Supplementary Materials for

The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4

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Published 25 September 2015, *Science* **349**, aaa5612 (2015)
DOI: 10.1126/science.aaa5612

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Figs. S1 to S6

Other supplementary material for this manuscript includes the following:

Table S1 (Excel format)

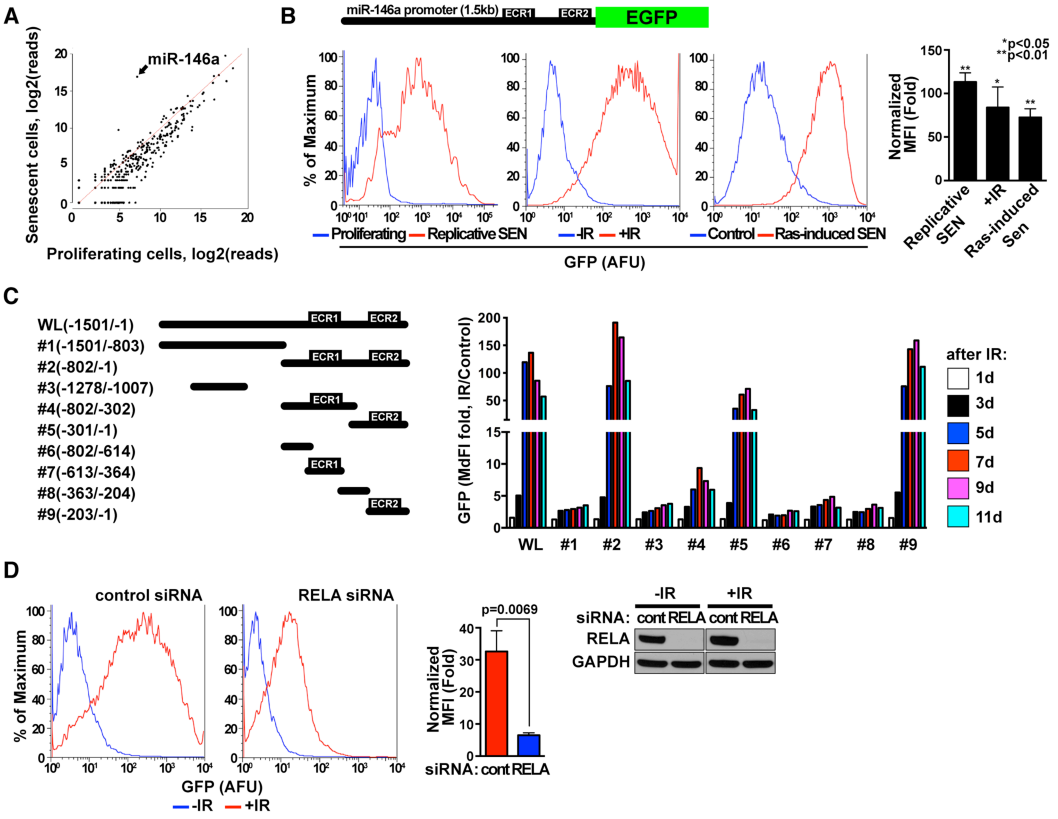

Fig. S1.

