CAPG enhances breast cancer metastasis by competing with PRMT5 to modulate STC-1 transcription

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Content:

Supplementary Figure Legends

Figure S1. CAPG protein detection data from iTRAQ-nano-HPLC-MS/MS. (A) CAPG protein detection data by MS/MS in MDA-MB-231 HM cells and MDA-MB-231 cells.

Figure S2. CAPG increased BC metastasis. (A) Migration and (B) invasion assays comparing MDA-BM-231 BO-control and MDA-MB-231 BO-shCAPG cells after 14 or 24 h.

Figure S3. Affymetrix Human Gene Expression Array detection and Go analysis of mRNA regulated by CAPG. (A) A heat map of the mRNA levels of 397 genes derived from control and MCF-7-CAPG cells in the Affymetrix Human Gene Expression Array. con., control. The scale bar depicts the change in standard deviation. (B) GO analysis of mRNAs from the Gene Expression Array according to biological processes. (C) BT474 cells were transfected with control or CAPG as shown. STC-1 expression was determined by western blot. GAPDH was used as loading control.

Figure S4. CAPG promotes STC-1 promoter activity in HEK 293T cells. Dual-luciferase reporter assay analyzing luciferase activity in HEK 293T cells transiently co-transfected with FLAG-CAPG, Renilla luciferase, and various STC-1 promoter fragments in the pGL3-basic reporter vector as indicated. ** $p<0.01$, *** $p<0.001$.

Figure S5. Coomassie staining of purified proteins in SDS-PAGE gels. (A) Coomassie staining showed purified His (lane 2), His-tagged CAPG (lane 3), GST (lane 4) and GST-tagged PRMT5 (lane 5) from BL21 cells induced with IPTG. Molecular weight markers are shown in lane 1. (B) PRMT5 protein levels in MCF-7-CAPG, MDA-MB-231 HM-shCAPG and MDA-MB-231 BO-shCAPG and their control cells.

Figure S6. Correlation between PRMT5 expression and overall survival (OS) was analyzed with Kaplan–Meier curves in BC patients. Kaplan-Meier analysis of OS (p=0.117) associated with different PRMT5 expression levels (PRMT5-: N=92, blue line; PRMT5+: N=152, green line) based on the BC tissue microarray.

Figure S7. CAPG competes with PRMT5 to bind the exact location of the STC-1 promoter. (A) qPCR-ChIP analysis of CAPG binding to the STC-1 ChIP-2 promoter in MDA-BM-231 HM transfected with pWPI or with pWPI-PRMT5 separately. (B) qPCR-re-ChIP analysis of H3R8me2s and H4R3me2s cross-linked the protein-DNA complex eluted from PRMT5 in MDA-MB-231 BO-control and MDA-MB-231 BO-shCAPG cells. qPCR was performed to amplify the STC-1 ChIP-2 promoter.

Supplementary Table S1-4

Table S1 Clinicopathological variables and the expression of CAPG and PRMT5 in the studied cases

Variables	CAPG expression		p[a] value	PRMT5 expression		p[a] value
	Negative n (%)	Positive n (%)		Negative n (%)	Positive n (%)	
Age			1.000			0.424
≤35 years	3 (1.2)	13 (5.3)		12 (4.9)	4 (1.6)	
>35 years	45 (18.5)	183 (75.0)		140 (57.4)	88 (36.1)	
Menopausal status			0.936			1.000
Premenopause	21 (8.6)	87 (35.7)		68 (28.0)	40 (16.5)	
Postmenopause	27 (11.1)	109 (44.6)		84 (34.4)	51 (18.8)	
Tumor size			0.868			0.586
≤2 cm	18 (9.3)	79 (37.6)		64 (26.9)	33 (13.9)	
>2 cm	28 (10.1)	112 (43.0)		88 (37.0)	53 (22.2)	
Grade			0.492			0.100
I-II	35 (17.1)	113 (55.1)		110 (53.9)	38 (18.6)	
III	10 (4.9)	47 (22.9)		42 (20.6)	14 (6.9)	
Lymph node status			0.918			0.290
Negative	29 (11.9)	120 (49.2)		78 (32.0)	54 (22.1)	
Positive	19 (7.8)	76 (31.1)		74 (30.3)	38 (15.6)	
ER status			**0.002**			0.228
Negative	19 (7.8)	125 (51.2)		85 (34.8)	59 (24.2)	
Positive	29 (11.9)	71 (29.1)		67 (27.5)	33 (13.5)	
HER-2/neu status			**0.005**			0.280
Negative	38 (15.6)	111 (45.5)		97 (39.8)	52 (21.3)	
Positive	10 (4.1)	85 (34.8)		55 (22.5)	40 (16.4)	

Abbreviations: CAPG, macrophage-capping protein; PRMT5, protein arginine methyltransferase 5; ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2; a. p is based on Fisher's exact test.

