Supplemental Materials

Molecular Biology of the Cell

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Supplemental Material

Supplementary Figure 1

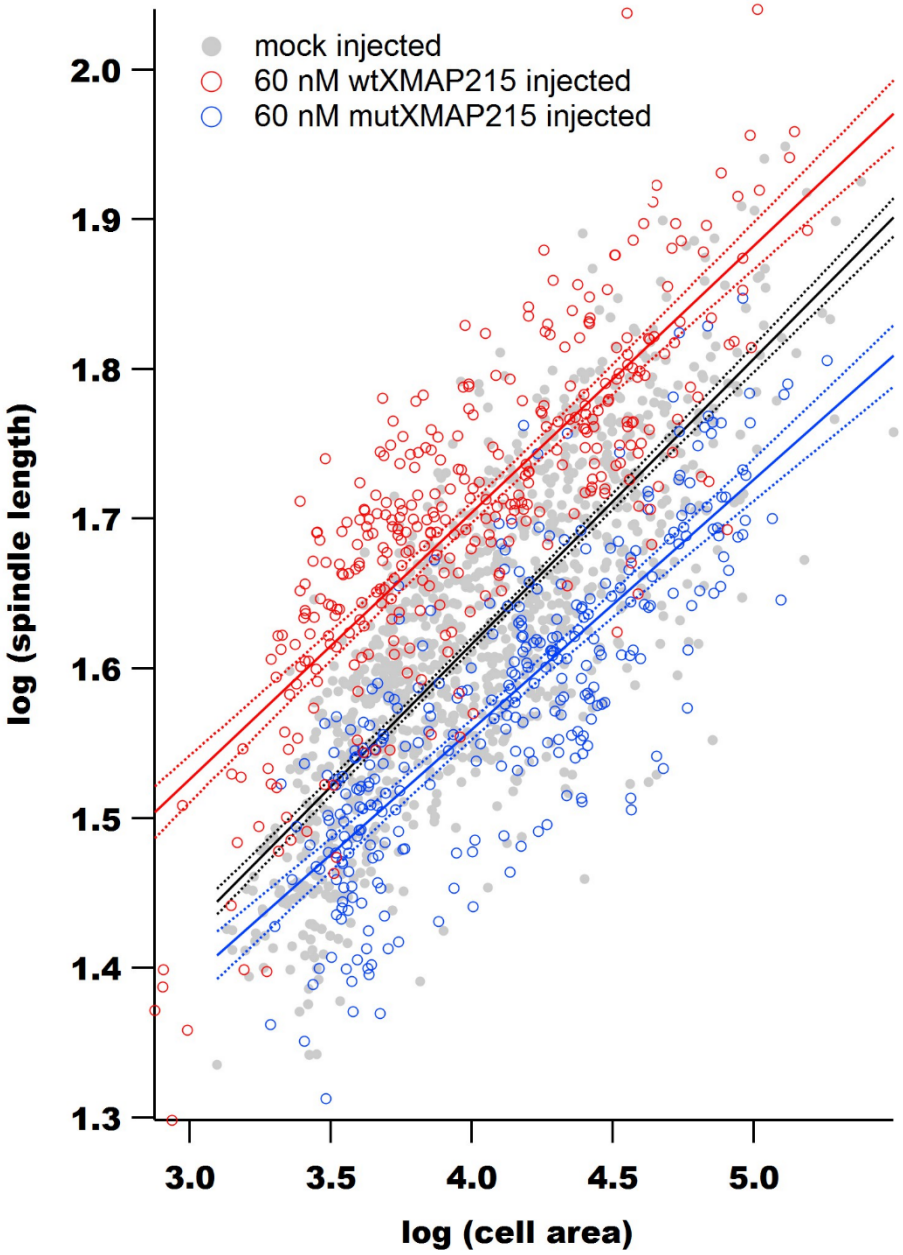

Figure S1. Comparison of spindle scaling in control, 60 nM wild-type and 60 nM mutXMAP215 injected embyos. Spindle length is shown plotted as a function of cell area in a log-log graph. Non-overlapping 95% confidence intervals for linear regressions indicate statistically significant shifts in the correlation between spindle and cell area. Best-fits for regressions were generated in Igor Pro 7 using

an iterative method that employs the Levenberg-Marquardt algorithm to search for the coefficient values that minimize chi-square, a form of nonlinear, least-squares fitting. Chi-square is defined as

$$\sum_i \left(\frac{y - y_i}{\sigma_i}\right)^2$$

where y = fitted value for a given point,
y_i = the measured data value for the point,
σ_i = an estimate of the standard deviation for y_i.

