Expanded View Figures

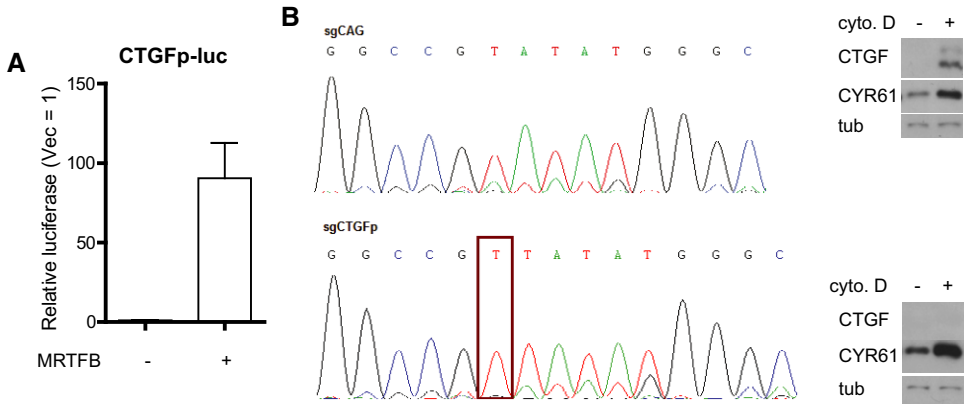

Figure EV1. SRF activity-independent regulation of TEAD-YAP target genes by MRTF.

A CTGF luciferase reporter assay in 293T cells expressing MRTFB. Data are presented as means ± s.e.m. (n = 3).

B (left) Genomic sequencing results for MCF-10A cells treated with CRISPR-Cas9 against control (sgCAG) or SRF-binding sequences in the CTGF promoter. A successful mutation in the SRF-binding motif (CArG box) is outlined in red. (right) Western blot confirming CTGF promoter mutant MCF-10A cells are unresponsive to SRF by treating them with cytochalasin D (cyto. D, 1 μM, 30 min).

