



Figure S1 Sample allele-specific PCR gel. (*) indicates a precise excision of the insertion shown. Both PCR products are around 400 bp (see Materials and Methods). Sizes of molecular weight markers are indicated. (A) Allele-specific PCR gel for *cyp6d2^{SD}* mutation. *P{SUPorP}*KG02287* serves as a positive control. (B) Allele-specific PCR gel for *cyp6d2^{NT}* mutation. *P{GT1}*CG42565* serves as a positive control.

WT -277 AAGAACCACATTCTATTTGGGGTCAACATATTGAAAGCATAATGATATGCTGTTGATATC
SD AAGAACCACATTCTATTTGGGGTCAACATATTGAAAGCATAATGATATGCTGTTGATATC
NT AAGAACCACATTCTATTTGGGGTCAACATATTGAAAGCATAATGATATGCTGTTGAAATC
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WT -217 ATATACCTTTGAAATTAGTTGTGCATTTCTGTAATGTGCTCAACAGGTAGTCTCTTTGCT
SD ATATACCTTTGAAATTAGTTGTGCATTTCTGTAATGTGCTCAACAGGTAGTCTCTTTGCT
NT ATATACCTTTGAAATTAGTTGTGCATTTCTGTCATGGGCTCAACAGGTAGTCTCTTTGCT
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WT -157 TTTTGCCGCCCTGCTTCCCTTGAAGAGTCGAAAGCTTTTTCCAGCTGAGAAGTCACTTGT
SD TTTTGCCGCCCTGCT--CCCTGAAAGAGTCGAAAGCTTTTTCCAGCTGAGAAGTCACTTGT
NT TTTTGCCGCCCTGCTTCCCTTGAAGAGTCGAAAGCTTTTTCCAGCTGAGAAGTCACTTGT

WT -97 TGATTTGCTGGAAGAGCAGCAGAGTCCAGAGAAAAAGACTCGGTTGTTGTAGCGCTTAT
SD TGATTTGCTGGAAGAGCAGCAGAGTCCAGAGAAAAAGACTCGGTTGTTGTAGCGCTTAT
NT TGATTTGCTGGAAGAGCAGCAGAGTCCAGAGAAAAAGACTCGGTTGTTGTAGCGCTTAT

WT -37 CGCGGGTTCTACGTCTATATAAGACCGATGCTCATGCAGTCTGAATTGTACAGTTTGAAG
SD CGCGGGTTCTACGTCTATATAAGACCGATGCTCATGCAGTCTGAATTGTACAGTTTGAAG
NT CGCGGGTTCTACGTCTATATAAGACCGATGCTCATGCAGTCTGAATTGTACAGTTTGAAG

WT 24 GTGTGCATCGAGTAGTTTCGCAAATATTTACCACCTTTTAGAAATTGTTCGATTTTCAATAC
SD GTGTGCATCGAGTAGTTTCGCAAATATTTACCACCTTTTAGAAATTGTTCGATTTTCAATAC
NT GTGTGCATCGAGTAGTTTCGCAAATATTTACCACCTTTTAGAAATTGTTCGATTTTCAATAC

WT 84 CAAAAGTTAGACTAGAGATTCTCAATATGTGGACCATTCCTCGACGATCCTAATCGCGG
SD CAAAAGTTAGACTAGAGATTCTCGATATGTGGACCATTCCTCGACGATCCTAATCGCGG
NT CAAAAGTTAGACTAGAGATTCTCAATATGTGGACCATTCCTCGACGATCCTAATCGCGG

WT 144 GTCTGCTGTACAGATATGTGAAGCGGCACTACACTCACTGGCAGCGATTGGGAGTCGATG
SD GTCTGCTGTACAGATATGTGAAGCGGCACTACACTCACTGGCAGCGATTGGGAGTCGATG
NT GTCTGCTGTACAGATATGTGAAGCGGCACTACACTCACTGGCAGCGATTGGGAGTCGATG

WT 204 AGGAGCCGGCAAAGATTCCCTTTGGCGTAATGGATACTGTCATGAAGCAGGAACGAAGTT
SD AGGAGCCGGCAAAGATTCCCTTTGGCGTAATGGATACTGTCATGAAGCAGGAACGAAGTT
NT AGGAGCCGGCAAAGATTCCCTTTGGCGTAATGGATACTGTCATGAAGCAGGAACGAAGTT

