Supplementary Methods

Strains and reporter transgenes

N2 Bristol wild-type [1] and CB4856 Hawaiian wild-type isolates [2] were used. The transgenic animals used were *vtIs1 [dat-1::gfp]* [3] in the manual screen and *vtIs1* plus *vsIs33 [dop-3::rfp]* [4] for the worm sorter screen.

Screening process

In all screens, animals were mutagenized with EMS according to standard protocols [1]. Worms were kept at 25˚C at all times. In the manual screen 5 parental (P0) mutagenized animals were placed in each of 10 founder P_0 plates. Three days later, 400 F_1 progeny of the mutagenized P_0 animals were singled. Their ensuing progeny (F_2 and F_3 generation) were screened under a stereomicroscope equipped with a fluorescent light source.

In the worm sorter screen, 5 large (150 mm) founder P0 plates were each seeded with 5 to 10 mutagenized P_0 worms. After 6 days the F_2/F_3 generation was directly screened by rinsing the 5 P_0 plates and passing half of the worm population through the COPAS biosorter.

The sorting process for 5 P_0 plates lasted on average 5 hours. The work involved mostly waiting time during sample sorting and short washing steps in between plates. The reported total time of 25 days for performing the worm sorter screen includes all stages of the procedure, i.e., mutagenesis, worm handling, actual sorting, manually inspecting the sorted animals and lastly mutant handling (confirm the heritability of the phenotype, filing and freezing). The worm sorter screened all larval and adult stages.

In total we screened in the worm sorter screen 110 plates that translate to over 2,000,000 worms. Mutants were recovered in 21 of the plates. The mutants presented here are all independent isolates, i.e. mutants coming from different plates or mutants coming from the same plate showing different phenotype. When multiple mutants of the same phenotype were retrieved from the same P0 plate they were considered as multiple isolates of the first isolated mutant and

are not reported in this paper. Note that this may lead to a slight underestimation of the number of mutants retrieved from the screen. The number of genomes screened with the worm sorter was estimated based on the initial number of P_0 worms used (5 to 10) and the average number of F_1 progeny our strain produced after mutagenesis (about 50). Therefore we estimated that we screened 500 to 1000 haploid genomes per P_0 plate depending on whether the initial number of P0 worms was 5 or 10 respectively.

To calculate the false-positive and false-negative rates of recovering non-*gfp* animals from a *vtIs1* population (presented in **Supplementary Table1**) we mixed 50 N2 individuals with 10,000 *vtIs1* worms. For comparison we made the same experiment using a transgene that carries *vsIs33* in the background. The results are summarized in **Supplementary Table 1**.

In our experimental setup we would get on average 60 false positives from each plate of 25,000 F_2s sorted. It would take us about 5 minutes to go through this number of worms under the dissecting scope and this would add a total of 30 minutes in the daily sorting routine; this number is included in the overall time effort calculation shown in **Table 2**.

Worm sorter settings

The sorting process was performed according to the manufacturer's instructions and (Union Biometrica). The worms were gated to exclude eggs, small L1s and debris using as gating parameters Time of Flight vs. Extinction. Then they were sorted based on fluorescence parameters, in particular Red Peak Height vs. Green Peak Height. We set the parameters for the fluorescence signal amplification as follows:

Full scale: Tof 2048, Ext 2048, RedPH 65536, GreenPH 65536

Gains (Signal): Ext 3, Green 4, Red 4 Trigger: Ext

Thresholds: Singal 30, TOF Minimum: 10

PMT Control: Green 750, Red 750

For the test runs in which we determined the efficiency of sorting non-*gfp* animals when *rfp* transgene (*vsIs33*) was present or not present in the

background (**Supplementary Table 1**), we used as sorting parameters Tof vs. GreenPH.

Mapping and allele identification

We first tested mutants for linkage to the X chromosome by crossing them with wild-type males and examining a potential mutant phenotype in hemizygous, male F_1 cross-progeny. If a phenotype was observed in the F_1 male progeny, it was tested whether this is due to dominance rather than to X-linkage.

