

SUPPLEMENTAL INFORMATION

Resurfaced ZIKV EDIII nanoparticle immunogens elicit immune-focused protective responses *in vivo*

George I. Georgiev¹, Ryan J. Malonis¹, Ariel S. Wirchnianski^{1,2}, Alex W. Wessel³, Helen S. Jung¹, Sean M. Cahill¹, Elisabeth K. Nyakatura^{1,6}, Olivia Vergnolle^{1,6}, Kimberly A. Dowd⁷, David Cowburn¹, Theodore C. Pierson⁷, Michael S. Diamond^{3,4,5}, Jonathan R. Lai^{1,8,*}

¹Department of Biochemistry, New York, NY 10461, USA

²Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY 10461, USA

³Department of Medicine, Washington University in St. Louis, School of Medicine, St. Louis, MO 63110, USA

⁴Department of Molecular Microbiology, Washington University in St. Louis, School of Medicine, St. Louis, MO 63110, USA

⁵Department of Pathology & Immunology, Washington University in St. Louis, School of Medicine, St. Louis, MO 63110, USA

⁶Present address: Tri-Institutional Therapeutics Discovery Institute, New York, NY 10021, USA

⁷Viral Pathogenesis Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

⁸Lead Contact

*Correspondence: jon.lai@einsteinmed.edu

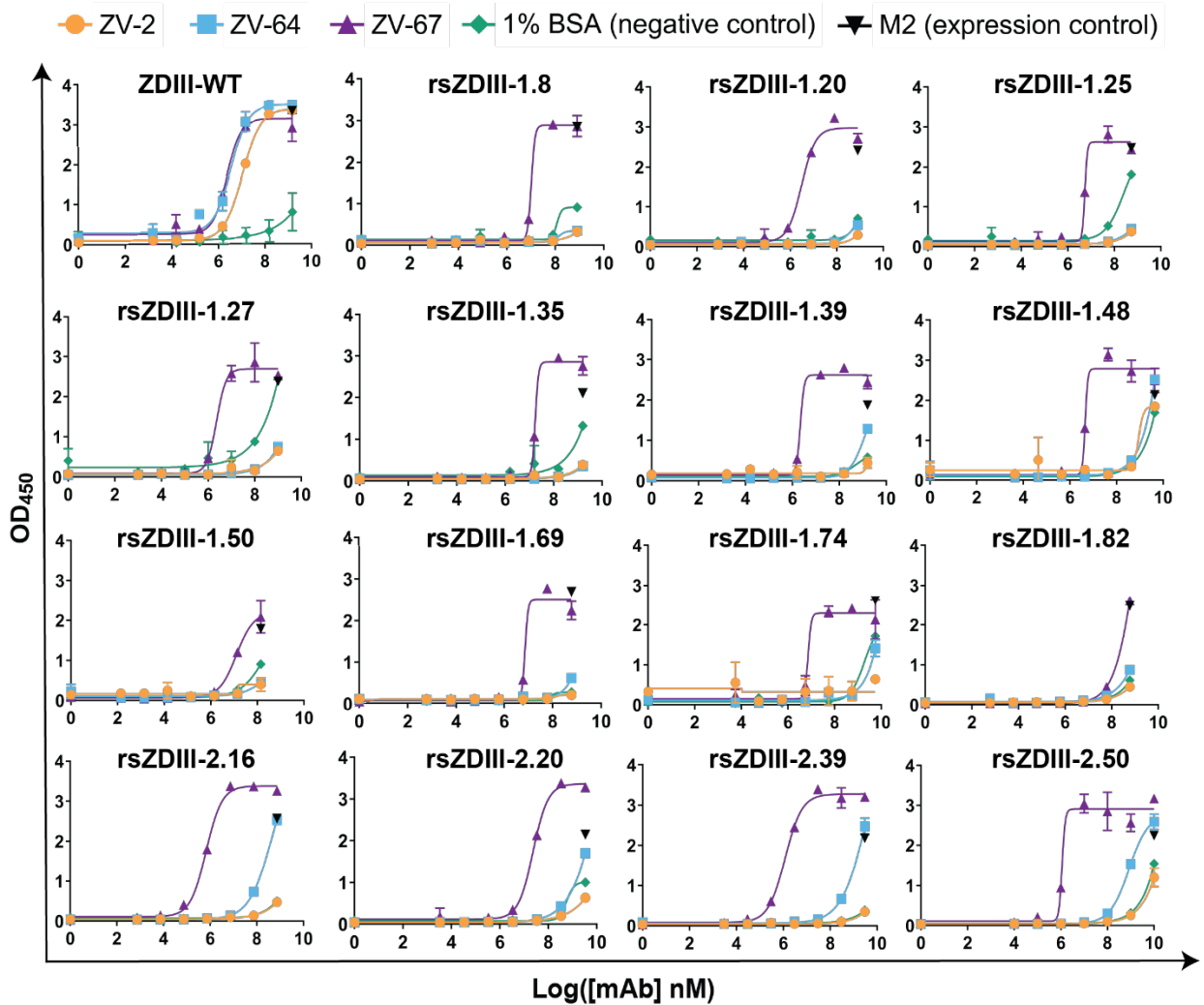


Figure S1, related to Figure 1. rsZDIII phage clone reactivity toward ZV-mAbs (ZV-2, ZV-64, and ZV-67) determined by ELISA. Data from a single experiment completed in duplicate are plotted as (mean OD₄₅₀) ± SD.

