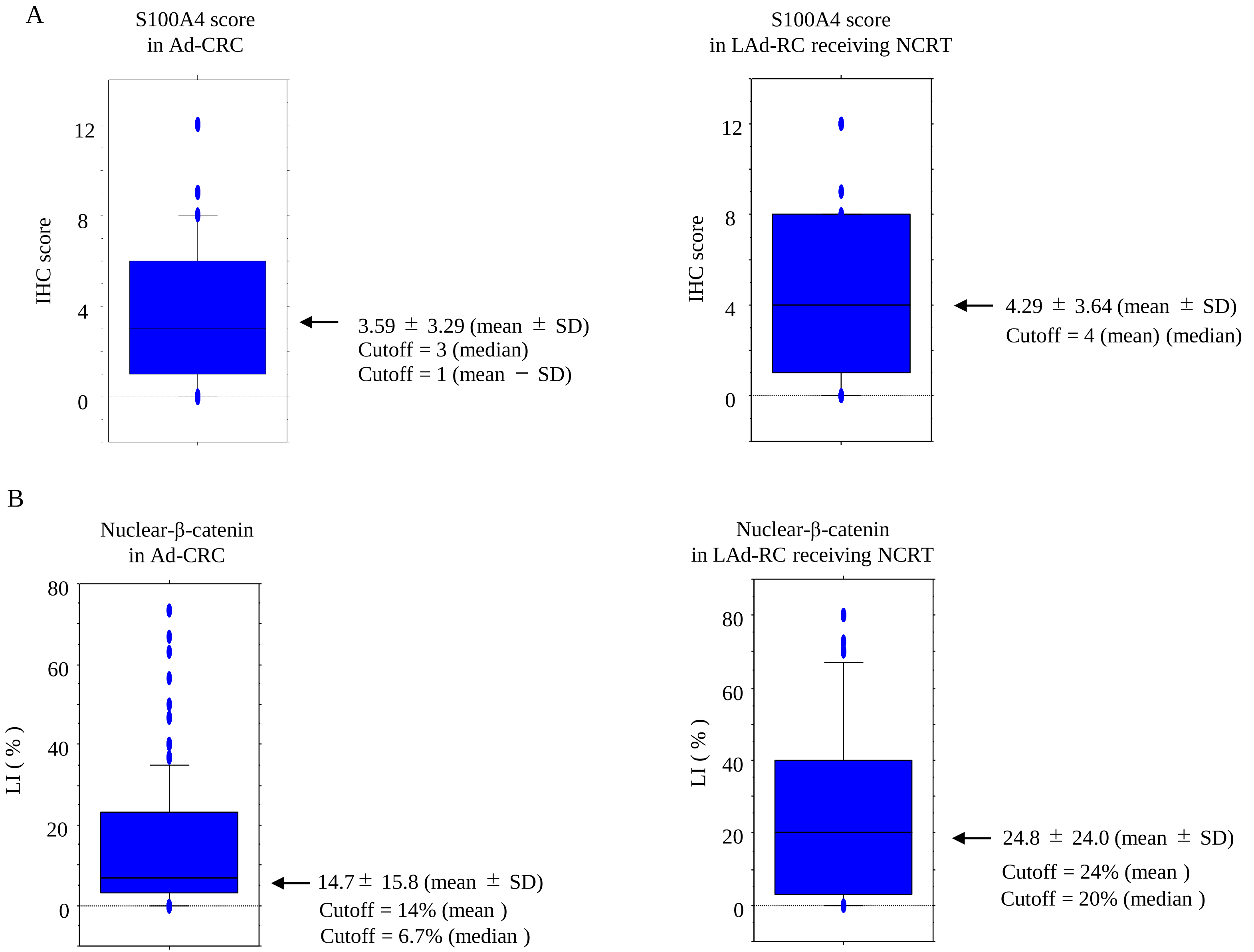
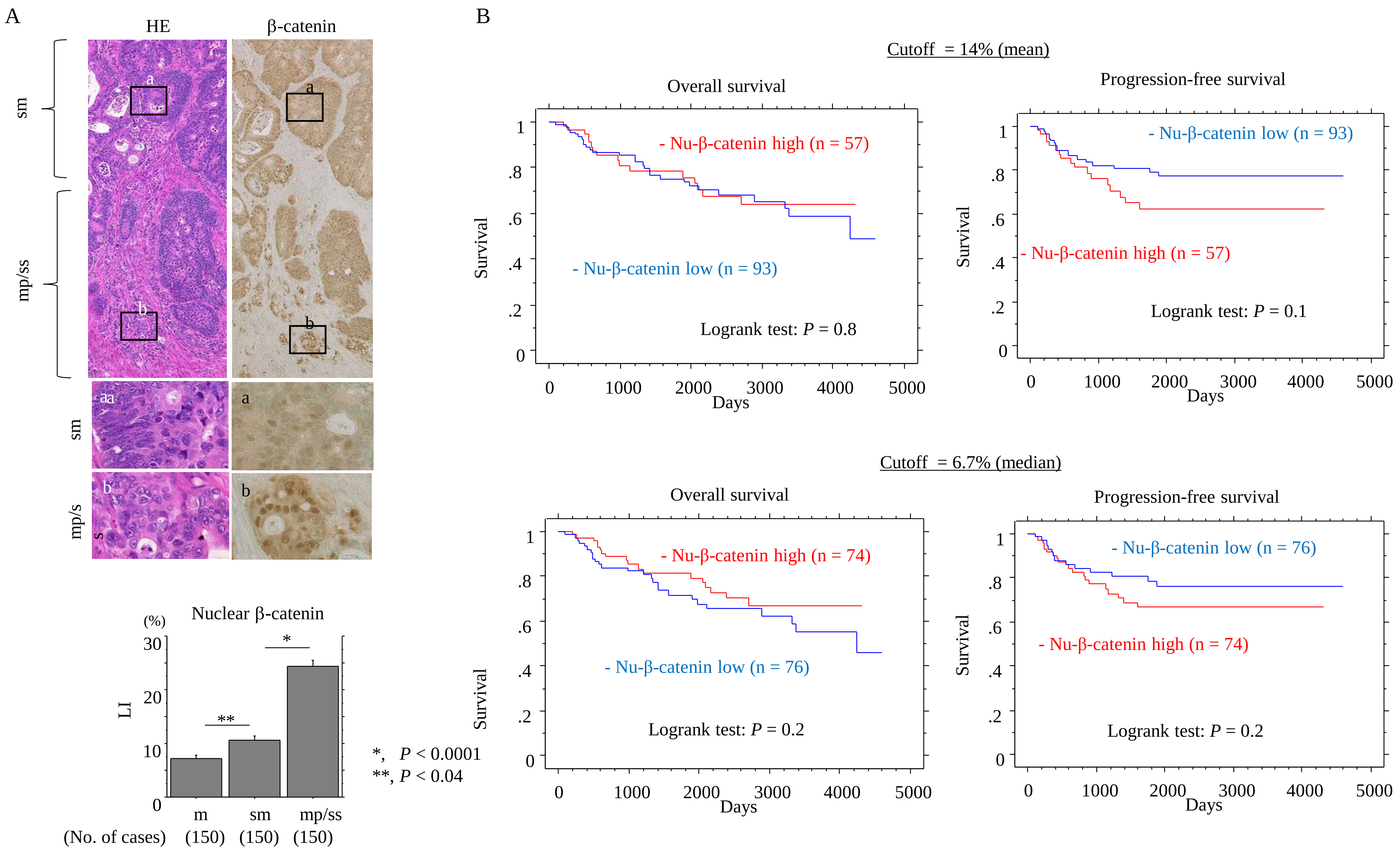
