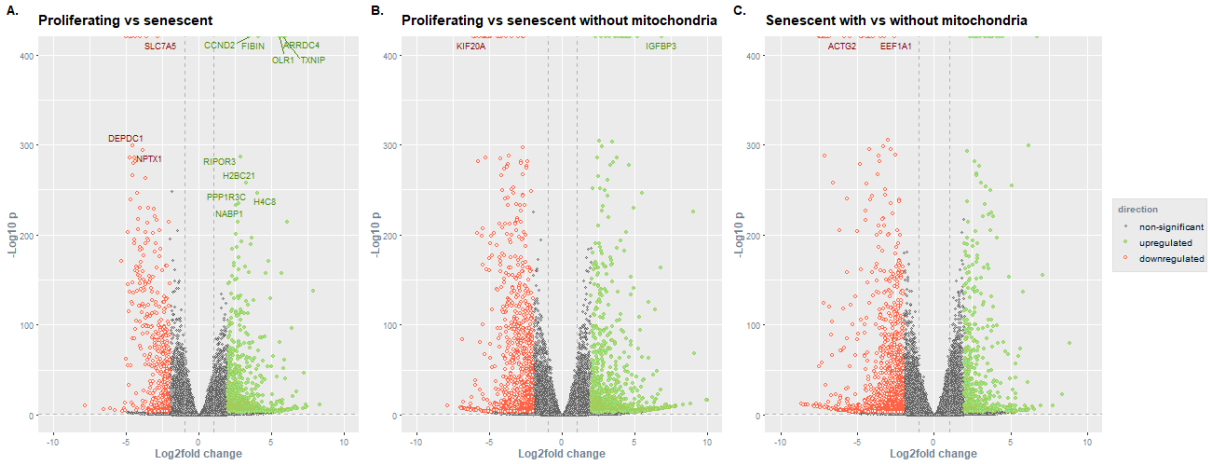


Figure 1. Change of gene expression proliferating and senescent cells with or without mitochondria of human IMR90 fibroblasts. The genes with the most significant difference in expression had lower p values in comparisons involving senescent cells without mitochondria (B. and C.), consistent with the magnitude of such change. More of the most significant genes are also overlapping between those groups, being involved in the functions performed by the mitochondria. For all comparisons, more downregulated genes reach higher absolute difference values than the upregulated genes. A total of 46 values (A: 10, B: 19, C: 17) were excluded due to the p value equal 0. *Direction – direction of change.*

