

Cell Genomics, Volume 4

Supplemental information

Complementation testing identifies genes

mediating effects at quantitative trait loci

underlying fear-related behavior

Patrick B. Chen, Rachel Chen, Nathan LaPierre, Zeyuan Chen, Joel Mefford, Emilie Marcus, Matthew G. Heffel, Daniela C. Soto, Jason Ernst, Chongyuan Luo, and Jonathan Flint

Supplemental Tables

Study ID	Name of measure in original data	EPM-open	FC-context	FC-cue	Source	Used	Sample size	Reference
Brown1	open_ent_EPM	1			JAX	YES	328	1
Flaherty1	EPM_open	1			JAX	YES	318	2
Milner-2008	EPM-open	1			Literature	YES	112	3
Schalkwyk1	entry_open_EPM	1			JAX	YES	86	4
Shin1	EPM_open	1			JAX	YES	123	5
UCLA-2023	EPM-open	1			UCLA	YES	333	This publication
Wahlsten1	EPM_open	1			JAX	YES	325	6-7
Brigman-2009	EPM-open	1			Gene Network		123	8
Brown1	open_ent_EZM	1			JAX		380	1
Carhuatanta-2014	EPM-open	1			Gene Network		307	9
Philip-2010	EPM-open	1			Gene Network		617	10
Yang-2008	EPM-open	1			Literature		142	11
Brigman-2009	FC-context		1		Gene Network	YES	111	3
Carhuatanta-2014	FC-context		1		Gene Network	YES	282	9
Owen-1997	FC-context		1		Literature	YES	300	12
UCLA-2023	FC-context		1		UCLA	YES	332	This publication
Yang-2008	FC-context		1		Literature	YES	150	11
Bolivar2	act_context_mean		1		JAX		289	13
Bothe-2005	FC-context		1		Literature		500	14
Knoll-2016	FC-context		1		Literature		721	15
Palmer1	freezing to context		1		JAX		462	16
Palmer3	freezing to context		1		JAX		319	17
Shin1	FC_memory		1		JAX		362	5
Smith-2011	ctx_imob		1		JAX		669	18
Brigman-2009	FC-cue			1	Gene Network	YES	116	8
Carhuatanta-2014	FC-cue			1	Gene Network	YES	301	9
Knoll-2016	FC-cue			1	Literature	YES	708	4
Owen-1997	FC-cue			1	Literature	YES	300	12
Smith-2011	cue_imob			1	JAX	YES	669	18
UCLA-2023	FC-cue			1	UCLA	YES	332	This publication
Yang-2008	FC-cue			1	Literature	YES	150	11
Bolivar2	act_cued_mean			1	JAX		289	13
Bothe-2005	FC-cue			1	Literature		500	14
Palmer1	freezing to tones			1	JAX		462	16
Palmer3	freezing to tones			1	JAX		319	17

Supplemental Table S2: Data identified from the published literature and websites for use in the meta-analysis for mapping fear related behavior, related to Figure 1. For the meta-analysis, this gives study ID, phenotype name used in the study, phenotype to which it was assigned in the meta-analysis (where EPM means elevated plus maze and FC means fear conditioning), source of the data, whether the data were included into the meta-analysis, and number of animals and a reference to the original publication.