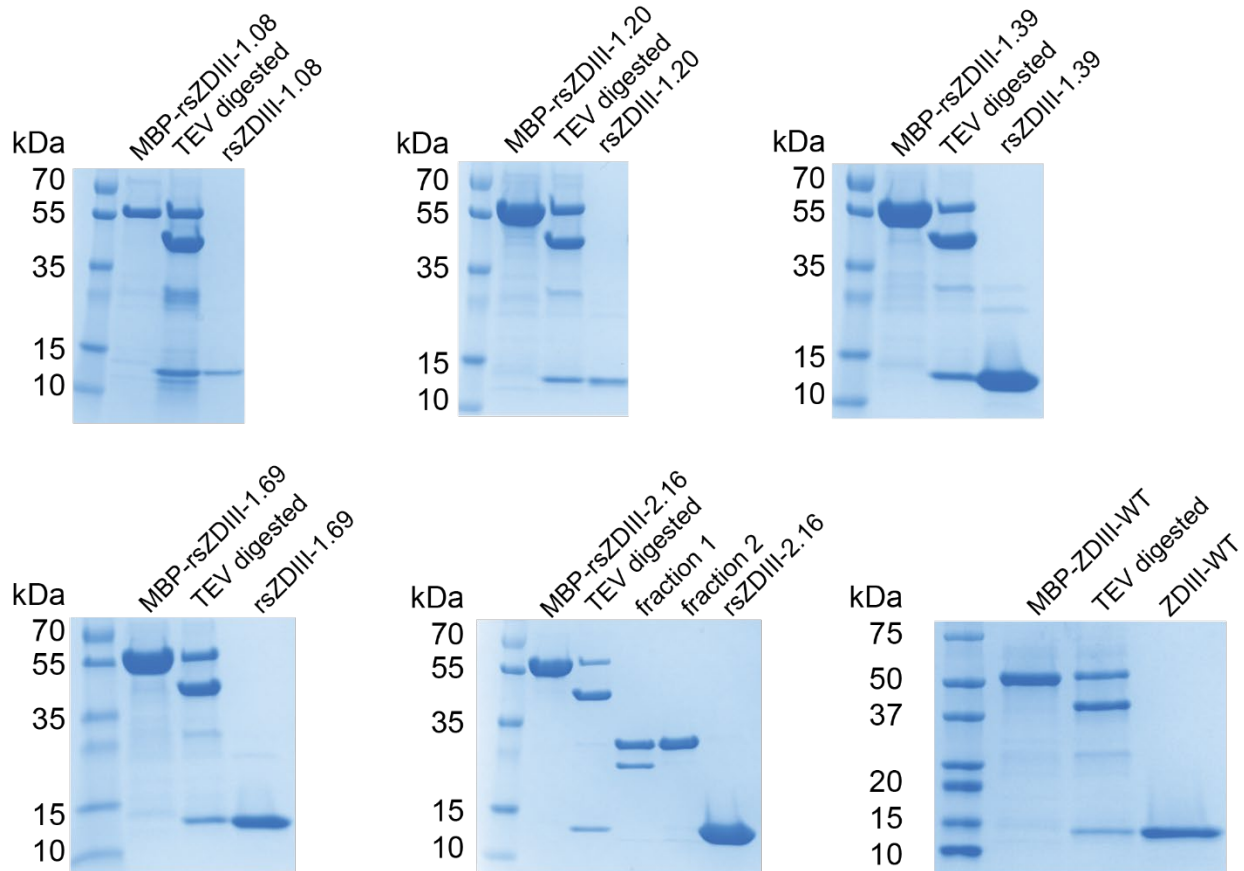


Figure S2, related to Figure 2. Coomassie blue-stained gels for recombinant expressed rsZDIII variants following TEV cleavage of MBP-rsZDIII fusion proteins and purification by anion exchange chromatography.

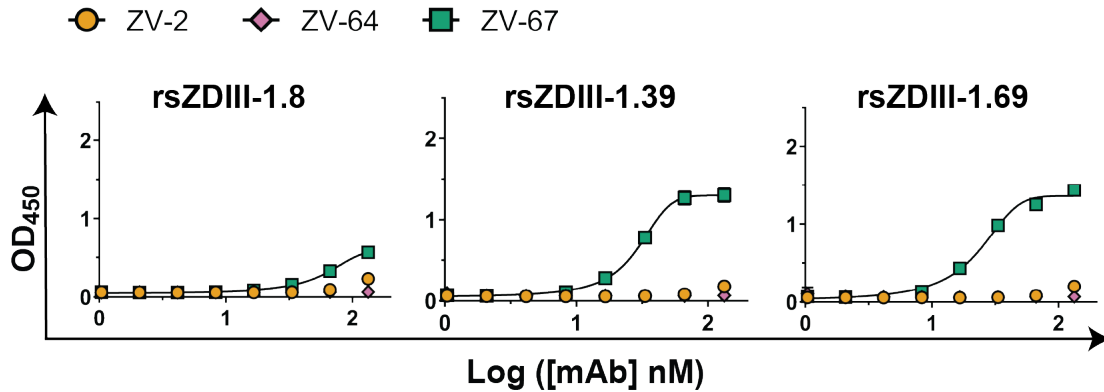
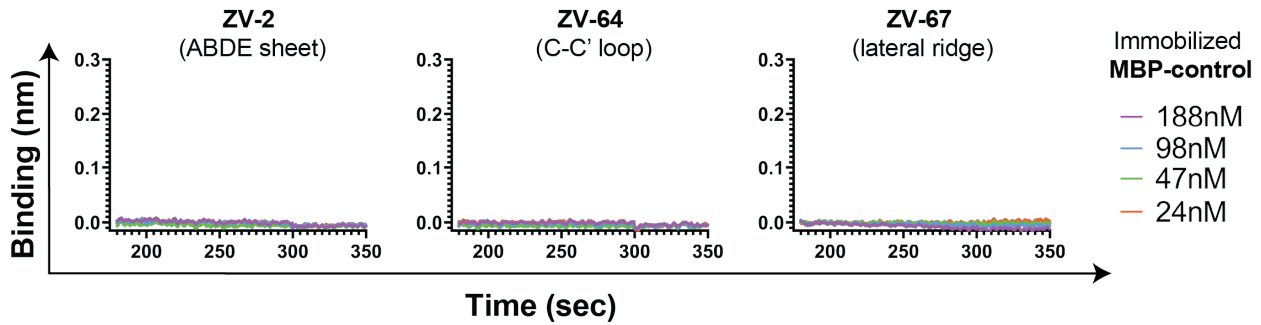
A**B**

Figure S3, related to Figure 2 and STAR methods. Biochemical characterization of rsZDIII variants. (A) Binding reactivity of ZV-mAbs (ZV-2, ZV-64, and ZV-67) to soluble rsZDIII variants determined by ELISA. Representative data from two independent experiments completed in triplicate are plotted as the (mean OD₄₅₀) ± SD. (B) Binding interactions between MBP and ZV-mAbs (ZV-2, ZV-64, and ZV-67) by BLI. Representative data from two independent experiments is shown.

