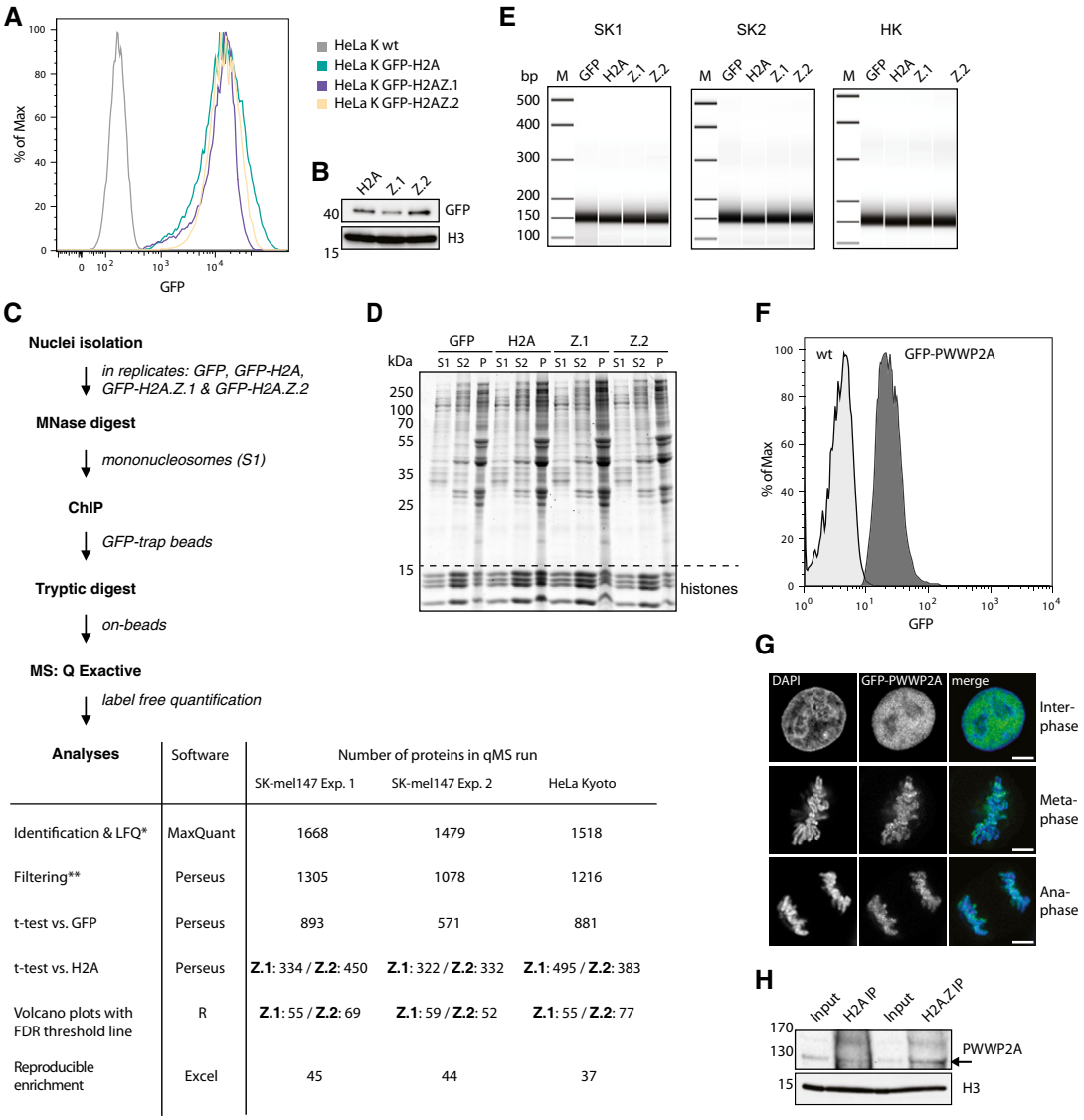
Expanded View Figures



Analyses	Software	Number of proteins in qMS run		
		SK-mel147 Exp. 1	SK-mel147 Exp. 2	HeLa Kyoto
Identification & LFQ*	MaxQuant	1668	1479	1518
Filtering**	Perseus	1305	1078	1216
t-test vs. GFP	Perseus	893	571	881
t-test vs. H2A	Perseus	**Z.1**: 334 / **Z.2**: 450	**Z.1**: 322 / **Z.2**: 332	**Z.1**: 495 / **Z.2**: 383
Volcano plots with FDR threshold line	R	**Z.1**: 55 / **Z.2**: 69	**Z.1**: 59 / **Z.2**: 52	**Z.1**: 55 / **Z.2**: 77
Reproducible enrichment	Excel	45	44	37

Figure EV1. Mononucleosome generation and LFQ-MS analysis workflow.

A Flow cytometry analysis of HeLaK cells either non-transfected (wt, gray histogram) or stably transfected with GFP-H2A (green histogram), GFP-H2A.Z.1 (blue histogram), and GFP-H2A.Z.2 (orange histogram). Notice that expression levels of tagged histones are similar.

B Immunoblots of nuclear extracts derived from HeLaK cells stably expressing GFP-tagged histones (see also A). Anti-H3 antibody was used as loading control.

