	Mos99 NA	Bil69 NA	SD93 NA + Zanamivir
Data collection			
Wavelength (Å)	0.97856	0.97872	1.12723
Resolution (Å)	1.397	1.537	1.645
Resolution Range ^a	43.458-1.397 (1.401-1.397)	46.049-1.537 (1.563-1.540)	76.340-1.700 (1.650-1.645)
Space group	4 2 2	C 2	P 4 2 ₁ 2
Cell dimensions			
a, b, c (Å)	136.15, 136.15, 150.77	116.34, 137.94, 138.25	107.95, 107.95, 78.62
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 92.16, 90.00	90.00, 90.00, 90.00
Total reflections	3,233,337	2,400,688	520,061
Unique reflections	138,645	322,494	54,981
Multiplicity ^a	23.0 (14.7)	7.5 (7.5)	9.5 (6.6)
Completeness (%) ^a	99.9 (99.2)	100.0 (100.0)	96.8 (97.3)
$< I/\sigma_I > a$	20.2 (2.2)	12.1 (2.1)	11.5 (2.1)
R _{merge} (%) ^a	10.8 (126.0)	10.4 (98.9)	12.7 (77.4)
R _{meas} (%) ^a	11.3 (130.7)	11.1 (106.4)	13.8 (83.4)
CC _{1/2} ^a	0.999 (0.674)	0.998 (0.778)	0.998 (0.895)
Refinement			
Resolution (Å)	43.46-1.40	25.00-1.54	25.00-1.65
No. reflections	138,634	304,302	51,828
R _{work} ^c / R _{free} ^d	0.151/0.164	0.166/0.180	0.188/0.215
No. atoms			
Protein	3,055	12,156	3,016
Water	583	2,004	317
Sugars/Inhibitor	124	408	149
B-factors			
Protein	14.2	14.9	18.0
Sugars/Inhibitor	25.1	25.5	33.1
Water	30.8	29.8	26.2
RMSD from ideal geo	metry		
Bond lengths (Å)	0.007	0.003	0.005
Bond angles (°)	1.12	0.995	1.210
Ramachandran statis	tics (%)		
Favored	99.7	95.7	96.1
Outliers	0.00	0.00	0.00
PDB code	7U4F	7U4E	7U4G

Supplementary Table 1. X-ray data collection and refinement statistics.

^a Numbers in parentheses refer to the highest resolution shell.

^b $R_{merge} = \Sigma |I_i - \langle I_i \rangle | / \Sigma |I_i where |I_i = the intensity of the$ *i* $th reflection and <math>\langle I_i \rangle = mean intensity.$

 $^{\circ}$ $R_{work} = \Sigma |F_{o} - F_{c}| / \Sigma |F_{o}|$, where F_{o} and F_{c} are the observed and calculated structure factors, respectively.

 $^{d}R_{\text{free}}$ was calculated as for R_{work} , but on a test set comprising 5% of the data excluded from refinement.

Primer name	Sequence
SD93lib-N387K-VF	5'-CGT ACG TCT CAC TGG TCC AAA CCT AAA TCC AAA
	TTG CAG-3'
SD93lib-VR	5'-CGT ACG TCT CAA AGC ACT TCC ATC AGT CAT TAC
	TAC TGT-3'
SD93lib-E248G-R249K-F	5'-CGT ACG TCT CAG CTT CAG RAA RAG CTG ATA CTA
	AAA TAC TAT TCA TTG AGG AGG GGA AA-3'
SD93lib-I265T-F	5'-TAA AAT ACT ATT CAT TGA GGA GGG GAA AAT CGT
	TCA TAY TAG CCC ATT GTC AGG AAG TGC-3'
SD93lib-336-346-5mut-F	5'-YAT TGC CKG RAT CCT AAC AAT GAG RAA GGG RGT
	CAT GGA GTG AAA GGC TGG GCC-3'
SD93lib-336-346-5mut-R	5'-ACY CCC TTY CTC ATT GTT AGG ATY CMG GCA ATR
	GCT ACT GCT GGA GCT GTC GTT-3'
SD93lib-E369K-R	5'-ACT TTG AAG GTT TCA TAA CCT GAG CGT AAC TYC
	TCG CTG ATC GTT CTT CCC ATC CA-3'
SD93lib-G381E-R	5'-CGT ACG TCT CAC CAG CCT YCA ATG ACT TTG AAG
	GTT TCA TAA CCT GAG CGT-3'
SD93lib-recover-F	5'-CAC TCT TTC CCT ACA CGA CGC TCT TCC GAT CTA
	GTA ATG ACT GAT GGA AGT GCT-3'
SD93lib-recover-R	5'-GAC TGG AGT TCA GAC GTG TGC TCT TCC GAT CTA
	CTT GCC TAT TTA TCT GCA ATT-3'

Supplementary Table 2. Primers for SD93 combinatorial mutagenesis experiment.

