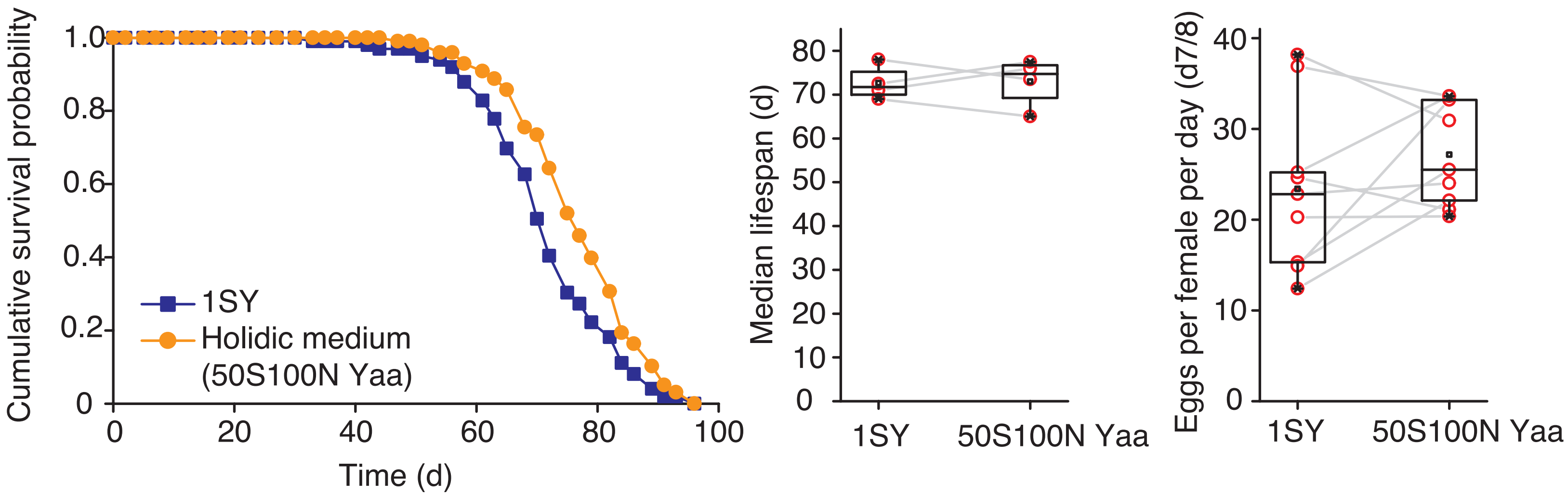
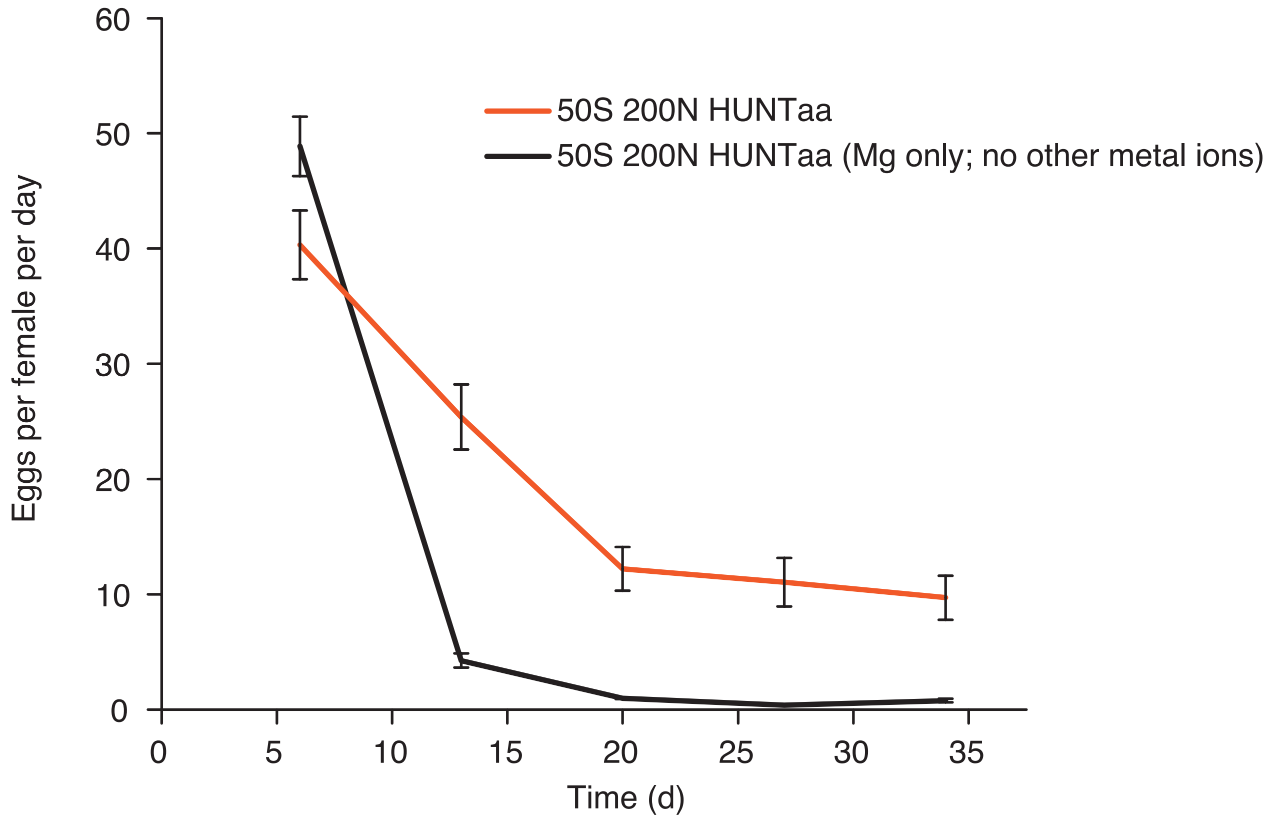
Supplementary Figure 1



