Figure S1. Interactions of the RGDKGE-containing collagen peptide P2 with tumor cells. (A) Quantification of B16F10 melanoma cell binding to control peptide CP and collagen peptide P2. (B) Quantification of 4T1 mammary carcinoma cell binding to control peptide CP and collagen peptide P2. (C) Quantification of C32 melanoma cell binding to control peptide CP and collagen peptide P2. (D) Quantification of YUMM1.7 melanoma cell binding to control peptide CP and collagen peptide P2. (E) Quantification of YUMM1.7 cell binding to collagen peptide P2 in the presence of non-specific control antibody (Ab Cont) or anti-RGDKGE antibody (Mab XL313). (F) Quantification of C32 cell binding to collagen peptide P2 in the presence of non-specific control antibody (Ab Cont) or anti-RGDKGE antibody (Mab XL313). Data bars represent mean cell binding (± SEM) from triplicate wells. Experiments were completed 2-3 times with similar results. *P<0.05.

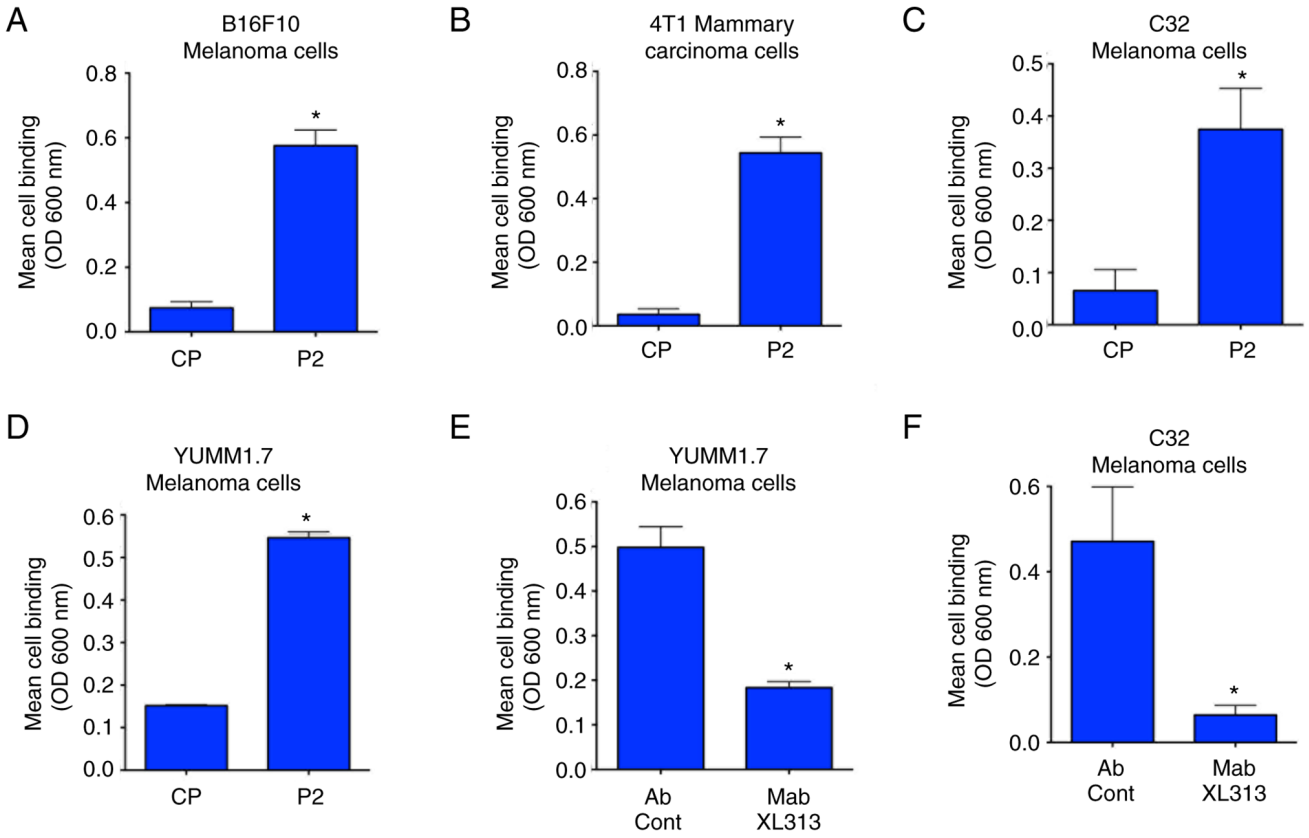

Figure S2. Effects of Brefeldin A and Cathepsin inhibitor on the levels of RGDKGE-containing collagen fragment in tumor cells. (A) Western blot analysis of whole cell lysates (LY) and serum free conditioned medium (CM) for the 16Kd RGDKGE-containing collagen fragment or loading control tubulin in C32 melanoma cells treated with Brefeldin-A. (B) Western blot analysis of whole cell lysates (LY) and serum free conditioned medium (CM) for the 16Kd RGDKGE-containing collagen fragment or loading control tubulin in YUMM1.7 melanoma cells treated with Brefeldin-A. (C) Western blot analysis of whole cell lysates for the 16Kd RGDKGE-containing collagen fragment or loading control tubulin in C32 melanoma cells treated with cathepsin inhibitor. (D) Western blot analysis of whole cell lysates for the 16Kd RGDKGE-containing collagen fragment or loading control tubulin in YUMM1.7 melanoma cells treated with cathepsin inhibitor. Experiments were completed twice with similar results.

