Figure S1: Heatmaps for overall data, including caged bees, Time 0 bees and bees in-hive for all target genes. Both heatmaps are generated in order to visualize the highest similarity per target genes and samples, respectively.

Fig. S1

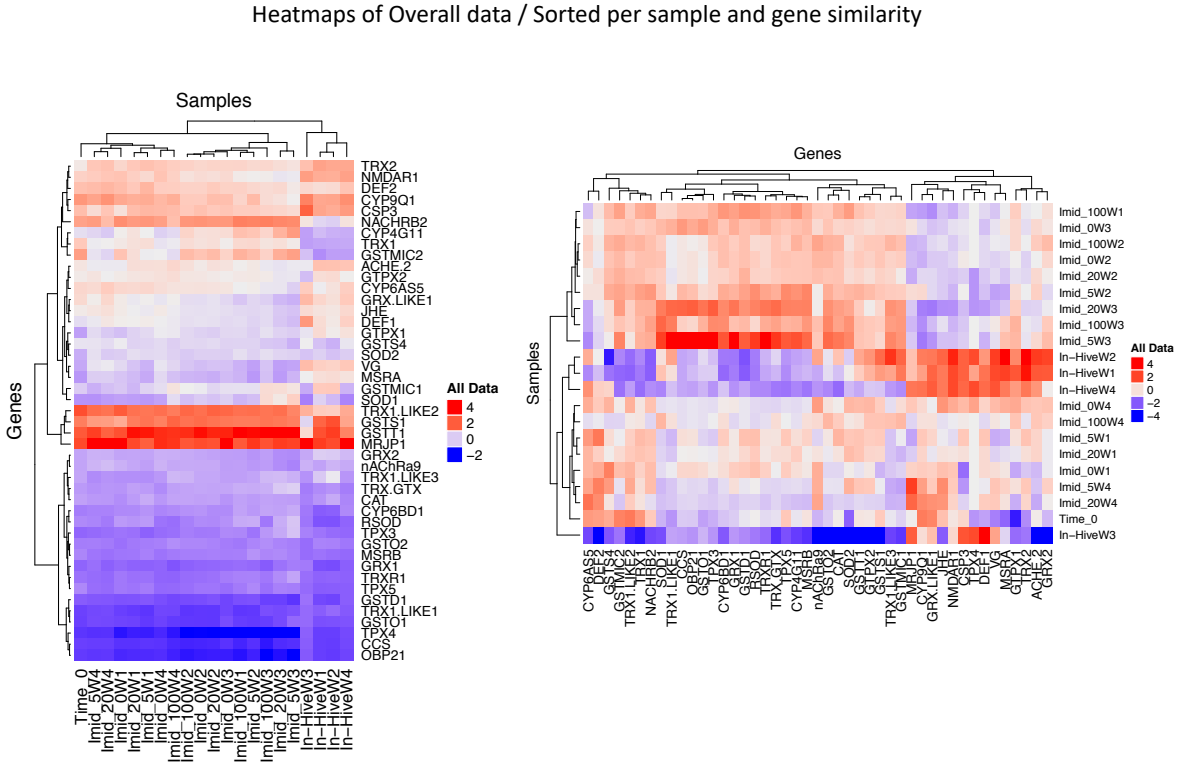


Figure S2 Weekly analysis of gene expression for caged bees exposed to imidacloprid compared with the control (0 PPB). MDS plots are given per week as well as hierarchical clustering plots. Right set of plots shows no DEGs at any time point.

