

Supplementary Fig. 1 Phenotypic analysis of methionine-restricted flies.

a, **b**, Oil red O staining of female guts of Canton-S flies fed with or without a methioninerestricted diet for nine days. Whole gut (**a**) or magnified view of the anterior midgut (**b**). Scale bar: 1 mm (**a**), or 100 μ m (**b**). **c**, Lifespans of female Canton-S flies fed with or without a methionine-restricted diet that contained three times as much cholesterol. Sample sizes (n) are shown in the figure. For the statistics, a log-rank test was used. **d**, Survivability of female Canton-S flies upon complete starvation after feeding with or without a methionine-restricted diet that contained three times as much cholesterol for a week. Sample sizes (n) are shown in the figure. For the statistics, a log-rank test was used. **e**, Climbing abilities of female Canton-S or w^{Dah} flies fed with or without a methioninerestricted diet for four weeks. **f**, Quantification of amino acids other than methionine in female w^{Dah} flies upon methionine restriction. The relative amount of each amino acid upon methionine restriction compared to the control diet is shown. n = 6. For the statistics, a two-tailed Student's *t* test was used. For the graph, the mean and SEM are shown. Data points indicate biological replicates. Source data are provided as a Source Data file.

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GO term "determination of adult lifespan"

Gene Name	baseMean	log2 fold change
dawdle(daw)	195	2.15
CG8846 gene product from transcript CG8846-RA(Thor/4E-BP)	12164	2.09
Methionine sulfoxide reductase A (MsrA)	1529	2.06
Peptidoglycan recognition protein LF(PGRP-LF)	42	1.90
period(per)	568	1.85
midkine and pleiotrophin 1(miple1)	21	1.48
CG10383 gene product from transcript CG10383-RB(CG10383)	1750	1.15
bunched(bun)	6576	0.99
puckered(puc)	1544	0.94
Death regulator Nedd2-like caspase(Dronc)	253	0.75
pointed(pnt)	667	0.71
CG42663 gene product from transcript CG42663-RB(CG42663)	639	0.64
Histone deacetylase 1(HDAC1)	546	-0.61
Coenzyme Q biosynthesis protein 2(Coq2)	141	-0.76
Phosphoribosylamidotransferase(Prat)	254	-0.77
Catalase(Cat)	13162	-0.80
CG31148 gene product from transcript CG31148-RA(CG31148)	3351	-0.93
Heat shock protein 68(Hsp68)	90	-1.89



Supplementary Fig. 2 Transcriptomic responses to methionine restriction in young and aged flies.

a, List of genes termed "determination of adult lifespan" by Gene Ontology analysis. **B**-**k**, Read counts of genes induced by methionine restriction at a young age from the RNAseq analysis. N = 3. For the statistics, one-way ANOVA with Holm-Šídák's multiple comparison test was used. For all graphs, the mean and SEM are shown. Data points indicate biological replicates. Source data are provided as a Source Data file.



Supplementary Fig. 3 MsrA induction upon methionine restriction.

a, Quantification of Met and MetSO in the hemolymph of female Canton-S flies that were fed with a standard yeast-based diet for four days post-eclosion. n = 3. For the statistics, a two-tailed Student's *t* test was used. **b-e**, Quantitative RT–PCR analysis of *MsrA* expression levels in female guts (**b**), male guts (**c**), female abdomens (**d**) and male abdomens (**e**) of Canton-S flies fed with or without a methionine-restricted diet for three days. n = 4. For the statistics, a two-tailed Student's *t* test was used. **f**, Quantitative RT–PCR analysis of *MsrA* expression in various tissues of female Canton-S flies fed with or without a methionine-restricted diet for three days. n = 4. For the statistics, one-way ANOVA with Holm-Šídák's multiple comparison test was used. **g**, Quantitative RT–PCR analysis of *MsrA* expression in female guts of *ovo*^{D1/+} fed with or without a methionine-restricted diet for three days. n = 6. For the statistics, a two-tailed Student's *t* test was used. For all graphs, the mean and SEM are shown. Data points indicate biological replicates. Source data are provided as a Source Data file.



Supplementary Fig. 4 Characterisation of the MsrA mutant.

a, Quantitative RT–PCR analysis of *MsrA* in the whole bodies of w^{Dah} or *MsrA*^{EY05753} flies. n = 4. For the statistics, a two-tailed Student's *t* test was used. **B**, Survivability of female flies of w^{Dah} or *MsrA*^{EY05753} upon 3% H₂O₂ treatment. Sample sizes (n) are shown in the figure. For the statistics, a log-rank test was used. For the graph, the mean and SEM are shown. Data points indicate biological replicates. Source data are provided as a Source Data file.



