PROJECT TOLERA · TARGET-MINING REPORT

Programming T-cell tolerance in eosinophilic esophagitis

Reprogramming the pathogenic CD4⁺ T cell by two routes: a personalized pMHC-II tolerance construct, and drug targets that dampen Th2 output or improve amenability to pMHC-induced tolerance — nominated from a genome-scale CD4⁺ T-cell Perturb-seq resource.

Dataset

Genome-scale CRISPRi Perturb-seq in primary human CD4⁺ T cells (11,526 gene knockdowns × three culture conditions: Rest, Stim 8 hr, Stim 48 hr; 10,282 measured genes). Produced by the laboratories of **Alex Marson** (Gladstone Institutes / UCSF) and his collaborator **Jonathan Pritchard** (Stanford University), and made publicly available through the CZI Virtual Cells Platform. This report uses the perturbation-level differential-expression resource (`GWCD4i.DE_stats`) to nominate T-cell-state drug targets.

All results are computational (in-silico) and derived from a healthy-donor perturbation atlas; they are hypotheses that require validation in EoE-patient T cells. Not medical advice.

1 · From pMHC to reprogramming: how the idea was generated

Project Tolera's lead program is a **personalized pMHC-II construct** that re-educates the food-specific T-cell clone toward tolerance based purely on its T-cell receptor — independent of the cell's activation state. While scoping how to strengthen that program, we asked a second, complementary question: **can we reprogram the T cell's state as well as its specificity?** Two openings motivated mining a T-cell perturbation atlas.

(A) Dampen the pathogenic output. EoE tissue damage is driven by type-2 cytokines — IL-13, IL-5 and IL-4 — produced by pathogenic Th2 cells. These are the upstream drivers of the epithelial CCL26 (eotaxin-3) and fibroblast POSTN (periostin) axis that recruits eosinophils and remodels the esophagus. Neither CCL26 nor POSTN is expressed in T cells (they were absent from the dataset, as expected), but the *T-cell regulators that switch the type-2 cytokines on and off are measured and perturbed*. Knockdowns that selectively lower IL-13/IL-5/IL-4 nominate state-modulation drug targets.

(B) Improve amenability to pMHC-induced tolerance. A tolerogenic pMHC construct works best on a T cell that is convertible. Committed, terminally differentiated effector clones can resist tolerogenic signals. If a co-target makes effector clones more likely to adopt a regulatory (TR1/iTreg) or anergic fate, it becomes a rational *combination adjunct* to the pMHC platform — the one place where state-modulation biology and the antigen-specific platform genuinely connect.

Scope and honest caveat

Perturb-seq is a healthy-donor, genome-scale regulatory map — it is **not** an EoE disease model and does not recapitulate patient T-cell phenotypes. It is used here strictly as a *regulator-nomination* resource: it tells us which genes control the Th2 and tolerance programs in human CD4⁺ T cells. Every nomination is a hypothesis to be confirmed in EoE-patient cells.

2 · Approach and screen quality

From the perturbation-level differential-expression resource we assembled a frozen 47-gene readout panel spanning six axes: Th2 effector cytokines (IL13/IL5/IL4/IL4R and receptors), the Th2 master transcription factors (GATA3/STAT6/IRF4/BATF), and three tolerance programs — TR1 (IL10/LAG3/HAVCR2/CTLA4/…), iTreg (FOXP3/IL2RA/IKZF2/…) and anergy (EGR2/3, NR4A1/2/3, CBLB, DGKA, TOX, …) — plus a Th1 guard set (IFNG/TBX21) to score selectivity. For every knockdown we extracted the log-fold-change, z-score and adjusted p-value against each readout, then scored two programs on top of the same matrix.

The screen is well-powered: ~11,300 gene perturbations per culture condition, a median of ~540 cells per perturbation, and significant on-target knockdown in 61–64% of perturbations (median ~6 $\log_2$FC reduction). Type-2 cytokines are stimulation-induced (IL13 mean expression rises ~50-fold from Rest to Stim 48 hr), so dampening is only interpretable in the stimulated conditions — the scoring restricts Program A accordingly.

