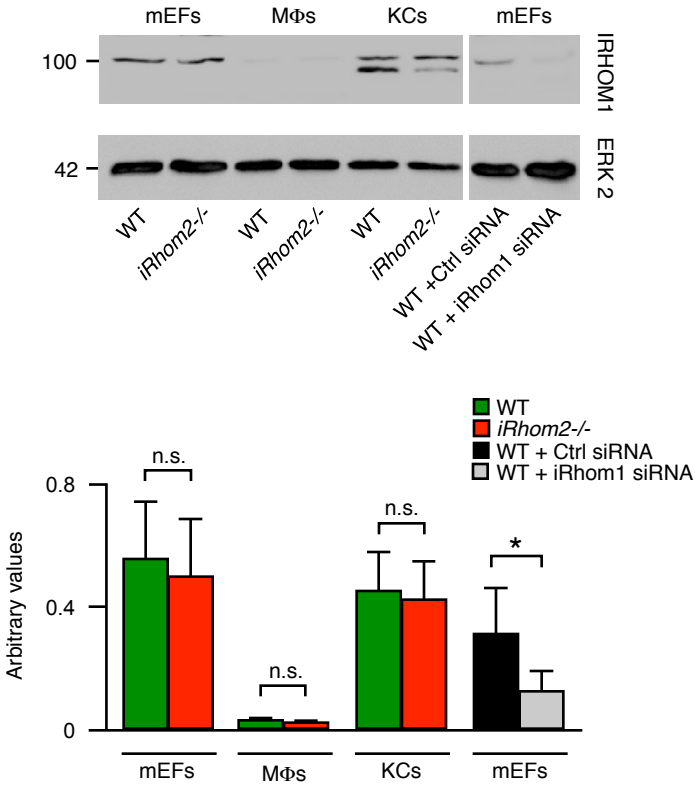
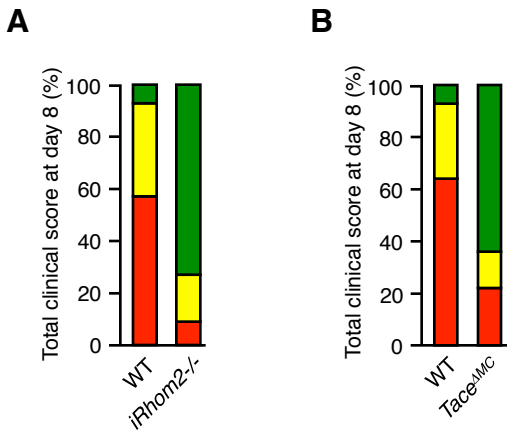
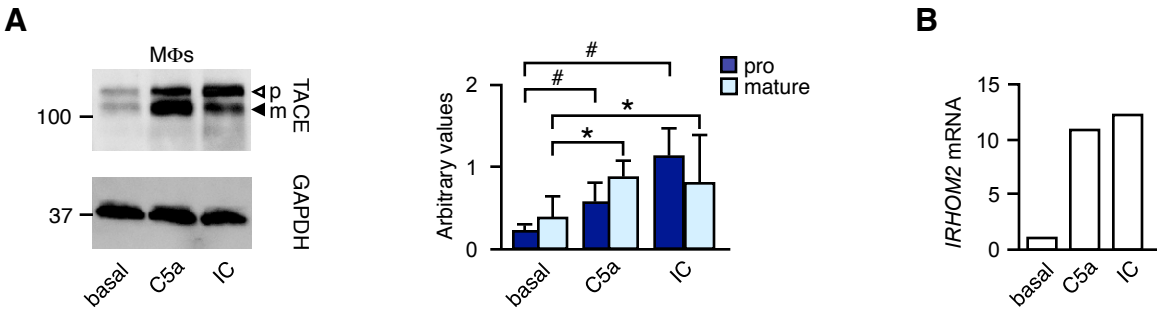
Supplementary Figures for the manuscript entitled:
"Novel role for IRHOM2 in the pathogenesis of inflammatory arthritis"
by Issuree et al.



