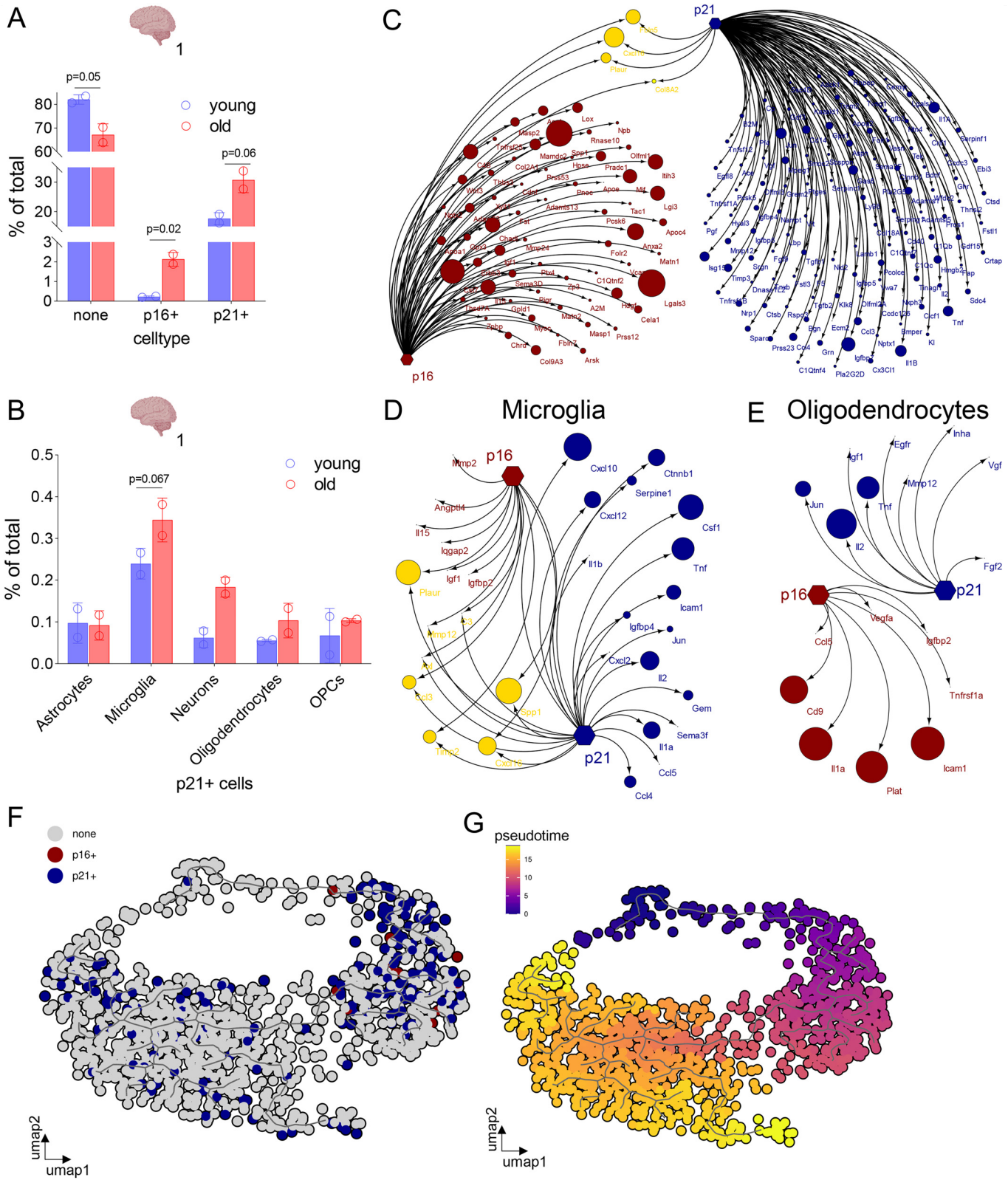


Expanded View Figures

Figure EV1. Comprehensive examination of the mRNA expression profiles for the whole secretome and SenMayo in aging murine hippocampus.

(A) $p16+$ cells are significantly more frequent in the old brain. (B) Microglia has more $p21+$ cells in the old brain. (C) In the murine hippocampus, there is minimal overlap between the mRNA expression of the whole secretome + SenMayo in $p21+$ and $p16+$ cells. (D) SenMayo composition in $p16+$ vs. $p21+$ microglia shows some common secretory genes like *Plaur* and *Cxcl16*, while in (E) oligodendrocytes, there is no common secretory phenotype. (F) The velocity trajectory of $p16+$ cells, $p21+$ cells and the remaining brain cells shows no overlap or common origin (G) Monocle3 applied to the backbone from f shows that there is no common “ancestor” for these cell types. A: unpaired t -test, $n = 2$ per condition, error bars: sd, B: multiple paired t -tests, $n = 2$ per condition, error bars: sd.



