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Supplemental Information

SILAC Analysis Reveals Increased Secretion of Hemostasis-Related Factors by Senescent Cells

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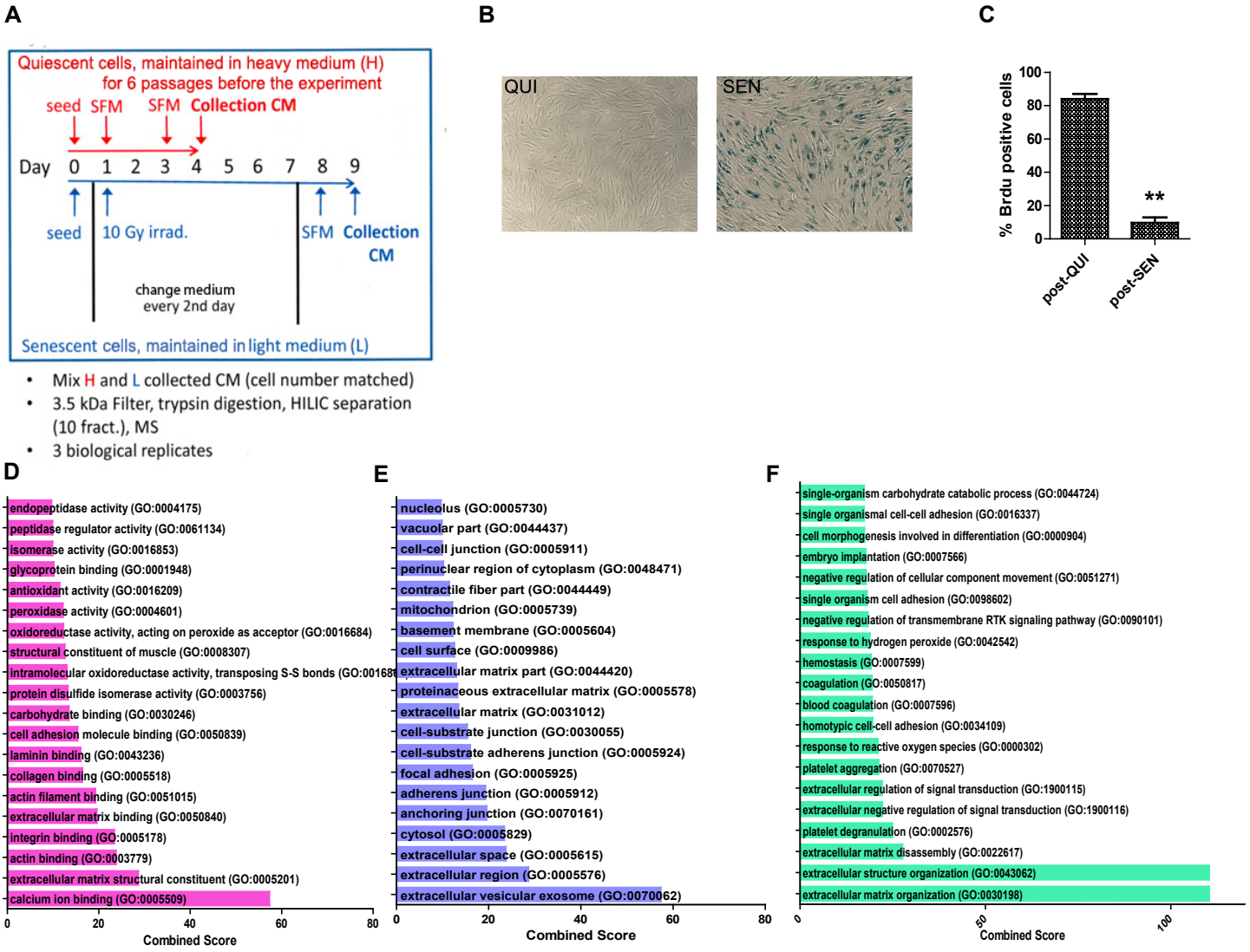

