

**Suppl. Fig. 1**

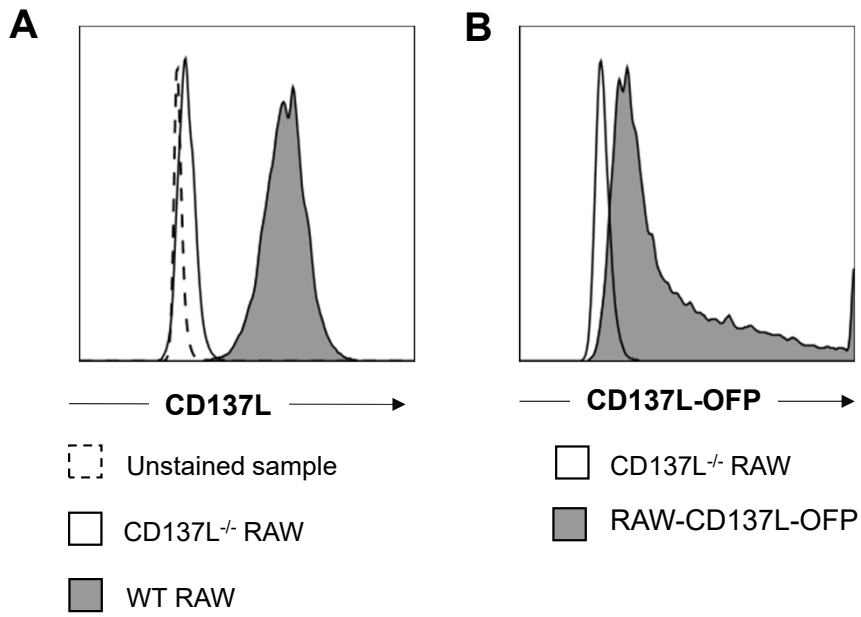


Figure S1. CD137L expression on RAW264.7 cells. (A) WT and CD137L<sup>-/-</sup> RAW264.7 cells were analyzed by flow cytometry for CD137L expression. (B) CD137L<sup>-/-</sup> RAW were transfected with CD137L-OFP to yield RAW-CD137L-OFP cells which express high level of CD137L-OFP.

## Suppl. Fig. 2

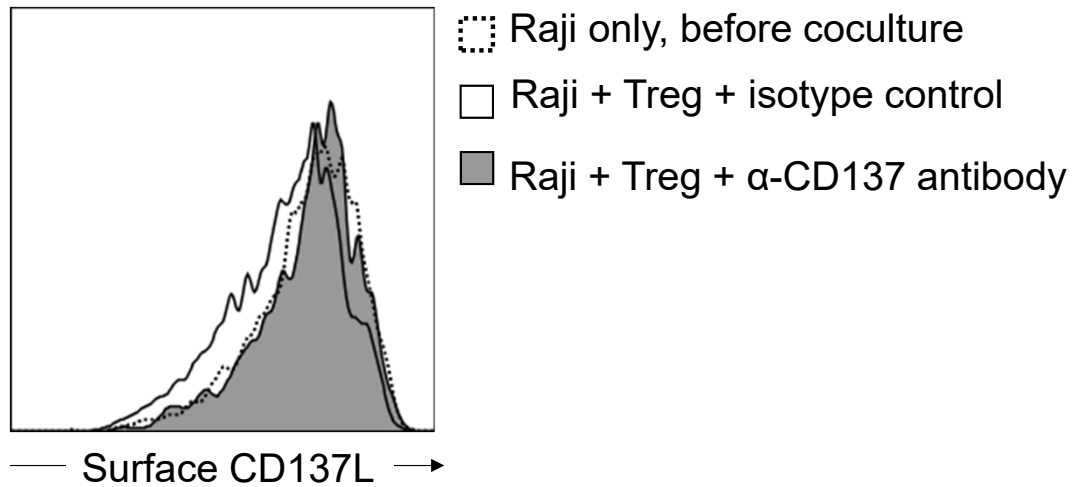
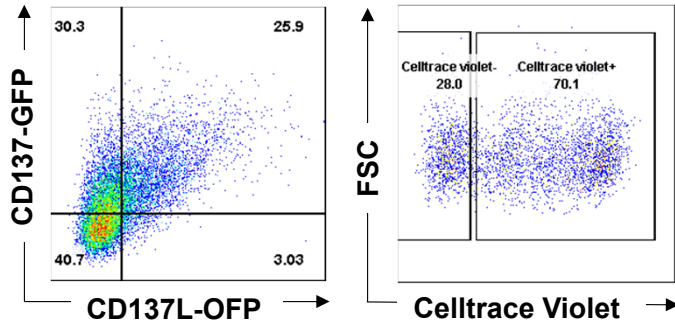