The miR-146a-GFP reporter, a new molecular marker for cellular senescence. (A) Total RNA was isolated from proliferating and replicative senescent IMR90 cells and total RNA-seq was performed. (B) Schematic representation of the miR-146a-GFP reporter. ECR1 and ECR2 are Evolutionary Conserved Region 1 and 2, respectively, identified by ECR browser (ecrbrowser.dcode.org) (upper). IMR90 cells expressing the miR-146a GFP reporter were induced to senesce by replicative exhaustion (bottom, left), IR (bottom, center, 7 d after 12 Gy IR), and oncogenic-Ras^{V12} (bottom, right, 6 d after H-Ras^{V12} induction) and GFP fluorescence was analyzed by FACS. Arbitrary Fluorescence Units (AFU). Median Fluorescence Intensity (MFI) was computed and normalized to control in each sets. Data are mean ± SEM. (C) Schematic representation of truncated versions of the miR-146a-GFP reporter (left). IMR90 cells expressing the indicated reporter constructs were irradiated with 12 Gy IR and GFP fluorescence was examined by FACS on the indicated day. (D) IMR90 cells expressing the miR-146a-GFP reporter were irradiated at 12 Gy. One day after IR, cells were transfected with the indicated siRNAs. Three days after the siRNA transfection, cells were transfected with same siRNAs to reinforce depletion. Three days after the 2nd siRNA transfection, GFP fluorescence was analyzed by FACS (left). Arbitrary Fluorescence Units (AFU). Median Fluorescence Intensity (MFI) was computed and normalized to –IR control. Data are mean ± SEM. cont refers to the firefly luciferase siRNA control. Immunoblotting analysis was performed to determine the efficiency of the depletion (right). Data are representative of three (B, D) or two (C) independent experiments.

