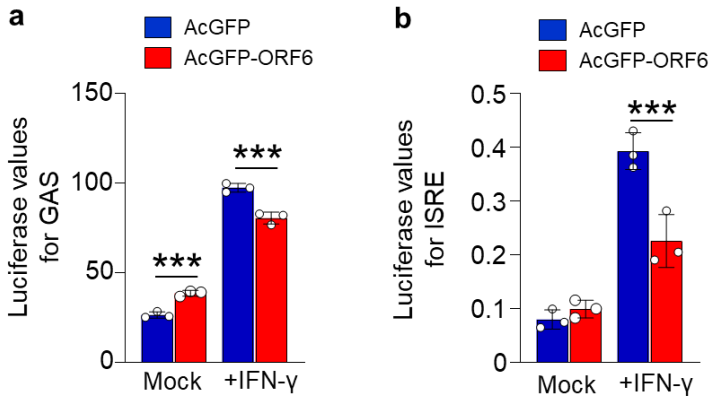
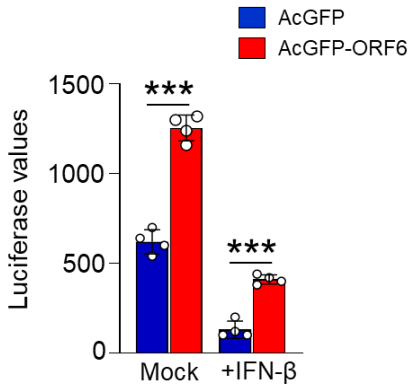


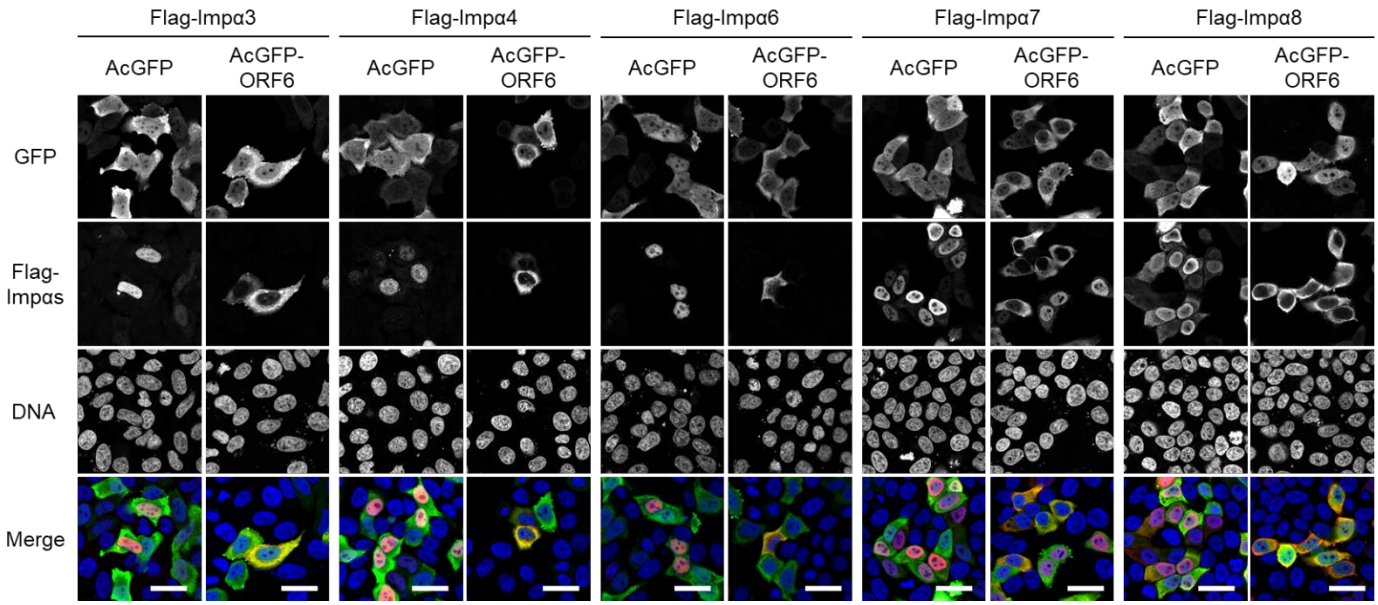
Supplementary Figure 1. Raw luciferase values in ORF6-transfected cells following IFN-γ stimulation

a-b) Raw luciferase values for the GAS-Luc (**a**) or the ISRE-luc (**b**) in AcGFP or AcGFP-ORF6-transfected Huh7 cells following IFN-γ stimulation (n=3). Fig. 1e and f are relative luciferase values based on the graphs **a** and **b**, respectively. ***P < 0.001, one-way ANOVA. error bars represent SD.



