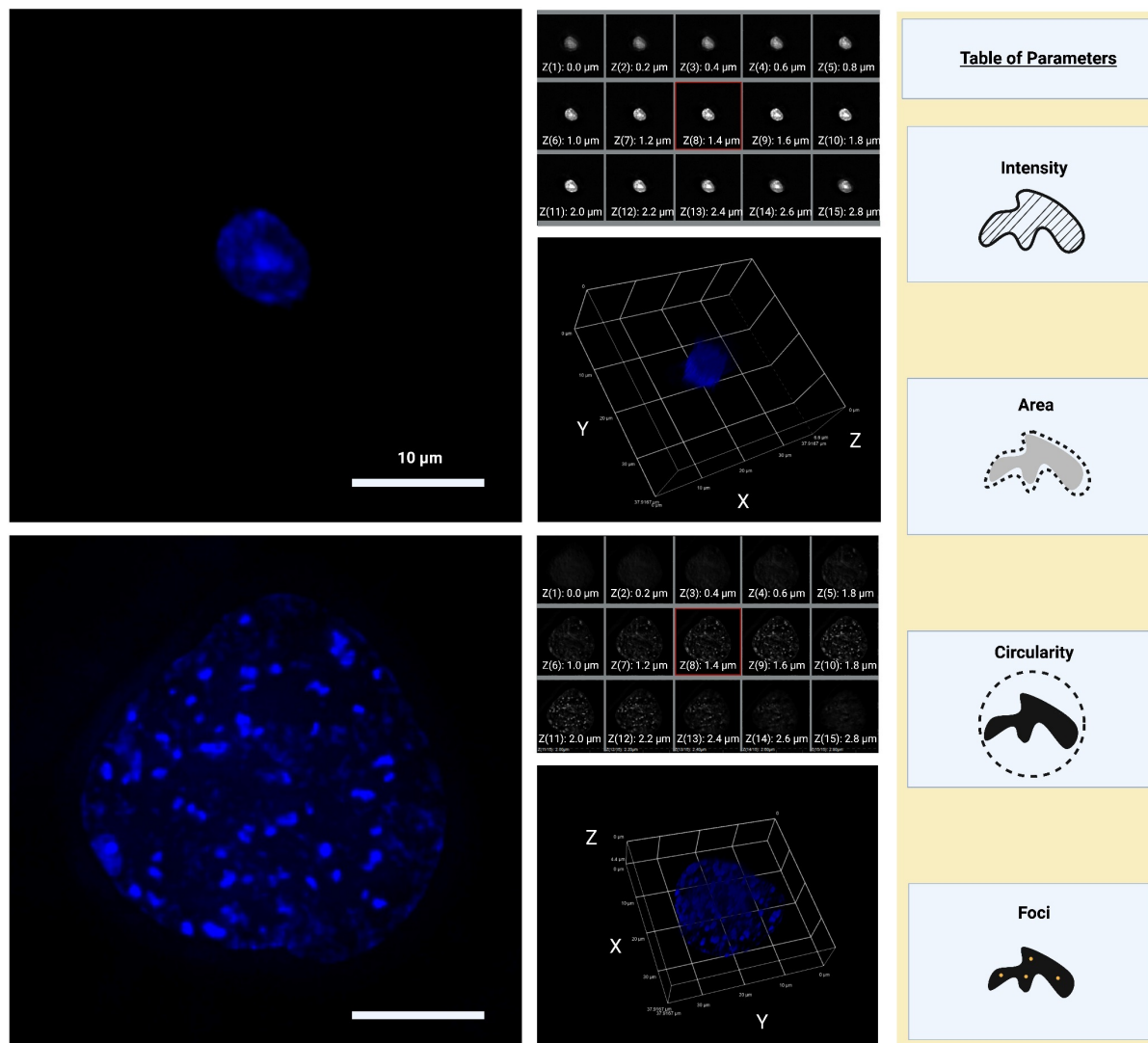
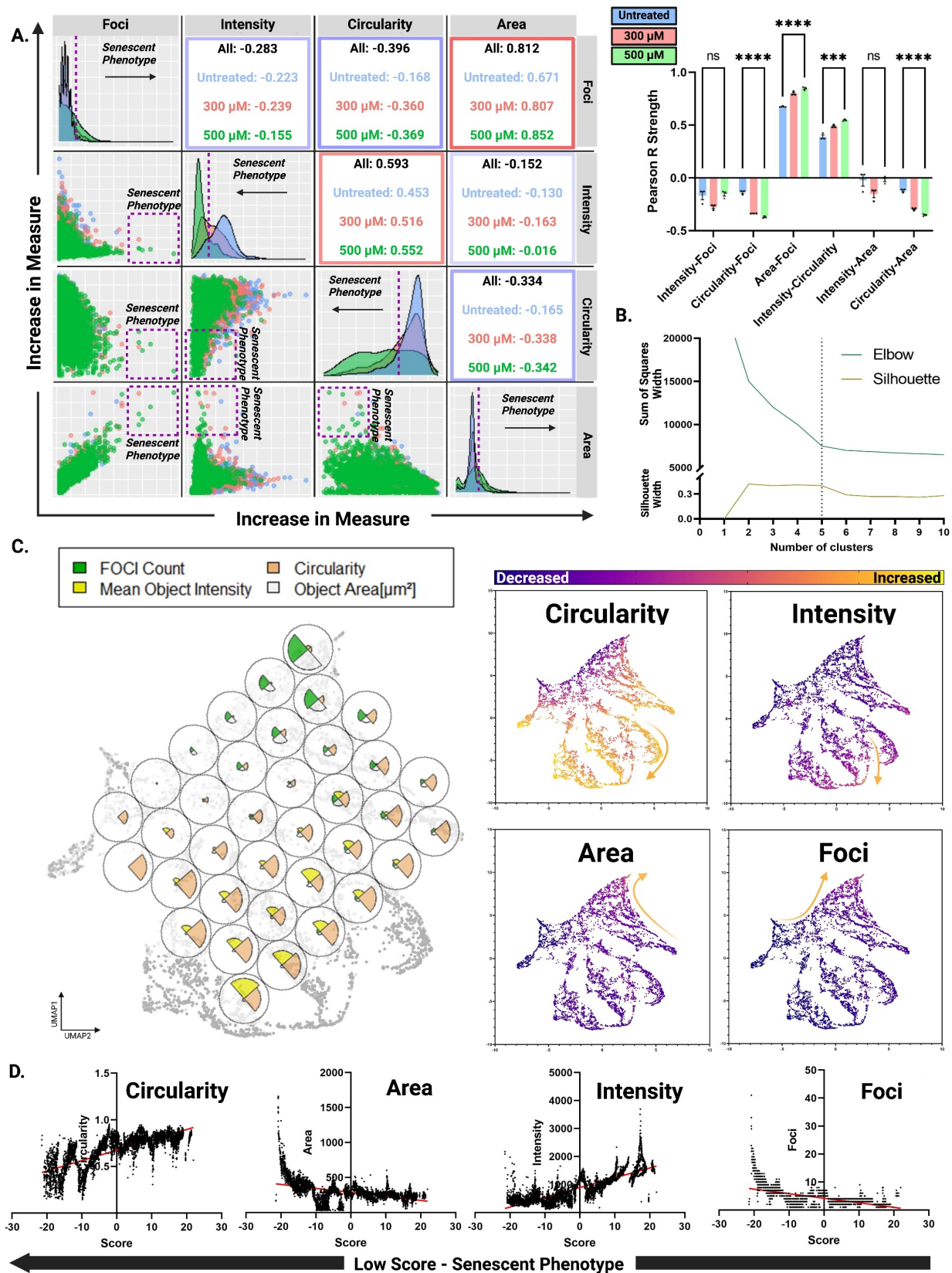


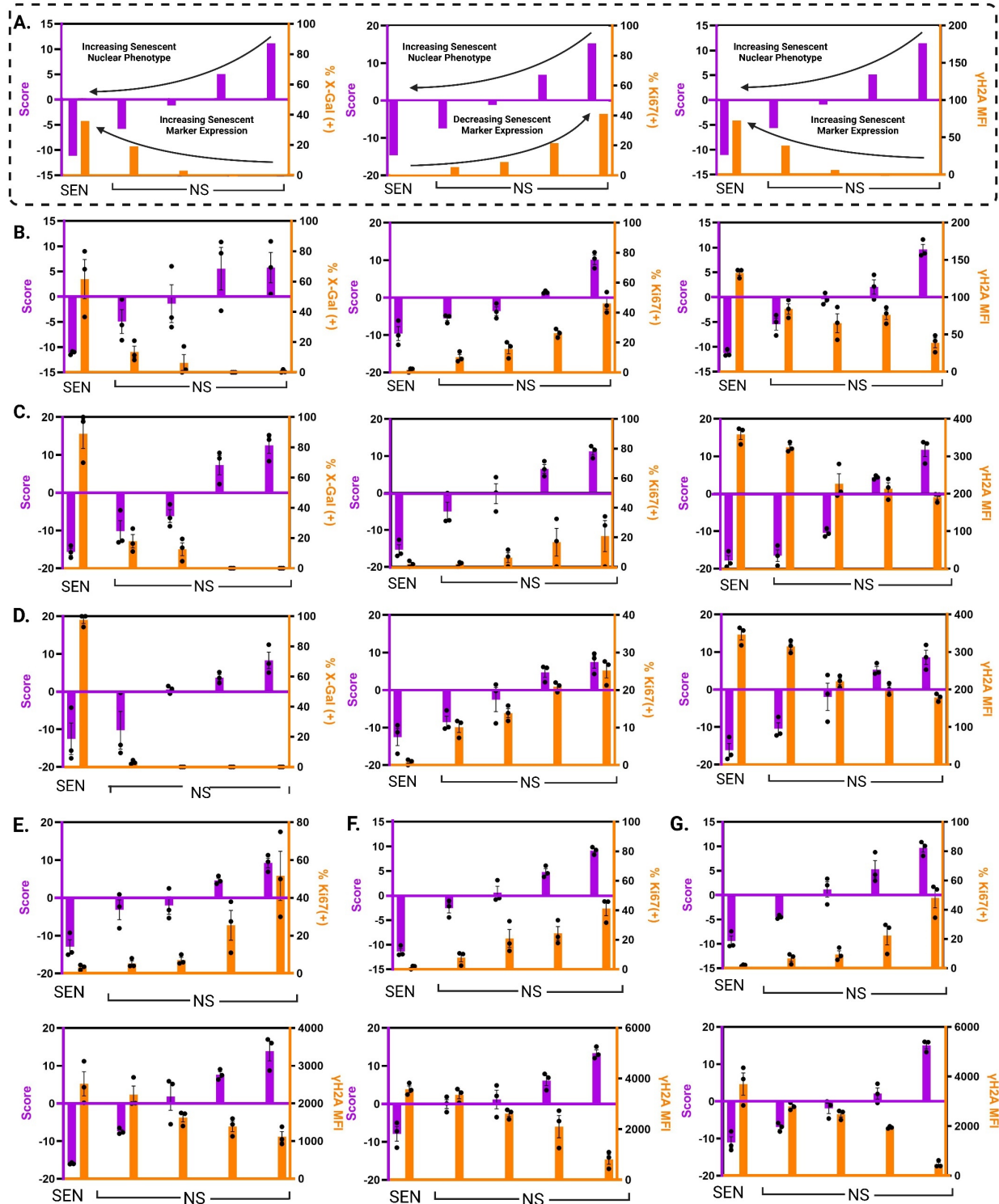


Supplementary Figure 1: Representative high-magnification images of DAPI stained H_2O_2 treated C2C12 nuclei from non-SnCs and SnCs. Top – non-SnC, Bottom – SnC. Large nuclear images are at the level noted by a red box in Z-stack images that are shown in the black-and-white panels. Volumetric representations of nuclei for each cell type are shown in the 3D panels. Variable LUTs were needed for clarity. Scale bars – 10 μm . Table of parameters (right) diagram the 4 nuclear morphometrics used in the NMP (Created in BioRender. Mapkar, S. (2025) <https://BioRender.com/qmm2ysb>).



