SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. **Determination of the rate of microtubule nucleation.** Cells expressing RFP-EB3 and GFP-γ-tubulin were adhered by either the WT or YA mutant integrin. The position of the centrosome was determined by GFP-γ-tubulin and newly nucleated microtubules emanating from the centrosome microtubules were marked by RFP-EB3. Images were taken every 3 seconds for a total of 30 seconds. *A*, Shown is the localization of EB3 comets and γ-tubulin at time zero for the representative cells shown in Figure 1D. *B*, An example of how newly nucleated microtubules are counted. The first six frames are shown for both WT and YA with the newly nucleated microtubules numbered in each frame.

FIGURE S2. **Additional experiments demonstrating the YA mutation inhibits the association of the androgen receptor with Src.** *A*, WT and YA cells were co-transfected with FLAG-tagged androgen receptor (FLAG-AR) and wild type Src (WT-Src). Transfected cells were replated onto fibrinogen in CCM1 to assay the association of AR and Src. An antibody directed against the FLAG tag was used for immunoprecipitation. *Top panel*, Immunoprecipitates were analyzed by Western blotting with antibodies specific for activated Src. Blots were stripped and reprobed for total Src and AR. *Bottom panel*, Lysates were also analyzed for each condition by Western blotting for the AR, phosphorylated Src and total Src levels and reprobed for total FAK as a loading control. Shown are the additional two experiments from Figure 2. *B*, Untransfected WT cells (lane 1) and WT cells transfected with FLAG-tagged AR (lane 2) were analyzed for expression of the AR by western blot. The lysate from transfected cells was diluted by a factor of ten with lysate from untransfected cells.

Figure S3. **Src signaling is required downstream of androgen.** *A-B*, WT and YA cells were replated onto fibrinogen (Fg) or fibronectin (Fn) in CCM1, SF F12 or SF F12 plus androgen, with androgen added only during the 5-min regrowth period. The Src-kinase inhibitor, SU6656, was added to WT cells during the last half hour of nocodazole treatment and in all solutions thereafter. *A*, Micrographs of representative cells stained for α-tubulin (red) and γ-tubulin (green). *B*, Quantification of the microtubule density at the centrosome where the values represent the normalized fluorescence intensities of the α-tubulin signal ± S.D. calculated from 150 cells per condition (n=3). *$p<0.05$ compared to cells adhered by the WT integrin. $^{\#}p<0.05$ compared to both the CCM1 control and SF F12 plus androgen. *C*, Src activation was analyzed by Western blotting using a phospho-specific antibody and reprobed for total Src as a loading control. *D*, Quantification of Src activation in response to androgen ± S.D. (n=3). Androgen increases Src activity when cells are adhered by the WT integrin ($p=0.151$). Scale bar, 3 μm.

Figure S4. **Restoring integrin function rescues microtubule nucleation and Src activity.** *A-B*, Adhering YA cells to fibronectin through their endogenous α5β1 fibronectin-binding integrins restores microtubule nucleation at the centrosome. WT and YA cells were replated onto either fibrinogen (Fg) or fibronectin (Fn) in CCM1, SF F12 or SF F12 plus androgen, with androgen only added during the 5-min regrowth. *A*, Micrographs of representative cells stained for α-tubulin (red) and γ-tubulin (green). *B*, Quantification of the microtubule density at the centrosome. Values are the normalized fluorescence intensities of the α-tubulin signal ± S.D. calculated from 150 cells per condition (n=3). *$p<0.05$ compared to WT cells adhered to fibrinogen. Scale bar, 3 μm.

Figure S5. **MEK/ERK signaling is both required and sufficient to promote microtubule nucleation.** *A-B*, Time-lapse imaging was used to track newly nucleated microtubules in WT or YA cells replated onto fibrinogen in CCM1. Shown are images taken every 3 sec of EB3 comets emanating from the centrosome in a representative cell. *A*, MEK signaling was suppressed by treating WT cells stably co-expressing RFP-EB3 and GFP-γ-tubulin with U0126. *B*, YA cells stably expressing RFP-EB3 were co-transfected with GFP-centrin with or without ca-RAF to activate MEK signaling. Scale bar, 3 μm. *C*.