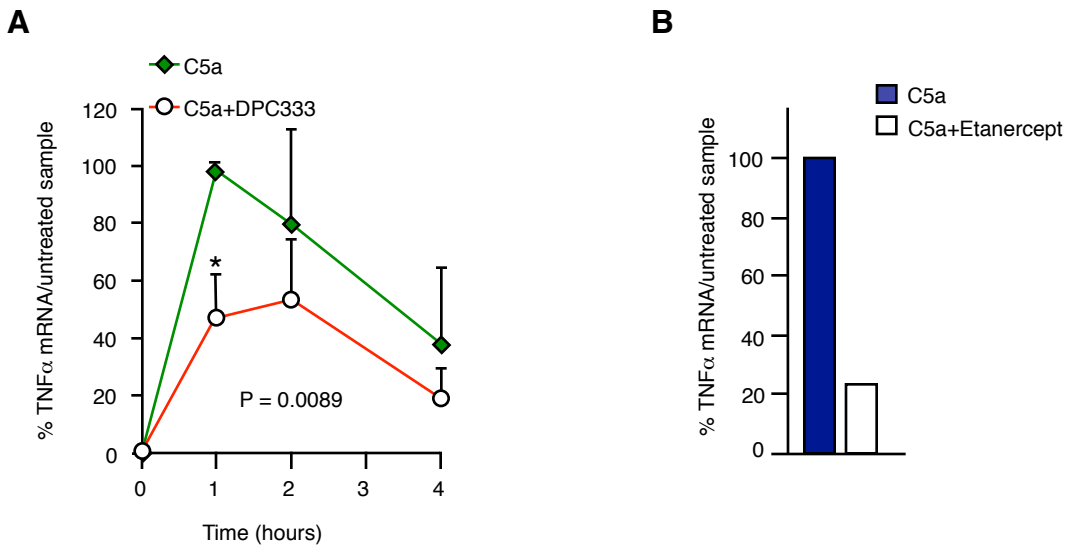
Supplementary Figure S1. Expression of IRHOM1 in mouse embryonic fibroblasts, macrophages or keratinocytes. Western blot of IRHOM1 in mouse embryonic fibroblasts (mEFs), primary macrophages (MΦs), primary keratinocytes (KCs, IRHOM1 runs as a doublet in these cells), and WT mEFs treated with control siRNA (Ctrl siRNA) or IRHOM1 siRNA. The WT + Ctrl siRNA and WT + iRhom1 siRNA samples were included to corroborate that the anti-IRHOM1 antibodies are specific. The lower panel shows the results of a densitometric quantification of Western blots with the same order of samples loaded that were generated from 3 separate experiments. ImageJ software (http://rsb.info.nih.gov/ij/) was used to quantify the bands obtained via Western blot analysis. The area under curve (AUC) for the specific signal was corrected for the loading control AUC. mean + SD, *$p<0.05$.



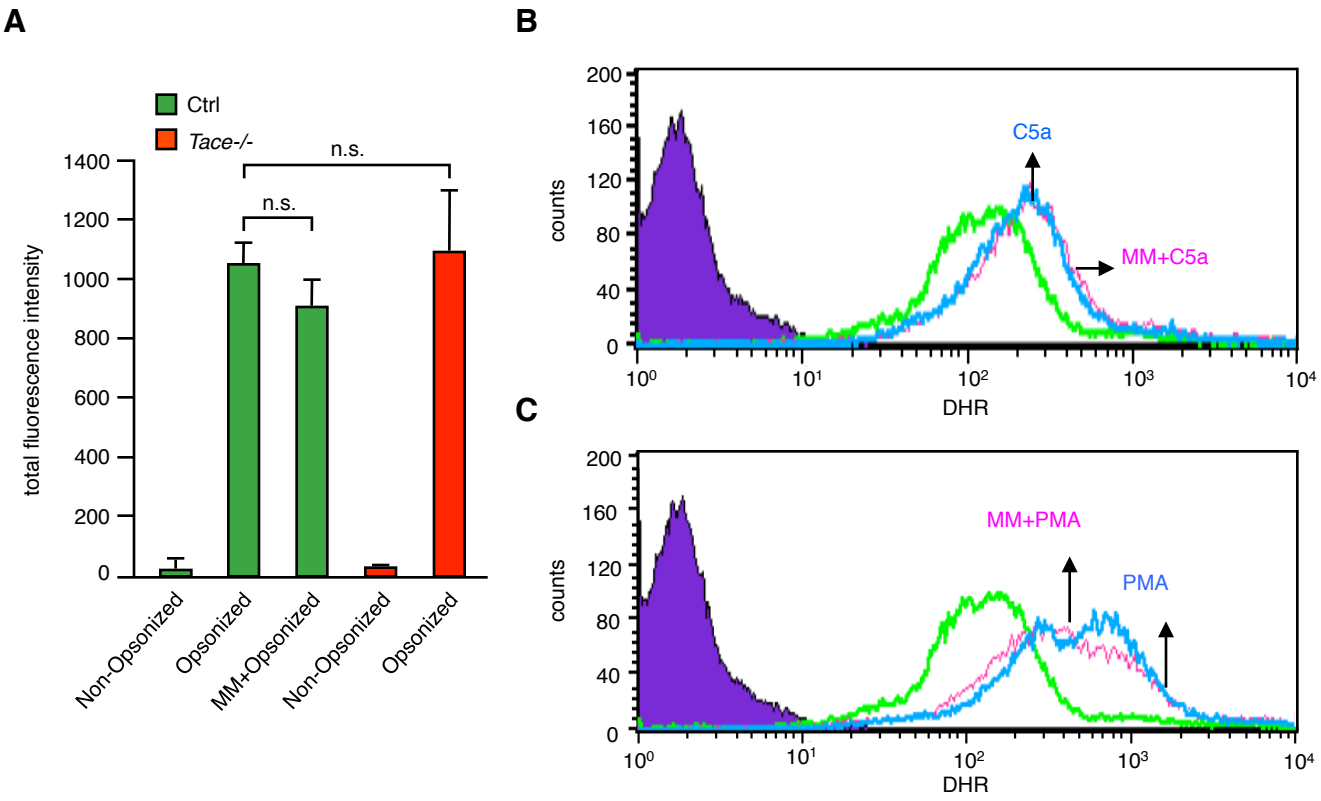
