#### **Supplementary Data**

IP/a       AdATCIGGTGAGAGATAGAGTGGC       GAATCIGGTAAGAGGATGGAC       [318] OR genomic DNA JoAL 408 [1872]       BT8.1       (a)         IP/a       AGATCIGGGTAAGAGGATGGAC       GAATCIGGTAAGAGGTTCGGTGGAAAGG       FS8 OR genomic DNA JoAL 408 [1872]       BT14.1       (b)         IP/a       AGATCIGGATGAAGGAAGCAATGGA       AGATCIGGATGAAGGAAGCAA       GAATCIGGATGAAGGAAGCAA       GAATCIGGATGAAGGAAGCAA       GAATCIGGATGAAGGAAGCAA       GAATCIGGATGAGGAAGCAA       GAATCIGGATGAGGAAGCAA       GGAACGAAGGAAGCAAGGAAGCAA       GAATCIGGATGAGGAAGCAA       GGAAGGAAGCAAGGAAGCAAGGAAGCAA       GGAAGGAAGCAAGGAAGCAAGGAAGCAA       GGAAGGAAGCAAGGAAGCAAGGAAGCAAGCAAGGAAGTGATGAGGAAGGTGATGAGAAACCGGA       GGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGG	Gene <sup>1</sup>	Forward primer (5'-3') <sup>2</sup>	Reverse primer (5'-3') <sup>2</sup>	Length (bp)	Template	Vector	Integration site	Transgenic line code	Source <sup>3</sup>
IP/ID         ADATCTGGGATGGGAGAACGACATCGAT         GAATCTGGGTGGATGGAAAA         GAATCTGGCTGGGTGAAAAA         GAATCTGGCTGGCTAAAGATGGCAAAAAAA         GAATCTGGCTGGCGAAATAAAAAAAAAAAAAAAAAAAAA	lr7a	AGATCTGGTGAAGAATAGAGTGTGGC	GAATTCTTTGAAACGAAACTGTTGCG	2318	OR genomic DNA	pGAL4 attB	attP2	BT58.1	(a)
IP/E         CICGAGGCGGTAGTGGTCCAAATA         AGACTATCGTTGTCLGGTGGTAATTGGGTCGTTTCCGGGGCGT         1958 OR genomic DNA <i>joGAL att</i> gl gttP2         BT10.1         E)           IP/A         AGACTCTACTGGTCGAATGCGGAC         GAATCTGCGCGCGAAGGAAATCGG         600 OR genomic DNA <i>joGAL att</i> gl gttP2         BT93.1         (b)           IP/A         AGACTCTACTGGCGCGAAATCGG         GAATCTACGCACCACAATCGA         600 OR genomic DNA <i>joGAL att</i> gl gttP2         BT98.1         (c)           IP/A         AGACTCTACGACACCACACACCCCGAAATCGG         600 OR genomic DNA <i>joGAL att</i> gl gttP2         BT98.1         (c)           IP/A         AGACTCTACGAATCCGA         AGACTCTACGAAGGACCCCCACAATCGGAAGGAATCGAAGGAAT         2488 OR genomic DNA <i>joGAL att</i> gl gttP2         BT91.1         (c)           IP/B         AGACTCTACGAAGGAACCAC         AGACTCTACGAAGGAACAC         2488 OR genomic DNA <i>joGAL att</i> gl gttP2         BT93.1         (c)           IP/B         AGACTCTACGAAGCAATCA         AGACTCTACGAAGGAATCA         2488 OR genomic DNA <i>joGAL att</i> gl gttP2         BT97.1         (c)           IP/B         AGACTCTACGAAGCCAATCA         AGACTCTCAAGCATGCAATCA         2488 OR genomic DNA <i>joGAL att</i> gl gttP2         BT97.1         (c)           IP/B         AGACTCTACGAAGCCAATCA         AGACTCTACGCAAGTCCAAGGAAGTAC         AGACTCTACGCAAGTCCAAGGAAGTAC         AGACTCTACGCAAGTCCAAGGAGTAGCA         AGACTCTACGCAAGTCCAAGTCGAATCA	lr7b	AGATCTGGGATGAGAACGACATCGAT	GAATTCGGCTAAAGAGTTGCCAAAGG	578	OR genomic DNA	pGAL4 attB	attP2	BT47.1	(b)
IPId       AGATCTACTIGGTCAATGCGATC       AGATCTGGCGAATGTCAAACATTTGG       1010 [OR genomic DNA pGAL4 attB gHP2       BT79.1       (b)         IPId       AGATCTGCCGGTCATCGCAAGGAGACTAG       600 [OR genomic DNA pGAL4 attB gHP2       BT99.1       (b)         IPId       AGATCTGTCCGGATTCGCAATTCGCA       AGATCTGTCGGCGCGCGCGCGTCAGATTCGCAATGCCG       (b)       (c)	lr7c	CTCGAGGCCGGTTAGTGGTCCAAATA	AGATCTATCGTGTTTCATCGGTGGCT	1595	OR genomic DNA	pGAL4 attB	attP2	BT110.1	
If*a         ABATCTTACTGGGCAGAGAGAACTAG         GAATCTTACTGGCCGCACATACTGGAATCGGA         F58.1         (b)           If*a         AAATCTACTGGAATCGGAATCGGAATCGGAATCGGAATTGCGAATTCGAA         766 (DR genomic DNA pGAL4 attB gtP2         8798.1         (c)           If*a         CAATCTATGGAATCGGAATCGGAATTGCGAATTCGAATTCGGA         7766 (DR genomic DNA pGAL4 attB gtP2         8798.1         (c)           If*a         CAATCTATGGAATCGGAATTGCGAATTGCGAATTGCGAATTGCGAATTCGAC         2789 (DR genomic DNA pGAL4 attB gtP2         8798.1         (c)           If*a         AAATCTGTGCAGCACGCGAAG         CAATCTGTGCCGACCGCGAATCGAAGGAAT         2789 (DR genomic DNA pGAL4 attB gtP2         879.1         (c)           If*a         AAATCTGCAGGAATCGAATTGAATTGGAAGAGAAT         2488 (DR genomic DNA pGAL4 attB gtP2         879.1         (c)         (c)           If*a         AAATCTGCAGGAATC         CAATCTGCAGGAATC         2488 (DR genomic DNA pGAL4 attB gtP2         879.1         (c)	lr7d	AGATCTAACTTGTGTCAATGCGATCC	AGATCTGGCGAATGTGAAACATTTGG	1010	OR genomic DNA	pGAL4 attB	attP2	BT79.1	(b)
IPT         AGATCIGECGETATICGAATICCGG         AGATCIGECGAATICCGA         T68 [OR genomic DNA pGAL 4 attB atP2         BT98.1         [b]           IPT         AGATCIGECGAATICCGA         AGATCIGECGETATICGAATICCGG         T68 [OR genomic DNA pGAL 4 attB atP2         BT98.1         [b]           IPT         AGATCIGECACTACC         CCGSCCCCCATCGAAGGACTIGGAATICCG         2499 [OR genomic DNA pGAL 4 attB atP2         BT51.1         [a]           IPT         AGATCIACTATIGGTACCACTACC         CCGSCCCCATCGAAGGACT         2488 [OR genomic DNA pGAL 4 attB atP2         BT51.1         [a]           IPT         AGATCIACGATIGGACGACC         CAATICGACTAGACGACTICGAAGGACT         2488 [OR genomic DNA pGAL 4 attB atP2         BT51.1         [a]           IPT         CAATCICCAGTIGCCAGATICC         CAATICIGCAGATICGAATICGAATICGAATICGAATICGACTICCAGATICGAATICGAATICGAATICGACTICCA         2488 [OR genomic DNA pGAL 4 attB atP2         BT97.1         [b]           IPT         CAATCICCAGATICCAGTICCAGATICGAATICGAATICGAAGGACTICATATICGAATICCA         2489 [OR genomic DNA pGAL 4 attB atP2         BT97.1         [b]           IPT         CAATCICCAGATICCAGTICCAGATICGAATICGAAGGACTICATICGAATICCA         2489 [OR genomic DNA pGAL 4 attB atP2         BT97.1         [b]           IPT         CAATCICAGAATICCAGATICGAATICGAAGGACTICATICGACTICGAAGGACTICATICGACTICAGATICGAAGGACTICATICGACTICAGATICGAAGGACTICAATICGAGGACTICACACTICAGGACGAGAAGGATICAATICGAGGACTICAATICGAGGACTICACACTICAGATICGAAGG	lr7e	AGATCTTACTTCGGCAGAGGAACTAG	GAATTCTTGCTCCCCGGACAAATCGT	600	OR genomic DNA	pGAL4 attB	attP2	BT59.1	(b)
