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**Fig. S1: Number of immunogenic epitopes in healthy donors vaccinated with viral antigens.** The plot indicates the number of immunogenic epitopes detected in healthy donors (black dots) vaccinated with viral antigens from HCV and HIV viruses. The length of each antigen (black) and the mean number of immunogenic epitopes (red) are reported.



**Fig. S2: Size Distribution of FSPs in Nous-209.** In plot is shown the distribution of the length in amino acids for FSPs encoded by Nous-209 vaccine



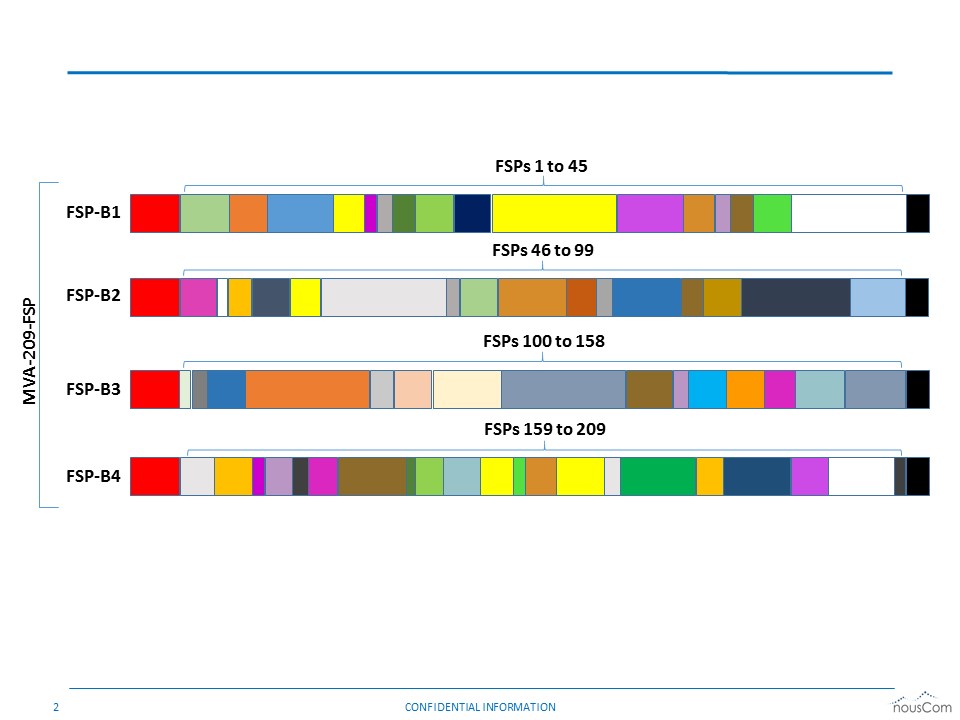
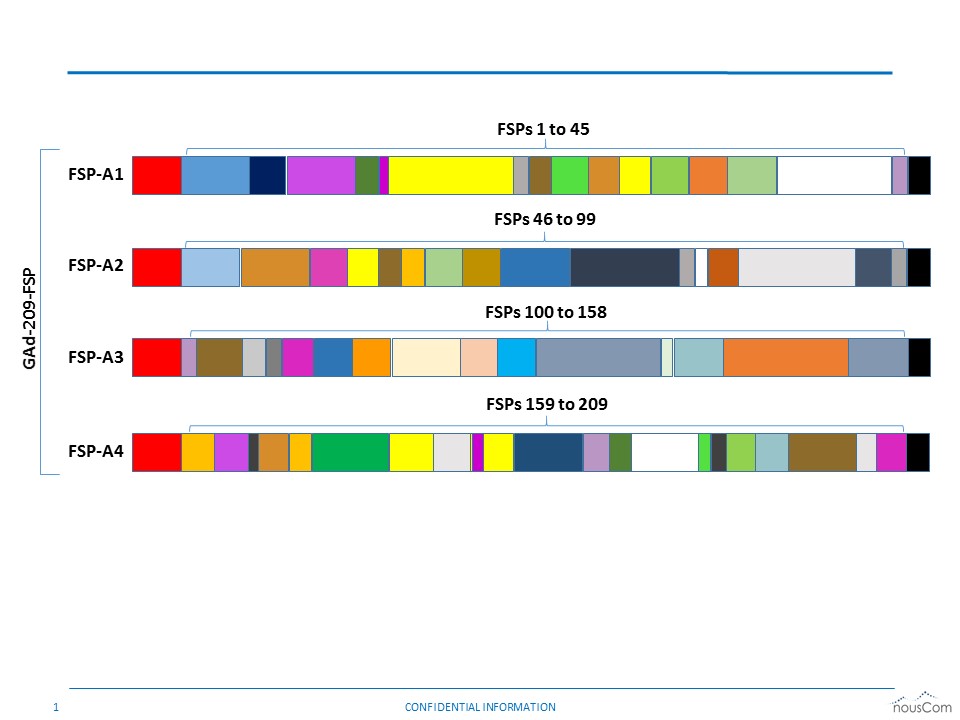
**Fig. S3: Detection of Nous-209 FSMs in early and late stage MSI tumors:** Scatter plot of frequencies of detection in early and in late stage tumors for the 204 FSMs selected for the vaccine. Regression line is shown in blue together with confidence intervals representing the deviations from the ideal correlation



**Fig. S4: Mutation allele frequency of Nous-209 FSMs detected in tumor and healthy mucosa of 20 CRC MSI patients**. **A,** Dots indicate the Mutation allele frequency of FSMs detected in healthy mucosa (green) and in tumor (black). Red bars indicate the mean mutation allele frequency for each patient. **B,** Variant calling versus look-up analysis on Nous-209 FSMs detected in MSI CRC patients**.** The number of vaccine-encoded FSMs detected in tumor tissues from the somatic variant calling analysis is represented in light gray. In green is reported the number of mutated FSMs detected according to the look-up analysis.

**A**

**B**



**GAd20-209-FSP**

**MVA-209-FSP**

**Fig. S5: Schematic of Nous-209 vaccine.**  **A,** Scheme of the four FSP transgenes encoded by the Gad20-209-FSPs vaccine. **B,** Scheme of the four FSP transgenes encoded by the MVA-209-FSPs vaccine. Each box represents a different FSP. Note that the number of boxes is only representative and do not correspond to the real number of FSP in each transgene (indicated on the top of each transgene scheme). Red box: TPA enhancer sequence. Black box: HA tag.



**Fig. S6: Overview of the procedure to isolate T cells recognizing peptide Ly4\_24.** The numbers correspond to the number of cells obtained during the different isolation/enrichment steps. After two weekly rounds of ex-vivo expansion using irradiated peptide-pulsed dendritic cells, cells from each of the 96 microcultures were stained with 10 nM of each of the two fluorescent multimers followed by staining with an anti-CD8ß antibody. The HLA-A2 control multimer was folded with the Ly55 peptide. Three microcultures (F1, H3, D5) were considered as positive because a clear cluster of multimer-positive cells was visible on the dotplot. A fourth microculture is shown as an example of negative well. The multimer-positive cells were sorted by flow cytometry from each of the three positive microcultures and expanded by 5-6 additional weekly rounds of stimulation with Ly4\_24 peptide-pulsed irradiated T2 cells, in the presence of LG2-EBV feeder cells and 50U per ml of IL-2. We obtained for each clonal populations (F1, H3, D5) several millions of T cells that were either frozen for further use or tested for tetramer staining and ability to secrete cytokines upon stimulation. Representative results of these experiments are shown in Figure 5.