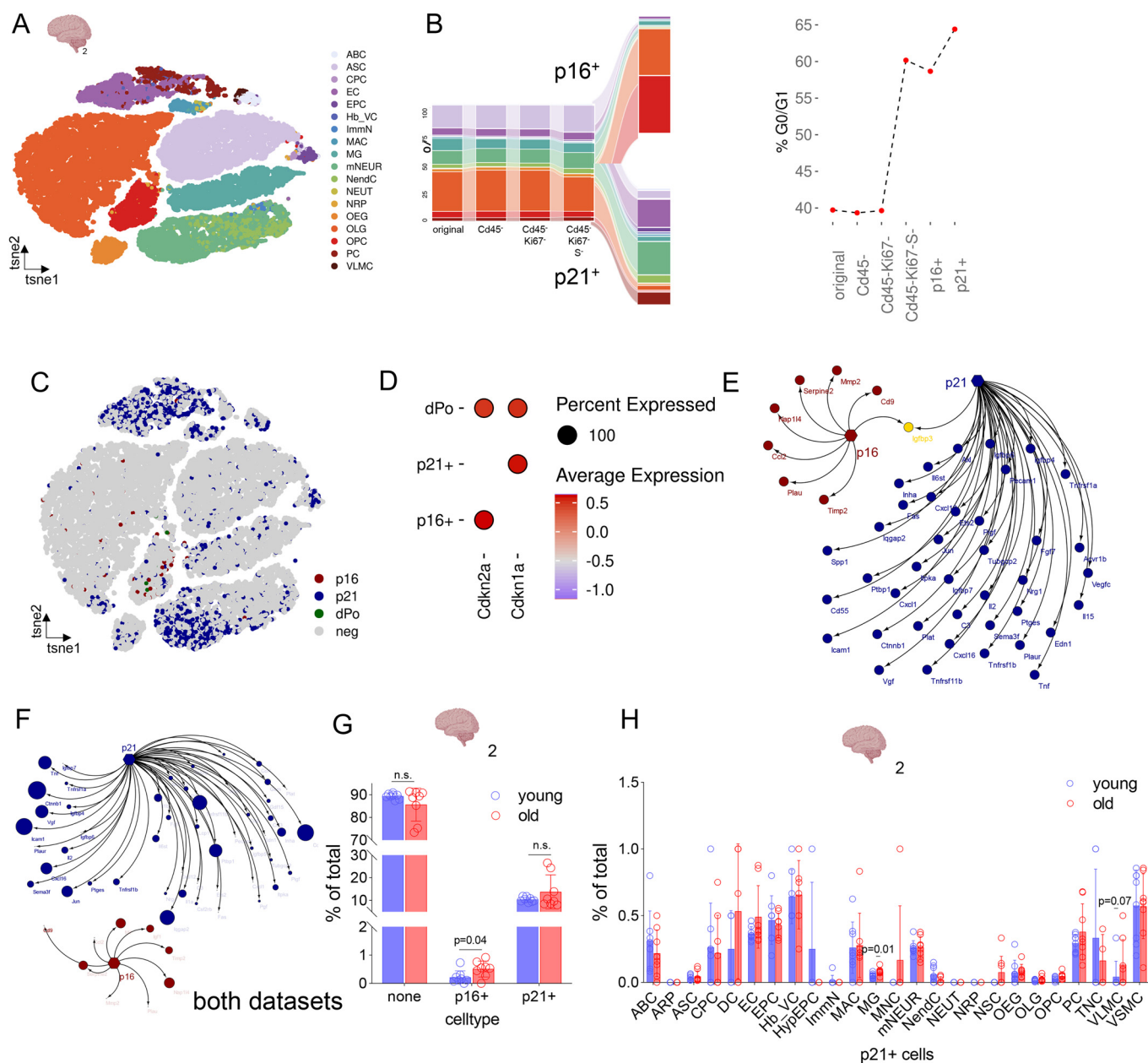


Figure EV2. A second brain sample (Ximerakis et al, 2019) was used to confirm the findings from the first brain sample.

(A) A t-SNE plot indicating all cell types. (B) The selection of $p16^+$ cells focuses on oligodendrocyte precursors, while $p21^+$ cells were mostly endothelial cells. (C) The t-SNE plot demonstrates that most cells are either $p16^+$ or $p21^+$, but double-positive cells are rare. (D) The expression of $p16^+$, $p21^+$ cells, and the rare dPo population. (E) There is only a very small overlap between the $p16^+$ and $p21^+$ secretory phenotype, namely just one gene (*Igfbp3*). (F) When combining both brain datasets, we observed no consistent overlap between the secretory profiles of $p16^+$ and $p21^+$ cells, underscoring that they represent distinct populations. Shared genes across both datasets are shown in solid color, whereas genes unique to a single dataset are displayed transparently. (G) $p16^+$ cells are significantly more frequent in the old brain. (H) Microglia (MG) has more $p21^+$ cells in the old brain. (G): unpaired *t*-test, $n = 8$ per condition, error bars: sd, (H) multiple paired *t*-tests, $n = 8$ per condition, error bars: sd.

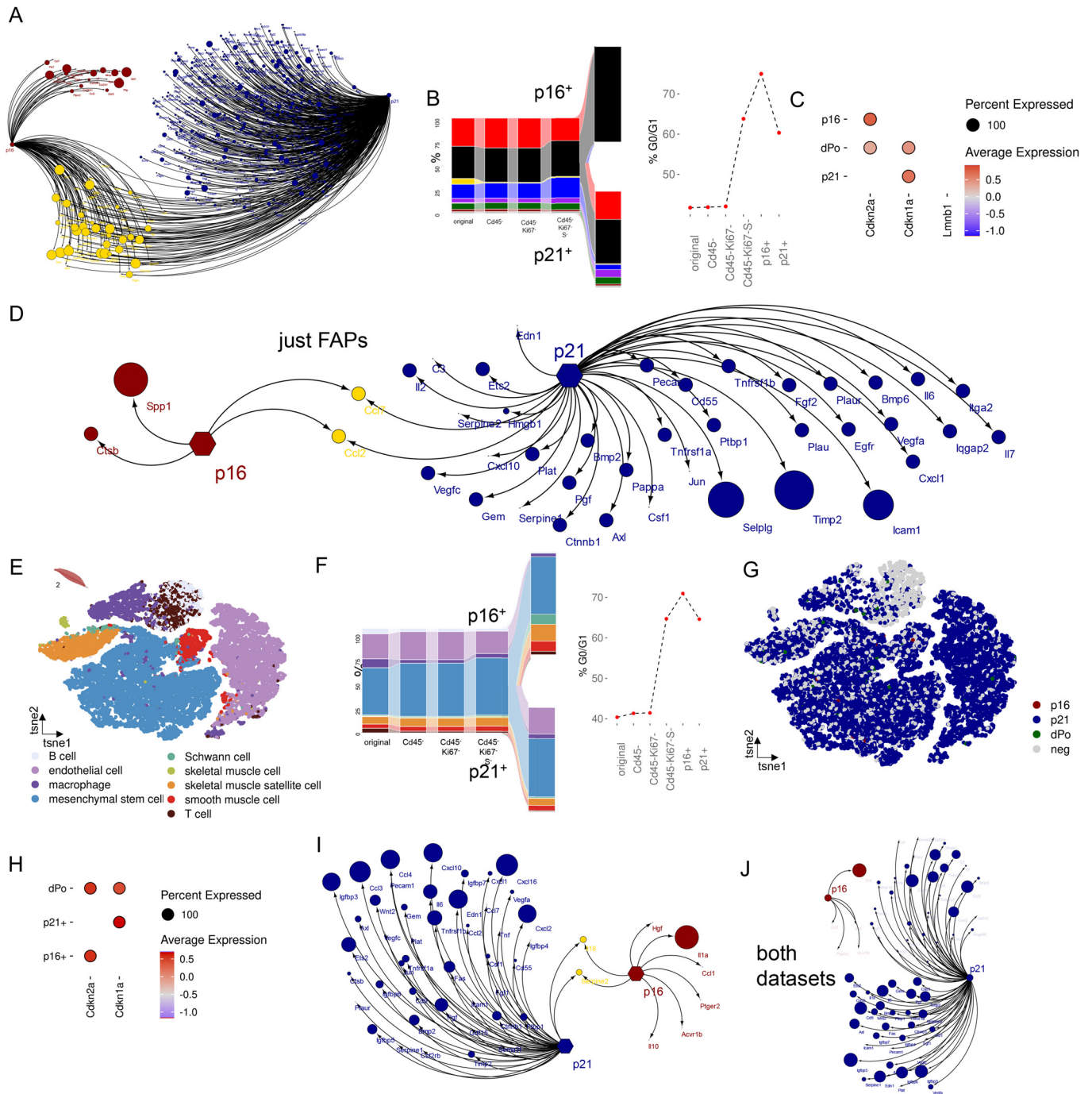


Figure EV3. Comprehensive analysis of mRNA expression profiles in aging murine muscle.

