

## Supplementary Material

**Supplementary Table 1:** Length of coding region and numbers of highly mutable and conserved positions identified in every influenza mRNA.

Gene name	Non-pandemic						Pandemic					
	Coding region length	Non-mutating positions	Highly mutable positions		Mutable third codon positions		Coding region length	Non-mutating positions	Highly mutable positions		Mutable third codon positions	
			Number	Percentage of total length, %	Number	Percentage of highly mutable, %			Number	Percentage of total length, %	Number	Percentage of highly mutable, %
PB2	2280	1763	262	11.5	211	80.5	2280	1717	289	12.7	232	80.3
PB1	2274	1762	277	12.2	237	85.6	2274	1679	286	12.6	226	79.0
PA	2151	1682	244	11.3	207	84.8	2151	1583	266	12.4	209	78.6
HA	1698	1254	241	14.2	173	71.8	1701	1164	269	15.8	179	66.5
NP	1497	1187	164	11.0	145	88.4	1497	1180	150	10.0	125	83.3
NA	1413	1008	219	15.5	154	70.3	1410	1000	201	14.3	128	63.7
M1	759	635	60	7.9	51	85.0	759	606	67	8.8	57	85.1
M2	294	232	28	9.5	18	64.3	294	230	20	6.8	10	50.0
NS1	693	515	82	11.8	46	56.1	660	467	82	12.4	44	53.7
NS2	366	296	38	10.4	28	73.7	366	297	27	7.4	18	66.7

**Supplementary Table 2:** Non-pandemic and pandemic H1N1 influenza A strains used for the analysis.

#	Non-pandemic	Pandemic
1	A/England/192/2000	A/Minneapolis/INS3_675/2012
2	A/California/VRDL209/2009	A/Helsinki/720M/2014
3	A/Alabama/UR06-0455/2007	A/Aalborg/INS132/2009
4	A/Arkansas/WRAIR1249P/2009	A/Aarhus/INS254/2009
5	A/Auckland/579/2000	A/Aarhus/INS3_653/2011
6	A/Auckland/580/2000	A/Aarhus/INS3_654/2011
7	A/Auckland/605/2001	A/Aarhus/INS609/2011
8	A/Auckland/619/2005	A/Aarhus/INS612/2011
9	A/Boston/1/2009	A/Alabama/01/2010
10	A/Boston/10/2009	A/Alaska/38/2009
11	A/Boston/12/2007	A/Arizona/20/2009
12	A/Boston/23/2009	A/Athens/INS163/2009
13	A/Boston/34/2008	A/Athens/INS271/2009
14	A/Boston/46/2009	A/Athens/INS345/2009
15	A/Boston/49/2008	A/Athens/INS398/2010
16	A/Boston/6/2009	A/Athens/INS412/2010
17	A/Boston/67/2009	A/Athens/INS416/2010
18	A/Boston/93/2009	A/Athens/INS571/2011
19	A/California/UR06-0125/2007	A/Bangkok/INS3_681/2012
20	A/California/UR06-0393/2007	A/Bangkok/INS424/2010
21	A/California/VRDL134/2009	A/Bangkok/INS425/2010
22	A/California/VRDL151/2009	A/Bangkok/INS478/2010
23	A/California/VRDL152/2009	A/Bangkok/INS481/2010
24	A/California/VRDL175/2009	A/Bangkok/INS490/2010
25	A/California/VRDL191/2009	A/Bangkok/INS505/2010
26	A/California/VRDL193/2009	A/Bangkok/INS511/2010
27	A/California/VRDL252/2009	A/Bogota/WRAIR0435N/2009

28	A/California/VRDL256/2009	A/Boston/685/2009
29	A/Canada/591/2004	A/Boston/DOA08/2011
30	A/Canterbury/01/2001	A/Boston/DOA14/2011
31	A/Canterbury/126/2001	A/Boston/DOA2-099/2012
32	A/Canterbury/27/2000	A/Boston/DOA40/2011
33	A/Chile/8885/2001	A/Boston/DOA90/2012
34	A/Christchurch/1/2003	A/Boston/YGA_00037/2013
35	A/Colorado/UR06-0053/2007	A/Boston/YGA_01185/2013
36	A/DaNang/DN238/2008	A/Boston/YGA_01217/2013
37	A/DaNang/DN345/2008	A/Brooklyn/INS549/2011
38	A/DaNang/DN431/2008	A/California/VRDL107/2009
39	A/England/493/2006	A/California/VRDL4/2010
40	A/England/494/2006	A/California/VRDL81/2009
41	A/England/545/2007	A/California/WR1316P/2009
42	A/England/593/2006	A/Cambridge/INS528/2010
43	A/Florida/UR06-0208/2007	A/Changchun/01/2009
44	A/Florida/UR07-0022/2008	A/Chicago/YGA_04019/2012
45	A/HaNoi/Q421/2006	A/Chile/115/2010
46	A/HaNoi/TX200/2008	A/Chile/6/2010
47	A/Hanoi/ISBM31/2005	A/Darlinghurst/INS3_643/2011
48	A/Hong Kong/1870/2008	A/District of Columbia/INS525/2010
49	A/Hue/H386/2008	A/District of Columbia/INS600/2011
50	A/Illinois/UR06-0146/2007	A/District of Columbia/WRAIRO313/2011
51	A/Johannesburg/159/1997	A/England/05120538/2010
52	A/Kentucky/UR06-0057/2007	A/England/280/2010
53	A/Kentucky/UR06-0539/2007	A/England/859/2009
54	A/Kentucky/UR07-0061/2008	A/Finland/102/2014
55	A/Kyoto/08K056/2009	A/Finland/1520N/2011
56	A/Malaysia/11641/1997	A/Finland/30/2014

57	A/Malaysia/14075/2000	A/Finland/61/2014
58	A/Malaysia/15042/1998	A/Gainesville/05/2014
59	A/Malaysia/1686034/2006	A/Georgia/T51700/2012
60	A/Malaysia/1706215/2007	A/Guangzhou/GIRD74/2010
61	A/Malaysia/1798564/2007	A/Gunma/267/2009
62	A/Malaysia/2143035/2009	A/Gunma/287/2009
63	A/Malaysia/30025/2004	A/Hamburg/INS92/2009
64	A/Malaysia/32110/2005	A/Helsinki/100/2013
65	A/Malaysia/33132/2005	A/Helsinki/1127/2014
66	A/Malaysia/34450/2006	A/Helsinki/11IH2213/2011
67	A/Malaysia/35164/2006	A/Helsinki/1289/2013
68	A/Managua/1038.01/2008	A/Helsinki/147/2013
69	A/Managua/107.01/2008	A/Helsinki/220M/2014
70	A/Managua/2055.01/2008	A/Helsinki/2430/2012
71	A/Managua/254.01/2008	A/Helsinki/39/2013
72	A/Managua/3153.01/2008	A/Helsinki/473N/2014
73	A/Managua/3759.02/2008	A/Helsinki/490/2013
74	A/Memphis/1/2001	A/Helsinki/771M/2014
75	A/Memphis/15/2000	A/Houston/JMM_131/2013
76	A/Memphis/6/2001	A/Hubei/75/2009
77	A/Memphis/7/2001	A/Hubei/76/2009
78	A/Mississippi/UR06-0378/2007	A/India/Nag132467/2013
79	A/Nagasaki/07N035/2008	A/India/Nsk12388/2012
80	A/Nanchang/11/1996	A/India/P1112874/2011
81	A/Nanchang/16A/1999	A/India/P1114854/2011
82	A/Nanchang/8/1996	A/India/P121773/2012
83	A/New Caledonia/20-JY2/1999	A/India/P121778/2012
84	A/New South Wales/26/2000	A/India/P12946/2012
85	A/New York/08-1253/2008	A/India/P131027/2013

86	A/New York/1062/2007	A/India/P131845/2013
87	A/New York/1104/2008	A/India/P132194/2013
88	A/New York/1159/2009	A/Iowa/04/2010
89	A/New York/1692/2009	A/Jiangsu/1/2009
90	A/New York/205/2001	A/Kazan/CRIE-02/2013
91	A/New York/281/2001	A/Khon Kaen/INS3_649/2012
92	A/New York/306/2001	A/Liaoning/1/2009
93	A/New York/442/2001	A/Lima/INS3_671/2012
94	A/New York/494/2002	A/Lima/WRAIR0672F/2009
95	A/New York/UR06-0199/2007	A/Managua/0305_10/2010
96	A/Shanghai/2/1997	A/Managua/1244.01/2009
97	A/Singapore/14/2001	A/Managua/3246.01/2010
98	A/South Australia/58/2005	A/Melbourne/INS471/2010
99	A/St. Petersburg/8/2006	A/Mexico/InDRECTRLA/2010
100	A/Taiwan/123/2002	A/Moscow/WRAIR1627T/2009
101	A/Taiwan/5072/1999	A/Nepal/VIROAF5/2012
102	A/Tennessee/UR06-0080/2007	A/New York/2372/2010
103	A/Texas/UR06-0026/2007	A/New York/3230/2010
104	A/Thailand/CU-B589/2009	A/New York/6530/2010
105	A/Thailand/CU-H223/2009	A/New York/7480/2010
106	A/Waikato/11/2005	A/New York/INS150/2009
107	A/Western Australia/18/2001	A/New York/WC-LVD-13-001/2013
108		A/New York/WC-LVD-13-007/2013
109		A/New York/WC-LVD-13-028/2013
110		A/New York/WC-LVD-14-001/2014
111		A/New York/WC-LVD-14-027/2014
112		A/New York/WC-LVD-14-034/2014
113		A/New York/WC-LVD-14-044/2014
114		A/New York/WC-LVD-14-058/2014

115		A/Nicaragua/4136_07/2013
116		A/Nicaragua/4211_07/2013
117		A/Nicaragua/6123_01/2011
118		A/Nicaragua/6263_05/2013
119		A/Nicaragua/AGA2-52/2011
120		A/North Carolina/05/2010
121		A/North Fitzroy/INS464/2010
122		A/Northern Ireland/04380108/2010
123		A/Novosibirsk/KSH/2011
124		A/Odense/INS143/2009
125		A/Ontario/720545/2010
126		A/Quito/WRAIR0617N/2009
127		A/San Diego/INS101/2009
128		A/San Diego/INS105/2009
129		A/Santiago/p13d2/2013
130		A/Santiago/p15d1/2011
131		A/Santiago/p18d0/2013
132		A/Santiago/p9d1/2011
133		A/Singapore/EN193/2010
134		A/Singapore/GP10/2010
135		A/Singapore/GP3839/2009
136		A/Singapore/GP413/2010
137		A/Singapore/GP4406/2010
138		A/Singapore/GP489/2010
139		A/Singapore/GP496/2011
140		A/Singapore/GP868/2011
141		A/Singapore/GP908/2011

142		A/Singapore/ON2136/2009
143		A/Singapore/SS34/2010
144		A/Singapore/TT157/2011
145		A/Sydney/DD3-10/2010
146		A/Sydney/DD3-11/2010
147		A/Sydney/DD3-27/2010
148		A/Sydney/DD3-37/2010
149		A/Tallinn/INS182/2010
150		A/Tennessee/F1093A/2010
151		A/Texas/JMS393/2009
152		A/Thailand/CU-B2357/2010
153		A/Thailand/CU-H2176/2010
154		A/Thailand/H1255/2010
155		A/Uganda/MUWRP-059/2009
156		A/Uganda/MUWRP-102/2009
157		A/Uganda/MUWRP-176/2010
158		A/Uganda/MUWRP-212/2010
159		A/Uganda/MUWRP-224/2010
160		A/Uganda/MUWRP-240/2011
161		A/Utah/02/2010
162		A/Viet Nam/11032010/2009
163		A/Viet Nam/15032004/2009
164		A/Warsaw/INS312/2009
165		A/Warsaw/INS3_657/2011
166		A/Wisconsin/629-D00134/2009
167		A/Wisconsin/629-D02424/2009
168		A/Wurzburg/INS382/2009

169		A/Yaroslavl/IIV-196/2009
170		A/Zhejiang/3/2009
171		A/Zhejiang/88/2009
172		A/Zhejiang/X1/2009
173		A/Zhejiang/X2/2009



**Supplementary Table 3:** Coordinates of identified structured RNA regions.

Gene Name	Non-pandemic		Pandemic	
	Start Position	End Position	Start Position	End Position
M1	8	12	59	63
	26	30	113	117
	50	55	138	156
	57	63	185	214
	89	93	247	254
	112	142	284	293
	149	189	361	366
	208	217	372	379
	232	288	449	454
	320	337	512	518
	340	344	520	531
	350	367	535	540
	371	393	610	628
	397	410	678	699
	492	503	707	717
	512	520		
	539	547		
	610	614		
	620	628		
	646	660		
667	676			
678	697			
M2	2	8	59	72
	17	50	148	153
	187	200	184	192

	237	251	223	227
	258	264	236	278
NS1	198	220	537	541
	270	298	577	602
	337	343		
	377	412		
	439	443		
	469	483		
	505	550		
	563	584		
NS2	9	129	7	30
	208	221	33	38
	347	353	40	87
			104	136
			210	214
			311	318
			321	334
			343	350
PA	262	273	99	125
	278	286	147	152
	307	317	312	318
	513	517	407	415
	855	868	914	930
	874	885	953	959
	1321	1326	967	976
	1332	1338	989	1002
	1384	1388	1004	1011
	1471	1480	1113	1117

	1551	1556	1549	1556
	1575	1579	1832	1842
	1682	1699	2047	2055
	1728	1764		
	1797	1801		
	1818	1822		
	1824	1852		
	1892	1896		
	1944	1950		
	2061	2114		
NP	3	7	100	166
	72	83	183	187
	187	210	200	213
	224	230	225	234
	244	248	241	247
	262	267	264	276
	284	288	323	344
	290	297	369	374
	325	329	384	389
	369	377	434	439
	419	423	504	508
	452	463	512	520
	637	656	539	543
	662	666	588	592
	680	688	672	678
	740	745	736	743
	751	760	769	777
	762	767	808	815
	1034	1038	824	832

	1053	1069	1048	1057
	1072	1087	1071	1076
	1090	1101	1183	1187
	1127	1139	1218	1222
	1189	1202	1310	1314
	1329	1344	1324	1340
	1423	1438	1368	1372
	1443	1451	1384	1392
	1460	1482	1396	1400
			1408	1440
			1444	1450
			1461	1483
	45	51	67	169
	314	332	552	563
	338	349	579	598
	351	356	707	713
	412	416	812	825
	550	561	854	858
	606	611	1067	1071
	619	632	1229	1241
	1066	1071	1288	1296
	1151	1159	1326	1330
	1386	1417	1482	1489
	1429	1433	1498	1519
	1592	1598	1861	1865
	1947	1951	2020	2035
	1956	1966	2048	2054
PB1				
	6	12	8	14
PB2	38	55	200	233

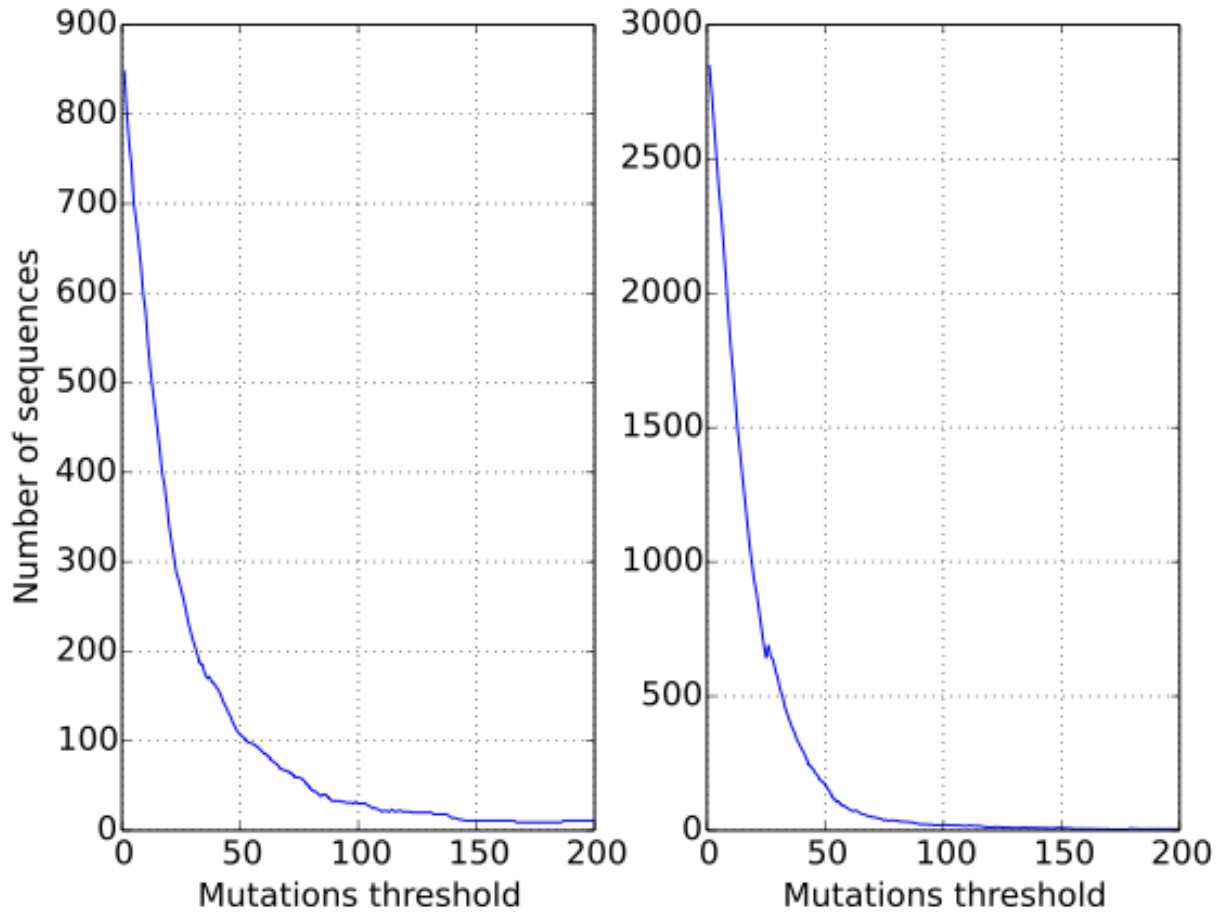
	95	99	464	476
	121	126	501	505
	281	286	788	794
	361	365	962	967
	372	378	1102	1124
	384	390	1450	1454
	417	422	1456	1465
	594	599	1498	1521
	779	789	1534	1555
	843	873	1618	1622
	909	922	1666	1676
	929	949	1682	1688
	978	986	1694	1708
	988	993	1844	1848
	998	1006	1938	1964
	1233	1239	2048	2053
	1443	1473		
	1844	1848		
	1973	1977		
	2014	2040		
	2113	2120		
HA	349	353	135	140
	367	371	224	228
	559	564	470	482
	645	653	703	707
	1048	1056	767	772
	1065	1074	898	905
	1120	1124	943	954
	1141	1147	966	981

	1200	1208	1090	1094
	1266	1275	1140	1144
	1601	1610	1174	1185
			1217	1222
			1224	1243
			1255	1278
			1280	1288
			1291	1300
			1303	1307
			1313	1320
			1328	1346
			1570	1574
			1661	1666
NA	63	77	13	23
	83	92	564	585
	97	102	590	596
	550	561	1047	1056
	564	568	1064	1073
	643	660	1268	1291
	1226	1234		

**Supplementary Table 4:** Pearson correlation coefficients between the mutability value (i.e. Shannon entropy) for every nucleotide position and corresponding value of moving average of individual standard deviations of the base-pairing probabilities of nucleotides. The p-value column shows the measure of statistical significance of the corresponding correlation coefficient.

	<b>Gene Name</b>	<b>Correlation Coefficient</b>	<b>P-value</b>
<b>Non pandemic</b>	PB2	0.120	8.86E-009
	PB1	0.102	1.20E-006
	PA	0.106	7.85E-007
	HA	0.103	2.04E-005
	NP	0.118	5.16E-006
	NA	0.067	0.0118
	M1	0.081	0.0265
	M2	0.140	0.0171
	NS1	0.110	3.97E-003
	NS2	0.129	0.0143
<b>Pandemic</b>	PB2	0.123	3.63E-009
	PB1	0.095	5.91E-006
	PA	0.059	6.35E-003
	HA	0.098	5.73E-005
	NP	0.149	7.76E-009
	NA	0.090	7.44E-004
	M1	0.074	0.0423
	M2	0.006	0.9240
	NS1	0.155	7.01E-005
	NS2	0.222	2.02E-005

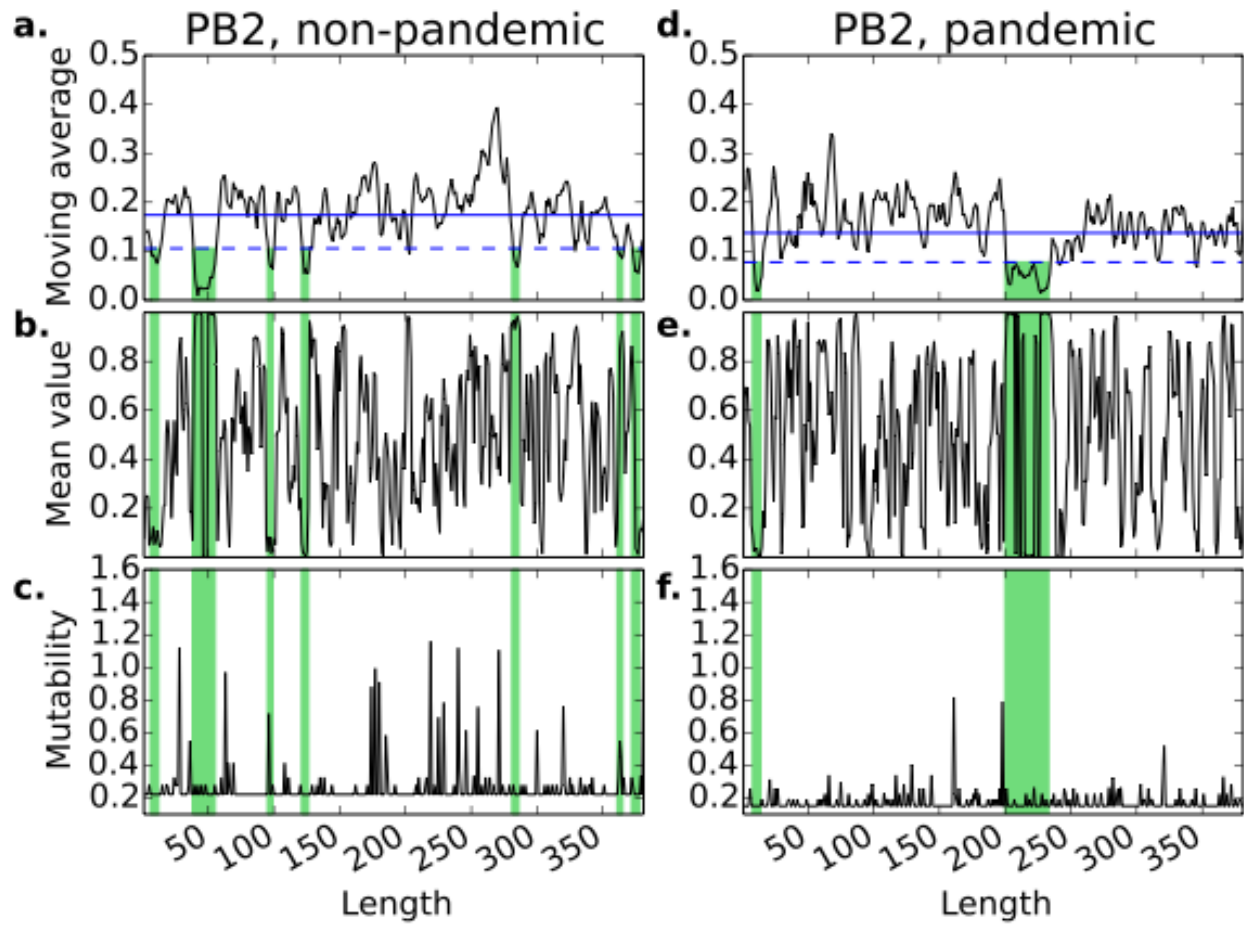
**Supplementary Figure 1:** Number of non-pandemic and pandemic influenza strains left after filtering very similar sequences depending on the identity threshold.



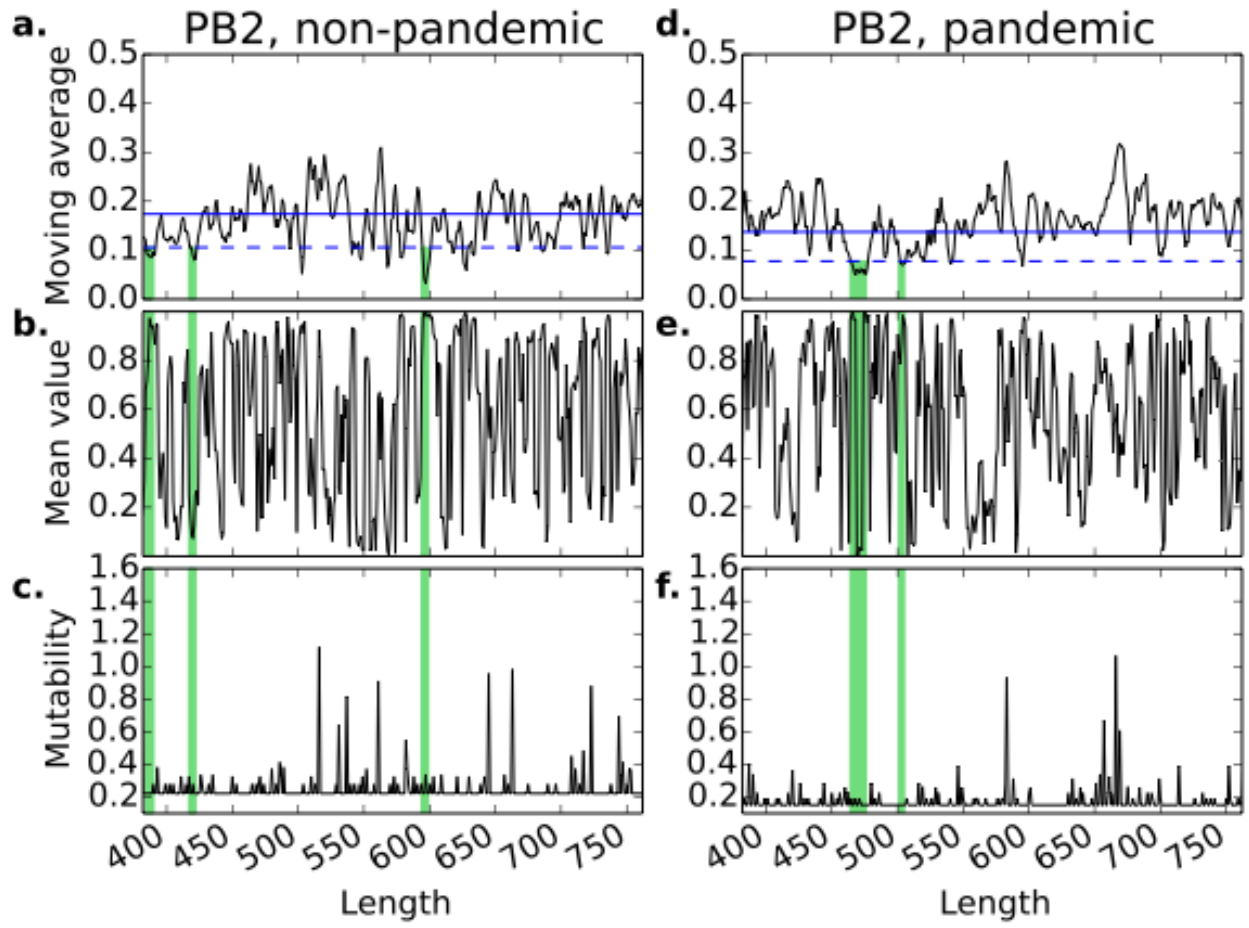


**Supplementary Figures 2-38:** Structure variability and mutability profiles for non-pandemic (a, b, and c) and pandemic (d, e, and f) influenza mRNAs. Plots a and d demonstrate structure conservation profiles; namely, they show the moving average that was calculated by applying a sliding window approach to smooth individual fluctuations of standard deviations of nucleotide base pairing probabilities. The blue solid line demonstrates the mean level of all moving average values, and the blue dashed line demonstrates the level equal to the mean of all moving average values decreased by the standard deviation of all moving average values. In this case, the mean and the standard deviation were computed based on all moving average values from all mRNAs of a particular type (pandemic or non-pandemic) of influenza strains. According to our definition, when the moving average goes below the blue dashed line, it is a structured RNA region. Such regions are colored with green across the plots. Plots b and e demonstrate profiles of the mean values of probabilities of nucleotide positions to be in a double-stranded conformation. If this value is close to 1, it means that in most strains in the dataset the correspondent nucleotide has a very high probability to be paired; and, if this value is close to 0, the correspondent nucleotide is very likely to be unpaired in most strains in the dataset. Plots c and f demonstrate mutability profiles for influenza mRNAs. Mutability of every nucleotide position is computed as a value of Shannon entropy which is calculated based on frequency of every ribonucleotide in a particular position.

