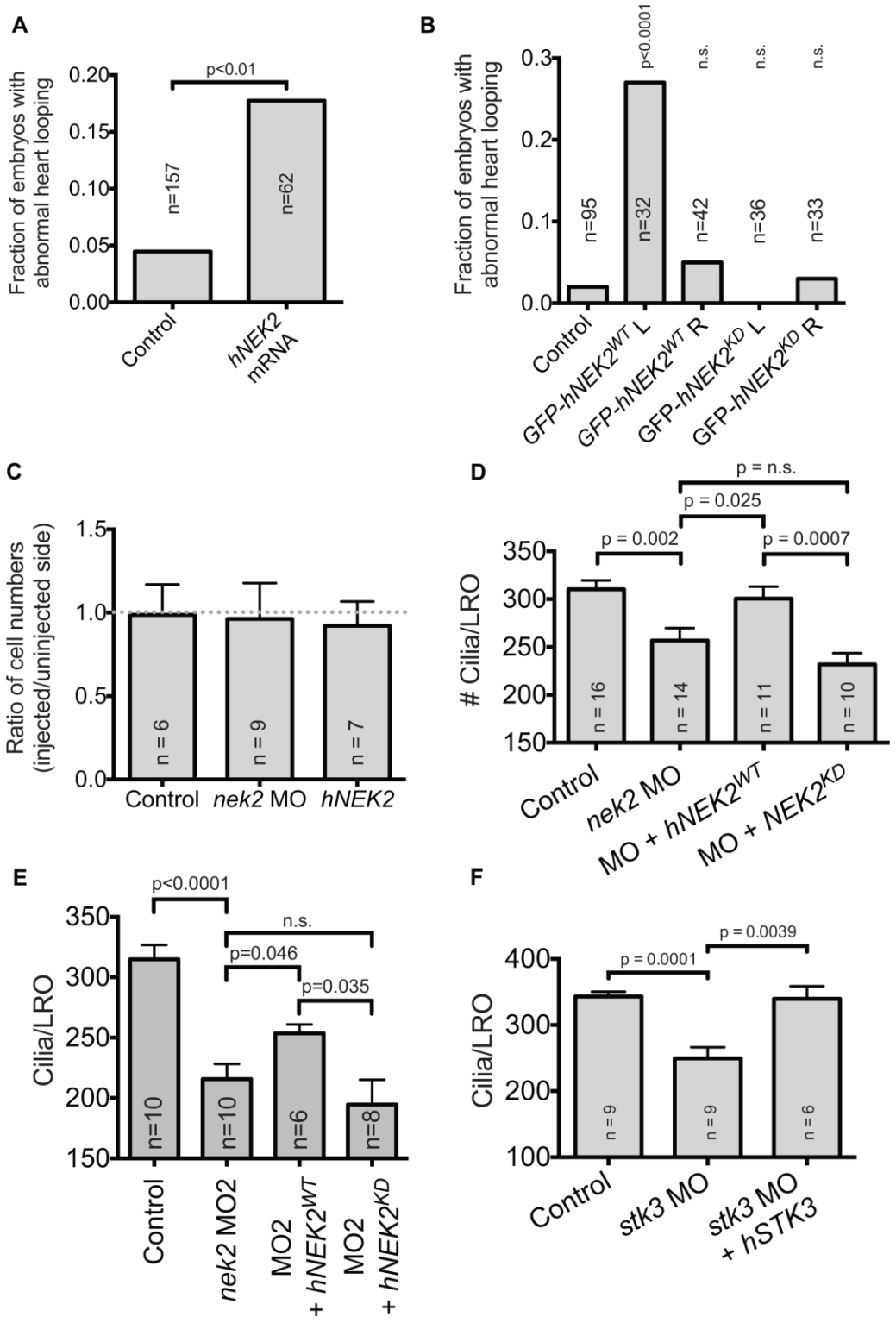

Fig. S1. Nek2 overexpression causes a heart-looping phenotype in *Xenopus*, dependent on its kinase activity. (A). Nek2 overexpression in *Xenopus* results in abnormal heart-looping. (B) Overexpression of Nek2 on the left causes abnormal heart-looping, while overexpression on the right does not, consistent with an LRO cilia phenotype.

