**Cell Reports Medicine, Volume *5***

## Supplemental information

# Combination of compound screening with an animal model identifies pentamidine to prevent C*hlamydia trachomatis* infection

**Katja Knapp, Romana Klasinc, Anna Koren, Magdalena Siller, Ruth Dingelmaier-Hovorka, Mathias Drach, Juan Sanchez, David Chromy, Marlene Kranawetter, Christoph Grimm, Andreas Bergthaler, Stefan Kubicek, Hannes Stockinger, and Georg Stary**

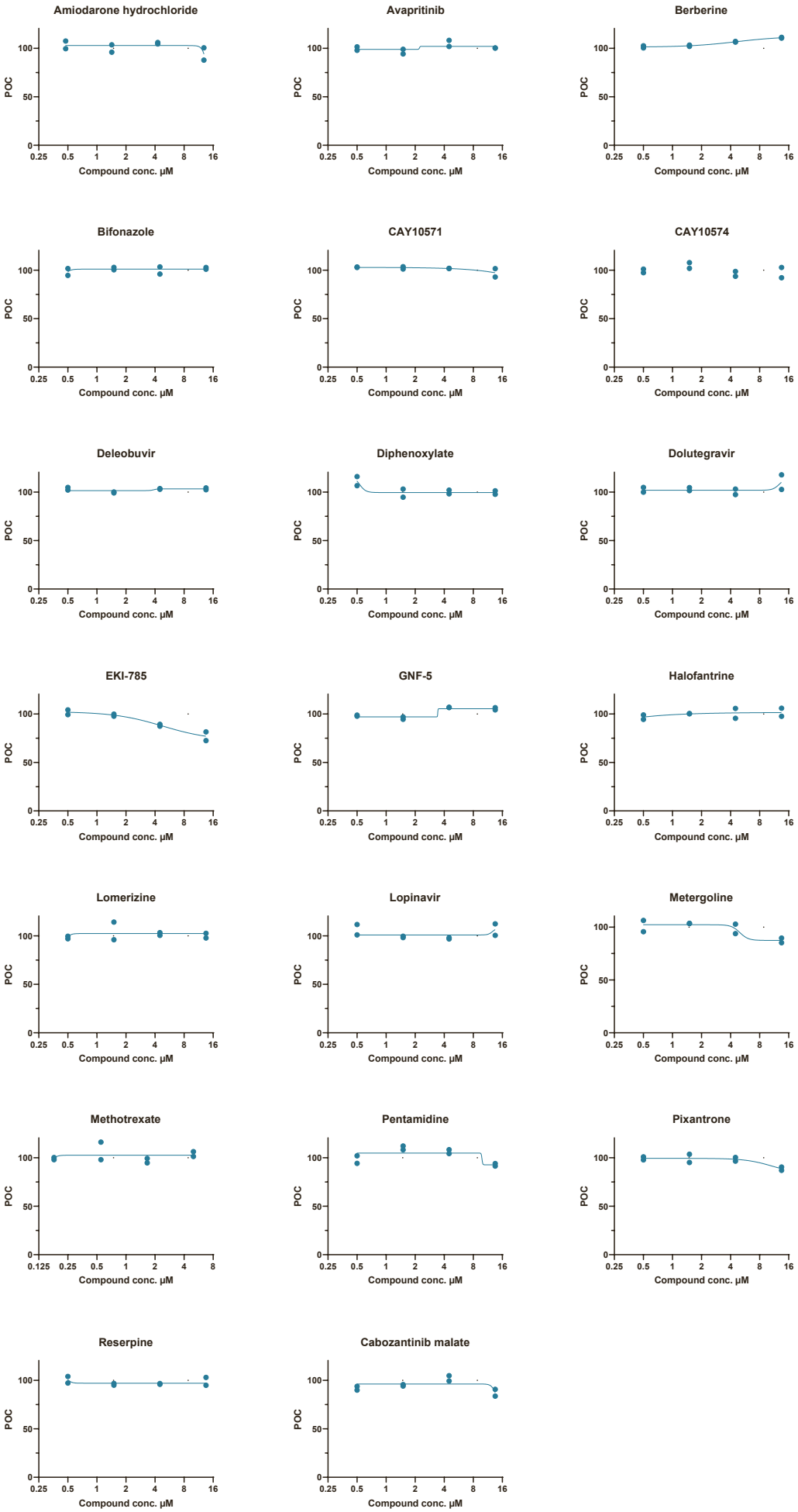



