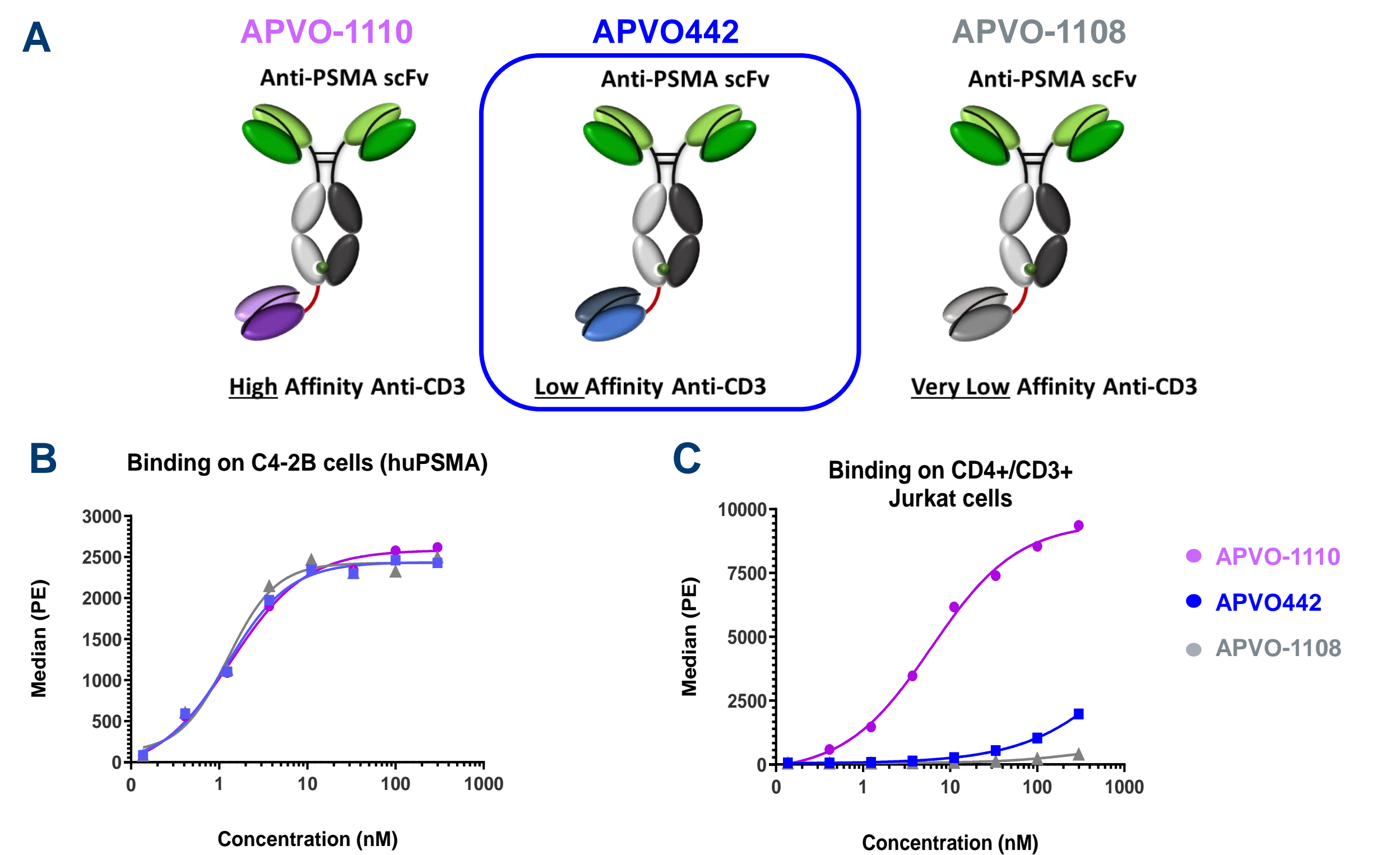




## APVO442 is designed for optimal safety and activity against prostate cancer

	Therapeutic Candidate	<ul style="list-style-type: none"> <li>ADAPTIR-FLEX™ (anti-CD3 x anti-PSMA) T cell engager</li> <li>Mutated IgG1 Fc; No ADCC or CDC; retains FcRn binding</li> </ul>
	Function/Mechanism of Action	<ul style="list-style-type: none"> <li>Engages T cells via CD3 to lyse PSMA<sup>+</sup> tumor cells</li> <li>Low-affinity monovalent CD3 binding</li> <li>High-avidity bivalent PSMA binding</li> <li>Reduces potential binding to circulating T cells</li> <li>Enables potential for better tumor biodistribution</li> <li>Low levels of cytokines in the absence of targets (pre-clinical)</li> </ul>
	Indications	<ul style="list-style-type: none"> <li>Metastatic castration-resistant prostate cancer (mCRPC)</li> <li>Other PSMA<sup>+</sup> tumors</li> </ul>
	Half-life	<ul style="list-style-type: none"> <li>9.3 days in murine models</li> </ul>
	Manufacturability	<ul style="list-style-type: none"> <li>Highly efficient heterologous "knob-in-hole" chain pairing</li> <li>Robust manufacturability profile comparable to mAbs</li> <li>Utilizes standard mAb production processes</li> </ul>
Development Stage	<ul style="list-style-type: none"> <li>Lead candidate selected</li> <li>CMC activities initiated</li> <li>Pre-clinical studies ongoing</li> </ul>	

