Supplementary Information

Expanding the CRISPR base editing toolbox in *Drosophila melanogaster*

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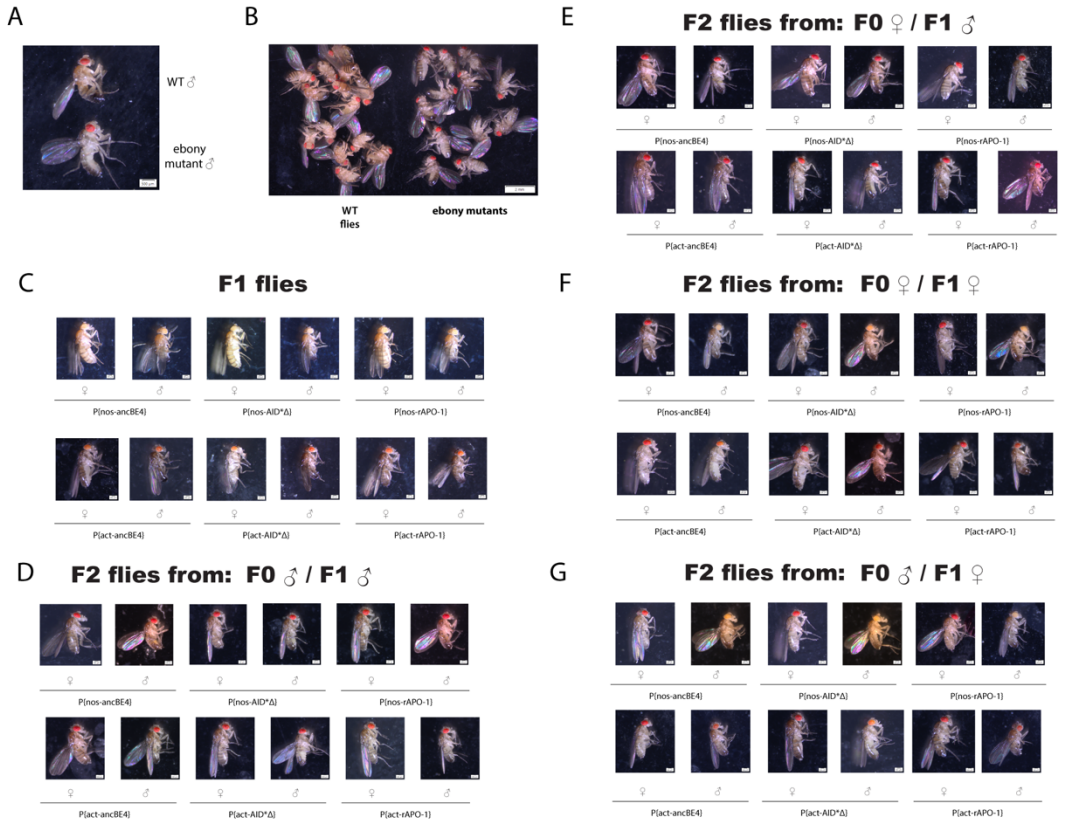
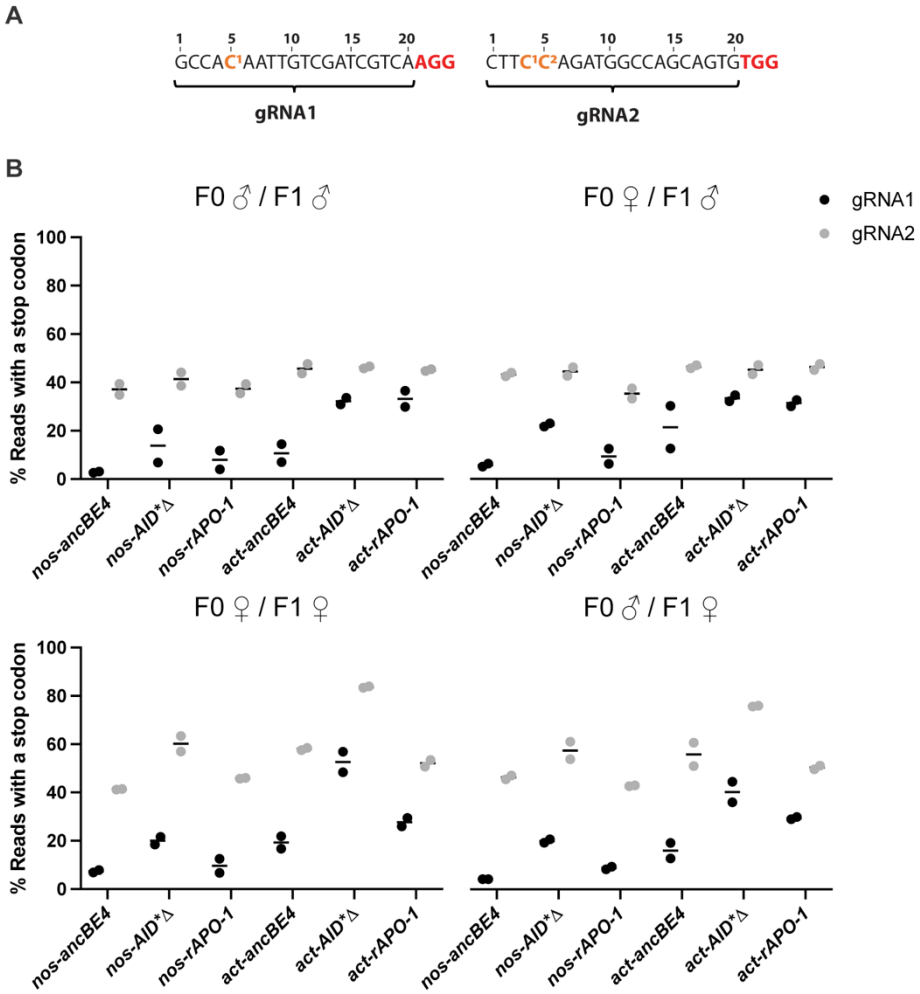
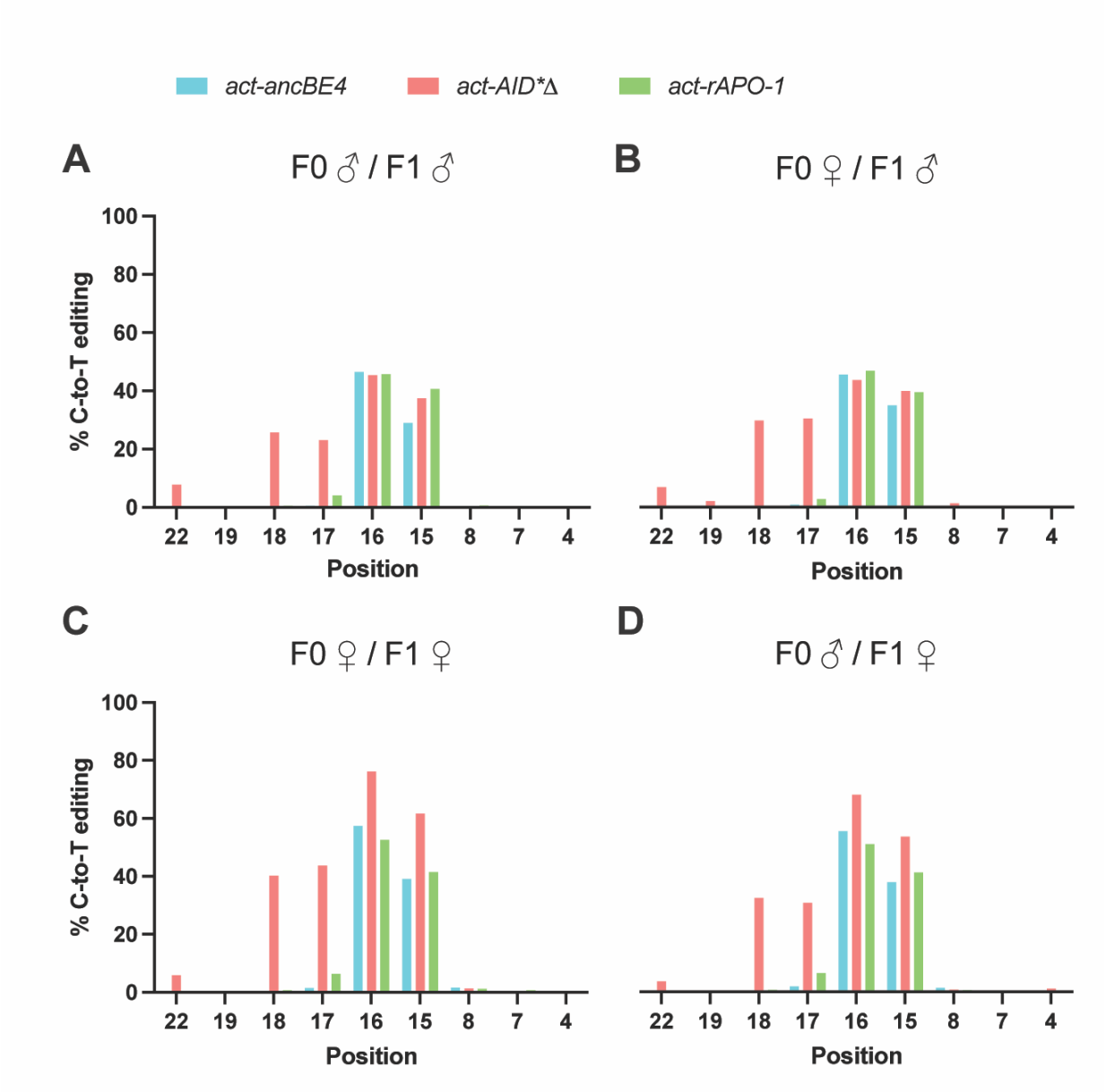


