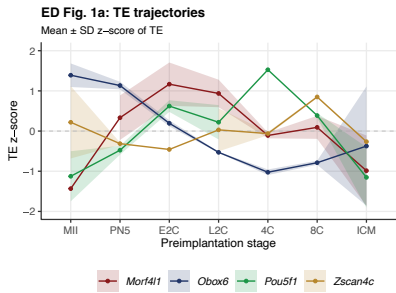
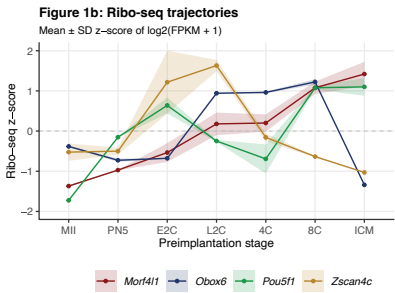
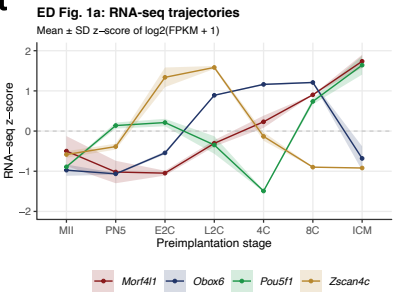
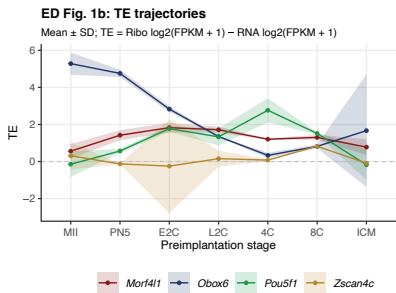
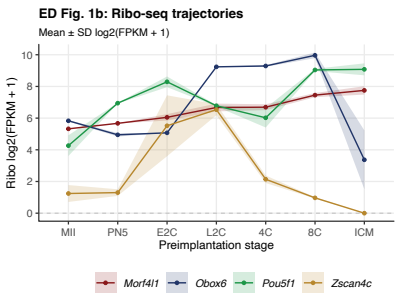
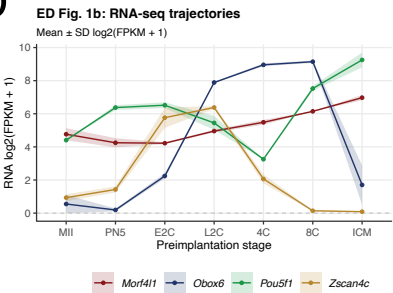


a



b



Extended Data Figure 1 | RNA, ribosome occupancy, and translational-efficiency trajectories of representative developmental regulators during mouse preimplantation development.

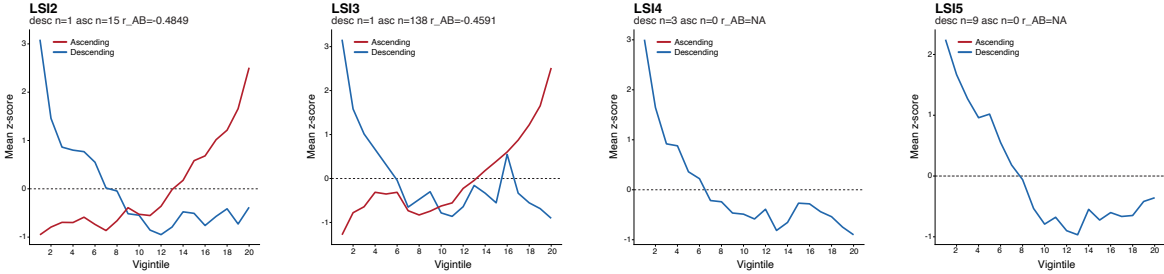
(a) Z-score normalized developmental trajectories for *Zscan4c*, *Obox6*, *Morf4l1*, and *Pou5f1* across mouse preimplantation stages. RNA abundance, ribosome occupancy, and translational efficiency (TE) values were calculated from $\log_2(\text{FPKM}+1)$ measurements and z-scored within each gene and molecular layer to facilitate comparison of developmental dynamics independent of expression magnitude. Data are shown as mean $\pm$ SD across biological replicates.

