SUPPORTING INFORMATION:

RNA Polymerase Tags To Monitor Multidimensional Protein-Protein Interactions Reveal Pharmacological Engagement of Bcl-2 Proteins

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Table S1 | List of plasmids used in this work.

Vector Name	Antibiotic resistance	Origin	Purpose	Map
p2-22	Carb	SC101	P_{T7} luciferase reporter plasmid	a*
p2-64	Carb	SC101	P_{CGG} luciferase reporter plasmid	a
pJin242	Carb	SC101	P_{K1F} luciferase reporter plasmid	a
pJin243	Carb	SC101	P_{CTGA} luciferase reporter plasmid	a
pJin244	Carb	SC101	P_{T3} luciferase reporter plasmid	a
p2-39	Chlor	CloDF13	ZB-linker-T7 $RNAP_C$ expression plasmid	b
p3-13	Chlor	CloDF13	ZB-linker-CGG $RNAP_C$ expression plasmid	b
pJin 245	Chlor	CloDF13	ZB-linker-T3 $RNAP_C$ expression plasmid	b
pJin 246	Chlor	CloDF13	ZB-linker-T3-R $RNAP_C$ expression plasmid	b
pJin 247	Chlor	CloDF13	ZB-linker-K1F-a $RNAP_C$ expression plasmid	b
pJin 248	Chlor	CloDF13	ZB-linker-K1F-b $RNAP_C$ expression plasmid	b
pJin 249	Chlor	CloDF13	ZB-linker-K1F-c $RNAP_C$ expression plasmid	b
pJin 252	Chlor	CloDF13	ZB-linker-CTGA $RNAP_C$ expression plasmid	b
p5-74	Spec	P15a	T7 $RNAP_N$ (N-29-1)-linker-ZA (I13I, L20I) expression plasmid	c
p9-02	Spec	P15a	T7 $RNAP_N$ (N-29-1) expression plasmid	d
p10-54	Chlor	CloDF13	(Bcl-2)-linker-T7 $RNAP_C$ expression plasmid	e
p7-77	Chlor	CloDF13	(Mcl-1)-linker-T7 $RNAP_C$ expression plasmid	e
p7-78	Chlor	CloDF13	(Bcl-W)-linker-T7 $RNAP_C$ expression plasmid	e
p7-79	Chlor	CloDF13	(Bcl-X_L)-linker-T7 $RNAP_C$ expression plasmid	e
p8-1	Spec	P15a	T7 $RNAP_N$ (N-29-1**)-linker-tBID expression plasmid	f
p8-35	Spec	P15a	T7 $RNAP_N$ (N-29-1)-linker-NOXA expression plasmid	f
p9-27	Spec	P15a	T7 $RNAP_N$ (N-29-1)-linker-deadBID expression plasmid	f
p9-49	Kan	pBR322	T7 $RNAP_N$ (N-29-1)-linker-tBID mammalian expression plasmid	g
p9-51	Kan	pBR322	T7 $RNAP_N$ (N-29-1)-linker-NOXA mammalian expression plasmid	g
p9-52	Kan	pBR322	T7 $RNAP_N$ (N-29-1)-linker-deadBID mammalian expression plasmid	g
p11-19	Kan	pBR322	T7 $RNAP_N$ (D5-18)-linker-tBID mammalian expression plasmid	g
p10-50	Kan	pBR322	T7 $RNAP_N$ (D5-18)-linker-NOXA mammalian expression plasmid	g
p11-20	Kan	pBR322	T7 $RNAP_N$ (D5-18)-linker-deadBID mammalian expression plasmid	g

p9-54	Kan	pBR322	(Bcl-X_L)-linker-T7 $RNAP_C$, P_{T7}-GFP expression plasmid	h
p9-61	Kan	pBR322	(Bcl-W)-linker-T7 $RNAP_C$, P_{T7}-GFP expression plasmid	h
p9-65	Kan	pBR322	(Mcl-1)-linker-T7 $RNAP_C$, P_{T7}-GFP expression plasmid	h
p10-53	Kan	pBR322	(Bcl-2)-linker-T7 $RNAP_C$, P_{T7}-GFP(RNA-1) expression plasmid	h
p9-53	Kan	pBR322	(Mcl-1)-linker-CGG $RNAP_C$, P_{CGG}-RFP expression plasmid	i
p10-52	Kan	pBR323	(Mcl-1)-linker-T3 $RNAP_C$, P_{T3}-RNA-2 expression plasmid	j
p12-75	Kan	pBR324	(Bcl-X_L)-linker-K1F $RNAP_C$, P_{K1F}-RNA-4 expression plasmid	l
p11-63	Kan	pBR325	(Bcl-W)-linker-CTGA $RNAP_C$, P_{CTGA}-RNA-3 expression plasmid	k

* Vector maps for each construct type shown in Figure S1.

** $RNAP_N$ mutation sites:

N-29-1: L32S, E35G, K98R, Q107K, T122S, A144T

D5-18: L24R, L32S, E35G, R57C, E63K, K98R, Q104K, Q107K, T122S, A144T

Table S2 | List of sequences of split RNAP fusions. The structures and amino acid sequences of the pro- and anti-apoptotic protein fusions used in this study.

