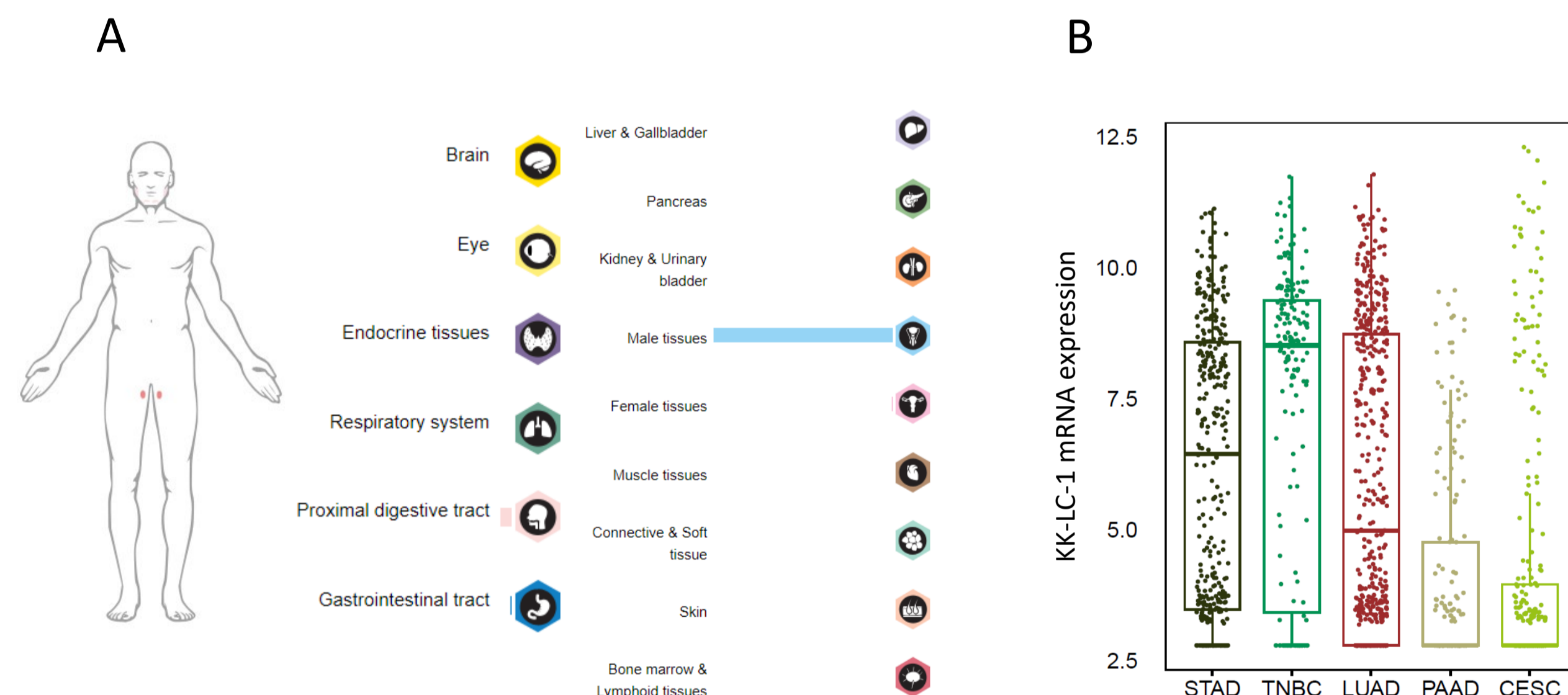


Background

- Intracellular tumor antigens presented as peptides on MHC (pMHC) class I molecules are attractive targets for development of highly tumor-targeted T-cell engager (TCE) therapies.
- There are a growing number of agents targeting pMHCs, most of which are restricted to HLA-A*02. There is an urgent need to identify effective pMHC-targeting therapies for other common HLA alleles to increase access for patients.
- The Kita-Kyushu lung cancer antigen-1 (KK-LC-1/CT83) peptide NTDNNLAVY presented on HLA-A*01:01 is a promising tumor target as its presentation is restricted to cancer cells such as gastric, lung, breast and cervical cancer.
- However, targeting the KK-LC-1 peptide NTDNNLAVY/HLA-A*01:01 complex is challenging due to the high levels of non-target peptides that are similar to the KK-LC-1 peptide and presented in normal tissues.
- A tailored screening and lead optimization process was undertaken to generate a safe and potent TCE against KK-LC-1/HLA-A*01:01.

