63 EPISCAN: A SYNTHETIC BIOLOGY PLATFORM FOR TARGETED IMMUNOPEPTIDOMICS

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Background Identification of $CD8^+$ T-cell epitopes is critical for the development of immunotherapeutics. Existing methods for empirical determination of peptide binding are time-intensive, expensive and highly specialized. Mass spectrometry, the predominant high-throughput approach for MHC-I ligand discovery, is unable to easily interrogate defined subsets of proteins. Thus, we sought a high-throughput, accessible method for the identification of MHC-I ligands derived from user chosen, synthetically encoded sources of peptides. Here we present EpiScan, a programmable genetic strategy to identify MHC-I ligands amongst predetermined starting pools comprising >100,000 peptides.

Methods To accomplish this, we used CRISPR-Cas9 to create 'EpiScan cells' that lack both endogenous MHC-I and short peptides in ER. Then, separate lentiviral introduction of an MHC-I allele and a single exogenous short peptide into the ER restores cell surface MHC-I levels according to the affinity of the peptide to the chosen MHC-I allele. We exploited the programmability of EpiScan to screen 12 different MHC-I alleles with large peptide libraries including the entire SARS-CoV-2 proteome.

Results These screens uncovered an unappreciated role for cysteine that increases the number of predicted ligands by 12–21%, revealed affinity hierarchies by analysis of biased-anchor peptide libraries, and identified conserved, high-affinity, T-cell reactive SARS-CoV-2 epitopes. Using these data, we generated and iteratively refined peptide binding predictions to create EpiScan Predictor, or ESP. ESP performed comparably to other state-of-the-art MHC-I peptide binding prediction algorithms while not suffering from underrepresentation of cysteine-containing peptides. Overall, the new specificities identified by EpiScan and ESP increase the number of peptides predicted to bind MHCs by over 15% on average. This significantly expands the potential human epitope landscape, facilitating epitope discovery efforts and the design of immunotherapeutics.

Conclusions Our work significantly expands the potential human epitope landscape, facilitating epitope discovery efforts and the design of immunotherapeutics.

Ethics Approval Peripheral blood was provided by collaborators from Ragon Institute of MGH that were PCR-confirmed COVID-19 cases. All study participants provided verbal and/or written informed consent. Participation in these studies was voluntary and the study protocols have been approved by the Partners Institutional Review Board.

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