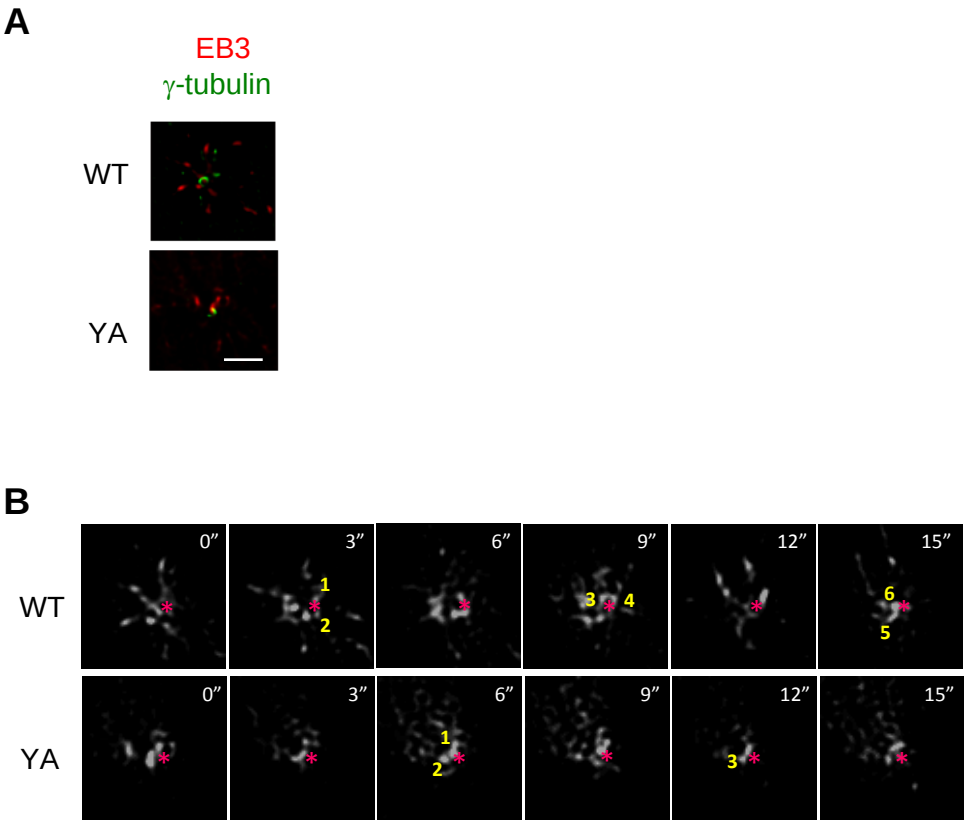
WT and YA cells transfected with GFP-centrin alone (WT and YA) or GFP-centrin + caRAF (YA+RAF) or cRAF alone (YA+RAF[1]) were replated onto fibrinogen-coated coverslips for 1 hr. Cell area for ~50 cells/sample was measured using NIS Elements software. Only transfected cells were analyzed. The box plot represents the data from 3 independent experiments. *p <0.05. Cells that are not spread have an area $\leq$ 600 μm^2 [1].

Figure S6. **Androgen promotes ERK activation in a Src-dependent manner.** WT cells were replated onto fibrinogen in SF F12 for 3 h. Src activation was inhibited by treating cells with the Src-kinase inhibitor, SU6656, for the last hour of incubation. Where indicated, cells were stimulated with androgen for only 5 min. ERK and Src activation were analyzed by Western blotting using phospho-specific antibodies. Blots were reprobed for total protein levels as loading controls.

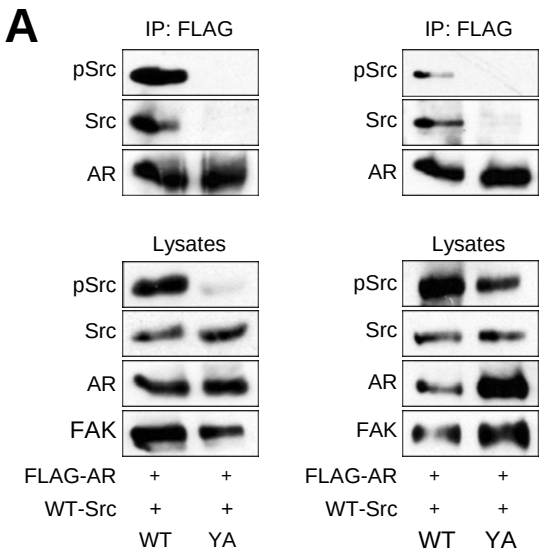
References

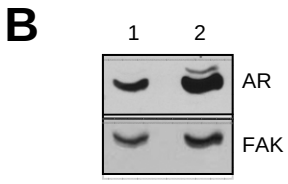
1. Berrier, A. L., Mastrangelo A. M., Downward J., Ginsberg, M. H., LaFlamme S.E. (2000) *J. Cell Biol.* **151**, 154901560.

