

Supplementary Material

Overexpression of a cytochrome P450 and a UDP-glycosyltransferase is associated with imidacloprid resistance in the Colorado potato beetle, *Leptinotarsa decemlineata*

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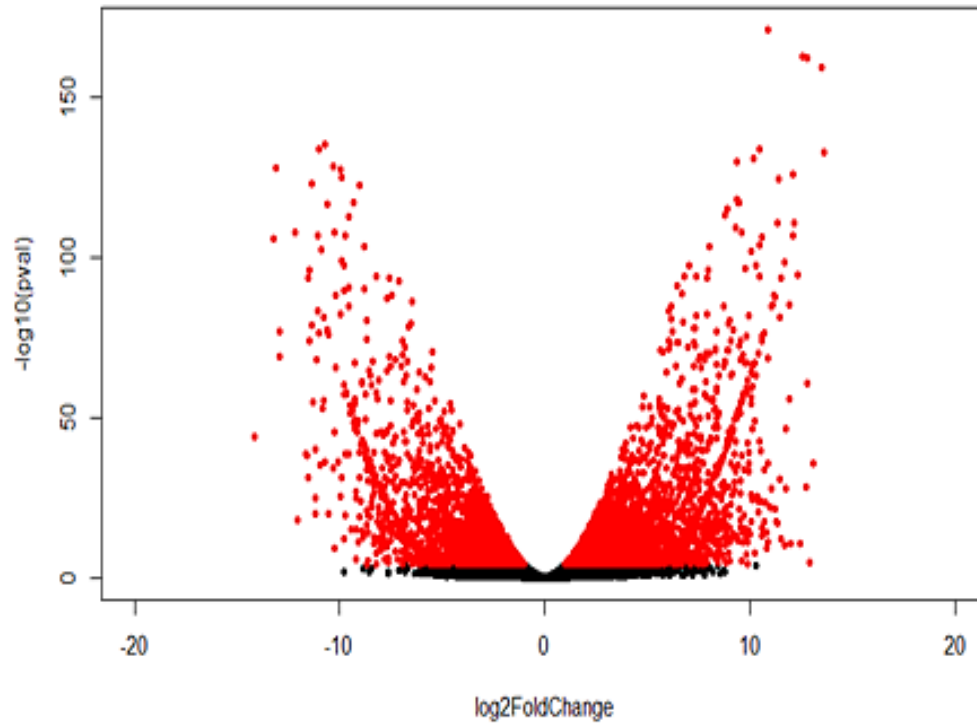


Figure S1. Volcano plot showing differentially expressed contigs between the RS and SS strains of the Colorado potato beetle. 7572 contigs showed differential expression, and of these 4220 showed increased and 3352 showed decreased transcript levels in the RS beetles. Contigs that were differentially expressed at FDR of ≤ 0.001 and fold change of $|\log_2| \geq 1$ are colored red.

