Supporting Information

Rosenstein et al. 10.1073/pnas.1418959111

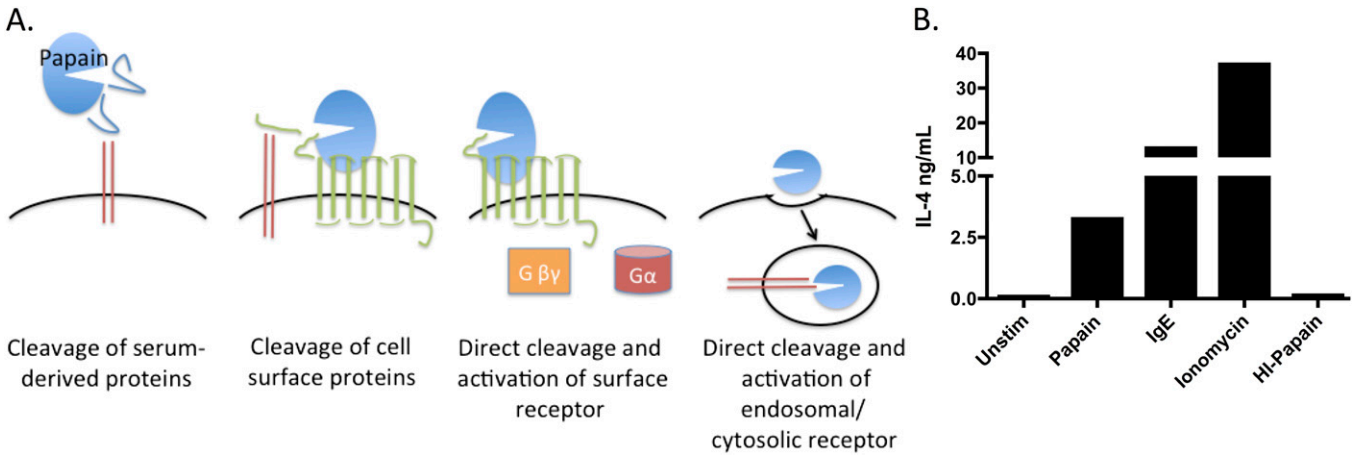

Fig. S1. Papain stimulation of basophils is TLR4 independent. (*A*) Schematic of possible mechanisms for papain stimulation of basophils. (*B*) IL-4 secretion by TLR4-deficient BMBs stimulated with indicated stimuli.

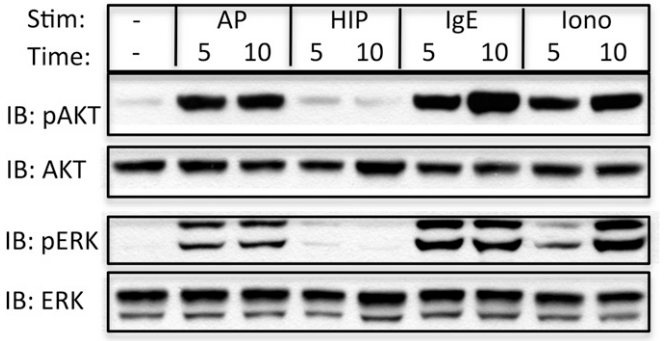

Fig. S2. The proteolytic activity of papain stimulates AKT and ERK activation and does not require TLR4 expression. Immunoblot analysis of pAKT/AKT and pERK/ERK prepared from TLR4-deficient BMBs stimulated as noted with active papain (AP), heat-inactivated papain (HIP), IgE cross-linking, or Ionomycin (Iono).

