

Supplementary

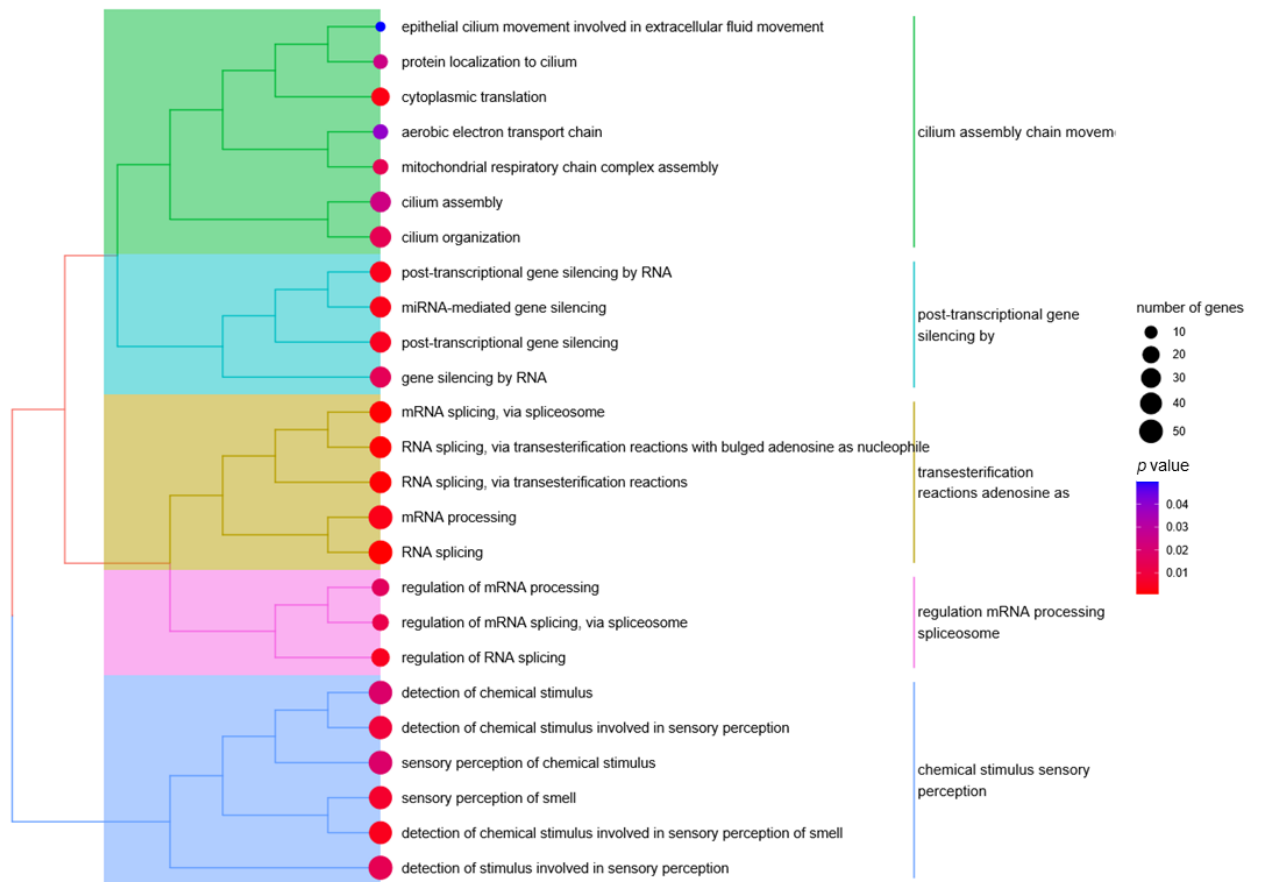


Figure S1: Tree plot of top 30 gene ontology (GO) terms enriched in upregulated genes in high $\Delta 40p53$ versus low invasive ductal carcinomas (IDCs).