Table S2 shRNA for genes

Gene symbol	shRNA
CAPG	5’-CCGGGCTGATATCTGATGACTGCTT-3’
	5’-CCGGGCATTTCACAAGACCTCCACA-3’
PRMT5	5’- AGTACCAGCAGGCCATCTATA-3’
	5’-CCCATCAGAGAGGAGCATTTC-3’
	5’-CCCATCCTCTTCCCTATTAAG-3’
STC-1	5’- CAACAGGAGACGCACCAATGA-3
	5’-TGCTGGCCATGGACGAATATG-3’

Table S3 Primers for plasmid construction

Gene symbol	Inserted plasmid name	Primers	Restriction enzyme
CAPG	pCDH-puro	F：5’ CGGCTAGCATGTACACAGCCATTCCCCAGA 3’	Nhel I
		R：5’ AAATATGCGGCCGCTCAGATTACAAGGATGACGACGATAAGTAATTTCCAGTCCTTGAAAAAT 3’	Not I
CAPG	pcDNA3-HA	F：5’ CGGAATTCTGATGTACACAGCCATTCCCCAGA 3’	EcoR I
		R：5’ TCCCTCGAGTCATTTCCAGTCCTTGAAAAAT 3’	Xho I
CAPG	pCMV-FLAG	F：5’ CGGAATTCATGTACACAGCCATTCCCCAGA 3’	EcoR I
		R：5’ TCCCTCGAGTTTCCAGTCCTTGA AAAAT 3’	Xho I
CAPG	pET28a	F：5’ CGGAATTCATGTACACAGCCATTCCCCAGA 3’	EcoR
		R：5’ TCCCTCGAGTTTCCAGTCCTTGA AAAAT 3’	Xho I
PRMT5	pCMV-FLAG	F：5’ ATAGGATCCATGCGGGGTCCGAACTCGGGGA 3’	BamHI
		R：5’ ATACTCGAGGAGGCCAATGGTATATGAGC 3’	Xho I
PRMT5	pWPI.1	F：5’ ATAGGATCCGCCACCATGCGGGGTCCGAACTCGGGGA 3’	BamHI
		R：5’ ATCACGCGT TTACTTATCGTCGTCATCCTTGT 3’	Mul I
PRMT5	pGEX-4T-1	F：5’ ATAGGATCCATGCGGGGTCCGAACTCGGGGA 3’	BamHI
		R：5’ ATACTCGAGCTAGAGGCCAATGGTATATGAGC 3’	Xho I
STC-1 promoter	pGL3-basic	F：5’ CGACGCGTCTTTGGCGTTTTACAGATGGG 3’	Mlu I
(-724 to 225 bp)		R：5’ GAAGATCTTTTCCCTCTCCTGGCTTGAGTG 3’	Bgl II
STC-1 promoter	pGL3-basic	F：5’ CGACGCGTACTGCTGTAAGCAGGTTAAG 3’	Mlu I
(-416 to 225 bp)		R：5’ GAAGATCTTTTCCCTCTCCTGGCTTGAGTG 3’	Bgl II

