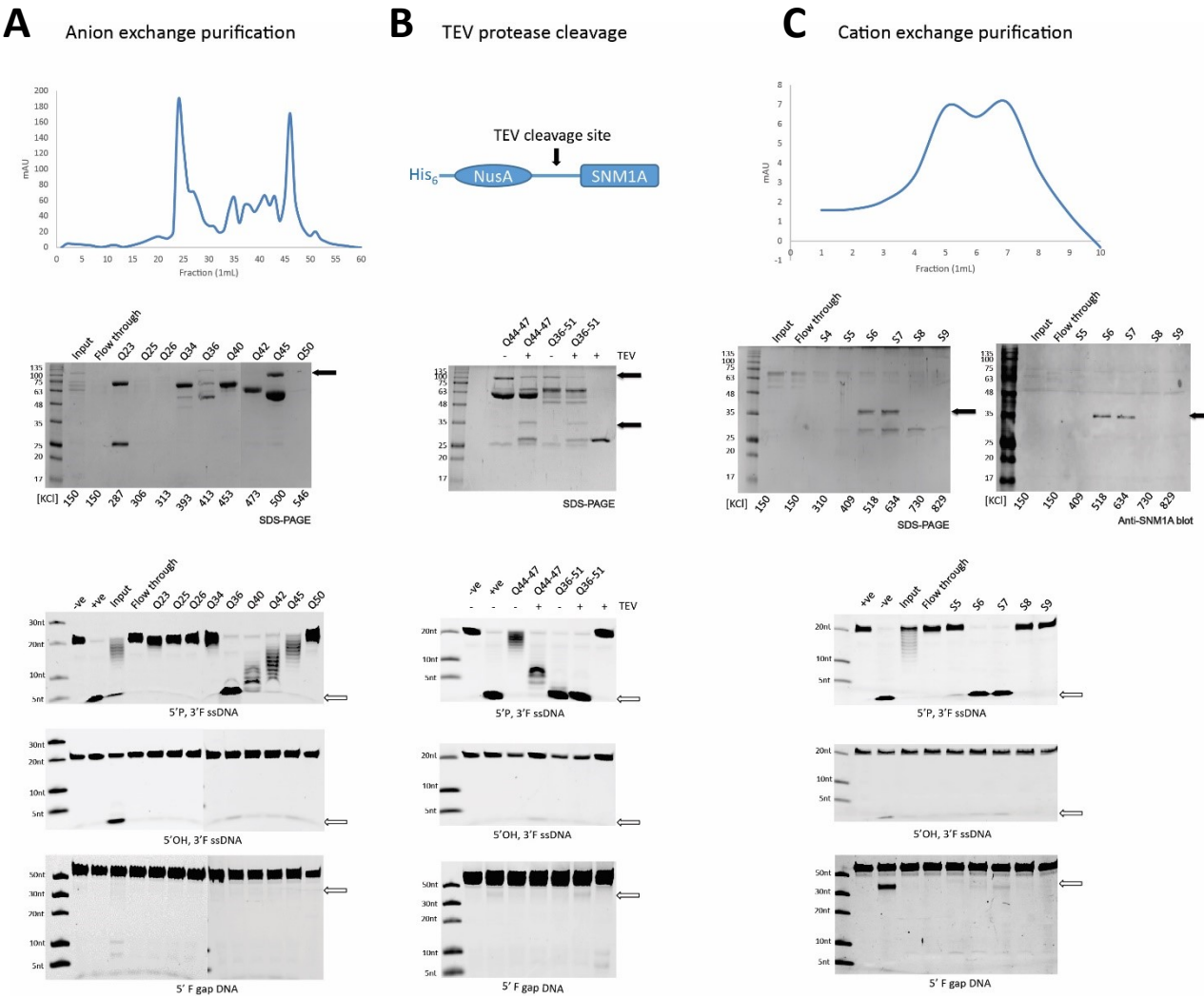
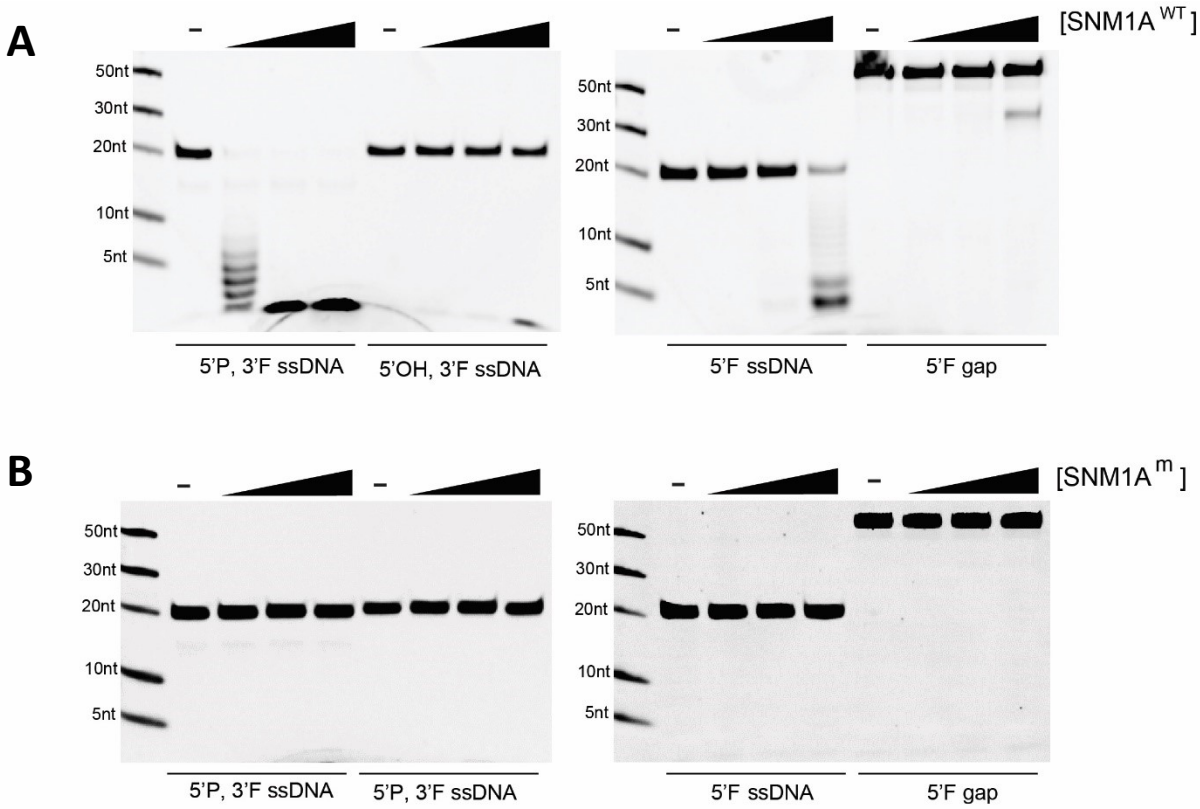
Substrate	Oligo	Sequence	Bases	Modifications
Gap	gap.T	G*AGG GCGAGCCCGATTTTTTCCGCTTGACCCAAGTAAGATTTTTTGCAGATACTTAAC*A*C	60	5'6FAM
	gap.B1	A*GCGGAAAAAATCGGGCTCGCCC*T*C	25	--
	gap.B2	G*TGTTAAGTATCTGCAAAAAATC *T*T	25	--
5' overhang	5'overhang.T	T*TTTTTTTTTTTTTTACTGAGTCCTACAGAAGG*A*T	35	5'6FAM
	overhang.B	G*AT CCT TCT GTA GGA CTC A*G*T	21	--
5' pseudo Y	5'overhang.T	T*TTTTTTTTTTTTTTACTGAGTCCTACAGAAGG*A*T	35	5'6FAM
	5'O/H.polyT.B	G*ATCCTTCTGTAGGACTCAGTTTTTTTTTTTTTT*T*T	36	
5' flap	5'overhang.T	T*TTTTTTTTTTTTTTACTGAGTCCTACAGAAGG*A*T	35	5'6FAM
	5'O/H.B	G*ATCCTTCTGTAGGACTCAGTCATATTTATAACA*C*C	36	--
	SJG 5'flap.B3	G*GTGTTATAAATA*T*G	15	--
3' overhang	3'overhang.T	A*CTGAGTCCTACAGAAGGATCTTTTTTTTTTTTT*T*T	36	5'6FAM
	overhang.B	G*ATCCTTCTGTAGGACTCA*G*T	21	--
3' pseudoY	3'overhang.T	A*CTGAGTCCTACAGAAGGATCTTTTTTTTTTTT*T*T	36	5'6FAM
	3'O/H.polyT.B	T*TTTTTTTTTTTTTTGATCCTTCTGTAGGACTCA*G*T	36	--
3' flap	3'overhang.T	A*CTGAGTCCTACAGAAGGATCTTTTTTTTTTTT*T*T	36	5'6FAM
	3'O/H.B	C*ATATTTATAACACCGATCCTTCTGTAGGACTCA*G*T	36	--
	SJG 5'flap.B3	G*GTGTTATAAATA*T*G	15	--
Symmetrical bubble	bubble.T	A*GGCTGTGTTAAGTATCTGG-TTTTTTTTTTGCTCGCCCTCAGGTCGAC*A*A	50	5'6FAM
	bubble.B	T*TG TCGACCT-GAGGGCGAGCCCGATGAATTCCAGA TAC TTAACACAGC*C*T	50	--
Heterologous loop	bubble.T	A*GGCTGTGTTAAGTATCTGG-TTTTTTTTTTGCTCGCCCTCAGGTCGAC*A*A	50	5'6FAM
