

# Evaluating the role of B7-H4 as a suppressor of tumor infiltrating lymphocytes and a target for immunotherapy in breast cancer

Elizabeth C. Wescott<sup>1,3</sup>, Paula I. Gonzalez-Ericsson, M.D.<sup>3</sup>, Violeta Sanchez<sup>3</sup>, Justin M. Balko, Pharm D, Ph.D.<sup>1,2,3</sup>

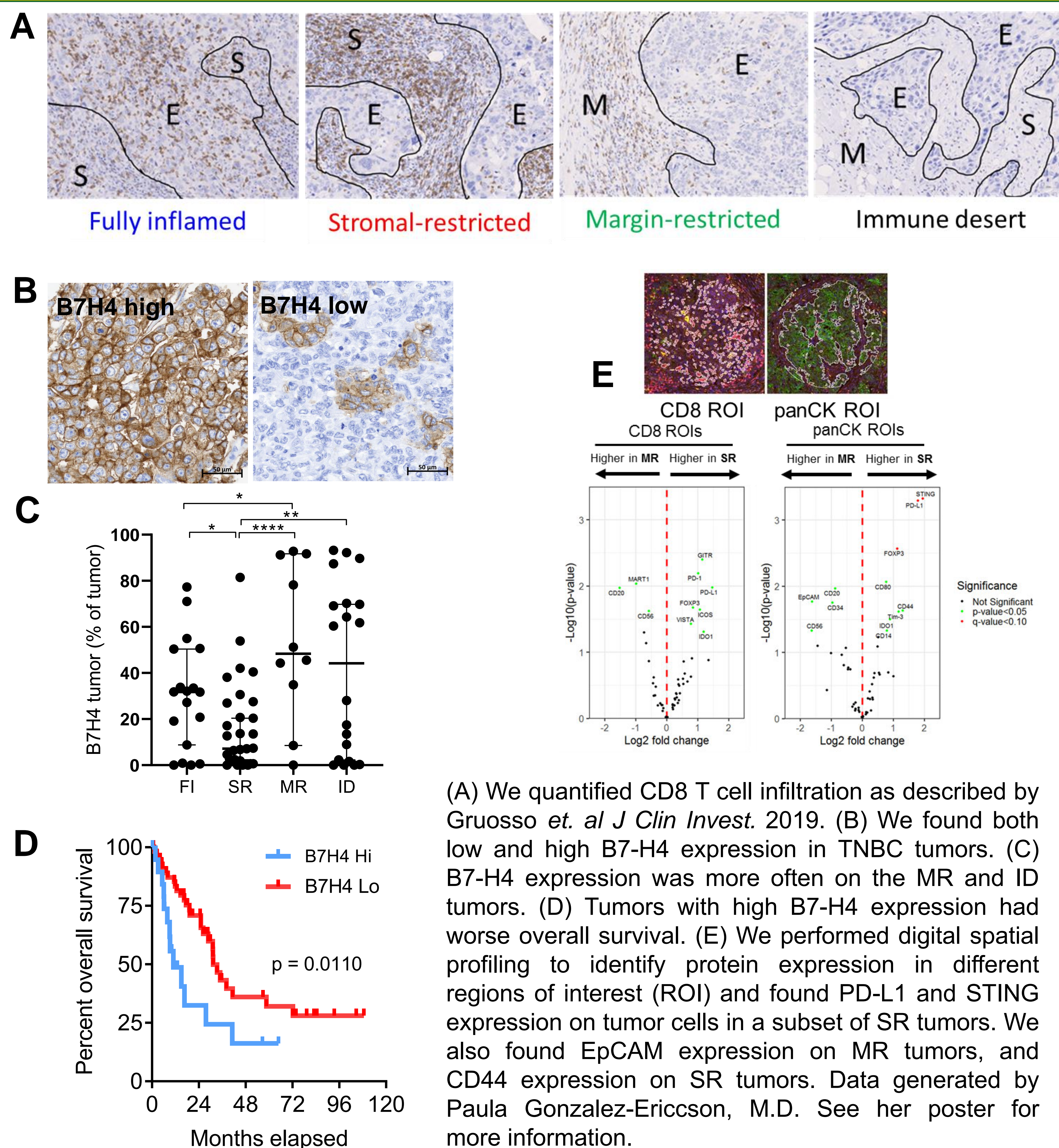
Departments of Pathology, Microbiology, and Immunology<sup>1</sup>, Medicine<sup>2</sup>, Vanderbilt University Medical Center, Nashville, TN;  
Breast Cancer Research Program<sup>3</sup>, Vanderbilt-Ingram Cancer Center; Vanderbilt University  
elizabeth.c.wescott@vanderbilt.edu