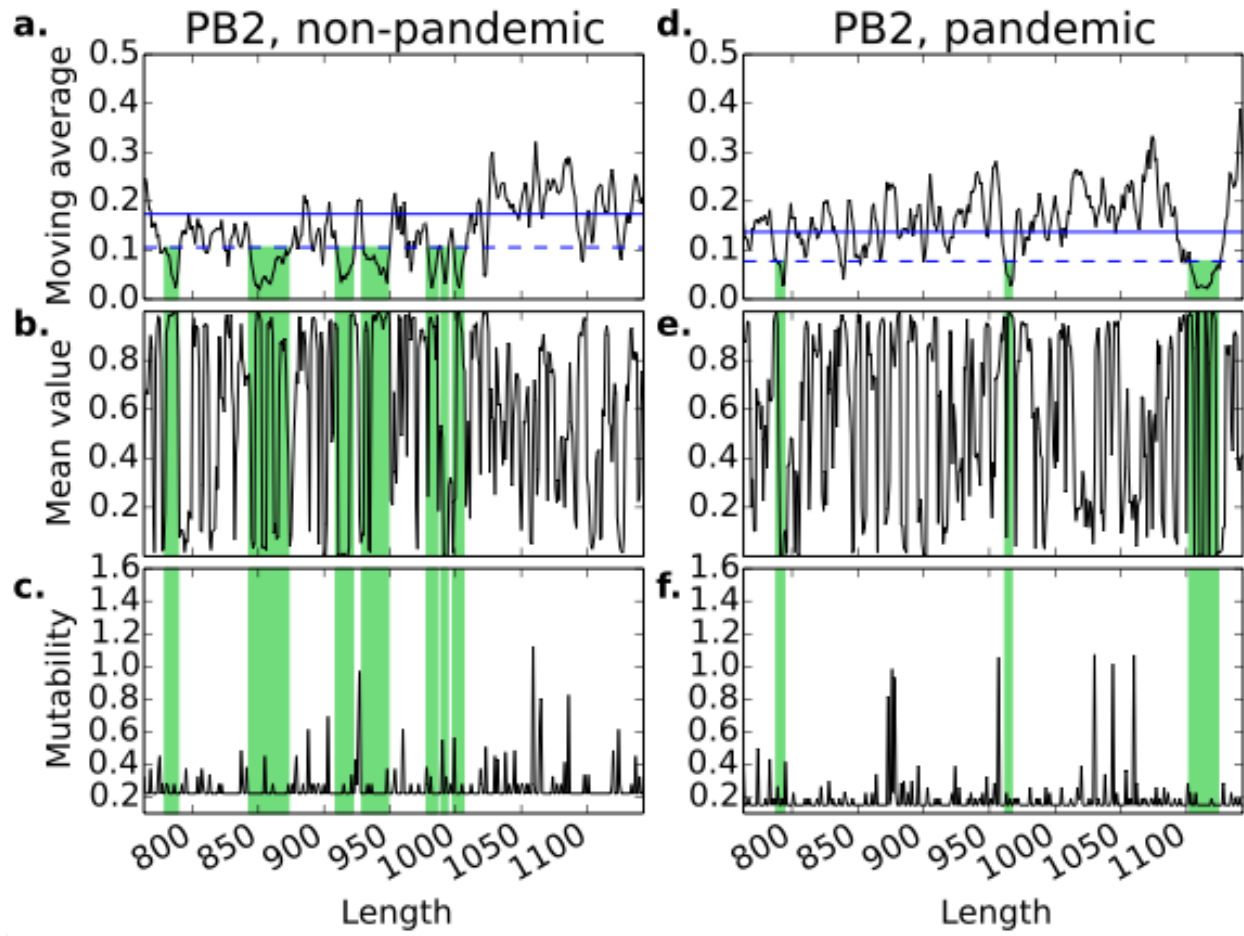
Supplementary Figure 2



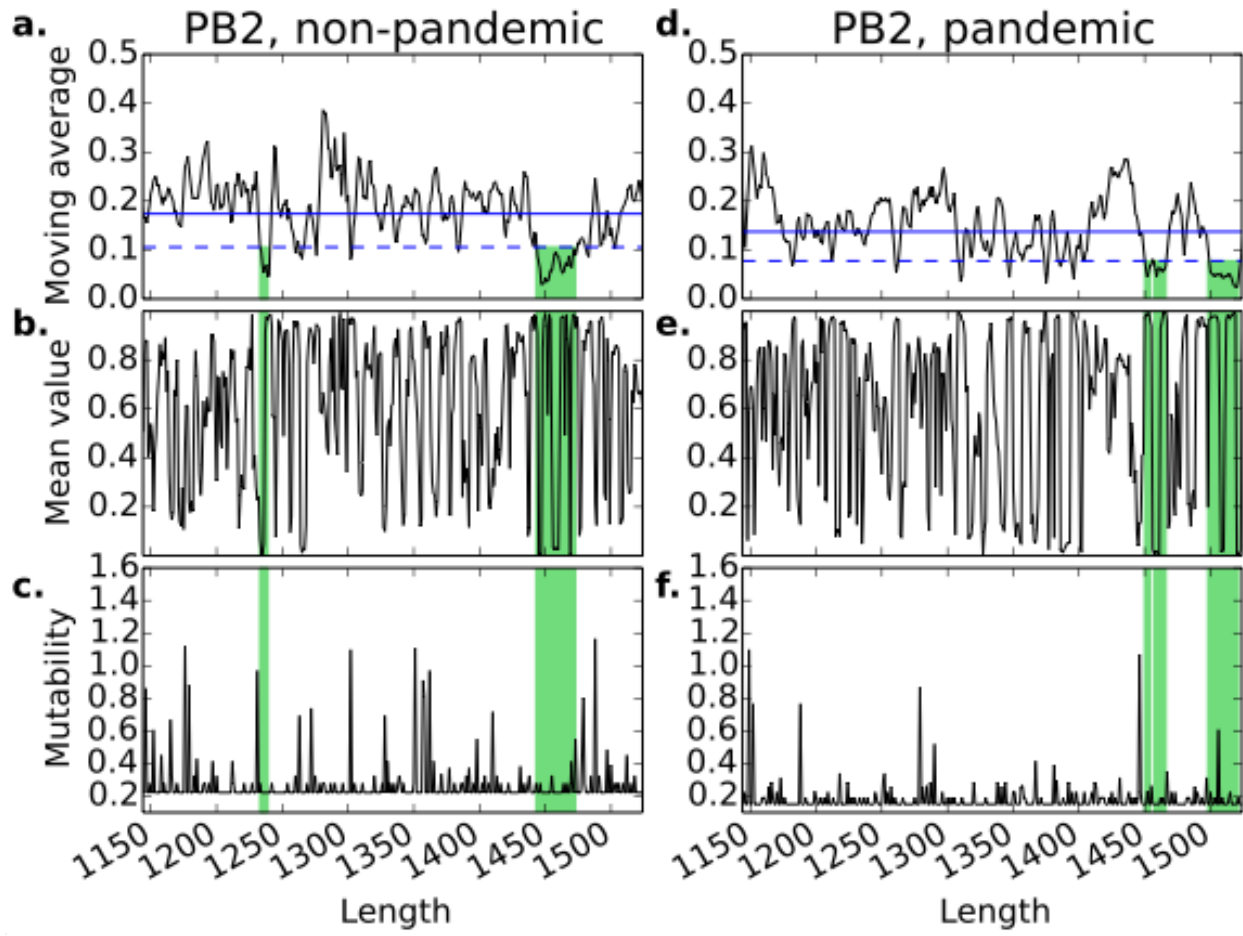
Supplementary Figure 3



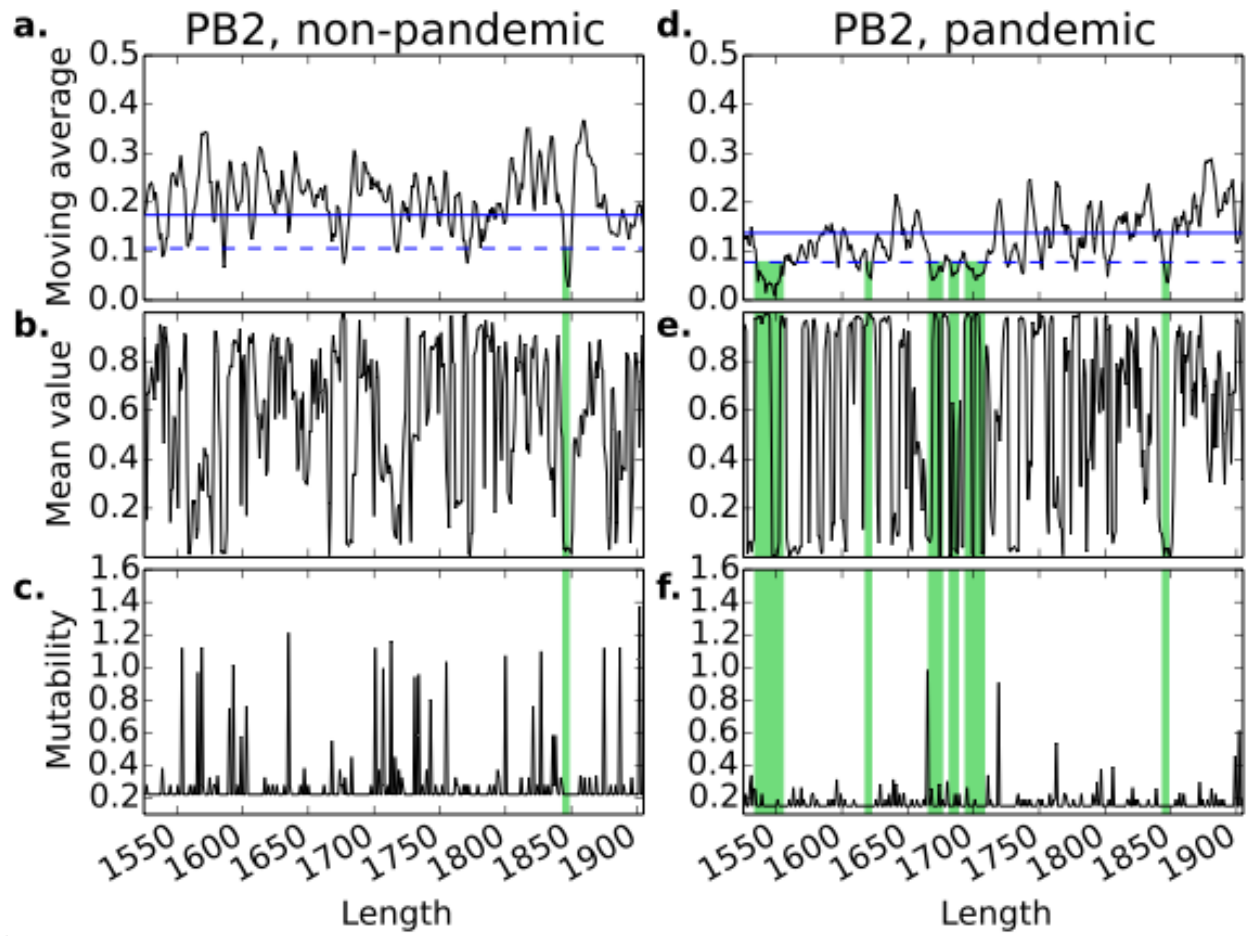
Supplementary Figure 4



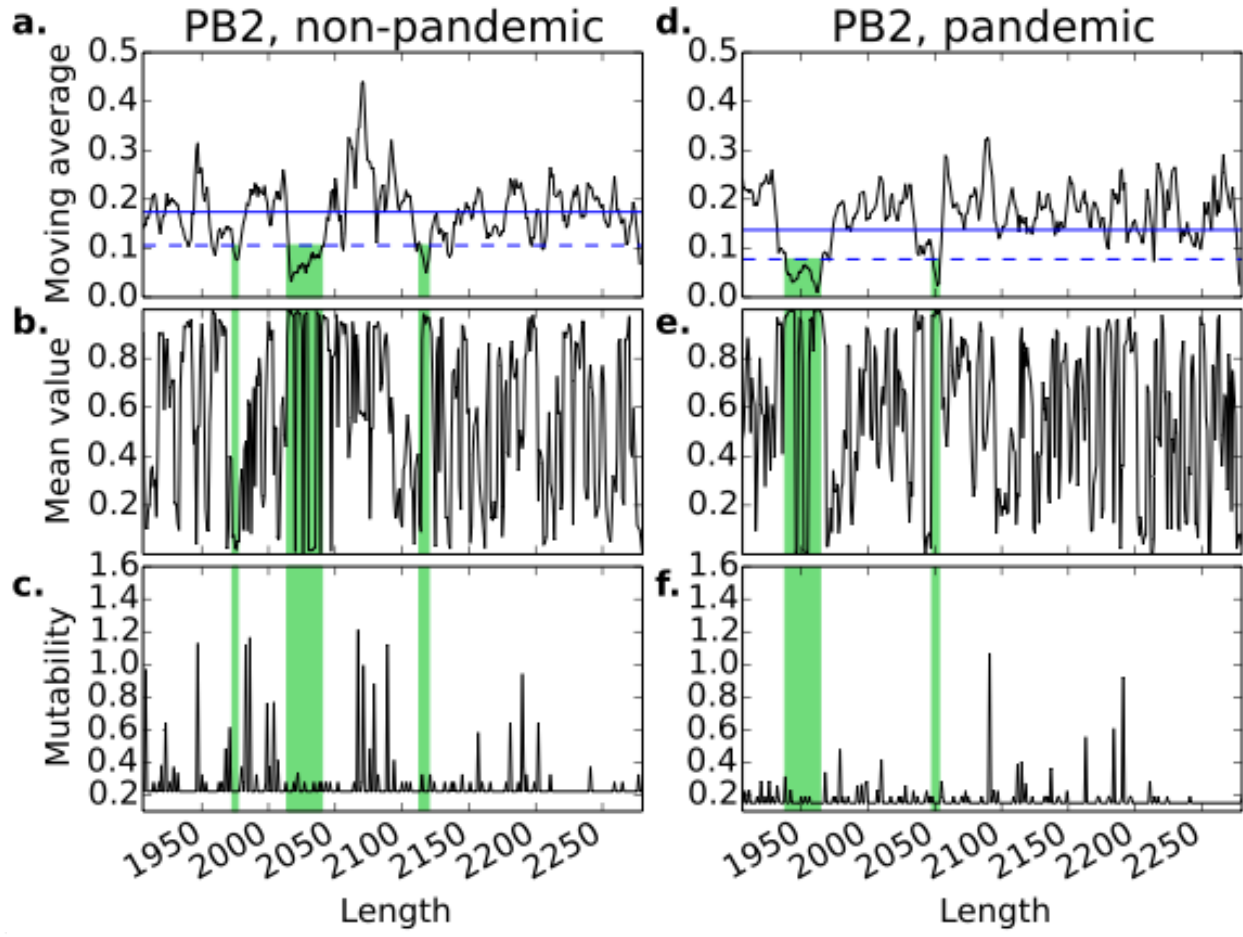
Supplementary Figure 5



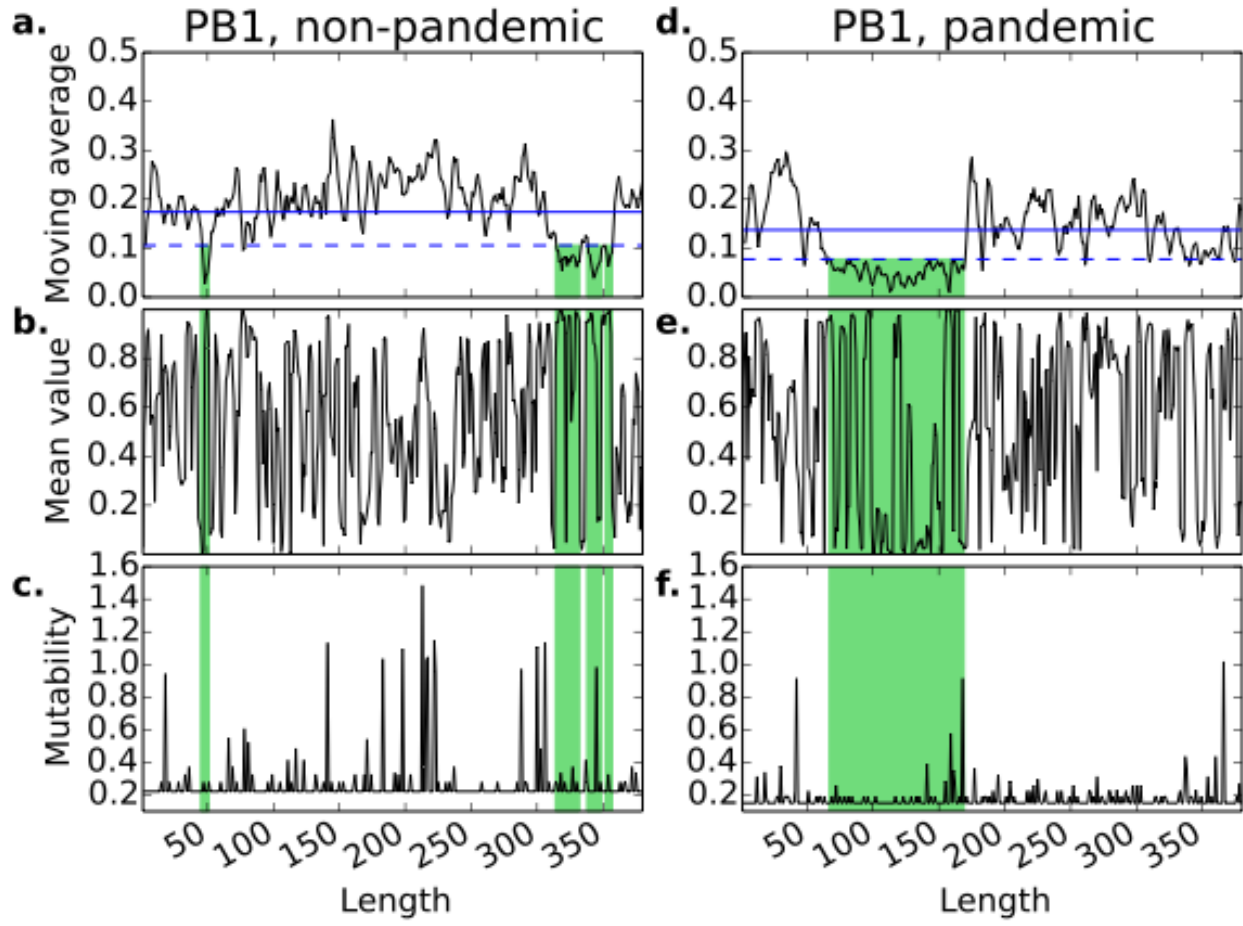
Supplementary Figure 6



Supplementary Figure 7

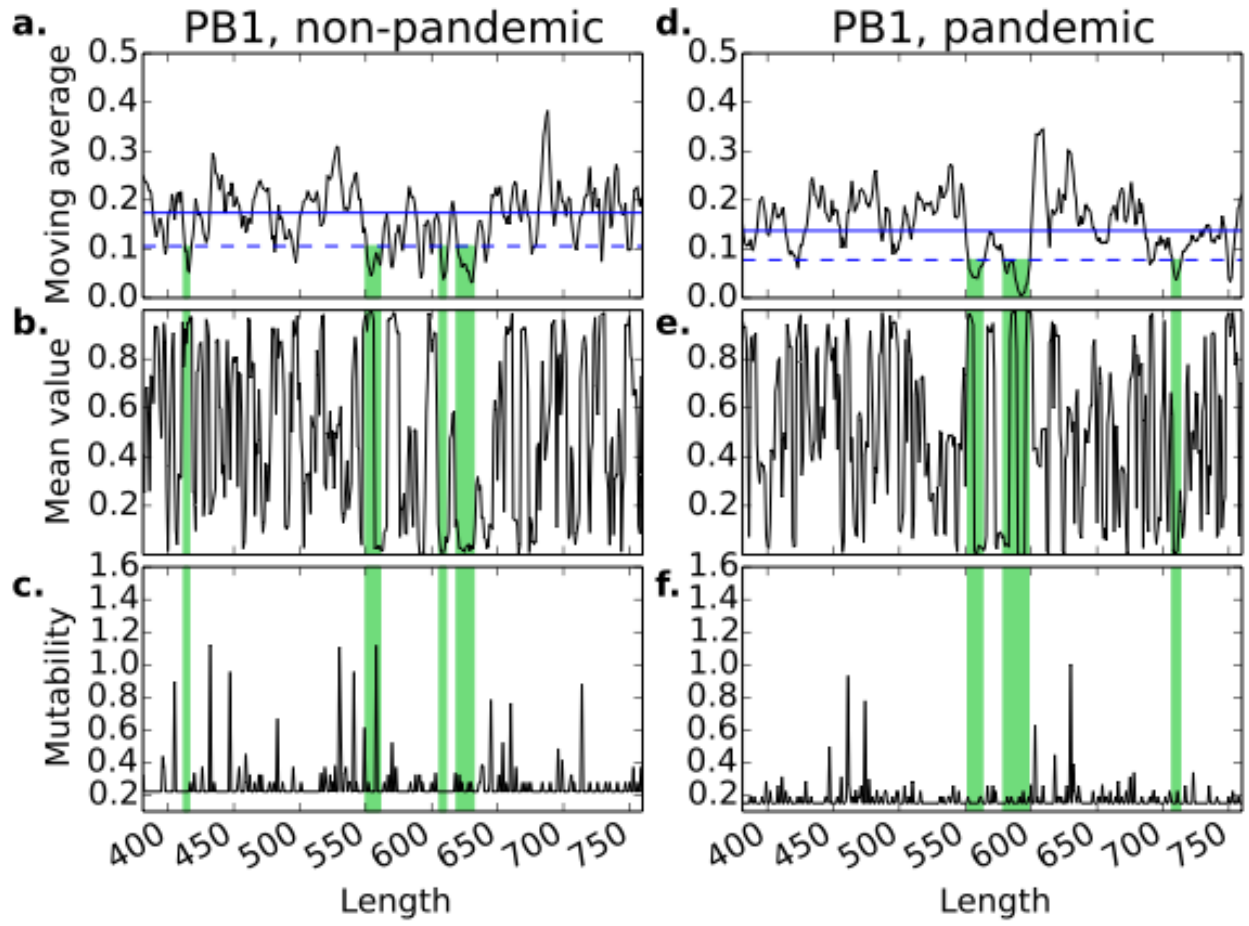


Supplementary Figure 8

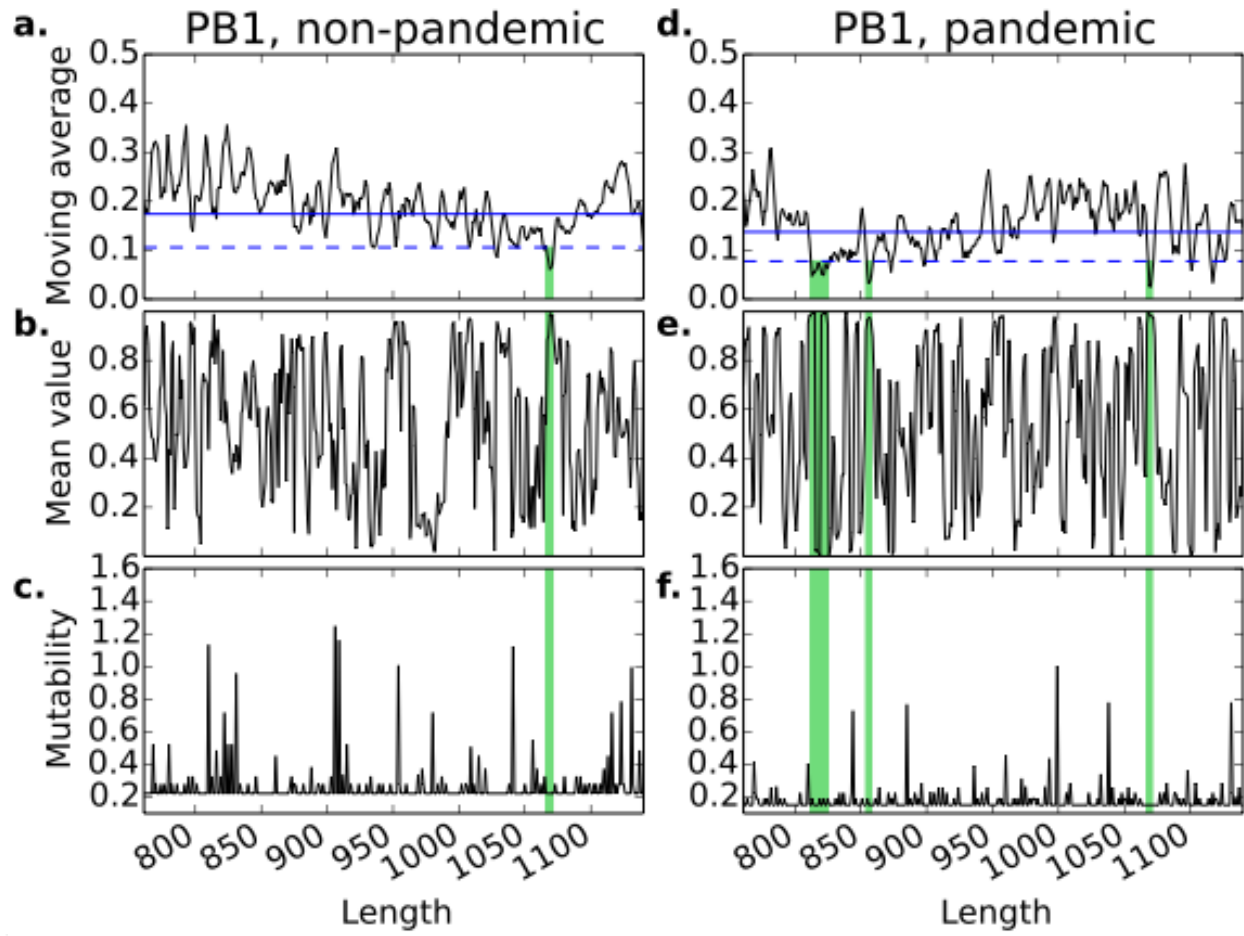




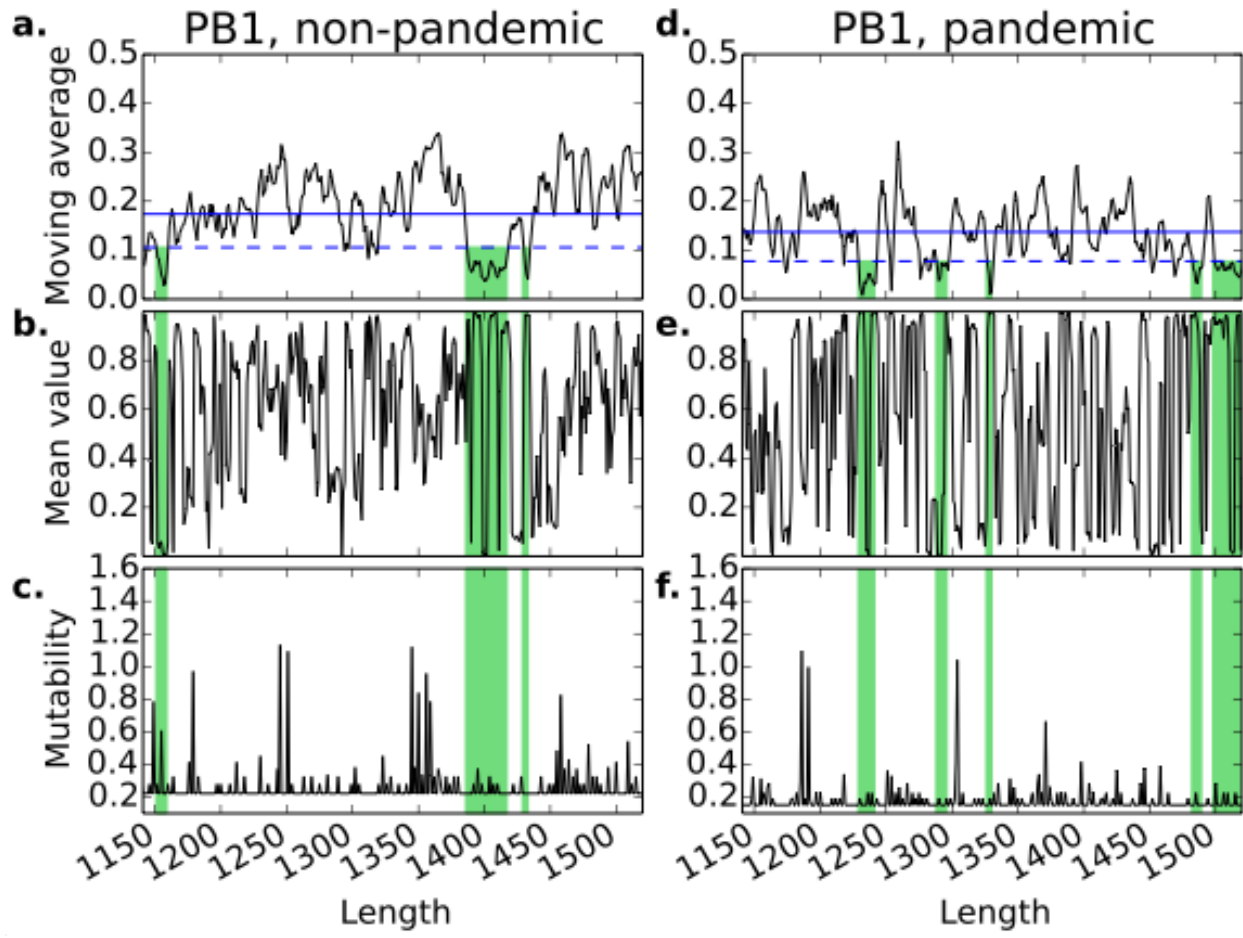
Supplementary Figure 9



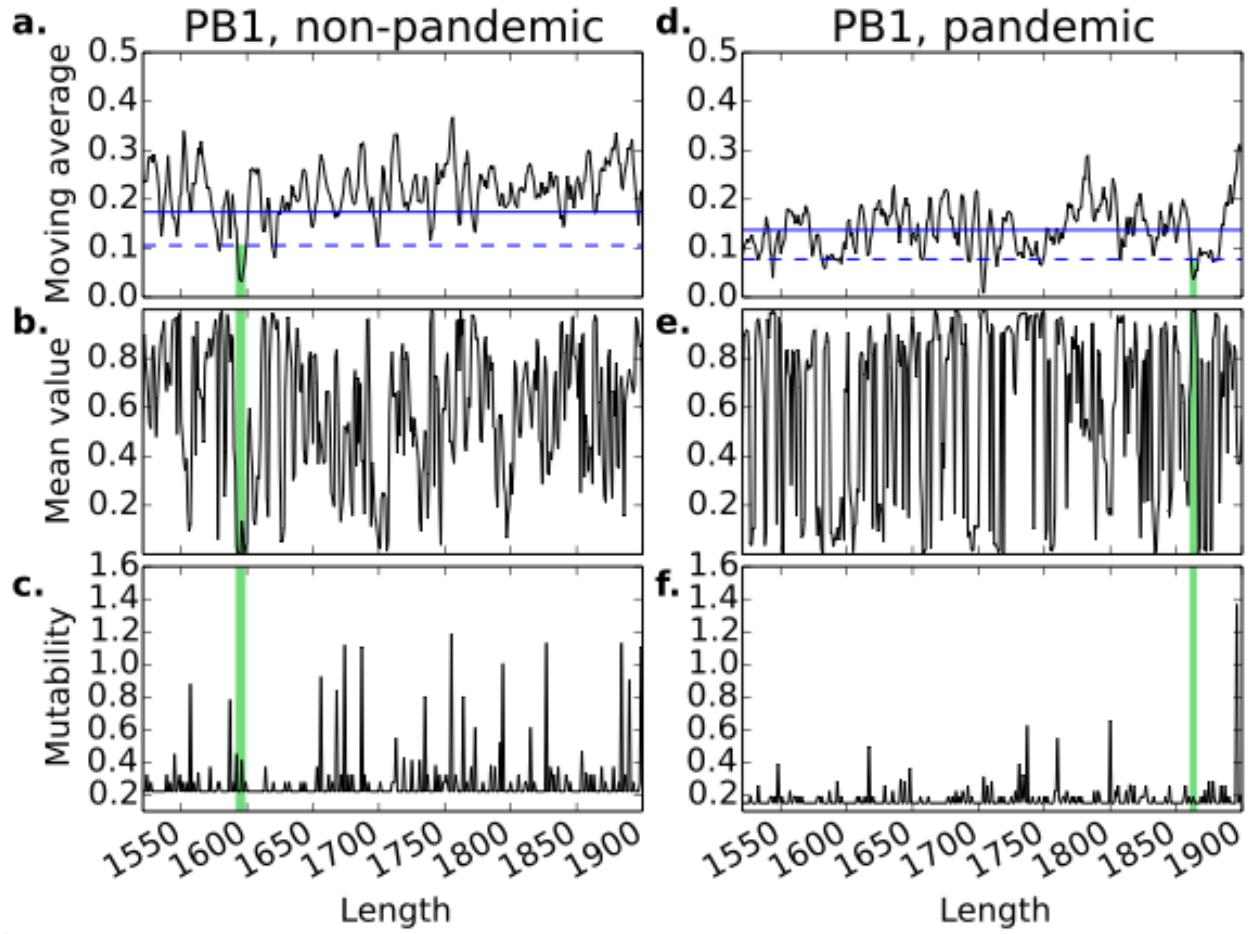
Supplementary Figure 10



