

September 17<sup>th</sup>, 2018

The Editors  
Journal of Visualized Experiments

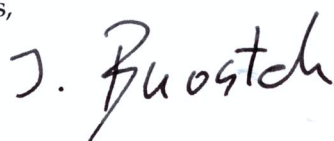
Dear Editors,

We wish to submit an original article entitled "Use of single chain MHC technology to investigate co-agonism in human CD8<sup>+</sup> T cell activation" for consideration by Journal of Visualized Experiments.

We have recently shown that non-stimulatory peptide MHC class I complexes can enhance human CD8<sup>+</sup> T cell activation through process termed co-agonism (Zhao et al., 2018 Nonstimulatory peptide-MHC enhances human T-cell antigen-specific responses by amplifying proximal TCR signaling. Nature Communications 13:2716. doi: 10.1038/s41467-018-05288-0). Our methods manuscript describes an experimental system to investigate co-agonism during human CD8<sup>+</sup> T cell activation by expressing human MHC class I molecules presenting pre-determined peptides as single polypeptides (single chain MHC) in a xenogeneic cell line. The protocol describes: cell line transfection with single chain MHC constructs, generation of stable cell lines, culture of hepatitis B virus-specific human CD8<sup>+</sup> T cells and T cell activation experiments simultaneously quantifying cytokine production and degranulation. The presented methods and the single chain MHC class I antigen presentation system can be used for research on different aspects of CD8<sup>+</sup> T cell activation in human T cell systems with known peptide MHC specificity.

We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

Sincerely yours,



Joanna Brzostek, Ph.D.  
Senior Research Fellow  
Prof. N. Gascoigne laboratory  
Department of Microbiology and Immunology  
Yong Loo Lin School of Medicine,  
National University of Singapore  
micjmb@nus.edu.sg