#7/g         AGATCIATCGAATCTCGAATCTCGAATCTCGAAATCGG         7766 (Mg genomic DNA p64.4 attB attP2         BT99.1         (b)           #11/a         GCGCGCCGCACACCTACTAGGCCGCCGACTGATAGGAATTCTGACCGC         2429 (Mg genomic DNA p64.4 attB attP2         BT19.1         (a)           #20a         ASATCIATGTATGCCACCAGC         GAATCGACTGAATGGCCGGCGCGACGAAGGAAT         2499 (Mg genomic DNA p64.4 attB attP2         BT11.1         (a)           #20a         ASATCIGCGAGTGCGAATC         GAATCIGCCGGCGCCGCACTGCAG         GAATCIGCGGCGCCGCACTGCAG         GAATCIGCGGCGCCGCACTGCAG         GAATCIGCGGCGCCGCACTGCAG         GAATCIGCGGCGCCGCACTGCAGGCGCGCCGCCGCCGCCGCGCGCCGCGCGCG	lr7f	AGATCTGTCCGTCTATCGAAATCCGG	AGATCTATCGATCCTCGAATTCTCCA	766	OR genomic DNA	pGAL4 attB	attP2	BT98.1	
Ir10a         GCGCCCCCACACTATAGGTCCACTACC         GCGGCCCCCACACATATAGGTCCACTACC         GCGGCCCCACACATATAGGTCCACTACCCCCCCACTAGAAGCACT         2299 (DR genomic DNA pGAL 4 attB attP2         BT15.1         (a)           Ir20a         AGATCTACATGCACCACAC         GAATCTGCCCCGCAC         GAATCTGCCACTACGACGAACT         2488 (DR genomic DNA pGAL 4 attB attP2         BT15.1         (a)           Ir27a         AGATCTGCCAGTCCAGTCCAGATCC         GAATCTGTGCGCGCGCACCGAAGGAAT         2488 (DR genomic DNA pGAL 4 attB attP2         BT97.1         (a)           Ir47a         AGATCTCCAGTTCCAGTTCCAGATGC         AGATCTGCGGAGGAACGAAT         2499 (DR genomic DNA pGAL 4 attB attP2         BT97.1         (a)           Ir48a //         AGATCTCCAGTTCCAGATCCA AGATCTGGAGGTTGATTAATAGAGCGT         2575 (DR genomic DNA pGAL 4 attB attP2         BT97.1         (a)           Ir37a //         AGATCTCCAGATCCAGATCCA AGATCTGGACGAACTGCACACACTCC         2931 (DR genomic DNA pGAL 4 attB attP2         BT97.1         (a)           Ir37a //         AGATCTCAGAGATATGGAGGAACTGC         GAATCTGGCGGAGGAACTGC         GAATCGGCGGAGGAACTGC         (a)         (a)           Ir37a //         AGATCTAGCGAGGAACTGC         GAATCGGCGGAGGAACTGC         GAATCGGCGGCGACTAGATTGC         (a)         (a)         (a)           Ir37a //         AGATCTAGCGAGGAACTGC         GAATCGGCGGGAGGAACTGC         GAATCGGGGAGGAACTGC         (a)         (a) </td <td>lr7g</td> <td>AGATCTATCGATCCTCGAATTCTCCA</td> <td>AGATCTGTCCGTCTATCGAAATCCGG</td> <td>766</td> <td>OR genomic DNA</td> <td>pGAL4 attB</td> <td>attP2</td> <td>BT99.1</td> <td>(b)</td>	lr7g	AGATCTATCGATCCTCGAATTCTCCA	AGATCTGTCCGTCTATCGAAATCCGG	766	OR genomic DNA	pGAL4 attB	attP2	BT99.1	(b)
Intel         AGAICICATOTATICACATGCACACGC         CAATICGACGTATICGCACGACACACACA         2028 OR genomic DNA pCAL 4 attB         pPP2         BT51.1         (a)           Int700         AGAICICACATGTCACGGCACACCACA         AGAICICACATGTCACAGGACA         2488 OR genomic DNA pCAL 4 attB         pPP2         BT53.1         (a)           Int70W         AGAICICACATGTCAGGTGACGAATC         CAATICITITATGGCCGTATGAACGAC         2488 OR genomic DNA pCAL 4 attB         pPP2         BT53.1         (a)           Int70W         AGAICICAGTGAGTGACGAATC         AGAICICAGGAAGCAC         22575 OR genomic DNA pCAL 4 attB         pP2         BT97.1         (a)           Int71W         AGAICICCAGGTCCAAGTGCACACACACACACACACACACACACAC	lr10a	GCGGCCGCGACACTATAGGTCCACTACC	GCGGCCGCTCGTATGGGATTTGTTAGCAC	2429	OR genomic DNA	pGAL4 attB	attP2	BT96.1	
HZ0a         AGATCTACATTGTCGGCACTGCGA         AGATCTTCGGCGCATTGCGCAGGCAT         2488         OR genomic DNA pCAL 4 attB         aPP2         BT6.1           H47a         AGATCTGCTGGTGACGATCC         GAATCTTTTATGGCCATTGAAAC         2488         OR genomic DNA pCAL 4 attB         aPP2         BT5.1           H48bW         AGATCTCCAAGTGCAAGTGCA         GAATCTCTGAAGAGTATATAGAGCGT         257         OR genomic DNA pCAL 4 attB         aPP2         BT9.1         E           H48b         AGATCTCCAAGTGCAAGTGCA         AGATCTCTGAAGATATATAGAGCGT         257         OR genomic DNA pCAL 4 attB         aPP2         BT8.1         E           H51a W         AGATCTCCAAGCTGAAGAC         AGATCTCGAGGATATATGGGAACTATGGGAAGATATATGGACAATCC         2493         OR genomic DNA pCAL 4 attB         aPP2         BT8.1         E           H52a         AGATCTCGAGAGTATTGCGGATGACATGCGACACTATGGA         C449         OR genomic DNA pCAL 4 attB         aPP2         BT5.1         (a)           H52a         AGATCTAGCGAGGAGTACCCATGGAGAGTATGCACTATGGA         644         OR genomic DNA pCAL 4 attB         aPP2         BT5.1         (a)           H52a         AGATCTAGCGAGGAGAGTACCACATGGAGGACTATGGA         644         OR genomic DNA pCAL 4 attB         aPP2         BT5.1         (a)           H52a         AGATCTATAGCGAGGAGGAGACTATGGAGGAGCCACACATGGAG <td< td=""><td>lr11a</td><td>AGATCTATGTATGTCATGCCACCAGC</td><td>GAATTCGACTGAATGGCCGTTGTGAA</td><td>2099</td><td>OR genomic DNA</td><td>pGAL4 attB</td><td>attP2</td><td>BT51.1</td><td>(a)</td></td<>	lr11a	AGATCTATGTATGTCATGCCACCAGC	GAATTCGACTGAATGGCCGTTGTGAA	2099	OR genomic DNA	pGAL4 attB	attP2	BT51.1	(a)
Int?bu         AGATCITECTGAGTIGGGTGACGAATC         CAATTCITTITATGGCCTITTGAAAC         248 B         OR genomic DNA pGAL 4 attB         BP2         BT53.1           Int?bu         AGATCITCCAGTICC	Ir20a	AGATCTACATTGTTCGGCACTCGCAG	AGATCTGTCGCCGGCATCGAAGGAAT	2488	OR genomic DNA	pGAL4 attB	attP2	BT61.1	
Int ToP         Interface	lr47a	AGATCTGCTGAGTTGGGTGACGAATC	GAATTCTTTTTATGGCCTTTTGAAAC	2488	OR genomic DNA	pGAL4 attB	attP2	BT53.1	
Intellation         Control Addition         Control Addition         Control Addition         Control Addition           1051ab         AGATCTCAAGTCCAGTCCAGATCA         AGATCTCGAGTGGTGGTGGTGGTGGTGGTGGTGGGTGGGT	lr47bΨ								
