Supplementary Materials for

A ubiquitin ligase mediates target-directed microRNA decay independently of tailing and trimming

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This PDF file includes:

Figs. S1 to S7

Other Supplementary Materials for this manuscript include the following:

Tables S1 and S2 (Excel)
MDAR Reproducibility Checklist

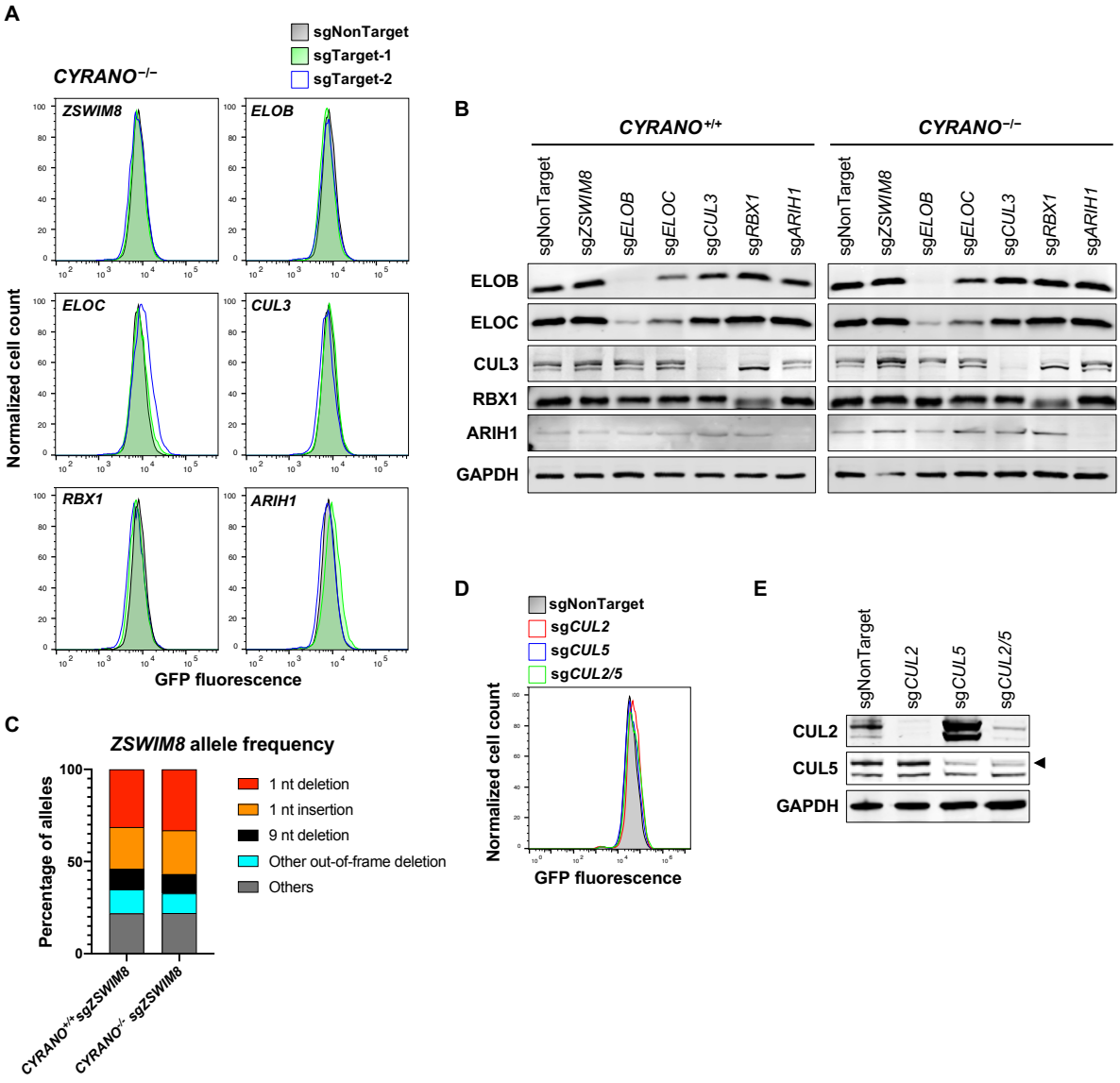

Fig. S1. Validation and additional analyses of cells with knockout of the ZSWIM8 complex. **(A)** Flow-cytometry analysis of EGFP expression in *CYRANO*$^{-/-}$ K562 *EGFP*$^{miR\text{-}7}$ cells after lentiviral expression of Cas9 and sgRNAs targeting individual ZSWIM8 complex components. **(B)** Western blot analysis showing depletion of ZSWIM8 complex components in K562 cells after expression of the indicated sgRNAs. **(C)** Due to a lack of an effective antibody, efficient *ZSWIM8* knockout