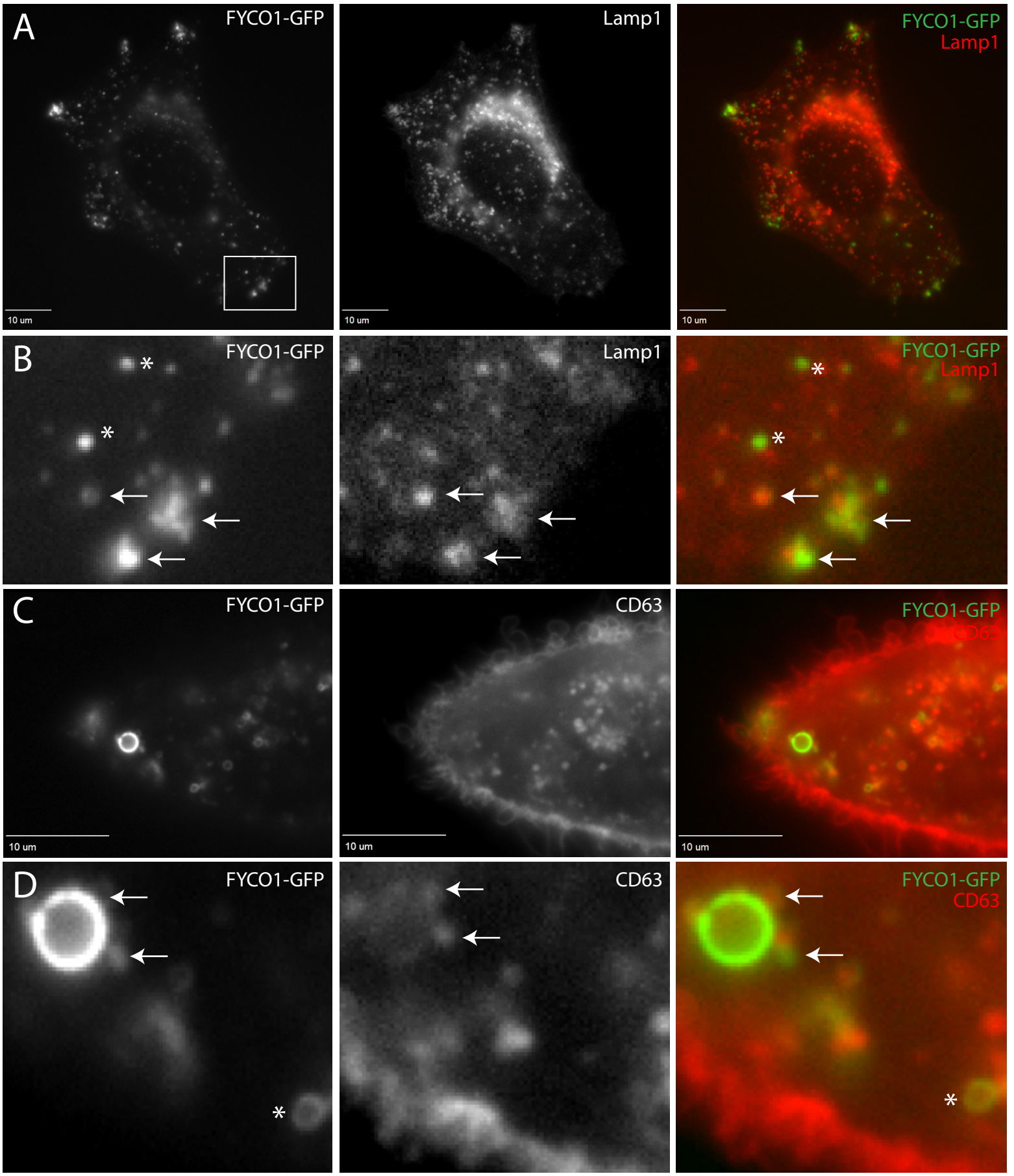
Supplemental Figure 1



Supplementary Figure 1. Partial FYCO1 co-localization with Lamp1.
HeLa cells were transiently co-transfected with FYCO1-GFP and then fixed and stained with two different lysosomal markers, ant-Lamp1 (A and B) and anti-CD63 (C and D) antibodies. The presence of lysosomal marker in FYCO1-associated organelles was the analyzed. Panels B and D show higher magnification image of the area boxed in panel A and C. Arrows point to organelles containing both FYCO1-GFP and Lamp1 or FYCO1-GFP and CD63. Asterisks mark organelle containing only FYCO1-GFP but not Lamp1 or CD63.