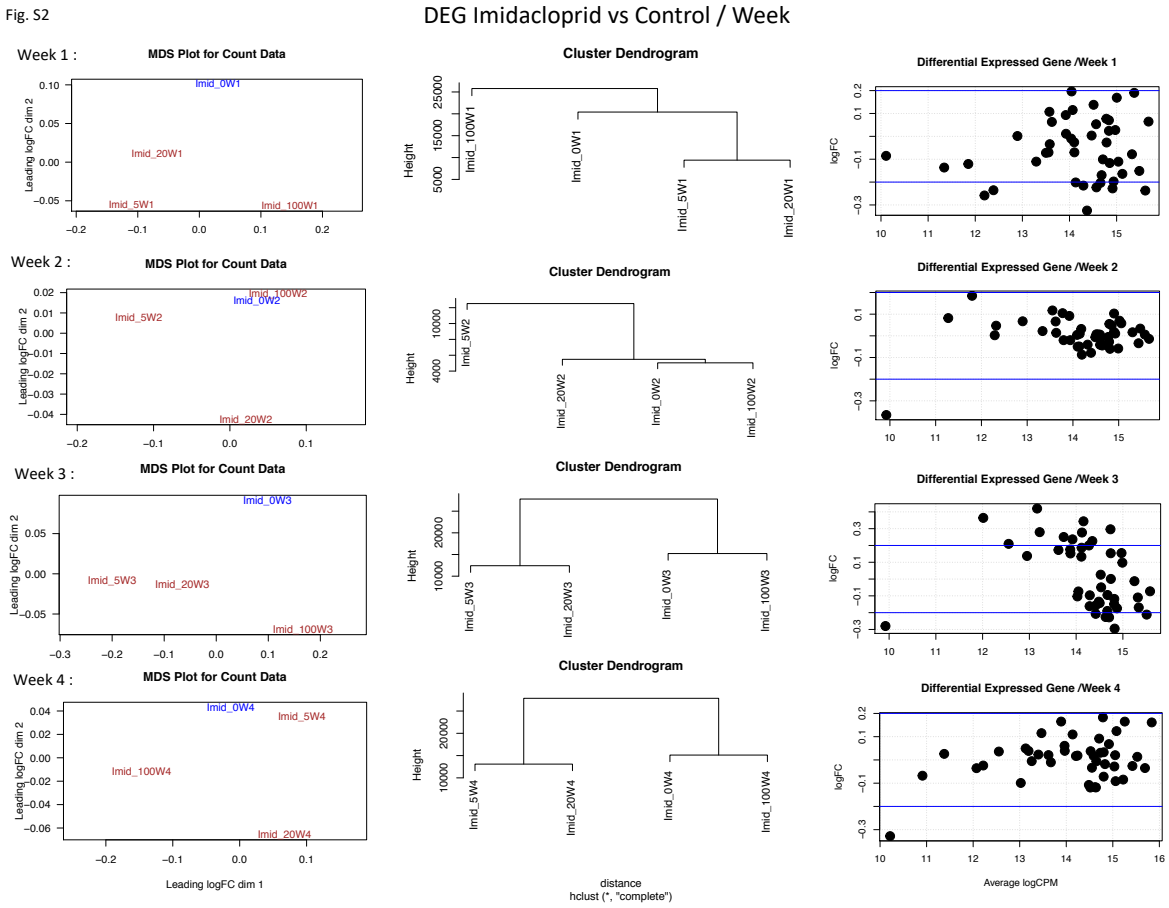


Figure S3 Shows a multi-dimensional scaling MDS of the DEG between Cage and Hive bees based on log-fold-changes between each pair of RNA samples as well as an hierarchical clustering plot of the same dataset.

