Supplemental material

Daytime feeding with prolonged fasting times reaps greater geroprotective effects when combined with calorie restriction in adult female mice

The document contains 5 supplemental figures and 5 supplemental tables.

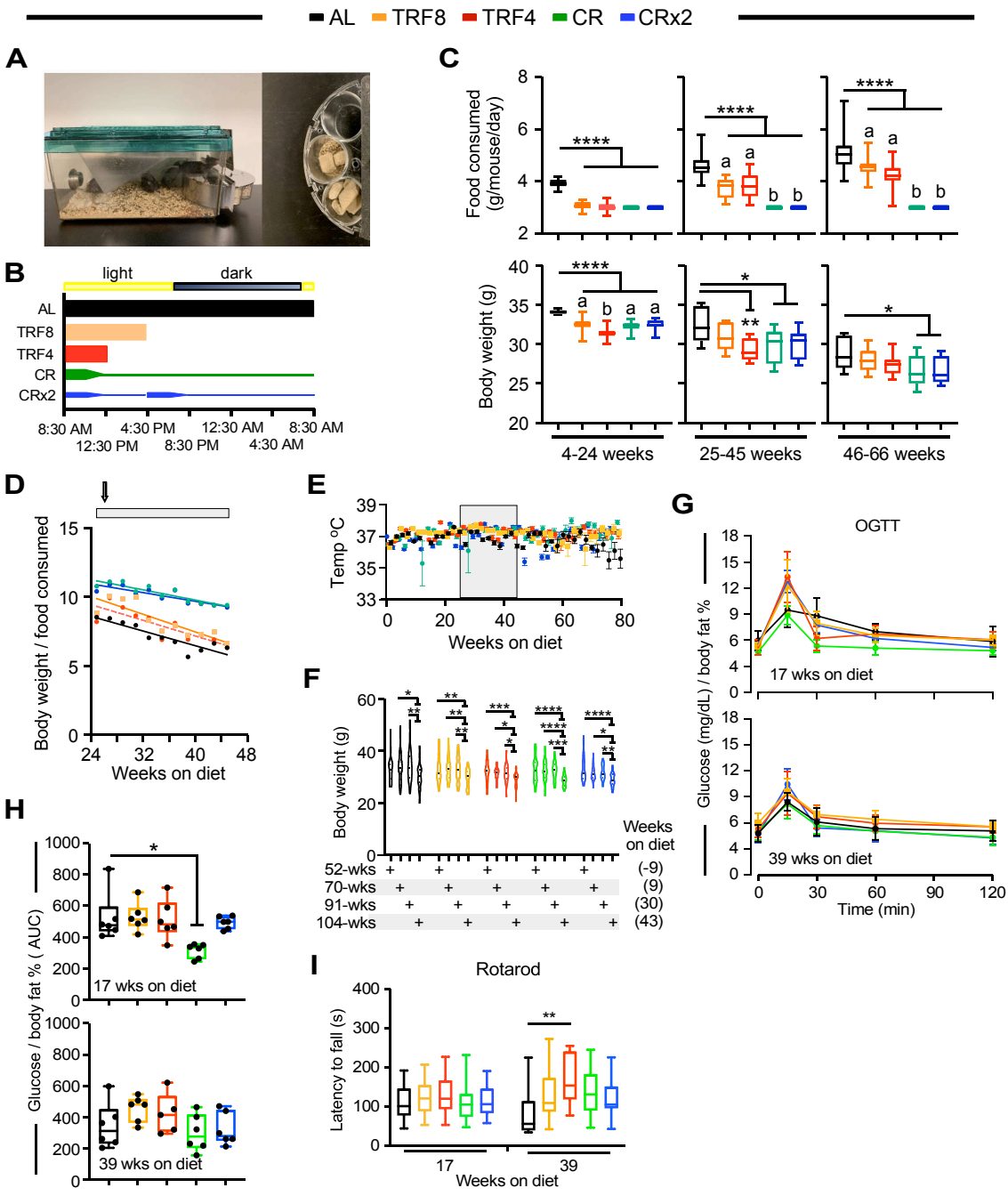

Figure S1. Effect of late onset feeding regime on food consumption, body weight, glucose metabolism, and physical performance in female mice.

(A) Configuration of manual 7-day feeder carousel onto mouse cage.

(B) Graphical representation of the experimental protocol.

(C) Discrete 20-week binning was performed to assess the effect of feeding regime on the amount of food consumed (upper panels) and body weight changes (lower panels): bin 1, 4-24 weeks (left panels); bin 2, 25-45 weeks (middle panels); bin 3, 46-66 weeks (right panels).

(D) Focus on trajectories of body weight (upper panel) and the ratio of 'body weight / amount of food consumed' (bottom panel) for the period between 25-45 weeks after diet switching. Arrow, bleed at 26 weeks for metabolomics analysis. See Data S1 for additional details.
(E) Trajectories of core temperature over the course of 80 weeks under the indicated feeding regimens. Data are expressed as means +/- 95% confidence intervals (CI). Gray box indicates a period of volitional increase in food consumption (25-45 weeks after diet switching) by mice fed AL, TRF8, and TRF4.
(F) Mouse body weight at the onset of body composition assessment by low-field nuclear magnetic resonance imaging. Data distribution is visualized as violin plots with a marker for the median.
(G) Trajectories of blood glucose clearance during an OGTT after normalization by body fat content. Values are represented as means +/- SD. n = 6 per group.
(H) Area under the curve (AUC) from the OGTT experiment depicted in panel F.
(I) Latency to fall off the rotarod.
(C,H,I) Values are represented as boxplots depicting minimum, lower quartile (Q1), median (Q2), upper quartile (Q3) and maximum values. Data have been analyzed using one-way ANOVA. *, **, **** $p < 0.05$, 0.01, 0.0001. Different lowercase letters indicate significant differences at $p < 0.05$ (panel C).
Related to Figure 1.

