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Supplemental information

Lentiviral interferon: A novel method for gene therapy in bladder cancer

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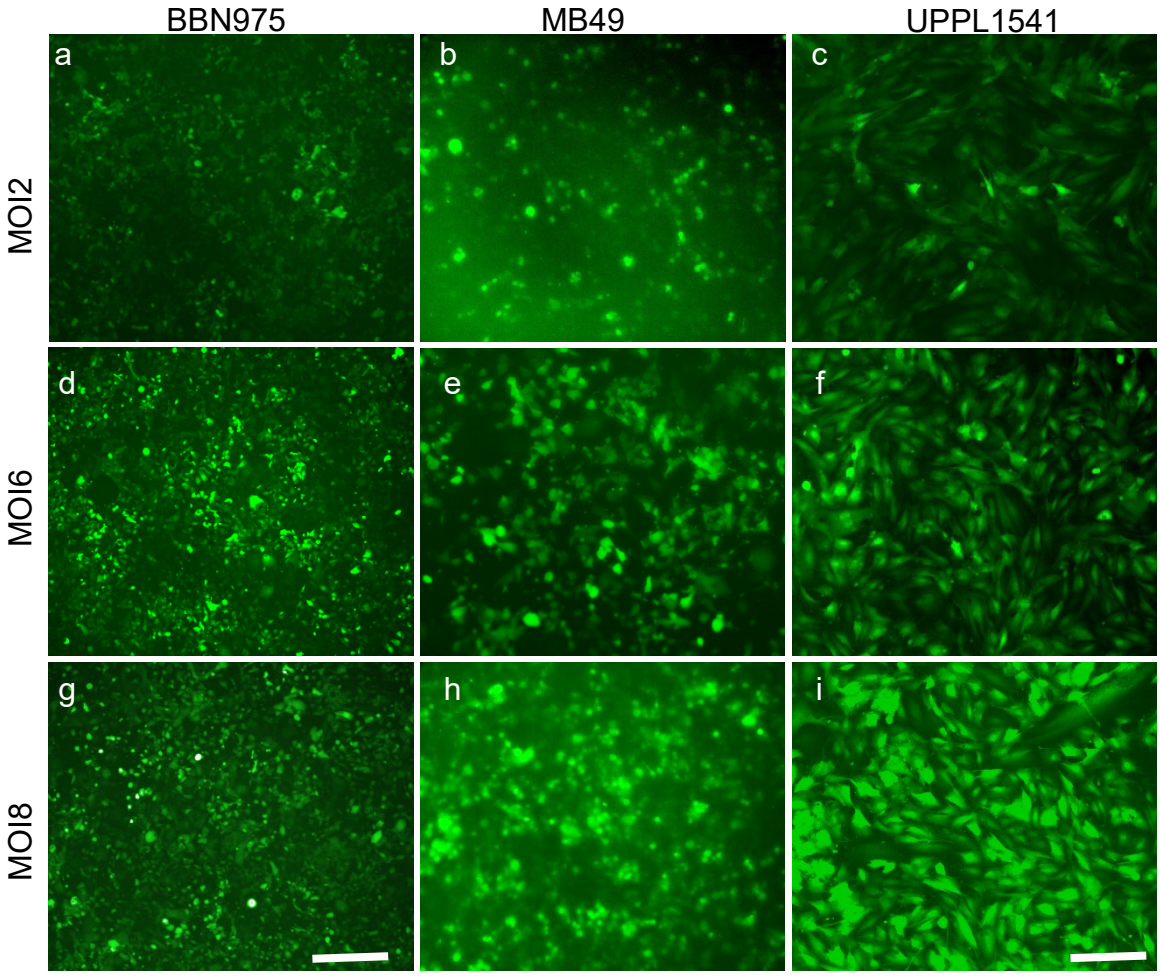

Figure S1. GFP expression in BLCA cell lines transduced with GFP lentiviral vectors at different MOIs. Dose dependent expression of GFP is observed in BBN975 (a, d, g), MB49 (b, e, h), UPPL1541 (c, f, i) at MOI2 (a, b, c), MOI6 (d, e, f) and MOI8 (g, h, i), respectively. Scale bar 150 µm (a, d, g) , 75 µM.(b, e, h, c, f, i).