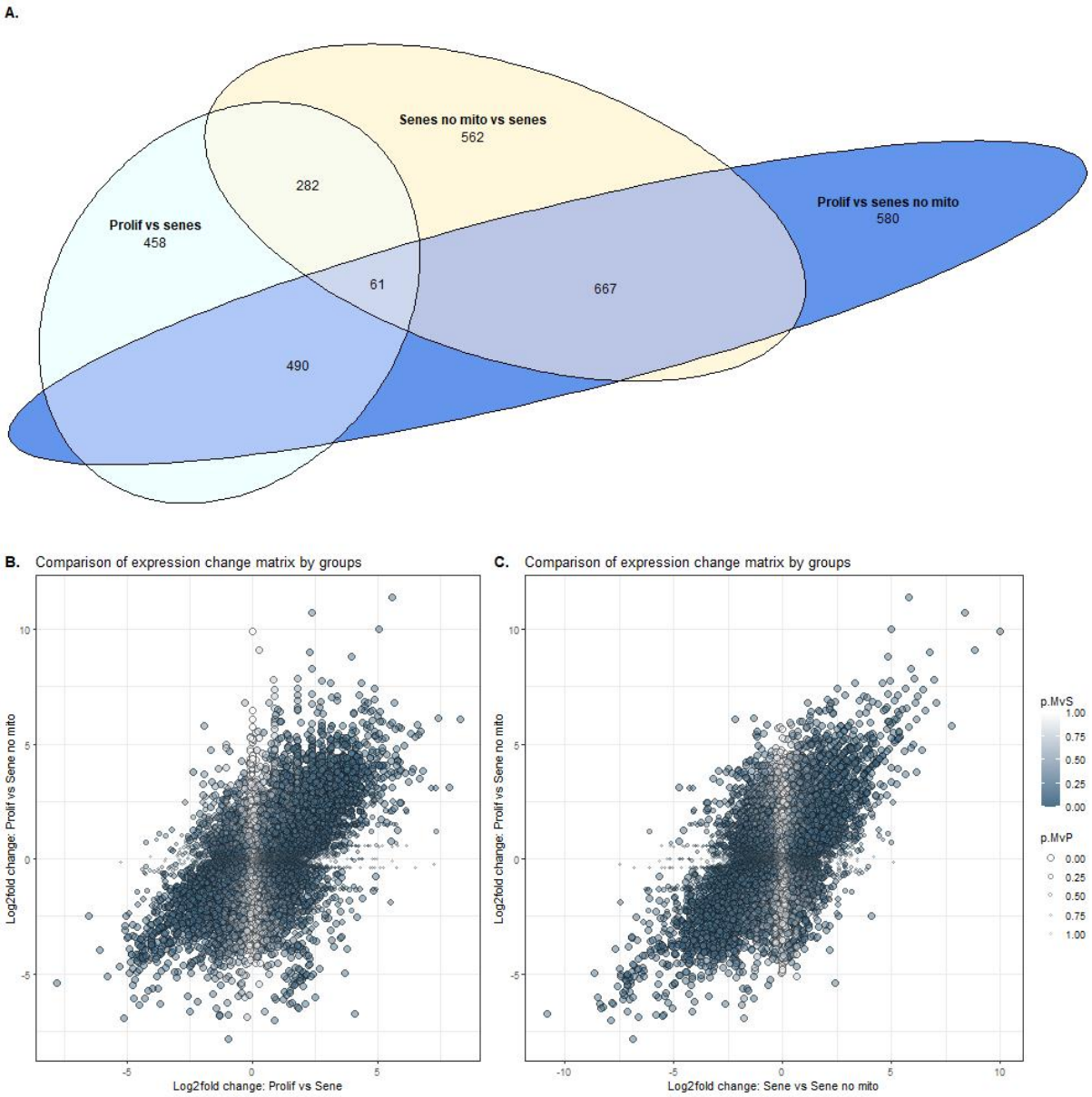


Figure 2. Relationships between genes significantly different between the test groups: proliferating (prof), senescent (senes) and senescent without mitochondria (senes no mito).

- ***A. Venn diagram of genes from the RNA-seq, that significantly vary in their expression between groups.*** *Most numerous were the groups involving the senescent cells without mitochondria, with more altered in comparison to proliferating cells (1798 vs 1572). A total of 1301 genes different in senescent cells vs. proliferating cells, including 458 unique for senescent cells with mitochondria. Simultaneously, 580 genes were uniquely different in senescent cells with depleted mitochondria than in the proliferating cells. 667 genes were shared for both comparisons involving cells without mitochondria, suggesting their independence on senescent state. Interestingly, 282 genes were commonly altered in proliferating and senescent mitochondria-depleted cells, validating further investigations into dividing state of those cells.*
- ***B. and C.*** *Expression change matrix of senescent cells without mitochondria vs. proliferating against proliferating (**B**) or against senescent cells (**C**). The change in gene expression appear to be more correlated in the latter comparison due to the lesser spread of data points; however, the correlation coefficient is similar for both (0.52 and 0.58, respectively).*

Figure 3. The comparison between the top 10 most significantly differently expressed genes between groups. A.Senescent vs proliferating cells. *Most of the most significantly altered genes are characterised by increased expression in proliferating cells than in other groups, with exceptions being CCND2 and ARRDC4, involved in the cyclin production and ubiquitination, respectively. SvP – proliferating vs senescent.* ***B. Mitochondria-depleted senescent vs proliferating cells.*** *Four genes (TPX2, BIRC5, KIF4A and KIF20A) have higher expression in the proliferating cells, due to their role in cell division and survival. Further four (SLC2A3, PGK1, NDRG1 and ENO2) are expressed highly in the group without mitochondria and are involved in energy homeostasis, cell differentiation and growth. Furthermore, the expression of the tumour suppressor gene SFRP1 is decreased in this group.* ***C. Mitochondria-depleted senescent vs senescent cells.*** *Expression of four genes (RIPOR3, CEMIP, ACTA2 and COL1A1) diverges from the level seen in senescent cells, resembling these in proliferating cells. Similarly to those stated before, those genes play role in cell proliferation and migration. Senes – senescent, senes_MtD – senescent with depleted mitochondria, prolif – proliferating, MvS – senescent vs senescent without mitochondria, MvP – proliferating vs senescent without mitochondria.*

