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Supplemental information

Oncolytic herpes virus G47Δ injected into tongue cancer swiftly traffics in lymphatics and suppresses metastasis

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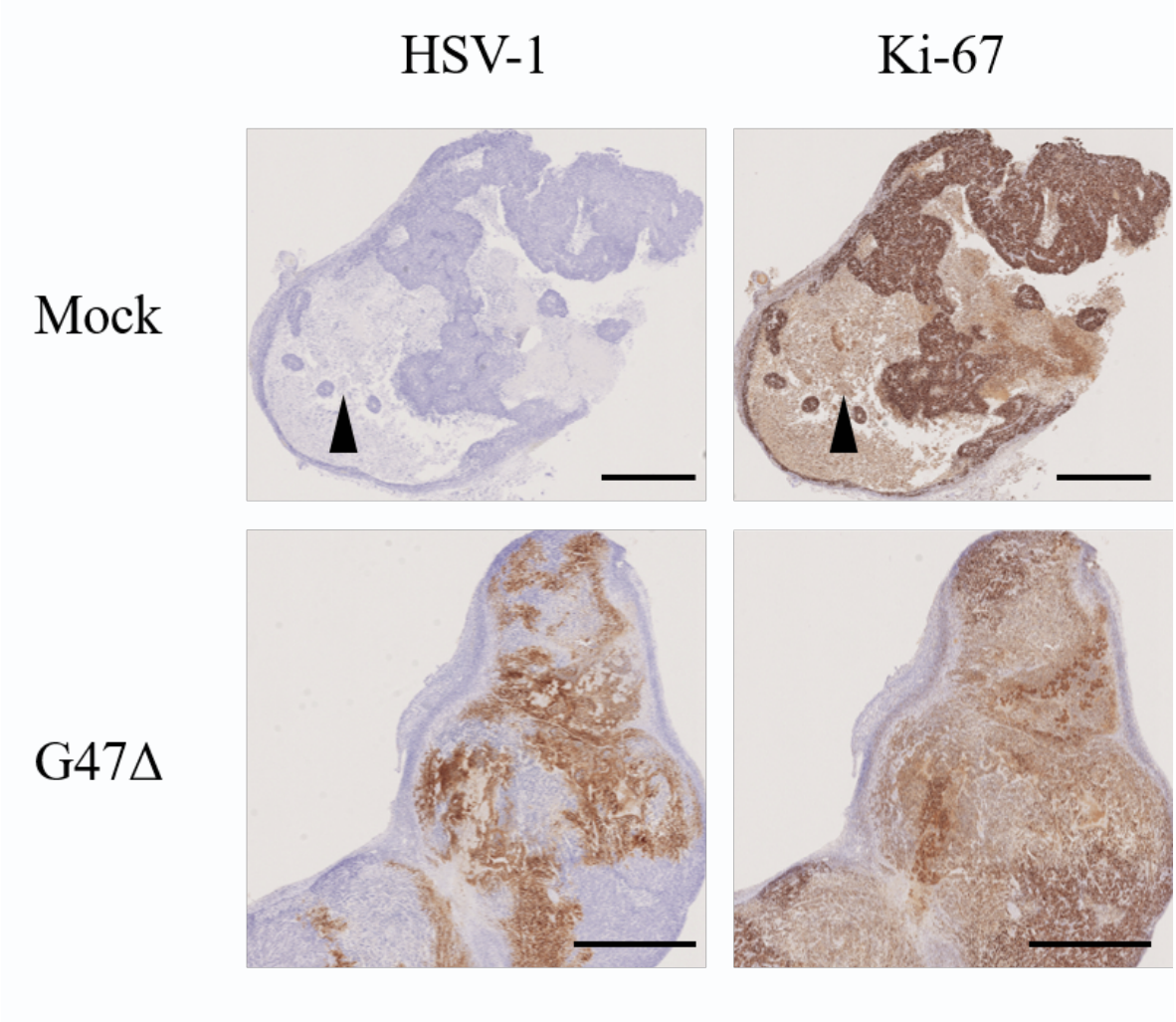
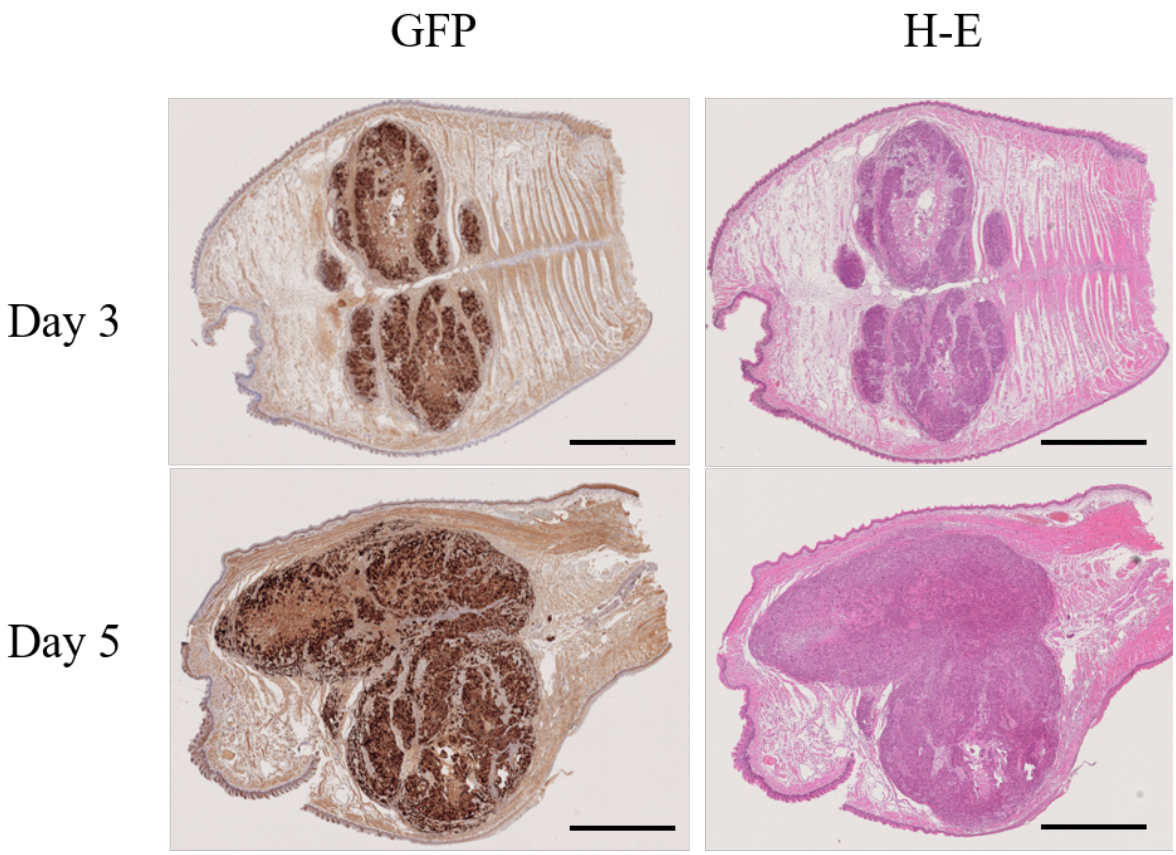