International problem         AGATCTCCAGTCCAGTCCAGTCCAGATGC         AGATCTCAGAGTCAGAGTGAGTAGC         AGATCTCAGAGTCAGAGTGAGTAGC         AGATCTCAGGAGTGGATAC         AGATCTCGGAGGTGATAC         AGATCTGGTGGTTGATCACC         2929         OR genomic DNA, pGAL 4 attll, attlP2         BT80.1           181b         AGATCTCGGAGAGTATTGGGTGGATAC         AGATCTCACCAGAAGTGTGC         2831 OR genomic DNA, pGAL 4 attll, attlP2         BT80.1           1822         AGATCTAGGAGATATTGCG         GAATCTGGTGGTCAAGGGGGTGGG         644 OR genomic DNA, pGAL 4 attlB         attlP2         BT80.1           1824         AGATCTAGCAGAGTGACTCG         GAATCTGGTGCTAAGGGGGGGGGGGGGGGGGGGGGGGGG	lr48aΨ								
1671aP         CATCTCAACCAATCAAGCTGGATAC         AGATCTGGTGGTTGATTCAATTGTGACA         2499         Org anomic DNA pGAL4 attB         BtP2         BT21.1           1182b         AGATCTCCGGACATTTCGTTGGCGAAAC         AGATCTGGTGGTTGATTCAATTGGTGTCAAACAACTGTTT         4730         Rg anomic DNA pGAL4 attB         BtP2         BT21.1         Imp32           1182b         AGATCTACTGGAGAATTTCCG         GAATCTGGTGCTAAAGTGACAACAACTGTTT         4730         Rg anomic DNA pGAL4 attB         BtP2         BT55.1         (a)           1182b         AGATCTGAGATACTGGAGGAACTGC         GAATCTGGAGGACTGCG         GAATCTGGAGGACTGCG         GAATCTGGAGGACTGC         GAATCGGAGGACTGCA         BtP2         BT55.1         (a)           1182b         AGATCTGAGATCGACTGGAGGACTGC         GAATTCGGTGCATACTGGAGGACTGC         GAATCGGACGCCCAATTGGAGGACTGC         GAATCGGACGCCCAATTGGGAGGACTGC         GAATCGGACGCCCAATTGGCGAGATGGC         GAATCGACTGACAGTGGACGACTGACTGG         GAATCGACTGGACGACGCCCAATTGG         GAATCCGACTGCACAGTGGAGGACTGC         AGATCTAATGGACGCCCAATTGGCGACTGCACTGACGACTGCC         2461         OR genomic DNA pGAL4 attB         BtP2         BT52.1         (a)           1186a         GAATCTGGAGGACGACGCCCAATGTG         AGATCTAATGAGGCGGCAATATGCACCAGTGGACGGCGCACTGCC         2461         OR genomic DNA pGAL4 attB         BtP2         BT52.1         (b)         Im56a         GAATCCATGGAGGACGACGCCCCAATGCAGGCGCGGACTGCCCCAGCGGGCACTGCCC	lr48b	AGATCTCCAGTCCAGTTCCAGATTGC	AGATCTCTGAAAGATATATAGAGCGT	2575	OR genomic DNA	pGAL4 attB	attP2	BT97.1	
Insta         AGATCTCAACCAATCAACCATCAACCTAGATAC         AGATCTCGAACAATCATCGCTGAC         AGATCTACCCACACATTCTTCGCGGTAC         AGATCTACCACACATTCTTCGCGTAC         AGATCTACGACAATTCTTCGCGTAC         AGATCTACGACAATTCTGCGATGAC         Insta         Insta<	lr51aΨ								
Instant         AGATCTCCCGACATTTCCTTCGCGTAAC         AGATCTCACGGACATTTCGCTTCGGC         AGATCTCACTGGACATTTCGGTGCG         AGATCTGACTGACGACATTTCGGTGCG         AGATCTGACGACAGATTTCGGTGCG         AGATCTGACGACAGATTCGGTGCG         AGATCTGACGACAGATTCGGTGCG         AGATCTGACGACAGATGCG         AGATCTGACGACAGATGCG         AGATCTGACGACAGATGCG         AGATCTGACGACAGCACATTGG         GAATCGGACGACATGGC         GAATTCGGACGACAGTGCG         GAATTCGGACGACAGTGCG         GAATTCGGACGCACATGGC         GAATTCGGACGACAGTGCGACATGGC         GAATTCGGACGACAGTGCACATGG         GAATTCGGACGACAGTGCACATGGC         GAATTCGGACGACAGTGCACATGGC         GAATTCGGACGACGCCCACTGGCGCGCGCGCAGAATAATTCGCACTGCA         2493 OR genomic DNA pGAL4 attB attP2         BT50.1         BT50.1         BT56.1         BT56.1         BT56.1         BT56.1         BT56.1         BT56.1         BT56.1         BT56.1         BT56.1         BT52.1         GA         GATCTGACAGCGCCACATGGC         CAATTCGGACGCCCACAGTGCCCACAGTGCACAGTGCACAGTGCCC         C44.00 R genomic DNA pGAL4 attB attP2         BT57.1         (b)         BT56.1         BT56.1         BT56.1         BT56.1         BT56.1         BT56.1         BT57.1         (b)         D         D         GAATTCATGACGACGCCACAGTGCC         AGATCTATAGGACGCACAGGCCACAGTGCC         AGATCTATAGGACGCACAGGCCAACAGGCC         AGATCTATAGGACGCACAGGCCAACAGCCC         AGATCTATAGGACGCACAGGCCAACAGCCC         AGATCTATAGGACGACGCACAGGCCGACAGTCCTCGCGCGACAGCGCGGACAACGCCGGGACAATCGACGCGGGACAATCGGACGCGCGCG	lr51b	AGATCTCAACCAATCAAGCTGGATAC	AGATCTGGTGGTTGATTCAATTGTGACA	2499	OR genomic DNA	pGAL4 attB	attP2	BT82.1	
Instant         AdsArctactored August         Bits 1         (a)           Instant         AdsArctactored and Antroced august         AdsArctactored and Antroced august         Bits 1         (a)           Instant         AdsArctactored august         AdsArctactored august         Bits 1         (b)           Instant         AdsArctactored august         AdsArctactored august         Bits 1         (b)           Instant         AdsArct	lr52a	AGATCTCCGACATTTCTTCGCGTAAC	AGATCTCACGAAACTGTTGACAATCC	2931	OR genomic DNA	pGAL4 attB	attP2	BT100.1	
Instant         AdartCrtaAAcGCrGGATGAAATTCCG         AdartCrGGGCAAAGTGACATTAGG         644 OR genomic DNA pGAL4 attB attP2         BT80.1           Instant         AdartCrtGGGAGGAACTGC         GAATTCGGTGCAAAGTTACTATTGC         644 OR genomic DNA pGAL4 attB attP2         BT80.1           Instant         AdartCrtGAGGAAGTCGACACTCATTTG         AGATCTGGCAAGGTCATTGC         CAATTCGGTCCCTTACCACTTGCC         2493 OR genomic DNA pGAL4 attB attP2         BT52.1         (a)           Instant         AGATCTGACAGGCCAATTGC         GAATTCGGCTCCCTTACCACTTGCA         2440 OR genomic DNA pGAL4 attB attP2         BT52.1         (a)           Instant         CGGGCCGCATATCCTCGGGCGACATGG         GAATTCGGCTCACAGGCG         2451 OR genomic DNA pGAL4 attB attP2         BT57.1         (b)           Instant         CGGGCCGCATGCATGGC         AGATCTATATTGGGCGCGCGCGCGCGCGCGCGCGCGCGCG	lr52b	AGATCTACTGGAGATATTGCGTTGCG	GAATTCTGTTTTCAAACAAACTGTTT	473	OR genomic DNA	pGAL4 attB	attP2	BT55.1	(a)
Instal         AGATCTEGAGATACTGGAGGAACTGC         GAATTCGGTGCAAGAGTTACTATTGC         664 OR genomic DNA         pGAL 4 attB         altP2         BTs6.1           Insta         AGATCTGACGCAAGTCGACTCATTTG         AGATCTGTCCTTTTCAATTATTGTCCC         2493 OR genomic DNA         pGAL 4 attB         altP2         BT101.1         Instal           Insta         AGATCTGACGCAAGTCGACTGCATTGC         GAATTCGGCGCCCTTACCACTTGCC         2493 OR genomic DNA         pGAL 4 attB         attP2         BT52.1         (a)           Insta         Insta         GGCCCCCTATCTCCTCTGCGCGAAGTGC         GGCCCCCCTAACATGCT         2400 OR genomic DNA         pGAL 4 attB         attP2         BT52.1         (b)           Insta         I	lr52c	AGATCTAAACGCTGGATGAAATTCCG	AGATCTGGTGCTAAAGTGACTAATGG	644	OR genomic DNA	pGAL4 attB	attP2	BT80.1	
