

# Next-generation multi-specific and conditionally activated CD3 Switch-DARPin with CD2 co-stimulation to tackle current limitations of T cell engagers in solid tumors

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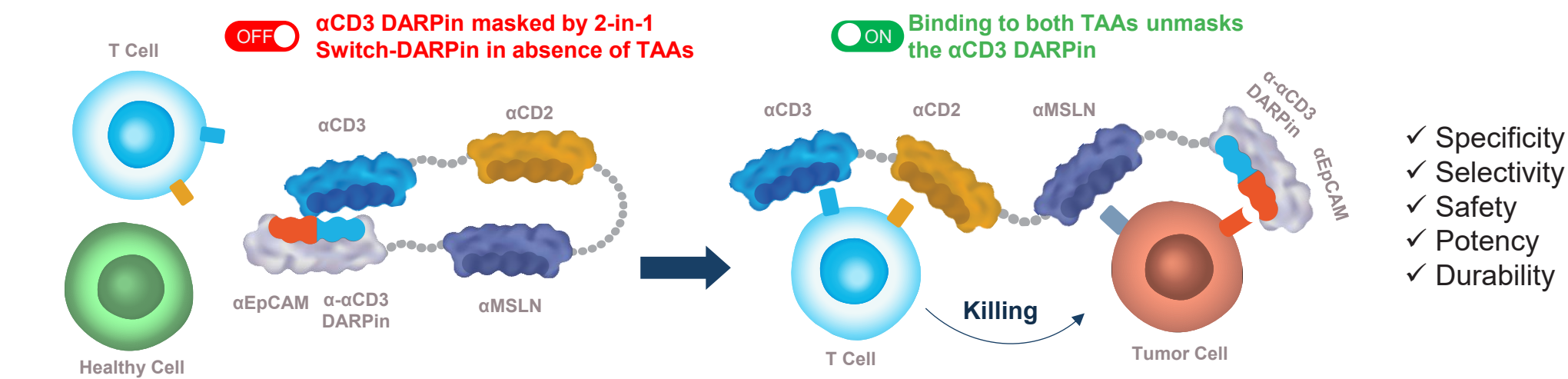
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Poster/abstract  
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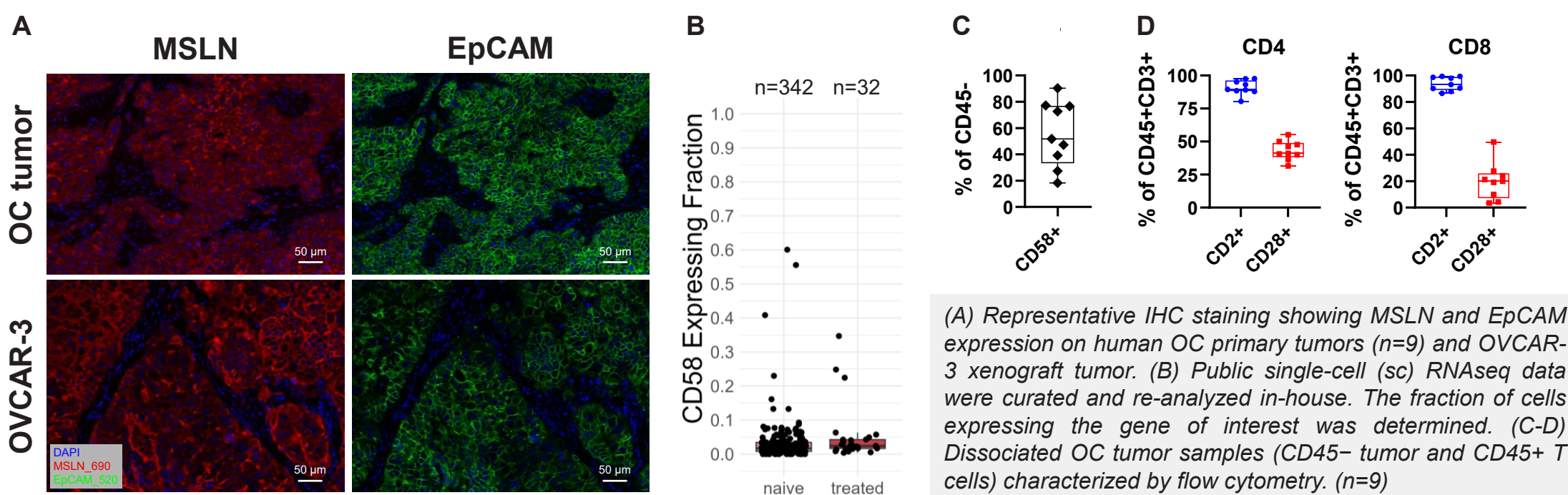
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## Introduction