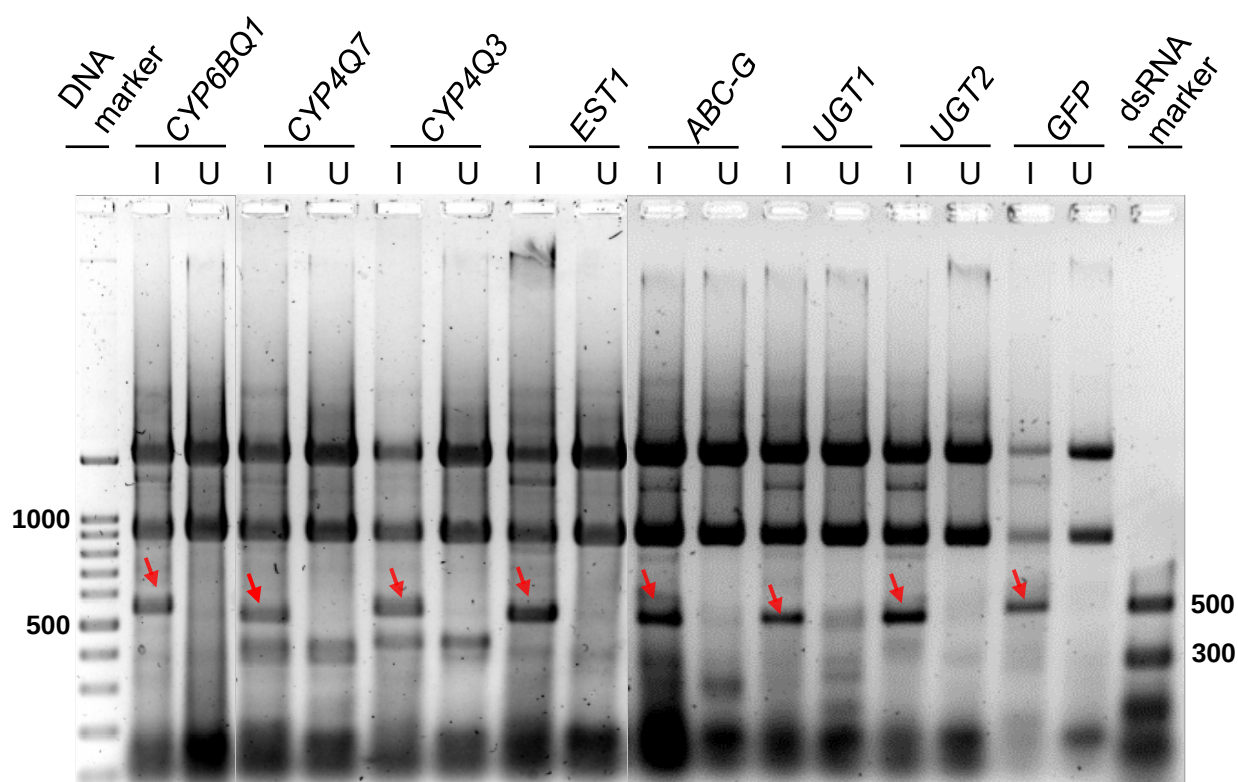


Figure S2. Production of dsRNA in *E. coli* HT115 for target genes. I and U indicate lanes loaded with a total RNA sample extracted from bacteria that were induced or not induced with IPTG, respectively. The positions of dsRNA species are marked with red arrows. Sizes of nucleic acid markers are as indicated.

