Angiomotins stimulate LATS kinase autophosphorylation and act as scaffolds to promote Hippo signaling

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Figure S1

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Figure S1

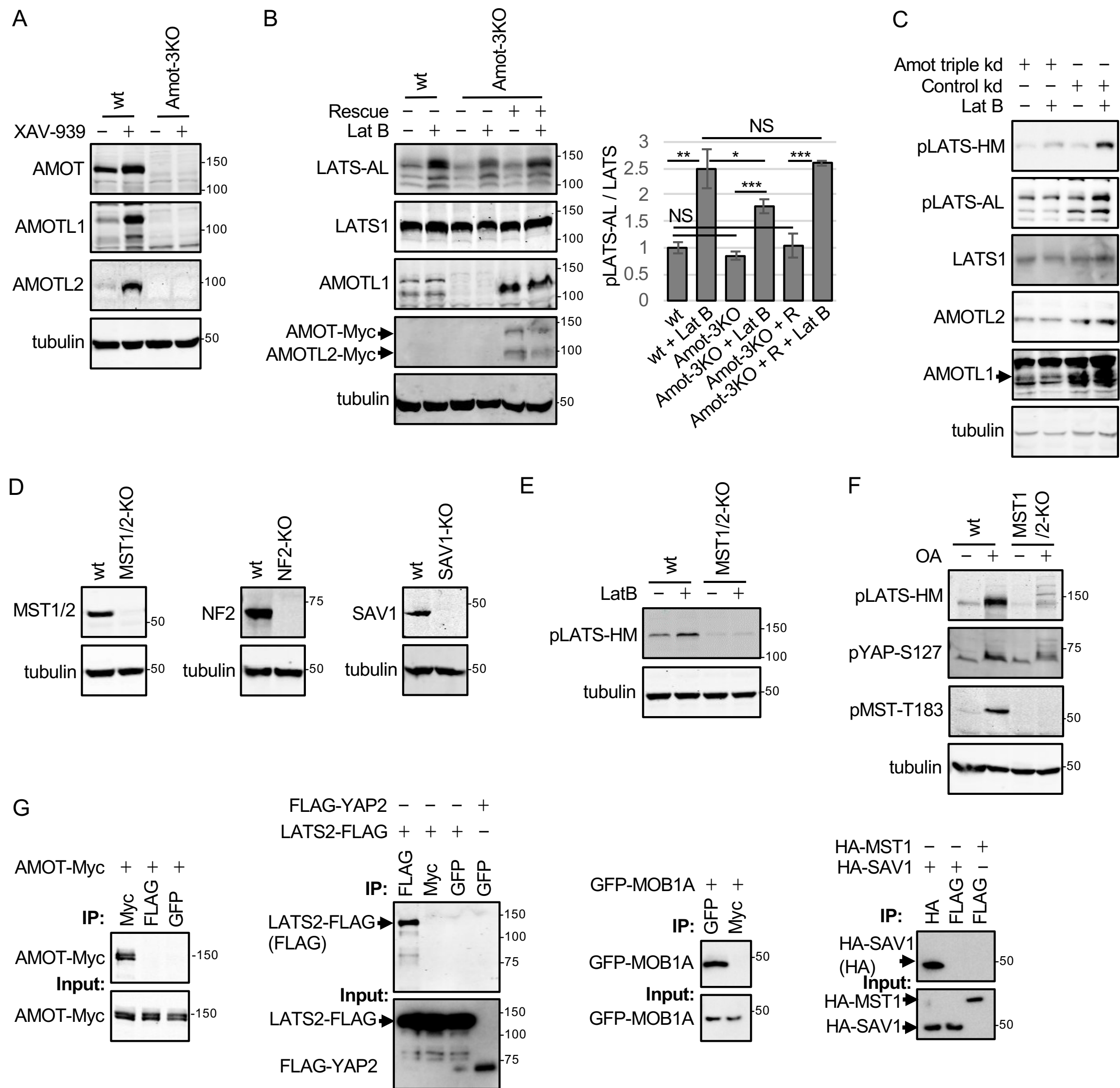

Figure S1. Characterization of knockout cell lines and epitope tag antibody cross-reactivity controls. A) Lysates from HEK293 (wt) or Amot-3KO HEK293 cells that had been incubated overnight with or without drug XAV-939 were analyzed by western blotting using antibodies against AMOT, AMOTL1, AMOTL2, and tubulin. (Note that XAV-939) was used to stabilize angiomotins for easier detection (1-4). B) HEK293, Amot-3KO HEK293, and Amot-3KO HEK293 cells that had been transfected with rescue plasmids (Amot-3KO +R) for expressing each of the angiomotins (AMOT, AMOTL1, AMOTL2) (Rescue) were treated with or without latrunculin B (Lat B). Lysates from these cells were analyzed by western blotting using antibodies against the LATS1/2 HM phosphorylation site, LATS1, Myc (AMOT-Myc, AMOTL2-Myc), AMOTL1, and tubulin. Quantification of LATS1/2-AL phosphorylation is shown. (Mean ± SD; n=3; NS=$P \geq 0.05$, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, T-test). C) HeLa cells were transfected with control siRNA, or siRNA against all three angiomotins (AMOT, AMOTL1, and AMOTL2) and treated or not treated with latrunculin B (Lat B). Cell lysates were analyzed by western blotting using antibodies against the LATS1/2 HM and AL phosphorylation sites, LATS1, AMOTL1, AMOTL2, and tubulin as indicated. We were unable to detect expression of AMOT in HeLa cells consistent with an earlier study (5). D) Lysates from HEK293 (wt) or CRISPR generated HEK293 lines with inactivated MST1 and MST2 (MST1/2-KO), NF2 (NF2-KO), and SAV1 (SAV1-KO) were analyzed by western blotting using antibodies against MST1/2, NF2, SAV1, and tubulin