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Supplemental information

MicroRNA function can be reversed by altering target gene expression levels

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Table S1: Literature Analysis of Random MicroRNAs, Related to Main Text and STAR Methods

MicroRNA	Apoptosis		Cell Proliferation		Invastion/Metastasis		# of References
	Increase	Decrease	Increase	Decrease	Increase	Decrease	
miR-25		x	x		x	x	458
miR-451	x	x	x	x	x	x	462
miR-185	x	x		x		x	313
miR-195	x			x		x	628
miR-202	x	x	x	x	x	x	159
miR-30a	x	x	x	x	x	x	283
miR-411	x	x	x	x		x	69
miR-8	x			x		x	73
miR-22	x	x	x	x		x	668
miR-324	x	x	x	x	x	x	149
miR-148a	x	x	x	x	x	x	105
miR-372	x	x	x	x	x	x	135
miR-92	x	x	x	x	x	x	169
miR-184	x	x	x	x	x	x	236
miR-218	x			x		x	466
miR-223	x	x	x	x	x	x	1195
miR-153	x	x	x	x	x	x	202
miR-409	x		x	x	x	x	111
miR-322	x	x	x	x			65
miR-139	x	x	x	x	x	x	365

# of microRNAs that exhibit both a promoting effect and a suppressive effect in one or more categories (apoptosis, cell proliferation, and/or invasion/metastasis): 17 (85%)

# of microRNAs that exhibit both a promoting effect and a suppressive effect in all three categories: 11 (55%)

Table S2: List of Model Equations, Related to STAR Methods

Protein/Protein Complex	Equation Governing Concentration Over Time (i.e., Equation for Rate of Change in Concentration)
Unbound, Inactivated BAK1	$\frac{d[inbak1]}{dt} = k_{bak1} * (1-f) - d_{bak1} * [inbak1] - k_{act} * (AddAct + [puma]) * [inbak1] + k_{deact} * [bak1]$
Unbound, Activated BAK1	$\frac{d[bak1]}{dt} = k_{act} * (AddAct + [puma]) * [inbak1] - k_{deact} * [bak1] - b_{bak1}^{mcl1} * [bak1] * [mcl1] + u_{bak1}^{mcl1} * [bak1.mcl1] - b_{bak1}^{bcl2} * [bak1] * [bcl2] + u_{bak1}^{bcl2} * [bak1.bcl2] - b_{bak1}^{bclw} * [bak1] * [bclw] + u_{bak1}^{bclw} * [bak1.bclw] - 2 * b_{bak_{dimer}} * [bak1]^2 + 2 * u_{bak_{dimer}} * [bak_{dimer}] - d_{bak1} * [bak1] + 2 * d_{bak1} * [bak_{dimer}] + d_{mcl1} * [bak1.mcl1] + d_{bcl2} * [bak1.bcl2] + d_{bclw} * [bak1.bclw]$
BAK1 Homodimer	$\frac{d[bak_{dimer}]}{dt} = b_{bak_{dimer}} * [bak1]^2 - u_{bak_{dimer}} * [bak_{dimer}] - 2 * d_{bak1} * [bak_{dimer}]$
MCL1•BAK1	$\frac{d[bak1.mcl1]}{dt} = b_{bak1}^{mcl1} * [bak1] * [mcl1] - u_{bak1}^{mcl1} * [bak1.mcl1] - d_{bak1} * [bak1.mcl1] - d_{mcl1} * [bak1.mcl1]$
BCL2•BAK1	$\frac{d[bak1.bcl2]}{dt} = b_{bak1}^{bcl2} * [bak1] * [bcl2] - u_{bak1}^{bcl2} * [bak1.bcl2] - d_{bak1} * [bak1.bcl2] - d_{bcl2} * [bak1.bcl2]$
BCL-w•BAK1	$\frac{d[bak1.bclw]}{dt} = b_{bak1}^{bclw} * [bak1] * [bclw] - u_{bak1}^{bclw} * [bak1.bclw] - d_{bak1} * [bak1.bclw] - d_{bclw} * [bak1.bclw]$
Total BAK1	$\frac{d[bak1_{total}]}{dt} = \frac{d[inbak1]}{dt} + \frac{d[bak1]}{dt} + \frac{d[bak1.mcl1]}{dt} + \frac{d[bak1.bcl2]}{dt} + \frac{d[bak1.bclw]}{dt} + 2 * \frac{d[bak_{dimer}]}{dt}$
BAX mRNA	$\frac{d[mRNA_{bax}]}{dt} = k_{mRNA_{bax}} \left(0.5 + \frac{([p53]/2)^{1.8}}{73^{1.8} + ([p53]/2)^{1.8}}\right) - d_{mRNA_{bax}}[mRNA_{bax}]$
Unbound, Inactivated BAX	$\frac{d[inbax]}{dt} = k_{bax} * [mRNA_{bax}] - d_{bax} * [inbax] - k_{act} * (AddAct + [puma]) * [inbax] + k_{deact} * [bax]$
Unbound, Activated BAX	$\frac{d[bax]}{dt} = k_{act} * (AddAct + [puma]) * [inbax] - k_{deact} * [bax] - b_{bax}^{mcl1} * [bax] * [mcl1] + u_{bax}^{mcl1} * [bax.mcl1] - b_{bax}^{bcl2} * [bax] * [bcl2] + u_{bax}^{bcl2} * [bax.bcl2] - b_{bax}^{bclw} * [bax] * [bclw] + u_{bax}^{bclw} * [bax.bclw] - 2 * b_{bax_{dimer}} * [bax]^2 + 2 * u_{bax_{dimer}} * [bax_{dimer}] - d_{bax} * [bax] + 2 * d_{bax} * [bax_{dimer}] + d_{mcl1} * [bax.mcl1] + d_{bcl2} * [bax.bcl2] + d_{bclw} * [bax.bclw]$

