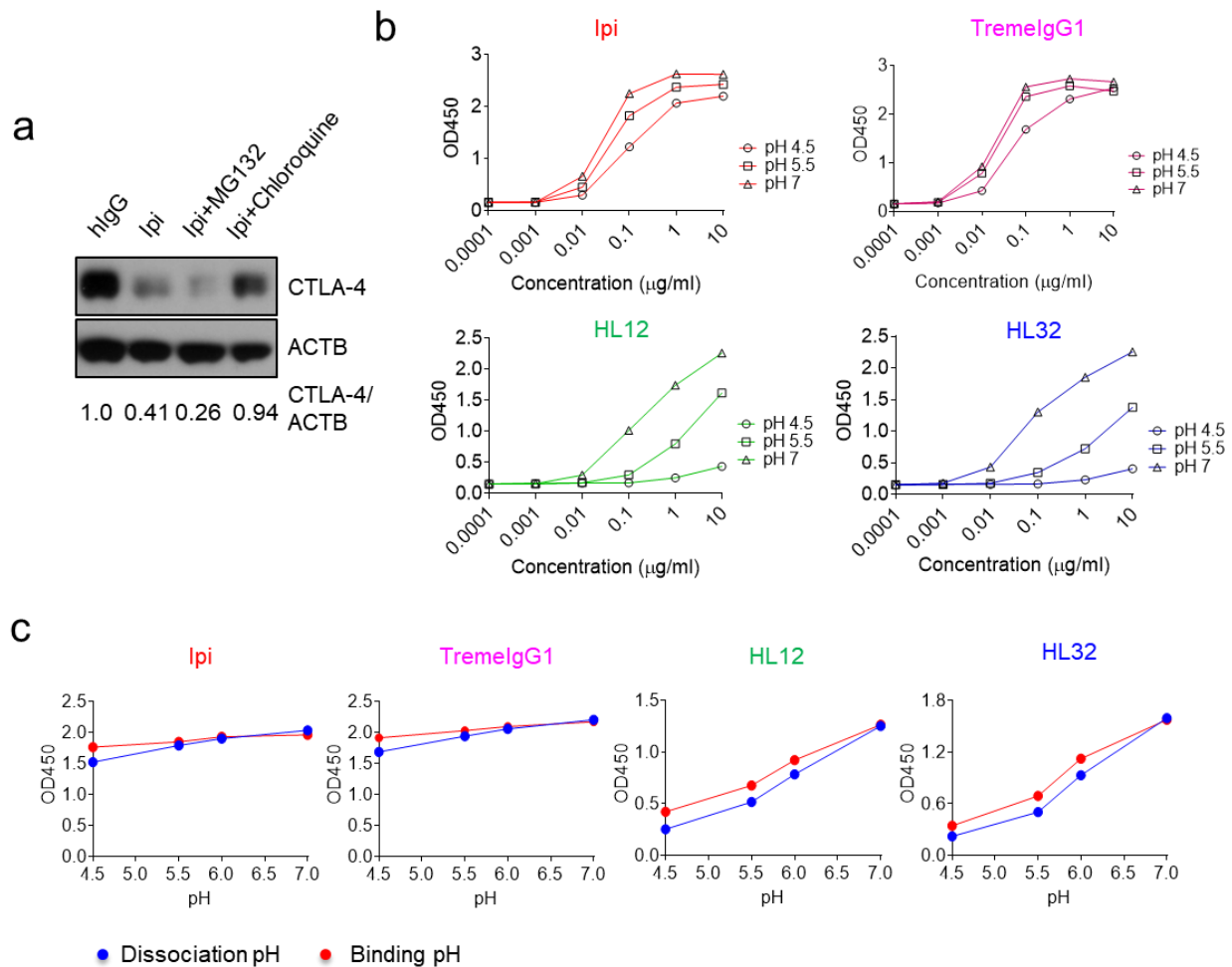




**Figure S3. pH-insensitive target binding of irAE-prone anti-CTLA-4 mAbs triggers lysosomal degradation of CTLA-4, Related to Figure 3.** (a) HEK293T cells transfected with hCTLA-4 were treated with either control hlgG Fc or Ipilimumab, with or without MG132 (1  $\mu\text{M}$ ) or chloroquine (50  $\mu\text{M}$ ), for 4hrs. The CTLA-4 protein levels were analyzed by Immunoblot. (b) His-hCTLA-4 (0.5  $\mu\text{g/ml}$ ) was coated onto ELISA plates and anti-CTLA4-mAbs with different dilutions were added in the buffer at pH 4.5, 5.5 and 7.0. Then antibodies binding with CTLA-4 were detected. (c) His-hCTLA-4 (0.5  $\mu\text{g/ml}$ ) was bound with different anti-CTLA4-mAbs at 10  $\mu\text{g/ml}$  at pH 7.0. After extra antibodies were washed

away, binding of CTLA-4 was detected followed by 2hrs incubation at lower pH buffer (pH 4.5, 5.5, 6 and 7). Data are means of duplicate optical density at 450 nm. Representative data of two independent experiments are shown in (a), (b) and (c).