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Supplemental information

Tubulin acetylation promotes penetrative capacity of cells undergoing radial intercalation

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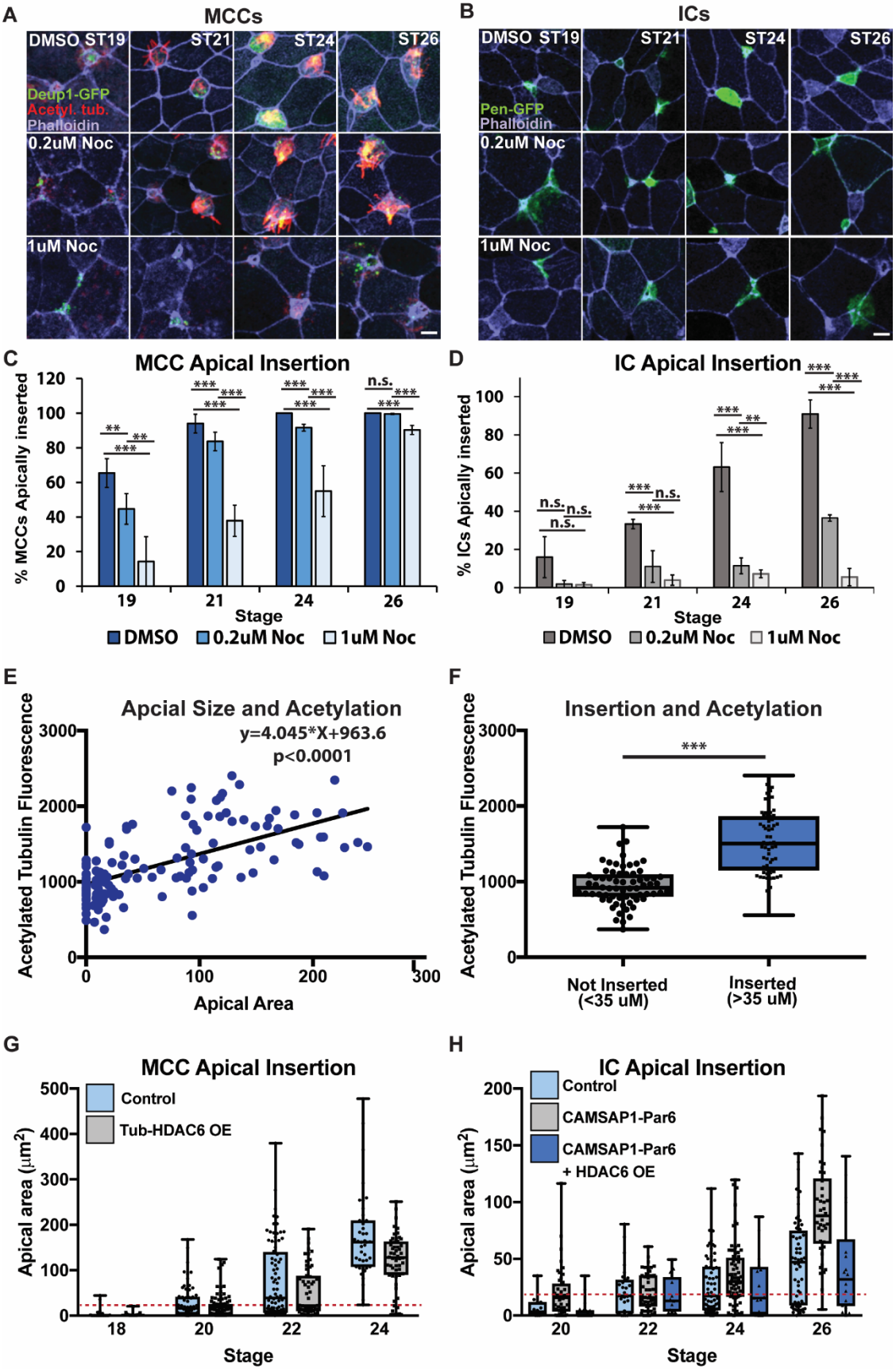


