Title: Dynamic organization of transcription compartments is dependent on functional nuclear architecture

Author Affiliations:

Shovamayee Maharana[†‡], Divya Sharma [†] , Xianke Shi[‡], and G.V. Shivashankar[†‡§]

[†]National Centre for Biological Sciences, TIFR, Bellary Road, Bangalore-560065, India;

[‡] Mechanobiology Institute and Department of Biological Sciences, National University of Singapore, 117411 Singapore

[§]Corresponding author (email: shiva.gvs@gmail.com)

Supplementary Information

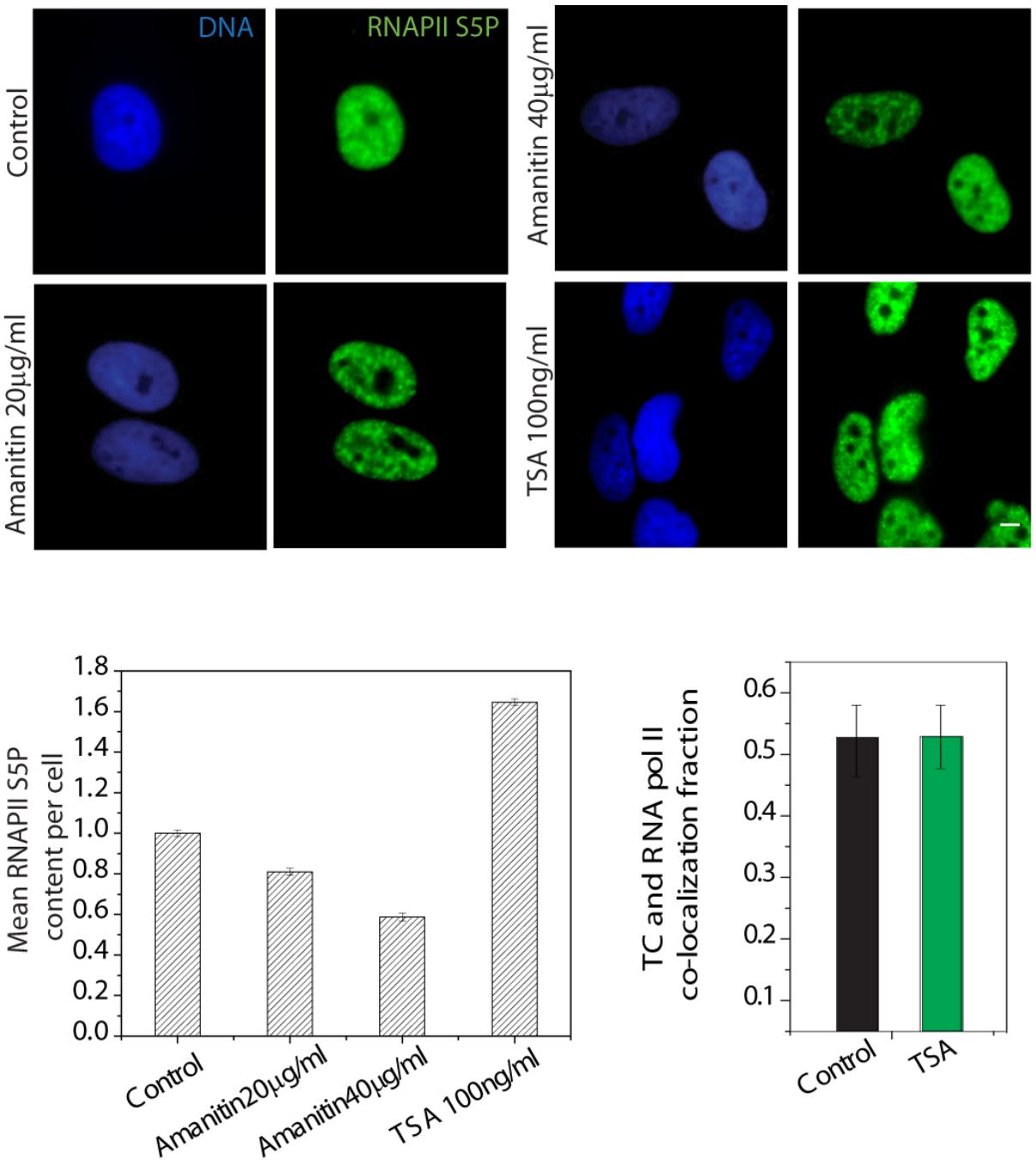

Figure S1. Quantification of RNAPII S5P in control (untreated), α-amanitin and TSA treated cells. The graph (left) shows decrease in amount of phosphorylated RNAPII S5 in widefield imaging of cells treated with 20µg/ml (1hr), 40µg/ml (1hrs) of α-amanitin and an increase in phosphorylated RNAPII S5 in case of TSA 100ng/ml (16hrs). The colocalization between TC and the phosphorylated RNA pol II remains similar between control and TSA treatment (right graph).