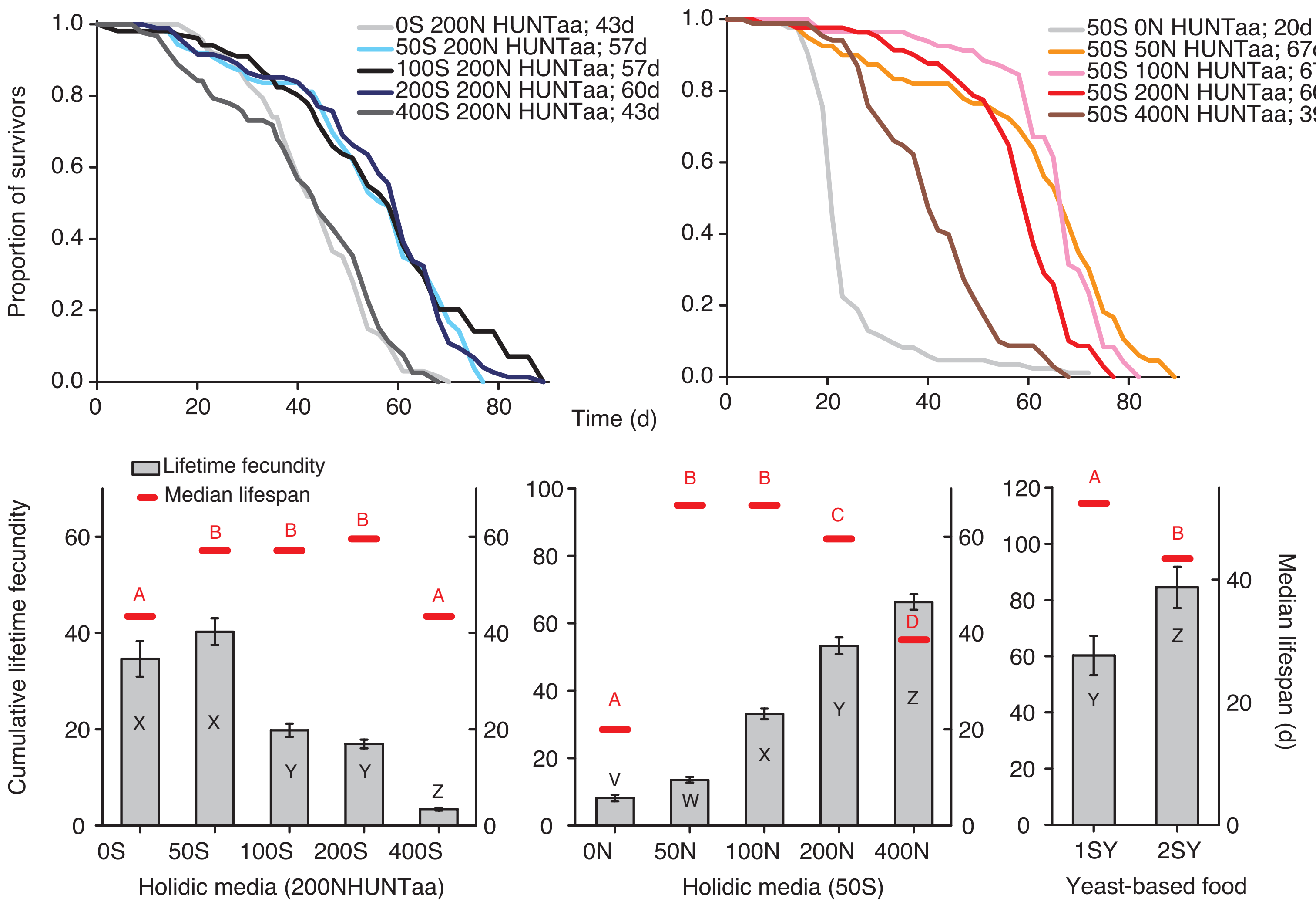
Supplementary Figure 1 I Lifespan and egg-laying of flies on 50S100N Yaa. Defined medium with an amino acid ratio similar to yeast (Yaa) supports adult lifespan ($P = 0.77$, n = 4, Wilcoxon rank sum test) and egg-laying ($P = 0.31$, n = 10, Wilcoxon rank sum test) to the same extent as a longevity-promoting yeast based diet (1SY). In this trial, flies on defined medium (76 d median) were longer lived than those on yeast-based food (71 d median; $P < 0.001$, n = 100, Log-rank test).

