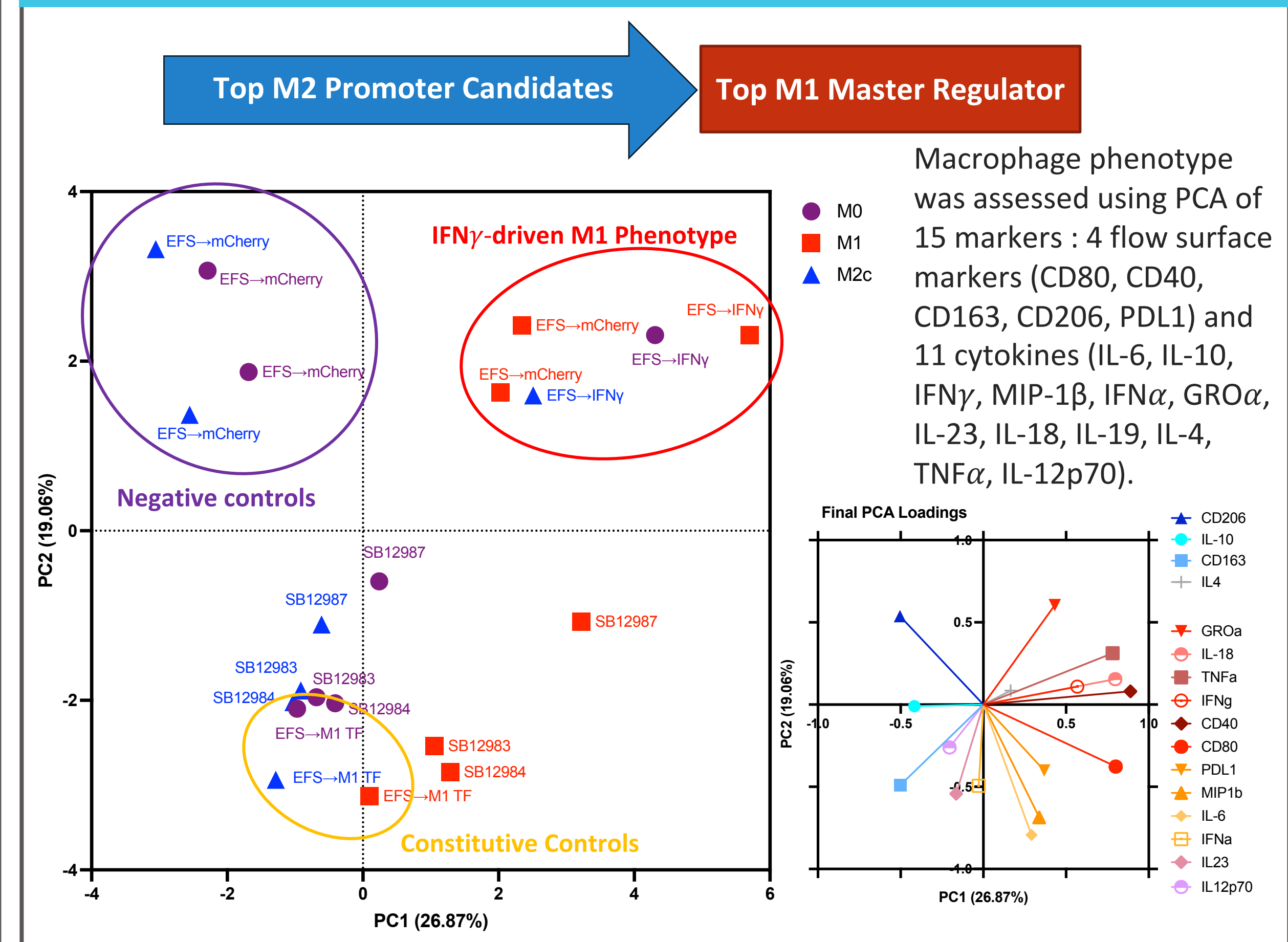
Promoter Optimization



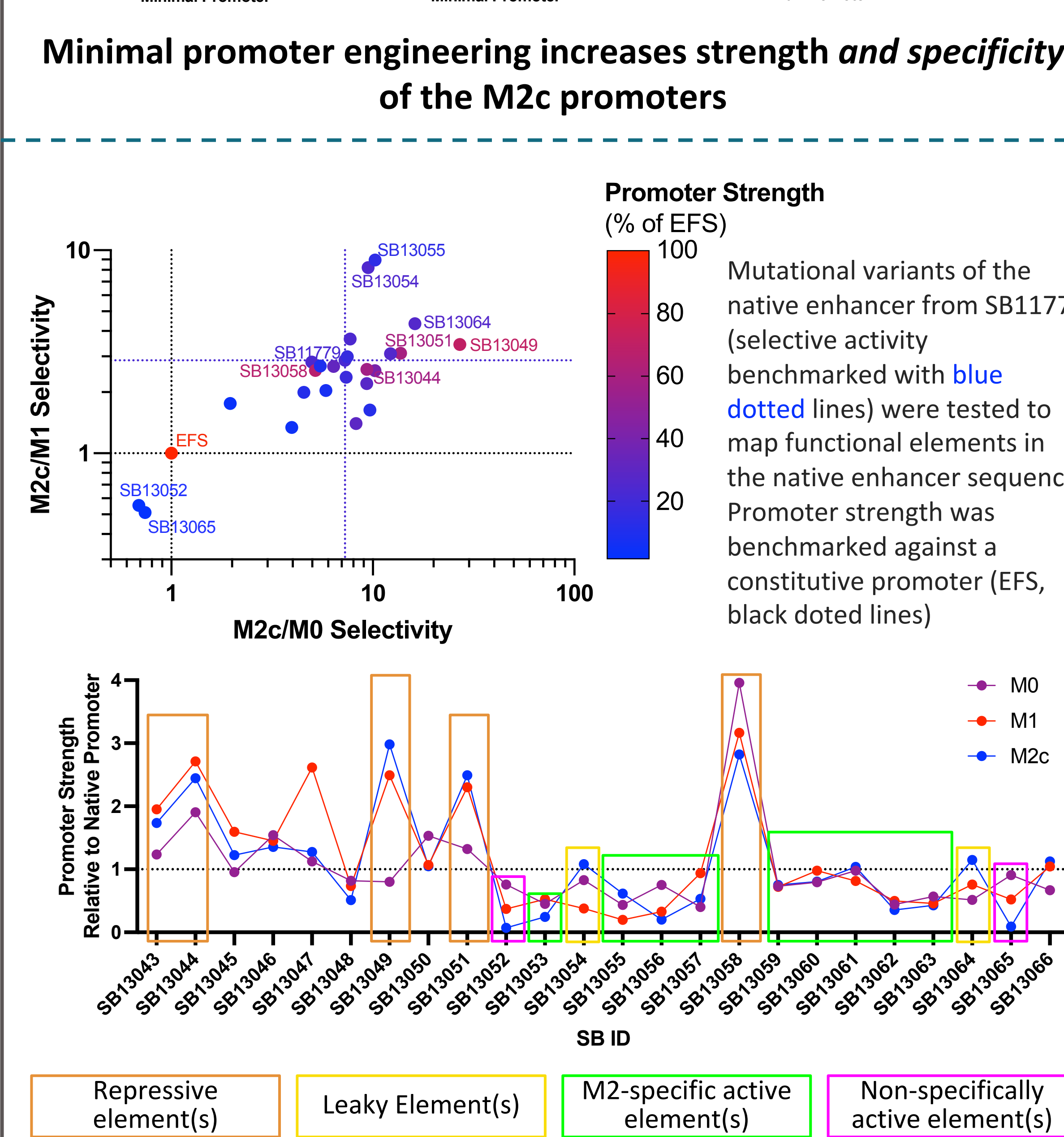
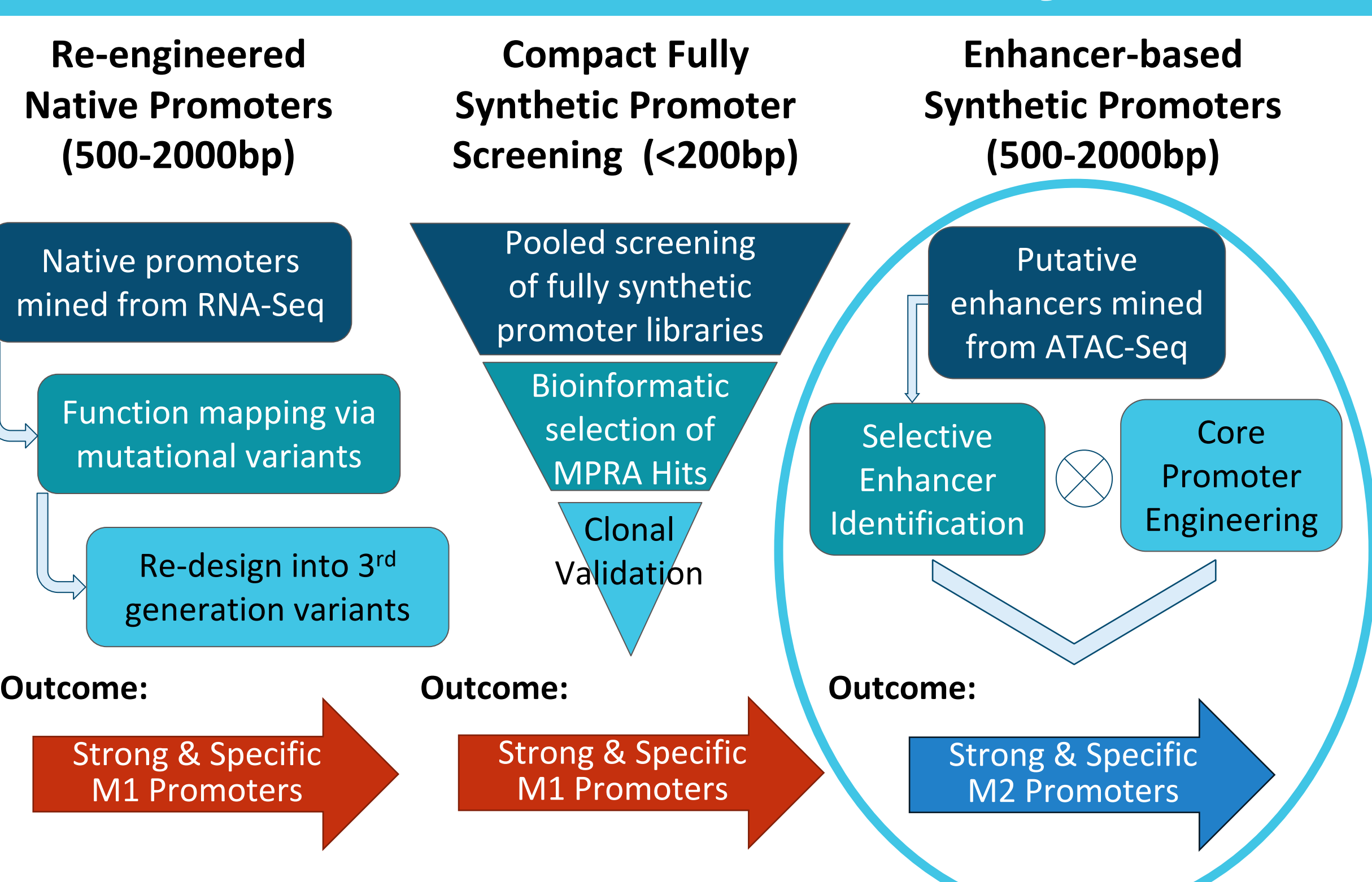
Master Regulator Activity



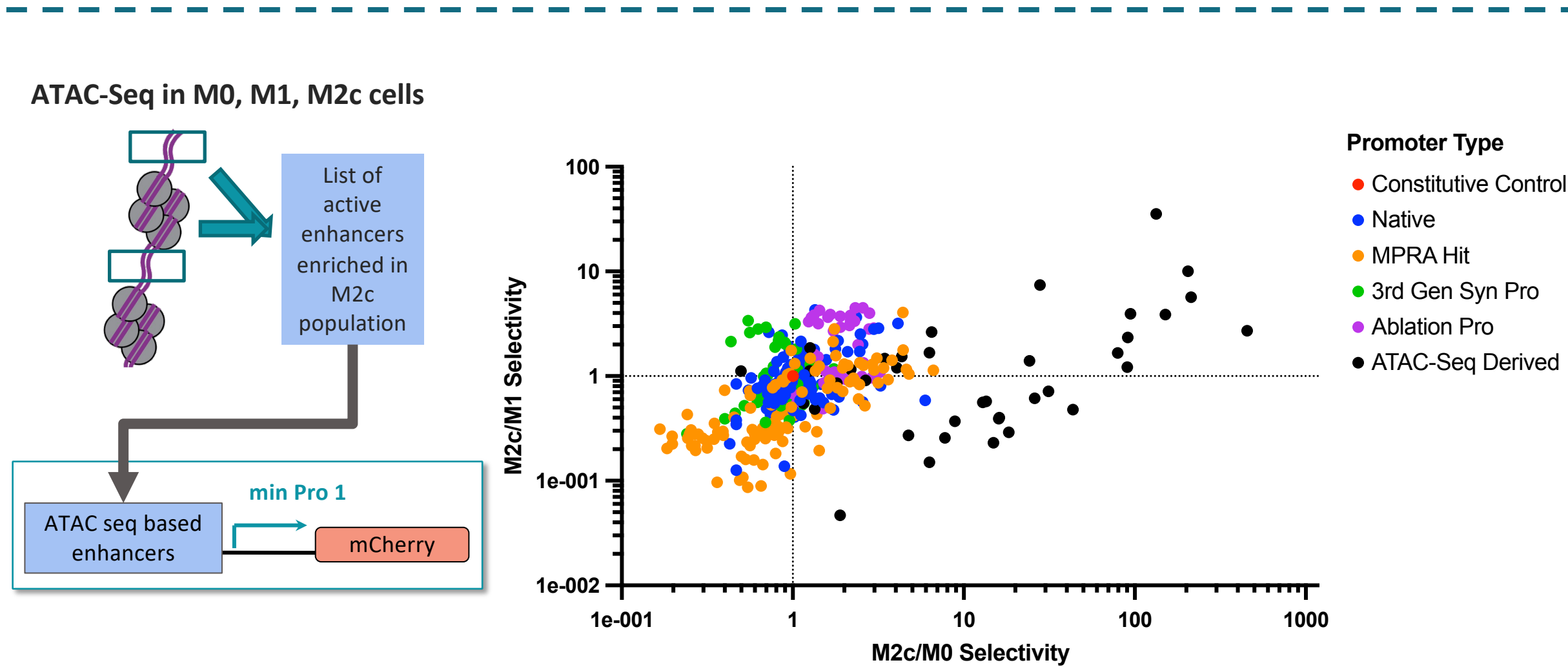
