Supporting Information

Krebs et al. 10.1073/pnas.1117835108

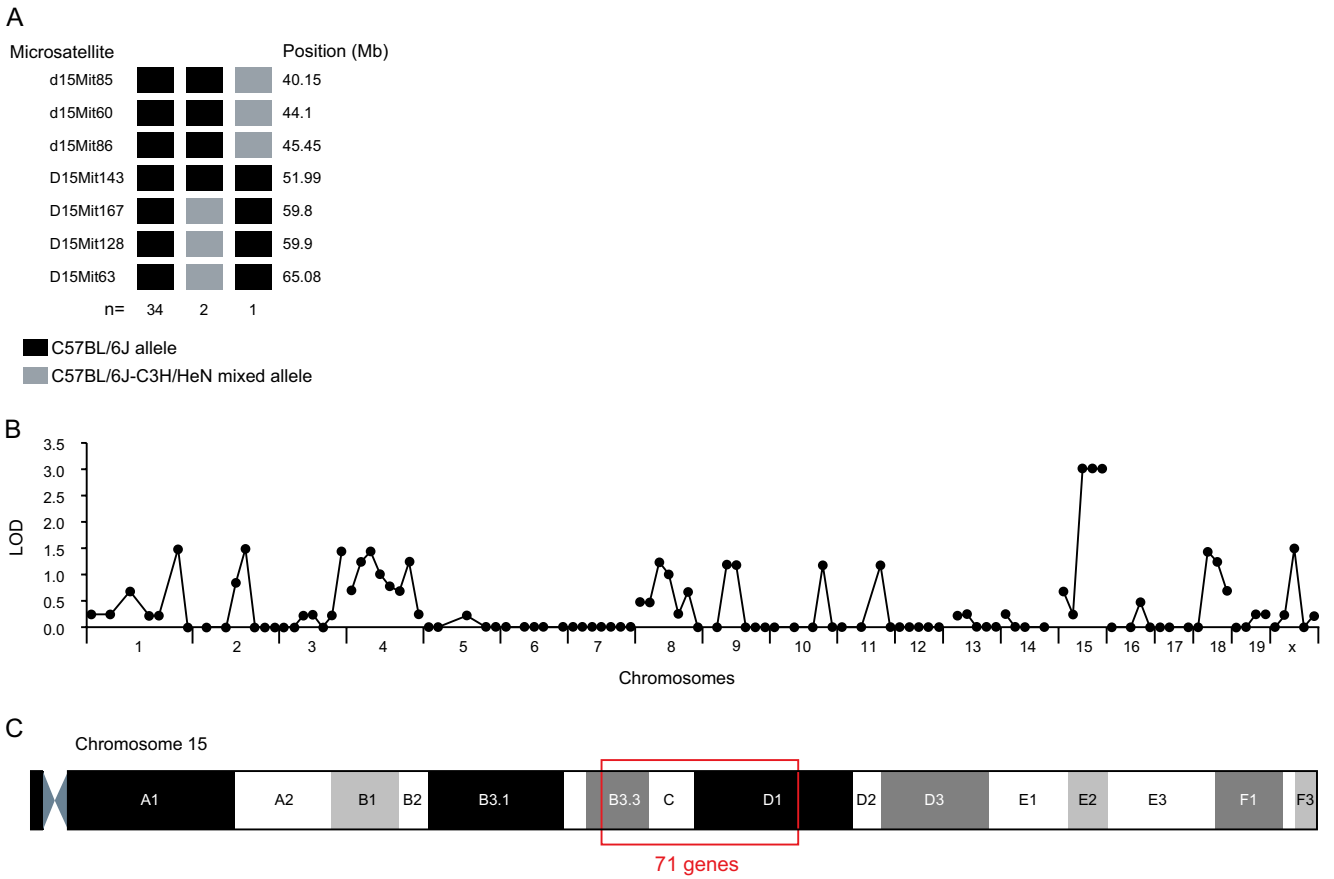

Fig. S1. Whole-genome linkage analysis of *zeitgeist*. (*A*) Meiotic mapping of the *Med30*^{zg} phenotype on chromosome 15 using F2 backcross progeny of C57BL/6J$^{zg/+}$ × C3H/HeN F1 hybrids. Haplotypes of affected *Med30*$^{zg/zg}$ mice are shown. Black squares indicate C57BL/6J homozygosity, gray squares C57BL/6J-C3H/HeN heterozygosity. Number of mice used is indicated. Mb, megabases. (*B*) Ten meioses were used to confine *zeitgeist* to the distal region of chromosome 15 with a logarithm of odds (LOD) score of 3.0. (*C*) The critical region contains 71 annotated genes.

