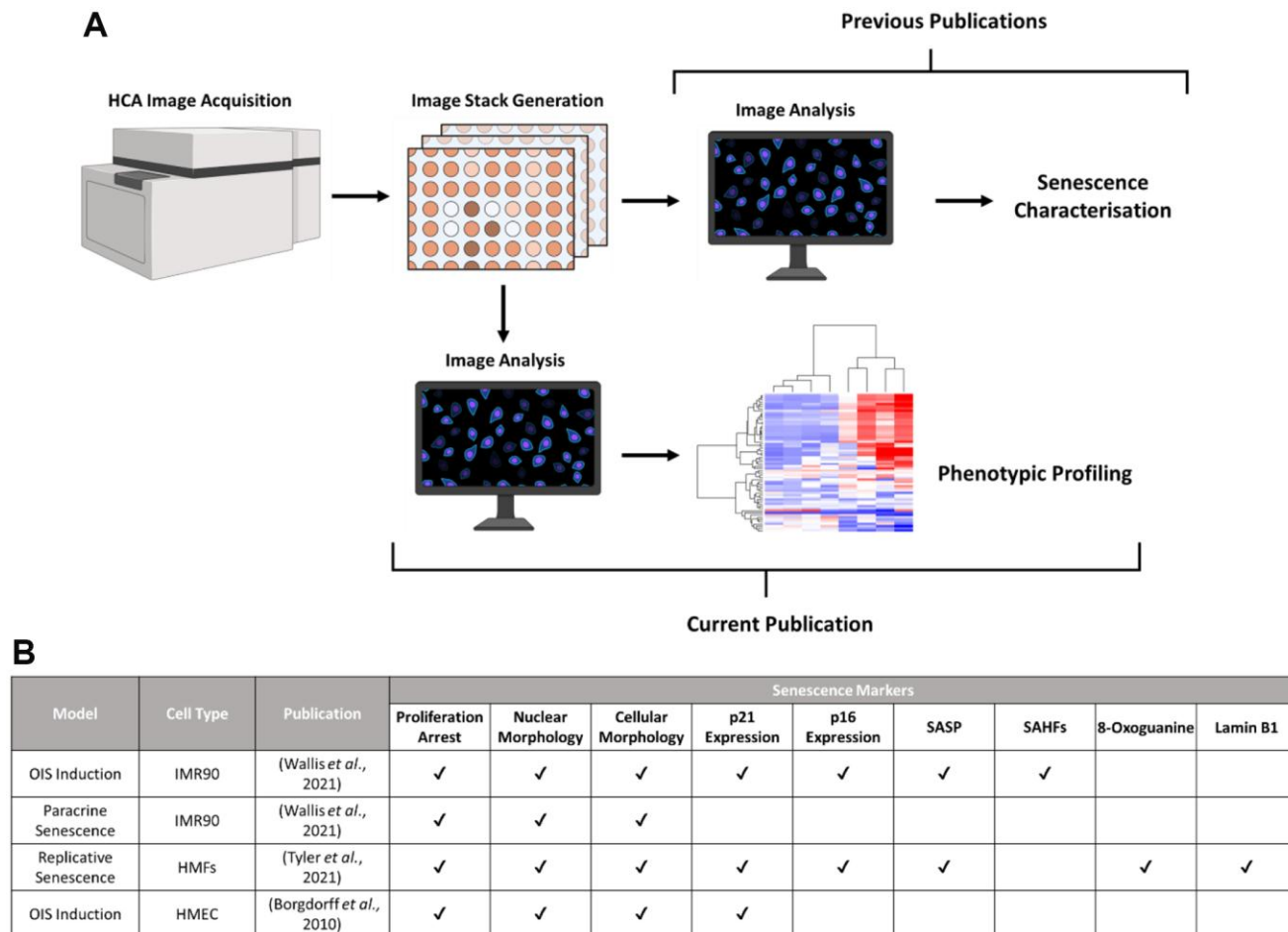
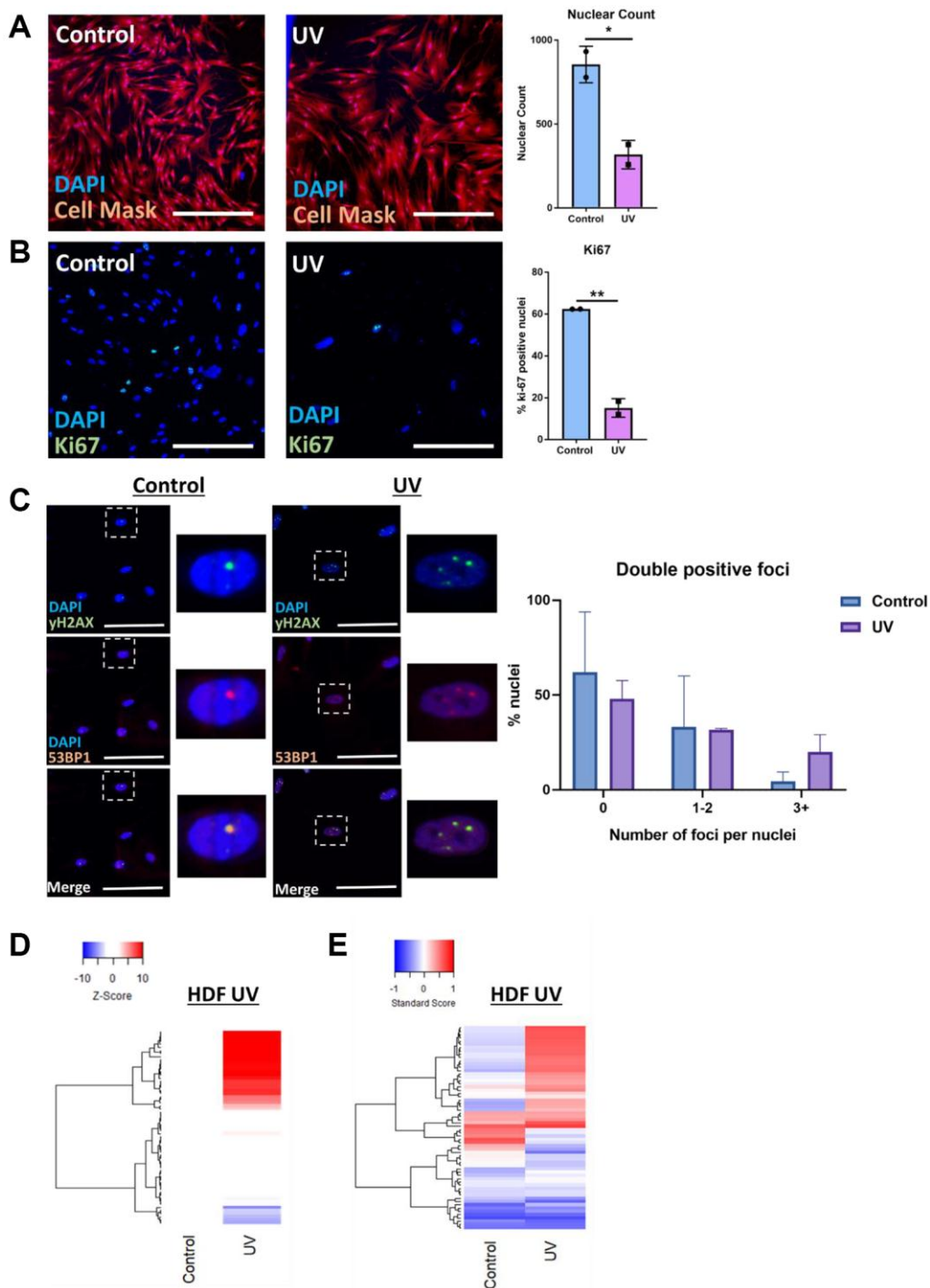


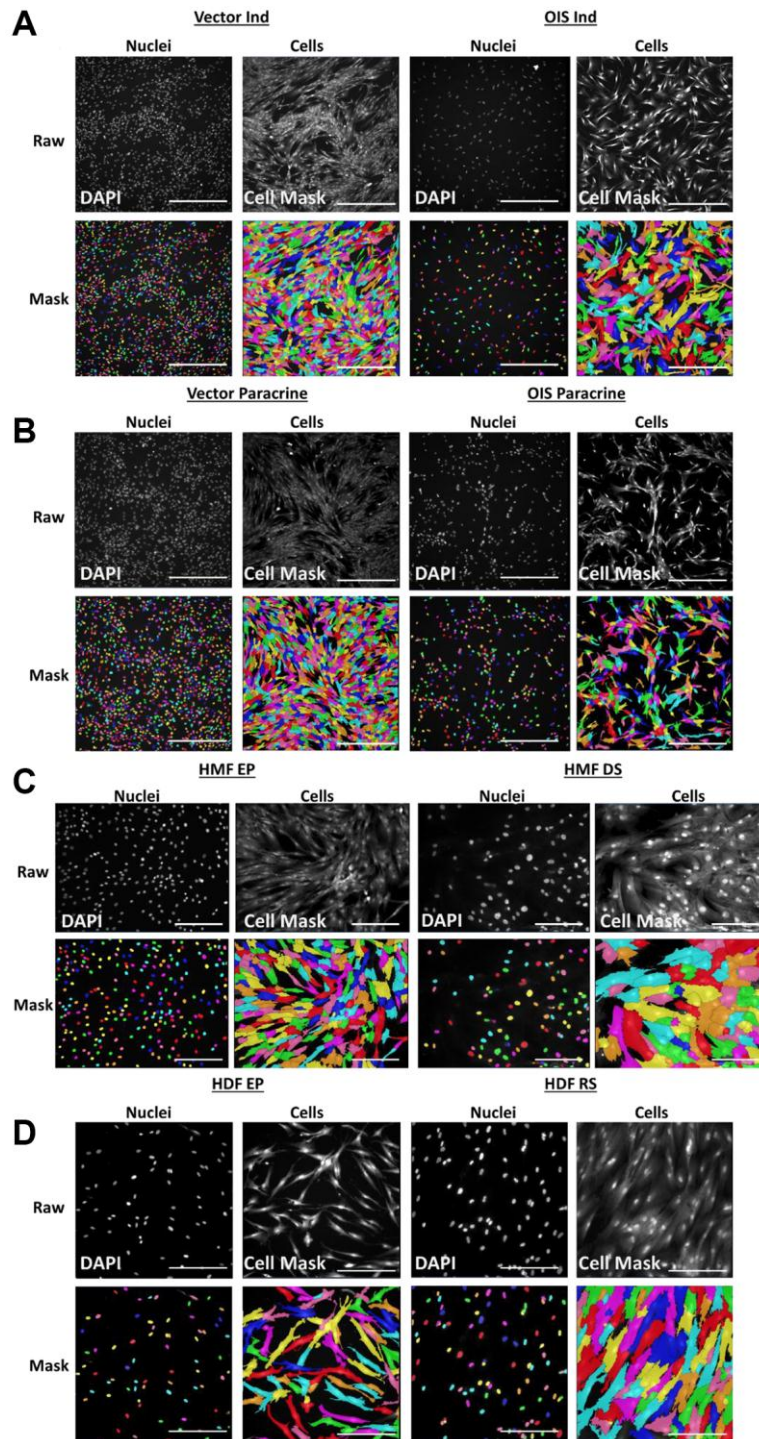
## SUPPLEMENTARY FIGURES



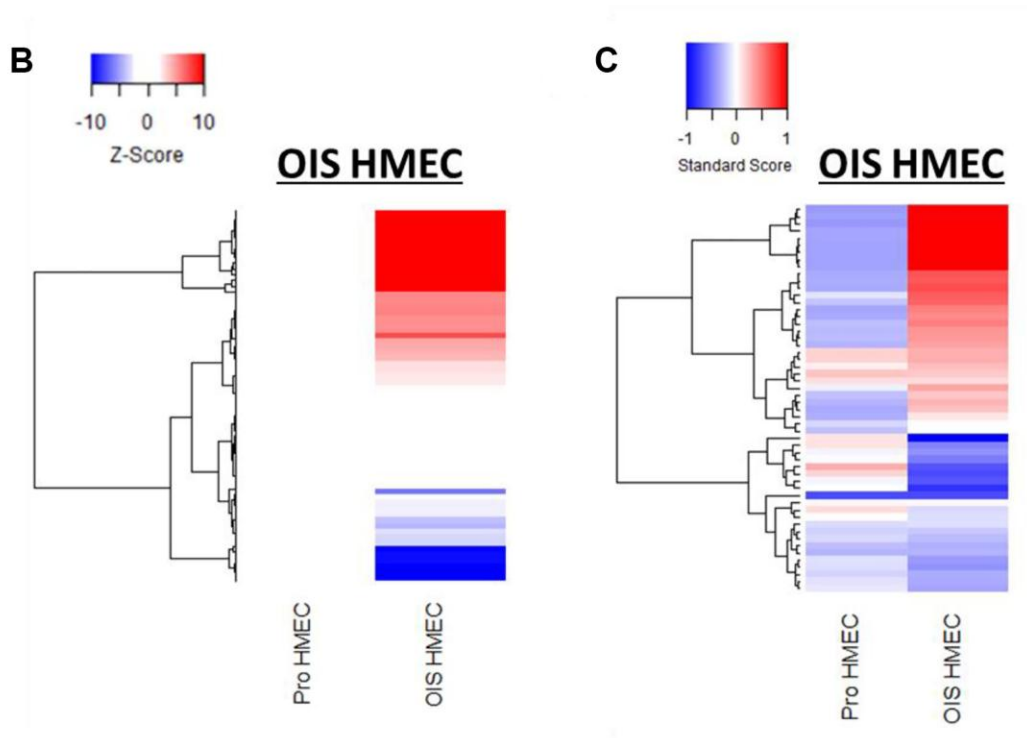
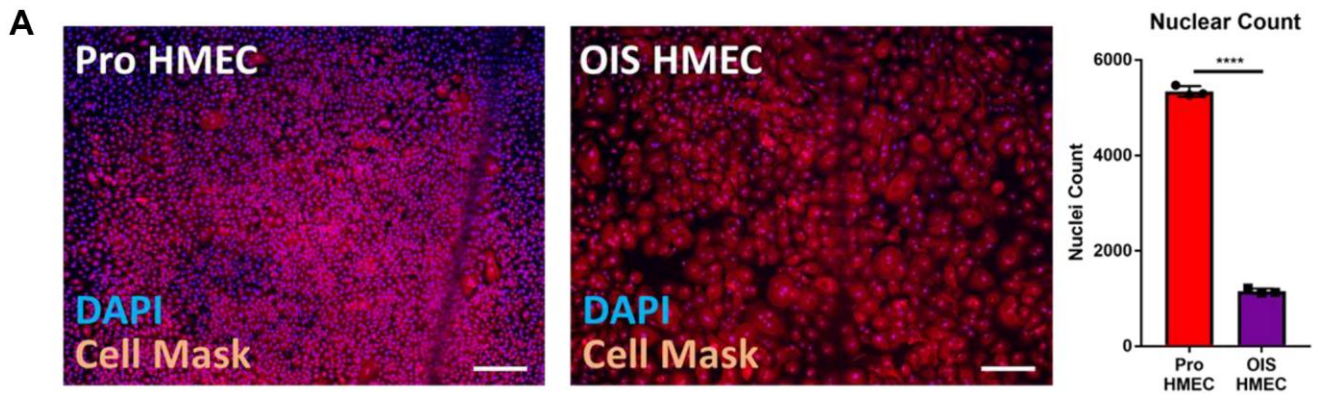
**Supplementary Figure 1. Workflow for use of established image stacks. (A)** Image stacks were previously generated through high content microscopy for oncogene-induced senescence (IMR90), paracrine senescence (IMR90), replicative senescence (HMFs) and oncogene-induced senescence (HMEC) models. These were then used to characterise a range of conventional senescence hallmarks. For this work, these image stacks were re-mined utilising phenotypic profiling for morphological assessment. Figure created with BioRender. **(B)** Summary of senescence markers used in previous publications for confirmatory senescence identification.