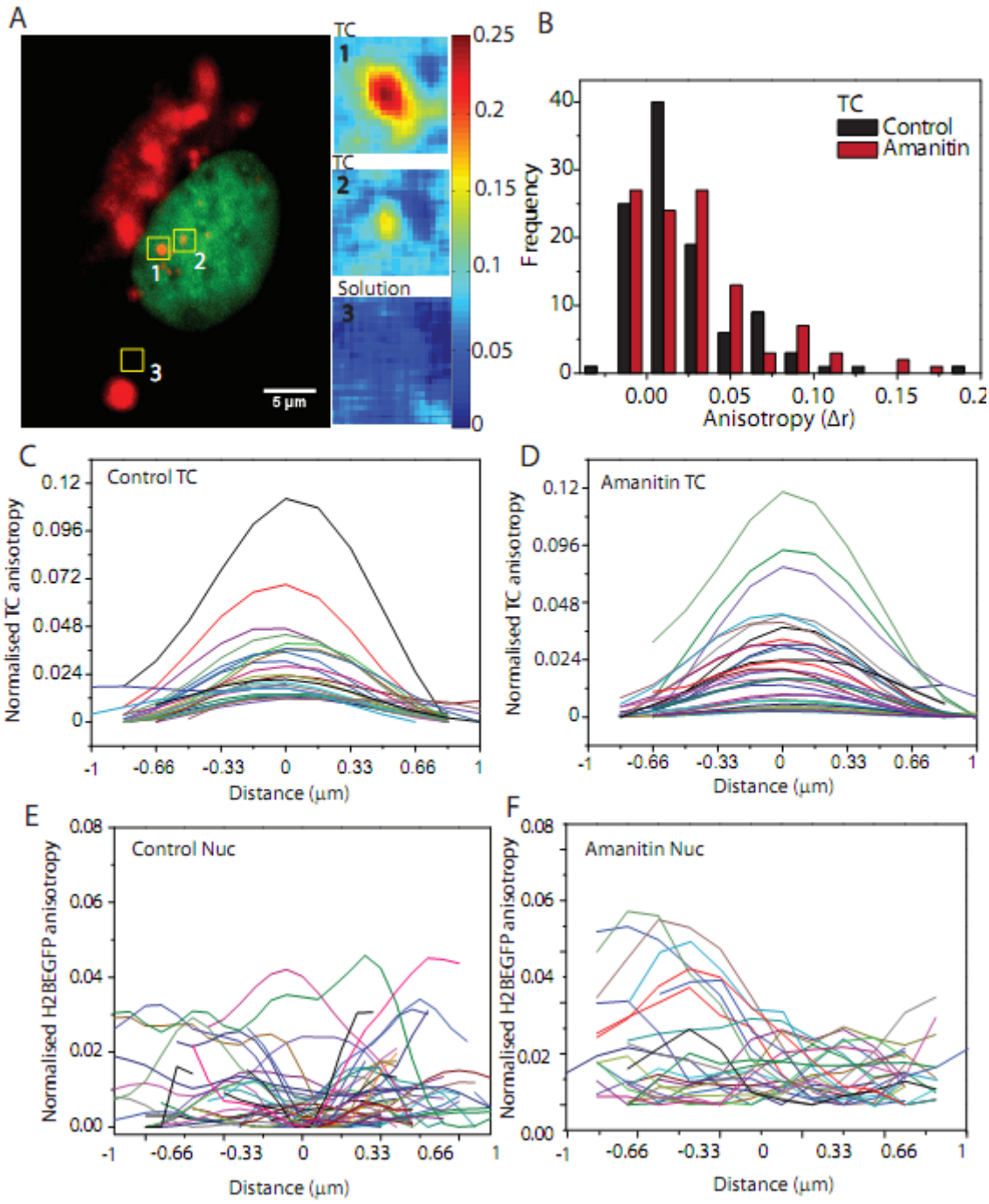

Figure S2. (A) Colour coded anisotropy profile for UTPs in solution and in TC. **(B)** Distribution of anisotropy values (Δr) for TC **(C** & **D)** Normalized (Minimum value was reduced to zero) anisotropy line profile of alexa546 UTP across multiple TCs in control and amanitin treatment respectively. The peak of the TC and H2BEGFP profiles was aligned at zero position (corresponding to centroid of TC). **(E** & **F)** Corresponding normalized (Minimum value was reduced to zero) anisotropy