Fig. S3

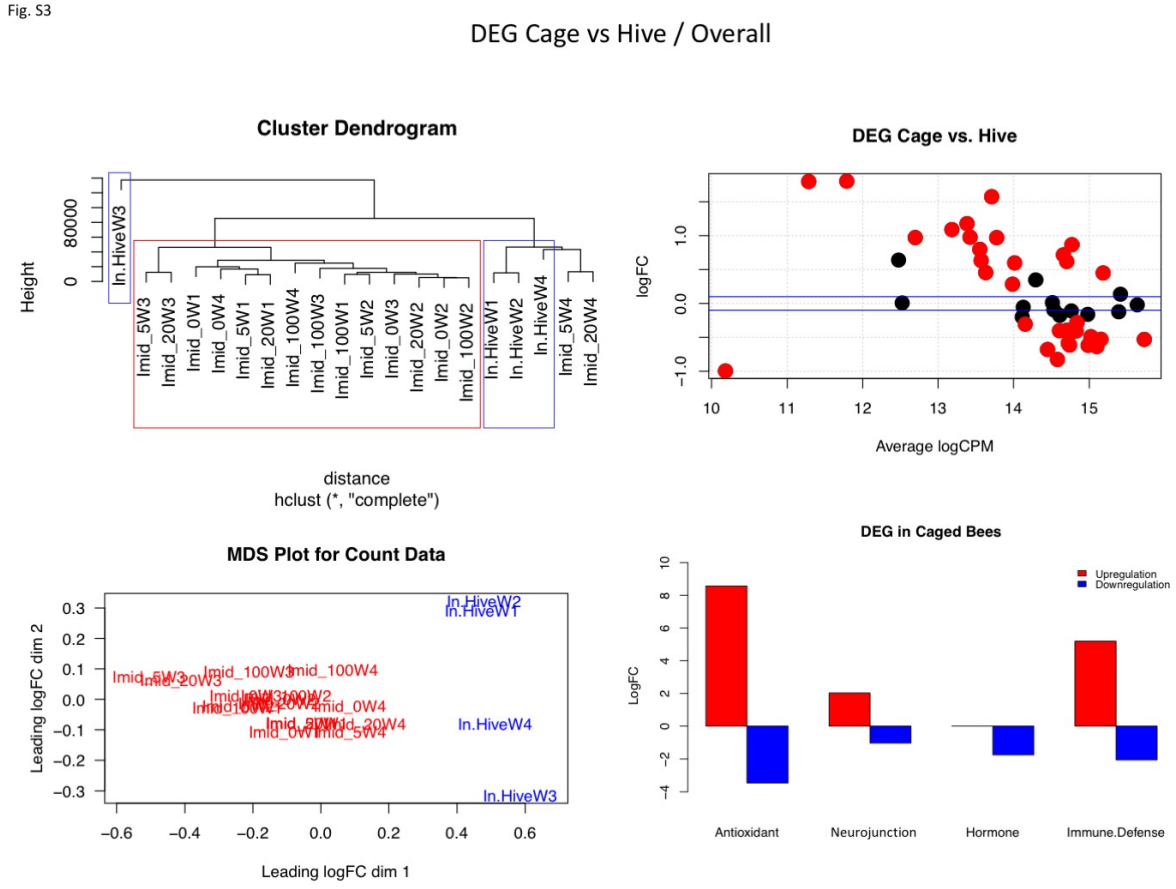
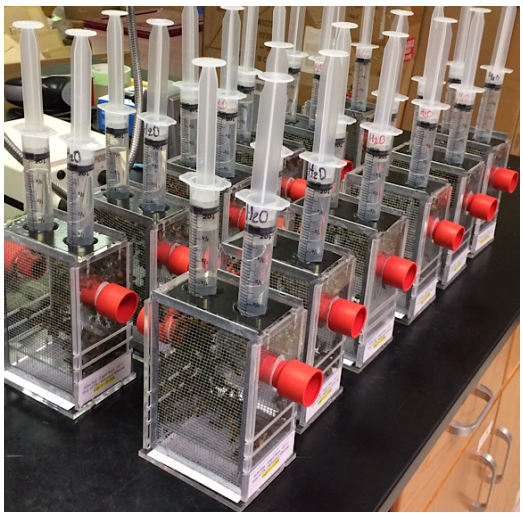
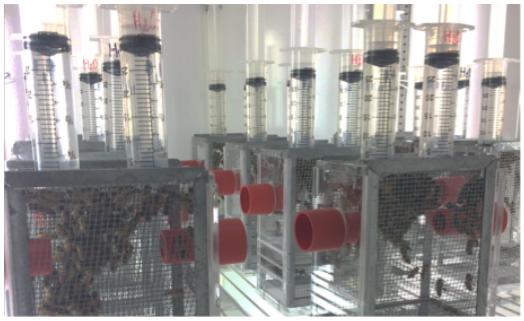


Figure S4: General view of the cages used in this experiment. Protein patties were provided to caged bees inside the red robber plugs, water and syrup treatment through both upper syringes. View of bees feeding on syrup and patty inside the cages.

