

In the format provided by the authors and unedited.

Longitudinal comparative transcriptomics reveals unique mechanisms underlying extended healthspan in bats

Zixia Huang ¹, Conor V. Whelan¹, Nicole M. Foley¹, David Jebb¹, Frédéric Touzalin^{1,2}, Eric J. Petit ³, Sébastien J. Puechmaille ^{1,4,5} and Emma C. Teeling ^{1*}

¹School of Biology and Environmental Science, Science Centre West, University College Dublin, Dublin, Ireland. ²Bretagne Vivante-SEPNB, Brest, France.

³Ecology and Ecosystem Health, Agrocampus Ouest, INRA, Rennes, France. ⁴Zoological Institute and Museum, University of Greifswald, Greifswald,

Germany. ⁵ISEM, University of Montpellier, Montpellier, France. *e-mail: Emma.Teeling@ucd.ie

Supplementary Information for

Longitudinal comparative transcriptomics reveals unique mechanisms underlying extended healthspan in bats

Zixia Huang¹, Conor V. Whelan¹, Nicole M. Foley¹, David Jebb¹, Frédéric Touzalin^{1,2}, Eric J. Petit³, Sébastien J. Puechmaille^{1,4,5} and Emma C. Teeling^{1*}

¹School of Biology and Environmental Science, Science Centre West, University College Dublin, Dublin 4, Ireland.

²Bretagne Vivante-SEPNB, 19 rue de Gouesnou, 29221 Brest Cedex 2, BP 62132, France.

³ESE, Ecology and Ecosystem Health, Agrocampus Ouest, INRA, 35042 Rennes, France.

⁴Zoological Institute and Museum, University of Greifswald, 17489 Greifswald, Germany.

⁵ISEM, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France.

*To whom correspondence should be addressed. Email: Emma.Teeling@ucd.ie

This file includes:

Supplementary Information

Supplementary Figure 1-14

Supplementary Table 1-9

Supplementary Data 1-6

Supplementary Information

1. Super transcriptome assembly and annotation

The ‘super’ transcriptome references were constructed by merging all reference-based assemblies. 97,629 assembled transcripts were initially generated, and 48,749 were retained after quality control. These transcripts were categorized as 31,460 protein-coding mRNA, 10,775 LncRNA and 6,514 misc-RNA, and the majority of these RNA were lowly expressed (TPM < 1; Supplementary Fig. 2A). Of all samples, the average number of expressed transcripts was 36,774 (Supplementary Fig. 2B). There were no significant differences in the number of transcripts expressed between the samples from different age cohorts (Wilcoxon signed-rank test, $P > 0.05$).

2. Gene co-expression network analyses

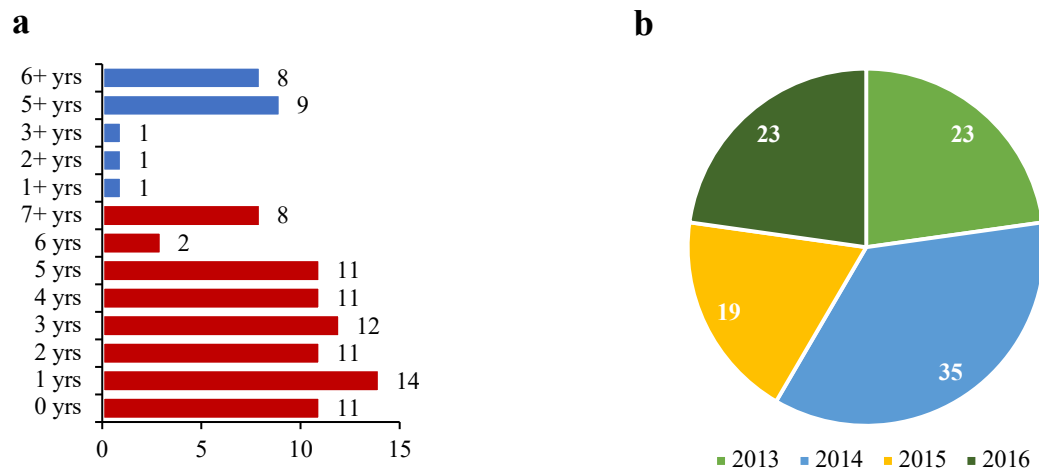
We maximized the differentially expressed (DE) genes detected by both *DESeq2* and *edgeR* in order to reduce false negatives and to obtain sufficient gene candidates for gene expression pattern analysis. Both methods reported a large overlap of common DE genes (Supplementary Table 4). It is noteworthy that fewer DE genes were detected between 6-year-old cohort and other cohorts, which possibly resulted from small sample size of 6-year-old cohort. All DE genes ($n = 6,692$) from pairwise differential expression analyses, which accounted for 54.6% of all genes examined in this study, were used in WGCNA analysis.

First of all, gene expression counts were TMM-normalized, and the outlier samples were detected and discarded using *hclust* and *cutreeStatic*, respectively. This removed 2 outlier samples (Supplementary Fig. 11). A power test was performed to determine the soft-threshold using *pickSoftThreshold*. The optimal soft-threshold power was set to 12 (Supplementary Fig. 12). This threshold was further used to compute gene expression distance for module detection. Based on the distance matrix, genes were subsequently clustered using the average linkage hierarchical clustering method using *hclust*, and the expression modules were detected using *dynamicTreeCut*. The modules with similar patterns were further clustered and merged into the consensus modules. The correlation between the consensus modules and age was calculated using *corPvalueStudent*. Collectively, 5,427 genes were clustered into 7 distinct modules while the rest of 1,265 genes were uncharacterized and were not part of any modules (Supplementary Fig. 13). In addition to module detection, the correlation between each module and age was also calculated (Supplementary Table 5). According to the threshold of significance ($P < 0.05$), 3 modules displayed positive correlation with age, 2 were negatively correlated and 2 had no correlation with age (Fig. 2). For each module, the top enriched GO terms were listed in Supplementary Fig. 14.

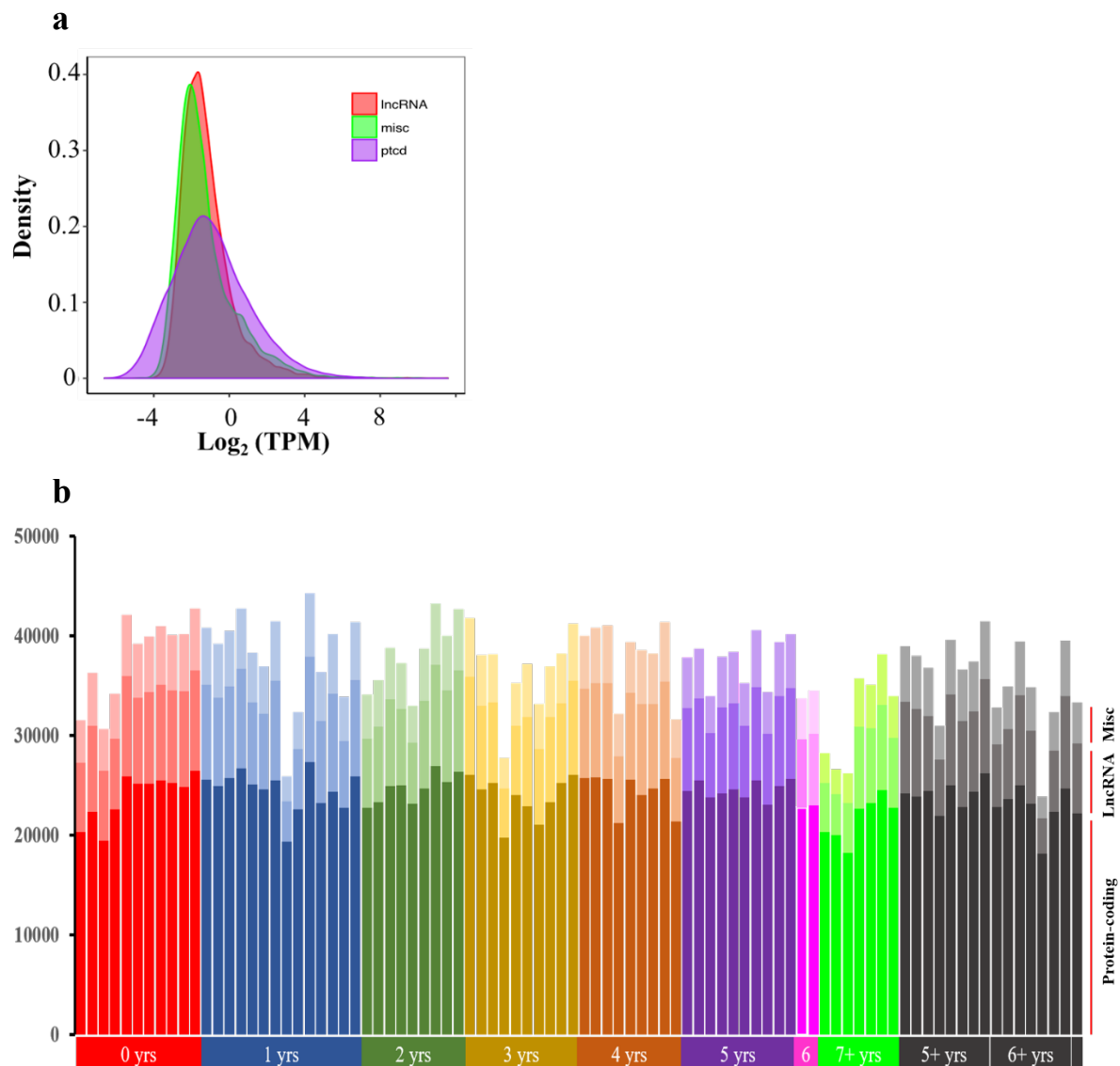
3. miRNome analyses

The summary of 50 miRNA-Seq samples is listed in Supplementary Table 7. In total, over 575 million 50 bp short RNA reads were generated. For each sample, on average 83,537 unique reads were obtained after quality control and duplication removal, approximately 27.5% of which could be unambiguously mapped to the reference genome (*M. lucifigus*). The miRNA identification pipeline predicted 117 known miRNA and 56 novel miRNA. The majority of DE miRNA between juvenile and adult participate in developmental processes. For instance, let-7d, let-7f and let-7g, which are the conserved miRNA proposed as critical regulators of development that control timing decisions, showed significant up-regulation (Supplementary Fig. 6).