Phenotype Switch Circuit



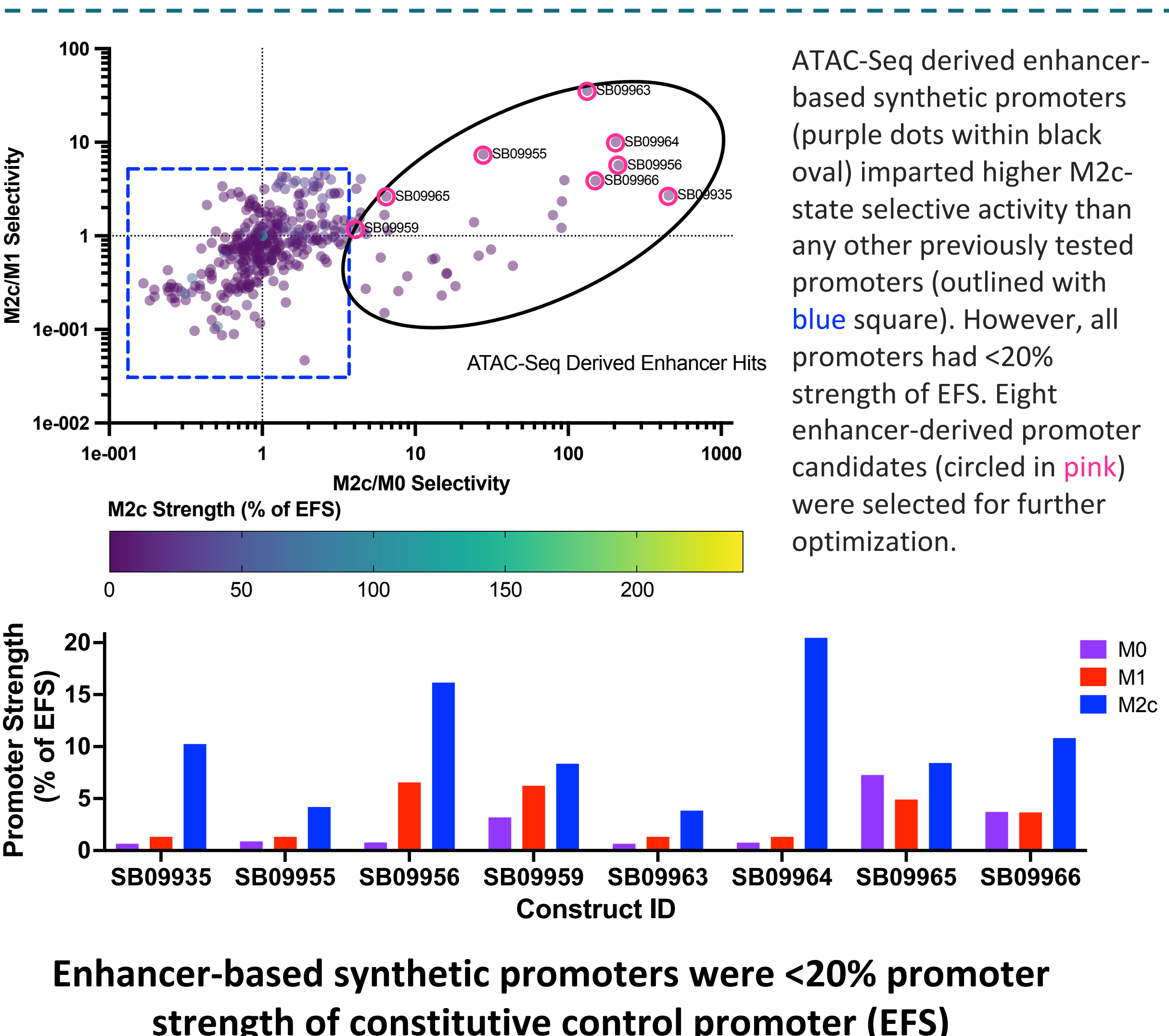
Promoter Discovery



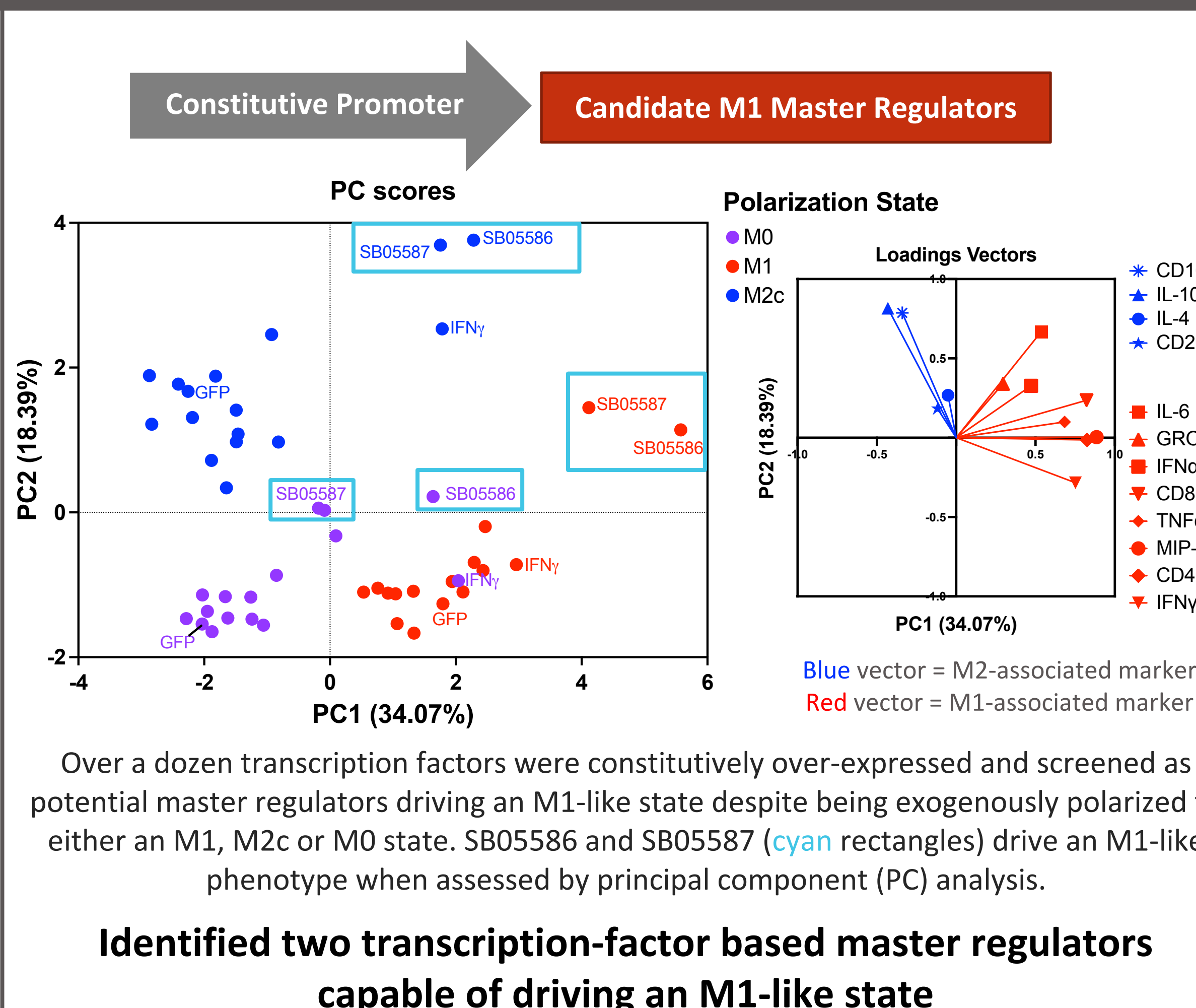
Enhancer-based synthetic promoters have higher M2c-selective activity than any other promoter discovery strategy