**Figure S1: Cytotoxicity assay with CellTiter-Glo - related to Figure 2.** HeLa cells were treated with compounds in 3-fold serial dilutions starting at 13.5 µM for 48 h to assess their cytotoxicity in the absence of Ct in duplicates. Cell viability was determined by measuring metabolic activity in each well using CellTiter-Glo. Percentage of control (POC) calculation was based on 0.1% DMSO-treated cells (100% viability anticipated).

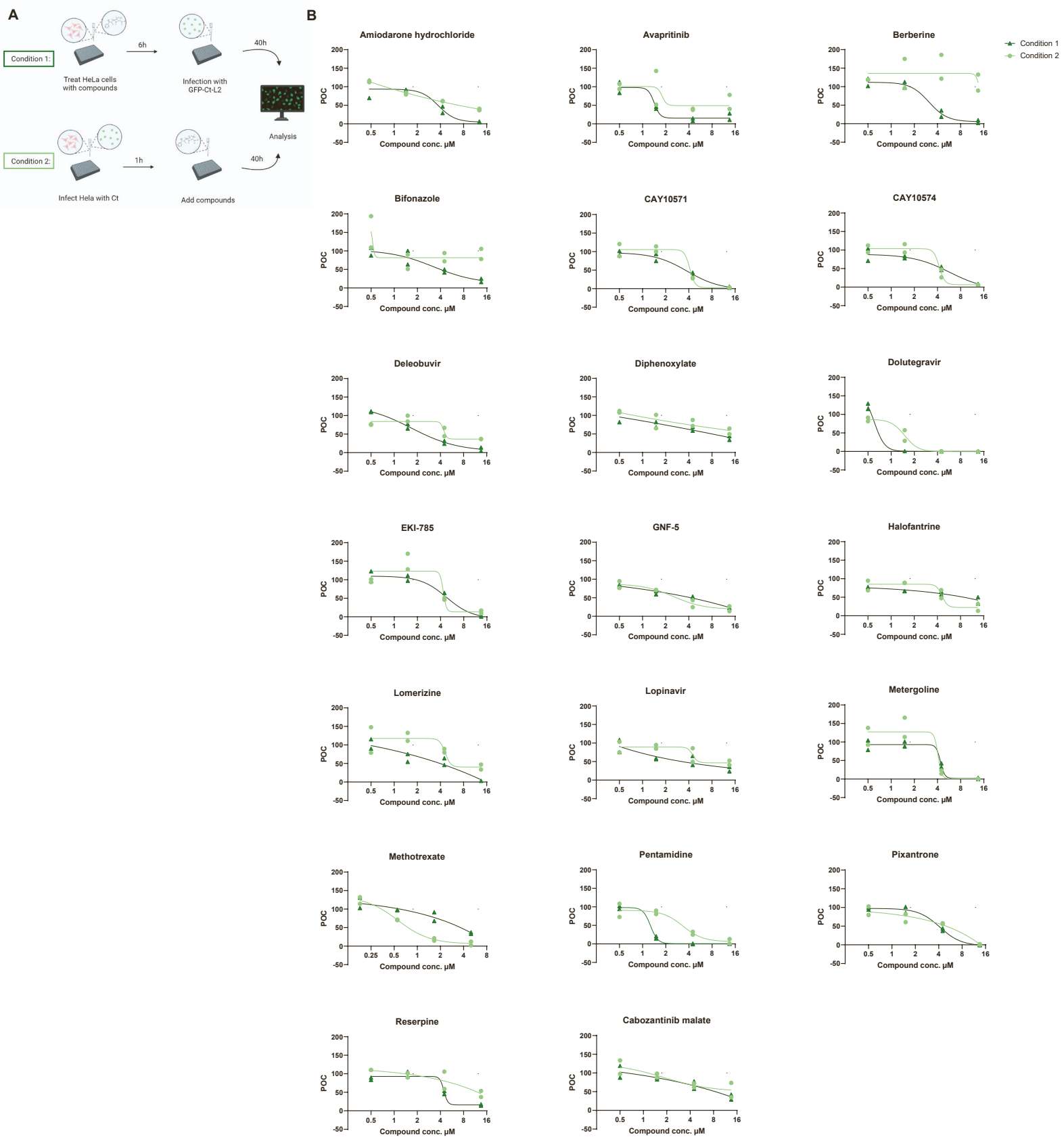
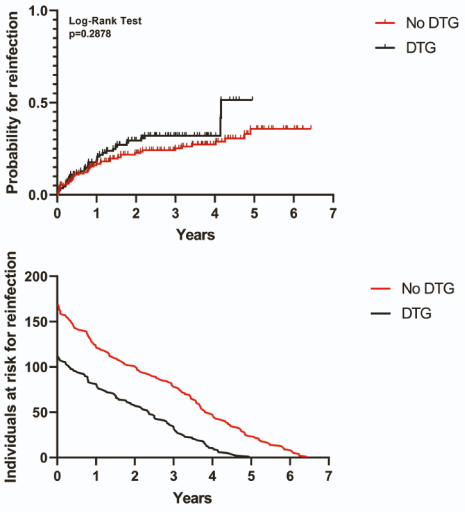



