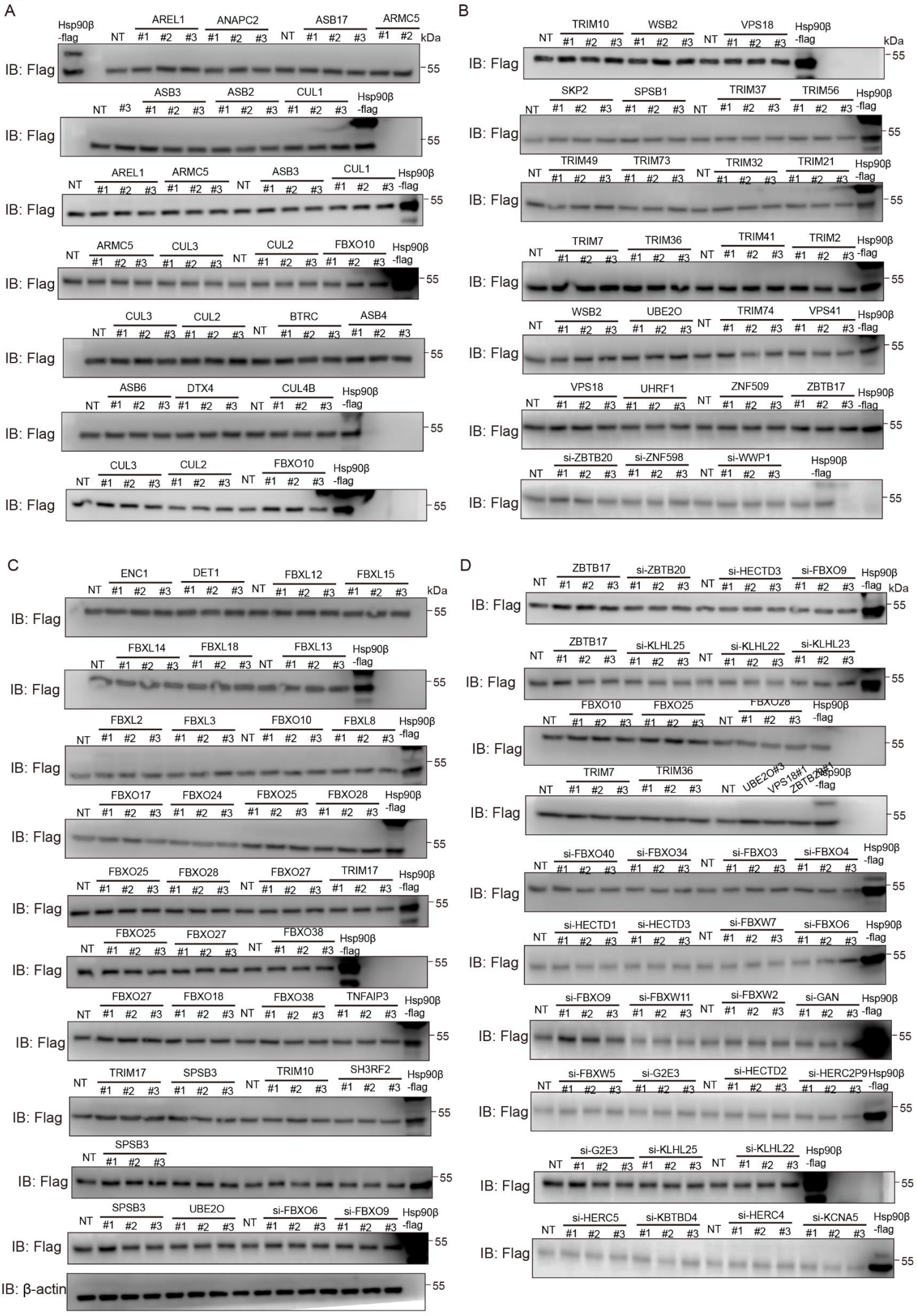
Supplementary Figure Legends

Supplementary Figure S1. Identification of the E3 ligase responsible for N protein degradation upon Hsp90β knockout by western blot. (A-H) 90β-KO-N-flag stable cells were seeded in 24-well plates for siRNA library screening. At 72 h post-transfection, cells were lysed and analyzed by western blot.

Supplementary Figure S2. Identification of the E3 ligase responsible for N protein degradation upon Hsp90β knockout by immunofluorescence. (A-B) HEK293T cells were seeded in 24-well plates, at 72 h post-transfection, cells were transfected with N-GFP plasmid for 48 h and observed by fluorescence microscopy. (C-D) 90β-KO-N-flag stable cells were seeded in 24-well plates. At 84 h post-transfection, cells were immunostained for N (green) and nuclei were stained with DAPI (blue). The cells were observed by fluorescence microscopy.

Supplementary Figure S3. Infection of Syrian hamsters by SARS-CoV-2 GFP/ΔN virus. (A) A549-ACE2 and Huh7-ACE2 cells were infected with $10^{8.5}$ PFU of Ad5-N in 100 μL for 48 h, then infected with SARS-CoV-2 GFP/ΔN virus. Lung tissue from Ad5-N/GFP-transduced hamsters was disrupted by grinding, and lysates were collected for western blot. (B) Ad5-N or Ad5-GFP-transduced hamsters were intranasally inoculated with $10^{6.5}$ PFU of SARS-CoV-2 GFP/ΔN virus in 100 μL of PBS. Body weight was monitored daily (n = 6 per group). (C) Left: SARS-CoV-2 Nsp4 mRNA copies per g of lung; right: SARS-CoV-2 M mRNA copies per g of lung were quantified by qRT-PCR. Results present mean and SD of three independent experiments.

