Supplementary Materials for

Identification of SARS-CoV2-mediated suppression of NRF2 signaling reveals a potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate

David P. Olagnier[1,*], Ensieh Farahani[1,†], Jacob Thyrsted[1,†], Julia B. Cadanet[1,†], Angela Herengt[1,†], Manja Idorn[1], Alon Hait[1,7], Bruno Hernaez[2], Alice Knudsen[1], Marie Beck Iversen[1], Mirjam Schilling[3], Sofie E. Jørgensen[1,7], Michelle Thomsen[1,7], Line Reinert[1], Michael Lappe[11], Huy-Dung Hoang[4], Victoria H. Gilchrist[4], Anne Louise Hansen[1], Rasmus Ottosen[10], Camilla Gunderstofte[1], Charlotte Møller[1], Jinrong Huang[9,12], Martin Jakobsen[1], Thomas B. Poulsen[10], Lydia Bartsch[6], Anne L. Thielke[1], Yonglun Luo[1,9], Tommy Alain[4], Jan Rehwinkel[3], Antonio Alcamí[2], John Hiscott[5], Trine Mogensen[1,7,8,††], Søren R. Paludan[1,††], Christian K. Holm[1,*].

Correspondence to: holm@biomed.au.dk or olagnier@biomed.au.dk

This PDF file includes:

Figs. S1 to S5.

Fig. S1.

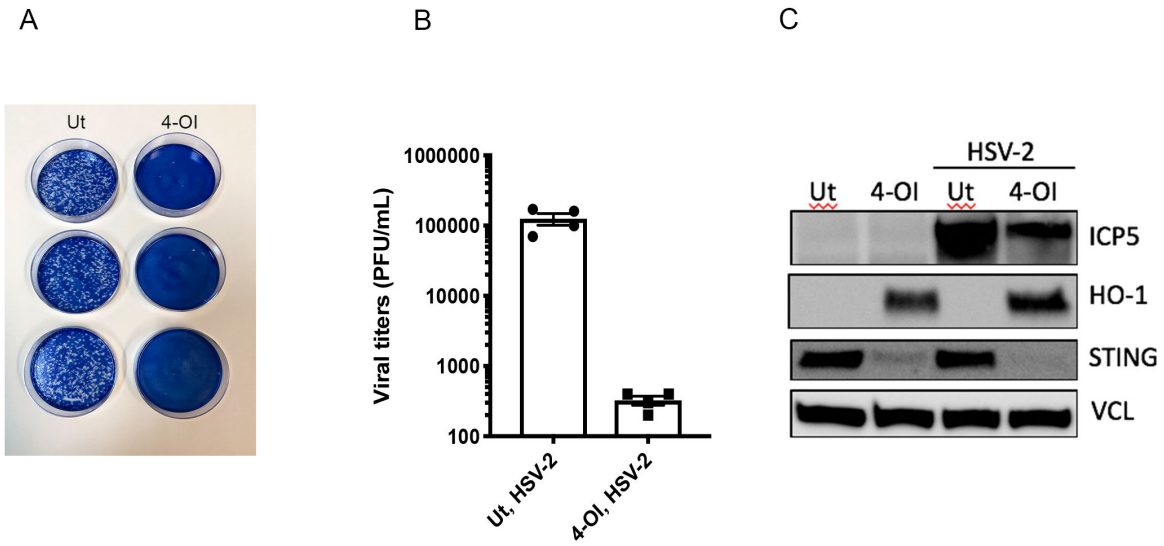

Fig. S1. 4-OI inhibits HSV2 replication
HaCaT cells were treated with 4-OI at 125μM for 48 hours before infection with HSV2 at MOI 0.01. Cell supernatants were then harvested to determine release of progeny virus by plaque assay (A+B) and cell pellets were lysed for immunoblotting using specific antibodies against HSV protein ICP5, Heme Oxygenase 1 (HO-1), STING, and Vinculin as loading control(C). A) is a photo of plaque assay performed on vero cells. B) is a quantification of the plaque assay. Experiements are representative of two independent experiments.