Supplementary Figure 2



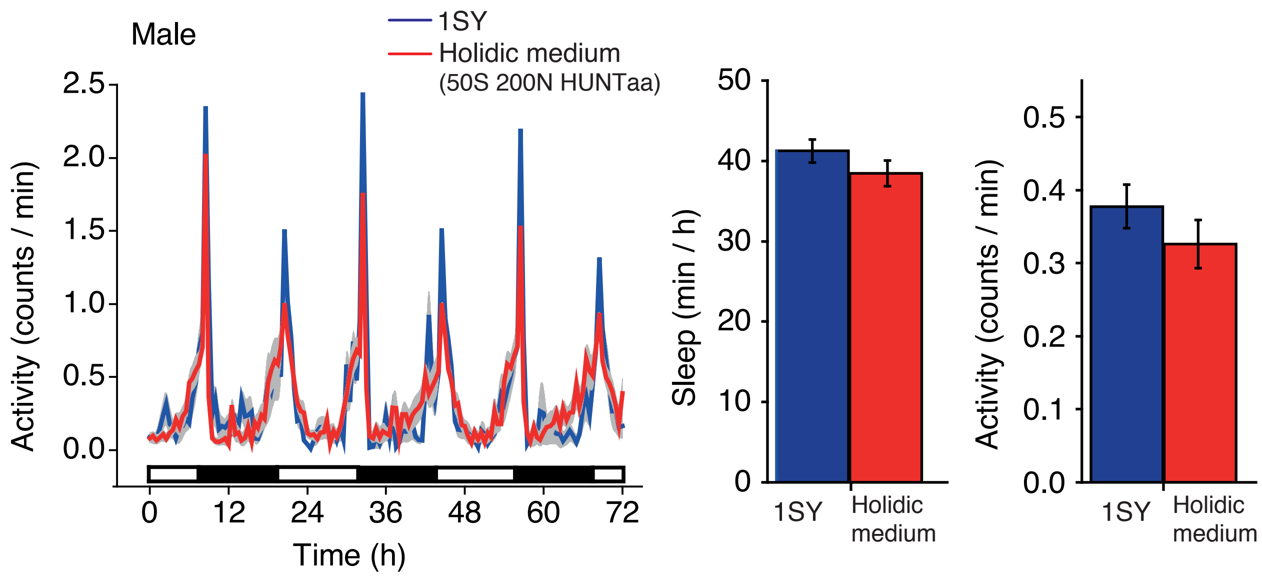
Supplementary Figure 2 | Effect on egg-laying of omission of metal ions from the defined medium. In order to sustain egg-laying beyond the first two weeks of adult life, flies required a source of Ca, Cu, Fe, Mn and Zn in addition to Mg. Cumulative egg-laying for this period differed ~2-fold (55.2 ± 2.3 eggs without metal ions versus 102 ± 4.4 with ($P = 0.009$, n = 5, Wilcoxon rank sum test). Data points represent mean ± s.e.m.