Figure S1. Related to Figure 1. MTs and tubulin acetylation are critical for apical insertion. **A**, Z-projections of Tub-Deup1-GFP embryos treated with DMSO, 0.2μM Nocodazole (Noc), or 1μM Noc fixed and stained with an α-acetylated tubulin antibody and phalloidin at the indicated stages. **B**, Z-projections of WT embryos injected with Pen-GFP and treated with DMSO, 0.2μM Noc, or 1μM Noc

fixed and stained with phalloidin at the indicated stages. **C-D,** Quantification of the percentage of MCCs (**C**) or ICs (**D**) apically inserted throughout the intercalation process. Embryos were treated with DMSO, 0.2μM Noc, or 1μM Noc from ST13 until fixation. For bar graphs, bars represent mean and error bars indicate SD, and *p<0.05, **p<0.01, ***p<0.001. Analysis includes n > 150 cells at least 9 embryos per condition and ST (**C**) and n > 100 cells from at least 9 embryos per condition and ST (**D**). **E**, Quantification of apical size versus acetylated tubulin fluorescent intensity in wild type Stage 22 embryos (n = 90 cells). **F**, Quantification of acetylated tubulin in ST22 embryos comparing cells that have or not apically inserted. **G-H**, Data from Figure 1F (**G**) and 1L (**H**) presented as dot blots of apical size, with the red dashed line indicating the 35μm^2 cutoff for apical insertion (for each condition and ST, n > 50 cells, $\geq$ 9 embryos). For box-and-whisker plots in **F-H** the box represents 25-75% range, the line is the median and the whiskers represent the total range. Scale bars in **A, B** is 10μm.