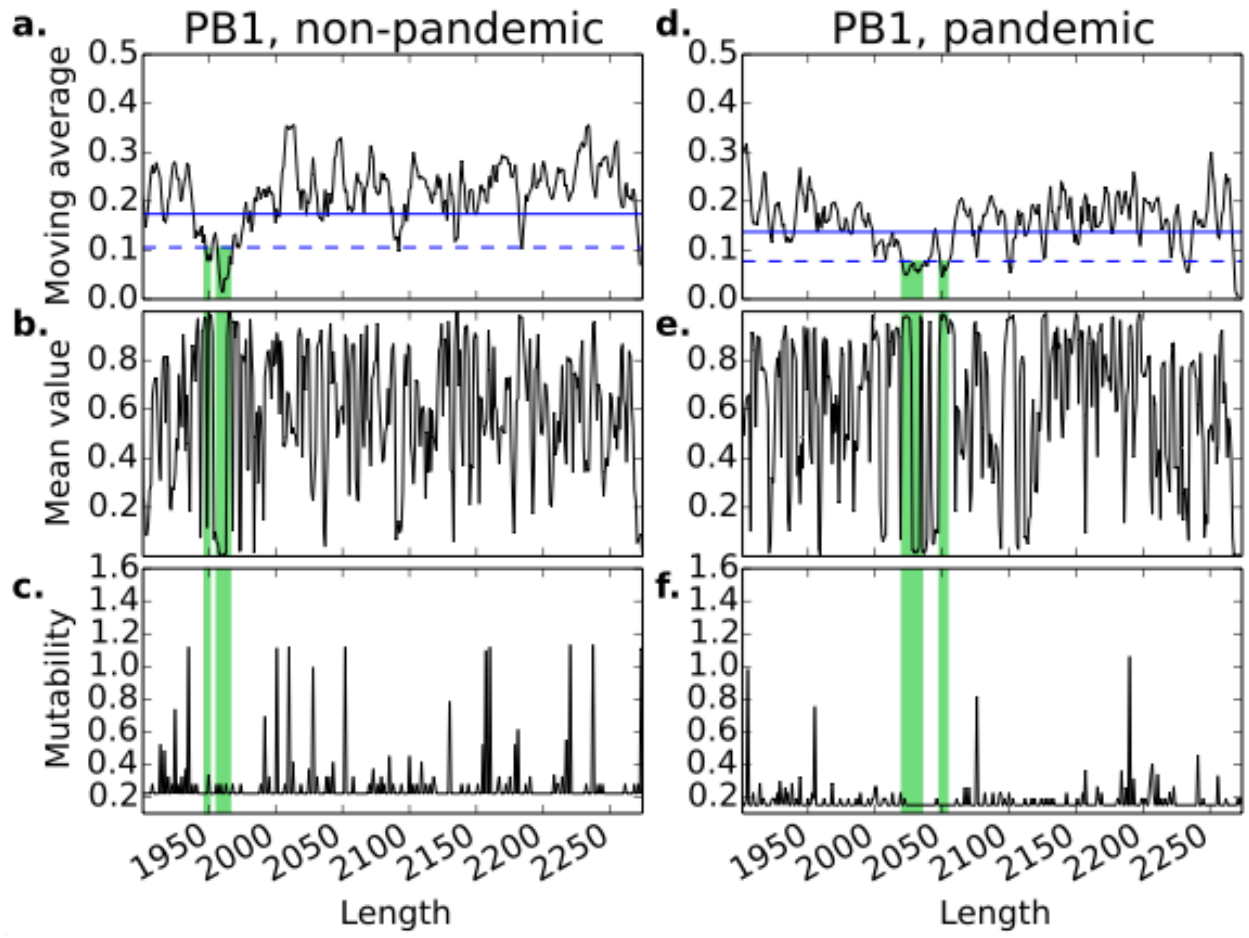
Supplementary Figure 11



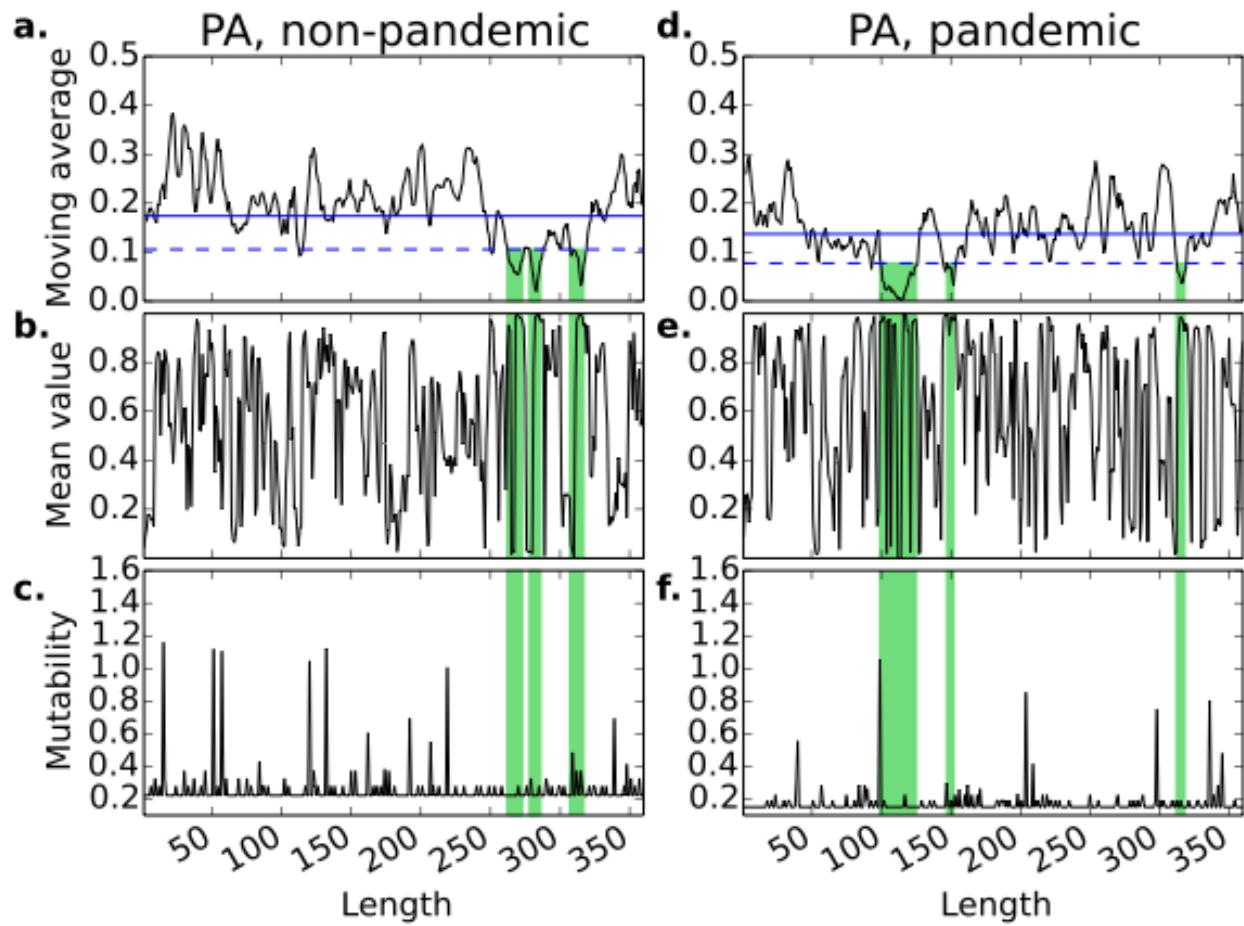
Supplementary Figure 12



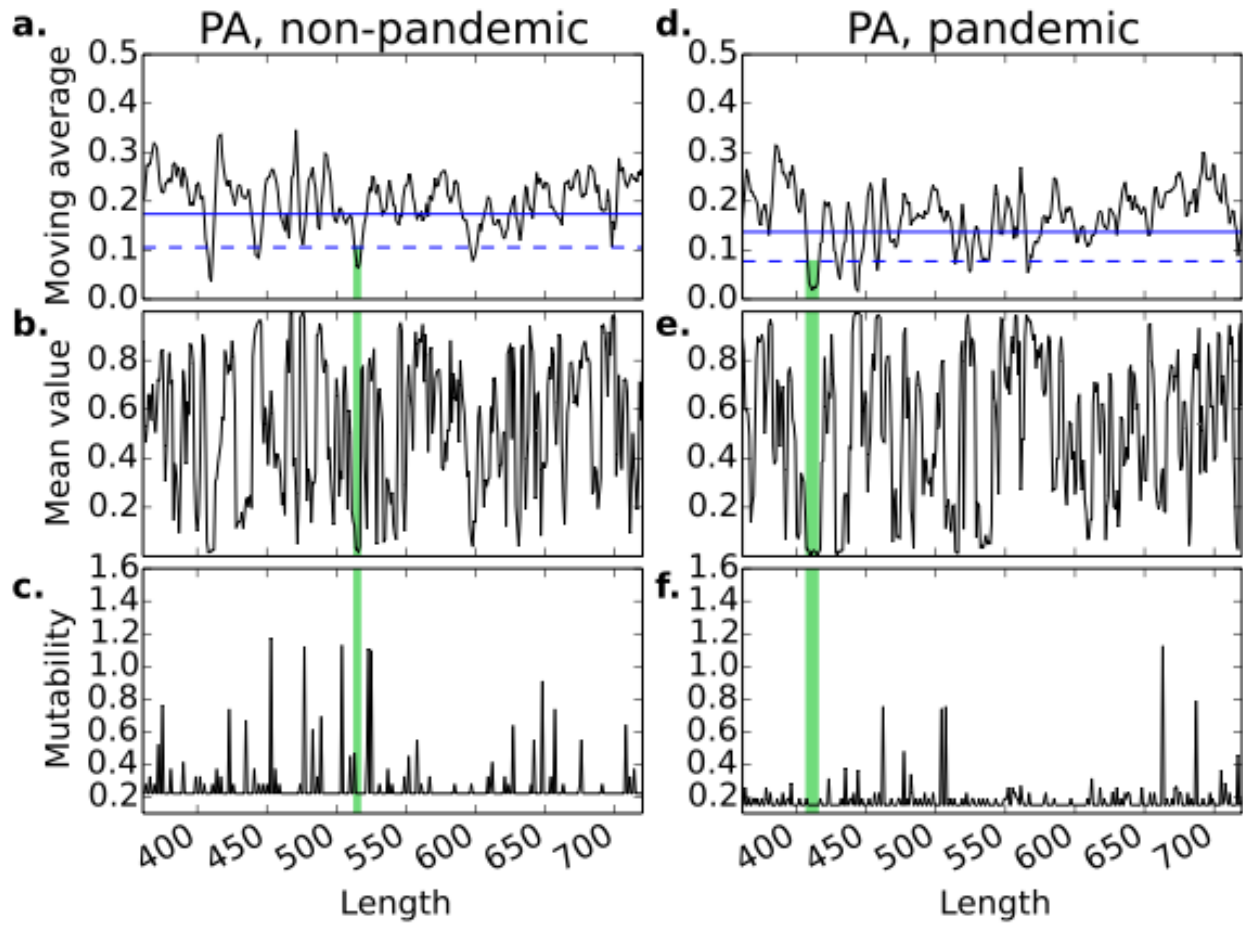
Supplementary Figure 13



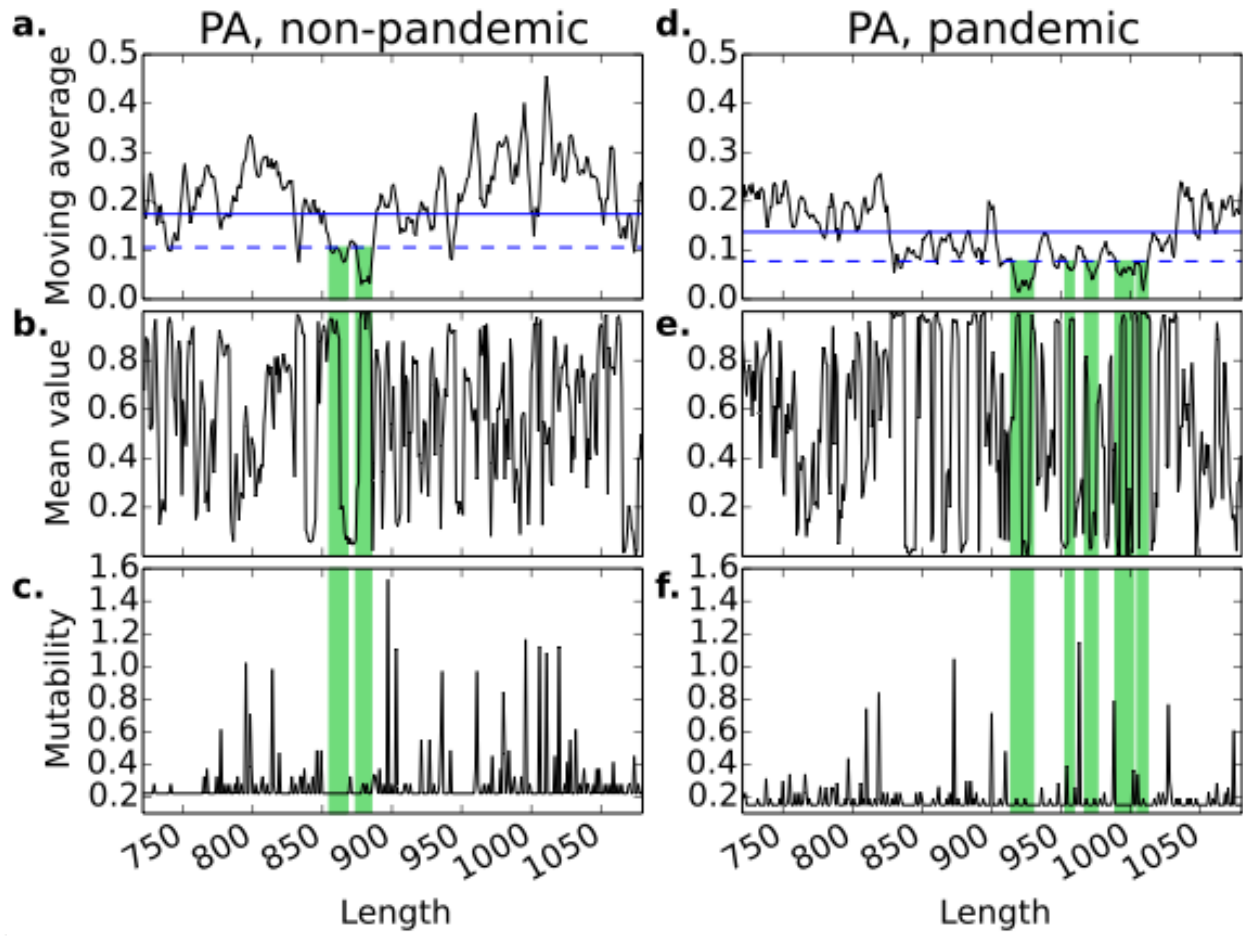
Supplementary Figure 14



Supplementary Figure 15

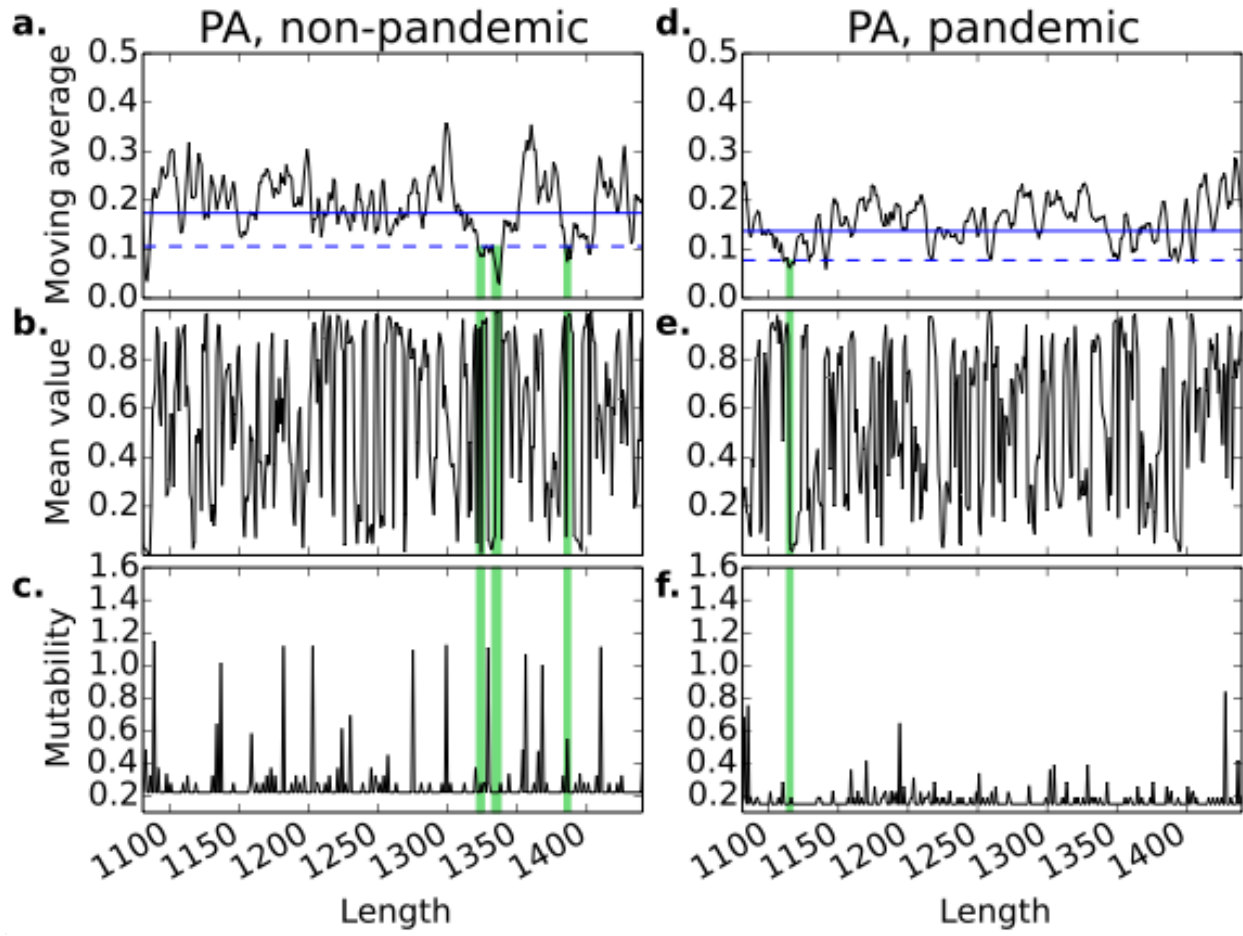


Supplementary Figure 16

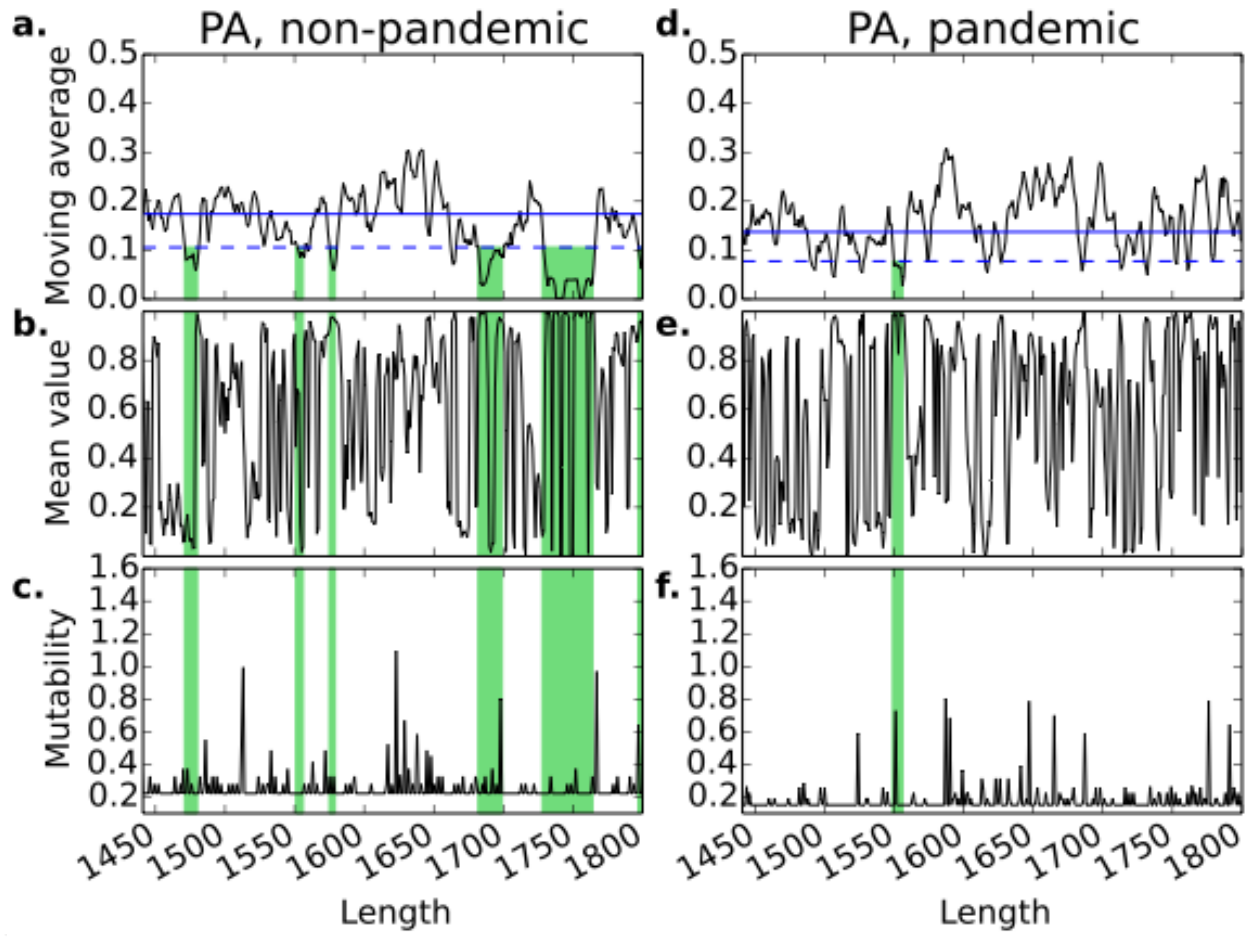




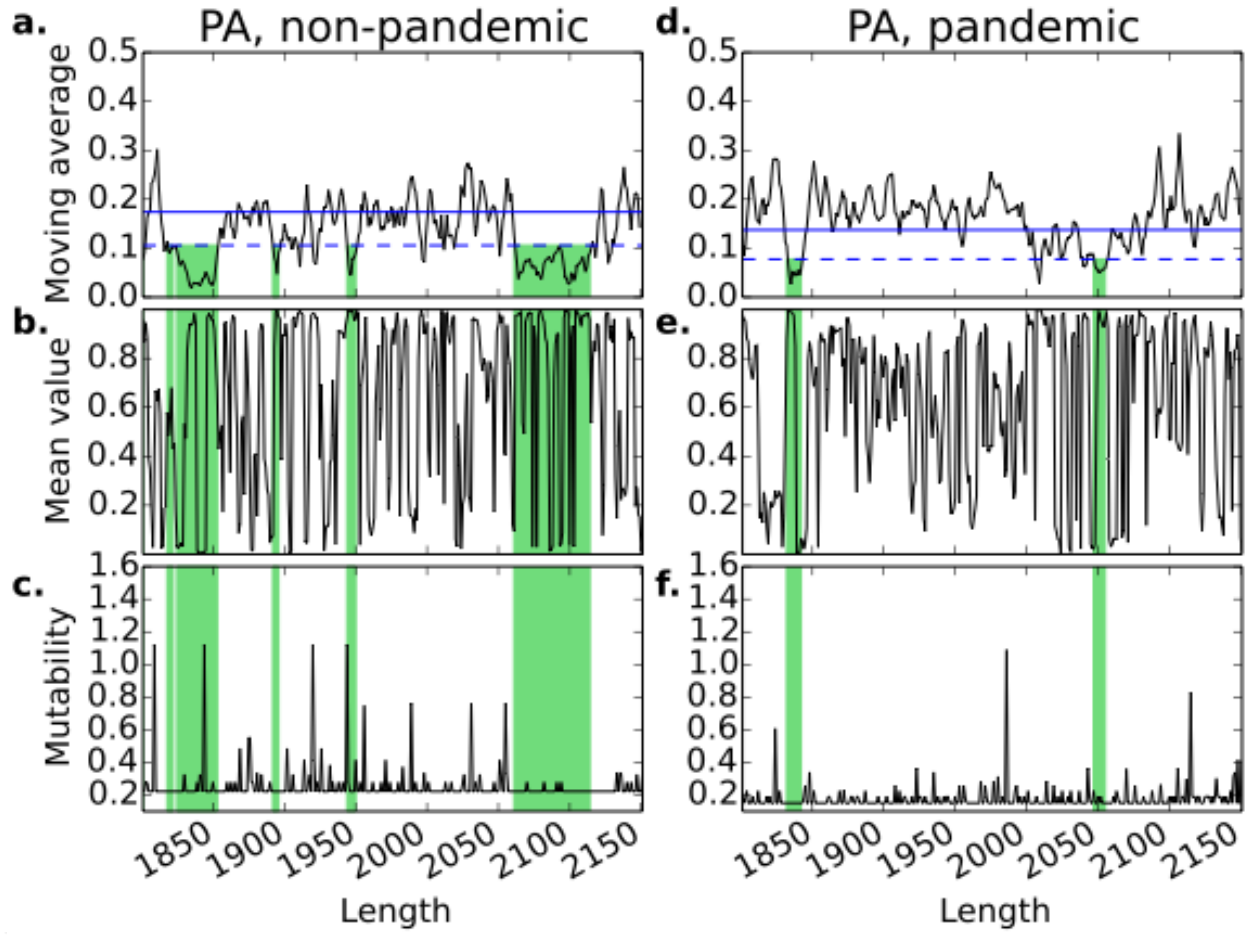
Supplementary Figure 17



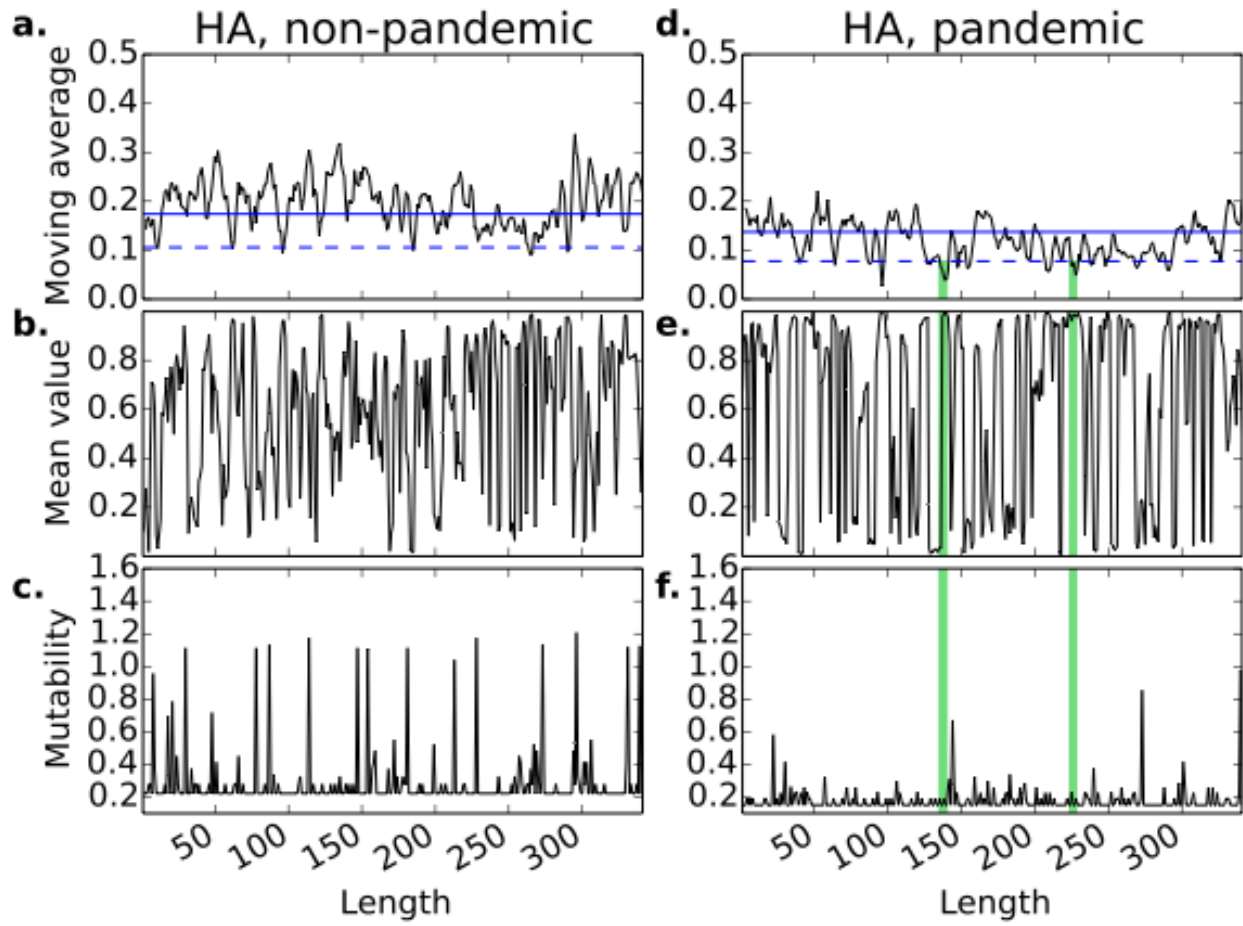
Supplementary Figure 18



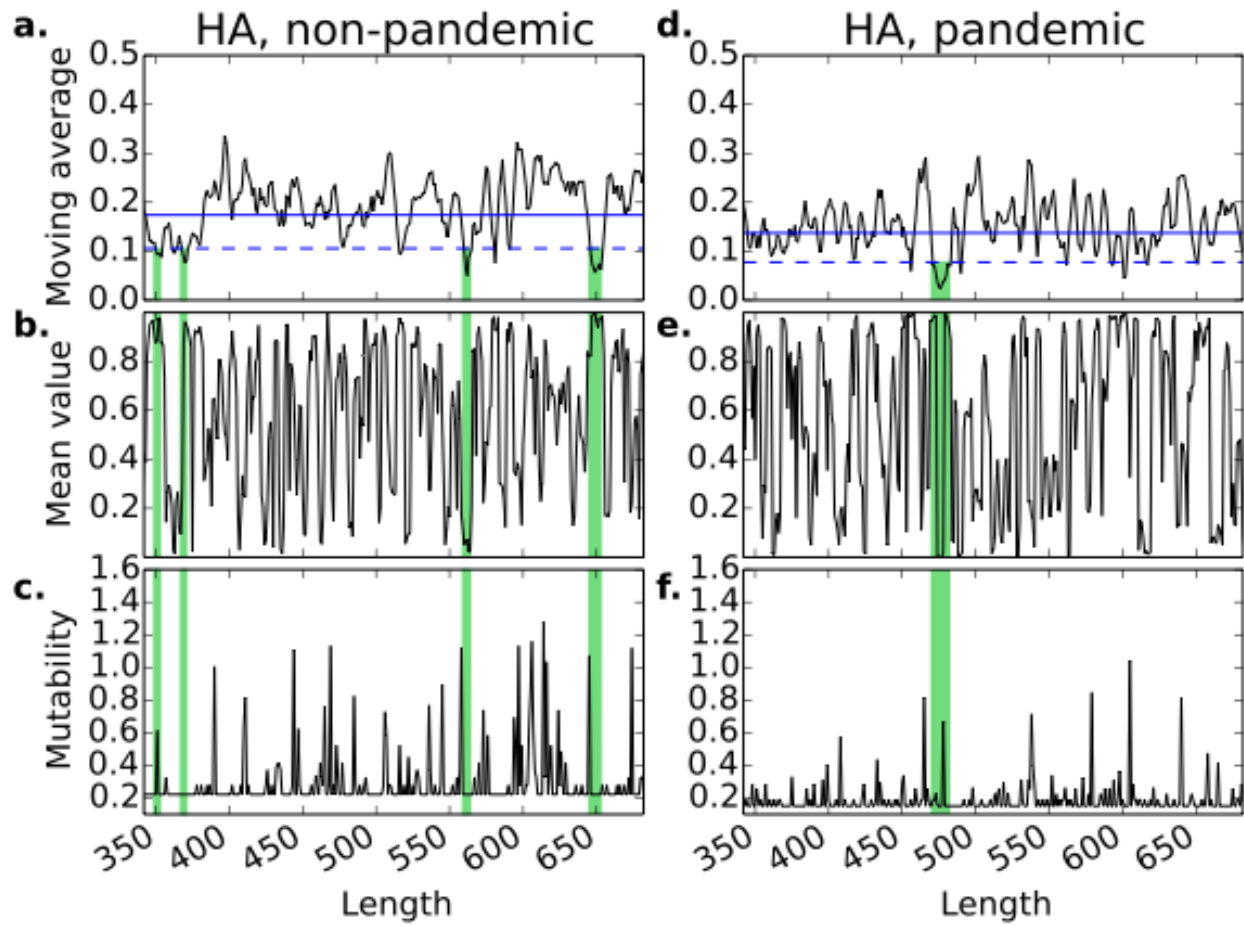