BAX Homodimer	$\frac{d[bax_{dimer}]}{dt} = b_{bax_{dimer}} * [bax]^2 - u_{bax_{dimer}} * [bax_{dimer}] - 2 * d_{bax} * [bax_{dimer}]$
MCL1•BAX	$\frac{d[bax.mcl1]}{dt} = b_{bax}^{mcl1} * [bax] * [mcl1] - u_{bax}^{mcl1} * [bax.mcl1] - d_{bax} * [bax.mcl1] - d_{mcl1} * [bax.mcl1]$
BCL2•BAX	$\frac{d[bax.bcl2]}{dt} = b_{bax}^{bcl2} * [bax] * [bcl2] - u_{bax}^{bcl2} * [bax.bcl2] - d_{bax} * [bax.bcl2] - d_{bcl2} * [bax.bcl2]$
BCL-w•BAX	$\frac{d[bax.bclw]}{dt} = b_{bax}^{bclw} * [bax] * [bclw] - u_{bax}^{bclw} * [bax.bclw] - d_{bax} * [bax.bclw] - d_{bclw} * [bax.bclw]$
Total BAX	$\frac{d[bax_{total}]}{dt} = \frac{d[inbax]}{dt} + \frac{d[bax]}{dt} + \frac{d[bax.mcl1]}{dt} + \frac{d[bax.bcl2]}{dt} + \frac{d[bax.bclw]}{dt} + 2 * \frac{d[bax_{dimer}]}{dt}$
Unbound MCL1	$\frac{d[mcl1]}{dt} = k_{mcl1} * (1-f) - d_{mcl1} * [mcl1] - b_{mcl1}^{puma} * [mcl1] * [puma] + u_{mcl1}^{puma} * [mcl1.puma] - b_{bak1}^{mcl1} * [bak1] * [mcl1] + u_{bak1}^{mcl1} * [bak1.mcl1] - b_{bax_{mcl1}} * [bax] * [mcl1] + u_{bax_{mcl1}} * [bax.mcl1] + d_{bak1} * [bak1.mcl1] + d_{bax} * [bax.mcl1] + d_{puma} * [mcl1.puma]$
MCL1•PUMA	$\frac{d[mcl1.puma]}{dt} = b_{mcl1}^{puma} * [mcl1] * [puma] - u_{mcl1}^{puma} * [mcl1.puma] - d_{mcl1} * [mcl1.puma] - d_{puma} * [mcl1.puma]$
Total MCL1	$\frac{d[mcl1_{total}]}{dt} = \frac{d[mcl1]}{dt} + \frac{d[bak1.mcl1]}{dt} + \frac{d[bax.mcl1]}{dt} + \frac{d[mcl1.puma]}{dt}$
Unbound BCL2	$\frac{d[bcl2]}{dt} = k_{bcl2} * (1-f) - d_{bcl2} * [bcl2] - b_{bcl2}^{puma} * [bcl2] * [puma] + u_{bcl2}^{puma} * [bcl2.puma] - b_{bcl2}^{bmf} * [bcl2] * [bmf] + u_{bcl2}^{bmf} * [bcl2.bmf] - b_{bak1}^{bcl2} * [bak1] * [bcl2] + u_{bak1}^{bcl2} * [bak1.bcl2] - b_{bax}^{bcl2} * [bax] * [bcl2] + u_{bax}^{bcl2} * [bax.bcl2] + d_{bak1} * [bak1.bcl2] + d_{bax} * [bax.bcl2] + d_{puma} * [bcl2.puma] + d_{bmf} * [bcl2.bmf]$
BCL2•PUMA	$\frac{d[bcl2.puma]}{dt} = b_{bcl2}^{puma} * [bcl2] * [puma] - u_{bcl2}^{puma} * [bcl2.puma] - d_{bcl2} * [bcl2.puma] - d_{puma} * [bcl2.puma]$
BCL2•BMF	$\frac{d[bcl2.bmf]}{dt} = b_{bcl2}^{bmf} * [bcl2] * [bmf] - u_{bcl2}^{bmf} * [bcl2.bmf] - d_{bcl2} * [bcl2.bmf] - d_{bmf} * [bcl2.bmf]$
Total BCL2	$\frac{d[bcl2_{total}]}{dt} = \frac{d[bcl2]}{dt} + \frac{d[bak1.bcl2]}{dt} + \frac{d[bax.bcl2]}{dt} + \frac{d[bcl2.puma]}{dt} + \frac{d[bcl2.bmf]}{dt}$