**Supplementary Figure 2. Characterisation of UV-induced senescence in human dermal fibroblasts.** (A) Immunofluorescence staining and quantitation of DAPI (blue) and Cell Mask (Red) in UV-induced senescence model.  $N = 2$ . Scale bar = 100  $\mu\text{m}$ . (B) Immunofluorescence staining and quantitation of DAPI (blue) and Ki67 (green) in UV-induced senescence model.  $N = 2$ . Scale bar = 100  $\mu\text{m}$ ; (C) Immunofluorescence staining of DAPI (blue),  $\gamma$ H2AX foci (green) and 53BP1 (red) in UV-induced senescence model.  $N = 2$ . Scale bar = 50  $\mu\text{m}$ . (D) Z-score profile heatmap of UV-induced senescence model. Y-axis comprises 62 morphological features (Red = positive modulation, Blue = negative modulation), White = no change) (E) Standard score profile heatmap of UV-induced senescence model. Y-axis comprises 62 morphological features (Red = positive modulation, Blue = negative modulation), White = no change). Proliferating condition (HDFs treated with 0  $\text{mJ}/\text{cm}^2$  UVB; Control). Senescent condition (HDFs treated with 6  $\text{mJ}/\text{cm}^2$  UVB; UV).

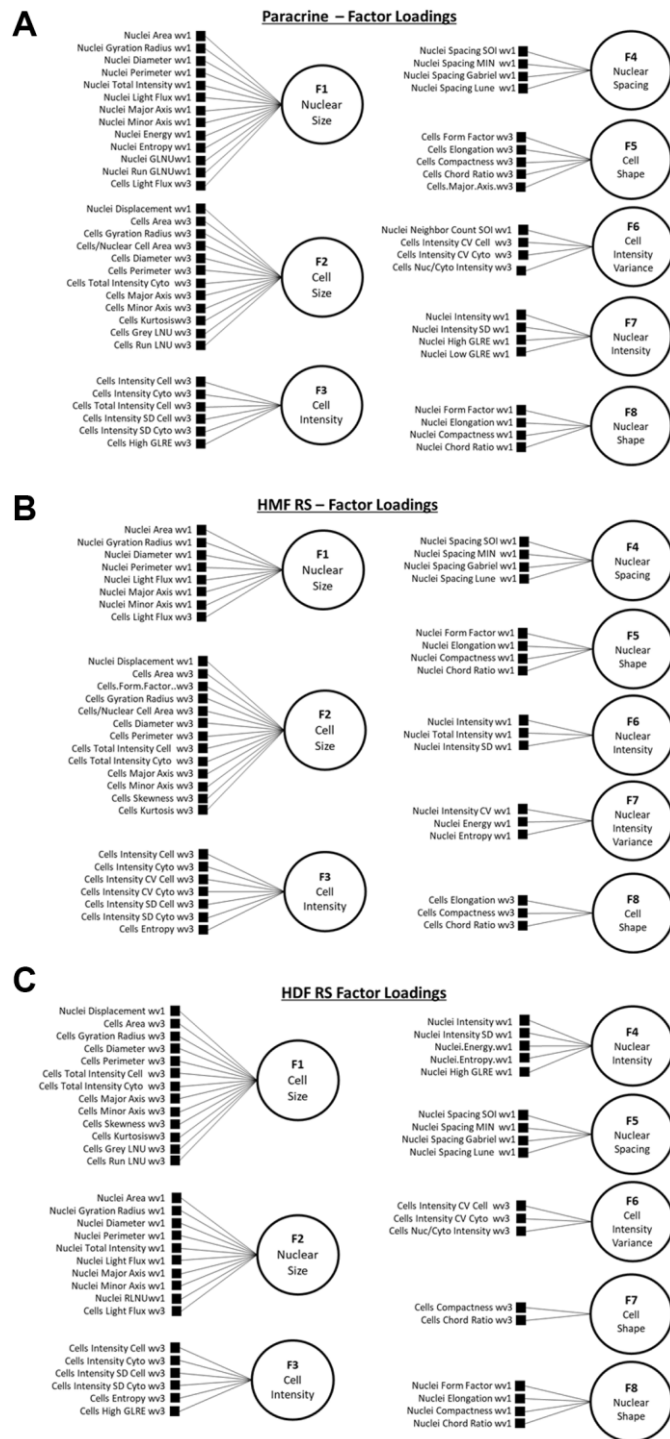


**Supplementary Figure 3. InCarta mask generation.** Representative raw images from each senescence model alongside nuclear and cellular masks generated via InCarta high content analysis (HCA) software. **(A)** Oncogene-induced senescence model. Proliferating condition: vector induction (Vector Ind), Senescence condition: OIS induction (OIS Ind). Scale bars = 500  $\mu\text{m}$  **(B)** Paracrine senescence model. Proliferating condition: Vector paracrine, Senescence condition: OIS Paracrine. Scale bars = 500  $\mu\text{m}$  **(C)** Human mammary fibroblast (HMF) replicative senescence model. Proliferating condition: Early proliferating HMFs (HMF EP), Senescence condition: Deep senescence HMFs (HMF DS). Scale bars = 250  $\mu\text{m}$  **(D)** Human dermal fibroblast (HDF) replicative senescence model. Proliferating condition: Early proliferating HDFs (HDF EP), Senescence condition: Deep senescence HDFs (HDF DS). Scale bars = 250  $\mu\text{m}$ .

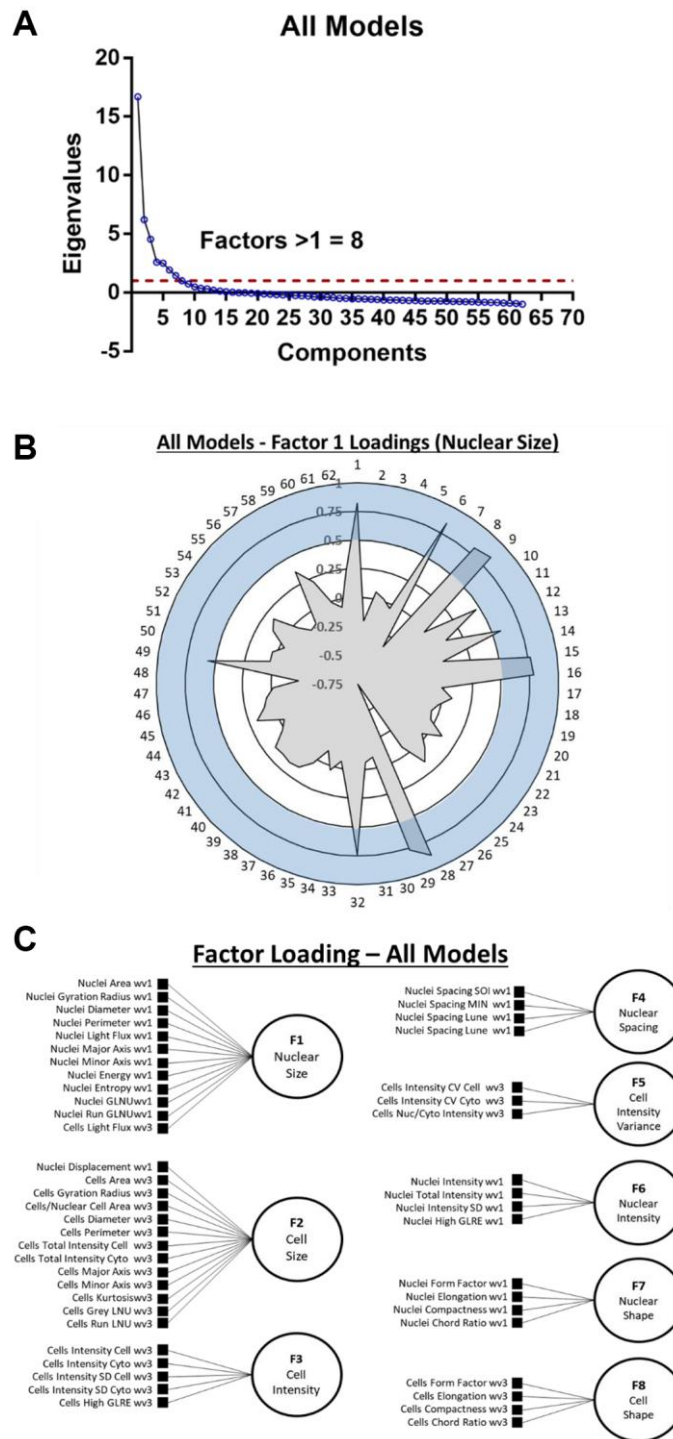


**Supplementary Figure 4. Characterisation of OIS in human mammary epithelial cells (HMEC).** (A) Immunofluorescence staining and quantitation of DAPI (blue) and Cell Mask (Red) in human mammary epithelial cell (HMEC) oncogene-induced senescence (OIS) model.  $N = 3$ . Scale bar = 500  $\mu\text{m}$ . (B) Z-score profile heatmap of HMEC OIS model. Y-axis comprises 62 morphological features (Red = positive modulation, Blue = negative modulation), White = no change) (C) Standard score profile heatmap HMEC OIS model. Y-axis comprises 62 morphological features (Red = positive modulation, Blue = negative modulation), White = no change). Proliferating condition (ER:RAS HMEC without tamoxifen induction; Pro HMEC). Senescent condition (ER:RAS HMEC with tamoxifen induction; OIS HMEC).



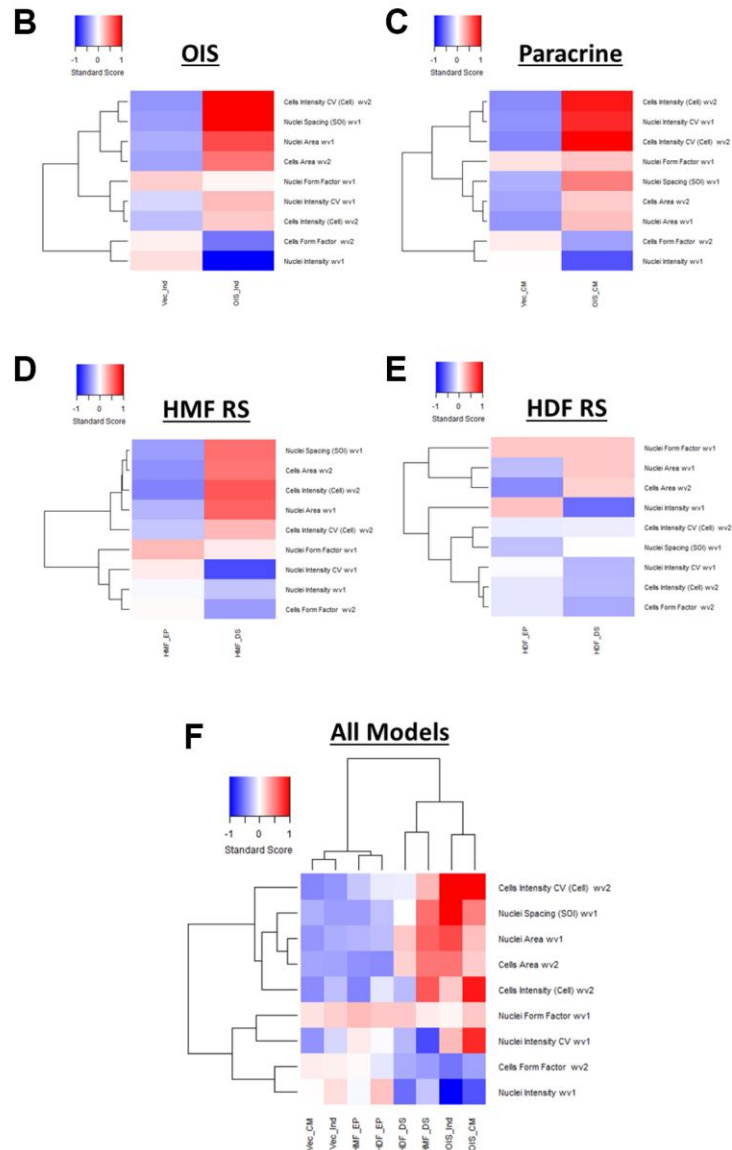