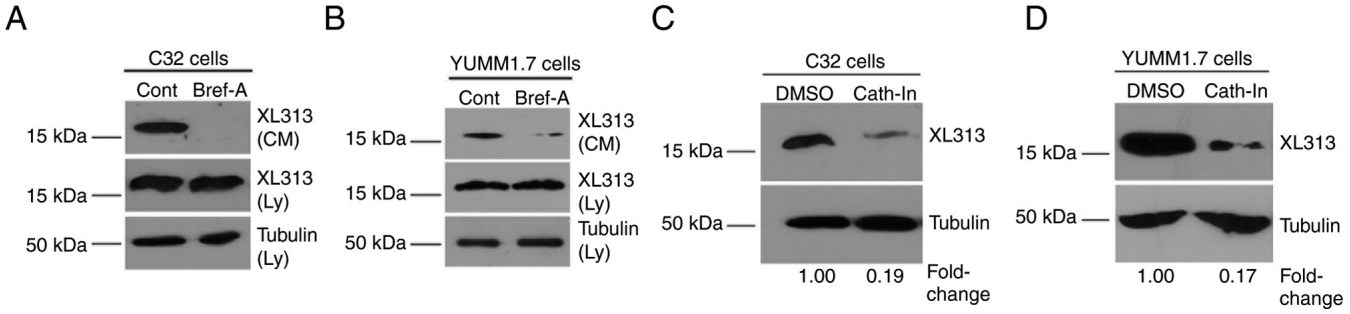

Figure S3. β3 integrin facilitates binding of B16F10 cells to the RGDKGE-containing collagen peptide P2 and blocking β3 integrin reduce levels of PD-L1. (A) Immunofluorescence staining analysis for the expression of PD-L1 in B16F10 melanoma cells. Example of the expression of PD-L1 (Red) in B16F10 melanoma cells. Photos were taken at a magnification of 400x. White bar indicates 20 microns. (B) Quantification of B16F10 cell binding to collagen peptide P2 in the presence of non-specific control antibody (Ab Cont), function blocking anti-β3-integrin antibody (Anti-β3) or function blocking anti-β1-integrin antibody (Anti-β1). Data bars represent mean cell binding (+ SEM) from 3 independent experiments with control antibody treatment set to 100 percent for comparison. P-value indicates comparison to antibody control. *P<0.05. (C) Analysis of the effects of 100 ug/ml treatment with anti-XL313 antibody (Mab XL313), non-specific control antibody (Ab Cont) or lysis buffer as a positive control (Positive Cont) on B16F10 cell cytotoxicity *in vitro* following 24 h incubation. Data bars represent mean induction of cytotoxicity + SE from triplicate wells. Experiments were completed 3 time with similar results. (D) Analysis of the effects of 100 ug/ml treatment with anti-XL313 antibody (Mab XL313) or non-specific control antibody (Ab Cont) on B16F10 cell growth *in vitro* using MTT assay following 24 h incubation. Data bars represent mean cell growth + SE from triplicate wells. Experiments were completed 3 time with similar results. *P<0.05. (E) Western blot analysis of whole cell lysates for PD-L1 or loading control tubulin in B16F10 cells treated for 15 min with anti-β3 integrin antibody (Anti-β3) or non-specific control (Ab Cont). Experiments were completed 3 time with similar results.

