

Brownian Bridging of Chromatin States in Leukemic Cells

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Chromatin mobility in living interphase nuclei provides a dynamical readout of genome regulation. From time-resolved trajectories of chromatin granules in primary acute lymphoblastic leukemia (ALL) samples (one B-ALL, one T-ALL), leukemic cell lines (U266, Jurkat), and healthy donor T lymphocytes, we resolve rare but reproducible transitions between extreme mobility states. We quantify these events with Brownian-bridge ensembles, i.e., trajectories conditioned on crossings between low- and high-mobility quartiles. Despite their low frequency, crossings reveal strong polarity: activation ($Q1 \rightarrow Q4$) concentrates on a superlinear, ballistic-like ridge, whereas reconfinement ($Q4 \rightarrow Q1$) returns more slowly toward confinement. The scarcity of extreme events is comparable in magnitude to reported bursting fractions, motivating future joint live-imaging tests that directly link mobility bridges to transcriptional state. These asymmetric pathways yield a compact dynamical signature of disrupted confinement and lineage-dependent nuclear deregulation in ALL, and generate testable predictions for cohort-level validation.

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I. INTRODUCTION

Eukaryotic genomes are not only biochemical repositories but mechanical objects constrained by nuclear architecture [1–3]. In interphase, chromatin operates in a metabolically sustained nonequilibrium steady state (NESS) [4,5]: ATP-driven remodeling continually injects motion against passive viscoelastic confinement [6–8]. Live-cell tracking now resolves locus dynamics with high precision [9–11], exposing a broad mobility spectrum—from confined subdiffusion to intermittent excursions [12,13]—a canonical active-matter NESS signature [14] that can encode functional organization beyond stochastic variability [3,15]. Yet chromatin motion is not free: fast two-point dynamics remain partly predictable from static 3D genome structure [16], implying

persistent architectural constraints even under drive [17]. In such nonequilibrium systems, broken detailed balance generically yields directional probability currents, i.e., irreversible transitions between dynamical states [4,18]. Here we develop a purely dynamical framework that isolates rare mobility-state transitions and quantifies their time-directional asymmetry directly from trajectories.

These physical cues become especially sharp in cancer, where nuclear architecture and regulatory programs are perturbed across scales [19–21]. Acute lymphoblastic leukemia (ALL) is a tractable benchmark because malignant transformation entails extensive chromatin reorganization [22–24], driven by dysregulated epigenetic modifiers and transcriptional programs that can actively reshape nuclear dynamics [8,25,26]. ALL is also molecularly diverse: B-ALL and T-ALL differ in developmental wiring [21] and immunophenotype [27], and have been reported to exhibit distinct genome organization [22,23], structural alteration patterns [28], and immune microenvironments [29]. These contrasts motivate a subtype-resolved biophysical characterization of chromatin dynamics. Yet, despite major advances in live-cell tracking [10,11], and active-polymer modeling [30–32], quantitative event-resolved frameworks for parametrizing rare state-switching remain scarce [8].

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In this work, we address a key biophysical issue in malignant leukemic transformation [33]: *are chromatin dynamics dominated by fluctuations within altered confinement landscapes, or does malignancy promote directed nonequilibrium transitions between metastable mobility states?* ALL provides an informative setting [21,27,28]. Malignant transformation is accompanied by extensive genome reorganization [22,23], a modulable secretome [24], and broad dysregulation of chromatin modifiers [25] and transcriptional programs [26] that can sustain active nuclear driving in the cells of origin [27]. The availability of cell lines and primary patient material enables subtype-resolved phenotyping under comparable interphase conditions, including B-ALL versus T-ALL. Although the primary dataset comprises one case per lineage, these contrasts serve as mechanistic exemplars for a general framework that infers rare activation dynamics from stochastic trajectories.

II. MOBILITY SPACES: BROWNIAN BRIDGES

To resolve dynamical transitions experimentally, we use a noninvasive multiple particle tracking (MPT) strategy that enables high-throughput tracking of chromatin granules in living, unstained interphase nuclei [9]. Chromatin granules are detected as intensity extrema in phase-contrast images using speeded-up robust features (SURF) [34]. As outlined in Fig. 1, trajectories are extracted, converted into time-lagged mean-squared displacement (MSD) traces, and used to construct mobility distributions. This framework is applied to cohorts spanning primary B-ALL and T-ALL samples, leukemic cell lines (U266, Jurkat), and healthy donor T lymphocytes. Within each nucleus, loci are stratified by early-lag MSD into mobility quartiles, defining low-mobility (Q_1) and high-mobility (Q_4) initial states. We then analyze MSD evolution conditioned on these classes, focusing on rare crossovers between Q_1 and Q_4 . A two-step procedure—*within-cell stratification followed by population aggregation*—controls for cell-to-cell variability while retaining statistics for low-probability transitions.

In stochastic theory, a Brownian bridge is a diffusion conditioned on fixed start and end points over a finite interval [35]. More generally, conditioned diffusions—including Ornstein-Uhlenbeck (OU) bridges—can be constructed using Doob’s h -transform [36–38]. We adopt this logic in a data-driven form: our “Brownian bridges” are event-conditioned mobility ensembles obtained by aligning chromatin MSD trajectories at mobility-state crossover events [37,38]. Conditioning is imposed on transitions between Q_1 and Q_4 quartiles across lag times rather than spatial endpoints. The resulting ensembles inhabit mobility space (MSD- τ) and quantify how chromatin dynamics reorganize between metastable confinement basins. This construction isolates rare transitions between latent mobility states and exposes pathway asymmetries and directional polarity directly from experimental trajectories.

Brownian bridging of chromatin loci mobility. Q_1/Q_4 denote mechanical mobility states inferred from locus dynamics; any association with transcriptional activation remains a working interpretation, not a measured label. Most live-cell studies summarize chromatin trajectories through MSD scaling and effective diffusivities [9,15], commonly

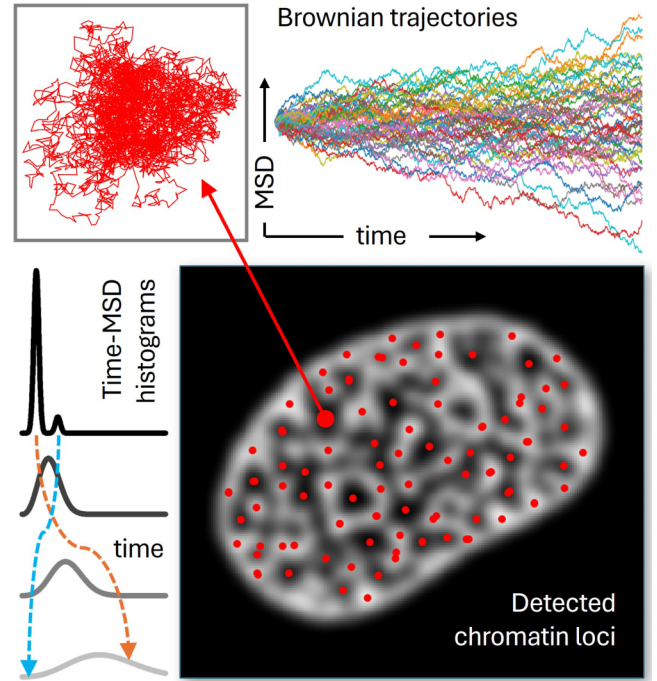


FIG. 1. Multiple particle tracking (MPT) of chromatin loci and event-conditioned mobility pathways. Chromatin granules (red markers) are detected in live, unstained interphase nuclei and tracked over time, yielding single-locus Brownian trajectories. Raw trajectories were corrected for rigid-body nuclear drift by frame-to-frame fiducial registration of chromatin keypoints, excluding stage and centroid-motion artifacts [9]. Rare transitions between low- and high-mobility states are used to define event-aligned histograms of MSD evolution (“Brownian-bridge” ensembles in mobility space; left panel). This conditioned-ensemble construction provides a structured description of activation (red) and reconfinement (blue) pathways that complements standard time-averaged diffusion summaries.

interpreted as anomalous subdiffusion from transient binding [12] and polymer viscoelasticity [13]. Complementary approaches characterize confinement by modeling fluctuations as OU processes [39–41], which capture mean-reverting dynamics within a mobility basin and arise naturally from linearized fluctuations around structure-imposed chromatin configurations [16]. While effective for quantifying heterogeneity, such OU-reduced descriptors are time-averaged and discard the temporal ordering needed to resolve rare transitions between mobility basins [10,11]. Conditioning instead on the initial mobility state and aligning trajectories at crossover events reconstructs event-conditioned pathways [4,18], revealing activation-reconfinement asymmetries that provide a dynamical signature of Brownian-bridge irreversibility.

Our central finding is that rare mobility-state bridges are strongly time-directional in mobility space (Fig. 1). Conditioning on $Q_1 \rightarrow Q_4$ crossovers reveals activation paths with superdiffusive to ballistic-like signatures, whereas $Q_4 \rightarrow Q_1$ conditioning shows slower reconfinement consistent with OU-like mean-reverting relaxation toward confinement [40]. This dynamical polarity is reproducible across cohorts and cell

types, separates leukemic from healthy nuclei, and provides a compact parametrization of nonequilibrium nuclear remodeling in ALL. The inferred crossover statistics remain stable under bootstrapping, subsampling, tracking-length thresholds, and trajectory-quality filters, arguing against selection artifacts from detection dropouts or segmentation [9]. Finally, the rarity of activation/deactivation events is comparable to reported transcriptional episode fractions [6,42], suggesting a plausible coupling between transient transcriptional activity and mobility remodeling. Together, these results identify event-conditioned chromatin mobility transitions as a quantitative biomarker of disrupted confinement and lineage-specific nuclear deregulation in acute leukemia.

III. MATERIALS AND METHODS

A. Cell culture: Cell lines and primary samples

1. Cell lines

Jurkat (T-lineage leukemia) and U266 (plasma-cell/myeloma-derived B-lineage malignancy) were used as stabilized lineage models to contextualize primary leukemic phenotypes. Cells were cultured in RPMI-1640 (Gibco) supplemented with 10% FBS and 1% penicillin–streptomycin at 37° C and 5% CO₂, and maintained in logarithmic growth.

2. Primary leukemic samples

Bone marrow aspirates were collected at diagnosis from two pediatric patients with acute lymphoblastic leukemia (T-ALL and B-ALL) at Hospital Infantil Universitario Niño Jesús (Madrid, Spain). The primary ALL cases belong to the private collection registered under code C.0005614. All donors (legal guardians) provided written informed consent, and the project was approved by the local Ethics Committee (reference R-0017-13), in accordance with the *Declaration of Helsinki*. Clinical profiles were: T-ALL, 11-year-old male (205 000 blasts/ μ l at diagnosis); B-ALL, 5-year-old female (2263 blasts/ μ l at diagnosis). Both patients were classified as intermediate risk under the SEHOP-PETHEMA 2013 protocol, showed favorable early response to induction therapy (day +8 and end-of-induction), completed treatment without major complications, and remain in complete remission > 10 years after diagnosis. Full clinical and genomic characterization is provided in Supplementary Note S1 of the Supplemental Material [43].

Mononuclear cells were isolated by Ficoll-Paque density-gradient centrifugation and cultured *ex vivo* in RPMI-1640 supplemented with 20% FBS and cytokines (IL-7, SCF, FLT3L) to support viability and preserve lineage-associated nuclear states. Primary cells were processed for live-cell imaging within 24 h of isolation to minimize *ex vivo* drift.

3. Optical genome mapping

Whole-genome architecture of the two primary diagnostic samples was profiled by Optical Genome Mapping (Bionano Genomics) using ultra-high-molecular-weight DNA, enabling simultaneous detection of structural variants (SVs) and copy-number variants (CNVs) [44,45]. Molecules were aligned to the hg38 reference genome and variants called using Bionano Access (v1.8). Detected SV classes included deletions,

duplications, insertions, inversions, and intra-/interchromosomal translocations, together with CNV gains/losses and aneuploidy. A detailed event-level summary, altered-span quantification, and derived structural indices used in the mechanistic interpretation are provided in Supplemental Material Note S1 [43].

Peripheral T-lymphocytes from healthy donors. Cells were cultured for 14 days at 10⁶ cells/ml in 24-well plates containing 2 ml of complete medium (CM) composed of 90% RPMI 1640 and 10% heat-inactivated human AB serum (HS; Sigma–Aldrich), supplemented with recombinant human IL-2 (Aldeleukin; Novartis; 6000 IU/ml), penicillin, streptomycin, and L-glutamine. T cells were activated for 48 h using anti-CD3/anti-CD28 stimulation. On days 7–9, expanded T cells were harvested and counted by trypan blue exclusion, and cell-cycle distribution was determined by propidium iodide staining (Cytognos) using a FACSCanto II flow cytometer.

B. High-speed phase-contrast videomicroscopy

Chromatin motion in live cells was imaged by high-speed phase-contrast microscopy. Suspension cells were washed in isotonic buffer, resuspended in TKM, and gently adhered to poly-L-lysine-coated coverslips to preserve structural integrity. Imaging was performed on a Nikon Eclipse Ti2000 inverted microscope with a 100 W TI-12 DH Pillar Illuminator, LWD 0.52 collimator, and 100 $\times$ Plan Apo VC oil-immersion objective (NA 1.4) in phase-contrast mode. Chromatin granule dynamics were recorded label-free using a FASTCAM SA3 camera (Photron) at 512 Hz for 10 s (5120 frames, 1 MP resolution), yielding an effective spatial resolution of 50 nm $\times$ 50 nm per pixel. Tracking was performed on recordings from $n = 350$ –600 nuclei per condition. Representative phase-contrast nuclei and corresponding MSD trajectories for all analyzed conditions are shown in Supplemental Material Fig. S1 [43]. As an immobile control, Jurkat nuclei were strongly fixed by methanol-dehydration, a procedure expected to immobilize chromatin in a structurally frozen state; these fixed nuclei displayed nearly flat MSD trajectories close to the localization baseline.