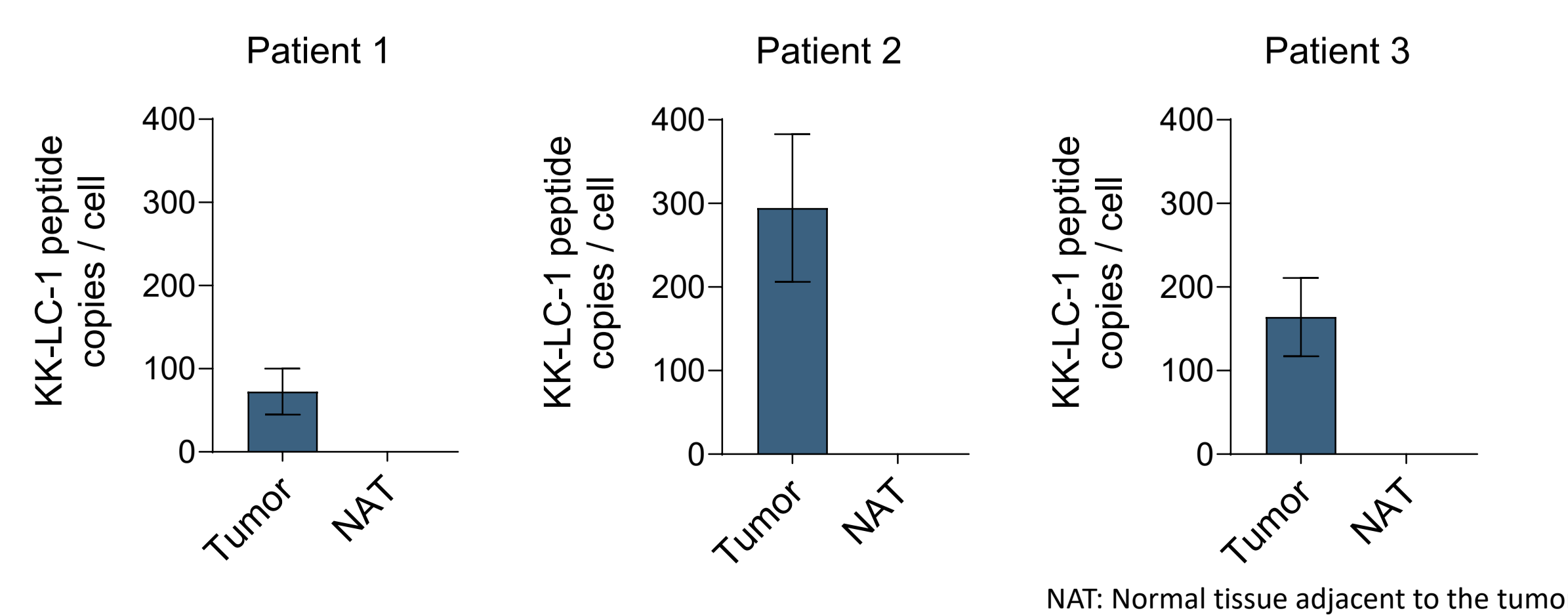
1 KK-LC-1/HLA-A*01:01 TCE has the potential to benefit a large patient population

KK-LC-1 is expressed in cancers of high unmet medical need including gastric cancer (STAD), triple negative breast cancer (TNBC), lung adenocarcinoma (LUAD), pancreatic adenocarcinoma (PAAD) and cervical squamous cell carcinoma (CESC). A In healthy subjects, KK-LC-1 mRNA expression (nTPM) is solely enriched in testis (left) (Source: www.proteinatlas.org; Uhlén M et al. 2005). B KK-LC-1 mRNA expression (log-transformed vst-normalized) was obtained (GeneVia Technology) from The Cancer Genome Atlas (TCGA).



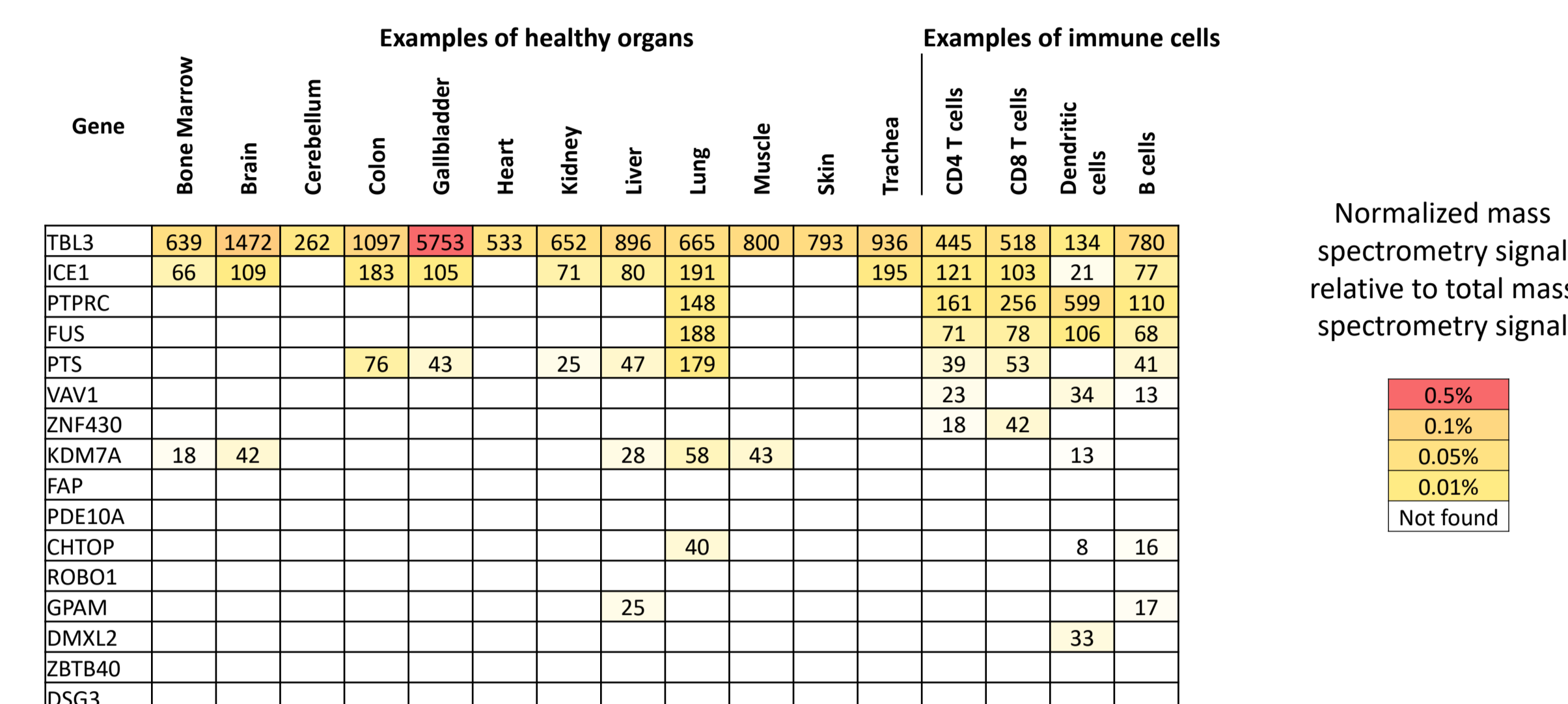
2 KK-LC-1 peptide NTDNNLAVY is presented at high density on the surface of NSCLC cells

