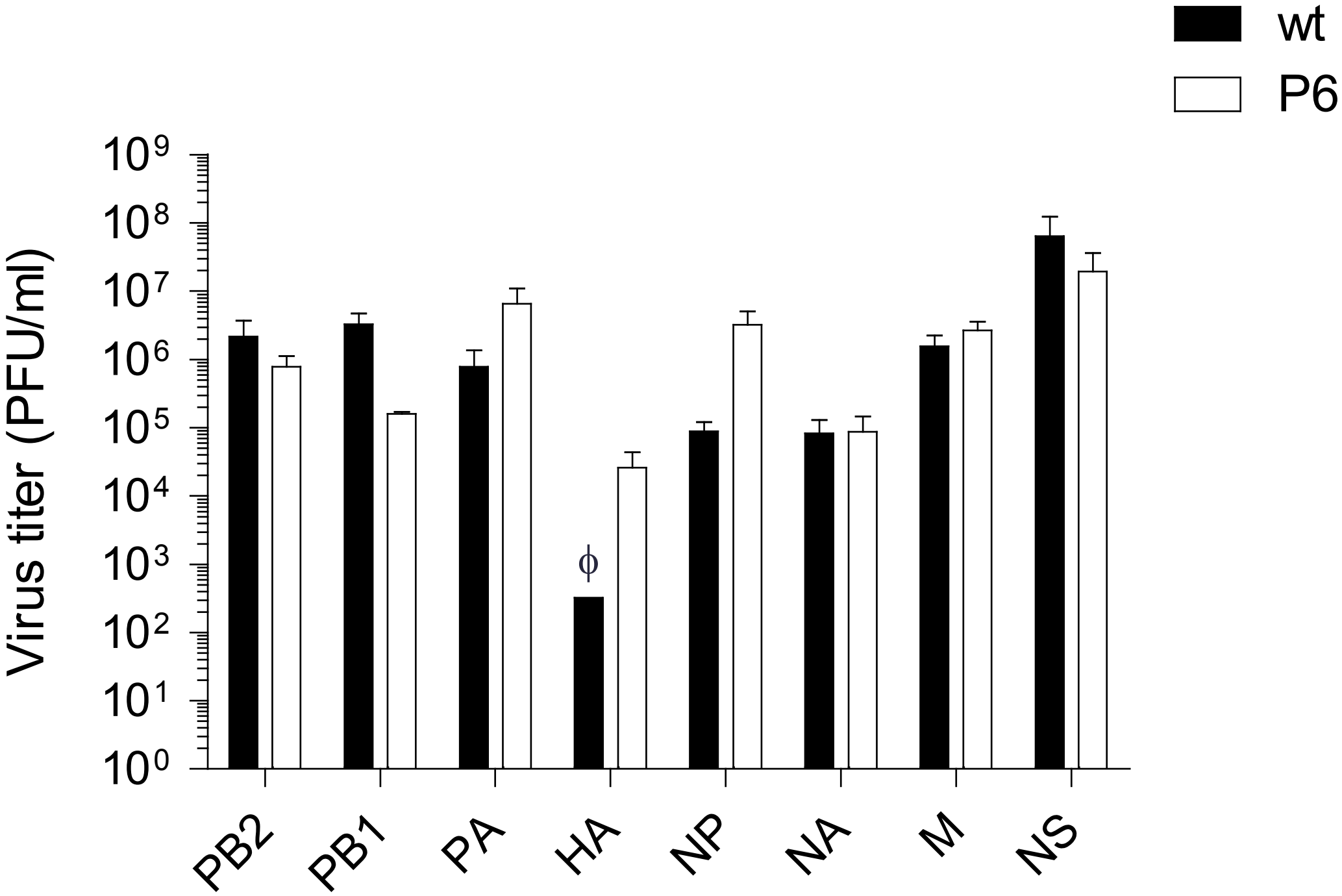


Figure S1: Functional analysis of cloned gene segments of HH/04 wt and HH/04 P6. Each cloned gene segment of HH/04 wt and HH/04 P6 was combined with gene segments of WSN/33 to test if they are able to produce infectious virus particles. 48 h after transfection of MDCK/HEK293T cells, supernatant was transferred onto MDCK cells to quantify virus titer via FFA. The results represent the mean SD for one experiment. φ no SD was determined.