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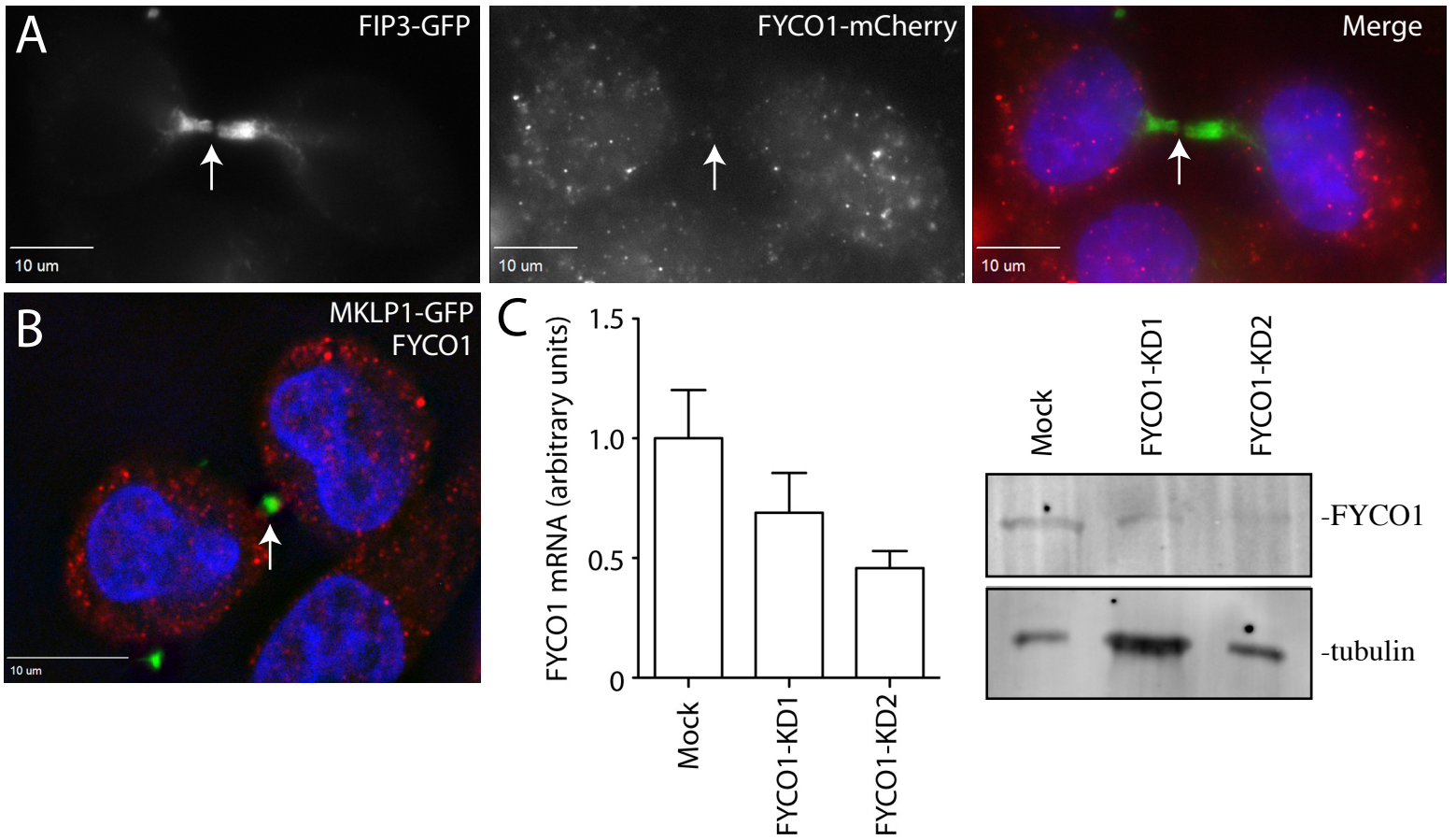
Supplemental Figure 2



Supplementary Figure 2. FYCO1 knock-down does not affect cytokinesis or autophagic flux.

(A) To test whether FYCO1 is present at the midbody during cytokinesis HeLa cells expressing FIP3-GFP (furrow endosome marker) and FYCO1-mCherry were fixed and analyzed for FYCO1 localization. Arrows mark the MB.

