

Synthetically-derived PD-1 antagonising VHHs bind in monomer and multimer formats to PD-L1-expressing breast cancer cells

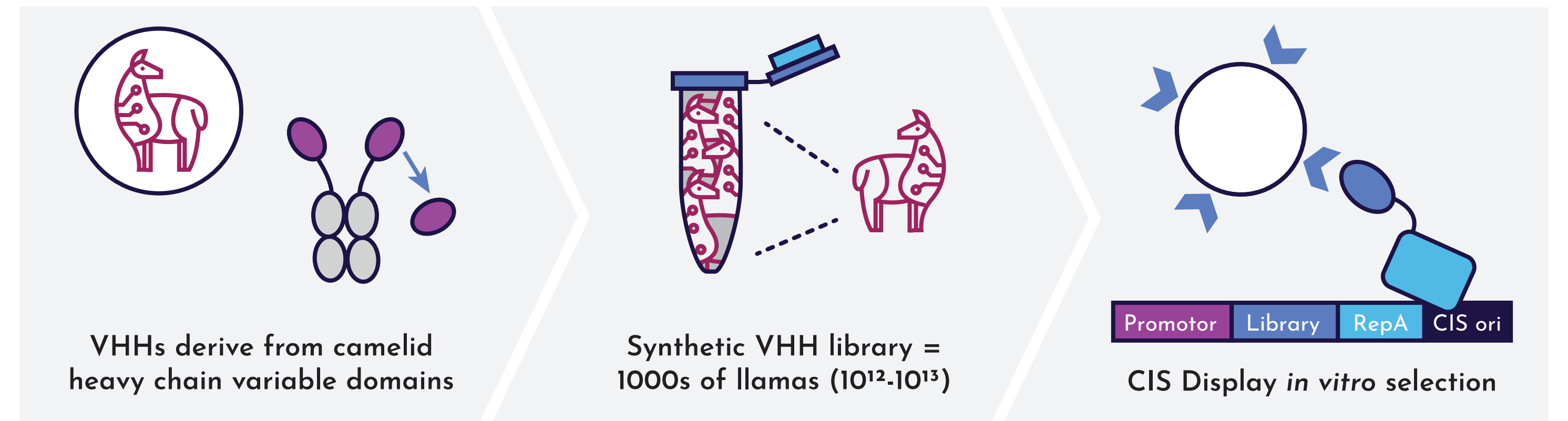
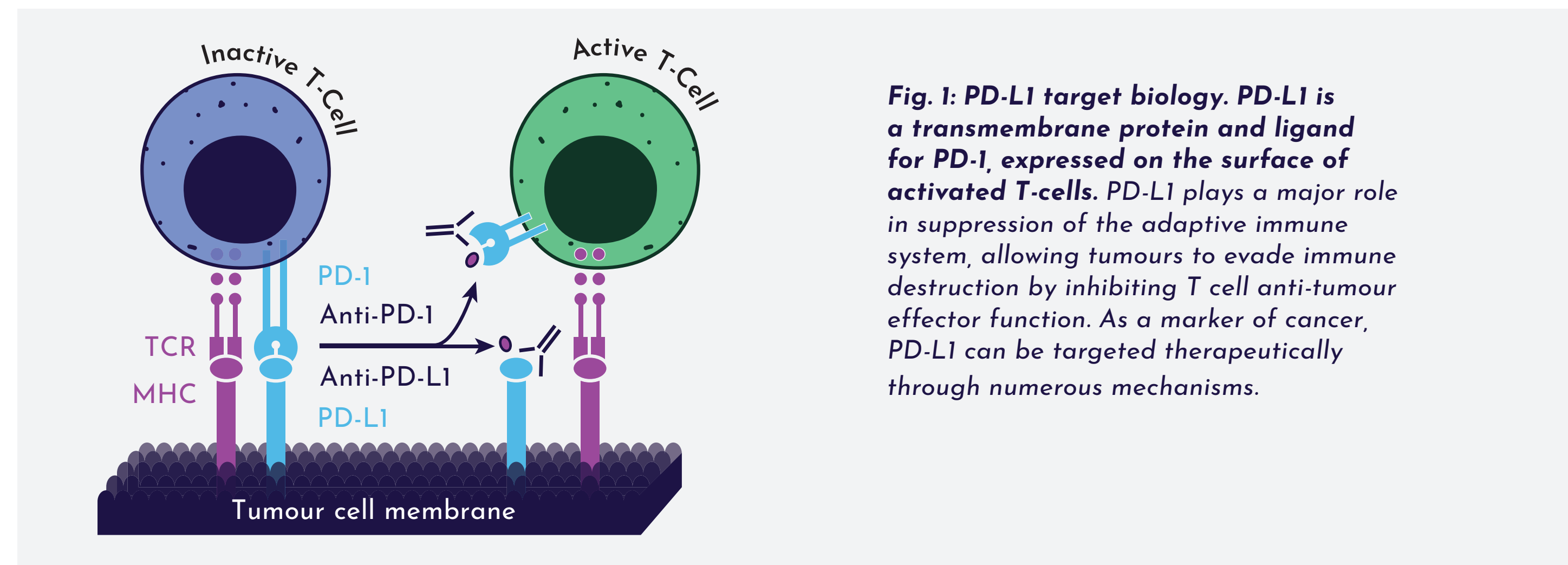
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ABSTRACT PD-L1 (programmed death-ligand 1) is expressed on the surface of many tumour cells. It is the ligand for PD-1, expressed on immune cells, and can contribute to T-cell inactivation, shutting down anti-tumour immune responses. Clinical mAbs against PD-L1 such as durvalumab (Imfinzi) block the PD-1/PD-L1 interaction, facilitating tumour cell killing (Figure 1). However, other therapeutic applications for anti-PD-L1 antibodies include tumour targeting of other moieties including ADC, imaging agents, other antibody fragments or CAR-Ts. Due to their small size and biophysical robustness, VHH single domain antibodies are ideally suited for targeting tumour-associated antigens such as PD-L1, as they can be formatted in many different ways.

