SUPPLEMETARY FIGURE TITLES AND LEGENDS

Figure S1. Related to Figures 2 and 3; Generation of SPCA1 KO cells.

(A) There are 9 consensus-coding sequence (CCDS) isoforms 1A-F and 2A-C of SPCA1. The isoforms 2A-C share the N-terminus (designated in dark grey); the different C-termini are shared by different isoforms (designated by the same color). Exon 7 was targeted for CRISPR editing by 2 different guide RNAs at indicated sites (#1 and #2). (B) CRISPR induced deletions in Hap1 cells targeting site #1 and #2. Hap1 WT clones #1-1 and #2-1 underwent the CRISPR editing but *ATP2C1* was not successfully disrupted. They were used in all downstream experiments as WT control clones with their respective KO clones. (C) Cell viability of Hap1 cells and CRISPR-generated Hap1 clones was determined by measuring cellular ATP levels. The daily replication was calculated over the course of 4 days, data represent the mean and SD of 15 technical replicates and are plotted as indicated. Cells are colored differently to indicate: parental WT = black; CRISPR WT clones = dark blue; and CRISPR KO clones = light blue**.**

Figure S2. Related to Figure 3; Paramyxovirus infections of Hap1 cells and CRISPR-generated Hap1 clones in the presence of a calcium ionophore and furin inhibitor.

Hap1 cells and CRISPR-generated Hap1 clones were infected with (A and B) MeV-GFP at a MOI of 0.1 or (C) hPIV-3-GFP at a MOI of 0.01. (A) The calcium ionophore A23817 was added to the culture at a concentration of 200 nM at 12 hpi and present throughout the infection. (B and C) The furin inhibitor Dec-RVKR-CMK was present throughout the

infection at a concentration of 20 μM. (A-C) Cells were fixed at 48 hpi for (A and B) microscopy (10x magnification) or (C) analyzed by flow cytometry and plotted as a percentage of GFP positive cells. Cells are colored differently to indicate: parental WT = black; CRISPR WT clones = dark blue and purple; and CRISPR KO clones = light blue and purple.

Figure S3. Related to Figures 5 and 7; Viral infections of Lovo cells to determine furin dependency.

Lovo cells were infected with different paramyxoviruses (A) RSV-GFP at a MOI of 0.5 (B) hPIV-3-GFP (WT) and (C) hPIV-3-GFP (passaged isolate P5) at a MOI of 0.1 and (D) MeV-GFP at MOI of 0.5. Infections with (E) VSV-GFP and (F) LCMV-GFP were performed at a MOI of 0.1. The togaviruses (G) VEEV-GFP, (H) CHIKV-GFP and (I) RRV-GFP were used at MOIs of 0.01, 0.1, and 500, respectively. Cells were also infected with different flaviruses (J) YFV-venus at MOI of 0.1, (K) WNV-GFP at a MOI of 10 and (L) DENV-GFP at a MOI of 0.2. The cells were (D) fixed for microscopy at 48 hpi (10x magnification) or harvested at 24, 48, 72, 96 hpi for all other virus infections to be analyzed by flow cytometry and plotted as a percentage of GFP positive cells. The data of virus infections, that were analyzed by flow cytometry (A-C and E-L), represent the mean and SD of 3 independent experiments.

Figure S4. Related to Figures 2 and 7; Viruses unaffected by SPCA1 deficiency.

Hap1 cells and CRISPR-generated WT and KO clones of SPCA1 infected with (A) VSV-GFP at a MOI of 0.01 (B) LCMV-GFP at a MOI of 0.05 (C) HSV-1 at a MOI of 0.05 and (D) BUNV at a MOI of 0.01. Cells were harvested (A) at 24 hpi and (B) at 48 hpi, analyzed by flow cytometry and plotted as a percentage of GFP positive cells. Data represent the mean and SD of 3 independent experiments. (C and D) Cells were harvested at (C) 48 hpi and (D) 72 hpi to determine cell viability via cellular ATP levels. The data represent the mean and SD of 9 technical replicates and are plotted as indicated as percentage relative to mock-infected cells. Cells are colored differently to indicate: parental WT = black; CRISPR WT clones = dark blue; and CRISPR KO clones = light blue.