Name	peptide sequence
N-tBID	RNAP(1-179)–GGSGSGSS–SQEDIIRNIARHLAQVGDSMDRSIPPG
N-NOXA	RNAP(1-179)–GGSGSGSS–PADLKDECAQLRRIGDKVNLRQKLLNM
N-dBID	RNAP(1-179)–GGSGSGSS–SQEDQVGDSMDRSIPPG
C-Bcl-2	MAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQPGHTPH PAASRDPVARTSPLQTPAAPGAAAGPALSPVPPVVHLTLRQAGDDFSRRYRRDFAEMS SQLHLTPFTARGRFATVVEELFRDGVNWGRIVAFFEFGGVMCVESVNREMSPLVDNIAL WMTEYLNRHLHTWIQDNGGWDAFVELYGPSMRPLFDFSWLSLK–TSGGSG–RNAP(180+)
C-Mcl-1	MEAPAADAIMSPEEELDGYEPEPLGKRPAVLPLLELVGESGNNTSTDGSLPSTPPPAEE EEDELYRQSLEIISRYLREQATGAKDTKPMGRSGATSRKALETLRRVGDGVQRNHETVF QGMLRKLDIKNEDDVKSLSRVMIHVFSDGVTNWGRIVTLISFGAFVAKHLKTINQESCIEP LAESITDVLVRTKRDWLVKQRGWDGFVEFFHVEDLEGGIR–TSGGSG–RNAP(180+)
C-Bcl-W	MATPASAPDTRALVADFVGYKLRQKGYVCGAGPGEGPAADPLHQAMRAAGDEFETRF RRTFSDLAAQLHVTPGSAQQRFTQVSDELFQGGPNWGRLVAFFVFGAALCAESVNKEM EPLVGQVQEWMVAYLETRLADWIHSSGGWAEFTALYGDGALEEARRLREGNWASVR–TSGGSG–RNAP(180+)
C-Bcl-X_L	MSQSNRELVVDFLSYKLSQKGYSWSQFSDVEENRTEAPEGTESEMETPSAINGNPSWH LADSPAVNGATGHSSSLDAREVIPMAAVKQALREAGDEFELRYRRAFSDLTSQLHITPGT AYQSFEQVVNELFRDGVNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAWMATYLND HLEPWIQENGGWDTFVELYGNNAAAESRKGQERFNR–TSGGSG–RNAP(180+)
ZA (L13I, L20I)	ALKKELQANKKEIAQLKWEIQALKKELAQ
ZB	MASEQLEKKLQALEKKLAQLEWKNQALEKKLAQ

Table S3 | List of sequences of RNA templates and primers for one-by-four qPCR assays.

Name	DNA sequence or oligo sequence
RNA-1 template	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGG GCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCA CCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTAC GGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTC TTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTC AAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGA CACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACG GCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCT ATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCC GCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAG AACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCT GAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACAT GGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACG AGCTGTACAAGTAA
RNA-1-primer-for	TGACCCTGAAGTTCATCTGC
RNA-1-primer-rev	GAAGTCGTGCTGCTTCATGT
RNA-2 template	ATGGCCTCCTCCGAGGACGTCATCAAGGAGTTCATGCGCTTCAAGGTGCGC ATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGA GGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGG GCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCCAGTACG GCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGC TGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACG GCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTC ATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTA ATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCC CGAGGACGGCGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAGG ACGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAG CCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAAGCTGGACATCACC TCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAGGG CCGCCACTCCACCGGCGCCTAA
RNA-2-primer-for	CGCGCAGCTTCACCTTGTAG
RNA-2-primer-rev	GGAGCGCGTGATGAACTTCG
RNA-3 template	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT CGAGCTGGACGGCGACAAACGTTTGGTTAAATTAGGTCGCATCTCTGAGGA GTGTGGTTTTGATACCGTATGGTTACTGGAGCATCATTTCACGGAGTTTGGT TTGCTTGGTAACCCTTATGTCGCTGCTGCATATTTACTTGGCGCGACTAAAA AATTGAATGTAGGAACTGCCGCTATTGTTCTTCCCACAGCCCATCCAGTACG CCAACTTGAAGATGTGAATTTATTGGATCAAATGTCAAAAGGACGATTTCGGT TTGGTATTTGCCGAGGGCTTTACAACAAGGACTTTCGCGTATTCGGCACAGA TATGAATAACAGTCGCGCCTTAGCGGAATGCTGGTACGGGCTGATAAAGAAT GGCATGACAGAGGGATATATGGAAGCTGATAATGAACATATCAAGTTCCATA AGGTAAAAGTAAACCCCGCGGCGTATAGCAGAGGTGGCGCACCGGTTTATG TGGTGGCTGAATCAGCTTCGACGACTGAGTGGGCTGCTCAATTTGGCCTAC CGATGATATTAAGTTGGATTATAAATACTAACGAAAAGAAAGCACAACTTGAG CTTTATAATGAAGTGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACT CTCGGCATGGACGAGCTGTACAAGTAA
RNA-3-primer-for	GTAGGAACTGCCGCTATTGT
RNA-3-primer-rev	TACGCGAAAGTCCTTGTTGTAA

RNA-4 template	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT CGAGCTGGACGGCGACGAAGACTGTTTATATTTGAACATCTATACTCCCGCT GATCTGACTAAGCGTGGTCGCCTGCCGGTAATGGTTTGGATTCACGGTGGA GGCCTGGTGCTTGGGGGTGCGCCCATGTACGACGGCGTTGTTCTTGCTGCA CATGAAAATGTGGTAGTCGTTGCCATCCAGTATCGCTTAGGGATCTGGGGCT TTTTCTCTACCGGAGACGAGCACAGCCGTGGTAATTGGGGACATTTAGATCA GGTCGCCGCCCTGCACTGGGTTCAAGAAAATATCGCCAATTTTGGTGGCGA TCCTGGCAGTGTCACGATTTTTGGGGAGTCCGCAGGCGGAGAGTCCGTATC TGTGTTAGTCCTTTCCCCGTTGGCAAAAAATTTATTCCATCGCGCCATTAGTG AATCAGGGGTCGCGCTGACGGTTGCTTTGGTACGTAAGGACATGAAGGCTG CTGCTAAGCAGATTGCTGTACTGGCTGGATGTAAAACTACTACCAGTGCGGT ATTTGTGCATTGCTTACGCCAAAAATCAGAGGACGAGCTTCTTGATTTAACGT TGAAGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATG GACGAGCTGTACAAGTAA
RNA-4-primer-for	AGCAATGCACAAATACCGCA
RNA-4-primer-rev	TGCTTTGGTACGTAAGGACA
GAPDH-primer-for	TGCACCACCAACTGCTTAGC
GAPDH-primer-rev	GGCATGGACTGTGGTCATGAG
T7 RNAP-primer-for	ACCAAAGGACTGCTTACGCT
T7 RNAP-primer-rev	TCAGGGAACGGAACCTTATC

