SUPPLEMENTAL EXPERIMENTAL PROCEDURES

PCR of porin Locus- Excisions of P element $porin^{EY2333}$ were analyzed by PCR using the oligonucleotides GGCCAAGCAGCGTCCCAGCACGGCGTC (primer 1 in Fig. S1A) and CTCCAGGGACAGCTTCAGAC (primer 2 in Fig. S1A) (whose sequences flank the P element insertion site) to generate a 2.7 kb wild type genomic PCR product. PCR products that appeared shorter than wild type on agarose gel electrophoresis (Fig. S1B) were isolated and directly sequenced to determine deletion breakpoints. Two excisions that gave a normally sized PCR product ($porin^{Ex341}$ and $porin^{ExS27}$) were also sequenced and confirmed as precise excisions for use as controls. For the imprecise excision allele, $porin^{Rev8}$, no PCR product was observed using primers 1+2 on genomic DNA isolated from $porin^{Rev8}$ homozygotes under regular PCR conditions (data not shown). However, when long-range PCR was performed using TaKaRa LA Taq (Takara Bio USA) and the oligoncleotides GGCCAAGCAGCGTCCCAGCACGGCGTC (primer 1 in Fig. S1A) and TCGCGTGGCTGGGGCAGGGAATGGGC (primer 3 in Fig. S1A), an approximately 7 kb insertion was detected (Fig. S1C).

Tissue Western Blot- 3-day-old male adult flies of each genotype were dissected into three parts: head, reproductive tract, and remaining body (i.e., thorax and remaining abdomen). Whole cell lysates were used for western blot analysis. Generation of the fly porin antibody has been described previously (1). Actin antisera was obtained from Santa Cruz Biotechnology, Inc.

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. Identification of *porin* Imprecise Excision Deletion Alleles by PCR. *A*. Genomic organization of *porin* locus on Chromosome 2L from is depicted. Black rectangles indicate exonic coding sequences and white rectangles indicate exonic untranslated regions. The gray rectangle represents the next upstream gene, *CG17085*. Genomic coordinates indicated are from Ensembl *Drosophila melanogaster* version 50.54a (BDGP 5.4) (the reverse strand relative to the Ensembl sequence is depicted) (2). The orientation of the arrows next to the gene names indicates the relative orientation of the coding sequences. The numbered arrows indicate the relative position of the oligonucleotides used for PCR as described in Supplemental Experimental Procedures. The breakpoints of three *porin* deletion alleles ($porin^{Ex75}$, $porin^{Ex78}$, $porin^{Ex365}$) derived from imprecise P element excision are indicated. The $porin^{Ex75}$ allele is recessive lethal (no F_1 $porin^{Ex75}$ homozygotes observed from $porin^{Ex75}$ heterozygote intercross, total F_1 progeny = 300). *B*. Agarose gel electrophoresis of PCR products from the *porin* locus of *porin* mutants demonstrates deletions. The $porin^{Ex341}$ allele is a precise excision control. *C*. Agarose gel electrophoresis of long-range PCR products from the *porin* locus demonstrates ~7 kb insertion in $porin^{Rev8}$. WT = *yw*; M1 = 1 kb DNA ladder; M2 = λ Hind III.

Fig. S2. *porin* Mutants Are Severe Hypomorphs. Western blot analyses of adult tissue lysates from *porin* controls (*yw*, $porin^{Ex341}$, $porin^{ExS27}$) and mutants ($porin^{Ex78}$, $porin^{Ex365}$, $porin^{Rev8}$) are shown. Blots were serially probed with *porin* and actin antisera. When blots are overexposed, all mutant samples show trace amounts of porin (data not shown).

