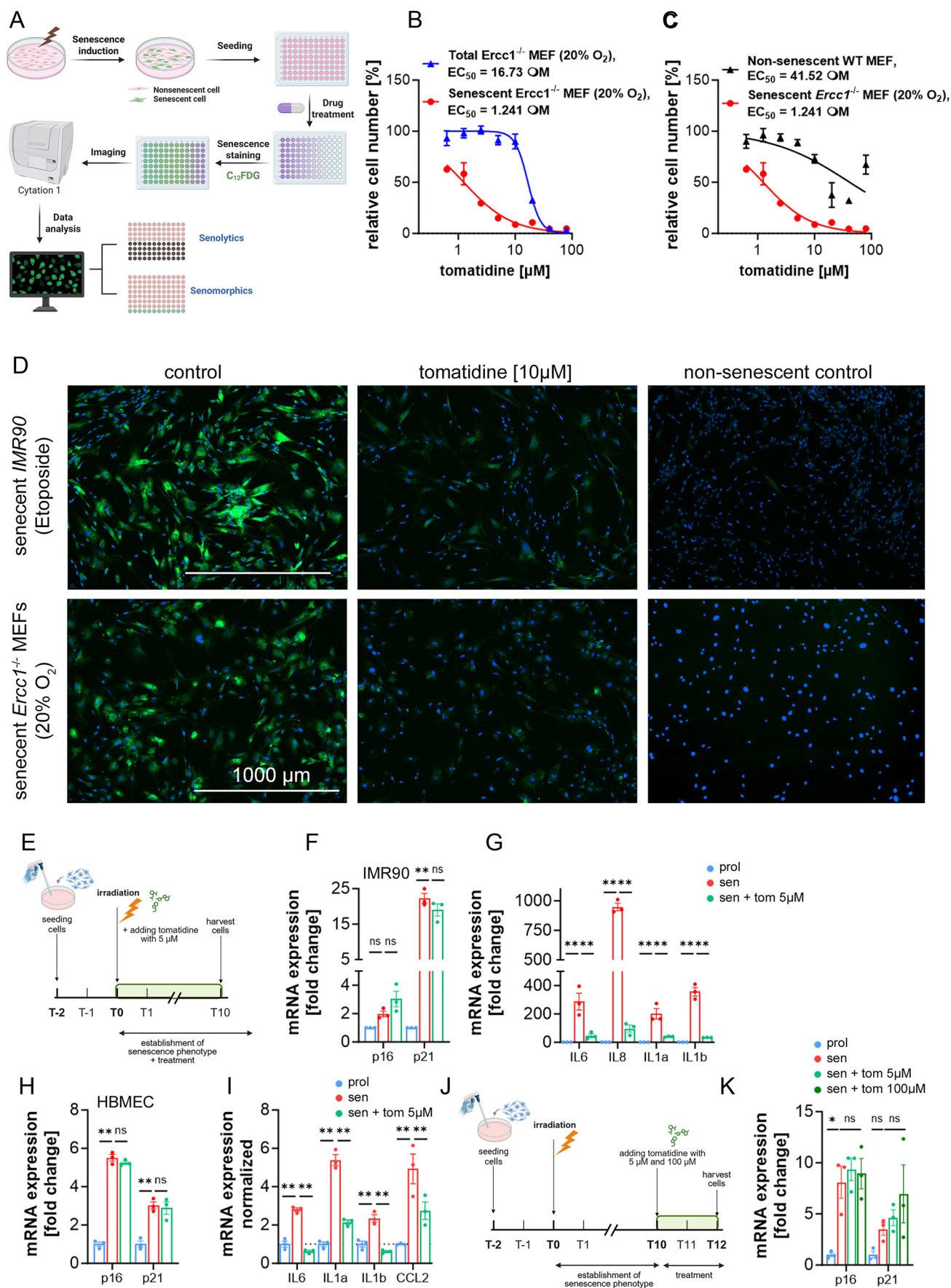


Expanded View Figures

Figure EV1. Identification of tomatidine as a novel senomorphic compound.