Supplementary figure 1. Ebony phenotypes observed in our F1 and F2 individuals. (a) A comparison between an individual ebony mutant and WT fly; these are flies from our regular stocks (WT and ebony mutant) and are not obtained from our experiments, they are shown as a reference. **(b)** A comparison between a group of ebony mutant and WT flies; these are flies from our regular stocks (WT and ebony mutant) and are not related with our experiments, they are shown as a reference. **(c)** Representative images of flies at the F1 stage, flies expressing base editors from the *actin* promoter displayed an ebony phenotype while those expressing from the *nos* promoter displayed a WT phenotype. This indicates the higher expression of the *actin* promoter in somatic tissues compared to *nos*, which is more germline specific **(d-g)** Representative images of flies at the F2 stage, all flies displayed an ebony phenotype.

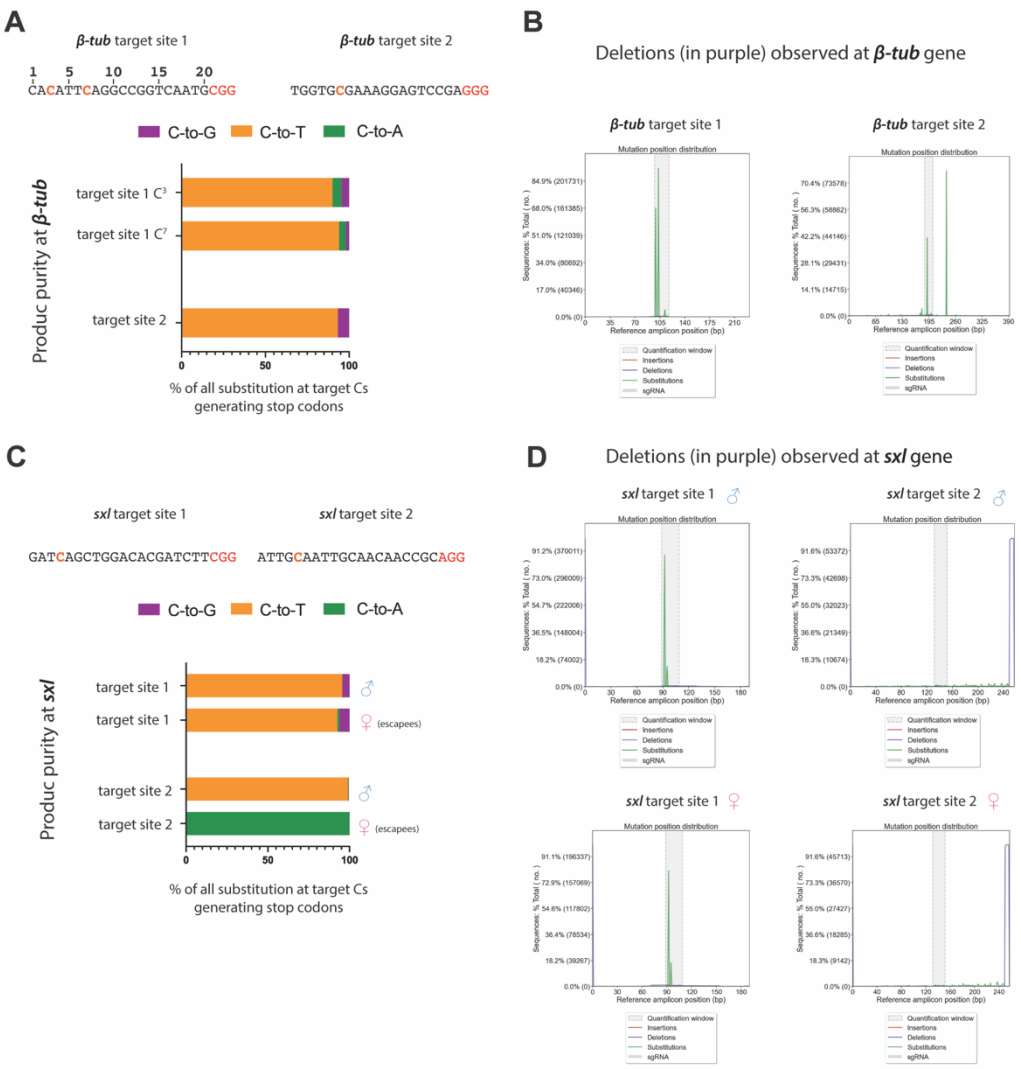


Supplementary figure 2. Stop codon rates at the *ebony* gene produced by two different gRNAs (**a**) Targeted Cs within the gRNAs that are susceptible to be edited and converted to a T. The C to T transition at position 5 on both gRNAs induces a stop codon. (**b**) The percentage of reads with a stop codon for gRNA1 and gRNA2 (n = 2, data is individual measurements (points); the total number of flies used per cross is detailed in **Supplementary table 4**, data is mean ± SD). The gRNA2 displayed higher rates of codon stop generation, indicating that gRNA2 should contribute more than gRNA1 to the observed ebony phenotype in the F2 generation within all conditions tested.



Supplementary figure 3. (**a-d**) The effect of different CBE transmission modes on the base editing window was assessed by examining the four transmission pathways. Position from left (22) to right (4) represents PAM-distal to PAM-proximal regions, respectively. The *AID*Δ* displayed a wider mutation window compared to the *ancBE4* and *rAPO-1* base editors.

Supplementary figure 4. C to T transition rates at *sxl* gene within surviving females (escapees) carrying both transgenes (actin/rAPO1). The gRNA1 introduced stop codons at the target C while the gRNA2 seemed to be almost inactive.