<i>trait</i>	<i>chr</i>	<i>start</i>	<i>end</i>	<i>max pos</i>	<i>size</i>	<i>logp</i>	<i>n. of genes</i>	<i>selected</i>
EPM-open	1	8,051,126	12,843,641	12822766	4.79	5.38	23	NO
EPM-open	1	81,170,369	90,915,537	90906140	9.75	4.71	114	NO
EPM-open	1	107,003,663	107,107,513	107039384	0.10	5.44	2	NO
EPM-open	1	134,517,231	142,991,595	142876925	8.47	5.17	67	NO
EPM-open	1	157,544,700	159,401,857	158161029	1.86	7.82	10	NO
EPM-open	2	86,964,644	87,228,108	87226901	0.26	9.73	13	NO
EPM-open	2	99,960,104	104,091,211	100845037	4.13	5.28	30	NO
EPM-open	2	114,378,383	117,549,383	114639431	3.17	7.64	15	NO
EPM-open	2	130,308,391	132,695,042	132656944	2.39	6.73	54	NO
EPM-open	2	178,967,969	181,729,773	178969014	2.76	5.07	71	NO
EPM-open	3	10,977,446	11,422,508	11421316	0.45	5.61	0	NO
EPM-open	3	38,981,096	45,775,379	38995330	6.79	6.23	22	NO
EPM-open	3	57,159,935	62,350,184	62350182	5.19	8.30	40	NO
EPM-open	3	105,097,575	112,000,682	111438974	6.90	5.03	87	NO
EPM-open	3	123,188,658	123,396,053	123276372	0.21	6.12	3	NO
EPM-open	3	145,929,813	150,609,181	148545481	4.68	4.51	22	NO
EPM-open	4	33,005,779	40,497,077	33095253	7.49	4.64	41	NO
EPM-open	4	57,671,803	62,857,853	57671910	5.19	4.73	62	NO
EPM-open	4	76,119,560	85,680,047	76447340	9.56	6.13	24	NO
EPM-open	4	115,165,660	121,522,914	117736252	6.36	5.49	149	NO
EPM-open	4	137,887,832	145,481,723	138501861	7.59	6.37	143	NO
EPM-open	4	155,544,804	156,238,419	155968187	0.69	4.55	48	NO
EPM-open	5	54,144,189	54,263,530	54234453	0.12	13.15	0	YES
EPM-open	5	108,100,558	110,251,292	108100635	2.15	10.25	47	NO
EPM-open	5	126,174,260	126,355,761	126192582	0.18	8.67	0	NO
EPM-open	5	144,223,539	144,987,290	144233030	0.76	4.98	11	YES
EPM-open	6	22,443,939	25,909,081	23092121	3.47	11.37	21	NO
EPM-open	6	88,721,203	98,441,406	92702629	9.72	5.54	84	NO
EPM-open	6	115,361,899	116,527,763	116527093	1.17	11.10	25	NO
EPM-open	6	140,493,700	148,611,826	148609800	8.12	5.19	74	NO
EPM-open	7	26,633,267	32,815,915	32807189	6.18	6.78	199	NO
EPM-open	7	92,714,690	100,590,709	99781176	7.88	4.81	78	NO
EPM-open	8	47,424,796	49,191,404	49189891	1.77	5.45	12	NO
EPM-open	8	117,219,229	117,238,740	117238740	0.02	7.91	0	NO
EPM-open	9	37,046,757	42,684,188	41699149	5.64	5.50	143	NO
EPM-open	9	59,269,502	60,576,659	60415574	1.31	5.04	18	NO
EPM-open	10	3,982,409	7,993,140	7460347	4.01	6.37	31	NO
EPM-open	10	62,407,026	68,117,393	66257850	5.71	4.97	44	NO
EPM-open	10	87,467,449	87,595,552	87595101	0.13	5.12	2	NO
EPM-open	10	99,702,519	100,643,112	100180799	0.94	9.25	10	NO
EPM-open	10	115,061,969	116,129,037	116101513	1.07	6.47	11	NO

EPM-open	10	127,597,093	128,207,380	128207149	0.61	7.15	26	NO
EPM-open	11	31,617,205	33,045,751	31650378	1.43	6.48	23	NO
EPM-open	11	73,152,512	78,258,400	74973330	5.11	4.75	140	NO
EPM-open	11	92,514,551	102,328,161	96517839	9.81	4.95	329	NO
EPM-open	12	10,903,674	12,325,756	12280611	1.42	10.29	12	NO
EPM-open	12	24,801,558	24,941,020	24940968	0.14	6.21	2	NO
EPM-open	12	72,397,728	80,142,472	79653619	7.75	4.76	71	NO
EPM-open	12	91,547,374	98,556,066	91724133	7.01	4.60	12	NO
EPM-open	12	108,589,687	116,579,257	113828296	7.99	9.45	176	NO
EPM-open	13	64,041,193	64,320,793	64314535	0.28	7.15	7	NO
EPM-open	13	96,546,053	106,493,266	96581488	9.95	5.45	83	NO
EPM-open	14	76,541,966	77,890,188	76807687	1.35	5.42	9	NO
EPM-open	14	120,012,537	122,241,245	121836360	2.23	4.76	18	NO
EPM-open	15	4,581,923	5,987,339	5910696	1.41	11.75	10	NO
EPM-open	15	58,665,949	59,903,946	59903856	1.24	11.96	13	NO
EPM-open	15	71,640,419	71,928,936	71799081	0.29	5.59	2	NO
EPM-open	15	95,805,247	96,231,856	96231261	0.43	8.20	6	NO
EPM-open	16	6,116,010	8,344,551	8160532	2.23	5.49	1	NO
EPM-open	16	41,370,396	41,397,117	41370402	0.03	9.48	1	YES
EPM-open	16	51,739,855	57,287,996	53176660	5.55	12.38	26	NO
EPM-open	17	17,525,875	18,122,485	18119717	0.60	10.79	12	NO
EPM-open	17	35,025,170	42,556,420	37885007	7.53	7.52	197	NO
EPM-open	17	71,861,284	80,410,865	73349005	8.55	5.49	59	NO
EPM-open	17	90,988,935	91,039,920	91039814	0.05	6.63	1	NO
EPM-open	18	19,237,701	19,467,138	19303086	0.23	6.69	0	NO
EPM-open	18	34,269,862	34,328,458	34323952	0.06	4.71	1	NO
EPM-open	18	45,056,349	54,799,081	52676655	9.74	5.11	52	NO
EPM-open	18	65,182,153	65,755,343	65182284	0.57	5.85	8	NO
EPM-open	18	75,473,013	76,395,866	76223188	0.92	8.47	6	NO
EPM-open	19	10,791,445	16,546,950	14581483	5.76	5.26	127	NO
EPM-open	19	46,663,249	47,371,106	46664076	0.71	11.33	16	NO
FC-context	2	125,868,001	129,363,717	128363526	3.50	5.13	61	NO
FC-context	2	131,054,562	132,419,076	131419005	1.36	5.46	24	NO
FC-context	4	54,968,565	58,974,284	55968578	4.01	6.56	28	NO
FC-context	4	63,558,947	65,303,376	64099832	1.74	6.14	9	NO
FC-context	4	70,154,809	78,177,088	73444653	8.02	7.39	25	YES
FC-context	4	78,766,185	85,257,786	84257660	6.49	8.23	21	YES
FC-context	5	111,026,691	113,811,974	112810193	2.79	5.43	39	NO
FC-context	10	92,603,234	94,876,655	93603389	2.27	6.03	31	NO
FC-context	10	102,470,556	105,577,476	105191184	3.11	5.99	12	NO
FC-context	11	113,211,188	113,375,870	113360130	0.16	5.54	1	NO
FC-context	13	79,653,177	84,780,850	80662886	5.13	7.07	17	NO
FC-context	13	88,050,130	92,556,190	89050599	4.51	5.97	13	NO