Primer name	Sequence
Bil69lib-VR	5'-CGT ACG TCT CAA CTC CCA TCA GTC ATT ACT ACT
	GT-3'
Bil69lib-VF	5'-CGT ACG TCT CAG CTC AGG TTA TGA AAC TTT CAA
	AG-3'
Bil69lib-R249K-F	5'-CGT ACG TCT CAG AGT GCT TCA GGG ARA GCC
	GAT ACT AGA ATA CTA TTC ATT-3'
Bil69lib-D286G-R	5'-CCT ATT AGA GCC TTT CCA GTT GTC TCT GCA GAT
	ACA TCT GAC GYC AGG ATA TCG AGG ATA-3'
Bil69lib-I302V-M307V-R	5'-CTA TAA TCT TTC AYA TTT ATG TCT ACG AYG GGC
	CTA TTA GAG CCT TTC CAG TTG TCT CTG-3'
Bil69lib-I302V-M307V-F	5'-CCC RTC GTA GAC ATA AAT RTG AAA GAT TAT AGC
	ATT GAT-3'
Bil69lib-D329N-K334S-1-R	5'-CTG CAA TGG CTC TTG CTA GAT CTG TCG TYG TTT
	CTA GGT GTG TCG CCA AC-3'
Bil69lib-D329N-K334S-2-R	5'-CTG CAA TGG CTA CTG CTA GAT CTG TCG TYG TTT
	CTA GGT GTG TCG CCA AC-3'
Bil69lib-D329N-K334S-1-F	5'-RAC GAC AGA TCT AGC AAG AGC CAT TGC AGG AAT
	CCT AAC AAT GAG AGA GG-3'
Bil69lib-D329N-K334S-2-F	5'-RAC GAC AGA TCT AGC AGT AGC CAT TGC AGG AAT
	CCT AAC AAT GAG AGA GG-3'
Bil69lib-N356D-R	5'-TTG CTG ATC GTT CTT CCC ATC CAC ACG TCA TTT
	CCA TYG TCA AAG GCC CAG CCT-3'
Bil69lib-L370S-R	5'-CGT ACG TCT CAG AGC GTR AAT CCT TGC TGA TCG
	TTC TTC CCA TCC ACA CGT-3'
Bil69lib-recover-F	5'-CAC TCT TTC CCT ACA CGA CGC TCT TCC GAT CTA
	CAG TAG TAA TGA CTG ATG GGA GT-3'
Bil69lib-recover-R	5'-GAC TGG AGT TCA GAC GTG TGC TCT TCC GAT CTC
	TTT GAA AGT TTC ATA ACC TGA GC-3'

Supplementary Table 3. Primers for Bil69 combinatorial mutagenesis experiment.