Int26 <sup>4</sup> AGATCTGACCAAGTCGACTCATTTG         AGATCTGTGACCAAGTCGACTCATTTG         AGATCTGTGACCAAGTCGACTCATTTG         AGATCTGTGACCAAGTCGACTCATTTG         AGATCTGTGACCAAGTCGACAGTCGC         GATTCGCTGCCACGCTGCCATGCC         2400         OR genomic DNA <i>pGAL4</i> attB attP2         BT52.1         (a)           1786a         AGATCTATCATCACTGGCTGACAGTCG         CGGCCCCCTGAAATATTTGTGCACGTTGAC         2400         OR genomic DNA <i>pGAL4</i> attB attP2         BT52.1         (a)           1786b         AGATCTATCCTTCCGGTGAACGTCG         CGGCCCCCCTGAAATATTTGTCGCCCTGACGTCC         2400         OR genomic DNA <i>pGAL4</i> attB attP2         BT57.1         (b)           1786b         GAATTCATGACGACGCACACATGGTC         AGATCTATATTGTGTGCGCGCACCC         389         OR genomic DNA <i>pGAL4</i> attB attP2         BT76.1           1786e         HCATTCAGCCGCGGGAACATGGTC         GAATTCATATGGTGCGCGCACGCCCACGCCGCGCGCGCCGCCGCGCGCG	lr52d	AGATCTTGAGATACTGGAGGAACTGC	GAATTCGGTGCAAGAGTTACTATTGC	664	OR genomic DNA	pGAL4 attB	attP2	BT56.1	
B54a         AGATCTACCCACGACTCATTIC         AGATCTGCTCTTTCATTAGTTGCC         2493 OR genomic DNA pGAL4 attB         BttP2         BT101.1           Inf6ba         AGATCTATCATCACTGGCTGCATGC         GAATTCGGCTGCATTGC         2400 OR genomic DNA pGAL4 attB         BttP2         BT52.1         (a)           Inf6bb         GCGGCCCACATATCCTTCGGTGCAAGTGC         GCGGCCCCACATATCCTTCGGTGCAAGTGC         GCGGCCCCACATATC         2440 OR genomic DNA pGAL4 attB         BttP2         BT52.1         (b)           Inf6bb         GCGGCCCACATATCCTTCGGTGCAACAGCT         GCATTCGAAGCACATGCTC         AGATCTGCAAGACAGTGCACACAGTCT         (b)         (b)           Inf6bd         GAATTCAGAGCAGTGGATATATC         GAATTCAGAGCAGTGGC         BSB OR genomic DNA pGAL4 attB         BttP2         BT61.1         (b)           Inf6bd         GAATTCAGAGTGCACACAGGTGGATGATATC         GAATTCCAGTGGTGGCACACAGGC         GAATTCCAGTGGTGCACACAGGTGGC         BSB OR genomic DNA pGAL4 attB         BttP2         BT61.1         (b)           Inf6bd         AGATCTAGATGGTACACAGAGTGG         GAATTCCAGTGGTGCCACAGAGTGGC         GAATTCCAGTGGTGCACACAGAGTGGC         GAATTCCAGTGGTGCACACAGGTGGC         GAATTCCAGTGGTGCACACAGGTGGCCGAATGCCTGCCCACAC         GS9 OR genomic DNA pGAL4 attB         BttP2         BT61.1         (b)         (b)         (b)         (b)         (b)         (b)         (b)         (c)         (c)	Ir52e <sup>4</sup>								
Inf56 <sup>3</sup> AGATCTATCATCACGECTGTCATEC         GAATTCGACCACTTTACC         2400         OR genomic DNA         GGAL 4 attB         attP2         BT52.1         (a)           If56b         GCGGCCCCATATCCTTCGGTCGAAGTGC         GCGGCCCCATATCCTTCGCTCAAGTGC         GCGGCCCCATATCCTTCGCACAGTGC         GCGGCCCCATATCCTGCAACATGCT         BT62.1         (b)           If56c         AGATCTGACAAGACGTCCACAGTGTC         GATTCGACTTTCCCTTAGAAGCACC         319 OR genomic DNA <i>pGAL 4 attB</i> attP2         BT57.1         (b)           If56d <sup>4</sup> GAATTCTGACACATGCTC         AGATCTATTTCCTTGCACGTCGC         858 OR genomic DNA <i>pGAL 4 attB</i> attP2         BT76.1         (c)           If56d <sup>4</sup> GAATTCTAAGTCCACGCGGCACTAATTATC         GAATTCTATGACCACGCGGCACTATTCCGAACGC         858 OR genomic DNA <i>pGAL 4 attB</i> attP2         BT83.1         (c)           If60e         AGATTCTAATGCACACAGGTGGC         GAATTCCATGGTAGCACACGAGGGGGGCACTGTGTCACCA         339 OR genomic DNA <i>pGAL 4 attB</i> attP2         BT81.1         (b)           If60e         AGATCTAAGATGGGTGCACACAG         GAATTCCATGGTAGCACGGGGGGGGGGGGGGGGGGGGGG	Ir54a	AGATCTGACGCAAGTCGACTCATTTG	AGATCTGTCCTTTCAATTATGTTGCC	2493	OR genomic DNA	pGAL4 attB	attP2	BT101.1	
TSBb         CCGGCCCCATATCCTTCGGTCGAAGTGC         GCGGCCCCGATATCCTTCGGTCGAAGTGC         GCGGCCCCATATCCTTCGGTCGAAGTGC         GCGGCCCCATATCCTTCGGTCGAAGTGC         GCGGCCCCACAGTTG         GATTCGAAAGACGTCCACAGTATG         GAATTCGAAAGACGTCCACAGTATG         GAATTCGAAAGACGTCCACAGTGTC         GATTCGAAAGACGTCCACAGTGCT         GATTCGAAAGACGTCCACAGTGCT         GATTCGAAAGACGTCCACAGTCC         GATTCGACAGTGCAACAGTCCT         GATTCGACAGTGCAACAGTCCT         GATTCATATCGCGGGACTGATTATC         GAATTCGAGCAGTGCACAGC         BTF.1         (b)           Ir66a <sup>4</sup> GAATTCCTAGCGAGTGCCACAGGC         AGATCTTAGCAGTGCCACAGGC         AGATCTAGCAGTGCCGGACAGCAC         AGATCTAGCAGTGCCGGACAGGC         AGATCTAGCAGTGCCGGACAGGC         AGATCTAGATGGTGCCCACAGGTGGC         AGATCTAGATGGTACCACAGGTGGC         AGATCTAGATGGTACCACAGGTGGC         AGATCTAGATGGTACCACAGAGTGG         AATTCCAAGGCAGCGCGGAAAATGCT         2466 0R genomic DNA <i>pGAL4 attB</i> attP2         BT18.1         (b)           Ir600         AGATCTAAATGAGCAGTCCCAAGTGG         GAATTCCAAGGCAGCGCGGAAAATGCT         2486 0R genomic DNA <i>pGAL4 attB</i> attP2         BT18.1         (b)           Ir600         AGATCTAAATAGCAGTCCCAAGTGG         GAATTCCAAGGCAACGCCGAAAATGCT         2486 0R genomic DNA <i>pGAL4 attB</i> attP2         BT17.1         (b)           Ir600         AGATCTAAATAGCAGTTCAATGGCGGGAATAGCTGGCGGGAAAATGCT         2496 0R genomic DNA <i>pGAL4 attB</i> attP2         BT17.1         (b)           Ir600         AGATCTAAAATGCAGTGCGAAAGGGGGAAATGCTGCGGGGAA	Ir56a <sup>5</sup>	AGATCTATCATCACTGGCTGTCATGC	GAATTCGGCTGCCTTACCACTTTGAC	2400	OR genomic DNA	nGAL4 attB	attP2	BT52 1	(a)