Figure S5. Related to Figure 2; Paramyxovirus infection of SPCA1 CRISPR WT and KO clones generated in A549 and HeLa cells.

Infections with hPIV-3-GFP at a MOI of 0.01 of (A) A549 and (B) HeLa cell clones generated by CRISPR editing using sgRNA #2 (previously used for editing of *ATP2C1* in Hap1 cells). Cells were harvested at 48 hpi, analyzed by flow cytometry and plotted as a percentage of GFP positive cells. (C and D) Western blot of CRISPR-generated (C) A549 and (D) HeLa cell clones probed for SPCA1 demonstrating protein knock-out as well as protein knock-down (A549 clones 2-3 and 2-8).

Figure S6. Related to Figure 6; SPCA1 protein levels in Hailey-Hailey disease (HHD) derived fibroblasts and Morpholino treated cultures.

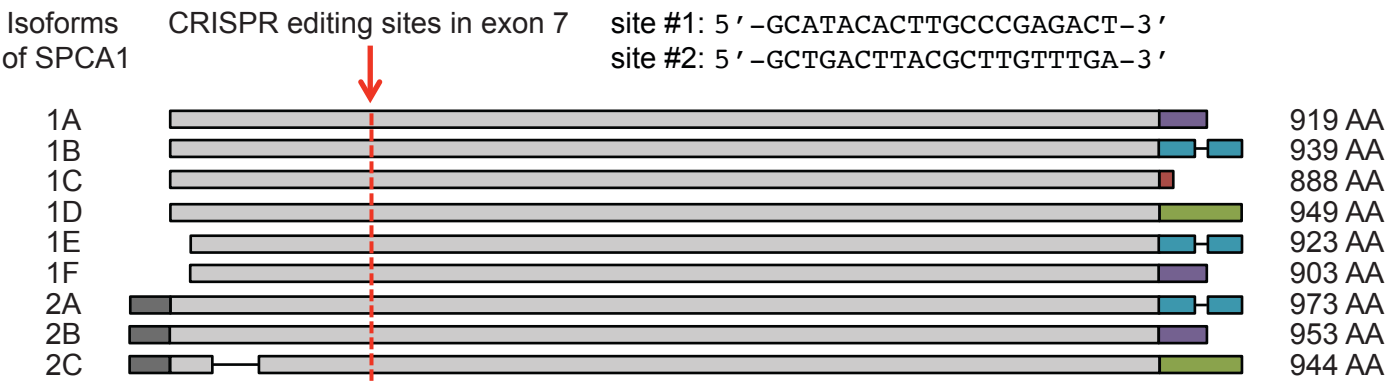
(A) Primary fibroblasts derived from healthy control individuals (C1, C2, C3, C4) and from HHD patients (H1, H2, H3, H4) were analyzed by western blot for SPCA1 and furin protein levels. (B) Hap1 cells and primary human airway epithelial (HAE) cultures were treated for 48 h with a control Morpholino or a Morpholino targeting *ATP2C1* and protein levels of SPCA1 were analyzed by western blot. The decrease of SPCA1 in HAE cultures is relatively modest compared to Hap1 cells. HAE cultures are multilayered cultures and only the apical layer, which is also being infected, receives direct access to the Morpholino treatment. The abundance of untreated cells beneath the apical layer might mask the Morpholino-induced decrease of SPCA1. Moreover, HAE cultures are differentiated, non-diving cultures, which may also impact the turnover rate of proteins (SPCA1) compared to rapidly replicating cell lines.

Figure S7. Related to Figure 7; Impaired spread of togaviruses in SPCA1 KO cells.