C. Chromatin trajectories: Multiple particle tracking via SURF detection

Chromatin dynamics were analyzed using a MPT pipeline [9] based on SURF [34]. Images were first binarized by adaptive local thresholding to isolate nuclear regions, then converted into grayscale intensity maps by Gaussian filtering to enable robust detection of chromatin granules [9]. Translational and rotational nuclear motion was removed by rigid-body drift correction obtained from frame-to-frame fiducial registration of the chromatin keypoint ensemble (see the Supplemental Material in Ref. [9]). SURF features were linked frame-to-frame using a nearest-neighbor algorithm with a maximum admitted displacement of ± 6 px ($\approx 0.1 \mu\text{m}^2$). Subpixel localization was refined by quadratic interpolation around each detected locus. Chromatin loci were identified as high-contrast, symmetric 2D Gaussian intensity peaks above the local background, and their positions were defined by the maximum of the fitted Gaussian profile. For each trajectory

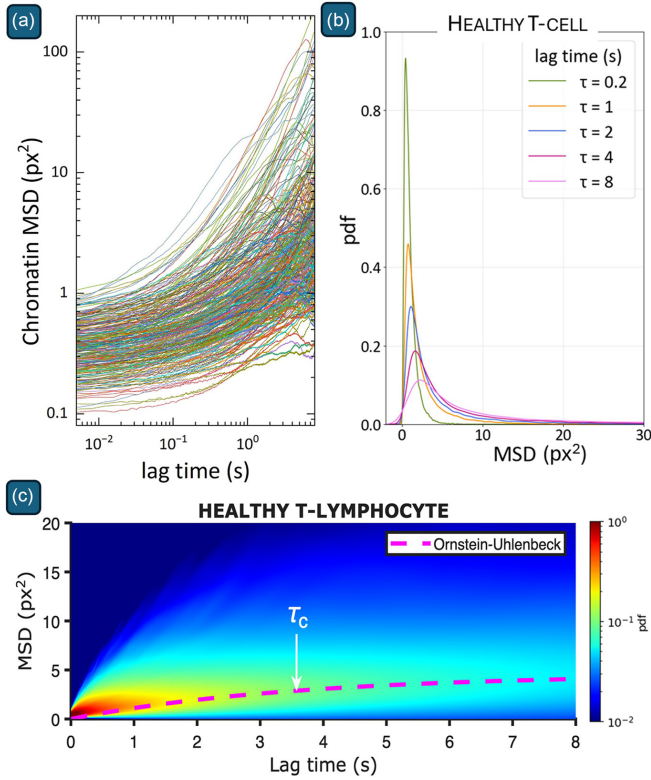


FIG. 2. Chromatin mobility in noncancerous cells: MSD ensembles and confinement mapping. Aggregated data from three healthy donors, comprising 200–250 nuclei per donor and $\simeq 10^5$ chromatin loci in total. (a) Ensemble of mean-squared displacement (MSD) curves from chromatin granules tracked in living, unstained interphase nuclei of healthy peripheral T lymphocytes. The black guideline indicates diffusive scaling $\text{MSD} \propto \tau$ (slope +1 in the log–log representation). Individual loci exhibit strong heterogeneity, with most trajectories remaining confined within the experimental observation window. (b) MSD probability density functions $P[\text{MSD}(\tau)]$ at selected lag times τ (legend), showing progressive broadening with increasing τ while remaining skewed toward low mobilities. (c) Time-resolved MSD probability density landscape $P[\text{MSD}(\tau)]$ (color scale) assembled from all trajectories. The dashed magenta curve shows the Ornstein-Uhlenbeck (OU) confinement model [Eq. (3)] fitted to the modal ridge of the MSD landscape, $M(\tau)$; the arrow indicates the characteristic confinement time τ_c . Units: MSD is reported in pixel-squared units (px^2), with $1 \text{ px} = 50 \text{ nm}$ ($1 \text{ px}^2 = 2500 \text{ nm}^2$). See Methods for analysis details.

$\mathbf{r}(t)$, the MSD was computed as

$$\text{MSD}(\tau) = \langle [\mathbf{r}(t + \tau) - \mathbf{r}(t)]^2 \rangle_t. \quad (1)$$

MSD values represent the area explored by each locus over lag time τ and are reported in pixel-squared units (px^2). Each pixel corresponds to $50 \times 50 \text{ nm}^2$ ($2500 \text{ nm}^2/\text{px}$), with subpixel localization precision of $\sim 1/5 \text{ px}$ ($\pm 5 \text{ nm}$). The full pipeline was implemented in MATHEMATICA v12.1 (Wolfram) as described in [9]. The SURF-MPT approach enabled simultaneous tracking of 50–400 chromatin loci per nucleus (Fig. 2), yielding 30 000–100 000 trajectories per cell type. Representative nuclei illustrating the chromatin texture used for granule tracking are provided in Supplemental Material Fig. S1 [43].

D. Statistical methods

All statistical analyses were performed in Python using NumPy, SciPy, pandas, and Matplotlib, with custom routines for advanced tasks. Unless stated otherwise, statistics were computed per cell and then aggregated to obtain population-level features. Chromatin dynamics were quantified using ensemble-averaged MSD trajectories and distributional analyses at fixed lag times τ . In addition to the MSD, we evaluated standard descriptors of the MSD distributions: *standard deviation* (SD), *skewness* (S), and *kurtosis* (K). The lag-dependent standard deviation of the MSD distribution reports the experimental dispersion of the underlying squared-displacement ensemble and is shown explicitly in Fig. 4. This dispersion increases with lag time in leukemic conditions and is also visible as broadening of the MSD probability-density landscapes in Fig. 3. To detect deviations from unimodal Gaussian behavior and identify mixed mobility states, Sarle’s modality coefficient was computed as

$$B = \frac{1 + S^2}{K}, \quad (2)$$

where larger B indicates increasing multimodality; for a Gaussian distribution $B_0 = 1/3$ ($S_0 = 0$, $K_0 = 3$).

Because MSD is continuous and its distributions are often multimodal, the most probable MSD at each lag time τ was estimated from the probability density function (PDF). Time-resolved MSD PDFs, $P[\text{MSD}(\tau)]$, were obtained by kernel density estimation (Parzen window) with a Gaussian kernel [46], using Silverman’s rule for bandwidth selection [47]. The statistical mode $M(\tau)$ was defined as the PDF maximum ridge at each τ , providing a robust reference for heterogeneous mobility distributions and underpinning the mobility-state definitions used throughout this work. For visualization, PDFs were assembled into a two-dimensional projected $\text{MSD}(\tau) - \tau$ landscape ordered by increasing τ [axes: τ and MSD; color: PDF amplitude; Fig. 2(c)]. Each PDF was normalized to unit area and concatenated along τ , with amplitudes capped at 1 in the heat map representation.

E. Stratification of chromatin granule dynamics

To control for cell-to-cell variability while preserving sensitivity to rare events, we use a two-step stratification scheme separating within-cell state definition from across-cell aggregation. Method 1 (within-cell stratification). Within each nucleus, loci are ranked by early-lag MSD and assigned to mobility quartiles ($Q_1 - Q_4$), defining relative mechanical mobility states independent of absolute mobility differences across cells. These quartiles are operational, within-cell mobility sectors rather than sharply separated microscopic states; the construction does not assume visible multimodal peaks at early lag times. Method 2 (population aggregation). Trajectories are then pooled across cells within each quartile label to construct population-level ensembles and estimate rare interquartile transitions (e.g., $Q_1 \rightarrow Q_4$ activation, $Q_4 \rightarrow Q_1$ reconfinement). This ordering prevents global thresholds from mixing between-cell variability (fast versus slow nuclei) with true within-cell state switching. Conditioned bridges are built from the per-cell transition fractions $f_{14}(Q_1 \rightarrow Q_4)$ and

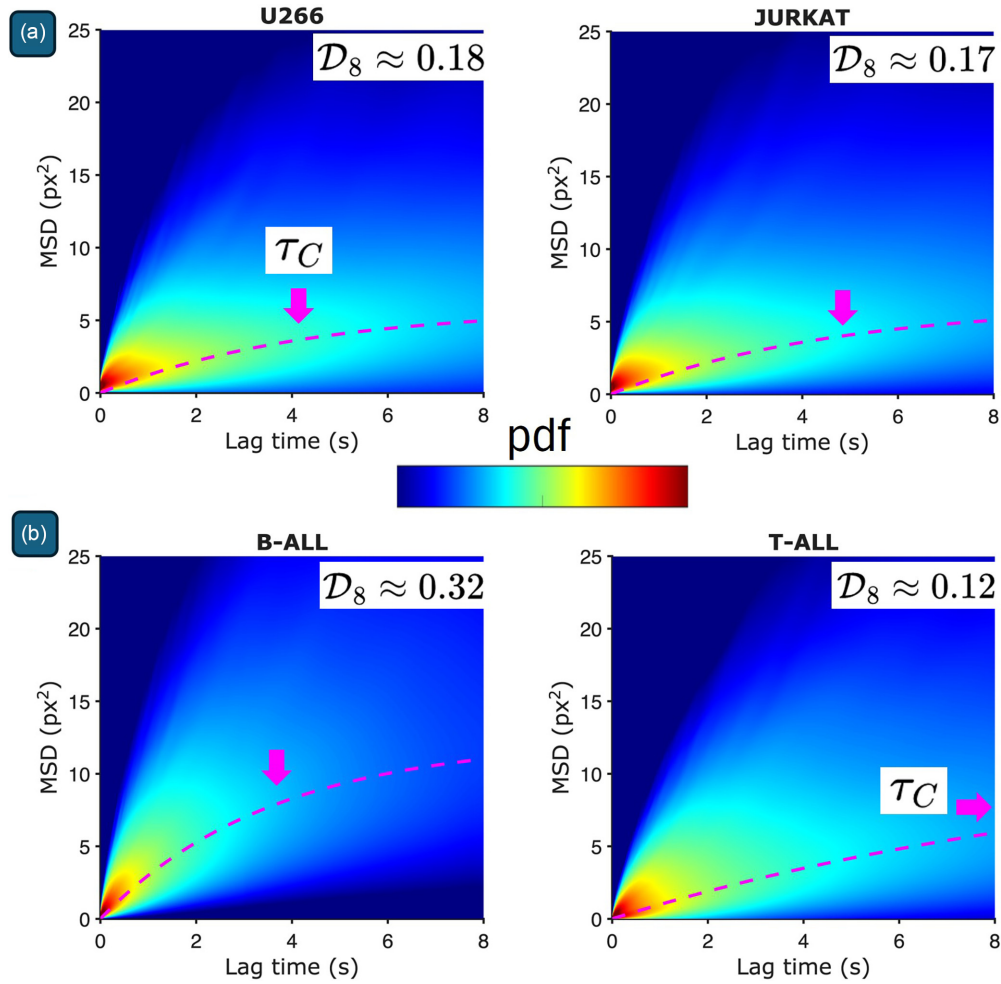


FIG. 3. Chromatin mobility landscapes in leukemic cell lines and primary ALL samples. (a) Time-resolved MSD probability density landscapes $P[\text{MSD}(\tau)]$ for U266 (B/plasma lineage) and Jurkat (T lineage). Relative to the nonleukemic benchmark (Fig. 2), both landscapes broaden and shift toward larger MSD values, indicating weaker confinement and increased mobility heterogeneity. (b) Primary B-ALL and T-ALL samples show stronger broadening, with B-ALL exhibiting the largest high-MSD weight at long lag times. In all panels, the dashed magenta curve is the best Ornstein-Uhlenbeck (OU) confinement fit [Eq. (3)] to the modal ridge, and the magenta arrow marks the crossover time τ_C . The boxed label reports the long-lag deconfinement index $D_8 \equiv \text{MSD}(8\text{ s})/r_C^2 - 1$, which quantifies the excess mobility above the OU confinement baseline at $\tau = 8\text{ s}$. Color scale: pdf (logarithmic, 10^{-2} to 1).

$f_{41}(Q_4 \rightarrow Q_1)$ and then aggregated across cells, so uncertainty in bridge parameters propagates from sampling error in these rare-event counts. Statistical significance and error bars were therefore estimated using a hierarchical bootstrap (cells as independent units), yielding 95% confidence intervals and two-sided p -values while accounting for intracell locus correlations and finite-sampling variability of f_{14} and f_{41} .

F. Time-resolved conditional MSD probability mapping: Brownian mobility bridges

To resolve directional switching between chromatin mobility states ($Q_1 \rightarrow Q_4$ activation; $Q_4 \rightarrow Q_1$ reconfinement), we constructed lag-time-resolved conditioned ensembles in MSD- τ space. Mobility states (Q_1 - Q_4) were defined within each cell from MSD quartiles (Method 1). A $Q_1 \rightarrow Q_4$ event was assigned when a trajectory belonged to Q_1 at the first ten lag points and Q_4 at the last ten; the reverse rule defined $Q_4 \rightarrow Q_1$ events. Using the first and last ten lag points stabilizes

the initial and final state assignments against local MSD fluctuations while retaining sensitivity to directional mobility switching. Event-conditioned trajectories were then pooled across cells (Method 2) to build population-level bridges. For each class we computed probability densities $P[\text{MSD}(\tau)]$ and represented them as mobility maps in (τ, MSD) space, from which the modal trajectory $M(\tau)$ —the most probable MSD at each τ —was extracted and fitted to classify regimes (diffusive, OU-like return, or superlinear/ballistic scaling). Bridge construction depends on the per-cell transition fractions f_{14} and f_{41} ; bridge shape (and parameters) propagate from these rare-event counts. Confidence intervals and significance tests were estimated across cells by hierarchical bootstrap (cells as independent units). We refer to these conditioned ensembles as *Brownian mobility bridges* [36], extending diffusion-bridge conditioning [48,49] to MSD- τ space. In our data, activation paths ($Q_1 \rightarrow Q_4$) show superlinear scaling [5], whereas reconfinement paths ($Q_4 \rightarrow Q_1$) follow OU-like relaxation [13], revealing directional asymmetry in chromatin mobility.

