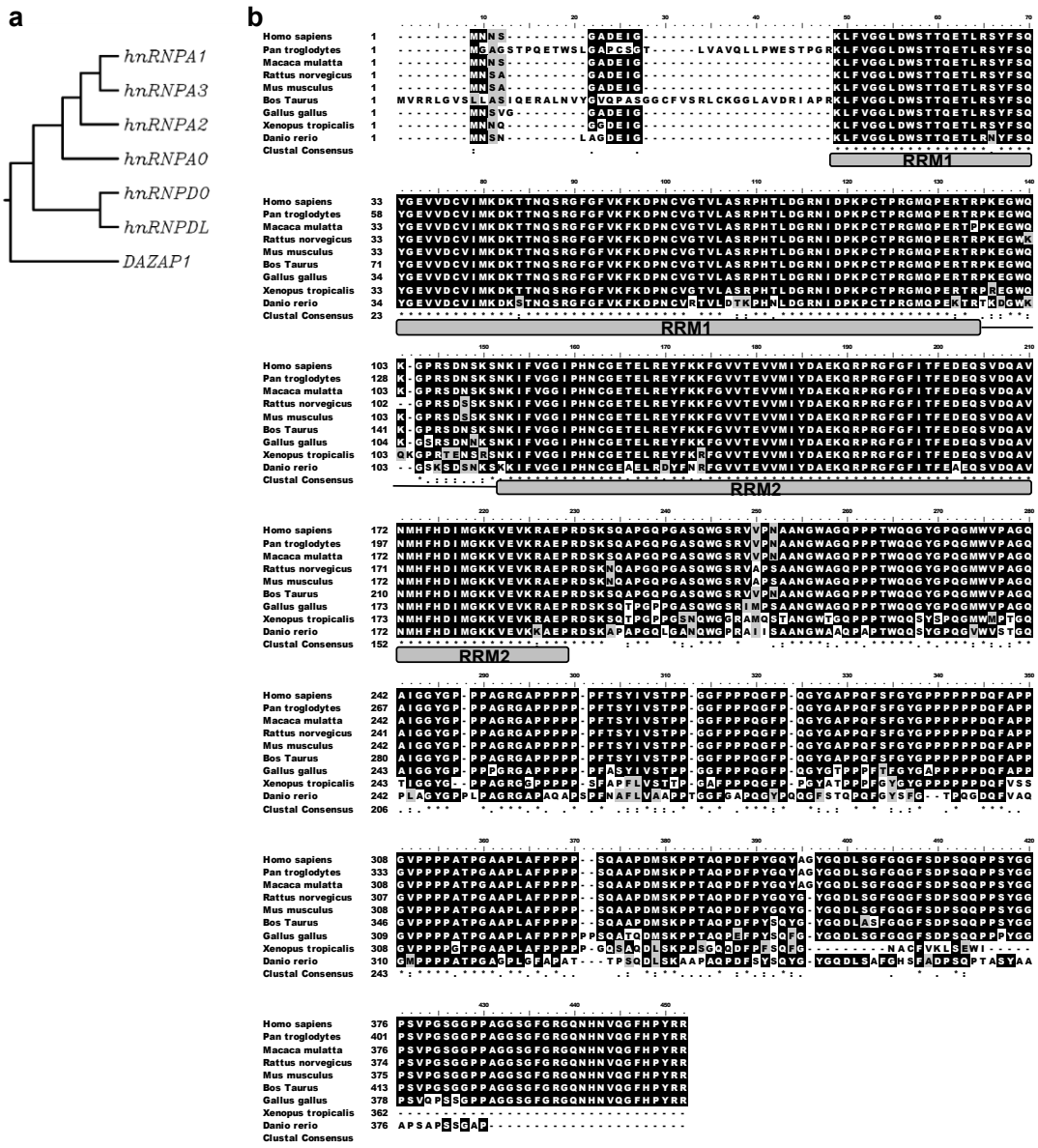
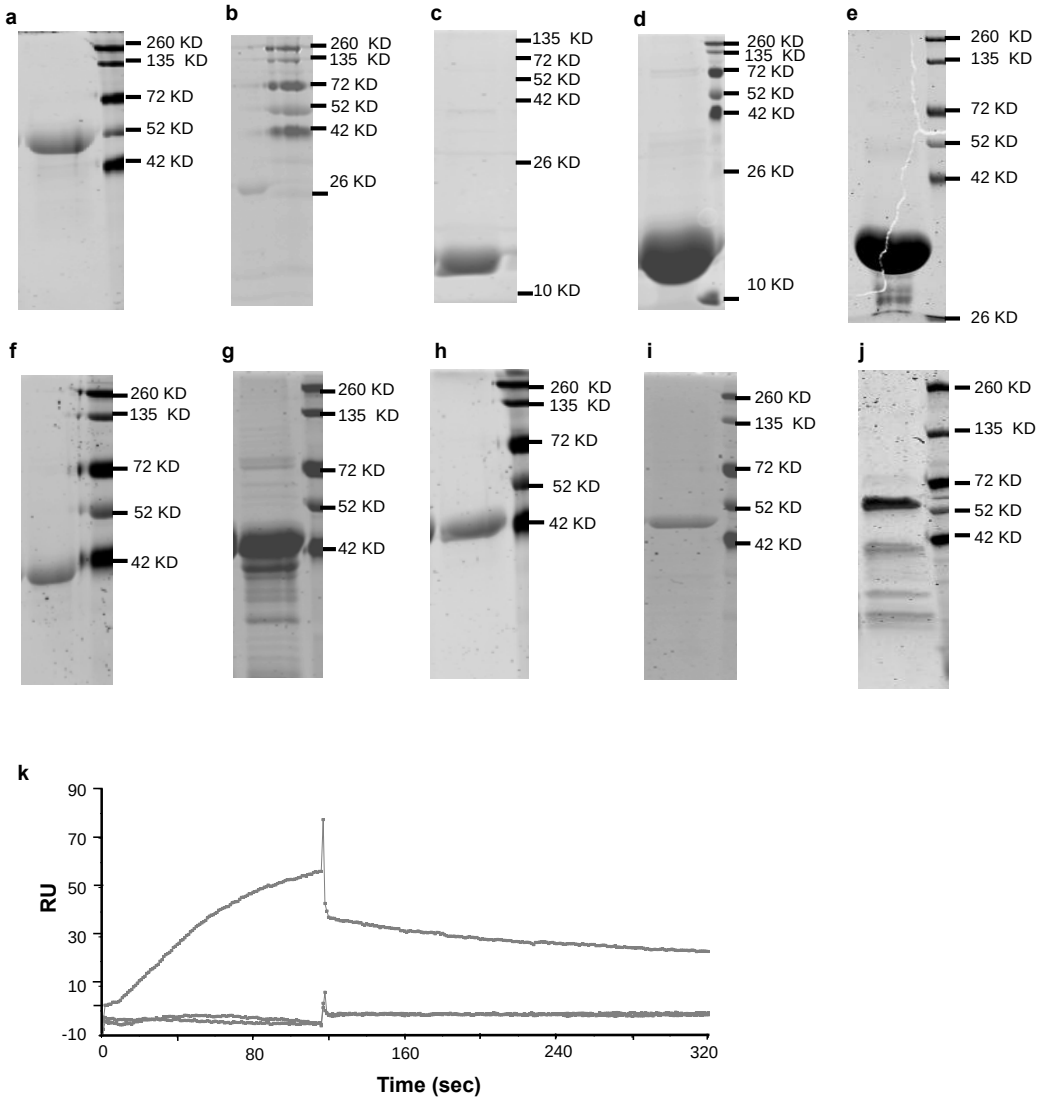
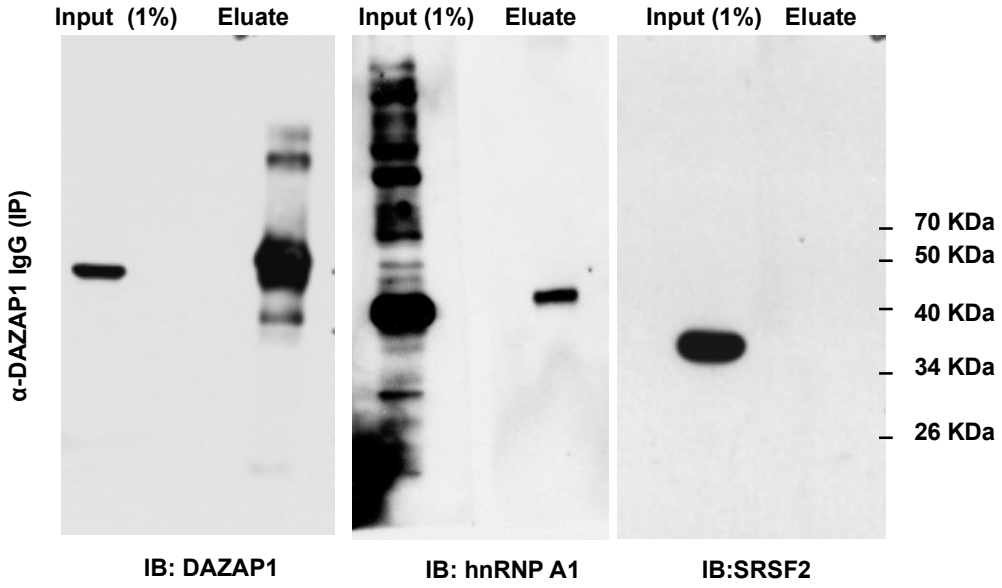