WT 264 TGGGCATGGCTCTGGCTGATATATACGCCCGGCACGAGGGCAAATCGTTGGCATATACA
SD TGGGCATGGCTCTGGCTGATATATACGCCCGGCACGAGGGCAAATCGTTGGCATATACA
NT TGGGCATGGCTCTGGCTGATATATACGCCCGGCACGAGGGCAAATCGTTGGCATATACA

WT 324 TGTGTAACAAACGAAGTATCCTGATCCGAGATGCCCAATTGGCTCGCCAAATCATGACCA
SD TGTGTAACAAACGAAGTATCCTGATCCGAGATGCCCAATTGGCTCGCCAAATCATGACCA
NT TGTGTAACAAACGAAGTATCCTGATCCGAGATGCCCAATTGGCTCGCCAAATCATGACCA

WT 384 GCGATTTCCGAGTTTCCACGATCGCGGGGTGTACGTGGACGAGGATAAGGACCCGTTGT
SD GCGATTTCCGAGTTTCCACGATCGCGGGGTGTACGTGGACGAGGATAAGGACCCGTTGT
NT GCGATTTCCGAGTTTCCACGATCGCGGGGTGTACGTGGACGAGGATAAGGACCCGTTGT

WT 444 CGGCCAACCTTTTTAACCTAAGAGGTGCTTCATGGCGAAATCTACGTCAGAACTTACGC
SD CGGCCAACCTTTTTAACCTAAGAGGTGCTTCATGGCGAAATCTACGTCAGAACTTACGC
NT CGGCCAACCTTTTTAACCTAAGAGGTGCTTCATGGCGAAATCTACGTCAGAACTTACGC

WT 504 CGTCGTTCTCCTCCGGCAAATAAAGGGCATGTTCCGGCACCATCGACGACGTGGGCGATA
SD CGTCGTTCTCCTCCGGCAAATAAAGGGCATGTTCCGGCACCATCGACGACGTGGGCGATA
NT CGTCGTTCTCCTCCGGCAAATAAAGGGCATGTTCCGGCACCATCGACGACGTGGGCGATA

WT 564 AGTTGGTGCAGCATTTGGAGGGGGCCCTTGACCAAAGCGATGAAGTGGAGATCAAGGATG
SD AGTTGGTGCAGCATTTGGAGGGGGCCCTTGACCAAAGCGATGAAGTGGAGATCAAGGATG
NT AGTTGGTGCAGCATTTGGAGGGGGCCCTTGACCAAAGCGATGAAGTGGAGATCAAGGATG

WT 624 TGATGACCACGTATGCGGTGGACATCATTGGGTCAGTCATTTTTGGCTTGGAGATAGACA
SD TGATGACCACGTATGCGGTGGACATCATTGGGTCAGTCATTTTTGGCTTGGAGATAGACA
NT TGATGACCACGTATGCGGTGGACATCATTGGGTCAGTCATTTTTGGCTTGGAGATAGACA

WT 684 GCTTCCGAAATCCAAAAAACGAGTTCCGCGAGATCAGCAGCTCTACGTCTAGGGATGAAT
SD GCTTCCGAAATCCAAAAAACGAGTTCCGCGAGATCAGCAGCTCTACGTCTAGGGATGAAT
NT GCTTCCGAAATCCAAAAAACGAGTTCCGCGAGATCAGCAGCTCTACGTCTAGGGATGAAT

WT 744 CTCTGCTGCTGAAGATTCACAATATGTCCATGTTTCATTTGTCCACCgtgagttcaaaaat
SD CTCTGCTGCTGAAGATTCACAATATGTCCATGTTTCATTTGTCCACCgtgagttcaaaaat
NT CTCTGCTGCTGAAGATTCACAATATGTCCATGTTTCATTTGTCCACCgtgagttcaaaaat

WT 804 aaccagggtatataatgggaatctttatctatatttataactaaatgttacagG
SD aaccagggtatataatgggaatctttatctatatttataactaaatgttacagG
NT aaccagggtatataatgggaatctttatctatatttataactaaatgttacagG

WT 864 ATTGCTAAGCTAATGAACCGCTTGGGCTACGAAAGCCGCATTCCTGACATCCCTGCGAGAC
SD ATTGCTAAGCTAATGAACCGCTTGGGCTACGAAAGCCGCATTCCTGACATCCCTGCGAGAC
NT ATTGCTAAGCTAATGAACCGCTTGGGCTACGAAAGCCGCATTCCTGACATCCCTGCGAGAC

