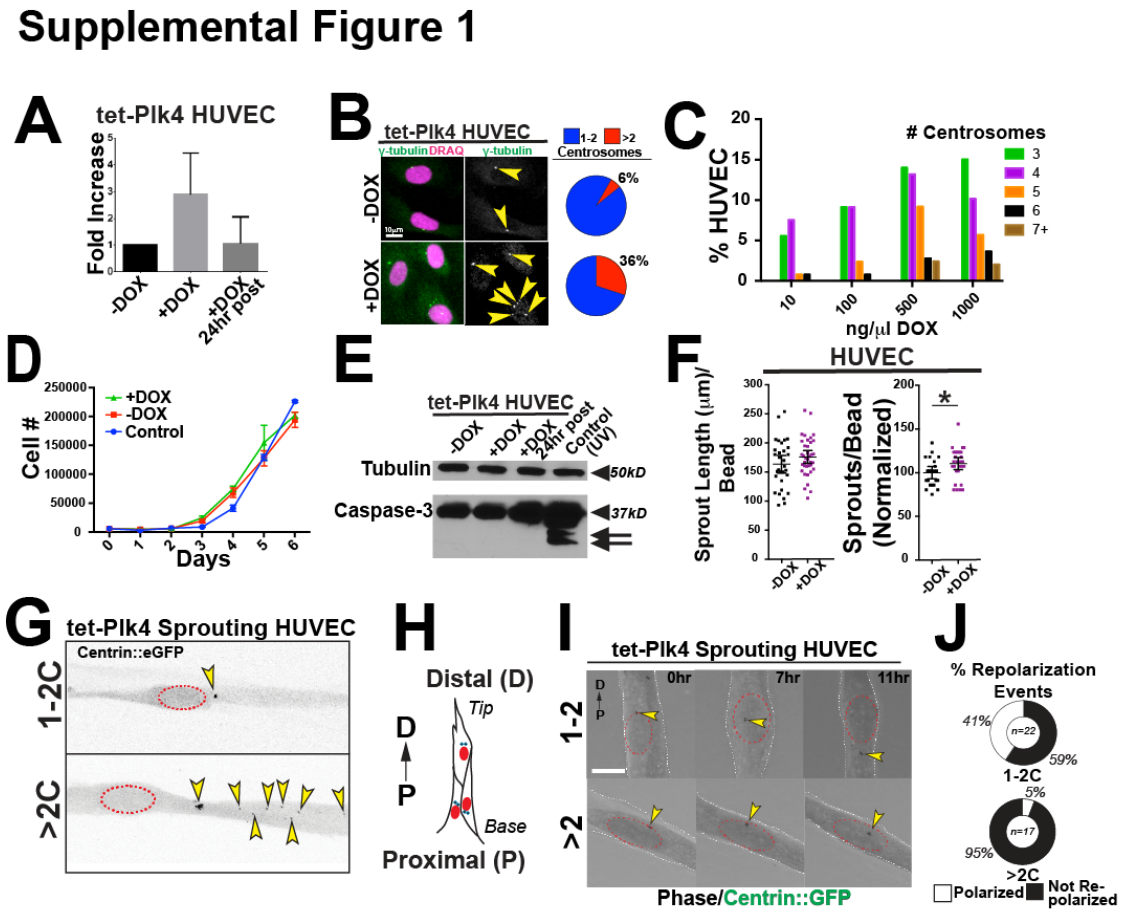
# Supplemental Materials

***Molecular Biology of the Cell***

Kushner et al.