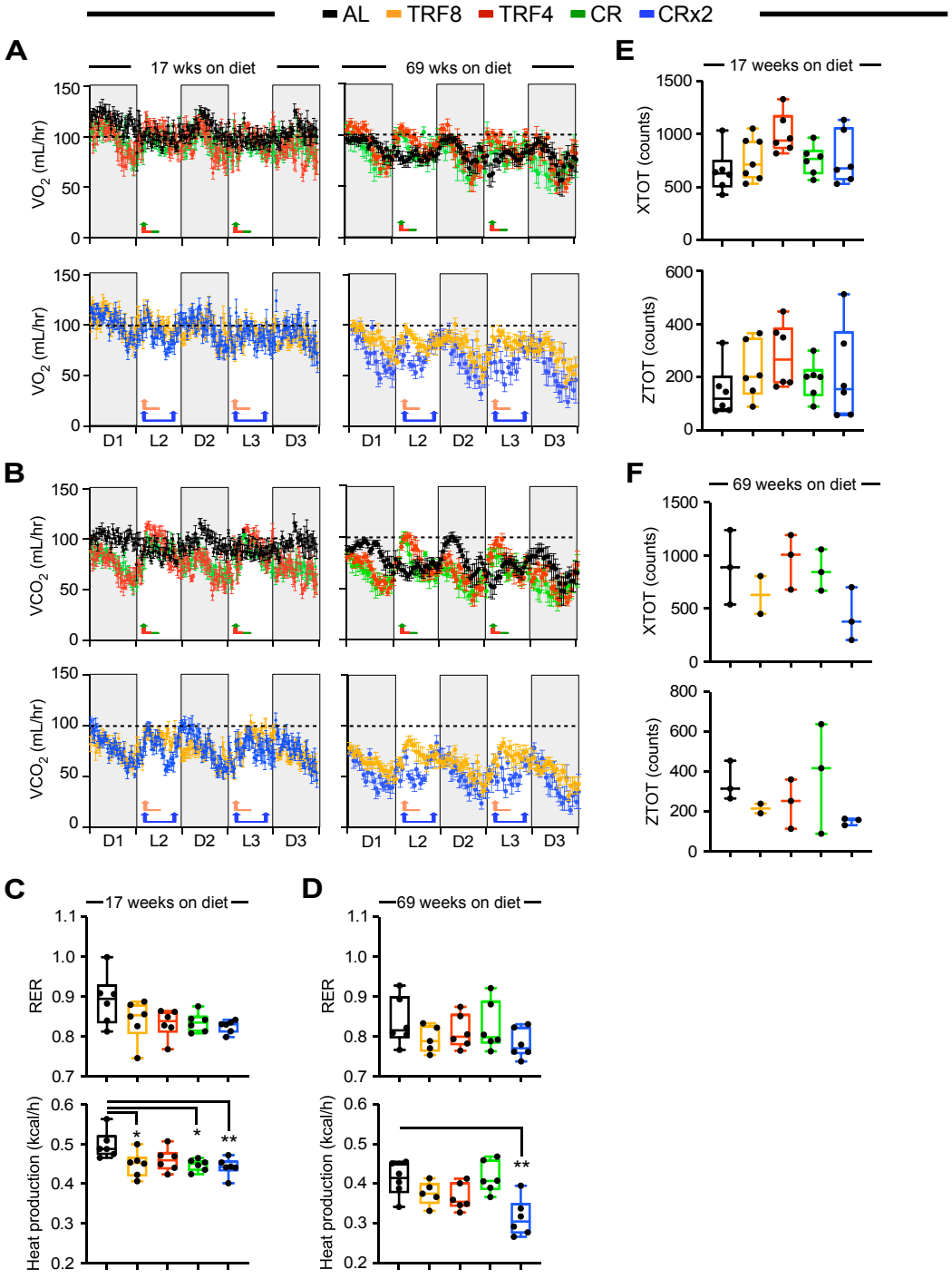

Figure S2. **Metabolic adaption to various feeding regimens in female mice**.
Mice exposed to the indicated feeding regime for 17 and 69 weeks were placed into metabolic cages for 72 h to measure VO_2, VCO_2, respiratory exchange ratio (RER), energy expenditure (EE), and locomotor activity. The values associated with the first 12 h acclimatization phase (L1) were discarded.

(A, B) Averaged hourly trajectories of O_2 consumption (A) and CO_2 production (B) were captured during 3 dark (D) and 2 light (L) cycles. Arrows indicate feeding time (8:30 AM for all groups and 4:30 PM for CRx2 only). Values represent average +/- SD, with n = 6-7 mice per group.
(C, D) The averaged RER (upper panels) and heat production (bottom panels) values were collected over 60 h from metabolic caged mice after a 17-week (C) or 69-week (D) intervention period. Data are represented as box and whisker plots, depicting minimum, lower quartile (Q1), median (Q2), upper quartile (Q3) and maximum values.
(E, F) Locomotor activity in x axis (XTOT, upper panels) and z axis (ZTOT, bottom panels) was measured in metabolic caged mice after a 17-week (E, n=6 mice per group) or 69-week (F, n=2-3 mice per group) intervention period.
One-way ANOVA coupled with Dunnett's post-hoc test was performed.
Related to Figure 2.