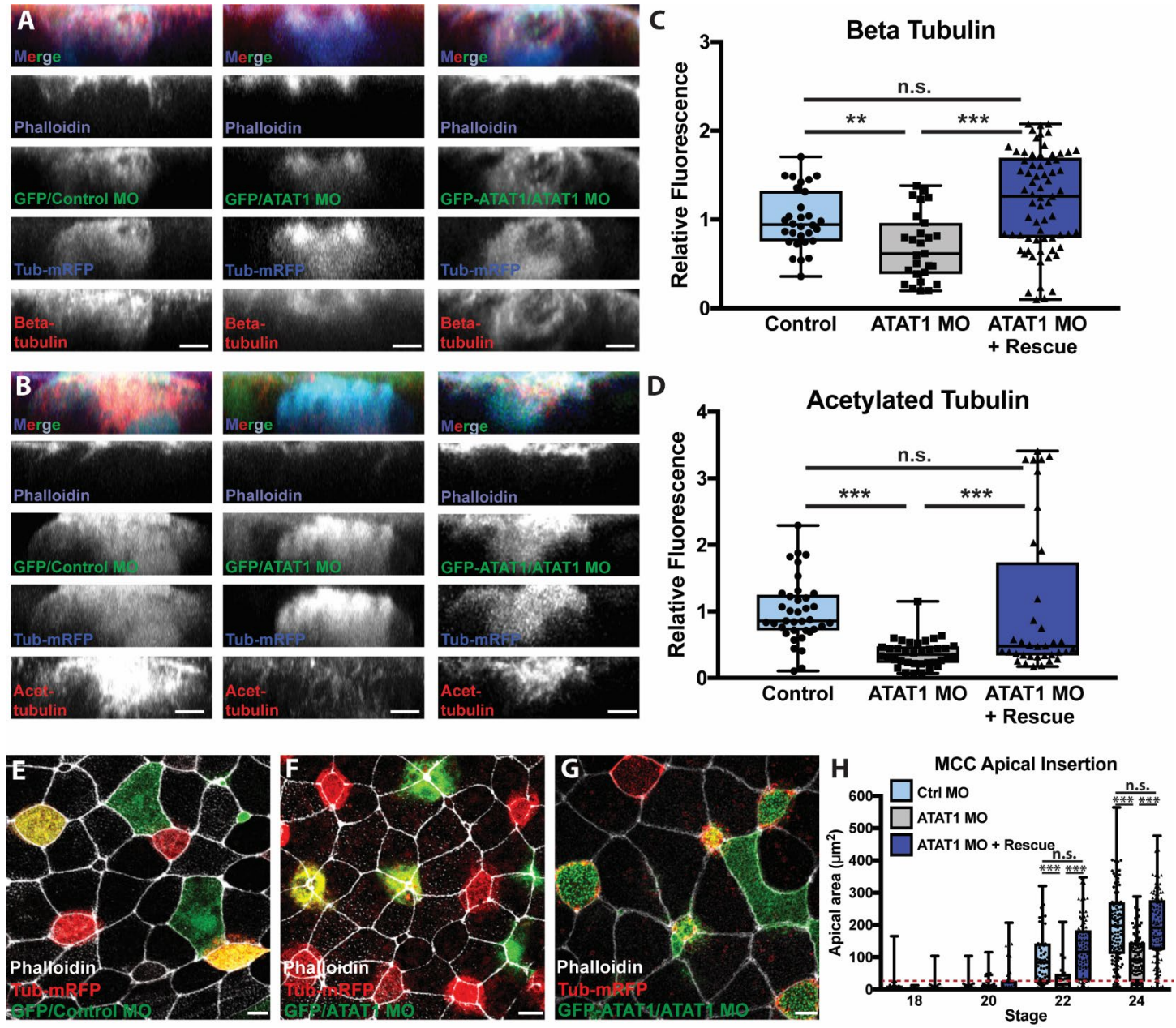


Figure S2. Related to Figure 1. ATAT1 MO delays apical insertion. **A-B**, Side projections of intercalating MCCs injected with GFP tracer with control MO, ATAT1 MO, and ATAT1 MO + GFP-ATAT1 fixed and stained with phalloidin and α-beta tub (**A**) or α-acetyl. tub. (**B**). **C-D**, Quantification of beta tub (**C**) and of acetyl. tub. (**D**) in control MO, ATAT1 MO and rescue MCCs (for each condition, n > 27cells from at least 2 embryos). Fluorescence was normalized relative to control MCCs for each experiment. **E-G**, Z-projections of ST22 Tub-mRFP embryos mosaically injected with GFP together with Control MO (**E**), ATAT1 MO (**F**) or ATAT1 MO + GFP-ATAT1 (**G**). **H**, Quantification of apical size at each ST in each treatment, with the dotted red line indicating the 35 μm cutoff for apical insertion (for each condition and ST, n > 25 cells from at least 5 embryos). For box-and-whisker plots in **C, D,** and **H** the box represents 25-75% range, the line is the median and the whiskers represent the total range. For all graphs, ** p<0.01 and ***p<0.001. Scale bar in **A, B** is 5μm and in **E-G** is 10μm.