(b) Corresponding developmental trajectories shown on their original scales. RNA abundance and ribosome occupancy are displayed as $\log_2(\text{FPKM}+1)$. Translational efficiency (TE) was calculated as $\log_2(\text{Ribo}+1) - \log_2(\text{RNA}+1)$. Data are shown as mean $\pm$ SD across biological replicates.

a

Reciprocity detection - LSI axes 2-5

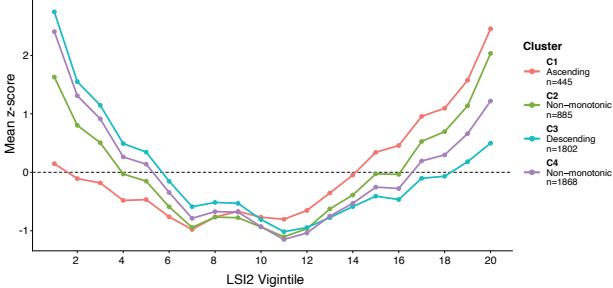
Row-normalized peaks | rho threshold=0.6 | Monotonicity>=70% | n=5000 variable peaks



b

K-means trajectory clusters - LSI2 (k=4)

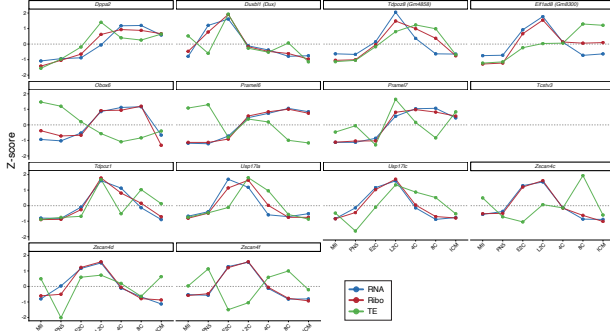
Row-normalized z-scores | 5000 peaks | Orthogonal check for reciprocity



c

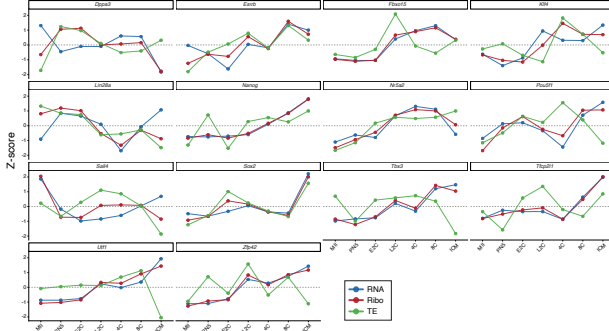
Totipotent module

Z-score of log2(FPKM+1) | per gene per layer | Stages: MII PN5 E2C L2C 4C 8C ICM | PN3 excluded



Pluripotent module

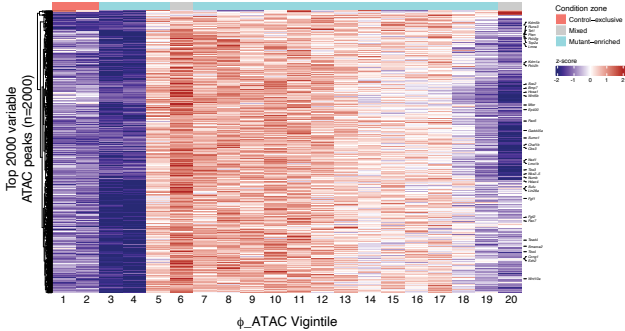
Z-score of log2(FPKM+1) | per gene per layer | Stages: MII PN5 E2C L2C 4C 8C ICM | PN3 excluded



