SUPPLEMNTARY INFORMATION

The transporters SLC35A1 and SLC30A1 play opposite roles in cell survival upon VSV virus infection

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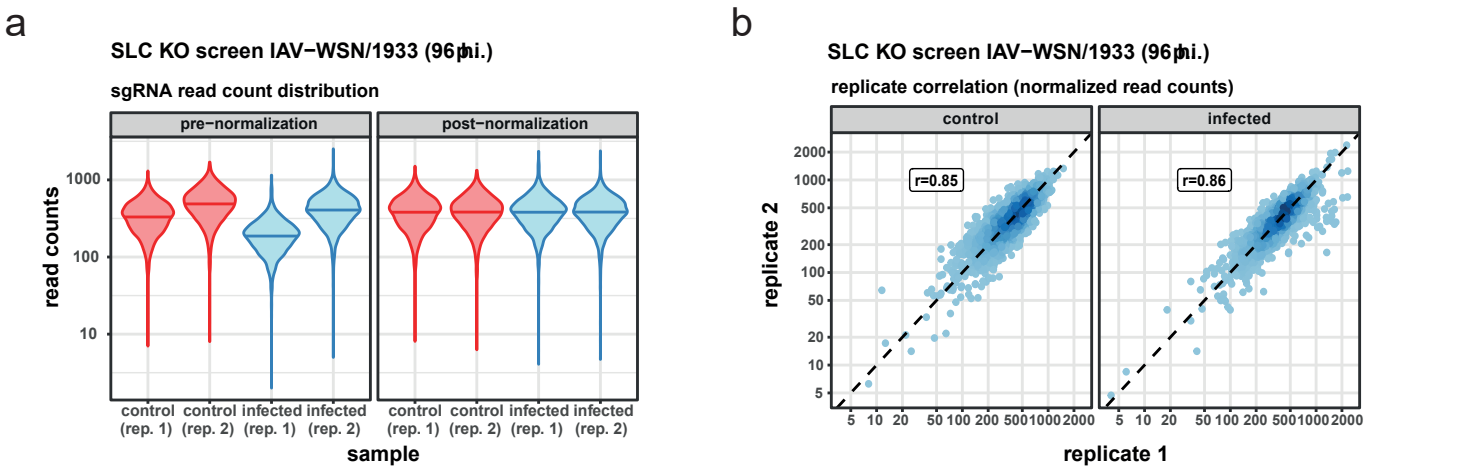
