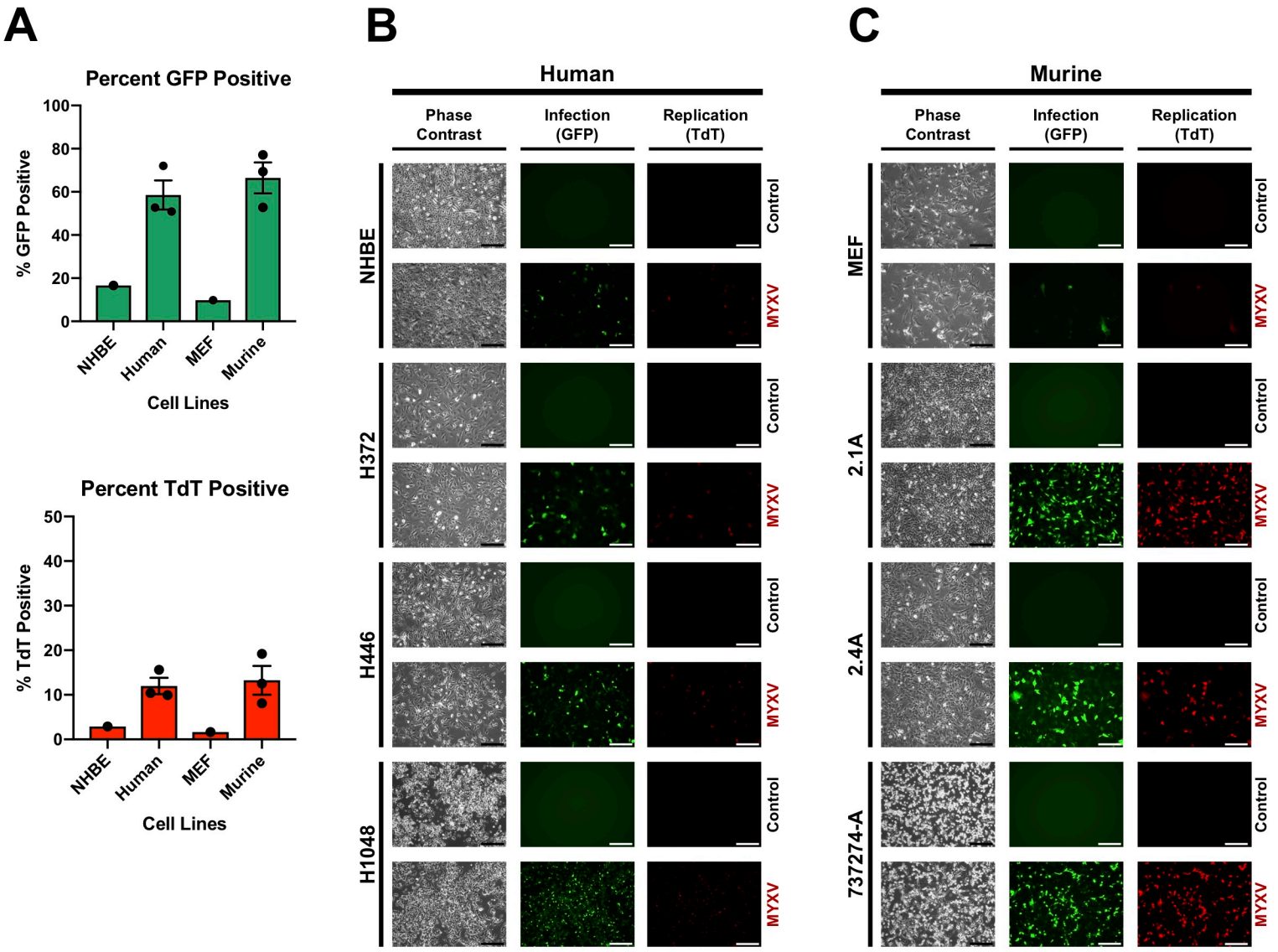

Figure S1. Quantification of positive populations determined by flow cytometry and corresponding fluorescence microscopy following MYXV infection.

Figure S1. Quantification of positive populations determined by flow cytometry and corresponding fluorescence microscopy following MYXV infection. (**A**) Quantification of cells positive for infection (GFP) and replication (TdT) determined by flow cytometry following 18 hour infection with 1 MOI (vMyx-GFP-TdT). Plot showing the average + SD with individual data points for the 3 human SCLC cell lines (H372, H446, and H1048) and 3 murine SCLC cell lines (2.1A, 2.4A, and 737274-A) compared to control cell lines NHBE and MEF. (**B & C**) Fluorescence microscopy of human cell lines (**B**) and murine cell lines (**C**) following 18 hour infection with 1 MOI (vMyx-GFP-TdT). Quantification from flow cytometry data correlates with the expected statistical probability based on a Poisson distribution that 63.2% of cells will observe viral attachment when infected at 1 MOI.

