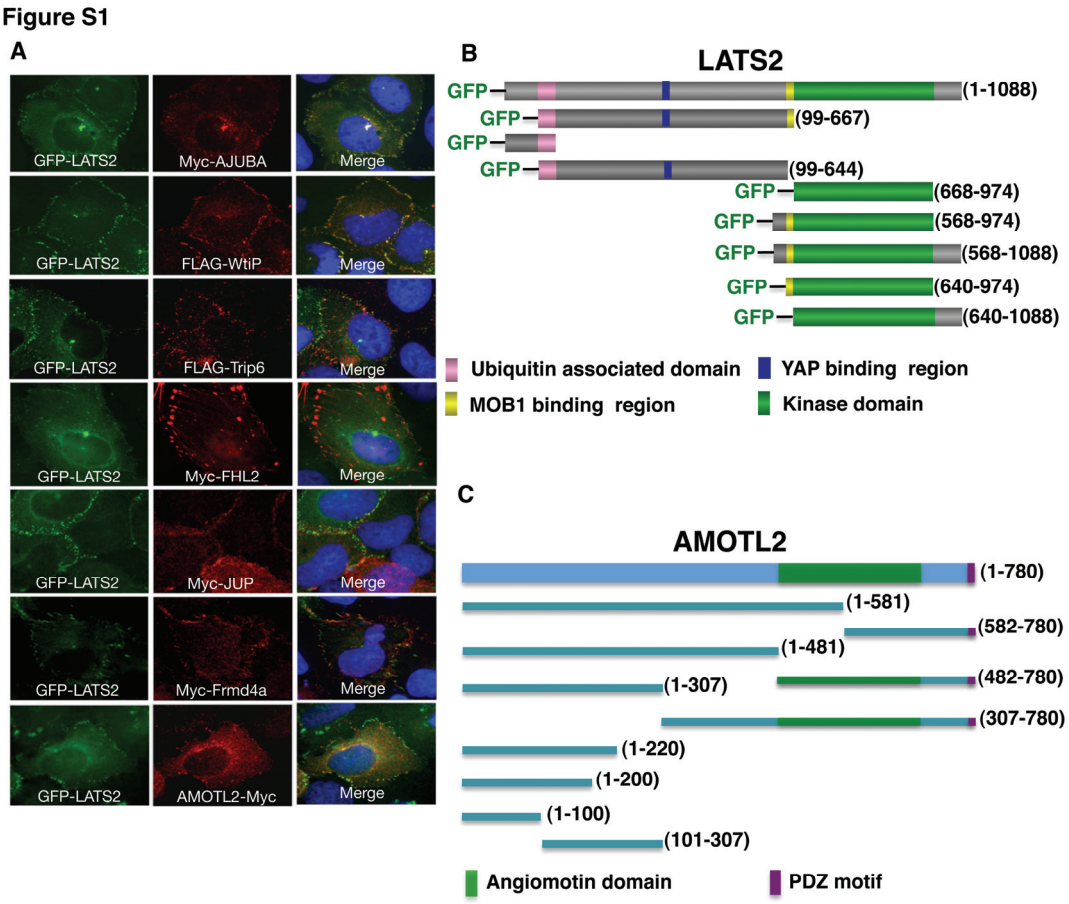

Figure S1. LATS2 Co-localizes at cell-cell contact sites with potential LATS2 binding partners.

(A) GFP-LATS2 was transfected into U2OS cells in combination with Myc or FLAG (red) tagged proteins as indicated, and cells were fixed and processed for immunofluorescence with anti-Myc or anti-FLAG antibodies. DNA was labeled with DAPI.

(B) A schematic representation of LATS2 mutant constructs used in this study. Predicted protein domains such as UBA, YAP binding region, MOB1 binding region, and kinase domains are shown.

(C) A schematic representation AMOTL2 mutant constructs used in this study. Predicted protein domains such as the angiomotin domain and PDZ motif are shown.

