**File S2 Supplemental methods**

Oligos used to construct plasmids are listed in Table S1. Plasmids used to knock-in *promoter::TIR1::F2A::mTagBFP2::AID\*::NLS* sequences are listed in Table S2. All other plasmids are listed in Table S3. Any unprovided cloning information is available upon request. All sequence files are available in Supplemental file S4.

*TIR::F2A::mTagBFP2::AID\*::NLS*

pDD356 and pDD357 were generated in parallel based on SEC-containing vectors for single-copy transgene insertion (Pani and Goldstein 2018). pDD356 is derived from pAP088 and mediates insertion near the *tiTi4348* site on LG I; pDD357 is derived from pAP087 and mediates insertion near the *ttTi5605* site on LG II. Note that in both vectors, the *LoxP* site present in the originally described SEC (Dickinson *et al.* 2015) has been replaced by *LoxN* to ensure compatibility with SEC vectors for endogenous gene editing (Pani and Goldstein 2018). pAP087 and pAP088 were linearized by digestion with NotI-HF and AflII, and each 11 kb vector backbone fragment was gel purified. Insert fragments comprising the *sun-1* promoter and codon-optimized TIR1 were PCR amplified from pZC3 (a gift from Abby Dernburg; (Zhang *et al.* 2015). The AID\* sequence was derived from the vector pUA77 (a gift from Oliver Hobert) and was amplified by PCR; a cMyc NLS sequences was added as part of the PCR primer. A codon-optimized (Redemann *et al.* 2011) mTagBFP2 sequence with an F2A peptide at the N-terminus was synthesized as a gBlock fragment (Integrated DNA Technologies). The P*sun-1*, TIR1, F2A::mTagBFP2, AID\*::NLS and vector backbone fragments were combined using Gibson assembly and transformed into *E. coli* strain DH10B. Correctly assembled clones were identified by restriction digestion and verified by Sanger sequencing.

pDD356 and pDD357 were linearized by restriction digestion with SphI-HF (NEB) and ClaI (NEB). Typically, 24 µl of a miniprep was mixed with 3 µl of 10xCutSmart, 1.5 µl of each enzyme. The reaction was incubated at 37ºC for 1-2 hours before running through DNA Clean and Concentrator kit (Zymo, D4013) and eluting in 30 µl of TE buffer. Unless otherwise noted, promoters were PCR amplified using Phusion polymerase ( from N2 genomic DNA using the primers in Table S1 and Gibson cloned into pDD356 and pDD357 to produce the repair templates listed in Table S2. We note that aside from the homology arms, pDD356 and pDD357 are identical; thus a single PCR product can be cloned into either vector. Correct assemblies for pJW plasmids was identified by colony PCR with oligos 9+2559

A 4,758 bp *cis*-regulatory element from the intergenic region immediately upstream of the *cdh-3* transcriptional start site to drive anchor cell expression (Matus *et al.* 2015) was cloned from pWZ126 (Table S3) and Gibson cloned into SphI/ClaI linearized pDD357 with Hifi DNA assembly mix (NEB, #E2621) to produce pWZ184. Correct clones were identified by colony PCR with oligos WZ118 and DQM515.

To generate the *SCMmin* TIR1 construct (pJW2024 and 2026) an *SCMmin* promoter reporter intermediate was generated by PCR amplifying the *SCMmin* sequence from pJM30 (Hajduskova *et al.* 2009) with oligos 4152+4153. pJW1947 was linearized using oligos 4606+4774, and DpnI digested as described for pJW2072. The *SCMmin* PCR product was Gibson cloned into linearized pJW1947 to generate pJW1940. Correct clones were identified by miniprepping and Sanger sequencing. The *SCMmin::pes-10* minimal promoter fragment was then PCR amplified from pJW1940 and Gibson cloned into SphI/ClaI linearized pDD356 and pDD357, as described above.

SCM enhancer-*pes-10* minimal promoter was cloned from an SV40 NLS::mScarlet-I (dpi)::tbb-2 3’UTR reporter (pJW1940) using oligos 4754+4755. Promoterless (pJW1841) and *pes-10 minimal promoter* (pJW1947) intermediates used to generate pJW1940 can be used to Gibson clone promoters or enhancers of interest and may be useful reagents to the community.

