Supporting Information

Maretzky et al. 10.1073/pnas.1302553110

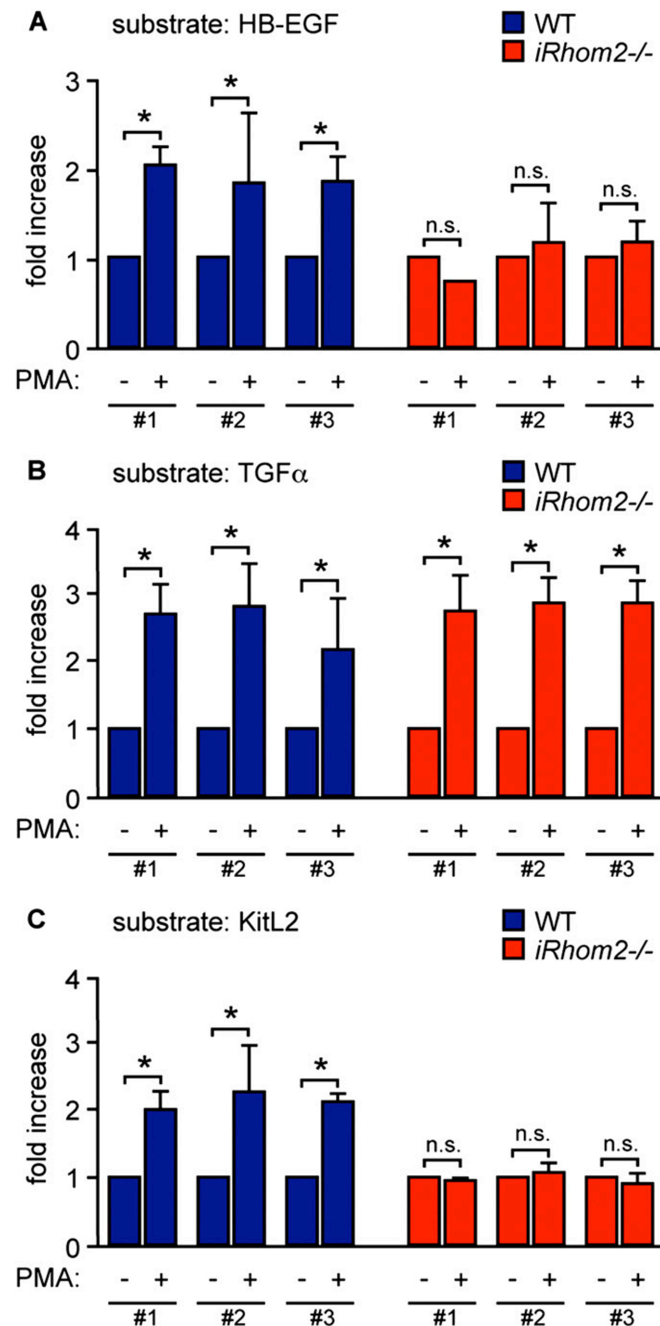

Fig. S1. Inactive rhomboid protein 2 (iRhom2) controls the stimulated shedding of heparin-binding (HB)-EGF and Kit ligand (KitL) 2, but not of TGFα in several independently isolated cultures of primary mouse embryonic fibroblasts (mEFs). (*A–C*) Three separately isolated cultures of primary wild-type (WT) or *iRhom2*$^{-/-}$ mEFs were transfected with the alkaline phosphatase (AP)-tagged ADAM17 (a disintegrin and metalloprotease 17) substrates HB-EGF (*A*), TGFα (*B*), or KitL2 (*C*) and stimulated for 30 min with 25 ng/mL phorbol-12-myristate-13-acetate (PMA). $^{*}P \leq 0.05$; ±SEM ($n = 3$).