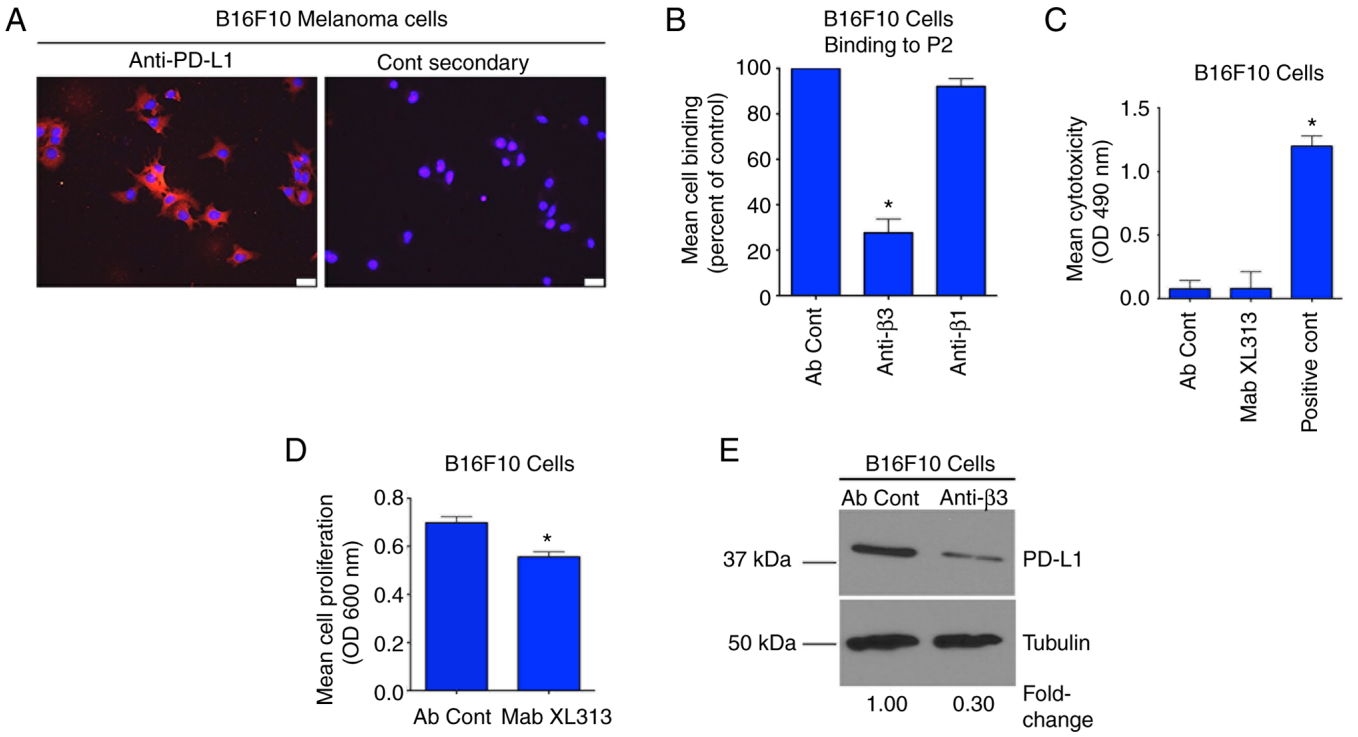

Figure S4. Mab XL313 regulates levels of PD-L1 in tumor cell. (A) Western blot analysis of B16F10 whole cell lysates for PD-L1 or loading control tubulin in B16F10 cells treated for 15 min with non-specific control (Ab Cont) or Mab XL313. Experiments were completed 3 time with similar results. (B) Quantification of the mean fold change (+ SEM) in PD-L1 mRNA levels in B16F10 cells treated with non-specific control antibody (Ab Cont) or anti-RGDKGE collagen fragment antibody (Mab XL313) for 1 h. Data bars represent mean (+ SEM) fold change in mRNA levels from 3 independent experiments. (C) Quantification of the mean fold change (+ SEM) in PD-L1 mRNA levels in B16F10 cells treated with non-specific control antibody (Ab Cont) or anti-RGDKGE collagen fragment antibody (Mab XL313) for 3 h. Data bars represent mean (+ SEM) fold change in mRNA levels from 3 independent experiments.

