Expanded View Figures

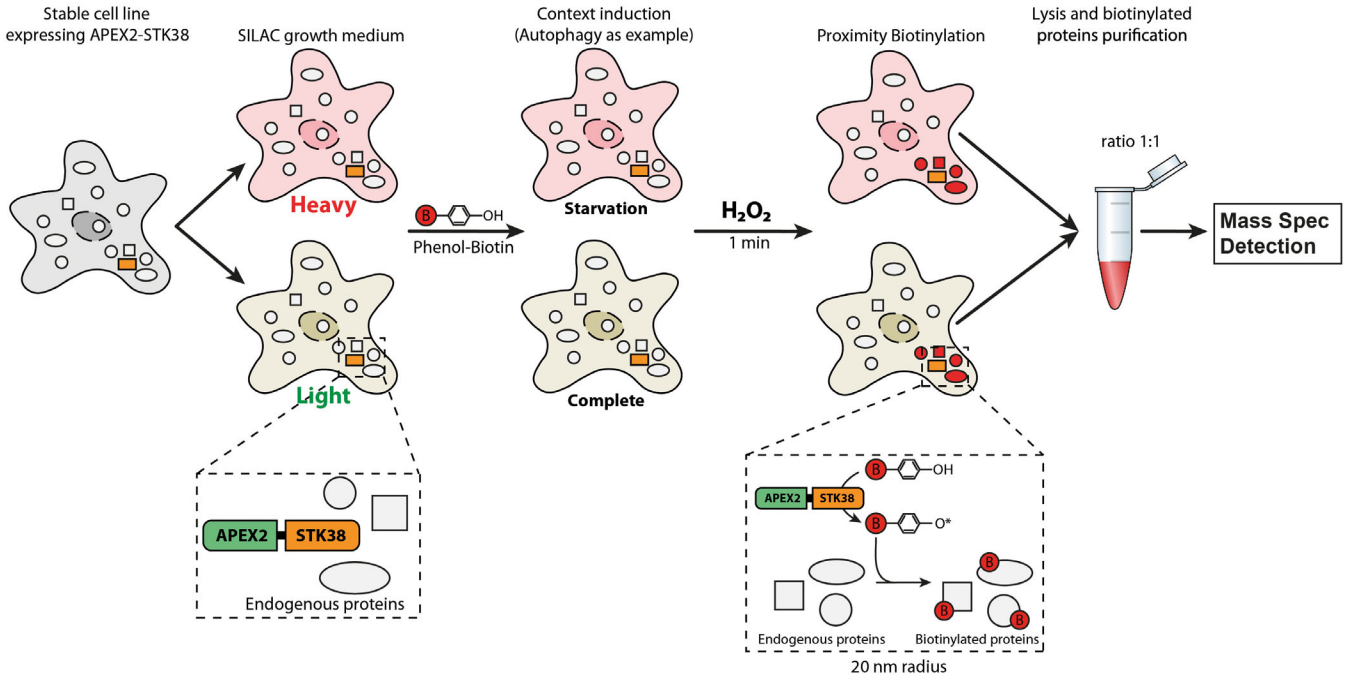

Figure EV1. STK38 partner identification strategy (related to Fig 1).
Figure indicating the strategy used to identify STK38 partner association dynamic depending on the context (example is shown for autophagy condition here). Stable cell lines expressing the fusion construct APEX2-STK38 were generated and then subdivided for amino acid replacement (SILAC). Context was then induced (4 h of EBSS incubation for autophagy induction and suspension growth) as well as a control condition (nutrient-rich medium incubation and attached growth, respectively). Proximity labeling of STK38 partners was performed as described [56]: Briefly, cells were incubated with phenol–biotin for a minimum of 30 min followed by H_2O_2 incubation for 1 min precisely. Finally, biotinylated proteins (=STK38 partners) were purified from whole-cell lysates using streptavidin-coated magnetic beads and subjected to mass spectrometry identification.