Overexpression of an equal amount of kinase-dead Nek2 does not cause a cardiac-looping phenotype. (C) Knockdown or overexpression of Nek2 does not affect cell numbers in the LRO of unilaterally-injected embryos. (D) Injection of WT h*NEK2* mRNA is able to rescue LRO cilia loss from MO1; however, injection of kinase-dead h*NEK2* is unable to rescue. (E) Injection of WT *hNEK2* mRNA is able to rescue LRO cilia loss from MO2; however, injection of kinase-dead *hNEK2* is unable to rescue. (F) Injection of *hSTK3* mRNA is able to rescue cilia loss from the LRO caused by MO-mediated knockdown of *stk3*. For (A-B) p-values were calculated by χ^2 test. For (C) all p-values are non-significant, calculated by paired t-test. For (D-F) error bars are S.E.M. p-values were calculated by unpaired Student's t-test.

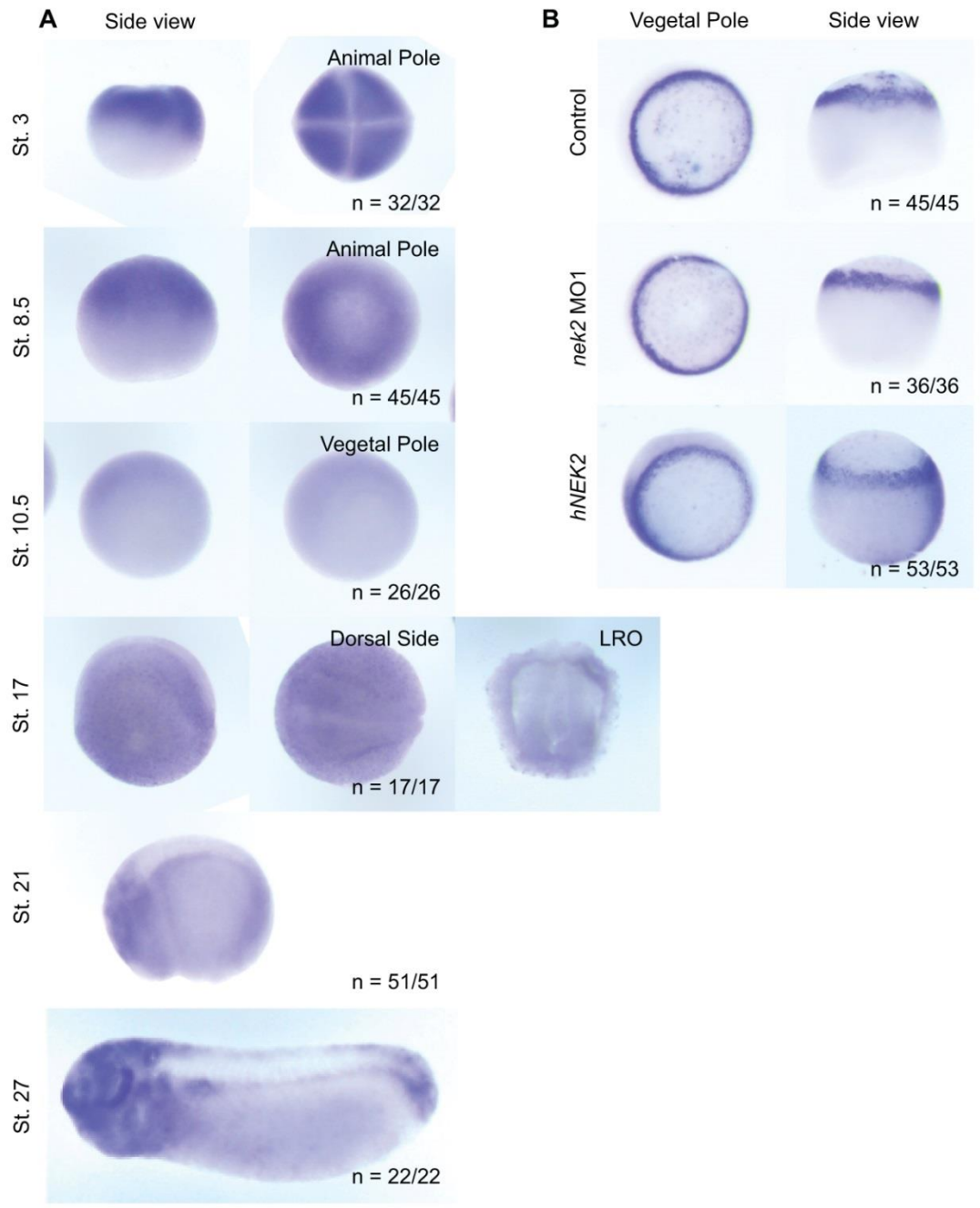

Fig. S2. *nek2* is expressed in many tissues involved in LR patterning. (A) *nek2* is expressed ubiquitously in early stage embryos, but at higher levels in the tissues with more rapidly dividing cells (the animal pole). *nek2* is expressed in the LRO (st. 16-17), the mediolateral midline (st. 21 & st. 27) and the lateral plate mesoderm (st. 21). (B) Neither knockdown nor overexpression of Nek2 affect mesoderm specification during gastrulation, suggesting that the LRO cilia defect does not arise from an improper specification of superficial mesoderm.

