

Supplementary Information

Long-term treatment with senolytic drugs Dasatinib and Quercetin ameliorates age-dependent intervertebral disc degeneration in mice

Supplemental Figure 1: Successful outcome of D+Q treatment in ameliorating age-dependent intervertebral disc degeneration depends on the baseline degeneration status. (a-b)

Representative histology of Veh and D+Q treated mice from 6-23M and 18-23M cohorts, respectively. Representative low (5X) and high (20X) magnification views of a whole disc and NP, AF, and CEP compartments are shown. (c-d) Level-by-level analysis of Modified Thompson Grading averages of NP and AF compartments from L3-L6 lumbar discs of 6-23M, and 18-23M cohorts. 6-23M Veh (n=13), D+Q (n=15); 18-23M Veh (n=11), D+Q (n=9); 3 lumbar levels per group were analyzed. Two-tailed t-test or Mann-Whitney test was used as appropriate for comparing differences between Veh and D+Q groups. Data are represented as mean \pm SEM. Low magnification scale bar = 200 μ m (first column: a, b); high magnification scale bar = 50 μ m. NP: nucleus pulposus; AF: annulus fibrosus; EP: endplate. Source data are provided as a Source Data file.

Supplemental Figure 2: Lack of disc cell proliferation following D+Q treatment and status of disc cell senescence burden correlates to outcomes of D+Q treatment regimen. (a-d)

Immunostaining and staining area quantifications of cell cycle and senescence markers in the disc compartments of 14-23M cohort: RB, pRB, and pH2AX. (e-g'') Immunohistological staining and staining area quantifications of senescence status in the disc compartments from 18-23M cohort: p16^{INK4a}, p19^{ARF}, RB, and IL-6. Two-tailed t-test or Mann-Whitney test was used as appropriated; n=6 mice/group, 2-3 levels per mouse were analyzed. Data are represented as mean \pm SEM. Scale bar =200 μ m. Source data are provided as a Source Data file.

Supplemental Figure 3: AF and NP tissues present distinct transcriptomic profiles in aged C57BL/6 mice. (a)

Representative percentage of upregulated DEGs in the AF and NP comparison from 23-month-old mice from the 14-23M Veh cohort. (b-c) Schematic summarizing the DEGs between AF and NP of 23-month-old mice related to focal adhesion and endochondral bone ossification pathways. Source data are provided as a Source Data file.

Supplemental Figure 4: D+Q treatment promoted unique transcriptomic profiles in the AF and NP compartments. (a) Representative percentage of up- and downregulated DEGs between D+Q and Veh AF tissues from the 14-23M cohort, $p \leq 0.05$ (b) Hierarchical clustering analysis of DEGs between D+Q and Veh AF tissues. (c) Representative DEGs from selected GO processes from upregulated DEGs between D+Q and Veh tissues (d) Representative DEGs from selected GO processes from downregulated DEGs between D+Q and Veh AF tissues (e) Representative percentage of up- and downregulated DEGs between D+Q and Veh NP tissues, $p \leq 0.05$. (f) Hierarchical clustering analysis of DEGs between D+Q and Veh NP tissues (g) Representative DEGs from selected GO processes from downregulated DEGs between D+Q and Veh. NP tissues. GO process enrichment analysis was performed using the PANTHER Overrepresentation Test with GO Ontology database annotations and a binomial statistical test with $FDR \leq 0.05$. (h) Representative DEGs from selected GO processes from upregulated DEGs between D+Q and Veh. NP tissues (i) Representative Top 50 upregulated DEGs between D+Q and Veh NP tissues, $p \leq 0.05$. Source data are provided as a Source Data file.

Supplemental Figure 5: D+Q treatment prevented the age-associated systemic increase in proinflammatory molecules, cytokines, and Th17-related proteins. Multiplex analysis of the (a-a') proinflammatory molecules, (b-b') cytokines, and (c-c') Th17-related proteins in serum from 6-23M Veh (n=12) and D+Q (n=12) and 18-23M Veh (n=9) and D+Q (n=8) cohorts. Two-tailed t-test or Mann-Whitney test was used as appropriate, n=8-12 mice/group. Data are represented as mean \pm SEM. Source data are provided as a Source Data file.

Supplemental Figure 6: Long-term D+Q treatment was well tolerated by mice. (a) Survival curve from 6-23M Veh and D+Q cohorts. (b) Weight progression in males from 6-23M Veh (n=6) and D+Q (n=7) cohorts. (c) Weight progression in females from 6-23M Veh (n=7) and D+Q (n=8) cohorts. (d) Survival curve from 18-23M Veh and D+Q cohorts. (e) Weight progression in males from 18-23M Veh (n=5) and D+Q (n=5) cohorts. (f) Weight progression in females from 18-23M Veh (n=6) and D+Q (n=4) cohorts. (g) Grip test comparison of 6-23M Veh (n=15) and D+Q (n=15) cohorts. Data are represented as mean \pm SEM. Source data are provided as a Source Data file.

Supplemental Figure 7: Effect of long-term D+Q treatment regimens on disc height and DHI in mice. (a-c) Disc height, vertebral height, and disc height index (DHI) comparisons between (a) 6-23M Veh (n=13) and D+Q (n=15), (b) 14-23M Veh (n=8) and D+Q (n=7), and (c) 18-23M Veh (n=11) and D+Q (n=9) cohorts, L4-L6 /mouse. Two-tailed t-test or Mann-Whitney test was used as appropriate, n=6-13 mice/group. Data are represented as mean \pm SEM. Source data are provided as a Source Data file.

Supplemental Figure 8: Effects of D+Q on trabecular bone quality parameters in 6-23M and 18-23M treatment cohort. (a-b) 3D reconstruction showing a transverse section through representative male and female lumbar vertebrae from 6-23M and 18-23M Veh and D+Q cohorts. Scale bar E-F = 500 μ m. (c-f) Analysis of trabecular bone parameters (c-c') BV/TV (d-d') trabecular thickness (Tb.Th), (e-e') trabecular number (Tb.N), and (f-f') trabecular space (Tb.Sp) between Veh and D+Q males and females during aging and in the 6-23M and 18-23M cohorts. Males 6-23M: Veh n=6, D+Q n=7; Females 6-23M: Veh n=7, D+Q n=8. Males 18-23M: Veh n=5, D+Q n=5. Females 18-23M: Veh n=6, D+Q n=4. Vertebrae L4-L6 /mouse. Two-tailed t-test or Mann-Whitney test was used as appropriate. Data are represented as mean \pm SEM. Source data are provided as a Source Data file.