Here, we present the isolation of synthetic VHHs binding to PD-L1 expressed on the breast cancer cell line MDA-MB-231. These VHHs were isolated from a vast 10^{12} - 10^{13} library using CIS display technology identifying 97 unique hits. The final panel of clones displayed good biophysical properties and specific low nM binding to PD-L1 expressed on MDA-MB-231 cells, retaining binding after formatting to bi- and tri-specific constructs. Varying antagonism profiles were observed indicating targeting of different PD-L1 epitopes, and many clones showed stronger antagonism compared with a Fab fragment of durvalumab. This demonstrates the utility of synthetic VHH libraries in therapeutic antibody discovery.

1. LLAMDA™ LIBRARY AND CIS DISPLAY SELECTIONS

Rapid isolation of high affinity antibodies to biologically relevant epitopes is critical for biologic discovery, and developability of candidates can make the difference for molecule progression. Less standard is isolation of antibodies pre-screened and depleted for manufacturing liabilities and developability issues, such as glycosylation and isomerization, as is the case for Isogenica's Llamda™ library. This vast, liability-depleted library ($>10^{12}$) was enriched for PD-L1 binders using Isogenica's cell-free CIS Display technology based on ITT and PCR using an in-house Fc-tagged version of the human PD-L1 extracellular domain.

