Intrinsically disordered N-terminal and structured DNA-binding domains jointly regulate progesterone receptor transcriptional condensates

Louna Raluy[1], Dominik Saul[2, 3], Catalina Romero-Aristizabal[4], Borja Requena[5], Lara I. de Llobet Cucalon[4], Robyn Laura Kosinsky[3], Nadine Youssef[1], Carla Garcia-Cabau[6], Maciej Lewenstein[5,7], Xavier Salvatella[6,7], Gorka Muñoz-Gil[8], Maria F. Garcia-Parajo[5,7,*], Juan A. Torreno-Pina[5,9,*], Priyanka Sharma[1,*]

[1]Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, Toulouse, France.

[2]Division of Endocrinology, Mayo Clinic, Rochester, MN 55905, USA
Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN 55905, USA.

[3]Robert Bosch Center for Tumor Diseases, Stuttgart, Germany.

[4]Centre de Regulació Genomica (CRG), The Barcelona Institute of Science and Technology (BIST), Dr. Aiguader 88, Barcelona, Spain.

[5]ICFO-Institut de Ciències Fotòniques, The Barcelona Institute for Science and Technology (BIST), 08860 Barcelona, Spain.

[6]Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Spain.

[7]ICREA (Institució Catalana de Recerca i Estudis Avançats), Pg. Lluís Companys 23, 08010 Barcelona, Spain.

[8]University of Innsbruck, Department for Theoretical Physics, Technikerstr. 21a, A-6020 Innsbruck, Austria.

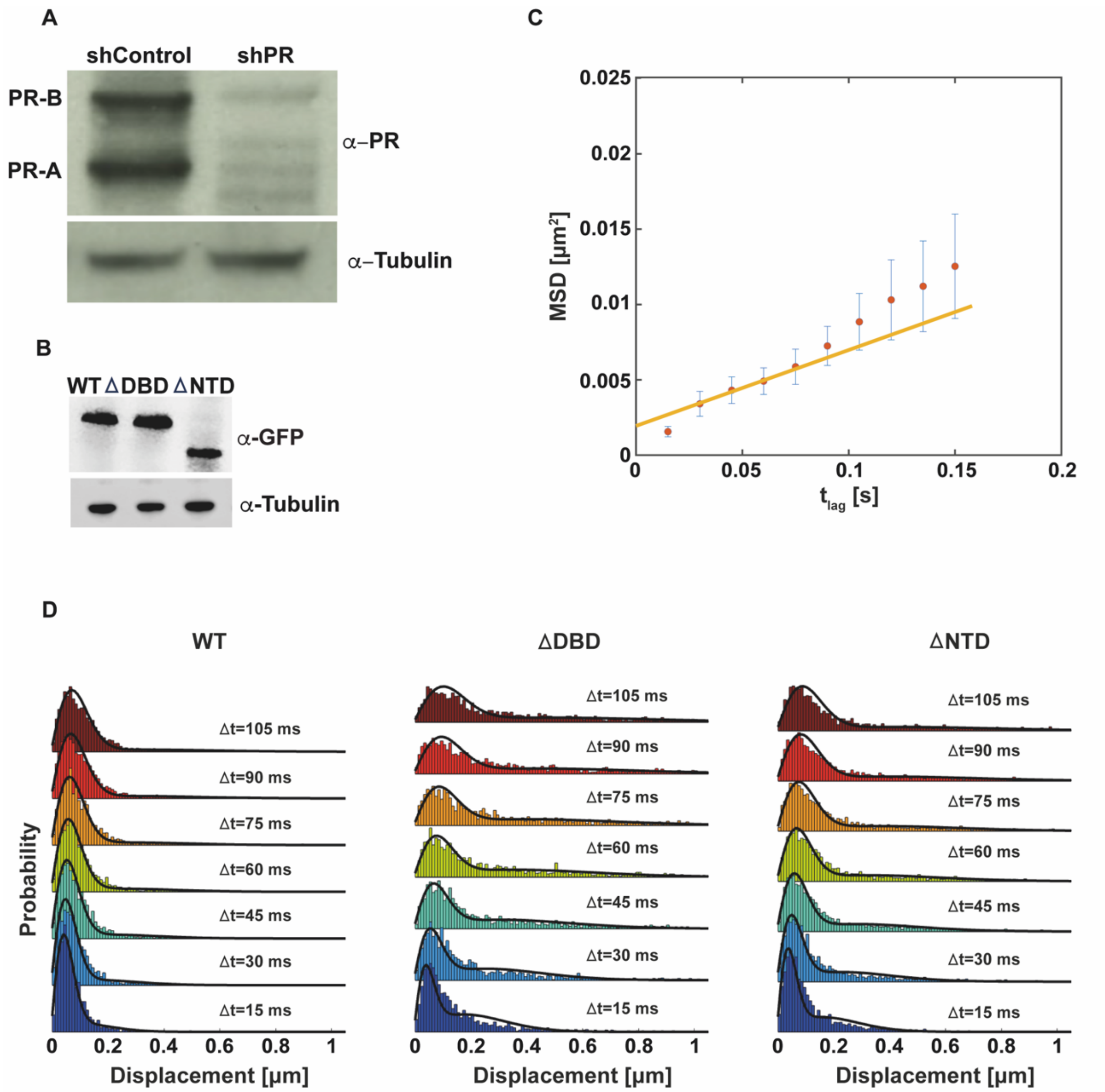
[9]Present address: Department of Macromolecular Structure, Centro Nacional de Biotecnologia-CSIC, C/ Darwin, 3, 28049 Cantoblanco Madrid, Spain.

