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Supplementary Materials for

Deletions in the cytoplasmic domain of iRhom1 and iRhom2 promote shedding of the TNF receptor by the protease ADAM17

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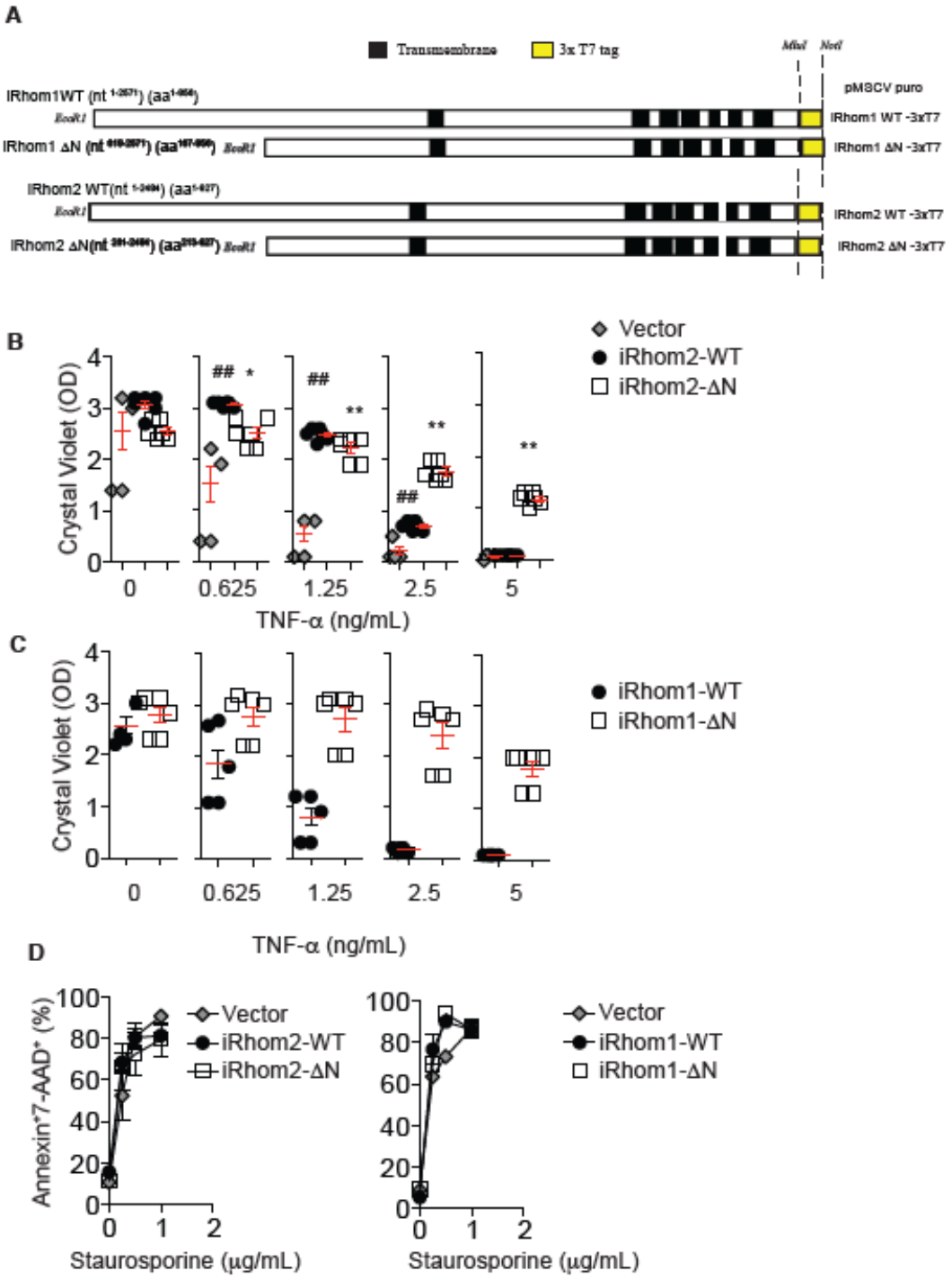