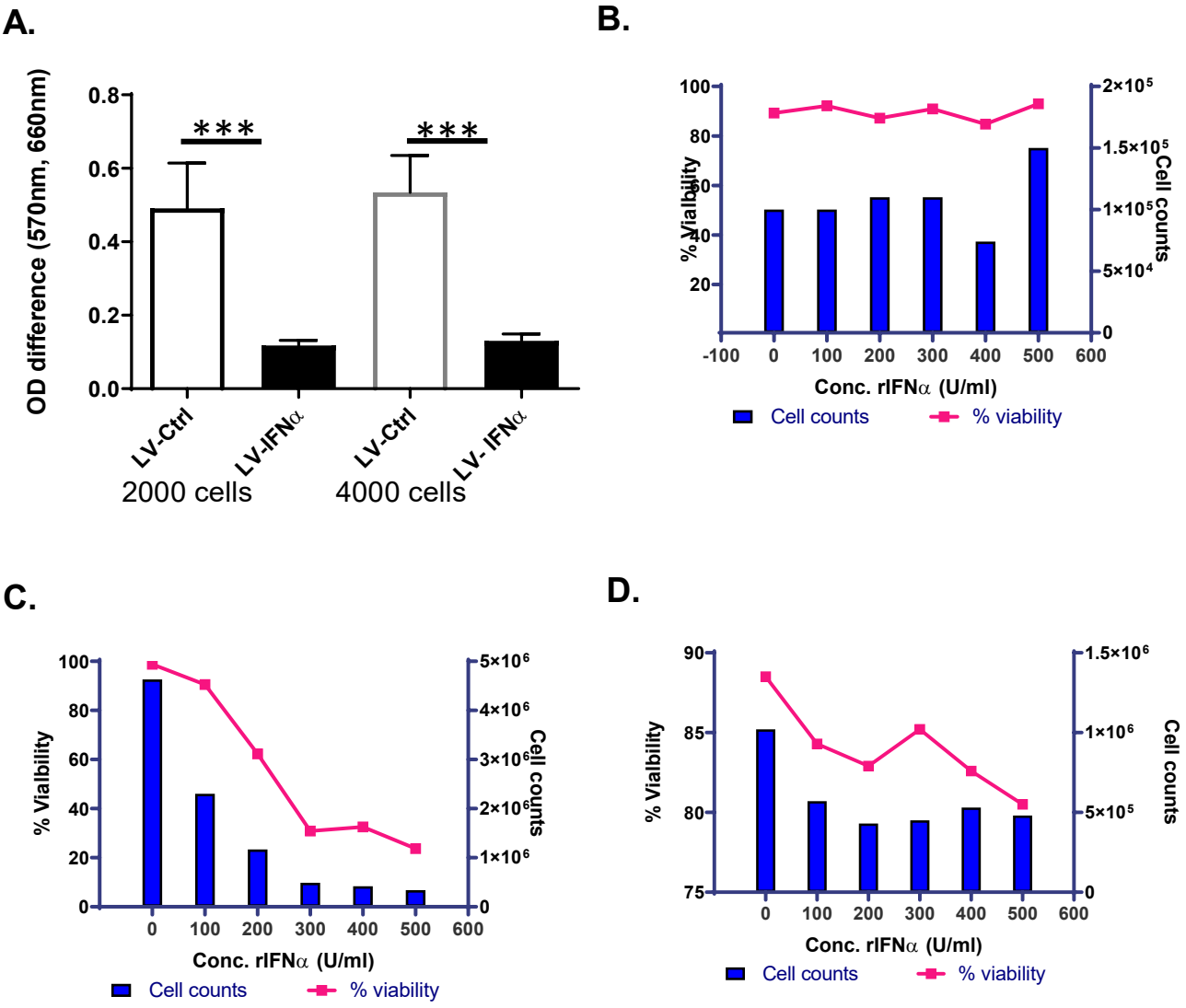

Figure S2. Cytotoxic effect of recombinant IFNα on BLCA cell lines. **A)** In MB49 cells, MTT assay was performed at 72h post transduction. Compared to LV-Ctrl, LV-IFNα cells showed significant reduction in cell numbers $p<0.001$. **B-D)** Murine cell lines were exposed to recombinant murine IFNα and cell counts and % viability were measured using Trypan blue dye exclusion method. At 72h, MB49 **(C)** and UPPL1541 **(D)** showed marked reduction in cell counts and % viability, BBN975 **(B)** cells showed no change in cell numbers or % viability.

