Supplemental Material

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The human PMR1 endonuclease stimulates cell motility by down regulating miR-200 family microRNAs

Supplemental Materials and Methods

***In vitro* analysis of hPMR1 activity against pre-miR-200c** (related to Supplemental Fig. S4)**.** Fluorescent forms of pre-miR-200c or pre-miR-200c bearing mutations in the apical loop YUGA element (U34A, G35C) were generated by *in vitro* transcription with T7 RNA polymerase (MEGAscript® T7 transcription Kit, Ambion). The DNA templates were prepared using primer pairs in Supplement Table S2 and gel purified. For in vitro transcription, each 20 µl reaction contained 0.5 mM ATP, CTP, GTP, 0.1mM UTP and 10 µM aminoallyl-UTP-ATTO-680 (Jena Bioscience), 5 µl template, 2 µl enzyme mix. Transcripts were then purified on 20% polyacrylamide/8M urea gels prior to use. The C-terminal biotinylation tag on hPMR1 was used to recover protein for activity assay. Dynabeads MyOne Streptavidin T1 (Invitrogen) were preincubated in 100mM NaOH and 50mM NaCl for 2 min, washed with 100 mM NaCl and PBS. These were incubated with gentle rotation for 30 min at 25°C with 100 µg of cytoplasmic extract from uninduced or doxycycline-treated cells in a 200 µl volume. Beads was washed 3-times with 20 mM Tris-HCl, pH 7.5, 1.5 mM $MgCl_2$, 25 mM NaCl, 1% glycerol and 1 mM DTT, and suspended in 50 µl of the same buffer followed by addition of 1 µl of pre-miR-200C. The reaction mixture was incubated with constant agitation for 60 min at 37°C, after which the beads were removed. 20 µl of the reaction was mixed with an equal volume of 95% deionized formamide/5 mM EDTA and separated on 20% polyacrylamide/8M urea gels. The gels were visualized directly on LI-COR Odyssey imaging system.