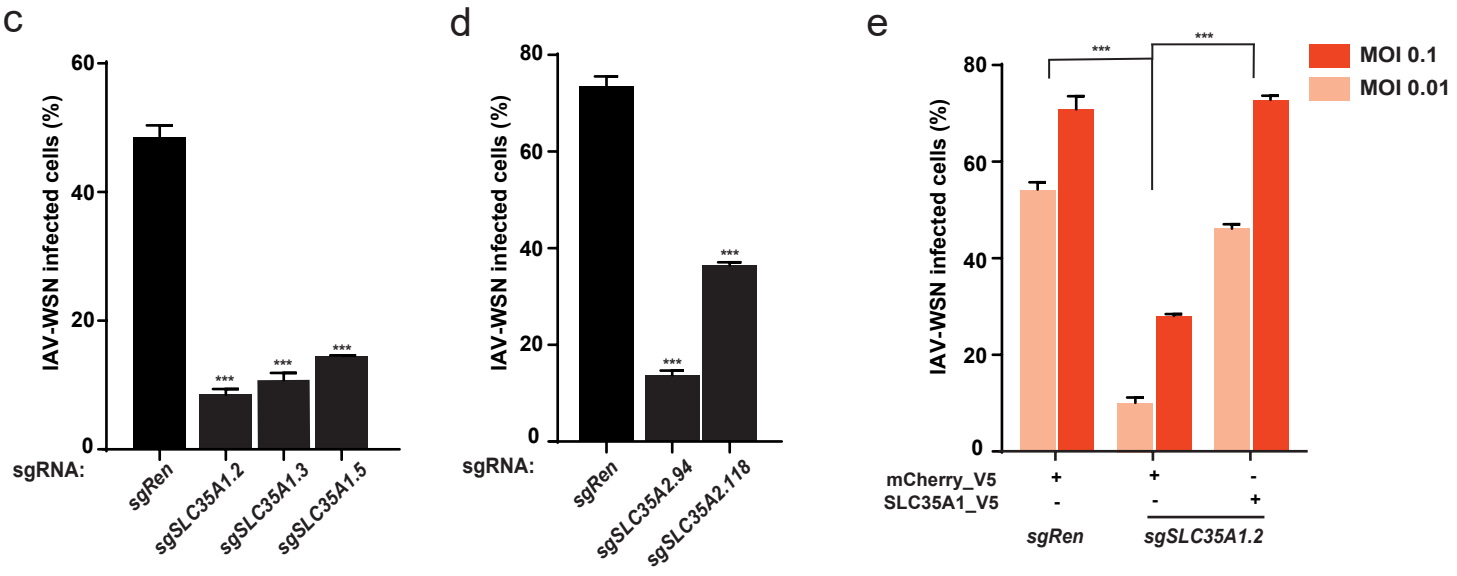
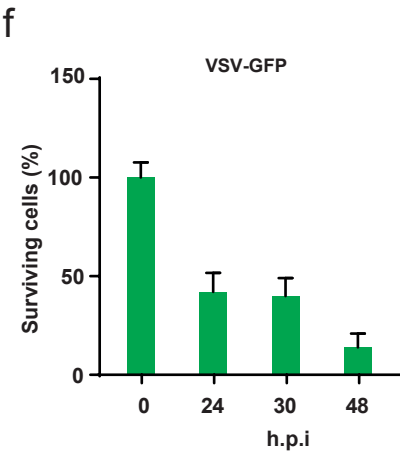
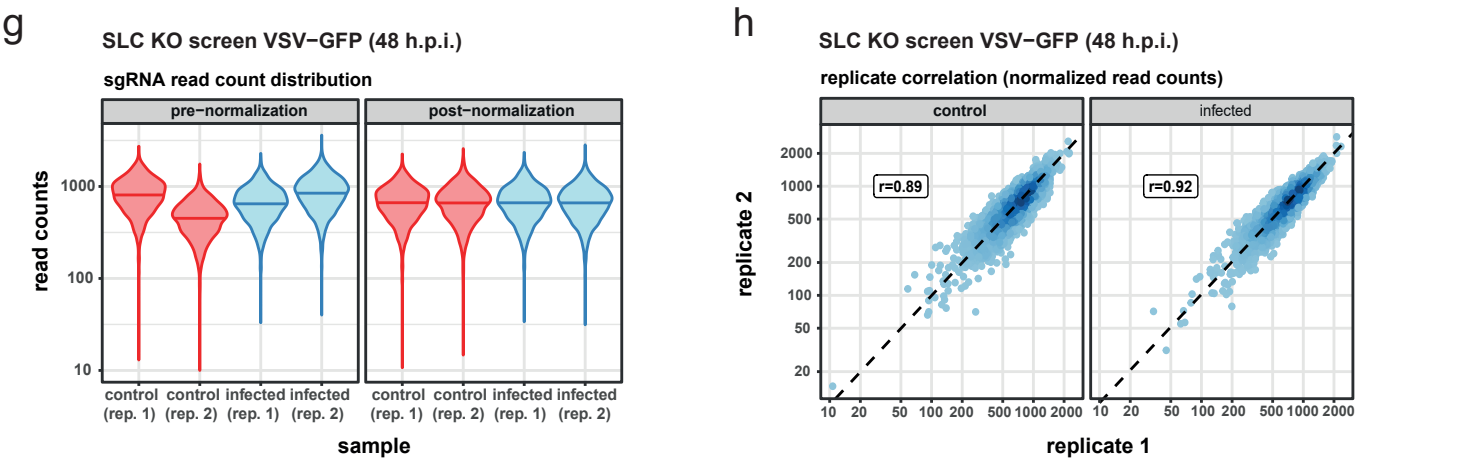
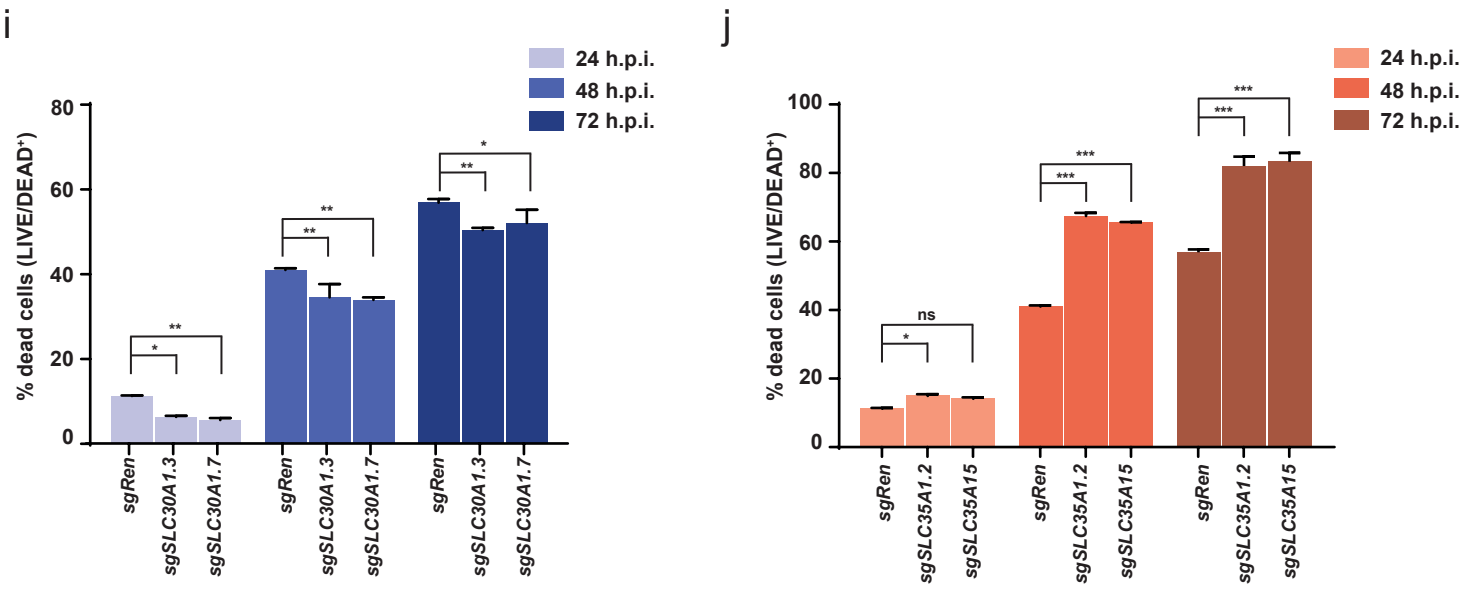
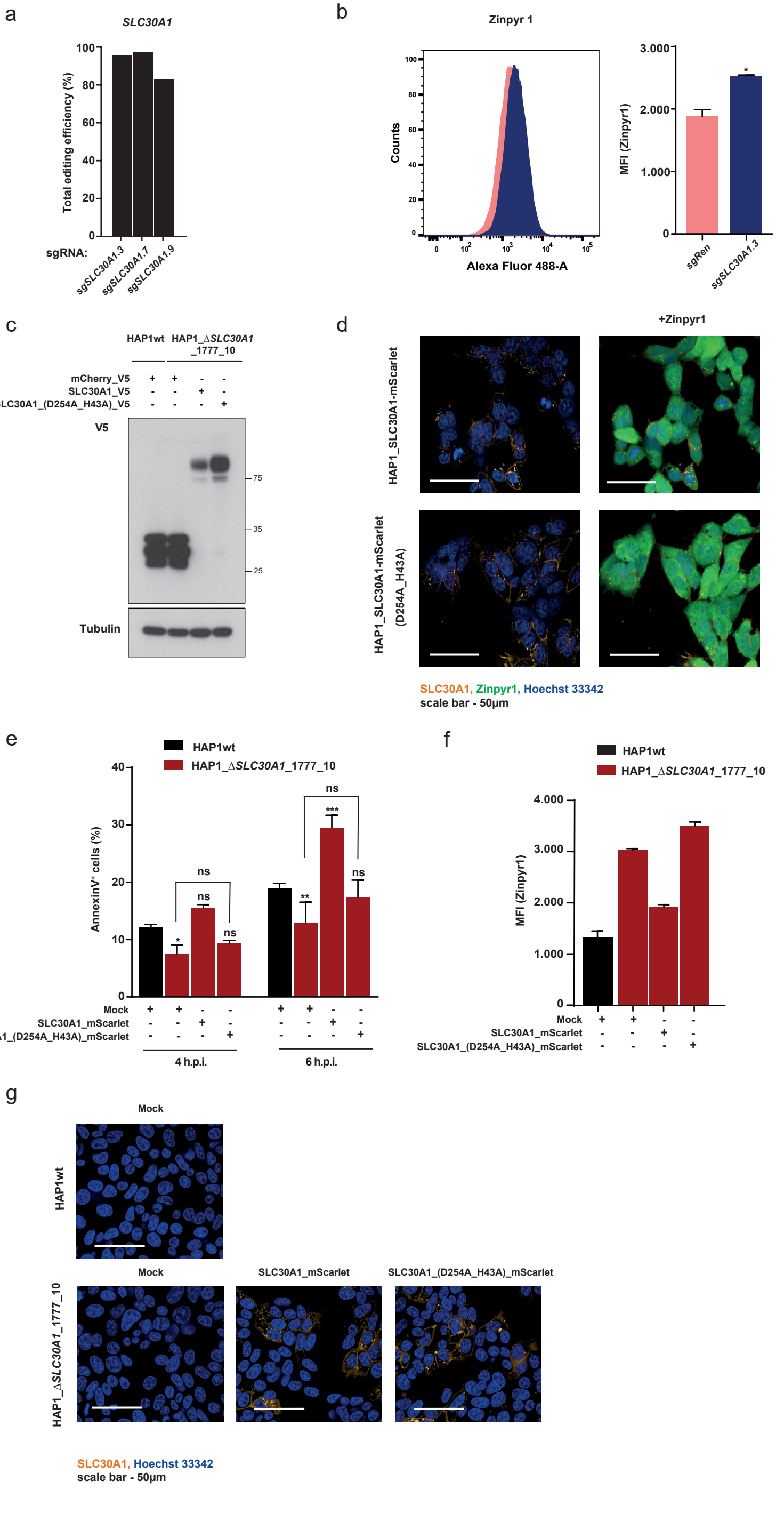