Fig. S4





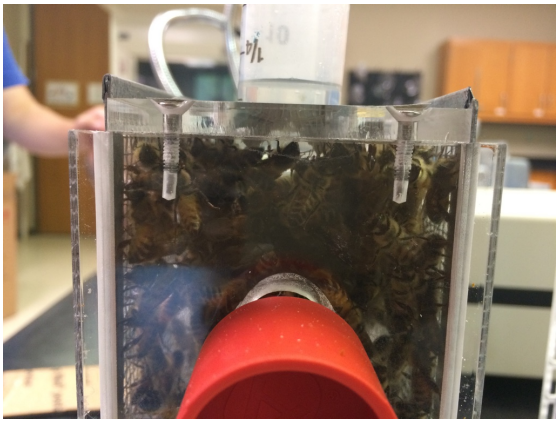


Table S1. Descriptive list of the studied genes targeted by RNA-seq

N	Gene code	Description/known gene function	Gene ID: NCBI/Beebase	References
Target genes				
Antioxidant				
1	Cat	Catalase	443552	Alburaki et al., 2017a
2	GstO1	Pyrimidodiazepine synthase	552118	Corona and Robinson, 2006
3	GstO2	Glutathione S-transferase omega-1	726823	Corona and Robinson, 2006
4	GstS1	Glutathione S-transferase S1	552304	Corona and Robinson, 2006; Liu et al., 2016
5	GstS4	Glutathione S-transferase S4	411045	Alburaki et al., 2017a; Corona and Robinson, 2006
6	GSTmic1	Microsomal glutathione S-transferase 1	410837	Corona and Robinson, 2006
7	GSTmic2	Uncharacterized LOC725853/Protein coding	725853	Corona and Robinson, 2006
8	GstT1	Glutathione S-transferase theta-1	552314	Corona and Robinson, 2006
9	Gtpx1	Glutathione peroxidase-like 1	494523	Corona and Robinson, 2006
10	Gtpx2	Probable phospholipid hydroperoxide glutathione peroxidase	726269	Corona and Robinson, 2006
11	GstD1	Glutathione S-transferase D1	409490	Alburaki et al., 2017a; Corona and Robinson, 2006
12	Sod1	Superoxide dismutase 1	409398	Corona and Robinson, 2006
13	Sod2	Superoxide dismutase 2	410082	Corona and Robinson, 2006
14	MsrA	Methionine sulphoxide reductase A	409097	Corona and Robinson, 2006
15	MsrB	Methionine sulphoxide reductase B	724494	Corona and Robinson, 2006
16	Trx-1	Thioredoxin, mitochondrial	410120	Corona and Robinson, 2006
17	Trx-2	Thioredoxin-2	409451	Corona and Robinson, 2006
18	Trx/Gtx	Glutaredoxin 3	409355	Corona and Robinson, 2006
19	Trx1-like1	Thioredoxin-like protein 1	550734	Corona and Robinson, 2006
20	Trx1-like2	Uncharacterized LOC725664/Protein coding	725664	Corona and Robinson, 2006
21	Trx1-like3	Endoplasmic reticulum resident protein 44	552191	Corona and Robinson, 2006

N	Gene code	Description/known gene function	Gene ID: NCBI/Beebase	References
22	Trxr-1	Thioredoxin reductase 1	410032	Corona and Robinson, 2006
23	Tpx3	Thioredoxin peroxidoase 3	408540	Corona and Robinson, 2006
24	Tpx4	Thioredoxin peroxidoase 4	551975	Corona and Robinson, 2006
25	Tpx5-6	Peroxiredoxin-6	411852	Corona and Robinson, 2006
26	CCS	Copper chaperone for superoxide dismutase	552629	Corona and Robinson, 2006
27	Grx-like1	Uncharacterized LOC411159/Protein coding	411159	Corona and Robinson, 2006
28	Grx1	Glutaredoxin-C4	727309	Corona and Robinson, 2006
29	Grx2	Glutaredoxin-related protein 5, mitochondrial	552835	Corona and Robinson, 2006
30	Rsod	Uncharacterized LOC413369/Protein coding	413369	Corona and Robinson, 2006
Immune defense/sensory				
31	Def1	Defensin-1	406143	Aronstein and Saldivar, 2005; Richard et al., 2012
32	Def2	Defensin-2	413397	Aronstein and Saldivar, 2005; Richard et al., 2012
33	CSP3/ASP	Chemosensory protein 3	406094	Briand et al., 2002
34	Cyp4g11	Cytochrome P450 4G11	409469	Gong and Diao, 2017
35	Cyp6as5	Cytochrome P450 6AS5	409677	Alptekin et al., 2016
36	Cyp6bd1	Cytochrome P450 6k1	551560	Li et al., 2014
37	Cyp9q1	Cytochrome P450 9e2	410492	Mao et al., 2011
38	Obp21	Odorant binding protein 21	551935	Iovinella et al., 2011
Development/hormone				
39	Vg	Vitellogenin	406088	Amdam et al., 2006; Bordier et al., 2017; Nelson et al., 2007
40	Jhe/Est	Juvenile hormone esterase	406066	Bordier et al., 2017
41	Mrjp1	Major royal jelly protein1		Buttstedt et al., 2014
Neuro-junction				

N	Gene code	Description/known gene function	Gene ID: NCBI/Beebase	References
42	AChE-2	Acetylcholinesterase 2	406104	Alburaki et al., 2017a
43	nAChRa9	Nicotinic acetylcholine receptor alpha9 subunit	411303	Alptekin et al., 2016
44	nAChRb2	Nicotinic acetylcholine receptor beta2 subunit	726079	Alptekin et al., 2016
45	Nmdar1	NMDA receptor 1	406079	Mussig et al., 2010
Housekeeping genes				
1	Ancr1	AncR-1 non-coding nuclear RNA	100049571	Alburaki et al., 2017a
2	Camkii	Calcium/calmodulin-dependent protein kinase II	551691	Alburaki et al., 2017a
3	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase 2	XM_393605.6	Alburaki et al., 2017a
4	E2F	Transcription factor E2F4	XM_006566781.2	Alburaki et al., 2017a

Genes included are involved in various processes: antioxidant, immune defense, nervous system regulation and hormone production. Target genes were standardized against four housekeeping genes.

