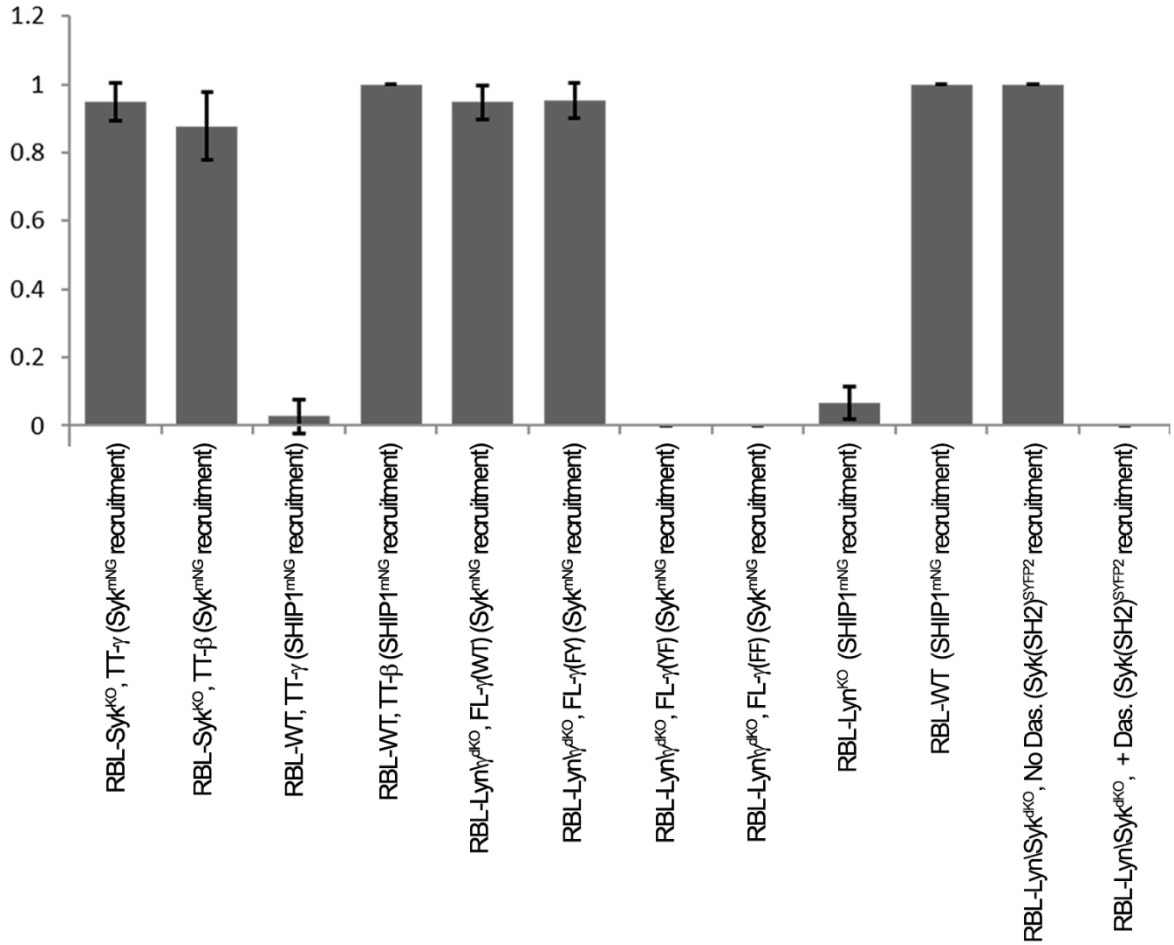