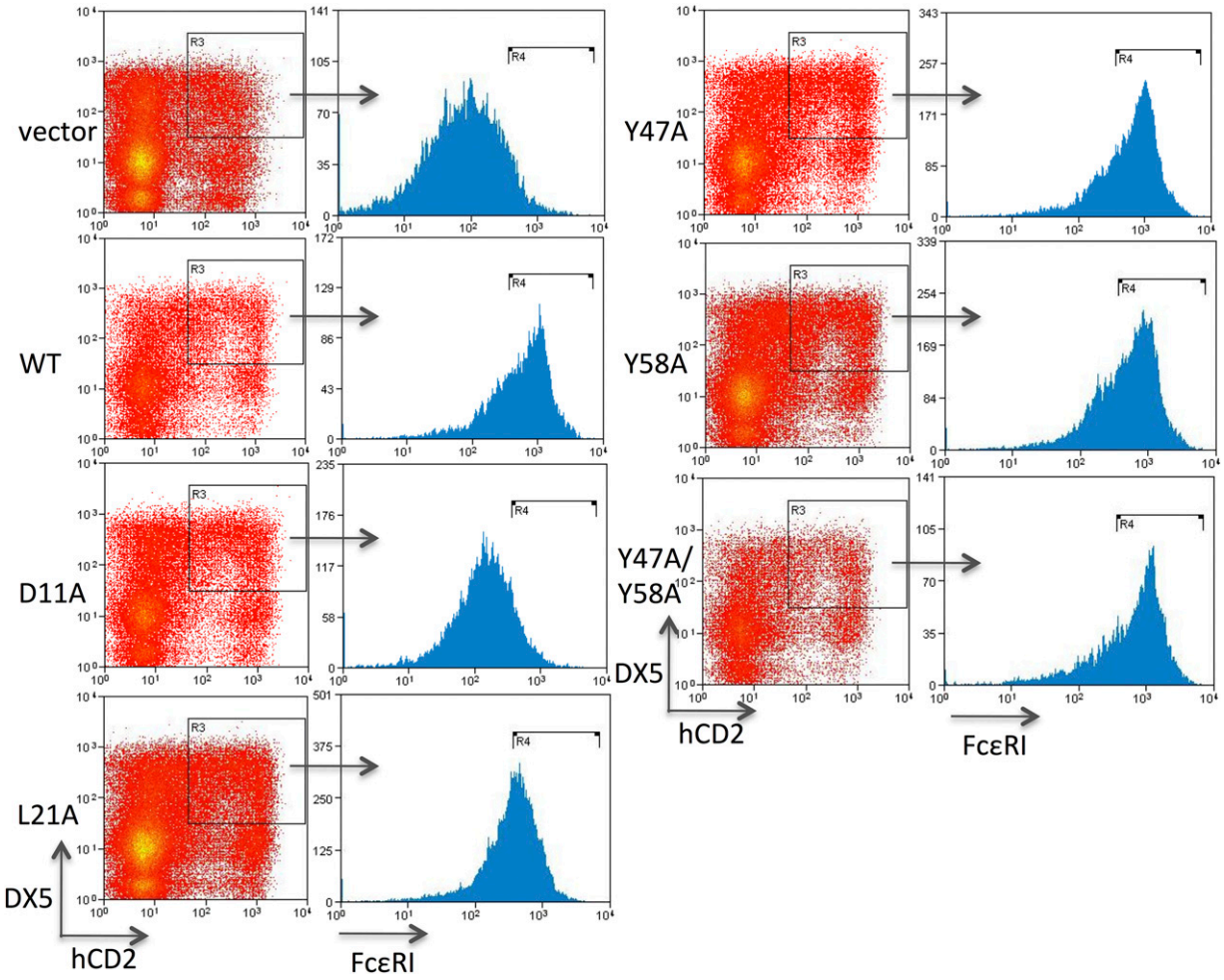

Fig. S3. Sorting method for transduced FcRγ$^{-/-}$ basophils and expression of FcεRI on sorted population. FcεRI expression of FACSorted DX5^{+} hCD2^{+} transduced FcRγ$^{-/-}$ basophils.

