PNAS

Supporting Information for

Contextual modifiers of healthspan, lifespan and epigenome in mice under chronic social stress

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Supporting Methods

Diets

As previously described (1) after the first week of CPS, half the dyads were randomized to the standard of the high fat diet using a simple randomization procedure of flipping a coin. After the conclusion of the CPS phase, the mice entered the aging phase of the study during which, all mice were fed standard diet. Finally, from the age of 10 months, all mice were switched to the mature rodent maintenance diet (D10012M) because of its better balance of essential nutrients tailored for aged rodents.

DNA methylation sample preparation

DNA was purified from the liver tissue using the Qiagen's DNAeasy Blood & Tissue kit (http://qiagen.com) on the QIAcube system. Nucleic acid quality was checked using a NanoDrop spectrophotometer (http://www.nanodrop.com). As a reference, a control mouse sample external to this experiment was also included.

DNA methylation and array processing

The DNA methylation assay was performed at the University of Minnesota Genomics Center (https://genomics.umn.edu/) according to the standard manufacturer's protocol (http://illumina.com/). Raw intensity data files (.idat files) were processed using either the GenomeStudio software (Illumina, San Diego, CA) or the R package SeSAMe (version 1.2.4). GenomeStudio (2011.1) was used to preprocess the raw data and evaluate the performance of the Illumina Illumina BeadChip kit (Avg Loci Detection: 99.8%; Avg P95 Green: 8771; Avg P95 Red: 12187; % Loci Detection of Control Sample: 99.56%). In all, genome-wide DNA methylation profiling returned 287,050 total targets from our samples, of which 284,860 CpG probes were retained after processing to remove any CpH methylation sites, SNPs, or unknown regions due to their low representation in our samples. Furthermore, we also excluded CpG loci which had missing data for > 5% of individuals in the sample. We then used GenomeStudio to obtain the intensity and β-values on the full set of 284,860 identified cg probes with color correction/background subtracted preprocessing. For analytical purposes, we also excluded any CpG loci with missing data for > 5% of individuals in the sample. The methylation status for each probe was recorded as a β-value that ranged between 0 and 1, where values close to 1 represent high levels of methylation and where values close to 0 represent low levels of methylation. Annotations of CpG markers are obtained from Illumina's annotation file and enhanced annotation to the UCSC Known Gene. All annotations use the mouse June 2020 (GRCm39/mm39) assembly.

Statistical design and analysis

Throughout this manuscript we are interested in assessing causal effects of variables including both treatment assignment, which can be randomized, and postulated mediating variables including behaviors, anatomical changes, and physiologic changes. Only those variables to which we can randomly assign experimental units

are variables for which we can confidently make causal inferences. Yet at the same time, while we only definitively estimate associations with other variables, including those aforementioned postulated mediating variables, we are indeed doing so with the hope of assessing their causal effects. Thus, any use of causal language on our part may be interpreted as a strong indicator of causal effects in the case of the randomized treatment assignments we use and as postulated or suggested causal effects inferred from associations in other cases. Analyses were performed using R (2021.09.1 Build 372 © 2009-2021) or Statistica 13.0 (Dell Inc., Tulsa, OK). Data are expressed as means ± standard error of the mean, unless otherwise specified.

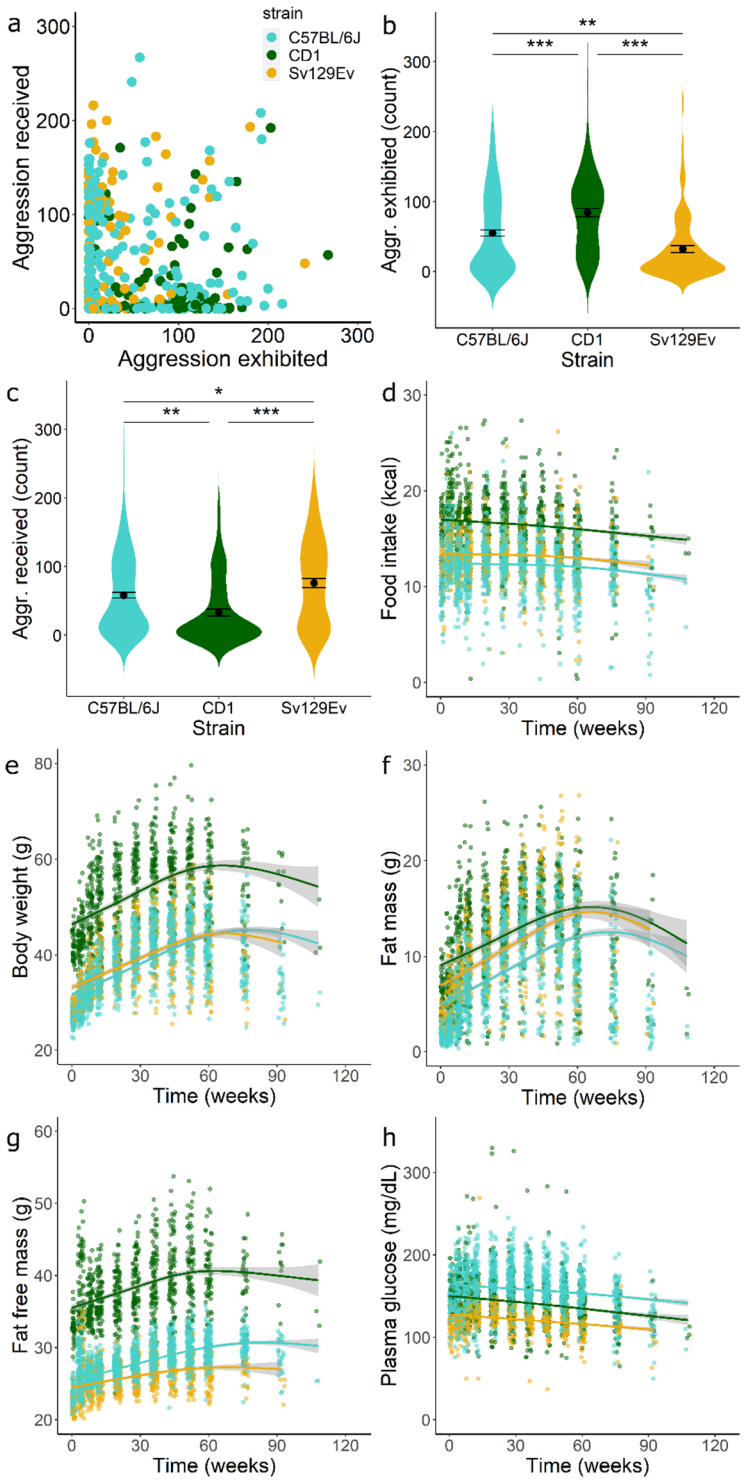

Figure S1. Characterization of C57BL/6J, CD1, and Sv129Ev male mice in the LCPS protocol. a) Scatterplot of contextual aggression exhibited and received during the course of the CPS phase of the protocol by all mice. Distribution of the aggression exhibited (b) or received (c) within each strain, indicating average and standard error of group means. (d-h) Healthspan profiling of each strain, as illustrated by individual values as well as solid lines representing “loess” smoothing method and grey areas represent 95% confidence intervals around smoothing for the entire duration of the study, and including food intake (d), body weight (e), fat mass (f), fat free mass (g), plasma glucose after 4h fasting (h). Asterisks represent significant differences from ANOVA with pairwise comparisons tested with Tukey’s HSD test, * $p<0.05$, ** $p< 0.01$, ***$p<0.001$.