Supplementary Figure 2

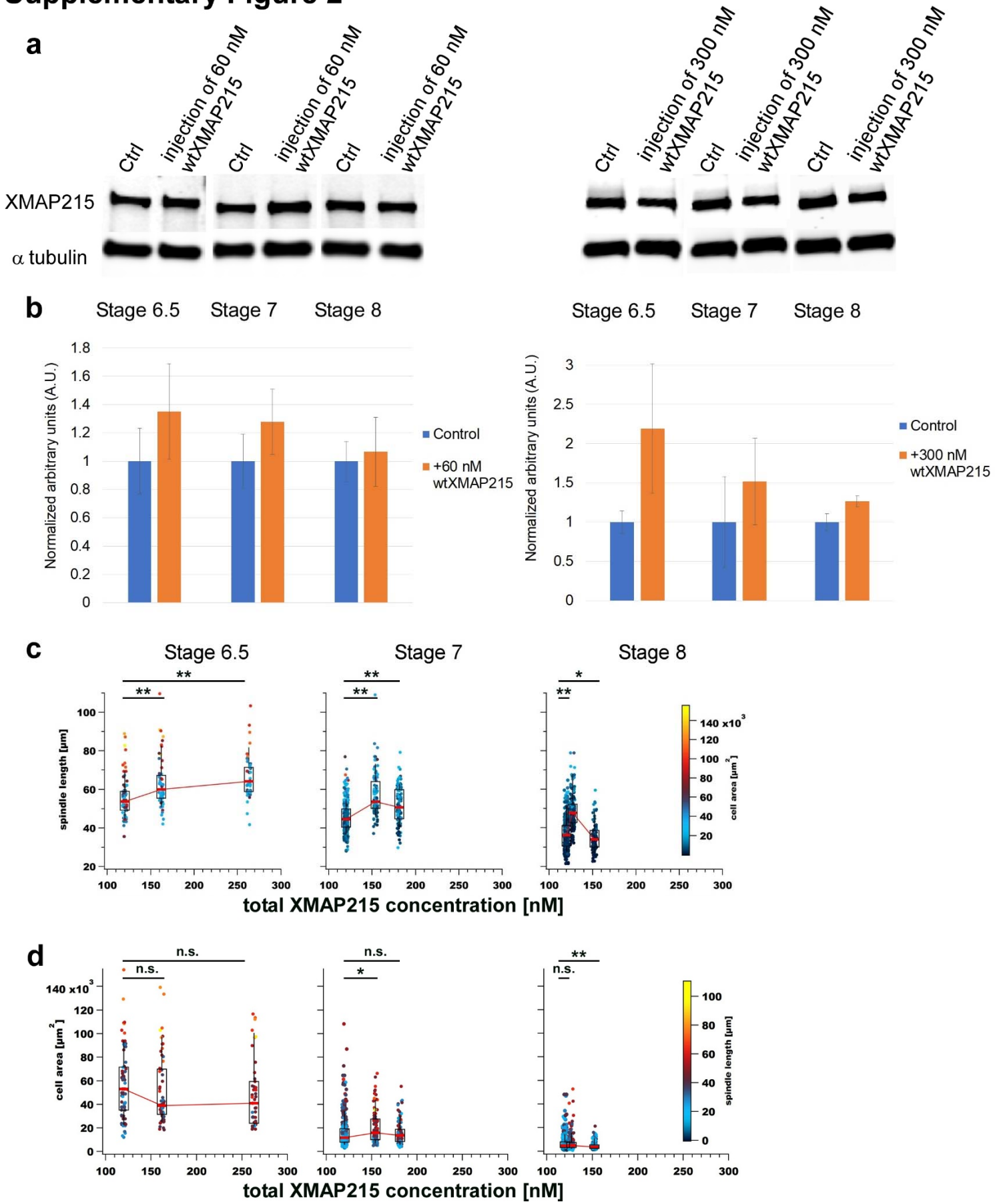

Figure S2. Stage-specific quantification and characterization of injected wild-type XMAP215. a, Western Blot analyses from stage 6.5, 7 and 8 embryos injected at one-cell stage with either 60nM or 300nM wtXMAP215. The panels show representative western blot bands of XMAP215 and α tubulin (loading control) at indicated stages. The graphs in panel **b** represent densitometric quantification of XMAP215 levels at indicated stages post-injection of additional XMAP215. Each graph represents an average of 3-4 independent experiments. 30-60 embryos were injected in each experiment and 10-20 embryos were used for western blot sample preparation for each stage. **c** and **d**, Plots show distributions of spindle length and cell size measurements as a function of total XMAP215 concentration at different developmental stages as determined via western blot analyses shown in **a**. All concentration measurements were taken from embryos at the indicated stages after initial injections at the one-cell-stage. Embryos were either mock injected (assumed to contain 120 nM XMAP215; left-most cluster of data points) or injected with 60 nM or 300 nM wtXMAP215 (middle and right-most cluster of data points, respectively). The boxplot box encompasses data from the 75^{th} to 25^{th} percentiles, whereas the upper and lower whiskers extend to the 90^{th} and 10^{th} percentiles, respectively. Mean values are indicated as central red lines. Statistical significance was determined using two-tailed T-tests with $p > 0.5$ = "n.s.", $0.005 < p \leq 0.5$ = "*", and $p \leq 0.005$ = "**". Heat map of spindle length data indicates corresponding cell areas whereas that of cell area data indicates spindle length (panel **c** and **d** respectively).