FC-context	15	3,062,020	6,754,143	5753680	3.69	6.34	26	NO
FC-context	16	69,443,083	73,061,157	72061114	3.62	5.67	9	NO
FC-cue	2	157,505,101	159,522,386	158521915	2.02	5.96	26	NO
FC-cue	7	81,429,834	101,030,310	89412852	19.60	12.88	186	NO
FC-cue	7	125,083,067	127,084,850	126084800	2.00	5.07	57	NO
FC-cue	9	106,693,528	108,694,995	107693870	2.00	5.89	77	NO
FC-cue	10	13,700,072	15,703,556	14700119	2.00	5.16	8	NO
FC-cue	11	3,370,877	9,544,314	8532018	6.17	5.5	100	NO
FC-cue	13	78,196,122	82,295,567	81923446	4.10	8.22	10	NO
FC-cue	13	82,924,324	89,583,537	88583892	6.66	7.06	14	NO
FC-cue	13	92,130,558	95,554,450	93126201	3.42	7.46	35	NO
FC-cue	13	116,386,705	118,387,377	117819025	2.00	7.57	7	YES
FC-cue	14	40,646,127	43,530,172	41646248	2.88	5.39	37	NO
FC-cue	14	61,757,581	64,255,785	62757647	2.50	5.31	40	NO
FC-cue	14	71,846,334	72,909,269	72846442	1.06	6.21	4	NO
FC-cue	16	26,422,169	28,281,084	27304476	1.86	6.71	10	NO
FC-cue	19	32,762,251	35,307,221	34269128	2.54	5.44	30	NO

Supplemental Table S3: Quantitative trait loci for fear-related behavior, related to Figure 1. The table shows the traits mapped (EPM-open: entries into the open arms of the elevated plus maze, FC-context: freezing to the context in a fear conditioning experiment; FC-cue: freezing to the cue in a fear conditioning experiment); *chr*: chromosome; *start*: start of empirically defined 95% confidence interval of the locus; *end*: end of the 95% confidence interval; *max pos*: position of the marker that gave the maximum negative logarithm (base 10) of the association P-value at the locus; *size* : length of the QTL; *logp*: value of the maximum negative logarithm (base 10) of the association P-value; *n. of genes*: number of genes in the interval; *selected*: whether or not this interval was chosen for testing by quantitative complementation