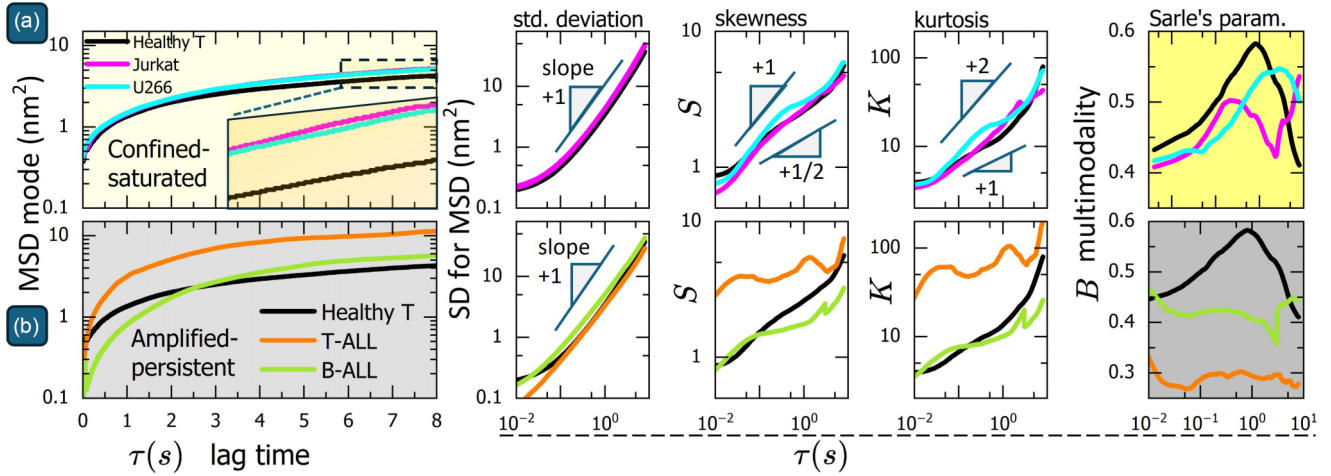


FIG. 4. Time-resolved higher-order statistics of chromatin mobility distributions. (a) Stabilized cell lines (Jurkat, U266) and Healthy T cells. Left: modal trajectory $M(\tau)$ extracted from the full MSD distribution $P[\text{MSD}(\tau)]$; inset, magnified long-lag regime. Middle panels: standard deviation $\text{SD}(\tau)$, skewness $S(\tau)$, and kurtosis $K(\tau)$ as functions of lag time. Right: Sarle's multimodality coefficient $B(\tau)$ [Eq. (2)]. (b) Primary leukemias (T-ALL and B-ALL) compared to Healthy T cells (black reference). Blue slope guides indicate empirical long-lag scaling references used to orient the eye and motivate the two-state framework (Supplemental Material Note N1 [43]): $\text{SD}(\tau) \sim \tau^{+1}$, $S(\tau) \sim \tau^{+1}$ to $\tau^{+1/2}$, and $K(\tau) \sim \tau^{+2}$ to τ^{+1} . These guides are visual benchmarks rather than fitted exponents. While the modal MSD exhibits smooth saturation consistent with effective confinement, higher-order moments reveal subtype-specific deviations from Gaussianity. In primary leukemias, $\text{SD}(\tau)$ retains an approximately linear envelope at long lags, whereas $S(\tau)$, $K(\tau)$, and $B(\tau)$ display oscillatory and attenuated scaling behavior, indicating redistribution of probability mass across mobility states and altered multimodal structure.

IV. RESULTS

A. Global MSD landscapes: Viscoelastic chromatin confinement in noncancerous nuclei

We begin by quantifying chromatin mobility at the single-cell level. For each nucleus, we track chromatin granules and compute the MSD trajectory of each observable locus (Method 1), yielding per-locus MSD traces. As a nonleukemic benchmark, Fig. 2 reports MSD trajectories for healthy donor peripheral T lymphocytes, providing a control reference for comparison with leukemic conditions.

In Fig. 2(a), representative single-particle MSD trajectories exhibit pronounced heterogeneity and predominantly subdiffusive scaling over several orders of magnitude in lag time τ . Figure 2(b) shows that, as τ increases, the distribution $P[\text{MSD}(\tau)]$ progressively broadens while remaining strongly weighted toward low mobilities, consistent with a spectrum of confinement strengths across loci within each healthy T-cell nucleus. Aggregating these cell-resolved MSD ensembles yields a robust nonmalignant baseline for chromatin confinement and heterogeneity (Method 2). In Fig. 2(c), the time-resolved MSD probability density landscape $P[\text{MSD}(\tau)]$ remains concentrated at small displacements even at long τ , indicating sustained confinement with limited large-exursion events. At the coarse-grained level, this confined dynamics is captured by an OU process, i.e., Brownian motion in an effective harmonic potential [39–41]. In two dimensions the OU model predicts

$$\text{MSD}(\tau) = 4D \tau_C (1 - e^{-\tau/\tau_C}), \quad (3)$$

where D is an effective diffusion coefficient (short-time mobility) and τ_C is the characteristic confinement time. The MSD saturates to the plateau $\text{MSD}_{\text{OU}}(\tau \gg \tau_C) = 4D \tau_C$, which sets the maximal area explored under harmonic confinement and

compactly summarizes nuclear viscoelastic restriction in this nonleukemic benchmark.

Henceforth, we use (D, τ_C) as baseline descriptors to compare leukemic conditions and test whether deviations from OU-like confinement arise from altered landscapes or directed nonequilibrium mobility transitions. In the OU interpretation, confinement emerges from a linear restoring force with relaxation rate $\gamma = k/\zeta = \tau_C^{-1}$ [39], where k is an effective stiffness and ζ a friction coefficient. Combined with the Stokes-Einstein relation $D = k_B T / \zeta$, the fitted pair (D, τ_C) yields $\zeta = k_B T / D$ and $k = \zeta / \tau_C$, linking chromatin mobility to viscoelastic properties of the nuclear microenvironment [31,50]. For noncancerous healthy T cells [Fig. 2(c)], the OU fit yields an effective diffusion coefficient $D = (8.0 \pm 0.05) \times 10^{-4} \mu\text{m}^2 \text{s}^{-1}$ and a confinement time $\tau_C = (3.58 \pm 0.03) \text{s}$. From the Stokes-Einstein relation $D = k_B T / \zeta$ (with $k_B T \simeq 4.1 \times 10^{-21} \text{J}$), we infer a hydrodynamic resistance $\zeta \simeq 5.1 (\mu\text{N s})/\text{m}$. The corresponding effective confinement stiffness is $k = \zeta / \tau_C \simeq 1.4 \mu\text{N}/\text{m}$. These values are consistent with measurements of chromatin microrheology [31,50]. Together, these results show that chromatin dynamics in nonmalignant nuclei are well described by a soft yet confined viscoelastic landscape.

To quantify departures from OU-like confinement, we define the deconfinement index

$$\mathcal{D}_\tau \equiv \frac{\text{MSD}(\tau)}{r_C^2} - 1, \quad r_C \equiv \sqrt{4D \tau_C}, \quad (4)$$

which measures the fractional excess of chromatin mobility relative to the OU plateau (confinement radius r_C). In healthy nuclei, $\mathcal{D}_\tau \rightarrow 0$ for $\tau \gg \tau_C$, providing a baseline to quantify leukemic deconfinement [Fig. 2(c)].

TABLE I. Ornstein-Uhlenbeck (OU) one-exponential fits of the modal MSD trajectories for each cell family [dashed curves in Figs. 2(c) and 3], using Eq. (3). D_0 is the short-time diffusivity and τ_C the confinement time. The associated confinement (correlation) length, $r_C \equiv \sqrt{4D_0\tau_C}$, corresponds to the square-root of the OU plateau MSD. $\mathcal{D}_8$ denotes the deconfinement index at $\tau = 8$ s, quantifying long-time departures from OU-like confinement. Modal fits are consistently high quality (RMSE ~ 0.13 – 0.17 ; $R^2 \sim 0.98$ – 0.997).

Family	D_0 (nm ² /s)	τ_C (s)	r_C (nm)	$\mathcal{D}_8$
Healthy T	798 ± 5	3.58 ± 0.03	75.6 ± 0.6	≈ 0
Jurkat	833 ± 3	4.67 ± 0.04	88.2 ± 0.5	0.17
U266	863 ± 5	4.25 ± 0.04	85.7 ± 0.7	0.18
T-ALL (LT)	647 ± 2	11.4 ± 0.1	121.4 ± 0.8	0.12
B-ALL (LB)	2145 ± 5	3.60 ± 0.01	124.2 ± 0.3	0.32

B. Leukemic chromatin landscapes: Subtype-specific mobility and deconfinement

We next examine how chromatin mobility landscapes are reshaped in leukemic conditions. In Fig. 3, time-resolved probability density landscapes $P[\text{MSD}(\tau)]$ show a systematic upward expansion of MSD contours relative to the healthy T-cell benchmark [Fig. 2(c)], indicating relaxation of confinement beyond the OU limit (Table I).

1. Leukemic cell lines. In both lines, MSD landscapes broaden and shift upward relative to the healthy benchmark, indicating weaker confinement and locus-to-locus variability. The modal MSD saturates at a higher level and more slowly, consistent with a confining environment that is mechanically softened at short times.

2. Primary patient samples. Primary ALL nuclei show a stronger departure from the tightly confined control landscape. B-ALL exhibits a pronounced high-MSD tail at long lag times, reflecting greater weight of highly mobile loci and a more fluidized dynamical ensemble. In contrast, T-ALL evolves more gradually, with slower saturation consistent with extended relaxation dynamics and prolonged persistence within confinement basins.

To quantify these trends, we fit the modal MSD trajectories to the OU model [Eq. (3)]; parameters are summarized in Table I. Both leukemic cell lines (U266 and Jurkat) remain close to the healthy baseline in diffusivity ($D_0 \simeq 800$ nm²/s, $\tau_C \simeq 4$ s, and $r_C \simeq 75$ nm) but show mildly increased confinement times along larger distances, consistent with slower saturation and broader mobility distributions. Primary samples deviate more strongly: B-ALL shows the largest increase in short-time mobility ($D_0 \simeq 2100$ nm²/s) while keeping a control-like confinement time ($\tau_C \simeq 3.6$ s), in a much larger space ($r_C \simeq 120$ nm), whereas T-ALL is dominated by a markedly prolonged confinement time ($\tau_C = 11.4$ s) in a similar space at moderate diffusivity ($D_0 \simeq 650$ nm²/s), indicating slowed relaxation rather than enhanced diffusivity. In short: leukemia does not simply “mobilize” chromatin—it rewires confinement.

To quantify long-lag departures from harmonic confinement, we evaluate the deconfinement index [Eq. (4)] at $\tau = 8$ s. Healthy T-cells give $\mathcal{D}_8 \approx 0$, while leukemic lines show

TABLE II. Mode-based viscoelastic estimates from Eq. (5) (Healthy T as reference).

Family	η_{eff} (Pa s)	G_{eff} (Pa)	η/η_H	G/G_H
Healthy T	≈ 20	≈ 5.7	1	1
Jurkat	≈ 20	≈ 4.2	≈ 1.00	≈ 0.74
U266	≈ 19	≈ 4.5	≈ 0.95	≈ 0.79
T-ALL (LT)	≈ 25	≈ 2.2	≈ 1.25	≈ 0.39
B-ALL (LB)	≈ 7.6	≈ 2.1	≈ 0.38	≈ 0.37

mild excess mobility (U266: $\mathcal{D}_8 \approx 0.18$; Jurkat: $\mathcal{D}_8 \approx 0.17$). Primary samples deviate more strongly: B-ALL shows the largest departure ($\mathcal{D}_8 \approx 0.32$), consistent with erosion of confinement that sustains high-mobility loci at long lags, whereas T-ALL remains closer to the plateau ($\mathcal{D}_8 \approx 0.12$). Overall, B-ALL is dominated by increased mobility, while T-ALL is shaped by slowed relaxation, reflecting distinct alterations of the confinement landscape relative to healthy nuclei. In sum, B-ALL erodes confinement; T-ALL prolongs relaxation.