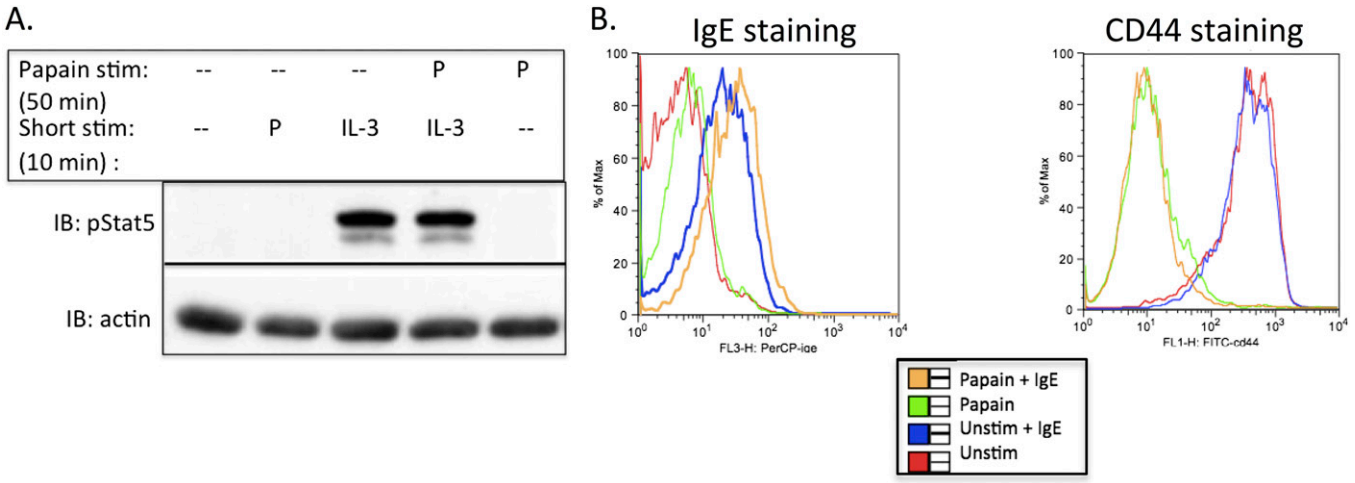

Fig. S4. Papain stimulation does not cleave the surface receptors for IgE or IL-3. (*A*) Immunoblot analysis of pStat5 and actin from BALB/c BMBs starved of IL-3 for 2 h and subsequently left unstimulated or stimulated with papain for 50 min; unstimulated and papain-stimulated samples were stimulated with IL-3 or papain or were not treated again. (*B*) Surface IgE and CD44 staining of BMBs treated with papain for 1 h and subsequently incubated with IgE for 20 min. CD44, a known target for papain cleavage of surface proteins, was stained as a positive control.

A.

Knock-out mice tested *in vitro* for basophil stimulation by papain:

ITAM receptors
DAP12

GPCR components/signaling
Gαi2
PLCβ2,3
PI3Kγ
B-arrestin 1
B-arrestin 2

Inflammasome components
Caspase 1
Asc

Trp channel
Trpa1
Trpv1

Adhesion Molecules
CD44
Ltb4r1
F2rl1

TLR components
TLR2/4
MyD88

Lineage-defining molecules
CD1d
Gata1

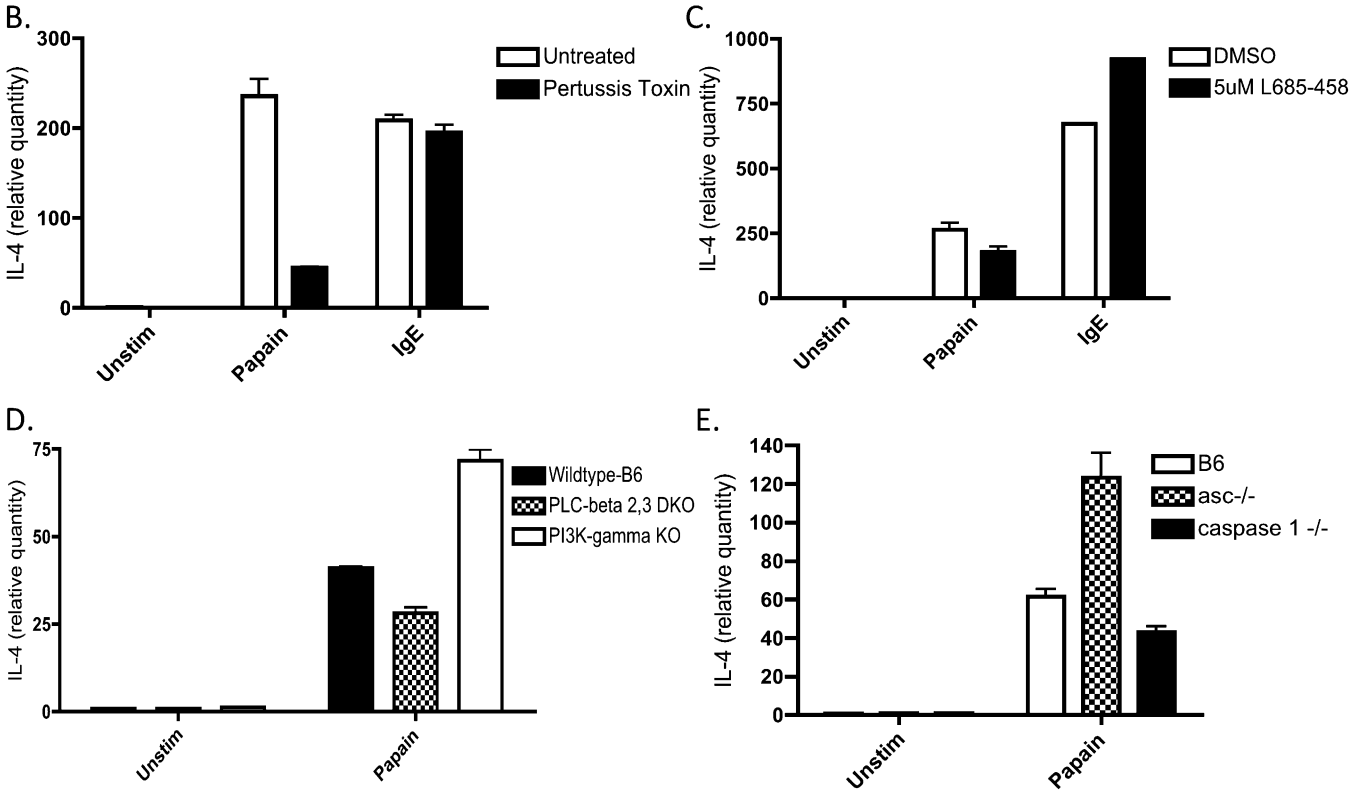

Fig. S5. Progress toward the identification of the sensor of papain stimulation of basophils. Papain stimulation of basophils is pertussis toxin dependent but is not dependent on the expression of PLCβ2,3 or PI3Kγ. (*A*) BMBs were derived from mice deficient in indicated proteins and from control wild-type mice, and IL-4 production was evaluated by ELISA or qPCR to determine the involvement of these receptors and/or signaling mediators in basophil sensing of papain. Each knockout mouse was tested in one or two experiments. (*B* and *C*) Relative abundance of IL-4 prepared from BALB/c BMBs pretreated overnight with pertussis toxin (*B*) or for 24 h with L-685,458 (*C*) and subsequently treated with papain or IgE. RNA was isolated after 4 h of stimulation. (*D* and *E*) Relative abundance of IL-4 prepared from BMBs derived from B6, PLCβ2,3$^{-/-}$, or PI3Kγ$^{-/-}$ mice (*D*) or B6, Asc$^{-/-}$, or caspase-1$^{-/-}$ mice (*E*) and stimulated as noted for 4 h. Stimulated samples were calibrated to the unstimulated sample of each respective strain. Error bars show SEM.

