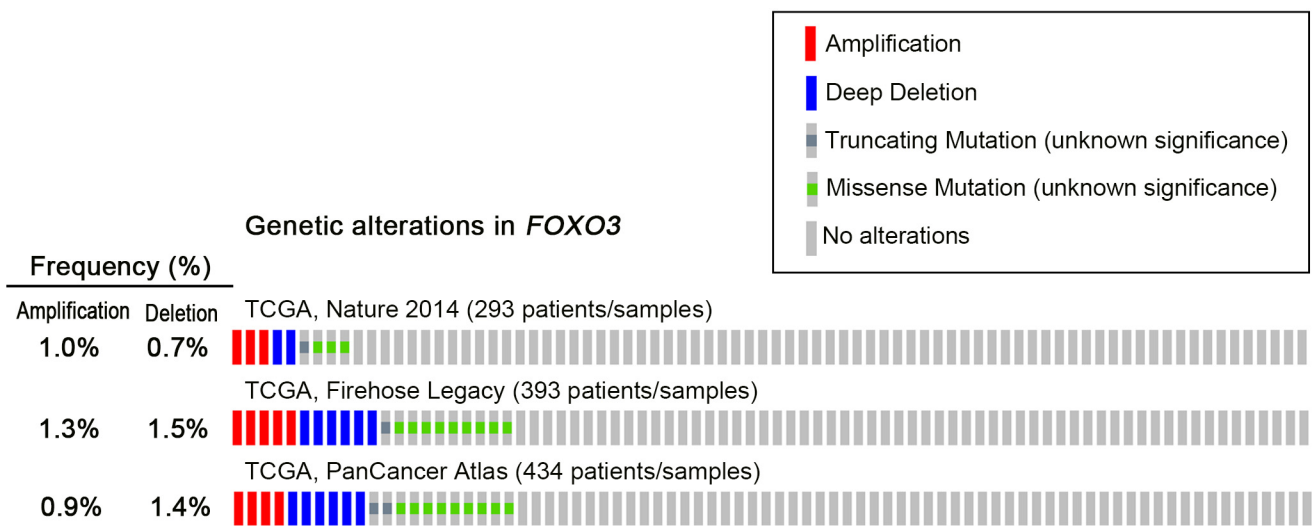
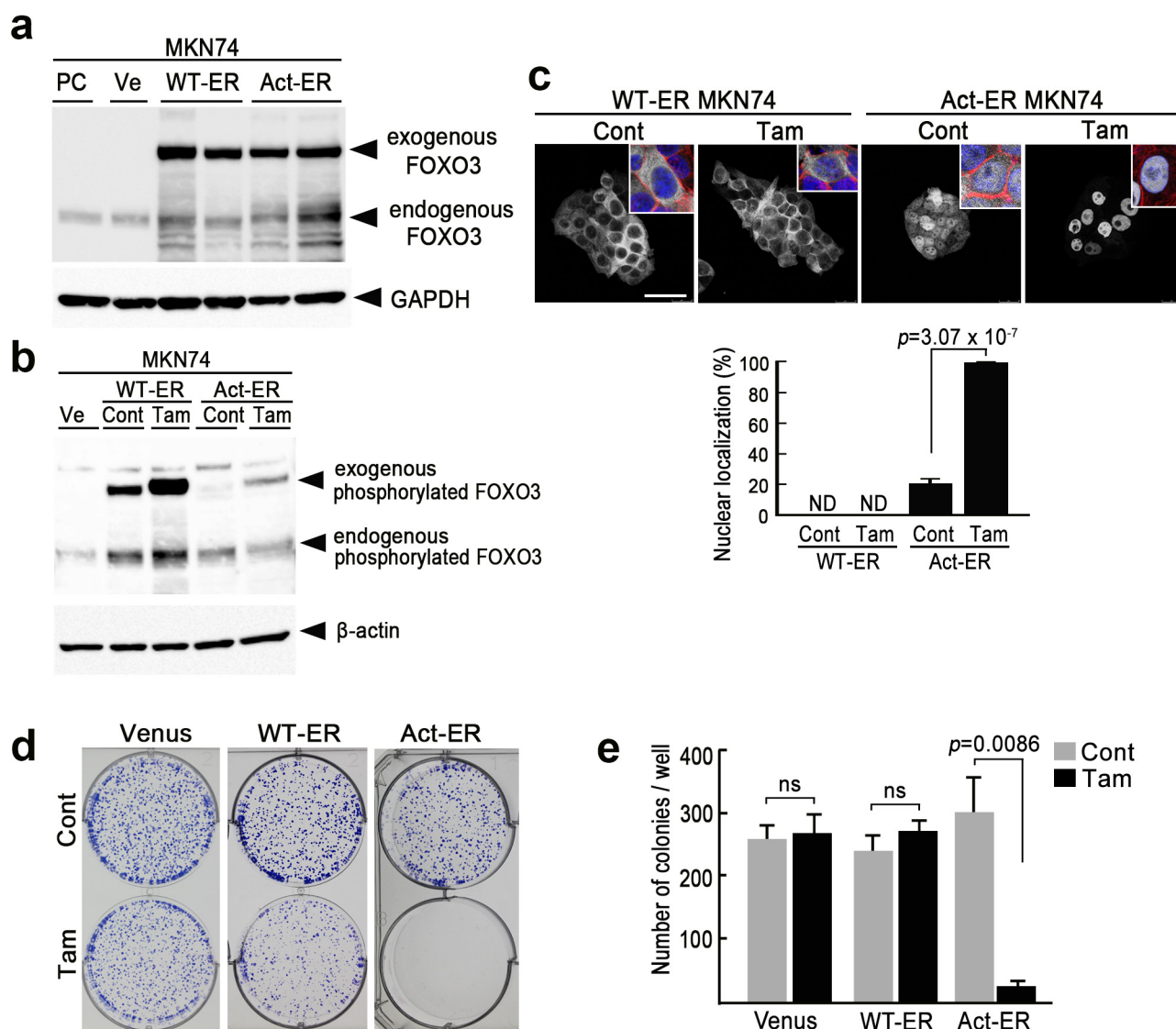


