Supplementary Information

Human coronaviruses activate and hijack the host transcription factor HSF1 to enhance viral replication

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Supplementary Table 1. Expression profile of 84 HSF1-target genes in HCoV-229E infected cells

GENE	Fold Change	*p*-value
ATF6	2,24	0,004042
BAG1	2,86	0,002787
BAG2	2,95	0,000159
BAG3	8,75	0,000143
BAG4	2,26	0,003037
BAG5	3,41	0,0003
CCS	1,41	0,007209
CCT2	2,43	0,006482
CCT3	3	0,006755
CCT4	3,51	0,000201
CCT5	2,34	0,000076
CCT6A	1,79	0,000517
CCT6B	1,97	0,00996
CCT7	1,52	0,003472
COQ8A	1,95	0,018829
CRYAA	0,93	0,972409
CRYAB	7,64	0,000045
DNAJA1	3,42	0,000447
DNAJA2	2,22	0,001657
DNAJA3	2,95	0,00021
DNAJA4	37,68	0,001242
DNAJB1	21,92	0,003122
DNAJB11	5,54	0,000045
DNAJB12	2,43	0,000333
DNAJB13	1,21	0,266541
DNAJB14	3,36	0,000008
DNAJB2	2,87	0,000061
DNAJB5	3,41	0,000003
DNAJB6	2,57	0,000329
DNAJB7	1,35	0,13324
DNAJB8	2,14	0,227409
DNAJB9	15,16	0,00002
DNAJC1	2,88	0,000067
DNAJC10	3,28	0,000933
DNAJC11	2,11	0,00018
DNAJC12	12,54	0,000116
DNAJC13	1,67	0,000871
DNAJC14	2,2	0,000264
DNAJC15	1,14	0,014643
DNAJC16	3,69	0,000304
DNAJC17	1,25	0,48577
DNAJC18	1,97	0,006605
DNAJC21	1,79	0,000057
DNAJC3	13,52	0,000123
DNAJC4	1,09	0,072608
DNAJC5	2,74	0,000118
DNAJC5B	1,94	0,433188
DNAJC5G	2,59	0,058501
DNAJC6	1,24	0,550764
DNAJC7	2,49	0,004597
DNAJC8	1,37	0,023288
DNAJC9	1,03	0,802655
HSF1	1,68	0,009516
HSF2	4,05	0,000862
HSF4	1,39	0,145565
HSP90AA1	6,53	0,00124
HSP90AB1	3,57	0,000081
HSP90B1	5,5	0,000083
HSPA14	2,58	0,000824
HSPA1A	26,45	0,005915
HSPA1B	54,66	0,000652
HSPA1L	1,49	0,423684
HSPA2	0,8	0,08965
HSPA4	2,61	0,001488
HSPA4L	10,03	0,000241
HSPA5	12,74	0,000184
HSPA6	1674,96	0,002617
HSPA8	2,05	0,001686
HSPA9	6,1	0,000005
HSPB1	1,62	0,181349
HSPB2	0,67	0,64913
HSPB3	0,88	0,584931
HSPB6	0,39	0,013922
HSPB7	0,64	0,160068
HSPB8	5,99	0,000064
HSPD1	4,46	0,000065
HSPE1	1,78	0,001644
HSPH1	13,63	0,000432
PFDN1	0,9	0,433629
PFDN2	1,36	0,002902
SERPINH1	3,74	0,00152
SIL1	2,49	0,001381
TCP1	3,14	0,000443
TOR1A	1,17	0,068212

MRC-5 cells infected with HCoV-229E (0.1 TCID$_{50}$/cell) for 24h. For details see legend Fig. 3A.

Supplementary Table 2. Expression profile of 84 HSF1-target genes in heat-shocked cells

GENE	Fold Change	*p*-value
ATF6	1,47	0,036919
BAG1	2,36	0,000475
BAG2	0,82	0,279689
BAG3	7,34	0,000124
BAG4	0,85	0,315173
BAG5	1,33	0,115042
CCS	2,01	0,00668
CCT2	1,13	0,372228
CCT3	1,34	0,221978
CCT4	1,53	0,078246
CCT5	0,74	0,009083
CCT6A	1,61	0,005258
CCT6B	1,75	0,067021
CCT7	1,08	0,890066
COQ8A	3,15	0,002652
CRYAA	10,5	0,06443
CRYAB	7,65	0,000006
DNAJA1	5,52	0,000065
DNAJA2	1,51	0,123754
DNAJA3	0,96	0,734253
DNAJA4	13,25	0,000001
DNAJB1	40,42	0,000005
DNAJB11	2,18	0,008582
DNAJB12	1,23	0,215473
DNAJB13	7,36	0,025194
DNAJB14	1,15	0,305747
DNAJB2	2,18	0,000273
DNAJB5	0,65	0,135038
DNAJB6	1,65	0,009224
DNAJB7	2,5	0,087585
DNAJB8	0,92	0,489083
DNAJB9	3,51	0,000137
DNAJC1	1,14	0,30654
DNAJC10	1,19	0,459702
DNAJC11	0,7	0,068169
DNAJC12	4,17	0,000144
DNAJC13	1,05	0,9834
DNAJC14	1,39	0,00831
DNAJC15	1,24	0,071779
DNAJC16	1,12	0,672599
DNAJC17	1,38	0,008337
DNAJC18	0,97	0,741256
DNAJC21	0,96	0,629384
DNAJC3	1,91	0,000467
DNAJC4	1,48	0,002066
DNAJC5	1,01	0,931602
DNAJC5B	1,39	0,176319
DNAJC5G	12,38	0,063994
DNAJC6	2,29	0,01689
DNAJC7	0,97	0,738533
DNAJC8	1,05	0,75302
DNAJC9	1,4	0,069386
HSF1	1,14	0,171801
HSF2	1,68	0,004032
HSF4	5,05	0,006061
HSP90AA1	3,48	0,000019
HSP90AB1	1,26	0,005016
HSP90B1	1,9	0,001505
HSPA14	0,67	0,027158
HSPA1A	95,02	0,000005
HSPA1B	155,67	0,000006
HSPA1L	8,16	0,010231
HSPA2	0	0,05966
HSPA4	1,48	0,002033
HSPA4L	23,25	0,000588
HSPA5	3,02	0,000101
HSPA6	312,55	0,000002
HSPA8	1,82	0,002618
HSPA9	0,65	0,007138
HSPB1	3,14	0,00004
HSPB2	1,56	0,154934
HSPB3	0,41	0,020257
HSPB6	1,04	0,60288
HSPB7	0,37	0,000011
HSPB8	1,36	0,219214
HSPD1	2,13	0,000165
HSPE1	2,36	0,000012
HSPH1	5,11	0,000044
PFDN1	1,13	0,356393
PFDN2	0,74	0,005894
SERPINH1	1,83	0,000028
SIL1	1,21	0,039457
TCP1	1,31	0,019544
TOR1A	0,79	0,166842