For placing the mutants into complementation groups, we first grouped them into phenotypic categories. Then we performed complementation tests among mutants of the same phenotypic category. Subsequently we mapped at least one -and in most cases more than one- mutant per complementation group, using a rapid SNP mapping procedure [5]. Last, we complemented mutants of different phenotypic categories that mapped in the same region.

The two retrieved mutants that showed no *gfp* expression (*ot344* and *ot373*) are likely array mutations based on the following analysis: mutant animals were crossed with N2 males and the cross progeny showed no recovery of the *gfp* expression indicating either a mutation on the array or a dominant mutation. Then mutants were crossed with wild-type *vtIs1* males. The cross progeny were not "off" for *gfp* as we would expect in the case of a dominant mutation but showed normal fluorescence levels.

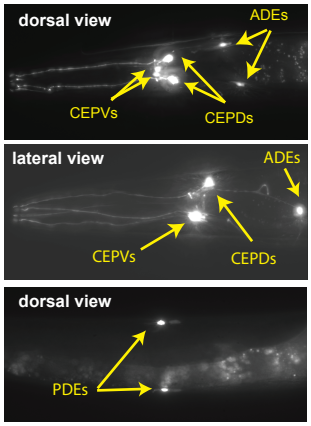
References

1. Brenner, S. The genetics of Caenorhabditis elegans. *Genetics* **77**, 71-94 (1974).
2. Hodgkin, J. & Doniach, T. Natural variation and copulatory plug formation in Caenorhabditis elegans. *Genetics* **146**, 149-64 (1997).
3. Nass, R. et al. A genetic screen in Caenorhabditis elegans for dopamine neuron insensitivity to 6-hydroxydopamine identifies dopamine transporter mutants impacting transporter biosynthesis and trafficking. *J Neurochem* **94**, 774-85 (2005).

4. Chase, D.L., Pepper, J.S. & Koelle, M.R. Mechanism of extrasynaptic dopamine signaling in Caenorhabditis elegans. *Nat Neurosci* **7**, 1096-103 (2004).
5. Davis, M.W. et al. Rapid single nucleotide polymorphism mapping in C. elegans. *BMC Genomics* **6**, 118 (2005).

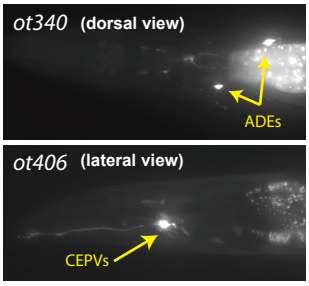
Supplementary Fig.1: Phenotypes of isolated dopy mutants.
Micrographs of representative animals and quantification of aberrant *dat-1::gfp* expression patterns are shown.

a *wild type*



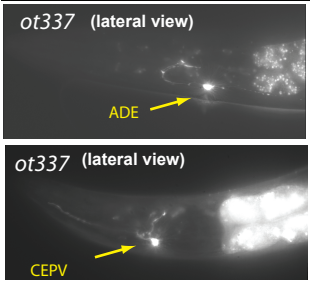
b *dopy-2*

% animals with *gfp* expression in:	fewer CEPVs	fewer CEPDs	extra CEPs	fewer ADEs	any phenotype	n
ot340	100	100	0	0	100	50
ot345	0	20	20	3	40	85
ot406	0	57	0	71	93	56



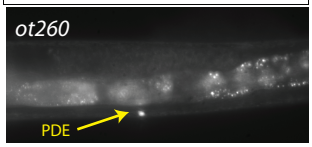
c *dopy-3*

% animals lacking *gfp* expression in:	CEPVs	CEPDs	ADEs	n
ot337	80	100	68	90



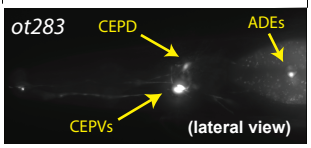
d *dopy-4*

% animals with *gfp* expression in:	fewer PDEs	more PDEs	n
ot260	28	15	40



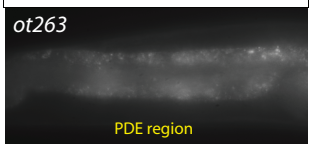
e *dopy-5*

% animals with *gfp* expression in fewer CEPDs:	n
ot283	58	12
ot284	20	40
ot296	43	28
ot298	39	26