Colello-Figure S1



Colello-Figure S2





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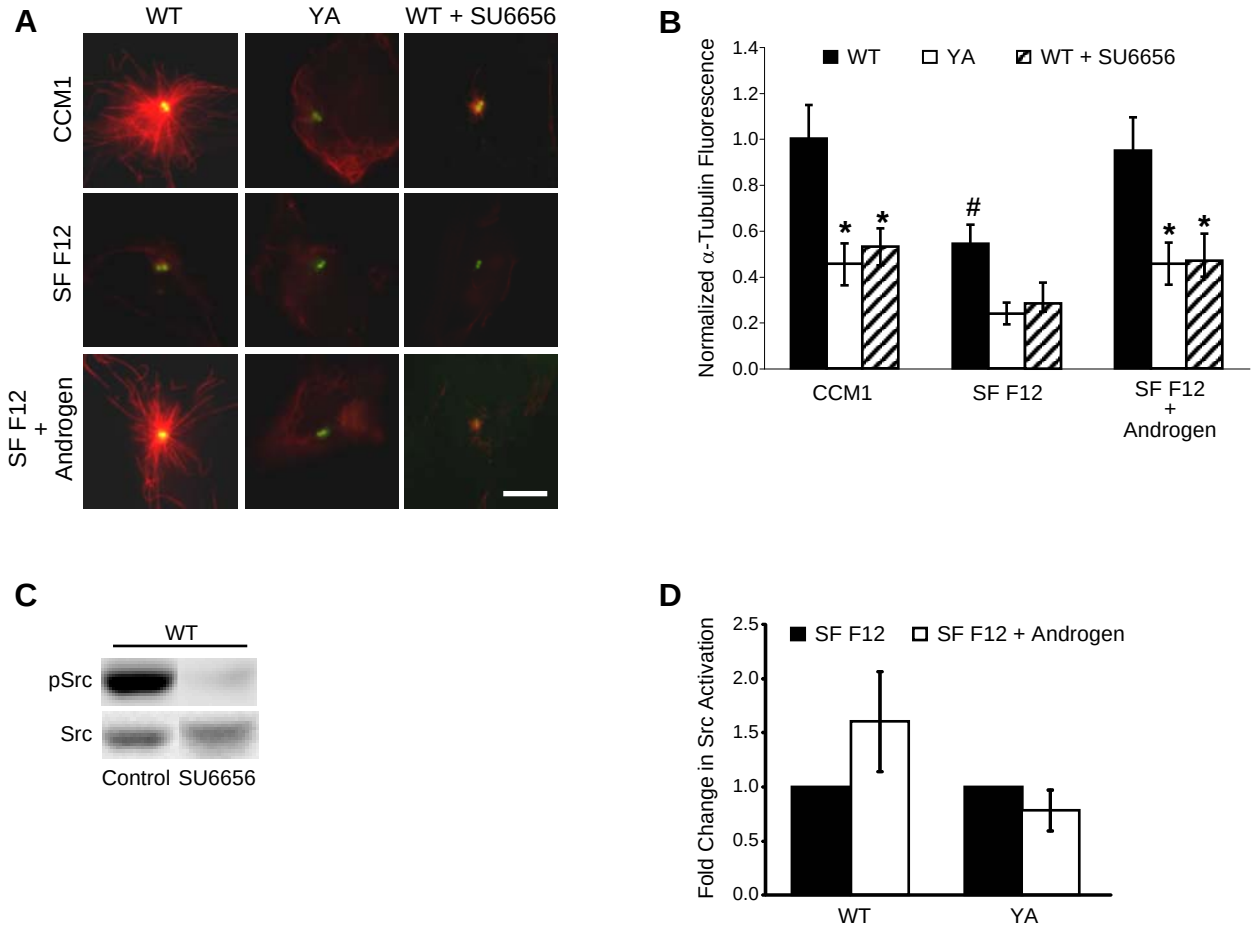
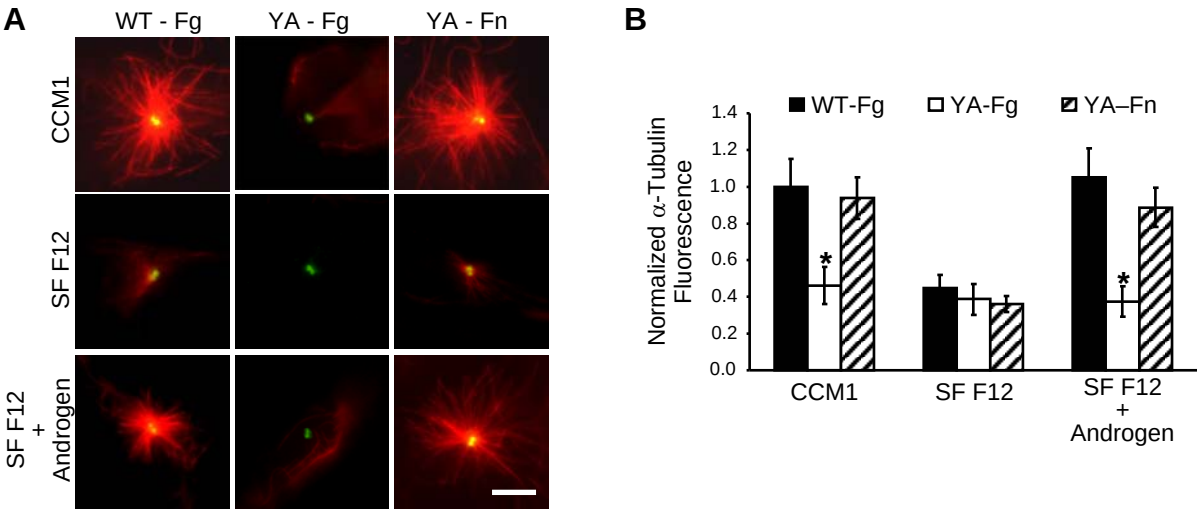