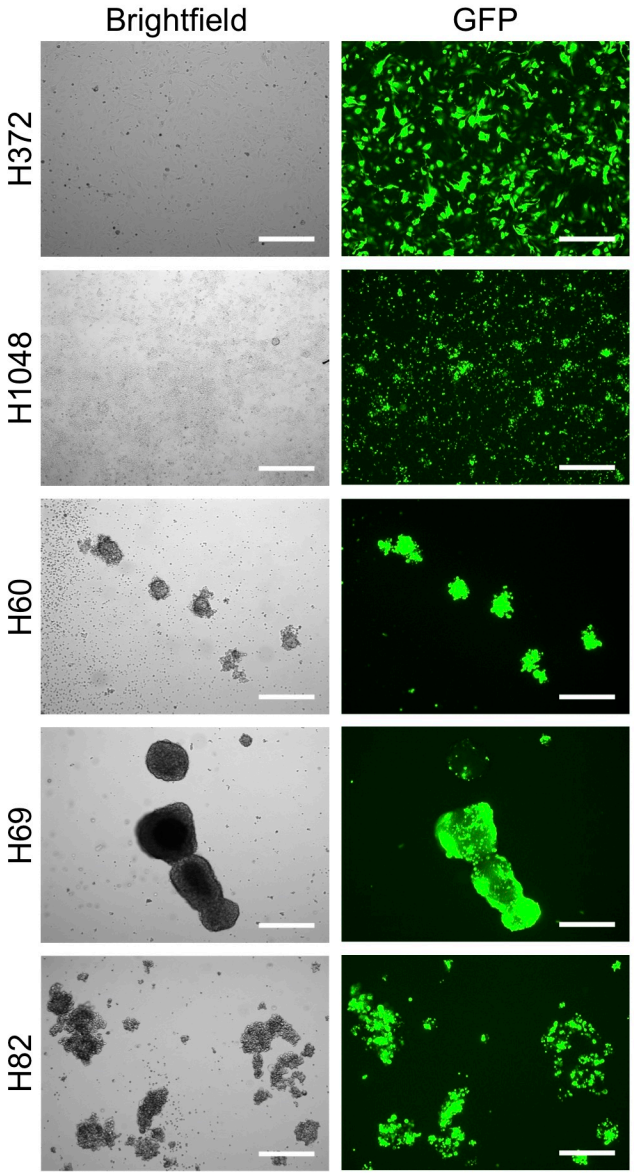

Figure S2. Human SCLC cell lines are permissive to vMyx-M135KO-GFP infection.

Figure S2. Human SCLC cell lines are permissive to vMyx-M135KO-GFP infection. Panel of 5 human SCLC lines infected with vMyx-M135KO-GFP at 10 MOI demonstrating infection (GFP) at 48 hours, scale bar = 50 um.

SCLC case	1	2	3	4	5	6	7	8	9	10	11	12	13
CD45 score	0	0	0	0	2	2	0	0	0	0	1	1	0
CD56 (Tumor)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
CD56 (NK cell)	0	0	0	0	0	0	rare	0	0	0	0	0	0
CD68 (Stroma)	1	rare	1	1	1	2	1	1	3	2	3	3	1
CD68 (Intratumoral)	1	0	0	0	1	1	1	1	2	1	1	1	1

SCLC case	14	15	16	17	18	19	20	21	22	23	24	25	26
CD45 score	1	0	0	0	0	0	0	3	2	1	0	0	0
CD56 (Tumor)	yes	yes	yes	yes	yes	faint	no	no	no	no	n/a	n/a	n/a
CD56 (NK cell)	0	0	0	0	0	0	0	1	1	rare	n/a	n/a	n/a
CD68 (Stroma)	2	2	1	1	1	2	0	1	3	2	n/a	n/a	n/a
CD68 (Intratumoral)	1	1	1	0	1	1	0	1	1	1	n/a	n/a	n/a

Tumor CD56 Immunostaining	
yes	Tumor CD56 positive
no	Tumor CD56 negative
n/a	not available

Immunostaining Scoring	
0	negative
1	mild
2	moderate
3	strong
n/a	not available

Figure S3. SCLC patient TMA immunostaining profiles.

