sFigure 1. Immunofluorescence, ELISA and mRNA profiles of different types of senescent cells with or without $p16^{INK4a}$ expression.

A. IL-6, IL-8, Ras^{V12}, $p21^{CIP1/WAF1}$, $p16^{INK4a}$ and p53 expression in presenescent (PRE) and senescent (SEN) cells. In the top panel WI-38 cells were analyzed by immunofluorescence and in the lower panels the indicated cells were analyzed by western blotting.

For immunofluorescence, each panel is a different field. Senescence inducers are shown on the left, and the SA-Bgal and growth status are shown on the right. Cultures were considered growth positive (+) when >70% of cells incorporated BrdU over a 1 d interval, and negative (-) when <10% of cells incorporated BrDU over the same period. Cultures were considered SA-Bgal positive (+) when >80% of cells stained for SA-Bgal and negative (-) when <5% of cells stained.

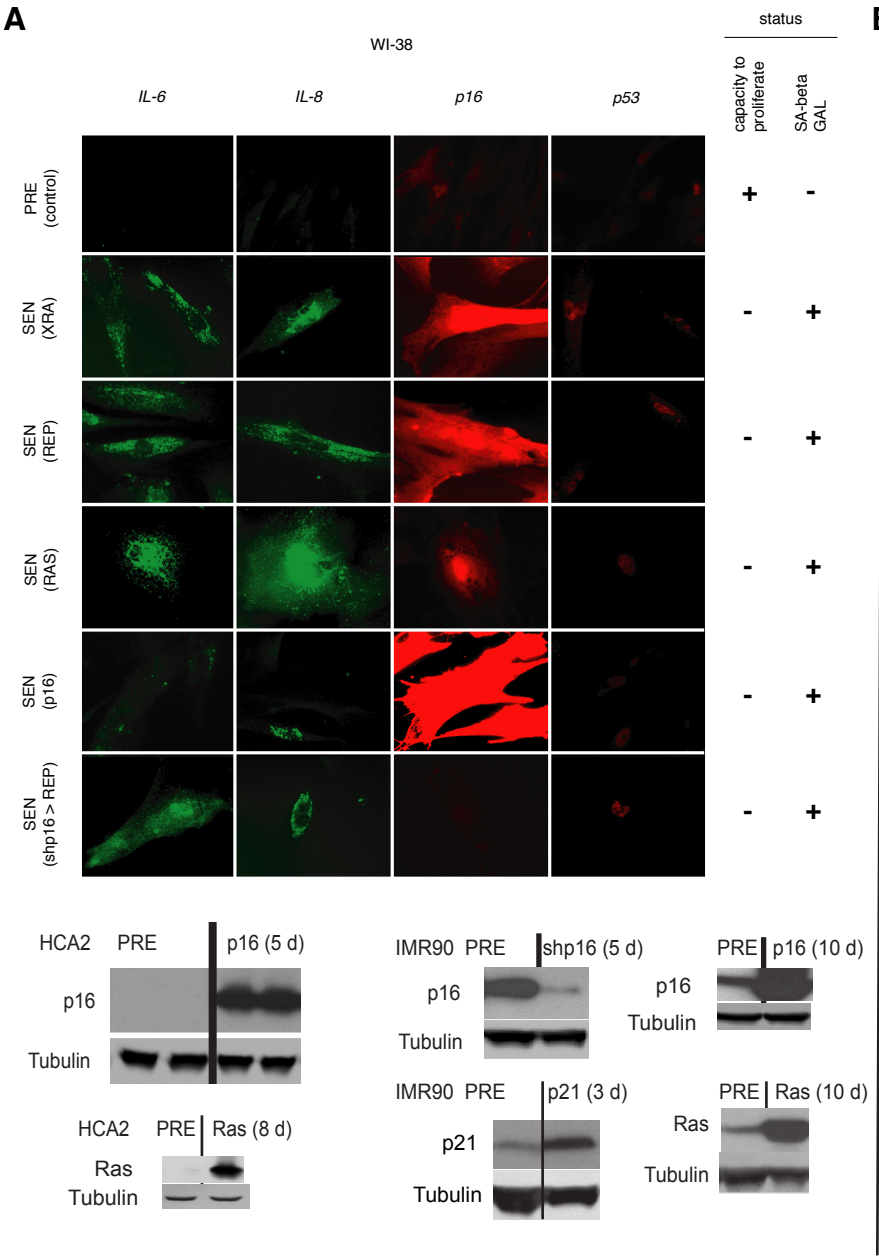
For western analysis, the two panels on the left show the indicated protein expression in HCA2 cells infected with control lentiviruses (PRE) or lentiviruses encoding $p16^{INK4a}$ (5 d after infection) or Ras^{V12} (8 d after infection). The four panels on the right show indicated protein expression in IMR90 cells infected with control lentiviruses (PRE) or lentiviruses encoding $p16^{INK4a}$ (10 d after infection), Ras^{V12} (10 d after infection), $p21^{CIP1/WAF1}$ (3 d after infection) or an shRNA targeting $p16^{INK4a}$ (5 d after infection).

