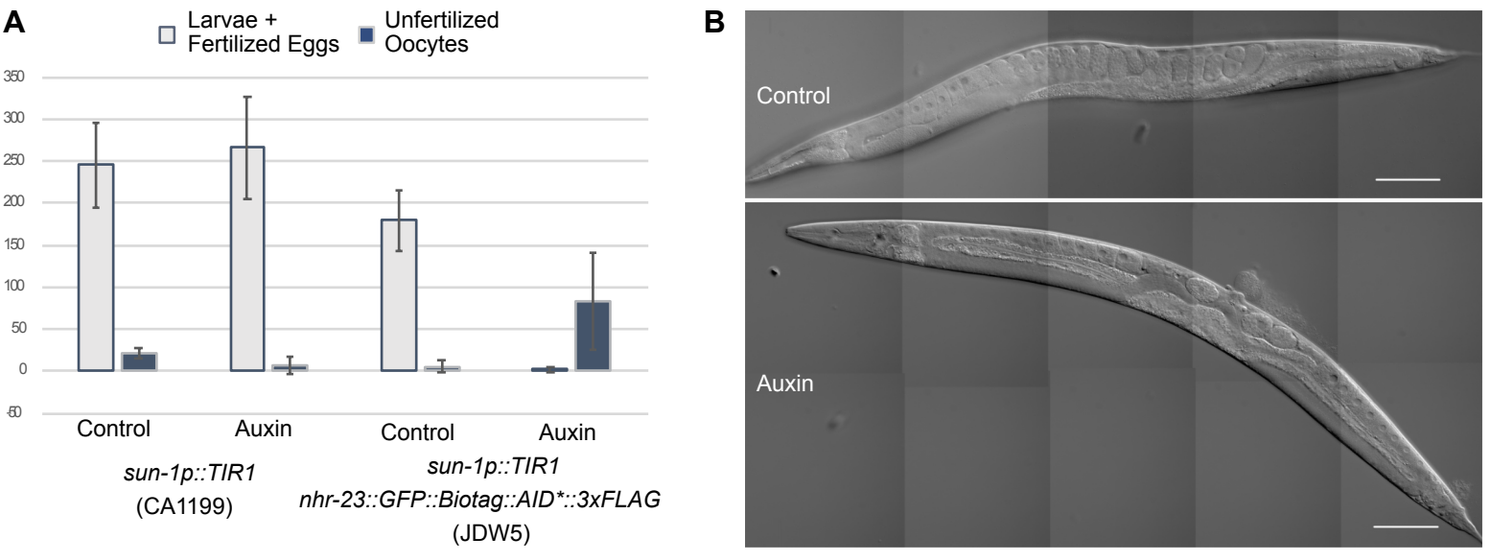


Fig. S1. NHR-23-depletion in worms expressing TIR1 from a *sun-1* promoter causes sterility similar to that observed when TIR1 is expressed from the *mex-5* promoter. (A) Combined count of L1 larvae/fertilized egg progeny or unfertilized oocyte from hermaphrodites grown from L1 onwards with or without 4 mM auxin. Error bars=standard deviation (n=12 for each condition) (B) Representative DIC images of L4 + 1 day *nhr-23::GFP::Biotag::AID*::3xFLAG; sun-1p::TIR1* hermaphrodites in the absence (top) or presence (bottom) of 4 mM auxin. Scale bars: 80 µm.