as indicated. E) Lysates from HEK293 (wt) or MST1/2-KO cells that had been treated or not treated with latrunculin B (Lat B) were analyzed by western blotting using antibodies against the LATS1/2 HM phosphorylation site, and tubulin as indicated. F) Lysates from HEK293 (wt) or MST1/2-KO cells that had been treated or not treated with Okadaic acid (OA) were analyzed by western blotting using antibodies against the LATS1/2 HM (pLATS1/2-HM), YAP S127 (pYAP S127), and MST1/2 T183 (pMST1/2 T183) phosphorylation sites, and tubulin as indicated. G) Various epitope tagged proteins used in co-immunoprecipitation studies were tested to ensure that they cannot be immunoprecipitated by antibodies against other epitope tags used in this study. The indicated epitope tagged proteins were expressed in HEK293 cells and then cell lysates were subjected to immunoprecipitation using the indicated antibodies and analyzed by western blotting using antibodies against their respective epitope tag. The levels of the tagged protein in the immunoprecipitates and the cell lysates (Input) are shown.

Figure S2

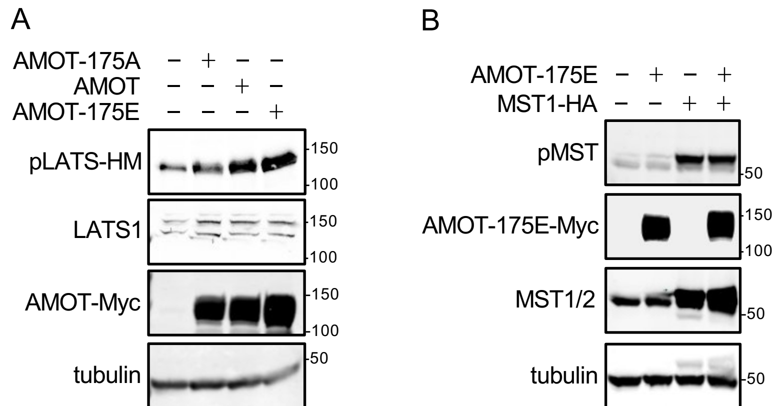

Figure S2. The phospho-mimetic S175E form of AMOT, which does not bind F-actin, promotes LATS1/2 but not MST1/2 activation. A) HEK293 cells were transfected with either a control plasmid or plasmids for expressing AMOT-Myc, AMOT-175A-Myc, or AMOT-175E-Myc as indicated. Cell lysates were analyzed by western blotting for LATS1/2-HM phosphorylation (pLATS1/2-HM), LATS1, Myc (AMOT-Myc), and tubulin levels. B) HEK293 cells were transfected with plasmids for expressing MST1-HA or AMOT-175E-Myc as indicated. Cell lysates were analyzed by western blotting to detect the levels of MST1/2 phosphorylation (pMST1) (note that this antibody detects phosphorylation on T183 and T180 in MST1 and MST2 respectively), Myc (AMOT-175E-Myc), MST1/2, and tubulin.