d

Chromatin State Space-phi_ATAC vigintiles 1-20

Ordered by toti/pluri accessibility ratio



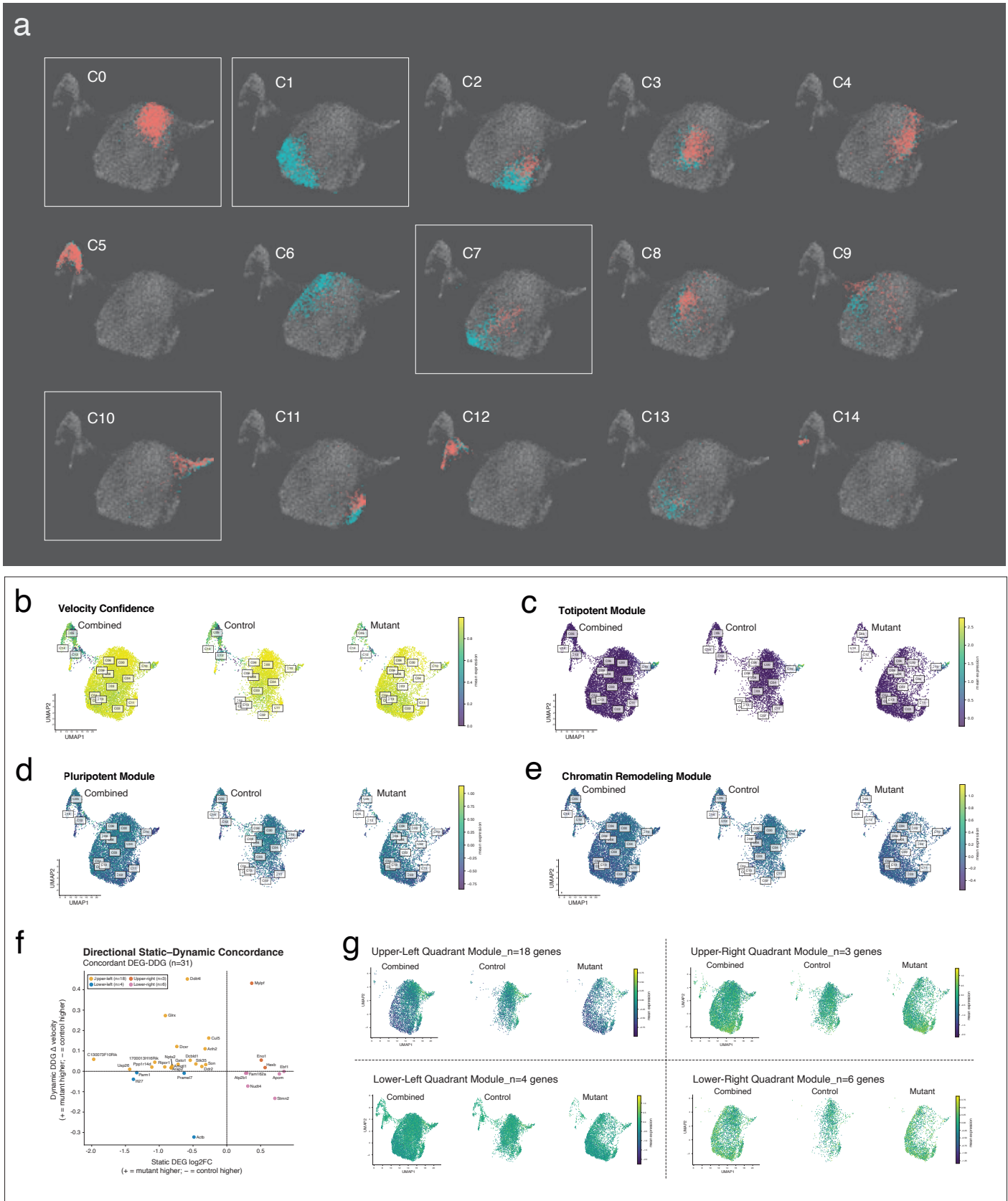
Extended Data Figure 2 | Developmental gene profiles and chromatin state space.

(a) Threshold sensitivity analysis of chromatin reciprocity detection. Mean z-scored accessibility per cluster across vigintiles shown for LSI axes 2, 3, 4, and 5 (four panels). Ascending and descending programs are detected at multiple signed Spearman rho thresholds (0.3-0.8) and monotonicity cutoffs. LSI4 and LSI5 capture predominantly closing programs; LSI2 and LSI3 show both ascending and descending programs at strict thresholds, confirming that the two programs occupy independent chromatin dimensions. (Supplementary Table S2d).

(b) K-means trajectory clustering of the top 5000 variable ATAC peaks across LSI2 vigintiles ($k=4$, $n=1,000$ random starts). Mean z-scored accessibility per cluster per vigintile is shown. Two programs are identified on the unbiased LSI2 axis: a descending program (C3: $n=1,802$ peaks, mean Spearman $\rho=-0.502$) and an ascending program (C1: $n=445$ peaks, mean $\rho=+0.677$). Program anti-correlation: Pearson $r=-0.485$. Statistical significance of the ascending program confirmed by vigintile-label permutation test ($p=0.002$, $n=1,000$ permutations). (Supplementary Table S2e).