Type:         AGATCTGCAAAGACGTCCACAGTATG         GAATTCGACTTTCCCTTAGAAGCACCC         319         OR genomic DNA pGAL attB         attP2         BT57.1         (b)           Irb6de <sup>4</sup> GAATTCAGACAGTCCACACATGCTC         AGATCTATTTTGTCGGCGCACCTGCC         888         OR genomic DNA pGAL attB         attP2         BT76.1         (b)           Irb6de <sup>4</sup> GAATTCAGACCAGCGCACACATGCTC         AGATCTATTTGTTGTCGTCGTCGCGCGCACTGCC         888         OR genomic DNA pGAL attB         attP2         BT76.1           Irb60         AGATTCATAGTCCGCGGACGTGACTATCC         GAATTCTATGTCTCGTCGCGGCACTGTGTGTGTCGTCTGGC         2523         OR genomic DNA pGAL attB         attP2         BT83.1         Irb60           Irb60         AGATTCTATAGTCCACAGCAGGGGGCGGGGACTGTCGCGGACGCGCGGACGCTGCGGACGGCGCGGACGGCCGGGACGGCGCGGGACGGCCGGGACGCGCGGGACGCCTGCG         2539         OR genomic DNA pGAL attB         attP2         BT14.1         (b)           Irb60         AGATCTAAATGACCGGGGTCCAAGT         GAATTCAATGCCGGGGGGACGCCTGCCGCGGGGGAAAGTCCT         2446         OR genomic DNA pGAL attB         attP2         BT14.1         (b)           Irb60         AGATCTAAATGCCCGAGTCAATGC         GAATTCAATGCCAGGGGGGGACGCCTGCT         2439         OR genomic DNA pGAL attB         attP2         BT14.1         Irb7.1         Irb7.1         Irb7.1         Irb7.1         Irb7.1         Irb7.1         Irb7.2	Ir56b	CCCCCCCCATATCCTTCCCTCCCAAGTCC	GCGGCCGCTGAAATAATTCTGCACTTGA	2461	OR genomic DNA	nGAL 4 attB	attP2	BT62.1	(u)
Instruction	Ir56c	AGATCTGCAAAGACGTCCACAGTATG	GAATTCGACTTTCCCTTAGAAGCACC	319	OR genomic DNA	nGAL4 attB	attP2	BT57 1	(h)
Bisket Fundsvection Concentration Concentration         Construction Concentration Concentration         Construction Concentration         Construction         Construction <thconstand in="" st<="" stand="" td="" the=""><td>Ir56d<sup>6</sup></td><td>GAATTCATGAGCAGTGCAACATGCTC</td><td></td><td>858</td><td>OR genomic DNA</td><td>nGAL4 attB</td><td>attP2</td><td>BT76 1</td><td>(0)</td></thconstand>	Ir56d <sup>6</sup>	GAATTCATGAGCAGTGCAACATGCTC		858	OR genomic DNA	nGAL4 attB	attP2	BT76 1	(0)
Display         GAATTCTAGTCCGCGGACTGATTATC         GAATTCTATTGCTTCTGTCACGTCGG         2523         OR genomic DNA         pGAL4         attP2         BT83.1           Ir60a         AGATTCTATACGATTCCCGAACGC         AGATTCTTTCGCGATTTCCCGAACGC         AGATCTTCGCTGCCGAACGC         AGATTCGTCGCGACGC         AGATTCGCTGCGC         2388         OR genomic DNA         pGAL4         attB         attP2         BT83.1         (b)           Ir60b         MGATTCGATGCGCGACGCGC         AGATTCGTCGCGACGACGCCC         339         OR genomic DNA         pGAL4         attB         attP2         BT83.1         (b)           Ir60b         AGATTCATAGATGGGATGCCC         GAATTCATTTCGCGACGCGCGACGTCGCTCACA         339         OR genomic DNA         pGAL4         attB         attP2         BT83.1         (b)           Ir60b         AGATCTAAATAATGATGGATGACGCGC         GAATTCATAGCGCGGGGAAAATGCTT         2466         OR genomic DNA         pGAL4         attB         attP2         BT72.1         (b)           Ir62a         GAATTCAAATGACGAGGACGTCATCATAGC         GAATTCATAATGACGCGAGCCCGAAATGCTT         2439         OR genomic DNA         pGAL4         attB         attP2         BT73.1         (b)	Ir56eW	CARITORICAGOACICORACITO	Adatoratarrietadoddddad1000	000	OIT genomic DINA	POAL4 allo	atu 2	5170.1	
Branding         Spanner         <	11000 T	CAATTCTAGTCCGCGGACTGATTATC	CANTECTATECTCICICACCICCC	2522	OR gonomic DNA	nGAL4 offR	o#P2	DT02 1	
Index         Index <th< td=""><td>1100a</td><td></td><td></td><td>2323</td><td>OR genomic DNA</td><td>pGAL4 allD</td><td>attr2</td><td>D103.1</td><td></td></th<>	1100a			2323	OR genomic DNA	pGAL4 allD	attr2	D103.1	
Macher         Shari Toski T	1/00D	GAATTCGATTGGATACCACACGGGGC	GAATTCGGCGACTATCCGAAACGAGC	2300	OR genomic DNA	pGAL4 allB	attP2	DT104.1	(b)
Monter Charanter Gostence Antropy         GANTECHARANTEGOSTECCOAT         GANTECHARANTEGOSTECCOATANTE         GANTECHARANTEGOSTECCOATANTECCOATANTECCOATANTECCOATANTECCOATANTECCOATANTECTOTGACACATANTEGOSTECOATANCE         GANTECHARANTEGOSTECOATANTEGOSTECOATANTECOATANTEGOSTECOATANTECCOATANTECOATANTEGOSTECOATANTECOATANTEGOSTECOATANTECOATANTEGOSTECOATANTECOATANTEGOSTECOATANTEGOSTECOATANTECOATANTEGOSTE	1100C+	AGATCTAGATTGGGTACCACAGGTGGC	GAATTCTTTTAAGGCGACTGCTCACA	300	OR genomic DNA	pGAL4 allD	attr2	D101.1	(D)
Index         Index <th< td=""><td>11000</td><td>AGATCTAGATIGGGIACCACAGATGG</td><td>GAATTCAAGGCAGCGGGAAAATGCTT</td><td>2466</td><td>OR genomic DNA</td><td>pGAL4 allD</td><td>attr2</td><td>D103.1</td><td></td></th<>	11000	AGATCTAGATIGGGIACCACAGATGG	GAATTCAAGGCAGCGGGAAAATGCTT	2466	OR genomic DNA	pGAL4 allD	attr2	D103.1	
Diff         Diff <thdiff< th="">         Diff         Diff         <thd< td=""><td>1/608U</td><td>AGATOTAATAATGAGGAGTOODGAT</td><td>CANTOANGOOAGOCCAAAATGOTT</td><td>2400</td><td>OIT genomic DIVA</td><td>POAL4 allo</td><td>atu 2</td><td>0172.1</td><td></td></thd<></thdiff<>	1/608U	AGATOTAATAATGAGGAGTOODGAT	CANTOANGOOAGOCCAAAATGOTT	2400	OIT genomic DIVA	POAL4 allo	atu 2	0172.1	
Initial         Initial <t< td=""><td>110017</td><td>CAATTCAAATCACCGAGTTCAATGGC</td><td>GAATTCATTTCGCTCGTCAACCATC</td><td>2420</td><td>OP gonomic DNA</td><td>nGAL4 offP</td><td>o#P2</td><td>DT110 1</td><td></td></t<>	110017	CAATTCAAATCACCGAGTTCAATGGC	GAATTCATTTCGCTCGTCAACCATC	2420	OP gonomic DNA	nGAL4 offP	o#P2	DT110 1	
International Construction         Construction <th< td=""><td>1102a Ir67a</td><td>AGATCTACAGACGTTTATCAGCAAAG</td><td>GAATTCATATCCTGGCTGAATGGCTG</td><td>2435</td><td>OR genomic DNA</td><td>pGAL4 attB</td><td>attP2</td><td>BTT10.1 BT73.1</td><td></td></th<>	1102a Ir67a	AGATCTACAGACGTTTATCAGCAAAG	GAATTCATATCCTGGCTGAATGGCTG	2435	OR genomic DNA	pGAL4 attB	attP2	BTT10.1 BT73.1	
Identication         IdenticationIdentication<	1107 a Ir67 b	AGATCTTCGTGTGTCACCACTATAGC	GAATTCTGAAATGTCTCTGAAATCCT	2430	OR genomic DNA	pGAL4 attB	attP2	BT74.1	