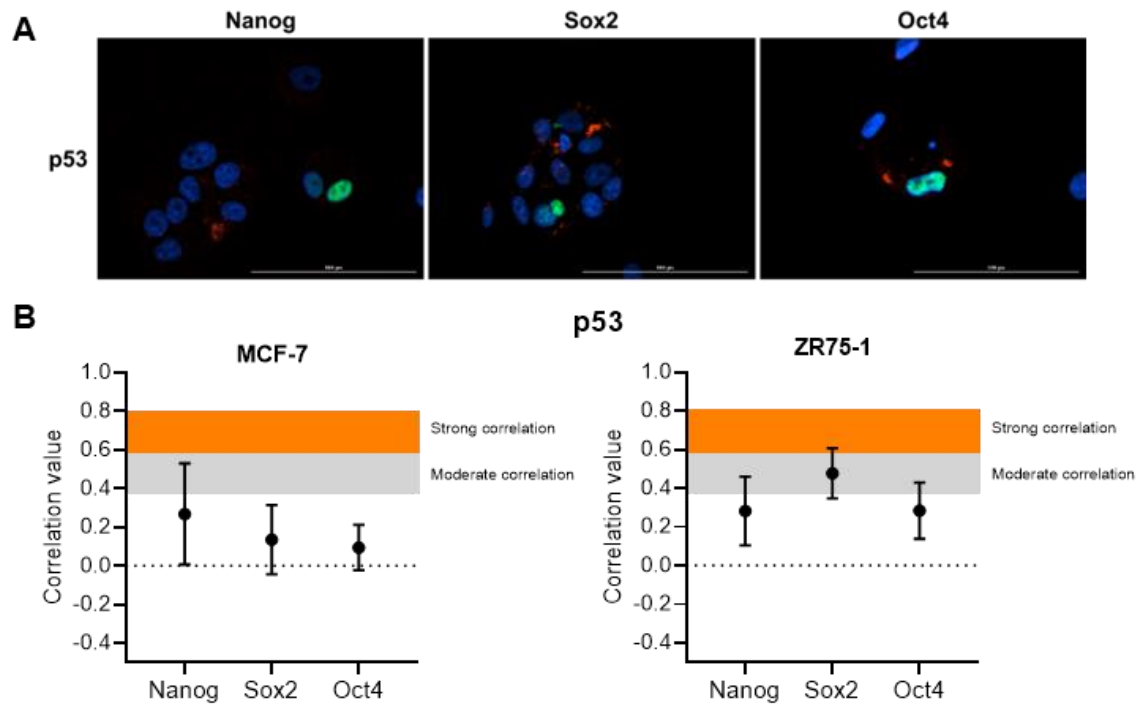


Figure S2: p53 does not co-localise with stem cell markers, Nanog, Sox2, and Oct4. (A) Immunofluorescence images of p53 and Nanog, Sox2, or Oct4 co-staining in the MCF-7 cell line. p53 (7F5) (1:800) and stem cell markers Nanog (1:50), Sox2 (1:200), and Oct4 (1:500) primary antibodies were used and cell nuclei were stained with DAPI. Scale bar indicates 100 μ m. (B) Correlation values of the co-localisation of p53 and Nanog, Sox2, or Oct4 in MCF-7 and ZR75-1 cells. Analyses were carried out using ImageJ (Coloc 2) and Spearman's rank correlation was used for the co-localisation analyses.

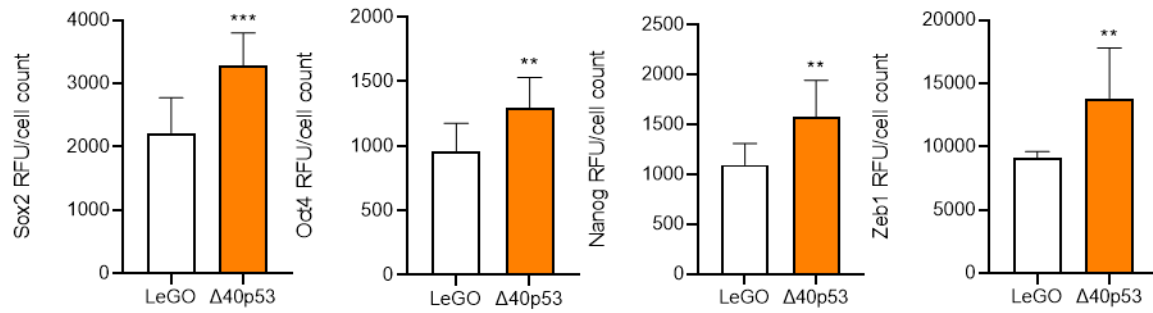


Figure S3: Sox2, Oct4, Nanog, and Zeb1 expression in relative fluorescence units (RFU) normalised to cell count in MCF-7-LeGO and MCF-7-Δ40p53 cells. Data shown represent three independent experiments of three technical replicates. Results are shown as the mean $\pm$ SD. Statistical analyses were carried out using an unpaired t-test. Results were considered significant at $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$.