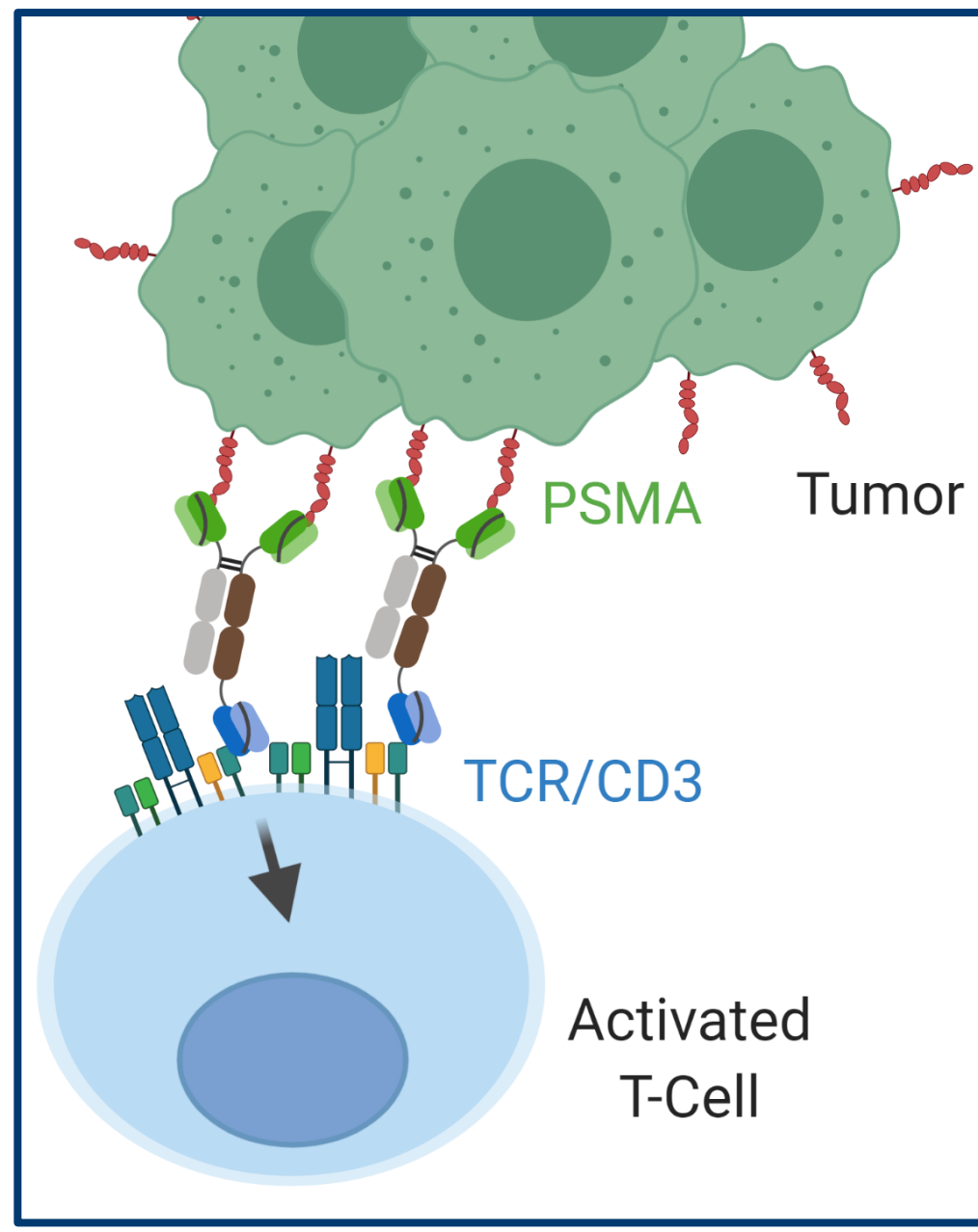
## Figure 1 : ADAPTIR-FLEX constructs with variable CD3 affinity validate *in vitro* and *in vivo* efficacy of APVO442



On-Cell Binding Parameters	APVO-1108		APVO442		APVO-1110	
	EC <sub>50</sub> (nM)	Max	EC <sub>50</sub> (nM)	Max	EC <sub>50</sub> (nM)	Max
Human PSMA binding affinity	1.3	2500	1.2	2400	1.4	2600
Human CD3 binding affinity	>> 200	409	> 200	2000	6.8	9000

