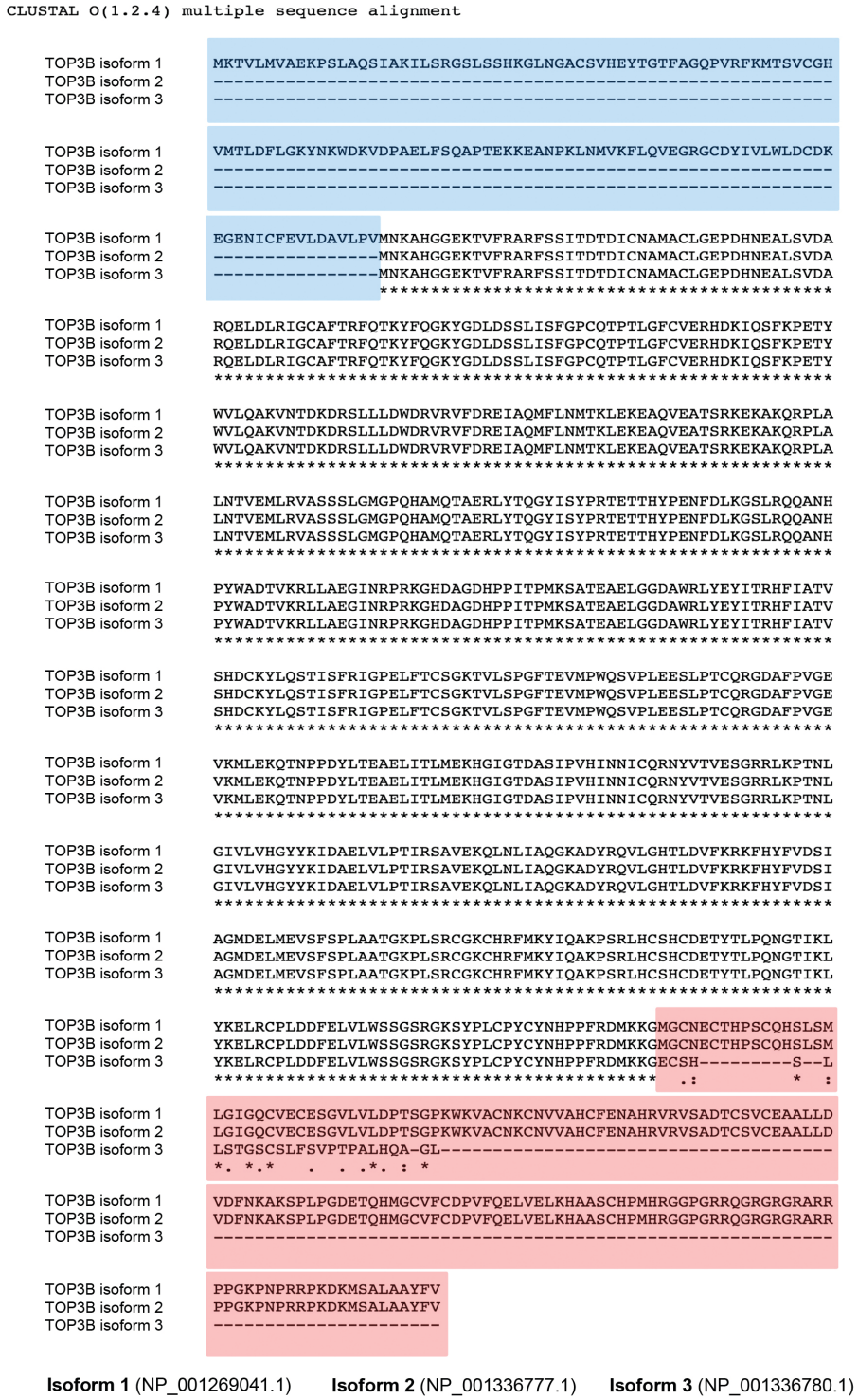
