**Additional file 2**



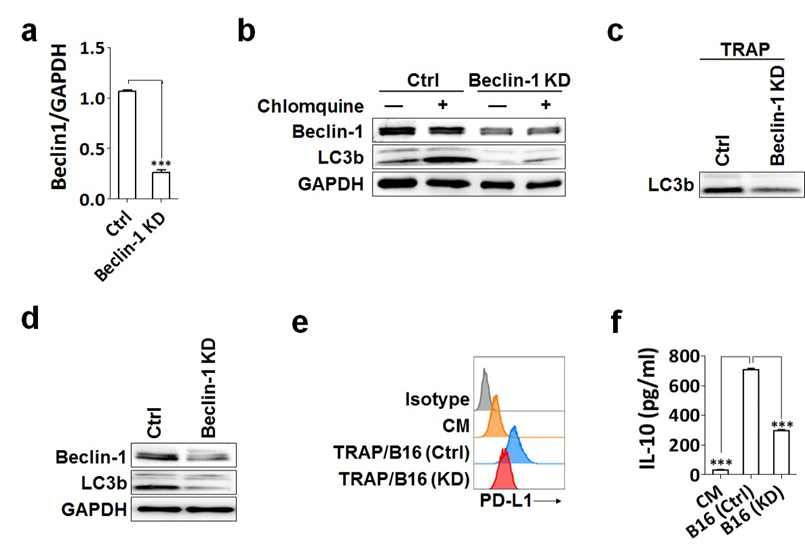
**Figure S1.** Characterization of TRAPs from tumor cell lines or cancer patients. **a-c** Characterization of B16F10 TRAPs. **a** Flow cytometry analysis of LC3B on TRAPs. **b** Western blot analysis of LC3-II for parental cells and TRAPs. **c** TEM images of TRAPs. Scale bar, 1 μm, 0.2 μm, 100 nm (from left to right). **d-f** Characterization of TRAPs derived from cancer patients. **d** Flow cytometry analysis of LC3B on TRAPs. **e** Western blot analysis of LC3-II for TRAPs from HepG2 cells and cancer patients. **f** TEM images of TRAPs. Scale bar, 1 μm, 0.2 μm, 100 nm (from left to right). Results are representative of three independent experiments.



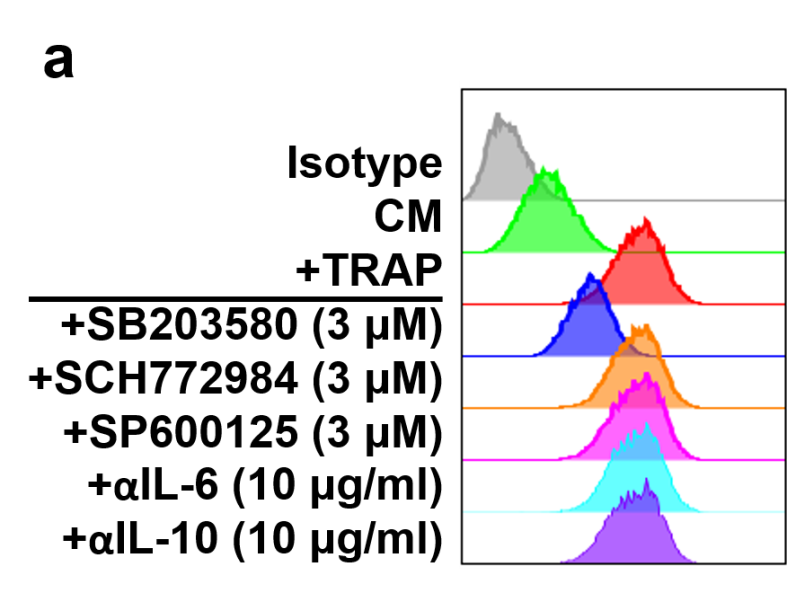
**Figure S2.** Phenotype determination of BMDMs stimulated by TRAPs with different doses and origin. **a** BMDMs were stimulated with TRAPs at indicated doses for 48 h. PD-L1 was evaluated by flow cytometry, and (**b**) IL-10 was measured by ELISA. **c** TRAPs were cultured with Raw264.7, peritoneal macrophage and BMDMs, respectively. PD-L1 and (**d**) IL-10 was detected after 48 h. **e** BMDMs were treated with TRAPs from EL4, B16F10, Hepa1-6 or 4T1 cells for 48 h, followed by assessment of PD-L1 and (**f**) IL-10. Data (mean ± SEM) are representative of three independent experiments. \*\*\**p* < 0.001 by unpaired *t* test (d and f).