Supplementary figure 5. The type of modifications introduced by the *r-APO1* base editor driven by the *nanos* promoter when targeting *β-Tub* and *sxl* loci. (a) The frequency of of base substitutions at the target C bases within the *β-Tub* locus is depicted. C bases that undergo substitution to T, inducing a stop codon, are highlighted in orange on both the gRNA sequences and product purity graphs. Data presented as the mean frequency of reads with the specified substitution out of all reads harbouring a non-C nucleotide at the target base. PAM sequences (NGG) are highlighted in red. **(b)** The frequency of different types of modifications within the amplicon are depicted. The Y-axis represents the percentage of a given modification, and number of total reads within each percentage are in parenthesis. The X-axis shows the amplicon size. The quantification window refers to the area covered by the gRNA sequence (in gray) where all modifications encountered during deep sequencing are shown. Substitutions (in green) are observed within the quantification window as expected. The green peak outside the quantification window on "target site 2" represents potential polymorphisms within our fly stocks. Insertions (in red) and deletions (in purple) were not observed at any of the target sites of the *β-Tub* gene. **(c)** The frequency base substitutions at the target C bases within the *sxl* locus is shown. C bases that undergo substitution to T, inducing a stop codon, are highlighted in orange on both the gRNA sequences and product purity graphs. Data presented as the mean frequency of reads with the specified substitution out of all reads harbouring a non-C nucleotide at the target base. PAM sequences (NGG) are highlighted in red. **(d)** The frequency of modifications within the amplicon are depicted. Substitutions (in green) are observed within the quantification window for the "target site 1" and no substitutions were observed at the "target site 2", as the gRNA2 was almost inactive. Insertions (in red) and deletions (in purple) are undetectable at any of the target sites of the *sxl* gene. The observed purple peak outside the quantification window (gRNA sequence) represents potential polymorphisms within our fly lines.