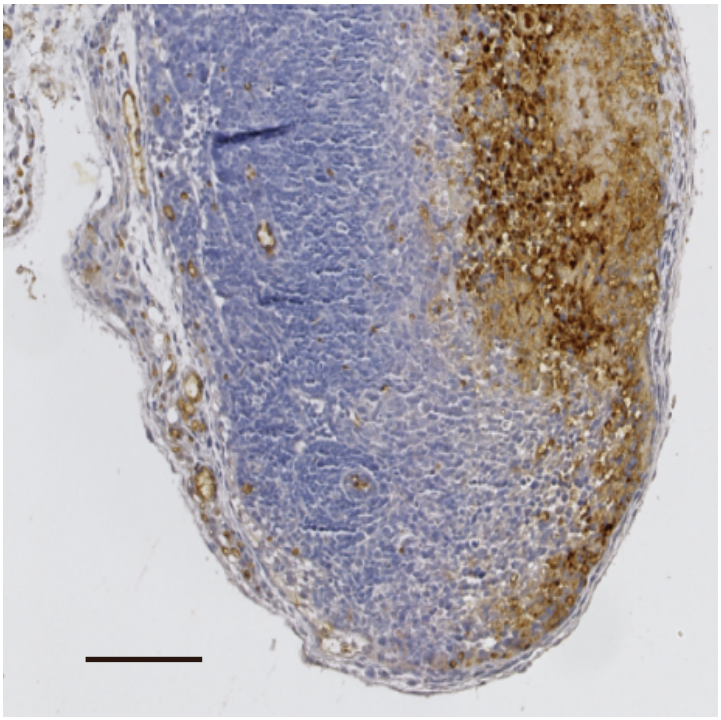
Supplementary Figure 1.