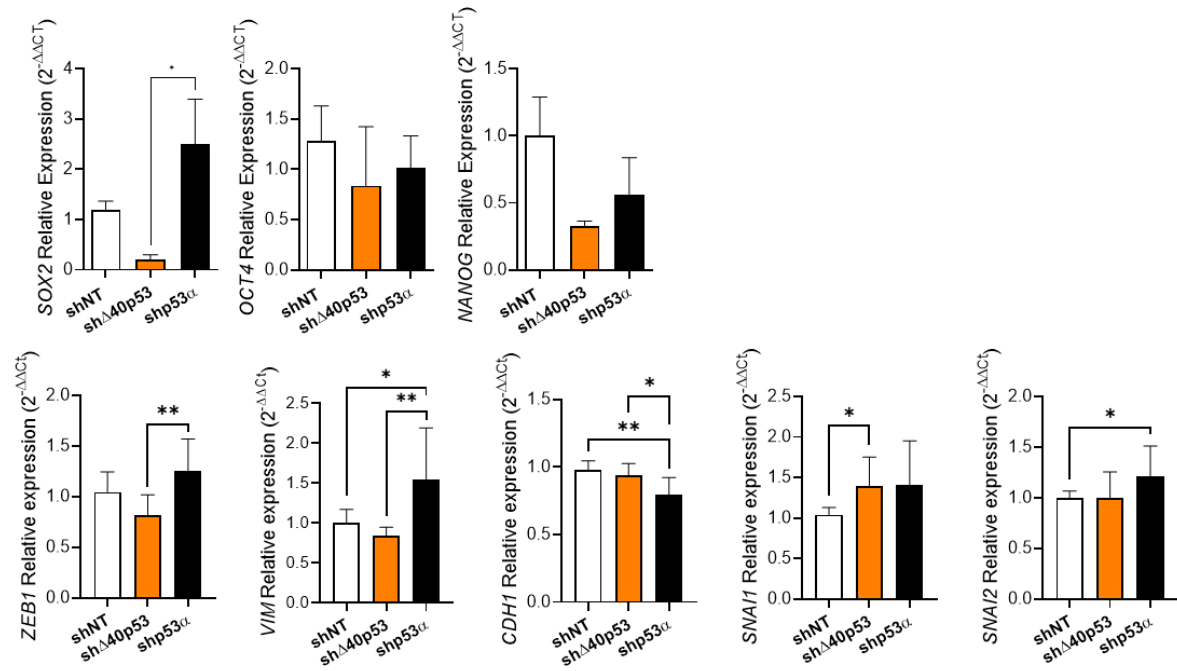


Figure S4: mRNA levels of *SOX2*, *OCT4*, *NANOG*, *ZEB1*, *VIM*, *CDH1*, *SNAI1*, and *SNAI2* in MCF-7-shNT, MCF-7-shΔ40p53, and MCF-7-shp53 sublines. Data shown represent three independent experiments of three technical replicates. Results are shown as the mean ± SD. Statistical analyses were carried out using one-way ANOVA followed by Tukey's post-test. Results were considered significant at $p < 0.05$; * $p < 0.05$, ** $p < 0.01$.