Current therapies for ovarian cancer (OC) lack durable response. Targeted immunotherapy has had limited success due to the absence of clean tumor-associated antigens (TAAs), poor efficacy/toxicity profile, and the presence of dysfunctional T cells. To overcome these challenges, we generated a conditionally activated CD3 Switch-DARPin T cell engager (TCE) boosted by a CD2-engaging costimulatory domain. In cancer, loss of CD58, the ligand of CD2, facilitates immune evasion and is linked to poor prognosis.<sup>1-3</sup> The CD3 Switch-DARPin triggers, tumor-specific T cell activation (TCA) only in the presence of mesothelin (MSLN) and epithelial cell adhesion molecule (EpCAM), two TAAs highly co-expressed on OC tumor cells, while the CD2 DARPin provides a co-stimulatory signal for sustained and potent T cell responses.

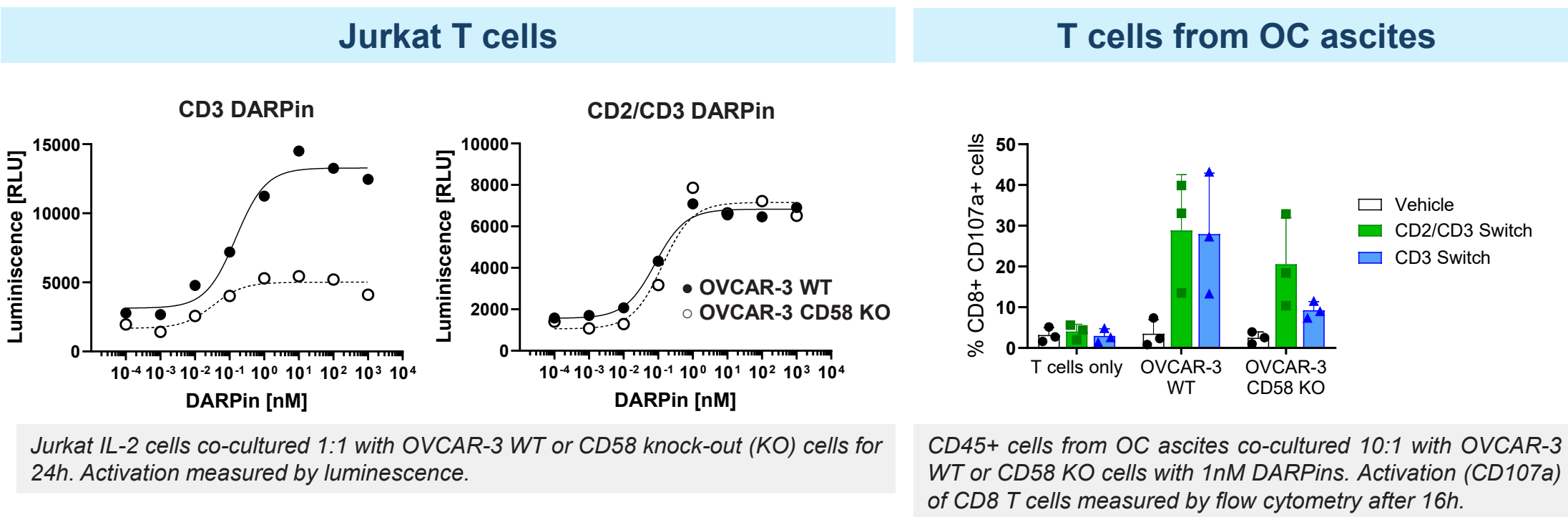


## MSLN/EpCAM are co-expressed in OC & CD2 is maintained on TILs



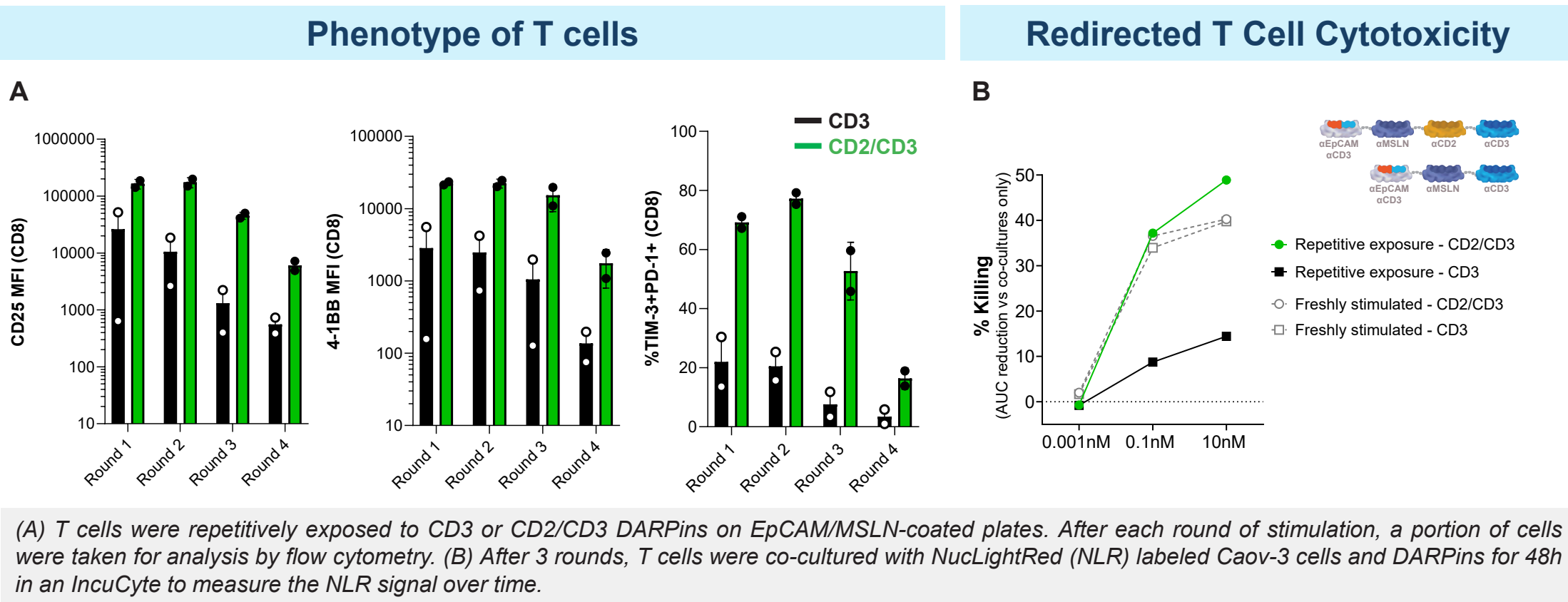
(A) High co-expression of MSLN and EpCAM in OC (scRNAseq data<sup>4</sup>) was confirmed by immunohistochemistry (IHC). (B-C) scRNAseq and flow cytometry data show that CD58, the ligand of CD2, is often down-regulated on OC tumor cells. (D) Analysis of tumor-infiltrating lymphocytes (TILs) shows that CD2 is expressed by >90% of CD4 and CD8 T cells.