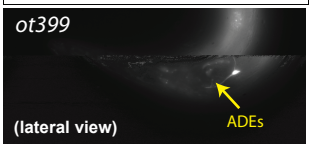
f *dopy-6*

% animals with *gfp* expression in:	fewer PDEs	more PDEs	n
ot263	49	3	158

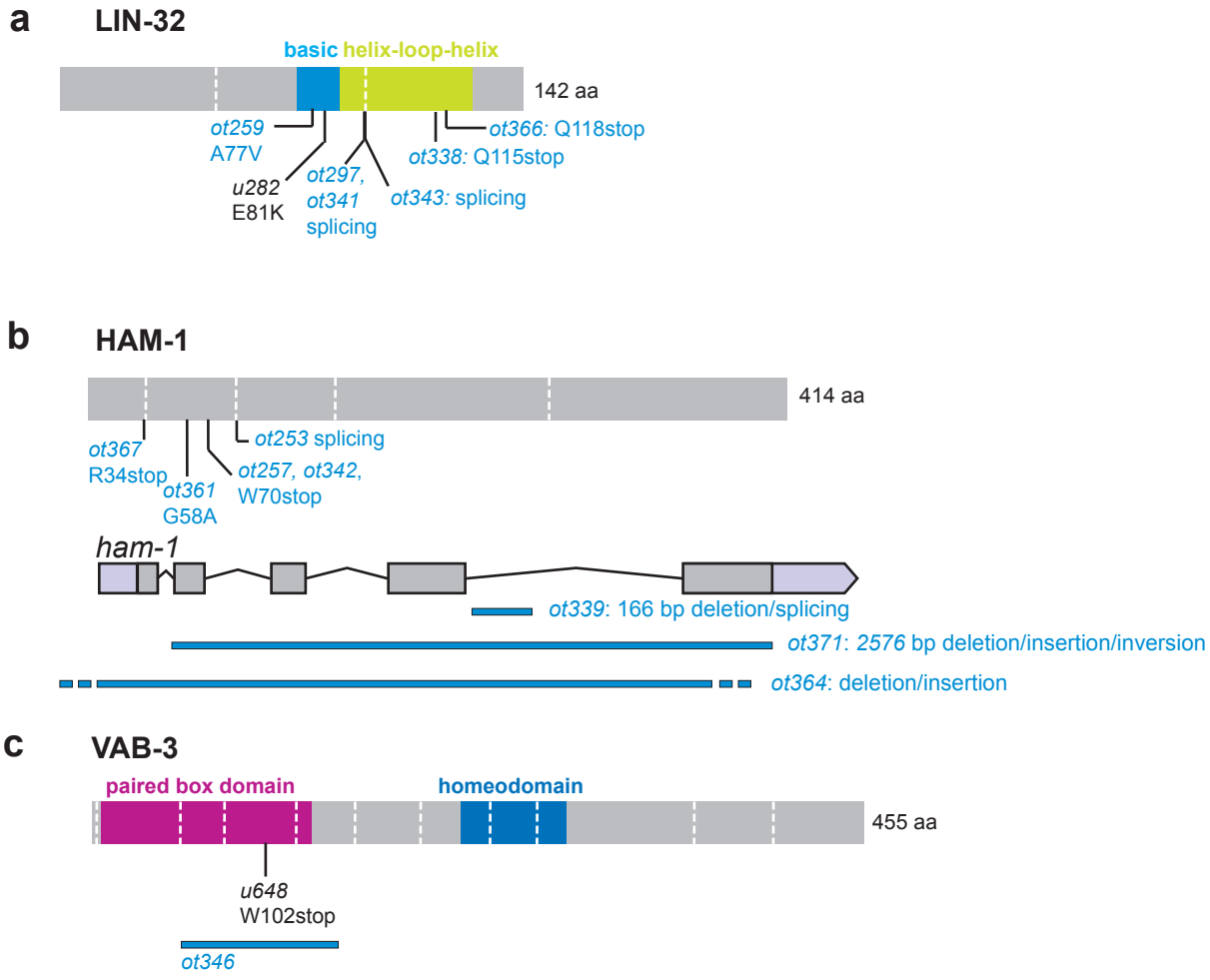


g *dopy-7*

% animals with *gfp* expression in:	no CEPs	n	no PDEs	n
ot399	77	52	61	26
ot347	25	64	38	32