Figure S3. SCLC patient TMA immunostaining profiles. Tissue microarray (TMA) data from 26 SCLC patients, samples were stained for CD45 (Abcam cat. # ab10558), CD56 (MRQ-42, Roche cat. # 760-4596), and CD68 (KP-1, Roche cat. # 760-4596). Staining utilized the Ventana Discovery XT automated platform and scoring performed by a board certified pathologist using the a scale 0-3 as defined in the experimental methods.

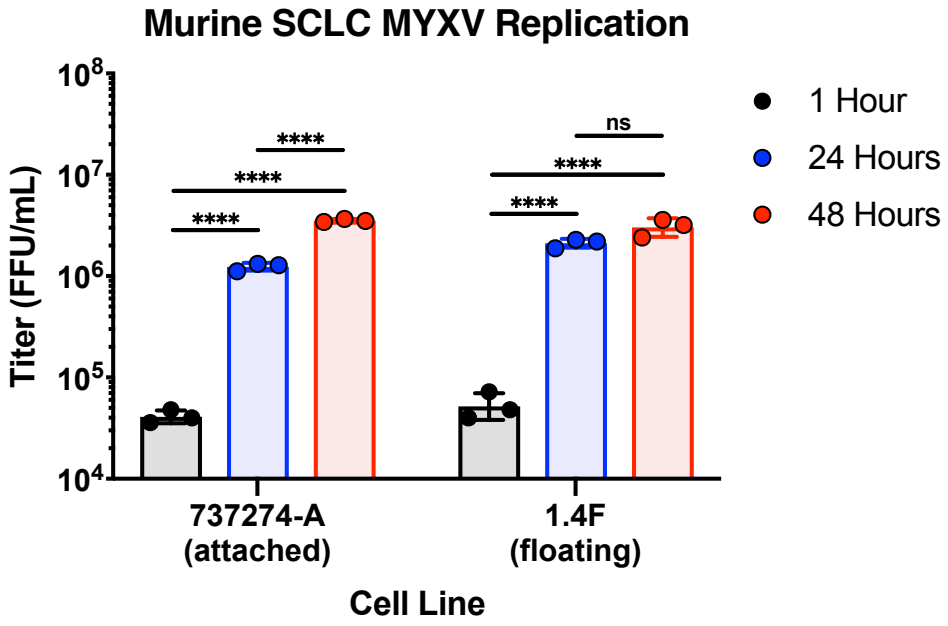

Figure S4. Replication and generation of infectious viral progeny in murine SCLC cell lines at 24 hours and 48 hours post infection (vMyx-GFP-TdT).

Figure S4. Replication and generation of infectious viral progeny in murine SCLC cell lines at 24 hours and 48 hours post infection (vMyx-GFP-TdT). Replication of MYXV in additional murine SCLC cells lines illustrated by the formation of viral progeny 24 hours and 48 hours post vMyx-GFP-TdT infection, results results are representative of 3 replicates per group, bar showing mean ± SD with all data points. ****P<0.0001, not significant (ns), by 1-way ANOVA and Tukey's multiple comparison test.

