Supplementary data

MATERIALS AND METHODS

Cell Culture

dIMR-32 and Wi-38 cells were grown in Eagle minimum essential medium, with Earle's salts (EMEM: Sigma, UK) supplemented with 10% heat-inactivated foetal bovine serum, 50 U/ml penicillin and 50 µg/ml streptomycin.

Preparation of standard stock virus

HRV-16 was grown and titred routinely in Wi-38 cells. Cell rounding was used as an indicator of virus infectivity and the virus stock was assessed by 50% tissue culture infectious dose end-point ($TCID_{50}$). Incubation was carried out at 33°C in 5% CO_2 and cytopathic effect (CPE) was examined using a (Nikon Eclipse TE-2000 U, Nikon, UK).

Virus UV inactivation

Virus pools or supernatants were UV inactivated at 12.11 joules for 10 min by utilizing a cross-linker 250 nm (Syngene, UK) in 1 ml volumes, in 35 mm petri dishes.

Preparation of virus free supernatants and virus pellets

Pools of virus stocks were ultra-centrifuged at 30,000 rpm in an Optima, Beckman Coulter centrifuge (USA) for 2 h at +4°C. The supernatant was collected and the virus pellet gently washed with phosphate buffered saline (PBS) prior to dilution in fresh medium. Aliquots of each preparation were UV inactivated and stored at -80°C. All preparations (with and without UV treatment) were titred in Wi-38 cells by $TCID_{50}$ to determine that they were free of infectious virus..

Immunofluorescence and flow cytometry

For immunofluorescence (IF) staining, cells were fixed with 4% paraformaldehyde and permeabilised with 0.1% Triton X-100 (Sigma). Cells were incubated with blocking solution (0.5% bovine serum albumin in PBS) prior to incubation with primary antibodies Coverslips were washed 3 times in PBS before incubation with secondary antibody. A further 3 washes were given before coverslips were mounted in vectashield mounting medium with DAPI (Vector Laboratories, USA) and examined using confocal laser scanning microscopy (TCS SP5 Leica, Germany).Staining for flow cytometry was carried out according to the BD Cytofix/Cytoperm Fixation/Permeablization Kit manufacturer's instructions (BD, UK) using TRP antibodies and non-immune rabbit serum as a control. The samples were examined in a FACS Canto II (BD) or CyFlow Space (Partec, UK) and analyzed by a FlowJo programme (Oregon, USA). TRP expression levels were quantified by geometric mean fluorescent intensity (GMFI)

RT-PCR

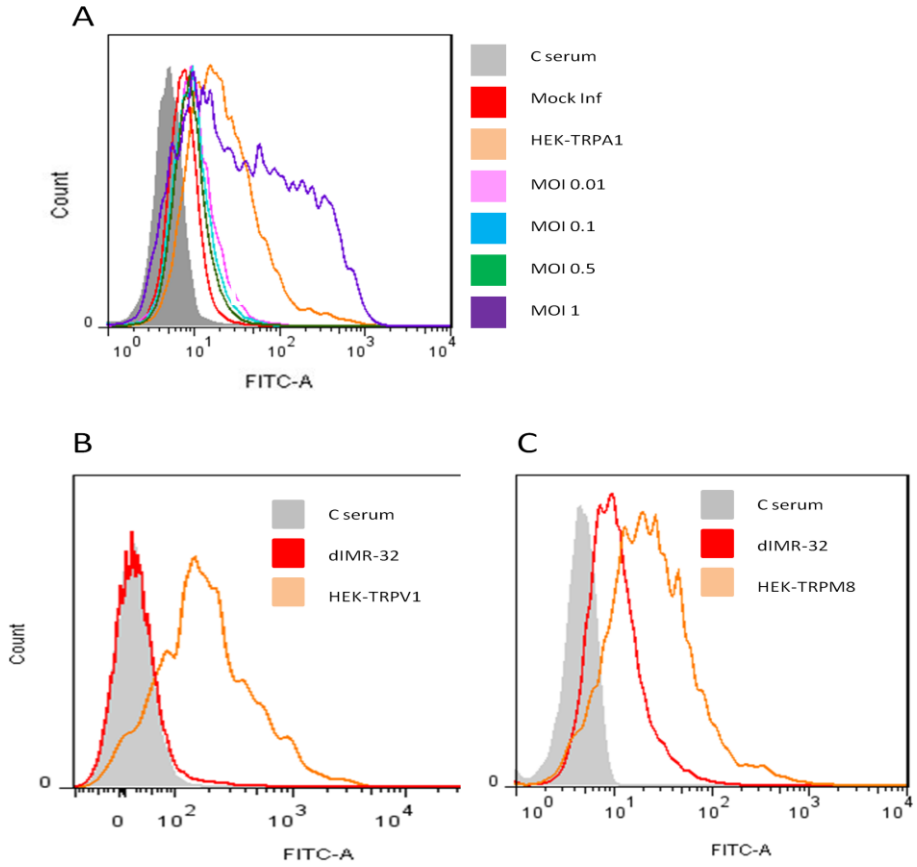
RNA was extracted using an RNeasy mini kit (Qiagen, UK) according to the manufacturer's instructions and measured in a nanodrop 1000 spectrophotometer (Thermo Scientific, USA). The 2 step RT-PCR was carried out using a Verso™ RT-PCR kit (Thermo scientific) and the reaction conditions; denaturation at 94°C for 20 sec, primer annealing at 55°C for 30 sec, primer extension at 72°C for 1 min and a final extension step at 72°C for 5 min (cycles: 30).HRV primers for the negative strand intermediate were used to avoid detecting the input virus:- Forward: 5' TTA CGA CCA GCT ACA C 3'; Reverse: 5' TTA TCC GCA AGA TGC C 3' (product=