Bits         Dist.         Dist.         Dist.         Dist.         Dist.           WBB         AGATCICCEGGTACTGAAAGATATG         GAATTCGTCTACGAGCACAACC         637         OR genomic DNA pGAL4 attB attP2         B148.1           WBB         AGATCICCEGGTACTGGAAAGATATG         GAATTCGTCTACGAGCACAACC         637         OR genomic DNA pGAL4 attB attP2         B148.1           WB7a         CTCGAGAGTTACCCAAGTAGCCG         GAATTCGTCCACACCATGTTGAGACCG         BAATTCGTCCACACACATAGCCATGTGACACCG         B149.1           WB7a         CTCGAGAGTTACAGCCAAGATAGCCG         GAATTCGTCCCAACACATGTGCC         GAATTCGTCCACACACATAGCCAAGATGTGCC         B149.2         B112.1           WB4a         AGATCTTAGAGTCAAGCCAAGATGAGCG         AGATCTTGCCGCAACACAATGAGCGGAGCTGTCACC         AGATCTTGCGCGAGCGTCCTATC         AGATCTTAGATCAAGCGAAGATGGCG         AGATCTTTTGCTGCGCGAGCGTCTCTATC         AGATCTTTGCTGCGCGAGCGTCTCTATC         AGATCTTTGCTGCGCGACACATAGGGGGATTACCCAAA         2230         OR genomic DNA pGAL4 attB attP2         B111.1           WB4d         CTCCAAACACATTGGTGCGGGATAGTTGGCGGGAATGATGGTGGT         2420         OR genomic DNA pGAL4 attB attP2         B178.1         D194           WB4d         CTCCAAACACATTGGTGGCGACTACAGGGGATTGACGAAA         322         DR genomic DNA pGAL4 attB attP2         B171.1         D194           WB4d         CTCCAAAACACTTGGCGACACATAGGGGGGATTGCCGAACACAAA         322	1670	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	2726	OR genomic DNA	pGAL4 attB	o#P2	DT0/1	-
Index         Index <th< td=""><td>1107C</td><td>AGATCTCCCGCTTACTCCGAAAGATATC</td><td>GAATTCGTTCTACGAGCAGACTAACC</td><td>2730</td><td>OR genomic DNA</td><td>pGAL4 allD</td><td>attr2</td><td>DT/04.1</td><td>-</td></th<>	1107C	AGATCTCCCGCTTACTCCGAAAGATATC	GAATTCGTTCTACGAGCAGACTAACC	2730	OR genomic DNA	pGAL4 allD	attr2	DT/04.1	-
Instruction         Description         Description <thdescription< th=""> <thdescription< th=""></thdescription<></thdescription<>	Ir85a	AGATCTAAGTCCTCCTCAGTGTCC	GAATTCTGCAATGCCAACTGTTTGAG	1369	OR genomic DNA	pGAL4 attB	attP2	BT40.1	
Bit         Dist         Dis         Dist         Dist         D	1103a Ir87a	CTCGAGAGTTACCCATATGGACACCG		2007	OR genomic DNA	pGAL4 attB	attP2	BT122.1	
Montechargenergy         Acate Chargenergy         Acate Chargenergy         Acate Chargenergy         Acate Chargenergy         Acate Chargenergy         Acate Chargenergy         Bits - 1           Model         Acate Chargenergy         Acate Chargenergy         Acate Chargenergy         Bits - 1         Bits - 1           Model         Acate Chargenergy         Acate Chargenergy         Acate Chargenergy         Bits - 1         Bits - 1           Model         Acate Chargenergy         Acate Chargenergy         Acate Chargenergy         Bits - 1         Bits - 2         Bits - 2 <td>1010</td> <td>AGATCTGCACACAGATAGATTCGCAG</td> <td>AGATOTTTCTACTTTACCCAACAAT</td> <td>2001</td> <td>OR genomic DNA</td> <td>pGAL4 attB</td> <td>o#P2</td> <td>DT122.1</td> <td></td>	1010	AGATCTGCACACAGATAGATTCGCAG	AGATOTTTCTACTTTACCCAACAAT	2001	OR genomic DNA	pGAL4 attB	o#P2	DT122.1	
Instru         Instruction         Instruction <t< td=""><td>1194a 1r04b</td><td>AGATCTAAGATCAAGCGAAGATGACG</td><td>GAATTCATTTCGTAATTCACGTAGTG</td><td>192</td><td>OR genomic DNA</td><td>pGAL4 allD</td><td>attr2</td><td>D104.1</td><td>-</td></t<>	1194a 1r04b	AGATCTAAGATCAAGCGAAGATGACG	GAATTCATTTCGTAATTCACGTAGTG	192	OR genomic DNA	pGAL4 allD	attr2	D104.1	-
Instrument         Description         Description         Distribution         Distribution <td>11940 IrQAc</td> <td>AGATCTTTCTGGCGAGCGTCTCTATC</td> <td>AGATCITITIAGITAGCCTIGGGTTA</td> <td>402</td> <td>OR genomic DNA</td> <td>DGAL 4 allD</td> <td>attP2</td> <td>BT65 1</td> <td><u>├</u></td>	11940 IrQAc	AGATCTTTCTGGCGAGCGTCTCTATC	AGATCITITIAGITAGCCTIGGGTTA	402	OR genomic DNA	DGAL 4 allD	attP2	BT65 1	<u>├</u>
Used         AGATCITITEGCGACATAAGATGTGGC         GAATTCITTCCCAGGGGATTACGAA         322 (DR genomic DNA pGAL4 attB attP2         BTT1.1           US4d         AGATCITTGGCGACATAAGATGTGGC         GAATTCITTCCCAGGGGATTACGAAA         322 (DR genomic DNA pGAL4 attB attP2         BTT8.1         (b)           US4d         AGATCITTGGCACCATAAGATGTGGC         GAATTCITTCCCAGGGGATTACACAAA         322 (DR genomic DNA pGAL4 attB attP2         BTT0.1           US4g         AGATCCATGTGGAGCGATCATGG         GAATTCCTTCTCAGGGGATGATGATG         2433 (DR genomic DNA pGAL4 attB attP2         BT102.1           US4g         GAATTCGAGGGGCATATAGG         GAATTCCTTCTTCATT         388 (DR genomic DNA pGAL4 attB attP2         BT175.1           US4g         GAATTCGAGGGGCATTACG         GAATTCGTAGCGAGCTATACGGAGCGATGGCGAACCGAGG         2000 (DR genomic DNA pGAL4 attB attP2         BT60.1         (b)           US40n         GAATTCGTCTGGCGAGCTATGCG         GAATTCGTAGCGGAGTGCTGGGGAGCTGGCGAGCGATGGCGAGGCG         2000 (DR genomic DNA pGAL4 attB attP2         BT60.1         (b)	IrQAd	CTCGAGACATTGTGTTCGGGTACGTG		2303	OR genomic DNA	pGAL4 allB	attP2	BT111 1	<u> </u>
Instruction         Sector         Sector <thsector< th=""> <thsector< th="">         Sect</thsector<></thsector<>	11040		CANTECTCCCACCCATTACACAAA	2420	OR genomic DNA	pGAL4 allB	ottD2	DT70 1	(b)
Instruction         Description         Description         Description         Description         Description         Difference         Difference <thdifference< th="">         Difference         Di</thdifference<>	11 340 Ir0 Af	AGATCTCATTGTCCACCCATCCATTC	GAATTCCTGTGCAGAGGGGGATTACACAAA	322	OR genomic DNA	pGAL4 allB	attr 2	DT102.1	(0)
Instance of the second of the	IrQAa	GAATTCGAGCTCACTGTTCACTATCC	GAATTCCTTATAATACTGACTTCATT	2493	OR genomic DNA	DGAL4 allb	attP2	BT75.1	-
	lrQAh	GAATTCTTGTTCACGCGCCAATTACC	GAATTCGACTTATACCGAAACCGACG	2000	OR genomic DNA	pGAL4 allb	attr2	BT60 1	(b)
	Ir100a	AGATCTTTCATCGGAGTCGTAGCTAG	GAATTCGTCAGGAGTTACTGAACCGT	2000	OR genomic DNA	pGAL4 attB	attP2	BT50.1	(2)