## CD2 co-stimulation prevents loss of activity against CD58 KO cells



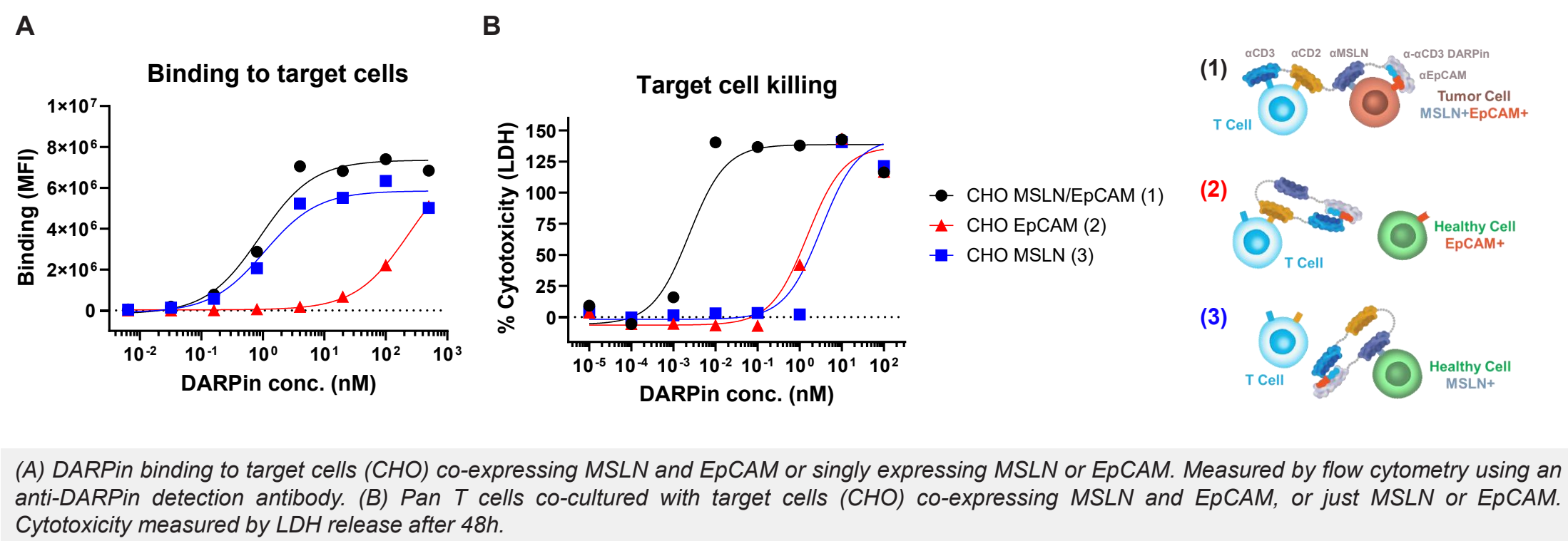
Loss of CD58 on tumor cells results in reduced efficacy of the CD3 Switch-DARPin whereas CD2/CD3 co-stimulation maintains efficacy, thus compensating for the lack of CD58.

## CD2 co-stimulation enables continued polyfunctionality of T cells



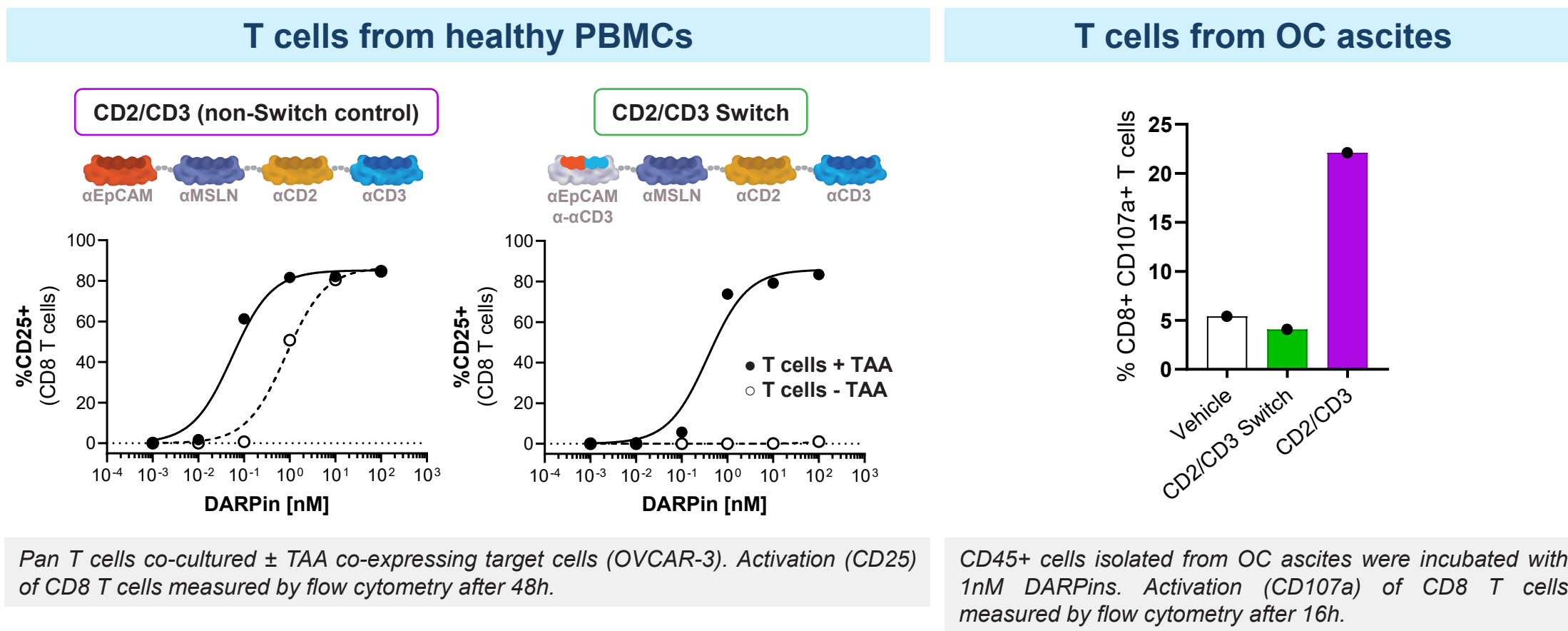
T cells repetitively exposed to CD2/CD3 DARPin maintain a more activated phenotype as well as a higher cytotoxic capacity compared to exposure to CD3-only DARPin.