Hap1 cells, CRISPR-generated Hap1 clones and SPCA1-reconstituted clones infected with (A) CHIKV-GFP at a MOI of 0.005, (B) RRV-GFP at a MOI of 20 and (C) VEEV-GFP at a MOI of 0.001. Cells were harvested at 34 hpi, 48 hpi and 28 hpi, respectively, analyzed by flow cytometry and plotted as a percentage of GFP positive cells. Data represent the mean and SD of 3 independent experiments. Cells are colored differently to indicate: parental WT = black; CRISPR WT clones = dark blue and green; and CRISPR KO clones = light blue and green.

Table S1. Related to Figure 1; Genes selected with RSV in a genome-wide KO screen performed in the Hap1 cell line.

A



B

CRISPR clones generated targeting site #1:

```
WT 1-1: GGAGCATACACTTGCCCGAGACTTGGTT//TAA – no INDEL
KO 1-1: GGAGCATACACTTGCCCGAG--------//TAA – 244nt del
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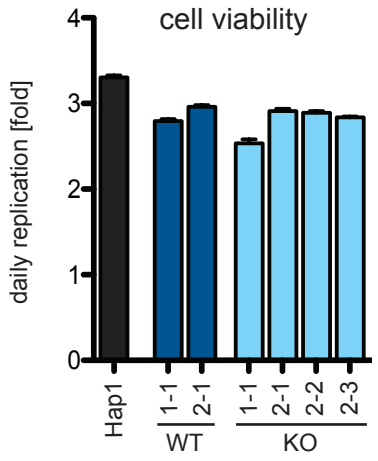
CRISPR clones generated targeting site #2:

```
WT 2-1: ATAGAGTTCCTGCTGACTTACGCTTGTTTGAGG – no INDEL
KO 2-1: ATAGAGTTCCTGCT----------TGTTTGAGG – 10nt del
KO 2-2: ATAGAGTTCCTGC----TTACGCTTGTTTGAGG – 4nt del
KO 2-3: ATAGAGTTCCTGCTGAC----GCTTGTTTGAGG – 4nt del
```

C



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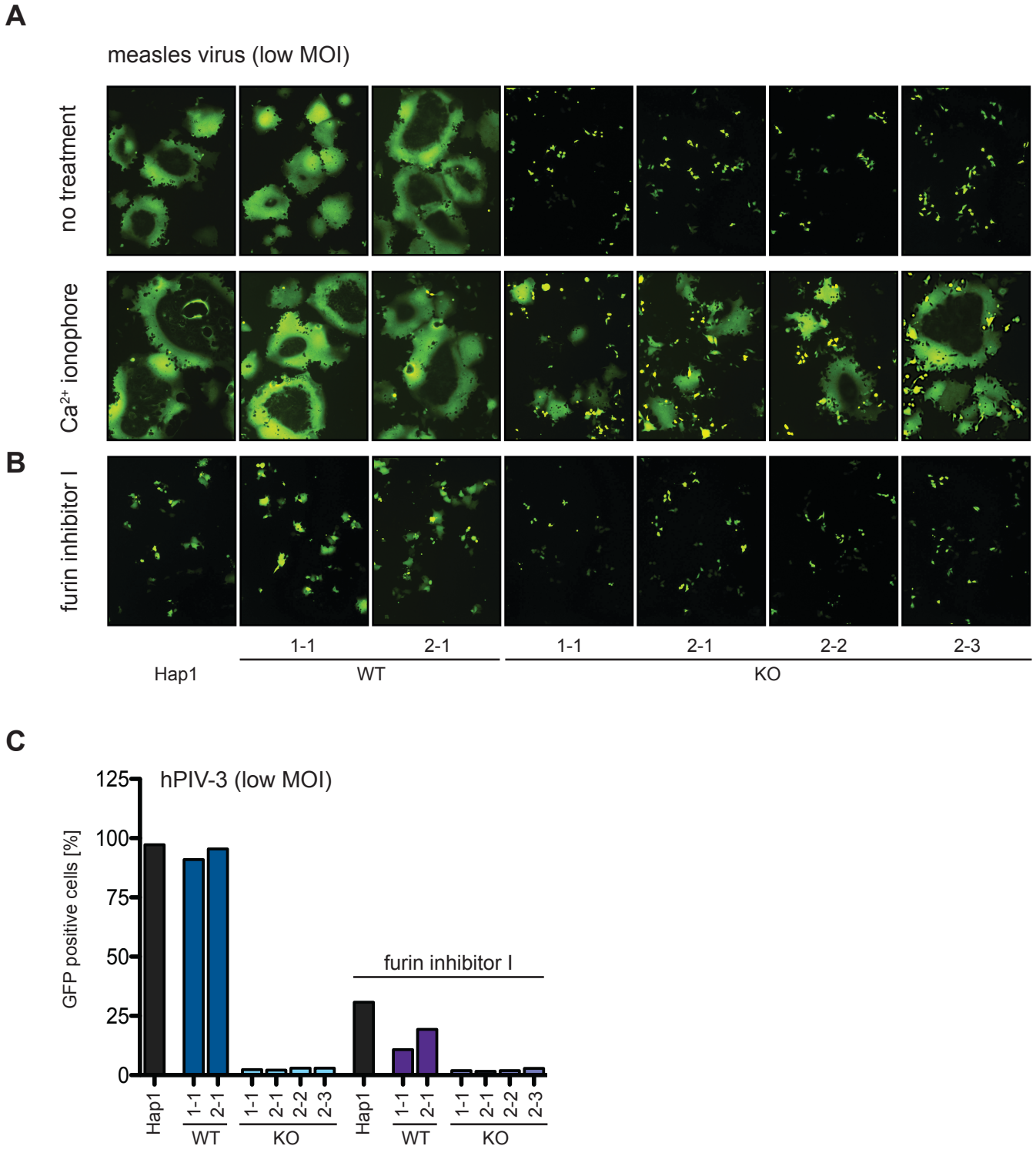