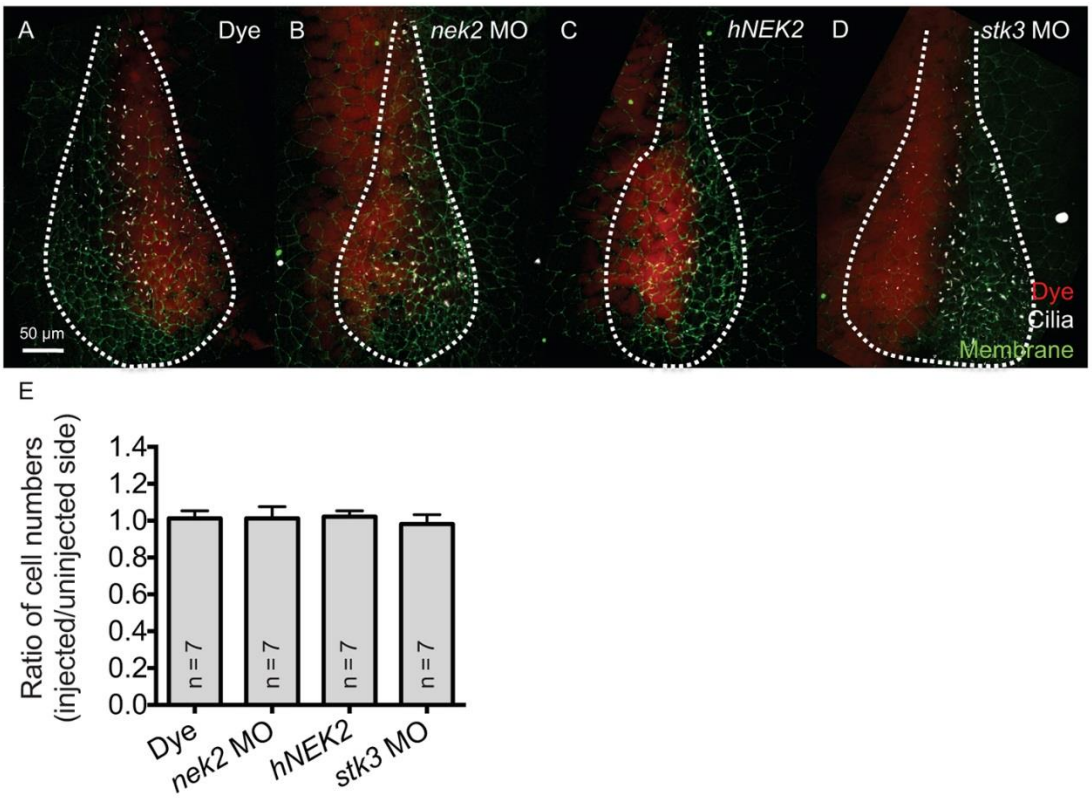

Fig. S3. *stk3* knockdown and *nek2* knockdown and overexpression do not affect cell numbers in the LRO. 2-cell *Xenopus* embryos were injected unilaterally with dye (A), *nek2* MO1 (B), *hNEK2* mRNA (C), or *stk3* MO (D), and immuno-labeled for acetylated tubulin (cilia) and cadherin (membrane). The LRO tissue is outlined in a white dotted line. There were no changes in cell number between the injected and uninjected sides (E).

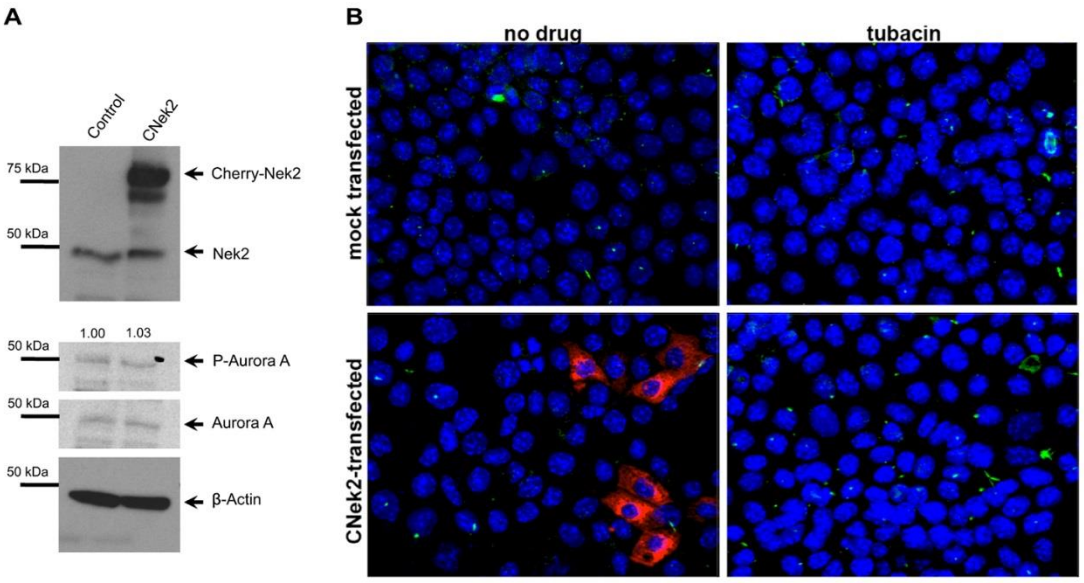

Fig. S4. Overexpression of Cherry-Nek2 in cultured cells does not affect levels of Aurora A phosphorylation. (A) Western blot showing levels of Nek2, Aurora A, P-Aurora A, with a GAPDH loading control. The quantification shown is the intensity of the PAA band divided by the intensity of the AA band, and then normalized to the mock transfected control. This experiment was completed 5 times with similar results. (B) Representative panels of cultured IMCD3 cells treated with tubacin showing level of ciliation. Cells are immuno-labeled for acetylated tubulin (cilia, green) and counterstained with Hoechst nuclear dye (blue). CNek2 transfected cells are immuno-labeled for DS-Red in the lower left panel.