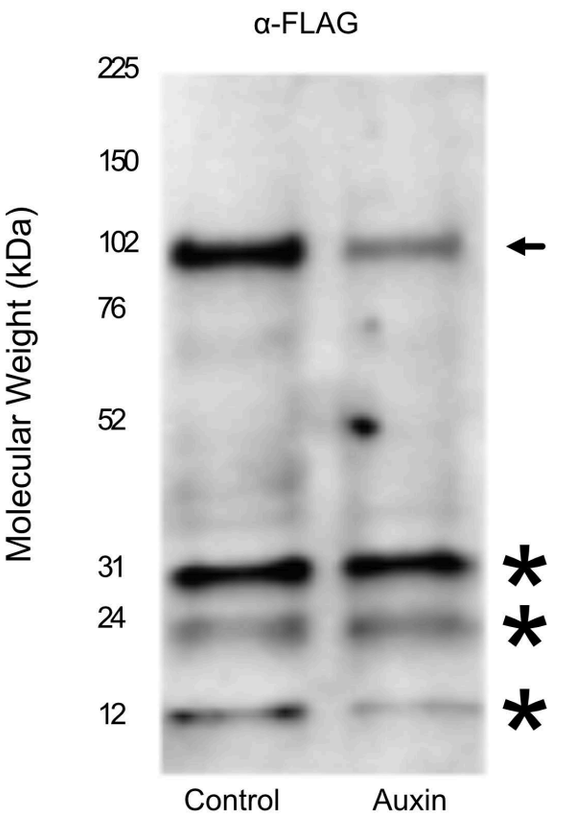
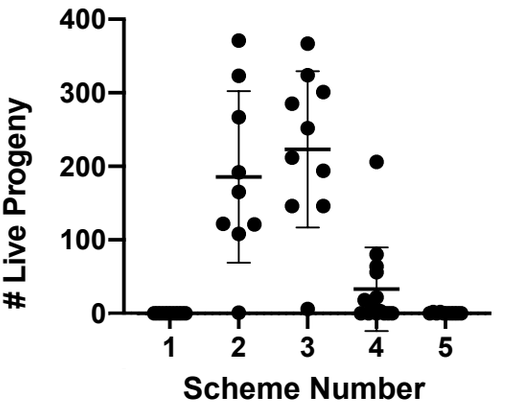


Fig. S2. Assessing NHR-23 depletion by western blot. Anti-FLAG immunoblot analyses as in Fig. 3 showing the entire blot. Arrow indicates NHR-23::GFP::AID*::3xFLAG band. Stars indicate background bands previously reported (Ward, 2015).

A

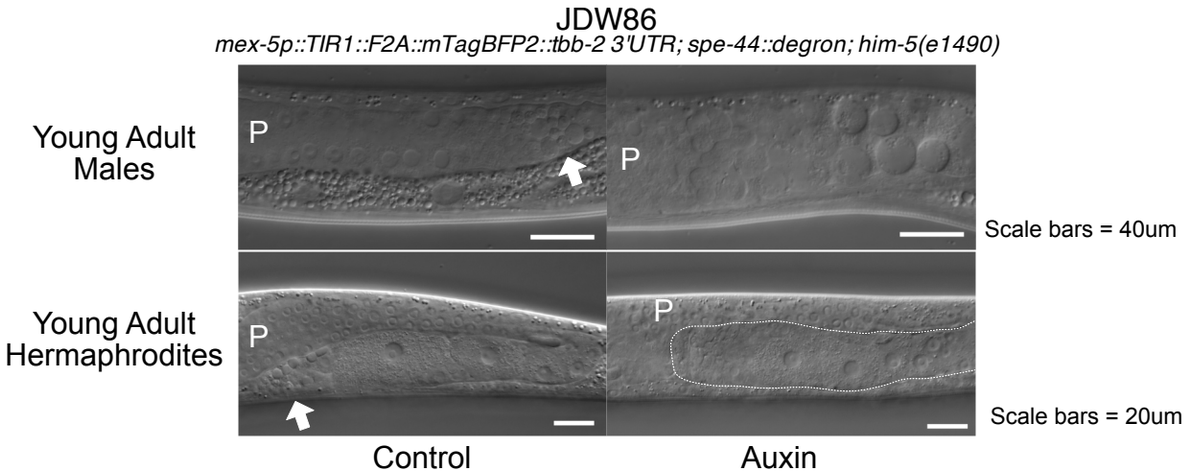
Crossing Scheme					# Live Progeny	
Scheme Number	Male Genotype [crossed to *spe-8 (hc50); him-8 (e1489)*]	Life Stage of Male	Media	N	Mean (SD)	Median
1	None (self)	-	Control	10	0	0
2	*him-5 (e1490)*	L4	Control	9	185.6 (±116.5)	165
3	JDW92 *wrdSi19(mex-5p::TIR1), nhr-23(kry61(nhr-23::AID*-TEV-3xFLAG)); him-5(e1490)*	L4	Control	10	223.3 (±106.3)	232
4	JDW92 *wrdSi19(mex-5p::TIR1), nhr-23(kry61(nhr-23::AID*-TEV-3xFLAG)); him-5(e1490)*	L4	Auxin	14	32.9 (±56.7)	7.5
5	JDW92 *wrdSi19(mex-5p::TIR1), nhr-23(kry61(nhr-23::AID*-TEV-3xFLAG)); him-5(e1490)*	Young Adult	Auxin	10	0.4 (±0.8)	0



