Supplementary Materials

Supplemental figures and legends

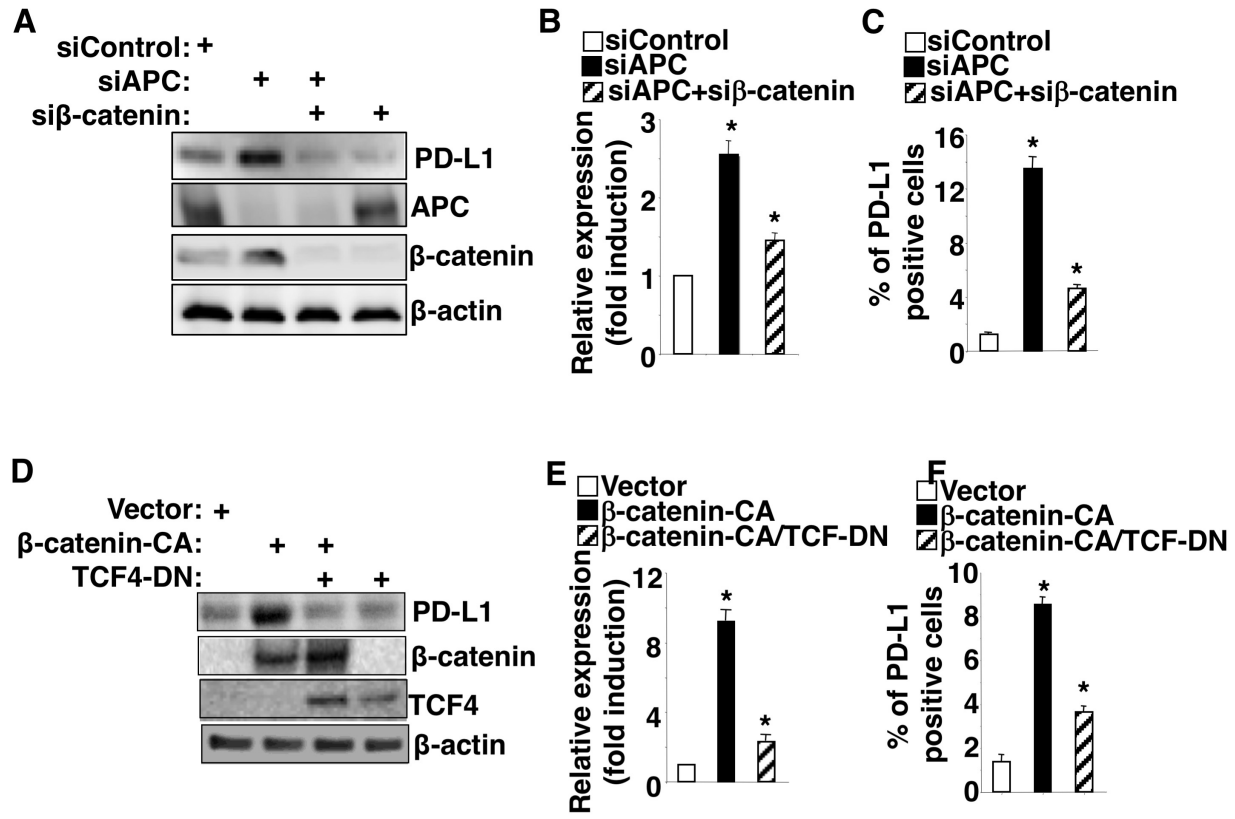

Fig. S1. **Knockdown of *APC* induces PD-L1 expression via a β-catenin/TCF4 pathway**

293T cells were transiently transfected with indicated siRNA or expression plasmid. After transfection, cells were cultured for 48 hr and subjected to Western blot, q-PCR, and flow cytometry. (**A** and **D**) PD-L1 protein levels in 293T cells transfected with siControl, siAPC, siβ-catenin, constitutively active β-catenin (β-catenin-CA) plasmid, and/or dominant negative TCF4 (TCF4-DN) plasmid were measured by Western blot. (**B** and **E**) PD-L1 mRNA levels in 293T cells transfected with siControl, siAPC, siβ-catenin, β-catenin-CA plasmid, and/or TCF4-DN plasmid were measured by q-PCR. (**C** and **F**) The cell surface PD-L1 protein levels in 293T cells transfected with siControl, siAPC, siβ-catenin, β-catenin-CA plasmid, and/or TCF4-DN plasmid were measured by flow cytometry. Each Western blot image is representative of three

independent experiments with similar results. Data are presented as mean ± SEM of three independent experiments. *p<0.05.