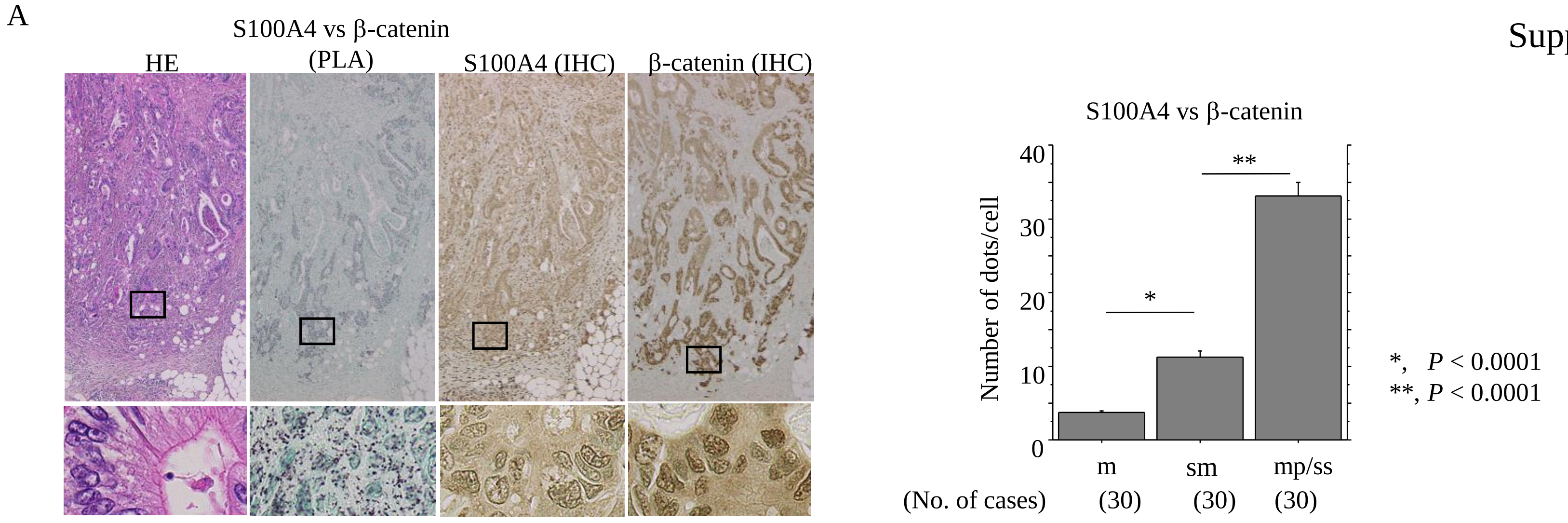
Supplementary Figure S1, Harada

