Supplementary Material

Title: TSLP-Stimulated CD4+ T Cells Induce Senescence in Advanced Breast Cancer

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Supplementary Figures

**Figure S1. TSLP induction protects against advanced breast cancer in an apoptosis-independent manner.**

Representative images of TUNEL assay on PyMt cell line-derived breast tumors in Tslp\textsuperscript{tg} and WT mice. TUNEL-stained dorsal skin from a Xpc\textsuperscript{-/-} mouse that is irradiated with 100 mJ/cm\textsuperscript{2} ultraviolet B (UVB) is shown as a positive control. Nuclei are stained with DAPI in blue. Scale bar: 100 μm.
Figure S2. TSLP induction protects against advanced breast cancer growth by inducing senescence in cancer cells.

(A) Western blot for senescence markers (p21 and p53) on PyMt cell line-derived breast tumor lysates from Tslp\textsuperscript{tg} and WT mice. GAPDH is used as the control housekeeping protein. Please note that the low levels of GAPDH in a few samples is due the very small size of tumors that developed in Tslp\textsuperscript{tg} mice. (B) Quantification of p21 and p53 protein bands in western blot of PyMt cell line-derived breast tumors from Tslp\textsuperscript{tg} (n=5) and WT (n=5) mice. Mann Whitney \textit{U} test, bar graphs show mean ± s.d.
Figure S3. Impact of cytokine blockade on PyMt cell growth-suppressing effect of TSLP-activated CD4\(^+\) T cell supernatant.

Quantification of crystal violet-stained 2D PyMt cell culture in TSLP-activated WT CD4\(^+\) T cell supernatant (test, n=4), test CD4\(^+\) T supernatant + αIL-3 (n=2), test CD4\(^+\) T supernatant + αIL-5 (n=2), test CD4\(^+\) T supernatant + αGM-CSF (n=2), test CD4\(^+\) T supernatant + αIL-4 (n=2), test CD4\(^+\) T supernatant + αIL-13 (n=2), test CD4\(^+\) T supernatant + αIL-4 and αIL-13 (n=1), Tslpr\(^{K0}\) CD4\(^+\) T supernatant (control, n=5), and media alone (n=8). Relative intensity of crystal violet stain in each well is determined using ImageJ. Bar graphs show mean + s.d.
Figure S4. TNF-α and IFN-γ blockade effect on PyMt breast tumor growth in WT mice.

PyMt cell line-derived tumor growth in WT mice treated with anti-TNF-α and anti-IFN-γ blocking antibodies (test, n=7) versus IgG control (control, n=7, P< 0.0001, two-way ANOVA).
Supplementary Table

Table S1. Primers used for genotyping mice in the study.

| Gene     | Forward primer            | Reverse primer            |
|----------|---------------------------|---------------------------|
| Tslp<sup>tg</sup> | TCATCCTGCAAGTACTAGTGGA TGGGGC | TGTTTTGGACTTCTTGTGCCATTTC CTGAG |
| PyMt<sup>tg</sup> | ATAATGCTGGAAGAAGACGAAATC CTTG | CTCTGTGAGTAGCTCTCATTCTGCTC ACTC |
| TSLPR<sup>KO</sup> | AGCGTTGGCTACCCTGATATTGC TGAAGAG | TCATGAACGACCACTCCTATGT GGACACG |