

Appendix

Canonical and non-canonical miRNA degradation shapes state transitions and stemness in breast cancer

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Table of Content

Appendix Figure S1, page 3

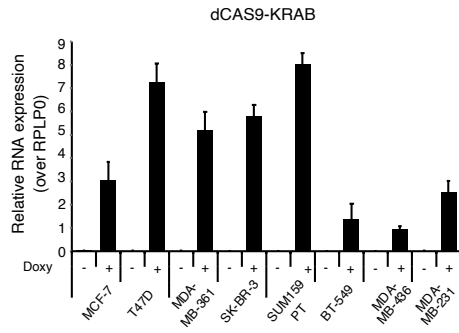
Appendix Figure S2, page 5

Appendix Figure S3, page 8

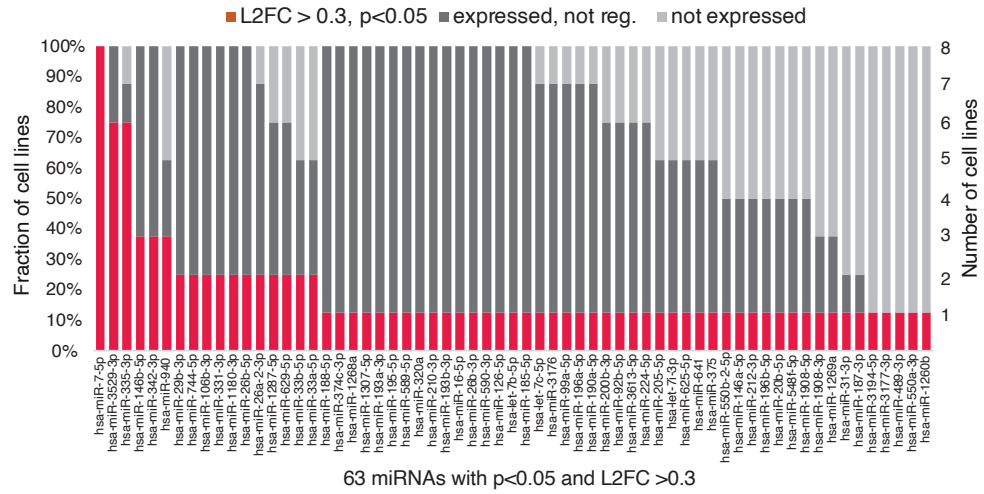
Appendix Figure S4, page 10

Appendix References, page 12

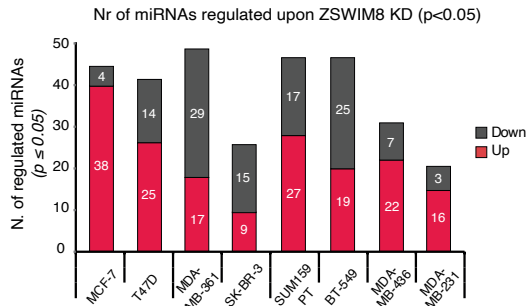
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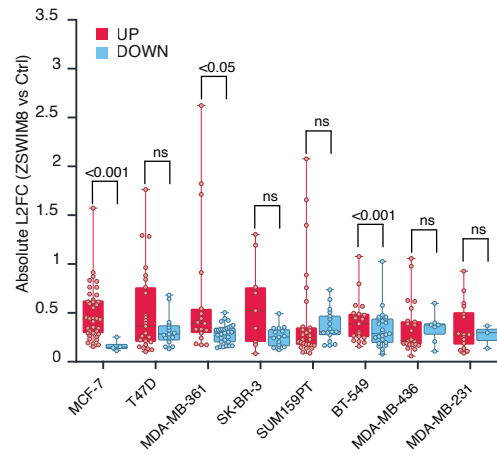
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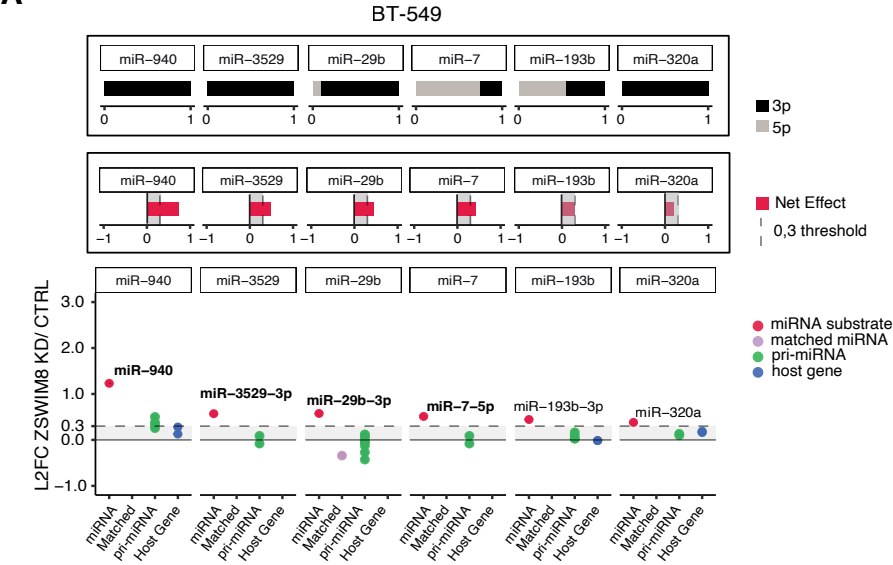
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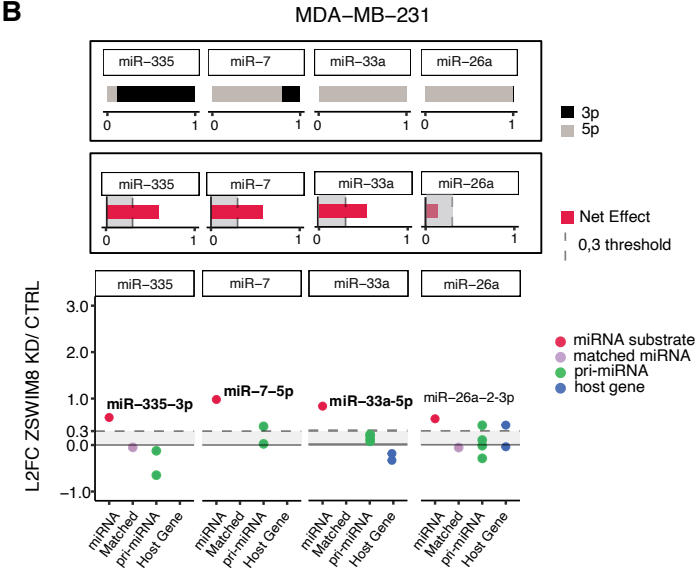
Appendix Figure S1: Analysis of miRNAs regulated upon ZSWIM8 KD.