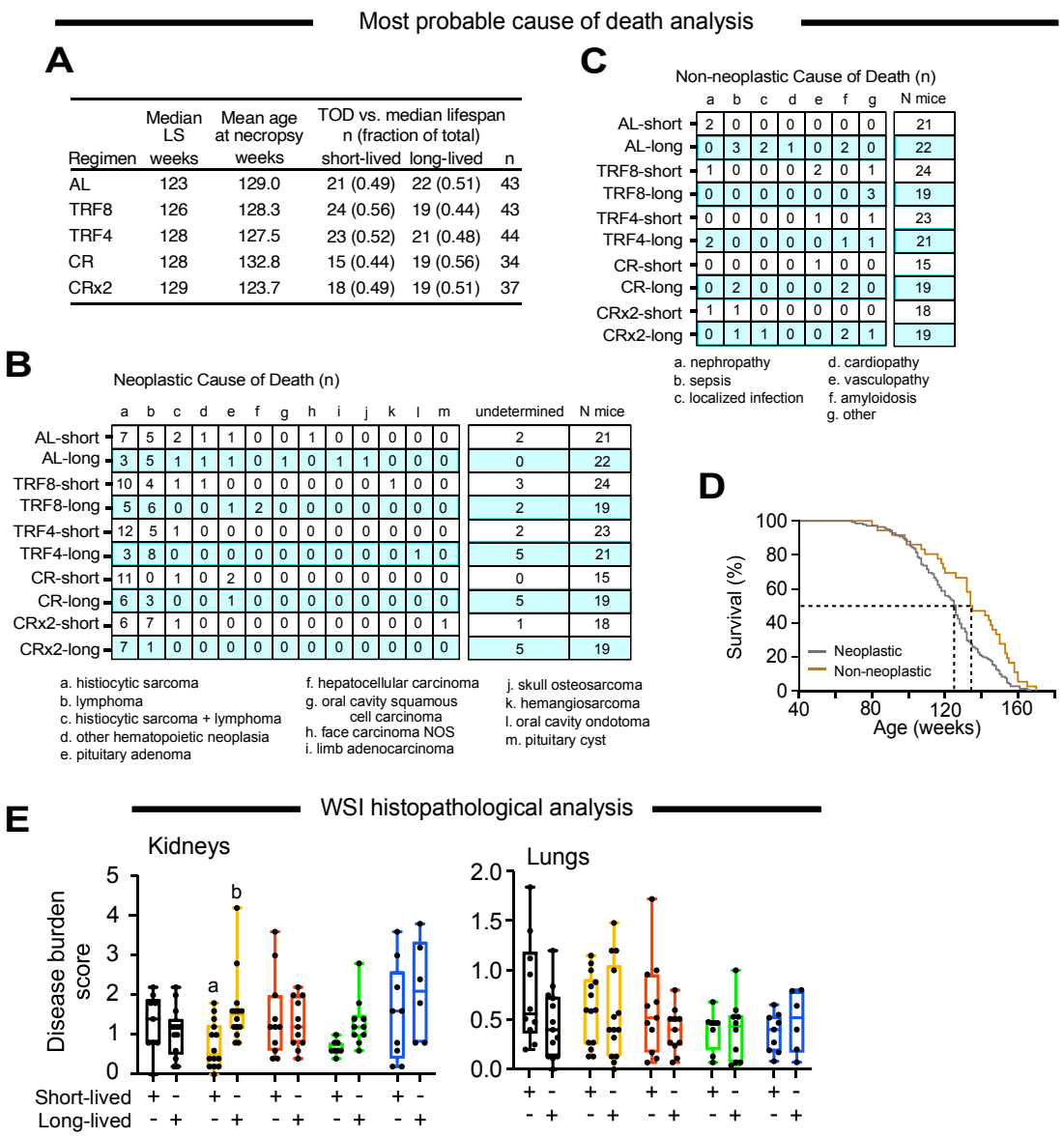

Figure S3. Most probable cause of death assessment at necropsy.

(A) Number, median lifespan (LS), and mean age of female mice at necropsy.

(B, C) Neoplastic (B) and non-neoplastic (C) most probable cause of death. The list of various pathologies and tumor histotypes is represented. (AL, n=43; TRF8, n=43; TRF4, n=44; CR, n=34; CRx2, n=37).

(D) Kaplan-Meier survival curves for female mice from all feeding groups that died with neoplastic (n=141) and non-neoplastic (n= 36) diseases, or from undetermined cause (n = 25). Log-rank (Mantel-Cox) test, $p = 0.0083$.

(E) Whole-slide image (WSI) histopathological analysis of the kidneys and lungs. Two-way ANOVA coupled with Sidak's post-hoc test was performed to assess the effect of feeding regimen, survival [short- vs. long-lived) and their interaction. Data are represented as box and whisker plots, depicting minimum, lower quartile (Q1), median (Q2), upper quartile (Q3) and maximum values. Different letters indicate statistical significance between short- and long-lived mice at $p < 0.05$.

Related to Figure 3B-C.