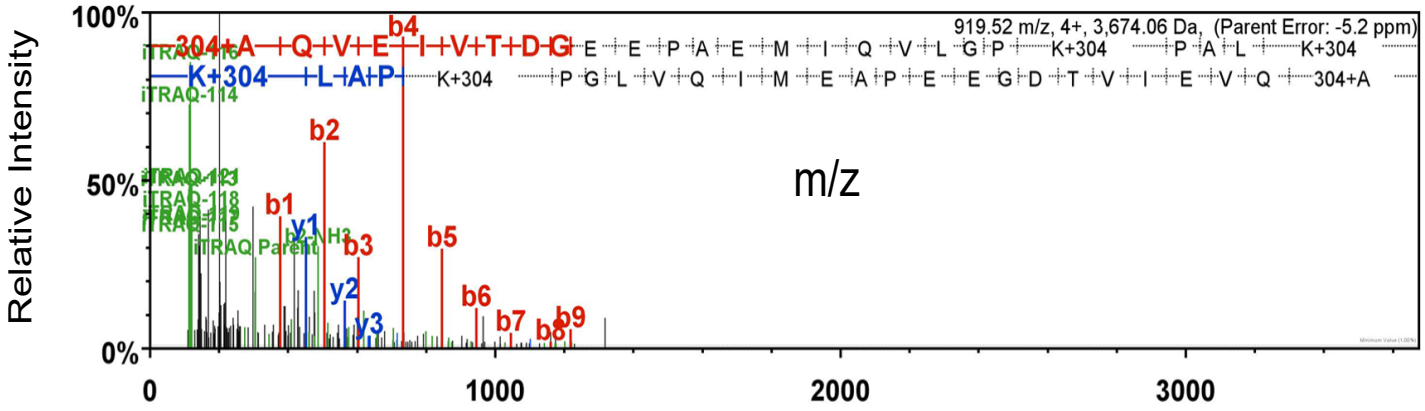
Table S3 Primers for plasmid construction (continued)

Gene symbol	Inserted plasmid name	Primers	Restriction enzyme
STC-1 promoter	pGL3-basic	F：5’ CGACGCGTCAGAGTTATCTCTTACTTCCACG 3’	Mlu I
(-253 to 225 bp)		R：5’ GAAGATCTTTTCCCTCTCCTGGCTTGAGTG 3’	Bgl II
STC-1 promoter	pGL3-basic	F：5’ CGACGCGTGGCAGCAGGTACTTTTAA 3’	Mlu I
(-174 to 225 bp)		R：5’ GAAGATCTTTTCCCTCTCCTGGCTTGAGTG 3’	Bgl II
STC-1 promoter	pGL3-basic	F：5’ CGACGCGTGACCTGATTGGTCCTTGATC 3’	Mlu I
(-74 to 225 bp)		R：5’ GAAGATCTTTTCCCTCTCCTGGCTTGAGTG 3’	Bgl II
STC-1 promoter	pGL3-basic	F：5’ CGACGCGTA AGCAGCGACTGCAGCAGCAG 3’	Mlu I
(25 to 225 bp)		R：5’ GAAGATCTTTTCCCTCTCCTGGCTTGAGTG 3’	Bgl II
STC-1 promoter	pGL3-basic	F：5’ CGACGCGTAGCAGCATCACCAGCAACAAC 3’	Mlu I
(119 to 225 bp)		R：5’ GAAGATCTTTTCCCTCTCCTGGCTTGAGTG 3’	Bgl II

Table S4 Primers for qPCR

Gene symbol	Primers
CAPG	F：5’ AAGCTGAAGCC GGTGCCTGT 3’
	R：5’ TGCTGGCCTATCCACAGGTGCA 3’
STC-1	F：5’ AAATGCATCGCCAACGGGGTCA 3’
	R：5’ TGATGGCTTCAGGGTTCCGCTT 3’
PRMT5	F：5’ CCTGTGGAGGTGAACACAGT 3’
	R：5’ AGAGGATGGGAAACCATGAG 3’
CCDC41	F：5’ CATCAAACTGGAGACAGCAAGAG 3’
	R：5’ GAGCACTTTGTGATGTTCAACAGC 3’
CXCR4	F：5’ GCCTTATCCTGCCTGGTATTGTC 3’
	R：5’ GCGAAGAAAGCCAGGATGAGGAT 3’
EPHX	F：5’ TCGATAAGTTCCGTTTCACCC 3’
	R：5’ AATTCATTCCGCCAGTAGGAGA 3’
HER2	F：5’ CTCTGAGACTGATGGCTACGTTGC 3’
	R：5’CGTCTTTGACGACCCCATTCTTCC 3’
MMP13	F：5’ TGCTGGCTCATGCTTTTCCTCCT 3’
	R：5’ CCGAACTCATGCGCAGCAACAA 3’
MMP9	F：5’ GCTGTGCGTCTTCCCCTTCACTTT 3’
	R：5’ AAGCCCCACTTCTTGTCGCTGT 3’
NM23	F：5’ AGACCAACCCTGCAGACTCCAA 3’
	R：5’ TCAGGGTGAAACCACAAGCCGA 3’
SDF-1	F：5’ AAAGCCATGTTGCCAGAGCCA 3’
	R：5’ GCTTCGGGTCAATGCACACTTGTCT 3’
VEGFA	F：5’ CCTGGTGGACATCTTCCAGGAGTACC 3’
	R：5’ GAAGCTCATCTCTCCTATGTGCTGGC 3’
GAPDH	F：5’ GAGCTGAACGGGAAGCTCACTG 3’
	R：5’ TGGTGCTCAGTGTAGCCCAGGA 3’
STC-1 promoter 1	F：5’ GCTCCAGCTG AATTGCATG 3’
(-126 to 225 bp)	R：5’ TTTCCCTC TCCTGGCTTG AGTG 3’
STC-1 promoter 2	F：5’ ACAGAAC CGAGAATGTG CTG 3’
(-451 to -75 bp)	R：5’ GTC CCTCAACTGG TCTGGTGA 3’
STC-1 promoter 3	F：5’ GGT ATACAGAATG TCAGAGTC 3’
(-747 to -414 bp)	R：5’ CTTAACCTG CTTACAGCAG 3’