Representative images of subcutaneous SAS tumors immunostained for HSV-1 or Ki-67. Established subcutaneous SAS tumors were inoculated with G47Δ (1×10^6 pfu) or mock on days 0 and 3. On day 15, tumors were extracted and used for HSV-1 or Ki-67 immunohistochemistry. In mock-treated tumors, viable tumor cells show strong immunostaining for Ki-67, except for the area with necrosis (black arrowhead). By contrast, in G47Δ- treated tumors, the rate of Ki-67 positivity of tumor cells drastically decreased in the areas with high HSV-1 positivity. Scale Bar, 1 mm.



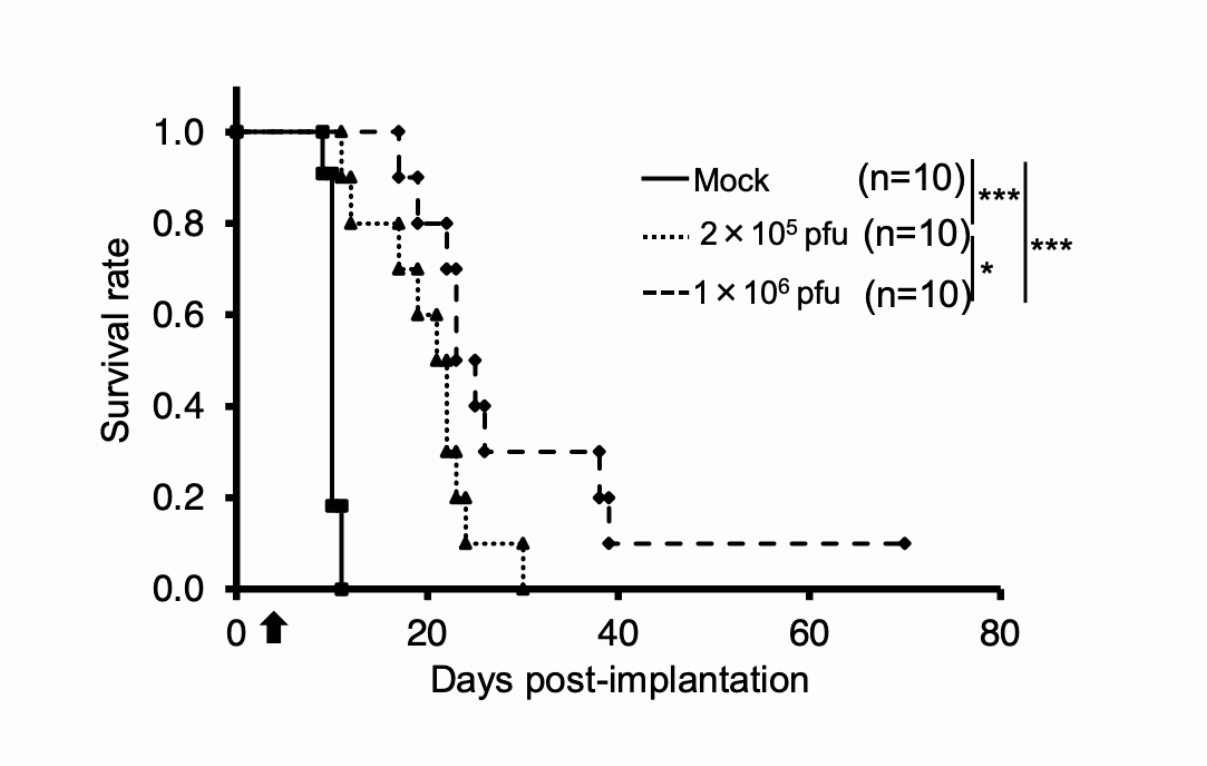
Supplementary Figure 2.