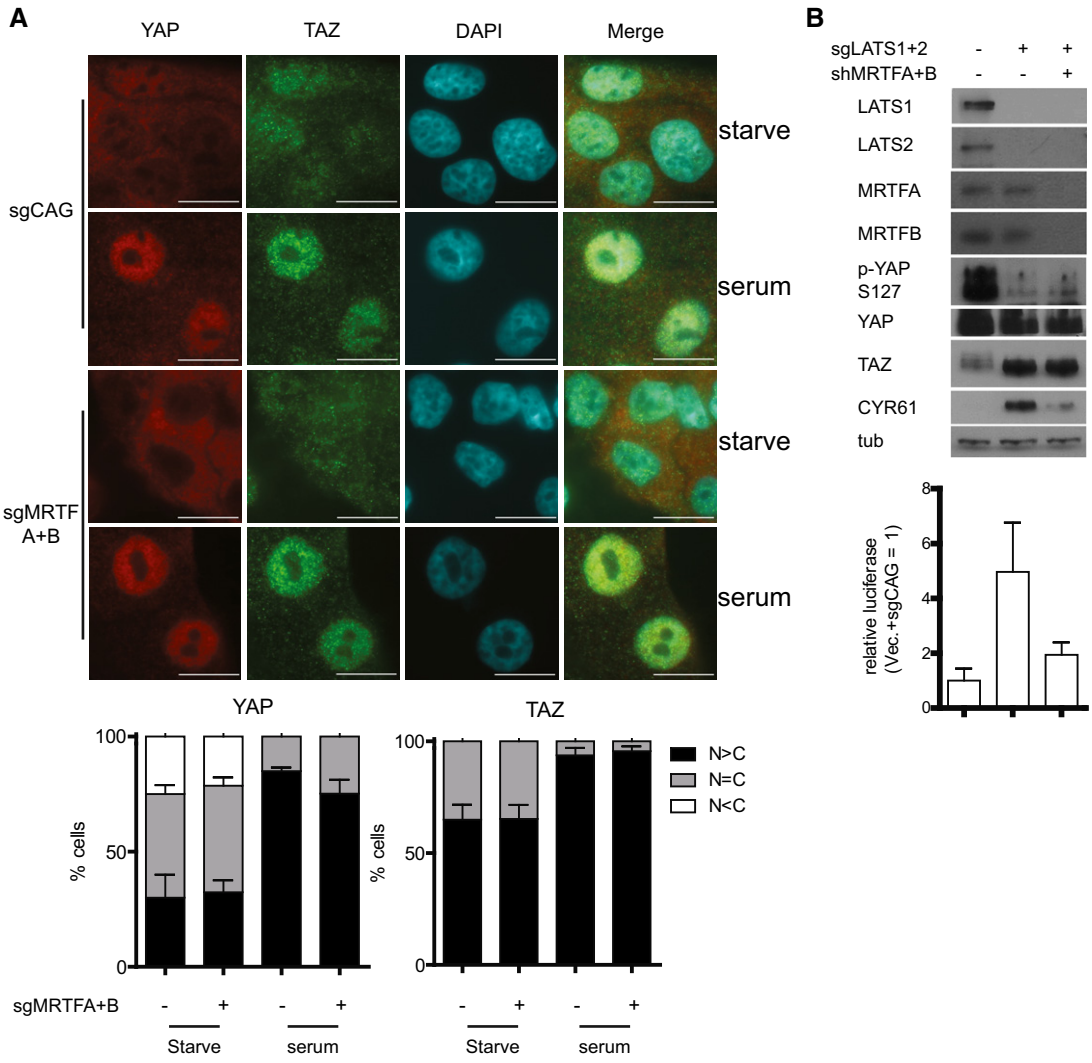

Figure EV2. MRTF-mediated regulation of TEAD-YAP activity is independent of LATS.

A Control (sgCAG) or MRTF-knockout (sgMRTFA+B) MCF-10A cells were serum-starved for 24 h and stimulated with complete medium for 1 h. Then, YAP and TAZ localization was examined with immunofluorescence. Quantifications for YAP (left) and TAZ (right) are shown below (n = 3, Scale bars: 10 μm).

B Western blots and TEAD-YAP luciferase reporter assays with control (sgCAG) or LATS1/2-knockout (sgLATS1 + 2) 293T cells were treated with shRNAs against MRTFA and MRTFB (n = 3).

Data Information: Data are presented as means ± s.e.m.

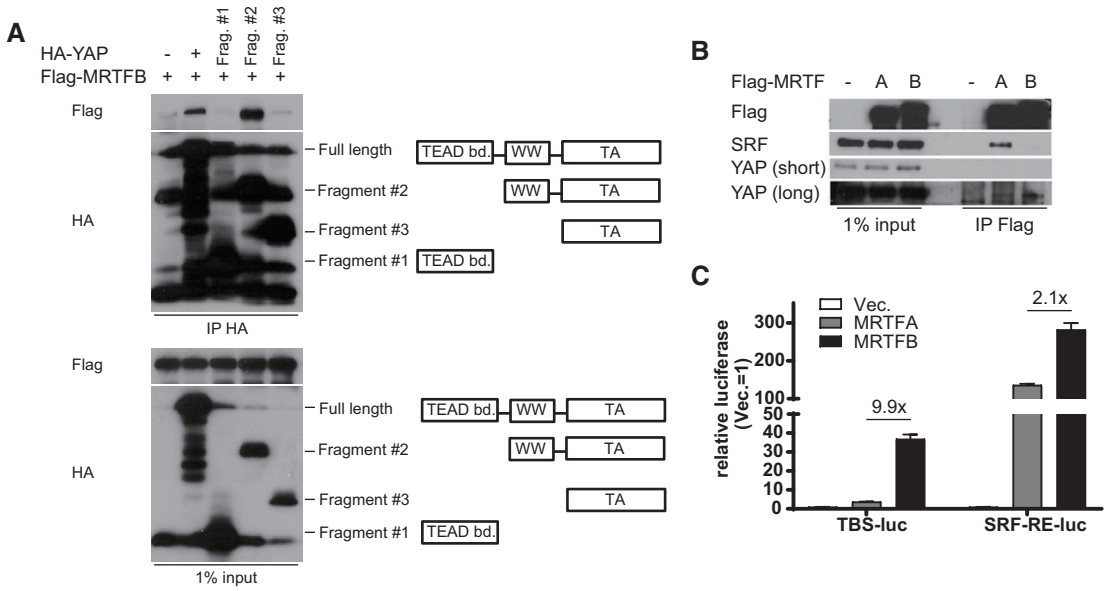

Figure EV3. Characterization of MRTF binding to YAP.