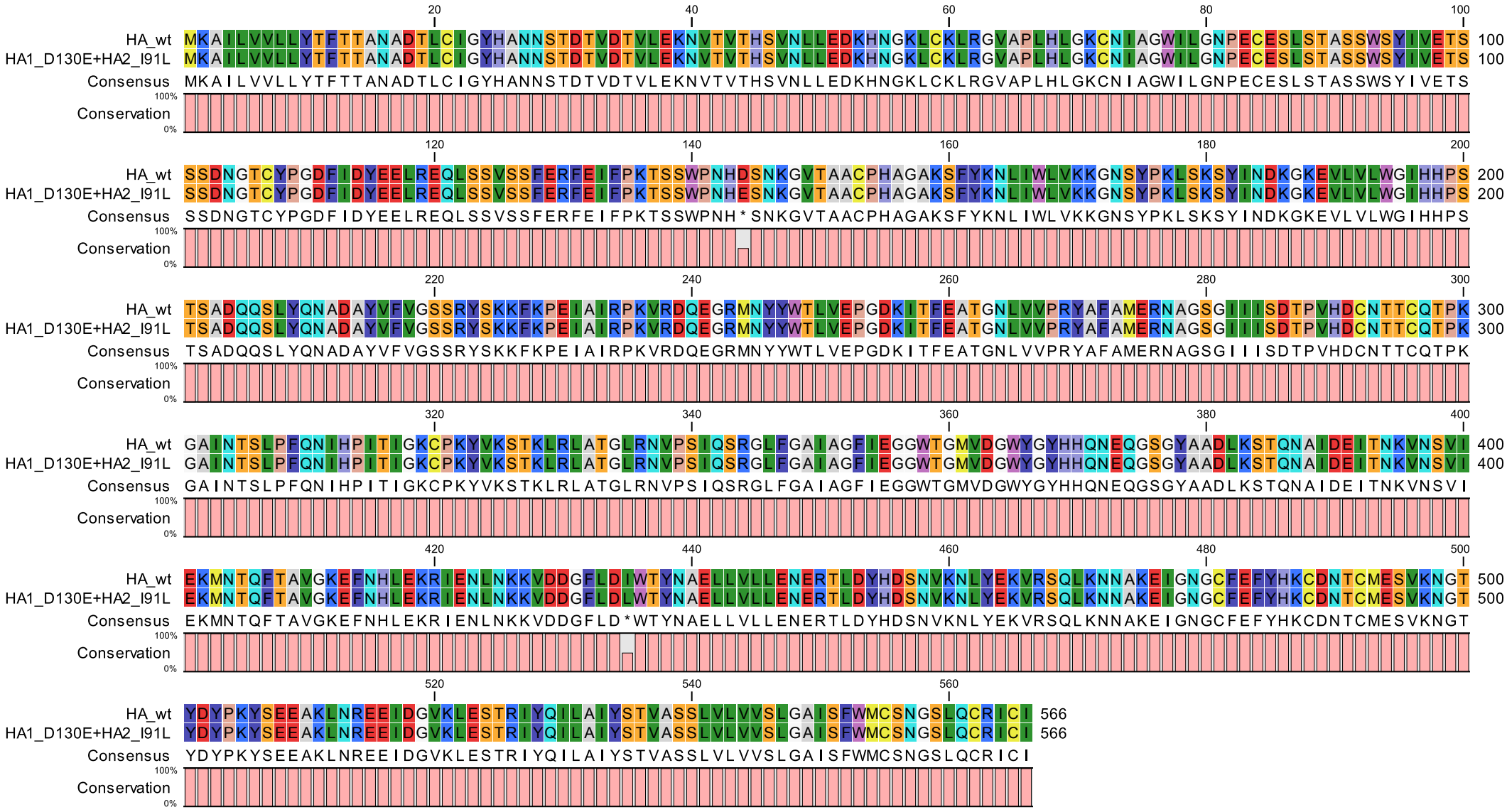


Figure S2: Sequence alignment of the HA gene segment. Alignment of the HA sequences of the HH/04 wt and the double mutant (HH/04 HA1 D130E + HA2 I91L) viruses using the 'CLC Sequence Viewer' (www.clcbio.com).