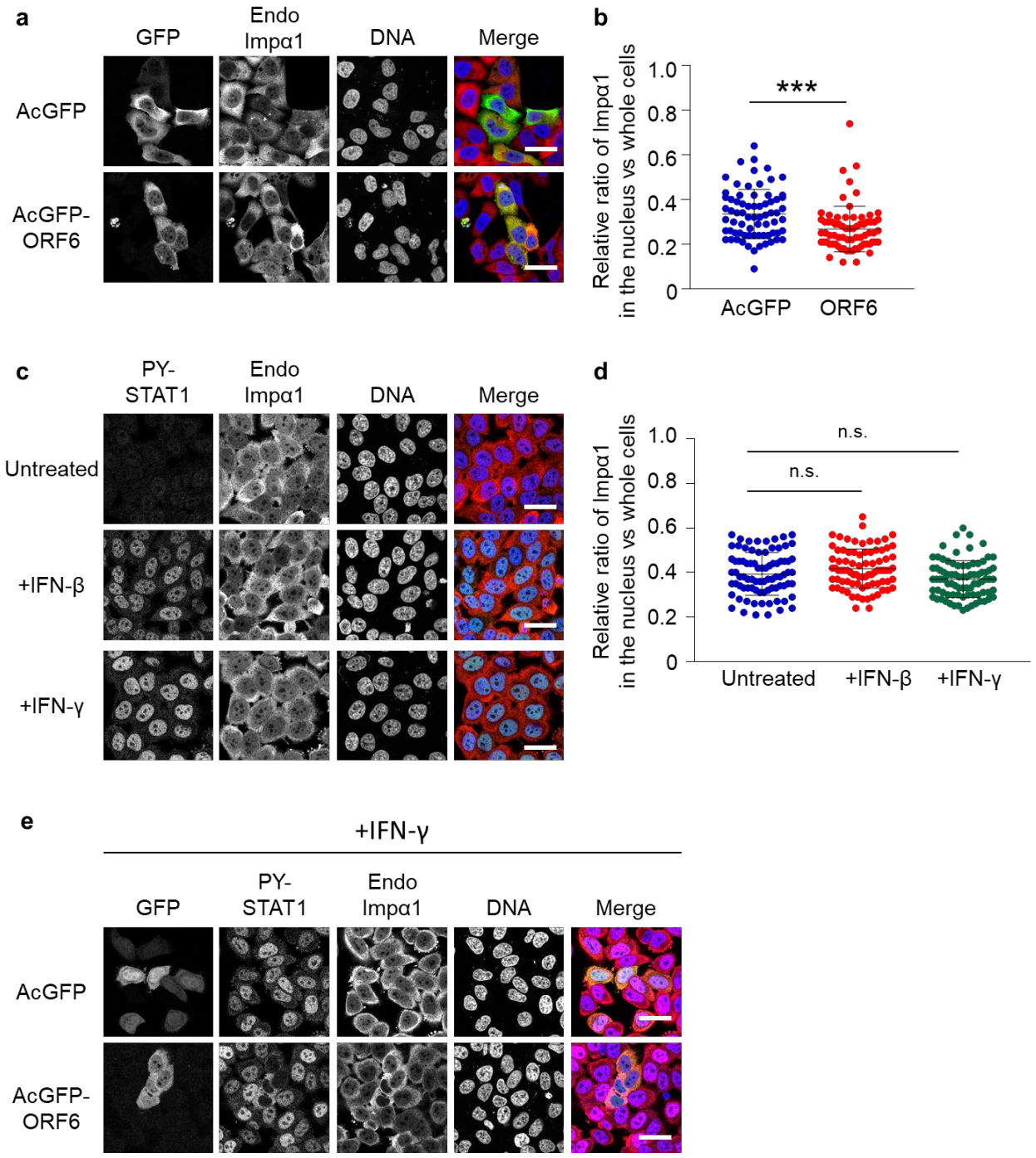
Supplementary Figure 2. ORF6 suppresses IFN-β-mediated down-regulation of the viral replication

Vero E6 replicon stable cell lines were transfected with AcGFP or AcGFP-ORF6, and then the cells were treated with IFN-β (final conc. 50 ng/mL) for 32 h. Luciferase values were determined by luciferase assay (n=4). ***P < 0.001, one-way ANOVA. error bars represent SD.



Supplementary Figure 3. Subcellular distribution of Flag-importin α subtypes

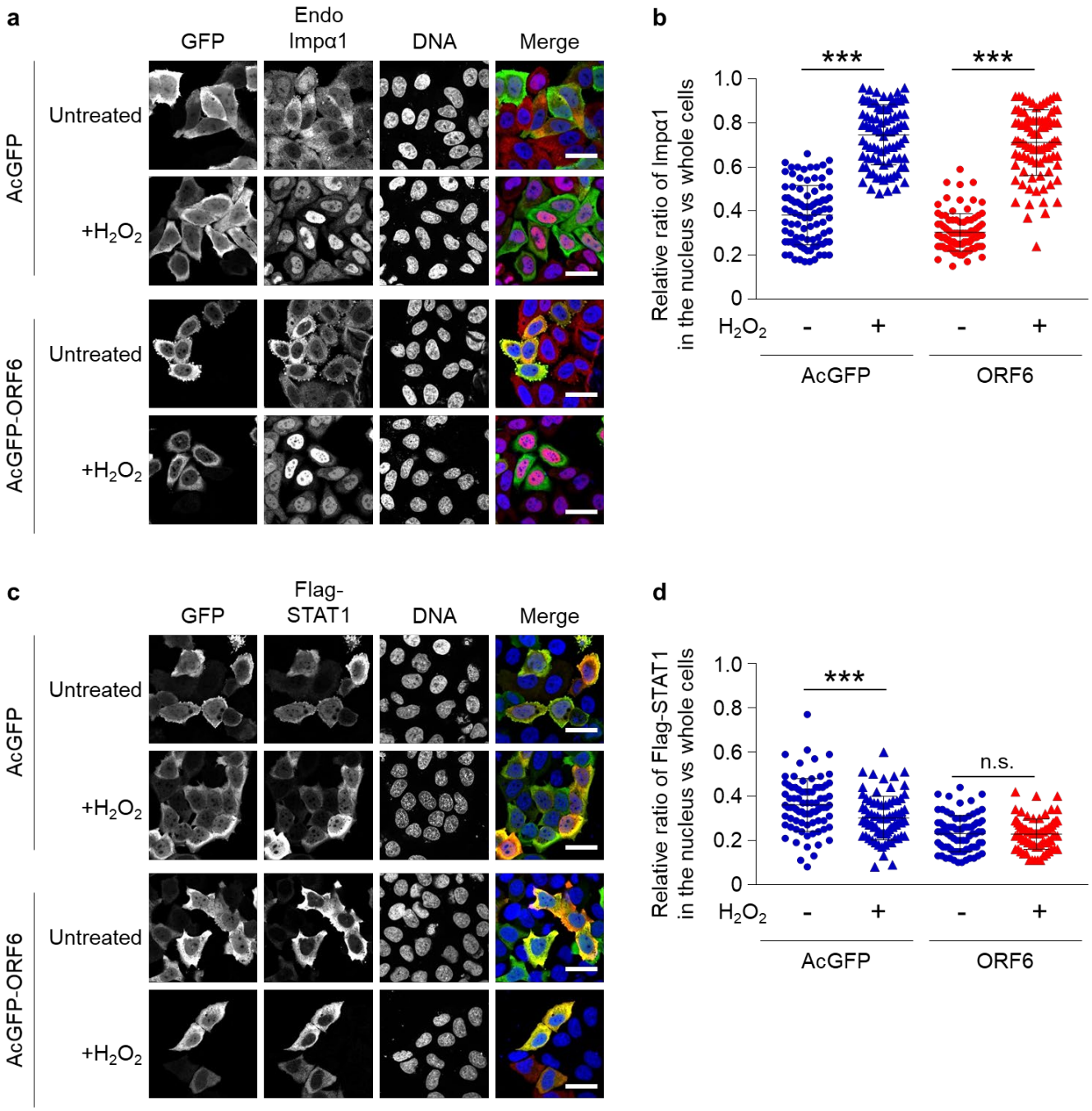
Subcellular localization of Flag-importin α3, α4, α6, α7, and α8 in HeLa cells expressed with AcGFP or AcGFP-ORF6. The GFP protein (green) and the Flag-importin α proteins (red) were detected using the anti-GFP or anti-Flag antibodies, respectively. DNA was stained with DAPI (blue). Scale bars: 30 μm.



