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Supplementary Materials for

TREM2- and DAP12-Dependent Activation of PI3K Requires DAP10 and Is Inhibited by SHIP1

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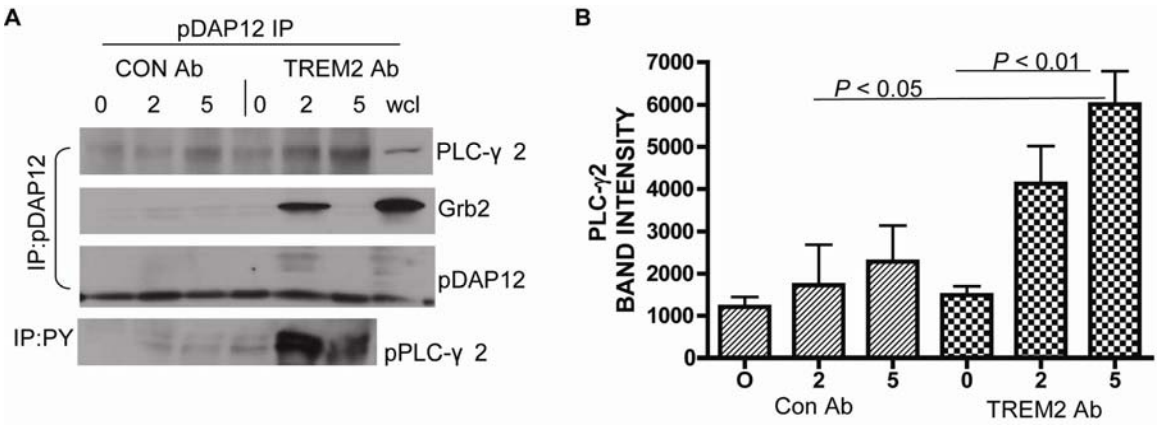

Fig. S1. Recruitment of PLC-γ2 and Grb2 to DAP12 occurs upon ligation of TREM2. (**A**) Lysates from RAW 264.7 cells stimulated with antibody against TREM2 or control antibody were subjected to immunoprecipitation with antibody against pDAP12 and analyzed by Western blotting with antibodies against PLC-γ2, Grb2, and pDAP12. Additional lysates were subjected to immunoprecipitation with antibody against pTyr residues and analyzed by Western blotting for pPLC-γ2. (**B**) Band intensity of PLC-γ2 that coimmunoprecipitated with pDAP12. Results are representative of three experiments.

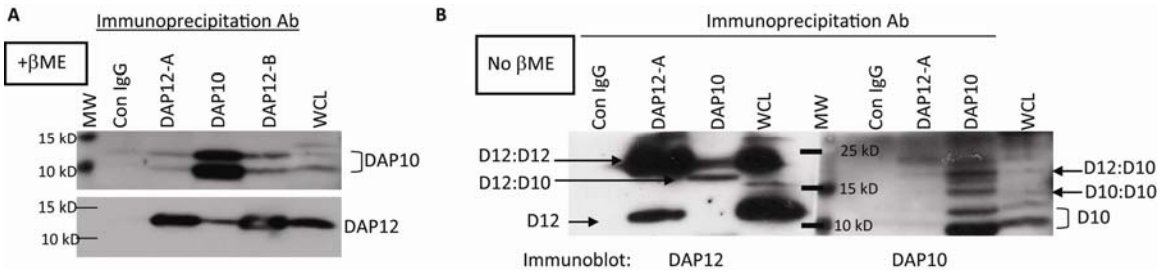

Fig. S2. DAP10 and DAP12 form homodimers and heterodimers in macrophages. DAP12 and DAP10 immunoprecipitations were performed from whole-cell lysates of J774 cells. (**A**) Precipitated complexes were analyzed under reducing conditions and by Western blotting with antibodies against DAP12 (antibody A and B) or DAP10. Antibody against DAP12 precipitated both DAP12 and DAP10, and antibody against DAP10 precipitated both DAP10 and DAP12. (**B**) DAP12 and DAP10 immunoprecipitates were analyzed under nonreducing conditions. Antibody against DAP10 precipitated a complex with an apparent molecular mass of ~20 kD that was detected with antibodies against DAP12 and DAP10. This complex may represent a DAP12-DAP10 heterodimer. Results are representative of two experiments.

