

Ginsenoside Rg3 prevents oncogenic long noncoding RNA ATXN8OS from inhibiting tumor-suppressive microRNA-424-5p in breast cancer cells

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Supporting Information

Table S1. PCR primers, siRNA, miR-mimic, and miR-inhibitor used in this study.

Gene		Sequence (5'-3')	Supplier
qRT-PCR			
GAPDH	F :	ACATCGCTCAGACACCATG	IDT
	R :	TGTAGTTGAGGTCAATGAAGGG	
ATXN8OS	F :	GATTCTGGAGGCTGGGAAGT	Bionics
	R :	TCTTGCCCTTCTGCCTTCTA	
DACH1	F :	TGAACAAGCAGAACAGACGC	
	R :	ATCTGTTCTGCCGCCACT	
CHRM3	F :	GTGGCACCTGGTCTCTTTCT	
	R :	AGACCAGCTCCACCTGTT C	
U6		Hs_RNU6-2_11 miScript Primer Assay (MS00033740)	
has-miR-424-5p		Hs_miR-424_11 miScript Primer Assay (MS00004186)	
Methylation Specific PCR			
ATXN8OS	Methyl F :	ATG TTT GGA AGT TTC GTG GG	Bionics
	Unmethyl F :	ATG TTT GGA AGT TTT GTG GG	
	R :	ACTAATTCTACAAAACTCAA A	
siRNA			

ATXN8OS #1	Sense :	CUCACAGAGUUUGAGUGUAtt	Bioneer
	Antisense :	UACACUCAAACUCUGUGAGtt	
ATXN8OS #2	Sense :	CUGAACACACAUAAGUAGAAtt	
	Antisense :	UUCUACUAUGUGUGUUCAGtt	
control		Negative control siRNA (SN-1003)	
miRNA mimic			
has-miR-424-5p	Sequence :	CAGCAGCAAUUCAUGAA	Bioneer
control		miRNA Negative control, mimic #1 (SMC-2002)	
miRNA inhibitor			
has-miR-424-5p	Sequence :	CAGCAGCAAUUVAUGUUUUGAA	Bioneer
control		miRNA Negative control, inhibitor #1 (SMC-3102)	

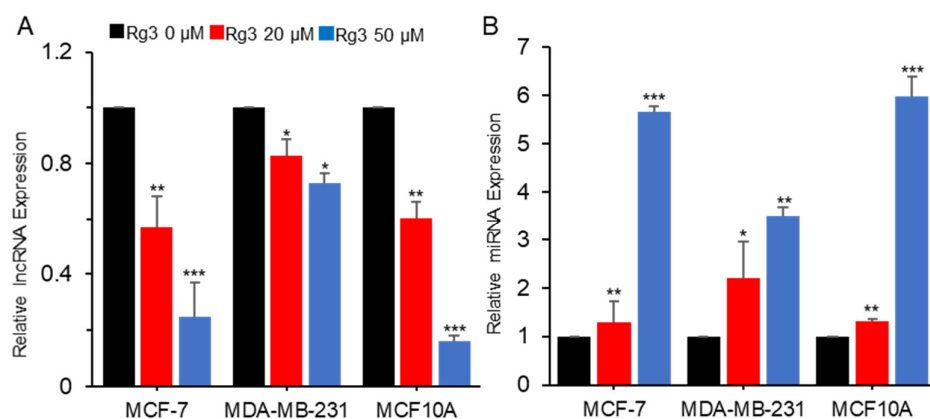


Figure S1. Regulation of ATXN8OS and miR-424-5p by Rg3 in mammary gland-derived cell lines. Breast cancer cell lines, MCF-7 (ER-positive) and MDA-MB-231 (ER-negative), and a normal breast cell line (ER-positive) were treated with Rg3, and the expression of ATXN8OS (A) and miR-424-5p (B) was examined by qRT-PCR. All experiments were performed in triplicate, and the values are presented as the mean $\pm$ SE. *P < 0.05, **P < 0.01, ***P < 0.001