Supplementary Figure 3



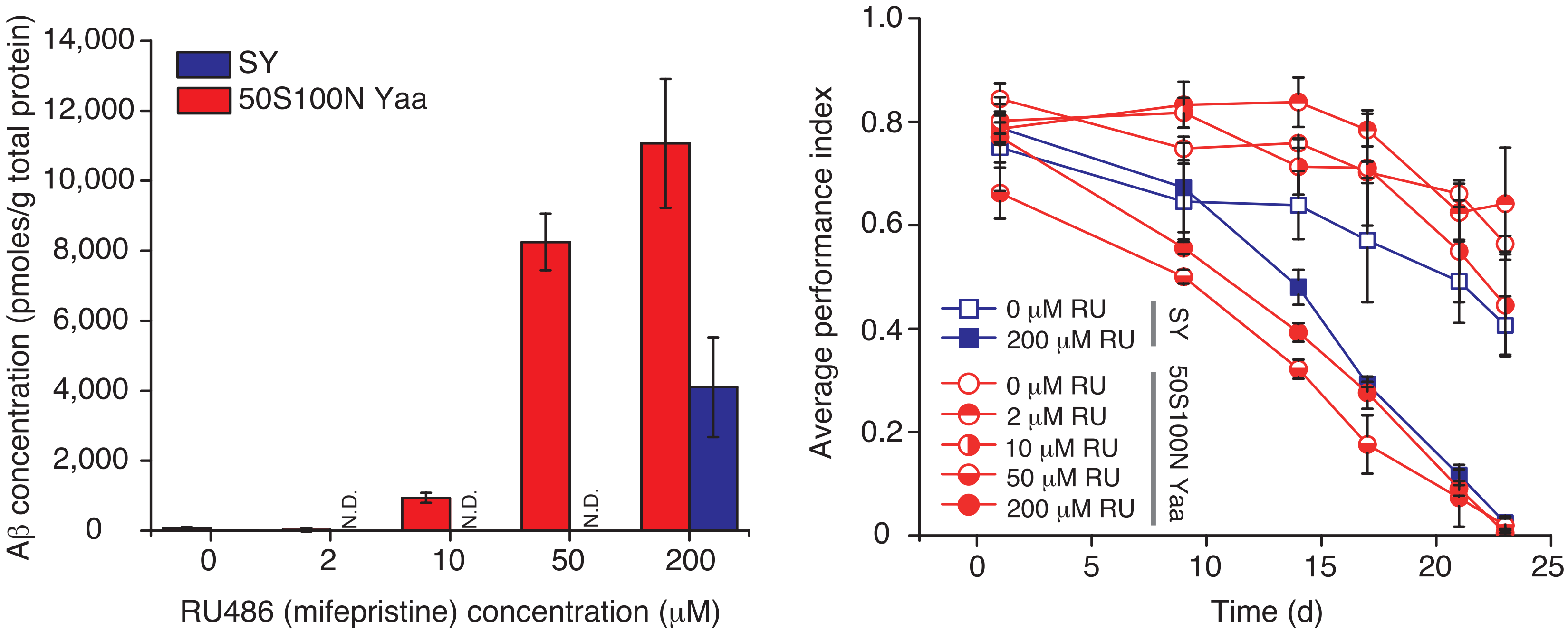
Supplementary Figure 3 I Egg-laying and lifespan were affected by amino acid and sugar concentration changes in holidic media. From 0 mM sugar to 50 mM sucrose in the medium, lifespan significantly increased ($P < 0.0001$, n = 100, Log-rank test), but remained constant for additions of 50mM, 100mM and 200mM (for each comparison $P > 0.3$, n = 100, Log-rank test), whereupon it was reduced by addition of 400 mM sucrose ($P < 0.0001$, n = 100, Log-rank test). For the same series, egg-laying was unaffected from 0 mM to 50 mM sucrose ($P = 0.24$, n = 10, Wilcoxon rank-sum test), but was decreased by increasing to either 100 mM, 200 mM or 400 mM (for each comparison $P < 0.001$, n = 10, Wilcoxon rank-sum test). When the sucrose concentration was fixed at 50 mM, increasing the amino acid concentration from 0 mM to 50 mM biologically available nitrogen increased lifespan ($P < 0.0001$, n = 100, Log-tank test), but was unaffected by further increasing to 100 mM ($P = 0.65$, n = 100, Log-rank test). Additional increases from 100 mM to 200 mM shortened lifespan ($P < 0.0001$, n = 100, Log-rank test) as did the further increase to 400 mM (200 mM v 400 mM, $P < 0.0001$, n = 100, Log-rank test). Egg-laying significantly increased for each addition of amino acids (for each increment comparison $P < 0.002$, n = 10, Wilcoxon rank-sum test). This inverse relationship between lifespan and egg-laying conforms to the definition of dietary restriction as seen on oligidic diets (1SY v 2SY: lifespan, $P < 0.0001$, n =100, Log-rank test; egg-laying, $P = 0.041$, n = 10, Wilcoxon rank-sum test). Unique letters within each plot represent significant differences. Bars represent mean ± s.e.m.