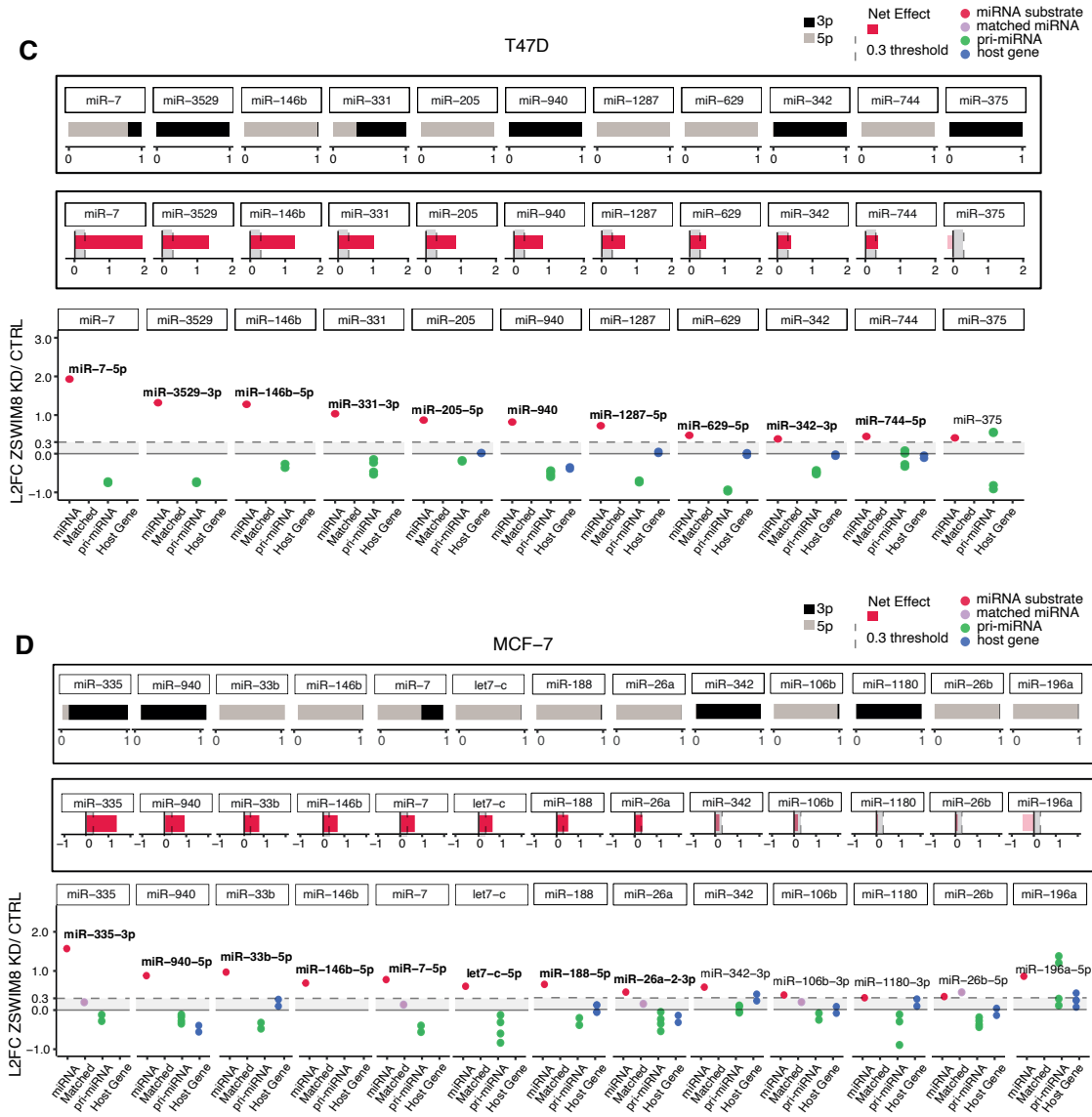
(A) dCas9-KRAB expression in breast cancer cell lines. RT-qPCR was performed upon administration of 1 µg/ml of Doxycycline (+) for 72h. The bar chart shows the relative RNA expression (n=3; +/- st.dev) using RPLP0 as reference. (B) The stacked bar chart reports the number of cell lines (and the proportion) in which each of the 63 TDMD miRNA substrates was found as upregulated (red, L2FC >0.3, pvalue <0.05), expressed but not upregulated (dark grey, >10 CPM) or not expressed (light grey, <10 CPM). MiRNAs are ordered according to the number of lines where they were found as upregulated. (C) Number of significantly upregulated (red) and downregulated (grey) miRNAs (ZSWIM8 KD vs Ctrl) in every cell line (Student's t-test, $p \leq 0.05$). (D) Effect size of the regulation of miRNAs upon ZSWIM8 KD. The boxplots show the L2FC values of upregulated (red) and downregulated (grey) miRNAs in every cell line (Mann-Whitney nonparametric t-test, *** $p \leq 0.001$; * $p \leq 0.05$).