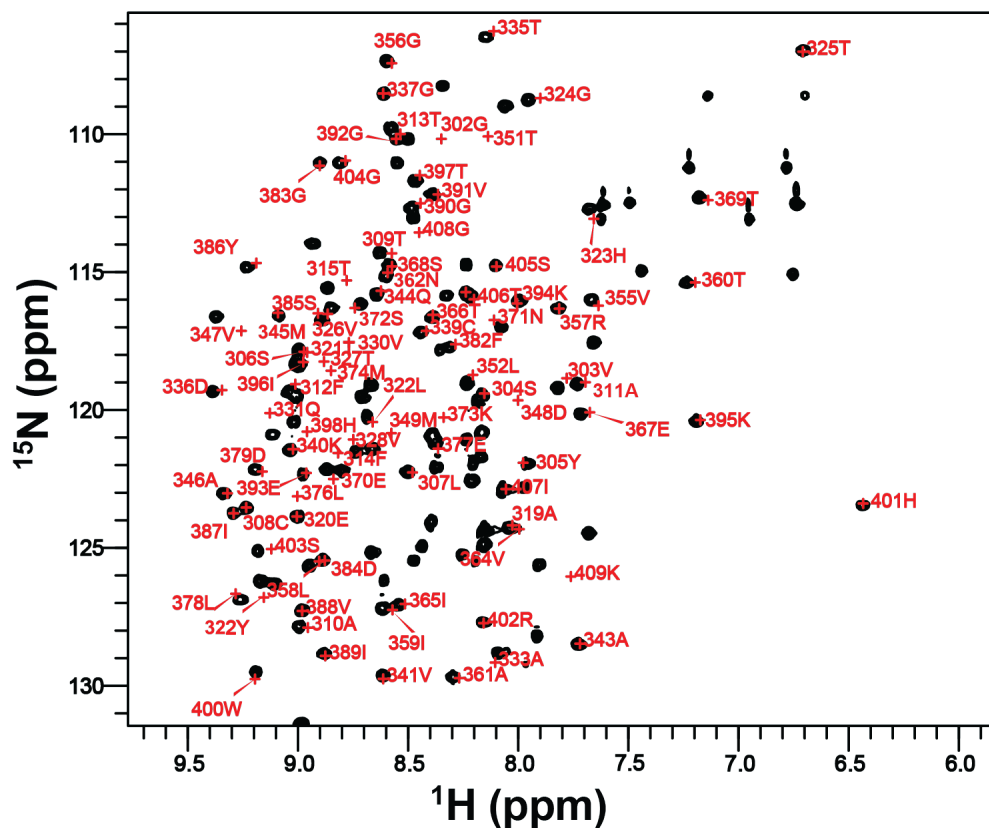


Figure S4, related to **Figure 3**. HSQC spectrum of rsZDIII-2.39 (shown in black contours) and the ZDIII-WT (BMRB 34167, shown as red crosses). Spectra shown was acquired from a single labelled protein preparation.

