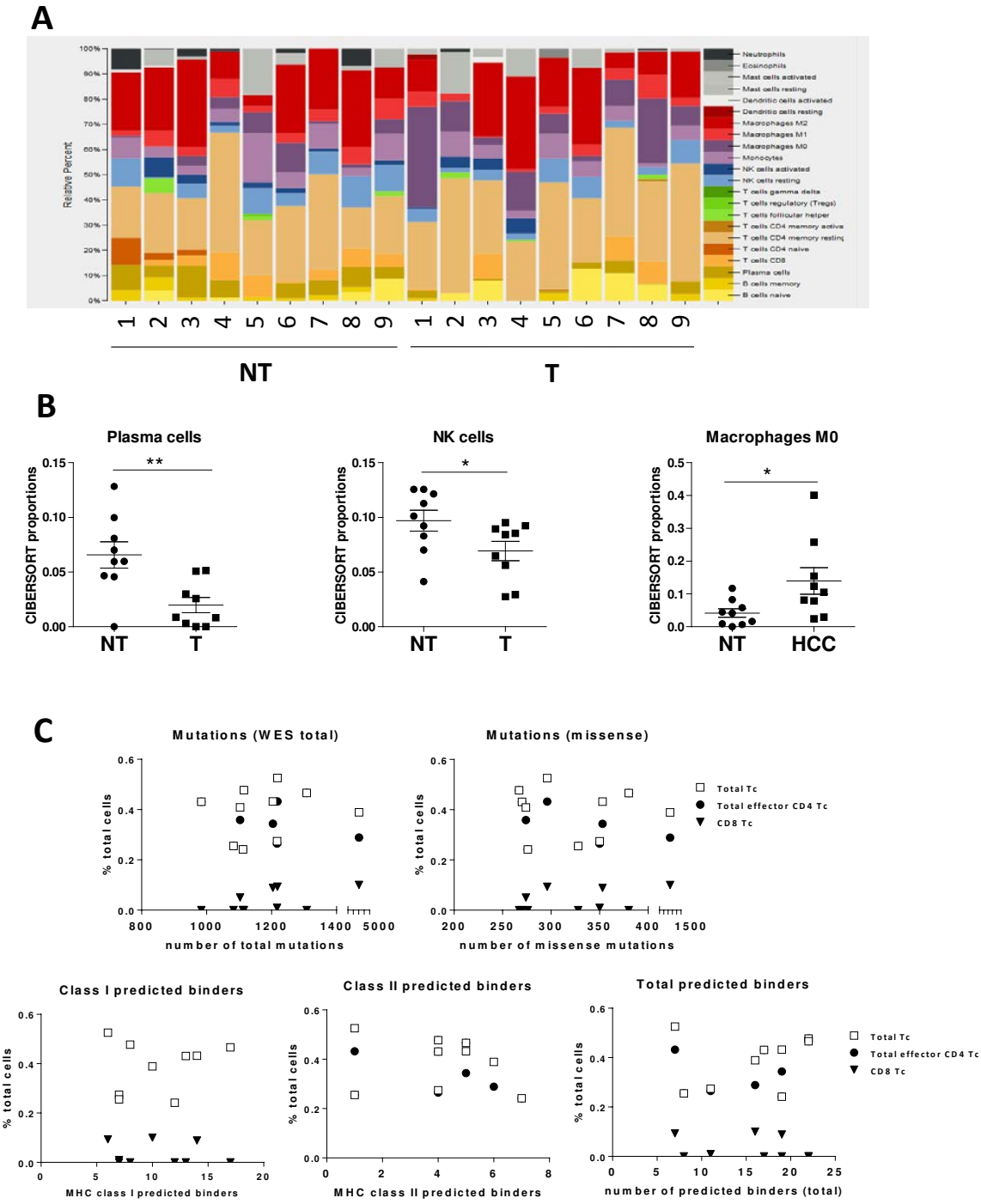
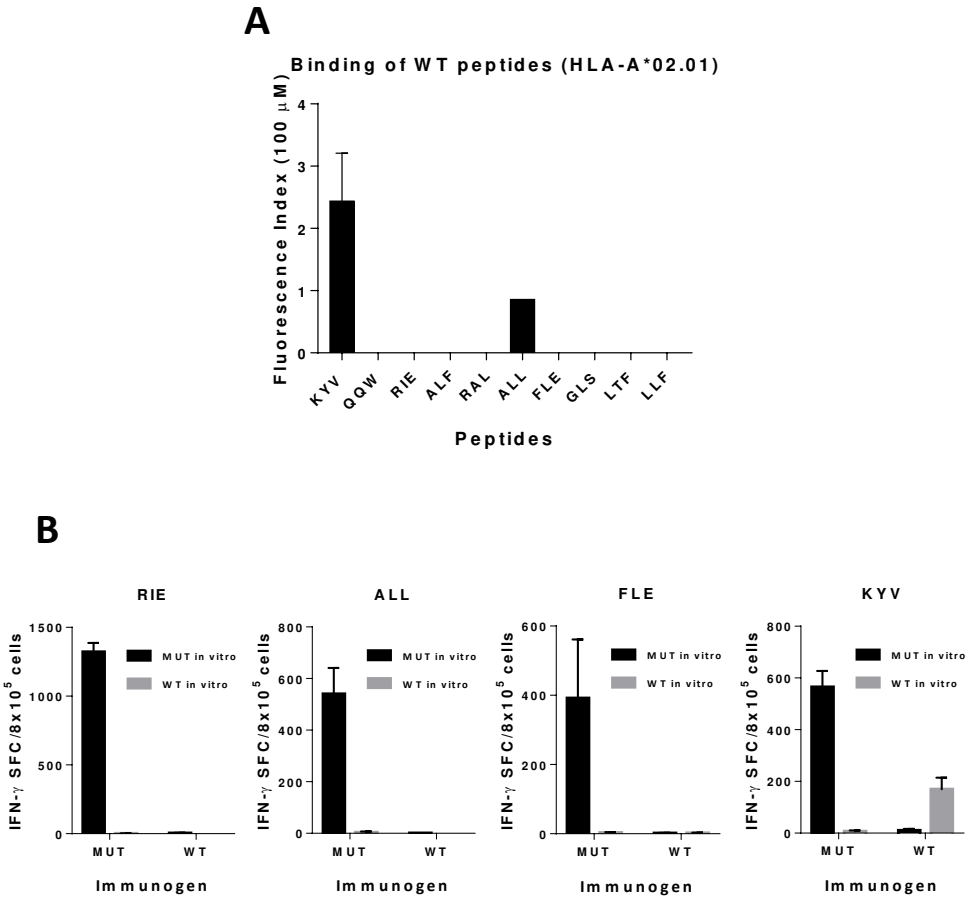
Supplementary methods for Supplementary Figure S1:

CIBERSORT analysis
Characterization of immune cell types in T and NT samples was carried out using CIBERSORT (https://cibersort.stanford.edu) [1]. By applying the original CIBERSORT gene signature file LM22, the percentage of 22 immune cell subtypes was calculated in the sample dataset. Data were analyzed with a number of permutations set to 100. Immune cell profile was calculated for each sample and T test was used to compare between T and NT samples.

[1] Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 2015;12:453–7. https://doi.org/10.1038/nmeth.3337.



Supplementary Figure S1. Immune infiltration in HCC tumors. (A) Data from RNAseq from 9 patients were analyzed using CIBERSORT and the proportion of leukocyte subsets in T and NT samples calculated. (B) Comparison of Plasma cells, NK cells and M0 macrophages in T and NT samples. (C) Association between mutations (total and missense) and predicted neoAgs and infiltrating T cells (total, effector CD4 and CD8). (*) $p < 0.05$.

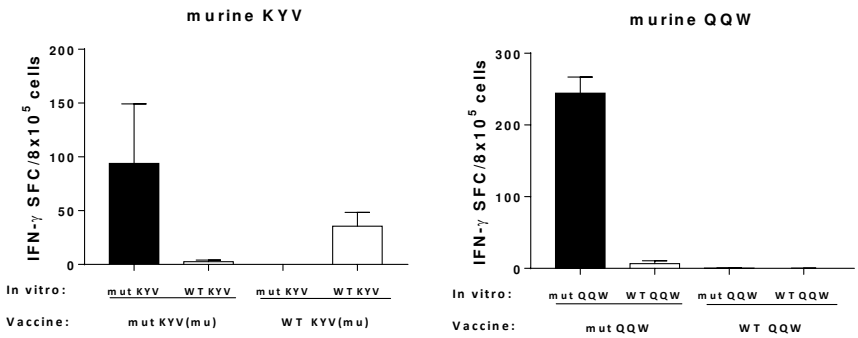


Supplementary Figure S2. Binding to HLA-A*02.01 molecules and immunogenicity of the WT versions of predicted neoAgs. (A) Binding of WT versions (at 100 μM) of mutated neoAgs was tested using T2 cells. Resulst are expressed as Fluorescence Index (FI) and peptides with FI > 0.5 were considered positive. (B) HHD-DR1 mice (n=4/group) were immunized with mutated or WT versions of neoAgs and immune response against both peptides were evaluated by ELISPOT.

A

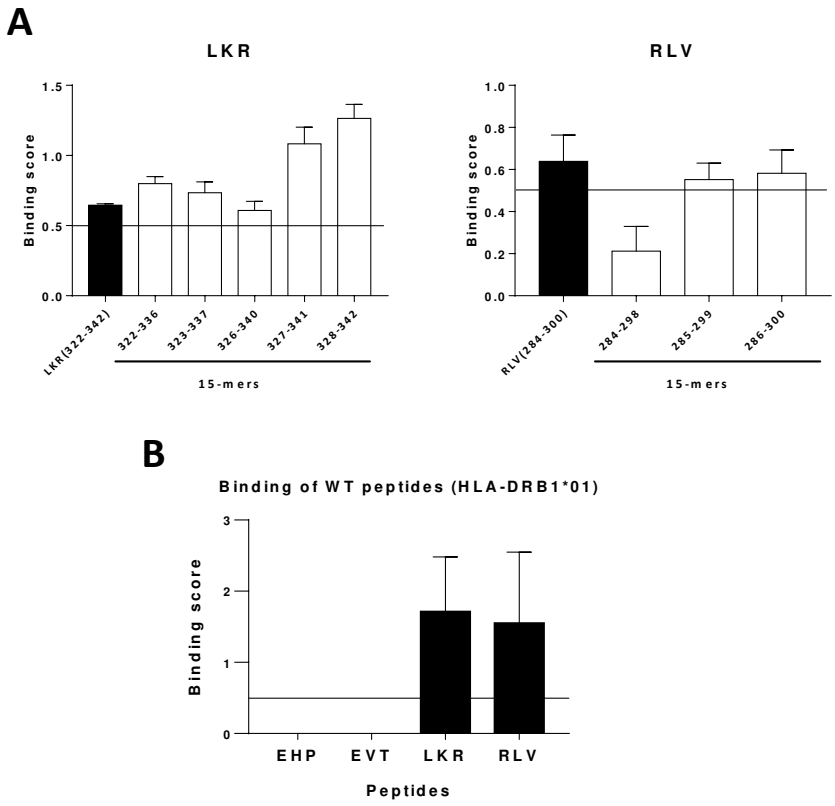
Peptide	WT sequence (hu)	WT sequence (mu)	Homology	mut sequence (hu)	mut sequence (mu)
KYV	KYVDDFGVSV	KYVDDAGVPV	80	KYV**Y**DFGVSV	KYV**Y**DAGVPV
QLD	QLDIIPASI	QADKTTASC	44	QLDIIPAS**V**	QADKTTAS**V**
QQW	QQRLALFFV	QQRLVVFSV	66	QQ**W**LALFFV	QQ**W**LVVFSV
CQQ	CQQRLALFFV	CQQRLVVFSV	70	CQQ**W**LALFFV	CQQ**W**LVVFSV
RIE	RIECIFFSE	RIECIFFSE	100	RIECIFFS**V**	RIECIFFS**V**
ALF	ALFALLEIP	ALFALLEVP	89	ALFALLEI**L**	ALFALLEV**L**
RAL	RALFALLEIP	RALFALLEVP	90	RALFALLEI**L**	RALFALLEV**L**
ALL	ALLFPESEL	SLLFPESEL	90	ALLF**L**ESEL	SLLF**L**ESEL
FLE	FPESELSIRI	FPESELSIRI	100	F**L**ESELSIRI	F**L**ESELSIRI
GLS	GVSFFILSL	GVSFFILSL	100	G**L**SFFILSL	G**L**SFFILSL
LTF	LKFNQCYGV	LKSNQCYGV	89	L**T**FNQCYGV	L**T**SNQCYGV
LLF	LLFGYSFAK	LLFGYSLAK	89	LLFGYSFA**T**	LLFGYSLA**T**

B



Supplementary Figure S3. Immunogenicity of mutated and WT peptides bearing murine sequences. (A) Comparison of the human and murine sequences of mutated and WT peptides indicating the % of homology human/murine. Underlined residues correspond to changes between human and murine peptides. Mutations present in tumor cells are shown in bold. (B) HHD-DR1 mice were immunized with the murine mutated or WT version of peptides KYV and QQW and recognition of mutated and WT versions of murine peptides was evaluated by ELISPOT.

Supplementary Figure S3



Supplementary Figure S4. Binding to HLA-DRB1*01 molecules of neoAg peptides. Binding to HLA-DRB1*01 molecules of 15-mer peptides contained in the 21- and 17-mer longer peptides (A) and of the WT versión of selected immunogenic neoAgs (B) was evaluated using HOM2 cells. Results are expressed as Binding Score (BS) and peptides with BS > 0.5 were considered positive.

Supplementary Figure S4