**Figure S2: Addition of compounds before or after Ct infection - related to Figure 2.** To assess the effect of compounds if applied after infection, the top 20 compounds were added in 3-fold serial dilutions starting at 13.5 µM to HeLa cells 1 h after infection with Ct-L2-GFP. The cells were incubated for 42 h and POC was compared with results from the previous dose-response validation screen with compounds being present already before Ct infection. A) Scheme of experimental setup. Created with BioRender. B) Dose response curves in technical duplicates.

A

| | N | Infections per year including reinfections during follow-up | 95%-CI |
|---|---|---|---|
| Overall episodes of *Ct* infection | 847 | 127.05 | 120.24-134.05 |
| HIV negative individuals Episodes of *Ct* infection | 436 | 60.01 | 60.01-71.15 |
| HIV positive individuals Episodes of *Ct* infection | 411 | 61.65 | 56.39-67.28 |
| HIV positive individuals with DTG Episodes of *Ct* infection 42.57% | 175 | 26.25 | 22.72-30.24 |
| | Standardized | 30.83 | |
| HIV positive individuals without DTG Episodes of *Ct* infection 57.42% | 236 | 35.55 | 56.39-67.28 |
| | Standardized | 30.96 | |

C




B

| HIV+ with serovar analysis available | | | DTG No | DTG yes | Total frequency of serovar in our cohort |
|---|---|---|---|---|---|
| Serovar | E | Count | 26 | 23 | 49 |
| | | % within serovar | 53.1% | 46.9% | 24,7% |
| | F | Count | 4 | 3 | 7 |
| | | % within serovar | 57.1% | 42.9% | 3,5% |
| | G | Count | 28 | 22 | 50 |
| | | % within serovar | 56% | 44% | 25,3% |
| | J | Count | 11 | 15 | 26 |
| | | % within serovar | 42.3% | 57.7% | 13,1% |
| | K | Count | 0 | 1 | 1 |
| | | % within serovar | 0% | 100% | 0,5% |
| | L1-L3 | Count | 32 | 33 | 65 |
| | | % within serovar | 49.2% | 50.8% | 32,8% |
| Total | | | | | 198 |
| | | | | | 100% |

**Figure S3: Chlamydia incidence in HIV+ and HIV- patients with and without dolutegravir (DTG) treatment - related to Figure 3.** A) Table shows the chlamydia infections per year in HIV negative and positive men. HIV positive patients with and without DTG treatment were assessed separately. B) Ct serovar typing of HIV+ patients with and without DTG treatment. (Fishers Exact test: p=0.815) C) Kaplan-Meier curves for the probability for Ct infection (upper chart) and the individual risk for Ct infection (lower chart) are presented.

