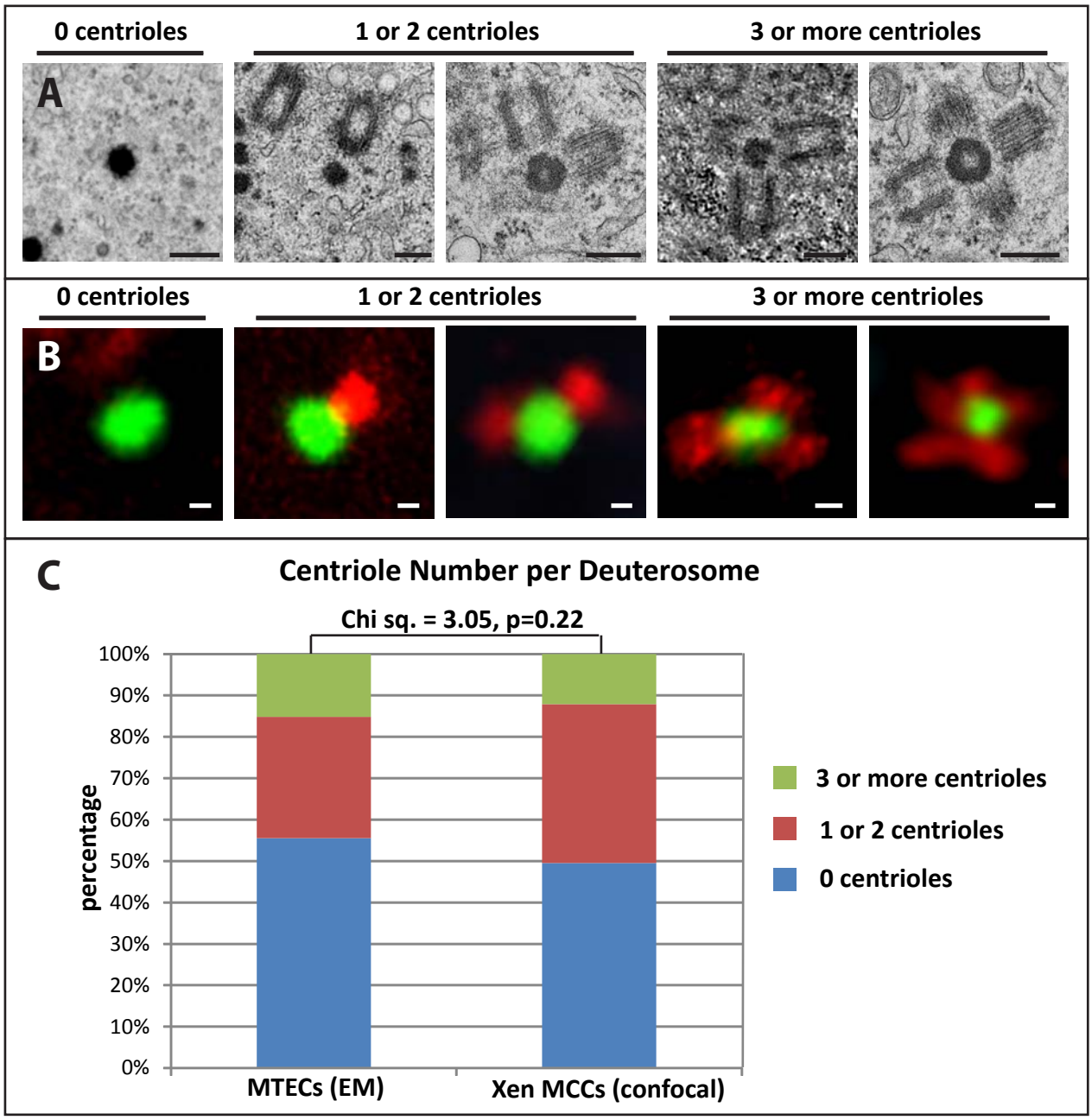
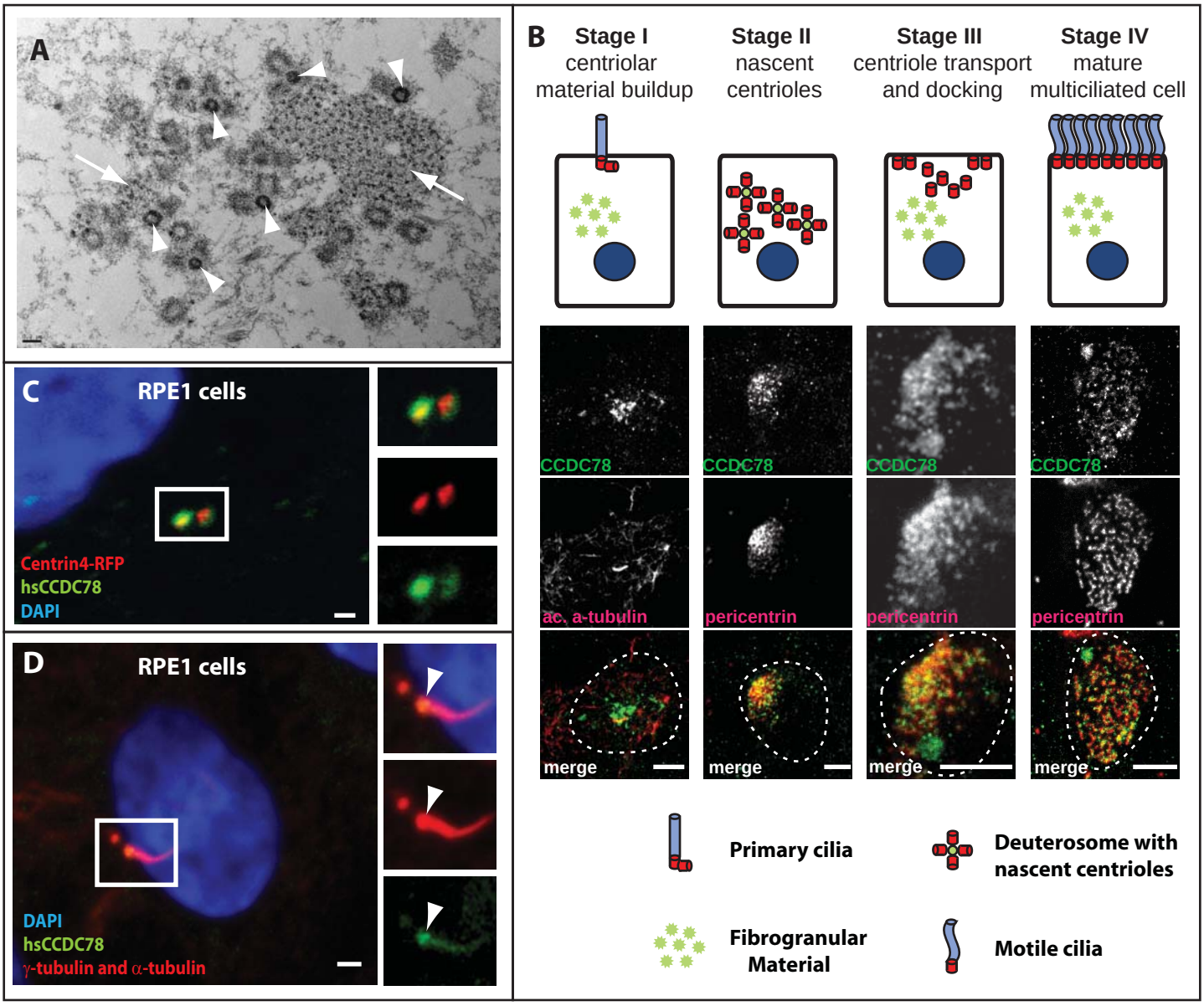
Klos-Dehring et al. Supplemental Figure 1.