Supplementary Figure 2 Molecular identity of mutant alleles of known genes retrieved from screens. Names of alleles isolated from our screens are in blue, previously described reference alleles are in black. Lines indicate deletions. For quantification see Supplementary Tables 1 to 3.



Supplementary Table 1: False positive/negative rates

Efficiency of sorting non-*gfp* (non-*vtIs1*) animals						
Input		**Recovery**			**Rates**	
# of *vtIs1* animals	# of N2 animals	# worms sorted	N2 recovered	False positive worms	% false negative (N2 *not* recovered)	% false positive
10,000	50	63	38	25	24% (12/50)	40% (25/63)
Efficiency of sorting non-*gfp* (non-*vtIs1*) animals with *rfp* transgene (*vsIs33*) in the background						
Input		**Recovery**			**Rates**	
# *vtIs1;vsIs33* animals	# *vsIs33* animals	# worms sorted	*vsIs33* recovered	False positive worms	% false negative (*vsIs33 not* recovered)	% false positive
10,000	50	71	50	21	0% (0/50)	30% (21/71)

Supplementary Table 2: Quantification of defects in *lin-32* mutants.

	% animals with *dat-1::gfp* expression:						
	missing in CEPVs	***missing in CEPDs***	***missing in ADEs***	***missing in PDEs***	***in extra cells near ADEs***	***% animals showing any phenotype***	**n**
wild type	0	0	0	0	0	0	100
ot259	0	61	0	77	7	95	55
ot297	0	94	17	100	74	100	42
ot341	0	84	1	99	93	100	38
ot343	0	71	4	100	14	100	38
ot338	0	100	7	100	60	100	30
ot366	0	100	20	100	76	100	42
tm2044	1	81	31	100	33	100	80
tm1446	0	100	8	100	95	100	42
u282	49	100	40	100	2	100	34

lin-32(u282) was used as reference allele. The *tm* alleles are putative null alleles (deleting parts of the bHLH domain; www.wormbase.org) and were provided by the *C. elegans* knockout consortium in Japan.

Supplementary Table 3: Quantification of defects in *vab-3* mutant.

	% animals with *dat-1::gfp* expression:						
	in extra cells	***in 1 extra cells***	***in 2 extra cells***	***in 3 extra cells***	***in 4 extra cells***	***missing in 1 CEPD***	**n**
wild type	0	0	0	0	0	0	0
ot346	30	22	8	0	0	21	52
u648	88	40	21	17	4	0	48

Supplementary Table 4: Quantification of defects in *ham-1* mutants.

	% animals with *dat-1::gfp* expression:							
	missing in 1 or 2 CEPDs	***in extra cells near CEPDs***	***missing in 1 or 2 ADEs***	***in extra cells near ADEs***	***missing in 1 or2 PDEs***	***in extra cells near PDEs***	***any phenotype***	***n***
wild type	0	0	0	0	0	0	0	100
ot253	23	13	0	95	0	15	98	40
ot257	40	14	0	80	0	0	94	50
ot342	47	17	0	83	0	23	90	30
ot361	17	0	3	90	0	30	93	30
ot367	40	0	3	90	0	20	100	30
ot339	47	20	3	90	0	13	97	30
ot371	40	10	3	73	27	6	100	30
ot364	45	13	0	80	0	26	94	31

Supplementary Table 5: Calculating the degree of saturation of the worm sorter screen.