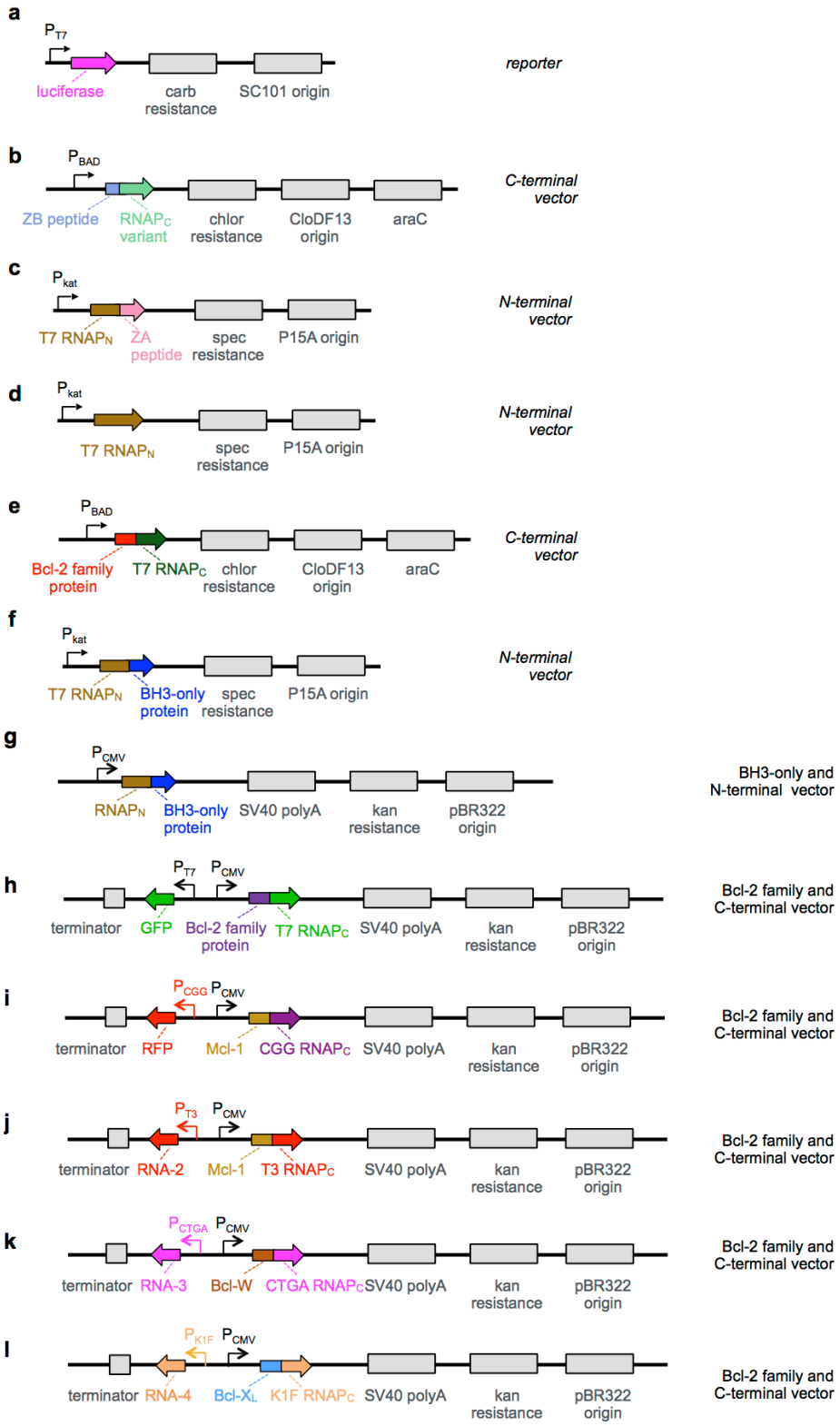

Figure S1 | Vector maps for all constructs used in this work. Vector maps corresponding to the vectors listed in Table S1.

