Hepatitis C virus NS3-4A protease regulates the lipid environment for RNA replication by cleaving host enzyme 24-dehydrocholesterol reductase

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** Contributed equally

Running title: HCV NS3-4A protease regulates desmosterol metabolism

Supporting Information

Figure S1. Mass spectrometry analysis of DHCR24 and DHCR24* isolated from Huh7.5 and Huh7.5-SGR cells. Samples were separated by SDS-PAGE. One half of the gel was silver-stained (left) and peptides (marked by arrows) were excised for mass spectrometry. The other half of the gel (right) was transferred to PVDF membrane and probed with anti-Flag antibody as described in Experimental Procedures. The molecular weight markers were used to align the two images. Mass spectrometry analysis of the excised bands, marked "a" and "b," detected multiple peptides derived from DHCR24. Lower signal was detected for peptides prior to residue 113, but a definitive cleavage site for the "b" sample could not be identified. Peak intensities for the indicated N-terminal charged peptide fragment ions in the "a" and "b" samples are reported in tables on pages 3 and 4. Raw data sets have been uploaded to MassIVE (accession number MSV000085492.

		peak intensity	
Peptide	**residues**	**a - 46739**	**b - 46740**
GLEFVLIHQR	22-31	8.01E+04	2.37E+04
AWVVFK	54-59	3.51E+06	1.56E+05
VRDIQK	71-76	1.53E+06	3.19E+05
NIMINLMDILEVDTKK	113-128	4.00E+06	3.94E+06
HVENYLK	307-313	1.08E+08	4.11E+07
LISEEDLAANDILDYKDDDDKV	526-547	7.97E+07	4.08E+07

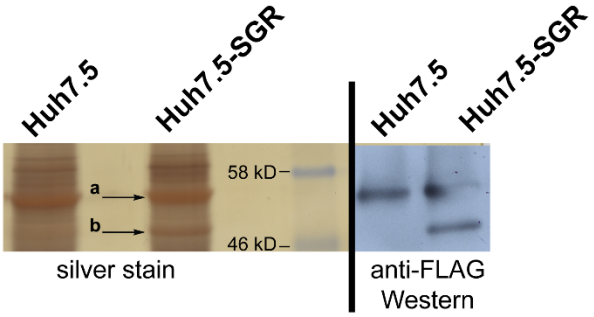


Figure S1 continued

DHCR24 Peak A

Protein ID	Obs m/z	z	XCorr	Peptide	Missed Cleavage	Modification
DHRC24	619.3264	2	3.572	R.YLFGWMVPPK.I	0	
DHRC24	603.3447	3	1.471	R.YLFGWM*VPPKISLLK.L	1	M - Oxidation
DHRC24	627.3240	2	3.550	R.YLFGWM*VPPK.I	0	M - Oxidation
DHRC24	571.3442	2	2.377	R.VRDIQKQVR.E	2	
DHRC24	379.7295	2	2.202	R.VRDIQK.Q	1	
DHRC24	443.7479	2	1.842	R.VKHFEAR.S	1	
DHRC24	436.2536	2	2.381	R.TRPLEQK.L	0	
DHRC24	669.9560	3	4.026	R.SVHGFQMLYADCYM*NR.E	0	M - Oxidation
DHRC24	669.9563	3	4.278	R.SVHGFQM*LYADCYMNR.E	0	M - Oxidation
DHRC24	675.2881	3	4.305	R.SVHGFQM*LYADCYM*NR.E	0	M - Oxidation
DHRC24	460.2716	2	1.387	R.QLEKFVR.S	1	
DHRC24	699.0340	3	4.124	R.KLYEQHHVVQDMLVPMK.C	0	
DHRC24	704.3654	3	3.116	R.KLYEQHHVVQDMLVPM*K.C	0	M - Oxidation
DHRC24	528.5256	4	2.666	R.KLYEQHHVVQDM*LVPMK.C	0	M - Oxidation
DHRC24	709.6968	3	3.195	R.KLYEQHHVVQDM*LVPM*K.C	0	M - Oxidation
DHRC24	388.1853	2	1.886	R.HYYHR.H	0	
DHRC24	431.2283	2	2.661	R.GLEAICAK.F	0	
DHRC24	497.2661	3	2.100	R.FEPVRGLEAICAK.F	1	
DHRC24	496.2462	2	1.677	R.EWKEQGSK.T	1	
DHRC24	734.0204	3	3.514	R.EKLGCQDAFPEVYDKICK.A	2	
DHRC24	600.2821	3	2.986	R.EKLGCQDAFPEVYDK.I	1	
DHRC24	747.3853	3	1.897	R.EGLEYIPLRHYYHRHTR.S	2	
DHRC24	615.9855	3	1.663	R.EGLEYIPLRHYYHR.H	1	
DHRC24	545.2997	2	3.095	R.EGLEYIPLR.H	0	
DHRC24	606.6004	3	1.368	R.EEFWEMFDGSLYHK.L	0	
DHRC24	611.9322	3	2.274	R.EEFWEM*FDGSLYHK.L	0	M - Oxidation
DHRC24	375.2207	2	2.412	R.AWVVFK.L	0	
DHRC24	436.2594	3	1.574	K.YVKLRFEPVR.G	2	
DHRC24	853.7638	3	3.039	K.YGLFQHICTAYELVLADGSFVR.C	0	
DHRC24	487.6003	3	3.601	K.TNREGLEYIPLR.H	1	
DHRC24	568.8072	4	3.260	K.THKNIMINLM*DILEVDTKK.Q	1	M - Oxidation
DHRC24	763.4065	3	2.966	K.THKNIM*INLM*DILEVDTKK.Q	1	M - Oxidation
DHRC24	687.8608	2	3.057	K.QVREWKEQGSK.T	2	
DHRC24	636.0069	3	2.129	K.NIMINLM*DILEVDTKK.Q	0	M - Oxidation
DHRC24	889.4600	2	5.273	K.NIMINLM*DILEVDTK.K	0	M - Oxidation
DHRC24	636.0068	3	1.705	K.NIM*INLMDILEVDTKK.Q	0	M - Oxidation
DHRC24	641.3383	3	2.652	K.NIM*INLM*DILEVDTKK.Q	0	M - Oxidation
DHRC24	897.4567	2	5.556	K.NIM*INLM*DILEVDTK.K	0	M - Oxidation
DHRC24	656.3351	3	4.274	K.LYEQHHVVQDMLVPMK.C	0	
DHRC24	661.6669	3	4.128	K.LYEQHHVVQDMLVPM*K.C	0	M - Oxidation
DHRC24	661.6665	3	4.083	K.LYEQHHVVQDM*LVPMK.C	0	M - Oxidation
DHRC24	666.9984	3	4.001	K.LYEQHHVVQDM*LVPM*K.C	0	M - Oxidation
DHRC24	523.3033	2	2.648	K.LTQGETLRK.L	0	
DHRC24	459.2550	2	2.387	K.LTQGETLR.K	0	
DHRC24	586.9951	3	3.231	K.LRFEPVRGLEAICAK.F	2	
DHRC24	458.7714	2	2.138	K.LRFEPVR.G	1	
DHRC24	592.9760	3	1.404	K.LNSIGNYYKPWFFK.H	0	
DHRC24	837.0683	3	4.708	K.LISEEDLAANDILDYKDDDDKV.-	2	
DHRC24	804.0453	3	3.100	K.LISEEDLAANDILDYKDDDDK.V	1	
DHRC24	911.4618	2	5.347	K.LISEEDLAANDILDYK.D	0	
DHRC24	863.7204	3	2.851	K.LIS#EEDLAANDILDYKDDDDKV.-	2	S - Phosphoryl.
DHRC24	561.0259	4	2.029	K.LGCQDAFPEVYDKICKAAR.H	2	
DHRC24	648.3071	3	3.928	K.LGCQDAFPEVYDKICK.A	1	
DHRC24	771.3503	2	4.288	K.LGCQDAFPEVYDK.I	0	
DHRC24	533.9984	3	2.429	K.ISLLKLTQGETLRK.L	1	
DHRC24	491.3000	3	3.121	K.ISLLKLTQGETLR.K	1	
DHRC24	425.2260	3	1.964	K.HVENYLKTNR.E	1	
DHRC24	451.7391	2	3.191	K.HVENYLK.T	0	
DHRC24	933.9473	2	5.450	K.GNEAELYIDIGAYGEPR.V	0	
DHRC24	404.5680	3	2.328	K.GLEFVLIHQR.W	0	
DHRC24	452.7174	2	2.318	K.FTHESQR.Q	0	

Figure S1 continued

DHCR24 Peak B

Protein ID	Obs m/z	z	XCorr	Peptide	Missed Cleavage	Modification
DHRC24	452.7172	2	2.329	K.FTHESQR.Q	0	
DHRC24	404.5678	3	2.239	K.GLEFVLIHQR.W	0	
DHRC24	933.9495	2	5.510	K.GNEAELYIDIGAYGEPR.V	0	
DHRC24	451.7399	2	3.277	K.HVENYLK.T	0	
DHRC24	491.2999	3	2.517	K.ISLLKLTQGETLR.K	1	
DHRC24	771.3510	2	4.475	K.LGCQDAFPEVYDK.I	0	
DHRC24	863.7235	3	3.200	K.LIS#EEDLAANDILDYKDDDDKV.-	2	S - Phosphoryl.
DHRC24	863.7207	3	2.941	K.LISEEDLAANDILDY#KDDDDKV.-	2	Y - Phosphoryl.
DHRC24	911.4619	2	5.262	K.LISEEDLAANDILDYK.D	0	
DHRC24	804.0450	3	3.418	K.LISEEDLAANDILDYKDDDDK.V	1	
DHRC24	837.0670	3	3.944	K.LISEEDLAANDILDYKDDDDKV.-	2	
DHRC24	592.9760	3	1.972	K.LNSIGNYYKPWFFK.H	0	
DHRC24	665.8485	4	1.975	K.LNSIGNYYKPWFFKHVENYLK.T	1	
DHRC24	458.7719	2	2.164	K.LRFEPVR.G	1	
DHRC24	459.2557	2	2.306	K.LTQGETLR.K	0	
DHRC24	523.3037	2	2.156	K.LTQGETLRK.L	0	
DHRC24	666.9981	3	4.060	K.LYEQHHVVQDM*LVPM*K.C	0	M - Oxidation
DHRC24	661.6667	3	3.766	K.LYEQHHVVQDM*LVPMK.C	0	M - Oxidation
DHRC24	656.3352	3	4.429	K.LYEQHHVVQDMLVPMK.C	0	
DHRC24	897.4580	2	4.341	K.NIM*INLM*DILEVDTK.K	0	M - Oxidation
DHRC24	641.3384	3	3.143	K.NIM*INLM*DILEVDTKK.Q	0	M - Oxidation
DHRC24	953.5079	2	3.457	K.NIM*INLMDILEVDTKK.Q	0	M - Oxidation
DHRC24	889.4602	2	5.421	K.NIMINLM*DILEVDTK.K	0	M - Oxidation
DHRC24	636.0068	3	1.548	K.NIMINLM*DILEVDTKK.Q	0	M - Oxidation
DHRC24	487.6007	3	3.485	K.TNREGLEYIPLR.H	1	
DHRC24	853.7640	3	2.916	K.YGLFQHICTAYELVLADGSFVR.C	0	
DHRC24	436.2593	3	1.673	K.YVKLRFEPVR.G	2	
DHRC24	375.2209	2	2.346	R.AWVVFK.L	0	
DHRC24	611.9324	3	2.331	R.EEFWEM*FDGSLYHK.L	0	M - Oxidation
DHRC24	606.6003	3	2.839	R.EEFWEMFDGSLYHK.L	0	
DHRC24	545.3007	2	3.068	R.EGLEYIPLR.H	0	
DHRC24	747.3849	3	1.921	R.EGLEYIPLRHYYHRHTR.S	2	
DHRC24	600.2829	3	2.361	R.EKLGCQDAFPEVYDK.I	1	
DHRC24	497.2664	3	1.203	R.FEPVRGLEAICAK.F	1	
DHRC24	431.2285	2	2.736	R.GLEAICAK.F	0	
DHRC24	388.1852	2	1.758	R.HYYHR.H	0	
DHRC24	532.5242	4	2.542	R.KLYEQHHVVQDM*LVPM*K.C	0	M - Oxidation
DHRC24	704.3655	3	2.772	R.KLYEQHHVVQDM*LVPMK.C	0	M - Oxidation
DHRC24	524.5266	4	2.084	R.KLYEQHHVVQDMLVPMK.C	0	
DHRC24	1162.214	3	2.873	R.QENHFVEGLLYSLDEAVIM*TGVM*TDEAEPSK.L	0	M - Oxidation
DHRC24	675.2881	3	4.015	R.SVHGFQM*LYADCYM*NR.E	0	M - Oxidation
DHRC24	1004.433	2	4.883	R.SVHGFQM*LYADCYMNR.E	0	M - Oxidation
DHRC24	664.6248	3	4.176	R.SVHGFQMLYADCYMNR.E	0	
DHRC24	436.2535	2	2.203	R.TRPLEQK.L	0	
DHRC24	379.7295	2	1.522	R.VRDIQK.Q	1	
DHRC24	627.3239	2	3.264	R.YLFGWM*VPPK.I	0	M - Oxidation
DHRC24	619.3265	2	3.722	R.YLFGWMVPPK.I	0	

Figure S2. Recombinant NS3-4A cleaves MBP-DHCR24 in an *in vitro* biochemical assay. The MBP-DHCR24 protein was produced by heterologous expression in an *E. coli*- derived i*n vitro* translation system. Incubation of the MBP-DHCR24 fusion protein with recombinant NS3-4A resulted in formation of a faster-migrating species (red arrow head). This reaction was blocked in the presence of telaprevir ("inh").

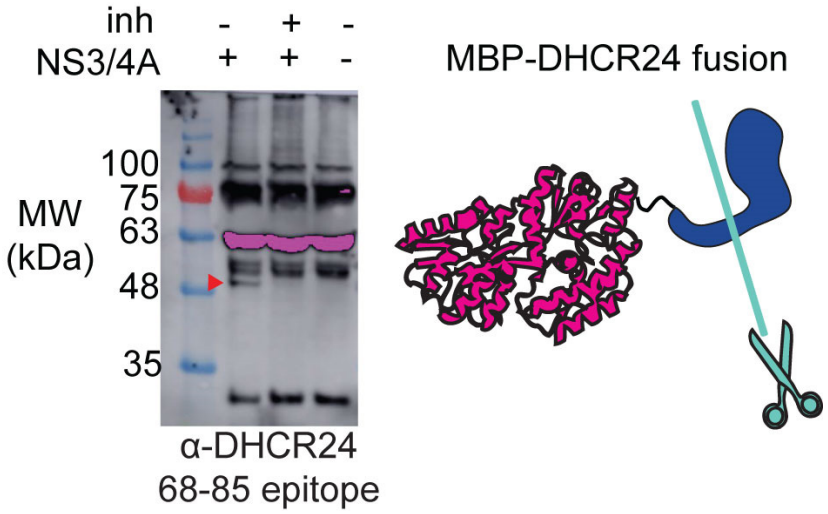


Figure S3. Characterization of Huh7.5 DHCR24^{KO} cells by PCR, immunoblot, and Sanger sequencing. A) Genomic DNA from Huh7.5 CRISPR-Cas9 knockout of DHCR24 (clone 3) shows no band corresponding to wild type (WT) DHCR24 in PCR assays using forward primer: 5'-AGA TGC CTA GTG TGT TGG GAA T-3' and reverse primer: 5'-AAA GGG TTC CCC TGA CCT ATT A-3'. B) Immunoblot analysis of lysates from candidate Huh7.5 DHCR24^{KO} clones using an N-terminal DHCR24 antibody (residues 68-85). No band was observed for clone 3. C) Nucleotide region of WT DHCR24 between exon 1 and 2. D) Region between exon 1 and 2 excised by CRISPR-Cas9. E) Sanger sequencing results of clone 3 band from agarose gel (shown in A) depicting removal of the designated region, resulting in removal of ~280 nucleotide bases in DHCR24 in clone 3. This clonal cell line is referred to as Huh7.5-DHCR24^{KO} in the text.