Supplementary Figure 4. Subcellular distribution of endogenous importin α1 in ORF6-transfected HeLa cells

a) Subcellular localization of endogenous importin α1 (Endo Impα1) in AcGFP or AcGFP-ORF6 HeLa cells. GFP (green) and importin α1 (red) were detected using specific antibodies. DNA was stained with DAPI (blue). Scale bars: 30 μm. **b)** The graph represents relative values of importin α1 in the nucleus against the whole cells in **a**. Signal intensities of total 80 different nuclei from two independent experiments were measured, and statistically analyzed using student's t-test. ***P<0.001. error bars represent SD. **c)** Subcellular localization of PY-STAT1 and endogenous importin α1 (endo Impα1) in HeLa cells treated with IFN-β or IFN-γ. PY-STAT1

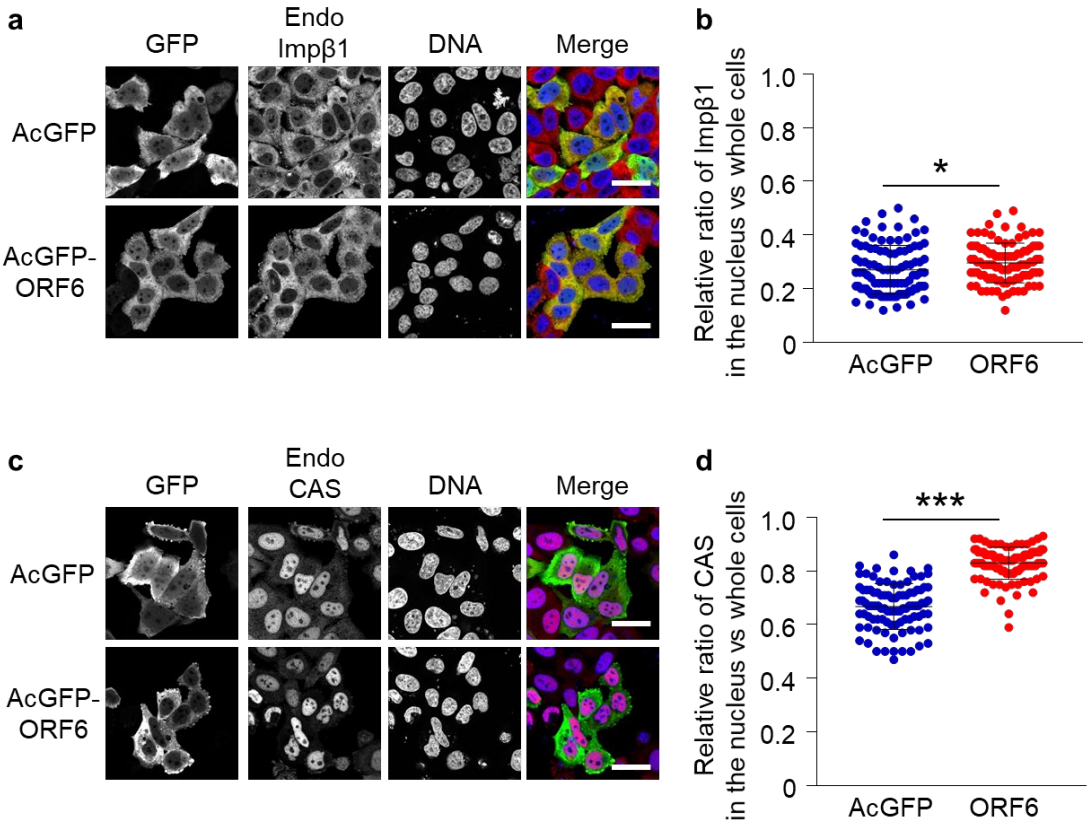
(green) and importin α1 (red) were detected using the specific antibodies. DNA was stained with DAPI (blue). Scale bars: 30 μm. **d)** The graph represents relative values of the fluorescent of importin α1 in the nucleus against the whole cells in **c**. Signal intensities of total 80 different nuclei from two independent experiments were measured, and statistically analyzed using a one-way ANOVA. n.s.: not significant. error bars represent SD. **e)** Subcellular localization of PY-STAT1 and endogenous Impα1 in AcGFP or AcGFP-ORF6-transfected HeLa cells treated with IFN-γ. Anti-PY-STAT1 (magenta) and anti-importin α1 (red) antibodies were used for detection. DNA was stained with DAPI (blue). Scale bars: 30 μm.