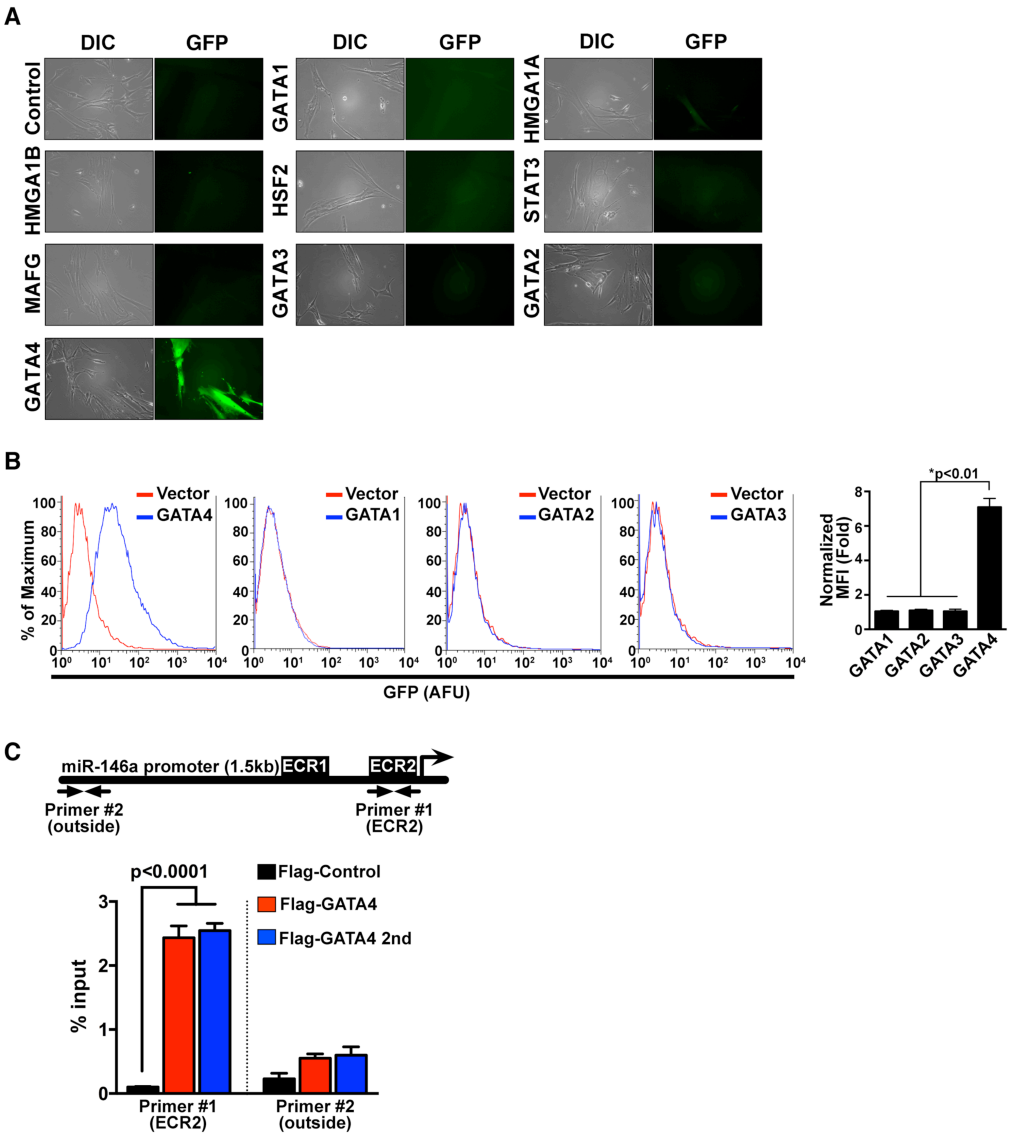

Fig. S2

(A and B) Ectopic expression of GATA4 activates the miR-146a-GFP reporter. IMR 90 cells containing an integrated miR-146a-GFP reporter were infected with virus ectopically expressing the indicated ORFs and GFP fluorescence was examined by fluorescence microscope (A) or FACS (B). DIC, differential interference contrast. Arbitrary Fluorescence Units (AFU). Median Fluorescence Intensity (MFI) was computed and normalized to Vector control. Data are mean ± SEM. One-way ANOVA was used for the statistical analysis. (C) Schematic representation of the miR-146a-GFP reporter with the indicated qPCR primers (upper). IMR90 cells expressing either HA/FLAG-control or HA/FLAG-tagged GATA4 were croslinked with formaldehyde and protein extracts were immunoprecipitated with Flag antibodies and DNA was eluted and the cross-links were reversed. The DNA was analyzed by real-time PCR using the indicated qPCR primers. The 'Flag-GATA4 2nd' data are from an independent ChIP-qPCR experiment. Data are mean ± SEM. One-way ANOVA was used for the statistical analysis. Data are representative of two (A, B) independent experiments.

