



Figures and figure supplements

Vg1-Nodal heterodimers are the endogenous inducers of mesendoderm

Tessa G Montague and Alexander F Schier

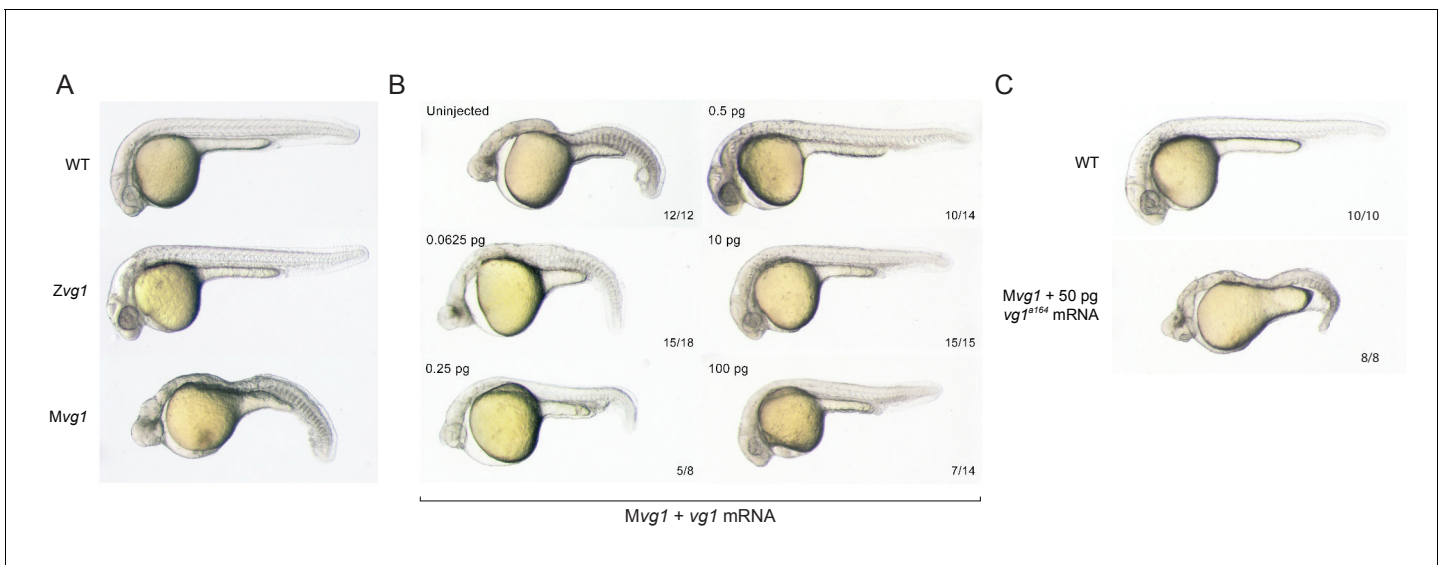


Figure 1. Maternal *vg1* is required for mesendoderm formation. (A) Zygotic and maternal *vg1* (*Zvg1* and *Mvg1*) mutants and wild-type (WT) embryo at 28 hours post-fertilization (hpf). See **Figure 1—figure supplement 1** for information about the *vg1* mutant alleles, and **Figure 1—figure supplement 2** for analysis of left-right asymmetry in WT and *Zvg1* embryos. (B) *Mvg1* embryos injected with 0.0625–100 pg of *vg1* mRNA. (C) *Mvg1* embryo injected with 50 pg of *vg1* mRNA containing the 8 bp deletion found in the genetic mutants (*vg1*^{a164}).

DOI: <https://doi.org/10.7554/eLife.28183.003>

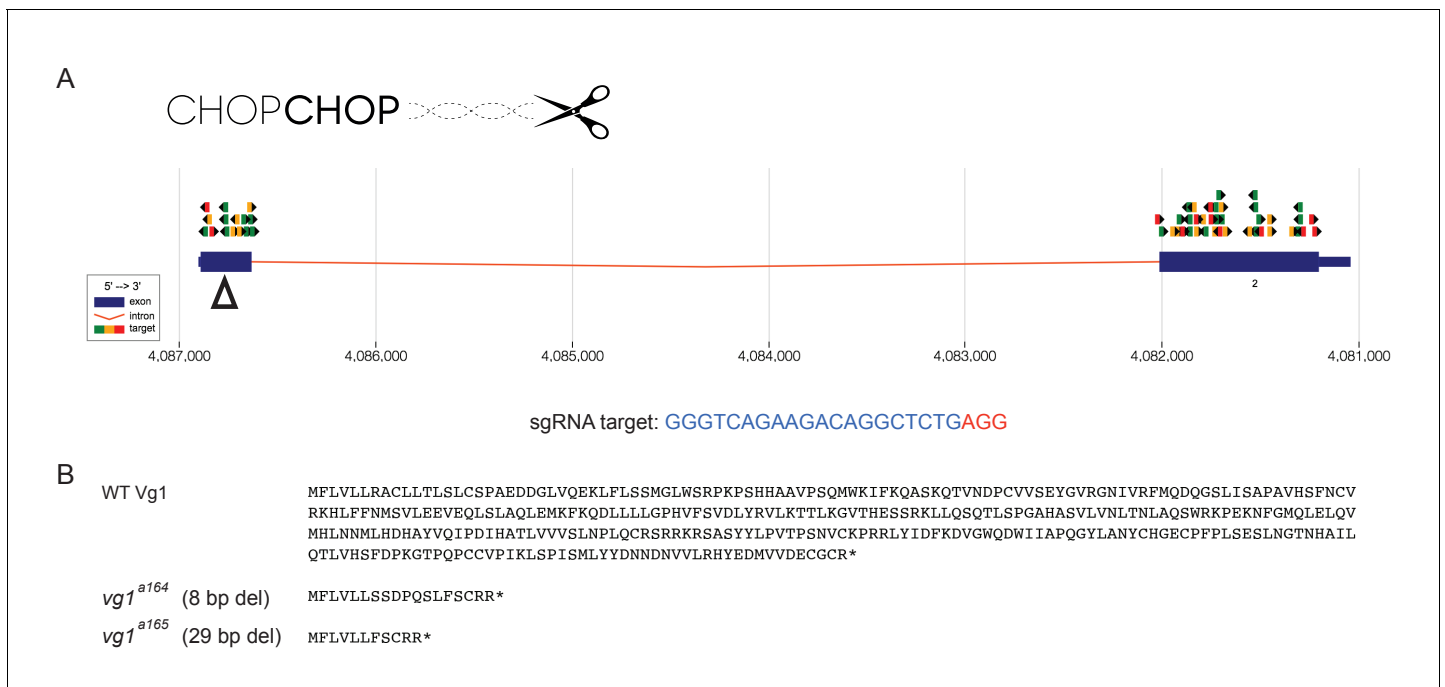


Figure 1—figure supplement 1. *vg1* mutant alleles. (A) Single guide RNAs (sgRNAs) targeting the *vg1/dvr1/gdf3* gene were designed using CHOPCHOP (Labun et al., 2016; Montague et al., 2014). Colored arrows indicate possible sgRNA targets, color-coded according to predicted efficiency (green: good to red: bad). Open arrowhead indicates the location of the sgRNA used in this study. The sgRNA sequence is provided in blue, with the PAM motif in red. (B) 8 bp (*vg1*^{a164}) and 29 bp (*vg1*^{a165}) deletion alleles cause frameshifts in *Vg1*, truncating it from a 355 amino acid protein to predicted 18 and 11 amino acid peptides, respectively.