(A) In the muscle, there is some overlap with a noticeably larger p21-associated secretome compared to p16. (B) The selection criteria for p21+ and p16+ cells. While most of the p16+ cells are FAPs (black), in the p21+ cells there are also some endothelial cells (red). (C) Key markers for the abovementioned populations. (D) Secretory phenotype composition in p16+ and p21+ FAPs. (E) A second muscle dataset (Tabula_Muris_Consortium, 2020) showed nine key cell populations, with (F) most of the p16+ and p21+ cells originating from the mesenchymal stem cell compartment. (G) The majority of cells is p21+. (H) Key markers for these three populations. (I) The secreted factors between these two populations are heterogeneous, and (J) Integration of both datasets shows no overlap in secreted factors between these two populations.

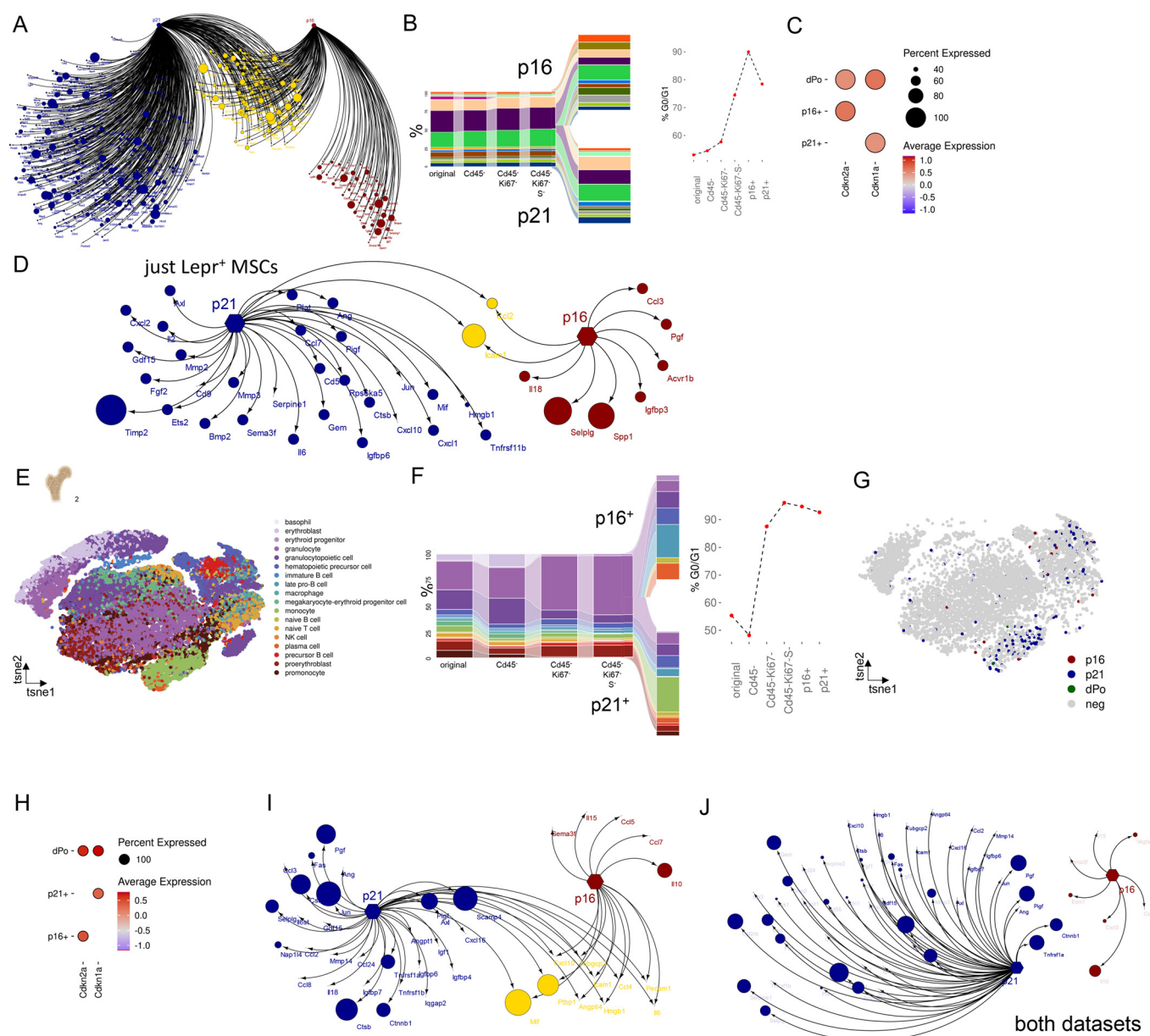


Figure EV4. Analysis of the secretome in bone during aging.

(A) Similar to the muscle, in the bone, there is some overlap but a more pronounced p21-associated secretome. (B) The selection criteria and key populations (C) for p16⁺ and p21⁺ cells. (D) Just Lepr⁺ MSCs and p16⁺ and p21-associated SenMayo phenotype, respectively. (E) A second bone dataset (Tabula_Muris_Consortium, 2020) shows a (F) high amount of B cells in p16⁺ cells and monocytes in p21⁺ cells. (G, H) There are a few p21⁺ cells, and almost no dPo cells. (I) There is some overlap between p16⁺ and p21⁺ cells, while a summary of both bone datasets (J) shows no single gene overlapping for p16⁺ and p21-associated secretory factors.