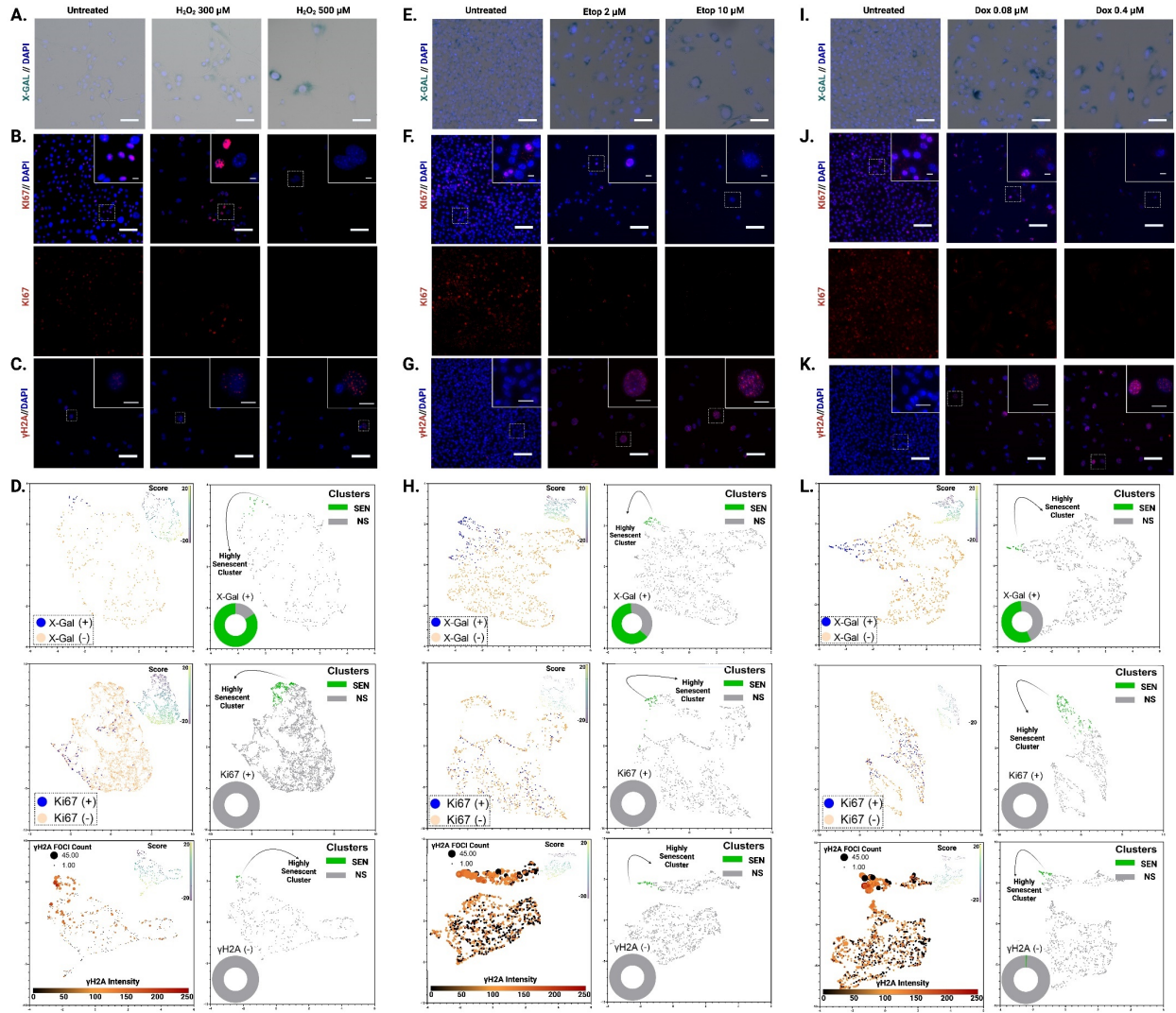
Supplementary Figure 2: Mechanism in which the NMP clusters and scores nuclei using nuclear morphometrics. Data is a revisualization of Figures 2A, B. A. Expanded correlogram visualizes

histograms of each parameter to dictate a shift of phenotypes found in senescence with increasing H₂O₂ treatments. Two-way interaction between parameters using scatterplot shows increased density with senescent phenotype and increasing H₂O₂ treatments. Quantifications use Pearson R coefficients to demonstrate strength of interaction between parameters. B. Clustering methods include Elbow and Silhouette plots, each of which resolves to 5 clusters. C. Visualization of how UMAPs colocalize measured parameters related to senescent phenotype. Larger areas within pie charts (left) and yellow arrows (right) indicate greater measures. D. Line graphs visualize the strength of score vs. each parameter, demonstrating that a low score correlates with the senescent phenotype. Error bars represent the mean $\pm$ standard error of the mean (SEM) and statistical tests were conducted using unpaired two-sided t-tests with no adjustments. ***p<0.001, ****p<0.0001. Source data are provided as a Source Data file which also includes all precise p-values.

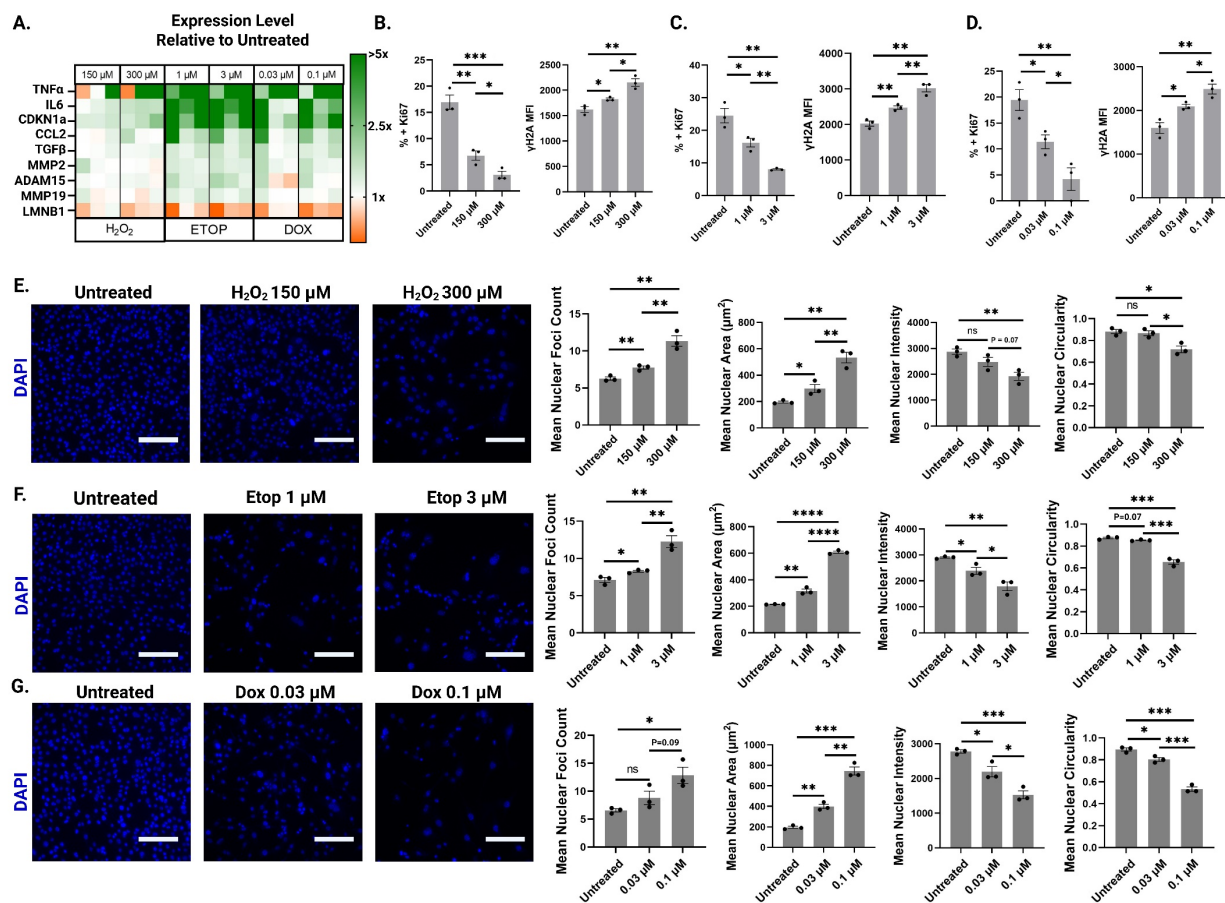


Supplementary Figure 3: Scores for NMP clusters correlate with senescent markers. (A) Demonstrates the theoretical relation between nuclear morphological associated score (purple) and an associated senescent marker (orange). Data is a revisualization of C2C12s treated with (B) H₂O₂-Figure 2D, (C) Etoposide-Figure 3E, (D) Doxorubicin-Figure 3F and 3T31Ls treated with (E) H₂O₂-Supplementary Figure 6A, (F) Etoposide-Supplementary Figure 6B, (G) Doxorubicin-Supplementary Figure 6C. Bar graphs show clusters defined by the NMP (x-axis)