Supplementary Figure 3

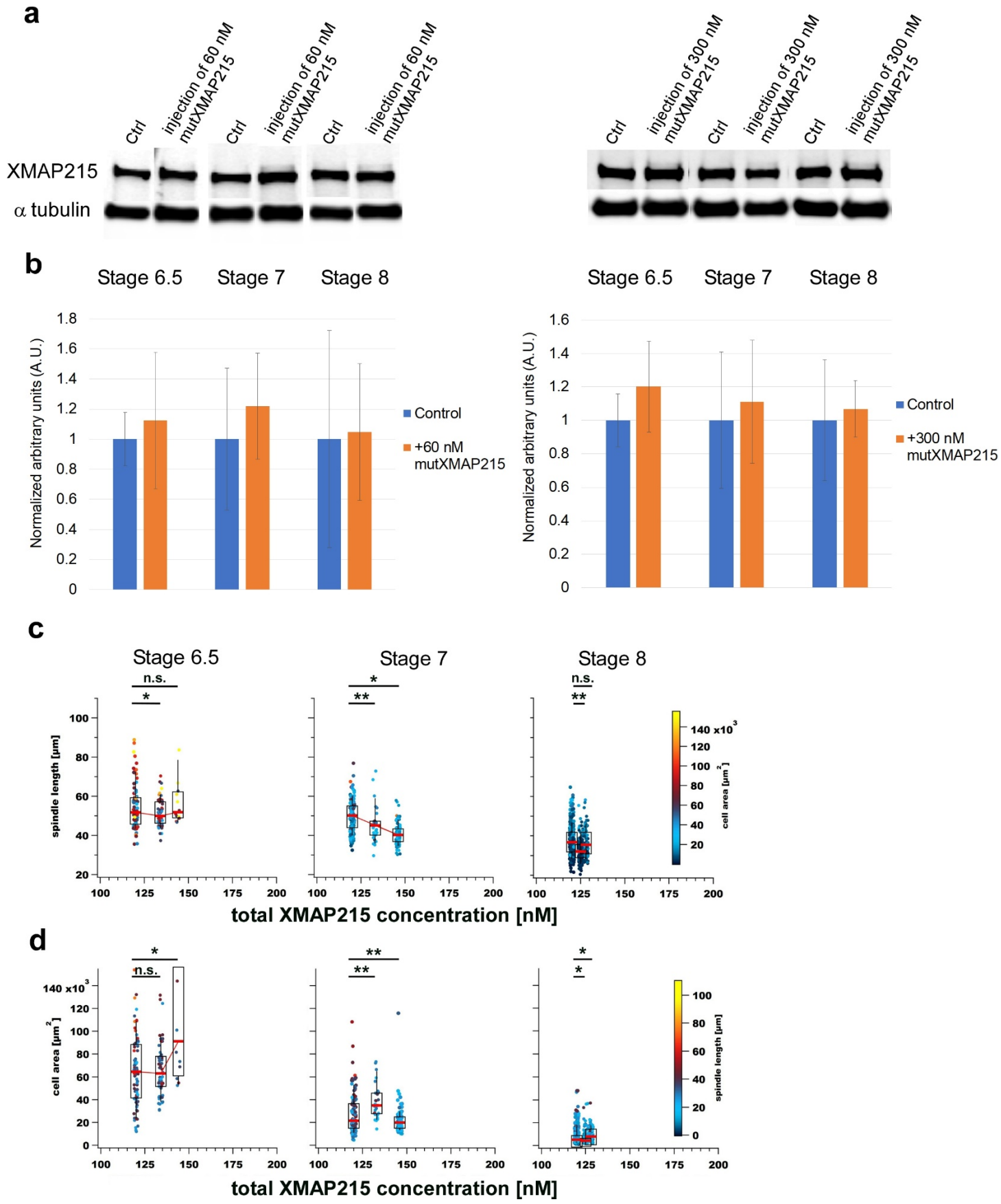

Figure S3. Stage-specific quantification and characterization of injected mutXMAP215. a, One-cell stage embryos were injected with either 60nM or 300nM mutXMAP215 and total protein levels were measured at stage 6.5, 7 and 8 via western blot. The panels show the representative western blot bands of XMAP215 and α tubulin (loading control) at the indicated stages. The graphs in lower panel represent quantification of total XMAP215 levels at indicated stages upon injection of mutXMAP215. Each graph represents an average of 3-4 independent experiments. 30-60 embryos were injected in each experiment and 10-20 embryos were used for western bot sample preparation for each stage. **c** and **d**, Plots show distributions of spindle length and cell size measurements as a function of measured total XMAP215 concentration (wild-type endogenous plus the injected mutant form) at different developmental stages as determined via western blot analyses shown in **a**. All concentration measurements were taken from embryos at the indicated stages after initial injections at the one-cell-stage and reflect total XMAP215 protein levels. Embryos were either mock injected (assumed to contain 120 nM wtXMAP215; left-most cluster of data points) or injected with 60 nM or 300 nM mutXMAP215 (middle and right-most cluster of data points, respectively). The boxplot box encompasses data from the 75^{th} to 25^{th} percentiles, whereas the upper and lower whiskers extend to the 90^{th} and 10^{th} percentiles, respectively. Mean values are indicated as central red lines. Statistical significance was determined using two-tailed T-tests with $p > 0.5$ = "n.s.", $0.005 < p \leq 0.5$ = "*", and $p \leq 0.005$ = "**". Heat map of spindle length data indicates corresponding cell areas whereas that of cell area data indicates spindle length (panel **c** and **d** respectively).