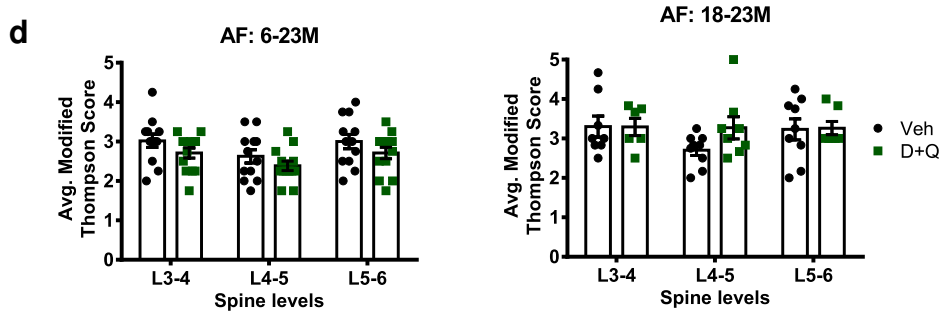
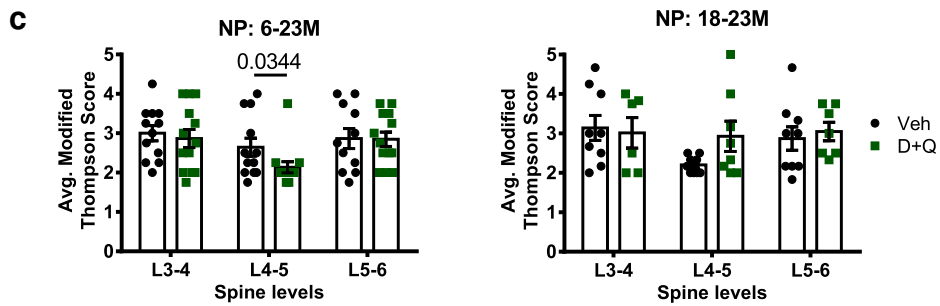
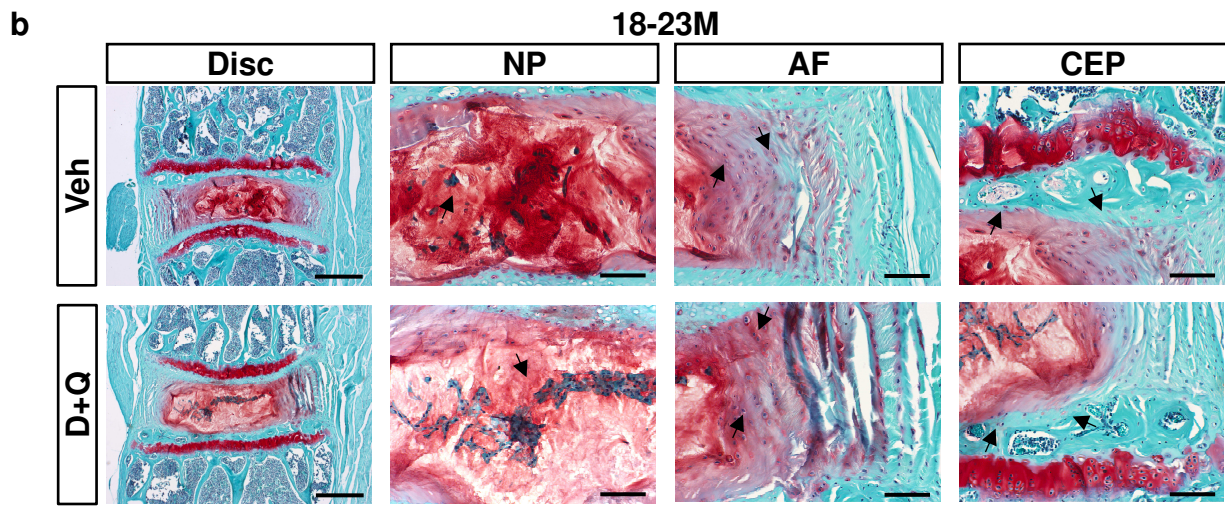
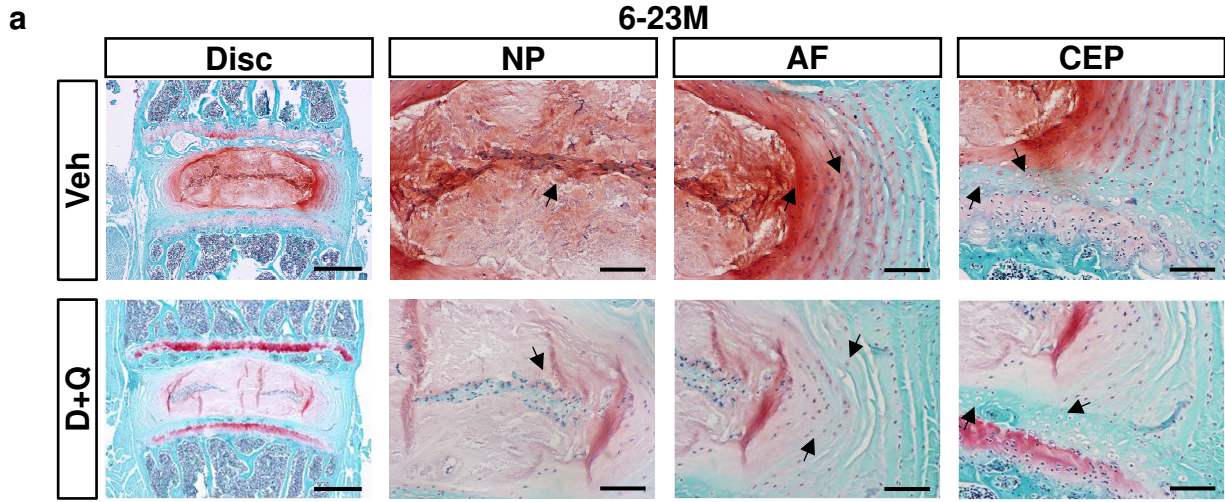
Supplemental Figure 9: Effect of D+Q on cortical bone parameters in 6-23M and 18-23M treatment cohort. (a-b) 3D reconstruction showing surface view and hemisection through representative male and female lumbar motion segment from 6-23M and 18-23M cohorts. Scale bar e-f = 500 μ m. (c-f) Analysis of cortical bone parameters (c-c') Bone volume (BV) (d-d') bone area (B.Ar) (e-e') cortical bone thickness (Cs.Th), and (f-f') eccentricity cross-sectional thickness (Ecc) between Veh and D+Q males and females during aging and in the 6-23M and 18-23M cohorts. Males 6-23M: Veh n=6, D+Q n=7; Females 6-23M: Veh n=7, D+Q n=8. Males 18-23M: Veh n=5, D+Q n=5. Females 18-23M: Veh n=6, D+Q n=4. Vertebrae L4-L6 /mouse. Two-tailed t-test or Mann-Whitney test was used as appropriate. Data are represented as mean \pm SEM. Source data are provided as a Source Data file.

Supplemental Figure 10: Effect of aging and D+Q treatment on BMD. (a-d) Bone mineral density of aged male and female mouse lumbar spines for Veh- and D+Q-treated mice from 6-23M, 14-23M, and 18-23M cohorts. Males 12m: n=5, 23m n=5. Females 12m n=4, 23m n=5. Males

6-23M: Veh n=6, D+Q n=7. Females 6-23M: Veh n=7, D+Q n=8. Males 18-23M: Veh n=5, D+Q n=5. Females 18-23M: Veh n=6, D+Q n=4. Vertebrae L4-L6 /mouse. Two-tailed t-test or Mann-Whitney test was used as appropriate. Data are represented as mean \pm SEM. Source data are provided as a Source Data file.