Supplementary Figure 5. Importin α1 shuttles in ORF6-transfected cells

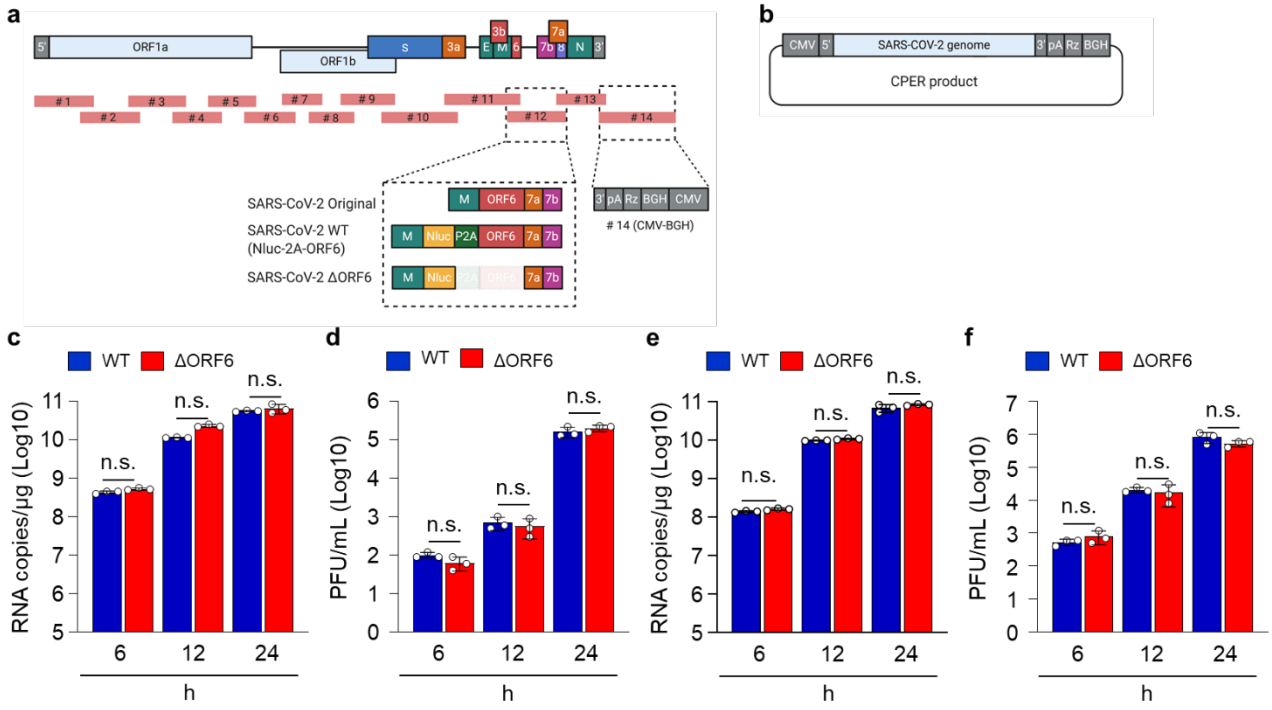
a) Subcellular localization of endogenous importin α1 (Endo Impα1) in HeLa cells expressed AcGFP or AcGFP-ORF6 following hydrogen peroxide (200 μM H_2O_2) treatment. GFP (green) and importin α1 (red) were detected using the anti-GFP or anti-importin α1 antibodies, respectively. DNA was stained with DAPI (blue). Scale bars: 30 μm. **b)** The graph represents the relative values of the fluorescent of importin α1 in the nucleus against the whole cells in **a**. Signal intensities of total 90 different nuclei from two independent experiments were measured, and statistically analyzed using a one-way ANOVA. ***P<0.001. error bars represent SD. **c)** Subcellular localization of Flag-STAT1 in HeLa cells transfected AcGFP or AcGFP-ORF6 following hydrogen peroxide (200 μM H_2O_2) treatment. GFP and Flag-STAT1 were detected using anti-GFP (green) or anti-Flag (red)

antibodies. DNA was stained with DAPI (blue). Scale bars: 30 μm. **d)** The graph represents the relative values of Flag-STAT1 in the nucleus against the whole cells in **c**. Signal intensities of total 80 different nuclei from two independent experiments were measured, and statistically analyzed using a one-way ANOVA. ***P<0.001, n.s.: not significant. error bars represent SD.



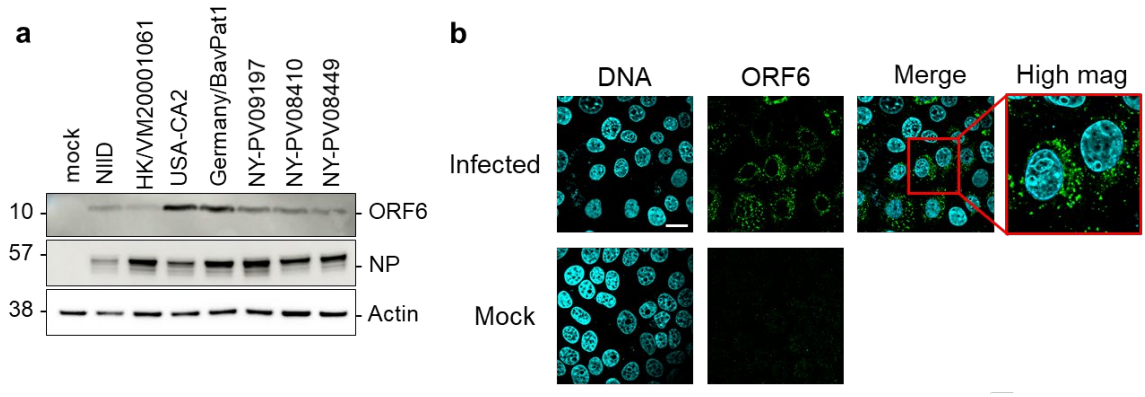
Supplementary Figure 6. Subcellular distribution of endogenous importin β1 or CAS in ORF6-transfected HeLa cells

a-d) Subcellular localization of endogenous importin β1 (Impβ1; **a**) or endogenous CAS (**c**) in AcGFP or AcGFP-ORF6 HeLa cells. GFP (green) and importin β1 (red) or CAS (red) were detected using specific antibodies. DNA was stained with DAPI (blue). Scale bars: 30 μm. The graph represents the relative values of the fluorescent of importin β1 (**b**) or CAS (**d**) in the nucleus against the whole cells. Signal intensities of total 70-100 different nuclei from two independent experiments were measured, and statistically analyzed using student's t-test. *P<0.05, ***P<0.001. error bars represent SD.