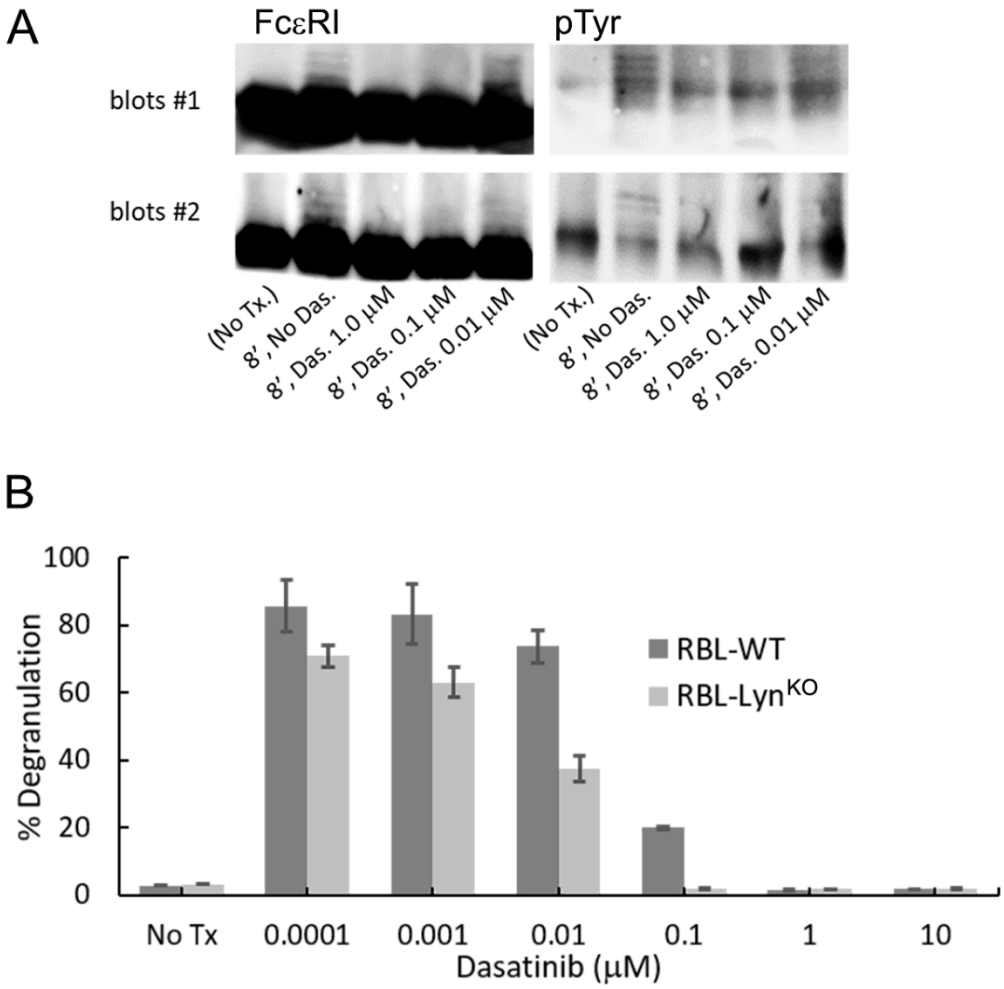
# Supplementary Materials