Fig. S3. porin Mutants Exhibit Increased Bang Sensitivity and Male Infertility/Subfertility. *A*. Bang sensitivity assay. The mean recovery time in seconds for each of the indicated genotypes is depicted. 341 = $porin^{Ex341}$ homozygotes; S27 = $porin^{ExS27}$ homozygotes; 78 = $porin^{Ex78}$ homozygotes; 365 = $porin^{Ex365}$ homozygotes; Rev8 = $porin^{Rev8}$ homozygotes. The error bars represent standard error of the mean (SEM). Statistical significance was evaluated by the Student's t Test with Welch correction. *$p < 0.001$. Sample size for each genotype is: *yw* (n = 21); $porin^{Ex341}$ homozygotes (n = 20); $porin^{ExS27}$ homozygotes (n = 20); $porin^{Ex78}$ homozygotes (n = 23); $porin^{Ex365}$ homozygotes (n = 28); and $porin^{Rev8}$ homozygotes (n = 22). *B*. Male fertility assay. The mean number of progeny per male is indicated. The genotypes are the same as

in *A*. The error bars represent standard error of the mean (SEM). Statistical significance was evaluated by the Student's t Test with Welch correction. *$p < 0.05$; **$p < 5x10^{-12}$. Sample size for each genotype is: *yw* (n = 20); $porin^{Ex341}$ homozygotes (n = 10); $porin^{ExS27}$ homozygotes (n = 10); $porin^{Ex78}$ homozygotes (n = 10); $porin^{Ex365}$ homozygotes (n = 10); and $porin^{Rev8}$ homozygotes (n = 10).

SUPPLEMENTAL REFERENCES

1. Graham, B. H., and Craigen, W. J. (2005) *Mol Genet Metab* **85**(4), 308-317

2. Hubbard, T. J., Aken, B. L., Ayling, S., Ballester, B., Beal, K., Bragin, E., Brent, S., Chen, Y., Clapham, P., Clarke, L., Coates, G., Fairley, S., Fitzgerald, S., Fernandez-Banet, J., Gordon, L., Graf, S., Haider, S., Hammond, M., Holland, R., Howe, K., Jenkinson, A., Johnson, N., Kahari, A., Keefe, D., Keenan, S., Kinsella, R., Kokocinski, F., Kulesha, E., Lawson, D., Longden, I., Megy, K., Meidl, P., Overduin, B., Parker, A., Pritchard, B., Rios, D., Schuster, M., Slater, G., Smedley, D., Spooner, W., Spudich, G., Trevanion, S., Vilella, A., Vogel, J., White, S., Wilder, S., Zadissa, A., Birney, E., Cunningham, F., Curwen, V., Durbin, R., Fernandez-Suarez, X. M., Herrero, J., Kasprzyk, A., Proctor, G., Smith, J., Searle, S., and Flicek, P. (2009) *Nucleic Acids Res* **37**(Database issue), D690-697

Table S1. Respiration Rates of Isolated Mitochondria from Wild Type and *porin* Mutant Flies

	Glutamate+Malate[a]									Succinate[a]								
	III[b]			IV[c]			UC[d]			III			IV			UC		
Genotype	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**
yw	181.3	54.7	7	100.2	27.3	7	112.6	41.8	7	299.3	73.9	8	158.7	43.8	8	185.0	58.4	8
***porin*^{Rev8}**¶	94.0	38.4	8	77.8	20.5	8	66.7	33.8	8	209.8	73.2	9	149.6	59.5	9	156.1	84.8	9

[a]Respiration rates expressed as ng atomic O/min/mg mitochondrial protein
[b]III = State III (ADP-stimulated) respiration
[c]IV = State IV (ADP-independent) respiration
[d]UC = Uncoupled respiration
¶*yw*; *porin*^{Rev8}/*porin*^{Rev8}

Table S2. Respiratory Chain Enzyme Activities of Isolated Mitochondria from Wild Type and *porin* Mutant Larvae