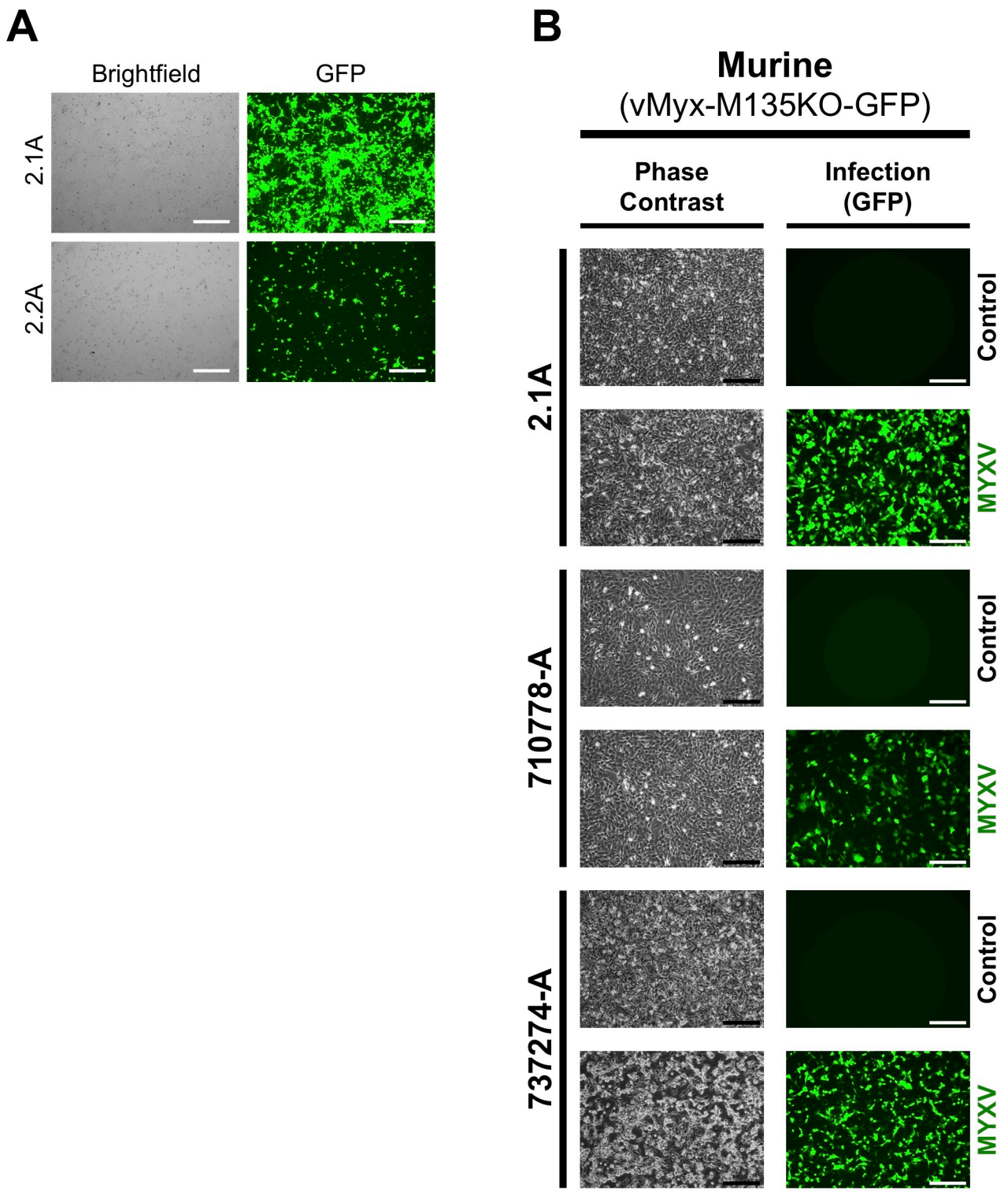

Figure S5. Murine SCLC cell lines are permissive to vMyx-M135KO-GFP infection.

Figure S5. Murine SCLC cell lines are permissive to vMyx-M135KO-GFP infection. (**A**) Murine SCLC cell lines infected with vMyx-M135KO-GFP at 10 MOI demonstrating infection (GFP) at 48 hours, scale bar = 50 um. (**B**) Murine SCLC cell lines infected with vMyx-M135KO-GFP at 10 MOI demonstrating infection (GFP) at 18 hours, scale bar = 250um.