a
SLC KO screen IAV−WSN/1933 (96 h.p.i.)
sgRNA read count distribution
pre−normalization
post−normalization
read counts
1000
100
10
control (rep. 1)
control (rep. 2)
infected (rep. 1)
infected (rep. 2)
sample
b
SLC KO screen IAV−WSN/1933 (96 h.p.i.)
replicate correlation (normalized read counts)
control
infected
r=0.85
r=0.86
replicate 2
replicate 1
2000
1000
500
200
100
50
20
10
5


c
IAV-WSN infected cells (%)
sgRNA:
sgRen
sgSLC35A1.2
sgSLC35A1.3
sgSLC35A1.5
d
IAV-WSN infected cells (%)
sgRNA:
sgRen
sgSLC35A2.94
sgSLC35A2.118
e
IAV-WSN infected cells (%)
MOI 0.1
MOI 0.01
mCherry_V5
SLC35A1_V5
sgRen
sgSLC35A1.2

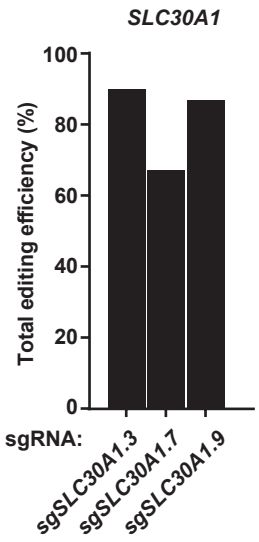

f
VSV-GFP
Surviving cells (%)
h.p.i


g
SLC KO screen VSV−GFP (48 h.p.i.)
sgRNA read count distribution
pre−normalization
post−normalization
read counts
sample
h
SLC KO screen VSV−GFP (48 h.p.i.)
replicate correlation (normalized read counts)
control
infected
r=0.89
r=0.92
replicate 2
replicate 1

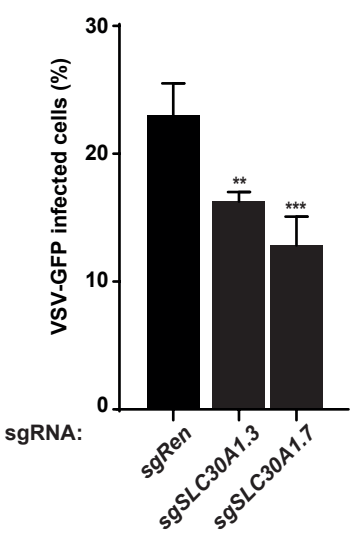

i
% dead cells (LIVE/DEAD+)
24 h.p.i.
48 h.p.i.
72 h.p.i.
sgRen
sgSLC30A1.3
sgSLC30A1.7
j
% dead cells (LIVE/DEAD+)
24 h.p.i.
48 h.p.i.
72 h.p.i.
ns
sgRen
sgSLC35A1.2
sgSLC35A15


a
SLC30A1
Total editing efficiency (%)
100
80
60
40
20
0
sgRNA:
sgSLC30A1.3
sgSLC30A1.7
sgSLC30A1.9
b
Zinpyr 1
Counts
Alexa Fluor 488-A
MFI (Zinpyr1)
3.000
2.000
1.000
0
sgRen
sgSLC30A1.3
c
HAP1wt
HAP1_ΔSLC30A1_1777_10
mCherry_V5
SLC30A1_V5
SLC30A1_(D254A_H43A)_V5
V5
75
35
25
Tubulin
d
+Zinpyr1
HAP1_SLC30A1-mScarlet
HAP1_SLC30A1-mScarlet (D254A_H43A)
SLC30A1, Zinpyr1, Hoechst 33342
scale bar - 50μm
e
HAP1wt
HAP1_ΔSLC30A1_1777_10
AnnexinV+ cells (%)
ns
*
**
***
Mock
SLC30A1_mScarlet
SLC30A1_(D254A_H43A)_mScarlet
4 h.p.i.
6 h.p.i.
f
HAP1wt
HAP1_ΔSLC30A1_1777_10
MFI (Zinpyr1)
4.000
3.000
2.000
1.000
0
Mock
SLC30A1_mScarlet
SLC30A1_(D254A_H43A)_mScarlet
g
Mock
HAP1wt
HAP1_ΔSLC30A1_1777_10
SLC30A1_mScarlet
SLC30A1_(D254A_H43A)_mScarlet
SLC30A1, Hoechst 33342
scale bar - 50μm

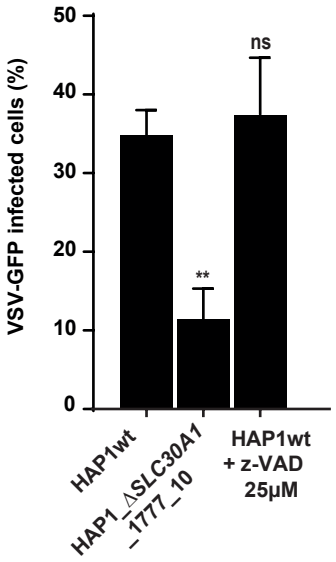
a


SLC30A1
Total editing efficiency (%)
100
80
60
40
20
0
sgRNA:
sgSLC30A1.3
sgSLC30A1.7
sgSLC30A1.9

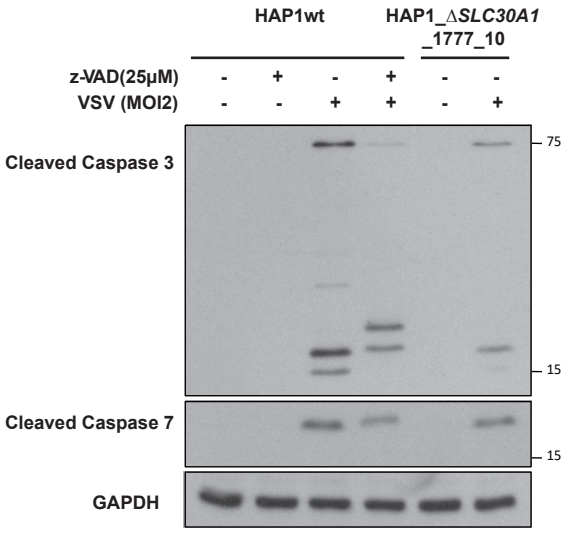
b


VSV-GFP infected cells (%)
30
20
10
0
**
***
sgRNA:
sgRen
sgSLC30A1.3
sgSLC30A1.7

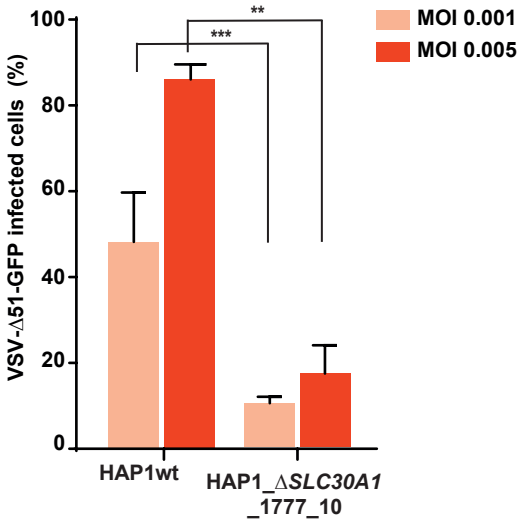
c


VSV-GFP infected cells (%)
50
40
30
20
10
0
ns
**
HAP1wt
HAP1_ΔSLC30A1_1777_10
HAP1wt + z-VAD 25μM

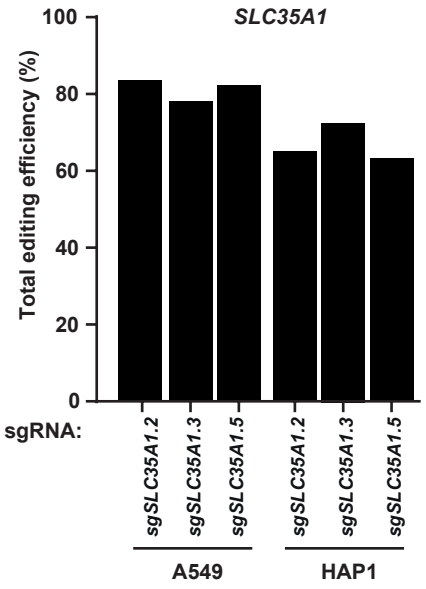
d


HAP1wt
HAP1_ΔSLC30A1_1777_10
z-VAD(25μM) - + - + - -
VSV (MOI2) - - + + - +
Cleaved Caspase 3
Cleaved Caspase 7
GAPDH
75
15
15

