SUPPLEMENTAL INFORMATION FOR

Gaining insights into cellular senescence – the tools it takes

A consensus reference from the International Cell Senescence Association (ICSA)

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SUPPLEMENTAL VIDEO LEGENDS

Suppl. Video 1: Non-induced (OFF) HBEC-CDC6 Tet-ON cells, present features of normal epithelial cells. (HBEC: Human Bronchial Epithelial Cells)

Suppl. Video 2: Induction of CDC6 expression (ON) in the HBEC CDC6 Tet-ON system, results in a progressive decrease of proliferation and acquisition of an -oncogene induced-senescence phenotype (reaching its pick at day 6 post-induction and remaining active up to day 26). During this period, senescent cells exhibit cellular enlargement, irregular shaping, elongated projections and increased granularity, compared to the non-induced (video 1) counterparts. Blue circles and frame depict representative senescent cells with elongated cytoplasmic projections while red circles correspond to cells with S/M phase dissociation, presenting also large size and irregular shape.

SUPPLEMENTAL TEXT

SeneQuest Site Construction

The entrez gene database was downloaded locally according to the instructions in http://barc.wi.mit.edu/entrez_gene/. All other scripting has been performed with the R-Language [R Core Team (2018). R: A language and environment for statistical computing. R Foundation for statistical Computing, Vienna, Austria. URL https://www.R-project.org/]. The following entrez gene tables where utilised in the SeneQuest database which was setup on a MySQL Server:

-gene2go

-gene_info

-generifs_basic

-interactions

-tax 2 name

The following tables where created:

-gene2senescence from **Supplementary Table 1**.

-go_name and go_tree from the R script 'make_go_tables.R' which utilises the R-Language package "ontologyIndex" [Greene D, Richardson S, Turro E. ontologyX: a suite of R packages for working with ontological data. Bioinformatics. 2017 Apr 1;33(7):1104-1106. doi: 10.1093/bioinformatics/btw763]. go_name connects GO-codes with GO-terms. go_tree describes the whole GO genealogical tree. This table is utilised in searching for genes related to senescence that have a specific GO-code. The search returns not only senescence related genes with that specific GO-code but also with the descendants of the GO-code.

-senegenes2entrezgenes from the R-script 'Pop_senegenes2entrezgenes.R'. This table links the genes present in gene2senescence with the gene present in gene_info.

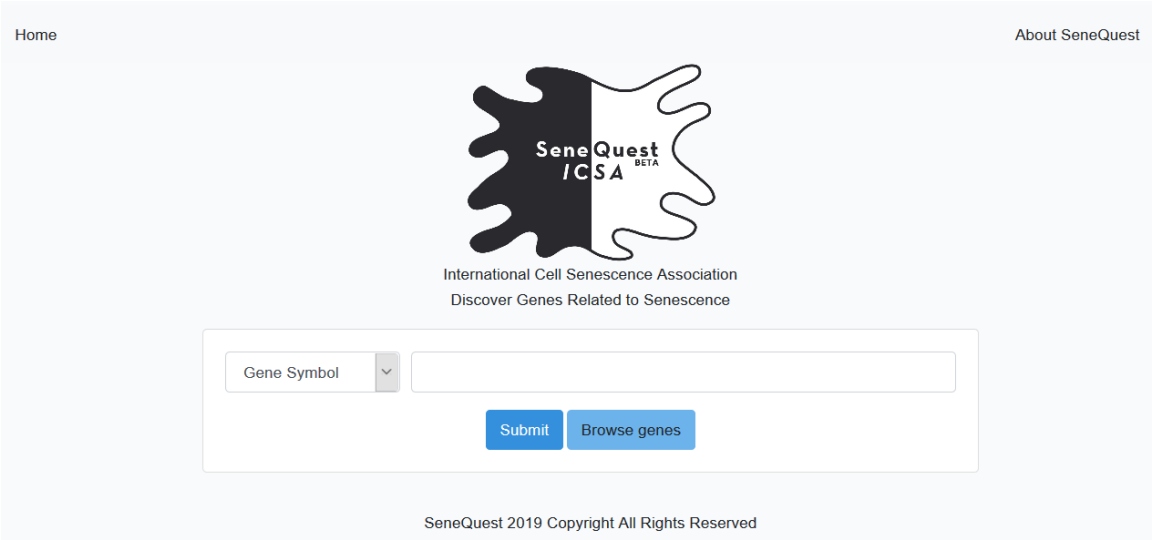
The SeneQuest database is available through http://www.senequest.net

