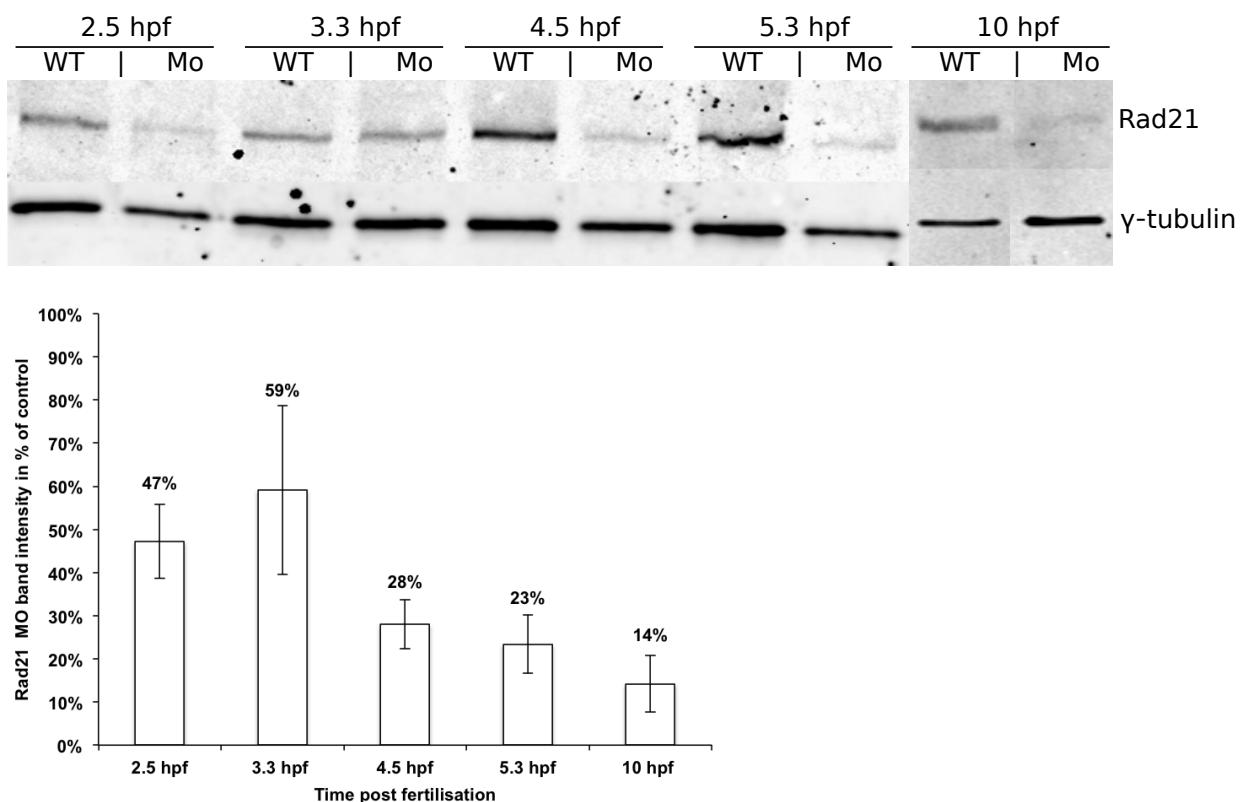
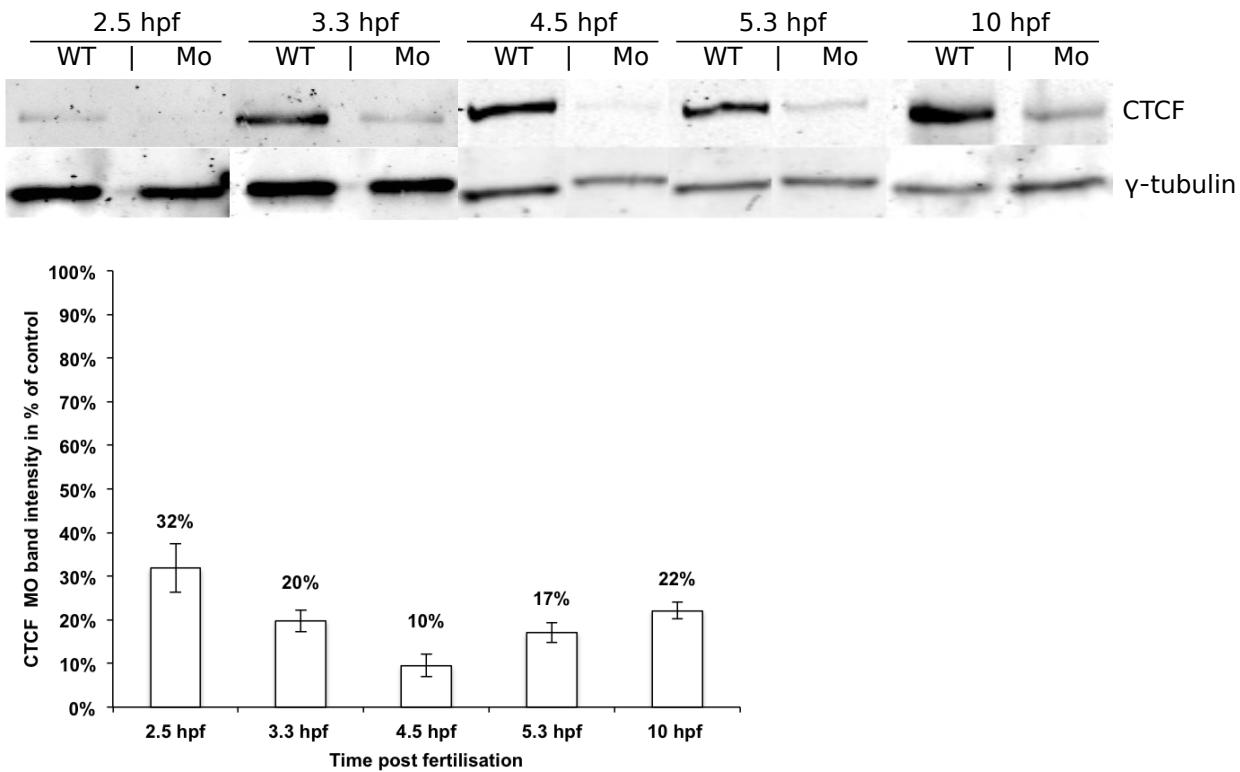