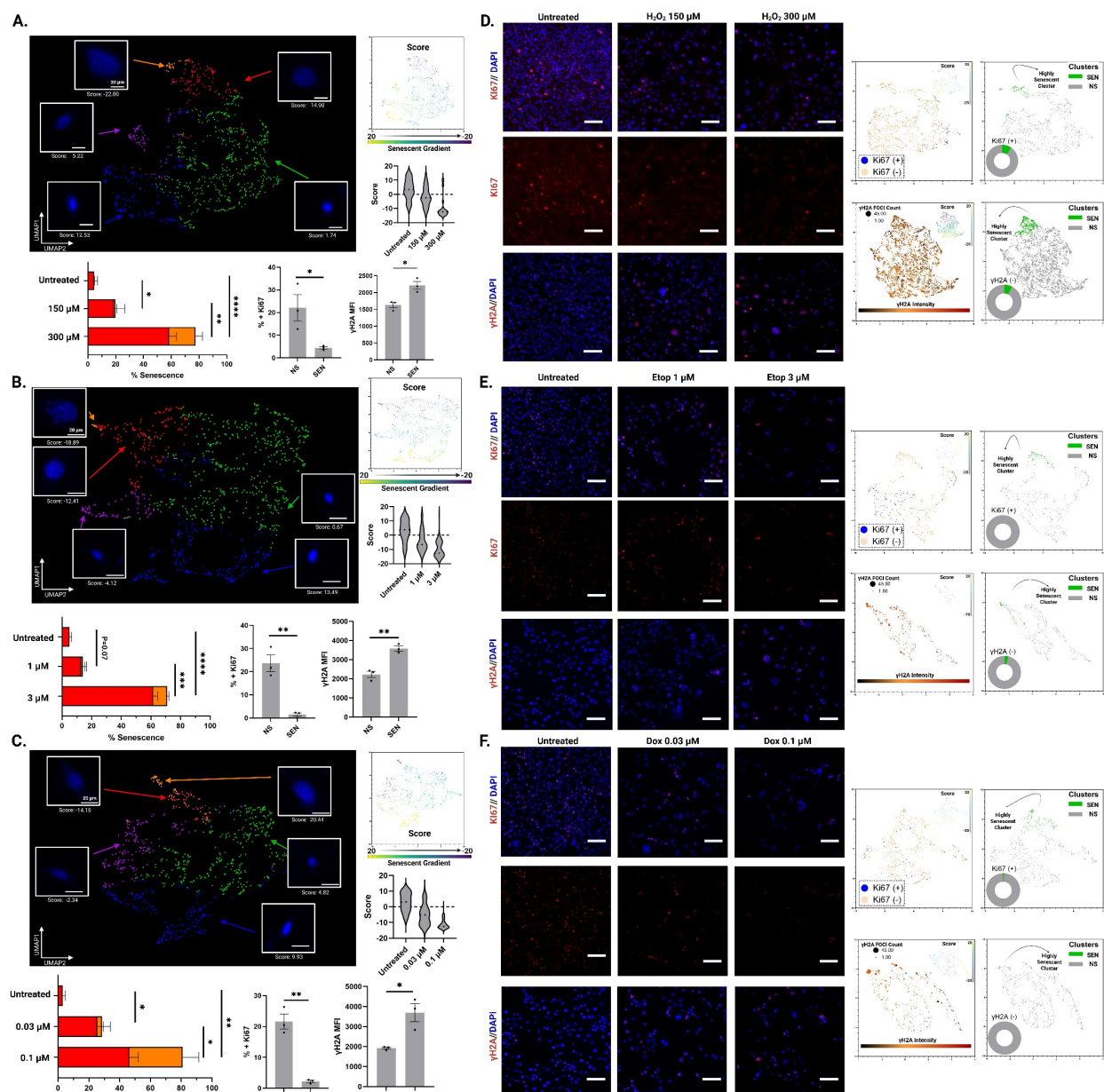
with the % stain of each noted marker (left y-axis) and the NMP-defined score (right y-axis). Source data are provided as a Source Data file.



Supplementary Figure 4: Visualization of nuclear phenotypes after in vitro senescence induction in C2C12 cells. A-C, E-G, and I-K. Representative images demonstrating senescence conversion following treatment with (A–C) H_2O_2 , (E–G) etoposide - Etop, or (I–K) doxorubicin - Dox. Scale bars – 100 μm, inset scale bars – 10 μm. All image Look-Up Tables (LUTs) and exposure times are equivalent between treatment groups. DAPI is less intense with increasing treatment due to the induction of the senescence phenotype. D, H, and L. Representative UMAPs of (D) H_2O_2 , (H) etoposide, and (L) doxorubicin-treated C2C12s, correlated with staining to confirm SnC clusters are genuinely senescent (SnCs = X-Gal+, Ki-67-, γH2A+). NS – non-senescent; SEN – senescent. Source data are provided as a Source Data file.

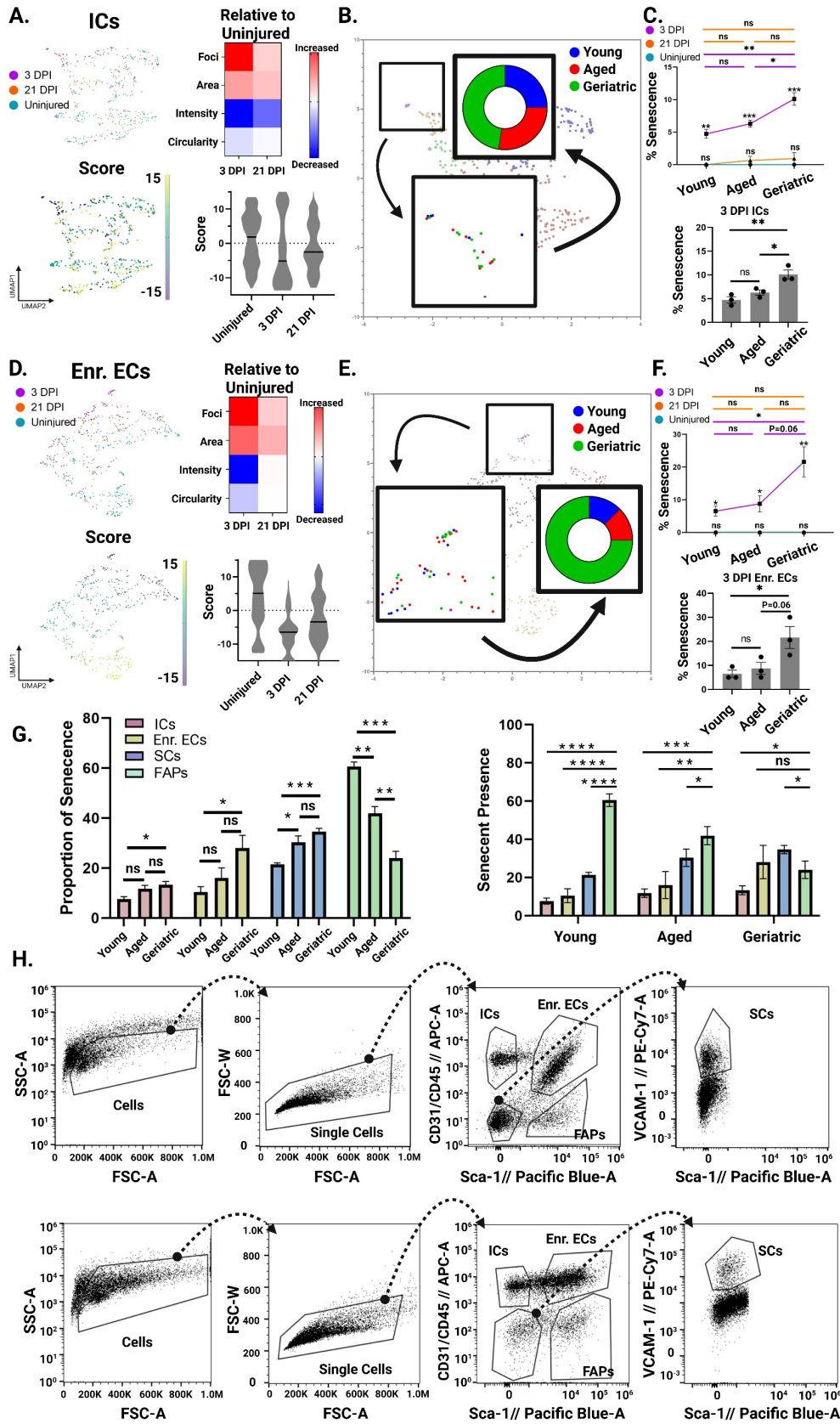


Supplementary Figure 5: The NMP effectively defines SnCs in 3T3-L1 preadipocytes treated with H₂O₂, etoposide, or doxorubicin. A. SASP assessment by qRT-PCR for 3T3-L1s treated with the noted senescence inducer. White indicates equivalency to untreated. n=3 independent experimental replicates per condition, where each column represents one replicate. B-D. Quantification of senescent phenotype markers in 3T3-L1s treated with (B) H₂O₂, (C) etoposide - Etop, or (D) doxorubicin - Dox. Note the dose-dependent response to senescence inducers with decreasing Ki67 and increasing γH2A, phenotypic markers of SnCs. MFI – mean fluorescence intensity. n=3 independent experimental replicates per condition. E-G. Representative images and quantifications of nuclear morphometrics of nuclei treated with (E) H₂O₂, (F) etoposide, or (G) doxorubicin. Between inducers image Look-Up Tables (LUTs) and exposure times are equivalent. n=3 independent experimental replicates; scale bar – 200 μm. Error bars represent the mean ± standard error of the mean (SEM) and statistical tests were conducted using unpaired two-sided t-tests with no adjustments. *p≤0.05, **p<0.01, ***p<0.001, ****p<0.0001. Source data are provided as a Source Data file which also includes all precise p-values.

