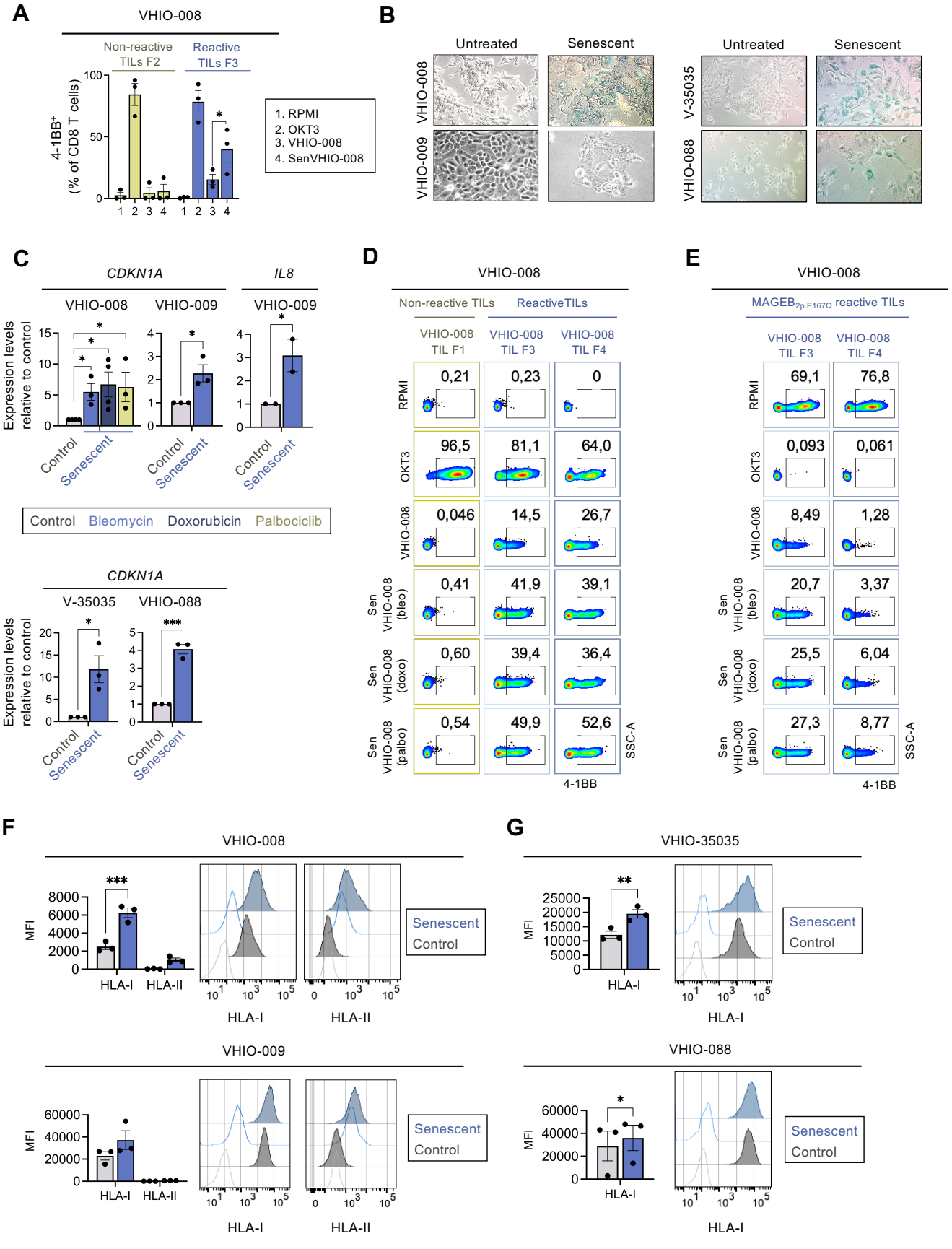
Supplementary Figure S5: Senescent cancer cells from human patients hyper-stimulate autologous reactive TILs