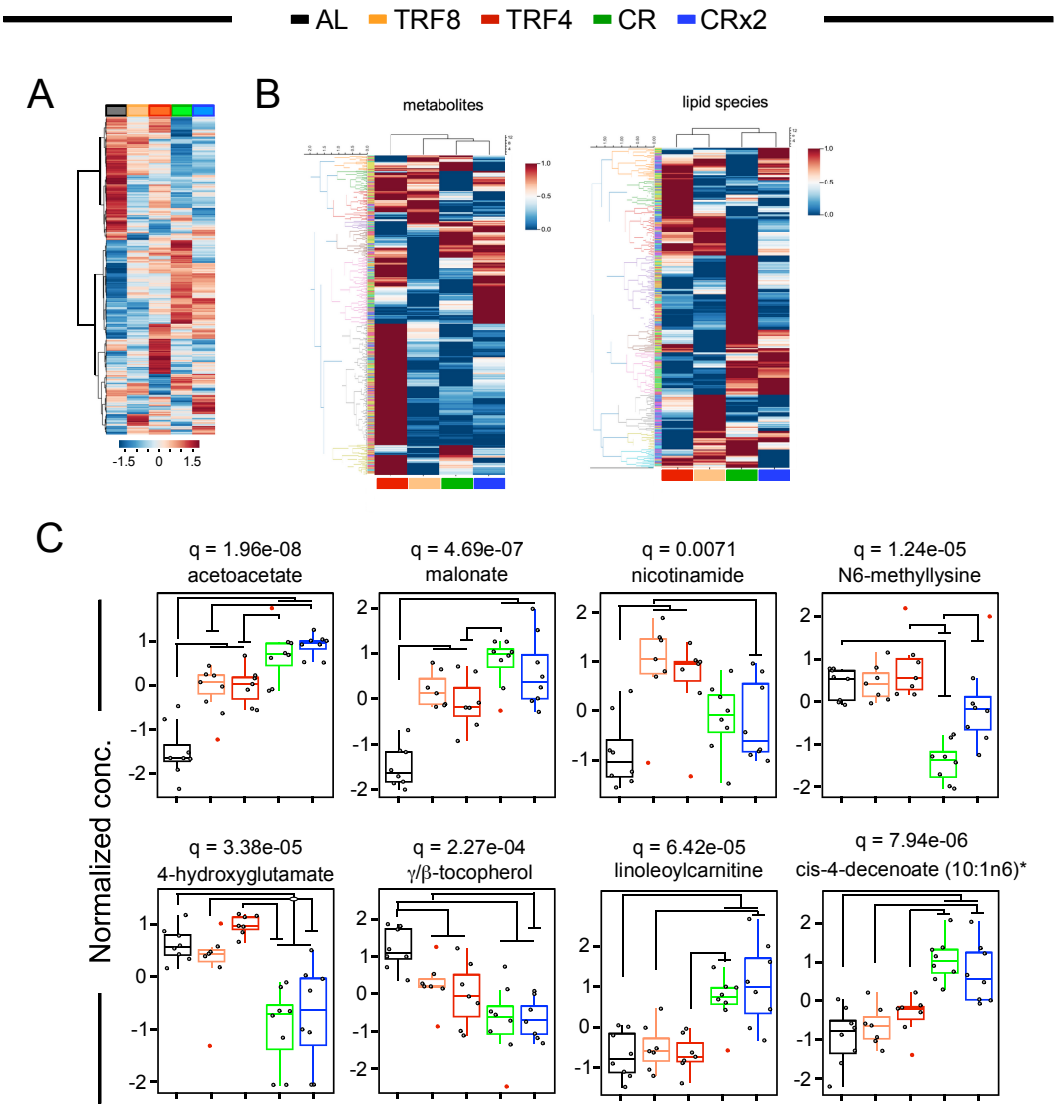

Figure S4. Impact of long-term feeding regimen on serum metabolomics signature in female mice.
The metabolomics data set was analyzed with MetaboAnalyst 5.0.
(A) Group averages of the normalized data using autoscale features. AL, n=8; TRF8, n=7; TRF4, n=7; CR, n=8; CRx2, n=8.
(B) Clustering analysis of metabolites (left panel) and lipid species (right panel) present in the serum of TRF4, TRF8, CR, and CRx2 mice vs. AL-fed controls
(C) Boxplots of select metabolites and lipid species present in the five experimental groups (AL, TRF8, TRF4, CR, CRx2). Data are represented as box and whisker plots, depicting minimum, lower quartile (Q1), median (Q2), upper quartile (Q3) and maximum values. q-values describe false discovery rate (FDR) derived from one-way ANOVA.
Related to Figure 4.