C Schematic workflow of LFQ-MS experiment (see also Fig 1A and Materials and Methods). Table depicts the number of proteins identified by indicated method in each step of the procedure.

D Coomassie staining of proteins found in S1, S2, and pellet (P) fractions after MNase digest of chromatin derived from SKmel-147 cells stably expressing GFP, GFP-H2A (H2A), GFP-H2A.Z.1 (Z.2), or GFP-H2A.Z.2 (Z.2). Notice that all fractions contain histones, but remaining protein content is different.

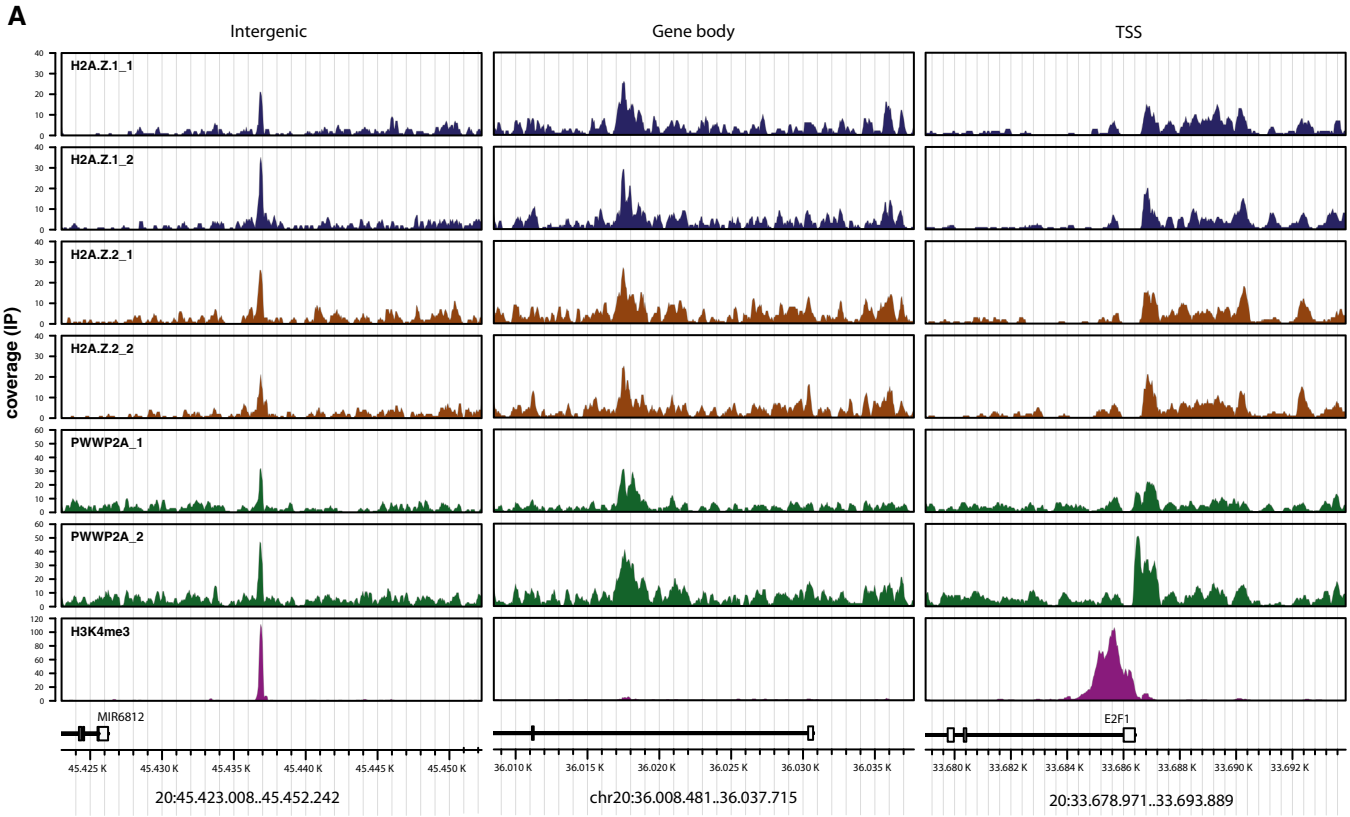
E Bioanalyzer separated DNA sizes of S1-containing nucleosomes after MNase digest of two independent SKmel-147 replicates (SK1 and SK2) or HeLaK (HK) cells stably expressing GFP, GFP-H2A (H2A), GFP-H2A.Z.1 (Z.2), or GFP-H2A.Z.2 (Z.2) used in LFQ-MS experiments shown in Fig 1A. Notice that S1 fractions that were used for LFQ-MS IP experiments contain > 90% pure mononucleosomes as seen by DNA sizes of around 150 bp.