4-OI inhibits replication of HSV2.

<insert page break then Fig. S2 here>

Fig. S2.

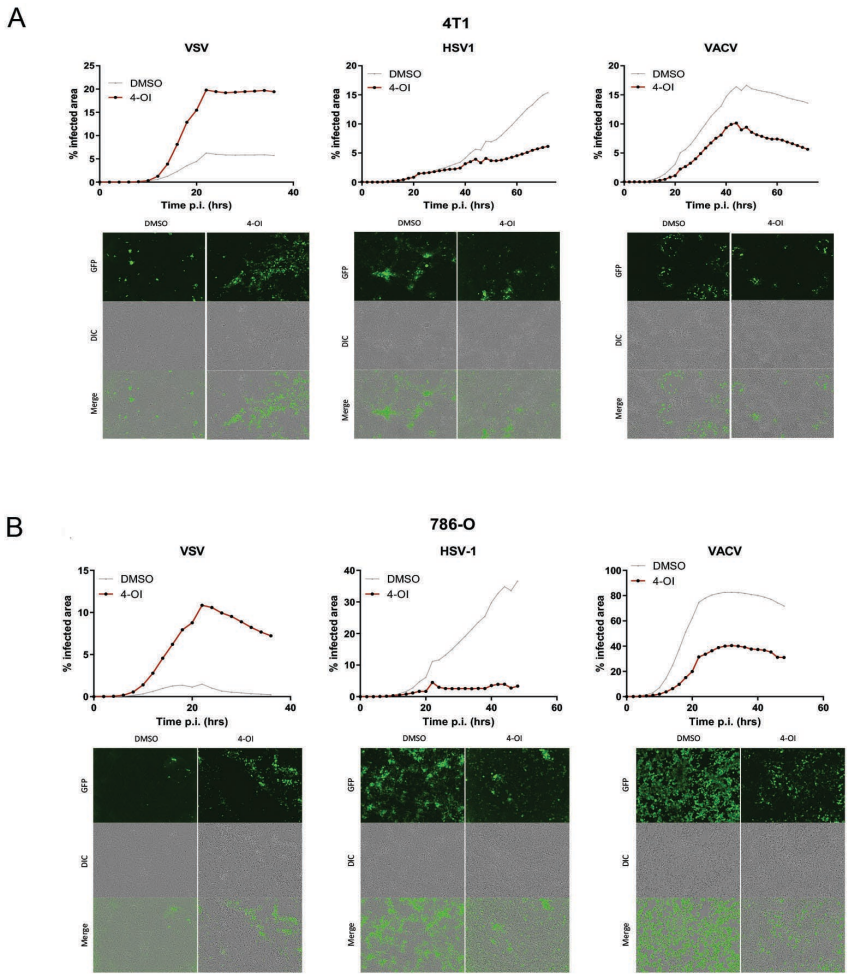

Fig. S2. 4-OI impairs HSV and VACV spread in cancer cells but enhances VSV. (A) Mouse breast cancer cells (4T1) or (B) human renal carcinoma cells (786-O) were treated with 4-OI (150 μM) for 48 hours, then infected with VSV-Δ51-GFP, HSV-ΔICP0-GFP or VACV-GFP at MOI of 0.1. Upper line charts: Live virus spreads were monitored via GFP fluorescence signal using the Incucyte Live-Cell imaging system, images were taken very 2 hours. Lower images: representative fluorescence microscopy images of virus infection at 24 hours p.i. for VSV and 48 hours p.i. for HSV-1 and VACV. Data are representative of two independent experiments.

4-OI impairs HSV and VACV spread in cancer cells but enhances VSV.

Fig. S3.