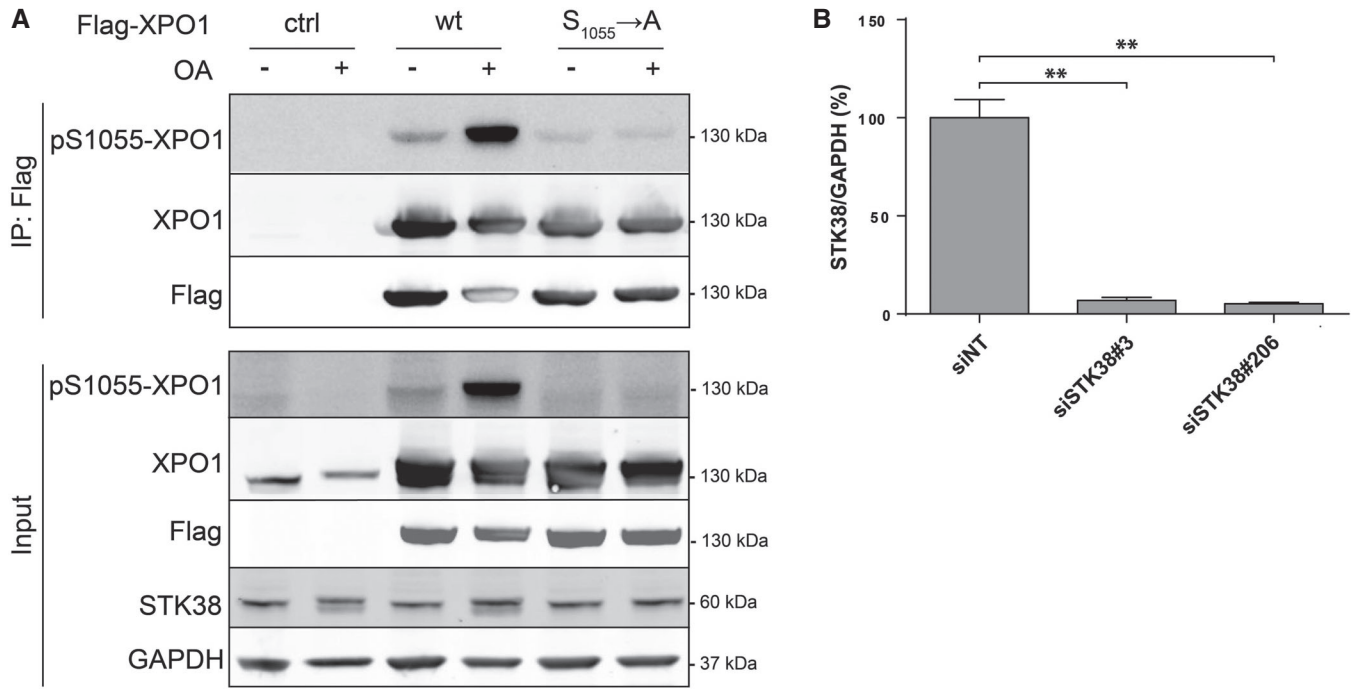

Figure EV2. Validation of pS1055_XPO1 antibody (related to Fig 4).

A HeLa cells were transiently transfected with the indicated Flag-XPO1 mutants expressing plasmids (wt or S1055A mutation) or without DNA. The next day, cells were treated with 1 μM OA or with vehicle (DMSO) for 1 h. Flag fusions were immunoprecipitated, and pulled-down proteins were analyzed by Western blotting. The upper panel displays immunoprecipitated proteins, and the lower panel represents whole-cell lysates.

B Graphical representation of STK38 protein level for experiment presented in Fig 4A and B (n = 3 independent experiments, mean ± SEM; $^{**}P < 0.01$, Mann–Whitney test).

