Supplementary Information

A-to-I RNA editing uncovers hidden signals of adaptive genome evolution in animals

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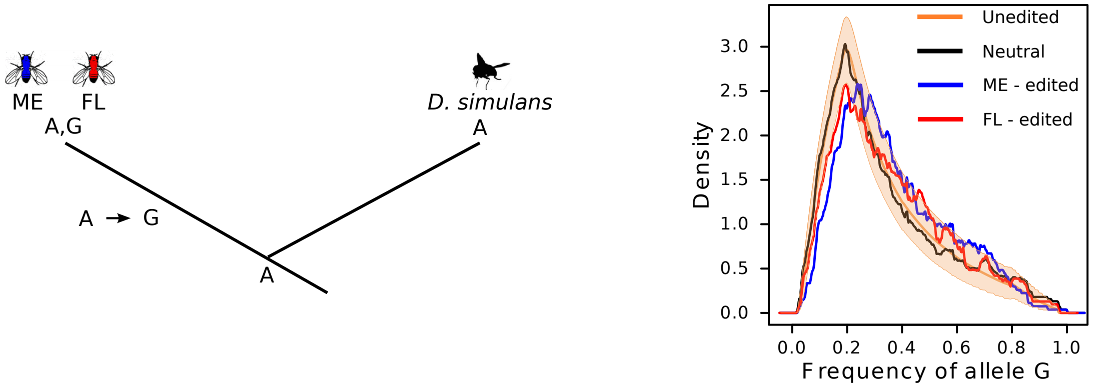
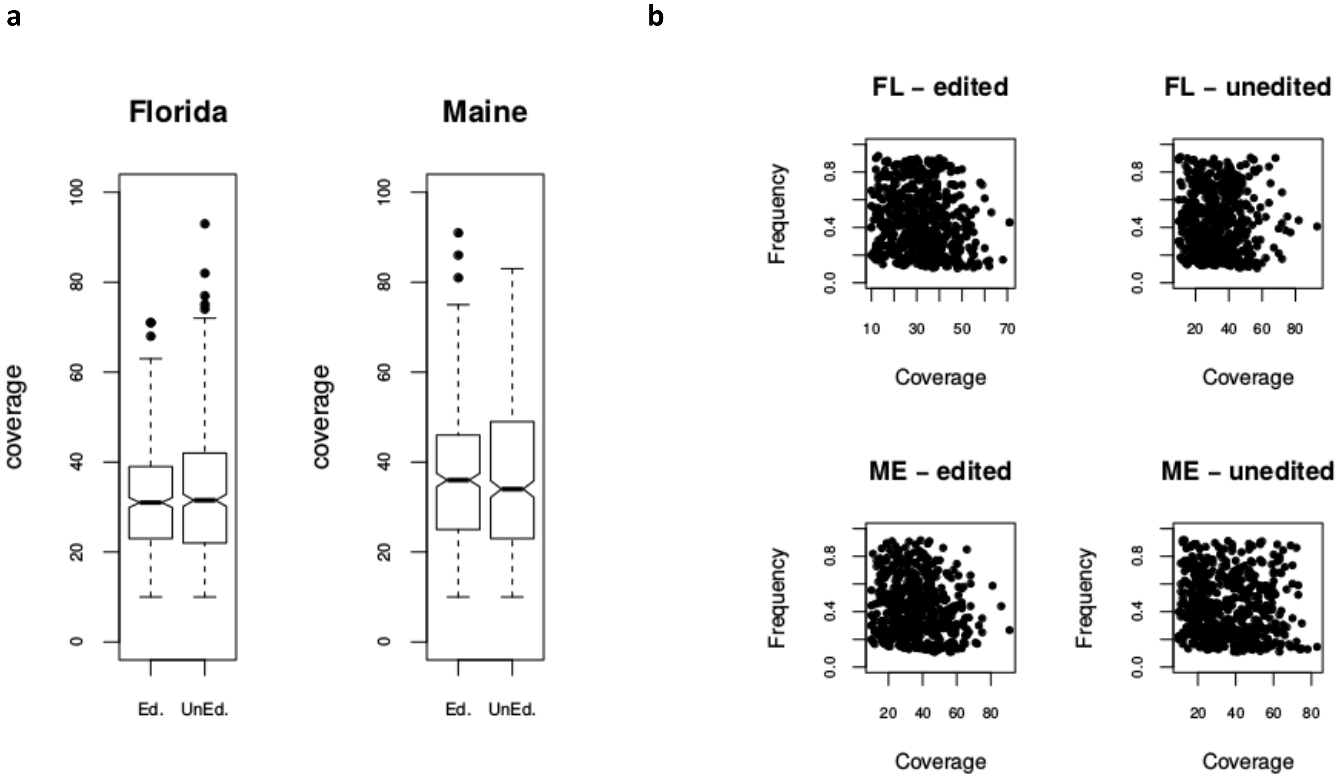
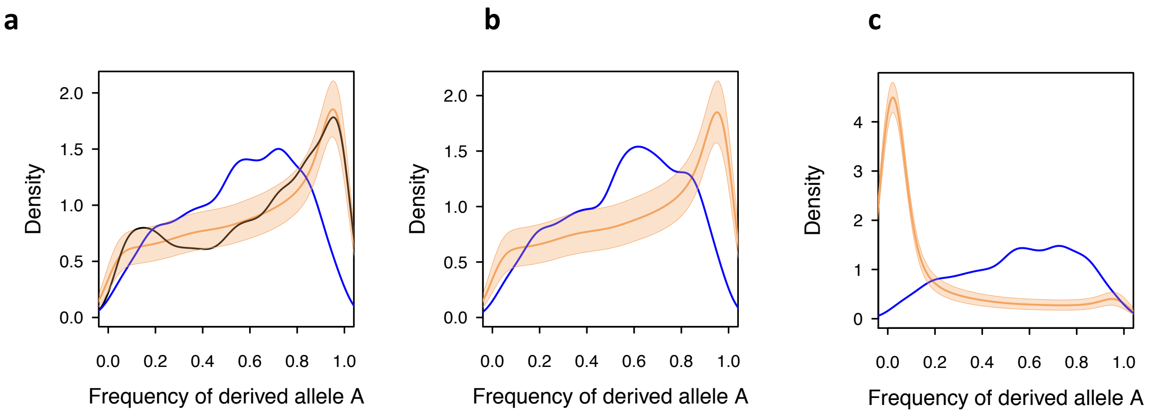