(A) Schematic of the senescent cell-based screening workflow. Senescent cells were generated, seeded into 96-well plates, treated with compounds, stained with C12FDG (SA- β -gal) and DAPI, imaged on a high-content system, and analyzed. (B) Concentration-response curves of tomatidine in senescent *Ercc1*^{-/-} MEFs and in total *Ercc1*^{-/-} MEF cultures. (C) Concentration-response curves comparing senescent *Ercc1*^{-/-} MEFs to non-senescent WT MEFs. Responses were normalized to untreated controls and fit by nonlinear regression using a variable-slope model (GraphPad Prism). The IC_{50} for senescent *Ercc1*^{-/-} MEFs was 1.24 (95% CI: 1.03–1.46), whereas the IC_{50} for non-senescent WT MEFs was 41.52 (95% CI: 21.42–140.5). The selectivity index (SI), calculated as IC_{50} (WT) / IC_{50} (senescent *Ercc1*^{-/-}), was 33.5. Data were shown as mean $\pm$ SD from $n = 3$ independent experiments, technical replicates. “Total” denotes mixed cultures following the senescence-induction protocol. The non-senescent control data presented in panels (B, C) derive from the same experimental dataset. (D) Representative C12FDG fluorescence images (green: C12FDG/SA- β -gal; blue: Hoechst). Scale bar, 100 μ m. (E) Illustration of the experimental setup for senomorphic assays. (F, G) Relative mRNA expression of p16, p21, and SASP-related genes in senescent IMR90 fibroblasts treated with tomatidine (in F) $p < 0.0001$ and in (G) IL6 $p = 0.021$ and $p = 0.005$, IL8 $p < 0.0001$ and $p < 0.0001$, IL1a $p = 0.001$ and $p = 0.031$, IL-1b $p < 0.0001$ and $p < 0.0001$). (H, I) Relative mRNA expression of p16, p21, and SASP-related genes in senescent HBMECs treated with tomatidine. (for both p16 and p21 in H): $p < 0.0001$, in (I): IL-6 $p = 0.0006$ and $p < 0.0001$, IL1a: $p < 0.0001$ and $p < 0.0001$, IL-1b: $p = 0.083$ and $p = 0.0008$, CCL2: $p < 0.0001$ and $p < 0.0001$). (J) Illustration of the experimental setup for senolytic assays. (K) Relative mRNA expression of p16 ($p = 0.0102$) and p21 in senescent HBMECs after 48 h of tomatidine treatment. Data were presented as mean $\pm$ s.e.m. (F–K). Statistical significance was determined using two-way ANOVA followed by Tukey’s multiple comparisons test. $n = 3$, technical replicates. Source data are available online for this figure.