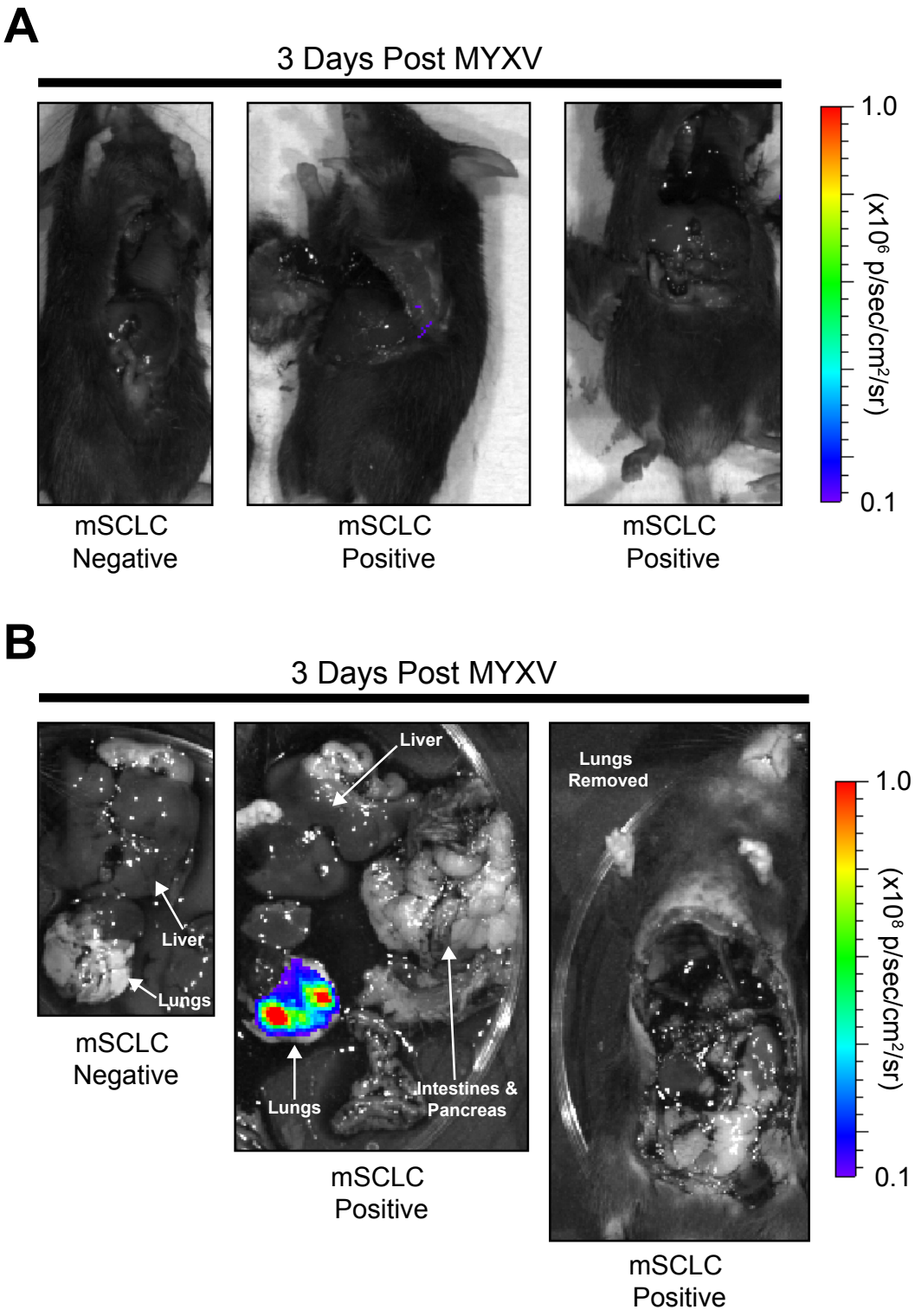

Figure S6. MYXV does not infect extrapulmonary organs and is rapidly cleared in immunocompetent conditional *p53*$^{-/-}$/*Rb1*$^{-/-}$/*p130*$^{-/-}$ SCLC GEMM.

Figure S6. MYXV does not infect extrapulmonary organs and is rapidly cleared in immunocompetent conditional *p53$^{-/-}$/Rb1$^{-/-}$/p130$^{-/-}$* SCLC GEMM. (**A**) Extrapulmonary organ bioluminescence imaging of conditional *p53$^{-/-}$/Rb1$^{-/-}$/p130$^{-/-}$* (SCLC Positive) and mouse lacking *p53/Rb1/p130* knockouts (SCLC negative) 3 days post infection with MYXV expressing firefly luciferase under poxvirus early/late promoter (vMyx-FLuc). Resected lungs are shown in Fig. 3 of the main text. (**B**) Bioluminescence imaging of resected lungs and liver from mouse lacking *p53/ Rb1/p130* knockouts (SCLC negative) 3 days post vMyx-FLuc (left), bioluminescence imaging of resected organs from conditional *p53$^{-/-}$/Rb1$^{-/-}$/p130$^{-/-}$* (SCLC Positive) mouse 3 days post vMyx-FLuc (middle), and extrapulmonary organ bioluminescence imaging of an additional conditional *p53$^{-/-}$ /Rb1$^{-/-}$/p130$^{-/-}$* (SCLC Positive) 3 days post vMyx-FLuc (right). For all animals vMyx-FLuc was administered by intranasal instillation ($5x10^7$ FFU in 60 uL PBS).

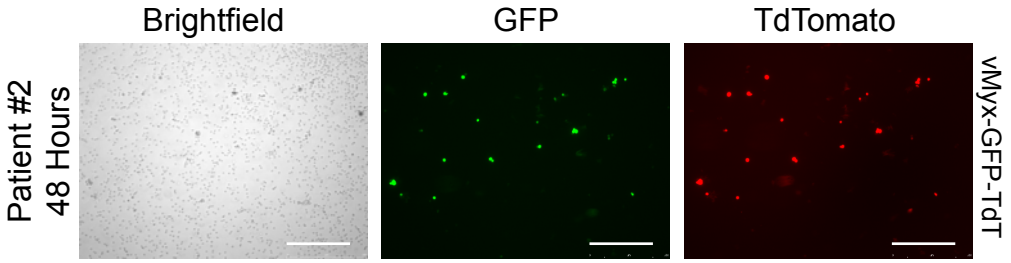

Figure S7. Additional primary SCLC sample obtained from different patient is permissive to MYXV.