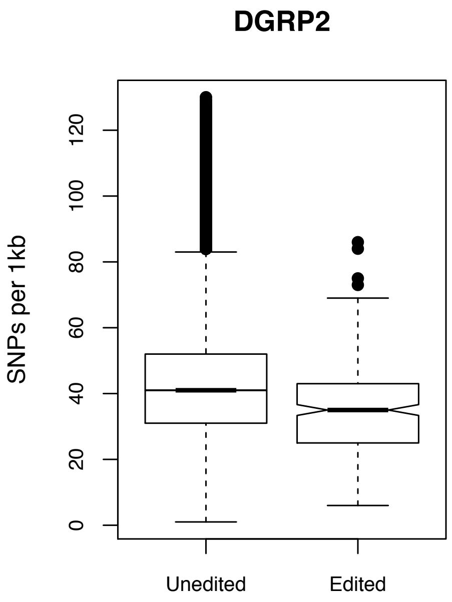
Supplementary Fig. 1. Allele frequency spectrum of the G alleles originated from A nucleotide sites in *D. melanogaster* lineage. We used *D. simulans* as an outgroup to infer the ancestral state of the A,G polymorphisms in *D. melanogaster*. Blue and red lines correspond to the frequency spectrum of the G alleles in edited sites in ME and FL populations, respectively. In black, we show the expected distribution of the G allele in edited sites if they were neutral in both populations. In peach, we show the allele frequency spectrum of the G allele in unedited sites (average and 95% confidence interval). Because the number of sequenced lines are low (39 and 86 lines for FL and ME populations, respectively), the differentiation between the genomic background and edited site is less obvious than in DGRP2 population.