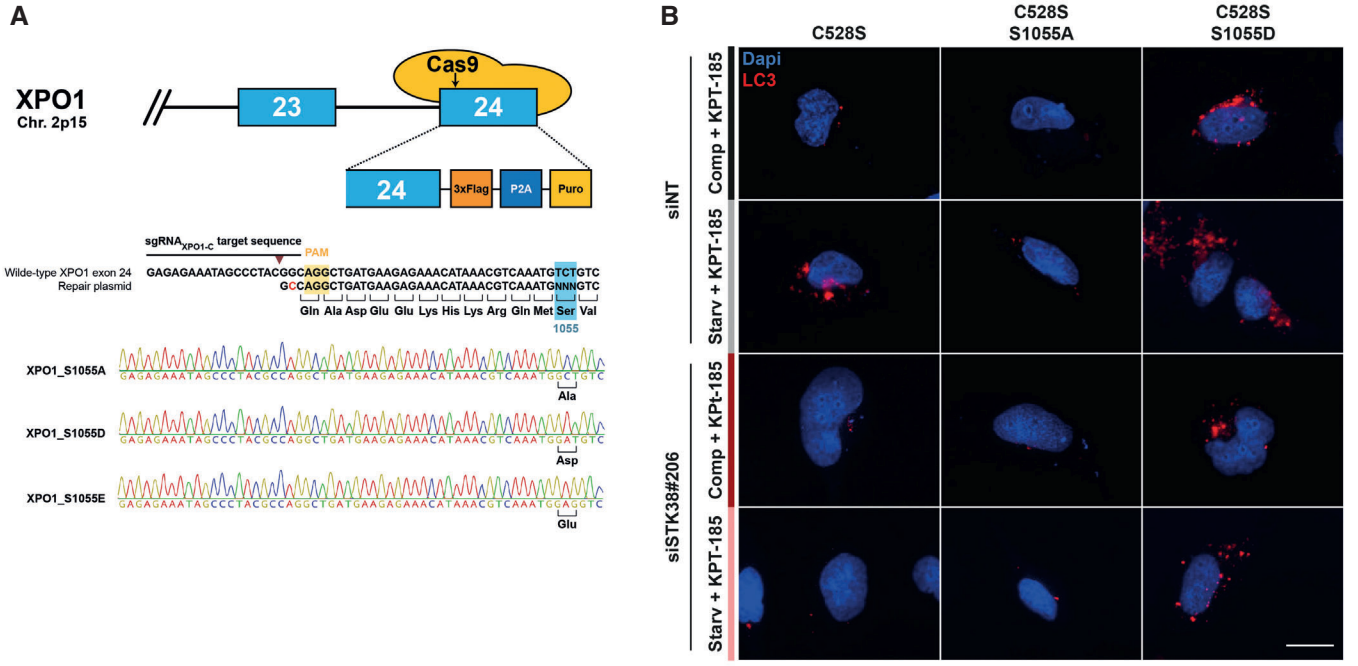

Figure EV3. CRISPR/Cas9 knock-in HAP1 cell lines and representative images of LC3 dots (related to Fig 5).

A Schematic overview of CRISPR/Cas9 genome editing of XPO1 in HAP1 cell lines. Exons are shown as blue boxes and introns are visualized as a thick black line, while Cas9 is represented by the yellow oval shapes. The DNA sequence and corresponding amino acid of the region around the Ser1055 residue are enlarged. The Cas9 PAM sequence is indicated in yellow, the sgRNA sequence is highlighted by a black line above the DNA sequence, and the Cas9 cutting site is indicated by the brown arrowhead. The wild-type sequence is shown together with the sequence of the donor repair plasmid used to generate the S1055 mutations, and the additional silent mutation to prevent recutting of the mutagenized gene is marked in red. Sanger sequencing chromatograms of the generated XPO1 S1055 mutant cell lines are shown with the desired mutations highlighted in bold.

B HEK-HT-iRFP-LC3 cells were transiently transfected with siRNA targeting endogenous STK38 (or with non-targeting siRNA (siNT)). After 2 days, cells were transiently transfected with the indicated Flag-XPO1 mutant plasmids. Twenty-four hours later, cells were incubated with DMEM or EBSS for 4 h, both supplemented with KPT-185 (final concentration = 1 μM) in order to inhibit endogenous XPO1 activity and with chloroquine (final concentration = 10 μM). Cells were then fixed, and the number of iRFP-LC3 dots per cell was recorded only in cells positive for Flag-XPO1 mutants. Representative images are shown, and scale bars are 20 μm.

