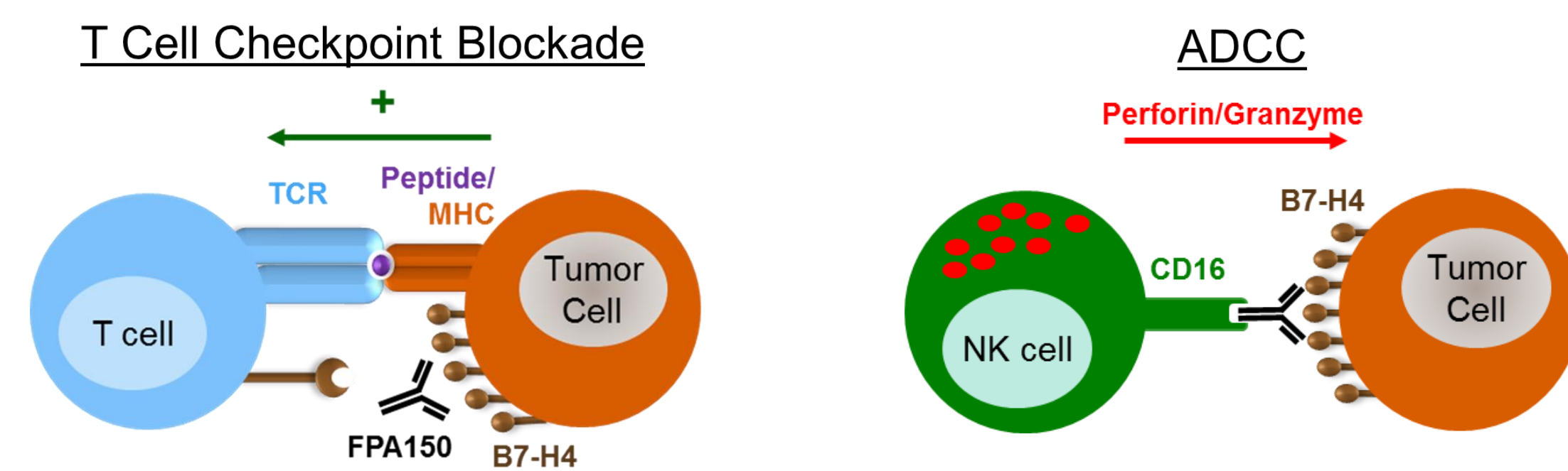




## Introduction

B7-H4 (B7x, B7-S1 and VTCN1) is a type I transmembrane protein comprised of both IgV and IgC ectodomains. While B7-H4 expression in healthy tissues is relatively limited at the protein level, B7-H4 is expressed in several solid tumors such as carcinomas of the breast, ovary and endometrium, and expression tends to correlate with poor prognosis. The receptor for B7-H4 is unknown yet is believed to be expressed on T cells as B7-H4 is described as a ligand capable of directly inhibiting T cell activity. Given its preferential overexpression pattern in solid tumors, its negative correlation with patient outcome, and its role as a T cell checkpoint, B7-H4 appears to be an ideal target for the development of a therapeutic antibody. Here we sought to generate B7-H4 monoclonal antibodies (mAbs) that would both block the inhibitory activity of B7-H4 against T cells as well as directly lead to the depletion of B7-H4 expressing cells via antibody-dependent cell-mediated cytotoxicity (ADCC).