MRC-5 cells exposed to heat-shock (43°C, 40 min, 1.5 h recovery). For details see Fig. S2 legend.

Supplementary Table 3. Antibodies used

Antibody	Source	Catalogue Number
HCoV-229E Spike (P)	LGC NAC Company	PAB21477-100
HCoV-229E Nucleocapsid (P)	Sino Biological	40640-T62
HCoV-OC43 Nucleocapsid (P)	Sino Biological	40643-T62
KDEL (M)	Enzo Life Sciences	ADI-SPA-827
HCoV-NL63 Nucleocapsid (P)	Sino Biological	40641-T62
VSV-G protein (M)	Sigma-Aldrich	V5507
SARS-CoV-2 spike (M)	LGC NAC Company	MAB12444
Puromycin (M)	Sigma-Aldrich	MABE343
dsRNA (M)	SCICONS	10010200
α-Tubulin (M)	Sigma-Aldrich	T5168
HSF1 (P)	Enzo Life Sciences	ADI-SPA-901
HSF1 (phospho-S121) (P)	Tebubio	A8041
HSF1 (phospho-S303) (P)	abcam	ab47369
HSF1 (phospho-S326) (P)	abcam	ab76076
β-Actin (P)	Sigma-Aldrich	A2066
ZFAND2A (P)	Sigma-Aldrich	HPA019469
GAPDH (P)	Cusabio	CSB-PA00025A0Rb
HSP70 (M)	Enzo Life Sciences	ADI-SPA-810
Histone H3 (P)	abcam	ab1791
HSP90β (P)	Santa Cruz Biotechnology	sc-1057
HSP90 (P)	Cell Signaling	4877
HSP60 (P)	StressMarq	SPC-105
GFP (M)	CUSABIO	CSB-MA000051M0m
ACE2 (M)	Santa Cruz Biotechnology	sc-73668
Alexa Fluor 488 goat anti-mouse	Invitrogen	A11001
Alexa Fluor 555 goat anti-rabbit	Invitrogen	A21428
Goat Anti-Mouse IgG (H+L), HRP	Jackson ImmunoResearch	115-035-003
Goat Anti-Rabbit IgG (H+L), HRP	Jackson ImmunoResearch	111-035-003
Mouse Anti-Goat IgG (H+L), HRP	Santa Cruz Biotechnology	sc-2354

(**M**): Monoclonal; (**P**): Polyclonal

Supplementary Table 4. Primers and siRNAs used

Primers used for mRNA gene expression

Gene	Primer direction	Primer sequence
AIRAP	Forward	5′-TCATTTTCCATACGCTGCAC-3′
	Reverse	5′-CTGTGGTCCAAAGGGTGTCT-3′
COX-2	Forward	5′-TTGCTGGCAGGGTTGCTGGTGGTA-3′
	Reverse	5′-CATCTGCCTGCTCTGGTCAATGGAA-3′
HSP701A	Forward	5′-CTACAAGGGGGAGACCAAGG-3′
	Reverse	5′-TTCACCAGCCTGTTGTCAAA-3′
NKRF	Forward	5′-CCAAACCTTCCAAAGGTCAA-3′
	Reverse	5′-CAGGGTTCCCACTGTCAAAA-3′
HCoV-229E	Forward	5′- TTCCGACGTGCTCGAACTTT-3′
	Reverse	5′- CCAACACGGTTGTGACAGTGA-3′
L34	Forward	5′-GGCCCTGCTGACATGTTTCTT-3′
	Reverse	5′-GTCCCGAACCCCTGGTAATAGA-3′

siRNAs sequences

siRNA	Target sequence
siHSF1$_1$	5′-TACCCAAGTACTTCAAGCACA-3′
siHSF1$_2$	5′-CAGTGACCACTTGGATGCTAT-3′