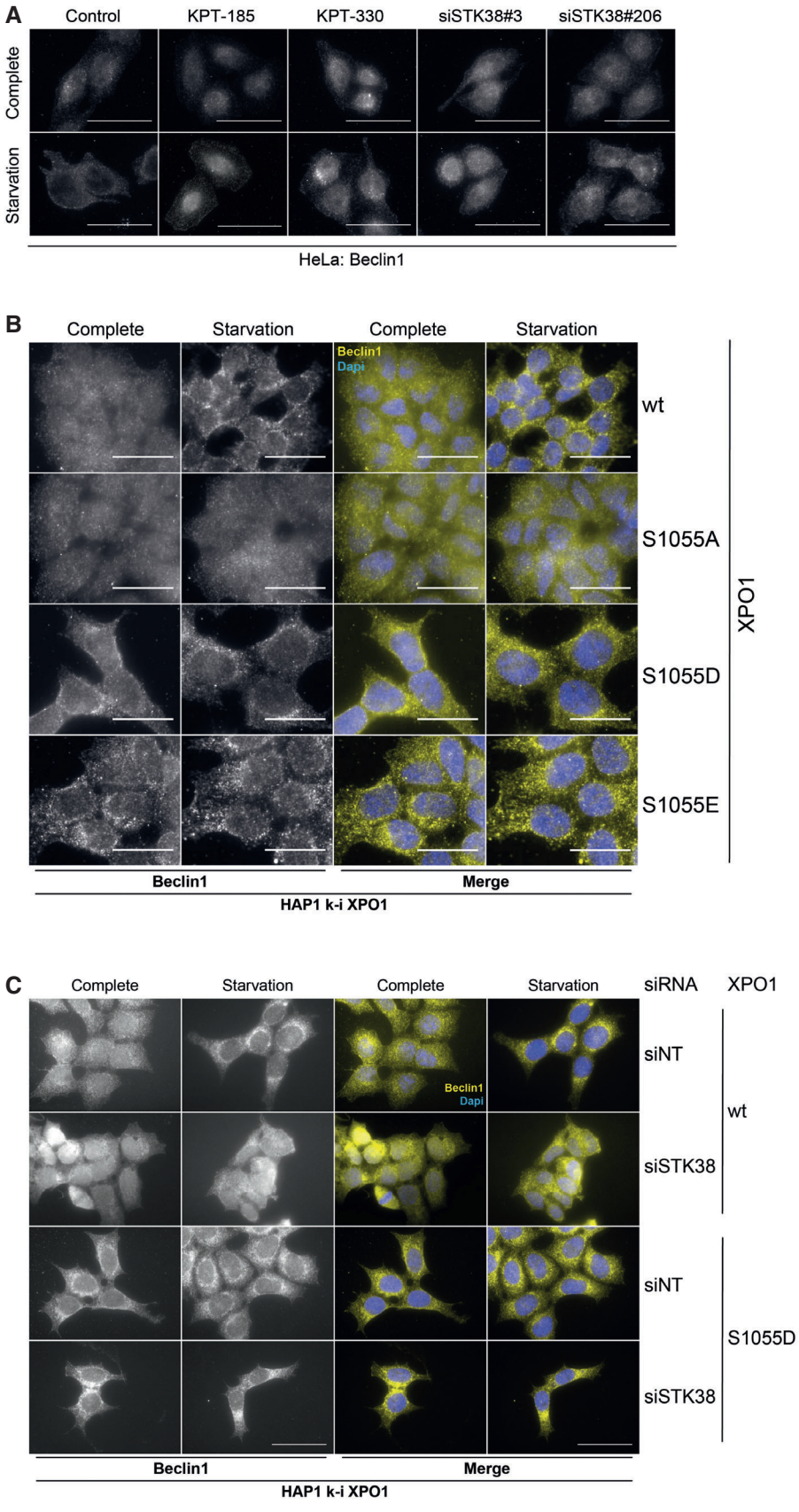

Figure EV4. Beclin1 subcellular localization (related to Fig 6).

A HeLa cells were transiently transfected with the indicated siRNA (control and KPT conditions = siNT). Seventy-two hours later, cells were incubated with XPO1 inhibitor KPT-185 or KPT-330 as indicated (final concentration = 1 μM) or with DMSO for all other conditions for 2 h prior to incubation with DMEM or EBSS supplemented (or not) with inhibitors for 2 h. Cells were fixed and stained for endogenous Beclin1. Representative images are shown, and scale bars are 40 μm (see Appendix Fig S6A for STK38 silencing).

B Genome-edited XPO1 mutant HAP1 cells were incubated with IMDM (complete) or EBSS (starvation) for 2 h. Cells were then fixed and stained for endogenous Beclin1. Representative images are shown, and scale bars are 20 μm.

C Genome-edited XPO1 mutant HAP1 cells were transiently transfected with siRNA targeting endogenous STK38 (siSTK38#206 or with non-targeting siRNA (siNT)). Seventy-two hours later, cells were incubated with IMDM (complete) or EBSS (starvation) for 2 h. Cells were then fixed and stained for endogenous Beclin1. Representative images are shown, and scale bars are 20 μm (see Appendix Fig S6C for STK38 silencing).