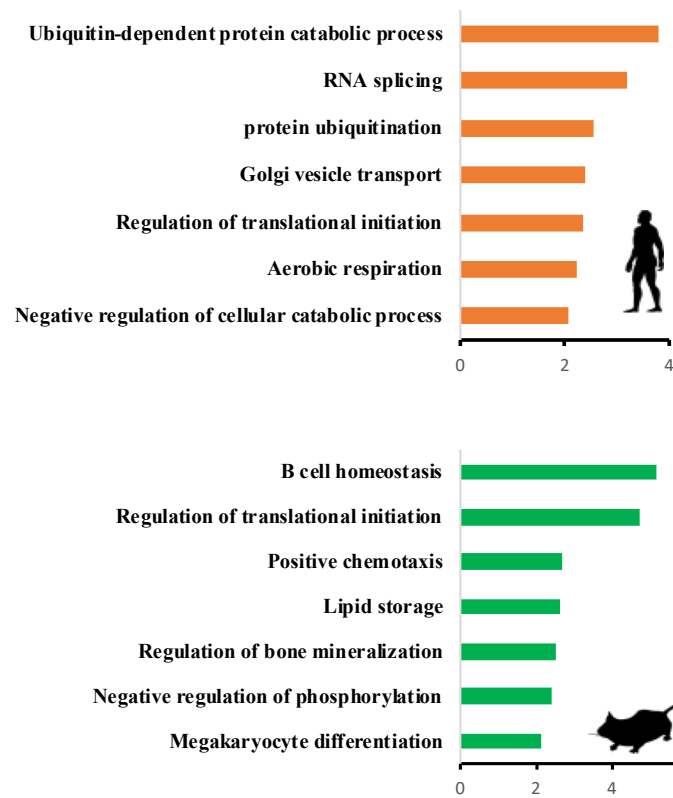
To gain insights into how miRNA regulated the age-related genes and pathways in *M. myotis*, we *in silico* predicted the ‘targetome’ of the miRNA. To achieve this, 17,459 3’-UTR sequences were predicted from the ‘super’ transcriptome assembly, 7,051 of which were confirmed in the UTRdb database, and were therefore used in miRNA target prediction. The average number of mRNA targets per miRNA was 86, with miR-330 having the largest number of targets ($n = 563$). To further characterize the miRNA-mRNA pairs with negative expression correlation, the Spearman’s ranked correlation coefficient for each pair was computed. These miRNA-mediated transcripts were mainly involved in the pathways associated with leukocyte differentiation, chromatin modification, viral process and cell cycle (Supplementary Fig. 7). The genes, which were involved in cell cycle regulation and DNA repair and were predicted to be regulated by miRNA, were listed in Supplementary Table 8. The top 50 miRNA-mRNA pairs with significant negative correlation were listed in Supplementary Table 9.



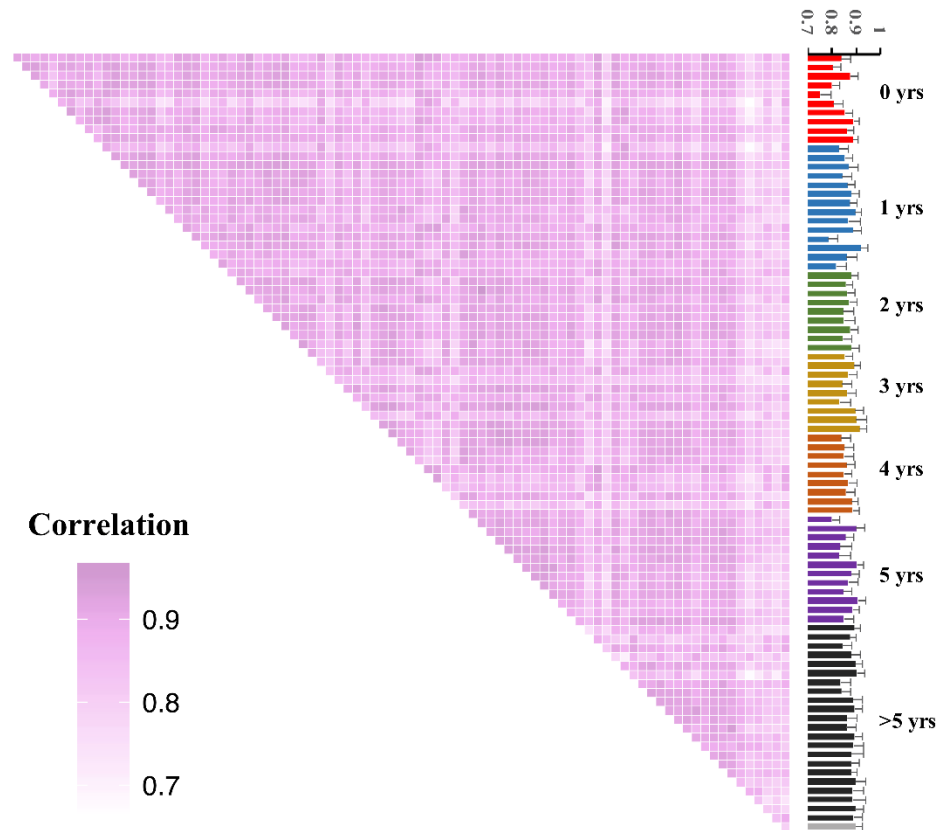
Supplementary Figure 1. Distribution of samples. **a)** Cohort distribution of samples. The samples in red were used in age cohort analyses. All samples contributed to the super transcriptome assembly. **b)** Year-of-capture distribution of samples.



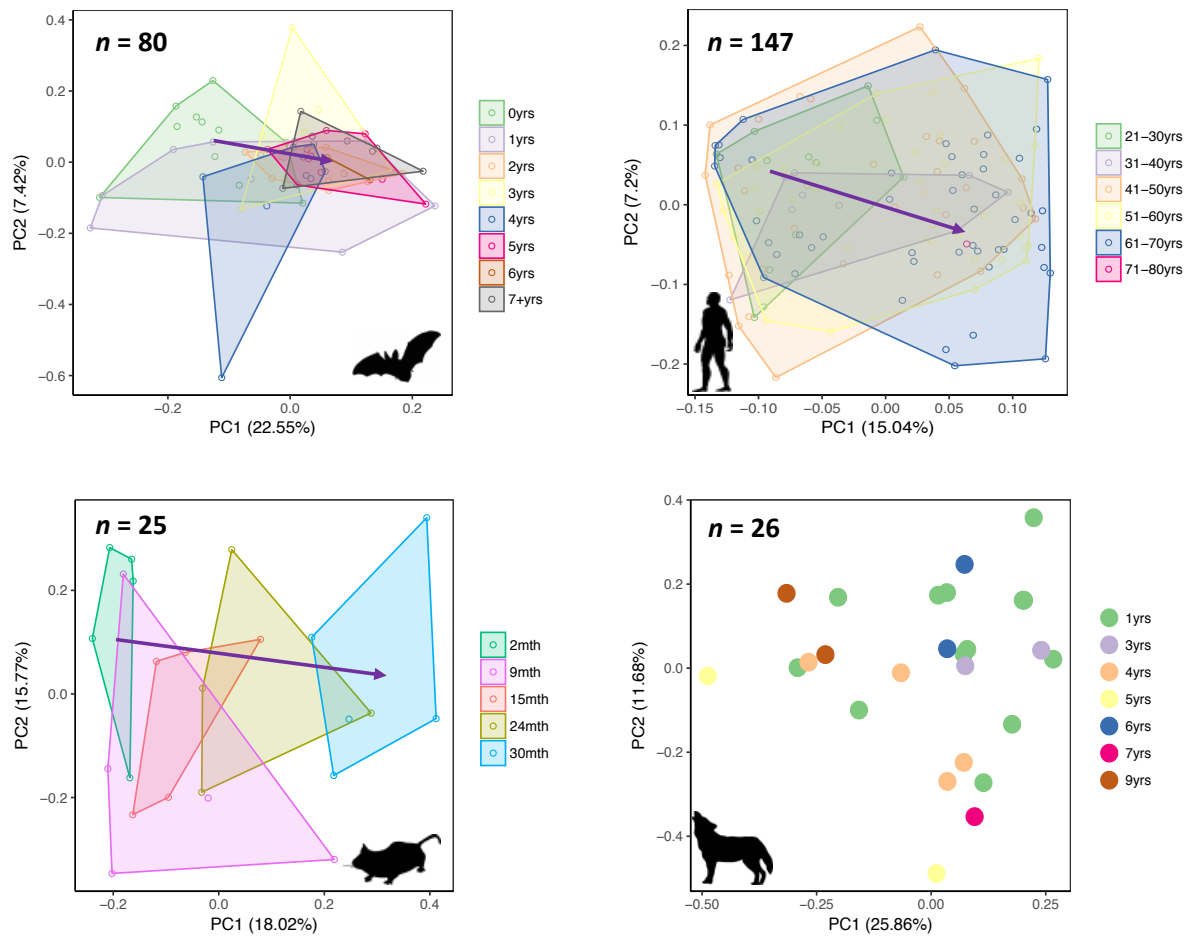
Supplementary Figure 2. Summary statistics for the assembled transcripts. a) Expression distribution of protein-coding transcripts, LncRNA and miscellaneous RNA respectively. The x-axis represents transcript expression values in TPM, and the y-axis represents the frequency of transcripts. **b) The number of transcripts expressed in each sample.** For each sample, the color indicates different types of transcripts. The last sample was HWJ-33 (3+ - year-old).