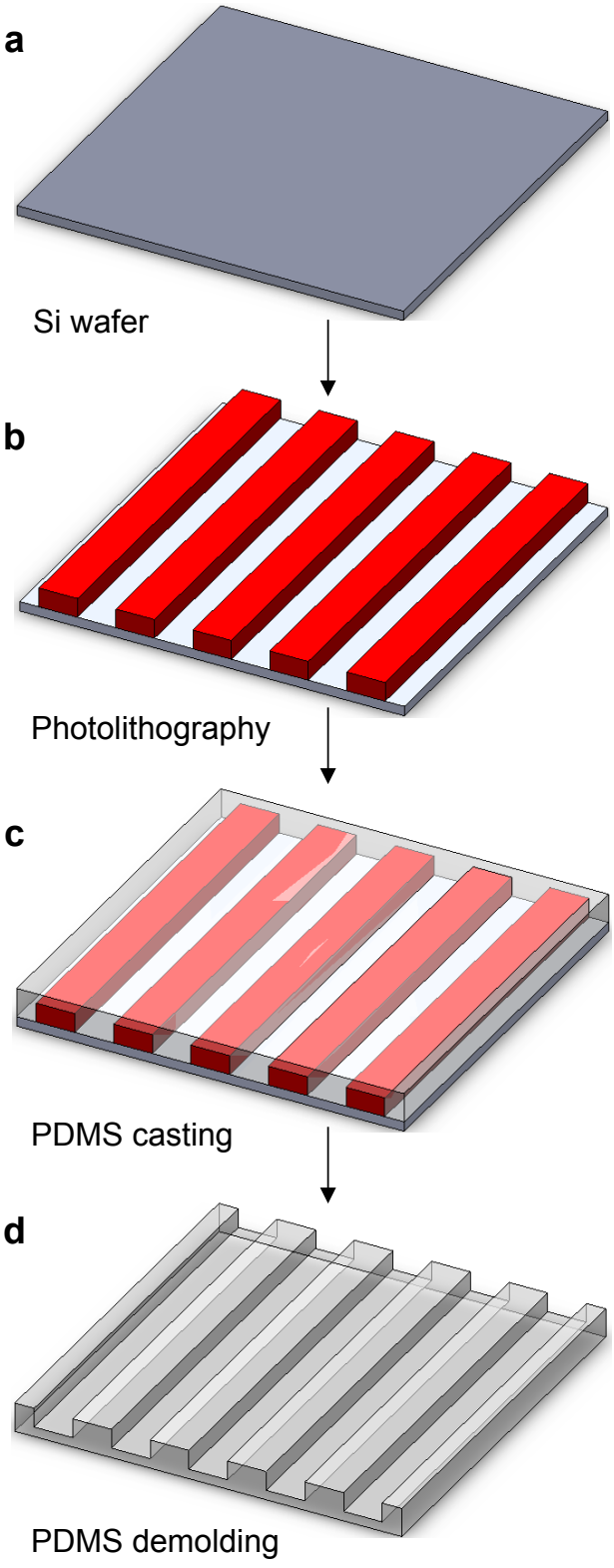

Figure S1. Protocol for generating biomimetic micropatterned polydimethylsiloxane matrices. (a) Silicon wafer. **(b)** Microscale lines are patterned on the surface via standard photolithography. **(c)** PDMS:curing agent mixture (10:1) is cast on the patterned wafer surface and allowed to cure. **(d)** Textured PDMS is demolded and readied for cell migration experiments.

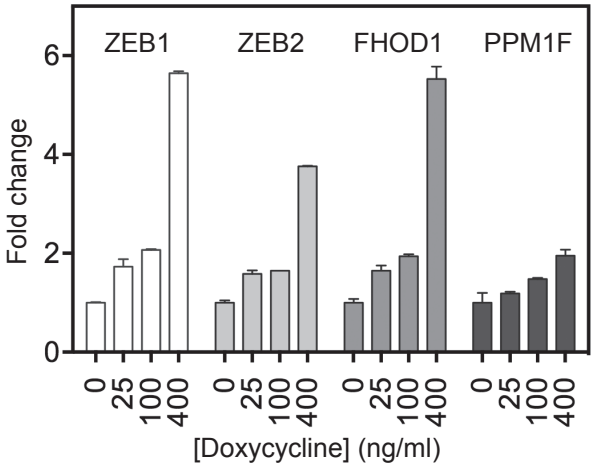

Figure S2. Concentration-dependent changes in miR-200 target gene expression. MCF-7 cells carrying an inducible hPMR1 transgene were treated for 24 hr with the indicated concentrations of docycycline. Cytoplasmic RNA recovered from each culture was analyzed by RT-qPCR for ZEB1, ZEB2, FHOD1 and PPM1F, and the results were normalized to RPLP0 mRNA. Results are presented as fold-increase over uninduced cells and represent the mean ± standard deviation (n=6).