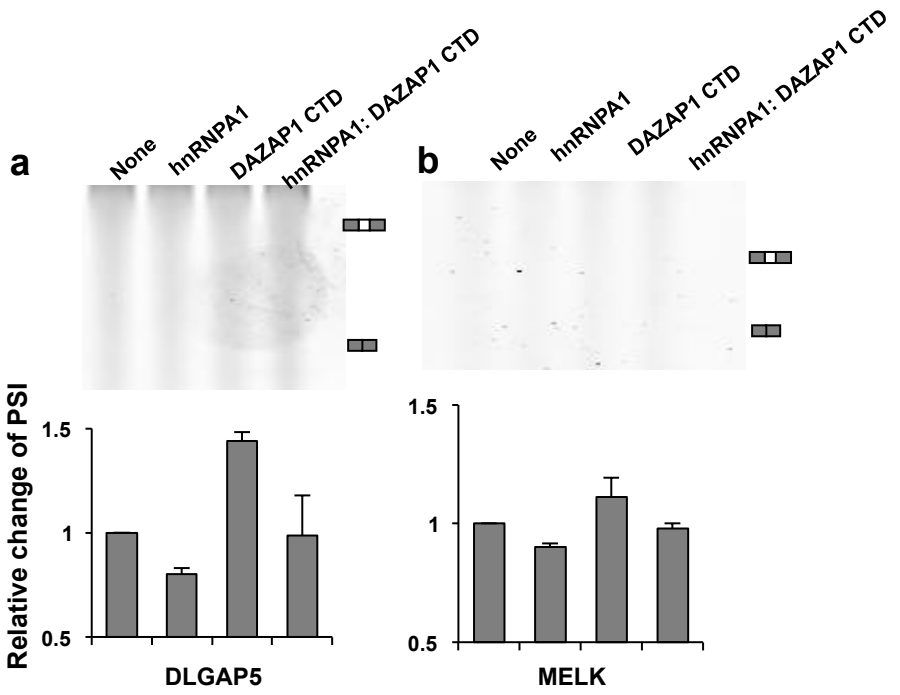
Supplementary Figure 1: Conservation of DAZAP1. **(a)** Evolutionary tree of the human hnRNPs homologous to DAZAP1 (generated by alignment of 2xRRM domains). **(b)** Multiple sequence alignment of vertebrate DAZAP1 proteins using Human (NP_061832.2), chimpanzee (XP_001147948.2), monkey (XP_001094635.2), rat (NP_001020913.1), mice (NP_001116076.1), cow (NP_001179960.1), chicken (NP_001026599.1), frog (NP_001006737) and fish (NP_001166975.1) genes. Identical sequences are shaded black while similar sequences are in grey. CLUSTALW consensus is shown at the bottom. The RRM domains are marked.



