

## Supplementary Material

### **Supplementary Figure S1: Schematic diagram of Defensin-ACE2 constructs. Panel**

**A: Schematic diagram of h-deface2 and p-deface2 primary structure.** Trx-Tag, Histidine-tag and S- tag are highlighted in gray, cyan and green, respectively. h-deface and p-deface amino acid sequences are highlighted in orange. ACE2 inserted residues are shown in red. Cysteine involved in disulfide bridges are indicated with an asterisk. **Panel B: Predicted Three-dimensional structure of h-deface2 and pdeface2.** The hypothetical three-dimensional structure of defensin-based peptides were created using The Phyre2 web portal for protein modeling, prediction, and analysis.  $\beta$ -sheets are represented in yellow,  $\alpha$ -helices are represented in blue and helix-stabilizing disulfides are shown in red.

### **Supplementary Figure S2: Binding of Defensin-ACE2 proteins to SARS-C2V-2**

**Spike protein in the presence and absence of Trx-tag.** Elisa assays were performed using 50 ng immobilized SARS-CoV-2 Spike protein and biotinylated defensin-ACE mimetic in the presence and absence of Trx-tag.

### **Supplementary Figure S3: Effect of liophilization and reconstitution on deface2**

**ability to detect SARS-CoV-2 spike protein.** Elisa assays were performed using 50 ng immobilized SARS-CoV-2 Spike protein and biotinylated defensin-ACE mimetic before and after lyophilization.

**Supplementary Figure S4: Soluble ACE2 binds SARS-CoV2 Spike protein expressing HEK293 cells.** Histogram overlay plots of the Flow-cytometry performed on HEK293 cell-lines expressing either A) Wuhan-Hu-1 (D614) or B) Delta variant (B.1.617.2) SARS-CoV2 Spike proteins using soluble hACE2-Fc and secondary-antibody (PE anti-human IgG-Fc) (Red) or only the PE anti-human IgG-Fc antibody (Blue) as the secondary-antibody control. The parental HEK293 cells were used as control cell line. All cells were also stained with Propidium Iodide (PI) for live-dead cell staining.

**Supplementary Figure S5: Binding titrations using ACE2-defensin peptidomimetics to HEK293 SARS-CoV2 Spike expressing cells.** HEK293 cell-lines expressing Wuhan-Hu-1 (D614) SARS-CoV2 Spike proteins were incubated with either A) h-deface2 or B) p-deface2 biotinylated proteins conjugated to Streptavidin-Phycoerythrin (SA-PE) fluorophore at the indicated concentrations. Histogram overlay plots (upper panel) and the Geometric Mean (FL-2:PE) plotted against the protein concentrations (lower panel) from the Flow-cytometry measurements are presented. SA-PE was used as secondary fluorophore control. All the cells were stained with Propidium Iodide (PI) for live-dead cell staining.

**Supplementary Figure S6: Flow-cytometry analysis of ACE2-defensin peptidomimetics binding to cells expressing Wuhan-Hu-1 (D614) SARS-CoV2 Spike protein.** HEK293 cell-lines expressing Wuhan-Hu-1 (D614) SARS-CoV2 Spike protein was incubated with either h-deface2 or p-deface2 biotinylated proteins (0.1  $\mu\text{g}/\mu\text{L}$ ) conjugated to Streptavidin-Phycoerythrin (SA-PE) fluorophore (upper panel). Parental HEK293 cell-line were also treated similarly with SA-PE conjugated h-deface2 or p-deface2 biotinylated proteins (0.1  $\mu\text{g}/\mu\text{L}$ ) to test the binding specificity of the engineered

proteins (lower panel). Histograms, Geometric mean, and the dot-plots data of the flow-cytometry analysis are presented. SA-PE was used as secondary fluorophore control. All the cells were stained with Propidium Iodide (PI) for live-dead cell staining.