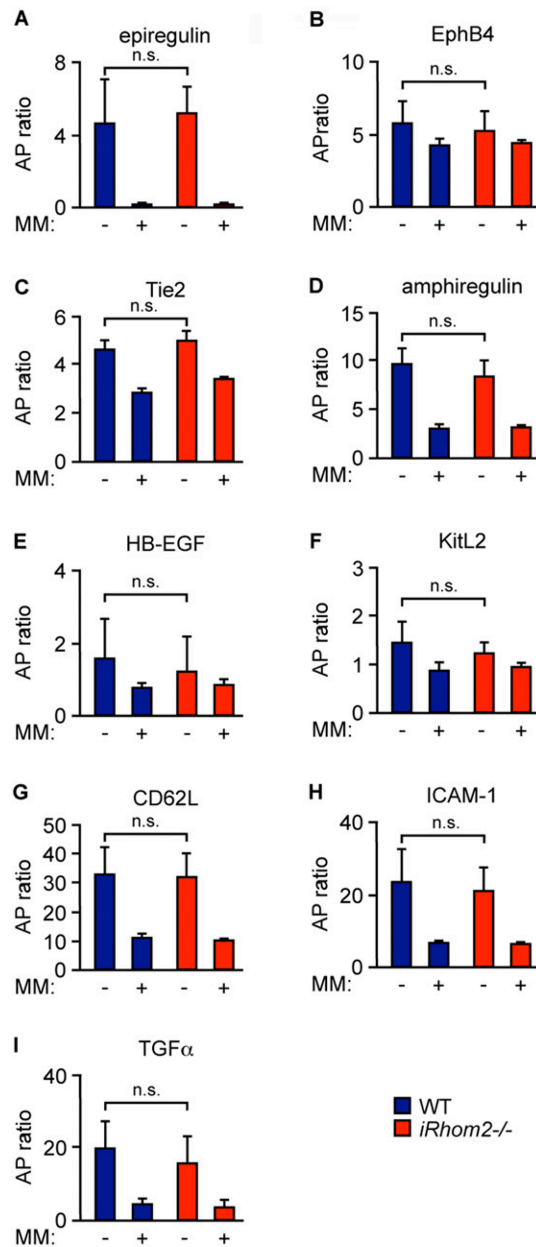

Fig. S2. The constitutive shedding of ADAM17 substrates is not significantly affected by the lack of iRhom2. WT or *iRhom2*$^{-/-}$ mEFs were transfected with the AP-tagged ADAM17 substrates epiregulin (*A*), Eph receptor B4 (EphB4) (*B*), Tie2 (*C*), amphiregulin (*D*), HB-EGF (*E*), KitL2 (*F*), CD62 ligand (CD62L) (*G*), intercellular adhesion molecule (ICAM)-1 (*H*), or TGFα (*I*), and constitutive shedding from cells incubated in the presence or absence of the metalloprotease inhibitor marimastat (MM) was measured after 4 h, as described in *Materials and Methods* ($n = 3$; ±SEM; n.s., not significantly affected).