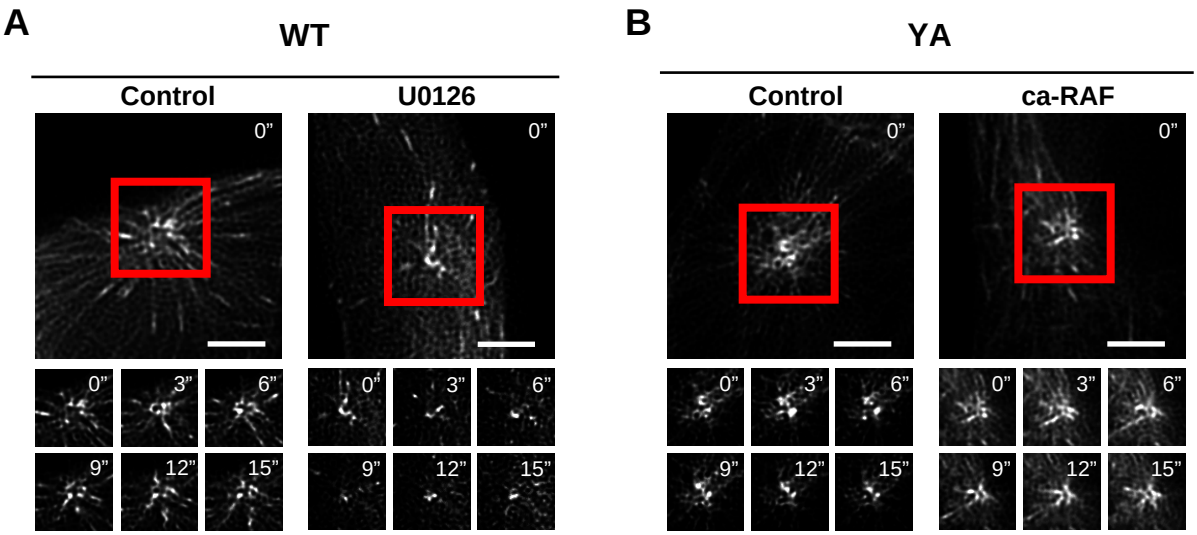
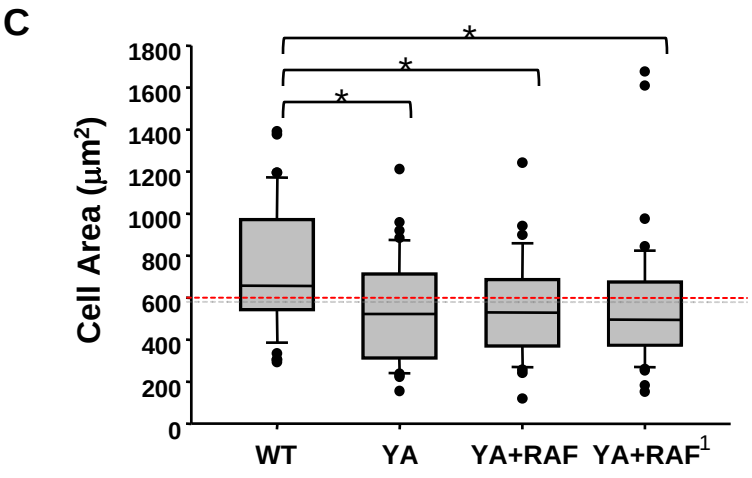


Colello-Figure S4



Colello-Figure S5





Colello-Figure S6