A



B

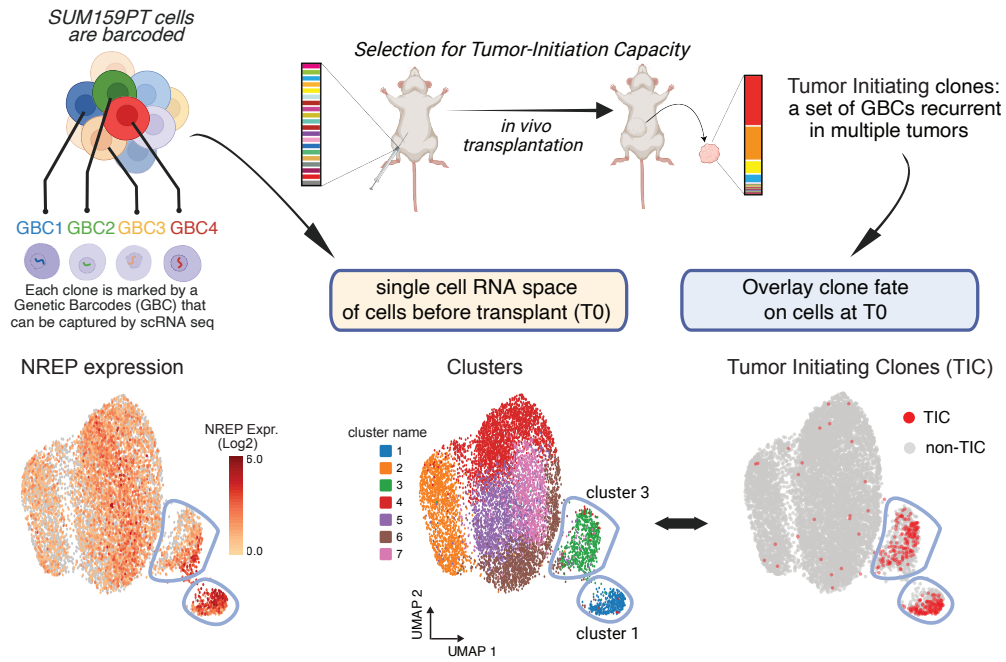




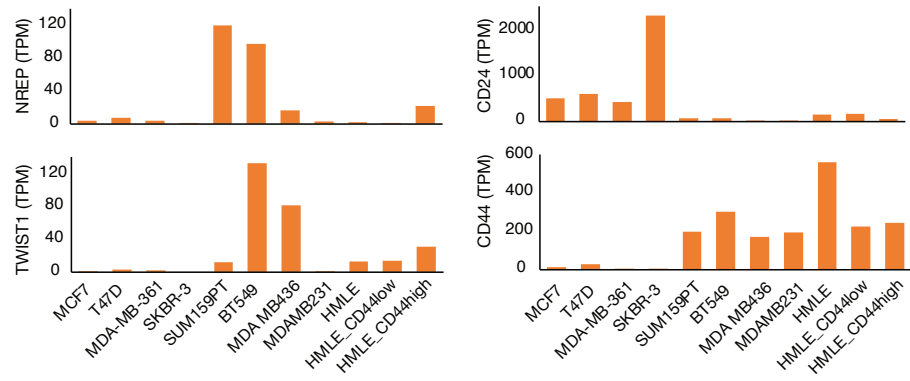
Appendix Figure S2: Identification of high confidence TDMD miRNA substrates.

(A-D) For each miRNA found as upregulated upon ZSWIM8 KD (see **Fig 2E**) is reported the distribution of reads from the 5p/3p strands, the TDMD net effect (red bar) and the values (L2FC values upon ZSWIM8-KD) of miRNA substrate (red dots) and matched miRNA (pink) obtained by smallRNA-Seq analysis, together