



Targeting mutant KRAS proteins with novel TCR-mimic fully human antibodies

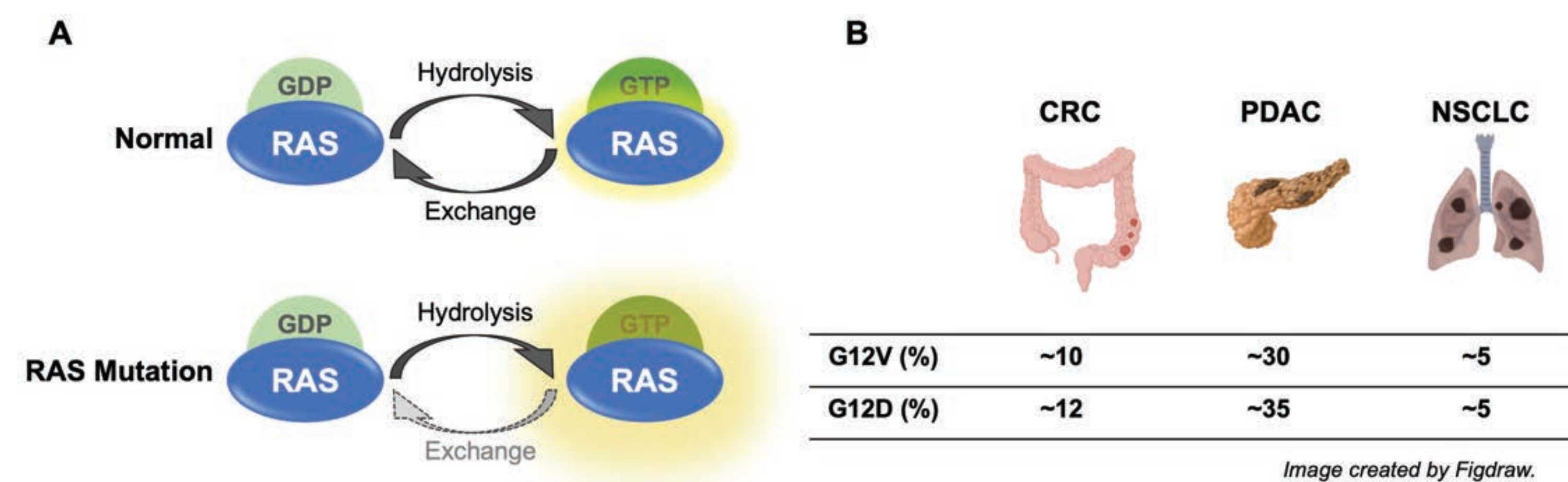
Jun Du, Wanbo Tang, Xin Jiao, Limin Zhao, Yue Zhang, Baihong Liu, Chaoshe Guo, W. Frank An, Yi Yang