Fig S1: Cloning of CPR screen–identified iRhom versions. (**A**) Diagram of cloning strategy used to generate iRhom1-WT, iRhom2-WT, and CPR screen-identified iRhom1-ΔN and iRhom2-ΔN constructs using EcoRI and NotI digestion into a modified version of MSCVpuro (Clontech) containing a C-terminal T7 tag vector (see Materials and Methods). **(B and C)** 10^5 L-

929 cells were treated with recombinant TNF for 48 hours followed by washing and crystal violet staining. Optical density values of crystal violet elution are shown for cells transduced with vector or (B) iRhom2-WT or iRhom2-ΔN, or (C) iRhom1-WT or iRhom1-ΔN (n=6) * $p<0.05$, ** = $p<0.01$, and *** = $p<0.001$; ### $p<0.001$ between iRhom2-WT and vector). **(D)** Cell death in cultures of 10^5 L-929 cells treated with staurosporine (0.5, 1 or 1.5 μg/ml) for 24 hours, assessed by Annexin V binding and 7-AAD staining using flow cytometry (Data are means n=6).

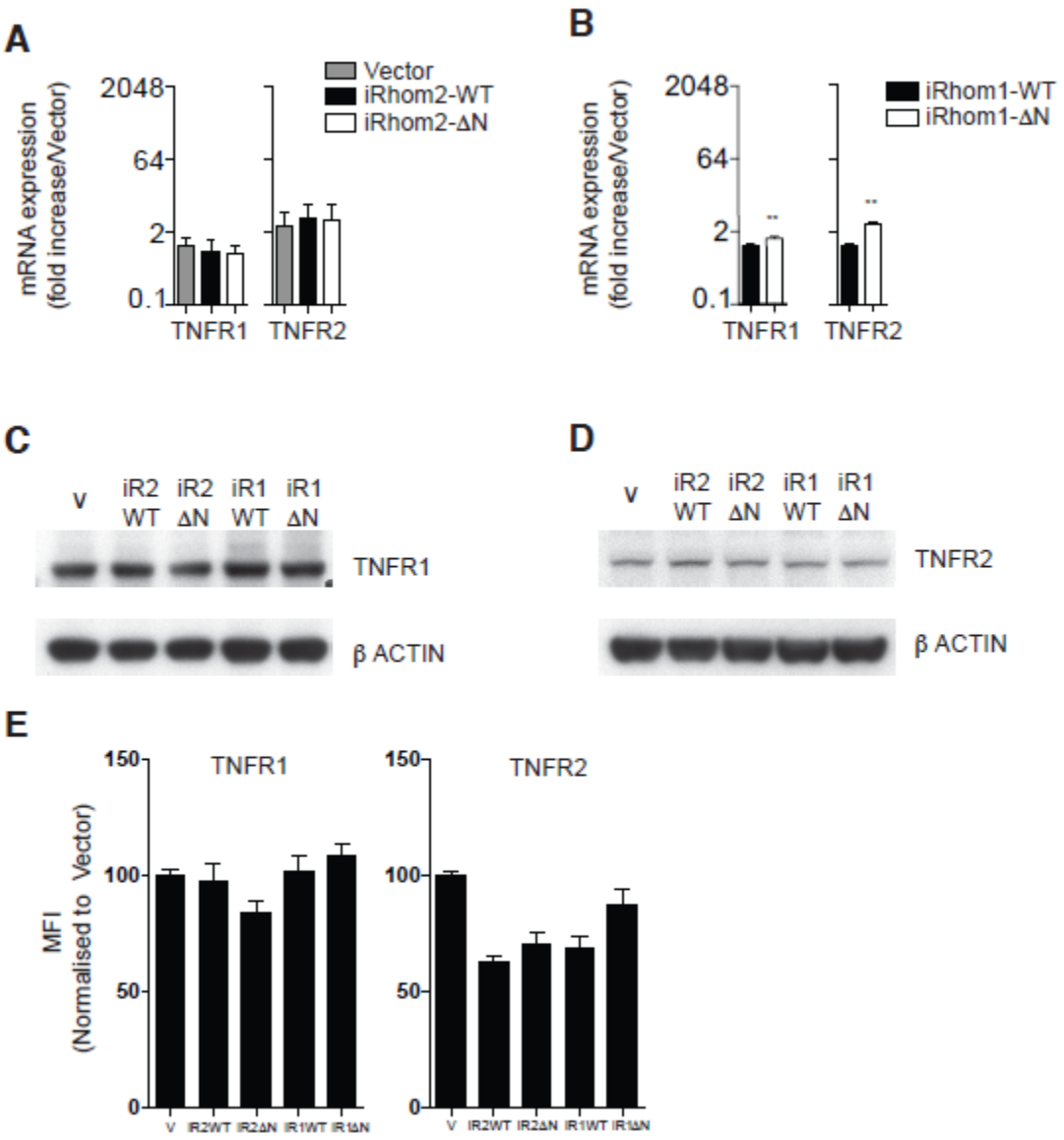

Fig S2: TNFR1 and TNFR2 abundance in iRhom-expressing cells. (A and B) RNA expression of *Tnfrsf1a* (left panel) and *Tnfrsf1b* (right panel) in L929 cells expressing (A) iRhom2-WT or iRhom2-ΔN, or (B) iRhom1-WT, or iRhom1-ΔN (n=6 experiments, ** $p<0.01$ between iRhom2-WT and iRhom2-ΔN). **(C and D)** TNFR1 (C) and TNFR2 (D) abundance by Western blotting whole cell lysates from L929 cells expressing vector control or wild-type (WT) or ΔN mutants of iRhom1 (iR1) or iRhom2 (iR2) (n=3). **(E)** TNFR1 and TNFR2 abundance using flow cytometery analysis of permeabilized L929 cells transfected as in (C and D) (TNFR1 n= 11, and TNFR2 n= (9-11)). Data are means ± SD.