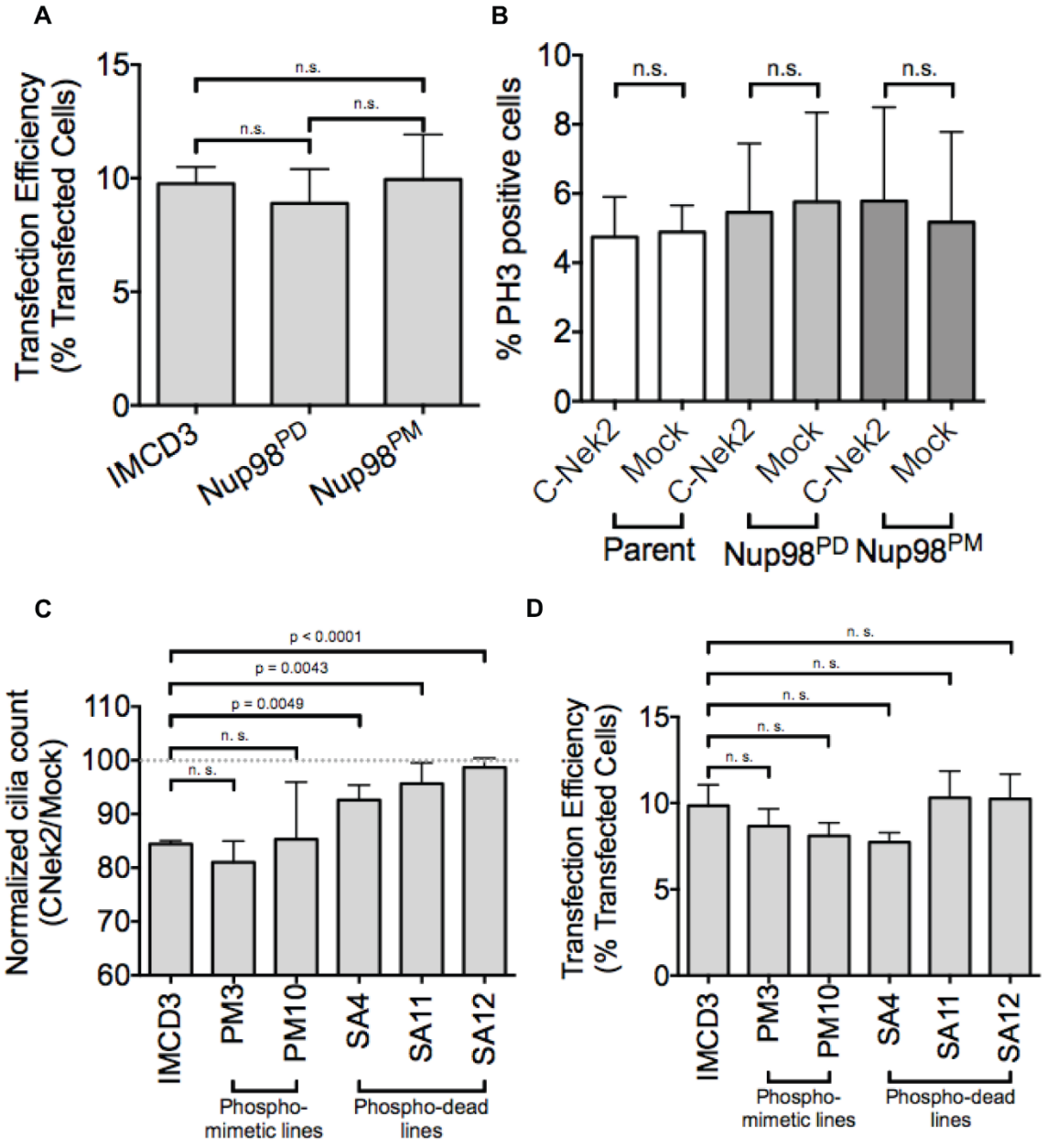

Fig. S5. NUP98 influences NEK2-mediated cilium resorption. (A) All cell lines in Figure 6F have the same transfection efficiency. (B) Transfection of the cell lines shown in Figure 6F does not influence mitotic index. (C) Multiple lines expressing PD Nup98 are resistant to Nek2-mediate cilium resorption, while lines expressing PM Nup98 are not. (D) None of the lines from (C) show different transfection efficiency from the parent line.