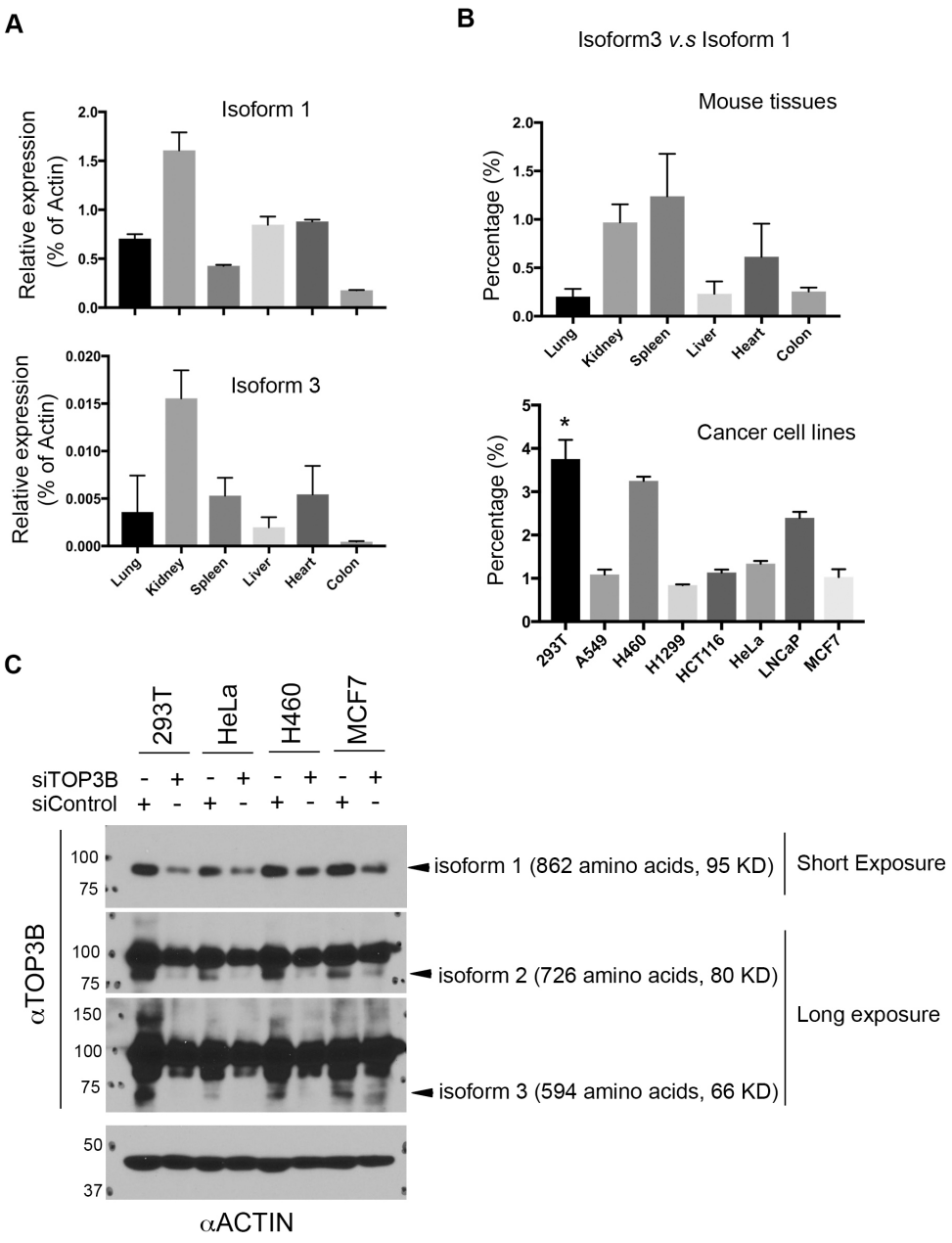
Supplementary Figure 1