S1 Fig A-D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Hsp90β -flag
AREL1
ANAPC2
ASB17
ARMC5
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 kDa
IB: Flag
55
ASB3
ASB2
CUL1
Hsp90β -flag
NT #3 #1 #2 #3 #1 #2 #3 #1 #2 #3
AREL1
ARMC5
ASB3
CUL1
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
ARMC5
CUL3
CUL2
FBXO10
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
CUL3
CUL2
BTRC
ASB4
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
ASB6
DTX4
CUL4B
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3
CUL3
CUL2
FBXO10
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3
B
TRIM10
WSB2
VPS18
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 kDa
SKP2
SPSB1
TRIM37
TRIM56
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
TRIM49
TRIM73
TRIM32
TRIM21
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
TRIM7
TRIM36
TRIM41
TRIM2
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
WSB2
UBE2O
TRIM74
VPS41
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
VPS18
UHRF1
ZNF509
ZBTB17
Hsp90β -flag
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si-ZBTB20
si-ZNF598
si-WWP1
Hsp90β -flag
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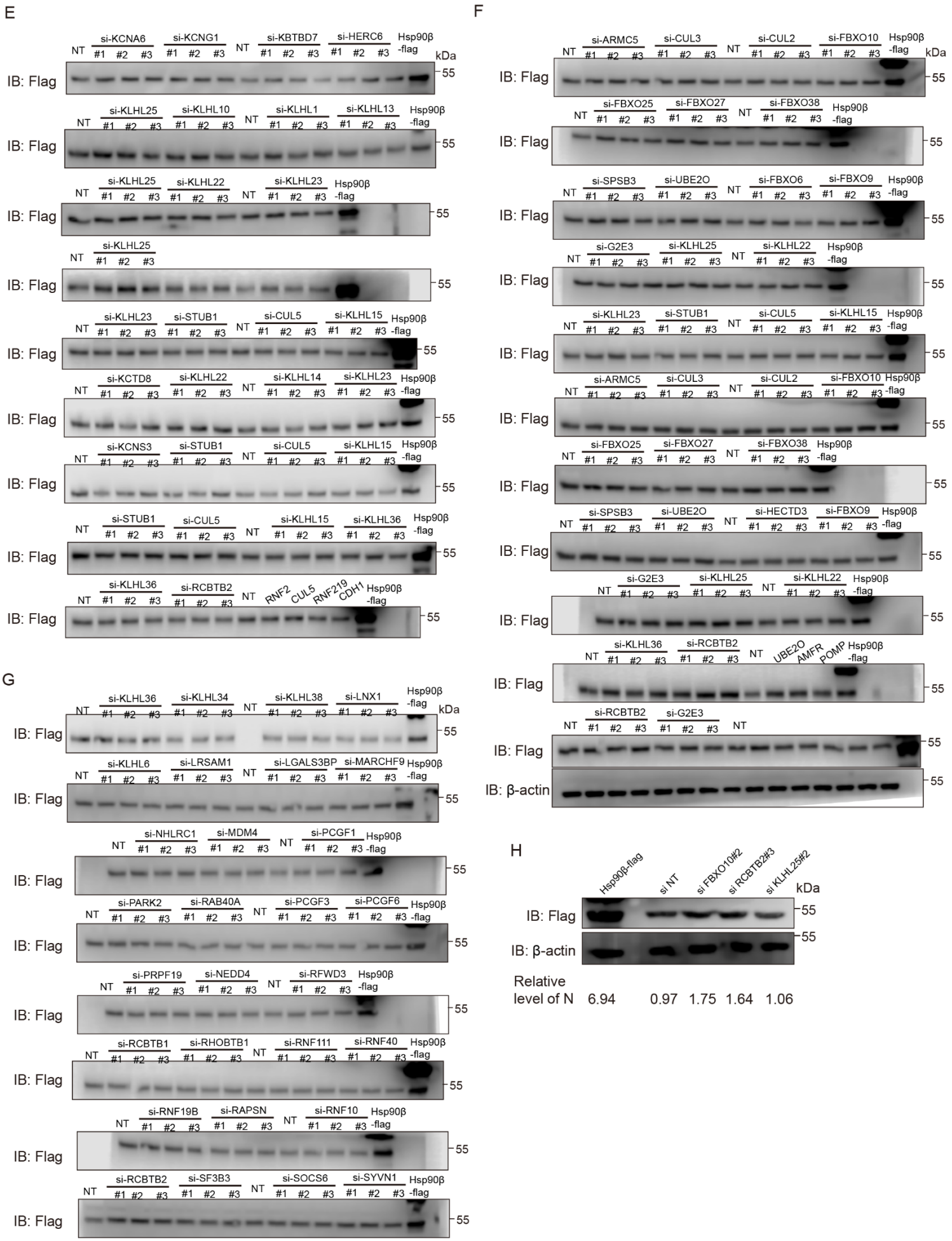