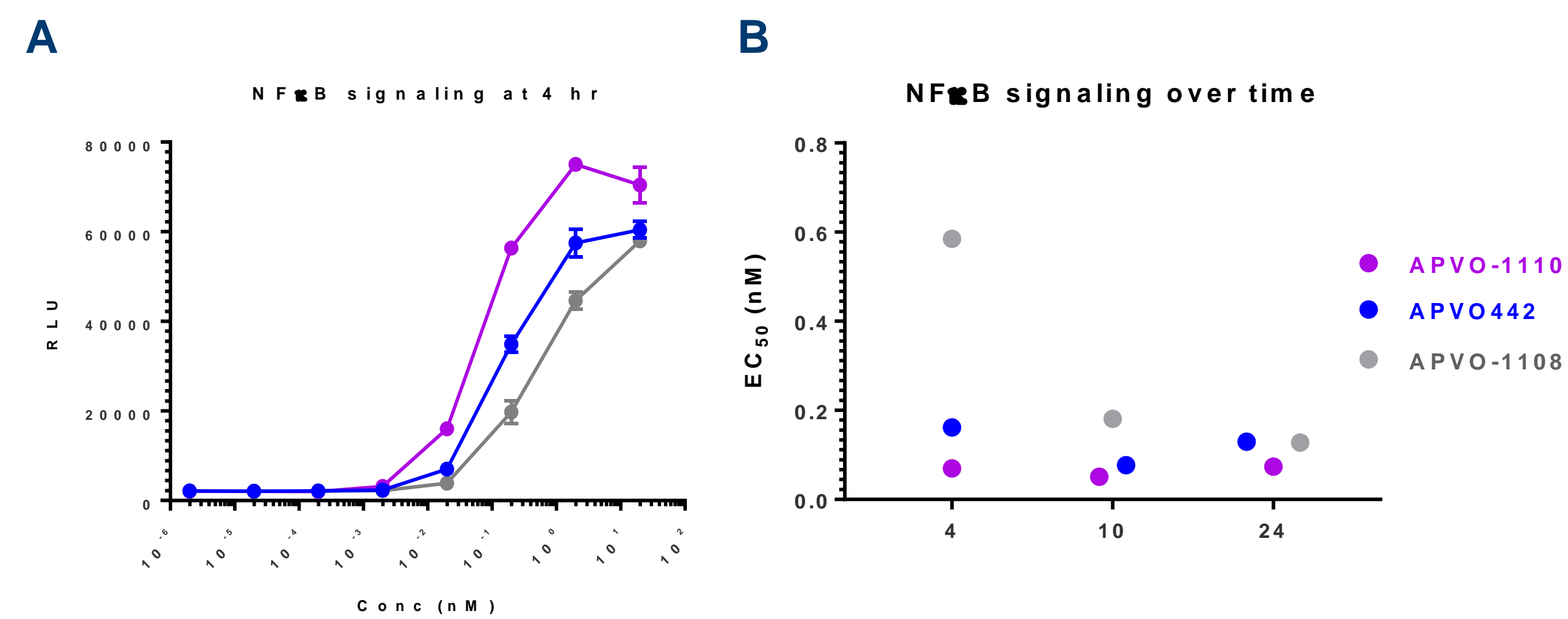
Design of ADAPTIR-FLEX constructs with distinct binding profiles. (A) ADAPTIR-FLEX constructs were designed with high-affinity, low-affinity, or very low-affinity monovalent CD3 binding properties and paired with bivalent PSMA to generate functional bispecific molecules.

APVO442 retains strong binding to PSMA and reduced affinity to CD3. Binding of ADAPTIR-FLEX molecules to conformational PSMA and CD3 targets was assessed by on-cell binding via flow cytometry on relevant target-expressing cell lines including (B) human PSMA-expressing C4-2B cells and (C) Jurkat CD4<sup>+</sup> T cell line expressing CD3. The on-cell binding values of EC<sub>50</sub> and Max binding RLU are listed for each of the cell lines (Table).