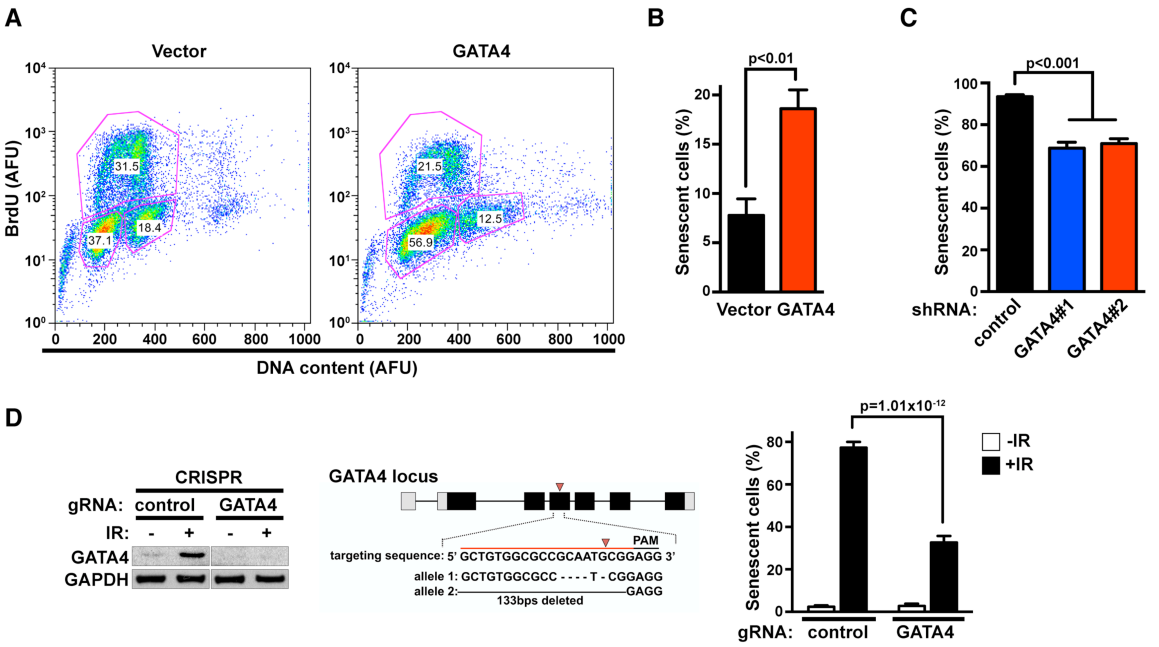

Fig. S3

(A) IMR90 cells expressing either empty vector control or GATA4 were analyzed for BrdU incorporation by FACS. (B) IMR90 cells expressing either Vector control or GATA4 were analyzed for SA-β-Gal staining. Data are mean ± SEM. (C) IMR90 cells expressing either control or GATA4 shRNAs were irradiated at 12Gy IR and 7 d after IR, SA-β-Gal staining was performed. Data are mean ± SEM. One-way ANOVA was used for the statistical analysis. (D) BJ-hTERT cells null for GATA4 were generated using CRISPR-Cas9 genome editing using a GATA4 gRNA designed to target the human GATA4 exon 4. A clone was isolated from a single cell, expanded and analyzed by western blotting (left). A successfully targeted clone was verified by PCR and sequencing of the target genomic region of GATA4 (middle). The targeting sequence is shown including the PAM. SA-β-Gal staining (right) was performed in proliferating (-IR) or IR-induced senescent cells (+IR, 7 d after 12 Gy IR). Data are mean ± SEM. Data are representative of three (A, B, C, D) independent experiments.