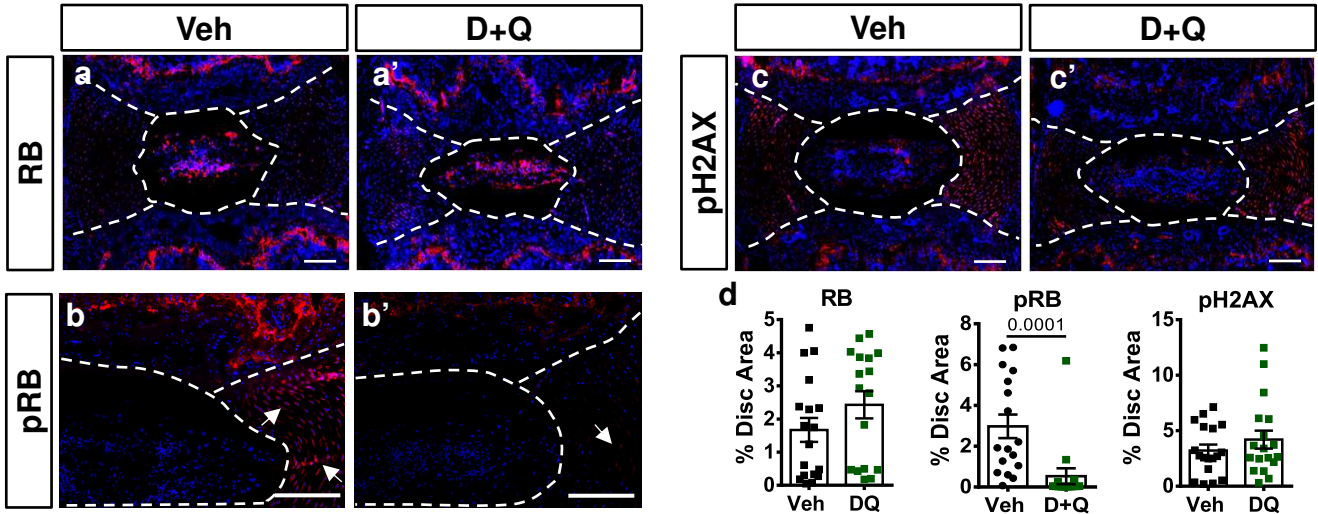
Supplemental Figure 11: Long-term systemic D+Q treatment did not ameliorate age-dependent knee articular cartilage degeneration and senescence burden in mice. (a) OARSI histological score (summed across 4 quadrants, 0-6 score for each) in 6-23M Veh (n=7) and D+Q (n=7) and 18-23M Veh (n=6) and D+Q (n=5) cohorts. **(b)** OARSI grading in males and females in the Veh and DQ groups across all treatment durations analyzed in the study. n=Veh: 12 male, 7 female; DQ: 14 male, 5 female. **(c-e’)** Staining and abundance analysis of key markers of senescence in the knee: p16^{INK4a}, p19^{ARF} and P21. M: Meniscus. n=5 mice/group. Two-tailed t-test or Mann-Whitney used as appropriate. Data are represented as mean \pm SEM. Source data are provided as a Source Data file.