[*]Co-corresponding authors:

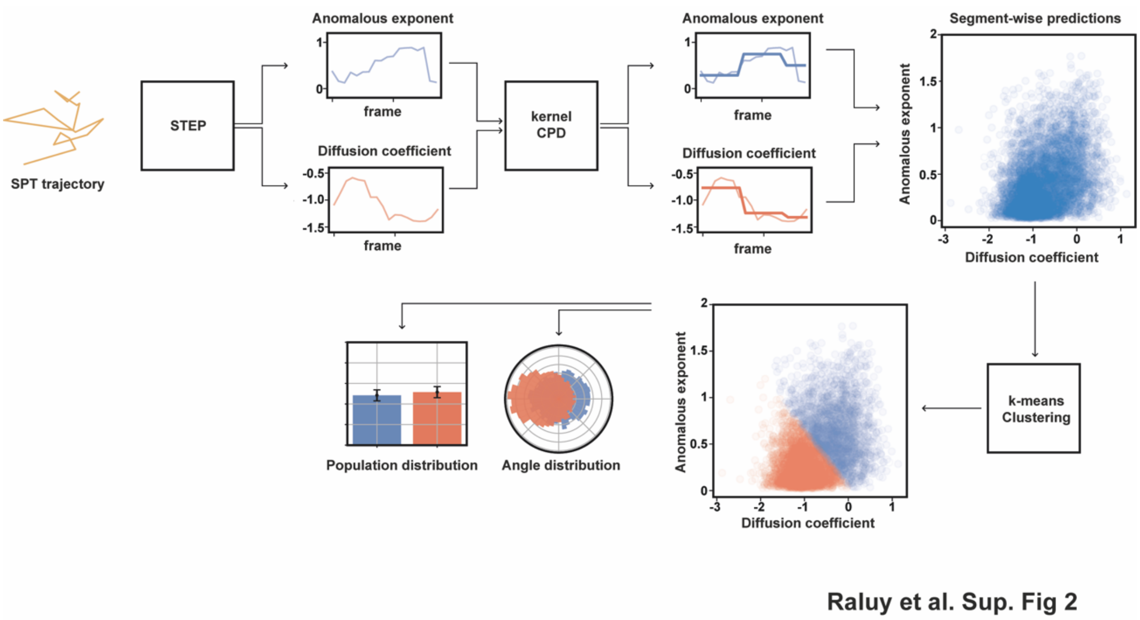
Maria F. Garcia-Parajo (maria.garcia-parajo@icfo.eu)
Juan A. Torreno-Pina (ja.torreno@cnb.csic.es)
Priyanka Sharma (priyanka.sharma@ipbs.fr)

