Regulation of BCL-X splicing reveals a role for the Polypyrimidine-tract binding protein (PTBP1/hnRNP I) in alternative 5′ splice site selection.

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Supplemental Table S1. List of the oligonucleotides used in this study.

#	Oligonucleotide name	sequence 5'→ 3'	Experiment
1	USP5/Ex14/XhoI/Fw	agctcgagaagaagttcaccttcggc	Cloning; Fig.4B,E; S5B
2	USP5/Ex16/HindIII/Rv	agaagcttatgacccagttcatggcgg	Cloning; Fig.4B,E; S5B
3	Δ1-500/XhoI/Fw	agctcgagatgtggaagagaacagg	Cloning
4	BGH/PolyA/Rv	cagtgggagtggcaccttc	Cloning; Fig.1D;3A,D-F;4B,E;5B-C
5	BCL-X/E2m1/Rv	ccgaaggagaccaaggccacaat	Cloning
6	BCL-X/E2m2/Rv	ccgaaggagaggaaggccacaat	Cloning
7	USP5/E15m1/Fw	agactccggctgctccc	Cloning
8	hnRNPF/HindIII/Fw	agaagctttatgatgctgggccctgaggga	Cloning
9	hnRNPF/BamHI/Rv	agggatccctagtcatagccacccatgctgttctg	Cloning
10	PTBP1/EcoRI/GFP/Fw	acgaattcaatggacggcattgtcccag	Cloning
11	PTBP1/SalI/GFP/Rv	acgtcgacctagatggtggacttggagaagg	Cloning
12	hSRp20/PSTI/Fw	agctgcagatgcatcgtgattcctgtcca	Cloning
13	hSRp20/BamHI/Rv	agggatccctatttcctttcatttgacctaga	Cloning
14	BCL-X/ATG/Fw	atgtctcagagcaaccgggagctg	Fig. 1B; S1A
15	BCL-X/TGA/Rv	tcatttccgactgaagagtgagcc	Fig. 1B: S1A
16	BCL-X/T7/Fw	attaatacgactcactatagggacagatatcagagcttt	Fig. 1C; 3C
17	BCL-X/Long/Rv	tgcgatccgactcaccaata	Fig. 1C; 3C
18	BCL-X-Exon2/Fw	ccatggcagcagtaaagcaa	Fig. 1D; 3A,E,F; 5B; S4A,B,D; S5A
19	BGH RV	cagtgggagtgcaccttc	Fig. 1D; 3A,E,F; 3D; 4B,D,E; 5B,C,F,G; S4A,B,D; S5A,B
20	BCL-XL/ExonJunct/Fw	aagagaacggcggctgggatac	Fig. 2A; S2C,E; S4C
21	BCL-Xs/ExonJunct/Fw	gggacagcatatcagagctttgaacaggatac	Fig. 2A; S2C,E; S4C
22	BCL-X/ExonJunct/Rv	tcatttccgactgaagagtgagccca	Fig. 2A; S2C,E; S4C
23	BCL-X/E2d/Fw	ccagggacagcatatcaga	Fig. 2B,D; 5A,D,E; S3C
24	BCL-X/E2d/RV	ccccatcccggaagagttcattca	Fig. 2B,D; 5A,D,E; S3C
25	BCL-X/E1/Fw	tggttcctgagcttcgcaat	Fig. 2D; 5A; S3C
26	BCL-X/E1/Rv	cggagggatcatgcgacc	Fig. 2D; 5A; S3C
27	BCL-X/I1/Fw	actgcccagggagtgacttt	Fig. 2D; 5A; S3C
28	BCL-X/I1/Rv	taccccgtcttctccgaaa	Fig. 2D; 5A; S3C
29	BCL-X/E2p/Fw	tcggatcgcagcttggatggc	Fig. 2D; 5A; S3C
30	BCL-X/E2p/RV	cagccgccgttctcctggat	Fig. 2D; 5A; S3C
31	BCL-X/I2/Fw	tgggcccagagtcacacccc	Fig. 2D; 5A; S3C
32	BCL-X/I2/RV	agggagcctctggtgggcag	Fig. 2D; 5A; S3C
33	BCL-X/E3/Fw/CLIPminig	gtggaactctatgggaacaatgca	Fig. 3D; 5C
34	USP5/T7/Fw	attaatacgactcactatagggcacccctggtcactccg	Fig. 4C
35	USP5/Rv/RNApulldown	agagtcactaacatgtcggag	Fig. 4C
36	USP5ex16/FW/Clip	gtctactacacgggcaacagcg	Fig. 4D; 5F,G
37	PTB tot FW	ggaaggtcaccaacctcctg	Fig. S2B
38	PTB tot RV	ggagagctgtcggtcttcag	Fig. S2B
39	siCTRL	ucuuucuucugcuuugcgg	Naro et al., 2014
40	siSRSF1	ccaaggacauugaggacgu	Naro et al., 2014