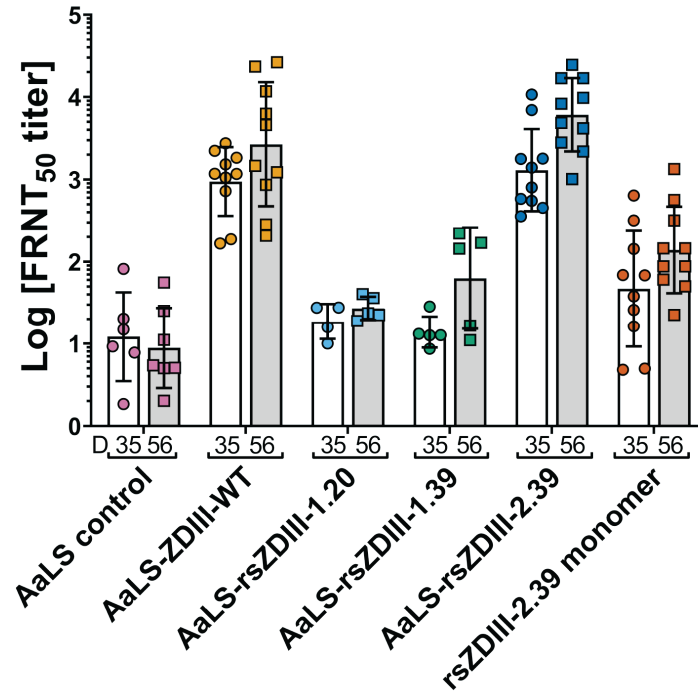


Figure S5, related to Figure 4. FRNT₅₀ neutralization titers of D35 and D56 serum collected from immunized mice. Data from two independent experiments, completed in triplicate, are plotted as (mean FRNT₅₀) \pm SD.