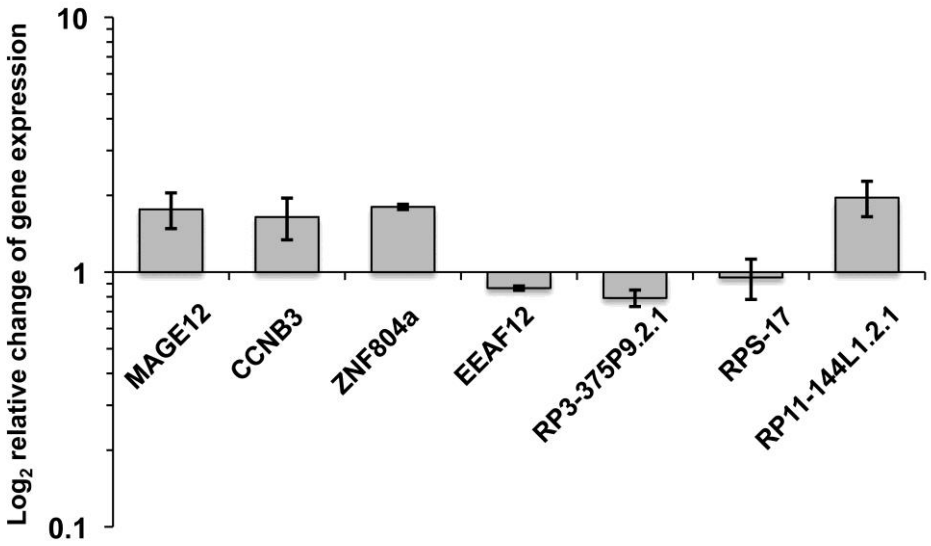
Supplementary Figure 2: Purification of different recombinant proteins to homogeneity for *in vitro* binding experiments. **(a)** Full length DAZAP1, **(b)** DAZAP1 2xRRM domain, **(c)** RRM1 of DAZAP1, **(d)** RRM2 of DAZAP1, **(e)** CTD of DAZAP1, **(f)** hnRNP A0, **(g)** hnRNP A1, **(h)** hnRNP A2, **(i)** hnRNP D0 and **(j)** hnRNP DL (D like). **(k)** The binding of DAZAP1 to a control sequence (CACACCA) was measured by SPR. The protein concentration were 0.1, 1.0, 3.0 µM from bottom to top. The apparent Kd value was calculated as >10 µM due to non-specific binding.



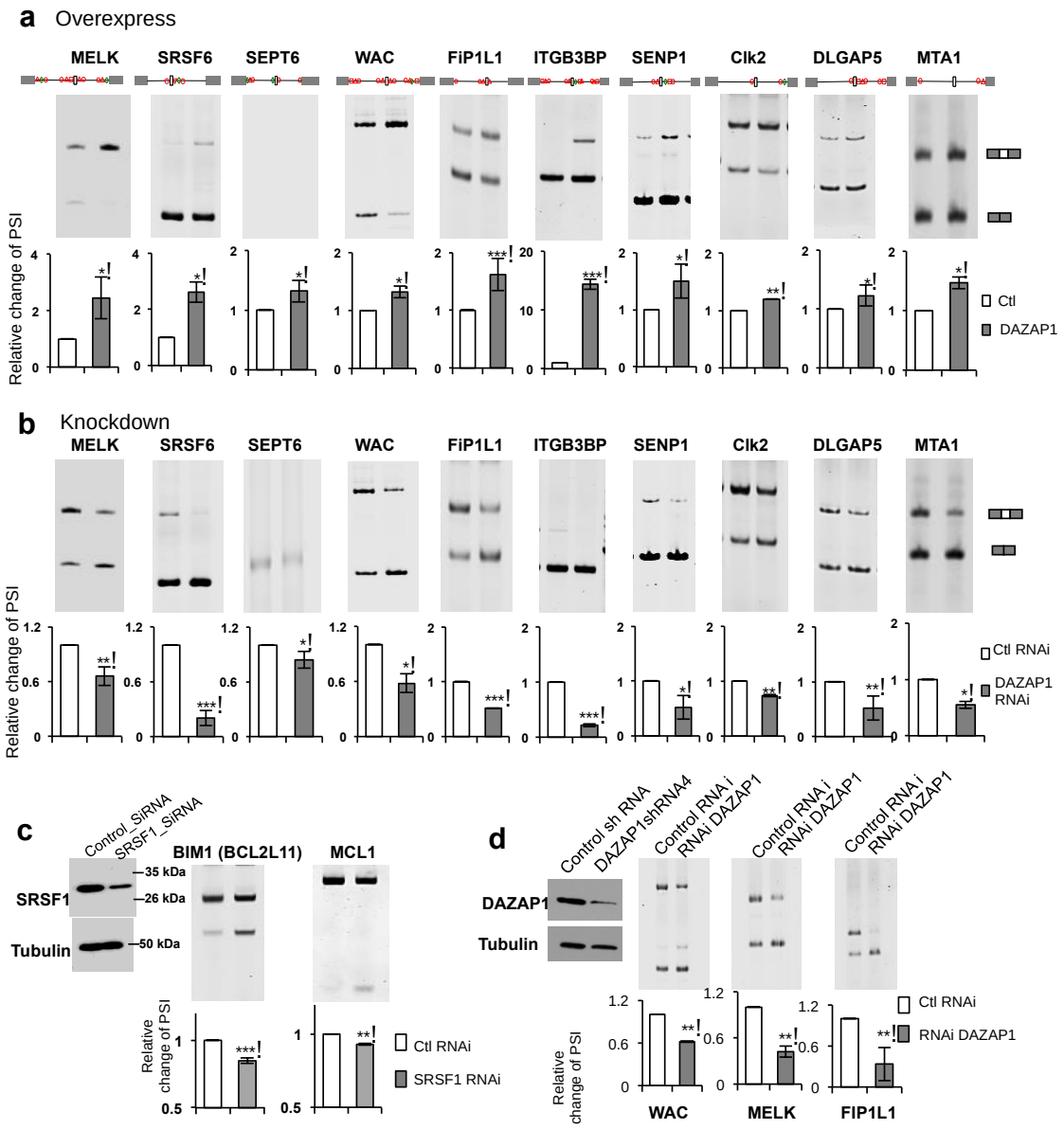
Supplementary Figure 3: Interaction of endogenous DAZAP1 and hnRNP A1 as detected by co-immunoprecipitation. DAZAP1 antibody was immobilized on protein AG beads and cell lysates form HEK293T cells were used for immuno-precipitation. DAZAP1 and hnRNP A1 was immuno-precipitated by DAZAP1 antibody (left and middle panel) whereas abundant splicing factor SRSF1(ASF/SF2) could not be pulled down by anti-DAZAP1 antibody (right panel) used as a control.