**Supplementary Figure S7: Flow-cytometry analysis of ACE2-defensin peptidomimetics binding to cells expressing Delta (B.1.617.2) SARS-CoV2 Spike protein.** HEK293 cell-lines expressing Delta (B.1.617.2) variant SARS-CoV2 Spike protein was incubated with either h-deface2 or p-deface2 biotinylated proteins (0.1  $\mu\text{g}/\mu\text{L}$ ) conjugated to Streptavidin-Phycoerythrin (SA-PE) fluorophore (upper panel). Parental HEK293 cell-line were also treated similarly with SA-PE conjugated h-deface2 or p-deface2 biotinylated proteins (0.1  $\mu\text{g}/\mu\text{L}$ ) to test the binding specificity of the engineered proteins (lower panel). Histograms, Geometric mean, and the dot-plots data of the flow-cytometry analysis are presented. SA-PE was used as secondary fluorophore control. All the cells were stained with Propidium Iodide (PI) for live-dead cell staining.

Figure S1

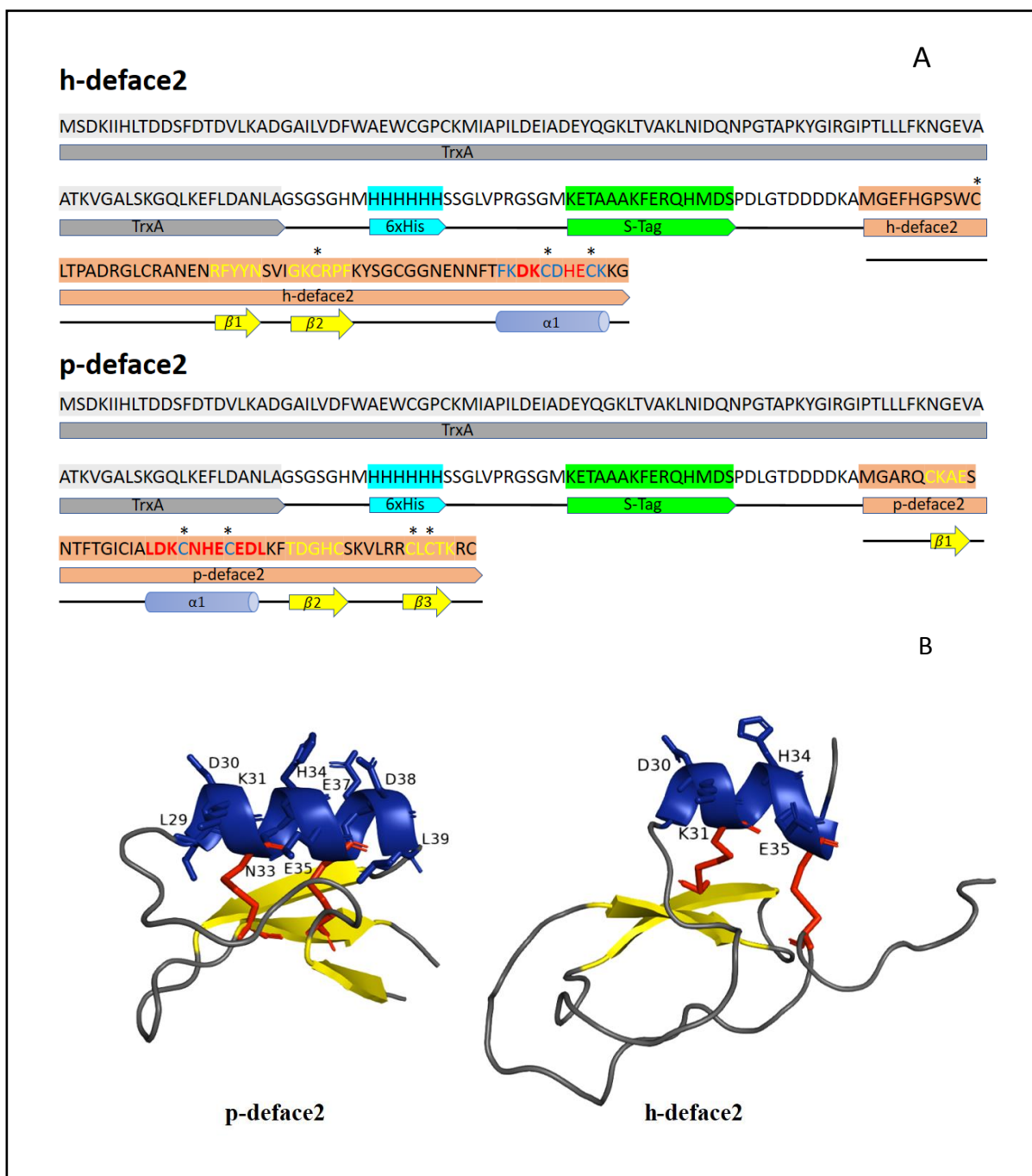


Figure S2

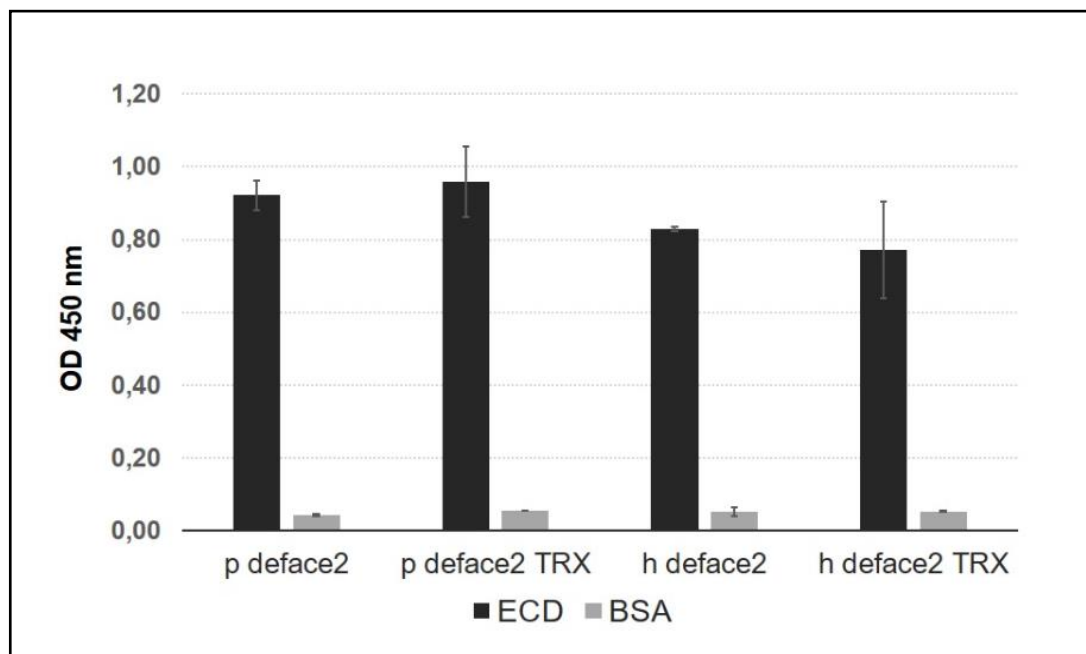