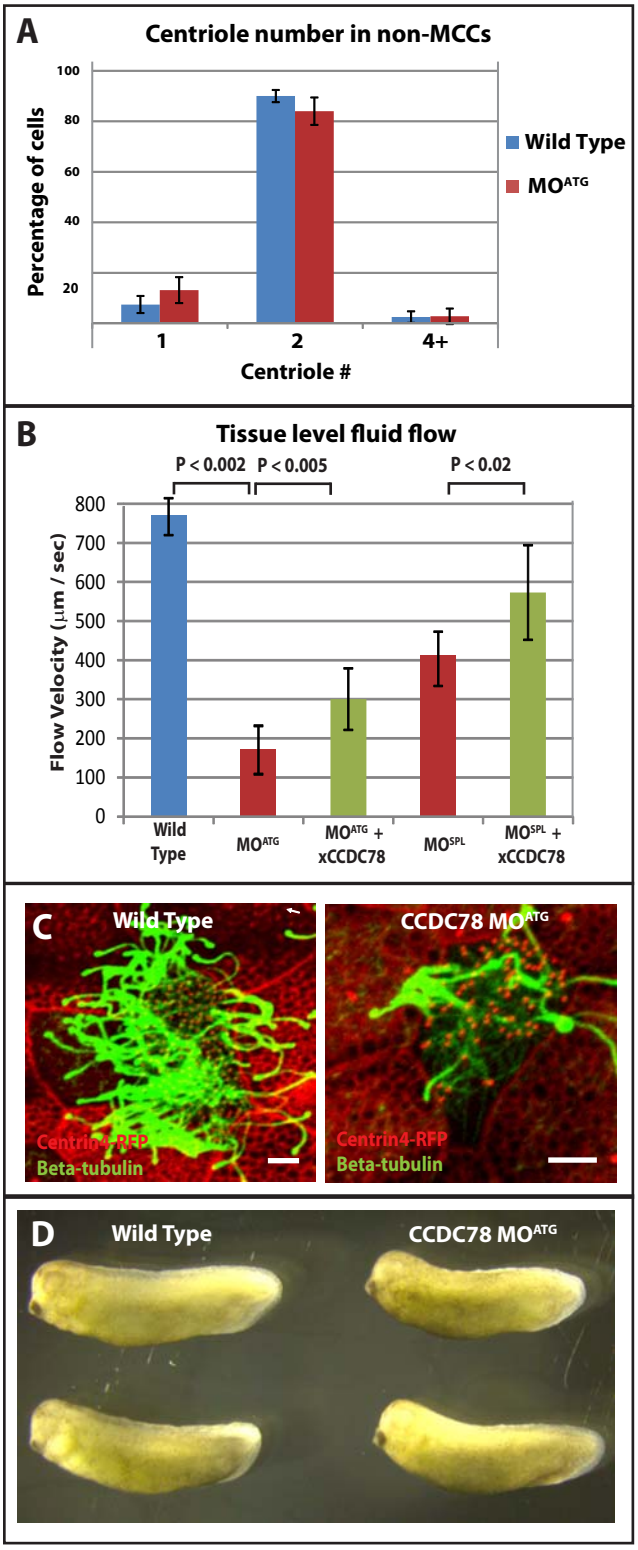
Supplemental Figure 1. Related to Figure 1. Comparison of deuterosome mediated centriole formation by EM in MTECs and by confocal light microscopy in Xenopus MCCs. (A) Series of EM images showing the range of centriole number budding off of deuterosomes (average distance from center of deuterosome to distal tip of nascent centriole is 0.34μm ± 0.08mm, n=98)(scale bars, 0.25μm). (B) Series of confocal images showing the range of centriole numbers budding off of deuterosomes (average distance from centroid of deuterosome to centroid of Centrin4-RFP is 0.49 ± 0.12μm, n=28)(scale bars, 0.2μm). (C) Quantification of centrioles budding off of deuterosomes in MTECs using EM and in Xenopus MCCs using GFP-xCCDC78 and Centrin-RFP, (chi square value of 3.05, p=0.22, n=221 for MTECs and n=136 for *Xenopus* MCCs).