HLA-A*01:01 positive tumor samples from non-small cell lung cancer (NSCLC) patients were used for immunoprecipitation followed by mass spectrometry analysis. The KK-LC-1 target peptide NTDNNLAVY was identified in three of six samples.



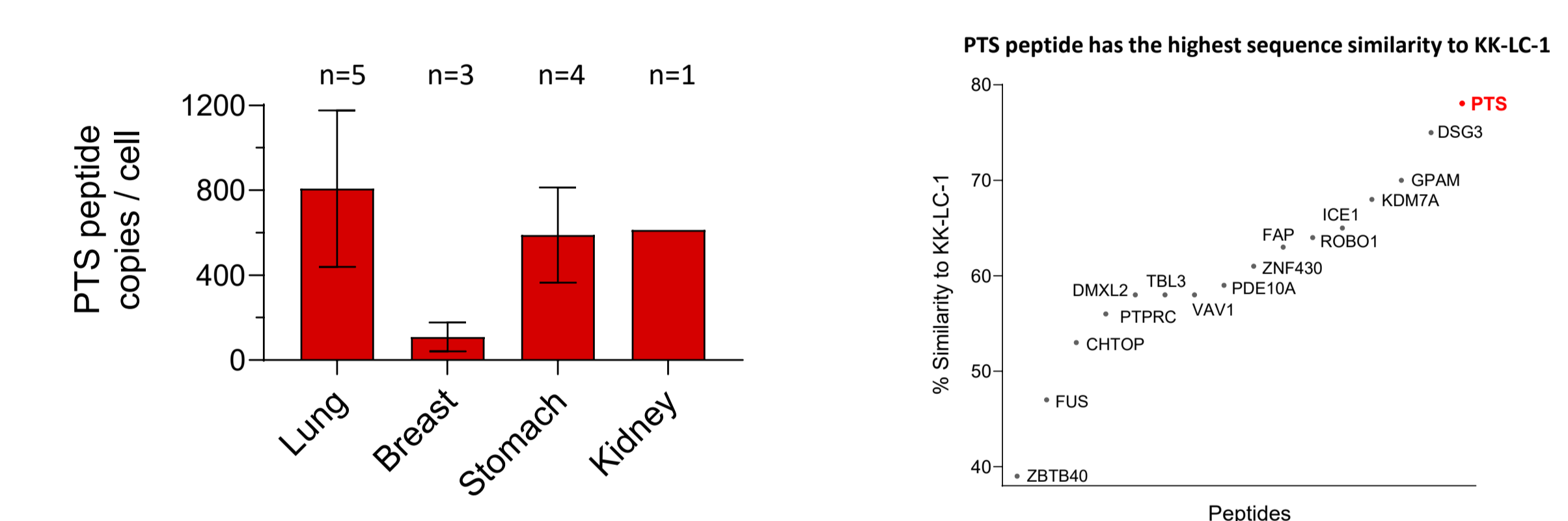
3 Identification of similar peptides in healthy tissue

An in silico analysis was performed (BLAST program; https://blast.ncbi.nlm.nih.gov) to identify peptides within the human proteome that share a high percentage of sequence identity with the KK-LC-1 peptide and are predicted to be loaded to HLA-A*01:01 (IEDB MHC-I prediction tool; http://tools.iedb.org/mhci/). Similar peptides identified in silico were cross-referenced against a database (Alithea Bio) containing mass spectrometry-identified peptides presented by an extensive range of healthy tissues. Several were found to be frequently present in healthy tissues.