Supplementary Information

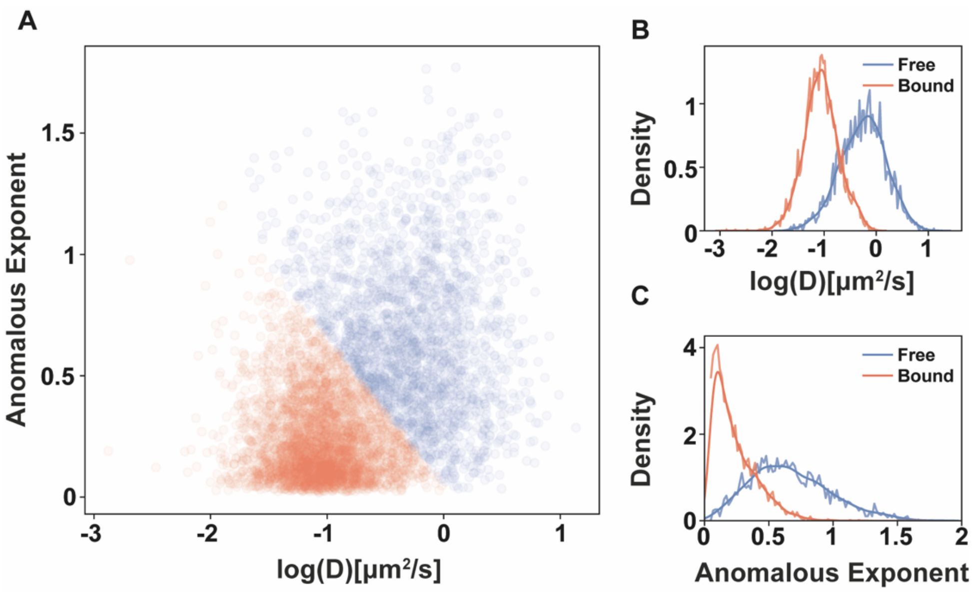
This file includes:
Supplementary Figures 1-5
Table S1



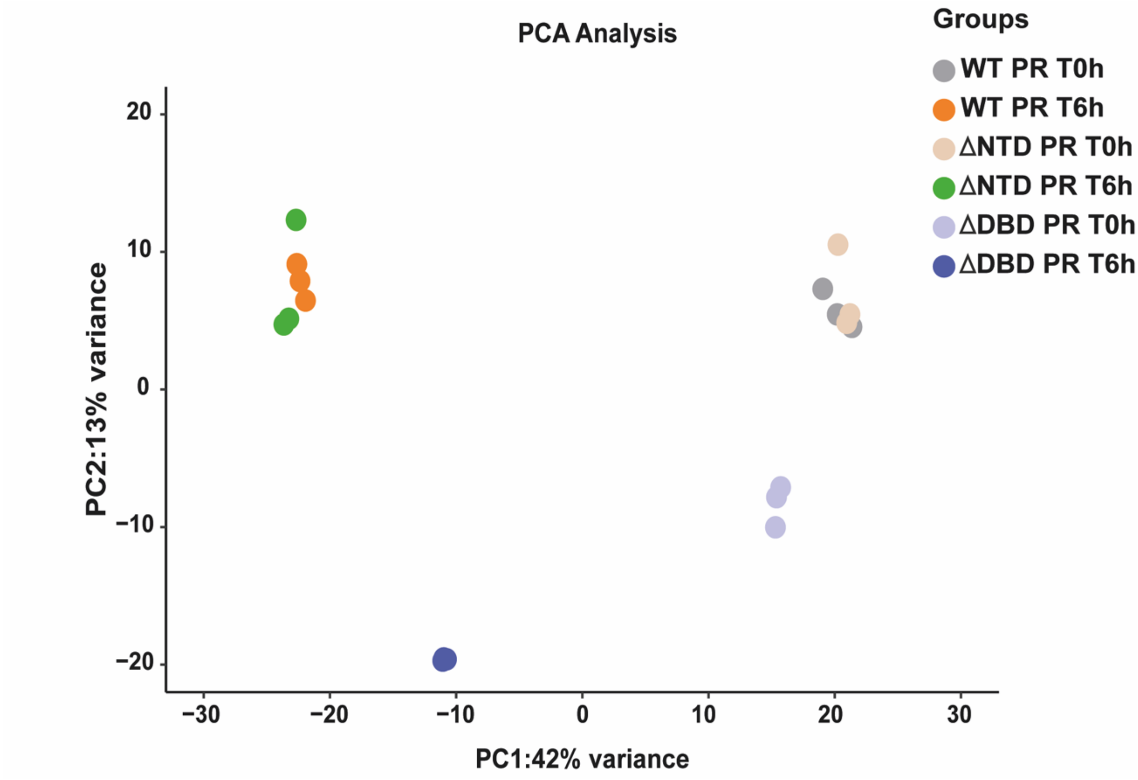
Supplementary Figure 1: **SPT analysis** (**A**) Extract from shControl and shPR showing the effective knockdown of endogenous PR as indicated by western blot for the indicated antibodies. (**B**) Extracts from WT, ΔDBD, or ΔNTD cells followed by the western blot of the indicated antibodies showing the knock-in expression of PR. (**C**) Representative MSD plot of an individual SPT trajectory. The MSD and the errors are represented as red dots and blue error lines, respectively. The fitting through the 2^{nd}-4^{th} points of the MSD plot is represented as a yellow line (see Methods). (**D**) Representative histograms of the displacements in dependence on time lags (insets) as extracted from Spot-On analysis (see Methods) of WT, ΔDBD, or ΔNTD. In the case of ΔDBD and ΔNTD, the distribution of the displacements is shifted towards longer displacements.



