A)

ADAR p150 variant 1

ADAR p110 variant 2

ADAR p150 variant 3

B)

Figure 1.

A)

B)

C)

D)

Rpa1
Chr11:75,300,577*

Wdr37
Chr13:8,805,663

Mdc1
Chr17:35,857,734*

gDNA

unmodified cDNA

inosine modified cDNA

edited PCR Products: 1/10   edited PCR Products: 1/10   edited PCR Products:1/10
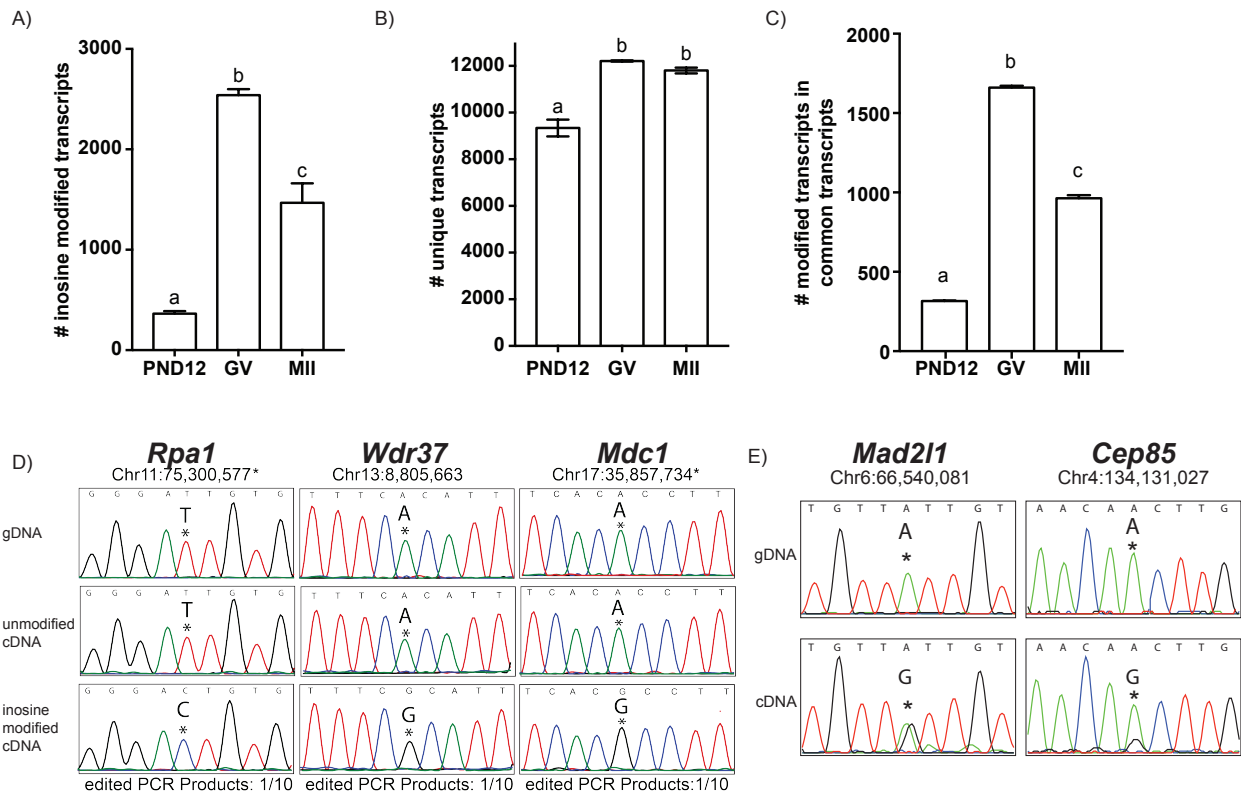
E)

Mad2l1
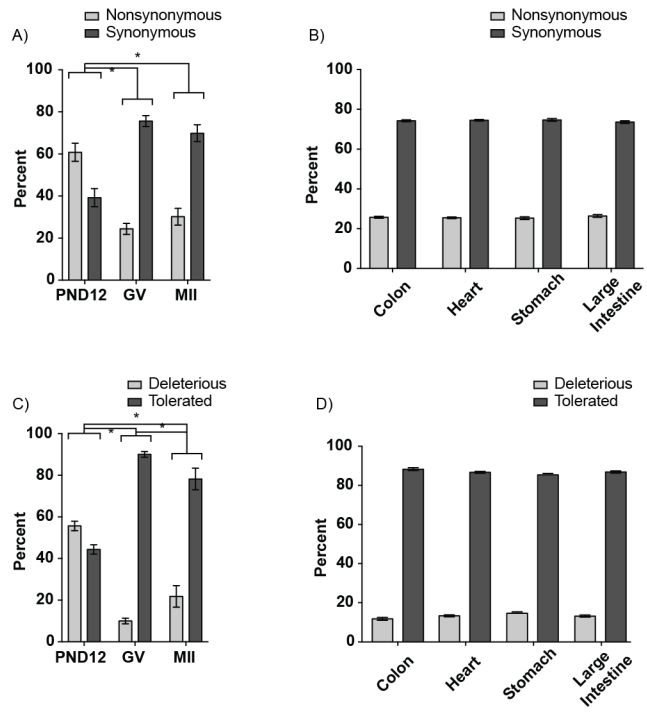Chr6:66,540,081

Cep85
Chr4:134,131,027

gDNA

cDNA

Figure 2.

Figure 3.

Figure 4.

Figure 5.