line profile of H2BEGFP across multiple TCs in control and α-amanitin treatment respectively.

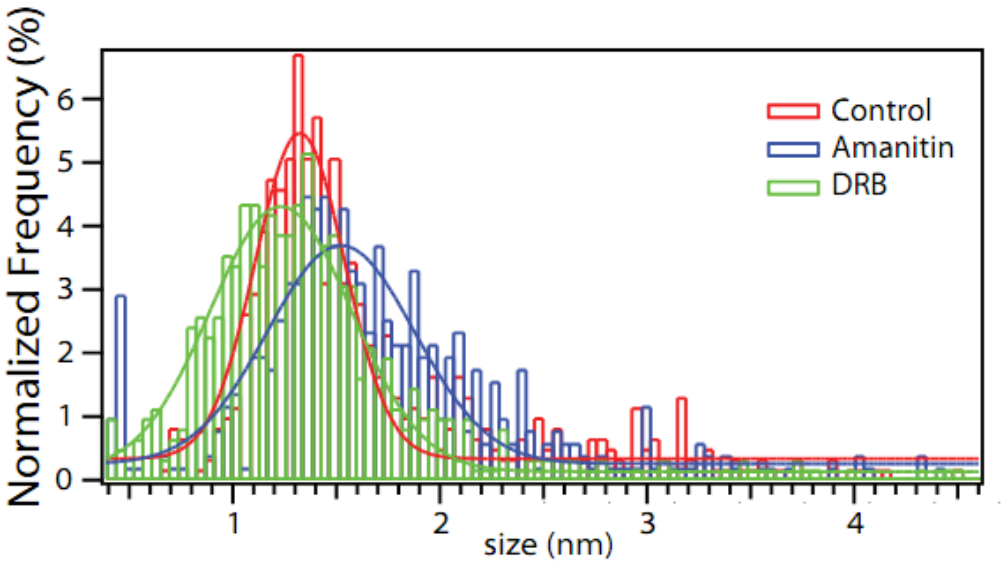

Figure S3. FCS reveals presence of sub-resolution UTP aggregates. Size distribution of sub resolution UTP aggregates measured by Fluorescence Correlation Microscopy with DRB which blocks transcription elongation leads to enrichment of smaller aggregates, whereas α-amanitin which blocks transcription initiation shows larger aggregates with a more heterogeneous distribution of sizes.

