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Supplemental information

MicroRNA-sensitive oncolytic measles virus for chemovirotherapy of pancreatic cancer

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Supplemental Information: Figure S1.

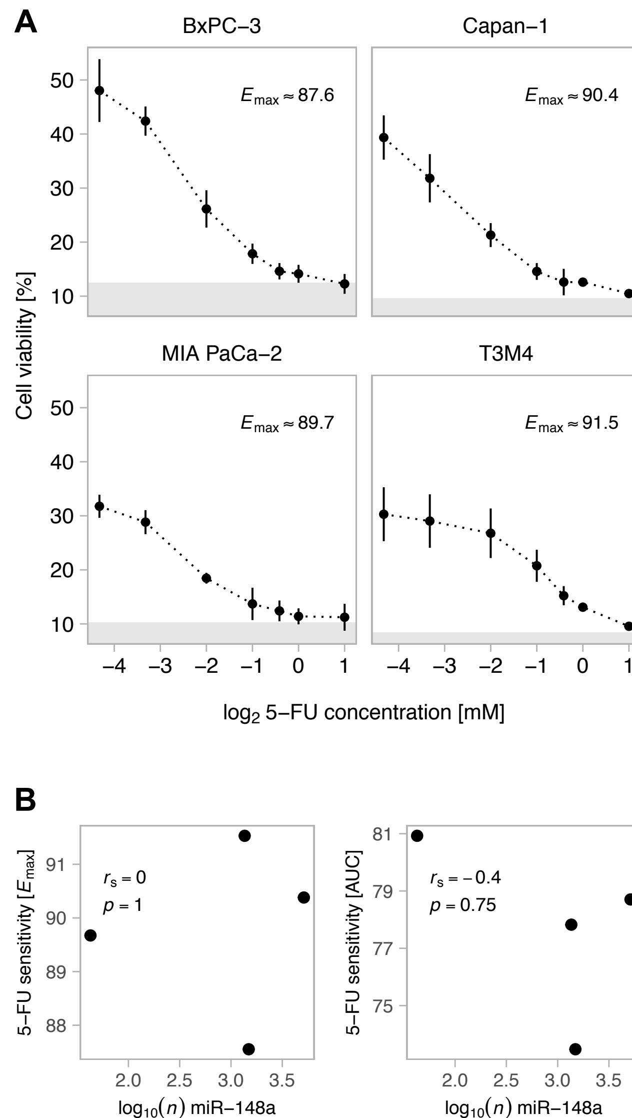

Figure S1. Sensitivity of PDAC Cell Lines to 5-FU

(A) Drug response profiles. PDAC cells were exposed to a dilution series of 5-FU and cell viability relative to mock-treated controls was determined after 72 hours. Plotted values represent median and standard deviation of each sample (n = 4). Viability data were used to fit a log-logistic dose-response model (with response defined as 100-viability). The maximum effect level (E_{max}, grey) and the area under the dose-response curve (AUC) were computed as drug sensitivity metrics for each cell line. (B) Relationship between 5-FU sensitivity and cellular miR-148a level in PDAC cell lines. MicroRNA expression was assessed as described in the main text. Scatter plots and Spearman's rank correlation coefficients (r_S) were computed twice using either E_{max} (*left*) or AUC (*right*). Each dot corresponds to one cell type analyzed in (A).

Supplemental Information: Figure S2.