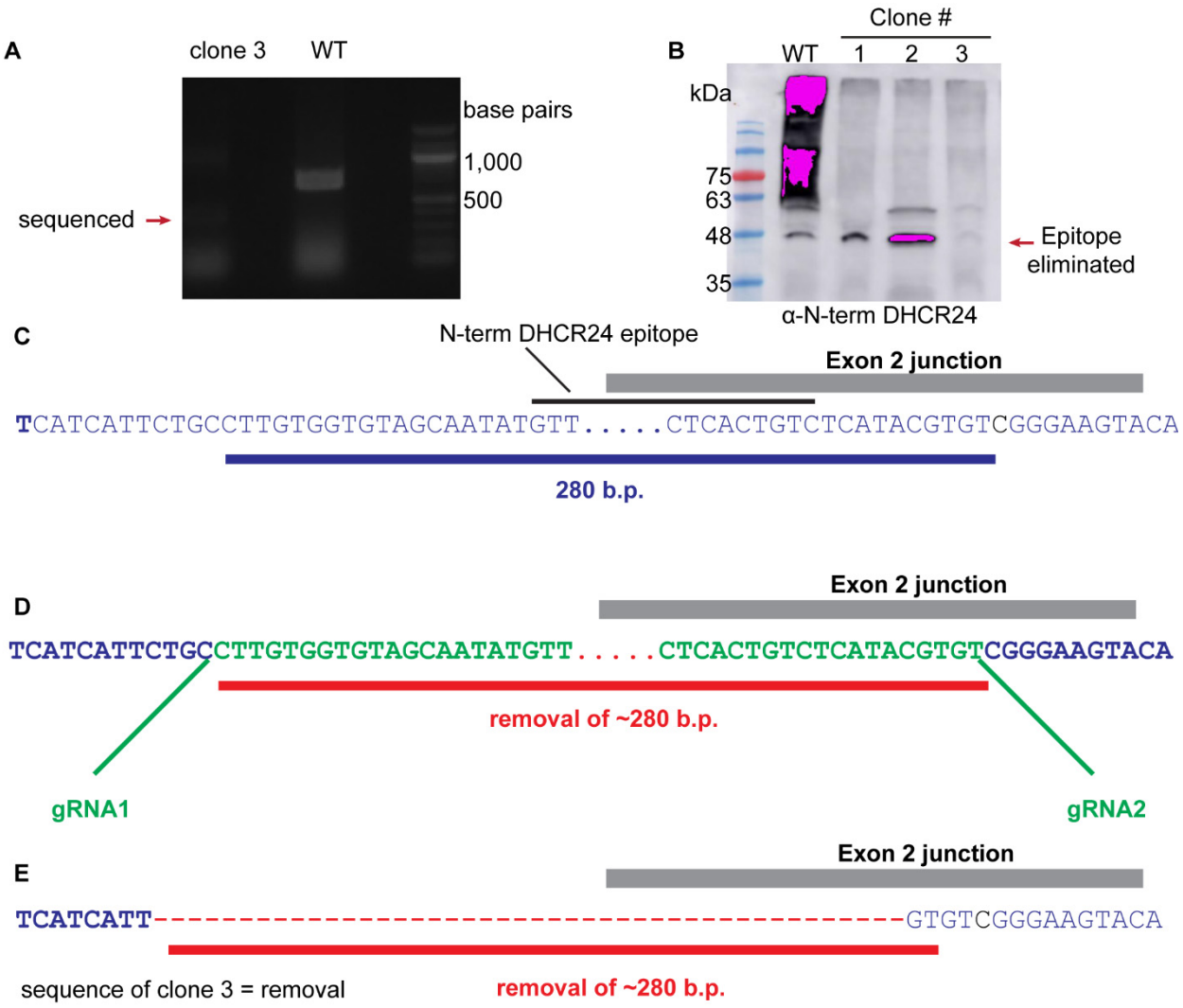


Figure S4. Co-occurrence of NS3-4A and DHCR24 associated with the ER of Huh-7.5[VEEV/NS3–5B] cells. Immunofluorescence detection of NS3-4A, DHCR24, and the ER marker protein disulfide isomerase (PDI) in Huh-7.5[VEEV/NS3–5B] cells. These cells have been transduced with a noncytopathic subgenomic replicon derived from Venezuelan equine encephalitis virus. They are an established system for expression of the NS3-5B polyprotein at physiological levels with appropriate polyprotein processing and allow for studies of protein expression, processing, and localization over several weeks when maintained under selective conditions (36). A) DHCR24 (magenta) and NS3-4A (green) were observed as a fraction co-occurring in the endoplasmic reticulum (ER). B) DHCR24 (magenta, gamma = 1.2) and the ER marker protein disulfide isomerase (PDI, green, gamma=1.4) co-occur in the ER. Insert scale bar = 10 µm.

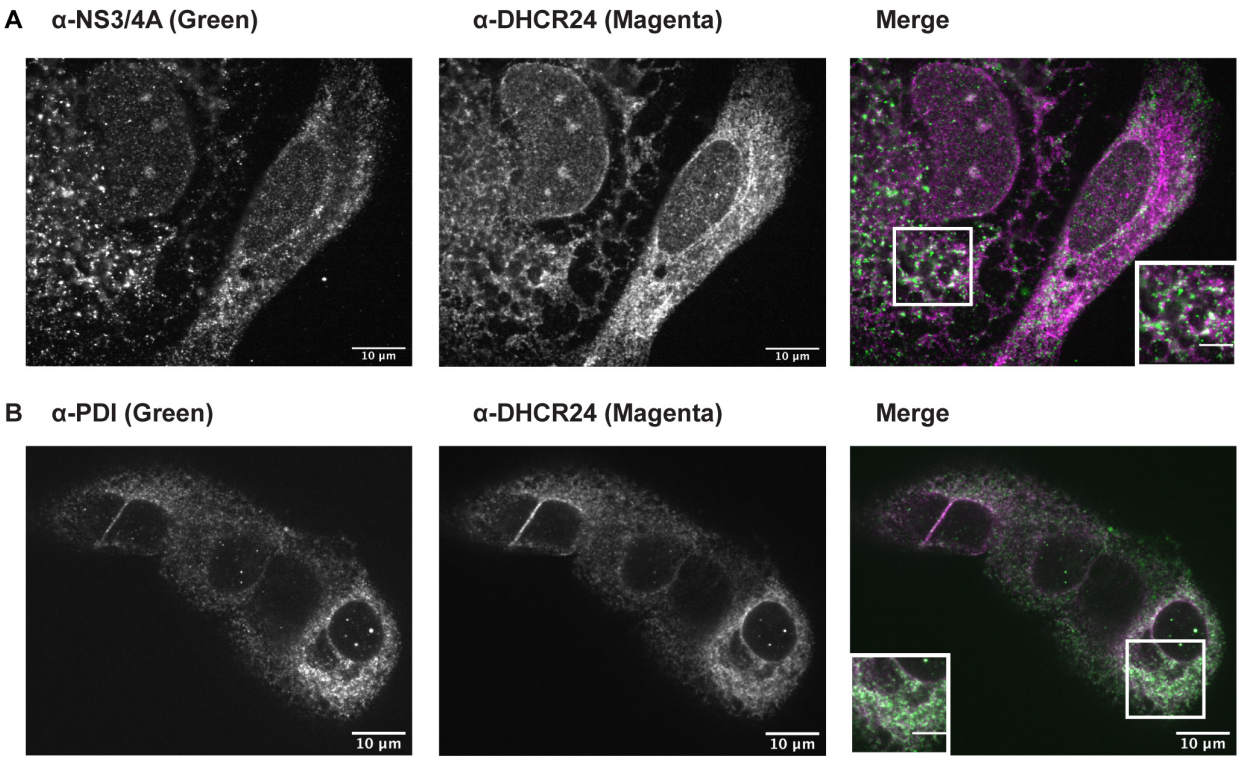
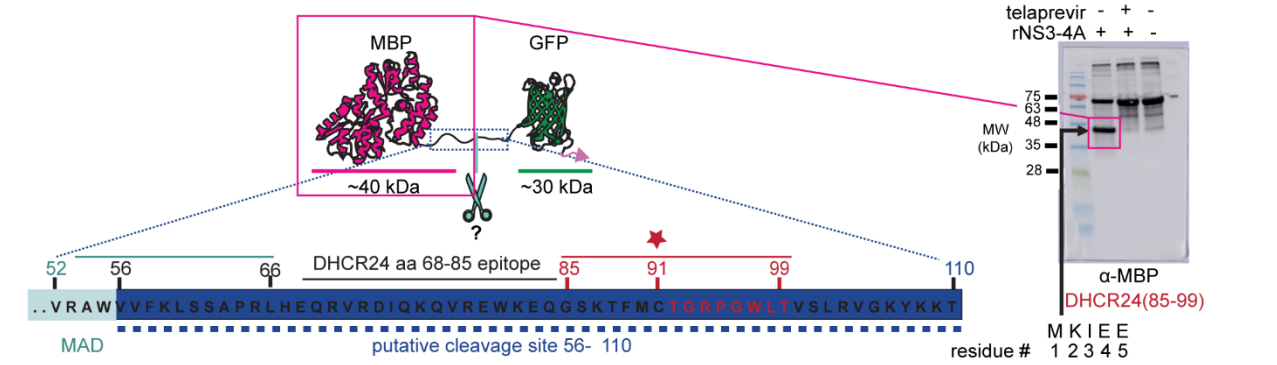
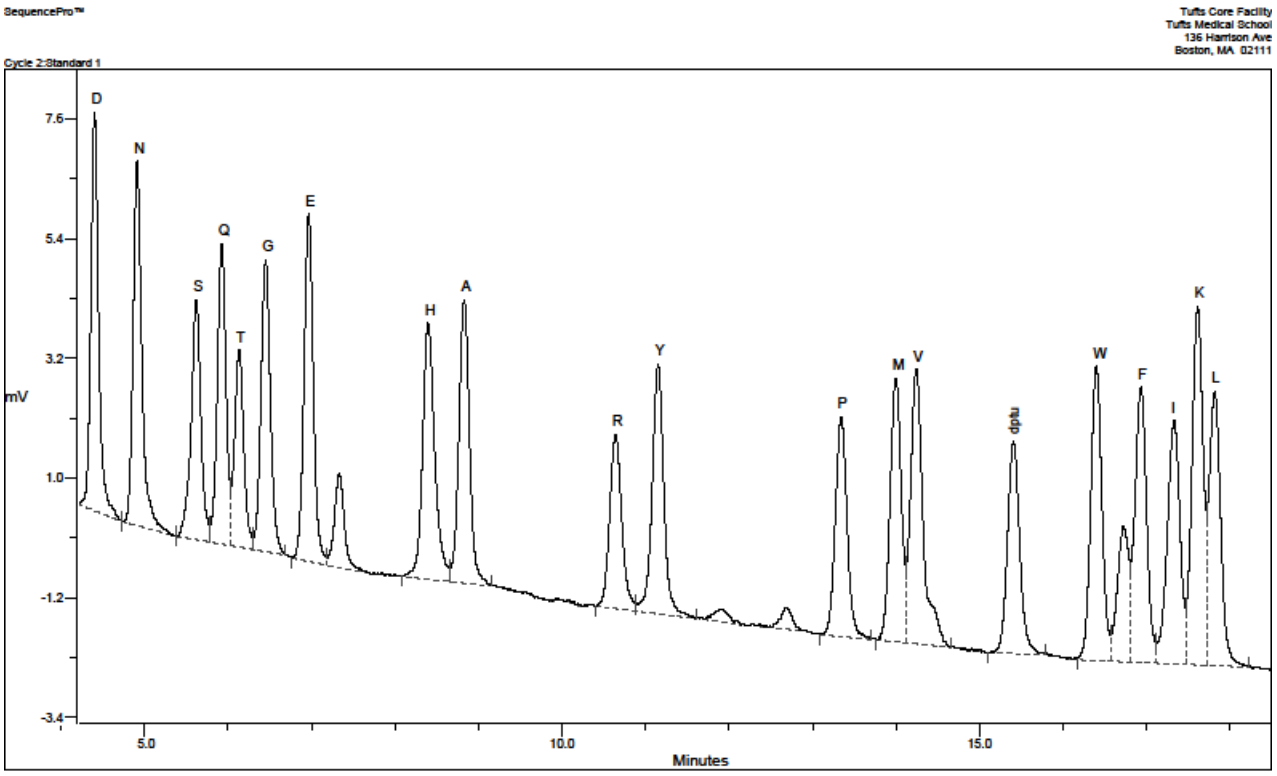


Figure S5. Edman degradation chromatograms of cleaved DHCR24 by NS3-4A. A) Shows MBP portion of MBP-85-99-DHCR24-GFP-FlagTag completed in 5 cycles with the residue calls in order from 1-5. Traces are shown on pages 8-13. B) GFP portion of MBP-85-99-DHCR24-GFP-FlagTag completed in 10 cycles with residue calls from 1-10. Cycle 6 and 10 are unassigned. B) begins on page 14 and continues through page 24. The peptide spanning map and immunoblots from Figure 3B have been reproduced here in order to orient the reader in viewing the Edman degradation traces.

A. STANDARD





PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.40	4.40	7.334	10.000
N	4.91	4.91	6.698	10.000
S	5.62	5.62	4.409	10.000
Q	5.92	5.92	5.525	10.000
T	6.13	6.13	3.618	10.000
G	6.45	6.45	5.375	10.000
E	6.97	6.97	6.384	10.000
H	8.40	8.40	4.708	10.000
A	8.83	8.83	5.207	10.000
R	10.64	10.64	3.205	10.000
Y	11.15	11.15	4.590	10.000
P	13.34	13.34	4.035	10.000
M	14.00	14.00	4.839	10.000
V	14.24	14.24	5.044	10.000
dptu	15.40	15.40	3.895	10.000
W	16.40	16.40	5.408	10.000
F	16.93	16.93	5.058	10.000
I	17.33	17.33	4.465	10.000
K	17.61	17.61	6.595	10.000
L	17.82	17.82	5.042	10.000

Wednesday, March 13, 2019 08:43:08

311LT-2 - Page 2 of 8

Figure S5. A) continued

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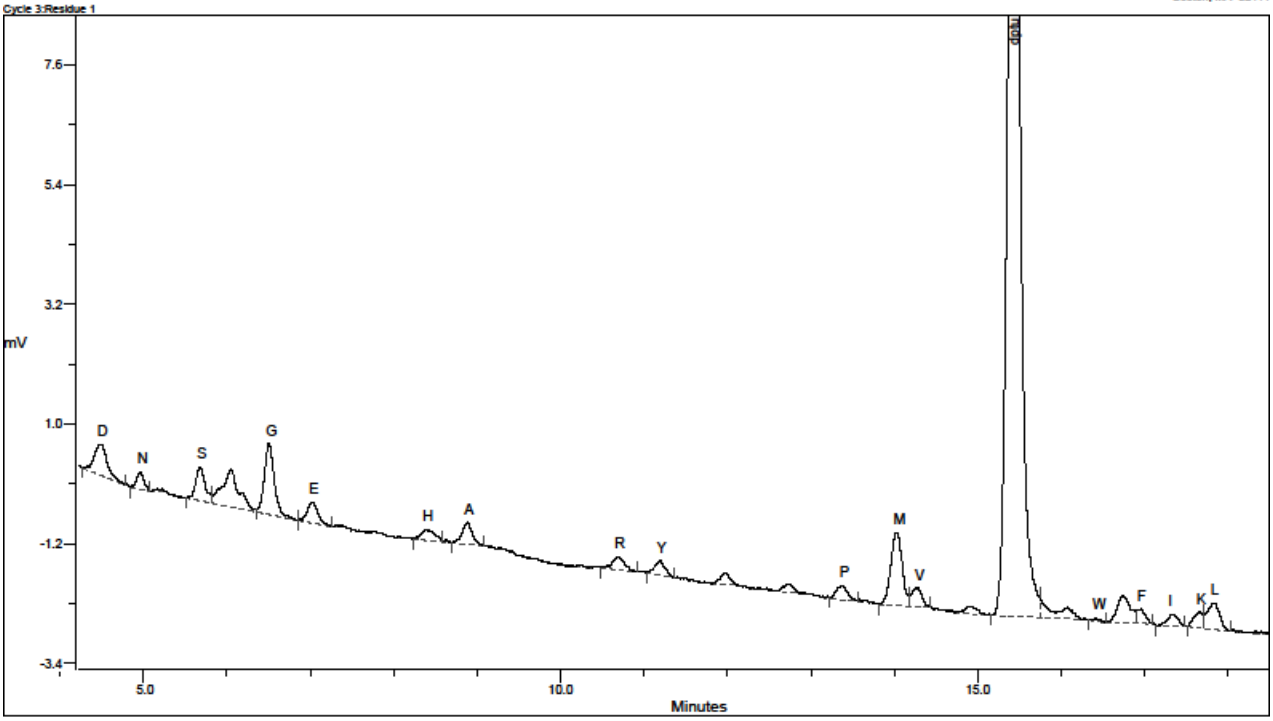
Cycle 3:Residue 1