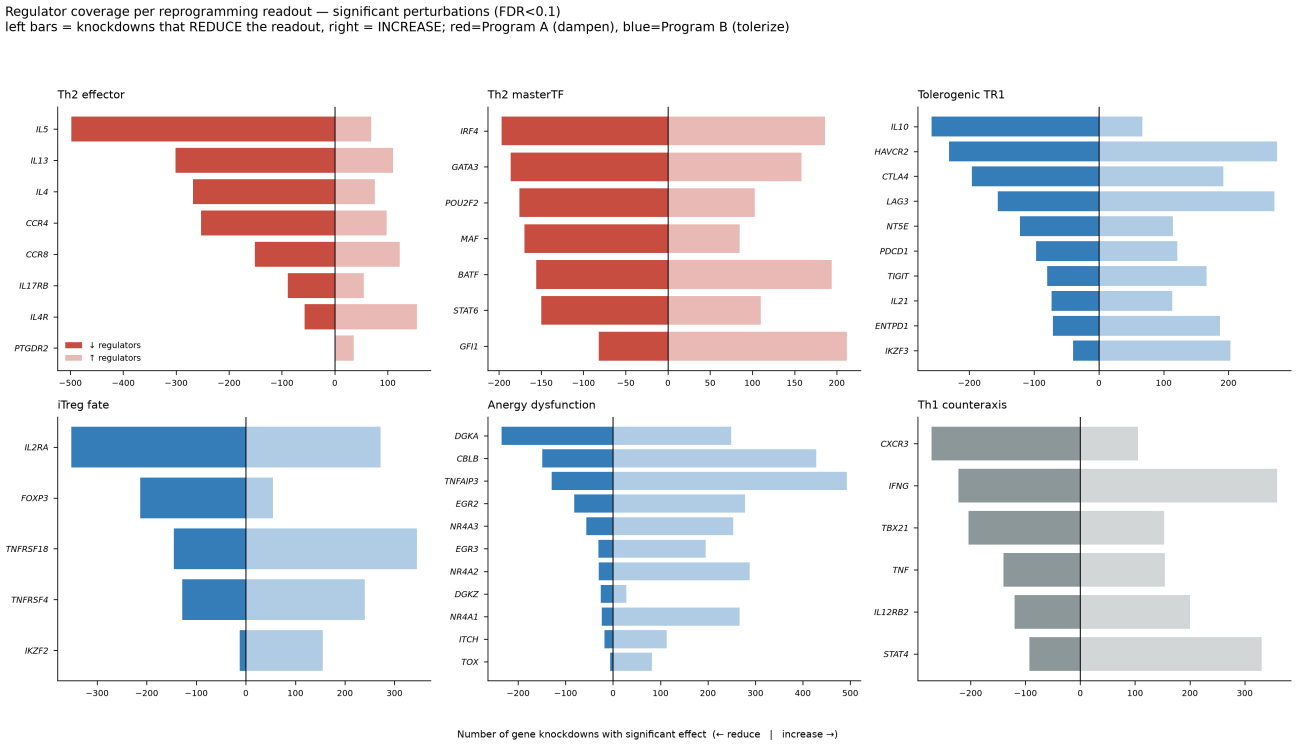

Figure 1 — Readout coverage. *Number of knockdowns that significantly move each readout gene (FDR<0.1), split by direction. The screen recovers hundreds of regulators per type-2 cytokine and per tolerance marker, confirming the atlas has the statistical depth to nominate state-modulation targets.*

3 · Program A — dampen pathogenic Th2 output

PROGRAM A · STATE MODULATION

Program A ranks knockdowns that **selectively** reduce the core type-2 cytokines (IL-13/IL-5/IL-4) without globally shutting the T cell down. The composite score combines the mean cytokine dampening (z-score), a selectivity term that penalizes collateral loss of the Th1 cytokine IFN-γ, and cross-donor reproducibility. **122 selective dampeners** passed the significance, effect-size and selectivity filters. GATA3 — the master Th2 transcription factor — recovers as a top hit, an internal positive control that the screen finds real EoE-relevant biology; IL4R (dupilumab's target) also appears, confirming the panel captures approved pharmacology.