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. HCoV-induced HSF1 phosphorylation and expression of HSF1-target genes is time- and m.o.i.-dependent, and is not mimicked by VSV infection. (**A**) Schematic representation of the experimental protocol (top panel). Whole-cell extracts (WCE) from samples mock-infected (Mock) or infected with HCoV-229E (0.1 $TCID_{50}$/cell) were analyzed for pHSF1 (Ser326), viral nucleocapsid (N)and α-tubulin protein levels at different times post infection (p.i.) by IB (bottom panels). (**B**) Immunoblot analysis of pHSF1 (Ser326), VSV-G glycoprotein and β-actin levels in HeLa cells mock-infected (0) or infected with VSV at different m.o.i. (0.1, 0.5 or 1 PFU/cell) or exposed to heat-stress (HS, 43°C, 40 min) (top panels). pHSF1-Ser326 protein levels were determined by densitometric analysis using ImageJ software (bottom panel). (**C**) MRC-5 cells were mock-infected or infected with HCoV-229E (1 $TCID_{50}$/cell) and levels of viral membrane (M-229E), HSP70 and AIRAP mRNA were analyzed at different times p.i. by qRT-PCR. (**D**) Total mRNA was extracted from MRC-5 cells mock-infected or infected with HCoV-229E (0.1 or 1 $TCID_{50}$/cell) for 24h and analyzed for M-229E, HSP70 and AIRAP gene expression by qRT-PCR. The fold increase was calculated by comparing the induction of M-229E, HSP70 and AIRAP in each sample to the relative control at 0h p.i. (C) or to the mock-infected control (D), which were arbitrarily set to 1. Error bars indicate means ± S.D. (n=3). * = $p < 0.05$; Student's *t*-test (B, C); ANOVA test (D).

Figure S2. Heat-shock and HCoV-229E infection turn on distinct HSF1-driven transcriptional programs in human lung cells. (**A-C**) Expression profile of selected HSF1-target genes affected by heat-shock (HS: 43°C, 40 min, 1.5h recovery) in MRC-5 cells as determined by qRT-PCR array (PAHS-076ZD-2-Qiagen). Heat Map (A) and Volcano plot (B) of 84 human HSPs and chaperones/cochaperones gene expression. In (A) each row represents a single gene, each column represents the mean of triplicate samples. The gradual color ranging from blue to red represents the mRNA expression level (Z-score). In the Volcano plot (B) fold regulation threshold is set to 2 and *p*- value cut off is 0.05; each dot represents a gene: red and blue dots indicate genes respectively up- and down-regulated more than 2-fold. Selected HSPs and chaperones/cochaperones genes whose expression is highly induced by HS are shown in (C). (**D,E**) Venn diagram (D) and comparison (E) of significantly ($p<0.05$) deregulated genes in MRC-5 cells heat-stressed as in (A) or infected with HCoV-229E (0.1 $TCID_{50}$/cell) for 24h.

Figure S3. **HCoV infection selectively induces canonical and non-canonical HSP expression in human lung cells.** (**A**) Levels of HSP90, GRP94, GRP78, HSP70, HSPA6, HSP60, AIRAP and viral spike protein shown in Fig. 3F were determined by densitometric analysis using ImageJ software, normalized to β-actin and expressed as arbitrary units (A.U.). (**B**) Levels of GRP94, GRP78, HSP70 and viral nucleocapsid (N-229E) protein shown in Fig. 3H were determined by densitometric analysis as in A. Error bars indicate means ± S.D. (n=3). * = $p < 0.05$; ** = $p < 0.01$; Student's *t*-test.

Figure S4. HCoV-OC43 and HCoV-NL63 infection induces the expression of HSF1-target genes in human cells. (**A, C**) Schematic representation of the experimental protocol (top panels). Immunoblot analysis of HSPA6, HSP60, AIRAP, viral nucleocapsid (N), α-tubulin and β-actin protein levels in MRC-5 (A) and Caco-2 hACE2 (C) cells mock-infected (-) or infected (+) with HCoV-OC43 (A) for 24h or HCoV-NL63 (C) for 72h at a m.o.i. of 0.1 $TCID_{50}$/cell (bottom panels). (**B, D**) Relative amounts of HSPA6, HSP60 and AIRAP proteins, normalized to α-tubulin (B) or to β-actin (D), were determined by densitometric analysis using ImageJ software. Error bars indicate means ± S.D. (n=3). * = $p < 0.05$; Student's *t*-test.

Figure S5. Effect of different SARS-CoV-2 variants on HSF1 phosphorylation in MRC5-hACE2 cells. (**A**) Schematic representation of the experimental protocol. (**B, C**) MRC5-hACE2 cells were mock-infected or infected with the Wuhan, Alpha and Delta SARS-CoV-2 variants (0.1 PFU/cell) for 40h. Equal amounts of whole-cell extracts (10 μl) were analyzed for levels of HSF1-Ser326, HSF1 and β-actin by IB (B). Levels of HSF1-Ser326 phosphorylation were determined after normalizing to β-actin and expressed as fold induction of the mock-infected control, which was arbitrarily set to 1 (C).

