Exercise prevents the negative impact of prolonged inactivity in *Drosophila*

Jodi Protasiewicz[1], Sarah Snider[1], Mousumee Khan[2,3], Li Tao[2], Robert J. Wessells[2], and Alyson Sujkowski[1,4*]

[1]Department of Pharmacology, Wayne State University School of Medicine, Detroit, MI 48201, USA

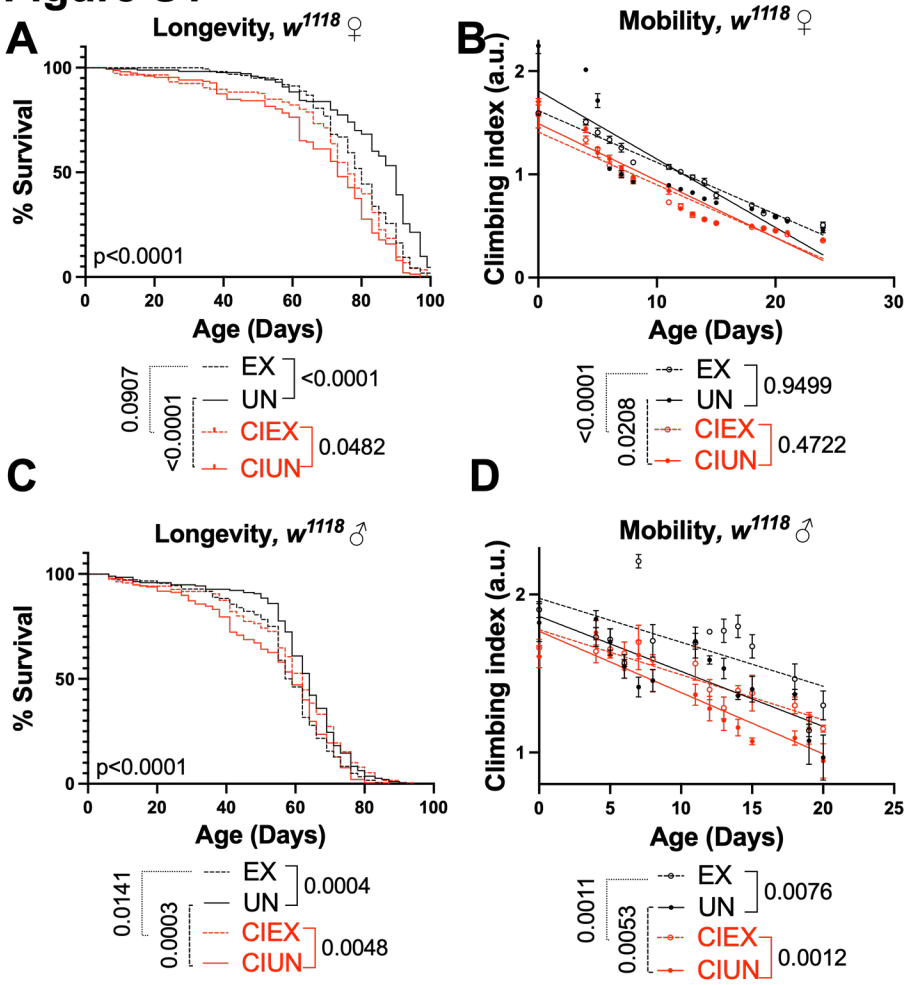
[2]Department of Physiology, Wayne State University School of Medicine, Detroit, MI 48201, USA

[3]Department of Ophthalmology, Visual, and Anatomical Sciences, Wayne State University School of Medicine, Detroit, MI 48201, USA

[4]Department of Physical Therapy, Wayne State University Eugene Applebaum College of Pharmacy and Health Sciences, Detroit, MI 48201, USA