Target	Score	Th2 dampening	Selectivity	Best condition	On-target KD
BHLHE40	0.977	6.05	5.20	Stim48hr	-13.1
GATA3	0.831	5.42	3.87	Stim8hr	-20.0
IL2RB	0.730	5.21	2.68	Stim48hr	-14.7
TMEM87B	0.712	4.05	4.05	Stim8hr	-5.3
CFAP20	0.700	4.83	2.88	Stim48hr	-7.9
WAC	0.699	4.69	3.04	Stim48hr	-6.9
ENO1	0.697	4.25	3.50	Stim48hr	-22.6
SMAD4	0.676	4.37	3.00	Stim8hr	-16.6
HEXIM1	0.673	3.91	3.91	Stim8hr	-13.4
STAT5A	0.657	3.93	3.93	Stim8hr	-23.8

Table 1 — Top 10 Program A dampeners. *Higher Th2-dampening and selectivity are better; on-target KD is the $\log_2$FC knockdown of the target itself. BHLHE40 (a non-canonical hit) and GATA3 (positive control) highlighted.*

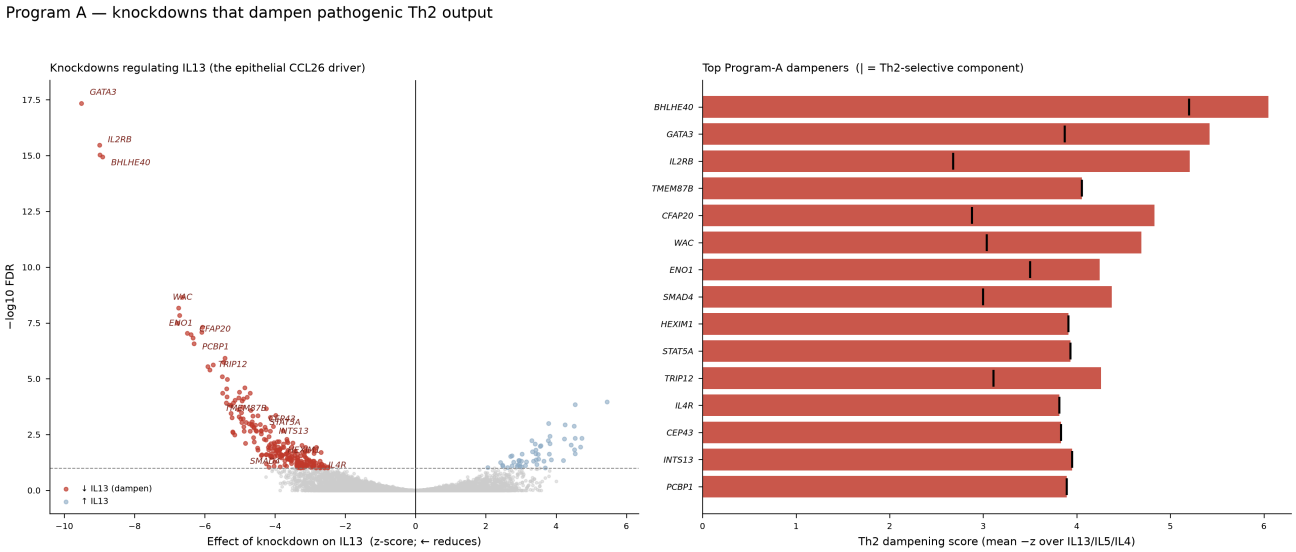

Figure 2 — Program A. *Left: IL13 regulator volcano (290 significant regulators; 238 reduce IL13, 52 increase it), GATA3 at the top-left extreme as the strongest, most significant reducer. Right: top dampeners ranked by composite score, marked by Th2-selectivity.*

4 · Program B — improve amenability to pMHC-induced tolerance

PROGRAM B · pMHC ADJUNCT

Program B ranks knockdowns that push a stimulated effector cell toward a tolerance-competent state — inducing TR1, iTreg and anergy markers while releasing the Th2 transcriptional lock. The amenability score sums induction across the three tolerance panels plus Th2-lock release; the composite adds program breadth and cross-donor reproducibility. **320 candidate co-targets** qualified, of which **151 are stimulation-relevant**. The top hits are mechanistically coherent: STK11 (LKB1), SIK3, PRKAR1A and ATG12 converge on the **LKB1→AMPK/SIK→CREB and cAMP/PKA axis** — an energy-sensing pathway with prior literature tying it to anergy susceptibility and regulatory differentiation. Multiple independent hits landing on one known pathway is a far stronger signal than any single gene score.