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.49	4.40	0.570	0.777
N	4.97	4.91	0.326	0.486
S	5.68	5.62	0.624	1.414
G	6.50	6.45	1.304	2.426
E	7.02	6.97	0.387	0.606
H	8.39	8.40	0.197	0.419
A	8.88	8.83	0.404	0.776
R	10.69	10.64	0.236	0.736
Y	11.19	11.15	0.263	0.574
P	13.38	13.34	0.262	0.650

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
M	14.03	14.00	1.349	2.788
V	14.28	14.24	0.345	0.684
dptu	15.43	15.40	19.343	49.666
W	16.41	16.40	0.051	0.095
F	16.95	16.93	0.265	0.524
I	17.32	17.33	0.215	0.482
K	17.66	17.61	0.288	0.436
L	17.83	17.82	0.482	0.956

Wednesday, March 13, 2019 08:43:08

311LT-2 - Page 3 of 8

RESIDUE #1 = M

Figure S5. A) continued

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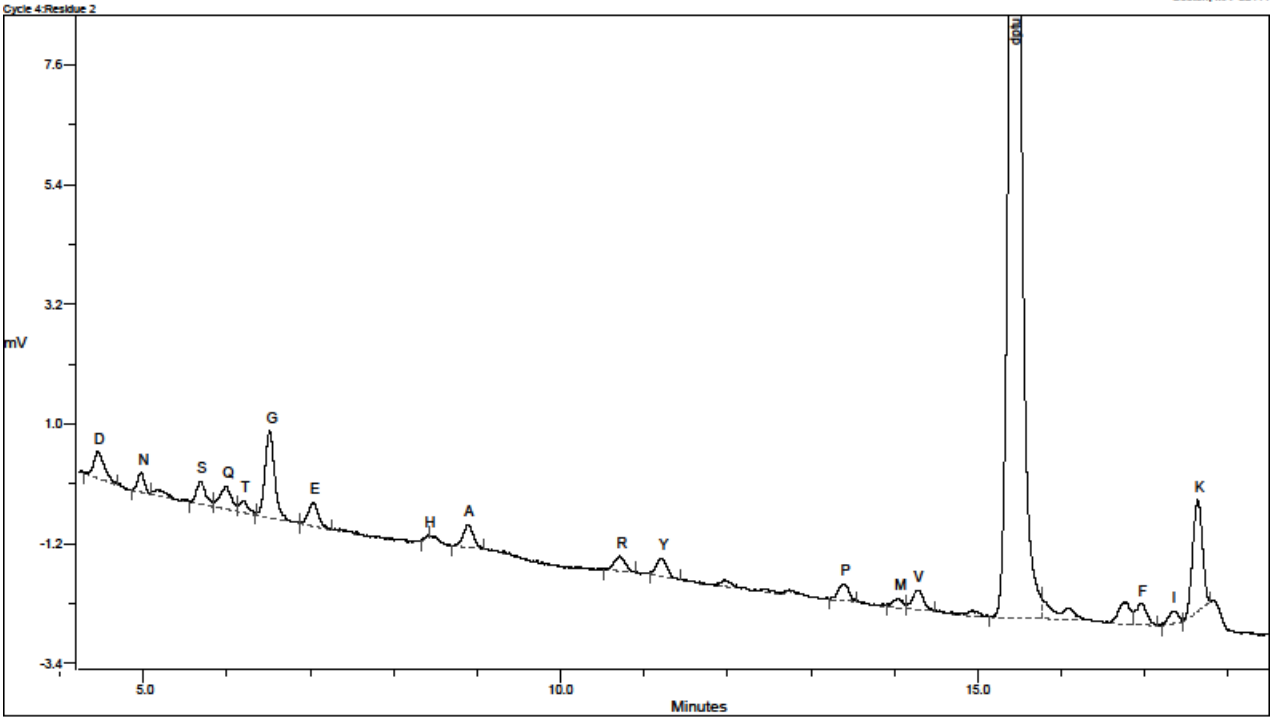
Cycle 4:Residue 2



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.45	4.40	0.479	0.653
N	4.97	4.91	0.357	0.533
S	5.68	5.62	0.411	0.932
Q	5.99	5.92	0.429	0.777
T	6.20	6.13	0.214	0.592
G	6.51	6.45	1.589	2.956
E	7.03	6.97	0.443	0.693
H	8.41	8.40	0.047	0.100
A	8.88	8.83	0.409	0.786
R	10.71	10.64	0.280	0.874

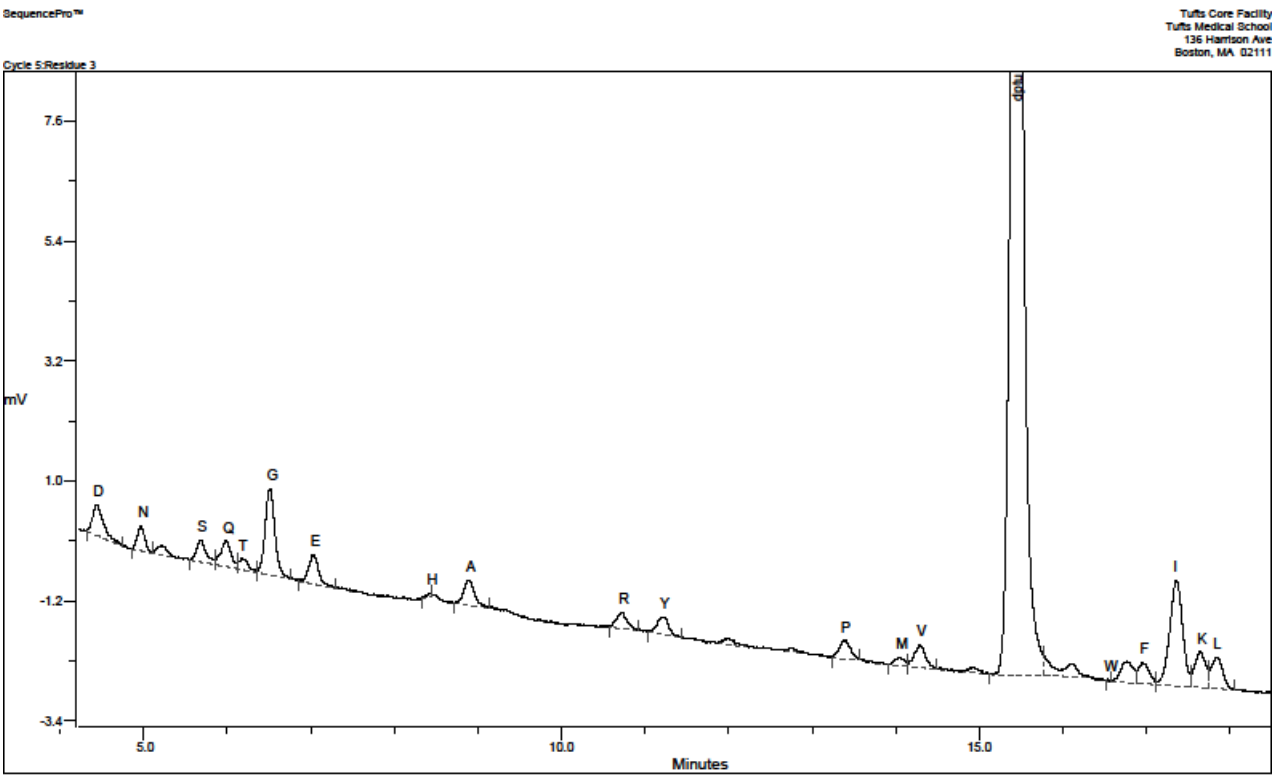
PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
Y	11.21	11.15	0.329	0.718
P	13.40	13.34	0.297	0.735
M	14.04	14.00	0.169	0.348
V	14.27	14.24	0.357	0.708
dptu	15.45	15.40	20.367	52.297
F	16.96	16.93	0.381	0.754
I	17.36	17.33	0.228	0.511
K	17.64	17.61	2.038	3.090

Wednesday, March 13, 2019 08:43:08

311LT-2 - Page 4 of 8

RESIDUE #2 = K

Figure S5. A) continued



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.43	4.40	0.566	0.772
N	4.97	4.91	0.452	0.674
S	5.68	5.62	0.405	0.918
Q	5.99	5.92	0.472	0.854
T	6.17	6.13	0.196	0.543
G	6.51	6.45	1.580	2.940
E	7.03	6.97	0.537	0.842
H	8.43	8.40	0.049	0.103
A	8.89	8.83	0.462	0.887
R	10.73	10.64	0.291	0.910

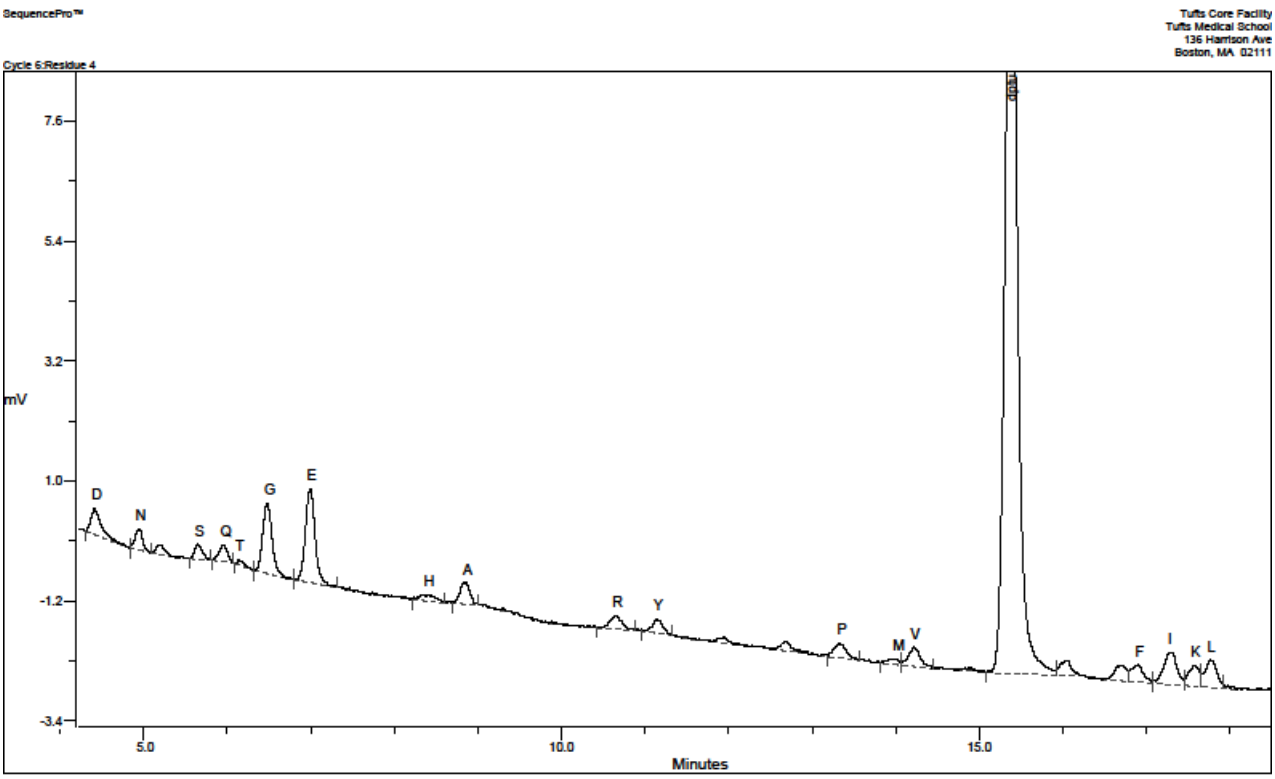
PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
Y	11.22	11.15	0.316	0.688
P	13.38	13.34	0.336	0.832
M	14.04	14.00	0.142	0.294
V	14.29	14.24	0.422	0.837
dptu	15.45	15.40	22.281	57.210
W	16.53	16.40	0.031	0.057
F	16.96	16.93	0.386	0.764
I	17.35	17.33	1.932	4.327
K	17.65	17.61	0.658	0.997
L	17.84	17.82	0.574	1.139

Wednesday, March 13, 2019 08:43:08

311LT-2 - Page 5 of 8

RESIDUE #3 = I

Figure S5. A) continued



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.42	4.40	0.483	0.659
N	4.94	4.91	0.387	0.577
S	5.65	5.62	0.272	0.617
Q	5.95	5.92	0.309	0.559
T	6.13	6.13	0.092	0.253
G	6.48	6.45	1.285	2.391
E	6.99	6.97	1.725	2.703
H	8.39	8.40	0.117	0.248
A	8.86	8.83	0.390	0.750
R	10.65	10.64	0.252	0.785

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
Y	11.14	11.15	0.254	0.553
P	13.34	13.34	0.270	0.670
M	14.00	14.00	0.113	0.233
V	14.21	14.24	0.362	0.718
dptu	15.38	15.40	15.832	40.650
F	16.90	16.93	0.323	0.639
I	17.29	17.33	0.597	1.337
K	17.57	17.61	0.388	0.589
L	17.76	17.82	0.509	1.009

Wednesday, March 13, 2019 08:43:08

311LT-2 - Page 6 of 8

RESIDUE #4 = E

Figure S5. A) continued

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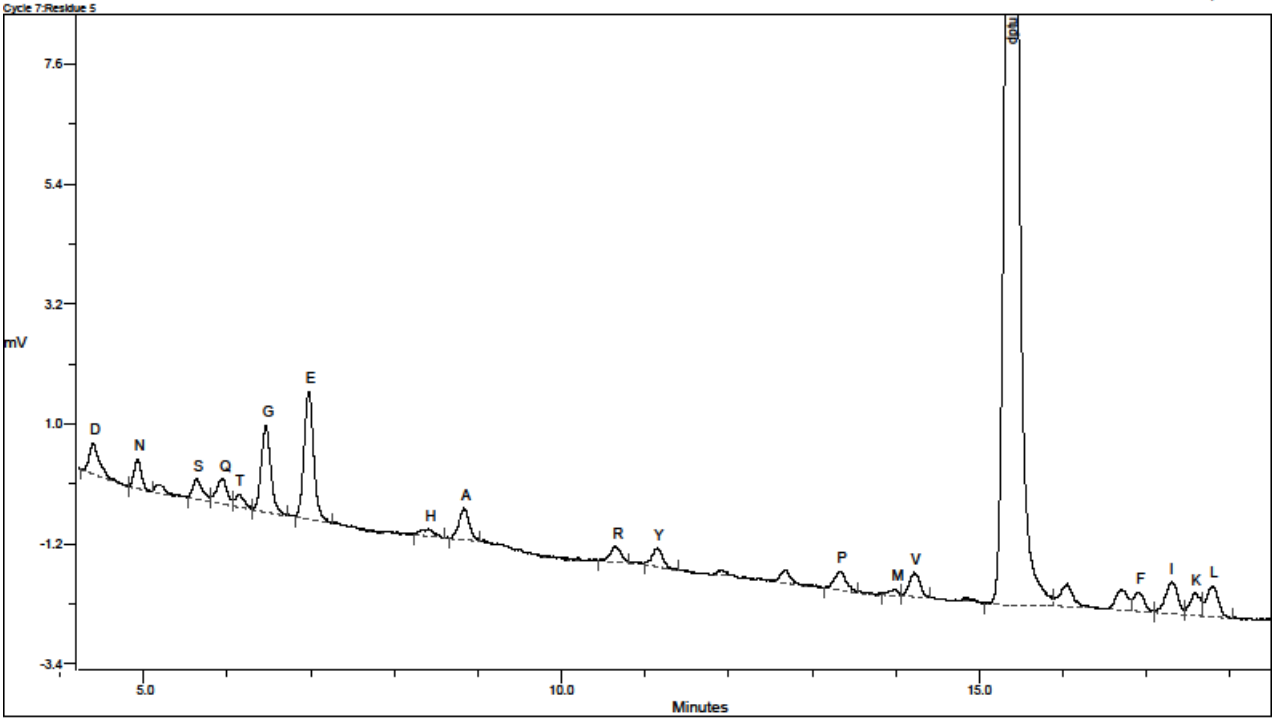
Tufts Core Facility
Tufts Medical School
136 Harrison Ave
Boston, MA 02111

Cycle 7:Residue 5



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.40	4.40	0.566	0.771
N	4.92	4.91	0.540	0.806
S	5.63	5.62	0.369	0.837
Q	5.95	5.92	0.456	0.825
T	6.14	6.13	0.226	0.625
G	6.46	6.45	1.594	2.966
E	6.97	6.97	2.334	3.656
H	8.41	8.40	0.129	0.272
A	8.84	8.83	0.578	1.111
R	10.65	10.64	0.278	0.867