 Footnates

 1: We predicted pseudogene in the reference D. melanogaster genome (Adams et al., Science 2000); for most of these we therefore did not construct a driver line. For Ir48a-Gal4 we observed very variable expression in the central and peripheral nervous system (data not shown). For Ir60c, this locus is predicted to be intact in a w<sup>178</sup> strain

 2: Restriction enzyme sites in cloning primers are highlighted in blue

 3: Lines previously published: (a) Croset et al., PLOS Genetics (2010); (b) Croset et al., Scientific Reports (2016)

 4: Ir52e is not present in the reference D. melanogaster genome, so no driver line was constructed for the locus in this study (see also Koh et al., Neuron 2014)

 5: Ir56a-Gal4 displays expression in several olfactory sensory neuron populations; this is likely to be non-specific as there is no evidence for antennal expression of Ir56a (Menuz et al., PLOS Genetics (2014); as Ir56a is located within the intro of another gene (5-H714) this may reflect overlapping regulatory elements of these genes

 6: Ir56d-Gal4 was also detected in some larval head chemosensory neurons, but this expression wask and was not confirmed in the Ir56d <sup>God4</sup> reporter allele.

 7: Ir60a-Gal4 displays extensive and variable non-neuronal expression (data not shown), which is likely to be non-specific; as Ir60a is located within the intro of another gene (nord), this may reflect overlapping regulatory elements of these genes

Supplementary Table 1. Oligonucleotide sequences and construction of Ir-Gal4 lines

		Calcium imaging Figure 5c	
Tastant	CAS	Concentration	Notes
water	-	-	
glycerol	56-81-5	10% (v/v)	
fructose	57-48-7	1 M	
trehalose	6138-23-4	1 M	
sucrose	57-50-1	1 M	
caffeine	58-08-2	15 mg/ml	
denatonium	3734-33-6	10 mM	
arginine	74-79-3	100 mM	
histidine	71-00-1	100 mM	
lysine	56-87-1	100 mM	
aspartic acid	56-84-8	100 mM	
glutamic acid	56-86-0	100 mM	
acetic acid	64-19-7	1% (v/v)	
NaCl (high)	7647-14-5	1 M	
NaCl (low)	7647-14-5	10 mM	
NaHCO <sub>3</sub> (pH 5)	144-55-8	200 mM	0.5 ml of 200 mM NaHCO <sub>3</sub> (pH 6.5) + 50 μl of 5 M H <sub>2</sub> PO <sub>4</sub>
CsHCO <sub>3</sub> (pH 7)	29703-01-3	200 mM	pH set with HCl immediately before use
NaH <sub>2</sub> PO <sub>4</sub>	13472-35-0	500 mM	
PBS pH 4	-	100%	
PBS pH 7	-	100%	7.8 mM NaH <sub>2</sub> PO <sub>4</sub> + 12.2 mM Na <sub>2</sub> HPO <sub>4</sub> + 153.8 mM NaCl (pH set with HCl or NaOH)
PBS pH 10	-	100%	
carbonated water	-	-	Aproz ® (in mg/ml: Ca <sup>2+</sup> 360; Mg <sup>2+</sup> 70; Na <sup>+</sup> 6; K <sup>+</sup> 2.5; HCO <sub>3</sub> <sup>-</sup> 250; NO <sub>3</sub> <sup>-</sup> 1.5; SO <sub>4</sub> <sup>2-</sup> 930; SiO <sub>2</sub> 7) + gaseous CO <sub>2</sub>
non-carbonated water	-	-	Aproz ® (in mg/ml: Ca <sup>2+</sup> 360; Mg <sup>2+</sup> 70; Na <sup>+</sup> 6; K <sup>+</sup> 2.5; HCO <sub>3</sub> <sup>-</sup> 250; NO <sub>3</sub> <sup>-</sup> 1.5; SO <sub>4</sub> <sup>2-</sup> 930; Si0 <sub>2</sub> 7)
hexanoic acid	142-62-1	-	

