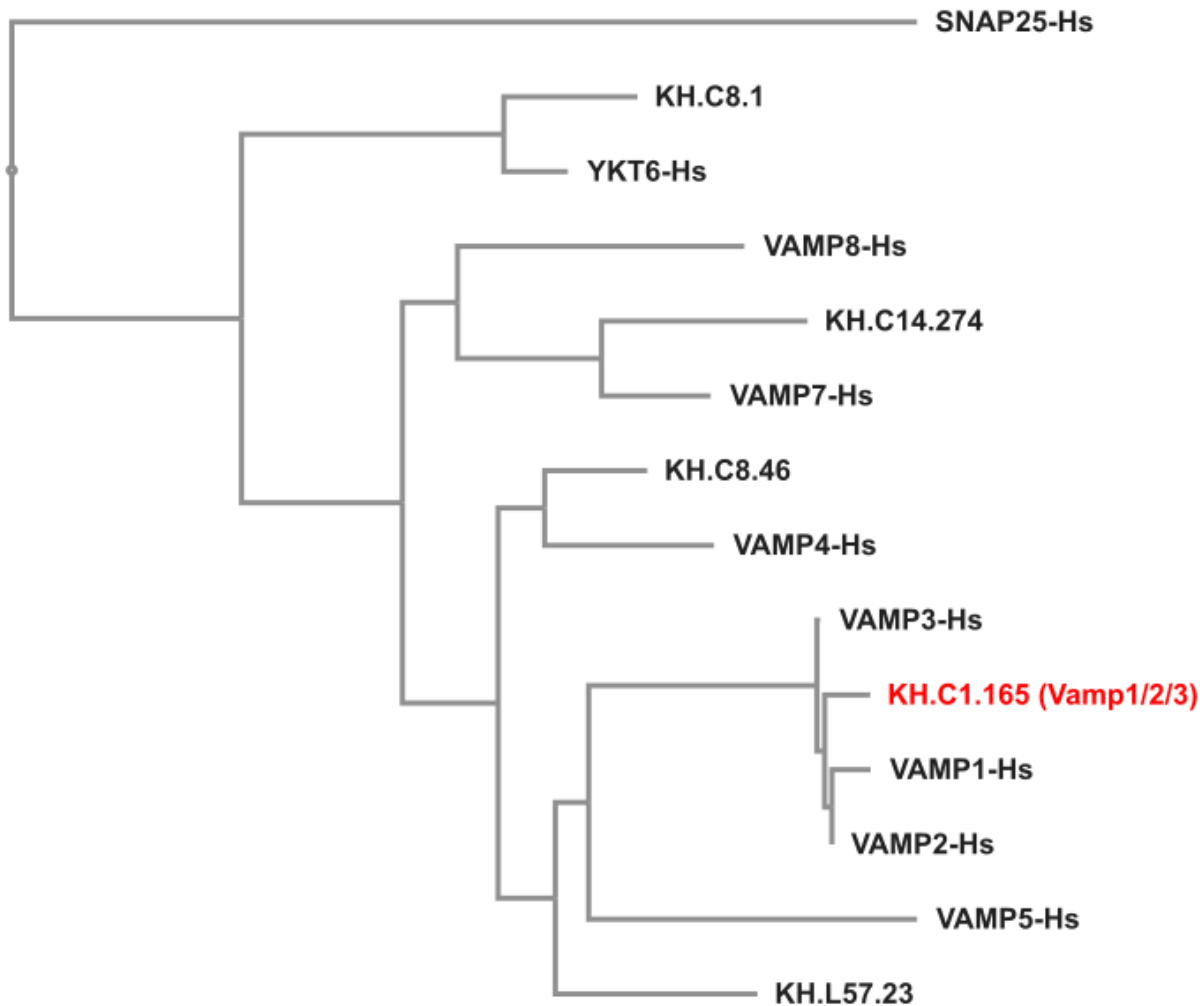


341



342

343 Figure S1. Phylogenetic tree of Vamp proteins.

344 Tree showing phylogenetic analysis of predicted proteins encoded by *VAMP* family genes from

345 human (Hs) and *Ciona robusta* (KH gene models). See methods for details and supplemental

346 sequences file for protein sequences used.