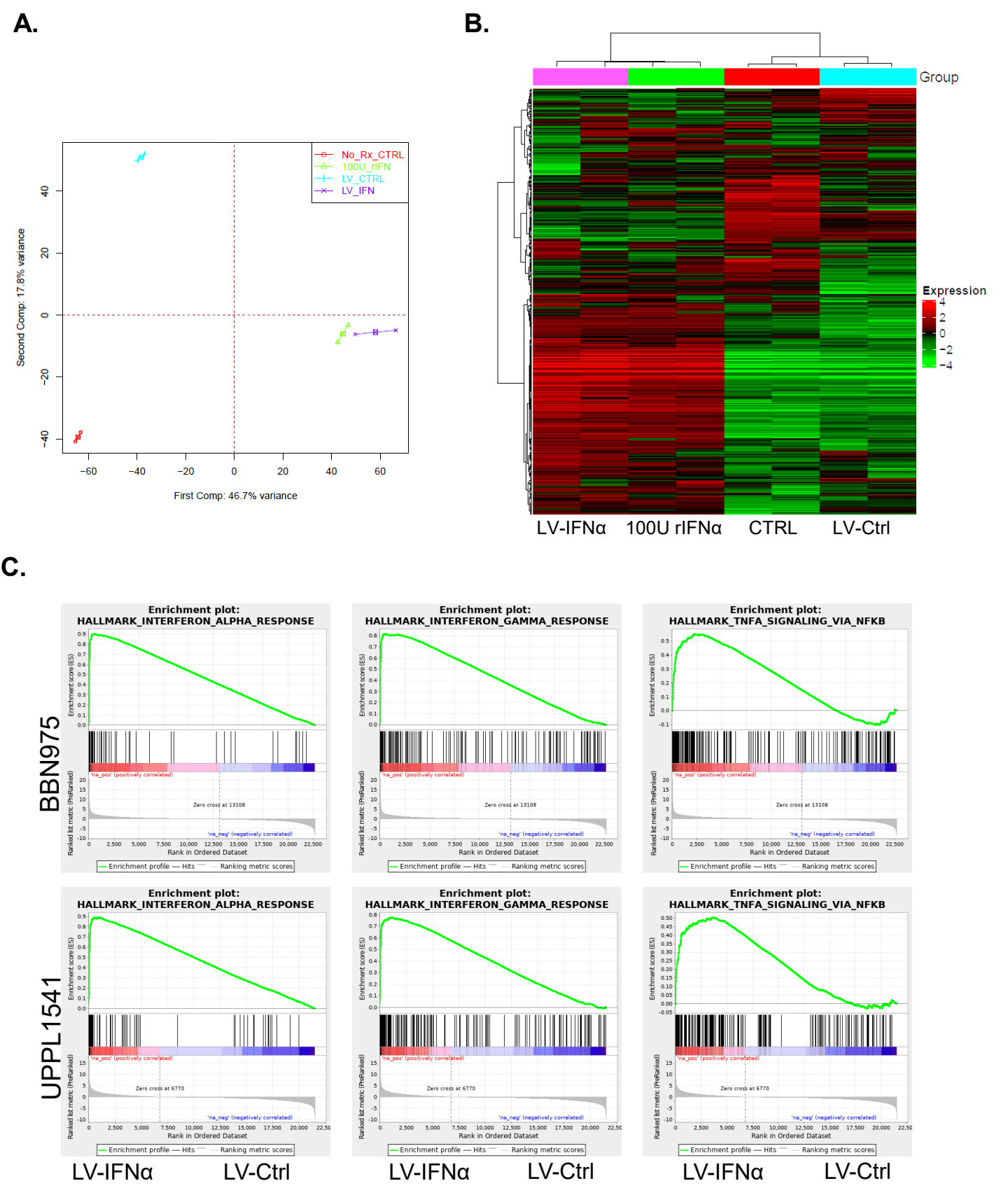

Figure S3. RNAseq analysis of mouse BLCA cell lines treated with recombinant IFNα or LV-IFN. **A)** Principal component analysis showing different groups in MB49 cell line RNAseq data. **B)** Heatmaps of significant genes (FDR cut off 5% and fold change 2) between the four groups, No treatment control, LV-Ctrl, 100 U recombinant IFNα and LV-IFNα in MB49 cells is shown. **C)** GSEA analysis showing enrichment of Interferon alpha and gamma response pathway and TNF alpha signaling pathway in BBN975 and UPPL1541 cells treated with LV-IFNα when compared to LV-Ctrl is shown.

