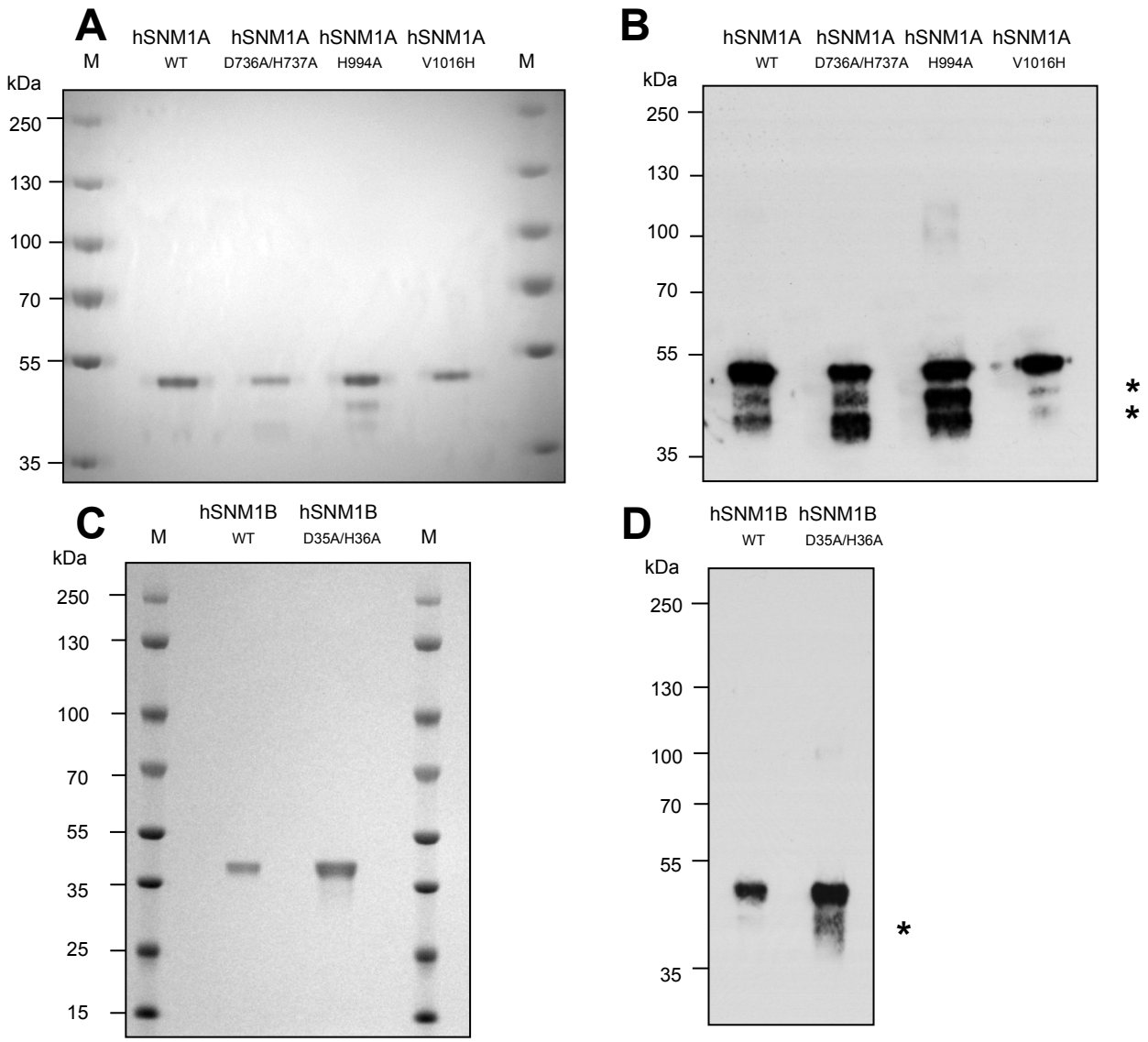
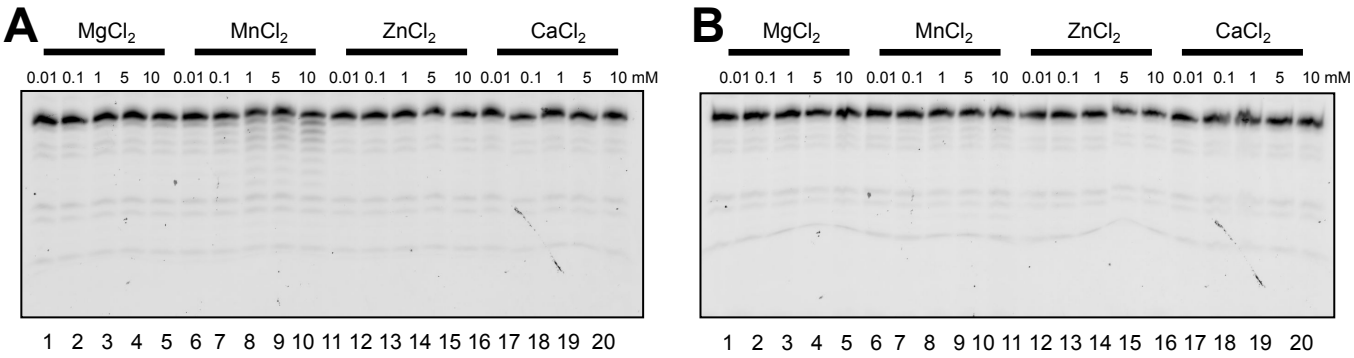
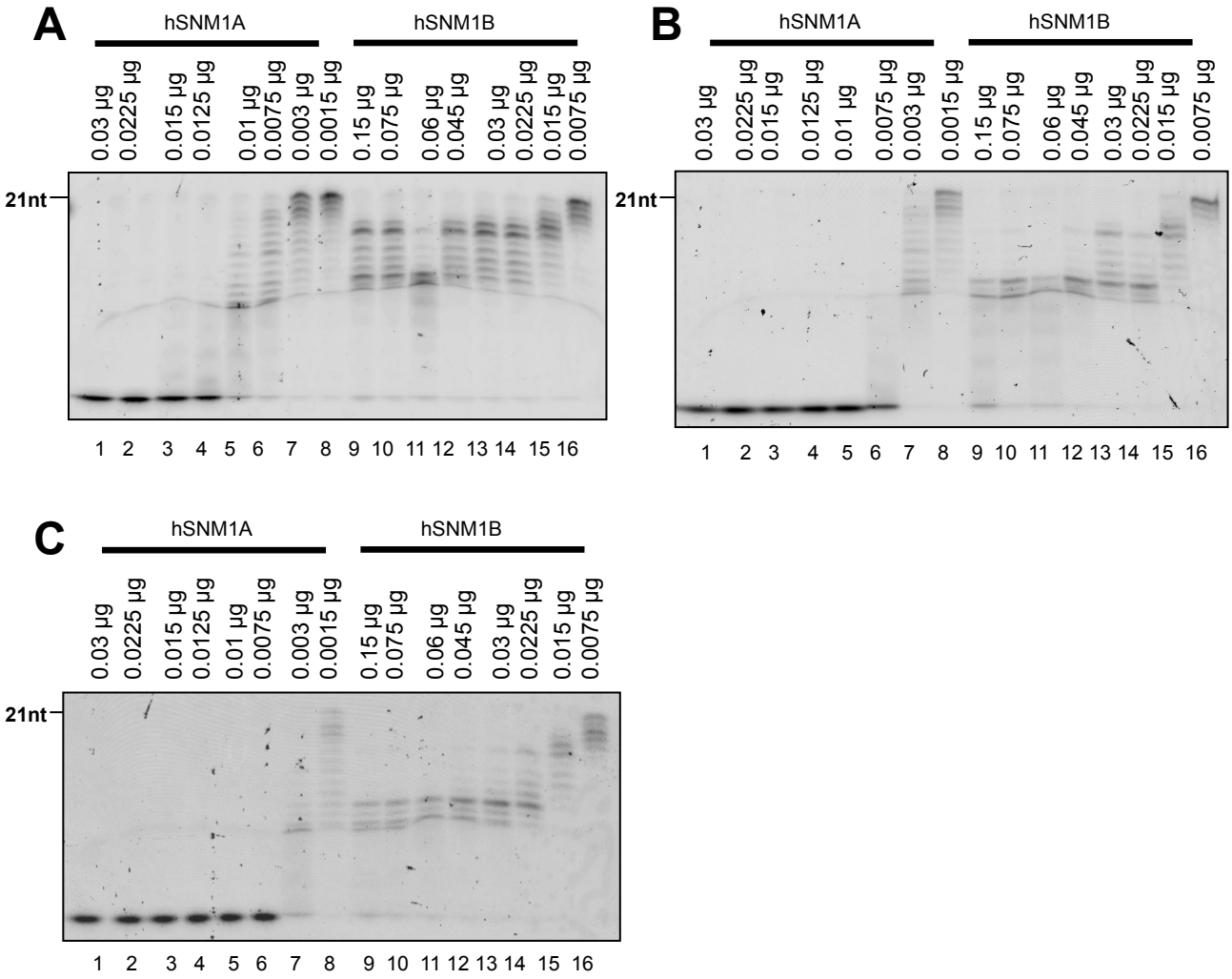


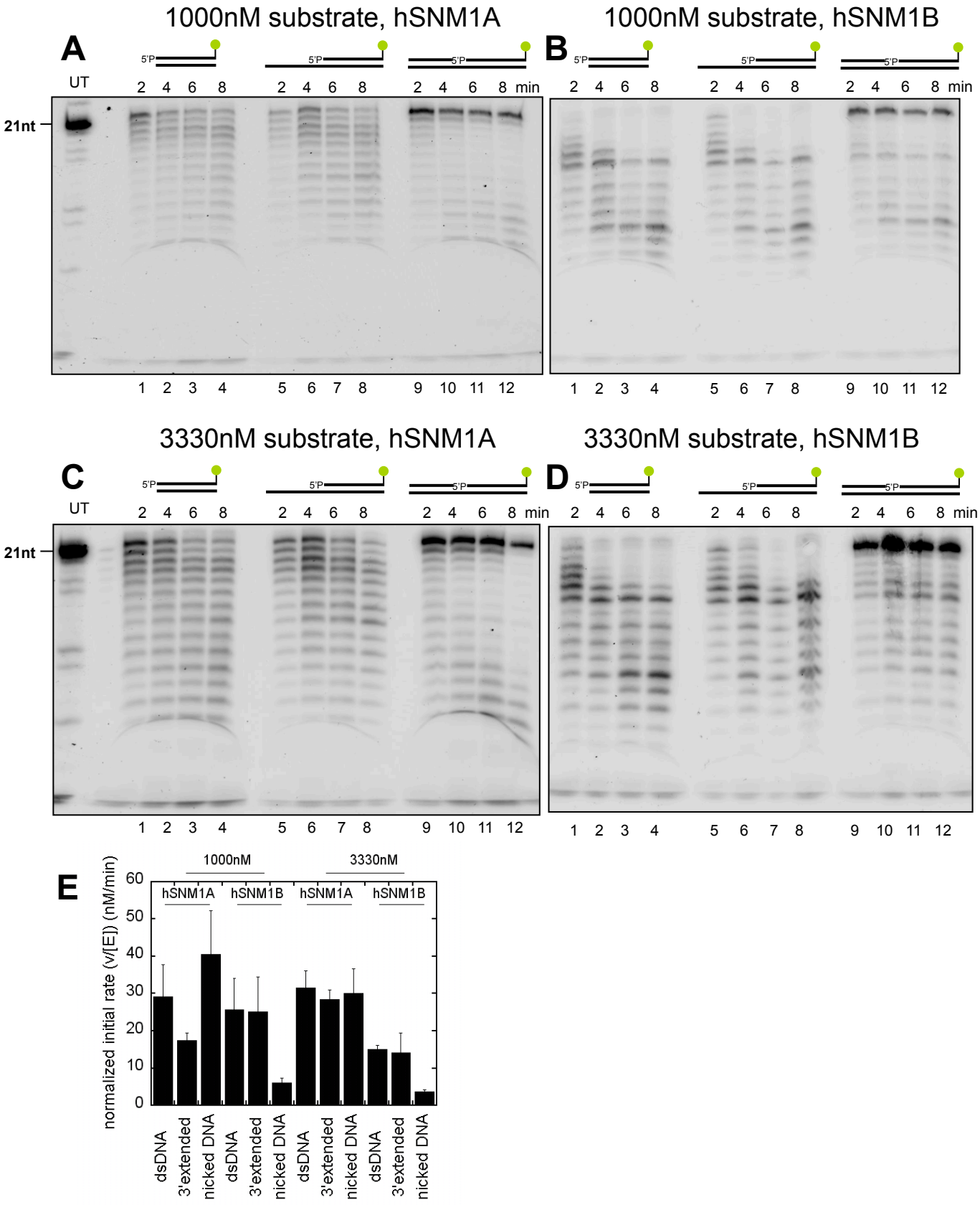
Supplementary Figure S1. (A) InstantBlue stained PAGE gel and (B) immunoblot (Abcam anti-hSNM1A antibody, cat. number ab14805, used at 1:1000) of the different preparations of hSNM1A used in this study, specifically hSNM1A WT, hSNM1A-D736A/H737A, hSNM1A-H994A and hSNM1A-V1016H. Lane (M) PageRuler Plus Prestained protein ladder (Fermentas). (C) InstantBlue stained PAGE gel and (D) immunoblot (Abgent anti-hSNM1B antibody, cat. number ap5426c, used at 1:500) of the different preparations of hSNM1B used in this study, specifically hSNM1B WT and hSNM1B-D35A/H36A. Lane (M) PageRuler Plus Prestained protein ladder (Fermentas). Presumed degradation products are marked with an asterisk.