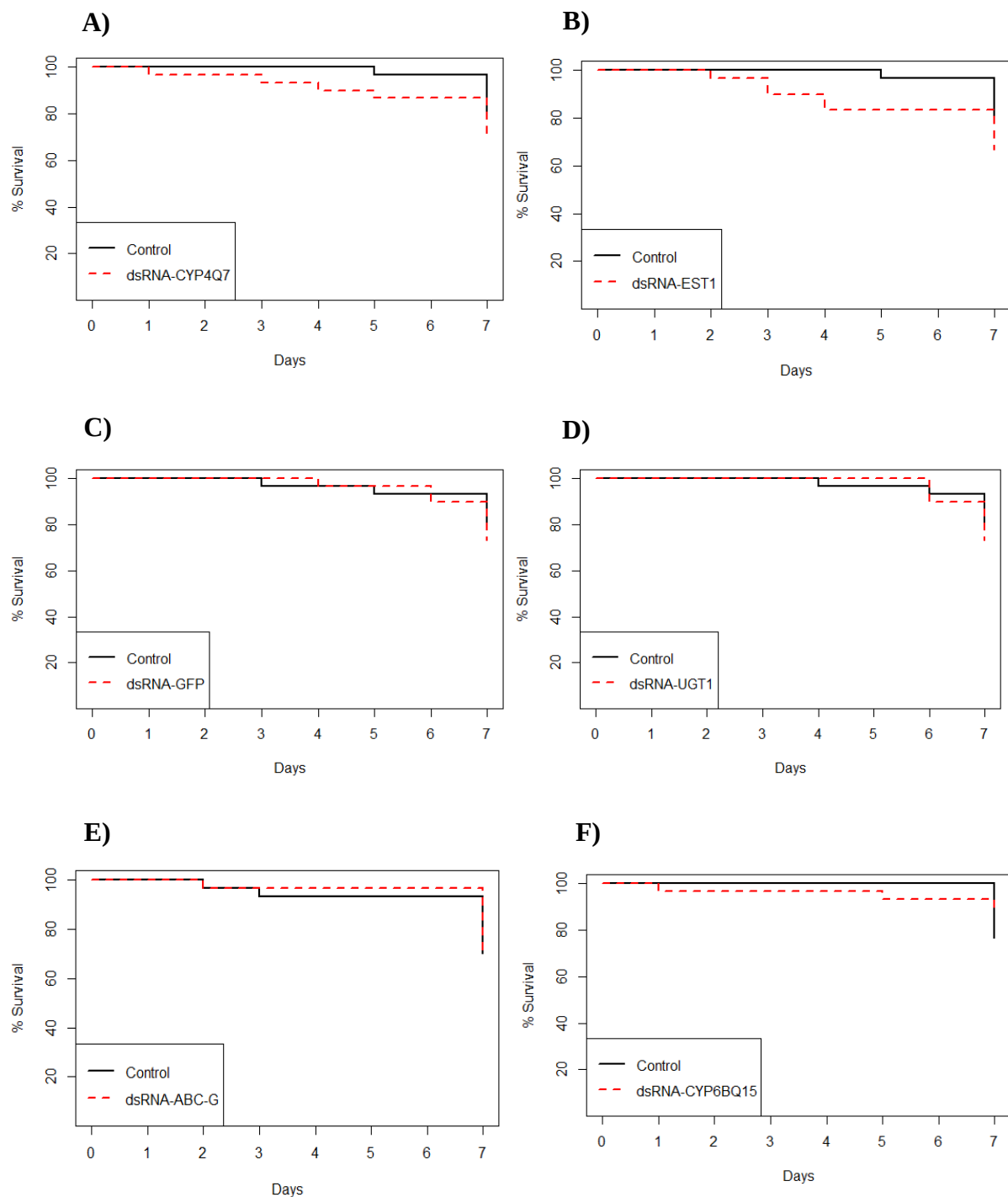


Figure S3. Kaplan-Meier survival curves illustrating the percent survival of the RS beetles exposed to LD₂₀ of imidacloprid after RNAi of target genes. Beetles either ingested *E. coli* HT115 (control) or *E. coli* HT115 producing dsRNA for **A) CYP4Q7**, **B) EST1**, **C) GFP**, **D) UGT1**, **E) ABC-G**, and **F) CYP6BQ15** genes.

Table S1. Summary of mRNA-seq data before and after mapping.

Sample name	Total raw reads	Mapped reads	% mapped	Uniquely mapped	% uniquely mapped
RS biorep1	58,892,932	51,250,746	87.0	22,586,506	44.1
RS biorep2	62,045,496	53,781,131	86.7	22,718,902	42.2
RS biorep3	55,059,038	48,065,489	87.3	20,613,349	42.9
SS biorep1	59,130,951	51,889,523	87.7	21,596,640	41.6
SS biorep2	61,911,228	53,098,114	85.8	22,677,904	42.7
SS biorep3	53,963,077	46,221,323	85.6	20,124,899	43.5

Table S2. List of significantly differentially expressed contigs encoding detoxifying enzymes (CYP, EST, UGT, and GST) and ABC transporters in the RS beetles compared to the SS beetles.

Contig ID ¹	Sequence Description ²	Read count SS ³	Read count RS ³	Log2 Fold change	P-adj ⁴	Regulation In RS
CYPs						
Ld_rep_c34031	CYP6BQ15	8.33	943.26	6.82	1.04E-32	Up
Ld_rep_c51084	CYP6K1	0.32	25.86	6.32	1.21E-09	Up
Ld_c20712	CYP4G57	1.61	108.33	6.07	8.18E-28	Up
Ld_rep_c41850	CYP6BJ1	0.65	40.4	5.97	8.94E-05	Up
Ld_rep_c61559	CYP6EF1	0.33	16.29	5.61	7.99E-06	Up
Ld_rep_c91876	CYP9Z12V1	0.32	10.78	5.06	7.62E-04	Up
Ld_rep_c33314	CYP4Q3	61.04	960.36	3.98	2.77E-38	Up
Ld_rep_c48733	predicted CYP	5.07	73.43	3.86	1.45E-12	Up
Ld_rep_c25417	CYP4Q	113.41	1592.4	3.81	7.08E-22	Up
Ld_rep_c36308	CYP6BU1	2.92	34.48	3.56	6.57E-08	Up
Ld_rep_c27085	CYP412A2	31.1	210.53	2.76	1.40E-15	Up
Ld_c756	CYP412A2	59.35	354.61	2.58	3.02E-16	Up
Ld_rep_c45335	CYP9Z14V3	14.79	81.61	2.46	1.59E-05	Up
Ld_rep_c63019	CYP413A1	13.15	67.8	2.37	1.48E-07	Up
Ld_c981	CYP12A4	504.67	2435.68	2.27	2.83E-16	Up
Ld_c259	CYP6BQ15	1995.9	7776.35	1.96	5.78E-06	Up
Ld_rep_c30474	CYP301B1	62.9	240.41	1.93	4.53E-06	Up
Ld_c20506	CYP6EH1	823.72	3049.34	1.89	2.55E-04	Up
Ld_c72702	CYP12H2	261.14	910.62	1.8	3.68E-10	Up
Ld_rep_c75503	CYP6BQ16	104.44	341.18	1.71	3.90E-04	Up
Ld_rep_c24490	CYP6BQ15	802.12	2476.07	1.63	4.88E-09	Up
Ld_c22309	CYP314A1	161.76	445.14	1.46	7.85E-06	Up
Ld_c55986	CYP314A1	240.19	617.71	1.36	5.60E-05	Up
Ld_rep_c68743	CYP4G57	0	228.6	NA	9.88E-34	Up
Ld_rep_c34317	CYP6BQ15	145.65	30.33	-2.26	3.65E-10	Down