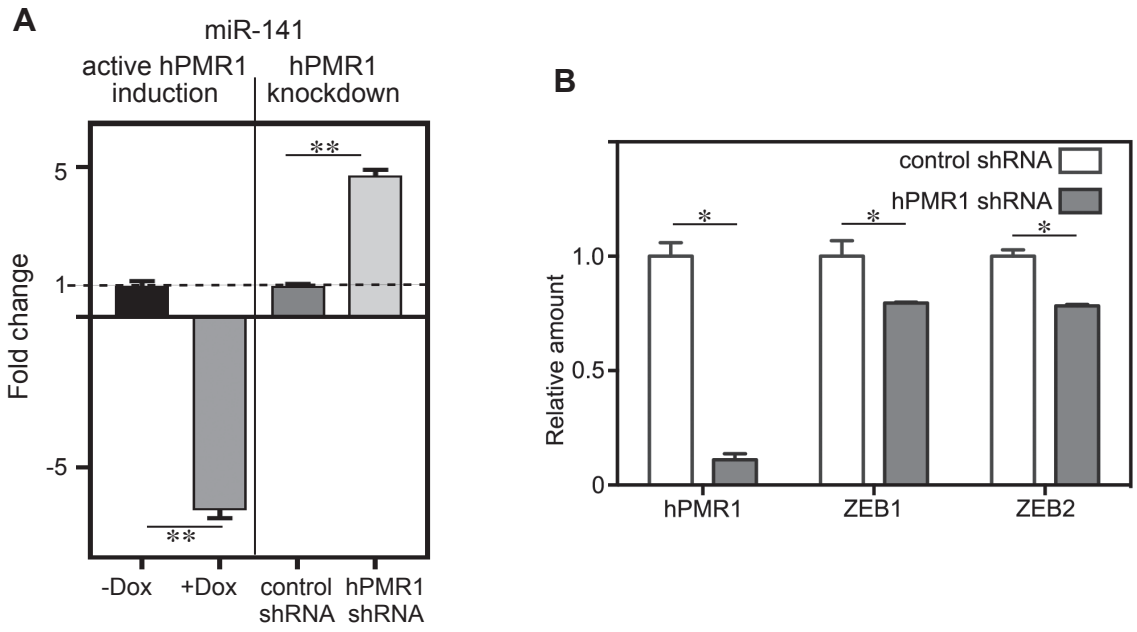

Figure S3. hPMR1-mediated changes in miR-141 and miR-200 target mRNAs. A. Taqman assay for miR-141 was performed on cytoplasmic RNA from uninduced and doxycycine-treated MCF-7 cells carrying the active hPMR1 transgene (left lanel) and on cytoplasmic RNA of MCF-7 cells that were transduced with lentivirus expressing control or hPMR1 shRNAs. The data were normalized to RNU48 and represent the mean ± standard deviation (n=3). The double asterisk (**) indicates $p<.0001$ by Student's T-test. **B.** Cytoplasmic RNA from MCF-7 cells expressing control or hPMR1 shRNA was analyzed by RT-qPCR for hPMR1, ZEB1 or ZEB2, and normalized to RPLP0 mRNA. The asterisk (*) indicates $p<.05$ by Student's T-test. The results are presented as the amount relative to control shRNA and represent the mean ± standard deviation (n=3).