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
Y	11.15	11.15	0.338	0.737
P	13.34	13.34	0.353	0.875
M	13.99	14.00	0.121	0.250
V	14.22	14.24	0.447	0.887
dptu	15.39	15.40	24.740	63.523
F	16.92	16.93	0.354	0.700
I	17.31	17.33	0.582	1.304
K	17.57	17.61	0.410	0.621
L	17.80	17.82	0.574	1.138

Wednesday, March 13, 2019 08:43:08

311LT-2 - Page 7 of 8

RESIDUE #5 = E

5 cycles total

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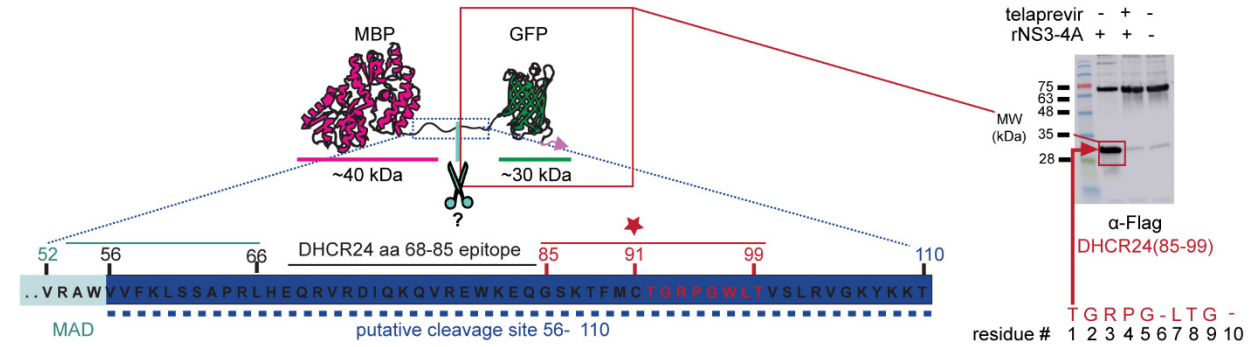
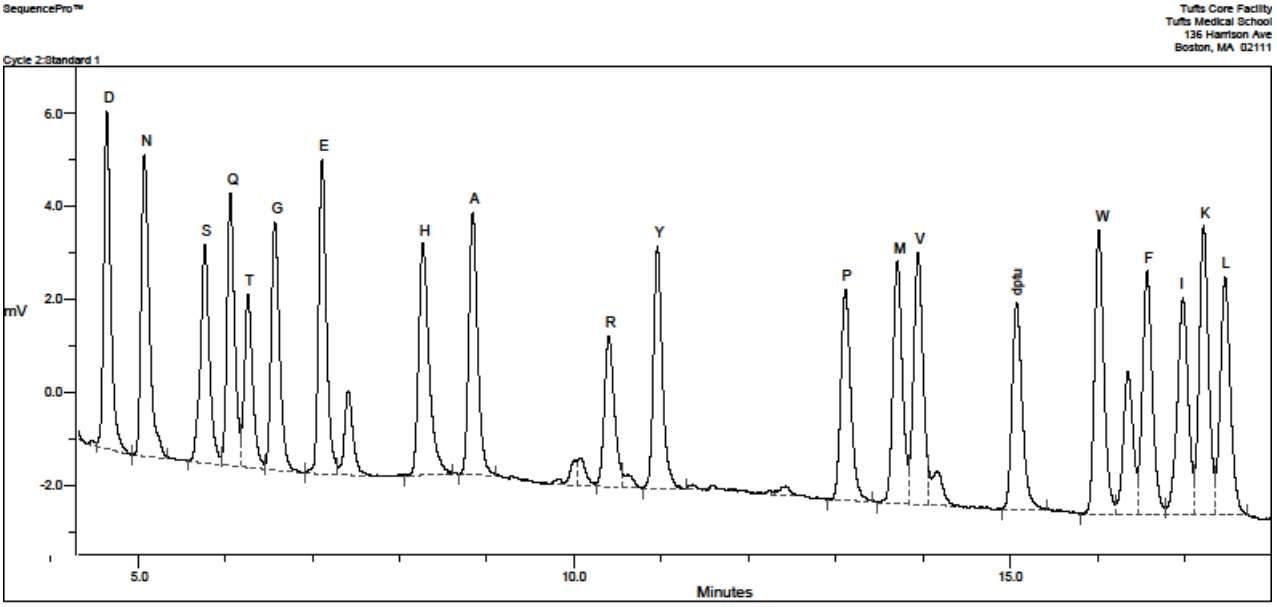
Tufts Core Facility
Tufts Medical School
136 Harrison Ave
Boston, MA 02111

Raw Pmol Table

	ASP	ASN	SER	GLN	THR	GLY	GLU	HIS	ALA	ARG	TYR	PRO	MET	VAL	TRP	PHE	ILE	LYS	LEU
1	0.777	0.486	1.414	0.000	0.000	2.426	0.606	0.419	0.776	0.736	0.574	0.650	2.788	0.684	0.095	0.524	0.482	0.436	0.956
2	0.653	0.533	0.932	0.777	0.592	2.956	0.693	0.100	0.786	0.874	0.718	0.735	0.348	0.708	0.000	0.754	0.511	3.090	0.000
3	0.772	0.674	0.918	0.854	0.543	2.940	0.842	0.103	0.887	0.910	0.688	0.832	0.294	0.837	0.057	0.764	4.327	0.997	1.139
4	0.659	0.577	0.617	0.559	0.253	2.391	2.703	0.248	0.750	0.785	0.553	0.670	0.233	0.718	0.000	0.639	1.337	0.589	1.009
5	0.771	0.806	0.837	0.825	0.625	2.966	3.656	0.272	1.111	0.867	0.737	0.875	0.250	0.887	0.000	0.700	1.304	0.621	1.138

Figure S5 B.





PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.11		14.413	
	4.48		0.105	
D	4.65	4.65	7.261	10.000
N	5.08	5.08	6.499	10.000
	5.50		0.029	
S	5.77	5.77	4.706	10.000
Q	6.06	6.06	5.866	10.000
T	6.26	6.26	3.726	10.000
G	6.57	6.57	5.347	10.000
E	7.11	7.11	6.781	10.000
	7.41		1.827	
	7.74		0.023	
	8.01		0.040	
H	8.27	8.27	4.987	10.000
A	8.84	8.84	5.658	10.000
	9.82		0.126	
	10.00		0.547	
	10.07		0.590	
R	10.40	10.40	3.240	10.000
	10.63		0.184	
Y	10.96	10.96	5.216	10.000
	11.37		0.095	
	11.60		0.090	
	11.80		0.041	
	12.25		0.084	
	12.43		0.191	
	12.78		0.037	
P	13.11	13.11	4.552	10.000
M	13.70	13.70	5.221	10.000
V	13.95	13.95	5.438	10.000
	14.16		0.574	
	14.68		0.029	
	14.86		0.028	
dptu	15.07	15.07	4.453	10.000
W	16.02	16.02	6.128	10.000
	16.35		3.079	
F	16.57	16.57	5.242	10.000
I	16.98	16.98	4.676	10.000
K	17.22	17.22	6.224	10.000
L	17.46	17.46	5.137	10.000
	18.02		0.045	
	18.15		0.044	
	18.44		0.042	
	18.54		0.047	
	18.83		0.039	

Wednesday, March 13, 2019 08:45:43

311LT-3 - Page 2 of 13

STANDARD

Figure S5. B) continued

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Tufts Medical School
136 Harrison Ave
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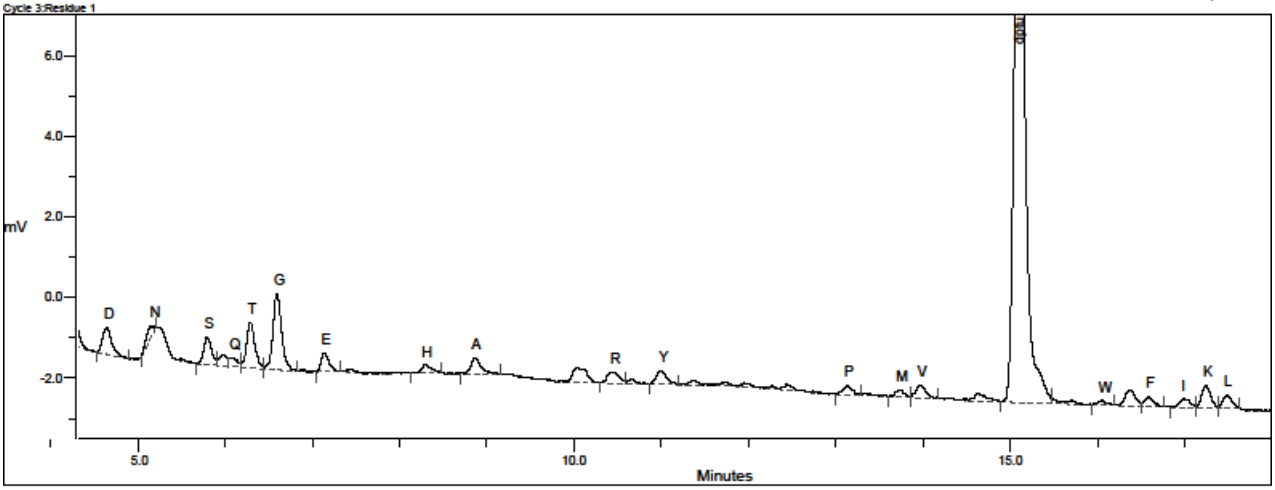
Cycle 3:Residue 1



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.13		14.525	
D	4.65	4.65	0.666	0.917
N	5.17	5.08	0.189	0.292
	5.50		0.061	
S	5.80	5.77	0.693	1.472
	5.97		0.272	
Q	6.08	6.06	0.224	0.383
T	6.29	6.26	1.130	3.034
G	6.59	6.57	1.888	3.531
	6.86		0.045	
	7.02		0.023	
E	7.14	7.11	0.470	0.694
	7.46		0.083	
	7.80		0.031	
	7.91		0.023	
H	8.29	8.27	0.200	0.400
	8.57		0.032	
	8.68		0.021	
A	8.86	8.84	0.418	0.739
	9.16		0.036	
	9.62		0.033	
	9.85		0.040	
	10.03		0.368	
R	10.45	10.40	0.283	0.874
	10.66		0.115	
	10.80		0.037	
Y	11.01	10.96	0.332	0.637
	11.25		0.033	
	11.37		0.118	
	11.73		0.091	
	11.95		0.098	
	12.28		0.068	
	12.44		0.132	
	12.74		0.047	
P	13.13	13.11	0.227	0.500
	13.55		0.046	
M	13.73	13.70	0.168	0.322
V	13.97	13.95	0.315	0.579
	14.23		0.031	
	14.29		0.023	
	14.48		0.058	
	14.64		0.194	
dptu	15.10	15.07	16.794	37.709
	15.70		0.081	
W	16.05	16.02	0.113	0.185
	16.37		0.403	
F	16.59	16.57	0.237	0.452
I	17.00	16.98	0.219	0.470
K	17.25	17.22	0.581	0.934
L	17.49	17.46	0.331	0.645
	17.75		0.044	
	17.84		0.058	
	18.00		0.042	
	18.45		0.029	
	18.60		0.030	

Wednesday, March 13, 2019 08:45:43

311LT-3 - Page 3 of 13

RESIDUE #1 = T

Figure S5. B) continued

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Tufts Core Facility
Tufts Medical School
136 Harrison Ave
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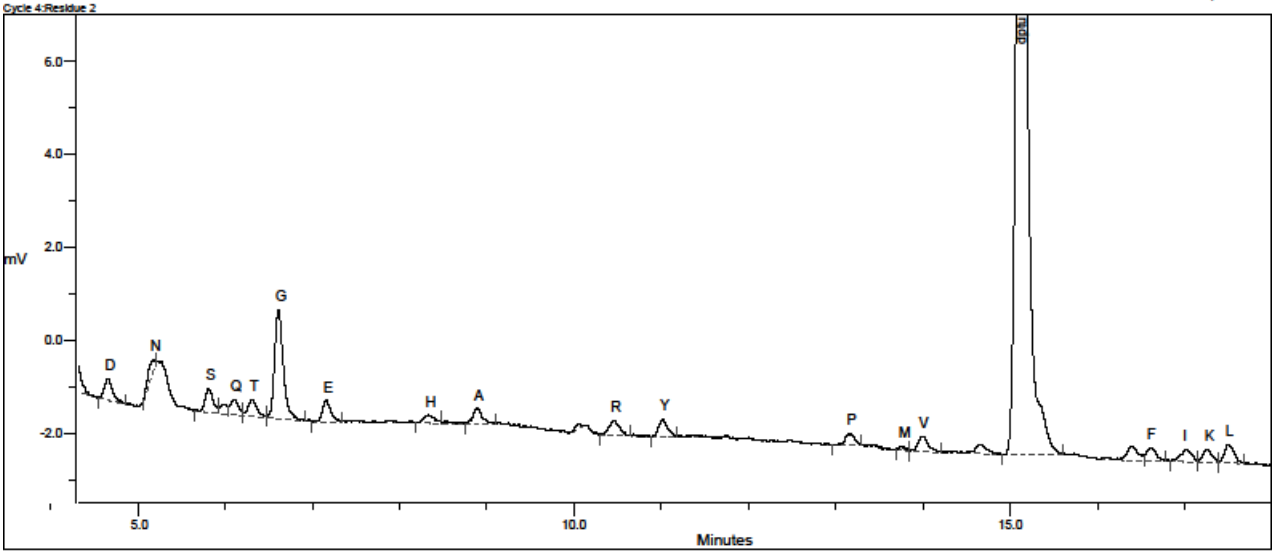
Cycle 4:Residue 2



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.15		14.125	
D	4.66	4.65	0.461	0.635
N	5.18	5.08	0.251	0.386
S	5.82	5.77	0.523	1.112
	6.00		0.201	
Q	6.10	6.06	0.322	0.548
T	6.32	6.26	0.370	0.994
G	6.61	6.57	2.356	4.407
	6.95		0.039	
E	7.17	7.11	0.461	0.679
	7.85		0.028	
	7.95		0.024	
H	8.33	8.27	0.184	0.370
	8.62		0.036	
	8.72		0.039	
A	8.89	8.84	0.342	0.604
	9.13		0.044	
	9.42		0.039	
	9.74		0.027	
	10.07		0.085	
R	10.46	10.40	0.317	0.978
	10.68		0.038	
	10.83		0.034	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
Y	11.02	10.96	0.376	0.720
	11.22		0.032	
	11.42		0.044	
	12.16		0.034	
	12.49		0.047	
	12.89		0.028	
P	13.16	13.11	0.249	0.547
M	13.75	13.70	0.089	0.170
V	13.99	13.95	0.332	0.610
	14.30		0.048	
	14.67		0.197	
dptu	15.13	15.07	21.124	47.434
	16.15		0.049	
	16.23		0.035	
	16.39		0.313	
F	16.60	16.57	0.281	0.536
I	17.02	16.98	0.278	0.595
K	17.27	17.22	0.279	0.449
L	17.50	17.46	0.398	0.775
	18.06		0.017	
	18.30		0.021	
	18.64		0.037	
	18.70		0.042	

Wednesday, March 13, 2019 08:45:44

311LT-3 - Page 4 of 13

RESIDUE #2 = G

Figure S5. B) continued

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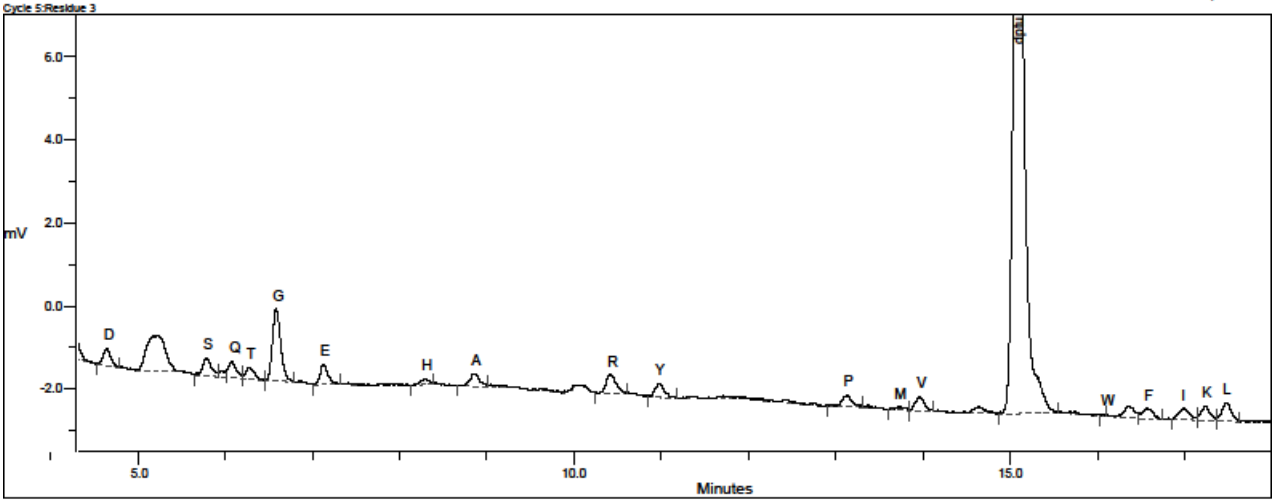
Cycle 5:Residue 3



