

Supplemental Material

Primer	Sequence
tetR-5	5'-CTCGAGATGTCCAGACTGGACAAGAGCAA-3'
tetR-3	5'-GGATCCTCAAGAGCCAGACTCACATTTTCAGCTGC-3'
tetO-mRFP-5	5'-GTCGACT <u>CCCTATCAGTGATAGAGA</u> AATGGTGAAGCGTGAGAAAAATGTCATC-3'
mRFP-3	5'-TTAATTAATTAGGCGCCGGTGGAGT-3'
tetO-mTyr-5	5'-GTCGACTCCCTATCAGTGATAGAGAATGTTCTTGGCTGTTTTGTATTGCCTTC-3'
mTyr-3	5'-TTAATTAATCACAGATGGCTCTGATACAGC-3'
Promoter	Sequence
P7.5	5'- TCACTAATTCCAAACCCACCCGCTTTTTATAGTAAGTTTTTCACCCATAAATAATAAATA CAATAATTAATTTCTCGTAAAAGTAGAAAATATATTCTAATTTATTGCACGGTAAGGAAGT AGATCATAA-3'
SEL	5'-AAAAATTGAAATTTTATTTTTTTTTTTTTTTGGGAATATAAATA-3'
SL	5'-TTTTTTTTTTTTTTTTTTTTTTGGCATATAAATA-3'

Table S1. Primer and promoter sequences.

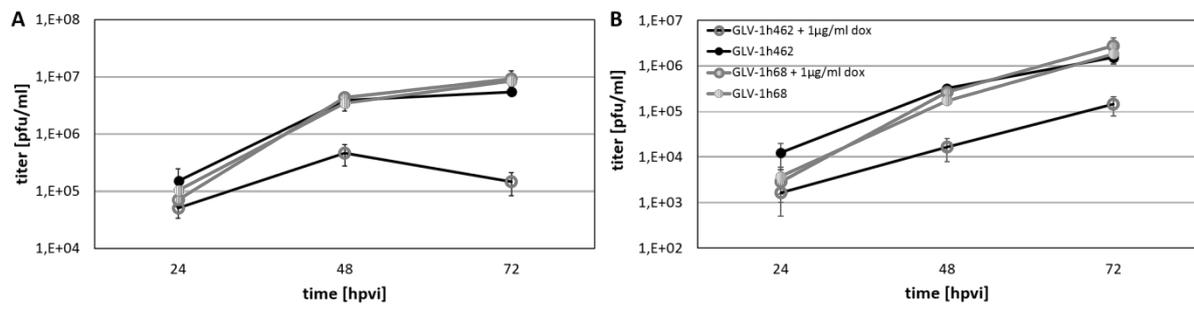


Figure S1: Viral replication assay. Replication of the control viruses GLV-1h68 and GLV-1h462 in absence or presence of dox (1µg/ml) over time in A549 (A) and PANC-1 (B) cancer cells.