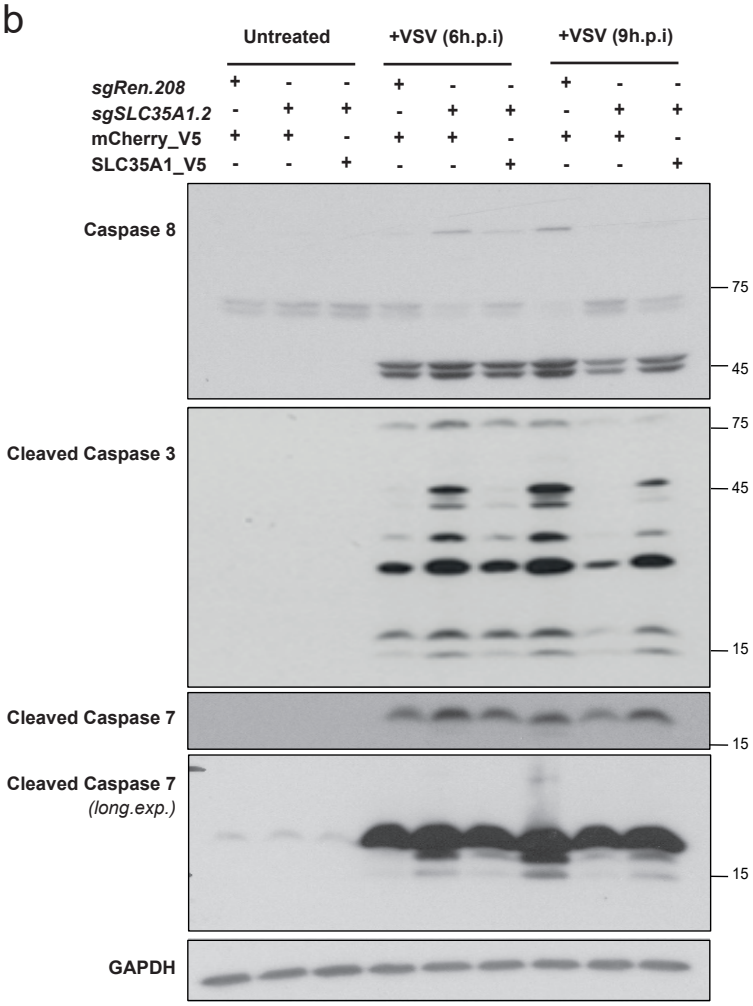
e


VSV-Δ51-GFP infected cells (%)
100
80
60
40
20
0
**
***
MOI 0.001
MOI 0.005
HAP1wt
HAP1_ΔSLC30A1_1777_10

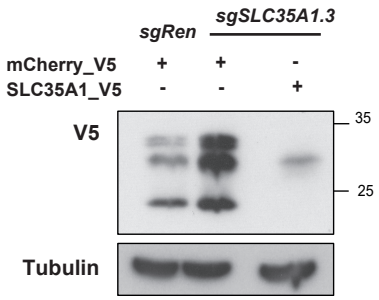
a


SLC35A1
Total editing efficiency (%)
100
80
60
40
20
0
sgRNA:
sgSLC35A1.2
sgSLC35A1.3
sgSLC35A1.5
sgSLC35A1.2
sgSLC35A1.3
sgSLC35A1.5
A549
HAP1

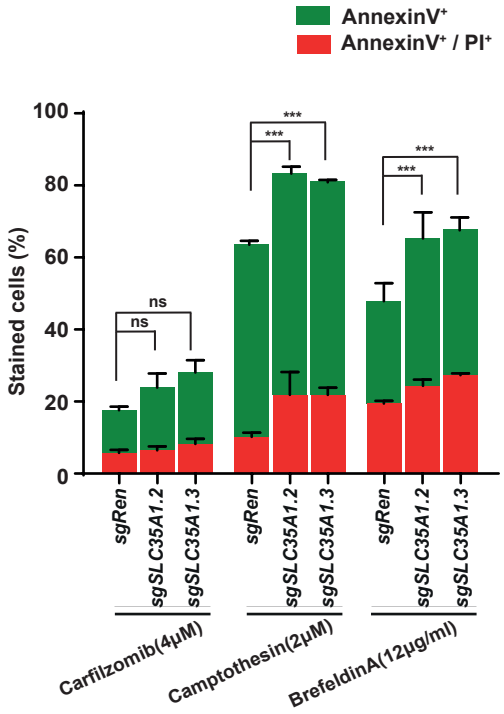
b


Untreated
+VSV (6h.p.i)
+VSV (9h.p.i)
sgRen.208
sgSLC35A1.2
mCherry_V5
SLC35A1_V5
Caspase 8
Cleaved Caspase 3
Cleaved Caspase 7
Cleaved Caspase 7
(long.exp.)
GAPDH
75
45
75
45
15
15
15

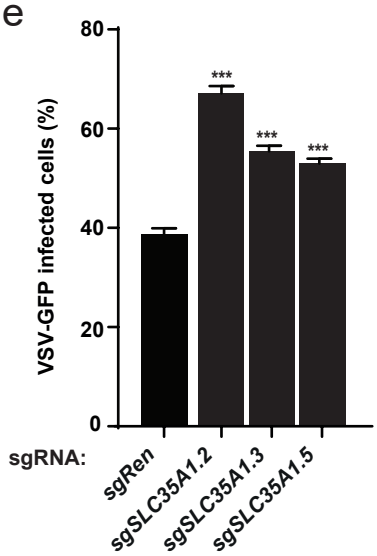
c


sgRen
sgSLC35A1.3
mCherry_V5
SLC35A1_V5
V5
Tubulin
35
25

d

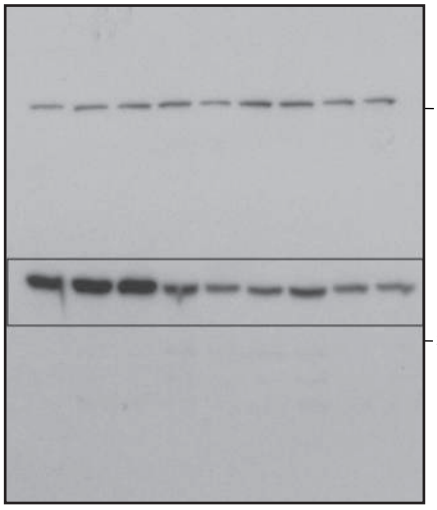

AnnexinV+
AnnexinV+ / PI+
Stained cells (%)
100
80
60
40
20
0
ns
ns
***
***
***
***
sgRen
sgSLC35A1.2
sgSLC35A1.3
Carfilzomib(4μM)
Camptothesin(2μM)
BrefeldinA(12μg/ml)

e

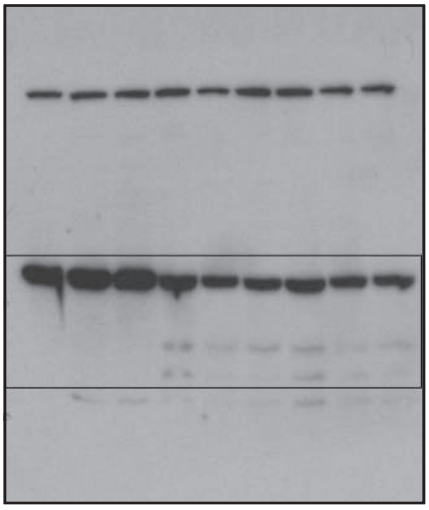

VSV-GFP infected cells (%)
80
60
40
20
0
***
***
***
sgRNA:
sgRen
sgSLC35A1.2
sgSLC35A1.3
sgSLC35A1.5

Full lenght immunoblots from Fig 2b

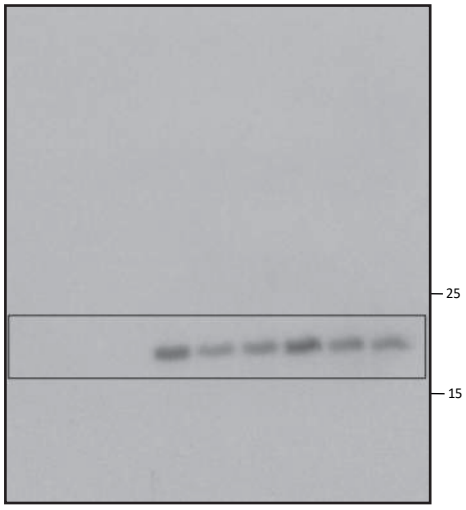
Caspase 9



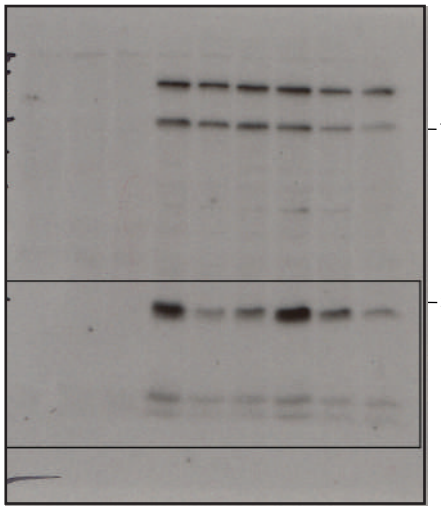
Caspase 9
(long. exp.)