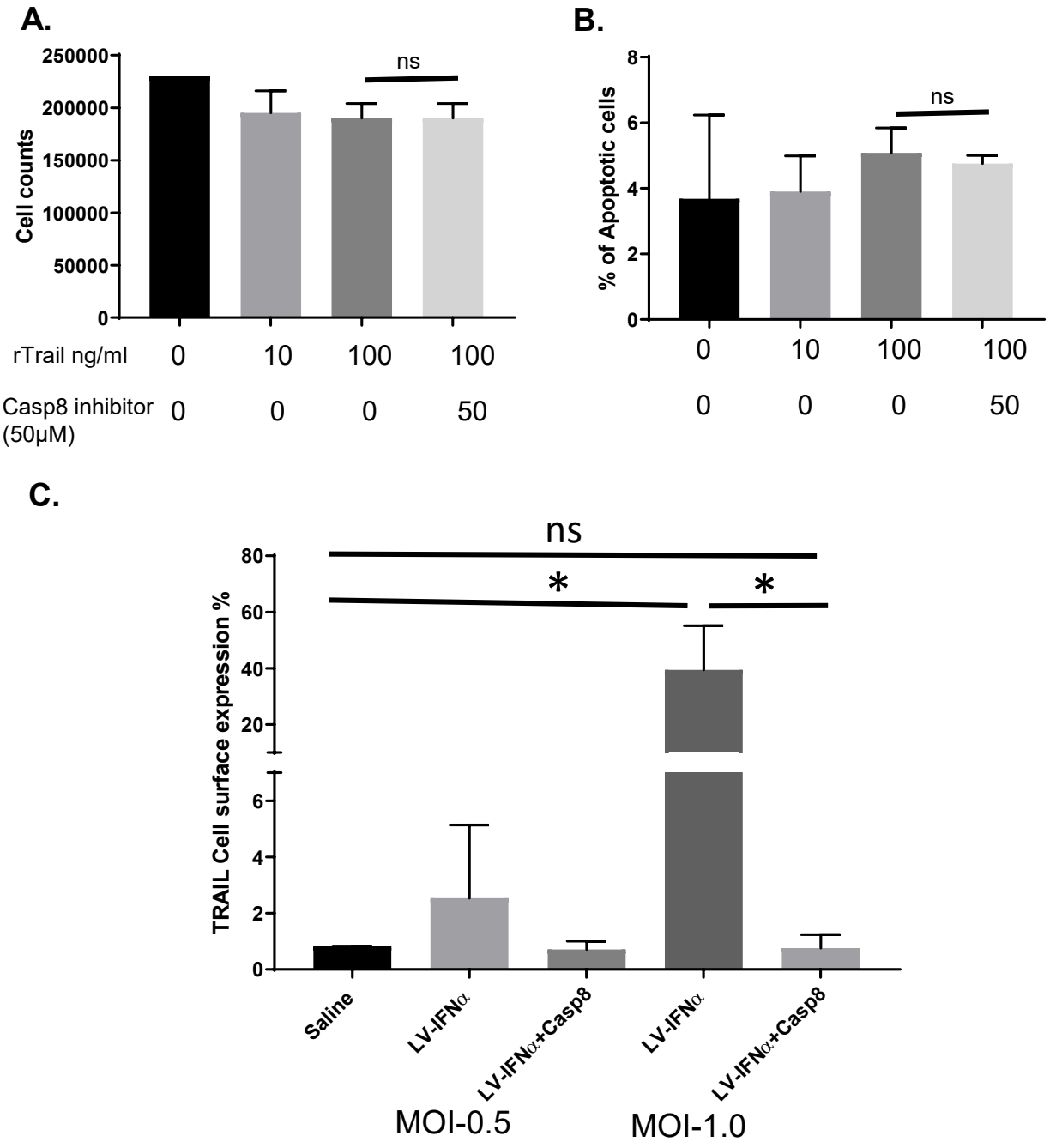

Figure S4. TRAIL-mediated cell death in presence of caspase 8 inhibitor in BBN975. A) Cell counts by trypan blue dye exclusion method in BBN975 cells treated with recombinant TRAIL (rTRAIL, 10ng/ml and 100ng/ml) and Caspase 8 inhibitor (50μM) showing no significant change in numbers. **B)** Annexin V staining showing no significant change in apoptotic cells following treatment with TRAIL and/or caspase 8 inhibitor. **C)** Increased cell-surface expression of TRAIL in cells treated with LV-IFNα rescued by addition of caspase 8 inhibitor. p-values ns>0.05; * <0.05.