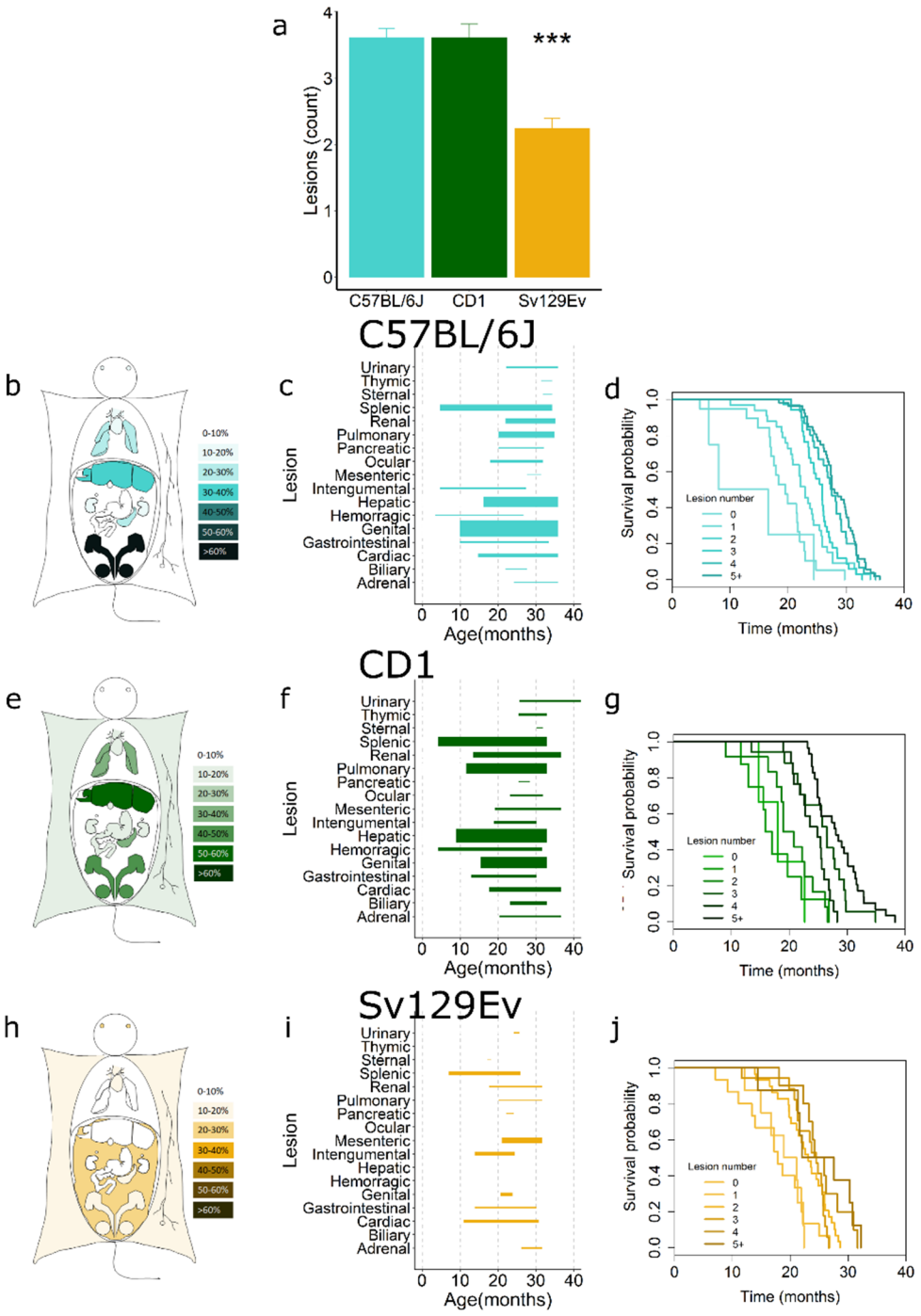

Figure S2. Mouse strain association with lesion burden, onset of aging-associated organ lesion, and lesion-dependent mortality. a) The number of lesions was significantly dependent on genetic strain ($F(2,334)=18.5$, $p<0.001$, $\eta_p^2= 0.098$, obs. power=0.999), with Sv129Ev mice developing a significantly lower number of lesions detectable at necropsy (Pair-wise comparisons: Sv129Ev-C57BL/6J: -1.37, CI=(-1.93, -0.80), $p<0.001$; Sv129Ev-CD1: -1.37, CI=(-2.02, -0.72), $p<0.001$). b-j) Heat-map of organ specific lesions, strain- and age-dependent distribution of macroscopic dissectible lesions in proportion to their prevalence within each organ within each strain, and strain survival probability as a function of the number of lesions detectable at necropsy in C57BL/6J (b-d), CD1 (e-g), and Sv129Ev mice (h-j). Data in (a) represent group mean ± standard error of mean. P-value represents level of significance in both pair-wise comparisons (Tukey's HSD test).

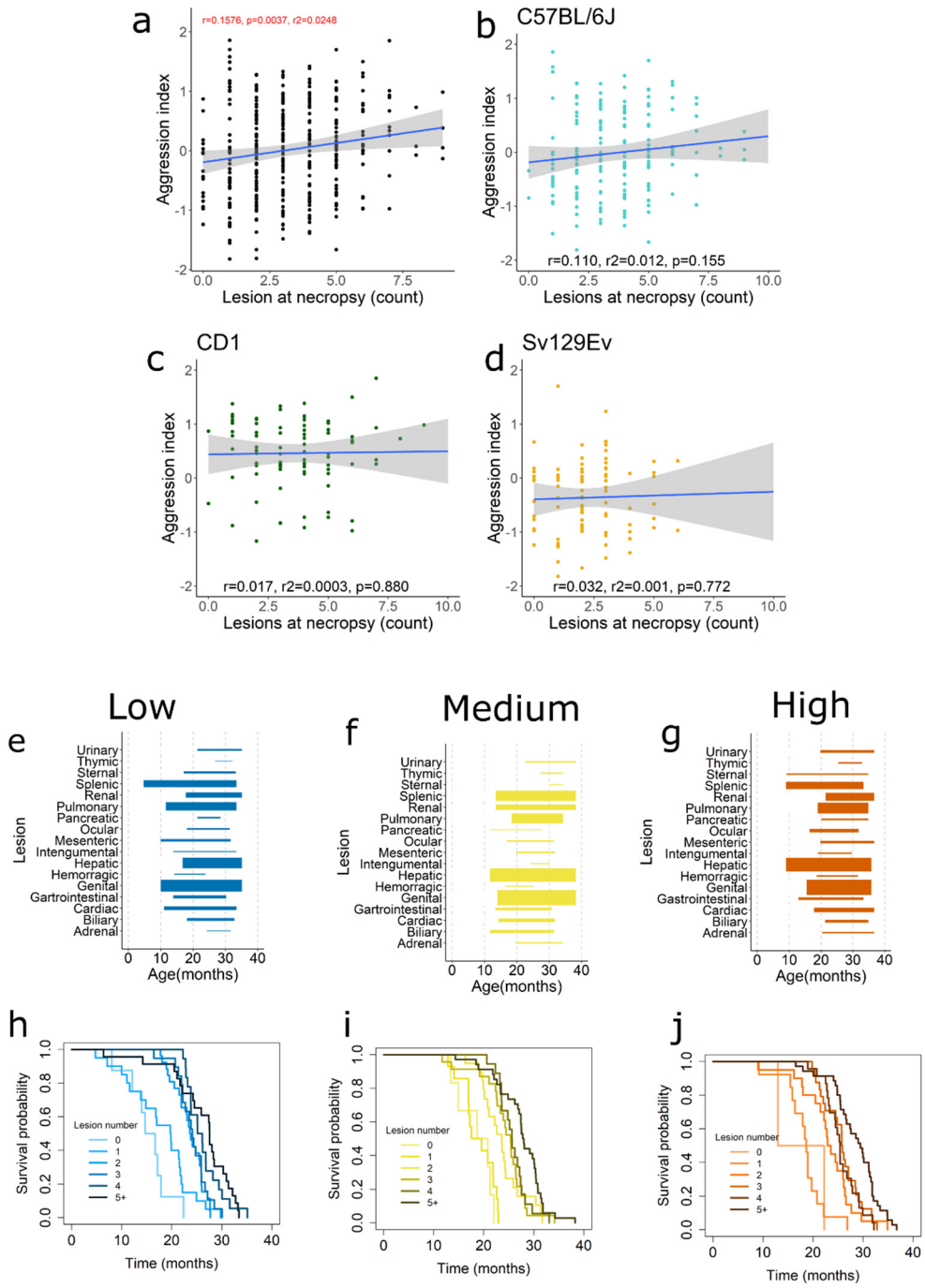

Figure S3. Aggression index association with lesion burden, onset of aging-associated organ lesion, and lesion-dependent mortality. a) Scatterplot of the correlation of the aggression index and number of lesions at necropsy in the general population (a), and within C57BL/6J (b), CD1 (c), and Sv129Ev (d) mice. e-g) DAI- and age-dependent distribution of macroscopic dissectible lesions in proportion to their prevalence within each organ within each of the DAI level. Within each strain, DAI significantly affected the number of lesions within C57BL/6J (low=3.09±0.25, medium=3.86±0.25, high=3.84±0.25, $F(2,165)=3.01$, $p=0.048$), but not within CD1 (low=3.42±0.55, medium=3.87±0.39, high=3.53±0.27, $F(2,81)=0.32$, $p=0.728$) or Sv129Ev mice (low=2.23±0.23, medium=2.25±0.07, high=2.22±0.50, $F(2,82)=0.002$, $p=0.998$). h-j) Survival probability as a function of the number of lesions detectable at necropsy within each of the DAI groups, a phenomenon that was recapitulated within each of the three strains (C57BL/6J: $\chi^2=65.71$, $p<0.0001$; CD1: $\chi^2=35.05$, $p<0.0001$; Sv129Ev: $\chi^2=30.00$, $p<0.0001$). In figures g through j, $p<0.05$ are noted in red, p value > 0.1 is noted in black.