## CD3 Switch-DARPin enables preferential targeting of tumor cells

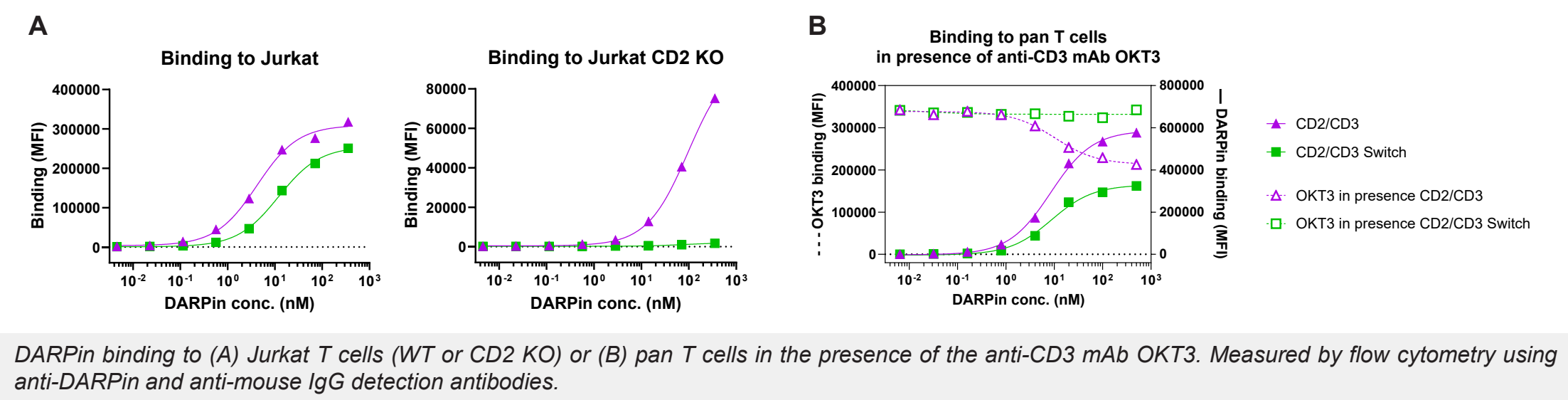


In absence of MSLN, binding to EpCAM is strongly reduced with the Switch-DARPin format. CD2/CD3 Switch induces selective targeting of MSLN/EpCAM co-expressing cells (AND-gate), while activity against single TAA-expressing cells is strongly reduced.