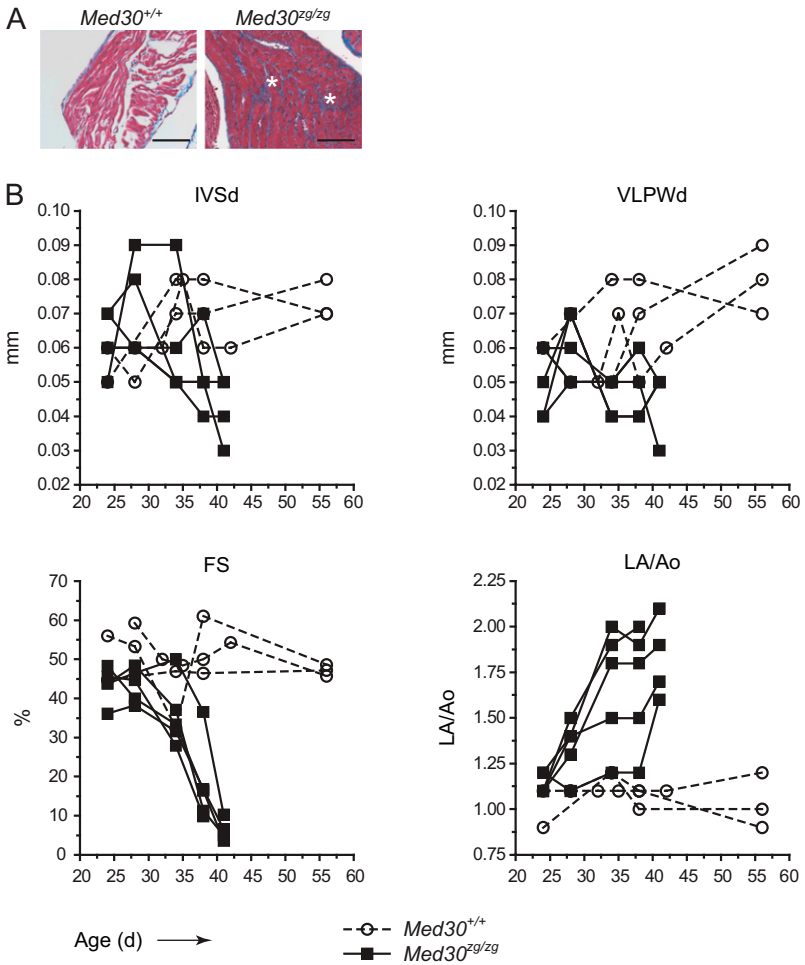

Fig. S2. Myocardial fibrosis and functional impairment of the heart in *zeitgeist* homozygotes. (*A*) Masson's trichrome staining of atrium. Asterisks show areas of prominent fibrosis. (Scale bars, 100 μm.) (*B*) Time-course analysis of interventricular septal thickness at diastole (IVSd, *Upper Left*), left ventricular posterior wall dimensions (LVPWd, *Upper Right*), fractional shortening (FS, *Lower Left*), left atrium/aorta ratio (LA/Ao, *Lower Right*). $n = 3$ for wild type and 5 for mutant mice.

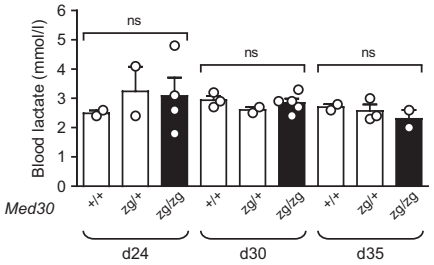

Fig. S3. Normal blood lactate concentration in *Med30*$^{zg/zg}$ mice. Analysis of blood lactate at days 24, 30, and 35 in *Med30*$^{zg/zg}$, *Med30*$^{zg/+}$, and *Med30*$^{+/+}$ mice. ns, nonsignificant. Values of quantitative results are expressed as mean ± SEM and overlapped with results for single mice. Lactate concentration was measured using a Lactate Scout Analyzer device. For each time point, the statistical significance of differences among the three genotypes was calculated using a one-way ANOVA test.