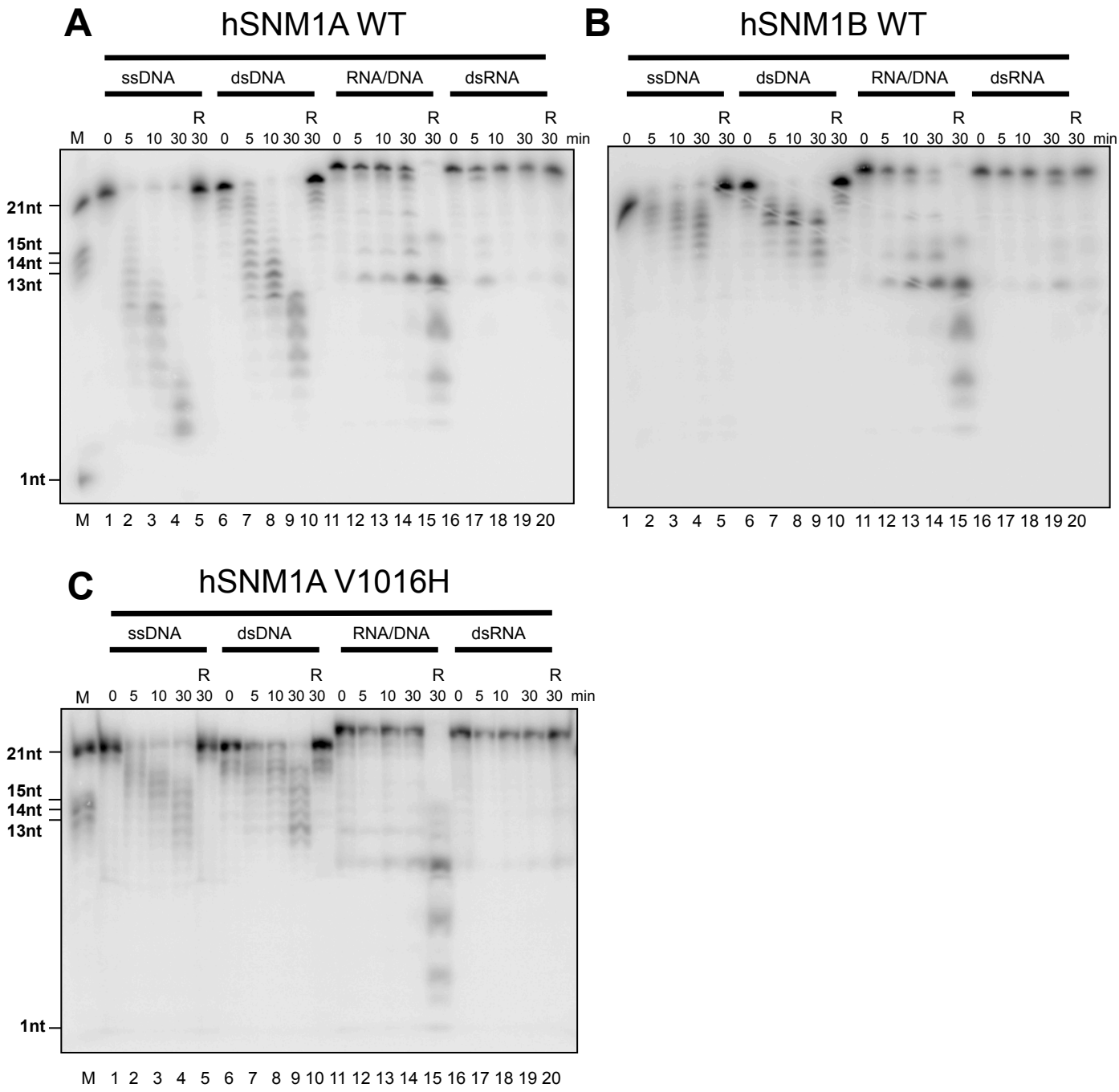
Supplementary Figure S2. Hydrolysis of dsDNA by hSNM1A-D736A/H737A and hSNM1B-D35A/H36A active site mutants in the presence of a variety of divalent metal ion cofactors, which is significantly reduced compared to wild-type proteins. (A-B) The extent of hydrolysis in 30 minutes after addition of (A) hSNM1A-D736A/H737A (0.002 µg), or (B) hSNM1B-D35A/H36A (0.02 µg) to 1000 nM 3' fluorescently labelled dsDNA in the presence of 0.01, 0.1, 1, 5 or 10 mM $MgCl_2$ (lanes 1-5), 0.01, 0.1, 1, 5 or 10 mM $MnCl_2$ (lanes 6-10), 0.01, 0.1, 1, 5 or 10 mM $ZnCl_2$ (lanes 11-15), or 0.01, 0.1, 1, 5 or 10 mM $CaCl_2$ (lanes 16-20).