## CD3 Switch-DARPin prevents TAA-independent T cell activation

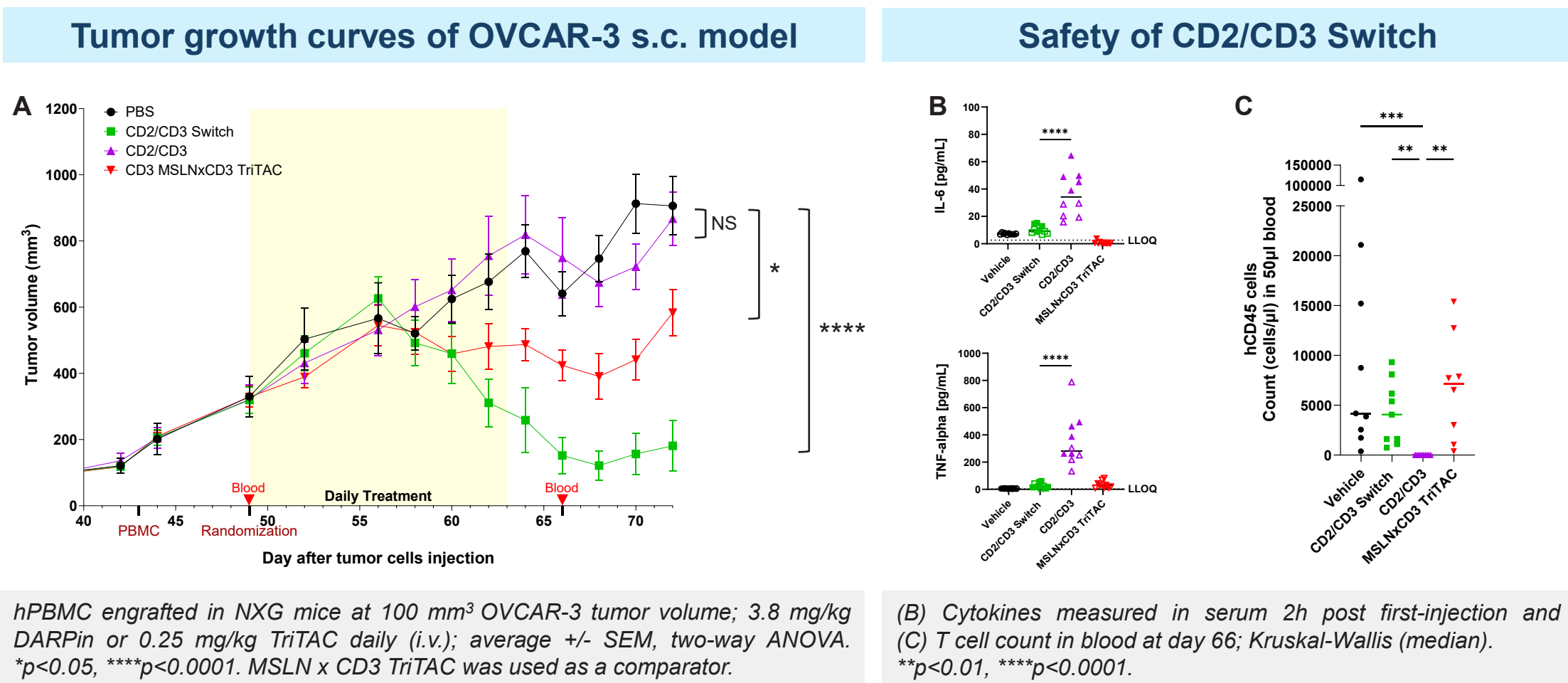


## CD3 Switch-DARPin prevents binding to CD3 in absence of TAAs



In the absence of TAA-expressing target cells, binding to T cells is exclusively mediated by the CD2 DARPin. This does not trigger the opening of the Switch-DARPin, thus preventing binding to CD3. Only the non-Switched control DARPin can bind to T cells when CD2 is knocked out or compete with OKT3 for binding to CD3 in the absence of target cells.

## CD2/CD3 Switch-DARPin induces tumor regression *in vivo*



(A) CD2/CD3 Switch-DARPin led to significant tumor regression (B) and lower cytokine levels in serum vs. the non-Switched control DARPin. (C) CD2/CD3 Switch-DARPin effectively prevented the loss of T cells in the periphery, unlike the non-Switch DARPin. This suggests that masking the CD3 domain allows the integration of a CD2 costimulatory domain, ensuring that the activity is targeted only in the presence of TAA-expressing tumor cells.

## Conclusions

We present a preclinical proof-of-concept for a conditionally activated CD3 Switch-DARPin TCE with CD2 co-stimulation, designed to increase the therapeutic window against MSLN and EpCAM co-expressing tumors as in OC. Our findings demonstrate that:

- The CD3 Switch-DARPin activates T cells specifically in the presence of cells co-expressing MSLN and EpCAM, increasing tumor specificity.
- CD2 co-engagement leads to sustained T cell activation and cytotoxic capacity, preventing T cell dysfunction.
- The CD3 Switch-DARPin with CD2 co-stimulation effectively induces significant tumor regression *in vivo*, without signs of T cell activation in the periphery, indicating a favorable safety profile.
- Our CD3 Switch-DARPin platform provides a novel approach for sustained tumor-specific T cell engagement, by combining a logic-gated on/off Switch mechanism with CD2 co-stimulation.