DOI: <https://doi.org/10.7554/eLife.28183.004>

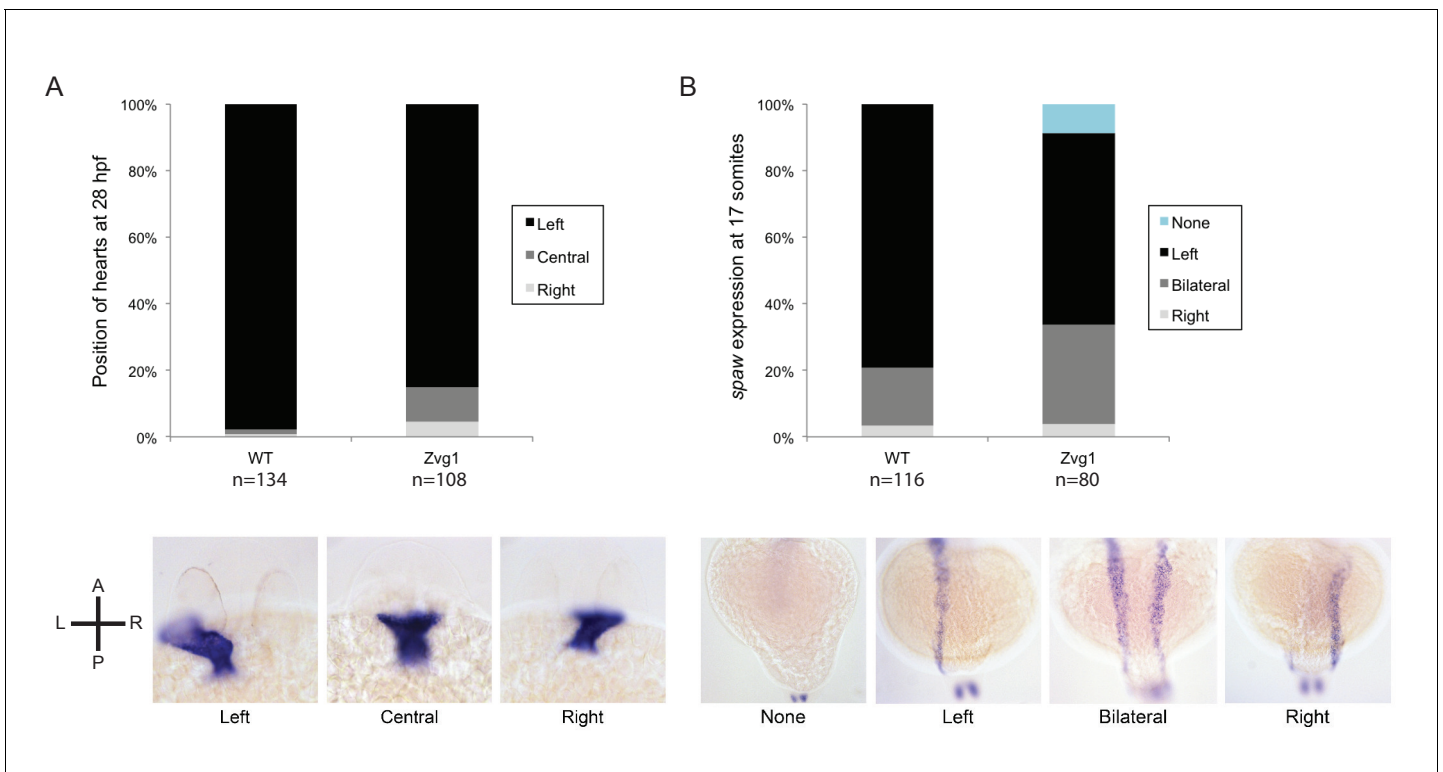


Figure 1—figure supplement 2. Left-right asymmetry in *Zvg1* mutants. (A) Heart positioning at 28 hpf in WT and *Zvg1* embryos, detected by expression of *cmlc2* by *in situ* hybridization. (B) Expression of *southpaw* (*spaw*), a Nodal ligand involved in left-right asymmetry, at the 17-somite stage in WT and *Zvg1* embryos. *spaw* is expressed in the lateral plate mesoderm.

