Supplementary information

The role of NSP6 in the biogenesis of the SARS-CoV-2 replication organelle

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Supplementary Video 8. Electron-tomogram of HeLa cells transfected with HA-NSP3 and mCherry-NSP4. White arrows show connections between ER and DVMs.

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Supplementary Video 11. Electron-tomogram of HeLa cells transfected with HA-NSP3, mCherry-NSP4, and GFP-NSP6ΔSGF. White arrows show numerous connections of zippered ER to DVMs. Black arrow shows connection of zippered region to regular ER cisternae.

Supplementary Video 12. 3D reconstruction of the tomogram shown in EM Video 11. DMVs, zippered ER, and regular ER are shown in red, green and yellow, respectively.

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Supplementary Tables

Supplementary Table 1. Nucleotide sequence of synthetic SARS-CoV-2 NSP6/NSP7 and IBV NSP6

SARS-CoV-2 NSP6/7

GGGGACAAGTTTGTACAAAAAAGCAGGCTGCATGAGCGCCGTGAAGAGAACCATCAAGGGAACCCACCACTGGCT
GCTGCTGACCATCCTGACCTCCCTGCTGGTGCTGGTGCAGTCTACCCAGTGGAGCCTGTTCTTCTTCCTGTACGA
GAACGCCTTCCTGCCCTTCGCTATGGGTATCATCGCCATGTCCGCCTTCGCTATGATGTTCGTGAAGCACAAGCA
CGCTTTCCTGTGCCTGTTCCTGCTGCCATCCCTGGCCACCGTGGCTTACTTCAACATGGTGTACATGCCCGCCTC
TTGGGTCATGCGCATCATGACCTGGCTGGACATGGTGGACACCAGCCTGTCCGGCTTCAAGCTGAAGGACTGCGT
GATGTACGCCTCTGCTGTGGTGCTGCTGATCCTGATGACCGCTAGAACCGTGTACGATGATGGTGCTAGAAGGGT
GTGGACCCTGATGAACGTGCTGACCCTGGTGTACAAGGTGTACTACGGCAACGCCCTGGACCAGGCTATCAGCAT
GTGGGCTCTGATCATCTCTGTGACCAGCAACTACTCCGGAGTGGTGACCACCGTGATGTTCCTGGCCAGAGGTAT
CGTGTTCATGTGCGTGGAGTACTGCCCTATCTTCTTCATCACCGGCAACACCCTGCAGTGCATCATGCTGGTGTA
CTGCTTCCTGGGTTACTTCTGCACCTGCTATTTCGGCCTGTTCTGCCTGCTGAACAGATACTTCCGTCTGACCCT
GGGCGTGTACGACTACCTGGTGTCCACCCAGGAATTCCGTTACATGAACTCTCAGGGACTGCTGCCCCCTAAGAA
CAGCATCGACGCTTTCAAGCTGAACATCAAGCTGCTGGGAGTGGGCGGAAAGCCATGCATCAAGGTGGCCACCGT
GCAGAGCAAGATGAGCGACGTGAAGTGCACCAGCGTGGTGCTGCTGTCTGTTCTGCAGCAGCTGAGAGTGGAAAG
CAGCAGCAAACTGTGGGCCCAGTGTGTCCAGCTGCACAACGATATCCTGCTGGCCAAGGATACCACCGAGGCCTT
CGAGAAGATGGTGTCCCTGCTGAGTGTGCTGCTGAGCATGCAGGGCGCCGTGGACATTAACAAGCTGTGCGAGGA
AATGCTGGACAACCGGGCCACACTGCAGTAGGACCCAGCTTTCTTGTACAAAGTGGTCCCC

