Rift Valley Fever Virus (RVFV; genus *Phlebovirus*, family *Bunyaviridae*) is an important zoonotic pathogen transmitted by mosquitoes that causes large outbreaks among ruminants and humans in Africa and the Arabian Peninsula. Human patients typically develop an acute febrile illness, which may be followed by fatal hemorrhagic fever, encephalitis or ocular disease (1;2). RVFV is known to be sensitive to the interferons (IFNs) (3;4), and an antibody response is critical for protection against the virus (5;6). In a rodent model, wild type RVFV infection causes acute death with fatal hepatic disease at 2 to 3 days post-infection (dpi), whereas infection with a mutant RVFV strain lacking NSm protein
expression results in delayed death with fatal neurological disease as well as hepatic failure (7).

This screen is designed to identify genes required for the control of RVFV infection in vivo. Since wild type RVFV is categorized as an enhanced animal biosafety level 3 (ABSL-3) containment agent, a mutagen-attenuated recombinant strain (arMP-12) that can be handled in ABSL-2 containment is used here (8). Preliminary experiments indicate that C57BL/6J mice injected intraperitoneally with 1x10⁵ pfu of arMP-12 control the infection with no signs of illness, but Ifnar⁻/⁻ and Stat1domino/domino mice succumb to death with high viremia and viral loads in the liver and spleen within 3 dpi (Figure 1). Mutagenized G3 mice that display severe sickness or death after intraperitoneal challenge with 1x10⁵ pfu of arMP-12 are identified as potential mutants in this screen. The Ex Vivo Macrophage Screen for Control of Viral Infection is also employed to discover components important for the control of RVFV infection, but utilizes thioglycolate-elicited peritoneal macrophages, thus saving the animal from infection-induced death.

Reagents and Solutions
In Vivo RVFV Susceptibility Screen

**Vero E6 medium**
Dulbecco’s modified eagle medium (DMEM) (GIBCO Invitrogen, Carlsbad, CA)
10% (v/v) heat-inactivated fetal bovine serum (FBS) (Atlanta Biologicals, Lawrenceville, GA)
100 IU/ml of penicillin and 100 μg/ml of streptomycin (GIBCO Invitrogen, Carlsbad, CA)

**Virus stock diluent**
Hank’s Balanced Salt Solution (HBSS) (HyClone, Logan, UT)
2% (v/v) FBS
100 IU/ml of penicillin and 100 μg/ml of streptomycin

**Tissue diluent**
HBSS
5% (v/v) FBS
200 IU/ml of penicillin and 200 μg/ml of streptomycin

**Plaque assay medium**
Modified Eagle Medium (MEM) (GIBCO Invitrogen, Carlsbad, CA)
0.6% (w/v) tragacanth gum (MP Biomedicals, Solon, OH)
2.5% (v/v) FBS
5% (w/v) tryptose phosphate broth (TBP) (BD Biosciences, San Jose, CA)

**Cell fixation solution**
Phosphate-buffered saline (PBS)
10% (w/w) formaldeyde

**Cell staining solution**
distilled H₂O
0.25% (w/v) crystal violet
2.5% (v/v) ethanol in distilled water

**Method**
In Vivo RVFV Susceptibility Screen

RVFV stock (arMP-12) preparation
1. Infect VeroE6 cells with a seed stock of arMP-12.
2. Collect culture supernatant at 2 to 3 dpi when most cells show severe cytopathic effect (CPE).
3. Centrifuge supernatant at 1500g for 5 min to remove cell debris.
4. Store clear supernatant at -80°C until use for titration and infection.

RVFV titration
5. Prepare confluent Vero E6 cells in 24- or 6-well plates. For each virus dilution, plan to infect triplicate wells.
6. Serially dilute (from 1:10 to 1:10^7) RVFV stock in Vero E6 medium.
7. Infect Vero E6 cells in triplicate wells with each virus dilution. (Use 100 ?l for each well of a 24-well plate; 400 ?l for each well of a 6-well plate). Incubate at 37°C/5%CO_2 in a humidified incubator for 1 hour.
8. Wash infected cells with PBS three times.
9. Add plaque assay medium to each well and incubate at 37°C/5%CO_2 in a humidified incubator for 4 days.
10. At 4 dpi, remove plaque assay medium and fix cells with cell fixation solution for at least 10 minutes at room temperature (RT).
11. Remove cell fixation solution and stain cells with cell staining solution for 5 to 10 min.
12. Remove cell staining solution and wash cells with water.
13. Count plaques and calculate titers according to dilution factors.

In vivo screening
14. Prepare the virus inoculum (5x10^5 pfu/ml) by diluting the titrated virus stock with virus stock diluent.
15. Challenge each G3 mouse intraperitoneally with 200 ?l of the inoculum (1x10^5 pfu/mouse). Save leftover inoculum for re-titration.
16. Check mice daily for sickness and death up to 2 to 3 weeks.
17. Once affected mice are identified, collect blood, liver, spleen and brain from the mice.
18. Make 10% (w/v) tissue homogenates in tissue diluent and determine virus titers as described in

Mutagenetix (http://mutagenetix.utsouthwestern.edu:80)
In Vivo RVFV Susceptibility Screen

RVFV titration (steps 5-13).
19. Check for RVFV susceptibility in siblings of affected mice.
20. Fix and map mutations using general genetic mapping protocol.

Critical Parameters and Troubleshooting

Leftover inoculum from step 15 may be re-titrated in case unexpected results are observed after infection, such as widespread death of wild type control mice.

Alleles Identified

domino
macro-2
maladaptive
tortellini