

Supplementary Figure S1: Release of sCD137 is mediated by ADAM10 in HT29 cells. (A) Transfection of CD137 was controlled by Simple Western using anti-tGFP antibodies. Tubulin was used as loading control. (B, C) One representative experiment out of three ELISAs (shown normalized in Figure 1A and B) is depicted to show the absolute levels of CD137 in cell lysates and supernatants, respectively (mean +/- SD). (B) Incubation with ADAM10 inhibitor GI (3 μ M), ADAM10/17 inhibitor GW (3 μ M) or broad-spectrum metalloprotease inhibitor marimastat (MM, 10 μ M) resulted in reduced amounts of sCD137 in the supernatants. (B) Cells were stimulated with ionomycin (IO; 1 μ M), melittin (Mel; 1 μ M) for 30 min or with PMA (200 ng/ml) for 2 h. Cell lysates and supernatants were analyzed by CD137 ELISA.



Supplementary Figure S2: ADAM10 and ADAM17 mediate CD137 release in HEK cells. (A) Transfection of CD137 in wild-type (WT) HEK and ADAM17/ADAM17 double-knockout HEK cells (dKO) was controlled by Simple Western using anti-tGFP antibodies. Tubulin was used as loading control. (B-D). One representative experiment out of three ELISAs (shown normalized in Figure 2A-C) is depicted to show the absolute levels of CD137 in cell lysates and supernatants of WT (black bars) and dKO cells (grey bars), respectively (mean +/- SD). (B) Incubation with ADAM10 inhibitor GI (3 μM), ADAM10/17 inhibitor GW (3 μM) or broad-spectrum metalloprotease inhibitor marimastat (MM, 10 μM) resulted in reduced amounts of sCD137 in the supernatants of WT cells. (C) Cells were stimulated with ionomycin (IO; 1 μM) or melittin (Mel; 1 μM) for 30 min or with PMA (200 ng/ml) for 2 h. (D) HEK293T dKO A10/A17 cells were co-transfected with CD137 and ADAM10, ADAM17 or mock vector and analyzed by CD137 ELISA.



Supplementary Figure S3: lonomycin-induced sheding of CD137 depends on ADAM-phosphatidylserine (PS) interaction. One representative experiment (shown normalized in Figure 3A/B) is depicted to show the absolute levels of CD137 in cell lysates and supernatants of (A) HT29 (n=4) and (B) HEK cells (n=5), respectively. CD137-transfected HT29 cells or HEK293T WT cells were stimulated with ionomycin (IO; 1 μ M) for 30 min in the presence of OPS or lactadherin (LA, 2 μ M). Cells and supernatants were analyzed by CD137 ELISA (mean +/- SD).



Supplementary Figure S4: Anoctamin-6 (ANO6) modulates CD137 shedding. One representative experiment out of five (A) and three (B) ELISAs (shown normalized in Figure 4A/B) is depicted to show the absolute levels of CD137 in cell lysates and supernatants of HT29 (A) and HEK cells (B), respectively (mean +/- SD). ANO6 and mock-transfected HT29 cells or HEK293T WT cells were stimulated with ionomycin (IO; 1 μ M) for 30 min. In addition, cells co-transfected with CD137 and hyperactive ANO6 were analyzed in the absence of any stimulus for 30 min by CD137 ELISA.



Supplementary Figure S5:

Representative agarose gel images showing mRNA expression of CD137 and CD137 Ligand (CD137L) in activated CD4+/CD8+ T cells. Total RNA was extracted and subjected to cDNA synthesis. The PCR products were amplified using specific primers and analyzed using 2,5 % agarose gel electrophoresis. THP1 cells were used as positive control. The housekeeping gene HPRT served as internal control.