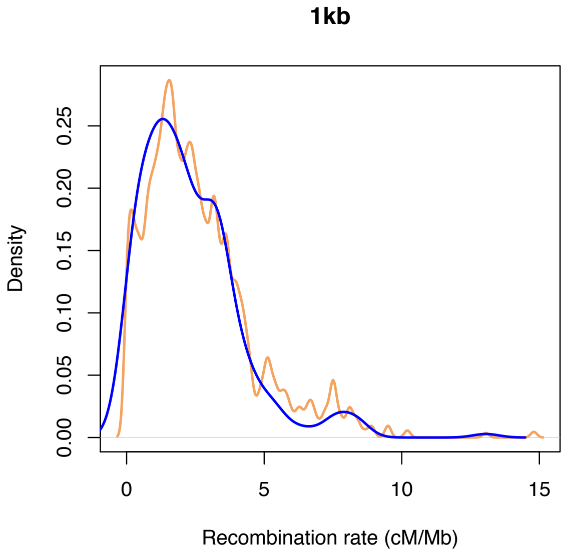
Supplementary Fig. 2. Differences in allele frequencies between edited and unedited sites is not affected by differences in sequencing coverage in *Drosophila*. a, The coverage distribution of edited sites in FL and ME populations is not different from unedited sites (*P* >> 0.05 for each paired comparison; two-sided Mann-Whitney-U test). **b,** The frequency of the minor allele and sequencing coverage at the polymorphic site do not correlate.



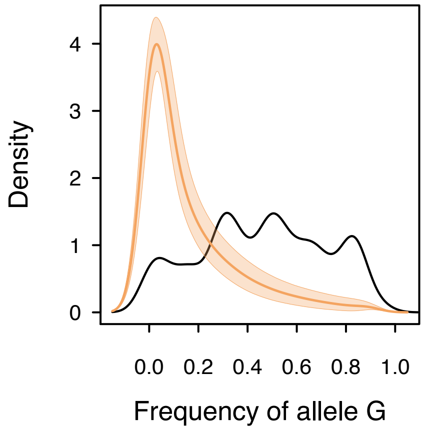
Supplementary Fig. 3. Allele frequency spectrum of the A alleles originated from G nucleotide sites in *D. melanogaster* lineage. Blue line corresponds to the frequency spectrum of the A alleles in edited sites in DGRP2 population. In black, we show the expected distribution of the A allele in edited sites if they were neutral. In peach, we show the allele frequency spectrum of the A allele in unedited sites (average and 95% confidence interval). **a,** Allele frequency spectrum of derived A alleles constrained to A sites in reference genome. It can be seen how derived A alleles segregate at lower frequencies at edited sites than at unedited sites. **b,** Same as **a**, but only for silent sites. **c,** Allele frequency spectrum of derived A alleles constrained to G sites in reference genome for genomic background and constrained to A sites in reference genome for edited sites. Comparison between **a** and **b** with **c** shows the importance of selecting sites from the same reference nucleotide type.