Supplementary Figure 4



Supplementary Figure 4 | Activity patterns of male flies housed on holidic and oligidic diets. Male flies were maintained on either holidic medium (50S 200N HUNTaa) or oligidic medium (1SY). Activity patterns (summed into 30 minute bins) were recorded for 72 h following a 24 h acclimation period. Average sleep amount and total waking activity were summed across the whole recording period and did not significantly differ between the holidic medium and 1SY ($P > 0.21$, n = 16, Wilcoxon rank-sum test). Mean ± s.e.m. is plotted for each graph. Black and white boxes represent light and dark periods.

Supplementary Figure 5



Supplementary Figure 5 | Enhanced bioavailability of RU486 in holidic medium. Flies transgenic for neuronally-driven, RU486-inducible Aβ42 expression were exposed to different concentrations of RU486 in their food and after 14 days, assessed by ELISA for levels of Aβ42 and for climbing ability. Flies in holidic medium containing 200 μM RU486 expressed higher levels of Aβ42 than those in SY food with the same dose of drug ($P = 0.0084$, n = 3, Student's t-test), however, there was no significant difference for SY + 200 μM RU486 versus either 10 μM or 50 μM RU486 in holidic food ($P > 0.13$, n = 3, Student's t-test) indicating an intermediate dose was required to achieve the same expression between food types. Climbing ability of flies fed 50 μM or 200 μM RU486 on holidic diet was worse than for 200 μM RU486 on SY food ($P < 0.005$, n = 3, 2-way ANOVA). Together, this prompted us in further experiments to test a dose of RU486 on holidic diet between 10–50 μM in order to match the effect of 200 μM RU486 on SY food (see Fig. 4a). Bars represent mean ± s.e.m. N.D. - not determined.