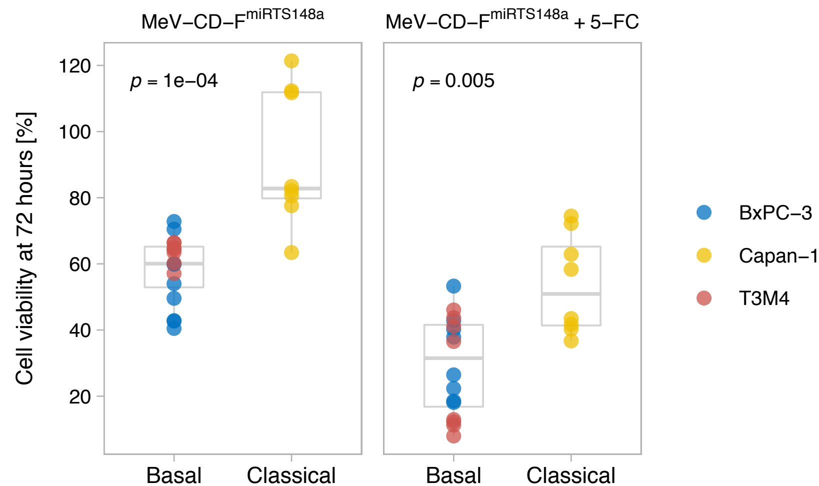

Figure S2. Efficacy of MeV Chemovirotherapy by Molecular PDAC Subtype
PDAC cell lines were infected with MeV-CD-F^{miRTS148a} (MOI = 0.1), followed by addition of 5-FC (1 mM) or mock treatment after 36 hours. Cell viability at 72 hours post treatment was determined by XTT assay. Data represent a subset of results summarized in Figure 6. To explore the association of tumor genetic profiles and chemovirotherapy efficacy, PDAC cell lines were stratified by molecular subtype ('Basal' vs. 'Classical') based on the transcriptomic classification by Yu *et al.* and the original nomenclature by Moffit *et al.* (see main text for details). Only cell lines with conclusive subtype profiles (false discovery rate <0.05) were included. Indicated *p*-values were obtained by Mann–Whitney *U* test. Biological replicates ($n = 8$ per group) are plotted.

Supplemental Information: Figure S3.