Supplementary Figure 1

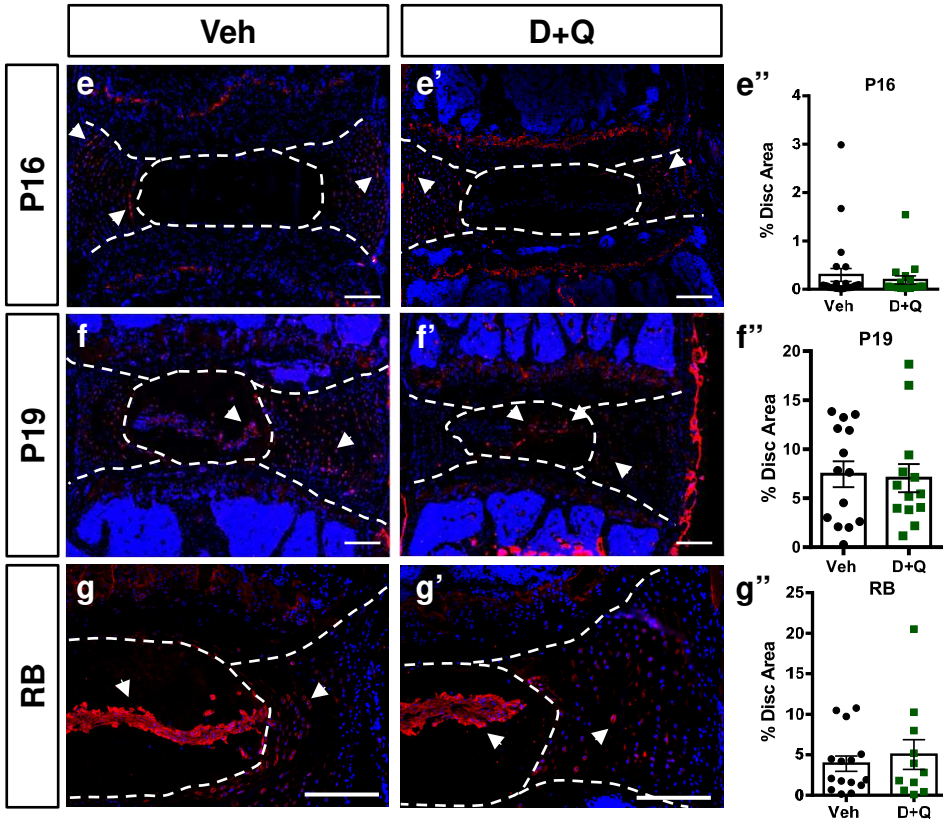


Supplementary Figure 2

14-23M

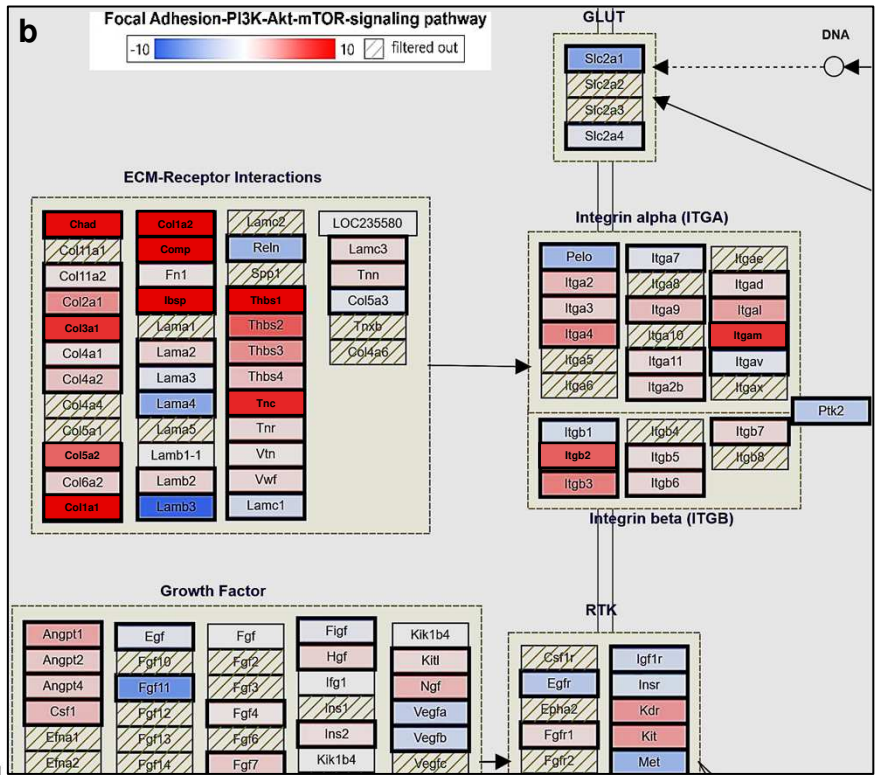
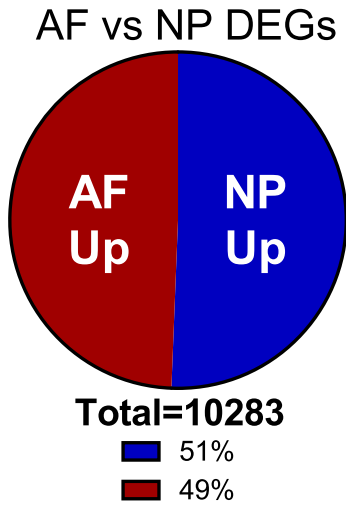


18-23M

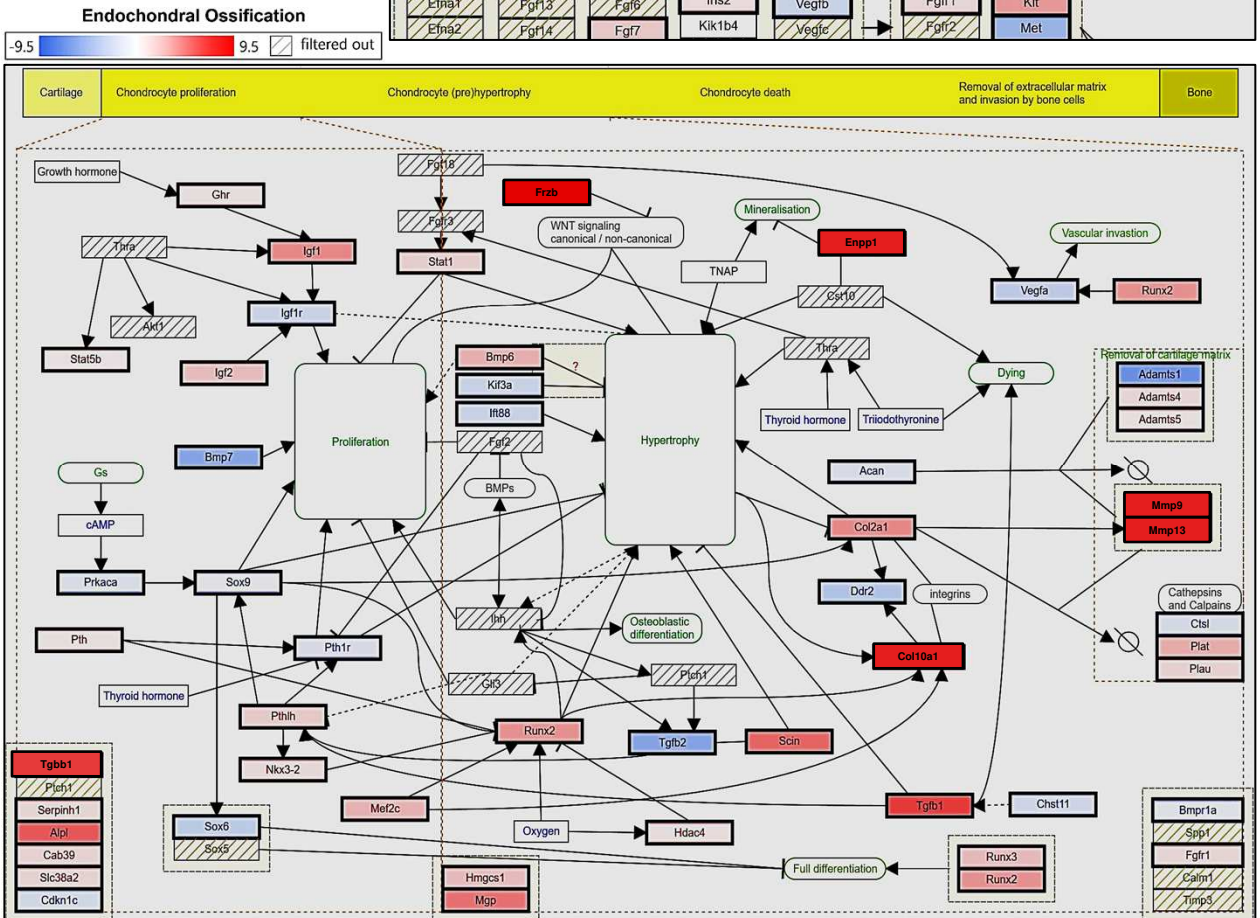


Supplementary Figure 3

a



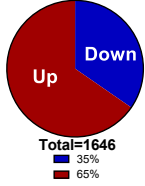
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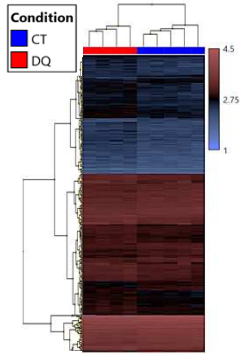
Supplementary Figure 4

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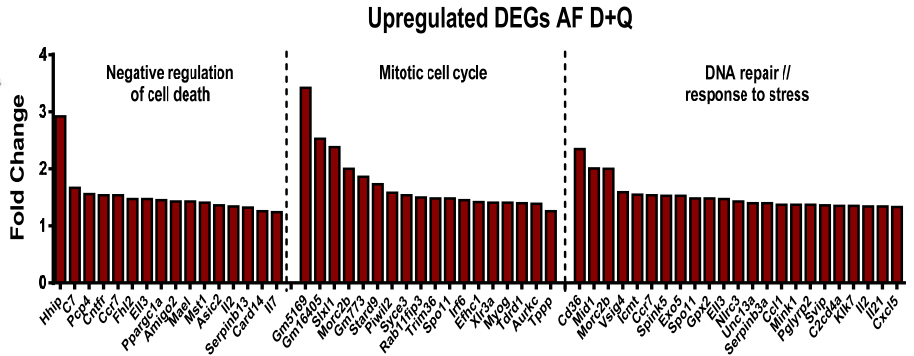
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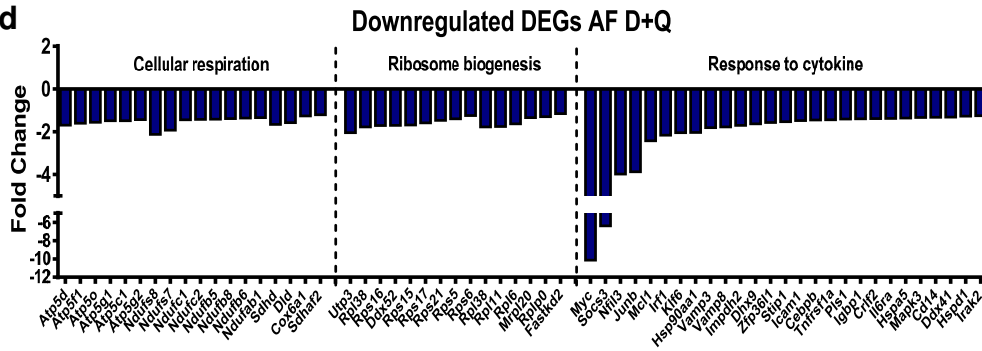
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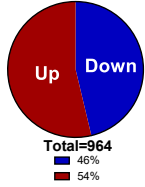