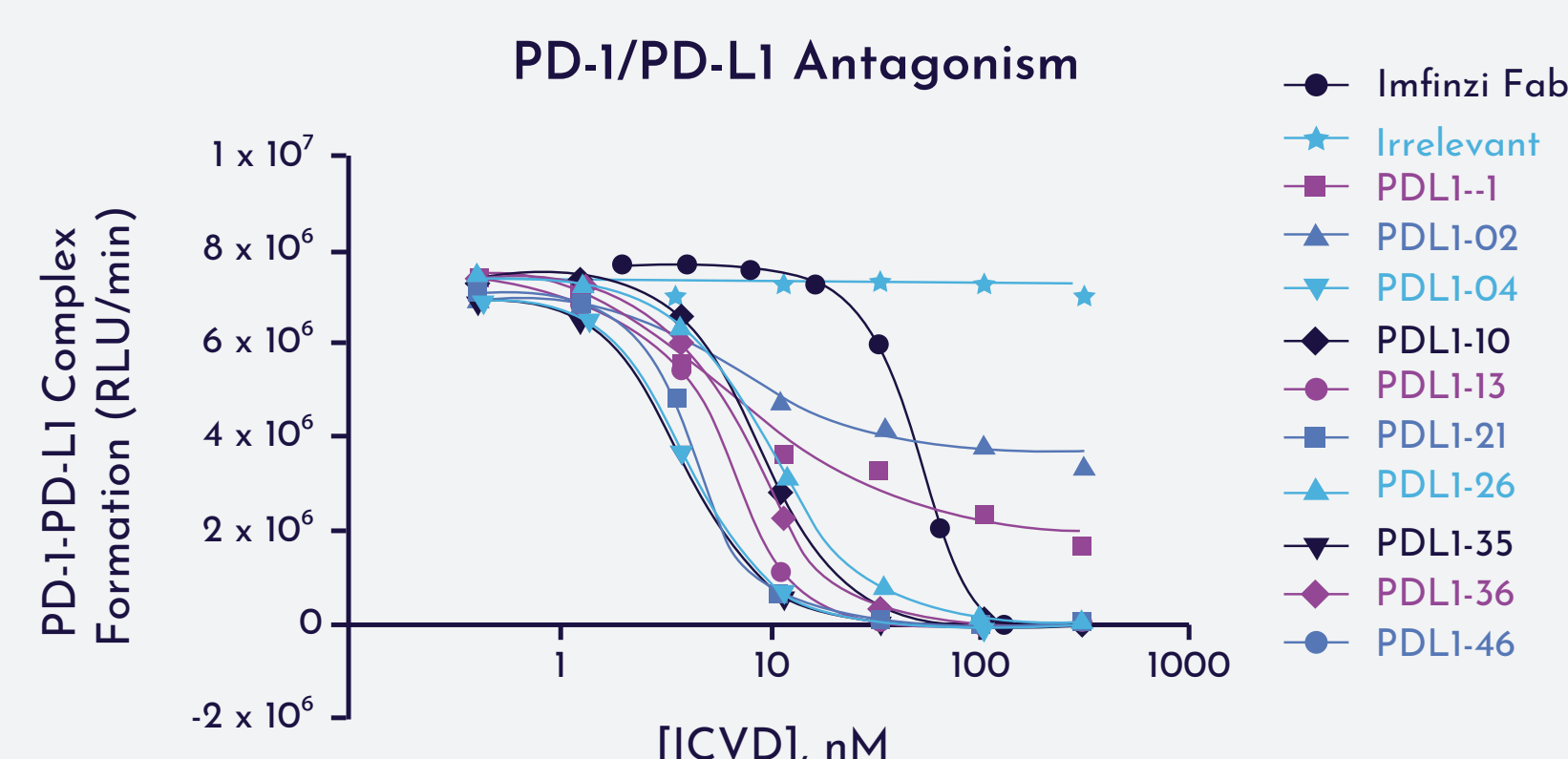
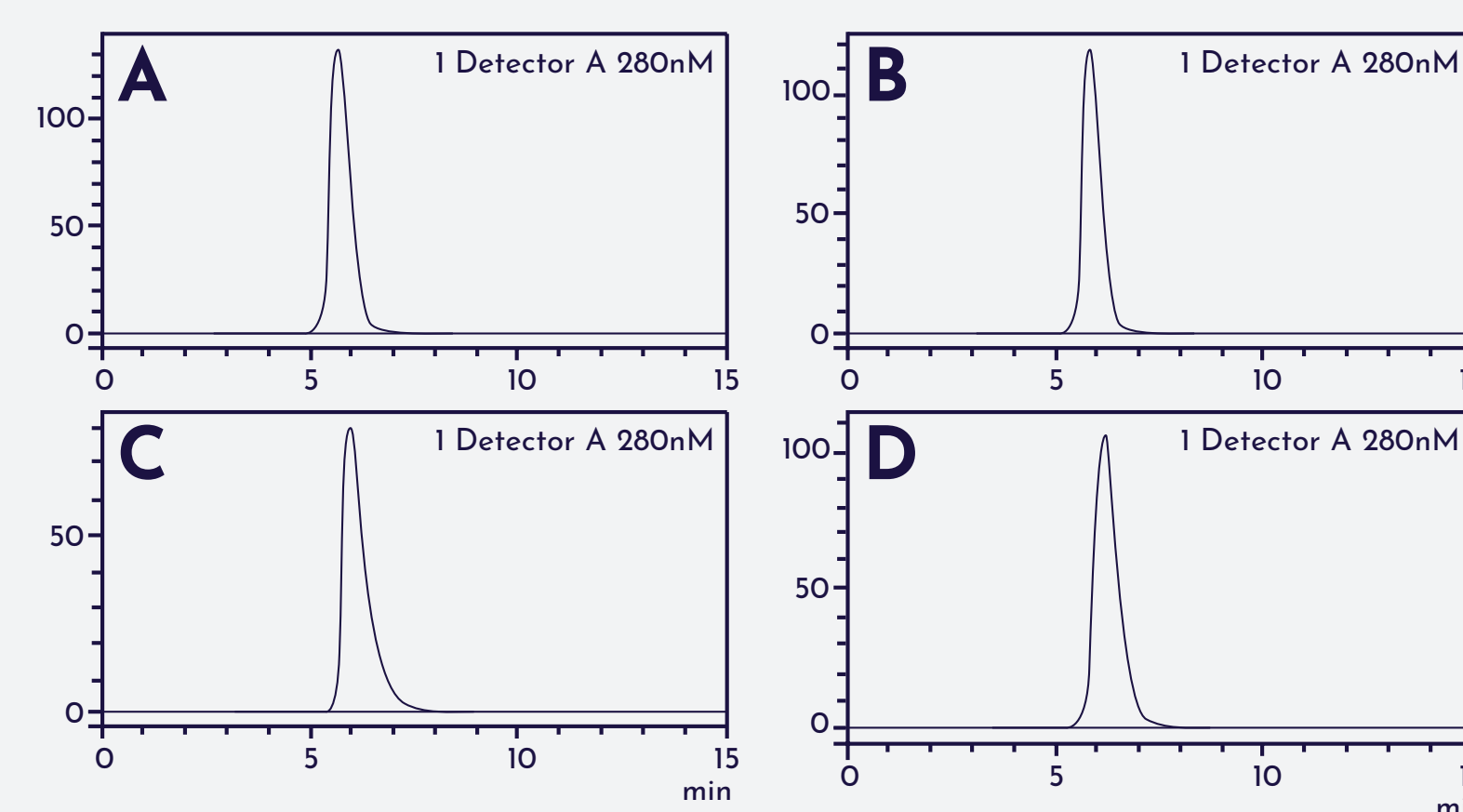