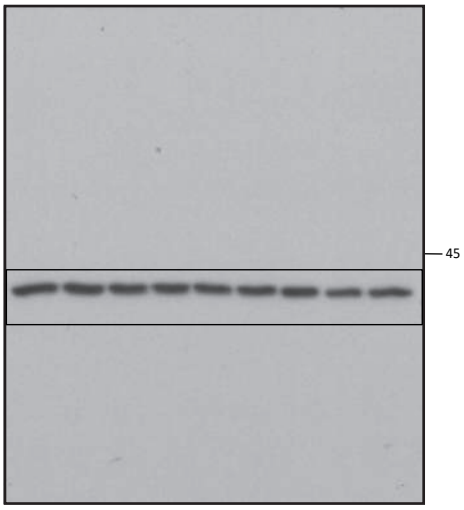
Cleaved Caspase 7



Cleaved Caspase 3

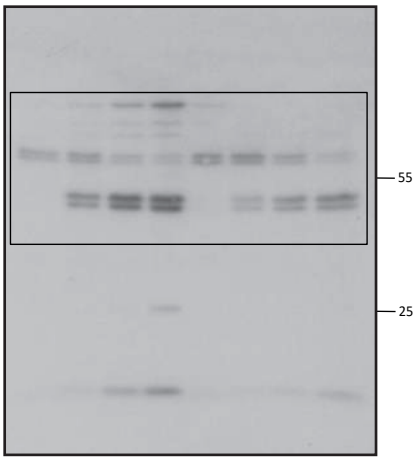


GAPDH

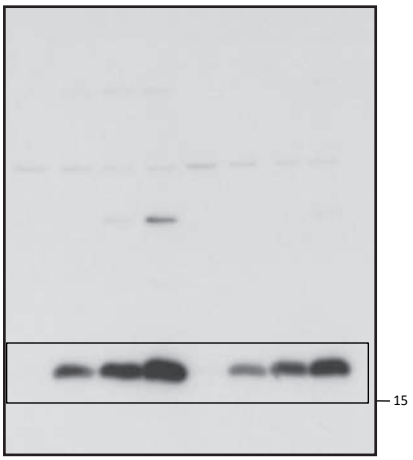


Full lenght immunoblots from Fig 2c

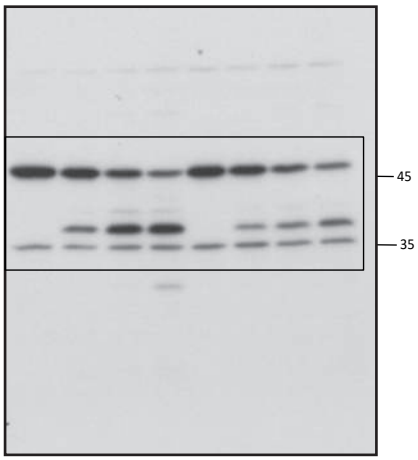
Caspase 8

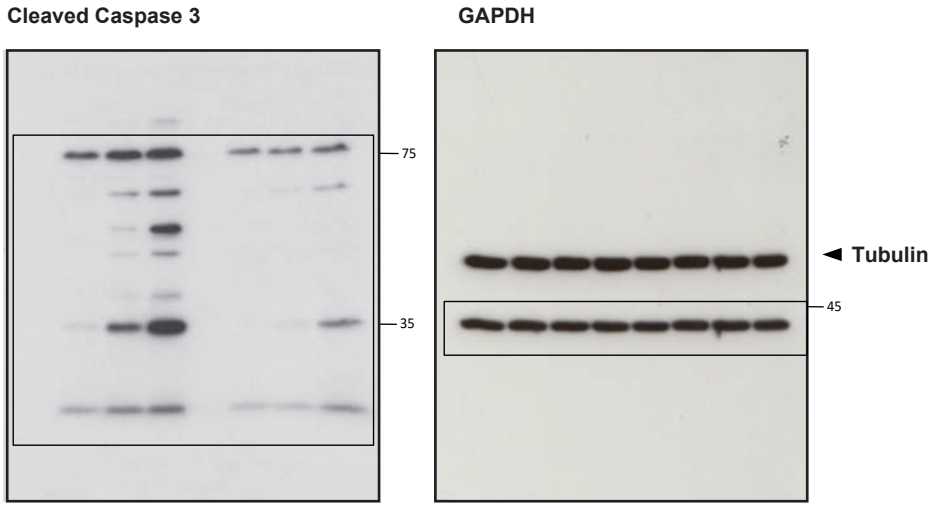


Cleaved Caspase 7

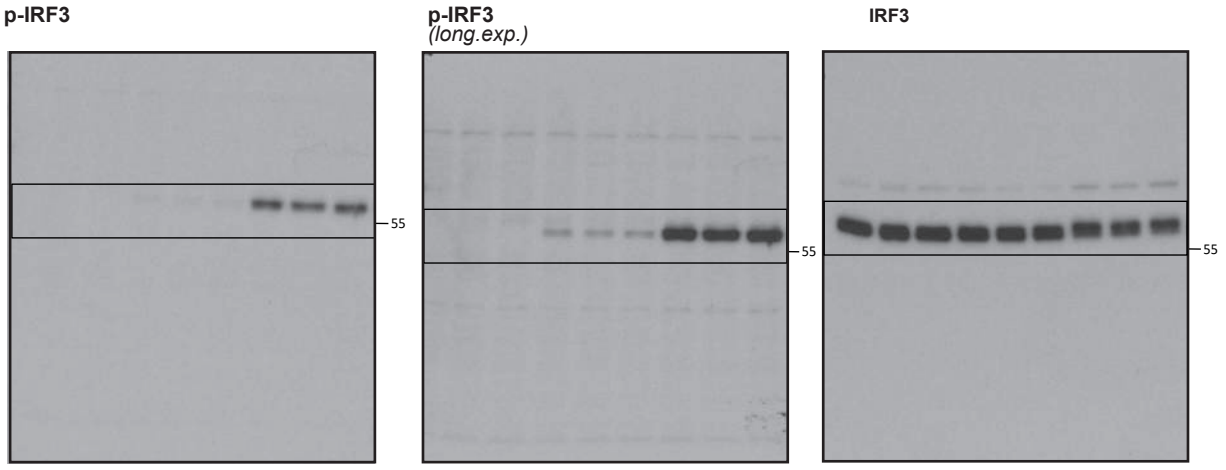


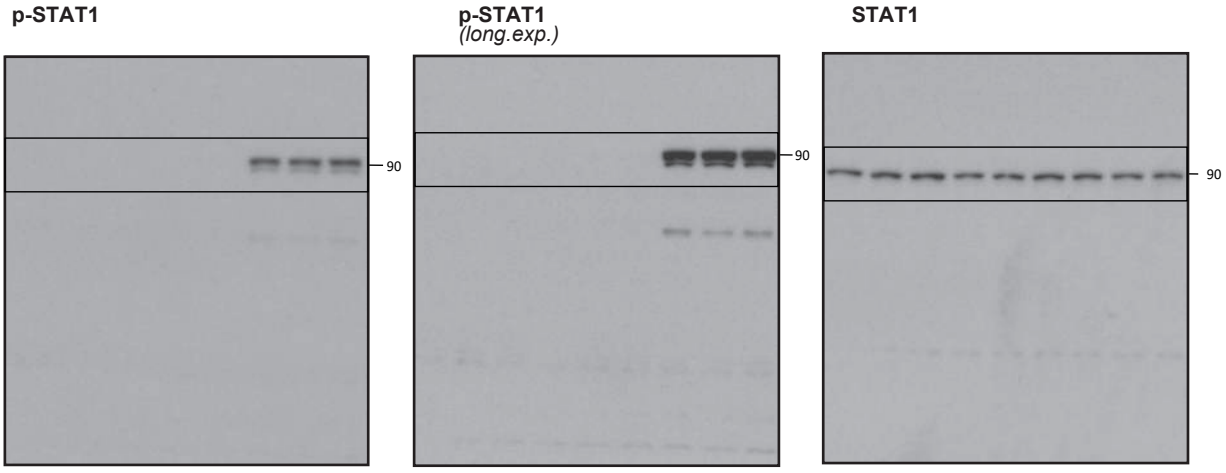
Caspase 9