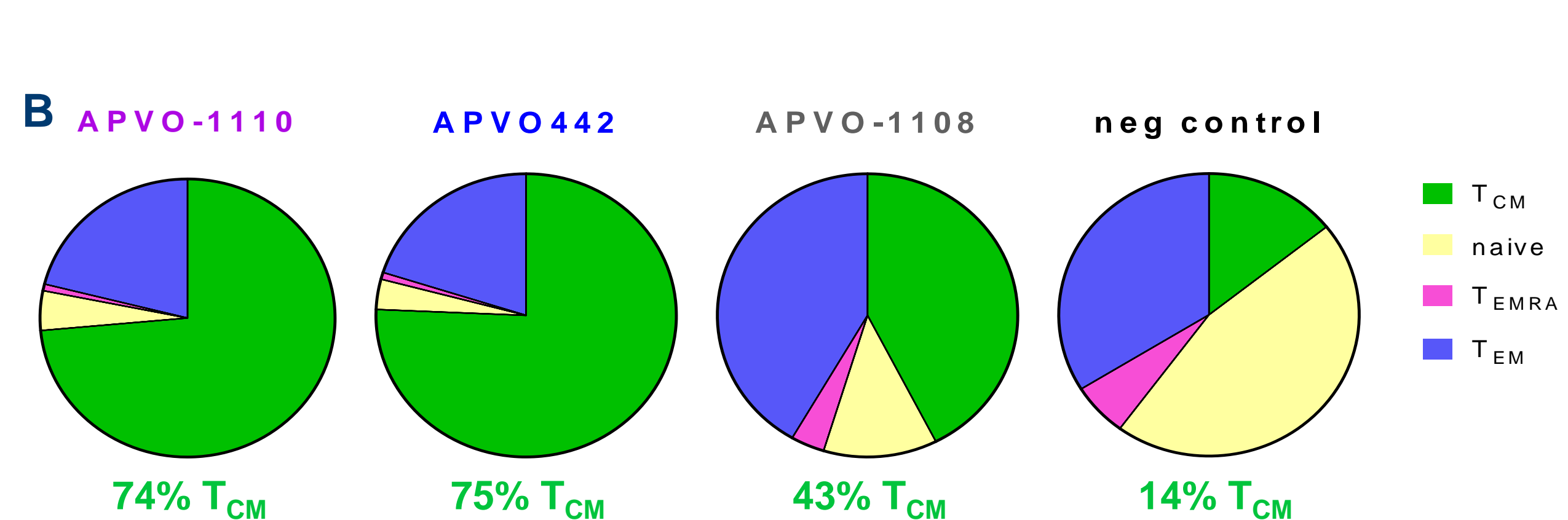
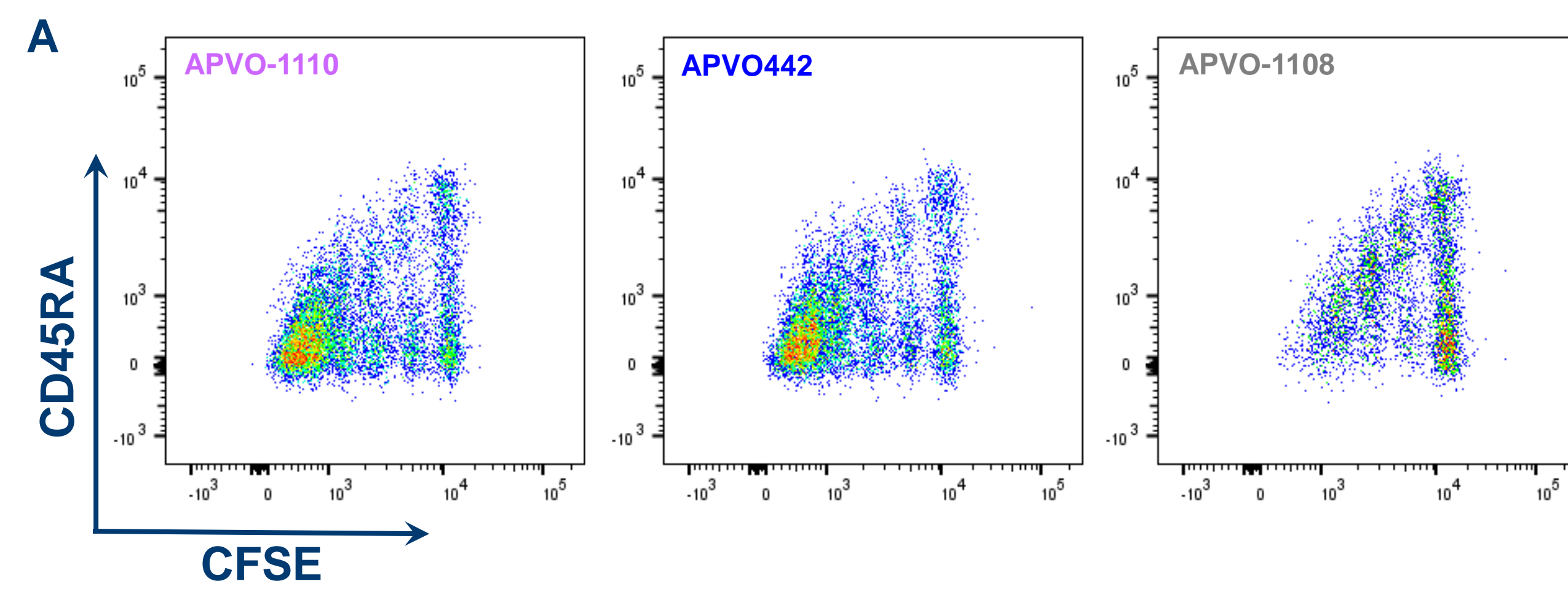
APVO442 displays a distinct high-affinity PSMA binding (<2 nM) and low-affinity CD3 (> 200 nM) binding profile.

