RESEARCH ARTICLE

Supplementary Information

Genome-scale CRISPRi identifies an LKB1–PKA–SIK module controlling an ENTPD1-centred transcriptional programme in primary human CD4⁺ T cells

Authors: Hongyu Ying, Dandan Yun, Lisha Ran and Dan Liu*

Affiliation: Department of Rheumatology and Immunology, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, Shanghai 201399, China

Corresponding author: Dan Liu, Department of Rheumatology and Immunology, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, Shanghai 201399, China; email: danliu600@126.com

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Supplementary Table S1

Core candidate registry. CREB percentiles use the 48-hour stimulated z-residual metric against 1,254 candidates.

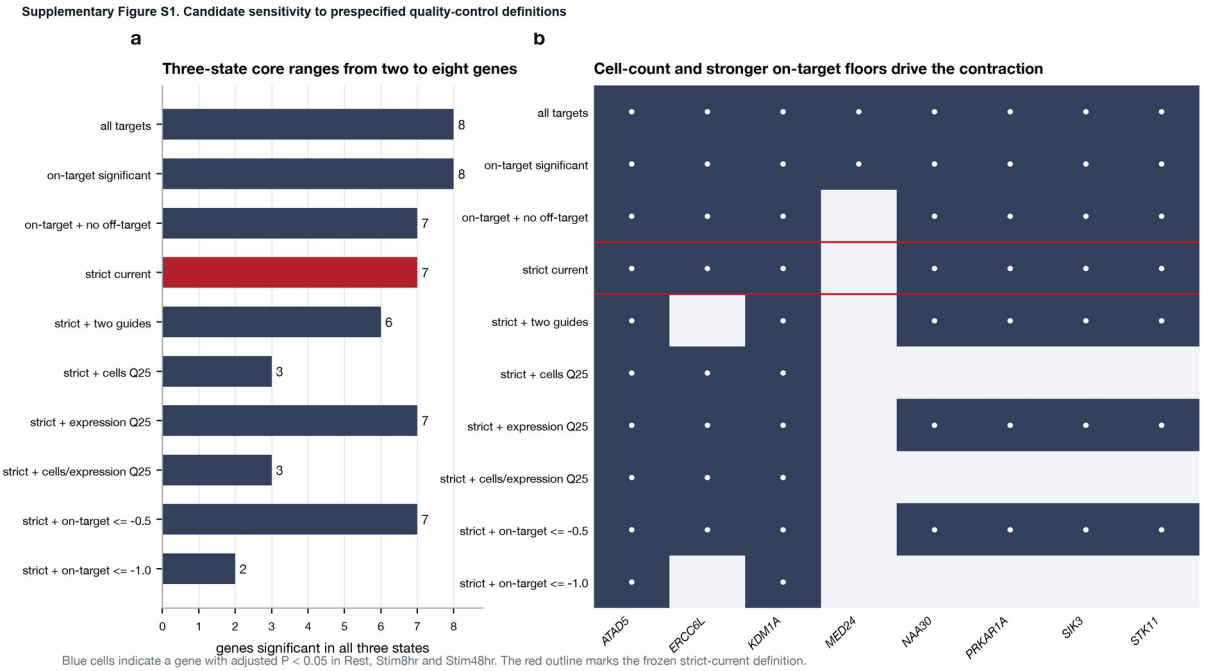
Gene	Classification	Panel breadth	CREB percentile	Interpretation
STK11	AXIS-1	8/16	98.3	LKB1–PKA–SIK module
PRKAR1A	AXIS-1	9/16	99.8	LKB1–PKA–SIK module
SIK3	AXIS-1	6/16	96.4	LKB1–PKA–SIK module
KDM1A	HIT-1	6/16	90.8	independent high-confidence hit
ATAD5	HIT-2	6/16 (5 direction robust)	19.4	independent high-confidence hit
NAA30	Tier 2	secondary observation	29.1	recurrent three-state observation
ERCC6L	Tier 2	secondary observation	40.0	recurrent three-state observation

Supplementary Table S2

Independent sorted-CD4$^+$ cohort programme validation. Values show paired baseline-to-month-3 change in the prespecified 10-gene Treg-suppression score. Parentheses contain global BH FDR values. The maximum-mean-expression and annotation-priority rules selected the same probes.