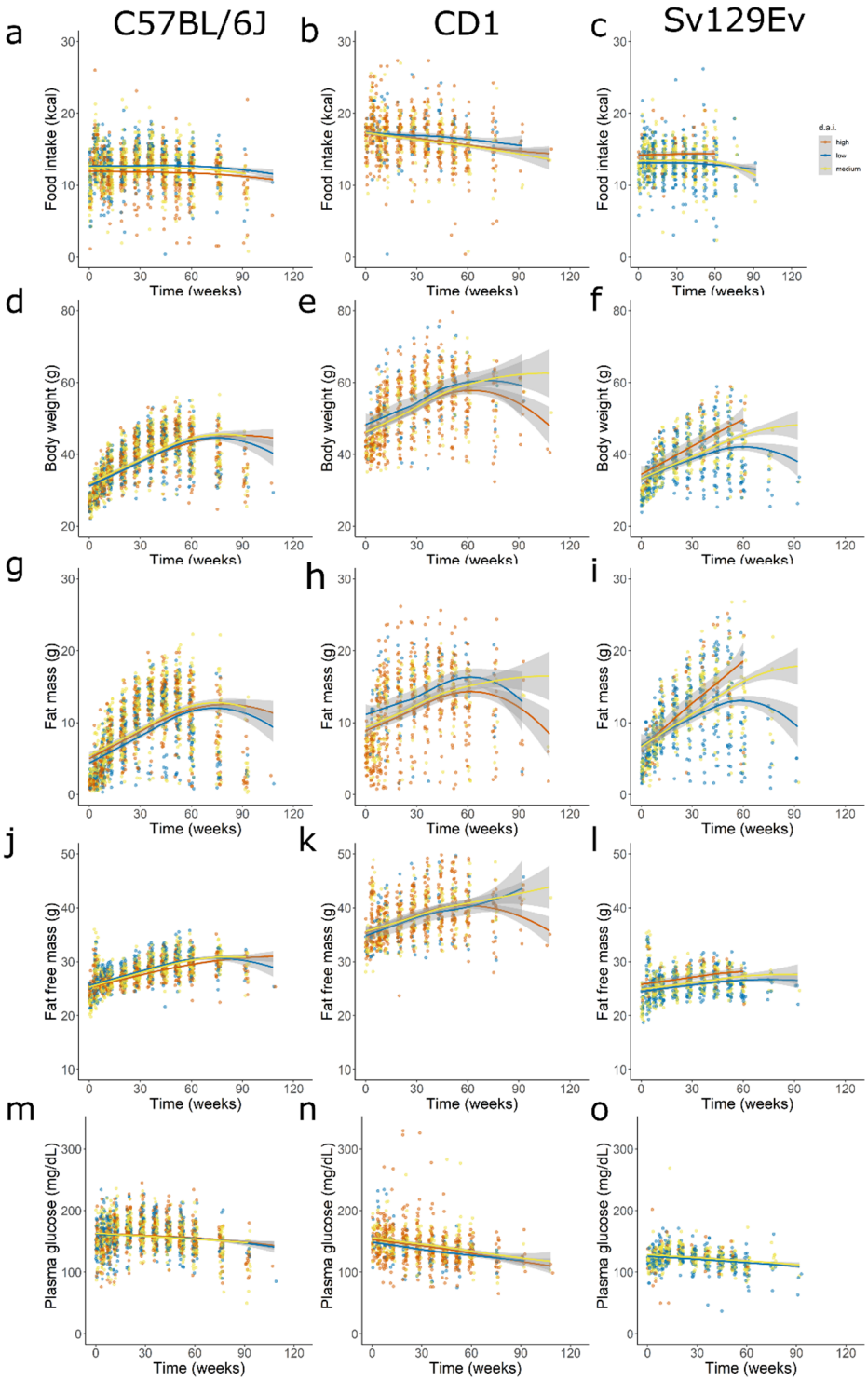

Figure S4. Healthspan profiling depending on DAI within each strain, as illustrated by individual values as well as linear mixed model predictors (solid lines) for the entire duration of the study, and including food intake (a-c), body weight (d-f), fat mass (g-i), fat free mass (j-l), plasma glucose (m-o).

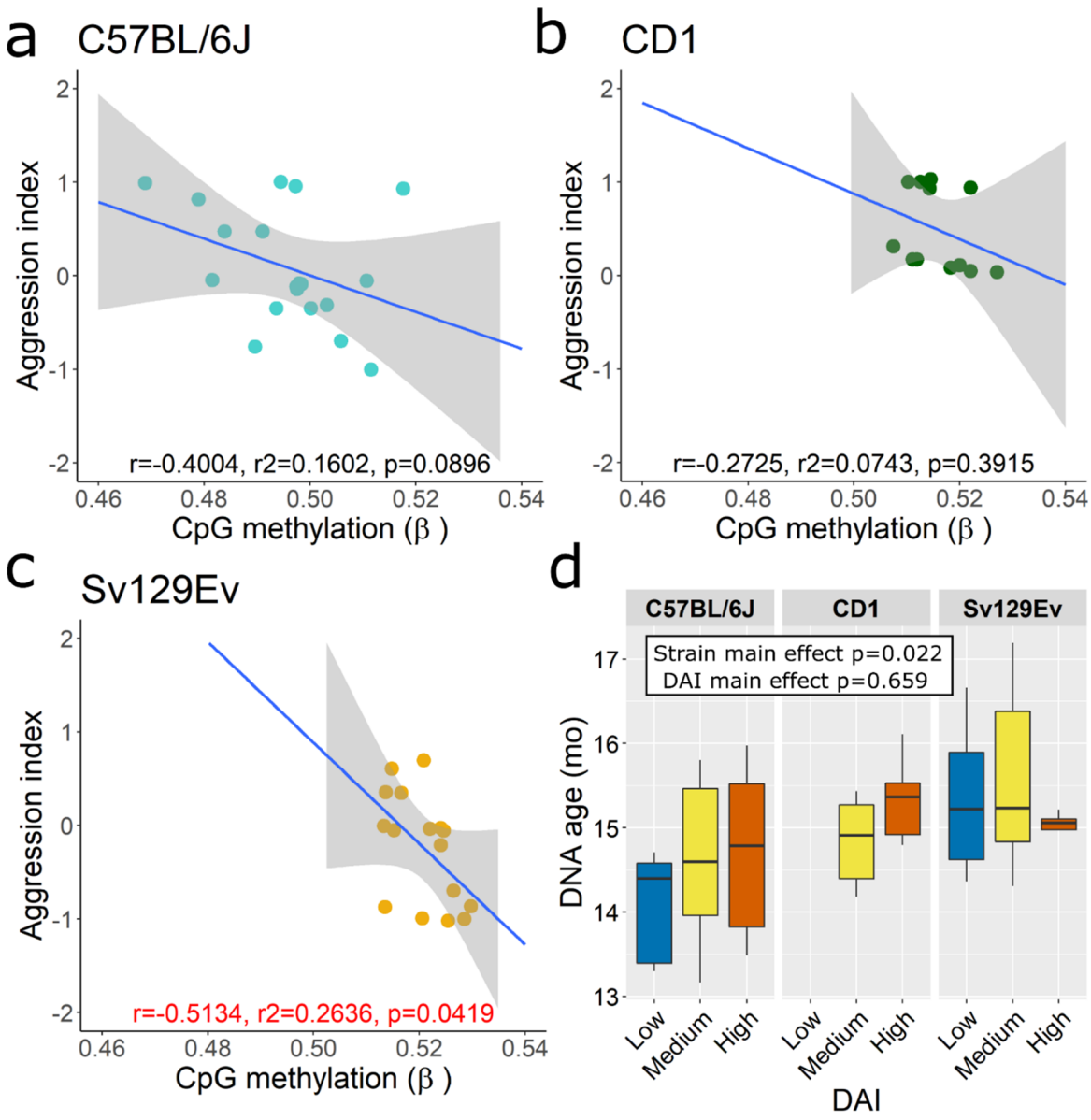

Figure S5. a-c**)** Scatterplots of the correlation between global CpG methylation level (β) and aggression index in the C57BL/6J (a), CD1 (b), and Sv129Ev (c) strain. In figures a through c, p<0.05 are noted in red. d) Tukey box-and-whisker plot of the average epigenetic age (in months) by strain and DAI (strain: η_p^2= 0.173, obs. power=0.705; DAI: η_p^2= 0.021, obs. power=0.113). Box plot shows median, upper and lower quartiles, minimum and maximum values. Data from 2 animals that exceeded 2 standard deviations from the average were excluded from the analysis.

