

Supplementary Materials for

Metformin prevents age-associated ovarian fibrosis by modulating the immune landscape in female mice

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Sci. Adv. **8**, eabq1475 (2022)
DOI: 10.1126/sciadv.abq1475

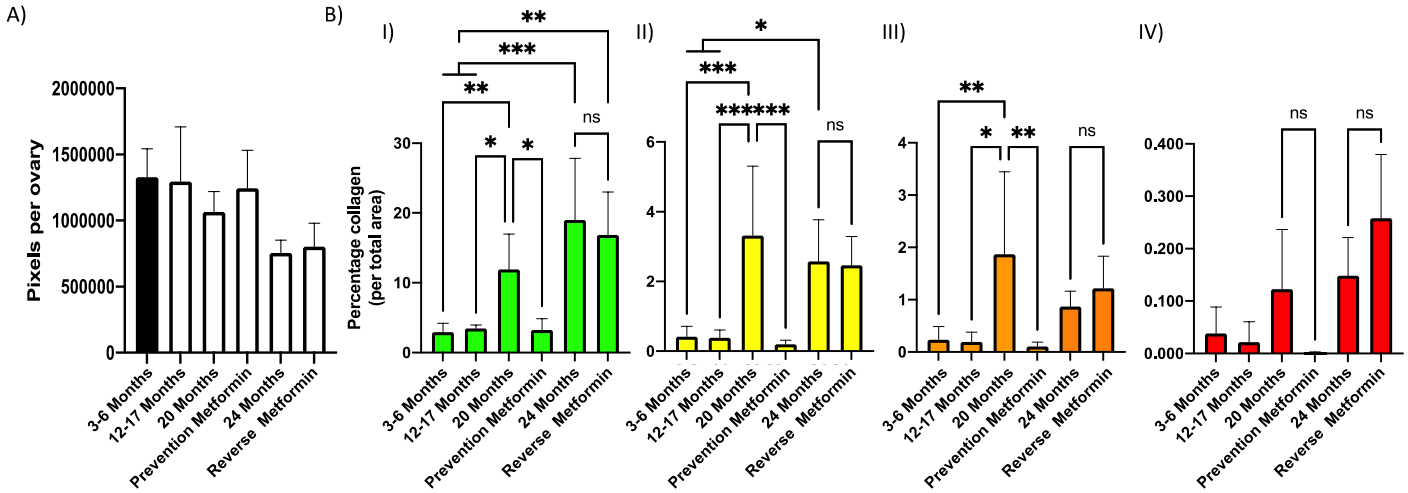
The PDF file includes:

Figs. S1 to S3
Table S1
Legends for tables S2 to S5

Other Supplementary Material for this manuscript includes the following:

Tables S2 to S5

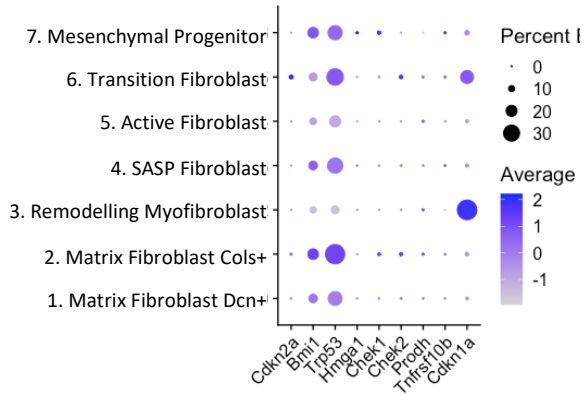
Supplementary Materials:



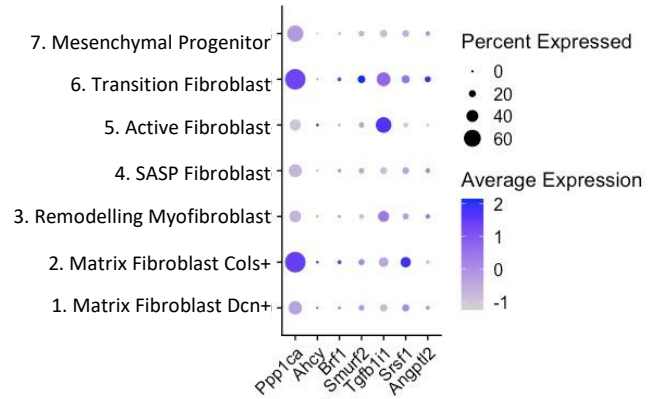
Supplemental Figure 1: Histological analysis of PSR-POL staining pixels and thickness

(A) The average number of total pixels for each whole ovary was analyzed by PSR-POL imaging. (B) Quantification of each HUE pixel color group, (I) green (thin fibers), (II) yellow, (III) orange, and (IV) red (thick fibers) pixels in association with age and treatment. Total positive pixels were normalized to total pixel count and averaged within each group. Data are means \pm SEM, (ns=notsignificant, * P <0.05, ** P <0.01, *** P <0.001).