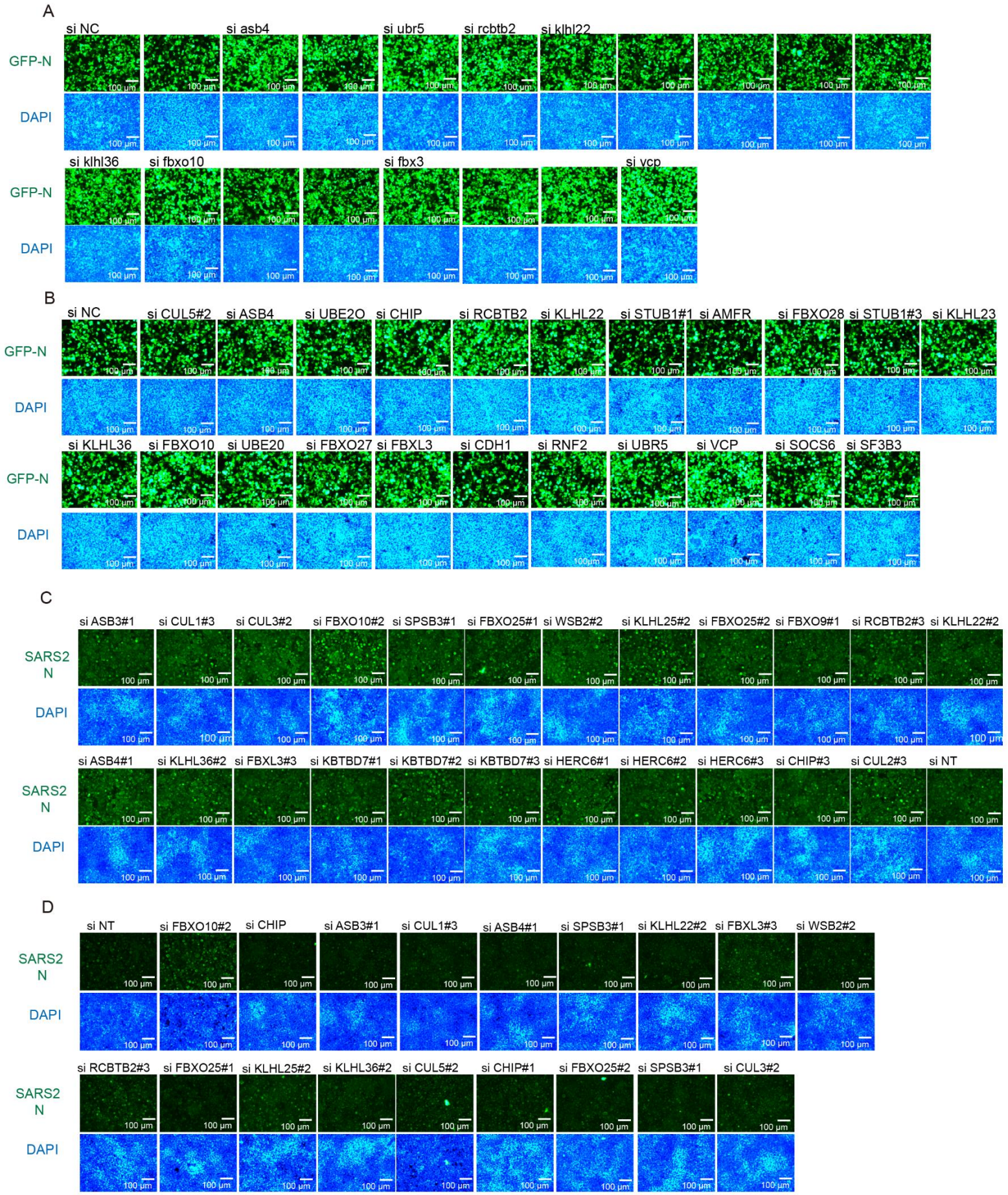
C
ENC1
DET1
FBXL12
FBXL15
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3 kDa
FBXL14
FBXL18
FBXL13
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3
FBXL2
FBXL3
FBXO10
FBXL8
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
FBXO17
FBXO24
FBXO25
FBXO28
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 #1 #2 #3 #1 #2 #3
FBXO25
FBXO28
FBXO27
TRIM17
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
FBXO25
FBXO27
FBXO38
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3
FBXO27
FBXO18
FBXO38
TNFAIP3
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
TRIM17
SPSB3
TRIM10
SH3RF2
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
SPSB3
NT #1 #2 #3
SPSB3
UBE2O
si-FBXO6
si-FBXO9
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
IB: β-actin
D
ZBTB17
si-ZBTB20
si-HECTD3
si-FBXO9
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3 kDa
ZBTB17
si-KLHL25
si-KLHL22
si-KLHL23
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
FBXO10
FBXO25
FBXO28
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3
TRIM7
TRIM36
UBE2O#3
VPS18#1
ZBTB20#1
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT
si-FBXO40
si-FBXO34
si-FBXO3