C. Modal OU parameters map to effective viscoelastic scales

To connect MSD landscapes to material parameters, we interpret the modal MSD trajectory as an effective background described by confined Brownian motion in a harmonic environment [39]. The fitted OU pair (D , τ_C) maps to an effective friction ζ and stiffness k through $D = k_B T / \zeta$ and $\tau_C = \zeta / k$. Taking a characteristic confinement length ℓ on the order of the chromatin-granule size ($\ell \simeq 250$ nm) [9], we define

$$\eta_{\text{eff}} \equiv \frac{\zeta}{\ell} = \frac{k_B T}{D \ell}, \quad G_{\text{eff}} \equiv \frac{k}{\ell} = \frac{k_B T}{D \tau_C \ell}. \quad (5)$$

Accordingly, the OU parameters in Table II provide mode-based estimates of background viscoelasticity and do not describe the high-MSD tails. The Healthy T-cell benchmark lies within published ranges of nuclear viscoelasticity from chromatin tracking and microrheology [9,31,50]. Relative to this reference, cell lines show similar η_{eff} but reduced G_{eff} , whereas primary samples shift more strongly: B-ALL shows η_{eff} and G_{eff} reduced by $\sim 60\%$, while T-ALL shows approximately threefold higher η_{eff} than B-ALL with similarly reduced G_{eff} . The next subsections resolve mobility subpopulations and quantify conditioned transitions beyond this modal background.

Figure 4 summarizes the lag-dependent MSD landscapes via the mode $M(\tau)$ and standardized moments $\text{SD}(\tau)$, skewness $S(\tau)$, and kurtosis $K(\tau)$ computed from $P[\text{MSD}(\tau)]$. The mode $M(\tau)$ tracks the dominant confinement basin and follows OU-like growth saturated for $\tau \gtrsim \tau_C$ [Sec. B and C; Eq. (3)] [40], with Healthy cells and clonal lines saturating earlier. Deviations from the confined background are captured by SD – S – K , reporting distributional broadening, right-tail asymmetry, and tail weight, respectively.

D. Statistical dissection: Mode confinement, higher-order heterogeneity, and a two-state mobility structure

1. Cell lines. Figure 4(a) compares Healthy T cells with Jurkat and U266. Despite malignant origin, all three show the

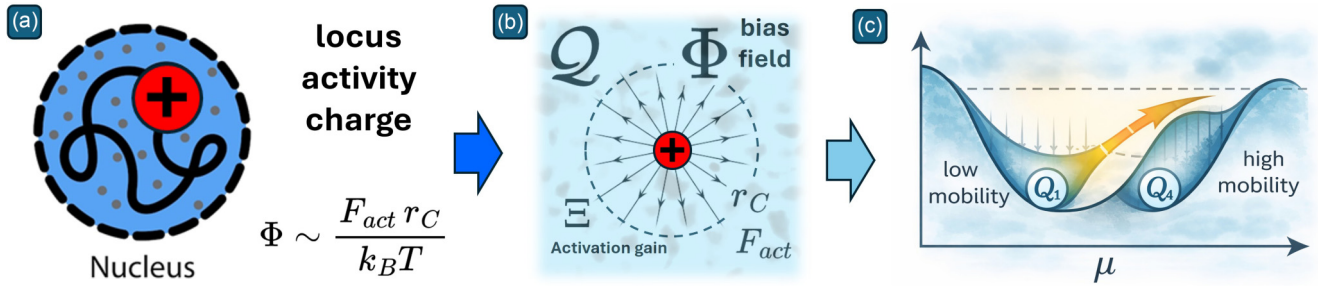


FIG. 5. Activity-source picture of nonequilibrium activation in the two-state mobility model. (a) A chromatin locus is modeled as a localized *activity source* (dimensionless “charge”) Q that sets the susceptibility of mobility switching. The resulting tilt enters through $Q\Phi$, where Φ is an effective bias (in thermal units). (b) The source induces a finite-range bias field over the confinement scale r_C , setting a characteristic tilt $\Phi \sim F_{\text{act}} r_C / k_B T$ with F_{act} an activation-force scale. The *activation gain* $\Xi = v^2 / 4D_{\text{rec}} \gamma \simeq (v \tau_C / r_C)^2$ summarizes how effectively short-time drive (speed v) is converted into a sustained excursion against reconfinement (rate γ and diffusivity D_{rec}). (c) Tilted double-well schematic for the mobility coordinate μ , with low- and high-mobility basins (operationally Q_1 and Q_4 , proxies for L and H). Here, μ is a coarse-grained scalar coordinate in MSD space. The mobility contrast $\Delta\mu$ separates confined and activated sectors; weighted by their occupancy, it contributes to the long-lag excess mobility summarized by $\mathcal{D}_8 = \text{MSD}(8 \text{ s}) / r_C^2 - 1$. Nonequilibrium activation biases $Q_1 \rightarrow Q_4$ relative to $Q_4 \rightarrow Q_1$, breaking detailed balance.

same core architecture: a confined modal trajectory together with comparable growth of $\text{SD}(\tau)$ and increasing $S(\tau)$ and $K(\tau)$ at long lags. Sarle’s bimodality coefficient indicates persistent multimodality across lag times in both control and leukemic lines, implying that heterogeneity is largely intrinsic in these stable clonal programs, with lines producing mainly quantitative shifts on a shared confined background.

2. Primary samples. Figure 4(b) compares primary B-ALL and T-ALL to Healthy T cells. Both elevate $M(\tau)$ and delay saturation, indicating higher typical mobility, with the largest shift in B-ALL. Higher moments separate subtypes: B-ALL shows a sustained long-lag right tail (persistently elevated S and K), consistent with a durable high-MSD sector, whereas T-ALL exhibits stronger short-to-intermediate-lag distortion but relaxes toward Healthy-T tail behavior at long lags, consistent with lag-selective excess mobility. This pattern is compatible with genomic burden (Supplemental Material Note S1 [43]), as recent single-cell genome analyses report a larger structural-alteration load in B-ALL than in T-ALL [51].

Finally, Sarle’s modality $B = (1 + S^2) / K$ supports subtype differences in state mixing: Healthy cells and clonal lines typically show an intermediate- τ rise followed by a long- τ decrease, consistent with mixture mixing and effective coarse-graining of the dominant class. B-ALL retains elevated $B(\tau)$ over a broad lag window, whereas T-ALL drops to lower $B(\tau)$ at long lags, consistent with a more unimodal late-time population.

E. Activity-charge model for intermittent mobility switching under nonequilibrium bias

Figure 5 casts locus activity as an effective *activity charge* Q that generates a bias field Φ in mobility space, with drive $\Phi \sim F_{\text{act}} r_C / k_B T$ [Figs. 5(a) and 5(b)]. This charge-field coupling biases switching between a confined sector L (typical OU-like fluctuations) and a rare activated sector H (high-mobility excursions) [Fig. 5(c)]. At each lag time τ , we model the MSD distribution as a two-sector mixture (Supplemental

Material Note N1 [43]):

$$P(\text{MSD}; \tau) = (1 - p_H(\tau)) P_L(\text{MSD}; \tau) + p_H(\tau) P_H(\text{MSD}; \tau), \quad (6)$$

where $p_H(\tau)$ is the activated-sector occupancy and $\Delta(\tau) \equiv \mu_H(\tau) - \mu_L(\tau)$ the mobility contrast. In the separation-dominated regime,

$$\text{Var}(\tau) \simeq p_H(\tau)(1 - p_H(\tau)) \Delta(\tau)^2, \quad (7)$$

so SD reflects both contrast and occupancy, while S and K preferentially weight rare H -excursions. Intermittency corresponds to $p_H(\tau) \ll 1$ with large $\Delta(\tau)$, implying $\text{Var}(\tau) \simeq p_H(\tau) \Delta(\tau)^2$: sporadic entries into H control higher moments while the confined modal basin persists. To connect to the long-lag envelopes in Fig. 4, we use minimal effective scalings over windows where $\mu_L(\tau)$ varies slowly (Supplemental Material Note N1 [43]): $\Delta(\tau) \sim \tau^\alpha$ and $p_H(\tau) \sim \tau^{-\beta}$. In the intermittent ($p_H \ll 1$), separation-dominated limit this yields the explicit moment scalings

$$\text{SD}(\tau) \sim \tau^{\alpha-\beta/2}, \quad S(\tau) \sim \tau^{\beta/2}, \quad K(\tau) \sim \tau^\beta, \quad (8)$$

so the SD envelope mixes contrast growth (α) with activation rarity (β), while skewness and kurtosis isolate the occupancy exponent β .

1. Elastic activity charge, bias field, and nonequilibrium tilting. Biased switching is encoded through

$$p_H(\tau) = \frac{k_{LH}(\tau)}{k_{LH}(\tau) + k_{HL}(\tau)}, \quad \frac{k_{LH}(\tau)}{k_{HL}(\tau)} = \exp(Q\Phi(\tau)), \quad (9)$$

where k_{LH} and k_{HL} are $L \rightleftharpoons H$ switching rates and $\Phi(\tau)$ is a lag-dependent bias (Supplemental Material Note N2 [43]). Under intermittency ($p_H \ll 1$; $k_{LH} \ll k_{HL}$), $Q\Phi$ mainly biases the flux of rare $L \rightarrow H$ attempts rather than producing a large occupation of H . As summarized in Fig. 5, a localized activity source is coarse-grained into a dimensionless elastic charge Q that sets switching susceptibility to Φ , tilting the mobility landscape toward $L \rightarrow H$ over $H \rightarrow L$. In the passive (detailed-balance) limit $Q_0 = \frac{1}{2}$, and activity increases

TABLE III. Intermittent two-sector model: minimal mapping between quartiles and moment scaling. For a rare activated sector $p_H(\tau) \ll 1$ with $\Delta(\tau) \sim \tau^\alpha$ and $p_H(\tau) \sim \tau^{-\beta}$, the separation-dominated closure yields $SD(\tau) \sim \tau^{\alpha-\beta/2}$, $S(\tau) \sim \tau^{\beta/2}$, and $K(\tau) \sim \tau^\beta$ (Fig. 4). Quartiles operationalize L (Q_1) and H (Q_4) for bridge distillation (Fig. 6).

Quartile/Sector	Model cue	Moment cue (Fig. 4)	Bridge role
Q_1-L	Confined bulk (dominant mode)	Saturating $M(\tau)$; low tails (S, K low)	$Q_1 \rightarrow Q_4$ source
Q_2-Q_3 -mixed	Bulk mixture controls dispersion	$SD(\tau) \sim \tau^{\alpha-\beta/2}$; $B = \mathcal{O}(1)$	Discarded
Q_4-H	Rare activated tail (p_H) with large Δ	Tail growth: $S(\tau) \sim \tau^{\beta/2}$, $K(\tau) \sim \tau^\beta$	$Q_4 \rightarrow Q_1$ sink

susceptibility via (Supplemental Material Note N2 [43])

$$\mathcal{Q} \equiv \mathcal{Q}_0 + \Xi = \frac{1}{2} + \Xi, \quad (10)$$

where $\Xi \geq 0$ is the activity-induced gain, yielding a high-gain/low-occupancy regime.

Within this two-state representation the ensemble mobility can be written as $MSD(\tau) \simeq \mu_L(\tau) + p_H(\tau) \Delta\mu(\tau)$, where $\Delta\mu = \mu_H - \mu_L$ is the mobility contrast and p_H the occupancy of the activated sector [Fig. 5(c)]. The long-lag deconfinement index introduced above therefore reflects this population-weighted contrast, $D_8 = MSD(8\text{ s})/r_C^2 - 1$. Accordingly, larger D_8 values imply a larger effective separation between confined and activated mobility sectors, whether through an increase in the sector contrast $\Delta\mu$, an increase in activated-state occupancy p_H , or both. In this sense, D_8 provides a macroscopic long-lag readout of the microscopic nonequilibrium mobility contrast encoded by the two-state model. In the weak-bias limit ($Q\Phi \ll 1$), where p_H responds linearly to the activity charge, the excess mobility scales as $\delta MSD \propto \mathcal{Q} \Delta\mu$, such that D_8 reflects the combined effect of sector contrast and activity-controlled switching susceptibility.

F. Lineage-specific intermittency of high-mobility chromatin states

Guided by the activity-charge two-sector model (Fig. 5; Sec. IV E), we read the statistics in Fig. 4 through two lag-dependent ingredients: the mobility contrast $\Delta(\tau)$ and activated occupancy $p_H(\tau)$. In the separation-dominated intermittent regime ($p_H \ll 1$), effective scalings $\Delta(\tau) \sim \tau^\alpha$ and $p_H(\tau) \sim \tau^{-\beta}$ yield $SD(\tau) \sim \tau^{\alpha-\beta/2}$, while tail moments isolate rarity, $S(\tau) \sim \tau^{\beta/2}$ and $K(\tau) \sim \tau^\beta$ (Supplemental Material Note N1 [43]). Table III summarizes the corresponding sector-level interpretation ($Q_1 \simeq L$, $Q_4 \simeq H$) and the moment-to-switching map used below to benchmark these inferences against conditioned transitions.

1. Stable benchmarks: Healthy and clonal lines. Before saturation, Healthy T cells and clonal lines (Jurkat, U266) share a common envelope [Fig. 4(a)]: $SD(\tau) \sim \tau$ ($\alpha - \beta/2 \simeq 1$), with steadily increasing $S(\tau)$ and $K(\tau)$ and $B(\tau) = \mathcal{O}(1)$. This defines an intermittent baseline with mixing: a dominant confined sector ($Q_1 - Q_3$) coexists with a sparse activated sector (Q_4) that enhances the right tail without collapsing multimodality, $B \geq B_0$.

2. Primary samples: enhanced tails with reduced mixing. Primary leukemias deviate systematically [Fig. 4(b)]. Both B-ALL and T-ALL show stronger tail amplification (elevated S and K), while Sarle's coefficient drops and remains suppressed—most strongly in T-ALL—indicating reduced

state mixing despite enhanced intermittency. In two-sector terms, H becomes more consequential while the distribution spends longer intervals dominated by a single effective class.

