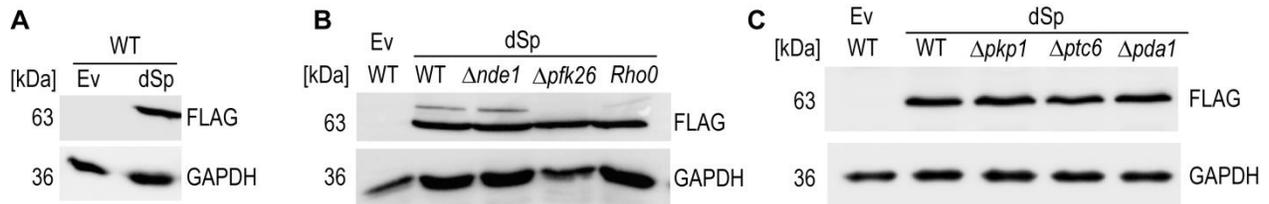


Supplementary Figures

Supplementary Figure 1



Supplementary Figure 1. Spartin expression is similar in all strains. *A.* Western blot analysis of dSp expression in WT and $\Delta ptc6$ cells, *B.* WT, $\Delta nde1$, $\Delta pfk26$ and $rho0$ cells and *C.* WT, $\Delta pkp1$, $\Delta ptc6$ or $\Delta pda1$ cells. The blots were probed with FLAG and as a loading control with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) antibodies and the corresponding secondary antibodies.