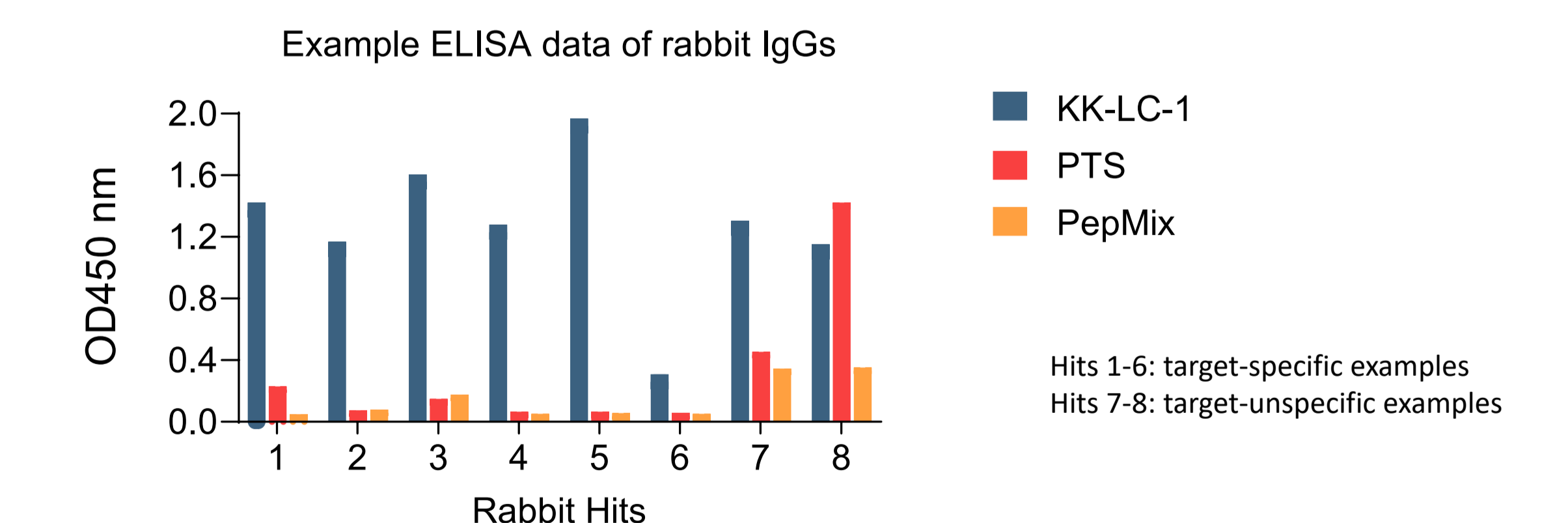
4 High natural levels of most similar human peptide PTS

HLA-A*01:01 positive tumor-adjacent tissue samples from 13 patients were used for HLA immunoprecipitation followed by mass spectrometry analysis. The most similar human peptide PTS was identified in very high numbers in each sample.



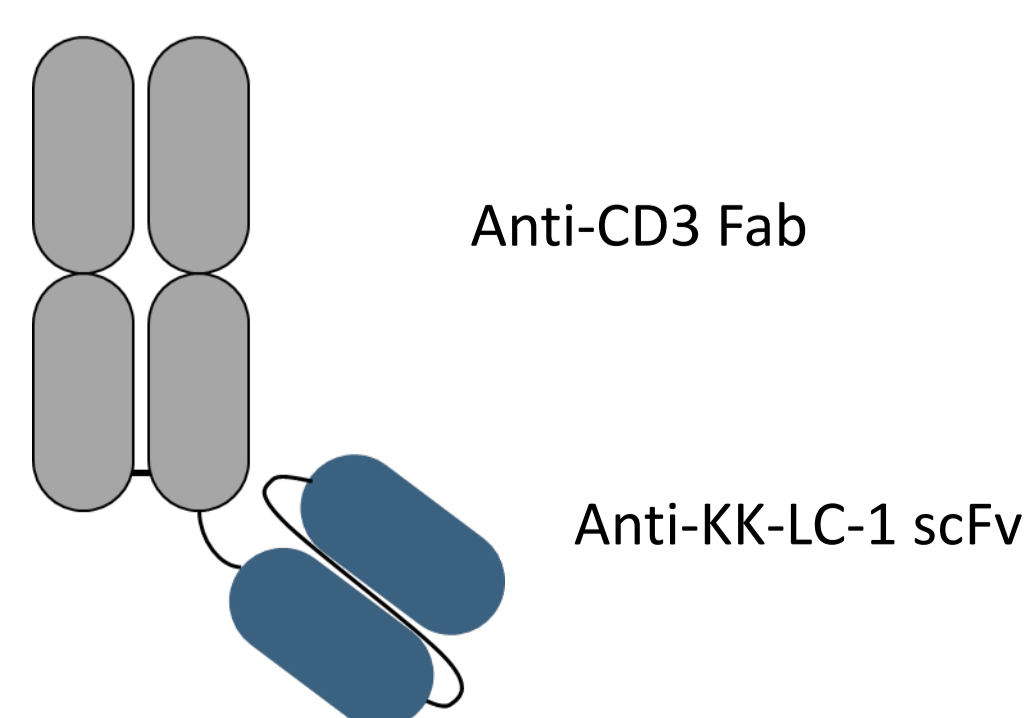
5 Screening identified antibodies against KK-LC-01/HLA-A*01:01 with no binding to PTS