(c) Z-scored $\log_2(\text{FPKM}+1)$ expression profiles across mouse preimplantation stages (MII, PN5, E2C, L2C, 4C, 8C, ICM) for RNA (blue), Ribo-seq (red), and translational efficiency ($\text{TE} = \log_2(\text{Ribo}+1) - \log_2(\text{RNA}+1)$; green). Left panel: 14 totipotent module genes. Right panel: 14 pluripotent module genes. Z-score computed per gene per layer across all stages. Values represent mean of two biological replicates per stage. Of the 14 totipotent genes, 10 shows neither rising RNA nor ribosomal occupancy from 4C to 8C, consistent with ZGA-associated expression peaking at E2C-L2C; *Obox6* shows a biphasic ribosomal occupancy profile with a second rise at 4C-to-8C (Ribo slope= $+376$) coinciding with rising *Morf4l1* and *Pou5f1* ribosomal levels (Fig. 1b); and *Tctv3* showed no detectable expression across the analyzed developmental stages and is therefore displayed as an empty profile. Of the 14 pluripotent genes, 9 show rising RNA and ribosomal occupancy from 4C to 8C (*Pou5f1*, *Nanog*, *Tfcp2l1*, *Esrrb*, *Tbx3*, *Lin28a*, *Utf1*, *Zfp42*, *Fbxo15*). Gene counts confirmed by explicit Gene_ID matching. (mm9 annotation aliases: *Eif1ad8*=Gm8300, *Tdpz8*=Gm4858, *Duxbl1*=Dux). (Supplementary Tables S2f).

(d) Chromatin state space heatmap. Z-scored mean accessibility of the top 2000 most variable ATAC peaks across phi_ATAC vigintiles V1-V20 (all cells integrated per vigintile). Rows clustered hierarchically by Ward's method on Euclidean distance (left dendrogram); columns ordered by phi_ATAC vigintile (high toti/pluri ratio at V1 to low at V20; no column clustering). Top annotation bar shows condition zone composition per vigintile derived from confirmed cell counts. Right annotation marks peaks within 100kb of 37 developmental regulatory genes (italic; full coordinates in Supplementary Table S2m). Peak-to-gene distance threshold: 100kb.



Extended Data Figure 3 | Developmental-state organization and directional transcriptional concordance.

(a) Cluster-specific UMAP projections for Leiden clusters C00–C14. Individual panels highlight a single cluster while all remaining cells are shown in gray. Cells are colored by genotype (Control, salmon; *Morf4l1*-deficient, teal). Clusters discussed in subsequent trajectory analyses (C00, C01, C07, and C10) are outlined for reference. These projections provide spatial context for cluster composition statistics reported in Supplementary Table S3a and Figure 3a.

(b) Velocity-confidence organization across the transcriptional manifold. Combined, Control-only, and Mutant-only UMAP projections colored by velocity confidence. Structured velocity-confidence landscapes remained evident in both conditions despite altered occupancy of trajectory states.

(c) Totipotent/2C developmental module organization. Combined, Control-only, and Mutant-only UMAP projections colored by totipotent/2C module scores. Totipotent-associated programs localized preferentially to root-associated trajectory regions in both Control and Mutant cells, indicating preservation of organized totipotent-state structure despite redistribution across the manifold.

(d) Pluripotent developmental module organization. Combined, Control-only, and Mutant-only UMAP projections colored by pluripotent module scores. Pluripotent programs remained spatially organized across the shared transcriptional manifold in both conditions.

(e) Chromatin-remodeling module organization. Combined, Control-only, and Mutant-only UMAP projections colored by chromatin-remodeling module scores. Chromatin-associated programs exhibited structured distribution across developmental trajectory states in both control and mutant cells.