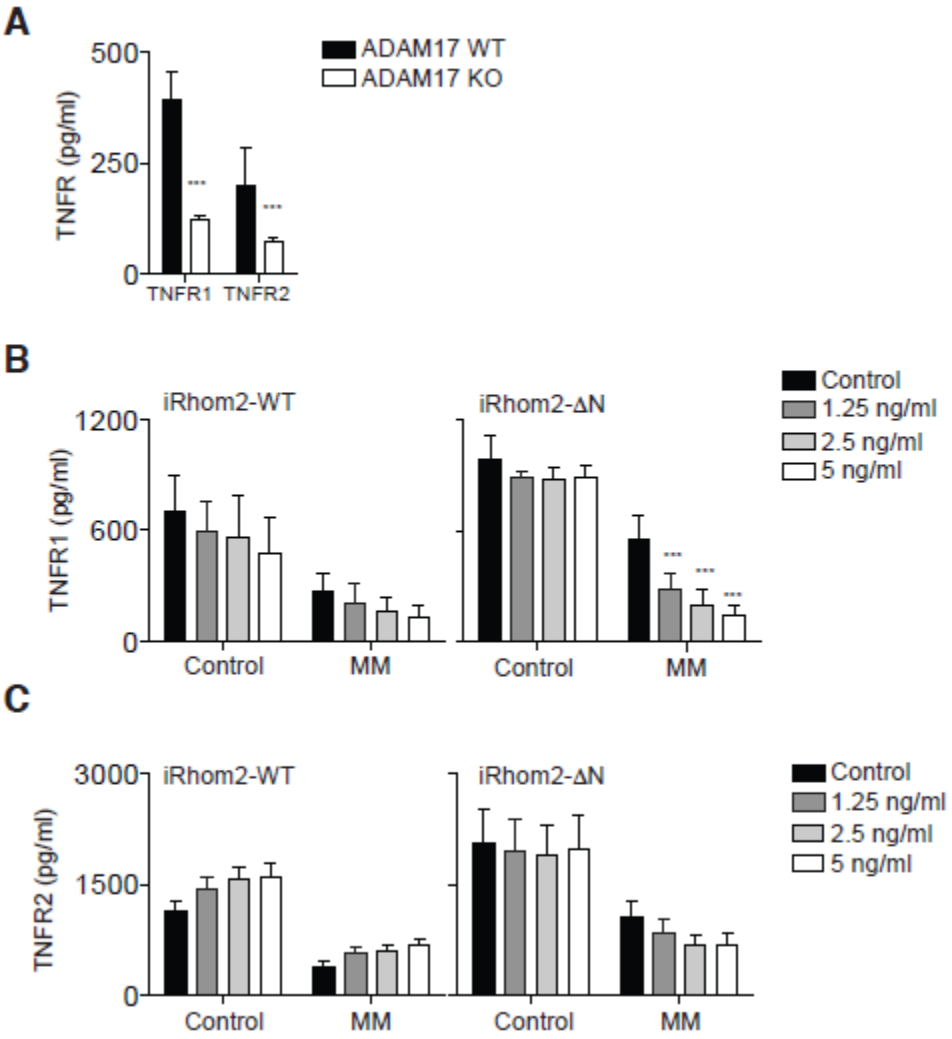

Fig S3: ADAM17 mediates cleavage of TNFRs. (A) TNFR1 and TNFR2 concentrations were determined in the supernatant of control and ADAM17-deficient iMEFs (n=6 experiments). Data are means ± SEM *** $p < 0.001$ **(B and C)** TNFR1 (B) and TNFR2 (C) abundance in cultures of 10^5 L-929 cells expressing different versions of iRhom2 treated with recombinant TNF in the presence or absence of marimastat (MM, 20 µM), assessed by TNFR ELISA (n=6). Data are means ± SD; *** $p < 0.001$.