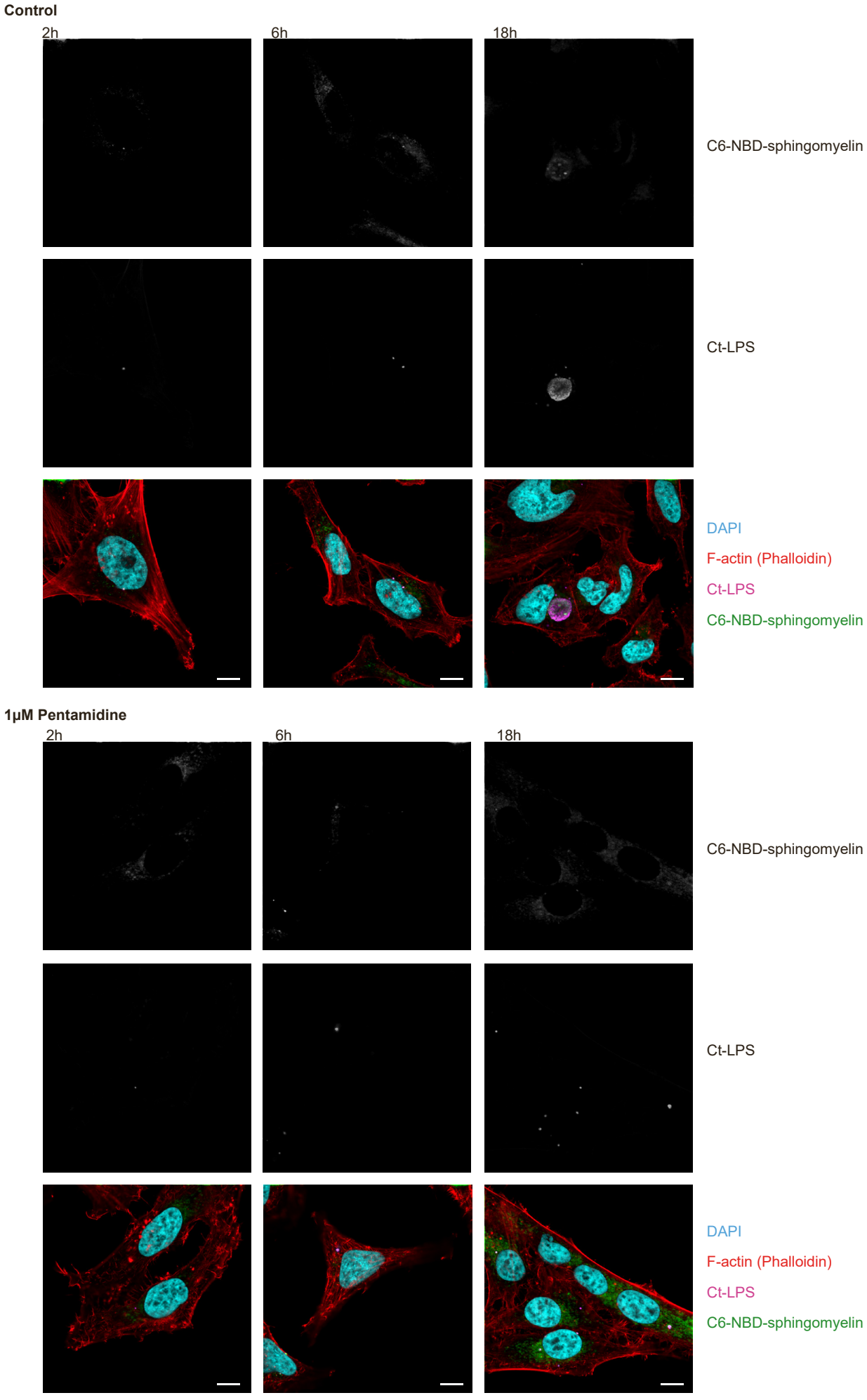



**Figure S4: C6-NBD-sphingomyelin is recruited to inclusions in pentamidine treated cells, but inclusions do not develop further - related to Figure 4.** HeLa cells were treated with 1 µM pent or DMSO (Control) for 6 h before infection with Ct-L2 MOI 2.5 (no GFP-expressing). 2 h, 6 h or 18 h after infection, cells were harvested and stained with C6-NBD-Ceramide, anti-Ct-LPS, phalloidin and DAPI. Representative images are shown. Scale bar = 10µm.