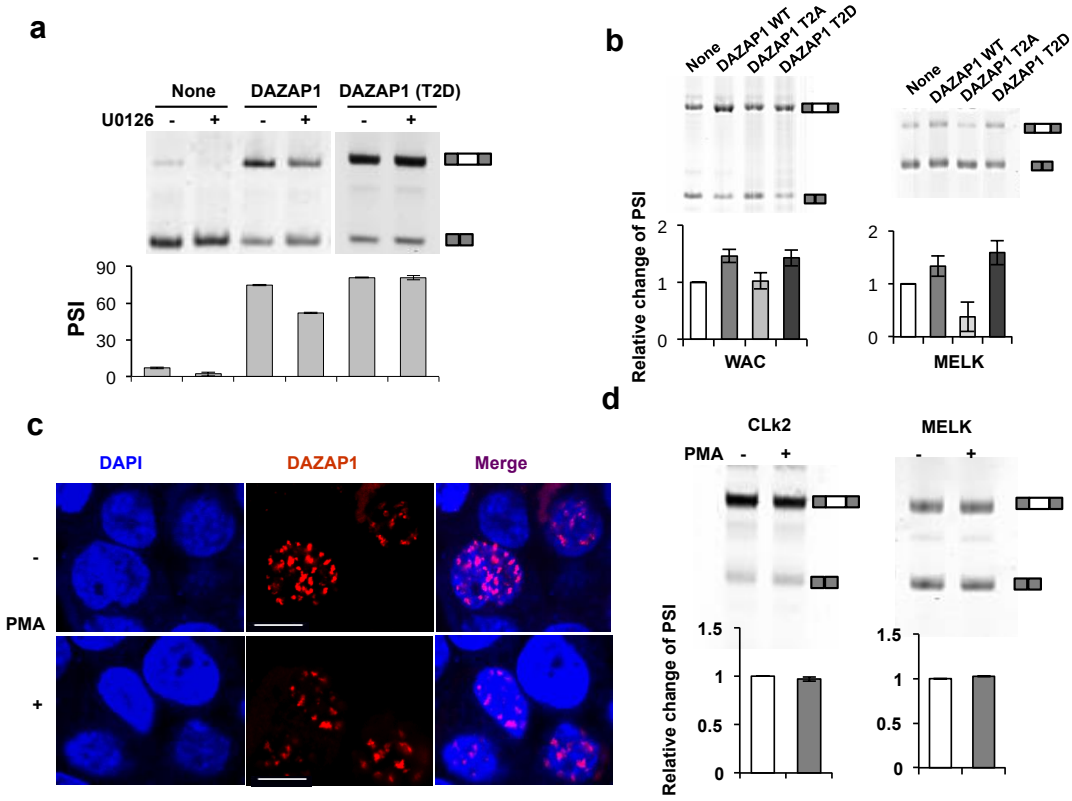
Supplementary Figure 4: DAZAP1 CTD can neutralize hnRNPA1 inhibitory effect on endogenous genes. DAZAP1-CTD and hnRNPA1 was overexpressed in HEK293T cells as described in Fig. 4b. Total RNA was reverse transcribed and PCR amplified using gene specific primers. The means and s.d from two independent experiments were shown**.** Overexpression of hnRNPA1 led to decrease in exon inclusion in DLGAP5 (panel a) and MELK (panel b), DAZAP1 CTD has opposite effect on these genes. Co-expression of hnRNPA1:DAZAP1 CTD in plasmid ratio of 1:15 partially neutralizes inhibitory affect of hnRNPA1.