Figure S6. DTHIB treatment inhibits HCoV-OC43 replication. (**A**) MRC-5 cells mock-infected or infected with HCoV-OC43 (0.1 $TCID_{50}$/cell) were treated with different concentrations of DTHIB immediately after the adsorption period. (**B, C**) MRC-5 cells were treated with 10 μM DTHIB (filled bars) or control vehicle (C, empty bars) at 3h before infection (PRE), only during the adsorption period (ADS), immediately after the adsorption period (POST 0h) or at 10h after infection (POST 10h) with HCoV-OC43 at a m.o.i. of 0.1 $TCID_{50}$/cell (B) or 1 $TCID_{50}$/cell (C). (**A-C**) Virus yield was determined at 24h p.i. by $TCID_{50}$ infectivity assay. Data, expressed as $TCID_{50}$/ml, represent the mean ± S.D. (n=3). * = $p < 0.05$, ** = p 0.01, *** = $p < 0.001$; ANOVA test.

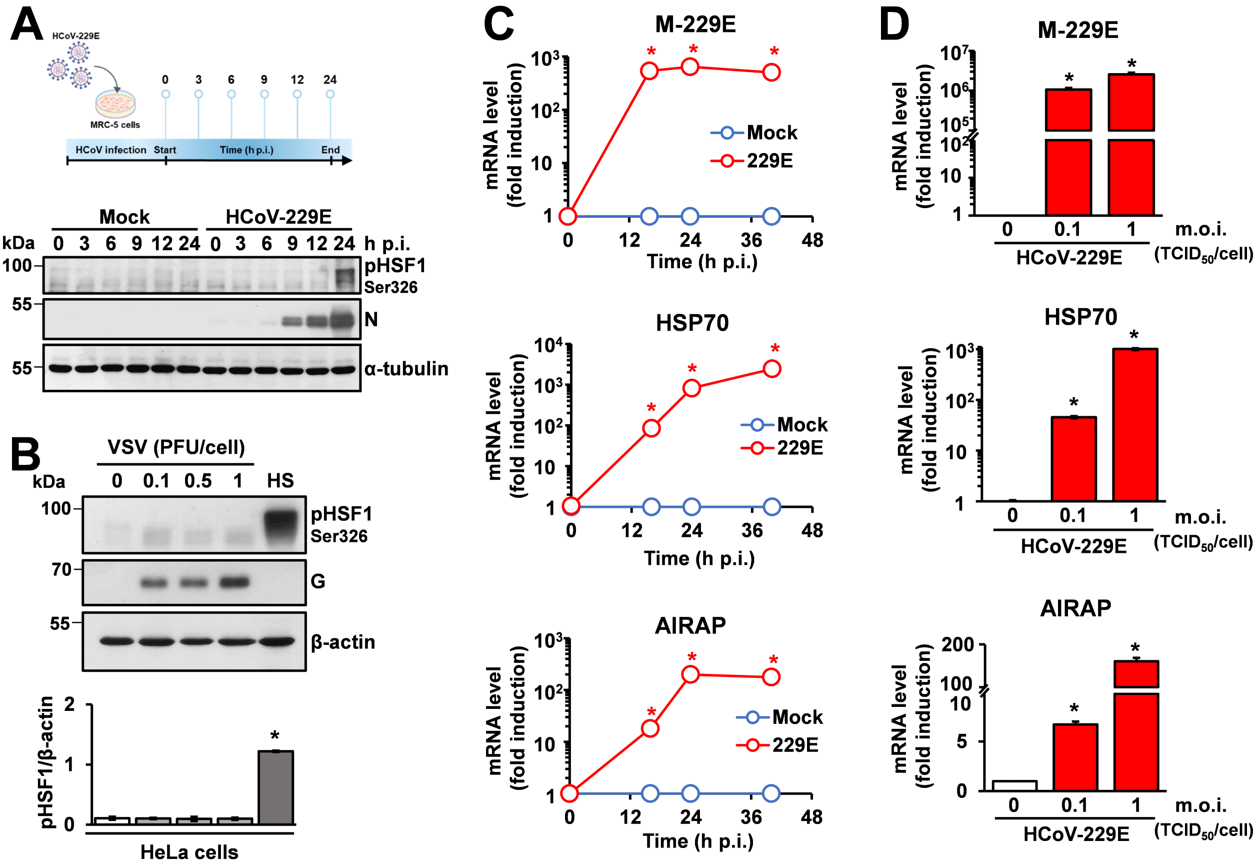
A
HCoV-229E
MRC-5 cells
0
3
6
9
12
24
HCoV infection
Start
Time (h p.i.)
End
Mock
HCoV-229E
kDa
0 3 6 9 12 24 0 3 6 9 12 24 h p.i.
100
pHSF1 Ser326
55
N
55
α-tubulin
B
VSV (PFU/cell)
kDa
0 0.1 0.5 1 HS
100
pHSF1 Ser326
70
G
55
β-actin
pHSF1/β-actin
2
1
0
*
HeLa cells
C
M-229E
mRNA level (fold induction)
10^3
10^2
10
1
Mock
229E
0 12 24 36 48
Time (h p.i.)
HSP70
10^4
10^3
10^2
10
1
Mock
229E
0 12 24 36 48
Time (h p.i.)
AIRAP
10^3
10^2
10
1
Mock
229E
0 12 24 36 48
Time (h p.i.)
*
D
M-229E
mRNA level (fold induction)
10^7
10^6
10^5
10^2
10
1
0 0.1 1 m.o.i.
HCoV-229E
(TCID$_{50}$/cell)
HSP70
10^3
10^2
10
1
0 0.1 1 m.o.i.
HCoV-229E
(TCID$_{50}$/cell)
AIRAP
200
100
10
5
0
0 0.1 1 m.o.i.
HCoV-229E
(TCID$_{50}$/cell)
*