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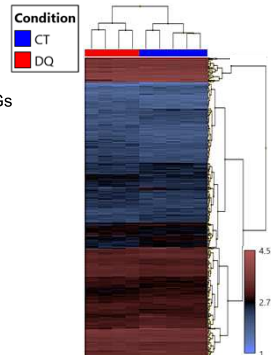


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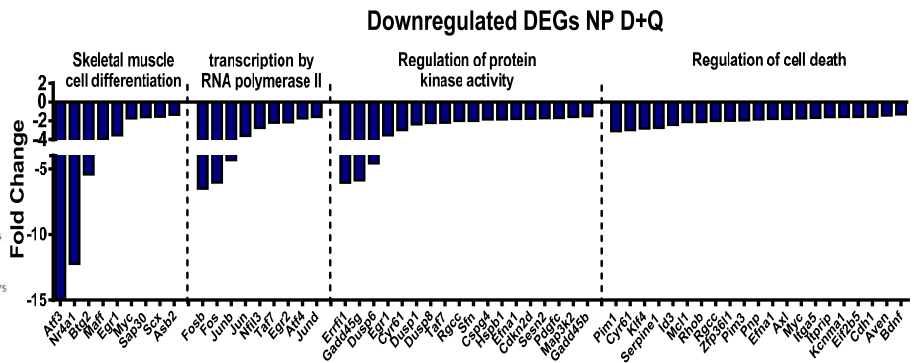
NP: DQ vs Veh DEGs



f

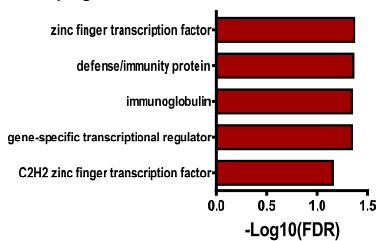


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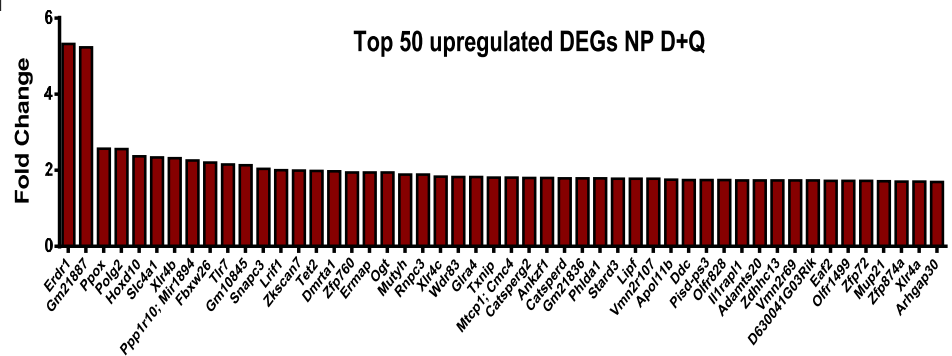