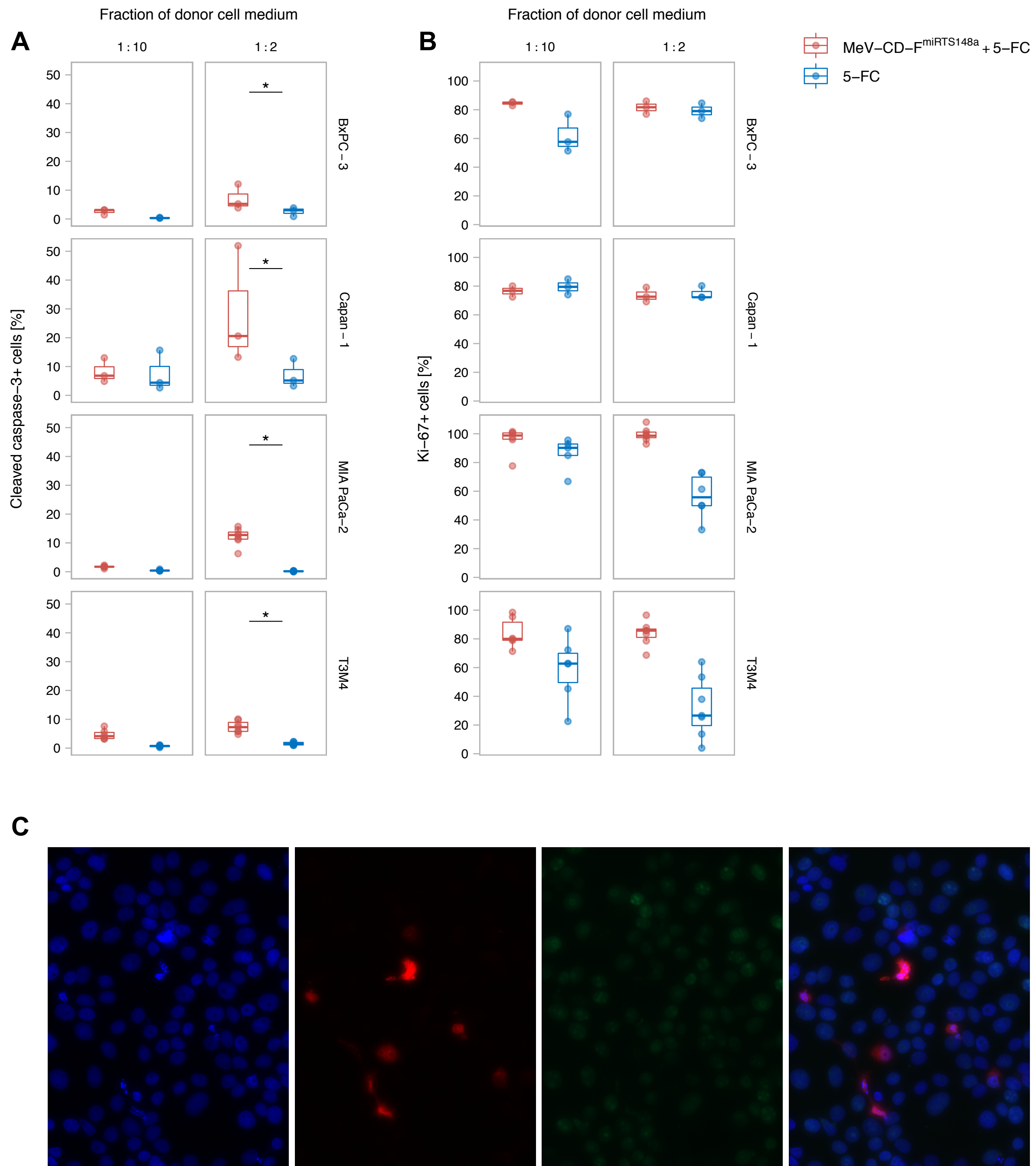

Figure S3. Apoptosis and Proliferation Marker Expression in Bystander PDAC Cells

PDAC cells were exposed to different dilutions of cell culture medium derived from cells undergoing either chemovirotherapy with MeV-CD-F^{miRTS148a} plus 5-FC or treatment with 5-FC alone. Details of the protocol are given in the main text. After 48 to 72 hours of treatment, immunofluorescence staining of cells was performed with antibodies against (A) cleaved caspase-3 or (B) Ki-67. Automated segmentation of digital immunofluorescence images was used to determine the fraction of labeled cells relative to the total number of DAPI-positive cells per

image. Plotted dots correspond to individual images (biological replicates). (C) Representative immunofluorescence images of T3M4 cells exposed to conditioned medium (1:2 dilution) derived from chemovirotherapy-treated cells. Images were acquired at 60 hours post treatment using a 20× objective. Images of the same field of view are shown for each channel (from *left* to *right*: DAPI, cleaved caspase-3, Ki-67, merged).

Supplemental Information: Figure S4.

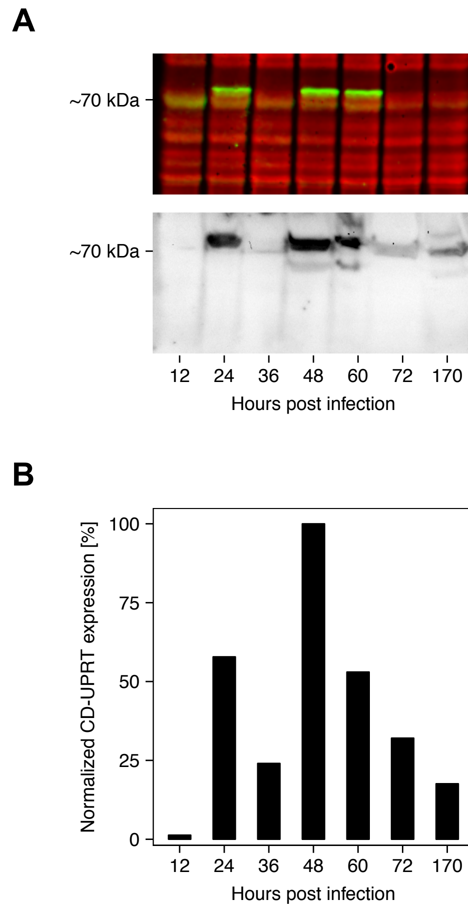

Figure S4. Transgene Expression in PDAC Xenograft Tumors
NOD/SCID/gamma mice with subcutaneous T3M4 tumors were treated by one intratumoral injection of MeV-CD-$F^{miRTS148a}$ (5×10^6 ciu). At the designated time points post injection, tumors were obtained for protein isolation, electrophoresis, and CD-UPRT detection. (A) Anti-CD immunoblot. *Top:* Fluorescence-based detection of CD in tumor lysates. Samples were prelabeled (Cy5, red) to allow for normalization of CD-specific signals (Cy3, green) to total protein. *Bottom:* Conventional immunoblot of tumor lysates with equal loading (139 µg of total protein per sample). (B) Intratumoral CD-UPRT protein levels over time. Each chemiluminescence signal was normalized to the corresponding tumor mass. Data represent protein levels from individual tumors with maximum expression set to 100%.