Figure S1

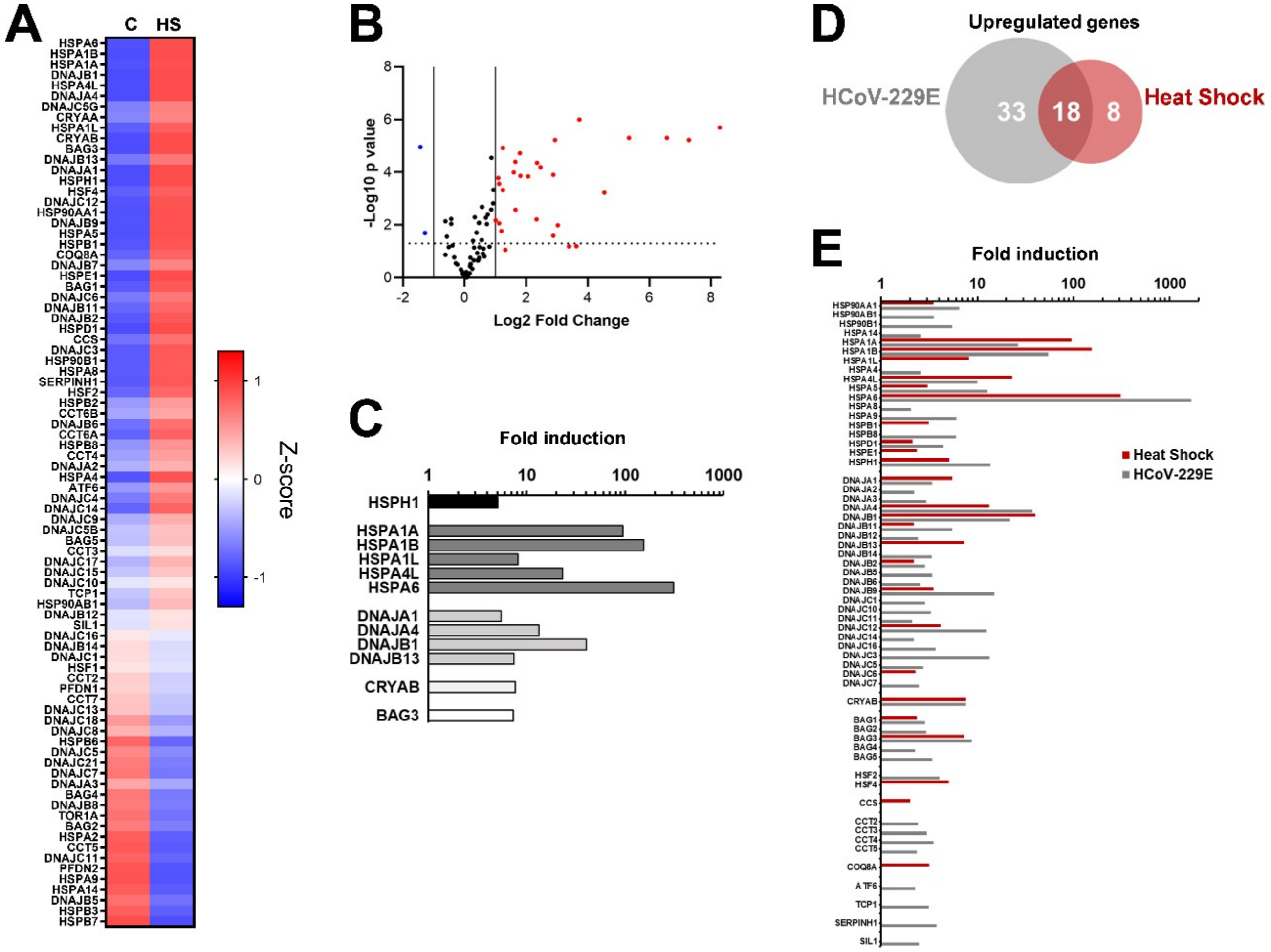
A
C
HS
HSPA6
HSPA1B
HSPA1A
DNAJB1
HSPA4L
DNAJA4
DNAJC5G
CRYAA
HSPA1L
CRYAB
BAG3
DNAJB13
DNAJA1
HSPH1
HSF4
DNAJC12
HSP90AA1
DNAJB9
HSPA5
HSPB1
COQ8A
DNAJB7
HSPE1
BAG1
DNAJC6
DNAJB11
DNAJB2
HSPD1
CCS
DNAJC3
HSP90B1
HSPA8
SERPINH1
HSF2
HSPB2
CCT6B
DNAJB6
CCT6A
HSPB8
CCT4
DNAJA2
HSPA4
ATF6
DNAJC4
DNAJC14
DNAJC9
DNAJC5B
BAG5
CCT3
DNAJC17
DNAJC15
DNAJC10
TCP1
HSP90AB1
DNAJB12
SIL1
DNAJC16
DNAJB14
DNAJC1
HSF1
CCT2
PFDN1
CCT7
DNAJC13
DNAJC18
DNAJC8
HSPB6
DNAJC5
DNAJC21
DNAJC7
DNAJA3
BAG4
DNAJB8
TOR1A
BAG2
HSPA2
CCT5
DNAJC11
PFDN2
HSPA9
HSPA14
DNAJB5
HSPB3
HSPB7
1
0
-1
Z-score
B
8
6
4
2
0
-Log10 p value
-2
0
2
4
6
8
Log2 Fold Change
C
Fold induction
1
10
100
1000
HSPH1
HSPA1A
HSPA1B
HSPA1L
HSPA4L
HSPA6
DNAJA1
DNAJA4
DNAJB1
DNAJB13
CRYAB
BAG3
D
Upregulated genes
HCoV-229E
33
18
8
Heat Shock
E
Fold induction
1
10
100
1000
HSP90AA1
HSP90AB1
HSP90B1
HSPA14
HSPA1A
HSPA1B
HSPA1L
HSPA4
HSPA4L
HSPA5
HSPA6
HSPA8
HSPA9
HSPB1
HSPB8
HSPD1
HSPE1
HSPH1
DNAJA1
DNAJA2
DNAJA3
DNAJA4
DNAJB1
DNAJB11
DNAJB12
DNAJB13
DNAJB14
DNAJB2
DNAJB5
DNAJB6
DNAJB9
DNAJC1
DNAJC10
DNAJC11
DNAJC12
DNAJC14
DNAJC16
DNAJC3
DNAJC5
DNAJC6
DNAJC7
CRYAB
BAG1
BAG2
BAG3
BAG4
BAG5
HSF2
HSF4
CCS
CCT2
CCT3
CCT4
CCT5
COQ8A
A TF6
TCP1
SERPINH1
SIL1
🟥 Heat Shock
⬛ HCoV-229E

Figure S2

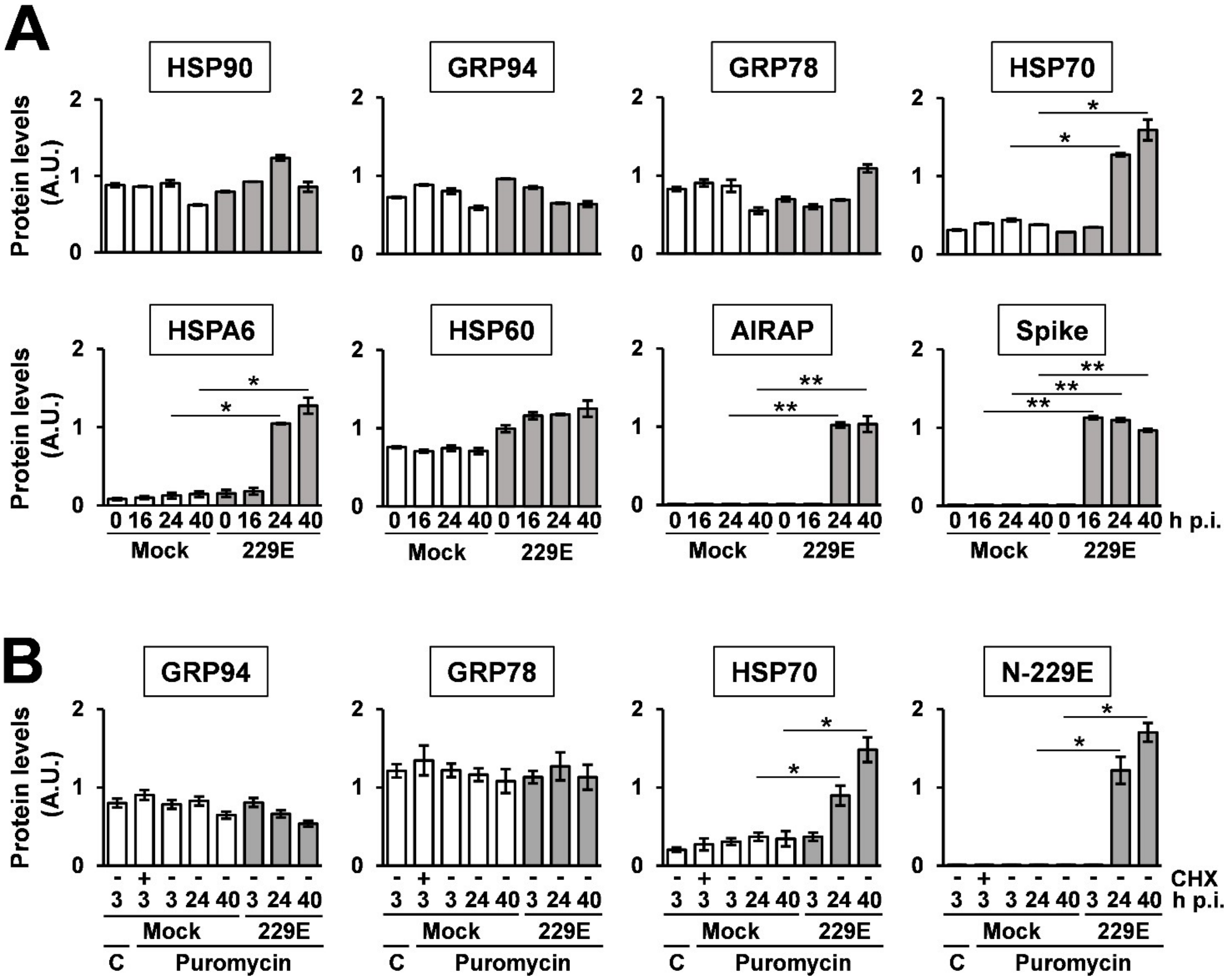
A
HSP90
GRP94
GRP78
HSP70
HSPA6
HSP60
AIRAP
Spike
Protein levels (A.U.)
0
1
2
*
**
0 16 24 40 0 16 24 40 h p.i.
Mock
229E
B
GRP94
GRP78
HSP70
N-229E
- + - - - - - - CHX
3 3 3 24 40 3 24 40 h p.i.
Mock
229E
C
Puromycin

Figure S3

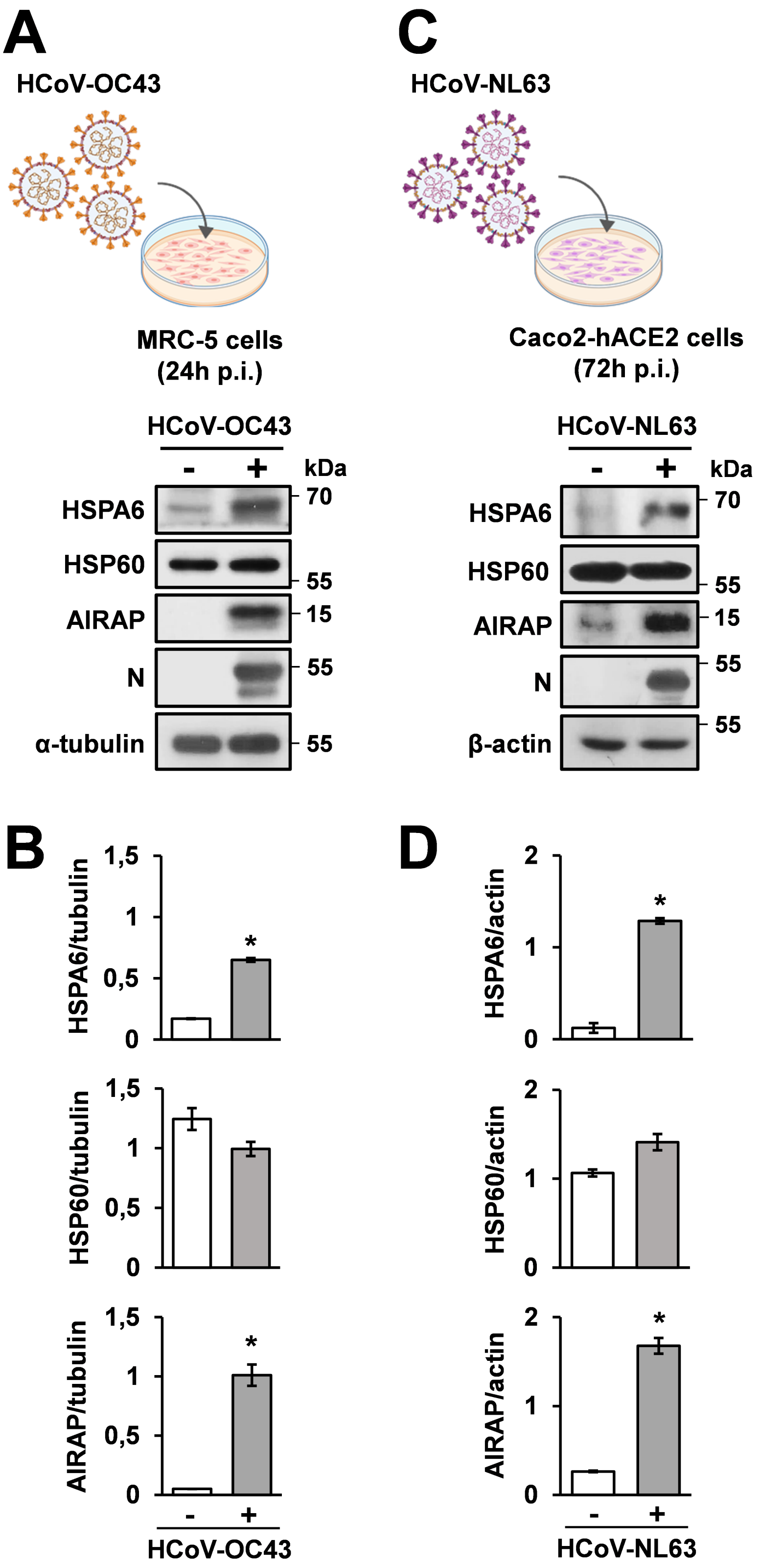
A
HCoV-OC43
MRC-5 cells
(24h p.i.)
HCoV-OC43
-
+
kDa
HSPA6
70
HSP60
55
AIRAP
15
N
55
α-tubulin
55
C
HCoV-NL63
Caco2-hACE2 cells
(72h p.i.)
HCoV-NL63
-
+
kDa
HSPA6
70
HSP60
55
AIRAP
15
N
55
β-actin
55
B
HSPA6/tubulin
1,5
1
0,5
0
*
HSP60/tubulin
1,5
1
0,5
0
AIRAP/tubulin
1,5
1
0,5
0
*
-
+
HCoV-OC43
D
HSPA6/actin
2
1
0
*
HSP60/actin
2
1
0
AIRAP/actin
2
1
0
*
-
+
HCoV-NL63