Supplementary Figure S2. Graphical representation of the clinical scores of *iRhom2-/-* and *Tace*$^{\Delta MC}$ mice and controls subjected to K/BxN arthritis. Clinical scores of *iRhom2-/-* (n=11) and controls (n=14) (A), or of *Tace*$^{\Delta MC}$ and controls (B, n=14 each) subjected to K/BxN arthritis. The percent of mice with low damage (green), moderate damage (yellow) or severe damage (red) was determined by the total clinical score at day 8.



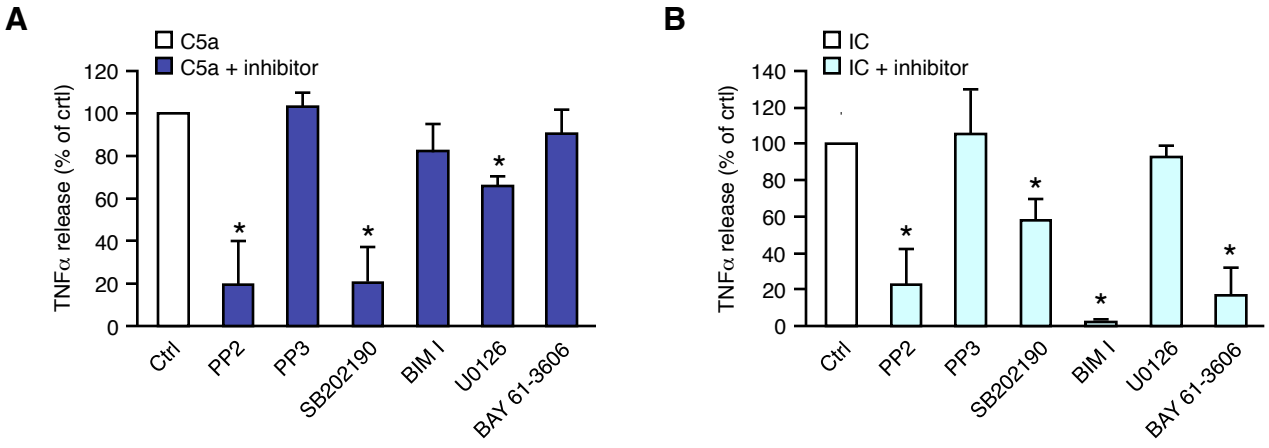
Supplementary Figure S3. Western blot for TACE and qPCR for *IRHOM2* on C5a- or IC-treated human monocytes. (A) Representative TACE Western blot (left) and quantification of the relative levels of pro- and mature TACE on Western blots of 4 separate experiments using ImageJ software, as described in Supplementary Figure S1. # indicates $p<0.05$ for levels of pro-TACE in basal vs. C5a- or IC-treated samples, * indicates $p<0.05$ for levels of mature TACE in basal vs. C5a or IC-treated samples, mean + SD. (B) Representative *IRHOM2* qPCR analysis of samples from human monocytes treated with or without 1µg/mL C5a or 0.25mg/mL IC, n=4.