*Molecular Biology of the Cell*

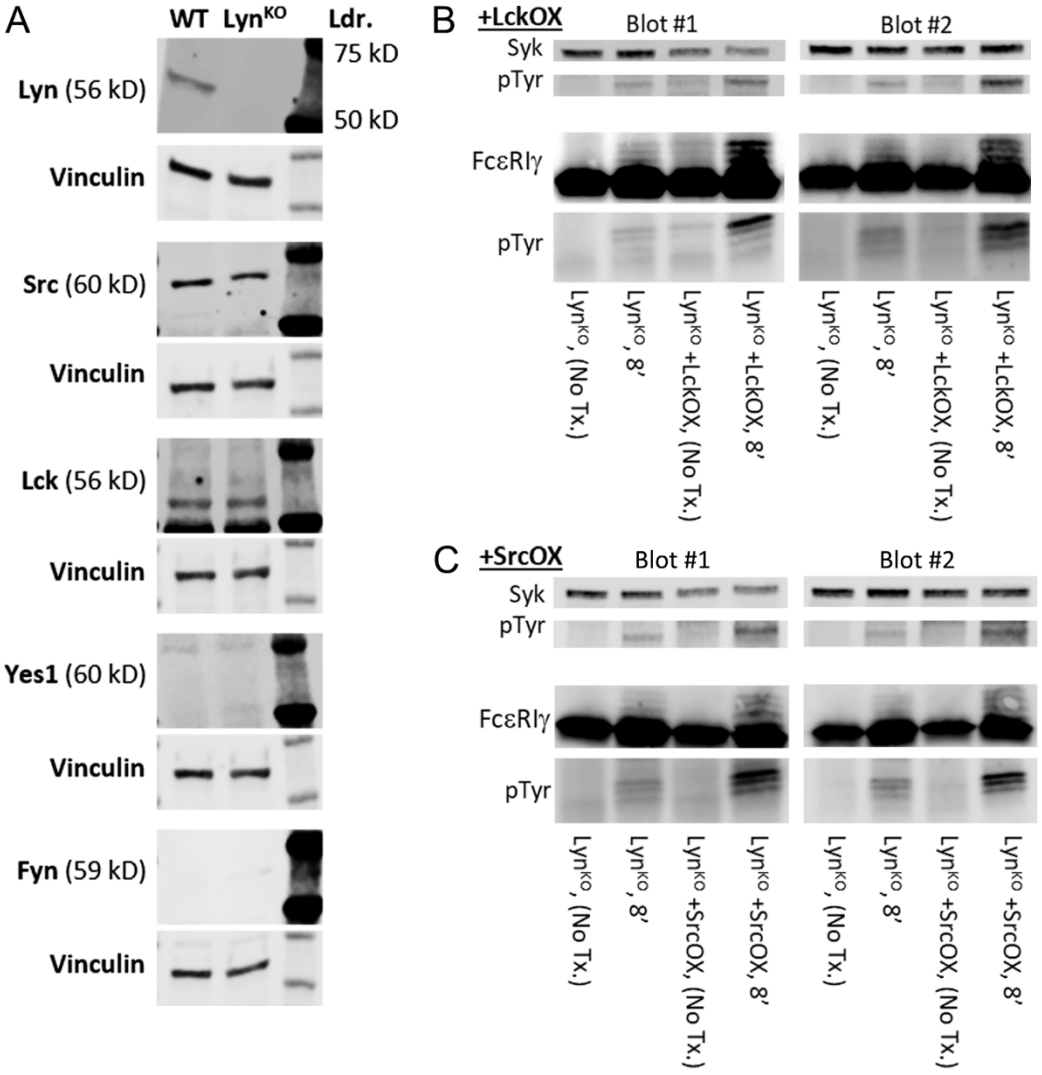
Kanagy *et al*.