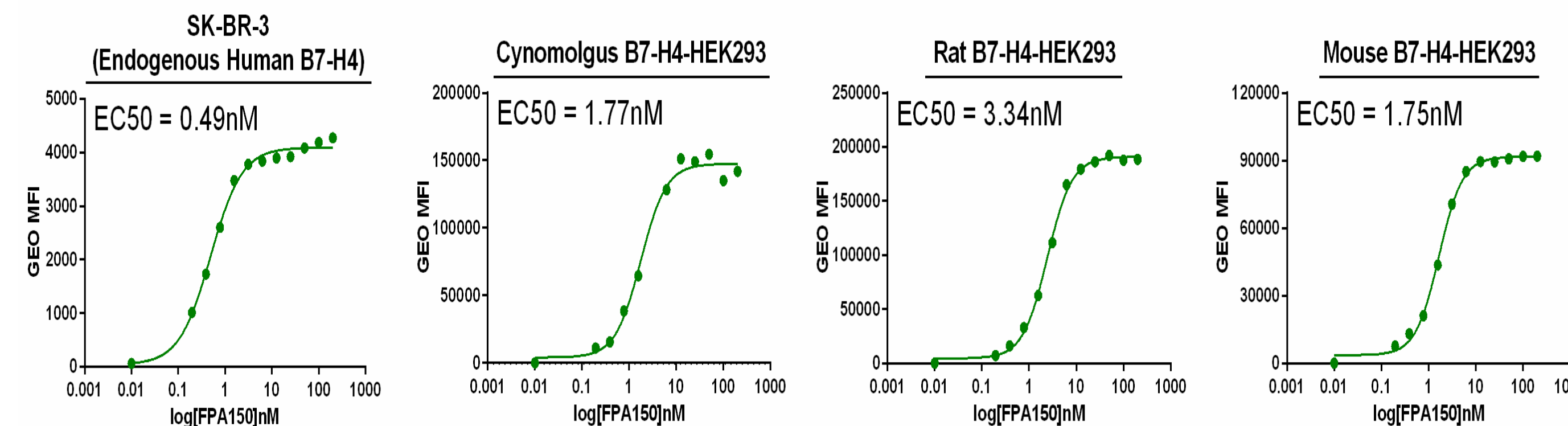


**Figure 2.** FPA150 is a High Affinity, Epitope Bin 2/3 Derived mAb that Binds the B7-H4 IgV Ectodomain and is Fully Species Cross-Reactive

**FPA150 B7-H4 Protein Binding Affinity (SPR)**

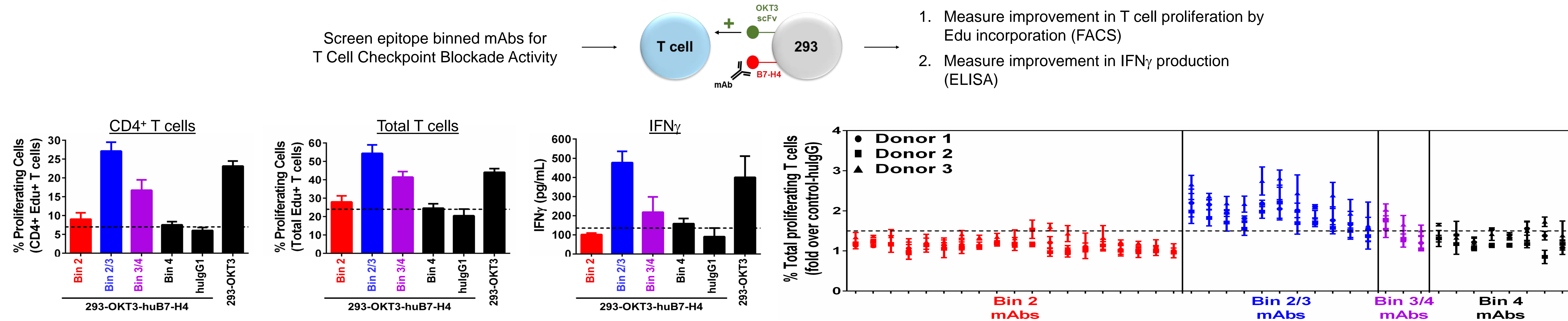
Species	Target	ka (1/Ms)	kd (1/s)	K <sub>D</sub> (nM)
Human	B7-H4 IgV	1.78E+05	3.61E-04	2.0
Human	B7-H4 ECD	7.16E+05	1.70E-03	2.4
Cynomolgus Monkey	B7-H4 ECD	9.62E+05	1.12E-03	1.2
Rat	B7-H4 ECD	7.84E+05	2.35E-03	3.0
Mouse	B7-H4 ECD	6.32E+05	2.13E-03	3.4

