

Supplementary Information

# Acetylation, Methylation and Allysine Modification Profile of Viral and Host Proteins during Influenza A Virus Infection

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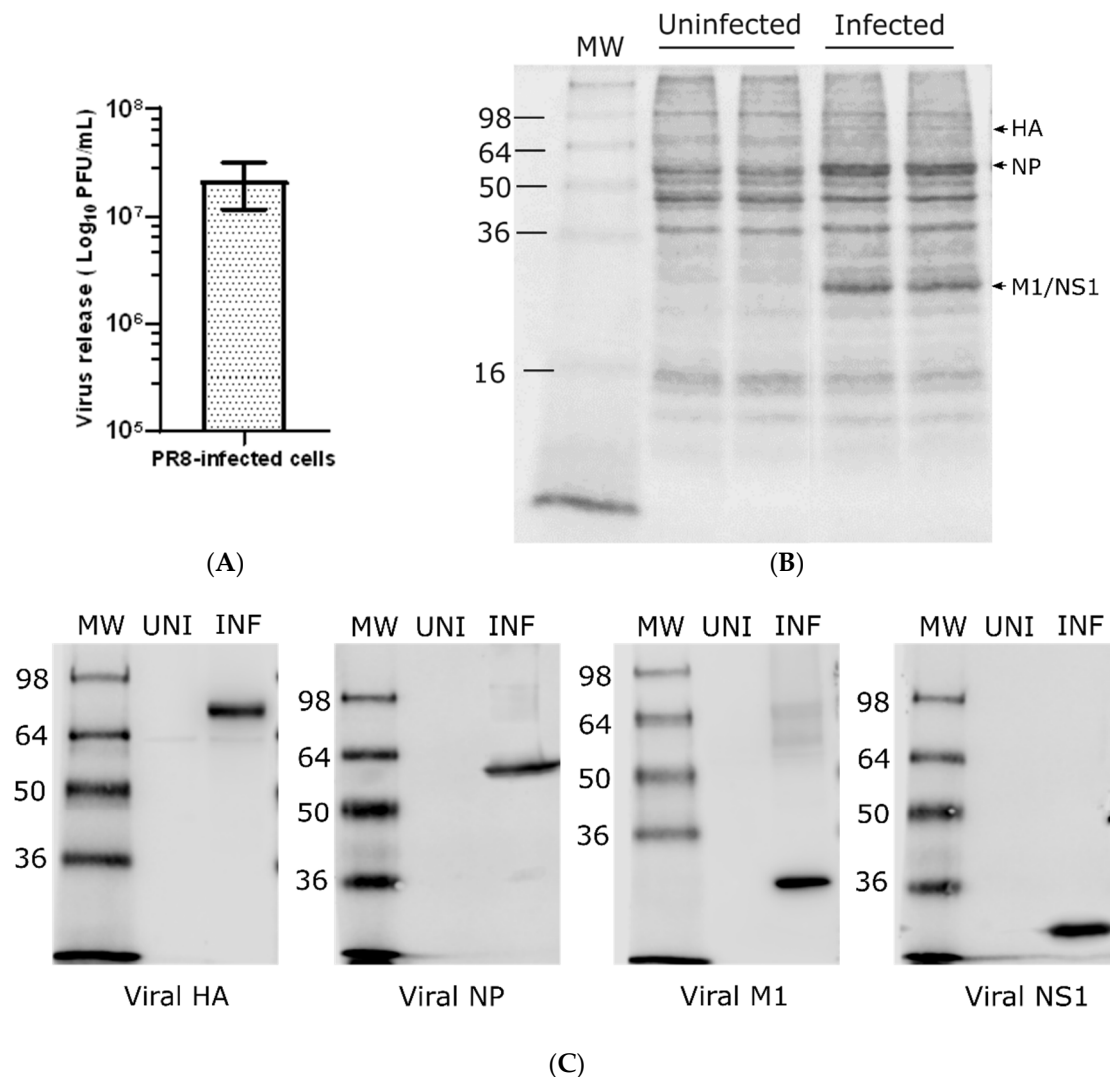
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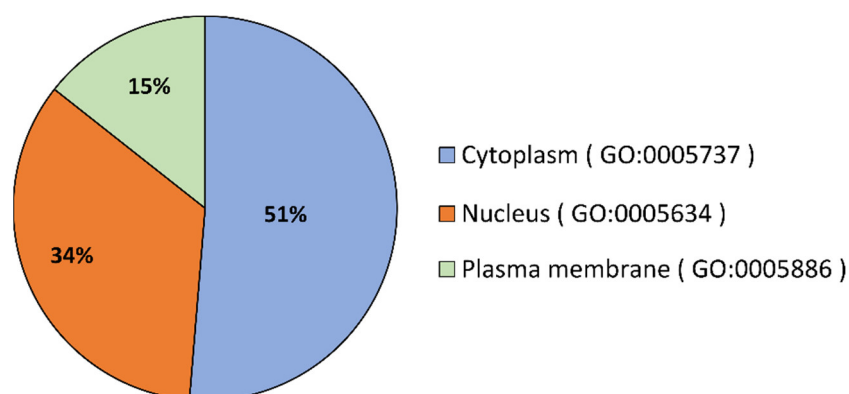
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**Figure S1.** Influenza A virus (IAV) titer and polypeptide profile of infected cells used for mass spectrometry. A549 cells were grown in 25 cm<sup>2</sup> cell culture flasks to 95% confluency and infected with IAV at MOI of 1.0 for 24 hours. The cells and the media were harvested separately. The media was titrated on MDCK cells by plaque assay to determine the titer of released viral progeny (A). Whereas the cells were lysed, and total cell lysates were resolved on 15% SDS-PAGE (B). The gel was stained with Coomassie blue and imaged on Odyssey Fc imager (Li-COR). (C) Total cell lysates from uninfected (UNI) and infected (INF) A549 cells were resolved on 10% SDS-PAGE, and viral HA (80 kDa), NP (56 kDa), M1 (28 kDa), and NS1 (26 kDa) polypeptides were detected by western blotting using the method described elsewhere (ref. 15). The antibodies to HA (NR-3148), NP (NR-19868), and NS1 (NR-44426) were obtained through BEI Resources (NIAID, NIH, USA) and antibody to M1 (G122) was kindly provided by Richard Webby (St Jude Children's Research Hospital, USA). MW, molecular weight in kDa.