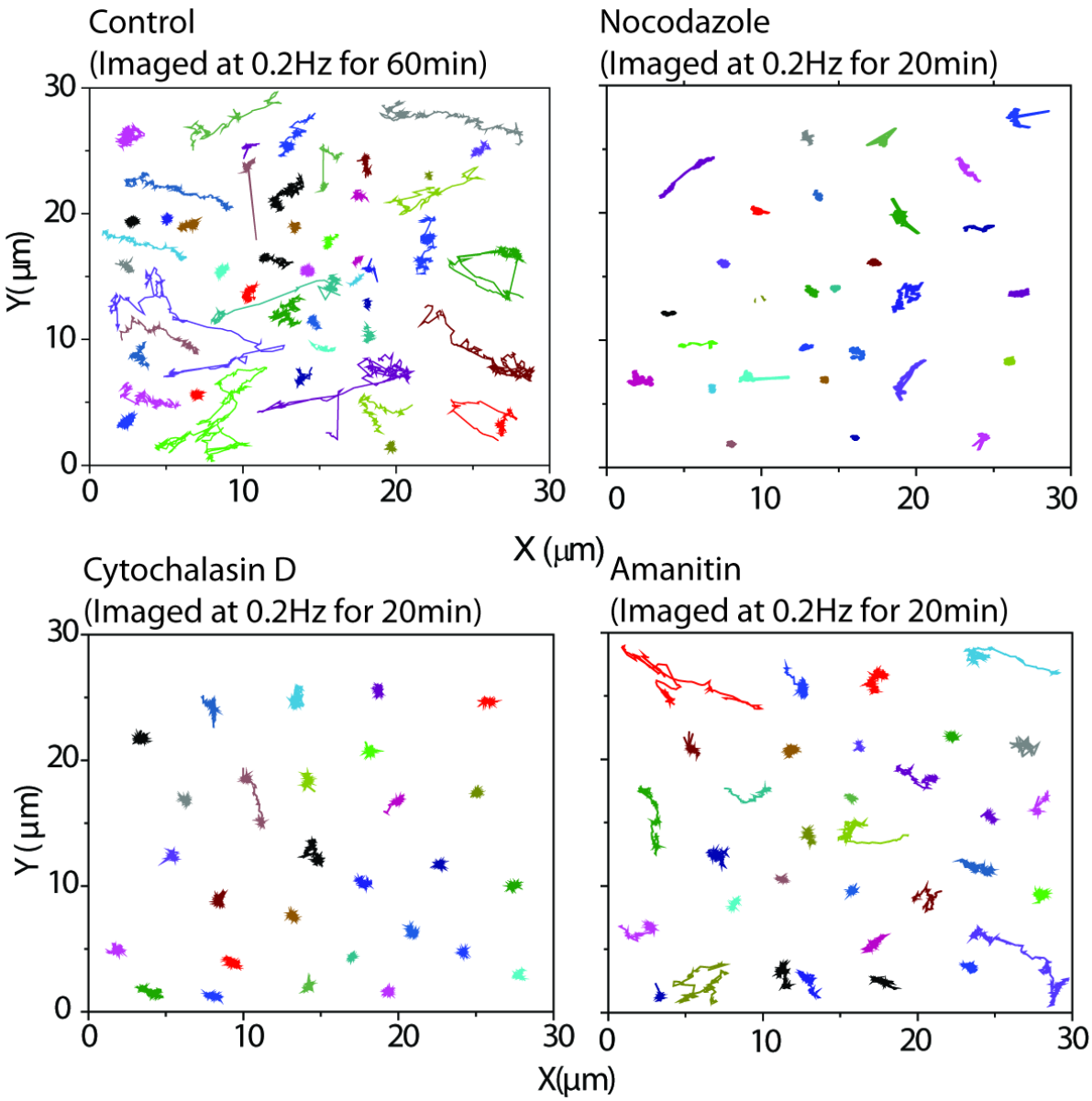

Figure S4 Slow tracking trajectories of TC with no treatment (control) or with treatment of Nocodazole, Cytochalasin D and α-amanitin.

