Aging and Viscoelasticity Affect Multiscale Tendon Properties and Tendon Derived Cell Behavior

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Supporting Information

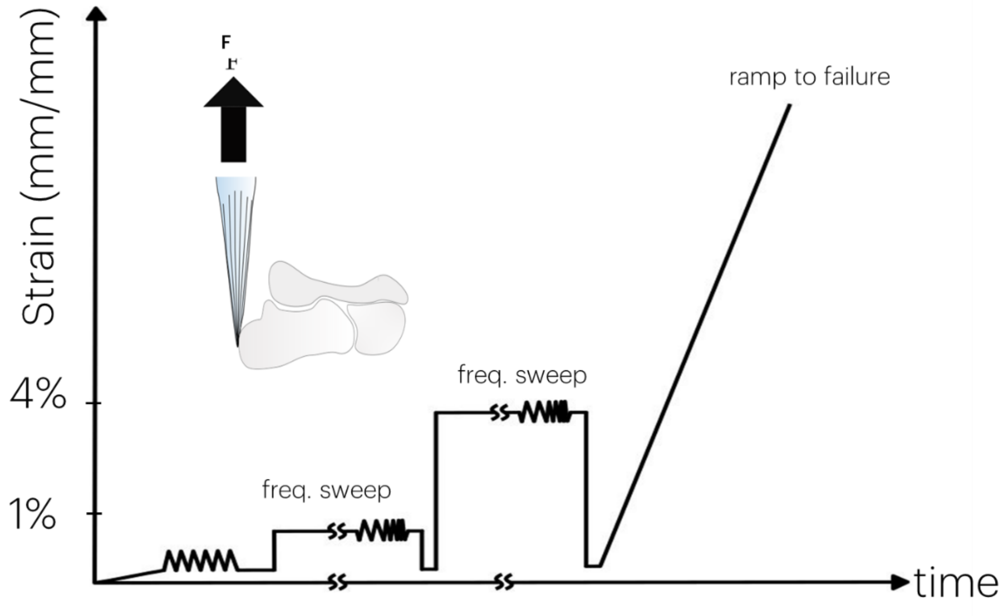

Figure S1 | Mechanical Testing Protocol. Tendons were mechanically tested in tension with stress relaxations at 1% and 4% strain, dynamic loading (0.125% strain at 0.1, 1, 5, and 10Hz) and a quasi-static ramp to failure.

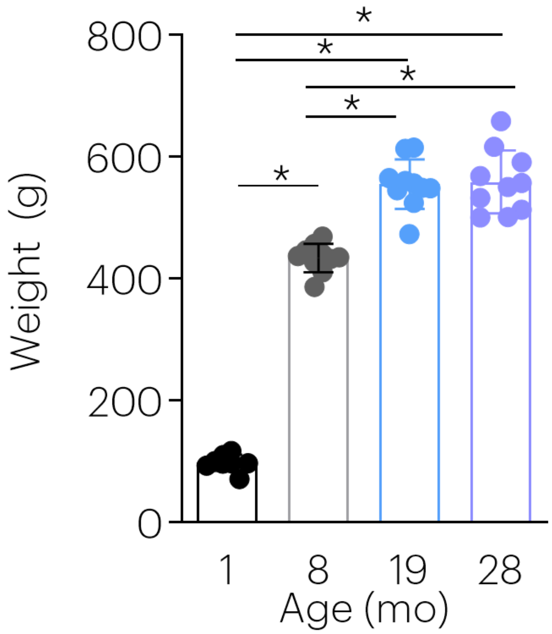

Figure S2 | Rat Weight with Aging. The effect of age on rat body weight. Data shown as mean±s.d. with N=10 rats/group. ***P** < 0.0083 indicates significant differences, as analyzed by a one-way ANOVA with post hoc tests with Bonferroni corrections.