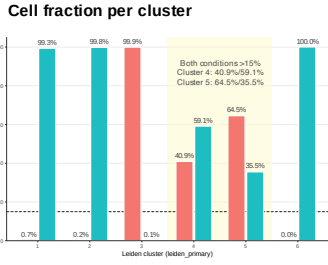
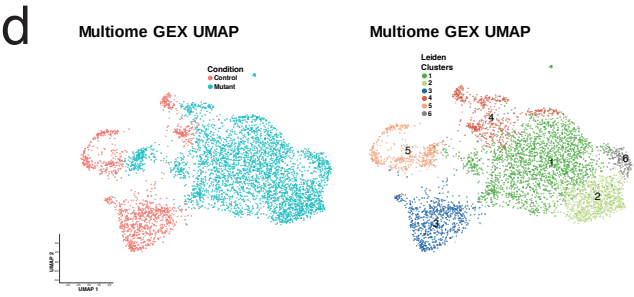
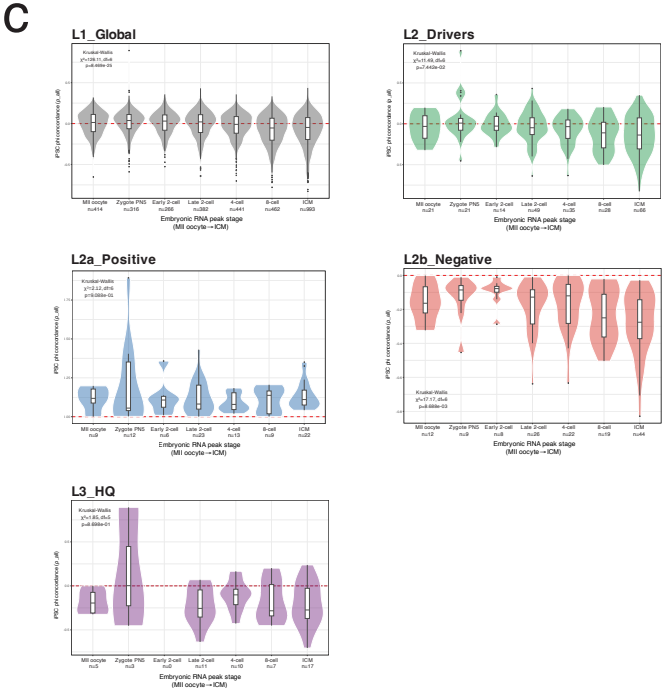
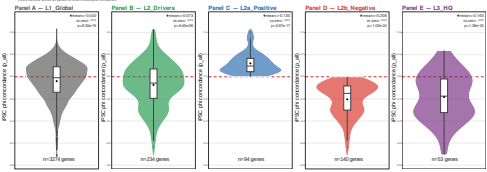
(f) Directional static–dynamic concordance analysis. Scatter plot comparing differential expression (DEG log2 fold-change) and differential dynamical velocity (DDG Δ velocity) for the concordant overlap gene set ($n = 31$ genes). Dashed lines indicate zero differential abundance and zero differential velocity. Genes were distributed across all four quadrants, demonstrating that static transcriptional differences and trajectory-associated dynamical differences can exhibit both concordant and discordant directional relationships. Gene identities and associated statistics are provided in Supplementary Table S3e.

(g) Trajectory projection of directional concordance classes. Genes from panel f were grouped according to quadrant membership and aggregated into four module scores corresponding to the upper-left ($n = 18$ genes), upper-right ($n = 3$ genes), lower-left ($n = 4$ genes), and lower-right ($n = 6$ genes) quadrants. UMAP projections show the spatial distribution of each quadrant-defined module across the shared transcriptional manifold. These analyses were used to visualize the trajectory localization of directional concordance classes and were interpreted as exploratory because they were derived from a filtered overlap set.

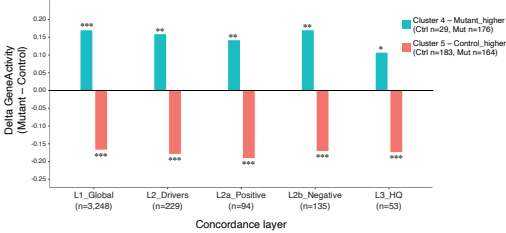
a **Six-Layer Phi Concordance Validation Framework**
Three independent experiments | At least four independent *Mor4i1*-deficient clones from different mice



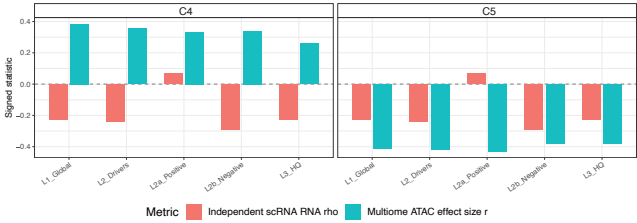
b **IPSC phi concordance (ρ_{all}) distributions per concordance layer**



e **Multiome ATAC GeneActivity - phi Concordance Layers**



f **Layer-level scRNA concordance vs multiome ATAC complementarity**



Extended Data Figure 4 | Phi concordance framework validation across six independent analytical layers.

(a) Six-layer phi concordance validation framework schematic. Each layer represents an independent analytical comparison performed on data from at least one of three independent experiments (Experiment 1a: scRNA-seq; Experiment 1b: scATAC-seq; Experiment 2: GEX/ATAC).