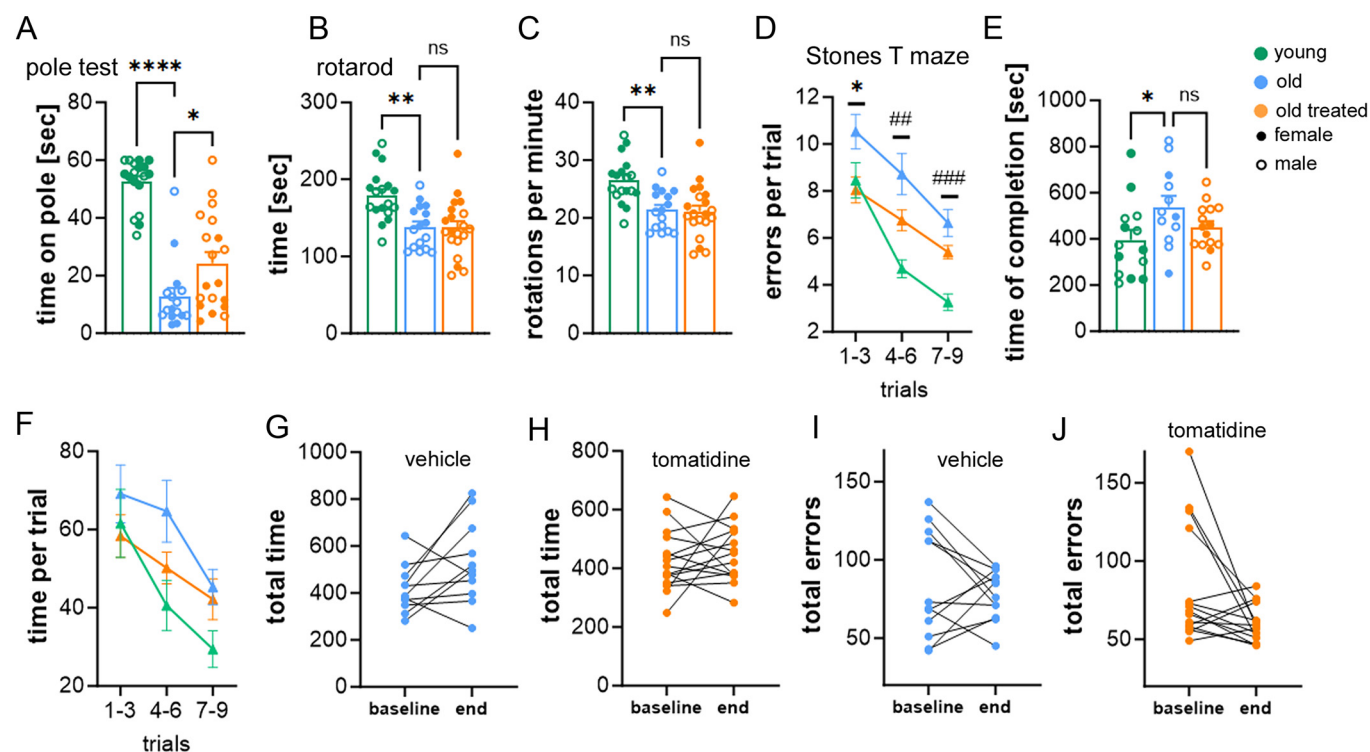


Figure EV2. Healthspan and cognitive assessments in aged mice treated with tomatidine.

(A) Performance on the pole test, measuring motor coordination and balance. (B, C) Rotarod testing showing (B) time spent on the rod and (C) maximal speed achieved. (D) Number of errors made in the Stone T maze across trials. Symbols: (*) indicates a significant difference between old and old tomatidine-treated groups, (##) indicates significant differences between young and old groups. Significant improvements in errors made between trial 1-3 vs 7-9 ($p = 0.0003$) and 4-6 vs 7-9 ($p = 0.0092$) in treated animals and in untreated animals, significant improvements between trial 1-3 vs 7-9 ($p = 0.0014$). (E, F) Time required to complete the Stone T maze in aged control and aged tomatidine-treated mice. (G, H) Comparison of baseline versus endpoint measurements for total time spent completing the maze. (I, J) Comparison of baseline versus endpoint measurements for total errors made prior to completing the maze. Data were presented as mean $\pm$ s.e.m. Statistical significance was determined using one-way or two-way ANOVA followed by Tukey's multiple comparisons test. $n = 15-23$, biological replicates. Males are represented by open circles, females by filled circles. Source data are available online for this figure.