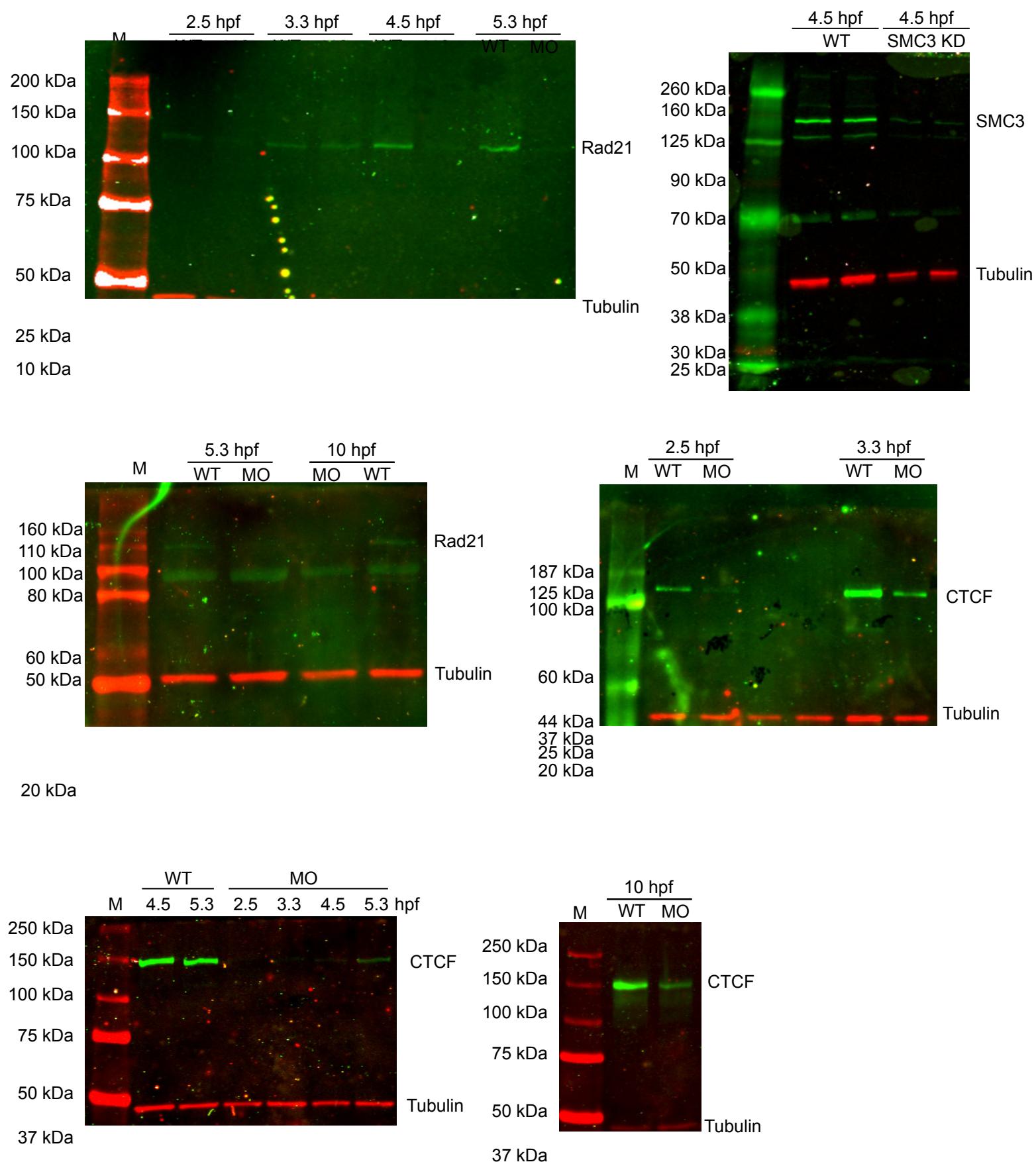