Figure S7. Additional primary SCLC sample obtained from different patient is permissive to MYXV. Primary patient SCLC specimen from patient #2 infected with vMyx-GFP-TdT, additional primary patient sample shows the ability for MYXV to infect and replicate by 48 hours, scale bar = 200 um. All samples were treated at a MOI of 10, and maintained in RPMI-1640 supplemented with 10% FBS, 100 units/mL penicillin, and 100 ug/mL streptomycin. Primary samples obtained from two different patients have been examined and show similar results, additional patient samples have not been examined solely due to the limited availability of primary SCLC patient samples.

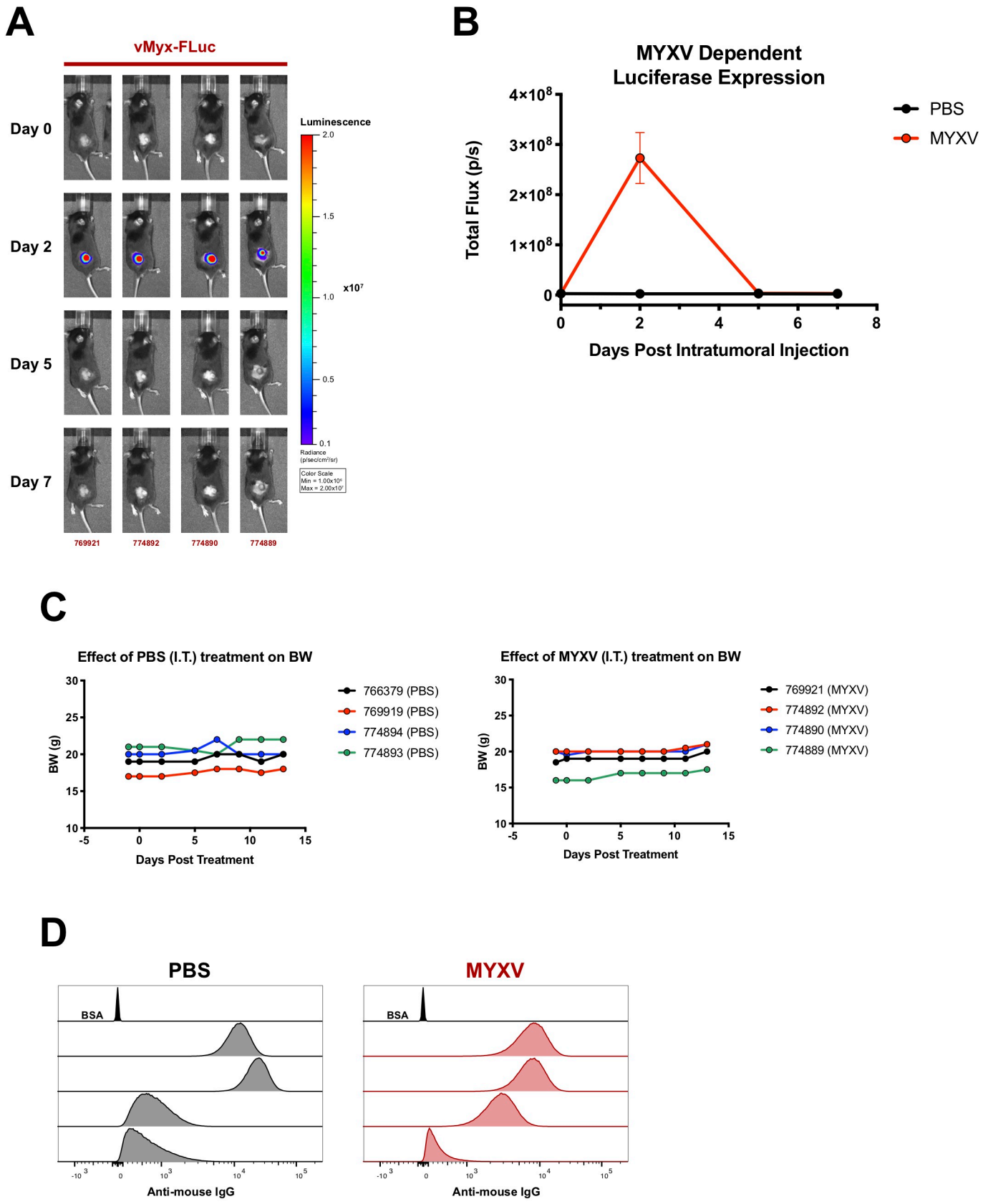

Figure S8. MYXV is rapidly cleared from immunocompetent SCLC allografts and treatment does not affect body weight.