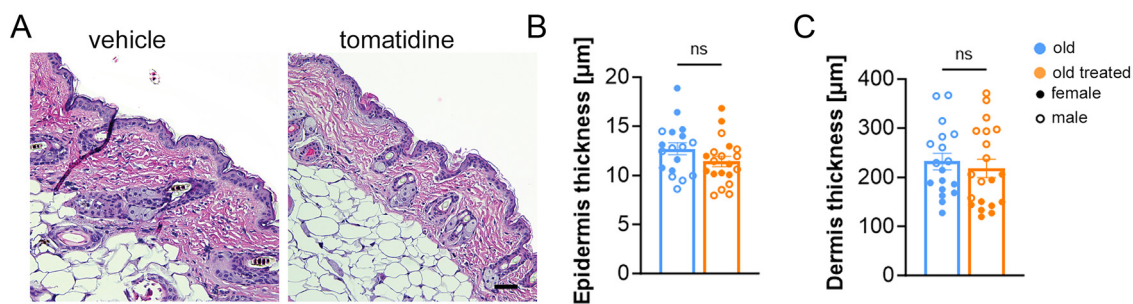


Figure EV3. Skin thickness measurements in aged mice treated with tomatidine.

(A) Representative H&E-stained skin sections from tomatidine-treated aged mice and untreated aged controls. (B) Quantification of epidermis thickness ($p = 0.1238$). (C) Quantification of dermal thickness ($p = 0.5839$). Data were presented as mean $\pm$ s.e.m. Statistical significance was determined using a t -test. Scale bar = 50 μ m, $n = 18$ –21, biological replicates. Males are represented by open circles, females by filled circles. Source data are available online for this figure.