Antibodies binding to the KK-LC-1/HLA-A*01:01 complex were isolated from phage display libraries and B cells of pMHC immunized rabbits that were counter-selected against a pool of similar off-target peptides in complex with HLA-A*01:01. More than 100 hits were measured in ELISA for binding to HLA-A*01:01 in complex with KK-LC-1, PTS and a pool of 20 unrelated peptides (peptide mix / PepMix).



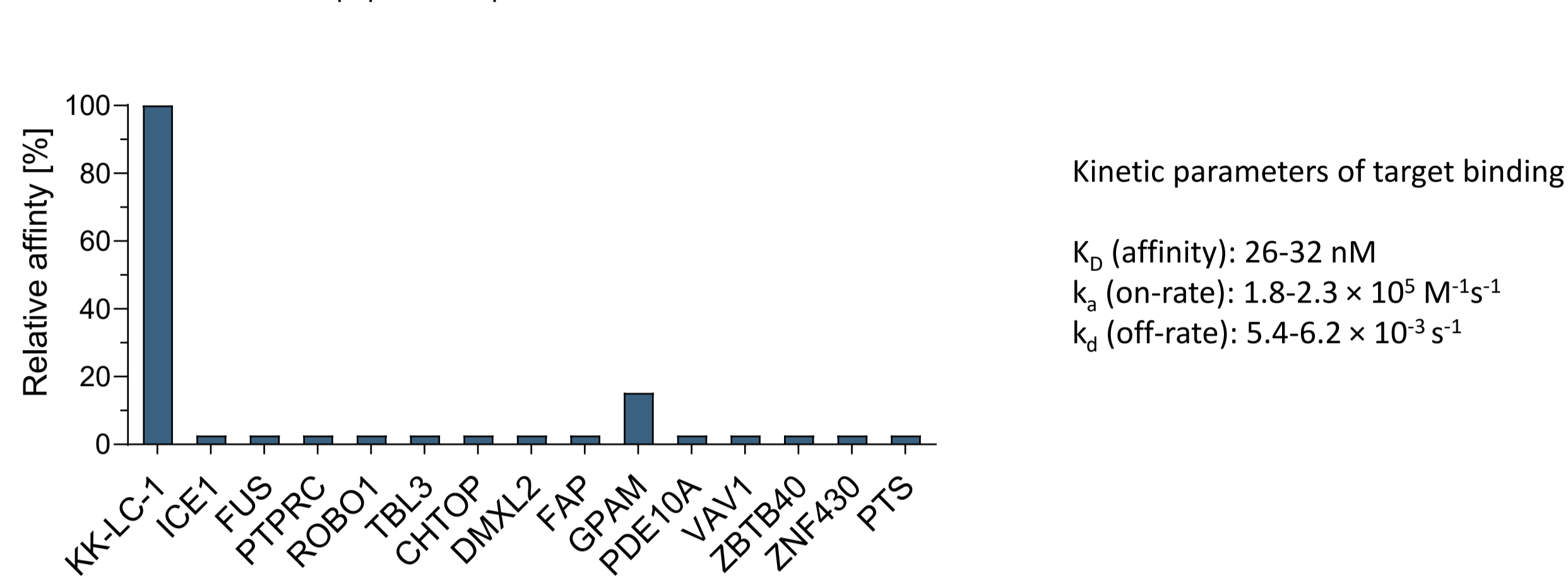
6 Most promising hits humanized as KK-LC-1xCD3 TCE using CDR-Life's M-Gager technology

The most specific antibodies were reformatted into a TCE by fusing the anti-KK-LC-1 antibody as scFv to an anti-CD3 Fab effector arm.



