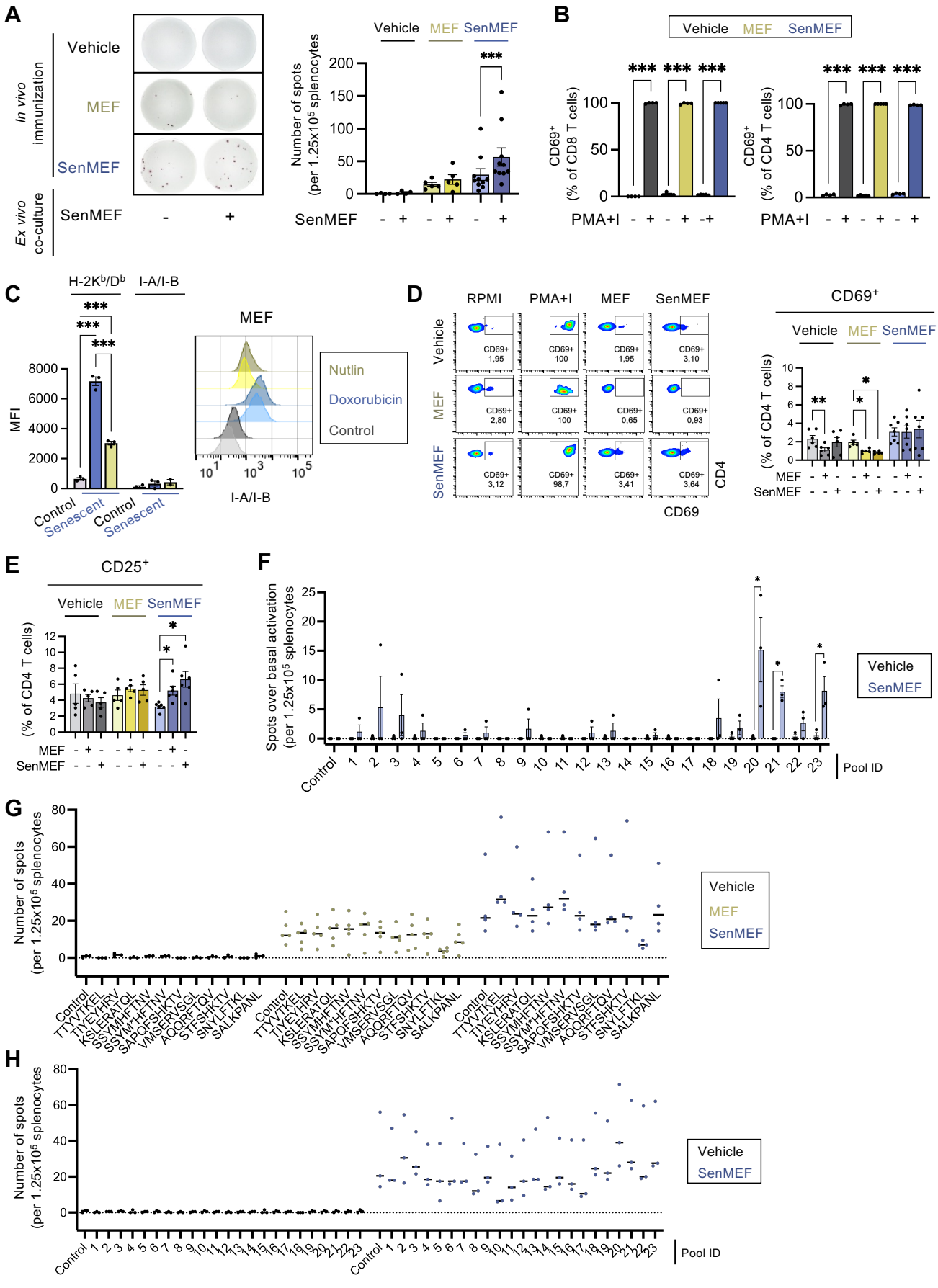
Supplementary Figure S2: Non-cancer senescent cells induce an adaptive immune response *in vivo* and present unique immunogenic peptides



Supplementary Figure S2: Non-cancer senescent cells induce an adaptive immune response *in vivo* and present unique immunogenic peptides

A. ELISpot assay to detect IFN-γ production in splenocytes isolated from non-immunized mice or animals immunized with senMEF (*n*=4 mice per group). $2.5x10^5$ or $1.25x10^5$ splenocytes were cultured in RPMI alone or with senFB (1:10 target-to-splenocyte ratio). Representative picture (left panel) and quantification (right panel) are shown. **p<0.01, *p<0.05; unpaired Student's t test, compared to RPMI alone treatment.

B. Measurement of CD8 (left panel) and CD4 (right panel) T cells activation, as monitored via CD69 expression upon PMA+Ionomycin treatment, used as a positive control of T cell activation.

C. Flow cytometry analysis of MHC-II (I-A/I-B) expression in control or senescent MEFs, treated with doxorubicin or nutlin. Representative histograms showing the fluorescence signal of each stained sample and its unstained control (lighter histogram) and quantification after autofluorescence of *n*=3 independent experiments are shown. Side comparison of MHC-I ($H\text{-}2K^b/D^b$) expression in the same conditions is also shown (representative histogram in Figure 1C). ***p<0.001; *p<0.05, two-way ANOVA test.

D. Flow cytometry analysis of the activation marker CD69 in CD4 T cells from naïve *versus* MEF or senMEF-immunized animals, after culture in RPMI medium either alone or with PMA+I, MEF or senMEF *ex vivo*. Representative pseudocolor plots and quantification of *n*=5-7 mice per group are shown. ***p<0.001, *p<0.05; two-way ANOVA test.

E. Flow cytometry analysis of the activation marker CD25 in CD4 T cells from naïve *versus* MEF or senMEF-immunized animals, after culture in RPMI medium either alone or with PMA+I, MEF or senMEF *ex vivo*. Quantification of *n*=5-7 mice per group are shown *p<0.05; two-way ANOVA test.

F. Pool of peptides validated using ELISpot assay to detect IFN-γ production in splenocytes isolated from non-immunized mice (vehicle) or animals immunized with senMEF (*n*=3 mice per group). Splenocytes were cultured in RPMI, either alone (control) or supplemented with the remaining peptides obtained from the immunopeptidome analysis pooled in combinations of 1-3 peptides as indicated. The number of spots for each condition above the control condition (background) was quantified and represented. *p<0.05; unpaired Student's t test, compared to controltreatment.

G. Raw number of spots from ELISpot assay to detect IFN-γ production in splenocytes isolated from non-immunized mice (vehicle), MEF or animals immunized with senMEF (*n*=3-6 mice per group). Splenocytes were cultured in RPMI, either alone (control), or supplemented with the selected different peptides obtained from the immunopeptidome analysis (as indicated).

H. Raw number of spots from ELISpot assay to detect IFN-γ production in splenocytes isolated from non-immunized mice (vehicle) or animals immunized with senMEF (*n*=3 mice per group). Splenocytes were cultured in RPMI, either alone peptide (control) or supplemented with the different peptides' pools obtained from the immunopeptidome analysis (as indicated).