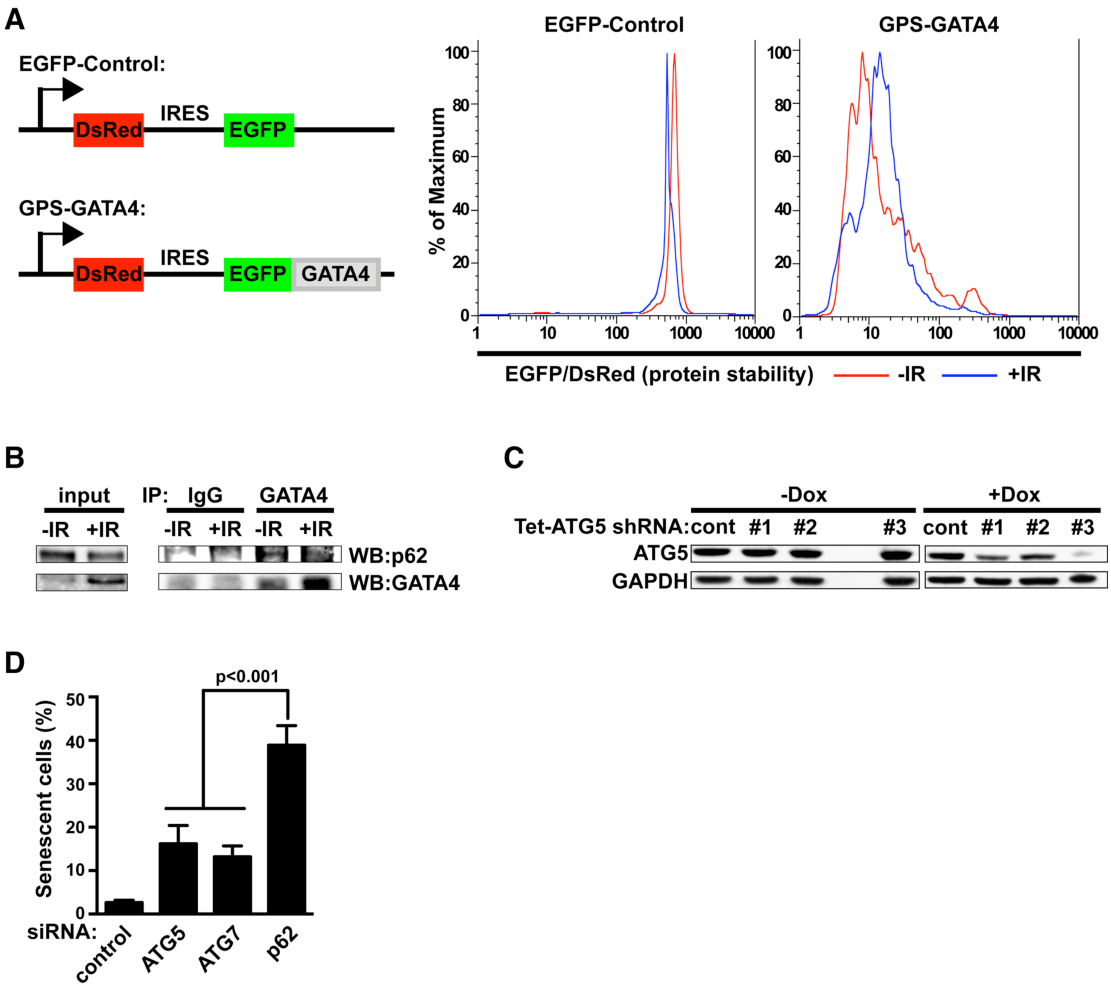

Fig. S4

(A) Determination of GATA4 protein stability using the Global Protein Stability (GPS) system. A schematic of the constructs (left). IMR90 cells expressing the DsRed-IRES-EGFP reporter cassette with either control (center) or GATA4 (right) were analyzed by FACS under proliferating (Red) or senescent (Blue, 7 d after 12 Gy IR) conditions. The EGFP/DsRed ratio is a readout for protein stability.(B) Immunoblotting of endogenous GATA4 immunoprecipitates from proliferating (-IR) or IR-induced senescent IMR90 cells (+IR, 7 d after 12 Gy IR). (C) Measuring the efficiency in IMR90 cells of Tet-inducible shRNA targeting the autophagy regulator ATG5. After treatment with or without Dox for 7 days, immunoblotting analysis was performed to determine the efficiency of the depletion. (D) p62 depletion induces senescence. IMR90 cells were transfected with the indicated siRNAs. Three days after the siRNA transfection, cells were transfected with same siRNA to reinforce knockdown. Three days after the 2nd transfection, cell were transfected with the same siRNA again. One and half days after the 3rd transfection, cells were quantified for SA-β-Gal staining. Data are mean ± SEM. One-way ANOVA was used for the statistical analysis. Data are representative of four (A), three (B, C), or two (D) independent experiments.