## Figure 2 : APVO442 stimulates NFκB signaling downstream of TCR similar to a monovalent high-affinity anti-CD3 construct



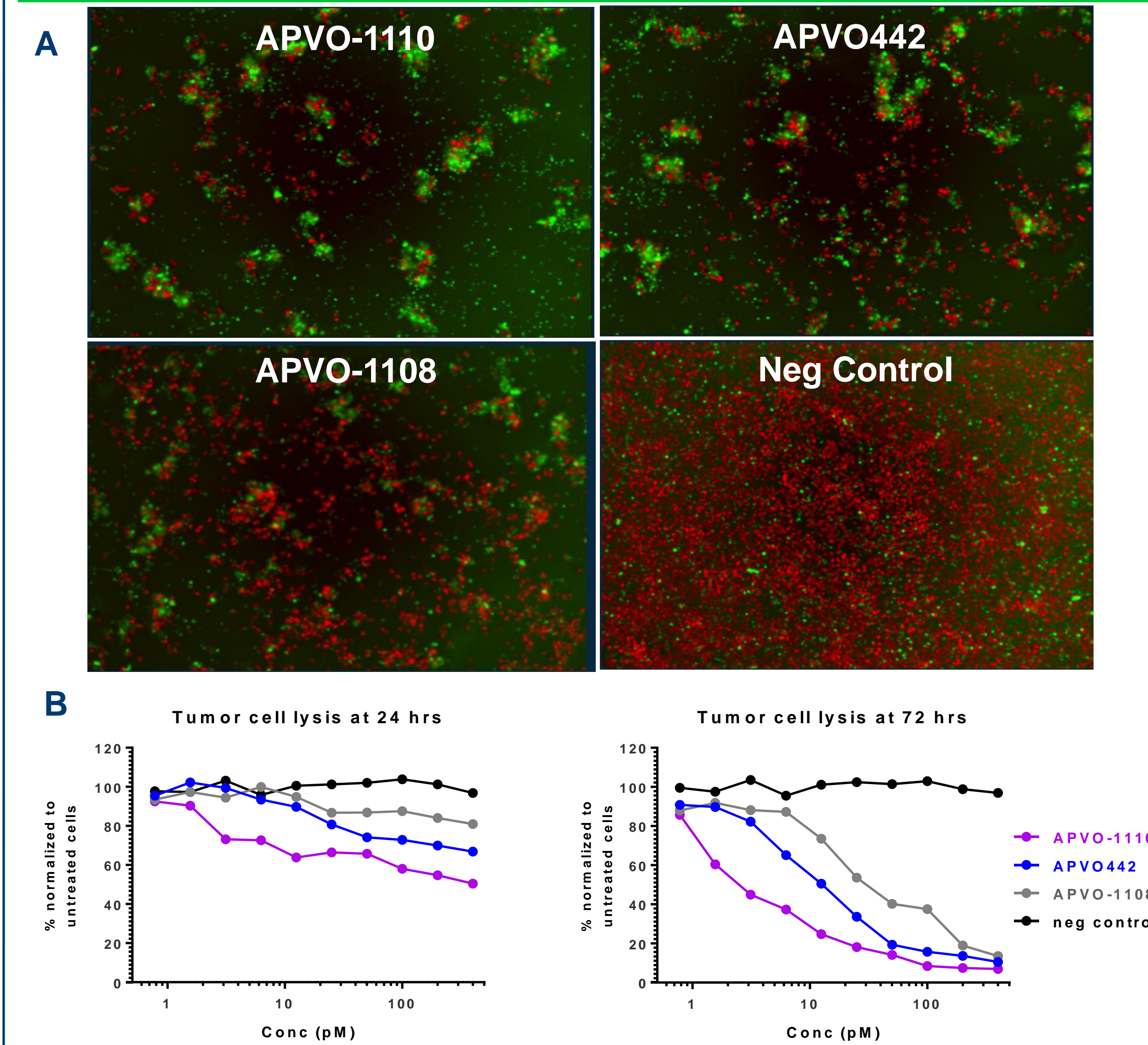
APVO442 stimulates strong signaling through NFκB, NFAT and ERK pathways. Jurkat/NFκB/luciferase reporter cells were incubated with a C4-2B PSMA<sup>+</sup> tumor cell line and a titration of constructs. At the desired timepoint, cells were lysed with BioGlo reagent (Promega) and luminescence (RLU) was read on a MicroBeta2 microplate counter (Perkin Elmer). (A) Example of NFκB signaling titration at 4-hour timepoint. (B) Comparison of EC<sub>50</sub> values for NFκB signaling at 4, 10 and 24 hours. Similar data was observed with both the NFAT and ERK signaling pathways.