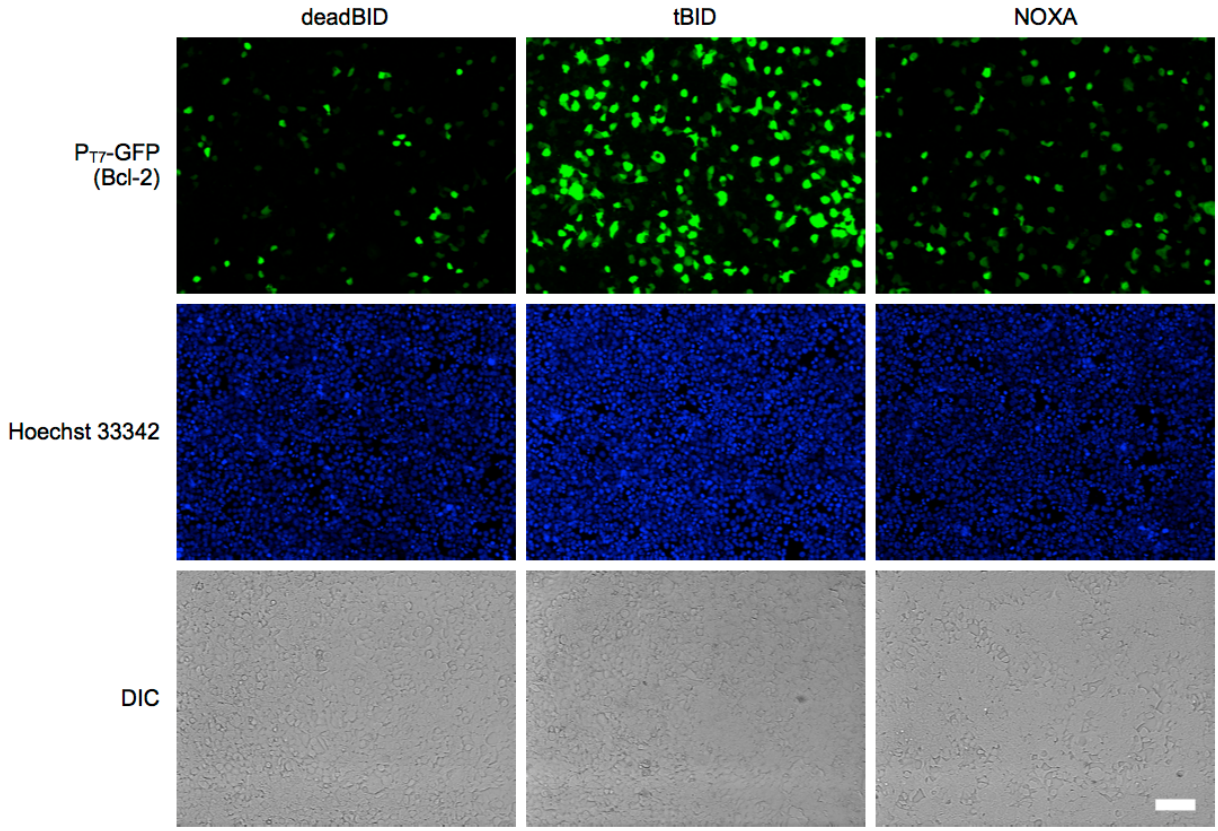

Figure S2 | Complete imaging series from Figure 2B for Bcl-2. HEK293T cells cotransfected with the plasmids shown in (Figure 2A) with $RNAP_N$-BH3-only protein and Bcl-2-$RNAP_C$. 30 h after transfection, the cells were loaded with 1 μM Hoechst 33342 and analyzed by fluorescence microscopy. 100 μm scale bar shown.

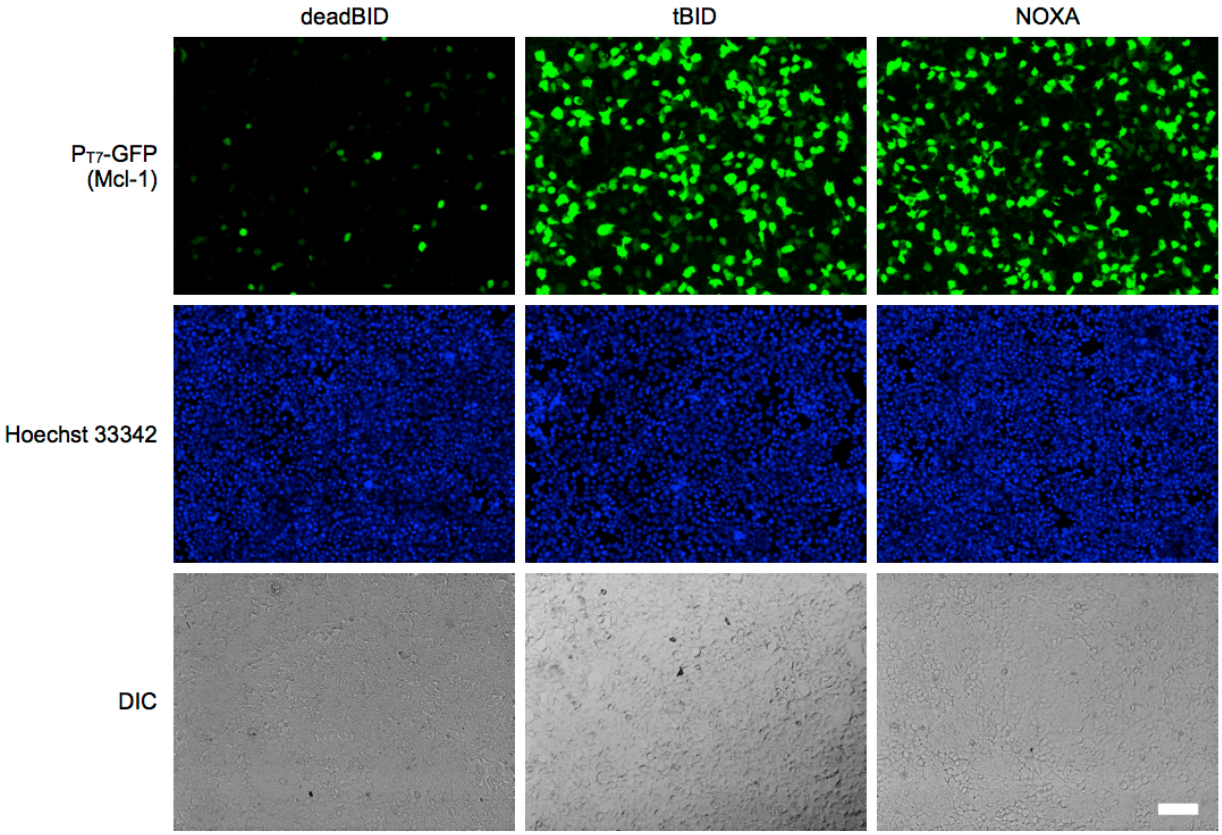

Figure S3 | Complete imaging series from Figure 2B for Mcl-1. HEK293T cells cotransfected with the plasmids shown in (Figure 2A) with $RNAP_N$- BH3-only protein and Mcl-1-$RNAP_C$. 30 h after transfection, the cells were loaded with 1 µM Hoechst 33342 and analyzed by fluorescence microscopy. 100 µm scale bar shown.