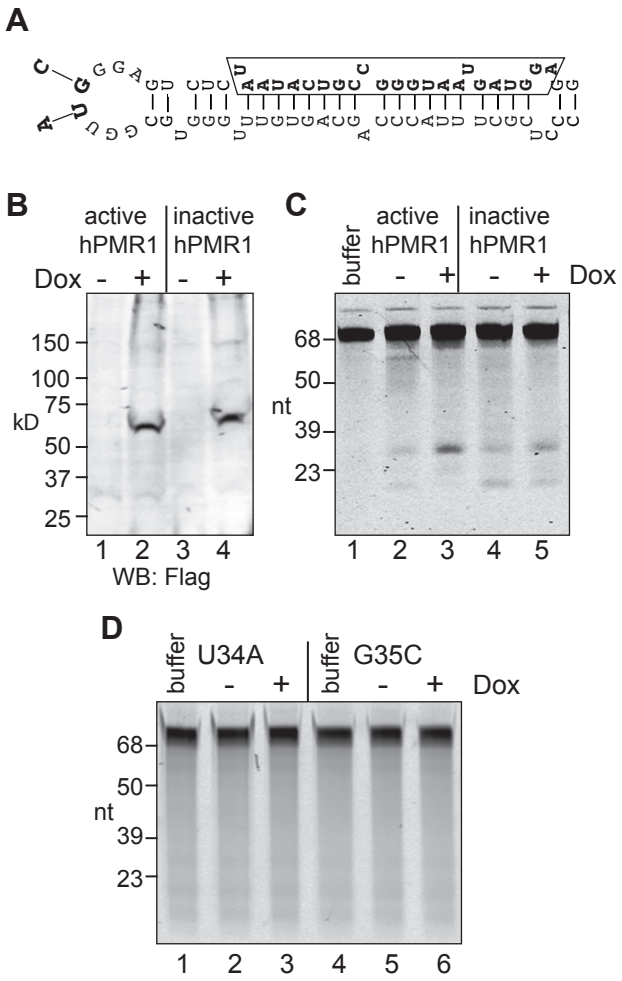

Figure S4. hPMR1 cleaves within the apical loop of pre-miR-200c. **A.** The secondary structure of pre-miR-200c is shown with the mature microRNA indicated by the box and the cleavage site UG dinucleotides identified along with mutations that inactivate cleavage. **B.** The induction of active and inactive hPMR1 was confirmed by Western blotting with anti-FLAG antibody after 24 hr treatment ± doxycycline. **C.** Cytoplasmic extracts from uninduced and induced MCF-7 cells were bound to streptavidin paramagnetic beads which were then washed to remove unbound protein. Fluorescent-labeled pre-miR-200c (see Supplementary Materials and Methods) was incubated for 1 hr at 37°C in buffer (lane 1), or with beads bound to protein from uninduced or induced cells expressing active (lanes 2 and 3) or inactive (lanes 4 and 5) hPMR1 transgenes. The reaction products were separated on a 20% polyacrylamide/urea gel and visualized by LiCor. **D.** The experiment in **C** was repeated with forms of pre-miR-200c indicated in A with U at position 34 changed to A (U34A) or G at position 35 changed to C (G35C). These changes were shown previously to prevent cleavage by PMR1 (1). Based on results in **D** we suspect that the residual activity seen in **C** is due to carryover of endogenous hPMR1.

1) Chernokalskaya,E., Dompenciel,R.E. and Schoenberg,D.R. (1997) Cleavage properties of a polysomal ribonuclease involved in the estrogen-regulated destabilization of albumin mRNA. Nucleic Acids Res. 25, 735-742.

Table S1. Primers used for RT-qPCR of hPMR1 and miR-200 target mRNAs

Transcript	NCBI ID	Primer name	Sequence
hPMR1	NM 144651	hPMR1 Forward	TTGTAAAGGCAGAATACCCACA
		hPMR1 Reverse	CAGCTTGTTTACTGCACAGC
RPLP0	NM 001002	RPLP0 Forward	GGAGAAACTGCTGCCTCATATC
		RPLP0 Reverse	CAGCAGCTGGCACCTTATT
ZEB1	NM 030751.5	ZEB1 Forward	GGCTCCTATAGCTCACACATAAG
		ZEB1 Reverse	TGCTGAAAGAGACGGTGAAG
ZEB2	NM 013241.2	ZEB2 Forward	AAGGTGTCAGAGGTGAAGGA
		ZEB2 Reverse	CAGGGCAGGGATTTCTGAATAG
FHOD1	NM 013241.2	FHOD1 Forward	GAAGAGCGGCAGAAGATTGA
		FHOD1 Reverse	AATGGAGGCAAGAGTCATCAG
PPM1F	NM 014634.3	PPM1F Forward	ATTCTGCCAAGGCTCCAAG
		PPM1F Reverse	CCGAGACGAACGCAGATAAA

Table S2. Primers used to generate pre-miR-200c transcripts. Shown are the DNA primers used for *in vitro* transcription to generate wild-type (WT) pre-miR-200c and cleavage site mutants. The locations of the TG dinucleotide clevage site and each of the single base mutantions are in red.

Primer name	**Sequence**
Pre-miR-200c WT	
Forward Primer	5'-GAAATTAATACGACTCACTATAGGGCCCTCGTCTTACCCAGCAGTGTTTGGGTGCGGTTGGGAGTCTCTA
Reverse primer	5'-CCTCCATCATTACCCGGCAGTATTAGAGACTCCCAACCGCACCCAAACAC
Pre-miR-200c U34A	
Forward Primer	5'-GAAATTAATACGACTCACTATAGGGCCCTCGTCTTACCCAGCAGTGTTTGGGTGCGGTAGGGAGTCTCTA
Reverse primer	5'-CCTCCATCATTACCCGGCAGTATTAGAGACTCCCTACCGCACCCAAACAC
Pre-miR-200c G35C	
Forward Primer	5'-GAAATTAATACGACTCACTATAGGGCCCTCGTCTTACCCAGCAGTGTTTGGGTGCGGTTCGGAGTCTCTA
Reverse primer	5'-CCTCCATCATTACCCGGCAGTATTAGAGACTCCGAACCGCACCCAAACAC