F Flow cytometry analysis of HeLaK cells either non-transfected (control, light gray histogram) or stably transfected with GFP-PWWP2A (dark gray histogram). Notice that a pure population of HeLaK cells expressing GFP-PWWP2A at low level was obtained.

G Confocal microscopy images of HeLaK cells stably expressing GFP-PWWP2A in interphase, metaphase, or anaphase. DNA was visualized by DAPI staining (blue), and GFP-PWWP2A is seen in green. Scale bars = 10 μm. Notice that some GFP-PWWP2A remains stably associated with condensed mitotic chromatin.

H Immunoblotting of endogenous PWWP2A upon mono-IPs using antibodies against endogenous H2A or H2A.Z. Arrow indicates endogenous PWWP2A.

Source data are available online for this figure.



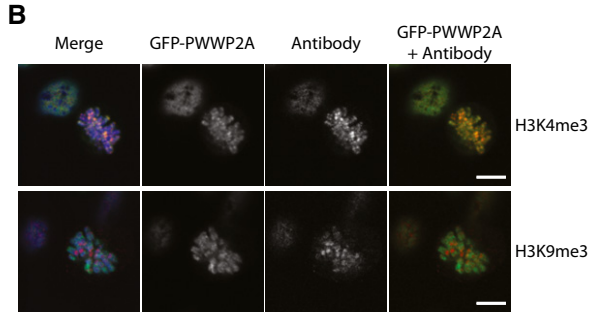


Figure EV2. PWWP2A localizes to euchromatic regions.

A Genome browser snap shot of a representative region in chromosome 20 displaying two independent replicates of HeLaK GFP-H2A.Z.1, GFP-H2A.Z.2, GFP-PWWP2A, and H3K4me3 nChIPs signals at intergenic, gene body, and TSS regions. Annotated genes are displayed below. See also Fig 4 for nChIP-seq analyses.

B Representative confocal IF pictures of HeLaK cells stably expressing GFP-PWWP2A (green), co-stained with DAPI (blue) and either H3K4me3 antibody (top, red) or H3K9me3 (bottom, red). Scale bars = 10 μm.