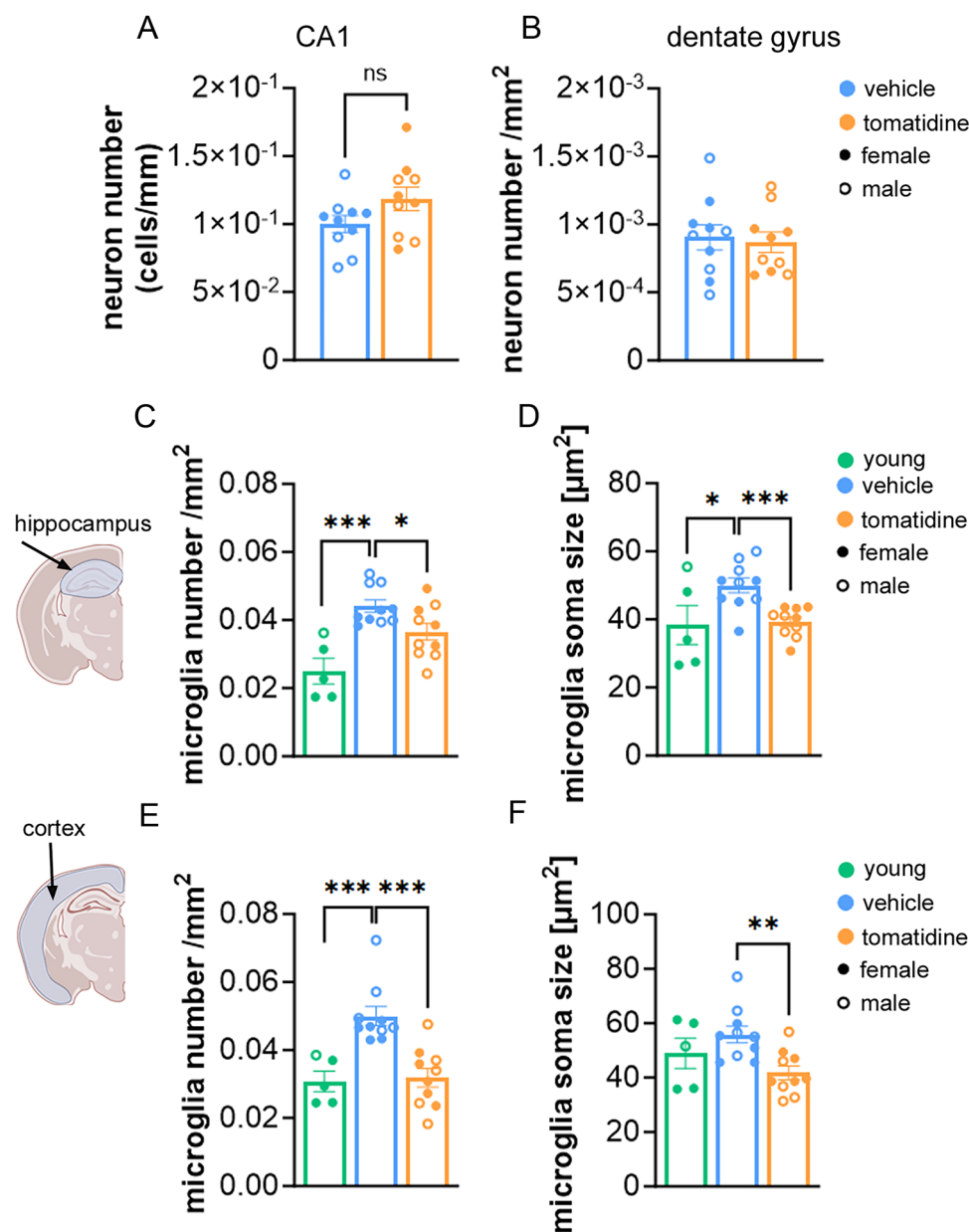
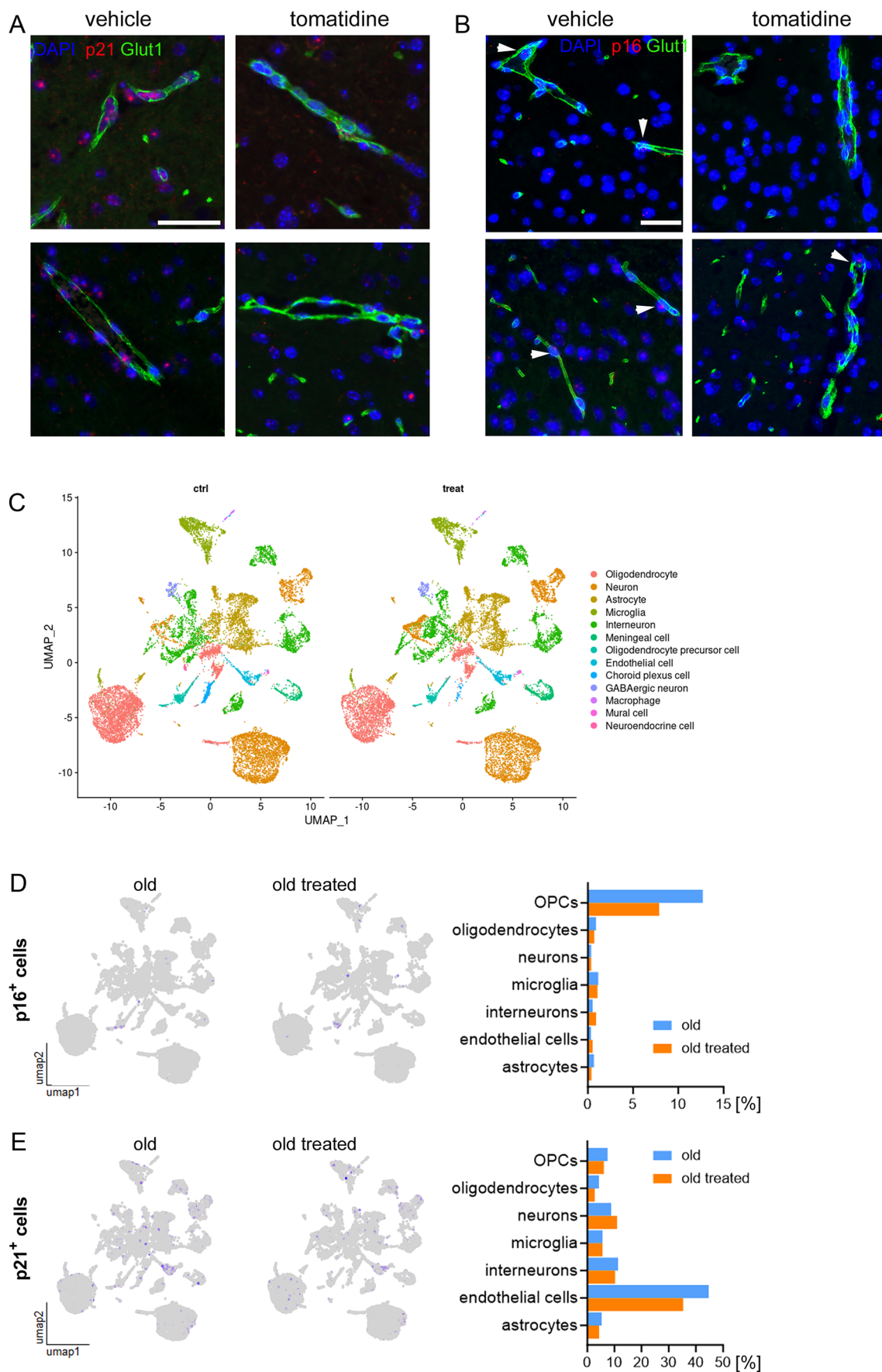