Supplementary table 1: plasmids used in this study

Diagram	Name	NCBI Accession	Description
	pMC-12-2-1	PP576063	*Nos* expressed ancBE4max cytidine deaminase
	pMC-12-2-2	PP576064	*Nos* expressed AID*Δ cytidine deaminase
	pMC-12-2-3	PP576065	*Nos* expressed rat-derived APOBEC-1 cytidine deaminase
	pMC-12-4-1	PP576061	*Actin* expressed ancBE4max cytidine deaminase
	pMC-12-4-2	PP576060	*Actin* expressed AID*Δ cytidine deaminase

	pMC-12-4-3	PP576062	*Actin* expressed rat-derived APOBEC-1 cytidine deaminase
	pMC-12-5-1	PP576066	Contains two gRNAs for targeting the *ebony* gene
	pMC-12-5-7	PP576068	Contains two gRNAs for targeting the *sxl* gene
	pMC-12-5-9	PP576067	Contains two gRNAs for targeting the *ß-tubulin* gene

Supplementary table 2: primers used in this study

Name	Sequence (5' → 3')	Comment
MC pMBO2744 3,194 F	GCAGAAGATAAATAAATTTCATTTAAC	Primers for removing paqCI sites from pMBO2744
MC pMBO2744 4,285 R	ACGAGTTTTTGATGTAAGGTATGCACG TGTGTAAGTCTTTTGGTTAGAAGA	
MC pMBO2744 4,287 F	TCTTCTAACCAAAAGACTTACACACGT GCATACCTTACATCAAAAACTCGTTT	
MC pMBO2744 4,936 R	ACGCTGCAGACAGTACTTACGTG	
pMC-12-1-1 4,953 F	CTGCAGCGTAAGCTTCGTACGTA GCAAGCTTCGACCGTTTTAACCT	Primers for constructing pMC-12-1-1
pMC-12-1-1-5,887 R	TTCGACGTGCAGGTGCACCTGCA CGTGGCGAAAATCCGGGTCGAA	
pMC-12-1-1 5,914 F	ACGTGCAGGTGCACCTGCACGTC GAAGCGAATCCAGCTCTGGAGCAGA	
pMC-12-1-1 6,827 R	TCTAGAGGTACCCTCGAGCCGCT TCCTGGCCCTTTTCGAGAAACG	
pMC-12-1-2 6,086 F	TCAAGAAGAACCTGATCGGAGCC	Primers for constructing pMC-12-1-2
pMC-12-1-2 9,901 R	GGGTAAACAGGTGGATGATATTCTCG	
pMC-12-2-1 5,891 F	GTAACTTTCGACCCGGATTTTCGCCAT GAGCAGTGAAACCGGACCAGTG	Primers for constructing pMC-12-2-1
pMC-12-2-1 6,574 R	CCGGGAGTCTCGCTGCCGCTCTTCAG GCCTGTAGCCCACAG	
pMC-12-4-1 F	CAGGATCGATCCCCGGGAATTCACCA TGAGCAGTGAAACCGGACCAGTG	Primer for constructing pMC-12-4-1
MC ebony NGS 2nd F	ACACTCTTTCCCTACACGACGCTCTT CCGATCTCCGACTGAGATTCTAAGCCCA	Primers for NGS of *ebony*
MC ebony NGS R	GACTGGAGTTCAGACGTGTGCTCTT CCGATCTTTCGCCTCCAGCAGTATGTG	
LA0095-F	ACACTCTTTCCCTACACGACGCTCTTCCGAT CTCCACTCAGACTGTTTTAAAAGCTCG	Primers for NGS of the first target site of *ß-tub*
LA0096-R	GACTGGAGTTCAGACGTGTGCTCTTCCGA TCTGCCAGAACGAACCGAATCCATG	