SeneQuest Site Description

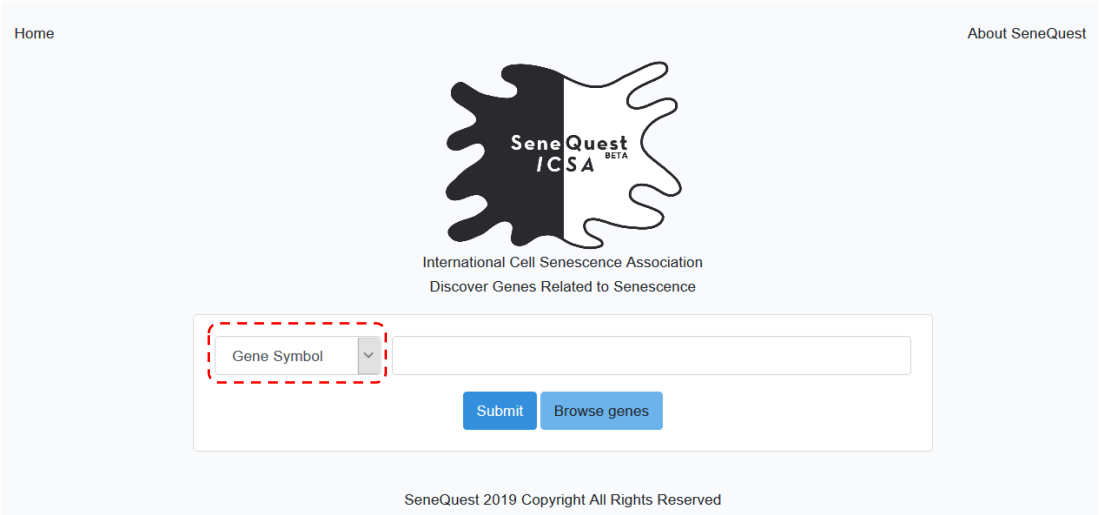
Short Description: *SeneQuest* is a literature-based evidence database of genes related to senescence. Each gene in the database is connected with multiple literature evidence, which is displayed in the form of PubMed IDs, showing the status of the gene in senescence (upregulated, downregulated or both). Traditional senescence markers such as SA-b-gal, p21WAF1/Cip1 and p16Ink4a applied solely in a study for senescence identification were not

included as an entry. Interactions of genes are also stored in the database and the user can search for interactants of a specific gene that are also connected with senescence. Finally Gene Ontology (GO) codes are associated with each gene. SeneQuest provides the ability for the user to search for senescence-associated genes that are linked to a specific GO-term or any of its decendants. All evidence is linked to one or multiple PubMed IDs that the user can immediately view by selecting the corresponding links.

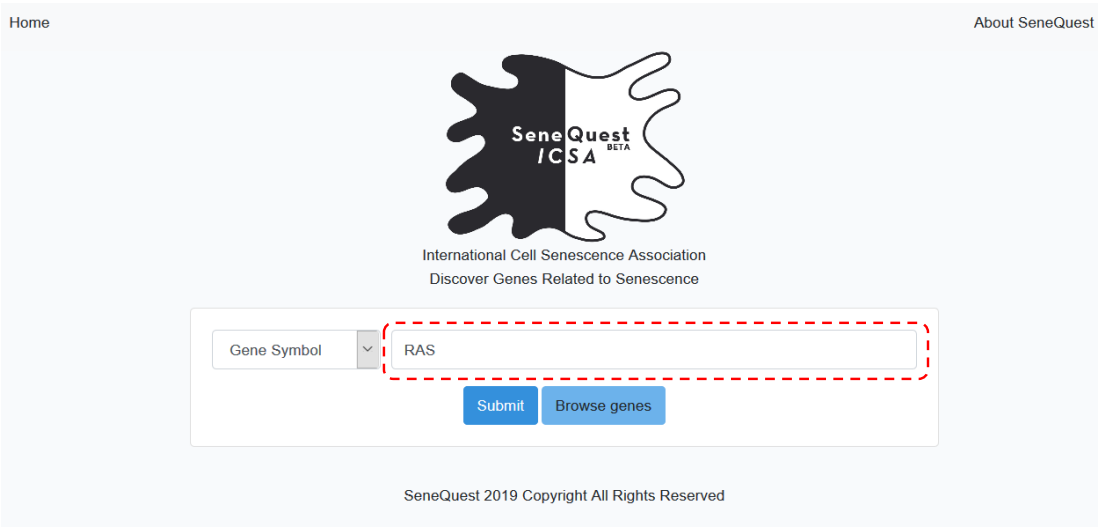
1. *SeneQuest* based web-application can be accessed through: **https://senequest.net**