	loop.B	T*TGTCGACCTGAGGGCGAGCCCAGA TA CTTAACACAGC*C*T	40	--
Stem loop	stemloop.T	GCTGACTGAGTCCTACAGAAGGATCTTTTTTTTTTGATCCTT CTGTAGGACTCAGTCAG	60	5'6FAM
Paired hairpin	hairpin20	G*AT TAC TAC GGT AG AGC TAC GTA GCT CTA CCG TAG TA*A*T	35	5'6FAM
Y-structure	5'Y.T	G*TACGTAACCTGACTGCTATCGACTGGACTTGATGCCG*T*C	40	5'6FAM
	Y.B1	G*ACGGCATCAAGTCCAGTCGCAGATGGCGTATAGCAGT*T*A	40	--
	Y.B2	T*AACTGCTATAC-GCCATCTGATAGCAGTCAGGTTACGT*A*C	41	--
5'P	5'P	TTTTTTTTTTTTTTTTTTT	20	3'6FAM, 5'P
5'OH	5'OH	TTTTTTTTTTTTTTTTTTT	20	3'6FAM
5'F (PolyT)	PolyT	T*TTTTTTTTTTTTTTTTT*T*T	23	5'6FAM
PolyA	PolyA	A*AAAAAAAAAAAAAAAAA*A*A	23	5'6FAM
PolyAC	PolyAC	A*AACCCAAACCCAAACCC*A*A	20	5'6FAM
SJG ICL 5' P	SJG(AT).5O/H.5'P.3F.T	TTTTTTTTTTTTTTTTATAATTTTAATTTGATCATTTATTATAAATTTTATTAT*A*T	56	3'6FAM, 5'P
SJG ICL 5'Y OH	SJG(AT).5O/H.3F.T	T*T*TTTTTTTTTTTTTATAATTTTAATTTGATCATTTATTATAAATTTTATTAT*A*T	56	3'6FAM
SJG ICL 5'Y F	SJG(AT).5O/H.5F.T	T*T*TTTTTTTTTTTTTATAATTTTAATTTGATCATTTATTATAAATTTTATTAT*A*T	56	5'6FAM
	SJG(AT:5'O/H).B	A*TATAATAAAATTTATAATAAATGATCAAATTAAAATTATCATATTTATAACA*C*C	56	--
	SJG 5'flap.B3	G*GTGTTATAAATA*T*G	15	--
Short ICL	pSJG-T-Cy3	ATAATTT GATC ATCTATTATA	21	3'CY3, 5'P
	SJG-B-Cy3	T*ATAATAGATGATCAAATT*A*T	21	--
Long ICL	SJG(AT).T	ATAATTT GATC ATTTATTATAAATTTTATTATAT	34	3'CY3, 5'P
	SJG(AT).B	A*TATAATAAAATTTATAATAAATGATCAAATT*A*T	34	--
		* denotes phosphorothioate substitution to prevent exonuclease cleavage		