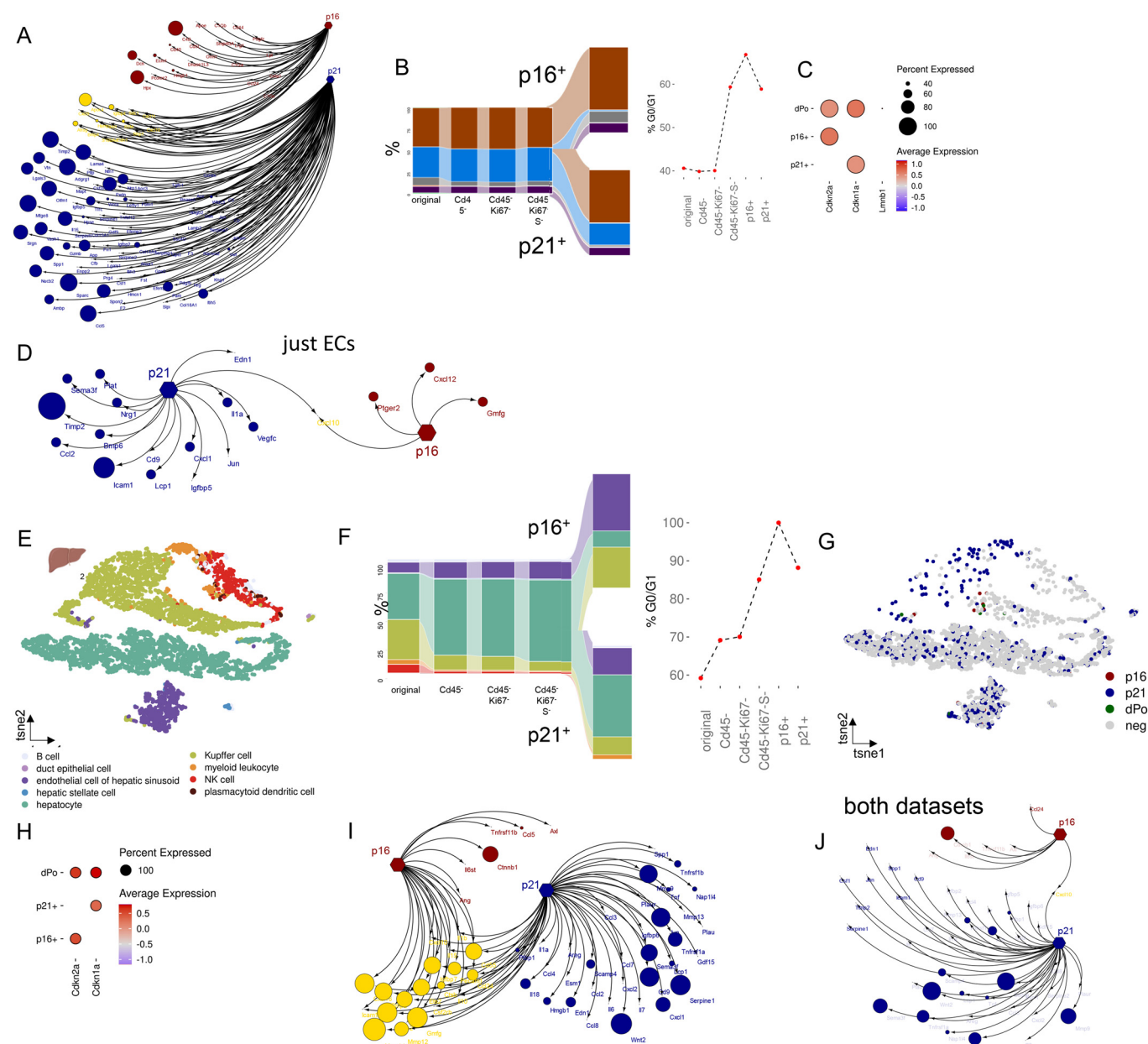


Figure EV5. Liver analysis demonstrates differences between $p21^+$ cells and $p16^+$ cells in aging.

(A) Within the liver, there is minimal overlap between $p21^+$ cells and $p16^+$ cells, with the $p21^+$ cells predominantly expressing the majority of factors. (B) The selection of $p16^+$ cells shows mostly endothelial cells, while in the $p21^+$ cells, there are also some hepatocytes. (C) The key markers for the three key populations. (D) $p21^+$ - and $p16^+$ -associated SenMayo secretory phenotype in ECs. (E) A second hepatic dataset (Tabula_Muris_Consortium, 2020) was analyzed, while (F) endothelial cells were the most abundant in $p16^+$ cells, and hepatocytes the most prominent in the $p21^+$ cells. (G, H) $p21^+$ cells were the majority compared to $p16^+$ cells, and (I) there was a substantial overlap between $p21^+$ - and $p16^+$ -associated secretory factors, although $p21^+$ cells expressed a greater number of factors than $p16^+$ cells. (J) Combining both datasets, there was just one overlapping factor (*Cxcl16*).

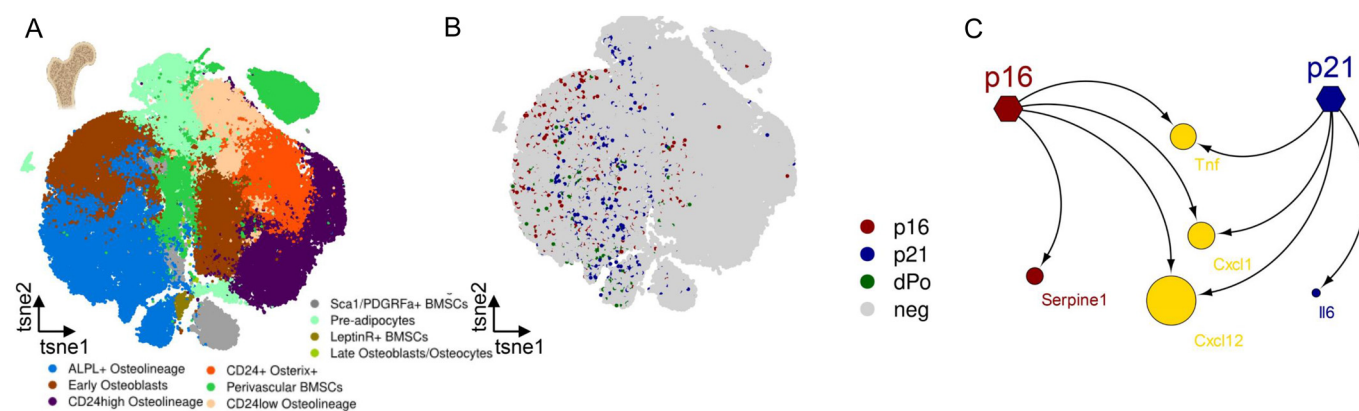


Figure EV6. CyTOF analyses of *p16*, *p21* and associated secreted factors in murine bone.

(A) CyTOF dataset from murine bone (Doolittle et al, 2023). (B) *p16*⁺ and *p21*⁺ cells are largely distinct, with minimal overlap. (C) The associated secretory phenotype of *p16*⁺ and *p21*⁺ cells shows some overlap but is limited to only a few factors, likely reflecting the limited antibody panel available.