Supplementary 1



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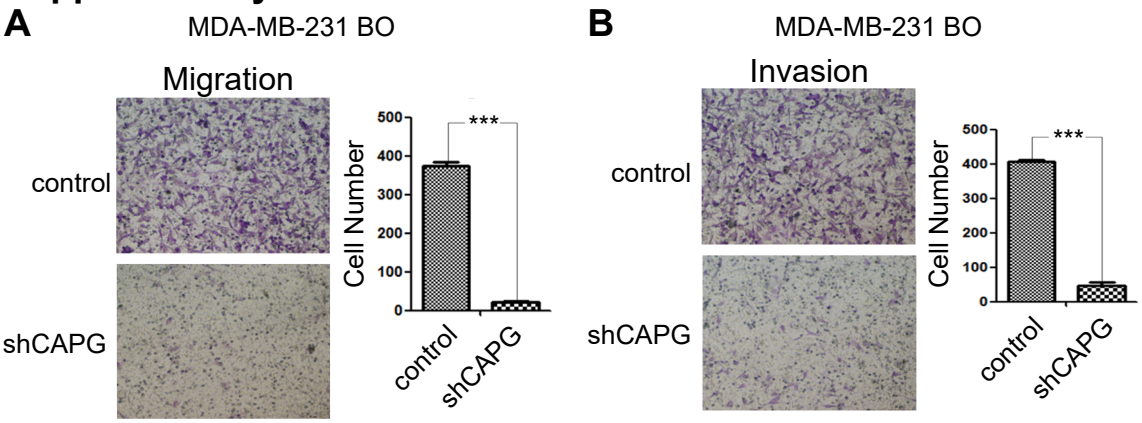

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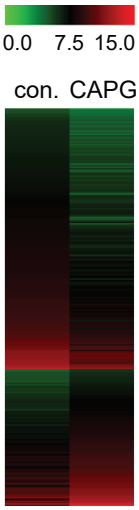