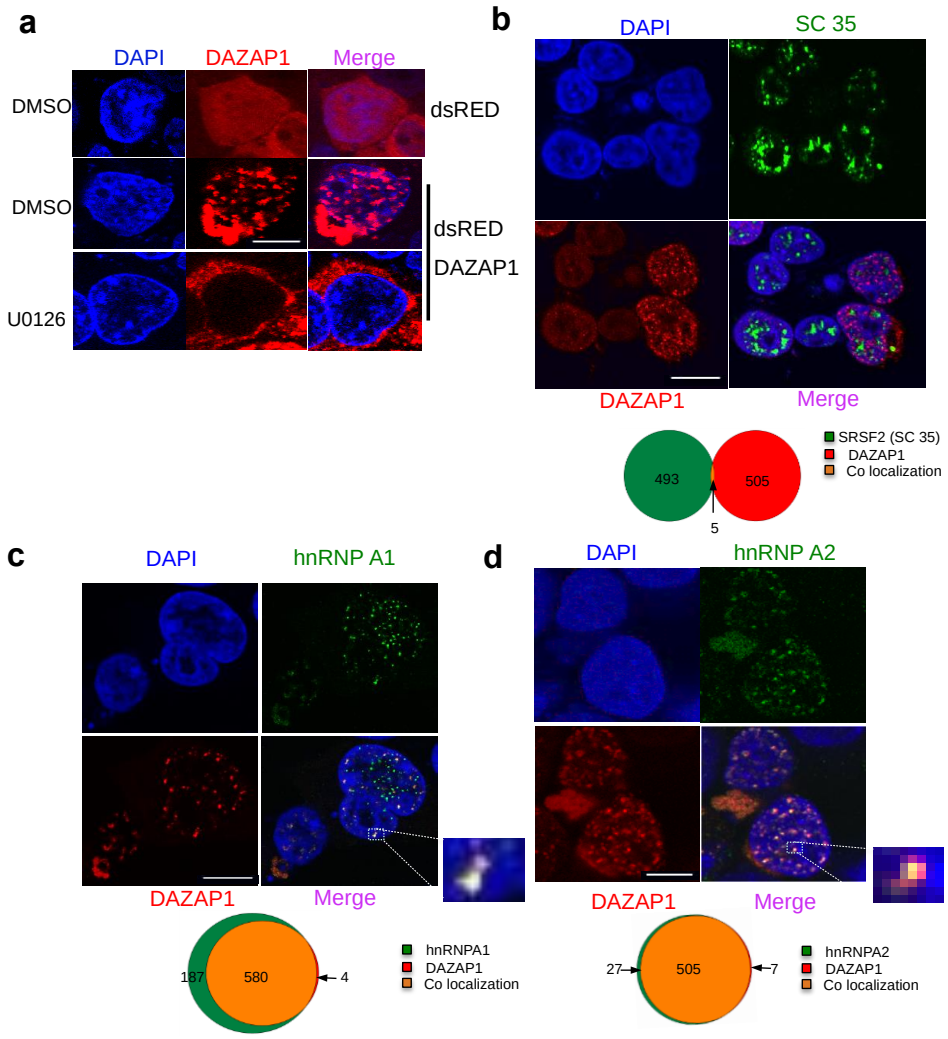
Supplementary Figure 5: Validation of DAZAP1 target genes by quantitative RT-PCR. The total RNAs from DAZAP1 depleted cell lines and control cells were used for the analysis. The genes were originally identified through mRNA-seq experiments (see main text). Expression of two pseudo genes: RP3-375P9.2.1 and RP11-144L1.2.1 are also tested, while ribosomal protein coding transcript RPS-17 did not show a significant change in expression compared control cells. Error bars indicated SE of mean across two different shRNA samples performed in triplicates.



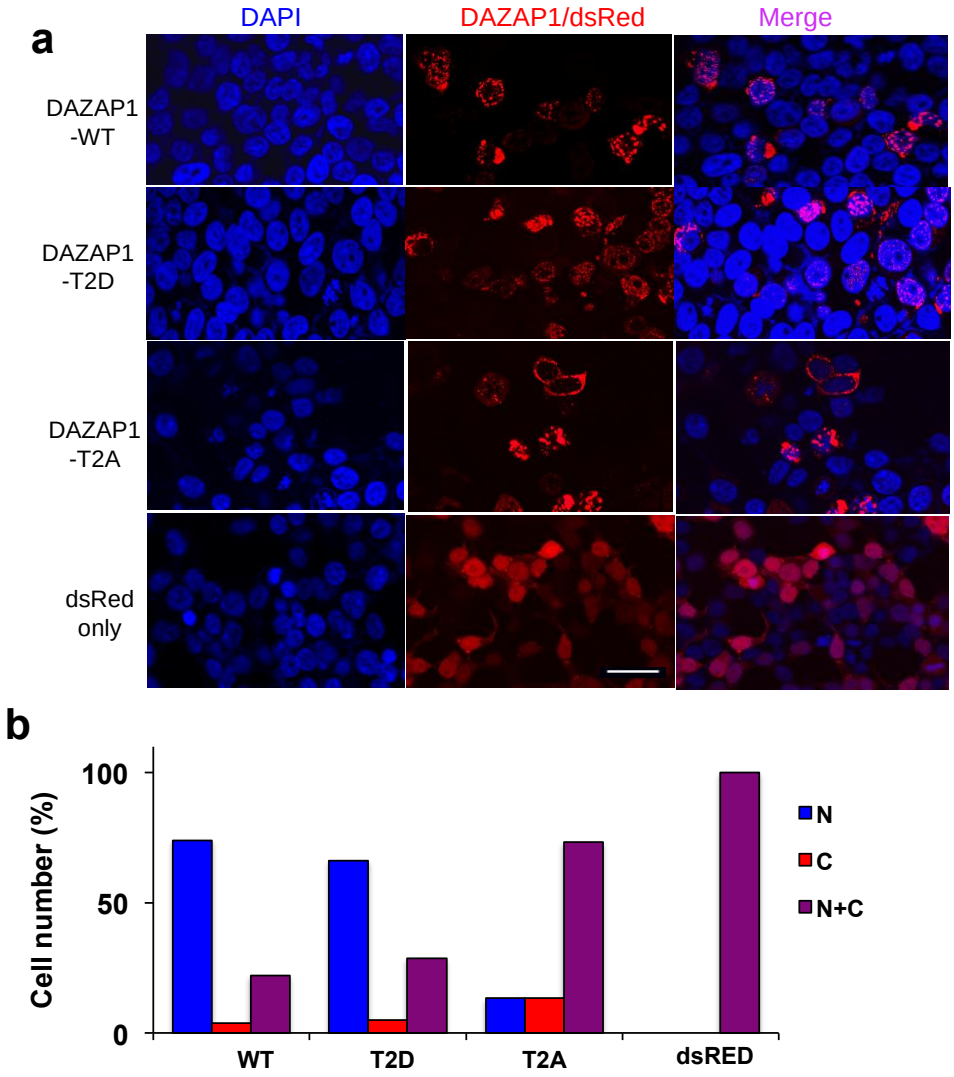
Supplementary Figure 6: Validation of endogenous splicing events affected by DAZAP1 using semi quantitative-RT-PCR in cells with over-expression **(a)** or knockdown **(b)** of DAZAP1. Relative fold changes of PSI compared to controls were plotted. The means and s.d from two independent experiments were shown. The regions around skipped exons are: MELK (chr9:36581641-36589888), SRSF6 (chr20:42087001-42088535), SEPT6 (chrX:118750908-118763471), WAC (chr10:28879649-28897691), FiP1L1 (chr4:54243812-54245431), ITGB3BP (chr1:63974199-63989178), SENP1 (chr12:48482946-48483076), Clk2 (chr1:155238499-155238586), DLGAP5 (chr14:55615312-55615402), MTA1 (chr14:105915696-105915746). P values were calculated with t-test,*p≤0.03;**p≤0.004; ***p≤0.0001. (c) Semi-quantitative RT-PCR of known splicing targets of SRSF1 on knock down cells was performed as a positive control. (d) RNAi of DAZAP1 in HeLa cells led to exon skipping in target genes. The means and s.d from two independent experiments were shown.