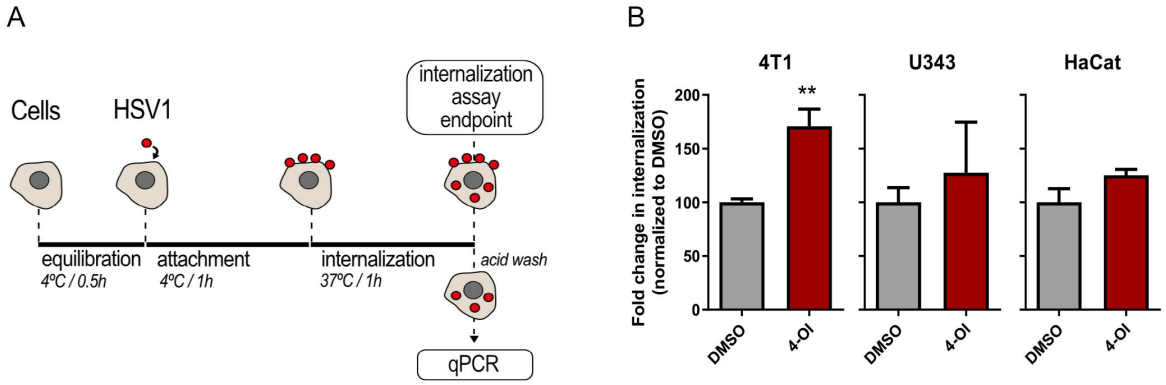

Fig. S3. 4-OI enhances HSV-1 internalization. (A) Schematic representation of the internalization assay performed in a breast cancer cell line (4TI), in human glioblastoma (U343), and in immortalized human keratinocyte (HaCaT). Cells were pre-treated with 4-OI (150μM) for 48 hours, then the viral entry assay was carried out with HSV-1 incubation at an MOI of 10. Bars indicate mean with SEM from at least 3 independent experiments. Statistical analysis was performed using unpaired student t-test, **:p<0.01.

4-OI enhances internalization of HSV1.

5

Fig. S4.

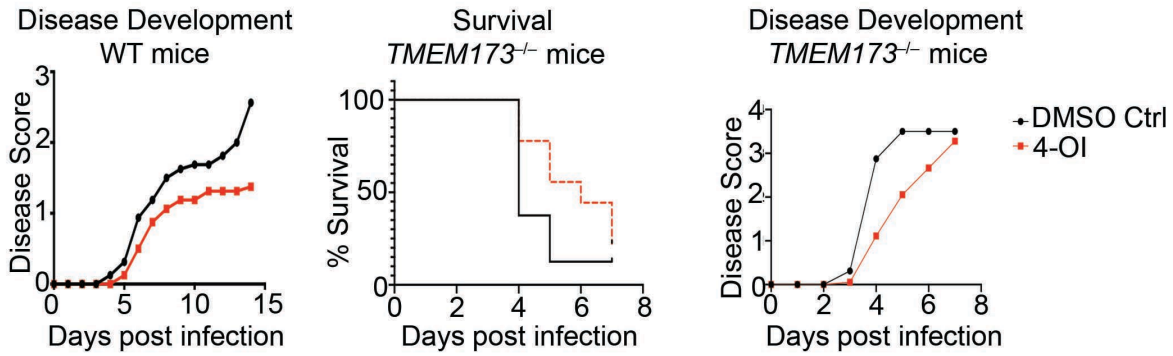

Fig. S4. *In vivo* anti-viral effect of 4-OI in model of HSV2
Eight week old wt or *TMEM173*$^{-/-}$ C57BL6J mice were treated intravaginally with 4-OI at 150μM before intravaginal inoculation with HSV2 (333 strain) 24 hours later. Disease progression and survival was monitored and scored for 15 days for WT and 8 days for *TMEM173*$^{-/-}$ mice. Data are representative of two independent experiments with 8 mice (n=8) in each of the experimental groups.

In vivo anti-viral effect of 4-OI in mouse model of HSV2

5

Fig. S5.