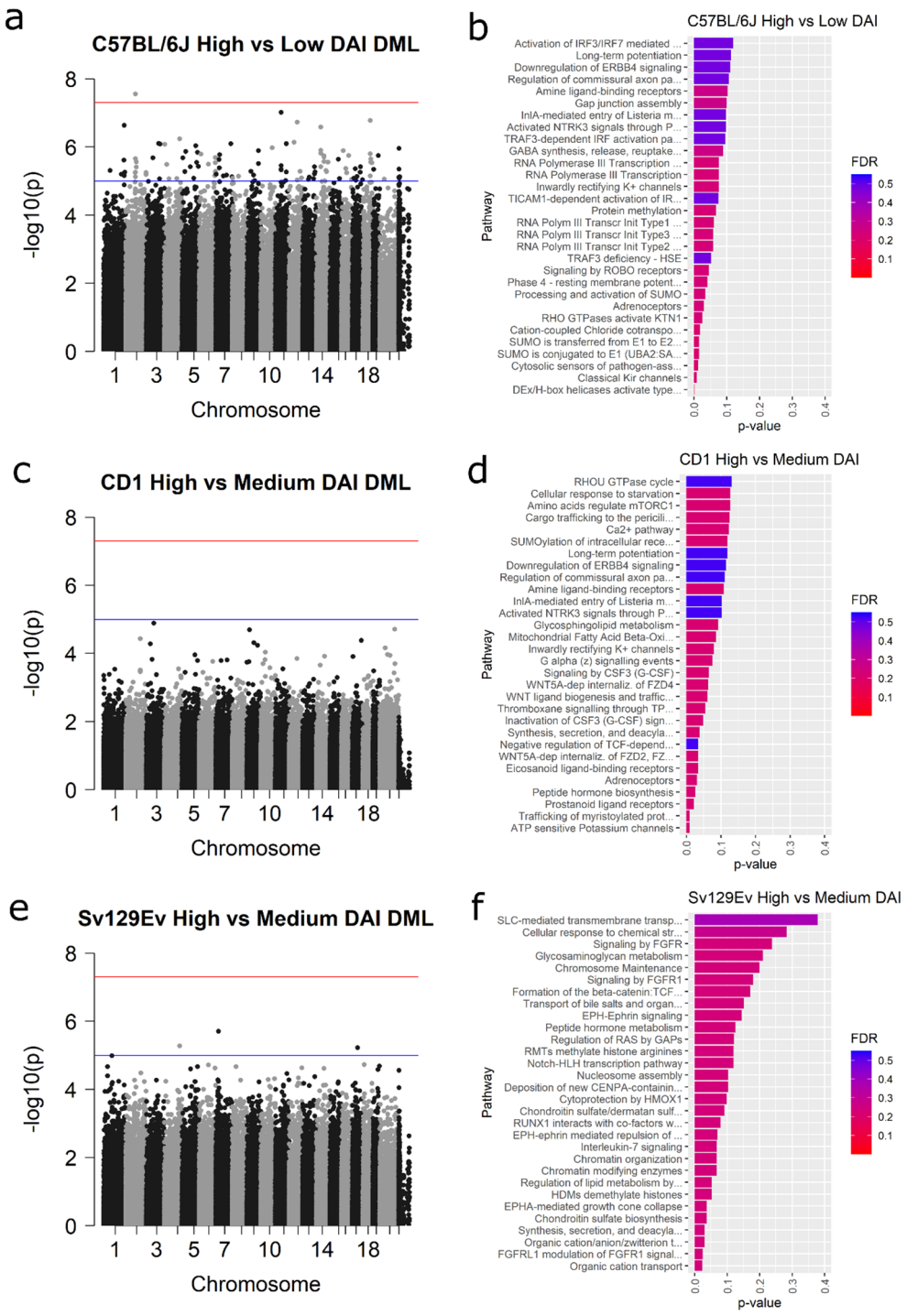

Figure S6. a,c,e) Features of differentially methylated CpG loci (DML). Each point in the Manhattan plots represents the location of a CpG locus (x-axis: autosomal chromosomes 1-19, chromosome X as 20 and chromosome Y as 21), and the association –log10p (y-axis) for the effect of DAI within each of the three strains (a) C57BL/6J, (b) CD1, and (c) Sv129Ev. The genome-wide significant threshold is set at –log10(5e-08) (red line) and the suggestive line threshold at –log10(1e-05) (blue line). b,d,f) Reactome gene pathways identified for each strain following the mapping of strain specific DMRs on the mouse genome. Reactome pathway browser version 3.7, database release 79; species Mus musculus pathways without projection to humans and with possible interactors (2).

Outcome	*Predictor*	*Estimate*	*Std. Error*	*t-value*	*p-value*	*Conditional R^2*	*Marginal R^2*	*Obs. power*
Food intake (kcal)	(Intercept)	12.49	0.12	106.30	<0.001	0.841	0.073	0.688
	Time (weeks)	-0.01	0.00	-2.50	0.013			
	CD1	4.41	0.18	25.20	<0.001			
	Sv129Ev	0.98	0.18	5.60	<0.001			
Body weight (g)	(Intercept)	31.49	0.26	120.50	<0.001	0.925	0.999	1.0
	Time (weeks)	0.22	0.01	29.70	<0.001			
	CD1	14.90	0.45	33.20	<0.001			
	Sv129Ev	1.71	0.45	3.80	<0.001			
Fat mass (g)	(Intercept)	4.87	0.19	26.30	<0.001	0.921	0.068	1.0
	Time (weeks)	0.12	0.01	22.40	<0.001			
	CD1	4.25	0.31	13.90	<0.001			
	Sv129Ev	2.05	0.31	6.70	<0.001			
Fat free mass (g)	(Intercept)	25.39	0.14	178.10	<0.001	0.966	0.227	1.0
	Time (weeks)	0.08	0.00	24.00	<0.001			
	CD1	10.14	0.23	44.50	<0.001			
	Sv129Ev	-0.91	0.23	-4.00	<0.001			
Plasma glucose (mg/dL)	(Intercept)	164.51	1.17	141.00	<0.001	0.515	0.233	1.0
	Time (weeks)	-0.21	0.02	-9.60	<0.001			
	CD1	-15.86	1.59	-10.0	<0.001			
	Sv129Ev	-36.47	1.60	-22.80	<0.001			

Table S1. Results of linear mixed models for each of the predicted healthspan variables (food intake, body weight, fat mass, fat free mass, and plasma glucose) with time and strain as the fixed effects, time and subject nested within pair as random effects, and with an autocorrelation correction for repeated individual sampling. C57BL/6J is the reference group. All the models were defined as it follows: mod =lme(dep.var ~ time.var + cat.var1, random = ~ time.var|pair/ID, corr=corAR1(form = ~ weeks1 |pair/ ID), control=lmeControl(opt='optim'), data=data). In separate and dedicated models food intake, body weight, fat mass, fat free mass, plasma glucose served as dependent variable, time and strain as fixed factors, time and subject nested in pair as random factors.