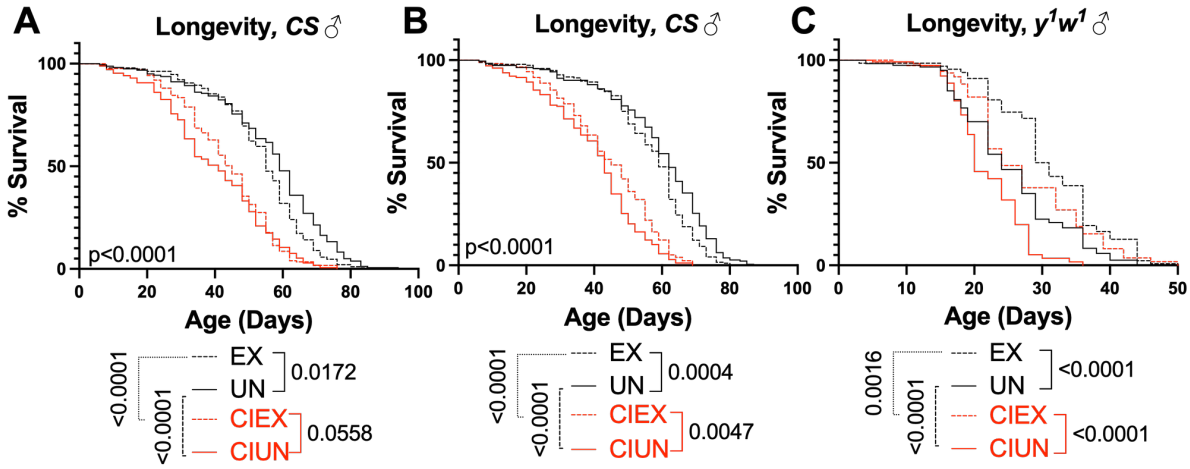
***Corresponding author** Alyson Sujkowski: Wayne State University School of Medicine, Department of Pharmacology, Detroit, MI 48201, USA. Email: asujkows@med.wayne.edu

Figure S1



Supplemental Figure S1: Additional confirmation of restraint and exercise effects in w^{1118} flies. (A) Lifespan is improved in restrained female flies that complete 3 weeks of exercise when compared to unexercised, restrained siblings. **(B)** In this biological cohort, female flies that undergo CI have worse mobility than freely mobile flies whether exercised or not. A second cohort of exercised male flies that undergo mobility restriction have better lifespan **(C)** and mobility **(D)** than unexercised restrained siblings. n>200 flies per experiment. Exercise, mobility and longevity experiments were repeated a minimum of 3 times and in 3 genetic backgrounds (w^{1118}, y^1w^1, and Canton S). Trends were similar in all genotypes, with representative cohorts depicted here. w^{1118}, and y^1w^1 males showed the most consistent phenotypes in response to both restraint and exercise and were therefore used to complete the remaining experiments. Longevity analyzed by log-rank, mobility analyzed by linear regression, looking for differences in slope and intercept.

Supplemental Figure S2



Supplemental Figure S2: Lifespan is improved in restrained exercised flies of multiple genetic backgrounds. Lifespan was assessed in duplicate biological replicates **(A, B)** of male *Canton S* flies under restraint stress. Exercise improved longevity in restrained flies but in one repetition **(A)** failed to achieve significance. **(C)** Exercise significantly improved lifespan in confined y^1w^1 males.

Supplemental Legends

Supplemental Figure S1: Additional confirmation of restraint and exercise effects in w^{1118} flies. (A) Lifespan is improved in restrained female flies that complete 3 weeks of exercise when compared to unexercised, restrained siblings. **(B)** In this biological cohort, female flies that undergo CI have worse mobility than freely mobile flies whether exercised or not. A second cohort of exercised male flies that undergo mobility restriction have better lifespan **(C)** and mobility **(D)** than unexercised restrained siblings. n>200 flies per experiment. Exercise, mobility and longevity experiments were repeated a minimum of 3 times and in 3 genetic backgrounds (w^{1118}, y^1w^1, and Canton S). Trends were similar in all genotypes, with representative cohorts depicted here. w^{1118}, and y^1w^1 males showed the most consistent phenotypes in response to both restraint and exercise and were therefore used to complete the remaining experiments. Longevity analyzed by log-rank, mobility analyzed by linear regression, looking for differences in slope and intercept.

Supplemental Figure S2: Lifespan is improved in restrained exercised flies of multiple genetic backgrounds. Lifespan was assessed in duplicate biological replicates **(A, B)** of male *Canton S* flies under restraint stress. Exercise improved longevity in restrained flies but in one repetition **(A)** failed to achieve significance. **(C)** Exercise significantly improved lifespan in confined y^1w^1 males.