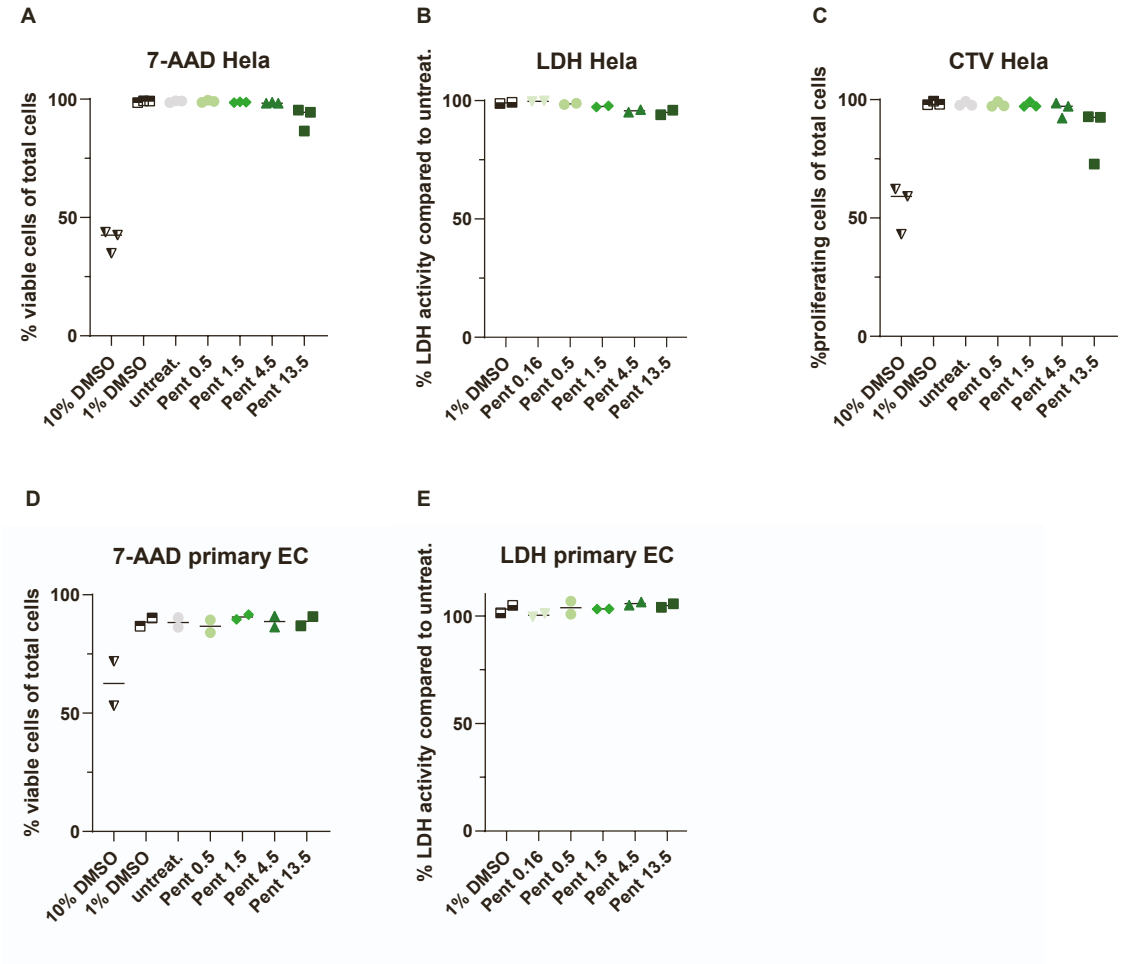
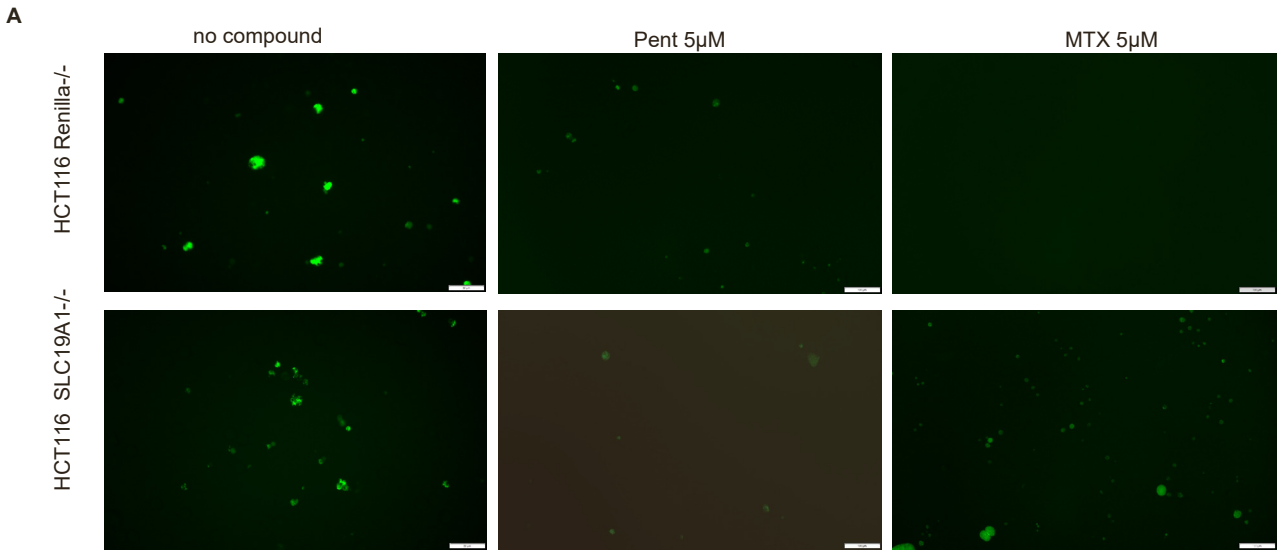