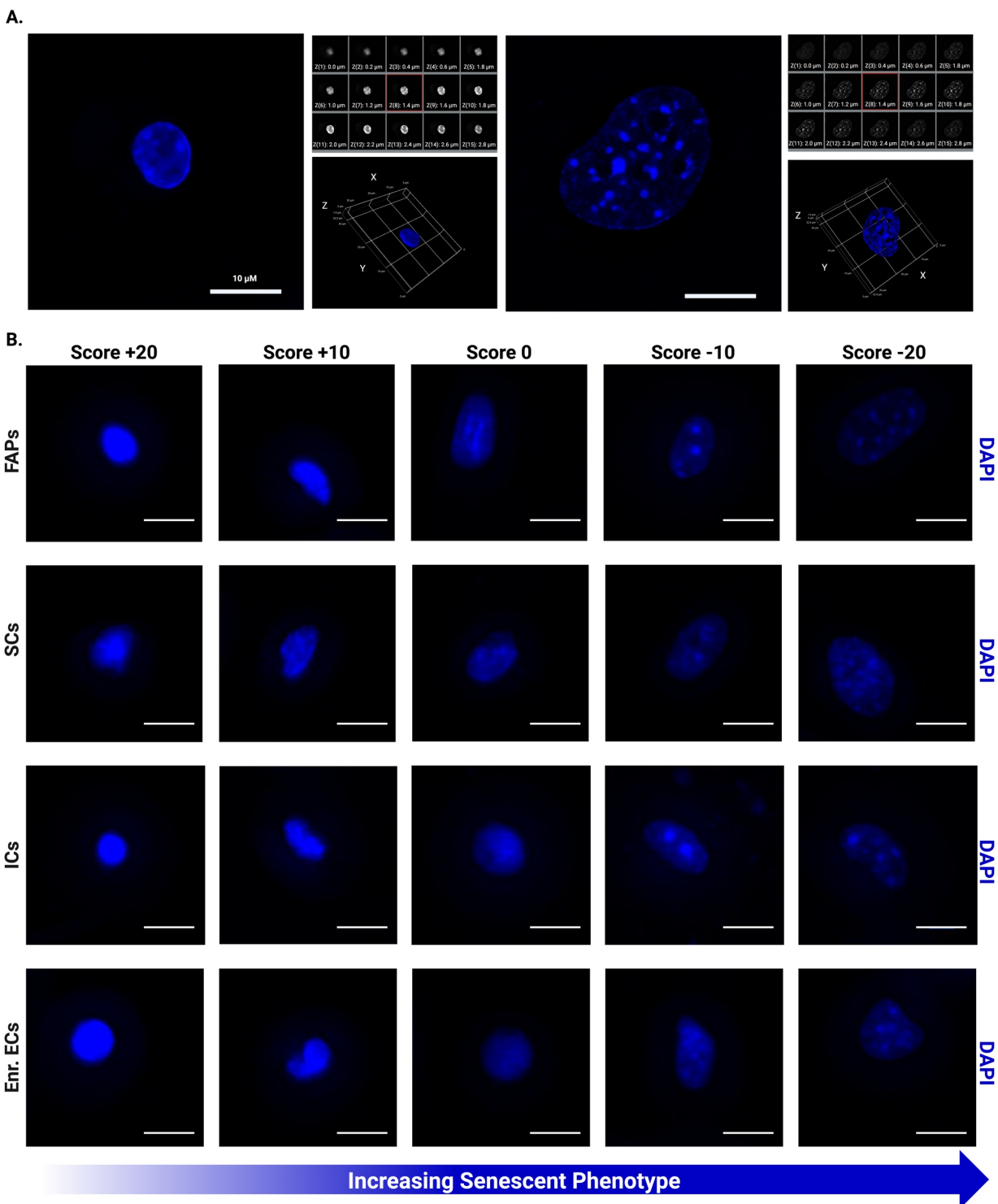


Supplementary Figure 6: Visualization of nuclear phenotypes after in vitro senescence induction in 3T3-L1 cells. A-C. Large UMAPs of reduced single-cell nuclear morphology with 5 groups resolved by the k-means clustering algorithm for 3T3-L1s treated (A) H_2O_2 , (B) etoposide, or (C) doxorubicin. Orange and red clusters exhibit a strong senescence phenotype and are quantified with corresponding colors in the horizontal bar graphs. $n=3$ independent experimental replicates. Inserts are representative nuclei from each cluster. Scale bars – 20 μm . MFI – mean fluorescence intensity. Further reduction creates a one-dimensional senescent “score” correlating with nuclear phenotype (small UMAP) and treatment condition (violin plots). Bar graphs quantify the noted marker in non-SnCs (NS) and SnCs (SEN). $n=3$ independent experimental replicates. D-F. Representative images of cells treated with (D) H_2O_2 , (E) etoposide - Etop or (F) doxorubicin - Dox and stained for senescence associated markers. Scale bars – 200 μm . Between inducers image Look-Up Tables (LUTs) and exposure times are equivalent. Corresponding UMAPs confirm expected staining for SnC clusters (SnCs = Ki-67-,

γ H2A+) identified by the NMP. NS – non-senescent; SEN – senescent. Error bars represent the mean $\pm$ standard error of the mean (SEM) and statistical tests were conducted using unpaired two-sided t-tests with no adjustments. * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Source data are provided as a Source Data file which also includes all precise p-values.

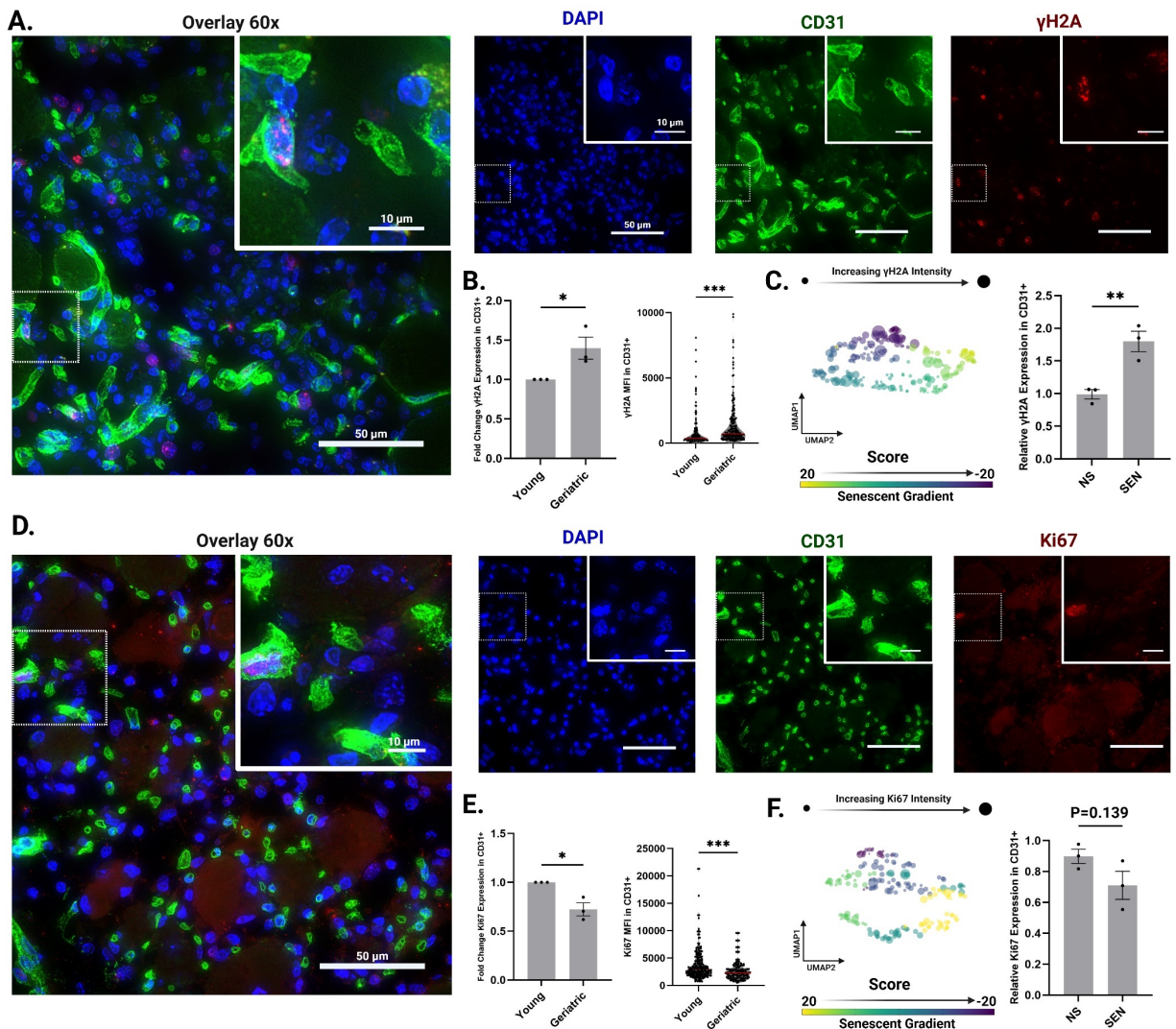


Supplementary Figure 7: The NMP identifies a dynamic state of senescence among the cell populations of regenerating SkM: A–C: Immune Cells – ICs, D–G: Enriched Endothelial Cells – Enr-ECs. A. and D. Representative UMAPs reducing nuclear phenotypes for the noted experimental conditions and the NMP-derived senescent score. Representative associated heat map displays phenotypic differences at noted days post injury (DPI). Representative associated violin plot visualizes differences in score. B. and E. Representative UMAPs to demonstrate the identification of senescence. C. and F. Quantification by % cells detected as senescent in different time points and age conditions. n=3 independent biological replicates. G. Quantification of senescence relative to FAPs and quantification of proportion of senescence by cell population between age groups. Fibroadipogenic progenitors – FAPs, Satellite Cells – SCs. H. Representative FACS plots demonstrating gating strategy to isolate cell populations; top row is from uninjured SkM while bottom row is from injured. Error bars represent the mean $\pm$ standard error of the mean (SEM) and statistical tests were conducted using unpaired two-sided t-tests with no adjustments. * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Source data are provided as a Source Data file which also includes all precise p-values.

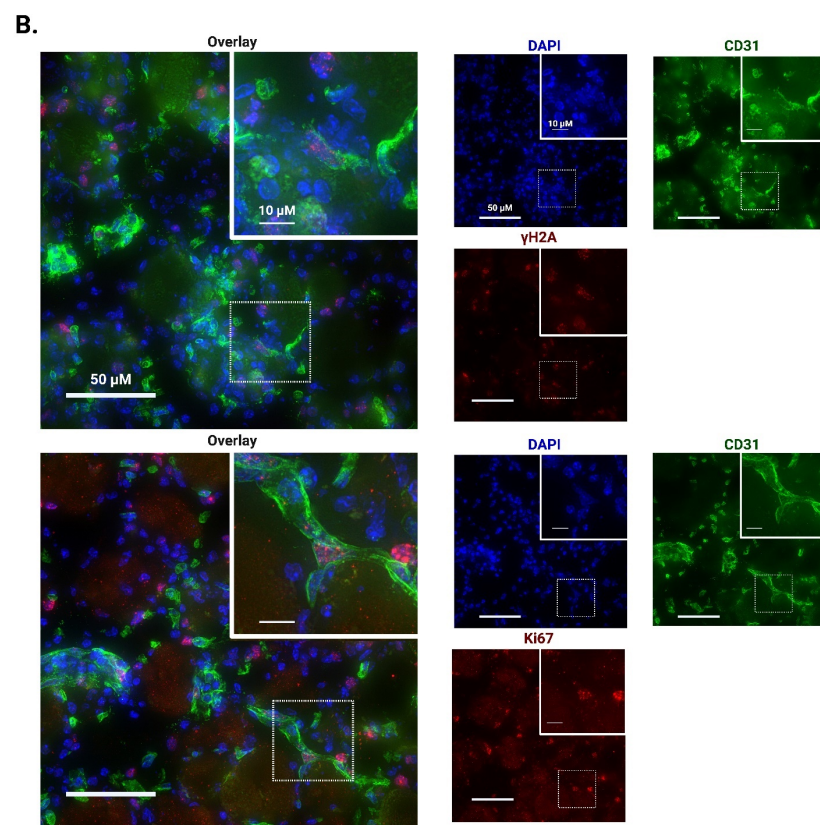
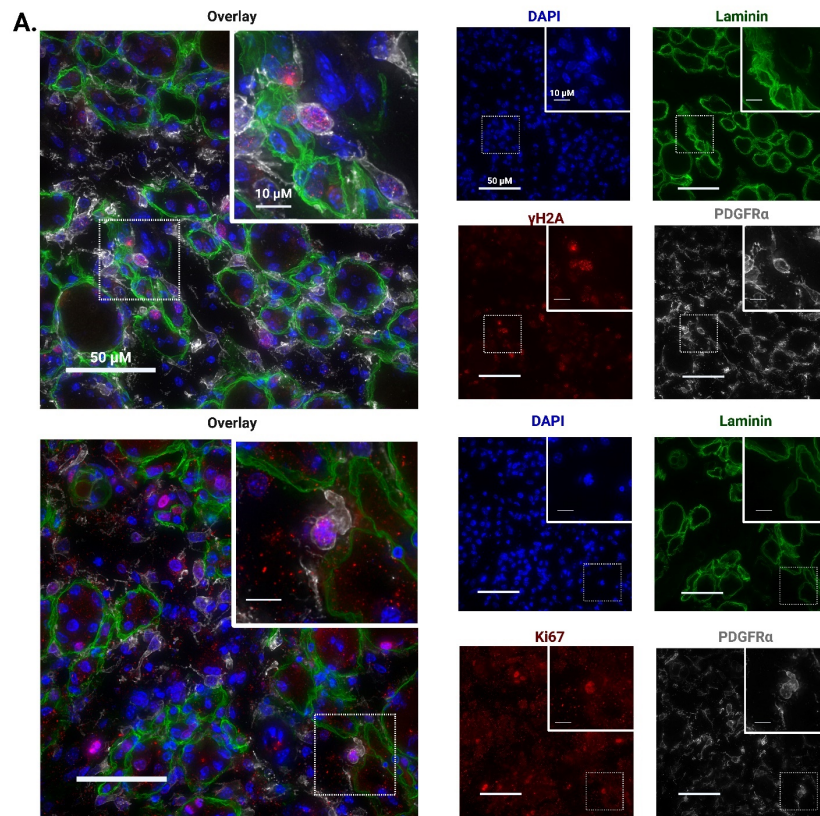