A Co-IP experiment with MRTFB and series of truncation mutants of YAP.

B Co-IP experiment with MRTFA or MRTFB with endogenous SRF and YAP. Note that MRTFA and MRTFB have different preferential binding partner.

C TEAD-activity reporter (TBS-luc) and SRF-activity reporter (SRF-RE-luc) assay with 293T cells expressing MRTFA or MRTFB (n = 3). Data are presented as means ± s.e.m.

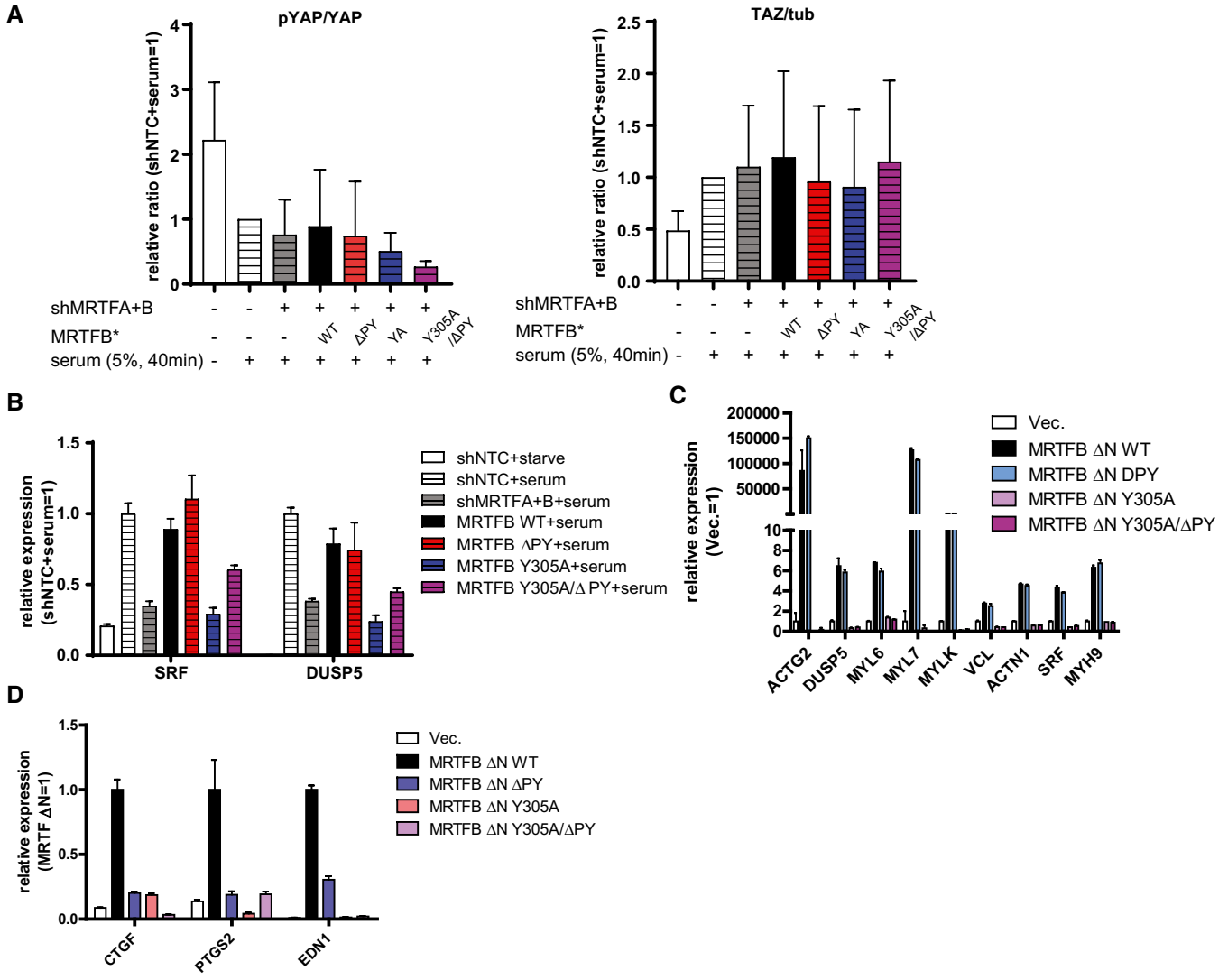

Figure EV4. Regulation of TEAD-YAP and SRF-MRTF targets by MRTF mutants.

A Quantifications of blot in Fig 4C (n = 4).