Supplementary Figure 2

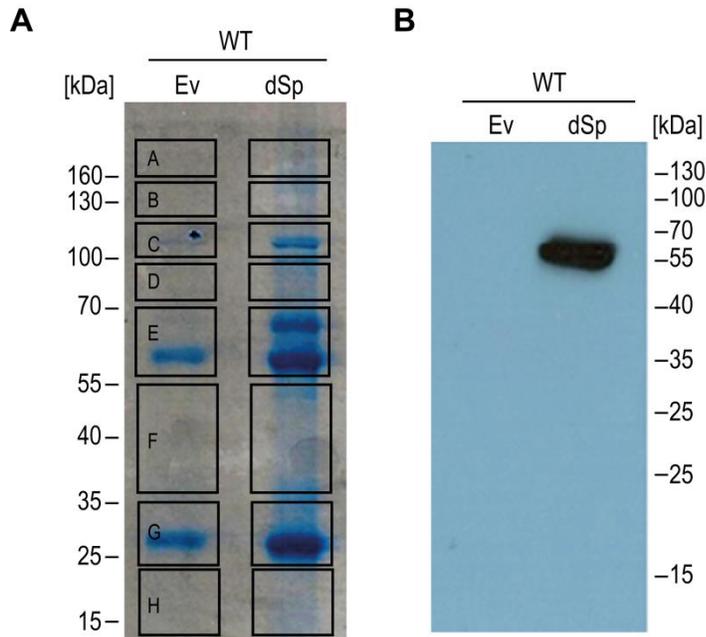
<i>Homo sapiens</i>	MEQEPQNGEPAEIKIIREAYKKAFLFVNKGLNTDELGQKEEAKNYKQIGHLLR--GIS
<i>D. melanogaster</i>	MAEEE-SEFLEAYAGIRTAAYKAAMTQVDLAVSHEEQESPQAIIVAYELALRMIEDTFGIP
<i>Homo sapiens</i>	IS-SKESEHTGPGWESARQMQQMKETLQNVTRLEILEKGLATSLQNDLQEVPKLYPEF
<i>D. melanogaster</i>	VGLPNKIDTVQAEWNDACALIQKLSAETELRYRLKVLRSQKQSIDDSAVEATEE-----
<i>Homo sapiens</i>	PPKDMCEKLPPEPQSFSSAPQHAEVNGNTSTPSAGAVAAPASLSLPSQSCPAAEAPPAYTPQ
<i>D. melanogaster</i>	SRAEMDTKRP--PLLAENPST-----QYGI---ANASGAPKTYREL
<i>Homo sapiens</i>	AAEGHYTVSYGTDSEGFSSVGEFFYRNHSQPPPLETLGLDADELILIPNGVQIFFVNPAG
<i>D. melanogaster</i>	AA-GLRELLAV---RDAKVLLELDFR-----AQVKMYRIEASG
<i>Homo sapiens</i>	EVSAPSYF-----GYLRIVRFLDNSLDTVLNRPFGFLQVCDWLYPLVPDRS
<i>D. melanogaster</i>	SVTTISGSSTMSLMVCTVGGKWKYLSGIYFIQC-----SMPNEGTAIWLPLYVPSIT
<i>Homo sapiens</i>	PVLKCTAGAYMFPDTMLQAGCFVGVVLSSELPEDDRE-LFEDLLRQMSDLRLQANWNRA
<i>D. melanogaster</i>	NCYQTEYGAFIFPDMCCQPGNAFGLMLTKEGQTSRTEDELEDLQQFFLDLLE-A-----
<i>Homo sapiens</i>	EEENEFQIPGRTRPSSDQLKEASGTDVKQLDQGNKDVRRHKGRKRAKDTSSSEEVNLSHI
<i>D. melanogaster</i>	-----VLAG-----TVVQLKSPT-----SQRAGL-----
<i>Homo sapiens</i>	VPCEPVPEEKPKELPEWSEKVAHNILSGASWVSWGLVKGAEITGKATQKGASKLRERIQP
<i>D. melanogaster</i>	-----ASDTVSGSEQVSRHIVSAADFIASNLVRGAEKTGGFMLRSTPYIISKMTP
<i>Homo sapiens</i>	EEKPVEVSPAVTKGLYIAKQATGGAAKVSQFLVDGVCIVANCVGKELAPHVKKHGSKLVP
<i>D. melanogaster</i>	ASMDAQVPSVQTSVEVAQKVTHAAAGMTGWIAGKVGITASMAVGRYLAPHIQEQGSKLLQ
<i>Homo sapiens</i>	ESLKKDK-DGKSPLDGAMVVAASSVQGFSTVWQGLECAAKCIVNNVSAETVQTVRYKGY
<i>D. melanogaster</i>	KGFGYDTSEANSTMEGAMTIAAGAVEGVSTVFDGLETSAKILGSSLSSENSVKIIEHKYGO
<i>Homo sapiens</i>	NAGEATHHAVDSAVNVGVTAYNINNIIGIKAMVKKTATQTGHTLLEDYQIVDNSQREN--Q
<i>D. melanogaster</i>	QTGNLASGTFDTVGNVVFVVSQNVNYITPKGIAKKMVKRTGEAVVSDYKRDLRKSESHYIN
<i>Homo sapiens</i>	EGAANVNRGEKDEQTEVKEAKKKDK
<i>D. melanogaster</i>	AGSLYPLDLRALKE-----

Domains: MIT UBR PSD

Conservation between groups of similar properties - roughly equivalent to scoring = > 0 in the Gonnet PAM 250 matrix

