Iterative Engineering of Polarization-State Responsive Synthetic Promoters for Autonomous Control of Macrophage Polarization Logic

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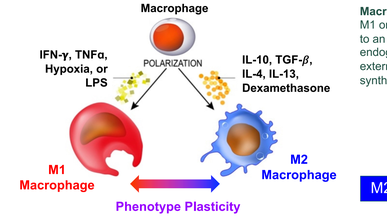
Macrophage Polarization Logic



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"**.

M2 Promoters

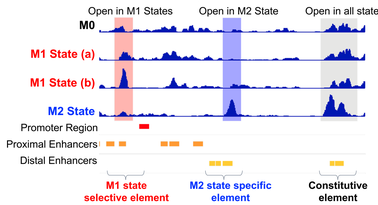
Applications: immuno-oncology; localized immune suppression

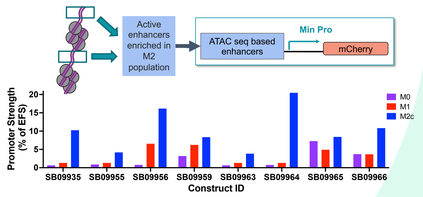
M2 Promoter → Therapeutic Payload

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

State-Specific Enhancer Discovery

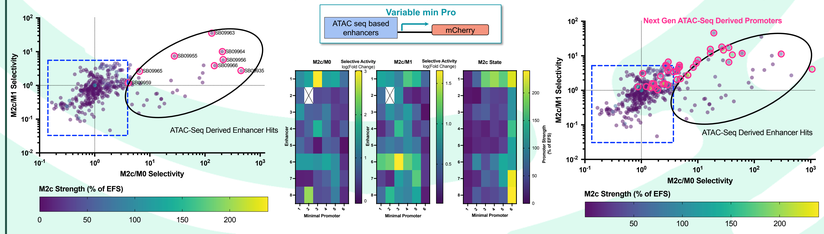
Genomic traces from ATAC-Seq in multiple polarization states (M0, M1, and M2) provide insight into chromatin accessibility in different cell states





Bioinformatics enables potent genomic loci selection with high M2c state selective activity

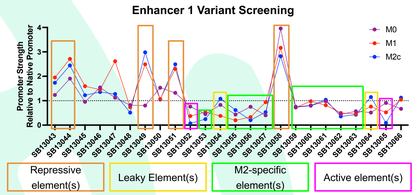
Top eight enhancer-derived promoters (pink circles, left) were re-engineered into next generation promoters by swapping out the original minimal promoter (min Pro 1) for five new variable minimal promoters (pink circles, right). All other promoter candidates screened are in dashed blue squares.

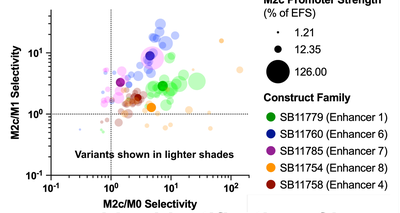


Core promoter engineering increases both strength and specificity of M2c-state specific promoters

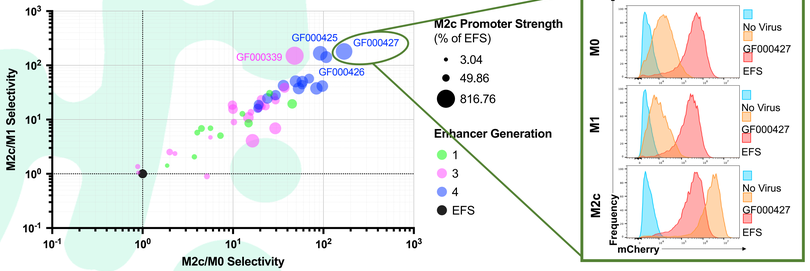
Synthetic Promoter Optimization

Mutational variants of five native enhancer families were screened and tested to map functional activity of entire native sequence





Variant screening of each native enhancer enables identification of key regulatory element activity in native enhancer sequences