Supplemental Figure Legends

Supplemental Figure S1. BCL-X alternative splicing pattern of minigene resembles pattern of the endogenous gene. A) RT-PCR analysis of in vivo splicing assays of endogenous BCL-X (left panel) and BCL-X minigene. The p values of Student's t-test are reported. ***, $p<0.001$. B) Evaluation of the indicated splicing factors expression level by Western-blot analysis.

Supplemental Figure S2. PTBP1 and BCL-Xs expression correlate with drug-induced apoptosis in glioblastoma cell lines. A) Western-blot analysis of PTBP1 expression in glioblastoma cell lines (A172, LN229, LN18). B,C) qPCR analysis of PTBP1 (B) and BCL-X isoforms (C) expression in glioblastoma cell lines (mean ± SD, n=3). D,E) Bar graph representation of immunofluorescence analysis of activated caspase-3 (D) and qPCR analysis of BCL-X isoforms (E) in glioblastoma cell lines treated for 72hrs with temozolomide (100μM) (TMZ 100μM) or DMSO (DMSO), as control (mean ± SD, n=3). B,C,E) The bar graph of fold variation of each sample was calculated by ΔΔCt method as described in the Methods section. B-E) The p values of Student's t-test are reported. *$p<0.05$, ***, $p<0.001$; n.s., not significant.

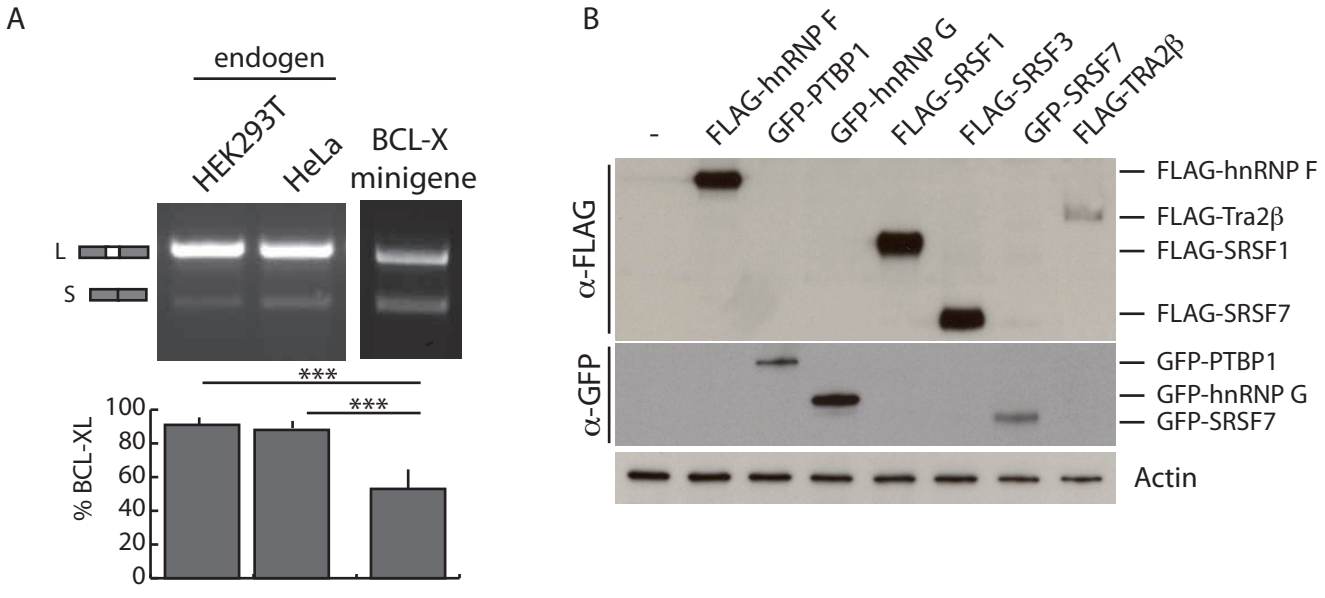
Supplemental Figure S3. Schematic representation of CLIP assay in presence of low concentration of RNaseI. A) Schematic representation of RNA isolation. B) Formaldheyde-agarose gel analysis of RNA digestion pattern after 2 hrs of treatment at 4°C with different doses of RNaseI (see Materials and Methods section). C) CLIP of PTBP1 performed in HEK293T, in presence of high concentration (1:5) of RNaseI. Associated BCL-X RNA was quantified by qPCR, using primers described in the scheme. Data are represent fold enrichment relative to the IgG sample (mean ± SD, n=3).

