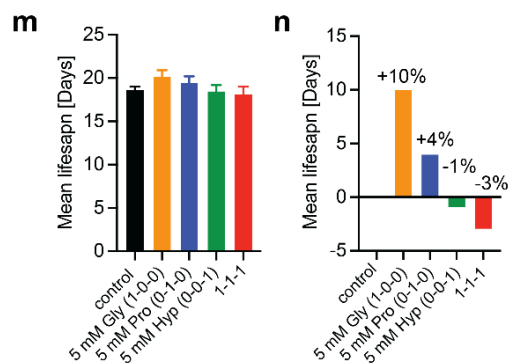
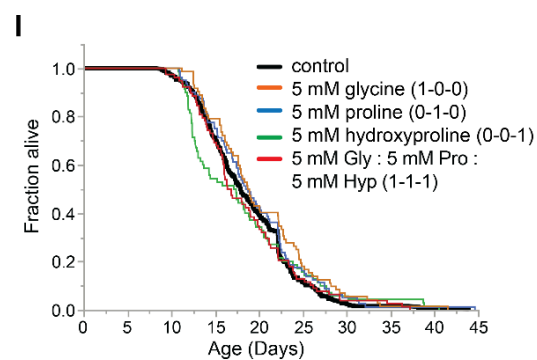
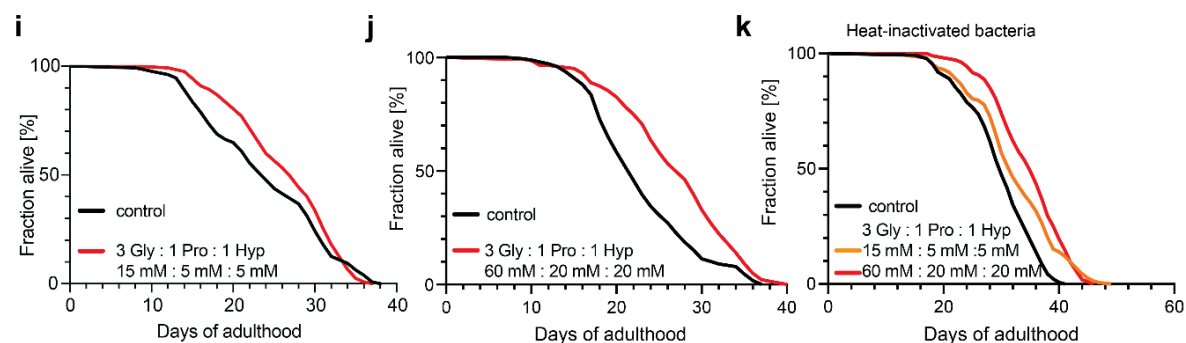
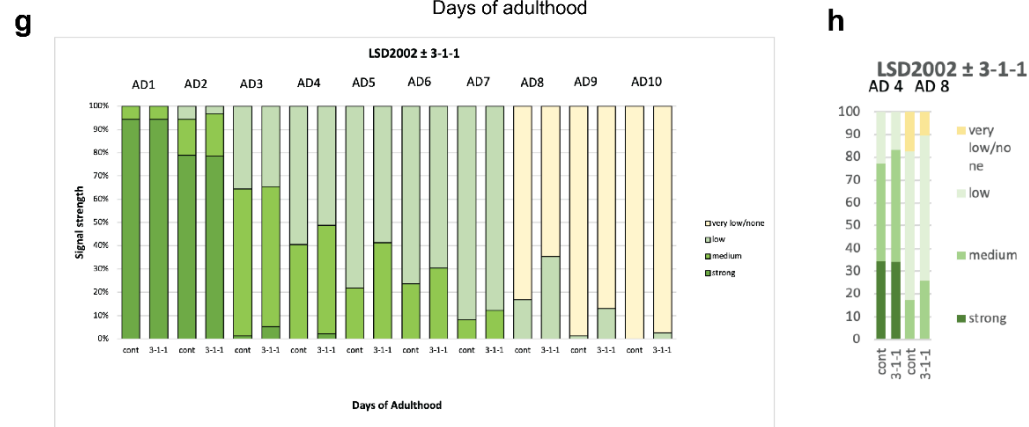