Klos-Dehring et al. Supplemental Figure 2.



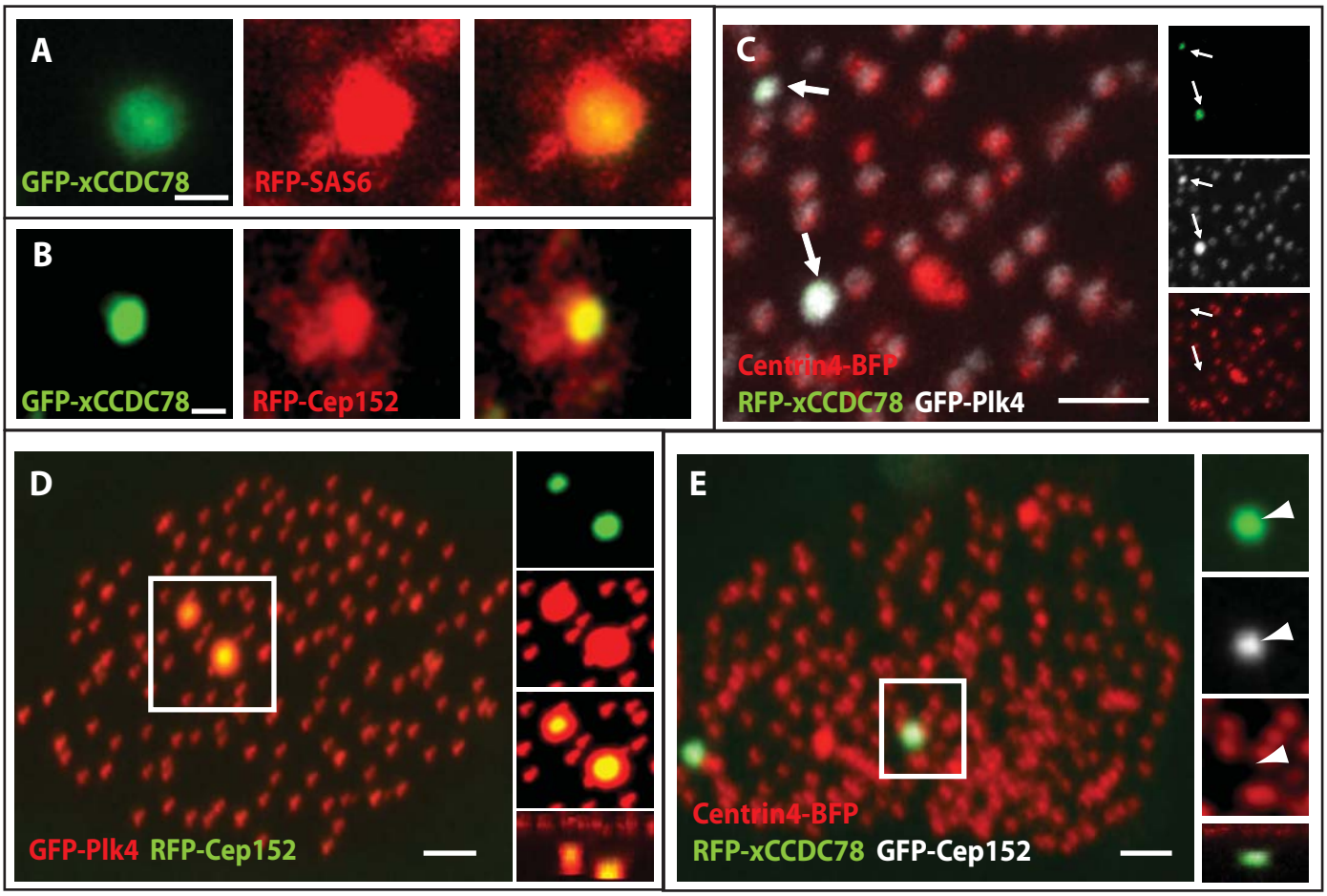
Supplemental Figure 2. Related to Figure 1. Localization of CCDC78 in MTECs and RPEs. (A) Fibrogranular material and deuterosomes in MTECs. Electron micrograph of MTECs undergoing centriole biogenesis, showing fibrogranular material (arrows) and deuterosomes (arrowheads) (scale bar, 0.2 μm). (B) Localization of mCCDC78 in MTECs. A developmental time course of MCC differentiation showing antibody staining of mCCDC78 together with pericentrin (or acetylated tubulin) including a diagram of the stages of centriole biogenesis (scale bars, 5μm). (C-D) Co -localization of hsCCDC78 in RPE-1 cells with one of two centrioles marked with Centin4-RFP (C) and co-localizing with g-tubulin (red) enriched at the mother centriole at the base of the cilia marked with a-tubulin (red)(D) (scale bar, 2μm).

Klos-Dehring et al. Supplemental Figure 3



Supplemental Figure 3. Related to Figure 2. CCDC78 morphant phenotype. (A) Quantification of centriole number in non-MCCs showing no statistical difference between wild type and morphant tissue (chi square value of 4.56, p=0.10, n=406 cell wild type and 299 morphant cells from 3 individual embryos). (B) Quantification of fluid flow velocity in wild type (n=11), morphant (MO^{ATG} n=12 p<0.002, MO^{SPL} n=11 p<0.002) and rescued embryos (ATG rescue n=10 p<0.005, SPL rescue n=15 p<0.02). Error bars, SD. (C) Morphant embryos have a decrease in cilia consistent with a decrease in centriole number (scale bars, 5 μm). (D) Morphant embryos exhibit no gross morphological defects.

Klos-Dehring et al. Supplemental Figure 4



Supplemental Figure 4. Related to Figure 3. Deuterosome localization of SAS6, Plk4 and Cep152. (A) Localization of RFP-SAS6 to the deuterosome marked with GFP-xCCDC78 and to nascent centrioles budding off the deuterosome (scale bars 0.5μm). (B) Localization of RFP-Cep152 to the deuterosome marked with GFP-xCCDC78 and to nascent centrioles budding off the deuterosome (scale bars 0.5μm). (C) Co-localization of GFP-Plk4 with both Centrin4-BFP and RFP-xCCDC78. (D) Co-localization of GFP-Plk4 with RFP-Cep152. (E) Co-localization of RFP-Cep152 with GFP-xCCDC78 but not Centrin4-BFP (pseudo-colored for consistency) (scale bar, 2μm).

