

Additional File 1

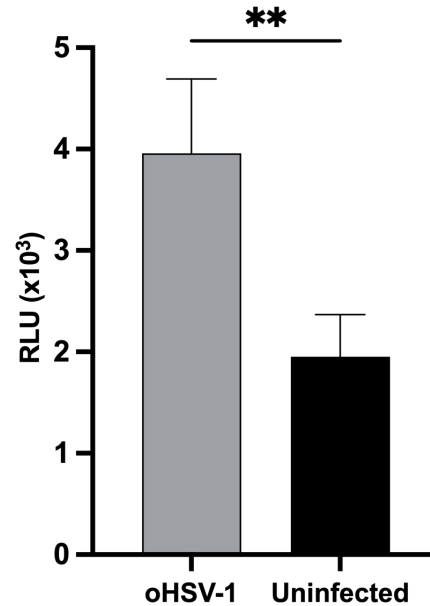
Antibody Specificity	CELL EXPRESSION	SOURCE	(CLONE) ^A	ANTIGEN RETRIEVAL ^B	WORKING DILUTION
CD3	T cells	Thermofisher (MA5-14524)	Rb (<i>SP7</i>)	E (20 min)	1/150
CD4	T-helper cells	Abcam (Ab183685)	Rb (<i>EPR19514</i>)	TC (20 min)	1/1000
CD8	Cytotoxic T-cells	Abcam (Ab209775)	Rb (<i>EPR20305</i>)	E (20 min)	1/1000
CD11b	Macrophages, Granulocytes and Dendritic cells	Abcam (Ab133357)	Rb (<i>EPR1344</i>)	E (10 min)	1/20000
FOXP3	Regulatory T-cells	Thermofisher (14-5773-82)	Rat (<i>FJK-165</i>)	TC (20 min)	1/100
GFAP	Astroglia cells	Genetex (GTX108711)	Rb	TC (20 min)	1/2000
Ki67	Proliferating cells	Abcam (Ab16667)	Rb (<i>SP6</i>)	TC (20 min)	1/100
MHC-II	Antigen presenting cells and CIITA-transfected tumor cells	Thermofisher (14-5321-82)	Rat (<i>M5/114.15.2</i>)	E (10 min)	1/100
Nestin	Neuro-epithelial stem cells, Activated Astroglia, GBM	Abcam (Ab221660)	Rb (<i>EPR22023</i>)	TC (20 min)	1/4000
Synaptophysin	Neurons	Abcam (Ab32127)	Rb (<i>YE269</i>)	TC (20 min)	1/6000
IBA-1	Pan Microglia and Macrophages	Abcam (Ab178847)	Rb (<i>EPR16589</i>)	E (10 min)	1/200
CD68	Macrophages and Monocytes	Abcam (Ab283654)	Rb (<i>EPR23917-164</i>)	E (10 min)	1/200
TIM3	Exhausted T-cells, tumor cells	Abcam (Ab241332)	Rb (<i>EPR22241</i>)	E (10 min)	1/300
PD1	Exhausted T-cells, tumor cells	Abcam (Ab214421)	Rb (<i>EPR20665</i>)	E (10 min)	1/300
HSV1	HSV-1 infected cells	Abcam (Ab9533)	Rb	TC (20 min)	1/100

^ARb,rabbit

^BE, EDTA buffer pH 8.0; TC, citrate buffer pH 6.0; in parenthesis, incubation time.

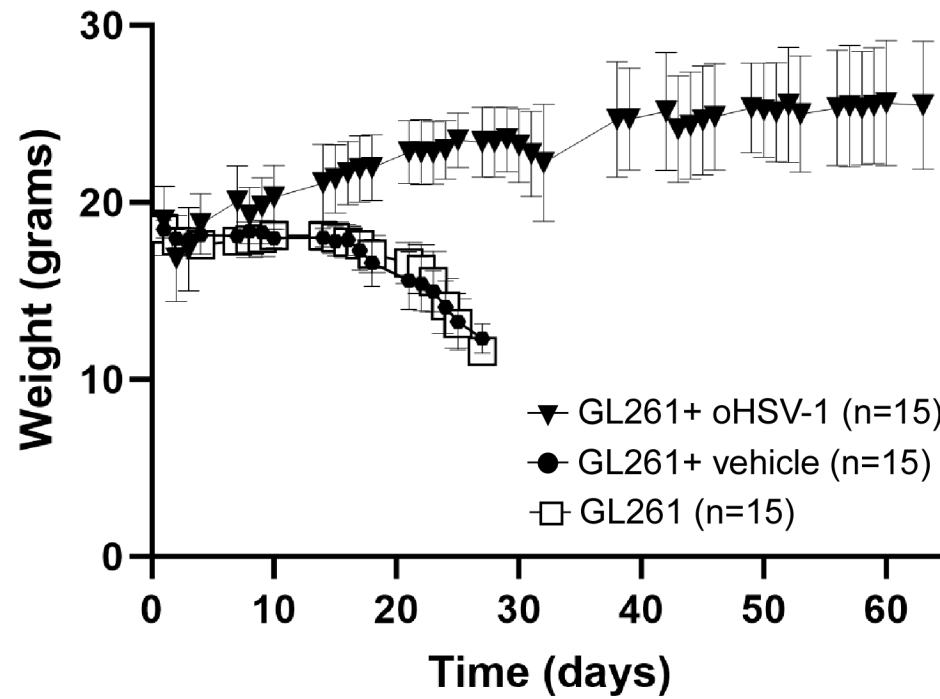
Additional File 1 Antibodies used for histopathological characterization of tumor sections

Additional File 2



Additional File 2. oHSV-1 induces extracellular ATP release in GL261 glioblastoma cells. Cells were seeded in a white, clear-bottom 96-well plate (5×10^3 cells/well), infected with oHSV-1 ($MOI=5$) for 1 hour in serum-free DMEM medium, then maintained in DMEM medium supplemented with 2% FBS. Three days post-infection, extracellular ATP release was determined using the RealTime-Glo™ Extracellular ATP Assay (Promega), which yields a luminescence signal that is proportional to the amount of ATP and was measured using a Varioskan™ LUX multimode microplate reader (ThermoScientific). Uninfected GL261 cells, seeded and cultured in the same conditions for 3 days, were assayed in a similar way. The experiment was performed in 4 replicates and the difference in luminescence between uninfected and oHSV-1-infected GL261 cells was evaluated by Student's t-test. $**P=0.003$. Error bars represent standard deviation. Values on the y axis indicate real luminescence units (RLU) as measured by the instrument.

Additional File 3



Additional File 3. Mean weight of oHSV-1- treated mice compared to control groups. Mice were weighed daily, and their weights are averaged over time. oHSV-1 intratumoral treatment (black triangles) compared to PBS (vehicle, black circles) and to parental GL261 (white squares).