Unbound BCL-w	$\frac{d[bclw]}{dt} = k_{bclw} * (1-f) - d_{bclw} * [bclw] - b_{bclw}^{bmf} * [bclw] * [bmf] + u_{bclw}^{bmf} * [bclw.bmf] - b_{bclw}^{puma} * [bclw] * [puma] + u_{bclw}^{puma} * [bclw.puma] - b_{bak1}^{bclw} * [bak1] * [bclw] + u_{bak1}^{bclw} * [bak1.bclw] - b_{bax}^{bclw} * [bax] * [bclw] + u_{bax}^{bclw} * [bax.bclw] + d_{bak1} * [bak1.bclw] + d_{bax} * [bax.bclw] + d_{bmf} * [bclw.bmf] + d_{puma} * [bclw.puma]$
BCL-w•BMF	$\frac{d[bclw.bmf]}{dt} = b_{bclw}^{bmf} * [bclw] * [bmf] - u_{bclw}^{bmf} * [bclw.bmf] - d_{bclw} * [bclw.bmf] - d_{bmf} * [bclw.bmf]$
BCL-w•PUMA	$\frac{d[bclw.puma]}{dt} = b_{bclw}^{puma} * [bclw] * [puma] - u_{bclw}^{puma} * [bclw.puma] - d_{bclw} * [bclw.puma] - d_{puma} * [bclw.puma]$
Total BCL-w	$\frac{d[bclw_{total}]}{dt} = \frac{d[bclw]}{dt} + \frac{d[bak1.bclw]}{dt} + \frac{d[bax.bclw]}{dt} + \frac{d[bclw.puma]}{dt} + \frac{d[bclw.bmf]}{dt}$
PUMA mRNA	$\frac{d[mRNA_{Puma}]}{dt} = k_{mRNA_{Puma}} * \left(\frac{([p53]/2)^{1.8}}{6.9^{1.8} + ([p53]/2)^{1.8}}\right) - d_{mRNA_{Puma}}[mRNA_{Puma}]$
Unbound PUMA	$\frac{d[puma]}{dt} = k_{puma} * [mRNA_{Puma}] * (1-f) - d_{puma} * [puma] - b_{mcl1}^{puma} * [mcl1] * [puma] + u_{mcl1}^{puma} * [mcl1.puma] - b_{bcl2}^{puma} * [bcl2] * [puma] + u_{bcl2}^{puma} * [bcl2.puma] - b_{bclw}^{puma} * [bclw] * [puma] + u_{bclw}^{puma} * [bclw.puma] + d_{mcl1} * [mcl1.puma] + d_{bcl2} * [bcl2.puma] + d_{bclw} * [bclw.puma]$
Total PUMA	$\frac{d[puma_{total}]}{dt} = \frac{d[puma]}{dt} + \frac{d[mcl1.puma]}{dt} + \frac{d[bcl2.puma]}{dt} + \frac{d[bclw.puma]}{dt}$
Unbound BMF	$\frac{d[bmf]}{dt} = k_{bmf} * (1-f) - d_{bmf} * [bmf] - b_{bcl2}^{bmf} * [bcl2] * [bmf] + u_{bcl2}^{bmf} * [bcl2.bmf] - b_{bclw}^{bmf} * [bclw] * [bmf] + u_{bclw}^{bmf} * [bclw.bmf] + d_{bcl2} * [bcl2.bmf] + d_{bclw} * [bclw.bmf]$
Total BMF	$\frac{d[bmf_{total}]}{dt} = \frac{d[bmf]}{dt} + \frac{d[bcl2.bmf]}{dt} + \frac{d[bclw.bmf]}{dt}$
P53	$\frac{d[p53]}{dt} = k_{p53} * (1-f) - d_{p53} * [p53]$