A)

Variant 1 (NM_019655)

Variant 2 (NM_001038587)

Variant 3 (NM_001146296)

Exons: 1   2   3   4 5 6 7 8   9 10 11   12 13 14   15

B)

Adar1 Exon 7 variations

Variant 1 (NM_019655)   GTTTGTTTACCAAGCAAAAGTTGGGGGCCGCTGGTTTCCAGCCGTGTGTGCACACAGCAAGAAACAGGGCAAGCAGGATGCAGCGGATGCAGCCCTCCGGGTCTTGATCGGGGAGAGCGA
Variant 2 (NM_001038587) GTTTGTTTACCAAGCAAAAGTTGGGGGCCGCTGGTTTCCAGCCGTGTGTGCACACAGCAAGAAACAGGGCAAGCAGGATGCAGCGGATGCAGCCCTCCGGGTCTTGATCGGGGAGAGCGA
Variant 3 (NM_001146296) GTTTGTTTACCAAGCAAAAGTTGGGGGCCGCTGGTTTCCAGCCGTGTGTGCACACAGCAAGAAACAGGGCAAGCAGGATGCAGCGGATGCAGCCCTCCGGGTCTTGATCGGGGAGAGCGA

Variant 1 (NM_019655)   GAAGGCAGAGCAGTTGGGTTTCGCAGAG--
Variant 2 (NM_001038587) GAAGGCAGAGCAGTTGGGTTTCGCAGAGGTAACCCCAGTAACAGGGGCCAGTCTCAGAAGAACTATGCTCCTCCTTTCCAGGTCCCCAGATGCACATCCAAAGACA
Variant 3 (NM_001146296) GAAGGCAGAGCAGTTGGGTTTCGCAGAGGTAACCCCAGTAACAGGGGCCAGTCTCAGAAGAACTATGCTCCTCCTTTCCAGGTCCCCAGATGCACATCCAAAGACA

C)

Variant 1 (NM_019655)
ADAR1 p150

Variant 2 (NM_001038587)
ADAR1 p110

Variant 3 (NM_001146296)
ADAR1 p150

■ Z-DNA binding domain
■ Nuclear export signal
■ dsRNA binding domain
■ Nuclear localization signal
■ Deaminase domain