- Layer 1: static RNA concordance vs RNA velocity within Experiment 1a.
- Layer 2: phi concordance from Experiment 1a vs phi vigintiling from Experiment 1b.
- Layer 3: iPSC phi concordance vs in vivo mouse embryo RNA, Ribo, and TE monotonicity across seven preimplantation stages.
- Layer 4: phi concordance layers vs Multiome GEX RNA expression (Experiment 2).
- Layer 5: phi concordance layers vs Multiome ATAC GeneActivity (Experiment 2).
- Layer 6: Multiome GEX RNA vs ATAC GeneActivity within the same cells (Experiment 2). No biological material is shared between Experiment 1a and Experiment 2.

(b) iPSC phi concordance (ρ_{all}) distributions per concordance layer. Five violin plots arranged left to right: L1_Global (n=3,274), L2_Drivers (n=234), L2a_Positive (n=94), L2b_Negative (n=140), L3_HQ (n=53).

- Layer identity indicated by title above each violin. y-axis: iPSC phi concordance (ρ_{all} ; Spearman correlation between per-cell gene expression rank and phi developmental trajectory rank, all cells).
- Red dashed horizontal reference line at $\rho=0$. Each violin shows kernel density estimate with embedded boxplot (median, IQR, $1.5 \times \text{IQR}$ whiskers).
- All five layers differ significantly from zero (one-sample Wilcoxon, all $p < 0.001$; Supplementary Table S3). L2a_Positive and L2b_Negative exhibited opposite concordance directions, resulting in a substantially weaker aggregate signal when combined within L2_Drivers. L2a_Positive vs L2b_Negative: Wilcoxon $p = 2.05 \times 10^{-38}$.

(c) iPSC phi concordance (ρ_{all}) by embryonic RNA peak stage per concordance layer. Five plots arranged left to right: L1_Global, L2_Drivers, L2a_Positive, L2b_Negative, L3_HQ.

- Each plot contains seven violins corresponding to embryonic RNA peak stage (x-axis: MII oocyte, PN5, E2C, L2C, 4C, 8C, ICM; PN3 excluded). y-axis: iPSC phi concordance (ρ_{all}). L3_HQ early 2C violin absent (n=0 genes with early 2C peak in this layer).
- Kruskal-Wallis test for stage-dependent variation: L1_Global $\chi^2 = 126.109$, $df = 6$, $p = 8.47 \times 10^{-25}$; L2b_Negative $\chi^2 = 17.167$, $df = 6$, $p = 0.0087$; L2a_Positive $p = 0.909$ ns; L3_HQ $p = 0.870$ ns.
- Post-hoc pairwise Wilcoxon BH-corrected for L1_Global: 8C and ICM show significantly more negative phi concordance than earlier stages (all $p < 0.001$); 8C vs ICM not significant ($p = 0.806$).
- No individual pairwise comparison survived BH correction within L2b_Negative (minimum BH-adjusted $p = 0.056$); the directional gradient toward more negative concordance at ICM-peaking genes is reported as an observation.
- Stage-dependent differences not significant within L2a_Positive or L3_HQ.

(d) Multiome GEX cell state landscape and cluster composition confirming mixed-condition clusters used for Layer 4 and Layer 5 validation.

