# Supplemental Materials

***Molecular Biology of the Cell***

Mana-Capelli et al.

**Figure S1.** Angiomotin family proteins localize to actin stress fibers through a conserved actin binding domain. (A) U2OS cells were transfected with plasmids for expression of amino acids 185-320 of AMOTL1 or 100-220 of AMOTL2 with or without the ABD sequence. Cells were stained for the Myc-tagged AMOTL1 and AMOTL2 using anti-Myc antibodies and for F-actin using phalloidin. Merged images with DNA in blue are shown. In all cases, nuclei were visualized with DAPI. Bar: 20 um.

**Figure S2.** (A) HEK293 cells were co-transfected with LATS2, its activators (MST1, MOB1, SAV) and Myc-tagged angiomotin constructs (AMOT130, AMOT130-(1-237), or AMOTL2-(1-307)) with or without the conserved LATS2 phosphorylation site mutated. LATS2 and each AMOT protein were immunoprecipitated on the same beads and immune-complex kinase assays were carried out ($^{32}$P). The levels of angiomotin proteins (Myc) and LATS2 (GFP) are shown. (B) Co-sedimentation of AMOT130 with actin. MBP-AMOT130 was incubated in the presence or absence of non-muscle F-actin and centrifuged. Pellets (P) were resuspended in a volume equal to the supernatant (S) and fractions were subjected to SDS-PAGE and Western blotting with antibodies against AMOT or actin.

**Figure S3.** AMOT130 mutants that cannot bind F-actin are more efficient at inhibiting YAP localization and activity and YAP overexpression outcompetes F-actin for AMOT130 binding. (A) AMOT-130 was co-expressed with LATS2 in U2OS cells, Cells were fixed and stained for AMOT130 (Myc) and endogenous YAP (note that the left cell is transfected and the untransfected cell on the right serves as a control). (B) Expression levels of selected AMOT130 constructs used in Figure 3A-B and S3A are shown. In addition, blots were also probed for endogenous YAP. All samples contained the same amount of total protein. (C) HeLa cells were transfected with the indicated AMOT130 plasmids, the 8xGTIIC-luciferase YAP-dependent promoter plasmid, and a plasmid with the SV40 promoter driving renilla luciferase. The next day, cell extracts were made and luciferase activity was measured for each sample. The levels of firefly luciferase (YAP activity) were normalized to the level of renilla luciferase in each sample. The experiment was done in triplicate, and the error bars indicate the standard deviation of the averages. Brackets on top of bars represent statistical significance (Student's test, $p<0.001$). (D) U2OS cells were transfected with the indicated AMOT130 and YAP2 plasmids and visualized after immuno-staining with FLAG (YAP2) and Myc (AMOT130). When indicated, DNA was stained with DAPI. Bar: 20 um.

**Figure S4.** Angiomotins and LATS are required to efficiently inhibit YAP after F-actin disturbance. (A) The effects of previously described treatments on the actin cytoskeleton are shown. Cells were treated with the same drug concentration and for the same time as used in Figure 5. All images of the same cell type were taken with the same exposure. (B) Knockdown levels for HEK293A and MCF10A cells are shown. We were unable to detect endogenous AMOT130 in MCF10A and endogenous AMOTL2 protein in HEK293A with the available antibodies. In separate experiments, the mRNA levels of AMOTL2 (HEK293A) and and AMOTL2, AMOTL1, and AMOT130 (MCF10A) were obtained by qPCR for control and triple knockdown cells (Triple kd). Bars represent the averages and standard deviation of three experiments. Values were normalized to GAPDH. (C) Example images of HEK293A cells treated with blebbistatin in Figure 4A are shown. (D) MCF10A cells stable for shRNA constructs targeting luciferase (control) or AMOTL2 were treated, stained, and scored, as described in Figure 5C. Bar: 20 um. N: nucleus. C: cytoplasm. (E,F) CTGF mRNA levels were determined for control cells or control and triple knockdown (Triple kd) cells after serum/growth factor withdrawal for HEK293A (E) and MCF10A (F) cells.