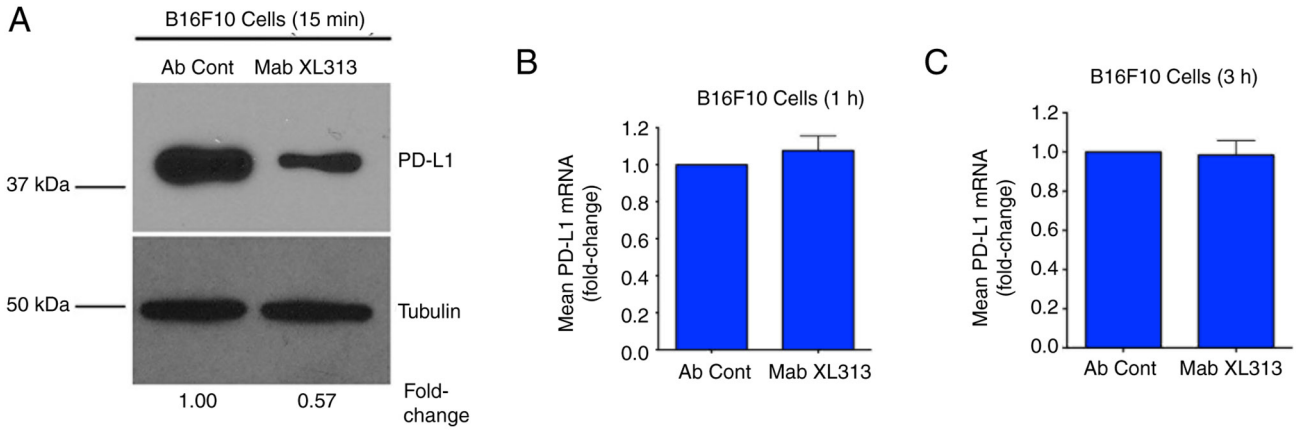

Figure S5. Proteasome inhibitor prevents Mab XL313 from reducing PD-L1 levels in tumor cells. (A) C32 cells were pre-treated with control buffer DMSO or proteasome inhibitor MG132 (10 uM) for 1 h. Cells were next washed and treated with control antibody or Mab XL313 for 15 min and lysates prepared. Example of western blot analysis for PD-L1 or loading control tubulin from each condition. (B) YUMM1.7 cells were pre-treated with control buffer DMSO or proteasome inhibitor MG132 (10 uM) for 1 h. Cells were next washed and treated with control antibody or Mab XL313 for 15 min and lysates prepared. Example of western blot analysis for PD-L1 or loading control tubulin from each condition. Experiments were conducted 2 to 3 times with similar results.