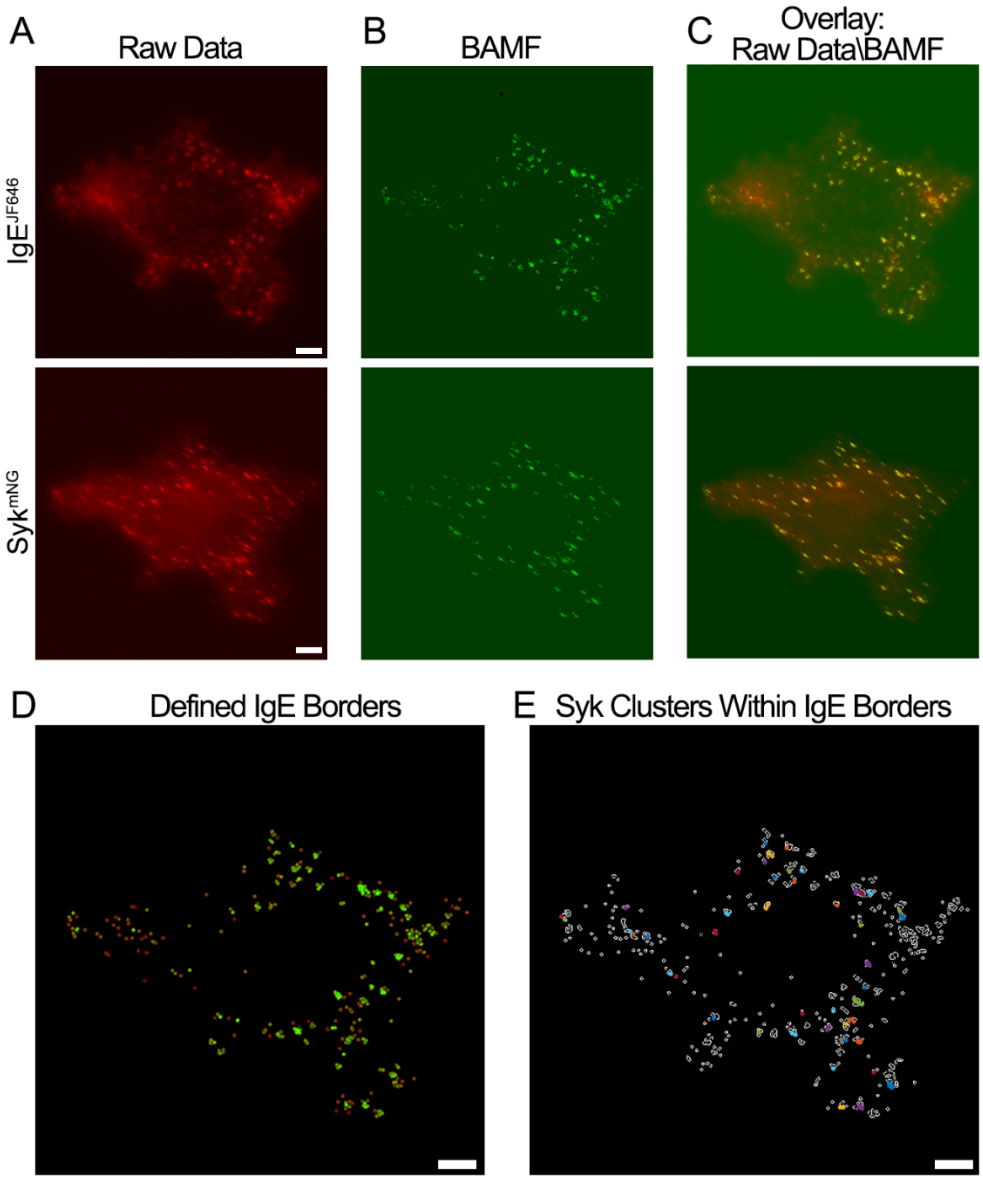

**Supplemental Figure S1. Quantification of frequency of protein recruitment.** Analysis of the recruitment of Syk-mNG or SHIP1-mNG to FcεRI or TT-ITAM aggregates as assessed by confocal microscopy. Images were acquired as described in the main text and visually assessed for protein recruitment. The state of recruitment is considered as a Bernoulli Trial where the bars represent the percentage of cells demonstrating protein recruitment for that condition (error is the 95% confidence interval). At least 5 independent coverslip preparations per condition. Number of cells per condition (left to right): n = 60, n = 41, n = 38, n = 59, n = 77, n = 65, n = 83, n = 65, n = 107, n = 103, n = 50, n = 73.