Supplementary Figure 19



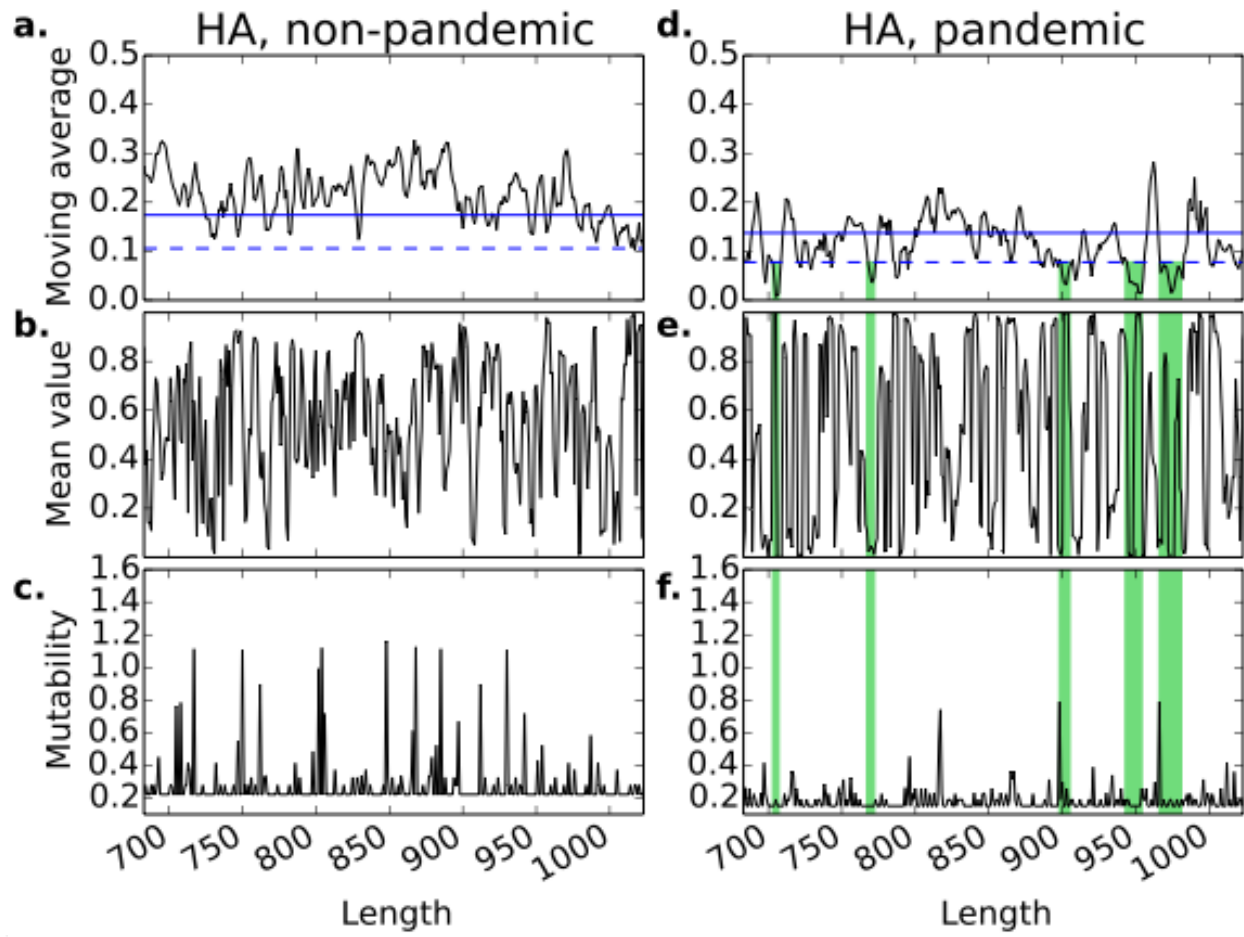
Supplementary Figure 20



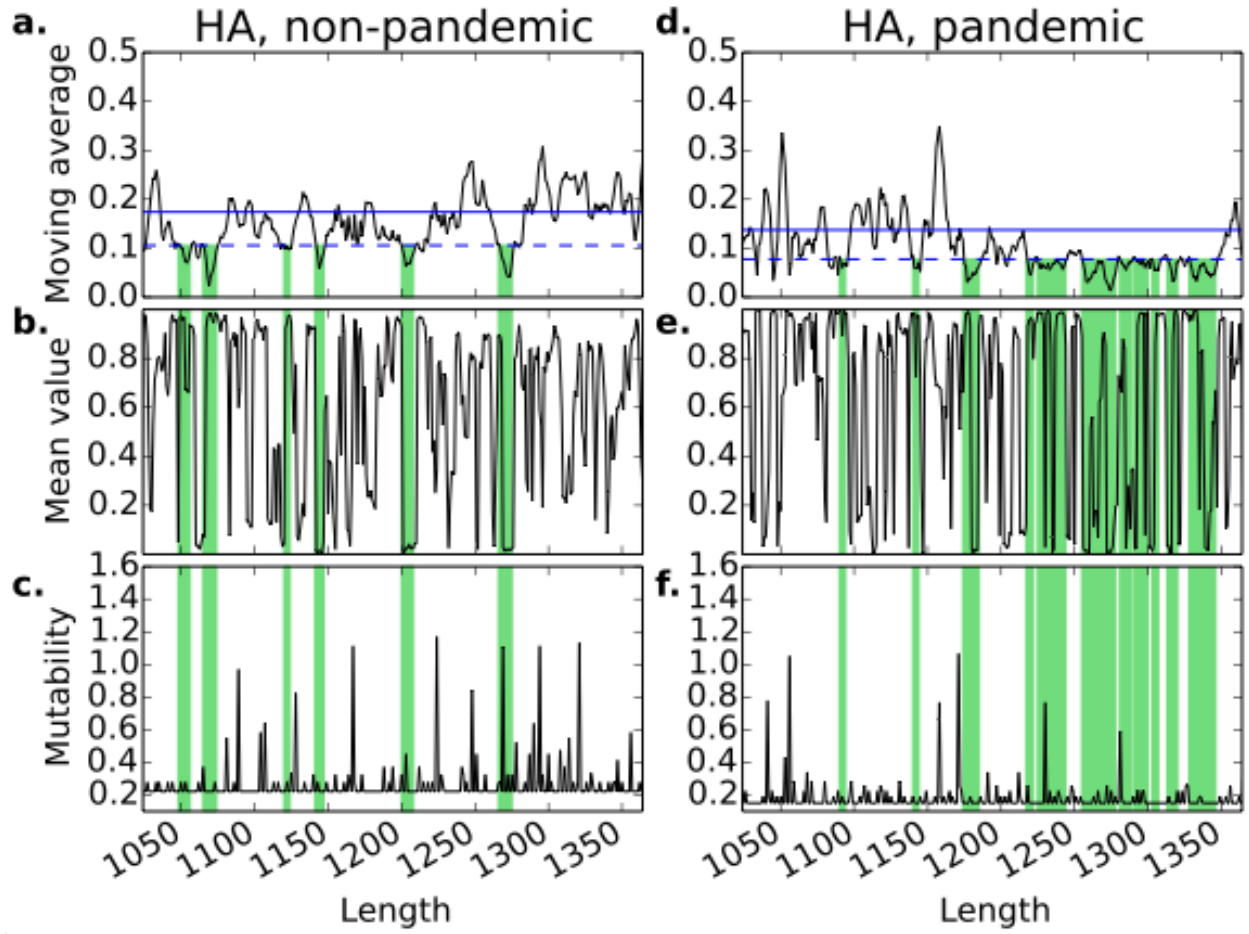
Supplementary Figure 21



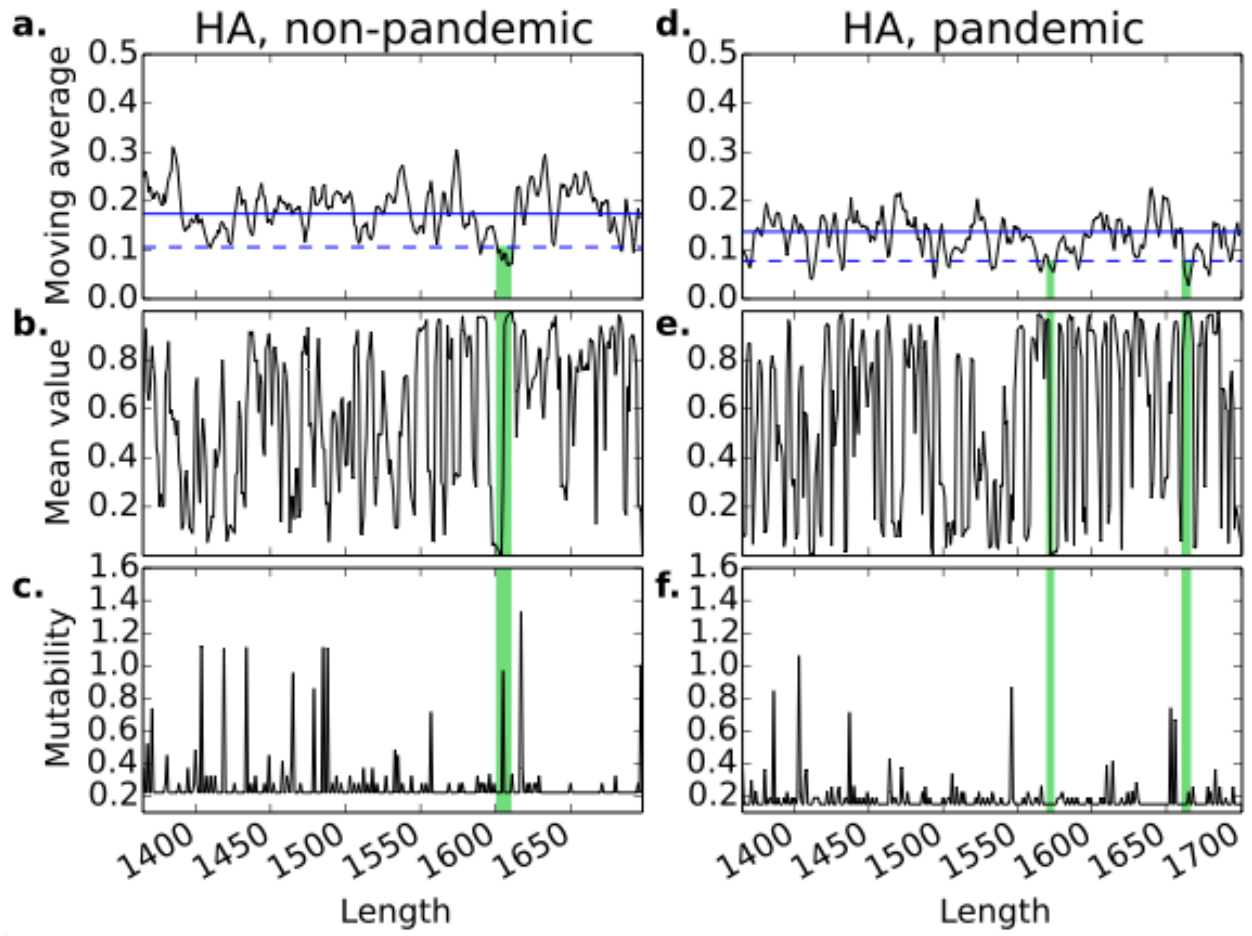
Supplementary Figure 22



Supplementary Figure 23

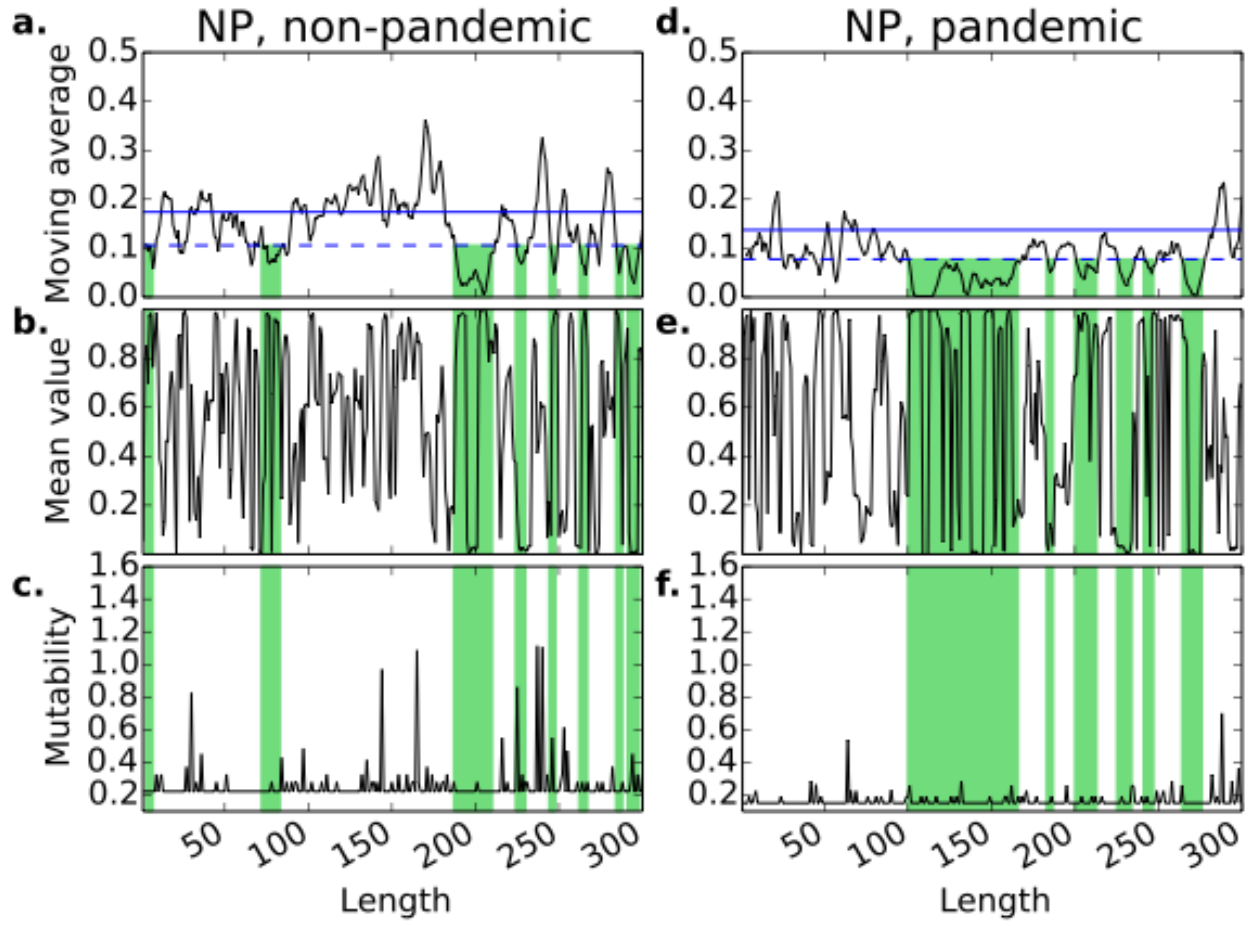


Supplementary Figure 24

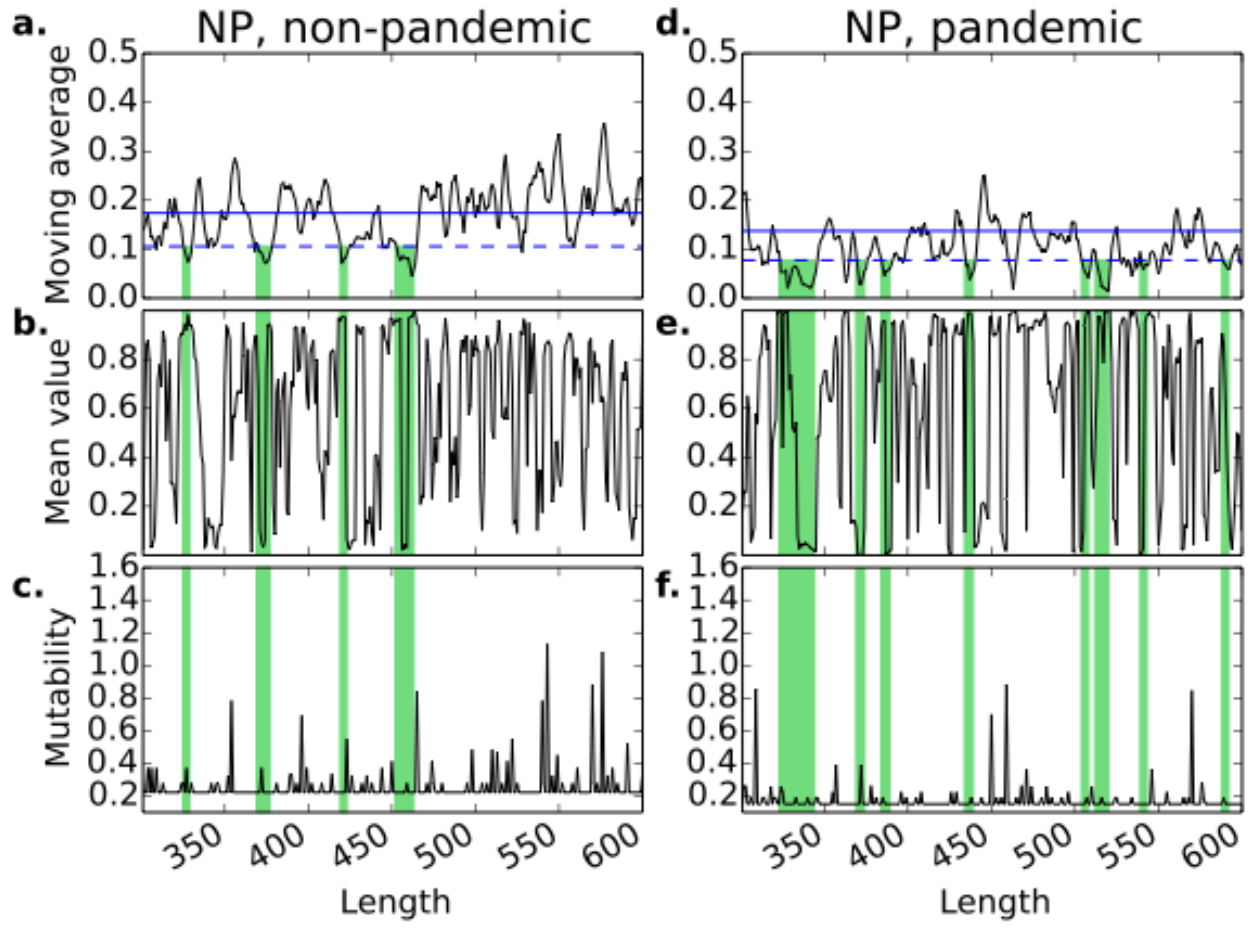




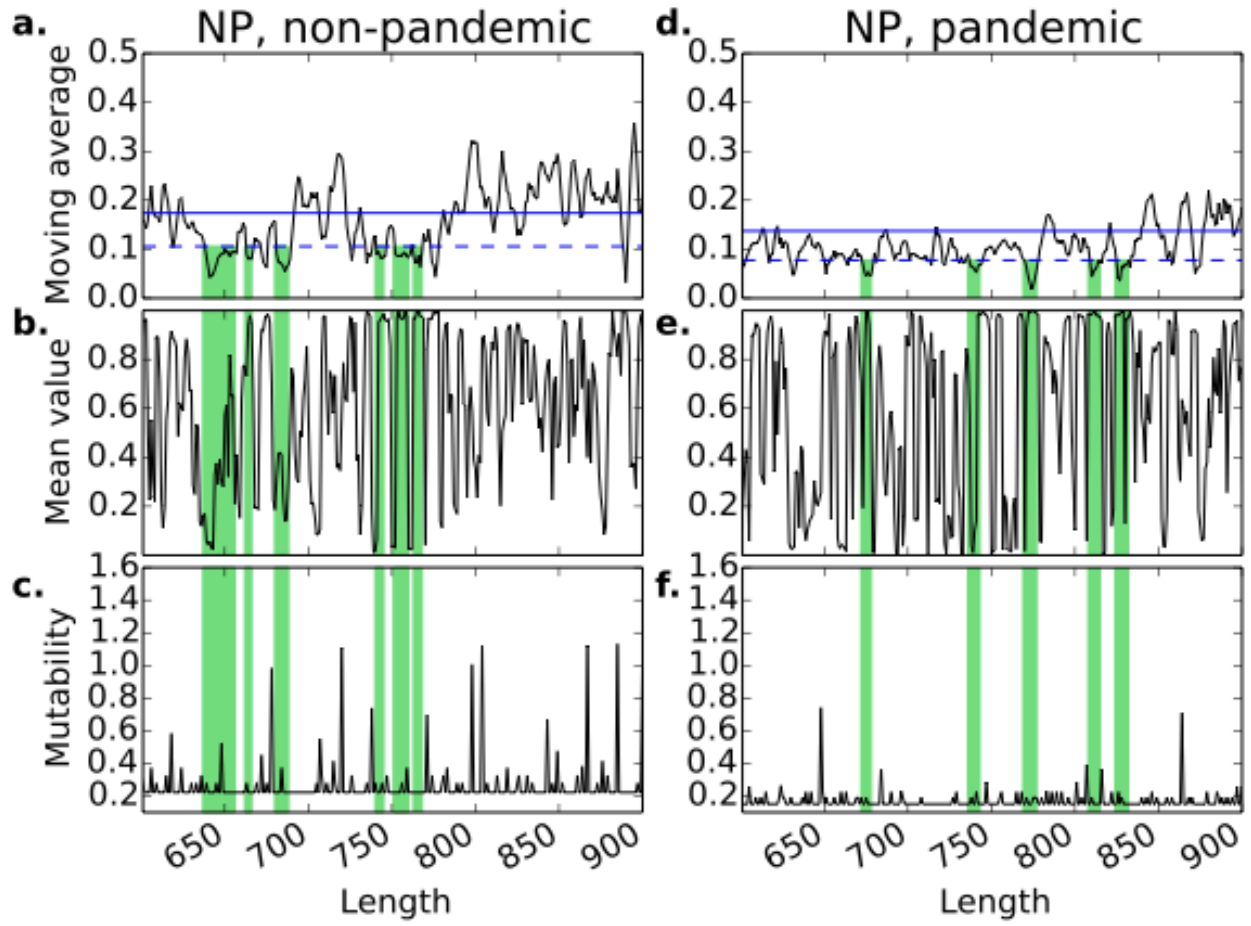
Supplementary Figure 25



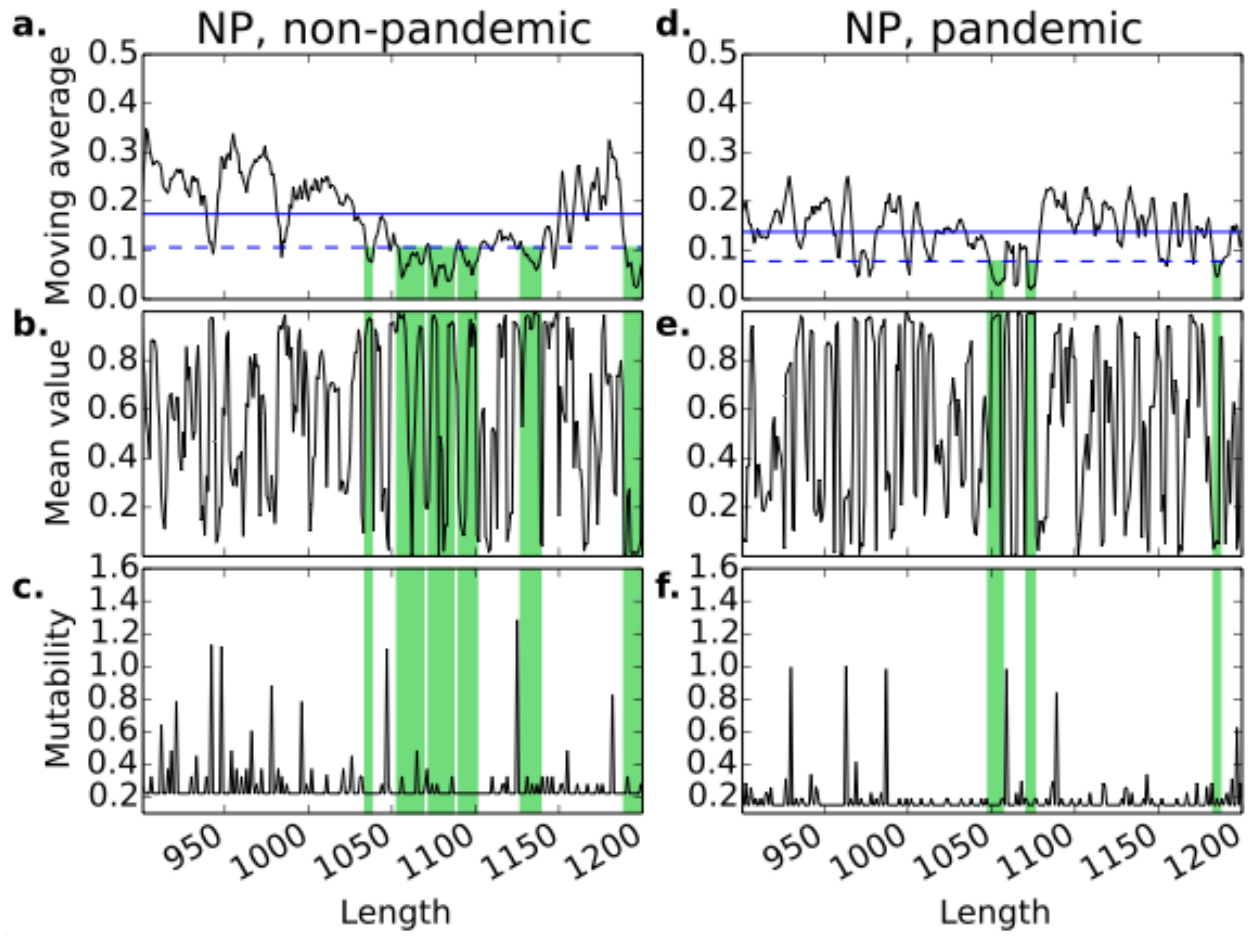
Supplementary Figure 26



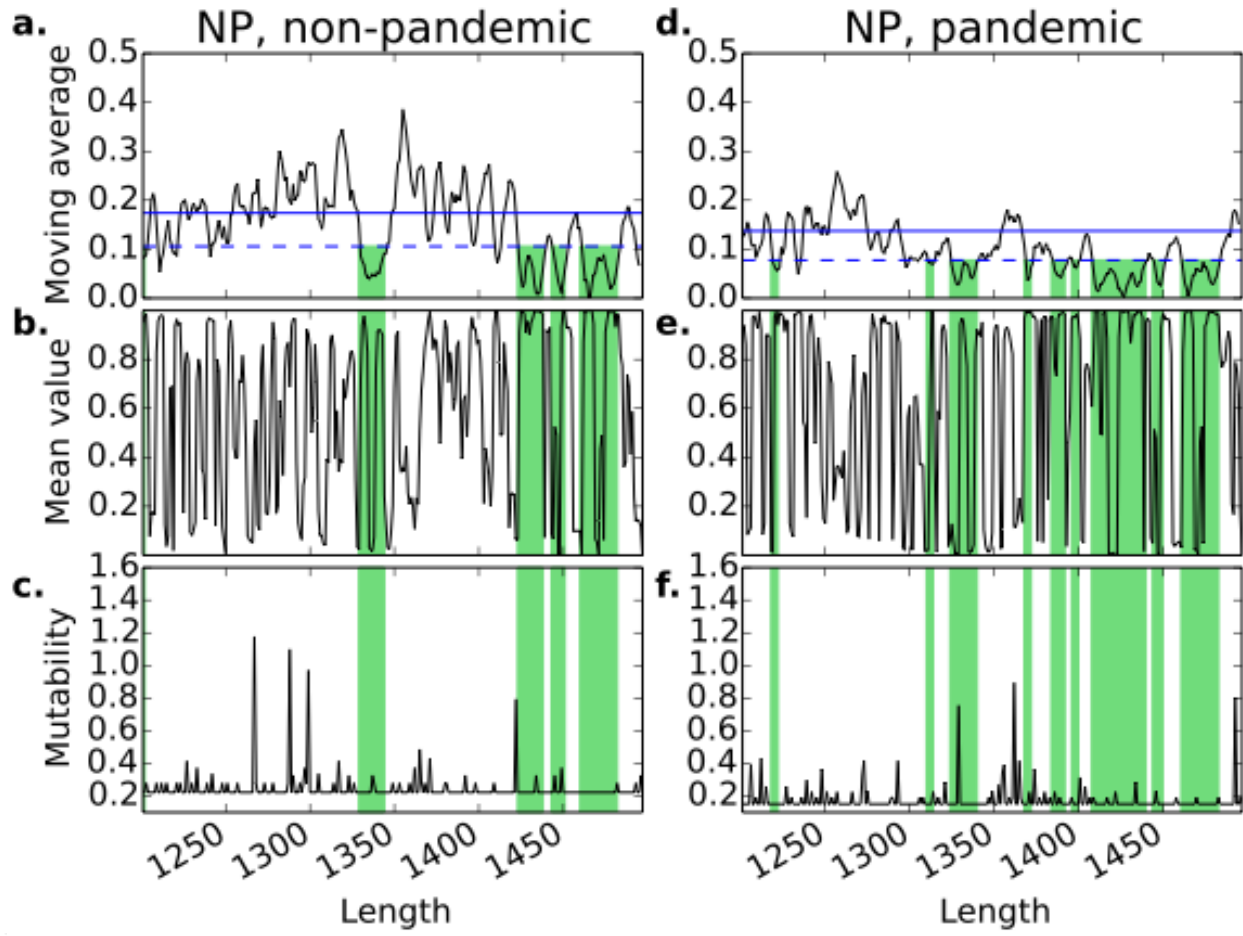
Supplementary Figure 27



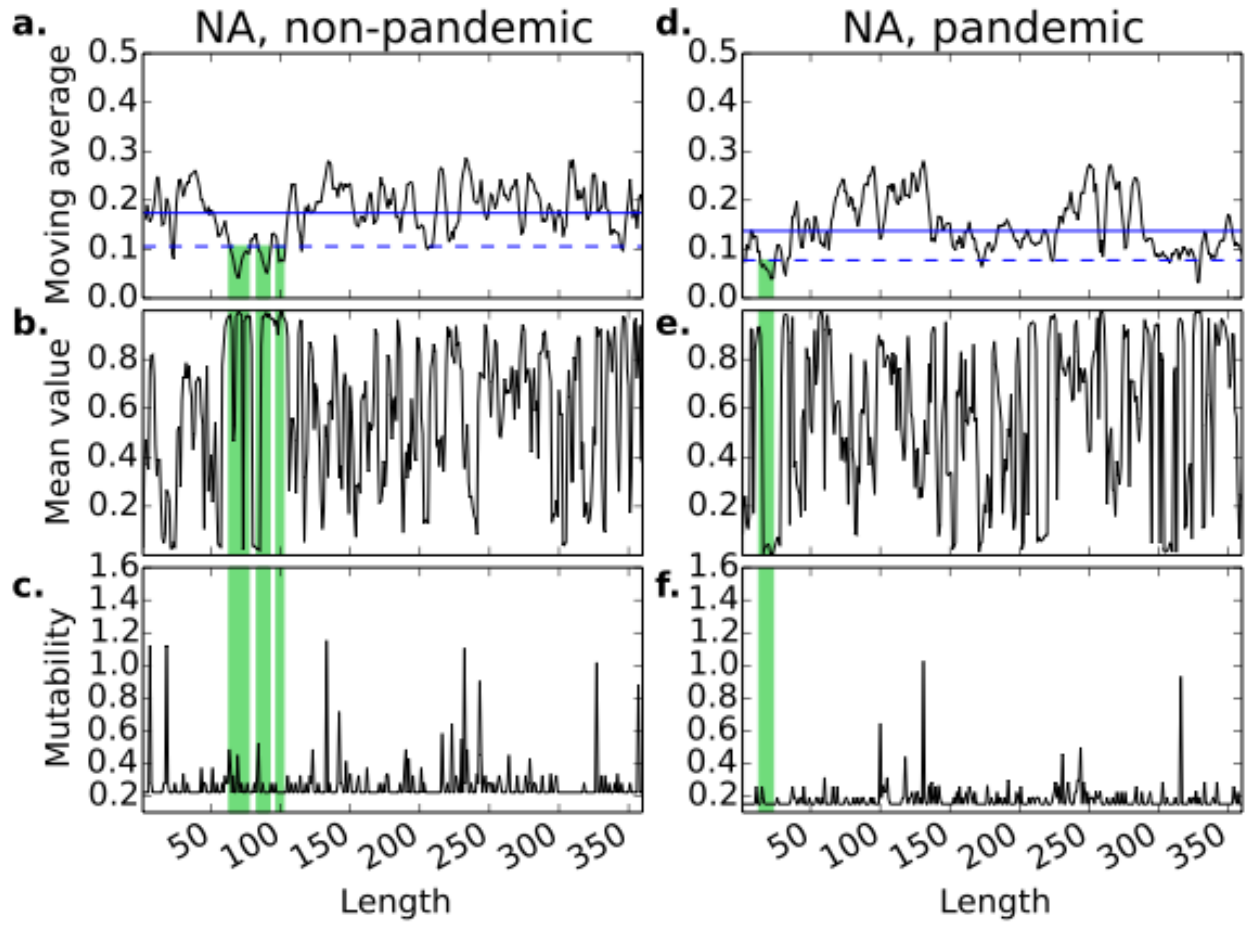