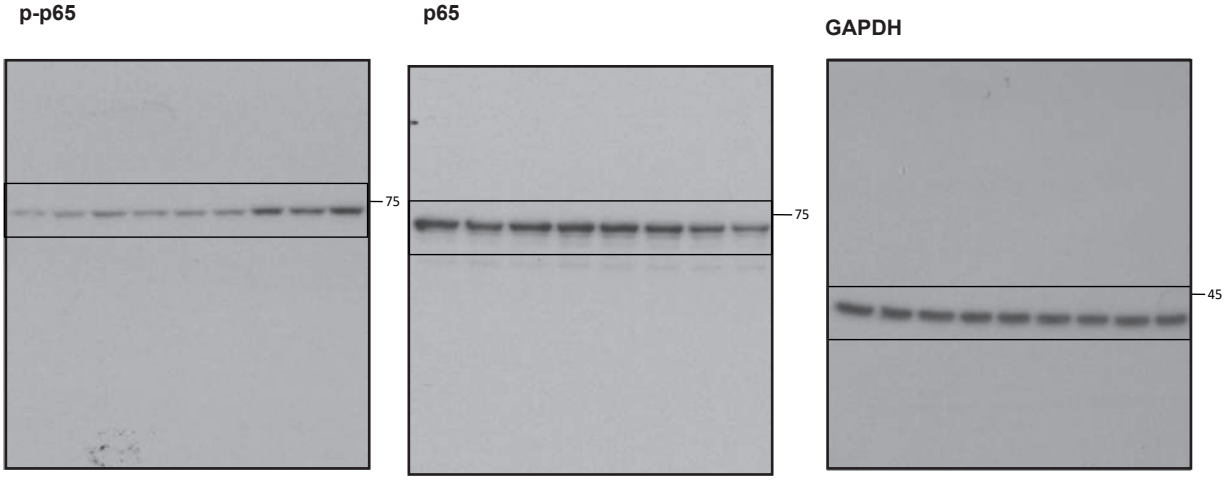




Full lenght immunoblots from Fig 3d

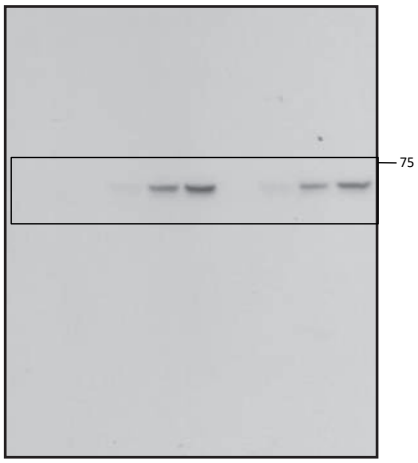




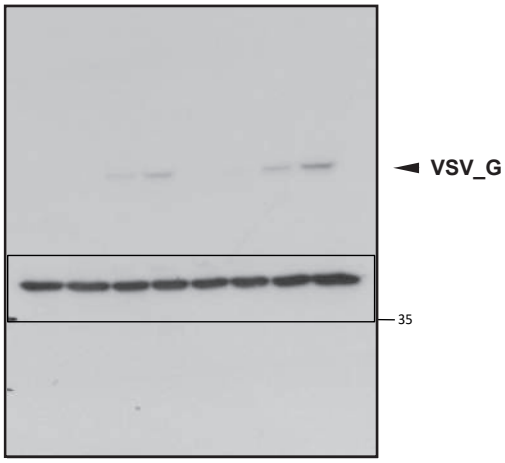


Full lenght immunoblots from Fig 3f

VSV_G

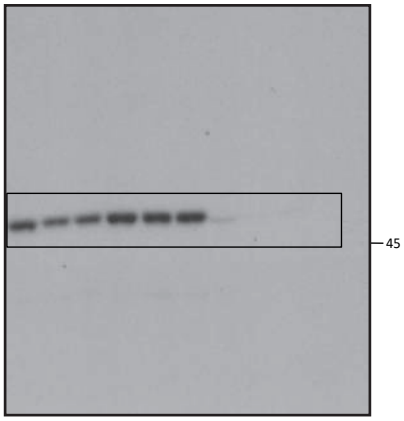


GAPDH

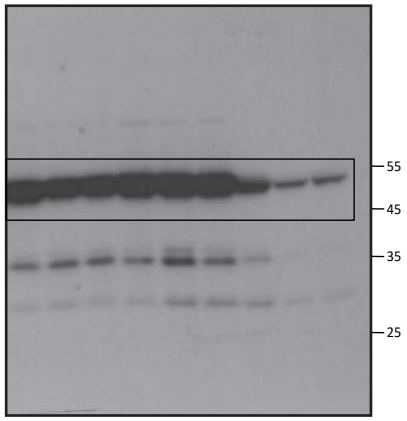


Full lenght immunoblots from Fig 4b

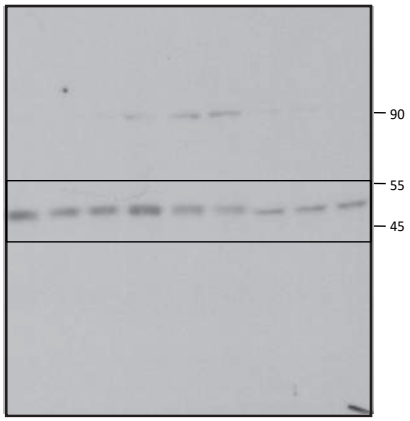
p-MEK



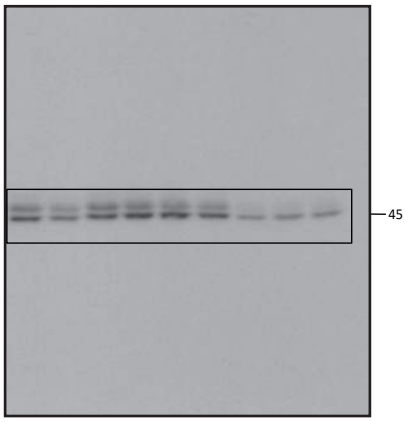
p-MEK
(long.exp)