**Figure S2.** Subcellular localization of the identified modified host proteins based on their GO term.

**Table S1.** Detected IAV proteins and their modifications.

No	Accession	Protein description	Coverage [%]	#Peptides	#PSMs	#Unique Peptides	MW [kDa]	Score SE-QUEST HT	Modifications	XCorr
1	ABP64734.1	Matrix protein 1 [Influenza virus A/PR/8/34(H1N1)]	89	34	2806	34	27.9	7192.23	Methyl [K95]	4.36
									Methyl [K98]	3.81
									Methyl [R160]	4.30
									Methyl [K230]	3.82
									Methyl [K242]	2.03
									Acetyl [S195]	4.61
									Acetyl [S196]	3.85
									Acetyl [S207]	3.68
									Acetyl [K95]	4.15
									Allysine [K35]	2.71
									Allysine [K98]	2.20
									Allysine [K230]	4.15
2	AAM75162.1	Matrix protein M2 [Influenza virus A/PR/8/34/Mt Sinai(H1N1)]	30	2	86	2	11	217.39	--	-
3	NP_040982.1	Nucleoprotein [Influenza virus A/PR/8/1934(H1N1)]	79	49	4167	6	56.1	10707.22	Methyl [R150]	2.44
									Methyl [R246]	4.20
									Methyl [R317]	2.56
									Methyl [K325]	2.56
									Methyl [R416]	2.27
									Methyl [R422]	4.44
									Acetyl [S274]	5.18
									Acetyl [S283]	6.23
									Acetyl [S287]	4.50
									Acetyl [S326]	3.73
									Acetyl [S403]	2.95
4	ABP64720.1	Polymerase acidic protein [Influenza virus A/hvPR8/34(H1N1)]	49	33	410	33	82.5	957.94	Acetyl [K325]	2.86
									Methyl [K102]	2.85
									Methyl [K104]	2.51
									Acetyl [K102]	3.15
									Acetyl [K104]	2.30
5	ABO21706.1	Polymerase basic protein 1 [Influenza virus A/PR/8/1934(H1N1)]	40	28	357	28	86.5	921.28	Acetyl [S631]	1.91
									Acetyl [N-Term]	3.10
6	ABP64718.1	Polymerase basic protein 2 [Influenza virus A/hvPR8/34(H1N1)]	52	39	523	39	86.1	1513.78	Allysine [K718]	4.02
7	NP_040984.1	Non-structural protein 1 [Influenza virus A/PR/8/1934(H1N1)]	68	20	1401	20	25.9	3886.3	Methyl [R193]	3.31
									Allysine [K110]	2.26
									Acetyl [N-Term]	4.03