## Figure 3 : APVO442 induces similar proliferative capacity and generation of central memory T cells compared to a monovalent high-affinity anti-CD3 construct



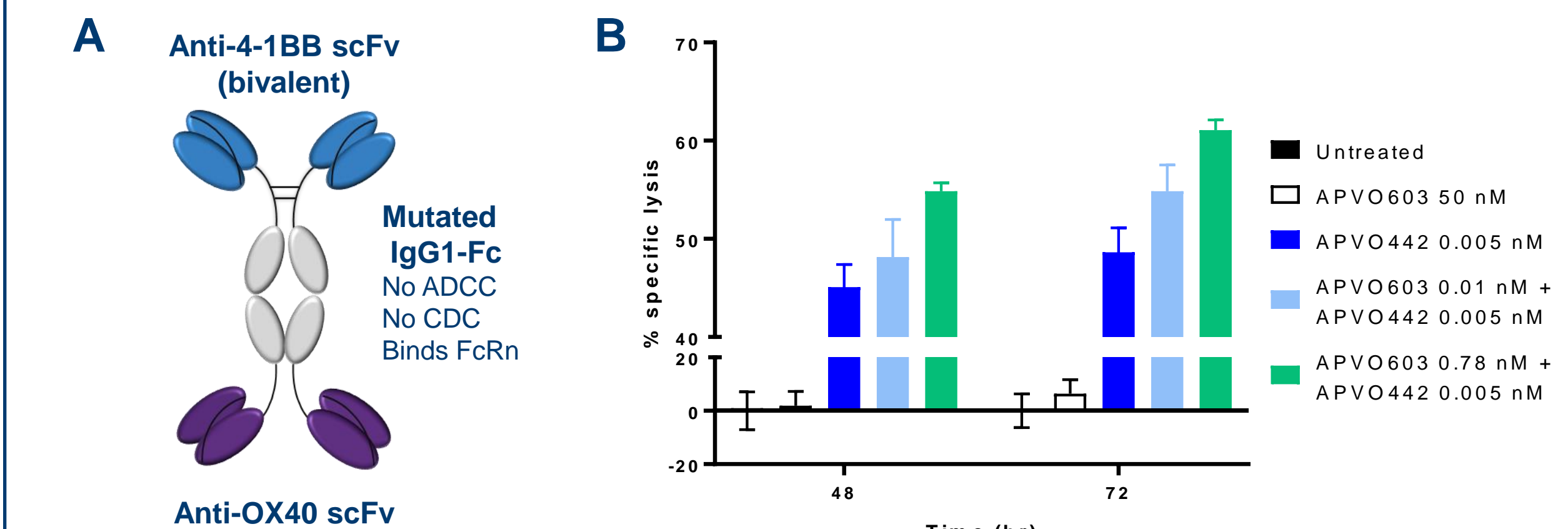
APVO442 induces proliferation of T cells toward generation of central memory cells. Despite lower CD3 affinity, APVO442 is engaging T cells as effectively as the high-affinity APVO-1110 comparator, stimulating the desired proliferation of T cells and increasing the percentage of central memory cell phenotype. CFSE-labeled primary T cells were co-cultured with a human PSMA<sup>+</sup> tumor cell line at a 3:1 ratio and constructs were titrated up to 2000 pM (shown) for 96 hours. Proliferation and memory cell phenotyping were assessed by flow cytometry. (A) Proliferation of CFSE-labeled T cells and decreased CD45RA expression. (B) Memory cell phenotypes based on CD45RA/CCR7 staining. T<sub>CM</sub> (CCR7<sup>+</sup>, CD45RA<sup>-</sup>), naive (CCR7<sup>+</sup>, CD45RA<sup>+</sup>), T<sub>EMRA</sub> (CCR7<sup>-</sup>, CD45RA<sup>+</sup>) and T<sub>EM</sub> (CCR7<sup>-</sup>, CD45RA<sup>-</sup>). Negative control construct was monovalent for CD3 and bivalent for germlined PSMA that specifically removes PSMA binding.