	Strain						
Lesion	**C57BL/6J**	**CD1**	**Sv129Ev**	**χ^2 (df=2)**	**p**	**d**	**Obs. power**
Urinary	6.40	8.14	4.60	0.98	0.611	0.107	0.411
Thymic	2.91	8.14	0.00	9.24	0.010	0.332	0.999
Sternal	1.74	3.49	1.15	1.52	0.500	0.133	0.592
Splenic	22.09	34.88	12.64	14.06	0.005	0.413	1.000
Renal	18.60	19.77	13.79	1.15	0.562	0.116	0.473
Pulmonary	24.42	38.37	21.84	5.61	0.061	0.257	0.993
Pancreatic	5.23	2.33	2.30	1.73	0.421	0.142	0.652
Ocular	11.05	8.14	0.00	10.25	0.006	0.350	0.999
Mesenteric	1.74	9.30	4.60	5.59	0.612	0.257	0.993
Intengumental	6.40	11.63	4.60	3.54	0.171	0.204	0.934
Hepatic	37.21	50.00	20.69	12.01	0.003*	0.380	0.999
Hemorragic	2.91	11.63	0.00	15.12	<0.001	0.429	1.000
Genital	61.05	36.05	5.75	44.74	<0.001	0.773	1.000
Gastrointestinal	6.40	11.63	11.49	1.81	0.405	0.145	0.672
Cardiac	13.37	16.28	5.75	5.01	0.082	0.243	0.987
Biliary	2.91	13.95	12.64	7.41	0.025	0.297	0.999
Adrenal	3.49	4.65	4.60	0.20	0.904	0.048	0.115

Cont'd

	C57BL/6J vs CD1				C57BL/6J vs SV129Ev				CD1 vs Sv129Ev			
Lesion	**χ^2 (df=1)**	**p<=**	**d**	**Obs. power**	**χ^2 (df=1)**	**p<=**	**d**	**Obs. power**	**χ^2 (df=2)**	**p<=**	**d**	**Obs. power**
Urinary	0.21	0.647	0.057	0.150	0.29	0.588	0.067	0.191	0.98	0.612	0.151	0.510
Thymic	2.48	0.115	0.197	0.887	2.91	0.089	0.214	0.932	9.24	0.010^	0.476	0.999
Sternal	0.58	0.446	0.095	0.333	0.12	0.727	0.043	0.107	1.39	0.499	0.180	0.658
Splenic	2.87	0.090	0.212	0.927	2.57	0.109	0.202	0.903	10.7	0.005	0.515	0.999
Renal	0.04	0.851	0.025	0.069	0.71	0.398	0.105	0.391	1.15	0.562	0.164	0.578
Pulmonary	3.10	0.078	0.221	0.945	0.14	0.705	0.047	0.118	5.61	0.061	0.367	0.998
Pancreatic	1.12	0.290	0.132	0.565	1.14	0.285	0.133	0.572	1.73	0.421	0.202	0.757
Ocular	0.44	0.507	0.083	0.267	11.05	<0.001^	0.423	0.999	10.2	0.006^	0.502	0.999
Mesenteric	5.17	0.023	0.286	0.996	1.28	0.257	0.141	0.621	5.59	0.061	0.367	0.998
Intengumental	1.52	0.218	0.154	0.698	0.29	0.588	0.067	0.190	3.54	0.171	0.289	0.967
Hepatic	1.88	0.171	0.171	0.786	4.71	0.030	0.273	0.992	12.0	0.002^	0.548	0.999

Hemorragic	5.23	0.022	0.288	0.996	2.91	0.088	0.214	0.931	15.1	<0.001	0.620	1.000
Genital	6.44	0.011	0.320	0.999	45.78	<0.001^	0.929	1.000	44.7	<0.001	1.185	1.000
Gastrointestinal	1.52	0.218	0.154	0.698	1.45	0.228	0.150	0.675	1.81	0.405	0.206	0.773
Cardiac	0.29	0.593	0.067	0.190	3.04	0.081	0.218	0.939	5.01	0.081	0.346	0.995
Biliary	7.24	0.007	0.340	0.999	6.10	0.014	0.311	0.999	7.41	0.025	0.424	0.999
Adrenal	0.17	0.684	0.051	0.130	0.15	0.697	0.048	0.121	0.20	0.904	0.068	0.145

Table S2. Percentage of mouse population presenting lesions detectable at necropsy**. *** Denotes significant differences after Bonferroni adjustment for multiple comparisons across the 17 tests (alpha=0.05/17 tests = 0.003). ^ Denotes significant differences after Bonferroni's adjustment for multiple comparisons between the indicated strains (alpha=0.05/3 pairwise comparisons = 0.017). Effect size d computed as standardized mean difference.

A. LDA Confusion matrix and statistics for genetic background

		Observed		
		C57BL/6J	**CD1**	**Sv129Ev**
Predicted	**C57BL/6J**	32	0	3
	CD1	0	16	0
	Sv129Ev	1	0	14

Overall Statistics	
Accuracy (95% CI)	0.9394 (0.852 - 0.9832)
No Information Rate	0.5
P-Value [Acc>NIR]	1.042e-14
Kappa	0.9019

ROC curve variable importance

Variable	**C57BL/6J**	**CD1**	**Sv129Ev**
Baseline Fat Free Mass (g)	100.00	100.00	100.00
Baseline Body Weight (g)	100.00	100.00	100.00
Baseline Fat Mass (g)	95.80	95.80	70.14
Baseline Food Intake (g)	84.13	84.13	67.20
Baseline Plasma Glucose (mg/dL)	71.91	81.82	81.82
Aggression Index	12.47	50.03	50.03
Lesions at death (count)	25.21	17.23	25.21
Age at death (mo)	17.77	0.00	17.77

B. LDA Confusion matrix and statistics for DAI category

		Observed		
		Low	**Medium**	**High**
Predicted	**Low**	17	5	3
	Medium	3	15	0
	High	2	2	14

Overall Statistics	
Accuracy (95% CI)	0.5909 (0.4629 - 0.7105)
No Information Rate	0.3333
P-Value [Acc>NIR]	1.584e-5
Kappa	0.3864

ROC curve variable importance

Variable	**Low**	**Medium**	**High**
Baseline Fat Free Mass (g)	100.00	85.354	100.00
Baseline Body Weight (g)	62.96	51.442	62.96
Baseline Plasma Glucose (mg/dL)	61.49	40.604	61.49
Lesions at death (count)	43.89	25.739	43.89
Age at death (mo)	35.87	12.994	35.87
Baseline Food Intake (g)	30.91	28.420	30.91
Baseline Fat Mass (g)	0.00	9.929	0.00

Table S3. Linear Discriminant analysis (LDA) of the importance of the healthspan, aggression index, lesions and age at death importance as predictors to classify mice genetic background (A) or DAI category (B). For DAI classification, aggression index was omitted from the model. CI = confidence interval; Acc = accuracy; NIR = No Information Rate.

Outcome	Strain	Predictor		Estimate	Std. Error	t-value	p-value	Conditional R^2	Marginal R^2	Obs. power
Food intake (kcal)	C57BL/6J	(Intercept)		11.90	0.18	68.23	<0.001	0.850	0.002	0.097
		Time (weeks)		-0.00	0.00	-0.22	0.828			
		DAI	medium	0.56	0.21	2.66	0.009			
			low	0.80	0.22	3.70	<0.001			
	CD1	(Intercept)		17.35	0.26	67.25	<0.001	0.863	0.006	0.967
		Time (weeks)		-0.03	0.01	-4.65	<0.001			
		DAI	medium	-0.11	0.40	-0.27	0.790			
			low	0.10	0.52	0.19	0.849			
	Sv129Ev	(Intercept)		14.23	0.45	31.92	<0.001	0.882	0.002	0.065
		Time (weeks)		0.00	0.01	0.17	0.908			
		DAI	medium	-0.86	0.49	-1.77	0.081			
			low	-1.14	0.47	-2.41	0.018			
Body weight (g)	C57BL/6J	(Intercept)		31.55	0.35	90.84	<0.001	0.915	0.113	1.0
		Time (weeks)		0.22	0.01	25.21	<0.001			
		DAI	medium	0.27	0.48	0.56	0.574			
			low	-0.35	0.49	-0.72	0.474			
	CD1	(Intercept)		45.92	0.67	68.23	<0.001	0.928	0.090	0.942
		Time (weeks)		0.23	0.02	13.80	<0.001			
		DAI	medium	0.19	1.18	0.16	0.873			
			low	2.50	1.53	1.64	0.106			
	Sv129Ev	(Intercept)		34.20	1.05	32.67	<0.001	0.917	0.077	1.0
		Time (weeks)		0.21	0.02	13.02	<0.001			
		DAI	medium	-1.02	1.17	-0.87	0.387			
			low	-1.05	1.15	-0.91	0.363			
Fat mass (g)	C57BL/6J	(Intercept)		4.89	0.26	19.18	<0.001	0.914	0.057	1.0
		Time (weeks)		0.12	0.01	19.43	<0.001			
		DAI	medium	0.50	0.35	1.42	0.158			
			low	-0.51	0.36	-1.42	0.157			

