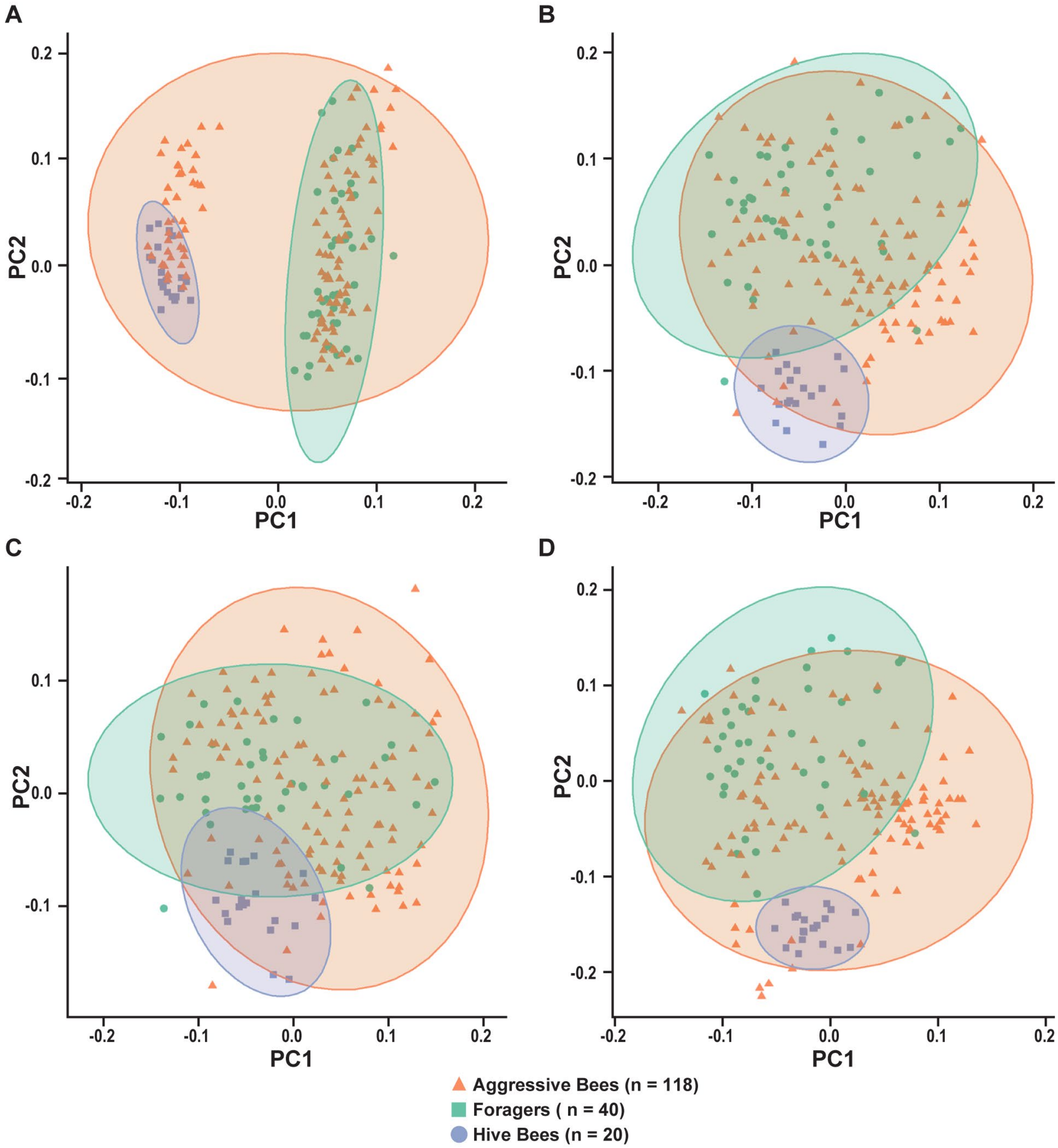

Figure S1: RUVcorr corrects for batch-wise variation prior to ANCOVA.