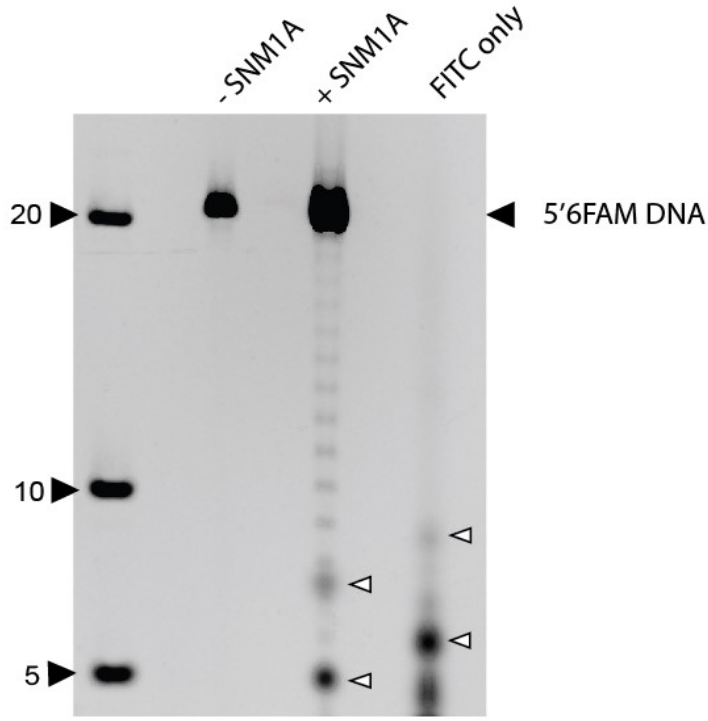
Supplementary 1: Oligonucleotides for nuclease assays.