WT 924 ATGATGAAGCGCACAATCGAGTTCCGGGAGGAGCACAACGTGGTGCGAAAGGACATGCTG
SD ATGATGAAGCGCACAATCGAGTTCCGGGAGGAGCACAACGTGGTGCGAAAGGACATGCTG
NT ATGATGAAGCGCACAATCGAGTTCCGGGAGGAGCACAACGTGGTGCGAAAGGACATGCTG

WT 984 CAGCTGCTGATCCGCCCTCCGAAATACGGGCAAGATTGGCGAGGACGACGACCAGGTGTGG
SD CAGCTGCTGATCCGCCCTCCGAAATACGGGCAAGATTGGCGAGGACGACGACCAGGTGTGG
NT CAGCTGCTGATCCGCCCTCCGAAATACGGGCAAGATTGGCGAGGACGACGACCAGGTGTGG

WT 1044 GACATGGAAACCGCACAGGAGCAGCTGAAGTCTATGTTCGATCGAGAAGATTGCCGCCAG
SD GACATGGAAACCGCACAGGAGCAGCTGAAGTCTATGTTCGATCGAGAAGATTGCCGCCAG
NT GACATGGAAACCGCACAGGAGCAGCTGAAGTCTATGTTCGATCGAGAAGATTGCCGCCAG

WT 1104 GCATTCTGTTCTACGTGGCGGGCTCGGAATCCACAGCTGCGGCGTCTGCCTTCACTCTC
SD GCATTCTGTTCTACGTGGCGGGCTCGGAATCCACAGCTGCGGCGTCTGCCTTCACTCTC
NT GCATTCTGTTCTACGTGGCGGGCTCGGAATCCACAGCTGCGGCGTCTGCCTTCACTCTC

WT 1164 TACGAGCTGTCCATGTATCCGGAAGTGTGAAGGAGGCGCAAGAAGAAGTGGATGCTGTG
SD TACGAGCTGTCCATGTATCCGGAAGTGTGAAGGAGGCGCAAGAAGAAGTGGATGCTGTG
NT TACGAGCTGTCCATGTATCCGGAAGTGTGAAGGAGGCGCAAGAAGAAGTGGATGCTGTG

WT 1224 CTGATGAAGCACAATCTGAAGCCCAAGGATAGGTTACCTACGAGGCGGTGCAGGACTTA
SD CTGATGAAGCACAATCTGAAGCCCAAGGATAGGTTACCTACGAGGCGGTGCAGGACTTA
NT CTGATGAAGCACAATCTGAAGCCCAAGGATAGGTTACCTACGAGGCGGTGCAGGACTTA

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WT 1284 AAGTTTCTGGACATCTGCATCATGGgtgggtttcacatagcattcagtttcaacgatcct
SD AAGTTTCTGGACATCTGCATCATGGgtgggtttcacatagcattcagtttcaacgatcct
NT AAGTTTCTGGACATCTGCATCATGGgtgggtttcacatagcattcagtttcaacgatcct
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WT 1344 tagttaaacgtatTTTTTgcagAAACCATTCGCAAATACCCCGGGCTACCATTCTCAATC
SD tagttaaacgtatTTTTTgcagAAACCATTCGCAAATACCCCGGGCTACCATTCTCAATC
NT tagttaaacgtatTTTTTgcagAAACCATTCGCAAATACCCCGGGCTACCATTCTCAATC
*****

WT 1404 GAGAGTGCACCGAAGATTATCCCGTACCTGGAAC TAATCACATCATAGCCAAGGGAACAC
SD GAGAGTGCACCGAAGATTATCCCGTACCTGGAAC TAATCACATCATAGCCAAGGGAACAC
NT GAGAGTGCACCGAAGATTATCCCGTACCTGGAAC TAATCACATCATAGCCAAGGGAACAC
*****

WT 1464 CCATTCTGATCTCGCTCTTTGGCATGCAGCGTGATCCCGTCTATTTTCCCAATCCCAATG
SD CCATTCTGATCTCGCTCTTTGGCATGCAGCGTGATCCCGTCTATTTTCCCAATCCCAATG
NT CCATTCTGATCTCGCTCTTTGGCATGCAGCGTGATCCCGTCTATTTTCCCAATCCCAATG
*****