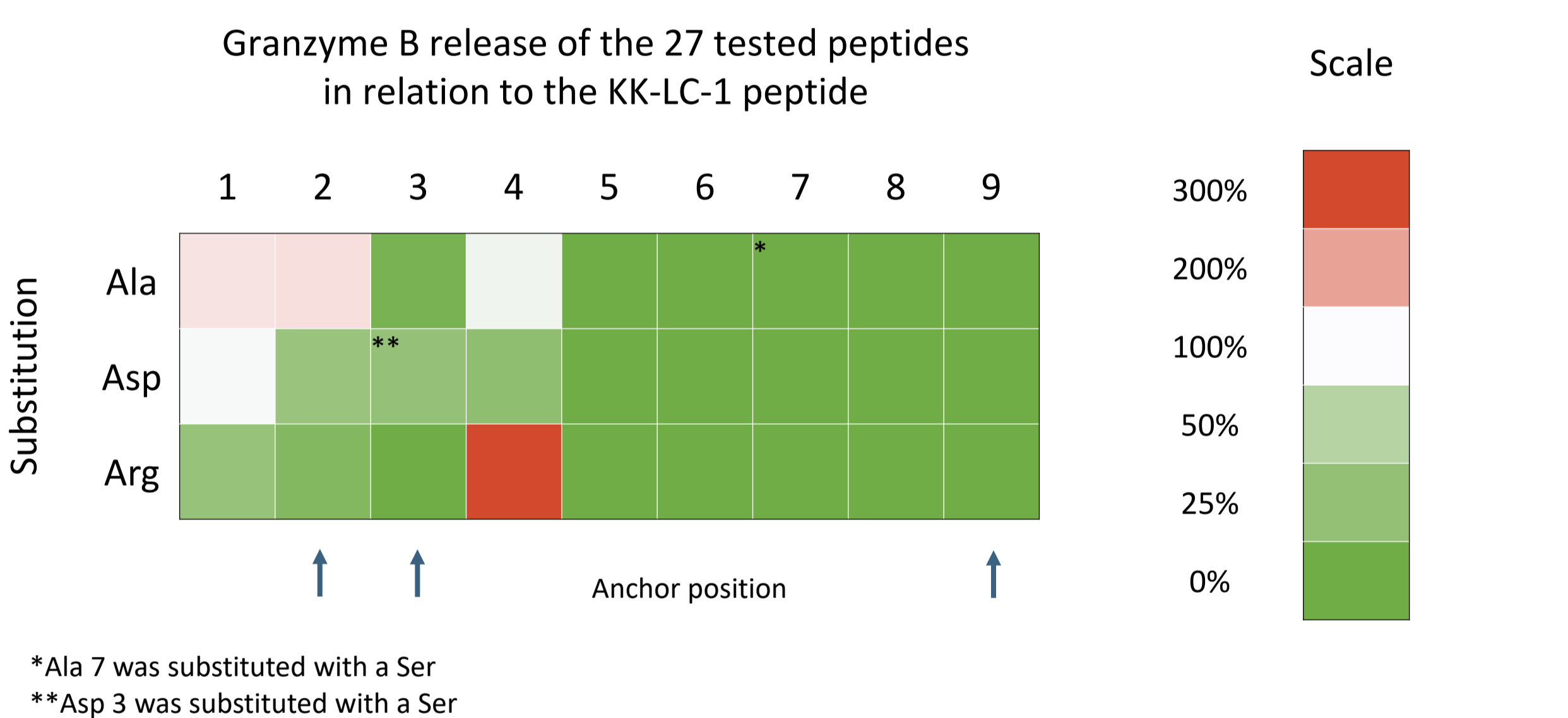
7 SPR measurements confirm highly-specific binding of KK-LC-1xCD3 TCE to KK-LC-1/HLA-A*01:01

Peptide/HLA-A*01:01 complexes were captured to the sensor surface and three consecutive injections of the KK-LC-1xCD3 TCE were performed. Relative affinities were calculated by dividing the K_D (affinity) of the KK-LC-1 interaction by the K_D obtained for the similar peptide complexes.



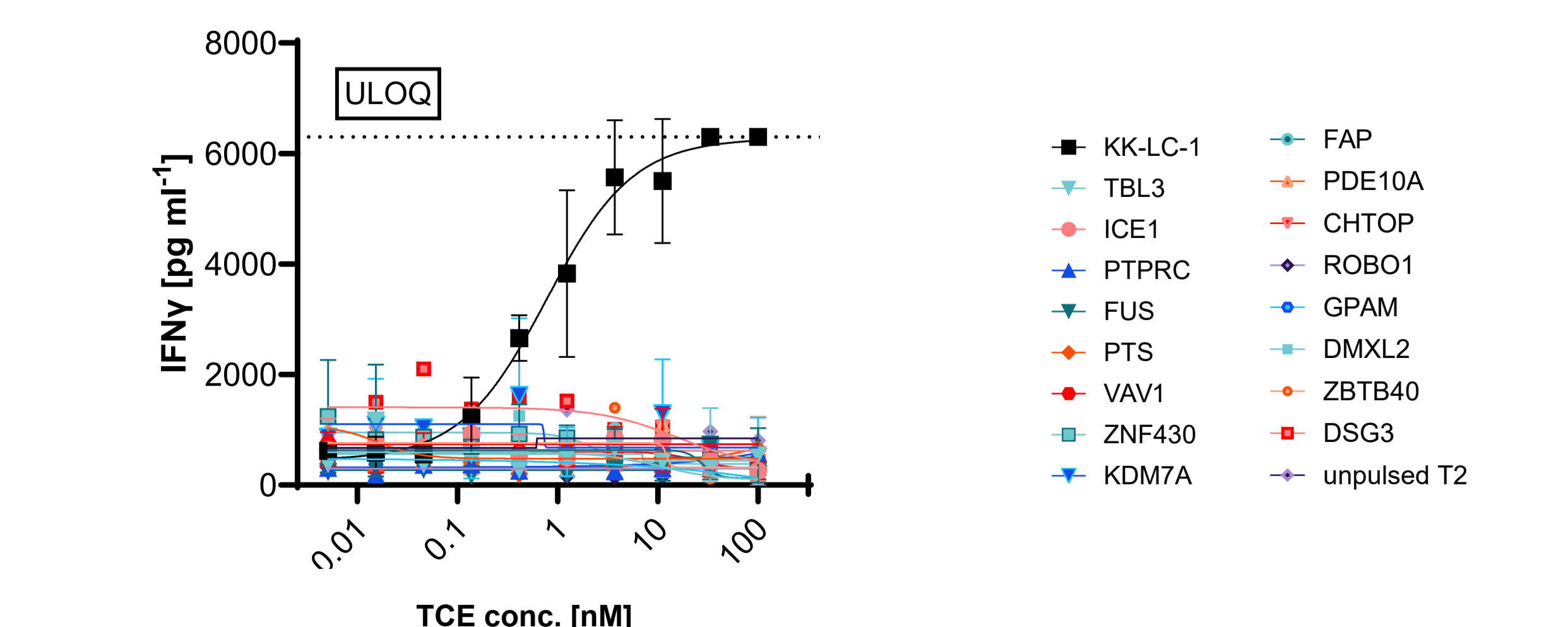
8 KK-LC-1xCD3 TCE shows excellent specificity to single-amino acid substitutions in the target peptide