A**B****Figure S1. Quantitation of protein levels in Rad21- and CTCF-depleted embryos.**

Immunoblot analysis of Rad21 (**A**) and CTCF (**B**) protein levels in wild type (WT) and morpholino (Mo) injected embryos using 1 pmol Mo for the stages until 5.3 hpf and 0.5 pmol MO for 10 hpf stage. Following dechorionation and deyolkling, zebrafish embryos were lysed and protein levels were quantified using BCA assay. Per lane, 10 μ g of protein was separated by electrophoresis on 10% polyacrylamide gels. The bands were visualized using a LiCor Odyssey imager, and the intensities for Rad21, CTCF and tubulin protein were quantified using Odyssey software. Rad21 and CTCF levels were normalized against those of tubulin (see graphs). Graphed data represent the means of 3 biological replicates \pm S.D.

**Figure S1 Extended.** Full immunoblots against Rad21, SMC3 and CTCF.

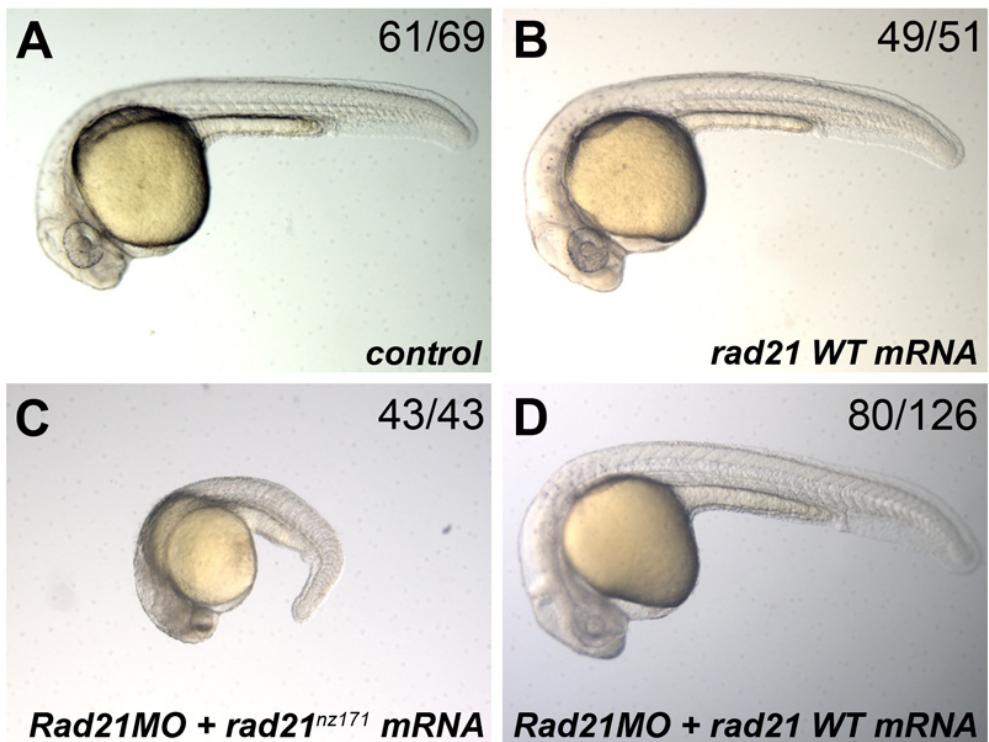


Figure S2

Rescue of Rad21 MO-treated embryos by wild type, but not mutant, *rad21* mRNA. Embryos were injected at the 1-cell stage. Images of representative embryos are shown, and the number of embryos exhibiting the displayed phenotype is shown at top left of each panel. **(A)** control embryo injected with 1 nl Danieu. **(B)** control embryo injected with 200 pg wild type (WT) *rad21* mRNA. **(C)** non-rescued embryo injected with 0.25 pmol Rad21 MO and 200 pg mutant *rad21* mRNA (*rad21*^{nz171}). **(D)** rescued embryo injected with 0.25 pmol Rad21 MO and 200 pg WT *rad21* mRNA.

