

**Exacerbation of Established Pulmonary Fibrosis in a Murine Model by
Gammaherpesvirus**

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On-Line Data Supplement

Lung Function Measurements

Briefly, mice were anesthetized with ketamine and xylazine. A tracheostomy tube was inserted into the trachea and secured. Mice were placed on a mechanical ventilator in a sealed plethysmograph as part of the Pulmonary Maneuvers System (Buxco Electronics). Measurement of functional residual capacity (FRC) was determined by occluding the airway while the mouse attempts to breathe spontaneously. The pressure that the animal exerted and the change in the animal's thoracic volume were recorded allowing the calculation of functional residual capacity using Boyle's law. A quasistatic pressure/volume maneuver was performed allowing measurements of lung compliance (slope of the pressure volume curve between 0 and 10 cm H₂O pressure) and total lung capacity (TLC). Data for all maneuvers was analyzed using Biosystem XA software (Buxco, Electronics)

Virus Plaque Assay

3T12 cells (American Type Culture Collection) were cultured in DMEM with 4% fetal calf serum (FCS), harvested using trypsin digestion, and plated on 12 well plates at 8.33×10^4 cells per well. The cells were incubated overnight to confirm 70-80% confluence. Two lung lobes of infected mice were homogenized in 2ml DMEM with Complete® protease inhibitor cocktail (1 tablet/7 ml media, Roche, Mannheim, Germany) and Triton X-100 (7 μ l/7ml media, Sigma, St. Louis, MO) and centrifuged at 1500 rpm for 5 minutes at 4°C. Dilutions were made in DMEM from the supernatant and the pellet was discarded. 250 μ l of each dilution was placed on the 3T12s; the plates were rocked 10 times; then incubated at 37°C and rocked every 15 min for one hour. The

inoculum was not removed and an overlay of 0.75% carboxymethylcellulose and 2X MEM with 10% FCS was placed on cell monolayers. The plates were incubated at 37°C. On day 8 the overlay was removed and the cells were fixed and stained with 70% methanol and 0.35% methylene blue for plaque enumeration.