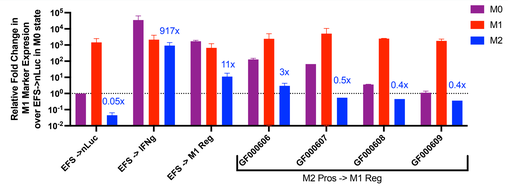


Iterative re-design by combining key regulatory elements improves strength & specificity (>100-fold) of synthetic promoters

Autonomous Phenotype Switch Circuit

M2 Promoter → M1 Regulator

- Constitutively expressed M1 master regulator maintains high M1 marker expression when not polarized (M0 state) or when polarized to an M2 state
- When macrophages are polarized M2c state, the state selective promoters driving the same master regulator resist decreases in M1 marker expression (0.4 to 3-fold changes in expression compared to 0.05-fold change)

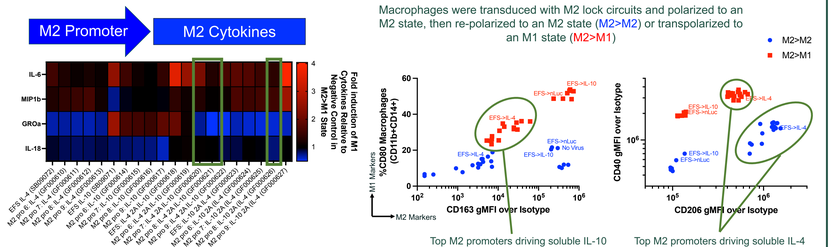


An M2-state specific promoter driving an M1 master regulator converts M2-polarized cell towards an M1-like phenotype

Autonomous Phenotype Lock Circuit

M2 Promoter → M2 Cytokines

Macrophages were transduced with M2 lock circuits and polarized to an M2 state, then re-polarized to an M2 state (M2>M2) or transpolarized to an M1 state (M2>M1)



M2 lock circuits (GF000620, GF000622, & GF000626) can have lowest expression of M1-associated cytokines (IL-6, MIP-1β, GROα, and IL-18) when transpolarized even compared to constitutive controls

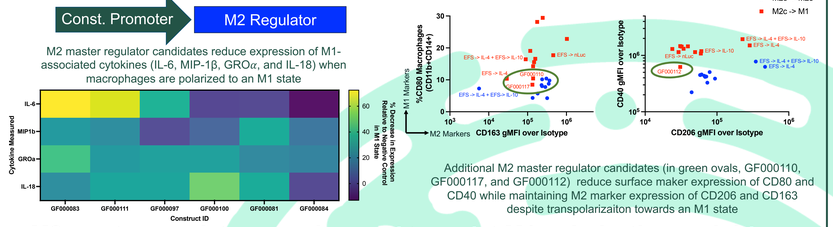
Top M2 promoters driving soluble IL-10 alone or in combination with IL-4 drive transpolarized cells to reduce CD80 expression (Mitigation of M1-like phenotype)

Top M2 promoters driving soluble IL-4 alone or in combination with IL-10 drive both transpolarized and re-polarized cells to increase CD206 expression (Increased M2-like phenotype)

State-specific M2 promoters expressing IL-4 in combination with IL-10 resist transpolarization away from an M2 state towards an M1 state

Const. Promoter → M2 Regulator

M2 master regulator candidates reduce expression of M1-associated cytokines (IL-6, MIP-1β, GROα, and IL-18) when macrophages are polarized to an M1 state



Additional M2 master regulator candidates (in green ovals, GF000110, GF000117, and GF000112) reduce surface maker expression of CD80 and CD40 while maintaining M2 marker expression of CD206 and CD163 despite transpolarizaiton towards an M1 state

M2 master regulators can be used to resist M1 polarization and drive additional phenotype changes towards an M2-like state

Conclusions

- Putative native enhancers mined from ATAC-Seq can be iteratively engineered into strong and M2c polarization state selective promoters with >100-fold specificity and 8-fold stronger than EFS
- Generation of mutational variants of native M2 enhancers enable functional identification and mapping of regulatory elements
- **State-specific promoters can be built into autonomous gene circuits to control macrophage polarization logic**
- **Next Steps**
  - ❖ Optimize pairs of engineered promoters and payloads for enhanced dynamic control of macrophage cell state
  - ❖ Validation in a therapeutically relevant model system