TAP-deficient, (empty) HLA-A*01:01 expressing T2A1 cells were pulsed with KK-LC-1 target peptide or with peptides containing a single amino acid substitution to Alanine, Aspartic acid, or Arginine on each peptide position. Peptide-pulsed T2A1 cells were co-cultured with human PBMCs and the KK-LC-1xCD3 TCE for 24 h and Granzyme B release was quantified by ELISA. Reduction of Granzyme B release indicates the relevance of the specific position for pMHC recognition by the TCE.



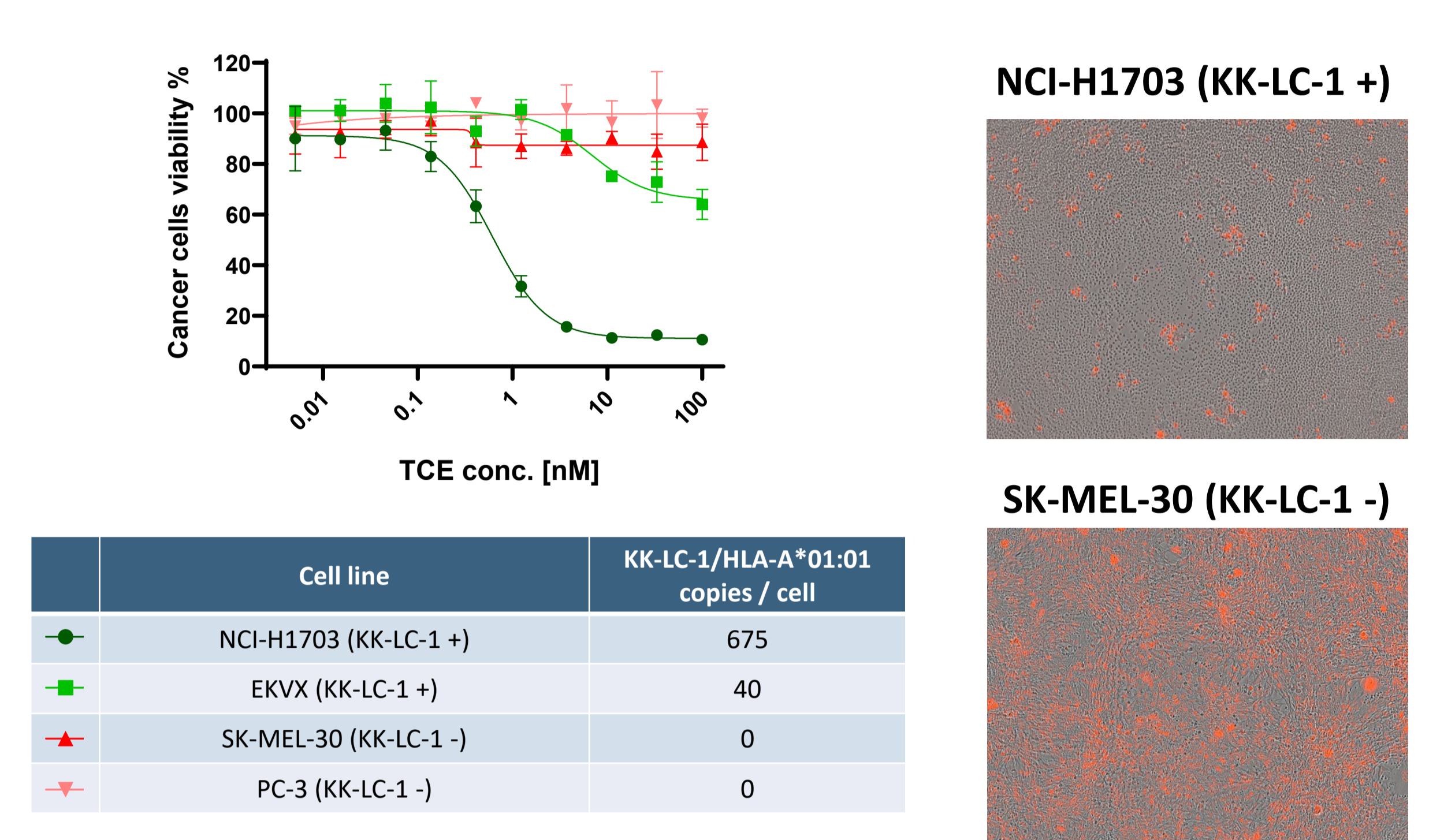
9 KK-LC-1xCD3 TCE does not trigger IFN γ release against T2A1 cells pulsed with similar peptides