DOI: <https://doi.org/10.7554/eLife.28183.005>

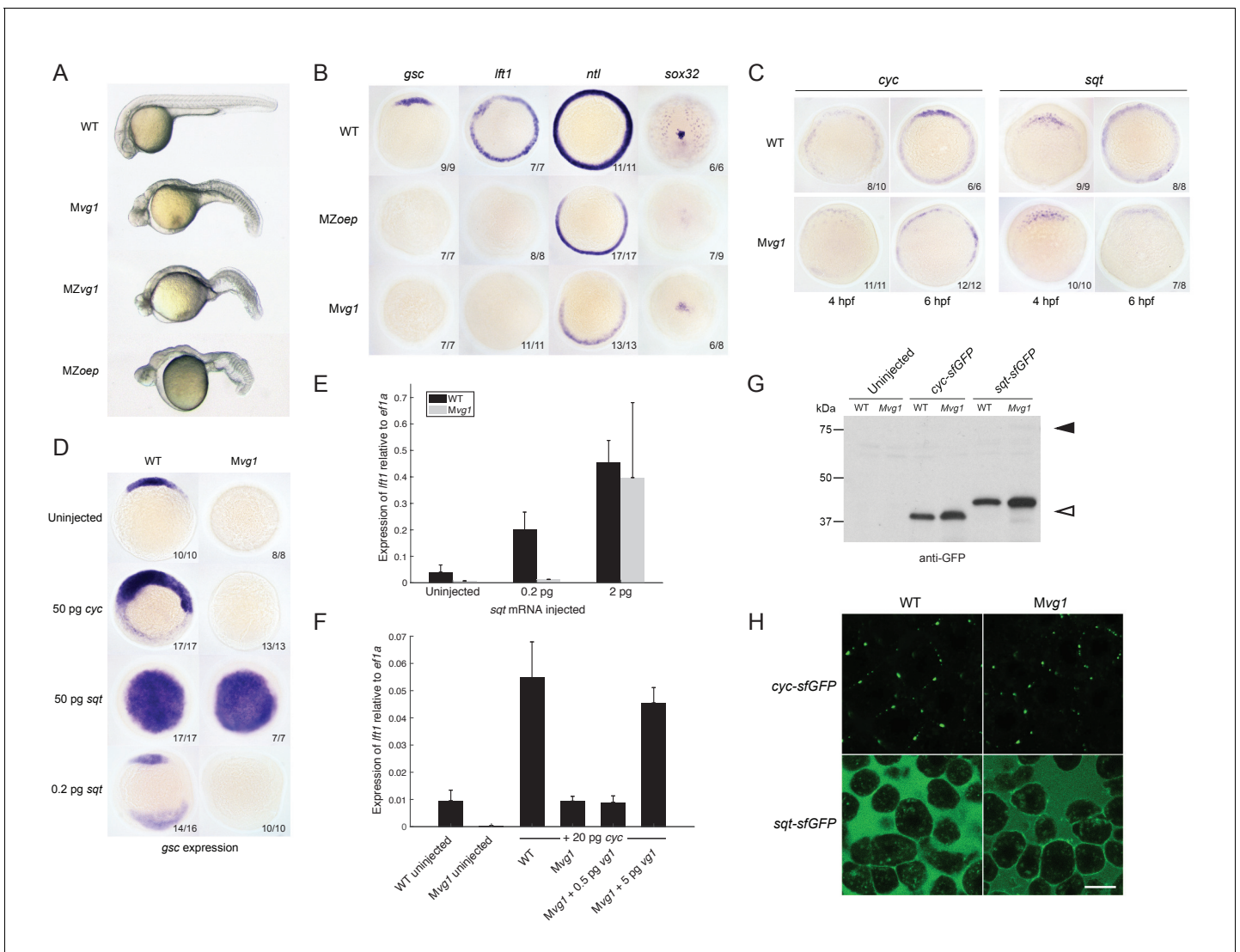


Figure 2. Endogenous Nodal signaling requires Vg1. (A) *Mvg1*, maternal-zygotic *vg1* (*MZvg1*) and maternal-zygotic *oep* (*MZoep*) mutants at 28 hpf. (B) Expression of Nodal target genes *gsc*, *lft1* and *ntl* at 50% epiboly and *sox32* at 90% epiboly in WT, *Mvg1* and *MZoep* embryos. (C) *cyc* and *sqt* expression at 4 and 6 hpf in WT and *Mvg1* embryos. (D) *gsc* expression at 50% epiboly in WT and *Mvg1* embryos injected with 50 pg of *cyc* mRNA, 50 pg or 0.2 pg of *sqt* mRNA. (E) qPCR of *lft1* expression at 50% epiboly relative to *ef1a* in WT and *Mvg1* embryos injected with 0.2 pg or 2 pg of *sqt* mRNA. The mean and standard error of the mean (SEM) was plotted. (F) qPCR of *lft1* expression at 50% epiboly relative to *ef1a* in embryos injected with 20 pg of *cyc* mRNA in combination with 0.5 pg or 5 pg of *vg1* mRNA. The mean and SEM was plotted. (G) Anti-GFP reducing immunoblot of WT and *Mvg1* embryos injected with 50 pg of *cyc*-sfGFP or *sqt*-sfGFP mRNA. Black arrowhead indicates the position of full-length protein, open arrowhead indicates processed protein. 8 embryos at 50% epiboly were loaded per well. (H) Live imaging of the animal cap of sphere-stage WT and *Mvg1* embryos injected with 50 pg of *cyc*-sfGFP or *sqt*-sfGFP mRNA. Scale bar, 17 μ m.

DOI: <https://doi.org/10.7554/eLife.28183.006>

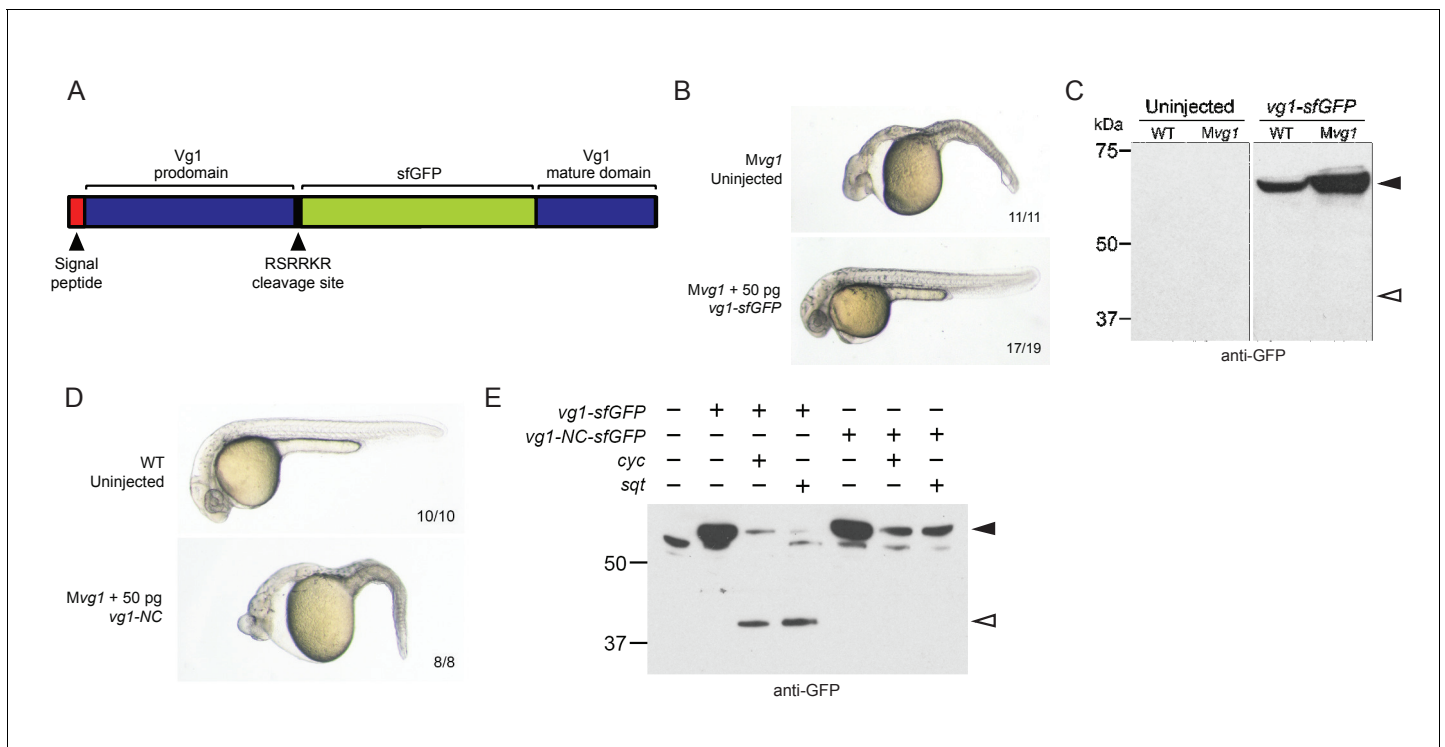


Figure 3. Vg1 processing requires Nodal. (A) *superfolderGFP* (*sfGFP*) was inserted into *vg1* downstream of the predicted basic cleavage site. (B) *Mvg1* embryo injected with 50 pg of *vg1-sfGFP* mRNA, shown at 28 hpf. (C) Anti-GFP reducing immunoblot of WT and *Mvg1* embryos injected with 50 pg of *vg1-sfGFP* mRNA. Black arrowhead indicates full-length Vg1-sfGFP; open arrowhead indicates the predicted size of cleaved Vg1-sfGFP. 8 embryos at 50% epiboly were loaded per well. (D) *Mvg1* embryo injected with 50 pg of non-cleavable *vg1* mRNA (*vg1-NC*, RSRRK->SQNTSN), shown at 28 hpf. Embryos were injected with up to 200 pg of *vg1-NC* mRNA with no rescue. (E) Anti-GFP reducing immunoblot of *Mvg1* embryos injected with 10 pg of *vg1-sfGFP* or *vg1-NC-sfGFP* mRNA and 10 pg of *cyc* or *sqt* mRNA. Black arrowhead indicates full-length Vg1-sfGFP, open arrowhead indicates cleaved Vg1-sfGFP. Molecular weights in kDa. 8 embryos at 50% epiboly were loaded per well. See also **Figure 3—figure supplement 1**.