with their pri-miRNAs (green), and host genes (blue dots) obtained by RT-qPCR, normalized with either RPLP0 or GADPH (two or more dots are shown, considering the two normalizations and different pair of primers used). A dashed bar indicates the threshold for the “TDMD net effect” (≥ 0.3 L2FC).

A

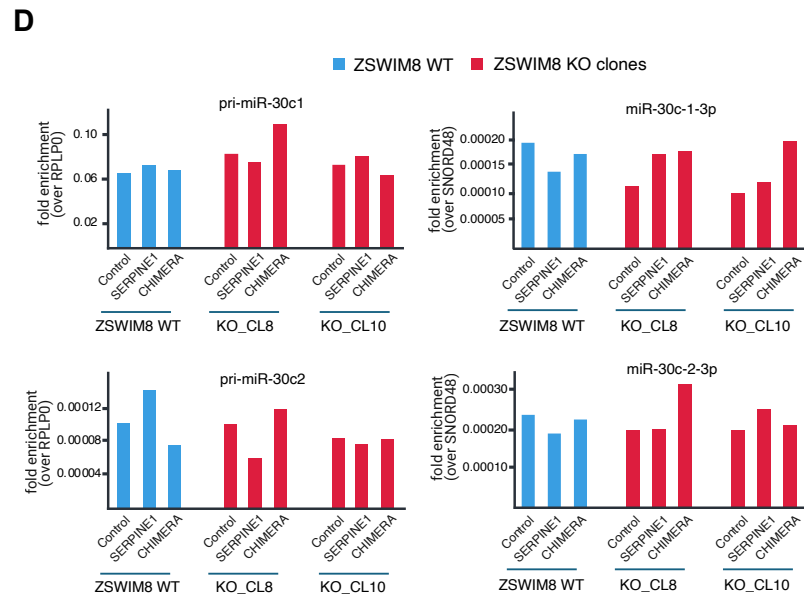
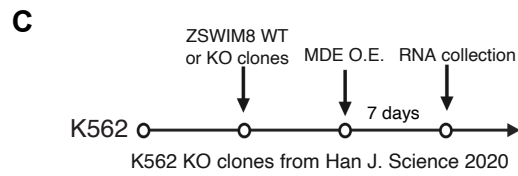
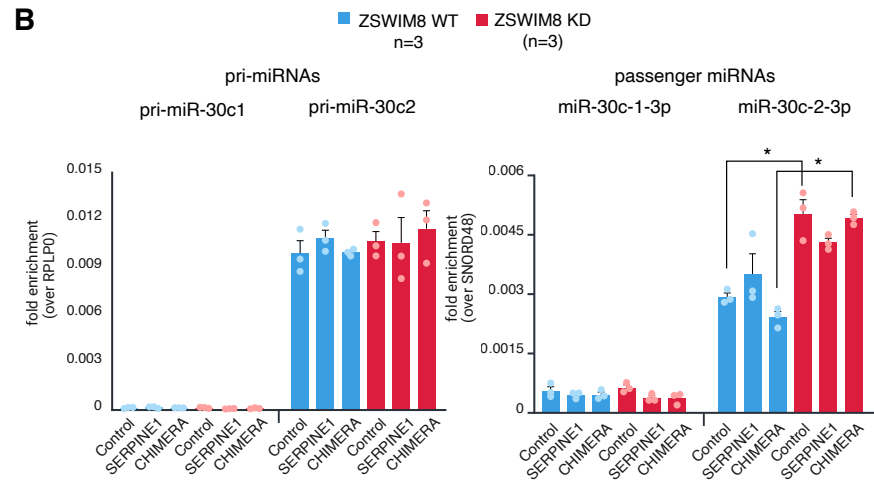
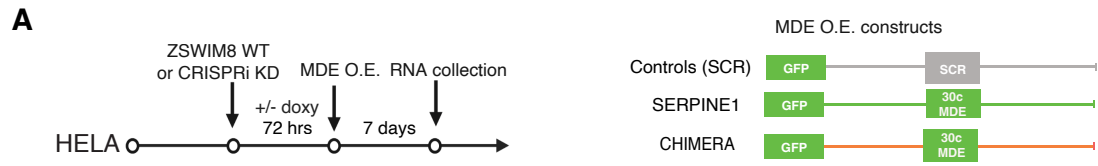