*PCR product gel purification using homemade crystal violet kit*

A 0.8% agarose gel was made using TAE buffer and allowed to cool to ~50ºC before adding 80 µl of 2 mg/mL crystal violet (CV) dye (Fisher, C581100) in ddH20. PCR reactions were mixed with a 6X homemade loading dye (30% glycerol, 20 mM EDTA, 100 µg/ml CV) and run on the 0.8% agarose gel containing CV. The gel was run until the CV dye front migrated ¼ of the way up the gel and the DNA bands were visible. Desired bands were excised manually, dissolved in a homemade NAI solution (6.6 M NaI (Fisher, AC202895000)/16 mM Na2SO3 (VWR, AA13454-36) through heating at ~42°C-50ºC and column purified using QIAprep Spin Miniprep Columns (Qiagen, 27115) and equivalent buffers (QG, PE, EB) to a QiaQuick gel extraction kit (Qiagen, 28704). Gennessee has provided the recipes for commonly used buffers in Qiagen kits here: <https://geneseesci.com/wp-content/uploads/2019/04/QiagenBufferRecipes.pdf> While we successfully used columns designed for minipreps, one could also use the columns designed for the gel extraction kit (Qiagen, 28115).

*Strain construction for mex-5p, myo-2, and cdh-3p strains*

DQM519 [*bmdSi175[^SEC^cdh-3pTIR1::F2A:: mTagBFP2::AID\*::NLS*] II: -0.77] was generated using pWZ126 and the reagents in Table S4 and crossed to JDW59 *nhr-25(wrd12*[*nhr-25::GFP^AID\*::3xFLAG]*) (Martinez *et al.* 2020). The SEC was excised as described (Dickinson *et al.* 2015) to generate DQM623 [*bmdSi176[^SEC^cdh-3pTIR1::F2A:: mTagBFP2::AID\*::NLS*] II:-0.77; *nhr-25(wrd12[nhr-25::GFP^degron:3xFLAG]) X*]. This strain was outcrossed against N2 twice and loss of the *nhr-25(wrd12)* allele was confirmed by genotyping PCR (primers available upon request).

JDW35 *nhr-23(wrd1[GFP^BioTag::degron::3xFLAG])* I; *wrdSi8[^SEC^mex-5p::TIR1::F2A::BFP::AID\*::NLS::tbb-2 3'UTR] II:-0.77* was generated by injecting pJW1726 into JDW1 *nhr-23(wrd1[GFP^BioTag::degron::3xFLAG])* I using the reagents in Table S4. The construction of JDW1 will be described elsewhere (Ragle *et al.* 2020). This strain was backcrossed twice against N2 animals two times to remove the *nhr-23(wrd1)* allele and produce JDW222 *wrdSi8[^SEC^mex-5p::TIR1::F2A::BFP::AID\*::NLS::tbb-2 3'UTR] II:-0.77*. The SEC was excised as described (Dickinson *et al.* 2015) to generate JDW221 *wrdSi35[mex-5p::TIR1::F2A::mTagBFP2::AID\*::NLS] II*:0.77.

JDW39 *wrdSi10[^SEC^mex-5p::TIR1:F2A:mTagBFP2:tbb-2 3'UTR]* I:-5.32*, nhr-23(wrd8[nhr-23::GFP^degron::3xFLAG])* I was generated by injecting pJW1357 into JDW29 *nhr-23(wrd8[nhr-23::GFP^degron::3xFLAG])* Iusing the reagents in Table S4. *wrd10* was subsequently outcrossed to N2 animals one time to remove the *nhr-23(wrd8)* allele and produce JDW222 *wrdSi8[^SEC^mex-5p::TIR1::F2A::BFP::AID\*::NLS::tbb-2 3'UTR]* II:-0.77. The SEC was excised as described (Dickinson *et al.* 2015), and outcrossed two more times to generate JDW223 *wrdSi35[mex-5p::TIR-1:F2A:mTagBFP2::AID\*::NLS]* II:-0.77.

LP870 *cpSi172 [myo-2p::TIR1::F2A::mTagBFP2::AID\*::NLS::tbb-2 3'UTR]* I:-5.32 was created by injecting into LP860 *daf-2(e1370) III; lea-1(cp431[mNG::3xFlag::AID\*::lea-1 loxP]) V)* and removing the *daf-2* and *lea-1* alleles by outcrossing against N2 animals. Loss of alleles was confirmed by PCR.

**Supplemental references**

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