si-FBXO4
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
si-HECTD1
si-HECTD3
si-FBXW7
si-FBXO6
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
si-FBXO9
si-FBXW11
si-FBXW2
si-GAN
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
si-FBXW5
si-G2E3
si-HECTD2
si-HERC2P9
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3
si-G2E3
si-KLHL25
si-KLHL22
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3
si-HERC5
si-KBTBD4
si-HERC4
si-KCNA5
Hsp90β -flag
NT #1 #2 #3 #1 #2 #3 NT #1 #2 #3 #1 #2 #3

S1 Fig E-H


E
si-KCNA6
si-KCNG1
si-KBTBD7
si-HERC6
Hsp90β -flag
kDa
IB: Flag
55
si-KLHL25
si-KLHL10
si-KLHL1
si-KLHL13
si-KLHL22
si-KLHL23
si-STUB1
si-CUL5
si-KLHL15
si-KCTD8
si-KLHL14
si-KCNS3
si-KLHL36
si-RCBTB2
RNF2
CUL5
RNF219
CDH1
F
si-ARMC5
si-CUL3
si-CUL2
si-FBXO10
si-FBXO25
si-FBXO27
si-FBXO38
si-SPSB3
si-UBE2O
si-FBXO6
si-FBXO9
si-G2E3
si-HECTD3
UBE2O
AMFR
POMP
IB: β-actin
G
si-KLHL34
si-KLHL38
si-LNX1
si-KLHL6
si-LRSAM1
si-LGALS3BP
si-MARCHF9
si-NHLRC1
si-MDM4
si-PCGF1
si-PARK2
si-RAB40A
si-PCGF3
si-PCGF6
si-PRPF19
si-NEDD4
si-RFWD3
si-RCBTB1
si-RHOBTB1
si-RNF111
si-RNF40
si-RNF19B
si-RAPSN
si-RNF10
si-SF3B3
si-SOCS6
si-SYVN1
H
Hsp90β-flag
si NT
si FBXO10#2
si RCBTB2#3
si KLHL25#2