347

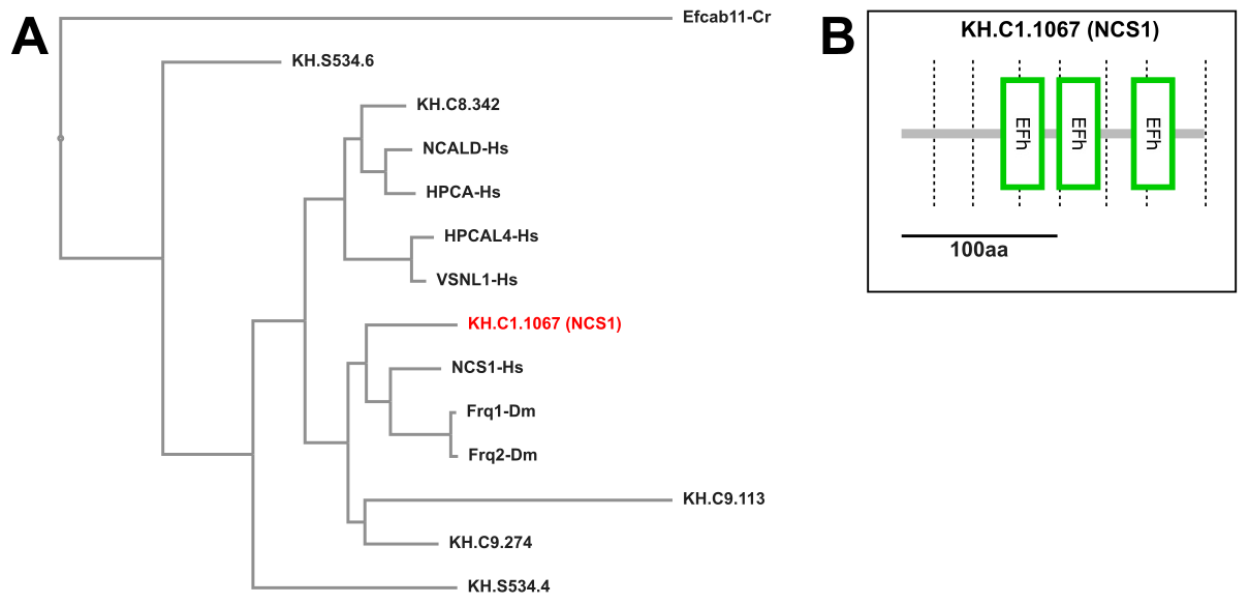
348

349

350

351

352



353

354 Figure S2. Phylogenetic tree of NCS proteins and diagram of *Ciona robusta* NCS1 domains.

355 A) Tree showing phylogenetic analysis of proteins encoded by *Neuronal Calcium Sensor* (NCS)

356 family genes from human (Hs), *Drosophila melanogaster* (Dm) and *Ciona robusta* (KH gene

357 models). Efcab11 from *C. robusta* (Cr) was used to root the tree. See methods for details and

358 supplemental sequences file for protein sequences used. B) Protein domain analysis diagram of

359 *Ciona robusta* NCS1 from SMART (Letunic et al. 2021) showing its predicted three EF-hand

360 (Efh) domains. Dashed lines indicate exon-exon junctions.