B, C qPCR analysis for selected SRF-MRTF targets with cells generated in (B) Fig 4C and (C) Fig 4F (n = 3).

D qPCR analysis for selected TEAD-YAP target genes in MDA-MB-231 cells expressing the indicated MRTFB mutant (n = 3).

Data Information: Data are presented as means ± s.e.m.

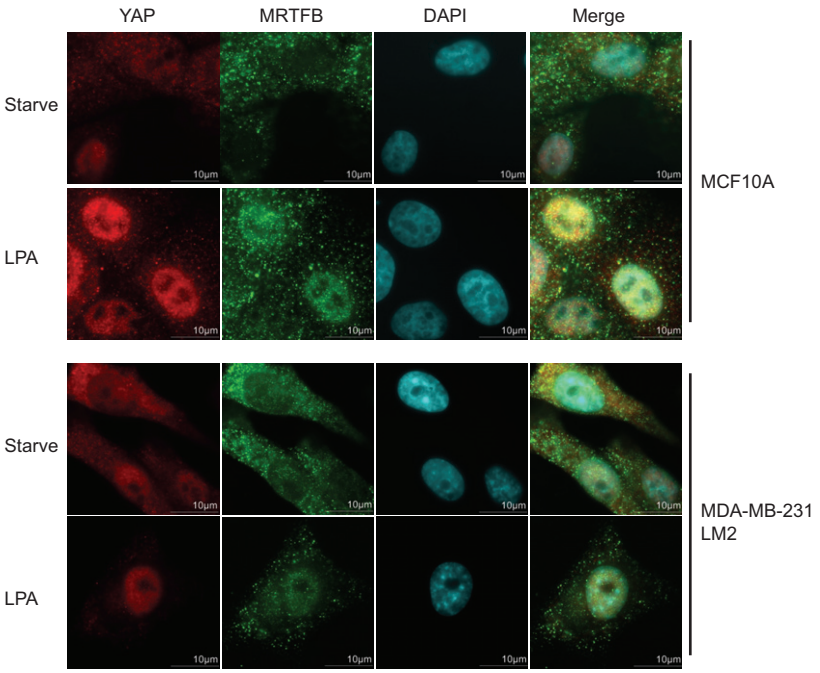

Figure EV5. LPA induces nuclear co-localization of YAP and MRTF.
Representative images of the indicated cell lines before and after LPA treatment.

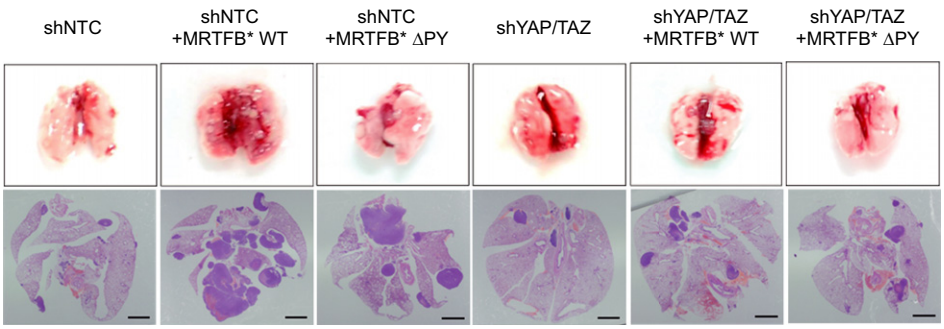

Figure EV6. Representative images of *in vivo* metastasis assay in Fig 6J.
Scale bars: 1 mm.