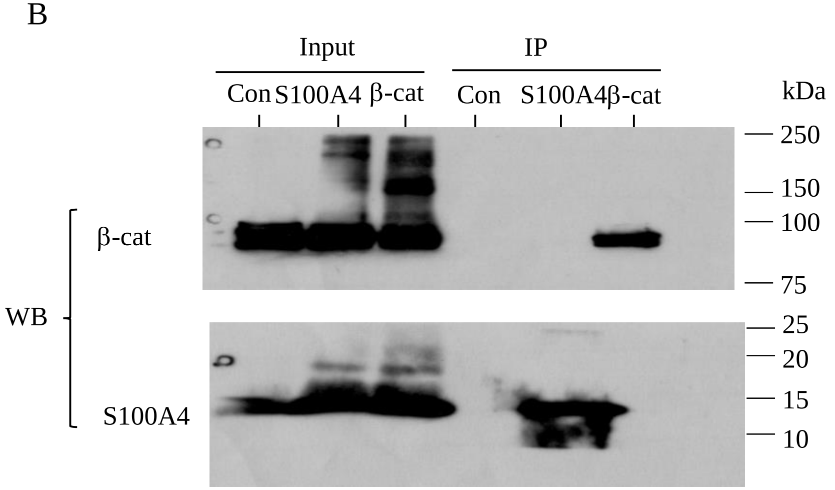


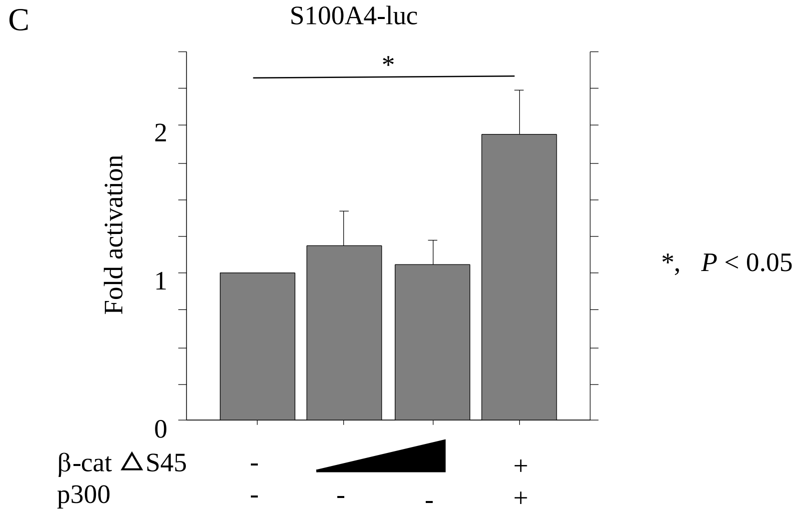
Supplementary Figure S1. (A,B) Dot plot analyses for S100A4 scores (A) and nuclear β-catenin LIs (B) in 150 Ad-CRC cases (left) and 150 NCRT-treated LAd-RC cases (right). The data are shown as mean ± SD. Based on the mean – standard deviation (SD), mean, or median values as cutoffs, cases are divided into two (high versus low expression) categories.



Supplementary Figure 2. Nuclear β-catenin accumulation in Ad-CRC. (A) Upper: staining with HE and IHC for β-catenin in Ad-CRC. Note the high levels of nuclear β-catenin at the invasive front in Ad-CRC. The closed boxes in the lower panels are magnified in the insets. Original magnification, x40 and x400 (insets). Lower: nuclear β-catenin LIs between mucosal (m), submucosal (sm), and muscularis propria (mp)/subserosal (ss) lesions of Ad-CRC. The data are presented shown as mean ± SD. Statistical analyses were carried out using the Mann-Whitney U-test. (B) OS (left) and PFS (roght) relative to nuclear β-catenin LI values (low versus high accumulation) based on the mean (upper) and median values (lower) as cutoffs in Ad-CRC. n, number of cases.