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.13		14.342	
D	4.65	4.65	0.409	0.564
	4.92		0.029	
	5.22		0.846	
S	5.78	5.77	0.442	0.939
	5.97		0.156	
Q	6.09	6.06	0.393	0.670
T	6.28	6.26	0.286	0.769
G	6.58	6.57	1.732	3.239
E	7.13	7.11	0.489	0.721
	7.42		0.042	
	7.80		0.041	
	7.92		0.044	
	8.08		0.025	
H	8.29	8.27	0.134	0.268
	8.57		0.035	
A	8.86	8.84	0.307	0.543
	9.05		0.041	
	9.21		0.042	
	9.61		0.043	
	9.89		0.042	
	10.01		0.058	
R	10.42	10.40	0.451	1.392
Y	10.97	10.96	0.320	0.613
	11.31		0.028	
	11.71		0.060	
	12.11		0.059	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	12.28		0.052	
	12.41		0.057	
	12.61		0.037	
	12.73		0.039	
P	13.13	13.11	0.281	0.618
	13.37		0.072	
M	13.71	13.70	0.075	0.144
V	13.96	13.95	0.350	0.644
	14.34		0.033	
	14.48		0.032	
	14.64		0.147	
dptu	15.09	15.07	17.097	38.390
	15.70		0.049	
	15.98		0.023	
W	16.08	16.02	0.028	0.045
	16.36		0.275	
F	16.57	16.57	0.255	0.486
	16.77		0.030	
I	17.00	16.98	0.268	0.573
K	17.23	17.22	0.343	0.551
L	17.47	17.46	0.440	0.857
	17.68		0.038	
	17.75		0.028	
	17.84		0.028	
	18.27		0.028	
	18.60		0.040	

Wednesday, March 13, 2019 08:45:44

311LT-3 - Page 5 of 13

RESIDUE #3 = R

Figure S5. B) continued

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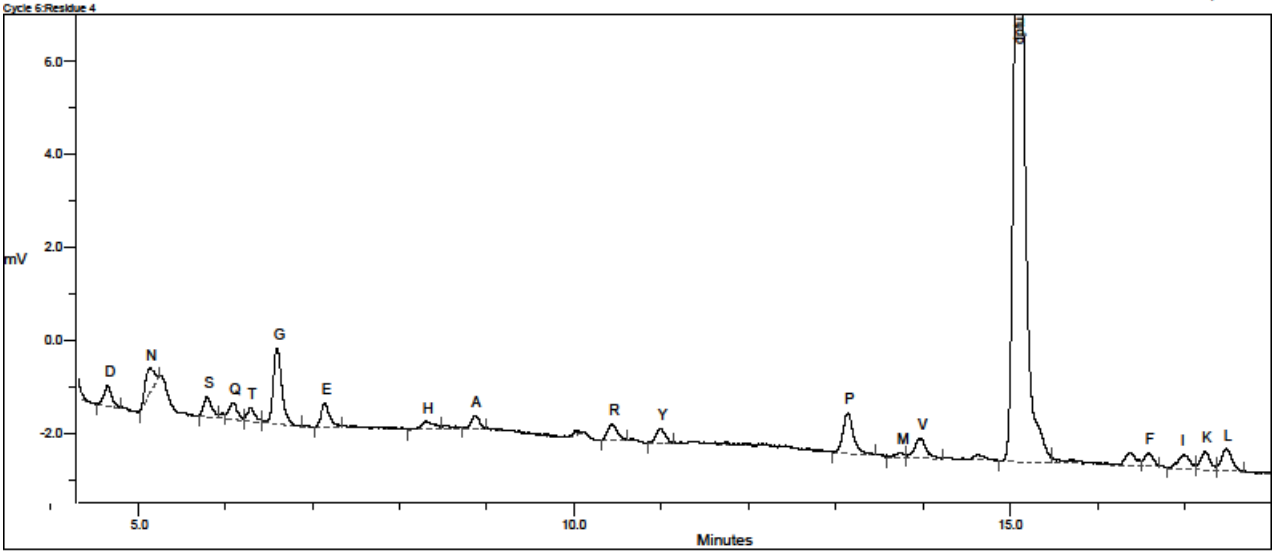
Boston, MA 02111

Cycle 6:Residue 4



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.13		13.724	
D	4.66	4.65	0.441	0.608
N	5.13	5.08	0.567	0.872
S	5.80	5.77	0.445	0.945
	5.97		0.130	
Q	6.09	6.06	0.361	0.615
T	6.29	6.26	0.285	0.764
G	6.60	6.57	1.643	3.073
	6.90		0.038	
E	7.14	7.11	0.525	0.774
	7.45		0.029	
	7.85		0.029	
	8.03		0.048	
H	8.30	8.27	0.178	0.358
	8.54		0.078	
	8.64		0.058	
A	8.86	8.84	0.292	0.516
	9.16		0.052	
	9.68		0.032	
	10.02		0.106	
R	10.44	10.40	0.358	1.105
Y	11.00	10.96	0.308	0.590
	11.23		0.030	
	11.33		0.023	
	11.74		0.057	
	11.89		0.032	
	11.96		0.047	
	12.07		0.042	
P	13.14	13.11	0.878	1.930
M	13.74	13.70	0.109	0.210
V	13.98	13.95	0.432	0.794
	14.45		0.041	
	14.63		0.128	
dptu	15.10	15.07	15.947	35.807
	15.73		0.078	
	16.20		0.037	
	16.37		0.290	
F	16.58	16.57	0.282	0.538
I	16.99	16.98	0.304	0.651
K	17.23	17.22	0.398	0.639
L	17.48	17.46	0.478	0.930
	17.92		0.030	
	18.14		0.039	
	18.35		0.027	
	18.43		0.042	

Wednesday, March 13, 2019 08:45:44

311LT-3 - Page 6 of 13

RESIDUE #4 = P

Figure S5. B) continued

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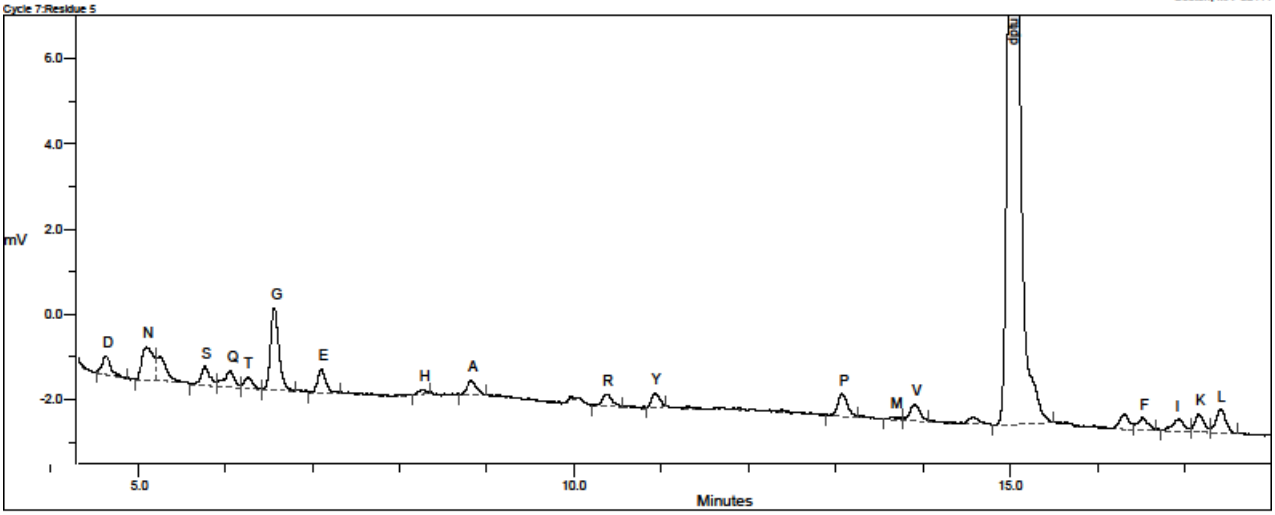
Tufts Core Facility
Tufts Medical School
136 Harrison Ave
Boston, MA 02111

Cycle 7:Residue 5



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.11		13.317	
D	4.63	4.65	0.449	0.618
N	5.10	5.08	0.778	1.198
	5.25		0.572	
S	5.77	5.77	0.447	0.951
Q	6.06	6.06	0.385	0.657
T	6.25	6.26	0.265	0.710
G	6.56	6.57	1.937	3.623
E	7.11	7.11	0.558	0.822
	7.42		0.036	
	7.55		0.046	
	7.75		0.021	
	8.03		0.039	
	8.11		0.028	
H	8.27	8.27	0.105	0.210
	8.52		0.033	
	8.68		0.037	
A	8.82	8.84	0.351	0.621
	9.24		0.043	
	9.48		0.032	
	9.96		0.083	
R	10.37	10.40	0.286	0.883
	10.60		0.040	
	10.80		0.044	
Y	10.93	10.96	0.328	0.630
	11.15		0.030	
	11.30		0.052	
	11.64		0.026	
	11.83		0.040	
	11.90		0.053	
	12.24		0.031	
	12.37		0.054	
P	13.07	13.11	0.536	1.177
	13.30		0.026	
M	13.67	13.70	0.075	0.144
V	13.91	13.95	0.390	0.718
	14.10		0.035	
	14.31		0.041	
	14.57		0.151	
dptu	15.03	15.07	22.251	49.964
	15.58		0.020	
	15.76		0.028	
	16.31		0.353	
F	16.52	16.57	0.286	0.546
	16.70		0.044	
I	16.93	16.98	0.285	0.609
K	17.16	17.22	0.419	0.674
L	17.41	17.46	0.543	1.056
	18.10		0.030	
	18.25		0.038	
	18.39		0.040	
	18.72		0.035	

Wednesday, March 13, 2019 08:45:44

311LT-3 - Page 7 of 13

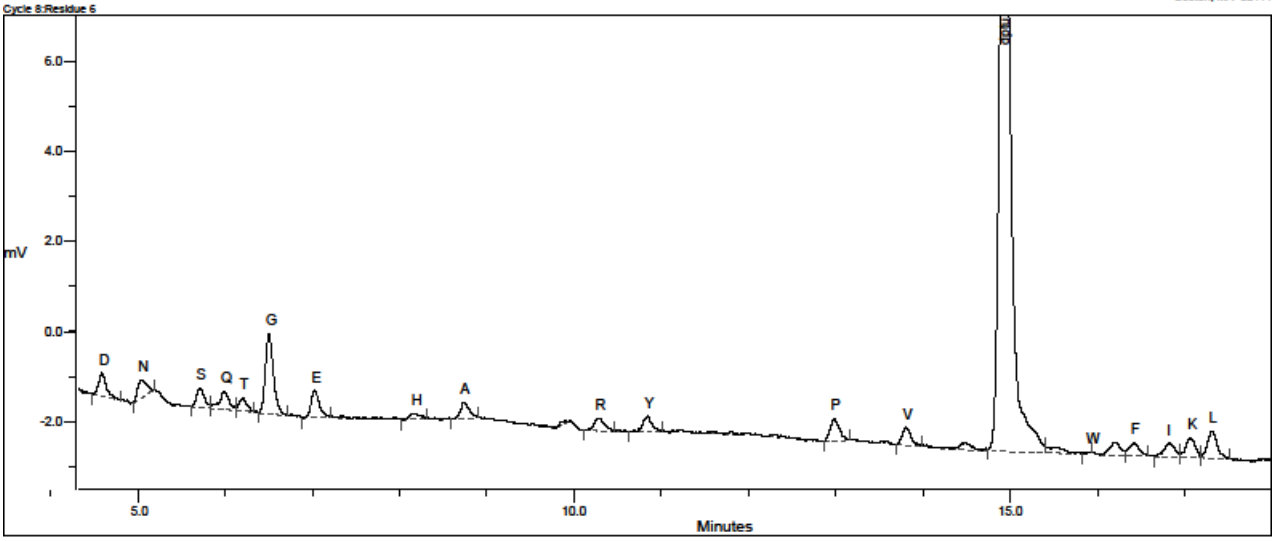
RESIDUE #5 = G

Figure S5. B) continued

SequencePro™

Tufts Core Facility
Tufts Medical School
136 Harrison Ave
Boston, MA 02111

Cycle 8:Residue 6



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.08		12.434	
D	4.59	4.65	0.514	0.708
N	5.04	5.08	0.410	0.631
S	5.70	5.77	0.431	0.917
Q	5.99	6.06	0.398	0.678
T	6.20	6.26	0.283	0.760
G	6.50	6.57	1.768	3.307
	6.75		0.038	
E	7.03	7.11	0.604	0.891
	7.33		0.044	
	7.53		0.042	
	7.58		0.049	
	7.74		0.039	
	7.93		0.036	
H	8.17	8.27	0.128	0.256
	8.51		0.033	
	8.57		0.034	
A	8.73	8.84	0.353	0.623
	9.75		0.023	
	9.91		0.037	
R	10.28	10.40	0.282	0.871
	10.50		0.040	
	10.58		0.029	
Y	10.85	10.96	0.342	0.657

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.13		0.035	
	11.58		0.047	
	11.80		0.064	
	12.29		0.062	
P	12.98	13.11	0.494	1.085
V	13.80	13.95	0.405	0.745
	14.25		0.038	
	14.49		0.151	
	14.72		0.056	
dptu	14.93	15.07	17.263	38.764
	15.53		0.104	
W	15.91	16.02	0.021	0.034
	16.20		0.292	
F	16.42	16.57	0.282	0.538
I	16.83	16.98	0.304	0.651
K	17.07	17.22	0.447	0.718
L	17.31	17.46	0.599	1.166
	17.59		0.038	
	17.78		0.036	
	17.95		0.029	
	18.22		0.031	
	18.34		0.032	
	18.75		0.034	
	18.88		0.043	

Wednesday, March 13, 2019 08:45:44

311LT-3 - Page 8 of 13

RESIDUE #6 = -

Figure S5. B) continued

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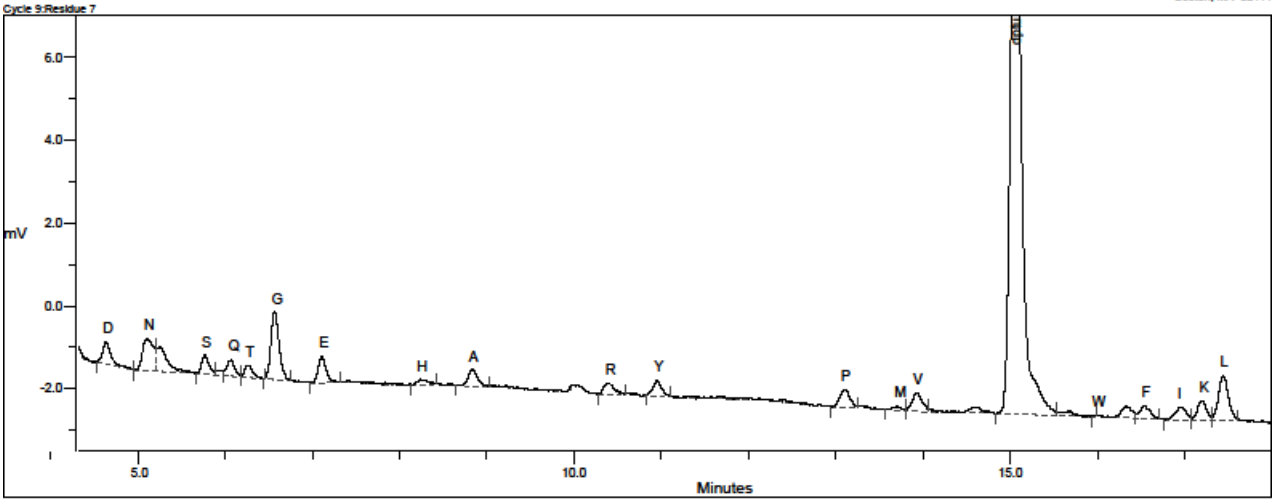
Cycle 9:Residue 7