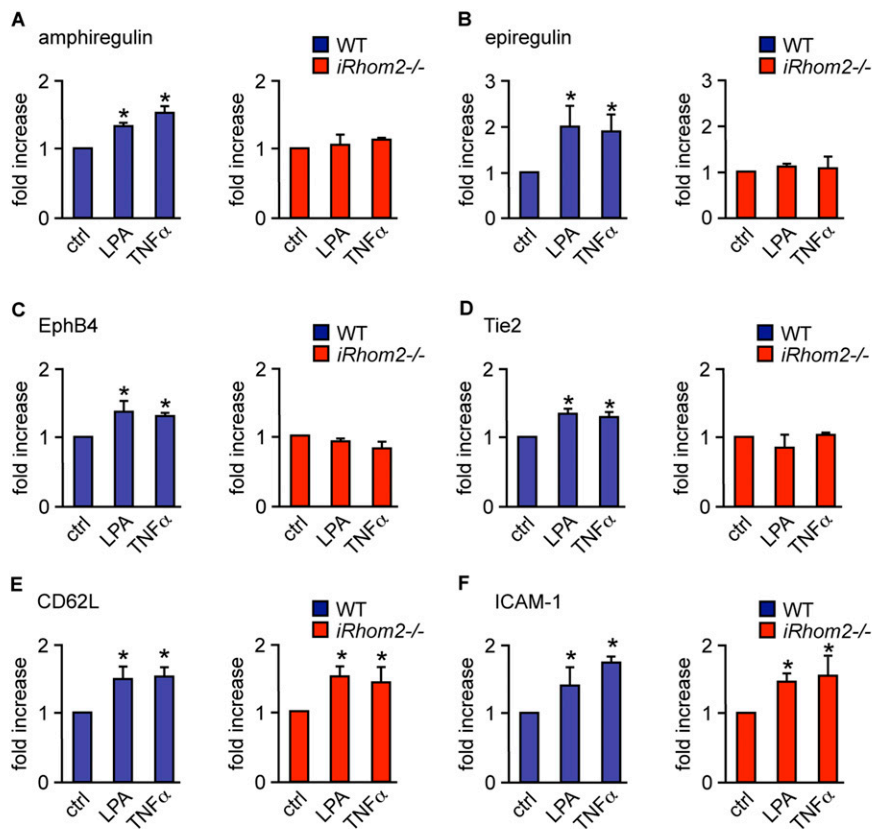

Fig. S3. iRhom2 controls the LPA and TNFα-stimulated shedding of some substrates of ADAM17 (amphiregulin, epiregulin, EphB4, and Tie2) but not of other substrates of ADAM17 (CD62L, ICAM-1). WT or *iRhom2*$^{-/-}$ mEFs were transfected with the AP-tagged ADAM17 substrates amphiregulin (*A*), epiregulin (*B*), EphB4 (*C*), Tie2 (*D*), CD62L (*E*), or ICAM-1 (*F*) and stimulated for 30 min with LPA (10 μM) or TNFα (10 ng/mL). Treatment with LPA or TNFα activated ADAM17-mediated shedding, as evidenced by the significantly increased cleavage of all of the tested substrates in WT mEFs. Identical experiments were performed with *iRhom2*$^{-/-}$ mEFs, in which stimulation for 30 min with LPA or TNFα did not increase the shedding of epiregulin, EphB4, Tie2, or amphiregulin but activated the release of CD62L and ICAM-1. Shedding was determined as described in *Materials and Methods*. $^{*}P \leq 0.05$; ±SEM (n = 3).

A

TGFα/HB-EGF ▮ = Transmembrane * = AP-tag

```
MVPSAGQLAL FALGIVLAAC QALENSTSPL SADPPVAAAV VSHFNDCPDS HTQFCFHGTC
RFLVQEDKPA CVCHSGYVGA RCEHADLLAV VAASQKKQTI LAVVAVVLSS VCLLVIVGLL
MFRYHRRGGY DVENEEKVKL GMTNSH
```

HB-EGF/TGFα ▮ = Transmembrane * = AP-tag

```
MKLLPSVVLK LFLAAVLSAL VTGESLERLR RGLAAGTSNP DPPTVSTDQL LPLGGGRDRK
VRDLQEADLD LLRVTLSSKP QALATPNKEE HGKRKKKGKG LGKKRDPCLR KYKDFCIHGE
CKYVKELRAP SCICHPGYHG ERCHGLSLPV ENRLYTYDHT AITALVVVSI VALAVLIITC
VLIHCCQVRK HCEWCRALIC RHEKPSALLK GRTACCHSET VV
```

TGFα/HB-EGF JM ▮ = Transmembrane * = AP-tag

```
MVPSAGQLAL FALGIVLAAC QALENSTSPL SADPPVAAAV VSHFNDCPDS HTQFCFHGTC
RFLVQEDKPA CVCHSGYVGA RCHGLSLPVE NRLYTYDHTT ILAVVAVVLS SVCLLVIVGL
LMFRYHRRGG YDVENEEKVK LGMTNSH
```

HB-EGF/TGFα JM ▮ = Transmembrane * = AP-tag

```
MKLLPSVVLK LFLAAVLSAL VTGESLERLR RGLAAGTSNP DPPTVSTDQL LPLGGGRDRK
VRDLQEADLD LLRVTLSSKP QALATPNKEE HGKRKKKGKG LGKKRDPCLR KYKDFCIHGE
CKYVKELRAP SCICHPGYHG ERCEHADLLA VVAASQKKQA ITALVVVSIV ALAVLIITCV
LIHCCQVRKH CEWCRALICR HEKPSALLKG RTACCHSETV V
```

B

Chimera primers:
Universal

```
EX FOR:           CTTAACTGGCTTATCGAAATTAATAC
EX FOR Nested:    ATACGACTCACTATAGGGAGACCCAAGCTT
Cyto REV:         GTCGAGGCTGATCAGCGAGCTCTAGCA
Cyto REV Nested:  GTGACACTATAGAATAGGGCCCTCTAGA
```

TGFα/HB-EGF

```
EX REV:     CACCACAGCCACCACGGCCAGGATGGTCTGCTTCTTCTGGCTGGCAGCCACCAC
Cyto FOR:   GTGGTGGCTGCCAGCCAGAAGAAGCAGACCATCCTGGCCGTGGTGGCTGTGGTG
```