Supplementary Figure 28



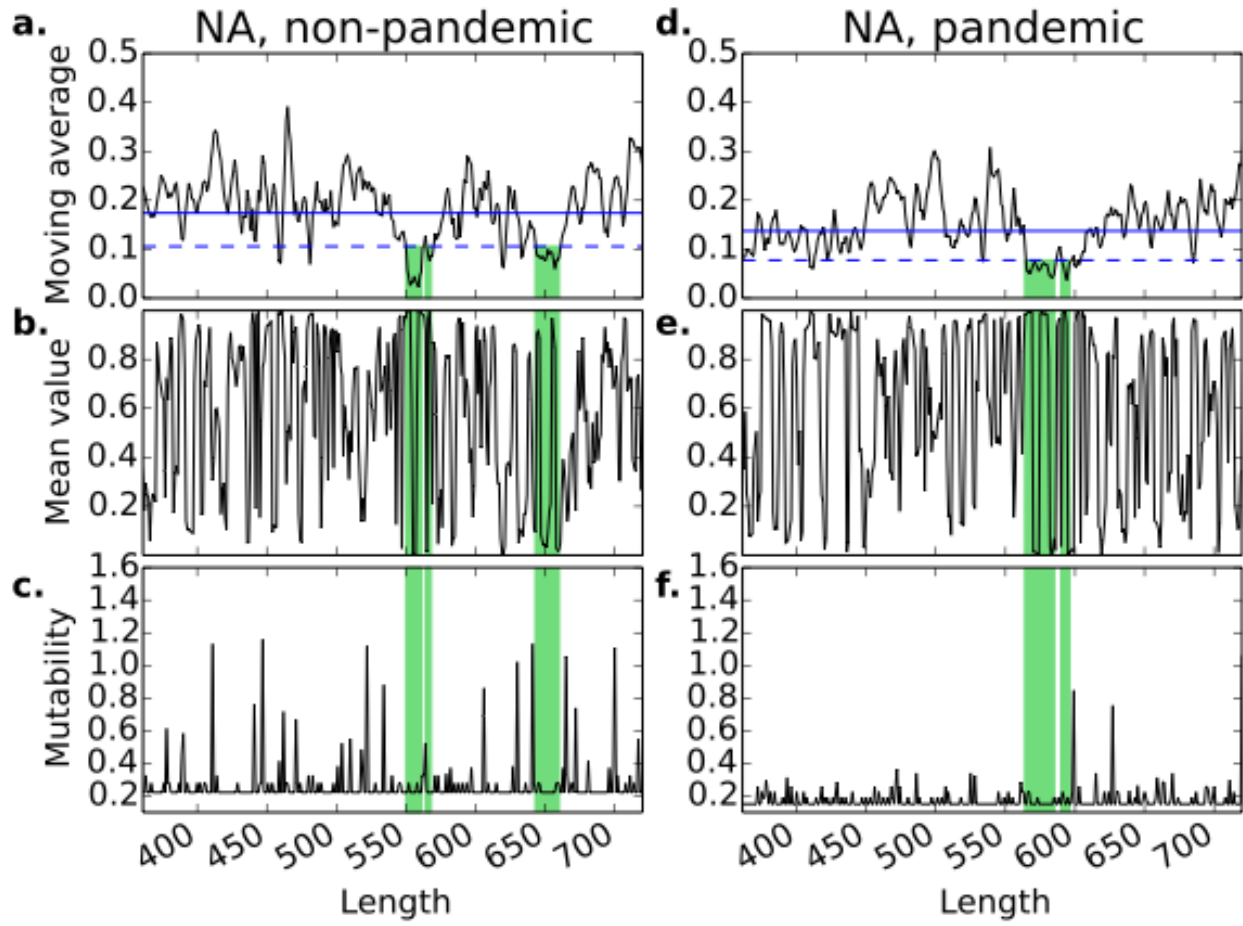
Supplementary Figure 29



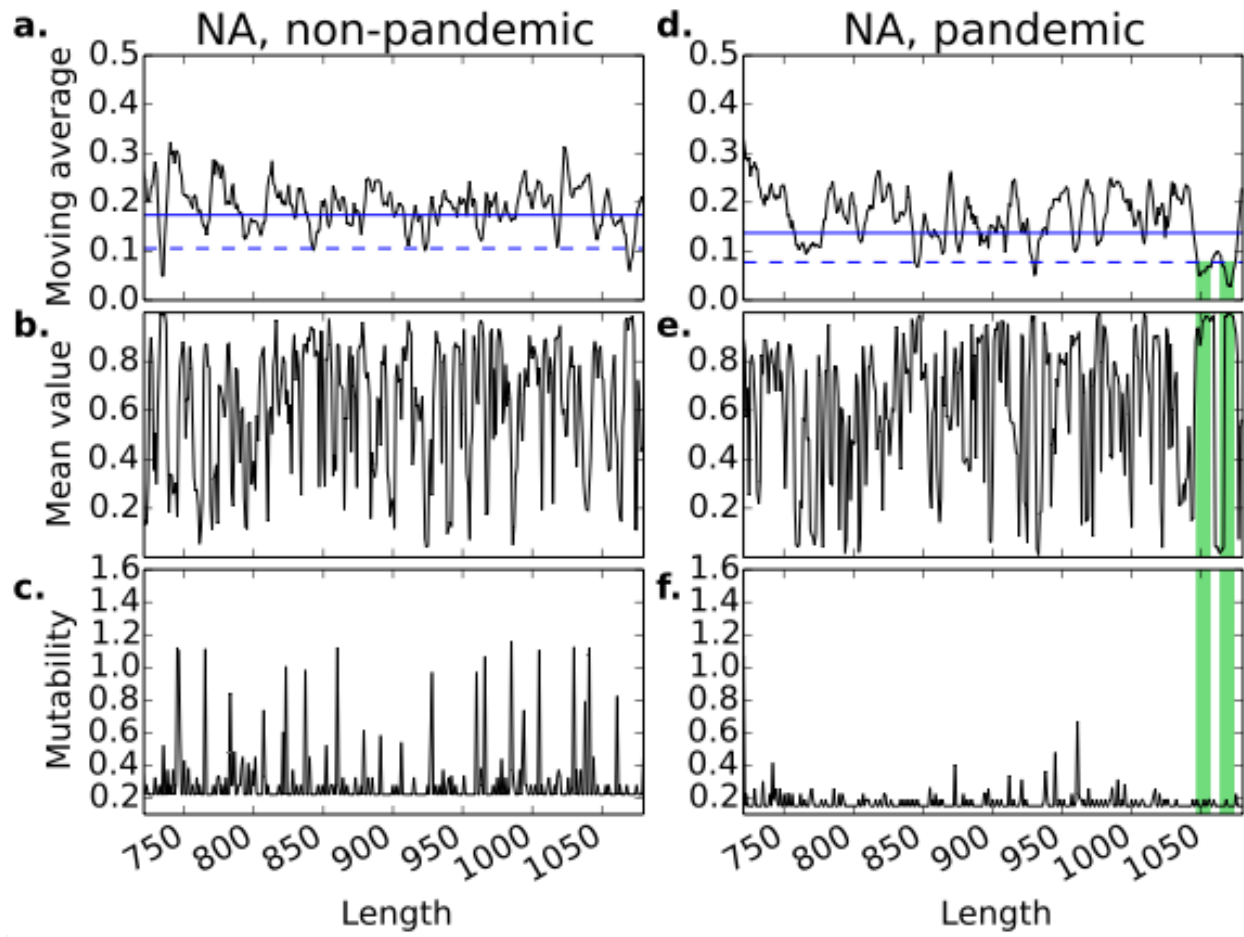
Supplementary Figure 30



Supplementary Figure 31

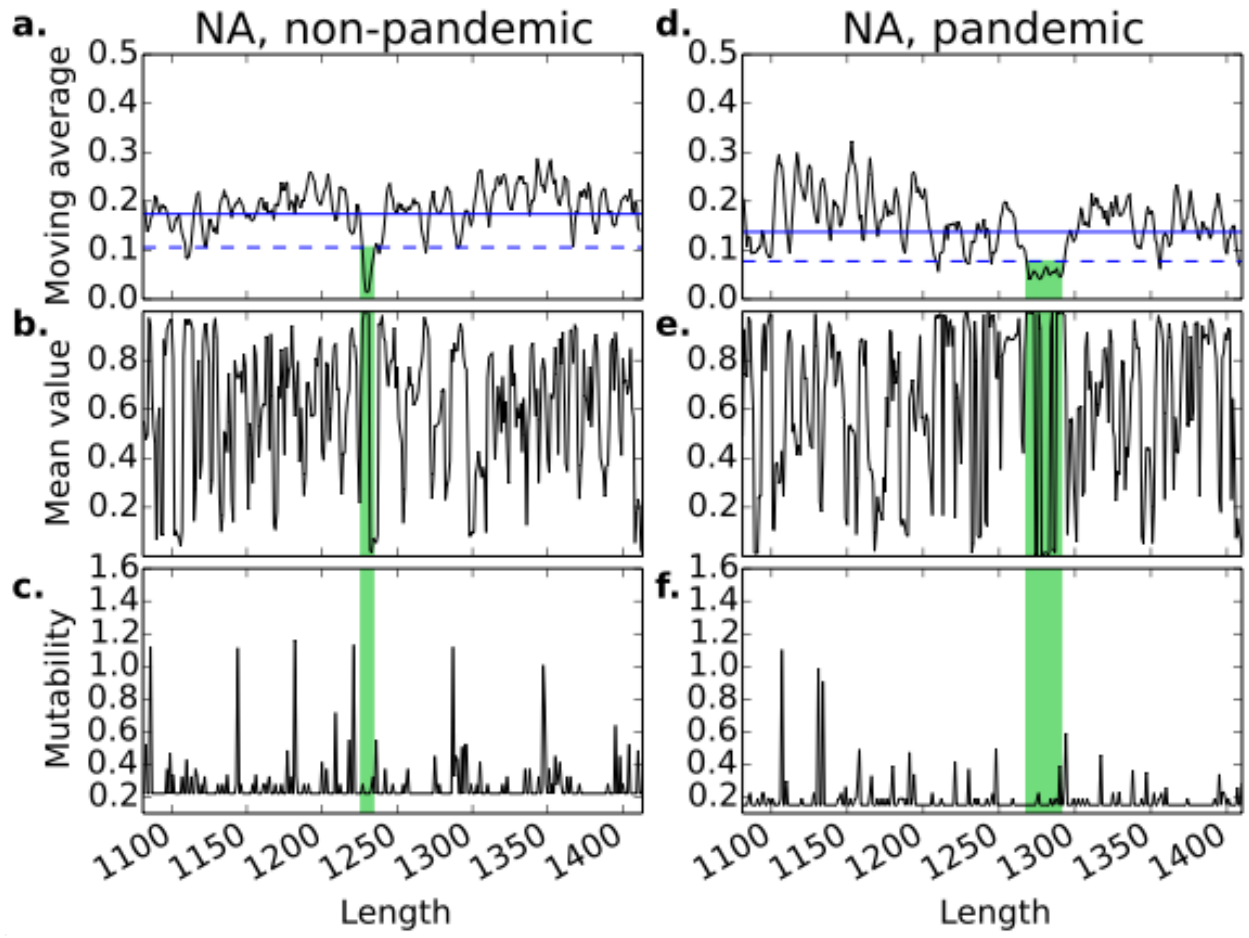


Supplementary Figure 32

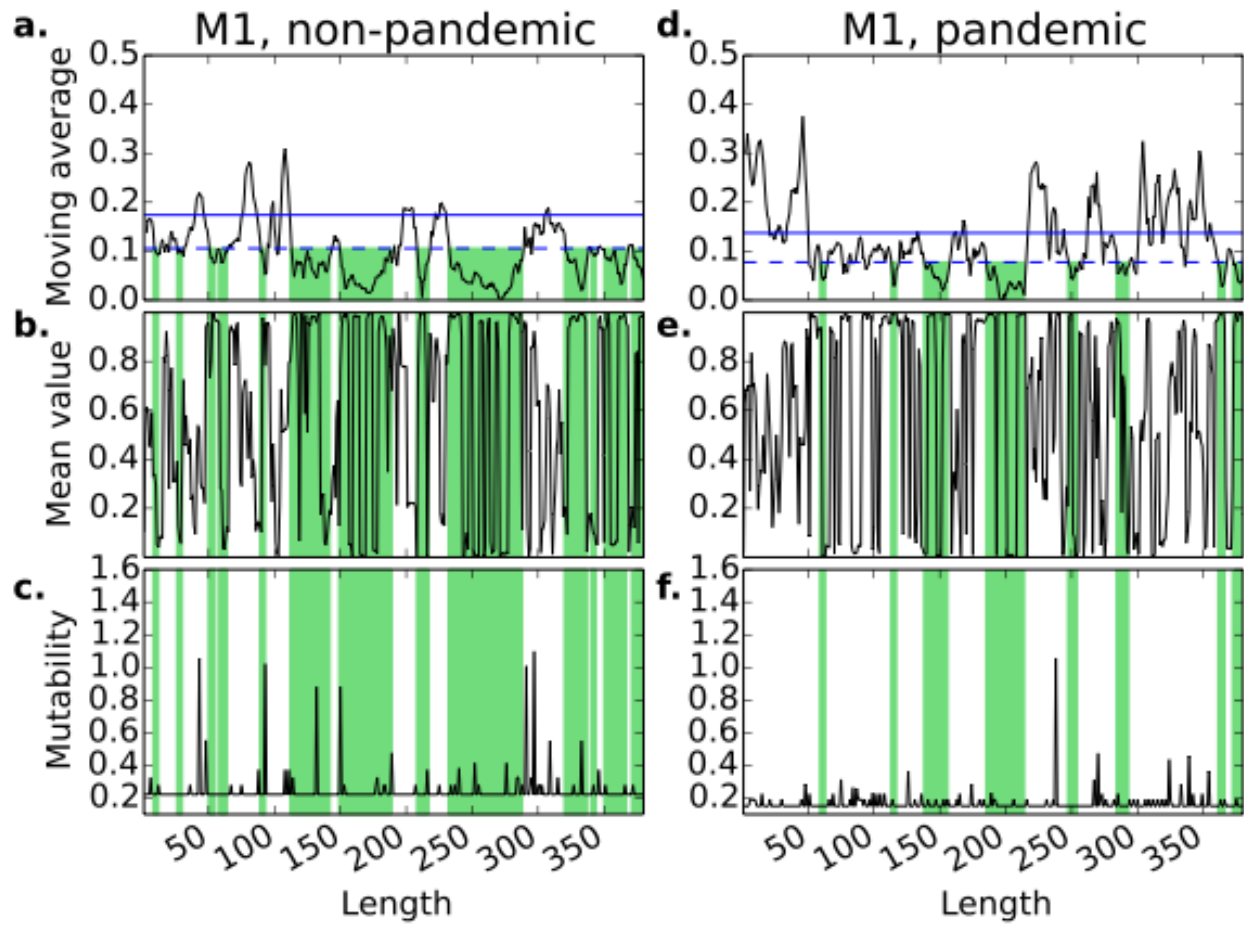




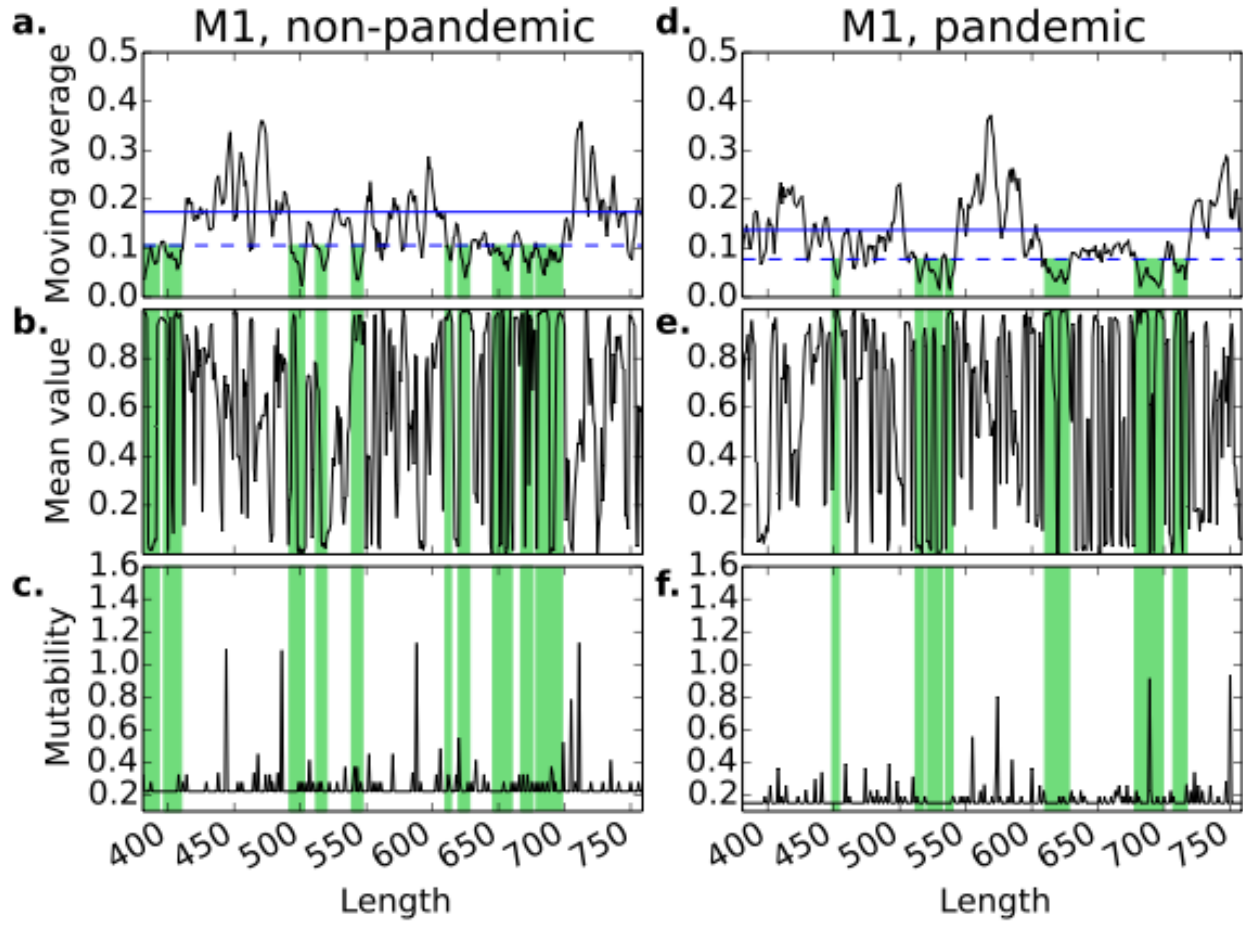
Supplementary Figure 33



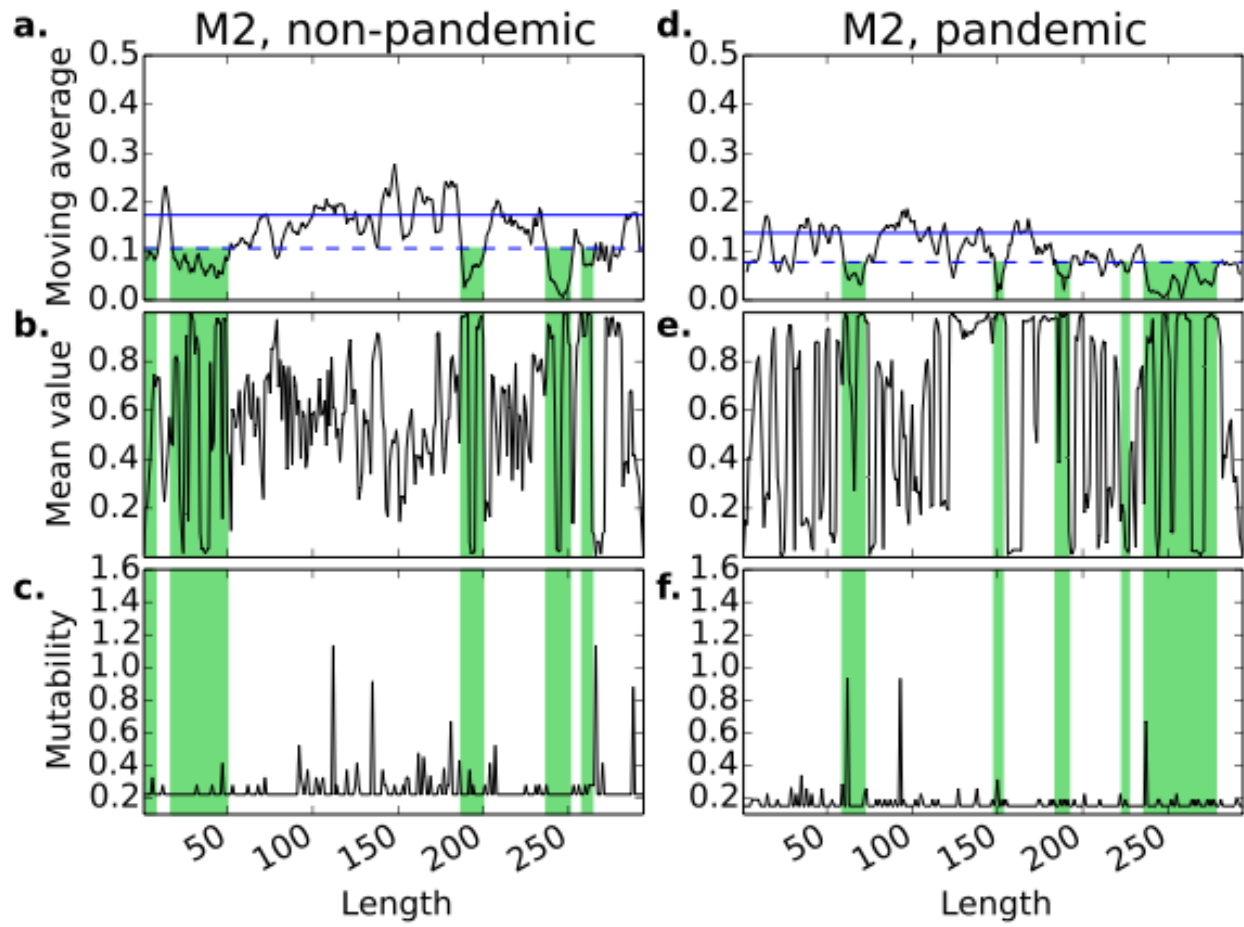
Supplementary Figure 34



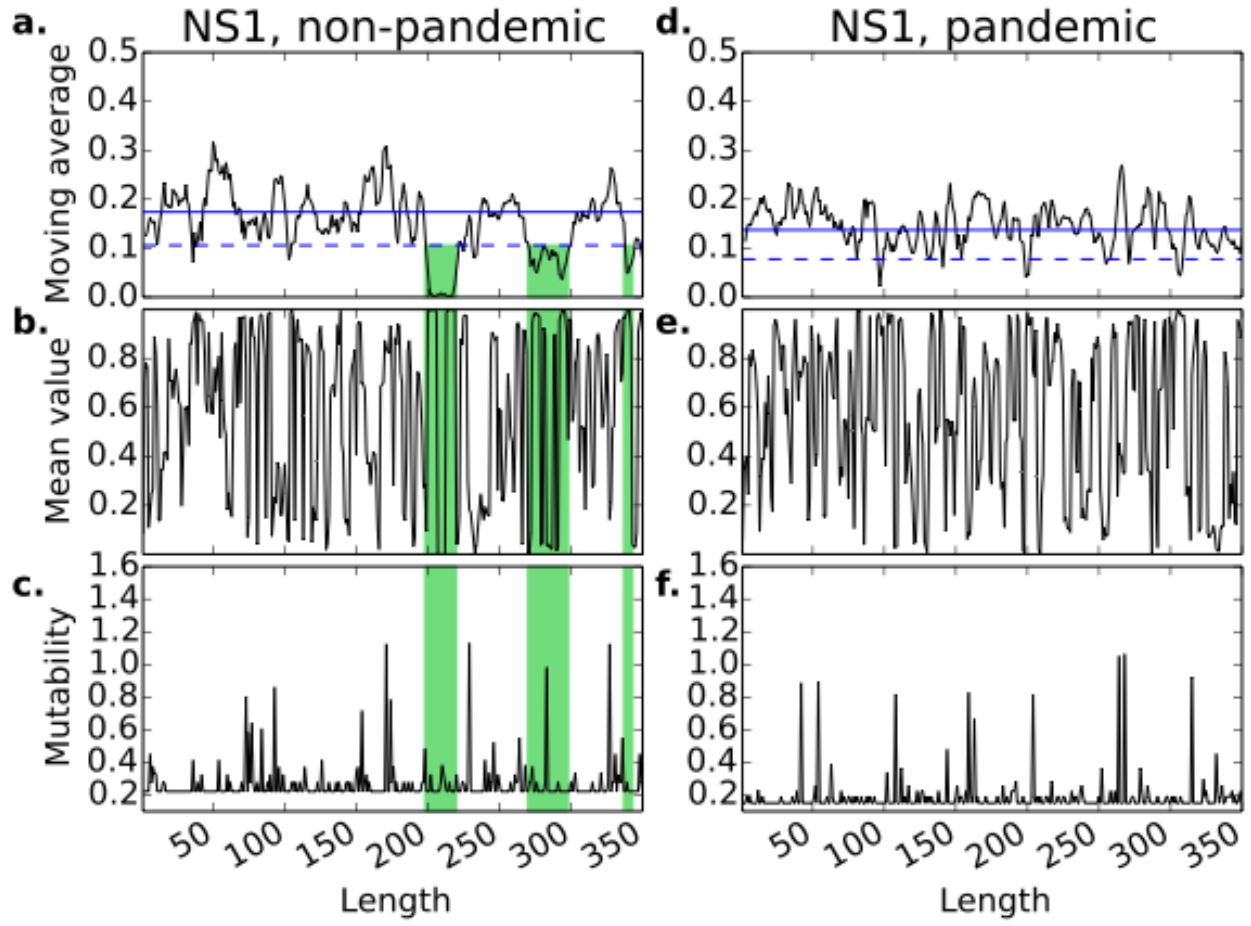
Supplementary Figure 35



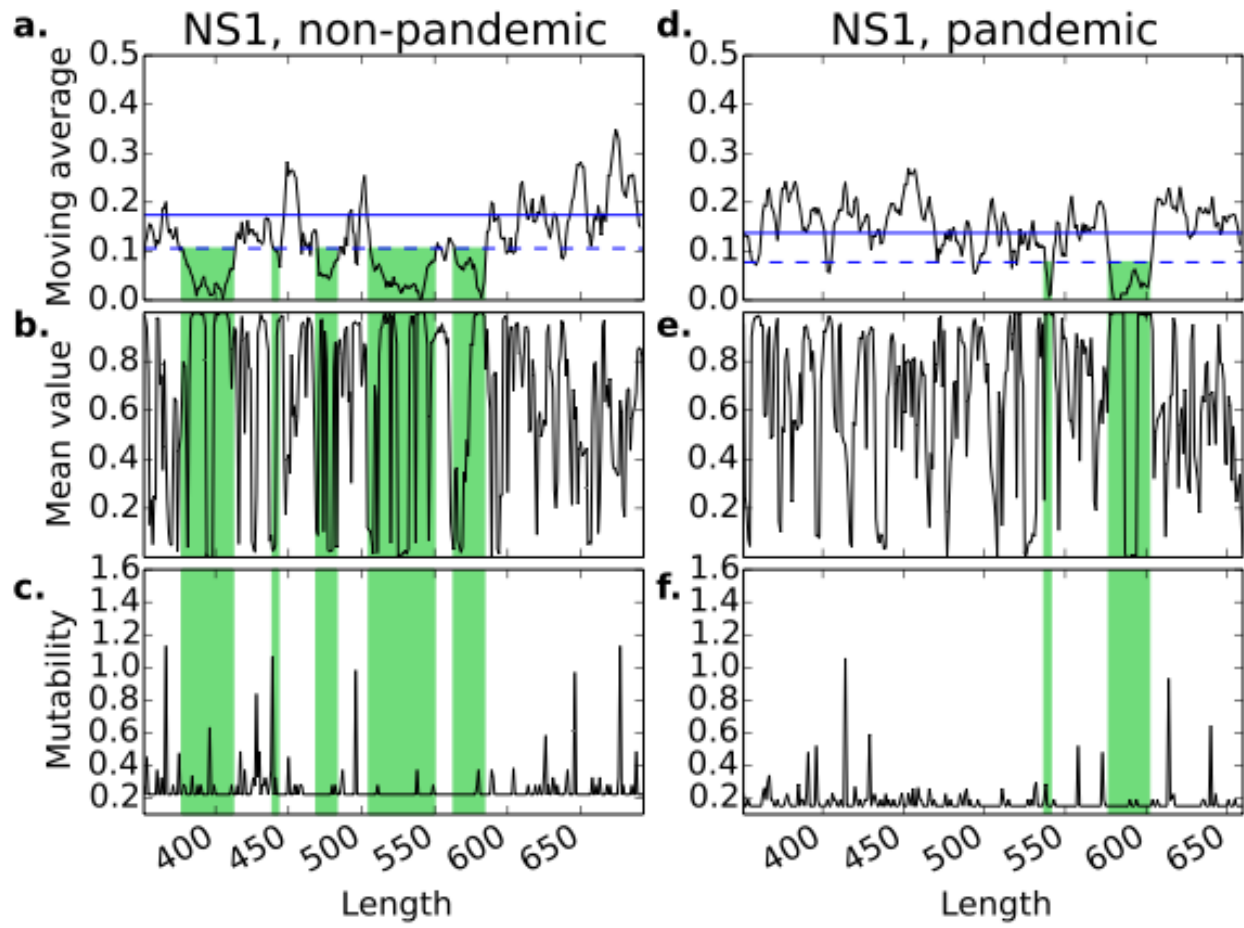
Supplementary Figure 36