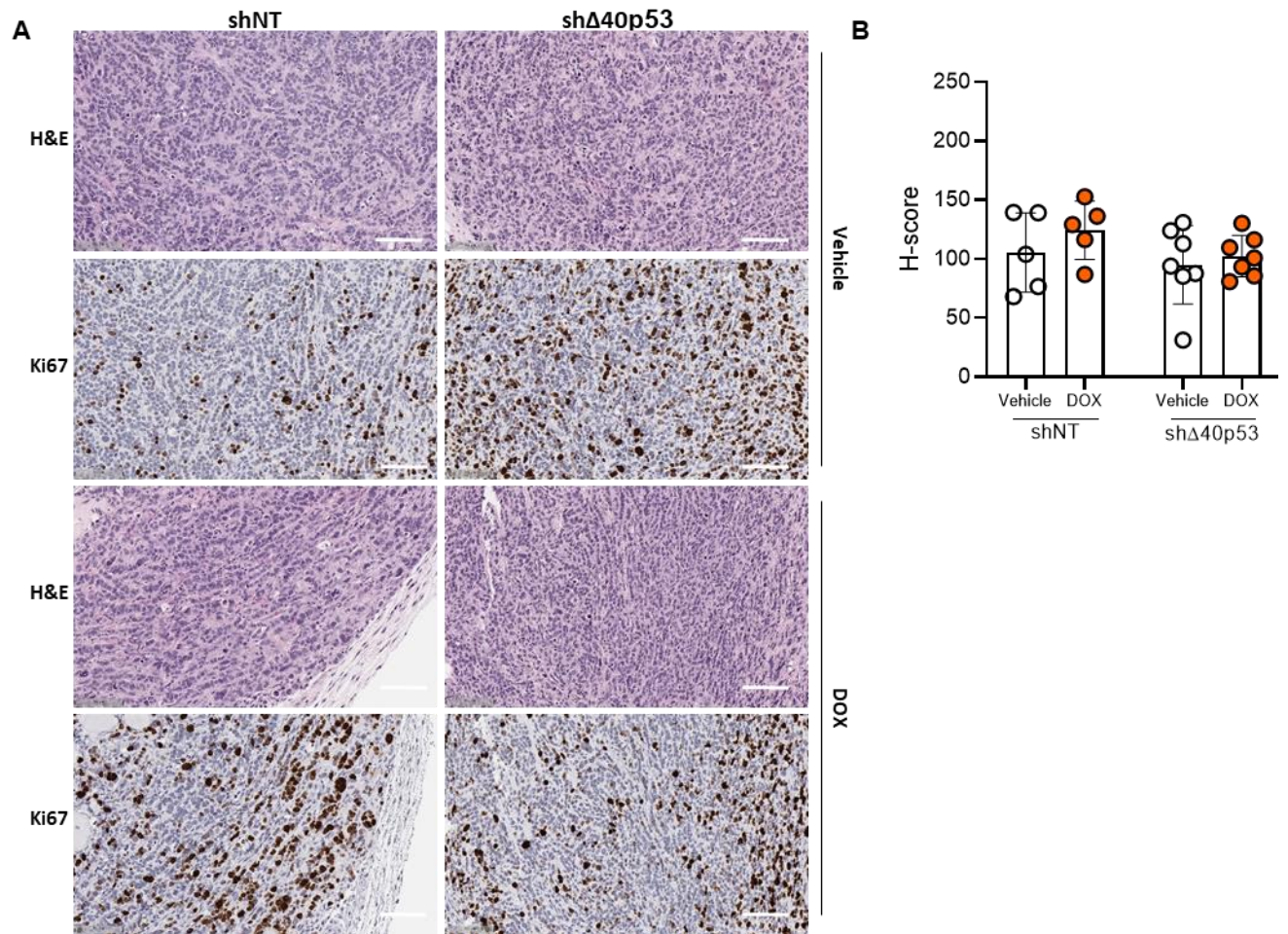


Figure S5: Ki67 levels in $\Delta 40p53$ knockdown tumour xenografts. (A) Representative images of H&E slides and slides stained for Ki67 of MCF-7-shNT or MCF-7-sh $\Delta 40p53$ tumour-bearing mice treated with vehicle or DOX. Scale bars represent 100 μ m. (B) H-score for Ki67 in MCF-7-shNT or MCF-7-sh $\Delta 40p53$ xenograft tumours. Results are shown as the mean $\pm$ SD. Statistical analysis were carried out using two-way ANOVA followed by Sidak's post-test. Results were considered significant at $p < 0.05$.