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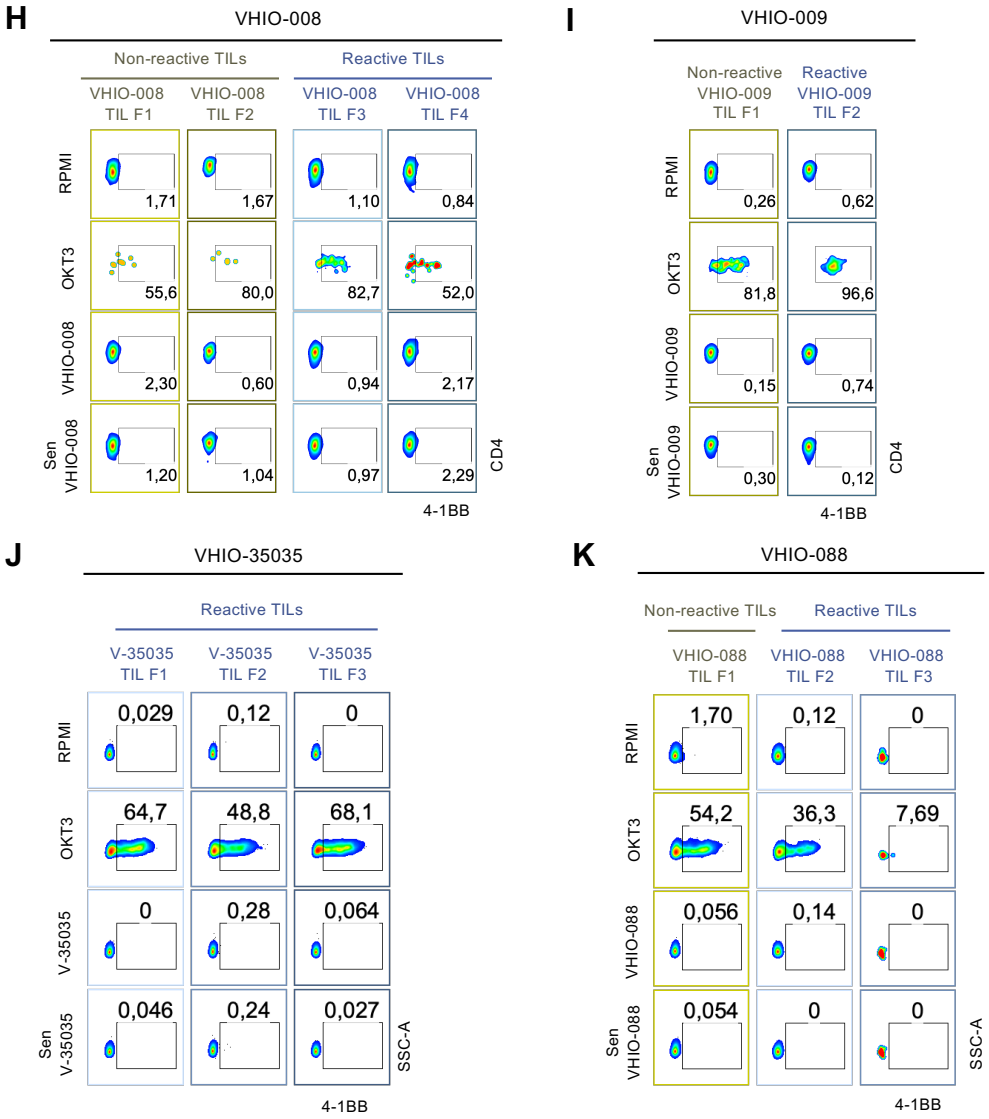

Supplementary Figure S5: Senescent cancer cells from human patients hyper-stimulate autologous reactive TILs

A. Flow cytometry analysis of 4-1BB activation marker in CD8 non-reactive (F2 fragment) and reactive (F3 fragment) autologous TILs from patient VHIO-008 after culture in RPMI medium either alone or with anti-CD3 (OKT3), control or bleomycin-treated senescent VHIO-008 cells (as indicated). Quantification of a total of 3 independent replicates with F2 and F3 TILs is shown in the right side. **p<0.01; unpaired Student's t test.