	Complex I[a]			Complex II[b]			Complex III[c]			Complex IV[d]			Citrate Synthase[e]		
Genotype[¶]	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**
***porin*^{341}**	223.6	39.1	6	9.6	2.3	6	48.5	7.7	6	29.0	7.5	6	252.8	27.3	6
***porin*^{S27}**	206.1	24.0	6	13.6	5.9	6	51.5	11.7	6	33.4	19.0	6	274.6	38.5	6
***porin*^{Rev8}**	56.4	13.5	6	15.5	3.1	6	84.5	9.1	6	34.5	6.2	6	309.5	32.2	6
***porin*^{365}**	70.2	12.2	6	11.9	2.9	6	85.7	6.9	6	28.1	2.6	6	312.6	27.0	6
***porin*^{78}**	66.2	10.7	6	12.2	5.0	6	78.0	8.8	6	34.3	11.5	6	293.1	38.4	6

[¶]Each genotype indicates homozygosity for the allele specified. *porin*^{341} and *porin*^{S27} are precise-excision wild type alleles, while *porin*^{Rev8}, *porin*^{365}, and *porin*^{78} are imprecise-excision mutant alleles

[a]NADH:Ubiquinol Oxidoreductase activity in nmol/min/mg protein

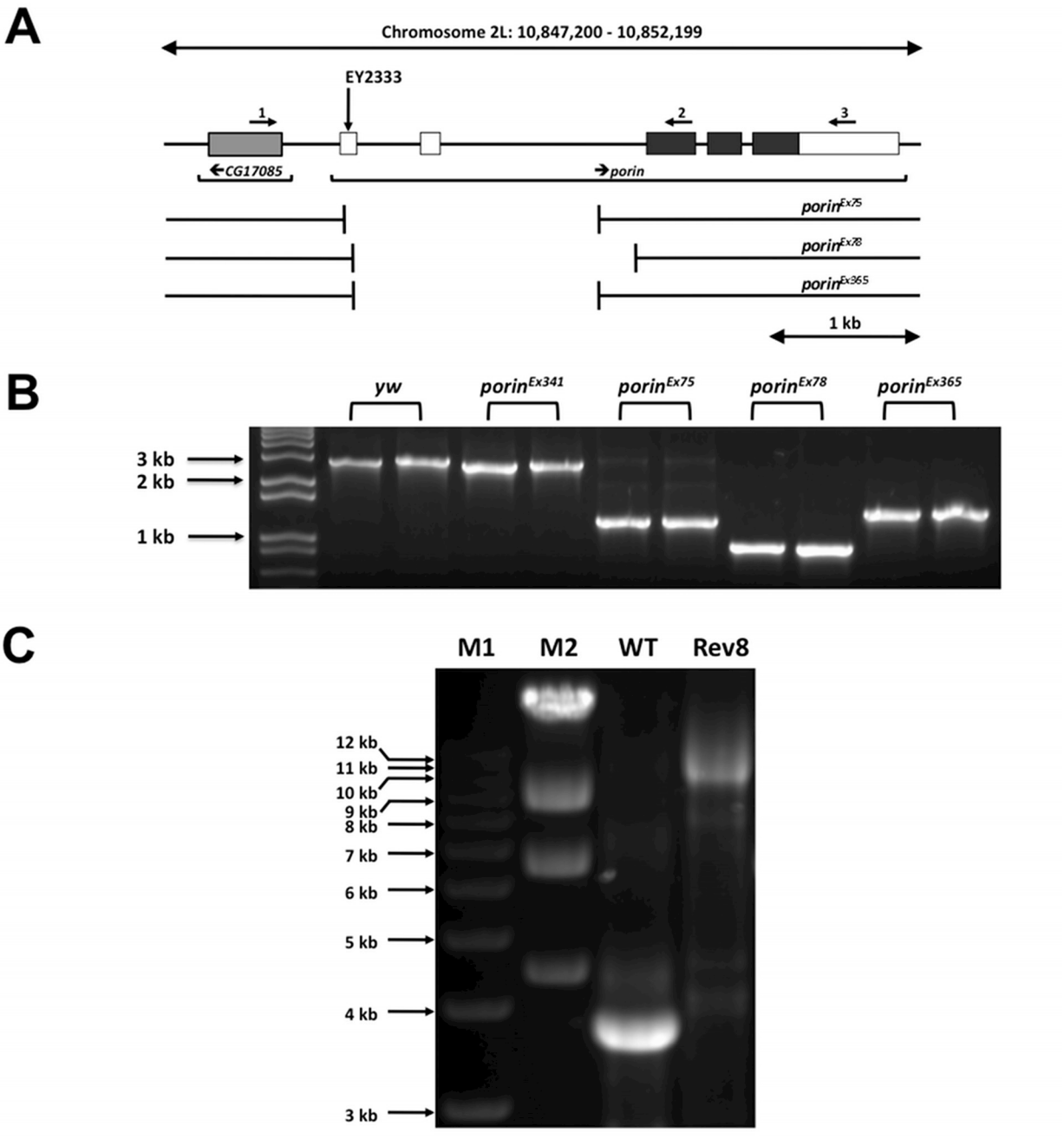
[b]Succinate Dehydrogenase activity in nmol/min/mg protein

