Figure EV1. USP9X knockdown impairs cell proliferation in a YAP-dependent manner.
RPE cells were transduced with vector (control) or Flag-YAP S5A retroviruses, and then transfected with control or USP9X-targeting siRNAs. Cells were quantified three days after siRNA transfection and normalized with respect to control siRNA-transfected cells. USP9X knockdown impaired cell proliferation in control cells, but not in Flag-YAP S5A-expressing cells (n = 4). Error bars indicate the SEM (compared with siControl cells; **P < 0.01, paired Student’s t-test).

Figure EV2. RhoA activity is not affected by USP9X depletion.
Sparsely cultured RPE cells were transfected with the indicated siRNAs, harvested, lysed and incubated with 5 μg of GST-Rhotekin pre-bound to 20 μl of GST beads. After incubation for 1 h at 4°C, the beads were collected and the bound proteins were analyzed by Western blotting.

Figure EV3. Analysis of the AMOTL2 K408R mutant.
RPE or MCF10A cells were transduced with Flag-AMOTL2 WT or the K408R mutant and immunoprecipitated with an anti-Flag antibody. Immunoprecipitated and WCL samples were analyzed by Western blotting for the indicated proteins.
Figure EV4. Interaction between full-length AMOTL2 and LATS2 WT or ΔUBA mutant. 293T cells were transfected with the indicated combinations of DNAs and subjected to co-immunoprecipitation.

Figure EV5. Interaction between USP9X and YAP.
A. 293T cells were transfected with the indicated combinations of DNAs and subjected to co-immunoprecipitation with the indicated antibodies.
B. Densely cultured RPE or MCF10A cells were lysed and subjected to co-immunoprecipitation with the indicated antibodies.