Gene	Description	Sequence 5'-3'	Product (bp)
Megf9	Megf9-M-F	GGTCAGGCTCTGAGGGTAGA	Mu: 206
	Megf9-M-R	GGAATCCGTTCCAATGAACA	(WT: 817)
	Megf9-W-F	CACAGGGCAGTGTGATTGTC	WT: 474
Emb	Emb W-F	CTCCCTCTGCTGGCTCTTTA	WT: 384
	Emb M-R	TCGAAACTGTCATCCAGTGC	Mu: 246
	Emb W-R	ATGGACATTTTTCCACGTC	
Hcn1	Hcn1-M-F	ATTTGGATCAAAGGCAAACA	Mu: 240
	Hcn1-M-R	CAGTTGGCTCAAATGATTCAGA	(WT: 1167)
	Hcn1-W-F	AAAGCTGGTTTGTGGTGGAC	WT: 377
Ptprd	Ptprd-M-F	GGGTTGGGCACTGAGAAGTA	Mu: 229
	Ptprd-M-R	R TTTTGACATCCATTTCCAAGG	(WT: 673)
	Ptprd-W-R	R TCTCCAAAAGTCCCCAAATG	WT: 475
Lsamp	Lsamp-M-F	GCCCTCTCAGAAATGTCACG	Mu: 176
	Lsamp-M-R	TCACCAGCACATGGAGTTTC	WT: 539
Nptx2	Nptx2-M-F	GAAGCCAGTTTCCTTTCT	Mu: 190
	Nptx2-M-R-2	GGAGGCCATCACAGTAAGGT	(WT: 976)
	Nptx2-W-R	GCTGACCTGTGTGCTCACTT	WT: 403
Stim2	Stim2-M-F	ATGGGATCCCTTCTCTCTGG	Mu: 233
	Stim2-M-R	CAACTTCACCTTCCGGCTAC	(Wt: 1486)
	Stim2-W-R	TGAGATGCCTCGGTTACCTC	Wt: 490
Mrps30	Mrps30 WT-F	TTCCATTGGTGGCTTAGAAAA	
	Mrps30 Mut-R	GCCATCATGAACAACATGG	Mu: 219
	Mrps30 WT-R	AGGAATGCGTTCTTCACCAC	WT: 442
Parp8	Parp8 WT-F	CACCGAATCTCCAAAATGG	
	Parp8 Mut-R	TTTACAATTTTCCAGGCTCCA	Mu: 246
	Parp8 WT-R	CGTGGTGATCCAGCTAACAG	WT: 396
Psip1	Psip1 WT-F	GGGAAGATGGGAGACAGACA	
	Psip1 Mut-R	CCAGTCCAGGTATGGCATT	Mu: 218
	Psip1 WT-R	TGTCACATCCCCTTAAATTTG	WT: 422
Sh3gl2	Sh3gl2 WT-F	TGGAGGAGAAACCTGTCACTG	
	Sh3gl2 Mut-R	ATGTGGCACCAAGCCTTAC	Mu: 250
	Sh3gl2 WT-R	CTTACCTGGATTGGGTTGGA	WT: 397
Snapc3	Snapc3 WT-F	GGGATGGCTCAGTGGTTAGA	Mu: 215
	Snapc3 Mut-R	GGAACAGGGTGGTAGACAGG	
Ttc39b	Ttc39b WT-F	GGTGAATGAAACGGAGCAGT	
	Ttc39b Mut-R	TTTGCTGGAGAGCATTCTTTT	Mu: 237
	Ttc 39b WT-R	GCCCTTTCTGTCCAGGAACT	WT: 392
4933413L06Rik	Rik-KI-F	GTCACAAGGGCACATGTTCCACT	Mu: 556
	Rik-KI-R	CCCCCACTGCTCACCAC	WT: 434

Supplemental Table S4: Genotyping primers for mouse knock outs, related to STAR Methods. The table gives the sequence of primers used to genotype the CRISPR-engineered mouse knockouts, providing the names of the target genes, forward and reverse primers, and the sizes of the amplification products (Mu: knockout ; WT: wildtype).

Target Gene	Description	Sequence 5'-3'	Tm	Product Size (bp)	Target Exons	Deleted Exon in knock out
Ptpd	Ptpd-23-24-25-F	GCACTTTATAAGAATGGATCGAG	57.15	198	23-24-25	#22
	Ptpd-23-24-25-R	AATGAAGCAGCCAGTTCTGC	60.55			
Megf9	Megf9-4-5-F	CGTGTTCGCGAGTGAAGTCT	61.45	105	4-5	#2
	Megf9-4-5-R	TATTGCAGTTCTGGCCTGTG	59.86			
Lsamp	Lsamp-3-4-F	ATGAGGGCAGCAATGTAACC	59	168	3-4	#2
	Lsamp-3-4-R	GGCAGCCTTGCACTCATATT	60			
Stim2	Stim2-9-10-F	TCCTCCCTGGACGAAGTAGA	59	226	9-10	#6-#7
	Stim2-9-10-R	CAGCAATAGGGTAGGGTGA	60			
Nptx2	Nptx2-2-3-F	CGGAGCTGGAAGATGAGAAG	60	229	2-3	#1
	Nptx2-2-3-R	AGGCAGATGGTAAAGGCGTA	59			
Hcn1	Hcn1-4-5-F	GCCACAGCTTTGATCCAGTC	60.81	159	4-5	#2
	Hcn1-4-5-R	ATCGAAGATCTTGCCTTGGT	58.74			
Psip1	Psip1-4-5-F	GCAAACCAAATAAACGGAAAGGT	58.63	196	4-5	#3
	Psip1-4-5-R	GTCACATCCTCATTGCTGGC	59.26			
Emb	Emb-6-7-F	GAATGAGCTGGTTGTGCTGA	59.99	211	6-7	#5
	Emb-6-7-R	GTACCGGGGGACATTGTTTT	60.84			
Parp8	Parp8-24-25 F	GACGAGCCAGCTTCCAGTAG	60.16	164	24-25	#20,21,22
	Parp8-24-25 R	TTCGGGACAACCCATATCTC	59.75			
Mrps30	Mrps 30-4-5 F	CCATTGTTTCAGACGGCAGTA	59.72	200	3-4	#2
	Mrps 30-4-5 R	GCTCCAGTCCAAGCAAAAAG	59.99			
Ttc39b	Ttc39b-3-4-F	TTGTACCATGCGAGACCAAA	60.11	124	3-4	#2
	Ttc39b-3-4-R	TTCAGTGCCTCTGCACACTC	60.19			
Sh3gl2	Sh3gl2-4-5-F	CAGAGGCCATGCTCAAGTTC	60.94	157	4-5	#3
	Sh3gl2-4-5-R	AGATTCTGAAGGGGGTCGAT	59.9			
Snapc3	Snapc3-4-5-F	CCTGAAAACCCAGCAGACAT	60.11	192	4-5	#3
	Snapc3-4-5-R	GAATTCTCCACCGATCTGGA	60.01			
4933413L06Rik	RIK(5)-4-F-1	CAGCCTGTTTCCCAGTCATC	60.6	192	4	#1
	RIK(5)-4-R-1	AATGCTCTTTGGTGGCATT	59.5			