LA0097-F	ACACTCTTTCCCTACACGACGCTCTTCCG ATCTCCAAGTATGTGCCACGCGCAATTC	Primers for NGS of the second target site of *ß-tub*
LA0098-R	GACTGGAGTTCAGACGTGTGCTCTTCCGA TCTGAGAAGGTGTTCATGATGCGGTCC	
LA0124-F	ACACTCTTTCCCTACACGACGCTCTTCCG ATCTCGAACAGGTTTCCTATGCACG	Primers for NGS of the first target site of *sxl*
LA0125-R	GACTGGAGTTCAGACGTGTGCTCTTCCG ATCTCCGAACAAAGGCCACACCAC	
LA0126-F	ACACTCTTTCCCTACACGACGCTCTTCC GATCGGGCAATGTTGCTGCTCACA	Primers for NGS of the second target site of *sxl*
LA0127-R	GACTGGAGTTCAGACGTGTGCTCTTCC GATCCCAGAGTGCTACTGCTGCCA	

Supplementary table 3: fly strains used in this study.

Strain	**Description**	**Reference**	**BDSC #**
Wild type	Canton S	[3]	9515
White eye	w^{1118}	[4]	5905
Ebony	e^{1}	[5]	1658
2nd chromosome AttP3	y[1] w[1118]; PBac{y[+]-attP-3B}VK00037	[6]	9752
Dm/act-rAPO-1	y^{1} w^{1118} ; P{*actin*::-rAPO-1}attP-3B	This study	
Dm/act-ancBE4	y^{1} w^{1118} ; P{*actin*::-ancBE4}attP-3B	This study	
Dm/act-AID*Δ	y^{1} w^{1118} ; P{*actin*::-AID*Δ}attP-3B	This study	
Dm/nos-rAPO-1	y^{1} w^{1118} ; P{*nos*::-rAPO-1}attP-3B	This study	
Dm/nos-ancBE4	y^{1} w^{1118} ; P{*nos*::-rAPO-1}attP-3B	This study	
Dm/nos-AID*Δ	y^{1} w^{1118} ; P{*nos*::-AID*Δ}attP-3B	This study	
Dm/pCFD4-2xebony	y^{1} w^{1118} ; P{*U6*::gRNA^{ebony}}attP-3B	This study	

Supplementary Table 4: The samples for the Amp-Seq analysis. WT, wild-type; no., number; CBE, cytosine base editor

Transmission mode	CBE	Sample	Ebony, no.	WT, no.	Total, no.	Ebony (%)
Male/Male	nos-ancBE4	A	70	14	84	83.3
		B	55	18	73	75.3
	nos-AID*Δ	A	78	14	92	84.8
		B	67	5	72	93.1
	nos-rAPO-1	A	78	26	104	75
		B	84	16	100	84
	act-ancBE4	A	88	6	94	93.6
		B	87	0	87	100
	act-AID*Δ	A	74	0	74	100
		B	84	1	85	98.8
	act-rAPO-1	A	81	1	82	98.8
		B	96	1	97	99
Male/Female	nos-ancBE4	A	61	4	65	93.8
		B	77	6	83	92.8
	nos-AID*Δ	A	64	1	65	98.5
		B	55	1	56	98.2
	nos-rAPO-1	A	49	5	54	90.7
		B	73	6	79	92.4
	act-ancBE4	A	58	5	63	92.1
		B	69	1	70	98.6
	act-AID*Δ	A	76	0	76	100
		B	41	0	41	100
	act-rAPO-1	A	100	1	101	99
		B	69	1	70	98.6
Female/Female	nos-ancBE4	A	33	6	39	84.6
		B	32	4	36	88.9
	nos-AID*Δ	A	109	0	109	100

		B	88	2	90	97.8
	nos-rAPO-1	A	90	7	97	92.8
		B	97	3	100	97
	act-ancBE4	A	83	0	83	100
		B	81	2	83	97.6
	act-AID*Δ	A	23	0	23	100
		B	16	0	16	100
	act-rAPO-1	A	105	1	106	99.1
		B	64	4	68	94.1
Female/Male	nos-ancBE4	A	69	8	77	89.6
		B	69	6	75	92
	nos-AID*Δ	A	93	3	96	96.9
		B	100	1	101	99
	nos-rAPO-1	A	60	21	81	74.1
		B	67	14	81	82.7
	act-ancBE4	A	64	1	65	98.5
		B	99	1	100	99
	act-AID*Δ	A	105	0	105	100
		B	81	2	83	97.6
	act-rAPO-1	A	81	2	83	97.6
		B	76	2	78	97.4

REFRENCES

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