361

362

363

364

365

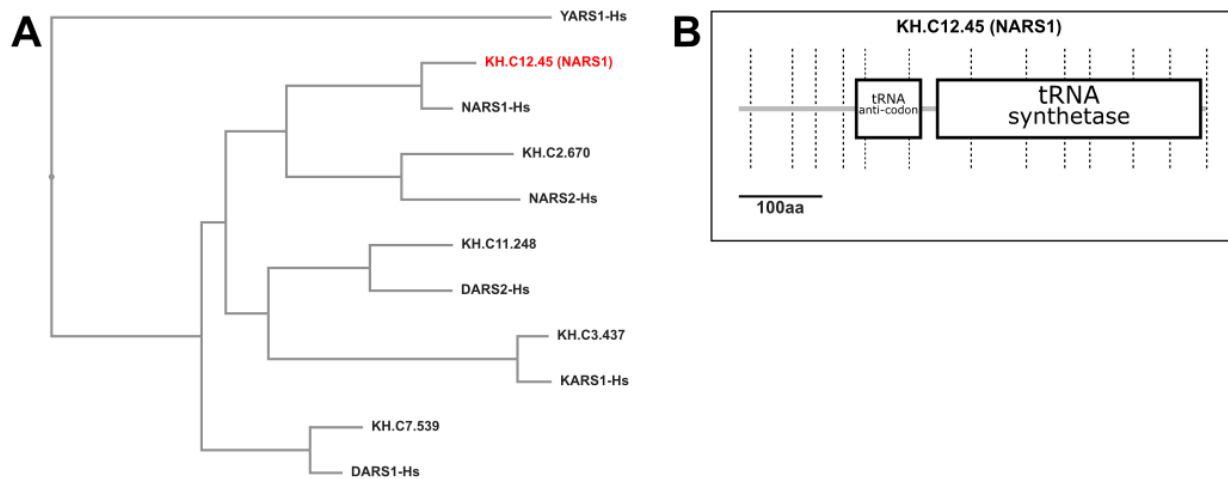
366

367

368

369

370



371

372 Figure S3. Phylogenetic tree of select aminoacyl-tRNA synthetases and *Ciona* NARS1 domains.

373 A) Tree showing phylogenetic analysis of proteins encoded by a sampling of aminoacyl-tRNA

374 synthetase genes from human (Hs) and *Ciona robusta* (KH gene models). See methods for

375 details and supplemental sequences file for protein sequences used. B) Protein domain analysis

376 diagram of *Ciona robusta* NARS1 from SMART (Letunic et al. 2021) showing its predicted

377 domains. Dashed lines indicate exon-exon junctions.

378

379

380

381

382

383

384

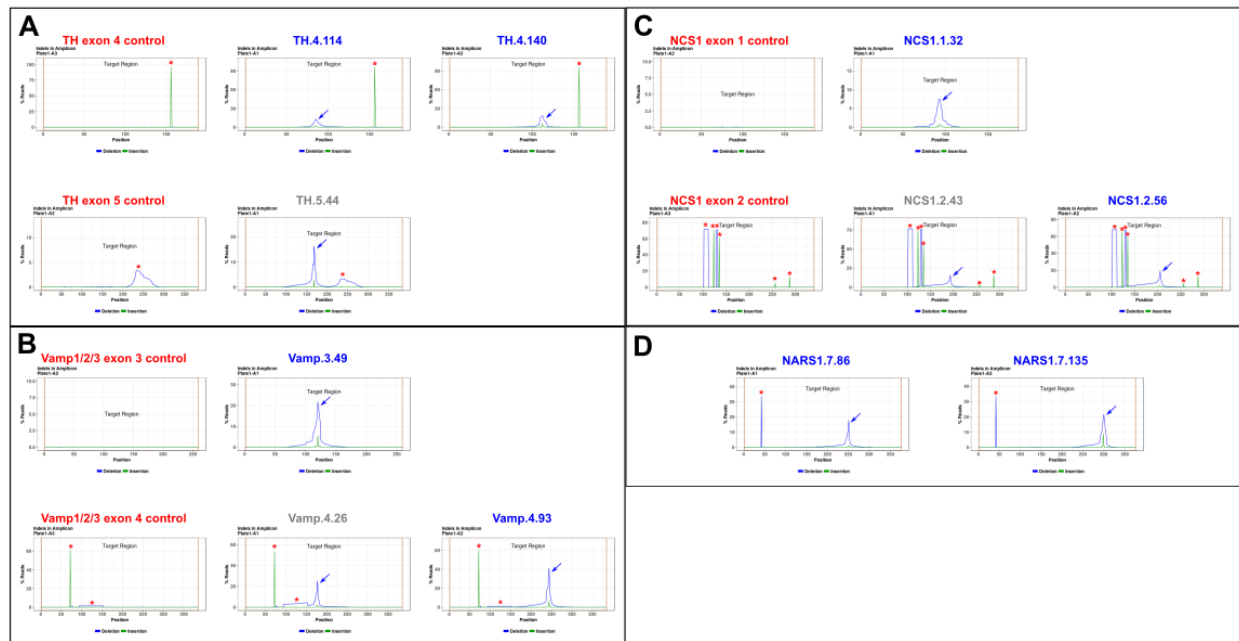
385

386

387

388

389



390

391 Figure S4. Indel plots for all sgRNAs tested.

392 A-D) NGS indel validation plots (including negative controls) for all the sgRNAs tested in this

393 study. No amplicon was obtained for the third *NARS1* sgRNA nor the *NARS1* negative control.

