

Identification and characterization of NanH2 and NanH3,
the enzymes responsible for sialidase activity in *Gardnerella vaginalis*

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Material included:

Figure S1: Sequence alignment of *B. longum* NanH2 with *G. vaginalis* NanH2 and NanH3
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 Figure S3: Genetic organization of the *nanH2* and *nanH3* gene clusters in *G. vaginalis*
 Figure S4: *G. vaginalis* NanH1 activity in the presence of divalent cations
 Figure S5: Release and consumption of sialic acids by *nanH3*-only and *nanH2*+ strains of *G. vaginalis*
 Figure S6: Presence of sialidase activity and *nanH* genes in *G. vaginalis*

B_longum_NanH2	VCGANH-----DGAMSLAAPGDYGVACY RIP ALAEAPNGWILAAFDARPHNCQDAPQAN	66
G_vaginalis_NanH2	VTGIKNTFESSLGLKTVKLTTRTNSVCY RIP AITQTANGWILAAAYDKRPGNCSDAPMPN	234
G_vaginalis_NanH3	VPRPRKGDPIRLATIGQTVGLTGKPY RIP AIAEANNNGWILTAWDYRPGAADAPNPN	145
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B_longum_NanH2	SIVQRI SKDGGRSF EPQHVVAAAGHDGV-----DKYGYSDPSYVVDROTGEVFLFFVKSY	120
G_vaginalis_NanH2	SIVQRI SKDGGKTF EAETVVQGHYGD-----NTRWGYSDPSYVLDRETNEIFLFSVKSY	290
G_vaginalis_NanH3	SIVQRI SKDGGKTF EKIQIVAKGKENSNDGLSNKYGFSDPSYVVDQETGNIFLFFVKSY	205
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B_longum_NanH2	DAGFGTSQAGVDPSAREVLQAAVTS SIDNGVTW SEPRIITADITN-SESWISRFASSGAG	179
G_vaginalis_NanH2	RNGWGGSSAGVDPENRNVLHASVTS SKDNGVTW STPKIITKDVTAADVGHSSRFAASGHG	350
G_vaginalis_NanH3	DGGVWDSTASTDKSDRHILDAAVTC SKDNGLTW SEPKVITKDITKYPKNERARFATSGHG	265
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B_longum_NanH2	IQLTYGEHAGRLIQQYTIKEL-----DGRYRAVSVF SDDHGATW HAGTPVG-----D	226
G_vaginalis_NanH2	IQLRHGKYKGRLLQQYTINN-----SGRIQAVTVY SDDHGKTW KVGKPVGFRSEKS	401
G_vaginalis_NanH3	IQLKYGKYKGRLLQQYAIVNSSNGSVQRNNEKWQAVSVY SDDHGVTW KAGNPVGLGKEQS	325
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B_longum_NanH2	HMDENKVVELSDGRVMLNSRSSDG--NGCRYVAI SRDGGATY GPVIRETQLPDPENNAQI	284
G_vaginalis_NanH2	HMDENKVVELSDGRVMLNSRPQN---FRHRLVAI SNDGGETY GEVKEEMQLPDPANNAQI	458
G_vaginalis_NanH3	HMDENKVVELSDGRIMLSNPHEGGANNHRIIAF SNDGGETY GRAYKDETLDPGNNAQI	385
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B_longum_NanH2	ARAFDAPEGSAQAKVLLYSSSSPSDRIDGLVRV SIDDGKTW SAGRRFTTGPMAYSVIAA	344
G_vaginalis_NanH2	TRAYPDAPMGSPKAVLVYSSSSAYGRSDGLIRV SFDDGATW DTGKLFKSGPMAYSCIEV	518
G_vaginalis_NanH3	TRAFPDAPIGSDAAKILLYSSSSGSGRANGLIRVSYNDGESWTEEGKFGKNGAMAYSTIQA	445
	:*:***** ** *:*:***** .* *:*:*** :*: * :*.*** ** *	
B_longum_NanH2	LSHKAGGGYGLLYEGDNNNIMYTRISLDWLNQQLNVDGIGGFPLSGEGGC-----	394
G_vaginalis_NanH2	LNKQHGGGFGLLYEGDNSNIYTHISEDWLGYPVTADNSVIDVPKNAKNEVKIKNLGS	578
G_vaginalis_NanH3	LSQKAGGGYGLLYEGDNDIMYTRISADWLSIRPNITLGSTTKINLSTQKISLPVNPPTS	505
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Figure S1. Sequence alignment of the *B. longum* NanH2 sialidase domain with those of *G. vaginalis* JCP8151B NanH2 and NanH3. Conserved RIP motifs are shown in bold red, and aspartate boxes are in bold blue. Alignment was performed with Clustal Omega.