Ld_rep_c75371	<i>CYP412A1</i>	52.29	9.77	-2.42	1.29E-06	Down
Ld_c20095	<i>CYP412A2</i>	41.63	7.6	-2.45	1.15E-05	Down
Ld_rep_c34168	<i>CYP4Q7</i>	100.01	15.34	-2.71	4.76E-11	Down
Ld_rep_c60423	<i>CYP4C1</i>	107.94	2.39	-5.5	2.42E-19	Down
Ld_rep_c48659	<i>CYP6BK17</i>	43.89	0.66	-6.06	2.11E-14	Down
ESTs						
Ld_rep_c71421	<i>EST4</i>	0.31	194.83	9.28	3.74E-32	Up
Ld_rep_c36657	<i>Carboxyl EST1</i>	0.32	82.38	7.99	8.41E-19	Up
Ld_rep_c34698	<i>EST5</i>	3.53	880.32	7.96	2.97E-31	Up
Ld_rep_c77075	<i>EST Beta</i>	0.32	51.99	7.33	2.29E-05	Up
Ld_rep_c35289	<i>Acetylcholin EST1</i>	5.15	292.39	5.83	7.48E-42	Up
Ld_rep_c53802	<i>EST FE4</i>	1.92	65.66	5.1	5.85E-16	Up
Ld_rep_c35399	<i>EST2</i>	14.51	389.18	4.75	1.44E-22	Up
Ld_rep_c46562	<i>EST3</i>	5.26	121.55	4.53	5.34E-15	Up
Ld_c2942	<i>EST1</i>	299.34	4078.42	3.77	7.68E-39	Up
Ld_rep_c24217	<i>EST6</i>	730.27	2934.8	2.01	3.25E-10	Up
Ld_c2931	<i>Carboxyl EST2</i>	134.3	530.97	1.98	2.49E-09	Up
Ld_rep_c25830	<i>Acetyl Cholinest2</i>	1409.01	3374.43	1.26	5.83E-04	Up
Ld_c5150	<i>EST7</i>	1084.67	2203.66	1.02	7.14E-04	Up
Ld_rep_c28597	<i>EST8</i>	0	181.41	NA	5.25E-46	Up
Ld_rep_c36550	<i>EST FE4</i>	60.88	0	NA	1.27E-21	Down
Ld_rep_c34853	<i>Venom Carboxyl EST6</i>	73.02	0	NA	7.20E-25	Down
Ld_rep_c68979	<i>Carboxyl EST4</i>	373.43	107.2	-1.8	5.46E-06	Down
Ld_rep_c33690	<i>EST10</i>	245.23	47.19	-2.38	1.58E-11	Down
Ld_rep_c33908	<i>EST FE4</i>	353.71	51.71	-2.77	6.86E-09	Down
Ld_rep_c36417	<i>EST FE4</i>	528.18	74.76	-2.82	2.35E-09	Down
Ld_rep_c24505	<i>Acetyl Cholinest</i>	1924.85	185.92	-3.37	7.24E-06	Down
Ld_rep_c24395	<i>EST11</i>	47.12	1.01	-5.55	1.59E-14	Down
Ld_rep_c26610	<i>Alpha EST</i>	469.03	1.73	-8.08	4.67E-21	Down

GSTs						
Ld_rep_c33018	<i>GST Sigma1</i>	3.24	2034.78	9.3	1.00E-106	Up
Ld_rep_c40253	<i>GST Sigma2</i>	1.58	91.86	5.86	1.99E-13	Up
Ld_rep_c24170	<i>GST1</i>	38.51	2058.82	5.74	1.88E-68	Up
Ld_rep_c41971	<i>GST Sigma3</i>	4.2	105.26	4.65	2.79E-22	Up
Ld_rep_c24256	<i>GST Delta1</i>	232.16	1275.78	2.46	4.29E-18	Up
Ld_rep_c26032	<i>GST Epsilon6</i>	96.75	449.15	2.21	2.92E-13	Up
Ld_rep_c44006	<i>GST2</i>	27.08	122.07	2.17	6.89E-09	Up
Ld_c19072	<i>GST2C1-Like</i>	12.51	50.78	2.02	4.86E-05	Up
Ld_rep_c24751	<i>GST Sigma4</i>	2715.74	7397.42	1.45	1.02E-04	Up
Ld_rep_c38387	<i>GST Epsilon</i>	574.55	1438.38	1.32	5.21E-06	Up
Ld_rep_c33334	<i>GST Omega1</i>	389.81	892.55	1.2	6.62E-05	Up
Ld_rep_c46479	<i>GST Theta</i>	158.96	359.43	1.18	2.62E-04	Up
Ld_rep_c25066	<i>GST Epsilon3</i>	799.76	1757.33	1.14	1.25E-04	Up
Ld_rep_c50771	<i>GST3</i>	0	36.27	NA	1.38E-04	Up
Ld_rep_c48065	<i>GST Delta2</i>	0	107.98	NA	2.25E-33	Up
Ld_rep_c54053	<i>GST Epsilon7</i>	474.9	171.67	-1.47	1.18E-06	Down
Ld_rep_c43735	<i>GST5</i>	939.47	148.33	-2.66	2.79E-20	Down
Ld_rep_c34301	<i>GST</i>	249.18	38.28	-2.7	2.72E-13	Down
Ld_rep_c38198	<i>GST7</i>	815.64	0.69	-10.2	7.68E-86	Down
UGTs						
Ld_rep_c84840	<i>UGT2C1</i>	0.33	356.14	10.06	1.31E-52	Up
Ld_rep_c41594	<i>UGT1</i>	0.65	192.9	8.22	7.10E-30	Up
Ld_rep_c83152	<i>UGT7</i>	0.64	124.34	7.61	5.06E-20	Up
Ld_rep_c45975	<i>Antennal enriched UGT</i>	2.61	70.11	4.75	1.46E-17	Up
Ld_rep_c28339	<i>UGT2B15</i>	23.96	95.58	2	6.09E-07	Up
Ld_rep_c58571	<i>UGT3</i>	16.15	63.67	1.98	1.37E-05	Up
Ld_c190	<i>UGT2</i>	147.93	522.5	1.82	9.03E-10	Up
Ld_c269	<i>UGT4</i>	409.05	1174.08	1.52	3.64E-04	Up
Ld_rep_c39043	<i>UGT2B23</i>	54.94	144.31	1.39	1.23E-04	Up
Ld_rep_c33389	<i>UGT5</i>	576.6	1281.78	1.15	1.08E-04	Up

