Supporting Information

Antibody-Mediated Neutralization of Perfringolysin O for Intracellular Protein Delivery

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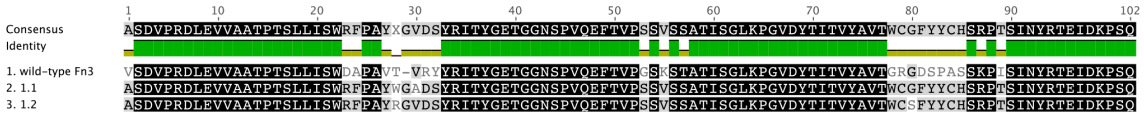

Supplementary Figure 1. Amino acid sequences of the wild-type Fn3 and the engineered PFO binders 1.1 and 1.2. Sequence alignment was performed using Geneious software (Biomatters).

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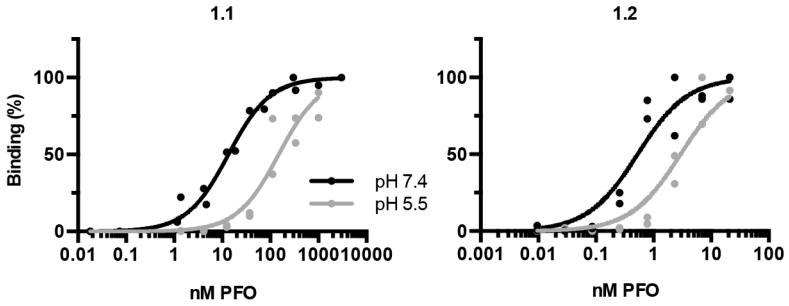
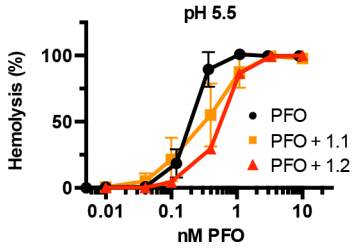
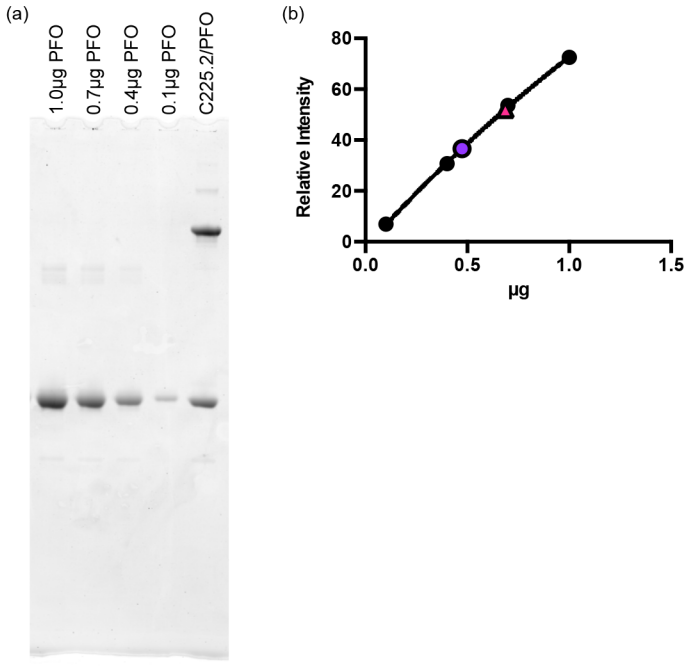


Fn3 clone	Kd, pH 7.4 (nM)	Kd, pH 5.5 (nM)	Fold difference of Kd
1.1	13.5 ± 2.9	129.1 ± 60.6	11
1.2	0.53 ± 0.26	3.0 ± 1.2	6