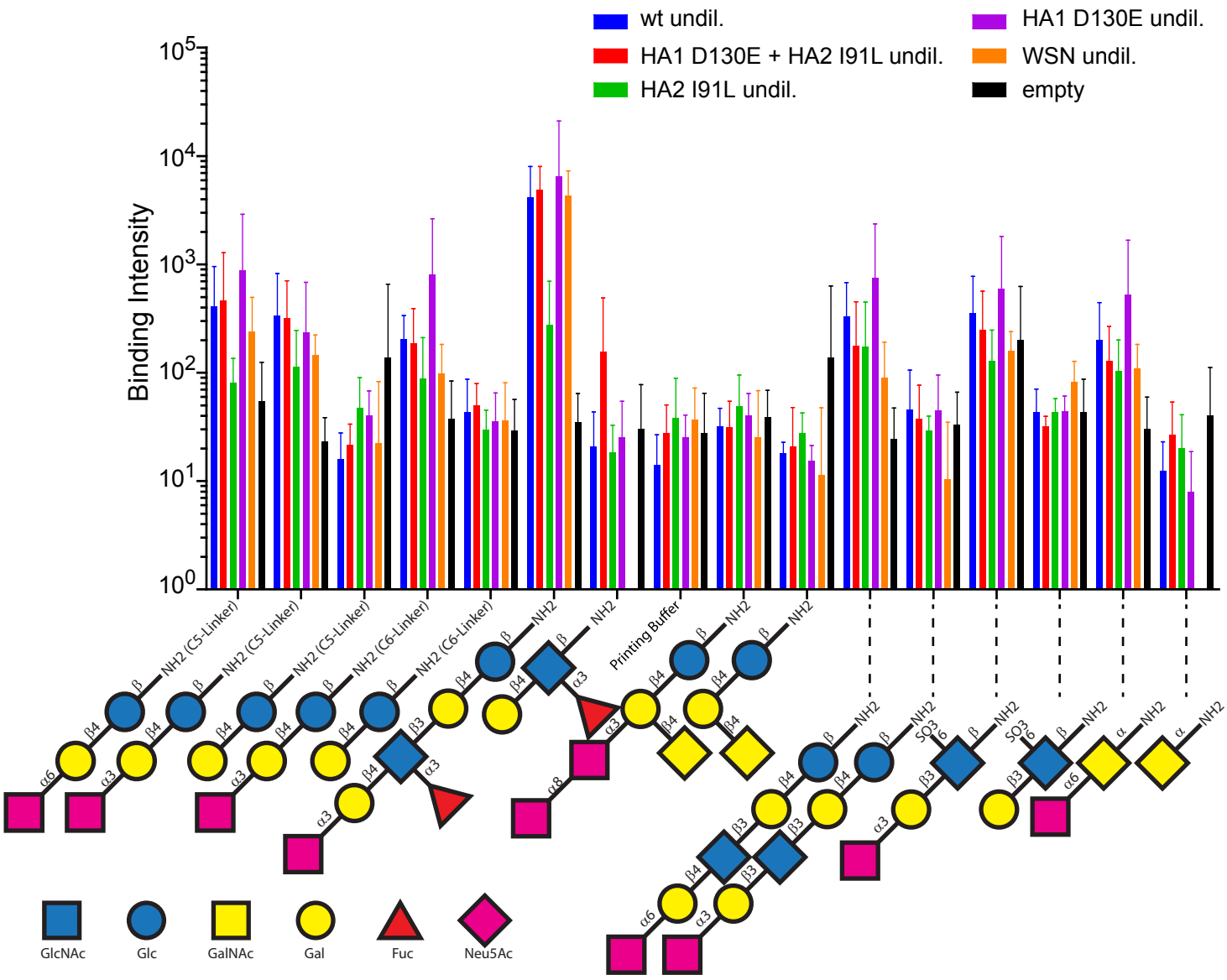


Figure S3: Binding characteristics of the indicated viruses to sialic acid receptors quantified by glycan arrays. Equal amounts of indicated recombinant viruses were bound to glycan arrays previously spotted with 15 different sialic acids or printing buffer as control. Staining of bound viruses was achieved using a NP-specific primary antibody and a Cy3-coupled secondary antibody. The results represent the mean + SD for two independent experiments.

