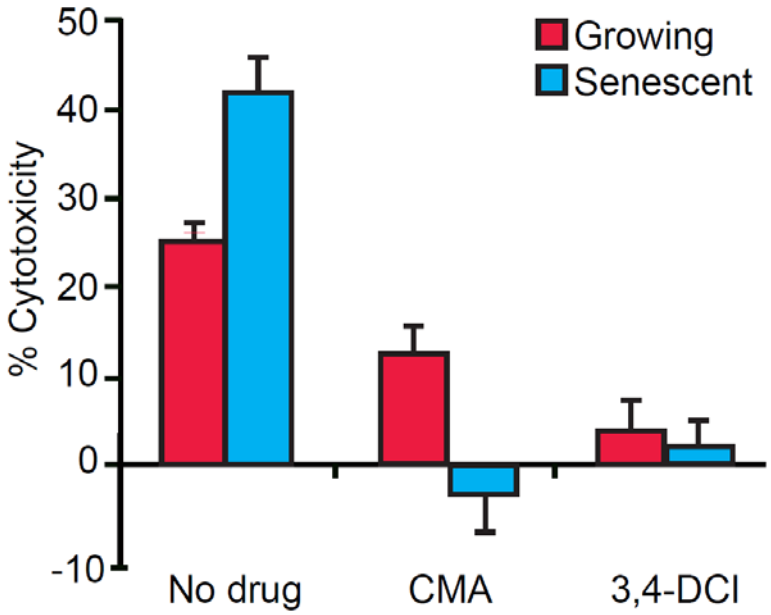
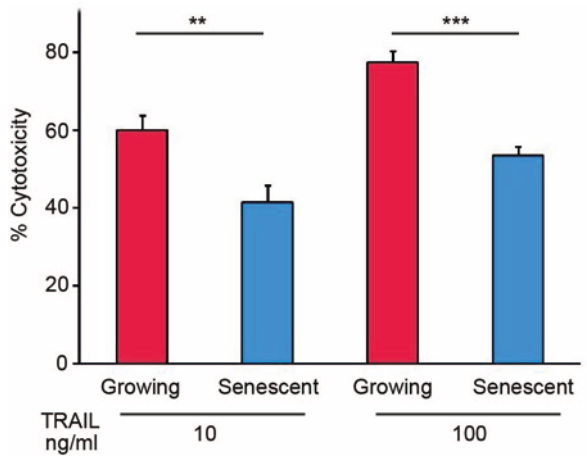
Sagiv et al.