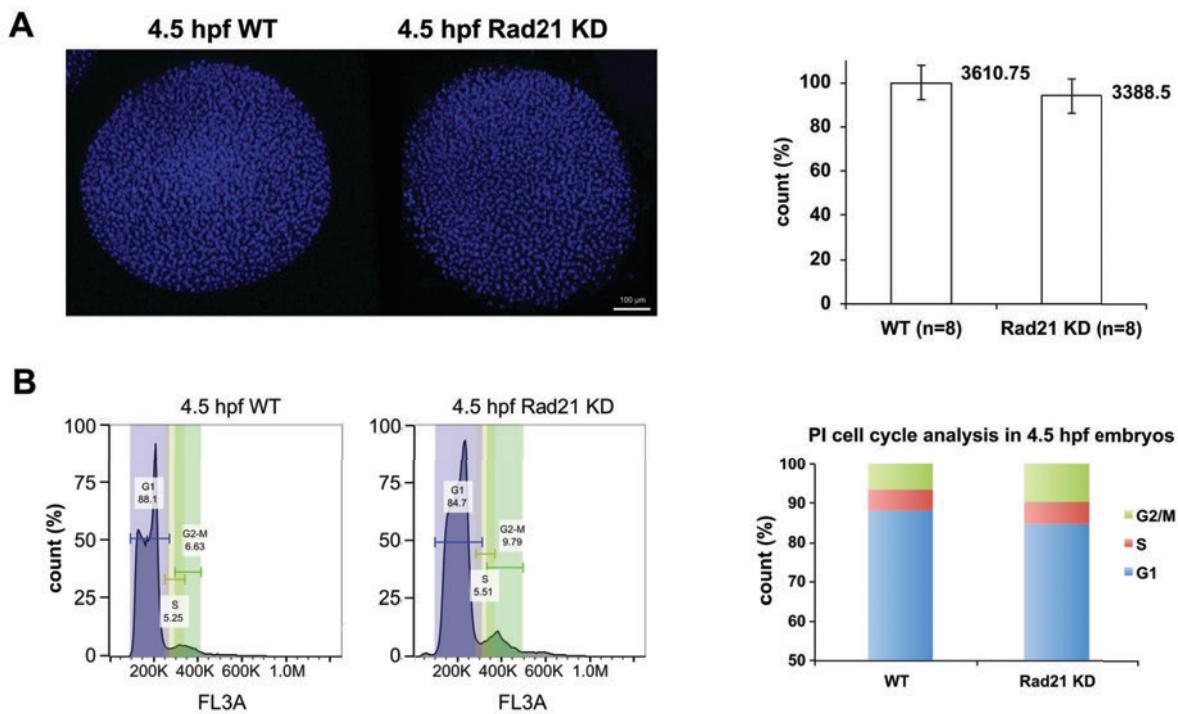


Figure S3. Cell cycle analysis of Rad21-depleted embryos

(A) Left, whole mount wild type (WT) and Rad21-depleted (Rad21 KD) embryos at 4.5 hpf. Embryos were fixed in 4 % formaldehyde, dechorionated, and dehydrated in methanol. Nuclei were stained with Hoechst, and confocal Z-stacks of embryos were obtained (see methods). Right, nuclei were quantified using Imaris software. No significant difference was observed in nuclei numbers between Rad21 KD and WT ($n=8$, $p=0.1138$, un-paired t-test).

(B) Cell cycle analysis of WT and Rad21 KD embryos. Left, representative propidium iodide staining profiles of 4.5 hpf WT and Rad21 KD embryos, with G1, S, G2/M phases indicated. Right, Rad21 KD embryos had ~50% more cells in G2/M phase compared to WT controls.

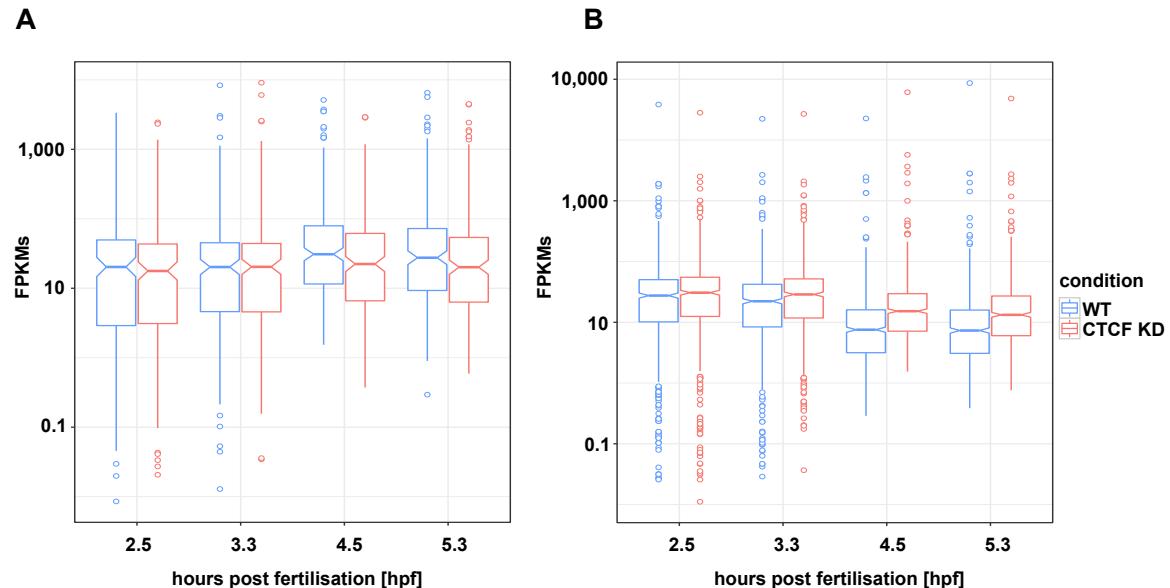
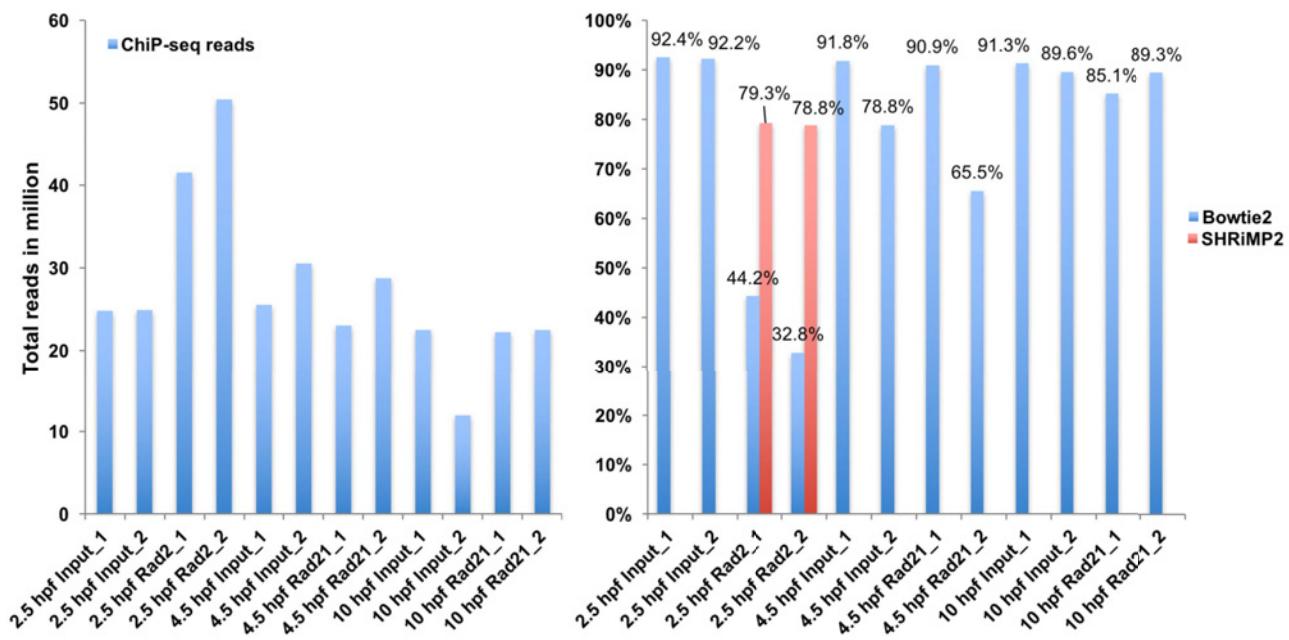