(D) Proteins identified in LATS2 purifications. The spectrum counts and percent sequence coverage for proteins identified in LATS2 complexes by mass spectrometry, which co-localize with LATS2 at sites of cell-cell contact are listed.

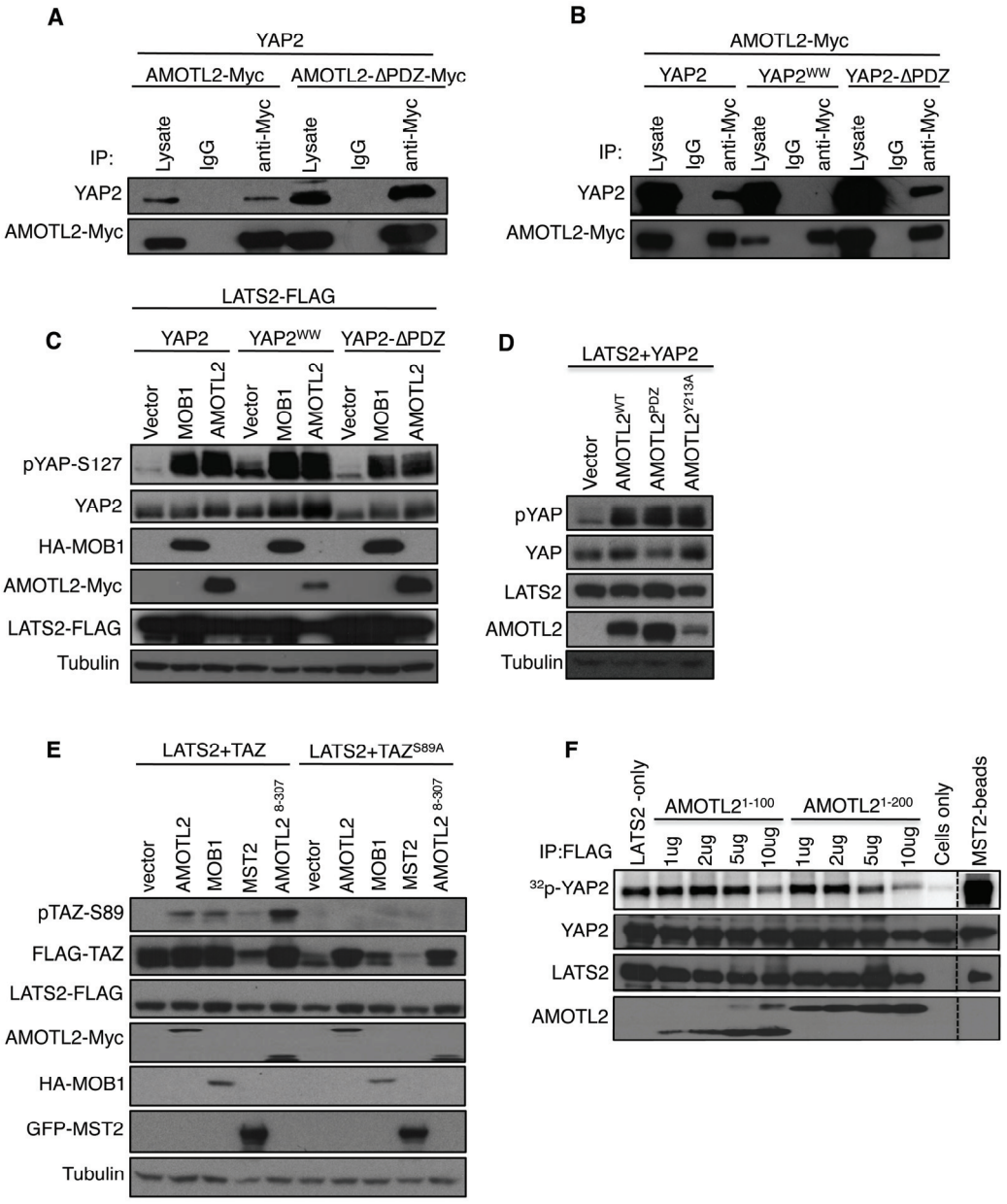

Figure S2. AMOTL2 interacts with YAP2 and promotes phosphorylation of YAP2 and TAZ. (A) YAP2 was co-expressed with AMOTL2-Myc or a version of AMOTL2 lacking the C-terminal PDZ domain (AMOTL2-ΔPDZ-Myc) in HEK 293 cells. Cell lysates were immunoprecipitated with anti-Myc or IgG antibody as indicated and analyzed by western blotting.