¹Biocytogen Pharmaceuticals (Beijing) Co., Ltd., Beijing, Beijing, China

ABSTRACT

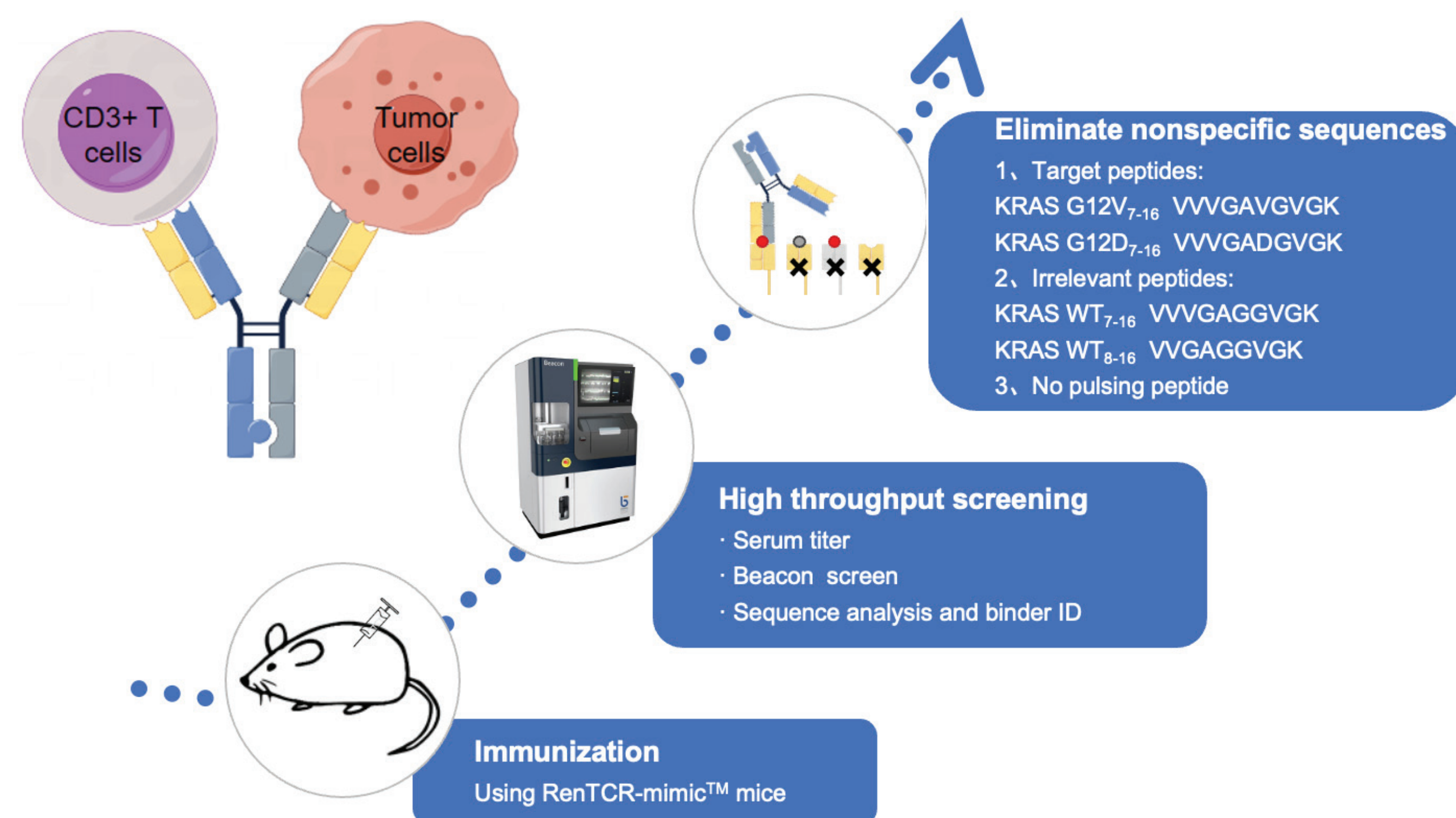
Mutated KRAS proteins are ideal cancer targets, as they are expressed frequently and specifically in certain solid tumors. A large proportion of human colorectal cancer and pancreatic ductal adenocarcinoma express the tumor driver KRAS gene mutations G12V/G12D, but drugs targeting G12V/G12D are not available, revealing a huge unmet clinical need. While small molecules often fail to target the KRAS mutation G12V/G12D, T cell receptor-mimic (TCR-mimic) antibodies can specifically recognize KRAS mutations presented by human leukocyte antigen (HLA), opening up possibilities for targeting such intracellular antigens. Here, we discovered novel antibodies highly specific to G12V/HLA and G12D/HLA complexes by immunizing our proprietary RenTCR-mimicTM mice and using high-throughput Beacon-based screening. These TCR-mimic antibodies have higher affinities compared to endogenous TCRs, which may effectively reduce the possibility of tumor escape. Germline distribution analysis indicated their high sequence diversity, which suggests diverse epitope targeting. Although pancreatic cancer is extremely difficult to treat and has an extremely low KRAS mutant peptide-HLA complex density on the cell surface, our TCR-mimic antibodies exhibited potent in vitro tumor lysis activity when assembled into CD3 T cell engagers. Furthermore, these antibodies demonstrated convincing lack of off-target binding. Together, our results indicate promising therapeutic potential of these KRAS mutation-targeted TCR-mimic antibodies for the treatment of solid tumors.