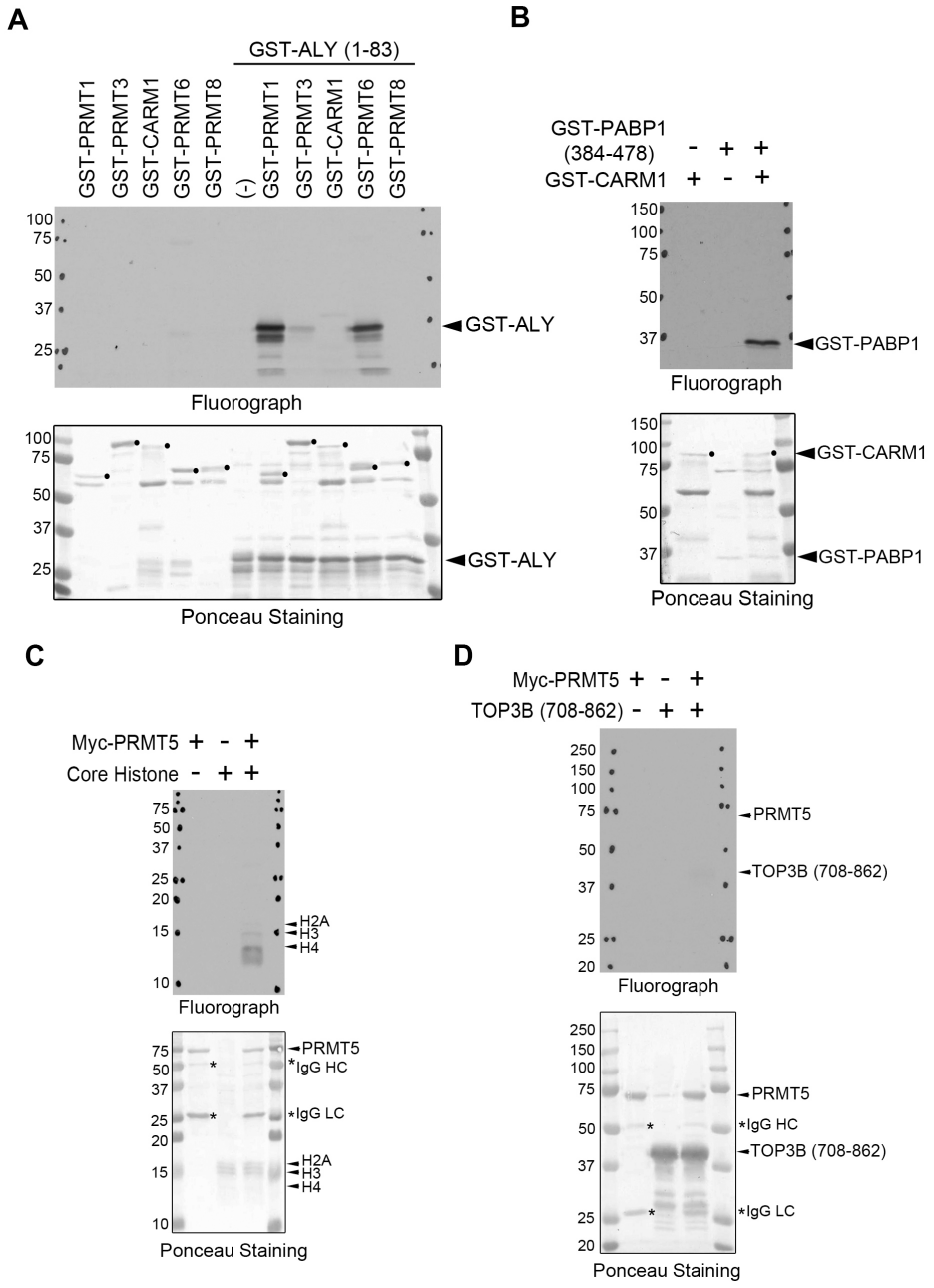
Supplementary Figure 1. Amino acid sequence alignment of three TOP3B isoforms. Clustal Omega alignment was performed using protein sequences of human TOP3B isoforms. The N- and C-terminus of three isoforms are highlighted in blue and red.