Figure S1. Confirmation of quiescence and senescence and pathway analysis, related to Figure 1. (A) The experimental timeline for SILAC-based identification of the secretome of senescent cells. Cells (HCA2 human fibroblasts) were made quiescent by serum deprivation (0.2%) for 3 days, then incubated in serum free medium for 24 hours (quiescent CM). Cells were made senescent by ionizing radiation (IR; 10 Gy X-irradiation), incubated in complete medium (10% serum), with changes every 2-3 days, for 8 days, then incubated in serum free medium for 24 hours (senescent CM).
(B) HCA2 cells were made quiescent or induced to senesce by IR. Three and 9 days later respectively, the cells were counted and re-seeded in complete medium, and stained for senescence-associated beta-galactosidase activity (SA-β-gal).
(C) HCA2 cells were made quiescent or senescent, as described in **(A)**. Three and 9 days later respectively, cells were counted and re-seeded in 0.2% serum (for quiescent cells) or complete medium (for both quiescent and senescent cells). The next day, cells were given BrdU for 24 h, fixed and immunostained for nuclear BrdU. **, $p<0.01$. Data reflect means of 3 experiments.
(D-F) Shown are the top 20 enriched non- redundant gene ontology (GO) categories from the original SILAC data (and represent QUI vs SEN): **(D)** GO molecular function analysis; **(E)** GO cellular components analysis; and **(F)** GO biological processes analysis.