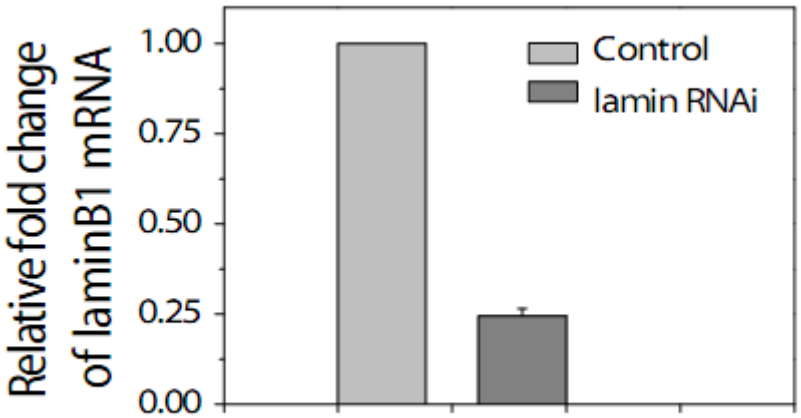

Figure S5 Relative mRNA concentration of laminB1 in RNAi treated cells as measured by Ct values obtained in Real time PCR using Sybr green.

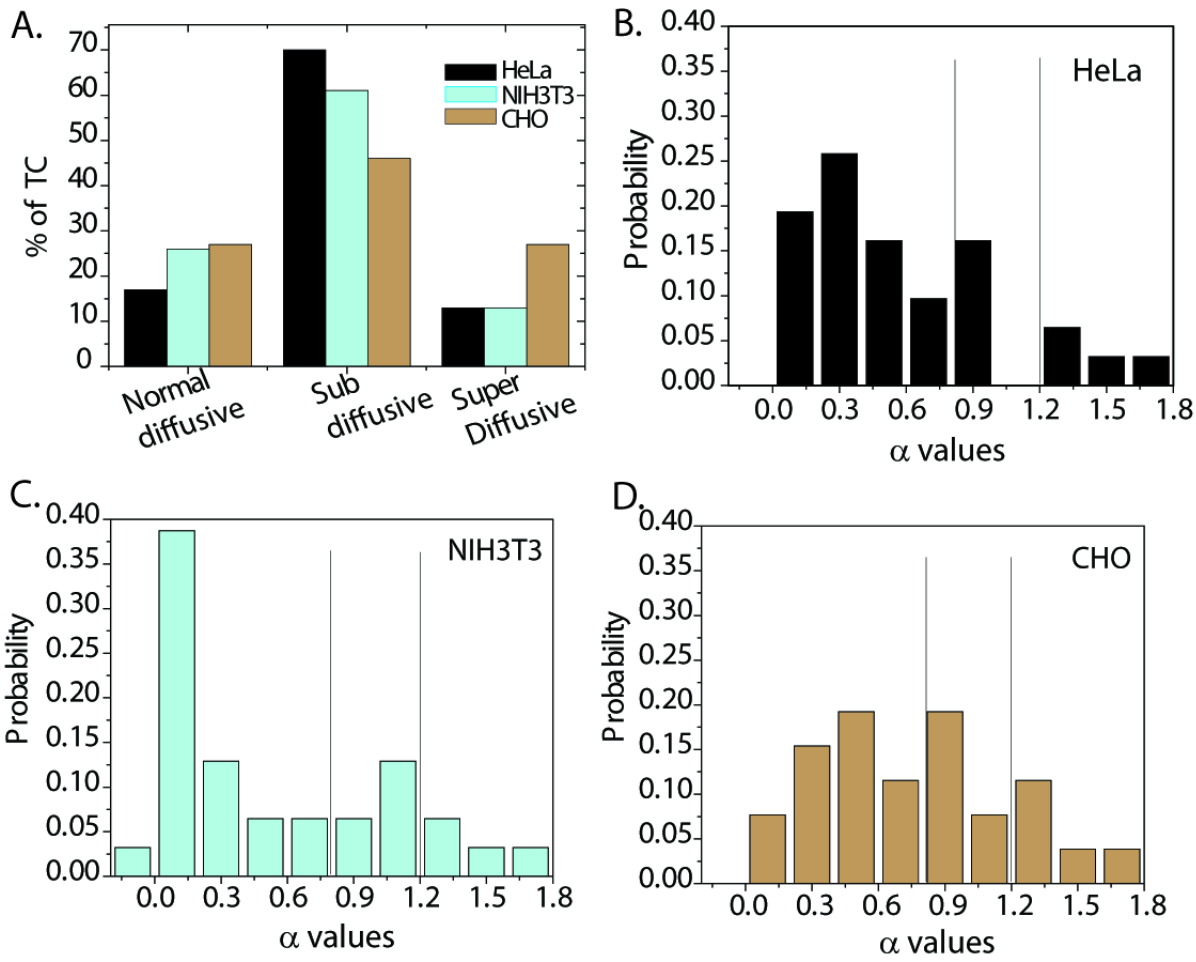