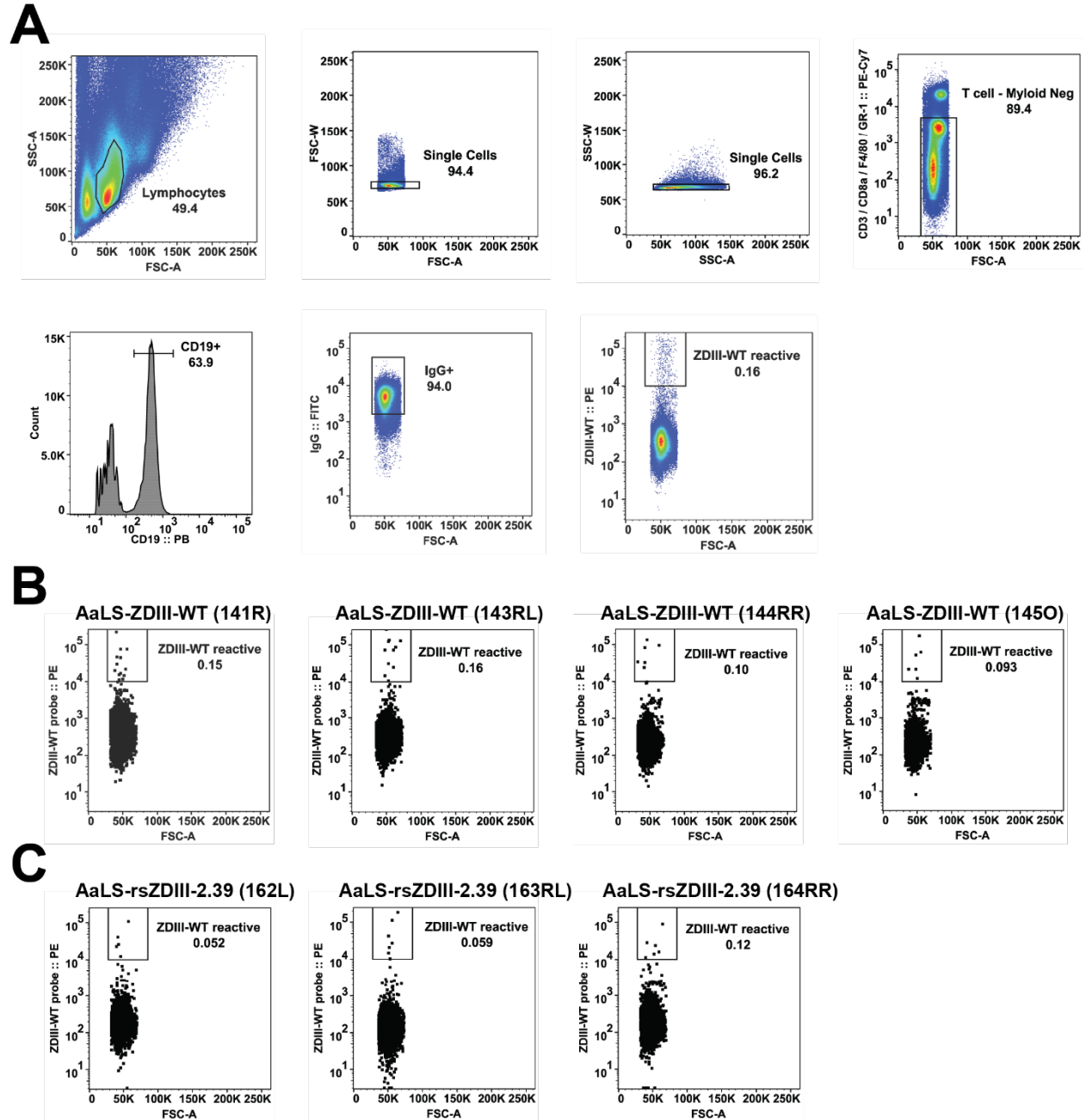


Figure S6, related to Figure 6. Flow cytometry analysis of the frequency of ZDIII-WT antigen-reactive CD19+ / IgG+ splenic B cells in vaccinated animals.

(A) Gating strategy used for analysis. (B) ZDIII-WT reactive B cells in AaLS-ZDIII-WT immunized animals collected at D56, each plot represents an independent biological sample and experiment. (C) Frequencies of ZDIII-WT reactive B cells in AaLS-rsZDIII-2.39 immunized animals collected at D56, each dot plot represents an independent biological sample and experiment.

Table S1, related to Figure 1 and Star Methods. Amino acid sequences of proteins and peptides described in this study.

Protein/ peptide	Amino acid sequence
SpyTag (SpT)	MAHIVMVDAYKPTK
SpyCatcher (SpC)	GAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWIS DGQVKDFLYPGKYTFVETAAPDGYEVATAITFTVNEQQQVTVNGKATKGAHI
ZDIII-WT-SpT	GVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMMELEDPFPGDSYIVIGVGEKKITHHWHRSGSTIGKSGSMAHIVMVDAYKPTK
AaLS-SpC	MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDCIVRHGGREEDITLVRVPGSWEIPVAAG ELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLANLSLELRKPITFGVITADTLEQAIERAGTK HGNKGWEAALSIAIEMANLFKSLRSGGGSGGGGAMVDTLSGLSSEQGQSGDMTIEEDSATHI KFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFLYPGKYTFVETAAPDGYEVATAI TFTVNEQQQVTVNGKATKGAHI