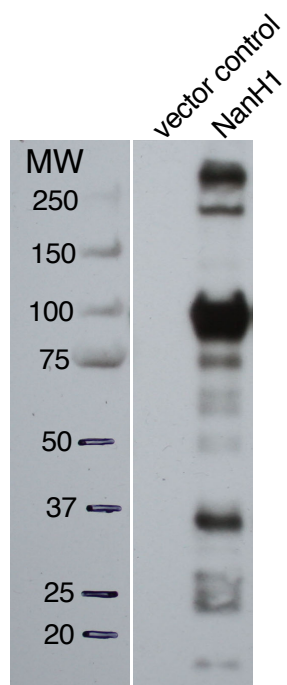


Figure S2. Expression of *G. vaginalis* NanH1. Western blot of *E. coli* whole cell lysates following expression of 6-histidine tagged JCP8151B NanH1 or vector control. The blot was probed with anti-6-histidine monoclonal antibody.

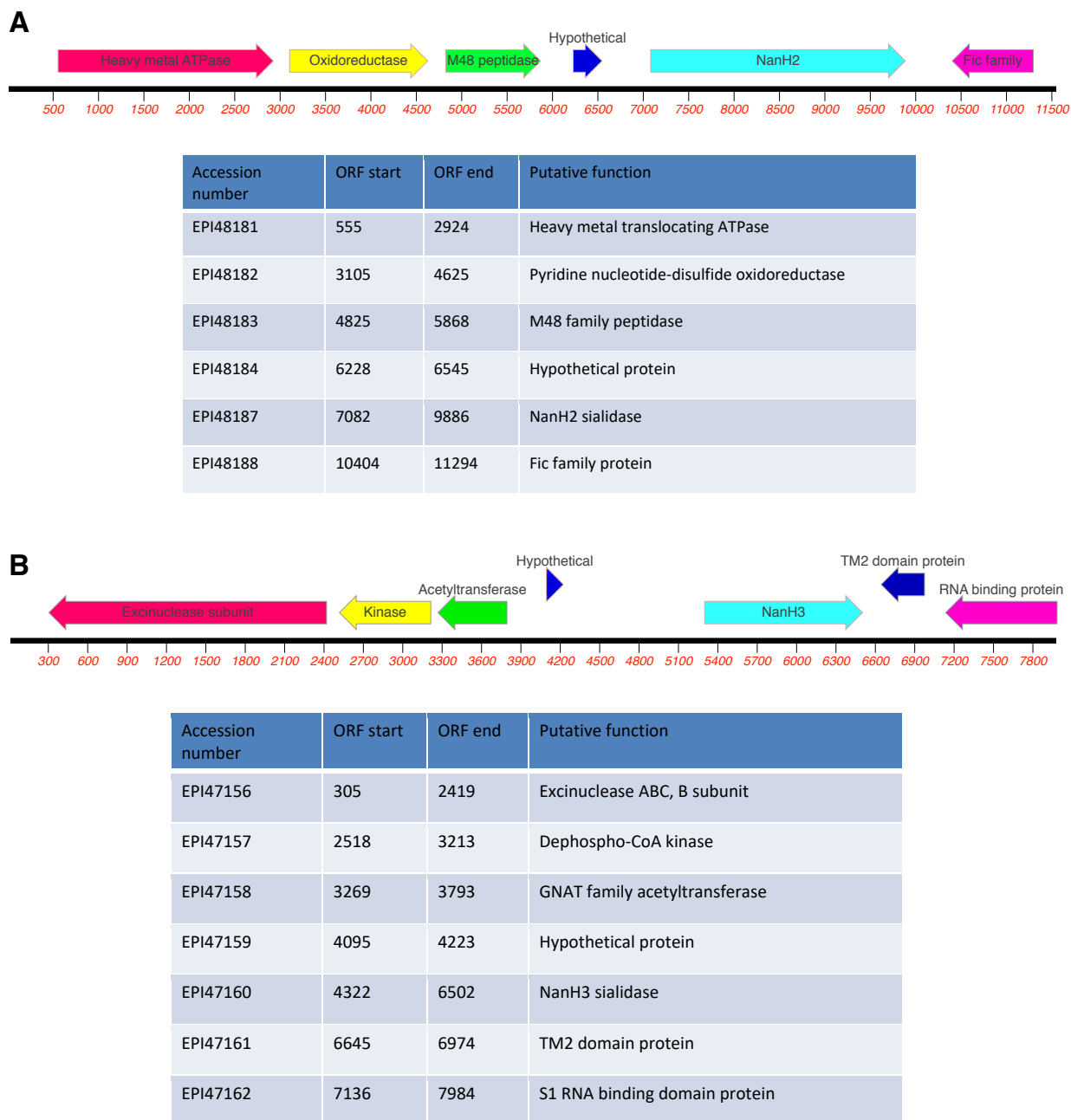


Figure S3. Genetic organization of the *nanH2* (A) and *nanH3* (B) gene clusters in *G. vaginalis*. In the published JCP8151B genome sequence, the 2.8 kb *nanH2* gene appears within a 3.2 kb contig with no other complete open reading frames. Therefore, the closely related JCP8151A genome sequence is shown