а		
HK68	MNPNQKIITIGSVSLTI <mark>ATV</mark> CF <mark>L</mark> MQIAIL <mark>V</mark> TTVTLHFKQYE <mark>CD</mark> SP <mark>AS</mark> NQVM <mark>P</mark> CEP <mark>I</mark> IIERNITEIVYL <mark>N</mark> NTTIEKEICPK	80
Vic11	MNPNQKIITIG\$V\$LTI <mark>\$</mark> T <mark>I</mark> CFFMQIAIL <mark>I</mark> TTVTLHFKQYE <mark>FN</mark> \$P <mark>PN</mark> NQVMLCEPTIIERNITEIVYL <mark>T</mark> NTTIEKEICPK	80
HK68	VVEYRNWSKPQCQITGFAPFSKDNSIRLSAGGDIWVTREPYVSCDHGKCYQFALGQGTTLDNKHSNDTIHDRIPHRTLLM	160
VICTI		100
HK68 Vic11	NELGVP FHLGTRQVC I AWSSSSCHDGKAWLHVC I TGDDKNA TASFI YDGRLVDSI GSWSQN I LRTQESECVC I NGTCTVV NELGVP FHLGT <mark>K</mark> QVC I AWSSSSCHDGKAWLHVC I TGDDKNA TASFI YNGRLVDS <mark>VV</mark> SWS <mark>KE</mark> I LRTQESECVC I NGTCTVV	240 240
HK68	MTDGSASGRADTRILFIEEGKIVHISPLSGSAQHVEECSCYPRYPGVRCICRDNWKGSNRPVVDINMEDYSIDSSYVCSG	320
Vic11	MTDGSASG <mark>K</mark> ADT <mark>K</mark> ILFIKEGKIVH <mark>TST</mark> LSGSAQHVEECSCYPRYPGVRC <mark>V</mark> CRDNWKGSNRP <mark>I</mark> VDIN <mark>IKDH</mark> SI <mark>V</mark> SSYVCSG	320
HK68	LVGDTPRNDDRSSNSNCRNPNNERGNQGVKGWAFDNGDDVWMGRTISKDLRSGYETFKVIGGWSTPNSKSQINRQVIVDS	400
Vic11	L VGDTPR <mark>KTD</mark> \$\$\$\$\$HCLDPNNEEGGHGVKGWAFDDGNDVWMGRTINET\$RLGYETFKVIEGW\$NPK\$KLQINRQVIVDR	400
HK68 Vic11	DNRSGYSGIFSVEGKSCINRCFYVELIRGRKQETRVWWTSNSIVVFCGTSGTYGTGSWPDGANINFMPI GDRSGYSGIFSVEGKSCINRCFYVELIRGRKEETEVLWTSNSIVVFCGTSGTYGTGSWPDGADLNLMPI	469 469
b		
Mos99	MNPNQK	80
Wy03	MNPNQKIITIGSVSLTI <mark>S</mark> TICF <mark>F</mark> MQIAIL <mark>I</mark> TTVTLHFKQYE <mark>F</mark> NSPPNNQVMLCEPTIIERNITEIVYLTNTTIEKEICPK	80
Mos99 Wy03	LAEYRNWSKPQCN I TGFA PFSKDNS I RLSAGGD I WVT REPYVSCDPDKCYQFAL GQGTTLNNGHSNDTVHDRTPYRTLLM	160 160
Magoo		
Wy03	NELGVFFHLGTKQVCTAWSSSSCHDGKAWLHVCVTGDDENA TASFTTNGKLVDSTGSWSKKTLKTQESECVCTNGTCTVV NELGVFFHLGTKQVCTAWSSSSCHDGKAWLHVCVTGDDENA TASFTYNGKLVDSTGSWSKKTLKTQESECVCTNGTCTVV	240
Mos99	M T D G S A S G K A D T K I L F I E E G K I V H T S <mark>P</mark> L S G S A Q H V E E C S C Y P R Y P G V R C V C R D N W K G S N R P I V D I N <mark>V</mark> K D Y S I V S S Y V C S G	320
Wy03	MTDGSASGKADTKILFIEEGKIVHTSTLSGSAQHVEECSCYPRYPGVRCVCRDNWKGSNRPIVDINI 385	320
Mos99	L V G D T P R K N D S S S S S H C L D P N N E E G G H G V K G W A F D D G N D V W M G R T I S E K L R S G Y E T F K V I E G W S K P N S K L Q I N R Q V I V D R	400
Wy03	L V G D T P R K N D S S S S S H C L D P N N E E G G H G V K G WA F D D G N D V W M G R T I S E K L R S G Y E T F K V I E G W S <mark>N</mark> P N S K L Q I N R Q V I V D R	400
Mos99 Wy03	G N R S G Y S G I F S V E G K S C I N R C F Y V E L I R G R K Q E T E V L W T S N S I V V F C G T S G T Y G T G S W P D G A D I N L M P I G N R S G Y S G I F S V E G K S C I N R C F Y V E L I R G R K Q E T E V L W T S N S I V V F C G T S G T Y G T G S W P D G A D I N L M P I	469 469
с		
SD93 Mos99	MNPNQKIITIGSVT NNPNQKIITIGSVSITIATICFLMQIAILVTTVTLHFKQYECNSPPNNQVMLCEPTIIERNITEIVYLTNTTIEKEICPK	80 80
6002		460
Mos99	LAEYRNWSKPQC <mark>N</mark> I TGFAPFSKDNS I RLSAGGD I WVT REPYVSCDPOKCTQFALGQGTTLNNKHSND I VHDRTPYRTLLM	160
SD93	N E L G V P F H L G T K Q V C I A W S S S S C H D G K A W L H V C V T G <mark>H</mark> D E N A T A S F I Y <mark>D</mark> G R L V D S I G S W S K <mark>N</mark> I L R T Q E S E C V C I N G T C T V V	240
Mos99	NELGVPFHLGTKQVCIAWSSSSCHDGKAWLHVCVTGDDENATASFIYNGRLVDSIGSWSKKILRTQESECVCINGTCTVV 2488249 265	240
SD93	MTDGSASERADTKILFIEEGKIVHISPLSGSAQHVEECSCYPRYPGVRCVCRDNWKGSNRPIVDINVKDYSIVSSYVCSG	320
Mos99	MIDGSASGRADIKILFIEEGKIVHTSPLSGSAQHVEECSCYPRYPGVRCVCRDNWKGSNRPIVDINVKDYSIVSSYVCSG 336 338&339 344 346 369 381	320
SD93	L V G D T P R K N D S S S S S <mark>Y C R N</mark> P N N E <mark>K G S</mark> H G V K G W A F D D G N D V W M G R T I S E <mark>E</mark> L R S G Y E T F K V I <mark>G</mark> G W S K P N S K L Q I N R Q V I V D R	400