Variant 1 (NM_019655)   FAE---LPL
Variant 2 (NM_001038587) FAEVTPVTGASLRRTMLLLSRSPDAHPKTLPL
Variant 3 (NM_001146296) FAEVTPVTGASLRRTMLLLSRSPDAHPKTLPL
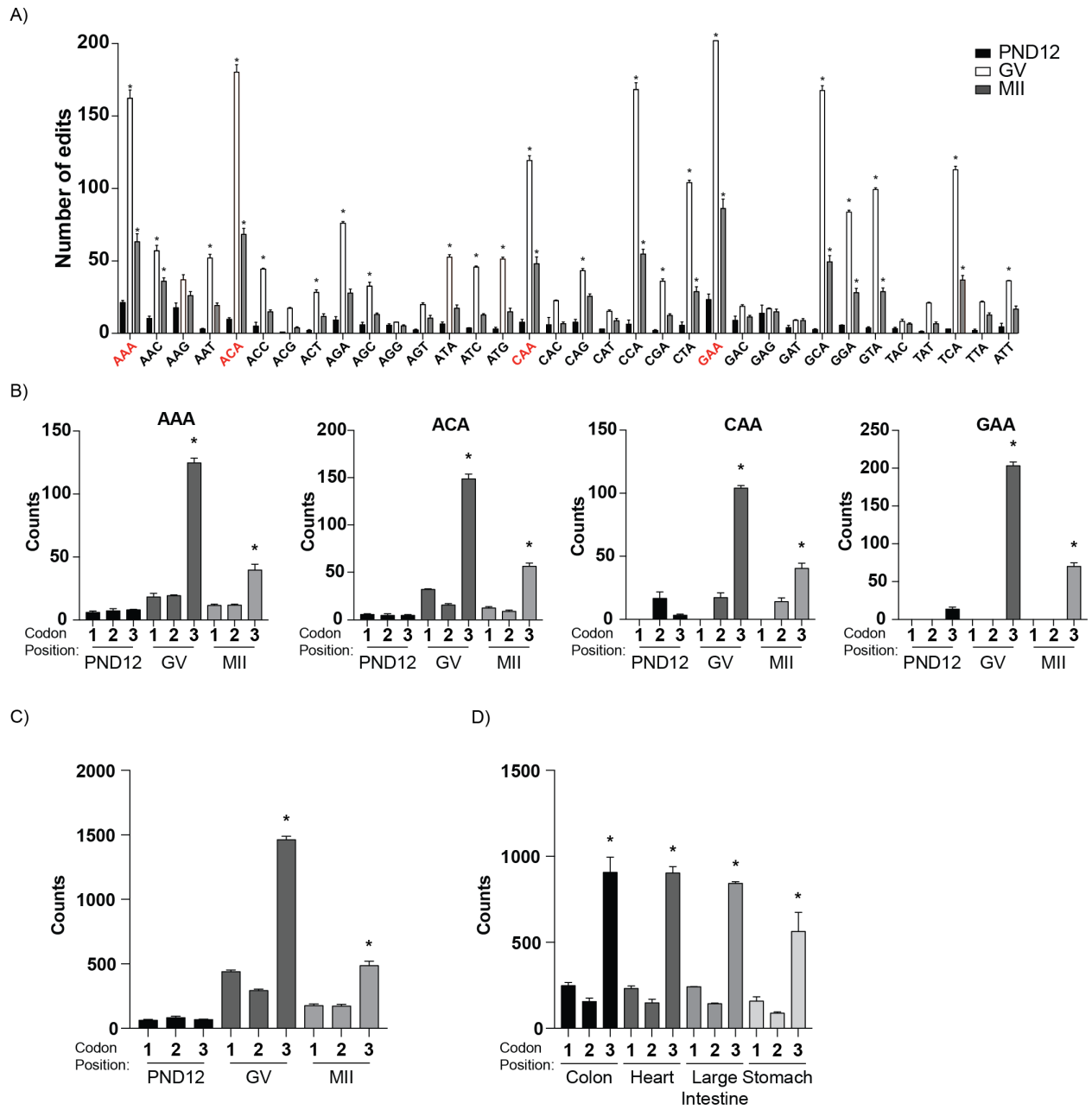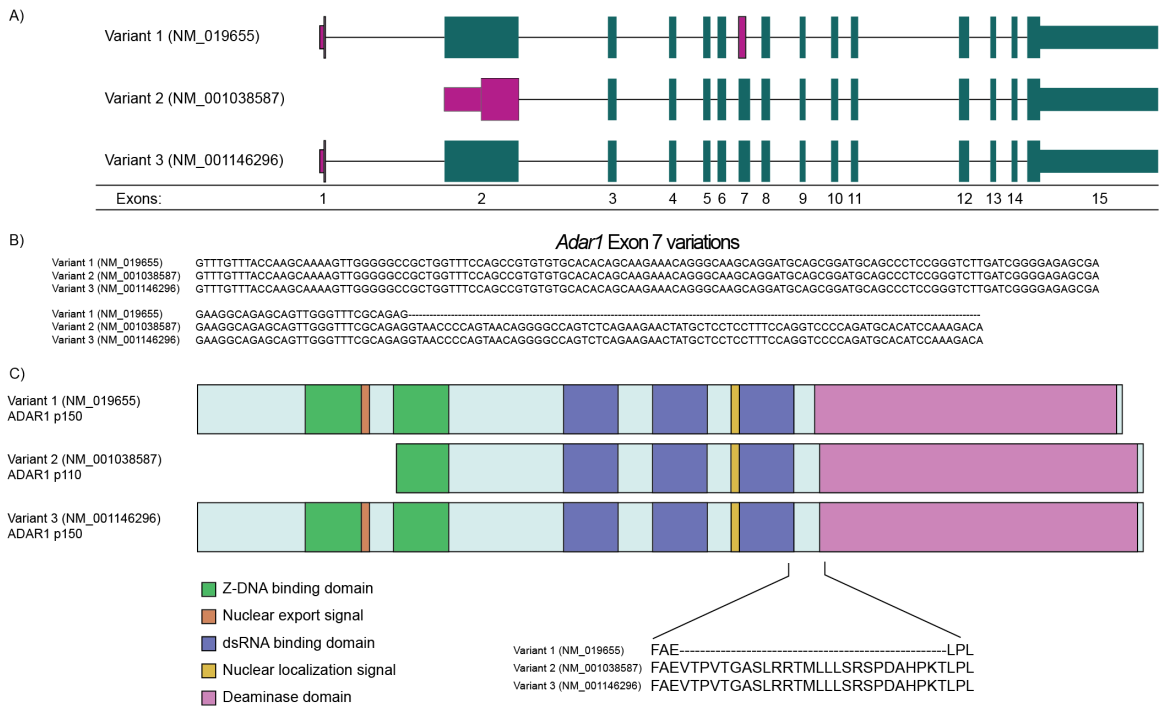
Fig. S1. Schematic diagrams showing ADAR1 variants. A) *Adar* transcript variants are labeled according to NCBI Reference Sequence. Variants 1 and 3 generate the longest mRNA isoforms, containing 15 exons. Variant 1, however, has a truncated exon 7 that is the result of alternative splicing. Variant 2 utilizes an alternative promoter and alternative start codon in exon 2. B) Exon 7 sequences of the three *Adar* variants detailing