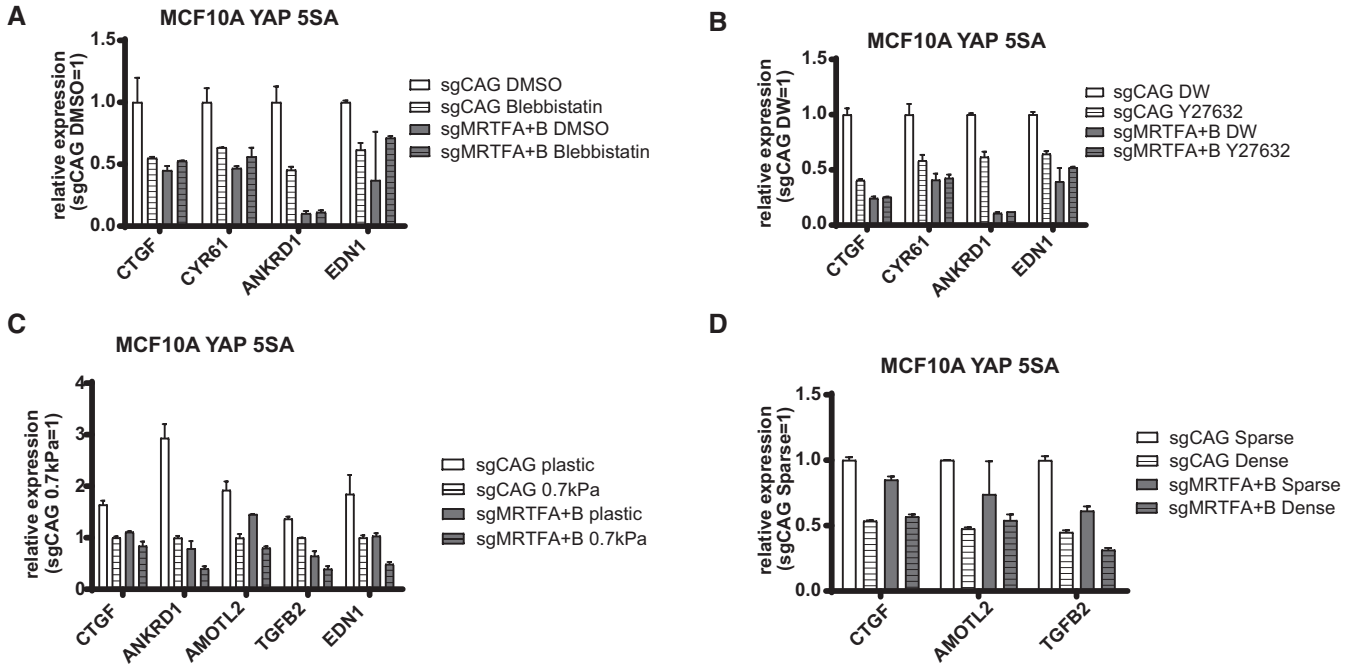

Figure EV7. MRTF mediates LATS-independent acute mechanical regulation of YAP activity.

A–D qPCR analysis of representative TEAD-YAP target genes for MCF-10A cells expressing YAP 5SA with sgRNA against control (sgCAG) and MRTFA and MRTFB (sgMRTFA+B) treated with (A) blebbistatin or solvent (DMSO), (B) Y27632 or solvent (distilled water, DW), (C) hard (tissue culture plastic) and soft (0.7 kPa) matrix, (D) sparse/dense cell density. Data are presented as means ± s.e.m. (n = 2 for each figure).