IBV NSP6

GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAGCAGCTTCGTGCGGAAGGCCACCTC
CTGGTTTTGGAGCAGATGTGTGCTGGCCTGCTTCCTGTTCGTGCTGTGTGCCATCGTGCT
GTTCACAGCCGTGCCTCTGAAGTTCTATGTGCACGCCGCCGTGATCCTGCTGATGGCCGT
GCTGTTTATCAGCTTTACCGTGAAGCACGTGATGGCCTACATGGACACCTTTCTGCTGCC
CACACTGATCACCGTGATTATCGGCGTGTGTGCCGAGGTGCCCTTCATCTACAACACCCT
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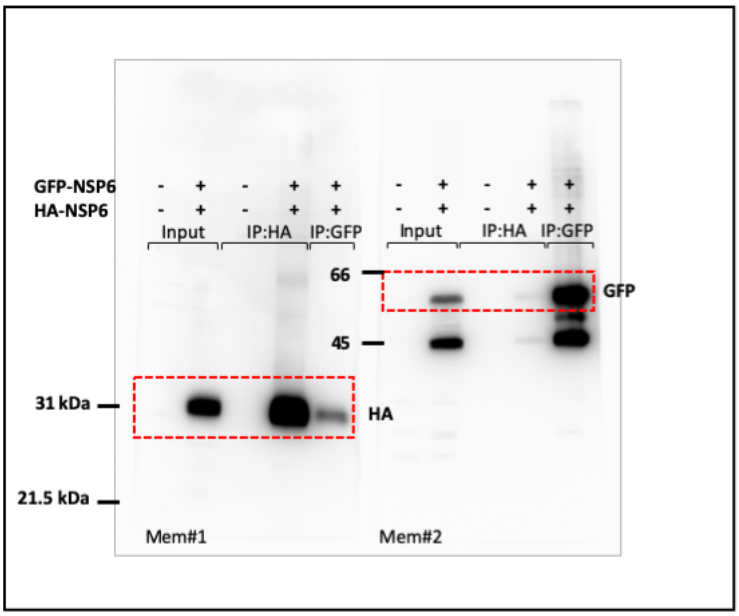
GTCCGTGGACCAGTACCGCTACATGTGCCTGCACAAAGTGAACCCTCCAAAGACCGTGTG
GGAAGTGTTCACCACCAACATCCTGATCCAAGGCATCGGCGGCGACAGAGTGCTGCCTAT
TGCTACCGTTCAGTAGGACCCAGCTTTCTTGTACAAAGTGGTCCCC

Supplementary Table 2. Oligonucleotides and siRNAs used in this study

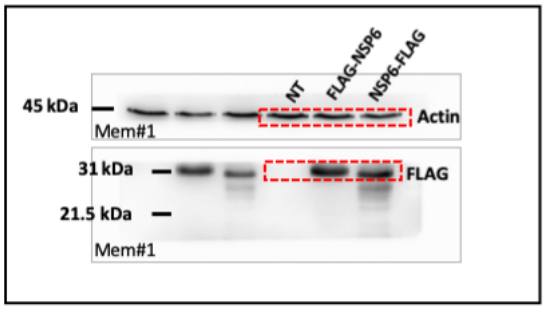
Oligos		Plasmid
NSP6 ns (+) NSP6 ns (-)	AAGGTGGCCACCGTGCAGAAATACCCAACTTTCTTGTAC GTACAAGAAAGTTGGGTATTTCTGCACGGTGGCCACCTT	pCDNA5-NSP6-3xFLAG
NSP6-Δ106-108(+) NSP6-Δ106-108(-)	GTGGACACCAGCCTGAAACTGAAGGACTGCGTGAT ATCACGCAGTCCTTCAGTTTCAGGCTGGTGTCCAC	pFLAG-NSP6-ΔSGF pHA-NSP6-ΔSGF pGFP-NSP6-ΔSGF
NSP6 Stop157 (+) NSP6 Stop157 (-)	GTGTACTACGGCAACGCCTAGGACCAGGCTATCAGCATG CATGCTGATAGCCTGGTCCTAGGCGTTGCCGTAGTACAC	pFLAG-NSP6-1-157
C80-NSP6 (+) C80-NSP6 (-)	TTGTACAAAAAAGTTGGCATGCTGGTGTACTGCTTCCTGG CCAGGAAGCAGTACACCAGCATGCCAACTTTTTTGTACAA	pFLAG-NSP6-C80
NSP6- F220Q/T222W(+) NSP6- F220Q/T222W(-)	CATGCTGGTGTACTGCTTCCTGGGTTATCAATGCTGGTGCTATT TCGGCCTGTTCTGCCTG CAGGCAGAACAGGCCGAAATAGCACCAGCATTGATAACCCAGG AAGCAGTACACCAGCATG	pFLAG/NSP6-F220Q/T222W and pFLAG-NSP6-C80 F220Q/T222W
NSP6-IBV ns (+) NSP6-IBV ns (-)	CTGCCTATTGCTACCGTTCAGGCCGACCCAGCTTTCTTGTAC GTACAAGAAAGCTGGGTCGGCCTGAACGGTAGCAATAGGCAG	pCDNA5-IBV-NSP6-3xFLAG
DFCP1-W543A (+) DFCP1-W543A (-)	CAGAGATTGTGCATGTGGCGCCTGGAACTGATGGGT ACCCATCAGTTCCAGGCGCCACATGCACAATCTCTG	pmCherry-DFCP1-W543A
DFCP1-C654S (+) DFCP1-C654S (-) DFCP1-C770S (+) DFCP1-C770S (-)	GTGCGGGTCTGTGACAACAGCTACGAAGCCAGGAACG CGTTCCTGGCTTCGTAGCTGTTGTCACAGACCCGCAC GTCCGAGTCTGCTTCAACAGCAATAAAAAGCCCGGTGA TCACCGGGCTTTTTATTGCTGTTGAAGCAGACTCGGAC	pmCherry-DFCP1-C654A-C770A
DFCP1-Δ1-416 (+) DFCP1-Δ1-416 (-)	CTCGAGGGTCGACCATGGCGCACAGCTCCTTTT AAAAGGAGCTGTGCGCCATGGTCGACCCTCGAG	pmCherry-DFCP1-Δ1-416
DFCP1-p223 (+) DFCP1-p223 (-)	GGGGACAAGTTTGTACAAAAAAGCAGGCTGCATGAGTGCCCAG ACTTCCCCAGCAG GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAAAGGTCACCG GGCTTTTTATTGCAG	pDONR223-DFCP1
siRNAs used		
siRNA-ZFYVE1 (DFCP1) #1	GGAUGGGUCUCGCAAAAUA[dT][dT]	
siRNA-ZFYVE1 (DFCP1) #2	GGAUGUAAGAAAAGCAUGA[dT][dT]	
siRNA-ZFYVE1 (DFCP1) #3	CACUAGGUCUGGUAAAGGA[dT][dT]	