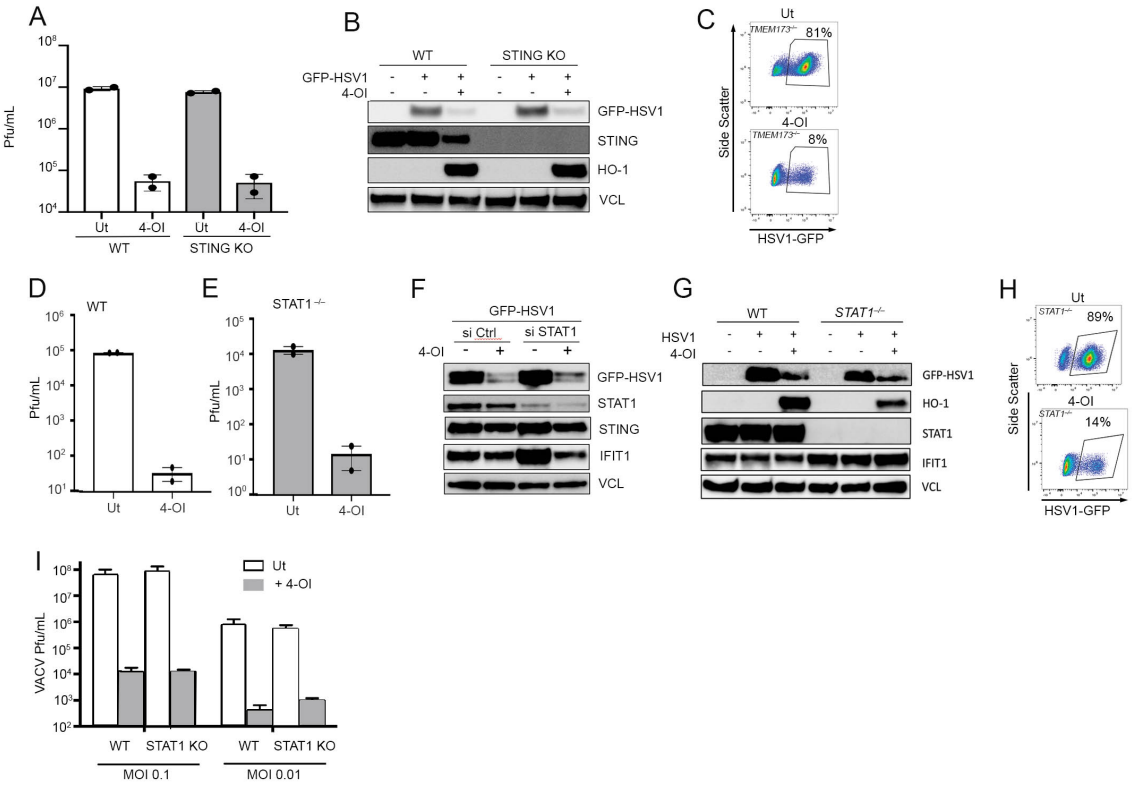

Fig. S5. 4-OI induces an anti-viral program independently of IFNs but dependent on NRF2. **A-I)** WT HaCaT cells or HaCaT cells deficient in STING (STING KO) or STAT1 (STAT1 KO were either treated with DMSO alone (Ut) or with 4-OI at 125μM before being infected with GFP-expressing HSV1 (GFP-HSV1) at MOI 0.01. After 20 hours, supernatants were collected for analysis by plaque assay (A, D, and E). In parallell experiment, cells were either lysed for immunoblotting (B, F, and G) using indicated antibodies or analyzed by flow cytometry (C and H). **I)** Cells were treated with 4-OI at 150μM before infection with VACV WR strain at MOI 0.01 and 0.1. After 20 hours, supernatants were collected and analyzed by plaque assay. Data are representative of a minimum of two independent experiments with bars indicating mean +/-s.e.m. of at least two biological replicates.

4-OI induces an anti-viral program independently of IFNs but dependent on NRF2

5