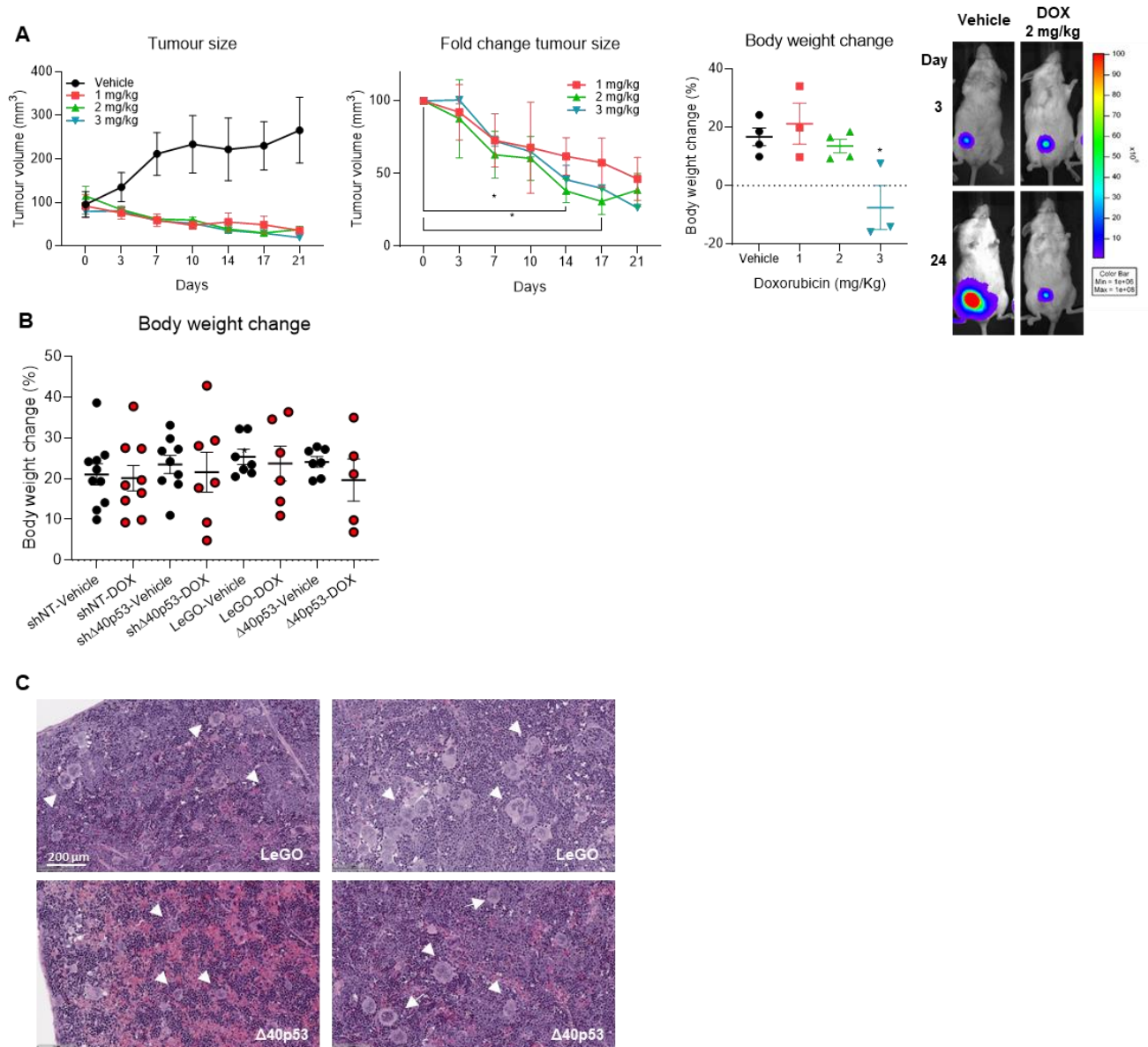


Figure S6: *In vivo* experiments. (A) Pilot *in vivo* study (see Methods – Treatment 1) to select the suboptimal dose of DOX. Tumour volume normalised to tumour size on day 0 (prior treatment) and body weight change of mice bearing the luciferase-labelled MCF-7-shNT subline treated with saline or three different doses of DOX (1 mg/kg, 2 mg/kg, or 3 mg/kg) ($n = 3$ in each treatment group). Representative images of luminescent imaging of mice treated with saline or DOX (2 mg/kg). DOX at 2 mg/kg was selected for the Treatment 2 (see Methods). (B) Body weight change of MCF-7-shNT, MCF-7-shΔ40p53, MCF-7-LeGO, and MCF-7-Δ40p53-derived xenografts treated with saline (vehicle) or DOX (2 mg/kg). (C) Representative H&E images of spleens that presented luciferin signal during mice imaging. Breast cancer cells were observed in the spleens (white arrows). These cells escaped the mammary fat pad during implantation of cells and were detected in the spleens due to splenic clearance. No differences were observed between MCF-7 sublines groups. No additional luminescence signals were detected during *in vivo* imaging. Scale bars represents 200 μ m. Results are shown as the mean $\pm$ SD. Statistical analyses were carried out using one-way ANOVA followed by Dunnett's post-test (body weight change) or two-way ANOVA followed by Tukey's post-test (tumour volume). Results were considered significant at $p < 0.05$; * $p < 0.05$.