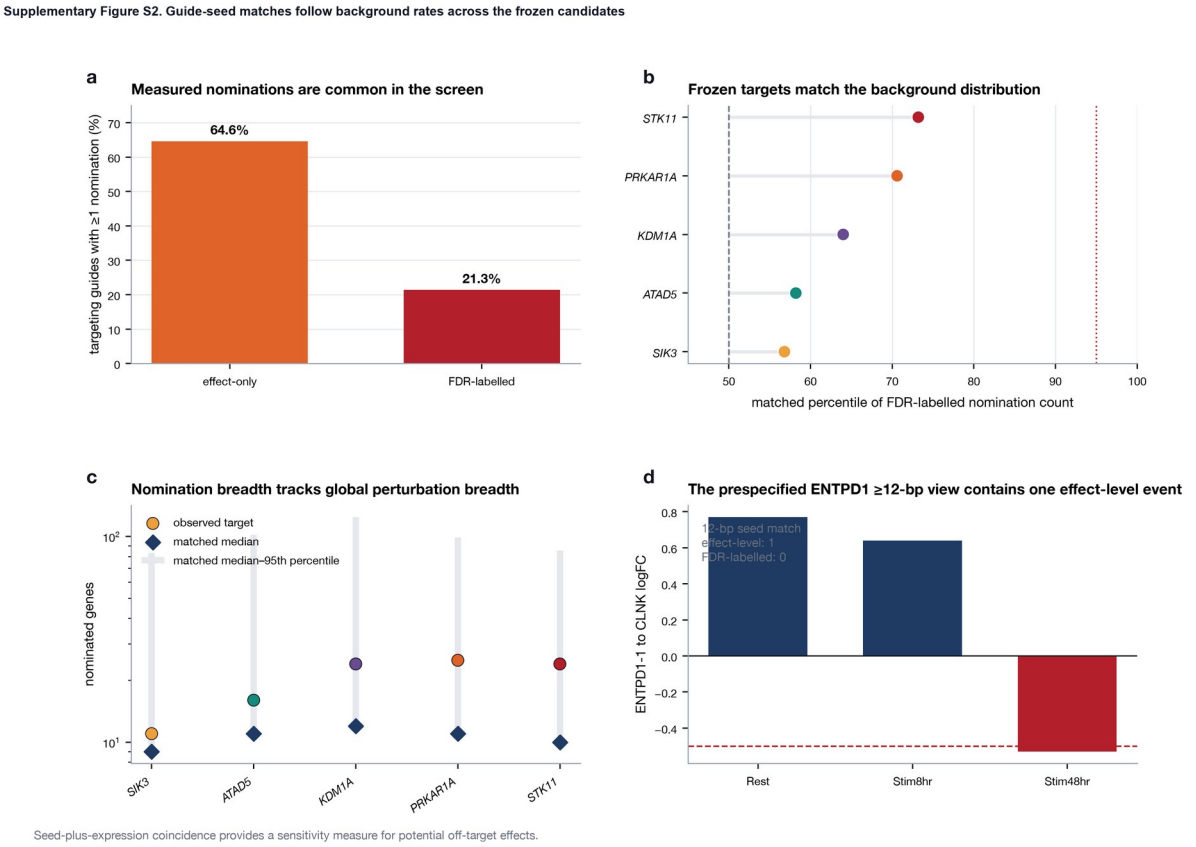
Probe-collapse rule	Mean change	Paired *t P* (FDR)	Signed-rank *P* (FDR)	Sign-flip *P* (FDR)	FDR tests <0.05
Maximum variance	−0.279	0.00508 (0.0500)	0.00475 (0.0475)	0.00508 (0.0508)	2/3
Maximum mean expression	−0.274	0.00331 (0.0397)	0.000816 (0.0106)	0.00298 (0.0358)	3/3
Annotation priority	−0.274	0.00331 (0.0397)	0.000816 (0.0106)	0.00298 (0.0358)	3/3
Median probe	−0.354	0.000510 (0.0153)	0.000321 (0.0106)	0.000520 (0.0156)	3/3