Model	Maximum likelihood	ΔAICc	Weight	% saturation	95% credible interval [lower - upper]
Poison	1.46×10^{-5}	0	.817	93.3%	77.9%-98.6%
Gamma	1.84×10^{-5}	4.53	.085	84.1%	51.4%-97.7%
2 Class	2.11×10^{-5}	4.27	.097	80.7%	42.1%-99.7%
3 Class	2.29×10^{-5}	14.10	.000	76.8%	72.5%-99.9%
4 Class	2.22×10^{-5}	14.10	.000	73.8%	85.1%-99.9%
2 Class Variable	2.21×10^{-5}	14.17	.000	79.8%	65.1%-99.6%

We calculated the degree of saturation based on the allele frequencies for the worm sorter isolated mutants *dopy-3 (*3 alleles), *dopy-3* (1 allele), *dopy-7* (2 alleles), *lin-32* (4 alleles), *ham-1* (6 alleles) and *vab-3* (1 allele). Summary of Maximum Likelihood, AICc (Aikaike Information Criterion) values and weights are shown. Poisson, multiple rates, and gamma distribution-based predictions for this mutagenesis were obtained as described (Pollock and Larkin, 2004). AICc, a measure of the quality of fit to an estimated statistical model, accounts for smaller data sets and therefore was used in this study to ascertain best fit to the given models (Akaike, 1978). ΔAIC represents the difference between the AIC for each model and the lowest AIC. The weight of evidence in favor of each model being the best fit is given as 'weight.' The "95% credible interval" indicates the upper and lower bounds of the saturation estimates and were determined by removing the highest and lower 2.5% of saturation values computed by SatMut. The Poisson model assumes an equal rate of mutability. The 2-, 3- and 4-class models are multiple rate models that assume two to four mutation rate classes which occur at equal frequencies, i.e., an equal number of genes that are highly mutable as are lowly mutable. The 2-class variable frequency models are multiple rate models which assume two to four mutation rate classes that occur at two frequencies throughout the genome, i.e., a few genes highly mutable and many lowly mutable. The Poisson model (shaded in grey) best fits this data.

Supplementary Table 6: Mutants retrieved from genetic screens

Gene name	Molecular identity	Manual screen	Sorter screen	Allele names	Affected cells	Dom/ Rec	Visible pleiotropies	Location
dopy-2	unknown	0 alleles	3 alleles	*ot340, ot345 ot406*	CEPs CEPDs, ADEs	R	-	III:–7 to –1
dopy-3	unknown	0 alleles	1 allele	*ot337*	CEPs, ADEs	D	-	I:+14 to +26
dopy-4	unknown	1 allele *	0 alleles	*ot260*	PDEs	R	-	I: left of –6
dopy-5	unknown	4 alleles **	0 alleles	*ot283, ot284, ot296, ot298*	CEPDs	R	sterile	III:–7 to –1
dopy-6	unknown	1 allele *	0 alleles	*ot263*	PDEs	R	-	X:–17 to –8
dopy-7	unknown	0 alleles	2 alleles	*ot399, ot347*	CEPs, PDEs	R	sick	X: left of –4
lin-32	bHLH	2 alleles	4 alleles	*ot259, ot297, ot341, ot343, ot338, ot366*	CEPDs, ADEs, PDEs	R	-	X:–16.01
ham-1	no homologies	2 alleles	6 alleles	*ot253, ot257, ot342, ot361, ot367, ot339, ot371, ot364*	CEPDs, ADEs, PDEs	R	-	IV:+6.02
vab-3	paired + homeodomain	0 alleles	1 allele	*ot346*	unknown	R	notched head	X:+2.22
Total allele number		10	17					
Genomes screened		11,000	80,000					
Allele frequency per genomes screen		1/1,100	1/4,700					
Time investment		100 days §	25 days §					
Allele frequency per time		1 allele / 10 days	1 allele / 1.5 days					

This is a more extensive version of Table 2, shown in the main body of the paper. Dom/Rec indicates “dominant/recessive”. § These days are differentially spent. 100 days dissecting scope work mean full time work at the microscope while 25 days of worm sorting involves mainly machine running and casual observation of functioning of sorter.