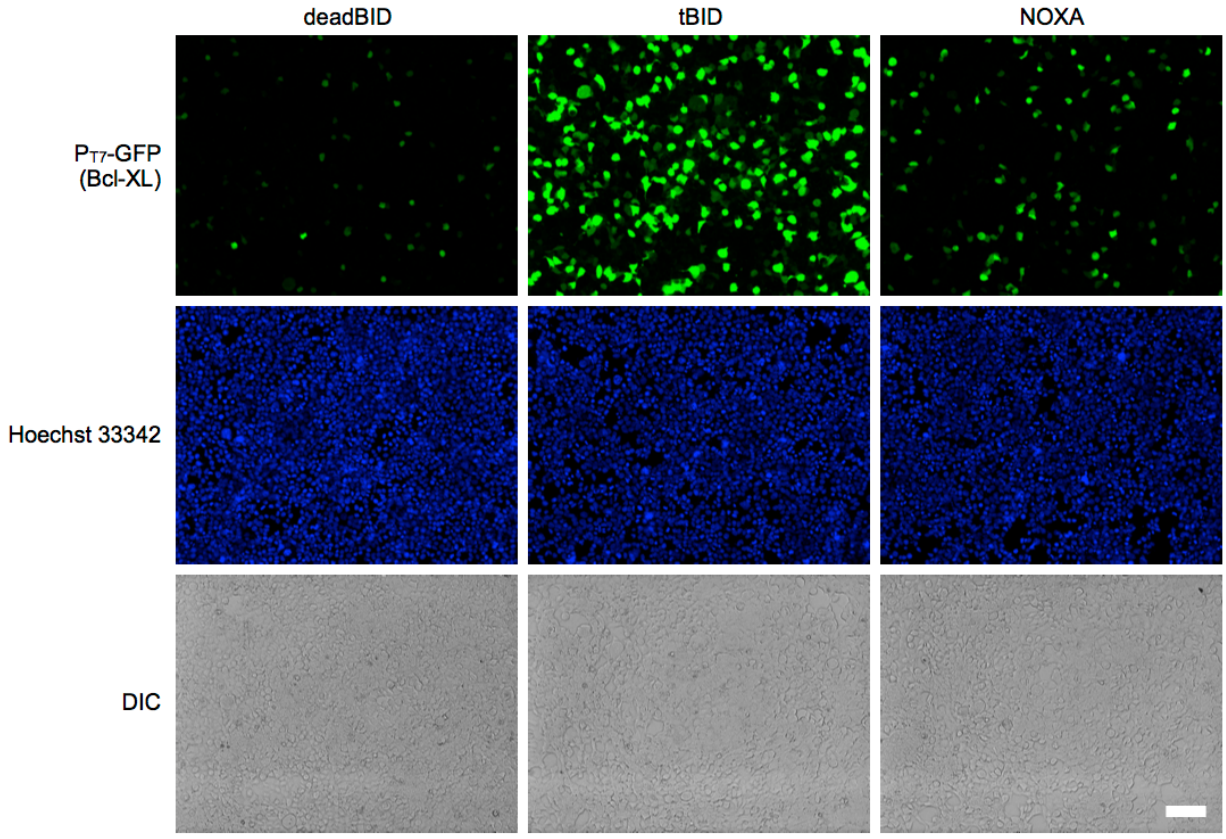

Figure S4 | Complete imaging series from Figure 2B for Bcl-X_L. HEK293T cells cotransfected with the plasmids shown in (Figure 2A) with $RNAP_N$- BH3-only protein and Bcl-X_L-$RNAP_C$. 30 h after transfection, the cells were loaded with 1 μM Hoechst 33342 and analyzed by fluorescence microscopy. 100 μm scale bar shown.