B. Senescence-associated beta-galactosidase staining of patient-derived VHIO-008, VHIO-009, VHIO-35035 (abbreviated as V-35035) and VHIO-088 cells, control or senescent after treatment with bleomycin.

C. mRNA expression levels of *CDKN1A* or *IL8* in patient-derived VHIO-008, VHIO-009, V-35035 and VHIO-088 cells, control or senescent after treatment with the indicated agent (*n=2*-3 independent experiments). Fold change relative to control cells. *p<0.05, **p<0.01; unpaired Student's t test, compared to control cells.

D. Flow cytometry analysis of 4-1BB activation marker in CD8 non-reactive (F1 fragment) and reactive (F3 and F4 fragments) autologous TILs from VHIO-008 patient after culture in RPMI medium either alone or with anti-CD3 (OKT3), control or senescent VHIO-008 cells treated with bleomycin, doxorubicin or palbociclib (as indicated).

E. Flow cytometry analysis of 4-1BB activation marker in CD8 reactive autologous TILs (reactive F3 and F4 fragments) enriched to be reactive against to $MAGEB2_{p.E167Q}$ (a neoantigen previously identified by whole exome sequencing of the autologous tumor cell line) after co-culture with RPMI medium either alone or with anti-CD3 (OKT3), control or senescent VHIO-008 cells treated with bleomycin, doxorubicin or palbociclib (as indicated).

F. Flow cytometry analysis of MHC-I (HLA-I) and MHC-II (HLA-II) expression in control or senescent VHIO-008 and VHIO-009, treated with bleomycin. Representative histograms (left panel) showing the fluorescence signal of each stained sample and its unstained control (uncolored) and quantification after autofluorescence subtraction (right panel) of *n*=3 independent experiments are shown. ***p<0.001; one-way ANOVA test, compared to control cells.

G. Flow cytometry analysis of MHC-I (HLA-I) expression in control or senescent V-35035 and VHIO-088, treated with bleomycin. Representative histograms (left panel) showing the fluorescence signal of each stained sample and its unstained control (uncolored) and quantification after autofluorescence subtraction (right panel) of *n*=3 independent experiments are shown. *p<0.05, unpaired Student's t test, compared to control cells.

H. Flow cytometry analysis of 4-1BB activation marker in CD4 cells from non-reactive (F1 and F2 fragments) and reactive (F3 and F4 fragments) autologous TILs from patient VHIO-008 after culture in RPMI medium either alone or with anti-CD3 (OKT3), control or bleomycin-treated senescent VHIO-008 cells (as indicated).

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I. Flow cytometry analysis of 4-1BB activation marker in CD4 cells from non-reactive (F1 fragment) and reactive (F2 fragment) autologous TILs from patient VHIO-009 after culture in RPMI medium either alone or with anti-CD3 (OKT3), control or bleomycin-treated senescent VHIO-009 cells (as indicated)..

J. Flow cytometry analysis of 4-1BB activation marker in CD4 cells from reactive (F1, F2 and F3 fragments) autologous TILs from patient VHIO-35035 (abbreviated as V-35035) after culture in RPMI medium either alone or with anti-CD3 (OKT3), control or bleomycin-treated senescent V-35035 cells (as indicated).

K. Flow cytometry analysis of 4-1BB activation marker in CD4 cells from non-reactive (F1 fragment) and reactive (F2 and F3 fragment) autologous TILs from patient VHIO-088 after culture in RPMI medium either alone or with anti-CD3 (OKT3), control or bleomycin-treated senescent VHIO-088 cells (as indicated).