Figure S6 (**A)** Percentage of TCs showing different diffusive behaviours in the three cell types. (**B-D**) Distribution of α values in Hela, NIH3T3 and CHO cells.

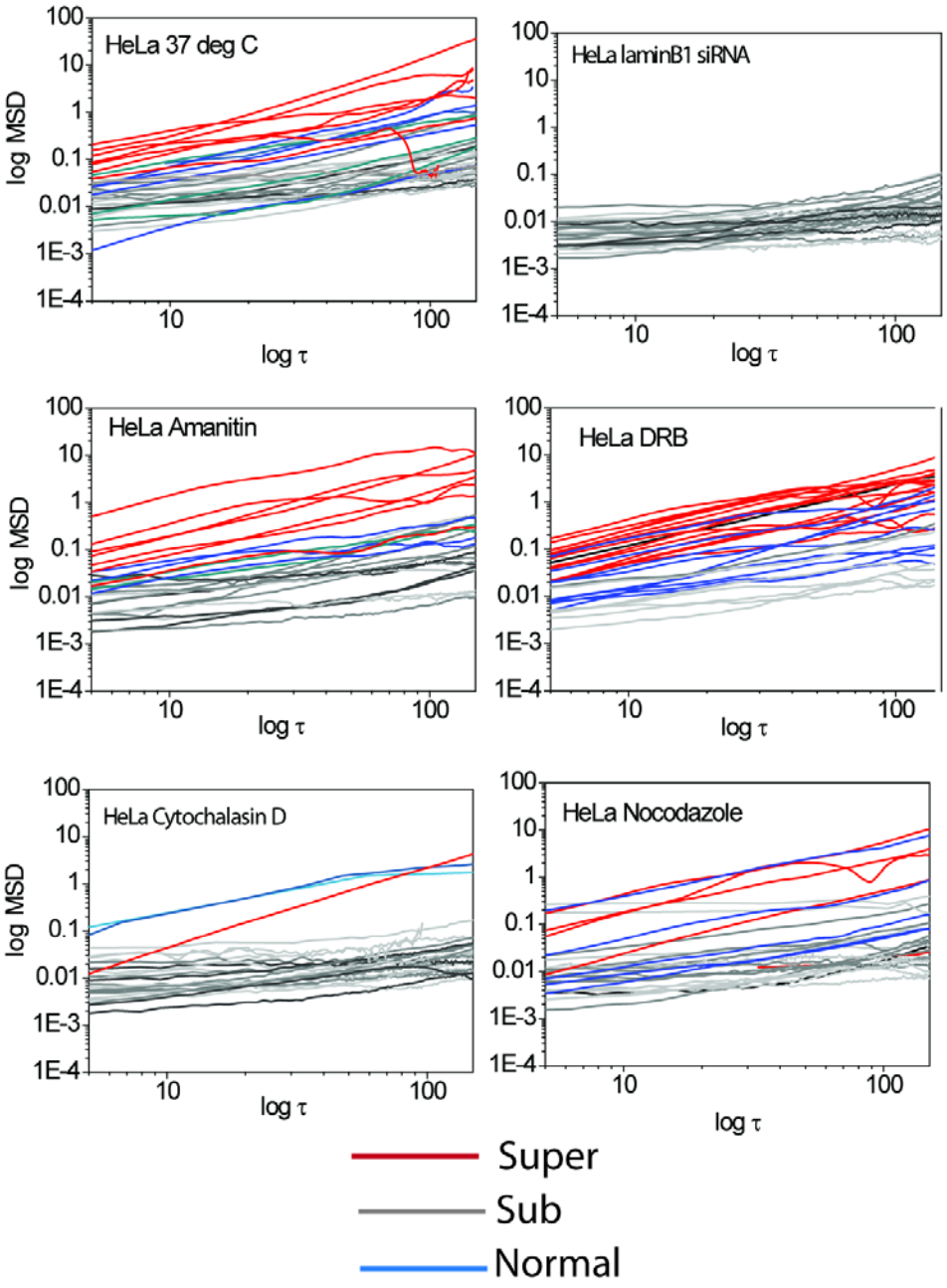

Figure S7 log MSD vs log τ plot for TC movement imaged at 0.2Hz for ~20min.

