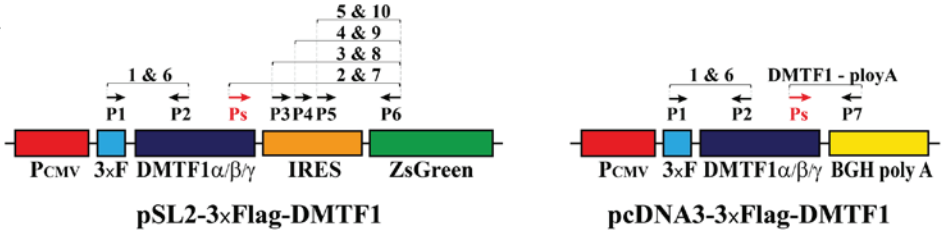
Figure S1. Primers used in quantitative and semi-quantitative PCR. (A) Schematic diagrams of the positions of different primer sets used in determining mRNA integrity. The predicted transcripts of pSL2- and pcDNA3-3xFlag-DMTF1α, β and γ are shown indicated. (B) The sequences of the primers pairs in (A), and the primers used in RT-qPCR to quantify ARF and GAPDH expression.

A



Ps: isoform specific primer, including P-α, P-β and P-γ

B **Primers used in quantitative and semi-quantitative PCR.**

Symbol	Primer name	Sequence (5' to 3')
P1	Flag-U	CGATTACAAGGATGACGATGAC
P2	DMTF1-338L	TTCTGCAAAATCTGTATCTGTG
P-α	DMTF1α-specific-U	ACAATACTTATCGTTCCTTCACC
P-β	DMTF1β-specific-U	ACAACTGTGGACCCCAAAAAAAG
P-γ	DMTF1γ-specific-U	GAAAAAGGCAATTGCTGCCTG
P3	IRES-113U	AAACCTGGCCCTGTCTTCT
P4	IRES-389U	CTCTCCTCAAGCGTATTCAACAAG
P5	IRES-424U	TGCCCAGAAGGTACCCCATTGT
P6	ZsGreen-94L	CGCCGGTGATCACGAACTTGT
P7	BGH-PolyA-L	GTGGGAGTGGCACCTTCCAGG
--	ARF-4U	GTGCGCAGGTTCTTGGTGACCC
--	ARF-92L	GTGAGCCGCGGGATGTGAACCA
--	GAPDH-530U	GGGAGCCAAAAGGGTCAT
--	GAPDH-710L	GAGTCCTTCCACGATACCAA

Figure S2. Examination of the specificity of DMTF1 isoform-specific primers. (A) Schematic diagram of alternative DMTF1 pre-mRNA splicing in intron 9 and positions of isoform-specific primers. The DMTF1α, β and γ specific primers cover the splicing junction sites of the corresponding isoforms and are shown in different colors (red, green and cyan, respectively, linked by dotted lines). The sequences of DMTF1α, β and γ isoform-specific primers are aca ata ctt atc gtt cct tca cc, aca act gtg gac ccc aaa aaa ag, gaa aaa ggc aat tgc tgc ctg, respectively. (B) Schematic diagrams of pcDNA3/3xFlag-DMTF1 reporter plasmids mimicking the spliced DMTF1α, β and γ isoforms in intron 9 (named as spliced isoform reporters). The isoform-specific primers of DMTF1α, β and γ are shown in red, green and cyan, respectively. A common downstream primer located in exon 10 (gtg ttg caa gta tcc ttc atc ag) is drawn in black. The stop codon shared by the DMTF1β and γ is indicated. (C) The DMTF1 spliced isoform reporters shown in (B) and an empty pcDNA3-3xFlag vector were individually transfected into HeLa cells. After 48 h, total RNA was extracted from the cells of each transfection, followed by reverse transcription using Poly(dT) as a primer. The samples were then analyzed by qPCR using the isoform- specific primers together with the common downstream primer. A primer pair of GAPDH was also used in qPCR and the data were used for normalization. *P<0.05. DMTF1, cyclin D binding myb-like transcription factor 1.