Ld_rep_c38005	<i>Antennal-enriched UGT</i>	0	143.76	NA	1.18E-34	Up
Ld_rep_c35232	<i>UGT6</i>	0	56.36	NA	1.96E-10	Up
Ld_rep_c30928	<i>UGT2C1</i>	0	458.59	NA	1.77E-72	Up
Ld_rep_c28388	<i>UGT7</i>	543.8	0.35	-10.62	5.63E-75	Down
Ld_rep_c84951	<i>UGT2C1-like</i>	71.06	21.57	-1.72	8.60E-05	Down
ABC transporters						
Ld_rep_c28427	<i>ABC-G</i>	0.66	22.29	5.09	5.20E-05	Up
Ld_rep_c27116	<i>MRP 4-1</i>	0.66	12.63	4.27	4.95E-04	Up
Ld_c11003	<i>ABC-B6</i>	3.88	61.31	3.98	1.53E-07	Up
Ld_c571	<i>MRP 4-2</i>	37.77	285.89	2.92	5.86E-08	Up
Ld_rep_c26545	<i>ABC-B6 mitochondrial</i>	241.68	615.56	1.35	6.66E-06	Up
Ld_c62808	<i>MRP-2</i>	0	80.57	NA	2.90E-27	Up
Ld_rep_c34742	<i>MRP-4-3</i>	0	291.03	NA	4.25E-59	Up
Ld_c24118	<i>MRP-4 like4</i>	0	14.12	NA	5.25E-06	Up
Ld_c7947	<i>MRP like</i>	123.94	47.61	-1.38	2.40E-04	Down
Ld_c12043	<i>MRP 4-like5</i>	275.31	91.46	-1.59	7.75E-07	Down
Ld_c73069	<i>MRP like</i>	399.13	121.75	-1.71	2.47E-08	Down
Ld_c24114	<i>MRP 4-like 5</i>	416.18	117.69	-1.82	2.03E-09	Down
Ld_c56678	<i>MRP</i>	334.54	38.75	-3.11	3.47E-07	Down
Ld_c6433	<i>MRP-1</i>	368.52	11.59	-4.99	2.73E-41	Down
Ld_rep_c91275	<i>MRP 4-4</i>	619.43	0.35	-10.81	6.23E-79	Down

¹Contig ID from the reference transcriptome³³

²Genes selected for qPCR validation of mRNA-seq data are bolded

³Read counts represent mean normalized counts from three biological replicates

⁴Adjusted *P*-value based on false discovery rate (FDR)⁵⁵-corrected α cut-off of 0.001

NA = not available

Table S3. Estimated fold change differences for seven genes in the RS beetles compared to the SS beetles from qPCR and DESeq analyses.

Contig ID ¹	Gene ²	Fold change in qPCR ³	Fold change in DESeq	Trend ⁴
Ld_rep_c33314	<i>CYP4Q3</i>	5.79	15.73	S
Ld_rep_c34031	<i>CYP6BQ15</i>	79.38	113.21	S
Ld_rep_c34168	<i>CYP4Q7</i>	7.24	0.15	O
Ld_c2942	<i>EST1</i>	6.22	13.62	S
Ld_c190	<i>UGT2</i>	3.91	3.53	S
Ld_rep_c41594	<i>UGT1</i>	1565.56	298.79	S
Ld_rep_c28427	<i>ABC-G</i>	35.67	33.98	S

¹Contig ID from the reference transcriptome³³

²Genes selected for RNAi studies are bolded

³qPCR fold changes are means from independent biological samples

⁴S = same trend and O = opposite trend in estimated transcript levels of the genes from two methods

Table S4. Nucleic acid sequences used for production of dsRNA in *E. coli* HT115.