Supplementary Figure 8: Visualization of sorted primary cells and their associated senescence score. A. Representative (60x) high-magnification images of DAPI stained nuclei from non-senescent (Left) and senescent (Right) FAPs of 3 DPI tissue. Red boxed Z-stack image shown in the black-and-white panels is displayed as large nuclear image. Volumetric representations are shown in the 3D panels. Scale bars – 10 µm. Image Look-Up Tables (LUTs) are variable to achieve clarity. B. Representative images of DAPI stained nuclei of the noted cell types labeled

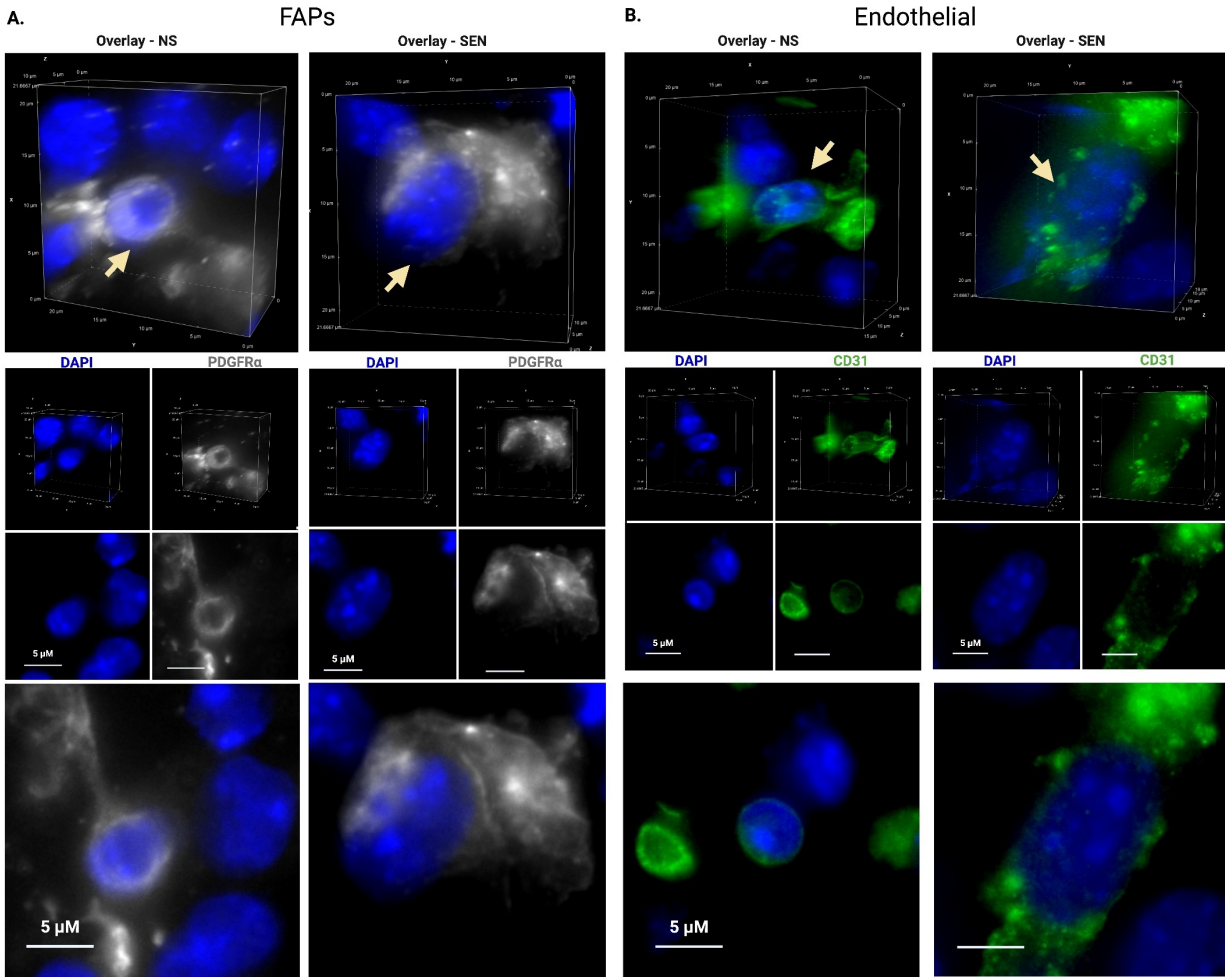
with senescence score generated by the NMP. LUTs and exposure times are equivalent for these images. Scale bars – 10µm.



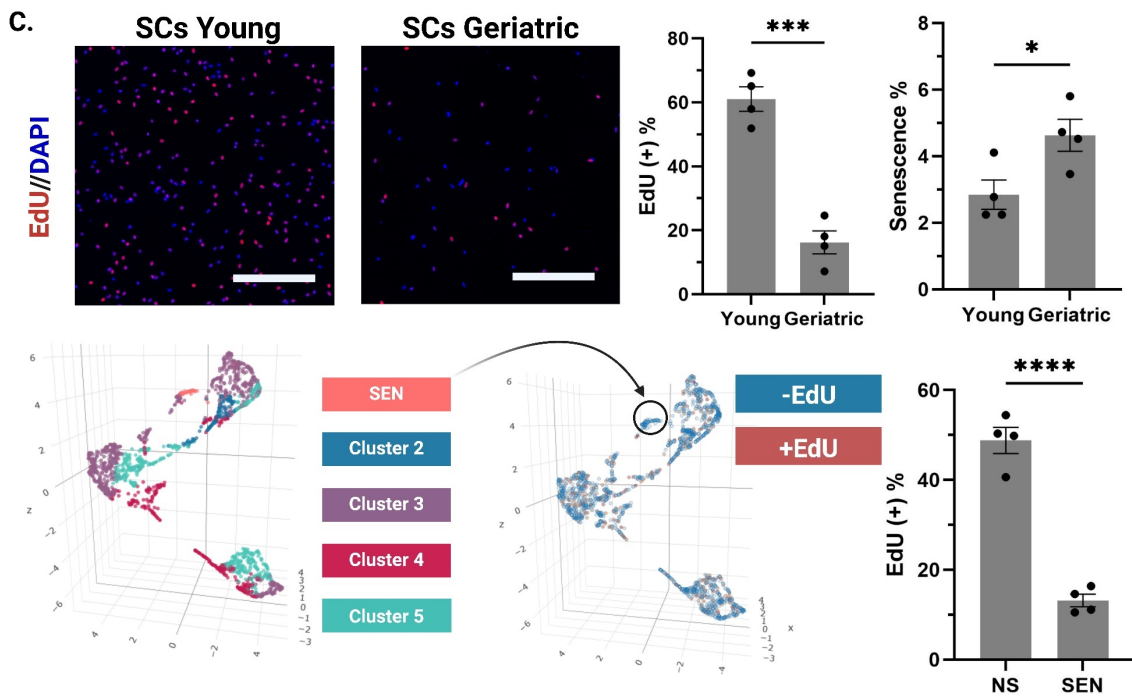
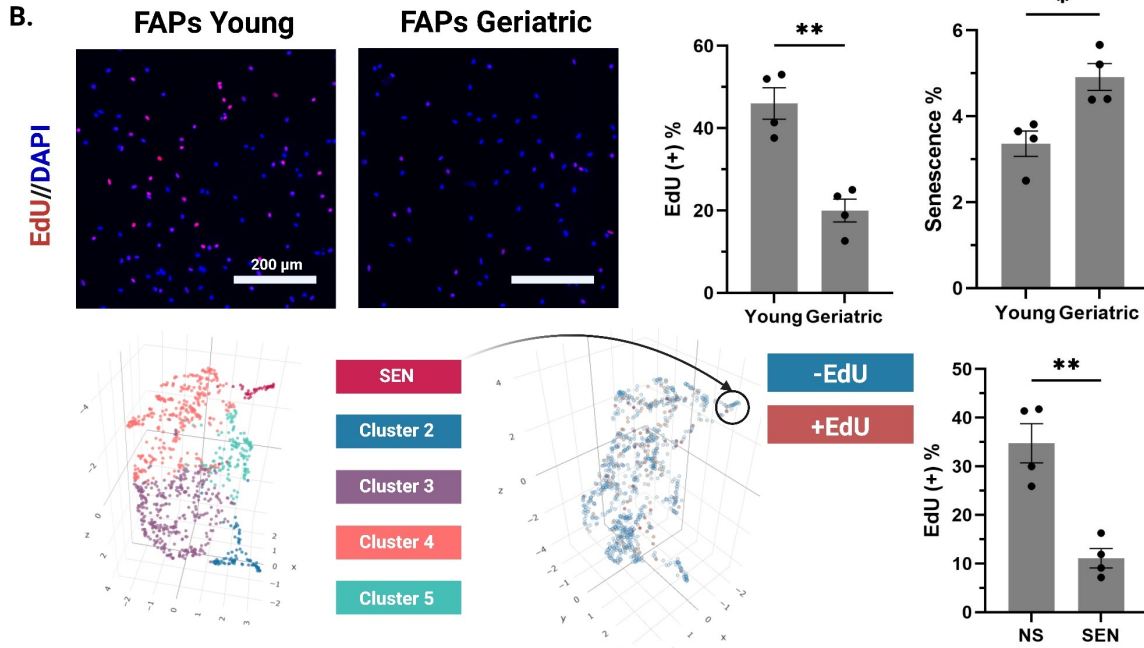
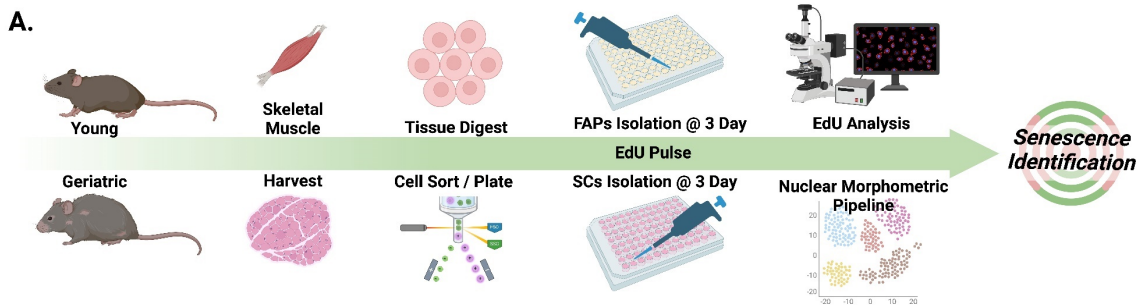
Supplementary Figure 9: The NMP identifies endothelial cells (ECs) with senescent characteristics in vivo in the regenerating muscles of young and geriatric mice. A, D. Representative images of geriatric SkM cross-sections identifying CD31+ endothelial cells, (A) γ H2A, (D) Ki67, and DAPI to quantify nuclear morphology. B, E. Quantification of (B) γ H2A+ damaged or (E) Ki67+ proliferating endothelial cells relative to young. Violin plots quantify the noted marker per single cell (each dot). MFI – mean fluorescence intensity. n=3 biological independent replicates per age. C, F. UMAPs showing the clustering of ECs by the NMP with senescent scores indicated by color (lower scores are highly senescent cells), and expression level of the noted marker by the size of the circle. n=3 biological independent replicates per age. Bar graphs quantify the amount of the noted marker in the non-SnC (NS) or SnCs (SEN). Scale bars – 50 μ m, inset scale bars – 10 μ m. Error bars represent the mean $\pm$ standard error of the mean (SEM) and statistical tests were conducted using unpaired two-sided t-tests with no adjustments. * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$. Source data are provided as a Source Data file which also includes all precise p-values.