the truncated region of variant 1. C) Protein domains of ADAR1 variants. Variant 1 encodes a 26 amino acids shorter protein than variant 3; those amino acids reside between the dsRNA binding domain and deaminase domain.
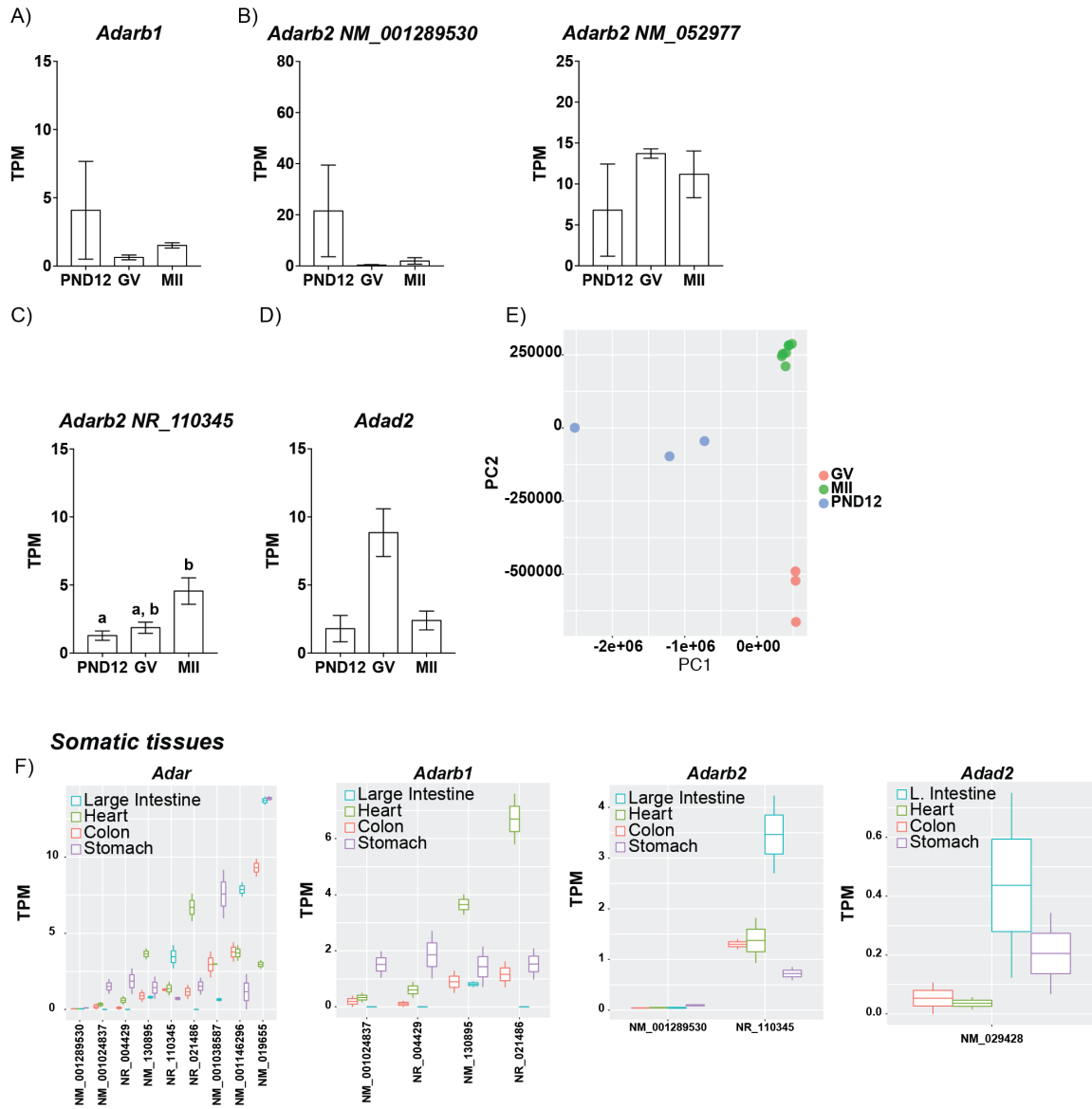
Fig. S2. Other adenosine deaminase genes expression levels in mouse oocytes and eggs. Adarb1 (A), Adarb2 (B-C), and Adad2 (D) isoform abundance in PND12 oocytes, GV oocytes, and MII eggs. E) Principal components analysis on RNA-seq datasets that were utilized in our studies. Expression of adenosine deaminase genes in somatic tissues (F). TPM: transcripts per million. a,b Means ± SEM within a panel that have different superscripts were different ($p < 0.05$); Kruskal-Wallis test.
