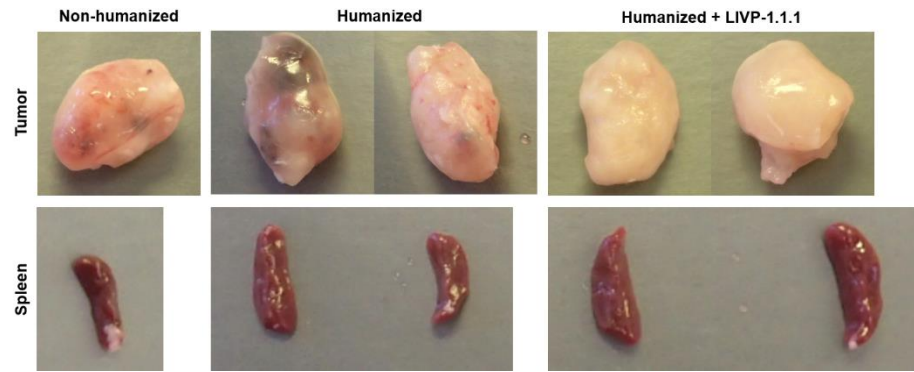


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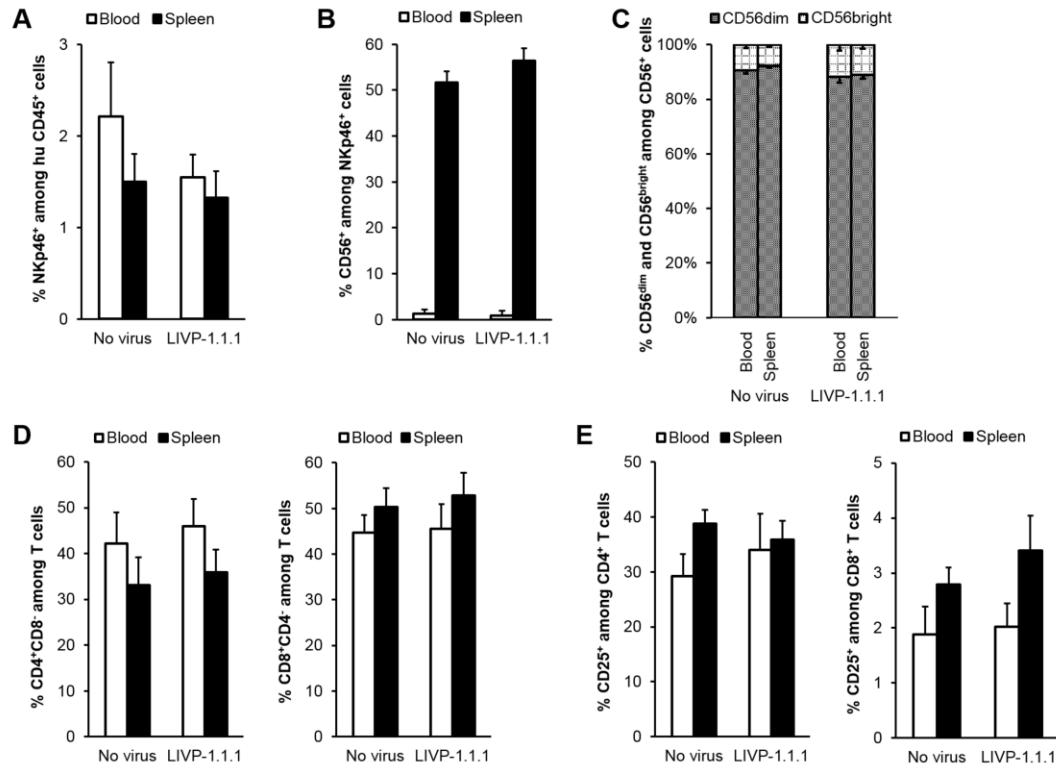
**Supplemental Information**

**Humanized Mice with Subcutaneous Human  
Solid Tumors for Immune Response Analysis  
of Vaccinia Virus-Mediated Oncolysis**

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**Figure S1. Macroscopic analysis of excised tumors and spleens.** Tumors and spleens were excised from three different A549 tumor-bearing NSG mouse groups: non-humanized, humanized and LVP-1.1.1 virus-treated humanized mice, at 18 weeks post humanization, 5 weeks after tumor implantation and 8 days after virus administration. All spleens are photographed at same magnification. Representative pictures are shown.



**Figure S2. Flow cytometric analysis of human NK and T cell populations in non-tumorous humanized NSG mice.** Blood (no fill) and spleen (solid fill) samples from untreated (n=8 (A, B and C) or n=6 (D and E)) or LIVP-1.1.1 virus-injected (n=9) humanized NSG mice were analyzed by flow cytometry for presence of human NK and T cells. Analysis were performed 8 weeks post humanization and 7 days after virus administration. Cells were stained with mouse anti-human monoclonal antibodies and gated first on human CD45 and then the respective lineage-specific marker. (A) NK cells were gated on NKp46 marker. (B) CD3<sup>+</sup>NKp46<sup>+</sup> subset was additionally examined for CD56 expression. (C) Percentage of CD56<sup>dim</sup> (grey pattern) and CD56<sup>bright</sup> (dotted grid) among CD3<sup>+</sup>NKp46<sup>+</sup>CD56<sup>+</sup> NK cells. Gating strategy: CD45<sup>+</sup>→ CD3<sup>+</sup>NKp46<sup>+</sup>→CD56<sup>+</sup>→(CD56<sup>dim</sup> or CD56<sup>bright</sup>). (D) T cells were gated on CD3 and then the respective CD4 (left panel) or CD8 (right panel) lineage-specific marker. (E) CD4 (left panel) and CD8 (right panel) single positive cells were additionally examined for CD25 expression. Gating strategy: CD45<sup>+</sup>→CD3<sup>+</sup>→(CD4<sup>+</sup>CD8<sup>-</sup> or CD8<sup>+</sup>CD4<sup>-</sup>)→CD25<sup>+</sup>. Mean values and standard error of the mean (SEM) are plotted. Differences between corresponding samples of untreated and virus-treated groups were tested using a two-tailed t-test for two samples with unequal variance (\* p<0.05, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001).