Supplementary Fig. 5 Contribution of lipid metabolism to lifespan extension upon methionine restriction.

a, Lipid staining of the female guts of bmm^{wt} and bmm^{1} flies fed with or without a methionine-restricted diet for one week using LipixTOX. Scale bar: 1 mm. Arrowheads indicate lipid accumulation. **b**, Survivability of female bmm^{wt} and bmm^{1} flies upon complete starvation after feeding with or without a methionine-restricted diet for one week. Sample sizes (n) are shown in the figure. For the statistics, a log-rank test was used. **c**, Lifespans of female flies of bmm^{WT} and bmm^{1} fed with or without a methionine-restricted diet in early life. Sample sizes (n) are shown in the figure. For the statistics, a log-rank test was used. I log-rank test was used. Source data are provided as a Source Data file.



Supplementary Fig. 6 Inhibition of Transsulfuration pathway did not abrogate lifespan extension upon methionine restriction.

a, Methionine metabolic and transsulfuration pathways, which can be inhibited by propargylglycine (PPG). **b-f**, Quantification of methionine metabolites and their oxidative products upon methionine restriction. n = 3. For the statistics, one-way ANOVA with Holm-Šídák's multiple comparison test was used. **g**, Lifespans of female Canton-S flies fed with or without a methionine-restricted diet supplemented with 0.5 mM PPG. Sample sizes (n) are shown in the figure. For the statistics, a log-rank test was used. For all graphs, the mean and SEM are shown. Data points indicate biological replicates. Source data are provided as a Source Data file.

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b	Cluster	Cell type	Key genes
	0	EB/ISC	DI, Tet, esg
	1	EB1	klu, esg, Tet
	2	EB2	klu, esg, Tet
	3	aEC1	alphaTry, betaTry
	4	EB3	klu, esg, Tet
	5	LFC	mag, CG10472, Jon99Fii
	6	pEC1	LManV, LManVI
	7	aEC2	alphaTry, beta⊺ry, Bace
	8	EB4	klu, esg, Tet
	9	pEC2	LManVI
	10	ISC	DI, HmgD, hoip, Awd
	11	AstA-EE	AstA, AstC, CCHa1
	12	pEC3	nub, Gs2, zetaTry
	13	mEC	Vha16-1, Vha13, CG5767
	14	Orcokinin-EE1	Orcokinin, AstC, IA-2
	15	EB5	klu, esg, Tet
	16	pEC4	nub, Npc2e
	17	IA-2/Tk-EE	IA-2, Tk, DH31
	18	copper/iron cell	MtnD, CG5399
	19	AstC-EE	AstC, CCHa2
	20	NPF-EE	NPF, IA-2
	21	CCHa2-EE	CCHa2, IA-2
	22	unknown	whe, CG34212
	23	Orcokinin-EE2	Orcokinin, AstC, IA-2
	24	cardia	Pgant4, Muc68D



Supplementary Fig. 7 Cell type clustering analysis and marker gene expression in each cluster.

a, Heatmap of clustered marker genes from the results of single-cell RNAseq of the *Drosophila* female midgut. **b**, Cell type names in clusters. **c**, UMAP plot of characteristic marker genes.



Supplementary Fig. 8 Heatmap analysis of DEGs from single-cell RNAseq.

a-c, Heatmap of DEGs in each cell type classified as progenitor cells (**a**), enterocytes (**b**), and enteroendocrine cells (**c**) upon methionine restriction with ageing. Source data are provided as a Source Data file.



Supplementary Fig. 9 PCA of single-cell RNAseq results upon methionine restriction.

a-e, PCA of DEGs in each cell type classified as progenitor cells (a), anterior enterocytes
(b), middle enterocytes (c), posterior enterocytes (d), and enteroendocrine cells (c) upon methionine restriction with ageing.

	Dependent variable:				
	Age (days)				
	w^{iso31} w^{Dah}				
	coefficient (SE)	coefficient (SE)			
Timing of diet treatment	-0.156 (0.125)	-0.048 (0.119)			
MetR diet	-0.753*** (0.130)	-0.524*** (0.120)			
Timing of diet treatment: MetR diet	0.589*** (0.178)	0.191 (0.167)			
Observations	528	576			
Score (Logrank) Test (df = 3)	38.854***	27.841***			
	**** <i>p</i> <0.001				

Supplementary Table 1. Cox PH analysis for w^{iso31} and w^{Dah} female flies.

Supplementary Table 2. Cox PH analysis for $MsrA^{EY05753}$ flies compared to w^{Dah} flies.

	Dependent variable:
	Age (days)
	coefficient (SE)
Genotype	-0.367*** (0.109)
MetR diet	-1.240*** (0.110)
Genotype: MetR diet	1.264*** (0.153)
Observations	703
Score (Logrank) Test (df = 3)	143.7***
	*** <i>p</i> <0.001

	Dependent variable:
	Age (days)
	coefficient (SE)
Genotype	0.946*** (0.117)
MetR diet	-0.790**** (0.116)
Genotype: MetR diet	-0.695*** (0.160)
Observations	663
Score (Logrank) Test ($df = 3$)	248***
	**** p <0.001

Supplementary Table 3. Cox PH analysis for *bmm¹* flies compared to *bmm^{WT}* flies.