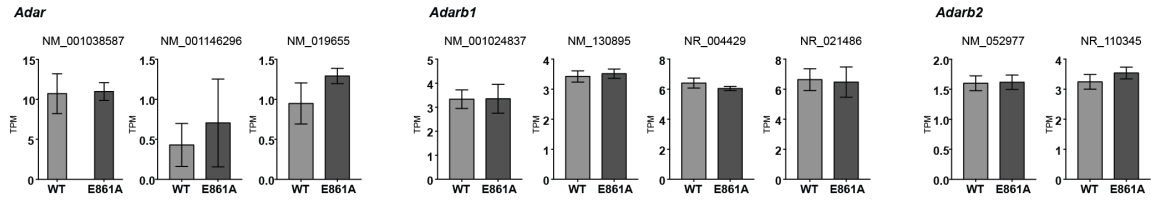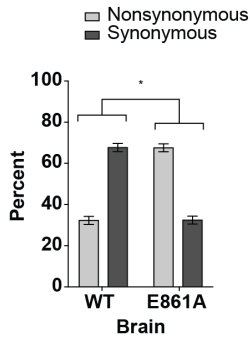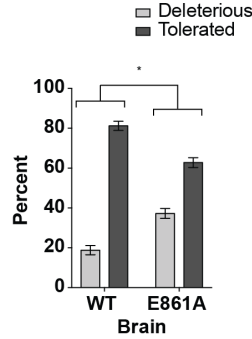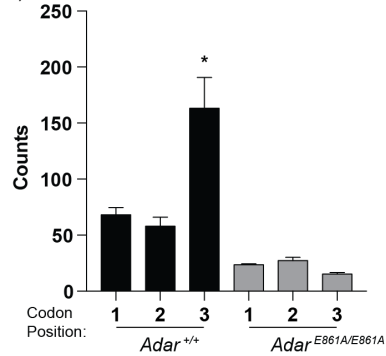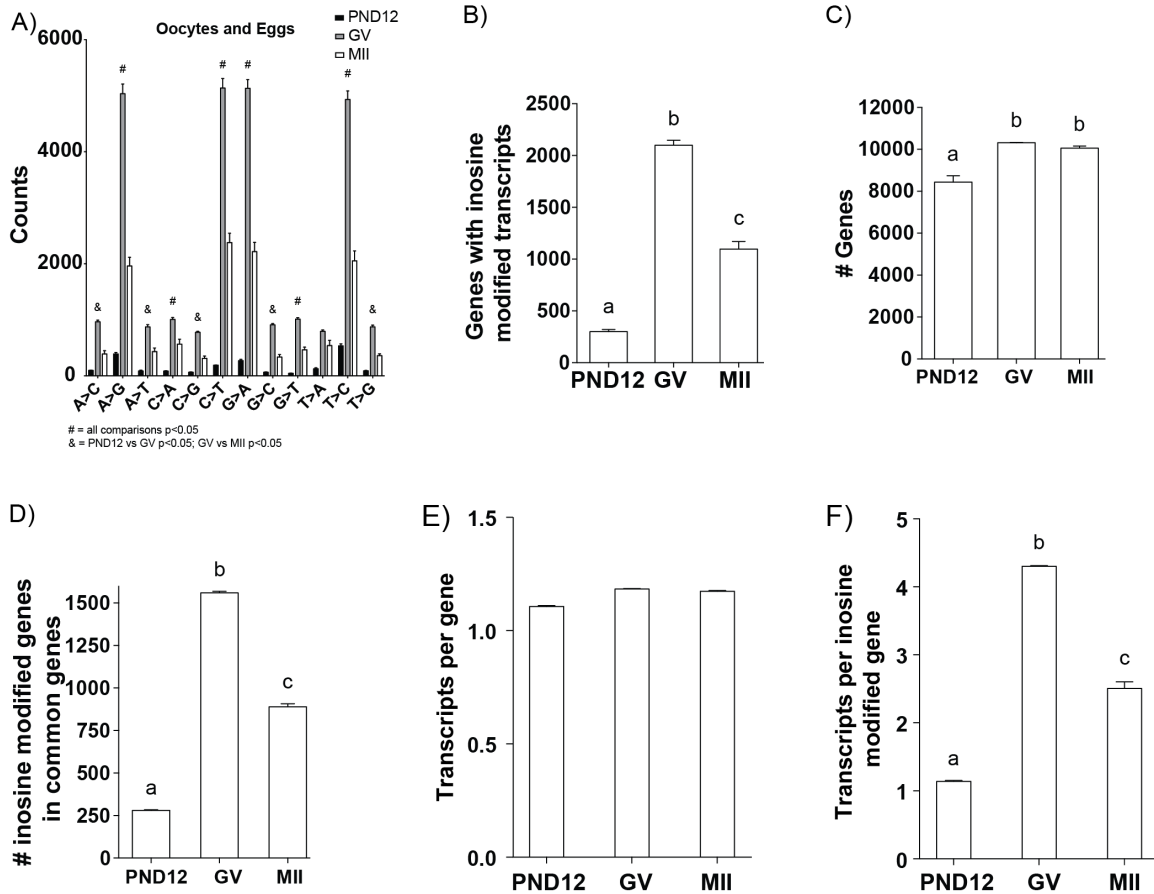
Fig. S3. Levels of individual adenosine deaminase family member transcript levels in WT (Adar$^{+/+}$) and Adar$^{E861A/E861A}$ adult brain tissues (A). Adad1 was undetected in all tissues examined. TPM: transcripts per million. B-D) Consequence of inosine RNA modifications and location of inosines within codons of Adar$^{+/+}$ and Adar$^{E861A/E861A}$ adult brain tissue.