Figure S4. Delay in transcript profiles of CTCF-depleted embryos.

(A, B) Distribution of differentially represented transcripts in CTCF-depleted (KD) over developmental time points (2.5–5.3 hpf). The bottom and top of the boxes are the first and third quartiles, and the line within represents the median, notches represent confidence intervals. The whiskers denote the interval within 1.5 times the interquartile range (IQR) from the median. (A) 203 transcripts with significantly (FDR 0.05) reduced levels in CTCF KD embryos at dome stage (4.5 hpf) embryos increase in FPKM levels over developmental time. (B) 695 transcripts with elevated levels in CTCF KD embryos decrease over developmental time.

A**B****Pearson correlation**

	2.5hpf_Rad21_1	2.5hpf_Rad21_2
2.5hpf_Rad21_1	1.00	0.97
2.5hpf_Rad21_2	0.97	1.00

Pearson correlation p-value

	2.5hpf_Rad21_1	2.5hpf_Rad21_2
2.5hpf_Rad21_1	0	0
2.5hpf_Rad21_2	0	0

Pearson correlation

	4.5hpf_Rad21_1	4.5hpf_Rad21_2
4.5hpf_Rad21_1	1.00	0.85
4.5hpf_Rad21_2	0.85	1.00

Pearson correlation p-value

	2015_4.5hpf_Rad21	2012_4.5hpf_Rad21
2015_4.5hpf_Rad21	0	0
2012_4.5hpf_Rad21	0	0

Pearson correlation

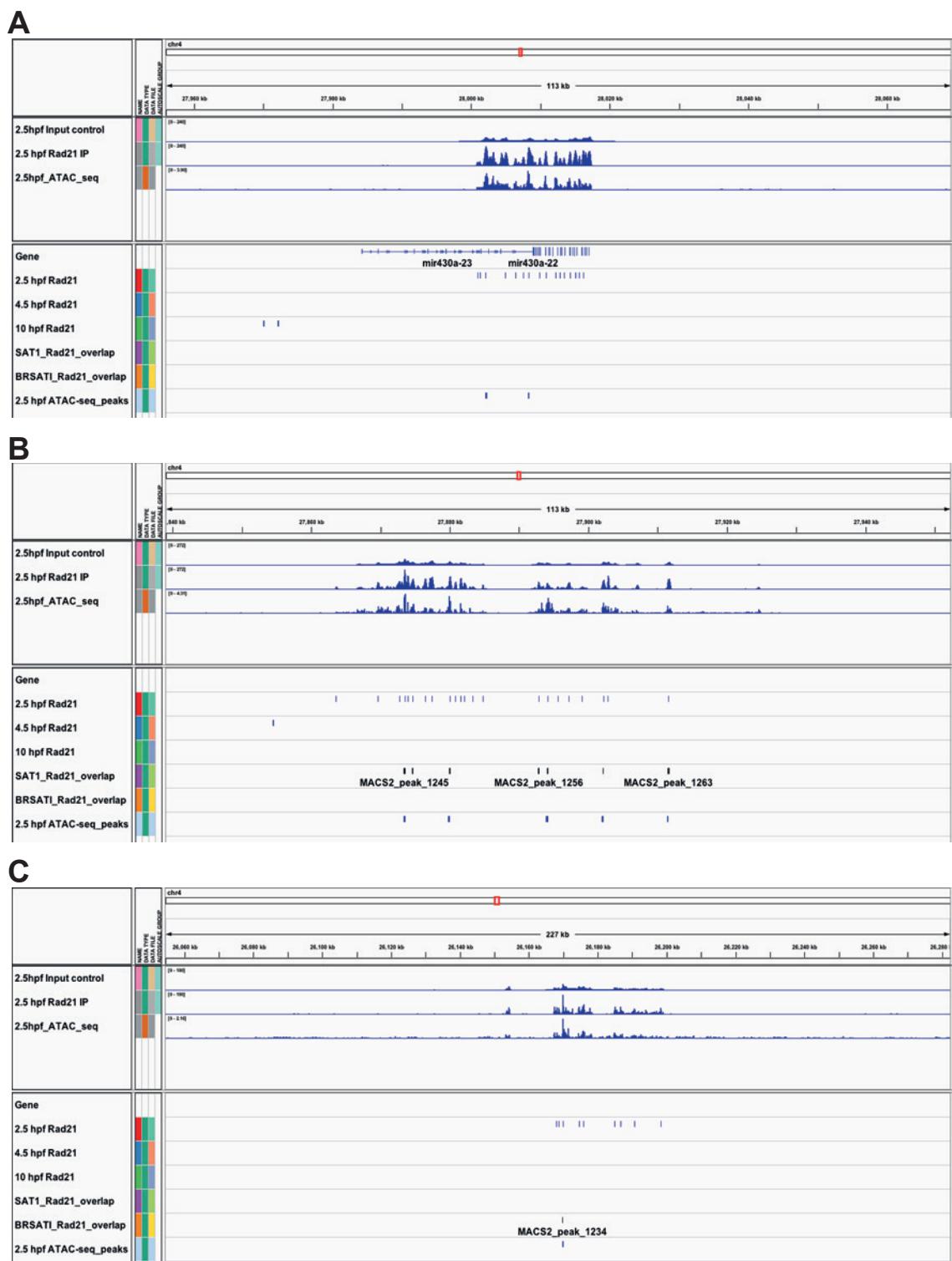
	10hpf_Rad21_1	10hpf_Rad21_2
10hpf_Rad21_1	1.00	0.90
10hpf_Rad21_2	0.90	1.00