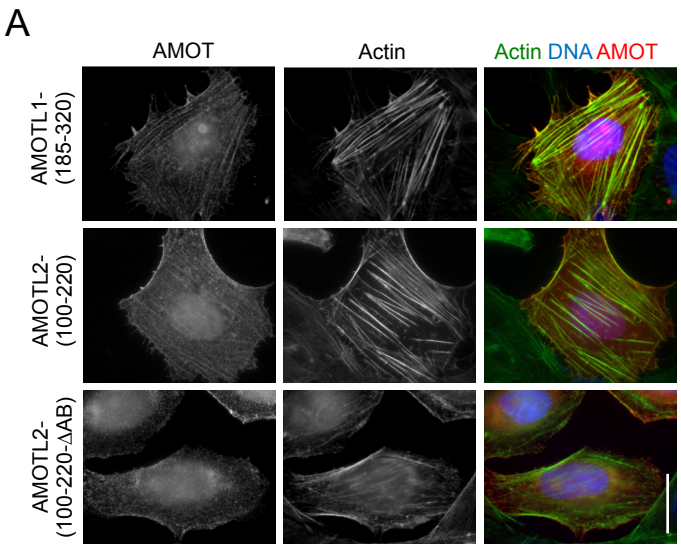
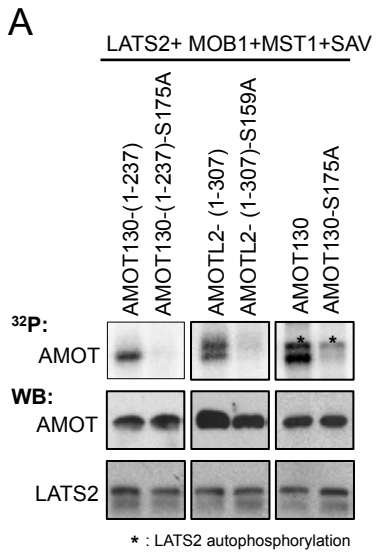

A
AMOT
Actin
Actin DNA AMOT
AMOTL1-
(185-320)
AMOTL2-
(100-220)
AMOTL2-
(100-220-ΔAB)

Figure S1


A
LATS2+ MOB1+MST1+SAV
AMOT130-(1-237)
AMOT130-(1-237)-S175A
AMOTL2- (1-307)
AMOTL2- (1-307)-S159A
AMOT130
AMOT130-S175A
32P:
AMOT
WB:
AMOT
LATS2
* : LATS2 autophosphorylation