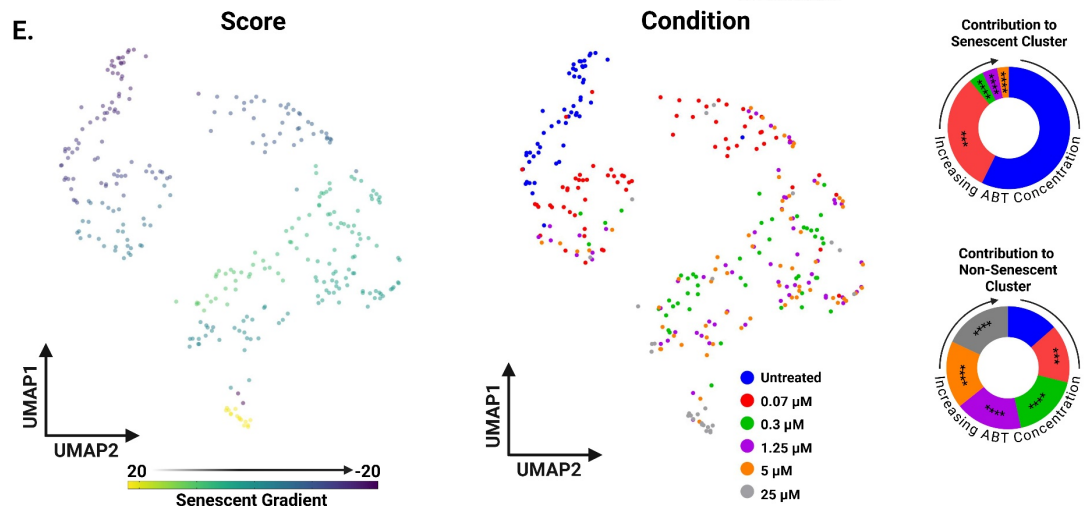
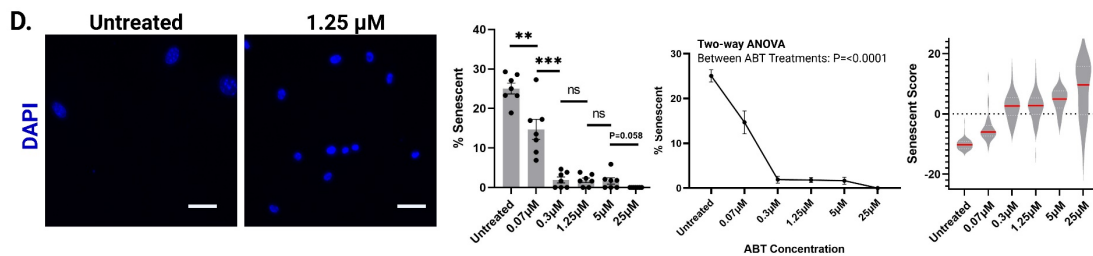
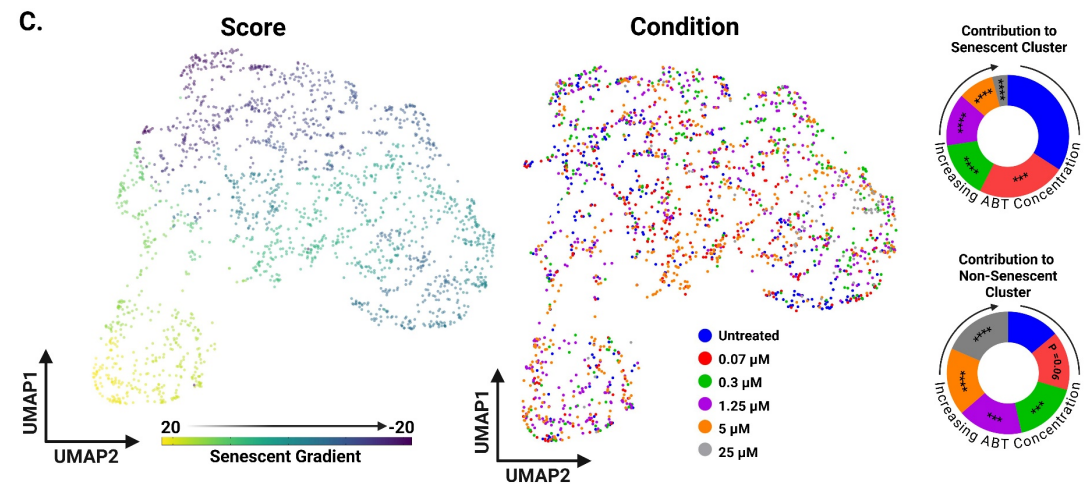
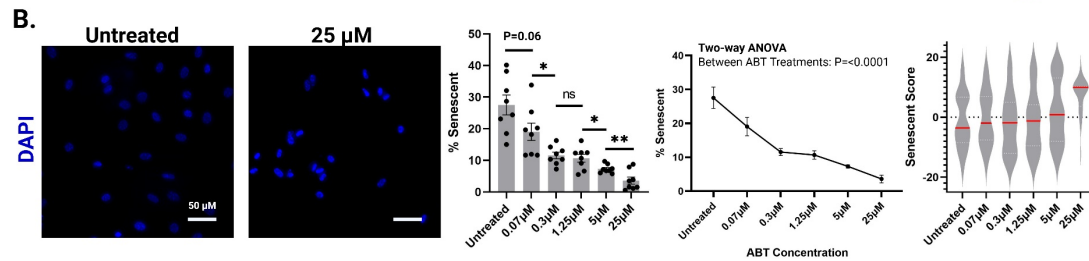
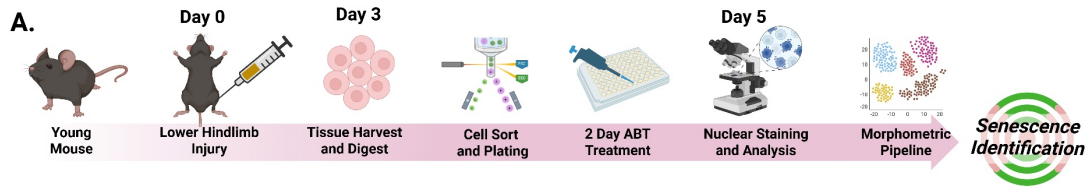
Supplementary Figure 10: The NMP identifies FAPs and endothelial cells (ECs) with senescent characteristics in vivo in young regenerating muscles. Representative images of young skeletal muscle (SkM) cross-sections identifying (A) PDGFR α + FAPs and Laminin+ encircled muscle fibers, or (B) CD31+ endothelial cells, stained with (top) γ H2A, or (bottom) Ki67. Scale bars – 50 μ m, inset scale bars – 10 μ m.