Figure EV4. Neuronal density and microglial morphology in hippocampus and cortex of aged mice treated with tomatidine.

(A) Quantification of neuronal density in CA1, showing the number of NeuN⁺ neurons per mm length ($p = 0.0501$). (B) Neuronal density in the dentate gyrus (DG) region of the hippocampus ($p = 0.3802$). (C) Number of Iba1⁺ microglia in the hippocampus; Exact p values for number Iba1⁺ (Young vs. vehicle, $p = 0.0002$; vehicle vs. tomatidine, $p = 0.0230$). (D) Microglial soma size in the hippocampus, reflecting changes in activation state; Exact p values for soma size in hippocampus (young vs. vehicle, $p = 0.037$; vehicle vs. tomatidine, $p = 0.0003$). (E) Number of Iba1⁺ microglia in the cortex; Exact p values for number Iba1⁺ (Young vs. vehicle, $p = 0.0009$; vehicle vs. tomatidine, $p = 0.0002$). (F) Microglial soma size in the cortex; Exact p values for soma size in cortex (Young vs. vehicle, $p = 0.2525$; vehicle vs. tomatidine, $p = 0.0022$). Panels (C-F) present the same datasets included in the main figure; here, we additionally show the values from young control mice to allow direct comparison between young, aged, and tomatidine-treated aged groups. Data were presented as mean $\pm$ s.e.m. Statistical significance was determined using a t -test or one-way ANOVA. $n = 5-10$, biological replicates. Males are represented by open circles, females by filled circles. Source data are available online for this figure.



**Figure EV5. RNA-ISH and scRNA-seq analysis of p16 and p21 expression in brain endothelial cells.**

(A) Representative RNA-ISH images showing p21 mRNA expression in GLUT1-positive brain endothelial cells (blue: DAPI; red: p21; green: GLUT1). Scale bar 50 μ m. (B) Representative RNA-ISH images showing p16 mRNA expression in GLUT1 positive BBB endothelial cells (blue: DAPI; red: p16; green: GLUT1). White arrows indicate positive endothelial cells. Scale bar 30 μ m (C) UMAP representation of all cell types colored by class from tomatidine treated and untreated controls. scRNA-seq was performed on hippocampus pooled from four animals per group. (D) UMAP representation and quantification of p16⁺ cells from tomatidine treated and untreated controls. (E) UMAP representation and quantification of p21⁺ cells from tomatidine treated and untreated controls. Source data are available online for this figure.