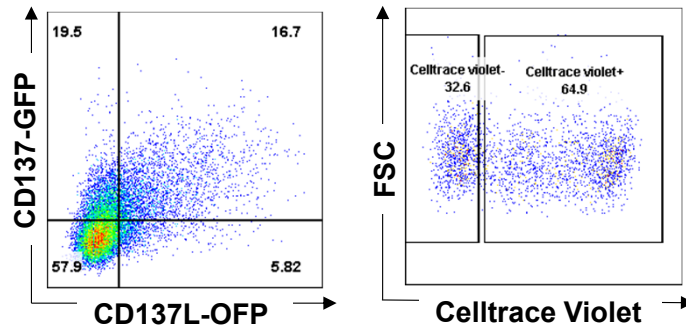
Figure S2. Example from one donor for the downregulation of surface CD137L on Raji cells upon coculture with activated Treg cells and how anti-CD137 antibody rescues the downregulation by blocking CD137 on Treg. Raji cells were labelled with CFSE before the coculture. Treg isolated from PBMC from healthy donors were activated with 100  $\mu$ g/ml PMA and 1 ng/ml ionomycin for 24 h and cocultured with Raji for 1 h at a ratio of 2 Treg : 1 Raji, with the addition of 5  $\mu$ g/ml of isotype antibody (clone MOPC-21), or anti-CD137 antibody (clone BBK-2). Cells were analysed by flow cytometry for surface CD137L levels after gating for single, live CFSE<sup>+</sup> cells.

Suppl. Fig. 3

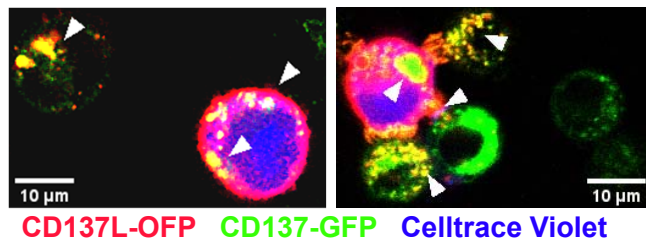
**A** CHO-FL-CD137-GFP + CHO-CD137L-OFP



**B** CHO-TR-CD137-GFP + CHO-CD137L-OFP



**C** CHO-FL-CD137-GFP + CHO-CD137L-OFP



**D** CHO-TR-CD137-GFP + CHO-CD137L-OFP

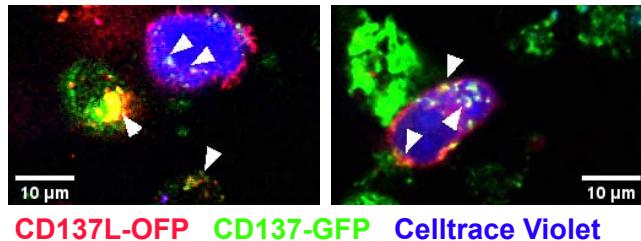


Figure S3. CD137 signalling is not involved in murine CD137 trogocytosis and the transfer is bi-directional. CHO-FL-CD137-GFP, CHO-TR-CD137-GFP or CHO-CD137L-OFP were obtained by transfecting CHO cells with the plasmid containing fluorescent murine full length CD137, truncated CD137 or CD137L, respectively. CHO-FL-CD137-GFP (A) or CHO-TR-CD137-GFP (B) cells were cocultured with CellTrace Violet-labelled CHO-CD137L-OFP cells for 3 h at a 1:1 ratio (left panel). Cells were analyzed by flow cytometry after gating for live and single cells. CellTrace Violet signals of CD137<sup>+</sup>CD137L<sup>+</sup> double-positive cells are depicted in the right panels. Data are representative of three different experiments. (C, D). Internalisation of the CD137-CD137L complex. CHO-CD137L-OFP cells were labelled with CellTrace Violet and cocultured for 24 h at a 1:1 ratio with (C) CHO-FL-CD137-GFP cells or (D) CHO-TR-CD137-GFP cells. Yellow spots indicate the complex of CD137 (green) and CD137L (red). White arrows indicate the location of the complex in cells.