Contig ID ¹	Sequence Description	Sequence ²
Ld_rep_c34031	CYP6BQ15	<u>AACATCCTCACGGACCATTCCATTATTGGCGATGC</u> CATAGACATCAAGGATGTCGTATCCCGATTACAA CTGACGTGATAGGTTCTGTAGCTTTCGGAATAGAY TGTAATAGTCTCAAGGATCCCGACTCCGAATTCAG GCATTGGGGAAAAAGGATATTCACGTTTCGATTTTA TGAGGAGAATCAAGAACAATATAACAATGTTGATT CCGAGGGATATTGTGATCAAGACAGGCATAAAATT GATGTCACGTGACTTGAAGACTTCTTCATGAACG TGGTCAGAAGCACAGTTCAGTTCAGAGAACTCAC AACGTCCATAGGAAGGATTTTCATGCACTTGCTGTT ACAACCTGAAGAATAAAGGACAAATTGCTGAGGAC GATAGCACTGATAAAGAAATCGAAATTAAGGCAC <u>CC</u>
Ld_rep_c34168	CYP4Q7	<u>CATCTCCTGACGTCCGAATCCACTAAAATTGTAGT</u> AAATACATGAGATGTATAGCCATGGTTTCTTCAAC CTCTGGARAAATATGTGTCCCATTTTATTAATGGAA GTTATGTATTCTTTATCCTTTTTAGTTTTCTGGTTCA GTTTGGTCCCCATGGATGATTCTGCGATAGTATTCA ATGTAAACTCTGATATCAATGGAAAGACATTGGTC GAGGTTTTTTCAGTTTCATTTCTAAGAACTTCCACT AATTTTTTCGTTTCATTATTGAAAACCTCCAACAAAT TGCTGCAAAATACTGRAATGAAAGGCTGGTGTTCAM GATTTTTTCGACGAGTCTGCCATTTGGAACCTGTACT TGTCAACAAGCCTTCTCCTAGCCAGCGATTCA
Ld_rep_c33314	CYP4Q3	<u>TTGGACCAGCAATCGCCTCAAATTCCTCTGCACCT</u> GTAATGTTACACCAGGGTAGAGAGAAGTCATGTC AATTCTGTAAATTGGTCCGTATGTTCTAGCCCAGTA TCTCACCTTGCGGAATAACGTTATATCGTCGCCCA AAAATTCAAGAACATTTTTCAAGACGGGTATAGGT TTTGGCCCAGGCAGTCCTTTCAAAATTTTCAACGTT CTCAAATGCTCGATGAAACCTCTCACAAGAAAAAA TAATATCACACATCCTGCCACCACAACCTGAAAAAT TTACTATAAACATTTTCAGTATATTCAATTCAAAAGT TCTTTGTCAAGAACAATCGTCTGAATAAATCTACT ACGCGTTGTGAACGCTGTCAATAATTTTTCTATTCTG AACAACCTTTTGAAGTGTTTTCGTGCGA

Ld_c2942	<i>EST1</i>	<u>TCGAATCCAACAAGTGGTGATCCACTTTCCATGAT</u> AGCTCCACGGAAGAGCTCTTCTCCTCCATTTTCTG TGCTAATACATGCAGACTCACAGATAATGAACCTG AACTTTCACCTACAAGGGTAACTTTTGCAGGATCT CCTCCAAATAAATGAATATTTTCATTCACCCATTTA ATTGCAAACCTTTTGATCTTTTCAGGCCGATGTTGGCC GGTATAATGTCATCTTCTGTTGTGGTGAATCCAAAT GGCCCCAATCGGTAATTGAAAGTTACTACAATTAT CTCATGATCCATGATGTATTTTGGTCCAGAATATTC ATATGTACTTGATTCTGATGATAAACCTCCACCATG TATCCAAAGTAAAAGTGGCAATTTCTTGATT <u>ACT</u> <u>ACCAGGTTTCAGCGGC</u>
Ld_rep_c28427	<i>ABC-G</i>	<u>TGGTGACTTTTCCACTGGGAATCGATTCTTTGTCTT</u> GTGGGGTCAACATGTCTCCCCTCGTTACACTGAGT CTCCCCTCATCTTGCTCTTTACTGAGCATCAGAAAA ACGTCTTCTAATGTTTGACTATTGTA AAAAAGCCAA AAGCCTTTCCGGGTTCTCTTGAGCCAAAATGATTCC CCCTCTCAACAAACAGATCTTATCAGTCTGCCTAC ATTCCTCAATATAGTGTGTGGTTATTATCACAGAA GTTCCTAGTTTCTTCGTCATATCGACTAGATAGTTC CATATTCTCTCCCTCAAAAGTGGATCAACTCCCACC GTTGGTTCATCCAGTATTAACAACCTCTGGTTTGTGG ATAACTGCCGAGGCAAAGGACACTCTTC
Ld_rep_c41594	<i>UGT1</i>	<u>TCACTCATGGCGGTTTGTGAGTACAACAGAAACA</u> ATCTATCATGGTGTACCAATACTCGCTATTCTGTT TTTGGGGATCAGAAGATGAATGCTGCAAAGGCGGT TGCAGCAGGATATGGTTTATCTTTATCAATAAACG AGTTGAGCGAGGAAAACCTTATCTAACAGCATAAAT GAACTTTTGAATAATTCTGAAGTATAGGGACAATGC AAAAAGAAGATCCGCAATCATGCACGATCGAAAA GTGAAGCCCATGGATCTTGCAACATATTGGATCGA ATTTGTAGTCCGACATAAAGGAGCACCACATTTGA GGGTAGCTGCCCTTGATTTAACTTGGTACCAATATT TCCTCGTGGATATTGTTCTTCTTGTGGGAGCTGTTG TTGCCAGTTTGATGCTAGCGTC
Ld_c190	<i>UGT2</i>	<u>TTTCCATCTCCGCATGAAATATTGCTGAAACTTGGA</u> GAACAACCTATTTATAATGTAGCAGAATAAGAATG AAGTGAAGAATATAAATCCCAAAATATCCAACAA