Supplementary Figure S1



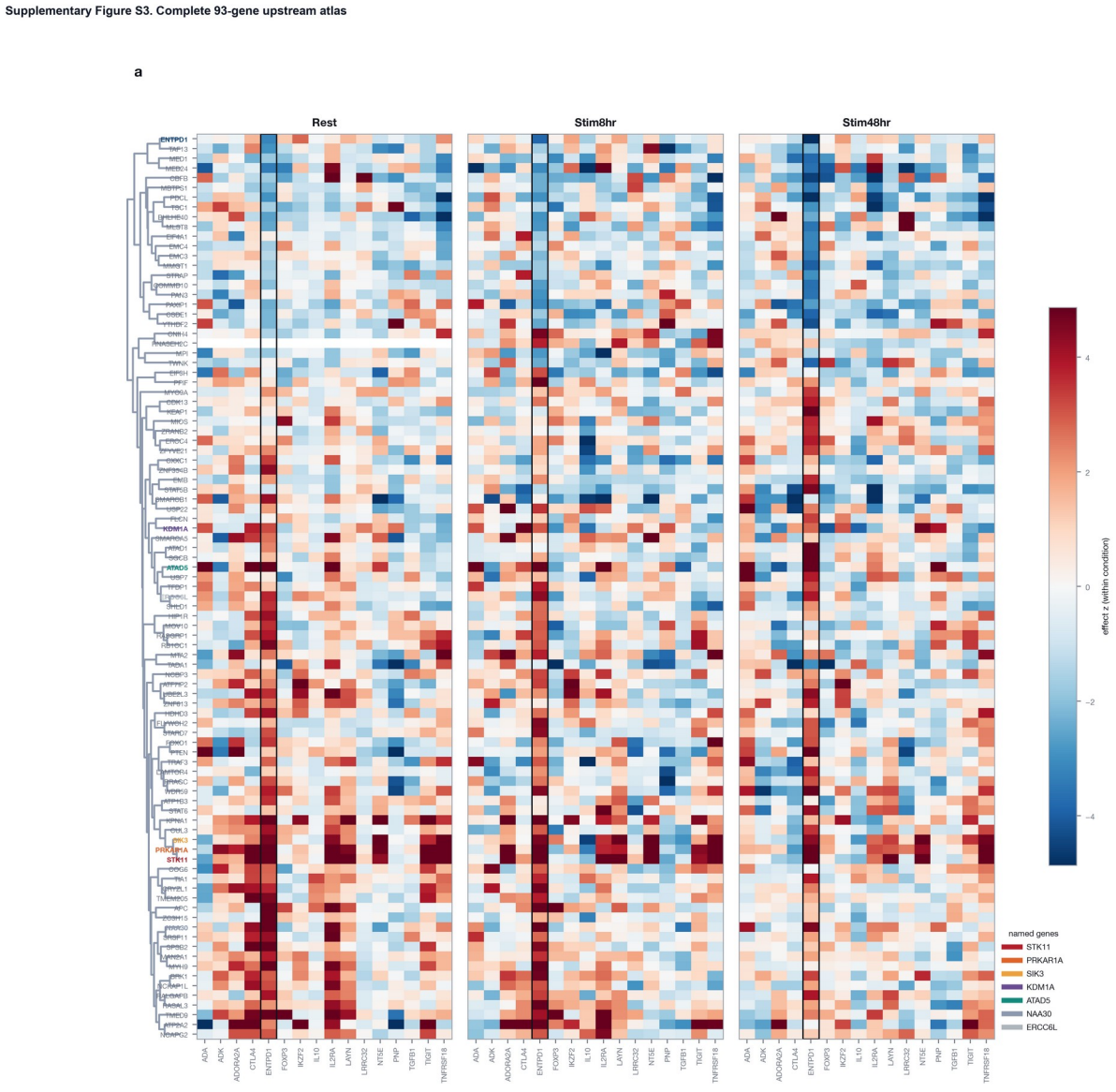
Supplementary Figure S1. Candidate sensitivity to prespecified quality-control definitions. (a) Number of genes significant for *ENTPD1* in all three states under ten QC regimes. (b) Gene-level retention matrix. The strict-current definition retains seven genes and the additional gates show candidate retention across increasing cell-count and guide-count requirements.