Supplementary Figure 3. The top enriched GO terms of top 100 highly expressed genes in human and mouse.



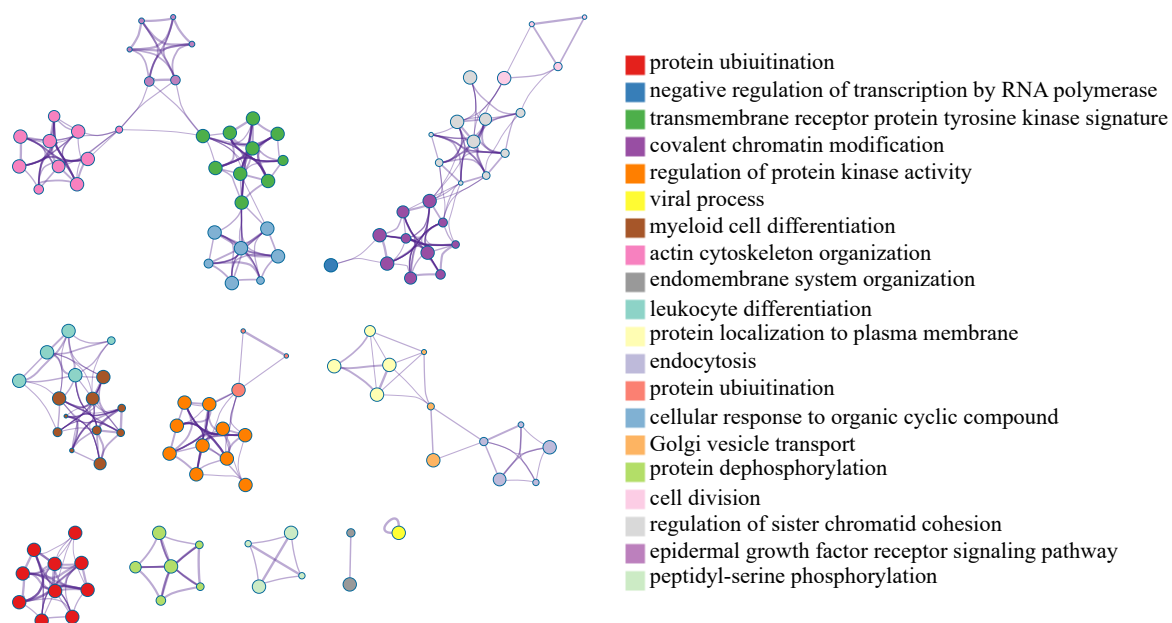
Supplementary Figure 4. The pairwise transcript expression correlation between samples. Spearman's rank correlation coefficient tests were performed based on the expression levels of 48,749 transcripts. The mean correlation coefficient of each sample to all other samples was calculated, with standard deviation depicted as an error bar. Different colors indicate the samples from different age cohorts. The >5 group (black) included the samples marked with age 5+, 6, 6+ and 7+. The last gray bar was the sample HWJ-33 (3+ -year-old).



Supplementary Figure 5. Principal Component Analysis (PCA) based on the expression data of all expressed genes in bat, human, mouse and wolf. n indicates the number of samples used in the analysis and the color frames indicate different age cohorts. Framing samples by age did not apply to wolf due to biased sample distribution.

	0yrs	1yrs	2yrs	3yrs	4yrs	5yrs	6yrs	7+yrs
0yrs		10	12	8	18	29	18	29
1yrs	-9		6	0	10	21	16	22
2yrs	-19	-10		10	0	8	3	7
3yrs	-12	0	-3		10	22	23	26
4yrs	-25	-13	0	-15		5	5	7
5yrs	-30	-21	-11	-24	-5		0	0
6yrs	-25	-17	-5	-20	-3	0		0
7+yrs	-25	-25	-14	-27	-7	0	0	

Supplementary Figure 6. The number of DE miRNA detected by pairwise differential expression analyses. The positive values indicate the number of the up-regulated DE miRNA while the negative values indicate the number of the down-regulated DE miRNA.



Supplementary Figure 7. The network of GO terms that were regulated by miRNA. Each circle represents a GO term. The circles with the same color belong to a common parental term. The connectivity between GO terms indicates that they shared the common genes.

Before

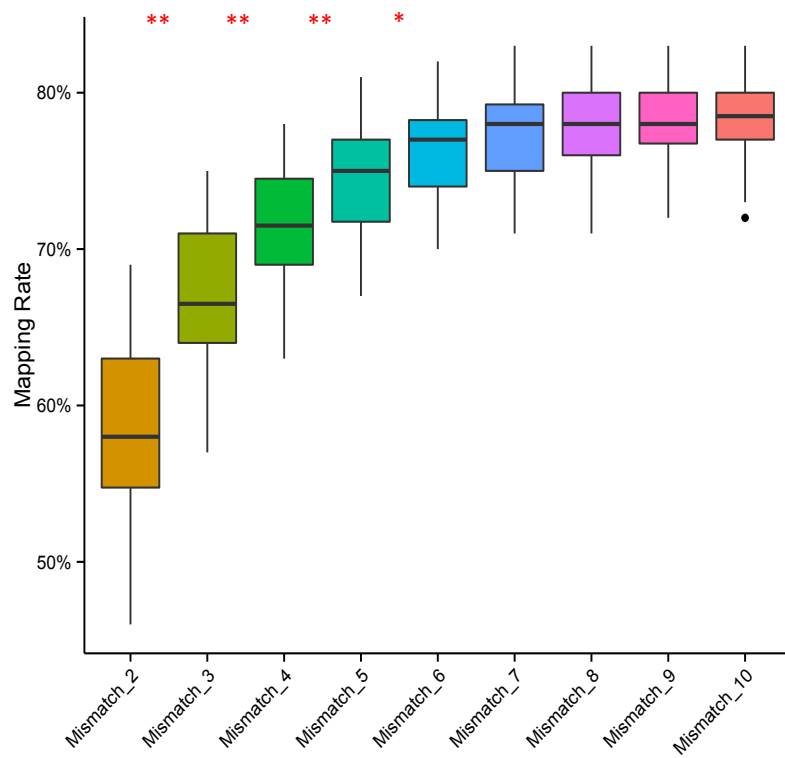
After

0.00	0.13	CGGGGCTCGGCTATGTTGCCAGGCTGGAGTGCAGTGGCATTATCCACAGGC
0.12	0.34	GGGGTCTCGCTATGTTGCCAGGCTGGAGTGCAGTGGCATTATCCACAGGCG
0.00	0.13	GGTCTCGCTATGTTGCCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGG
0.00	0.28	GTCTGCTATGTTGCCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGA
0.00	0.28	CTGCTATGTTGCCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATC
0.00	0.14	TGCTATGTTGCCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCC
0.00	0.33	CGCTATGTTGCCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCC
0.00	0.12	GCTATGTTGCCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCA
0.00	0.23	GTTGCCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACT
0.13	0.13	GTCCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACTGAT
0.13	0.70	GGCCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACTGAT
0.00	0.47	CCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACTGATC
0.00	0.19	CCGGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACTGATCA
0.20	1.99	CCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACTGATCA
0.00	0.84	CAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACTGATCAG
0.00	0.23	AGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACTGATCAGC
0.00	0.44	GGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACTGATCAGCA
0.00	0.12	GCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACTGATCAGCAG
0.00	0.30	CTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACTGATCAGCAG
0.10	0.00	GGAGTGCATTATCCACAGGCGGATCCCACTACTGATCAGCAGGGAGCTT
0.33	0.30	GTGGCTATCCACAGGCGGATCCCACTACTGATCAGCAGGGAGCTTTGA
0.16	0.25	TGGCTATCCACAGGCGGATCCCACTACTGATCAGCAGGGAGCTTTGAC
0.16	0.21	GGCTATCCACAGGCGGATCCCACTACTGATCAGCAGGGAGCTTTGAC
0.23	0.26	GCTATCCACAGGCGGATCCCACTACTGATCAGCAGGGAGCTTTGACCT
0.10	0.23	CTATCCACAGGCGGATCCCACTACTGATCAGCAGGGAGCTTTGACCTGT
0.00	0.19	ATCCACAGGCGGATCCCACTACTGATCAGCAGGGAGCTTTGACCTGCT
0.00	0.13	CACCTACTGATCAGCAGGGAGCTTTGACCTGCTCGCTCCGACTGGGC
0.00	0.17	CTACTGATCAGCAGGGAGCTTTGACCTGCTCGCTCCGACTGGGCGG
0.00	0.12	CAGCAGGGAGCTTTGACCTGCTCGCTCCGACTGGGCGGGTTCACCC
0.12	0.23	CTGCTCGCTCCGACTGGGCGGGTTCACCCCTCTTAGGCAACTGGTG
0.89	0.47	GTCCTGCTCCGACTGGGCGGGTTCACCCCTCTTAGGCAACTGGTG
0.32	0.36	CTCCTGCTCCGACTGGGCGGGTTCACCCCTCTTAGGCAACTGGTG
0.18	0.22	TCCTGCTCCGACTGGGCGGGTTCACCCCTCTTAGGCAACTGGTG
0.17	0.29	CCCTGCTCCGACTGGGCGGGTTCACCCCTCTTAGGCAACTGGTG
0.13	0.12	GTCCTCCGACTGGGCGGGTTCACCCCTCTTAGGCAACTGGTG
0.63	0.47	CTCCGACTGGGCGGGTTCACCCCTCTTAGGCAACTGGTG
0.13	0.00	CCGACTGGGCGGGTTCACCCCTCTTAGGCAACTGGTG
0.13	0.33	GGGCGGTTCACCCCTCTTAGGCAACTGGTG
0.00	0.24	GGGCGGTTCACCCCTCTTAGGCAACTGGTG
1.46	0.17	CACCCCTCTTAGGCAACTGGTG
0.00	0.00	CGCCCTCTCTTAGGCAACTGGTG
0.62	0.00	GGCCCTCTCTTAGGCAACTGGTG
0.23	0.00	ACCCCTCTCTTAGGCAACTGGTG
0.12	0.00	CGCCCTCTTAGGCAACTGGTG
6.32	0.84	CGCCCTCTTAGGCAACTGGTG
4.90	0.78	TCCTCTTAGGCAACTGGTG
2.94	0.49	CTCTCTTAGGCAACTGGTG
3.19	0.62	CTCTCTTAGGCAACTGGTG
1.30	0.24	CTCTCTTAGGCAACTGGTG
4.86	1.75	CTCTCTTAGGCAACTGGTG
1.36	0.89	CTCTCTTAGGCAACTGGTG