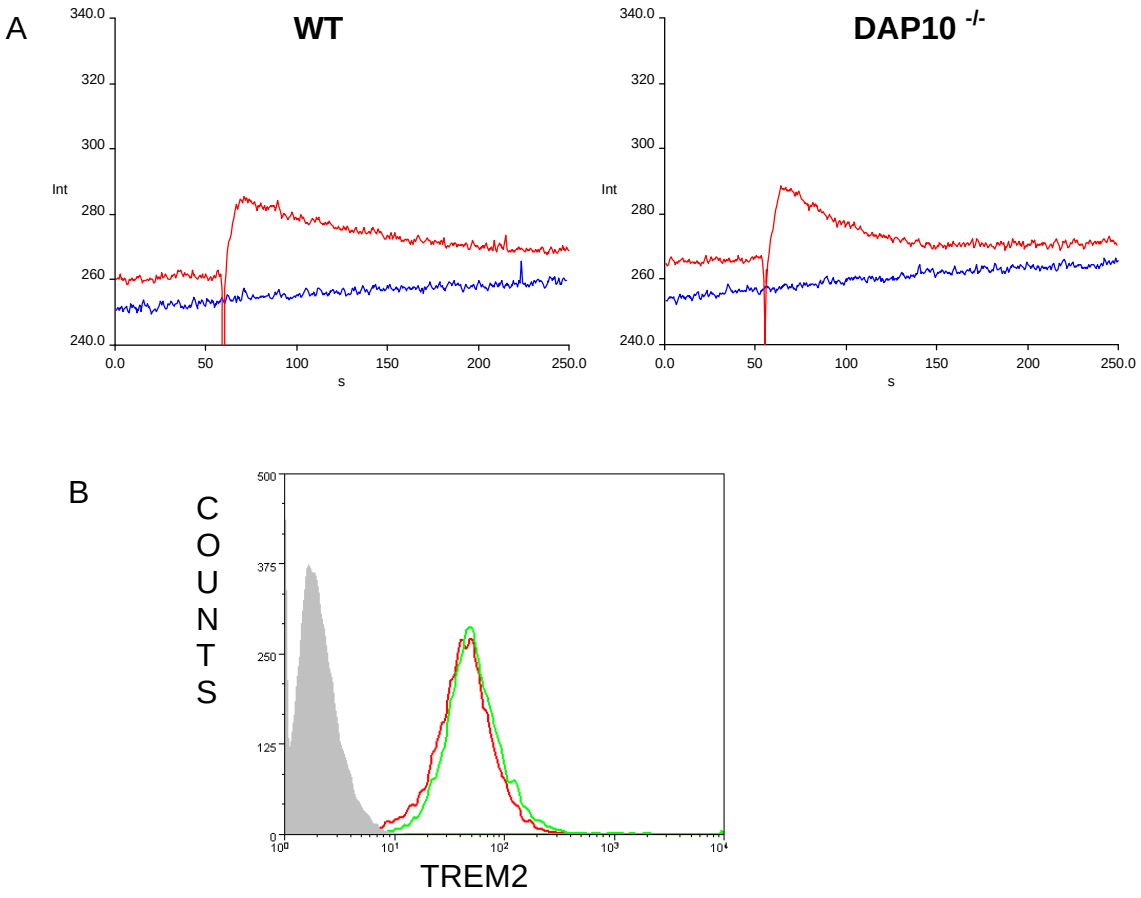

Fig. S3. DAP10 is not required for the expression of TREM2 at the cell surface or for TREM2-induced Ca^{2+} flux. (**A**) WT and DAP10-deficient BMMs were stimulated with antibody against TREM2 or with control antibody and Ca^{2+} flux was measured. (**B**) Flow cytometry confirms the similar abundance of TREM2 on the surface of WT (green) and DAP10-deficient (red) BMMs compared to staining with an isotype-matched control antibody (gray). Data are representative of two experiments.