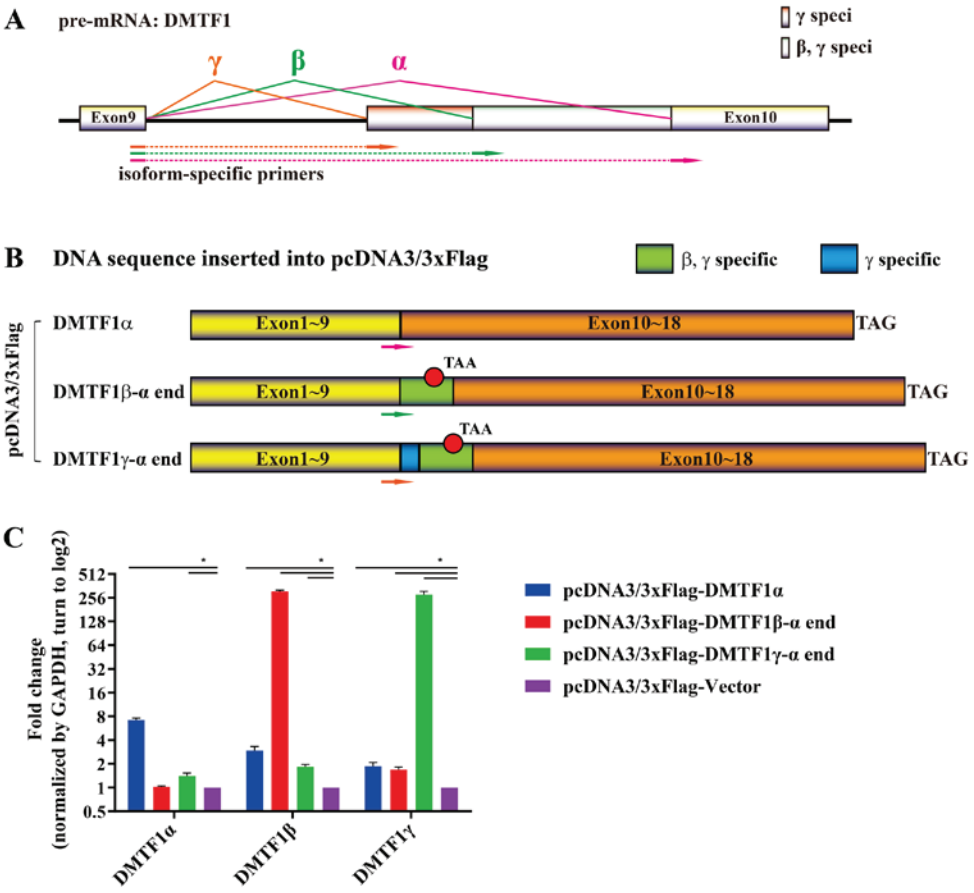


Figure S3. Exhibition of semi-quantitative analysis of mRNA integrity. (A) Schematic diagrams of the positions of different primer sets used in determining mRNA integrity. The predicted transcripts of pSL2- and pcDNA3-3xFlag-DMTF1α, β and γ are shown at the left and right, respectively. The primers pairs are indicated and their sequences are presented in Table SI. (B) Representative DNA agarose electrophoresis of a semi-quantitative PCR for the transcripts. For each DMTF1 isoform, lanes 1-5 are controls using the corresponding plasmids as PCR templates, and lanes 6-10 are samples using their corresponding cDNAs as templates. (C) Quantification of DNA bands of PCR1 to PCR5 in (B). DMTF1, cyclin D binding myb-like transcription factor 1.