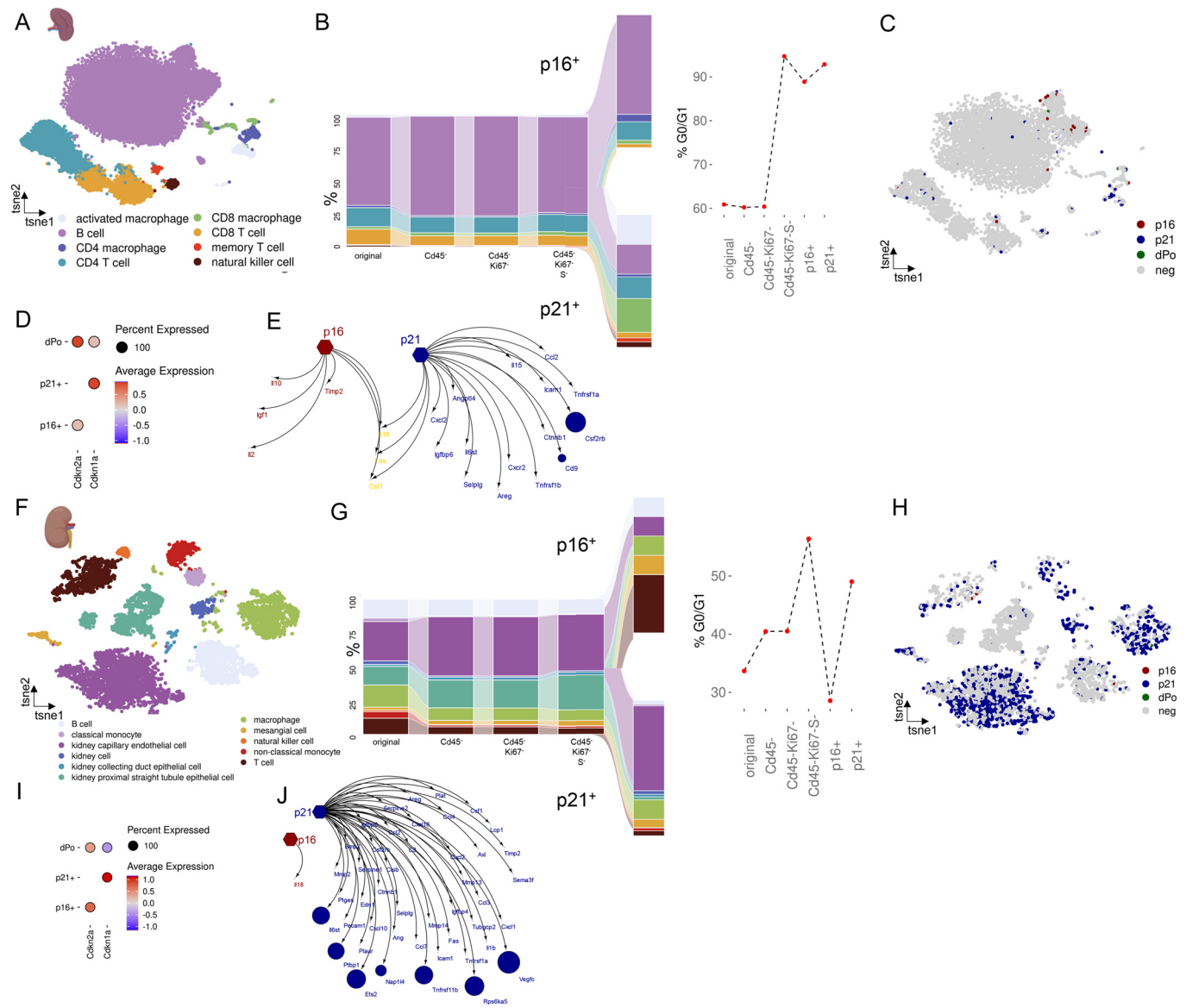


Figure EV7. Spleen and kidney display p16- and p21-associated differences consistent with previous murine datasets.

(A) In the spleen calico dataset (Kimmel et al, 2019, GSE 132901), eight different populations were identified and (B) analyzed according to their Cd45-negativity, Ki67-negativity and S-phase exclusion, followed by p16- (mostly B cells) and p21-positivity (mostly Cd8 macrophages). (C) The majority of cells were p21+, although (D) only a small fraction of total cells were positive for either marker. (E) Secreted factors showed limited overlap, with most associated with p21+ cells. (F) The kidney dataset consisted of 11 different cell types, out of which (G) T cells were the most abundant in p16+ cells and capillary endothelial cells were the most abundant in p21+ cells. (H, I) The majority of cells was p21+. (J) A vast majority of secreted factors was associated with p21+ cells.

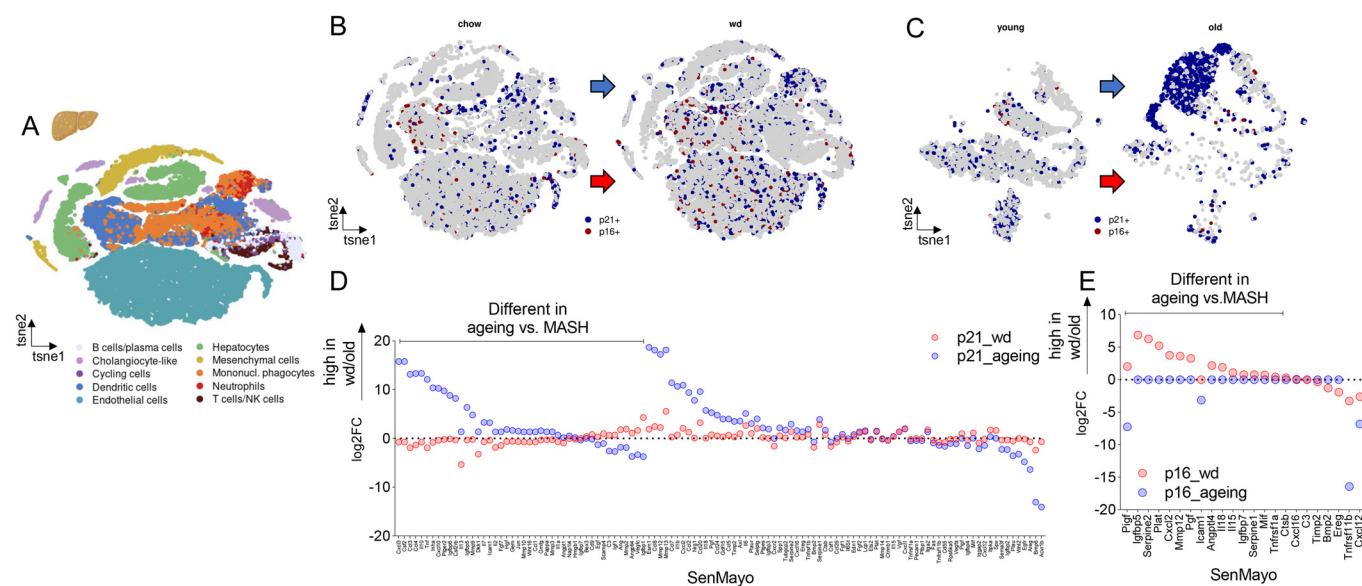


Figure EV8. Distinct secretory phenotypes of $p16+$ and $p21+$ cells in MASH and aging liver.

(A) Analysis of a diseased liver (MASH, [GSE218300](#), (Bendixen et al, 2024)), (B) a western-style diet (wd) compared to chow resulted in a moderate increase of $p21+$ cells, while (C) aging resulted in a profound increase of $p21+$ cells. (D) The secretory phenotype in $p21+$ cells was different to a large degree in aging vs. MASH, with a higher amount of genes upregulated in aging. Similarly, the (E) secretory phenotype in $p16+$ cells was markedly different in aging vs. MASH, but with more genes upregulated in the western diet compared to aging.

