A mutagenic screen reveals NspS residues important for regulation of *Vibrio cholerae* biofilm formation

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List of materials:

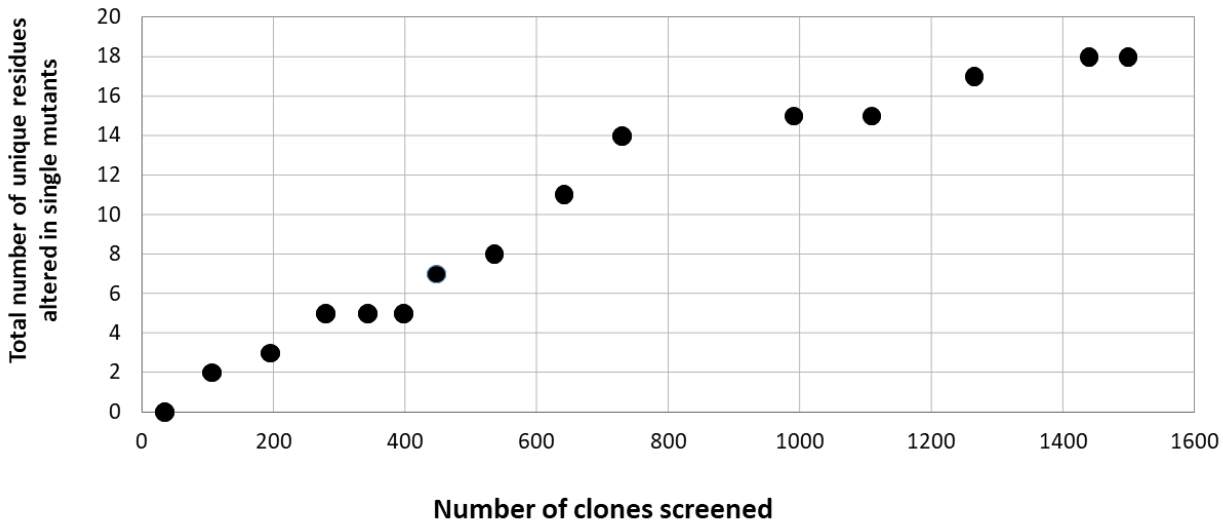

Figure S1. Saturation of the mutagenic screen. Number of unique hits that were obtained throughout the screening process versus the number of clones screened.

Table S1: Conservation of twenty binding site residues across functional and structural homologs of NspS. Residues that comprise the proposed NspS polyamine binding site were compared across functional homologs (shown with UniProt IDs) and structural homologs (indicated with PDB IDs). Homology models of the functional homologs and structural homologs were used to confirm presence of residues in the proposed binding site. The number of conserved binding site residues, percent identity of binding site residues, and percent identity of the total protein sequence for each homolog compared to NspS are shown at the bottom of the table. The titles "% conserved functional homologs" and "% conserved structural homologs" refer to the conservation of each individual residue within the binding site for each specific type of homolog. These values were used to generate **Figure 9B**.

	Functional homologs					Structural homologs						% conserved functional homologs	% conserved structural homologs
NspS	**A1SUA3**	**A4VGH6**	**A8FVY0**	**Q2S7Q6**	**Q92RK9**	**1A99**	**3TTM**	**3TTN**	**1POT**	**4GL0**	**2V84**		
W41	W48	W40	W43	W40	W30	W37	W35	W34	W34	W42	W37	100%	100%
E42	G49	E41	S44	E41	E31	S38	S36	T35	T35	G43	T38	60%	0%
D43	D50	E42	E45	D42	D32	D39	D37	D36	E36	D44	Y39	60%	67%
T44	Y51	Y43	Y46	Y43	Y33	Y40	Y38	Y37	Y37	Y45	Y40	0%	0%
N68												0%	0%
D69	S75	S67	S70	D67	S57	S64	S62	S61	S61	S69	S64	20%	0%
D70	G76	G68	D71	D68	D58	N65	N63	N62	N62	N70	N65	60%	0%
D90	D97	E88	S92	D89	D78	S85	S83	S82	S83	S90	S85	60%	0%
V92	E99	I90	V94	L91	N80	S87	S85	N84	Y85	Y92	D87	20%	0%
S93	V100	S91	D95	M92	G81	F88	F86	F85	Y86	A93	F88	20%	0%
F131	A140	L131	F138	A135	F121	M134	Y131	L131	I130	F137	Y133	40%	17%
V172	I181	E172	R179	S176	S162	E184	T182	D180	R170	R177	R172	0%	0%
E173	D182	D173	D180	T177	E163	E185	E183	E181	E171	E178	E173	20%	100%
Y233	Y242	Y233	Y240	Y237	Y223	W244	Y241	Y239	W229	F236	F232	100%	33%
S234	S243	S234	N241	N238	S224	A245	S242	S240	N230	S237	A233	60%	50%
G235	D244	G235	G242	G239	G225	G246	G243	G241	G231	G238	E234	80%	83%
D236	D245	D236	D243	D240	D226	D247	D244	D242	S232	E239	A235	100%	50%
Y259	L268	L261	A264	G261	L250	M274	G271	N269	I253	N260	P259	0%	0%
W261	F270	W263	W266	W263	W252	F276	F273	W271	W255	W262	Y261	80%	50%
D263	D272	D265	D268	D265	D254	D278	D275	D273	D257	D264	D263	100%	100%
# residues conserved binding site	7	9	9	11	13	6	8	9	5	8	3		
% identity binding site	35%	45%	45%	55%	65%	30%	40%	45%	25%	40%	15%		
Entire protein sequence % identity	28%	33%	22%	24%	31%	23%	26%	25%	20%	25%	17%		