Representative images of immunostaining for GFP and H&E staining of SAS-GFP tumors in the orthotopic tongue cancer model. Three days or 5 days after implanting SAS-GFP cells (1×10^6) in the tongue of athymic mice, the tongues were excised for histological analysis. The tumor is well established at day 3 and grows rapidly to occupy a large portion of the tongue by day 5. Scale Bar, 1 mm.



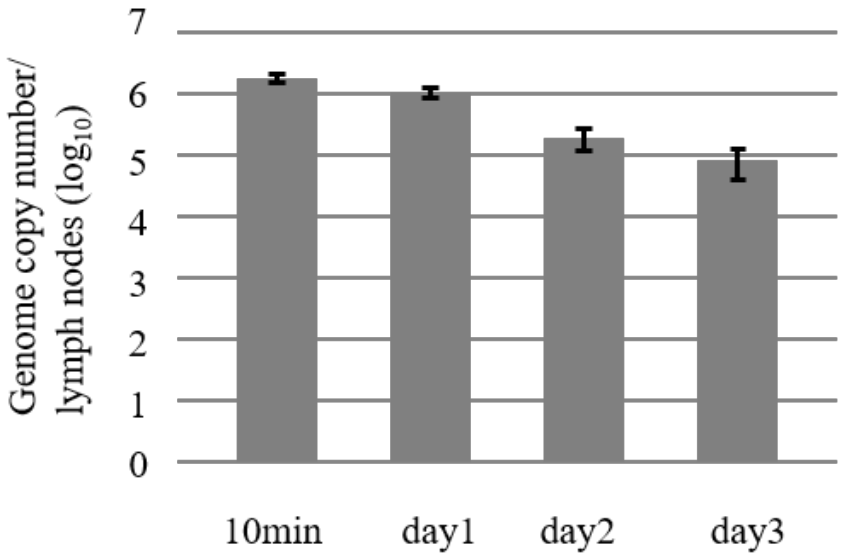
Supplementary Figure 3.

A representative image of HSV-1 immunostaining of a cervical lymph node in the HSC-3 tongue cancer model. HSC-3 cells (1×10^6) were implanted in the tongues of athymic mice. After 7 days, G47Δ was intratumorally inoculated at a dose of 1×10^6 pfu. The cervical lymph nodes were resected 3 days after virus inoculation. HSV-1 immunostaining revealed that G47Δ infection was evident in the metastatic lymph nodes. Scale Bar, 100 μm.



Supplementary Figure 4.

In vivo evaluation of intratumoral G47Δ treatment in the SCCVII orthotopic tongue cancer model. SCCVII (2×10^5) cells were implanted in the tongues of C3H/He mice. After 3 days, G47Δ was intratumorally inoculated at a dose of 2×10^5 pfu or 1×10^6 pfu. Intratumoral G47Δ treatment significantly prolonged the survival in a dose dependent manner. Arrow indicates the timing of G47Δ injection. *, $P < 0.05$; ***, $P < 0.001$ (Log-rank test).



Supplementary Figure 5.

Viral DNA copy numbers in the cervical lymph nodes after G47Δ injections to the primary tongue tumors in immunocompetent mice. KLN205-MUC1 cells (2×10^5) were implanted in the tongues of DBA/2 mice. After 7 days, G47Δ was intratumorally inoculated at a dose of 1×10^6 pfu. The cervical lymph nodes were removed 10 min, 1, 2, or 3 days after virus inoculation, and G47Δ DNA copy numbers were quantified by quantitative real-time PCR. The copy numbers of G47Δ DNA in the lymph nodes gradually decreased with time. N = 4 at each time point. Bars, ±SEM.