instead. For the *nanH3* locus, the JCP8151B sequence is shown. Predicted coding regions are represented by open arrows indicating the direction of transcription. Accession numbers, open reading frame start and stop positions, and putative functions are shown under the arrows.

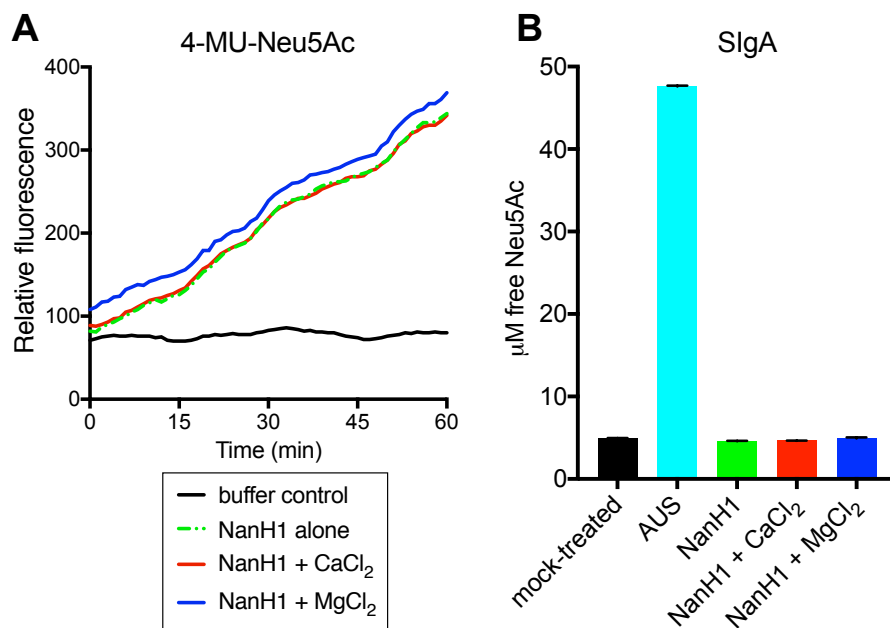


Figure S4. Purified recombinant NanH1 activity in the presence of 1 mM calcium or magnesium. *A*, 4-MU-Neu5Ac assay. *B*, end point assay showing release of Neu5Ac from colostrum IgA. AUS was used as a positive control. Samples were incubated at 37 °C for 2 hrs and free sialic acid was measured by DMB-HPLC.