DOI: <https://doi.org/10.7554/eLife.28183.008>

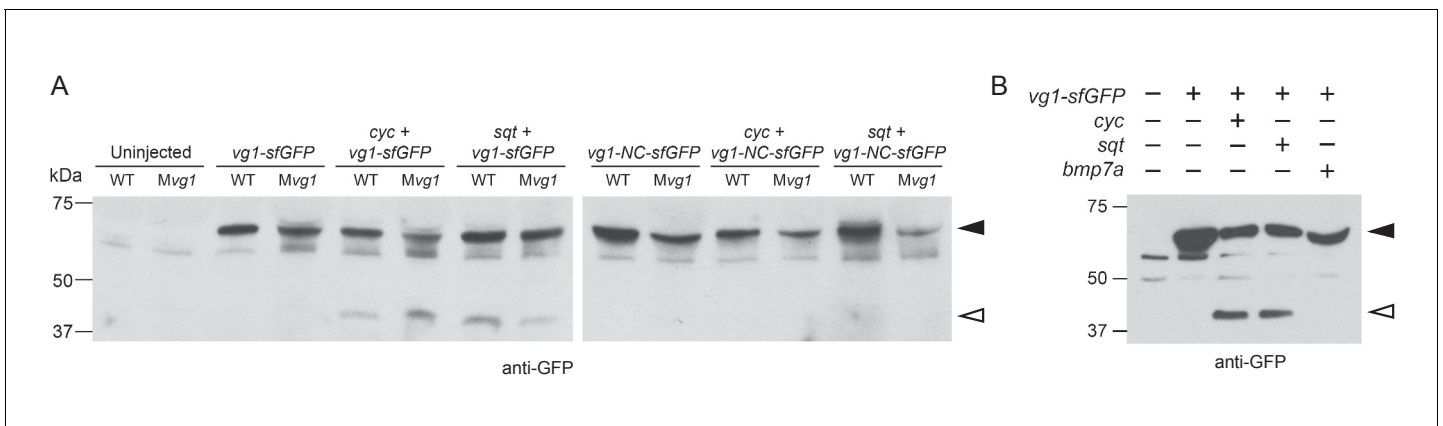


Figure 3—figure supplement 1. Vg1 processing requires Nodal. (A) Anti-GFP reducing immunoblot of WT and *Mvg1* embryos injected with 50 pg of *vg1-sfGFP* or *vg1-NC-sfGFP* mRNA and 50 pg of *cyc* or *sqt* mRNA. Black arrowhead indicates full-length Vg1-sfGFP, open arrowhead indicates cleaved Vg1-sfGFP. 8 embryos at 50% epiboly were loaded per well. (B) Anti-GFP reducing immunoblot of *Mvg1* embryos injected with 50 pg of *vg1-sfGFP* mRNA and 50 pg of *cyc*, *sqt* or *bmp7a* mRNA. Black arrowhead indicates full-length Vg1-sfGFP, open arrowhead indicates processed Vg1-sfGFP. Molecular weights in kDa. 8 embryos at 50% epiboly were loaded per well.

DOI: <https://doi.org/10.7554/eLife.28183.009>

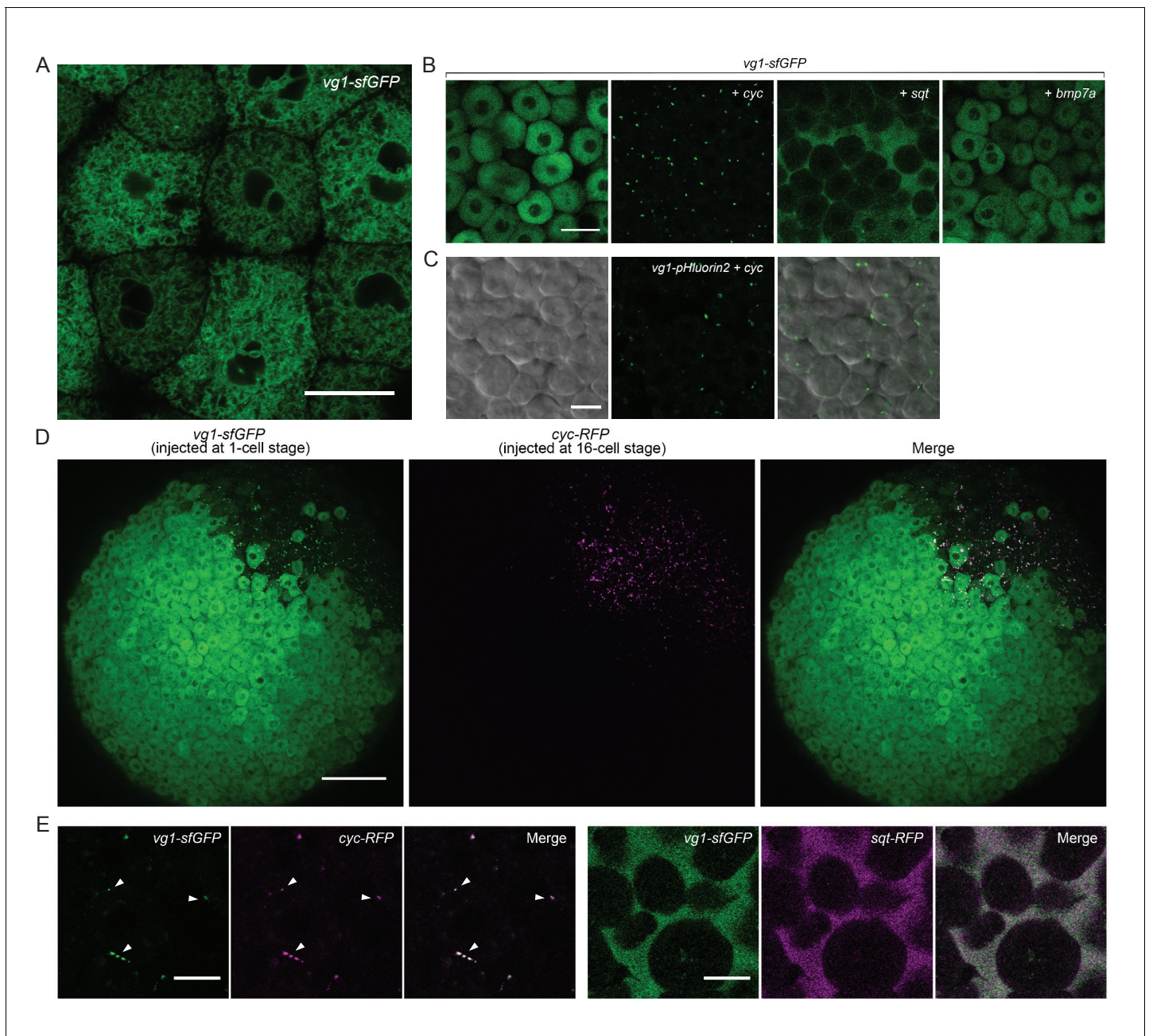


Figure 4. Vg1 secretion requires Nodal. (A) Live imaging of Mvg1 embryo injected with 50 pg of *vg1-sfGFP* mRNA. Scale bar, 17 μ m. (B) Mvg1 embryos co-injected with 50 pg of *vg1-sfGFP* mRNA and 50 pg of *cyc*, *sqt* or *bmp7a* mRNA. Scale bar, 17 μ m. See also **Figure 4—figure supplement 1A**. (C) Mvg1 embryo co-injected with 50 pg of pH-sensitive fluorescent Vg1 (*vg1-pHluorin2*) and 50 pg of *cyc* mRNA. Scale bar, 17 μ m. See also **Figure 4—figure supplement 1B**. (D) Mvg1 embryo co-injected with 50 pg of *vg1-sfGFP* mRNA at the 1-cell stage and 10 pg of *cyc-RFP* mRNA into 1 cell at the 16-cell stage. Scale bar, 100 μ m. (E) Mvg1 embryos co-injected with 50 pg of *vg1-sfGFP* mRNA and 50 pg of *cyc*- or *sqt-RFP* mRNA. Arrowheads indicate examples of co-localization. Scale bar, 17 μ m. See also **Figure 4—figure supplement 1C**.

