Figure S2 Results of quantitative complementation tests. We carried out tests using a series of deficiencies and insertional mutants. The "Interaction" P-value presented assesses the significance of the Founder × Mutant interaction, determining whether there is a significant guantitative failure to complement. Information regarding the positions of deficiencies/insertions is reported based on Release 6 of the Drosophila melanogaster reference genome. Information regarding the genes deleted was taken from FlyBase on March 11, 2017.

2L Deficiencies

7497

BDSC:

7497.Df

Exelixis Deficiency Nicotine Resistance w1118; Df(2L)Exel6011/CyO 0.8 Deletes: 2L:5,147,258..5,305,646 0.6 13 protein-coding genes including: Cyp28d1, Cyp28d2, Cyp4ac1, 0.4 Cyp4ac2, and Cyp4ac3 Interaction: $P < 10^{-9}$ 0.2





- BDSC: 26545 **BSC Deficiency** w1118; Df(2L)BSC693/SM6a
- Deletes: 2L:5,209,495..5,305,646 11 protein-coding genes including: Cyp28d1, Cyp4ac1, Cyp4ac2, and Cyp4ac3

 $P < 10^{-10}$ Interaction:



7957.Df

3R Deficiencies

BDSC:	7957
	Exelixis Deficiency
	w ¹¹¹⁸ ; Df(3R)Exel7306/TM6B, Tb ¹

- Deletes: 3R:10,871,007..11,156,829 18 protein-coding genes including: Ugt86Dd, Ugt86Di, and Ugt86Dc
- $P < 10^{-8}$ Interaction:





- BDSC: 7958 **Exelixis Deficiency** w1118; Df(3R)Exel8152/TM6B, Tb1
- Deletes: 3R:11,154,150..11,200,280 11 protein-coding genes including: Ugt86Dc, Ugt86Da, Ugt86Dg, Ugt86De, Ugt35b, Ugt35a, Ugt86Dj, and Ugt86Dh

Interaction: $P < 10^{-6}$





- BDSC: 9083 **DrosDel Deficiency** w1118; Df(3R)ED5506/TM6C, cu1 Sb1
- Deletes: 3R:10,884,998..11,172,748 19 protein-coding genes including: Uqt86Dd, Uqt86Di, Uqt86Dc, Ugt86Da, Ugt86Dg, Ugt86De, Ugt35b, and Ugt35a

Interaction: $P < 10^{-9}$



2

23530.ins

- BDSC: 23530 Minos Insertion w¹¹¹⁸; Mi{ET1}Cyp28d1^{MB03293}
- Insertion: 2L:5,211,244 Inserts within *Cyp28d1* coding exon

Interaction: $P < 10^{-5}$



23587.ins

- BDSC: 23587 Minos Insertion w¹¹¹⁸; Mi{ET1}Cyp28d2^{MB02776}
- Insertion: 2L:5,208,263 Inserts within *Cyp28d2* coding exon

Interaction: $P < 10^{-14}$



24834.ins

- BDSC: 24834 Minos Insertion w¹¹¹⁸; Mi{ET1}Ugt86Dj^{MB04890}
- Insertion: 3R:11,173,796 Inserts within *Ugt86Dj* coding exon
- Interaction: P < 0.001

Note that there is a larger difference between founder alleles in the 'Control' background than in the 'Mutant' background. This observation is not consistent with an allelic failure to complement, and more likely indicates epistasis.



27861.ins

- BDSC: 27861 Minos Insertion w¹¹¹⁸; Mi{ET1}Ugt86Dh^{MB11311}
- Insertion: 3R:11,177,673 Inserts within the 3'UTR of one of the two *Ugt86Dh* isoforms
- Interaction: P < 0.01

Note that there is a larger difference between founder alleles in the 'Control' background than in the 'Mutant' background. This observation is not consistent with an allelic failure to complement, and more likely indicates epistasis.