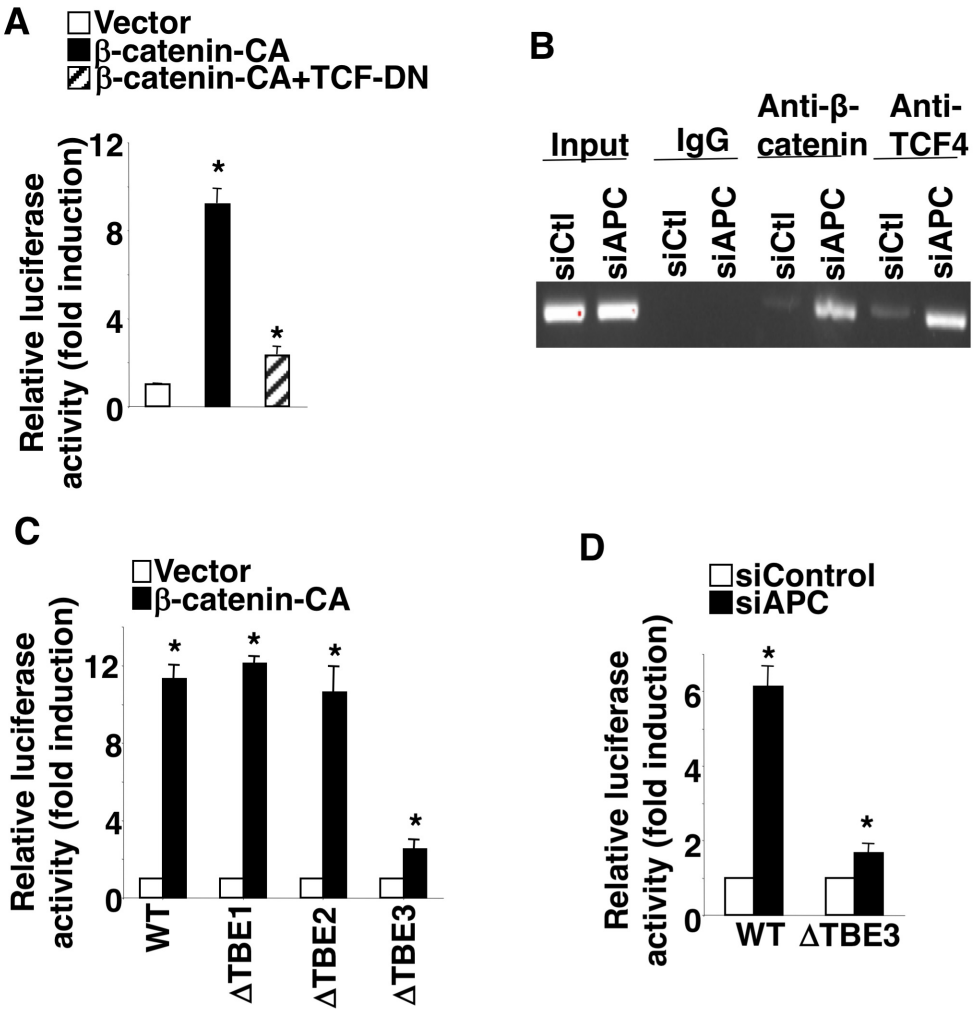

Fig. S2. Loss of APC induces PD-L1 transcription by binding of β-catenin/TCF4 complex to PD-L1 promoter

(**A**) 293T cells were transiently co-transfected with PD-L1 promoter luciferase reporter and pRL-SV40 *Renilla* luciferase plasmids plus control vector, constitutively active β-catenin (β-catenin-CA), and/or dominant negative TCF4 (TCF4-DN) plasmids. (**B**) A representative image of three independent ChIP assays for binding of the β-catenin/TCF4 complex to TBE3 of the PD-L1

promoter in 293T/si*Control* and 293T/si*APC* cells. Immunoprecipitation with IgG antibody was used as a control. (**C-D**) Luciferase activity of the wild-type and mutant PD-L1 promoters was measured in 293T/vector, 293T/β-catenin-CA, and 293T/si*APC* cells. ΔTBE1, ΔTBE2, and ΔTBE3 indicate mutant PD-L1 promoter with deletion of either TBE1, TBE2, or TBE3. Data are presented as mean ± SEM of relative luciferase activity from three independent experiments. *$p<0.05$.