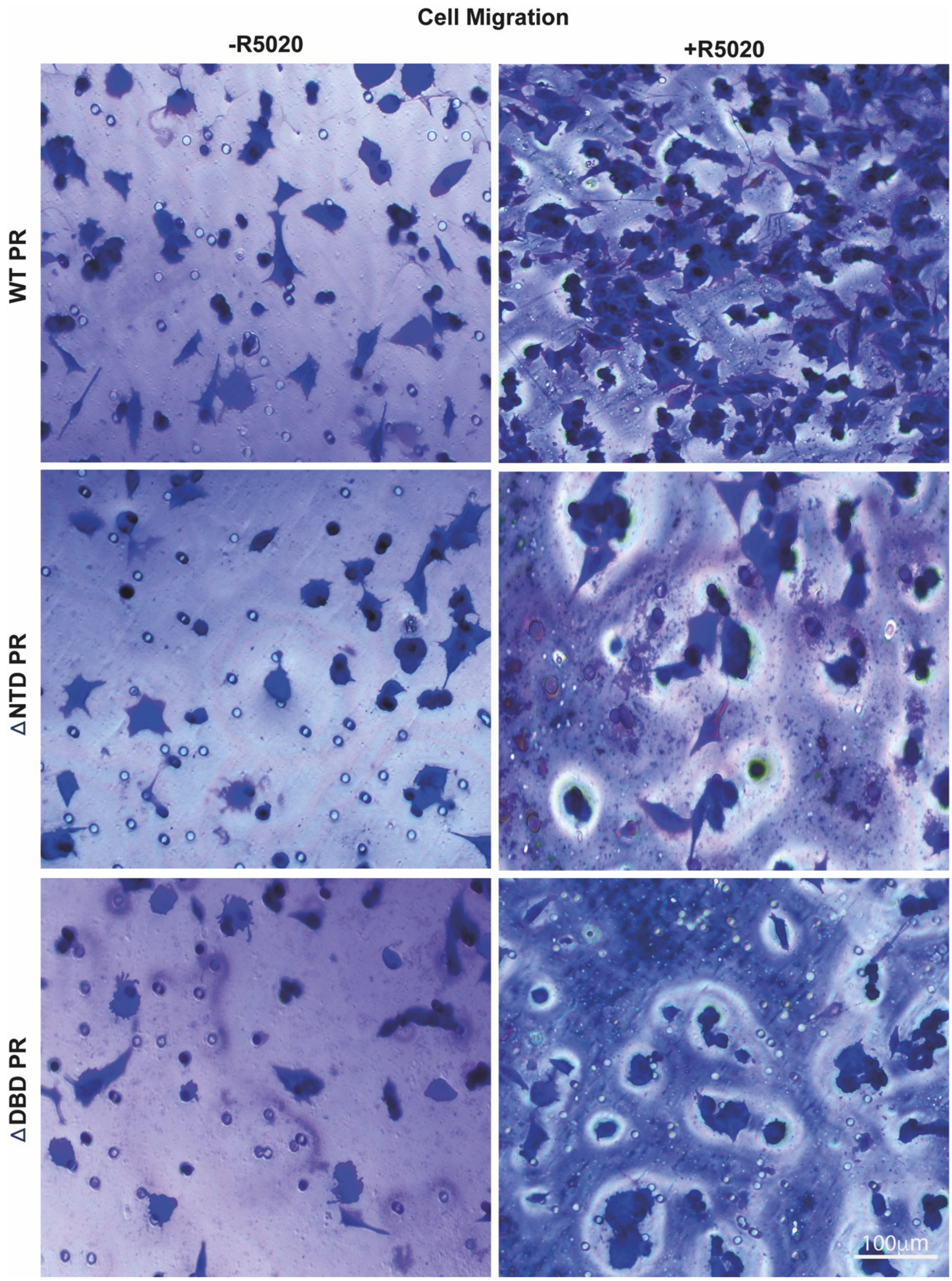
Supplementary Figure 2: Deep learning pipeline: Given a trajectory, STEP predicts diffusive properties for each frame. We then use a kernel changepoint detection algorithm to segment these predictions. From the obtained segment, we perform clustering via k-means. The resulting clusters are then used to compute population and angle distribution as well as the confinement radius of the bound state.