Supplemental Table S5: qPCR primers used to determine expression of the CRISPR targeted genes, related to STAR Methods. The table gives the sequence of primers used in the quantitative PCR testing of the knock-out animals, providing the names of the target genes, forward and reverse primers, the melting temperature (Tm), the product size, the exons amplified and the exon that is deleted in the CRISPR engineered mouse line.

RNA	Hippocampus			Amygdala			
	Cell type	total	significant	pct	Cell type	total	significant
Exc-CA1-Camk2d	18015	587	3.258	Exc-Abi3bp	18785	473	2.518
Exc-CA1-Fras1	18152	1149	6.330	Exc-Ebf2	13090	111	0.848
Exc-CA1-Galnt16	19739	1993	10.097	Exc-Strip2	5714	7	0.123
Exc-CA3	19562	1511	7.724	Exc-Tafa1	19527	940	4.814
Exc-Cdh12	13249	155	1.170	Exc-Tfap2c	8494	19	0.224
Exc-DG	20271	2236	11.031	Exc-Vwa5b1	19592	767	3.915
Exc-ENT	18376	1123	6.111	Inh-Drd2	13046	35	0.268
Exc-Gpc6	15946	526	3.299	Inh-Htr3a	14074	126	0.895
Inh-CGE	16845	399	2.369	Inh-Lamp5	12786	74	0.579
Inh-Lamp5	14426	229	1.587	Inh-Lypd6b	12946	78	0.603
Inh-MGE	17820	365	2.048	Inh-Nxph2	19945	164	0.822
NN-Astro	18340	466	2.541	Inh-Sst	18321	340	1.856
NN-MGC	13338	164	1.230	Inh-Tshz1	17989	28	0.156
NN-ODC	15986	601	3.760	Inh-Zfhx3	18812	107	0.569
NN-OPC	15579	384	2.465	NN-Astro	19206	570	2.968
NN-VLMC	11062	63	0.570	NN-Endo_SMCPeri	5506	6	0.109
CR-CR	11082	45	0.406	NN-Endo_VLMC	4373	86	1.967
				NN-MGC	13709	188	1.371
				NN-ODC	14315	348	2.431
				NN-OPC	16216	364	2.245
				NN-VLMC	8814	18	0.204

ATAC	Hippocampus			Amygdala			
	Cell type	total	significant	pct	Cell type	total	significant
Exc-CA1-Camk2d	274444	1176	0.429	Exc-Abi3bp	244313	325	0.133
Exc-CA1-Fras1	268808	1242	0.462	Exc-Ebf2	197530	52	0.026
Exc-CA1-Galnt16	277994	3637	1.308	Exc-Strip2	143706	9	0.006
Exc-CA3	277897	3296	1.186	Exc-Tafa1	251656	2755	1.095
Exc-Cdh12	162588	32	0.020	Exc-Tfap2c	82406	8	0.010
Exc-DG	277754	4282	1.542	Exc-Vwa5b1	248416	918	0.370
Exc-ENT	261482	690	0.264	Inh-Drd2	141913	22	0.016
Exc-Gpc6	230447	888	0.385	Inh-Htr3a	121437	19	0.016
Inh-CGE	218934	95	0.043	Inh-Lamp5	111249	23	0.021
Inh-Lamp5	158758	40	0.025	Inh-Lypd6b	100583	8	0.008
Inh-MGE	254528	32	0.013	Inh-Nxph2	246554	424	0.172
NN-Astro	51716	3	0.006	Inh-Sst	206133	85	0.041
NN-MGC	55630	9	0.016	Inh-Tshz1	243662	393	0.161
NN-ODC	38625	2	0.005	Inh-Zfhx3	242906	474	0.195
NN-OPC	47529	3	0.006	NN-Astro	48911	3	0.006
				NN-MGC	46732	5	0.011
				NN-ODC	28016	2	0.007

