Supplementary Data for A.C.-K.Chung et al.
CAN-06-3168 Version 2

Supplementary Fig. 1, Fig. 2, and Fig. 3

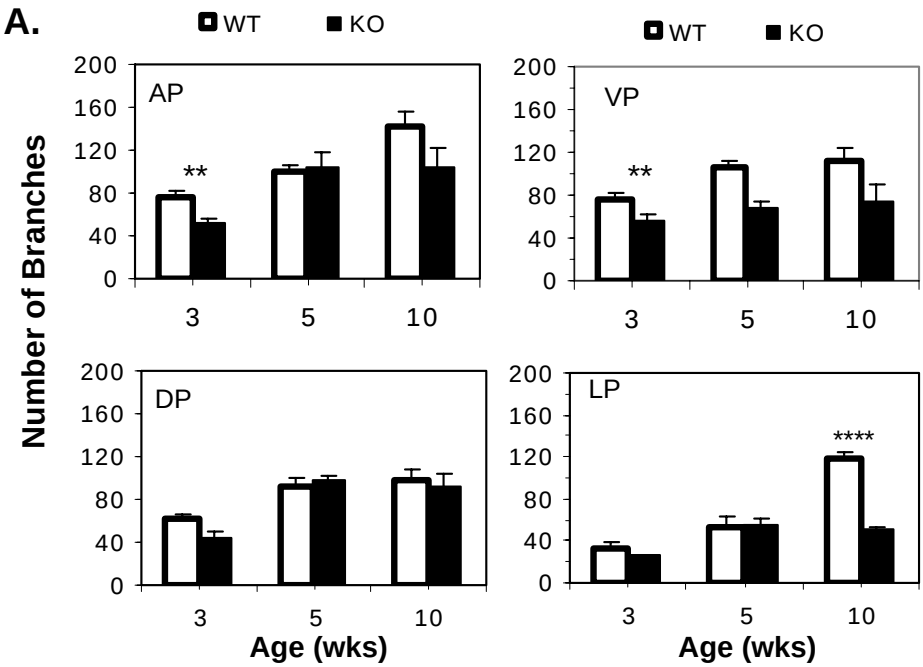
Supplementary Fig. 1. Comparison of androgen-regulated prostate morphogenesis in WT and AIB1$^{-/-}$ mice.

A. Comparison of branching points in WT and AIB1$^{-/-}$ (KO) mice with indicated ages. For each time point, at least three animals were analyzed. Data are presented as Mean ± SE. ** or ****, $p < 0.05$ or $p < 0.0005$ by unpaired t test.

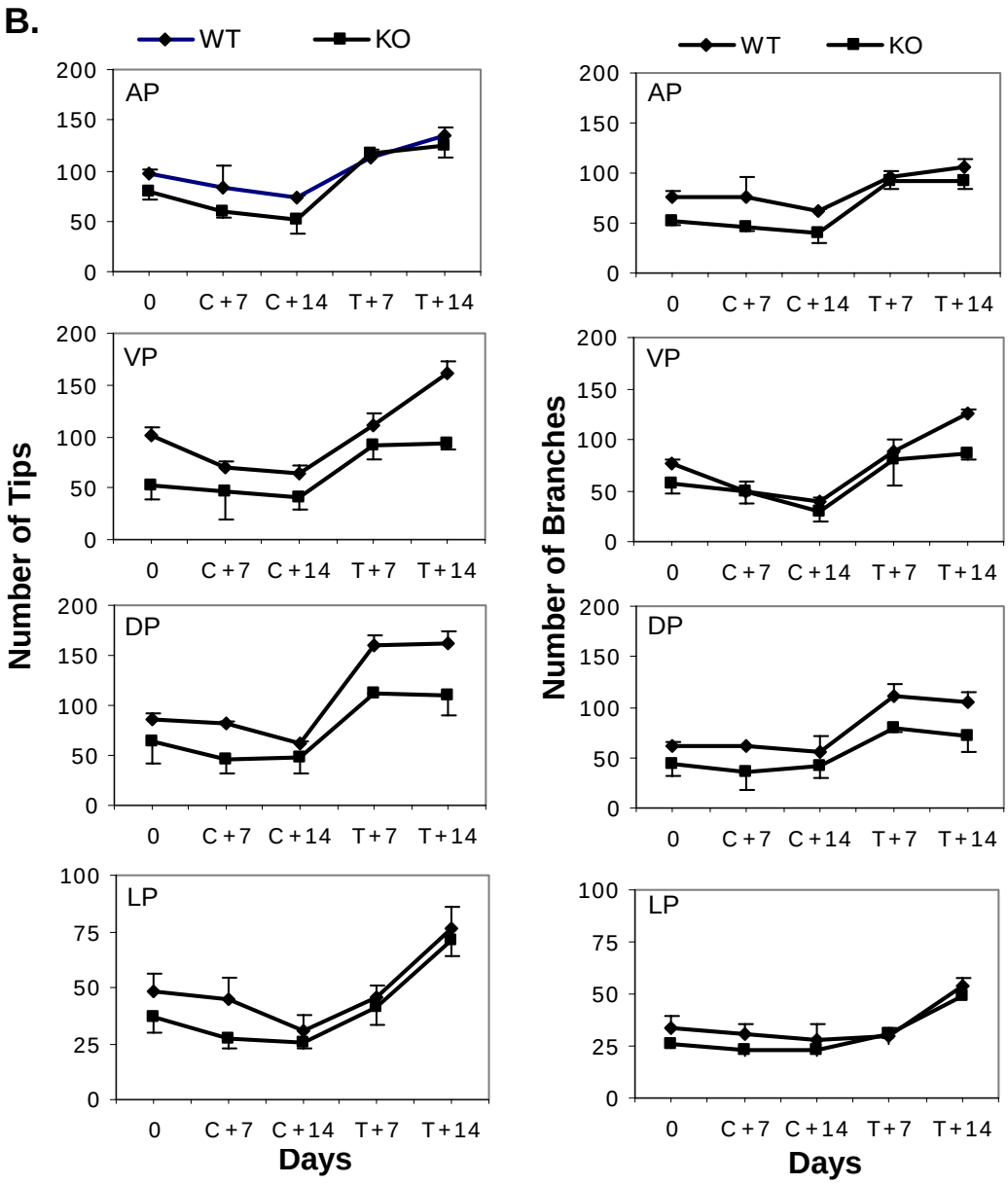
B. Comparison of androgen-mediated prostate growth in young WT and AIB1$^{-/-}$ (KO) mice. Mice were castrated at the age of 3 weeks and testosterone was replaced for 2 weeks from castration day 14 (C+14) to testosterone treatment day 14 (T+14). Prostates were micro-dissected and examined at indicated time points. For each time point, at least five animals were used. There were no statistical differences at all time points.

