Figure S1. Temperature effects on Tet-Off/tetO expression.

The influence of temperature on expression in ALM TRNs in animals homozygous for both *mec-4* promoter Tet-Off drivers (as illustrated in schematic above the graphs) and *tetO* reporters (see Fig. 1 for schematics) with differing numbers of *tetO* sites integrated on Chr I (n=13-20). The data was collected from the same set of animals as Fig. 1C. Arbitrary units are defined identically to those in Fig. 1B and 1C and comparable. Genotypes of the strains used are listed in Table S1. Summary statistics are provided in Table S2.

Figure S2. Influence of binding site complexity on expression.

**A)** Quantification of expression in ALM TRNs from reporters with varying number of *lexO*, *UAS* and *QUAS* binding sites driven by their cognate driver expressed under a *mec-4* promoter. The structures of the drivers are illustrated above the graphs. Refer to Fig. 2A and 2B for schematics of the structure of the drivers and reporters (n=14-20). **B)** Influence of chromatin modification defects on expression of *tetO* reporters in ALM TRNs. Quantification of expression from animals homozygous for a 14X *tetO* reporter, a *mec-4p* Tet-Off driver, and for the chromatin modification mutations listed (n=13-21). **C)** Quantification of expression from reporters with varying number of minimally repetitive binding sites (shown in Fig. 2A) driven by their cognate Tet-Off driver expressed under a *mec-4* promoter (n=25-35). Genotypes of the strains used are listed in Table S1. Summary statistics are provided in Table S2.

Figure S3. Influence of *tetO* site spacing on expression.

Quantification of expression in ALM TRNs from reporters with varying spacing between *tetO* binding sites upstream of a *mec-7* basal promoter. The *tetO* reporters are driven by a *mec-4p* Tet-Off driver schematized above the graph. **A)** Left) Schematic of the structure of *4X tetO* promoters with varied spacing between *tetO* sites. Right) Quantification of the expression of GFP in ALM under control of a TRN driver (n=9-10). **B)** Left) Schematic of the structure of *13X tetO* promoters with varied spacing between *tetO* sites. Right) Quantification of the expression of GFP in ALM under control of a TRN driver (n=14). **C)** Quantification of expression from 13X reporters. 13 X *lexO* and *QUAS* reporters with sites separated by 43 bp analogous to the reporter shown in Fig S3B driven by their cognate mec-4p drivers was quantified in L4 animals grown at 22.5°C. Genotypes of the strains used are listed in Table S1. Summary statistics are provided in Table S2.

Figure S4. Quantification of tissue type influence on tetO promoter expression levels.

Shown are the expression levels obtained from a series of reporters containing varying numbers of tetO binding sites driving expression of GFP using a *mec-7* basal promoter. Distinct tissue specific promoters (labelled over each graph) were used to examine the expression profile as a function of tissue type [*ehs-1p* (n=12-15), *cup-4p* (n=12-19), *dpy-7p* (n=10-21), *vha-6p* (n=11-17), *myo-3p* (n=10-18), *myo-2p* (n=12-16), *nhx-2p* (n=12-16), *phat-5p* (n=14-17), *mec-4Sp* (n=25-33)]. These data are the individual tissue data presented in aggregate in Figure 4. Genotypes of the strains used are listed in Table S1. Summary statistics are provided in Table S2.

Figure S5. Influence of basal promoters on expression levels from *tetO* promoters.

**A)** Left) A diagram of basal promoter regions used for expression studied. The horizontal line represents the genomic region used as a basal promoter. The arrow above the line represents the major transcriptional start site. The red triangle below the line represents the position of a trans-splicing signal sequence. The numbers represent the position of defined elements in bases upstream of the ATG. The position of the elements is only shown for the first of several basal promoter fragments derived from the same gene. Except for the *pes-10* basal promoter (-23) all sequences from -1 were used. p10m7bp is a dual basal promoter contain both the *pes-10* and *mec-7* basal promoters head to tail. Right) Images of the head and tail of L4 animals carrying a homozygous *7X tetO* GFP transgene containing the specified basal promoter. All images shown were taken under identical conditions and treated similarly in assembling the figure. Scale bar: 25 µm for both image columns. **B)** Quantification of GFP expression levels in the ALM TRN of L4 animals carrying both a *mec-4* promoter Tet-Off driver and a *tetO 7X* GFP reporter using a specified basal promoter (n=11-22). The presence of a *cis* marker varied depending on the methodology used to create the transgenes and is shown below the basal promoter. Genotypes of the strains used are listed in Table S1. Summary statistics are provided in Table S2.

Figure S6. Efficacy of random and truncated basal promoters of *tetO* promoter expression.

Left) A schematic of the structure of insertions with random 200 bp (200-R1 to 200-R3), 400 bp (400-R1), or small sequence motifs in place of a traditional basal promoter. The motif (k) consists of a *C. elegans* consensus Kozak sequence (AAAA). The small motif ‘t’ consists of the 24 bp directly upstream of the *tbb-2* ATG and contains a consensus Kozak sequence, a SL1 trans-splice signal, and a major *tbb-2* transcriptional start site. The motif ‘l’ consists of the 17 bp directly upstream of the *lys-8* ATG and contains an SL1 trans-splice signal and a major transcriptional start site. Right) Quantification of expression in ALM from transgenes homozygous for both the reporters and a *mec-4p* driver schematized above the graph (n=11-29). Summary statistics are provided in Table S2.

Figure S7. Influence of 3' UTRs on *tetO* expression.

**A)** Structure of 3' UTRs. Shown a representation of the last exon (pink or teal) and 3' UTR(brown) and associated transcriptional termination region of the listed genes. In blue is the region used as the 3' UTR in the transgenic constructs. The exact sequence of the 3' UTRs is denoted in Table S1. **B)** The structure of the synthetic compound ter-222 3' UTR constructed using three 3' UTRs as illustrated in the schematic. **C)** Top) Structure of the construct used to test for promoter/enhancer activity of 3' UTR and other insert sequences. Bottom) Images of whole L4 animals testing the listed inserts for enhancer activity. Only the *tbb-2* 3' UTR exhibited detectable activity as assayed by detectable GFP signal. Scale bar 100 µm.