f = fraction of gene's mRNA inhibited by miR-125b (value between 0 and 1)

k = production rate constant

d = degradation rate constant

b = binding (association) rate constant

u = unbinding (dissociation) rate constant

k_{act} = BAK1/BAX activation rate constant

k_{deact} = BAK1/BAX deactivation rate constant

[*X*] = concentration of unbound protein X

[*X.Y*] = concentration of protein X-protein Y complex

AddAct = baseline additional activator

Model Parameters

Table S3: Production and degradation rate constants, Related to STAR Methods

Protein	Concentration (nM)	Half-Life (hrs)	k_{deg} ($min.^{-1}$)	k_{prod} ($nM\ min.^{-1}$)	Source References
P53	140	0.33	0.03466	4.852	(Gaglia et al., 2013; Ma et al., 2005; Maltzman and Czyzyk, 1984)
PUMA	400	3.4	0.003398	1.359	(Fricker et al., 2010; Lindner et al., 2013)
BMF	400	3.4	0.003398	1.359	(Grespi et al., 2010)
MCL1	400	1	0.01155	4.621	(Dai et al., 2009; Lindner et al., 2013; Stewart et al., 2010)
BCL2	400	20	0.0005776	0.23104	(Lindner et al., 2013; Rooswinkel et al., 2014)
BCL-w	400	20	0.0005776	0.23104	(Rooswinkel et al., 2014)
BAX	600	11.6	0.0009959	0.5975	(Lindner et al., 2013; Wang et al., 2010)
BAK1	600	48	0.0002407	0.1444	(Ferrer et al., 2012; Lindner et al., 2013)

Table S4: Protein complex association and dissociation rate constants, Related to STAR Methods

Protein Complex	K_D (nM)	Association Rate ($nM.^{-1}\ min.^{-1}$)	Dissociation Rate ($min.^{-1}$)	Source References
MCL1•PUMA	2.62	0.005954	0.0156	(Chen et al., 2005; Ku et al., 2011; Lindner et al., 2013)
MCL1•BAX	39.5	0.00039492	0.0156	(Chen et al., 2005; Ku et al., 2011; Lindner et al., 2013)
MCL1•BAK1	1.33	0.01173	0.0156	(Chen et al., 2005; Ku et al., 2011; Lindner et al., 2013)
BCL2•BMF	13.9	0.0006042	0.0084	(Chen et al., 2005; Ku et al., 2011; Lindner et al., 2013)
BCL2•PUMA	3.04	0.002763	0.0084	(Chen et al., 2005; Ku et al., 2011; Lindner et al., 2013)
BCL2•BAX	15.1	0.00055629	0.0084	(Chen et al., 2005; Ku et al., 2011; Lindner et al., 2013)
BCL2•BAK1	70.4	0.00011934	0.0084	(Chen et al., 2005; Ku et al., 2011; Lindner et al., 2013)
BCL-w•BMF	10.9	0.014862	0.162	(Chen et al., 2005; Ku et al., 2011)
BCL-w•PUMA	5.13	0.031578	0.162	(Chen et al., 2005; Ku et al., 2011)
BCL-w•BAX	22.9	0.007074	0.162	(Chen et al., 2005; Ku et al., 2011)
BCL-w•BAK1	114	0.0014208	0.162	(Chen et al., 2005; Ku et al., 2011)
BAX Homodimer	15	0.000768	0.01158	(Lindner et al., 2013)
BAK1 Homodimer	15	0.000768	0.01158	(Lindner et al., 2013)

Table S5: Rate constants for BAX/BAK1 activation and deactivation, Related to STAR Methods

Reaction	Activation Rate ($nM.^{-1}\ min.^{-1}$)	Deactivation Rate ($min.^{-1}$)	Source References
BAX Activation	0.0001542	0.01542	(Dai et al., 2011; Lindner et al., 2013)
BAK1 Activation	0.0001542	0.01542	(Dai et al., 2011; Lindner et al., 2013)