Supplementary Figure 7. Establishment of recombinant SARS-CoV-2 by the circular polymerase extension reaction

a) Schematic representation of recombinant SARS-CoV-2. A total of 13 fragments (#1-#13) that cover the viral genome were amplified with 40-60-nt overlapping ends. The fragments were mixed with the #14 fragment, which contained the CMV promoter (CMV) followed by the first 20 nt of SARS-CoV-2 genome and, at the other end, the 3' UTR of SARS-CoV-2, a synthetic poly(A) tail (pA), hepatitis delta virus ribozyme (Rz), and bovine growth hormone polyadenylation sequence (BGH). NLuc, NanoLuc gene; P2A, Porcine teschovirus 2A peptide. **b)** The genetic structure of the CPER product of the recombinant SARS-CoV-2. A total of 14 fragments were assembled by CPER. **c-f.** Huh7-ACE2 cells (**c**, **d**) or Vero-TMPRSS2 cells (**e**, **f**) were infected with SARS-CoV-2 WT (Nluc-2A-ORF6) or ΔORF6 and supernatants were collected at 6, 12 and 24 h post infection. Viral RNA in the supernatants was quantified using qRT-PCR (**c**, **e**), and the viral titer was quantified using plaque forming assay (**d**, **f**). Two-tailed Student's t-test, n.s.: not significant. error bars represent SD (n=3).



Supplementary Figure 8. Identification of ORF6 in different SARS-CoV-2 strains by a newly established antibody

a) VeroE6/TMPRSS2 cells were infected with several SARS-CoV-2 strains, and cell lysates were collected for detection of the ORF6 protein using western blotting. NP and Actin were used as an infection control and internal control, respectively. **b)** Immunofluorescence of ORF6 in VeroE6/TMPRSS2 cells infected with or without SARS-CoV-2 from NIID. The squares with a red line show a magnified marge image. Scale bars: 20 μm.

Supplemental Table 1. Oligo sequences for plasmid constructions

	Name	Sequence	Vectors
Mammalian expression vector	ORF6_Fw	5'-GCTGTACAAGGAATTCATGTTTCATCTCGTTGACTTTCAGG-3'	pCAG AcGFP-HA
	ORF6_Rv	5'-GGTATCCTCTGAATTCATCAATCTCCATTGGTTGCTC-3'	
	ORF6-M1_Fw	5'-GCTGTACAAGGAATTCATGTTTCATCTCGTTGACTTTCAGG-3'	
	ORF6-M1_Rv	5'-GGTATCCTCTGAATTCATCAATCTCCATTGGTTGCTCTTCATC TGCTGCTGCTGCTTTATTCTCAGTTAGTGACT-3'	
	ORF6-M2_Fw	5'-GCTGTACAAGGAATTCATGTTTCATCTCGTTGACTTTCAGG-3'	
	ORF6-M2_Rv	5'-GGTATCCTCTGAATTCATCAATCTCCATTGGTTGTGCTGCTG CTAATTGAGAATATTTATTCT-3'	
	ORF6-M3_Fw	5'-GCTGTACAAGGAATTCATGTTTCATCTCGTTGACTTTCAGG-3'	
	ORF6-M3_Rv	5'-GGTATCCTCTGAATTCTGCTGCTGCTGCTGCTGCCTCTTCA TCTAATTGAGAAT-3'	
	ORF6 Δ9-N_Fw	5'-GCTGTACAAGGAATTCATGTTTCATCTCGTTGACTTTCAGG-3'	
	ORF6 Δ9-N_Rv	5'-ATGATGTAAGTCCTCATAATAATTAGTAATATC-3'	
	ORF6 Δ9-C_Fw	5'-TGAGGACTTACATCATAAACCTCATAATTAAAA-3'	
	ORF6 Δ9-C_Rv	5'-GGTATCCTCTGAATTCATCAATCTCCATTGGTTGCTC-3'	
	STAT1_Fw	5'-CGATGACAAGGGATCCATGTCTCAGTGGTACGAAC-3'	pcDNA5/FRT/3x FLAG
	STAT1_Rv	5'-GGCTCTCGCTGAATTCCTATACTGTGTTCATCATACT-3'	
	SV40T-NLS_Fw	5'-GATCTCCTCCAAAAAAGAAGAGAAAGGTAGAAGACG-3'	mCherry-C1
	SV40T-NLS_Rv	5'-TCGACGTCTTCTACCTTTCTCTTCTTTTTTGGAGGA-3'	
Bacteria expression	ORF6_Fw	5'- GACGATATCAGGATCCATGTTTCATCTCGTTGACTTTCAGG-3'	pGEX6P2-GFP
	ORF6_Rv	5'- GATGCGGCCGCTCGAGATCAATCTCCATTGGTTGCTC-3'	
	M0_Fw	5'-GATCCGAATTCTATTCTCAATTAGATGAAGAGCAACCAATGG AGATTGATCCC-3'	pGEX2T-GFP
	M0_Rv	5'-GGGATCAATCTCCATTGGTTGCTCTTCATCTAATTGAGAATAG AATTCG-3'	
	M1_Fw	5'-GATCCGAATTCGCAGCAGCAGCAGATGAAGAGCAACCAATGG AGATTGATCCC-3'	
	M1_Rv	5'-GGGATCAATCTCCATTGGTTGCTCTTCATCTGCTGCTGCTGC GAATTCG-3'	
	M2_Fw	5'-GATCCGAATTCTATTCTCAATTAGCAGCAGCACAACCAATGGA GATTGATCCC-3'	
	M2_Rv	5'-GGGATCAATCTCCATTGGTTGTGCTGCTGCTAATTGAGAATA GAATTCG-3'	
	M3_Fw	5'-GATCCGAATTCTATTCTCAATTAGATGAAGAGGCAGCAGCAG CAGCAGCACCC -3'	
	M3_Rv	5'-GGGTGCTGCTGCTGCTGCTGCCTCTTCATCTAATTGAGAATA GAATTCG-3'	

Supplemental Table 2. Primers for qRT-PCR

Name	Sequence
IP-10_Fw	5’-GGCCATCAAGAATTTACTGAAAGCA-3’
IP-10_Rv	5’-TCTGTGTG GTCCATCCTTGGAA-3’
β-actin for IP-10_Fw	5'-TTCCAGGAGCGAGATCCCT-3'
β-actin for IP-10_Rv	5'-CACCCATGACGAACATGGG-3'
SARS-CoV-2_N2_Fw	5’-AAATTTTGGGGACCAGGAAC-3’
SARS-CoV-2_N2_Rv	5’-TGGCAGCTGTGTAGGTCAAC-3’
β-actin for N2_Fw	5’-TTGCTGACAGGATGCAGAAG-3’
β-actin for N2_Rv	5’-GTACTTGCGCTCAGGAGGAG- 3’