h

Upregulated PANTHER Classes in DQ - NP

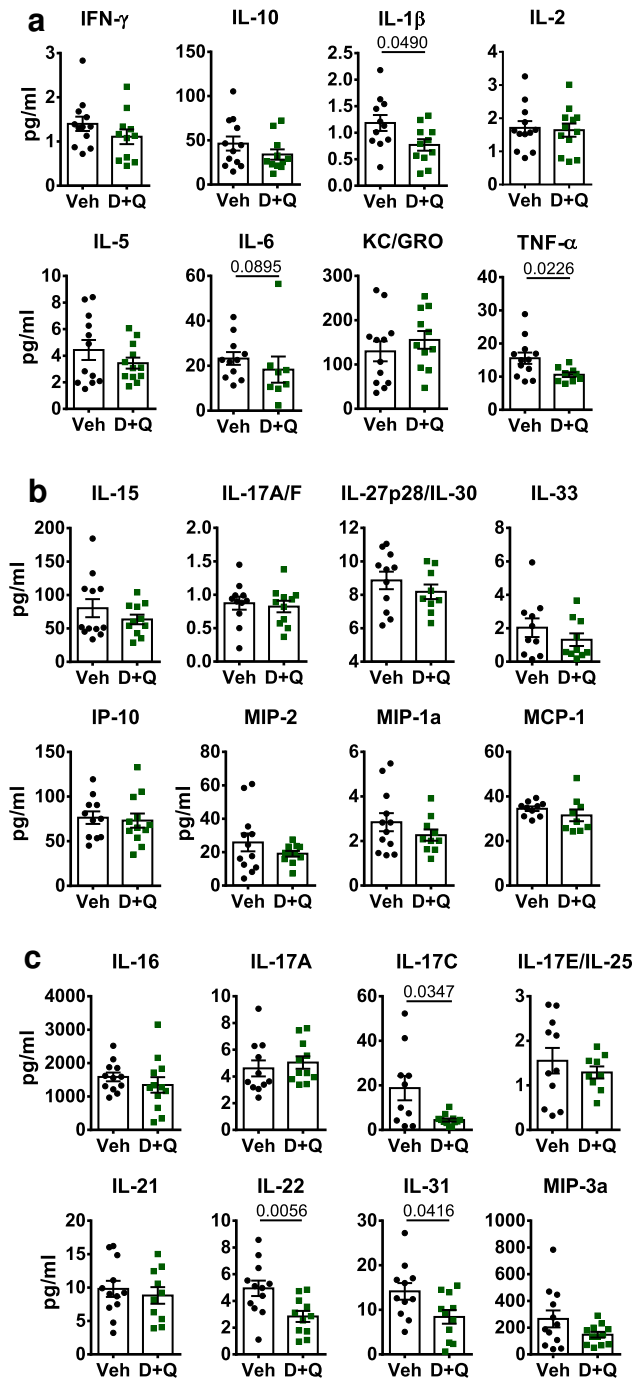


i

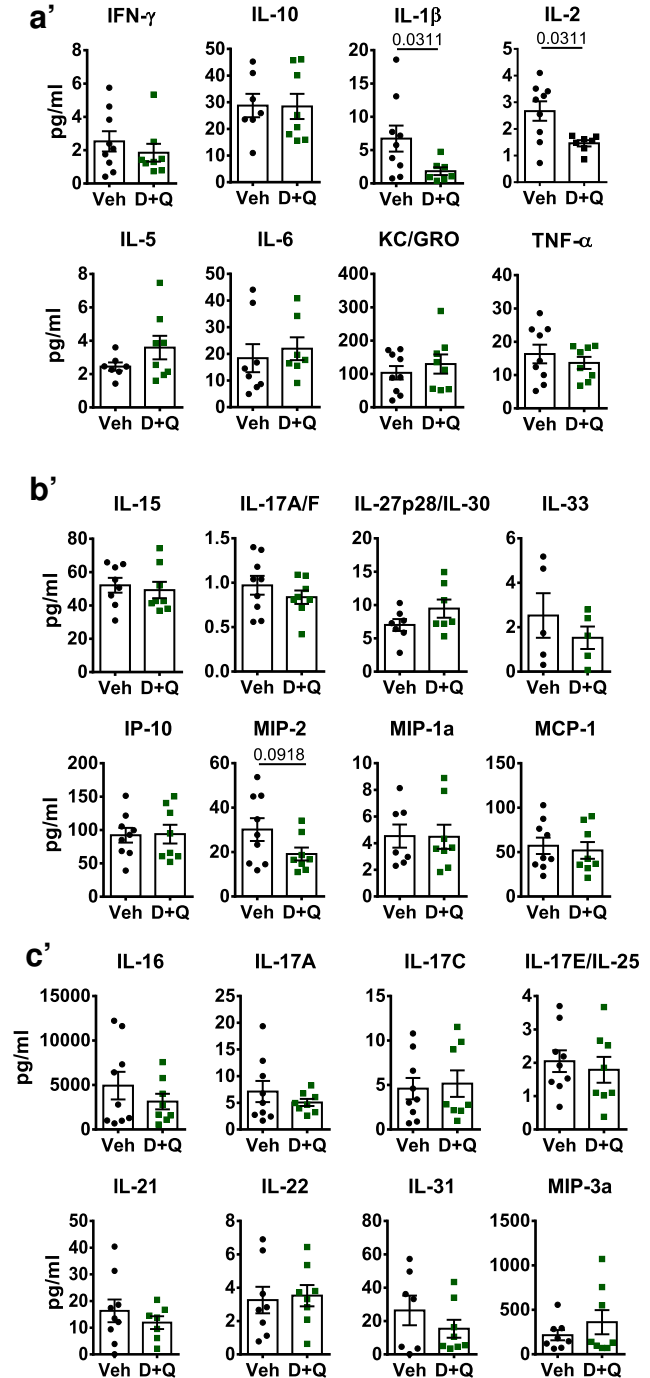


Supplementary Figure 5

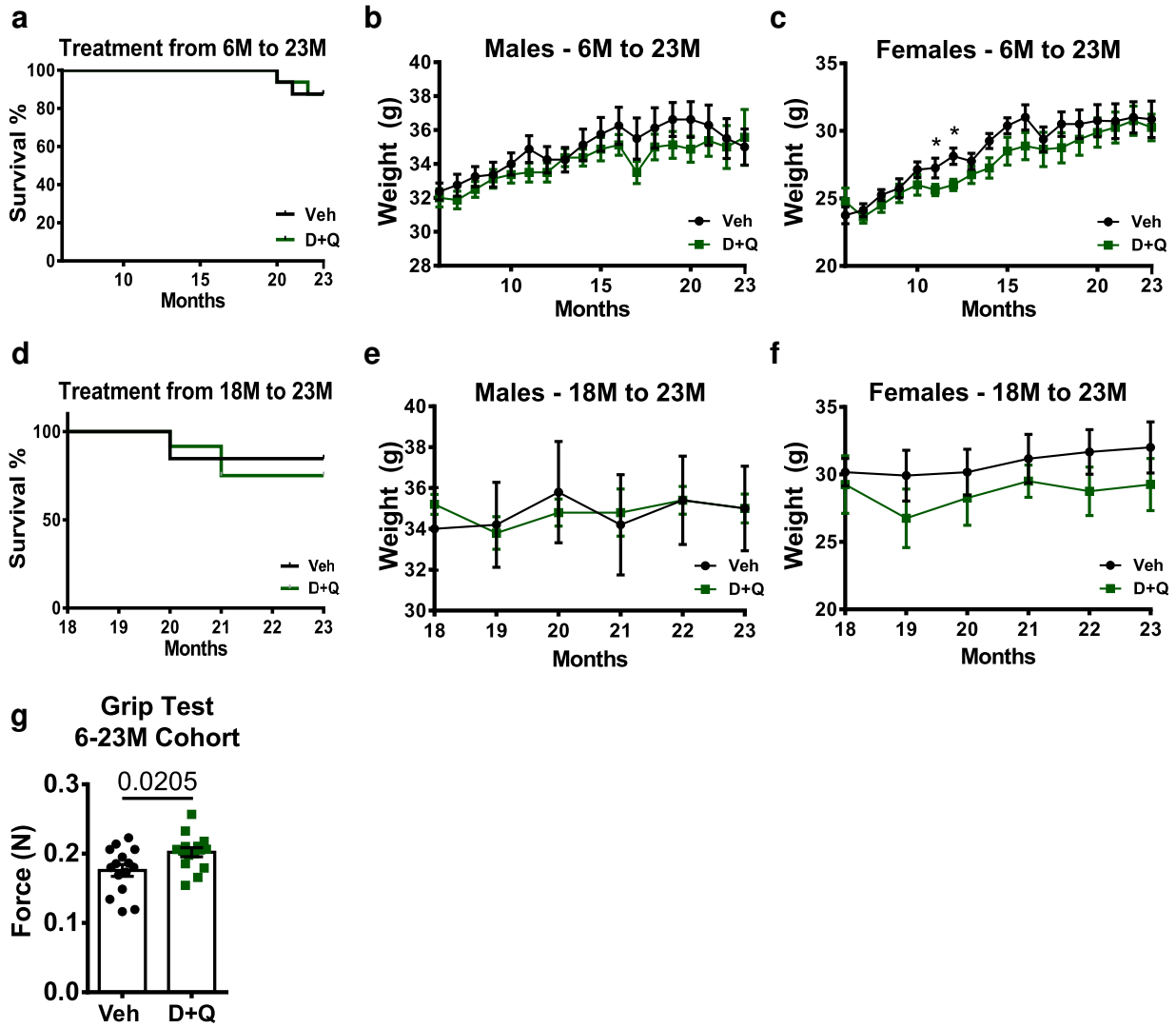
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6-23M D+Q treatment