**FPA150 Cell Binding Potency (FACS)**



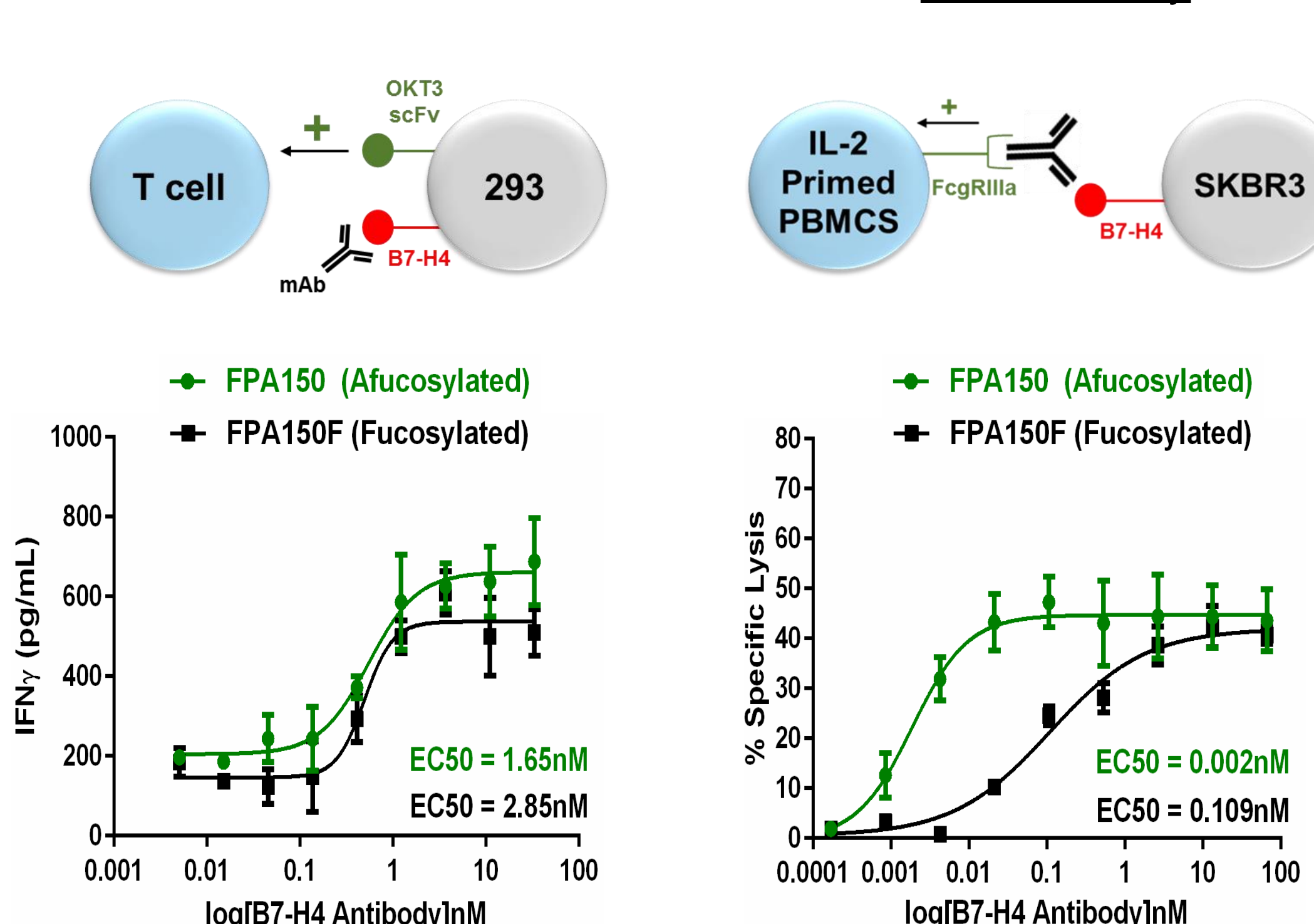
## Figure 1. Identification B7-H4 Monoclonal Antibodies with T Cell Checkpoint Blockade Activity

B7-H4-specific antibodies were isolated from full-length human IgG1 naïve antibody libraries using an *in vitro* yeast-based platform. After multiple rounds of selection the resulting IgG were sequenced and unique antibodies produced and evaluated for binding affinity to recombinant B7-H4 ectodomain and epitope binning by Surface Plasmon Resonance (SPR), and for target-specific cell binding by flow cytometry.