Supplementary Figure S2



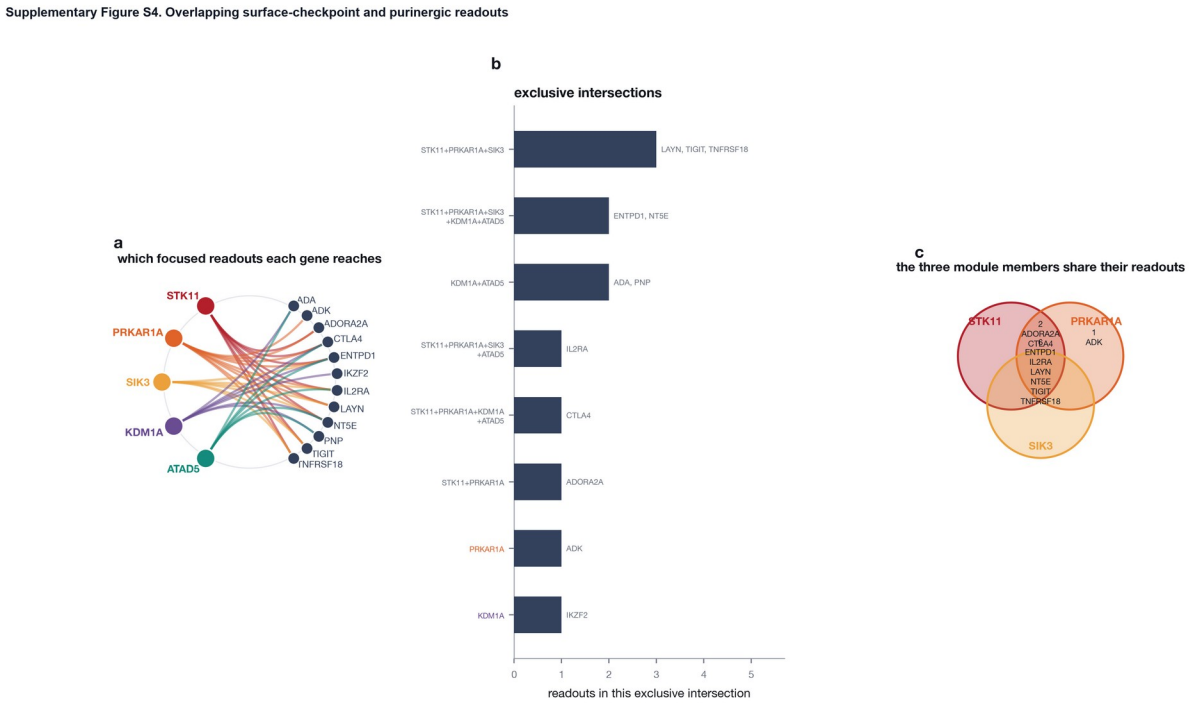
Supplementary Figure S2. Guide-seed matches follow background rates across the frozen candidates. (a) Background frequency of effect-level and FDR-labelled seed nominations. (b) Matched percentiles for the frozen targets. (c) Observed FDR-labelled nominations versus matched distributions. (d) Prespecified *ENTPD1* ≥12-base view.