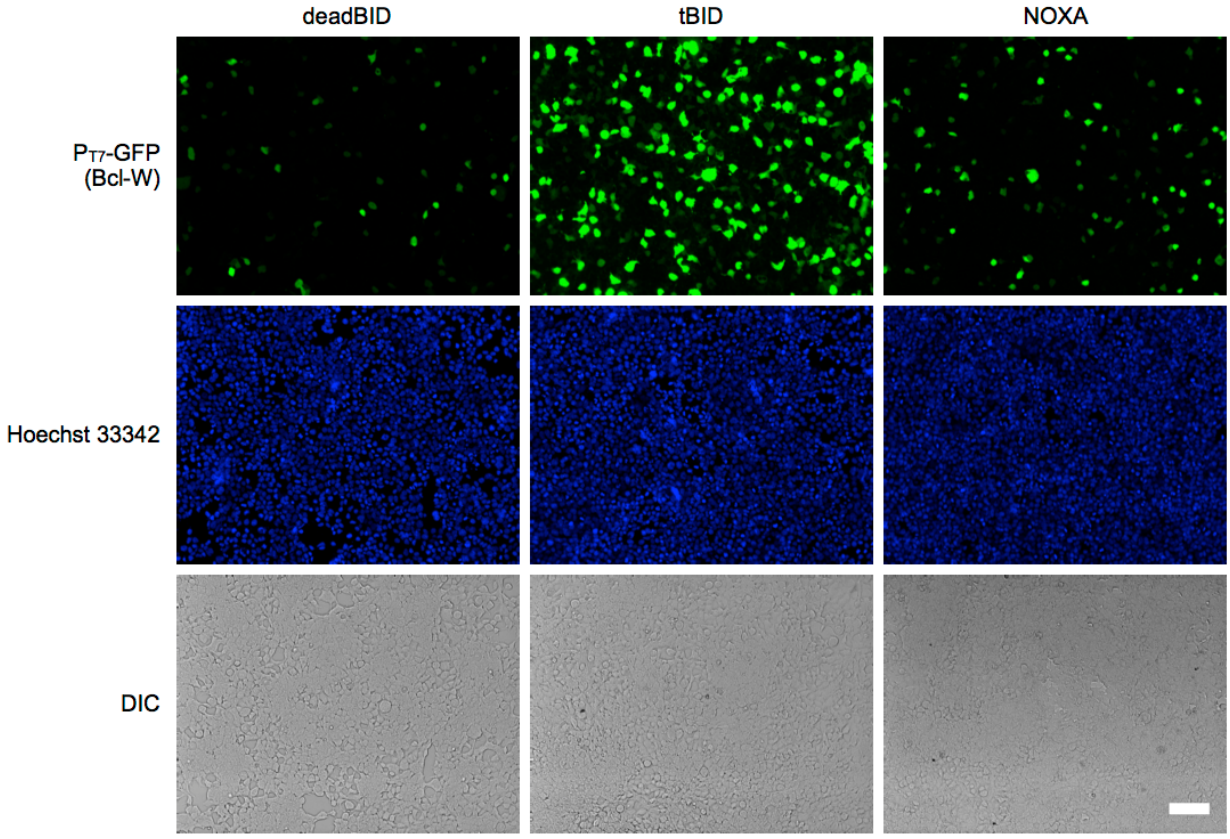

Figure S5 | Complete imaging series from Figure 2B for Bcl-W. HEK293T cells cotransfected with the plasmids shown in (Figure 2A) with $RNAP_N$- BH3-only protein and Bcl-W-$RNAP_C$. 30 h after transfection, the cells were loaded with 1 μM Hoechst 33342 and analyzed by fluorescence microscopy. 100 μm scale bar shown.

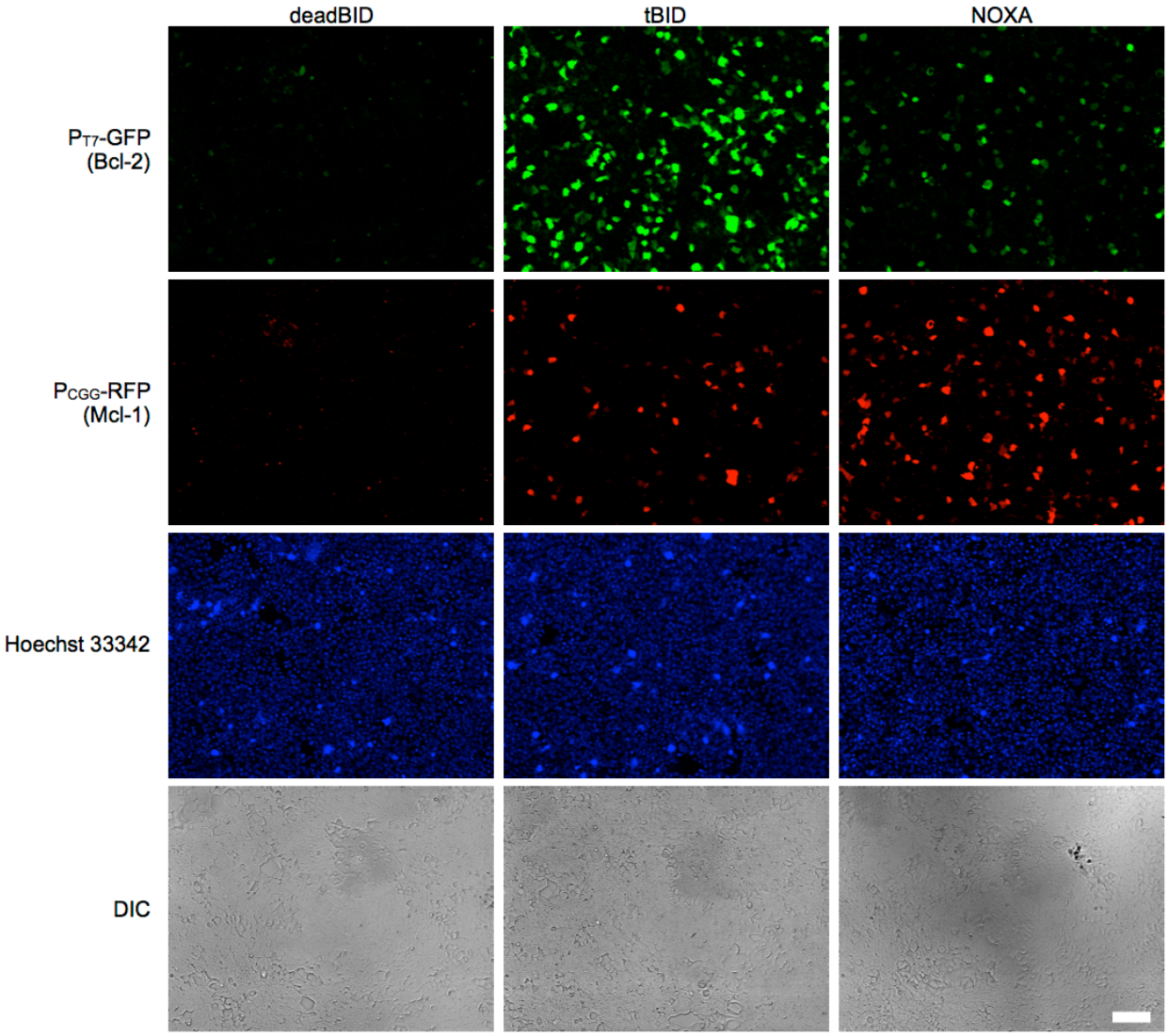

Figure S6 | Complete imaging series from Figure 3B. HEK293T cells cotransfected with the plasmids shown in (Figure 3A). 30 h after transfection, the cells were loaded with 1 μM Hoechst 33342 and analyzed by fluorescence microscopy. 100 μm scale bar shown.