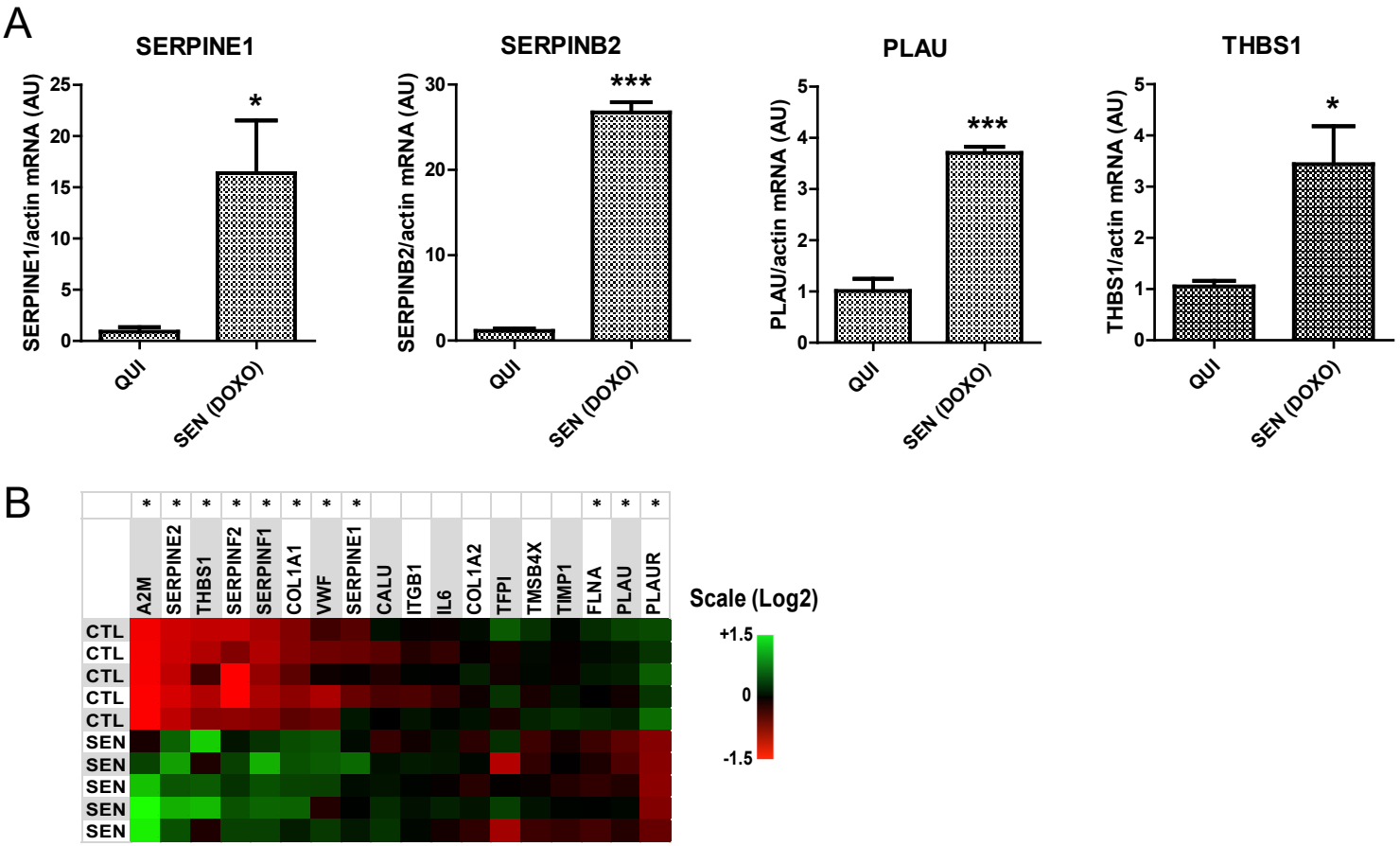

Figure S2. Expression of hemostasis-related SASP genes in WI-38 human fibroblasts and human umbilical vascular endothelial cells (HUVEC), related to Figure 2.

(A) We induced senescence in WI-38 human fetal lung fibroblasts treated using DOXO and extracted RNA 9 d later, and analyzed for the indicated mRNAs by quantitative PCR. Bars reflect means of 3 experiments + SEM. * = $p<0.05$; *** = $p<0.001$.

(B) We induced HUVECs to senesce via 10 Gy of IR or treated with mock irradiation, and extracted mRNA from both treatment groups 10 days later. We analyzed mRNA levels of hemostasis factors by quantitative PCR. mRNA levels for all groups were normalized to actin mRNA. All values of $p<0.05$ are indicated by * in the heat map.