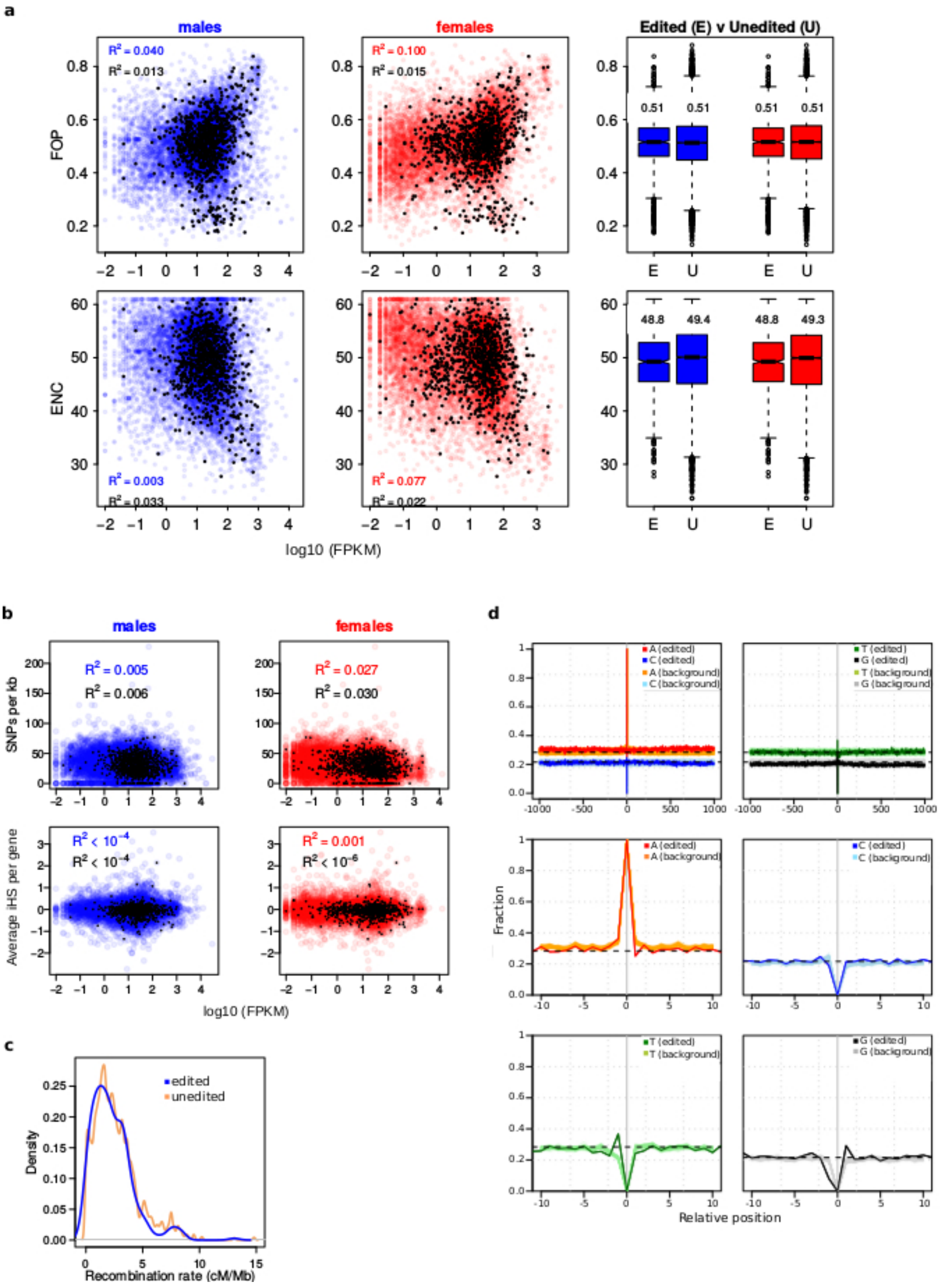
Supplementary Fig. 4. Diversity at edited and unedited sites in *Drosophila*. Windows centered on polarized A-to-G polymorphic sites have lower diversity (in SNPs per kb) for edited SNPs than for unedited SNPs (*P* = 8.4 x 10^{-16}; one-sided Mann-Whitney-U test).