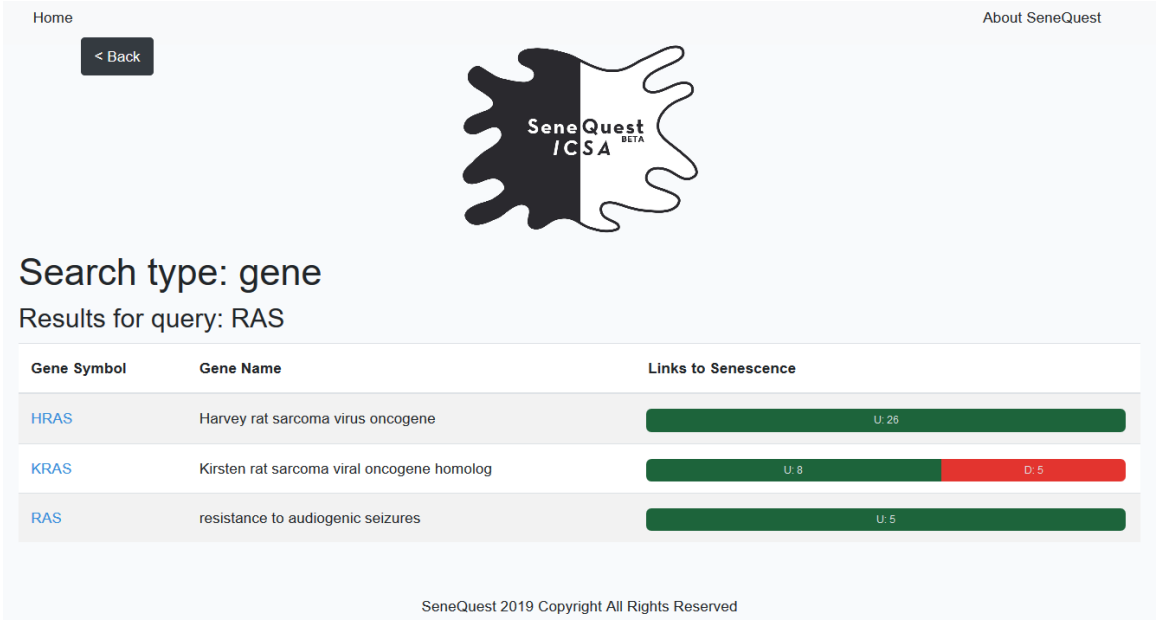
2. On the left hand side, centrally positioned, the user can select from a drop-down menu one of following terms: i) *Gene Symbol*, ii) *GO Term*, iii) *Cell-line*, iv) *Tissue* (see red dashed line in figure).



3. In the adjacent line on the right, the user must enter the official name or an alias name of a gene and press Submit. If you are interested in genes which are related to cellular senescence in a cell line you should insert the official name of the cell line according to the ATCC culture collection (https://www.atcc.org/).