Supplemental Figure S4. Mutations in polypirimidine tract in the B2 element did not affect hnRNP F and SRSF1 splicing activity. A,B) RT-PCR analysis of the splicing assays performed in HEK293T cells transfected with wt and mutated (E2m1 and E2m2) minigenes in presence of either hnRNP F (A) or SRSF1 (B). The bar graph shows the percentage of BCL-XL (mean ± SD, n=3). C) qPCR analysis of BCL-X isoforms in HEK293T cells transfected with scramble (CTRL), PTBP1 and PTBP2 (PTBP1/2), and hnRNP F siRNAs. The fold variation was calculated as in Supplemental Figure S2. D) RT-PCR analysis of the splicing assays performed as in

A,B) in presence of suboptimal amount of hnRNP F and PTBP1. A,B) The p values of Student's t-test are reported. **, $p<0.01$; ***, $p<0.001$; n.s., not significant.

Supplemental Figure S5. Competition between PTBP1 and SRSF1 regulates *BCL-X* and *USP5* splicing. A) RT-PCR of *in vivo* splicing assay performed in HEK293T transfected with the indicated minigenes, GFP-PTB1 and increasing amounts of Flag-SRSF1. Bar graph (*bottom panel*) shows the percentage of BCL-XL (mean ± SD, n=3). B) RT-PCR of *in vivo* splicing assay performed in HEK293T cells transfected with the USP5 minigene and the indicated SR proteins. The bar graph shows the percentage of isoform 2 of USP5 (mean ± SD, n=3). A,B) The p values of Student's t-test are reported. *$p<0.05$, **, $p<0.01$; ***, $p<0.001$; n.s., not significant.

Bielli_Supplemental Figure 1



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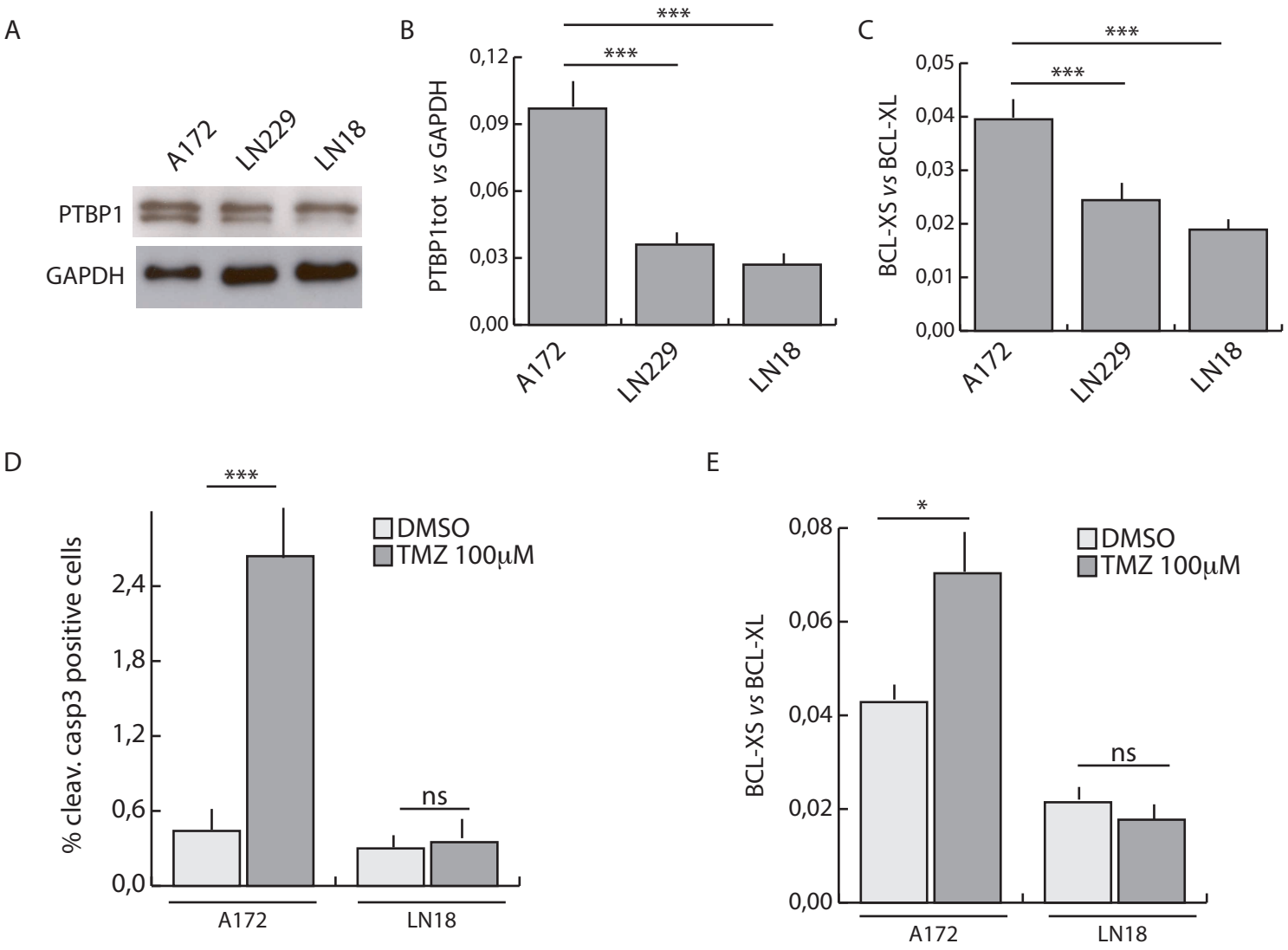