Relative level of N
6.94
0.97
1.75
1.64
1.06

S2 Fig


A
si NC
si asb4
si ubr5
si rcbtb2
si klhl22
GFP-N
DAPI
si klhl36
si fbxo10
si fbx3
si vcp
B
si NC
si CUL5#2
si ASB4
si UBE2O
si CHIP
si RCBTB2
si KLHL22
si STUB1#1
si AMFR
si FBXO28
si STUB1#3
si KLHL23
si KLHL36
si FBXO10
si UBE20
si FBXO27
si FBXL3
si CDH1
si RNF2
si UBR5
si VCP
si SOCS6
si SF3B3
C
si ASB3#1
si CUL1#3
si CUL3#2
si FBXO10#2
si SPSB3#1
si FBXO25#1
si WSB2#2
si KLHL25#2
si FBXO25#2
si FBXO9#1
si RCBTB2#3
si KLHL22#2
SARS2 N
DAPI
si ASB4#1
si KLHL36#2
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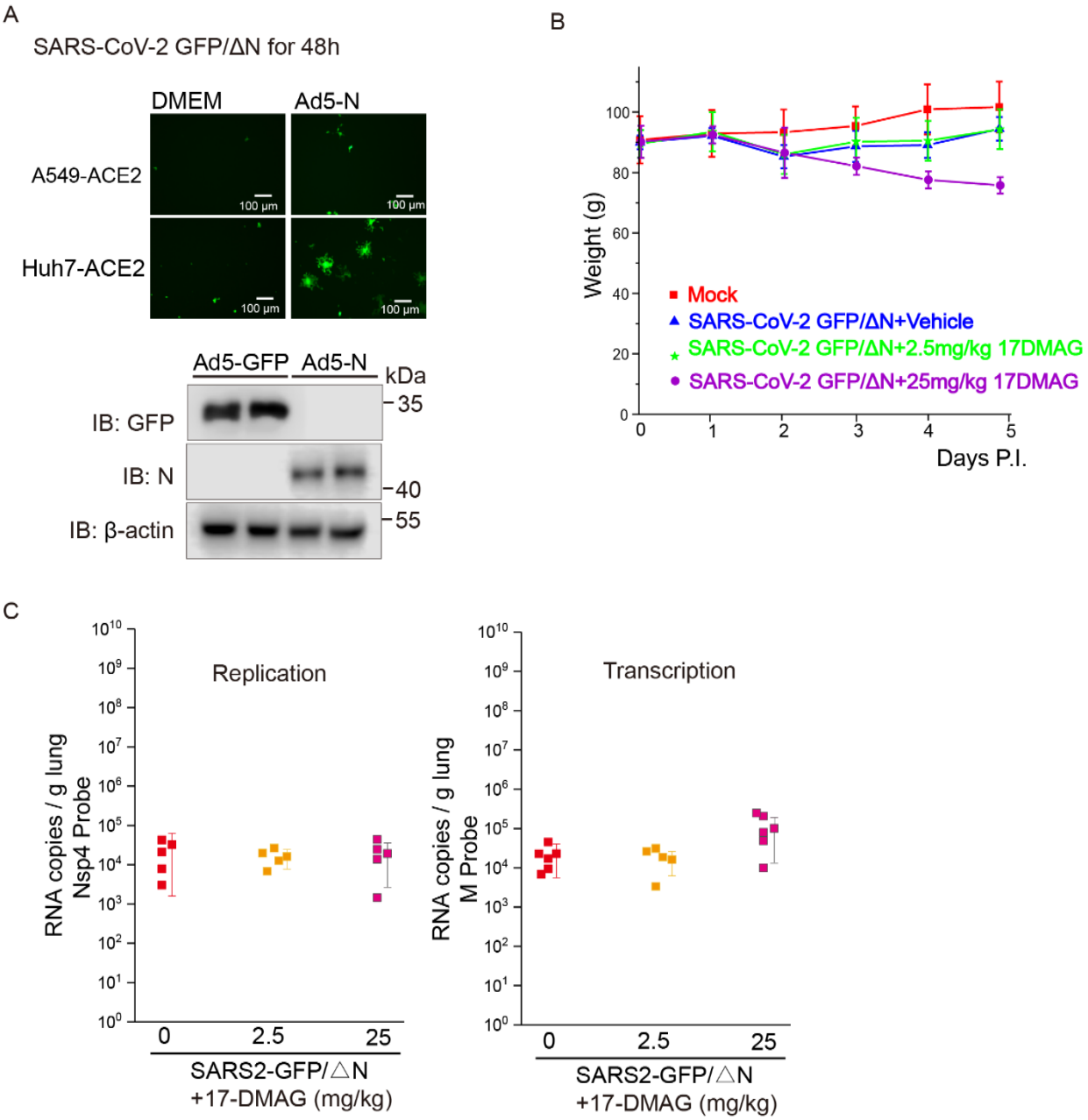
si HERC6#1
si HERC6#2
si HERC6#3
si CHIP#3
si CUL2#3
si NT
D
si NT
si FBXO10#2
si CHIP
si ASB3#1
si CUL1#3
si ASB4#1
si SPSB3#1
si KLHL22#2
si FBXL3#3
si WSB2#2
si RCBTB2#3
si FBXO25#1
si KLHL25#2
si KLHL36#2
si CUL5#2
si CHIP#1
si FBXO25#2
si SPSB3#1
si CUL3#2
100 μm

S3 Fig


A
SARS-CoV-2 GFP/ΔN for 48h
DMEM
Ad5-N
A549-ACE2
Huh7-ACE2
100 μm
Ad5-GFP
Ad5-N
kDa
IB: GFP
IB: N
IB: β-actin
35
40
55
B
Weight (g)
Days P.I.
Mock
SARS-CoV-2 GFP/ΔN+Vehicle
SARS-CoV-2 GFP/ΔN+2.5mg/kg 17DMAG
SARS-CoV-2 GFP/ΔN+25mg/kg 17DMAG
C
Replication
Transcription
RNA copies / g lung
Nsp4 Probe
M Probe
SARS2-GFP/△N
+17-DMAG (mg/kg)