**Figure S3.** TRAPs treated BMDMs inhibit T cell proliferation. **a** CFSE-labeled T cells were left untreated or were activated in the presence of plate-bound anti-CD3 plus soluble anti-CD28 mAb and were either cultured alone or were incubated with control or TRAPs (10 μg/ml) stimulated BMDMs at indicated ratios (20:1, 20:2 and 20:4). Cells were harvested after 72 h, and T cells division was analyzed by flow cytometry. **b** BMDMs were loaded with peptide SIINFEKL (1 μg/ml) for 2 h, washed, and were then cocultured with B3Z cells for 18 h at a ratio of 1:3, in the presence of anti-PD-L1 (10 μg/ml), anti-IL-10 (10 μg/ml), or IgG isotype control (10 μg/ml). B3Z T cell activation was measured by CPRG assay. Data are representative of three independent experiments. \**p* < 0.05, \*\**p* < 0.01 and \*\*\**p* < 0.001 by unpaired *t* test (a and b).

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**Figure S4.** Genetic inhibition of autophagy by targeting Beclin1 reduces TRAPs production. **a** qRT-PCR analysis of *Beclin1*mRNA expression in B16F10 (Ctrl) and B16F10 (BECN1 KD) cells. **b** Tumor cells were treated with or without chlomquine (30 μM) for 24 h, lysates were detected for Beclin1 and LC3B expression by western blot. **c** TRAPs were obtained from equal number of B16F10 (Ctrl) and B16F10 (BECN1 KD) cells, suspended in same volume of PBS, then were detected for LC3-II by western blot. **d** Lysates from resected tumors were assessed for Beclin1 and LC3B by western blot (n = 6 per group). **e, f** BMDMs were treated with TRAPs from equal number of BECN1-KD B16F10 (B16 KD) and Ctrl-B16F10 (B16 Ctrl) cells for 24 h, expression of PD-L1 and IL-10 was detected by flow cytometry and ELISA, respectively. Data are representative of three independent experiments.



**Figure S5.** TRAPs induced PD-L1 upregulation on BMDMs was mainly dependent on p38 activation. **a** BMDMs were preincubated with p38 inhibitor SB203580 (3 μM), Erk1/2 inhibitor SCH772984 (3 μM), JNK inhibitor SP600125 (3 μM), anti-IL-6 mAb (10 μg/ml) and anti-IL-10 mAb (10 μg/ml) for 1 h, and then treated with TRAPs (10 μg/ml) for 48 h. Expression of PD-L1 was determined by flow cytometry.



**Figure S6.** Comparison of LC3B+ EVs and LC3B- EVs in converting monocytes. **a** LC3B+ EVs and LC3B- EVs were sorted from total EVs of a lung cancer patient, and LC3B expression was determined by flow cytometry. **b-d** Purified CD14+ monocytes from healthy donors were treated for 3 d with total EVs, LC3B+ EVs, LC3B- EVs (5 μg/ml) and magnetic beads, respectively. Expression of CD163, HLA-DR, CD86 (**b**) and PD-L1 (**c**) was detected by flow cytometry. IL-10 production (**d**) in the supernatant was assessed by ELISA.