Supplemental Methods

Plasmids and Morpholinos

CCDC78 was amplified by PCR from *Xenopus* cDNA using the primers 5'-GCGGATCCATGGATTCTACAGAAGATCG-3' and 5'-CGCTCGAGACATCTCATGGCTGTTTC-3' and cloned into pCS2+ using BamHI and XhoI and was N-terminally fused to GFP or RFP. Cep152 (Unigene Xl.13956) was amplified from an Open Biosystems clone using primers 5'-CCATCGATGTTCTATCGACTTTGATAGTGGAGCACTGCAGACT-3' and 5'-CCATCGATTTAGTTGAAGTTATTTAAGTTGGGAAATGGGCT6GTC-3' and cloned into pCS2+ using ClaI and N-terminally fused to RFP. Sas6 (Unigene Xl.33005) was amplified from *Xenopus* cDNA using primers 5'-GCGAATTCATGGCCGACGAGTTGTTC-3' and 5'-GCCTCGAGGGAAGCAGGAAGCCTGG-3' and cloned into pCS2+ using EcoRI and XhoI and was N-terminally fused to RFP. Plk4 (Unigene Xl.56605) was amplified by PCR from *Xenopus* cDNA using the primers 5'-GCGAATTCATGGGGGGCAGCATAGG-3' and 5'-GCTCTAGAGCGACTGGACGAGCTGG-3' and cloned into pCS2+ using EcoRI and XbaI and N-terminally tagged with GFP. To allow for injection of DNA of this construct the CMV promoter was switched with the α-tubulin promoter. GFP-xCCDC78$^{1-210}$ and GFP-xCCDC78$^{205-559}$ were made by amplifying the fragments from the GFP-xCCDC78 clone using primers: 5'-GCCTCGAGATGGATTCTACAGAAGAT-3', 5'-GGGGTACCTGGCTTGATGTTGCG-3', 5'-GGGGTACCGCCCGCAACATCAAG-3', and 5'-CGCTCGAGACATCTCATGGCTGTTTC-3'. The fragments were blunted and ligated into pCS2+ and N-terminally tagged with GFP. GFP-hCCDC78 (Unigene Hs.381943) was amplified from a clone using primers 5'-GCGAATTCATGGAGCACGCAGCCAC-3' and 5'-GCCTCGAGGGATTTCGTGCTTGTACC-3' cloned into pCS2+ using EcoRI and XhoI and N-terminally tagged with GFP or RFP. For mammalian

cell transfections, RFP-CCDC78 was cut out with HindIII and XbaI and ligated into EGFP-N1 that was also digested with HindIII and XbaI to remove the GFP. All cloning was verified by sequencing.

Morpholino antisense oligonucleotides (GeneTools) were injected into two- or four-cell stage embryos to inhibit the expression of *Xenopus CCDC78* (Unigene Xl.4890) (Nasevicius and Ekker, 2000). One morpholino was used to target *CCDC78* at the initiation site or a splice site: Initiation Site: 5'-CATCAGTGTTACTAGGATAGGCAGG-3'; Splice Site: 5'-TTATATCCTTACTCCTACCTTTAGT-3'; Control: 5'-CCTCTTACCTCAGTTACAATTTATA-3'. Morpholinos were injected into each quadrant of the animal pole at the two- or four-cell stage using a total of 50-75ng per embryo.

Antibodies

Antibody	Dilution	Company
Rabbit anti-γ-tubulin(T6557)	1:300	Sigma-Aldrich
Beta-tubulin (E7)	1:500	DSHB
Rabbit anti-ZO1	1:100	Invitrogen
CCDC78 (AV53233)		Sigma-Aldrich
CCDC78 (HPA041186)		Sigma-Aldrich
Acetylated α-tubulin(ab24610)*		Abcam
Pericentrin (611814)		BD Biosciences
Cy-5-conjugated secondary antibodies	1:750	Jackson Immuno
Alexa fluor conjugated secondary antibodies		Life Technologies

* (Chu and Klymkowsky, 1989)

Supplemental References:

Chu, D.T., and Klymkowsky, M.W. (1989). The appearance of acetylated alpha-tubulin during early development and cellular differentiation in Xenopus. Dev Biol *136*, 104-117.
Nasevicius, A., and Ekker, S.C. (2000). Effective targeted gene 'knockdown' in zebrafish. Nat Genet *26*, 216-220.