(B) To test whether FYCO1 is present at the midbody during cytokinesis HeLa cells expressing MKLP1-GFP (MB marker) were fixed and stained with anti-FYCO1 antibody. Arrow marks the MB.

(C) Quantitative PCR and western blots assessing FYCO1 mRNA levels in HeLa cells stably expressing two different FYCO1 shRNAs. Data shown are the means and standard deviations.

(D) To test whether FYCO1 knock-down affects cytokinesis control cells or FYCO1 shRNA-expressing cells were stained with anti-acetylated tubulin and Hoechst 33342. Number of multi-nucleated cells or cells in the telophase were then counted. Data shown are the means and standard deviations from three independent experiments.

(E) To test effect of FYCO1 knock-down on autophagic flux cells were grown in the presence or absence of 40 μM chloroquine for 4 hours. Cells were then lysed and levels of LC3-II (activated LC3) was analyzed by western blotting.

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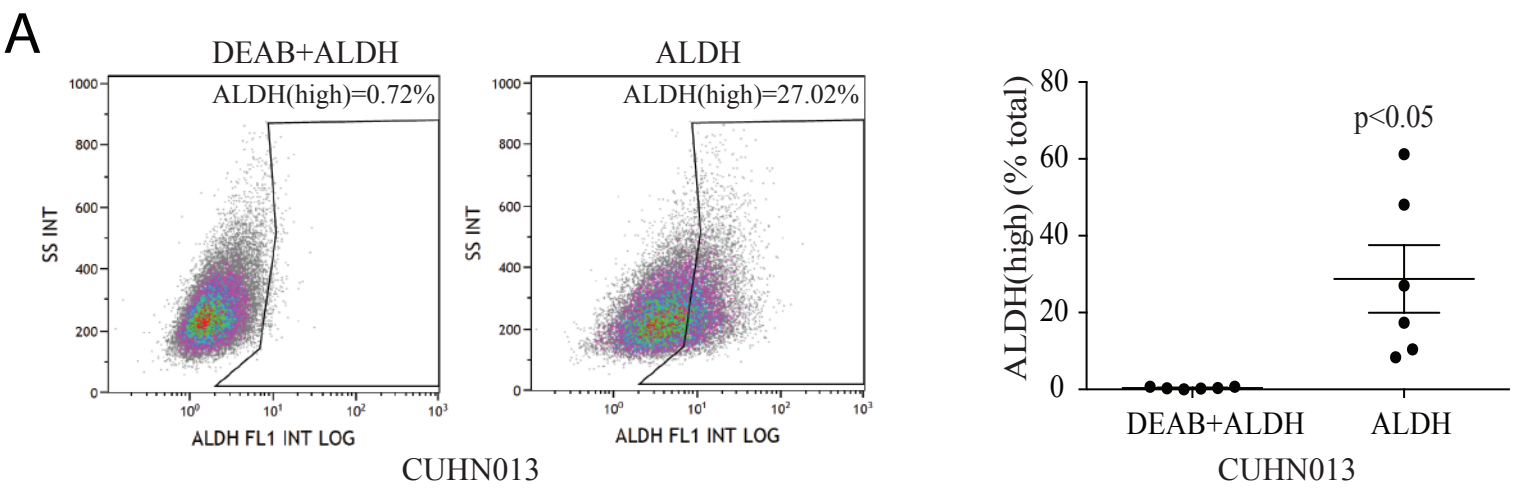
Supplemental Figure 3



Supplementary Figure 3. ALDH(high) population of CUHN013 squamous cell carcinoma accumulate post-mitotic MBs

(A) Flow cytometry analysis of ALDH(high) cells in CUHN013. Cells were incubated in the dark with ALDEFLUOR assay buffer containing the activated ALDEFLUOR substrate, with or without ALDH inhibitor diethylaminobenzaldehyde (DEAB). Cells were flow sorted to identify ALDH(high) and ALDH(low) populations. Data shown are the means and standard deviations from six independent experiments.

(B) Flow sorted ALDH(high) and ALDH(low) CUHN013 cells were cultured on Ultra Low Attachment six-well plates to allow formation of spheroids (top panels). Note that ALDH(high) form larger spheroids than ALDH(low). The spheroids were then fixed and stained with anti-MKLP1 antibodies to visualize post-mitotic MBs. Images shown are maximum intensity projection along the z-axis. Bar graph shows quantification of MB number in CUHN013 ALDH(high) and ALDH(low)-derived spheroids. Data shown are the average number of MBs per spheroid area. Data shown are the means and standard deviations from three independent experiments.