Figure S3

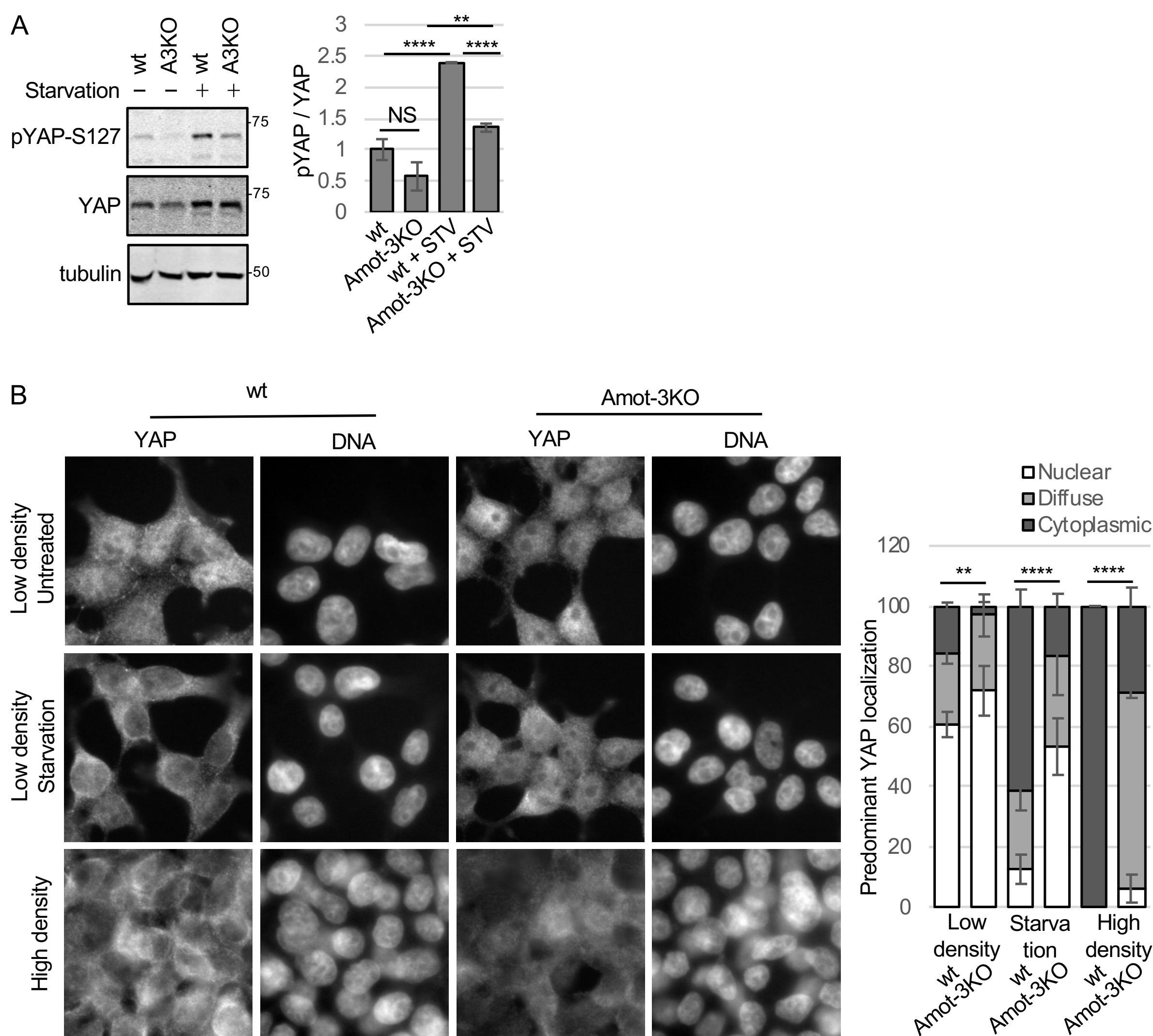

Figure S3. Angiomotins inhibit YAP nuclear localization after growth to high density of serum starvation. A) HEK293 (wt) and Amot-3KO HEK293 cells were grown to low density and left untreated or serum starved. Cells were analyzed by western blotting for YAP S127 phosphorylation (pYAP-S127), YAP, and tubulin levels. Quantification of YAP S127 phosphorylation relative to YAP levels is shown. STV: serum starved. (Mean ± SD; n=3; NS=P≥0.05, **P≤0.01, ****P≤0.0001, T-test). B) HEK293 (wt) and Amot-3KO HEK293 cells were grown to low density and left untreated or serum starved, or grown to high density. Cells were analyzed by immunofluorescence and stained for YAP and DNA. Individual cells were scored for whether YAP was predominantly in (Nuclear) or out (Cytoplasmic) of the nucleus or had similar concentrations in and out of the nucleus (Diffuse). (Mean ± SD; n=3 (100 cells each); **P≤0.01, ****P≤0.0001, T-test).

Figure S4

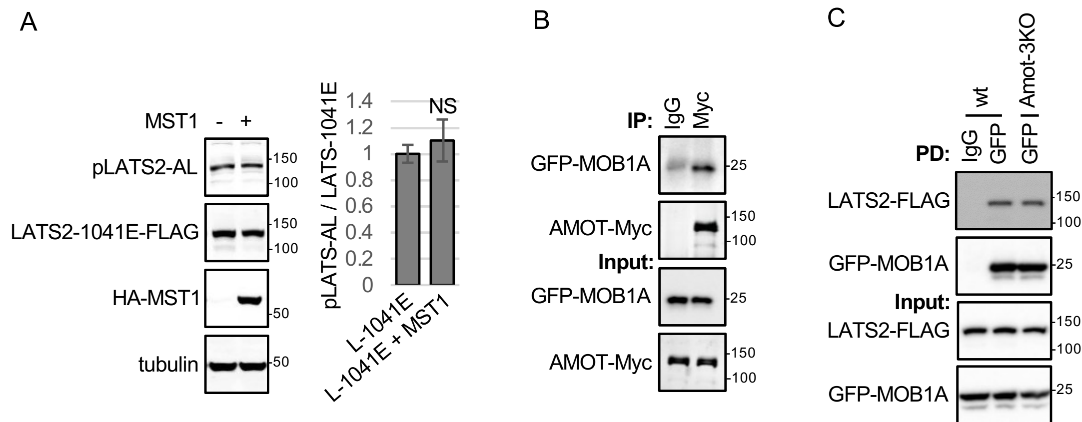