B. Soluble factors (IL-6, IL-8, GROα) produced by the indicated cells were determined by ELISA ($x10^{-6}$pg/cell in the top panel or pg/1000 cells in the lower panel).

C. The indicated mRNA levels expressed by various senescent (SEN) cell types (L3P in PRE cells = control vector).

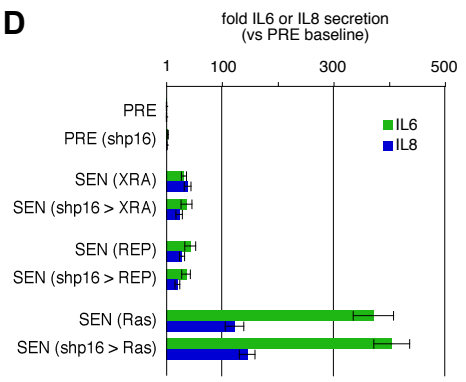
D. ELISA measurements of IL-6 and IL-8 secretion by cells expressing $p16^{INK4a}$ or depleted of $p16^{INK4a}$ (shp16) (complement to Fig. 3).

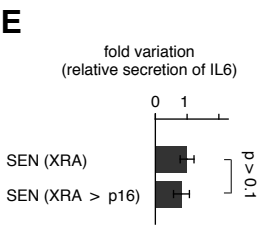
E. Fold variation for ELISA measurements of IL-6 secretion by irradiated HCA2 cells (SEN(XRA)) and similar HCA2 cells induced to ectopically express $p16^{INK4a}$ 48 hours after irradiation (SEN(XRA > p16)).



C

	mRNA levels (TaqMan)						
	PRE	PRE L3P	SEN p16	SEN p21	SEN XRA	SEN REP	SEN shp16 > REP
IL-6	1.0	1.1	1.8	1.5	74.0	104.5	81.4
IL-8 (CXCL-8)	1.0	1.1	1.8	-2.4	398.9	1022.4	787.7
GROa (CXCL-1)	1.0	1.0	-1.2	-3.7	268.7	135.2	129.2
GROg (CXCL-3)	1.0	1.2	-1.1	-2.1	130.7	212.3	95.8
GROb (CXCL-2)	1.0	-1.1	1.7	1.3	29.4	31.8	32.1
OPG (TNFRSF-II)	1.0	1.0	1.9	-2.0	5.1	8.0	2.3
IGFBP-3	1.0	1.1	1.4	5.5	12.6	17.5	6.9
EREG	1.0	1.0	2.5	1.6	12.8	7.6	6.5
AREG	1.0	-1.4	1.5	1.1	10.4	10.5	7.1
p16	1.0	1.2	**515.6**	1.2	2.5	8.1	**-12.8**
p21	1.0	-1.1	4.4	**33.4**	6.4	5.3	4.7
p53	1.0	1.3	1.2	-1.8	1.5	2.2	-1.8
CAV-1	1.0	1.1	2.6	1.6	1.8	5.0	-1.7
H1A	1.0	1.0	-1.4	-1.4	-1.9	-1.9	-1.3





sFigure 1 (supplement) _ Coppé et al