Table S2. Differentially expressed genes (DEGs) between caged bees and in-hive bees across 4 weeks

	Gene	FDR	logFC	Regulation
Week 1				
1	Rsod	8.79E-11	2.052154955	Up
2	GSTmic2	1.51E-10	1.283773182	Up
3	cyp6bd1	5.62E-07	1.502223105	Up
4	nAChRb2	1.41E-06	0.760356888	Up
5	Trx-1	1.79E-05	0.815020997	Up
6	Vg	2.85E-05	–0.68666675	Down
7	GSTmic1	0.000105929	–0.625977048	Down
8	Grx1	0.000147178	1.466580514	Up
9	MsrA	0.000147178	–0.640895526	Down
10	Tpx5	0.000326753	1.151666352	Up
11	GstD1	0.000529584	1.626628738	Up
12	Cyp4g11	0.003272353	0.538929922	Up
13	Trx1-like2	0.004426979	0.409447152	Up
14	Trxr-1	0.004426979	0.894300369	Up
15	Gtpx1	0.007143152	–0.45210316	Down
16	Trx1-like3	0.007416819	–0.53308333	Down
17	Trx-2	0.007416819	–0.396484705	Down
18	Nmdar1	0.010552677	–0.390262699	Down
19	AChE-2	0.013164542	–0.384722407	Down
20	CCS	0.013164542	1.789933422	Up
21	Sod1	0.022290396	–0.443546	Down
22	CSP3	0.029977795	–0.347780712	Down
23	Grx-like1	0.038464198	–0.345534644	Down
24	Grx2	0.044258894	–0.395457615	Down
25	GstS1	0.045643338	–0.303379857	Down

	Gene	FDR	logFC	Regulation
				Up=12 Down=13
Week 2				
1	GSTmic2	5.63E-10	1.125683012	Up
2	Rsod	1.38E-09	1.920755161	Up
3	nAChRb2	1.34E-07	0.816655652	Up
4	Cyp6bd1	2.17E-06	1.359483887	Up
5	Grx1	6.34E-06	1.676160098	Up
6	MsrA	6.34E-06	–0.750403485	Down
7	Trx1	1.50E-05	0.793851856	Up
8	Vg	1.50E-05	–0.691147669	Down
9	Cyp4g11	3.29E-05	0.736912478	Up
10	GstD1	4.01E-05	1.850627106	Up
11	Tpx5	0.000248075	1.141077705	Up
12	Nmdar1	0.001369615	–0.462428888	Down
13	Trx1-like2	0.001746155	0.435546394	Up
14	Trx1-like3	0.002126055	–0.540989241	Down
15	Trx2	0.003455925	–0.412143627	Down
16	GSTmic1	0.004504549	–0.442902891	Down
17	MsrB	0.005116035	0.773936843	Up
18	Trxr-1	0.008514979	0.807396999	Up
19	CCS	0.012821076	1.79540416	Up
20	GstS1	0.012821076	–0.29896178	Down
21	Tpx3	0.021384737	0.670350958	Up
22	AChE-2	0.022404618	–0.339632338	Down
23	Cyp9q1	0.022960491	–0.314596096	Down
24	CSP3	0.036059913	–0.295797369	Down
				Up=14 Down=10