Methylation	Hippocampus			Amygdala				
	Cell type	total	mutation	pct	Cell type	total	mutation	pct
	Exc-CA1-Camk2d	591460	1051	0.178	Exc-Abi3bp	822415	547	0.067
	Exc-CA1-Fras1	1012284	1737	0.172	Exc-Ebf2	2418	0	0.000
	Exc-CA1-Galnt16	6761056	26464	0.391	Exc-Strip2	2267	0	0.000
	Exc-CA3	3255884	9203	0.283	Exc-Tafa1	1.2E+07	46504	0.380
	Exc-Cdh12	7485	110	1.470	Exc-Tfap2c	280	3	1.071
	Exc-DG	14759665	81752	0.554	Exc-Vwa5b1	5264608	12172	0.231
	Exc-ENT	739955	1176	0.159	Inh-Drd2	3085	0	0.000
	Exc-Gpc6	200918	484	0.241	Inh-Htr3a	8419	1	0.012
	Inh-CGE	15833	40	0.253	Inh-Lamp5	4167	0	0.000
	Inh-Lamp5	12629	20	0.158	Inh-Lypd6b	5250	1	0.019
	Inh-MGE	754092	578	0.077	Inh-Nxph2	3860176	7128	0.185
	NN-Astro	5141	30	0.584	Inh-Sst	267290	205	0.077
	NN-EC	436	3	0.688	Inh-Tshz1	1021024	1466	0.144
	NN-MGC	93803	366	0.390	Inh-Zfhx3	1189382	1879	0.158
	NN-ODC	1620528	1073	0.066	NN-Astro	91417	104	0.114
	NN-OPC	29065	166	0.571	NN-MGC	17552	17	0.097
	NN-VLMC	954	8	0.839	NN-ODC	71576	96	0.134
					NN-OPC	16264	52	0.320
					NN-VLMC	1232	8	0.649

Supplemental Table S7: The number of RNA species and ATAC sites that differed significantly between C57BL/6J and DBA/2J, and the number of methylation sites containing mutations that removed the methylated site, related to Figure 6. Significant differences were determined by exceeding an adjusted p value below a 5% FDR cutoff value, the default from the output of DESeq2). Data for each cell type is shown below for the two brain regions (hippocampus and amygdala), with counts of the significant species, and percentages of the total.

Modality	Class	Hippocampus	Amygdala
RNA	Excitatory	6.47	2.72
	Inhibitory	2.02	0.74
ATAC	Excitatory	0.75	0.35
	Inhibitory	0.03	0.10
Methylation	Excitatory	0.45	0.32
	Inhibitory	0.08	0.17

Supplemental Table S8: The weighted means of significant strain differences, expressed as a percentage, for the excitatory and inhibitory classes of cell type in the hippocampus and amygdala, related to Figure 6. Results are means of the excitatory and inhibitory cell types from Supplemental Table S7, weighted by the number of observations, showing that there appear to be more excitatory than inhibitory variable species. All differences are highly significant by Fisher's exact test P-values $P < 2e-16$.

Modality	Tissue	Deviance	P-value
RNA	Hippocampus	1291.6	<2.2e-16
	Amygdala	1282.2	<2.2e-16
ATAC	Hippocampus	12895.3	<2.2e-16
	Amygdala	8771.2	<2.2e-16
Methyl	Hippocampus	28295.7	<2.2e-16
	Amygdala	517.86	<2.2e-16

Supplemental Table S9: Cell type contributes to the number of significant differences, related to Figure 6. The relative contribution of sequence coverage and cell type was assessed by comparing the fit of two models. In one model we used a single predictor, sequence coverage, for the number of significant species. In the second model we added cell type as a predictor. The improvement in fit was tested using ANOVA. Results are highly significant, as shown by the deviance and associated P-values in the table.

Modality	Tissue	Predictor	Estimate	z-value	P-value
RNA	Hippocampus	Sequence coverage	6.15E-05	30.80	<2e-16
		Class	-8.27E-01	-28.11	<2e-16
	Amygdala	Sequence coverage	6.72E-06	5.10	3.48E-07
		Class	-3.99E-01	-13.46	<2e-16
ATAC	Hippocampus	Sequence coverage	3.64E-04	30.91	<2e-16
		Class	-2.20E+00	-50.03	<2e-16
	Amygdala	Sequence coverage	1.65E-04	6.92	4.53E-12
		Class	-9.51E-01	-35.44	<2e-16
Methyl	Hippocampus	Sequence coverage	1.08E-01	102.61	<2e-16
		Class	-9.06E-01	-31.96	<2e-16
	Amygdala	Sequence coverage	8.39E-02	56.54	<2e-16
		Class	-6.29E-01	-61.35	<2e-16

Supplemental Table S10: Results from a generalized linear model (the glm function in R), testing whether sequence coverage and class (“Inhibitory” or “Excitatory”) predict whether RNA or ATAC sites were significant, and whether methylation sites were mutant or not (in each case coding outcomes as 1 or 0), related to Figure 6. The effect of class is relative to “Excitatory” and the direction for each modality is the same (negative, meaning that prediction is positive for “Excitatory”), and highly significant. Similar results were obtained using negative binomial distribution. P-values were well calibrated, as shown by creating an empirical distribution by randomly selecting the same number of transcripts as in the observed data for each cell type from the total set of transcripts in all cell types, while ensuring that a gene was selected only once for each cell type. Analysing the empirical data in the same way as the real data, using the generalized linear model to test whether sequence coverage and class (“Inhibitory” or “Excitatory”) predicted the strain differences, confirmed that the P-values we obtained for the real data were significantly different from the empirical null distribution ($P < 0.0001$ in all cases).

