

A pervasive RT-qPCR artifact inflates RNA knockdown by RNA-targeting CRISPR

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Supplementary Information Table of Contents

1. Supplementary Figure 1. Flow cytometry gating strategy
2. Supplementary Table 1. qPCR primer, spacer, and gRNA sequences
3. Supplementary Table 2. Statistical test information

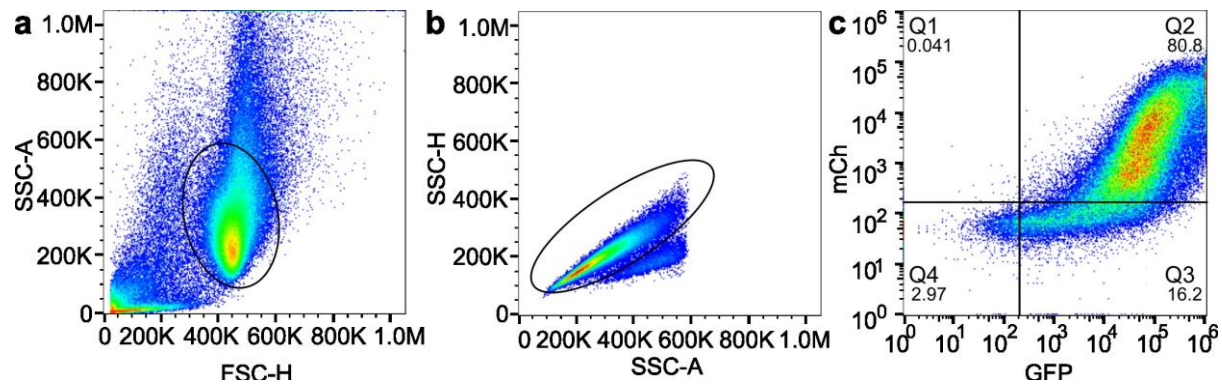


Figure S1. Flow cytometry gating strategy. **a)** Live cell gate (AU) **b)** Single cell population selected from the live cell population (AU) **c)** GFP (BL1-H) and mCherry (YL2-H) expression (AU) for single cell population. Data displayed is a WT Csm (GFP+) with NT gRNA co-transfected with the mCherry reporter. The sample is representative of all transfection efficiencies in the manuscript.

Supplementary Table 1. qPCR, spacer, siRNA, ddASO sequences.

Target	Distance from gRNA (nt)	qPCR primer F	qPCR primer R
BRCA1 spanning	0	TACATCAGGCCTTCA TCCTGAGGATTTAT C	ACAATTAGGTGGGCTTAGATT TCTACTGACTACTA
BRCA1 upstream	145	GCCTCCCCAACTTAA GCCAT	ACCATTCTGCTCCGTTTGGT
BRCA1 upstream	302	ACTGCCATGCTCAGA GAATC	GGATCACTGGCCAGTAAGTC
BRCA1 upstream	519	CAGATGGGCTGGAA GTAAGG	GATTCTCTGAGCATGGCAGT
BRCA1 upstream	633	ACAGCATGAGAACA GCAGTT	CCTTACTTCCAGCCCATCTG
BRCA1 downstream	177	GCACAGCAGAAACC TACAAC	CTGGGAAAGTATCGCTGTCA
BRCA1 downstream	4113	TGTTGGCTTAGGGCT GGAAG	CCTCCCCAATGTTCCACTCC
XIST spanning	0	GTTGTATCGGGAGG CAGTAAGAATCATCT TT	GAAAAGCACACAGCAAAGAC AAAGAGGC
XIST upstream	234	CCTACCGCTTTGGCA GAGAA	TCCTCTGCCTGACCTGCTAT
XIST downstream	37	TGGTTAGCATGGTGG TGGAC	ACATATGACAACGCCTGCCA
XIST downstream	89	AAGGTCTTGCCGCA GTGTAA	ACATATGACAACGCCTGCCA
XIST downstream	277	GGGCTTGCCGCATT GTAAA	GCCCCTCCACTTCTTTCTCC
XIST downstream	4777	CCCTCATCCCCACTT TTCCC	AAGGGGTCGGAGAGTAGGA C
XIST downstream	12485	AGCACCTGCCAGCA ACAGCT	AGCGTTGGCACAGTCCACCA
GAPDH	N/A	CCAGAACATCATCCC TGCCTCTACTG	GGAAATGAGCTTGACAAAGT GGTCGTTG
Cas13b/CasRx mCh spanning	0	GGGCGAGGAGGATA ACATGG	CAAGTAGTCGGGGATGTCGG
Csm mCh spanning, Cas13b/CasRx mCh downstream	976 downstream	GCGGCGTTTCGTGAA GGCATC	CGCCGACGAGCATCCAATCA