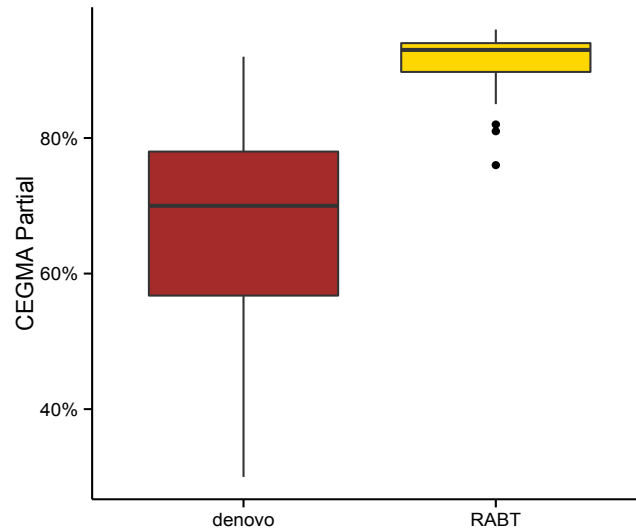
7sRNA

GGGGGCTCGGCTATGTTGCCAGGCTGGAGTGCAGTGGCATTATCCACAGGCGGATCCCACTACTGATCAGCAGGGAGCTTTGACCTGCTCGCTCCGACTGGGCGGGTTCACCCCTCTTAGGCAACTGGTG

Supplementary Figure 8. Evaluation of 7s rRNA depletion performance before and after the depletion step. The figure shows the mapping of short Illumina MiSeq reads to the 7s rRNA transcript. The values on the left indicate the percentages of short reads presented in the whole sequencing output. The highlighted values suggest that the number of short reads that mapped to the 3'-end of 7s rRNA significantly reduced after the depletion step.



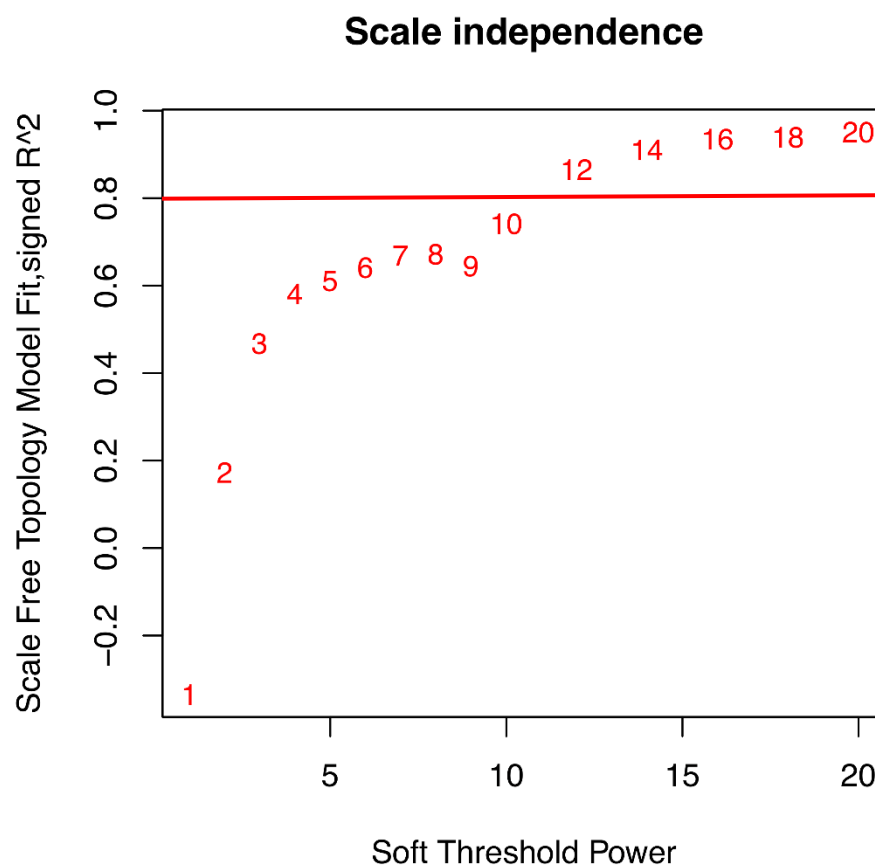
Supplementary Figure 9. Genome mapping tests with different mismatch numbers per alignment. The * represents the statistical significance between the adjacent groups using Student's *t*-tests ($p < 0.01$ **, $0.01 < p < 0.05$ *).



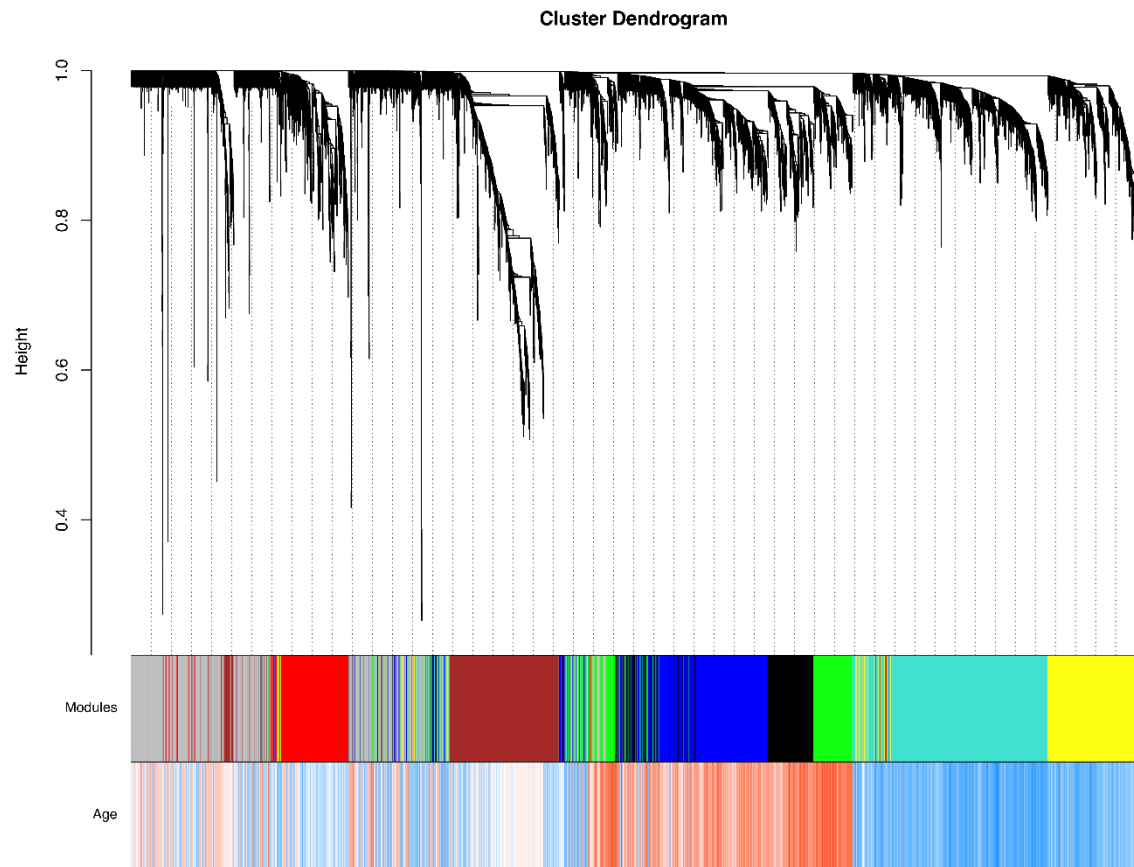
Supplementary Figure 10. The transcriptome completeness index (CI) assessed by CEMGA using both *de novo* and RABT methods. RABT: Reference Annotation-Based Transcript.

Detailed description: This dendrogram illustrates the hierarchical clustering of 150 samples. The vertical axis, labeled 'Height', represents the distance between clusters, with major ticks at 0e+00, 2e+07, 4e+07, 6e+07, and 8e+07. A prominent red horizontal line is positioned at the 4e+07 mark. The samples, listed along the horizontal axis, are: HWJ.96, HWJ.98, HWJ.104, HWJ.130, HWJ.32, HWJ.29, HWJ.102, HWJ.53, HWJ.63, HWJ.31, HWJ.135, HWJ.62, HWJ.4b, HWJ.38, HWJ.59, HWJ.42, HWJ.113, HWJ.118, HWJ.8b, HWJ.51, HWJ.34, HWJ.103, HWJ.111, HWJ.97, HWJ.128, HWJ.101, HWJ.105, HWJ.126, HWJ.136, HWJ.127, HWJ.131, HWJ.106, HWJ.2b, HWJ.11, HWJ.53, HWJ.46, HWJ.10, HWJ.153, HWJ.155, HWJ.58, HWJ.129, HWJ.143, HWJ.172, HWJ.35, HWJ.99, HWJ.114, HWJ.17, HWJ.39, HWJ.19, HWJ.37, HWJ.110, HWJ.156, HWJ.148, HWJ.3b, HWJ.51, HWJ.154, HWJ.152, HWJ.149, HWJ.90, HWJ.41, HWJ.95, HWJ.112, HWJ.40, and HWJ.150. The clustering shows that most samples join at heights below 2e+07, with a few outliers like HWJ.99 and HWJ.114 joining at around 2e+07. The samples HWJ.40 and HWJ.150 form a separate cluster that joins the main group at a height of approximately 4e+07, which is highlighted by the red line.