Supplementary Figure 1

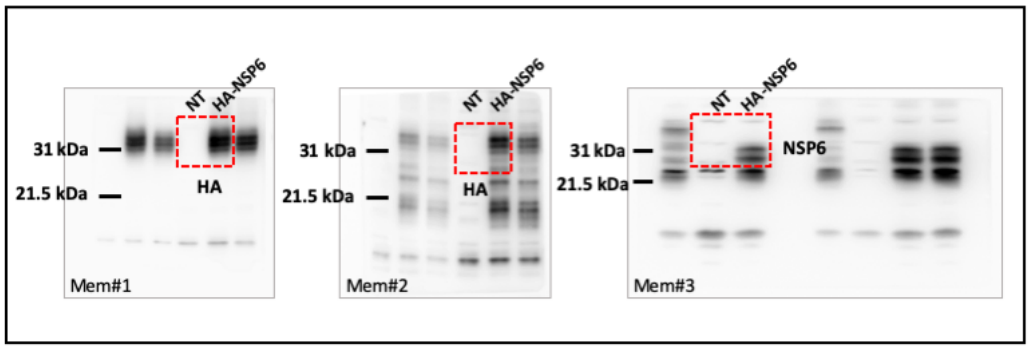
Figure 2d



Extended Data Figure 1b



Extended Data Figure 1c



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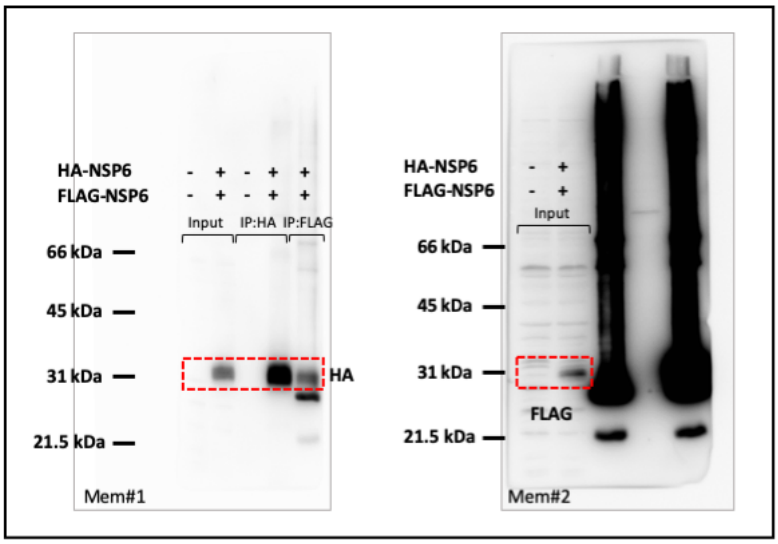