## Figure 4 : APVO442 augments T cell-enhanced PSMA<sup>+</sup> tumor cell lysis



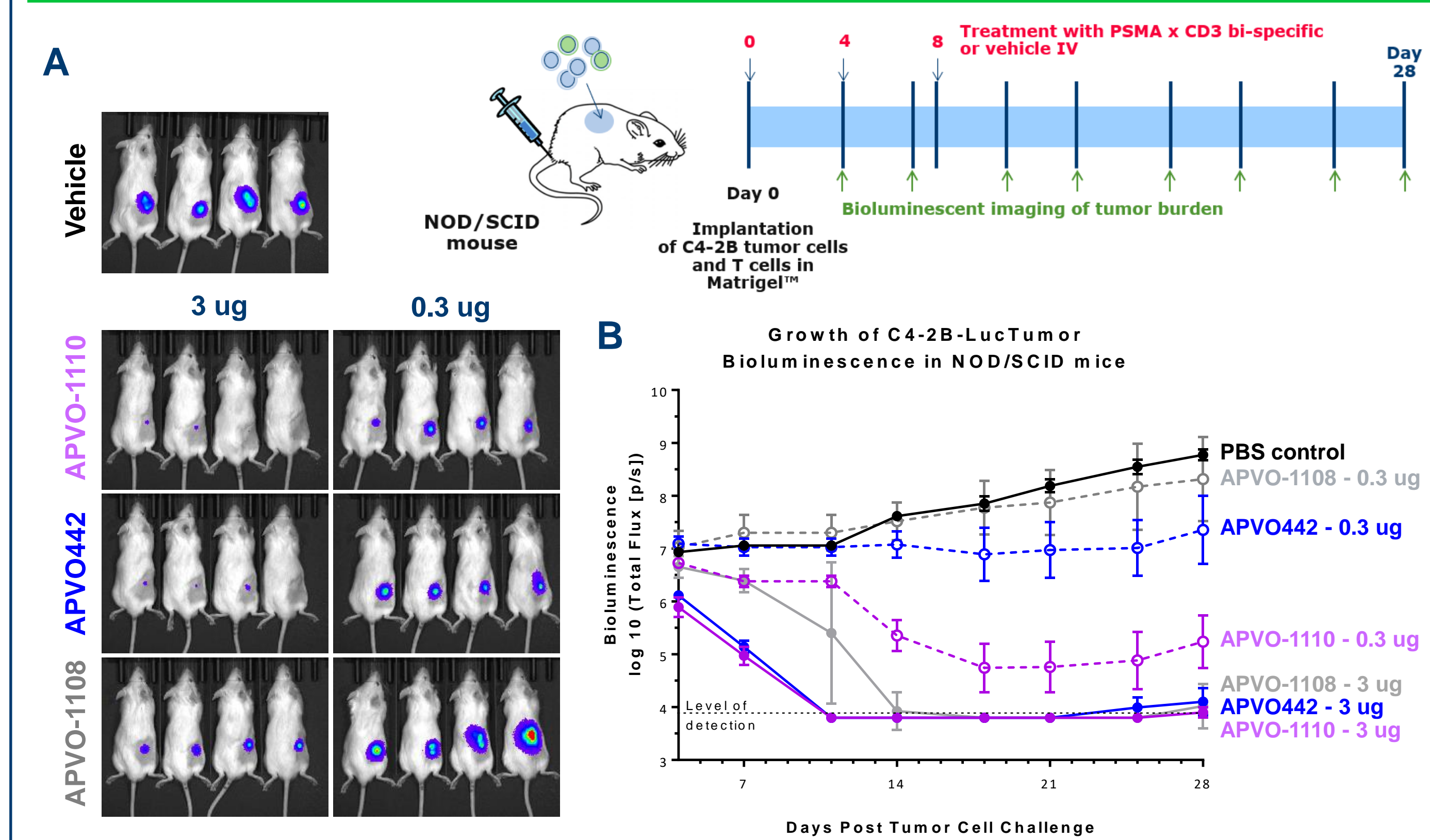
APVO442 promotes tumor lysis in an *in vitro* cytotoxicity assay. CFSE-labeled primary T cells (green) were co-cultured with human NuLight Orange (Sartorius) stably transfected PSMA<sup>+</sup> C4-2B tumor cell line at a 4:1 ratio and a titration of ADAPTIR-FLEX constructs. Serial Incucyte images were taken throughout culture. (A) Representative images taken at 72 hours (100 pM). (B) Cytotoxicity was quantified as count of NuLight Orange C4-2B tumor cells in treated wells normalized to untreated wells (24 and 72 hr timepoints).

## Figure 5 : APVO442 in combination with an OX40 x 4-1BB bispecific (APVO603) promotes increased tumor lysis



APVO442 promotes increased tumor lysis in an *in vitro* PBMC cytotoxicity assay when combined with an immune modulator, APVO603. APVO603 is a conditional 4-1BB and OX40 bispecific agonist antibody that strictly induces downstream signaling when it binds to both 4-1BB and OX40 on recently activated tumor infiltrating T cells and NK cells. It was designed with the potential to reduce toxicities and overcome clinical obstacles such as on-target toxicity and limited efficacy of the current monoclonal antibodies for the treatment of multiple solid tumors. Primary T cells were co-cultured with C4-2B PSMA<sup>+</sup> tumor cells at a 10:1 ratio. Purified T cells were treated with a titration of APVO603 in combination with 0.005 nM APVO442. (A) Design of APVO603 ADAPTIR. (B) Cytotoxicity was quantified throughout culture by measuring the total NuLight Orange object area of tumor cells with test reagents and comparing them to no treatment. APVO603-treated T cells do not induce tumor cell killing.