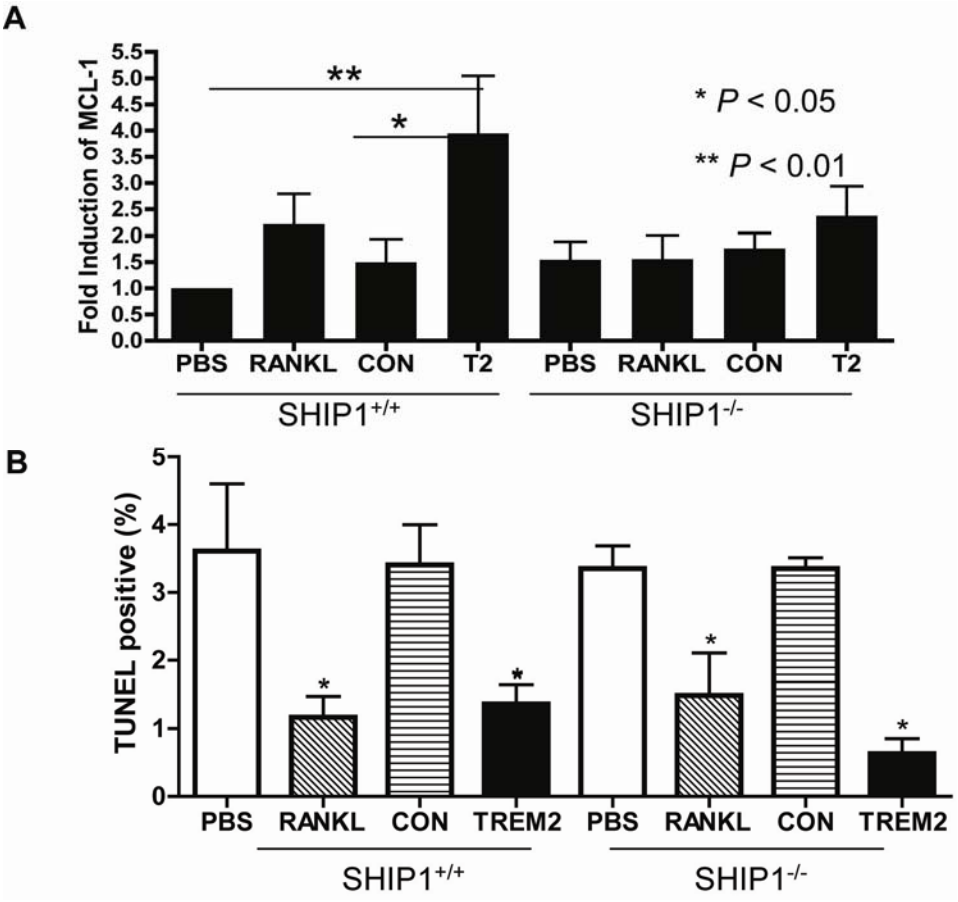

Fig. S4. Ligation of TREM2 leads to protection from apoptosis by inducing the production of MCL-1. (**A**) Osteoclasts were starved of serum overnight to induce apoptosis in the presence of PBS, RANKL, antibody against TREM2, or a control antibody. Cell lysates were analyzed by Western blotting for the anti-apoptotic protein MCL-1 and the intensities of bands from three experiments were quantified. (**B**) In parallel apoptotic assays, cells were fixed and TUNEL staining was performed. The number of TUNEL positive cells was quantified. Data are representative of three studies.