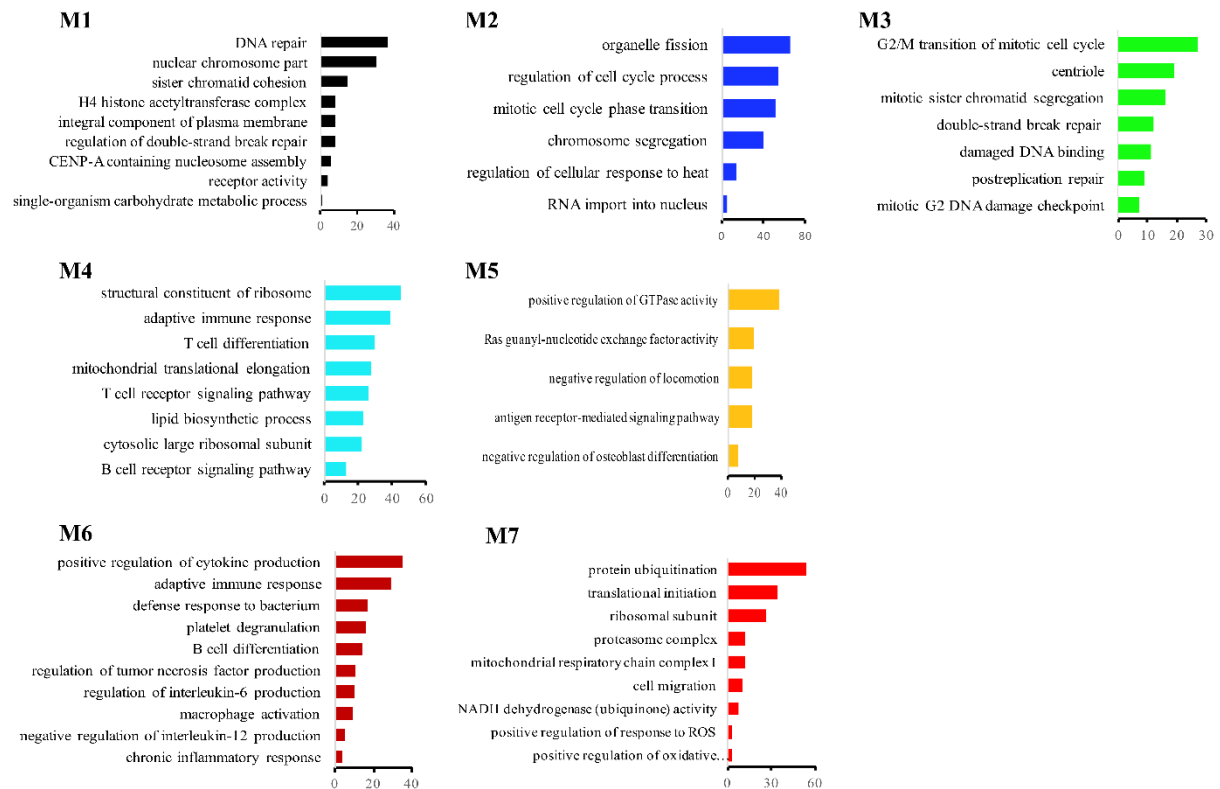
14



Supplementary Figure 12. The tests on the determination of the optimal soft threshold power. Based on the scale free topology model fit value, 12 was selected as the optimal soft threshold power.



Supplementary Figure 13. The Clustering tree of 6,692 genes and their correlation with age. In the first lane, the colors indicate the genes in different expression modules. In the second lane, the colors indicate the correlation between each gene and age. Blue: negative correlation; white: no correlation; red: positive correlation.



Supplementary Figure 14. The top GO terms enriched in each module. The x-axis indicates the number of genes involved in each GO term.

Supplementary Table 1. The information of 150 samples used in this study. MMY_ID: sample ID; RNA-Seq and miRNA-Seq: sequencing index; Age: the age of samples when collected; Individual: the transponder number for each individual bat; Site: the locations where the samples were collected; Year: the years when the samples were collected; Batch: sequencing batches.

MMY_ID	RNA-Seq	miRNA-Seq	Age	Individual	Colony	Year	Batch
MMY352	HWJ-1b		0	00072A069A	Férel	2013	A
MMY202	HWJ-2b	HWJ-72	0	000715E030	Béganne	2013	A
MMY171	HWJ-3b		0	000718D48D	Béganne	2013	A
MMY161	HWJ-4b		0	0007192882	Béganne	2013	A
MMY535	HWJ-7b	HWJ-74	0	0007287D32	Férel	2013	A
MMY283	HWJ-8b		0	000728A17A	Férel	2013	A
MMY330	HWJ-9	HWJ-73	0	000728D281	Férel	2013	A
MMY537	HWJ-10	HWJ-76	0	000728F304	Noyal-Muzillac	2013	A
MMY888	HWJ-11		0	000715A06F	La Roche-Bernard	2014	A
MMY577	HWJ-12		0	000719061D	Béganne	2014	A
MMY407	HWJ-13		0	00074EE4BE	Noyal-Muzillac	2013	A
MMY872	HWJ-51		1	000715AA2A	La Roche-Bernard	2014	A
MMY811	HWJ-52		1	00074EE4BE	Noyal-Muzillac	2014	A
MMY740	HWJ-53	HWJ-80	1	0007287D32	Férel	2014	A
MMY181	HWJ-54	HWJ-81	1	000715A4DE	Béganne	2013	A
MMY939	HWJ-55		1	00074F0B7B	Noyal-Muzillac	2014	A
MMY466	HWJ-57		1	0007190397	Béganne	2014	A
MMY256	HWJ-58		1	000715C719	Béganne	2013	A
MMY691	HWJ-59		1	00074EE4BE1	Férel	2014	A
MMY318	HWJ-60		1	000718D6B2	Férel	2013	A
MMY612	HWJ-61		1	000715A07B	Béganne	2014	A
MMY317	HWJ-62		1	0006E22EA6	Férel	2013	A
MMY897	HWJ-63		1	00074F018B	Noyal-Muzillac	2014	A
MMY582	HWJ-28	HWJ-77	1	000715E030	Béganne	2014	A
MMY730	HWJ-29	HWJ-79	1	000728D281	Férel	2014	A
MMY54	HWJ-31		3	000702E6B0	La Roche-Bernard	2013	A
MMY209	HWJ-32	HWJ-83	3	000702F397	Béganne	2013	A
MMY198	HWJ-34	HWJ-82	3	000702FF51	Béganne	2013	A
MMY670	HWJ-35	HWJ-85	3	0006B39389	Béganne	2014	A
MMY744	HWJ-36		3	0006B3B2E8	Férel	2014	A
MMY701	HWJ-37	HWJ-86	3	0006B3B5B8	Béganne	2014	A
MMY646	HWJ-38		3	0006B3BC85	Béganne	2014	A
MMY457	HWJ-39		3	0006B3D199	Béganne	2014	A
MMY458	HWJ-40		3	0006E23728	Béganne	2014	A
MMY465	HWJ-41		3	0006E2388C	Béganne	2014	A
MMY602	HWJ-42	HWJ-92	3	0006E248E6	Béganne	2014	A
MMY182	HWJ-48		3	000702EDA5	Béganne	2013	A
MMY726	HWJ-64		5+	000702FEF1	Béganne	2014	A

MMY_ID	RNA-Seq	miRNA-Seq	Age	Individual	Site	Year	Batch
MMY640	HWJ-65		5+	000702F849	Béganne	2014	A
MMY674	HWJ-66		5+	000702E600	Béganne	2014	A
MMY621	HWJ-67		5+	000702E4B6	Béganne	2014	A
MMY669	HWJ-45	HWJ-89	5+	0006F1EFEB	Béganne	2014	A
MMY641	HWJ-46	HWJ-88	5+	0007026EE7	Béganne	2014	A
MMY629	HWJ-47	HWJ-91	5+	000702873D	Béganne	2014	A
MMY445	HWJ-49	HWJ-90	5+	000703035B	Béganne	2014	A
MMY668	HWJ-50	HWJ-87	5+	0007030BAB	Béganne	2014	A
MMY104	HWJ-93	HWJ-116	2	0006E23728	Béganne	2013	B
MMY197	HWJ-94	HWJ-117	2	0006B39389	Béganne	2013	B
MMY107	HWJ-95	HWJ-118	2	0006B3B5B8	Béganne	2013	B
MMY2323	HWJ-96	HWJ-119	2	000715AA2A	La Roche-Bernard	2015	B
MMY180	HWJ-97	HWJ-120	2	0006B3D199	Béganne	2013	B
MMY1233	HWJ-98		2	0007190397	Béganne	2015	B
MMY733	HWJ-99		2	0006B38DA8	Férel	2014	B
MMY2314	HWJ-100		2	0007192882	Béganne	2015	B
MMY2369	HWJ-101		2	000715A07B	Béganne	2015	B
MMY890	HWJ-102		2	0007192881	La Roche-Bernard	2014	B
MMY706	HWJ-103		2	0007158EC8	Férel	2014	B
MMY2389	HWJ-105	HWJ-121	4	0006B3EB3E	Béganne	2015	B
MMY2406	HWJ-106	HWJ-122	4	0006E242EE	Limerzel	2015	B
MMY1094	HWJ-108	HWJ-124	4	0006B3B5B8	Béganne	2015	B
MMY2365	HWJ-109	HWJ-125	4	0006B3BC85	Béganne	2015	B
MMY566	HWJ-110		4	0007025E92	Béganne	2014	B
MMY2313	HWJ-111		4	000634B9B3	La Roche-Bernard	2015	B
MMY462	HWJ-112		4	000702FF51	Béganne	2014	B
MMY2296	HWJ-113		4	0006E24A1B	Béganne	2015	B
MMY664	HWJ-114		4	000702F397	Béganne	2014	B
MMY2426	HWJ-115		4	0006B39389	Béganne	2015	B
MMY2861	HWJ-126	HWJ-157	5	0006E24B4C	Limerzel	2016	C
MMY2857	HWJ-127	HWJ-158	5	0006B3A496	Limerzel	2016	C
MMY2363	HWJ-128	HWJ-159	5	000702F397	Béganne	2015	C
MMY3204	HWJ-129	HWJ-160	5	0006B3EB3E	Béganne	2016	C
MMY3050	HWJ-130	HWJ-161	5	000634B9B3	La Roche-Bernard	2016	C
MMY2555	HWJ-131	HWJ-162	5	0006B39389	Béganne	2016	C
MMY2860	HWJ-132	HWJ-163	5	0006E242EE	Limerzel	2016	C
MMY2780	HWJ-133		5	0006B3B5B8	Béganne	2016	C
MMY1230	HWJ-134		5	000702FF51	Béganne	2015	C
MMY2455	HWJ-135		5	000702E6B0	La Roche-Bernard	2015	C
MMY2298	HWJ-136		5	000702EA84	Béganne	2015	C
MMY2638	HWJ-137	HWJ-164	6+	0006B3AC86	Béganne	2016	C

