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Project-1918Mice

Treatment protocol : Lung tissue from each animal were harvested and briefly rinsed in cold (4ºC) PBS. Following the RNALater (Ambion) protocol, tissue was cut into small chunks (<0.5cm in any single dimension) and placed immediately into a 10-20 volumes (w/v) (e.g. 100mg/ml) RNALater. After a 4ºC incubation overnight, samples were stored at -80ºC until processing. Lung tissue was removed from RNALater, washed in a small volume of Trizol, homogenized in 10-20 volumes (w/v) Trizol and stored at -80°C until RNA isolation.

RNA Extraction : All Trizol lysates were processed simultaneously: they were phase-separated, and RNA was isolated from the aqueous phase (diluted 2 fold with RLT buffer) using Qiagen RNeasy Mini columns and the manufacturer’s recommended protocol (Qiagen Inc., Valencia, CA). RNA quality was assessed on an Agilent 2100 Bioanalyzer using the nanochip format, and only intact RNA was used for microarray analyses.

Microarray profiling : Transcriptomics profiling was performed using Agilent-014868 Whole Mouse Genome Microarray 4x44K G4122F (Probe Name version).

Project-H7N9Human

Treatment protocol : Confluent monolayers of polarized Calu-3 achieving stable transepithelial resistance were infected apically at a MOI of 1. After a 1-hour incubation, monolayers were washed to remove nonadherent virus and 2 ml of MEM-BSA was added to both apical and basolateral reservoirs of cells and left for the duration of the experiment.

RNA Extraction protocol : All Trizol lysates were processed simultaneously: they were phase-separated, and RNA was isolated from the aqueous phase (diluted 2 fold with RLT buffer) using Qiagen RNeasy Mini columns and the manufacturer’s recommended protocol (Qiagen Inc., Valencia, CA). RNA quality was assessed on an Agilent 2100 Bioanalyzer using the nanochip format, and only intact RNA was used for microarray analyses.

Microarray profiling : Transcriptomics profiling was performed using Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 (Probe Name version).

Project-SARSHuman

Treatment protocol : Cells were seeded in 6-well plates (1 x 10e6 cells/well) two days prior to infection. Immediately preceding infection, cell monolayers were washed with fresh medium and inoculated with either SARS viruses (MOI = 2) or A/CA/04/2009 (MOI = 1) and subsequently incubated at 37°C for 40 minutes. Mock-infected controls were inoculated with culture medium only. Following the incubation, cell monolayers were washed 3X with 1X PBS and fresh medium was added to the wells prior to time 0.

RNA Extraction protocol : At 0, 12, 24, 36, 48, 60, 72, 84 and 96 hours post-infection (hpi) (SARS viruses) or 0, 6, 12, 18, 24, 36 and 48 hpi (H1N1) triplicate/quadruplicate wells of infected cells or mock infected were washed with 1X PBS and lysed directly with 1 ml of Trizol (Invitrogen) according to the manufacturer’s recommendation. The resulting lysates were stored at -80°C until further processing. All Trizol lysates were processed simultaneously: they were phase-separated, and RNA was isolated from the aqueous phase (diluted 2 fold with RLT buffer) using Qiagen RNeasy Mini columns and the manufacturer’s recommended protocol (Qiagen Inc., Valencia, CA). RNA quality was assessed on an Agilent 2100 Bioanalyzer using the nanochip format, and only intact RNA was used for quantitative PCR (qPCR) and microarray analyses.

Microarray profiling : Agilent-014850 Whole Human Genome Microarray 4x44K G4112F (Probe Name version)

Project-SARSMice

Treatment protocol : Lung tissue from each animal were harvested and briefly rinsed in cold (4ºC) PBS. Following the RNALater (Ambion) protocol, tissue was cut into small chunks (<0.5cm in any single dimension) and placed immediately into a 10-20 volumes (w/v) (e.g. 100mg/ml) RNALater. After a 4ºC incubation overnight, samples were stored at -80ºC until processing. Lung tissue was removed from RNALater, washed in a small volume of Trizol, homogenized in 10-20 volumes (w/v) Trizol and stored at -80°C until RNA isolation.

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Microarray profiling : Transcriptomics profiling was performed using Agilent-014868 Whole Mouse Genome Microarray 4x44K G4122F (Probe Name version).