G. Functional segregation of mobility states by quartile-based distillation: Interquartile transitions

To operationalize the two-sector picture without absolute thresholds, we stratify each nucleus by MSD quartiles (Methods E), taking $Q_1 \simeq L$ (confined) and $Q_4 \simeq H$ (activated). We then distill *event-conditioned MSD bridges* for extreme transitions, $Q_1 \rightarrow Q_4$ (activation) and $Q_4 \rightarrow Q_1$ (reconfinement). Figure 6 illustrates the procedure in Healthy cells by constructing conditional MSD- τ probability maps and extracting the ridge. Activation bridges [Fig. 6(a)] show superlinear growth; we fit

$$MSD_{1 \rightarrow 4}(\tau) = 4D_{1 \rightarrow 4} \tau + v^2 \tau^2, \quad (11)$$

where $D_{1 \rightarrow 4}$ is the activation-channel diffusivity and v a propulsion speed. Reconfinement bridges [Fig. 6(b)] saturate and are fit by the OU-like form

$$MSD_{4 \rightarrow 1}(\tau) = \frac{4D_{4 \rightarrow 1}}{\gamma} (1 - e^{-\gamma\tau}), \quad (12)$$

with reconfinement diffusivity $D_{4 \rightarrow 1}$ and relaxation rate $\gamma = \tau_C^{-1}$ [Eq. (3)].

1. Event rarity. Extreme interquartile crossings are rare by construction, requiring switching between the lowest and highest mobility classes within the conditioning window ($Q_1 \leftrightarrow Q_4$), rather than remaining in the bulk quartiles ($Q_2 - Q_3$). Let N_{14} and N_{41} be the counts of activation ($Q_1 \rightarrow Q_4$) and reconfinement ($Q_4 \rightarrow Q_1$) events, and $f_{14} = N_{14}/N$ and $f_{41} = N_{41}/N$ the corresponding fractions among N loci. In Healthy T cells, crossings are subpercent [Fig. 6(c)]: $f_{14} = (0.51 \pm 0.04)\%$ and $f_{41} = (0.40 \pm 0.03)\%$. These low values ($f_{ij} \lesssim 1\%$) confirm that bridges isolate rare excursions rather than bulk fluctuations (Table III).

2. Two-scale rheology: Active elastic injection versus cage dissipation (Healthy reference). For Healthy T cells, the OU cage sets the viscoelastic screening scale $r_C^2 \simeq 4D_0\tau_C$ with $\tau_C = \gamma^{-1}$ (Table I), providing the background rheology ($\eta_{\text{eff}}, G_{\text{eff}}$) (Table II). Rare excursions are captured by a propulsion speed v persisting over τ_C , i.e., a run length $\lambda = v\tau_C = v/\gamma$. Using $v = 27.20 \pm 0.15$ nm/s and $\gamma = 0.917 \pm 0.008$ s $^{-1}$ (Table IV) gives $\lambda_H = 29.7 \pm 0.3$ nm, well below the cage size $r_C = 75.6 \pm 0.6$ nm: active injection is *subcage* and dissipates before spanning confinement. Traffic statistics show only a mild activation bias: $B = \ln(f_{14}/f_{41}) = 0.24 \pm 0.07$ (Table IV) implies an activation share among extreme crossings $p_H^{(\text{events})} = 1/(1 + e^{-B}) = 0.56 \pm 0.02$, while

TABLE IV. Event-conditioned bridge parameters. Activation ($Q_1 \rightarrow Q_4$): diffusive+propulsive fit ($D_{1 \rightarrow 4}, v$) [Eq. (11)]. Reconfinement ($Q_4 \rightarrow Q_1$): OU-like fit ($D_{4 \rightarrow 1}, \gamma$) with $\gamma \equiv \tau_C^{-1}$ [Eq. (3)]. All quantities are reported in physical units using 1 px = 50 nm. Mechanistic indices (dimensionless): traffic bias $B \equiv \ln(f_{14}/f_{41})$; elastic activity charge $Q \equiv \frac{1}{2} + \Xi$ with activation gain $\Xi \equiv v^2/(4D_{4 \rightarrow 1}\gamma)$; and normalized reconfinement rate $\Gamma \equiv \gamma/\gamma_0 = \gamma \tau_C^{(0)}$, where $\tau_C^{(0)}$ is the modal OU confinement time for the same family (Table I). Uncertainties are propagated from fit errors (and binomial counting for B).

Family	Activation $Q_1 \rightarrow Q_4$		Reconfinement $Q_4 \rightarrow Q_1$		Mechanistic (dimensionless)		
	$D_{1 \rightarrow 4}$ (nm ² /s)	v (nm/s)	$D_{4 \rightarrow 1}$ (nm ² /s)	γ (s ⁻¹)	$B = \ln\left(\frac{f_{14}}{f_{41}}\right)$	$Q = \frac{1}{2} + \frac{v^2}{4D_{4 \rightarrow 1}\gamma}$	$\Gamma = \gamma \tau_C^{(0)}$
Healthy T	1378 $\pm$ 13	27.20 $\pm$ 0.15	2195 $\pm$ 23	0.917 $\pm$ 0.008	0.24 $\pm$ 0.07	0.592 $\pm$ 0.002	3.28 $\pm$ 0.04
Jurkat	1585 $\pm$ 5	32.52 $\pm$ 0.05	2525 $\pm$ 25	0.769 $\pm$ 0.006	0.69 $\pm$ 0.08	0.636 $\pm$ 0.002	3.59 $\pm$ 0.04
T-ALL	723 $\pm$ 5	32.41 $\pm$ 0.04	2158 $\pm$ 18	0.575 $\pm$ 0.007	0.15 $\pm$ 0.09	0.712 $\pm$ 0.003	6.56 $\pm$ 0.10
U266	1510 $\pm$ 5	32.15 $\pm$ 0.05	2360 $\pm$ 18	0.833 $\pm$ 0.007	0.98 $\pm$ 0.09	0.631 $\pm$ 0.002	3.54 $\pm$ 0.05
B-ALL	2130 $\pm$ 8	35.40 $\pm$ 0.05	5575 $\pm$ 50	1.412 $\pm$ 0.002	0.55 $\pm$ 0.09	0.540 $\pm$ 0.001	5.08 $\pm$ 0.02

overall intermittency remains set by the subpercent event rates ($f_{ij} \lesssim 1\%$), i.e., a stable confined modal basin with sporadic, weakly biased entries into the high-mobility sector.

H. Event-conditioned MSD bridges reveal lineage-specific polarity in leukemic chromatin

Figure 7 applies the extreme-quartile conditioning to leukemic lines and primary samples (fit parameters in Table IV). Activation bridges ($Q_1 \rightarrow Q_4$; top row) remain sharply ridge-like across cohorts, with a narrow superlinear backbone, indicating that entry into H occurs through brief directed bursts rather than bulk drift. Lineages differ mainly in activation-channel loading: T-ALL shows a reduced diffusive component relative to Jurkat, consistent with a more friction-loaded activation gate, whereas B-ALL shows an enhanced diffusive component relative to U266, consistent with a more permissive activation channel. Reconfinement bridges ($Q_4 \rightarrow Q_1$; middle row) are lineage-sensitive. In clonal lines, return remains OU-like and channelized, with biased traffic ($f_{14} \neq f_{41}$). Primary leukemias erode this confinement channel: T-ALL broadens the return ridge and yields near-balanced traffic ($f_{14} \approx f_{41}$; n.s.), whereas B-ALL shows the strongest loss of ridge integrity (broad flume, slow saturation) while retaining imbalanced traffic. Thus, activation is conserved as a sharp injection process, while reconfinement degrades in a lineage-specific manner, reporting how confinement heterogeneity and return kinetics shape recovery from high-mobility excursions.

I. Quantitative bridge parametrization and mechanistic indices of nonequilibrium switching

The event-conditioned ridges in Figs. 6 and 7 yield a subtype-resolved parametrization of mobility switching within the activity-charge framework (Fig. 5; Sec. IV E). Activation bridges ($Q_1 \rightarrow Q_4$) are fit by the diffusive+propulsive form [Eq. (11)], giving ($D_{1 \rightarrow 4}, v$), while reconfinement bridges ($Q_4 \rightarrow Q_1$) are fit by an OU-like return [Eq. (12)], giving ($D_{4 \rightarrow 1}, \gamma$) with $\gamma = \tau_C^{-1}$. Best-fitting values and confidence intervals are listed in Table IV.

(1) Reduced indices from bridges and event fractions. We combine bridge parameters with the extreme-event fractions

(f_{14}, f_{41}) into

$$Q \equiv \frac{1}{2} + \Xi, \quad \Gamma \equiv \gamma \tau_C^{(0)}, \quad B \equiv \ln\left(\frac{f_{14}}{f_{41}}\right), \quad (13)$$

where Q is the elastic activity charge (via gain Ξ , Sec. IV E), Γ normalizes the return rate by the baseline confinement time $\tau_C^{(0)}$ (Table I), and B quantifies traffic asymmetry.

(2) Gain, charge, and retention. The gain compares drive injection to reconfinement retention (Supplemental Material Note N2 [43]),

$$\Xi \equiv \frac{v^2}{4D_{4 \rightarrow 1}\gamma}, \quad (14)$$

so $Q = \frac{1}{2} + \Xi$ measures activity-amplified switching susceptibility, while Γ reports return speed relative to the baseline OU cage.

(3) Reduced activation length. A complementary kinematic coordinate is

$$\Lambda \equiv \frac{\lambda_{1 \rightarrow 4}}{r_C^{(0)}}, \quad \lambda_{1 \rightarrow 4} \equiv \frac{v}{\gamma}, \quad (15)$$

so Λ measures activation persistence in units of the baseline cage size $r_C^{(0)}$ (Table I) and serves as the natural argument of the coarse-grained bias field $\Phi^*(\Lambda)$ (Supplemental Material Note N2 [43]).

(4) Irreversibility and channel asymmetry. Irreversibility is quantified by $B = \ln(f_{14}/f_{41})$. Bridge fits also define the channel diffusivity asymmetry

$$\mathcal{A}_D \equiv \frac{D_{4 \rightarrow 1}}{D_{1 \rightarrow 4}}, \quad (16)$$

with $\mathcal{A}_D > 1$ indicating a more diffusive reconfinement channel (Einstein-wise, lower effective friction) than activation. A minimal decomposition of traffic bias is then (Supplemental Material Note N4 [43])

$$B \simeq -\ln \mathcal{A}_D + Q \Phi^*(\Lambda), \quad (17)$$

separating intrinsic channel asymmetry from the residual nonequilibrium tilt on the bridge timescale.

Figure 8 condenses bridge dynamics into a reduced kinematic phase space spanned by the reduced activation length Λ and reduced reconfinement rate Γ (Table IV). By construction, $\Lambda = (v/\gamma)/r_C$ measures activation reach in units of the

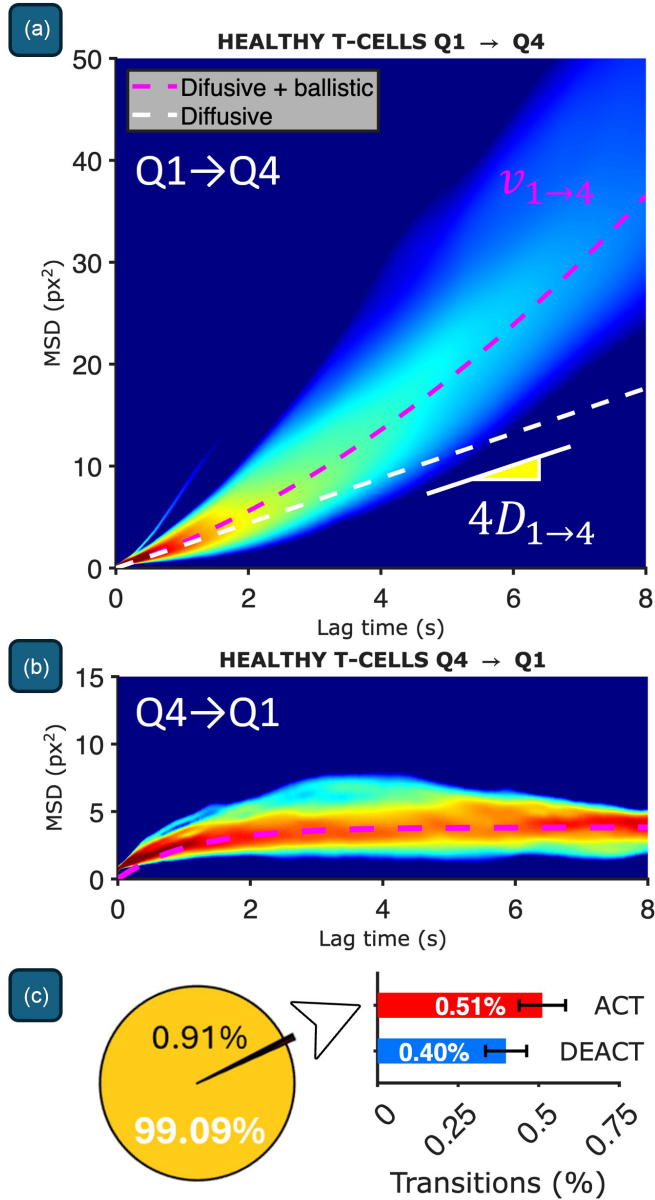


FIG. 6. Conditioned interquartile mobility bridges in healthy T-cell nuclei. (a) Activation bridge ($Q_1 \rightarrow Q_4$): conditional MSD probability map for loci starting in the lowest-mobility quartile and reaching the highest-mobility quartile within the conditioning window. The ridge is well captured by a diffusive+propulsive fit [magenta dashed, Eq. (11)]; the white dashed line indicates the purely diffusive guide. Color scale: normalized trajectory density. (b) Reconfinement bridge ($Q_4 \rightarrow Q_1$): conditional MSD map for loci starting in the highest-mobility quartile and returning to the lowest-mobility quartile. The ridge approaches a plateau and is consistent with mean-reverting OU relaxation [magenta dashed, Eq. (3)]. Color scale: normalized trajectory density. (c) Transition statistics and ensemble sizes. From $N = 98\,705$ loci tracked across $n \simeq 600$ healthy T-cell nuclei, extreme-quartile crossings occur in 0.91% of trajectories and define the conditioned ensembles: $N_{14} = 503$ activation events ($Q_1 \rightarrow Q_4$) and $N_{41} = 395$ reconfinement events ($Q_4 \rightarrow Q_1$) (bars: mean $\pm$ SEM).

intrinsic cage size, while $\Gamma = \gamma \tau_C$ measures return speed in units of the baseline relaxation time; lineage differences can therefore be read independently of absolute scales.