Figure S8. MYXV is rapidly cleared from immunocompetent SCLC allografts and treatment does not affect body weight. (**A**) Bioluminescence imaging of animals treated with MYXV expressing firefly luciferase under poxvirus early/late promoter (vMyx-FLuc). (**B**) Quantification of bioluminescent photon flux from MYXV treated animals compared to baseline signal from PBS treated animals. (**C**) Body weight measurements from PBS and MYXV treated animals showing intratumoral PBS and MYXV treatment does not affect body weight. (**D**) Endogenous anti-sera reactivity against the mSCLC line used to generate allograft tumors (cell line 737274-A) from PBS treated and MYXV treated animals.

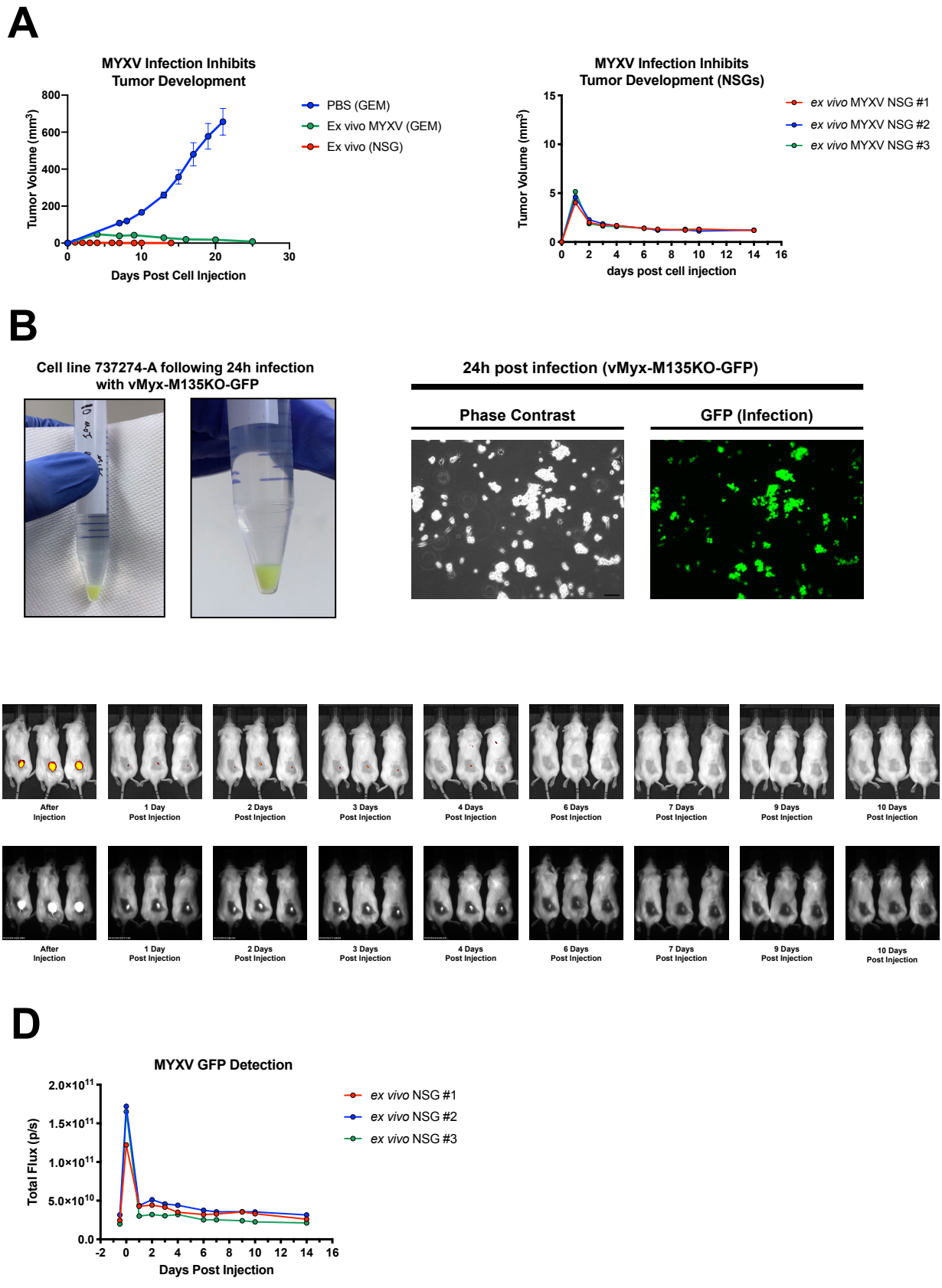

Figure S9. Ex vivo MYXV infection inhibits tumor development in immunocompetent animals and immunodeficient NSG mice.