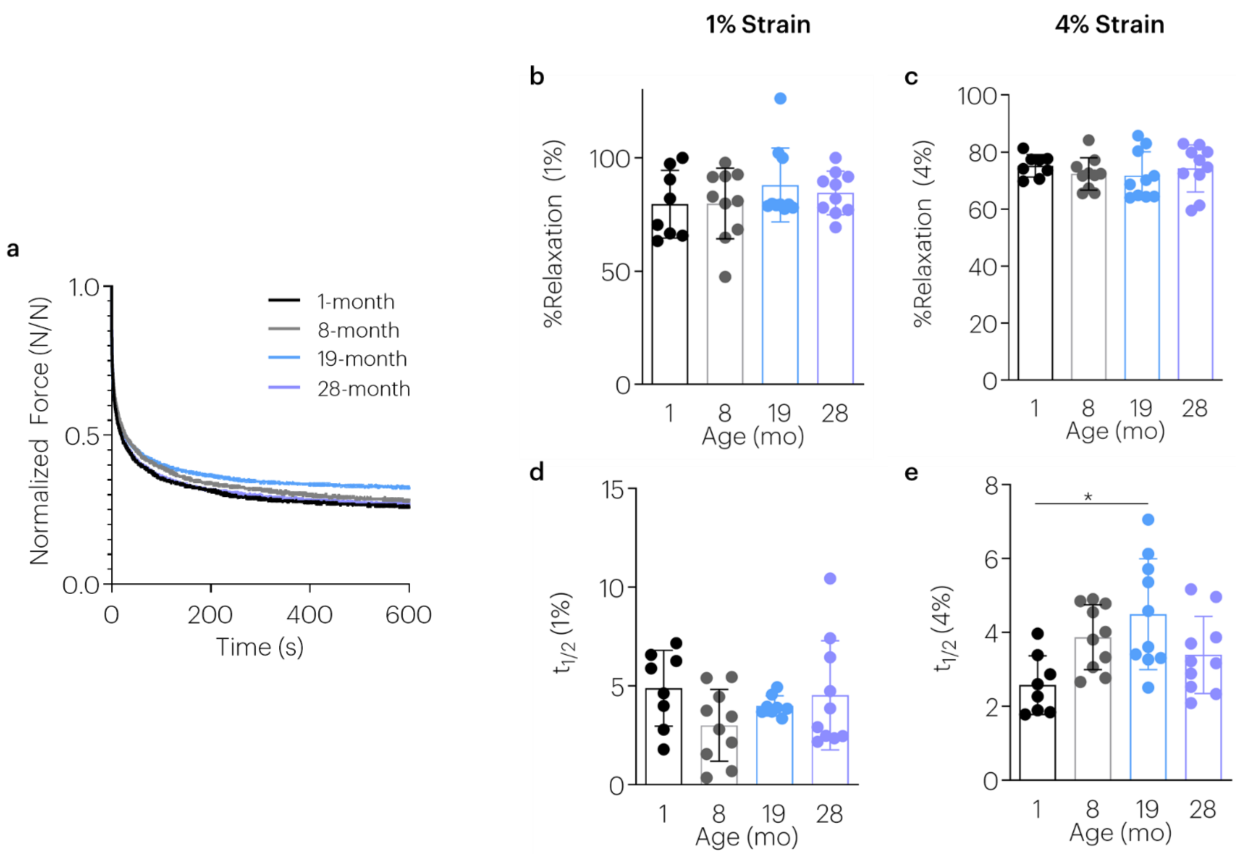

Figure S3 | Effect of Age on Achilles Tendon Viscoelastic Properties. (**a**) Representative stress relaxation curves following ramp to 4% strain. The effect of aging on (**b**) the percent relaxation at 1% strain, (**c**) the percent relaxation at 4% strain, (**d**) the relaxation half time at 1% strain, and (**e**) the relaxation half time at 4% strain were investigated. Data shown as mean±s.d. with N=7-10 tendons/group. ***$P < 0.0083$ indicates significant differences, as analyzed by a one-way ANOVA with post hoc tests with Bonferroni corrections.