MMY_ID	RNA-Seq	miRNA-Seq	Age	Individual	Site	Year	Batch
MMY2636	HWJ-138	HWJ-165	6+	0006B37F3D	Béganne	2016	C
MMY2379	HWJ-139	HWJ-166	6+	0007030CDF	Béganne	2015	C
MMY2403	HWJ-140	HWJ-167	6+	00070291D6	Limerzel	2015	C
MMY3119	HWJ-141	HWJ-168	6+	0006B3B98F	Limerzel	2016	C
MMY2368	HWJ-142	HWJ-169	6+	0007027D1E	Béganne	2015	C
MMY2793	HWJ-143		6+	0006B36FE3	Férel	2016	C
MMY3183	HWJ-144		6+	0006B3A9FD	Béganne	2016	C
MMY2675	HWJ-145	HWJ-170	6	000702EA84	Béganne	2016	C
MMY3120	HWJ-146	HWJ-171	6	000695849D	Limerzel	2016	C
MMY3127		HWJ-172	6	0007030043	Béganne	2016	C
MMY2855	HWJ-148	HWJ-173	7+	000702EDD6	Limerzel	2016	C
MMY3176	HWJ-149	HWJ-174	7+	000702E600	Béganne	2016	C
MMY2626	HWJ-150	HWJ-175	7+	0006F1EFEB	Béganne	2016	C
MMY2877	HWJ-151	HWJ-176	7+	000703035B	Béganne	2016	C
MMY2856	HWJ-152		7+	000702FC8C	Limerzel	2016	C
MMY2635	HWJ-153		7+	0007027A0E	Béganne	2016	C
MMY3118	HWJ-154		7+	000702DAA5	Limerzel	2016	C
MMY3153	HWJ-155		7+	000702E190	Béganne	2016	C
MMY2352	HWJ-156	HWJ-123	4	0006B3E42D	Béganne	2015	C
MMY345	HWJ-6b	HWJ-75	1+	00072847C2	Férel	2013	A
MMY689	HWJ-56	HWJ-78	2+	00072847C2	Férel	2014	A
MMY187	HWJ-33		3+	000702FD14	Béganne	2013	A

Supplementary Table 2. Geographical coordinates of the five colonies

Colony	Latitude	Longitude	No. of samples
Béganne	N47°596'	W2°239'	63
Noyal-Muzillac	N47°591'	W2°456'	5
Férel	N47°482'	W2°344'	15
La Roche-Bernard	N47°519'	W2°301'	8
Limerzel	N47°636'	W2°354'	10

Supplementary Table 3. The summary of 100 RNA-Seq samples. *J represents juvenile; A represents adult.

Sample	Age*	No. of raw reads	No. of reads after adaptors trimming	No. of reads after QC	No. of unique paired reads
HWJ-1b	J0	76,450,419	74,983,517 (98.1%)	63,289,652	11,791,832
HWJ-2b	J0	74,387,899	73,349,271 (98.6%)	61,375,764	12,140,052
HWJ-3b	J0	81,674,327	80,445,204 (98.5%)	67,598,375	12,203,452
HWJ-4b	J0	75,204,051	73,406,477 (97.6%)	59,989,782	14,838,401
HWJ-7b	J0	89,989,550	88,455,042 (98.3%)	73,550,112	19,165,999
HWJ-8b	J0	86,729,666	84,857,439 (97.8%)	70,541,099	18,165,848
HWJ-9	J0	72,521,477	71,704,263 (98.9%)	58,732,781	15,580,775
HWJ-10	J0	77,884,623	77,325,965 (99.3%)	64,417,155	17,925,378
HWJ-11	J0	70,240,155	69,633,811 (99.1%)	55,583,007	15,093,199
HWJ-12	J0	75,409,759	74,680,974 (99.0%)	62,003,007	15,742,287
HWJ-13	J0	71,361,124	70,818,576 (99.2%)	58,406,547	20,356,453
HWJ-51	A1	53,344,066	52,922,101 (99.2%)	45,901,154	12,168,873
HWJ-52	A1	66,971,252	66,463,611 (99.2%)	57,045,326	16,924,418
HWJ-53	A1	70,438,337	69,764,147 (99.0%)	60,452,113	14,601,896
HWJ-54	A1	67,386,495	66,744,092 (99.0%)	58,228,393	11,989,855
HWJ-55	A1	71,054,270	70,282,592 (98.9%)	61,748,904	14,841,085
HWJ-57	A1	70,030,944	69,315,495 (99.0%)	60,291,906	9,756,922
HWJ-58	A1	65,635,764	64,650,087 (98.5%)	55,898,734	11,179,108
HWJ-59	A1	71,014,344	70,203,138 (98.9%)	59,953,474	19,479,460
HWJ-60	A1	72,029,965	71,175,336 (98.8%)	62,265,819	11,915,657
HWJ-61	A1	82,410,258	81,457,375 (98.8%)	71,461,387	14,358,621
HWJ-62	A1	62,383,231	61,562,950 (98.7%)	52,044,323	7,431,884
HWJ-63	A1	72,966,645	72,286,835 (99.1%)	62,406,684	14,062,140
HWJ-28	A1	67,897,177	67,064,006 (98.8%)	55,078,019	16,000,467
HWJ-29	A1	76,875,451	76,213,497 (99.1%)	62,430,871	15,553,376
HWJ-31	A3	85,024,602	84,200,806 (99.0%)	70,152,779	20,829,204
HWJ-32	A3	61,718,371	61,097,859 (99.0%)	51,500,717	14,019,809
HWJ-33	A3+	72,862,233	71,893,531 (98.7%)	61,455,821	13,670,849
HWJ-34	A3	67,867,537	67,235,194 (99.1%)	56,512,501	14,484,673
HWJ-35	A3	92,953,999	92,052,676 (99.0%)	79,419,362	13,179,592
HWJ-36	A3	102,832,904	101,407,625 (98.6%)	87,601,223	13,805,562
HWJ-37	A3	59,724,137	59,026,270 (98.8%)	50,572,935	11,385,279
HWJ-38	A3	65,526,101	64,758,703 (98.8%)	54,766,930	12,342,160
HWJ-39	A3	82,010,133	81,041,383 (98.8%)	70,933,631	13,916,253
HWJ-40	A3	84,519,781	83,586,544 (98.9%)	71,885,027	13,564,989

Sample	Age*	No. of raw reads	No. of reads after trimming	No. of reads after QC	No. of unique paired reads
HWJ-41	A3	72,570,612	72,002,310 (99.2%)	61,938,849	13,561,955
HWJ-42	A3	67,527,339	66,990,168 (99.2%)	56,331,082	14,394,260
HWJ-48	A3	67,854,135	67,378,241 (99.3%)	57,084,317	15,861,542
HWJ-64	A5+	77,295,832	76,688,525 (99.2%)	67,399,696	14,903,300
HWJ-65	A5+	76,924,682	76,143,490 (99.0%)	67,655,353	12,278,304
HWJ-66	A5+	68,362,741	67,681,708 (99.0%)	58,321,160	10,971,516
HWJ-67	A5+	79,155,546	78,473,922 (99.1%)	67,975,397	15,383,292
HWJ-45	A5+	57,984,594	57,456,049 (99.1%)	49,274,283	13,049,779
HWJ-46	A5+	67,625,747	66,977,059 (99.0%)	56,931,589	12,590,619
HWJ-47	A5+	50,282,936	49,802,152 (99.0%)	41,798,516	11,011,689
HWJ-49	A5+	79,232,501	78,423,324 (99.0%)	68,227,515	11,337,222
HWJ-50	A5+	69,735,028	69,034,874 (99.0%)	58,798,385	12,127,387
HWJ-93	A2	61,106,022	58,720,120 (96.1%)	51,963,155	8,981,694
HWJ-94	A2	76,950,380	76,260,988 (99.1%)	67,979,641	9,205,323
HWJ-95	A2	70,667,088	69,744,364 (98.7%)	61,971,094	11,333,857
HWJ-96	A2	65,735,308	64,886,318 (98.7%)	57,778,482	14,757,133
HWJ-97	A2	63,984,705	63,062,826 (98.6%)	54,653,358	13,579,769
HWJ-98	A2	62,230,520	61,564,844 (98.9%)	54,075,470	11,766,634
HWJ-99	A2	69,894,296	68,942,572 (98.6%)	61,636,365	13,579,769
HWJ-100	A2	85,194,838	83,150,766 (97.6%)	75,027,859	8,573,728
HWJ-101	A2	61,593,162	60,524,051 (98.3%)	52,347,373	18,019,260
HWJ-102	A2	65,503,634	64,647,239 (98.7%)	57,561,539	16,347,453
HWJ-103	A2	67,392,731	66,656,125 (98.9%)	57,952,313	17,241,628
HWJ-105	A4	64,558,538	63,871,335 (98.9%)	55,722,942	15,545,660
HWJ-106	A4	63,430,478	62,497,176 (98.5%)	55,356,430	15,507,035
HWJ-108	A4	63,924,691	62,780,866 (98.2%)	55,121,275	15,555,447
HWJ-109	A4	73,913,216	72,516,479 (98.1%)	64,970,759	10,806,297
HWJ-110	A4	64,187,645	62,924,733 (98.0%)	55,922,926	11,206,416
HWJ-111	A4	54,037,383	53,279,708 (98.6%)	46,684,105	14,200,795
HWJ-112	A4	67,302,832	66,656,094 (99.0%)	59,537,563	13,644,202
HWJ-113	A4	63,558,438	62,799,588 (98.8%)	55,420,841	13,609,859
HWJ-114	A4	71,454,641	70,411,168 (98.5%)	60,638,409	8,354,925
HWJ-115	A4	86,135,144	84,907,681 (98.6%)	75,385,099	9,209,127
HWJ-126	A5	61,128,845	60,103,862 (98.3%)	53,250,847	11,711,997
HWJ-127	A5	60,383,163	59,435,359 (98.4%)	53,162,535	14,430,450
HWJ-128	A5	58,126,190	56,882,111 (97.9%)	50,258,456	12,077,438
HWJ-129	A5	58,440,603	57,478,049 (98.4%)	51,397,631	12,324,652
HWJ-130	A5	57,834,408	56,750,610 (98.1%)	49,919,332	11,847,874
HWJ-131	A5	59,494,313	58,634,077 (98.6%)	51,591,566	5,946,875
HWJ-132	A5	63,306,435	62,157,194 (98.2%)	55,325,730	12,751,583
HWJ-133	A5	68,729,028	67,430,722 (98.1%)	61,110,992	10,365,731