Mos99	L V G D T P R K N D S S S S S H C <mark>L D</mark> P N N E <mark>E</mark> G <mark>G</mark> H G V K G W A F D D G N D V W M G R T I S E <mark>K</mark> L R S G Y E T F K V I <mark>E</mark> G W S K P N S K L Q I N R Q V I V D R	400
SD93	G N R S G Y S G I F S V E G K S C I N R C F Y V E L I R G R K Q E T E V <mark>W</mark> W T S N S I V V F C G T S G T Y G T G S W P D G <mark>G</mark> D I N L M P I	469
Mos99	G N R S G Y S G I F S V E G K S C I N R C F Y V E L I R G R K Q E T E V <mark>L</mark> W T S N S I V V F C G T S G T Y G T G S W P D G <mark>A</mark> D I N L M P I	469

d

Bil69	MNPNQKIITIGS	V SL <mark>I</mark> IAT <mark>V</mark> CFLMQIAILVTTVT	L H F K Q <mark>H</mark> E C D S P <mark>S S</mark> N Q V M	LCEPIIIERNITEIVYL	NNTTIEKE <mark>T</mark> CPK 80
Bil71	MNPNQKIITIGS	I SLTIATICFLMQIAILVTTVT	L H F K Q <mark>Y</mark> E C D S P <mark>A N</mark> N Q V M	PCEPIIIERNITEIVYL	TNTTIEKE <mark>I</mark> CPK 80
Bil69	LVEYRNWSKPQC	K I T G F A P F S K D N S I R L S A G G D I	WVTREPYVSCDPGKCYQ	FALGQGTTLDNKHSNDT	IHDRIPHRTLLM 160
Bil71	LVEYRNWSKPQC	K I T G F A P F S K D N S I R L S A G G D I	WVTREPYVSCDPGKCYQ	FALGQGTTLDNKHSNDT	IHDRIPHRTLLM 160
Bil69	NELGVPFHLGTR	QVCIAWSSSSCHDGKAWLHVCV	TGDDKNATASFIYDGRL	VDSIGSWSQNILRTQES	ECVCINGTCTVV 240
Bil71	NELGVPEHLGTR	OVCIAWSSSSCHDGKAWLHVCV	TGDDKNATASELYDGRL	VDSIGSWSONILRTOFS	ECVCINGTCTVV 240
	249				
			286	417 417	
B.160	243			302 307	
Bil69	MTDGSASGRADT	RILFIEEGKIVHISPLSGSAQH	VEECSCYPRYPDVRCIC	302 307 R D N W K G S N R P I V D I N M K	DYSIDSSYVCSG 320
Bil69 Bil71	MTDGSASGRADT MTDGSASGKADT	R I L F I E E G K I V H I S P L S G S A Q H R I L F I E E G K I V H I S P L S G S A Q H	286 VEECSCYPRYPDVRCIC VEECSCYPRYPGVRCIC	302 307 R D N W K G S N R P I V D I N M K R D N W K G S N R P V V D I N V K	DYSIDSSYVCSG 320 DYSIDSSYVCSG 320
Bil69 Bil71	MTDGSASGRADT MTDGSASGKADT 329	R I L F I E E G K I V H I S P L S G S A Q H R I L F I E E G K I V H I S P L S G S A Q H 334 336	VEECSCYPRYPDVRCIC VEECSCYPRYPGVRCIC 356 370	302 307 RDNWKG S N R P I V D I N <mark>M</mark> K RDNWKG S N R P <mark>V</mark> V D I N <mark>V</mark> K	DYSIDSSYVCSG 320 DYSIDSSYVCSG 320
Bil69 Bil71 Bil69	MTDGSASGRADT MTDGSASGKADT 329 LVGDTPRNDDRS	R I L F I E E G K I V H I S P L S G S A Q H R I L F I E E G K I V H I S P L S G S A Q H 334 336 S K S N C R N P N N E R G N H G V K G W A F	286 VEECSCYPRYPDVRCIC VEECSCYPRYPGVRCIC 356 370 DNGNDVWMGRTISKDLR	302 307 RDNWKG S N R P I V D I N M K RDNWKG S N R P V V D I N V K SGY E T F K V I GGW ST P N S	DYSIDSSYVCSG 320 Dysidssyvcsg 320 Ksqinrqvivds 400