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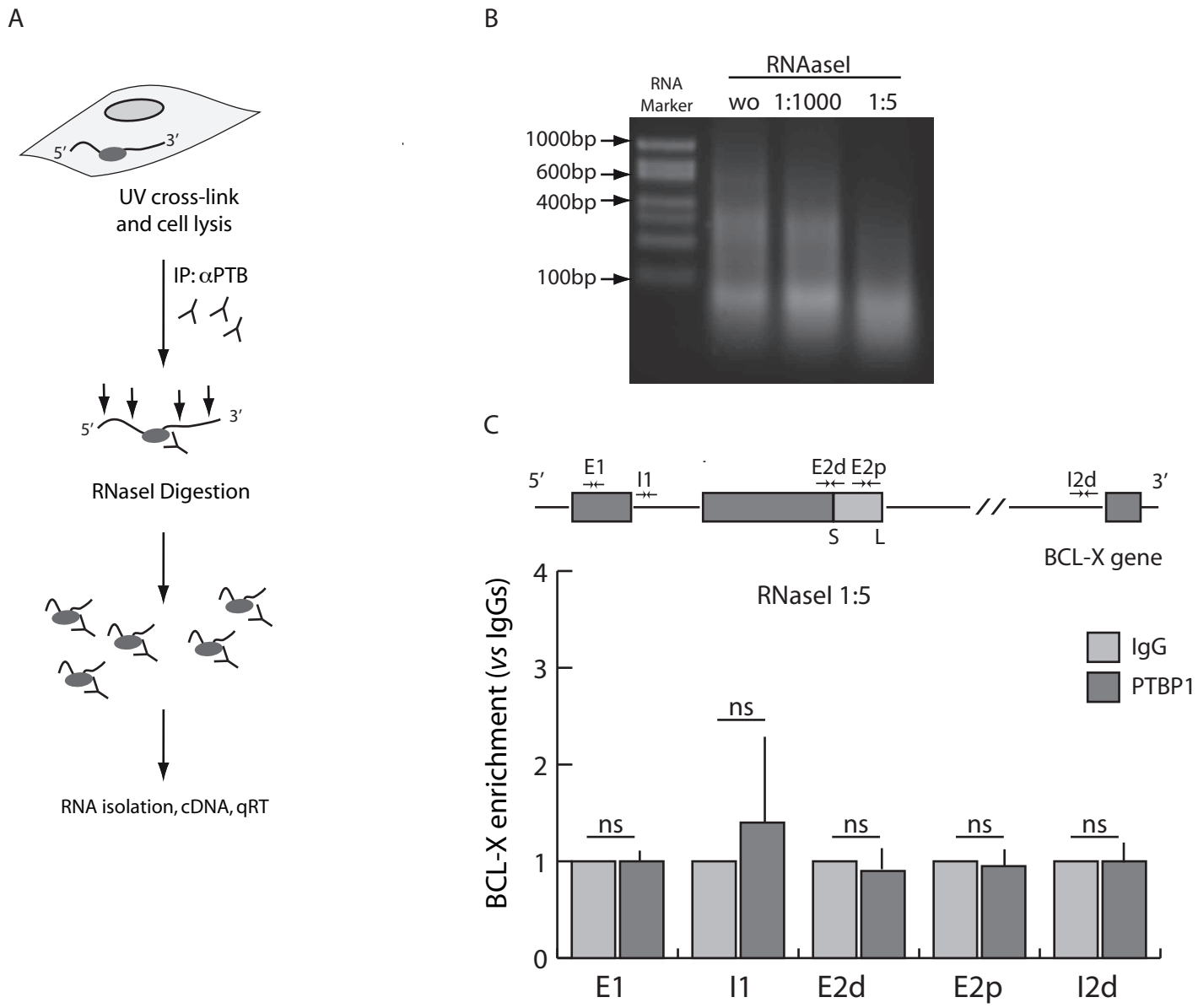
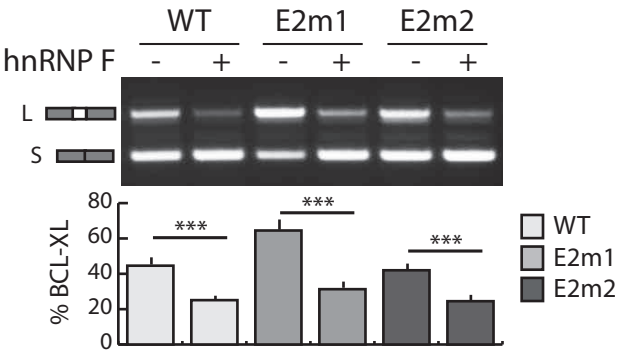