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.12		13.677	
D	4.63	4.65	0.540	0.744
N	5.11	5.08	0.769	1.183
	5.26		0.598	
S	5.77	5.77	0.453	0.964
	5.95		0.123	
Q	6.07	6.06	0.395	0.674
T	6.27	6.26	0.300	0.804
G	6.57	6.57	1.638	3.064
	6.92		0.031	
E	7.11	7.11	0.655	0.967
	7.42		0.060	
	7.61		0.033	
	7.83		0.050	
H	8.24	8.27	0.134	0.269
	8.58		0.043	
A	8.83	8.84	0.402	0.711
	9.05		0.025	
	9.22		0.042	
	9.82		0.039	
	9.99		0.063	
R	10.40	10.40	0.270	0.833
	10.80		0.043	
Y	10.95	10.96	0.370	0.710
	11.35		0.069	
	11.57		0.033	
	11.69		0.055	
	11.88		0.043	
	12.12		0.034	
	12.35		0.044	
	12.42		0.062	
	12.64		0.038	
	12.80		0.040	
P	13.10	13.11	0.417	0.915
M	13.71	13.70	0.098	0.189
V	13.93	13.95	0.458	0.843
	14.20		0.044	
	14.28		0.044	
	14.34		0.032	
	14.61		0.125	
dptu	15.06	15.07	15.700	35.253
	15.68		0.102	
	15.91		0.019	
W	15.98	16.02	0.024	0.039
	16.34		0.280	
F	16.54	16.57	0.311	0.593
I	16.95	16.98	0.308	0.659
K	17.20	17.22	0.471	0.757
L	17.44	17.46	1.063	2.069
	17.90		0.046	
	18.09		0.027	
	18.28		0.035	
	18.48		0.022	
	18.68		0.029	

Wednesday, March 13, 2019 08:45:44

311LT-3 - Page 9 of 13

RESIDUE #7 = L

Figure S5. B) continued

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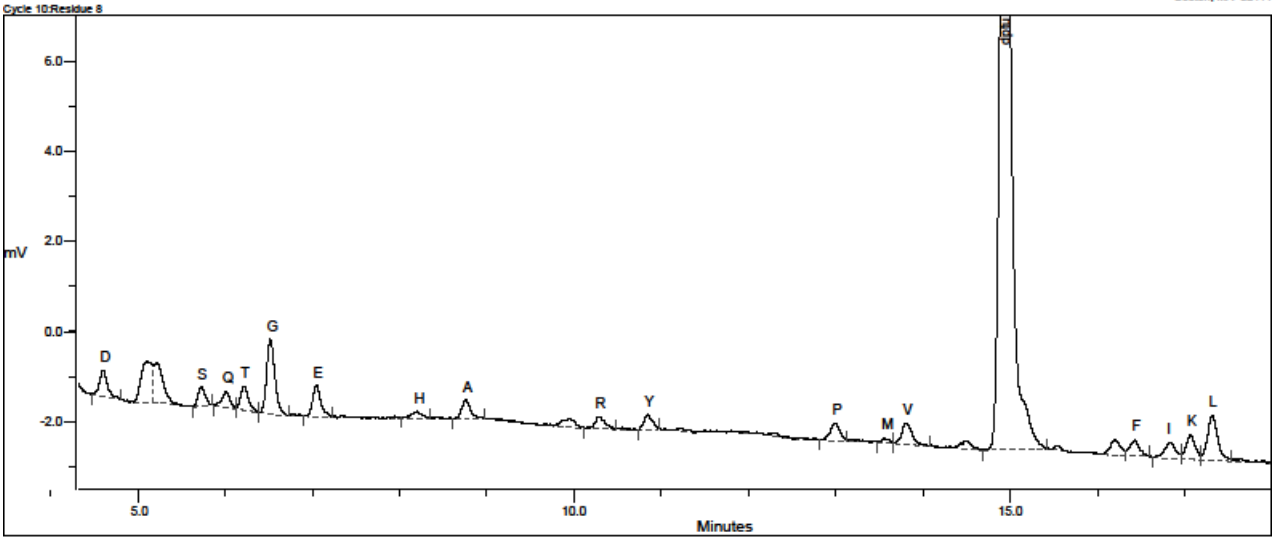
Cycle 10:Residue 8



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.09		14.082	
D	4.60	4.65	0.567	0.781
	5.10		0.912	
	5.21		0.895	
S	5.72	5.77	0.422	0.896
Q	6.01	6.06	0.363	0.619
T	6.22	6.26	0.516	1.386
G	6.52	6.57	1.656	3.098
	6.78		0.043	
E	7.05	7.11	0.686	1.012
	7.34		0.046	
	7.81		0.047	
	7.95		0.047	
H	8.20	8.27	0.150	0.302
	8.57		0.044	
A	8.76	8.84	0.426	0.753
	9.94		0.182	
R	10.28	10.40	0.259	0.799
	10.55		0.059	
	10.63		0.027	
Y	10.84	10.96	0.333	0.638
	11.03		0.028	
	11.19		0.050	
	11.50		0.032	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.95		0.050	
	12.29		0.076	
	12.60		0.043	
	12.77		0.039	
P	13.00	13.11	0.390	0.858
	13.20		0.038	
M	13.56	13.70	0.108	0.207
V	13.81	13.95	0.480	0.882
	14.26		0.028	
	14.49		0.180	
dptu	14.94	15.07	19.670	44.169
	15.56		0.091	
	16.20		0.328	
F	16.43	16.57	0.352	0.672
I	16.83	16.98	0.354	0.757
K	17.06	17.22	0.551	0.885
L	17.32	17.46	1.003	1.952
	17.65		0.067	
	17.82		0.044	
	18.03		0.031	
	18.11		0.034	
	18.49		0.050	
	18.59		0.046	
	18.75		0.030	

Wednesday, March 13, 2019 08:45:45

311LT-3 - Page 10 of 13

RESIDUE #8 = T

Figure S5. B) continued

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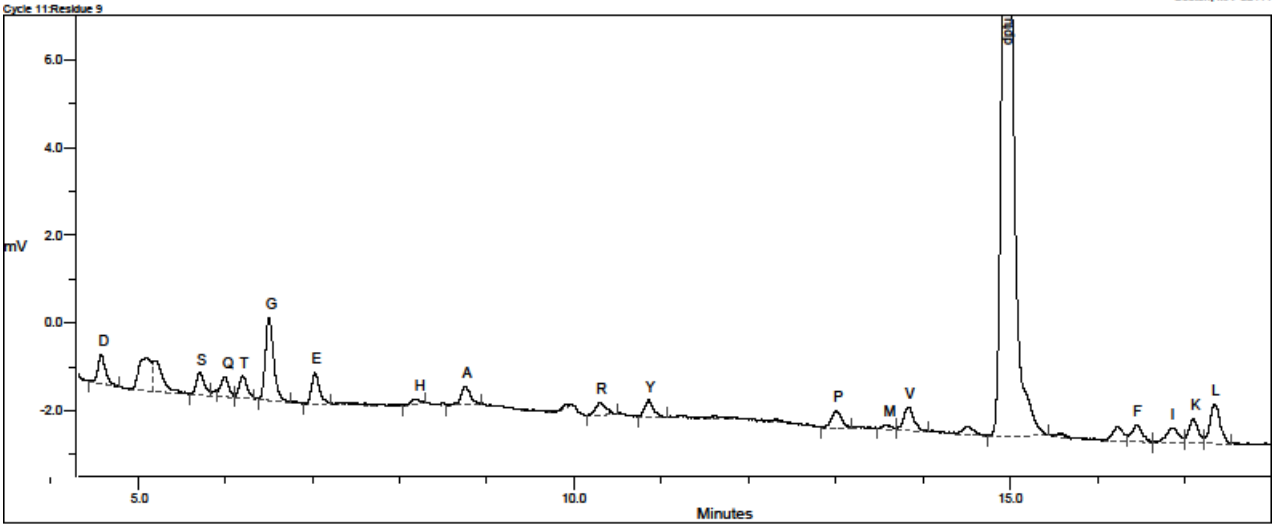
Cycle 11:Residue 9



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.07		14.041	
D	4.58	4.65	0.668	0.919
	5.09		0.762	
	5.19		0.695	
S	5.71	5.77	0.507	1.077
	5.90		0.142	
Q	6.00	6.06	0.449	0.766
T	6.20	6.26	0.505	1.358
G	6.50	6.57	1.884	3.524
E	7.03	7.11	0.717	1.057
	7.35		0.037	
	7.61		0.047	
	7.71		0.044	
	8.02		0.045	
H	8.21	8.27	0.111	0.223
	8.50		0.046	
A	8.75	8.84	0.424	0.749
	9.04		0.021	
	9.41		0.026	
	9.54		0.030	
	9.72		0.026	
	9.91		0.070	
R	10.29	10.40	0.283	0.875
Y	10.86	10.96	0.385	0.739
	11.11		0.030	
	11.52		0.036	
	11.62		0.052	
	11.84		0.042	
	11.98		0.025	
	12.31		0.065	
P	13.01	13.11	0.391	0.859
	13.27		0.033	
M	13.59	13.70	0.104	0.199
V	13.83	13.95	0.538	0.989
	14.30		0.038	
	14.51		0.180	
dptu	14.96	15.07	19.453	43.680
	15.58		0.100	
	16.23		0.336	
F	16.44	16.57	0.388	0.741
I	16.87	16.98	0.346	0.740
K	17.10	17.22	0.551	0.886
L	17.35	17.46	0.912	1.775
	17.73		0.049	
	17.86		0.030	
	18.07		0.035	
	18.23		0.039	
	18.35		0.035	
	18.60		0.036	
	18.89		0.035	

Wednesday, March 13, 2019 08:45:45

311LT-3 - Page 11 of 13

RESIDUE #9 = G

Figure S5. B) continued

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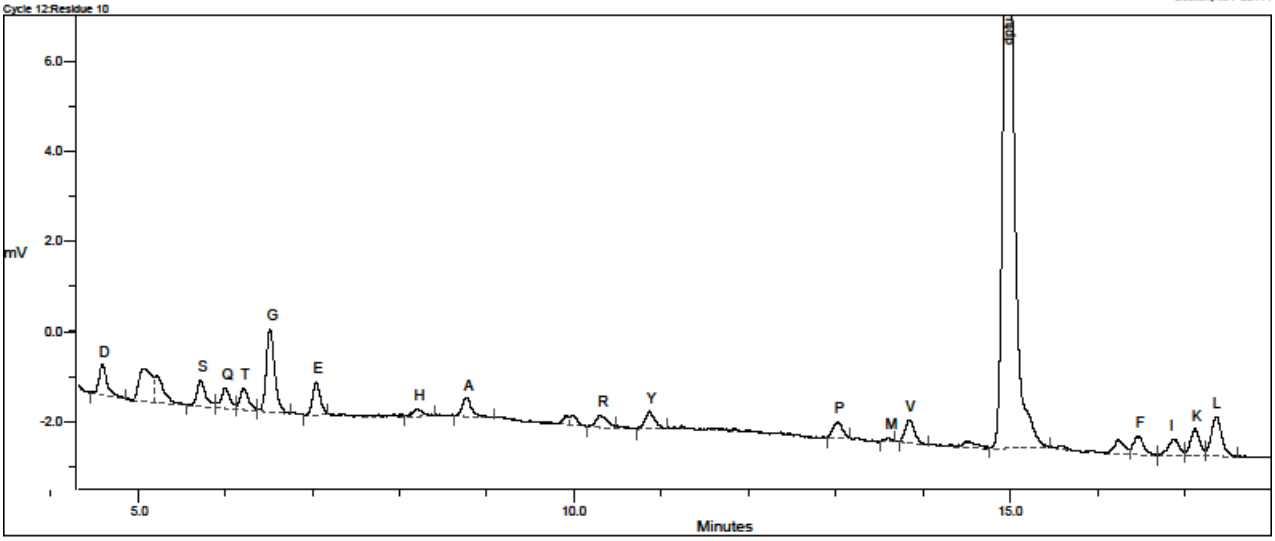
Cycle 12:Residue 10



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.08		13.828	
D	4.59	4.65	0.673	0.927
	5.08		0.732	
	5.22		0.607	
S	5.72	5.77	0.581	1.235
Q	6.00	6.06	0.462	0.787
T	6.21	6.26	0.489	1.314
G	6.51	6.57	1.850	3.460
E	7.05	7.11	0.742	1.094
	7.65		0.036	
	7.76		0.047	
	8.03		0.048	
H	8.20	8.27	0.167	0.334
	8.42		0.038	
A	8.77	8.84	0.403	0.712
	9.50		0.039	
	9.90		0.183	
	9.98		0.207	
R	10.31	10.40	0.266	0.822
	10.56		0.040	
	10.70		0.029	
Y	10.87	10.96	0.392	0.751
	11.15		0.026	
	11.23		0.064	
	11.45		0.026	
	11.62		0.039	
	11.83		0.061	
	12.86		0.039	
P	13.03	13.11	0.373	0.820
M	13.60	13.70	0.083	0.158
V	13.84	13.95	0.503	0.924
	14.33		0.039	
	14.53		0.153	
	14.74		0.057	
dptu	14.98	15.07	15.201	34.132
	15.57		0.079	
	16.25		0.313	
F	16.48	16.57	0.405	0.773
I	16.86	16.98	0.375	0.802
K	17.12	17.22	0.605	0.972
L	17.36	17.46	0.883	1.720
	17.68		0.038	
	18.07		0.036	
	18.22		0.033	
	18.53		0.046	
	18.60		0.030	
	18.73		0.032	
	18.90		0.035	

Wednesday, March 13, 2019 08:45:45

311LT-3 - Page 12 of 13

RESIDUE #10 = -

10 cycles total

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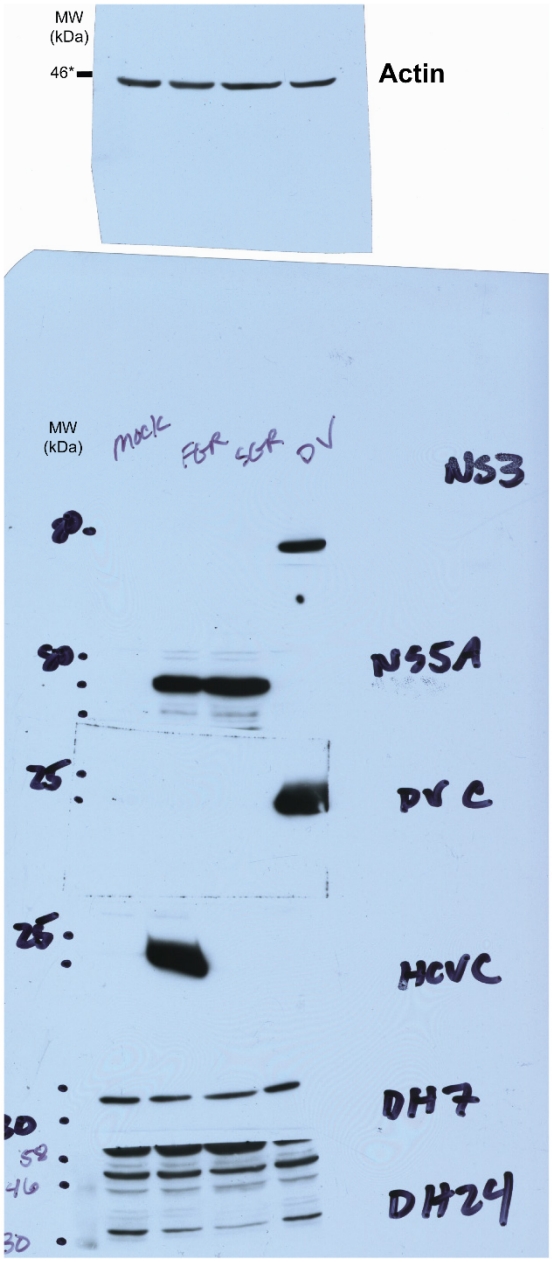
Raw Pmol Table