No	Accession	Protein description	Coverage [%]	#Peptides	#PSMs	#Unique Peptides	MW [kDa]	Score SE-QUEST HT	Modifications	XCorr
8	ABP64727.1	Non-structural protein 2 [Influenza virus A/hvPR8/34(H1N1)]	65	7	89	7	14.4	236.82	Acetyl [N-Term]	2.28
9	CAA24272.1	Haemagglutinin [Influenza virus (A/PR/8/1934(H1N1))]	54	31	1908	4	63.3	4720.2	Methyl [R91] Methyl [R269] Methyl [K252] Allysine [K62]	1.82 3.52 3.53 3.41
10	ABP64723.1	Neuraminidase [Influenza virus (A/hvPR8/34(H1N1))]	35	18	550	18	50.1	1672.05	--	-

Coverage: The percentage of full protein sequence that is covered by the identified peptides, #Peptides: The number of distinct peptide sequences in a protein group, #PSMs (Peptide Spectra Matches): The total number of identified peptide sequences for the target protein, #Unique peptides: The number of peptide sequences unique to a protein group, Score SEQUEST HT: The sum of the individual scores of each peptide, Xcorr (cross correlation): The number of fragment ions that are common to two different peptides with same precursor mass.

**Table S2.** Number of viral protein sequences and IAV subtypes used for alignments.

IAV Proteins	Number of Sequences	IAV Subtypes
M1	800	H1N1, H1N2, H1N3, H2N2, H2N3, H2N5, H2N9, H3N2, H3N6, H3N8, H4N5, H4N6, H4N7, H4N9, H5N1, H5N2, H5N3, H5N8, H5N9, H6N1, H6N2, H6N3, H6N5, H6N6, H6N8, H7N1, H7N2, H7N3, H7N7, H9N2, H9N9, H10N3, H10N4, H10N7, H11N7, H11N8, H11N9, H12N5, H13N6
NP	1124	H1N1, H1N2, H1N9, H2N2, H2N3, H2N5, H2N9, H3N2, H3N6, H3N8, H4N2, H4N5, H4N6, H4N7, H4N9, H5N1, H5N2, H5N3, H5N8, H5N9, H6N1, H6N2, H6N3, H6N5, H6N6, H6N8, H7N1, H7N3, H7N7, H7N8, H7N9, H8N4, H9N2, H9N9, H10N3, H10N4, H10N7, H11N7, H11N9, H12N2, H12N4, H12N5, H14N6
PA	800	H1N1, H1N2, H1N3, H1N9, H2N2, H2N3, H2N5, H2N9, H3N2, H3N6, H3N8, H4N2, H4N6, H4N5, H4N7, H4N9, H5N1, H5N2, H5N3, H5N8, H5N9, H6N1, H6N2, H6N5, H6N8, H7N1, H7N3, H7N7, H7N8, H7N9, H8N4, H9N2, H9N9, H10N3, H10N4, H10N5, H10N6, H10N7, H10N8, H11N2, H11N7, H11N8, H11N9, H12N2, H12N4, H12N5, H13N9, H14N6
PB2	986	H1N1, H1N2, H1N3, H2N2, H2N3, H2N5, H2N9, H3N2, H3N6, H3N8, H4N2, H4N6, H4N5, H4N7, H4N9, H5N1, H5N2, H5N3, H5N8, H5N9, H6N1, H6N2, H6N4, H6N5, H6N8, H7N1, H7N3, H7N7, H7N8, H7N9, H8N4, H9N2, H9N9, H10N3, H10N4, H10N5, H10N6, H10N7, H10N8, H11N2, H11N7, H11N8, H11N9, H12N2, H12N4, H12N5, H13N9, H14N6
NS1	998	H1N1, H1N2, H1N3, H2N2, H2N3, H2N5, H2N9, H3N2, H3N6, H3N8, H4N2, H4N6, H4N5, H4N7, H4N9, H5N1, H5N2, H5N3, H5N8, H5N9, H6N1, H6N2, H6N5, H6N8, H7N1, H7N3, H7N7, H7N8, H7N9, H8N4, H9N2, H9N9, H10N3, H10N4, H10N5, H10N6, H10N7, H10N8, H11N2, H11N7, H11N8, H11N9, H12N2, H12N4, H12N5, H13N6, H13N9, H14N6
HA	480	H1N1, H1N2, H1N3, H1N5, H1N6, H1N7, H1N9