Supplementary Figure 2



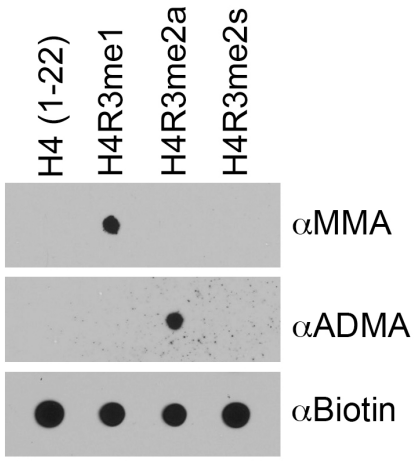
Supplementary Figure 2. Expression analysis of TOP3B isoform1 and isoform3 in mouse tissues and human cancer cell lines. (**A**) The mRNA expression of TOP3B isoform 1 and isoform 3 in mouse tissues were examined by RT-qPCR using isoform-specific primers. (**B**) The relative expression of isoform 3 compared to isoform 1 was examined in mouse tissues and human cancer cell lines. * 293T is isolated from human embryonic kidneys. (**C**) Detection of isoform 1 and isoform 3 in several human cancer cell lines. The cells were transfected with control siRNA or TOP3B specific siRNA. Western blot was performed using anti-TOP3B mouse monoclonal antibody. The protein expression of isoform 3 can be detected in all the cell lines tested.

Supplementary Figure 3



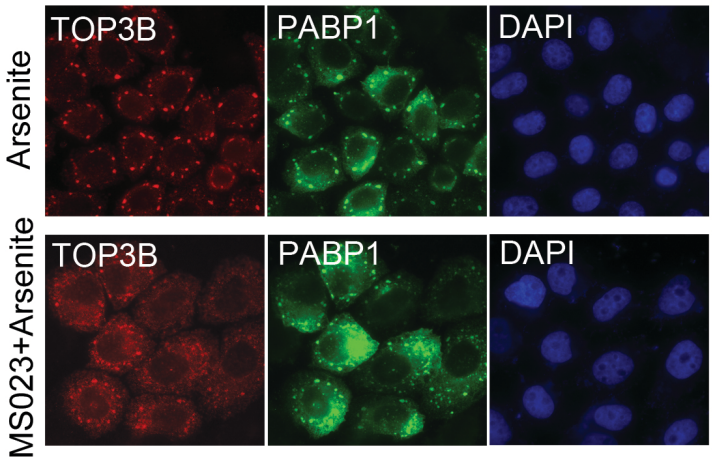
Supplementary Figure 3. Recombinant PRMTs used in the *in vitro* methylation assays are active and TOP3B C-terminal domain is not methylated by PRMT5. (**A**) *In vitro* methylation was performed on a known arginine methylated protein REF/ALY to confirm that recombinant PRMT1, PRMT3 and PRMT6 are active. (**B**) Recombinant CARM1 methylates a known substrate PABP1. (**C**) Myc-tag PRMT5 was purified from 293T cells and incubated with HeLa cell core histone in an *in vitro* methylation reaction. PRMT5 preferentially methylates H4. H3 and H2A are also methylated but to a lesser extent. (**D**) *In vitro* methylation was performed by incubating Myc-PRMT5 with TOP3B C-terminal domain (708-862). For the same exposure time as histone substrates (one week), no obvious methylation signal was detected.