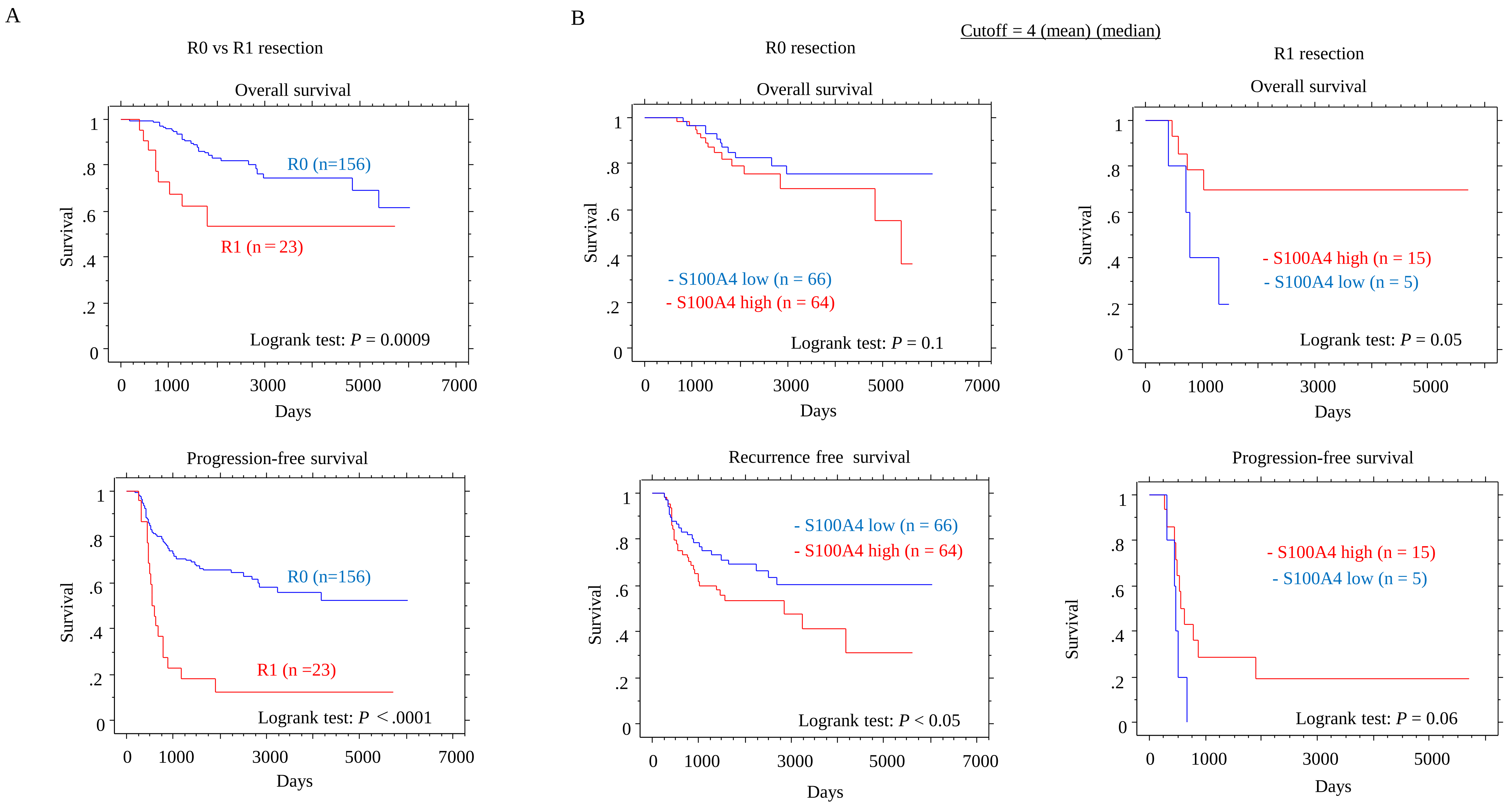




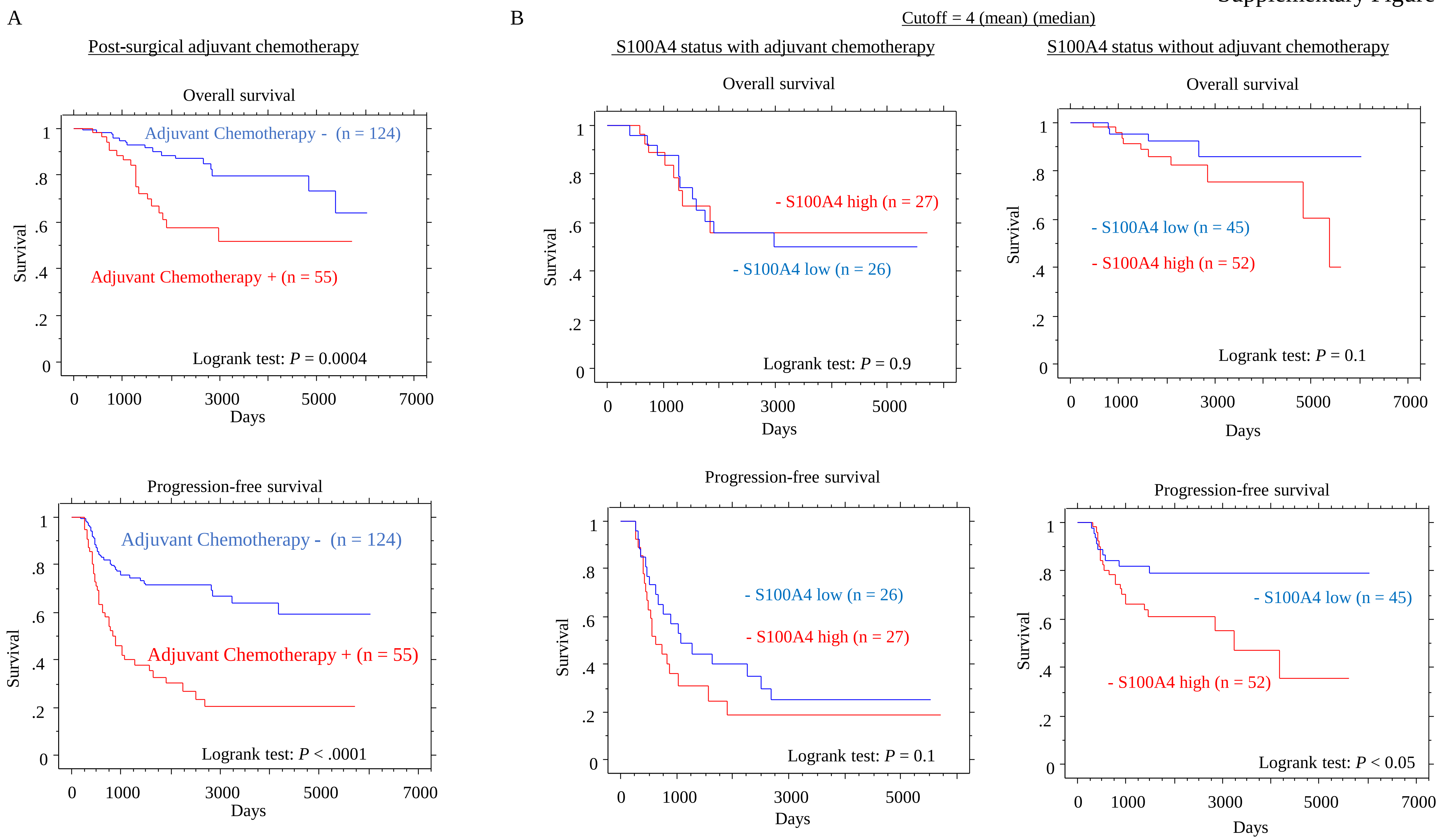


Supplementary Figure S3. Interaction between S100A4 and β-catenin in CRC cells. (A) Left: PLA assay for the S100A4/β-catenin interaction, as well as IHC for S100A4 and β-catenin, in Ad-CRC. Note the small aggregated dots in nuclear/cytoplasmic compartments of the tumor cells. The closed box in the PLA panel is magnified in the inset. Original magnification, x100 and x400 (inset). Right: numbers of PLA scores for S100A4/β-catenin combination between mucosal (m), submucosal (sm), and muscularis propria (mp)/subserosal (ss) lesions of Ad-CRC. The data are shown as mean ± SD. Statistical analyses were carried out using the Mann-Whitney U-test. (B) After immunoprecipitation (IP) with the indicated antibodies using HCT116 cell lysates, western blot assay (WB) with anti-β-catenin (upper panel) and anti-S100A4 antibodies (lower panel) was carried out. Input was 5% of the total cell extract. Normal rabbit IgG was used as a negative control. The reconstructed images of all blots with membrane edges visible are shown because some of the original full-length bots were cut prior to hybridization with antibodies. The experiments were performed in triplicate. (C) HCT116 cells were transfected with *S100A4* promoter luciferase (luc), together with β-catenin delS45 and p300, using LipofectAMINE PLUS. Relative activity was determined based on arbitrary light units of luciferase activity normalized to pRL-TK activity. The activities of the reporter plus the effector relative to that of the reporter plus empty vector are shown as means ± SDs. The experiments were performed in triplicate.