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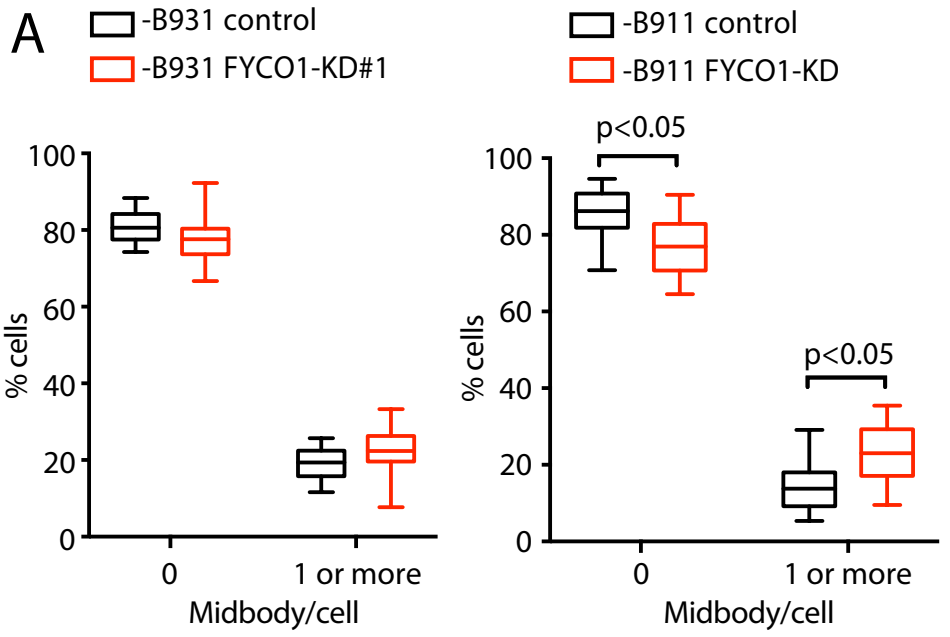
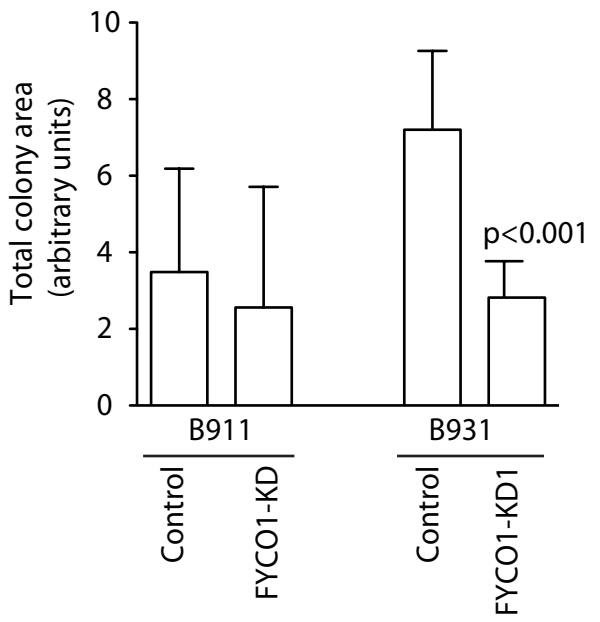
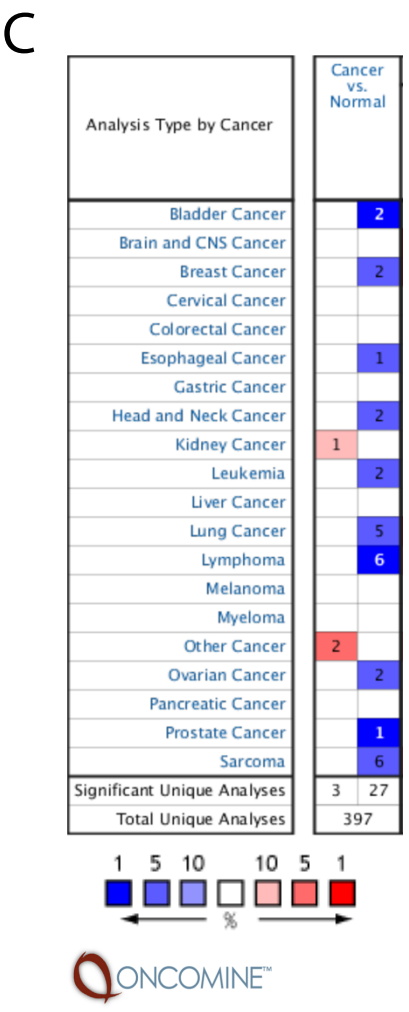
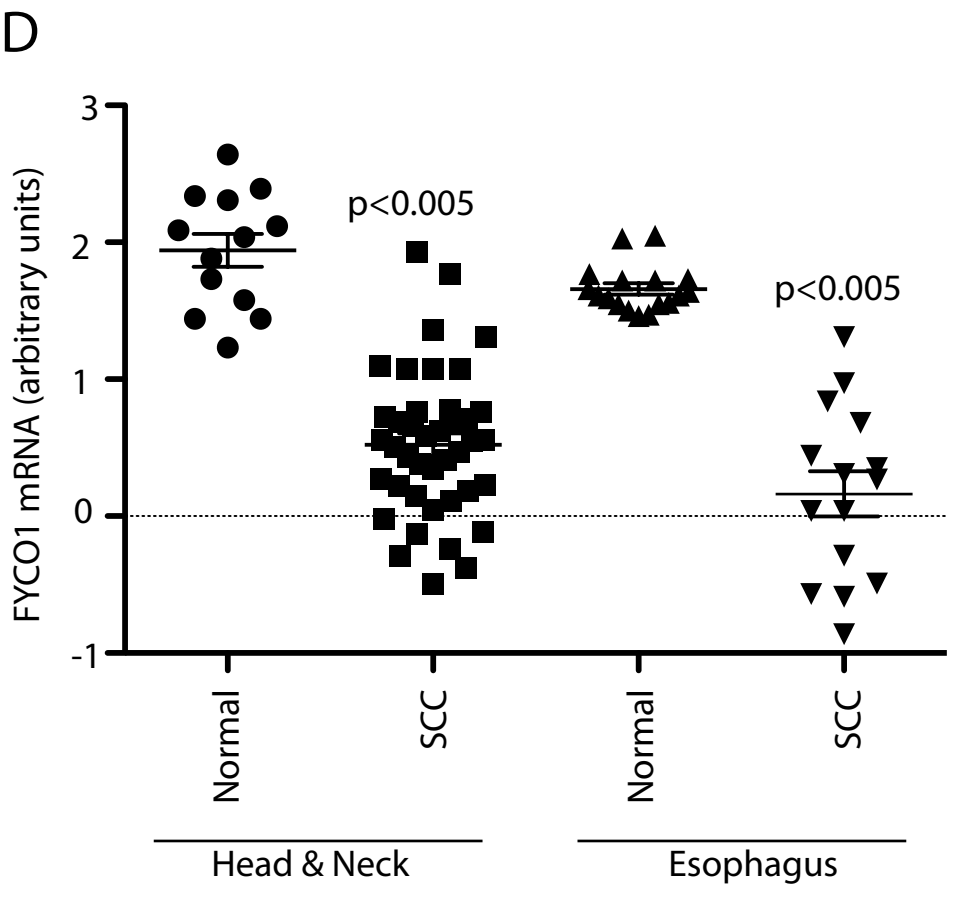
Supplemental Figure 4









Supplemental Figure 4. FYCO1 is down-regulated in many cancers including squamous cell carcinomas.
(A) B911 and B931 cells stably expressing FYCO1 shRNAs cells were analyzed for the absence or presence of post-mitotic MBs as determined by staining with anti-MKLP1 antibody. The vertical segments in box plots show the first quartile, median, and third quartile. The whiskers on both ends represent the maximum and minimum values for each dataset analyzed from three independent experiments.
(B) To test the effect of FYCO1 knock-down on stemness of B911 and B931 cells, mock or FYCO1 shRNA-expressing cells were plated in 6 well plates at the dilution of 100 cells per well. Cells were allowed grow for one week and then stained with 0.1% crystal violet. The number and size of colonies and then quantified by Metamorph. Data shown are the means and standard deviations from three independent experiments.
(C) Messenger RNA levels of FYCO1 in various cancers as compared to normal tissue. The data shown was obtained using data mining by Oncomine.
(D) Messenger RNA levels of FYCO1 in head & neck and esophagus squamous cell carcinomas as compared to normal tissue. The data shown was obtained using data mining by Oncomine.