KRAS G12 mutations are prevalent in multiple types of solid tumors



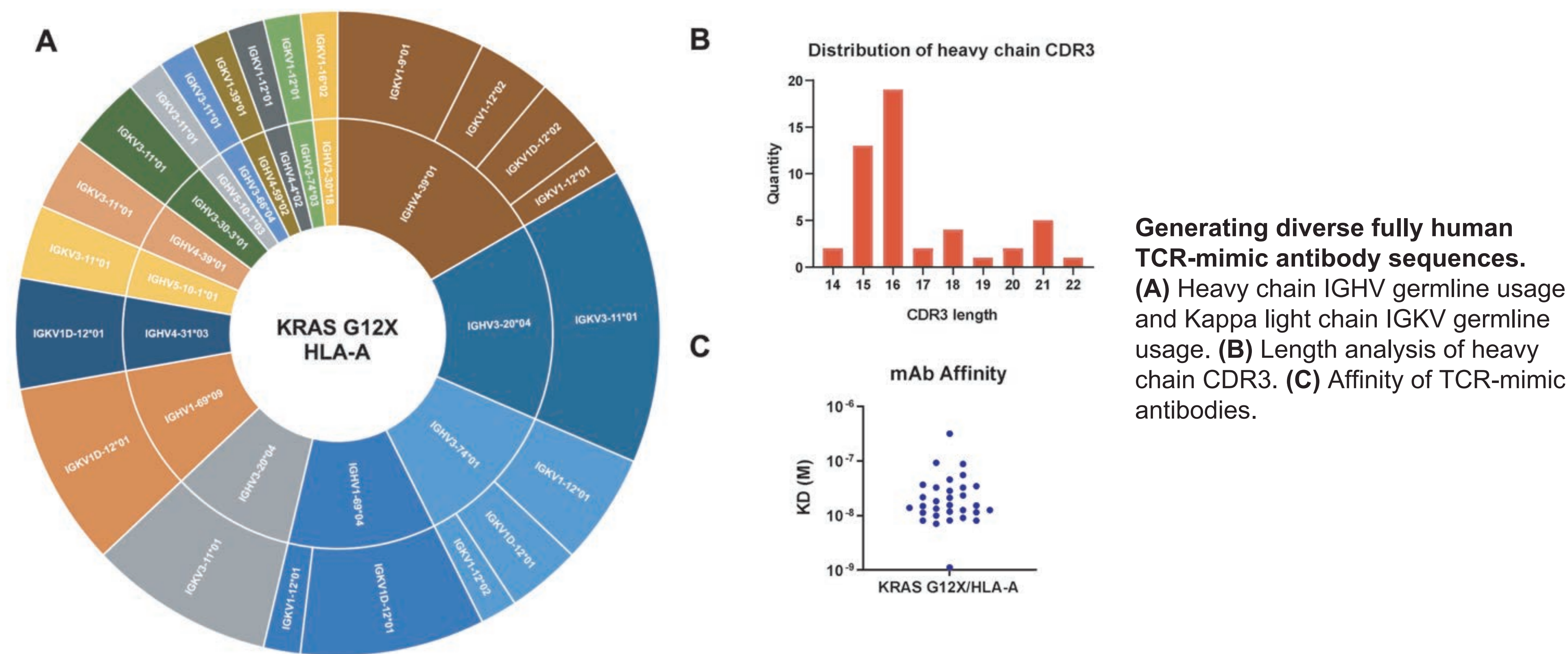
KRAS mutations drive tumorigenesis. (A) KRAS functions as a membrane-bound protein that switches from an inactive state when bound to GDP to an active state when bound to GTP. In its active form, KRAS can trigger downstream signaling pathways that promote cell growth and differentiation. (B) Mutations in the KRAS gene are the primary driver of various cancer types, such as colorectal cancer (CRC), pancreatic ductal adenocarcinoma (PDAC), non-small cell lung cancer (NSCLC), as well as certain hematological disorders. Despite this, there is currently no approved medication specifically targeting the KRAS G12V or G12D mutation.

