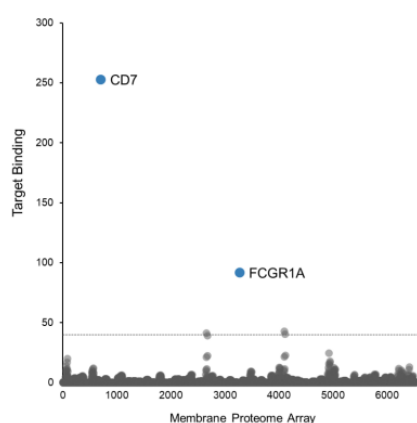


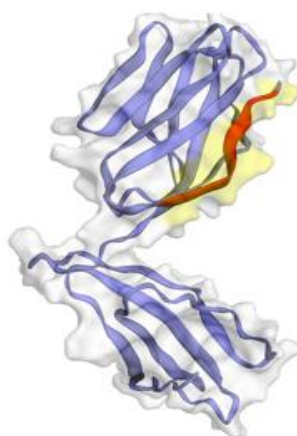
A

Clone	$k_a M^{-1}s^{-1}$	$k_d s^{-1}$	KD M
VHH6	$4.925 \times 10^5$	$7.685 \times 10^{-5}$	$1.560 \times 10^{-10}$
HuVHH6	$2.129 \times 10^5$	$1.223 \times 10^{-4}$	$5.745 \times 10^{-10}$

B



C



**Figure S1. Kinetic binding properties and specificity profiling of humanized CD7 nanobody (HuVHH6)**

(A) Binding and dissociation kinetics ( $k_a$  and  $k_d$ ) of HuVHH6 and equilibrium dissociation constants (KD) of the CD7 binders. Experiments were repeated independently, and representative data is shown in the table. (B) Specificity of HuVHH6 profiled using Membrane Proteome Array (MPA), which includes 5,344 membrane proteins (representing over 90% of the human membrane proteome). The platform uses flow cytometry to directly detect antibodies binding to membrane proteins expressed in unfixed cells, which contains three procedures: determination of assay screening conditions, membrane proteome array screen and validation of antibody targets. (C) Tertiary structures of HuVHH6 have been predicted through Protein Homology/analogy Recognition Engine V 2.0 (Phyre<sup>2</sup>) with amino acid sequences. Mapped epitope is highlighted in red.