

## Supplementary Information

### Figure S1. Luciferase expression is not reduced in transfected PIK3R5 and PI3K $\gamma$

**knockout SVG-A cells.** Parental SVG-A cells and PI3K $\gamma$  and PIK3R5 knockout (KO) cells were transfected with phGluc plasmid DNA. Luciferase activity was measured in the medium 72 h post-transfection.

**Figure S2. Sequences of targeted genes in knockout cell lines.** Sequences surrounding the sgRNA binding sites are shown. The top line in each set shows the wild-type sequence with the sgRNA binding site boxed in red. The other lines show the sequences obtained from deep sequencing of cloned knockout cell lines. Note that PIK3R5 knockout cell line R5-3 has a single mutant cell strain; the other knockout cells have two mutant cell strains, each with a different mutation. No wild-type sequences were observed in the cloned knockout cell lines.

**Figure S3. Representative two-dimensional flow cytometry histograms for PI3K $\gamma$  rescue experiment.** Parental SVG-A cell and PI3K $\gamma$  knockout (clone P $\gamma$ -3) SVG-A cells were infected with LEX-PI3K $\gamma$  or empty LEX vector lentivirus and selected in puromycin. Pooled puromycin-resistant cells were mock-infected or infected in duplicate with JCPyV. Three days later, cells were fixed, immunostained for intracellular VP1 and PI3K $\gamma$  and assessed by flow cytometry. Panels show two-dimensional histograms. The percentage of cells that are VP1 positive in the PI3K $\gamma^{\text{hi}}$  and PI3K $\gamma^{\text{lo}}$  quadrants in the infected samples are shown in the red boxes.

### Figure S4. PIK3R5 knockdown cells are refractory to infection by Merkel Cell

**Polyomavirus.** SVG-A cells were transduced with scrambled control shRNA or shRNA (clone

B8) and selected with puromycin. Puromycin-resistant cells were infected with the indicated number of genome equivalents of JCPsV and MCPsV expressing *Gaussia* luciferase. Gluc activity in the culture medium was measured 72 h.p.i.

Fig. S1

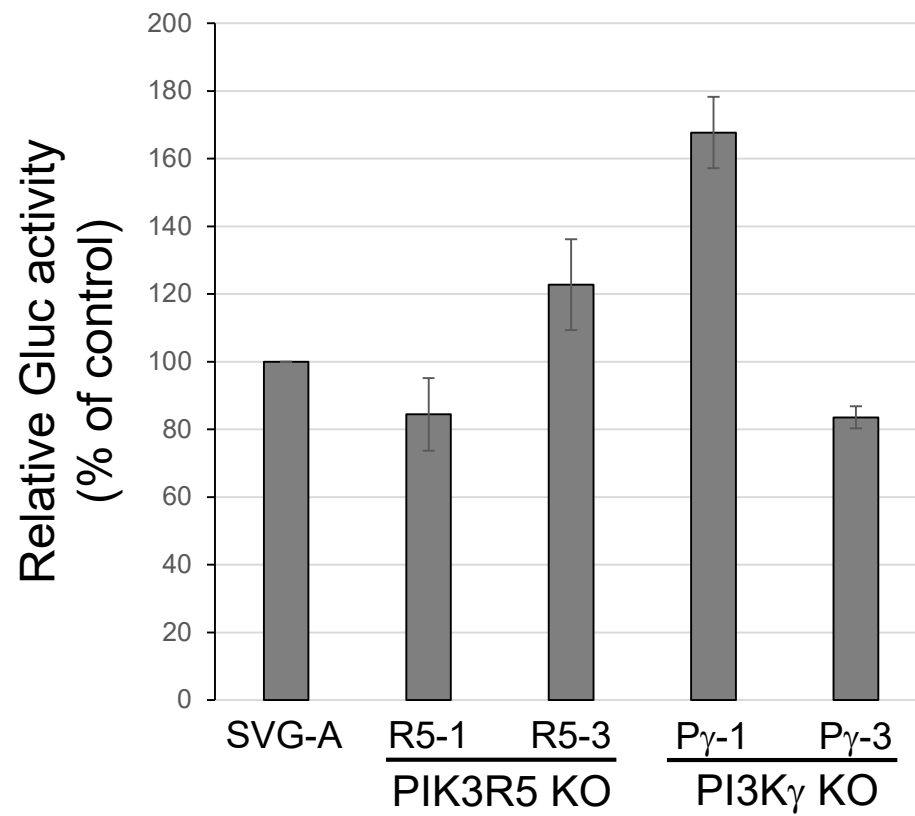
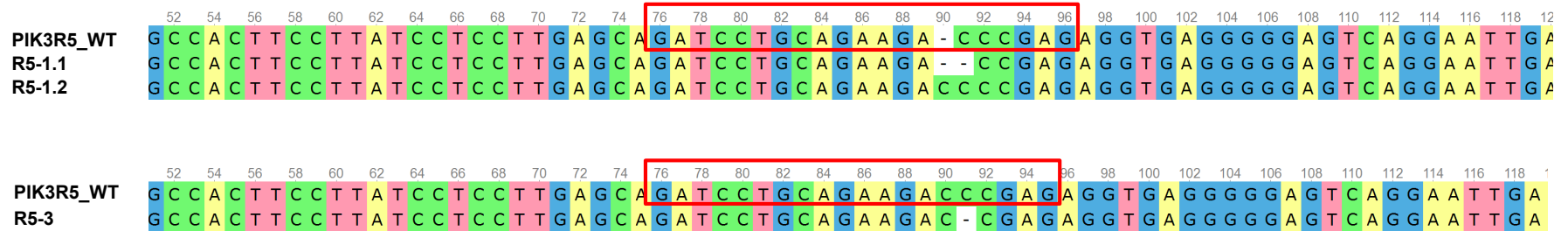