289bp). Primers used for β-actin mRNA were;- Forward 5' TCA TGA AGT GTG ACG TTG ACA TCC GTA AAG 3', Reverse 5' CCT AGA AGC ATT TGC GGT GCA CGA TGG AGG 3' as previously described[21] Primers were supplied by Invitrogen (Life technologies, Invitrogen, UK). The PCR products were examined on a 1% agarose gel.

qRT-PCR

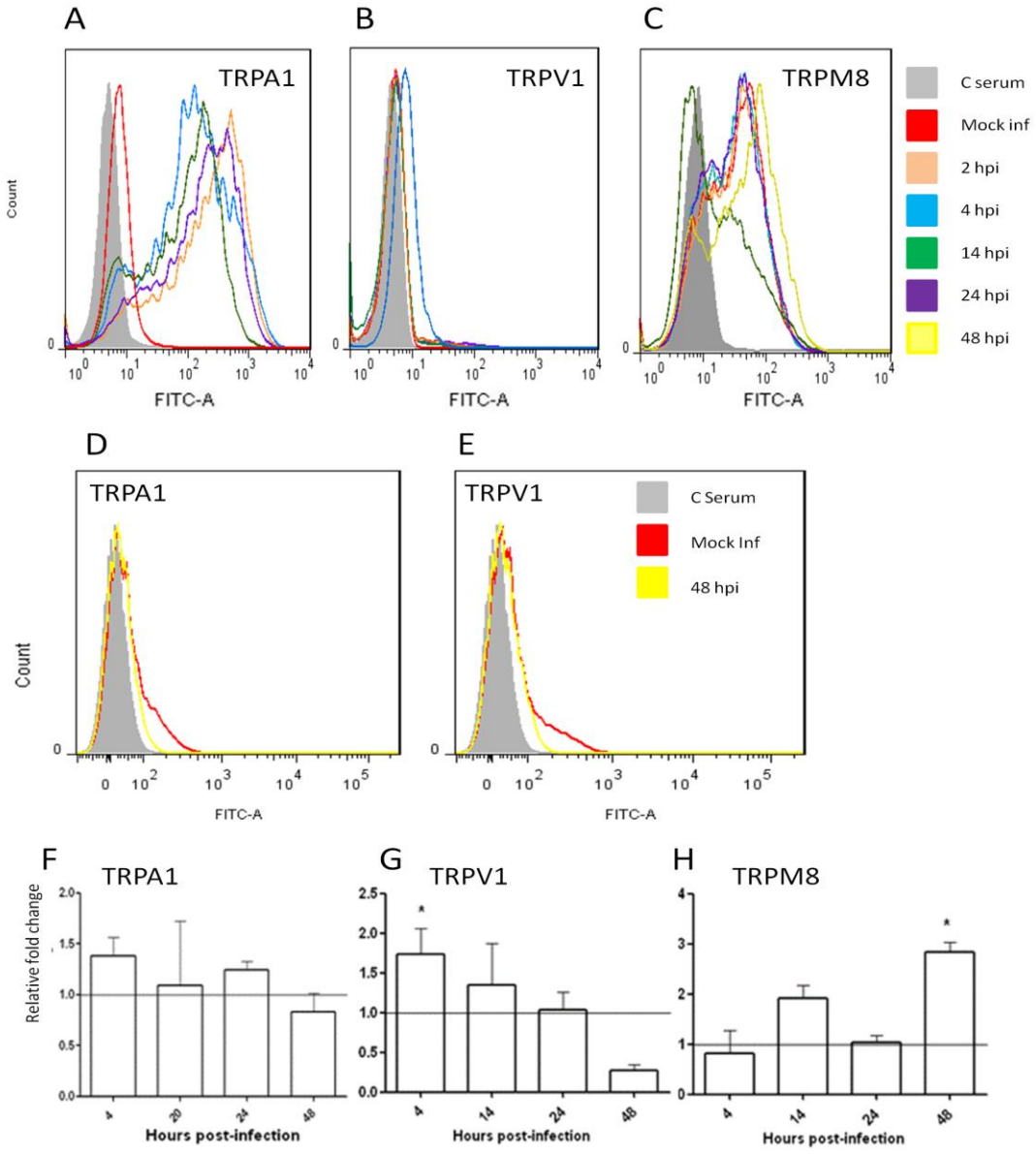
Quantitative RT-PCR was carried out using a QuantiTect Reverse Transcription Kit (Qiagen) for cDNA production and analyzed using a Mx3005P qRT-PCR machine (Agilent Technologies, USA). Taqman primers and probes for human TRPA1, TRPM8 and TRPV1 were obtained from Life Technologies (UK) and used according to the manufacturer's instructions. Relative copy numbers of the TRP receptors were normalized against ACTB and PPIA house-keeping genes (Roche, UK) as determined and validated by the Human Reference Gene Panel, 96 (Roche). cDNA prepared from either TRPA1 or TRPV1 transfected HEK cells was used to generate a standard curve. The copy number of HRV was quantified using HRV all subtypes (generic) genesig advanced kit (PrimerDesign, UK) according to manufacturer's instruction for a one step detection protocol. Absolute HRV copy numbers in infected cells were normalized against ACTB as supplied with the kit.