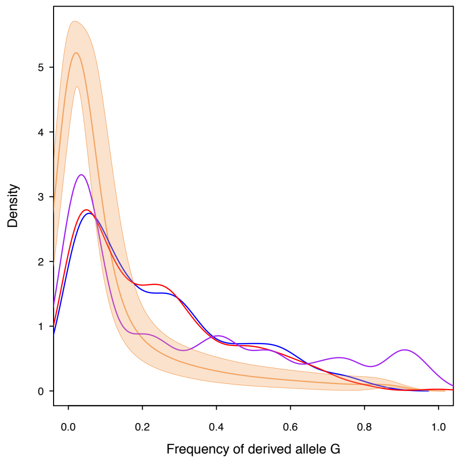
Supplementary Fig. 5. Distribution of local recombination rates at edited and unedited sites in *Drosophila*. Recombination rates were measured for windows of 1kb centered in edited (blue) and unedited (peach) sites. Both distributions are virtually identical.



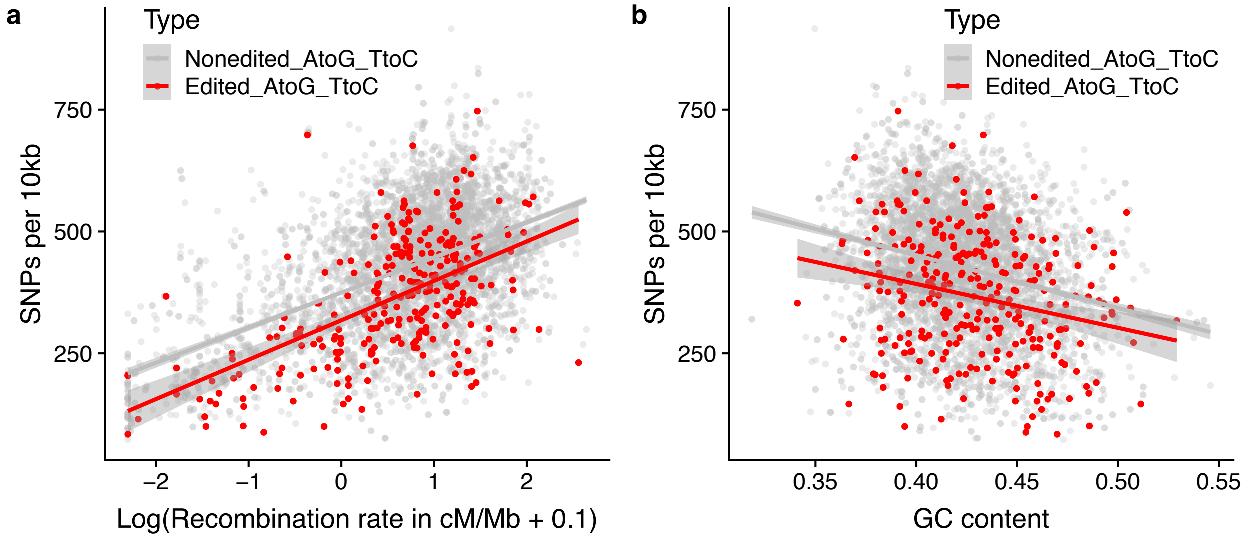
Supplementary Fig. 6. Allele frequency spectrum of intergenic G alleles originated from A nucleotide sites in human lineage. Intergenic G alleles segregate at higher frequencies in edited sites (black line) than in unedited sites (peach).