Supplementary Figure 1



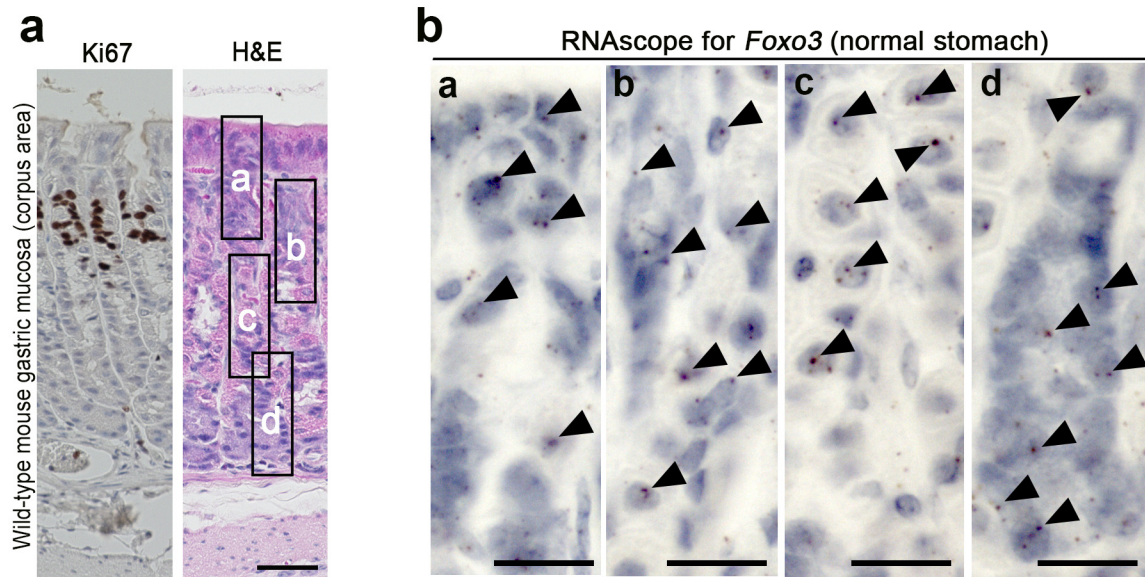
Supplementary Figure 1. Database analyses for *FOXO3* genetic alterations in human gastric cancer. The frequencies (%) of amplification and deletion of *FOXO3* gene in each database are indicated.