VANDERBILT UNIVERSITY  
MEDICAL CENTER

## Abstract

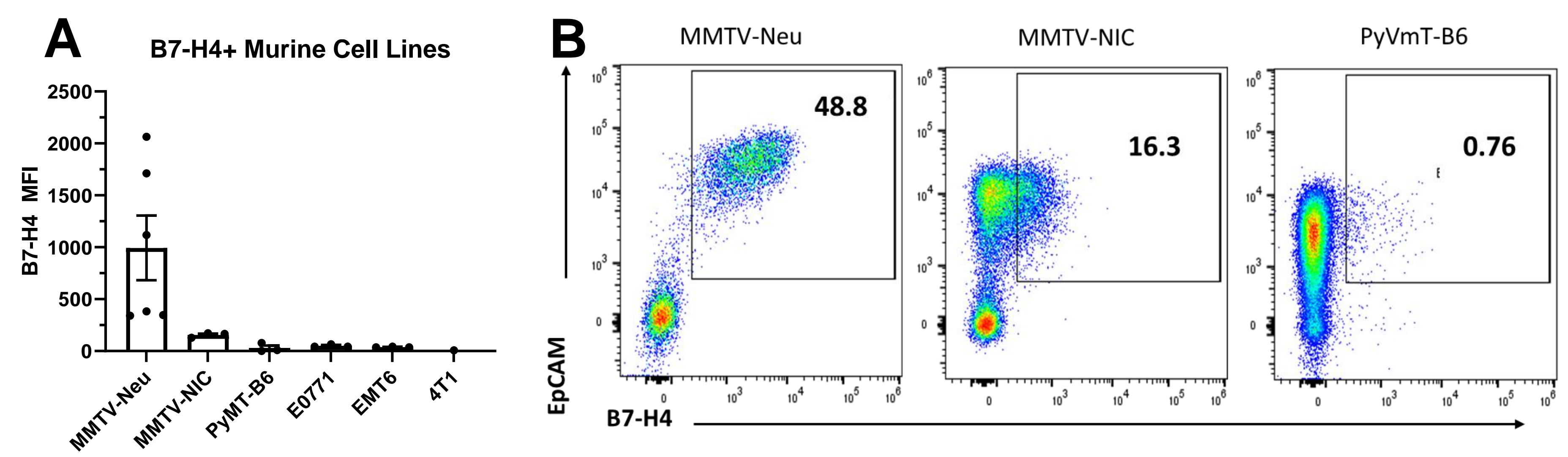
- Immunotherapy has seen broad success across cancer types and has become a key aspect of TNBC treatment, but some patients fail to respond
- B7-H4 (*VTCN1*) is an alternative immune checkpoint ligand in the CD28/B7 family of molecules, like PD-L1
- We observed both high and low B7-H4 expression on several mammary cancer cell lines
- B7-H4 is exclusively expressed on cells bearing epithelial markers
- mRNA expression data from a variety of human breast cancer cell lines corroborate this strong positive correlation of B7-H4 expression on epithelial cells