Modality	Tissue	Level	Predictor	Estimate	Est. Error	Lower 95% CI	Upper 95% CI
RNA	HIPPOCAMPUS	Group	Class	0.68	0.56	0.02	2.09
			Class:celltype	0.52	0.15	0.32	0.89
		Population	Coverage	0.50	0.01	0.49	0.51
RNA	AMYGDALA	Group	Class	0.85	0.79	0.02	2.85
			Class:celltype	1.10	0.25	0.73	1.68
		Population	Coverage	0.38	0.01	0.36	0.39
ATAC	HIPPOCAMPUS	Group	Class	2.54	1.31	0.73	5.81
			Class:celltype	1.26	0.41	0.69	2.27
		Population	Coverage	0.60	0.02	0.56	0.63
ATAC	AMYGDALA	Group	Class	1.33	1.45	0.04	5.19
			Class:celltype	0.42	0.32	0.02	1.23
		Population	Coverage	0.70	0.14	0.44	0.97
Methylation	HIPPOCAMPUS	Group	Class	1.37	1.37	0.04	4.87
			Class:celltype	0.95	0.47	0.36	2.13
		Population	Coverage	2.12	0.31	1.52	2.74
Methylation	AMYGDALA	Group	Class	0.86	0.79	0.04	3.07
			Class:celltype	0.49	0.33	0.13	1.39
		Population	Coverage	2.45	0.44	1.62	3.30

Supplemental table S11: Contribution of cell type to the number of significant differences between excitatory and inhibitory neurons, related to Figure 6. The table shows the results of testing whether the number of strain differences are significantly different between the two cell type classes (excitatory and inhibitory) while considering the coverage difference and accounting for the different numbers of cell types in each class. Results are from a Bayesian model using the *brms* package¹⁹ with a negative binomial distribution for the number of significant differences (for RNA and ATAC data) or the number of mutations (for methylation data). The model includes predictors for sequence *Coverage* and *Class* of cell type (italicized words refer to the terms in the table). Results are given for the two tissues (hippocampus and amygdala) and three epigenetic modalities (RNA, methylation and ATAC), reporting standard deviations for group-level effects (class and cell type nested within class) and estimates for population-level effects (sequence coverage). The two-sided 95% credible intervals (lower and upper 95% confidence interval (CI) based on quantiles are given. Results are from eight chains, each of 2,000 iterations of which the first 1,000 are warmup to calibrate the NUTS sampler. To reduce divergent transitions, *adapt_delta* was set to 0.95. Chain convergence was 1, as estimated from the potential scale reduction factor on split chains ($R_{hat} = 1$). Results show that the estimate for class for each tissue and each modality has confidence intervals that do not overlap zero and therefore can be regarded as significant.

Strain	Cell type	Total	Mutant sites	Percentage
A/J	Excitatory	13,085,362	1,285,640	9.83
	Inhibitory	2,700,028	53,309	1.97
	Non-neuronal	1,435,768	23,415	1.63
C57BL/6J	Excitatory	12,639,259	758,120	6
	Inhibitory	944,284	14,485	1.53
	Non-neuronal	989,433	16,513	1.67
BALB/cJ	Excitatory	12,388,844	1,213,199	9.79
	Inhibitory	2,811,830	59,446	2.11
	Non-neuronal	1,807,105	29,817	1.65
DBA/2J	Excitatory	12,424,446	1,013,242	8.16
	Inhibitory	1,232,573	20,534	1.67
	Non-neuronal	2,267,190	37,280	1.64
FVB/J	Excitatory	12,494,856	682,230	5.46
	Inhibitory	349,790	8,262	2.36
	Non-neuronal	979,704	16,955	1.73
PWK/PhJ	Excitatory	13,766,132	985,463	7.16
	Inhibitory	983,129	15,771	1.6
	Non-neuronal	270,084	9,609	3.56
WSB/EiJ	Excitatory	12,046,029	1,257,213	10.44
	Inhibitory	2,654,718	58,372	2.2
	Non-neuronal	2,382,166	41,153	1.73

Supplemental Table S12: Proportion of variant methylation sites in multiple strain comparison, related to Figure 6. Results from seven strain comparisons are shown (column 1), each time with CAST/EiJ as the outgroup, for excitatory neurons, inhibitory neurons and non-neuronal cell types. The total number of methylated sites is given for each strain comparison ('Total') in each cell type. The column headed 'mutant sites' lists the number of sites fully methylated in one strain but ablated in the other due to the presence of a mutation. The column headed 'pct' gives the percentage of mutant sites.