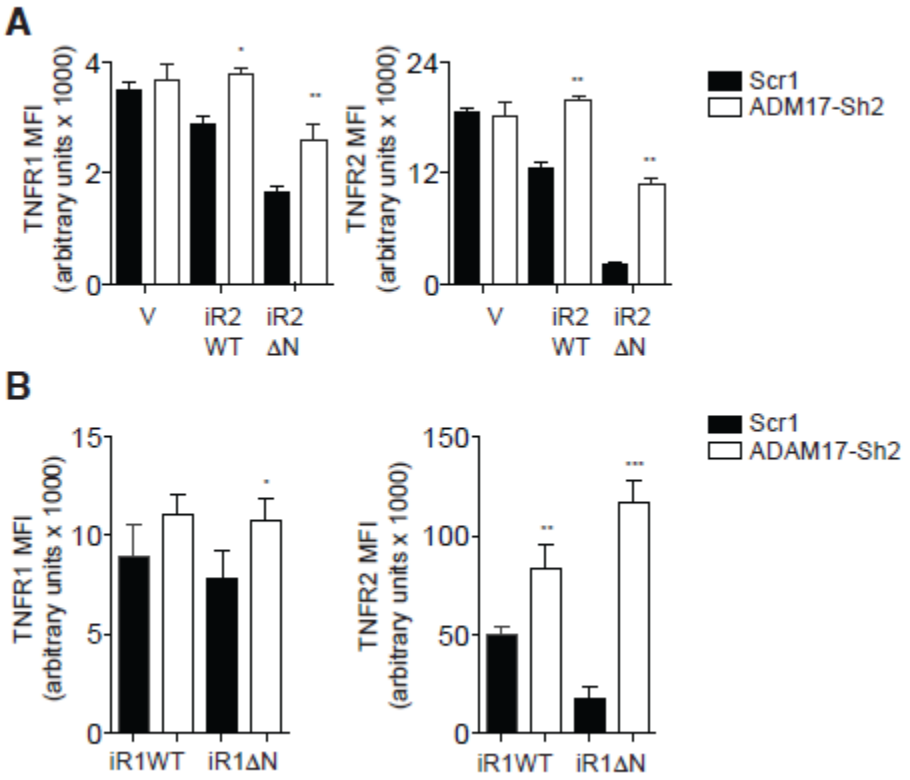

Fig S4: Stable knockdown of ADAM17 prevents ΔN-iRhom–dependent TNFR shedding. **(A)** Surface abundance of TNFR1 and TNFR2 determined by flow cytometry in L929 cells expressing a vector control (V), full length iRhom2 (WT) or iRhom2-ΔN in the presence of either scrambled shRNA (Scr1) or shRNA against *Adam17* (Sh2) (n=6). **(B)** Surface abundance of TNFR1 (n=6) and TNFR2 (n=4) determined by flow cytometry in L929 cells expressing a full length iRhom1 or iRhom1-ΔN in presence of either scrambled (Scr1) or *Adam17* shRNA. Data are means ± SD; * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