Table S2. Effect of norspermidine on biofilm formation of mutant clones. Biofilms were formed in the absence or presence of norspermidine and quantified using the optical density biofilm assay. Raw data for all of the biological replicates, averages, and standard deviations where it could be calculated are shown. We were only able to complete two biological replicates with the five mutants listed at the bottom of the table due to restrictions resulting from COVID-19.

Biofilm cell density without norspermidine							**Biofilm cell density with norspermidine**					
	Rep 1	Rep 2	Rep 3	Biofilm Average	Standard Deviation			Rep 1	Rep 2	Rep 3	Biofilm Average	Standard Deviation
Positive Control	0.230	0.194	0.227	0.217	0.020		Positive Control	0.271	0.320	0.292	0.294	0.024
Negative Control	0.028	0.018	0.025	0.024	0.005		Negative Control	0.013	0.019	0.028	0.020	0.008
R219S	0.017	0.022	0.018	0.019	0.002		R219S	0.017	0.019	0.016	0.017	0.002
R219H	0.031	0.033	0.026	0.030	0.003		R219H	0.098	0.147	0.151	0.132	0.030
L89W	0.038	0.040	0.044	0.041	0.003		L89W	0.037	0.043	0.029	0.036	0.007
R219C	0.037	0.032	0.021	0.030	0.008		R219C	0.040	0.060	0.043	0.048	0.010
S216I	0.016	0.013	0.018	0.016	0.002		S216I	0.014	0.012	0.014	0.014	0.001
S216N	0.025	0.018	0.024	0.022	0.004		S216N	0.024	0.015	0.020	0.020	0.005
V218L	0.038	0.025	0.044	0.036	0.010		V218L	0.244	0.241	0.267	0.251	0.014
F84V	0.022	0.072	0.032	0.042	0.027		F84V	0.041	0.034	0.046	0.040	0.006
V218A	0.087	0.017	0.108	0.071	0.047		V218A	0.335	0.248	0.310	0.298	0.045
S79C	0.047	0.053	0.034	0.044	0.010		S79C	0.132	0.120	0.127	0.126	0.006
L40H	0.056	0.042	0.035	0.044	0.011		L40H	0.321	0.330	0.307	0.319	0.011
L82R	0.027	0.025	0.041	0.031	0.009		L82R	0.030	0.030	0.063	0.041	0.019
L82P	0.045	0.026	0.030	0.034	0.010		L82P	0.175	0.153	0.180	0.169	0.014
S79G	0.048	0.036	0.044	0.042	0.006		S79G	0.210	0.234	0.223	0.222	0.012
L40R	0.041	0.025	0.019	0.028	0.011		L40R	0.073	0.070	0.066	0.070	0.004
D43N	0.028	0.024	0.024	0.025	0.002		D43N	0.148	0.153	0.169	0.157	0.011
S216R	0.016	0.013	0.042	0.024	0.016		S216R	0.019	0.017	0.014	0.016	0.003
N268D	0.016	0.016	0.035	0.023	0.011		N268D	0.027	0.030	0.022	0.026	0.004
F243V	0.032	0.023	0.034	0.030	0.006		F243V	0.206	0.215	0.164	0.195	0.027
D263V	0.048	0.019	0.035	0.034	0.015		D263V	0.033	0.020	0.016	0.023	0.009
N268S	0.034	0.042	0.037	0.038	0.004		N268S	0.111	0.123	0.132	0.122	0.010
F66V	0.027	0.029	0.021	0.026	0.004		F66V	0.056	0.056	0.058	0.057	0.001
L40P	0.036	0.035	0.054	0.042	0.011		L40P	0.188	0.238	0.326	0.251	0.070
F66Y	0.047	0.036	0.031	0.038	0.008		F66Y	0.117	0.124	0.080	0.107	0.024
P83L	0.021	0.035		0.028			P83L	0.211	0.252		0.231	
N68I	0.025	0.044		0.035			N68I	0.293	0.334		0.313	
R242L	0.009	0.021		0.015			R242L	0.279	0.304		0.292	
F64L	0.032	0.049		0.041			F64L	0.101	0.303		0.202	
L281F	0.038	0.048		0.043			L281F	0.251	0.267		0.259	