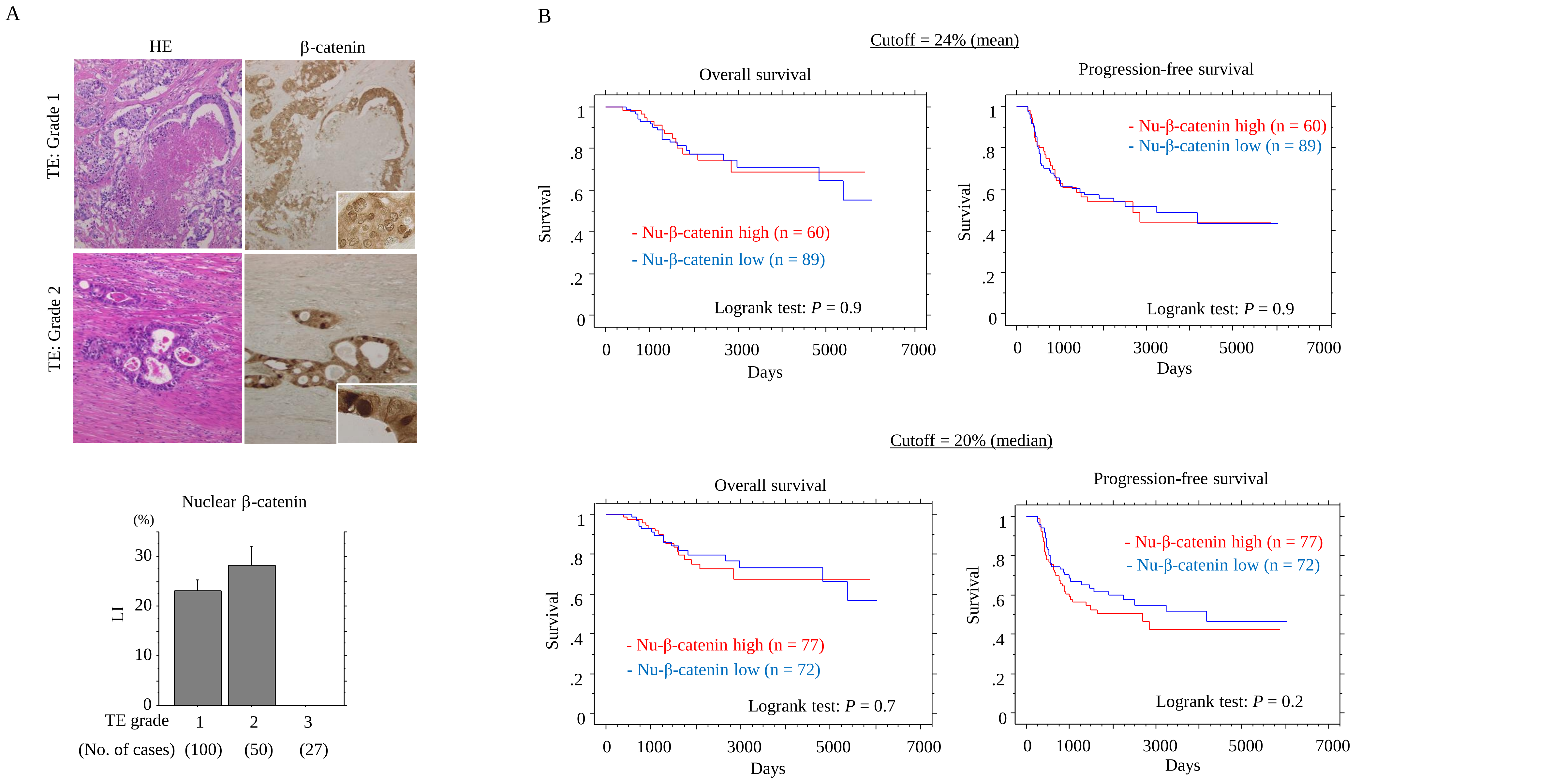
Supplementary Figure S4, Harada



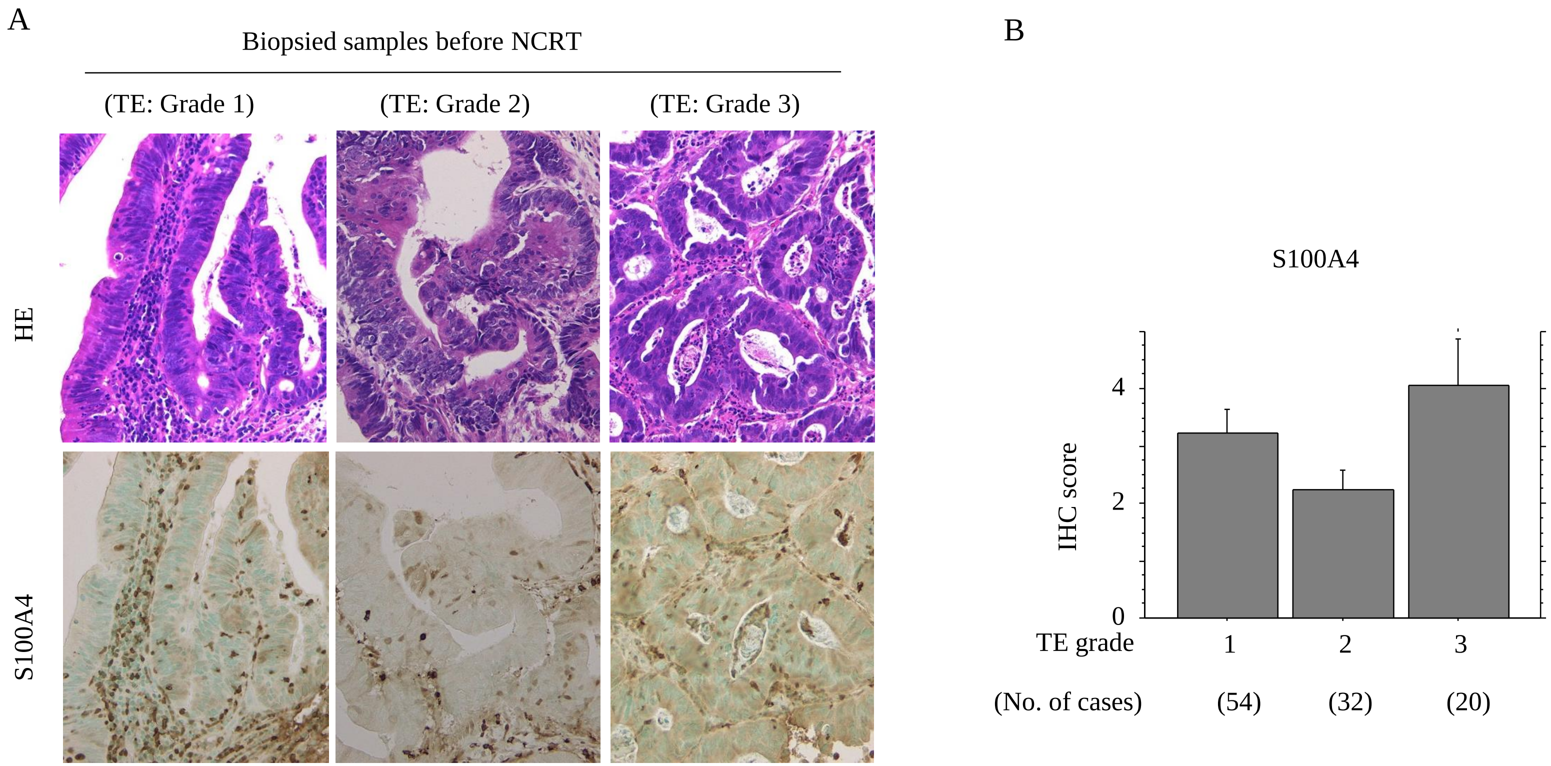
Supplementary Figure S4. Prognostic significance of residual (R) tumor status in NCRT-treated LAd-RC. (A) OS (upper) and PFS (lower) relative to residual tumors (R0 versus R1 resection) in NCRT-treated RC. (B) OS (upper) and RFS (lower-left) or PFS (lower-right) relative to S100A4 scores (low versus high expression) based on the mean and median values as cutoff in NCRT-treated RC with R0 (left) and R1 resection (right). n, number of cases.



