**SUPPLEMENTARY MATERIAL**

RO0504985 is an Inhibitor of CMGC Kinase Proteins and has

Anti-Human Cytomegalovirus Activity

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**Compound treatment and infection of cells for screening.** Screening was based on previous genetic and compound screens performed by our laboratory ([Khan et al., 2017](#_ENREF_2); [Polachek et al., 2016](#_ENREF_3)). The Roche Kinase Inhibitor library (stock concentration of 3.33 mM of each compound in DMSO) was screened in duplicate. Twenty four hours before infection 2 × 103 HFF cells were seeded in each well of each Corning 384 plate. Unless stated otherwise, liquid was added to wells using a WellMate apparatus. At the time of infection, medium was removed with a suction manifold and 30 l of complete medium was added to each well. Compounds were added to the plate containing HFF cells using a 100 nl pin transfer on a liquid handling robot. Negative and positive controls (water+0.3% DMSO or heparan sulfate (5 g/ml) + 0.3% DMSO, respectively) were added to plates by hand (12 wells of each). Cells were then infected with HCMV strain AD169 (multiplicity of infection (MOI) of 1 plaque-forming unit (p.f.u.) per cell) in a total volume of 5l. Infection of cells at MOI 1 was the amount of virus found necessary to return robust, statistically relevant, differences in viral protein production when comparing positive and negative controls ([Khan et al., 2017](#_ENREF_2); [Polachek et al., 2016](#_ENREF_3)). The final concentration of compound in each well was 9.4 M. Infected cells were incubated for 72 hours at 37oC and then prepared for microscopy analysis.

**Preparation of screening plates for microscopy analysis.** Cell culture medium was removed from infected cells and replaced with 20 l Hoechst 33342 (SIGMA) diluted in PBS to a final concentration of 10g/ml. After incubation for 1 hour at 37oC, 20 l of Deep Red Cell Mask (Invitrogen) (diluted in PBS to a concentration of 5 g/ml) was added to each well. Cells were incubated for a further 5 min at 37oC. Cells were then fixed by removing PBS containing Hoechst and Cell Mask and adding 50l of 3.5%Formaldyhyde (SIGMA) in PBS to each well. After incubating at room temperature for 10 min, fixative was removed and 50l of PBS containing 0.5% TritonX-100 was added per well to permeablise cells. After 10 min incubation at room temperature, PBS containing detergent was removed, and cells were washed once with PBS. PBS was removed and replaced with 20 l MAb P207 recognizing pp28 (Virusys) (dilution 1:1000) and anti-mouse secondary antibody conjugated to fluorophore Alexa488 (Molecular Probes) (dilution 1:1000). Plates were incubated at 37oC for 1 hour. After incubation, PBS containing antibodies was removed and replaced with 50l of PBS. Plates were then analysed using automated microscopy for the presence of pp28 protein.

**Microscopy analysis of screening plates.** Infected cells stained with antibody to detect pp28 were imaged on an Image Express Micro (IXM) microscope (Molecular Devices) at 10x magnification to detect 3 wavelengths; 488 nm to detect antibody recognizing pp28, 568nm to detect Deep Red CellMask and 350 nm to detect Hoechst 33342 stain bound to nuclear DNA. Three images were captured from each wavelength in each well of the 384-well plate. The number of cells positive at all 3 wavelengths and percentage of pp28 positive cells in each well were determined using the Metamorph Multiwavelength Cell Scoring software (Molecular Devices). Typically, approximately 60% of cells were infected in wells treated with negative control, DMSO (data not shown).

**Analysis of screening results.** To assess the quality of data that could be returned from the screening protocol we calculated the Z’-factor ([Birmingham et al., 2009](#_ENREF_1); [Zhang et al., 1999](#_ENREF_4)) derived from the positive (heparan sulphate treated infected cells) and negative (DMSO treated infected cells) control wells. The screening controls returned Z’-factors of greater than or equal to 0.5, indicating a robust separation of difference in the data derived from positive and negative controls (data not shown). Thus, the screening protocol could be reliably used to screen the compound collection.

After screening of the compound collection, data were discarded from any well in which the number of cells stained with Hoechst 33342 fell below 2-fold of the mean of the number of cells in each well of the plate. The data from the remaining wells from each plate was converted to a z-score (the number of standard deviations from the mean of the data ([Birmingham et al., 2009](#_ENREF_1); [Zhang et al., 1999](#_ENREF_4))) and the average z-score from data in duplicate plates was determined. Images chosen at random were visually inspected throughout image capture and analysis to ensure raw data were consistent with z-scores.

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