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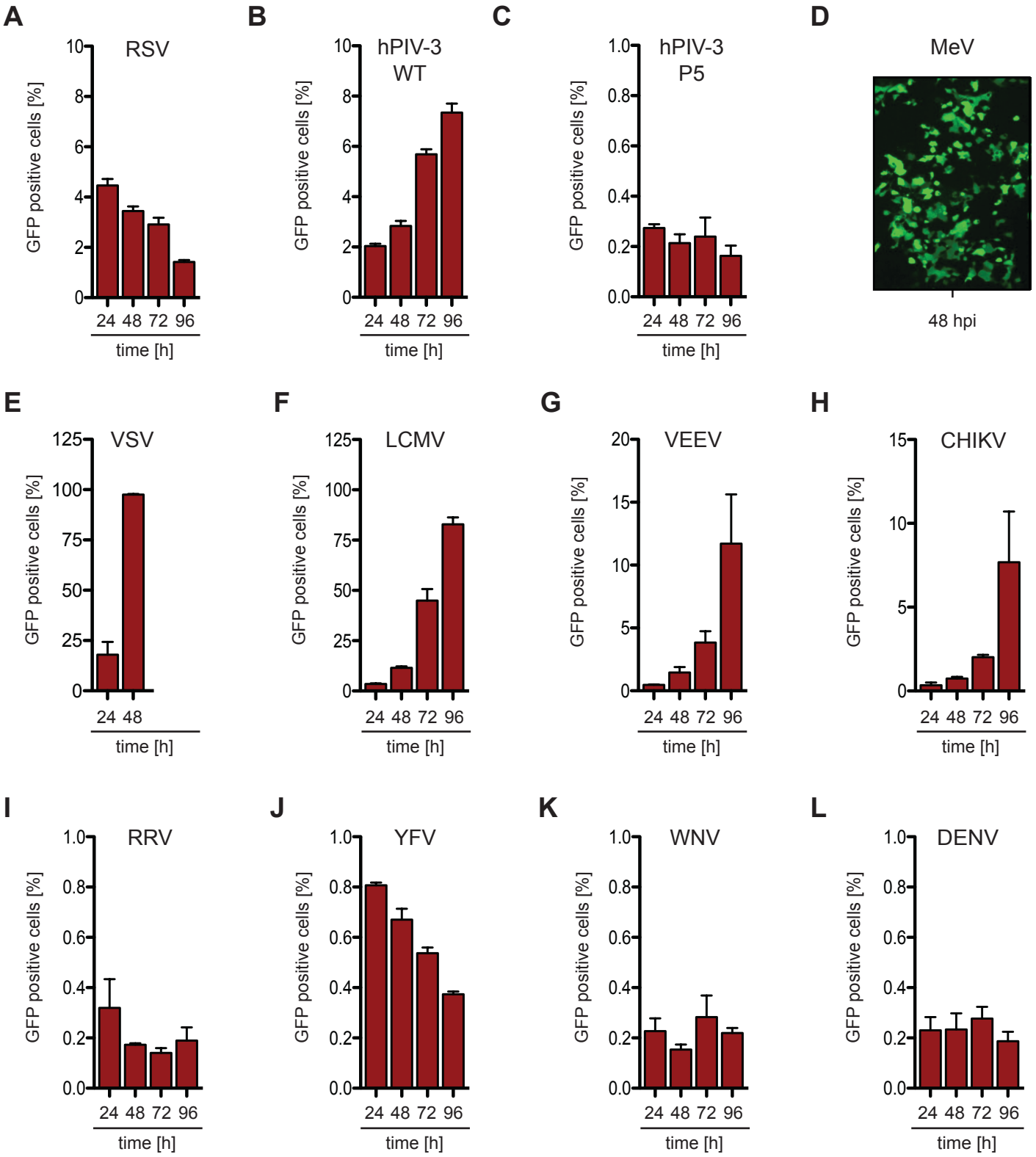

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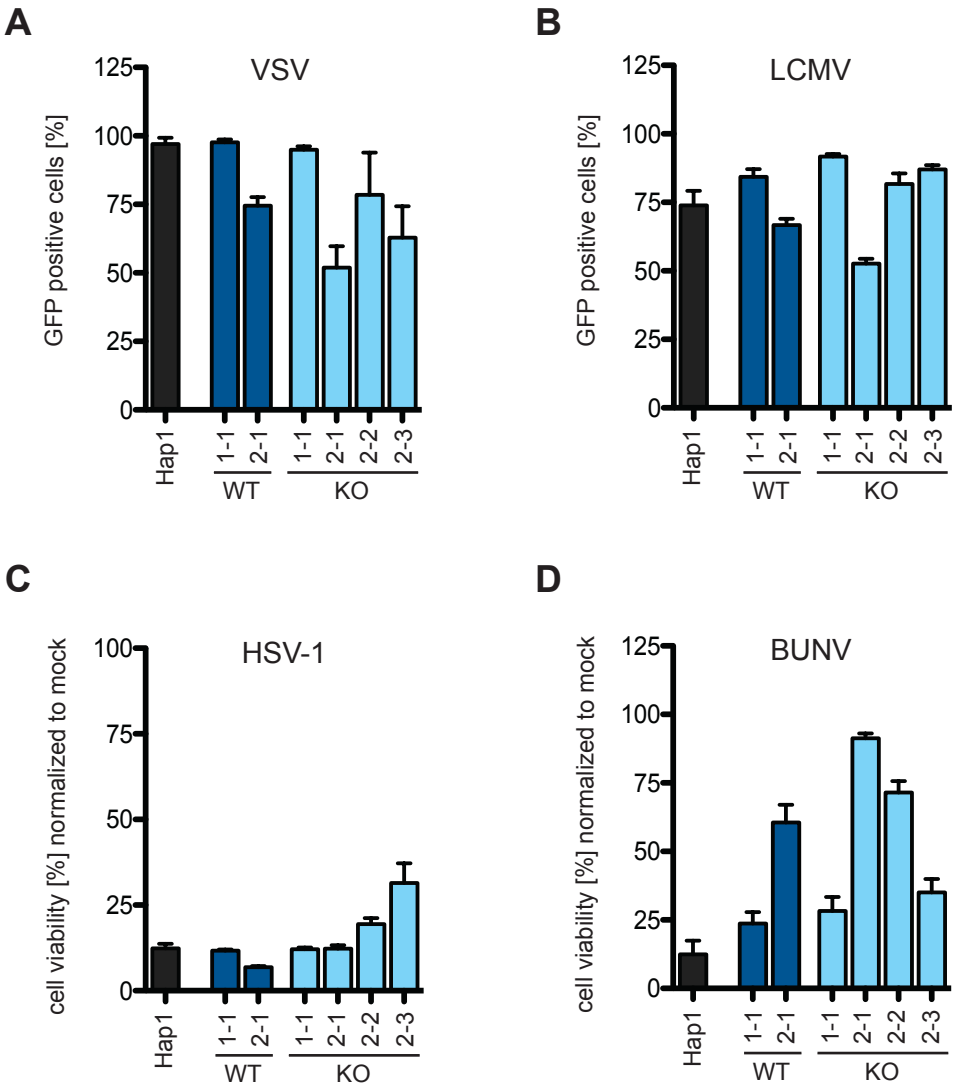