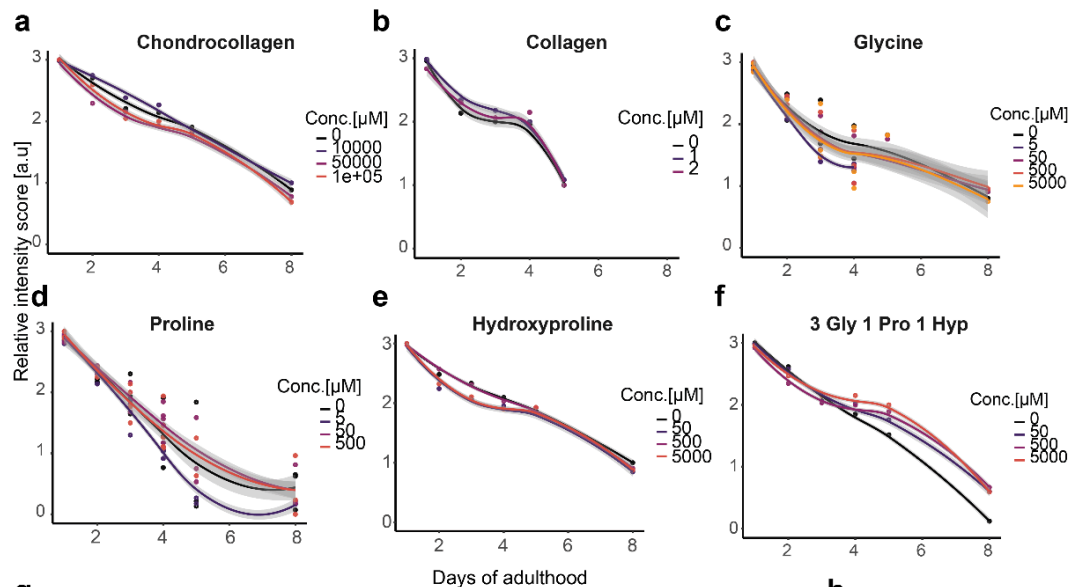
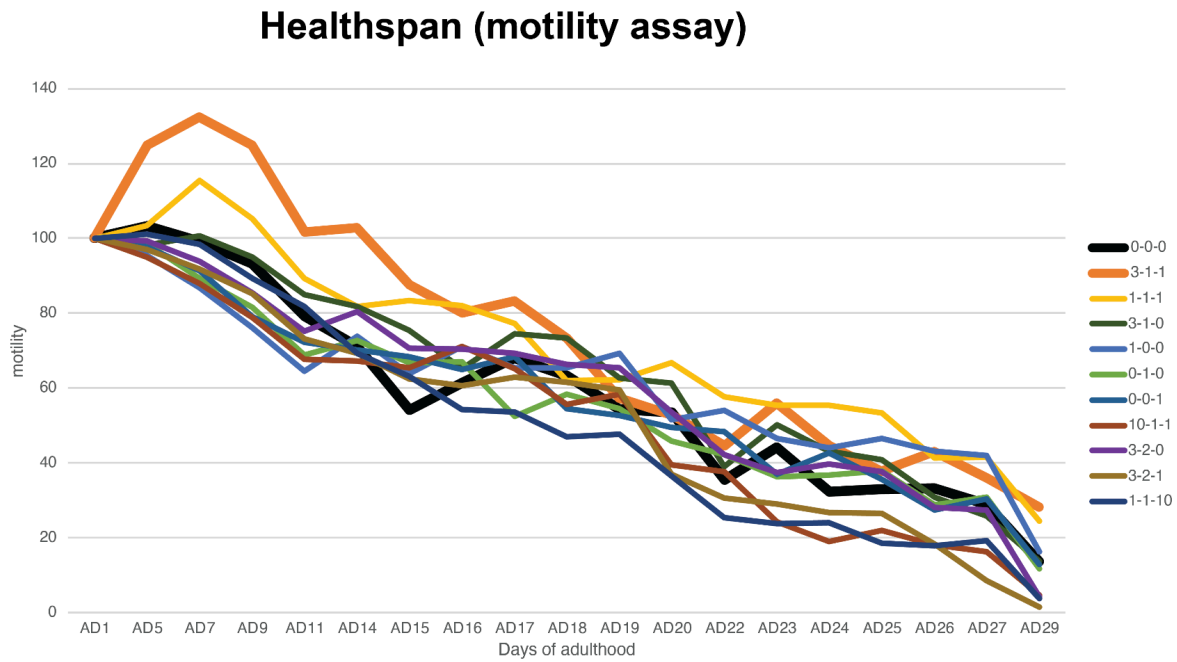
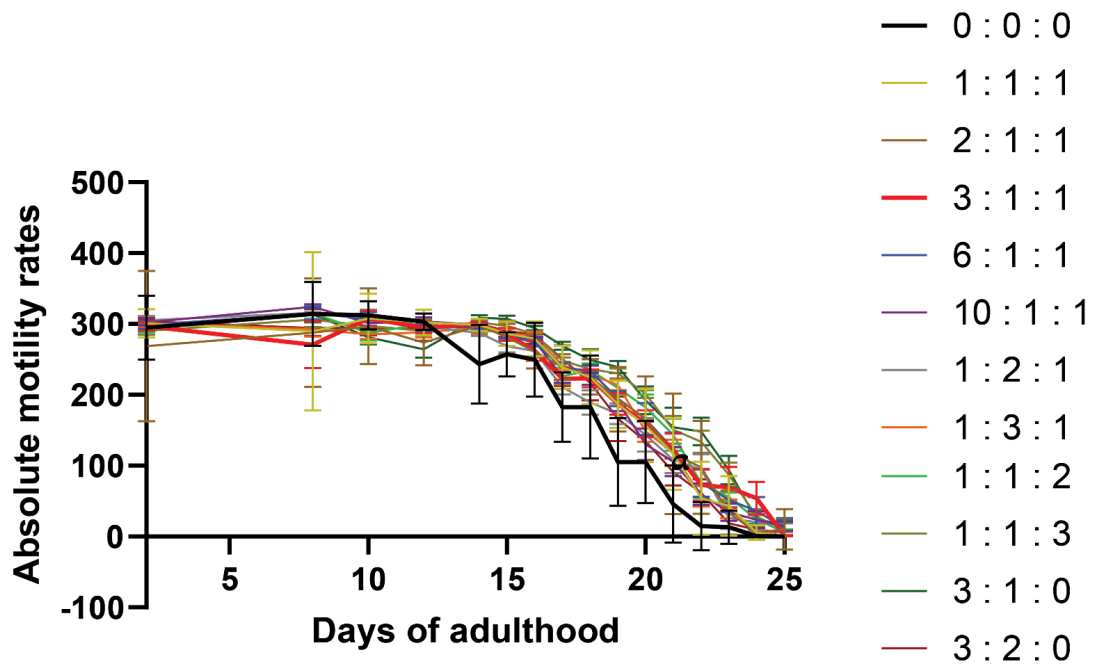