	CD1	(Intercept)		8.85	0.48	18.614	<0.001	0.921	0.037	0.717
		Time (weeks)		0.10	0.01	9.910	<0.001			
		DAI	medium	0.49	0.81	0.602	0.549			
			low	2.22	1.05	2.104	0.038			
	Sv129Ev	(Intercept)		6.70	0.65	10.244	<0.001	0.935	0.053	1.0
		Time (weeks)		0.15	0.01	11.502	<0.001			
		DAI	medium	-0.06	0.73	-0.084	0.933			
			low	0.15	0.71	0.206	0.838			
Fat free mass (g)	C57BL/6J	(Intercept)		25.15	0.19	135.512	<0.001	0.970	0.054	0.998
		Time (weeks)		0.08	0.00	26.422	<0.001			
		DAI	medium	0.23	0.25	0.928	0.355			
			low	0.46	0.25	1.824	0.070			
	CD1	(Intercept)		35.39	0.34	105.525	<0.0001	0.960	0.035	0.958
		Time (weeks)		0.10	0.01	10.853	<0.001			
		DAI	medium	0.29	0.59	0.499	0.6187			
			low	-0.57	0.76	-0.744	0.459			
	Sv129Ev	(Intercept)		25.86	0.62	41.66	<0.001	0.888	0.025	0.928
		Time (weeks)		0.04	0.00	9.57	<0.001			
		DAI	medium	-1.00	0.69	-1.46	0.149			
			low	-1.39	0.67	-2.07	0.042			
Plasma glucose (mg/dL)	C57BL/6J	(Intercept)		163.53	1.86	86.28	<0.001	0.427	0.011	0.989
		Time (weeks)		-0.13	0.03	-4.18	<0.001			
		DAI	medium	-1.87	2.13	-0.88	0.381			
			low	-1.19	2.20	-0.54	0.590			
	CD1	(Intercept)		153.35	2.56	59.91	<0.001	0.333	0.085	1.0
		Time (weeks)		-0.37	0.04	-9.11	<0.001			
		DAI	medium	2.74	4.11	0.67	0.506			
			low	-4.82	5.35	-0.90	0.370			
	Sv129Ev	(Intercept)		126.72	2.62	48.40	<0.001	0.429	0.031	0.998

		Time (weeks)		-0.17	0.03	-5.30	<0.001			
		DAI	medium	1.79	2.81	0.64	0.527			
			low	-0.83	2.75	-0.30	0763			

Table S4. Linear mixed model analysis of healthspan variables for each of the 3 strains, with DAI and time as fixed factors, time and ID nested within pair as random factors, and with an autocorrelation correction for repeated individual sampling. High DAI is the reference group. All the models were defined as it follows: mod =lme(dep.var ~ time.var + cat.var1, random = ~ time.var|pair/ID, corr=corAR1(form = ~ weeks1 |pair/ID), control=lmeControl(opt='optim'), data=data). In separate and dedicated models for each strain food intake, body weight, fat mass, fat free mass, plasma glucose served as dependent variable, time and DAI as fixed factors, time and subject as random factors.

A.

DAI	Survival	Strain			K-W (df=2)	p<=	η^2[H]	C57BL/6J vs CD1			C57BL/6J vs Sv129Ev			CD1 vs Sv129Ev		
		C57BL/6J	CD1	Sv129Ev				W (df=1)	p<=	r	W (df=1)	p<=	r	W (df=1)	p<=	r
Low	**50%** (23.8mo)	51%	58%	42%	2.27	0.321	0.002	298	0.466	0.089	1424.5	0.301	0.103	336	0.162	0.187
	10% (29.6mo)	12%	8%	9%	0.36	0.835	<0	355	0.722	0.045	1326	0.590	0.054	270.5	1	0.003
Medium	**50%** (24.3mo)	61%	46%	33%	9.68	0.008*	0.069	839	0.096	0.186	1277	0.003*	0.316	448	0.342	0.127
	10% (30.7mo)	16%	8%	3%	3.93	0.140	0.017	734	0.390	0.096	1065	0.057	0.201	418	0.369	0.122
High	**50%** (25.5mo)	58%	46%	22%	6.20	0.045*	0.037	1619.5	0.200	0.136	376.5	0.018*	0.295	297	0.094	0.211
	10% (31.8mo)	14%	8%	0%	1.92	0.384	<0	1500	0.396	0.079	292.5	0.242	0.144	243	0.397	0.113

B.

Strain	Survival	DAI			K-W (df=2)	p<=	η^2[H]	Low vs Medium			Low vs High			Medium vs High		
		Low	Med.	High				W (df=1)	p<=	r	W (df=1)	p<=	r	W (df=1)	p<=	r
C57BL/6J	**50%** (25.7mo)	39%	56%	54%	6.06	0.048*	0.02	1976	0.031*	0.202	1974	0.032*	0.206	1597.5	0.875	0.018
	10% (31.8mo)	5%	7%	14%	2.85	0.240	0.005	1651	0.723	0.07	1761	0.132	0.179	1512	0.233	0.123
CD1	**50%** (24.8mo)	50%	42%	52%	0.99	0.611	<0	1240	0.472	0.123	300	1	0	524	0.348	0.110

	10% (31.3mo)	8%	8%	12%	0.31	0.855	<0	144	1	0	312	0.709	0.05	578	0.645	0.06
Sv129Ev	**50%** (22.2mo)	56%	45%	33%	1.56	0.458	<0	687.5	0.495	0.08	155	0.247	0.159	170.5	0.465	0.115
	10% (27.7mo)	11%	12%	0%	1.14	0.564	<0	745	0.971	0.005	180	0.310	0.141	165	0.292	0.167

Table S5. Percent survival at median and maximum (10%) survival as a function of the strain and discretized aggression index (DAI)**.**
Data were analyzed as counts (percentages) surviving using the Gao-Allison methods for testing differences in maximum lifespan (3) with tau for median (50%) and maximum (top 10%) survival calculated (A) per DAI group, and (B) per strain ; K-W, Kruskal-Wallis test; W, Wilcox rank sum test. * $p<0.05$.

Strain	DAI	HR	95% CI	p-value
C57BL/6J	low	1.51	0.99-2.31	0.031
	high	0.87	0.57-1.32	
CD1	low	0.98	0.48-1.99	0.853
	high	0.88	0.53-1.45	
Sv129Ev	low	0.85	0.54-1.35	0.513
	high	1.28	0.61-2.71	

Table S6. Cox regression time to death analysis hazard ratios (HR) and confidence interval (CI) with the medium DAI group as reference for each of the strains. The model was adjusted for pair as random factor.

				C57BL/6J			
Order	**cpg**	**Chr**	**Position**	**Gene ID**	**p-value**	**Level of significance**	**Direction of association with ref group**
1	cg38646594	2	86669066	NA	2.80E-08	significant	+
2	cg29535263	11	52447690	NA	9.69E-08	suggestive	+
3	cg35850660	18	66431979	NA	1.66E-07	suggestive	+
4	cg30799690	12	74075255	Syt16	1.87E-07	suggestive	+
5	cg37865611	1	1.82E+08	Enah	2.31E-07	suggestive	+
6	cg32412206	14	43787293	Gm5799	2.58E-07	suggestive	+
7	cg41542652	4	1.36E+08	Lypla2	5.77E-07	suggestive	-
8	cg30595132	12	44391610	Nrcam	7.19E-07	suggestive	+
9	cg40183908	3	1.1E+08	Ntng1	7.86E-07	suggestive	+
10	cg45178315	7	1.32E+08	Fgfr2	7.95E-07	suggestive	+
11	cg29993401	11	97329068	Arhgap23	8.00E-07	suggestive	+
12	cg40271573	3	1.23E+08	Sec24	8.20E-07	suggestive	+
13	cg40569998	4	5264455	NA	8.39E-07	suggestive	+
14	cg42744710	5	1.22E+08	NA	9.13E-07	suggestive	+
15	cg28919112	10	1.01E+08	NA	1.05E-06	suggestive	+
16	cg48218845	21	2266017	NA	1.10E-06	suggestive	+
17	cg32414003	14	44116955	NA	1.25E-06	suggestive	+
18	cg33952050	16	17054469	Hic2	1.27E-06	suggestive	+
19	cg47188237	9	1.04E+08	NA	1.27E-06	suggestive	+
20	cg42016915	5	33953778	NA	1.34E-06	suggestive	+
21	cg42924664	5	1.38E+08	NA	1.44E-06	suggestive	+
22	cg32413404	14	43991839	Gm6526	1.52E-06	suggestive	+
23	cg35851396	18	66491663	NA	1.58E-06	suggestive	+