Fig. S4. The number of genes with inosine RNA modifications within PND12 oocytes, GV oocytes, and MII eggs. A) Single nucleotide substitution counts identified by our RNA modification discovery pipeline in PND12 oocytes, GV oocytes, and MII eggs in the 5′ UTR, CDS, intron, and 3′ UTR. B) Total number of genes with inosines, TPM $\geq$ 1. C) Number of genes detected, TPM $\geq$ 1. D) Number of genes with inosine modifications among genes in common among samples, TPM $\geq$ 1. E) Number of transcripts per gene detected in PND12 oocytes, GV oocytes, and MII eggs. F) Number of transcripts per inosine modified gene in PND12 oocytes, GV oocytes, and MII eggs.
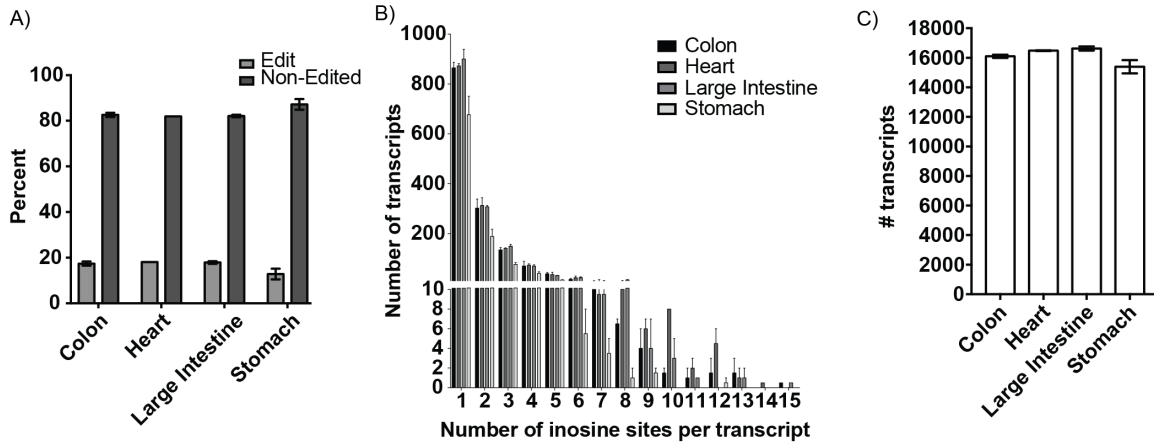
**Fig. S5**. Patterns of inosine RNA modifications in somatic cells. A) Proportion of the transcriptome that contains inosine modification sites within colon, heart, large intestine, and stomach. B) Counts of inosine modified transcripts exhibiting one or multiple inosine sites per transcript in somatic tissues. C) Total number of unique mRNA transcripts per sample. Only transcripts with TPM $\geq$ 1 were analyzed.
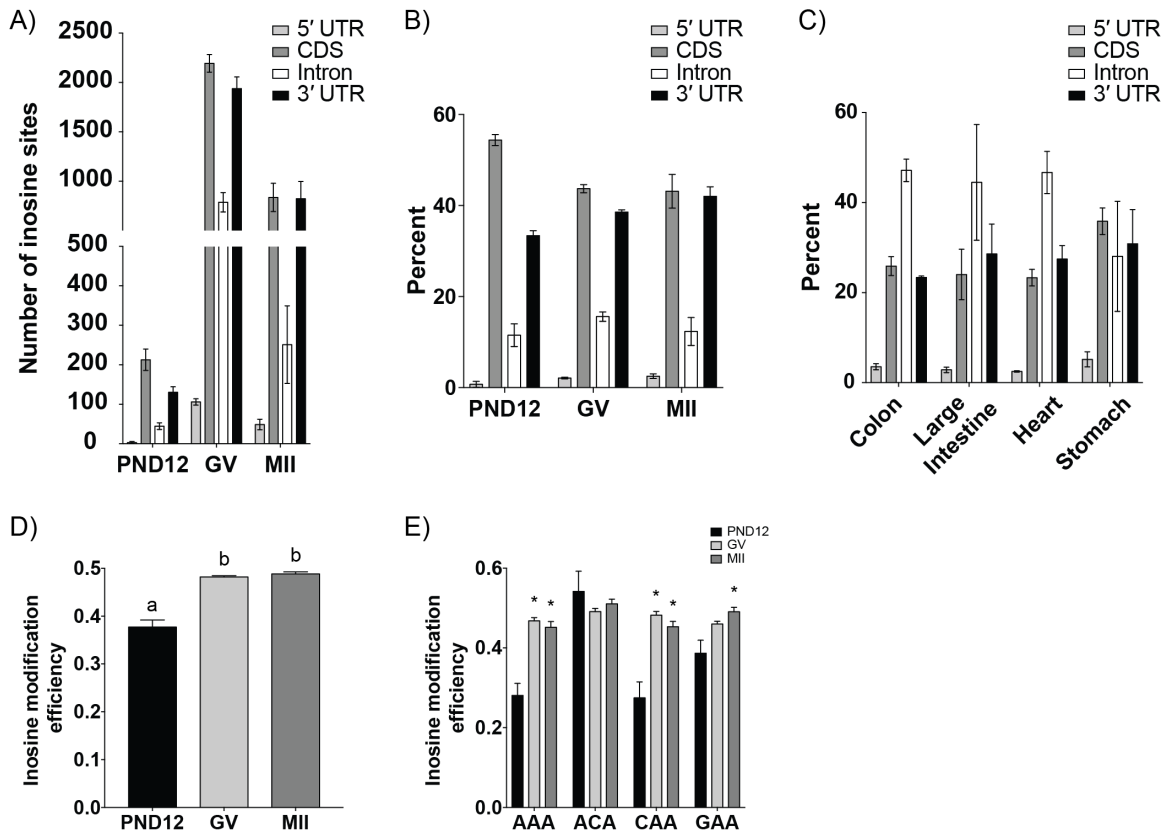