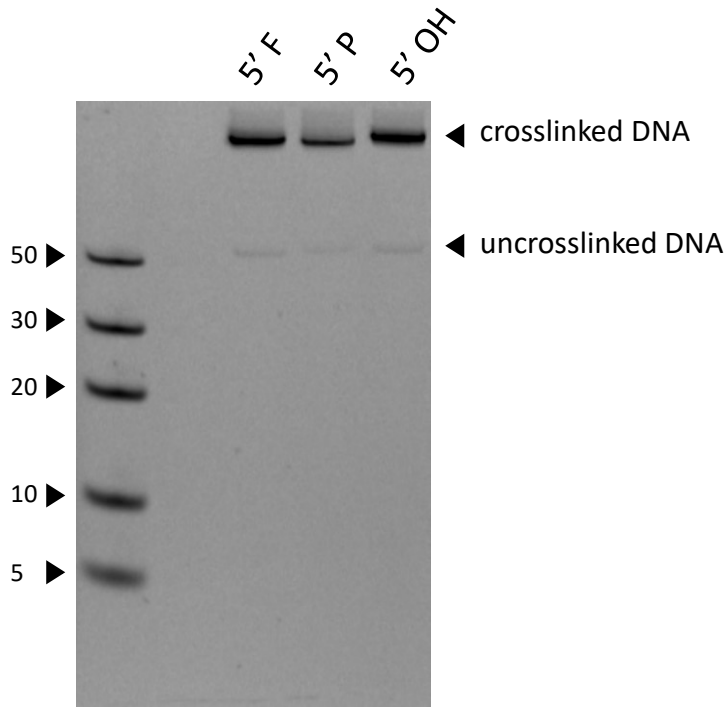
Supplementary 2: Analysis of nuclease activity in SNM1A purification fractions. Following nickel affinity purification, fractions containing SNM1A were pooled and further purified using a MonoQ anion exchange column (GE Healthcare) and linear salt gradient (0.1-1M). Proteins present in collected fractions (20uL) were resolved with 9% SDS PAGE and stained with Coomassie. Nuclease activity present within these fractions was analyzed using indicated substrates. Nuclease assays were performed using 1uL of protein from a given fraction. (B) TEV protease removal of the N-terminal NusA fusion enhances SNM1A nuclease activity. Anion exchange fractions containing His-NusA SNM1A were pooled and digested with TEV protease overnight. Products were resolved with 9% SDS PAGE and stained with Coomassie. Nuclease activity was determined as in (A). (C) Analysis of nuclease activity in fractions from cation exchange purification of SNM1A. SNM1A was further purified using a MonoS cation exchange column (GE Healthcare) and a linear salt gradient (0.1-1M). Fractions (40uL) were resolved with 9% SDS PAGE and stained with Blue Silver. The identity of SNM1A was confirmed using an anti-SNM1A polyclonal antibody (sc-367631, Santa Cruz Biotechnology). Nuclease activity was determined as in (A). Fractions from different chromatographic steps are indicated with Q, S to indicate MonoQ and MonoS, respectively. The number following Q or S denotes the fraction or pool of fractions analyzed. Black arrows indicate SNM1A protein (fusion and/or cleaved); open arrows indicate expected nuclease products.