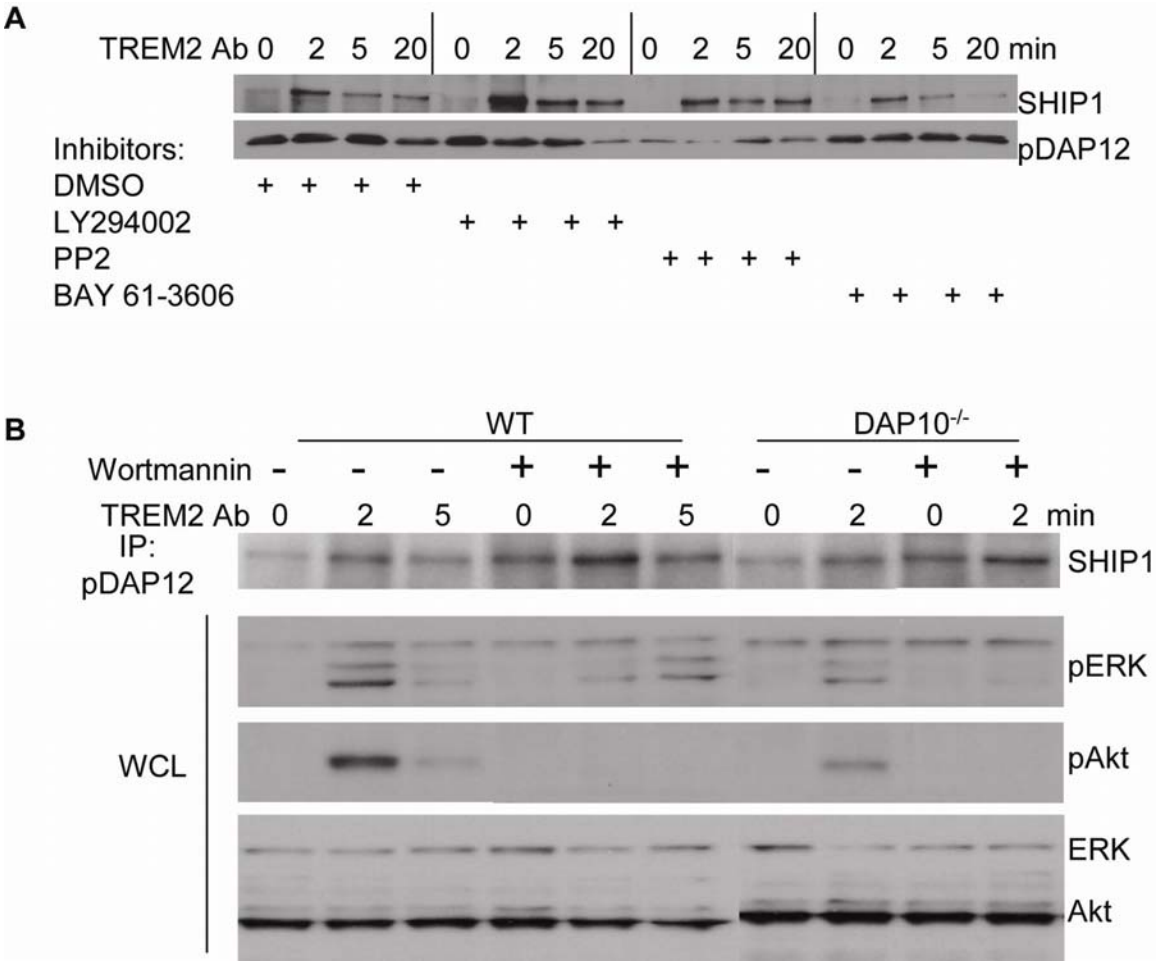

Fig. S5. Inhibition of PI3K, but not of Src or Syk, increases the amount of SHIP1 recruited to DAP12. (**A**) TREM2 was crosslinked in J774 cells treated with DMSO, the PI3K inhibitor LY294002, the Src inhibitor PP2, or the Syk inhibitor BAY 61-3606. Samples were subjected to immunoprecipitation with antibody against pDAP12. The association of SHIP1 with DAP12 was substantially increased in LY294002-treated cells compared to that in cells treated with DMSO, but was not changed in cells treated with Src or Syk inhibitors. The amount of pDAP12 was reduced in cells treated with the Src inhibitor. (**B**) WT and DAP10-deficient BMMs were treated with DMSO or wortmannin during TREM2 ligation. Enhanced binding of SHIP1 binding to DAP12 was seen in the wortmannin-treated cells (which was independent of DAP10) compared to that in DMSO-treated cells. Western blotting analysis of whole-cell lysates for the presence of pERK and pAkt confirmed that wortmannin inhibited these signals. Results are representative of two experiments.