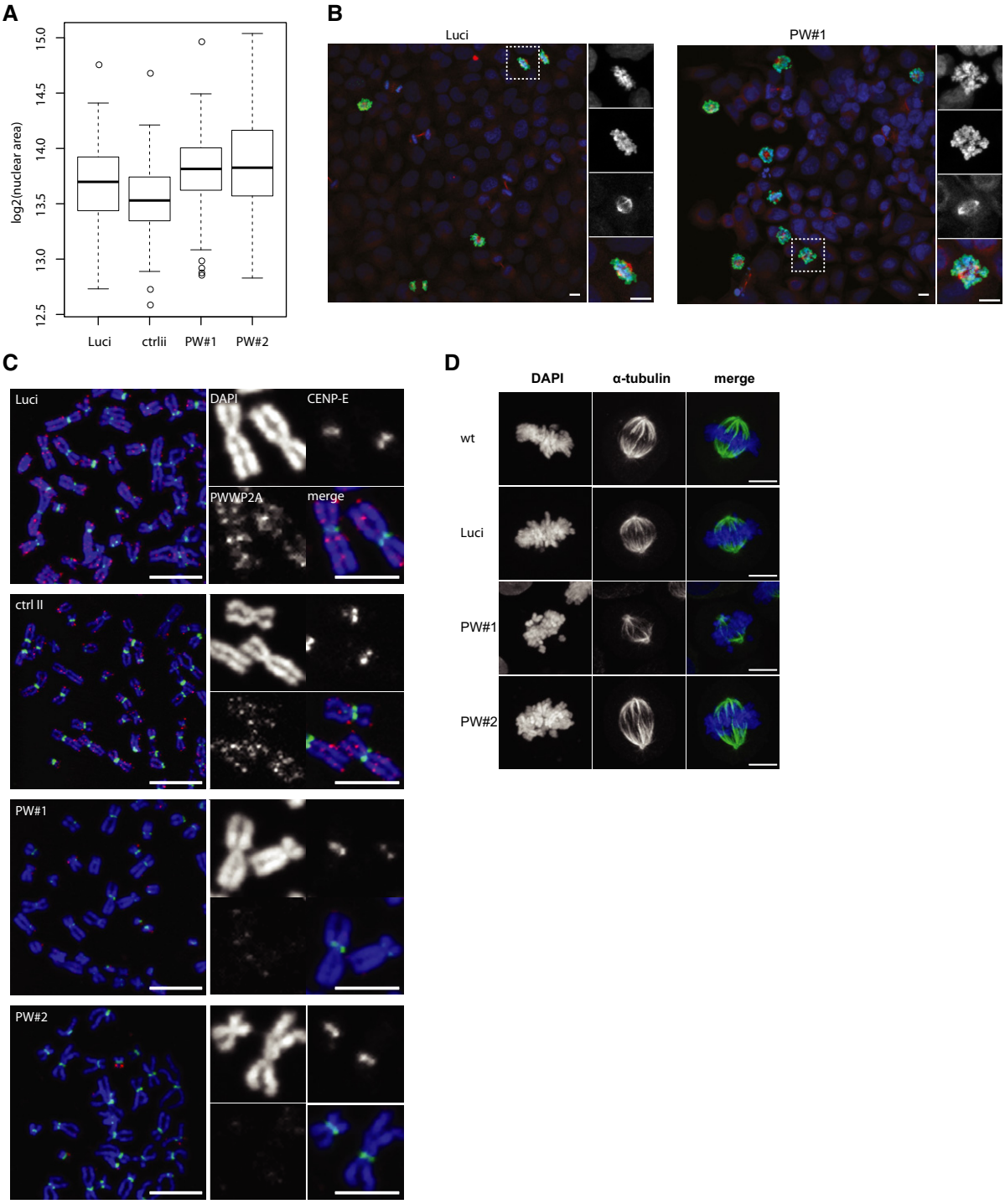


Figure EV3. Role of PWWP2A during mitosis.

A Standard Tukey boxplots of nuclear sizes of control (ctrlii, luci) and PWWP2A-depleted (PW#1, PW#2) cells (n = 3). Horizontal line = median, box = interquartile range.