Fig. S6. Distribution of inosine RNA modifications in oocyte and egg samples. Total number (A) and total percent (B-C) of inosine RNA modifications within specific regions (5′ UTR, CDS, intron, and 3′ UTR) in mRNA of oocytes and eggs, and somatic tissues of C57BL/6 wild-type mice. D) Transcriptome-wide inosine RNA modification efficiency at the third codon position in oocyte samples. E) Inosine RNA modification efficiency at the four codons with highest enrichment for wobble position inosine modifications. a,b Means ± SEM within a panel that have different superscripts were different ($p < 0.05$); significance was determined using one-way ANOVA, followed by Tukey's multiple comparison tests. Means ± SEM within a panel that have superscripts * were different ($p < 0.05$); significance was determined using two-way ANOVA tests. Only codons from transcripts with TPM $\geq$ 1 were analyzed.
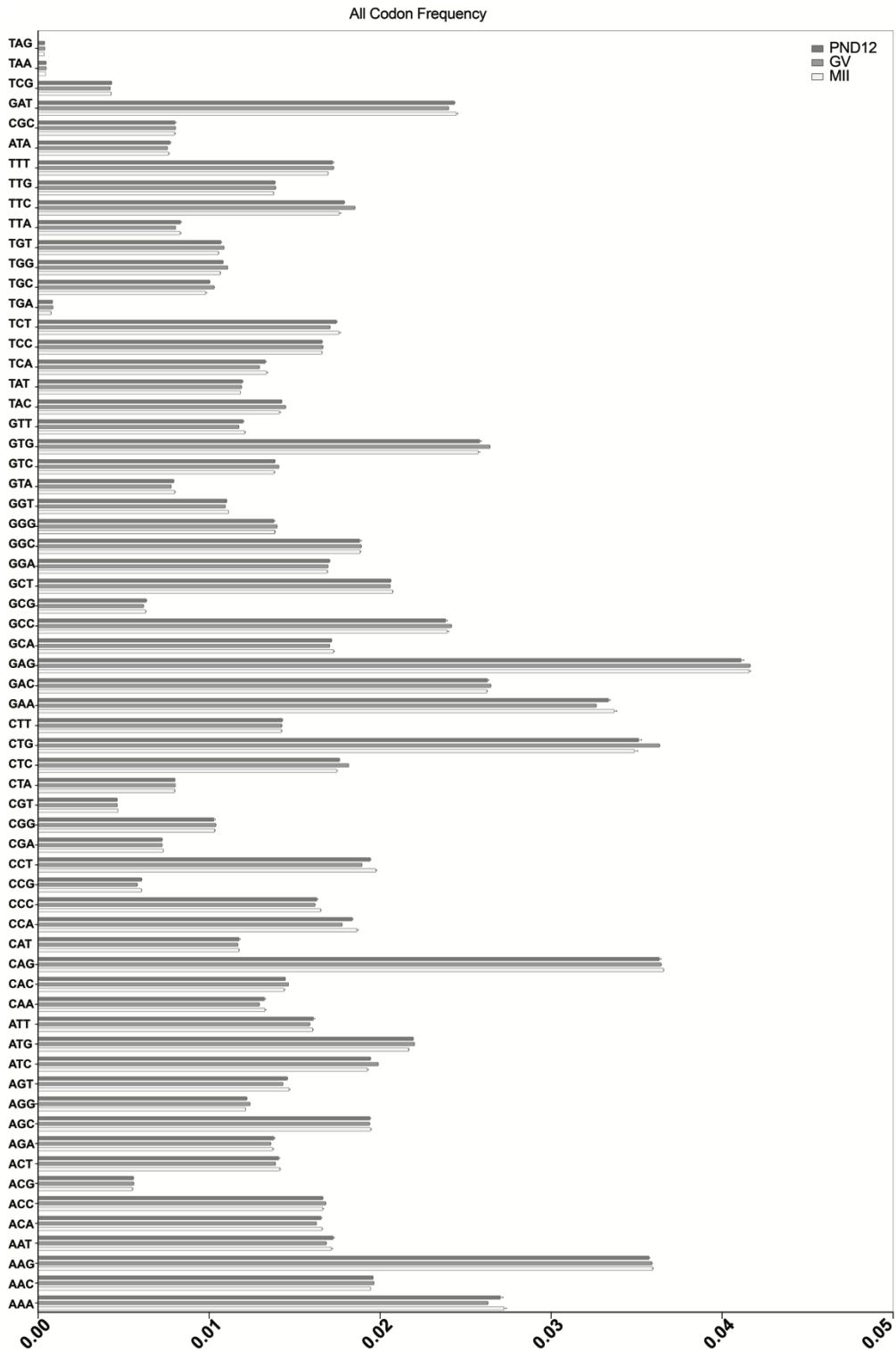
Fig. S7. Codon usage in PND12 oocytes, GV oocytes, and MII eggs. The frequency of codons occurring in all transcripts with a TPM ≥ 1.
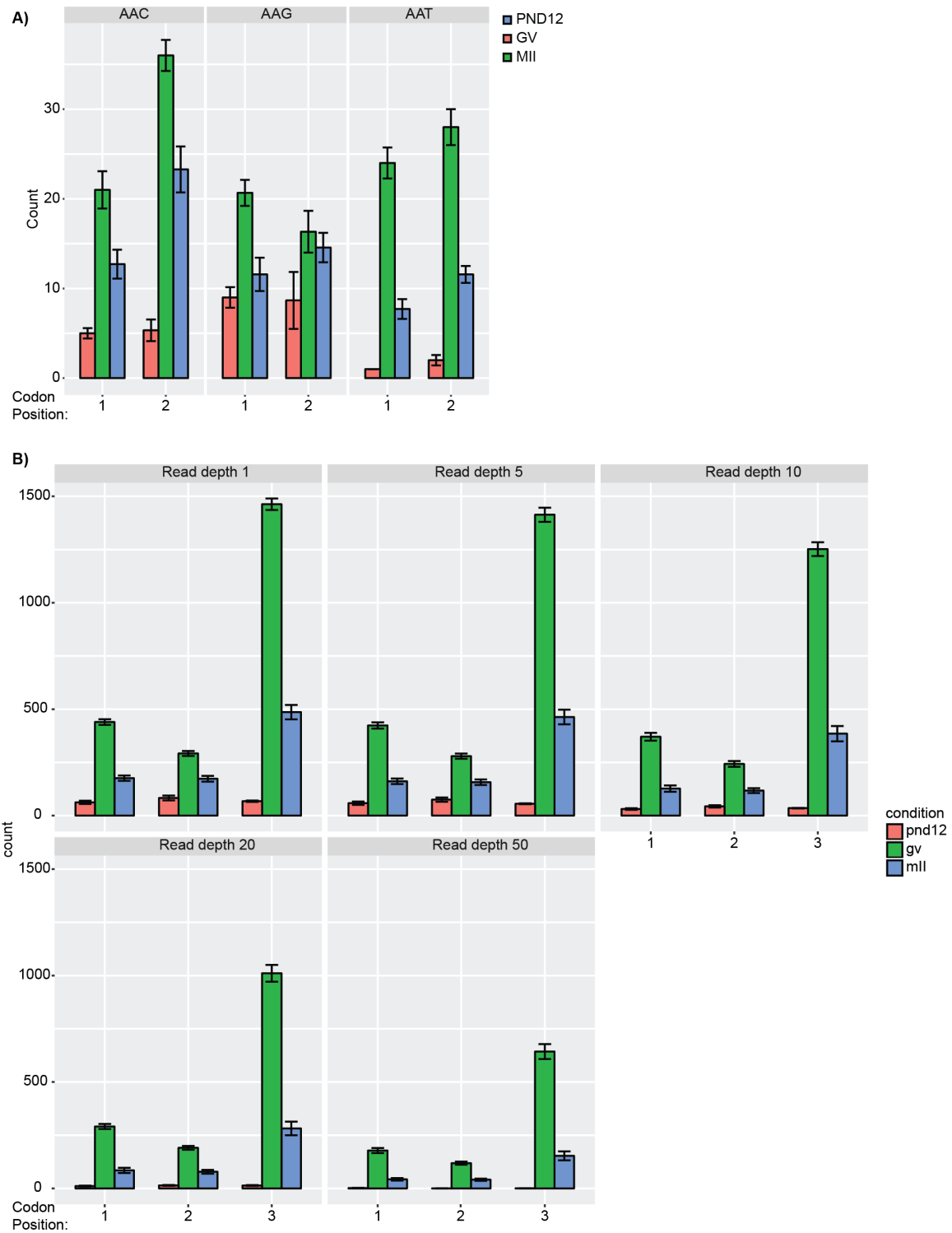
Fig. S8. A) Global frequency of inosine sites occurring at the first, second, or third codon position in PND12, GV, and MII samples within codons with adenosines occurring in the first two codon positions. B) Global frequency of inosine sites occurring at the first, second, or third codon position in PND12, GV, and MII samples at various read depths (number of reads covering an inosine site).

