SUPPLEMENTARY METHODS

DNA constructs

The original sensor construct that we previously described was generated by PCR fusion using a 5 kb fragment of the *ceh-36* promoter [1]. For internal consistency, we have based all constructs used in the present study on a cloned *ceh-36^{prom2}::gfp* plasmid, which shows the exact same expression pattern as the previous fragment, but due to its smaller size is easier to handle. This plasmid was generated by cloning 1.8 kb of the *ceh-36* promoter (1883 bp to 36 bp upstream of the ATG start codon) into the Hind III/BamH1 sites of the canonical pPD95.75 *gfp* vector, kindly provided by Andy Fire. EcoRI and EagI digestion releases the *unc-54* 3'UTR and all other 3'UTRs were inserted into these two sites. All these 3'UTRs were amplified from genomic wild-type DNA. All 3'UTR mutations were introduced by fusion PCR, in which two pieces of the 3'UTR were separately amplified with overlapping primers that contained the mutation of interest, and fused in a second PCR reaction. All mutations were confirmed by sequencing. The *lsy-6* target site from *cog-1* (see **Fig.1**) was inserted into position 370 of the 700 bp *unc-54* 3'UTR and it replaced the *let-7* target site at position 250 in the 467 bp *lin-28* 3'UTR. A list of primers and DNA constructs used in this study can be found in Suppl. Table 1.

Generation of transgenic lines

A list of transgenic arrays and lines can be found in **Supplementary Table 1**. Stable transgenic lines were generated by injecting the sensor *gfp* constructs (at 5 ng/µl) with *rol-6(d)* (at 100 ng/µl) as an injection marker into the gonad of wild-type N2 animals. The relatively low amount of injected sensor DNA ensures that the *gfp* signal is in the sub-saturation range thereby allowing to observe even slight variations in expression levels.

Scoring of transgenic lines

All strains were maintained at 20°C prior to scoring. All lines were scored blind to genotype under a Zeiss Axioplan 2 microscope. To minimize the inclusion of mosaic

animals which may have lost the reporter in either ASEL, ASER or both lineages, only those animals were scored in which expression in the AWCL and AWCR neurons was observed (see *gfp* image in **Fig.1a**).

The fluorescence intensity of the 3' UTR sensor constructs was compared between ASEL and ASER in each individual animal. This assay is quantitative in that it quantifies the percentage of animals whose *gfp* expression pattern falls into one of three categories: "ASEL > ASER", "ASEL = ASER", and "ASEL < ASER". Therefore, our assay quantifies expression levels over a population based on qualitative differences in individual animals. As computerized measurements in individual animals for a large-scale study involving thousands of individual specimens are impracticable, we have spot-checked our qualitative assessment of *gfp* expression using AxioVision imaging software (Zeiss, Inc.) that measures the pixilation intensity of ASEL vs. ASER. We found that our qualitative scoring of each individual animal is sensitive to 25% differences in *gfp* expression between ASEL and ASER. Importantly, this 25% limit of sensitivity is well below the inherent noise levels of the *in vivo* system, in which variation of *gfp* expression from an extra-chromosomal array occurs in a random and chaotic manner. This noise is apparent in non-regulated control 3'UTRs (*unc-54*, *lin-28* or seed-mutated *cog-1* 3'UTR; **Fig.1**); ~20-30% of animals show "ASEL>ASER" *gfp* expression but about the same number of animals also shows "ASER>ASEL" *gfp* expression. This chaotic noise in the system makes the assay extremely sensitive to regulation; whenever a 3'UTR is *lsy-6*-regulated, these "noise categories" are transformed into striking levels of differences in *gfp* intensity in ASEL vs. ASER; meaning, there are still wild-type levels of fluorescence intensity in ASER, but fluorescence is virtually undetectable in ASEL. This is very easy and reliable to score and eliminates the necessity for computerized scoring of every single of the thousands of animals we scored. In contrast, if a 3' UTR sensor fails to be regulated by *lsy-6* then the noise levels are maintained in both cells and a characteristic "un-regulated" distribution is observed (~20-30% of animals in "ASEL > ASER" category, ~50-60% of animals in "ASEL = ASER" category, ~20-30 % of animals in "ASEL < ASER" category).

Due to variability in DNA copy number on transgenic arrays [2], the levels of noise vary from line to line and construct to construct, thereby preventing a comparison of

absolute numbers of animals in each category in different lines. For example, despite tightly controlled injection concentrations, expression levels may vary from one array to another by several fold. Assume that copy number of DNA on array #1 expression is 2X that of array #2 and assume that both arrays contain DNA that is regulated by *lsy-6*. Scoring by our presented method (which does not compare array to array, but compares the “ASEL>ASER” and “ASER>ASEL” category within a transgenic line) will reveal that both DNAs are regulated. In contrast, if one were to compare absolute fluorescence intensity from different lines, one would reach the inaccurate conclusion that one construct is not regulated as well as the other since the absolute fluorescence intensity in the ASEL cell would be smaller in the lines with less DNA copy numbers on the array.

3’UTR selection

3’UTR seed matches to *lsy-6* were identified using the partially overlapping predictions of several algorithms [3]. All but two targets (C48D5.2A, F59A6.1) were predicted by PicTar [4] and three targets (C48D5.2A, F59A6.1, ZK637.13) were predicted both by miRBase [5] and a new unpublished algorithm (http://cbcsrv.watson.ibm.com/rna22.html; I. Rigoutsos, IBM Watson Research Center, pers.comm.). All predicted *lsy-6* sites are located in between 30 and 482 bp 3’ from the predicted stop codon and in each cases where experimental evidence of the 3’UTR is available (EST clones) the *lsy-6* sites lies within the 3’UTR. Moreover, the *lsy-6* sites are conserved between *C.elegans* and *C.briggsae* orthologs (**Supplementary Fig.2**).

ΔG values have been proposed to be important determinants of miRNA/target interaction [6]. As determined by RNAhybrid [7], ΔG values for tested *lsy-6* targets have a range of –13.3 to –19.4 kcal/mol, which is comparable to the predicted value of the *lsy-6/cog-1* interaction (-17.6 kcal/mol).

Note that some of the predicted *lsy-6* targets display considerable pairing in the 3' region of the heteroduplex with *lsy-6*, while others, in analogy to the *lsy-6/cog-1* heteroduplex (**Fig.1a**), display little 3' complementarity (**Fig.2b** and **Supplementary Fig.1**). Since neither type of predicted target is regulated by *lsy-6*, we do not consider 3' end pairing as a critical parameter in *lsy-6* target recognition.

REFERENCES FOR SUPPLEMENTARY METHODS

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