B Confocal IF microscopy analysis of HeLaK RNAi cells to visualize mitotic phases. Two days after RNAi cells were stained with DAPI (blue), H3S10ph (green), and tubulin (red) antibodies. Scale bars = 10 μm. See also Fig 6E for quantitation of mitotic stages.

C Representative confocal IF pictures of chromosomes of control (ctrlii, Luci) and PWWP2A-depleted (PW#1, PW#2) HeLaK cells co-stained with DAPI (blue), CENP-E (green), and PWWP2A (red). Scale bars = 10 μm (enlarged chromosomes in boxes; scale bars = 5 μm).

D Representative confocal IF pictures of mitotic HeLaK cells two days after control (wt, Luci) and PWWP2A (PW#1, PW#2) siRNA transfections. HeLaK cells were co-stained with DAPI (blue) and tubulin (green) antibodies. Scale bars = 10 μm.

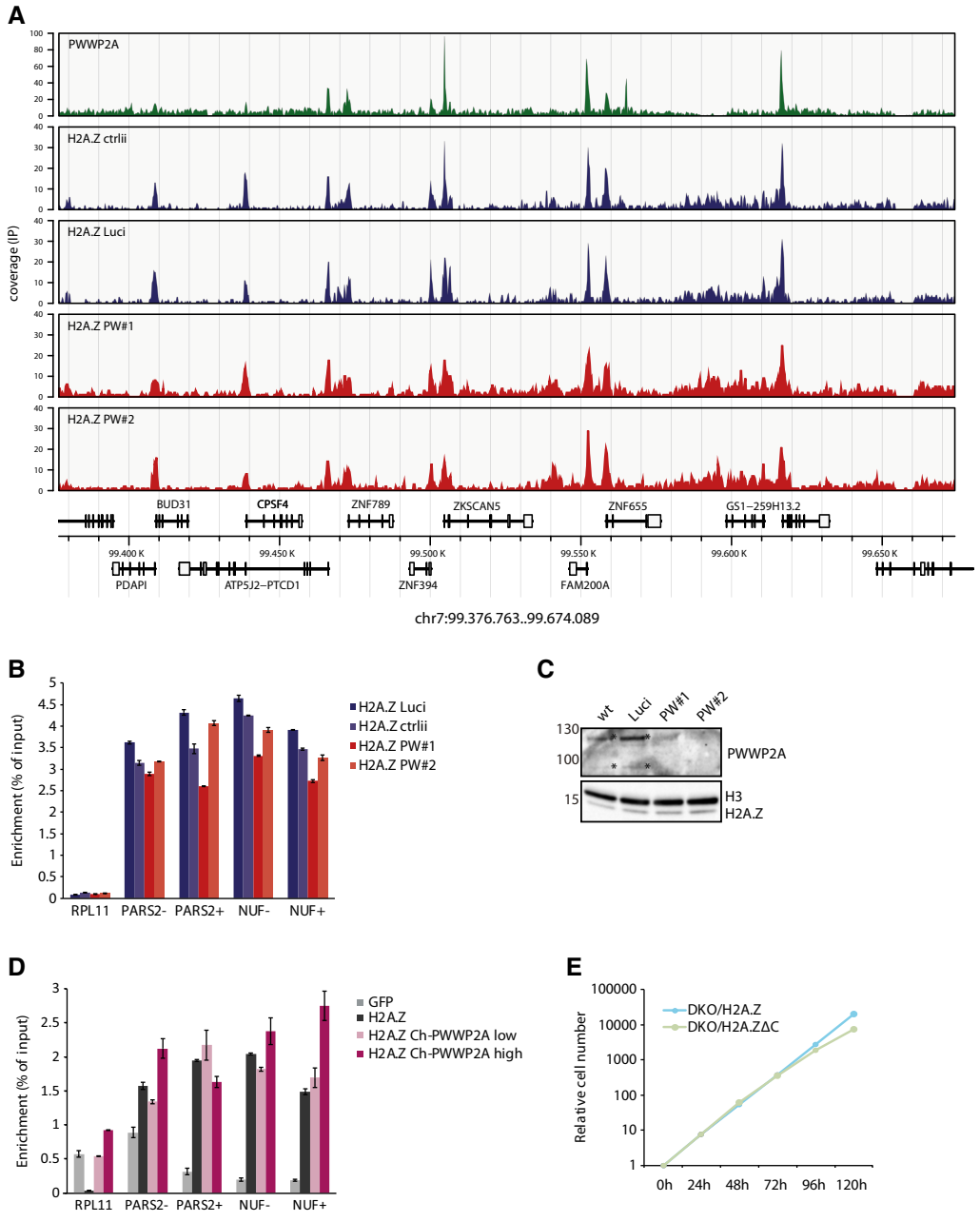


Figure EV4. PWWP2A does not affect H2A.Z genomic localization.

A Genome browser snap shot of a representative region in chromosome 7 displaying GFP-PWWP2A (green, taken from Fig 4) and GFP-H2A.Z nChIP-seq signals 2 days after control (ctrlii, Luci, blue) or PWWP2A depletion (PW#1, PW#2, red). Annotated gene structures are shown below. Notice that H2A.Z peaks do not significantly change upon PWWP2A knockdown and in comparison with PWWP2A-containing or PWWP2A-missing sites. See also Fig 7B for Venn diagrams.