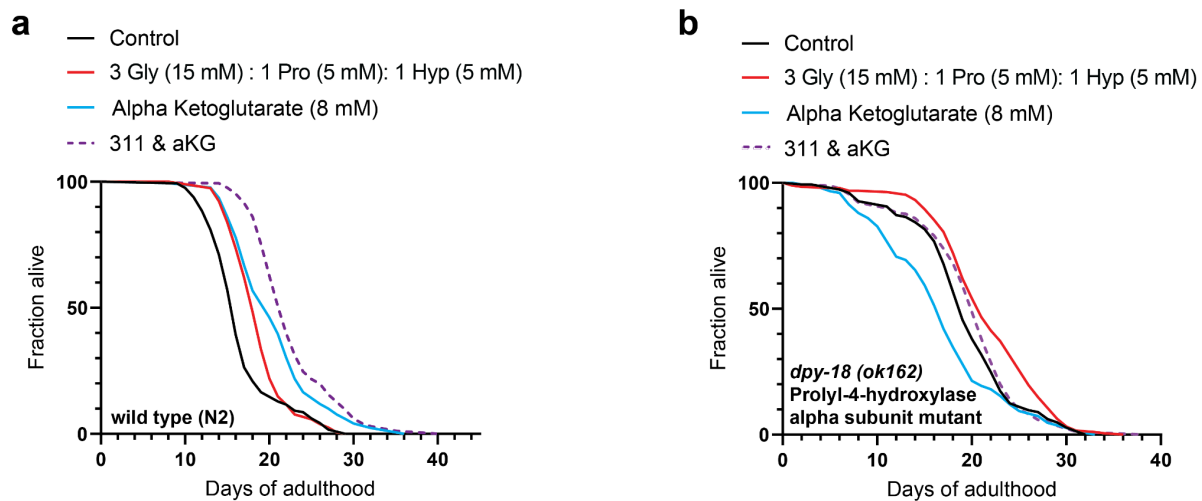
Supplementary Fig.1: Supplementation of 3 Gly : 1 Pro : 1 Hyp prolonged endogenous collagens expression during aging and promoted longevity.

