

Supplementary Figure S1. Viral burst sizes of KM100 in TUBO cells that were either treated with KM100 alone or in combination with 1 μ M or 5 μ M MTX. TUBO cells were plated in 12-well plates and the next day infected with KM100 at an MOI of 10. After 1 hour of infection wells were washed with PBS and medium with or without MTX was applied. Infected cells were incubated for 48 hours before samples were harvested for viral titers. The average and standard deviations are from three independent experiments each run with three biological replicates.

Supplementary Figure S2. Efficacy of KM100 and/or 1 μ M MTX in BALB/c mice bearing subcutaneous TUBO tumors. A total of 5×10^5 TUBO cells were implanted into BALB/c mice by subcutaneous injection into the left flank. Palpable tumors were treated with 2×10^7 total pfu of KM100 and/or 1 μ M MTX (1.3 mg/kg). Tumors were measured every three days for 40 days or until the tumors reached end point. (A) Kaplan-Meier estimates of survival of BALB/c mice bearing subcutaneous tumors after different treatments. (B) The fold change in tumor volume following various treatments.

Supplementary figure S3. Tumor infiltrating immune cell population in subcutaneous TUBO tumors. Tumors were treated with three doses of KM100, 5 μ M MTX or KM100+5 μ M MTX. Seven and ten days after treatment the tumors were isolated and CD45⁺ immune cells isolated using magnetic bead positive selection. Samples were stained for the surface markers Gr1, CD11c, and F4/80. The average and standard deviation of cell numbers were generated from 5 mice per each treatment group.