Supplementary Figure 4



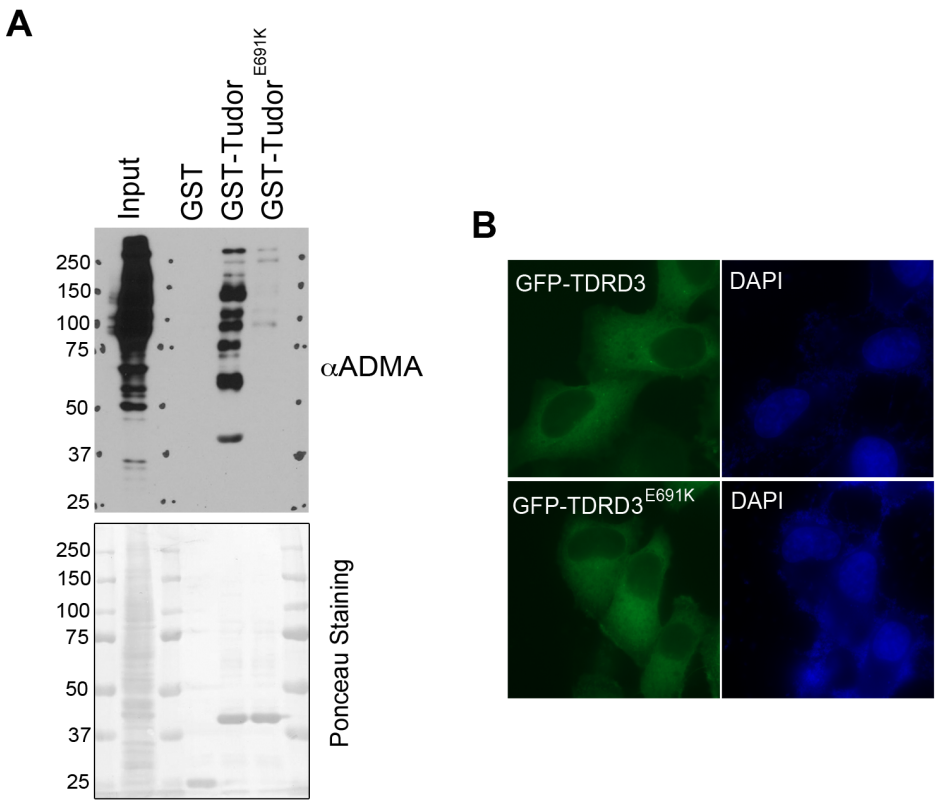
Supplementary Figure 4. Assess the specificity of MMA and ADMA antibodies. Dot-blot assays were performed to assess the specificity of pan-MMA and pan-ADMA antibodies using synthetic biotinylated Histone H4 peptides (N-terminus 22 amino acids) carrying defined methylation at arginine 3 site: monomethylation (H4R3me1), asymmetrical dimethylation (H4R3me2a), and symmetrical dimethylation (H4R3me2s). The loading of the peptides was examined using anti-Biotin antibody.

Supplementary Figure 5



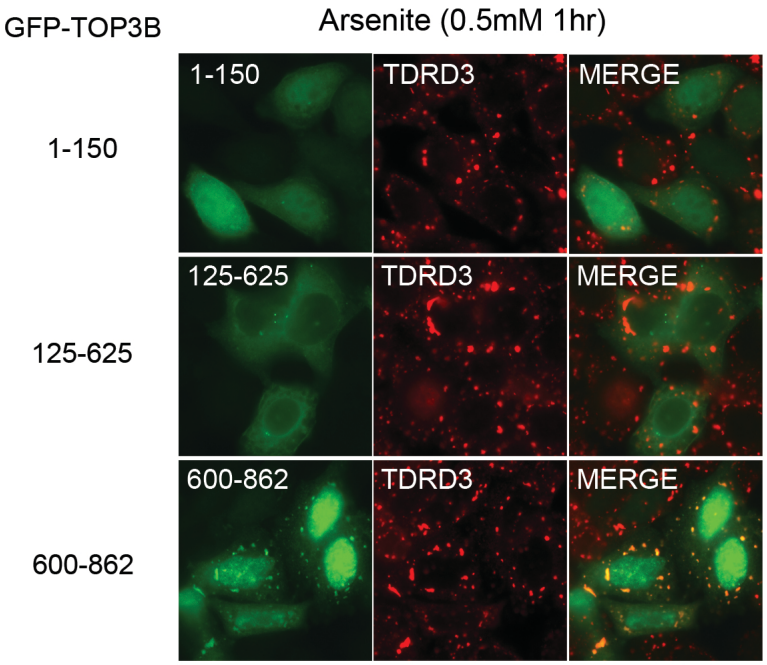
Supplementary Figure 5. Inhibition of protein arginine methylation reduces TOP3B stress granule localization. HeLa cells cultured on glass cover slides were untreated or treated with MS023 for 2 days before the cells were treated with 0.5 mM Arsenite for 1 hour. The samples were processed as described in **Figure 5** (**A**), except that anti-TOP3B and anti-PABP1 (marker for stress granules) antibodies were used to examine the endogenous protein localization.