a-f, Synchronized L4 transgenic *C. elegans* driving GFP with the collagen *col-144* promoter (LSD2002 *Pcol-144::GFP*) were placed on culturing plates containing heat-killed OP50 bacteria food with different concentrations of either chondrocollagen (**a**), rat tail collagen (**b**), glycine (**c**), proline (**d**), hydroxyproline (**e**), or a mixture of [3 Gly : 1 Pro : 1 Hyp] (**f**), and GFP intensity was scored during aging at 25°C. See Supplementary Table 1 for raw data. **g,h**, LSD2002 *Pcol-144::GFP* L4 were placed on heat-killed OP50 bacteria food with [3 Gly (15 mM) : 1 Pro (5 mM) : 1 Hyp (5 mM)] (annotated as 311) and *col-144* expression was scored from 1-10 adulthood days (AD). For data and statistics, see Supplementary Table 1. **i-k**, Supplementation of [3 Gly : 1 Pro : 1 Hyp] starting from young adulthood increased the lifespan of temperature-sensitive wild-type background TJ1060 *C. elegans*. Concentration in mM given. TJ1060 *C. elegans* were placed on heat-inactivated bacteria treated with carbenicillin to exclude bacterial changes in the supplemented amino acids (**k**). **l-n**, Supplementation of individual amino acids or in a 1/1/1 ratio [1 Gly : 1 Pro : 1 Hyp] starting from young adulthood was insufficient to increase the lifespan of temperature-sensitive wild-type background TJ1060 *C. elegans*. **j**, Survival curves. **k**, Graph of mean $\pm$ SEM lifespan. **l**, Graph of percent mean lifespan increase. For (**i-n**) statistical analysis, replicates and raw data are in Supplementary Table 2.