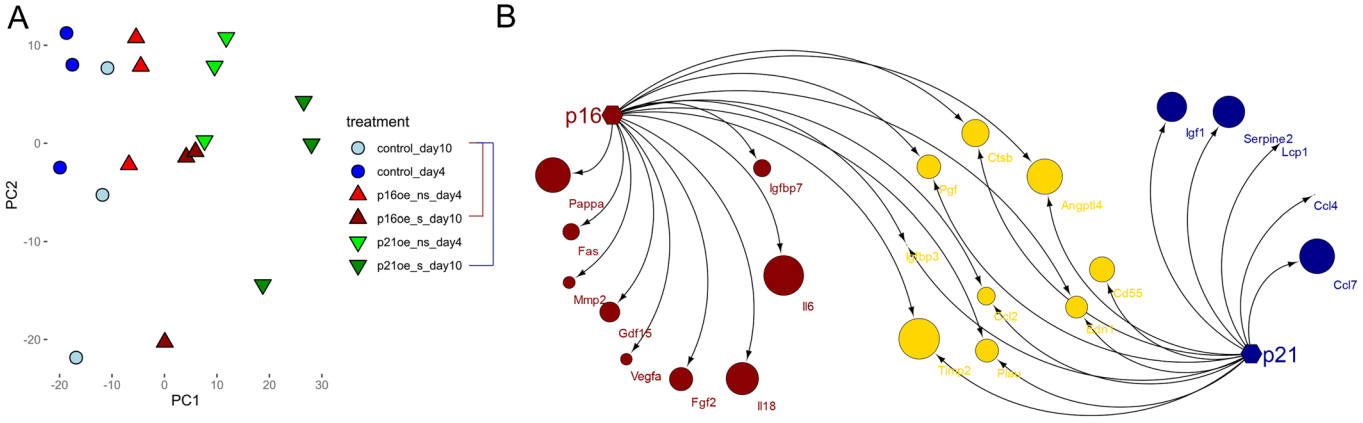


Figure EV9. Overexpression of p16 and p21 reveals distinct secretory profiles (GSE117278).

(A) A PCA plot illustrating control-MEFs on day 4 and 10, as well as p16-overexpressing cells (adeno-Cre-EGFP virus Ai14;L-p16 injection into the tail) on day 4 and 10, compared to p21-overexpressing cells (Ai14;L-p21) on day 4 and 10. (B) A comparable number of factors are expressed in p16+ (red)- vs. p21+ (blue) cells, with overlap indicated in yellow.

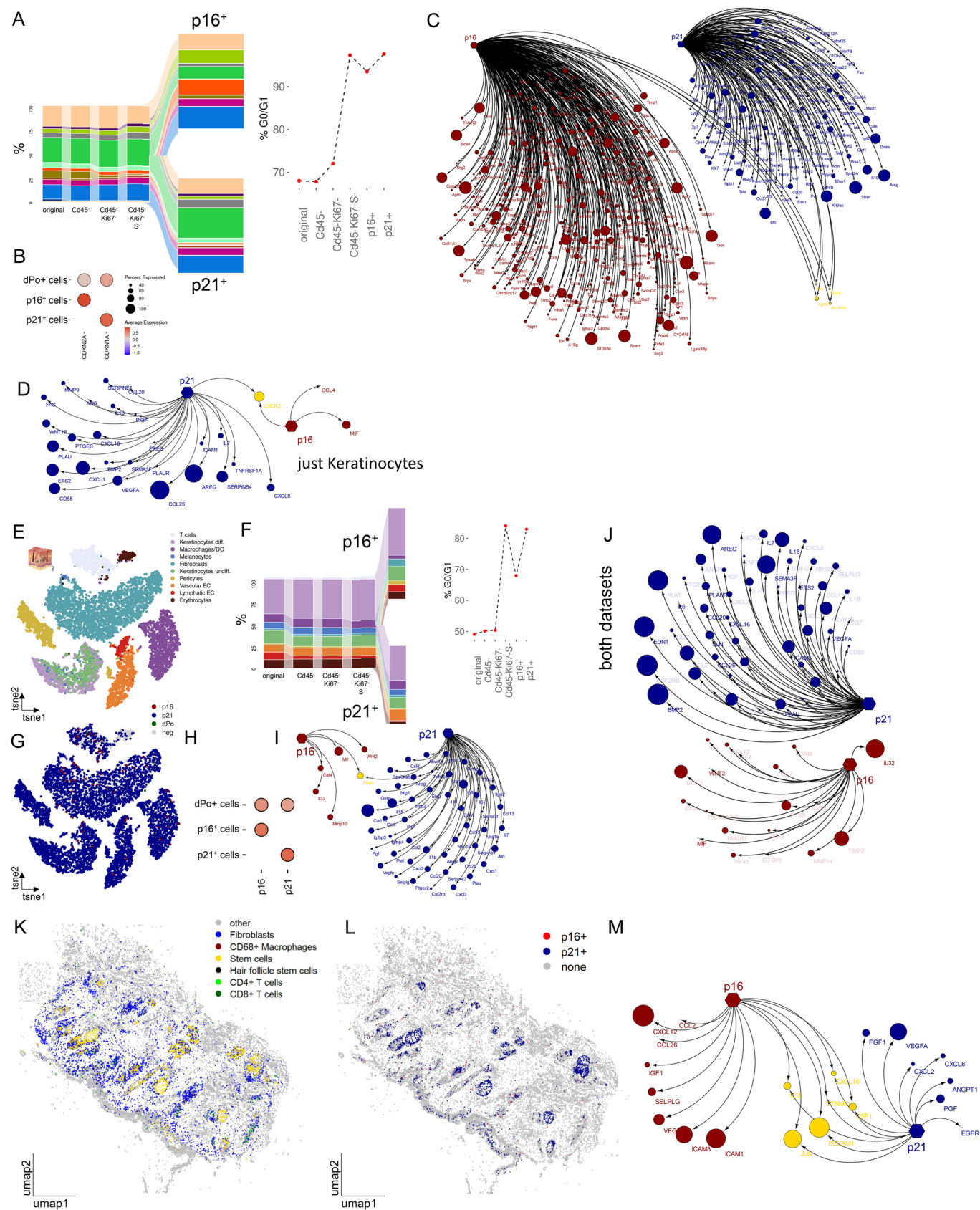




Figure EV10. Human skin secretome, associated with p21 and p16, incorporating datasets from Zou et al, (2021) and Solé-Boldo et al, (2020).

(A) In the skin, there is minimal overlap between *p16*⁺ and *p21*⁺ cells in terms of mRNA expression of the whole secretome. Unlike the majority of examined tissues, the mRNA expression of the entire secretome associated with p16 is as extensive as that associated with p21. (B) CD45-negativity, followed by Ki67-negativity and exclusion of S-phase cells were followed by p21- or p16-positivity for selection of proposedly senescent cells, which key markers are depicted in (C). (D) SenMayo in *p16*⁺ and *p21*⁺ keratinocytes, respectively. (E) Five human skin samples ([GSE130973](#)) were likewise (F) applied these selection criteria, but *p16*⁺ cells were lacking, resulting in an abundance of (G) *p21*⁺ cells and (H) no *p16*⁺ cells. (I) Subsequently, there is just a p21-associated SASP, and no overlap (J) in both datasets for *p16*⁺ and *p21*⁺ cells. (K) Multiplexed error-robust fluorescence in situ hybridization (MERFISH) analysis on human skin samples, with different cell types within a human skin sample. Within these cell types, (L) *p16*⁺ (red) and *p21*⁺ (blue) cells are largely distinct, with minimal overlap between these markers. (M) *p16*⁺ and *p21*⁺-associated secretory profiles within the MERFISH dataset.