(A) Principal Component Analysis (PCA) revealed substantial batch effects between microarray datasets, which are conflated with the behavioral states (aggressive bees, hive bees and foragers) assayed. **(B)** To prevent this from affecting the ANCOVAs, we applied the RUVcorr package,

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which largely eliminated the batch effects along the first two principal components. To determine whether remaining batch effects were likely to bias analyses of transcriptional regulatory plasticity, we performed PCA separately on putative target genes (C) and the transcriptomic background (the rest of the probes on the original microarrays) (D). Both sets of genes resulted in similar patterns of variance distribution, suggesting that neither set was unduly biased by batch effects. Shaded areas represent the 95% confidence intervals for each group.

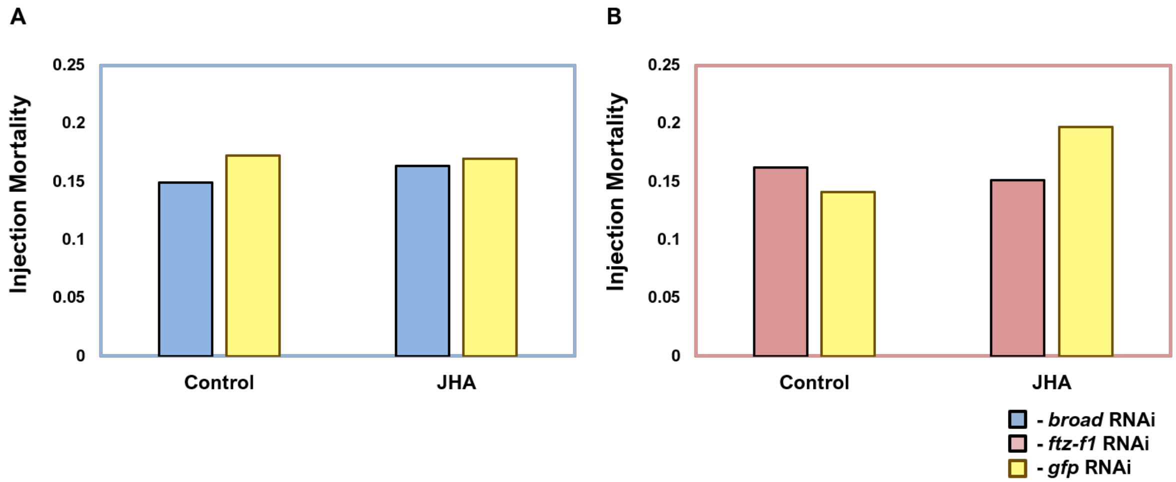

Figure S2: Effect of RNAi brain injection on mortality.

Low mortality was observed on the day of behavioral testing (48 hours after RNAi injection) for experiments contrasting *br* and *gfp* RNAi **(A)**, and *ftz-f1* and *gfp* RNAi **(B)**. No significant differences in mortality were found between RNAi or JHA treatment groups (Kruskal-Wallis test).

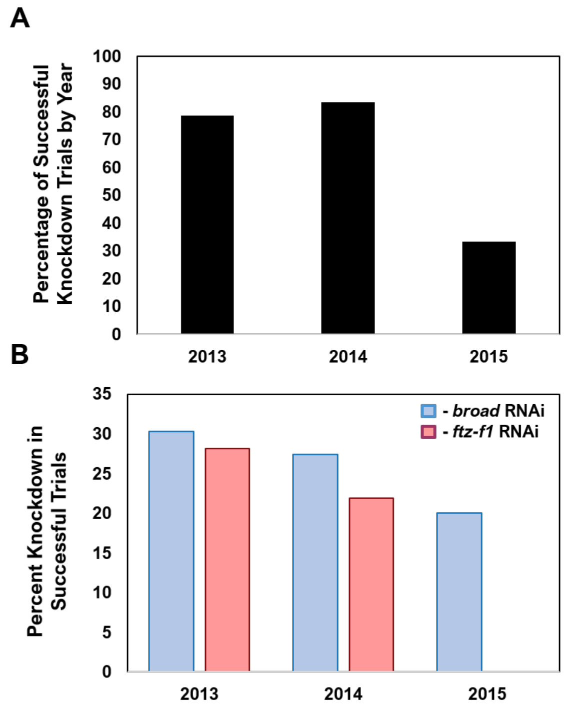

Figure S3: Diminished efficacy of RNAi treatment in trials conducted in 2015 relative to 2013 and 2014.