Supplementary 3: Observed SNM1A nuclease activities are not due to contaminating nucleases. Observed SNM1A nuclease activities are not due to contaminating nucleases. Comparison of nuclease activities for wild-type and catalytically inactive SNM1A mutant. 1-100nM of wild-type SNM1A (A) and catalytic mutant SNM1A^{m} (B) were incubated with indicated substrates for 60 minutes. Products were resolved using 20% denaturing PAGE and imaged with ChemiDoc XRS (BioRad) at 526nm.

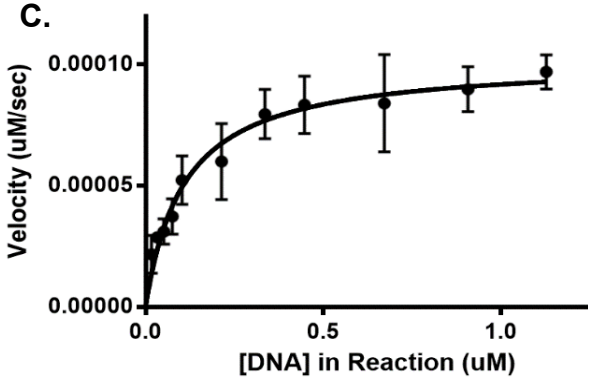


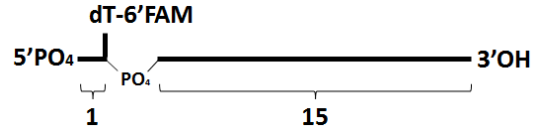
Supplementary 4: Aberrant processing of 6FAM label by SNM1A. Activity of SNM1A on 5'fluorophore-labelled DNA results in distinct shadows between five and ten nucleotides (indicated by white triangles). Free fluorescein isocynate (FITC) fluorophore also has similar distinct shadows, suggesting aberrant processing of the 6FAM fluorophore label. Products were resolved using 20% denaturing PAGE and imaged with Typhoon Imager (GE Healthcare) at 526nm.



Supplementary 5: Analysis of purity of SJG-136 crosslinked 5' flap DNA substrates. (A) 5' flap cross-linked DNA substrates ((100nM) used in Figure 4 were incubated without protein for 60 minutes at 37°C . Substrates were resolved using 20% denaturing PAGE and imaged with the ChemiDoc XRS (BioRad) at 526nm. Approximately 3% of substrate exists as uncross-linked DNA.

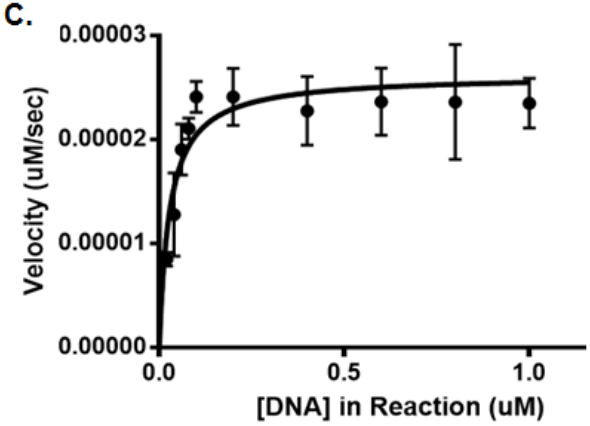
C.

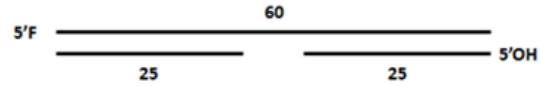




V_{max}	101.8	pM/sec
K_M	109.1	μM
K_{cat}	0.53	s^{-1}
Catalytic Efficiency	4.85	$\mu M^{-1}s^{-1}$