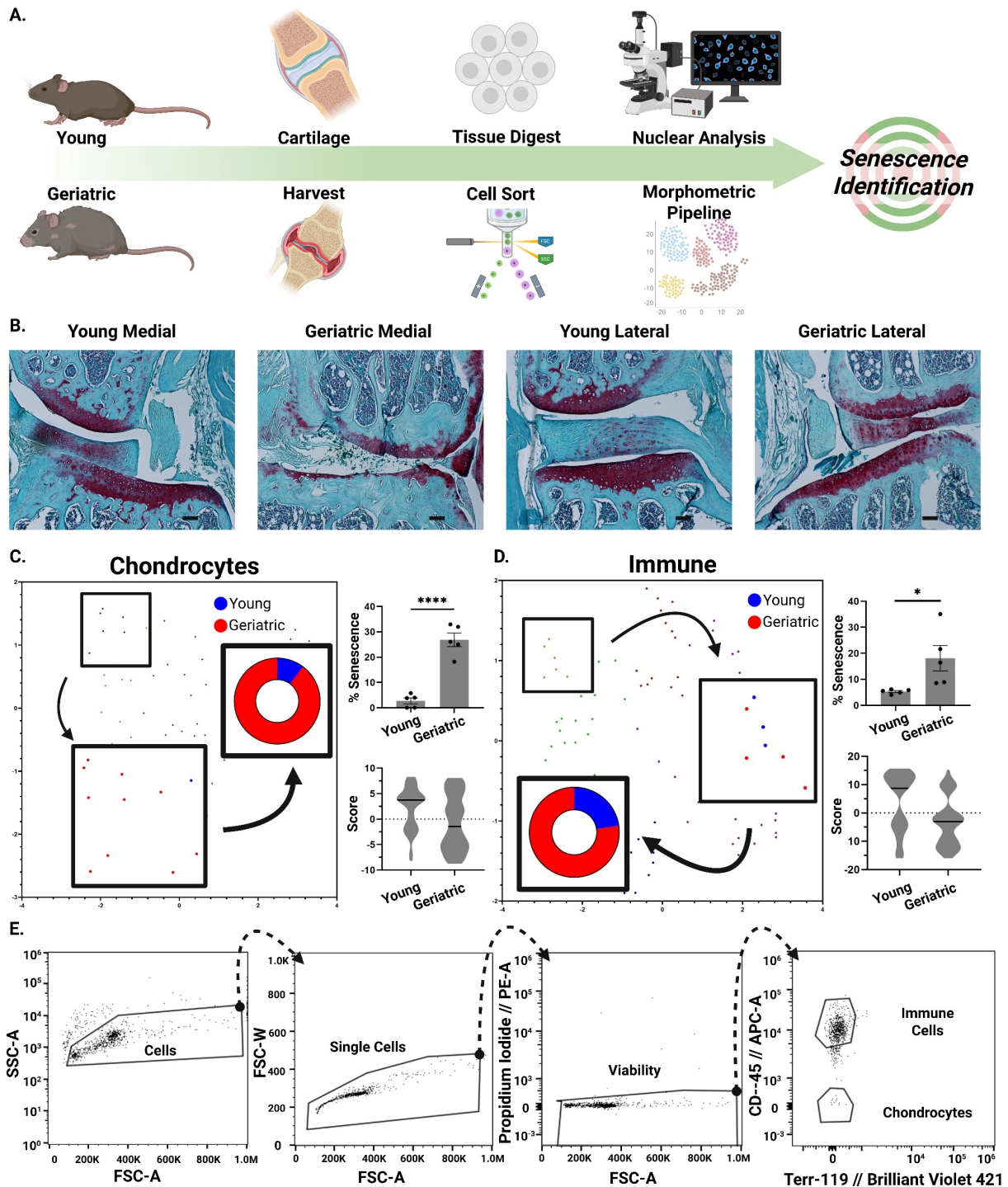
Supplementary Figure 11: In vivo visualization of nuclei at 3 DPI in SkM tissue determined to be senescent (SEN) and non-senescent (NS) by the NMP. (A) PDGFR α + FAPs and (B) CD31+ Endothelial cells. Top - Representative three-dimensional high-magnification images of DAPI stained nuclei from. Bottom – Single image from Z-stack three-dimensional images. Scale bars – 5 μ m. Image Look-Up Tables (LUTs) are variable to achieve clarity and exposure times are equivalent.



Supplementary Figure 12: The NMP effectively identifies SnCs from primary cells grown in culture. A. Schematic demonstrating the process of cell harvest to senescence identification (Created in BioRender. Mapkar, S. (2025) <https://BioRender.com/mj1i554>). B, C. Representative images of the noted cells, stained for EdU incorporation, with bar graphs quantifying differences between young and geriatric cells for proliferation (%EdU+), % SnCs as defined by the NMP, and %EdU+ in NMP-defined non-senescent cells (NS) and senescent cells (SEN). Image Look-Up Tables (LUTs) and exposure times are equivalent. Scale bars – 200 μ m. n=4 independent biological replicates per age. Representative UMAPs. Error bars represent the mean $\pm$ standard error of the mean (SEM) and statistical tests were conducted using unpaired two-sided t-tests with no adjustments. *p $\leq$ 0.05, **p<0.01, ***p<0.001, ****p<0.0001. Source data are provided as a Source Data file which also includes all precise p-values.

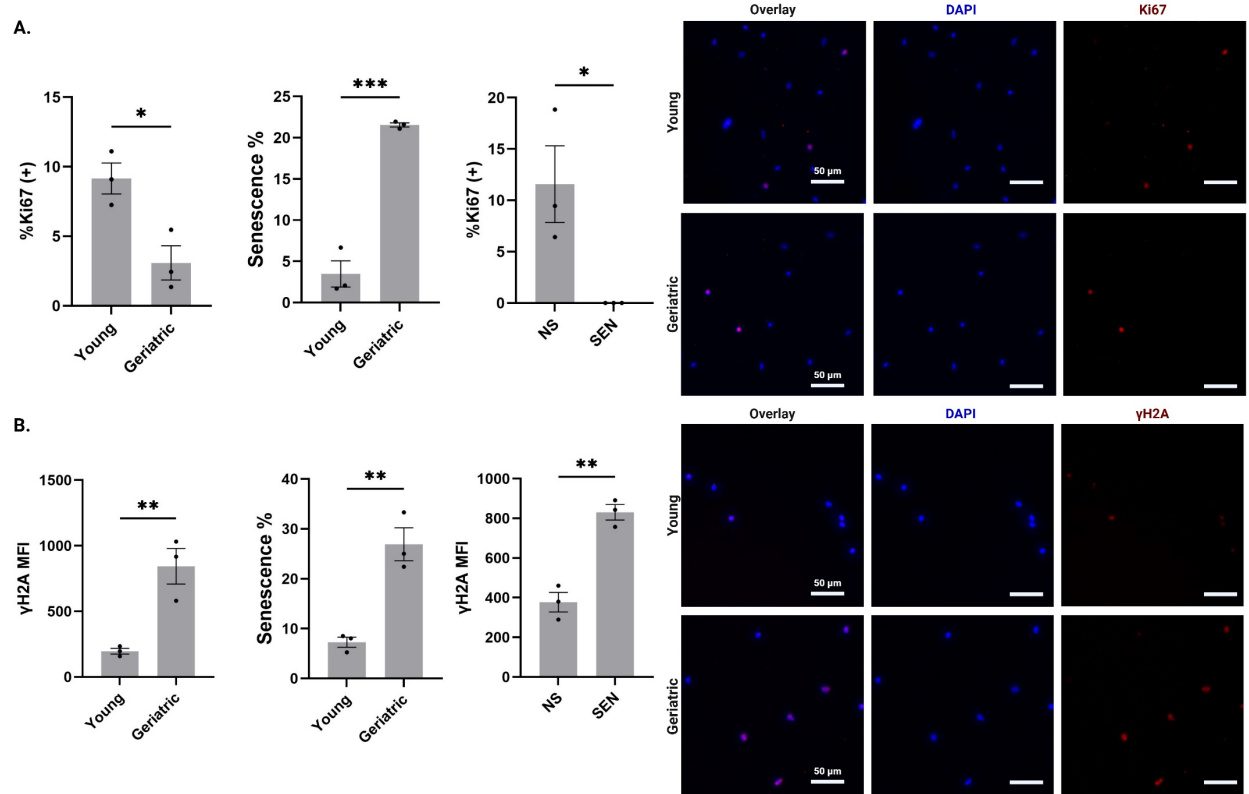


Supplementary Figure 13: Senolysis of SnCs in freshly sorted primary cells confirms the efficacy of the NMP in detecting genuine senescence. A. Schematic for the experimental approach, from cell harvest to senescence identification (Created in BioRender. Mapkar, S. (2025) <https://BioRender.com/vrb4qji>). B – FAPs, D – SCs. Representative images and quantification demonstrating dose response of senolytic treatment removing NMP-defined SnCs in sorted cells collected from injured muscle. Image Look-Up Tables (LUTs) and exposure times are equivalent. Violin graphs visualize the decrease in density of cells with low scores that reflect SnC presence. Scale bars – 50 μ m. B, $n = 8$ experimental replicates with cell input pulled from 2 mice to make 4 replicates, this was done twice; D, $n = 7$ experimental replicates with cell input pulled from 2 mice to make 4 replicates, and 2 mice to make 3 replicates. C – FAPs, E – SCs. Representative UMAPs with the senescence “score” derived from the NMP and nuclei noted per the treatment condition. Donut charts plot the relative contribution of each treatment group (by color) to the senescent or non-senescent clusters. Significance is relative to the untreated group. Error bars represent the mean $\pm$ standard error of the mean (SEM) and statistical tests were conducted using unpaired two-sided t-tests with no adjustments. * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Source data are provided as a Source Data file which also includes all precise p-values.

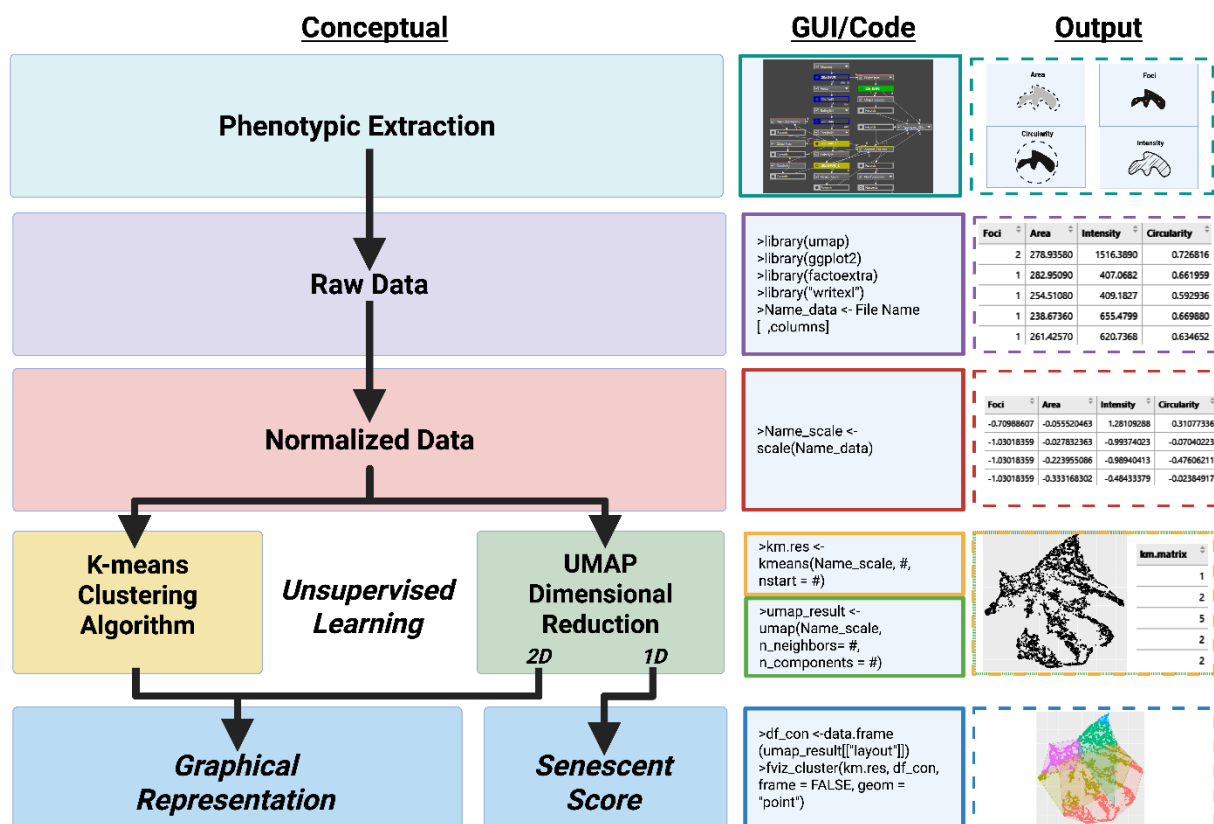


Supplementary Figure 14: The NMP successfully identifies SnCs in an osteoarthritic model. A. Schematic demonstrating the process of cartilage extraction to senescence identification (Created in BioRender. Mapkar, S. (2025) <https://BioRender.com/5gviyx6>). B. Representative histological images of Safranin O staining (SO, red area) to visualize arthritic changes in cartilage that occur with aging. Note the loss of SO staining in the geriatric samples. Scale bar – 200 μ m C. and D. Representative UMAPs of the noted cell populations from the NMP demonstrating identification of senescence. Violin plots reveal the differences in senescent

scores. Quantification by % cells detected as senescent in different ages. n=5 independent biological replicates per age. E. Representative FACS plots demonstrating gating for isolation of associated cell populations. Note, immune cells come from bone underlying the articular cartilage, but not bone marrow, since Ter119 staining is negative. Error bars represent the mean $\pm$ standard error of the mean (SEM) and statistical tests were conducted using unpaired two-sided t-tests with no adjustments. * $p \leq 0.05$, **** $p < 0.0001$. Source data are provided as a Source Data file which also includes all precise p-values.