FIGURES



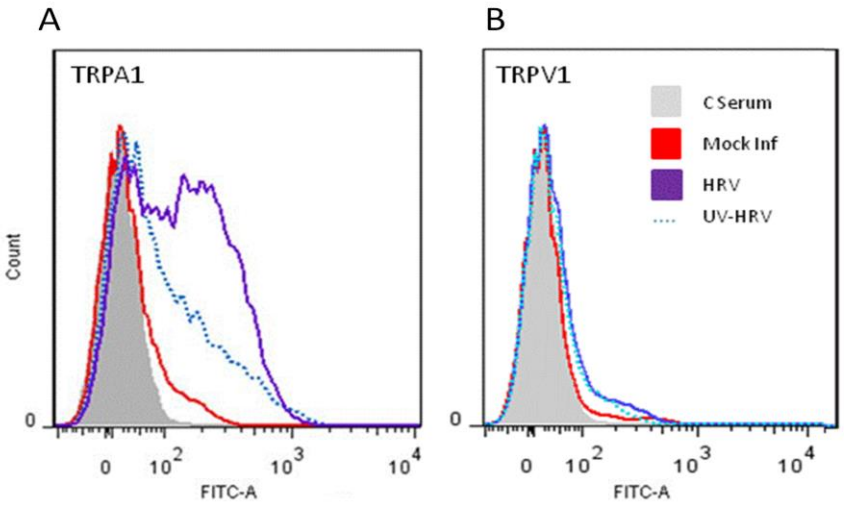
Supplement Fig 1

TRP expression is dependent on MOI used. dIMR-32 and HEK-transfected cells were stained for TRP channels at 24 hpi/seeding. Transfected HEK cells were used as positive controls for flow cytometry. (A) dIMR-32 cells were infected with different MOIs of 0.01, 0.1, 0.5 and 1 at 24 hpi and stained for TRPA1. HEK-TRPA1 was stained for TRPA1 as positive control. (B) HEK-TRPV1 and dIMR-32 cells stained for TRPV1 at 4 h post-seeding and (C) HEK-TRPM8 and dIMR-32 cells stained for TRPM8 at 48 h post-seeding.



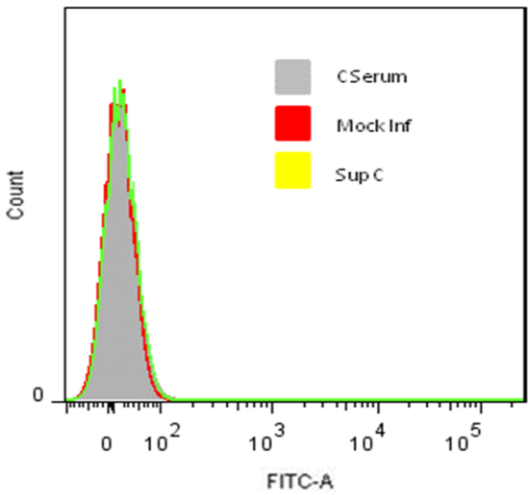
Supplement Fig 2

TRP expression is dependent on time after infection. TRP expression was determined by FACs analysis and qRT-PCR. The cells were infected using MOI of 1 up to 48 hpi and stained for (A) and (D) TRPA1, (B) and (E) TRPV1 and (C) TRPM8. The level of TRP channel mRNA following infection at a MOI of 1 was measured by qRT-PCR for (F) TRPA1 (G) TRPV1 and (H) TRPM8.