In Figure 8(a), healthy cells and stabilized lines (Jurkat, U266) fall on an approximately linear manifold in (Λ, Γ) , consistent with near-proportional coupling between activation reach and conditioned return. Primary leukemias break this scaling: T-ALL occupies a high- Λ , high- Γ sector (cage-spanning excursions with rapid return), whereas B-ALL shifts to lower Λ at elevated Γ (limited reach despite strong reconfinement). Thus, primary disease states reorganize the activation-reconfinement coupling rather than simply rescaling it.

Figure 8(b) reparametrizes the same fits by two orthogonal nonequilibrium controls: activation gain Ξ and channel diffusivity asymmetry $\mathcal{A}_D$. Healthy cells and lines cluster at low gain and moderate asymmetry, while primary leukemias separate primarily along $\mathcal{A}_D$, identifying channel asymmetry as the dominant discriminator of switching topology. Because $\mathcal{Q} = \frac{1}{2} + \Xi$, T-ALL sits in a high-charge (high-gain) sector, whereas B-ALL remains near the passive baseline charge despite pronounced asymmetry; the archetypes therefore differ mainly in amplification of intermittent drive versus retention/asymmetry, not in propulsion speed.

To anchor the superlinear excursions energetically, we estimate the dissipative force sustaining activation. In an overdamped medium, $F_{\text{act}} = \zeta v$ and, using $\zeta = k_B T / D_{1 \rightarrow 4}$,

$$F_{\text{act}} \simeq \frac{k_B T}{D_{1 \rightarrow 4}} v. \quad (18)$$

Forces are sub-pN [Fig. 8(c)], consistent with single-locus nuclear force scales [17]. Since $F_{\text{act}} \propto v / D_{1 \rightarrow 4}$, ordering is set mainly by activation-channel diffusivity: B-ALL combines fast propulsion with high $D_{1 \rightarrow 4}$ (lower effective friction), whereas T-ALL operates at lower $D_{1 \rightarrow 4}$, implying higher effective friction and larger force demand at comparable v , consistent with increased nucleoplasmic friction in T-ALL (Table II). Notably, the condition with the largest inferred force (T-ALL) also exhibits the largest $\mathcal{Q}$ via elevated Ξ , linking higher switching susceptibility to higher dissipative cost.

V. DISCUSSION

A biological reading of the gain-contrast phase space is that chromatin mobility intermittency reports *how* the leukemic genome reorganizes: through localized regulatory-circuit activation bursts versus broader structural remodeling driven by compartment-scale reconfiguration [2,22] and copy-number variation [23,28]. In T-lineage ALL, oncogenic programs centered on NOTCH1 frequently engage MYC-driven transcriptional networks [52–54]. By contrast, B-lineage differentiation and transformation involve extensive 3D genome reorganization, including compartment switching and immunoglobulin-locus nuclear architecture [55–57]. In B-ALL, this architectural layer is often amplified by recurrent copy-number variation across B-cell developmental genes [58–60].

The two pediatric ALL samples analyzed here therefore serve as lineage-informed mechanistic archetypes rather than cohort representatives (Supplemental Material Note S1 [43]). One case was T-ALL and one B-ALL; both were intermediate-risk and achieved durable remission. This

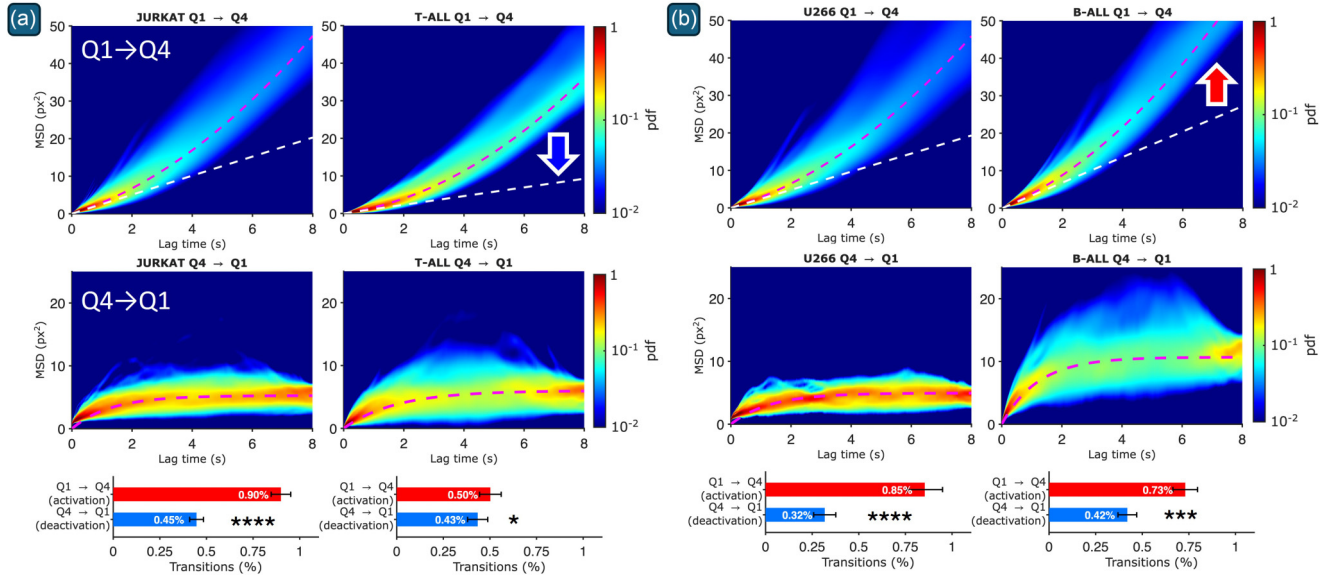


FIG. 7. Conditioned MSD bridges for activation and reconfinement in leukemia lineages. Conditional probability-density maps in the MSD-lag-time plane for interquartile transitions between the lowest-mobility state (Q_1) and the highest-mobility state (Q_4) across cell families [columns: (a) Jurkat and primary T-ALL; (b) U266 and primary B-ALL]. Sample sizes per condition: Jurkat ($n = 410$ nuclei; $N = 61\,500$ loci), U266 ($n = 460$; $N = 55\,200$), T-ALL ($n = 530$; $N = 47\,700$), and B-ALL ($n = 512$; $N = 49\,500$). Top row: activation bridges ($Q_1 \rightarrow Q_4$). Dashed magenta curves show the best diffusive+propulsive fit [Eq. (11)]. The white dashed line indicates the purely diffusive reference $MSD_0 \approx 4D_{14}\tau$. Middle row: reconfinement bridges ($Q_4 \rightarrow Q_1$). Dashed magenta curves show the best OU-like reconfinement fit [Eq. (3)]. Bottom row: transition frequencies for activation ($Q_1 \rightarrow Q_4$, red) and reconfinement ($Q_4 \rightarrow Q_1$, blue), reported as percentage of all trajectories for each condition (mean $\pm$ SEM). Statistical significance was estimated using a two-sided Student's t -test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

shared clinical course reduces the likelihood that the observed separation reflects relapse-associated evolution and supports a diagnostic interpretation in which T-ALL reflects localized regulatory-circuit stress with comparatively fragmented structural remodeling, whereas B-ALL reflects broader architecture- and CNV-driven reorganization (Supplemental Material Note S1 [43]).

To place these archetypes against stabilized lineage backgrounds, each primary sample was paired with a lineage-consistent model providing a chromatin baseline. Jurkat, derived from pediatric T-ALL, retains core T-cell regulatory circuitry and remains a standard system for TCR-linked signaling and chromatin regulation [61,62]. U266, an IgE-secreting plasma-cell/myeloma line, provides a stable B-lineage epigenetic context where immunoglobulin programs and compartment-scale organization dominate [63]. Although these lines do not reproduce the full mutational spectrum of primary pediatric ALL, they bracket primary cases in gain-contrast space and support a lineage-conditioned interpretation of nuclear remodeling. Within this framework, Jurkat clustering with the T-ALL primary sample along an activation-dominated sector (high gain, modest diffusivity-limited force) is consistent with NOTCH1/MYC circuitry increasing the probability of transient activation bursts and biasing entry into rare high-mobility excursions [52,53]. Conversely, U266 aligning with the B-ALL case along a contrast-amplified regime is consistent with broader architectural remodeling—compartment reorganization, immunoglobulin-locus organization, and CNV burden—enhancing mobility-state separation and deconfinement

without maximal activation gain [56,59]. This pairing does not imply molecular identity but provides a scaffold where stabilized lineage baselines help map genome-scale remodeling modes onto the nonequilibrium mechanical coordinates extracted from mobility-conditioned Brownian bridges.

A. From intermittent mobility landscapes to mechanistic control planes

Our central object is a *conditioned mobility bridge* in MSD-lag space: an event-aligned ensemble of MSD trajectories conditioned on extreme mobility-state crossings (e.g., $Q_1 \rightarrow Q_4$ activation, $Q_4 \rightarrow Q_1$ reconfinement). In this restricted sense, “Brownian bridges” denote empirical, event-restricted pathways in (MSD, τ) space rather than Doob-conditioned coordinate-space diffusions, with classical bridge theory providing the conditioning template [36–38]. Because lag time τ sets the interrogation scale, the landscape $P[\text{MSD}(\tau)]$ resolves mobility sectors and their NESS switching structure beyond mean-MSD descriptions. Our Results support a two-sector picture in which a dominant confined basin (L), captured by the OU-like modal background, coexists with a rare activated sector (H) that drives the right tail and higher moments. Extreme-quartile bridges isolate these excursions and parametrize activation and reconfinement kinetics. Using bridge parameters together with the rheological baselines inferred from the OU mode (Table II), we reorganize the Results into mechanistic control planes (Fig. 9) and thermodynamic control planes (Fig. 10) that con-

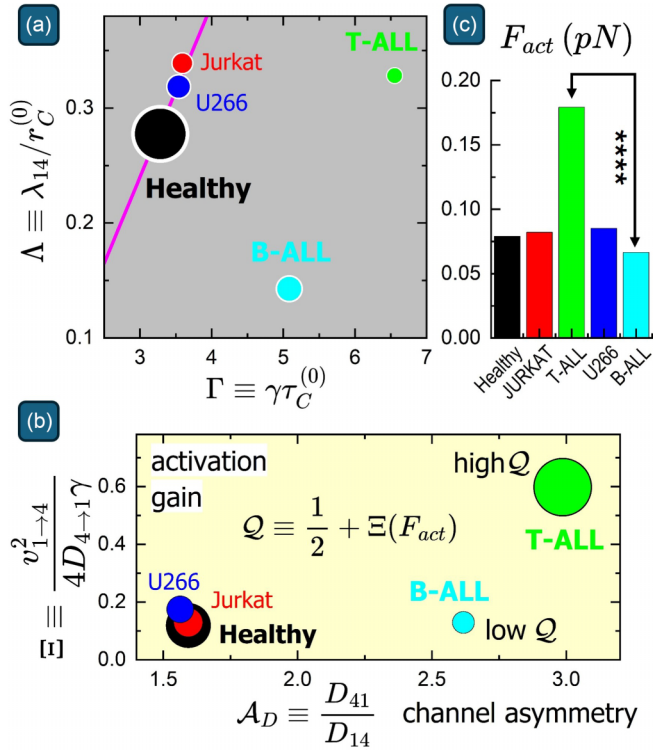


FIG. 8. Mechanistic planes for nonequilibrium mobility switching. Points are computed from bridge-fit parameters and event fractions (Table IV). Shaded circles around each point denote propagated multiplicative uncertainty of the derived coordinates; circle radii represent the combined fractional error of the contributing parameters (v , D , γ) and read as uncertainty envelopes. (A) Reduced bridge kinematics. Reduced propulsion length $\Lambda \equiv \lambda_{14}/\tau_C^{(0)}$, with $\lambda_{14} = v_{1 \rightarrow 4}/\gamma$, plotted against reduced reconfinement rate $\Gamma \equiv \gamma \tau_C^{(0)}$. Here $\tau_C^{(0)}$ is the modal OU caging length (Table I) and γ is obtained from $Q_4 \rightarrow Q_1$ bridges. (b) Orthogonal nonequilibrium controls. Activation gain $\Xi \equiv v_{1 \rightarrow 4}^2/(4D_{4 \rightarrow 1}\gamma)$ versus channel asymmetry $A_D \equiv D_{41}/D_{14}$ (Table IV). Gain sets the activity-induced charge $Q = \frac{1}{2} + \Xi$. (c) Dissipative force sustaining activation excursions (Fig. 7). $F_{act} \simeq (k_B T/D_{1 \rightarrow 4}) v_{1 \rightarrow 4}$ [Eq. (18)]. Bars: mean $\pm$ 95% CI (hierarchical bootstrap; Methods E).

dense activation, retention, and dissipation into a small set of nonequilibrium coordinates. This directly resolves the central distinction posed in the Introduction. The unconditioned MSD landscapes and OU modal fits show that leukemia reshapes the effective confinement background, producing softened, delayed, or partially deconfined mobility landscapes. However, these background descriptors do not encode temporal ordering. The event-conditioned Brownian bridges reveal the second layer: directed nonequilibrium transitions between mobility sectors, with superlinear activation and OU-like reconfinement following different pathways. Thus, leukemic chromatin dynamics are not described by altered confinement alone, but by confinement remodeling plus directional rare-event switching superimposed on that landscape.