**Supplementary Figure 5. Exploratory factor analysis (EFA) factor loading diagrams. (A–C)** Factor loading diagrams for EFA models constructed using data from paracrine senescence, HMF replicative senescence and HDF replicative senescence models. Factor designations are also indicated.



**Supplementary Figure 6. EFA model using single target data from all senescence models. (A)** Scree plot for combined single target data from all senescence models. Red line indicates eigenvalue = 1. **(B)** Polar plot of factor loading values for factor 1 from all senescence models EFA model (designated Nuclear Size). 1–62 refer to features (Table 2). Blue shaded area indicates factor loading threshold of 0.5. **(C)** Factor loading diagram for all senescence models EFA model with factor designations.

Latent Factor	Extracted Feature	Literature Precedent
Nuclear Size	Nuclear Area (1)	(Zhao <i>et al.</i> , 2010; Studencka and Schaber, 2017)
Nuclear Shape	Nuclear Form Factor (2)	(Matsumura, Zerrudo and Hayflick, 1979; Sadaie <i>et al.</i> , 2015)
Nuclear Intensity	Nuclear Intensity (10)	(Zhao and Darzynkiewicz, 2013; Roukos <i>et al.</i> , 2015)
Nuclear Intensity (Variance)	Nuclear Intensity CoV (12)	(Narita <i>et al.</i> , 2003; Di Micco <i>et al.</i> , 2011)