	ASP	ASN	SER	GLN	THR	GLY	GLU	HIS	ALA	ARG	TYR	PRO	MET	VAL	TRP	PHE	ILE	LYS	LEU
1	0.917	0.292	1.472	0.383	3.034	3.531	0.694	0.400	0.739	0.874	0.637	0.500	0.322	0.579	0.185	0.452	0.470	0.934	0.645
2	0.635	0.386	1.112	0.548	0.994	4.407	0.679	0.370	0.604	0.978	0.720	0.547	0.170	0.610	0.000	0.536	0.595	0.449	0.775
3	0.564	0.000	0.939	0.670	0.769	3.239	0.721	0.268	0.543	1.392	0.613	0.618	0.144	0.644	0.045	0.486	0.573	0.551	0.857
4	0.608	0.872	0.945	0.615	0.764	3.073	0.774	0.358	0.516	1.105	0.590	1.930	0.210	0.794	0.000	0.538	0.651	0.639	0.930
5	0.618	1.198	0.951	0.657	0.710	3.623	0.822	0.210	0.621	0.883	0.630	1.177	0.144	0.718	0.000	0.546	0.609	0.674	1.056
6	0.708	0.631	0.917	0.678	0.760	3.307	0.891	0.256	0.623	0.871	0.657	1.085	0.000	0.745	0.034	0.538	0.651	0.718	1.166
7	0.744	1.183	0.964	0.674	0.804	3.064	0.967	0.269	0.711	0.833	0.710	0.915	0.189	0.843	0.039	0.593	0.659	0.757	2.069
8	0.781	0.000	0.896	0.619	1.386	3.098	1.012	0.302	0.753	0.799	0.638	0.858	0.207	0.882	0.000	0.672	0.757	0.885	1.952
9	0.919	0.000	1.077	0.766	1.358	3.524	1.057	0.223	0.749	0.875	0.739	0.859	0.199	0.989	0.000	0.741	0.740	0.886	1.775
10	0.927	0.000	1.235	0.787	1.314	3.460	1.094	0.334	0.712	0.822	0.751	0.820	0.158	0.924	0.000	0.773	0.802	0.972	1.720

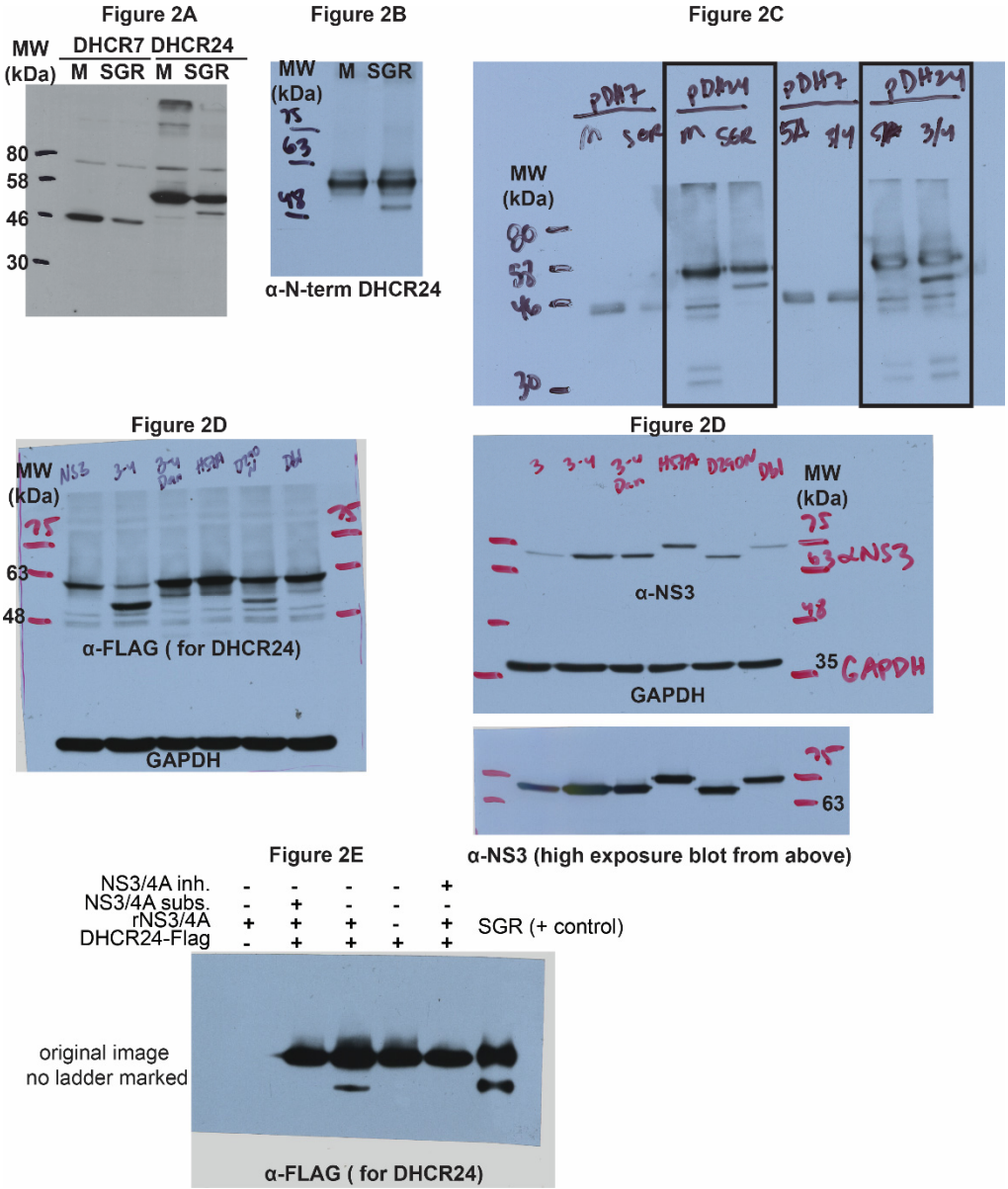
Figure S6. Original scans of immunoblots from Figures 1D, 2, and 4B showing MW ladder.

Original scanned immunoblot for Figure 1D with ladder. Note that MW marks were unfortunately not preserved for the actin blot. The location of the actin band relative to core and NS5A was consistent between this and other immunoblots in which the MW markers were recorded. We have therefore indicated the approximate location of the 46 kDa marker relative to actin on comparable immunoblots and indicated this with an "*" mark.

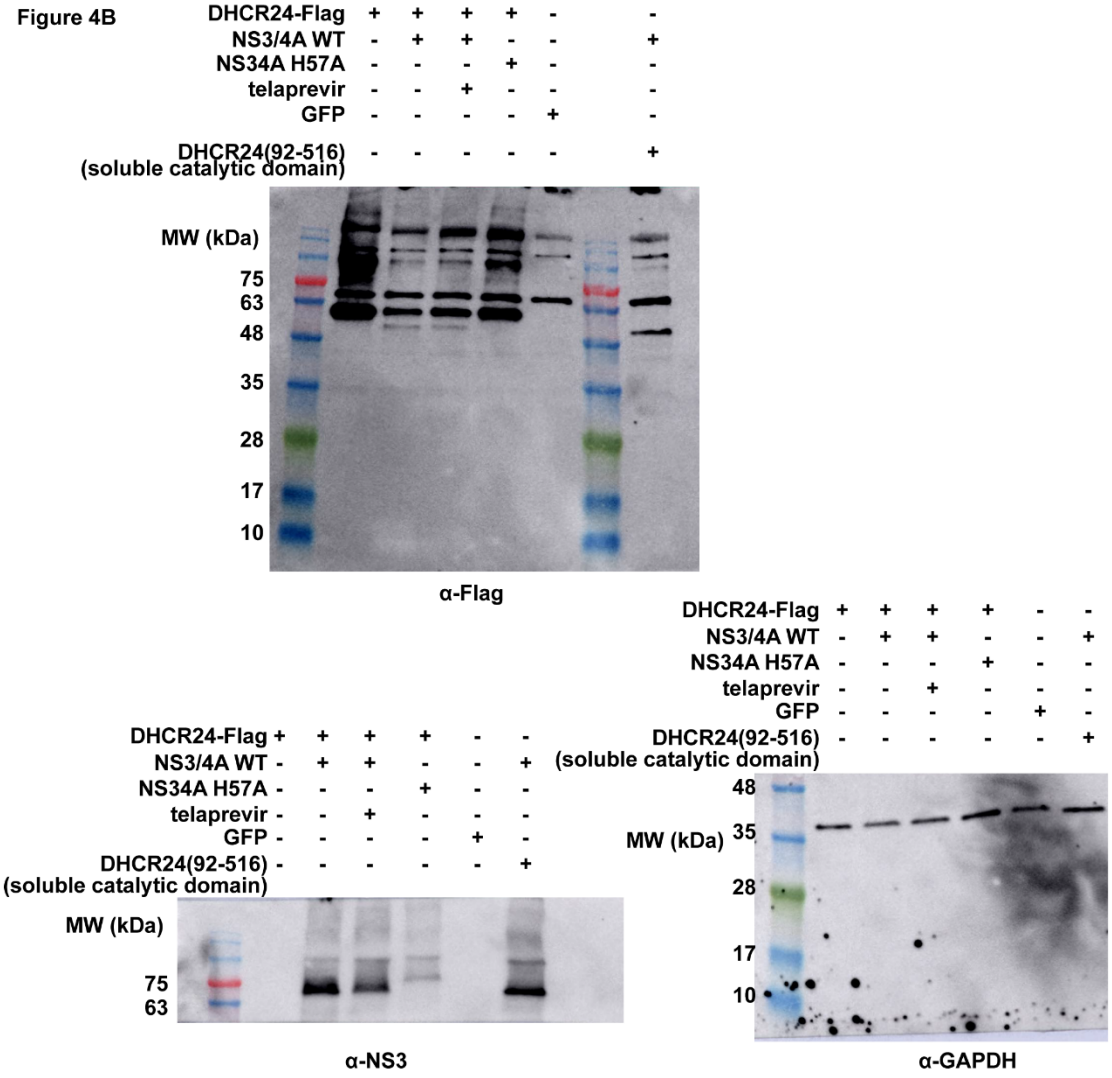
Figure 1D



Original scanned immunoblots for Figure 2 with respective MW ladders. The areas highlighted by boxes in Figure 2C were discussed in the main study. While there is no marker indicated in the image presented in Figure 2E (excised during processing), the relative locations of the upper and lower bands are consistent with DHCR24 (higher) and DHCR24* (lower) observed in other experiments.



Original scanned immunoblots for Figure 4B with respective MW ladders.



DETAILED EXPERIMENTAL PROCEDURES

A. Cells

A1. Huh7.5 cells wild-type. Huh7.5 cells were obtained from Charles Rice (Rockefeller University) and cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with nonessential amino acids and 10% fetal bovine serum (FBS, Invitrogen) at 37°C incubator with 5% CO_2 (53).

A2. Huh7.5-SGR cells-JFH1 (2a). Huh7.5-SGR cells stably replicating the HCV JFH1 (2a) subgenomic replicon Huh7.5 cells were electroporated with 7.5 µg of JFH1 subgenomic replicon *in vitro* transcript as previously described [Kato 2003]. Twenty-four hours after electroporation, cells were maintained in Huh7.5 cell in DMEM supplemented with nonessential amino acids, 10% FBS (FBS, Invitrogen) supplemented with 750 ug/mL G418 sulfate (18).

A3. Generation of *DHCR24^{KO}* Huh7.5 cells. *DHCR24^{KO}* Huh7.5 cells were generated by CRISPR/Cas9-mediated genome editing as previously described (55). DHCR24 has two isoforms that largely differ in the *N*-terminal region encoded in Exon 1. For this reason, Exon 2 was chosen as the target sequence. The removal introduced a frame shift resulting in a non-functional gene and partial removal of the *N*-terminal DHCR24 antibody epitope (DHCR24 residues 68-84). Two guide RNAs targeting removal of the splicing junction between intron1/exon2 encoded in exon 2 of DHCR24 (gRNA1: 5'-GCTTGTGGTGTAGCAATATGT-3' and gRNA2: 5'-GCTCACTGTCTCACTACGTGTG-3') were employed.

The CRISPR-DHCR24 double gRNA construct was transfected into Huh7.5 cells using Lipofectamine 2000 (Thermo Fisher Scientific) according to manufacturer's protocolin a T-75 flask seeded at a density of 2.1 x10^6 cells/mL following the manufacturer's protocol. The cells were cultured overnight at 37°C with 5% CO_2. Following transfection, the medium was replaced with medium containing a final concentration of 4 µg/mL puromycin (InvivoGen) to select for Huh7.5 cells containing transfected pCRISPR-DHCR24 2x-gRNA-*DHCR24^{KO}*. Cells viable after selection with puromycin over several days were subjected to monoclonal selection by dilution of the cells to 10 cells/10 mL resuspended in sterile-filtered 50% preconditioned media for monoclonal limiting dilution in a 96-well plate in the absence of puromycin. Medium in the wells was replaced every 7 days with non-conditioned media until the appearance of single colonies (~5 weeks) was observed. Upon observation of single colonies, wells containing single colonies were amplified and characterized by Sanger sequencing and immunoblot analysis for the elimination of the *N*-terminal DHCR24 epitope (Figure S3). Genome editing was confirmed by PCR. Diagnostic primers: Forward primer: 5'-AGA TGC CTA GTG TGT TGG GAA T-3' and Reverse primer: 5'-AAA GGG TTC CCC TGA CCT ATT A-3'.

A4. *DHCR24^{KO}* Huh7.5 cells. *DHCR24^{KO}* Huh7.5 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with nonessential amino acids and 10% fetal bovine serum (FBS, Invitrogen) at 37°C incubator with 5% CO_2 without any supplementation of cholesterol.

A5. VEEV-NS3-5B cells Huh7.5 cells. VEEV-NS3-5B Huh7.5 cells were obtained from Brett Lindenbach (Yale University) and cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with nonessential amino acids and 10% fetal bovine serum (FBS, Invitrogen) at 37°C with 5% CO_2 (36) with puromycin (5 µg/mL).This experimental model was utilized for immunofluorescence studies because it supports physiological expression of the nonstructural proteins in a system not dependent upon HCV RNA replication.

B. Plasmids and cloning

B1. *In cellulo* plasmids. pCMV-DHCR24-MYC-DYKDDDDK and pCMV-DHCR7-MYC-DYKDDDDK were purchased from Origene. NS3, NS3-4A, NS4B, NS5A, and NS5B were amplified from pJFH1 and subcloned into pCMV-Entry6 (Origene) using standard restriction cloning. Site-directed mutagenesis was applied to introduce a stop codon to eliminate inclusion of MYC- DYKDDDDK for viral proteins and to introduce mutations (H57A, D290A, and double mutant H57A/D290A) to NS3-4A by QuikChange II Site-Directed Mutagenesis (Agilent). pIRES_DHCR24-Flag constructs containing a hygromycin resistant cassette were made using NEBuilder and NEBuilder HiFi DNA Assembly Master Mix (NEB). A silent mutation at Arg103 was made to prevent any possible guide RNAs from recognizing newly transfected pIRES_DHCR24-Arg103-Flag constructs and mutants in *DHCR24*^{KO} Huh7.5 cells. pIRES_DHCR24-Flag mutants (C91P, C91N, C91F, C91A-T92A, C91V-T92V, C91S, C91T, and ΔC91-92) were designed and generated using In-Fusion Cloning Primer Design Tool online software, CloneAmp HiFi PCR Premix for PCR amplification of the linearized template (pIRES_DHCR24-Arg103-Flag) (Takara), and In-Fusion HD Cloning Plus (Takara).

B2. *In vitro* plasmids. pMALc5x_maltose binding protein (MBP)-DHCR24 and pMALc5x-DHCR24 (full-length)-super folded green fluorescent protein (sfGFP) containing an ampicillin resistant cassette were purchased from GenScript USA Inc. The super-folded (sf)GFP cassettes were based on constructs that have previously been expressed and described (56). The various surrogate DHCR24 peptide constructs spanning residues 56-110, 52-66, and 85-99, and the 85-99-C91P mutant were expressed as fusions between an *N*-terminal MBP and sfGFP with a *C*-terminal DYKDDDDK (Flag-Tag). Expressions constructs were generated by overlapping extension PCR using Q5 High-Fidelity DNA Polymerase (NEB) and were subcloned into a pET28b plasmid containing a kanamycin resistance cassette. These plasmids were transformed into Rosetta2 (DE3) pLysS host strain (Novagen) following the manufacturer's protocol.

C. Immunoprecipitation

C1. Preparation of samples for immunoprecipitation of DHCR7-Flag and DHCR24-Flag. Transfection of Huh7.5 cells, or Huh7.5 cells stably harboring SGR or SGR-GFP under G418 selection (750 µg/mL), were transfected with plasmids using Lipofectamine 2000 (Thermo Fisher Scientific) within 30 minutes of seeding. For a T75 flask, Huh7.5 cells were seeded at 60% confluency and transfected with 20 µg of either DHCR7-Flag or DHCR24-Flag, 20 µL of Lipofectamine 2000 (Thermo Fisher Scientific), and one mL of Opti-MEM (Gibco) with incubation times followed according to manufacturer's protocol. Sixteen hours later, the cell culture medium was removed and replaced with fresh medium. Forty eight hours after the addition of transfection reagents, the cells were lysed with RIPA buffer (Boston BioProducts) and HALT protease inhibitor cocktail (Roche) and stored at -20ºC until analysis.