Figure S9. *Ex vivo* MYXV infection inhibits tumor development in immunocompetent animals and immunodeficient NSG mice. (**A**) Tumor volume over time following injection of murine SCLC cells infected with 10 MOI of vMyx-M135KO-GFP for 24 hours prior to cell injection (*ex vivo* infection) in both immunocompetent (GEM) animals (n=3) and immunodeficient (NSG) mice (n=3) compared to PBS treated control cells injected immunocompetent (GEM) animals (n=4). (**B**) Microscopy prior to cell collection confirms MYXV infection and expression of GFP with cell pellets displaying noticeable green color due to high levels of MYXV GFP expression permitted in SCLC cells. (**C**) Live animal GFP imaging detects strong GFP signal from MYXV infected cells immediately following injection of *ex vivo* infected cells in NSG mice (n=3), within 24 hours only weak GFP signal is detected and 2 weeks post injection signal returns to baseline levels with no tumor growth detected. (**D**) Quantification of live animal GFP imaging.

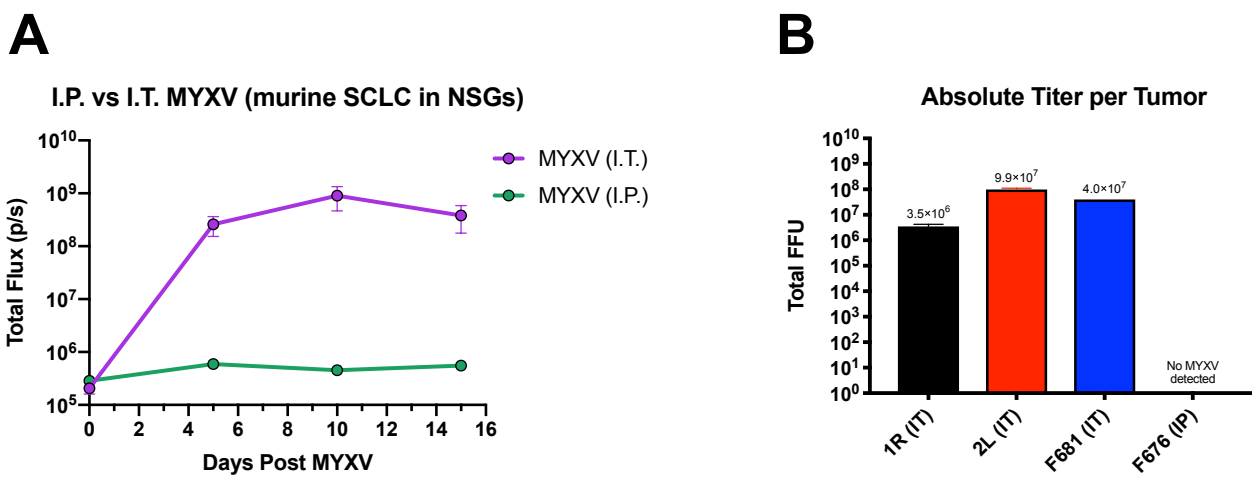

Figure S10. MYXV delivered by I.P. injection does not localize in subcutaneous SCLC allograft tumors and in immunodeficient NSG mice MYXV delivered by I.T. is detectable for at least 15 days.

Figure S10. MYXV delivered by I.P. injection does not localize in subcutaneous SCLC allograft tumors and in immunodeficient NSG mice MYXV delivered by I.T. is detectable for at least 15 days. (**A**) Quantification of bioluminescent photon flux from NSG mice harboring murine SCLC allograft tumors derived from murine SCLC (cell line 510986-2A) treated with MYXV (vMyx-FLuc, $5x10^7$ FFU in 50 uL PBS) by either intratumoral injection (I.T. injection, n=3) or Intraperitoneal injection (I.P. injection, n=1). (**B**) Total titer of virus from whole tumor homogenates collected from animals 15 days post MYXV treatment (either I.T. or I.P. injection).