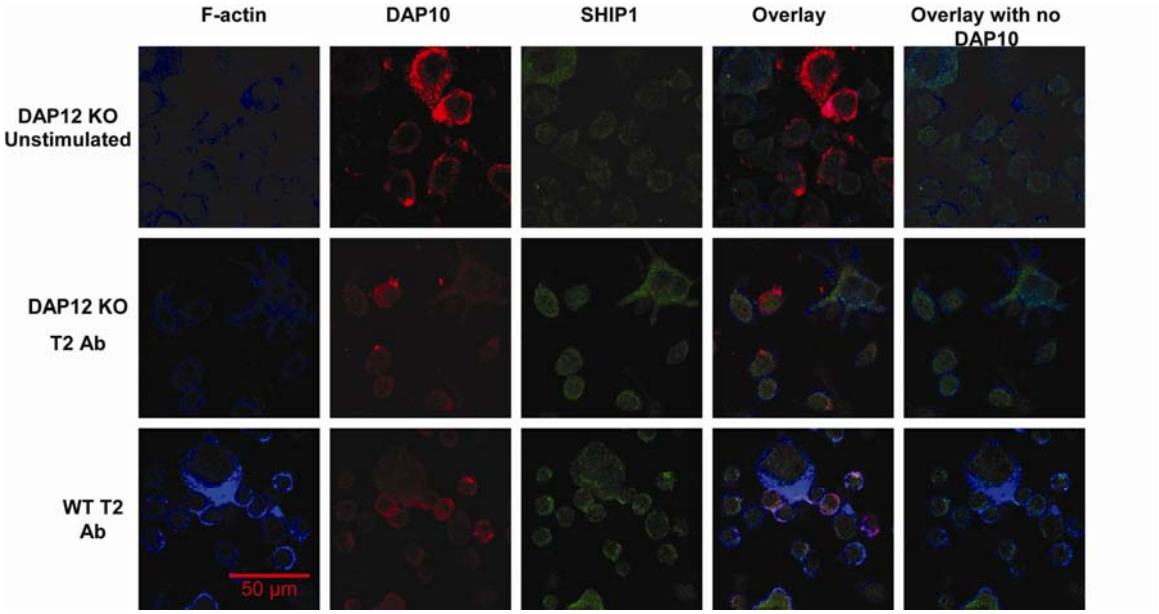

Fig. S6. SHIP1 and DAP10 colocalize with actin in cap-like structures after ligation of TREM2. Osteoclasts generated from DAP12-deficient and FcRγ-deficient (DAP12 KO) BMMs or from WT BMMs were starved of serum and treated with antibody against TREM2. DAP10 was both cytoplasmic and membrane-localized at rest. Ligation of TREM2 induced the formation of cap-like DAP10-containing structures in both WT and DAP12 KO cells. In WT cells, SHIP1, DAP10, and F-actin colocalized in these structures, whereas DAP10 and SHIP1 failed to colocalize in DAP12 KO cells. Results are representative of three experiments.