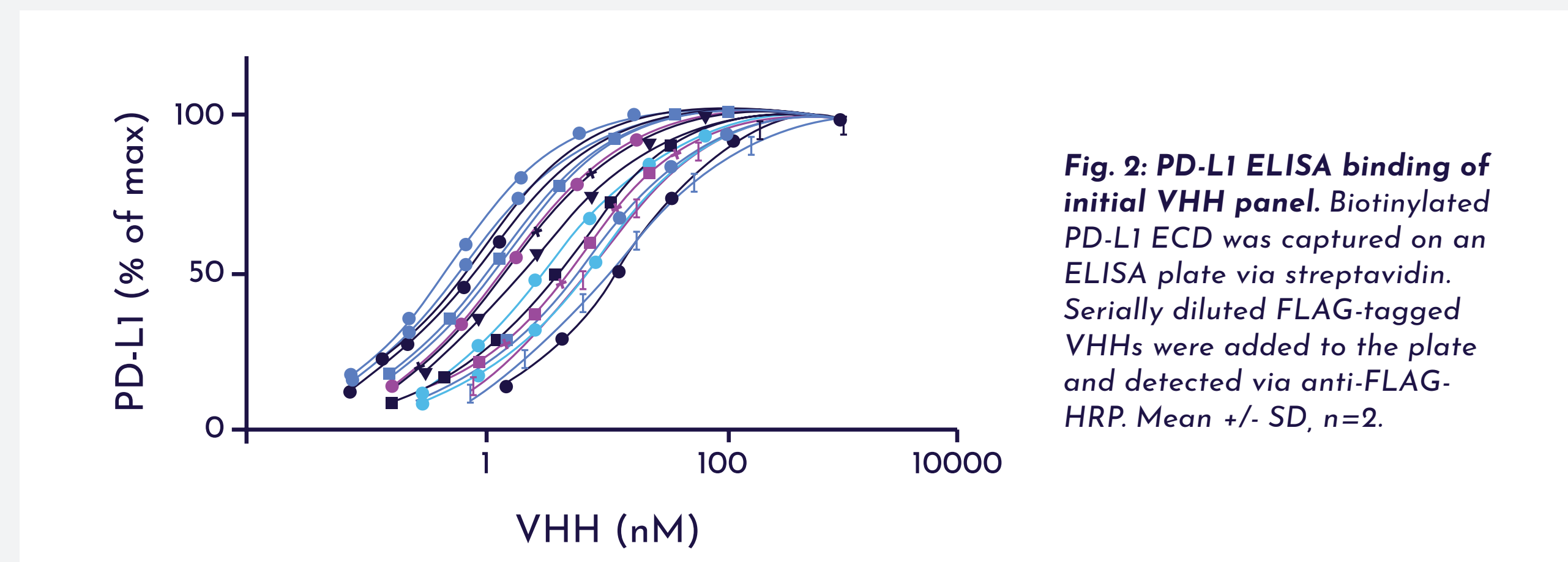