DOI: <https://doi.org/10.7554/eLife.28183.010>

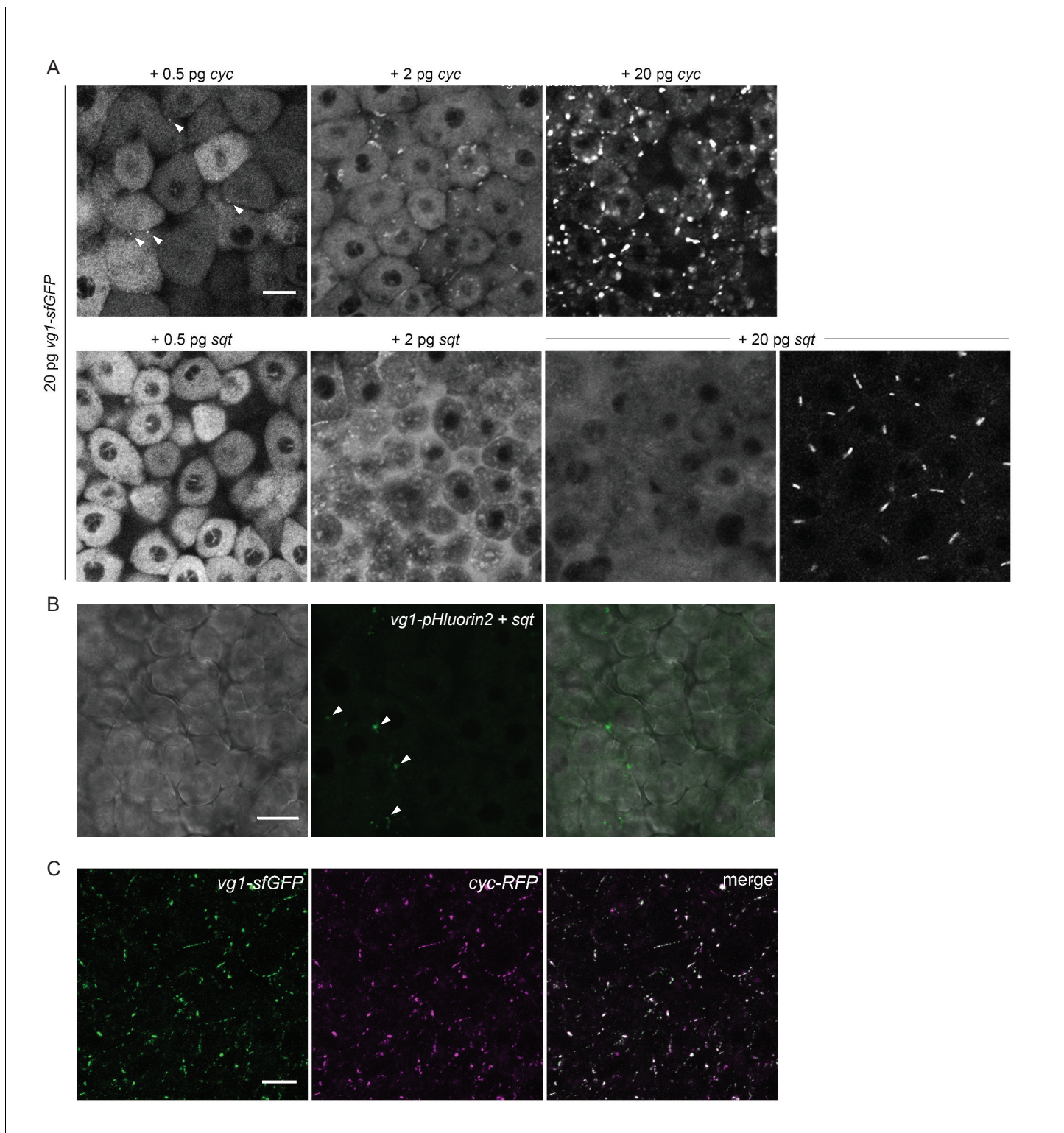


Figure 4—figure supplement 1. *Vg1* secretion requires Nodal. (A) *Mvg1* embryos co-injected with 20 pg of *vg1-sfGFP* mRNA and 0.5 pg, 2 pg or 20 pg of *cyc* or *sqt* mRNA. These images were all captured under the same microscope settings. Arrowheads indicate small extracellular puncta. Note that the puncta increase in size with increasing concentrations of *cyc* mRNA. Co-expression of *vg1-sfGFP* mRNA and *sqt* mRNA can result in extracellular diffuse signal and/or extracellular puncta. Scale bar, 17 μ m. See **Table 1** for quantification. (B) *Mvg1* embryo co-injected with pH-sensitive fluorescent *vg1* (*vg1-pHluorin2*) and *sqt*. Arrowheads indicate fluorescent puncta. Scale bar, 17 μ m. (C) Z-stack of *Mvg1* embryo co-injected with 50 pg of *vg1-sfGFP* and 50 pg of *cyc-sfGFP*. Scale bar, 17 μ m.