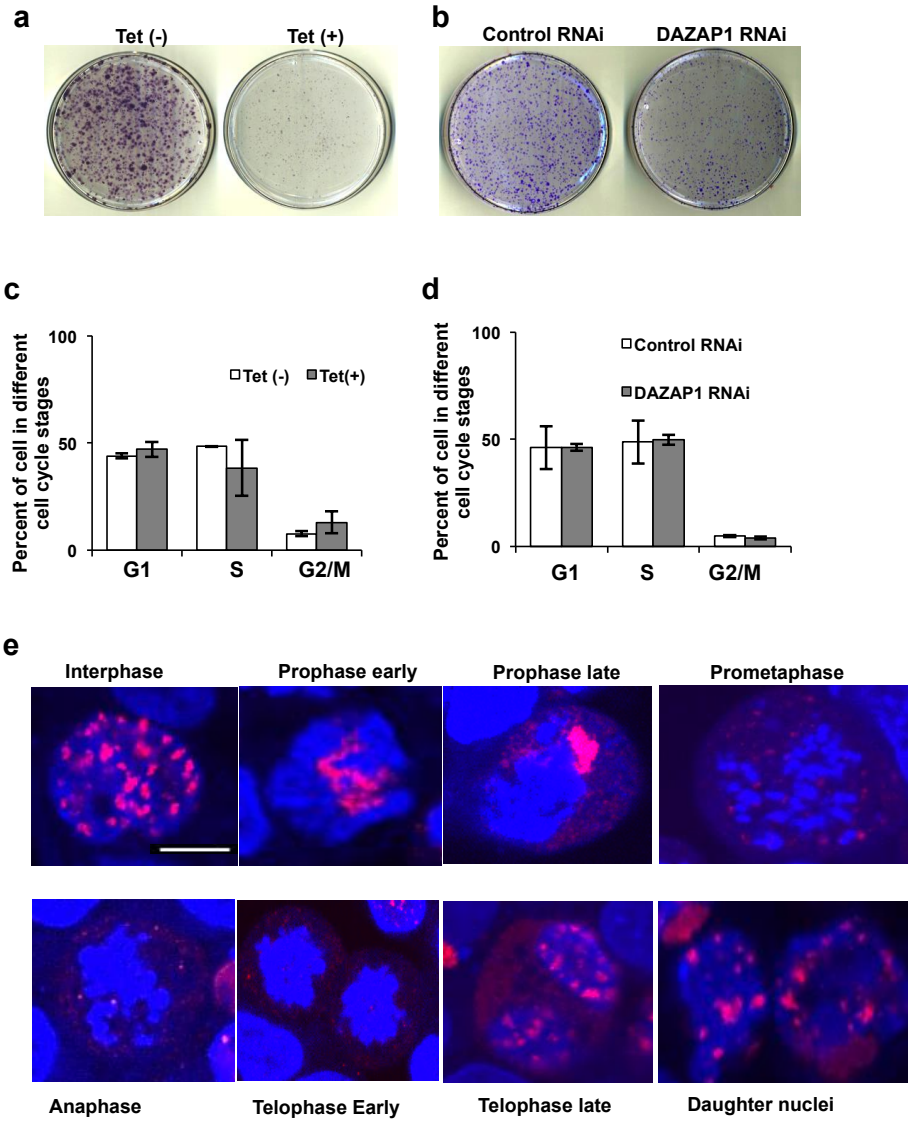
Supplementary Figure 7: Effect of DAZAP1 phosphorylation on splicing. **(a)** Addition of MEK inhibitor (U0126) inhibited the activity of Wt DAZAP1 but not the T2D mutant that mimics the phosphorylated DAZAP1. The lanes 1-4 are controls that is the same as the main Fig 6b. The splicing reporter with MS2 site was used, and the experiment condition are similar to Fig 6b. For all panels, the experiments were repeated three times with mean PSI and s.d. shown below a represented gel. **(b)** Expression of DAZAP1(WT), T2A or T2D in 293 cells have different effects in splicing of endogenous targets of DAZAP1 as judged by quantitative RT-PCR. Experiment conditions are identical to that in Supplementary Fig. 6. The splicing of DAZAP1 targets were was promoted by wild type or T2D form of DAZAP1, but not by T2A. Overexpression of T2A inhibited MELK splicing, likely due to competition with wildtype DAZAP1. **(c)** PMA activation did not affect DAZAP1 localization. Scale bar is 5μm. **(d)** PMA activation did not affect splicing of endogenous genes as exemplified by two representative genes.



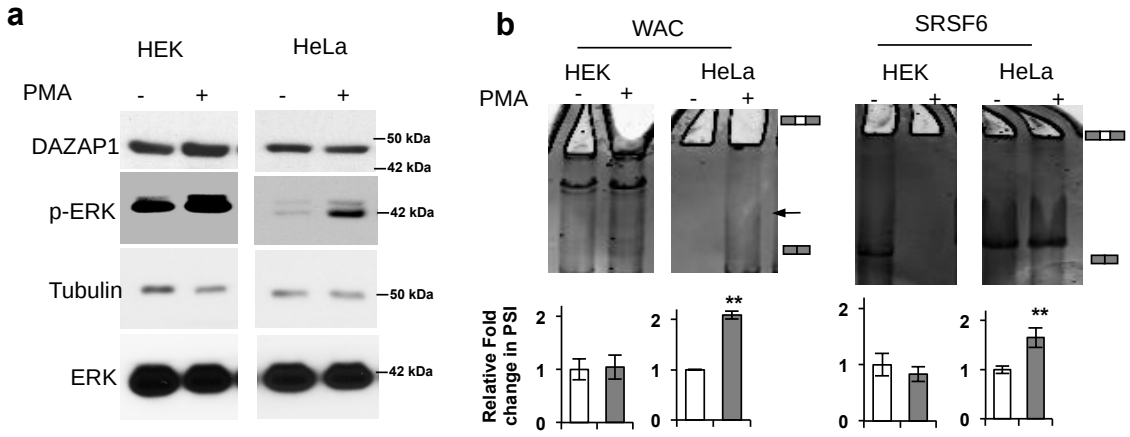
Supplementary Figure 8: **(a)** MEK/Erk phosphorylation of DAZAP1 changed its sub-cellular localization. The 293 cells were treated with the MEK inhibitor (U0126), and the dsRED-DAZAP1 were detected. **(b)** Localization of endogenous DAZAP1 to nuclear structures that are distinct from SC 35 containing nuclear speckles as detected with confocal microscopy. **(c,d)** Co-localization of endogenous DAZAP1 with hnRNP A1 (c) and hnRNP A2 (d) are detected with confocal microscopy. All experiments were conducted in HEK293T cells. The co-localization of endogenous DAZAP1 with sc35 (SRSF2), hnRNPA1 and hnRNPA2 were shown in Venn Diagram. At least 500 foci from duplicated experiments were counted using Olympus viewer software and the diagram was generated using Venn Diagram plotter from Pacific North West National Laboratory. Scale bar is 5μm in all panels.