Supplementary Figure 6



Supplementary Figure 6. Confirmation of Tudor domain functional mutation and GFP-TDRD3 subcellular localization. (**A**) GST-tag recombinant Tudor domain and methylarginine-binding deficient Tudor (E691K) were incubated with HeLa cell lysates. After pull-down, the samples were subjected to western blot detection using ADMA antibody. (**B**) Both GFP-TDRD3 and GFP-TDRD3 (E691K) were transiently transfected into HeLa cells. The subcellular localization of both proteins was visualized by fluorescence microscope.

Supplementary Figure 7



Supplementary Figure 7. The interaction between TDRD3 OB-fold and TOP3B catalytic domain is not sufficient to mediate their respective recruitment to SGs. Three TOP3B truncations were expressed as GFP-fusion proteins. After Arsenite treatment, the co-localization of GFP-fusion proteins with TDRD3 (Red) was examined by immunofluorescence. Note that TOP3B (125-625), which is sufficient to interact with TDRD3, doesn't exhibit strong stress granule localization.

Supplementary Table 1 (RT-qPCR primers)

NRAS	CTACAGGGAGCAGATTAAGCG
	TAACTCTTGGCCAGTTCGTG
DDX5	TGATTTGGAGAGAGGTGTGG
	TTCAAAGCCCATATCAAGCA
c-MYC	TTCTCTCCGTCCTCGGATTCTCTG
	TCTTCTTGTTCCTCCTCAGAGTCG
Human *TOP3B* isoform 1	CGTCCTTGGCACAGTCAATTG
	ATCTTGAAGCGCACTGGCTGG
Human *TOP3B* isoform 3	GTCCTGTGGTCATCAGGCTCT
	GACTGCAGCTACCTGTGGAC
Mouse *Top3b* isoform 1	GCAACACCTGCGAGGCTGCC
	GCTGCATGCTTAAGCTCCACCA
Mouse *Top3b* isoform 3	GAACTGGTCCTGTGGTCCTC
	CTCTCAGCTCACCTGTTACTTG

Supplementary Table 2 (DRIP-qPCR primers)

pFC53 R loop fragment	TTTAGAGCTTGACGGGGAAA
	CAACAGTTGCGTAGCCTGAA
c-MYC	GAGGCTATTCTGCCCATTTG
	GGTGCTTACCTGGTTTTCCA
DDX5	GTGTCATCGGTGTCCTTCCT
	ACTCGAATAACCCGACATGG
NRAS	CGTTTCACTGATGCCAGAAA
	TCCTTCCCATTCTCCCTTCT

Supplementary Table 3 (TOP3B site mutagenesis primers)

R824K	ctgccaccccatgcac AAA ggtggaccagggagaag
	cttctccctggtccaccTTTgtgcatggggtggcag
R829/830K	cgcggtggaccaggg AAAAAG cagggtcgagggcgg
	ccgccctcgaccctgCTTTTTccctggtccaccgcg
R833/835K	gggagaaggcagggt AAAgggAAG ggccgggccaggagg
	cctcctggcccggccCTTcccTTTaccctgccttctccc
R837/839/840K	ggtcgagggcggggc AAGgccAAGAAG ccccctgggaagccc
	gggcttcccaggggggCTTCTTggcCTTgccccgccctcgacc
R848/849K	gggaagcccaacccc AAAAAG cccaaggacaagatg
	catcttgtccttgggCTTTTTggggttgggcttccc