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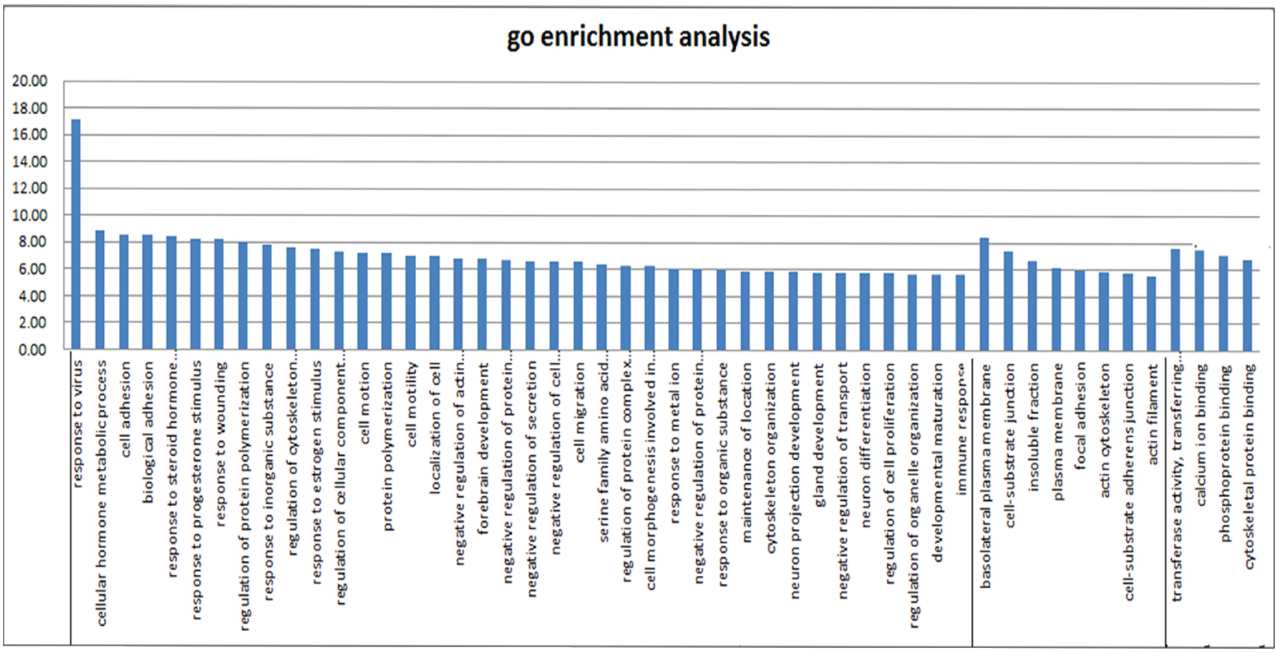

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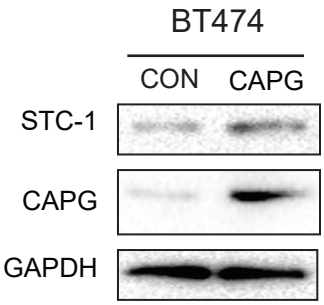

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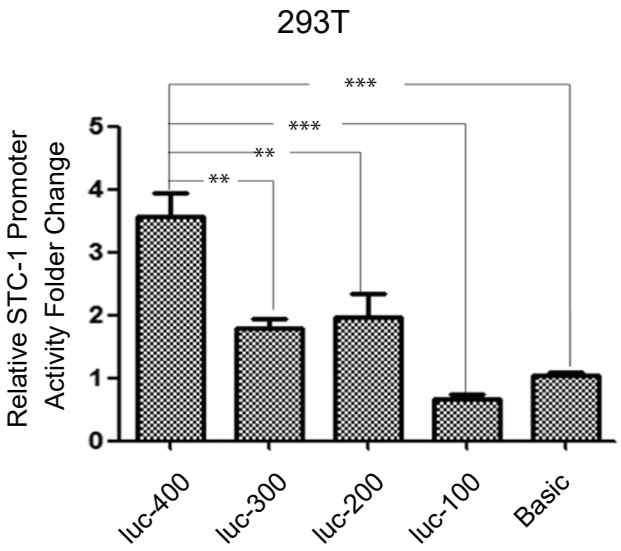