394 Blue arrows indicate CRISPR-generated indels, red asterisks indicate naturally occurring indels.

395

396

397

398

399

400

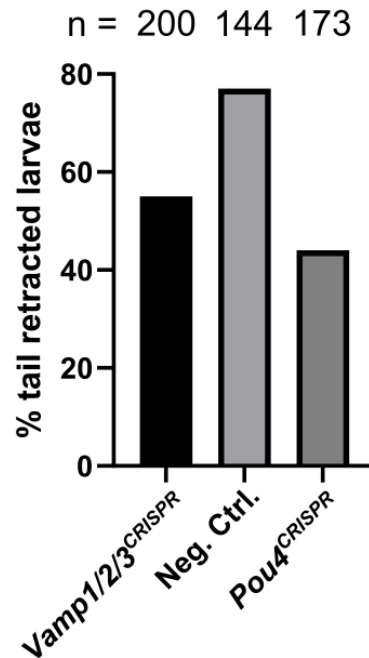
401

402

403

404

405



406

407 Figure S5. Replicate of *Vamp1/2/3* CRISPR.

408 Independent replicate of papilla-specific *Vamp1/2/3* CRISPR in *Ciona* larvae. Embryos were  
409 electroporated with 40 µg/700 µl *Foxc*>*Cas9* and gene-specific pairs of sgRNA vectors (40  
410 µg/700 µl each sgRNA vector). Negative control embryos were electroporated with 40 µg/700 µl  
411 *Foxc*>*Cas9* alone. Tail retraction was scored at 48 hours post-fertilization without screening for  
412 mCherry+ individuals.

413

414

415

416

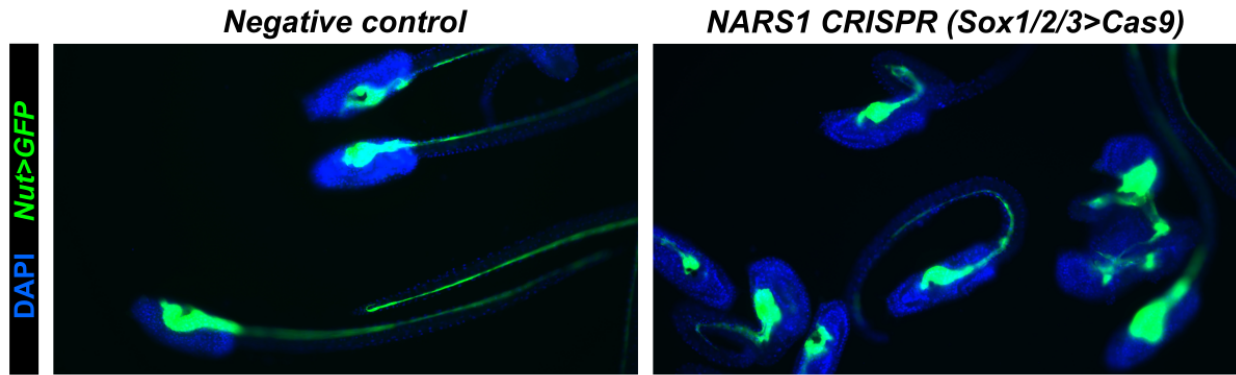
417

418

419

420

421



422

423 Figure S6. Neurectoderm-specific knockout of *NARS1* impairs neurulation.

424 Negative control larvae showing normal neural tube and tail morphogenesis, compared to  
425 *NARS1* CRISPR larvae. *Nut>Unc-76::GFP* (green) labels the central nervous system. Nuclei  
426 counterstained by DAPI (blue). See text for quantification.

427

428

429

430

431

432

433

434

435

436

437

438

439

440