B. Mechanical control: Rheology as regulator of activation gain

Figure 9 links bridge-derived nonequilibrium parameters to nuclear rheology across two coarse-grained scales: a short-time injection scale, governed by the activation chan-

nel (v_{14} , D_{14}), and a long-time dissipation/retention scale, governed by reconfinement (γ , r_C) and the OU background [Fig. 9(d)]. In this framework, the elastic activity charge $Q \equiv \frac{1}{2} + \Xi$ quantifies amplification, with

$$\Xi \equiv \frac{v_{14}^2}{4D_{41}\gamma},$$

whereas return efficiency is captured by the reduced reconfinement index

$$\Gamma \equiv \gamma \tau_C^{(0)}.$$

Because Ξ depends on injection relative to retention, while Γ depends only on the conditioned return rate relative to the OU baseline, gain and return are mechanically nonredundant. This separation is reflected in Fig. 9(a), where larger OU-inferred viscoelastic stiffness G/G_H is associated with lower Q , consistent with stronger elastic reconfinement suppressing activation gain. The same reduction clarifies the role of friction. The bridge-inferred activation-force scale,

$$F_{act} \simeq \frac{k_B T}{D_{14}} v_{14},$$

increases when the activation channel is more friction-loaded (smaller D_{14}) and/or propulsion is faster (larger v_{14}). Figure 9(c) shows that higher elastic charge generally coincides with higher inferred force demand, defining a frictional-activation axis complementary to the elastic-reconfinement axis in Fig. 9(a). Primary T-ALL occupies the high-charge, high-force regime, consistent with activation under dissipative loading, whereas primary B-ALL lies in a lower-charge, lower-force regime, indicating a more permissive activation channel despite clear deconfinement events. Healthy cells and stabilized lines occupy intermediate positions. Together, these relations define a mechanical control structure for rare mobility bursts.

Elasticity, encoded by OU-inferred stiffness and reconfinement rate, primarily sets activation retention, whereas viscosity and channel diffusivity set the dissipative cost of reaching the activated state. Figure 9 thus recasts bridge parameters into a rheology-anchored space where lineage differences reflect distinct balances among injection, cage retention, and frictional dissipation. Because these contributions enter multiplicatively, energetic cost and directional polarity remain separable: large activation work does not imply strong irreversibility unless transition traffic is biased, whereas substantial bridge directionality can arise at moderate energetic burden if activation remains accessible. In this reduced description, the bias

$$B \equiv \ln(f_{14}/f_{41})$$

acts as a coarse-grained indicator of broken detailed balance: nonzero B signals biased transitions between confined (L) and activated (H) sectors, evidencing sustained probability currents in the reduced mobility space. This motivates the thermodynamic control planes discussed next.

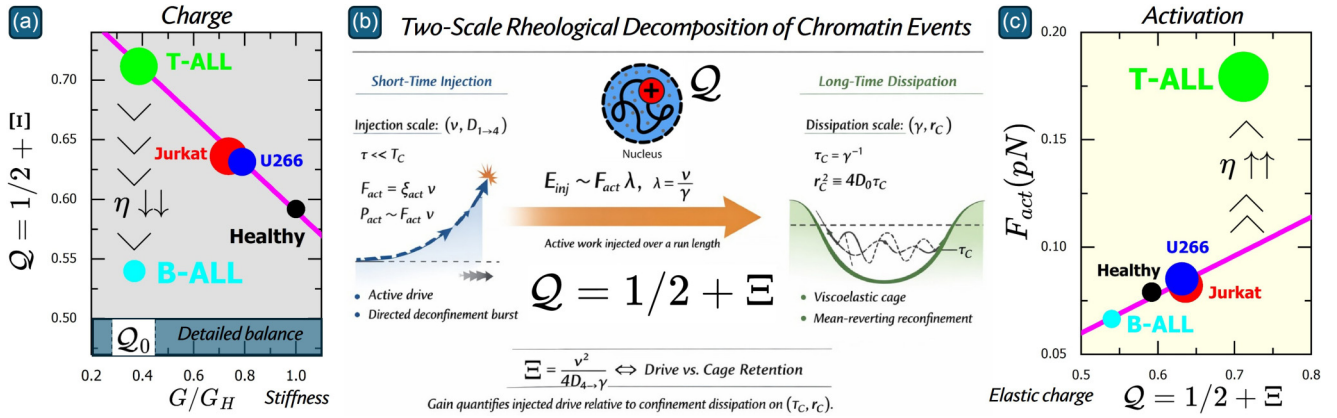


FIG. 9. Rheological synthesis of elastic charge, OU-inferred stiffness, and activation force across leukemia lineages. (a) Elastic charge versus OU-inferred viscoelastic stiffness. The activity-dependent elastic charge $Q \equiv \frac{1}{2} + \Xi$ (Table IV) is plotted against the normalized viscoelastic stiffness G/G_H , where G is inferred from the Ornstein-Uhlenbeck modal mapping of the confinement ridge, $G \simeq k_B T / (D_0 \tau_C l)$, and normalized by the healthy reference G_H (Tables I and II). Across families, stiffer nuclei tend to exhibit lower gain/charge, consistent with stronger elastic reconfinement limiting activation amplification. Conversely, under reduced viscous loading ($\eta \downarrow$), B-ALL shows the largest shift of Q toward the equilibrium limit $Q_0 = 1/2$ expected at detailed balance. (b) Two-scale rheological decomposition of chromatin events. Short-time activation reflects drive injection in the activation channel, parameterized by $(v_{1 \rightarrow 4}, D_{1 \rightarrow 4})$, with injected work over a run length $\lambda = v/\gamma$ scaling as $E_{inj} \sim F_{act} \lambda$. Long-time dissipation and retention are governed by the reconfinement scale (γ, r_C) , with $\tau_C = \gamma^{-1}$ and $r_C^2 \equiv 4D_0 \tau_C$. The activity gain $\Xi \equiv v_{1 \rightarrow 4}^2 / (4D_{4 \rightarrow 1} \gamma)$ quantifies injected drive relative to cage retention and sets the elastic activity charge $Q = 1/2 + \Xi$. (c) Frictional activation: activation force versus elastic charge. The effective activation-force scale $F_{act} \simeq (k_B T / D_{1 \rightarrow 4}) v_{1 \rightarrow 4}$ [Eq. (18)] is plotted against Q . Higher charge generally corresponds to higher inferred force demand. T-ALL occupies the high-charge, high-force regime, indicating the strongest active forcing against friction and the largest departure from the equilibrium limit $Q_0 = 1/2$ (equivalently $\Xi_{eq} = 0$). By contrast, B-ALL combines lower charge with a more weakly friction-loaded activation channel, while Jurkat and U266 lie in an intermediate regime near the healthy reference. Together, panels (a)–(c) show that lineage separation emerges from the interplay between OU-inferred viscoelastic confinement, activity-controlled gain, and frictional loading of the activation channel, rather than propulsion speed alone. Shaded circles indicate propagated multiplicative uncertainty of the plotted coordinates; radii correspond to the combined fractional error of the parameters entering each derived quantity (v, D, γ, G), added in quadrature.

C. Energetics versus irreversibility: Separating cost from directionality

Activation bursts admit a natural energetic normalization based on dissipation in the activation channel (Supplemental Material Note N4 [43]). Using $\zeta_{14} = k_B T / D_{14}$, we define the dimensionless drive-energy scale

$$\mathcal{E} \equiv \frac{\zeta_{14} v_{14}^2}{k_B T \gamma} = \frac{v_{14}^2}{D_{14} \gamma} = 4 \mathcal{A}_D \Xi, \quad (19)$$

where $\mathcal{A}_D \equiv D_{41} / D_{14}$ is the diffusivity asymmetry and $\Xi = v_{14}^2 / (4D_{41} \gamma)$ the gain index (Table IV). Thus, $\mathcal{E}$ compares injected drive with reconfinement dissipation and provides a channel-resolved measure of activation strength, independent of traffic statistics.

Figure 10 reorganizes the bridge-resolved kinetics into two complementary nonequilibrium coordinates. Figure 10(a) reports the activation-weighted energetic burden,

$$\mathcal{E}_{eff} = p_H \frac{F_{act} r_C}{k_B T},$$

which estimates the typical activation work, in thermal units, required to sustain deconfinement excursions. Since $F_{act} \simeq (k_B T / D_{14}) v_{14}$, this burden increases with frictional loading of the activation channel.

Figure 10(b) provides an internal closure of the two-state reduction: the bias-based estimate

$$p_H(B) = \frac{1}{1 + e^{-B}}, \quad B \equiv \ln(f_{14}/f_{41}),$$

agrees with the direct event estimator $p_H = f_{14}/(f_{14} + f_{41})$. We therefore use event-based p_H as primary and reserve the logistic form for analytic scaling (SN4).

Figures 10(c) and 10(d) isolate directionality through the coarse-grained entropy-production proxy

$$\frac{\dot{S}}{k_B} = \underbrace{\frac{v_{14}^2}{D_{14}}}_{\text{kinetic dissipation scale}} \times \underbrace{p_H}_{\text{sector accessibility}} \times \underbrace{B}_{\text{traffic bias}}, \quad (20)$$

which separates dissipation, accessibility, and directional asymmetry [40]. This is a coarse-grained proxy, not an exact entropy-production rate, and relies on a two-state Markov reduction, overdamped activation dynamics, and traffic bias as an indicator of broken detailed balance. Here B quantifies the imbalance between activation and reconfinement traffic; thus $B \neq 0$ signals biased transitions between confined (L) and activated (H) sectors and sustained probability currents in the reduced mobility landscape. Absolute values should therefore be read comparatively rather than thermodynamically. Because these coordinates are built from conditioned rare events, they are also model-dependent and sensitive to the analysis bandwidth and event-window definition (robustness is assessed in Supplemental Material Note N4 [43]).

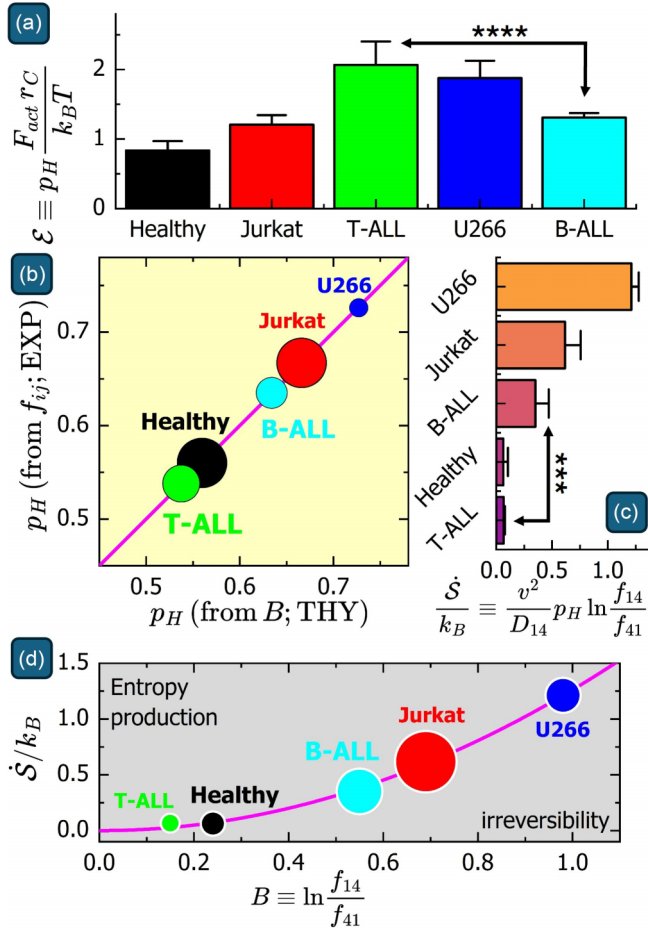


FIG. 10. Energetic and nonequilibrium metrics derived from the two-state activation model (Supplemental Material Note N3 [43]). (a) Activation-weighted energetic burden. Dissipative work in thermal units, $\varepsilon \equiv p_H (F_{act} r_C) / (k_B T)$, with $F_{act} \simeq (k_B T / D_{14}) v_{14}$ and r_C the OU confinement length (SN3.1). (b) Occupancy closure from traffic statistics. Activation probability p_H estimated directly from event fractions, $p_H = f_{14} / (f_{14} + f_{41})$, compared with the bias-based estimate inferred from $B \equiv \ln(f_{14} / f_{41})$ (SN3.4). The diagonal indicates equality. (c) Coarse-grained irreversibility proxy. Entropy-production-like coordinate, $\dot{S} / k_B \equiv (v_{14}^2 / D_{14}) p_H \ln(f_{14} / f_{41})$, combining activation dissipation with directional traffic asymmetry (SN3.2–N3.3). Bars show mean $\pm$ SEM; significance as indicated. (d) Entropy-production proxy vs traffic bias. $\dot{S} / k_B$ plotted against $B \equiv \ln(f_{14} / f_{41})$; the magenta curve is a quadratic guide to the data. Shaded circles indicate propagated multiplicative uncertainty of derived metrics.