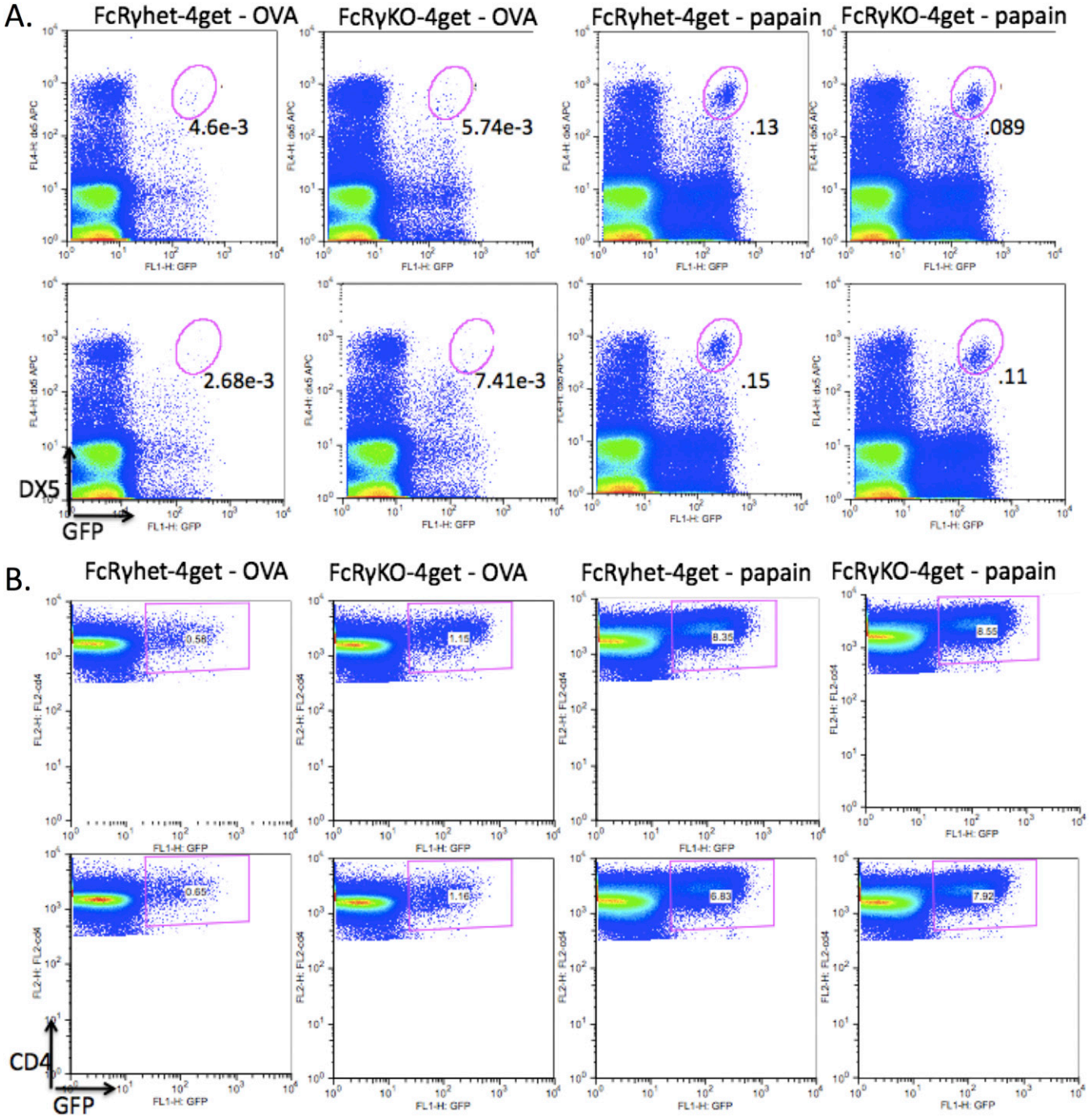

Fig. S6. FcRγ expression is not required for basophil migration to the lymph node or for T-cell IL-4 production in the lymph node. (*A*) $DX5^+$ GFP^+ basophils and (*B*) $CD4^+$ GFP^+ T cells from $FcR\gamma^{-/-}$ 4get and $FcR\gamma^{+/-}$ 4get (IL-4–GFP reporter mouse) popliteal lymph nodes 3 d after s.c. immunization with ovalbumin (OVA) or papain.