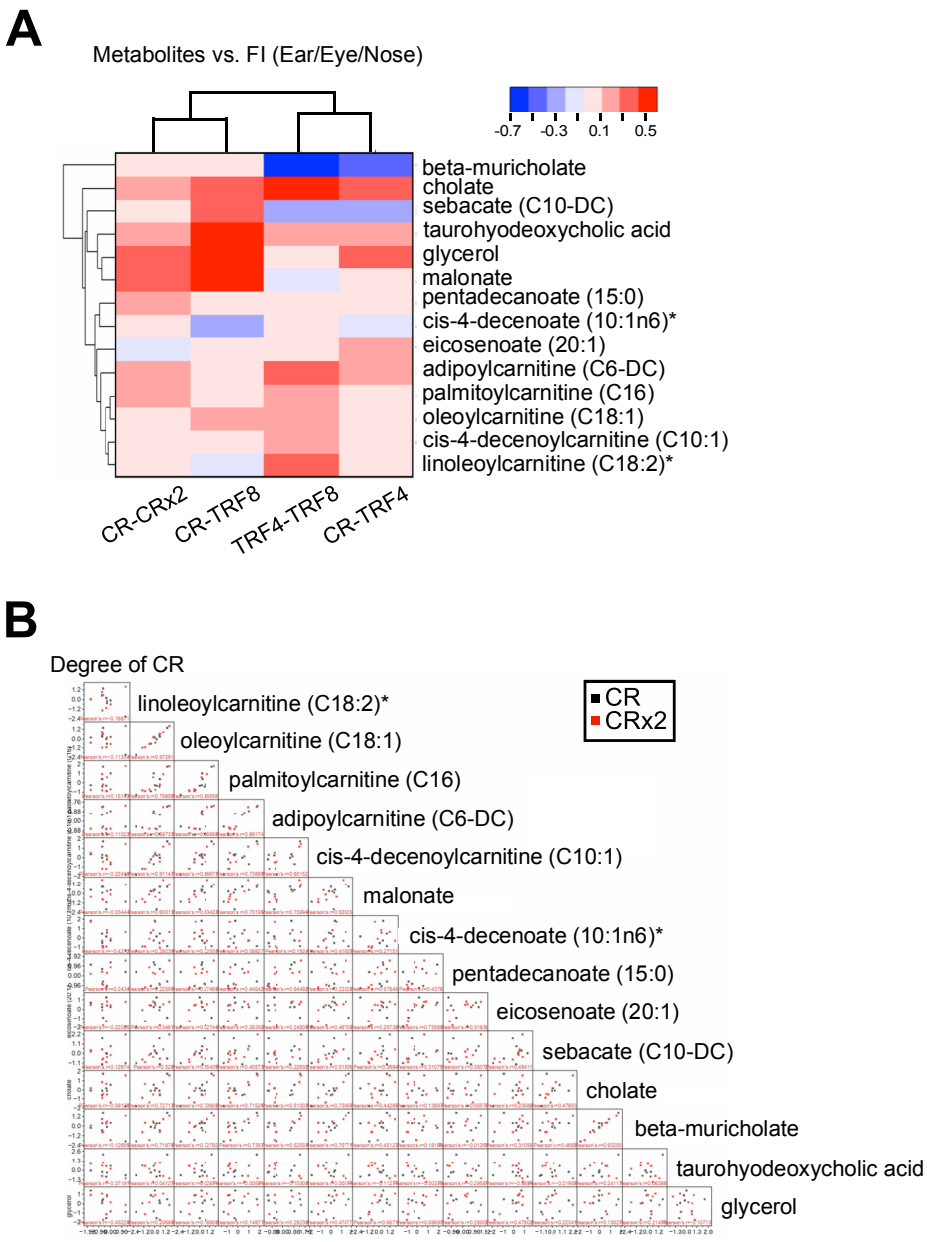

Figure S5. Integrated interpretation of various data sets that includes functional signatures of physiological and metabolic outcomes.

(A) Heatmaps of hierarchically clustered correlation coefficients of metabolites vs. Ear/Eye/Nasal FI component

(B) An example of scatter plot matrix of 14 metabolites vs. degree of CR (1st column) and of each metabolite with respect to each one of the other metabolites; the first column of the matrix shows the Pearson correlation coefficients displayed in the Source Data file for Figure 6A-D.

Related to Figure 6.

Table S1, Related to Figures 1-3 and S3E. Statistical analysis on the effects of feeding regimens on various physiological and energetic outcomes, and disease burden score.

	Physiological data (Figures 1 and S1) -two-way ANOVA[1]			
Figure panel	Measured outcome	Interaction	Feeding regime	Time on diet
Fig. 1D top	% fat -MRI	F (12, 832) = 0.6460 P = 0.8034	F (4, 832) = 4.266 P = 0.0020, **	F (3, 832) = 44.45 P < 0.0001, ****
Fig. 1D bottom	Lean/fat ratio -MRI	F (12, 822) = 1.707 P = 0.0606	F (4, 822) = 9.535 P < 0.0001, ****	F (3, 822) = 41.24 P < 0.0001, ****
Fig. S1F	Body weight -MRI	F (12, 841) = 0.9003 P = 0.5463	F (4, 841) = 5.358 P = 0.0003, ***	F (3, 841) = 26.41 P < 0.0001, ****
	Physiological data (Figures 1 and S1) -one-way ANOVA[2]			
		F (DFn, DFd)	P value	Kruskal-Wallis
Fig. 1E, baseline	6-h FBG	F (4, 35) = 1.444	0.2402	
Fig. 1E, 26 wks	6-h FBG	F (4, 47) = 40.78	<0.0001, ****	
Fig. 1G, 17 wks	AUC OGTT	F (4, 25) = 3.067	0.0348, *	
Fig. 1G, 39 wks	AUC OGTT	F (4, 25) = 2.753	0.0503	
Fig. 1H, 39 wks	Cagetop (Newtons)		<0.0001, ****	5, 64 = 23.86
Fig. 1I	Frailty index score	F (4, 48) = 7.013	0.0002, ***	
Fig. 1J	Frailty_ integumentary	F (4, 48) = 2.581	0.0489, *	
Fig. 1J	Frailty_musculo-skeletal	F (4, 48) = 5.190	0.0015, **	
Fig. 1J	Frailty_ear-eye-nose	F (4, 48) = 3.624	0.0117, *	
Fig. 1J	Frailty_Digestive urogenital	F (4, 48) = 1.189	0.3279	
Fig. 1J	Frailty_Breathing	F (4, 48) = 2.772	0.0375, *	
Fig. 1J	Frailty_Discomfort	F (4, 48) = 7.075	0.0001, ***	
Fig. S1C, 4-24 wks	Food consumed	F (4, 100) = 200.5	<0.0001, ****	
Fig. S1C, 25-45 wks	Food consumed	F (4, 97) = 85.52	<0.0001, ****	
Fig. S1C, 46-66 wks	Food consumed	F (4, 100) = 107.3	<0.0001, ****	
Fig. S1C, 4-24 wks	Body weight	F (4, 50) = 19.82	<0.0001, ****	
Fig. S1C, 25-45 wks	Body weight	F (4, 50) = 4.752	0.0025, **	
Fig. S1C, 46-66 wks	Body weight	F (4, 50) = 4.235	0.0050, **	
Fig. S1H, 17wks	AUC OGTT/fat %		0.0081, **	5, 30 = 13.75
Fig. S1H, 39 wks	AUC OGTT/fat %	F (4, 24) = 1.832	0.1556	
Fig. S1I, 17 wks	Rotarod (s)	F (4, 111) = 0.7381	0.5680	
Fig. S1I, 39 wks	Rotarod (s)	F (4, 63) = 3.499	0.0121, *	
	Metabolic cage data (Figures 2 and S2) -One-way ANOVA[2]			
Figure panel	Measured outcome	F (DFn, DFd)	P value	
Fig. 2A left, 17 wks	ΔBW (g)	F (4, 25) = 1.806	0.1592	
Fig. 2A right, 69 wks	ΔBW (g)	F (4, 24) = 6.226	0.0014, **	
Fig. 2B left, 17 wks	Food consumed	F (4, 25) = 9.264	<0.0001, ****	
Fig. 2B right, 69 wks	Food consumed	F (4, 24) = 15.58	<0.0001, ****	
Fig. 2C left, 17 wks	Food efficiency	F (4, 25) = 2.281	0.0889	
Fig. 2C right, 69 wks	Food efficiency	F (4, 24) = 3.219	0.0300, *	
Fig. S2C top, 17 wks	RER	F (4, 25) = 2.357	0.0810	
Fig. S2C bot, 17 wks	Heat production	F (4, 25) = 3.963	0.0126, *	
Fig. S2D top, 69 wks	RER	F (4, 24) = 1.214	0.3307	
Fig. S2D bot, 69 wks	Heat production	F (4, 24) = 6.457	0.0011, **	
Fig. 2E top, 17 wks	X-TOT	F (4, 25) = 2.482	0.0686	