Energetic cost and directional irreversibility are structurally separable [41]. Large activation work does not imply strong irreversibility unless traffic is biased ($B \neq 0$), whereas marked directionality can arise at moderate energetic burden if activation remains accessible. Panel D shows that $\dot{S} / k_B$ grows superlinearly with B . This curvature reflects coupling between bias and gain: at fixed activation-channel dissipation, $\varepsilon \propto 4A_D \Xi$, while the switching tilt gives $B \sim Q\Phi$ with $Q = \frac{1}{2} + \Xi$ (Supplemental Material Notes N2 and N4 [43]). Irreversibility thus increases with diffusivity asymmetry and charge amplification, reproducing the curved trend in Fig. 10(d) without added phenomenology. The same plane

separates two activation regimes. A diffusion-permissive channel (large D_{14}) lowers effective friction and suppresses ε even at strong contrast, whereas a resistive channel (small D_{14}) requires higher deterministic drive, increasing both ε and v_{14}^2 / D_{14} . The projection therefore distinguishes *channel softening* from *forced activation*. More generally, Fig. 10 defines a constrained nonequilibrium manifold in $(B, \dot{S})$: because B , p_H , and v_{14}^2 / D_{14} are jointly set by bridge-fit parameters and event statistics (Table IV), viable nuclear states populate a curved “lifeline” rather than the full phase plane.

Closure. Together, Fig. 9 and Table V define a hierarchical control structure for rare chromatin bursts. Figure 9 identifies the mechanical layer, showing that OU-inferred rheology sets activation gain and dissipative cost through the balance between retention and frictional loading, whereas Table V summarizes the thermodynamic layer, where traffic bias governs how that cost is directionally deployed between metastable mobility sectors. The two-state bridge reduction thus maps intermittent chromatin activation onto coupled mechanical, energetic, and irreversibility coordinates, showing that leukemia subtypes differ not only in drive magnitude but also in how that drive is organized along a constrained nonequilibrium manifold. Here the entropy-production proxy should be read as a coarse-grained irreversibility coordinate rather than an exact stochastic-thermodynamic estimator [64].

D. Clinical lineage phenotypes in the gain-retention-bias space

Taken together, Figs. 9 and 10 define a three-axis mechanical phenotype in which leukemic states are organized by (i) amplification gain $Q = \frac{1}{2} + \Xi$, (ii) reconfinement efficiency Γ , and (iii) directional traffic bias B . Importantly, entropy production depends not on energetic cost alone, but on the bias-weighted dissipation scale

$$\frac{\dot{S}}{k_B} = \left(\frac{v_{14}^2}{D_{14}} \right) p_H B$$

so that activation cost and irreversibility are structurally separable observables (Discussion II; SN4). The qualitative ordering of cases by irreversibility and entropy production is summarized in Table V.

This hierarchy helps resolve an apparent clinical tension. B-lineage ALL is frequently associated with a substantial burden of copy-number alterations and structural genomic rearrangements affecting B-cell developmental regulators and chromatin architecture [58–60]. By contrast, T-ALL, although not necessarily carrying the largest structural alteration load, has historically been associated with higher rates of early treatment resistance and severe relapse in several clinical series [65–67]. In the present study, the molecular profiles of the two primary cases are consistent with this lineage-specific distinction (Supplemental Material Note S1 [43]): the B-ALL genome exhibits broader copy-number-like remodeling, whereas the T-ALL case shows a more focal, regulatory-circuit-linked structural pattern. The mechanical phenotype separates these dimensions. Primary B-ALL loads predominantly a fluidization-bias axis: elevated activation-channel diffusivity and structural permissiveness increase accessibility to high-mobility states, producing moderate but sustained entropy generation. In contrast, primary T-ALL oc-

TABLE V. Hierarchy of irreversibility across chromatin mobility phenotypes. Cases are ranked qualitatively by the entropy-production proxy $\dot{S}/k_B$ (Fig. 10). Gain Ξ , retention Γ , and bias B are taken from Table IV.

Case	Gain Ξ	Bias B	$\dot{S}/k_B$	Regime classification
Healthy	Low gain, balanced retention	≈ 0	Minimal	Near-equilibrium switching
Primary T-ALL	High cost, moderate gain	Small / moderate	Low	Friction-loaded, weakly irreversible
Primary B-ALL	Low cost, permissive	Moderate	Intermediate	Structurally fluidized, moderately irreversible
Jurkat	Amplified gain and sustained activation accessibility	High	High	Strongly irreversible nonequilibrium
U266	Amplified gain + high bias	Highest	Maximal	Extremal nonequilibrium phenotype

copies a friction-loaded drive regime: activation bursts are energetically costly due to reduced D_{14} , yet directional bias remains limited. As a result, entropy production remains comparatively low despite elevated activation force demand. In this sense, T-ALL behaves as a dynamically constrained but weakly polarized system, closer to near-equilibrium switching than its energetic cost alone would suggest.

The stabilized leukemic lines extend this hierarchy into nonequilibrium extremes. Jurkat, a canonical T-lineage leukemia model derived from pediatric T-ALL [62], displays amplified gain and persistent directional bias, consistent with sustained regulatory drive in T-ALL programs. In particular, NOTCH1-centered oncogenic circuitry engages MYC and enhancer control layers that promote high transcriptional throughput and chromatin remodeling [52,54]. U266, an IgE-secreting plasma-cell/myeloma line [63,68], exhibits the largest entropy-production proxy, reflecting both high activation accessibility and pronounced traffic asymmetry. Biologically, such extremal nonequilibrium behavior is consistent with stabilized *in vitro* states dominated by persistent biosynthetic and proteostasis fluxes: plasma-cell differentiation and myeloma maintenance rely on an expanded secretory apparatus and unfolded-protein-response (UPR) control (e.g., XBP1/IRE1 signaling), which impose sustained energy dissipation through chronic secretory load [69–71]. Together, these lineage-appropriate high-throughput programs provide plausible cellular correlates for elevated bias-weighted dissipation without invoking additional mechanical parameters [70]. Thus, the gain-retention-bias decomposition separates structural permissiveness from dynamically driven irreversibility. B-ALL emerges as structurally fluidized yet energetically economical, T-ALL as friction-loaded but weakly polarized, and stabilized lines as high-throughput nonequilibrium extremes. The resulting entropy hierarchy is not a scalar measure of instability, but a directional organization of activation work across metastable mobility basins.

E. Nonequilibrium phase-space displacement: Clinical scores and predictability

The present primary dataset (one case per lineage) is not designed for statistical stratification. However, the gain-retention-bias framework is intrinsically scalable because it compresses live-cell mobility trajectories into a small set of dimensionless, patient-level scores that can be tracked longitudinally and compared across cohorts. Each patient maps to a point in (Gain, Retention, Bias) space with an associated

entropy-production proxy, while Fig. 10 provides the corresponding nonequilibrium “lifeline”—the accessible manifold imposed by rheological and metabolic constraints. As n increases, cohorts can be stratified by (i) displacement along the lifeline (accessible dynamic range) and (ii) direction in score space, separating fluidization-dominant from drive/bias-dominant phenotypes within and across lineages.

1. Scorecard for dynamic stratification

For each patient (or sample), the model yields three primary coordinates and two derived projections:

- (1) Gain score (Ξ or Q): amplification of rare activation relative to cage retention (drive persistence).
- (2) Retention score (Γ): efficiency of reconfinement (return competence) relative to the background cage.
- (3) Bias score (B): directional asymmetry of extreme-state traffic (polarity of switching).
- (4) Cost score ($\mathcal{E}$): activation burden set by viscous gating of the activation channel (force–diffusion partition).
- (5) Irreversibility score ($\dot{S}$ proxy): bias-weighted dissipation integrating accessibility, dissipation scale, and traffic polarity (Discussion II; SN4).

Together these scores define a mechanical phenotype vector that can be reported per patient as a compact clinical signature (Table V) and used to map disease state, treatment phase, and risk group onto a common nonequilibrium phase portrait.

2. Rheology-to-score therapeutic predictions

The score structure implies specific, testable directions of motion under interventions:

- (1) Viscous gating predominantly moves the Cost score. Perturbations that alter nucleoplasmic viscosity are predicted to change activation-channel permissiveness (D_{14}) and therefore $\mathcal{E}$, shifting samples along a force–diffusion partition axis, with secondary effects on irreversibility only if accessibility and bias are concurrently altered.
- (2) Elastic retention predominantly moves the Retention and Gain scores. Perturbations that alter chromatin stiffness or confinement heterogeneity primarily reshape Γ and Ξ , shifting samples along a fluidization–retention axis, with bias changing only insofar as occupancy is reweighted.
- (3) Directional organization predominantly moves the Bias and Irreversibility scores. Because irreversibility requires net traffic polarity, interventions that reduce regulatory-circuit asymmetry are predicted to decrease B and the irreversibility score even when activation remains energetically costly.

3. From molecular route to mechanical score

Distinct molecular routes to leukemic remodeling (Supplemental Material Note S1 [43]) should map onto distinct score patterns: genome-wide copy-number/compartiment remodeling elevates accessibility and weakens retention (fluidization-dominant signatures), whereas regulatory-circuit rewiring enhances gain and/or bias (drive–polarity signatures). This provides a physically constrained bridge between static alteration load and dynamic nuclear function.

4. Clinical outlook

Applied to prospective cohorts spanning risk groups and treatment phases (diagnosis, remission, relapse), the framework predicts score trajectories rather than single-point biomarkers. Remission should correspond to contraction toward low bias and improved retention, whereas relapse may manifest as bias amplification and increased irreversibility even without maximal fluidization. Because the scores are extracted directly from live-cell trajectories, they provide a quantitative route toward dynamic stratification that complements genomic classification with a functional nonequilibrium layer.

Closure. Leukemic phenotypes are not defined solely by alteration load, but by how the nuclear medium redistributes amplification, retention, and directional flux. The gain-retention-bias scorecard therefore links molecular route (Supplemental Material Note S1 [43]), viscoelastic control, and outcome-relevant nonequilibrium behavior.

VI. CONCLUSION

We have shown that rare chromatin mobility bursts in leukemia can be reduced to a minimal two-state switching framework closed by event-conditioned bridges. Within this reduction, three measurable controls—activation gain (Ξ), reconfinement efficiency (Γ), and traffic bias (B)—are sufficient to organize heterogeneous MSD landscapes into a nonequilibrium mechanical phase space. This construction separates structural confinement erosion from dissipative regulatory drive and reveals that energetic burden and irreversibility are mechanistically orthogonal observables. In this sense, the data support a two-layer answer to the question posed in the Introduction: leukemia remodels the confinement landscape, but also promotes directed nonequilibrium mobility transitions that become visible only after event-conditioned bridge analysis.

Anchoring bridge-derived parameters to independent rheological measurements demonstrates that chromatin intermittency is not governed by propulsion magnitude alone, but by frictional gating and cage retention. Stiffness primarily suppresses amplification by accelerating return dynamics, whereas viscosity reweights the force–diffusion partition of activation. In this active rheology-controlled picture, leukemic phenotypes occupy distinct regions of gain-retention-bias space: B-ALL is consistent with structural fluidization and a permissive activation channel, whereas T-ALL is consistent with friction-loaded activation and elevated energetic burden despite more modest deconfinement. This separation provides a mechanistic route by which structural alteration load and dynamical drive can decouple, offering a physical explanation

for why extensive genomic remodeling need not imply maximal nonequilibrium polarity.

More broadly, the intermittent two-sector reduction places nuclear chromatin within a measurable nonequilibrium rheological framework. The activation charge, energetic burden, and entropy-production proxy introduced here are not phenomenological add-ons, but emerge directly from bridge-resolved kinematics and extreme-state traffic statistics. Because these quantities are extracted from live-cell mobility trajectories, they naturally define a compact scorecard for functional stratification of nuclear states that complements genomic classification.

Future work should connect these coarse-grained mechanical coordinates to molecular drivers of chromatin remodeling and transcriptional regulation (Supplemental Material Note S1 [43]), and test whether targeted perturbations of viscosity, elasticity, or active-force generation displace cells predictably within the gain-retention-bias phase portrait. Such integration may establish chromatin mobility not merely as a readout of nuclear organization, but as a quantitative nonequilibrium biomarker of regulatory state and disease progression.

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COMPUTATIONAL REPRODUCIBILITY

All numerical analyses were performed using Python (version 3.12) with NumPy (v2.1), SciPy (v1.14), and scikit-image (v0.24), together with Wolfram Mathematica (version 14.1) for symbolic derivations and nonlinear fits. Randomized procedures used for bootstrap uncertainty estimation employed fixed pseudorandom seeds to ensure reproducibility. Bootstrap confidence intervals reported throughout the manuscript were obtained using hierarchical resampling over cells and loci with 10^4 resamples, unless otherwise stated. All figure scripts reproduce the experimental results directly from the archived processed datasets.

DATA AVAILABILITY

The datasets generated and analyzed in this study include: (i) raw phase-contrast videomicroscopy recordings of chromatin granule dynamics, (ii) extracted chromatin trajectory

ries obtained from the SURF-based multiple-particle tracking pipeline, and (iii) derived statistical observables, including MSD ensembles, probability density landscapes $P[\text{MSD}(\tau)]$, and bridge-conditioned MSD datasets used for activation and reconfinement analysis. A curated dataset including repre-

sentative trajectories, MSD distributions, bridge-conditioned ensembles, and derived statistical arrays used to produce the figures is archived at Zenodo [72]. Additional processed trajectory data are available from the corresponding author upon reasonable request.

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