#### Supplementary Table 2. Gustatory stimuli

Oligonucleotide	Sequence (5'-3')	Notes
CRISPRSgR	AAAAGCACCGACTCGGTGCCACTTTTCAAGTTGATGACGGACTAGCCTTATTTTAACTTGCTAGTTCTAGCTCTAAAAC	
CRISPRsgF-Ir56d <sup>7</sup>	GAAATTAATACGACTCACTATA GGTCATCACGGAGCGCATGTGTTTAGAGCTAGAAATAGC	T7 promoter sequence is italicised; Ir56d target sequence is underlined
CRISPRsgF-Ir56d <sup>2</sup>	GAAAT7AA7ACGAC7CAC7A7A <u>GGTCAGCTATAGCTATCCCAT</u> GTTTTAGAGCTAGAAATAGC	T7 promoter sequence is italicised; Ir56d target sequence is underlined
Ir56d sgRNAs-fwd	GCGGCCCGGGTTCGATTCCCGGCCGATATATGGATAATCGTGCTGCTTGCT	sgRNA cloning into <i>pCFD5</i>
Ir56d sgRNAs-rev	ATTITAACTTGCTATTTCTAGCTCTAAAACGCAAGCCCAGATCGTTTCTCTGCACCAGCCGGGAATCGAACCC	sgRNA cloning into <i>p CFD5</i>
Ir56d Gal4-HA1-fwd	GATCCACCTGCGATCCCCACGCACCTGTGCATCCTTGAAGTGC	Homology Arm 1-Ga/4 ORF fusion
Ir56d Gal4-HA1-rev	GATCCACCTGCGATCCTAGGATCCAGTAGTCAGGGCAC	Homology Arm 1-Ga/4 ORF fusion
Ir56d Gal4-HA1-internal-	ACTGGCAGTCGCCGTACAAATATGAAGCTACTGTCTTCTATCGAACAAGC	Homology Arm 1-Ga/4 ORF fusion
Ir56d Gal4-HA1-internal-	CGATAGAAGACAGTAGCTTCATATTTGTACGGCGACTGCCAGTGGGTAAC	Homology Arm 1-Ga/4 ORF fusion
Ir56d-HA2-fwd	GATCGCTCTTCGTATAGCCCAGATCGTTTCTCAGGCGCGTTCATG	Homology Arm 2
Ir56d -HA2-rev	[GATCGCTCTTCGGACGATGCCTTGCAATTGATACGTGAACG	Homology Arm 2

Supplementary Table 3. Oligonucleotide sequences used for CRISPR/Cas9-mediated editing of the *Ir56d* locus



## Supplementary Figure 1. Projections of *Ir-Gal4* expressing sensory neurons in the larval central nervous system

Immunofluorescence with anti-GFP (green) and nc82 (magenta) on wholemount brains of third instar larvae, revealing the projection patterns of *Ir-Gal4*expressing neuron populations in the brain and ventral nerve cord (as schematised in the cartoon at the top left). Images for *Ir7a*, *Ir7b*, *Ir7g*, *Ir94*e and *Ir94h* drivers have been adapted from Croset et al., 2016<sup>28</sup>. Genotypes are of the form: *w;UAS-mCD8:GFP;IrX-Gal4*. SEZ: Subesophageal Zone; VNC: Ventral Nerve Cord. Scale bars: 20 µm.



## Supplementary Figure 2. Projections of *Ir-Gal4* expressing sensory neurons in the adult central nervous system

Immunofluorescence with anti-GFP (green) and nc82 (magenta) on wholemount adult brains and ventral nerve cords (schematised top left), revealing the projection patterns of *Ir-Gal4*-expressing neuron populations. Grey panels indicate no expression was detected for that Gal4 driver. Genotypes are of the form: *UAS-mCD8:GFP;IrX-Gal4*. Scale bars: 50  $\mu$ m (brain), 100  $\mu$ m (ventral nerve cord).



## Supplementary Figure 3. An *Ir56d*<sup>Gal4</sup> allele shows a similar expression pattern to *Ir56d-Gal4*

(a) Schematic representing the generation of the *Ir56d*<sup>Gal4</sup> allele. The entire *Ir56d* exon was substituted with the *Gal4* sequence. In an additional step, the *DsRed* marker used for screening of positive recombination events was removed with Cre recombinase. HA: Homology Arm. Unfilled arrowheads point to the sgRNA targets.

(b) Immunofluorescence with anti-GFP (green), overlaid on a bright-field image, of a whole mount proboscis of a *w;Ir56d*<sup>Ga/4</sup>;UAS-mCD8:GFP animal. Scale bar: 25  $\mu$ m.

(c) Immunofluorescence with anti-GFP (green) and anti-nc82 (blue) on a whole mount brain of a *w;Ir56d*<sup>Gal4</sup>;UAS-mCD8:GFP animal. Scale bar: 50  $\mu$ m (right).



# Supplementary Figure 4. Analysis of carbonation-evoked feeding by Expresso

(a) Feeding success, latency to first bout, total consumption per fly, number of meal bouts and average bout volume for male  $w^{1118}$  flies (n=30 per tastant) feeding from water, 100 mM NaHCO<sub>3</sub> pH 8.5 or 100 mM NaHCO<sub>3</sub> pH 6.5 solutions.

(b) Feeding success, latency to first bout, total consumption per fly, number of meal bouts and average bout volume for male  $w^{1118}$  flies (n=30 per tastant) feeding from solutions containing 5 mM sucrose, 5 mM sucrose + 100 mM NaHCO<sub>3</sub> pH 8.5 or 5 mM sucrose + 100 mM NaHCO<sub>3</sub> pH 6.5 solutions. *ns*: non-significant (pairwise comparisons using Tukey and Kramer (Nemenyi) test with Tukey-Dist approximation for independent samples).



#### Supplementary Figure 5. IR56d is required for physiological and behavioural responses to hexanoic acid

(a) Colour-coded images (reflecting the maximal GCaMP3 fluorescence intensity changes) in *w;Bl/+;UAS-GCaMP3,Ir56d-Gal4/+* animals stimulated with distilled water, 1 M sucrose and 1% (v/v) hexanoic acid.

(b) Quantification of changes in GCaMP3 fluorescence ( $\Delta$ F/F) upon stimulation with the indicated chemicals (as in (a)) to the proboscis of the flies. Genotypes: Control: *w;Bl/+;UAS-GCaMP3,Ir56d-Gal4/+* (n=7); Mutant: *w;Ir56d<sup>1</sup>/Ir56d<sup>1</sup>;UAS-GCaMP3,Ir56d-Gal4/+* (n=8); Rescue: *w;Ir56d<sup>1</sup>,UAS-Ir56d/Ir56d<sup>1</sup>;UAS-GCaMP3,Ir56d-Gal4/+* (n=11). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 (Wilcoxon rank sum test with Bonferroni correction for multiple comparisons).

(c) Fraction of  $w^{1118}$  (n=36) or *Ir56d*<sup>1</sup> mutant (n=33) flies showing proboscis extension reflex (PER) to 1% (v/v) hexanoic acid and 100 mM sucrose. Error bars represent the ±95% binomial confidence intervals \*\*\*P<0.001 (Fisher exact test).