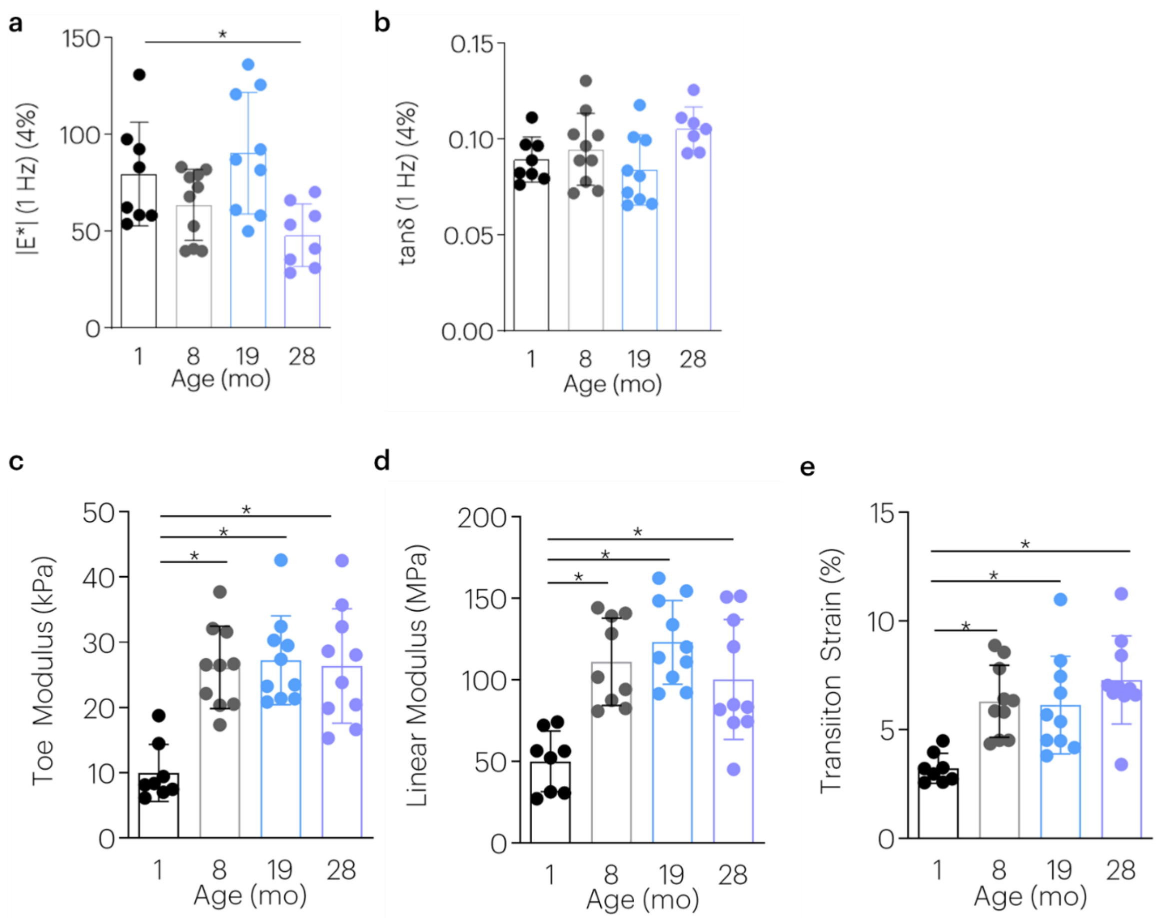

Figure S4 | Effect of Age on Dynamic and Quasi-Static Achilles Tendon Mechanics. The effect of age on the (**a**) dynamic modulus at 4% strain, (**b**) tan(δ) at 4% strain, (**c**) toe modulus, (**d**) linear modulus, and (**e**) transition strain. Data shown as mean±s.d. with N=7-10 tendons/group. ****P*** < 0.0083 indicates significant differences, as analyzed by a one-way ANOVA with post hoc tests with Bonferroni corrections.