CDR3 length	#uniques
5-9	33
10-14	14
15-18	6
19-22	20
Pooled	24
TOTAL	97

Libraries with CDR3 regions varying in length (5-9, 10-14, 15-18, or 19-22 amino acids) each generated high hit rates (29-95%) against the target in a binding ELISA, compared with an off-target control, resulting in a pool of 97 unique hits with varied CDR3 lengths (see Table).

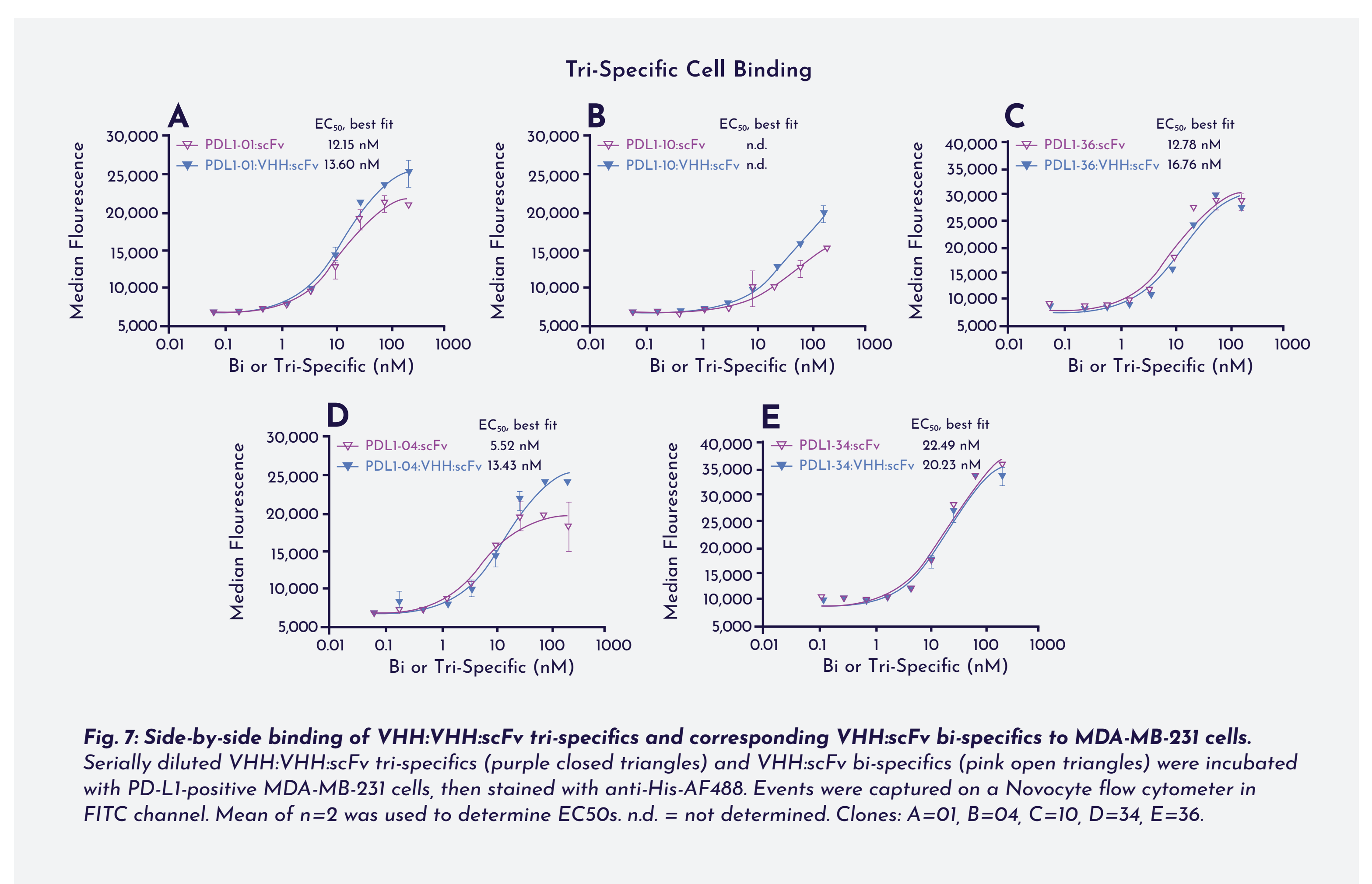
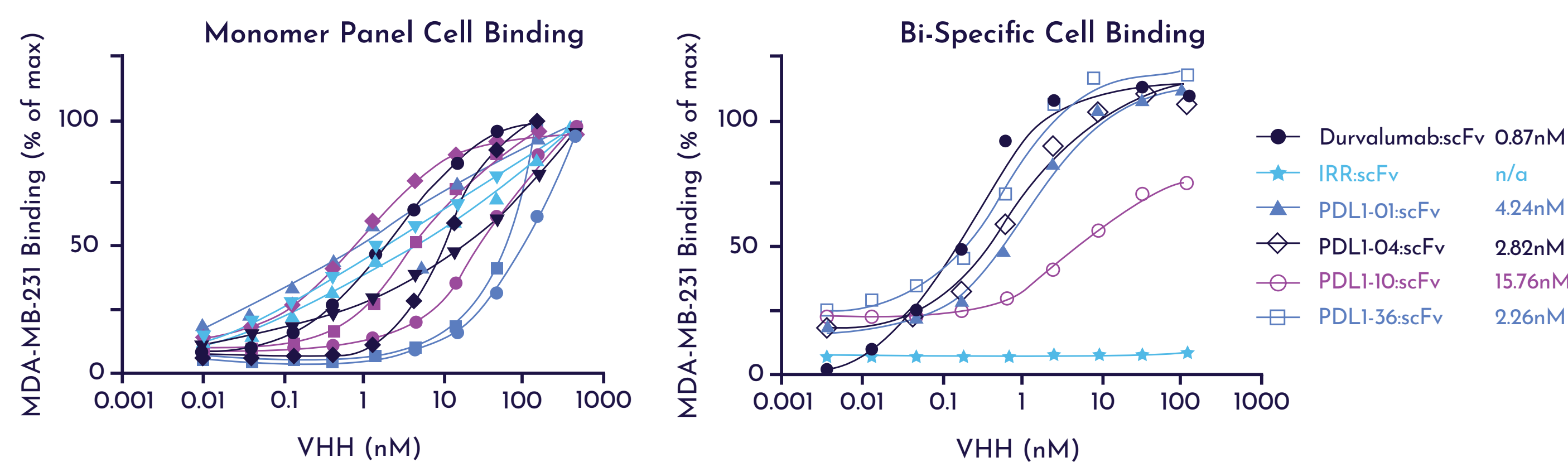
2. SYNTHETIC LIBRARY HTP SCREENING GENERATED POTENT, DEVELOPABLE ANTI-PD-L1 VHH MONOMERS