Bil69 Bil71 Bil69 Bil71	MTDGSASGRADT MTDGSASGRADT 329 LVGDTPRNDDRS LVGDTPRNDRS	R I L F I E E G K I V H I S P L S G S A Q H R I L F I E E G K I V H I S P L S G S A Q H 334 336 S K S N C R N P N N E R G N H G V K G W A F S S S Y C R N P N N E R G N H G V K G W A F	286 VEECSCYPRYPDVRCIC VEECSCYPRYPGVRCIC 356 370 DNGNDVWMGRTISKDLR DGNDVWMGRTISKDSR	302 307 RDNWKG SNRP I V D I NMK RDNWKG SNRP V V D I N V K SGY E T F K V I GGWSTP N S SGY E T F K V I GGWSTP N S	DYSIDSSYVCSG 320 DYSIDSSYVCSG 320 KSQINRQVIVDS 400 KSQINRQVIVDS 400
Bil69 Bil71 Bil69 Bil71	MTDGSASGRADT MTDGSASGKADT 329 LVGDTPRNDDRS LVGDTPRNNDRS	R I L F I E E G K I V H I S P L S G S A Q H R I L F I E E G K I V H I S P L S G S A Q H 334 336 S K S N C R N P N N E R G N H G V K G W A F S <mark>S S Y</mark> C R N P N N E R G N H G V K G W A F	286 VEECSCYPRYPDVRCIC VEECSCYPRYPGVRCIC 356 370 DNGNDVWMGRTISKDLR DDGNDVWMGRTISKDSR	SGYETFKVIGGWSTPNS	DYSIDSSYVCSG 320 DYSIDSSYVCSG 320 KSQINRQVIVDS 400 KSQINRQVIVDS 400
Bil69 Bil71 Bil69 Bil71 Bil69	MTDGSASGKADTI MTDGSASGKADTI 329 LVGDTPRNDDRS LVGDTPRNNDRS DNRSGYSGIFSV	RILFIEEGKIVHISPLSGSAQH RILFIEEGKIVHISPLSGSAQH 334 336 SKSNCRNPNNERGNHGVKGWAF S <mark>SSY</mark> CRNPNNERGNHGVKGWAF EGKSCINRCFYVELIRGREQET	286 VEECSCYPRYPDVRCIC VEECSCYPRYPGVRCIC 356 370 DNGNDVWMGRTISKDLR DDGNDVWMGRTISKDSR RVWWTSNSIVVFCGTSG	302 307 RDNWKG S NRP I V D I N M K RDNWKG S NRP V V D I N V K SGY E T F K V I GGW ST P N S SGY E T F K V I GGW ST P N S TYG T G SWP D G A N I N F M P	DYSIDSSYVCSG 320 DYSIDSSYVCSG 320 KSQINRQVIVDS 400 KSQINRQVIVDS 400
Bil69 Bil71 Bil69 Bil71 Bil69	MTDGSASGRADTI MTDGSASGRADTI 329 LVGDTPRNDDRS LVGDTPRNDDRS DNRSGYSGIFSV	RILFIEEGKIVHISPLSGSAQH RILFIEEGKIVHISPLSGSAQH 334336 SKSNCRNPNNERGNHGVKGWAF SSYCRNPNNERGNHGVKGWAF EGKSCINRCFYVELIRGREQET	286 VEECSCYPRYPDVRCIC VEECSCYPRYPGVRCIC 356 370 DNGNDVWMGRTISKDLR DDGNDVWMGRTISKDSR RVWWTSNSIVVFCGTSG	302 307 RDNWKGSNRPIVDINMK RDNWKGSNRPVVDINVK SGYETFKVIGGWSTPNS SGYETFKVIGGWSTPNS TYGTGSWPDGANINFMP	DYSIDSSYVCSG 320 DYSIDSSYVCSG 320 KSQINRQVIVDS 400 KSQINRQVIVDS 400 I 469

Supplementary Figure 1. Sequence alignment of influenza virus H3N2 neuraminidase (NA).