B



Appendix Figure S3: Identification of tumor initiating cells in SUM159PT cells

(A) Schematics of the experimental design for the identification of tumor initiation clones in SUM159PT. Barcoded SUM159PT cells were injected orthotopically in NSG mice; after tumor formation, clonal identity and frequency were evaluated by retrieving GBC from gDNA. Barcodes recurrently found in multiple mice were defined as “tumor initiating clones” (TICs). TICs were then mapped on the different subpopulations defined by single cell RNA sequencing at parental state (T0). The two subpopulations that were strongly enriched for TICs (cluster 1 and cluster 3) display high expression of NREP. The figure is adapted from Nadalin et al, 2024. **(B)** Expression of NREP, TWIST1, CD24 and CD44 across BC cell lines and HMLE, either bulk or sorted CD44^{high}/CD24^{low} and CD44^{low}/CD24^{high} populations (TPM, by RNA sequencing).



Appendix Figure S4: Post-transcriptional and ZSWIM8 independent TDMD mechanism

(A) Schematic overview of the experimental strategy used in HeLa used to assess ZSWIM8 dependent and -independent TDMD mechanism. Cells were infected with sgRNA targeting ZSWIM8 (sg28, sg34, sg42), then treated or not with doxycycline to induce ZSWIM8 knockdown (WT vs KD) and finally transduced with different TDMD constructs. Samples were collected after 7 days for the analyses. Shown also the TDMD constructs that were used. (B) Expression levels of pri-miRNAs and passenger strands for miR-30c-5p were measured by RT-qPCR in HeLa cells. Data are normalized to *RPLP0* (for pri-miRNAs) and *SNORD48* (for passenger strands). Bar charts show the average and SEM (N=3). P-values were calculated through Student's t-test comparing WT and ZSWIM8 KD cells expressing the same TDMD construct. The only significant effect observed was an increase (rather than a decrease) in miR-30c-2-3p levels upon ZSWIM8 KD. * = $p < 0.05$. (C) Schematic of the experimental strategy used in K562 cells to assess ZSWIM8-dependent and -independent TDMDs, comparing wild-type or ZSWIM8 knock-out clones. (D) Expression levels of pri-miRNAs and passenger strands were measured by RT-qPCR in K562 WT or KO clones (KO_CL8 and KO_CL10) upon over-expression of the same TDMD constructs used in panel A and B. Bar charts report the expression levels normalized over *RPLP0* (for pri-miRNAs) and *SNORD48* (for passenger strands) for individual replicates.

Appendix References

Nadalin F, Marzi MJ, Pirra Piscazzi M, Fuentes-Bravo P, Procaccia S, Climent M, Bonetti P, Rubolino C, Giuliani B, Papatheodorou I *et al* (2024) Multi-omic lineage tracing predicts the transcriptional, epigenetic and genetic determinants of cancer evolution. *Nat Commun* 15: 7609