**Figure S5: Viability of Hela cells and primary EC is not impacted by pentamidine - related to Figure 4.** A-C) HeLa cells were treated with various concentrations of pentamidine (13.5 µM – 0.16 µM) and DMSO for 48 h. A) Flow-cytometric analysis of 7-AAD negative cells, 3 independent experiments. B) Analysis of LDH-activity in pentamidine treated samples in comparison to untreated cells, 2 independent experiments. C) Flow-cytometric analysis of proliferating cells by assessing dilution of CellTrace Violet (CTV), 3 independent experiments. D-E) primary cervical epithelial cells were treated with various concentrations of pentamidine and DMSO for 48 h. D) Flow-cytometric analysis of 7-AAD negative cells, 2 independent experiments. E) Analysis of LDH-activity in pentamidine treated samples in comparison to untreated cells, 2 independent experiments.



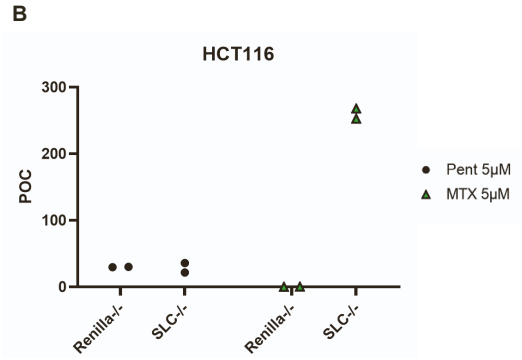




**Figure S6: Pentamidine, in contrast to methotrexate, does not inhibit Ct growth via SLC19A1 - related to Figure 4.** HCT116 Renilla-/- and SLC19A1-/- (SLC) cells treated with 5µM pentamidine (Pent) or 5 µM methotrexate (MTX) 6 h before infection were analyzed 48 h after infection. A) Representative images, scale bar = 100 µm. B) Percentage of control (n=2 independent experiments).