High-throughput screening for KRAS G12X/HLA-A specific TCR-mimic antibodies

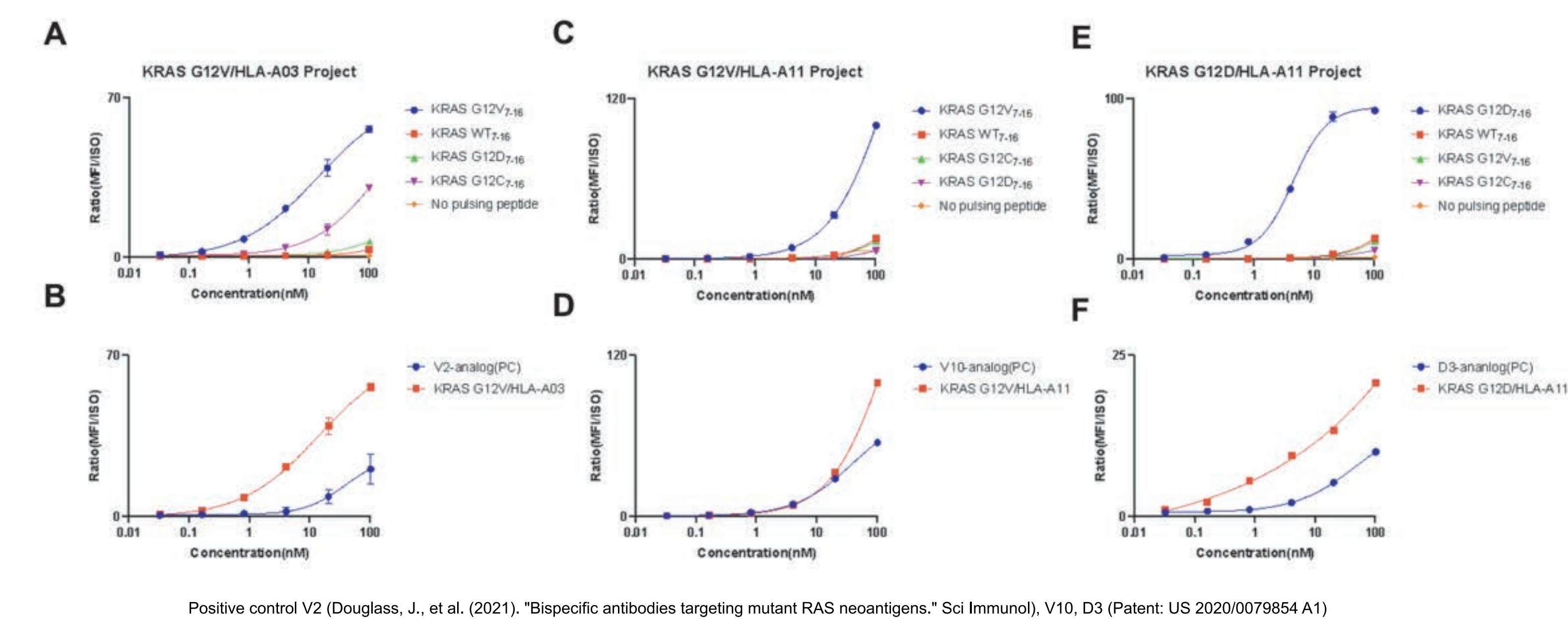


Screening process: Novel antibodies highly specific to KRAS G12V/HLA-A03, G12V/HLA-A11 or G12D/HLA-A11 complexes by immunizing our proprietary RenTCR-mimicTM mice and using high-throughput Beacon-based screening system.

KRAS G12X/HLA-A sequences exhibit abundant diversity and high affinity

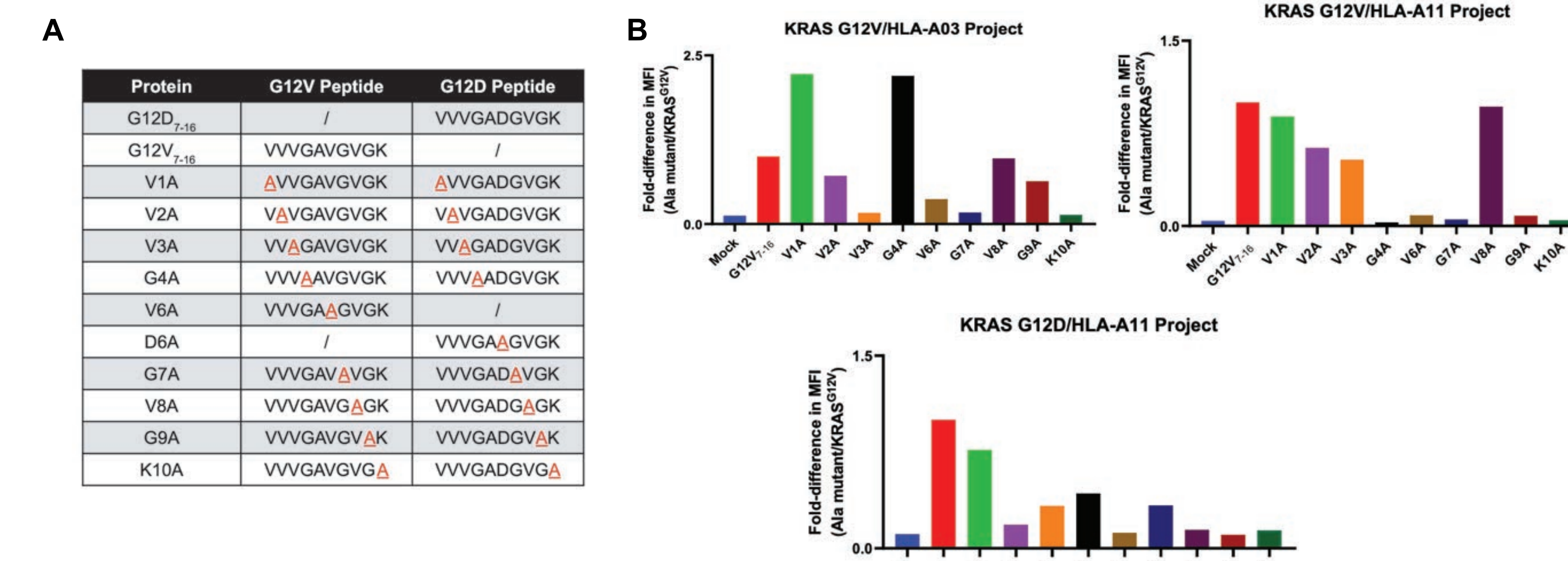


KRAS G12X/HLA-A antibodies exhibit specific binding

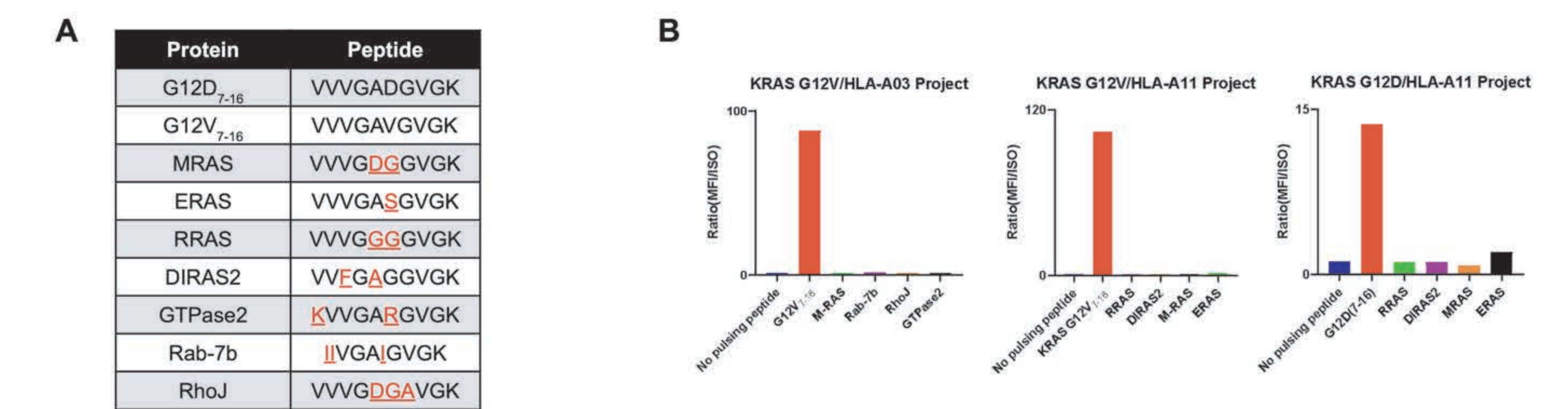


KRAS G12X/HLA-A antibody-specific binding assays. (A/C/E) TCR-mimic antibodies specifically bind their respective KRAS/HLA targets (G12V/HLA-A03, KRAS G12V/HLA-A11, and KRAS G12D/HLA-A11). (B/D/F) TCR-mimic antibodies all show better binding activity than that of positive control. (B) HLA-A03 overexpressing cells pulsed with G12V₇₋₁₆ peptide. (D) HLA-A11 overexpressing cells pulsed with G12V₇₋₁₆ peptide. (F) HLA-A11 overexpressing cells pulsed with G12D₇₋₁₆ peptide.

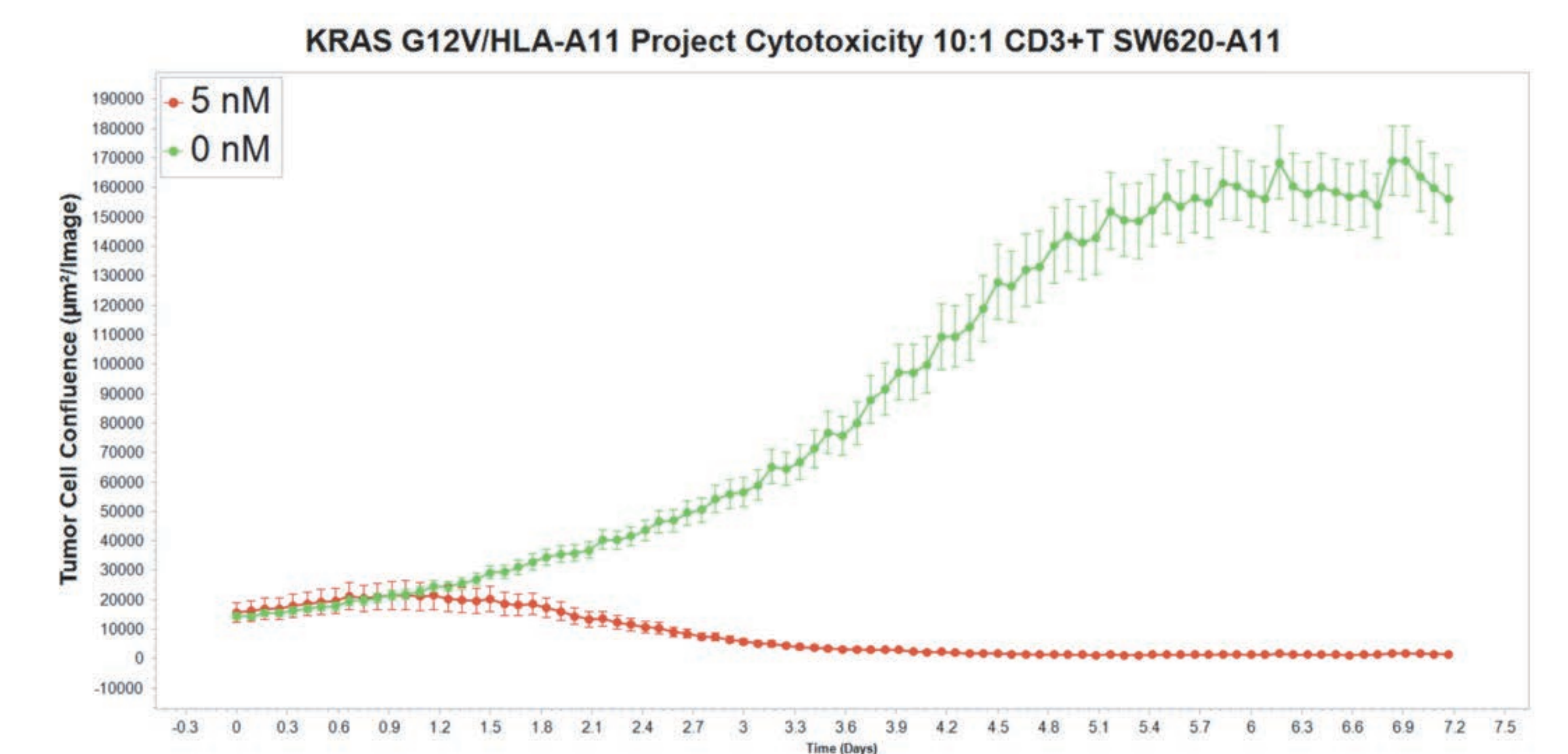
Alanine scan indicates residue 6 is a key binding site



TCR-mimic antibodies show specificity by distinguishing putative off-target peptide



TCR-mimic antibody engagers show potent cytotoxic activity



Cytotoxicity of KRAS G12V/HLA-A11 bispecific antibodies. The cytotoxic activity of KRAS G12V/HLA-A11 x CD3 T cell engager against SW620-A11 cells. Target cells were incubated with antibody and human CD3⁺ T cells in E:T=10:1. SW620-A11:SW620 cell line was engineered to express HLA-A*11:01 and EGFP. KRAS G12V/HLA-A03 bispecific antibody also showed similar cytotoxic activity (data presented before and not shown here).

SUMMARY

- We identified TCR-mimic antibodies specific for KRAS G12X peptide HLA-A03 or HLA-A11 complexes.
- TCR-mimic antibodies showed diverse germline gene usage diversity and high affinity.
- TCR-mimic antibodies did not cross-bind similar peptide-HLA complexes or potential off-target peptide.
- Alanine scan revealed residue 6 in KRAS G12X was required for antibody binding.
- Cytotoxicity assays demonstrated potent lysis of tumor cells by KRAS G12X/HLA x CD3 bispecific T cell engager.