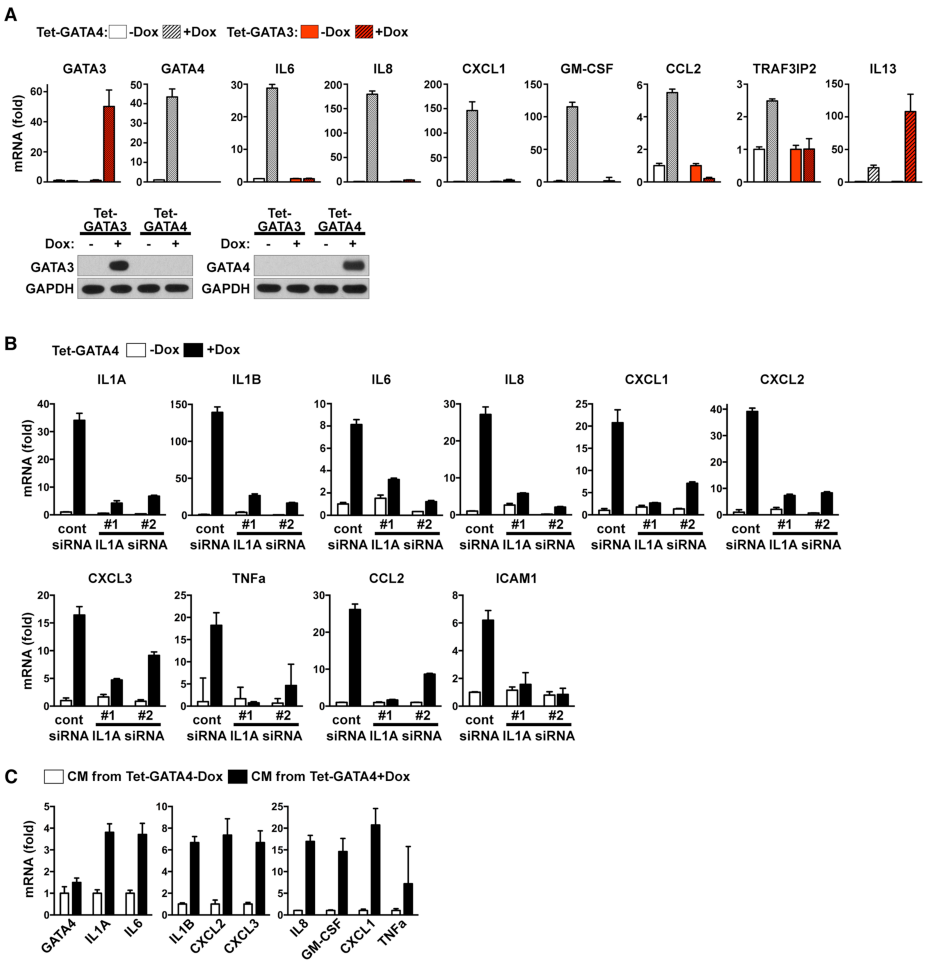

Fig. S5

(A) BJ cells expressing either inducible GATA3 or GATA4 were treated with Dox for 3 days and abundance of mRNA was quantified for the indicated genes using RT-qPCR (upper). Relative abundance of the indicated mRNA was expressed as fold-change compared to expression in cells without Dox treatment. Protein levels were examined by western blot (bottom). (B) One day after transfection of either control or IL1A targeting siRNAs, GATA4 was ectopically induced in IMR90 cells for 2 days using the Tet-ON system and abundance of the indicated mRNA was analyzed by RT-qPCR. cont represents the siRNA to firefly luciferase. Relative abundance of the indicated mRNA was expressed as fold-changes compared with expression in cells transfected with control siRNA without Dox treatment. (C) After 2 days of GATA4 induction using the Tet-On system in IMR90 cells, conditioned medium was transferred to IMR90 cells not expressing Tet-GATA4. Two days after the conditioned medium transfer, abundance of the indicated mRNA was analyzed by RT-qPCR. Relative abundance of the indicated mRNA was expressed as fold-change compared to expression in cells treated with conditioned medium from cells not expressing GATA4. Data are representative of two (A, B, C) independent experiments.