Table S1. Synonymous substitutions validated in the RADAR database. The validated substitutions occur at the third position of the codon in all cases when multiple adenosines are present.

Chromosome Position	Gene	Amino Acid	Nucleotide Sequence	Reference
chr12:72,063,017	*Actr10*	Ala>Ala	GCA>GCG	Gu et al. 2012
chr15:38,421,368	*Azin1*	Glu>Glu	GAA>GAG	Gu et al. 2012
chr16:91,656,315	*Son*	Ser>Ser	TCA>TCG	Danecek et al 2012
chr16:91,656,378	*Son*	Leu>Leu	TTA>TTG	Danecek et al 2012
chr16:91,656,471	*Son*	Leu>Leu	CTA>CTG	Danecek et al 2012
chr19:43,515,804	*Cnnm1*	Gln>Gln	CAA>CAG	Danecek et al 2012
chr3:32,460,407	*Mfn1*	Ala>Ala	GCA>GCG	Danecek et al 2012
chr3:96,024,186	*Hist2h2ab*	Leu>Leu	TTA>TTG	Cattenoz et al. 2013
chr8:109,409,504	uc009ngo.1	Leu>Leu	CTA>CTG	Gu et al. 2012

Table S2. RNA-seq alignment statistics for samples used.

Adult Brain ADAR E861A						
Name	SRR5223114	SRR5223115	SRR5223116	SRR5223117	SRR5223118	SRR5223119
# Reads	81245663	91095314	74985821	94478063	94284943	92826293
# paired reads	81245663 (100.00%)	91095314 (100.00%)	74985821 (100.00%)	94478063 (100.00%)	94284943 (100.00%)	92826293 (100.00%)
# aligned concordantly 0 time	11625083 (14.31%)	12905847 (14.17%)	11068350 (14.76%)	13329461 (14.11%)	15242188 (16.17%)	13728329 (14.79%)
# aligned concordantly exactly 1 time	64134835 (78.94%)	71641901 (78.64%)	59061846 (78.76%)	74891906 (79.27%)	71943133 (76.30%)	72709092 (78.33%)
# aligned concordantly >1 times	5485745 (6.75%)	6547566 (7.19%)	4855625 (6.48%)	6256696 (6.62%)	7099622 (7.53%)	6388872 (6.88%)
# pairs aligned concordantly 1 times	11625083	12905847	11068350	13329461	15242188	13728329
# aligned discordantly 1 time	113693 (0.98%)	119921 (0.93%)	85844 (0.78%)	130000 (0.98%)	120422 (0.79%)	133755 (0.97%)
# pairs aligned 0 times concordantly or discordantly	11511390	12785926	10982506	13199461	15121766	13594574
# mates make up the pairs	23022780	25571852	21965012	26398922	30243532	27189148
# aligned 0 times	19617312 (85.21%)	21936133 (85.78%)	18814406 (85.66%)	22403143 (84.86%)	26223922 (86.71%)	23200759 (85.33%)
# aligned exactly 1 time	2865353 (12.45%)	3121684 (12.21%)	2680245 (12.20%)	3358645 (12.72%)	3297205 (10.90%)	3350699 (12.32%)
# aligned >1 times	540115 (2.35%)	514035 (2.01%)	470361 (2.14%)	637134 (2.41%)	722405 (2.39%)	637690 (2.35%)
overall alignment rate	87.93%	87.96%	87.45%	88.14%	86.09%	87.50%
GV						
Name	SRR1986034	SRR1986035	SRR1986036			
# Reads	19398267	15032360	18001213			
# paired reads	19398267 (100.00%)	15032360 (100.00%)	18001213 (100.00%)			
# aligned concordantly 0 time	1048012 (5.40%)	1188986 (7.91%)	1004962 (5.58%)			
# aligned concordantly exactly 1 time	15759846 (81.24%)	11679622 (77.70%)	14674412 (81.52%)			
# aligned concordantly >1 times	2590409 (13.35%)	2163752 (14.39%)	2321839 (12.90%)			
# pairs aligned concordantly 1 times	1048012	1188986	1004962			
# aligned discordantly 1 time	37514 (3.58%)	88753 (7.46%)	47306 (4.71%)			
# pairs aligned 0 times concordantly or discordantly	1010498	1100233	957656			
# mates make up the pairs	2020996	2200466	1915312			
# aligned 0 times	1488461 (73.65%)	1466337 (66.64%)	1363330 (71.18%)			
# aligned exactly 1 time	350911 (17.36%)	503802 (22.90%)	367151 (19.17%)			
# aligned >1 times	181624 (8.99%)	230327 (10.47%)	184831 (9.65%)			
overall alignment rate	96.16%	95.12%	96.21%			
MII						
Name	SRR2878525	SRR2048236	SRR2878527	SRR2048236	SRR2048237	SRR2048238
# Reads	19476338	5199427	24546728	5199427	4779221	5637428
# paired reads	19476338 (100.00%)	5199427 (100.00%)	24546728 (100.00%)	5199427 (100.00%)	4779221 (100.00%)	5637428 (100.00%)
# aligned concordantly 0 time	8273947 (42.48%)	1704872 (32.79%)	9238046 (37.63%)	1704872 (32.79%)	805759 (16.86%)	58473 (17.00%)
# aligned concordantly exactly 1 time	10424248 (53.52%)	3279806 (63.08%)	14217528 (57.92%)	3279806 (63.08%)	3754696 (78.56%)	4421294 (78.43%)
# aligned concordantly >1 times	778143 (4.00%)	214749 (4.13%)	1091154 (4.45%)	214749 (4.13%)	218766 (4.58%)	257661 (4.57%)
# pairs aligned concordantly 0 times	8273947	1704872	9238046	1704872	805759	958473
# aligned discordantly 1 time	979797 (11.84%)	47529 (2.79%)	1329727 (14.39%)	47529 (2.79%)	67646 (8.40%)	80820 (8.43%)
# pairs aligned 0 times concordantly or discordantly	7294150	1657343	7908319	1657343	738113	877653
# mates make up the pairs	14588300	3314686	15816638	3314686	1476226	1755306
# aligned 0 times	7448569 (51.06%)	2285813 (68.96%)	8028167 (50.76%)	2285813 (68.96%)	1043334 (70.68%)	1228030 (69.96%)
# aligned exactly 1 time	5557838 (38.10%)	929112 (28.03%)	6478867 (40.96%)	929112 (28.03%)	386841 (26.20%)	468827 (26.71%)
# aligned >1 times	1581893 (10.84%)	99761 (3.01%)	1309604 (8.28%)	99761 (3.01%)	46051 (3.12%)	58449 (3.33%)
overall alignment rate	80.88%	78.02%	83.65%	78.02%	89.08%	89.11%
PND12						
Name	9pbCNTL1_SRR6749400	10pbCNTL2_SRR6749401	11pbCNTL3_SRR6749399			
# Reads	14903618	12908435	11208327			
# unpaired	14903618 (100.00%)	12908435 (100.00%)	11208327 (100.00%)			
# aligned 0 times	2981562 (20.01%)	2570856 (19.92%)	2305865 (20.57%)			
# aligned exactly 1 time	9397666 (63.06%)	8470318 (65.62%)	7313920 (65.25%)			
# aligned >1 times	2524390 (16.94%)	1867261 (14.47%)	1588542 (14.17%)			
overall alignment rate	79.99%	80.08%	79.43%			