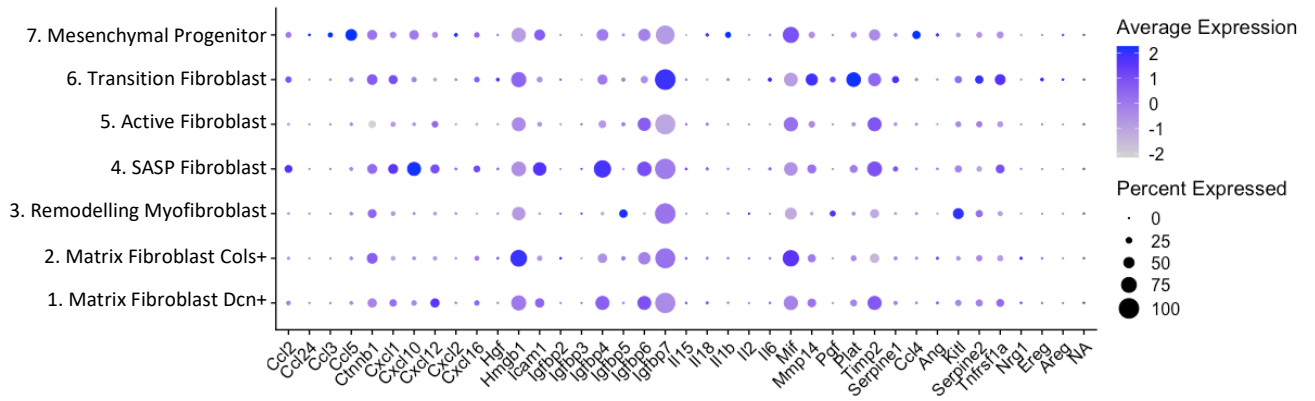
A) Senescence Core Genes



B) Senescence Effector Genes



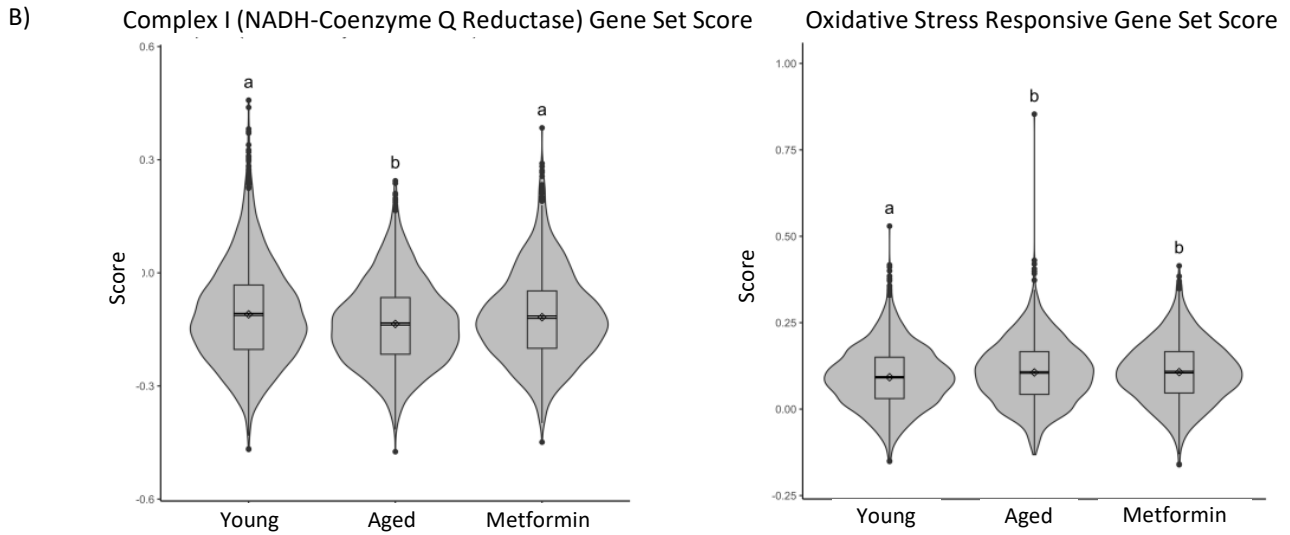
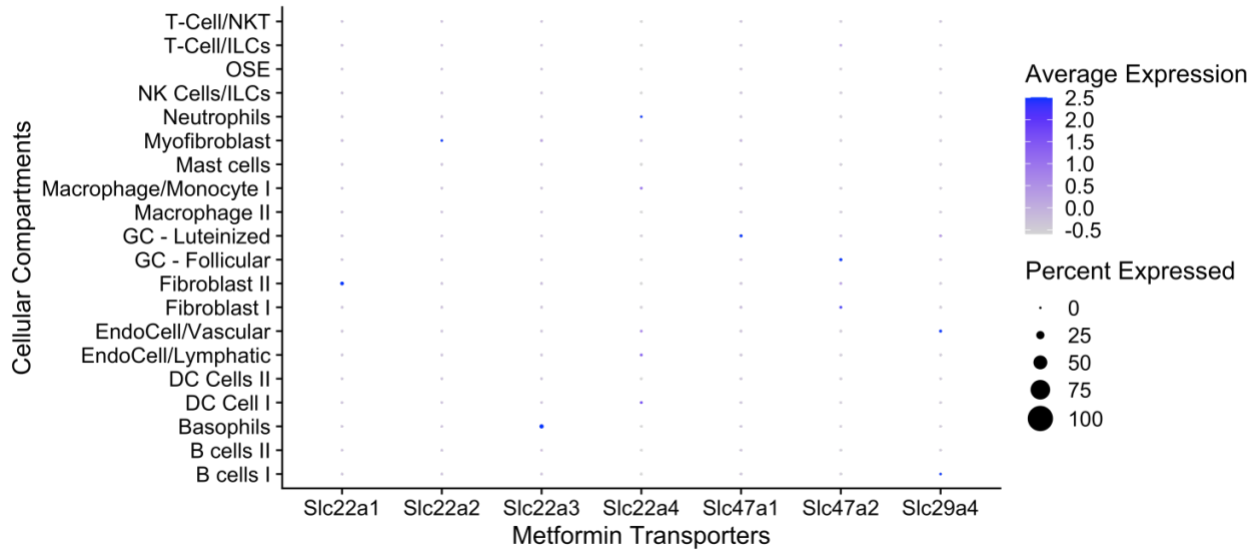
C) SASP Genes



Supplemental Figure 2: Expression of senescence-related genes in fibroblasts revealed higher SASP expression in fibroblast cluster 4

Analysis of specific genes associated with (A) senescence core genes, (B) senescence effector genes, and (C) senescence-associated secretory phenotype (SASP) genes in fibroblasts showing the average expression from grey to blue and the percentage of cells in each cluster that expressed the genes by the dot size.

A) Membrane Transporters Involved in Metformin Pharmacokinetics



Supplemental Figure 3: Effects of metformin may be indirect in the mouse ovary

(A) Gene expression of known membrane transporters involved in metformin transport showing the average expression from grey to blue and the percentage of cells in each cluster that expressed the genes by the dot size. (B) Module score for Complex I (NADH-Coenzyme Q Reductase) gene set, and Oxidative Stress Responsive gene set in fibroblasts. Different letters above error bars (means \pm SEM) indicate significant differences ($p < 0.05$) among groups (Tukey's posthoc test).

Supplemental Table 1: Flow sorting data of immune and non-immune cell proportion

Group	CD45- Cells	CD45+ Cells	Total Cells	Percentage CD45+ Cells
Young 3m	307000	33000	340000	9.7
Middle Aged 14m	80000	29000	109000	26.6
Aged 20m	45880	13200	59080	22.3
Aged 20m + Metformin	142000	28000	170000	16.5
Aged 24m	1750	1100	2850	38.6
Aged 24m + Metformin	76000	32000	108000	29.6

Supplemental Table 2: List of Differentially Expressed Genes in fibroblast clusters

Supplemental Table 3: List of PANTHER pathways from the overrepresentation test of fibroblast clusters

Supplemental Table 4: List of Differentially Expressed Genes in macrophage III

Supplemental Table 5: List of all Ligand-Receptors identified from LIANA analysis