Supplemental Table 3. Primers for CPER reaction

CPER#1-Fw	5’-GAGCTCGTTTAGTGAACCGTATTAAAGGTTTATACCTTCC-3’
CPER#1-Rv	5’-CTATCACAGTGTCATCACCAAAAGTAACCTTTGTTGGTGC-3’
CPER#2-Fw	5’-CCTTCACACTCAAAGGCGGTGCACCAACAAAGGTTACTTT-3’
CPER#2-Rv	5’-CTTCTACACCCTTAAGGGTTGTCTGCTGTTGTCCACAAGT-3’
CPER#3-Fw	5’-TCTTGAACGTGGTGTGTAAAACTTGTGGACAACAGCAGAC-3’
CPER#3-Rv	5’-TAACTTTAATTAACTGCTTCAACCAATTATTAACAATTTT-3’
CPER#4-Fw	5’-AGATAGCACTTAAGGGTGGTAAAATTGTTAATAATTGGTT-3’
CPER#4-Rv	5’-GAACCCTTAATAGTGAAATTGGGCCTCATAGCACATTGGT-3’
CPER#5-Fw	5’-TGGTTCACCATCTGGTGTTTACCAATGTGCTATGAGGCCC-3’
CPER#5-Rv	5’-TTTATGTCTACAGCACCCTGCATGGAAAGCAAAACAGAAA-3’
CPER#6-Fw	5’-TCACTACTTTCTGTTTTGCTTTCCATGCAGGGTGCTGTAG-3’
CPER#6-Rv	5’-ACCAGAAGCAGCGTGCATAGCAGGGTCAGCAGCATACACA-3’
CPER#7-Fw	5’-CTTAGTTTTAAGGAATTACTTGTGTATGCTGCTGACCCTG-3’
CPER#7-Rv	5’-TGGGTAAGCATCTATAGCTAAAGACACGAACCGTTCAATC-3’
CPER#8-Fw	5’-CAGATGGTACACTTATGATTGAACGGTTCGTGTCTTTAGC-3’
CPER#8-Rv	5’-AGGTGTGTAGGTGCCTGTGTAGGATGTAACCCAGTGATTA-3’
CPER#9-Fw	5’-AAGATTGTAGTAAGGTAATCACTGGGTTACATCCTACACA-3’
CPER#9-Rv	5’-GACTAGAGACTAGTGGCAATAAAACAAGAAAAACAAACAT-3’
CPER#10-Fw	5’-TGTTAACAACTAAACGAACAATGTTTGTTTTTCTTGTTTT-3’
CPER#10-Rv	5’-CAAATGAGGTCTCTAGCAGCAATATCACCAAGGCAATCAC-3’
CPER#11-Fw	5’-GCTTCATCAAACAATATGGTGATTGCCTTGGTGATATTGC-3’
CPER#11-Rv	5’-TAAGCTCTTCAACGGTAATAGTACCGTTGGAATCTGCCAT-3’
CPER#12-Fw	5’-TTGGAACTTTAATTTTAGCCATGGCAGATTCCAACGGTAC-3’
CPER#12-Rv	5’-TACATTCTTGGTGAAATGCAGCTACAGTTGTGATGATTCC-3’
CPER#13-Fw	5’-AAATTTCTTGTTTTCTTAGGAATCATCACAACTGTAGCTG-3’
CPER#13-Rv	5’-TTGTCATTCTCCTAAGAAGCTA-3’
CPER#14-Fw	5’-CTATCCCCATGTGATTTTAATAGCTTCTTAGGAGAATGAC-3’
CPER#14-Rv	5’-GGAAGGTATAAACCTTTAATACGGTTCACTAAACGAGCTC-3’

Unprocessed western blot/CBB gel data

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The area circled by the red square is the area shown in figures.

Fig. 2f

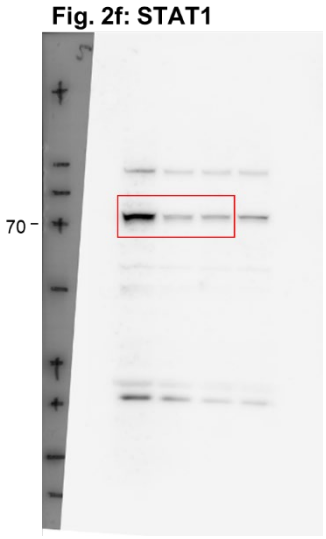
Fig. 2f: STAT1
70

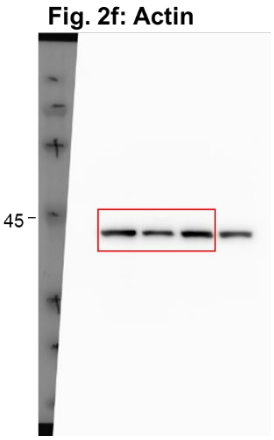
Fig. 2f: Actin
45

Fig. 3e

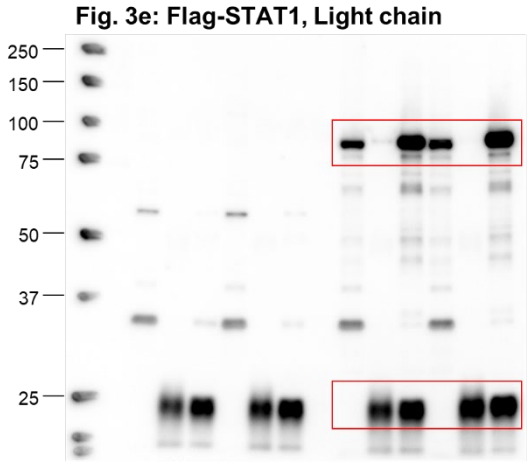
Fig. 3e: Flag-STAT1, Light chain
250
150
100
75
50
37
25