Supplementary Figure 15: NMP-defined SnCs from cartilage have a senescent phenotype. Bar graphs quantifying differences between young and geriatric chondrocytes for (A) proliferation (%Ki67+) and (B) DNA damage (γH2A). % SnCs as defined by the NMP. % Ki67 or mean fluorescence intensity (MFI) of γH2A in NMP-defined non-senescent cells (NS) and senescent cells (SEN). Representative images of young and geriatric cells stained for (A) Ki67 or (B) γH2A. Image Look-Up Tables (LUTs) and exposure times are equivalent. Scale bars – 50 μm. n=3 independent biological replicates per age, each with 100-200 nuclei from chondrocytes quantified per condition. Error bars represent the mean ± standard error of the mean (SEM) and statistical tests were conducted using unpaired two-sided t-tests with no adjustments. *p<0.05, **p<0.01, ***p<0.001. Source data are provided as a Source Data file which also includes all precise p-values.



Supplementary Figure 16: The NMP integrates biological, computational, and graphical components to extract and classify nuclear morphologies associated with cellular senescence. Nuclear phenotypic features, including area, circularity, intensity, and foci, are extracted from images acquired using the Nikon AR system (light blue) yielding raw quantitative data imported into RStudio (purple). Following normalization (red), the dataset undergoes unsupervised learning through two parallel approaches: K-means clustering to identify discrete nuclear phenotypes (yellow), and Uniform Manifold Approximation and Projection (UMAP) for dimensional reduction (green). The 2D UMAP output facilitates graphical visualization of nuclear phenotypic space, while the 1D embedding provides a continuous “senescence score” reflecting nuclear deviation from non-senescent morphology (blue). Final outputs include graphical cluster representation and senescence scores enabling robust detection and quantification of senescent cells across diverse biological conditions. (Created in BioRender. Mapkar, S. (2025) <https://BioRender.com/mzmqzsjsj>).

Supplementary Table 1.
Primer Sequences for qPCR

GenBank Accession	PrimerBank ID	Primer Name	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
NM_011333	141803162c1	mCCL2	TAAAAACCTGGATCGGAACCAAA	GCATTAGCTTCAGATTACGGGT
NM_001111099	6671726a1	mCDKN1A	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
NM_013693	133892368c1	mTNF	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
NM_001037722	109148516c2	mADAM15	ATGGCACCCGAATGGTCAG	CTCCAGTGTATAGCCTCTCTCTG
NM_031168	13624310c1	mIL6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
NM_001164197	255958311c3	mMMP19	CCTGGTCCCATGCCAAACC	CCCTTGAAAGCATAAGTCTTCCC
NM_011577	6755774c1	mTGF β 1	CCACCTGCAAGACCATCGAC	CTGGCGAGCCTTAGTTTGAC
NM_010721	188219588c2	mLMNB1	GAGTATGAGGCGGCACTAAAC	CATCTGCTAACTGCTTTTGGC
NM_008610	NA	mMMP2	TGCAGGAGACAAGTTCTGGA	GACGGCATCCAGGTTATCAG
NM_008084	NA	mGAPDH	TCAAGAAGGTGGTGAAGCAG	GTTGAAGTCGCAGGAGACAA

Supplementary Table 2.
Main Body Figures p-Values

Figure // Panel	Condition	Comparison			P-Value
1B	X-Gal	Untreated	v	300µm	0.000688
1B	X-Gal	300µm	v	500µm	0.052366
1B	X-Gal	Untreated	v	500µm	0.001696
1B	γH2A	Untreated	v	300µm	0.009585
1B	γH2A	300µm	v	500µm	0.03013
1B	γH2A	Untreated	v	500µm	0.000563
1B	Ki67	Untreated	v	300µm	0.00622
1B	Ki67	300µm	v	500µm	0.00481
1B	Ki67	Untreated	v	500µm	0.000877
1B	Caspase	Untreated	v	300µm	0.325025
1B	Caspase	300µm	v	500µm	0.442187
1B	Caspase	Untreated	v	500µm	0.889914
1C	Spider-Gal	Untreated	v	300µm	0.02831
1C	Spider-Gal	300µm	v	500µm	0.054592
1C	Spider-Gal	Untreated	v	500µm	0.030466
1E	Foci	Untreated	v	300µm	0.027277
1E	Foci	300µm	v	500µm	0.005821
1E	Foci	Untreated	v	500µm	0.001825
1E	Area	Untreated	v	300µm	0.002788
1E	Area	300µm	v	500µm	0.018119
1E	Area	Untreated	v	500µm	0.000769
1E	Intensity	Untreated	v	300µm	2.38E-07
1E	Intensity	300µm	v	500µm	0.000222
1E	Intensity	Untreated	v	500µm	1.84E-05
1E	Circularity	Untreated	v	300µm	0.000126
1E	Circularity	300µm	v	500µm	0.001389
1E	Circularity	Untreated	v	500µm	0.000406
2C	Senescent %	Untreated	v	300µm	0.005439
2C	Senescent %	300µm	v	500µm	0.002679
2C	Senescent %	Untreated	v	500µm	4.83E-05
2D	X-Gal	NS	v	SEN	0.01191
2D	Ki67	NS	v	SEN	0.003782
2D	γH2A	NS	v	SEN	0.001133
3A	X-Gal	Untreated	v	2µm	0.002365
3A	X-Gal	2µm	v	10µm	0.002538
3A	X-Gal	Untreated	v	10µm	0.000584
3A	γH2A	Untreated	v	2µm	0.001449
3A	γH2A	2µm	v	10µm	0.000492
3A	γH2A	Untreated	v	10µm	0.00028
3A	Ki67	Untreated	v	2µm	0.004924
3A	Ki67	2µm	v	10µm	0.01475
3A	Ki67	Untreated	v	10µm	0.001506
3B	X-Gal	Untreated	v	0.08 µm	0.017562
3B	X-Gal	0.08 µm	v	0.4 µm	0.005774
3B	X-Gal	Untreated	v	0.4 µm	0.000128
3B	γH2A	Untreated	v	0.08µm	0.005597

3B	γH2A	0.08μm	v	0.4μm	0.181026
3B	γH2A	Untreated	v	0.4μm	0.000689
3B	Ki67	Untreated	v	0.08μm	0.001781
3B	Ki67	0.08μm	v	0.4μm	0.028094
3B	Ki67	Untreated	v	0.4μm	0.000433
3C	Foci	Untreated	v	2μm	0.016866
3C	Foci	2μm	v	10μm	0.06445
3C	Foci	Untreated	v	10μm	0.004778
3C	Area	Untreated	v	2μm	0.001634
3C	Area	2μm	v	10μm	0.003505
3C	Area	Untreated	v	10μm	0.000211
3C	Intensity	Untreated	v	2μm	3.46E-07
3C	Intensity	2μm	v	10μm	0.000573
3C	Intensity	Untreated	v	10μm	2.02E-08
3C	Circularity	Untreated	v	2μm	0.014949
3C	Circularity	2μm	v	10μm	0.000847
3C	Circularity	Untreated	v	10μm	0.000148
3D	Foci	Untreated	v	0.08 μm	9.61E-05
3D	Foci	0.08 μm	v	0.4 μm	0.000856
3D	Foci	Untreated	v	0.4 μm	2.18E-05
3D	Area	Untreated	v	0.08 μm	0.001689
3D	Area	0.08 μm	v	0.4 μm	0.00079
3D	Area	Untreated	v	0.4 μm	0.000158
3D	Intensity	Untreated	v	0.08 μm	3.5E-07
3D	Intensity	0.08 μm	v	0.4 μm	0.000981
3D	Intensity	Untreated	v	0.4 μm	1.72E-07
3D	Circularity	Untreated	v	0.08 μm	0.000172
3D	Circularity	0.08 μm	v	0.4 μm	0.00335
3D	Circularity	Untreated	v	0.4 μm	0.000131
3E	X-Gal	NS	v	SEN	0.00036
3E	Ki67	NS	v	SEN	3.81E-05
3E	γH2A	NS	v	SEN	0.003032
3E	Senescent %	Untreated	v	2μm	0.038847
3E	Senescent %	2μm	v	10μm	0.001916
3E	Senescent %	Untreated	v	10μm	0.000152
3F	X-Gal	NS	v	SEN	6.88E-05
3F	Ki67	NS	v	SEN	0.001453
3F	γH2A	NS	v	SEN	0.00737
3F	Senescent %	Untreated	v	0.08um	0.002625
3F	Senescent %	0.08um	v	0.4um	0.014978
3F	Senescent %	Untreated	v	0.4um	0.00058
4D	Young	Uninjured	v	3DPI	3.9E-06
4D	Young	Uninjured	v	21DPI	0.36828
4D	Aged	Uninjured	v	3DPI	2.34E-05
4D	Aged	Uninjured	v	21DPI	0.366969
4D	Geriatric	Uninjured	v	3DPI	0.001041
4D	Geriatric	Uninjured	v	21DPI	0.151178
4D	3DPI	Young	v	Aged	0.000522
4D	3DPI	Aged	v	Geriatric	0.159686

4D	3DPI	Young	v	Geriatric	0.001391
4G	Young	Uninjured	v	3DPI	2.82E-06
4G	Young	Uninjured	v	21DPI	0.125069
4G	Aged	Uninjured	v	3DPI	0.000266
4G	Aged	Uninjured	v	21DPI	0.117462
4G	Geriatric	Uninjured	v	3DPI	1.48E-05
4G	Geriatric	Uninjured	v	21DPI	0.074126
4G	3DPI	Young	v	Aged	0.089146
4G	3DPI	Aged	v	Geriatric	0.004067
4G	3DPI	Young	v	Geriatric	0.00028
5B	Ki67 (box)	Young	v	Geriatric	0.000462
5B	Ki67 (violin)	Young	v	Geriatric	0.033050
5C	Ki67	Sen	v	NS	0.00064
5E	γH2A (box)	Young	v	Geriatric	0.003642
5E	γH2A (violin)	Young	v	Geriatric	0.014191
5F	γH2A	Sen	v	NS	0.021021