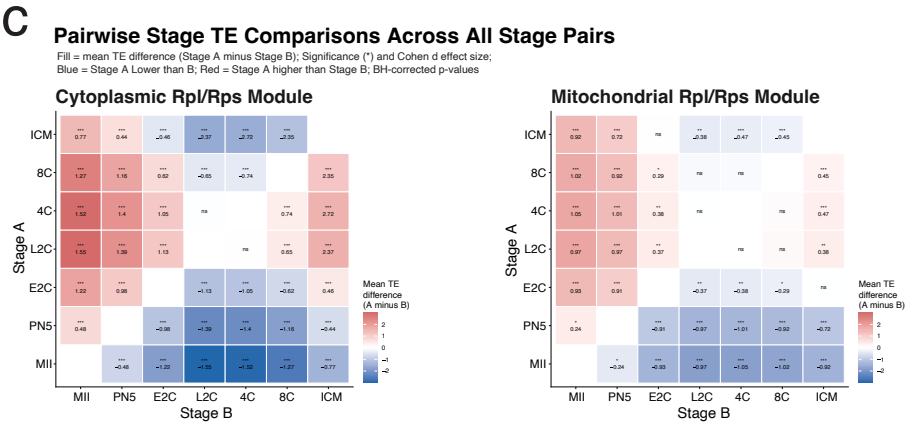
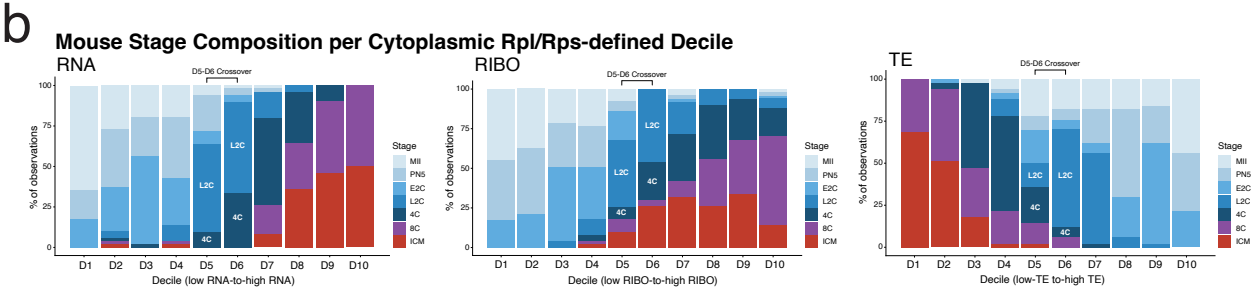
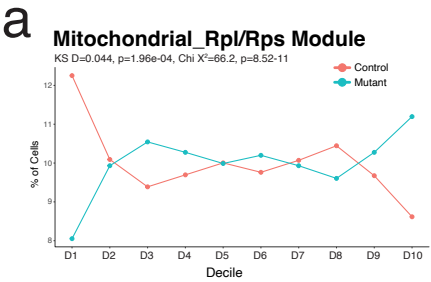
- Left: GEX UMAP colored by condition (Control n=1,280 salmon; Mutant n=3,596 teal; total n=4,876; Multiome GEX Experiment 2).
- Middle: GEX UMAP colored by Leiden cluster identity (clusters 1–6 labeled). Right: percentage of Control and Mutant cells per Leiden cluster with percentages labeled on each bar; yellow shading highlights clusters 4 and 5 — the only clusters exceeding the 15% mixed-condition threshold in both conditions and used for Layer 4 and Layer 5 genotype comparisons. Cluster 4: Control 40.9%, Mutant 59.1%. Cluster 5: Control 64.5%, Mutant 35.5%. Clusters 1, 2, 6: Mutant-predominant or exclusive (>97% Mutant). Cluster 3: Control-exclusive (99.9% Control).

(e) Multiome ATAC GeneActivity genotype differences per concordance layer — Layer 5 validation. y-axis: Δ GeneActivity (Mutant – Control). Five concordance layers on x-axis: L1_Global, L2_Drivers, L2a_Positive, L2b_Negative, L3_HQ.

- Cluster 4 bars (Mutant-predominant; Control n=221, Mutant n=320): all five layers above zero — higher accessibility in Mutant. L1_Global $\Delta=+0.171$ ($p=9.25 \times 10^{-4}$); L2_Drivers $\Delta=+0.160$ ($p=2.25 \times 10^{-3}$); L2a_Positive $\Delta=+0.143$ ($p=4.03 \times 10^{-3}$); L2b_Negative $\Delta=+0.171$ ($p=3.46 \times 10^{-3}$); L3_HQ $\Delta=+0.108$ ($p=0.025$).
- Cluster 5 bars (Control-predominant; Control n=309, Mutant n=170): all five layers below zero — higher accessibility in Control. L1_Global $\Delta=-0.168$ ($p=2.44 \times 10^{-11}$); L2_Drivers $\Delta=-0.180$ ($p=2.15 \times 10^{-11}$); L2a_Positive $\Delta=-0.192$ ($p=5.09 \times 10^{-12}$); L2b_Negative $\Delta=-0.172$ ($p=9.61 \times 10^{-10}$); L3_HQ $\Delta=-0.175$ ($p=8.50 \times 10^{-10}$).
- Accessibility changes were significant across all concordance layers in both mixed-condition clusters, indicating broad concordance-layer chromatin redistribution that tracked cluster-specific condition dominance.
- Bidirectional pattern across clusters argues against global chromatin collapse and is consistent with state-dependent redistribution of chromatin accessibility. All comparisons Wilcoxon rank-sum, BH-corrected. GeneActivity computed using Signac GeneActivity() with gene coordinates spanning 2 kb upstream of the TSS plus full gene body; TF-IDF normalized