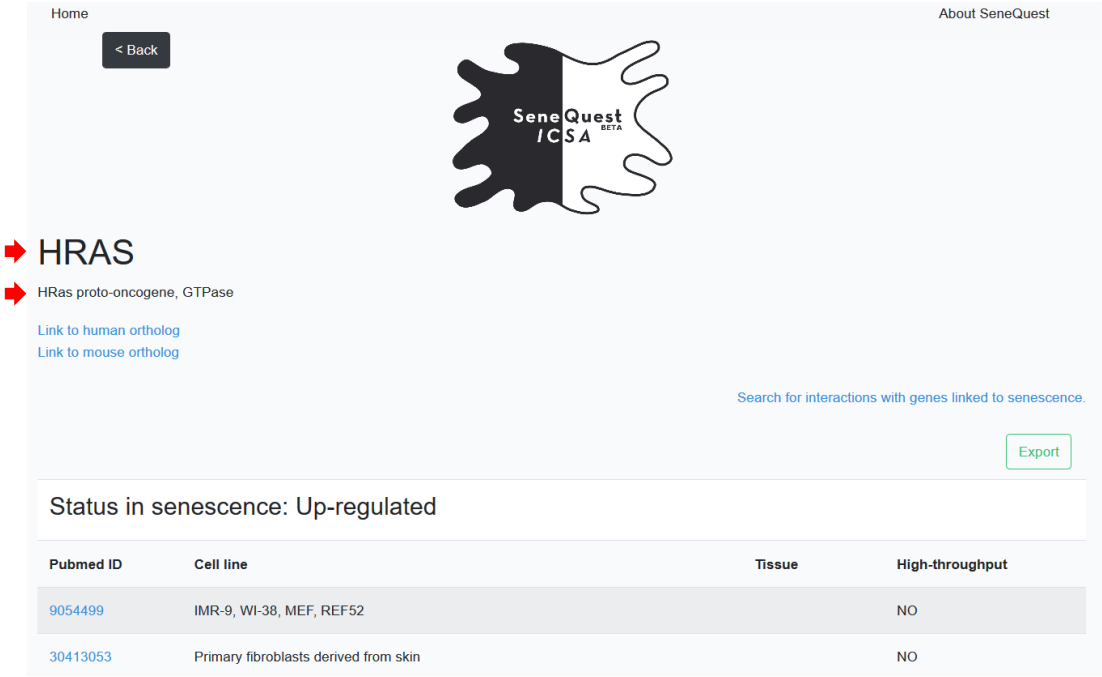


4. For each gene the output displays either a list of homologous genes, from which the user can further define the desired gene for interrogation, or the status of the selected gene in senescence.

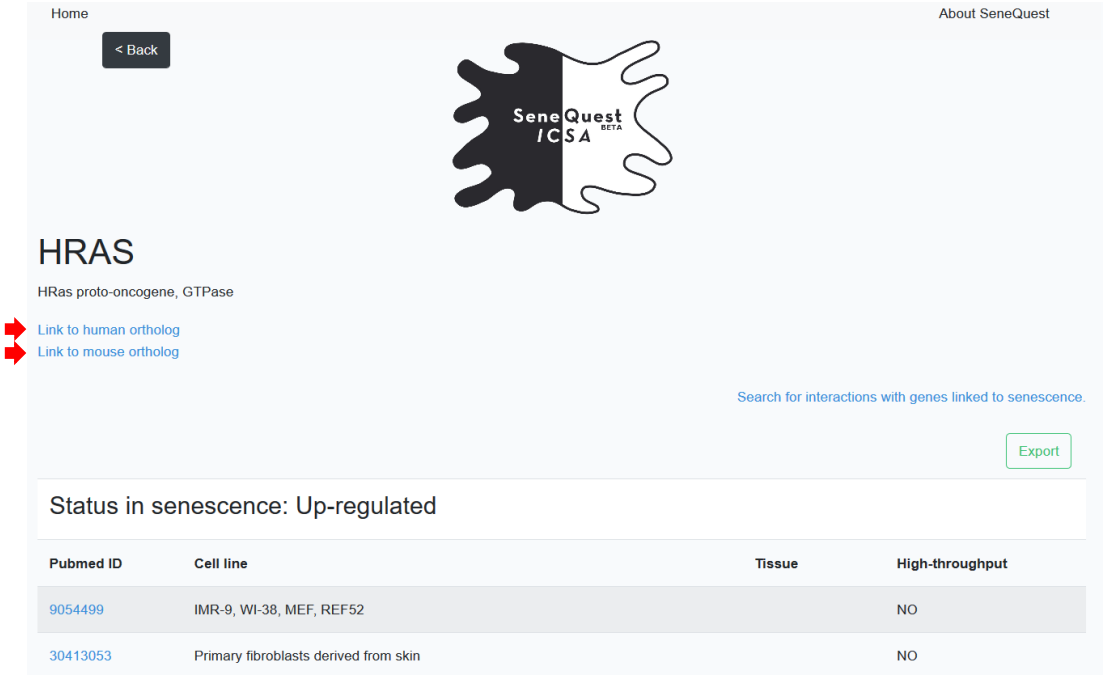


4. Any of the listed gene names under the “gene symbol” can be further selected and leads to a single specific gene entry page. This page contains the following items:

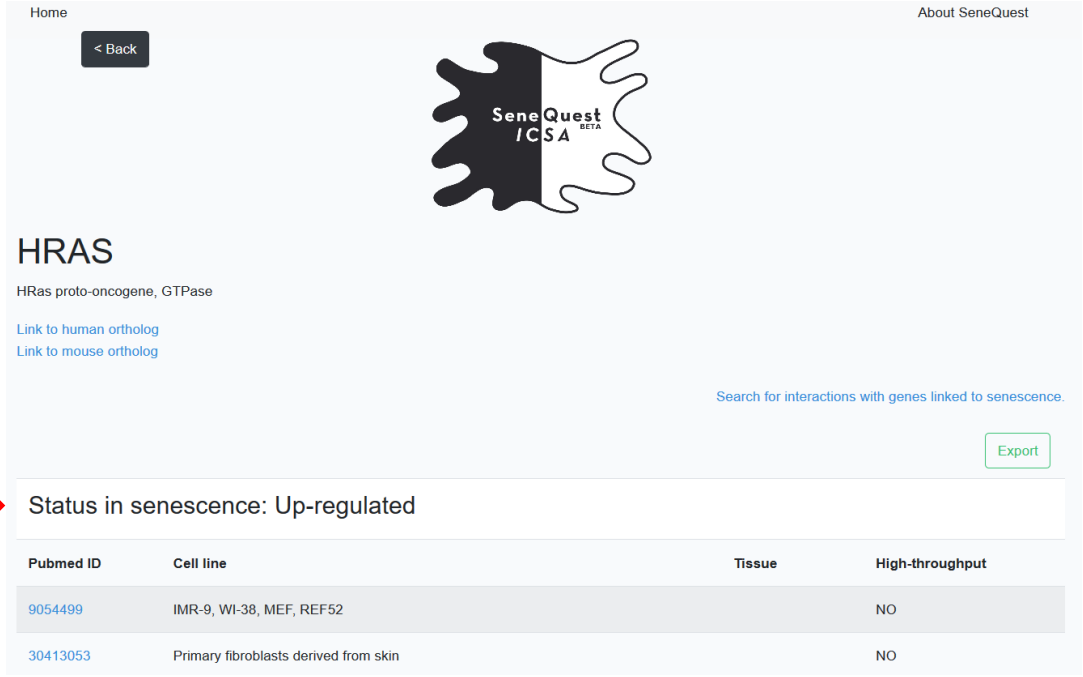
- 4a. In this page the selected gene symbol and gene name are displayed.



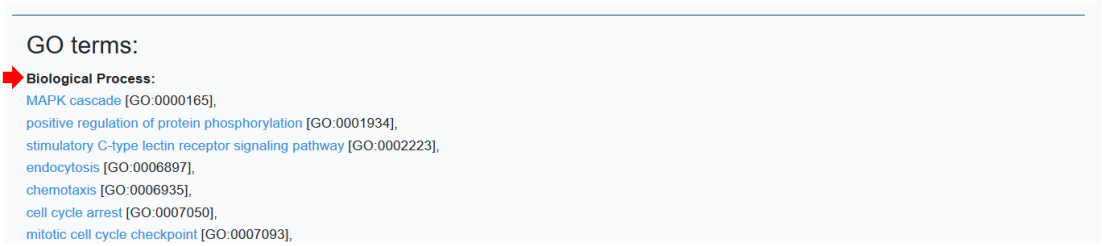
- 4b. Below this information two links are disclosed leading to the human and mouse ortholog entries (if available) in the Entrez gene database.