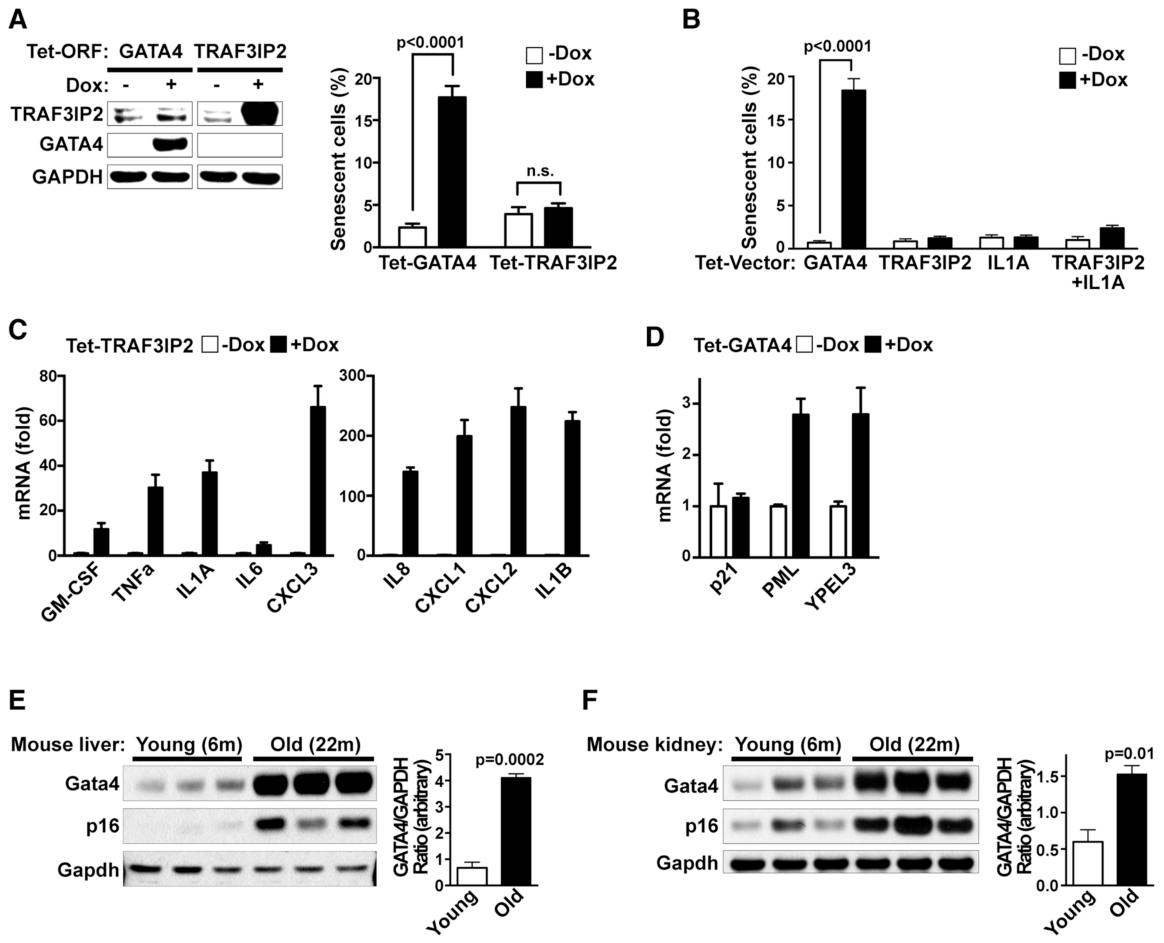

Fig. S6

(A) Either GATA4 or TRAF3IP2 were ectopically expressed using the Tet-ON system for 3.5 days in IMR90 cells. Immunoblotting was used to measure the expression of the indicated proteins (left) and quantification of SA-β-Gal staining (right). Data are mean ± SEM. n.s.=not significant. (B) The indicated genes were ectopically expressed using the Tet-ON system for 3.5 days in IMR90 cells. Quantification of SA-β-Gal staining and data are mean ± SEM. (C) IMR90 cells expressing inducible TRAF3IP2 were treated with Dox for 3.5 days and abundance of the indicated mRNA was analyzed by RT-qPCR. Relative abundance of the indicated mRNA was expressed as fold-changes compared with expression in cells without Dox treatment. (D) IMR90 cells expressing inducible GATA4 were treated with Dox for 3.5 days and the abundance of the indicated mRNA was analyzed by RT-qPCR. Relative abundance of the indicated mRNA was expressed as fold-change compared to expression in cells without Dox treatment. Data are representative of two (A, B, C) or four (D) independent experiments. (E and F) Liver (E) and kidney (F) tissues were taken from young (6-month-old) and old (22-month-old) C57BL/6 mice and analyzed by western blotting (left). Densitometric analysis was performed to determine the GATA4/GAPDH ratio. Data are mean ± SEM.