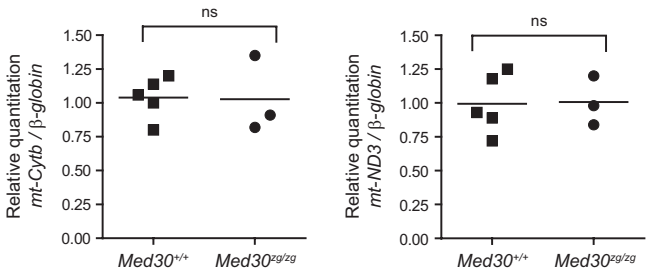

Fig. S4. Relative mitochondrial DNA copy numbers are similar in *Med30*$^{zg/zg}$ and wild-type hearts. Ratio of gene expression of either mitochondrial *mt-Cytb* or *mt-Nd3* over expression of nuclear β-*globin* is plotted. Each point represents an individual mouse.

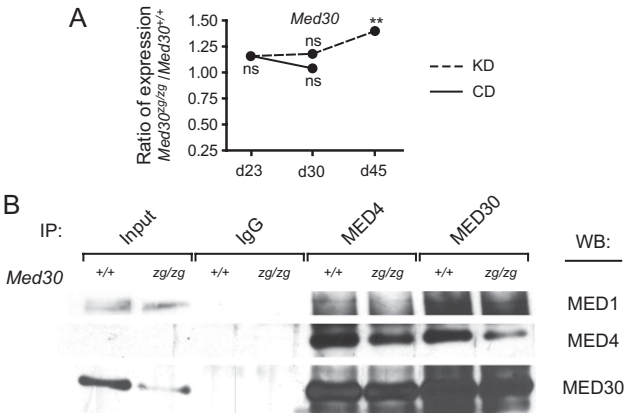

Fig. S5. (*A*) Relative expression of *Med30* at 23, 30 and 45 d of age as measured by quantitative PCR. Mice were fed either chow (CD; solid lines) or ketogenic (KD; dotted lines) diet beginning on day 23, when they were weaned. Gene expression in *Med30*$^{zg/zg}$ over that in *Med30*$^{+/+}$ whole hearts is represented. For each biological sample, quantitative PCR reactions were performed in duplicate, and expression was normalized to *Rpl32* expression. For each indicated time point and diet, *P* values were determined by comparing the relative expression of the indicated gene in *Med30*$^{zg/zg}$ vs. *Med30*$^{+/+}$ hearts using an unpaired Student's two-tailed *t* test. For *Med30*$^{zg/zg}$ hearts, $n = 3$ for day 23 CD; $n = 8$ for day 30 CD; $n = 5$ for day 30 KD; $n = 5$ for day 45 KD. For *Med30*$^{+/+}$ hearts, $n = 2$ for day 23 CD; $n = 3$ for day 30 CD; $n = 5$ for day 30 KD; $n = 4$ for day 45 KD. ns, nonsignificant. (*B*) MED30^{zg} does not cause any major perturbation of the Mediator complex integrity. Single-cell suspension was made from pooled thymi and spleens from littermate *Med30*$^{+/+}$ or *Med30*$^{zg/zg}$ mice. Nuclear extracts were isolated as previously described (1). Rabbit IgG, MED4- or MED30-specific immunoglobulins, respectively, were used for immunoprecipitation in binding buffer containing 100 mM NaCl and 0.15% Nonidet P-40, overnight at 4 °C. Immunoprecipitates were washed six times using the same buffer. After elution in Laemmli sample buffer, supernatants were separated by SDS/PAGE on a 4–12% gradient gel and analyzed by immunoblotting using MED1/TRAP220 antibody, anti-MED4, or anti-MED30 immune sera. Input represents 10% of the nuclear lysates used for immunoprecipitation reactions (20 μg and 200 μg of proteins, respectively). Samples shown were all processed in parallel.