[c]Ubiquinol:Cytochrome *c* Oxidoreductase activity in nmol/min/mg protein

[d]Cytochrome *c* Oxidase activity in nmol/min/mg protein

[e]nmol/min/mg protein

Figure 1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2
3
CG17085
porin
porin^Ex75
porin^Ex78
porin^Ex365
1 kb
B
yw
porin^Ex341
porin^Ex75
porin^Ex78
porin^Ex365
3 kb
2 kb
1 kb
C
M1
M2
WT
Rev8
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6 kb
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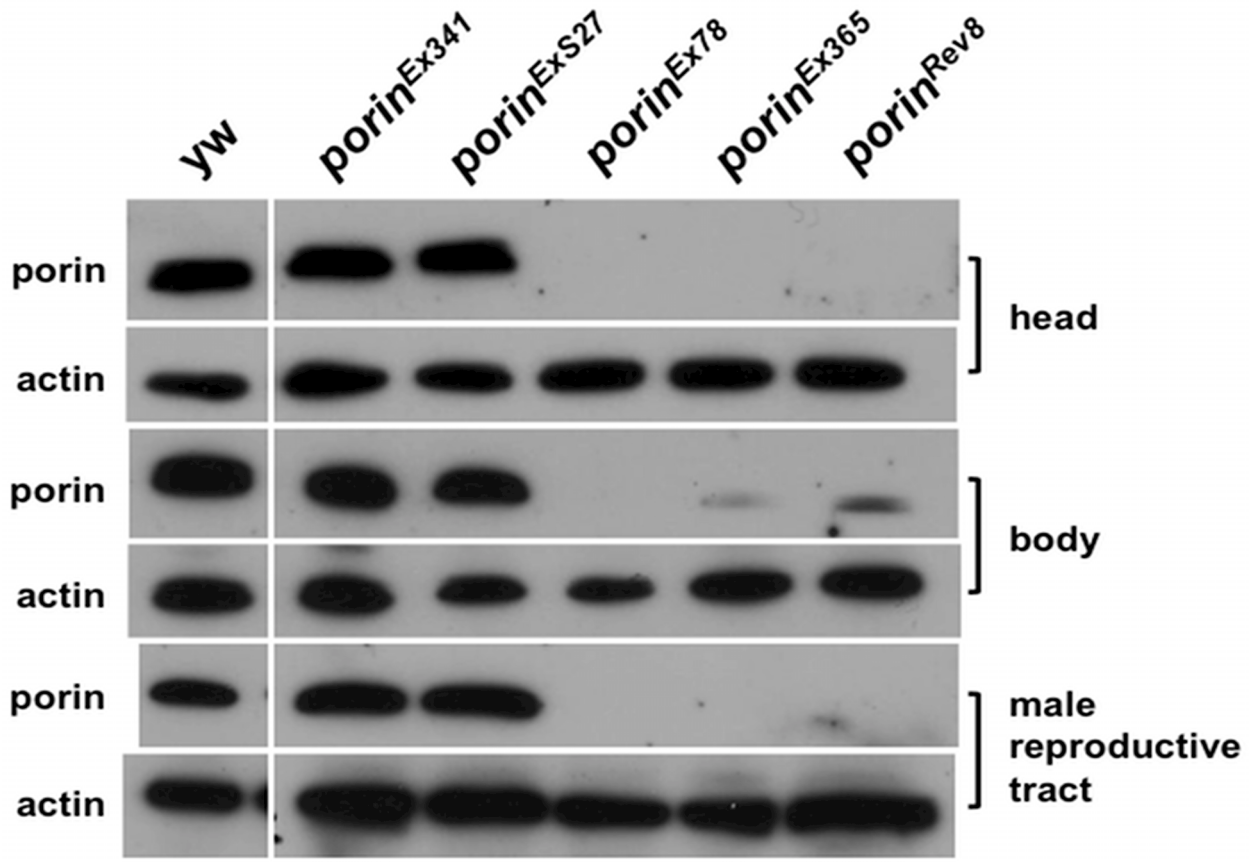
4 kb
3 kb