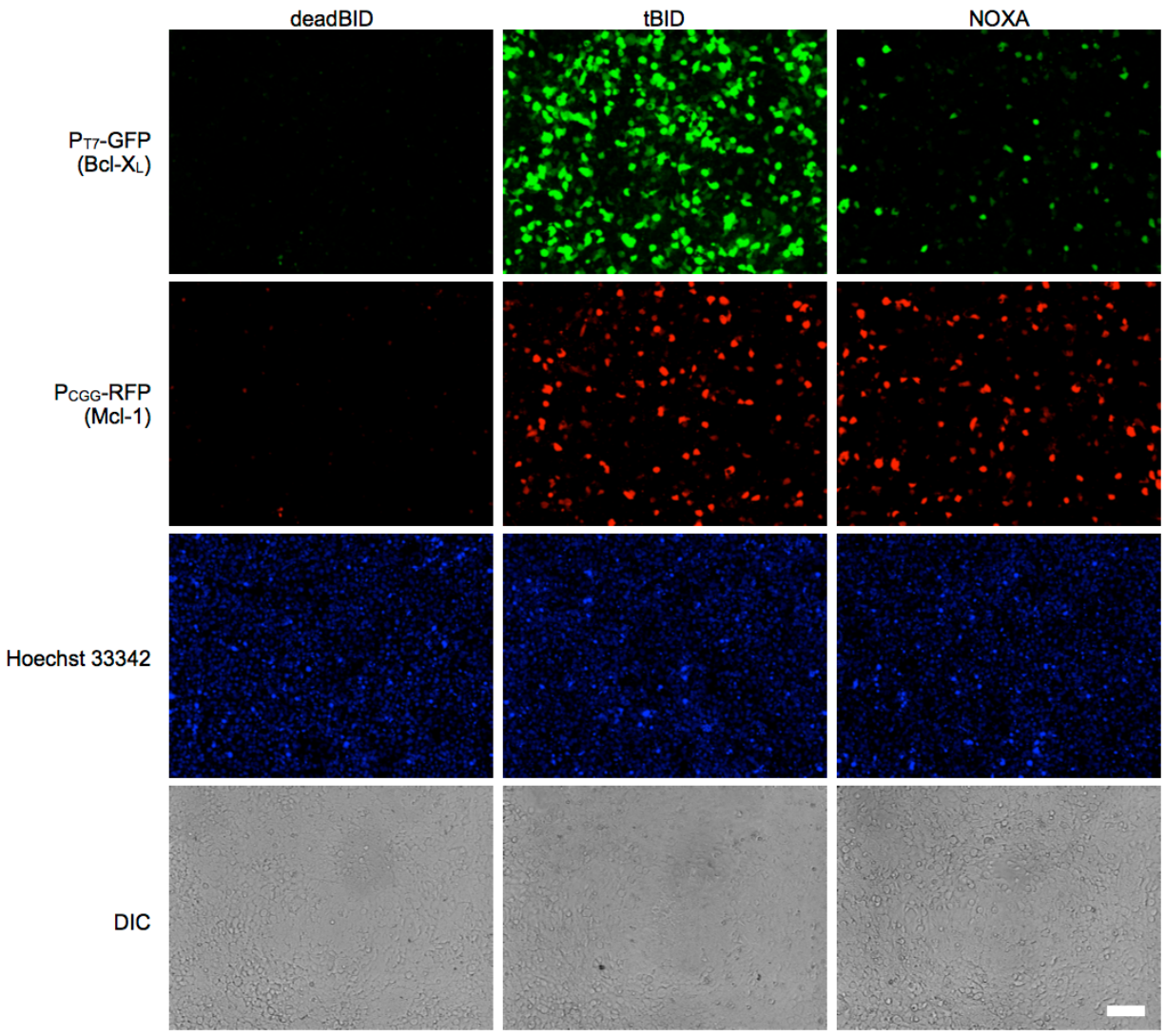

Figure S7 | Split RNAP biosensors to monitor Bcl-X_L and Mcl-1 simultaneously. HEK293T cells cotransfected with the plasmids shown in (Figure 3A) except with Bcl-X_L-T7-$RNAP_C$. 30 h after transfection, the cells were loaded with 1 μM Hoechst 33342 and analyzed by fluorescence microscopy. 100 μm scale bar shown.

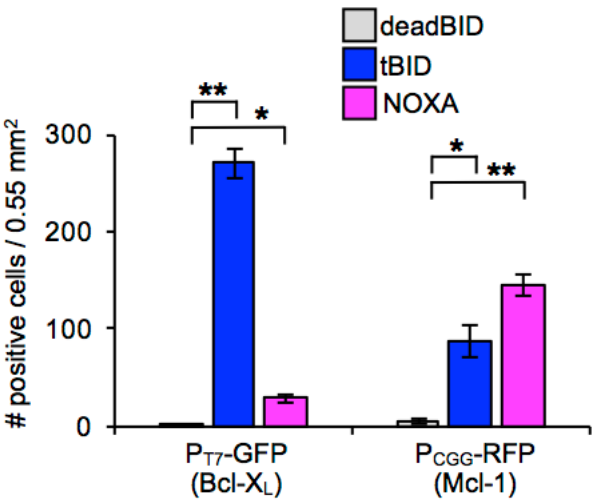

Figure S8 | Quantification of Fig. S7. (error bars are ± s.e.m, n = 5). Student's *t*-test; **P* < 0.01, ***P* < 0.001.