C2. Immuno precipitation and immunoblot analysis of immunoprecipitates. Samples for immunoprecipitation were thawed on ice and clarified by centrifugation (18,000 x *g*) for five minutes. Protein lysate was collected by vigorously pipeting cell pellets in RIPA buffer supplemented with Halt protease cocktail inhibitor (Roche), and protein content was quantified by Bradford assay (Pierce). 50 µg of protein (each of two biological replicate) was applied to the

magnetic anti-DYKDDDDK resin (Clontech), which was incubated with supernatant overnight at 4ºC with a tube rotisserie. After incubation, the resin was separated magnetically and washed six times with RIPA buffer. Sample was boiled and eluted with 1X SDS denaturing loading dye to elute immunoprecipitated protein and subjected to separation by SDS-PAGE followed by transfer to a PVDF membrane (semidry transfer at 10 volts for 1.5 hours). The membrane was washed with TBST followed by blocking in 5% milk (TBST, w/v). Post-blocking, the membranes were incubated with their respective primary antibodies overnight at 4°C rocking in the dark. The membranes were then washed with TBST, followed by incubation with a species-specific secondary antibody. After washing with TBST, the membrane was incubated with a chemiluminescent reagent (Pierce) and exposed to an X-ray film for imaging.

Samples containing DHCR24-MYC-DYKDDDDK and DHCR7-MYC-DYKDDDDK were detected with anti-FLAG-horseradish peroxidase M2 (Sigma). Additional antibodies were purchased or acquired for immunoblot analysis: Anti-NS3 (1:1000) (Abcam), anti-NS5A (1:1000) (9E10 from C. Rice), anti-GAPDH (1:25,000) (Genetex GTX28245), and anti-DHCR24 (1:500) (Sigma S8571). Each respective blot was incubated with their respective species-specific secondary horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (BioRad 170-6516) and anti-rabbit IgG (BioRad 170-6515) for 1 hour at room temperature.

C3. Co-transfection of viral genes and DHCR24-Flag. 3 x 10^5 Huh7.5 cells were seeded per well in a 6-well plate. Cells were co-transfected with 1 µg pCMV-NS3-4A (variants or NS3) + 2.6 µg pCMV-DHCR24-FLAG (WT) + 4 µL Lipofectamine and 250 µL Opti-Mem. Cells were allowed to recover at 37 °C for 20 min prior to addition of Lipo-DNA mix. Danoprevir, an NS3-4A inhibitor, (10 µM) was immediately added after delivery of Lipo-DNA mix. After 48 hours post-transfection, cells were lysed with RIPA buffer and frozen at 20°C. Thawed, clarified lysates were analyzed with anti-DHCR24, NS3 or GAPDH antibody. Immunoblot analysis is repeated as described in *Immunoblot analysis of immunoprecipitates.*

C4. Co-transfection studies of DHCR24-Flag with variants of NS3-4A. Analysis of ectopically expressed DHCR24-Flag: 90% confluent Huh7.5 cells or Huh7.5/SGR cells (750 µg/mL G418) were plated in a T75 flask (1:2 split) and allowed to recover in complete medium for 25 minutes prior to addition of Lipofectamine-DNA complexes containing Lipofectamine 2000 + DNA: 20 µL Lipofectamine + 20 µg pCMV-DHCR24-MYC- DYKDDDDK. For experiments involving NS5A and NS3: 40 µL Lipofectamine + 20 µg pCMV DHCR24-MYC- DYKDDDDK + 20 µg pCMV-NS5A or pCMV-NS3-4A. Danoprevir (10 µM) was immediately added after delivery of the Lipofectamine-DNA mix. After 18 hours, the medium was changed. Cells were then harvested 68 hours post-transfection with non-denaturing lysis buffer (10 mM phosphate, 150 mM NaCl, 1% NP40 pH 7.5). DHCR24-Flag was immunoprecipitated as previously described. Analysis of endogenous DHCR24: T150 flasks were seeded with Huh7.5/SGR (under 750 µg/mL G418 selection) or Huh7.5 cells. 72 hours later, cells were harvested with RIPA non-denaturing lysis buffer. ~1 mL supernatant was incubated with 2 µL of anti-DHCR24 antibody (Sigma S8571) overnight at 4° C. 15 hours later, 40 µL of goat anti-rabbit resin was added and incubated for four hours. The resin was washed with non-denaturing lysis buffer using gravity flow. Samples were eluted with 1X SDS denaturing loading dye. Thawed, clarified samples were analyzed by immunoblot using anti-DHCR24, NS3 or GAPDH antibodies.

D. Protein expression

D1. Recombinant expression. MBP-DHCR24-56-110-GFP, MBP-DHCR24-52-66-GFP, MBP-DHCR24-85-99, and MBP-DHCR24-85-99-C91P-GFP all have a *C*-terminal DYKDDDDK (Flag) tag and were recombinantly expressed in the Rosetta2 (DE3) pLysS (Novagen) *E. coli* host strain. Cells were grown in terrific broth media containing chloramphenicol (25 µg/mL) and kanamycin sulfate (50 µg/mL). When the optical density reached 0.8-1.0 at 600 nm, cells were induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG, 500 µM). After growing overnight at 16°C, the cells were harvested by centrifugation, resuspended in lysis buffer (50 mM HEPES, pH 7.8, 100 mM NaCl, 10% glycerol, 5 mM DTT), and supplemented with 0.1 mg/ mL lysozyme (Sigma) and 5 µg/ mL DNAse I (Sigma). The cell lysate was passed through an EmulsiFlex-C5 disrupter (Avestin) at 15,000 PSI three times and centrifuged for 30 minutes (15,000 x *g*, 4°C) to remove cell debris. A three-step purification process was followed to obtain purified test substrates. This included an initial purification on amylose resin (NEB) following the manufacturer's protocol, followed by size-exclusion chromatography on a Size Superdex 200 Increase 10/300 GL column (GE Healthcare), using degassed and 0.2 µm sterile filtered (Corning) SEC buffer (50 mM HEPES, pH 7.8, 100 mM NaCl, 10% glycerol, 5 mM DTT) and a protocol from the UNICORN6 software package (GE Healthcare) specific to the Superdex column. was used . Finally, the protein (~71.6 KDa) was concentrated in a 50 kDa Amicon ultra centrifugation filter (MilliporeSigma) and purified using a magnetic anti-Flag affinity resin (Pierce) following the manufacturer's protocol. The eluted sample was immediately buffer exchanged into SEC buffer using a 7K MWCO Zebra spin desalting column (Thermo Scientific) to eliminate any excess FLAG peptide following the manufacturer's protocol. The purified proteins were flash frozen in liquid nitrogen and stored at -80 °C.

D2. *In vitro* protein synthesis. MBP-DHCR24 (full-length) in a pET28b vector (Novagen) was expressed using the PURExpress *in vitro* synthesis kit (NEB E6800S) as previously described (Shimazu 2001). Briefly, the pET28_MBP-DHCR24 plasmid was prepared using a maxi-prep kit (Qiagen), eluted in ultra-pure water (Invitrogen), and subsequently used in the *in vitro* translation reaction. To the *in vitro* translation reaction mixture, RNAsin (Promega) and 450 ng of purified plasmid were added and incubated for 4 hours (37 °C, 250 RPM). The reaction mixture was concentrated using a 0.5 mL Ultra Centrifugal Filter MWCO of 50 kDa to eliminate transcription and translation proteins from the kit. The protein mixture was flash frozen in liquid nitrogen and stored at -80°C.

E. Biochemical assays

E1. *In vitro* experiments with recombinant NS3-4A. The various DHCR24 fusion constructs either purified from recombinant expression in *E.coli* or through *in vitro* translation were incubated with recombinant NS3-4A (AnaSpec) for 16 hours (30 °C, 250 RPM) containing: 50 µM DHCR24 test substrate and 18 µM NS3-4A in 50 mM HEPES (pH 7.5), 100 mM NaCl, 20% glycerol, 1 µM DTT. The reaction was quenched by the direct addition of 1X SDS denaturing loading dye.

E2. Immunoblot analysis to of NS3-4A cleavage assays. 100 µg of the reaction mixture (25 µL) was analyzed by SDS-PAGE using a 8-16% TGX Mini-protean gel (BioRad). The gel was transferred onto nitrocellulose membrane (BioRad Trans-blot Turbo). The membrane was

washed in TBST and blocked with 5% milk (TBST, w/v). After incubation overnight with primary antibodies at 4 °C, membranes were washed with TBST and incubated with their respective species-specific secondary antibodies. After washing with TBST, blots were developed with enhanced chemiluminescence reagents (Thermo Fisher Scientific), and the chemiluminescence signal was processed using the Amersham Imager 600 (GE Healthcare).

Primary antibodies and their dilutions were as follows: anti-DHCR24 (rabbit) (epitope region 68-85) (1:2000), anti-MBP-horseradish peroxidase (NEB E8038) (1:2000), and anti-FLAG-horseradish peroxidase M2 (Sigma A8592) (1:1000). Anti-rabbit IgG-HRP (BioRad 170-6515) was used to blot against the anti-DHCR24 (epitope region 68-85) (Sigma S8571) (1:2000).

F. Lipid analyses

F1. Lipid isolation. Cells were washed once with 1X PBS and harvested by trypsinization, addition of medium to quench trypsin, and centrifugation (1000 x *g*, 5 minutes, 4 °C). Cell pellets were washed twice with cold 1X PBS, resuspended in 1 mL of PBS and processed with a Dounce homogenizer using 2:1:1 $CHCl_3$:CH_3OH:PBS buffer. The organic and aqueous layers were separated by centrifugation (2000 x *g*, 5 minutes, 4°C). The organic layer was removed, concentrated under nitrogen gas (N_2), and then dissolved in 90 µL $CHCl_3$ prior to analysis by LC-MS or GC-MS. 30 µL of this sample was used for LC-MS. Absolute quantitation of sterol levels was accomplished by isotope dilution mass spectrometry and adding deuterated ketocholesterol-d7 (5 µL of 10 µM stock) as an internal quantitation standard. For GC-MS samples, samples were dried down with the internal standard (ketocholesterol-d7).

F2. Liquid Chromatography- Mass Spectrometry. LC-MS was performed on an Agilent 6530 QTOF mass spectrometer with 1290 Infinity Binary LC in positive ion mode. For the quantification of desmosterol, lipids were chromatographically separated on a UHPLC Phenomenex Kinetex 2.6 µM XBC18, 100 Å column. Gradients using mobile phase A (85% methanol, 15% water, 5 mM ammonium acetate, and 0.1% formic acid) and mobile phase B (100% methanol, 5 mM ammonium acetate, and 0.1% formic acid) were run as follows: 0−2 min 100% A, 2−15 min 100% B, 15−25.5 100% B, and 25.5−30 min 100% A.

F3. Gas Chromatography- Mass Spectrometry. GC/MS analysis was performed on a Thermo Scientific TRACE 1310 Gas Chromatograph equipped with a Thermo Scientific Q Exactive Orbitrap mass spectrometry system. 50 µL of the (BSTFA+10% TMCS)/pyridine (1/1 v/v) derivatized product was added into each vial, vortexed well, and heated at 70˚C for 30 min. One µL of the sample was injected into a Thermo fused-silica capillary column of cross-linked TG-5SILMS (30 m x 0.25 mm x 0.25 µm). The GC conditions were as follows: inlet and transfer line temperatures, 290˚C; oven temperature program, 50˚C for 0 min, 24˚C/min to 325˚C for 5.7 min; inlet helium carrier gas flow rate, 1 mL/min; split ratio, 5. The electron impact (EI)-MS conditions were as follows: ion source temperature, 310˚C; full scan m/z range, 30 - 750 Da; resolution, 60,000; AGC target, 1e6; maximum IT, 200ms. Data were acquired and analyzed with Thermo TraceFinder 4.1 software package.

G. Mass Spectrometry Analysis of Protein Bands Excised gel bands were cut into approximately 1 mm^3 pieces and submitted to the Taplin Mass Spectrometry Facility for subsequent processing and analysis. The samples were reduced with 1 mM DTT for 30 minutes

at 60ºC and then alkylated with 5 mM iodoacetamide for 15 minutes in the dark at room temperature. Gel pieces were then subjected to a modified in-gel trypsin digestion procedure (57). Gel pieces were washed and dehydrated with acetonitrile for 10 min, followed by removal of acetonitrile. Pieces were then completely dried in a Speed-Vac. Rehydration of the gel pieces was with 50 mM ammonium bicarbonate solution containing 12.5 ng/µl modified sequencing-grade trypsin (Promega, Madison, WI) at 4ºC. Samples were then placed in a 37 ºC room overnight. Peptides were later extracted by removing the ammonium bicarbonate solution, followed by one wash with a solution containing 50% acetonitrile and 1% formic acid. The extracts were then dried in a speed-vac (~1 hr). The samples were then stored at 4 ºC until analysis.

On the day of analysis the samples were reconstituted in 5 - 10 µL of HPLC solvent A (2.5% acetonitrile, 0.1% formic acid). A nano-scale reverse-phase HPLC capillary column was created by packing 2.6 µm C18 spherical silica beads into a fused silica capillary (100 µm inner diameter x ~30 cm length) with a flame-drawn tip (58). After equilibrating the column each sample was loaded via a Famos auto sampler (LC Packings, San Francisco CA) onto the column. A gradient was formed and peptides were eluted with increasing concentrations of solvent B (97.5% acetonitrile, 0.1% formic acid). As each peptide was eluted, they were subjected to electrospray ionization followed by entry into an LTQ Orbitrap Velos Pro ion-trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Eluting peptides were detected, isolated, and fragmented to produce a tandem mass spectrum of specific fragment ions for each peptide. Peptide sequences (and hence protein identity) were determined by matching protein or translated nucleotide databases with the acquired fragmentation pattern by the software program, Sequest (ThermoFinnigan, San Jose, CA) (59). The modification of 79.9663 mass units to serine, threonine, and tyrosine was included in the database searches to determine phosphopeptides. Phosphorylation assignments were determined by the Ascore algorithm (60). All databases include a reversed version of all the sequences and the data was filtered to between a 1-2% peptide false discovery rate.

H. Immunofluorescence Microscopy

VEEV-NS3-5B cells were seeded at a density of 0.5 x 10^5 cells/mL in µ-Slide 8 Well Glass Bottom: # 1.5H (170 µm +/- 5 µm) D 263 M Schott glass, chambered slides (Ibidi) and incubated for 48 hours under 5 µg/mL puromycin selection (5% CO_2, 37 °C). At approximately 70% cell confluency, the medium was replaced with pre-warmed DMEM without phenol red for 1 hour (5% CO_2, 37 °C). Cells were washed twice with filtered PBS and fixed with cold methanol for 1-2 hours at 4 °C, then were washed twice with PBS and permeabilized with 0.1% Triton-X 100 (15 minutes, room temperature). Cells were then washed with PBS and blocked in blocking solution (10% fetal bovine serum and 5% bovine serum albumin in PBS) (1 hour, 37°C). Cell were then sequentially stained for DHCR24 or PDI followed by staining for NS3. First, cells were incubated with anti-DHCR24 or anti-PDI primary antibodies in blocking buffer (overnight, 4 °C). Cells were washed in PBS and incubated with the appropriate secondary antibodies in a humidity chamber (1 hour, at 37 °C dark). After this first staining, cells were washed with PBS and stained with anti-NS3 in blocking buffer (1 hour, 37 °C, dark) followed by secondary antibody staining as described. Samples were mounted with ProLong Gold antifade reagent (Invitrogen P36930).

Primary and secondary antibody combinations and their dilutions were as follows: anti-DHCR24 (epitope region 68-85) (Sigma S8571) (1:1500) used with AlexaFluor647-anti-rabbit (Invitrogen A21244) (1:1000), anti-PDI (Abcam ab110195) (1:500) used with AlexaFluor555 anti-

goat (Invitrogen A32816) (1:1000), and anti-NS3 (Abcam ab65407) (1:500) used with AlexaFluor488 anti-mouse (Invitrogen A11001) (1:1000).

Images were acquired with a Nikon Ti-E inverted microscope system using a Plan Apo 100x/1.45 objective with a Hamamatsu Orca Flash sCMOS camera, SOLA light engine (Lumencor), Shutter and LUDL motorized excitation/emission filter wheels on a Nikon Ti-E inverted microscope equipped with a Yokogawa CSU-W1 spinning disk scanhead using laser lines 488, 514, 561 and 640 (Toptica) and a Plan Apo 100x/1.45 objective DIC. Images were acquired with the NIS-Elements software package.

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* = Denotes additional reference number after reference list located in the main text.