Granule exocytosis mediates immune surveillance of senescent cells

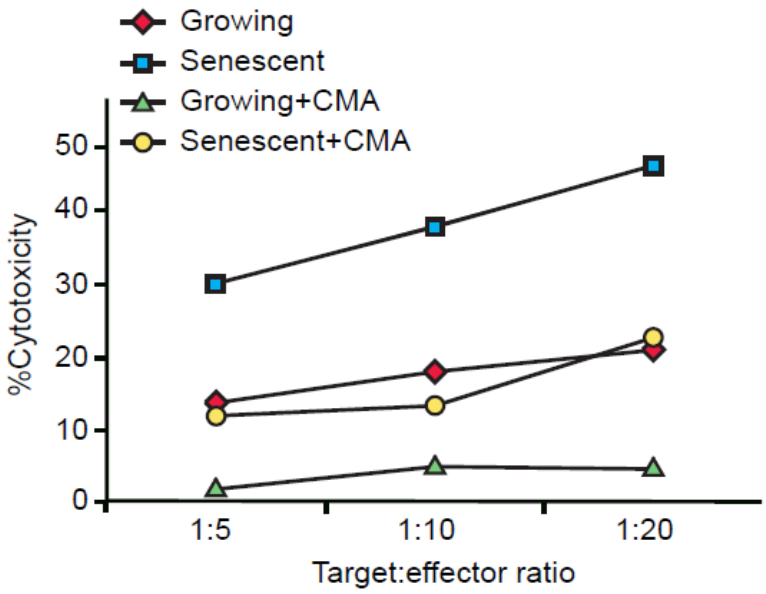
Supplementary Information



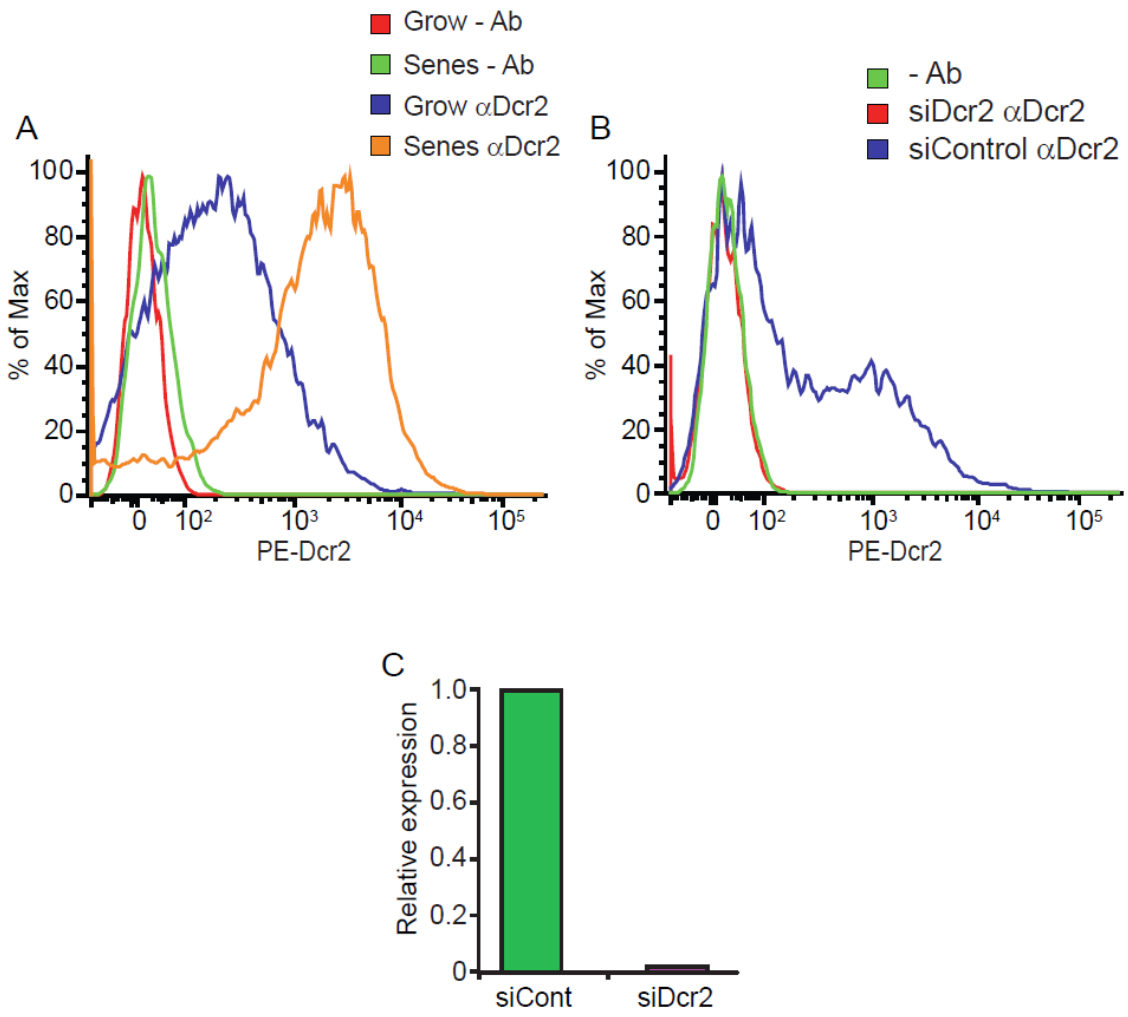
Supplementary Figure S1. Granule exocytosis pathway is necessary for the efficient killing of senescent cells by NK-92 cells. Senescent and growing IMR-90 fibroblasts were co-incubated with NK-92 cells. Cytotoxicity assays were performed either in the presence of 100nM granule exocytosis inhibitor, CMA or following pre-incubation of the NK-92 cells with 25 μM Granzyme B inhibitor 3,4-DCI.