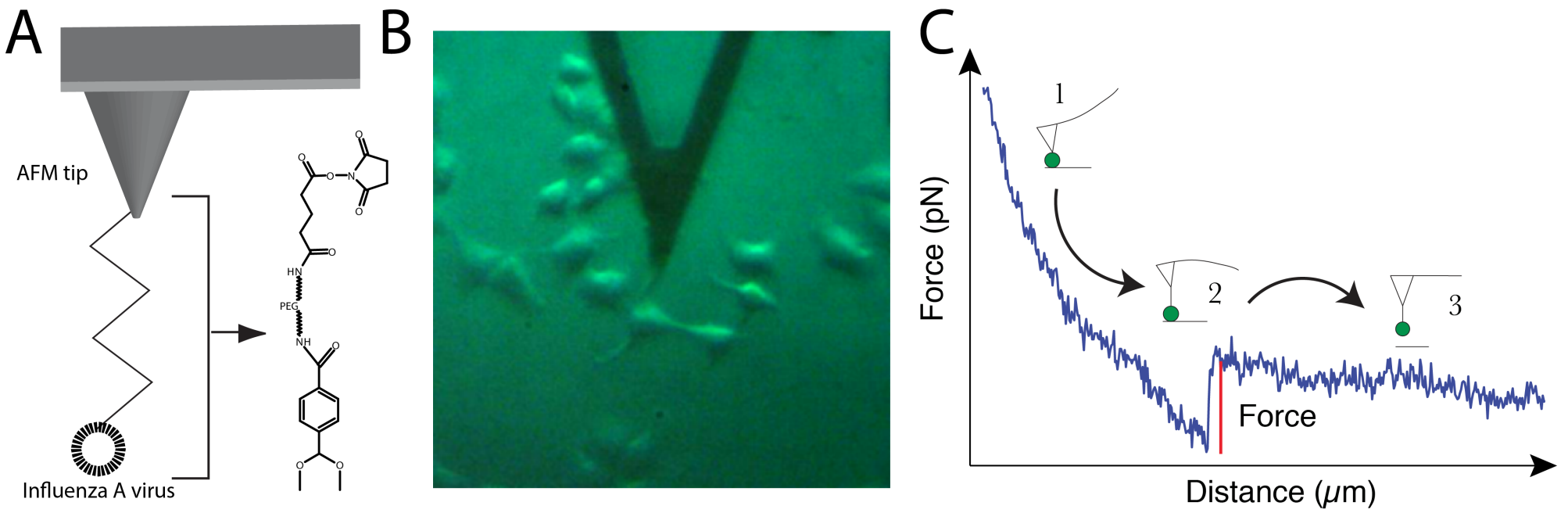
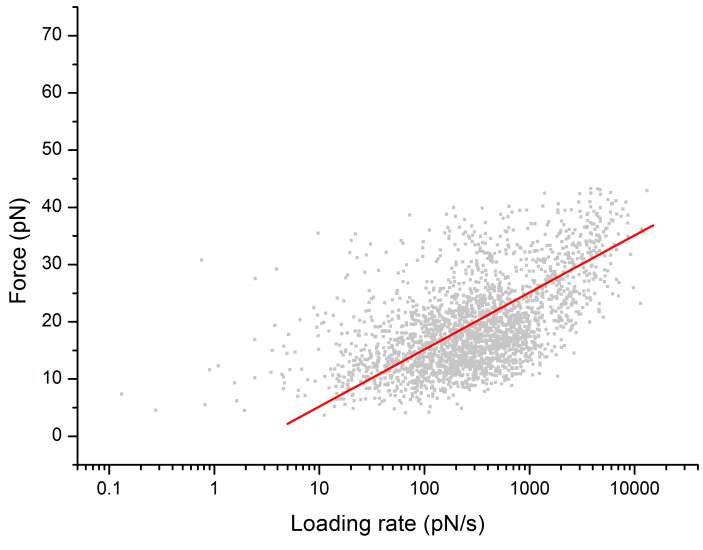
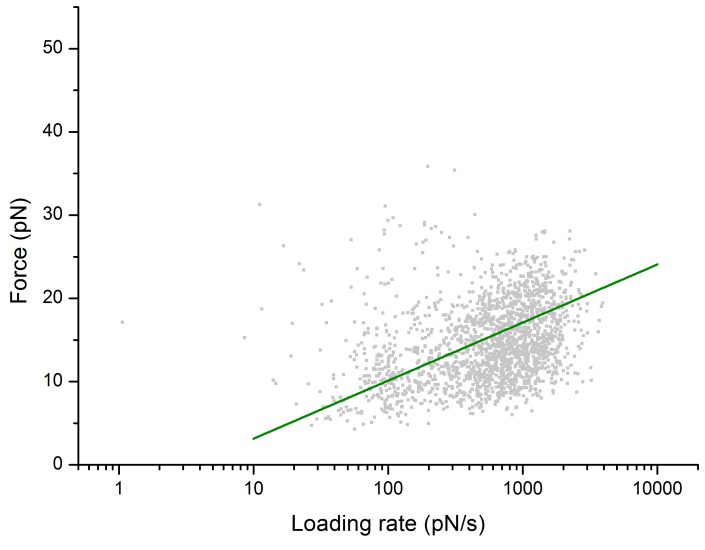
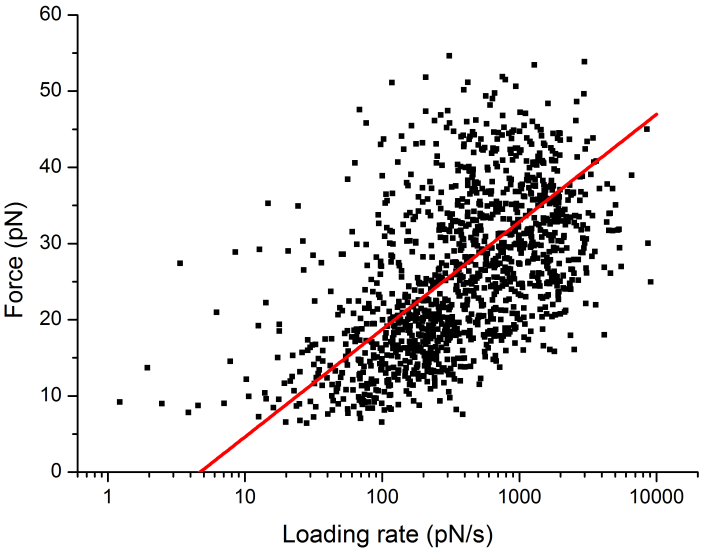