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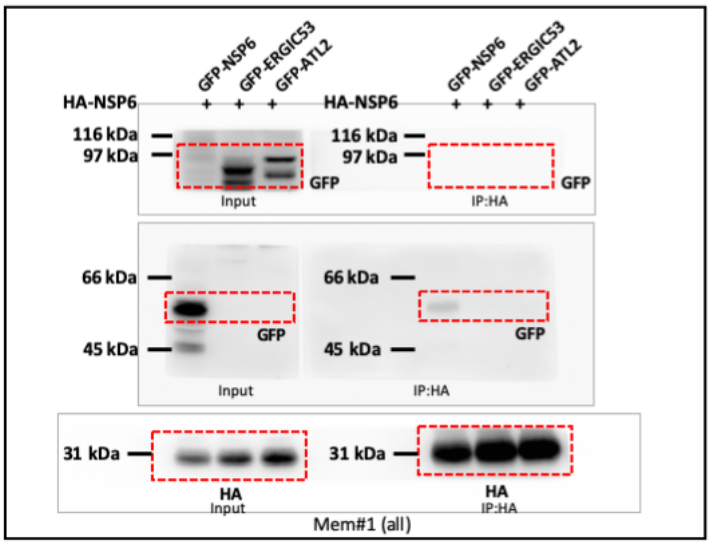

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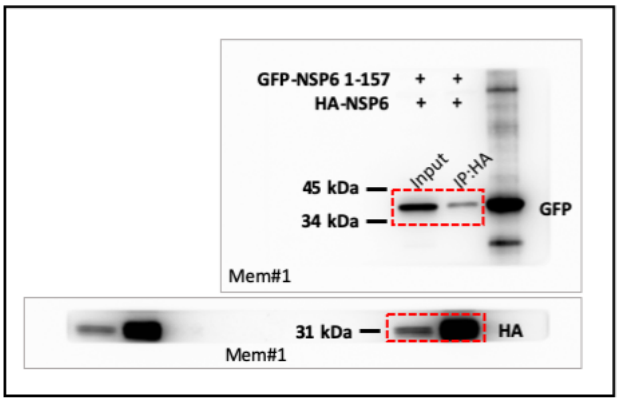

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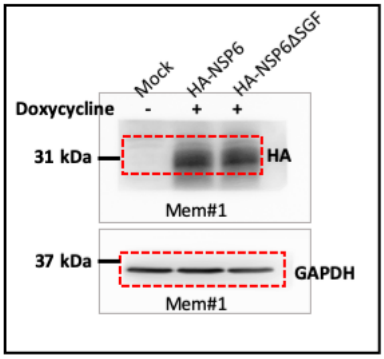

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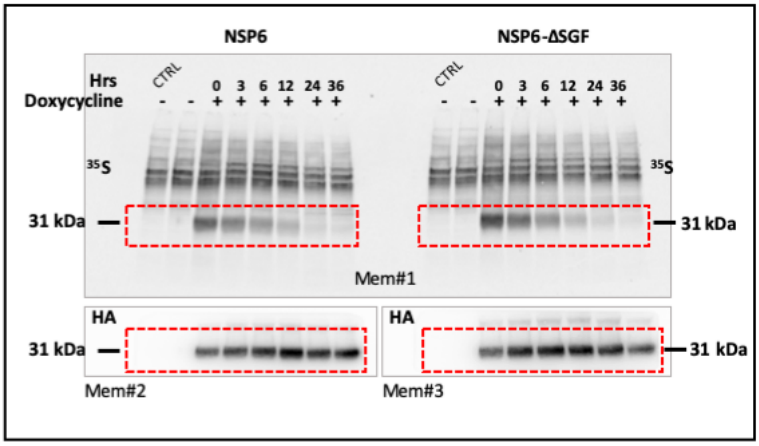