Supplementary Figure 37

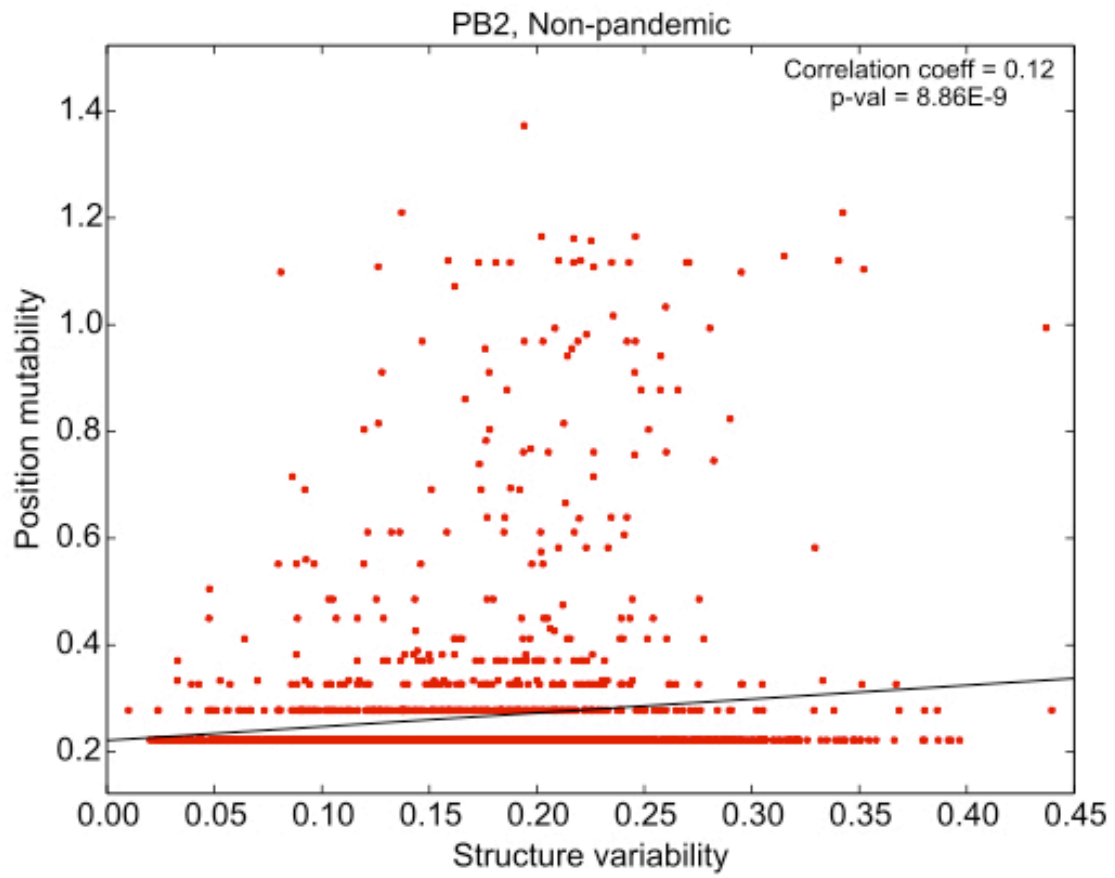


Supplementary Figure 38



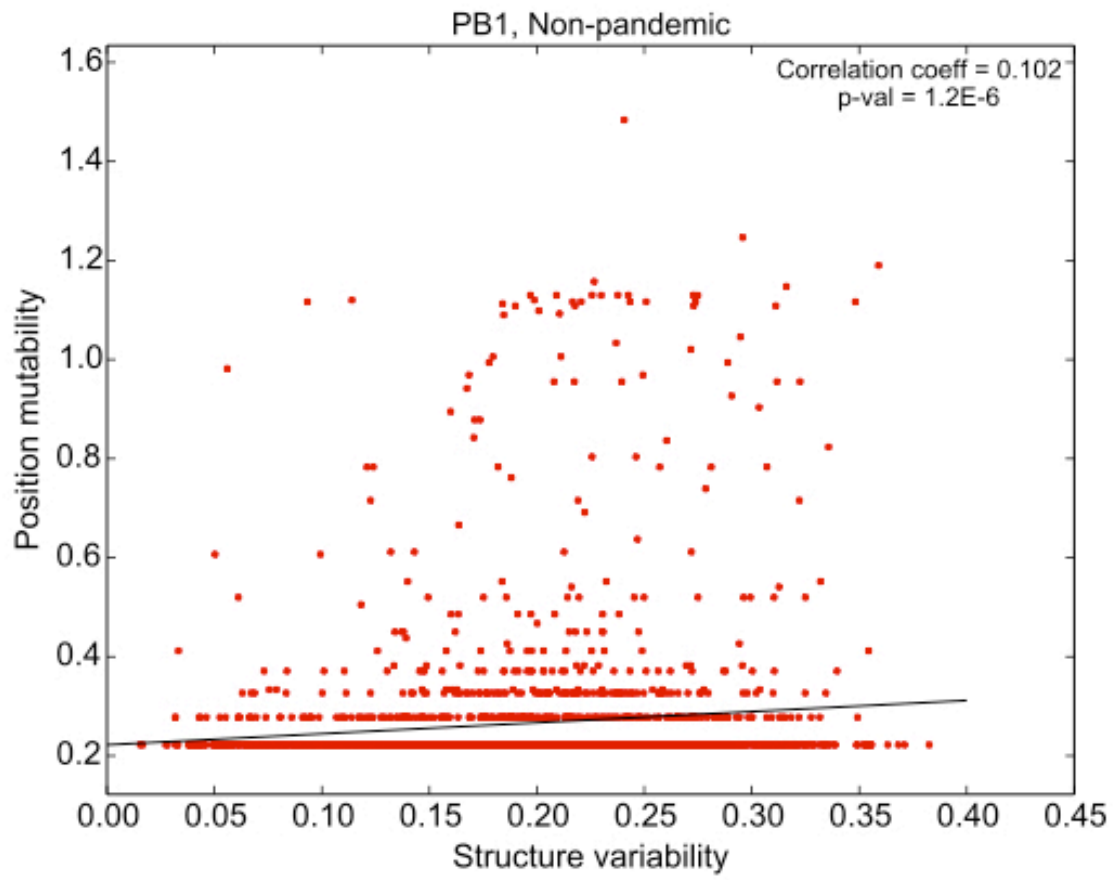
**Supplementary Figures 39-58:** Associations between mutability value for every nucleotide position (position mutability) and corresponding value of moving average of individual standard deviations of the probabilities of nucleotides to be paired (structure variability). X represents structure variability, while Y represents position mutability. The observed correlations are very low, which demonstrates the absence of relationship between these parameters.