(f) Layer-level scRNA concordance vs Multiome GEX complementarity — Layer 4 validation. Two barplots shown for Leiden cluster 4 (Control n=221, Mutant n=320; left) and Leiden cluster 5 (Control n=309, Mutant n=170; right). Each barplot shows mean normalized RNA expression difference (Mutant – Control) per concordance layer across five layers (L1_Global, L2_Drivers, L2a_Positive, L2b_Negative, L3_HQ) for Control and Mutant cells within the same Multiome cells.

- Selective transcriptional significance (L2a_Positive BH $p=1.49 \times 10^{-5}$; L3_HQ $p=0.026$; all others ns) coexists with broad ATAC significance across all five layers (panel e) within the same cell populations, ruling out cell-composition differences as an explanation for the modality discrepancy.
- In contrast to the broad ATAC response, transcriptional differences were selective and remained strongest within the L2a_Positive and L3_HQ gene sets, indicating that only a subset of concordance-layer accessibility changes are realized at the RNA level.



Extended Data Figure 5 | Mitochondrial ribosomal redistribution, developmental stage composition, and pairwise translational efficiency comparisons.

(a) phi_ribo decile distribution of Control (n=4,538; salmon) and *Morf4l1*-deficient (n=5,216; teal) iPSCs along the mitochondrial ribosomal protein axis (Mi_Rpl/Rps; n=78 structural genes). Cells partitioned into ten deciles by mean z-scored Mi_Rpl/Rps expression. Five crossovers at D2-D3 (exact=2.123), D4-D5 (exact=4.973, near-tangential), D5-D6 (exact=5.035, near-tangential), D6-D7 (exact=6.757), and D8-D9 (exact=8.583); oscillatory pattern indicates no systematic unidirectional redistribution. KS D=0.044, $p=1.96 \times 10^{-4}$; chi-square $X^2=66.17$, df=9, $p=8.52 \times 10^{-11}$. (See Supplementary Table S5b).

(b) Mouse embryo preimplantation stage composition per Cy_Rpl/Rps decile across seven stages (MII, PN5, E2C, L2C, 4C, 8C, ICM; PN3 excluded; n=2 replicates per stage; n=72 Cy genes). Gene-level deciles assigned independently per layer from lowest (D1) to highest (D10). Left: RNA-defined deciles (mean $\log_2(\text{RNA FPKM}+1)$). Middle: Ribo-defined deciles (mean $\log_2(\text{Ribo FPKM}+1)$). Right: TE-defined deciles (mean TE per gene per stage). RNA and Ribo panels show early stages (MII, PN5, E2C) dominating low deciles and late stages (8C, ICM) dominating high deciles, confirming developmental progression along the Cy ribosomal axis. TE panel shows an inverted gradient — early stages at high TE deciles, late stages at low TE deciles — consistent with cytoplasmic ribosomal translational efficiency peaking at early cleavage and declining toward the 4C–8C interval. Stage color legend: MII (lightest blue) through ICM (dark red); MII at top of legend. (See Supplementary Table S5c).

(c) Pairwise translational efficiency comparisons across all preimplantation stage pairs for cytoplasmic (left; Cy_Rpl/Rps; n=72 genes) and mitochondrial (right; Mi_Rpl/Rps; n=78 genes) modules. Paired t-tests, BH-corrected. x-axis: Stage_B (later stage); y-axis: Stage_A (earlier stage); both axes MII→ICM. Color: mean_diff = TE(Stage_A) – TE(Stage_B); anti-symmetric matrix (lower triangle values negated). Cell labels: significance tier and Cohen's d. Warm colors: TE declines Stage_A→Stage_B. Cool colors: TE rises. PN3 excluded; diagonal empty. Cytoplasmic module: rising zone (MII-E2C → L2C-4C; ***), non-significant plateau (L2C → 4C; ns), declining zone (4C→8C: mean_diff=+0.381, d=0.741, $P_{\text{adj}} < 0.001$; 4C→ICM: mean_diff=+1.535, d=2.720, $P_{\text{adj}} < 0.001$). Mitochondrial module: no significant 4C→8C transition (mean_diff=+0.083, $P_{\text{adj}}=0.164$, d=0.169 ns). (See Supplementary Table S5f, S5g).