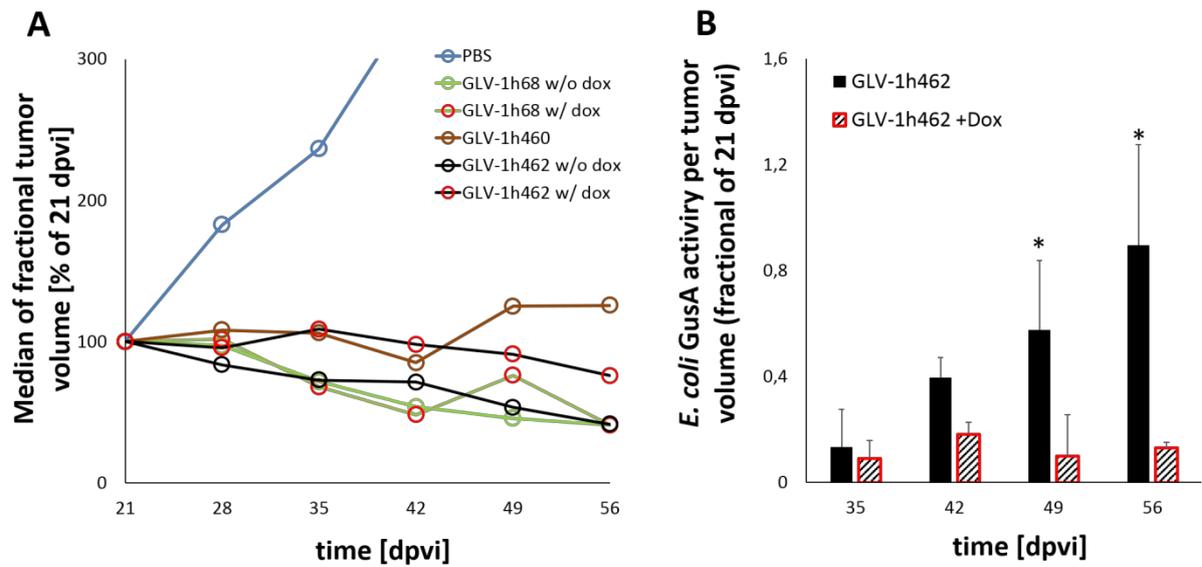


Figure S2: Tumor growth curve in athymic nude mice bearing A549 xenografts. A) Tumor size measurements represented as median fractional tumor volume at 21 dpvi (3 d post induction). At each time point n = 5-8. B) Fractional *Escherichia coli* β -glucuronidase activity of GusA-activity at 21 dpvi normalized by tumor volume. Significantly higher GusA activity of uninduced non-melanogenic GLV-1h462 infected blood serum samples at 49 and 56 dpvi compared to blood serum samples of induced melanin synthesizing GLV-1h462 colonized A549 tumor bearing mice (*; p < 0.05).

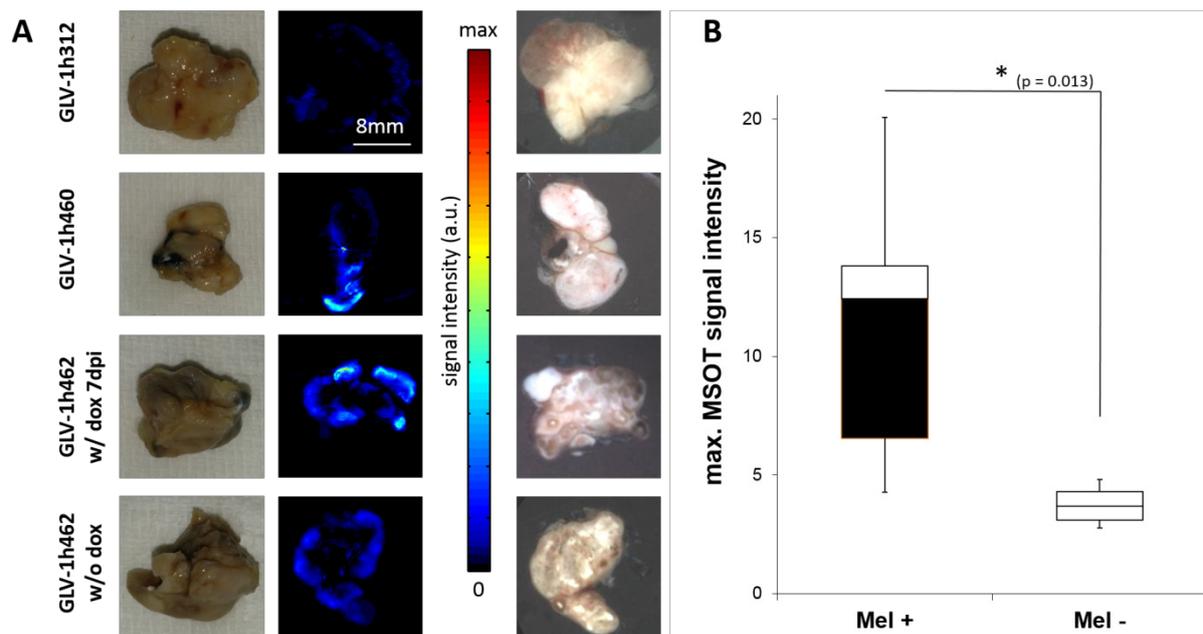


Figure S3: MSOT analysis of rVACV infected PANC-1 xenografts. A) Excised tumors colonized by the non-melanogenic rVACV GLV-1h312 (upper row), the constitutively melanin synthesizing GLV-1h460 (upper-middle row), the induced melanin producing GLV-1h462 (lower-middle row) and the un-induced non melanogenic GLV-1h462 (lower row). The same tumors are presented as photographic images (left), heat maps of MSOT signal intensities (middle) and cryosections (right). The orientation is not identical between the images. B) Quantitative analysis of the maximal MSOT signal intensities of PANC-1 tumors infected with melanogenic vaccinia viruses (GLV-1h460 and induced GLV-1h462 infected tumors) and not melanin producing rVACV colonized tumors (PBS, GLV-1h312 and uninduced GLV-1h462 infected tumors). The max. MSOT signal intensity of rVACV infected melanin producing tumors was significantly higher compared to non melanogenic rVACV colonized tumors ($p < 0.05$).