Fig. S2

## PIK3R5 knockout



## PI3K $\gamma$ knockout

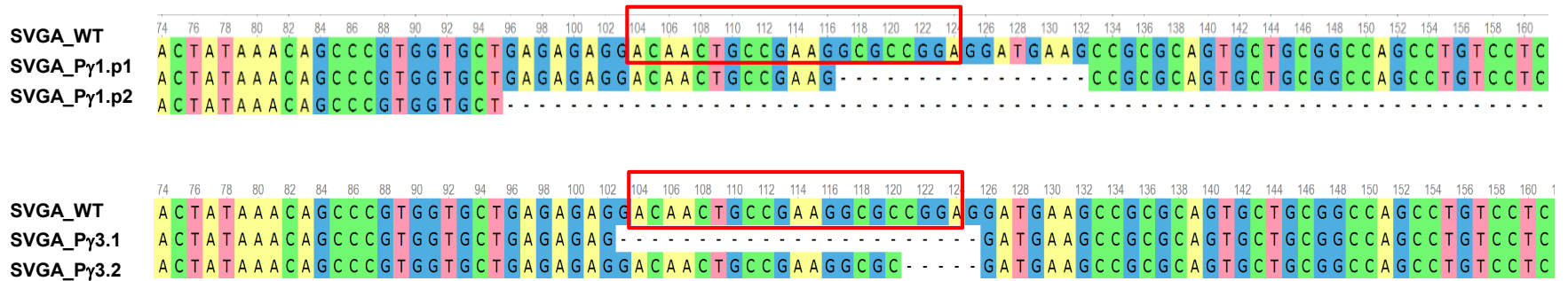


Fig. S3

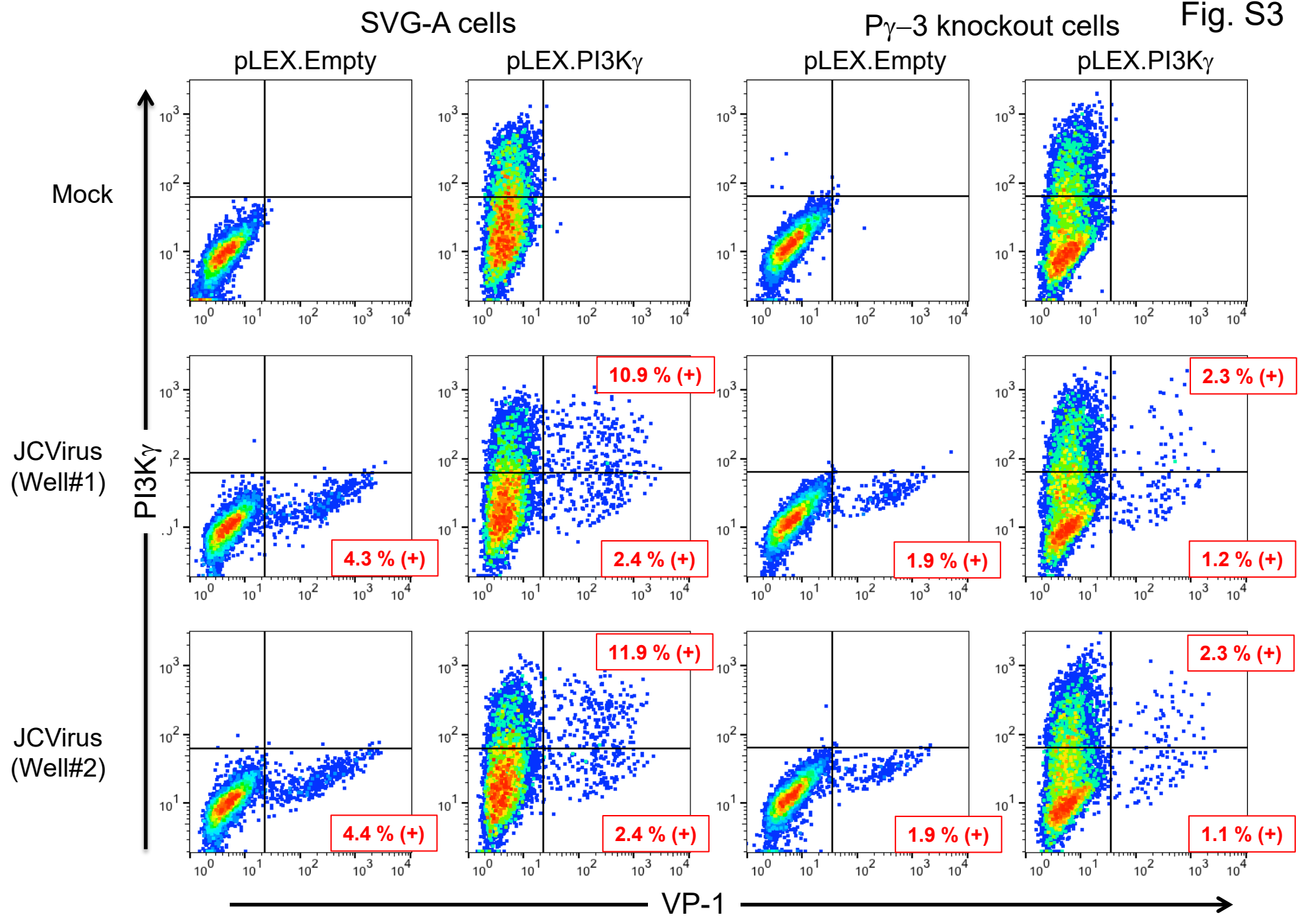


Fig. S4