Supplemental Information: Figure S5.

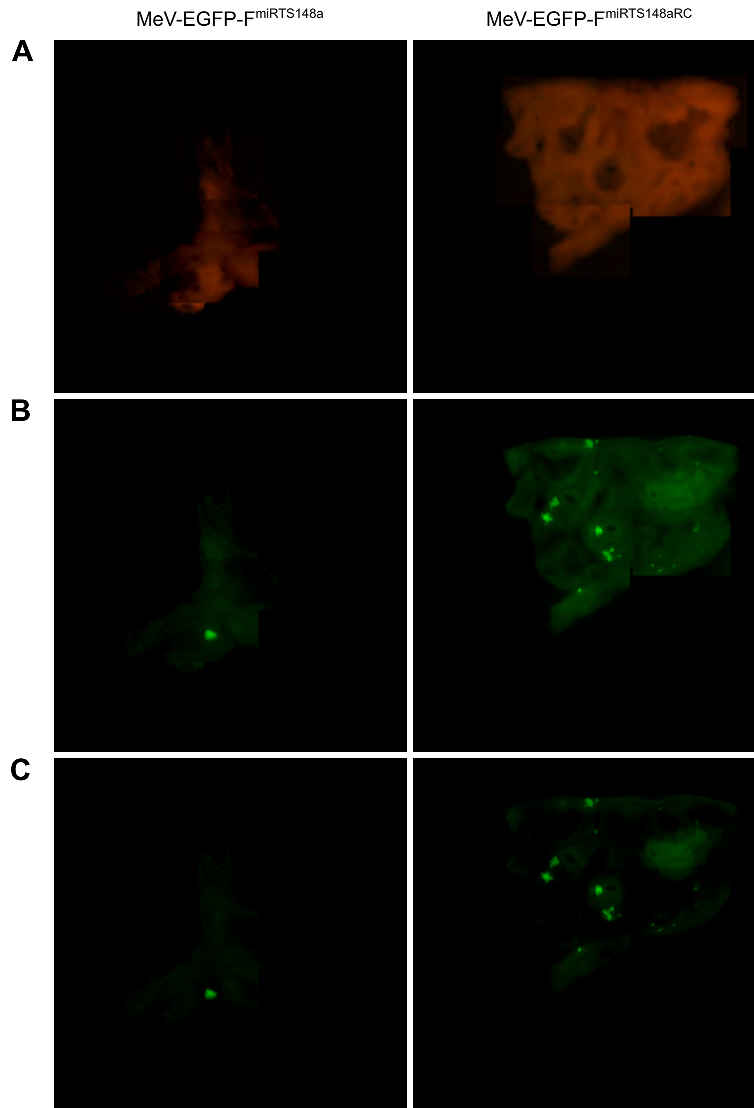

Figure S5. Infection of Primary Normal Liver Tissue

Primary human liver tissue sections were infected *ex vivo* with MeV-EGFP-F^{miRTS148a} or control MeV-EGFP-F^{miRTS148aRC} at 5×10^6 ciu per sample. Tissue specimens were maintained in culture using multi-well inserts, and EGFP expression and MeV syncytia formation were assessed in regular intervals by fluorescence microscopy. Multichannel images of each localization were acquired at 116 hours post infection using a 5× objective. Brightness and contrast of digital images was adjusted, and whole-slice representations were constructed from adjacent tiles. (A) Negative controls acquired with filter settings optimized for tdTomato to adjust for tissue autofluorescence. (B) Channel EGFP. (C) Channel EGFP with background correction.