(B) AMOTL2-Myc was co-expressed either with YAP2 or YAP2 mutants lacking the PDZ domain (YAP2-ΔPDZ) or with inactivating mutations in the WW domains ($YAP2^{WW}$) in

HEK 293 cells as indicated. Cell lysates were processed for immunoprecipitation with anti-Myc or IgG antibodies as indicated and analyzed by western blotting.
(C) Wild type and different YAP2 mutants described in (B) were transfected in HEK 293 cells along with LATS2-FLAG and either vector control, MOB1, or AMOTL2 as indicated, and subjected to western blot analysis for pYAP-S127 levels. Blots were reprobed for the indicated proteins.
(D) YAP2 and LATS2-FLAG were transfected along with either vector control, AMOTL2-Myc, AMOTL2-ΔPDZ-Myc or AMOTL2^{Y213A}-Myc into HEK 293 cells and subjected to western blot analysis for pYAP-S127 levels. Blots were reprobed for the indicated proteins.
(E) LATS2-FLAG and FLAG-TAZ or FLAG-TAZ^{S89A} were co-transfected with the indicated plasmids in HEK 293 cells. Cell lysates were prepared and subjected to western blot analysis for detection of pTAZ^{S89} using anti-pYAP-S127 antibody, which can recognize the Ser89 phosphorylation site because of sequence conservation (Lei *et al.*, 2008). Note that reduction of TAZ and corresponding pTAZ levels in MST2 overexpressing samples is consistent with results showing that hyper-phosphorylated TAZ becomes degraded (Zhao *et al.*, 2010).
(F) Lysates of HEK 293 cells expressing LATS2-FLAG alone were immunoprecipitated with FLAG antibody and bound to beads. GST-AMOTL2 aa1-100 and 1-200 purified from *E.coli* were added to LATS2 beads in increasing concentration along with GST-YAP2, and processed for in vitro kinase assays as described in 3A. EGFP-MST2 bound beads mixed with LATS2-FLAG beads were used as a positive control. Simultaneously, equal volumes of kinase assay samples were subjected to western blot analysis to detect the levels of LATS2-FLAG GST-YAP2 and GST-AMOTL2 . The dashed line indicates a gap where non-relevant lanes were excised.