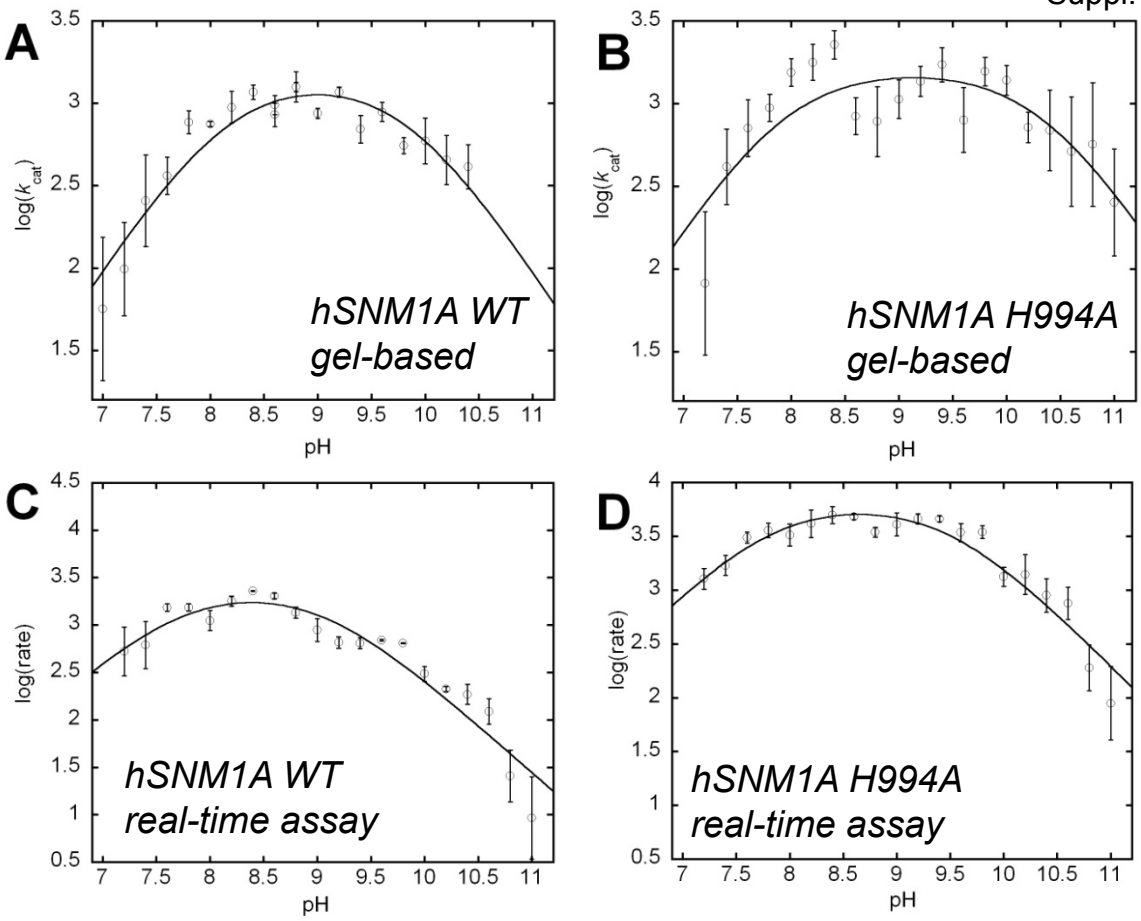
Supplementary Figure S3. Normalizing hSNM1A and hSNM1B activity on dsDNA using (A) 1000 nM, (B) 250 nM or (C) 100 nM substrate. Hydrolysis (30 min) of 1000 nM 3' fluorescein-labelled dsDNA in 10 µL in the presence of 0.03-0.0015 µg hSNM1A (lanes 1-8) or 0.15-0.0075 µg hSNM1B (lanes 9-16). The amount of protein present in the reactions was 0.03 µg (1), 0.0225 µg (2), 0.015 µg (3), 0.0125 µg (4), 0.01 µg (5), 0.0075 µg (6), 0.003 µg (7), 0.0015 µg (8) hSNM1A or 0.15 µg (9), 0.075 µg (10), 0.06 µg (11), 0.045 µg (12), 0.03 µg (13), 0.0225 µg (14), 0.015 µg (15), 0.0075 µg (16) hSNM1B.