was confirmed by determining the mutant allele frequency in cells expressing *ZSWIM8*-targeting sgRNAs by high-throughput sequencing. **(D)** EGFP expression in *CYRANO*$^{+/+}$ K562 *EGFP*$^{miR\text{-}7}$ cells after knockout of *CUL2*, *CUL5*, or both. **(E)** Western blot analysis of CUL2 and CUL5 in K562 cells expressing the indicated sgRNAs. CUL5-specific band indicated with arrow.

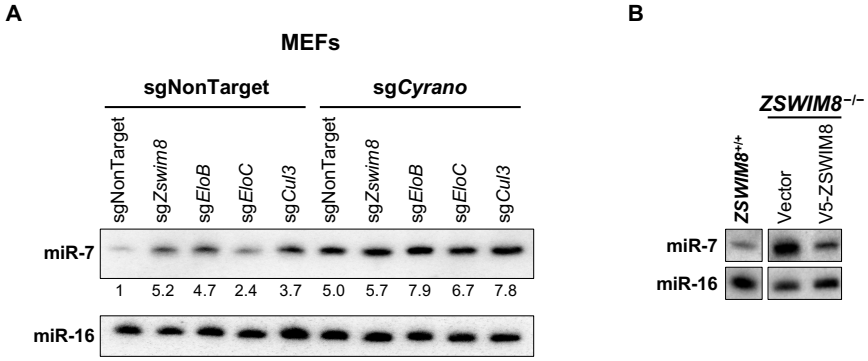

Fig. S2. Analysis of ZSWIM8 CRL components in MEFs and K562 cells. (A) Northern blot analysis of miRNA expression in MEFs after lentiviral expression of Cas9 and sgRNAs targeting ZSWIM8 ubiquitin ligase components. **(B)** Northern blot analysis of miRNA expression demonstrating restoration of TDMD of miR-7 by heterologous expression of V5-ZSWIM8 in K562 *ZSWIM8*$^{-/-}$ cells. Experiments were performed in biological triplicate (representative data shown).