**SUPPLEMENTAL FIGURE LEGENDS**

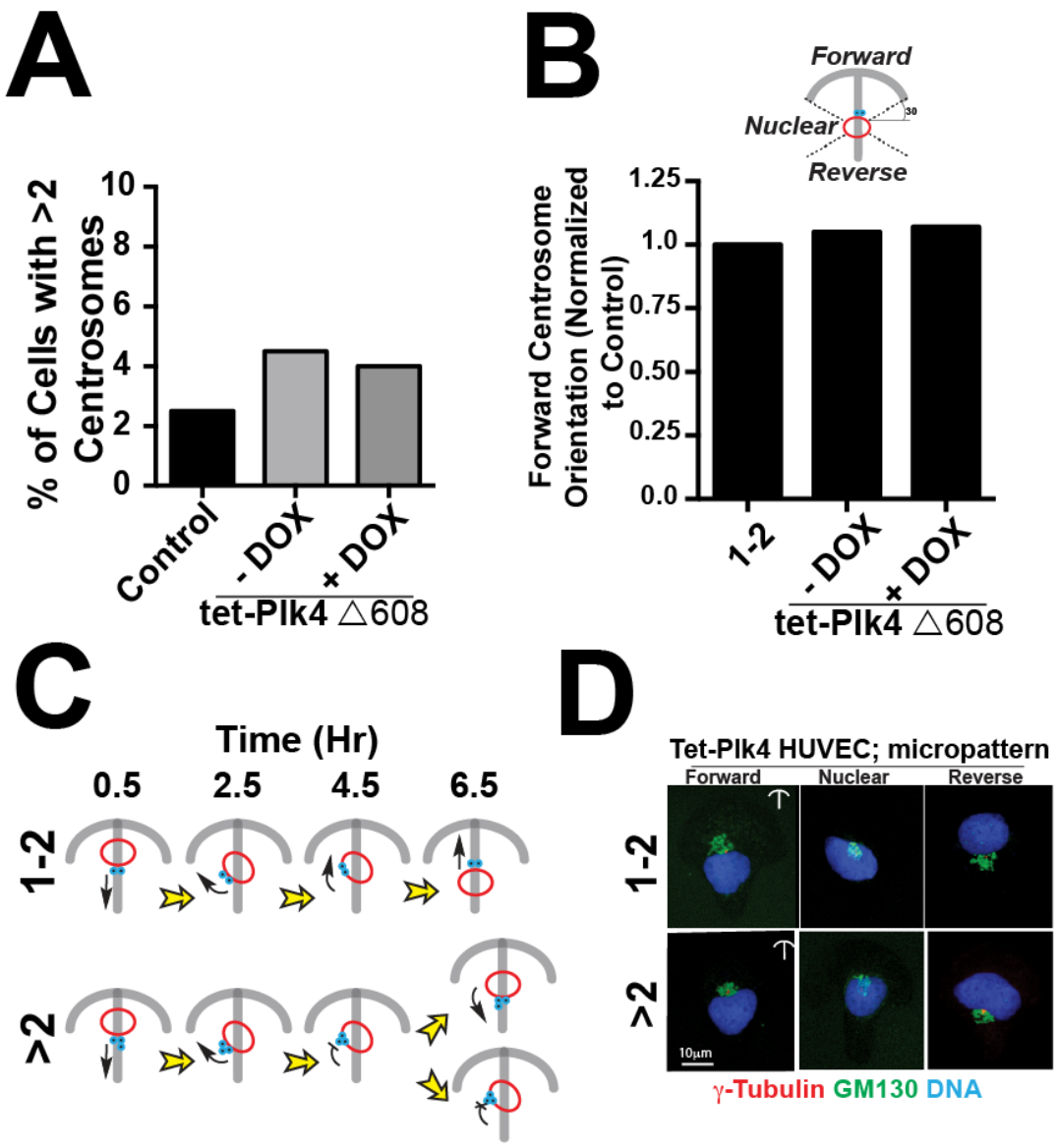



**Supplemental Figure 1 (Related to Fig. 1). Excess Centrosomes Promote Migration Defects in Angiogenic Sprouts.**

(**A**) Quantitative PCR expression levels of Plk4 mRNA relative to no DOX control between indicated groups. (**B**) Images of HUVEC without doxycycline (-DOX) and with DOX for 24 hr (+DOX), stained for centrosomes (γ-tubulin; green) and DNA (DRAQ, blue). Yellow arrowheads, centrosomes. Pie charts (right) show % centrosome over-amplification (>2 centrosomes (>2)) between indicated groups. (**C**) Graph of centrosome numbers in HUVEC by DOX concentration (24hr exposure). **(D)** Growth curves between indicated groups of HUVEC, cell # per well. **(E)** Western blot of caspase 3 levels in indicated groups. Control, uninfected with UV treatment. Arrows denote cleaved (active)