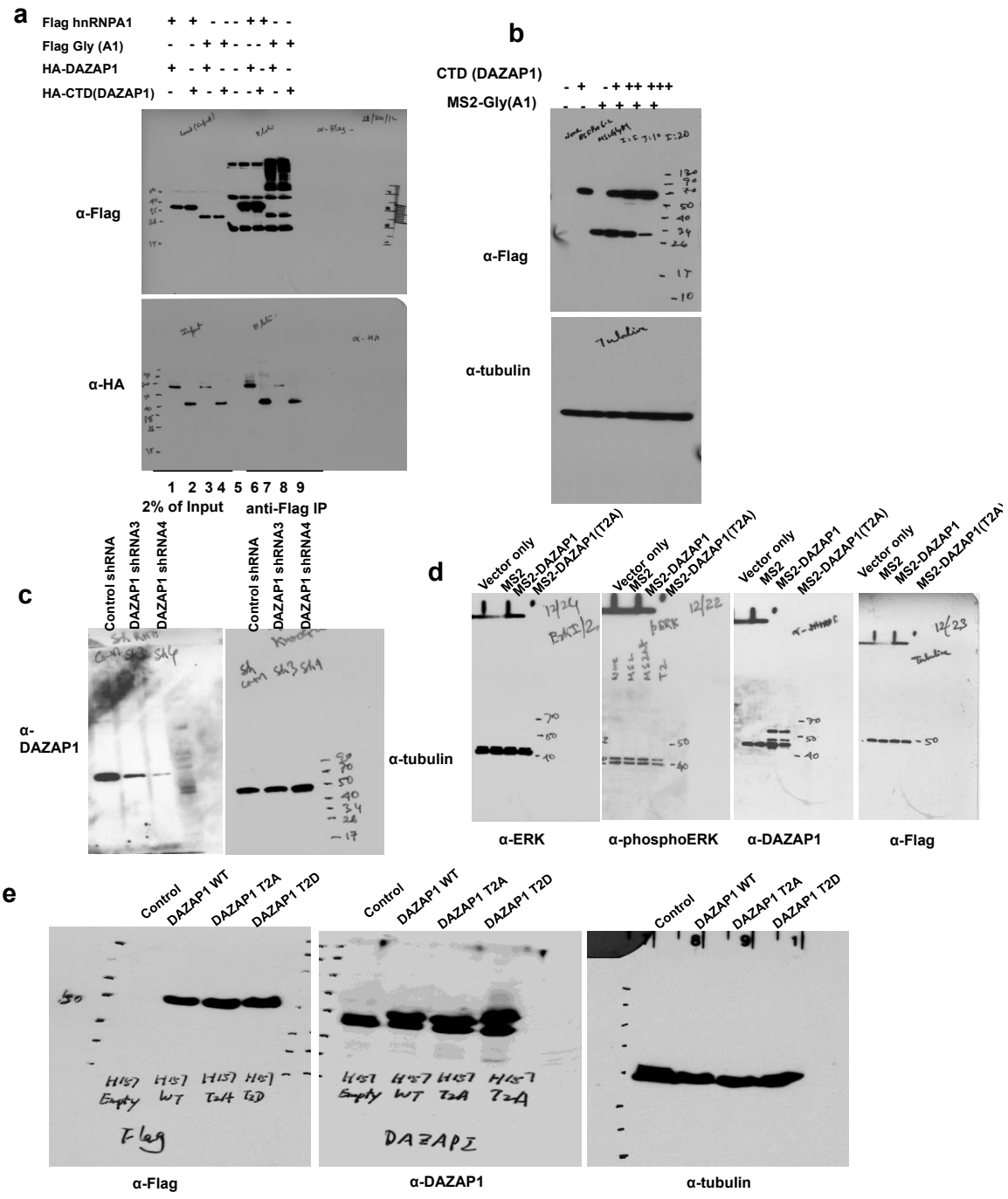
Supplementary Figure 9: **(a)** Sub-cellular localization of DAZAP1 is affected by the two Erk phosphorylation sites. The wild type (WT) and phosphomimetic (T2D) DAZAP1 are mostly nuclear, whereas mutation of the T269/T315 residues to alanine (T2A) leads to cytoplasmic translocation. **(b)** The quantification from above experiments showed percent of cells with DAZAP1 in nuclear (N), cytoplasmic (C) or both (N+C) compartments. The experiments were carried out twice, and more than 70 cells were counted for each sample. Scale bar is 10 µm.



Supplementary Figure 10: (a) Effect of DAZAP1 over expression on cell proliferation as judged by colony formation assay. The expression of DAZAP1 is induced by addition of tetracycline (tet). **(b)** Effect of DAZAP1 knockdown on cell proliferation as judged by colony formation assay. **(c-d)** The percent of cells in different cell cycle stages were determined using flow cytometry measurement of DNA contents. The cells lines with DAZAP1 over-expression **(c)** or knockdown **(d)** were shown. Experiments were carried out in duplicates and the means and s.d were shown. **(e)** DAZAP1 localization during mitosis. DAZAP1 was mainly detected in the nucleus before nuclear membrane disruption. Subsequently DAZAP1 became cytoplasmic and finally reappeared in nucleus in late telophase. Scale bar is 5µm.