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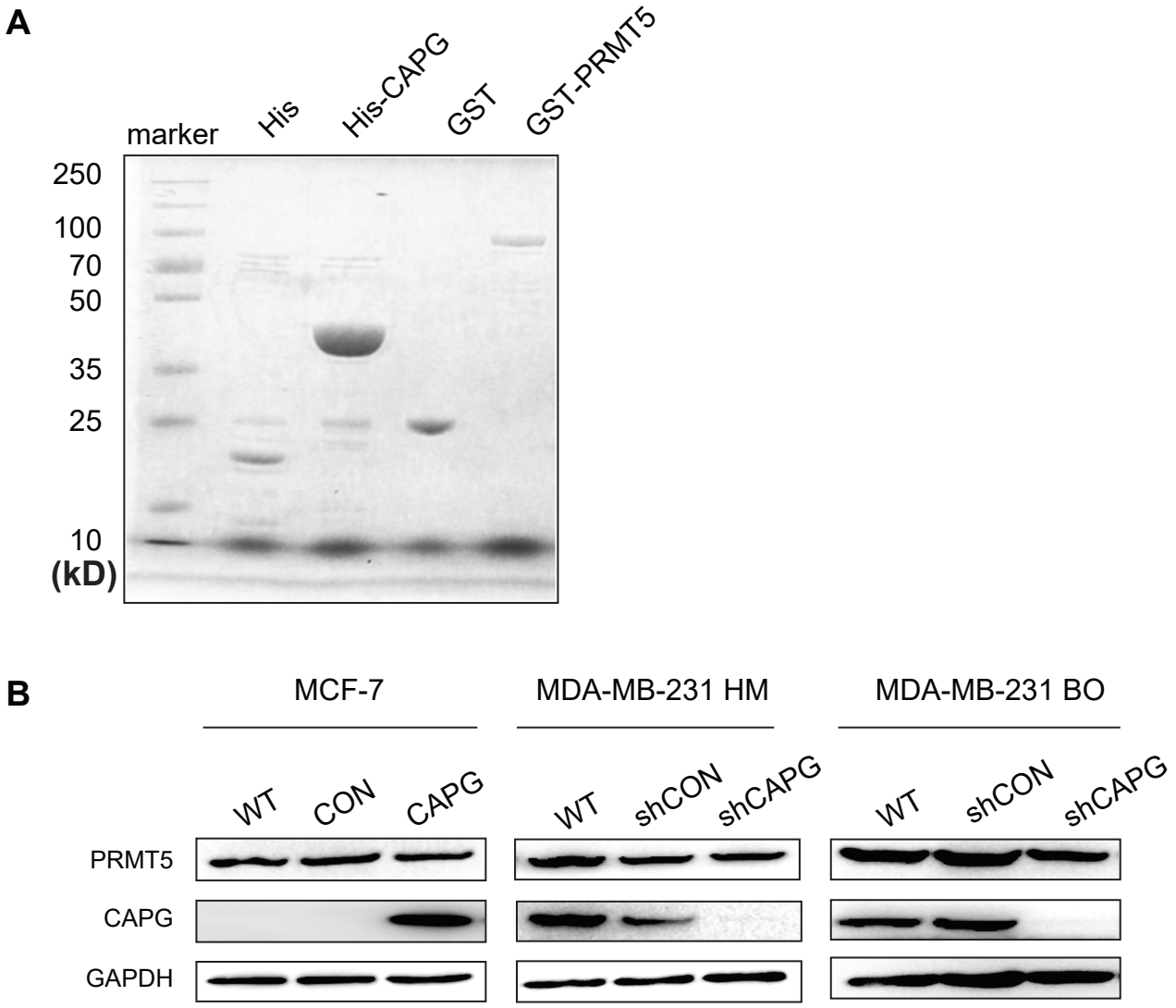

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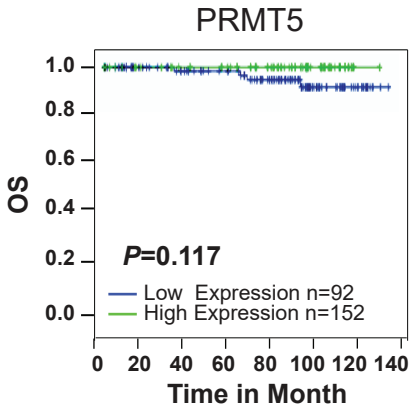

Supplementary 7

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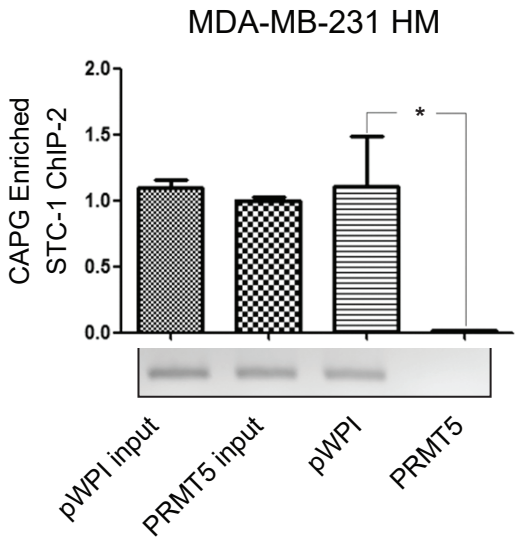

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