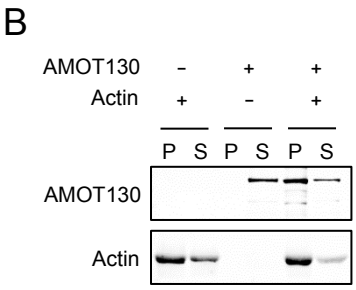

B
AMOT130 - + +
Actin + - +
P S P S P S
AMOT130
Actin

Figure S2

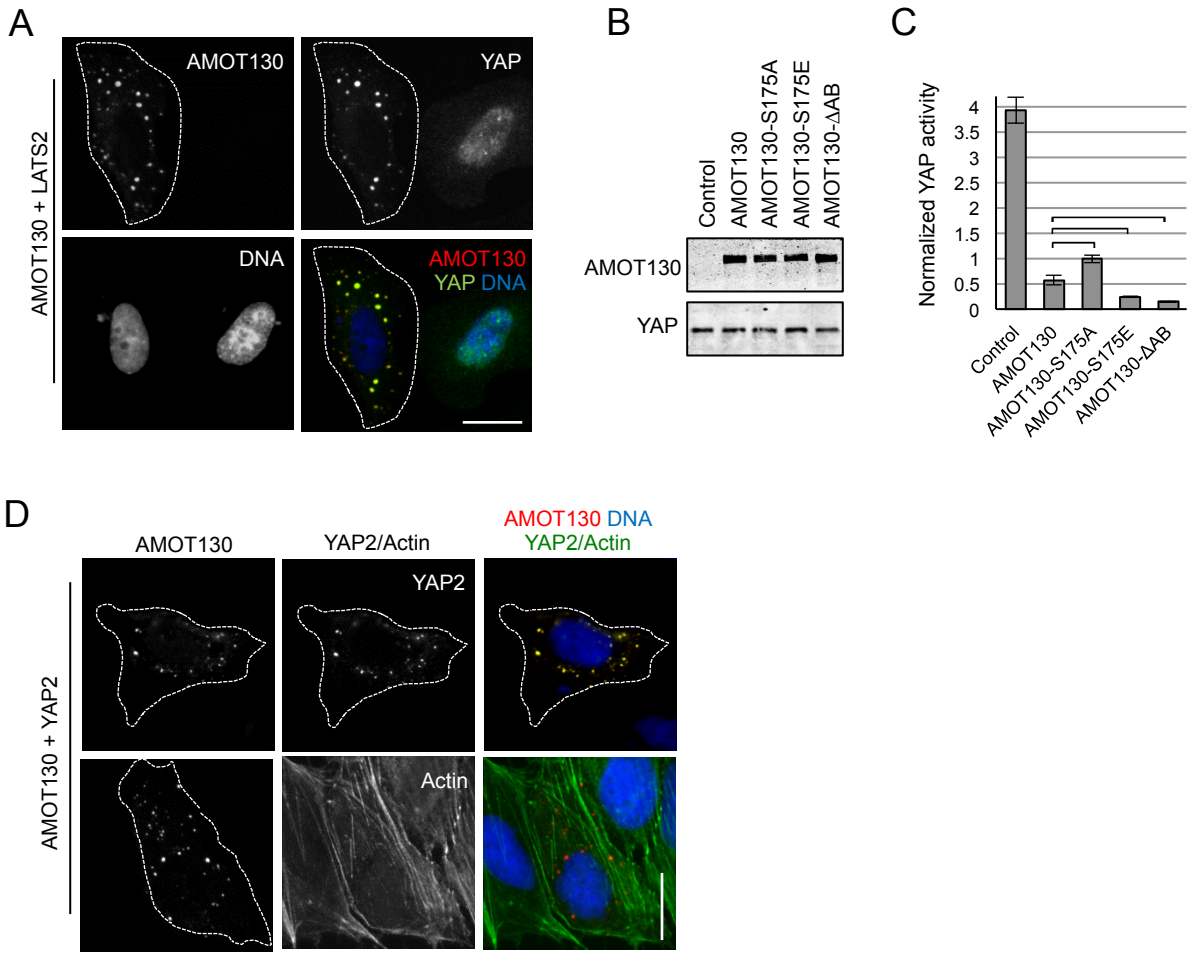

A
AMOT130
YAP
DNA
AMOT130
YAP DNA
AMOT130 + LATS2
B
Control
AMOT130
AMOT130-S175A
AMOT130-S175E
AMOT130-ΔAB
AMOT130
YAP
C
Normalized YAP activity
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
AMOT130
AMOT130-S175A
AMOT130-S175E
AMOT130-ΔAB
D
AMOT130
YAP2/Actin
AMOT130 DNA
YAP2/Actin
YAP2
Actin
AMOT130 + YAP2