caspase in UV-treated positive control. **(F)** Scatter plots of indicated sprouting parameters in uninfected HUVEC. –DOX , n=22; +DOX, n=29 beads. **(G)** Representative live imaging of ECs in angiogenic sprouts, expressing centrin::eGFP to visualize centrosomes. Dotted red circle, nucleus perimeter; yellow arrowheads, individual or groups of centrosomes. **(H)** Diagram of distal and proximal orientation in sprouting angiogenesis. **(I)** Time-lapse imaging of intrasprout EC with 1-2 (top panels) or >2 centrosomes (bottom panels). 1-2 centrosome EC reorients whereas >2 centrosomes EC does not during imaging. Yellow arrowheads, centrosomes; dotted red circles, nucleus perimeter. Scale bar, 10 μm. **(J)** % intrasprout centrosome re-polarization events between indicated groups. EC were imaged for 24-28 hr. P-values were derived from two-tailed Student's *t*-test from 3 experiments unless indicated otherwise. *, $p \leq 0.05$.

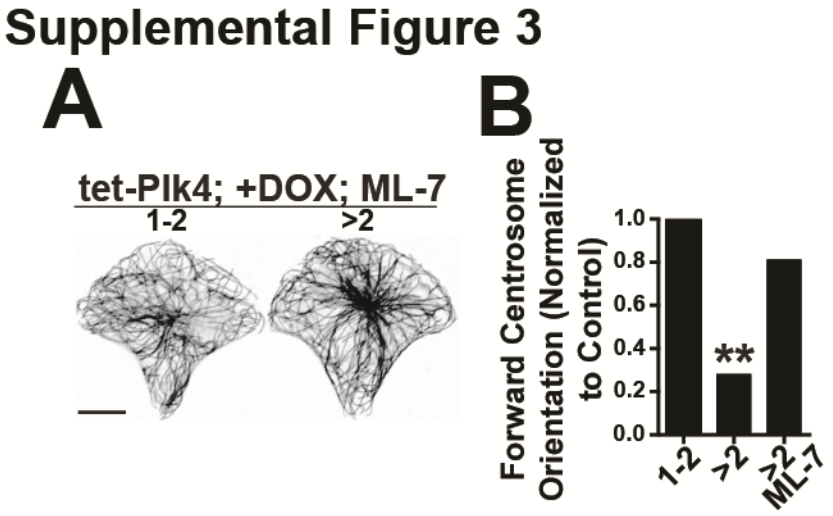
# Supplemental Figure 2




**Supplemental Figure 2 (Related to Fig. 2). Micropattern-induced Centrosome Orientation is Compromised in HUVEC with Excess Centrosomes.**

**(A)** % EC with >2 centrosomes in indicated groups. Control, untreated/uninfected (n=145 EC), tet-Plk4 Δ608 –DOX (n=139 EC), +DOX (n=179 EC). (**B**) % forward-oriented EC with indicated treatments for 5 hr. 1-2 Centrosomes (n=153 EC); tet-Plk4 Δ608 -DOX (n=191 EC), +DOX (n=130 EC). **(C)** Diagram summarizing orientation defects observed

between EC with 1-2 or >2 centrosomes. **(D)** Representative images of EC on micropatterns stained for Golgi (GM130, green), centrosomes (γ-tubulin, red) and DNA (DRAQ, blue) grouped by centrosome position and number.




**Supplemental Figure 3 (Related to Fig. 5). Inhibition of** Myosin Light Chain Kinase Activity **Rescues Microtubule Defects.**

(**A**) Representative images of HUVEC on micropatterns with ML-7 treatment stained for α-tubulin. Scale bar, 10 μm. **(B)** % forward-oriented EC in indicated groups. 1-2, n=32 EC; >2, n=32 EC; >2 +ML-7, n=42 EC. Comparisons are to 1-2 centrosome group analyzed by $X^2$. All p values derived from two-tailed Student's *t*-test from 3 experiments unless indicated otherwise. NS, not significant; **p≤0.01.