Pearson correlation p-value

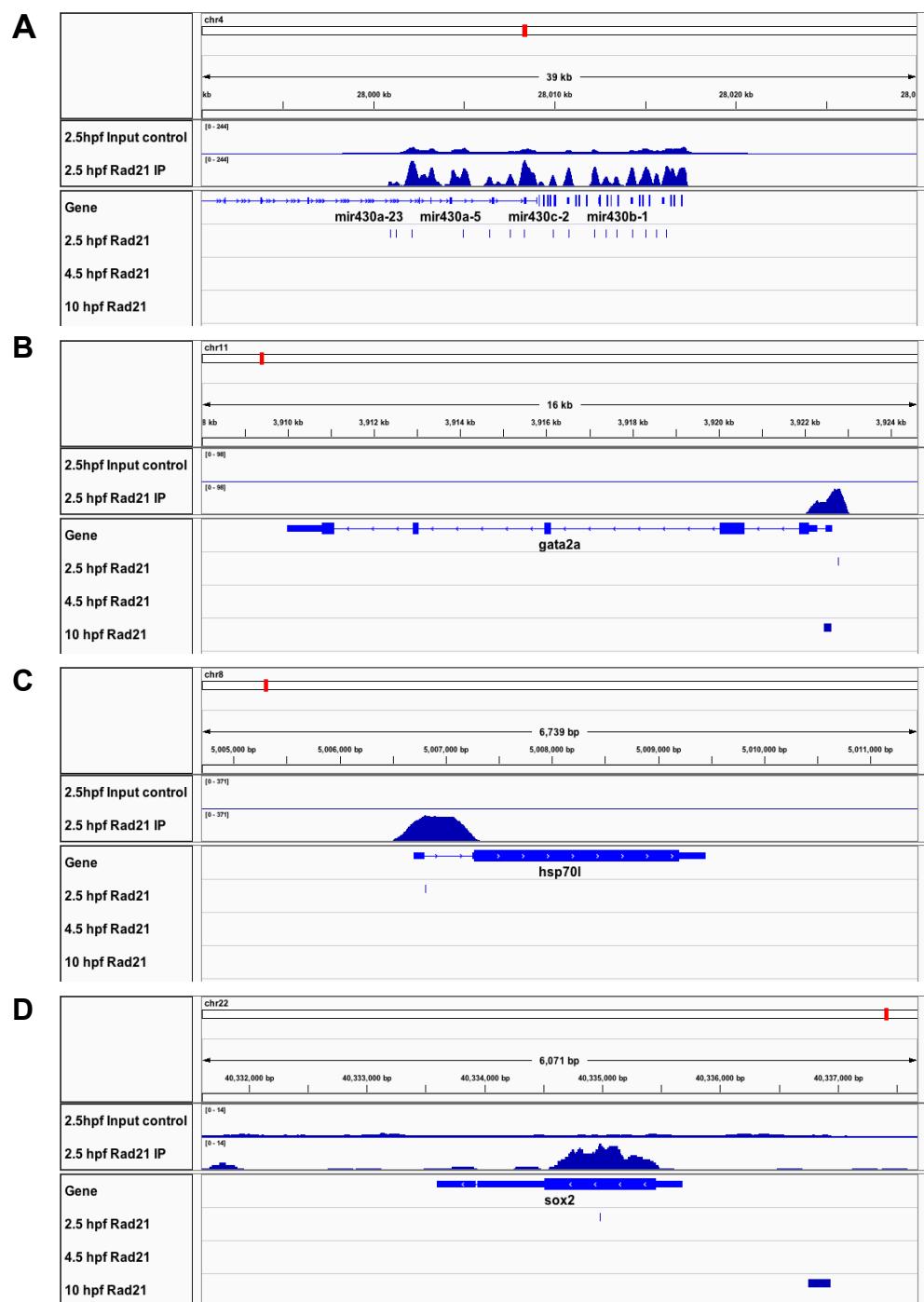
	10hpf_Rad21_1	10hpf_Rad21_2
10hpf_Rad21_1	0	0
10hpf_Rad21_2	0	0

Figure S5.