a
males
$R^2 = 0.040$
$R^2 = 0.013$
females
$R^2 = 0.100$
$R^2 = 0.015$
Edited (E) v Unedited (U)
0.51
0.51
0.51
0.51
FOP
0.8
0.6
0.4
0.2
-2 -1 0 1 2 3 4
E U E U
ENC
60
50
40
30
48.8
49.4
48.8
49.3
$R^2 = 0.003$
$R^2 = 0.033$
$R^2 = 0.077$
$R^2 = 0.022$
log10 (FPKM)
b
males
females
$R^2 = 0.005$
$R^2 = 0.006$
$R^2 = 0.027$
$R^2 = 0.030$
SNPs per kb
200
150
100
50
0
Average iHS per gene
$R^2 < 10^{-4}$
$R^2 < 10^{-4}$
$R^2 = 0.001$
$R^2 < 10^{-6}$
log10 (FPKM)
c
edited
unedited
Density
0.25
0.20
0.15
0.10
0.05
0.00
0 5 10 15
Recombination rate (cM/Mb)
d
A (edited)
C (edited)
A (background)
C (background)
T (edited)
G (edited)
T (background)
G (background)
-1000 -500 0 500 1000
A (edited)
A (background)
C (edited)
C (background)
Fraction
T (edited)
T (background)
G (edited)
G (background)
-10 -5 0 5 10
Relative position

Supplementary Fig. 7. Control analyses for differences in polymorphic rates and polymorphism types as a byproduct of gene expression level, recombination rate and local sequence composition in *Drosophila*. a, Bias in synonymous codon usage per gene is represented as a function of gene expression level in males (blue) and females (red). Gene expression level only explains 4% (males) to 10% (females) of the total variance in codon bias when measured as the frequency of optimal codons (FOP; the higher, the more biased) and 0.3% (males) to 7% (females) of the total variance in codon bias when measured as the effective number of codons (ENC; the lower, the more biased). The coefficient of determination for edited sites (black dots) is even lower than for unedited sites. Numbers in the boxplots refer to the mean. **b,** Nucleotide diversity (SNPs per kb per gene) and iHS (averaged per gene) does not correlate with gene expression level. Black dots: genes containing edited sites. Blue and red dots: unedited genes. **c,** Local recombination rates in 10 kb windows centered on edited (blue) and on unedited (peach) sites show identical distributions. **d,** Nucleotide profiles show that local sequence context around edited and unedited sites (±1000 bp and ±10 bp) are virtually identical.



Supplementary Fig. 8. Allele frequency spectrum of the G alleles originated from A nucleotide sites in *D. melanogaster* lineage for DGRP2 population. Blue line: validated data from St. Laurent et al. [8] Red line: data from RADAR [37]. Purple line: data form Yu et al. [11] In peach, we show the allele frequency spectrum of the derived G allele in unedited sites (average and 95% confidence interval).



Supplementary Fig. 9. Number of SNPs in 10 kb windows as a function of (a) recombination rate and (b) GC content. The 10 kb windows are centered on A-to-G mutations that are either edited (red) or unedited (grey). Note that even though SNP number is a function of both recombination rate and GC content, there is an effect of editing reducing the SNP number that cannot be explained by these two factors. Linear regression lines are fitted to the data.

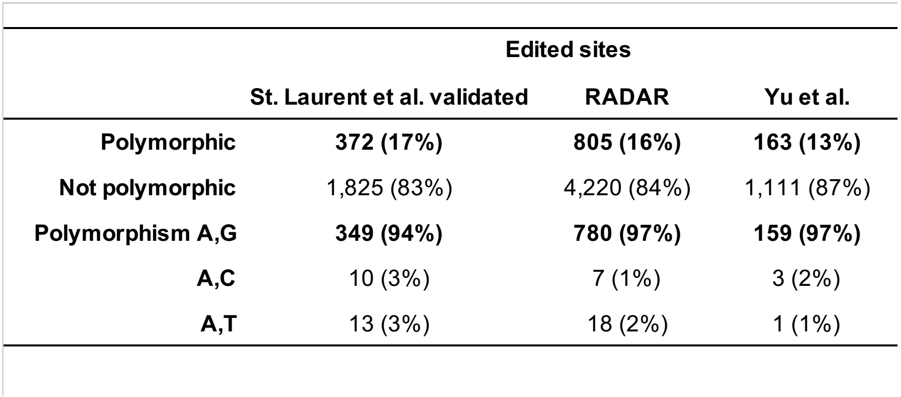
Supplementary Table 1. Number of A,G polymorphisms (and percentages) in coding regions of different *D. melanogaster* populations.