24	cg43184802	6	18793842	NA	1.60E-06	suggestive	+
25	cg44434125	7	35537781	Gm28514	1.68E-06	suggestive	-
26	cg36482058	19	47934973	Cfap58	1.76E-06	suggestive	+
27	cg34315332	16	65523530	NA	1.78E-06	suggestive	+
28	cg32670645	14	73142593	NA	1.81E-06	suggestive	+
29	cg32670651	14	73142812	NA	1.95E-06	suggestive	+
30	cg44111625	6	1.42E+08	NA	2.03E-06	suggestive	+

CD1

Order	cpg	Chr	Position	Gene ID	p-value	Level of significance	Direction of association with ref group
1	cg39852628	3	67442076	NA	1.29E-05	not significant	+
2	cg48019901	20	1.34E+08	Gm15015	1.95E-05	not significant	+
3	cg46534633	9	26992086	Ncapd3	2.00E-05	not significant	+
4	cg38936095	2	1.28E+08	Acox1	3.66E-05	not significant	+
5	cg35248666	17	79852800	NA	4.17E-05	not significant	+
6	cg46870232	9	64390801	Megf11	4.83E-05	not significant	+
7	cg39658225	3	37497626	Spata5	5.20E-05	not significant	+
8	cg47107617	9	95482225	NA	5.78E-05	not significant	+
9	cg47633187	20	51963222	NA	6.86E-05	not significant	+

10	cg28394239	10	42018312	NA	9.18E-05	not significant	-
11	cg47722149	20	70917118	NA	0.00011	not significant	+
12	cg42638860	5	1.15E+08	1500011B03Rik; 2610524H06Rik	0.00011	not significant	+
13	cg47838301	20	94865798	Zc3h1b	0.00011	not significant	-
14	cg32718185	14	78276445	Gm48954	0.00012	not significant	+
15	cg34602611	17	7190076	Rsph3b	0.00012	not significant	-
16	cg30756917	12	70074253	Nin	0.00014	not significant	+
17	cg39739084	3	51484100	NA	0.00015	not significant	-
18	cg28375764	10	40257654	Cdk19	0.00015	not significant	-
19	cg44914594	7	1E+08	Fam168a	0.00015	not significant	-
20	cg42901277	5	1.36E+08	Orai2	0.00016	not significant	+
21	cg44543118	7	47008189	NA	0.00016	not significant	-
22	cg43921097	6	1.2E+08	Wnk1	0.00017	not significant	-
23	cg46479420	9	20460086	NA	0.00017	not significant	-
24	cg46391587	9	4309388	Aasdhppt	0.00018	not significant	-
25	cg28265553	10	22729317	NA	0.0002	not significant	+
26	cg48036020	20	1.37E+08	Il1rapl2	0.0002	not significant	+

27	cg32403405	14	41087832	NA	0.00022	not significant	+
28	cg28738156	10	81320308	NA	0.00026	not significant	-
29	cg29681398	11	67625172	Glpr2	0.00028	not significant	+
30	cg41409042	4	1.26E+08	Grik3	0.00028	not significant	+

Sv129Ev

Order	cpg	Chr	Position	Gene ID	p-value	Level of significance	Direction of association with ref group
1	cg44315949	7	24333976	Gm26550	1.97E-06	suggestive	+
2	cg41542652	4	1.36E+08	Lypla2	5.27E-06	suggestive	+
3	cg34962073	17	46253657	NA	5.93E-06	suggestive	-
4	cg37092343	1	74882495	NA	1.02E-05	not significant	+
5	cg35443870	18	12974748	Osbpl1a	1.88E-05	not significant	+
6	cg43616393	6	82905144	Sema4f	1.91E-05	not significant	+
7	cg36571320	19	57702184	Atrnl1	2.04E-05	not significant	+
8	cg36810749	1	36782471	4933424G06Rik	2.15E-05	not significant	+
9	cg42910173	5	1.37E+08	4933404012Rik	2.15E-05	not significant	-
10	cg45580295	8	35825149	NA	2.15E-05	not significant	+

11	cg31162817	12	1.13E+08	Adssl1	2.30E-05	not significant	+
12	cg44090234	6	1.4E+08	Pik3c2g	2.33E-05	not significant	-
13	cg32669979	14	73061363	Gm41206	2.38E-05	not significant	+
14	cg42329931	5	77266773	Gm15831	2.48E-05	not significant	-
15	cg36440609	19	45006822	Lzts2	2.58E-05	not significant	-
16	cg48214177	21	1146940	Uty	2.75E-05	not significant	-
17	cg38995122	2	1.34E+08	Tmx4	3.25E-05	not significant	-
18	cg30050277	11	1.01E+08	Nbr1	3.40E-05	not significant	+
19	cg36810747	1	36782275	4933424G06Rik; Gm335533	3.95E-05	not significant	+
20	cg32045742	13	1.08E+08	Ndufaf2	4.24E-05	not significant	+
21	cg30873316	12	82171778	NA	4.52E-05	not significant	+
22	cg29084456	10	1.21E+08	Gns	4.84E-05	not significant	+
23	cg28581892	10	67102119	NA	5.14E-05	not significant	+
24	cg44299063	7	19950713	NA	5.22E-05	not significant	+
25	cg30282584	11	1.2E+08	NA	5.25E-05	not significant	+
26	cg45849815	8	75041924	NA	5.30E-05	not significant	+
27	cg39997317	3	88685688	5830417110Rik	5.31E-05	not significant	+

28	cg38600828	2	79161936	Itga4	5.36E-05	not significant	-
29	cg40638851	4	15268768	Tmem64	5.70E-05	not significant	-
30	cg37479939	1	1.33E+08	Sox13	5.73E-05	not significant	+

Table S7. Top differentially methylated loci (DMLs) within each strain in comparisons between DAI groups. Significant = p values below the genome-wide significant threshold (p<5e-08); suggestive = p values between the genome-wide significant threshold and the suggestive threshold (5e-08<p<1e-05); not significant = p values above the suggestive threshold (p>1e-05). Chr = chromosome; DML = differentially methylated locus.

C57BL/6J

Order	Chr	Start	Stop	Probes in DMR	p-value	BH-adjusted pvalue	level of significance	Gene name (UCSC)	Direction of association with ref group
1	12	1.1E+08	1.1E+08	21	4.91E-48	6.52E-43	significant	no transcript	-
2	7	1.43E+08	1.43E+08	16	3.31E-15	2.2E-10	significant	Mir675	-
3	11	51436440	51437601	3	3.53E-14	1.56E-09	significant	no transcript	+
4	1	40442427	40465902	8	1.61E-13	5.35E-09	significant	Il18r1; Il1rl1	+
5	7	67803849	67804129	4	5.95E-13	1.16E-08	significant	no transcript	-
6	3	81997016	81999791	4	4.98E-13	1.16E-08	significant	Asic5	-
7	18	66491595	66495636	4	6.13E-13	1.16E-08	significant	no transcript	+
8	16	94066300	94066426	5	8.41E-13	1.4E-08	significant	no transcript	-
9	9	45939275	45943660	8	9.78E-13	1.44E-08	significant	Mir7087	-
10	12	1.12E+08	1.12E+08	4	1.67E-12	2.22E-08	significant	Klc1	-
11	11	53772482	53784759	6	3.31E-12	3.99E-08	significant	Irf1	+
12	7	1.03E+08	1.03E+08	16	3.91E-12	3.99E-08	significant	Olfr859; Olfr856; Olfr855; Olfr854; Olfr853	+
13	4	1.27E+08	1.27E+08	5	3.88E-12	3.99E-08	significant	Gjb3	-
14	15	81925826	81926515	5	4.39E-12	4.16E-08	significant	Polr3h	-
15	14	70462819	70466883	4	5.38E-12	4.76E-08	significant	Phyhip	+
16	5	74702963	74703128	3	9.88E-12	8.2E-08	significant	no transcript	-
17	2	90782626	90783534	6	1.74E-11	1.36E-07	significant	Agbl2	-
18	2	87739264	87769271	3	3.69E-11	2.45E-07	significant	Olfr1140; Olfr1141	+
19	13	1.2E+08	1.2E+08	3	3.52E-11	2.45E-07	significant	cDNA sequence BC147527	+
20	12	1.1E+08	1.1E+08	4	6.73E-11	4.25E-07	significant	no transcript	-
21	15	80115299	80115989	4	8.64E-11	5.17E-07	significant	Syngr1	-