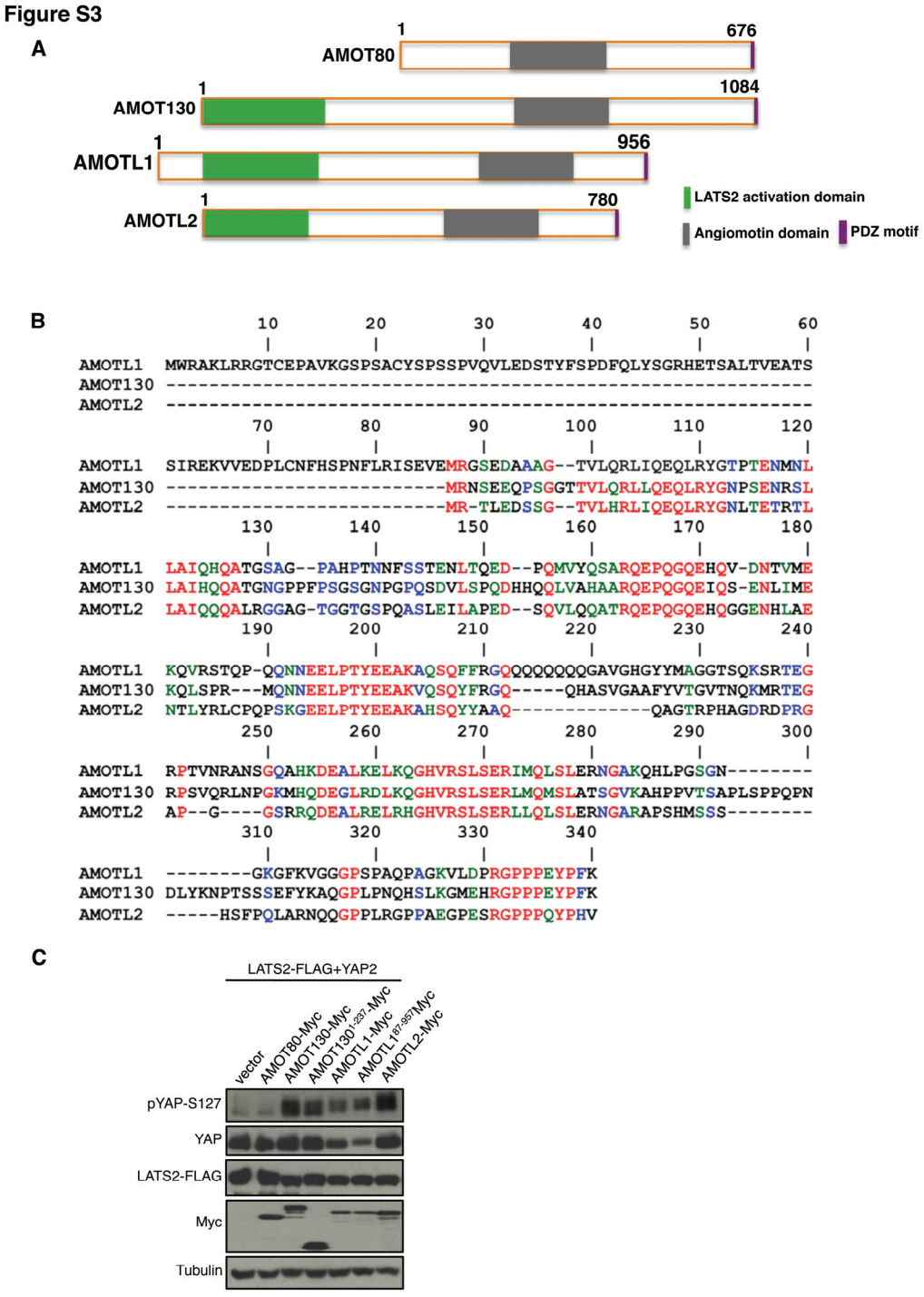

Figure S3. AMOT130 shares homology with AMOTL1 and AMOTL2 in the N-terminal domain. (A) A schematic diagram of AMOT80, AMOT130, AMOTL1, and AMOTL2 proteins is shown. The angiomotin, and C-terminal PDZ domains are

conserved in all four proteins. The N-terminal LATS2 activation domain is found in AMOT130, AMOTL1, and AMOTL2, but not in AMOT80.
(B) Amino acid sequence alignment of the N-terminal regions of AMOT130, AMOTL1 and AMOTL2 that are involved in LATS2 activation was generated using CLUSTALW and conserved amino acids are color coded according to the CLUSTALW program.
(C) LATS2-FLAG, YAP2, and either vector control, full-length AMOT80, AMOT130, AMOTL1, AMOTL2, the first 237 amino acids of AMOT130 ($AMOT130^{1-237}$), or AMOTL1 lacking the first 86 amino-acids of AMOTL1 ($AMOTL1^{87-957}$) were co-expressed in HEK 293 cells. Cell lysates were processed and subjected to western blot analysis for pYAP-S127 levels and the levels of the various proteins indicated.

Table S1

Proteins	Spectrum count	(%) Sequence coverage
RCN2	150	49.8
YAP	10	15.7
AJUBA	4	14.1
FHL2	5	14.3
PTPN14	4	14.1
AMOTL2	4	9.7
FMRD4A	4	3.2
WTIP	3	4.4
JUP	9	6.7