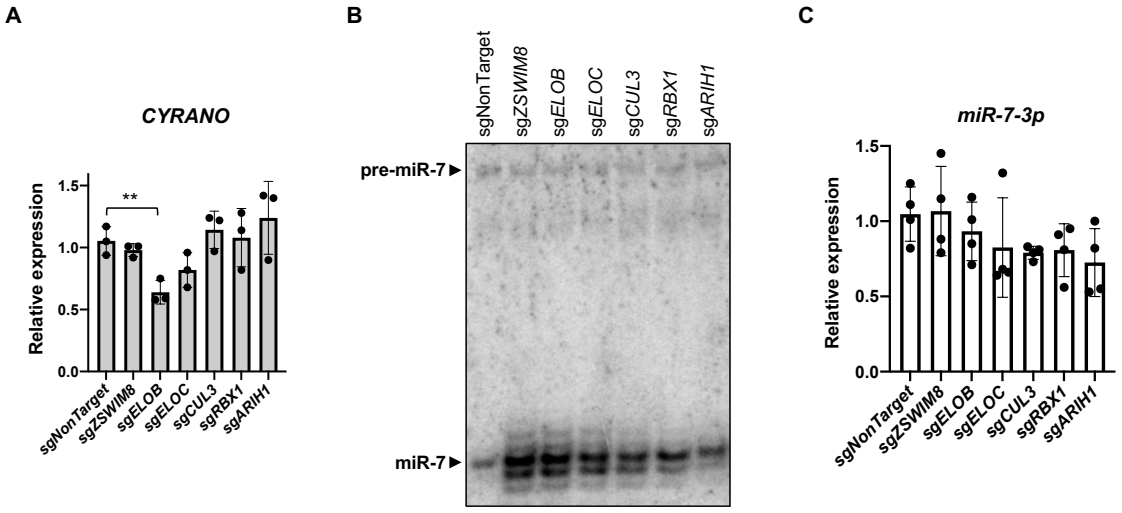

Fig. S3. Expression of *CYRANO* and miR-7 species after knockout of ZSWIM8 CRL components. (A) qRT-PCR analysis of *CYRANO* (normalized to *GAPDH*) in K562 cells expressing the indicated sgRNAs targeting ZSWIM8 ligase components. **(B)** Northern blot analysis of mature and pre-miR-7 in *CYRANO*$^{+/+}$ K562 cells expressing the indicated sgRNAs. This figure is identical to the blot shown in Fig. 2B, but uncropped to show the pre-miRNA levels. **(C)** qRT-PCR analysis of miR-7-3p, the miR-7 passenger strand, in *CYRANO*$^{+/+}$ K562 cells. Expression was normalized to U6 snRNA levels. n=3-4 biological replicates for all experiments (representative northern blot results shown). Mean +/- SD shown. $^{**}p<0.01$; Student's t test.

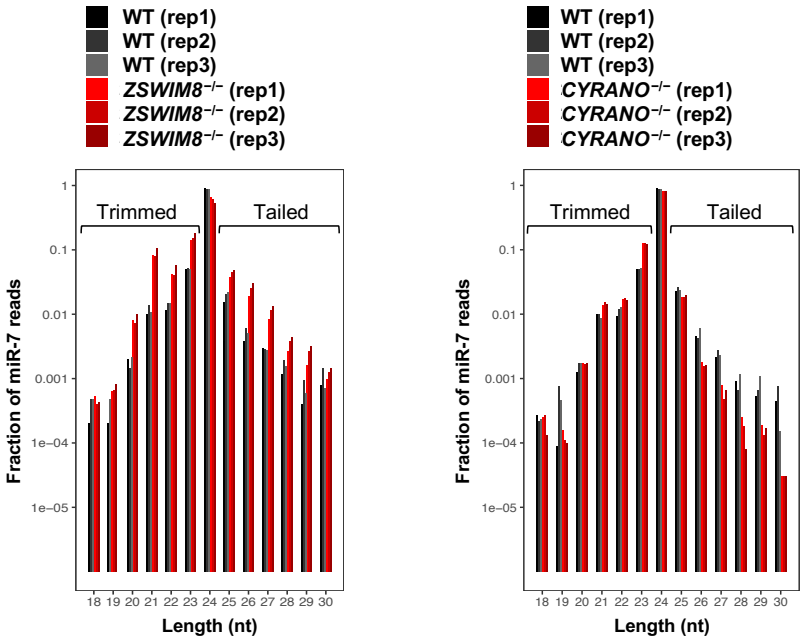

Fig. S4. Analysis of miR-7 tailing and trimming. miR-7 isoforms in *CYRANO*$^{-/-}$ or *ZSWIM8*$^{-/-}$ K562 cells were quantified by small RNA sequencing.