Fig. 2E bot, 17 wks	Z-TOT	F (4, 25) = 1.012	0.4202	
	WSI -histopathological analysis (Figures 3 and S3E) -two-way ANOVA[1]			
Figure panel	Measured outcome	Interaction	Feeding regime	Short vs. long-lived
Fig. 3B - combined	Disease burden score	p=0.0030, **	p=0.0443, *	p=0.0169, *
Fig. 3C – heart		p=0.0668	p=0.0481, *	p=0.0120, *
Fig. 3C – liver		p=0.0006, ***	p=0.1743	p=0.5664
Fig. S3E- kidneys		p=0.0780	p=0.0496, *	p=0.0315, *
Fig. S3E - lungs		p=0.3977	p=0.2575	p=0.2082

[1]The results were analyzed by two-way ANOVA with Sidak's post-hoc test.
[2]The results were analyzed by one-way ANOVA with Dunnett's post-hoc test

Table S2, Related to Figure 3A. Lifespan descriptive statistics and pairwise survival comparisons using Cox Proportional Hazard Regressions.

	Mice (n)	Lifespan (weeks)						
		Min.	P25%	Median	Mean	P75%	P90%	Max.
Overall								
Cohort*	201	69	110	127	125	143	152	170
Subgroup								
AL	43	71	107	123	123	143	149	155
CR	35	80	110	128	129	150	161	170
CRX2	37	69	115	129	127	150	155	167
TRF4	44	73	108	128	125	144	153	160
TRF8	42	83	118	126	125	135	141	154

Group	AL	CR	CRX2	TRF8
CR	HR=0.56 (0.35-0.90), pval = 0.026*			
CRX2	HR=0.70 (0.45-1.10), pval = 0.080	HR=1.26 (0.79-2.02), pval = 0.361		
TRF8	HR=0.88 (0.58-1.34), pval = 0.528	HR=1.61 (1.02-2.55), pval = 0.069	HR=1.27 (0.82-1.98), pval = 0.312	
TRF4	HR=1.06 (0.70-1.63), pval = 0.612	HR=1.95 (1.22-3.14), pval = 0.016*	HR=1.54 (0.98-2.43), pval = 0.073	HR=1.21 (0.79-1.86), pval = 0.296

* Only mice that died of natural causes. Abbreviations: P25%, P75%, and P90%, percentile 25, 75, and 90%, respectively; AL, ad libitum; CR, caloric restriction; TRF, time-restricted feeding.
HR and pval values were derived from Cox regressions and Log-Rank test, respectively. n= 244 mice (dead: 201; censored: 43).

Table S3, Related to Figure 3B-3D. Modification of the grading system performed according to the guidelines of the geropathology grading system for aging mouse studies set by the Geropathology Research Network*

Amyloid grading	Tumor grading
0: absent	0: absent
1: amyloid deposits in < 25% of the organ	1: benign tumor
2: amyloid deposits in 26-50% of the organ	2: malignant tumor in < 30% of the organ
3: amyloid deposits in 51-75% of the organ	3: malignant tumor in 31-75% of the organ
4: amyloid deposits in > 75% of the organ	2: malignant tumor in >75% of the organ
Disease burden score calculation in following tissues	
Heart	(Amyloid score + fibrosis score + all other lesions sum) / 3
Lung	(Alveolar histiocytosis score + eosinophilic crystalline pneumonia score + inflammation changes average + all other lesions sum) / 5
Kidney	(Glomerular changes sum + tubular changes sum + interstitial changes sum + vascular changes sum + all other lesions sum) / 5
Liver	(Cellular changes sum + inflammation changes sum + vascular changes sum + tumor sum) / 4

* From Snyder et al., 2019.

Table S4. Raw data of the top 25 metabolites responsible for group effects. Related to Figure 4B.