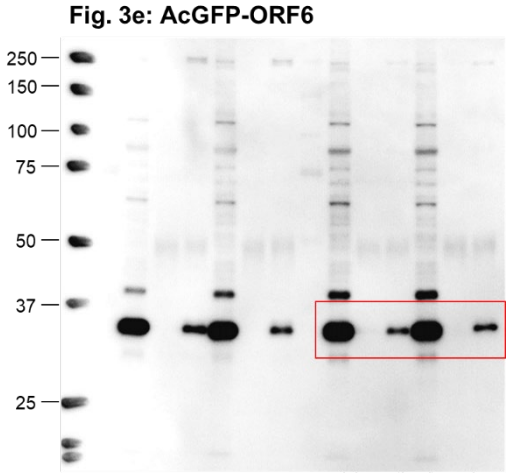
Fig. 3e: AcGFP-ORF6
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150
100
75
50
37
25

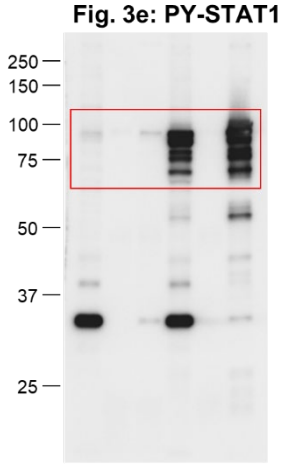


Fig. 3e: PY-STAT1
250
150
100
75
50
37
25

Fig. 4a,b

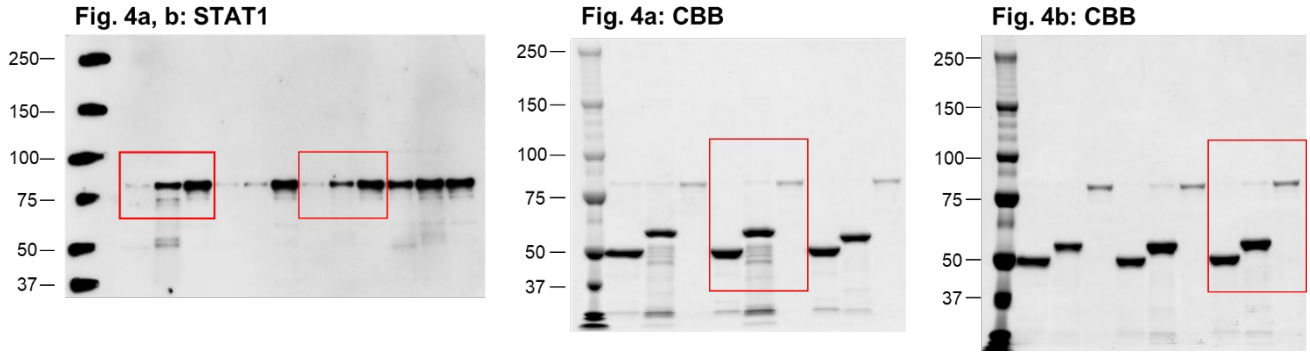


Fig. 4c

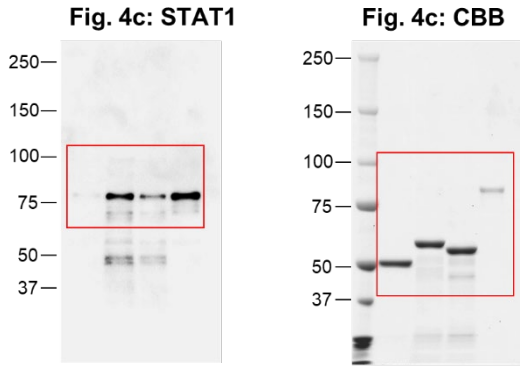


Fig. 4d

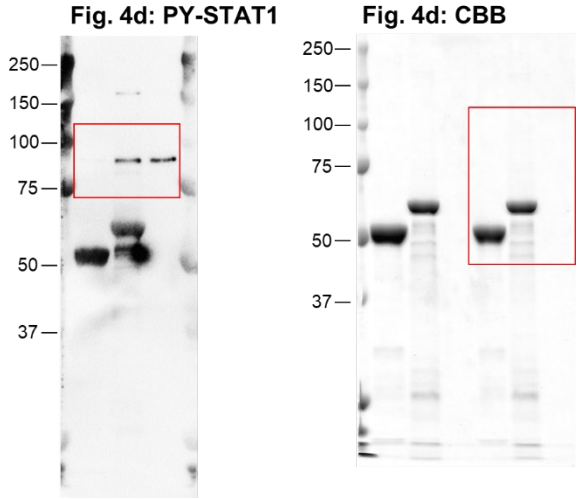


Fig. 4e

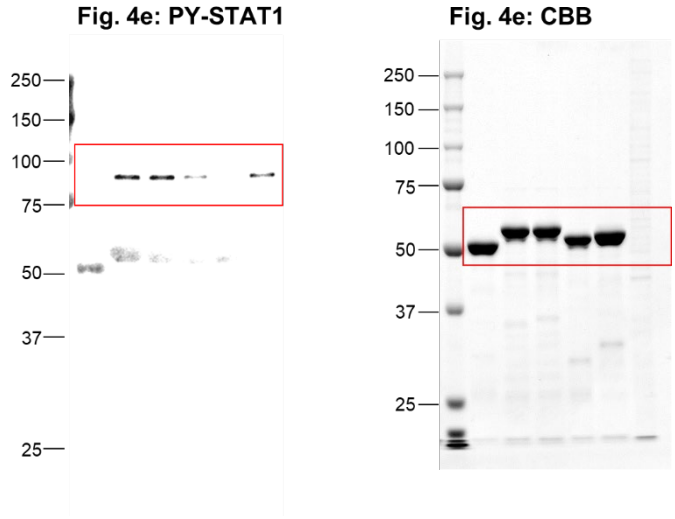


Fig. 5c

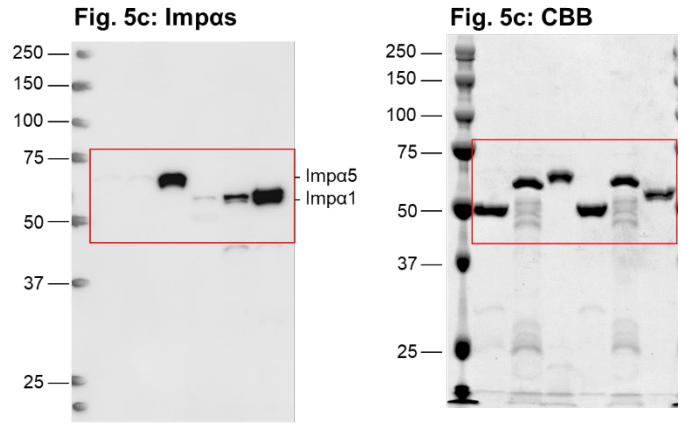


Fig. 5d

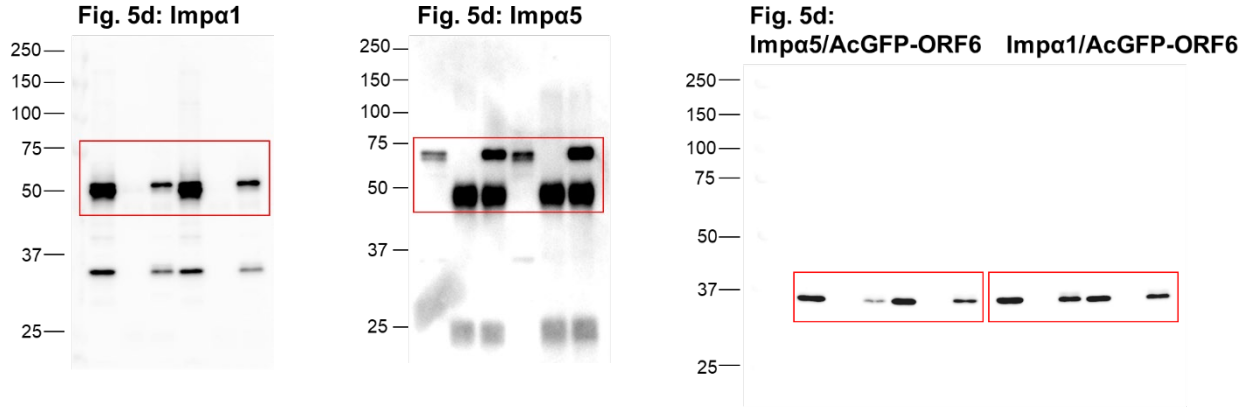