(A) RNAi was effective in 11 out of 14 trials performed in 2013 and 5 out of 6 performed in 2014. We wish to add to the literature the observation that effect strength declined in 2015, after the experiments for this study were performed. We speculate that the reason for this decline might be related to world-wide declines in bee health, especially the increase in bee viruses during this time, but the precise mechanism is unknown. Although RNAi in honey bees has been effectively administered to adult bees by multiple laboratories including our own using abdominal (Ament et al., 2012; Ament et al., 2011; Guidugli et al., 2005; Wang et al., 2012) and brain (Farooqui et al., 2003; Müßig et al., 2010; Rein et al., 2013) and targeting *ftz-f1* in pharate adults (Mello et al., 2018), potential users of this technique should be mindful of the above-noted variation.

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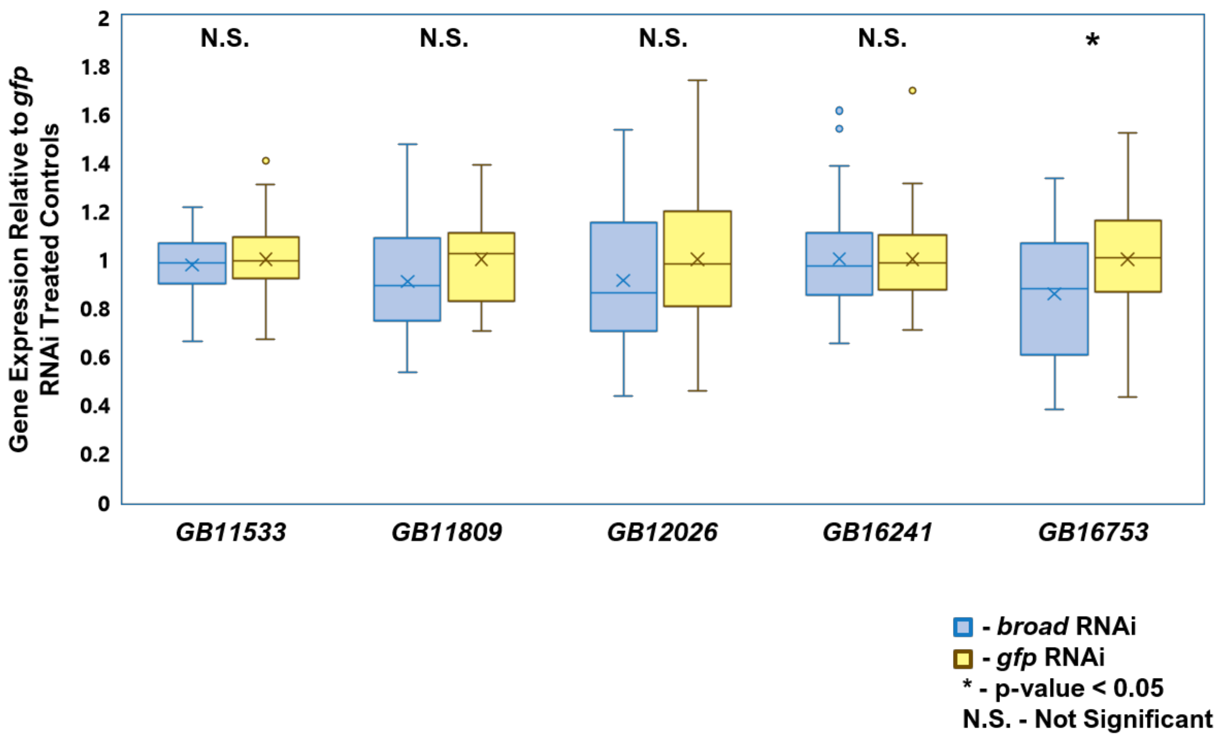

Figure S4: Broad RNAi has reduced influence on the expression of putative CG17912 target genes.