	AL		TRF8		TRF4		CR		CRx2	
sample name	AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD
trans-4-hydroxyproline	-0.011	0.092	-0.257	0.052	-0.189	0.151	-0.734	0.168	-0.469	0.192
equol sulfate	-0.131	0.314	-0.646	0.244	-0.283	0.425	-2.006	0.422	-1.386	0.277
equol glucuronide	-0.144	0.335	-1.063	0.181	-0.369	0.525	-2.397	0.768	-1.282	0.241
2-(4-hydroxyphenyl)propionate	0.058	0.238	-0.511	0.344	-0.277	0.369	-1.837	0.288	-1.496	0.372
gentisate	-0.329	0.274	-1.467	0.409	-0.935	0.371	-1.940	0.297	-1.730	0.429
genistein sufate*	-0.086	0.670	-3.419	0.831	-2.844	0.564	-4.336	0.464	-3.304	0.495
diazein sulfate (2)	-0.318	0.731	-3.460	0.608	-2.916	0.717	-4.039	0.488	-3.655	0.476
glycitein sulfate (2)	-0.427	0.668	-2.722	0.517	-1.718	0.343	-3.021	0.000	-2.954	0.190
2,6-dihydroxybenzoic acid	-0.137	0.650	-1.525	0.239	-1.041	0.315	-1.887	0.413	-1.718	0.245
docosahexaenoylcarnitine (C22:6)	1.072	0.230	-0.073	0.192	0.277	0.271	-0.299	0.539	-0.094	0.199
salicylate	0.155	0.510	-1.061	0.255	-0.644	0.323	-1.619	0.144	-1.496	0.149
lysine	0.245	0.070	-0.197	0.201	-0.016	0.160	-0.397	0.130	-0.277	0.054
acetoacetate	-1.339	0.491	-0.190	0.460	-0.061	0.332	0.447	0.494	0.619	0.210
3-hydroxybutyroylglycine**	-1.418	1.108	0.819	0.487	0.546	0.399	1.862	0.580	1.568	0.335
4-hydroxybutyrate (GBH)	-0.300	0.733	1.250	0.395	1.139	0.205	1.933	0.269	1.815	0.344
3-hydroxybutyrate (BHBA)	-0.333	0.744	1.236	0.328	1.109	0.250	1.851	0.283	1.764	0.346
palmitoleate (16:1n7)	-0.207	0.132	0.655	0.178	0.537	0.226	1.055	0.158	0.515	0.303
myristate (14:0)	-0.105	0.185	0.619	0.164	0.631	0.140	1.037	0.164	0.508	0.339
laurate (12:0)	-0.073	0.116	0.514	0.178	0.555	0.131	0.979	0.180	0.481	0.294
10-nonadecenoate (19:1n9)	-0.363	0.110	0.067	0.135	-0.060	0.123	0.365	0.112	0.299	0.229
oleate/vaccenate (18:1)	-0.168	0.090	0.192	0.120	0.133	0.163	0.409	0.121	0.217	0.109
linolenate [alpha or gamma; (18::	-0.359	0.087	0.294	0.154	0.105	0.179	0.709	0.137	0.363	0.346
hexadecadienoate (16:2n6)	-0.209	0.189	0.407	0.250	0.178	0.227	0.943	0.212	0.516	0.288
linoleate (18:2n6)	-0.284	0.060	0.105	0.157	-0.098	0.118	0.383	0.129	0.254	0.144
10-heptadecenoate (17:1n7)	-0.256	0.086	0.190	0.162	-0.035	0.219	0.593	0.143	0.327	0.250

Table S5, related to Figure 4 and Fig. S4. List of serum metabolites significantly and uniquely accumulated in the indicated pairwise comparison. Data were obtained from 4-way Venn diagrams.

		-Log(P)		
HMDB	**Metabolites**	**TRF4_AL**	**TRF8_AL**	**Shared_AL**
HMDB0000695	4-methyl-2-oxopentanoate	**3.644**		
HMDB0002064	N-acetylputrescine	**0.839**		
HMDB0002189	(N(1) + N(8))-acetylspermidine	**2.416**		
HMDB0003681	4-acetamidobutanoate	**1.495**		
HMDB0004089	N-formylanthranilic acid	**1.945**		
HMDB0000821	Phenylacetylglycine	**1.262**		
HMDB0041724	dihydroferulic acid sulfate	**1.361**		
HMDB0000012	2'-deoxyuridine	**1.483**		
HMDB0000930	Cinnamate	**1.658**		
HMDB0000622	Ethylmalonate		**2.940**	
HMDB0006275	dopamine 3-O-sulfate		**1.858**	
HMDB0240459	4-methylcatechol sulfate		**1.741**	
HMDB0001406	Nicotinamide			**3.571**
HMDB0000764	3-phenylpropionate (hydrocinnamate)			**3.180**
	Metabolites	**CR_AL**	**CRx2_AL**	**Shared_AL**
HMDB0000317	2-hydroxy-3-methylvalerate	1.488		
HMDB0011756	N-acetylleucine	1.493		
HMDB0000378	2-methylbutyrylcarnitine (C5)	**2.173**		
HMDB0002038	N6-methyllysine	**6.070**		
HMDB0038670	S-methylmethionine	**2.722**		
HMDB0003320	indole-3-carboxylate	**3.012**		
HMDB29737	3-formylindole	**2.714**		
HMDB0000232	Quinolinate		**2.573**	
HMDB0000273	Thymidine		**1.521**	
HMDB0000128	Guanidinoacetate		**3.023**	
HMDB0000729	2-hydroxybutyrate/2-hydroxyisobutyrate			**3.433**
HMDB0000396	3-hydroxy-2-ethylpropionate			**2.448**
HMDB0002035	4-hydroxycinnamate			**5.519**
HMDB0001344	4-hydroxyglutamate			**5.329**
HMDB0003464	4-guanidinobutanoate			**5.194**
HMDB0002108	S-methylcysteine			**2.612**
HMDB0002302	Indolepropionate			**2.857**
HMDB0000214	Ornithine			**2.983**
HMDB0006344	phenylacetylglutamine			**3.295**
HMDB0001870	Benzoate			**2.673**
HMDB0062551	4-ethylphenylsulfate			**2.162**
HMDB0029968	ethyl α-glucopyranoside			**4.544**
HMDB0240658	2,8-quinolinediol sulfate			**2.257**
	Lipid species	**CR_AL**	**CRx2_AL**	**Shared**
HMDB0061636	3-hydroxydecanoylcarnitine	**3.377**		
HMDB0000672	hexadecanedioate (C16-DC)	**3.250**		
HMDB0000872	tetradecanedioate (C14-DC)	**5.033**		
HMDB0000131	Glycerol	**2.658**		
HMDB0005320	1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	**3.458**		
HMDB0000761	lithocholate	**2.030**		
HMDB0000874	taurohyodeoxycholic acid	**1.976**		
HMDB00327	1-palmitoyl-GPA (16:0)	**3.920**		