Sample	Age*	No. of raw reads	No. of reads after trimming	No. of reads after QC	No. of unique paired reads
HWJ-134	A5	63,225,351	62,199,045 (98.4%)	55,709,312	12,776,924
HWJ-135	A5	58,825,322	57,774,319 (98.2%)	51,874,641	12,885,516
HWJ-136	A5	66,052,825	64,953,046 (98.3%)	56,088,784	12,995,793
HWJ-137	A6+	61,368,254	60,396,088 (98.4%)	52,675,628	10,068,864
HWJ-138	A6+	61,371,361	60,112,907 (97.9%)	54,114,803	8,722,774
HWJ-139	A6+	58,659,136	57,563,386 (98.1%)	50,617,260	10,521,818
HWJ-140	A6+	66,796,848	65,846,608 (98.6%)	58,424,002	13,037,646
HWJ-141	A6+	67,980,967	66,621,864 (98.0%)	59,141,718	11,538,346
HWJ-142	A6+	62,479,320	61,266,219 (98.1%)	54,951,210	9,062,412
HWJ-143	A6+	60,079,067	59,022,326 (98.2%)	48,074,541	9,267,070
HWJ-144	A6+	65,557,090	64,434,540 (98.3%)	56,116,128	12,813,992
HWJ-145	A6	67,065,189	65,822,480 (98.1%)	56,426,435	10,550,524
HWJ-146	A6	62,541,237	61,309,302 (98.0%)	52,342,358	10,088,077
HWJ-148	A7+	72,739,342	71,379,638 (98.1%)	61,771,964	10,940,913
HWJ-149	A7+	71,488,256	70,057,852 (98.0%)	60,974,535	10,259,788
HWJ-150	A7+	88,114,630	85,413,332 (96.9%)	75,995,605	10,958,569
HWJ-151	A7+	75,291,278	73,451,144 (97.6%)	65,029,731	9,067,255
HWJ-152	A7+	75,962,321	74,509,056 (98.1%)	65,647,118	11,411,875
HWJ-153	A7+	63,689,054	62,484,693 (98.1%)	55,587,545	10,641,034
HWJ-154	A7+	64,855,180	63,711,745 (98.2%)	56,621,787	12,598,093
HWJ-155	A7+	74,259,606	72,229,050 (97.3%)	64,282,251	10,772,018
HWJ-156	A4	73,572,657	72,255,980 (98.2%)	64,401,979	10,220,301
HWJ-6b	A1+	68,062,483	66,876,097(98.3%)	56,420,064	14,368,120
HWJ-56	A2+	68,170,672	67,498,956(99.0%)	58,307,061	16,803,200

Supplementary Table 4. The number of DE genes identified by both *DESeq2* and *edgeR* through pairwise differential expression analysis amongst age cohorts.

Comparison	<i>DESeq2</i>		<i>EdgeR</i>		Intersection of DE genes		Percentage of overlaps (%)	
	up	down	up	down	up	down	up	down
0 vs 1	45	67	248	135	42	60	93.3	89.6
0 vs 2	1180	1467	1214	1077	1098	1008	93.1	93.6
0 vs 3	929	1484	921	1338	823	1205	89.4	90
0 vs 4	634	814	888	614	578	537	91.2	87.5
0 vs 5	1571	2087	1552	1925	1473	1764	94.9	91.6
0 vs 6	349	426	241	114	211	113	87.6	99.1
0 vs 7+	1443	2092	1343	1845	1316	1807	98	97.9
1 vs 2	82	80	85	86	50	26	61	72.2
1 vs 3	231	420	208	442	159	345	76.4	82.1
1 vs 4	30	20	102	32	21	12	70	60
1 vs 5	81	375	78	284	55	236	70.5	83.1
1 vs 6	3	3	8	3	2	1	66.7	33.3
1 vs 7+	166	575	91	209	82	207	90.1	99
2 vs 3	314	250	46	121	42	115	91.3	95
2 vs 4	3	0	1	2	0	0	0	0
2 vs 5	78	60	8	12	7	11	87.5	91.7
2 vs 6	39	21	29	10	19	8	65.5	80
2 vs 7+	288	114	126	70	116	50	92.1	71.4
3 vs 4	111	95	36	25	30	20	83.3	80
3 vs 5	15	71	2	1	0	0	0	0
3 vs 6	14	20	13	3	6	3	46.2	100
3 vs 7+	279	294	155	168	148	151	95.5	89.9
4 vs 5	58	70	17	98	16	32	94.1	45.7
4 vs 6	34	21	9	3	4	3	44.4	100
4 vs 7+	426	455	246	305	236	215	95.9	70.5
5 vs 6	4	1	2	1	2	1	100	100
5 vs 7+	22	1	7	0	3	0	42.9	0
6 vs 7+	0	0	5	0	NA	NA	NA	NA

Supplementary Table 5. The eigengenes in each module and their correlation with age.
The eigengene is a central gene whose expression pattern can represent the whole module.

Module	Number of genes	Eigengene	R-square	P-value
M1	566	<i>SRP72</i>	0.59	6×10^{-8}
M2	501	<i>ATX2</i>	0.43	2×10^{-4}
M3	943	<i>NU155</i>	0.38	0.001
M4	1,415	<i>ELP1</i>	-0.58	10^{-7}
M5	734	<i>SPTB2</i>	-0.5	10^{-5}
M6	547	<i>SLN14</i>	-0.21	0.08
M7	882	<i>STAT6</i>	-0.11	0.4

Supplementary Table 6. Protein-protein interaction between the top novel candidates (Fig. 3B) and 207 human aging-associated genes based on the STRING database.

The top novel candidates (Fig. 3B)	Human aging-associated genes
<i>MRE11A</i>	<i>ATR, XRCC6, WRN, NBN, FEN1</i>
<i>MAGED2</i>	<i>CLU, VEGFA, TGFB1, EGF</i>
<i>ZEB1</i>	<i>VEGFA, SUMO1, PARP1</i>
<i>PAF15</i>	<i>PCNA, POLD1, TOP2A</i>
<i>NEIL1</i>	<i>POLB, FEN1, PCNA</i>
<i>TGIF2</i>	<i>SERPINE1, HDAC1</i>
<i>IPP2</i>	<i>PPP1CA</i>
<i>PLK4</i>	<i>CDK1</i>
<i>AKIRIN2</i>	<i>IL6</i>
<i>BTAF1</i>	<i>TBP</i>
<i>CD19</i>	<i>PIK3R1</i>
<i>WRAP53</i>	<i>TERT</i>
<i>COMMD4</i>	<i>TFDP1</i>
<i>MPZL1</i>	<i>PTPN11</i>
<i>CD72</i>	<i>PLCG2</i>

Supplementary Table 7. Sequencing summary statistics of 50 miRNA-Seq samples.