Figure S2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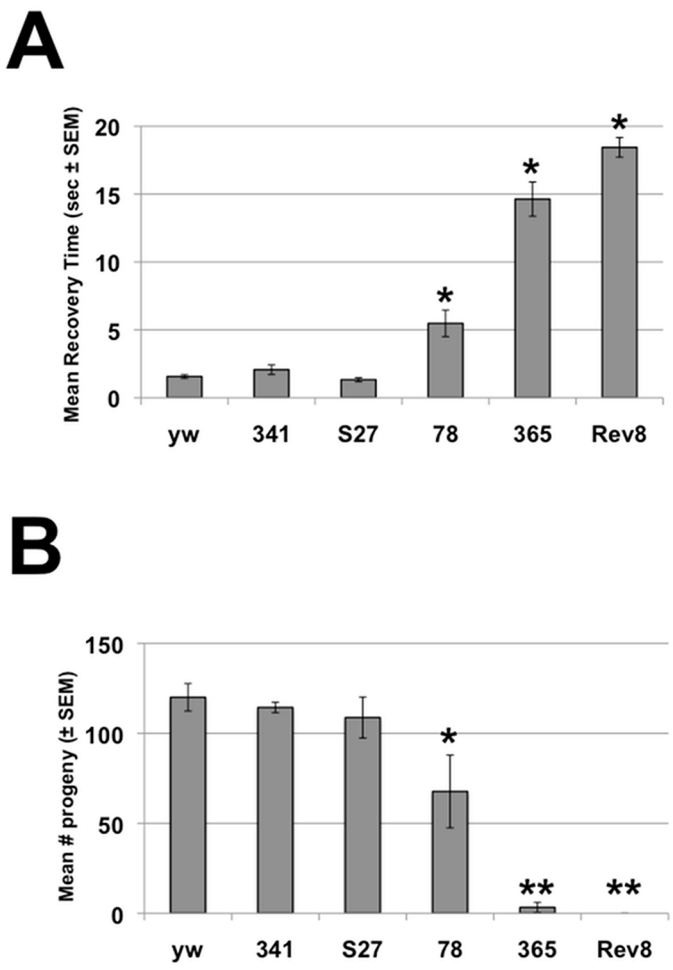
porin^ExS27
porin^Ex78
porin^Ex365
porin^Rev8
porin
actin
porin
actin
porin
actin
head
body
male
reproductive
tract

Figure S3


A
Mean Recovery Time (sec ± SEM)
20
15
10
5
0
*
*
*
yw
341
S27
78
365
Rev8
B
Mean # progeny (± SEM)
150
100
50
0
*
**
**
yw
341
S27
78
365
Rev8