## Background

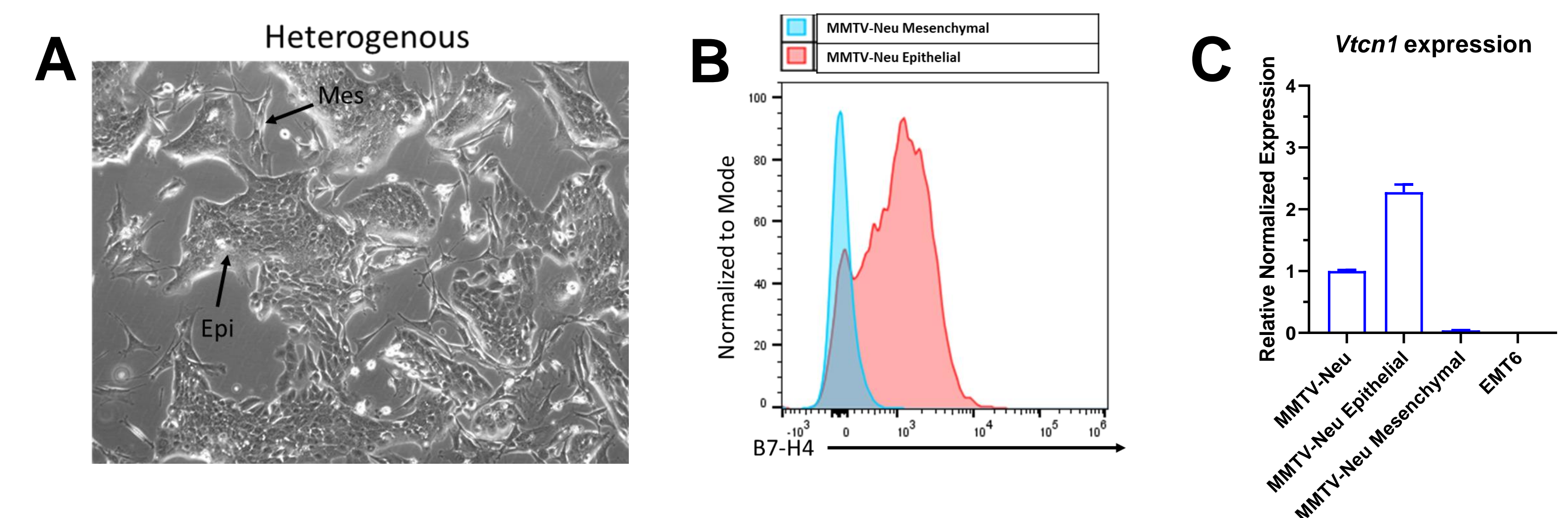


(A) We quantified CD8 T cell infiltration as described by Gruosso *et al J Clin Invest.* 2019. (B) We found both low and high B7-H4 expression in TNBC tumors. (C) B7-H4 expression was more often on the MR and ID tumors. (D) Tumors with high B7-H4 expression had worse overall survival. (E) We performed digital spatial profiling to identify protein expression in different regions of interest (ROI) and found PD-L1 and STING expression on tumor cells in a subset of SR tumors. We also found EpCAM expression on MR tumors, and CD44 expression on SR tumors. Data generated by Paula Gonzalez-Ericsson, M.D. See her poster for more information.

## B7-H4 is expressed on EpCAM+ murine mammary cancer cells

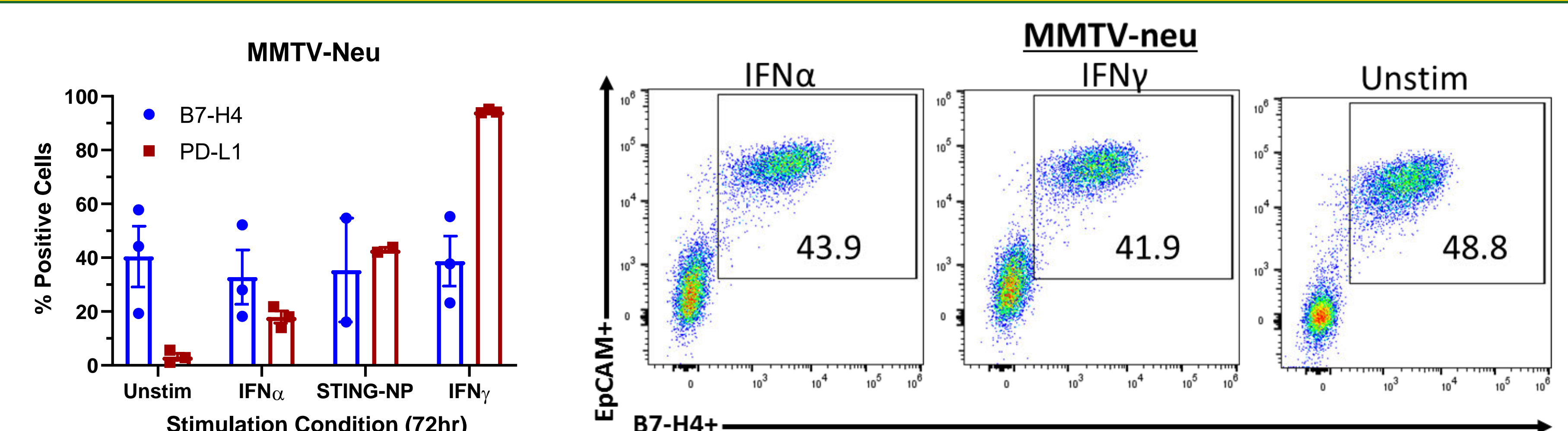


(A) We screened a panel of murine mammary cancer cell lines and found both high and low expression of B7-H4. (B) Those cells that were B7-H4+ were also EpCAM+ in multiple cell lines.