Supplementary Figure S3



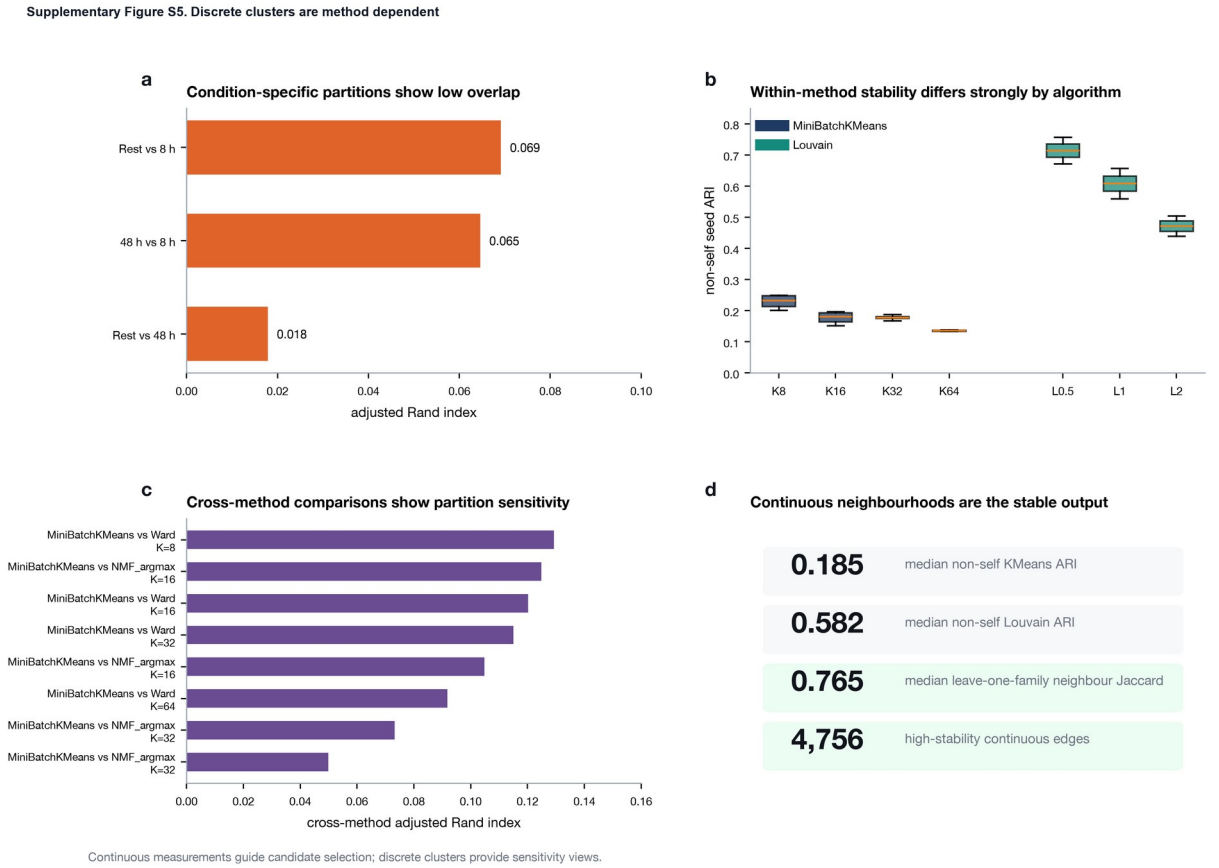
Supplementary Figure S3. Complete 93-gene upstream atlas. Rows are genes reaching *ENTPD1* significance in at least one state. Colour represents within-condition z scores.

Supplementary Figure S4



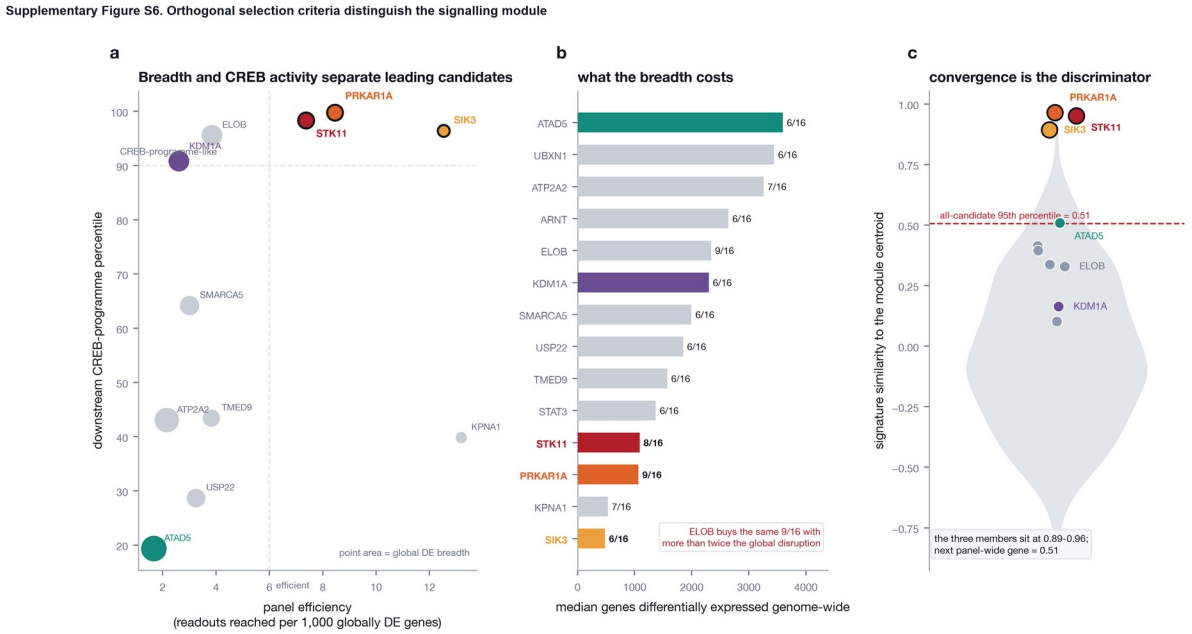
Supplementary Figure S4. Frozen genes act on an overlapping surface-checkpoint and purinergic readout set. The three module members share *ENTPD1*, *IL2RA*, *LAYN*, *NT5E*, *TIGIT* and *TNFRSF18* under identical QC and FDR rules.