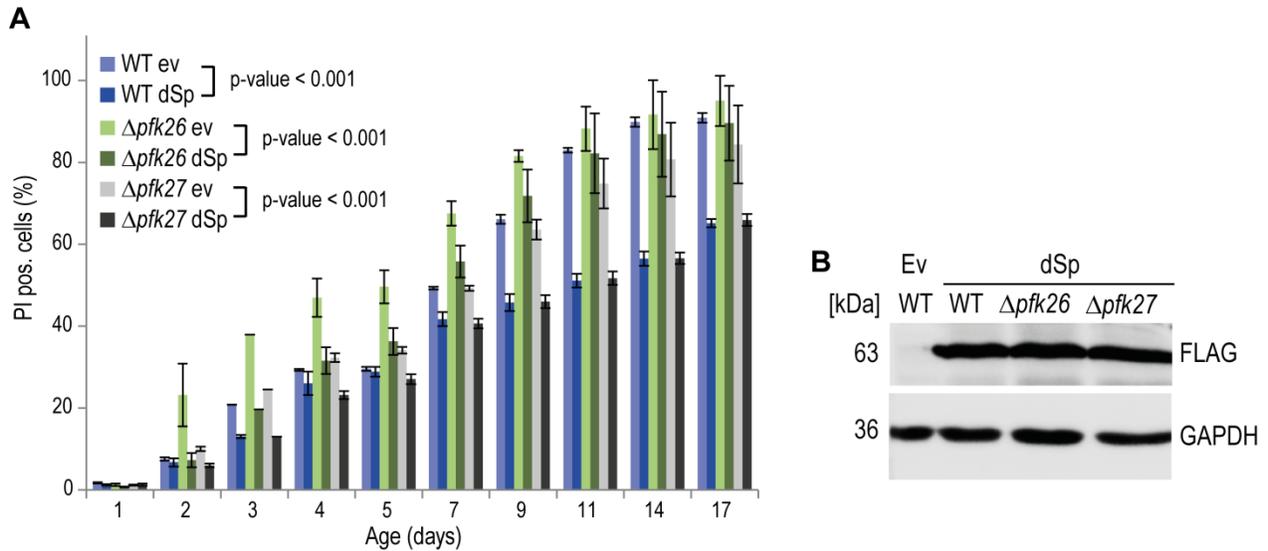
Positions of fully conserved residue

Supplementary Figure 2. Spartin is highly conserved from fly to human. Alignment of *Drosophila* and human Spartin. Identical residues are indicated with dark gray background. Similar residues are indicated by light gray background. The microtubule-interacting and trafficking (MIT) domain is marked green, ubiquitin binding region (UBR) in blue and plant related senescence domain (PSD) in red.

Supplementary Figure 3

Supplementary Figure 3. FLAG-Pulldown of dSpartin. A. Eluate from FLAG (FLAG beads) pull down on SDS gel coomassie stained. Letters correspond to bands on gel in Table B. and respective western blot analysis. The blot was probed with FLAG antibody and the corresponding secondary antibodies.

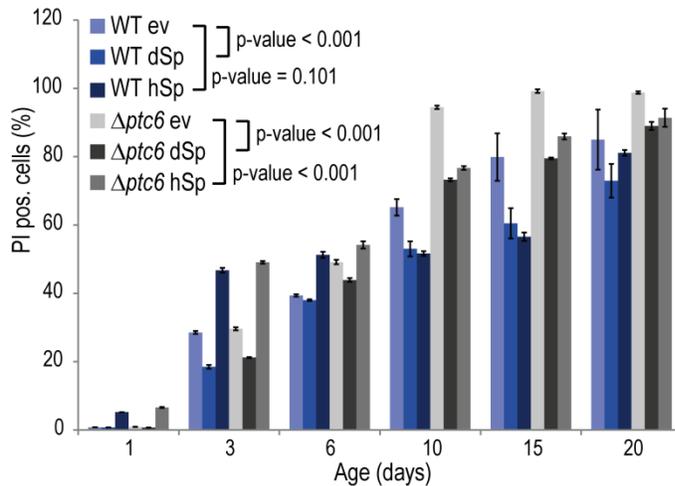
Supplementary Figure 4



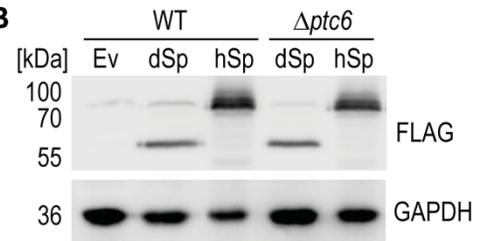
Supplementary Figure 4. Spartin displays a pro survival role depending on Pfk26 in old cells and complements $\Delta pfk26$ in young cells but is independent of Pfk27. *A.* Cell death during chronological aging of WT cells expressing dSp or cells harboring the corresponding empty vector control (ev). Dead cells are stained with propidium iodide (PI). Data represent mean values \pm SEM of at least four independent experiments performed at the same time. *B.* Western blot analysis of dSp expression in WT, $\Delta pfk26$ and $\Delta pfk27$ cells. The blot was probed with FLAG and as a loading control with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) antibody and the corresponding secondary antibodies.

