

# A pooled genome-wide screening strategy to identify and rank influenza host restriction factors in cell-based vaccine production platforms

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## Supplemental S2. FACS gating strategies

In all flow cytometry and FACS applications in this study, the first two gating steps were the same. **a)** whole cells are isolated from debris and large clumps followed by **b)** doublet discrimination based on pulse width. For cell sorting during the screen, two populations of cells were collected: **c)** a high yield fraction consisting of the top 10% of GFP expressing cells and **d)** a control fraction consisting of all infected (GFP positive) cells. **e)** For the purposes of titrating the PR/8GFPΔHA reporter virus, infected cells were compared to a mock infected control, with the threshold for GFP expression set at 1% of the control. **f)** For the purposes of titrating and monitoring wildtype PR/8 infection kinetics by means of influenza NP expression, infected cells were compared to a mock infected control, with the threshold for GFP expression set at 1% of the control.