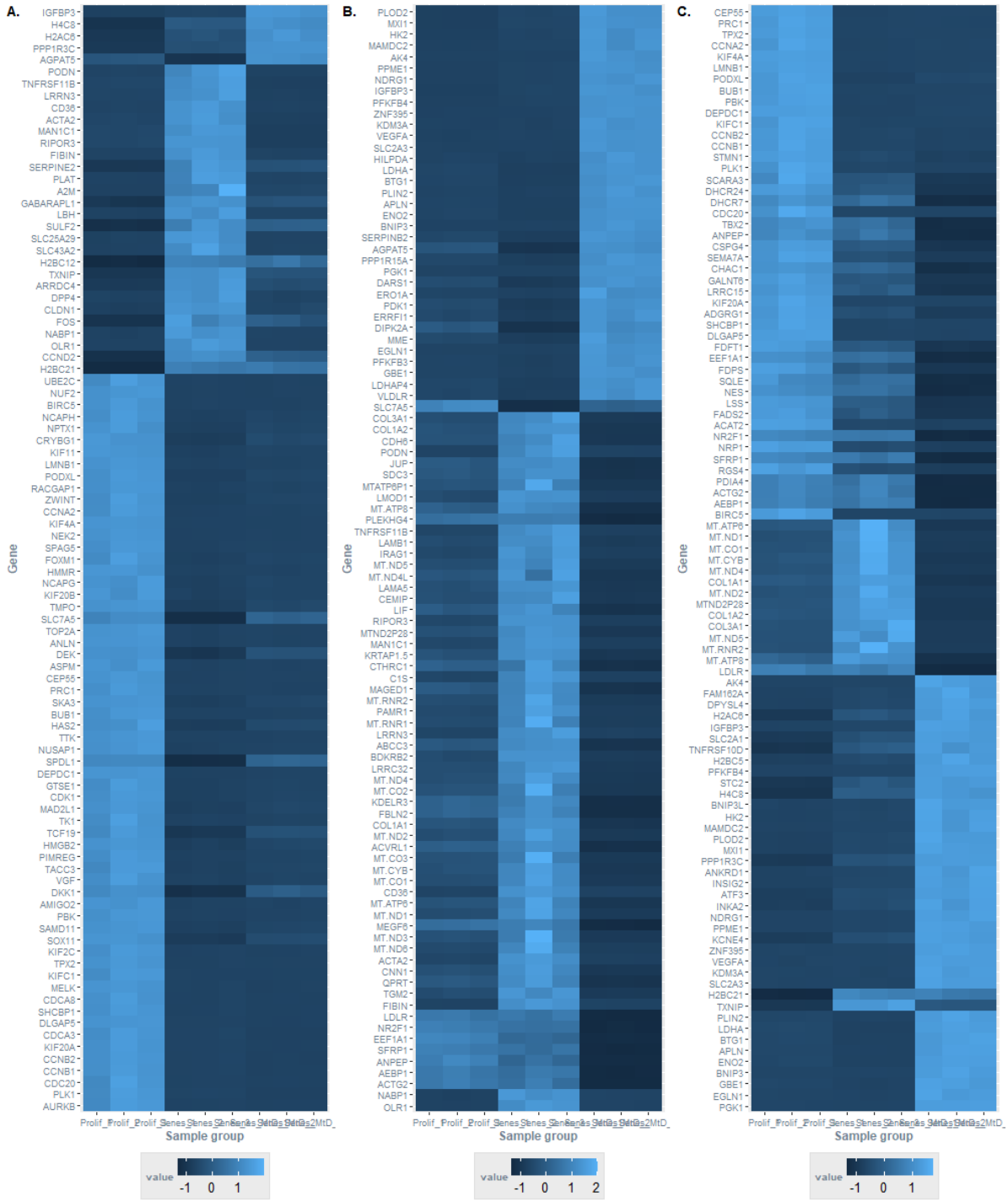


Figure 4. Heatmap of gene expression in tested samples for the hundred of most significantly differentially expressed genes in senescent vs. proliferating (A.), senescent without mitochondria vs. senescent (B) and senescent without mitochondria vs. proliferating cells (C). *A. Three clusters can be distinguished. Most numerous are the genes highly expressed in proliferating cells, with other two clusters being highly expressed uniquely in either MtD or senescent cells. B. Most genes have uniquely high expression in senescent cells or MtD cells, with few sharing this characteristic between senescent and proliferating cells. Many of the genes distinguished in comparisons on* ***B*** *and* ***C*** *are unique for the group without mitochondria, with similar expression patterns for senescent and proliferating cells. Order of samples: 3x proliferating, 3x senescent, 3x mitochondria-depleted (MtD).*