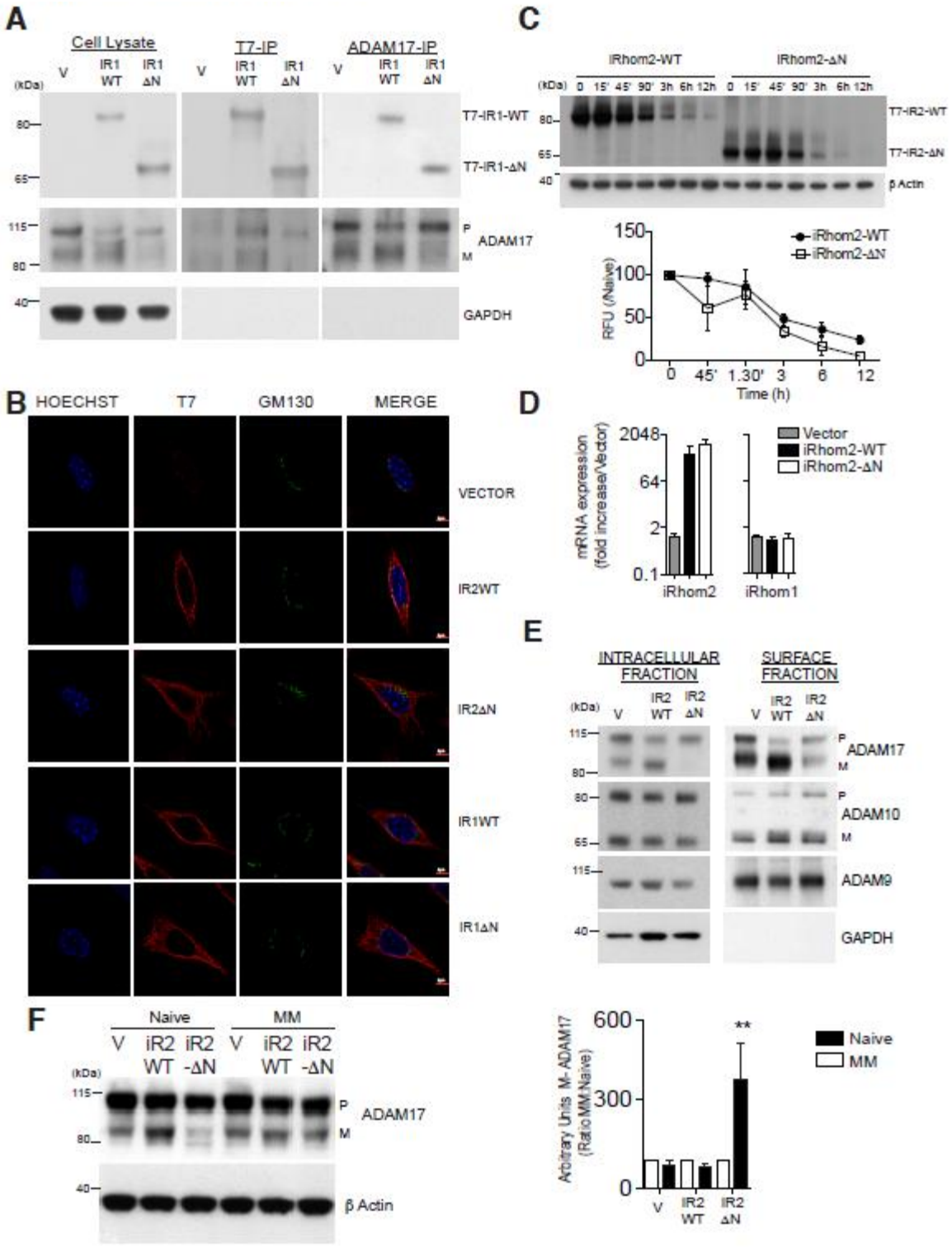

Fig S5: Localization patterns of truncated iRhoms and association with ADAM17. (A) L-929 cells stably expressing iRhom1-WT (T7-tagged), iRhom1-ΔN (T7-tagged), or a vector

plasmid were lysed followed by immunoprecipitation (IP) using T7 or ADAM17 antibodies. Immunoblots of lysates enriched using T7 or ADAM17 antibodies are shown, with GAPDH as a control. Bands representing pro-(P) and mature-(M) ADAM17 are indicated. Blots are representative of 3 experiments. **(B)** L-929 cells expressing vector control, iRhom2-WT, iRhom2-ΔN, iRhom1-WT or iRhom1-ΔN were fixed and permeabilized before staining for T7 (Cy3) and GM130 (Cy2) along with Hoechst (n=3). **(C)** 10^5 L-929 cells expressing different versions of iRhom2 were treated with cycloheximide (100 µg/mL) for the indicated time points. Whole cell lysates were probed for T7-tagged-iRhom2-WT or -iRhom2-ΔN abundance by immmunblotting and were quantified by densitometry (n=3). **(D)** iRhom2 and iRhom1 transcript expression was determined in L-929 cells expressing a vector control, iRhom2-WT, and iRhom2-ΔN (n=5). **(E)** Surface proteins from L-929 cells expressing full length iRhom2, iRhom2-ΔN, or a vector were enriched followed by immunoblotting for intracellular and surface fractions for ADAM17 (P = proform; M = mature form), ADAM10 (P = proform; M = mature form), and ADAM9; GAPDH served as loading control. Blots are representative of 3. **(F)** L-929 cells overexpressing a vector control, iRhom2-WT or iRhom2-ΔN were exposed to marimastat. Immunoblot of ADAM17 is shown before and after marimastat treatment (left panel). Bands representing pro-(P) and mature-(M) ADAM17 are indicated. Right, data are mean densitometry values ± SD of 3 blots; ** $p<0.01$ between MM-treated and naïve cells).