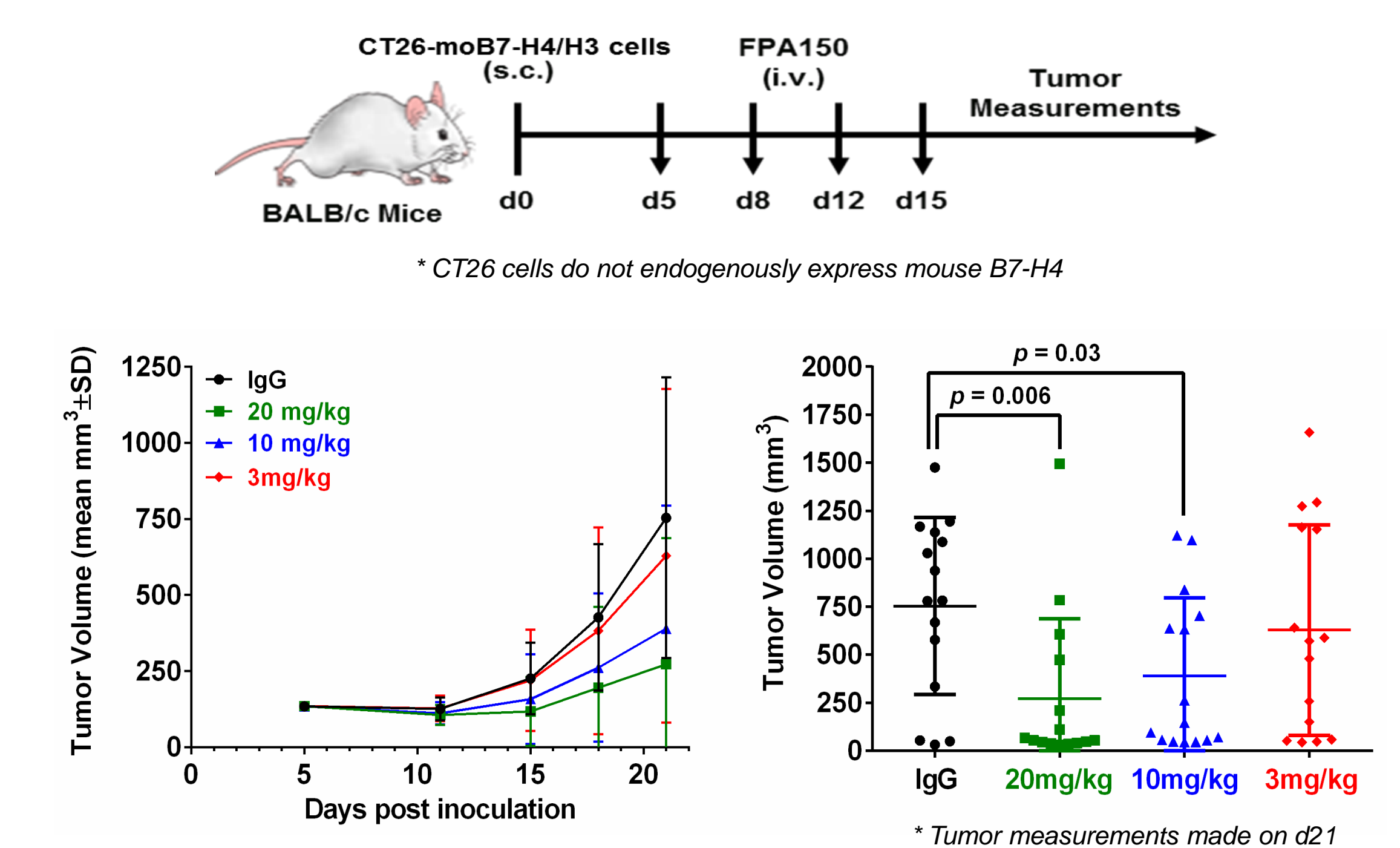
**Figure 3.** FPA150 is an Afucosylated hulgG1-based mAb that Demonstrates Potent T Cell Checkpoint Blockade and ADCC Activity *In Vitro*

**T Cell Checkpoint Blockade Assay** and **ADCC Assay**



**Figure 4.** FPA150 Demonstrates Potent Dose-Dependent Anti-Tumor Activity *In Vivo* in a CT26-mouseB7-H4/mouseB7-H3 Tumor Model

Mouse CT26 colorectal carcinoma cells were engineered to express a chimeric protein consisting of the extracellular domain of murine B7-H4 and the transmembrane domain of murine B7-H3



## Summary and Conclusions

- We successfully generated a therapeutic candidate B7-H4 antibody (FPA150) which possesses both T cell immune checkpoint blockade and ADCC activity *in vitro* and demonstrates significant dose-dependent anti-tumor activity *in vivo*.
- B7-H4 antibodies with T cell checkpoint blockade activity bind and block an evolutionarily conserved functional epitope within the B7-H4 IgV ectodomain.
- In rat and cynomolgus monkey pilot PK and toxicity studies, FPA150 demonstrates a typical antibody PK profile without any toxicity.
- These data suggest that the B7-H4 mAb FPA150, which possesses T cell checkpoint and ADCC activity, has the potential to be an effective therapeutic by improving anti-tumor immune responses in cancer patients.
- IND-enabling studies are ongoing.
- Corresponding author email address: charles.kaplan@fiveprime.com