DOI: <https://doi.org/10.7554/eLife.28183.011>

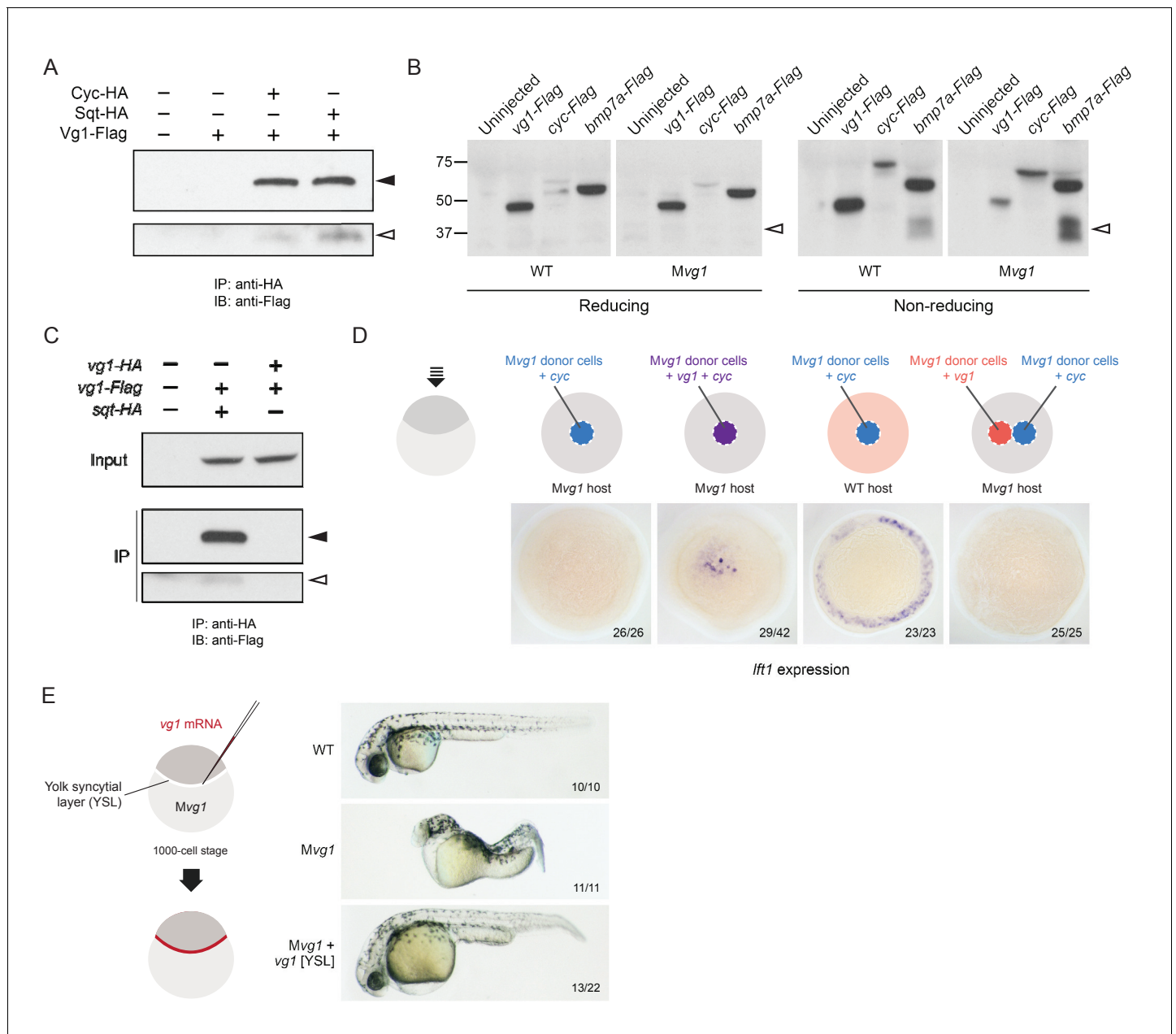


Figure 5. Vg1 and Nodal form heterodimers, and are only active when co-expressed. (A) Anti-Flag reducing immunoblot (IB) of anti-HA immunoprecipitates (IP) from lysates of *Mvg1* embryos injected with 50 pg of *cyc*-HA, *sqt*-HA and/or 50 pg of *vg1*-Flag mRNA. Black arrowhead indicates full-length Vg1-Flag; open arrowhead indicates cleaved Vg1-Flag. See also **Figure 5—figure supplements 1A and B**. (B) Anti-Flag reducing and non-reducing immunoblots of WT and *Mvg1* embryos injected with 50 pg of *vg1*-Flag, *cyc*-Flag or *bmp7a*-Flag, collected at 50% epiboly. Under reducing conditions the proteins migrated at sizes consistent with the theoretical molecular weights for full-length monomers: Vg1-Flag – 42 kDa; Cyc-Flag – 58 kDa; Bmp7a-Flag – 50 kDa. Open arrowhead indicates where mature Bmp7a-Flag homodimers are expected to migrate as two species under non-reducing conditions (Little and Mullins, 2009). For an annotated gel, see **Figure 5—figure supplement 1C**. (C) Anti-Flag reducing immunoblot of anti-HA IP from lysates of *Mvg1* embryos injected with 50 pg of *sqt*-HA and *vg1*-Flag mRNAs or *vg1*-HA and *vg1*-Flag mRNAs. Black arrowhead indicates full-length Vg1-Flag; open arrowhead indicates cleaved Vg1-Flag. (D) Transplantation of cells from donor embryos injected with *cyc*, *vg1* or *cyc* and *vg1* mRNAs into host embryos for analysis of Nodal target gene *lft1* expression at 50% epiboly. mRNAs were co-injected with *sfGFP* mRNA to verify successful transplantation using DAB staining (**Figure 5—figure supplement 2A**). (E) Injection of *vg1* mRNA into the yolk syncytial layer (YSL) of *Mvg1* mutants, shown at 32 hpf. For Nodal target gene expression in *vg1* mRNA YSL-injected embryos see **Figure 5—figure supplement 2B**. *vg1* was co-injected with a fluorescent dextran to verify YSL localization (**Figure 5—figure supplement 2C**).