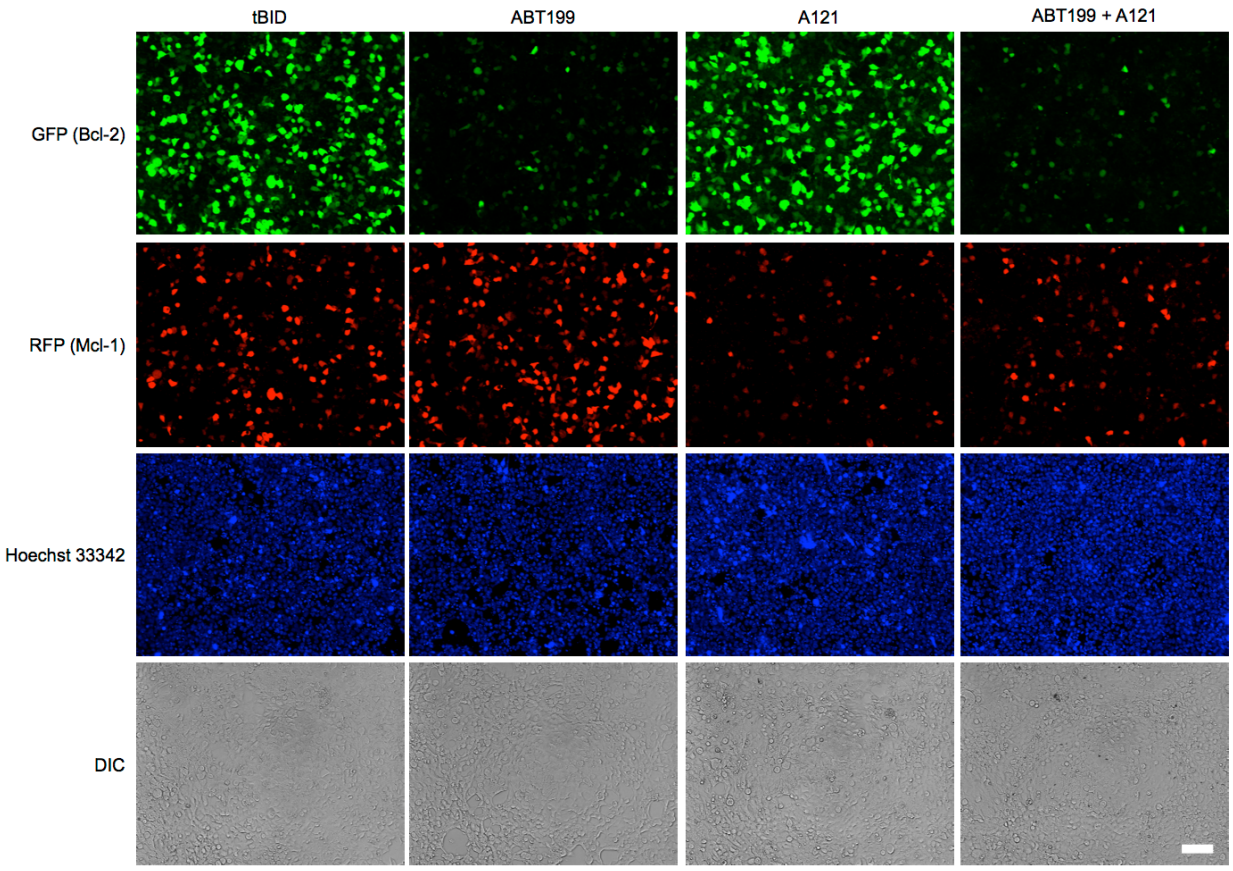

Figure S9 | Complete imaging series from Fig. 4A. HEK293T cells cotransfected with the plasmids shown in (Fig. 3A) along with DMSO carrier control, 0.5 μM ABT-199, 10 μM A1210477, or a combination of both 0.5 μM ABT-199 and 10 μM A1210447. 30 h after transfection, the cells were loaded with 1 μM Hoechst 33342 and analyzed by fluorescence microscopy. 100 μm scale bar shown.

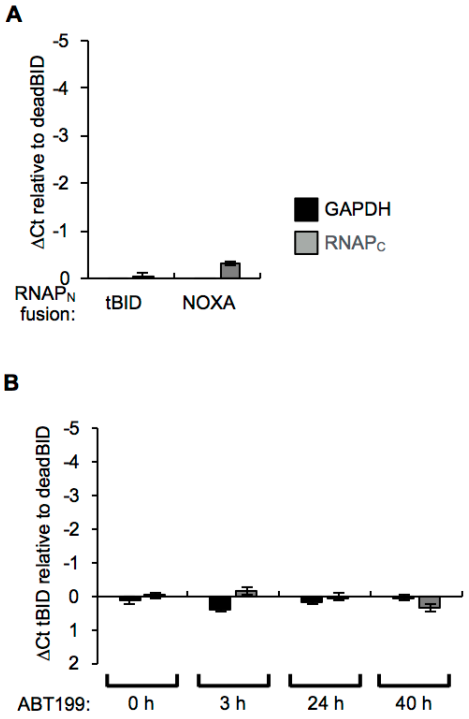

Figure S10 | Controls for RT-qPCR analysis in Figure 7. Controls (GAPDH and $RNAP_C$) for experiments shown in Figure 7A and 7B shown. (A) Briefly, HEK293T cells were cotransfected with the plasmids shown in Figure 6A with the fusions indicated, grown for 40 h, lysed, and then total RNA was isolated and quantified by RT-qPCR. PCR primers were generated for GAPDH and total $RNAP_C$. The data displayed is the delta-Ct value in comparison to cells transfected with the $RNAP_N$-deadBID "negative control". (B) HEK293T cells were cotransfected with the plasmids as in (A) with 0.5 μM ABT-199 added at different time points, lysed, and then total RNA was isolated and quantified by RT-qPCR as described in (A). The data displayed is the delta-Ct value in comparison to cells transfected with the $RNAP_N$-deadBID "negative control". Error bars are ± s.e.m., n = 4.