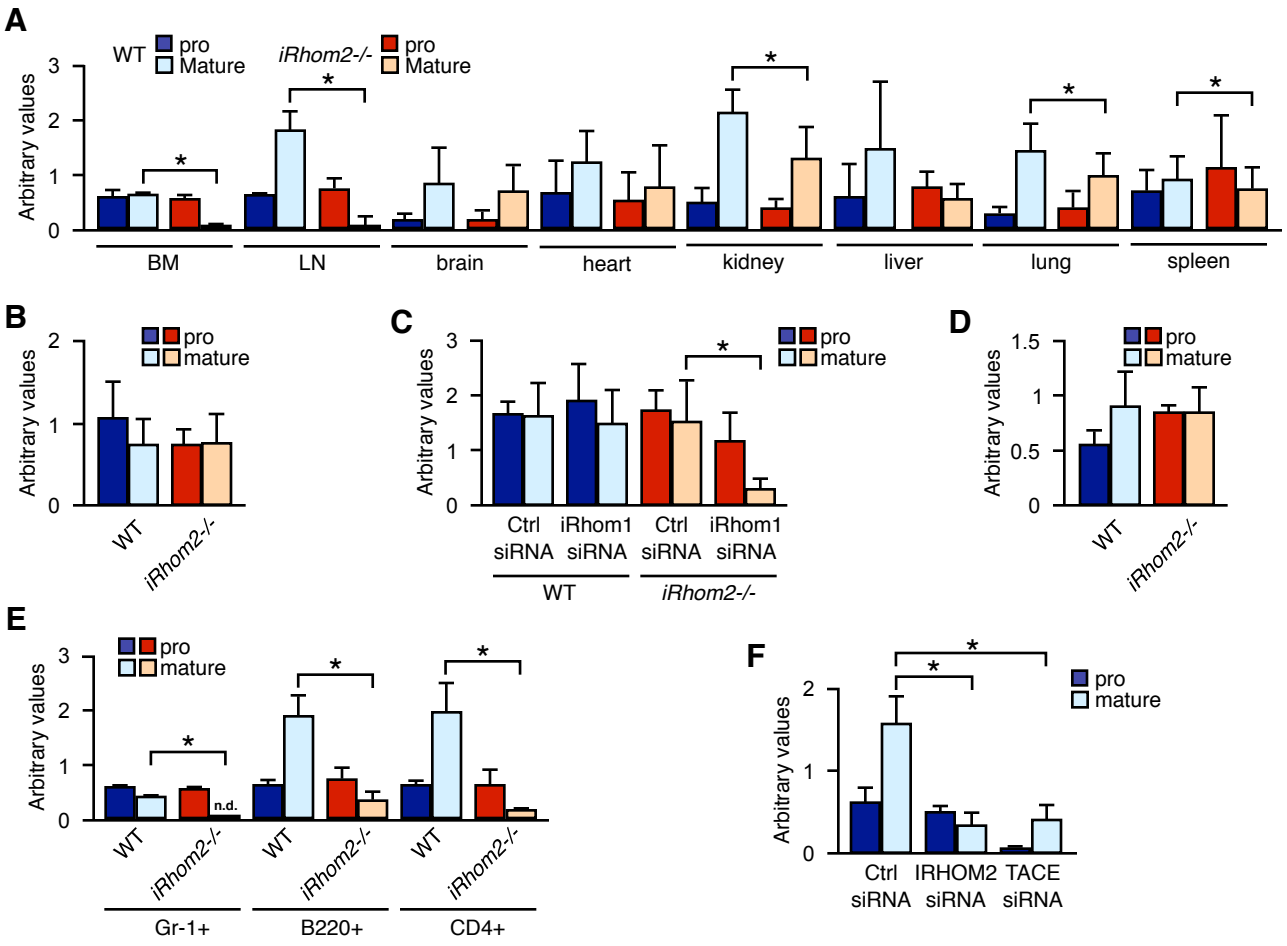
Supplementary Figure S4. Production of mRNA for TNFα is enhanced by TNFα shedding. (A) Quantification of the mRNA levels of TNFα by qPCR in human monocytes following stimulation with 1μg/mL C5a at different time points in the presence or absence of 10μM of the TACE-selective inhibitor DPC333 (n=4, mean + SD). (B) Quantification of mRNA levels of TNFα by qPCR following 30 min pre-treatment with or without 10μg/ml etanercept and stimulation with 1μg/mL C5a for 1 hr, in the presence or absence of 10μg/ml etanercept (representative of 3 experiments).



Supplementary Figure S5. Effect of inactivating TACE on C5aR- and FcγR-mediated phagocytosis and generation of reactive oxidants. (A) Quantification of total sheep red blood cells phagocytosed by wild type or *Tace-/-* fetal liver macrophages. Opsonized sheep red blood cells were stained with carboxyfluorescein diacetate succinimidyl ester and total fluorescence intensity (TFI) was measured by flow cytometry, n=3, mean + SD. (B,C) Release of reactive oxygen species (ROS) by human monocytes when stimulated with C5a (B) or PMA (C) in the presence or absence of the metalloproteinase inhibitor Marimastat (MM). ROS was quantified by FACS by measuring the mean intensity of the ROS-reactive dye dihydrorhodamine 123 (DHR). These results are representative of 4 independent experiments.