B nChIP-qPCR of GFP-H2A.Z to verify results obtained in (A) and Fig 7B. RPL11 represents a gene body site missing H2A.Z nucleosomes (negative control), while all other regions show H2A.Z-containing promoter sites (− = −1 nucleosome, + = +1 nucleosome). Error bars indicate SEM of two to three technical replicates. Notice that PWWP2A depletion does not lead to a significant change in H2A.Z occupancy.

C Immunoblot of cell extracts 2 days after control (wt, Luci) or PWWP2A (PW#1, PW#2) knockdown with antibodies against PWWP2A, H3, and H2A.Z. Notice that PWWP2A reduction does not influence global H2A.Z levels.

D nChIP-qPCR of GFP (negative control, light gray), GFP-H2A.Z without exogenous Cherry-PWWP2A (Ch-PWWP2A) expression (dark gray) or upon sorting of low (light red) or high (dark red) Ch-PWWP2A expression levels (see Appendix Fig S4B for FACS levels) using primer pairs shown in Fig EV4B. Error bars indicate SEM of two to three technical replicates. Notice that PWWP2A overexpression does not significantly affect H2A.Z occupancy.

E Growth curve of DKO cells rescued with either wt Flag-H2A.Z (red, see also Kusakabe *et al*, 2016) or Flag-H2A.ZΔC (green). Notice that although Flag-H2A.ZΔC transfected cells grow slower than cells expressing wt Flag-H2A.Z, they are able to partially rescue the severe mitotic defect observed in DKO cells implying chromatin deposition took place (see also Fig 7E).

