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# Macrophage Polarization Logic



Macrophage Polarization Phenotype and Plasticity. Macrophages can be polarized to M1 or M2 states by various extracellular cues (left) and are plastic with the ability to revert to an alternative phenotype if new cues are encountered. Macrophages contain endogenous DNA regulatory elements such as enhancers that are responsive to these external polarization cues. These regulatory elements can be leveraged and built into synthetic promoters and gene circuits to create "smart sensors".





#### **Promoter Discovery**

## **Promoter Optimization**







Master Regulator Activity

\* IL-10 is exogeneously added to polarize cells to M2c state

In an M2-polarized state, elevated M1 marker expression from transcription-factor based master regulators is concurrent with decreases in multiple M2-associated markers (IL-10, CD163, and CD206).

**Both M1 master regulators have distinct molecular signatures that** are stronger than a secreted IFN $\gamma$  payload at driving an M1-like state

# **Phenotype Switch Circuit**









Many of candidates M2>M1 Phenotype Switch circuits can drive a phenotype comparable to our constitutive controls (orange oval). This suggests that our state-selective promoters when paired with our TF-based master regulator are just as strong a constitutive promoter (EFS), but also have leaky activity in the M0 and M1 states.



1e-001

**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)

element(s)	Leaky Liement(3)	element(s)	active element(s)

Variant screening enables identification of regulatory element activity within native promoter sequence

## Master Regulator Screening



Over a dozen transcription factors were constitutively over-expressed and screened as potential master regulators driving an M1-like state despite being exogenously polarized to either an M1, M2c or M0 state. SB05586 and SB05587 (cyan rectangles) drive an M1-like phenotype when assessed by principal component (PC) analysis.

Identified two transcription-factor based master regulators capable of driving an M1-like state

M2c polarized macrophages engineered with our phenotype switch circuits (dashed cyan boxes) can drive strong expression of M1-associated cytokines (MIP-1 $\beta$ , IL-6, IFN- $\alpha$ , and IL-18) that far exceeds the phenotype changes due to constitutively expressed IFN $\gamma$  controls. These changes are robust across two biological replicates.

#### 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 M1like 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
- $\Rightarrow$  Demonstrate that an M2  $\rightarrow$  M1 phenotype switch gene circuit can turn a cold tumor hot