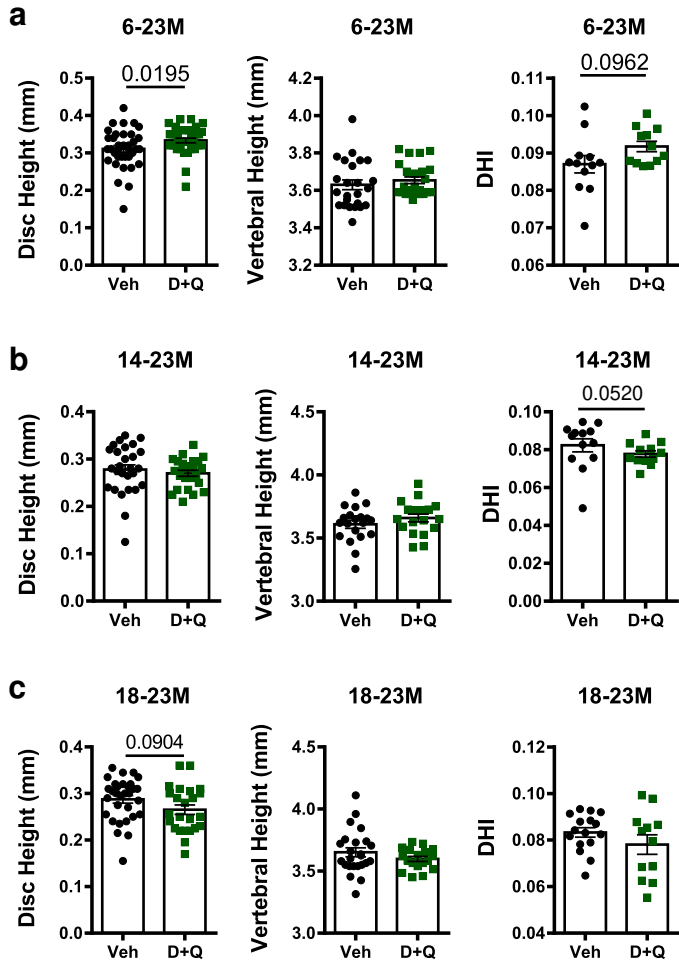
Blood Analysis
18-23M D+Q treatment



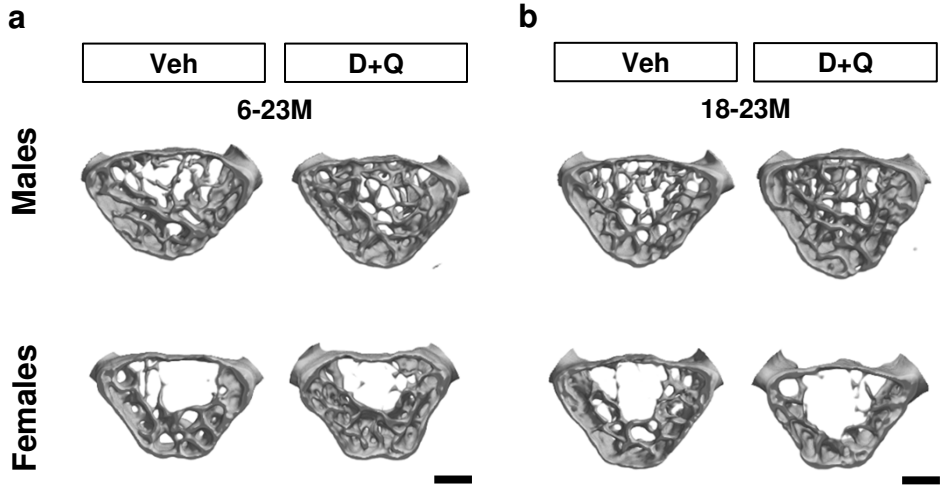
Supplementary Figure 6



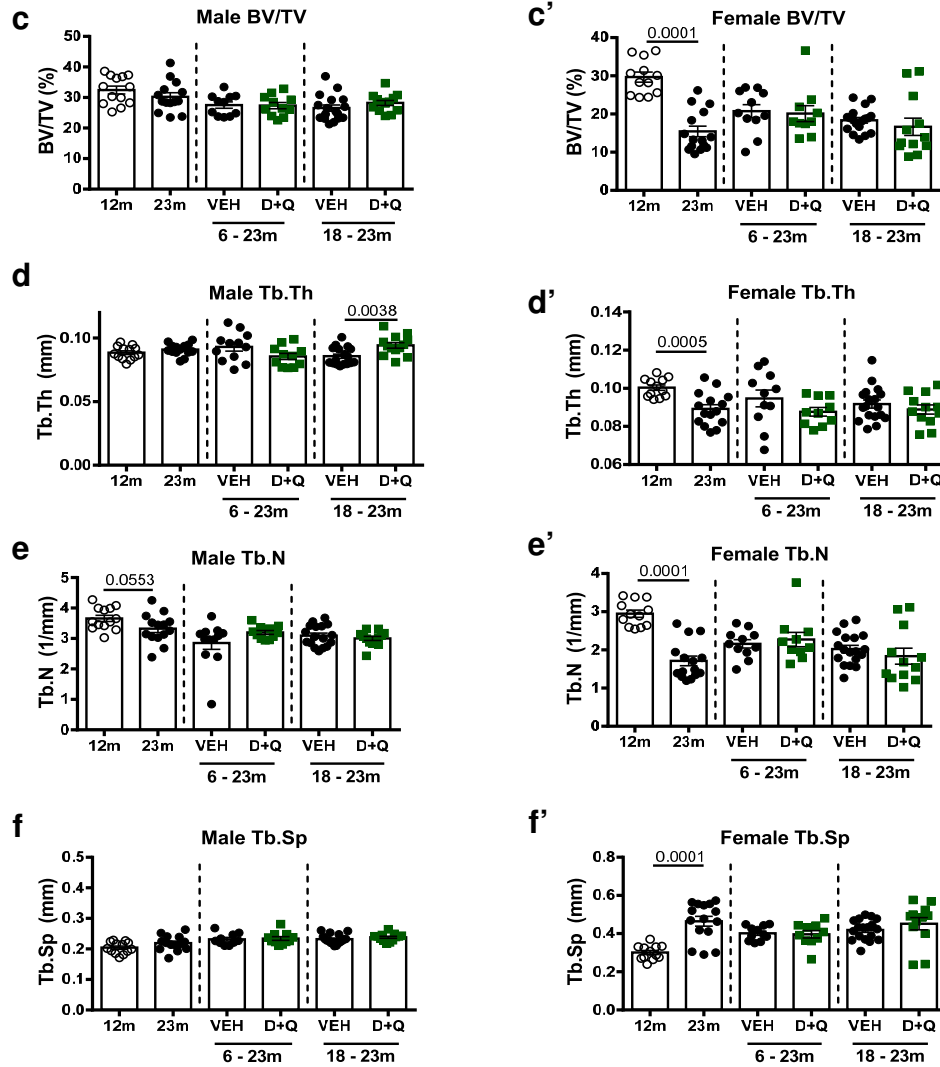
Supplementary Figure 7



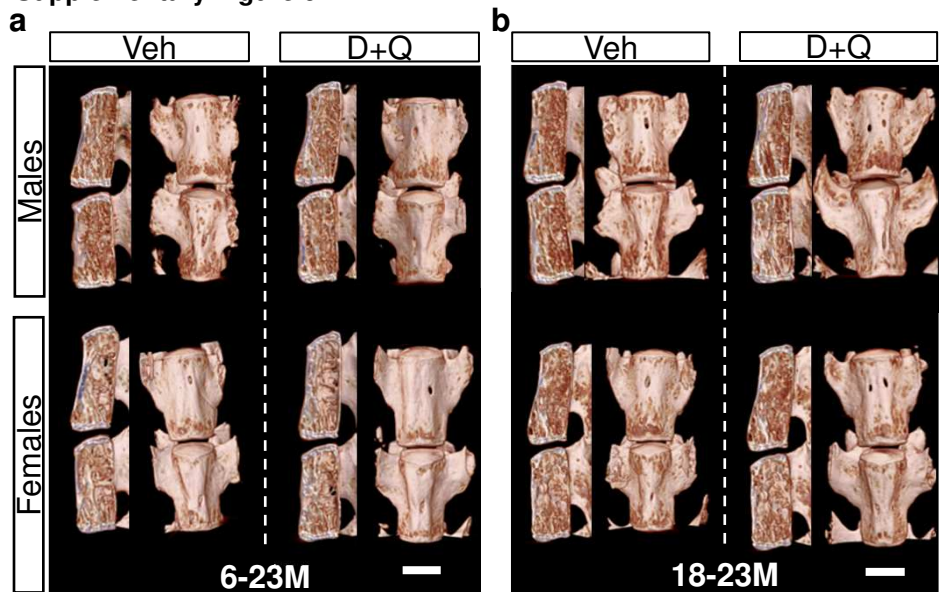
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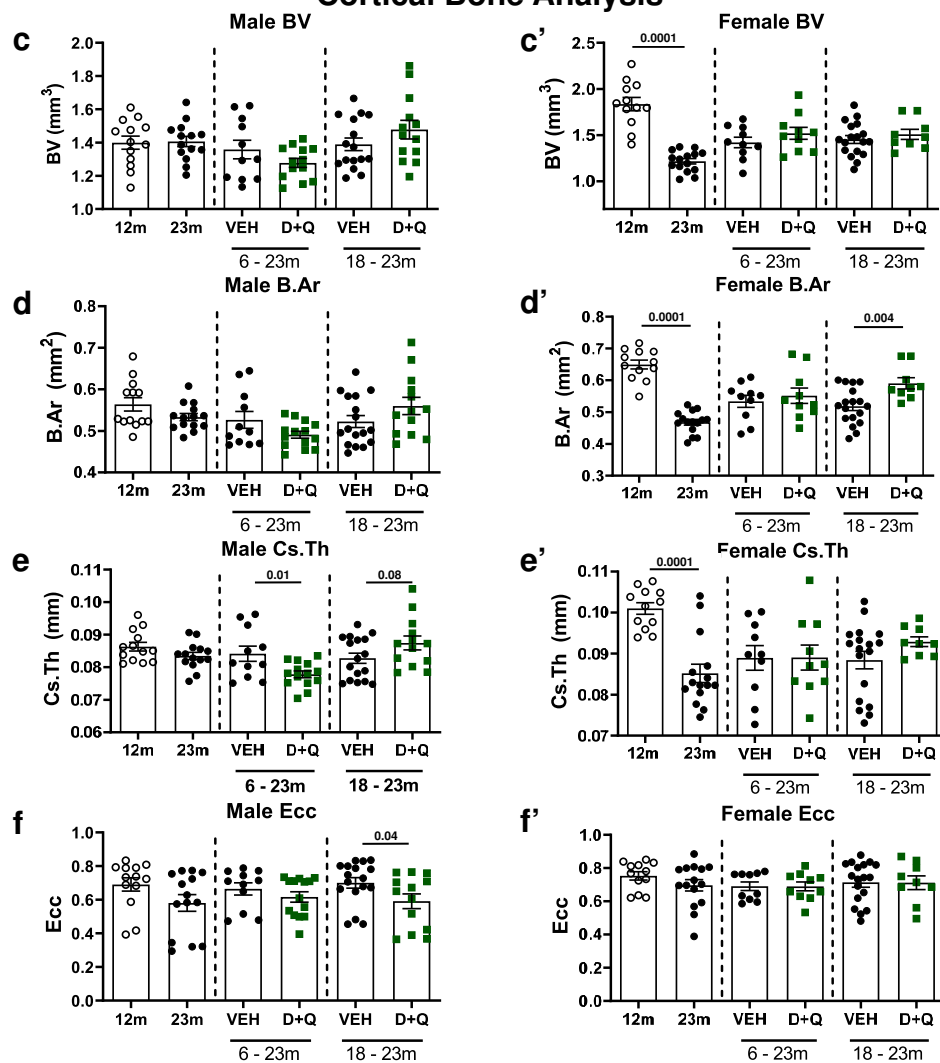
Trabecular Bone Analysis