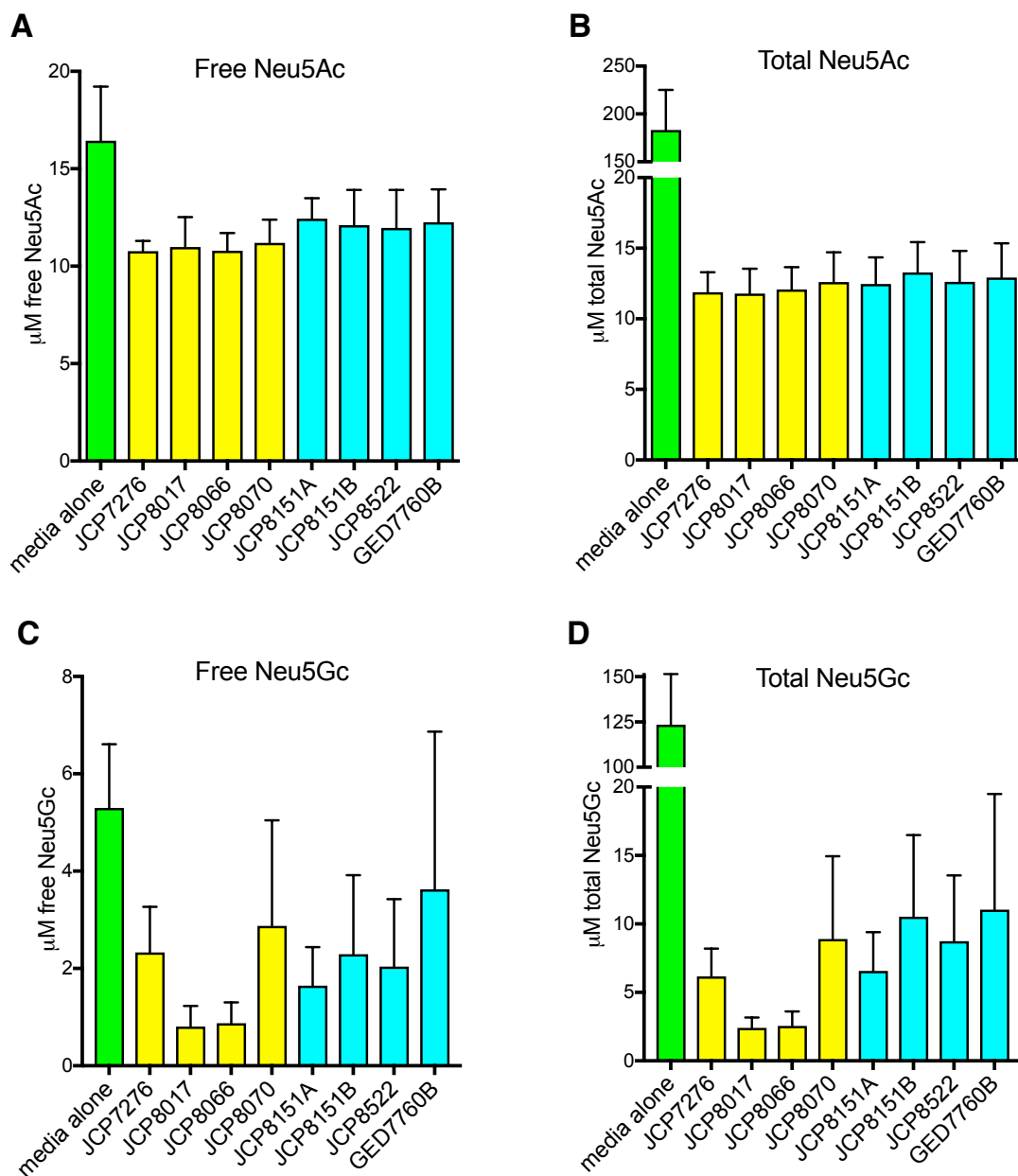


Figure S5. Release and consumption of Neu5Ac and Neu5Gc by *nanH3*-only and *nanH2*⁺ strains of *G. vaginalis*. Following overnight growth of *G. vaginalis* clinical isolates in NYCIII media supplemented with BSM, supernatants were collected and analyzed by DMB HPLC for Neu5Ac (A and B) and Neu5Gc (C and D) before (A and C) and after (B and D) treatment with AUS to release remaining bound sialic acids. Strains with *nanH3* only are shown in yellow; *nanH2*⁺ strains are shown in cyan.