ChIP-Seq mapping statistics. **(A)** Total reads obtained from ChIP-seq samples shown in the left diagram. Mapping statistics shown in the diagram on the right for each sample after raw sequencing reads were aligned to the zebrafish genome using bowtie2 (Langmead and Salzberg 2012). **(B)** Rad21 IP replicate statistics. Alignment files (BAM format) were sorted and indexed using samtools (Li et al. 2009). The program Ngs.plot (Shen et al. 2014) was used to assess the correlation (Pearson) on non-zero values in replicate samples.

**Figure S6.**

IGV genome browser views of alignment of Rad21 ChIP-seq peaks with ATAC-seq peaks at 2.5 hours post-fertilization. The Rad21 input control, Rad21 immunoprecipitation (IP) and ATAC-seq peaks visualized over chromosome 4 regions harboring (A) *miR-430*, (B) *SAT1* and (C) *BRSAT1*. Robust Rad21 peaks align with the strongest ATAC-seq peaks.

**Figure S7.**

Timecourse of Rad21 binding at selected loci from pre-ZGA to 10 hpf. miR-430 (**A**), gata2a (**B**), hsp70l (**C**), and sox2 (**D**) enrichment tracks for Rad21 binding visualized with the IGV browser, showing the coverage for 2.5 hpf for input control and Rad21 ChIP-seq peaks from 2.5 hpf, 4.5 hpf and 10 hpf.

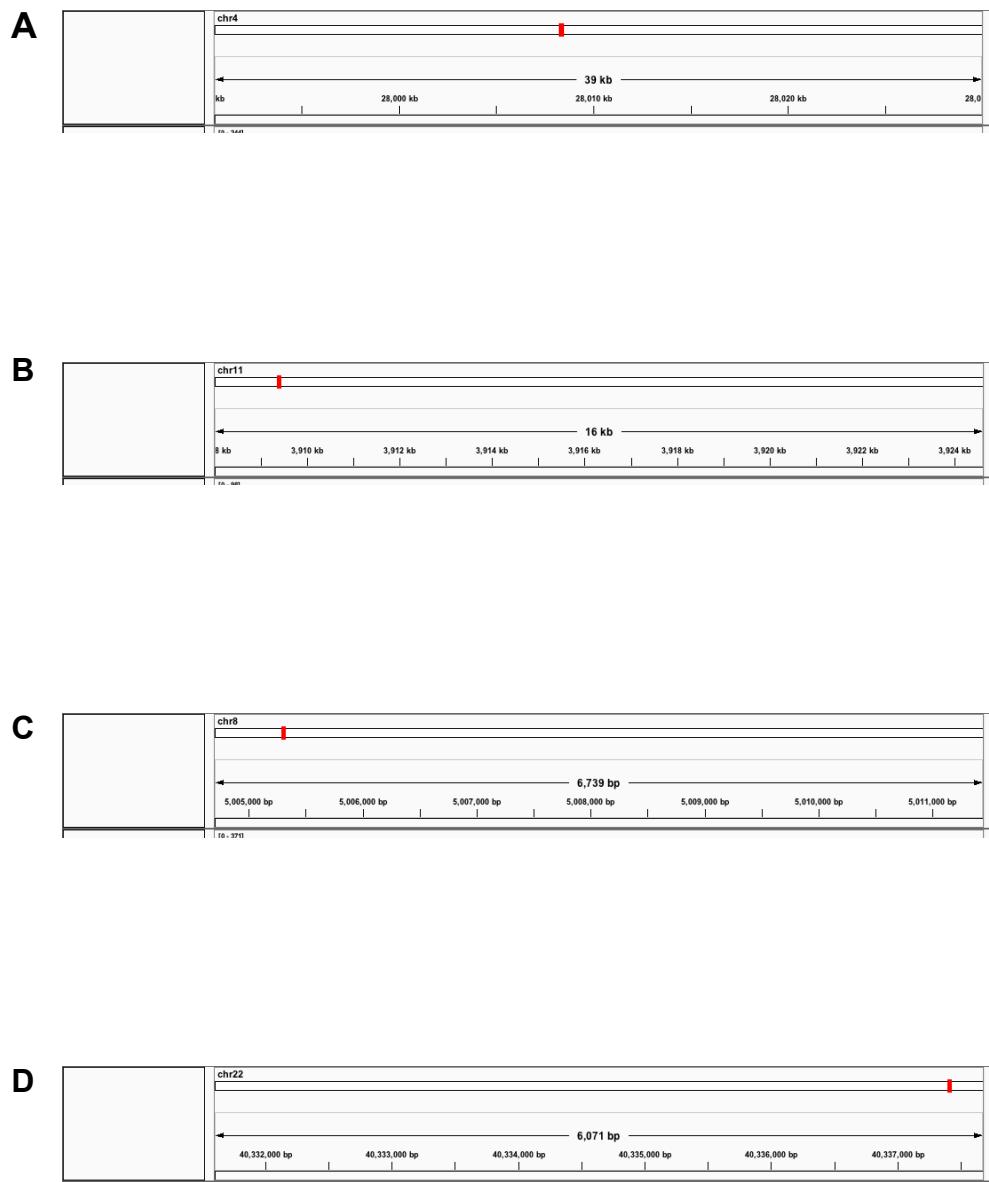
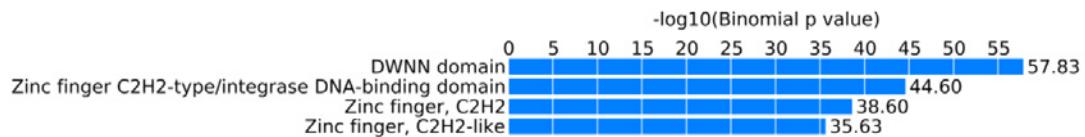
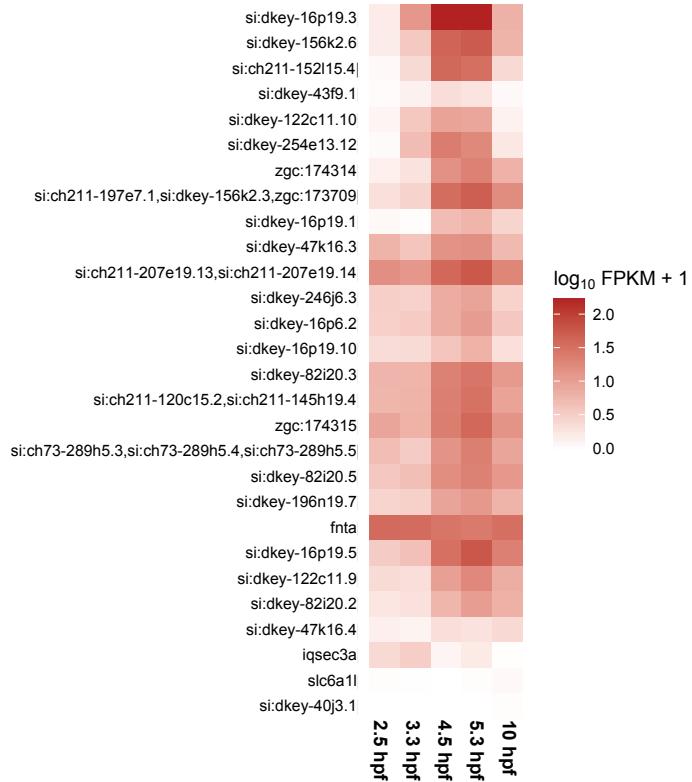