Figure S3

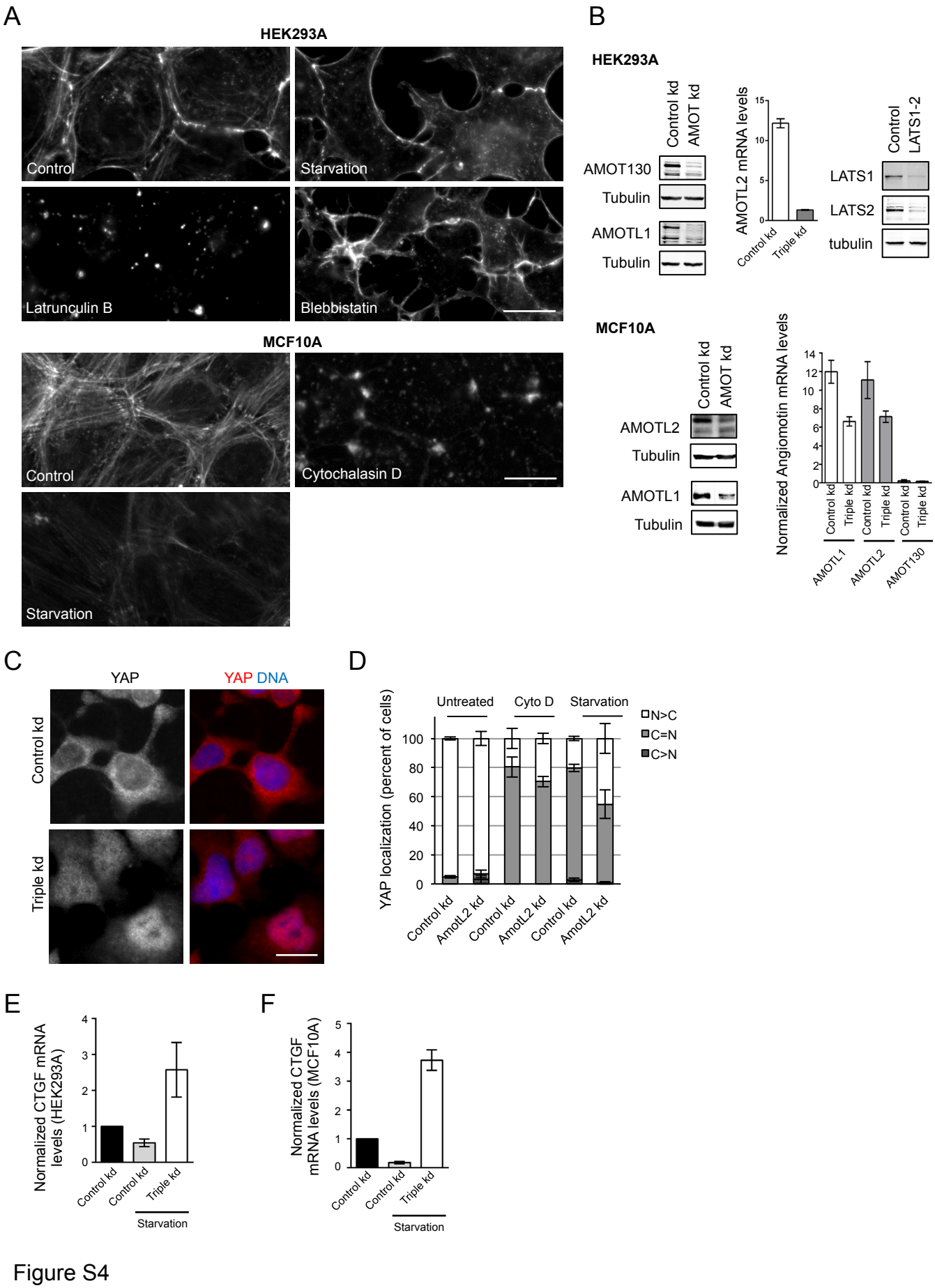

A
HEK293A
Control
Starvation
Latrunculin B
Blebbistatin
MCF10A
Control
Cytochalasin D
Starvation
B
HEK293A
Control kd
AMOT kd
AMOT130
Tubulin
AMOTL1
Tubulin
AMOTL2 mRNA levels
Control kd
Triple kd
Control
LATS1-2
LATS1
LATS2
tubulin
MCF10A
Control kd
AMOT kd
AMOTL2
Tubulin
AMOTL1
Tubulin
Normalized Angiomotin mRNA levels
Control kd
Triple kd
AMOTL1
AMOTL2
AMOT130
C
YAP
YAP DNA
Control kd
Triple kd
D
Untreated
Cyto D
Starvation
N>C
C=N
C>N
YAP localization (percent of cells)
Control kd
AmotL2 kd
E
Normalized CTGF mRNA levels (HEK293A)
Control kd
Control kd
Triple kd
Starvation
F
Normalized CTGF mRNA levels (MCF10A)
Control kd
Control kd
Triple kd
Starvation

Figure S4