Supplementary Figure S5



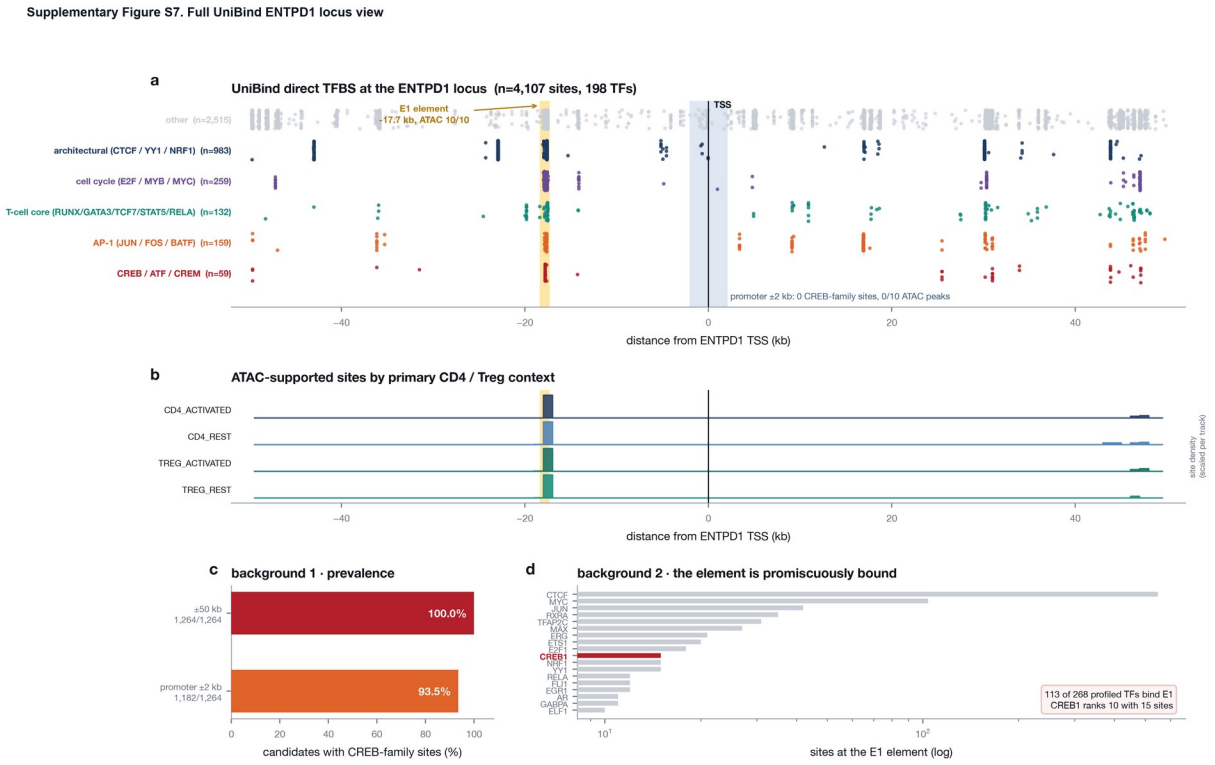
Supplementary Figure S5. Cluster membership varies across methods and continuous neighbourhoods remain stable. Continuous measurements guided candidate selection; discrete partitions provided sensitivity views.