Figure S4. MST1 expression does not affect activation loop (AL) phosphorylation of a LATS2-T1041E mutant, and AMOT and MOB1 can be co-immunoprecipitated. A) HEK293 cells were transfected with a plasmid for expressing LATS2-1041E-FLAG and either a control plasmid or a plasmids for expressing HA-MST1 as indicated. Cell lysates were analyzed by western blotting for LATS2-AL phosphorylation (pLATS2-AL), FLAG (LATS2-1041E-FLAG), HA (HA-MST1), and tubulin levels. Quantification of LATS2-AL phosphorylation relative to LATS2-1041E-FLAG levels is shown. L-1041E: LATS2-1041E. (Mean ± SD; n=3; NS=P≥0.05, T-test). B) HEK293 cells were transfected with plasmids for expressing GFP-MOB1A and AMOT-Myc. Immunoprecipitations were performed on each cell lysate with either Myc (AMOT-Myc) or control (IgG) antibodies, and immune complexes and cell lysates were analyzed by western blotting for GFP-MOB1A and AMOT-Myc levels in immune complexes and cell lysates. C) HEK293 cells or Amot-3KO HEK292 cells were transfected with plasmids for expressing GFP-MOB1A and LATS2-FLAG. Immunoprecipitations were performed on each cell lysate with either GFP (GFP=MOB1A) or control (IgG) antibodies, and immune complexes and cell lysates were analyzed by western blotting for GFP-MOB1A and LATS2-FLAG levels in immune complexes and cell lysates.

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