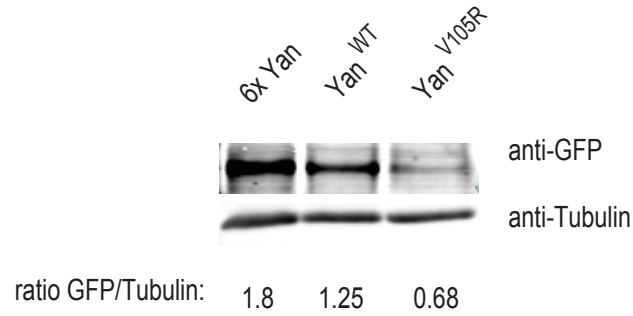
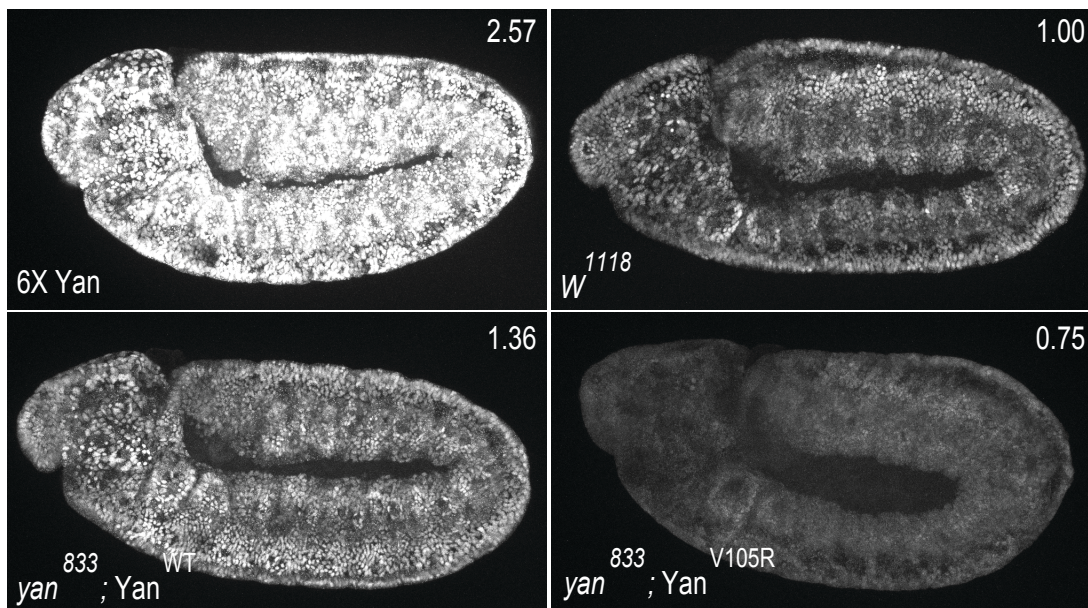


**A****B**

**Figure S5** Yan protein levels in 6X, WT and V105R embryos. A) Following dechoriation and GFP sorting, approximately 100 GFP-negative stage 11 *yan* null; Yan<sup>WT</sup> or Yan<sup>V105R</sup> embryos or 100 6x embryos were homogenized in 50ml SDS sample buffer (250mM Tris-Cl pH 8, 10% SDS, 50% Glycerol, 5% b-mercaptoethanol, 0.04% bromophenol blue). Samples were passed through a 27G needle 10 times and boiled for 10 minutes prior to running on an 8% acrylamide gel and transfer to PVDF. The blots were probed with anti-GFP (1:1000; Invitrogen) and anti-tubulin (1:1000; Sigma). 6x Yan carries four GFP-tagged *yan* genes while Yan<sup>WT</sup> and Yan<sup>V105R</sup> each carry two GFP-tagged *yan* genes. B) Embryos were fixed at room temperature for 20 minutes in a 1:1 mixture of n-heptane and 4% formaldehyde solution in PBS with vigorous shaking. The vitelline membrane was removed by replacing the formaldehyde solution with methanol. Embryos were blocked in PNT (PBS, 1% normal goat serum, 0.1% TX100) and stained with guinea pig anti-Yan (1:2000), followed by secondary antibody staining with cy3-conjugated anti-guinea pig antibody (1:2000). 6x Yan carries two endogenous copies of *yan* and four GFP tagged *yan* genes that are all detectable by anti-Yan while Yan<sup>WT</sup> and Yan<sup>V105R</sup> embryos only carry two GFP-tagged *yan* genes that will yield a protein product. Embryos were fixed, stained and imaged in parallel with identical confocal settings.