Supplementary Figure 4

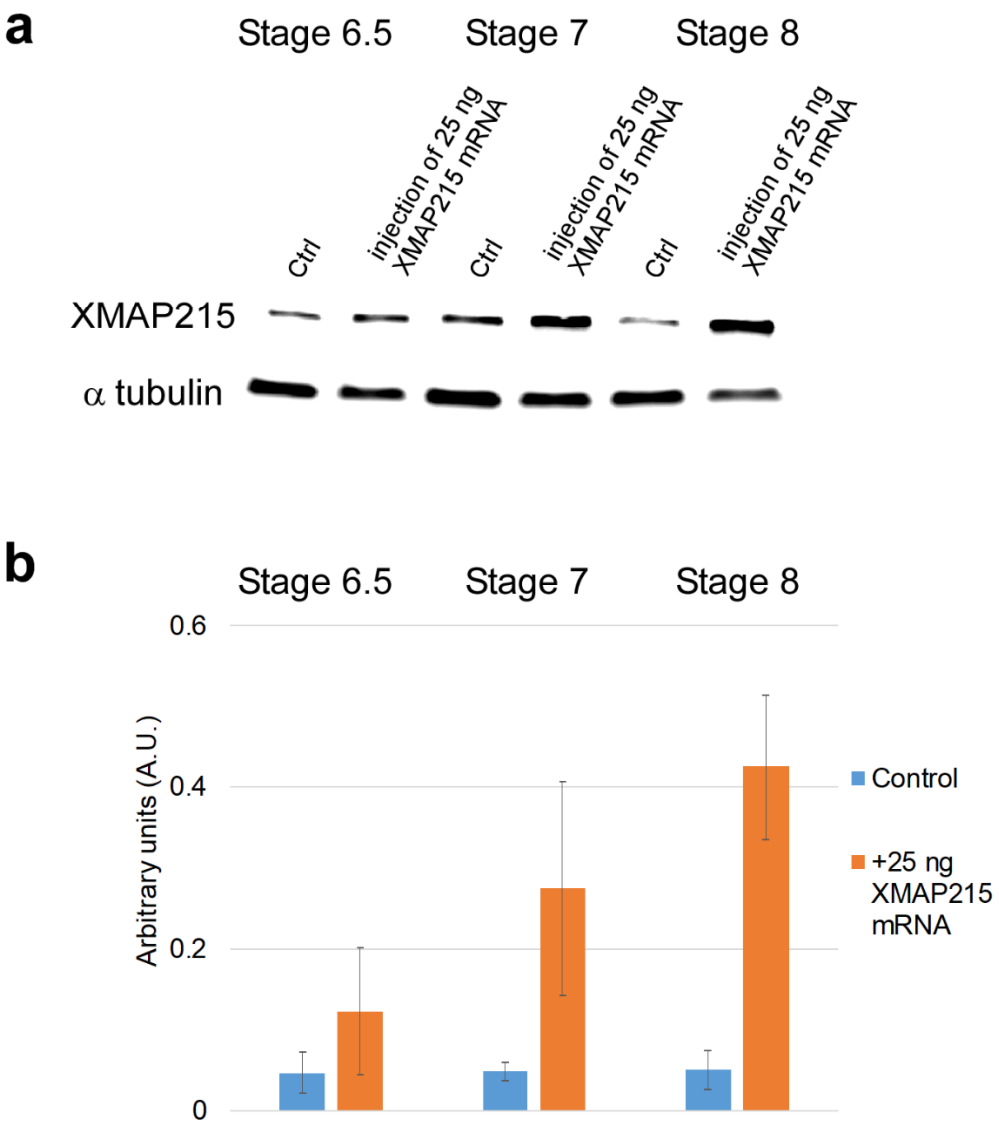

Figure S4. Quantification of XMAP215 levels in XMAP215 mRNA injected embryos. 25ng of mRNA encoding wild-type XMAP215 was injected into one-cell stage embryos and XMAP215 protein levels were determined at the indicated stages via densitometric analysis of western blots. **a**, representative western blot bands of XMAP215 and α tubulin (used as a loading control) at the indicated stages. Bar graph in **b** shows the results of densitometric quantification of XMAP215 levels produced by indicated stages. Each graph represents an average of 3 independent experiments. 30-60 embryos were injected in each experiment and 10-20 embryos were used for western blot sample preparation for each stage.