22	15	60164803	60297120	7	8.96E-11	5.17E-07	significant	no transcript	+
23	6	55676907	55677183	4	1.43E-10	7.91E-07	significant	no transcript	-
24	7	45822541	45827305	6	1.9E-10	9.42E-07	significant	Grwd1; Kcnj14	+
25	19	44649530	44649889	4	1.92E-10	9.42E-07	significant	no transcript	-
26	8	27227730	27228676	5	2.47E-10	1.13E-06	significant	Adrb3	-
27	7	16365024	16368610	3	2.82E-10	1.2E-06	significant	Sae1	-
28	11	35505042	35544982	7	2.86E-10	1.2E-06	significant	Slit3	+
29	Y	2664232	2743793	9	3.25E-10	1.2E-06	significant	H2al2b	+
30	5	97935268	97935581	3	3.45E-10	1.2E-06	significant	no transcript	-

CD1

Order	**Chr**	**Start**	**Stop**	**Probes in DMR**	**p-value**	**BH-adjusted pvalue**	**level of significance**	**Gene name (UCSC)**	**Direction of association with ref group**
1	9	1.04E+08	1.04E+08	7	2.87E-16	1.27E-11	significant	Uba5; Acad11	-
2	11	1.01E+08	1.01E+08	3	1.05E-15	3.5E-11	significant	no transcript	+
3	5	1.1E+08	1.1E+08	4	3.19E-12	8.46E-08	significant	Chfr	-
4	7	13261082	13261446	4	6.34E-12	1.2E-07	significant	Zswim9	-
5	6	56568439	56574052	6	2.1E-10	2.32E-06	significant	no transcript	-
6	7	19332833	19342527	3	3.71E-09	3.79E-05	significant	no transcript	-
7	8	27227730	27228676	5	5.15E-09	4.89E-05	significant	Adrb3	+
8	17	32891475	32891715	4	1.09E-08	9.35E-05	significant	Zfp870	-
9	3	96278226	96278548	3	1.24E-08	9.66E-05	significant	no transcript	+
10	6	1.43E+08	1.43E+08	3	4.34E-08	0.000274	significant	Kcnj8	+
11	10	81332257	81332762	4	7.93E-08	0.000458	suggestive	Tbxa2r	-

12	6	1.28E+08	1.28E+08	5	9.8E-08	0.000542	suggestive	Itfg2	-
13	11	88293992	88297947	4	1.36E-07	0.000724	suggestive	Ccdc182	-
14	11	1.21E+08	1.21E+08	3	3.71E-07	0.001587	suggestive	Rab40B	+
15	10	80172911	80173108	3	5.64E-07	0.002122	suggestive	Fam174c	-
16	9	1.04E+08	1.04E+08	4	7.58E-07	0.00272	suggestive	Nphp3	-
17	12	1.19E+08	1.19E+08	3	1.04E-06	0.003543	suggestive	D230030E09Rik	-
18	10	1.27E+08	1.27E+08	3	1.35E-06	0.004371	suggestive	B4galnt1	-
19	4	1.48E+08	1.48E+08	5	2.08E-06	0.006268	suggestive	Fv1; Miip	-
20	7	24585591	24585647	3	2.23E-06	0.006586	suggestive	Zpf575	-
21	2	26138476	26138848	4	3.41E-06	0.009056	suggestive	C33006A16Rik	-
22	5	92809348	92809617	3	5.2E-06	0.012116	suggestive	Shroom3	-
23	9	83441051	83447797	4	5.72E-06	0.012869	suggestive	Gm2087; Lca5	-
24	10	74991426	74992072	5	6.02E-06	0.013131	suggestive	Gnaz	-
25	18	37504353	37507000	3	6.41E-06	0.013319	suggestive	Pcdhb20	-
26	5	52515778	52516161	3	7.18E-06	0.014233	suggestive	no transcript	-
27	13	75089781	75090113	4	7.29E-06	0.014235	suggestive	Pcsk1	+
28	8	1.1E+08	1.1E+08	3	1.01E-05	0.018797	not significant	Atxn1	-
29	14	28509713	28511587	4	1.26E-05	0.02266	not significant	Wnt5a	-
30	15	28509713	28511587	4	1.26E-05	0.02266	not significant	Mir3080	-

Sv129Ev

Order	**Chr**	**Start**	**Stop**	**Probes in DMR**	**p-value**	**BH-adjusted pvalue**	**level of significance**	**Gene name (UCSC)**	**Direction of association with ref group**

1	2	1.2E+08	1.2E+08	9	1.41E-11	1.87E-06	significant	Nusap1; Oip5	+
2	4	53713974	53714290	4	2.70E-09	0.000179	significant	fukutin	-
3	Y	1244883	1246228	24	1.43E-08	0.000634	significant	Uty	-
4	18	7001626	7002659	4	1.13E-07	0.003758	suggestive	mohawk homeobox	+
5	11	8624579	8624816	3	1.96E-07	0.005207	suggestive	no transcript	+
6	17	43952988	43953329	3	5.28E-07	0.011685	suggestive	Rcan2	+
7	1	74881981	74882496	3	8.63E-07	0.016369	suggestive	Fev	+
8	11	19998090	19998317	3	3.78E-06	0.062761	suggestive	Spred2	+
9	4	54966184	54966261	3	7.63E-06	0.088525	suggestive	no transcript	+
10	11	1.01E+08	1.01E+08	3	0.000008	0.088525	suggestive	no transcript	+
11	5	24591271	24592735	6	9.68E-06	0.098884	suggestive	Smarcd3; Mir671; Chpf2	+
12	11	94149106	94149327	3	1.43E-05	0.126854	not significant	no transcript	+
13	17	20078475	20114625	4	2.75E-05	0.19152	not significant	no transcript	+
14	5	33953872	33954009	3	3.05E-05	0.19152	not significant	no transcript	+
15	10	93165836	93167508	3	3.08E-05	0.19152	not significant	no transcript	+
16	15	38471763	38472349	3	3.17E-05	0.19152	not significant	no transcript	+
17	4	1.4E+08	1.4E+08	3	3.88E-05	0.223723	not significant	no transcript	+
18	14	33348991	33348992	4	5.13E-05	0.283524	not significant	no transcript	+
19	8	34123616	34143338	3	5.49E-05	0.291366	not significant	Leprotl1; Mboat4	+
20	8	87093185	87158380	7	5.86E-05	0.299014	not significant	no transcript	+
21	17	26431938	26432317	4	6.48E-05	0.318454	not significant	Neurl1b	-
22	3	89316130	89316731	4	7.74E-05	0.350622	not significant	Efna3	+
23	12	20845590	20847186	5	8.32E-05	0.350622	not significant	no transcript	+
24	10	40548879	40570576	5	8.36E-05	0.350622	not significant	Slc22a16	-
25	2	1.31E+08	1.31E+08	5	1.01E-04	0.375577	not significant	1700037H04Rik	-
26	9	58077454	58079444	3	1.02E-04	0.375577	not significant	Ccdc33	+

27	14	73142650	73143769	6	1.16E-04	0.406927	not significant	Rcbtb2	+
28	Y	1285077	1286761	19	1.27E-04	0.41983	not significant	Ddx3y	-
29	4	1.41E+08	1.41E+08	3	1.31E-04	0.41983	not significant	no transcript	-
30	10	84760085	84760211	3	1.39E-04	0.429459	not significant	no transcript	+

Table S8. Top differentially methylated regions (DMRs) within each strain in comparisons between DAI groups.Significant = p values below the genome-wide significant threshold (p<5e-08); suggestive = p values between the genome-wide significant threshold and the suggestive threshold (5e-08<p<1e-05); not significant = p values above the suggestive threshold (p>1e-05). Chr = chromosome; DMR = differentially methylated region; BH = Benjamini-Hochberg.

Strain	Mean (mo)	Min (mo)	Max (mo)	Resource
129/J	22.32	Na	na	(4)
129/Sv	23.31	10	33.11	(5)
129S1/SvlmJ	28.86	17.56	36.23	Phenome.jax.org
129S1/SvlmJ	28.99	Na	na	(6)
129S1/SvlmJ	28.99	Na	34.32	(7)
C57BL/6J	29.39	23.21	37.48	Phenome.jax.org
C57BL/6J	29.62	Na	na	(6)
C57BL/6J	29.62	Na	34.88	(7)
C57BL/6J	22.22	Na	na	(4)
Harlan CD1	27.1	13	32	(8)
ICD-CD1	25.08	Na	na	(9)
Swiss-CD1	21.86	8.51	29.91	(10)

Table S9. Strain survival in standard housing

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