Co-target	Score	Amenability	TR1 ind.	Anergy ind.	Best condition
ATP2A2	0.977	8.12	3.78	0.13	Stim8hr
STK11	0.905	8.19	2.67	3.58	Stim8hr
SIK3	0.815	7.05	1.64	2.37	Rest
MTA2	0.772	6.42	2.34	1.38	Stim8hr
PRKAR1A	0.771	7.16	3.19	1.35	Rest
KPNA1	0.737	7.27	4.05	0.55	Rest
ATG12	0.691	5.81	2.39	1.82	Rest
CISD1	0.668	7.46	4.81	1.68	Rest
CCDC138	0.661	6.06	3.36	1.81	Rest
CPSF6	0.650	4.80	0.77	2.24	Stim8hr

Table 2 — Top 10 Program B co-targets. *Amenability is the summed tolerance-program induction; STK11 and SIK3 (the LKB1→SIK kinase axis, and the most pharmacologically tractable) highlighted.*

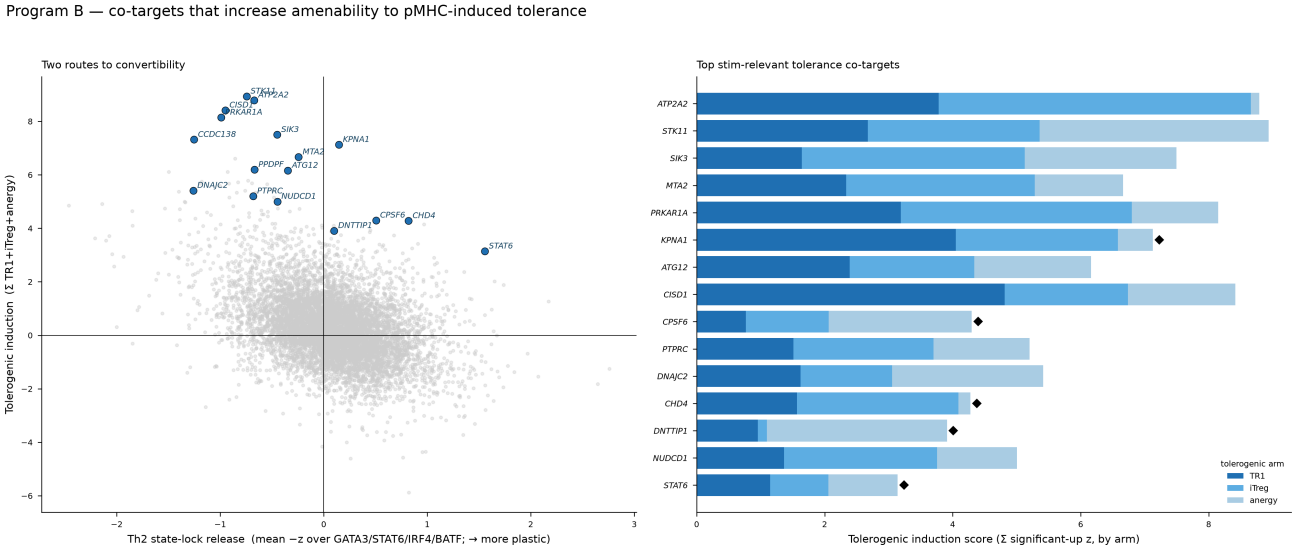

Figure 3 — Program B. *Tolerance-amenability co-targets; the metabolic LKB1/SIK/PKA cluster leads.*

5 · Integration: dual-program hits, corroboration and druggability

Merging the two programs surfaces **33 dual-program hits** — knockdowns that both dampen Th2 output *and* increase tolerance-amenability. We annotated every candidate with the study authors' own independent Th2/Th1 polarization model (an orthogonal corroboration axis) and with cross-donor robustness tiers. **SIK3** is the standout: strong in both programs (A = 0.55, B = 0.82), high cross-donor robustness, and independently corroborated by the authors' polarization model (rank 0.78) — and it is a kinase, the most tractable target class.