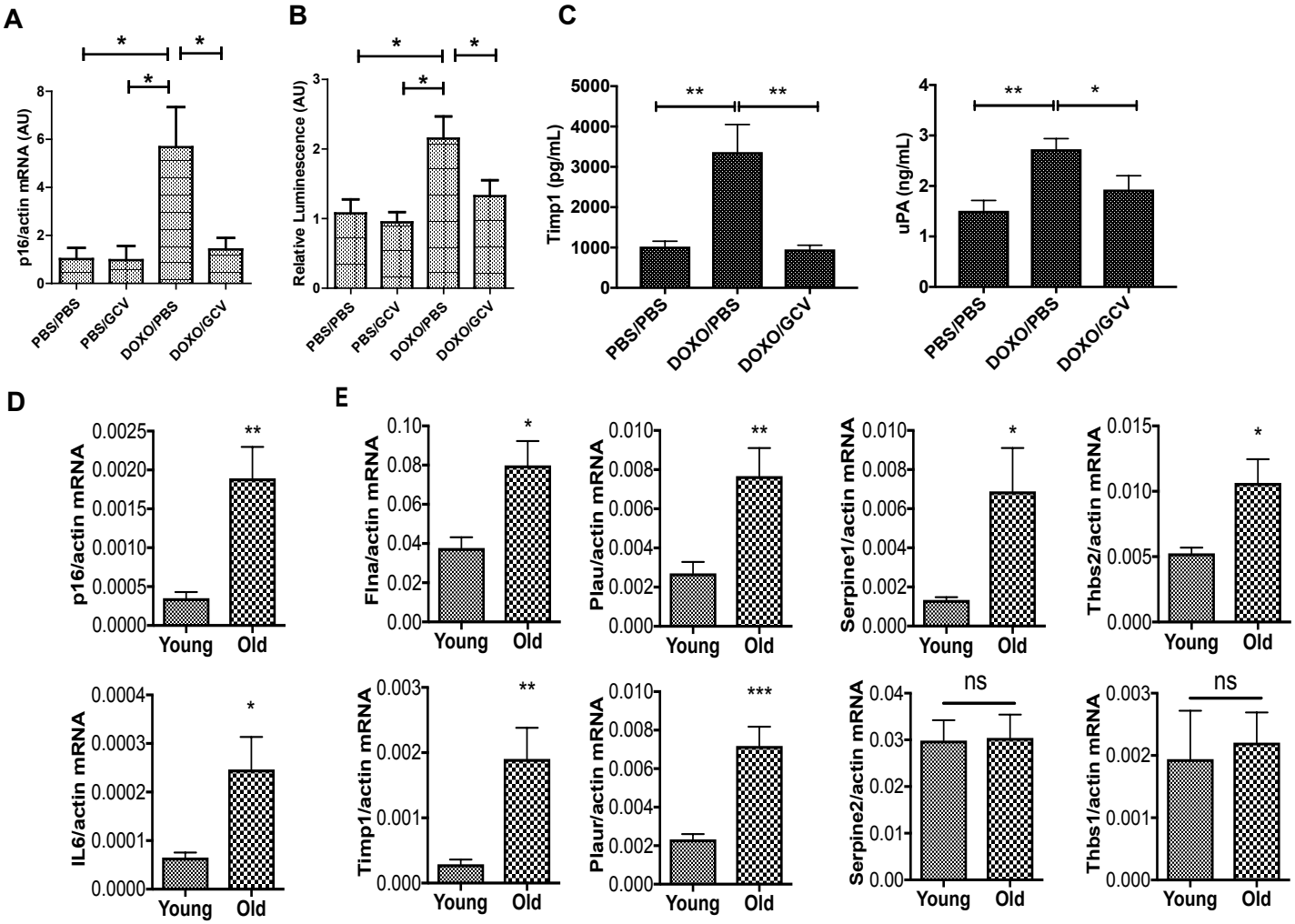

Figure S3. Evidence for the hemostatic SASP in mice, related to Figure 3. (A-C) 4-7 month old p16-3MR mice were given PBS (control) or DOXO (10mg/kg). Ten days later, they were treated with vehicle (PBS) or 25 mg/kg of GCV for 5 d (daily i.p. injections).

(A) RNA was extracted from the liver and analyzed by quantitative PCR for mRNA levels of p16INK4a, which were normalized to actin mRNA.

(B) 4-7 month old p16-3MR mice were treated with vehicle, DOXO and GCV as described in **(A).** Luminescence was analyzed in liver tissue extracts and normalized to total protein concentration.

(C) Platelet-poor plasma from PBS/PBS, DOXO/PBS, or DOXO/GCV mice was analyzed for Timp1 (left) or uPA (right) by competitive ELISA.

(D-E) C57BL/6 mice were aged for 4-6 months (young) or 24-25 months (old). mRNA was extracted from the livers of these mice and analyzed for biomarkers of senescence **(D)** or hemostasis factors **(E)** divided by actin. Bars show the mean± SEM from at least 7 mice. * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$ for all experiments.