Figure S4

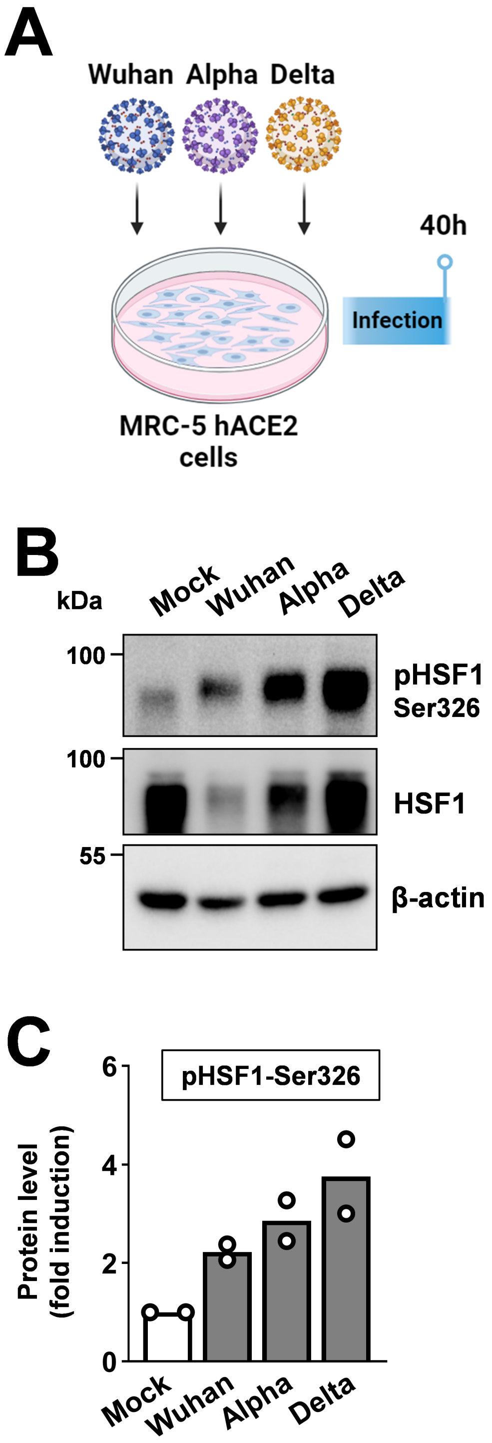
A
Wuhan
Alpha
Delta
40h
Infection
MRC-5 hACE2 cells
B
kDa
Mock
Wuhan
Alpha
Delta
100
pHSF1 Ser326
100
HSF1
55
β-actin
C
pHSF1-Ser326
Protein level (fold induction)
6
4
2
0
Mock
Wuhan
Alpha
Delta

Figure S5

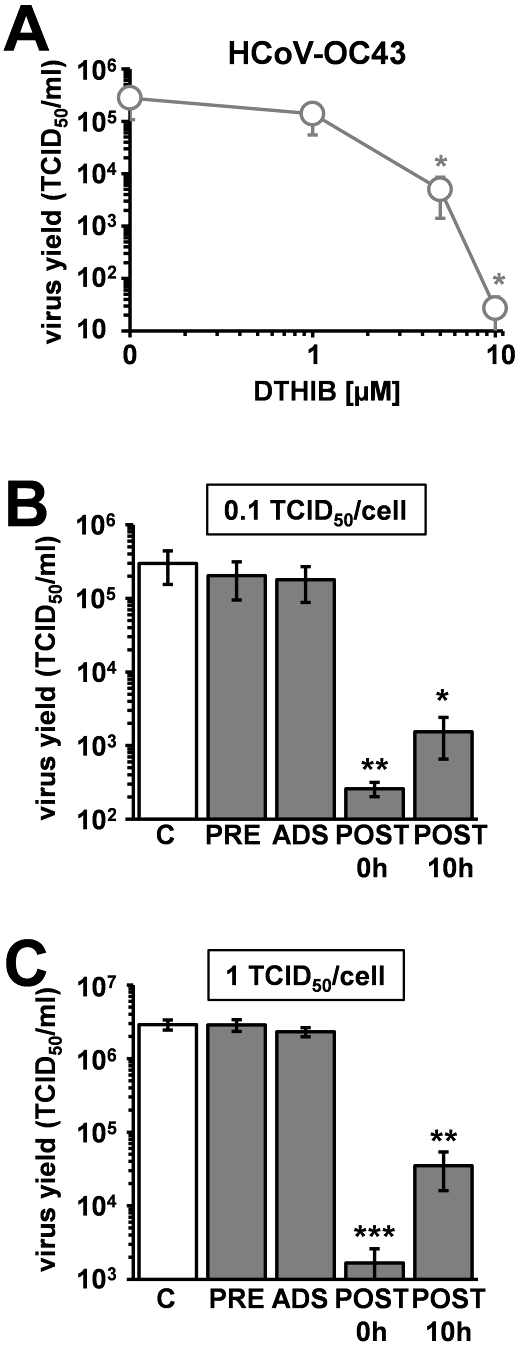
A
HCoV-OC43
virus yield (TCID$_{50}$/ml)
10^6
10^5
10^4
10^3
10^2
10
*
*
0
1
10
DTHIB [µM]
B
0.1 TCID$_{50}$/cell
virus yield (TCID$_{50}$/ml)
10^6
10^5
10^4
10^3
10^2
**
*
C
PRE
ADS
POST 0h
POST 10h
C
1 TCID$_{50}$/cell
virus yield (TCID$_{50}$/ml)
10^7
10^6
10^5
10^4
10^3
***
**
C
PRE
ADS
POST 0h
POST 10h

Figure S6