Supplementary Figure 3: **K-means clustering of trajectory segments.** (**A**) Clustering of the segments as predicted by k-means with two target clusters. (**B**, **C**) Diffusion coefficient and anomalous exponent distribution of the two found clusters. The actual distribution and a kernel density estimation are represented.



Supplementary Figure 4: **Principal Component Analysis (PCA) of transcriptional profiles across experimental groups**. PCA plot illustrates the variance in transcriptional profiles among six experimental groups of PR expressing cells with R5020 induction: WT (T0), WT (T6h), ΔNTD (T0), ΔNTD (T6h), ΔDBD (T0), and ΔDBD (T6h). PC1 accounts for 42% of the variance, and PC2 accounts for 13%. Each dot represents a biological triplicate, color-coded based on the group as indicated. The separation of clusters emphasizes the transcriptional impact of the WT-PR and PR mutants (ΔNTD or ΔDBD) in response to R5020 induction.



Supplementary Figure 5. **Cell Migration.** Representative Crystal Violet staining microscopy images of MCF7 cells expressing WT-PR and PR mutant (ΔNTD or ΔDBD) before (-R5020) and after (+ R5020) induction treatment during the transwell cell migration assay. Scale bar 100μm.

Supplementary Table S1- Primers used in this study.

Name	RT-qPCR Sequence (5′ to 3′)
SNAIL1, F.P.	CTGGGTGCCCTCAAGAT
SNAIL1, R.P.	GCCAGGGCCTAGAGAAG
PAX6, F.P.	TCTAATCGAAGGGCCAAATG
PAX6, R.P.	GTGCTGAAACTACTGCTGATA
SOX18, F.P.	GGCACTGGCCAAACTG
SOX18, R.P.	GATGCACGCGCTGTAATA
PHF19, F.P.	AGACTTGATGTCCAAACTGAC
PHF19, R. P	CCTTCCATAGGACCCAGTAT
CHECK2, F.P.	CTGCTGGAGTTTAGGAGTTATT
CHECK2, R.P.	GGGTGTCTTAAGGCTTCTTC
CADM1 F.P.	CCAACAGGCAGACCATTTA
CADM1 R.P.	GACTTCAATCTCCTCACCTTC
HSPA9, F.P.	CCTTTACAGCAGATGGTGAG
HSPA9, R.P.	GAGACGCTTGGTAGCATAAA
GLUD1, F.P.	CTGGAGGAGTGACAGTATCT
GLUD1, R. P	CCTGAGCAAGTGGTAGTTAG
P2RX4, F.P.	CTTCGACATCATTGTGTTTGG
P2RX4, R.P.	TGCTCGTAATCTTCCACATATT
RIBC1, F.P.	GGAAAGAGCAGGAAGTACAA
RIBC1, R.P.	CCCTTTGAAATACAGCACAC
LARP7, F.P.	GAGAAGTTCAGCTGTTCTA
LARP7, R.P	CGTTCATCCTCATCCTTTGG
HMOX1 F.P.	GCAGAGAATGCTGAGTTCAT
HMOX1 R.P.	ACATAGATGTGGTACAGGGA
GAPDH, F.P.	GAGTCAACGGATTTGGTCGT
GAPDH, R.P.	TTGATTTTGGAGGGATCTCG