Supplementary Figure 2



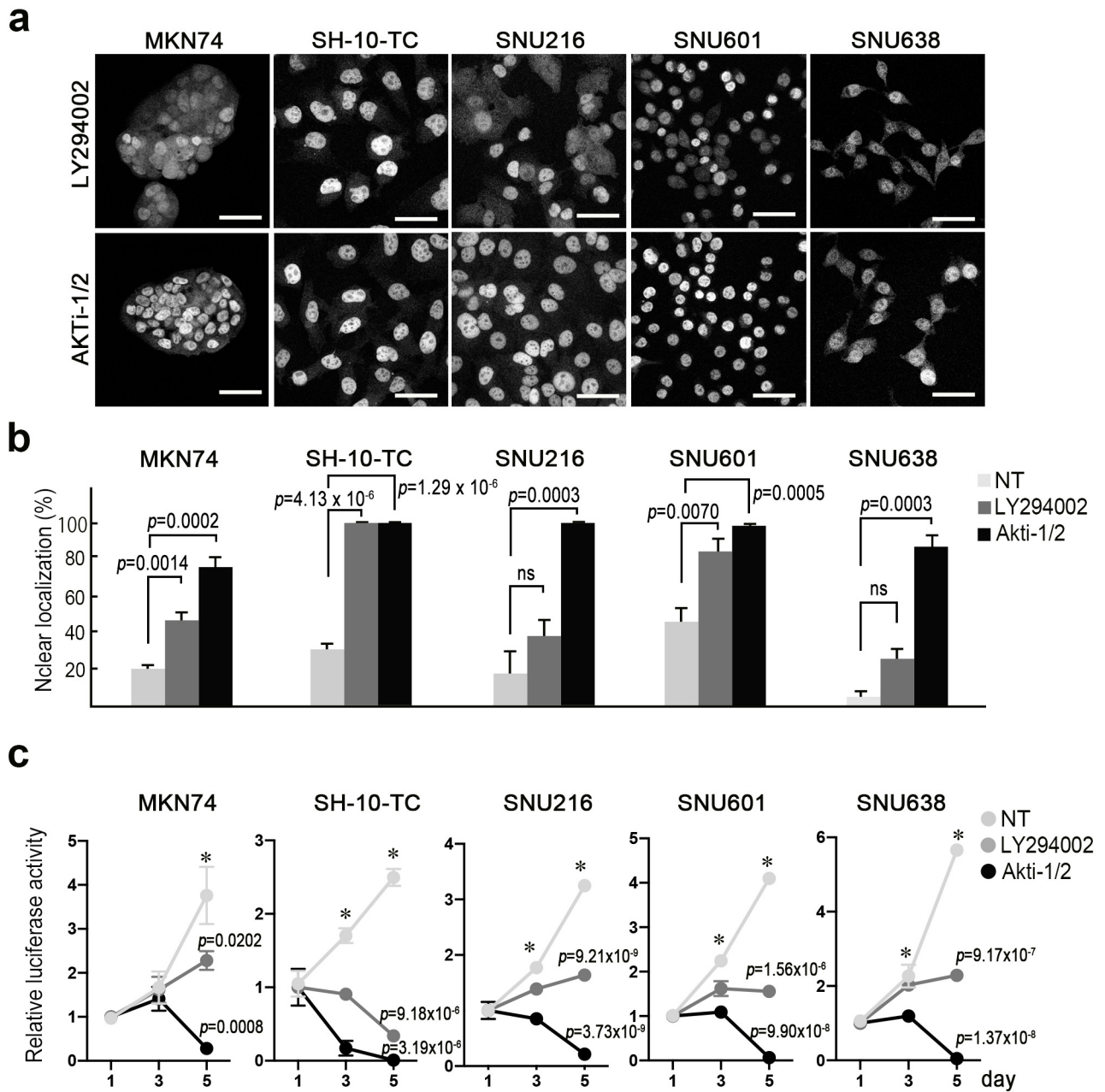
Supplementary Figure 2. Growth suppression of MKN74 gastric cancer cells by FOXO3 nuclear accumulation. **a** Immunoblotting results for exogenous FOXO3 (WT-ER and Act-ER) and endogenous FOXO3 in MKN74 cells are shown. PC, Parental cells; Ve, Venus-expressing cells; WT-ER and Act-ER, WT-ER and Act-ER FOXO3-expressing MKN74 cells, respectively. GAPDH was used as an internal control. **b** Immunoblotting results for phosphorylated FOXO3 in control and Tam-treated WT-ER or Act-ER MKN74 cells are shown. β -Actin was used as an internal control. **c** Representative photographs of immunocytochemistry for FOXO3 of the tamoxifen-treated (Tam) and untreated (Cont) WT-ER and Act-ER FOXO3-expressing MKN74 cells (*top*) (Representative images of $n=3$ independent cultures). Insets show enlarged images with Phalloidin (red) and DAPI (blue) staining. Bar, 50 μ m. The ratios of FOXO3-nuclear localized cells in the control and Tam-treated WT-ER and Act-ER FOXO3-expressing MKN74 cells are shown as a bar graph (mean $\pm$ s.d.) (*bottom*). A two-sided t -test was used to calculate statistical significance, and p value is provided. ND, not detected. **d** The results of a colony formation assay of Tam-treated (Tam) and untreated (Cont) MKN74 cells expressing Venus (*left*), WT-ER (*center*) and Act-ER (*right*) in 6-well plates are shown. The images are representative of $n=3$ independent experiments. **e** The mean colony numbers per well are shown in a bar graph (mean $\pm$ s.d.). A two-sided t -test was used to calculate statistical difference, and p value is provided. ns, not significant.