Figure S3

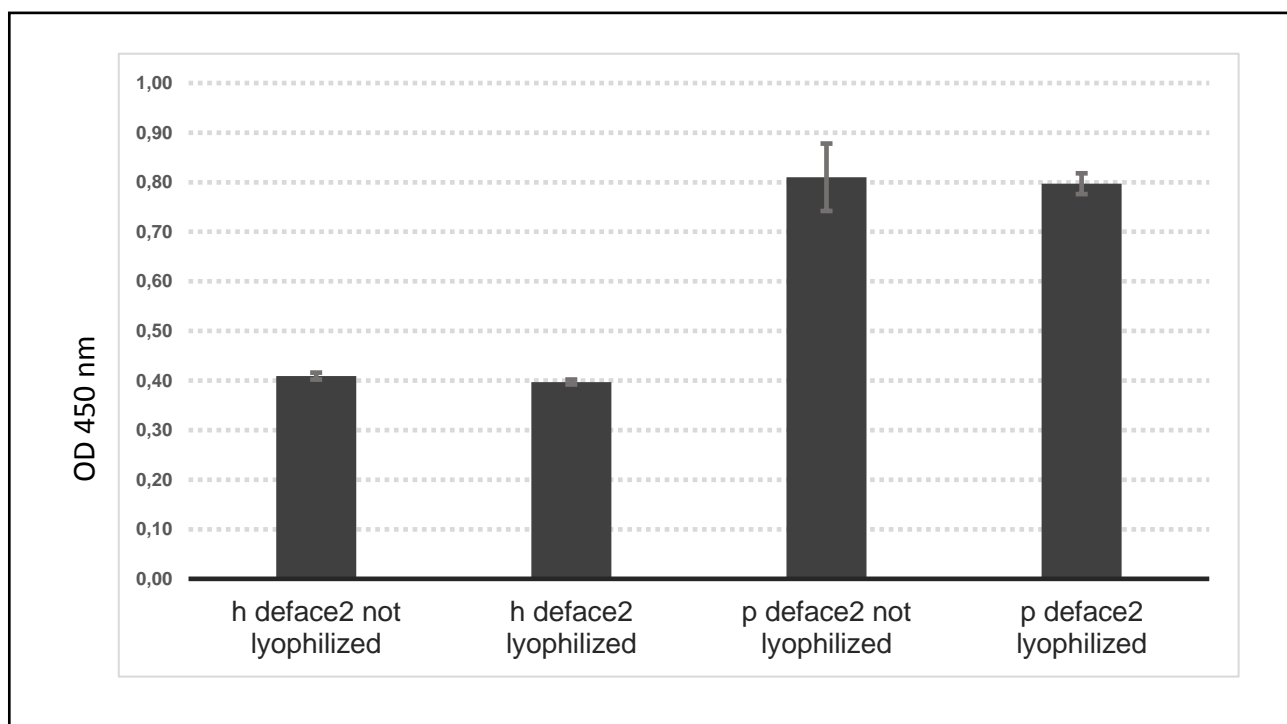


Figure S4

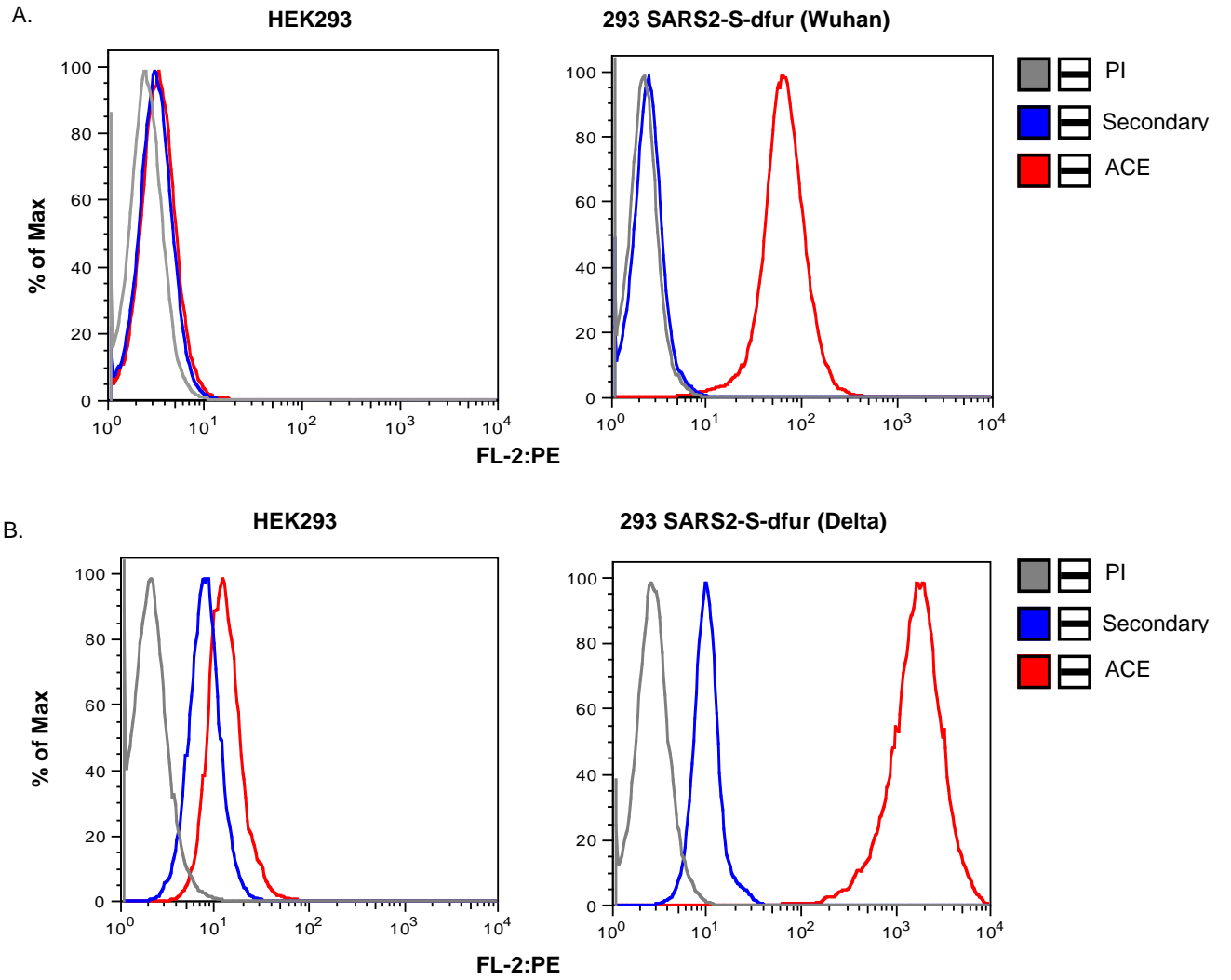


Figure S5

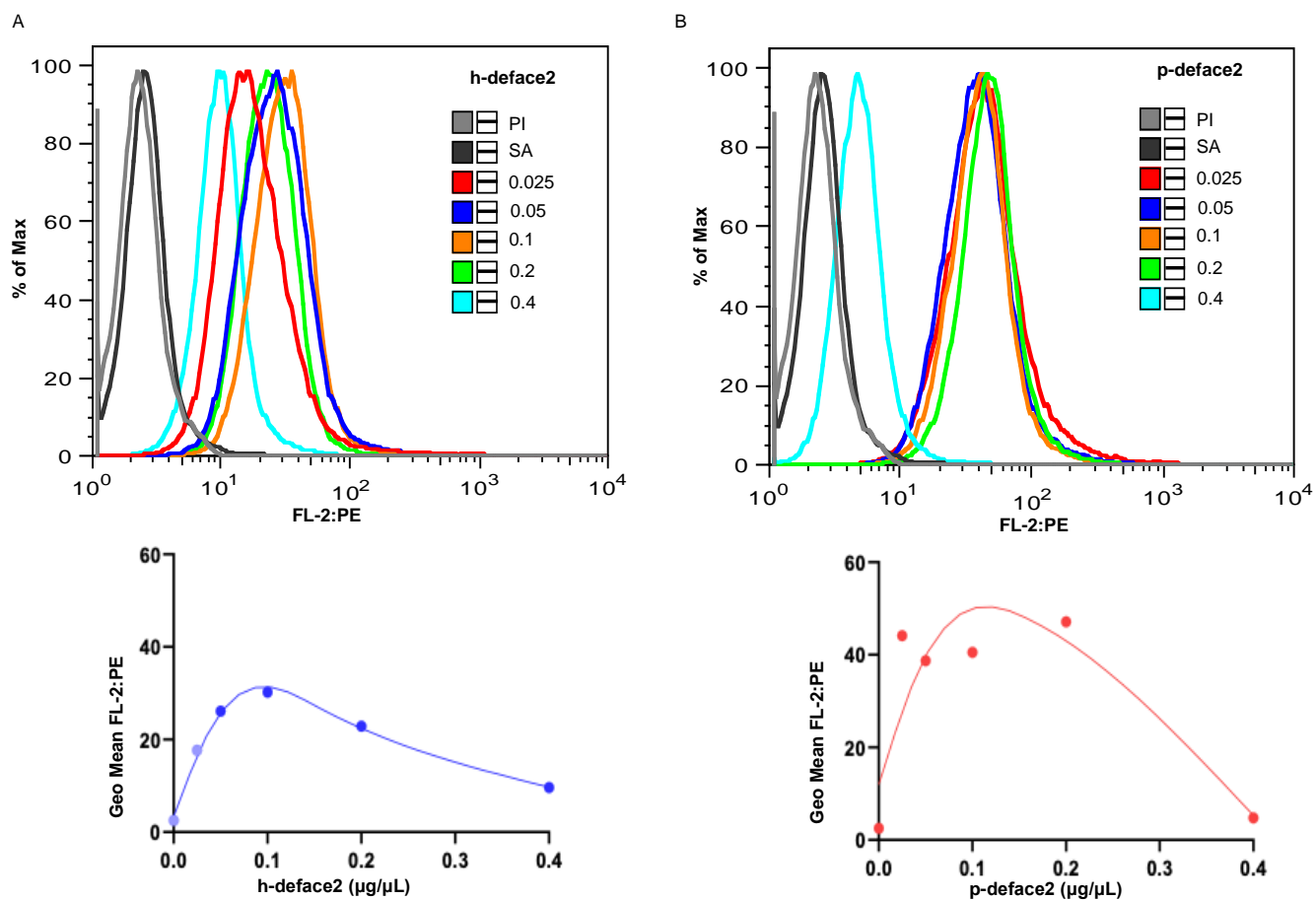




Figure S6

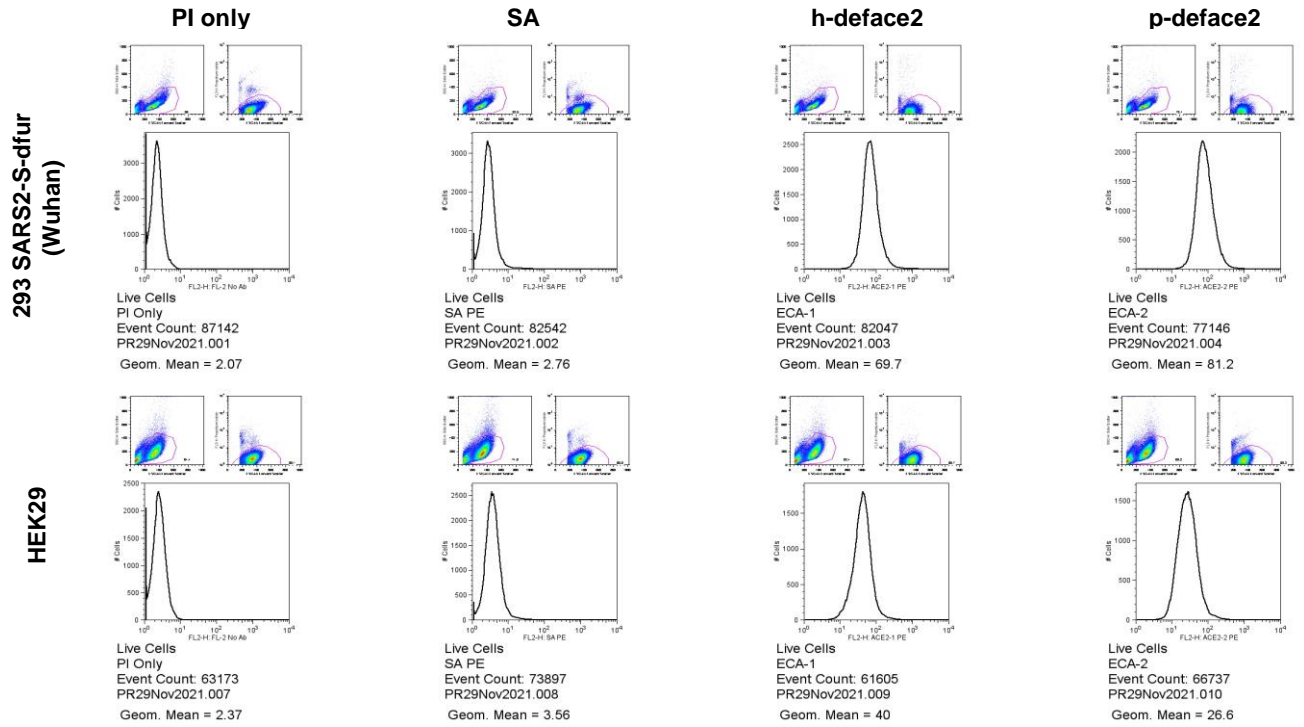


Figure S7