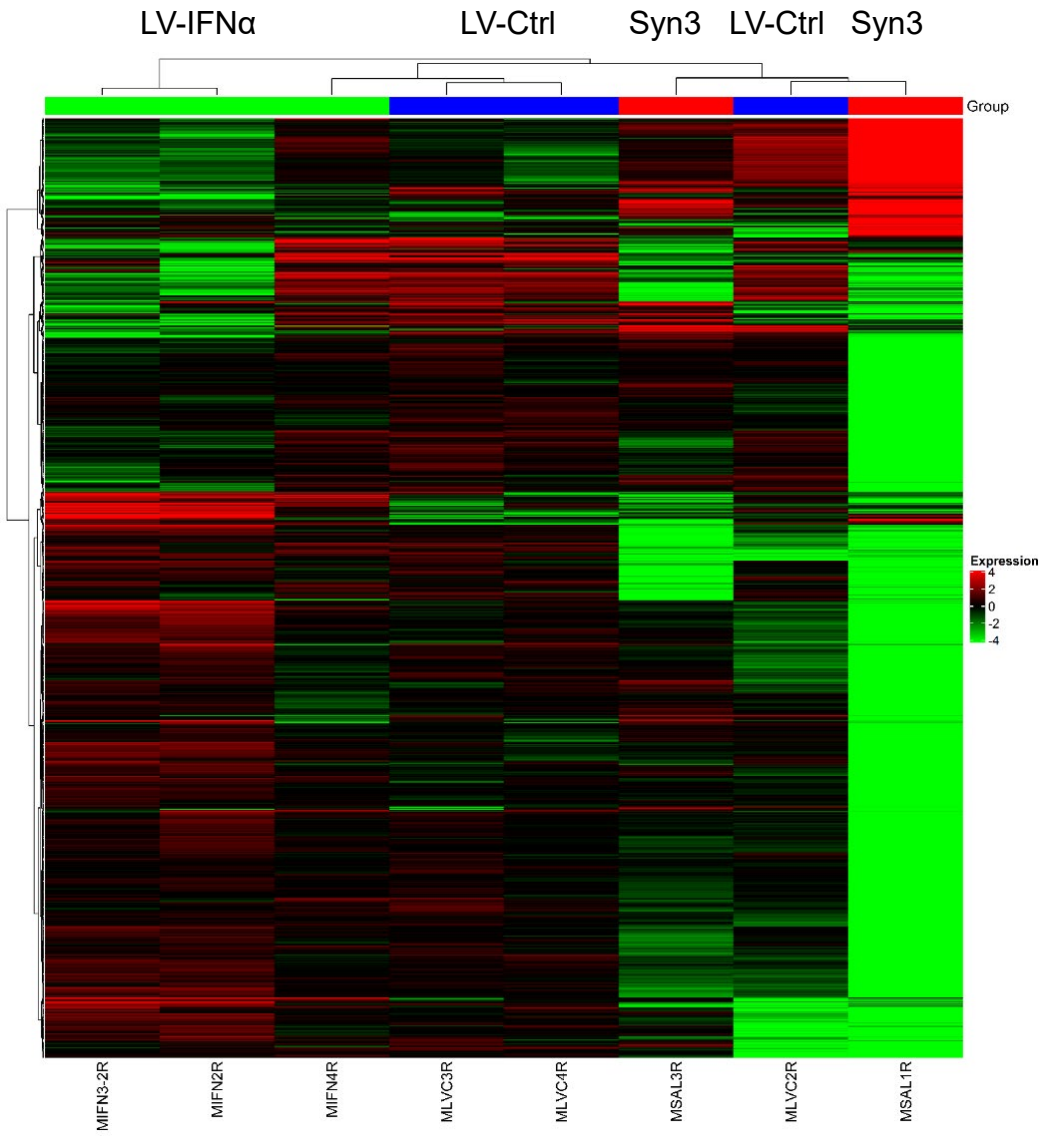

Figure S5. RNAseq analysis of mouse tumors treated with vehicle (sal) or LV-Ctrl or LV-IFNα vectors. Heatmap and hierarchical clustering of top candidate genes in MB49 intravesical tumors treated groups showing clear separation of LV-IFNα tumors from the LV-Ctrl or Sal groups.