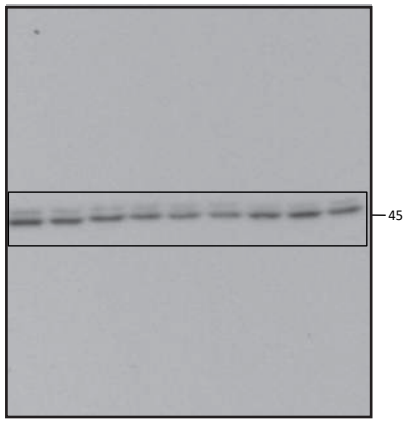
MEK



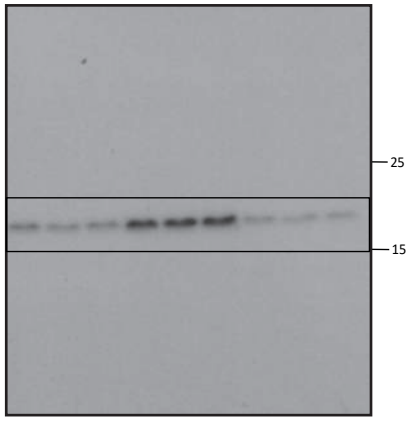
p-ERK



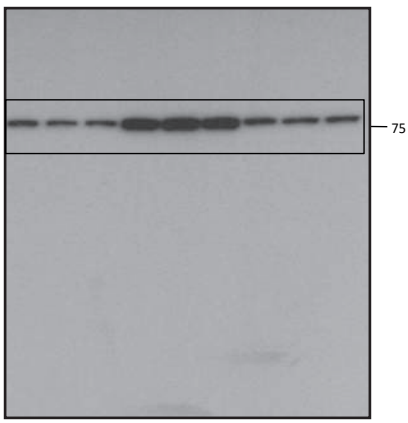
ERK



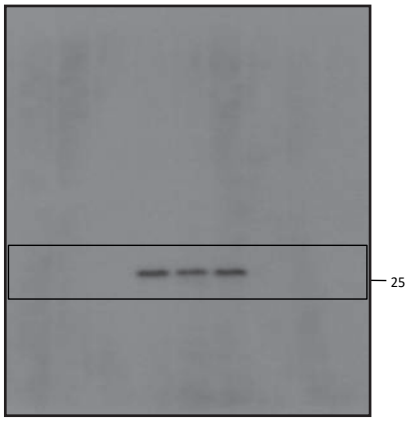
ARF4



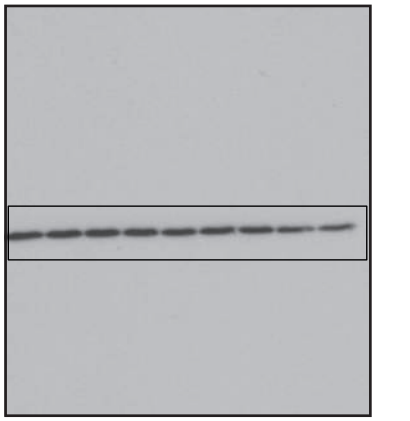
GRP78



CHOP

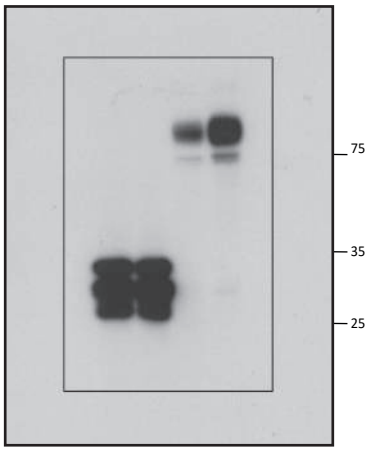


GAPDH

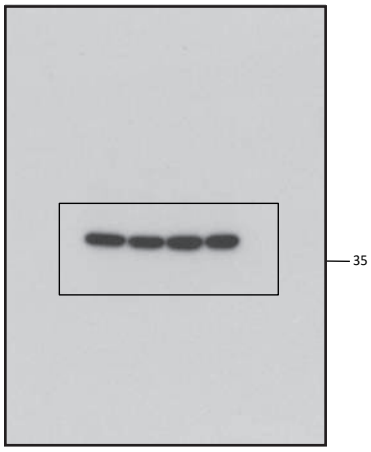


Full lenght immunoblots from Suppl Fig 2c

V5

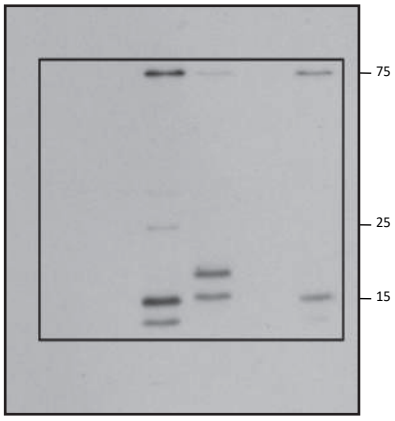


GAPDH

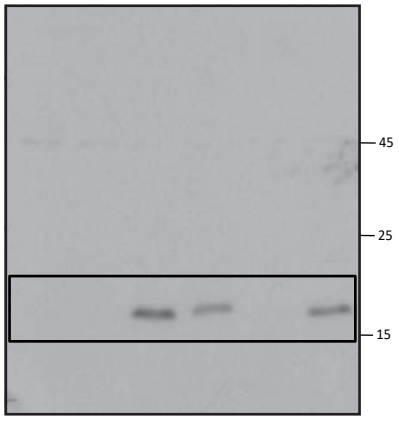


Full lenght immunoblots from Suppl Fig 3d

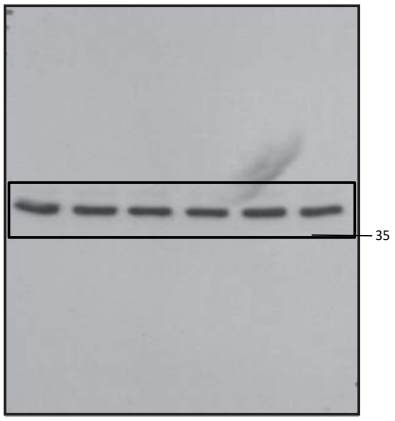
Cleaved Caspase 3



Cleaved Caspase 7

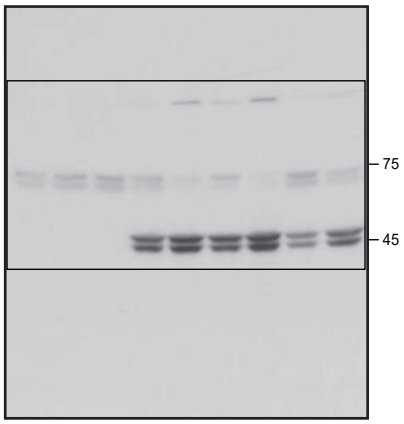


GAPDH

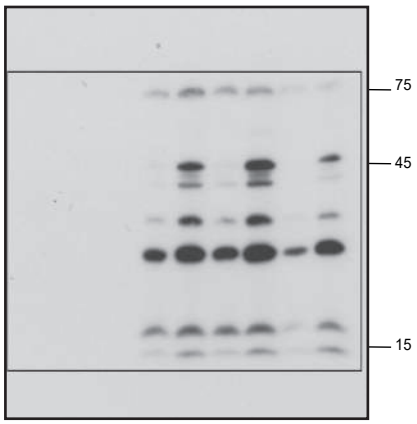


Full lenght immunoblots from Suppl Fig 4b

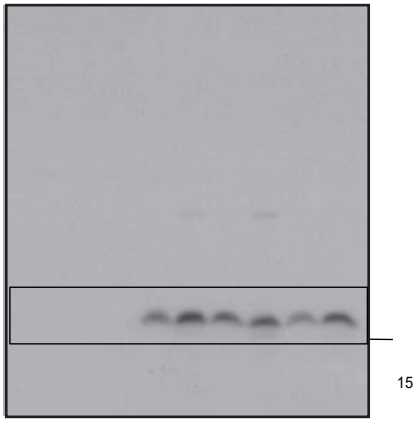
Caspase 8



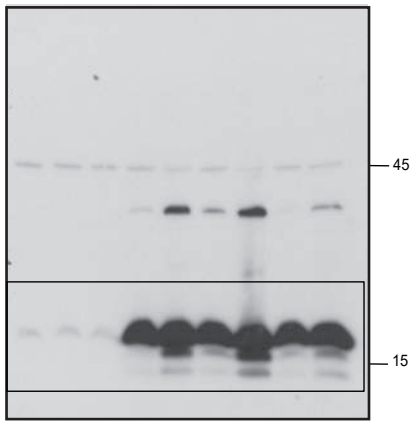
Cleaved Caspase 3



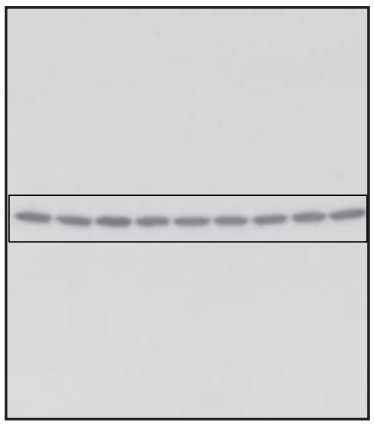
Cleaved Caspase 7



Cleaved Caspase 7
(long.exp.)