Figure S4: Principle of single-virus force spectroscopy (SVFS) using atomic force microscopy (AFM). (A) For SVFS, influenza A virions are covalently attached to the AFM cantilever using an acetal-PEG800-NHS crosslinker (22). (B) The triangular cantilever is moved on top of a suitable cell using brightfield microscopy allowing precise positioning. The cantilever is then lowered until touching the cell surface. To record a force-distance-cycle (C), the cantilever is retracted at a defined velocity. In case of an interaction, the cantilever will bend towards the sample until the underlying bond fails and the cantilever returns into the zero-force position. The underlying force can be extracted from the force-distance curve (C). C, modified from (34).







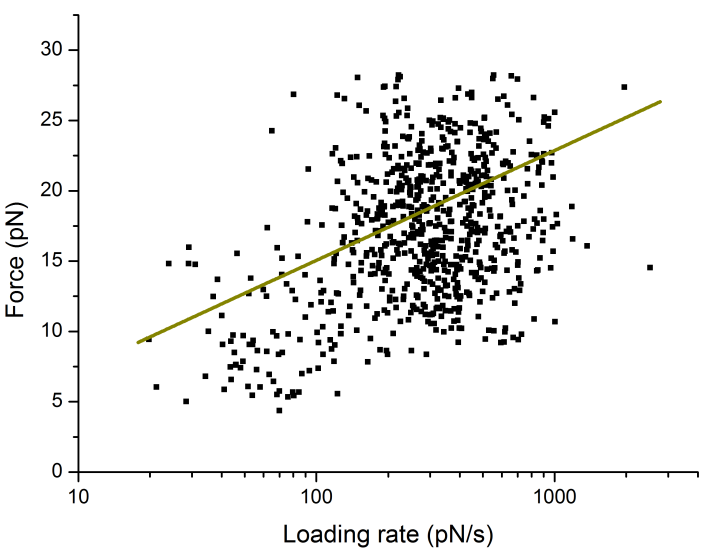


Figure S5: SVFS dynamic force spectra of Wt and HH/04 HA1 D130E + HA2 I91L (Mut) viruses interacting with single receptors on living A549 and CHO cells. Scatter plots showing the unbinding force F plotted against the loading rate r of each individual force curve. Shown are the scatter plots for Wt-A549 (top left), Wt-CHO (top right), Mut-A549 (bottom left) and Mut-CHO (bottom-right). From those data, the values for koff and χu were determined as described in Materials and Methods.