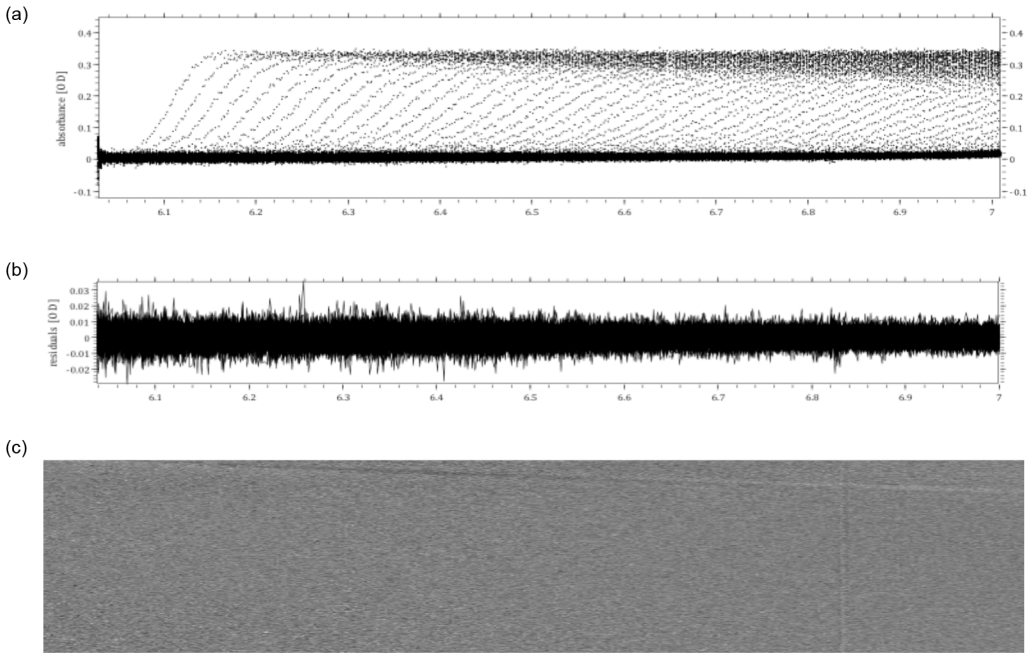
Supplementary Figure 2. The titration curves and fitted K_D values for clones 1.1 and 1.2 at pH 7.4 and 5.5, respectively. The titrations were performed on the yeast surface using biotinylated PFO as previously described [1]. The error ranges in the table denote the 95% confidence interval of the global fit to a monovalent binding isotherm.



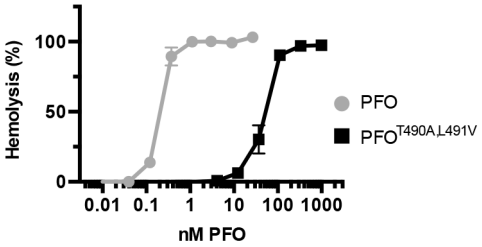
Supplementary Figure 3. Hemolytic activity of PFO at pH 5.5 in the presence of 1.1 (3μM) or 1.2 (300nM), following the same procedures as described for Figure 1b.



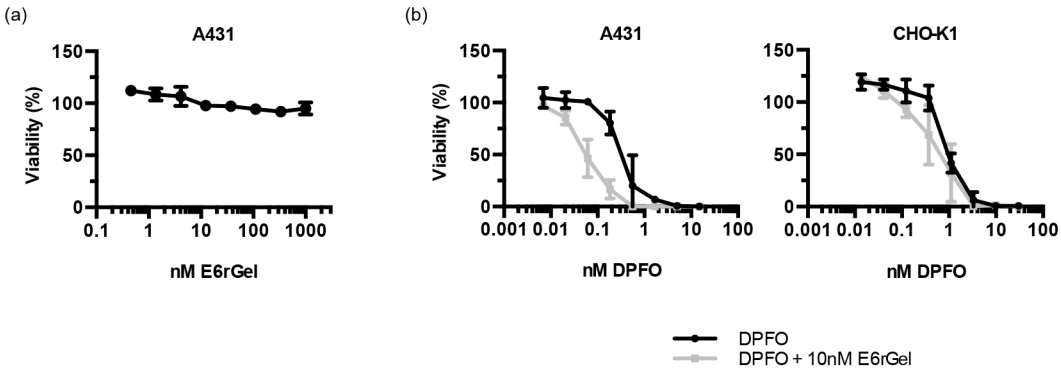
Supplementary Figure 4. (a) Non-reducing SDS-PAGE of C225.2/PFO and PFO stained with SYPRO orange and scanned on the Typhoon Trio imager. The lower bands are PFO, the upper band C225.2. **(b)** Quantification of the protein gel in panel a using Image J. The interpolated values for C225.2 (pink triangle) and PFO (purple circle) are plotted on the standard curve (black) generated from fitting the median intensities of the bands containing known amounts of PFO (black circles).