REFERENCES

1. O'Leary, T.P., Gunn, R.K., and Brown, R.E. (2013). What are we measuring when we test strain differences in anxiety in mice? *Behav Genet* *43*, 34-50.
2. Cook, M.N., Williams, R.W., and Flaherty, L. (2001). Anxiety-related behaviors in the elevated zero-maze are affected by genetic factors and retinal degeneration. *Behav Neurosci* *115*, 468-476.
3. Milner, L.C., and Crabbe, J.C. (2008). Three murine anxiety models: results from multiple inbred strain comparisons. *Genes Brain Behav* *7*, 496-505.
4. Lad, H.V., Liu, L., Paya-Cano, J.L., Parsons, M.J., Kember, R., Fernandes, C., and Schalkwyk, L.C. (2010). Behavioural battery testing: evaluation and behavioural outcomes in 8 inbred mouse strains. *Physiol Behav* *99*, 301-316.
5. Keum, S., Park, J., Kim, A., Park, J., Kim, K.K., Jeong, J., and Shin, H.S. (2016). Variability in empathic fear response among 11 inbred strains of mice. *Genes Brain Behav* *15*, 231-242.
6. Wahlsten, D., Metten, P., and Crabbe, J.C. (2003). Survey of 21 inbred mouse strains in two laboratories reveals that BTBR T/+ tf/tf has severely reduced hippocampal commissure and absent corpus callosum. *Brain Res* *971*, 47-54.
7. Wahlsten, D., Bachmanov, A., Finn, D.A., and Crabbe, J.C. (2006). Stability of inbred mouse strain differences in behavior and brain size between laboratories and across decades. *Proc Natl Acad Sci U S A* *103*, 16364-16369.
8. Brigman, J.L., Mathur, P., Lu, L., Williams, R.W., and Holmes, A. (2009). Genetic relationship between anxiety-related and fear-related behaviors in BXD recombinant inbred mice. *Behav Pharmacol* *20*, 204-209.
9. Carhuatanta, K.A., Shea, C.J., Herman, J.P., and Jankord, R. (2014). Unique genetic loci identified for emotional behavior in control and chronic stress conditions. *Front Behav Neurosci* *8*, 341.
10. Philip, V.M., Duvvuru, S., Gomero, B., Ansah, T.A., Blaha, C.D., Cook, M.N., Hamre, K.M., Lariviere, W.R., Matthews, D.B., Mittleman, G., *et al.* (2010). High-throughput behavioral phenotyping in the expanded panel of BXD recombinant inbred strains. *Genes Brain Behav* *9*, 129-159.
11. Yang, R.J., Mozhui, K., Karlsson, R.M., Cameron, H.A., Williams, R.W., and Holmes, A. (2008). Variation in mouse basolateral amygdala volume is associated with differences in stress reactivity and fear learning. *Neuropsychopharmacology* *33*, 2595-2604.
12. Owen, E.H., Christensen, S.C., Paylor, R., and Wehner, J.M. (1997). Identification of quantitative trait loci involved in contextual and auditory-cued fear conditioning in BXD recombinant inbred strains. *Behavioral Neuroscience* *111*, 292-300.
13. Bolivar, V.J., Pooler, O., and Flaherty, L. (2001). Inbred strain variation in contextual and cued fear conditioning behavior. *Mamm Genome* *12*, 651-656.
14. Bothe, G.W.M., Bolivar, V., Vedder, M.J., and Geistfeld, J.G. (2005). Behavioral differences among fourteen inbred mouse strains commonly used as disease models. *Comparative Medicine* *55*, 326-334.
15. Knoll, A.T., Halladay, L.R., Holmes, A.J., and Levitt, P. (2016). Quantitative Trait Loci and a Novel Genetic Candidate for Fear Learning. *J Neurosci* *36*, 6258-6268.
16. Ponder, C.A., Munoz, M., Gilliam, T.C., and Palmer, A.A. (2007). Genetic architecture of fear conditioning in chromosome substitution strains: relationship to measures of innate (unlearned) anxiety-like behavior. *Mamm Genome* *18*, 221-228.

17. Sittig, L.J., Carbonetto, P., Engel, K.A., Krauss, K.S., Barrios-Camacho, C.M., and Palmer, A.A. (2016). Genetic Background Limits Generalizability of Genotype-Phenotype Relationships. *Neuron* 91, 1253-1259.
18. Park, C.C., Gale, G.D., de Jong, S., Ghazalpour, A., Bennett, B.J., Farber, C.R., Langfelder, P., Lin, A., Khan, A.H., Eskin, E., *et al.* (2011). Gene networks associated with conditional fear in mice identified using a systems genetics approach. *BMC Syst Biol* 5, 43.
19. Bürkner, P.C. (2017). brms: An R Package for Bayesian Multilevel Models Using Stan. *Journal of Statistical Software* 80, 1-28.