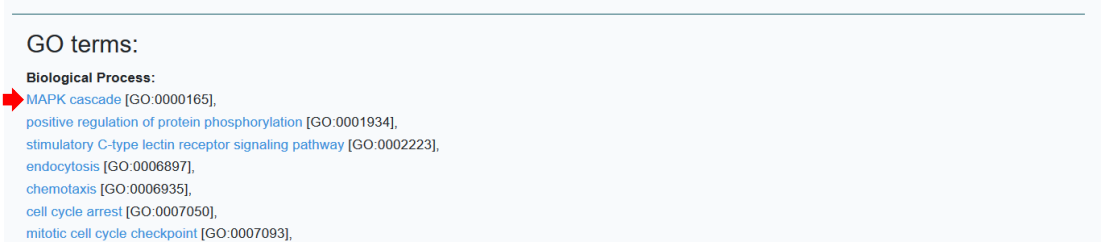
- 4c. Subsequently, there are entries for up-regulation or down-regulation in a specific senescence context for the specific gene that are shown along with the PubMed ID link leading to the actual PubMed entry from which was retrieved the original source information. Cell lines, tissues and/or high-throughput data examined in the selected publication ID are also provided along with the disease type that they represent.



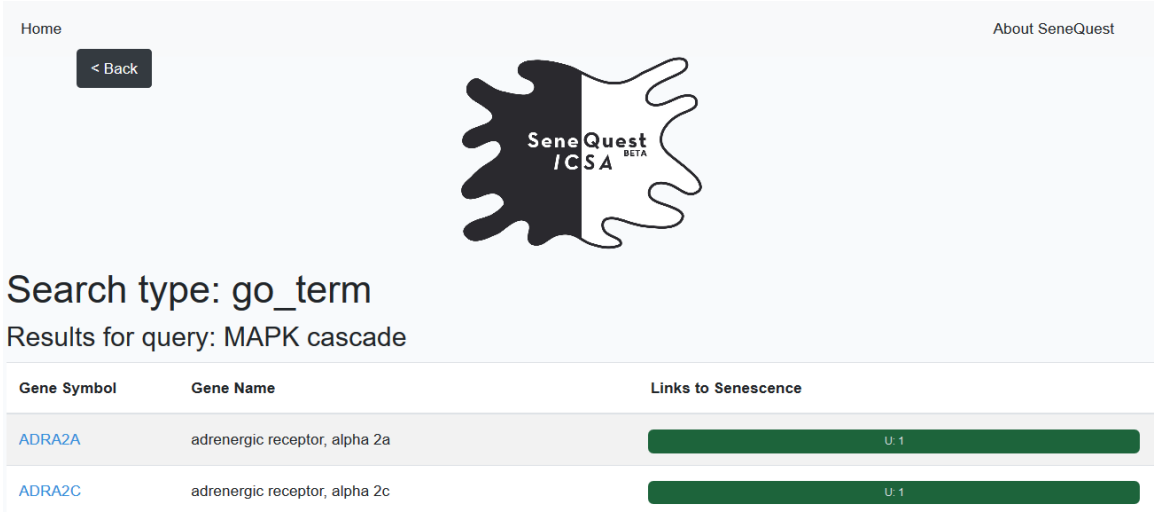
- 4d. Following on the same page, GO terms and codes linking the specific gene with the three main ontologies namely, “biological process”, “molecular function” and “cellular component” are presented.



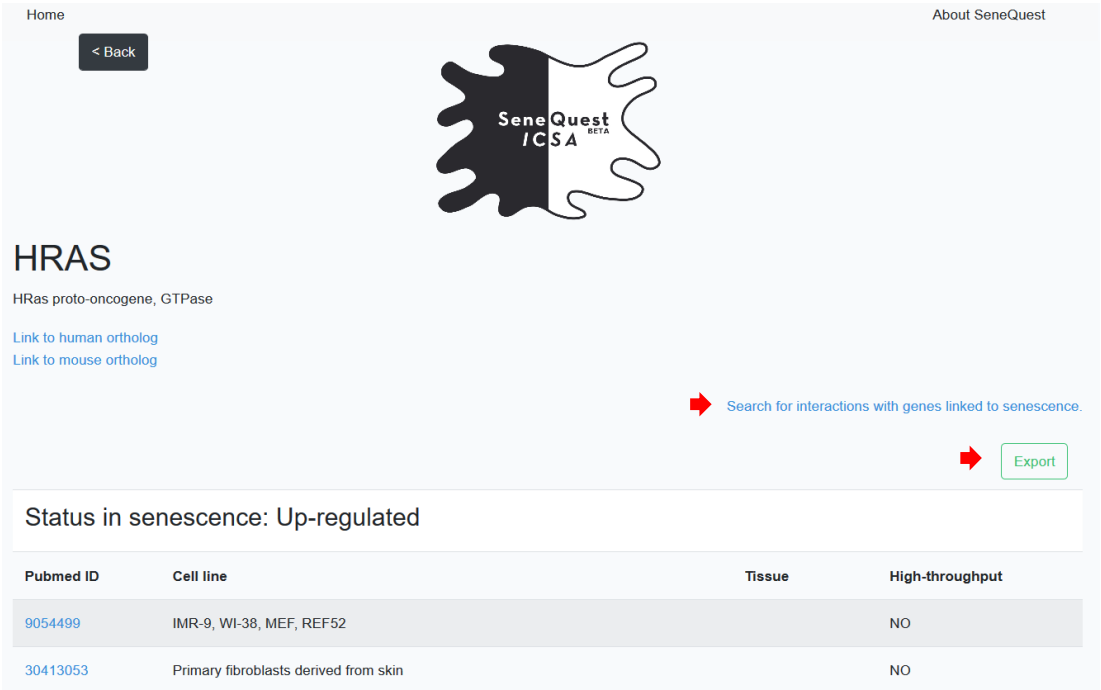
5. Each GO term, available in step (**4d**), can be further “selected”,