Bielli_Supplemental Figure 4

A



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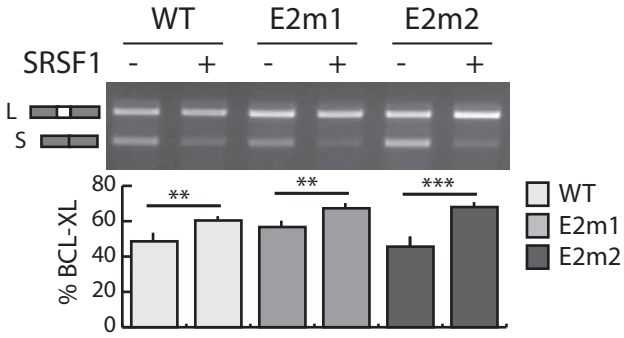

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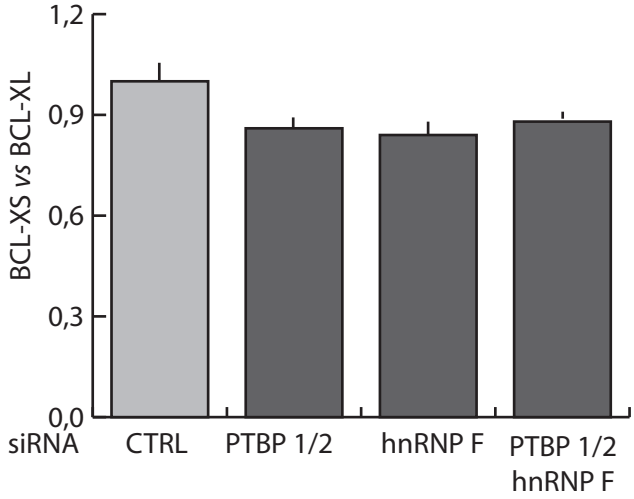

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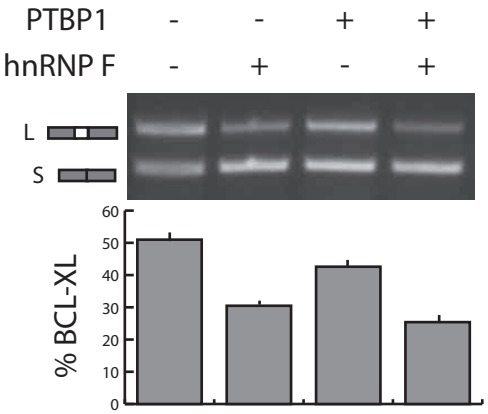

Bielli_Supplemental Figure 5