WT 1524 GCTACGATCCCCATCGCTTCGATTCAAATAACATGAACTACGATCAGGCAGCTTATATGC
SD GCTACGATCCCCATCGCTTCGATTCAAATAACATGAACTACGATCAGGCAGCTTATATGC
NT GCTACGATCCCCATCGCTTCGATTCAATAACATGAACTACGATCAGGCAGCTTATATGC
*****

WT 1584 CTTTGGAGAGGGTCCTAGGCACTGCATAGgtaaagatgtgtttgtaccagtggctcttg
SD CTTTGGAGAGGGTCCTAGGCACTGCATAGgtaaagatgtgtttgtaccagtggctcttg
NT CTTTGGAGAGGGTCCTAGGCACTGCATAGgtaaagatgtgtttgtaccagtggctcttg
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WT 1644 tcttcttctgatcctgttctcttaattctttgccccaaagCTCTTCGCATGGGAAAAGTC
SD tcttcttctgatcctgttctcttaattctttgccccaaagCTCTTCGCATGGGAAAAGTC
NT tcttcttctgatcctgttctcttaattctttgccccaaagCTCTTCGCATGGGAAAAGTC
*****

WT 1704 AACTCCAAGGTGGCGGTGGCTAAGATTTTGGCCAATTTTGATCTGGTTCAATCTCCACGC
SD AACTCCAAGGTGGCGGTGGCTAAGATTTTGGCCAATTTTGATCTGGTTCAATCTCCACGC
NT AACTCCAAGGTGGCGGTGGCTAAGATTTTGGCCAATTTTGATCTGGTTCAATCTCCACGC
*****

WT 1764 AAGGAGGTGGAGTTCCGTTTCGATGCCGCCCTGTTTTAGTGACCAAGGAGCCGTTGAAG
SD AAGGAGGTGGAGTTCCGTTTCGATGCCGCCCTGTTTTAGTGACCAAGGAGCCGTTGAAG
NT AAGGAGGTGGAGTTCCGTTTCGATGCCGCCCTGTTTTAGTGACCAAGGAGCCGTTGAAG
*****

WT 1824 CTGCGCTTGACCAAGAGAAAAGTAAATCAAAAATATGTGGCTATTGTATG-----TAA
SD CTGCGCTTGACCAAGAGAAAAGTAAATCAAAAATATGTGGCTATTGTATG-----TAA
NT CTGCGCTTGACCAAGAGAAAAGTAAATCAAAAATATGTGGCTAAAAGCGAATATACTATAT
*****

WT 1876 TTGCAATTTGTATGTATAGATATATATCGATACTCAAATAAATTAATACATTGGTTTTAT
SD TTGCAATTTGTATGTATAGATATATATCGATACTCAAATAAATTAATACATTGGTTTTAT
NT AATAAATTTTATGTATAGATATATATCGATACTCAAATAAATTAATACATTGGTTTTAT
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WT 1936 TGATCATCTACCAAGATAAT
SD TGATCATCTACCAAGATAAT
NT TGATCATCTACCAAGATAAT
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Figure S2 Nucleotide sequence of *Cyp6d2* alleles. Nucleotide positions relative to *Cyp6d2* transcription start site are indicated. Red: untranslated regions, Blue: protein coding regions, Black uppercase: upstream intergenic region, Black lowercase: introns. Asterisk below sequence indicates all three alleles are identical at this position. Highlighted SNPs predicted to change amino acid sequence. Two stocks for each allele were sequenced to confirm changes: WT (*P{SUPor-P}KG06046*, *P{SUPor-P}KG01596*), SD (*P{SUPor-P}KG02287*, *P{SUPor-P}KG06675*), NT (*Mi{ET1}CG3746*, *P{GT1}CG42565*). Alignment performed using Clustal Omega (Sievers et al. 2011) and boundaries are as defined by FlyBase.

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Ding JSO, et al. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology* 7: 1–6.

Table S1 Amino acid changes found in non-complementing region

Gene	Mutation
<i>CG30195</i>	I12S
<i>CG34445</i>	D26V
	E55K
	K128Q
<i>CG3746</i>	E20G
	S44A
<i>Cyp6d2</i>	A459P/R
<i>CG13511</i>	A49T