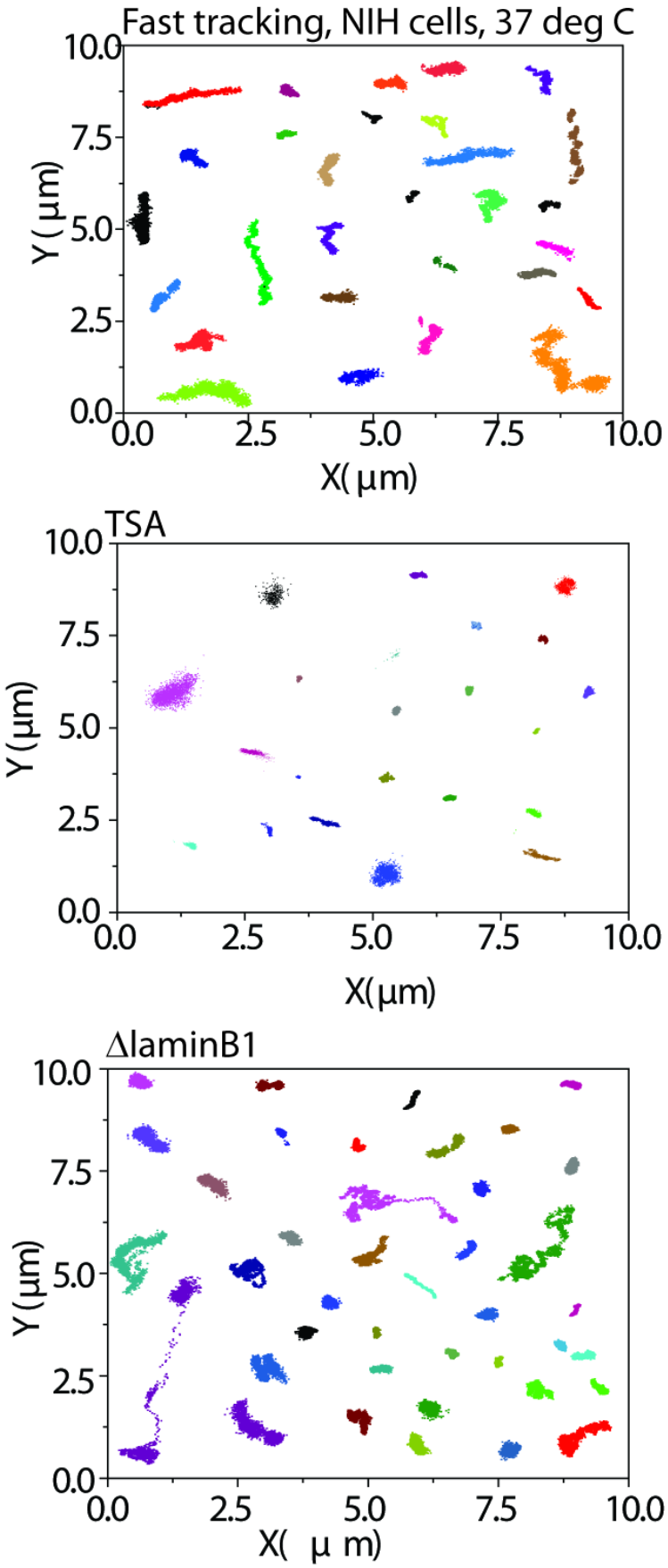

Figure S8 Trajectories for fast tracking of TCs imaged at 25Hz for 100seconds.