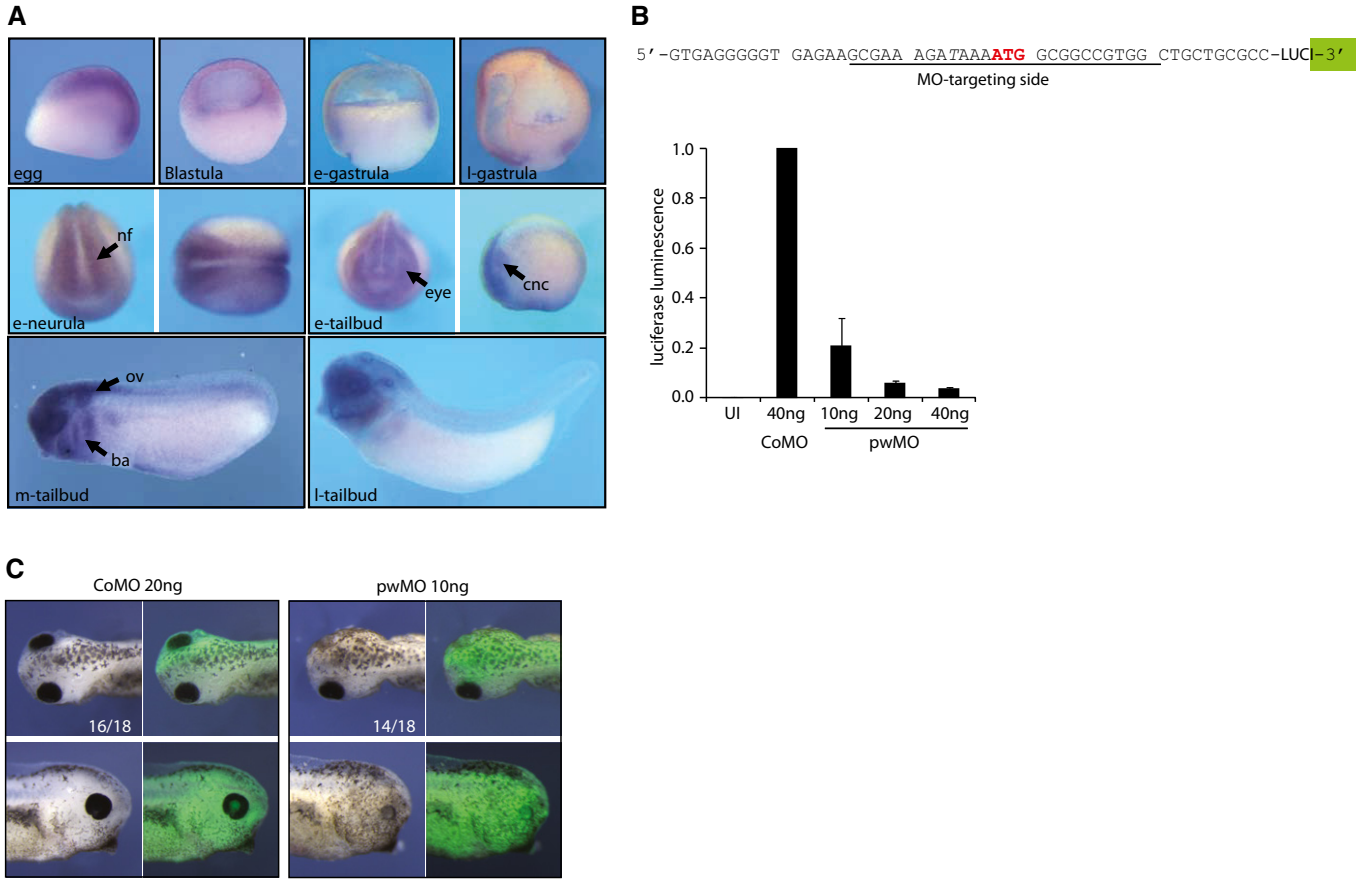


Figure EV5. Analysis of *Xenopus pwwp2a* expression and function.

A Expression pattern of endogenous *Xenopus tropicalis pwwp2a* mRNA during early development. Pictures show whole-mount RNA *in situ* hybridization patterns (purple color) for the following developmental stages: egg, blastula and early (e-)/late (l-) gastrula stages in lateral views of sagittal sections; e-neurula and e-tailbud stages show anterior views (left picture) or dorsal views (right picture, anterior to the left). Mid (m-) and l-tailbud stages are shown in lateral views (anterior left). Abbreviations: ba, branchial arches; cnc, cranial neural crest; eye, retinal Anlage; nf, neural folds; ov, otic vesicle.

B Top: Targeting region of the pwMO oligonucleotide on the *X. laevis pwwp2a* mRNA. The translational start site is highlighted in red. To determine the translational blocking efficiency of pwMO *in vivo*, a ~275-bp fragment of pwwp2a 5′ cDNA sequence, including the AUG, was cloned in frame upstream of the luciferase coding region. Bottom: The above depicted luciferase construct was injected in CoMO- or pwMO-loaded embryos and chemiluminescence measured at gastrula stage. Error bars indicate SEM of three independent biological replicates. UI, uninjected embryos.

C Morphology of *X. tropicalis* embryos injected with CoMO and pwMO together with lineage-tracer Alexa-488; numbers indicate penetrance of major morphological phenotype over total embryos inspected (n = 3 experiments).