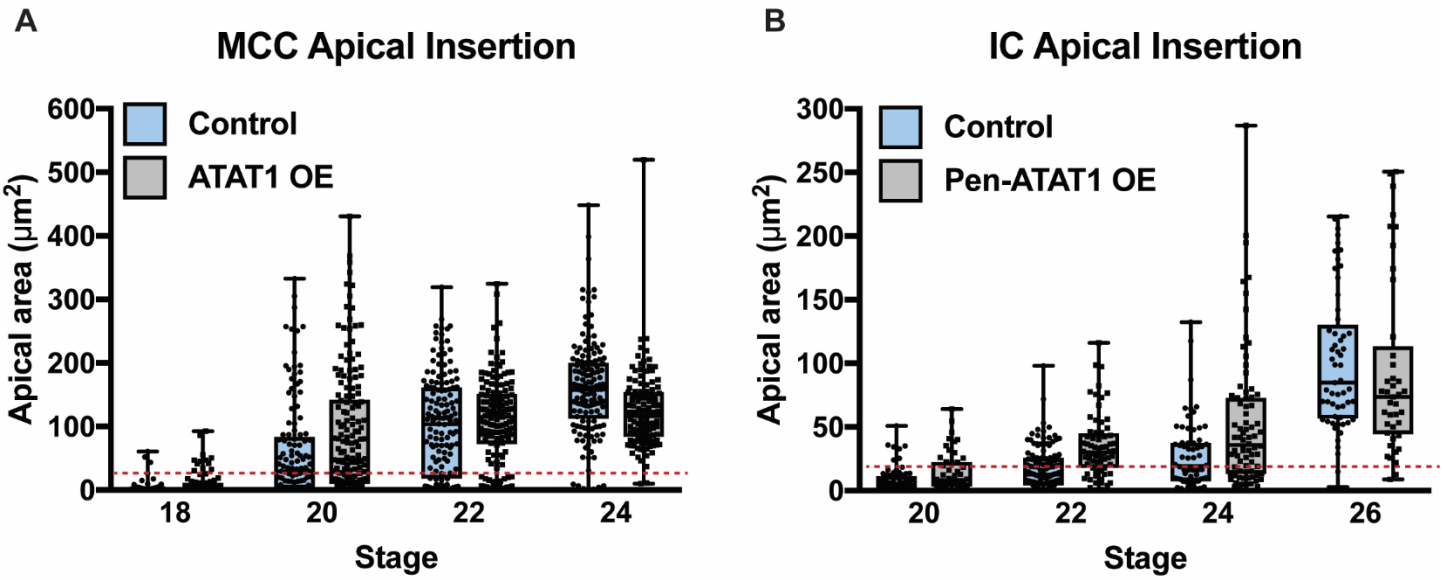
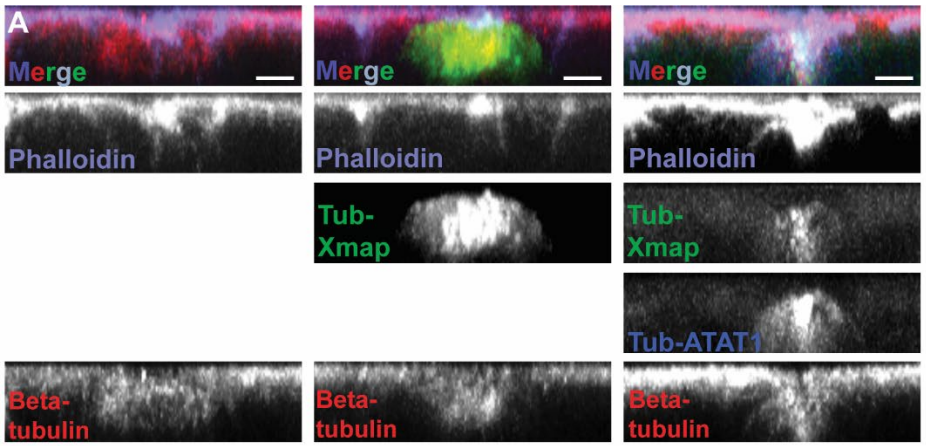
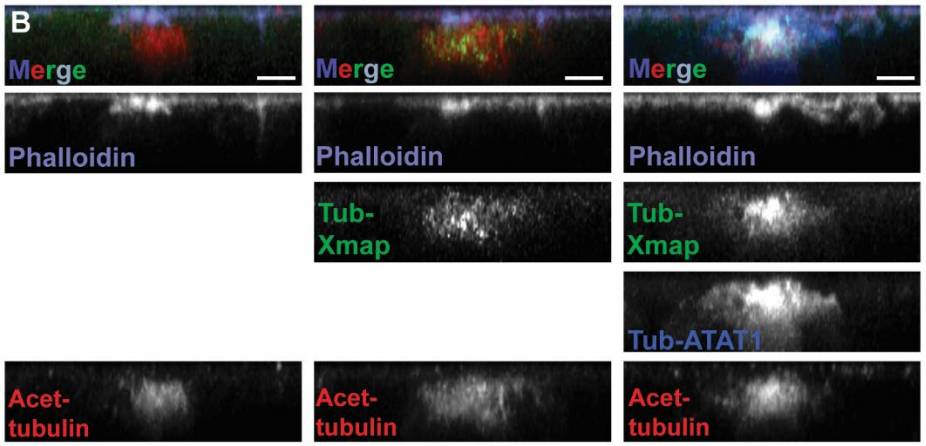
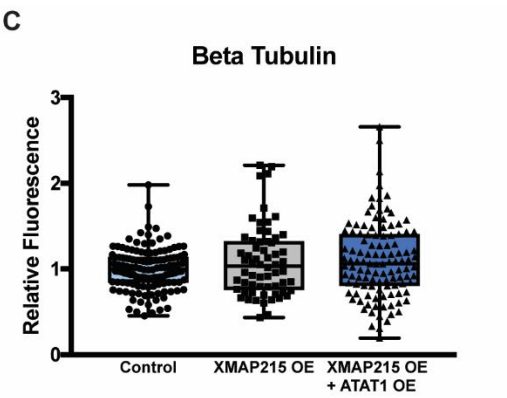
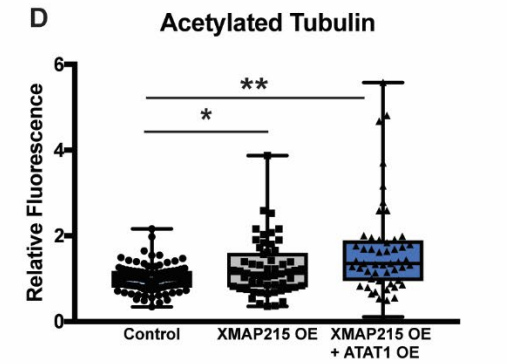