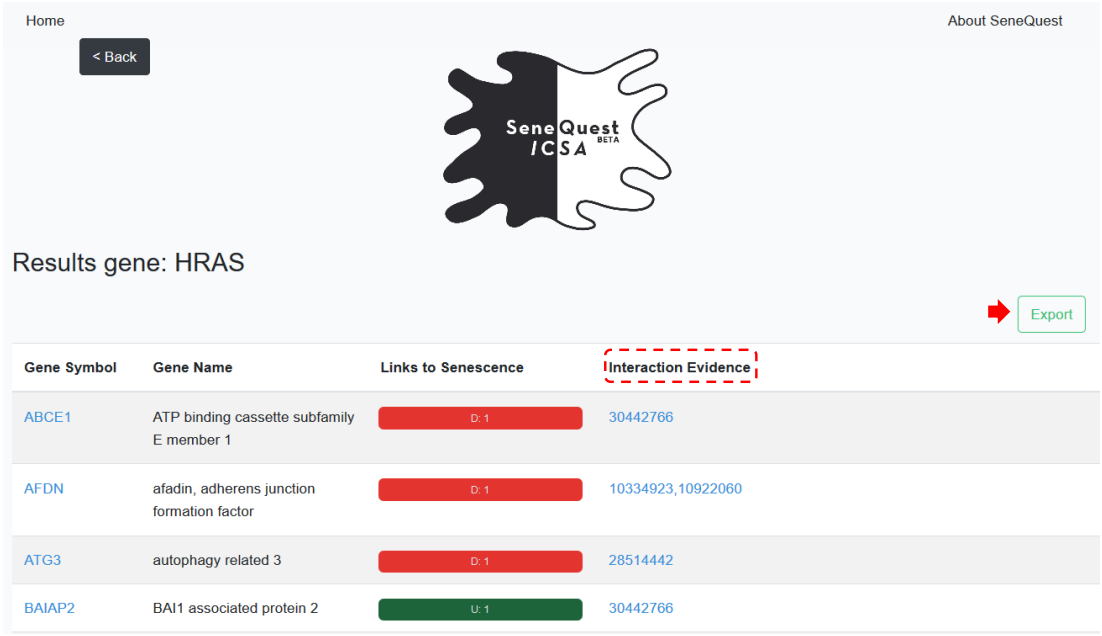
and upon “activation” a search is conducted retrieving senescence related genes linked to the specific GO term or to one of its descendants as defined in the GO tree.



6. Below the links leading to the human and mouse ortholog entries, available in step (**4b**) and located on the right side of the screen, there is a link termed "Search for interactions with genes linked to senescence". Pressing this selection will retrieve genes from the "database senescence-related genes" that interact with the specific gene specified in **step 3**. It must be noted that gene-to-GO and gene-to-gene relationships are retrieved from the Entrez gene database.



The outpout from the “Search for interactions with genes linked to senescence” option, as shown below, also provides “Interaction Evidence” in the form of PubMed IDs.



Moreover, selecting the “Export” option allows download (as a csv file) of the retrieved gene list.

In all pages the SeneQuest logo leads to home page, while selection of the “< Back” option returns to the previous page.