Alignments of NA amino acid sequences between **(a)** HK68 and Vic11, **(b)** Mos99 and Wy03 **(c)** SD93 and Mos99, and **(d)** Bil69 and Bil71. Residues that are not conserved between sequences are highlighted in pink. Mutations of interest are labeled in red. NA head domain is from residues 82 to 469.



Supplementary Figure 2. Fitness effects of NA mutations are largely independent of other segments. (a) The replication fitness of viruses that carry HK68 NA but different HAs (HK68 HA and Vic11 HA) was examined by a virus rescue experiment. Virus titer was measured by TCID₅₀. **(b)** The replication fitness of NA N387K and NA Y336H in authentic H3N2 A/Udorn/1972 (all eight segments were from A/Udorn/72) was examined by a virus rescue experiment. Virus titer was measured by plaque forming units (PFU). Each bar represents the mean of three independent biological replicates. Each dot represents one biological replicate. Source data are provided as a Source Data file.



Supplementary Figure 3. Virus replication fitness of permissive mutations for N387K. The replication fitness of different (a) Mos99 NA mutants, and (b) SD93 NA mutants was examined by a virus rescue experiment. Mutant 5 (-N387K): E248G/R249K/Y336N/K344E/E369K, Mutant 8 (-N387K): E248G/R249K/Y336N/K344E/E369K/K385N, Mutant 9 (-N387K): E248G/R249K/Y336H/K344E/E369K/K385N. Virus titer was measured by TCID₅₀. The green dashed line represents the lower detection limit. Each bar represents the mean of (a) two or (b) three independent biological replicates. Each dot represents one biological replicate. Source data are provided as a Source Data file.



Supplementary Figure 4. Gating strategies for flow cytometry in this study. Gating strategy for **(a)** surface expression, and **(b)** intracellular expression of NA. Mos99 N387K is shown as an example.



Supplementary Figure 5. Cellular expression and localization of Mos99 NA mutants. (a) Flow cytometry analysis of 293T cells that transiently expressed HA-tagged Mos99 NA mutants. (b) Confocal microscopy analysis of HA-tagged Mos99 NA mutants. Blue (DAPI), Green (GM130, Golgi), Magenta (NA). The orange box highlights the zoomed-in region, which is shown on the left. Scale bar for large image is 5 μ m and scale bar for zoomed-in image is 2 μ m. Below each micrograph is a cytofluorogram, in which each data point represents a pixel. The Pearson correlation coefficient between the green intensity (GM130, Golgi) and magenta intensity (NA) across all pixels in each image is indicated. The result of a representative experiment out of two independent biological replicates is shown.



Supplementary Figure 6. Measuring the thermal stability of different NA mutants using SYPRO orange dye-based thermal shift assay. The first differential curves for the relative fluorescence unit (RFU) with respect to temperature are shown for (a) Mos99 NA WT and mutants, (b) SD93 NA WT and mutants, and (c) Bil69 NA WT and mutants. For SD93, mutant 5 represents E248G/R249K/Y336N/K344E/E369K/N387K, whereas mutant 8 represents E248G/R249K/Y336N/K344E/E369K/K385N/N387K. The melting temperature (T_m) , which corresponds to the lowest point of the first derivative -d(RFU)/dT, is listed for each variant. The reported T_m is an average of six independent biological replicates. (a-c) The result of a representative experiment out of six independent biological replicates is shown.



Supplementary Figure 7. Tryptophan emission spectrum of SD93 NA WT and mutants. (a) Normalized steady-state emission spectrum of SD93 NA WT and mutants, using λ_{exc} = 295 nm. Of note, the blue (N387K) and green (K385N/N387K) curves almost completely overlap. Mutant 5 represents E248G/R249K/Y336N/K344E/E369K/N387K, whereas mutant 8 represents E248G/R249K/Y336N/K344E/E369K/K385N/N387K. The result of a representative experiment out of two is shown. (b) Two out of 12 tryptophans in the head domain of SD93 NA are located at the protomer-protomer interface. Tryptophans are shown as spheres on one protomer that is in white cartoon representation, while the other three protomers are show as semitransparent black surface. The two tryptophans at the protomer-protomer interface, namely Trp115 and Trp458, are shown in blue. Other tryptophans are in cyan.