C. Comparison of androgen status-dependent prostate regression and regeneration in adult WT and AIB1$^{-/-}$ (KO) mice. 10-week-old mice (n=6 for each time point) were castrated on day 0 and testosterone was replaced from day C+21 to day T+14 as indicated on X-axis. The AP, VP, DP and LP lobes were miscro-dissected at all time points. The numbers of prostatic distal tips and branching points were counted and presented as Mean ± SE. WT and AIB1$^{-/-}$ prostates showed similar regression profiles after castration and regeneration profiles after testosterone treatment.

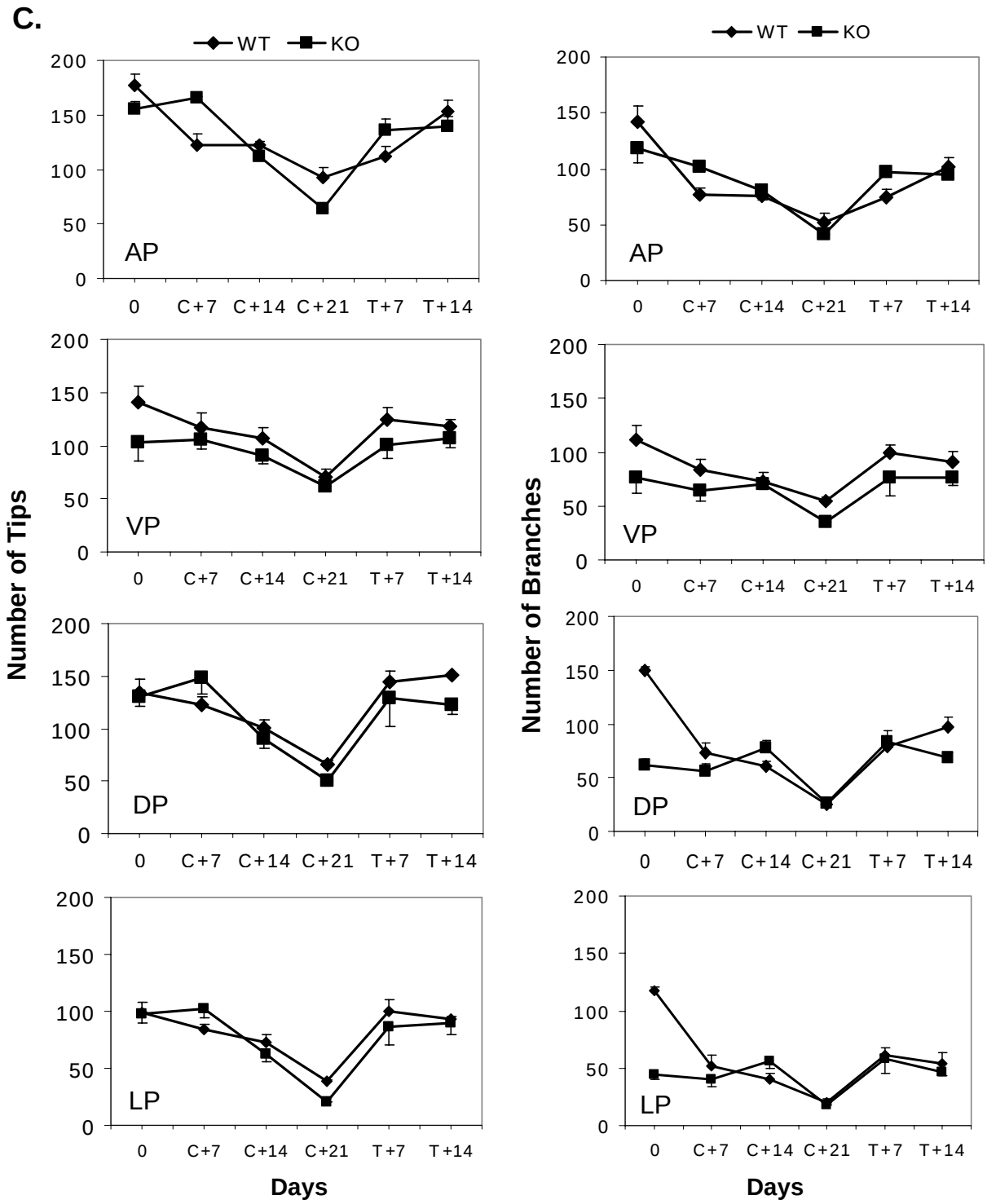
Supplementary Fig. 1A



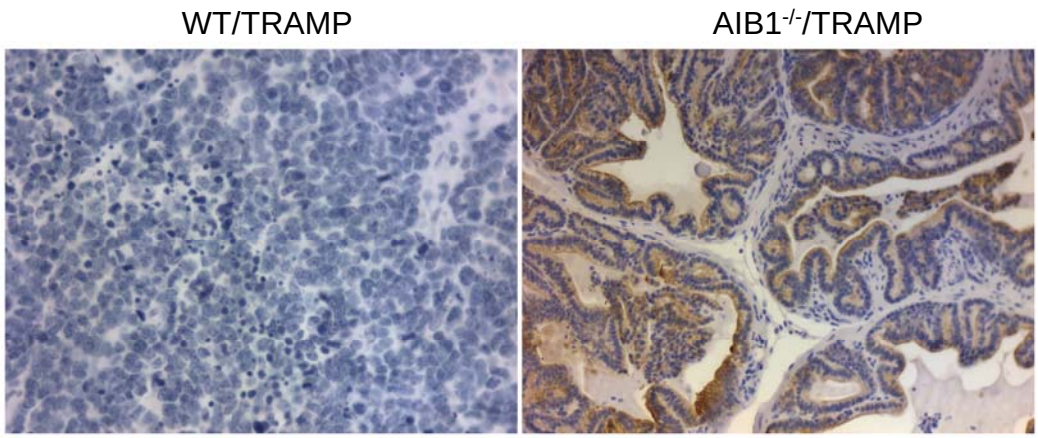
Supplementary Fig. 1B



Supplementary Fig. 1C



Supplementary Fig. 2. AIB1 deficiency protects cytokeratin 8 (CK8) expression in prostate tumors. Histological sections were prepared from tissues/tumors of DP lobes of WT/TRAMP and 30-week-old AIB1$^{-/-}$/TRAMP mice. IHC was performed with a CK8 antibody and images were taken at 200X magnification. The sections were counterstained with hematoxylin. Brown color reflects CK8 immunoreactivity, which is only seen in the WD carcinomas of AIB1$^{-/-}$/TRAMP prostates but not in the PD carcinomas of WT/TRAMP prostates.



Supplementary Fig. 3. Inactivation of AIB1 has no obvious effect on cell apoptosis in the prostate.

A. TUNEL assay of the DP lobes isolated from WT/TRAMP and AIB1$^{-/-}$/TRAMP mice with indicated ages. TUNEL assay was performed on deparaffinized and rehydrated tissue sections by using a TdT-FragEL DNA-fragmentation detection kit (EMD Biosciences, La Jolla, CA). Sections were counterstained with hematoxylin. Images were taken at 400X magnification. Arrows indicate representative apoptotic cells in brown color.

B. Quantitative analysis of apoptotic indices in WT/TRAMP (WT) and AIB1$^{-/-}$/TRAMP (KO) DP lobes. The percentage of apoptotic cells was determined by counting the number of TUNEL-positive cells and the number of total cells. For each age and genotype group, prostate sections were prepared from at least three animals, and at least 500 prostate cells were counted from at least three randomly selected areas from each section. **, $P < 0.05$ by unpaired t test. The “All” on the X-axis is the average of all AP, VP, DP and LP lobes.

Supplementary Fig. 3, A&B