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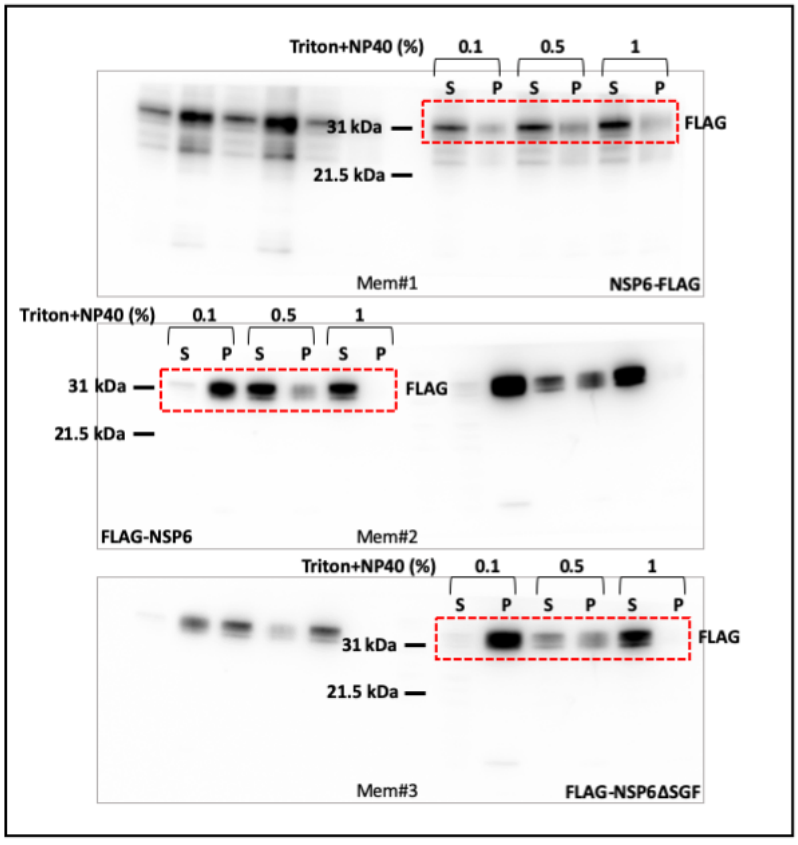

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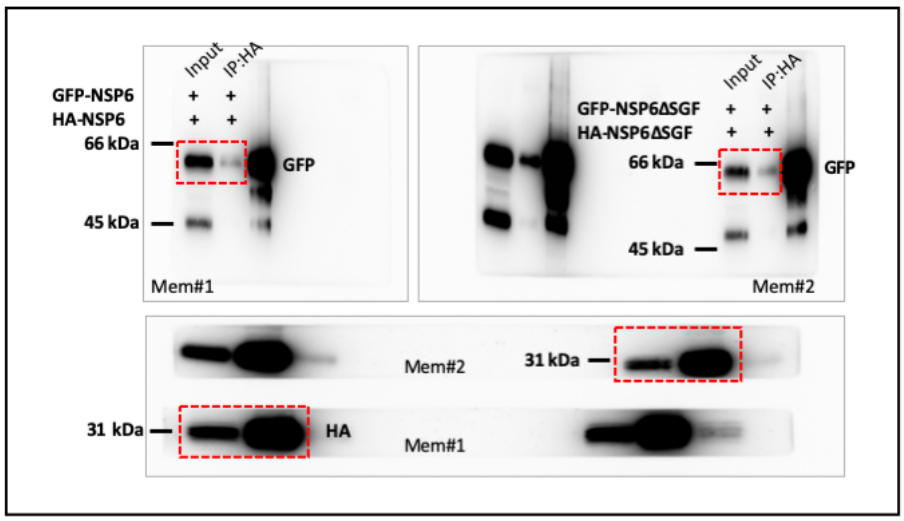