Dual-program hit	Program A	Program B	Polarization rank	Robustness
SIK3	0.545	0.815	0.78	high
CPSF6	0.622	0.650	0.42	high
PDSS2	0.406	0.551	0.72	high
HEXIM1	0.673	0.539	0.97	high
METTL1	0.570	0.529	0.41	high
BCL10	0.334	0.486	0.49	high
N6AMT1	0.460	0.451	0.56	high
IMPDH2	0.604	0.446	0.87	high
TADA2B	0.270	0.417	0.59	high
TUFM	0.477	0.417	0.53	high

Table 3 — Top dual-program hits *(ranked by Program B score; all high cross-donor robustness). Polarization rank is the authors' independent Th2/Th1 model. SIK3 highlighted.*

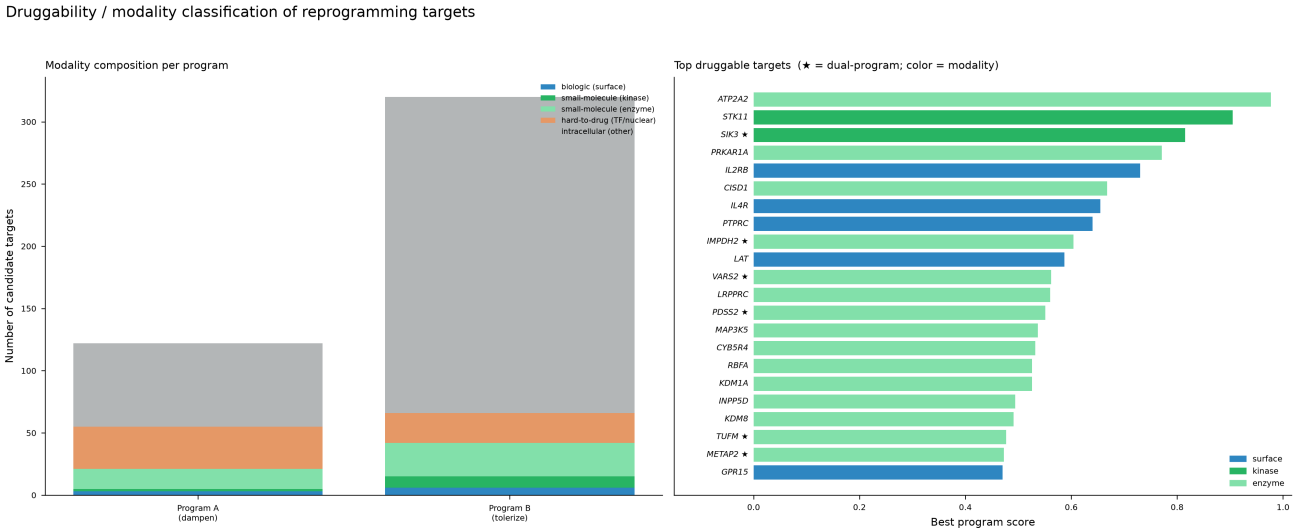

Figure 4 — Druggability lanes. *Candidates sorted into modality lanes from protein class and subcellular localization: biologic (surface/secreted, 9), small-molecule kinase (10), small-molecule enzyme/metabolic (35), hard-to-drug TF/nuclear (41, degrader/oligo territory), and intracellular scaffold/other (314). Program A's tractable hits are surface receptors (IL2RB, IL4R, LAT); Program B's lead node SIK3 sits in the tractable kinase lane.*

6 · Program recommendations

The mined targets were assessed with a drug-program-architect methodology (go / no-go, lead-target verdicts, modality fit, kill criteria). The two programs came out very differently.

Program A — do NOT pursue as a standalone drug

The biology is real but **redundant**. The tractable hits re-hit ground that approved or filed drugs already own: IL4R *is* dupilumab's target; IL2RB/LAT are blunt pan-suppression levers; GATA3 and SMAD4 are undruggable or unsafe transcription factors with no edge over existing biologics. Program A's real value is as a **positive control** proving the screen recovers genuine EoE pharmacology — not as a differentiated program.