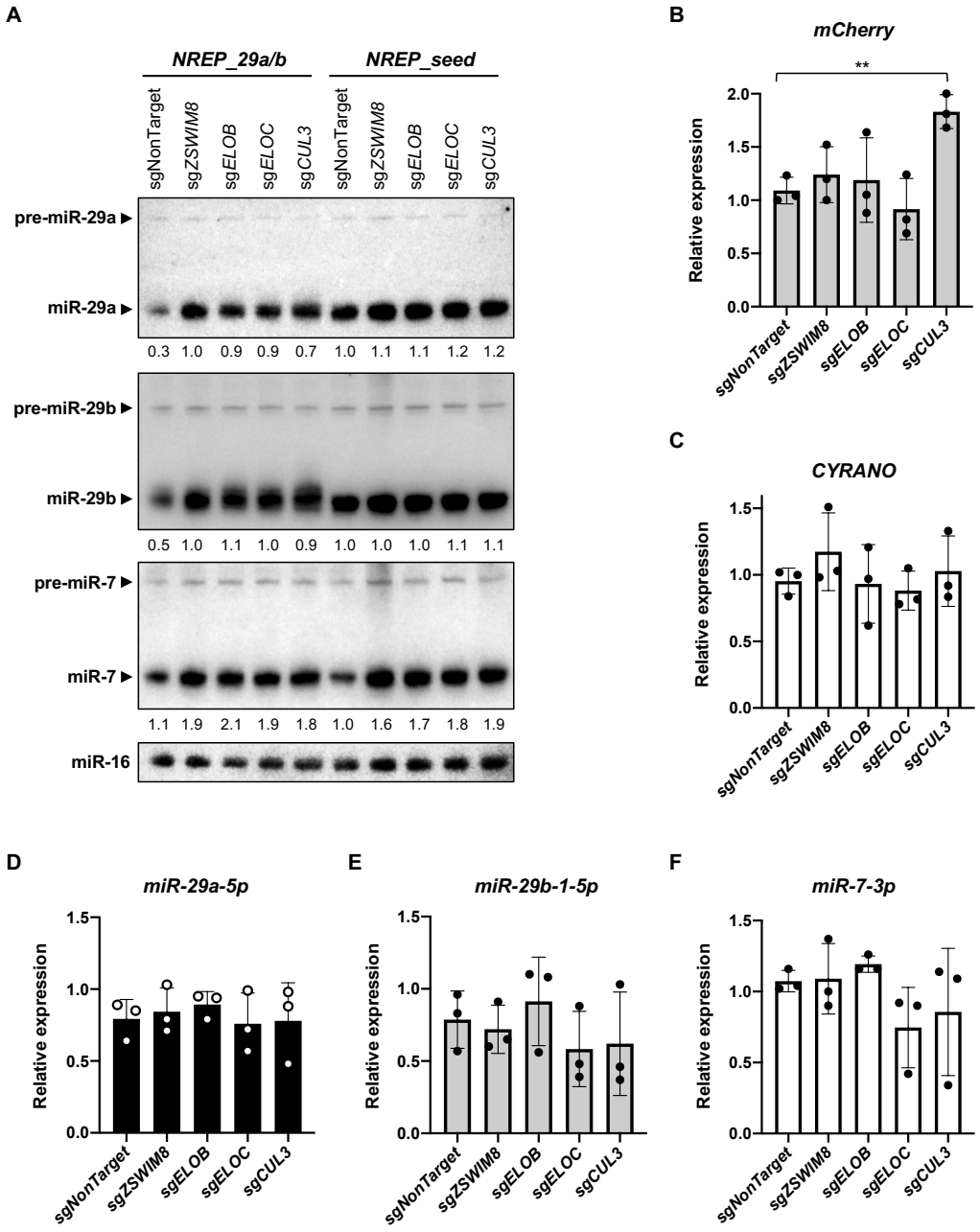

Fig. S5. ZSWIM8 CRL components are essential for *NREP*-mediated TDMD. (A) Northern blot analysis of miRNA expression in HCT116 cells expressing *NREP* transcripts and the indicated sgRNAs targeting ZSWIM8 ligase components. Note the unchanged expression of pre-miRNAs, consistent with regulation of mature miRNA stability in these experiments. **(B-F)** qRT-PCR analysis of *mCherry* (B), *CYRANO* (C), and miRNA passenger strand (D-F) expression in HCT116 cells expressing *NREP_29a/b* and the indicated sgRNAs. *GAPDH* (for *mCherry* and *CYRANO*), or miR-16 or U6 snRNA (for miRNA passengers) were used for normalization. n=3 biological replicates for all experiments (representative northern blot results shown). Mean +/- SD shown. $^{**}p<0.01$; Student's t test.