Supplementary Figure 39

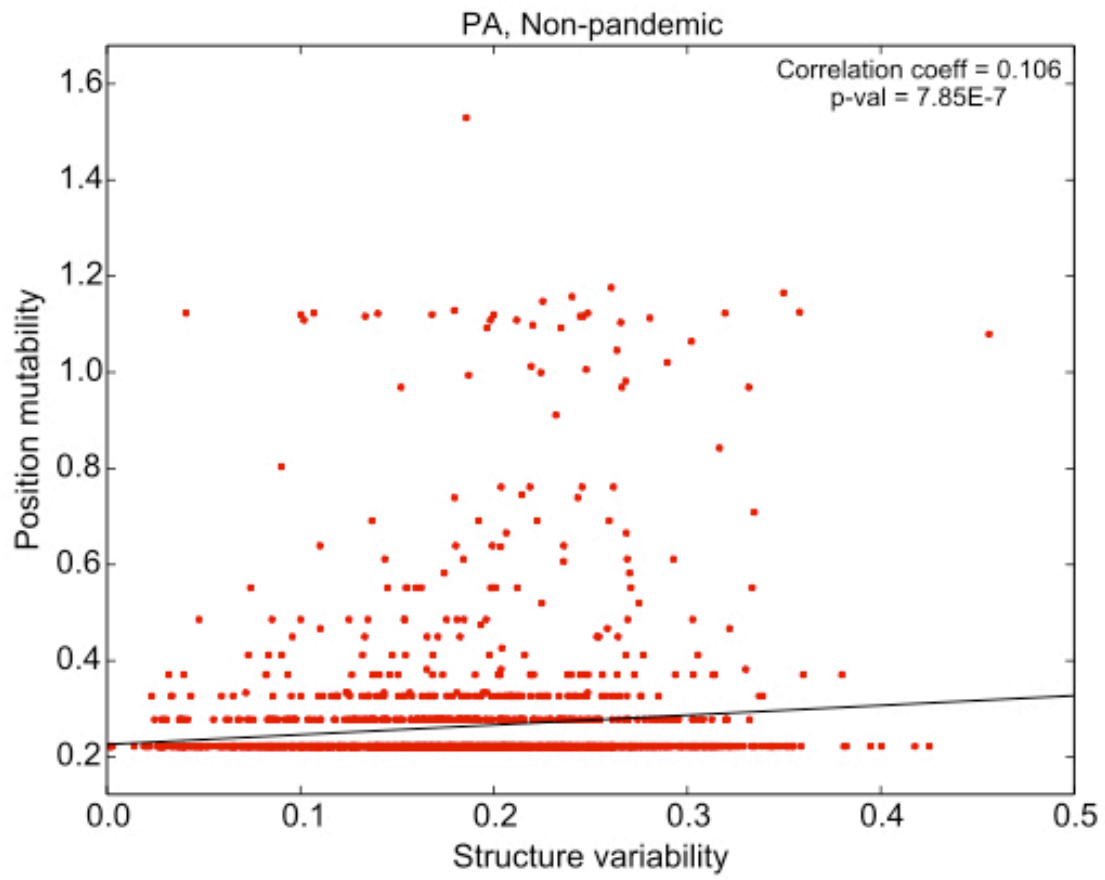




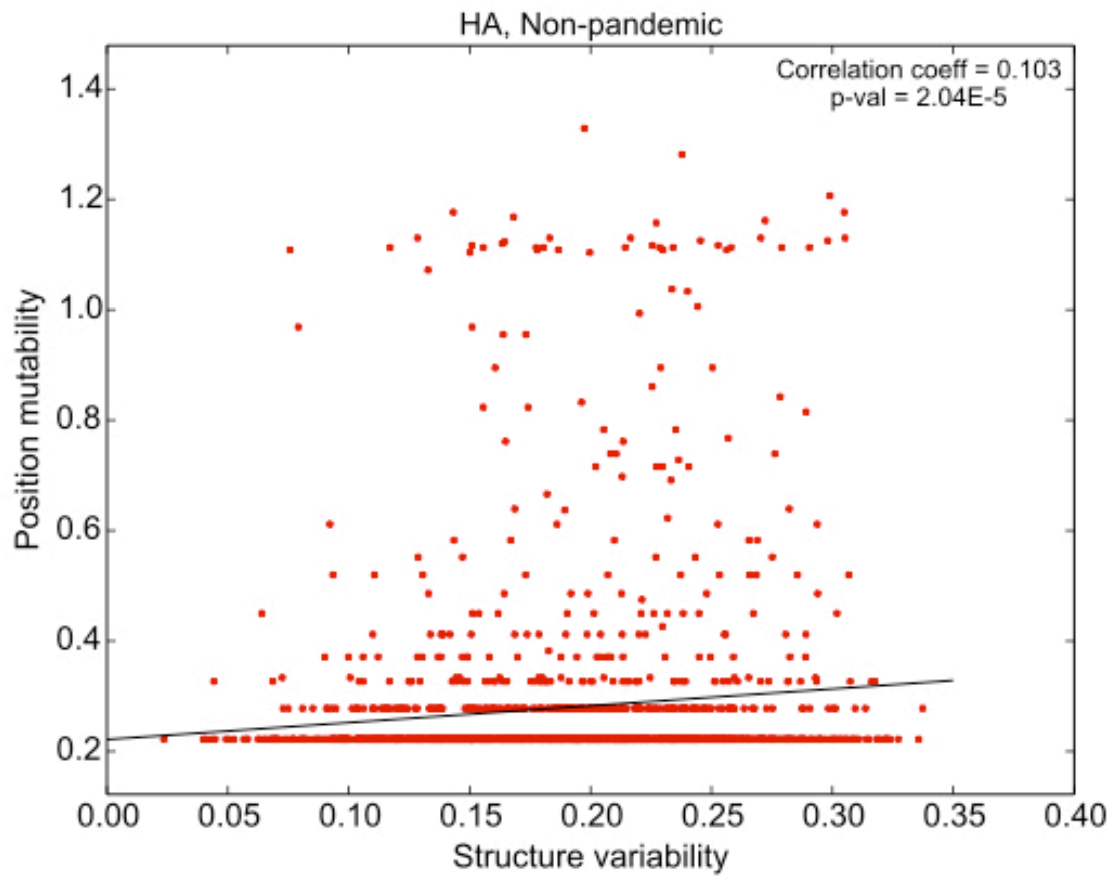
Supplementary Figure 40



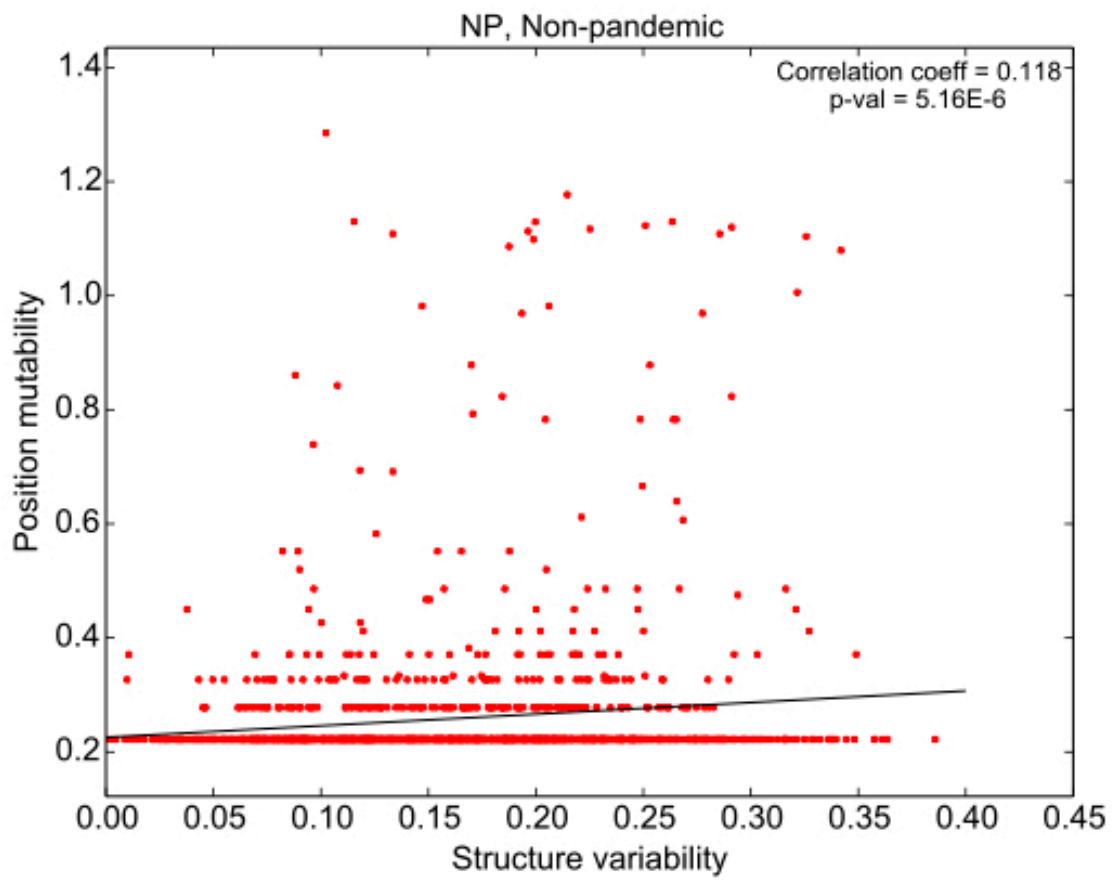
Supplementary Figure 41



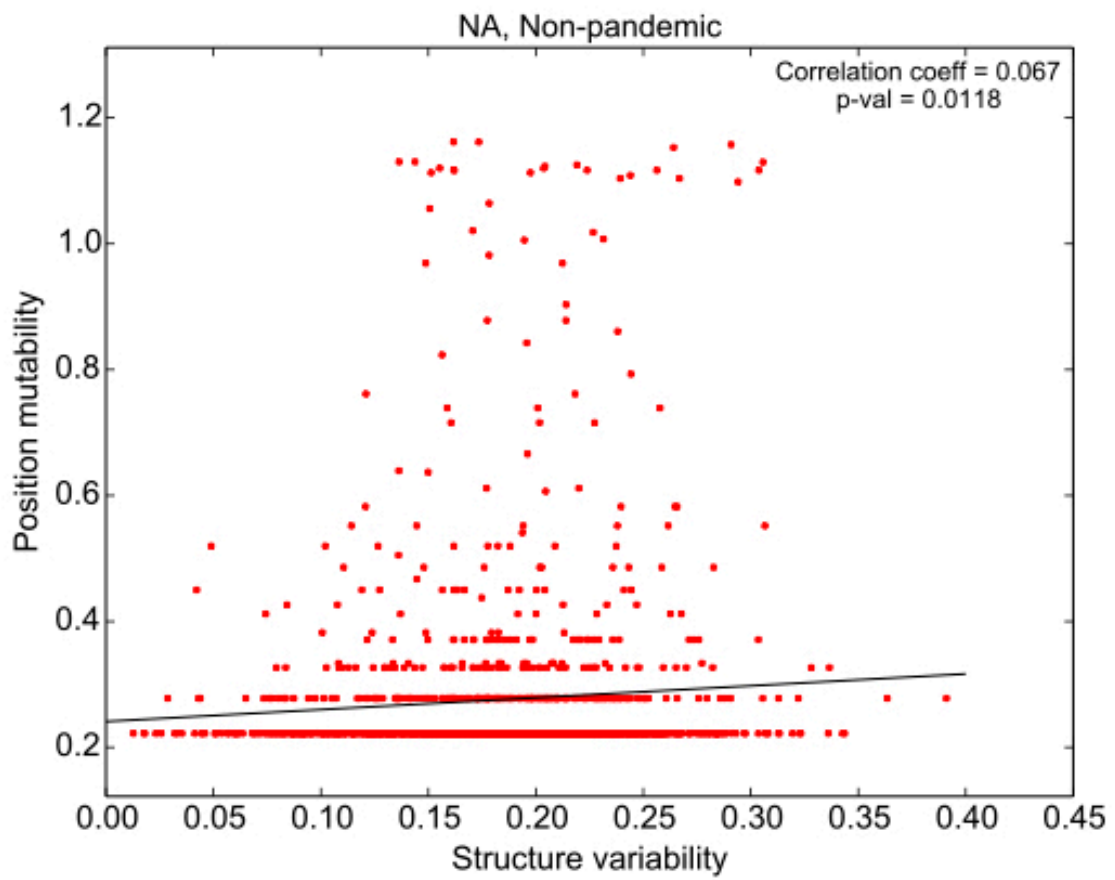
Supplementary Figure 42



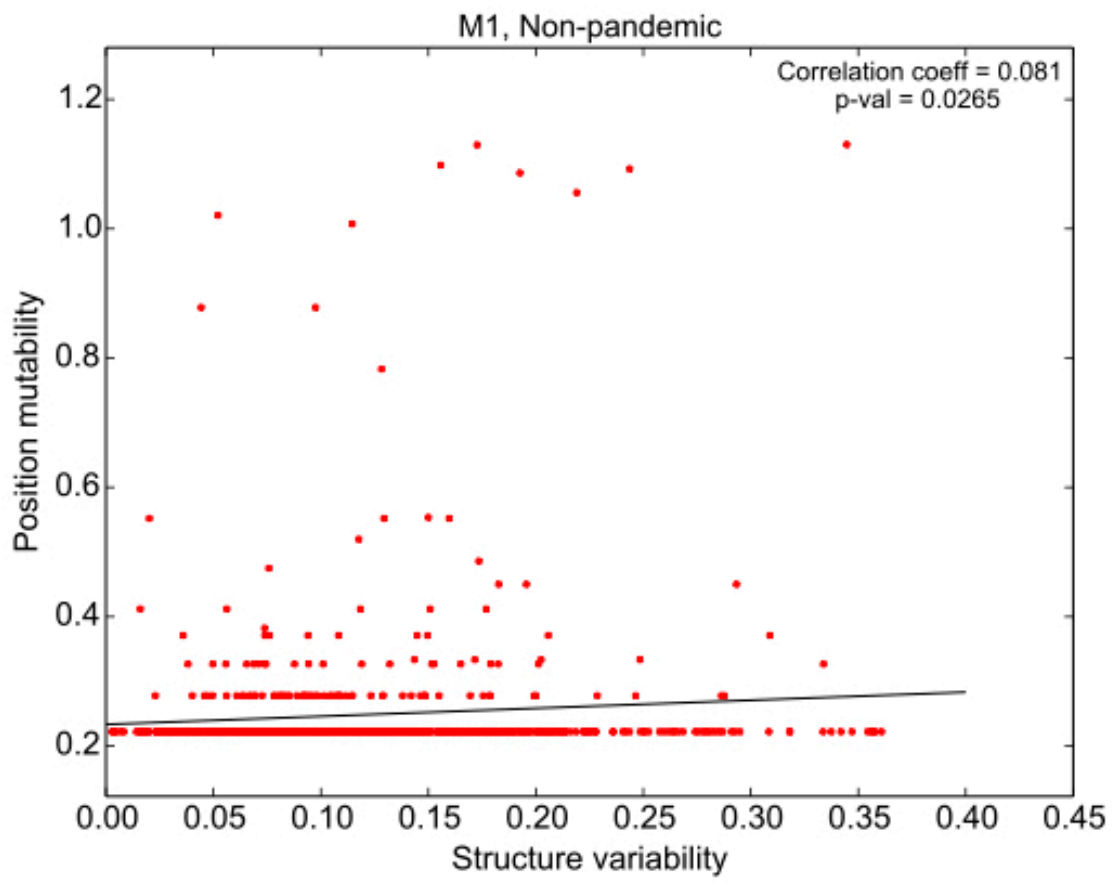
Supplementary Figure 43



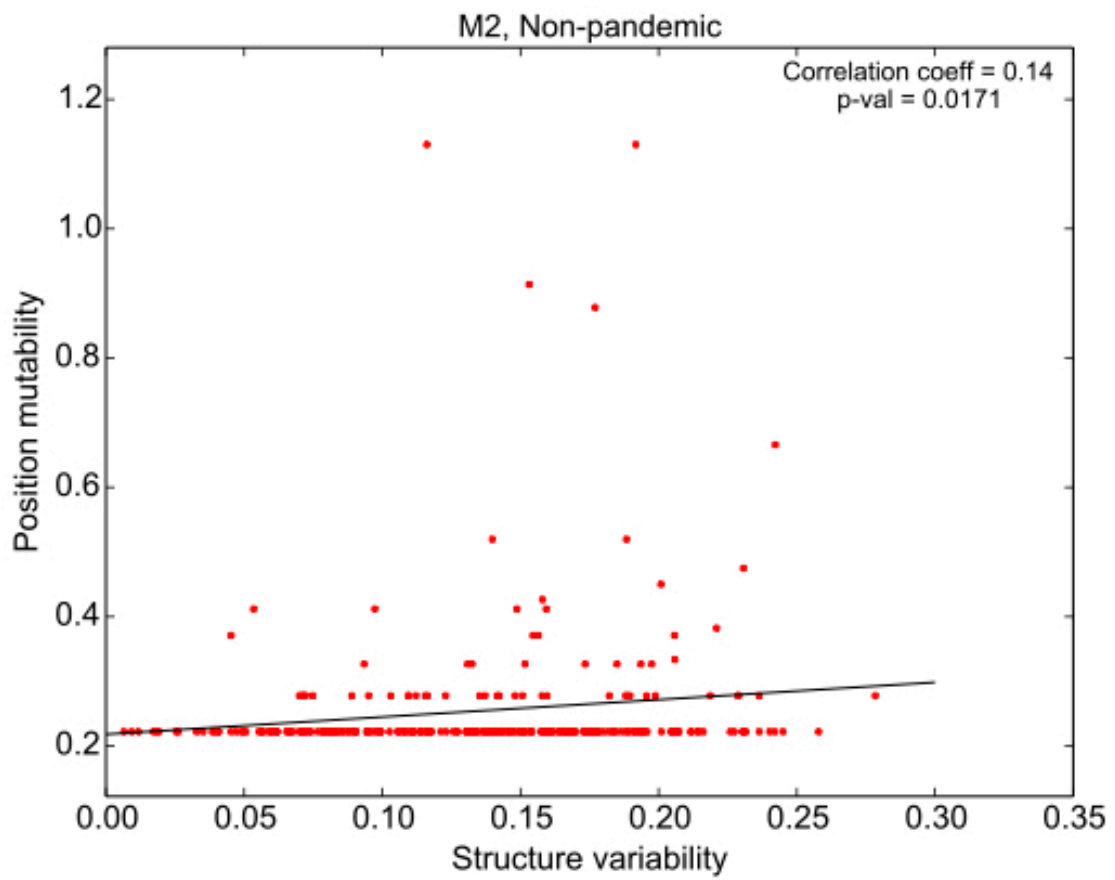
Supplementary Figure 44



Supplementary Figure 45



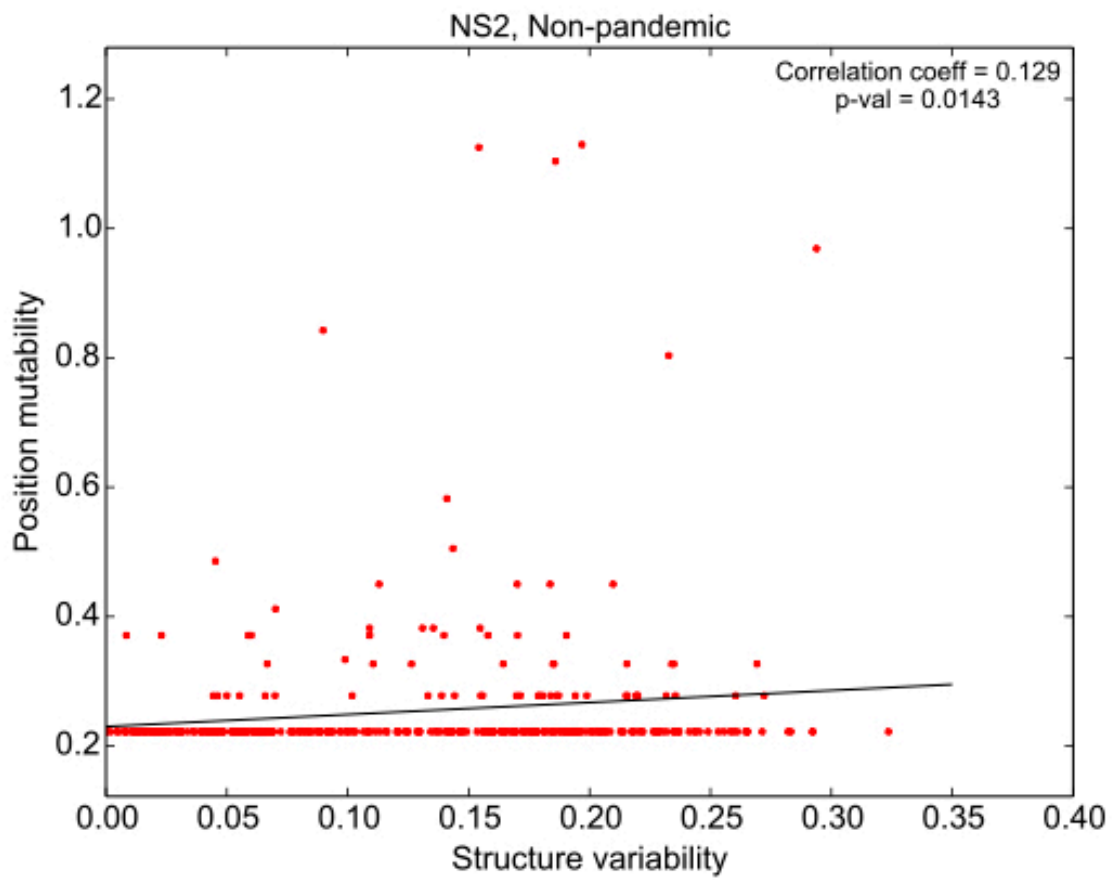
Supplementary Figure 46



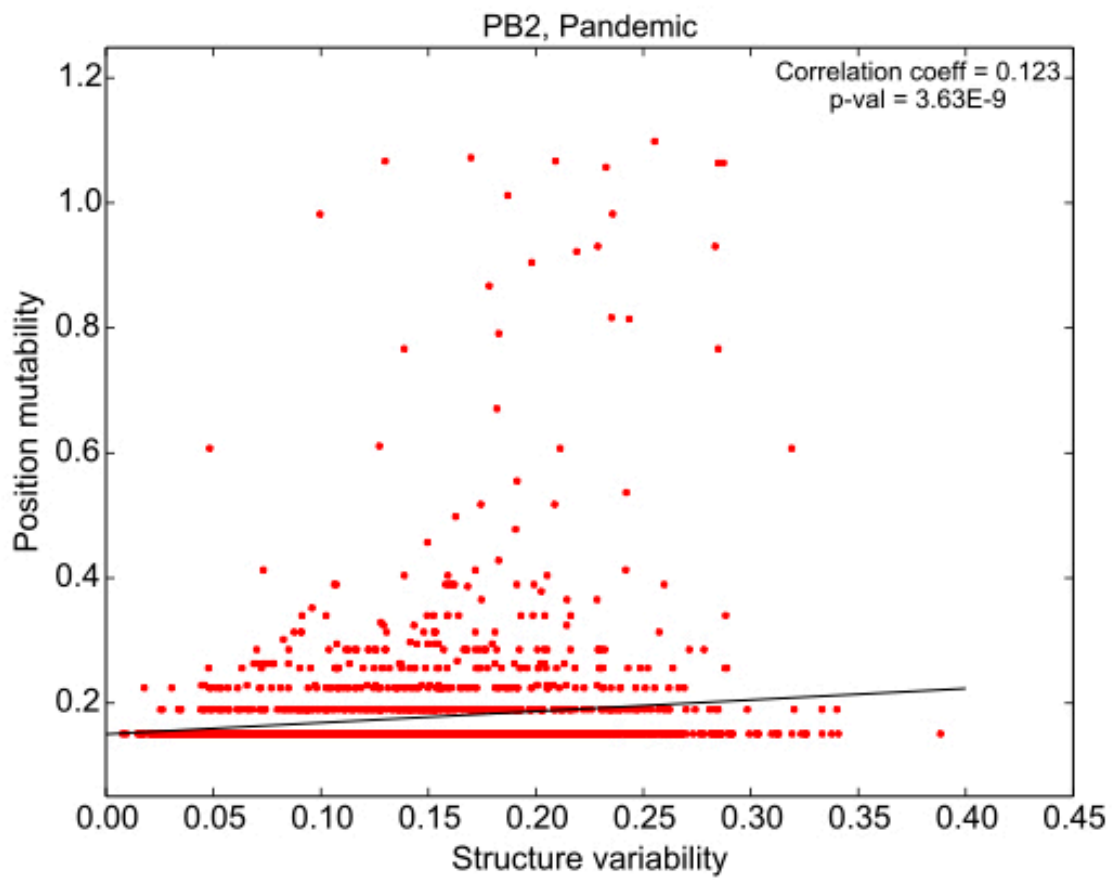




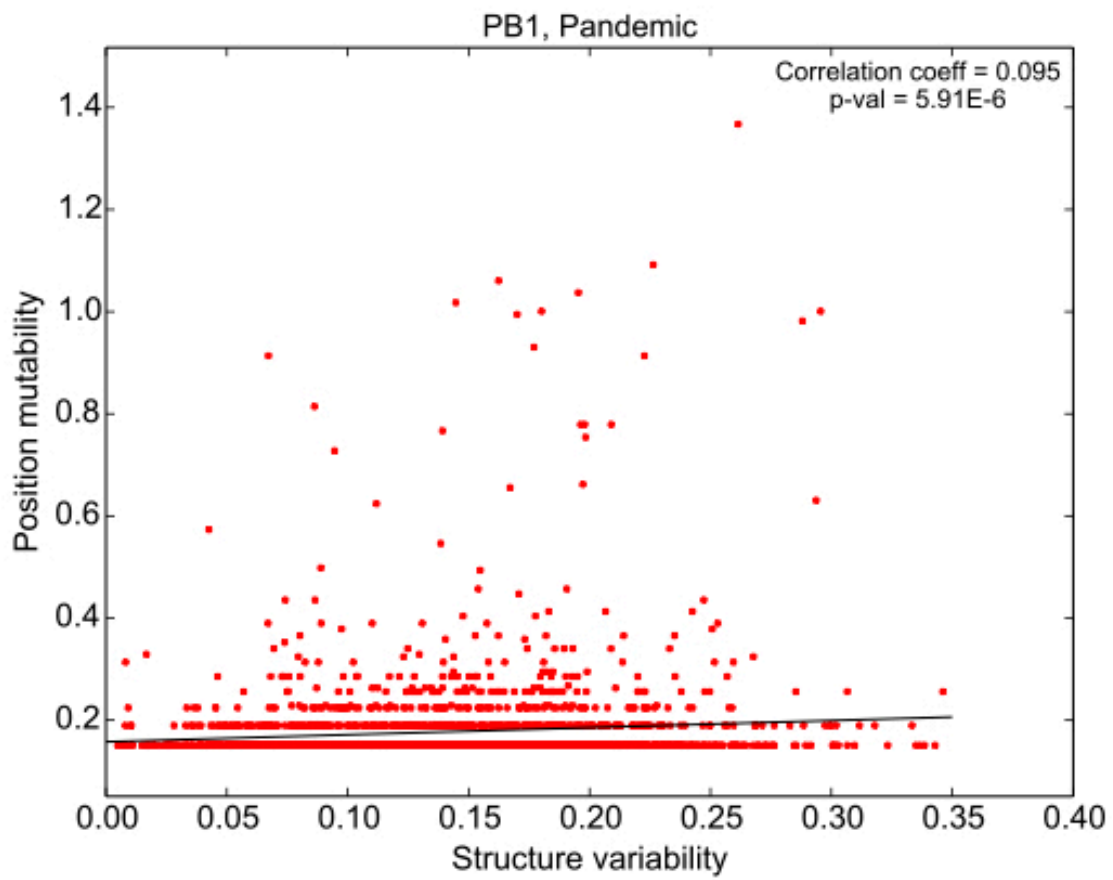