B

Fig. S3. Mating/Transactivation Assay. (A) Quantitation of live progeny produced from the indicated crosses to *spe-8(hc50); him-8(e1489)* hermaphrodites. Standard deviation (SD) is provided in the table. In the graph, the center horizontal lines for each crossing scheme represent mean and the whiskers represent standard deviation. (B) Image of JDW92 *wrdSi19[mex-5p::TIR1:F2A:mTagBFP2:tbb-2 3'UTR, I:-5.32], nhr-23(kry61(nhr-23::AID*::TEV::3xFLAG)) I; him-5(e1490) V* animals grown on auxin. The male in the image is engaging in mating behavior.

A



B

	Genotype	Media	N	Mean (SEM)	Median
N2	Wild-type	Control	12	273.5 (±11.4)	269
JDW101	*wrd20(spe-44::30xlinker::mScarlet::Lox511I::3xMyc)*	Control	12	264.8 (±8.6)	276
JDW86	*wrdSi15(mex-5p::TIR1:F2A:mTagBFP2:tbb-2 3'UTR); spe-44(fx110[spe-44::degron]); him-5(e1490)*	Control	14	126.6 (±9.5)	130
JDW86	*wrdSi15(mex-5p::TIR1:F2A:mTagBFP2:tbb-2 3'UTR); spe-44(fx110[spe-44::degron]); him-5(e1490)*	Auxin	12	0	0

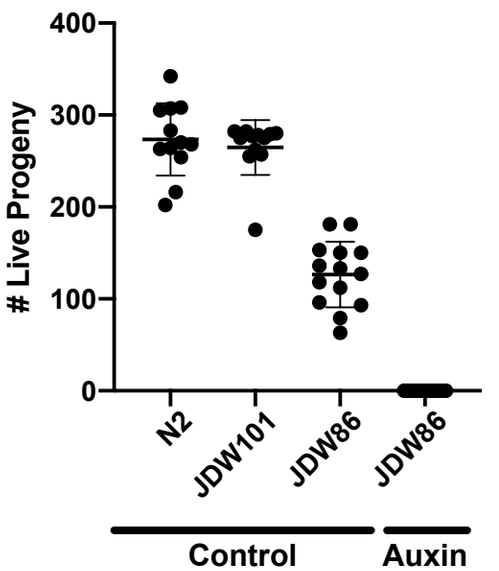


Fig. S4. Validation of *spe-44* alleles. (A) DIC images of JDW 86 *wrdSi15[mex-5p::TIR1:F2A:mTagBFP2:tbb-2 3'UTR]; spe-44(fx110[spe-44::AID*]); him-5(e1490)* young adult males and young adult hermaphrodites grown on control media or 4 mM auxin. The arrows point to examples of mature spermatids in control animal germlines. (B) Live progeny counts from animals of the indicated genotypes. Standard error of the mean (SEM) is provided in the table. In the graph, the center horizontal lines for each strain represent mean and the whiskers represent SEM.

