

Methods S1

Cell culture and virus infections

Primary MEFs were infected with MHV68-WT at a low (0.05) or high (5) MOI. Cells were infected for 1h, washed with PBS, then 8% CMEM was added. Bone marrow-derived macrophages (BMDMs) were generated by isolating bone marrow from 8-12 wk old mice from the femur and differentiation for 5d in DMEM with Glutamax (Life Technologies) containing 20% FBS and 30% L-supplement (BMM-Hi) in non-tissue culture treated plates. Cells were maintained in DMEM with Glutamax containing 10% FBS and 15% L-supplement (BMM-low) in non-tissue culture treated plates. BMDMs were infected with MHV68-eYFP at high MOI (10.0). At the indicated times post infection, cells were freeze-thawed and serial dilutions were titered as described for acute titers.

Pathogenesis

Mice were infected by intraperitoneal infection with 1000 PFU of MHV68 in 0.5 ml 10% CMEM, For acute titers, spleens were harvested from mice at 9 dpi and titered as described above. At 16 dpi, peritoneal exudate cells were isolated by injecting the peritoneum with 10 ml of 8% CMEM, followed by shaking the abdomen and then removing the media with a syringe. For latency and reactivation assays, spleens and peritoneal exudate cells were isolated and analyzed by the limiting dilution assays described above. For T cell co-culture assays, enriched T cells were added at a 5:1 or 25:1 ratio to the splenocytes or peritoneal exudate cells in the explant reactivation plates.

T Cell Transfer

Sts-1/2 dKO and C57BL/6 mice were infected as described above. Spleens were removed 28 dpi, homogenized in CMEM, red blood cell lysed, and filtered through a 100 μ M filter. T cells were isolated using positive selection with α CD90.2 microbeads (Miltenyi Biotech). Purity was analyzed by staining cells for CD3 and CD19 with flow cytometry, as described above. WT mice were anesthetized with 5% isoflurane via face mask, and 10^7 , 10^6 , and 10^5 purified Sts-1/2 dKO or C57BL/6 T cells were injected

retroorbitally in 5x C57BL/6 mice. 24 h later mice were infected with 1000 PFU WT MHV68 as described above. After 6 days, lungs were titered as described above. Bar represents geometric mean. $*=p<0.05$.