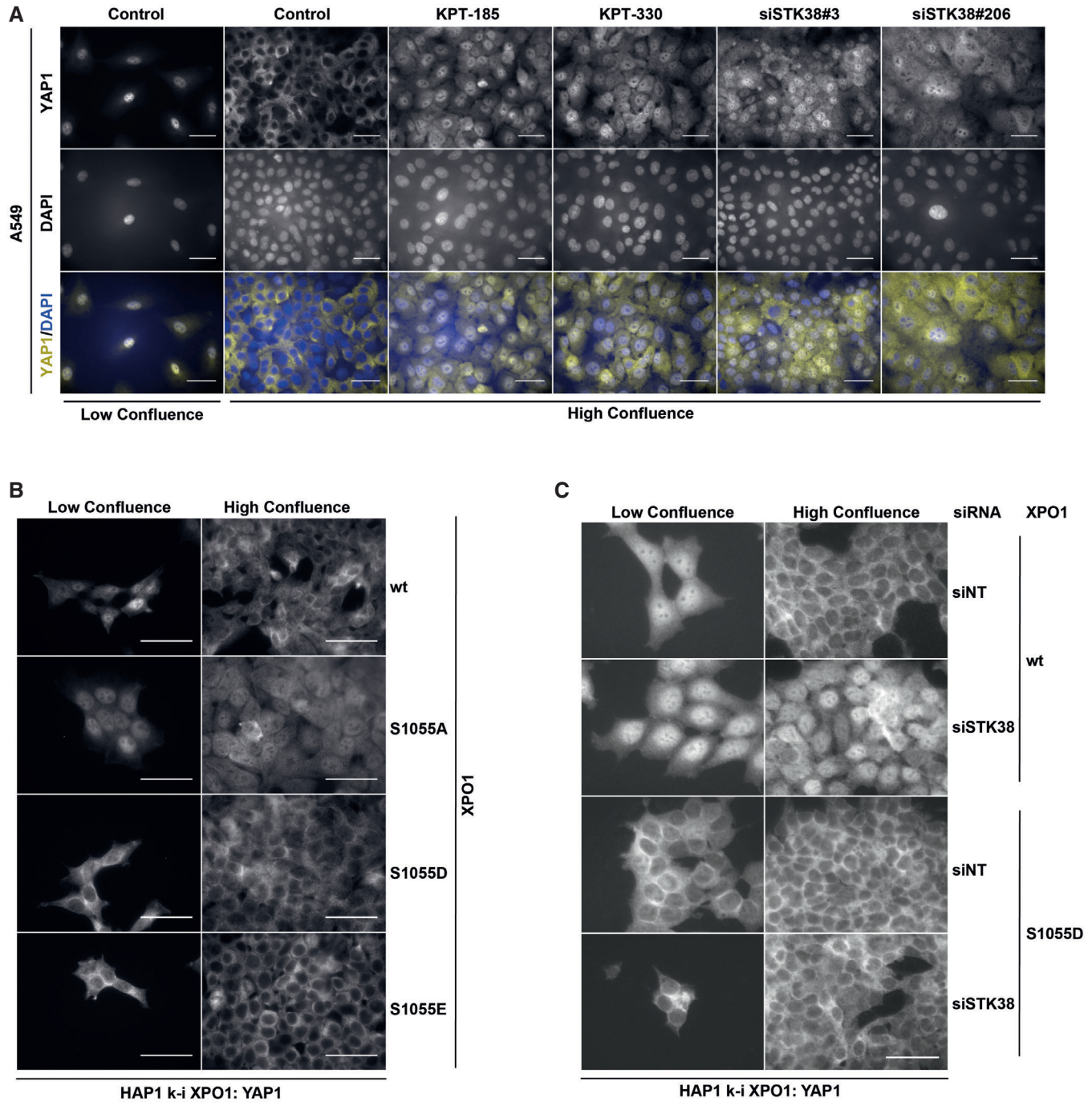

Figure EV5. YAP1 subcellular localization (related to Fig 7).

A A549 cells were transiently transfected with the indicated siRNA (control and KPT conditions = siNT) at low or high confluence. Forty-eight hours later, cells were incubated overnight in the presence of XPO1 inhibitors KPT-185 and KPT-330 (final concentration = 1 μM) or with DMSO for all other conditions. The next day, cells were fixed and stained for endogenous YAP1. Representative images are shown, and scale bars are 40 μm (see Appendix Fig S7A for STK38 silencing).

B Genome-edited XPO1 HAP1 cells were cultured for 2 days at low versus high confluency. Cells were then fixed and stained for endogenous YAP1. Representative images are shown, and scale bars are 40 μm.

C Genome-edited XPO1 mutant HAP1 cells were transiently transfected with siRNA targeting endogenous STK38 (siSTK38#206 or with non-targeting siRNA (siNT)) at low or high confluence. Seventy-two hours later, cells were fixed and stained for endogenous YAP1. Representative images are shown, and scale bars are 40 μm (see Appendix Fig S7D for STK38 silencing).