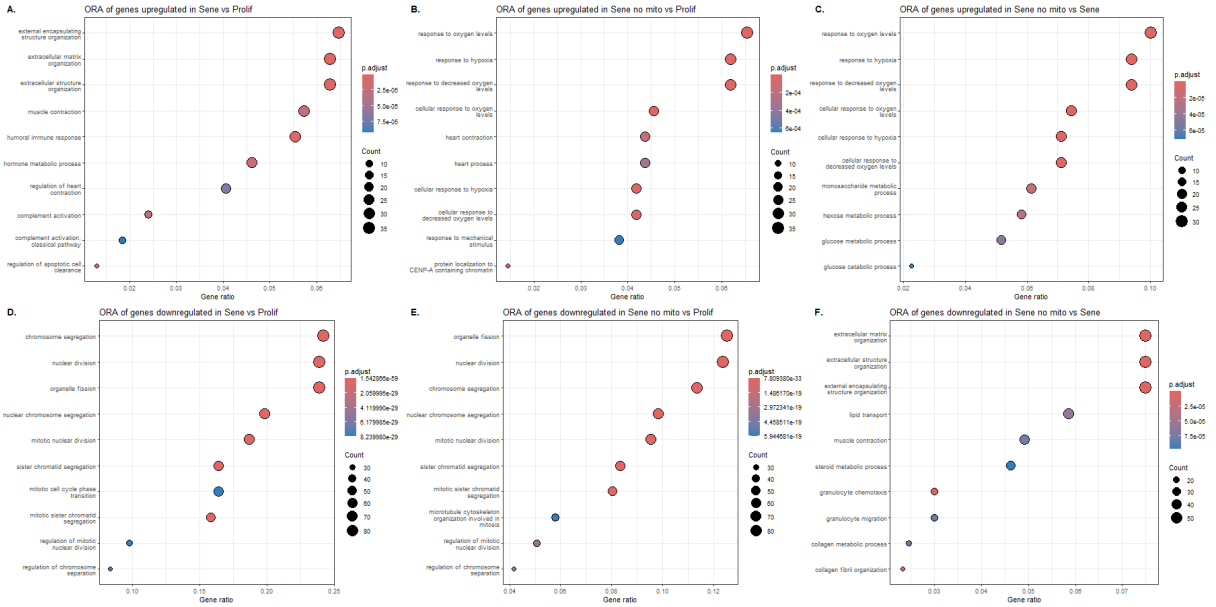
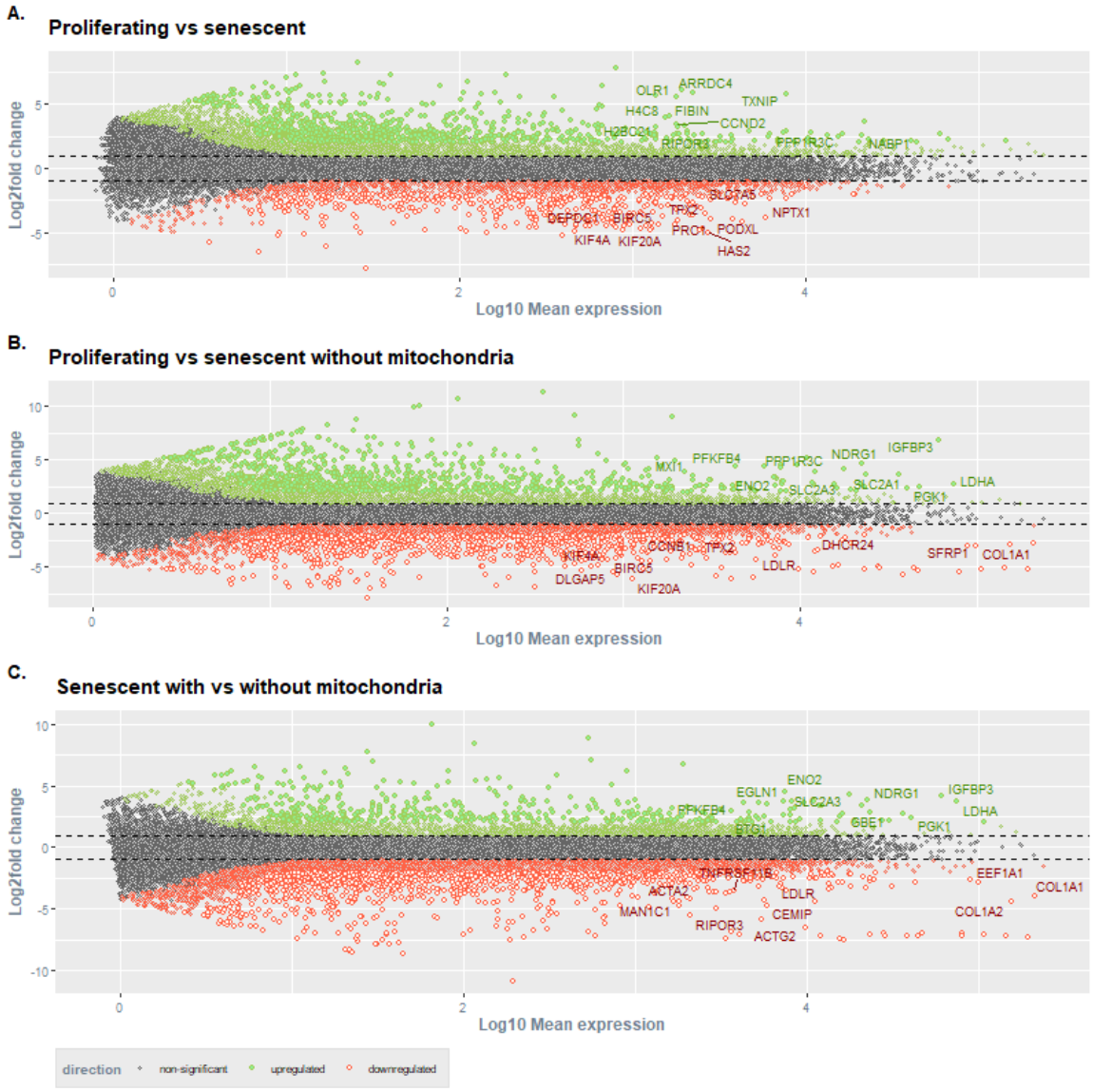
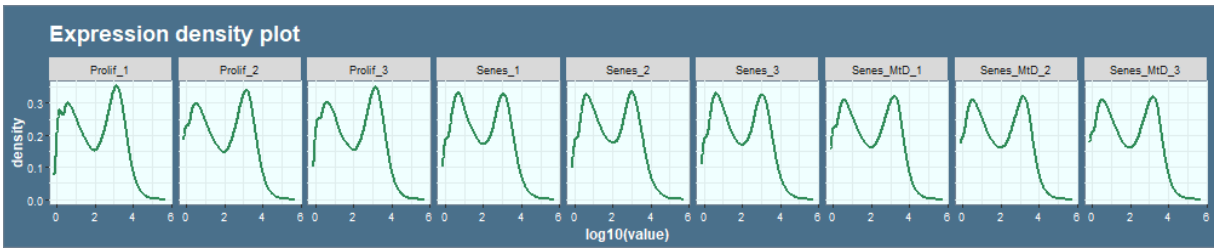


Figure 5. Up- and down-regulated genes differentially expressed between groups by biological function. A. and D. Senescent cells have upregulated expression of genes engaged in cell positioning and survival, whereas the genes engaged in cell division are downregulated. **B. and E.** When compared to the MtD cells, proliferating cells have upregulated expression of genes involved in cell respiration. The expression of genes important for cell division is inferior in this group to the proliferating cells. **C. and F.** Senescent cells without mitochondria have lower expression of genes involved in cell respiration, whereas the expression of genes important for cell attachment is significantly lower.

Supplemental figures



Supplementary Figure 1. Genes with the highest statistical significance of difference between groups were also characterised by high expression.



Supplementary Figure 2. The expression density plot shows a similar binomial distribution for all samples.