Supplementary Figure 5

A



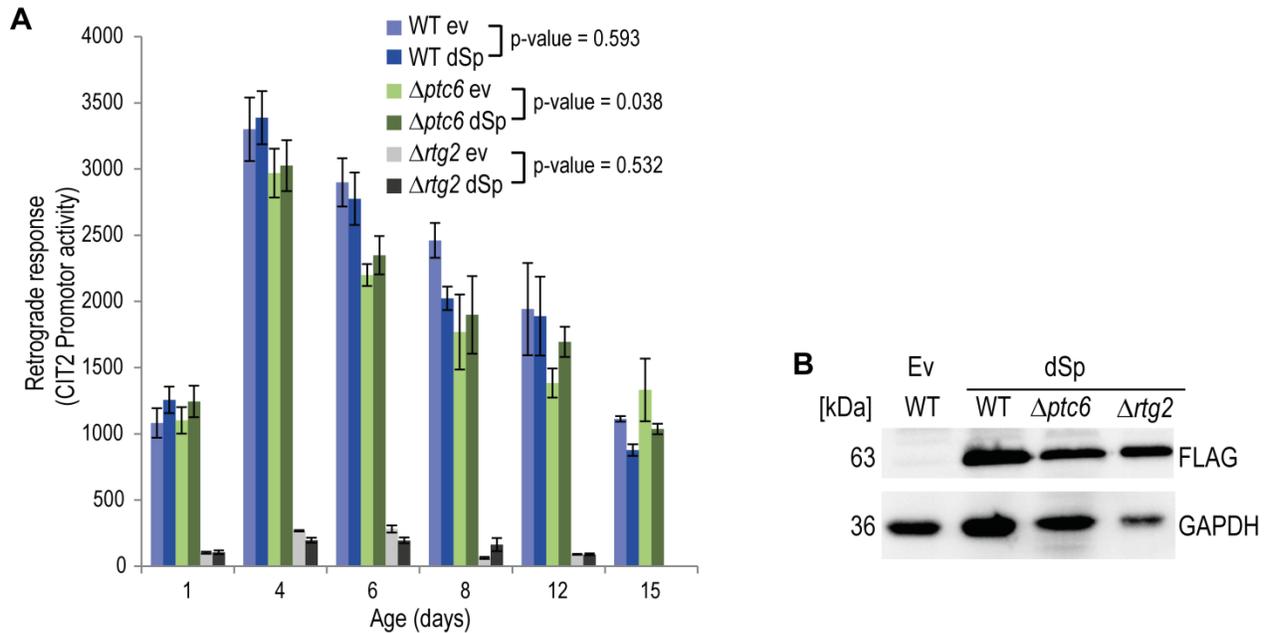
B



Supplementary Figure 5. *Drosophila* and human spartin promote longevity in yeast and complement the $\Delta ptc6$ survival phenotype.

A. Survival during chronological aging of WT cells expressing *Drosophila* (dSp), human spartin (hSp) or cells harboring the corresponding empty vector control (ev). Dead cells are stained with propidium iodide (PI). Data represent mean values \pm SEM of at least four independent experiments performed at the same time. **B.** Western blot analysis of dSp and hSp expression in WT and $\Delta ptc6$ cells. The blot was probed with FLAG and as a loading control with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) antibodies and the corresponding secondary antibodies.

Supplementary Figure 6



Supplementary Figure 6. Spartin does not influence retrograde response (RTG) signaling in yeast. *A.* Quantification of RTG activity by determining transcriptional activation of an integrated *CIT2-lacZ* reporter gene in WT, $\Delta ptc6$ and $\Delta rtg2$ cells expressing dSp or harboring the corresponding ev, additionally carrying pYEp355-*CIT2::lacZ*. β -Galactosidase activity was measured from cells at indicated time points during chronological aging. Data represent mean \pm SEM of at least three independent experiments performed at the same time. *B.* Western blot analysis of dSp expression in WT, $\Delta ptc6$ and $\Delta rtg2$ cells. The western blot was probed with FLAG and as a loading control glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) antibodies and the corresponding secondary antibodies.