Supplementary Figure S5. Prognostic significance of post-surgical adjuvant chemotherapy in NCRT-treated LAd-RC. (A) OS (upper) and PFS (lower) relative to post-surgical adjuvant chemotherapy [Yes (+) versus No (-)] in NCRT-treated RC. (B) OS (upper) and PFS (lower) relative to S100A4 scores (low versus high expression) based on the mean and median values as cutoff in NCRT-treated RC with (left) and without post-surgical adjuvant chemotherapy (right). n, number of cases.

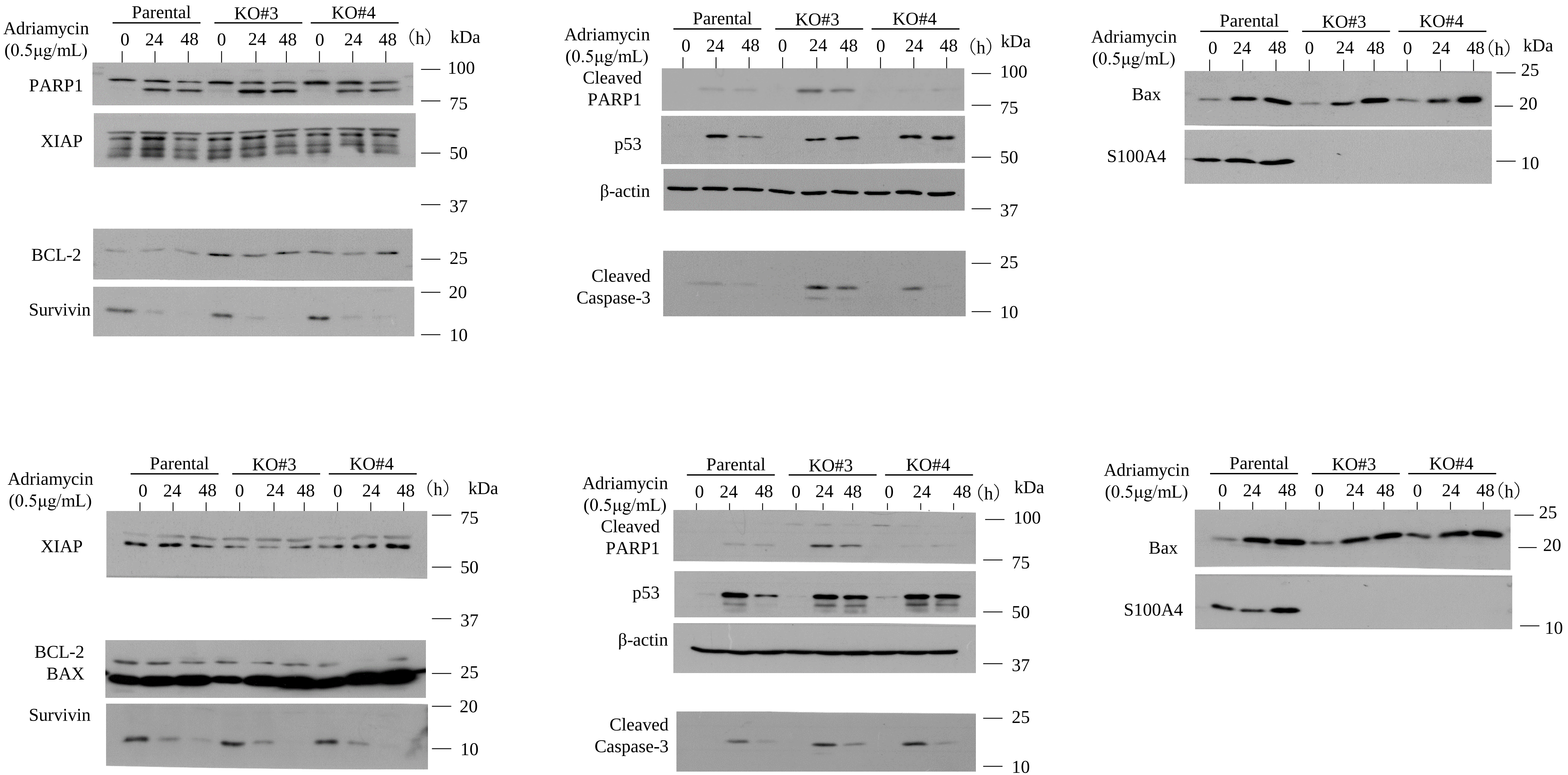


Supplementary Figure S6. Relationship between nuclear β-catenin accumulation and NCRT resistance in LAd-RC. (A) Upper: staining with HE and IHC for β-catenin in samples of LAd-RC cases that respond poorly to NCRT (TE: Grade 1) or respond moderately (TE: Grade 2). The closed boxes in the IHC panels are magnified in the insets. Original