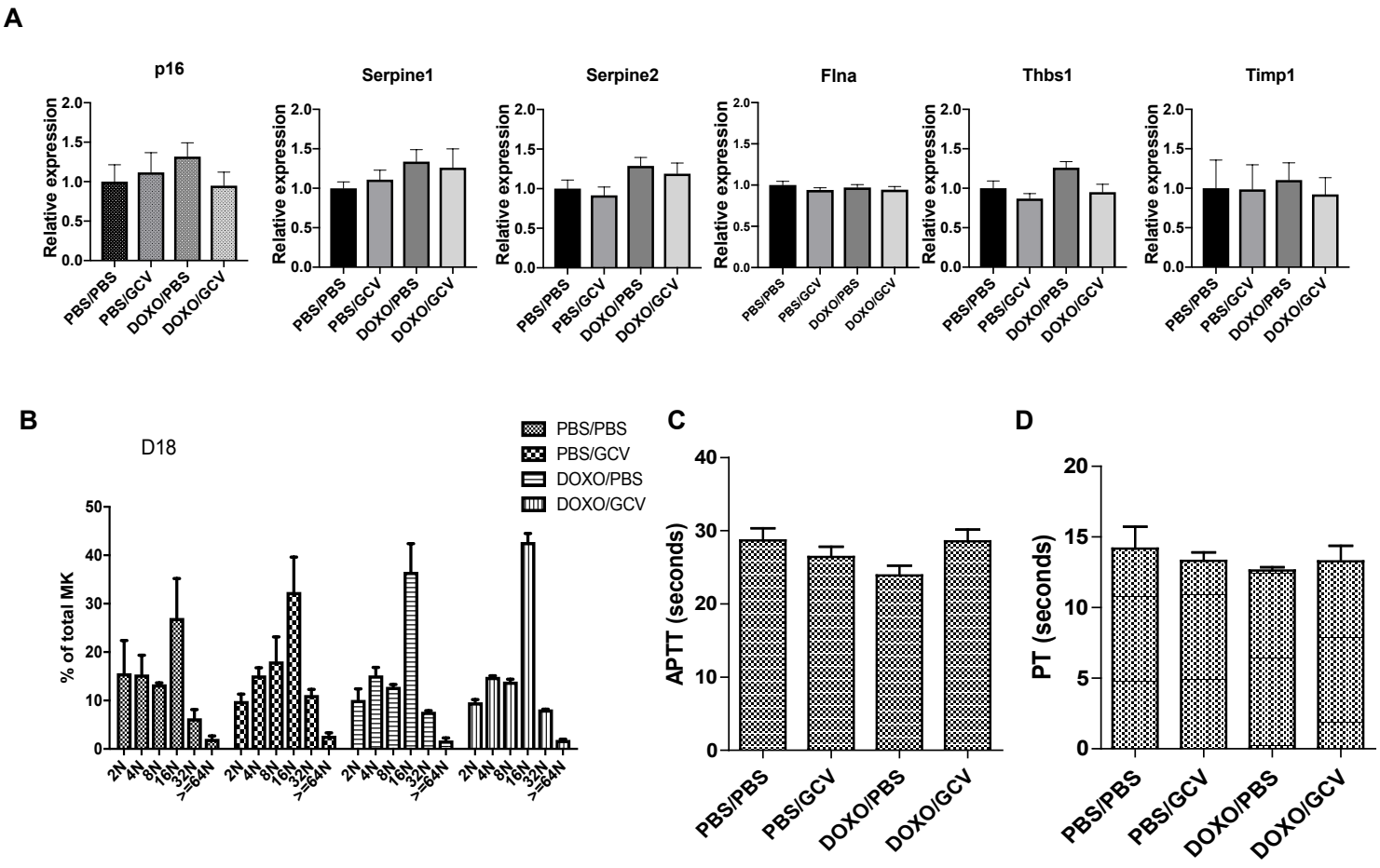

Figure S4. DOXO-induced senescence does not affect bone marrow, megakaryocyte maturation, or the coagulation cascade, related to Figure 4.

(A) Bone marrow was extracted from all treatment groups 15 days after DOXO administration and mRNA extracts were analyzed by qPCR for p16, Serpine1, Serpine2, Flna, Thbs1, or Timp1, normalized to actin. n = 9-10 for all experiments, no treatment groups were statistically significant.

(B) Megakaryocytes were extracted from bone marrow from treated mice 18 days after DOXO administration, cultured for 10 d, and ploidy was measured by double staining with FITC conjugated anti-CD61 antibody and propidium iodide, followed by flow cytometry analysis.

(C-D) 4-7 month old p16-3MR mice were treated as described for Figure S3A. Citrated plasma was collected for APTT **(C)**, and PT **(D)** assays. Bars show the mean± SEM from at least 7 mice. * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$ for all experiments.