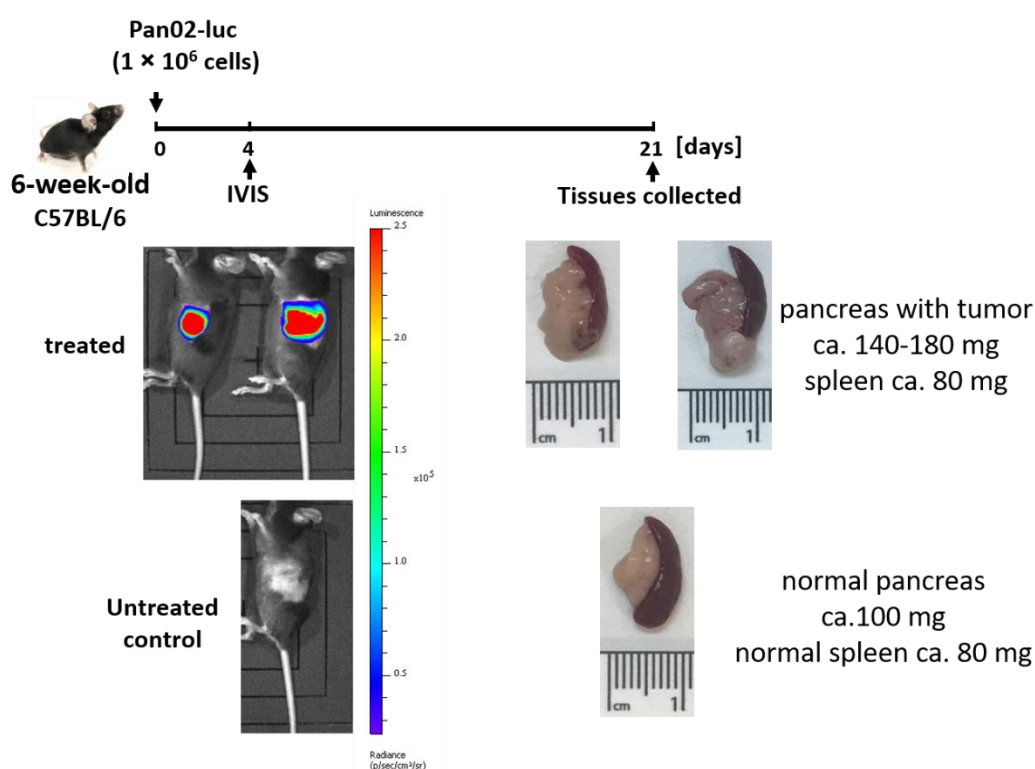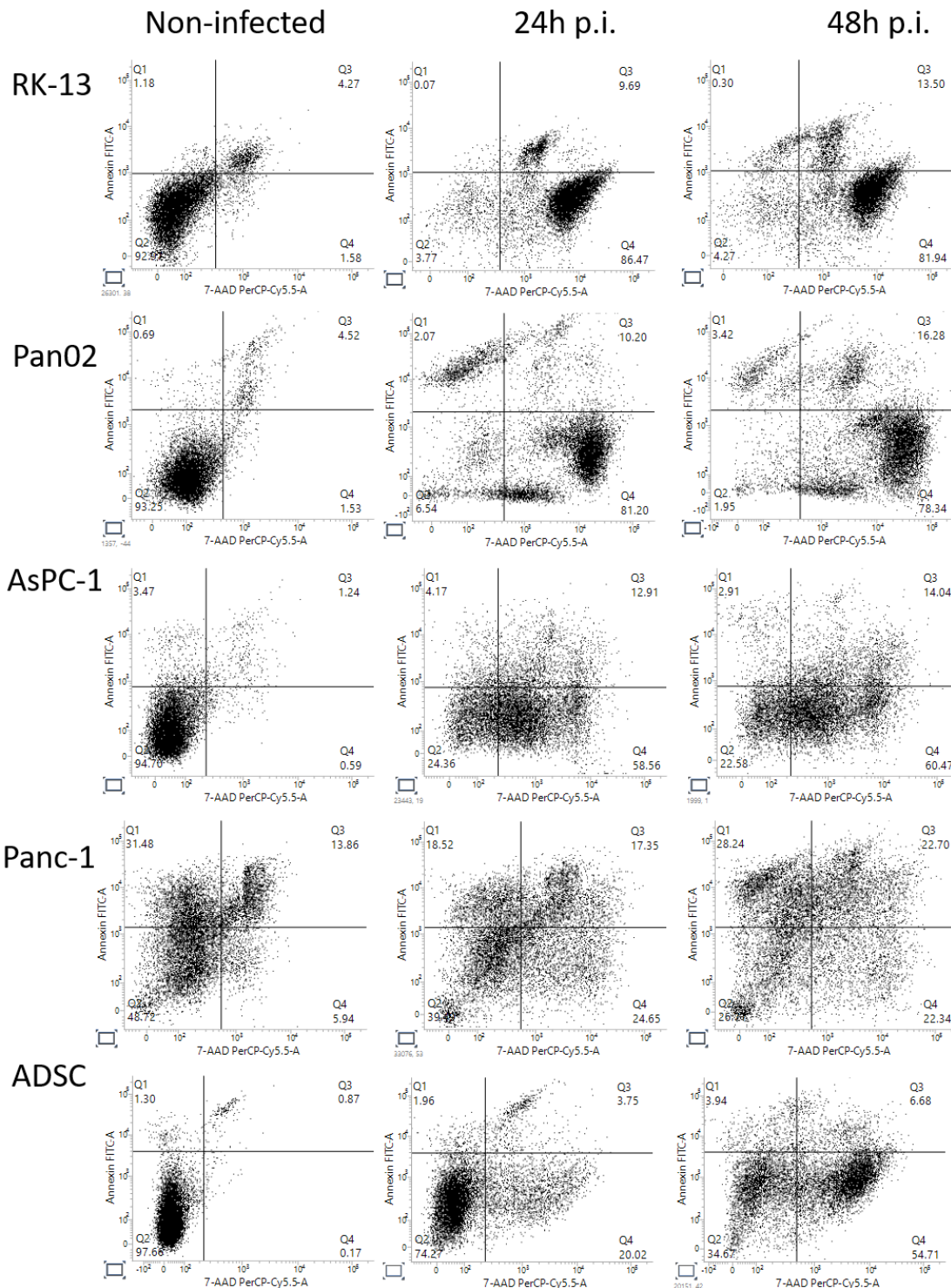# Supplementary Materials: Combination of LIGHT(TNFSF14)-Armed Myxoma Virus Pre-loaded into ADSCs and Gemcitabine in the Treatment of Experimental Orthotopic Murine Pancreatic Adenocarcinoma
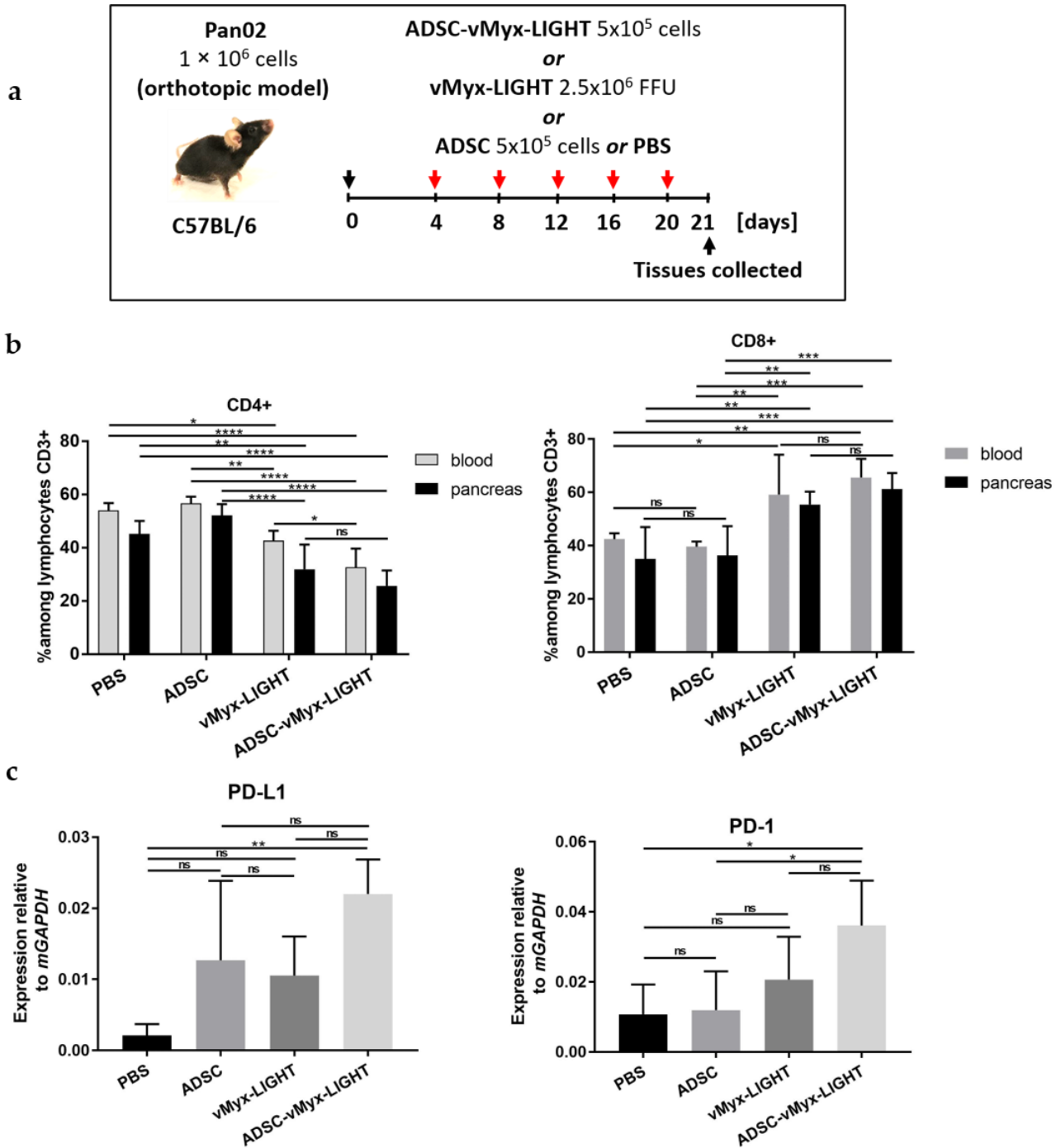
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Figure S1. Establishment of orthotopic pancreatic tumors. C57BL/6 mice were orthotopically injected (day 0) with 1×10^6 Pan02-luc cells suspended in 30 μL PBS⁻. Four days after implantation, mice were anesthetized with 2.5% isoflurane and intraperitoneally injected with 1.5 mg D-luciferin (Promega) and bioluminescence images were acquired. BLI expressed as radiance (photons/sec/cm²/sr). After 21 days the dissected organs (pancreata and spleens) were macroscopically examined for size as well as organ weight.