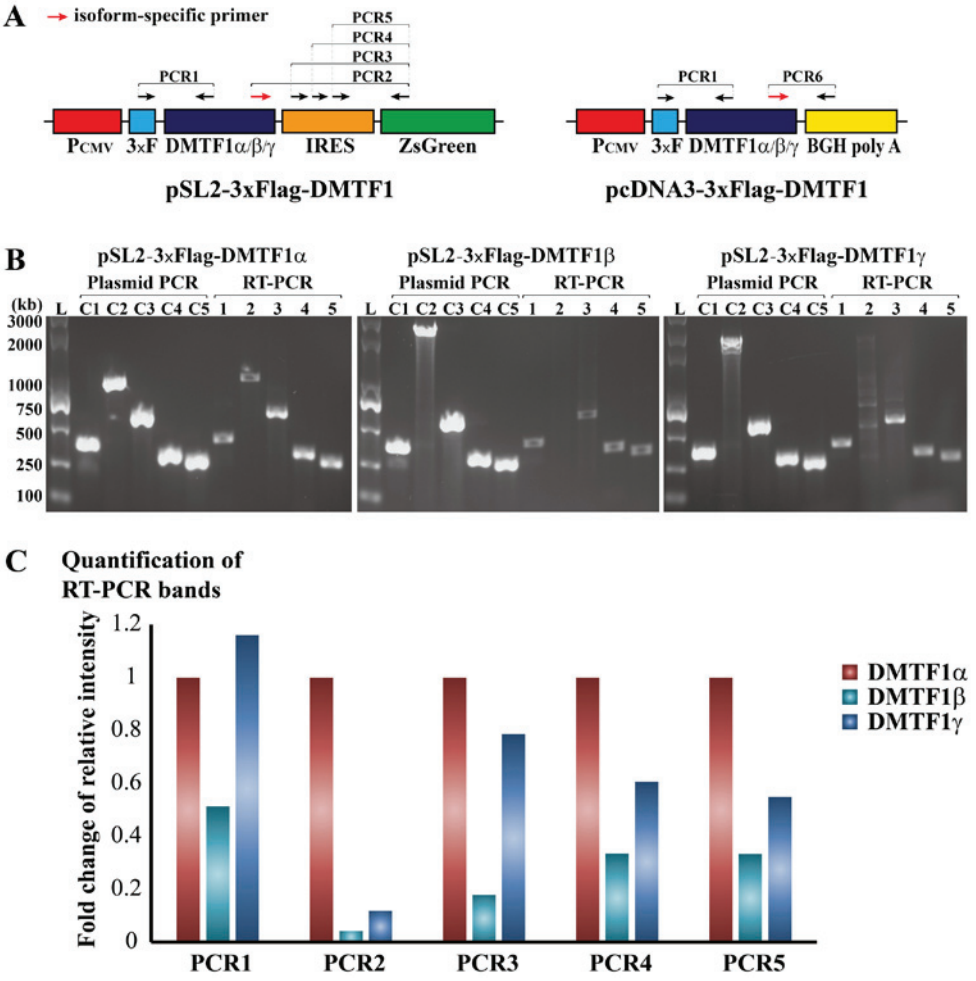


Figure S4. Detection of ZsGreen and RFP expression levels using flow cytometry. The pSL2-3xFlag-DMTF1 vectors expressing DMTF1α, β and γ isoforms, as well as ZsGreen, were individually co-transfected with the pSL3-RFP plasmid into HeLa cells. After 48 h of the transfection, the cells were collected and analyzed by flow cytometry. The cells with both green and red fluorescent signals were detected and quantified for the intensity of the two fluorescent signals. For comparison, the majority of the analyzed cells was denoted by red dashed lines in each graph.

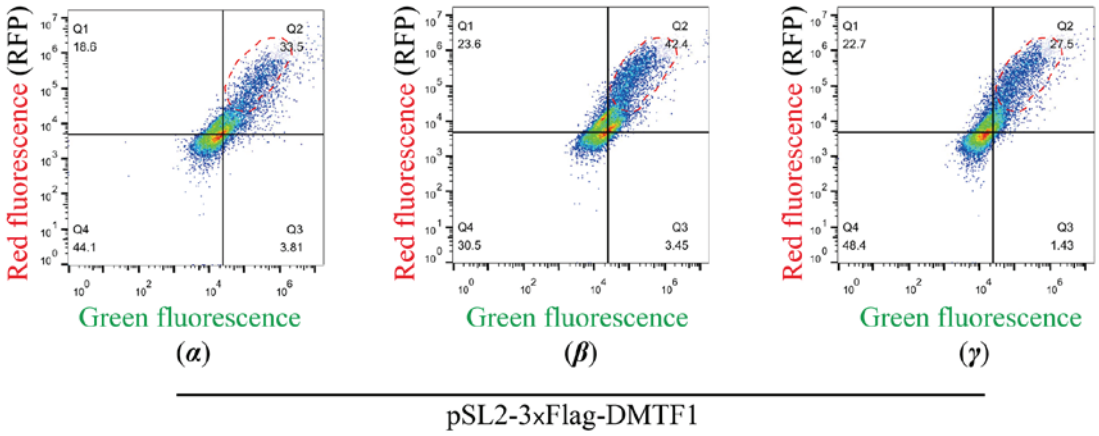


Figure S5. Subcellular localization of DMTF1 isoforms protein in MDA-MB-231 cells. (A and B) Immunostaining of (A) wild-type DMTF1 isoforms and (B) their K52/R53-to-2A (i.e., KR-2A) mutants in MDB-MB-231 cells. MDA-MB-231 cells were individually transfected by empty vector, pcDNA3-3xFlag-DMTF1α, β and γ wild-type, or KR-2A mutants together with an EGFP expression vector, and then immunostained using a Flag antibody. White arrow heads were used to indicate and align transfected cells. DMTF1, cyclin D binding myb-like transcription factor 1.