(A) The MMTV-Neu cell line consists of epithelial and mesenchymal cell phenotypes. (B) When these cell populations were separated by differential trypsinization, we found only the epithelial cells expressed B7-H4. (C) These data were further confirmed by quantitative PCR of the heterogenous, epithelial, and mesenchymal cells, compared to an EMT6 negative control.

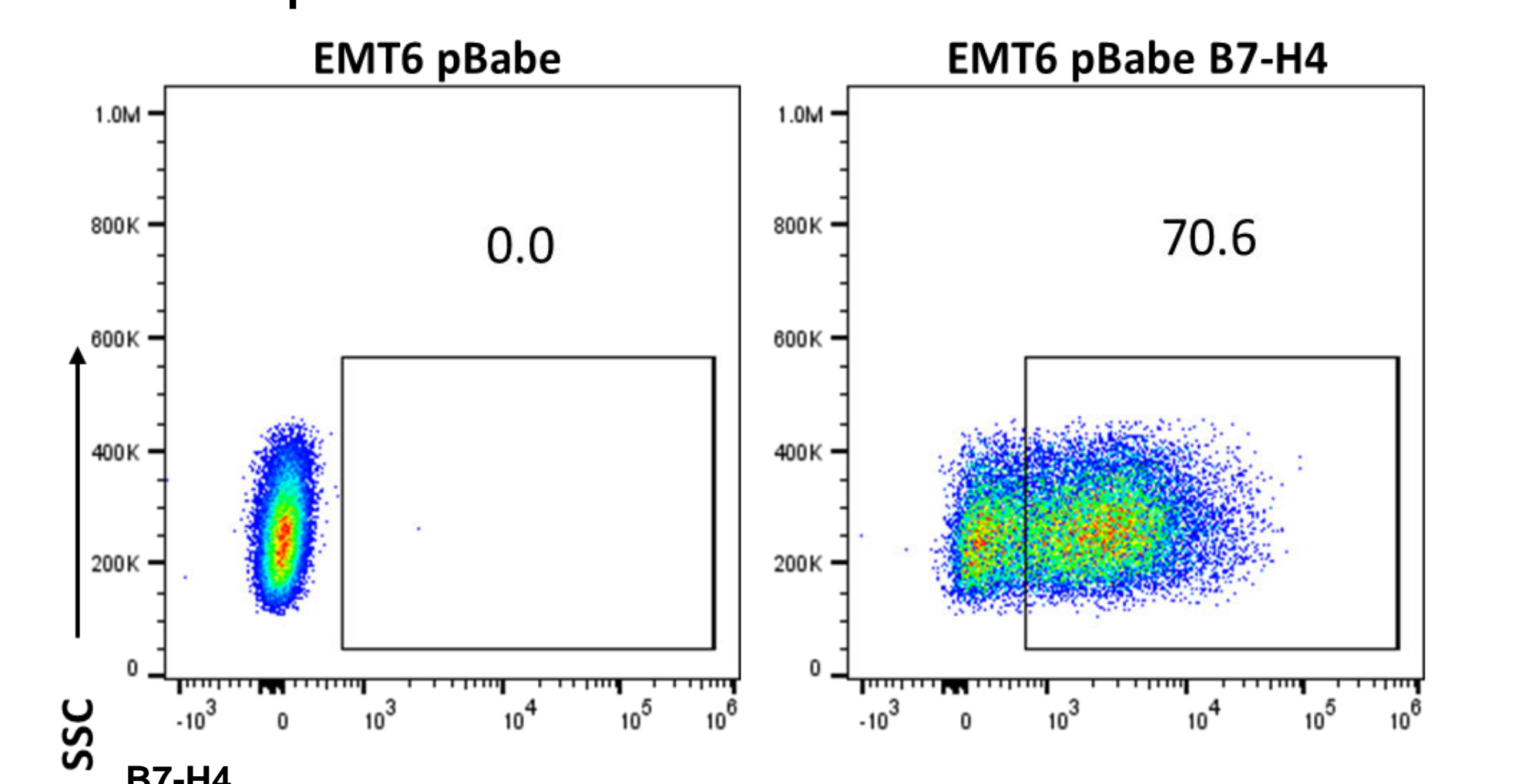
## B7-H4 expression is not regulated by interferon



We found B7-H4 expression was not induced by Type I or Type II interferon treatment, unlike PD-L1.