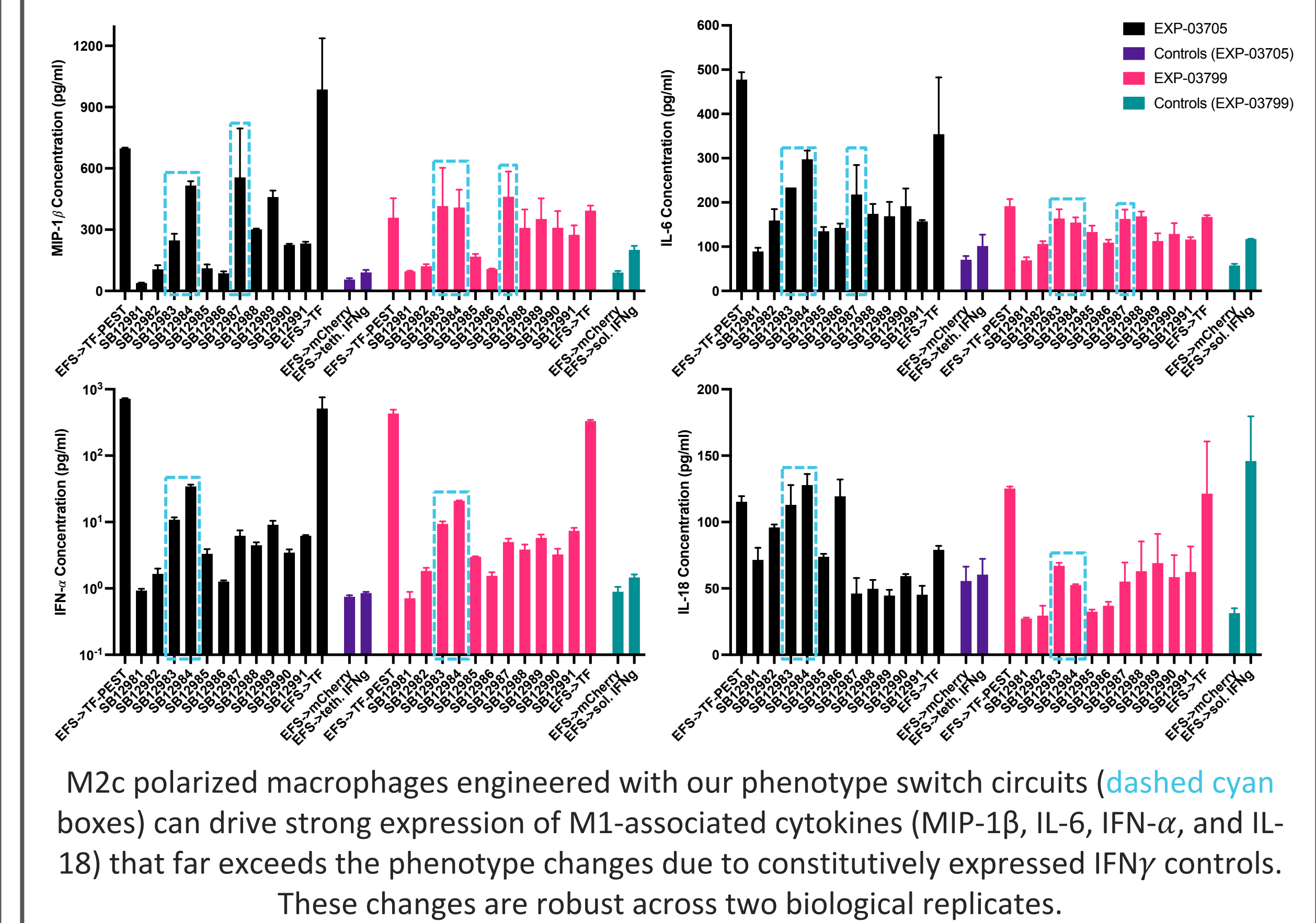
Enhancer-based synthetic promoters were <20% promoter strength of constitutive control promoter (EFS)



Master Regulator Screening



Pairing an M2-state responsive promoter with an M1 master regulator can switch an M2-polarized cell to an M1-like phenotype



Conclusions

- Putative native enhancers mined from ATAC-Seq can be engineered into strong and M2c polarization state selective promoters when paired with certain minimal promoters
- Generation of mutational variants of native M2 enhancers enable functional identification and mapping of regulatory elements
- Overexpression of transcription factors can be used as a master regulator to drive an M1-like phenotype
- State-specific promoters can be built into smart sensor circuits to control macrophage polarization logic
- Next Steps
 - Optimize pairs of engineered promoters and payloads for enhanced dynamic control of macrophage cell state
 - Further testing in a therapeutically relevant model system
 - Demonstrate that an M2 \rightarrow M1 phenotype switch gene circuit can turn a cold tumor hot