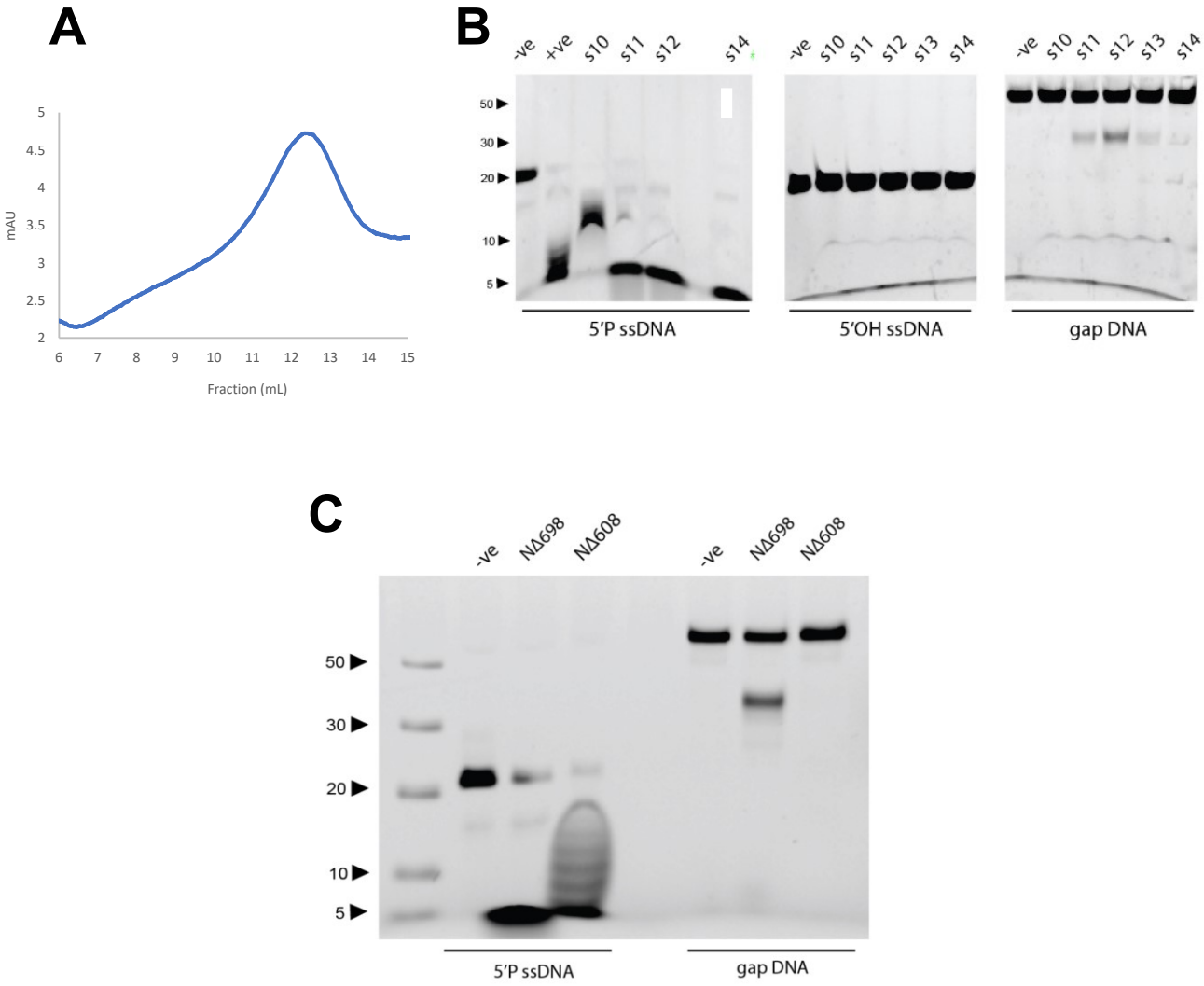
C.





V_{max}	26.2	pM/sec
K_M	28.01	μM
K_{cat}	0.00014	s^{-1}
Catalytic Efficiency	0.00487	$\mu M^{-1}s^{-1}$

Supplementary 6: Kinetic analysis of SNM1A. SNM1A (0.02, 2 μM) was incubated with ssDNA with 5' phosphate, internal fluophore label (A) for 60 minutes or gap DNA with 5'6-FAM label (B) 120 minutes, respectively. Products were resolved using 22.5% denaturing PAGE and imaged with the ChemiDoc XRS (BioRad) at 526nm. Gels were quantified in quadruplicate with ImageLab and analyzed with curve fitting software Prism GraphPad.



Supplementary 7: Analysis of nuclease activity in fractions from cation exchange purification of SNM1A$^{N\Delta608}$. (A) Chromatogram of SNM1A S-seph cation exchange linear salt gradient (0.1-1M). (B) Nuclease activity of S-seph fractions. 1.5µL of each fraction from (A) was incubated with ssDNA with 3'6-FAM label for 60 minutes or gap DNA with 5'6-FAM label 120 minutes. Products were resolved using 20% denaturing PAGE and imaged with the ChemiDoc XRS (BioRad) at 526nm. (C) Nuclease activity of flash frozen S12 fractions of SNM1A$^{N\Delta698}$ (1µL) and SNM1A$^{N\Delta608}$ (5µL). Protein was incubated with ssDNA with 3'6-FAM label for 60 minutes or gap DNA with 5'6-FAM label 120 minutes. Products were resolved using 20% denaturing PAGE and imaged with the ChemiDoc XRS (BioRad) at 526nm.