Figure S3. Related to Figure 2. ATAT1 overexpression increases the speed of apical insertion leading to larger apical size. A-B, Representative experiment from Figure 2F (**A**) and 2L (**B**) presented as dot blots of apical size, with the red dashed line indicating the 35μm^2 cutoff for apical insertion (for each ST and condition, n $\geq$ 80 cells **(A)** or 40 cells **(B)** from $\geq$ 2 embryos). Box-and-whisker plots where the box represents 25-75% range, the line is the median and the whiskers represent the total range.









E

This is a derivation of Eq. (1). Define A_k as the proposition that "one or more k-vertices exist in a given neighborhood" and B_k the proposition that no higher vertices exist in that same neighborhood". The intercalation probability is then $P(A_k \cap B_k)$:

$$\begin{aligned} P(A_k \cap B_k) &= P(A_k|B_k)P(B_k) \\ &= \left(1 - P(\overline{A}_k|B_k)\right) P(B_k) \\ &= \left(1 - \frac{P(\overline{A}_k \cap B_k)}{P(B_k)}\right) P(B_k) \\ &= P(B_k) - P(\overline{A}_k \cap B_k) \\ &= (1 - \sum_{k'>k}^{6} p_{k'})^q - (1 - \sum_{k' \geq k}^{6} p_{k'})^q \end{aligned} \tag{2}$$

where $q = m/n$. This is what is denoted $P_q(k)$ in the main part of this note.

Figure S4. Related to Figure 4. Beta tubulin and acetylated tubulin levels in MCCs expressing MT nucleating and acetylation constructs. A-B, Side projections of intercalating control, XMAP215 OE, and XMAP215 OE + ATAT1 OE MCCs fixed and stained with α-beta tub **(A)** or α-acetyl. tub. **(B)**. **C-D,** Quantification of beta tub **(C)** and of acetyl. tub. **(D)** in control, XMAP215, and XMAP215 OE + ATAT1 OE MCCs. Fluorescence was normalized relative to control (uninjected) MCCs in mosaic embryos for each experiment. For all bar graphs, bars represent the mean, error bars indicate SD, and *p<0.05, **p<0.01. Analysis includes n > 45 cells from at least 6 embryos per condition and ST **(C, D)**. Scale bars in **A-B**, 10μm. **(E),** The derivation of Eq (1).