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 **GSEA**

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We use this protocol and it's working

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Abstract

GSEA

- 1 GSEA of senescence-associated pathways was performed with GSEA software (version v4.3.2; <https://www.gsea-msigdb.org>) using the Molecular Signatures Database (MSigDB, version v6.2, RRID:SCR_016863)
- 2 RNA counts from control and CDDO-Me-treated samples were ranked based on the signal-to-noise ratio (SNR), calculated as $(\mu_{\text{treated}} - \mu_{\text{control}}) / (\sigma_{\text{treated}} + \sigma_{\text{control}})$.
- 3 Genes were sorted in descending order according to the SNR.
- 4 GSEA was used to calculate the enrichment score (ES), normalized enrichment score (NES), and corresponding false discovery rate (FDR).
- 5 Default GSEA settings were applied, with the permutation parameter set to "gene set" because of the small sample size ($n < 7$).
- 6 The gene set size was restricted to 10–500 genes.
- 7 Input data included RNA-seq read counts from all three cell types, a sample label mapping file, and senescence-related gene sets.
- 8 Gene sets were obtained from MSigDB (senescence, SASP, and cell cycle regulation) and the manually curated SenMayo SASP gene set (Ref. main text 7, 27–30, 90).