Table S2, related to Figure 3. Chemical shift perturbation (CSP) values for assigned residues in rsZDIII-2.39 compared to ZDIII-WT (5OMZ; BMRB 34167).

Position	Residue	H	NH	ΔH	ΔNH	CSP
303	Val	7.82	119.2	-0.04	-0.35	0.081
304	Ser	8.18	119.66	-0.03	-0.26	0.060
305	Tyr	7.96	121.94	0.02	-0.03	0.021
307	Leu	8.5	122.25	-0.02	0.02	0.020
308	Cys	9.24	123.54	-0.01	0	0.010
309	Thr	8.63	114.3	-0.06	0.02	0.060
310	Ala	8.99	127.84	-0.03	0.05	0.032
311	Ala	7.73	119.05	-0.03	-0.06	0.032
312	Phe	9.03	119.32	-0.02	-0.26	0.056
313	Thr	8.58	109.77	-0.04	0.22	0.059
314	Phe	8.74	121.51	0.08	0.05	0.081
315	Thr	8.87	115.58	-0.09	-0.28	0.106
320	Glu	9	123.84	0.01	0.02	0.011
323	His	7.68	112.72	-0.02	0.35	0.073
324	Gly	7.96	108.75	-0.06	-0.06	0.061
325	Thr	6.7	106.95	0.01	0.06	0.016
326	Val	8.89	116.76	-0.02	-0.24	0.052
328	Val	8.67	121.4	0.08	-0.33	0.104
331	Gln	9.12	120.9	0.01	-0.79	0.158
332	Tyr	9.17	126.18	-0.02	0.62	0.126
333	Ala	8.09	128.83	0.02	0.33	0.069
335	Thr	8.15	106.46	-0.04	-0.2	0.057
336	Asp	9.39	119.33	-0.04	-0.05	0.041
337	Gly	8.61	108.52	0	0	0.000
339	Cys	8.44	117.17	-0.02	-0.04	0.022
340	Lys	9.04	121.45	-0.02	-0.03	0.021
341	Val	8.62	129.67	-0.01	0.08	0.019
343	Ala	7.73	128.49	-0.01	-0.02	0.011
344	Gln	8.65	115.81	-0.03	-0.13	0.040
345	Met	8.85	116.3	0.06	0.19	0.071
346	Ala	9.34	123.03	-0.02	-0.01	0.020
347	Val	9.37	116.61	-0.11	0.52	0.151