Figure S2. Quantitative Analysis of Apoptosis by Flow Cytometry. Cells were seeded at a density of 1×10^5 cells/well using 6-well plates and vMyx-mLIGHT-FLuc/tdTr (MOI = 5) was added to the cultured cells. After 24 h and 48 h the cells were collected and washed twice with PBS^- and staining buffer. Then the cells were stained with anti-Annexin V antibody and 7-aminoactinomycin D (Bio-Legend 640922) and analyzed for apoptosis/necrosis using flow cytometry (BD FACS Canto II). Annexin V was detected using FITC channel and 7-AAD using PerCP-Cy5.5 channel and a region for live cells was defined. Non-infected cells were used as a control. Flow cytometry plots for non-infected and infected with vMyx-LIGHT cell lines: RK-13, Pan02, AsPC-1, Panc-1 and ADSCs. Q1 - early apoptotic cells; Q2 - viable cells; Q3 - late apoptotic cells and Q4 - necrotic cells.

Figure S3. Antitumor immune response after monotherapy of experimental pancreatic adenocarcinoma using LIGHT-armed MYXV construct. Pancreatic tumors were established in recipient C57Bl/6NCrl mice (*n* = 5/group) (day 0) by orthotopic implantation of 1×10^6 Pan02 cells/30 μL PBS⁻. For five-dose treatment regimens (days 4, 8, 12, 16 and 20), mice were injected IP with ADSCs (5×10^5 cells/100 μL PBS⁻) or ADSCs infected (MOI = 5) for 24 h with vMyx-LIGHT (5×10^5 cells/100 μL PBS⁻) or with unshielded vMyx-LIGHT (2.5×10^6 FFU/100 μL PBS⁻), or 100 μL PBS⁻ (control). After 21 days, mice were sacrificed and the pancreata were excised. (**a**) timeline of experiment; (**b**) flow cytometry data showing CD4+ and CD8+ cell percentage among CD3+ lymphocytes in blood and pancreata (21st day); (c) Analysis of PD-L1 and PD-1 gene expression (RT-qPCR) in pancreata. Changes in the gene expression were rendered as a ratio of target gene vs. reference gene (*mGAPDH*) relative to expression in control samples. The data (mean ± SD) were analyzed with one-way ANOVA; statistically significant differences are indicated (*$p \leq 0.05$; ** $p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$; ns: non-significant differences).

Table S1. Primers.

Gene name	Forward primer (5'-3')	Reverse primer (5'–3')
mTNF-α	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAACTGATGAGAGGGAG
mINFγ	CAGCAACAGCAAGGCGAAAAAGG	TTTCCGCTTCCTGAGGCTGGAT
mIL2	GCGGCATGTTCTGGATTTGACTC	CCACCACAGTTGCTGACTCATC
mIL-15	GTAGGTCTCCCTAAAACAGAGGC	TCCAGGAGAAAGCAGTTCATTGC
mIL10	CGGGAAGACAATAACTGCACCC	CGGTTAGCAGTATGTTGTCCAGC
mTGFβ	TGATACGCCTGAGTGGCTGTCT	CACAAGAGCAGTGAGCGCTGAA
mCD8a	ACTACCAAGCCAGTGCTGCGAA	ATCACAGGCGAAGTCCAATCCG
mCD4	GTTCAGGACAGCGACTTCTGGA	GAAGGAGAACTCCGCTGACTCT
mPD-L1	TGCGGACTACAAGCGAATCACG	CTCAGCTTCTGGATAACCCTCG
mPD-1	CGGTTTCAAGGCATGGTCATTGG	TCAGAGTGTCGTCCTTGCTTCC
mLight	GGAGACATAGTAGCTCATCTGCC	CCACCAATACCTATCAAGCTGGC
mHVEM	CCAGGCTACTTCTGTGAGAACC	CAGTCAGCACATACAGTGTCCTG
mLTβR	TCCTTGGAGGAAGTGGTGCTAC	CGGTCACATGAATGCCATTCGC
S18	CGGAAAATAGCCTTCGCCATCAC	ATCACTCGCTCCACCTCATCCT
mGAPDH	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG
hGAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA