

# Supplementary Figure 1

A.

## Entry Page

**Welcome to the ABPEPserver!**

Cancer cells experience tryptophan shortage as a consequence of interferon-gamma (IFN) signaling pathway. It has been recently demonstrated that such tryptophan shortage leads to tryptophan to phenylalanine codon reassignment (W>F) resulting into aberrant peptide product. ABPEPserver offers the visualization of a large-scale proteomics analysis of multiple human cancer types in which aberrant peptides are detected. In this analysis, tryptophan to phenylalanine (W>F) codon reassignment were found to be a vastly abundant phenomenon in multiple cancer types. Furthermore, these W>F mis-incorporations, called W>F substituents, were found to be enriched in tumors as compared with adjacent normal tissues, and often their appearance was associated with T-cell and oncogenic signaling activities. Proteomic cancer data from multiple cancer types, hosted by [PDC commons](#) database, have been used in this analysis. An in depth description of how the analysis was conducted is shown in the **'Methods'** tab. The **'Cancer types'** tab shows cancer types analyzed and includes information about each proteomic dataset. This server will enable ease and fast access to aberrant peptides, which are potent immunotherapeutic targets and hence of high value. Results are available in:

- Barplots depicting cumulative number of W-substitutants detected in tumour and adjacent normal tissue samples.
- Violin plots depicting the number of substitutions detected in low (intensity < 0) and high (intensity > 0) tumor and adjacent normal tissue samples.
- Scatter contour plots depicting for every gene the number of W>F substitutions when the gene is higher expressed (intensity > 0) on X-axis (High Class) and when the gene is lower expressed (intensity < 0) on Y-axis (Low Class).
- Excel file outputs with the list of aberrant peptides.

**Background information**

T-cells infiltrating tumour microenvironment secrete IFN-gamma (IFN $\gamma$ ) which activates Indoleamine 2,3-dioxygenase 1 (IDO1) enzyme expression in cancer cells. IDO1 stimulates pathways that subvert T-cell immunity while also breaking down tryptophan in its process, leading to a tryptophan deficiency.<sup>1</sup> When a healthy cell is deficient in tryptophan, ribosomes will stop translation at the codon for tryptophan. Therefore, protein synthesis will stop. Nevertheless, in Melanoma cells protein synthesis continues despite interferon gamma (IFN $\gamma$ )-mediated tryptophan depletion. They skip the first nucleotide of the codon for tryptophan. Consequently, the whole reading frame jumps from that point on leading to the synthesis of aberrant proteins as a result of ribosomal frameshifting at tryptophan codons.<sup>2</sup> In follow up studies, it was shown that IFN $\gamma$ -mediated tryptophan shortage also impacts protein quality by generating specific mis-incorporations of phenylalanine at tryptophan codons. This type of amino acid deprivation-induced codon reassignments are called 'substitutants' to distinguish them from somatic genetic substitutions. Therefore, the absence of the essential amino acid tryptophan causes ribosomes to induce errors of two-types, i.e., ribosomal frame-shifting and amino-acid substitution.

**Notes**

1. Champagne J, Pataskar A, Blommaert N, et al. Oncogene-dependent sloppiness in mRNA translation [published online ahead of print, 2021 Sep 21]. Mol Cell. 2021;S1097-2765(21)00736-X. doi:10.1016/j.molcel.2021.09.002.
2. Bartok, O., Pataskar, A., Nagel, R. et al. Anti-tumour immunity induces aberrant peptide presentation in melanoma. Nature 590, 332–337 (2021). <https://doi.org/10.1038/s41586-020-03054-1>.

B.

## Analyze Module

**Analyze W-Substitutants**

Select database:  
Substitutant database with wild type sequences

Select cancer type to analyze:  
Lung Squamous Cell Carcinoma

Select plots:  
 Barplot depicting number of W-Substitutants  
 Scatter contour plot  
 Violin plot

Plot

C.

## Browse Peptides

**Browse Peptides**

Select database:  
Substitutant database with wild type sequences

Select cancer type:  
Lung Squamous Cell Carcinoma

Load data

W>F Substitutants in the peptides are shown with a lowercase f.

The column 'Total' accounts for the total occurrences of the peptide in all samples. The column 'Σ Total' accounts for the total of occurrences in tumor tissue samples and the column 'Σ Normal' accounts for the total of occurrences in adj. normal tissue samples. The following columns showcases for each sample whether the peptide is found.