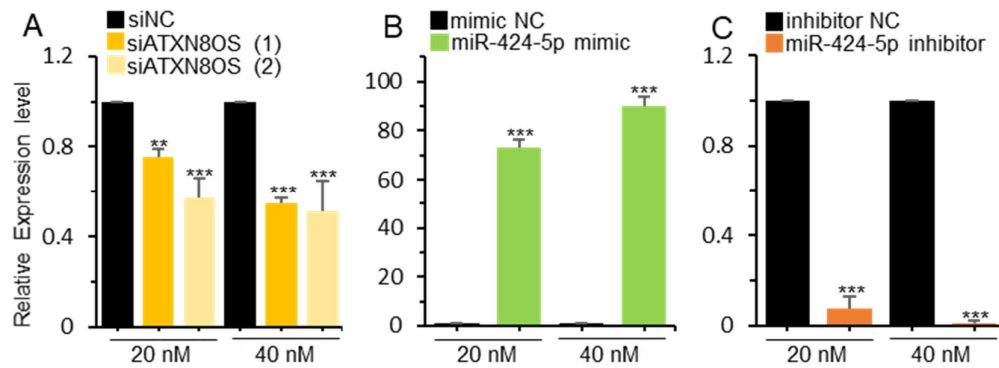


Figure S2. Induction of deregulation of ATXN8OS and miR-424-5p in MCF-7. Cultured cells were transiently transfected with one of the two siRNAs targeting different sites of ATXN8OS (A), miR-424-5p mimic (B), and miR-424-5p inhibitor (C). Gene expression was examined by qRT-PCR. siNC, control siRNA; mimic NC, negative control mimic for miR-424-5p; inhibitor NC, negative control inhibitor for miR-424-5p. All results are reported as the means $\pm$ SE of three experiments. ** $P < 0.01$, *** $P < 0.001$

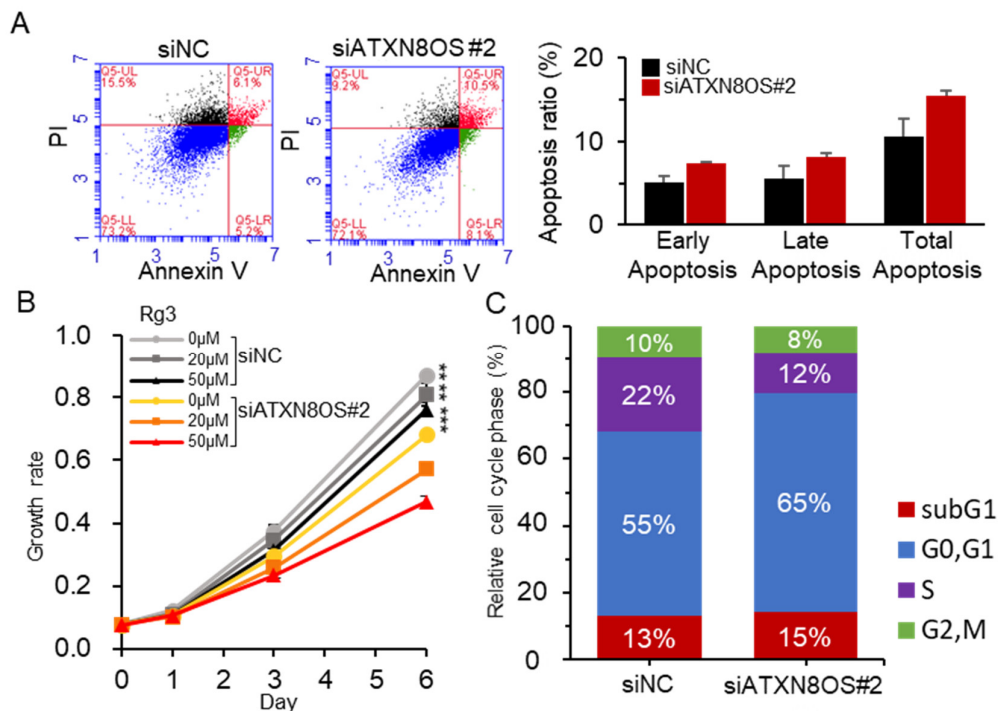


Figure S3. Effect of ATXN8OS on apoptosis, cell growth, and cell cycle. An siRNA of ATXN8OS (siATXN8OS#2) targeting different site from the one (siATXN8OS#1) used in the main manuscript was transiently transfected to MCF-7 cells and its effect on apoptosis (A), cell growth (B), and cell cycle (C) was monitored. Experiments were performed in triplicate, and the values are presented as the mean $\pm$ SE. siNC, control siRNA. ** $P < 0.01$, *** $P < 0.001$