Supplementary Figure S4. Substrate preference of hSNM1A and hSNM1B in the context of a variety of oligonucleotide (low molecular weight) substrates. (A-D) Timecourses of the hydrolysis of 1000nM substrate by hSNM1A (A) or hSNM1B (B) and of 3330nM substrate by hSNM1A (C) or hSNM1B (D), using varying oligonucleotide substrates: blunt-ended (lanes 1-4, hydrolysis after 2,4,6,8 min), recessed (lanes 5-8, hydrolysis after 2,4,6,8 min) and nicked (lanes 9-12, hydrolysis after 2,4,6,8 min) dsDNA. Lane (UT) sample of untreated 3'-fluorescein-containing 21mer DNA. Gels are representative of multiple experiments, which show qualitatively equivalent results, and the results are quantified in panel (E).

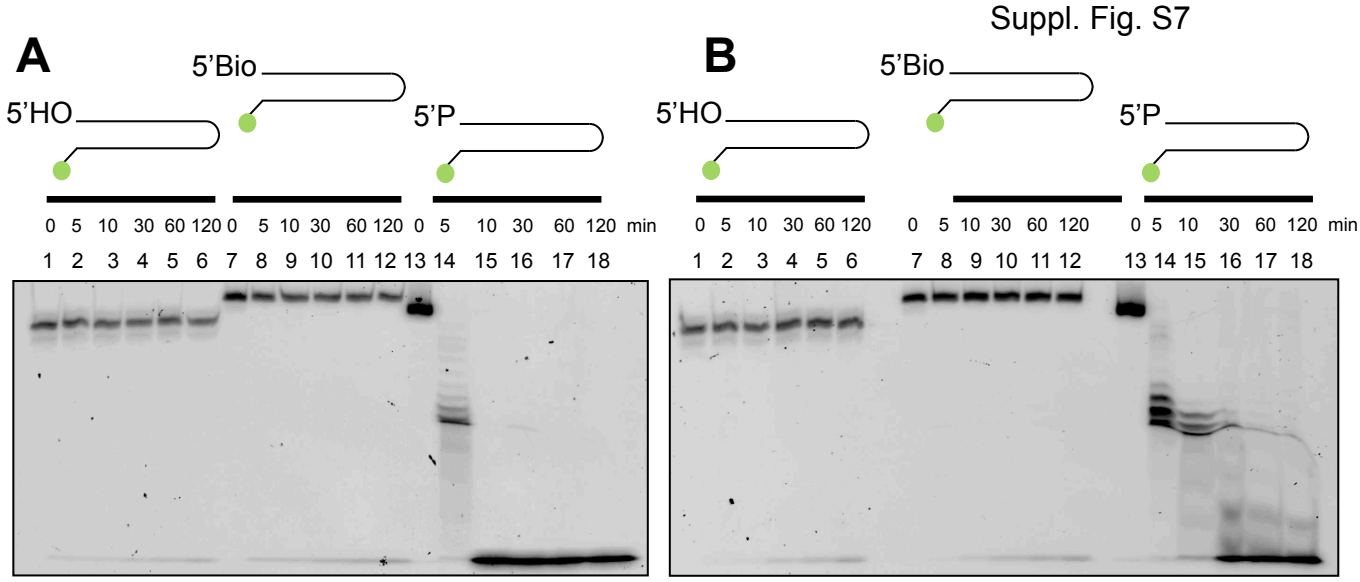


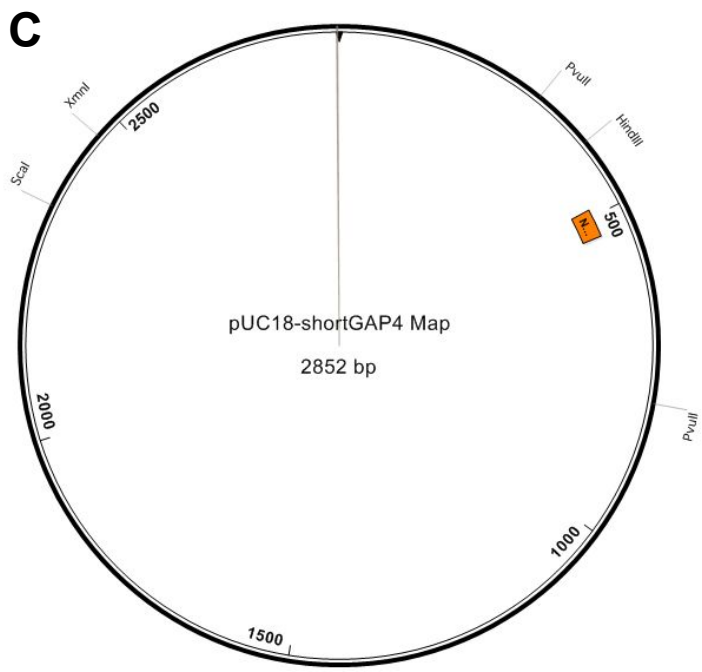
Supplementary Figure S5. hSNM1A and hSNM1B both show a preference for DNA over RNA hydrolysis. This specificity remains unchanged in the V1016H mutant of hSNM1A. Lane (M) 3'-labelled marker oligonucleotides of the size indicated. Timecourses (0, 5, 10, 30 minutes) of the hydrolysis reaction, by hSNM1A (A), hSNM1B (B) or hSNM1A-V1016H (C), on ssDNA (lanes 1-4), dsDNA, (lanes 6-9), RNA-DNA hybrid (lanes 11-14) or dsRNA (lanes 16-19). A control reaction with RNase H (30 min, 0.2 U, Promega) is shown with ssDNA (lane 5), dsDNA, (lane 10), RNA-DNA hybrid (lane 15) and dsRNA (lane 20). Reactions were carried out in 10 µL, with 100 nM 3'-labelled ($^{\alpha}$-32P-dATP) substrate and either 0.002 µg wild-type hSNM1A, 0.02 µg hSNM1B or 0.001 µg hSNM1A-V1016H. Gels are representative of multiple experiments, which show qualitatively equivalent results.