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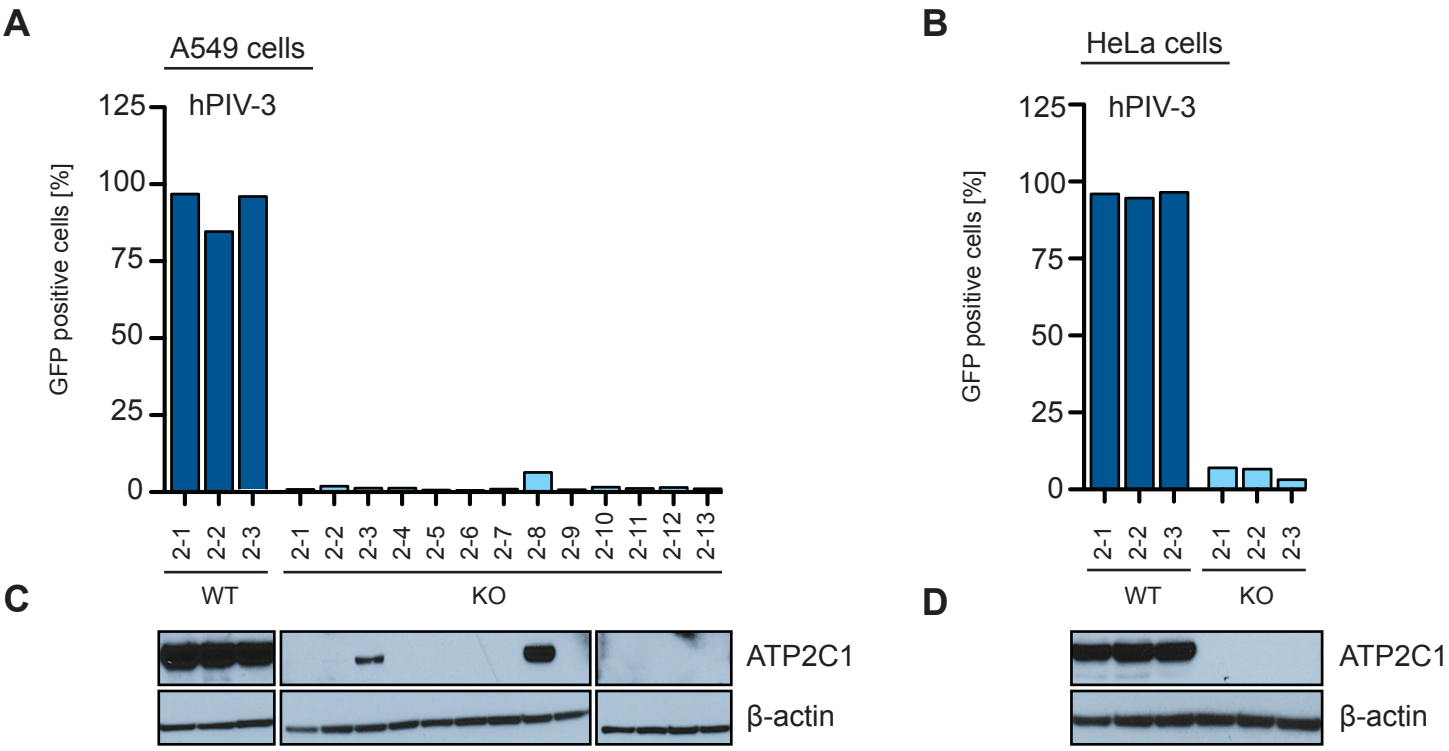

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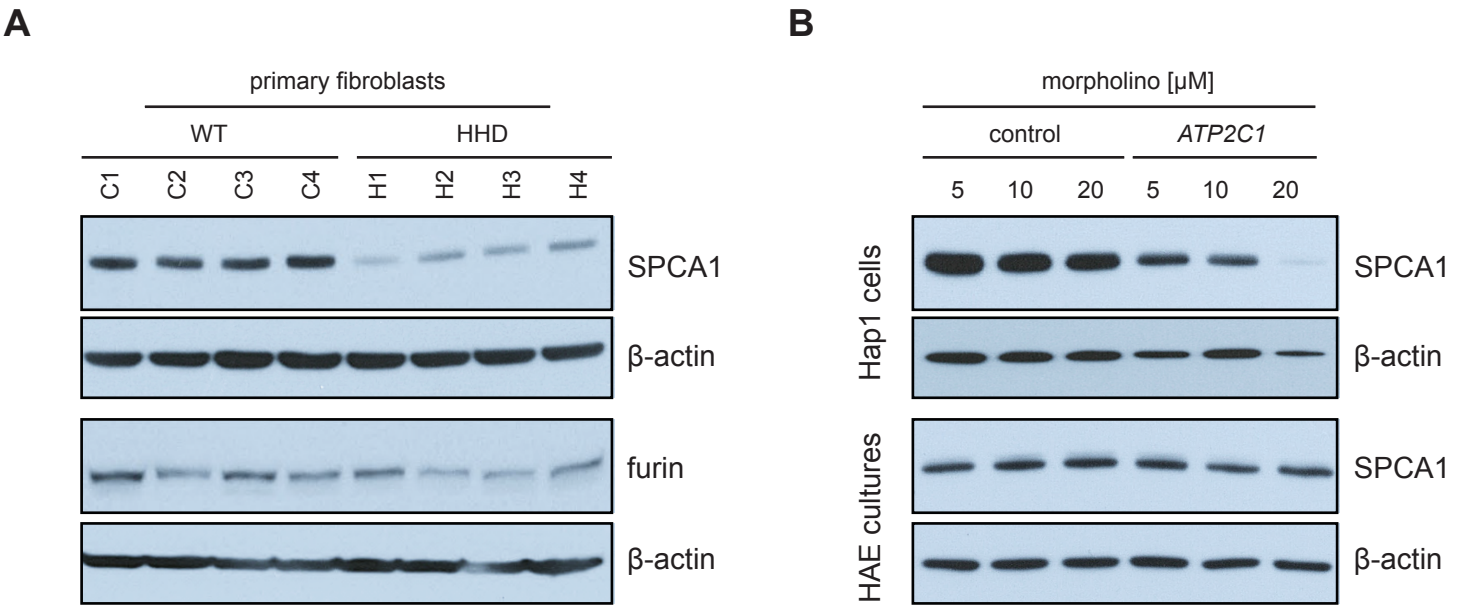