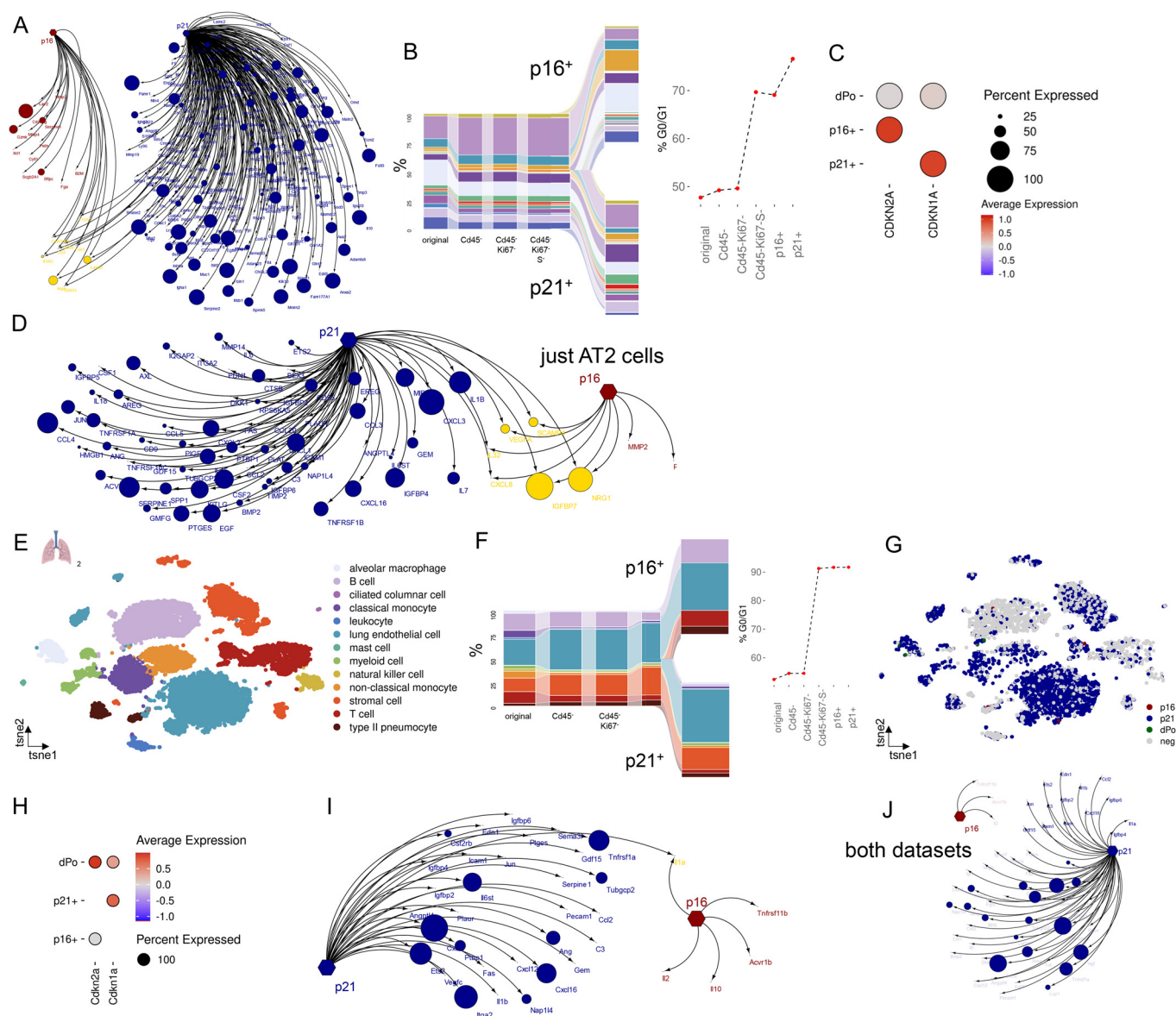


Figure EV11. Analysis of lung reveals limited overlap in the secretory phenotype and distinct developmental profiles of p16- and p21-positive cells.

(A) In the human lung, the p21+ cells express a large number of secreted factors, with a very small overlap with p16+ cells. (B) Selection criteria for human lung tissue include CD45-negativity, followed by Ki67-negativity and exclusion of S-phase cells, before the cells are distributed between p16+ or p21+. (C) The markers for these three key populations. (D) p16- and p21-SenMayo associated secretory phenotype in p16+ and p21+ AT2 cells, respectively. (E) The murine Calico lung dataset (Kimmel et al, 2019) provides 13 different cell types, which are selected (F) as abovementioned. (G, H) The vast majority of cells are p21+, (I) The secretory phenotype itself is more abundant in the p21+ cells and there is (I) a small overlap of p16+ and p21+ associated factors between both datasets.