	Edited			Unedited		
	DGRP2	ME	FL	DGRP2	ME	FL
Polymorphic A,G sites	319 (31%)	218 (22%)	227 (22%)	113,973 (2%)	25,547 (0.5%)	27,250 (0.5%)
Total A sites		1,015			5,225,594	

Supplementary Table 2. Number of polarized polymorphisms in the genome of three *Drosophila* populations.

	DGRP 2		Florida		Maine	
	Total	**A-to-G**	**Total**	**A-to-G**	**Total**	**A-to-G**
Edited	755	303	543	179	507	155
Unedited	3,951,070	462,498	1,367,160	125,628	1,235,454	110,689

Supplementary Table 3. Number of single nucleotide polymorphism sites and polymorphism types among edited sites in DGRP2.



	Edited sites		
	St. Laurent et al. validated	**RADAR**	**Yu et al.**
Polymorphic	**372 (17%)**	**805 (16%)**	**163 (13%)**
Not polymorphic	1,825 (83%)	4,220 (84%)	1,111 (87%)
Polymorphism A,G	**349 (94%)**	**780 (97%)**	**159 (97%)**
A,C	10 (3%)	7 (1%)	3 (2%)
A,T	13 (3%)	18 (2%)	1 (1%)

St. Laurent et al. validated: Ref. 8.
RADAR: Ref. 37.
Yu et al.: Ref. 11.
In bold: increased proportion in edited sites compared to unedited sites.

Supplementary Table 4. Potential A,G replacements at non-coding and intergenic regions in *Drosophila* populations.

Population	**Potential A,G replacements at non-coding and intergenic regions**				
	Edited (l^{edited} = 2,544)		Genome (l = 29,325,288)		Ratio
	Polymorphic	Rate (f_l^{edited})	Polymorphic	Rate (f_l)	f_l^{edited} / f_l
DGRP2	411	0.162	702,149	0.024	6.750
ME	281	0.110	1,180,129	0.040	2.750
FL	310	0.122	1,065,262	0.036	3.389

Supplementary Table 5. Testing for an effect of editing on the polymorphism to divergence ratio in 10kb surrounding window, and on iHS, using the DGRP2 data.

Polymorphism to divergence ratio in 10kb windows centered on A-to-G				
Filter on G allele frequency	Factor	Estimate	Std. Error	p-value
No filter	Intercept	0.538577	0.04198	< 2e-16 ***
	log(Recombination rate + 0.1)	0.127586	0.003301	< 2e-16 ***
	GC content	0.232169	0.098863	0.01889 *
	Editing status (edited)	-0.040343	0.012631	0.00141 **
> 5%	Intercept	0.505884	0.06575	2.15e-14 ***
	log(Recombination rate + 0.1)	0.122606	0.005303	< 2e-16 ***
	GC content	0.339607	0.154933	0.028488 *
	Editing status (edited)	-0.051553	0.014925	0.000563 ***
> 10%	Intercept	0.436508	0.07661	1.44e-08 ***
	log(Recombination rate + 0.1)	0.110643	0.006339	< 2e-16 ***
	GC content	0.513387	0.180688	0.004550 **
	Editing status (edited)	-0.057072	0.01673	0.000663 ***
iHS of A-to-G polymorphisms				
Filter on G allele frequency	Factor	Estimate	Std. Error	p-value
No filter	Intercept	0.60326	0.19705	0.00221 **
	log(Recombination rate + 0.1)	0.02242	0.01643	0.17253
	GC content	1.46664	0.46186	0.00150 **
	Editing status (edited)	-0.13301	0.06869	0.05286 .
> 10%	Intercept	0.42848	0.21843	0.0499 *
	log(Recombination rate + 0.1)	-0.01398	0.01855	0.451
	GC content	-0.98617	0.51237	0.0543
	Editing status (edited)	-0.14924	0.07467	0.0457 *