Supplementary Figure 48



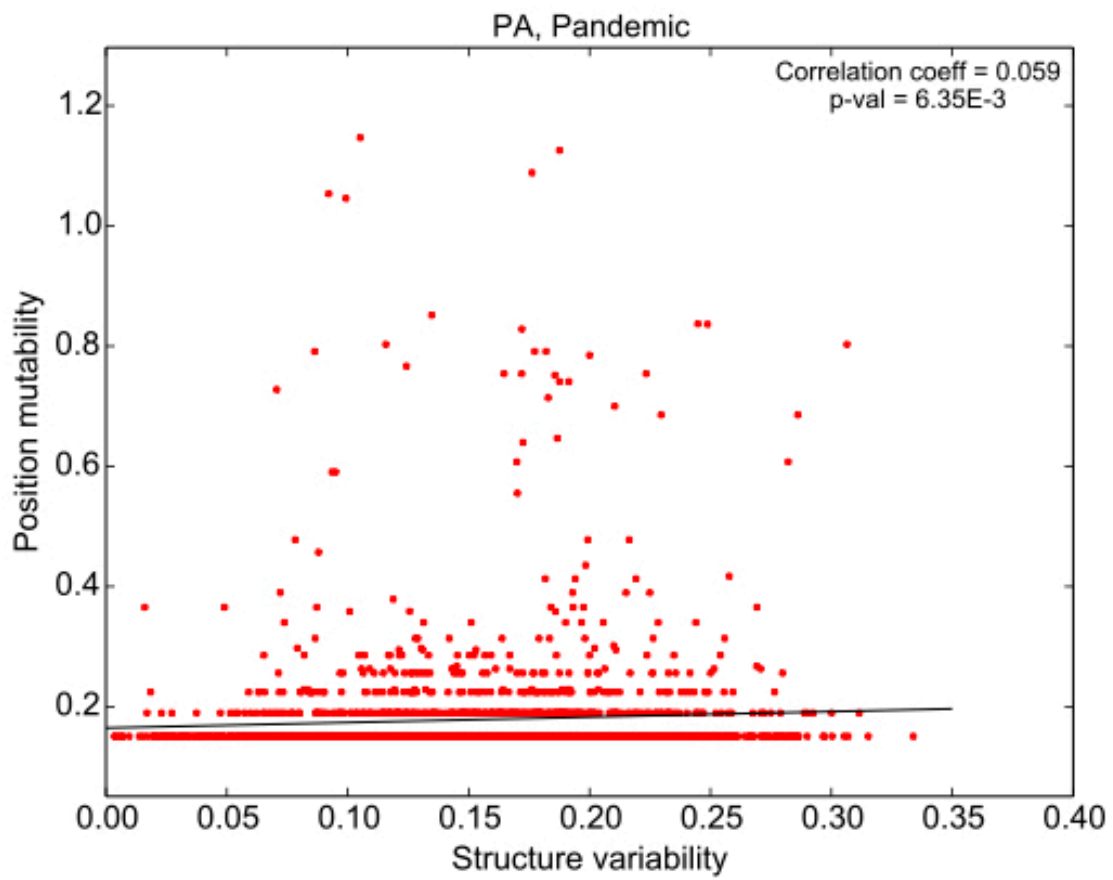
Supplementary Figure 49



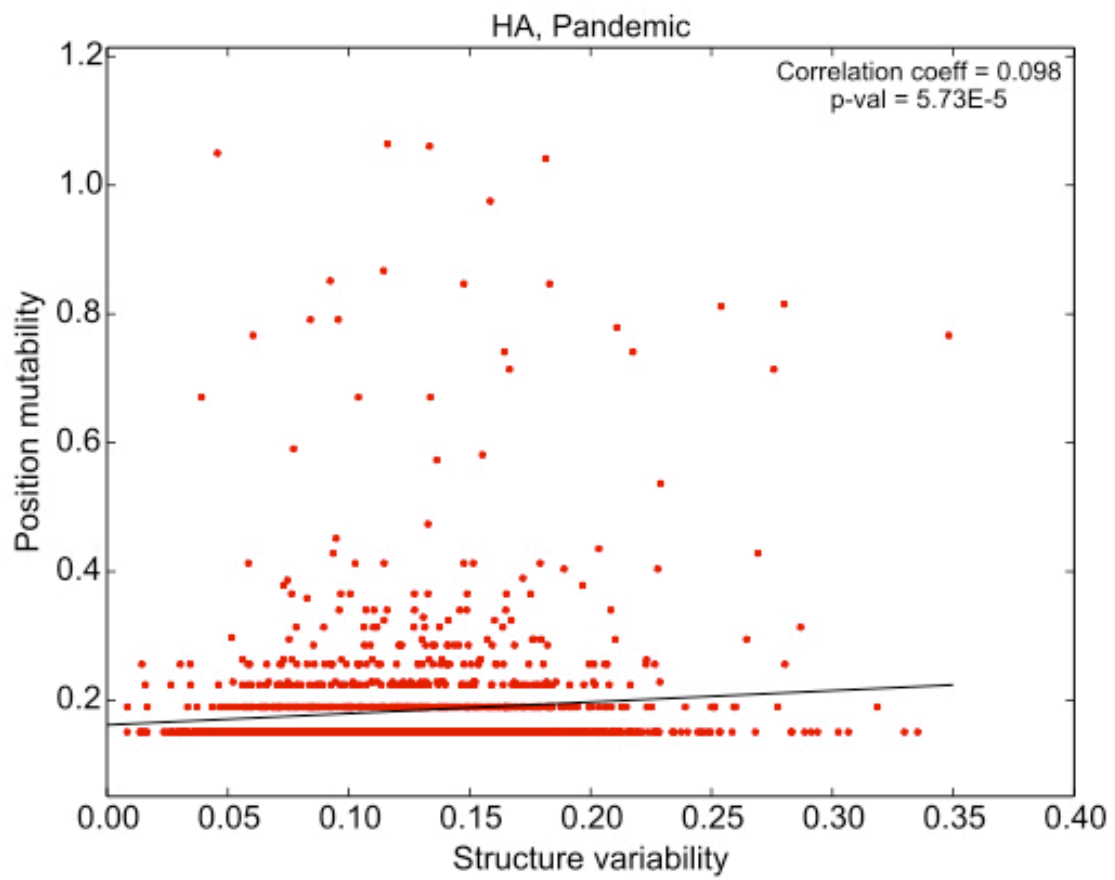
Supplementary Figure 50



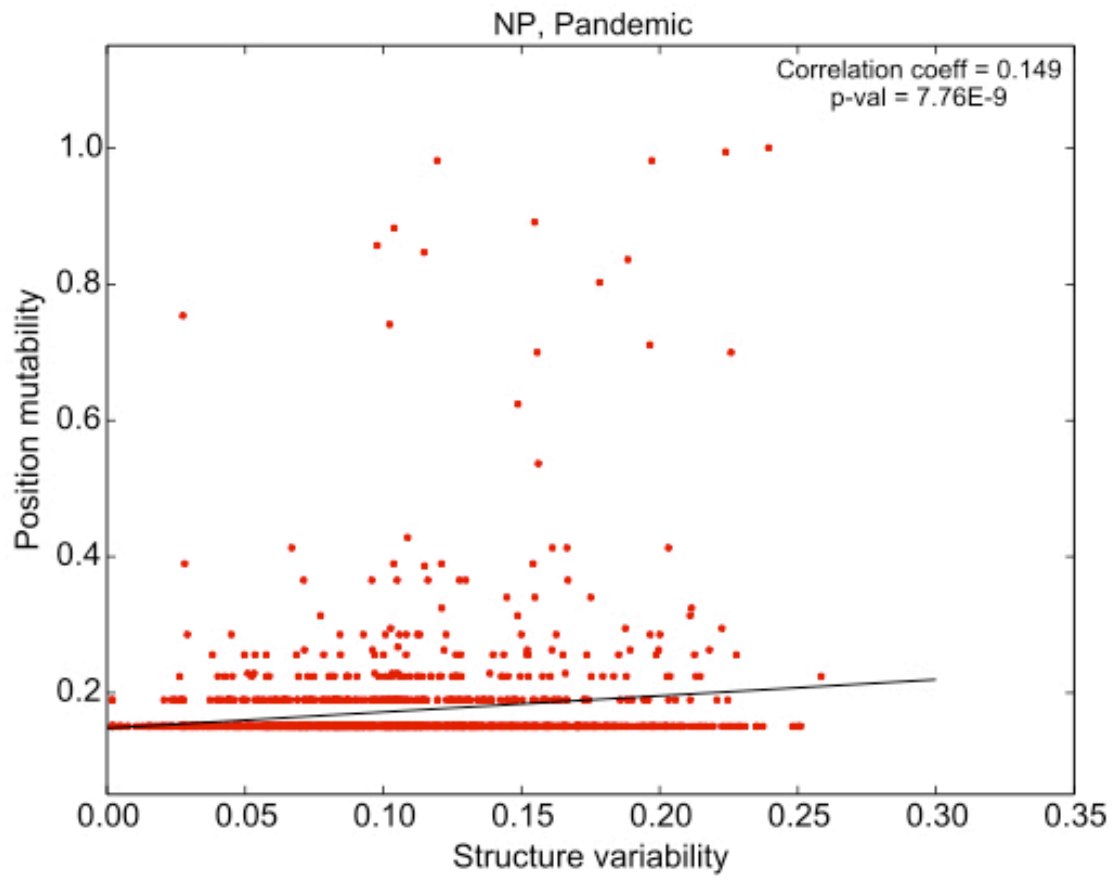
Supplementary Figure 51



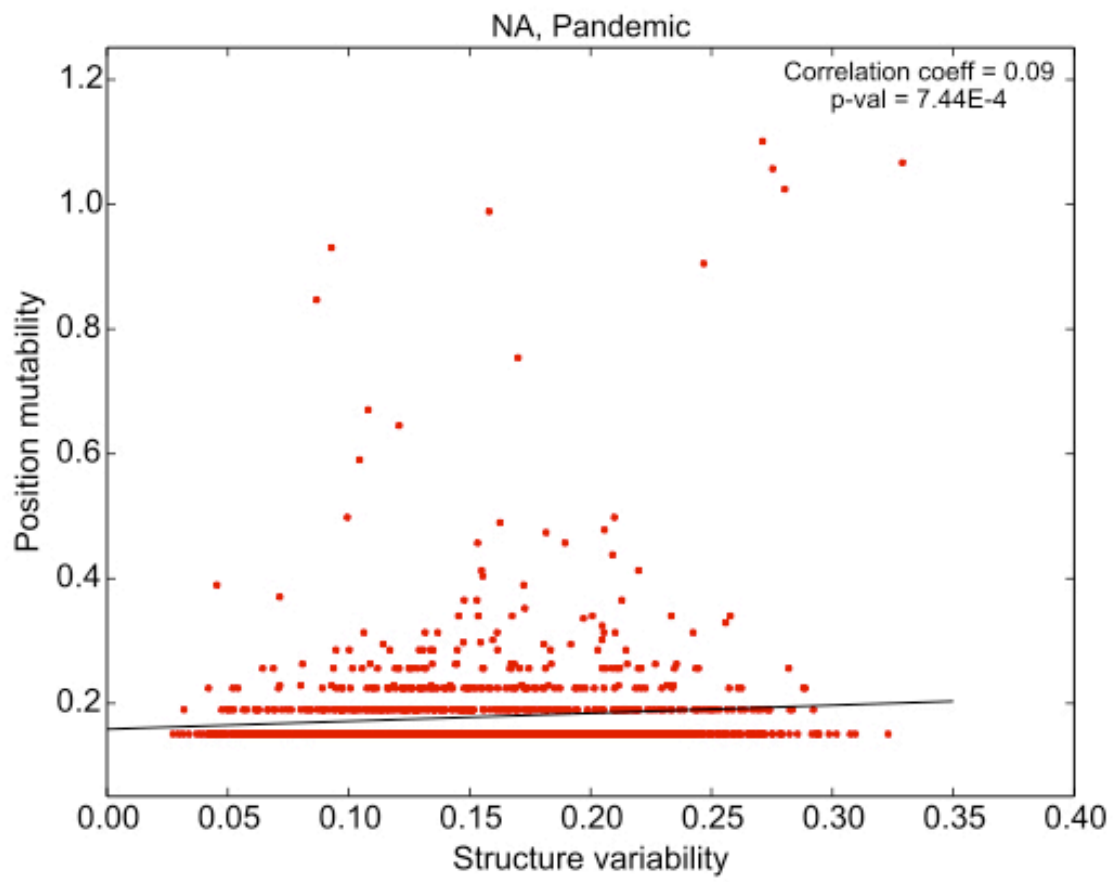
Supplementary Figure 52



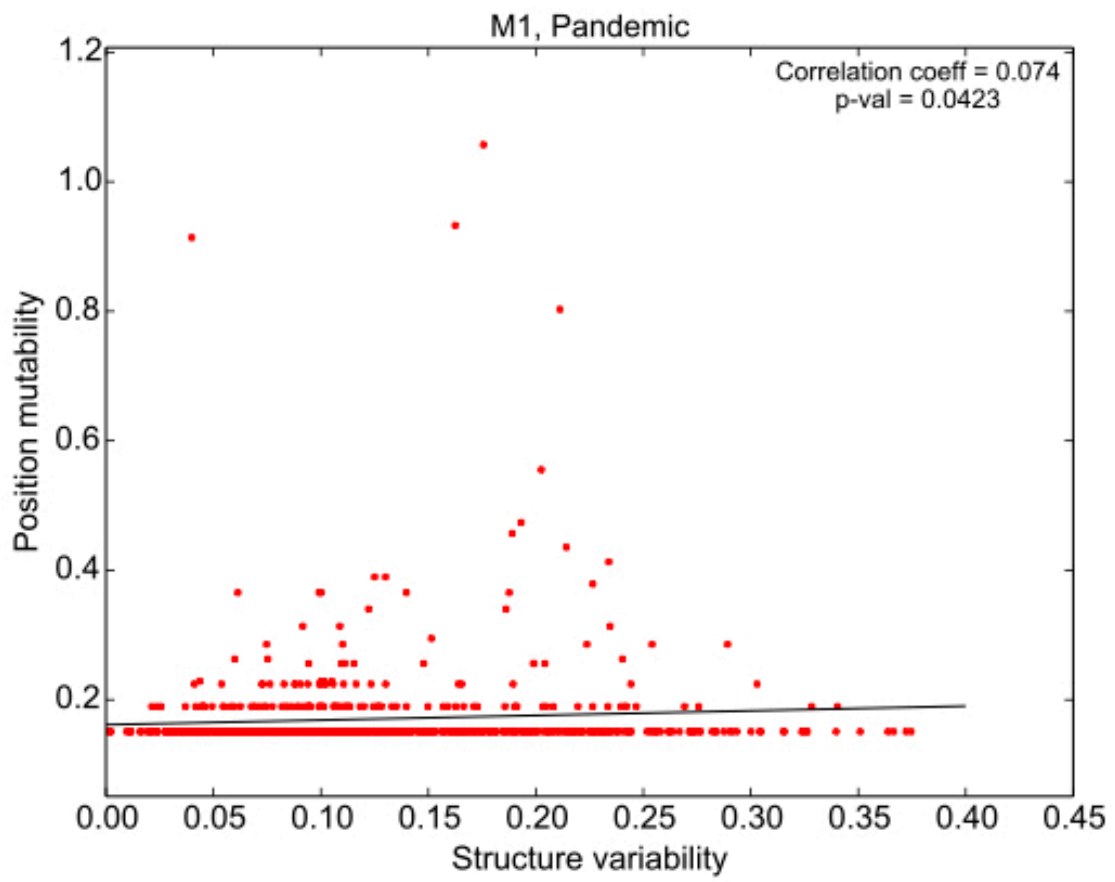
Supplementary Figure 53



Supplementary Figure 54

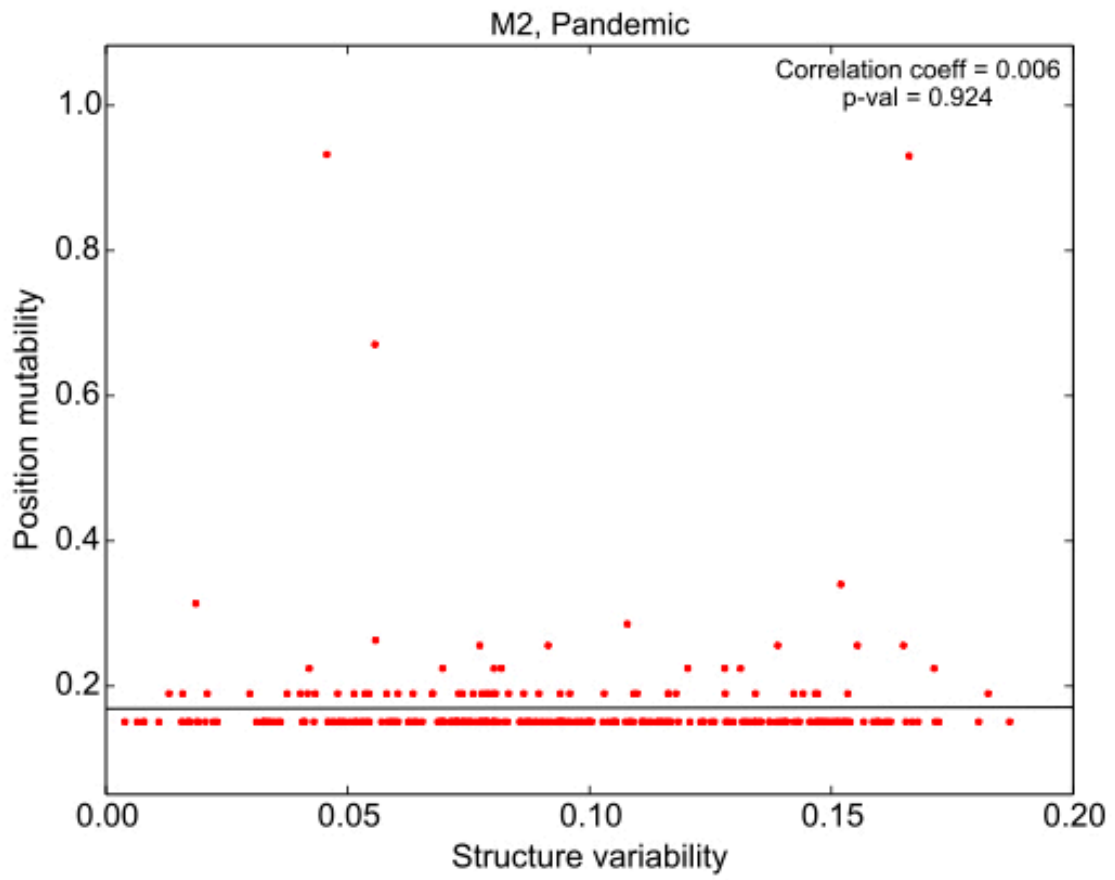


Supplementary Figure 55

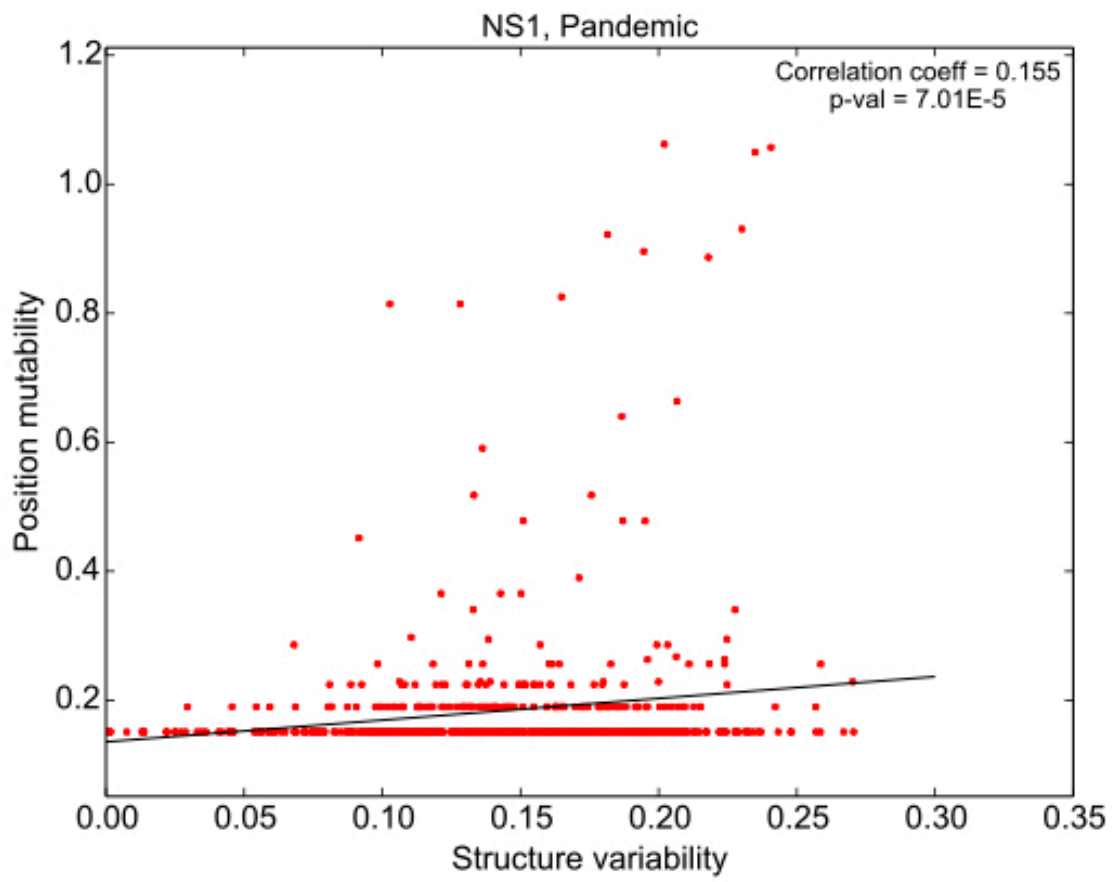




Supplementary Figure 56



Supplementary Figure 57



Supplementary Figure 58