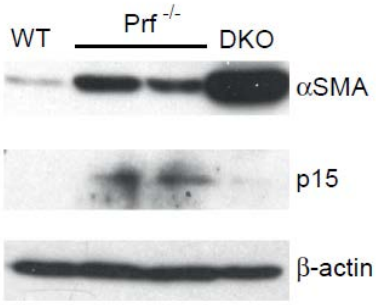
Supplementary Figure S2. Senescent cells are more resistant to TRAIL cytotoxicity. Senescent or growing IMR-90 fibroblasts were incubated for 24 hours with either 10 or 100 ng/ml TRAIL. Cytotoxicity was determined at the end of the co-incubation period. The graphs represent the average and standard error of triplicate measurements from at least three independent experiments. **-$p<0.001$, ***-$p<0.0001$.



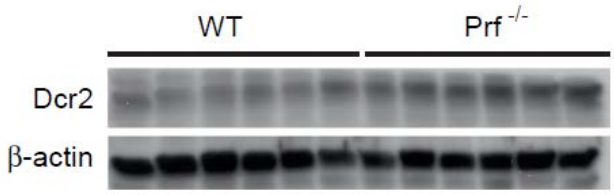
Supplementary Figure S3. Granule exocytosis is necessary for senescent cell killing in a wide range of effecter : target cell ratios. Senescent or growing IMR-90 fibroblasts were co-incubated with YT cells for 12 hours at the indicated ratios and cytotoxicity was determined. Were indicated cytotoxicity assays were performed either in the presence of 100nM CMA or following pre-incubation of YT cells with 25 μM granzyme B inhibitor 3,4-DCI.



Supplementary Figure S4. Expression of Dcr2 on the cell surface of senescent cells. Senescent or growing IMR-90 fibroblasts were stained with anti-Dcr2-PE and analyzed with flow cytometry (A). siDcr2 downregulates expression of Dcr2 on the cell surface (B). Senescent IMR-90 fibroblasts transfected with either siDcr2 or siControl were stained with anti-Dcr2-PE and analyzed with flow cytometry. (C) Downregulation of Dcr2 expression is stable for 5 days as assessed by quantitative RT-PCR.



Supplementary Figure S5. Expression of $p15^{ink4b}$ and aSMA is increased in *Prf*$^{-/-}$ fibrotic livers. Immunoblot analysis of expression of senescence marker $p15^{ink4b}$ and HSC marker αSMA was performed on lysates of fibrotic livers of *Prf*$^{-/-}$ and *p53*$^{-/-}$*;INK4a/ARF*$^{-/-}$ (DKO) mice.



Supplementary Figure S6. Dcr2 expression is increased in *Prf*$^{-/-}$ fibrotic livers.
Dcr2 expression was evaluated by immunoblot analysis preformed on lysates of fibrotic livers of *Prf*$^{-/-}$ and wild type (WT) mice. 6 mice of each genotype were used for the analysis.

Supplementary materials and methods

For detection of Dcr2 surface expression senescent, growing and senescent cells transfected with siDcr2 or siControl were stained with anti-Dcr2-PE antibody (BD Biosciences, Belgium) according to the manufactures protocol and analyzed in a LSRII flow cytometry unit (BD Biosciences, Belgium).