TAP-deficient, (empty) HLA-A*01:01 expressing T2A1 cells were pulsed with KK-LC-1 or similar peptides and co-cultured with human PBMCs and a serial dilution of the KK-LC-1xCD3 TCE for 24 h. Interferon γ (IFN γ) release from PBMCs was measured by ELISA.



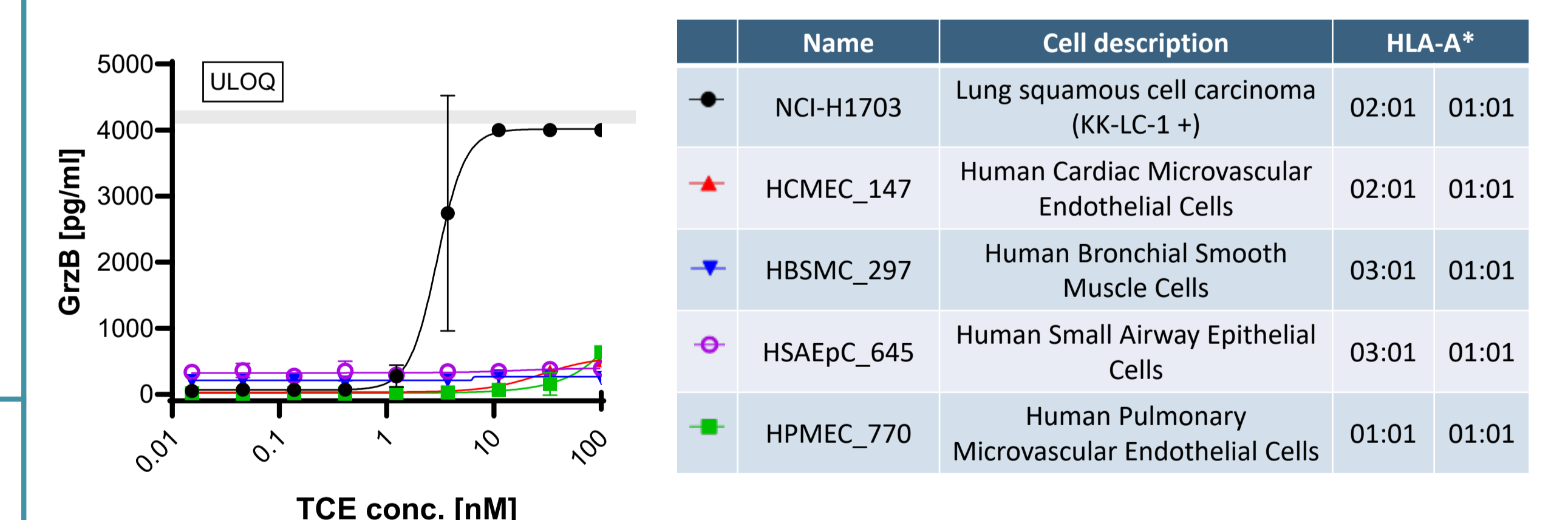
10 KK-LC-1xCD3 TCE demonstrates cell lysis of KK-LC-1 positive tumor cells and no killing of KK-LC-1 negative tumor cells in vitro

KK-LC-1xCD3 TCE was tested in a co-culture assay of HLA-A*01:01-expressing KK-LC-1 (+) and (-) cancer cell lines with human PBMCs. Cancer cell killing was evaluated with live-cell imaging in IncuCyte after 72 h (cells tagged with fluorescent, nuclear-restricted RFP). Good correlation of potency and target copy number was observed.



11 KK-LC-1xCD3 TCE demonstrates no cross-reactivity towards normal human primary cells

KK-LC-1xCD3 TCE was tested in co-cultures of HLA-A*01:01-expressing normal human primary cells or positive control cells (NCI-H1703) with human PBMCs. Granzyme B (GrzB) release was quantified by ELISA after 24 h. Mass spectrometry-based immunopeptidomics analysis of HCMEC_147 cells identified ~260 copies per cell of PTS peptide. No data are available for the other three primary cell samples.



Conclusion

- KK-LC-1 is an attractive tumor-specific target with a high prevalence in several common cancers.
- KK-LC-1-derived peptide NTDNNLAVY was identified in high copy number on NSCLC cells.
- Through selective screening, very specific antibodies against the target complex KK-LC-1/HLA-A*01:01 were identified.
- High potency with no off-tumor activity with KK-LC-1xCD3 TCE confirmed.
- Overall, these results support the further development of this TCE into a tumor therapy.