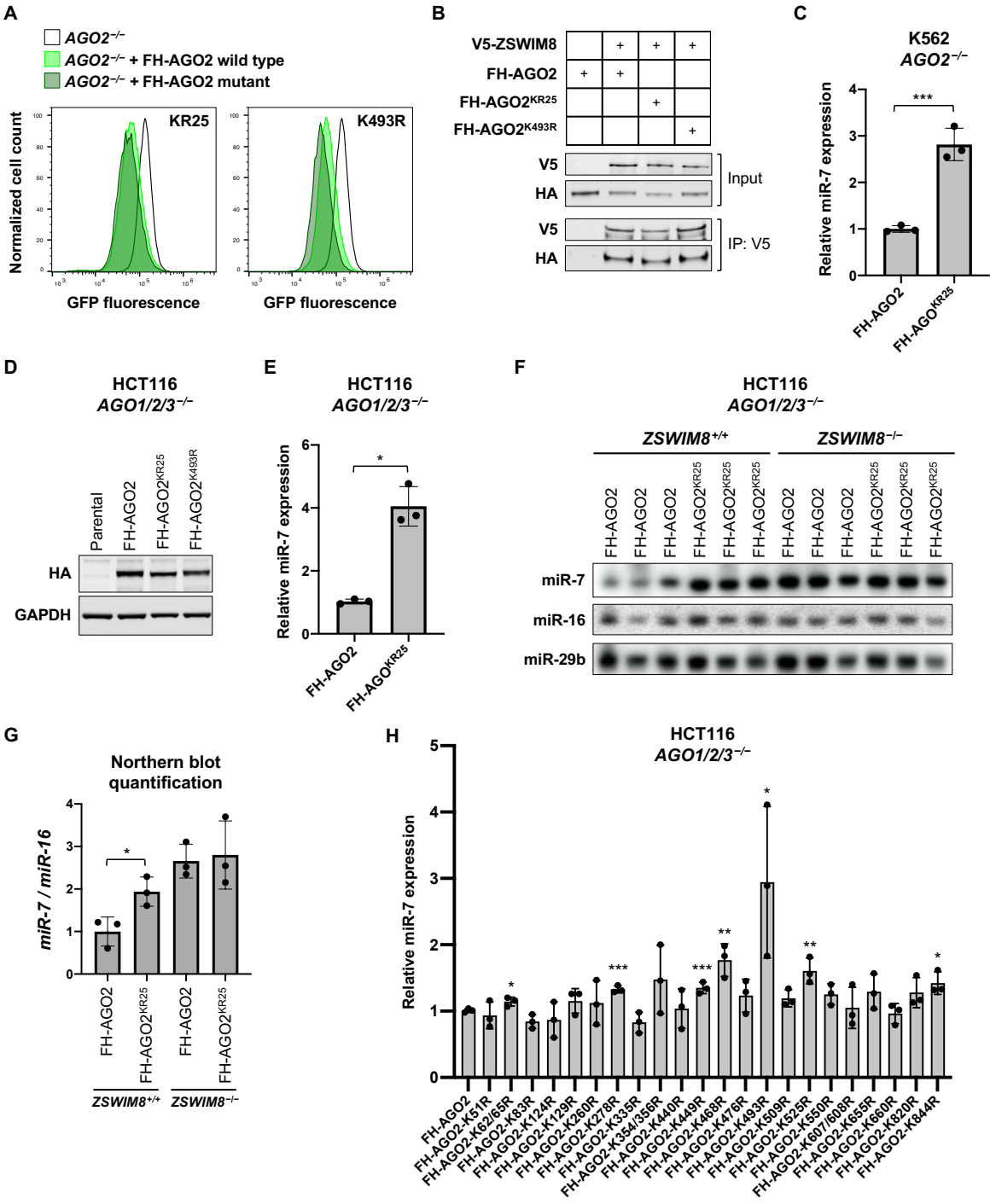

Fig. S6. Analysis of AGO2 lysine mutants. (A) Flow-cytometry analysis of EGFP expression in *AGO2*$^{-/-}$ K562 *EGFP*$^{miR\text{-}7}$ cells after lentiviral expression of wild-type FLAG-HA-tagged AGO2 (FH-AGO2) or the indicated mutants. **(B)** Co-immunoprecipitation of V5-ZSWIM8 and wild-type or mutant FH-AGO2. **(C)** qRT-PCR analysis of miR-7 (normalized to miR-16) in *AGO2*$^{-/-}$ K562 cells reconstituted with wild-type FH-AGO2 or FH-AGO2^{KR25}. **(D)** Western blot showing stable expression of wild-type or mutant FH-AGO2 proteins in HCT116 *AGO1/2/3*$^{-/-}$ cells. **(E)** qRT-PCR analysis of miR-7 (normalized to miR-16) in *AGO1/2/3*$^{-/-}$ cells reconstituted with