To explore whether *broad* RNAi brain injection had nonspecific effects on gene expression, we tested whether five putative target genes of the TF CG17912 responded to *broad* RNAi treatments. The y-axis represents gene expression of the *broad* RNAi-treated samples (n = 51) relative to the average expression of the *gfp* treated control samples (n = 52). Only 1 out of 5 genes was significantly affected by *broad* RNAi (ANOVA, $p < 0.05$), providing some indication of specificity. In all box plots, center lines show the group median and x's indicate the group means; the limits represent the 25th and 75th percentiles, and whiskers extend up to 1.5 times the interquartile range from these percentiles.

Table S1: Nucleotide sequences for qPCR probes and RNAi constructs used in this study

Gene Name	OGS 2.0	qPCR Forward Primer	qPCR Reverse Primer
RCP1	-	TCAATTAACTCGGAATCGGA	CCTGGATTTCCCTGCTGAT
RP49	GB10903	GGGACAATATTTGATGCCCAAT	CTTGACATTATGTACCAAAACTTTTCT
S8	GB12747	TGAGTGTCTGCTATGGATTGCA	TCGCGGCTCGTGGTAAA
GAPDH	GB14798	ACTGGTATGGCCTTCCGTGTAC	TGCCAAGTCTAACTGTTAAGTCAACA
EiF1a	GB16844	CAATTTCTGGTTGGCATGGA	CATCCCTTAAACCAAGGCATTT
broad	GB30150	AGAAGCCGAGGGTGCTGTC	CATCGAGAAATCGGTGGGC
ftz-f1	GB16873	ACCAACTACCACCTACTCATTACCAA	GCAGACCGGACAGAGTTCTTCT
ERBP	GB11484	TCACATTTGGATTATTATTCTGGACG	AGCATCAGTCATAAGTTCATCCACA
	GB11842	AGTCGACCGTGGGAGTGGT	TCTGTGGACCCAGCCTGTG
	GB11874	GTTTATGATGCAACGCAAAGCT	CATTGCTGGAGCCATGAAACT
oys	GB15142	ATGGCTTAGATCGATTGTGTACGA	GGCAGAAAGGGAGTATGTGAACA
	GB15320	AGTGGTTTGTCTGGAACATGGA	TGGTTGCAATTTGGTAGCTTCA
Nrk	GB15972	GTATGGGCGTTCGCGGTAT	TCTTCGTGCGTCATTCCATAAT
	GB16117	TCCTAAAACTTTGCGTCACACG	TGCCATCGTTTTAGAATTTGGA
TFIIeα	GB17516	GTACCAACTGTAACTGTTGCAGGAA	ACAGGAGTCATTTCTGCTATTAATGC
TyrR	GB17991	GGCCCCTTCGTATCGTTCA	TGAACGGCATCACGAGGAT
	GB14024	TCCAGAAGAAAAATACCAAGTATCGC	GAATCTGGAGTCACGACAAGACAA
unc79	GB15608	GGTTTCATCAAAGACAGGGACAA	TCCGGTATCGTGGTCTTGAATT
Toll-7	GB15177	CTGACCTTCCTCCCGTTACG	ACACCGGAAACGTCACCAA
Construct Name		**Antisense**	**Sense**
ftz-f1 Construct 1		CCACUUAGACGUUUCUUCUUGAUAGUU	CUAUCAAGAAGAAACGUCUAAGUGG
ftz-f1 Construct 2		UUUAAAUACUAAUUGAGGUUCUUUCUC	GAAAGAACCUCAAUUAGUAUUUAAA
ftz-f1 Construct 3		UUGCCAUAUCUUGUCUACGUUGUCUUU	AGACAACGUAGACAAGAUAUGGCAA
broad Construct 1		CGCAACAAUUGCUGCACGGUGGUCGGU	CGACCACCGUGCAGCAAUUGUUGCG
broad Construct 2		ACUGCUAAGGGAACGCUGAUGCACGUU	CGUGCAUCAGCGUUCCCUUAGCAGT
broad Construct 3		CGUUAUACUGCUUUGGUAAUUGUUCCA	GAACAAUUACCAAAGCAGUAUAACG

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