Supplementary Figure 9

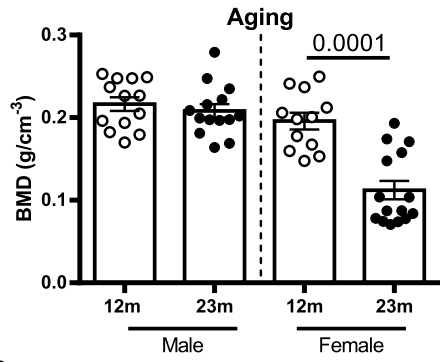


Cortical Bone Analysis

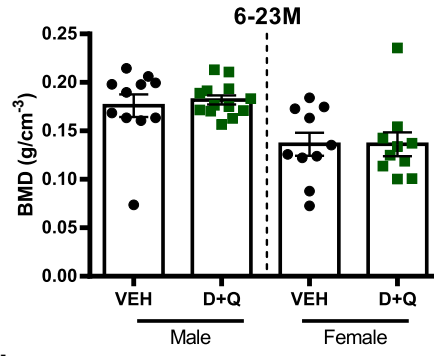


Supplementary Figure 10

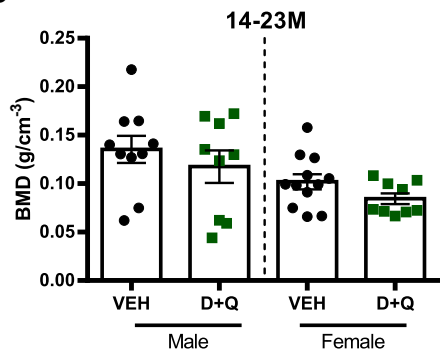
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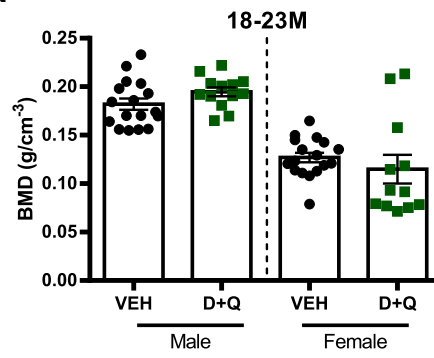
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c



d



Supplementary Figure 11