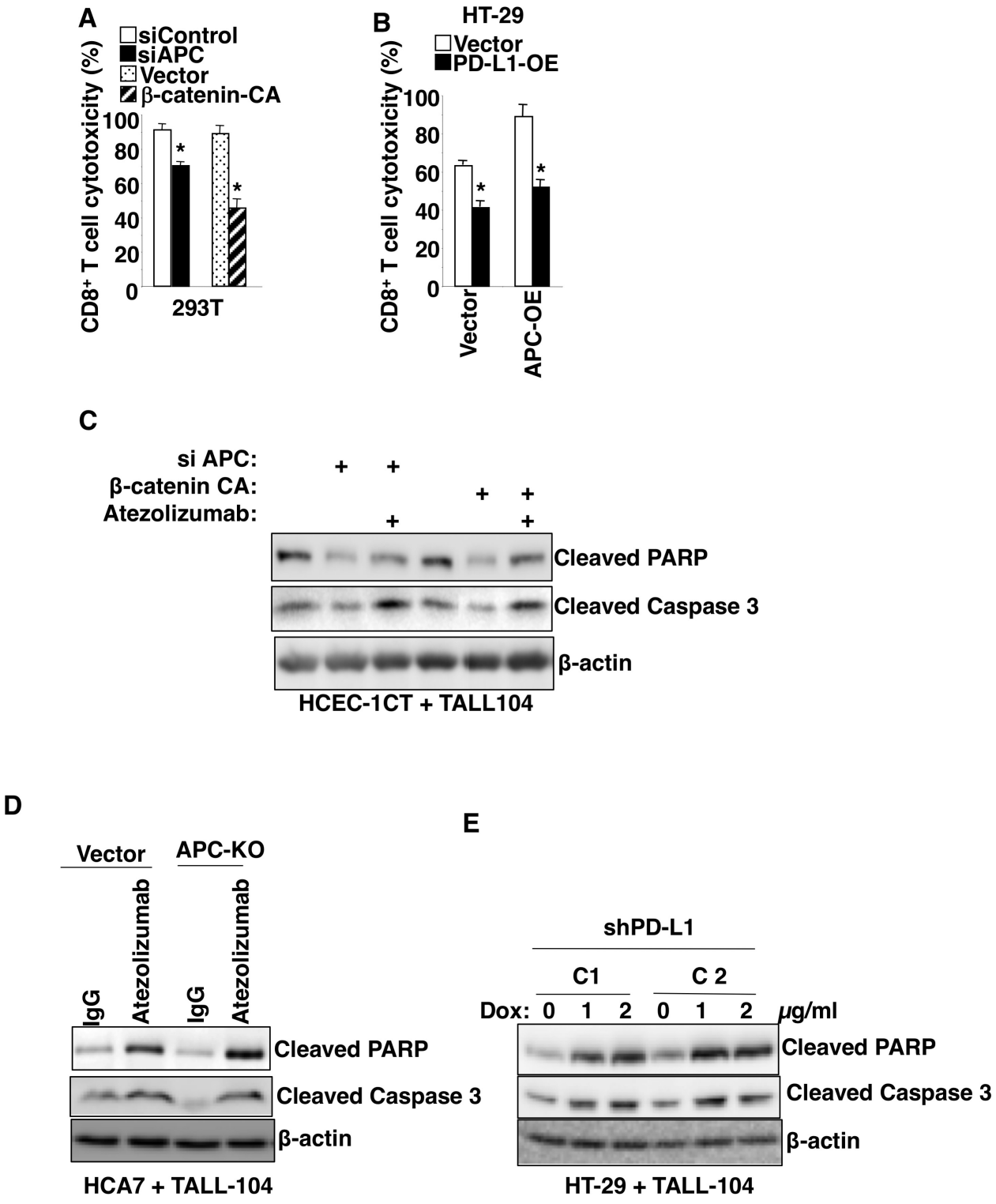

Fig. S3. Loss of APC induces cell resistance to CD8+ T cell cytotoxicity by induction of PD-L1 via the β-catenin/TCF4 pathway

(**A-B**) Cytotoxicity of TALL-104 cells against 293T/si*Control*, 293T/si*APC*, 293T/vector, HCEC-1CT/β-catenin-CA, 293T/vector, or 293T/PD-L1-OE cells was determined as described in the Method section. PD-L1-OE: PD-L1 overexpression. (C) The protein levels of cleaved PARP and caspase 3 in indicated cells after coculture with TALL-104. (D) The protein levels of cleaved PARP and caspase 3 in indicated cells after coculture with TALL-104 treated with IgG or atezolizumab. (E) The protein levels of cleaved PARP and caspase 3 in 293T/vector and 293T/sh*APC* cells after coculture with TALL-104.

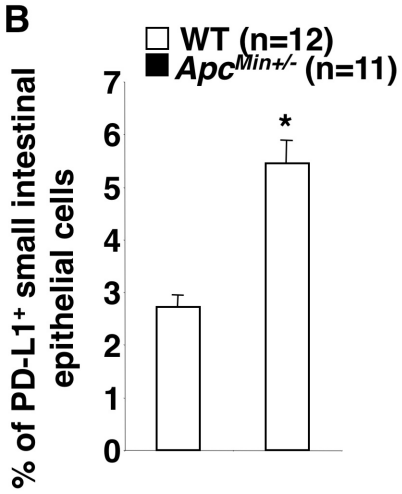

Fig. S4. PD-L1 expression is elevated in intestinal epithelial cells of *Apc*$^{Min+/-}$ mice as compared to WT mice

The percentage of PD-L1-positive intestinal epithelial cells was determined by flow cytometry in the indicated group of mice.