sample	age	raw reads	reads with adaptors	trimmed reads	unique reads	mapped reads	mapping rate
HWJ-72	0	8,894,904	8,801,964	7,581,458	72,287	22,052	30.51%
HWJ-73	0	9,724,576	9,622,557	9,252,561	63,987	19,716	30.81%
HWJ-74	0	10,045,885	9,931,603	9,156,033	64,945	19,569	30.13%
HWJ-76	0	9,269,610	9,127,303	8,842,741	63,248	19,865	31.41%
HWJ-77	1	12,579,011	12,452,183	11,674,274	74,899	21,570	28.80%
HWJ-79	1	12,589,547	12,462,959	12,001,006	72,476	22,325	30.80%
HWJ-80	1	13,609,592	13,479,694	12,454,350	68,911	20,506	29.76%
HWJ-81	1	14,150,705	14,019,077	13,365,130	73,524	22,662	30.82%
HWJ-116	2	12,594,788	12,159,024	11,392,061	108,540	22,188	20.44%
HWJ-117	2	11,982,103	11,605,390	11,170,285	114,938	23,368	20.33%
HWJ-118	2	10,108,041	9,749,835	9,367,265	94,073	18,674	19.85%
HWJ-119	2	11,949,393	11,550,364	11,098,177	112,732	24,015	21.30%
HWJ-120	2	11,117,431	10,770,886	10,501,994	102,862	21,396	20.80%
HWJ-82	3	10,637,365	10,503,873	10,174,538	62,231	18,694	30.04%
HWJ-83	3	10,092,743	9,991,180	9,523,756	68,176	20,908	30.67%
HWJ-85	3	13,286,799	13,160,729	12,662,422	78,900	22,263	28.22%
HWJ-86	3	18,235,632	18,042,158	16,934,269	121,326	31,406	25.89%
HWJ-92	3	12,518,623	12,373,656	11,863,235	64,487	20,263	31.42%
HWJ-121	4	10,227,598	9,955,031	9,399,556	95,709	20,241	21.15%
HWJ-122	4	9,521,398	9,217,281	8,749,065	89,703	18,370	20.48%
HWJ-123	4	9,480,511	9,151,219	8,924,255	87,685	18,653	21.27%
HWJ-124	4	10,750,283	10,351,432	10,058,185	86,663	17,095	19.73%
HWJ-125	4	10,560,288	10,243,766	9,999,430	94,400	20,061	21.25%
HWJ-75	1+	8,099,094	7,990,255	7,740,323	54,531	16,507	30.27%
HWJ-78	2+	8,718,923	8,611,060	8,241,628	55,426	17,123	30.89%
HWJ-157	5	10,703,419	10,402,860	9,662,211	71,826	17,803	24.79%
HWJ-158	5	10,365,030	10,065,864	9,715,458	84,975	21,621	25.44%
HWJ-159	5	11,332,527	11,032,564	10,543,003	80,579	19,650	24.39%
HWJ-160	5	11,329,341	10,993,005	10,271,374	83,341	19,804	23.76%
HWJ-161	5	10,708,510	10,395,981	9,798,997	88,724	21,949	24.74%
HWJ-162	5	10,682,931	10,358,862	10,014,149	79,160	19,642	24.81%
HWJ-163	5	11,130,430	10,808,179	10,275,481	88,674	20,681	23.32%
HWJ-87	5+	11,693,719	11,569,564	10,989,822	76,795	23,914	31.14%
HWJ-88	5+	11,544,960	11,422,108	10,735,946	73,059	20,728	28.37%
HWJ-89	5+	13,385,309	13,263,446	12,710,293	78,090	23,985	30.71%
HWJ-90	5+	15,218,647	15,052,636	14,411,092	74,612	22,612	30.31%
HWJ-91	5+	10,362,448	10,258,394	9,940,793	57,762	17,664	30.58%
HWJ-164	6+	11,442,549	11,098,799	10,786,159	94,492	23,691	25.07%
HWJ-165	6+	11,175,180	10,866,384	10,122,535	95,313	22,660	23.77%

sample	age	raw reads	reads with adaptors	trimmed reads	unique reads	mapped reads	mapping rate
HWJ-166	6+	11,610,252	11,285,984	10,434,419	93,509	21,824	23.34%
HWJ-167	6+	11,168,168	10,792,484	10,441,877	84,002	21,918	26.09%
HWJ-168	6+	11,631,942	11,301,306	10,450,400	74,826	18,776	25.09%
HWJ-169	6+	11,960,016	11,604,040	11,049,005	83,798	21,232	25.34%
HWJ-170	6	11,156,002	10,823,283	10,054,204	86,014	22,316	25.94%
HWJ-171	6	11,545,802	11,205,460	10,388,704	84,545	20,939	24.77%
HWJ-172	6	11,457,308	11,114,093	10,016,843	84,412	20,701	24.52%
HWJ-173	7+	11,359,195	11,029,673	10,584,650	92,277	21,889	23.73%
HWJ-174	7+	11,229,070	10,854,762	10,218,577	79,635	19,193	24.10%
HWJ-175	7+	11,846,711	11,474,890	9,904,792	87,489	22,105	25.27%
HWJ-176	7+	13,389,734	12,946,651	12,440,629	95,536	23,420	24.51%

Supplementary Table 8. The genes associated with cell cycle regulation/DNA repair that were regulated by miRNA. (FDR-adjusted p -value < 0.1)

Gene	miRNA	ρ (rho)	Gene	miRNA	ρ (rho)
<i>BLM</i>	miR-1306-3p	-0.358	<i>CDC27</i>	miR-15b-5p	-0.504
<i>EP300</i>	miR-148b-3p	-0.377	<i>EZH2</i>	let-7g-5p	-0.36
<i>EZH2</i>	let-7d-5p	-0.305	<i>EZH2</i>	let-7f-5p	-0.258
<i>ORC4</i>	miR-194-5p	-0.297	<i>RAB11A</i>	miR-532-5p	-0.302
<i>CTDSP2</i>	miR-34c-5p	-0.464	<i>CTDSP2</i>	miR-34b-5p	-0.439
<i>CTDSP2</i>	miR-185-5p	-0.273	<i>CLASP2</i>	miR-185-5p	-0.302
<i>PPME1</i>	miR-27b-3p	-0.309	<i>ALNL</i>	miR-342-5p	-0.352
<i>KMT2E</i>	let-7g-5p	-0.288	<i>UVRAG</i>	miR-423-5p	-0.337
<i>UVRAG</i>	miR-4657	-0.256	<i>ROCK2</i>	miR-671-3p	-0.355
<i>ROCK2</i>	miR-16-5p	-0.281	<i>WAPL</i>	miR-502b	-0.347
<i>RACGAP1</i>	miR-185-5p	-0.314	<i>PHC3</i>	miR-339-5p	-0.35
<i>CDK19</i>	miR-130a-3p	-0.385	<i>CDK19</i>	miR-330-5p	-0.314
<i>CDK19</i>	miR-148b-3p	-0.297	<i>FOPNL</i>	miR-186-5p	-0.639
<i>PDS5A</i>	miR-185-5p	-0.294	<i>WDR33</i>	miR-185-5p	-0.299
<i>WDR33</i>	miR-532-5p	-0.274	<i>DOTIL</i>	miR-532-5p	-0.317
<i>RNF169</i>	miR-500a-3p	-0.359			

Supplementary Table 9. The top 50 negatively correlated miRNA-mRNA pairs. (FDR-adjusted p -value < 0.05)

mRNA	miRNA	ρ (rho)	mRNA	miRNA	ρ (rho)
<i>FOPNL</i>	miR-186-5p	-0.639	<i>PPT1</i>	miR-331-5p	-0.517
<i>DYRK2</i>	miR-34c-5p	-0.635	<i>SNX6</i>	miR-15b	-0.516
<i>TAXB1</i>	miR-542-3p	-0.596	<i>UBN2</i>	miR-502b	-0.513
<i>FXL14</i>	let-7f-5p	-0.582	<i>ETS1</i>	miR-103a-3p	-0.513
<i>ICLN</i>	miR-502b	-0.576	<i>ATAD1</i>	miR-27b-3p	-0.51
<i>CD79B</i>	miR-532-5p	-0.575	<i>CDC27</i>	miR-15b-5p	-0.503
<i>AFTIN</i>	miR-185-5p	-0.571	<i>TUT7</i>	miR-15b-5p	-0.502
<i>TNR6A</i>	miR-30c-5p	-0.571	<i>CD37L</i>	miR-15b-5p	-0.502
<i>MA2A1</i>	miR-27b-3p	-0.570	<i>PRPF3</i>	miR-339-5p	-0.501
<i>ARI1</i>	miR-502b	-0.563	<i>KAISO</i>	miR-532-5p	-0.497
<i>BTG1</i>	miR-34b-5p	-0.562	<i>PCX3</i>	miR-34c-5p	-0.496
<i>UB2Q1</i>	miR-103a-3p	-0.561	<i>CALM</i>	miR-542-3p	-0.494
<i>RIR2B</i>	miR-30a	-0.558	<i>MGAP</i>	miR-378a-3p	-0.494
<i>ICLN</i>	miR-502b	-0.557	<i>HHAT</i>	miR-29c-3p	-0.494
<i>PDE4B</i>	miR-34c-5p	-0.553	<i>DDIT4</i>	miR-30a	-0.492
<i>NCOA3</i>	let-7g-5p	-0.552	<i>RNF10</i>	miR-34c-5p	-0.492
<i>BTG1</i>	miR-34c-5p	-0.543	<i>CAPR1</i>	miR-34c-5p	-0.491
<i>ROA2</i>	miR-330-5p	-0.541	<i>SSH2</i>	miR-34c-5p	-0.490
<i>ZFY27</i>	miR-361-5p	-0.539	<i>ZDHC5</i>	miR-29a-3p	-0.487
<i>DYRK2</i>	let-7g-5p	-0.538	<i>TAXB1</i>	miR-542-3p	-0.486
<i>SATB1</i>	miR-500a-3p	-0.531	<i>MTMR3</i>	miR-16-5p	-0.486
<i>PDE7A</i>	miR-30d	-0.531	<i>NF2L1</i>	miR-10a-5p	-0.485
<i>MOD5</i>	miR-23a-3p	-0.53	<i>LMBL3</i>	miR-138-5p	-0.484
<i>SSH2</i>	miR-34b-5p	-0.525	<i>UBR5</i>	miR-542-3p	-0.483
<i>EF2</i>	miR-92b-3p	-0.52	<i>GRK6</i>	miR-30a	-0.481

Supplementary Data 1: Spearman's correlation coefficients between gene expression and age (n=12,263).

Supplementary Data 2: Top 20 genes that exhibited the strongest correlation (both positive and negative) with age in *M. myotis*.

Supplementary Data 3: The full list of GO term expression pattern with age across 4 species.

Supplementary Data 4: Spearman's correlation coefficients between expression of 207 human aging-associated genes and age.

Supplementary Data 5: Raw gene expression counts (n=12,263).

Supplementary Data 6: Raw mature miRNA expression counts (n=117).