Supplementary Figure S6. Effect of signaling pathway inhibitors on the C5a- and IC-stimulated release of TNFα from primary human macrophages. (A,B) TNFα shedding from C5a-stimulated (A) or IC-stimulated (B) macrophages was measured in the presence or absence of the following inhibitors: 10 μM PP2 (Src-kinase family inhibitor), 10 μM PP3 (inactive control for PP2), 10 μM SB202190 (p38 MAPK inhibitor), 10 μM BIM I (PKC inhibitor), 10 μM U0126 (MEK inhibitor) or 5 μM BAY 61-3606 (Syk inhibitor). In each case, the amount of soluble TNFα released from control cells stimulated with 1μg/mL C5a or 0.25mg/mL IC in the absence of inhibitors was set to 100%, and the shedding from cells treated with the indicated inhibitors was calculated as a percentage of the control. n≥4, mean + SD, * p≤0.01.



Supplementary Figure S7. Quantification of the results of the Western blot analyses presented in the main figures. Quantification of Western blots for Figure 1A (A, * p<0.05 WT vs *iRhom2-/-* mature TACE), Figure 1C (B), Figure 1E (C, * p<0.05, levels of mature TACE in *iRhom2-/-* mEF compared to *iRhom2-/-* mEFs treated with iRhom1 siRNA), Figure 1G (D), Figure 2E (E, * p<0.05 WT vs *iRhom2-/-* mature TACE), Figure 3E (F * p<0.05 mature TACE levels in primary human macrophages treated with Ctrl siRNA vs. iRhom2 siRNA or TACE siRNA), mean + SD. ImageJ software was used, as described in Supplementary Figure S1. The Western blot in Figure 1A was generated from Clodronate-treated and PBS-perfused mice by lysing tissue samples as described (Weskamp et al., 1996, *J. Cell Biol*. 132:717-726). Clodronate (Encapsula Nanosciences) was used to deplete tissue-resident macrophages.