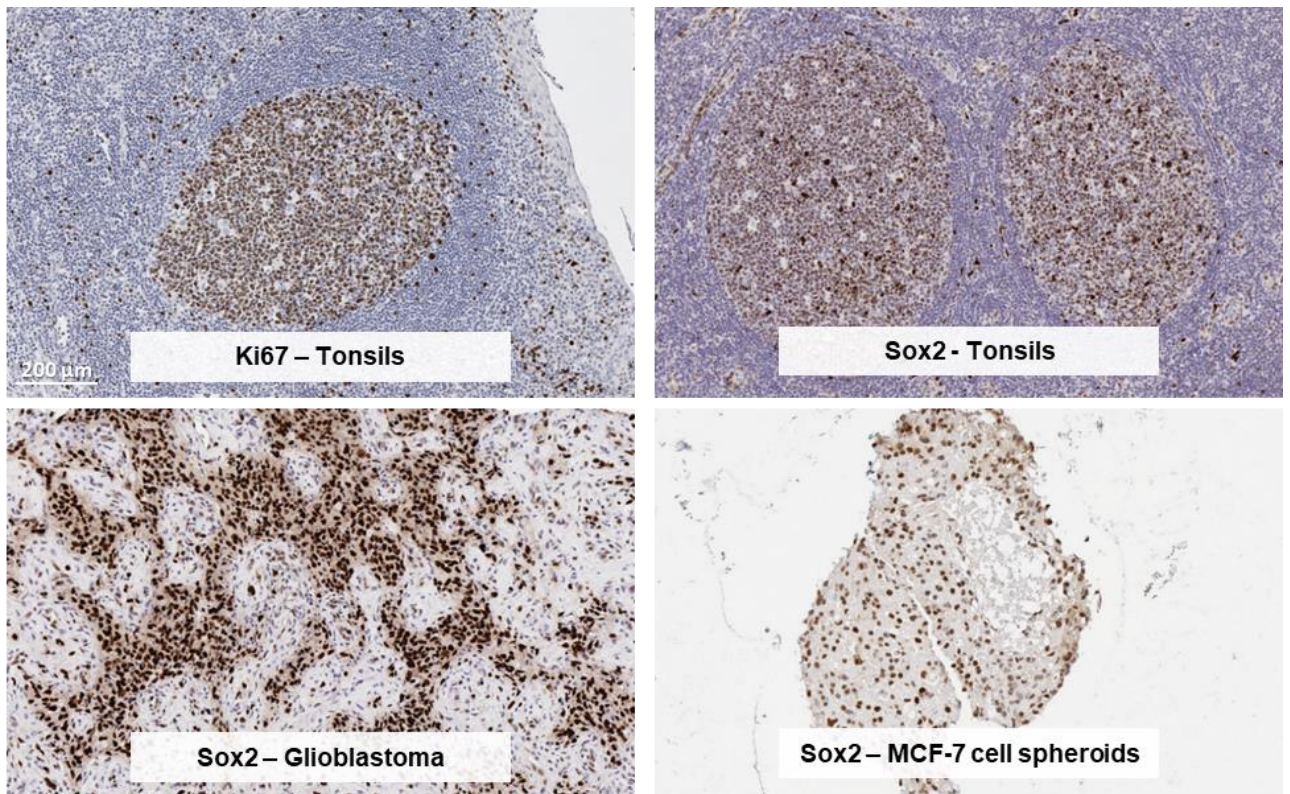


Figure S7: Representative images of control tissues used for Ki67 and Sox2 antibodies optimisation for immunohistochemistry. Scale bar represents 200 μm.