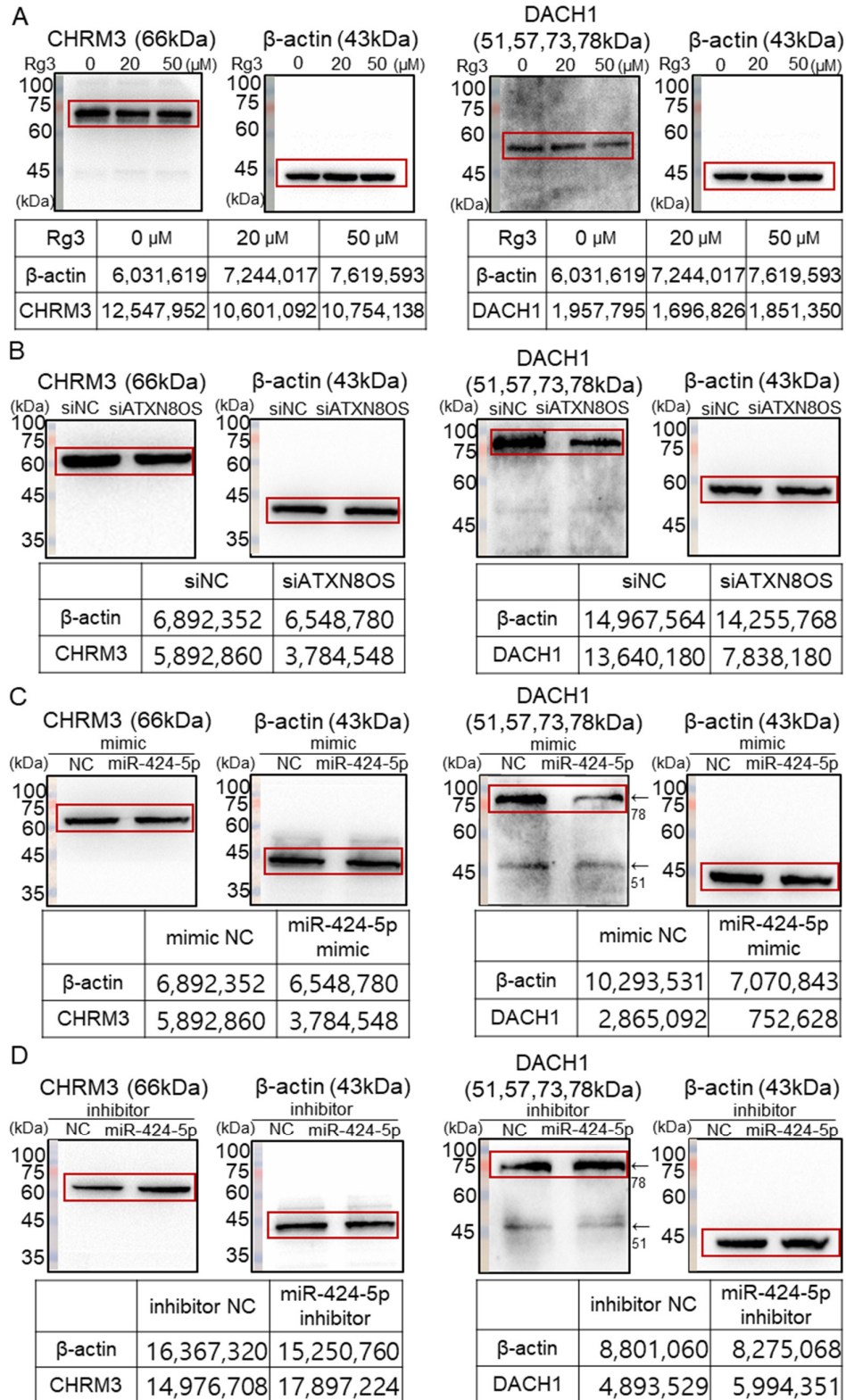


Figure S4. Uncropped Western blots. The boxed areas correspond to the images in the main text. Protein is extracted after Rg3 treated (A), transiently transfected with a siRNA to ATXN8OS (B), miR-424-5p mimic (C), and miR-424-5p inhibitor (D).