wild-type FH-AGO2 or FH-AGO2^{KR25}. **(F-G)** Northern blot (F) and associated quantification (G) of miRNA levels in *AGO1/2/3*$^{-/-}$ cells reconstituted with wild-type FH-AGO2 or FH-AGO2^{KR25}, with or without *ZSWIM8* knockout. Each set of three lanes represents biological triplicates. **(H)** qRT-PCR analysis of miR-7 (normalized to miR-16) in *AGO1/2/3*$^{-/-}$ cells reconstituted with wild-type or mutant FH-AGO2. n=3 biological replicates for all qRT-PCR experiments. Mean +/- SD shown. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; Student's t test.

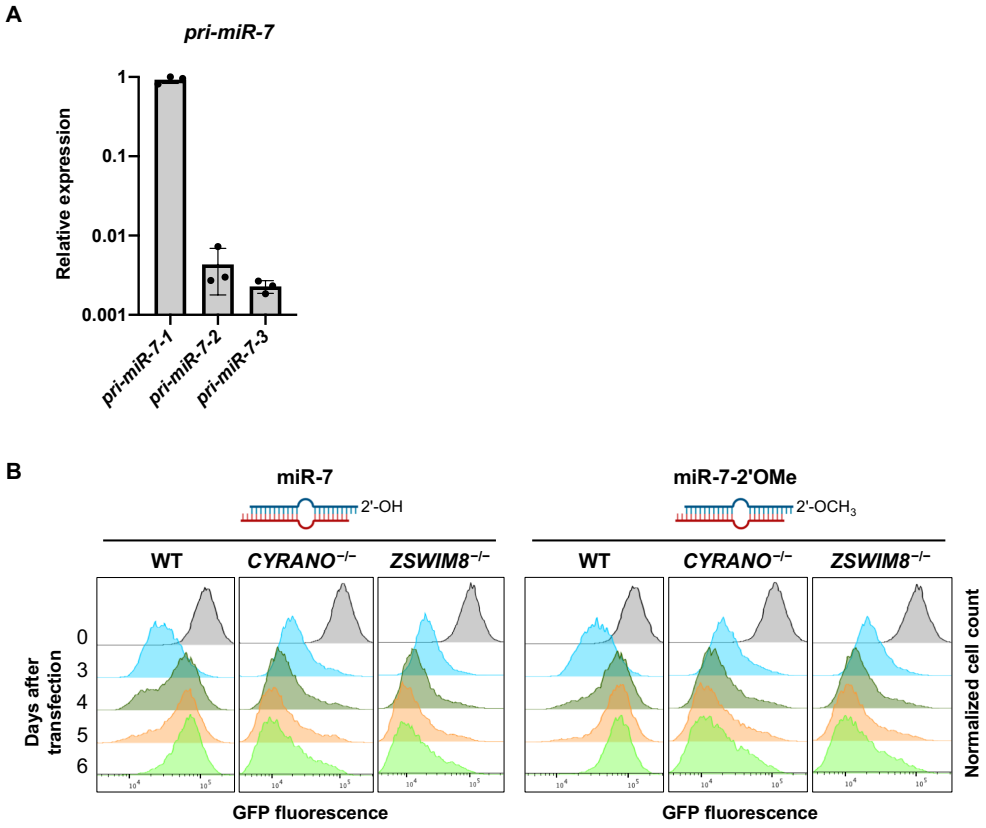

Fig. S7. miRNA tailing and trimming is not essential for TDMD. (A) qRT-PCR analysis of miR-7 primary transcripts demonstrates that miR-7-1 is the major source of miR-7 expression in K562 cells (note $\log_{10}$ scale). Mean +/- SD shown. **(B)** Flow cytometry analysis of EGFP expression in *miR-7-1*$^{-/-}$ K562 *EGFP*$^{miR\text{-}7}$ cells of the indicated *CYRANO* and *ZSWIM8* genotypes after transfection of unmodified or 2'-O-methylated miR-7 duplexes. All experiments were performed in biological triplicate.

Table S1. (separate .xlsx file). MAGeCK analysis of CRISPR screen

Table S2. (separate .xlsx file). Oligonucleotides and antibodies used in this study