Measurement method	WT hSNM1A	H994A hSNM1A
Gel-based (dsDNA)	pK_{a1} = 8.15 ± 0.12	pK_{a1} = 7.94 ± 0.20
	pK_{a2} = 9.84 ± 0.15	pK_{a2} = 10.32 ± 0.21
Real-time fluorescence based (ssDNA)	pK_{a1} = 7.72 ± 0.30	pK_{a1} = 7.81 ± 0.18
	pK_{a2} = 9.06 ± 0.19	pK_{a2} = 9.49 ± 0.12

Supplementary figure S6: Mutation of histidine-994 in hSNM1A does not significantly alter the pH-rate profile of the dsDNA hydrolysis reaction. A logarithmic plot of an estimate of k_{cat} for wild-type (A) and H994A (B) hSNM1A as it varies with pH. The value of k_{cat} was estimated as the normalized initial reaction rate (v/[E]) at 1000 nM dsDNA, a point at which the Michaelis-Menten curve in Figure 3A is saturated. Values were measured in at least triplicate and the standard error was calculated. The normalized reaction rate (v/[E]) for hydrolysis of 1000nM ssDNA was also measured using the real-time assay (Figure 5) and is shown as a logarithmic plot of the initial reaction rate (increase in fluorescence per minute per nM enzyme) for wild-type (C) and H994A (D) hSNM1A. All plots were fitted to the double ionization model with enzyme-substrate active in singly-ionized form (equation 2) to obtain values of pK_{a1} and pK_{a2} as summarized in the inset table.