Gene		FDR	logFC	Regulation
Week 3				
1	Mrjp1	8.48E-30	–1.334501094	Down
2	CSP3	8.01E-23	–1.378281782	Down
3	Def1	4.70E-21	–1.48284558	Down
4	Cyp9q1	7.19E-17	–1.26844048	Down
5	Vg	1.03E-16	–1.415441134	Down
6	Nmdar1	3.76E-15	–1.219803445	Down
7	Grx-like1	5.39E-15	–1.306168202	Down
8	Cyp6as5	9.79E-08	–0.932079671	Down
9	Sod1	3.25E-07	1.453492666	Up
10	Trx2	3.33E-07	–0.843916112	Down
11	Jhe	3.50E-07	–0.926640082	Down
12	MsrA	3.26E-06	–0.912448636	Down
13	GstS4	6.02E-05	–0.787091217	Down
14	MsrB	0.000167022	1.515519968	Up
15	Trx/Gtx	0.000168527	1.189757184	Up
16	GstO2	0.000209972	1.510206221	Up
17	DefF2	0.000292519	–0.640178689	Down
18	Cyp4g11	0.000370339	0.825928994	Up
19	Trxr-1	0.000606566	1.441878171	Up
20	Rsod	0.000842094	1.255522862	Up
21	Cyp6bd1	0.000895332	1.107718079	Up
22	Tpx5	0.000895332	1.490533886	Up
23	Gtpx1	0.004267108	–0.562430366	Down
24	Trx1-like2	0.004267108	–0.441305624	Down
25	Obp21	0.006305542	3.045853631	Up
26	CCS	0.007839115	2.109938747	Up

Gene		FDR	logFC	Regulation
27	Trx1	0.007928216	0.629539606	Up
28	AChE-2	0.013108948	–0.479519602	Down
29	Tpx3	0.014996185	0.803097403	Up
30	Cat	0.017555048	0.759332849	Up
31	GstS1	0.042206547	0.381846224	Up
32	GstO1	0.048847638	0.957056383	Up
				Up=16 Down=16
Week 4				
1	Vg	0.001242008	–0.606664482	Down
2	CSP3	0.00252203	–0.503672436	Down
3	Nmdar1	0.002523729	–0.489465881	Down
4	MsrA	0.006270594	–0.542183971	Down
5	AChE-2	0.007054297	–0.467422182	Down
6	GSTmic2	0.007054297	0.635050548	Up
7	Trx2	0.007054297	–0.430007244	Down
8	Grx-like1	0.012104495	–0.444598377	Down
9	Def1	0.01305566	–0.44637647	Down
10	Trx1	0.015380605	0.546124297	Up
11	Rsod	0.016919141	0.877252547	Up
12	Gtpx1	0.020410695	–0.422238441	Down
13	Cyp9q1	0.028824892	–0.374761958	Down
14	Tpx5	0.028824892	0.875701234	Down
				Up=3 Down=11
Overall weeks				
1	Trx1	2.31E-13	0.716399134	Up
2	MsrA	5.77E-13	–0.679235231	Down
3	Rsod	5.82E-12	1.574315294	Up

Gene		FDR	logFC	Regulation
4	Vg	5.82E-12	–0.826047318	Down
5	GSTmic2	2.35E-09	0.867077354	Up
6	Tpx5	2.98E-09	1.177112211	Up
7	Trx2	6.22E-09	–0.490134369	Down
8	Nmdar1	1.49E-08	–0.617713476	Down
9	CSP3	3.57E-08	–0.634702226	Down
10	Cyp6bd1	7.29E-08	0.970961205	Up
11	Cyp4g11	3.27E-07	0.618538837	Up
12	AChE-2	1.33E-06	–0.40628725	Down
13	Grx-like1	1.33E-06	–0.584585069	Down
14	Gtpx1	1.41E-06	–0.401699776	Down
15	MsrB	1.41E-06	0.798387461	Up
16	Def1	1.56E-06	–0.613786308	Down
17	Trxr-1	9.85E-06	0.977293279	Up
18	Grx1	1.33E-05	1.089440238	Up
19	Trx/Gtx	1.53E-05	0.598159979	Up
20	Cyp9q1	2.37E-05	–0.529039749	Down
21	CCS	6.18E-05	1.802497238	Up
22	GstD1	8.11E-05	0.971662373	Up
23	nAChRb2	9.14E-05	0.449655327	Up
24	Mrjp	9.30E-05	–0.530617812	Down
25	Jhe	0.000116388	–0.392980093	Down
26	Obp21	0.00012842	1.797821637	Up
27	GstO2	0.000806508	0.455560596	Up
28	Grx2	0.002324083	–0.305279916	Down
29	Tpx3	0.008824973	0.633043926	Up
30	Cat	0.009449226	0.284131552	Up

	Gene	FDR	logFC	Regulation
31	Cyp6as5	0.012545948	−0.281873672	Down
32	Tpx4	0.030356733	−0.995920357	Down
				Up=17 Down=15

Genes are sorted descending from most (false discovery rate, FDR<0.001) to least (FDR<0.05) significant differential. logFC is the log fold-change and the regulation is for bees in cages. Table generated by the EdgeR package.