Csm upstream	mCh	65	AAAGATCCAGCCAAA CCTCC	TTTCCTCAAGTACGGTGCTC
Csm upstream	mCh	207	AGATACAGTGGCTCC GGTAA	GGAGGTTTGGCTGGATCTTT
Csm upstream	mCh	376	AGCTGTACAAGGCG GATATG	GTTACCGGAGCCACTGTATC
Csm upstream	mCh	509	GCAGAAGAAGACCA TGGGCT	GGTGTAGTCCTCGTTGTGGG

Target	ddASO sequence
Anti-BRCA1 gRNA	GCAATATGGAACCTCGAATTAAATATCCACAATGTTTCCGT/3ddC/

Target	Csm spacer sequence
mCh reporter	CTTCTTCTCCTCCATGTTTGCCCATGGTAAAA
BRCA1	ATTGTGGATATTTAATTTCGAGTTCCATATTGC
XIST	GCCACTTGAACACTGCGACAGAACTGGATCCG
CKB	TTGATATGCACACCTGCCCGCAGCCCGGTGCC
NT	TCTCCGAACGTGTCACGTCTTTAGCGACTAAA

Target	PspCas13b/CasRx spacer sequence
mCherry (CasRx)	ACCTTGAAGCGCATGAACTCCT
mCherry (PspCas13b)	ACCTTGAAGCGCATGAACTCCTTGATGATG
NT	TCACCAGAAGCGTACCATACTC

Target	Synthetic gRNA sequence
BRCA1	rA*rC*rGr*rGrArArArCrArUrUrGrUrGrGrArUrArUrUrArArUrUrCrGrArGrUrUrCrCr ArUrArU*rU*rG*rC
NT	rA*rC*rGr*rGrArArArCrTrCrTrCrCrGrArArCrGrTrGrTrCrArCrGrTrCrTrTrArGrCrGr ArCrT*rA*rA*rA

Purple text indicates sequences designed by Colognori et al. /3ddC/ = 3' terminal dideoxycytidine. rN = RNA base. rN*= Phosphorothioate modified RNA.

Supplementary Table 2. Statistical test information.

Figure	Sample	Amplicon	Statistical test	Degrees of Freedom	p-value
1c	WT	BRCA1 spanning	One sample T-test	2	0.0002
1c	WT vs. RD	BRCA1 spanning	Two sample Welch's T-test	2.229	0.1013
1c	WT vs. RD	XIST spanning	Two-sample Welch's T-test	2.494	0.9632
1d	WT	N/A	One-sample T-test	2	<0.0001
1d	RD	N/A	One-sample T-test	2	0.2175
1e	WT	mCh Csm spanning	One-sample T-test	2	<0.0001
1e	WT	mCh Csm upstream	One-sample T-test	2	<0.0001
1e	RD	mCh Csm spanning	One sample T-test	2	0.0066
1e	RD	mCh Csm upstream	One sample T-test	2	0.6809
1f	WT	BRCA1 spanning	One-sample T-test	2	0.0002
1f	WT	BRCA1 upstream	One-sample T-test	2	<0.0001
1f	WT	BRCA1 downstream	One-sample T-test	2	0.0014
1f	WT	BRCA1 downstream vs. spanning	Two-sample Welch's T-test	2.952	0.0018
1f	RD	BRCA1 spanning	One-sample T-test	2	0.0040
1f	RD	BRCA1 upstream	One-sample T-test	2	0.0033
1f	RD	BRCA1 downstream	One-sample T-test	2	0.8575
1g	WT	XIST spanning	One-sample T-test	2	0.0004
1g	WT	XIST upstream	One-sample T-test	2	0.0012
1g	WT	XIST downstream	One-sample T-test	2	0.0113
1g	WT	XIST downstream vs. spanning	Two-sample Welch's T-test	2.909	0.0011
1g	RD	XIST spanning	One-sample T-test	2	<0.0001
1g	RD	XIST upstream	One-sample T-test	2	0.0135
1g	RD	XIST downstream	One-sample T-test	2	0.0143
1h	Cas13b WT	N/A	One-sample T-test	2	0.0004
1h	Cas13b RD	N/A	One-sample T-test	2	0.2130
1h	CasRx WT	N/A	One-sample T-test	2	0.0043
1h	CasRx RD	N/A	One-sample T-test	2	0.0796
1i	Cas13b WT	mCh Cas13b/CasRx spanning	One sample T-test	2	<0.0001
1i	Cas13b WT	mCh Cas13b/CasRx downstream	One sample T-test	2	0.0016
1i	Cas13b WT	mCh Cas13b/CasRx downstream vs. spanning	Two-sample Welch's T-test	2.072	0.0013