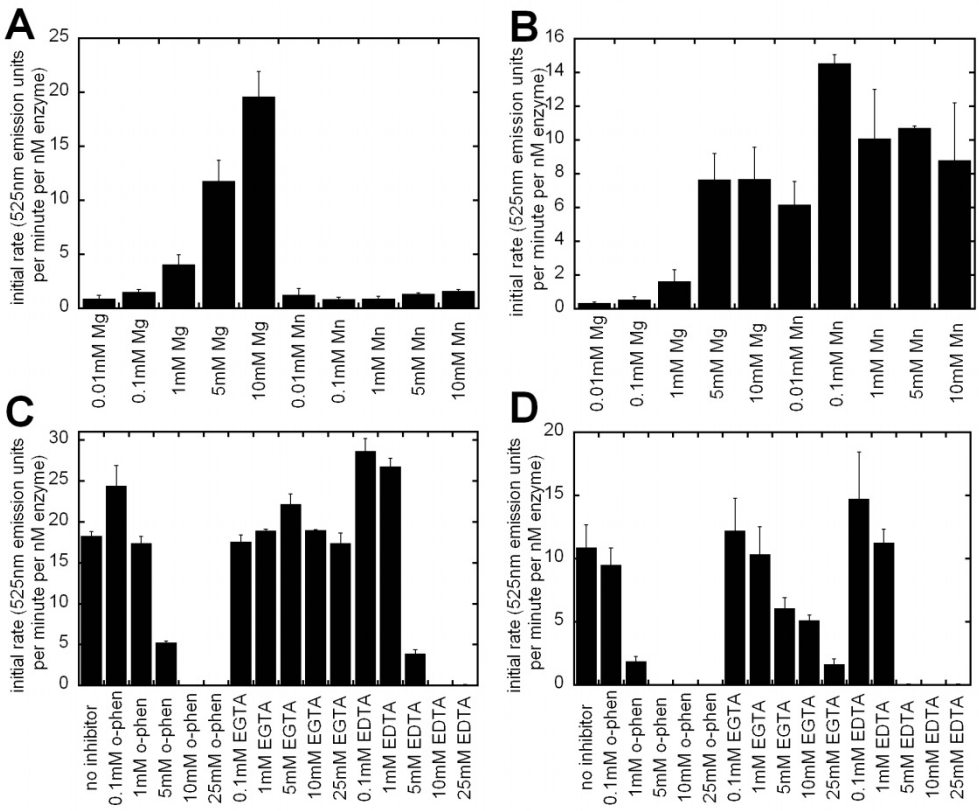
HindIII restriction site position 399-404:

5' aagctt 3'

Nb.Bbv series of nicking sites position 483-535:

5' cctcagcTACAcctcagcGATCcctcagcACTAGT
cctcagcTACGcctcagc 3'

Supplementary Figure S7. Neither hSNM1A nor hSNM1B exhibit hairpin opening endonucleolytic activity. Timecourses (0, 5, 10, 30, 60, 120 minutes) of the hydrolysis reaction, by hSNM1A (A) or hSNM1B (B), on 5'-OH hairpin (lanes 1-6), 5'-biotin hairpin (lanes 7-12), or 5'-phosphate hairpin (lanes 13-18) substrates. Reactions were carried out in 50 µL, with 1000 nM 3'-fluorescein containing substrate and either 0.01 µg (per 10 µL reaction) hSNM1A or 0.1 µg (per 10 µL reaction) hSNM1B. Gels are representative of multiple experiments, which show qualitatively equivalent results. (C) Map of the modified pUC18 plasmid used to carry out the experiments shown in Figure 4A-B, indicating the position of the HindIII and Nb.BbvCI restriction sites, marked with HindIII and N... respectively.



Supplementary figure S8: (A,B,C,D) Increase in fluorescence varies with the dose of magnesium and manganese, and can be abrogated by the addition of metal chelators *o*-phenanthroline, EGTA or EDTA. (A,B) Initial normalized rate of reaction, v/[E] in fluorescence increase per minute per nM enzyme, when 1000nM ssDNA substrate was hydrolyzed by hSNM1A (A) or hSNM1B (B) in the presence of 0.01-10 mM $MgCl_2$ or $MnCl_2$. (C,D) Initial rate of reaction when 1000nM ssDNA substrate was hydrolyzed by hSNM1A (C) or hSNM1B (D) in the presence of 10 mM $MgCl_2$ and increasing amount of *o*-phenanthroline, EGTA or EDTA. Reactions were all carried out in 50µL volume, in 20 mM HEPES-KOH pH7.5, 50 mM KCl, 0.5 mM DTT, 10 mM $MgCl_2$, 0.05 % Triton-X, 0.1 mg/mL BSA, 5 % glycerol, with 0.007 µg hSNM1A (or 0.015 µg hSNM1B) per 10µL reaction. All measurements were made at least in duplicate and standard errors are represented by the error bars