Figure S7.

Timecourse of Rad21 binding at selected loci from pre-ZGA to 10 hpf. Mir-430 (A), gata2a (B), Hsp70l (C), and sox2 (D) enrichment tracks for Rad21 binding visualized with the IGV browser, showing the coverage for 2.5 hpf for input control and Rad21 IP, as well as Rad21 ChIP-seq peaks from 2.5 hpf, 4.5 hpf and 10 hpf.

A**B****Figure S8**

Zinc finger domain protein encoding domains are enriched in pre-ZGA Rad21 binding and increase in expression at MZT. (A) Interpro domain enrichment derived from GREAT analysis of 2.5 hpf Rad21 peaks. (B). Expression of Zinc finger proteins located on Chromosome 4 at 2.5-10 hpf.

Table S1. List of significant differentially-represented transcripts from RNA-seq of Rad21- and CTCF-depleted embryos at 2.5 hpf, 3.3 hpf, 4.5 hpf, 5.3 hpf and 10 hpf.

[Click here to Download Table S1](#)

Table S2. Gene Ontology analysis of RNA-seq from Rad21 and CTCF-depleted embryos.

Table lists Gene Ontology analysis of significant ($p \leq 0.01$) differentially represented transcripts at 4.5 hpf following Rad21 or CTCF depletion.

[Click here to Download Table S2](#)

Table S3. Rad21 ChIP-seq and ATAC-seq peaks. Lists Rad21 ChIP-seq peaks at 2.5, 4.5 and 10 hpf, and ATAC-seq peaks at 2.5 hpf.

[Click here to Download Table S3](#)

Table S4. Overlap of Rad21 binding with histone marks and pluripotency factors.

[Click here to Download Table S4](#)

Table S5. Quantitative PCR primer sequences.

Gene name	Forward primer	Reverse primer	Product length (bp)
<i>cyp2aa4</i>	TTCCATTTCCTGGGGCTG	AGGAGAACAGTGGCGAACAA	82
<i>lpl</i>	CCTGGTCAACCCCAATCCAT	AAACATACCGTGACCGTCC	93
<i>mcm6</i>	TATTCGGGTTGAGACGCCTG	AGGTACATCGTGCCCCATTCA	97
<i>bmp7a</i>	GGAGACCTCACATGGTGCAA	AAAACGCCACCATAAACGGC	95
<i>apoeb</i>	CGCTTCTCAGGTGTTCTGTCT	GCCAGAACGGTCCACCATC	150
<i>ND3-ND4I</i> (reference)	CCTACGAATGAGCCCAAGG	CGGTGAAATGTAAGTCCTGCT	177