1i	Cas13b RD	mCh Cas13b/CasRx spanning	One-sample T-test	2	0.0031
1i	Cas13b RD	mCh Cas13b/CasRx downstream	One-sample T-test	2	0.2857
1i	CasRx WT	mCh Cas13b/CasRx spanning	One-sample T-test	2	<0.0001
1i	CasRx WT	mCh Cas13b/CasRx downstream	One-sample T-test	2	0.0050
1i	CasRx WT	mCh Cas13b/CasRx downstream vs. spanning	Two-sample Welch's T-test	2.063	0.655
1i	CasRx RD	mCh Cas13b/CasRx spanning	One-sample T-test	2	0.0068
1i	CasRx RD	mCh Cas13b/CasRx downstream	One-Sample T-test	2	0.0544
2a	WT	BRCA1 spanning	One sample T-test	2	0.0001
2a	WT	BRCA1 upstream	One sample T-test	2	0.0005
2a	WT	BRCA1 downstream	One sample T-test	2	0.0039
2a	RD	BRCA1 spanning	One sample T-test	2	0.0077
2a	RD	BRCA1 upstream	One sample T-test	2	0.3222
2a	RD	BRCA1 downstream	One sample T-test	2	0.9135
2b	All	BRCA1 spanning	One way ANOVA	8	<0.0001
2b	All	BRCA1 upstream	One way ANOVA	8	<0.0001
2b	All	BRCA1 downstream	One way ANOVA	8	0.5110
2c	All RD	BRCA1 spanning	One way ANOVA	11	0.0004
2c	All RD	BRCA1 upstream	One way ANOVA	11	0.0007
2c	All RD	BRCA1 downstream	One way ANOVA	11	0.6240
2c	WT 0 vs 10 μ M ddASO	BRCA1 spanning	Two-sample Welch's T-test	3.701	0.0097
2c	WT 0 vs 10 μ M ddASO	BRCA1 upstream	Two-sample Welch's T-test	2.824	0.0666
2c	WT 0 vs 10 μ M ddASO	BRCA1 downstream	Two-sample Welch's T-test	3.510	0.7130
2e	BRCA1 RD	BRCA1 spanning	One sample T-test	2	0.0254
2e	BRCA1 RD	BRCA1 upstream	One sample T-test	2	0.1340
2e	BRCA1 RD	BRCA1 downstream	One sample T-test	2	0.5003
2e	XIST RD	XIST spanning	One sample T-test	1	0.4702
2e	XIST RD	XIST upstream	One sample T-test	1	0.7206
2e	XIST RD	XIST downstream	One sample T-test	1	0.2308
2e	BRCA1 WT	All	Welch's ANOVA	W(2.000, 1.421) = 2.311	0.3575

2e	XIST WT	All	Welch's ANOVA	W(2, 1.344) = 0.4767	0.6975
EDF1a	BRCA1 RD	N/A	One sample T-test	2	0.4625
EDF1a	BRCA1 WT	N/A	One sample T-test	2	0.0646
EDF1c	WT vs DD	N/A	Two-sample Welch's T-test	3.987	0.0635
EDF1c	RD vs DM	N/A	Two-sample Welch's T-test	2.606	0.5040
EDF1d	DD	mCh Csm spanning	One-sample T-test	2	0.0002
EDF1d	DD	mCh Csm upstream	One-sample T-test	2	0.0007
EDF1d	DM	mCh Csm spanning	One-sample T-test	2	0.0261
EDF1d	DM	mCh Csm upstream	One-sample T-test	2	0.1306
EDF1h	Cas13b RD	mCh Cas13b/CasRx spanning	One sample T-test	1	0.5
EDF1h	Cas13b RD	mCh Cas13b/CasRx downstream	One sample T-test	1	0.5

All T-tests are Two-tailed.