352	Leu	8.24	119.02	-0.03	-0.29	0.065
355	Val	7.67	116.01	-0.03	0.21	0.052
356	Gly	8.6	107.35	-0.03	0.08	0.034
359	Ile	8.62	127.21	-0.05	0.04	0.051
360	Thr	7.23	115.35	-0.03	0.02	0.030
361	Ala	8.3	129.67	-0.03	0.05	0.032
362	Asn	8.6	115.13	-0.01	-0.11	0.024
364	Val	8.04	124.26	-0.04	0.06	0.042
365	Ile	8.55	127.08	-0.04	-0.05	0.041
366	Thr	8.39	116.64	0	-0.09	0.018
367	Glu	7.72	120.12	-0.05	-0.03	0.050
368	Ser	8.58	114.76	-0.01	0.03	0.012
369	Thr	7.18	112.31	-0.04	0.08	0.043
370	Glu	8.8	122.19	0.04	0.32	0.075
371	Asn	8.08	116.98	0.03	-0.24	0.057
372	Ser	8.72	116.14	0.02	0.16	0.038
377	Glu	8.38	121.17	0.62	1.96	0.734
378	Leu	9.27	126.87	0.01	-0.21	0.043
379	Asp	9.2	122.15	-0.04	0.08	0.043
382	Phe	8.31	117.7	-0.02	-0.08	0.026
383	Gly	8.9	111.03	0	0.11	0.022
384	Asp	8.95	125.66	-0.05	-0.17	0.060
385	Ser	9.09	116.57	0	-0.09	0.018
386	Tyr	9.23	114.83	-0.04	-0.16	0.051
387	Ile	9.3	123.75	-0.01	0	0.010
388	Val	8.98	127.27	0	0.02	0.004
389	Ile	8.88	128.82	0	0.1	0.020
390	Gly	8.48	112.68	-0.04	-0.18	0.054
391	Val	8.39	112.2	-0.03	-0.02	0.030
392	Gly	8.81	111.06	-0.03	-0.11	0.037
393	Glu	8.98	122.36	-0.02	-0.08	0.026
394	Lys	8	116.04	0	0.1	0.020
395	Lys	7.19	120.4	-0.02	-0.04	0.022
396	Ile	9	118.38	-0.02	-0.12	0.031
397	Thr	8.46	111.7	-0.01	-0.21	0.043
400	Trp	9.19	129.52	0.01	0.24	0.049

401	His	6.44	123.45	-0.01	-0.06	0.016
402	Arg	8.16	127.72	0	0.01	0.002
403	Ser	9.18	125.11	-0.06	-0.07	0.062
404	Gly	8.56	110.16	-0.01	0.01	0.010
405	Ser	8.11	114.77	-0.01	0.03	0.012
406	Thr	8.22	115.85	-0.02	0.14	0.034
407	Ile	8.07	122.87	-0.01	0	0.010
408	Gly	8.47	113.01	-0.02	0.55	0.112