Supplementary Figure 11: Effect of PMA activation on DAZAP1 has subtle difference in different cell types. (**a**) Addition of PMA to serum starved HEK 293T or HeLa cells led to elevation of p-ERK levels. The Erk activation in HEK cells is minimal as the basal level of Erk phosphorylation is hard to suppress by serum starvation. In HeLa cells, the Erk phosphorylation can be robustly suppressed by serum starvation and re-induced by PMA. (**b**) PMA activation did not significantly affect splicing of endogenous DAZAP1 targets in HEK cells, whereas in HeLa cells addition of PMA led to increased exon inclusion. The experiments were repeated twice and a representative gel was shown above the quantification. Error bars indicate s.d. *P* values are calculated with t-test ;**p≤0.004. Arrow indicates non specific bands.



Supplementary Figure 12:- Important full length scans of Western Blots used in this study. (a) used in Fig. 4a **(b)** used in Fig.4c, the last lane was cropped in the main figure **(c)** used in Fig.5a **(d)** used in Fig.6d right panel **(e)** used in Fig.7a.

Supplementary Table 1: Binding affinities of hnRNPs to different ISS and ISE sequence motifs

	K_d (M)			
hnRNP protein	**ISS (F) (AGAUAU)**	**ISS (H) (AAUUUA)**	**ISS (I) (AGUAGG)**	**ISE (F) (GUAACG)**
A0	25.5×10^{-9} (±12)	10.3×10^{-9} (±1.7)	22.2×10^{-9} (±10.0)	ND
A1	29.3×10^{-9} (±0.2)	2.11×10^{-9} (±1.2)	1.85×10^{-9} (±0.8)	ND
A2	159.0×10^{-9} (±35)	80×10^{-9} (±21)	22.1×10^{-9} (±5)	ND
D (AUF1)	20.2×10^{-9} (±4.0)	14.7×10^{-9} (±3.2)	16.7×10^{-9} (±7.0)	ND
D like	55.9×10^{-9} (±15)	12×10^{-9} (±2.0)	19.7×10^{-9} (±8.0)	ND
DAZAP1	31.3×10^{-9} (±0.03)	34.8×10^{-9} (±1.2)	72.5×10^{-9} (±10.1)	31.5×10^{-9} (±8.0)

Supplementary Table 2: Lists of oligonucleotide used in this study

Primer #	Name	Sequence	Notes
1	DAZAP1_NheF1	CACGCTAGCAACAACTCGGGCGCCGACGAG	The primer pair used for amplifying DAZAP1 coding region from c-DNA library
2	DAZAP_NotR1	GTCGCGGCCGC TAGCGTCGGTAGGGGTGGAACC	
3	DAZAP1_pt7BamF1	CACGGATCCGAACAACTCGGGCGCCGACGAG	Primer used for cloning DAZAP1 in bacterial expression vector as well as sub cloning to MS2 vector
4	DAZAP_pt7RRM1R	CACGTCGACTTACGGCCGTGTTCTCTCCGGCTGC	The reverse primer for RRM1 amplification
5	DAZAP_pt7 RRM2F	CACGGATCC AATAAGATATTTGTCGGTGGAATTCC	The forward and reverse primer pairs used for amplification of RRM2
6	DAZAP_pt7 RRM2R	CACGTCGACTTA AGGCTCAGCTCGTTTAACTTCC	
7	DAZAP_CTD_BamF1	CAC GGATCC CGGGACAGCAAGAGCCAAGC	Forward primer used for CTD amplification and cloning in pT7HTb
8	MS2 HindIII F1	CACAAGCTTGCCACCATGGGCGCCTCCAACTTC	Forward primer pairs for MS2 coat protein amplification
9	MS2 BamR1	CACGGATCCGTAGATGCCGGAGTTGGC	
10	MS2 sBamR1	CACGGATCTTACCGTAGATGCCGGAGTTGGC	Reverse primer used for cloning MS2 coat protein with a stop codon into pcDNA3 vector
11	MS2HAF1	CACAAGCTTGCCACCATGTACCCATACGATGTTCCAGA TTACGCTGGCGCCTCCAACTTC	Forward primer used for cloning of MS2 coat protein and fusion protein(s) into HA tagged pcDNA 3.1
12	670BamF1	CACGGATCC CCCACGTGGCAGCAAGGATATG	Primers used to generate CTD truncation mutants of DAZAP1
13	772BamF1	CACGGATCC CCACCCCCACCGTTCACCTCC	
14	877BamF1	CACGGATCCGGCTACGGGCCTCCACCTCC	
15	970 BamF1	CACGGATCCCCACCGCCTCCGTCTCAGGC	
15	1138 BamF1	CACGGATCCGGGTCGGGGGGCCCCCCCGCC	
17	SpEcoF1	CACGAATTCGAGCTCGGTACCCCG	Primers for sub cloning the MS2 splicing reporter constructs in pCDNA3
18	SpXbR1	CACTCTAGAGGATCCAAGAGTACTG	
19	MS2_intF1	TCGAG CGATCACCATCAGGGATCG GGGCC	Primer pair used for cloning MS2 stem loop into intronic splicing reporter pZW2C
20	MS2_intR1	CCGATCCCTGATGGTGATCGC	

21	DAZAPT269AF1	CCTACATCGTGTCCGCCCCTCCTGGAGGCTTTCCCCC	Mutation oligos used for mutating Thr^{269} to Ala
22	DAZAPT269AR1	GGGGGAAAGCCTCCAGGAGGGGCGGACACGATGTAGG	
23	DAZAPT315AF1	CCTCCACCAGCCGCTCCCGGGGCAGCACCTCTGGC	Mutation oligos used for mutating Thr^{315} to Ala
24	DAZAPT315AR1	GCCAGAGGTGCTGCCCCGGGAGCGGCTGGTGGAGG	