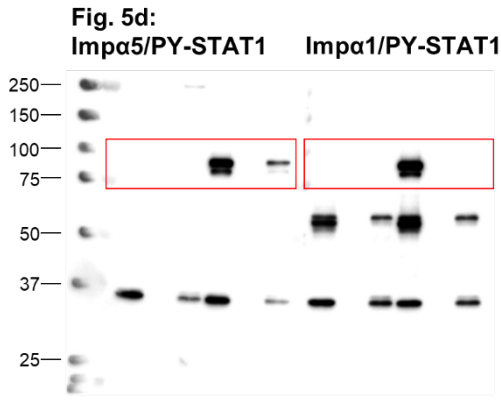


Fig. 5d



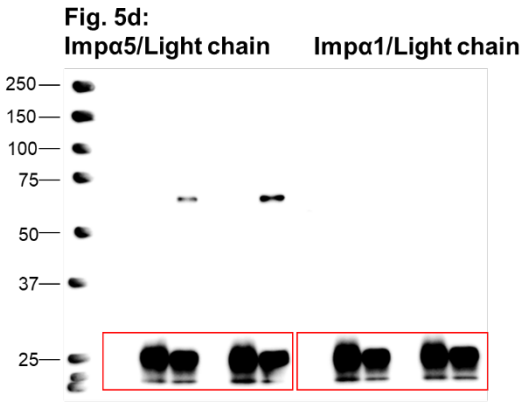
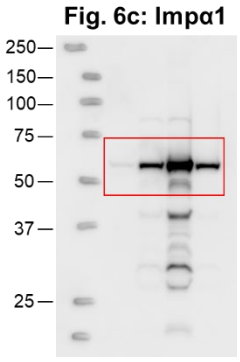


Fig. 6c



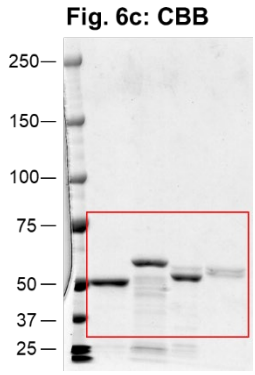
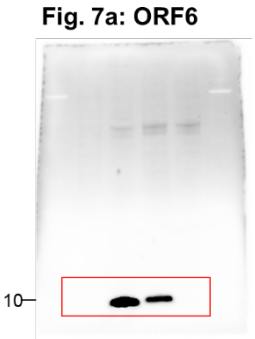
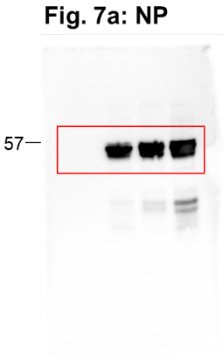
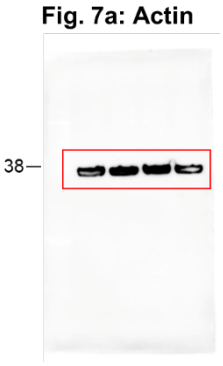


Fig. 7a

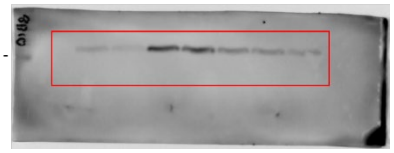




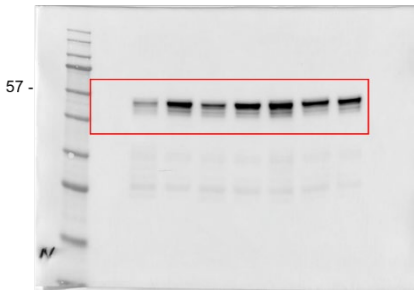


Suplemental Fig. 8a

Supple Fig. 8a: ORF6



Supple Fig. 8a: NP



Supple Fig. 8a: Actin