HMDB0011561	1-myristoylglycerol (14:0)	**3.269**		
HMDB0000114	glycerophosphoethanolamine	**1.563**		
HMDB0000946	ursodeoxycholate		**3.060**	
HMDB0013205	cis-4-decenoylcarnitine (C10:1)			**2.538**
HMDB0006469	linoleoylcarnitine (C18:2)*			**4.412**
HMDB0000792	sebacate (C10-DC)			**2.984**
HMDB0000409	5-hydroxyhexanoate			**2.728**
HMDB0002231	eicosenoate (20:1)			**2.552**
HMDB0007856	1-linoleoyl-GPA (18:2)*			**2.328**
HMDB0004980	cis-4-decenoate (10:1n6)*			**4.212**
HMDB0000619	Cholate			**3.233**
HMDB00502	3-dehydrocholate			**2.923**
HMDB0240600	1-linoleoyl-GPG (18:2)*			**2.564**
HMDB0240601	1-palmitoyl-GPG (16:0)*			**1.959**
HMDB10383	2-palmitoleoyl-GPC (16:1)*			**2.578**
HMDB0011565	1-palmitoleoylglycerol (16:1)*			**3.914**
HMDB0011534	2-palmitoleoylglycerol (16:1)*			**4.680**
HMDB0008105	1-oleoyl-2-linoleoyl-GPC (18:1/18:2)*			**2.096**
HMDB0008038	1-stearoyl-2-oleoyl-GPC (18:0/18:1)			**4.674**
	metabolites	**CR_TRF4**	**CRx2_TRF8**	
HMDB0000729	2-hydroxybutyrate/2-hydroxyisobutyrate	**2.822**		
HMDB0000532	N-acetylglycine	**3.057**		
HMDB0000317	2-hydroxy-3-methylvalerate	**1.999**		
HMDB0000396	3-hydroxy-2-ethylpropionate	**2.109**		
HMDB0000764	3-phenylpropionate (hydrocinnamate)	**0.806**		
HMDB0000128	guanidinoacetate	**0.870**		
HMDB0003464	4-guanidinobutanoate	**1.207**		
HMDB0038670	S-methylmethionine	**3.170**		
HMDB0003320	indole-3-carboxylate	**1.368**		
HMDB0240347	N6-carboxymethyllysine	**1.350**		
HMDB0000840	2-hydroxyhippurate (salicylurate)	**0.694**		
HMDB0061880	N-acetyl-beta-alanine		**2.384**	
HMDB0002035	4-hydroxycinnamate		**1.847**	
HMDB0001406	nicotinamide		**1.477**	
HMDB0006335	γ-tocopherol/β-tocopherol		**1.990**	
HMDB0004983	dimethyl sulfone		**1.249**	
	Lipid species	**CR_TRF4**	**CRx2_TRF8**	
HMDB0061677	adipoylcarnitine (C6-DC)	**1.949**		
HMDB0013207	palmitoleoylcarnitine (C16:1)*	**1.885**		
HMDB0000691	Malonate	**1.991**		
HMDB0061859	(14 or 15)-methylpalmitate (a17:0 or i17:0)	**2.267**		
HMDB0002231	eicosenoate (20:1)	**1.869**		
HMDB0000826	pentadecanoate (15:0)	**2.726**		
HMDB0005322	1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	**2.122**		
HMDB0001161	deoxycarnitine	**1.313**		
HMDB0000824	propionylcarnitine (C3)	**1.669**		
HMDB0002013	butyrylcarnitine (C4)	**3.487**		
HMDB0094649	2-aminoheptanoate	**1.367**		
HMDB0010384	1-stearoyl-GPC (18:0)	**1.613**		
HMDB0010395	1-arachidonoyl-GPC (20:4n6)*	**1.237**		
HMDB0011130	1-stearoyl-GPE (18:0)	**1.342**		
HMDB0000227	mevalonate	**1.603**		

HMDB0011577	1-dihomo-linolenylglycerol (20:3)	**1.243**		
HMDB0008036	1,2-distearoyl-GPC (18:0/18:0)	**1.096**		
HMDB0000114	glycerophosphoethanolamine	**1.431**		
HMDB0000222	palmitoylcarnitine (C16)		**0.931**	
HMDB0005065	oleoylcarnitine (C18:1)		**1.471**	
HMDB0000321	2-hydroxyadipate		**1.143**	
HMDB0000792	sebacate (C10-DC)		**1.199**	
HMDB0007856	1-linoleoyl-GPA (18:2)*		**1.213**	
HMDB0000355	3-hydroxy-3-methylglutarate		**1.373**	
HMDB0000415	beta-muricholate		**1.996**	
HMDB0000619	Cholate		**2.277**	
HMDB0000733	hyodeoxycholate		**1.298**	
HMDB0000811	6-beta-hydroxylithocholate		**2.097**	
HMDB0000946	ursodeoxycholate		**3.372**	
HMDB00502	3-dehydrocholate		**2.701**	
HMDB0004949	N-palmitoyl-sphingosine (d18:1/16:0)		**1.172**	
HMDB0240684	ceramide (d18:1/20:0, d16:1/22:0, d20:1/18.0)*		**1.570**	
HMDB0000126	glycerol 3-phosphate		**1.198**	
HMDB10383	2-palmitoleoyl-GPC (16:1)*		**2.713**	
HMDB0008038	1-stearoyl-2-oleoyl-GPC (18:0/18:1)		**1.745**	
HMDB0008147	1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*		**3.959**	

Typeset: AL, **black**; TRF4, red; TRF8, orange; CR, green; CRx2, blue.