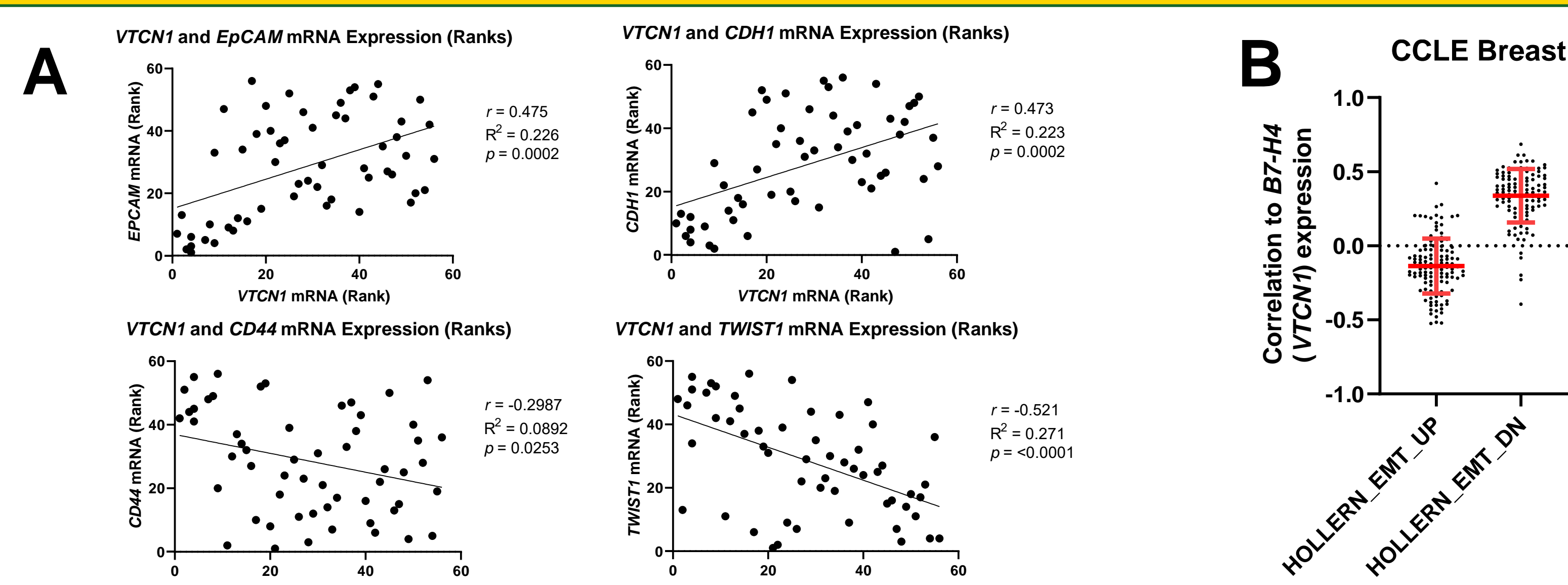
## Conclusions & Future Directions

- Immunotherapy has seen broad success in a variety of tumor types, including breast cancer.
- B7-H4 is expressed in breast cancers and correlates with epithelial markers.
- B7-H4 expression is not regulated by interferon stimulation, unlike PD-L1, but may be regulated by mesenchymal to epithelial transition in tumors.
- I will investigate models of enforcing cell transition into EMT and measure changes in B7-H4 expression.



- To determine whether B7-H4 is an alternative mechanism of immunologic escape in breast cancer, I will test whether enforced expression of B7-H4 causes EMT6 tumors to be resistant to anti-PD-L1 immunotherapy *in vivo*.

## VTCN1 expression correlates with epithelial cell markers in human breast cancer cell lines



(A) We looked at the CCLE breast dataset and found B7-H4 (*VTCN1*) expression correlates with epithelial cell markers but not mesenchymal cell markers. (B) Gene expression data from genes upregulated during epithelial-to-mesenchymal transition (EMT) are negatively correlated with *VTCN1* expression.