eLife's transparent reporting form

We encourage authors to provide detailed information *within their submission* to facilitate the interpretation and replication of experiments. Authors can upload supporting documentation to indicate the use of appropriate reporting guidelines for health-related research (see EQUATOR Network), life science research (see the BioSharing Information Resource), or the ARRIVE guidelines for reporting work involving animal research. Where applicable, authors should refer to any relevant reporting standards documents in this form.

If you have any questions, please consult our Journal Policies and/or contact us: editorial@elifesciences.org.

Sample-size estimation
- You should state whether an appropriate sample size was computed when the study was being designed
- You should state the statistical method of sample size computation and any required assumptions
- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

> No formal, explicit power analysis was used to determine the sample size for these experiments. Prior experiments of a similar nature done on the same cell and tissue type suggested that statistical significance was achievable with a minimum sample size of 3 animals. This information is found in the Methods section.

Replicates
- You should report how often each experiment was performed
- You should include a definition of biological versus technical replication
- The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

> Each experiment was performed at least 3 times (3 biological replicates) and 3 technical replicates for RT-qPCR experiments. Tendons from 1 mouse represent 1 biological replicate. This information is found in the Methods Section.

Statistical reporting

- Statistical analysis methods should be described and justified
- Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

1. For the RT-qPCR assays, we performed an ANOVA to test for differences among the six time points. We then computed post hoc pairwise comparisons using Tukey's Honestly significant difference test in order to test for differences between each pair of time points. All statistics were done on the ΔCT values with 3 biological replicates per time point and each qPCR reaction was run in technical triplicate. Sample size and dispersion measures (standard error) are reported in the figure legend for Fig. 4.
2. For each timepoint analyzed by flow cytometry, at least 3 biological replicates were used. We tested for differences between pairs of time points using a Welch's T test.
3. One way ANOVA was used for statistical analysis of cell density (Fig 5E).
4. Two-tailed t-test was used for statistical analysis of BrdU incorporation in sections (Fig 3F) and Achilles tendon length measurements (Fig. 5F).

All details regarding the statistical methods can be found in the Methods section under the "Statistics" subheading.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

Group allocation

- Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
- Indicate if masking was used during group allocation, data collection and/or data analysis

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

For the H2B-GFP, BrdU, and EdU experiments mice were randomly selected for administration of Dox, BrdU, or EdU respectively. For the RT-qPCR experiments, tendon measurements, and cell counting, samples were randomly collected from the unused littermates of the mice from the pulse-chase experiments. No formal randomization method was employed.

Additional data files ("source data")

- We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
- Where provided, these should be in the most useful format, and they can be uploaded as "Source data" files linked to a main figure or table
- Include model definition files including the full list of parameters used
- Include code used for data analysis (e.g., R, MatLab)
- Avoid stating that data files are "available upon request"

Please indicate the figures or tables for which source data files have been provided:

R scripts used for RT-qPCR calculations and statistical analysis are provided, along with the R markdown file used to generate statistical tables.
We also provided all the source files for the graphs in the figures as Graphpad Prism files.