Unique hits from primary ELISA screening were purified and titration ELISAs showed a range of EC50s in the lead panel from 14.4 to 0.5 nM (Figure 2). Size-exclusion chromatography (SEC) allowed identification of clones within the panel showing extremely strong monomeric peaks with little to no aggregation or fragmentation (Figure 3). Testing of the lead panel in a PD-1/PD-L1 functional ELISA identified subsets of clones capable of either full or partial antagonism of this interaction, with full antagonists more effective than a durvalumab (Imfinzi) Fab fragment (Figure 4). As well as being of therapeutic relevance, this highlights the targeting of multiple epitopes on PD-L1.



3. SPECIFIC BINDING TO MDA-MB-231 CELLS IS RETAINED IN BI- AND TRI-SPECIFIC FORMATS

Cell binding was assessed by flow cytometry on PD-L1 positive (MDA-MB-231) and PD-L1 negative (MCF7) cell lines. A panel of clones was identified showing strong and specific binding to MDA-MB-231 cells. These clones were formatted as bi-specifics, joined to an scFv portion, and as tri-specifics joined to another VHH and an scFv (both binding additional different targets) by 2xG4S linkers. A Fab fragment of durvalumab, and an irrelevant (off-target) VHH were included as positive and negative controls, respectively, illustrating retention of specific, PD-L1 binding functionality for a subset of clones in both bi- and tri-specific formats.



Anti-PD-L1 VHHs were identified from Isogenica's Llamda™ libraries with the ability to bind strongly and specifically to cell membrane-expressed PD-L1 with < 5 nM EC50s and potently inhibit PD-1/PD-L1 interactions. Due to their strong biophysical profiles and ability to format with other moieties, these clones have the potential to be used in a wide variety of diagnostic and therapeutic applications such as ADCs, radioimaging, or CAR-T.