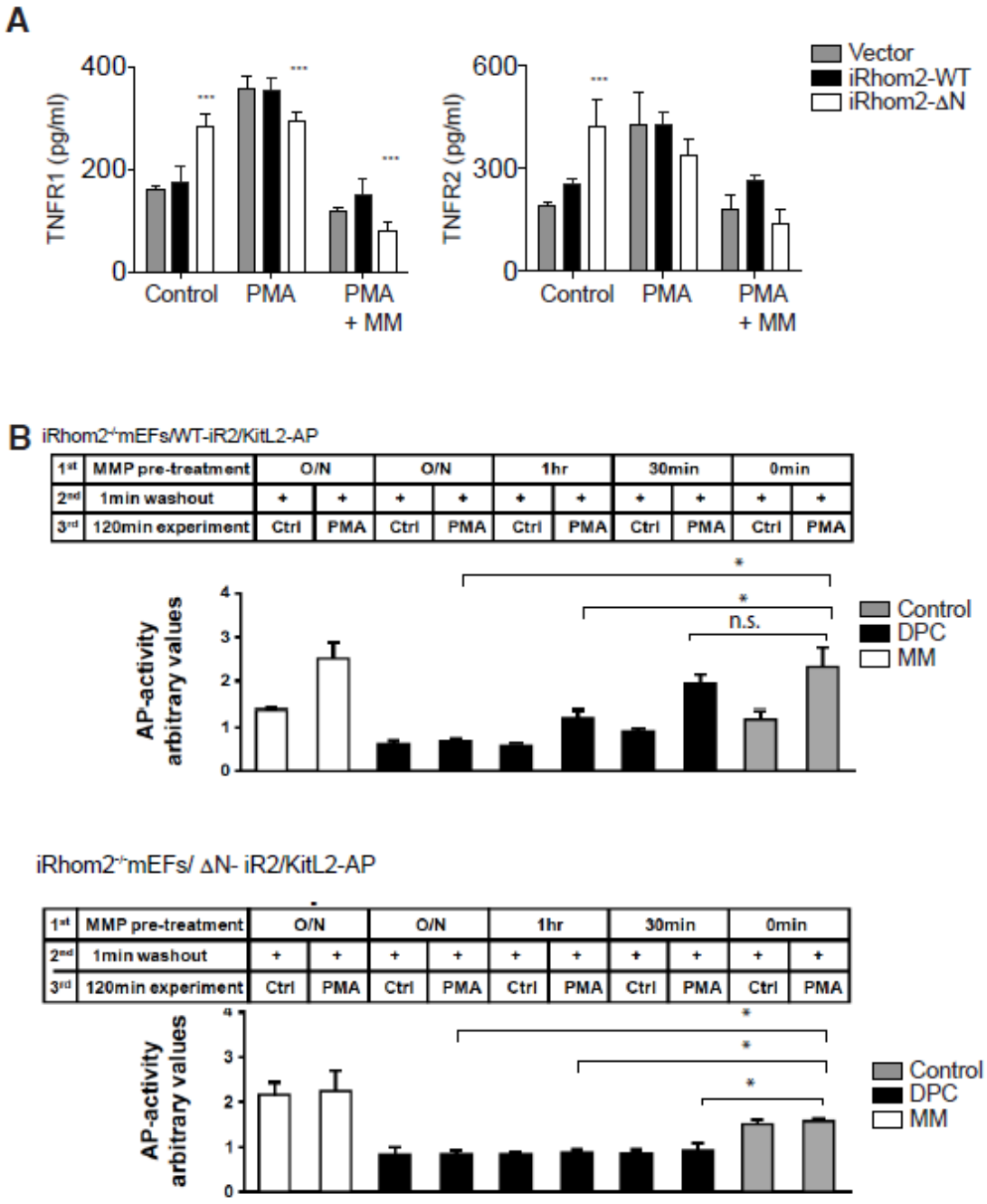

Fig S6: Increased ADAM17 activity associated with N-terminal truncated iRhom2. (A) TNFR1 ($n \geq 4$) and TNFR2 in supernatants from 10^5 L-929 cells expressing full length (WT) or -ΔN iRhom2 cultured with PMA (100 ng/ml) in the presence or absence of marimastat (MM, 20 μM) (*** $p<0.001$ compared to WT). **(B)** Mouse embryonic fibroblasts lacking iRhom2 (*iRhom2*$^{-/-}$ mEFs) were transfected with either iRhom2-wild-type (top panel) or iRhom2-ΔN

(lower panel) and KitL2-AP. Following treatment with the irreversible ADAM17 inhibitor DPC, or the reversible ADAM17 inhibitor MM for indicated time points (O/N = overnight), cells were washed and PMA dependent shedding of KitL2-AP was determined (n=3).