a**b**

Supplementary Fig. 2: Longitudinal motility improved by 3 Gly : 1 Pro : 1 Hyp supplementation.

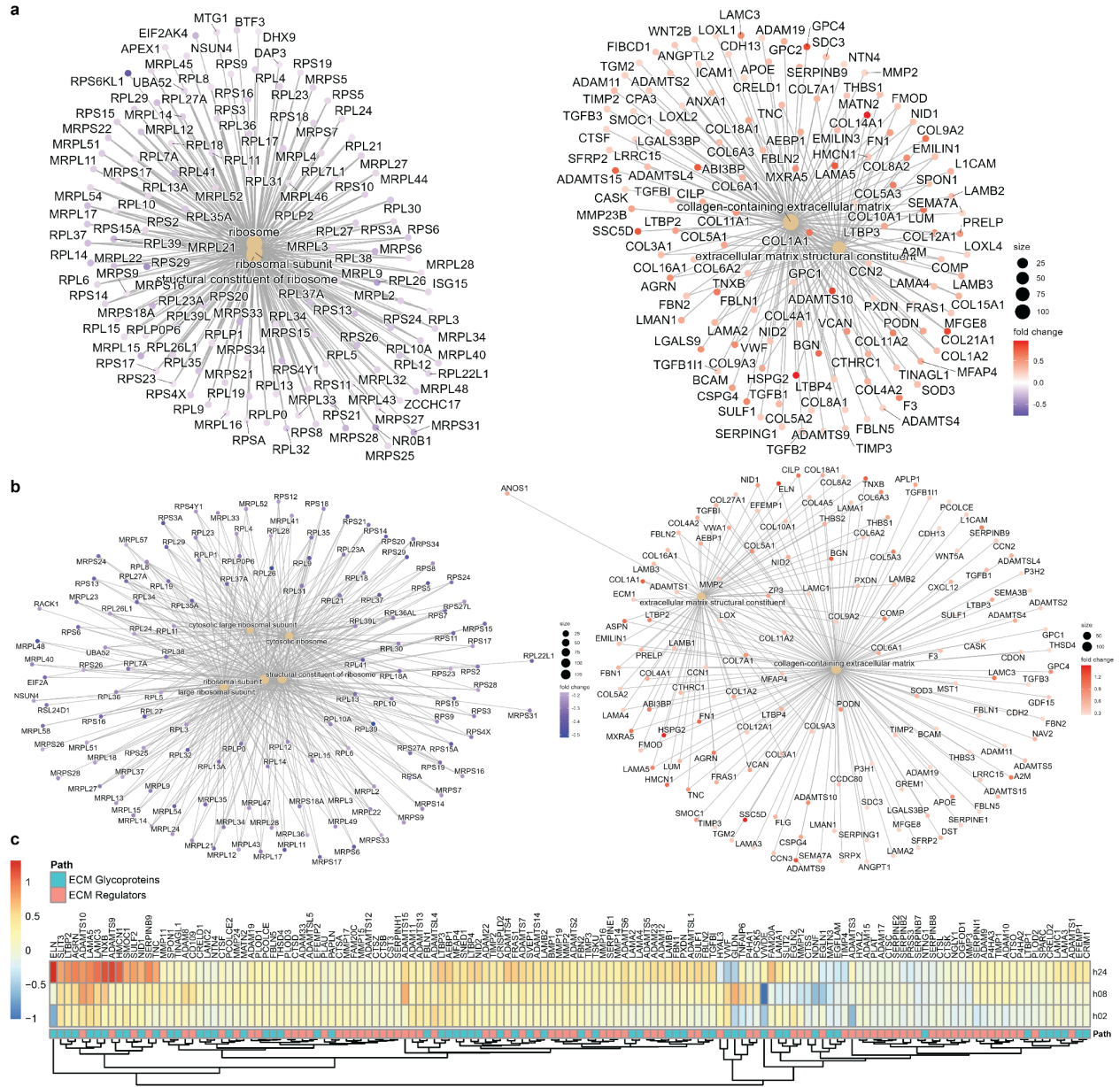
a-b, Synchronized day-1 adult *C. elegans* (**a**, TJ1060; **b**, N2) were placed into S-complete buffer containing heat-killed OP50 bacteria food with different ratios of [Gly : Pro : Hyp], and swimming speed (mobility) was measured as a proxy for healthspan. Raw data in Supplementary Table 3.