Program B — pursue WITH CONDITIONS (as a pMHC combination adjunct)

Program B is differentiated precisely because it is a **combination mechanism**, not a me-too cytokine blocker: transiently de-lock a committed Th2 effector so a tolerogenic pMHC-II construct can convert it to a regulatory fate. The convergence of independent top hits (STK11, SIK3, PRKAR1A, ATG12) on one known energy-sensing pathway is the strongest evidence the amenability phenotype is real. Advance conditional on the combination out-performing either arm alone in EoE-patient cells.

Lead-target verdicts

Target	Verdict	Rationale
GATA3	**DROP**	Textbook Th2 TF but undruggable, broadly required; no edge over approved biologics.
IL2RB	**DROP**	Shared IL-2/IL-15 machinery; risks depleting Tregs and NK function, no advantage.
IL4R	**DROP**	Dupilumab's own target; useful only as screen positive control.
BHLHE40	**ADVANCE (cond.)**	Non-canonical, differentiated hit; needs confirmed Th2-selectivity in patient-derived cells.
SMAD4	**DROP**	Central TGF-beta hub and tumor suppressor; unacceptable safety profile for chronic use.
STK11	**ADVANCE (cond.)**	Strong anergy/Treg biology but tumor suppressor; only viable as transient adjunct dosing.
SIK3	**ADVANCE (cond.)**	Most tractable, dual-program hit; needs isoform-selective chemistry to avoid metabolic liability.
PRKAR1A	**DROP**	Ubiquitous PKA node linked to Carney complex; informative pathway signal only, not a target.

Table 4 — Lead-target verdicts. *Only BHLHE40 (Program A's one non-redundant hit) and SIK3/STK11 (the dual-program kinase axis) advance, all conditionally.*

7 · Next steps — validate in EoE-patient T cells

Every nomination here comes from a healthy-donor perturbation atlas. The decisive question is whether these regulators behave the same way in **disease-relevant, antigen-experienced T cells from EoE patients**. The path forward is a single gating experiment plus the supporting workstreams to resource it.

The one de-risking experiment that gates everything

Combination assay (EoE-patient cells)

In antigen-experienced / EoE patient-derived CD4⁺ T cells, transiently knock down or inhibit **STK11 / SIK3**, then co-culture with the tolerogenic pMHC-II construct — against *construct-alone* and *knockdown-alone* controls. Read out TR1/iTreg conversion, suppressive function, and durability under antigen rechallenge. **The combination must beat either arm alone**; if kinase inhibition alone reproduces the tolerance signature, the "adjunct" framing collapses into generic immunosuppression.

Kill criteria

- Knockdown/inhibition fails to reproduce Th2 dampening or amenability gain in EoE patient PBMC or esophageal-biopsy T cells under allergen recall stimulation
- Counter-screens show comparable perturbation effects in Th1/Th17/Treg populations (selectivity ratio <2x vs Th2), indicating generic immunosuppression rather than selective reprogramming
- Converted cells revert to effector phenotype/cytokine output upon antigen rechallenge after extended rest, or marker induction does not correlate with actual suppression assay

Supporting workstreams

- Cross-validate BHLHE40, STK11, SIK3, PRKAR1A expression/regulation against public EoE patient transcriptomic datasets
- Compile Peutz-Jeghers, Carney complex, and SIK-inhibitor safety literature into a formal target-safety dossier
- Design and resource the STK11/SIK3 + pMHC combination de-risking assay, contingent on epitope/HLA-II restriction from the antigen pipeline
- Task protein-design/binder stream with SIK3 isoform-selective inhibitor or degrader design
- Flag TCR-specificity/clonotype identification as prioritization input before selecting clones for the combination experiment

Two hard dependencies

Program B's de-risking assay cannot run without (1) a defined EoE-relevant epitope and HLA-II restriction from the antigen/epitope pipeline, and (2) dominant EoE clonotypes from the TCR-specificity thread. The clean near-term ask for the protein-design stream is a **SIK3 isoform-selective inhibitor or degrader**.

Data: genome-scale CD4⁺ T-cell Perturb-seq — laboratories of Alex Marson (Gladstone / UCSF) and Jonathan Pritchard (Stanford), via the CZI Virtual Cells Platform. Analysis and figures produced during the Built with Claude: Life Sciences Hackathon, 2026, with substantial AI assistance under human direction. In-silico results only; not experimentally or clinically validated; not medical advice.