Table S1. Plasmids used in this study		
Plasmid	**Parent plasmid or Reference**	**Notes**
pCZGY2747	Unpublished, gift from Zhiping Wang	*eft-3*p::*Cas9*; *U6*p::sgRNA vector targeting LGI site where ttTi4348 is inserted
pDD363	Dickinson *et al.*, 2018	LoxP-flanked SEC donor for the Sap Trap cloning system
pDD372	Dickinson *et al.*, 2018	Codon-optimized GFP donor for the Sap Trap cloning system
pDD379	Dickinson *et al.*, 2018	SapTrap destination vector for building combined sgRNA expression + repair template vectors, using the F+E sgRNA scaffold
pJW1254	Zhang *et al.*, 2015	*eft-3*p::Cas9; *U6p*::sgRNA (F+E) targeting *nhr-23* 3' end
pJW1357	Ashley *et al.*, 2020	*mex-5*p::TIR1:F2A:mTagBFP2 construct for knock-in at LGI site where ttTi4348 is inserted
pJW1598	Ashley *et al.*, 2020	linker::GFP^SEC^BioTag::AID*::3xFLAG vector for C-terminally tagging NHR-23
pJW1659	Ashley *et al.*, 2020	9 amino acid flexible linker::AID*-3xFLAG donor for NT slot in the Sap Trap cloning system
pJW1725	pDD379	repair template for C-terminally tagging NHR-23 generated by Sap Trap: linker::GFP^SEC^AID*::3xFLAG with *nhr-23* homology arms; *U6*p::*nhr-23* sgRNA (F+E)
pJW1776	pDONR221	*nhr-23* 5' homology arm for Sap Trap cloning (Sap sites in arm are silently mutated)
pJW1781	pDONR221	*nhr-23* 3' arm for Sap Trap cloning
pJW1816	Ashley *et al.*, 2020	30 amino acid flexible linker::mScarlet^SEC (Lox511I)^3xMyc multicassette donor for the Sap Trap cloning system
pJW1838	Ashley *et al.*, 2020	Sap Trap sgRNA vector, U6 promoter and 3'UTR from Calarco paper with F+E sgRNA modifications
pJW1872	pJW1838	*U6*::*sgRNA* targeting *spe-44* 3' end
pJW1876	Ashley *et al.*, 2020	repair template for C-terminally tagging SPE-44 generated by SapTrap:: 30 amino acid flexible linker::germ line optimized mScarlet^SEC (Lox511I)^3xMyc with *spe-44* homology arms
pJW258	pDONR221	*nhr-23* cDNA in Gateway entry vector
pMLS257	Schwartz *et al.*, 2016	Sap Trap repair template backbone lacking the *U6p::sgRNA* cassette
pMLS287	Schwartz *et al.*, 2016	SapTrap flexible 12 amino acid linker C-terminal connector donor plasmid

Table S2. Oligonucleotides used in this study

Click here to Download Table S2

Table S3. sgRNAs and repair oligos used for co-CRISPR in this study		
Name	**Purpose**	**Sequence**
dpy-10 sgRNA	Co-CRISPR to inactivate *fog-3*	taatacgactcactataggctaccataggcaccacgaggtttaagagctatgctggaaac
fog-3 sgRNA	Co-CRISPR to inactivate *fog-3*	taatacgactcactatagtagacgagaaatgtgagacggtttaagagctatgctggaa
dpy-10 (cn64) repair oligo	Co-CRISPR to inactivate *fog-3*	cacttgaacttcaatacggcaagatgagaatgactggaaaccgtaccgcatgcggtgcctatggtagcggagcttcacatggcttcagaccaacagcctat
fog-3 repair oligo	Co-CRISPR to inactivate *fog-3*	aggtcggcatatttggcgctgaacttggaaactacgaattctaaagtctcacatttctcgtctacctgggatgttcatca