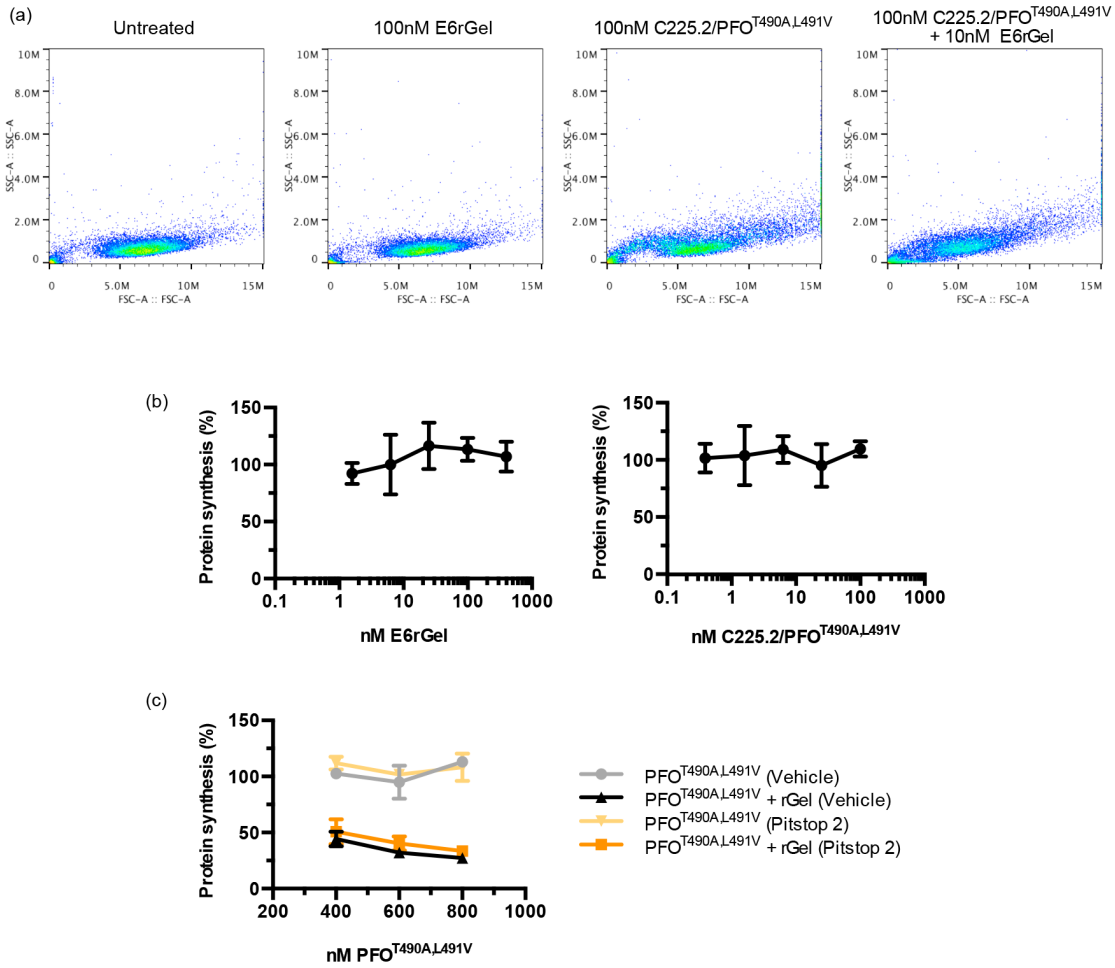
Supplementary Figure 5. The raw absorbance data collected from the sedimentation velocity AUC run of the C225.2/PFO complex **(a)**, with the residuals **(b)** and residual bitmap **(c)** of the fit. The rmsd was 0.004907.



Supplementary Figure 6. Comparison of the hemolytic activities of PFO and $PFO^{T490A,L491V}$, following procedures described in Materials and Methods. The measured values were normalized to that of the positive control (1% Triton X-100).



Supplementary Figure 7. Viability of the indicated cell lines treated with E6rGel alone **(a)**, or in combination with DPFO **(b)**, overnight at 37ºC. DPFO is a fusion protein consisting of the Fn3 clone D [2], which targets EGFR, and PFO [3]. Viability was measured using the WST-1 reagent following procedures described in Materials and Methods. The measured values were normalized to that of untreated cells.



Supplementary Figure 8. (a) Forward and side scatter of A431 cells treated with E6rGel (100nM), C225.2/PFO^{T490A,L491V} (100nM) or the combination of both (100nM C225.2/PFO^{T490A,L491V} + 10nM E6rGel) for 2 hours at 37ºC. The cells were washed with PBS, trypsinized and analyzed on an Accuri C6 cytometer. **(b)** Protein synthesis levels of A431 cells treated with E6rGel or C225.2/PFO^{T490A,L491V} alone for 2 hours at 37ºC, normalized to that of untreated cells. **(c)** Protein synthesis levels of A431 cells treated with PFO^{T490A,L491V} alone or in combination with 100nM rGel for 45 minutes at 37ºC, following procedures as described for Figure 5b.

References

(1) Hackel, B. J.; Kapila, A.; Dane Wittrup, K. Picomolar Affinity Fibronectin Domains Engineered Utilizing Loop Length Diversity, Recursive Mutagenesis, and Loop Shuffling. *J. Mol. Biol.* **2008**, *381*, 1238–1252.

(2) Hackel, B. J.; Neil, J. R.; White, F. M.; Wittrup, K. D. Epidermal Growth Factor Receptor Downregulation by Small Heterodimeric Binding Proteins. *Protein Eng. Des. Sel.* **2012**, *25*, 47–57.

(3) Liu, D. V.; Yang, N. J.; Wittrup, K. D. A Nonpolycationic Fully Proteinaceous Multiagent System for Potent Targeted Delivery of siRNA. *Mol. Ther. — Nucleic Acids* **2014**, *3*, e162.