		GTAAAACTGGTAGAGCGGTATGTCTGAAGCGGGGT TTCGTAGCTCTTTGGCTCCTCTATTCCTTATAACGT ATTCAGTCCACCATAACAGCCTTTTCCAAACCACTCA TTGGTTCATCACCAAGAAGACTGGCAAGCTTTCTA ATGGACGATTTATACTTCTCATTATGTGTAACCTCC ATGATGGCATCTCTCAAATCCTTATAGCTCAAAGC CGGTTTATGATAAATTTGTTTCCCAATATTTTTATT CTCTACGATTTCGAGCGTTTTTTCAGCTGATCTCCGAA <u>GAACGGCATAGCTA</u>
-	<i>GFP</i>	<u>CCATGCCCCGAAGGTTATGTACAGGAAAGAACTATA</u> TTTTTCAAAGATGACGGGAACACAAAGACACGTAA GTTTAAACAGTTCGGTACTAACTAACCATACATAT TTAAATTTTCAGGTGCTGAAGTCAAGTTTGAAGGT GATACCCTTGTTAATAGAATCGAGTTAAAAGGTAT TGATTTTAAAGAAGATGGAAACATTCTTGGACACA AATTGGAATACAACCTATAACTCACACAATGTATAC ATCATGGCAGACAAACAAAAGAATGGAATCAAAG TTGTAAGTTTAAACATGATTTTACTAACTAACTAAT CTGATTTAAATTTTCAGAACTTCAAAATTAGACAC AACATTGAAGATGGAAGCATTCAACTAGCAGACCA TTATCAACAAAATACTCCAATTGGCGATGGCCCTG <u>TCCTTTTACCAGACAACCATTACCTGTCC</u>

¹Contig ID from reference transcriptome³³

²Primers used (without restriction enzyme cut sites) are underlined

Table S5. Topical bioassay results data used to calculate resistance ratio at LD₅₀ of imidacloprid in adult CPB

CPB Strain	Dose (µg)	BR	N	Time period (days)												Total (M+D)	% mortality ¹	% corrected mortality ²	Approximate LD ₅₀ RR ³		
				1		2		3		4		5		6						7	
				M	D	M	D	M	D	M	D	M	D	M	D					M	D
SS	0.0 (acetone control)	1	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	53	25.3		
		2	10	0	0	0	0	0	0	0	0	0	0	0	0	0					
		3	10	2	0	0	0	0	0	0	0	0	0	0	0	0					
	0.19	1	10	8	0	5	1	3	2	4	2	4	2	3	2	1	3			4	
		2	10	9	0	8	0	5	0	5	0	7	0	4	2	3	3			6	
		3	10	7	1	3	1	6	2	4	2	5	2	5	2	4	2			6	
RS	0.0 (acetone control)	1	10	2	0	0	0	0	0	0	0	0	0	0	0	0	0	55			
		2	10	0	0	0	0	0	0	0	0	0	0	0	0	0				0	
		3	10	0	0	0	0	0	0	0	1	0	1	0	1	0	1			1	
	4.8	1	10	10	0	9	0	6	1	8	1	6	2	3	3	5	1			6	
		2	10	10	0	7	0	5	0	8	0	3	2	2	3	2	3			5	
		3	10	7	1	4	1	4	1	5	1	3	2	3	2	4	2			6	

M= total number of moribund beetles at given day

D= total number of dead beetles at a given day

BR = biological replicate

N= number of adult beetles used per biological replicate

¹Percent mortality was calculated by dividing total number of dead and moribund beetles at the end of 7th day by total number of beetles used in the bioassay

²Percent corrected mortality was calculated using Abbott's formula

³Approximate LD₅₀ resistance ratio (RR) was calculated by dividing approximate LD₅₀ of imidacloprid for RS by approximate LD₅₀ of imidacloprid for SS

Table S6. List of primers used in qPCR analysis.

