Supporting Information

Disease-Associated Mutations G589A and V590F Relieve RFTS-mediated Autoinhibition of DNA Methyltransferase 1

Emma K. Dolen[†,‖], James H. McGinnis[‡,§,‖], Rachel N. Tavory[‡], Jill A. Weiss[‡], and Rebecca L. Switzer[,†]*

[†]*Department of Chemistry, Bucknell University, Lewisburg, PA 17837, United States*

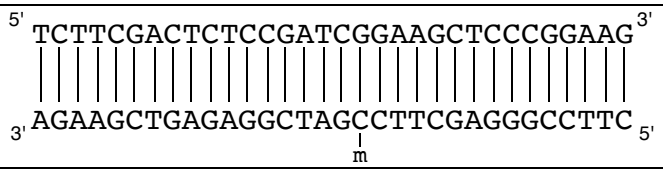
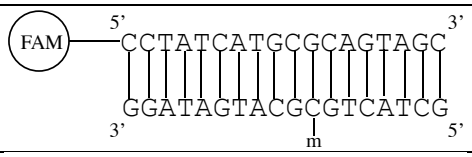
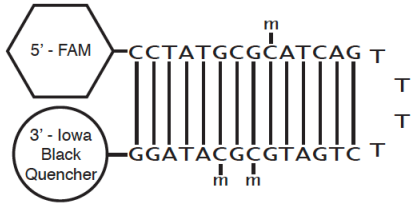
[‡]*Program in Cell Biology/Biochemistry, Bucknell University, Lewisburg, PA 17837, United States*

[*]Correspondence: rebecca.switzer@bucknell.edu

Table S1. Primers used for site-directed mutagenesis

	Sequence (5' → 3')
G589A_For	CTGATCAAGCTGGCTGCGGTCACGCTGGGACAG
G589A_Rev	CTGTCCCAGCGTGACCGCAGCCAGCTTGATCAG
V590F_For	CAAGCTGGCTGGGTTCACGCTGGGACAGAGG
V590F_Rev	CCTCTGTCCCAGCGTGAACCCAGCCAGCTTG

Table S2. DNA oligonucleotides used.

32bp duplex used in EMSA and activity assays	
18bp duplex used in FP assays	
Hairpin DNA for kinetics assays	

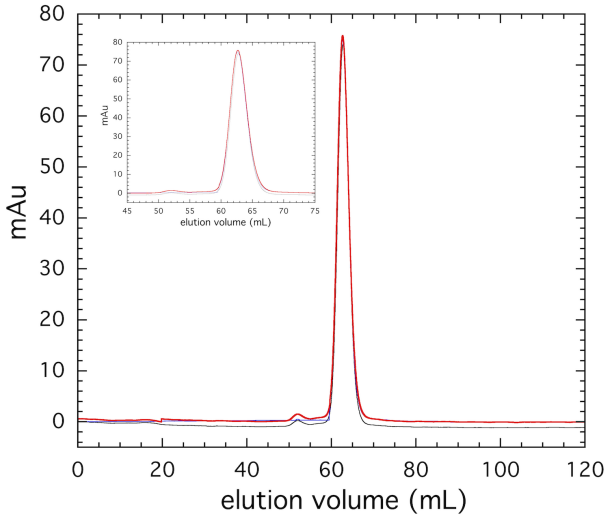


Figure S1: Size-exclusion chromatograms (Hi-Load 16/600 Superdex 75 column) of purified wild-type (black), G589A (blue), and V590F (red) RFTS domains. All RFTS domains eluted as a single peak with an elution volume of ~63 mL.

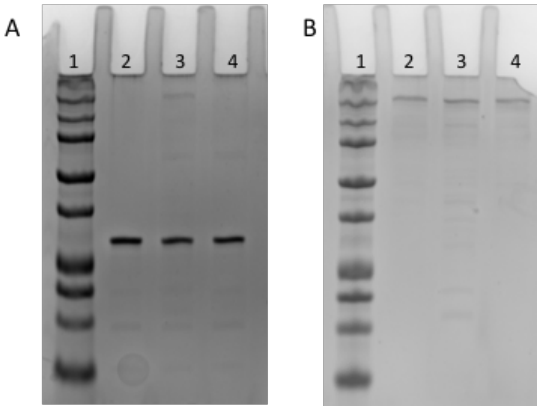


Figure S2: Roughly 1 μg of purified protein was loaded into 12.5% NEXT GEL (VWR Life Science) and electrophoresed at 200 V for 50 minutes. Gels were stained with GelCode Blue (Thermo Fisher Scientific) following manufacturer's instructors and imaged using a Bio-Rad Gel Doc EZ system. (A) Purified RFTS domains (30 kDa) - lane 1: Bio-Rad Precision Plus Protein All Blue Prestained Ladder; lane 2: Wild-type; lane 3: G589A; lane 4 – V590F. (B) Purified RFTS-containing DNMT1 protein (145 kDa) - lane 1: Bio-Rad Precision Plus Protein All Blue Prestained Ladder; lane 2: Wild-type; lane 3: G589A; lane 4 – V590F.