Supplementary Figure S6



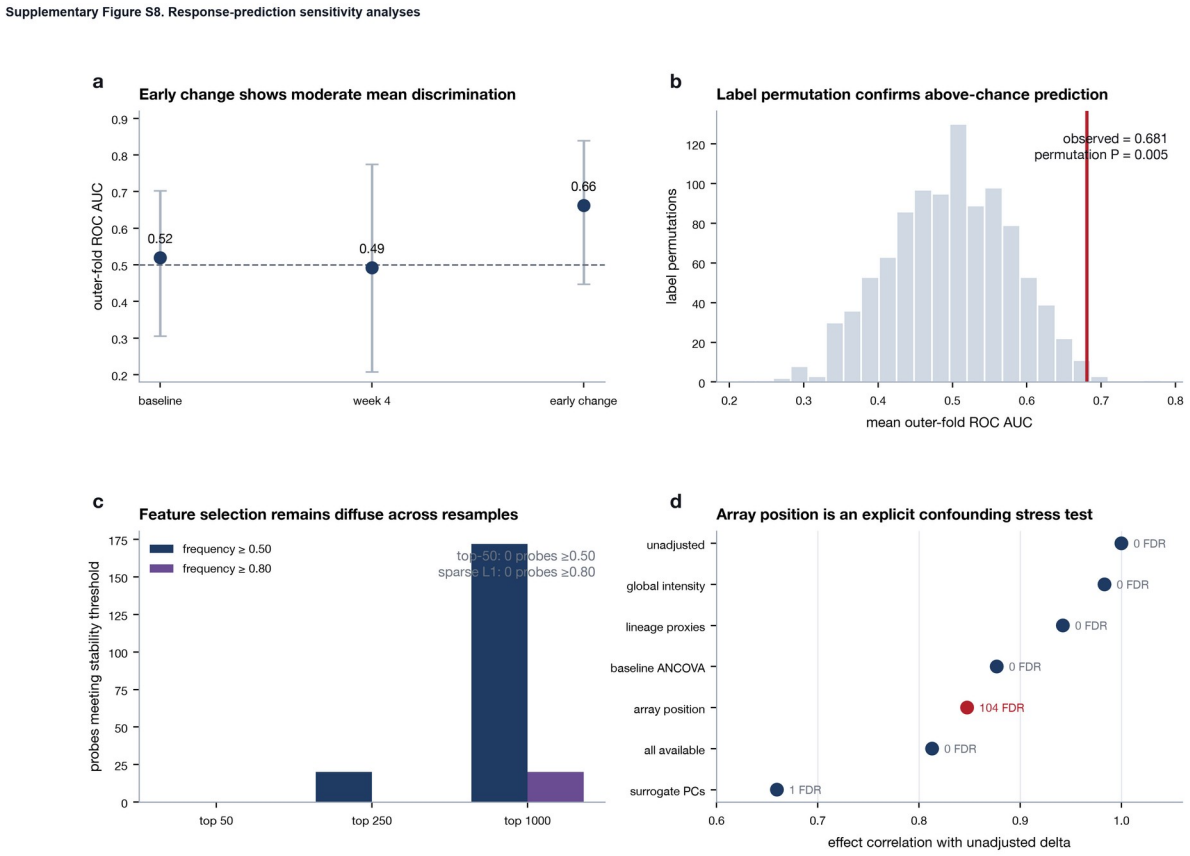
Supplementary Figure S6. Orthogonal selection criteria distinguish the signalling module. Panel breadth is interpreted with global perturbation cost, CREB-programme percentile and similarity to the module centroid.

Supplementary Figure S7



Supplementary Figure S7. Full UniBind locus view. CREB-family occupancy maps to an accessible element 17.7 kb upstream of *ENTPD1*. Background prevalence identifies this element as a shared transcription-factor-rich region.

Supplementary Figure S8



Supplementary Figure S8. Response-prediction sensitivity analyses. Nested cross-validation and label permutation show a moderate early-change signal. Feature selection remains diffuse across resamples, and covariate stress tests define technical sensitivity.