HB-EGF/TGFα

```
EX REV:     GGAGACCACCACCAAGGCGGTGATGGCTGTGTGGTCATAGGTATATAAGCGATT
Cyto FOR:   AATCGCTTATATACCTATGACCACACAGCCATCACCGCCTTGGTGGTGGTCTCC
```

TGFα/HB-EGF JM

```
EX REV:     TTCCACTGGGAGGCTCAGCCCATGACAGCGTGCACCAACGTACCCAGAATGGCA
CYTO FOR:   TGCCATTCTGGGTACGTTGGTGCACGCTGTCATGGGCTGAGCCTCCCAGTGGAA
```

HB-EGF/TGFα JM

```
EX REV:     CACGGCCAGGAGGTCCGCATGCTCACACCTCTCTCCATGGTAACCCGGGTGGCA
CYTO FOR:   TGCCACCCGGGTTACCATGGAGAGAGGTGTGAGCATGCGGACCTCCTGGCCGTG
```

Fig. S4. Chimera between TGFα-AP and HB-EGF-AP. (*A*) Table of the amino acid sequences of the TGFα-AP/HB-EGF-AP chimera expression constructs used in this study. Domain components for TGFα and HB-EGF are presented in blue and red, respectively. The transmembrane region is highlighted in yellow. Asterisks indicate the modified insertion site of the AP tag. (*B*) Primer sequences used for overlap extension PCR to generate the chimera between TGFα-AP and HB-EGF-AP.

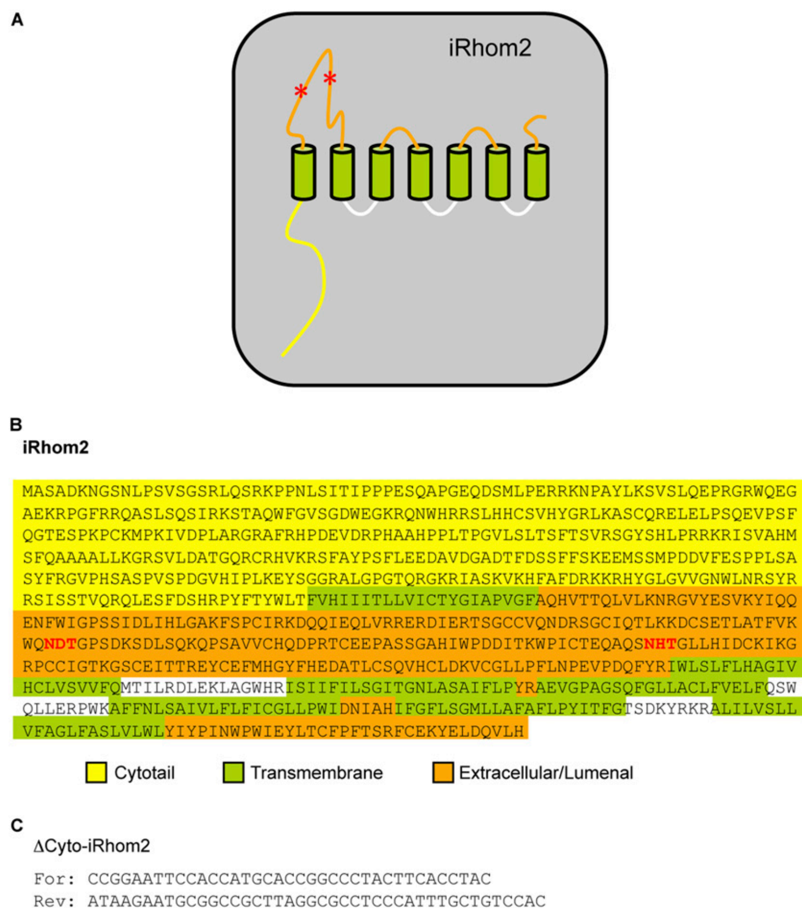

Fig. S5. Membrane topology and sequence of iRhom2. (A) Diagram of the predicted membrane topology of iRhom2, with asterisks indicating potential N-linked glycosylation sites in the extracellular loop of iRhom2. (*B*) Amino acid sequence of iRhom2 with the predicted N-terminal cytoplasmic domain highlighted in yellow, the predicted transmembrane domain highlighted in green, and the extracellular/luminal domains in orange, the potential N-linked glycosylation sites in the extracellular loop of iRhom2 in red, and the small cytoplasmic loops between transmembrane domains in white. (*C*) Primer sequences used for cytoplasmic domain-deletion mutant of iRhom2.