Supplementary Figure 3



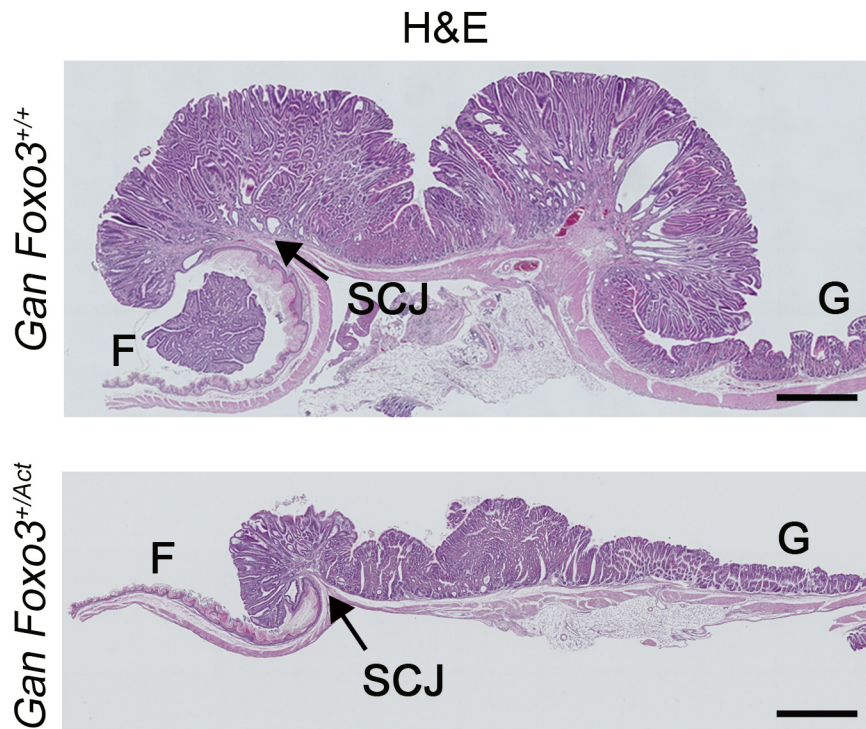
Supplementary Figure 3. FOXO3 expression in wild-type mouse stomach. **a** Representative photographs of corpus area of wild-type mouse stomach (H&E) (*right*) and Ki67 immunohistochemistry (*left*). Boxes (a-d) indicate the pit, neck, gland middle, and gland bottom, respectively. Bars, 20 μ m. **b** The results of RNAscope *in situ* hybridization for *Foxo3* mRNA in the boxed area in **a** are shown. Arrowheads indicate positive signals. Bars, 20 μ m. Images in (**a**) and (**b**) are representative of n=3 biologically independent animals.

Supplementary Figure 4



Supplementary Figure 4. Growth suppression of gastric cancer cells by PI3K-AKT inhibition. **a** Representative photographs of immunocytochemistry for FOXO3 of the gastric cancer cell lines treated with LY294002 (*top*) and AKTi-1/2 (*bottom*). Bars, 50 μ m. The images are representative of $n=3$ independent experiments. Immunocytochemistry of the untreated control cells are shown in Figure 2b. **b** The ratios of FOXO3 nuclear localization in the indicated gastric cancer cell lines are shown as bar graphs (mean $\pm$ s.d.). ($n=9$ independent microscopic fields for each cell line). NT, no treatment. A two-sided t -test was used to calculate statistical significance, and p values are provided. **c** Relative cell proliferations examined by luciferase activity in gastric cancer cells with no treatment (NT) and treated with LY294002 or Akti-1/2 are shown (mean $\pm$ s.d.). The data at each day point were analyzed by one-way ANOVA test. Asterisks, $p < 0.05$. A two-sided t -test was used to calculate statistical difference at day 5, and p values vs. NT are provided.