magnification, x100 and x400 (insets). Lower: nuclear β-catenin LIs in samples from NCRT-treated LAd-RC patients. The LIs are shown as mean ± SD. (B) OS (left) and PFS (right) relative to nuclear β-catenin (low versus high LIs) based on the mean (upper) and median LI values (lower) as cutoffs in LAd-RC receiving NCRT. N, number of cases.

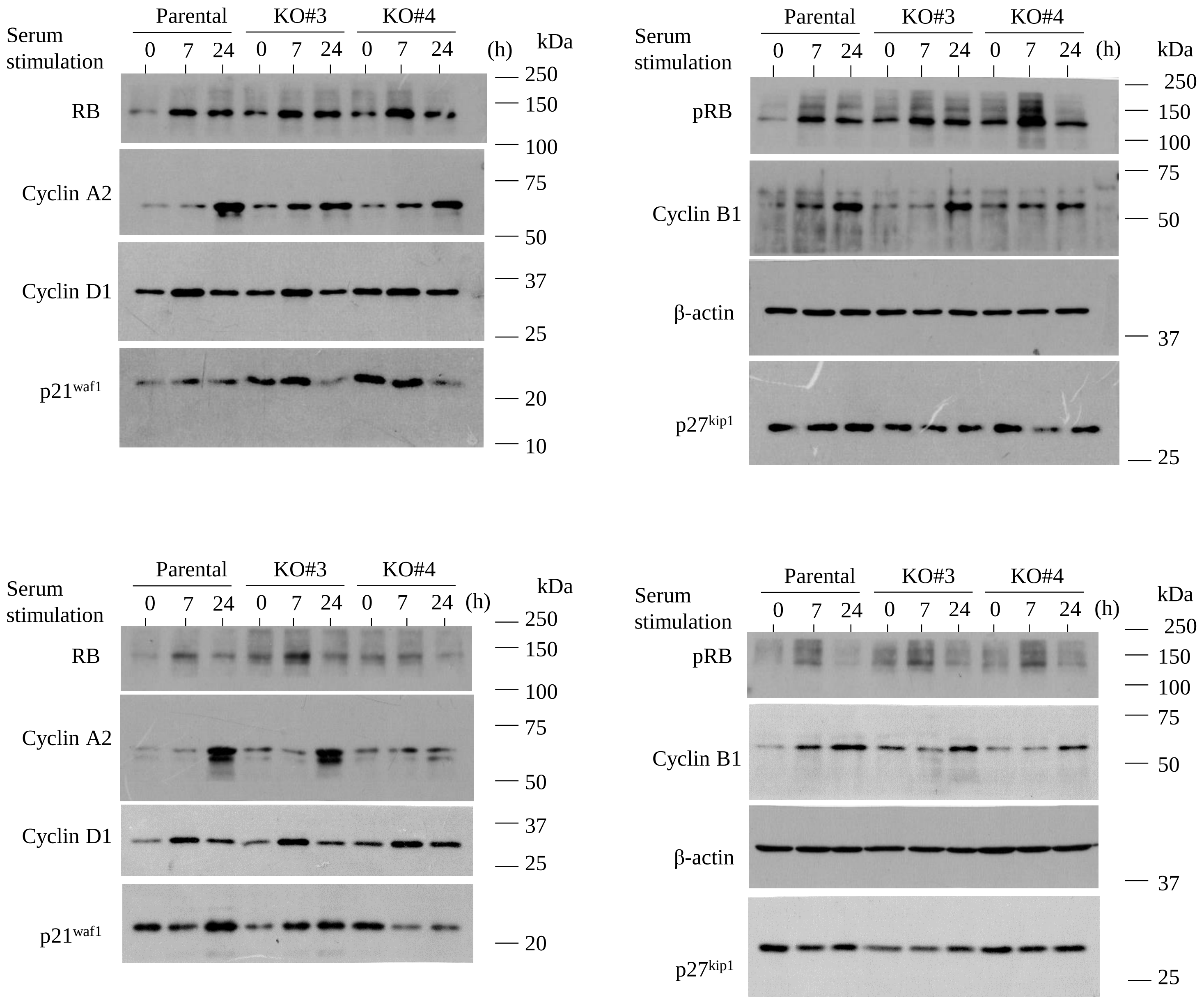


Supplementary Figure S7. Relationship between S100A4 expression in pretreatment-biopsied samples and TE grade in surgical specimens of NCRT-treated LAd-RC. (A) Staining with HE and IHC for S100A4 in pretreatment LAd-RC patient biopsies from TE: Grade 1 (left), TE: Grade 2 (middle), and TE: Grade 3 (right). Original magnification, x200. (B) IHC scores for S100A4 in pretreatment biopsied samples from TE grade 1, 2, and 3. The scores are shown as mean ± SD.