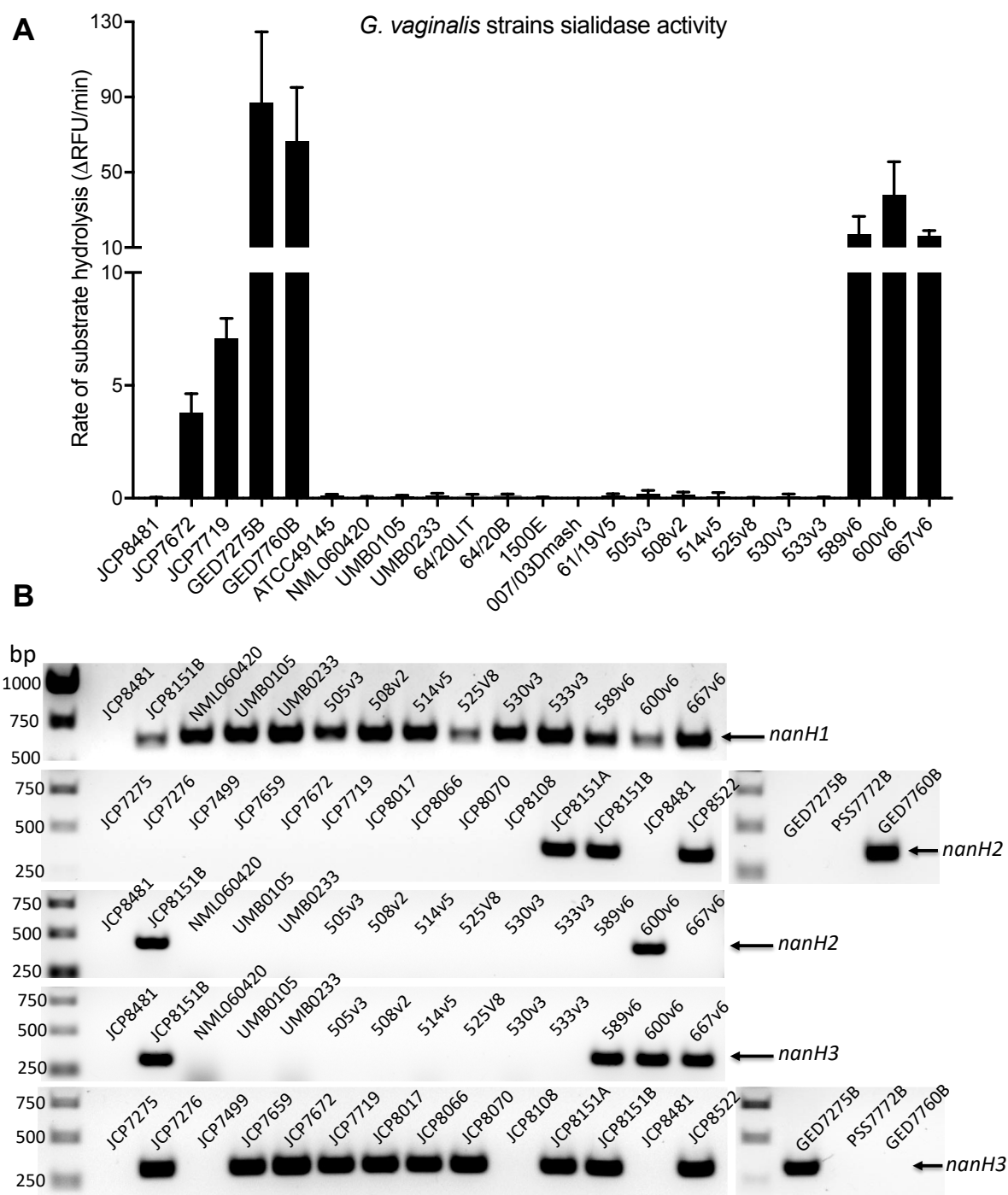


Figure S6. Presence of sialidase activity and *nanH* genes in *G. vaginalis* clinical isolates. 4-MU-Neu5Ac assays (A) and PCR amplification of *nanH1*, *nanH2*, or *nanH3* (B) in *G. vaginalis* strains.