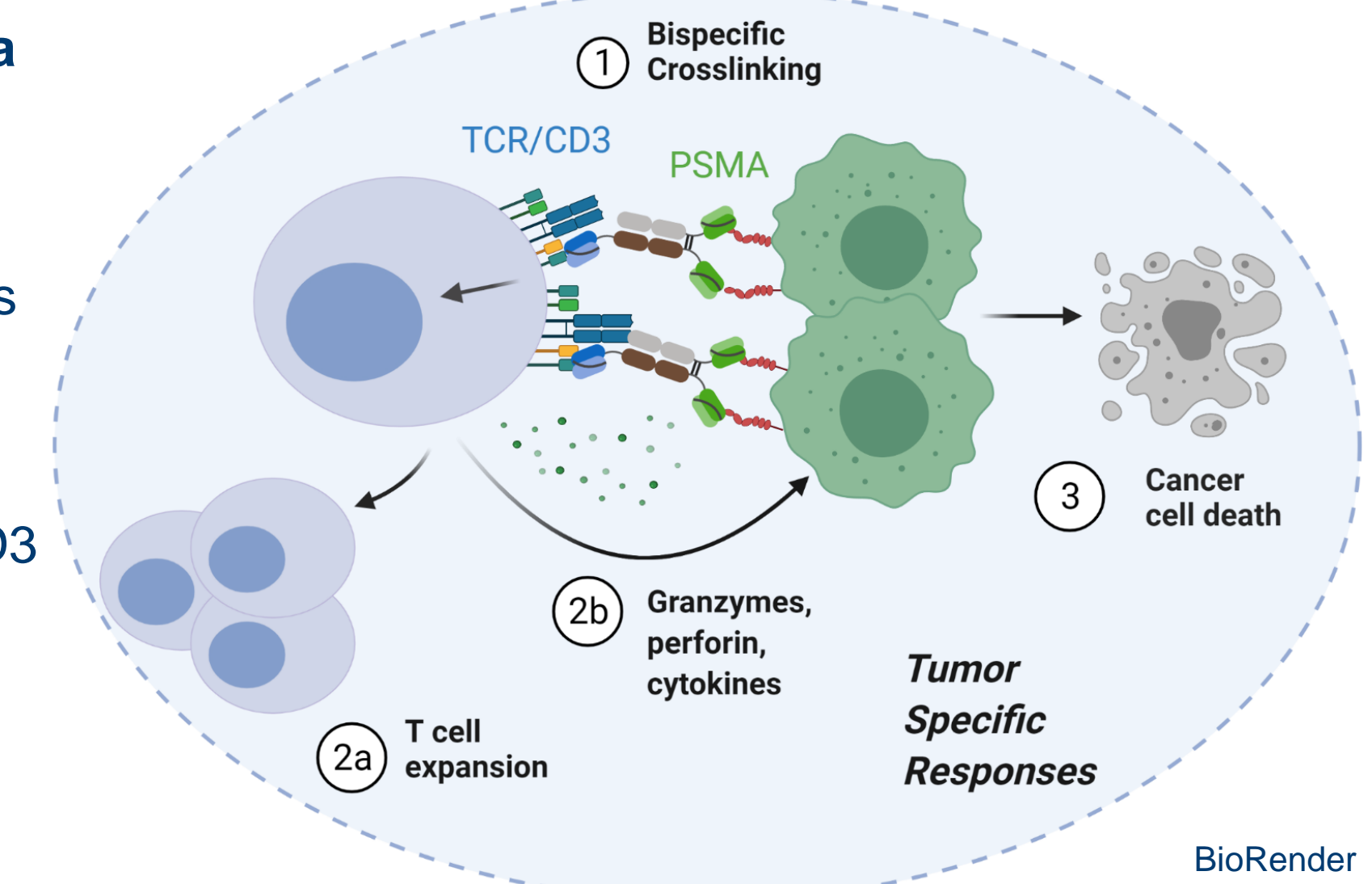
## Figure 6 : APVO442 therapy is effective at reducing PSMA<sup>+</sup> tumors in NOD/SCID mice at low doses



APVO442 therapy promotes increased prostate cancer tumor lysis in an *in vivo* cytotoxicity assay. NOD/SCID mice were implanted with a PSMA<sup>+</sup> C4-2B luciferase tumor cell line and purified T cells on Day 0. Mice were dosed with 0.3, 3, 100 or 300 μg on Day 0, 4 and 8. Bioluminescent tumor imaging occurred through Day 28. (A) Representative images at Day 28 of mice treated at 3 or 0.3 μg. (B) Graph of tumor bioluminescence from Day 4 through Day 28.

## Summary and Advantages of APVO442

APVO442 is designed to deliver a tumor directed T cell response against metastatic castration-resistant prostate cancer. The unique design of APVO442 reduces the potential for peripheral T cell engagement to limit on-target CD3 activation (cytokines) and sink effects. Low-affinity monovalent CD3 cooperates with a high-avidity bivalent PSMA to elicit tumor-specific T cell activation and cytotoxicity comparable with higher affinity T cell engager approaches.



Characteristic	APVO-1110 High CD3 affinity	APVO442 Low CD3 affinity	APVO-1108 Very low CD3 affinity	APVO442 Benefit
PSMA binding	++	++	++	Enriched tumor Targeting
CD3 binding	++	+	+/-	Reduced T cell sink/activation
T cell activation	++	++	+	Retained optimal T cell profile
T cell proliferation	++	++	+	
T cell mediated tumor killing	++	++	+	
T cell memory profile	++	++	+	Reduced toxicity profile
Cytokine production	+	+	+	
PK	++	++	++	Enriched Tumor distribution and potency
Tumor regression	++	++	+	

Aptevo's unique low-affinity CD3 targeting approach positions APVO442 for favorable outcomes compared to traditional high-affinity CD3 approaches