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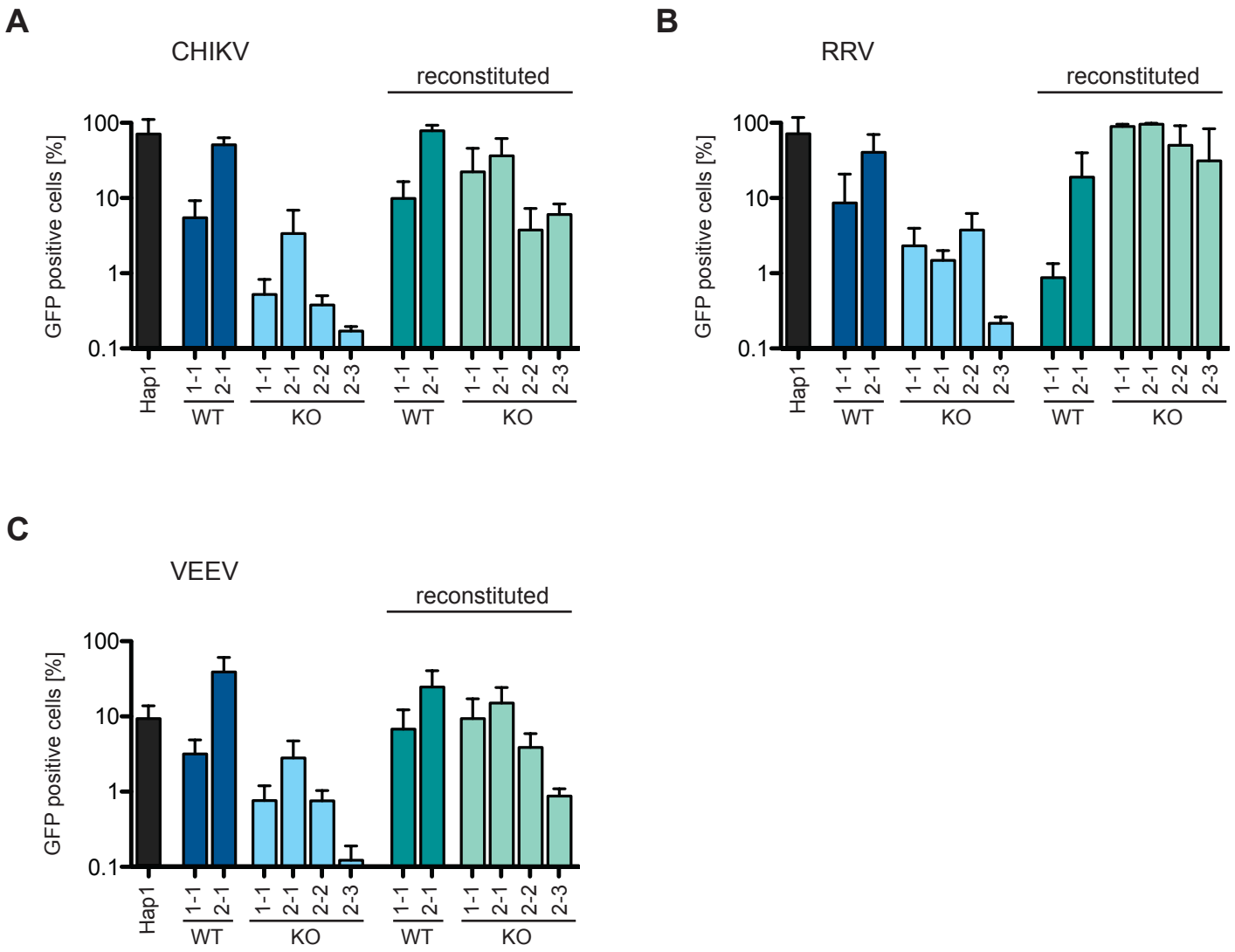

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