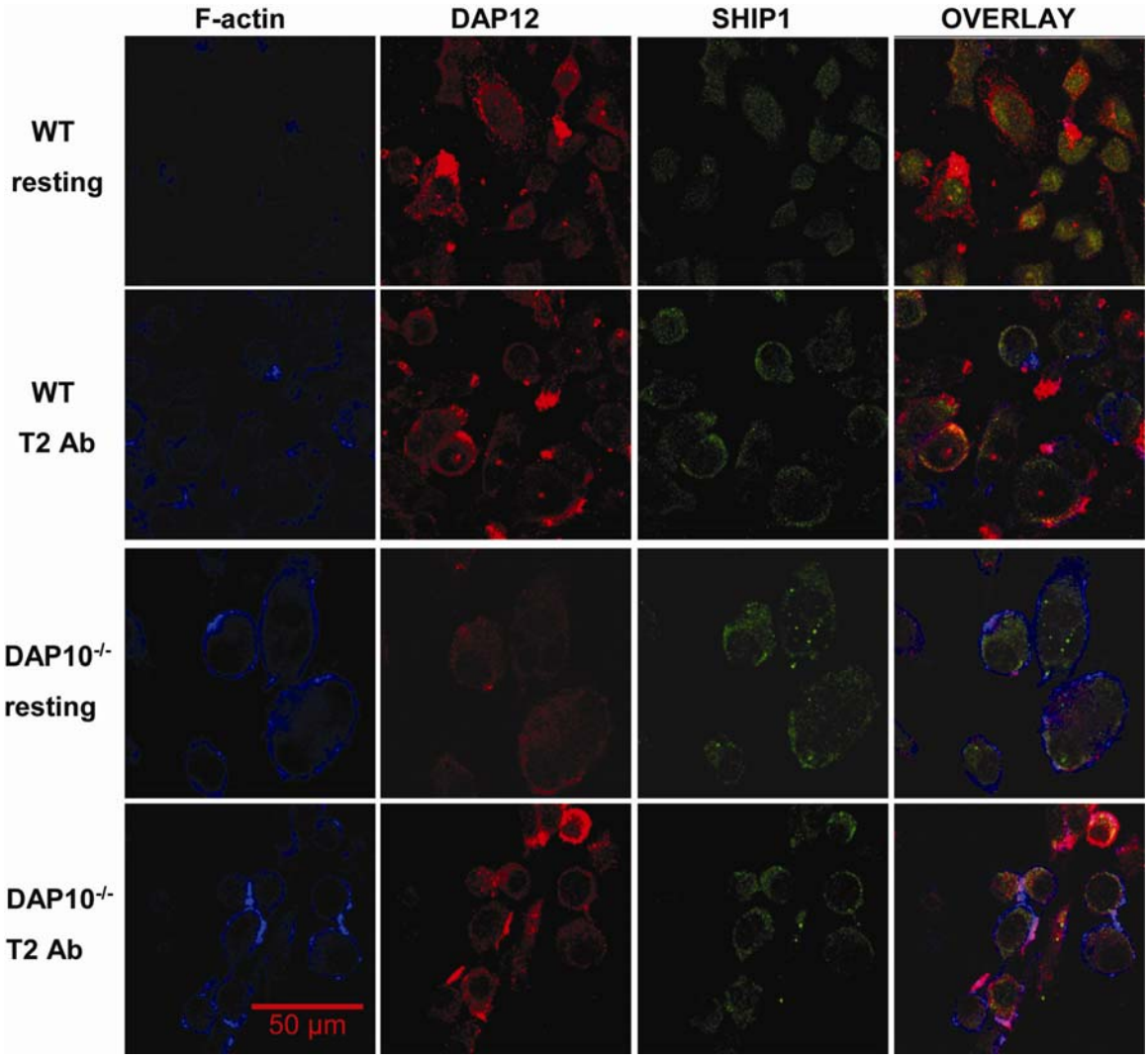

Fig. S7. DAP10 is not required for the translocation of SHIP1 to the plasma membrane. WT and DAP10-deficient osteoclasts were generated, starved of serum, and treated with antibody against TREM2. The abundance of F-actin was greater in DAP10-deficient cells at rest than in WT cells. Additionally, ligation of TREM2 induced colocalization of DAP12 and F-actin to a greater extent in DAP10-deficient cells than in WT cells. SHIP1 translocated to the periphery of the cell where it colocalized with F-actin and DAP12 in both WT and DAP10-deficient cells. Results are representative of two experiments.