Contig ID ¹	Gene	Forward and reverse primers (5'-3')	Primer Efficiency (%) ²	Amplicon size (bp) ³
Ld_rep_c33314	<i>CYP4Q3</i>	TACCCTGGTGTGAACATTAC AATGAAAGGCTGGTGTCAAG	97.1	178
Ld_rep_c34031	<i>CYP6BQ</i> <i>15</i>	TAGGCTGACCCCAACATTCA AATGGAATGGTCCGTGAGGA	93.4	103
Ld_rep_c34168	<i>CYP4Q7</i>	CAGCCTAAGACTTCCTTGATG GTTTCGAGGGATTTGACACTAC	96.1	193
Ld_rep_c28427	<i>ABC-G</i>	TCACCTCCACTACAGTCAAC GCTCTGGTGGAAAGTCTAAC	99.6	158
Ld_rep_c41594	<i>UGT1</i>	AGCACCACATTTGAGGGTAG GGTGAGTGAAGATGAGATCC	95.9	207
Ld_c190	<i>UGT2</i>	TCTCCGAAGAACGGCATAG GAGTCATCTCTCCCTTGAATGT	95.2	176
Ld_c2942	<i>EST1</i>	ACCCTGCCACTTTTCCACTT ACTGACACAATCGGTGACG	94.4	177
-	<i>L8E</i>	GGTAACCATCAACACATTGG TCTTGGCATCCACTTTACC	97.4	124
-	<i>ARF1</i>	GACTGCAAGTAGGAGAAGTTG TCGGCAGAGTCTACCACAT	94.1	181
-	<i>EF1A</i>	CAGGGCAAGGTTTGAAAGATAA CCATCAGCACAGTTCCCAT	99.6	168

¹Contig ID from reference transcriptome³³

²Primer efficiencies were tested by generating standard curves following the guidelines described⁵⁹

³PCR products were sequenced to confirm amplification of correct sequences

Table S7. List of primers used in cloning and sequencing of plasmid constructs.

Contig ID ¹	Gene	Forward and reverse primers (5'-3') ²	Product size (bp)
Ld_rep_c34031	<i>CYP6BQ15</i>	TAGCGGCCGCAACATCCTCACGGACCATTC ACAGGTCGACGGGTGCCTTAATTTTCGATTTC	420
Ld_rep_c34168	<i>CYP4Q7</i>	TAGCGGCCGCCATCTCCTGACGTCCGAATC ACAGGTCGACTGAATCGCTGGCTAGGAGAAG	387
Ld_rep_c33314	<i>CYP4Q3</i>	TAGCGGCCGCTTGGACCAGCAATCGCCT ACAGGTCGACTCGCACGAAAACACTTCAAA	413
Ld_c2942	<i>EST1</i>	TAGCGGCCGCTCGAATCCAACAAGTGGTGA ACAGGTCGACGCCGCTGAAACCTGGTAGTA	408
Ld_rep_c28427	<i>ABC-G</i>	TAGCGGCCGCTGGTGACTTTTCCACTGGG ACAGGTCGACGAAGAGTGTCTTTGCCTC	384
Ld_rep_c41594	<i>UGT1</i>	TAGCGGCCGCTCACTCATGGCGGTTTGTG ACAGGTCGACGACGCTAGCATCAAACCTGGC	409
Ld_c190	<i>UGT2</i>	TAGCGGCCGCTTTCCATCTCCGCATGAAAT ACAGGTCGACTAGCTATGCCGTTCTTCG	404
-	<i>GFP</i>	TAGCGGCCGCCCATGCCCCGAAGGTTATGTA ACAGGTCGACGGACAGGTAATGGTTGTCTGG	449
-	L4440 sequencing primers	GACCGGCAGATCTGATATCATC CTCACTGGCCGTCGTTTTAC	-

¹Contig ID from the reference transcriptome³³

²*NotI* and *SalI* restriction enzyme cut sites are underlined

Table S8. Plasmid constructs used in this study.

Plasmid¹	Description	Source
L4440, Amp^R	RNAi feeding vector, empty backbone	Addgene plasmid # 1654
GFP::L4440, Amp^R	Contains full length <i>GFP</i> sequence	Addgene plasmid # 11335
GFP-RNAi::L4440, Amp^R	dsRNA production for <i>GFP</i> control	This study
CYP4Q3::L4440, Amp^R	dsRNA production for <i>CYP4Q3</i>	This study
CYP4Q7::L4440, Amp^R	dsRNA production for <i>CYP4Q7</i>	This study
CYP6BQ15::L4440, Amp^R	dsRNA production for <i>CYP6BQ15</i>	This study
ABC-G::L4440, Amp^R	dsRNA production for <i>ABC-G</i>	This study
UGT1::L4440, Amp^R	dsRNA production for <i>UGT1</i>	This study
UGT2::L4440, Amp^R	dsRNA production for <i>UGT2</i>	This study
EST1::L4440, Amp^R	dsRNA production for <i>EST1</i>	This study

¹Amp^R is resistance to ampicillin (β -lactamase)