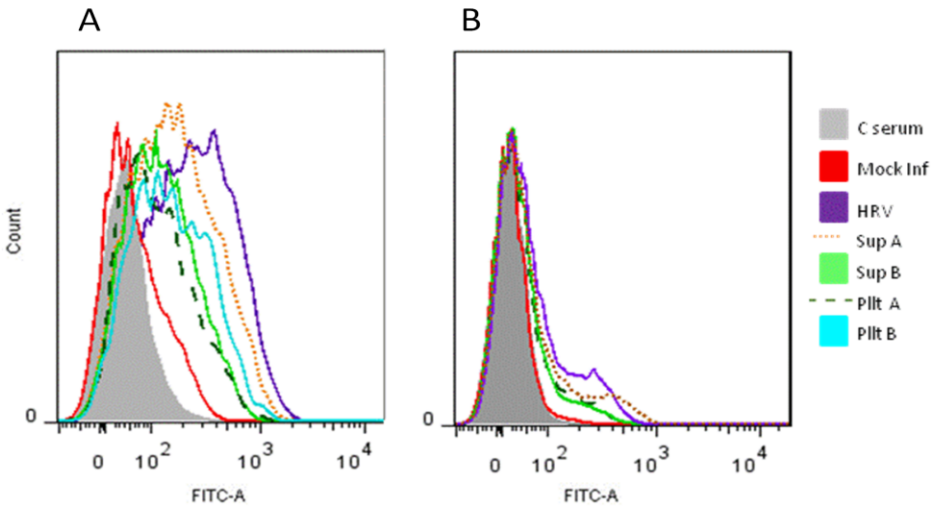
Supplement Fig 3

The expression of TRPA1 and TRPV1 are up-regulated by virus induced soluble factors. The cells were either infected at an MOI of 1 (HRV) or treated with UV-inactivated HRV (UV-HRV). TRP protein levels were measured by flow cytometry. (A) TRPA1 at 24 hpi and (B) TRPV1 at 4 hpi following infection or treatment with UV inactivated standard virus.



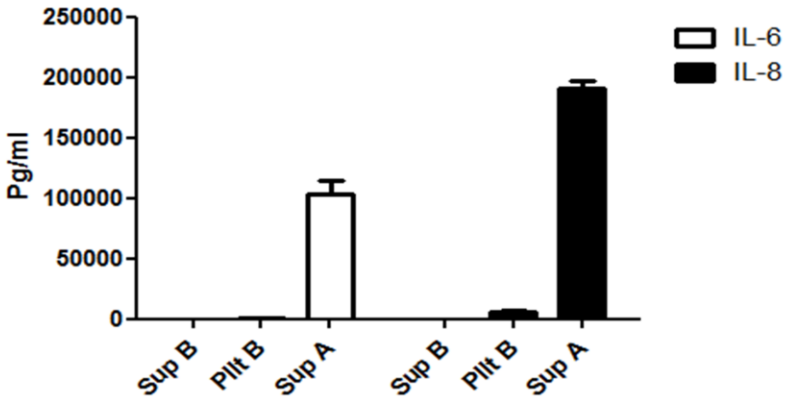
Supplement Fig 4

Treatment with HRV infected dIMR-32 supernatant (Sup C) does not increase the level of TRPA1 expression. Supernatant obtained from HRV infected dIMR-32 at 24 hpi (free from infectious virus particles) was added to fresh dIMR-32 cells and the level of TRPA1 was measured by flow cytometry at 24 h post treatment.



Supplement Fig 5

The expression of TRPA1 and TRPV1 is up-regulated by virus induced soluble factors. The cells were either infected at an MOI of 1 (HRV) or treated with UV-inactivated HRV (UV-HRV), virus free supernatant (Sup A), supernatant from uninfected Wi-38 cells (Sup B), UV inactivated pelleted virus (Pllt A) or non-UV inactivated pelleted virus (Pllt B) for (A) TRPA1 and (B) TRPV1.



Supplement Figure 6

Insignificant levels of IL-6 and IL-8 are found in pelleted virus preparations. IL-6 and IL-8 levels were examined in virus free supernatant (Sup A), supernatant from uninfected Wi-38 cells (Sup B), and in non-UV inactivated pelleted virus (Pllt B) preparations by multiplex assay according to the manufacturer's instructions by using Luminex 200 (Millipore, Germany).