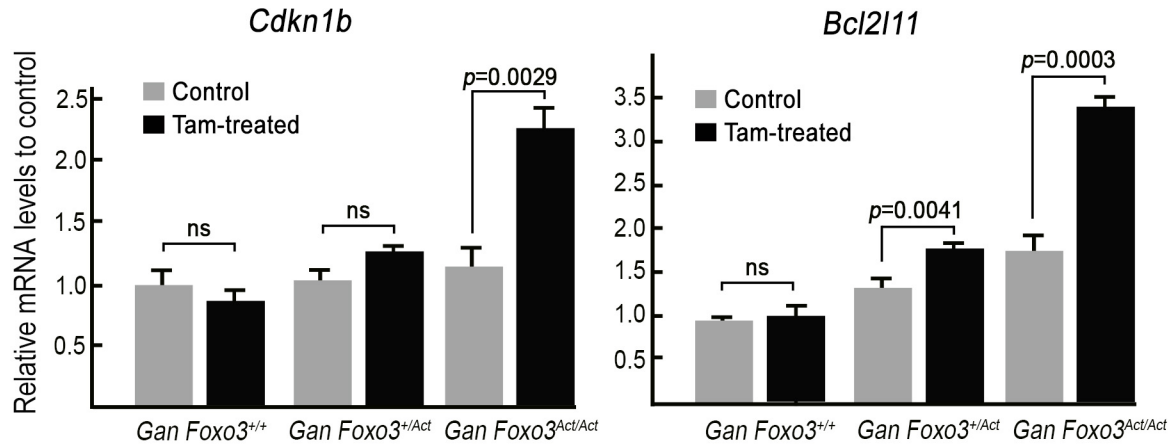
Supplementary Figure 5



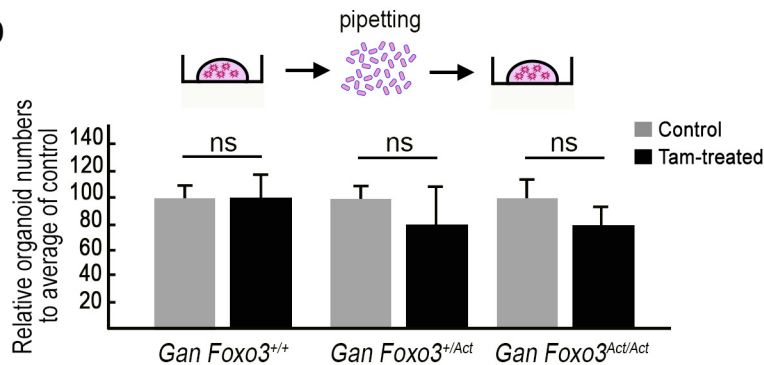
Supplementary Figure 5. Representative histology photographs of *Gan Foxo3^{+/+}* (top) and *Gan Foxo3^{+/Act}* (bottom) mouse gastric tumors (H&E) at 50 weeks of age. F, forestomach; G, glandular stomach; SCJ, squamocolumnar junction. Bars, 1 mm. The images are representative of n=9 and n=7 biologically independent *Gan Foxo3^{+/+}* and *Gan Foxo3^{+/Act}* mice, respectively.

Supplementary Figure 6

a



b



Supplementary Figure 6. Induction of FOXO3 target genes by expression of Act-ER FOXO3. **a** The relative mRNA levels of the FOXO3 target genes *Cdkn1b* (left) and *Bcl2l1* (right) in the control and Tam-treated tumor-derived organoids with indicated genotypes to the mean control level of *Gan FOXO3^{+/+}* organoids (mean $\pm$ s.d.). The RT-PCR experiments were repeated five times. **b** A schematic illustration of the mechanical dissociation of organoids by pipetting and passage (top). Relative organoid numbers developed after the mechanical dissociation of control and Tam-treated *Gan Foxo3^{+/+}*, *Gan Foxo3^{+/-Act}*, and *Gan Foxo3^{Act/Act}* tumor-derived organoids are shown as a bar graph (mean $\pm$ s.d.). The organoid passage experiments were repeated five times. For the data in (a) and (b), a two-sided *t*-test was used to calculate statistical significance, and *p* values are provided. ns, not significant.