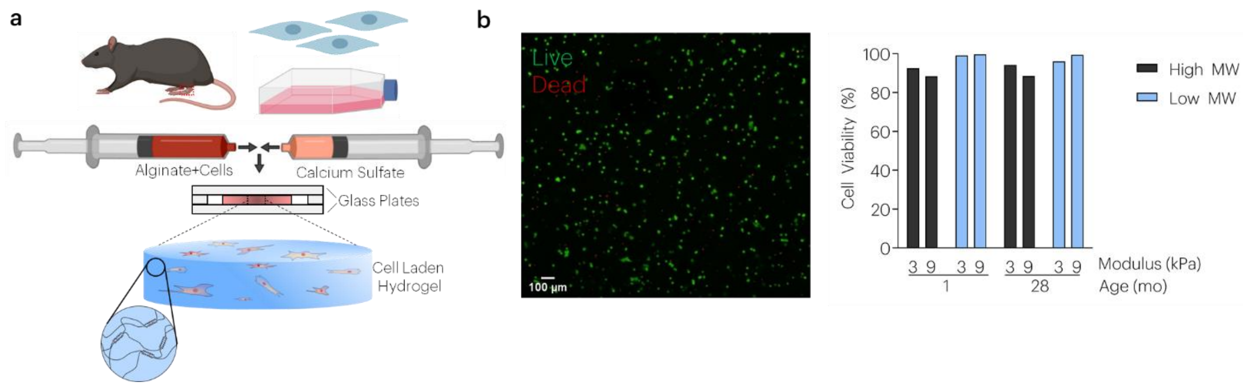

Figure S5 | Cell Viability in Alginate Hydrogels. (**a**) Cells isolated and expanded from rat Achilles tendons at 1 and 28 months of age were encapsulated in 3D into alginate hydrogels of varying stiffness and viscoelasticity. (**b**) Cells were examined for their viability following encapsulation after 24h, and cell viability quantified. Values represent the average viability of >1000 cells.

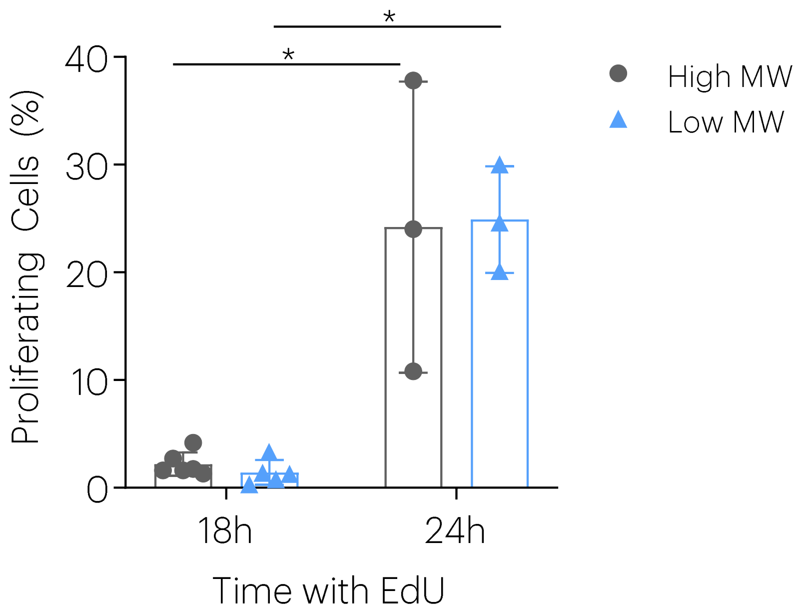

Figure S6 | Effect of EdU Incubation Time on Proliferation. The effect of EdU incubation time and alginate molecular weight on the percent of proliferating cells from 1-mo donors after 3-days in culture was examined. Data shown as mean ± s.d. with N=3-6 gels/group. *$P < 0.0125$ indicates significant differences, as analyzed by a two-way ANOVA with post hoc tests with Bonferroni corrections.

Table S1: Overview of the $CaSO_4$ concentrations (in mM) used for MVG and VLVG to tune their stiffness.

Alginate	Stiffness	$CaSO_4$ (mM)
MVG	high	42
MVG	low	16
VLVG	high	48
VLVG	low	24