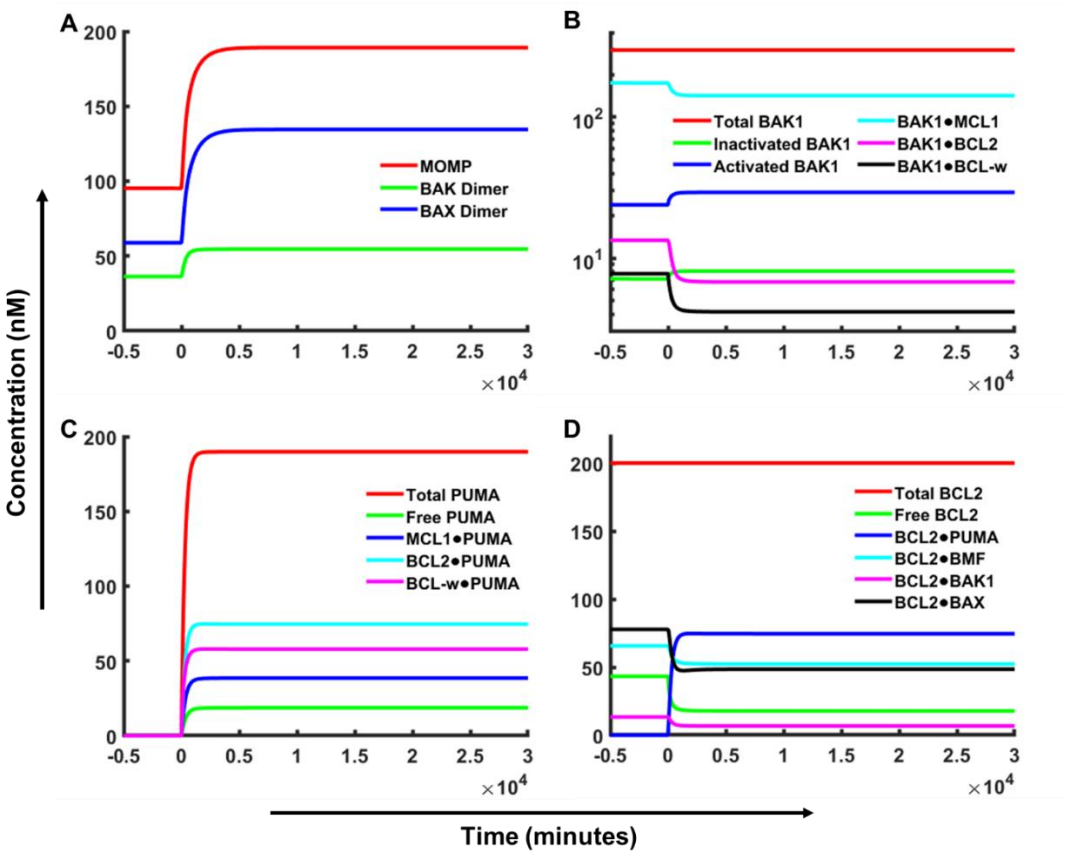

Figure S1: Typical concentration vs. time plots for several components of the model, Related to STAR Methods. The model was run with the default parameter values and 400 nM baseline activator. At time zero, after the model had reached steady-state, P53 was activated to generate an apoptotic response, and the model continued to run until steady-state was again achieved. The MOMP and respective BAK1 and BAX dimer concentrations are shown in (A). The total BAK1, PUMA, and BCL2 concentrations, and the concentrations of each of their respective states are shown in (B), (C), and (D), respectively.