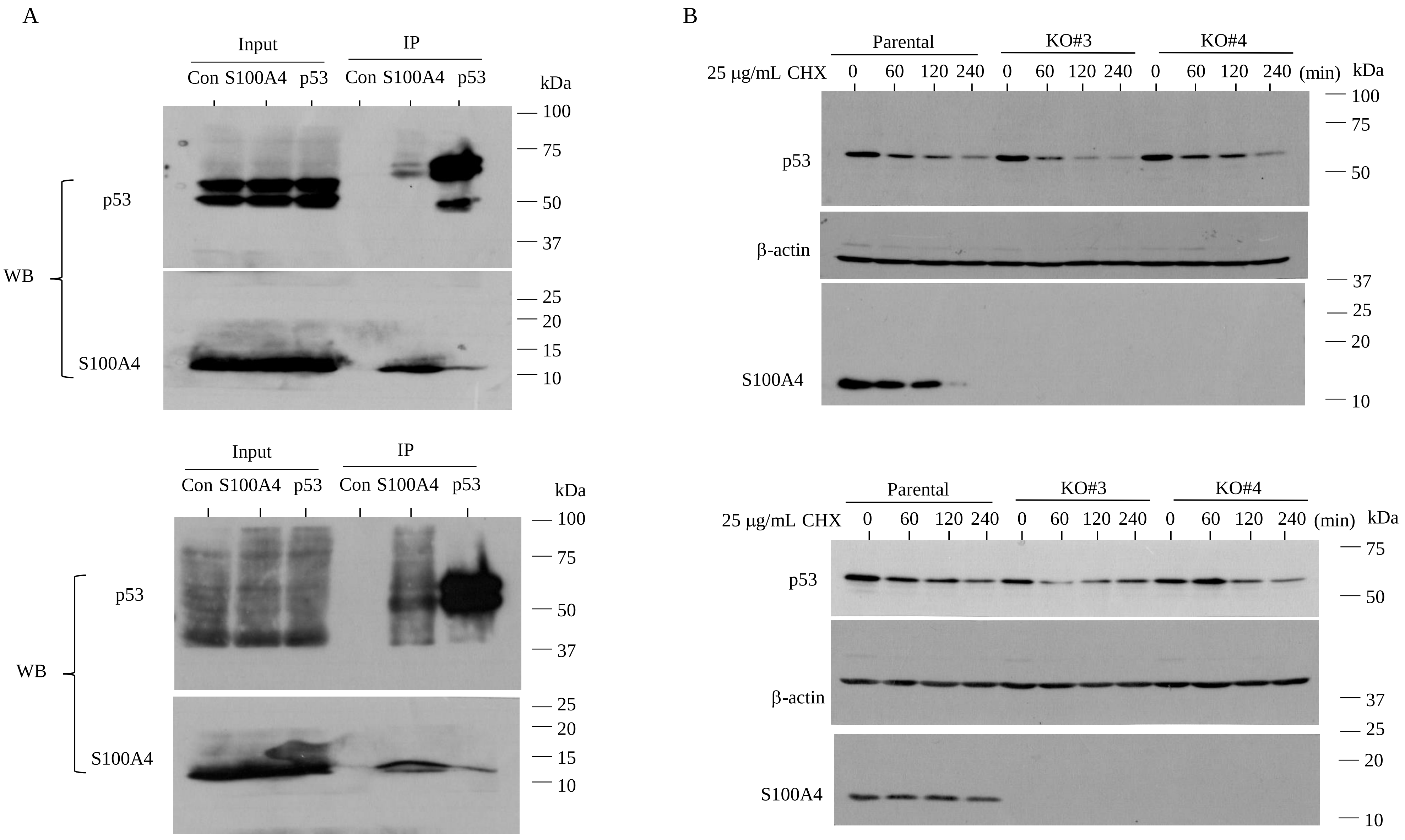
Supplementary Figure S8, Harada



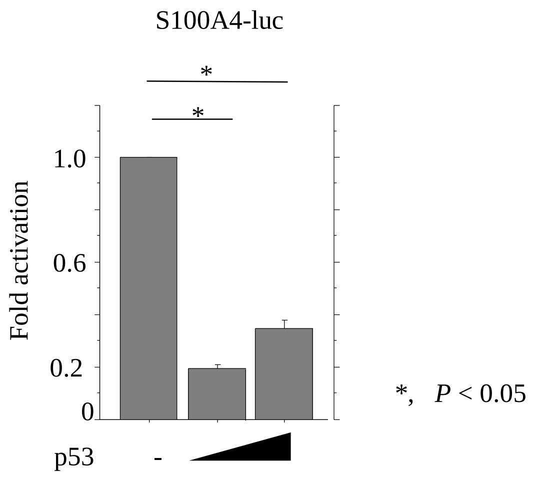
Supplementary Figure S8. Original images of western blot analysis for the indicated proteins in total lysates from HCT116-S100A4 KO and parental cells after 0.5 mg/mL ADR treatment.
The reconstructed images of all blots with membrane edges visible are shown because some of the original full-length blots were cut prior to hybridization with antibodies (upper) and the replicate blots (lower).



Supplementary Figure S9. Original images of western blot analysis for the indicated proteins in total lysates from HCT116-S100A4 KO and parental cells following re-stimulation of serum-starved (24 h) cells with 10% serum for the indicated times. The reconstructed images of all blots with membrane edges visible are shown because some of the original full-length blots were cut prior to hybridization with antibodies (upper) and the replicate blots (lower).



Supplementary Figure S10. Original images of western blot analysis for the indicated proteins in co-IP-western blot (A) and in total lysates from HCT116-S100A4 KO and parental cells treated with 25 mg/mL cycloheximide (CHX) at the indicated timepoints (B). The reconstructed images of all blots with membrane edges visible are shown because some of the original full-length blots were cut prior to hybridization with antibodies (upper) and the replicate blots (lower).



Supplementary Figure S11. p53-dependent repression of the *S100A4* promoter. HCT116 cells were transfected with *S100A4* promoter luciferase (luc), together with p53 using LipofectAMINE PLUS. Relative activity was determined based on arbitrary light units of luciferase activity normalized to pRL-TK activity. The activities of the reporter plus the effector relative to that of the reporter plus empty vector are shown as means ± SDs. The experiments were performed in triplicate.