1. Dignam JD, Lebovitz RM, Roeder RG (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res* 11:1475–1489.

Table S1. Noncomplementation between the *Med30*^{zg} allele and a *Med30*$^{\Delta exon4}$ trap allele

Med30$^{+/\Delta exon4}$ × *Med30*$^{+/zg}$ Pups	Observed	Expected
Med30$^{zg/\Delta exon4}$	0	12.5
Med30$^{+/zg}$	16	12.5
Med30$^{+/\Delta exon4}$	17	12.5
Med30$^{+/+}$	17	12.5
Total	50	

Distribution of the genotypes in progeny from intercrosses of *Med30*$^{zg/+}$ and *Med30*$^{\Delta exon4/+}$ mice. Significance was determined by Freeman-Halton extension of the Fisher's exact probability test ($P = 0.0032$).

Table S2. Distribution of genotypes in progeny from *Med30*$^{zg/+}$ intercrosses

Progeny	Observed	Expected
Pups		
Med30$^{zg/zg}$	53	91.5
Med30$^{+/zg}$	202	183
Med30$^{+/+}$	111	91.5
Total	366	
E15.5 embryos		
Med30$^{zg/zg}$	6	6.75
Med30$^{+/zg}$	12	13.5
Med30$^{+/+}$	9	6.75
Total	27	

Pups were genotyped at 2 wk of age ($P < 0.0001$, Chi-Square test). Litters were carefully counted on the day of birth and no postnatal deaths were observed before weaning. E15.5 embryos: $P = 0.799$, Freeman-Halton extension of the Fisher's exact probability test. Comparison of the observed distributions of genotypes among pups versus embryos, $P = 0.4152$ (Chi-Square test).

Table S3. Hemodynamic parameters of 35-d-old *Med30*$^{zg/zg}$ mice and control littermates

Hemodynamic parameter	*Med30*$^{+/+}$ ($n = 3$)	*Med30*$^{zg/zg}$ ($n = 4$)	*P* value
LVIDd (mm)	3.57 ± 0.21	4.78 ± 0.26	0.0013
IVSd (mm)	0.6 ± 0.02	0.5 ± 0.08	0.0624
LVPWd (mm)	0.77 ± 0.06	0.5 ± 0.17	0.0968
LA/Ao	1.5 ± 0.4	2.9 ± 0.8	0.0394
FS (%)	45.7 ± 4.0	13.9 ± 5.9	0.0004
EF of LV (%)	84.6 ± 5.9	27.2 ± 6.0	<0.0001

EF, ejection fraction; FS, fractional shortening; IVSd, interventricular septum, diastolic phase; LA/Ao, left atrial/aorta ratio; LV, left ventricle; LVIDd, LV chamber diameter, diastolic phase; LVPWd, LV posterior wall, diastolic phase.

Table S4. Blood chemistry values for three *Med30*$^{zg/zg}$ mutants with dilated cardiomyopathy

	Units	*Med30*$^{zg/zg}$ #1	*Med30*$^{zg/zg}$ #2	*Med30*$^{zg/zg}$ #3	Standard range mean	SD
Albumin	g/dL	1.1	<1.0	<1.0	1.4	0.2
Alkaline phos	IU/L	124	262	214	101.5	32.2
ALT (SGPT)	IU/L	14	78	104	38.7	25.9
AST (SGOT)	IU/L	72	54	82	72.7	36.3
Bicarbonate	mmol/L	15	15	19	15.9	3.2
Bilirubin, Direct	mg/dL	0.2	0.1	0.2	0.1	0.1
Bilirubin, Total	mg/dL	0.1	0.3	0.3	0.4	0.2
BUN	mg/dL	74	38	58	21.4	4.4
Calcium	mg/dL	8.0	9.5	8.8	9.0	0.4
Chloride	mmol/L	113	114	105	107.5	4.2
Creatinine	mg/dL	<0.2	0.2	<0.2	0.2	0.2
Glucose	mg/dL	130	266	280	196.7	91.2
Phosphorous	mg/dL	8.8	10.0	10.1	8.0	1.6
Potassium	mmol/L	6.8	7.0	5.7	5.0	1.1
Sodium	mmol/L	149	152	140	150.5	3.9
Total protein	gm/dL	3.1	3.1	3.0	4.2	0.3

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen. Standard range represents the mean values and SD measured in ~400 control C57BL/6 mice.