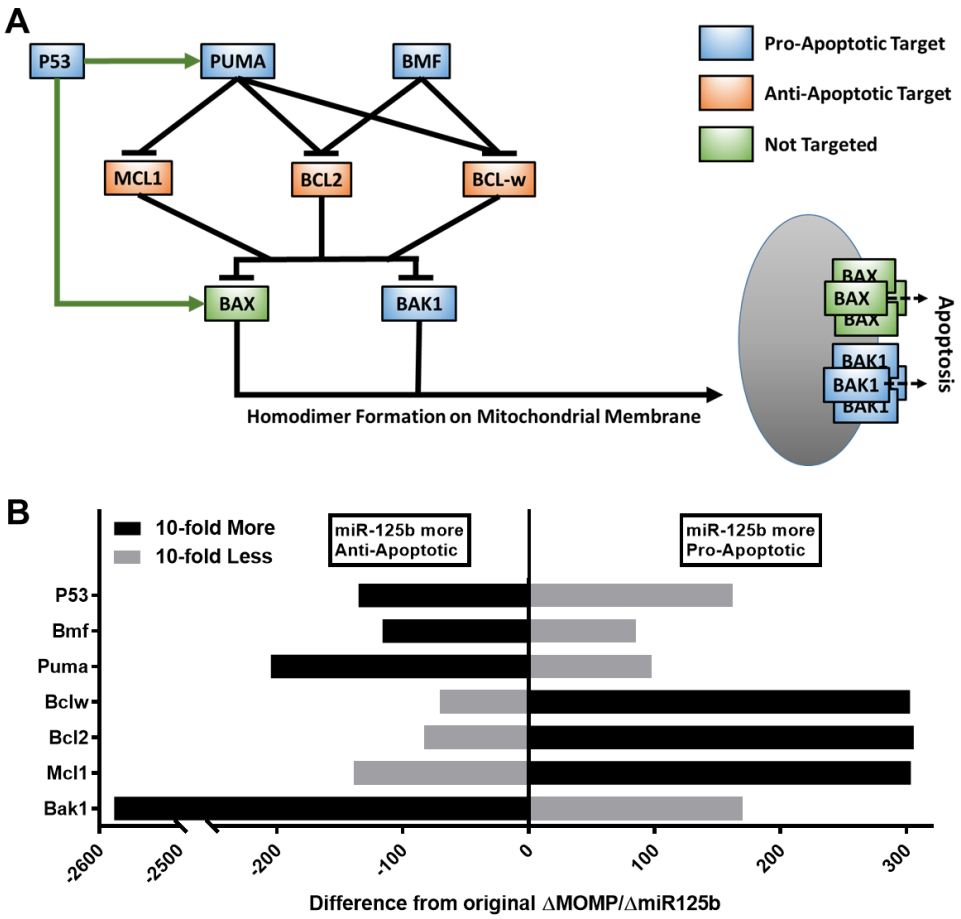

Figure S2: Results with indirect activation model of the apoptosis pathway, Related to Figure 3.

A) Diagram of the indirect activation model of the apoptosis pathway. Blue and orange boxes represent pro-apoptotic and anti-apoptotic members, respectively, of the pathway that are targeted by miR-125b. Green boxes represent closely interacting members of the pathway that are not targeted by miR-125b. Green arrowheads from P53 represent transcriptional activation. Hammerheads represent inhibition by direct binding. BAX and BAK1 are constitutively active, and, when not inhibited, form homodimers on the mitochondrial membrane, which are in turn linked together to form pores that result in apoptosis.

B) Difference in the apoptoticity (average slope of curve for MOMP vs. miR-125b) compared to default parameter values for each miR-125b-targeted component of the indirect activation model when the production rate was increased 10-fold (black) or decreased 10-fold (gray). Mir-125b is more anti-apoptotic left of the vertical axis and more pro-apoptotic right of the vertical axis.

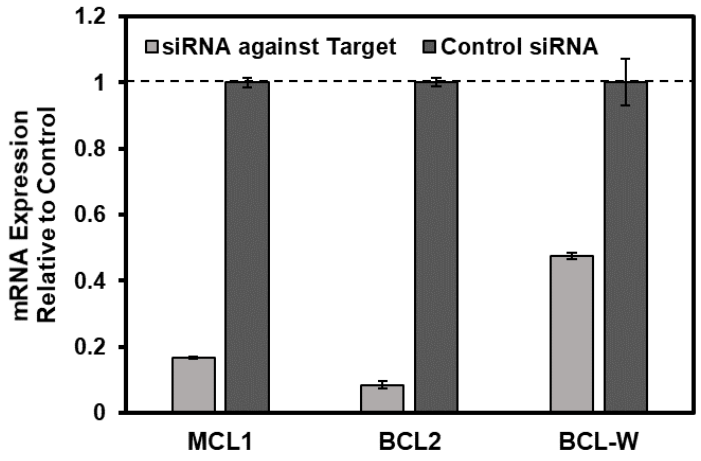

Figure S3: siRNA-mediated inhibition of target mRNAs, Related to Figure 4. RT-qPCR results for NCI-H23 cells co-transfected with the indicated siRNA and miRNA mimic negative control. Data are shown as mean ± S.E.; $P < 0.01$ for all samples.