DOI: <https://doi.org/10.7554/eLife.28183.013>

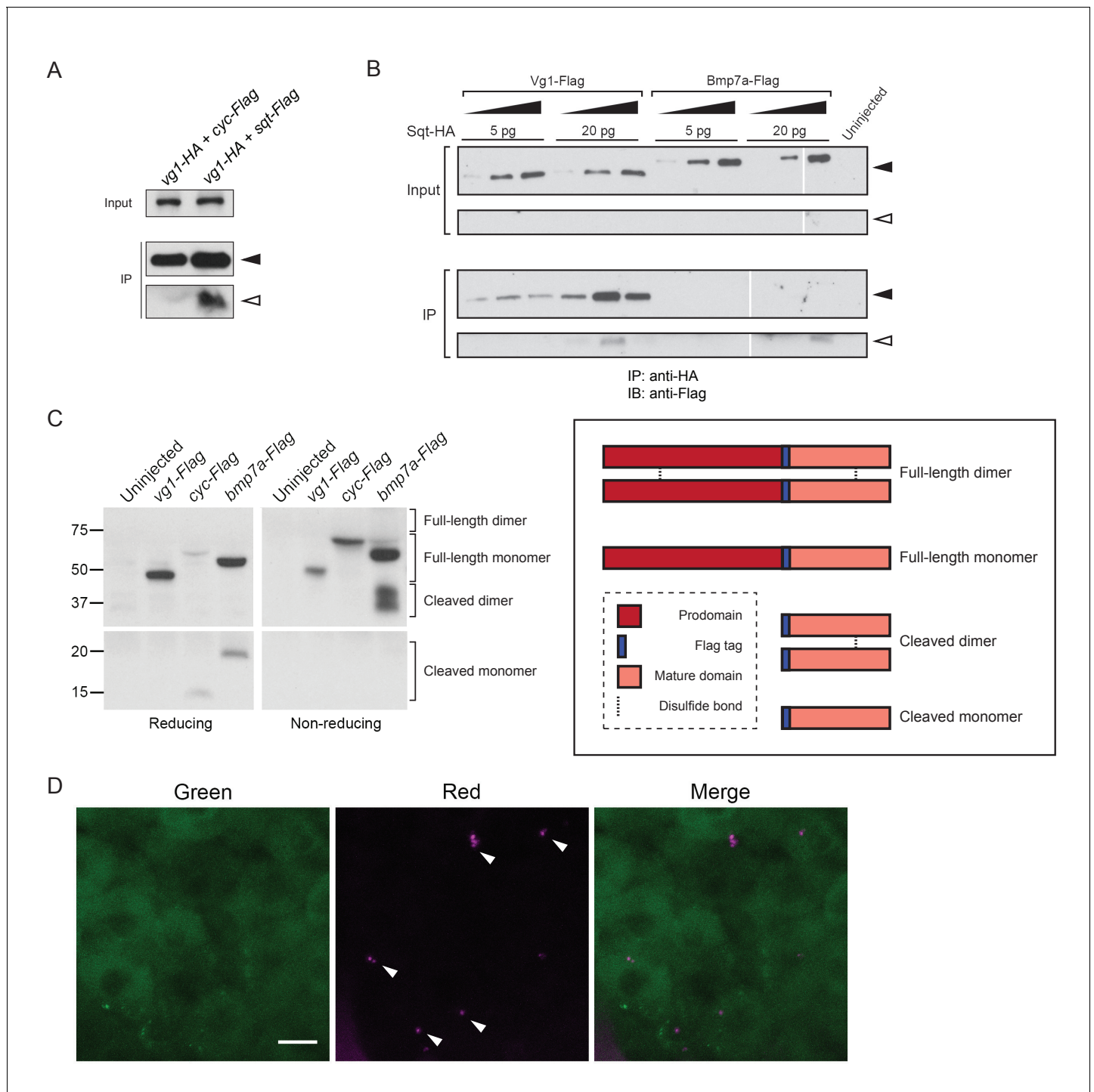


Figure 5—figure supplement 1. Vg1 and Nodal form heterodimers. (A) Anti-Flag reducing immunoblot of anti-HA IP from lysates of WT embryos injected with 50 pg of *vg1-HA* mRNA and 50 pg of *cyc-Flag* or *sqt-Flag* mRNA. Black arrowhead indicates full-length protein; open arrowhead indicates cleaved protein. (B) Anti-Flag reducing immunoblot of anti-HA IP from lysates of *Mvg1* embryos injected with either 5 pg or 20 pg of *sqt-HA* mRNA combined with increasing concentrations (5 pg, 20 pg or 50 pg) of *vg1-Flag* or *bmp7a-Flag* mRNA. Black arrowhead indicates full-length protein; open arrowhead indicates cleaved protein. In contrast to *Vg1-Flag*, the mature domain of *Bmp7a-Flag* is more readily detected than the full-length protein (Little and Mullins, 2009). The samples were loaded onto three gels run under the same conditions at the same time (gel boundaries are marked by white vertical lines). (C) Anti-Flag reducing and non-reducing immunoblots of *Mvg1* embryos injected with *vg1-Flag*, *cyc-Flag* or *bmp7a-Flag* mRNA (from Figure 5B). Approximate locations of expected monomers and dimers are indicated. TGF-beta family protein dimers are connected by disulfide bonds, which are broken under reducing conditions. (D) Z-stack of ~256-cell stage *Mvg1* embryo co-injected with 100 pg of *vg1-Dendra2* mRNA at the

Figure 5—figure supplement 1 continued on next page

Figure 5—figure supplement 1 continued

1-cell stage and 5 pg of *cyc* mRNA at the 64-cell stage immediately followed by photoconversion. The red puncta represent Vg1-Dendra2 protein synthesized before the 64-cell stage that has heterodimerized and been secreted with Cyc protein.

DOI: <https://doi.org/10.7554/eLife.28183.014>

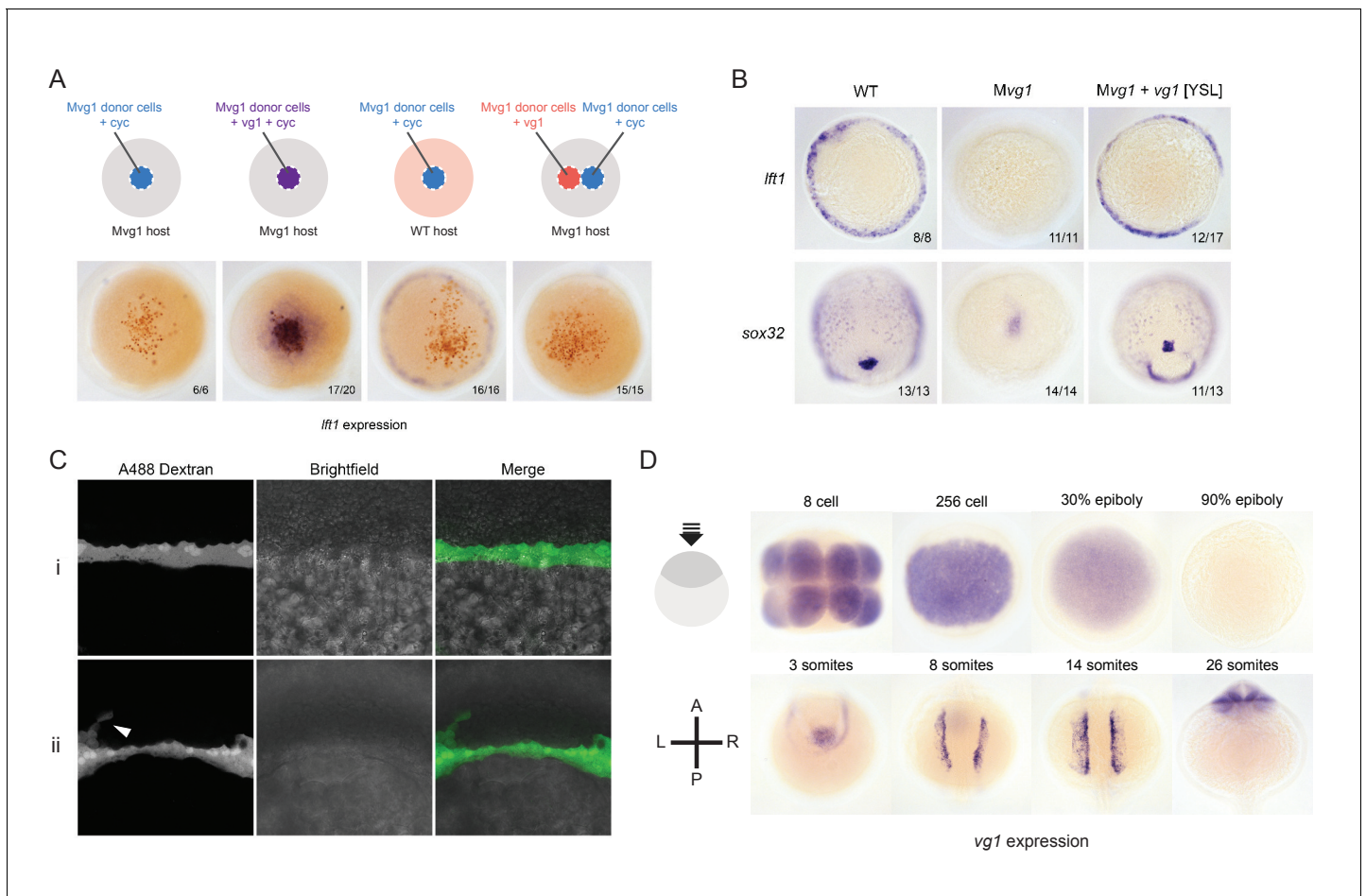


Figure 5—figure supplement 2. Vg1 and Nodal are only active when co-expressed. (A) Transplantation experiments with DAB staining to show the location of the transplanted cells (orange) and *lft1* expression (purple). (B) *in situ* hybridizations for a mesoderm marker (*lft1*) and an endoderm marker (*sox32*) in *vg1* mRNA YSL-injected embryos. (C) Confocal imaging of *Mvg1* embryos injected with 100 pg of *vg1* and 500 pg of Alexa 488 dextran into the embryonic YSL at the 1000-cell stage. The majority of fluorescent dextran localized to the YSL (i), but in some cases dextran was detectable in a few margin cells (ii, white arrowhead). (D) Endogenous *vg1* expression in WT embryos.