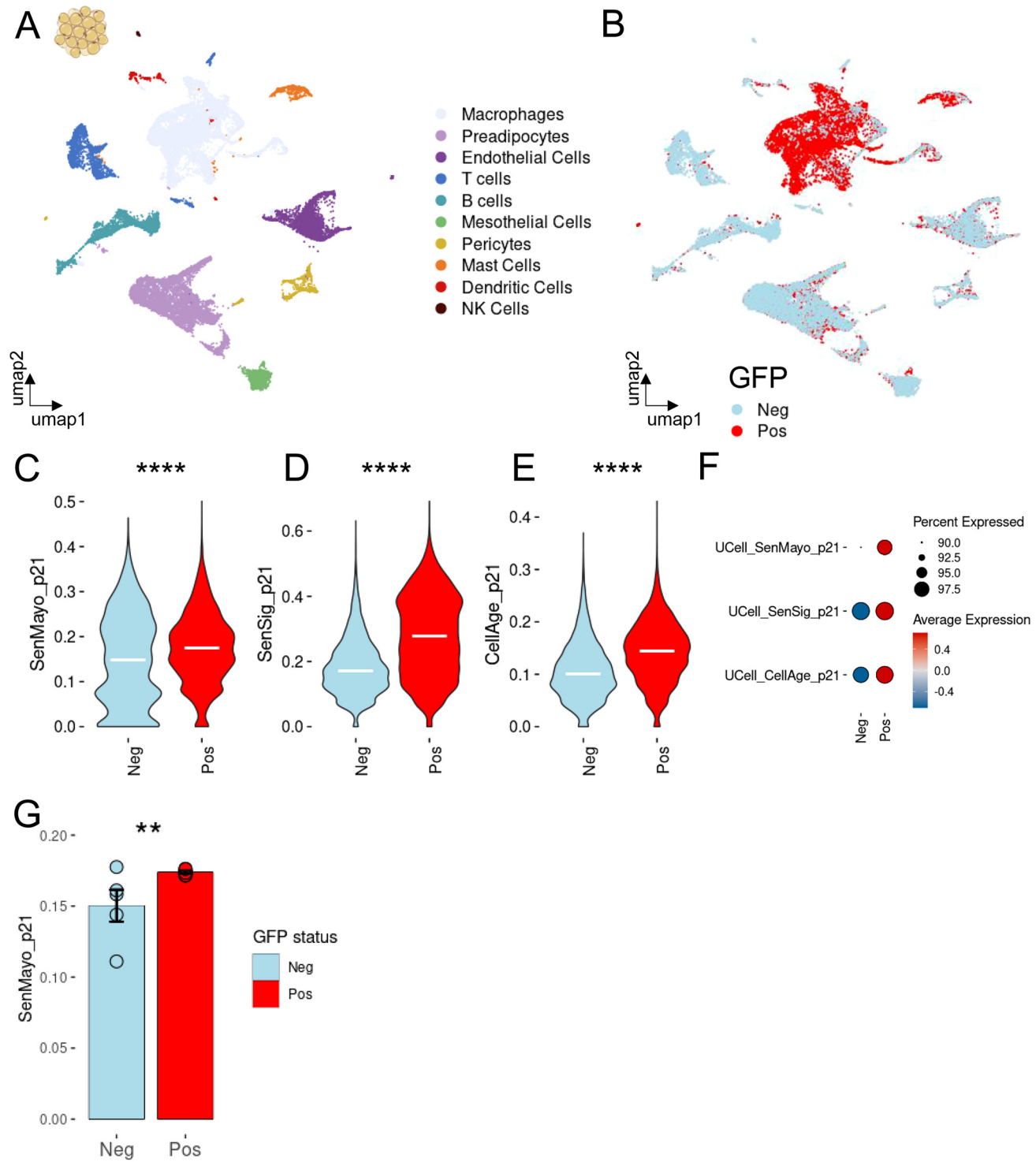


Figure EV12. In an adipose tissue dataset (Wang et al, 2024a, GSE269660), the GFP/Cdkn1a-positive cells exhibited an enrichment of the p21-associated secretory phenotype.

(A) In adipose tissue, ten different populations were identified, out of which the (B) macrophages were the highest in p21/GFP. (C) The p21-associated SenMayo, (D) SenSig, and (E) CellAge were all (F) significantly higher in the GFP-positive cells compared to the GFP-negative cells. (G) Likewise, a samplewise comparison ($n=10$) between GFP-positive ($n=5$) and GFP-negative ($n=5$) samples shows an increase of p21-associated SenMayo in the positive samples. **** p value <0.0001 , *** p value <0.001 , ** p value <0.01 , * p value <0.05 . (C-E) Exact p values = 2×10^{-16} , Wilcoxon test, (G) Exact p value = 0.004, t -test, error bars: 2 SE. $n=5$ per condition

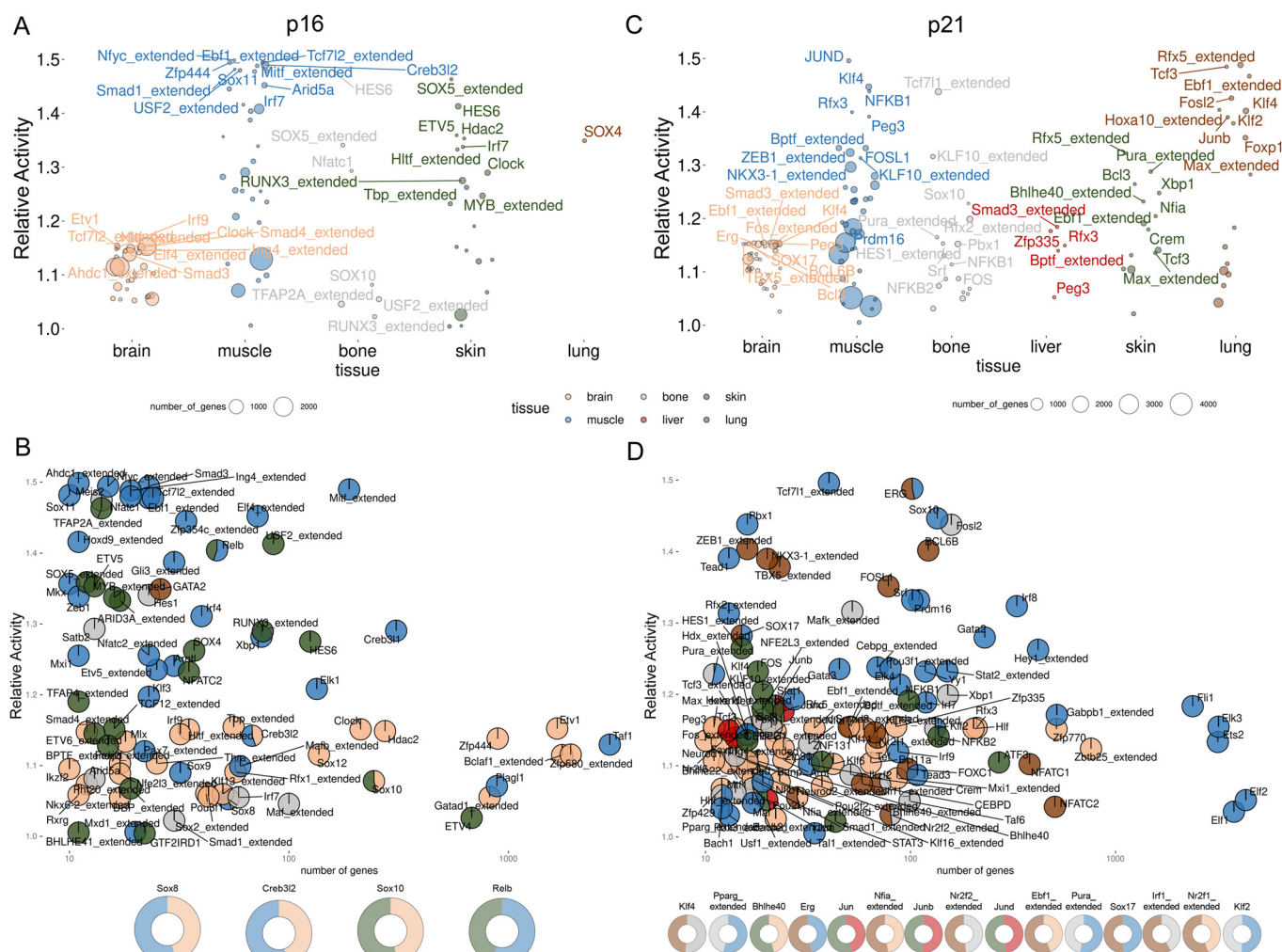


Figure EV13. SCENIC analysis of the regulating transcription factors of *p16*⁺ and *p21*⁺ cells.

(A) When analyzing five distinct tissues for regulatory factors in *p16*⁺ cells, each tissue exhibits a substantial array of transcription factors governing the behavior of *p16*⁺ cells. (Since the liver has very few *p16*-positive cells for proper calculation with SCENIC, these were excluded). (B) The x-axis represents the number of genes, plotted against the y-axis, illustrating the relative activity of the respective transcription factor, highlighting the significance of each factor. Interestingly, only four factors (*Sox8*, *Creb3l2*, *Sox10*, and *Relb*) exhibit consistency across multiple tissues in the context of *p16*⁺ cells. (C) In *p21*⁺ cells, the regulating transcription factors show a high heterogeneity across tissues. (D) The transcription factors associated with *p21* are predominantly specific to a single organ, but a few transcription factors, such as *Erg*, *Sox17*, *Klf4*, *Jun*, *Klf2*, and others, regulate *p21*⁺ cells in two tissues.