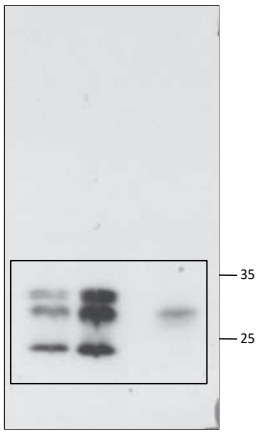


GAPDH



Full lenght immunoblots from Suppl Fig 4c

V5



Tubulin

Supplementary Figures

Suppl.Fig. 1 Genetic screens for cell survival upon IAV and VSV infection. a. Violin plots of raw and normalized read counts and **b.** Correlation of normalized read counts across biological replicates in the A549 IAV-WSN/1933 screen. **c-e.** Quantification of flow cytometry-based viral replication assay for HAP1 *SLC35A1* **(c),** *SLC35A2* **(d)** knockout cell lines and cells re-expressing SLC35A1 cDNA **(e)**. Cells were infected with IAV-WSN/1933 at MOI 0.01. At 24 h.p.i. the number of the infected cells was assessed after staining with anti-Influenza Nucleoprotein antibody coupled to AF488. **f.** Assessment of A549 cell death upon infection with wild type VSV-GFP (MOI of 10). Cells were incubated for the indicated time points and relative cell number was determined using crystal violet staining. **g.** Violin plots of raw and normalized read counts and **h.** Correlation of normalized read counts across biological replicates in the A549 VSV-GFP screen. **i** and **j**A549 cells expressing sgRNAs targeting *SLC30A1* (**i**) or *SLC35A1* (**j**) were infected with VSV-GFP at MOI of 5 and at the indicated time points stained with LIVE/DEAD cell viability dye. Positive cells were quantified with flow cytometry. Statistical significance was assessed using one-way ANOVA with Dunnett's (**c** and **d**) or two-way ANOVA with Tukey's (**e**) or Dunnett's (**i** and **j**) tests. Unless otherwise indicated, adjusted P-values in relation to to sgRen control are shown. Data are represented as mean ± SD of one representative experiment out of at least two independent replicates. *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ns: not significant.

Suppl.Fig. 2. SLC30A1 loss results in increased intracellular Zn^{2+} concentration and decreased virus-induced cell death. a. Editing efficiency of sgRNAs targeting the *SLC30A1* gene as quantified by the TIDE sequencing method in A549 cells. **b**. Representative FACS histogram and quantification of the A549 SLC30A1 knockout cells stained with Zn^{2+} reactive dye – Zinpyr1. **c.** Representative immunoblot of V5 tagged wild type SLC30A1 and SLC30A1(D254A_H43A) double mutant cDNA expressing cells. Cropped images are shown for conciseness. Full-length blots are presented in Supplementary Figure 5. **d.** Immunofluorescent image of HAP1 wild type cells overexpressing mScarlet-fused wild type SLC30A1 and SLC30A1(D254A_H43A) double mutant stained with Zn^{2+} reactive fluorescent dye Zinpyr1. **e**. Percentage of apoptotic cells in wild type or SLC30A1-deficient HAP1 cells in untrasfected (mock) cells or cells transiently transfected with wild type or transport-deficient SCL30A1 cDNA, upon infection with VSV (MOI 2), as measured with AnnexinV-AF647. Transiently transfected cells ($mScarlet^+$) were compared to the equivalent ($mScarlet^-$, mock) non-transfected cells. **f.** Intracellular Zn^{2+} levels determined using Zinpyr1 fluorescent dye in mock transiently transfected HAP1 cells. Experiment performed once. **g**. Immunofluorescent image of HAP1 wild type cells or HAP1 SLC30A1-deficient clone overexpressing mScarlet-fused wild type SLC30A1 and SLC30A1(D254A_H43A) double mutant. Statistical significance was assessed using two-tailed unpaired Student's t-test (**b**), two-way ANOVA with Sidak's correction **(e**) or one-way ANOVA with Tukey's test (**f**). Unless indicated otherwise P-values in relation to sgRen control (**b**) or

adjusted P-values in relation to mock transfected HAP1wt (**e** and **f**) cells are shown. Data are represented as mean ± SD of one representative experiment out of at least two independent replicates. *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ns: not significant.

Suppl.Fig. 3. SLC30A1 in apoptosis and VSV infectivity. a. Editing efficiency of sgRNAs targeting the *SLC30A1* gene as quantified by the TIDE sequencing method in HAP1 cells. **b.** Flow cytometry-based VSV-GFP virus replication in *SLC30A1* knockout HEK293T cells (MOI 0.001). **c.** VSV-GFP virus replication in *SLC30A1* knockout HAP1 cells treated with caspase inhibitor z-VAD and infected at MOI of 0.001. The number of GFP^+ cells was quantified with flow cytometry at 14 h.p.i. **d.** Immunoblot analysis of caspase 3 and 7 cleavage in HAP1wt cells stimulated with zVAD and SLC30A1 knockout clone upon infection with VSV-GFP (MOI 2). Cropped images are shown for conciseness. Full-length blots are presented in Supplementary Figure 5. **d.** VSV-Δ51-GFP virus replication in the HAP1 SLC30A1 knockout and wild type cells infected with MOI 0.001 and 0.005 for 14 hours. Statistical significance was assessed using one-way ANOVA with Dunnett's test (**b** and **c**) or two-way ANOVA with Sidak's correction (**e**). Unless otherwise indicated, adjusted P-values in relation to sgRen control or HAP1wt are shown. Data are represented as mean ± SD of one representative experiment out of at least two independent replicates. **: $p \leq 0.01$; ***: $p \leq 0.001$; ns: not significant.

Suppl.Fig. 4. SLC35A1 in apoptosis and VSV infectivity. a. Editing efficiency of sgRNAs targeting *SLC35A1* gene as quantified using the TIDE sequencing method in HAP1 and A549 cells. **b.** Pro-apoptotic caspases activation of VSV infected HAP1 *SLC35A1* knockout cells and SLC35A1 cDNA-expressing cells. Cropped images are shown for conciseness. Full-length blots are presented in Supplementary Figure 5. **c.** Representative immunoblot of HAP1 cells expressing V5-tagged wild type SLC35A1. Cropped images are shown for conciseness. Full-length blots are presented in Supplementary Figure 5. **d.** Percentage of apoptotic ($AnnexinV^+$) and necrotic ($AnnexinV^+/PI^+$) cells in the HAP1 cell line expressing sgRNAs against *SLC35A1* and *Renilla luciferase* and stimulation with Brefeldin A (12μg/ml), Carfilzomib (4μM) and Campthotesin (2μM) for 8 hours. **e.** Flow cytometry based VSV-GFP virus replication in *SLC35A1* knockout HEK293T cells (MOI 0.001). Statistical significance was assessed using one-way ANOVA with Tukey's (**d** and **f**) or Dunnett's (**e)** tests. Unless otherwise indicated, adjusted P-values in relation to sgRen control are shown. Data are represented as mean ± SD of one representative experiment out of at least two independent replicates. **: $p \leq 0.01$; ***: $p \leq 0.001$; ns: not significant.

Suppl.Fig. 5. Full blots used in this study.

Tables

- **Suppl Table1**: Guide RNA read count tables from SLC knoxkout screens
- **Suppl Table2**: Primers (qPCR, sequencing) and sgRNAs used in the study