Nuclear Spacing	Nuclear Spacing SOI (17)	(Hayflick and Moorhead, 1961)
Cell Size	Cell Area (33)	(Cristofalo and Kritchevsky, 1969; Biran <i>et al.</i> , 2017)
Cell Shape	Cell Form Factor (34)	(Druelle <i>et al.</i> , 2016; Cormenier <i>et al.</i> , 2018)
Cell Intensity	Cell Intensity Cell (42)	(Neurohr <i>et al.</i> , 2019)
Cell Intensity (Variance)	Cell Intensity CoV Cell (46)	-



**Supplementary Figure 7. Representative profiles.** (A) Table indicating senescence-associated latent factors and selected representative features. Literature precedent for use of feature in senescence characterisation included. (B–E) Standard score profile heatmaps for oncogene-induced senescence (OIS), paracrine senescence (Paracrine), HMF replicative senescence (HMF RS) and HDF replicative senescence (HDF RS) models. Y-axis comprises standard-scores for the 8 extracted features above (Red = positive modulation, Blue = negative modulation), White = no change). Proliferating conditions: vector induction (Vec\_Ind), vector conditioned media (Vec\_CM), HMF early proliferating (HMF\_EP) and HDF early proliferating (HDF\_EP). Senescence conditions: OIS induction (OIS\_Ind), OIS conditioned media (OIS\_CM), HMF deep senescence (HMF\_DS), HDF deep senescence (HDF\_DS). (F) Summary standard-score profile heat map and hierarchical clustering of all proliferating and senescence conditions. Y-axis comprises standard-scores for the 8 extracted features above.