Supplementary Fig.3: The synergistic lifespan effects of 3 Gly: 1 Pro: 1 Hyp with alpha-ketoglutarate supplementation were mediated through two distinct mechanisms.

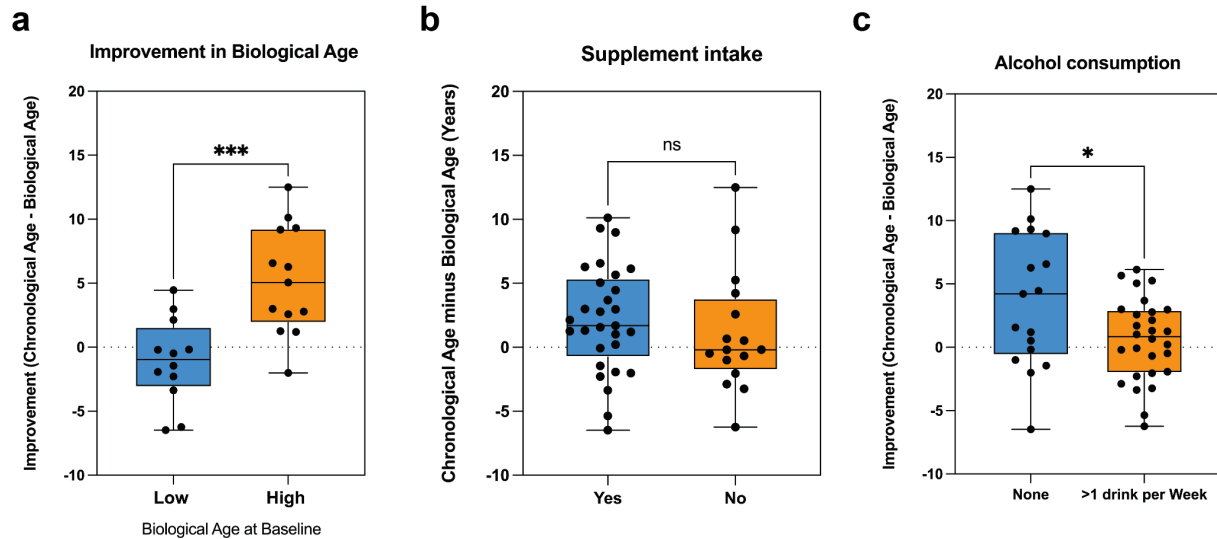
a, Supplementation of [3 Gly : 1 Pro : 1 Hyp], alpha ketoglutarate, or a combination starting from day 1 of adulthood increased the lifespan of wild type (N2) *C. elegans*.

b, Supplementation of [3 Gly : 1 Pro : 1 Hyp], but not alpha ketoglutarate, nor a combination starting from day 1 of adulthood, increased the lifespan of *dpy-18(ok162)* mutants. Lifespan data and statistics in Supplementary Table 2.



Supplementary Fig.4: Treatment of primary human dermal fibroblasts with 3 Gly : 1 Pro : 1 Hyp increased collagen-related gene expression.

a,b, Network plots of gene set enrichment analysis results after 8 hours (a) and 24 hours of treatment (b). **c**, Heatmap showing log₂ fold change vs respective timepoint-matched controls of matrisome genes annotated as extracellular matrix (ECM) glycoproteins or ECM regulators.



Supplementary Fig.5: Effects of initial biological age and lifestyle behavior on Collagen Activator's improvements on biological age.

a, Boxplot showing significant changes over 6 months in biological age for individuals with higher (25th percentile) and lower (75th percentile) biological age relative to their chronological age at baseline ($P = 0.0004$). **b**, Boxplot showing the distribution of biological age change over six months, stratified by supplement intake. No significant difference has been observed between the two groups. **c**, Boxplot showing the distribution of biological age change over six months, stratified by alcohol consumption. Alcohol drinkers showed less improvement in biological age than non-drinkers ($P = 0.016$).