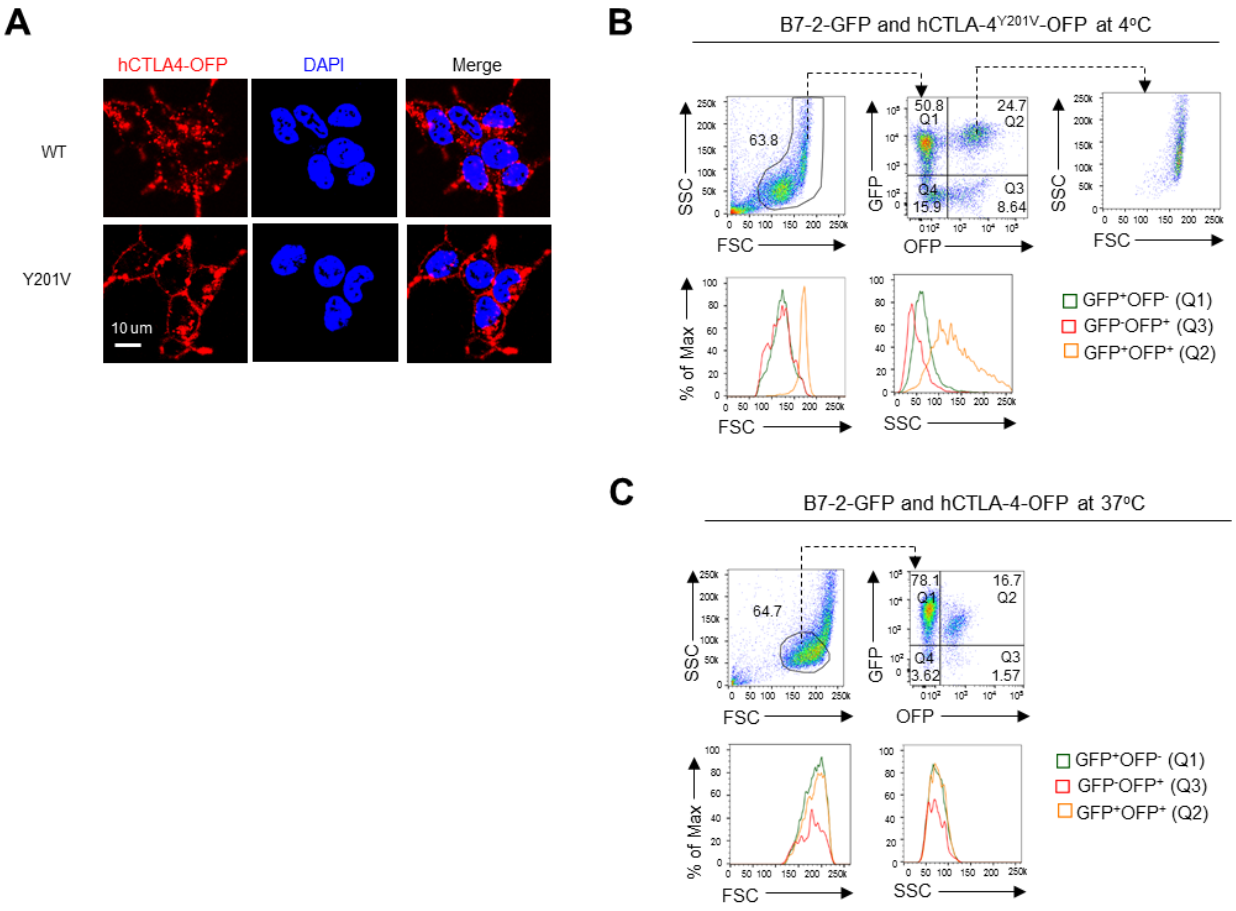
Figure S3



Supplementary information, Figure S3 Characterization of cellular assays for B7-CTLA-4 interaction. **(A)** Confocal images of 293T cells stably expressing wild-type (WT, top panels) and Y201V mutant (bottom panels) of human hCTLA-4-OFP proteins. Note that while WT hCTLA-4 is predominantly intracellular, mutant hCTLA-4 molecules show a clear pattern of plasma membrane distribution. **(B)** GFP^+OFP^+ cells in cell-cell binding assays used in Figure 3 are cell-cell aggregates based on their forward and side scatters. Representative flow profiles of hB7-2-GFP-CHO and hCTLA-4^{Y201V}-OFP-293T cells co-incubated at 4^oC for 2h. Top panels show forward vs. side scatters of the GFP^+OFP^+ cells, while the lower panels show comparisons of the forward scatters (left) and side scatters (right) of single vs. double positive cells. **(C)** Characterization of the

transendocytosis assay. The top panels show the gating used for data presented in Figure 4, while the lower panels show that after co-incubation at 37^{o}C for 4 hours, CTLA-4-OFP-CHO cells acquired GFP signals from hB7-2-GFP-CHO cells without alteration in the forward and side scatters.