DOI: <https://doi.org/10.7554/eLife.28183.015>

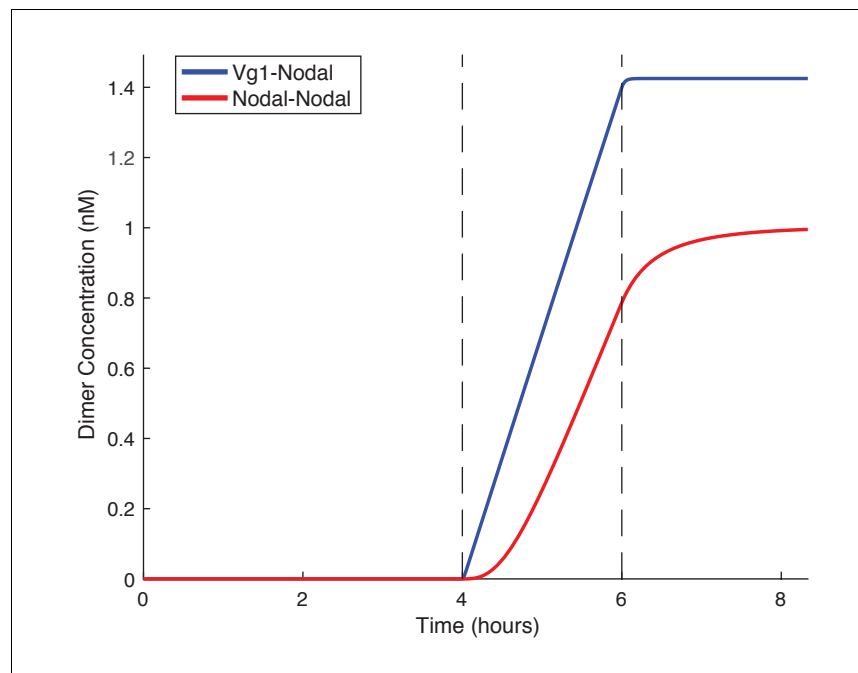


Figure 6. Vg1 can enable rapid response to low concentrations of Nodal. Kinetic model comparing Nodal homodimer formation in the absence of Vg1 (red line) and Vg1-Nodal heterodimer formation in the presence of a maternal Vg1 pool (blue line). For both conditions Nodal monomer production begins at 4 hpf (the onset of zygotic transcription and translation, first dotted line) and concludes after mesendodermal patterning (6 hpf, second dotted line). For the heterodimer simulation an excess of Vg1 is provided in the initial conditions.

DOI: <https://doi.org/10.7554/eLife.28183.016>

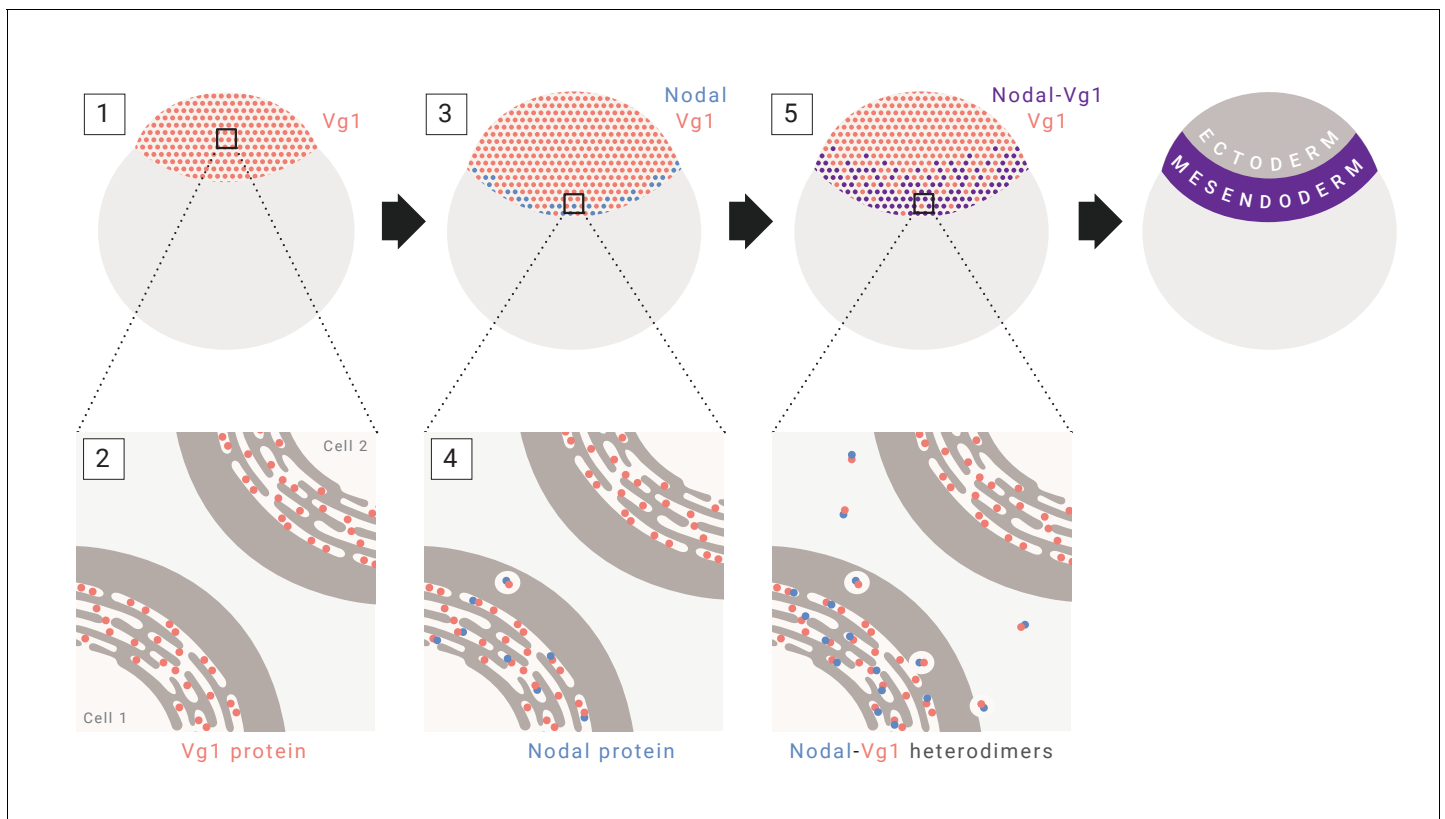


Figure 7. Model for mesendoderm induction in zebrafish. As described in the main text: (1) *vg1* mRNA is inherited from the mother, and is ubiquitous in the early embryo; (2) Vg1 protein is synthesized ubiquitously and retained predominantly in the ER; (3) *cyc* and *sqt* are transcribed and translated in the YSL; (4) Cyc and Sqt form heterodimers with Vg1, resulting in Vg1 secretion and cleavage; (5) Cyc-Vg1 and Sqt-Vg1 heterodimers activate the Nodal signaling pathway to induce mesendoderm.

DOI: <https://doi.org/10.7554/eLife.28183.017>