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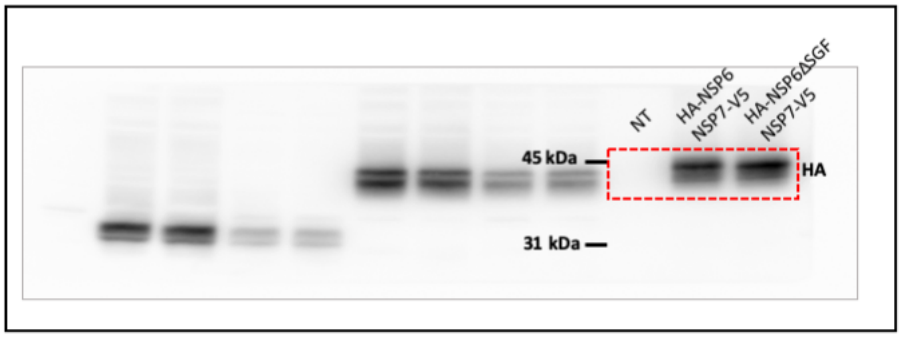

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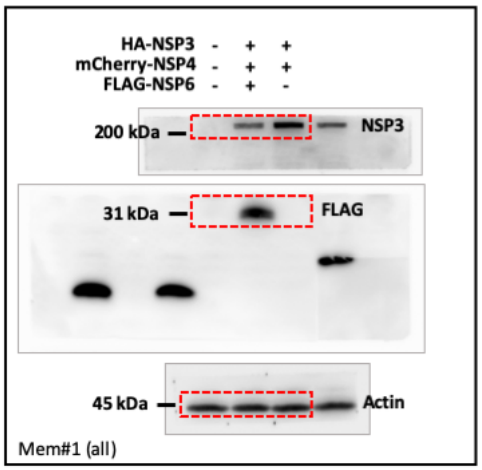

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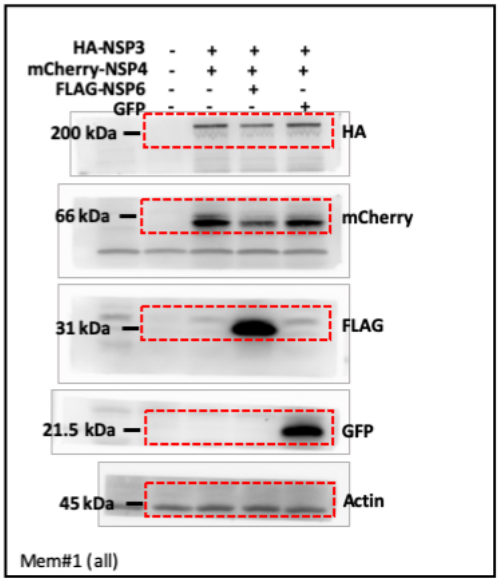

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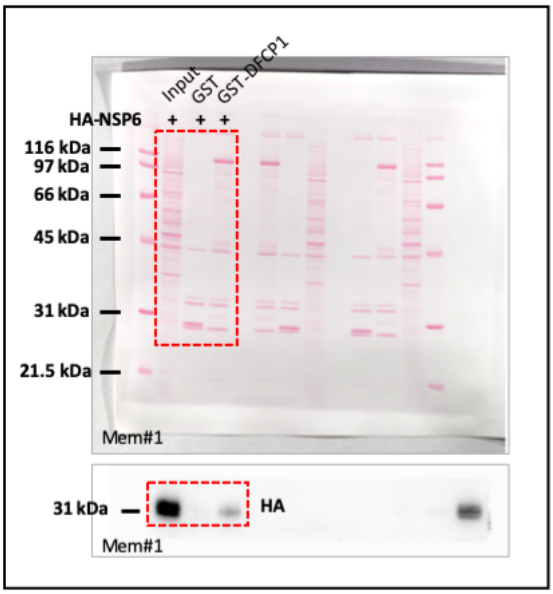

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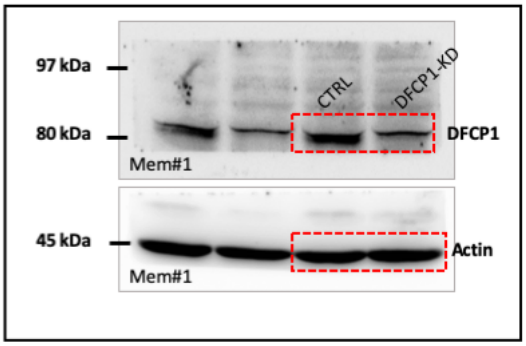

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