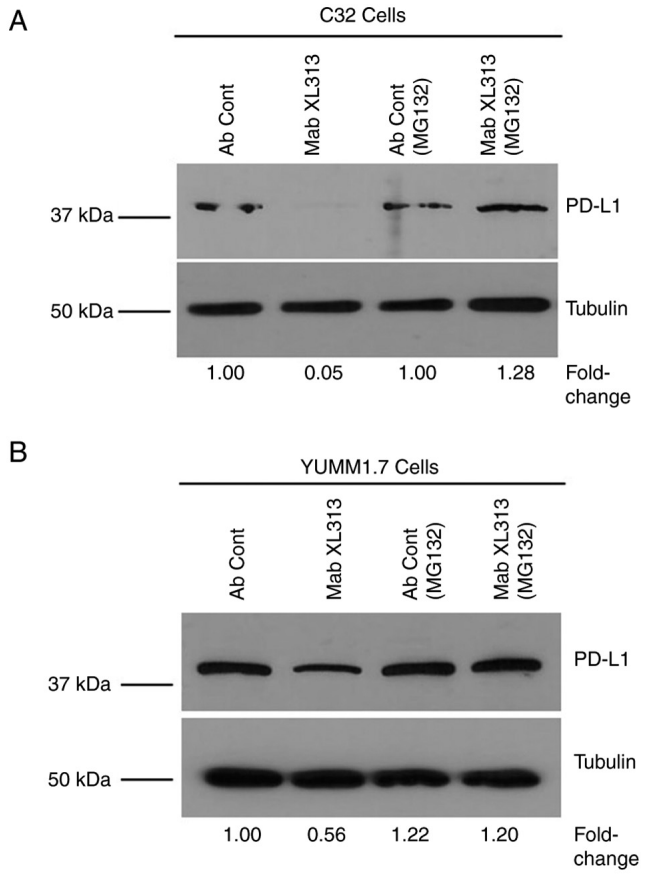

Figure S6. PKA inhibitor prevents Mab XL313 from reducing PD-L1 levels in tumor cells. (A) B16F10 cells were pre-treated with control buffer DMSO or PKA inhibitor KT5720 (5 uM) for 1 h. Cells were next washed and treated with control antibody or Mab XL313 for 15 min and lysates prepared. Example of western blot analysis for PD-L1 or loading control tubulin from each condition. (B) C32 cells were pre-treated with control buffer DMSO or PKA inhibitor H89 (10 uM) for 1 h. Cells were next washed and treated with control antibody or Mab XL313 for 15 min and lysates prepared. Example of western blot analysis for PD-L1 or loading control tubulin from each condition. (C) YUMM1.7 cells were pre-treated with control buffer DMSO or PKA inhibitor H89 (10 uM) for 1 h. Cells were next washed and treated with control antibody or Mab XL313 for 15 min and lysates prepared. Example of western blot analysis for PD-L1 or loading control tubulin from each condition. Experiments were conducted 2 to 3 times with similar results.

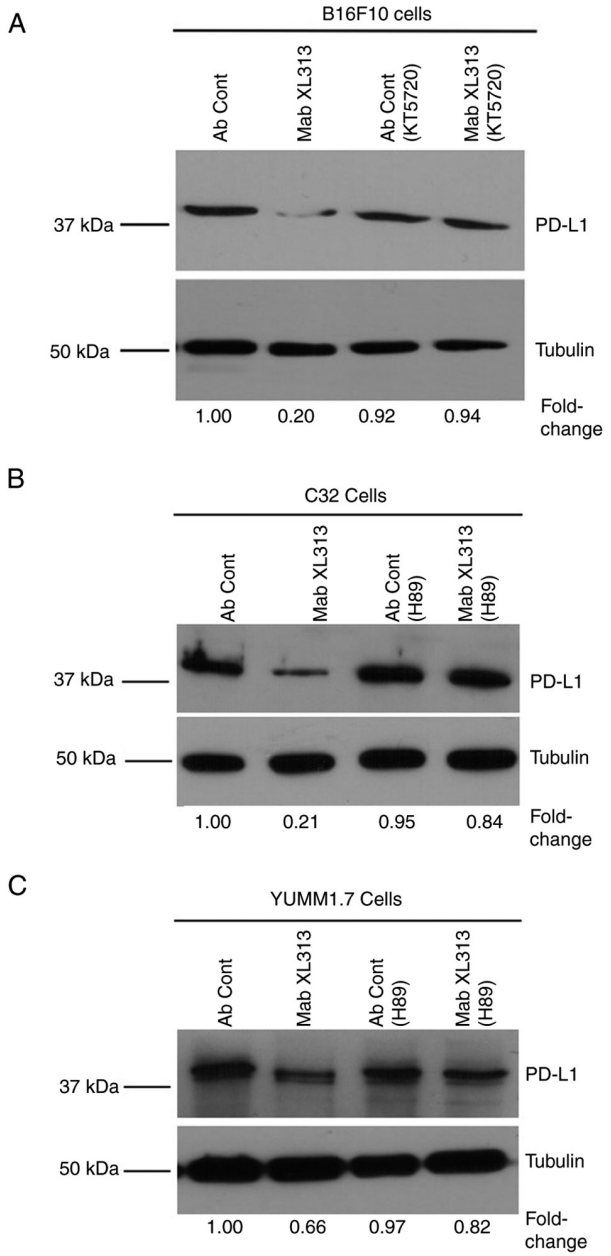

Figure S7. Knock-down of PD-L1 expression in B16F10 melanoma cells. Western blot analysis of whole cell lysates for PD-L1 or loading control tubulin in control shRNA transfected (NT-Cont) and pd-L1-shRNA transfected B16F10 cells (PD-L1-K/D). Relative fold change in levels of protein expression was determined by Image J version 5.