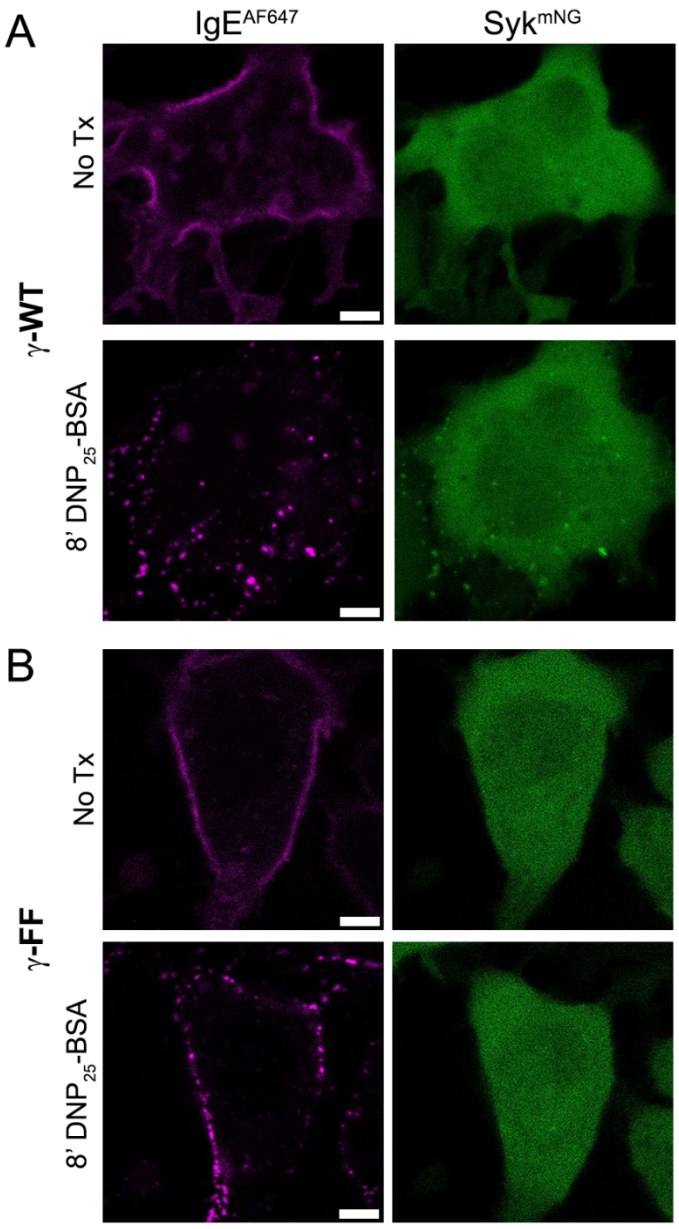

**Supplemental Figure S2.** Inhibition of SFK activity prevents FcεRIγ ITAM phosphorylation. (A) Immunoblots of RBL-Lyn$^{KO}$ cell lysates show that γ phosphorylation is reduced by dasatanib (Das.) in a dose dependent manner. Cells were lysed at 8' after addition of 0.1 μg/mL $DNP_{25}$-BSA. Lysates were probed for either total FcεRIγ (left) or phosphorylation (pTyr, right), and repeated in duplicate (upper and lower). (B) Degranulation was measured by relative β-hexosaminidase released after 30 min incubation with 0.1 μg/mL $DNP_{25}$-BSA. Cells were pre-treated with dasatinib for 30 min prior to degranulation assay. Average values from 3 replicate experiments; mean +/- SD. No treatment (No Tx).



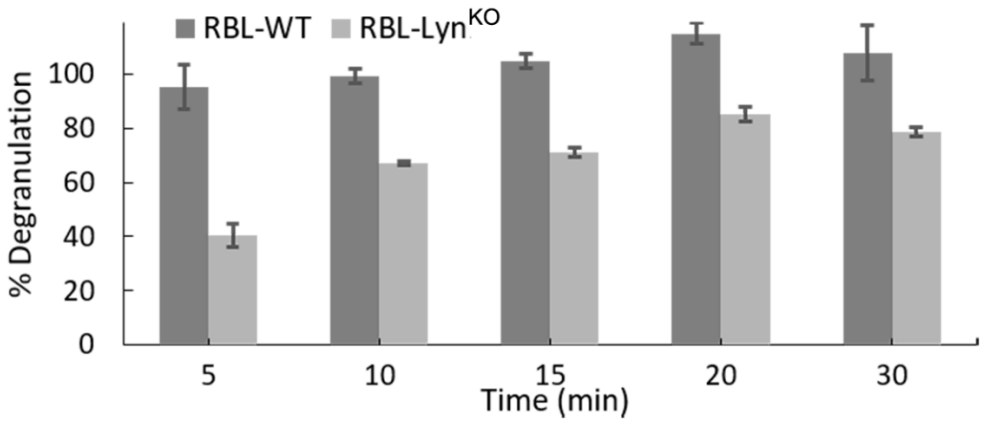

**Supplemental Figure S3.** Lck or Src are present in RBL cells and have the potential to compensate for Lyn. (A) Whole cell lysates were collected from RBL-WT and RBL-Lyn$^{KO}$ cells to test for the presence of known SFKs by western blot (n=2). Src and Lck are present in both cell lines. (B-C) Immunoblots of RBL-Lyn$^{KO}$ cell lysates showing that over-expression of Lyn-GFP (+LckOX) or Src-GFP (+SrcOX) enhances γ and Syk phosphorylation in RBL-Lyn$^{KO}$ cells. Lysates were probed for total Syk and pTyr (top panels). Samples were also immunoprecipitated using anti-IgE antibody and probed for total FcεRIγ and pTyr levels (bottom panels). Experiments repeated in duplicate (left and right).




**Supplemental Figure S4.** Application of Bayesian Multiple-emitter Fitting (BAMF) to identify FcεRI aggregates and Syk clusters. (A) Beginning with the raw data (red) of the IgE or Syk channel. (B) BAMF was used to reconstruct a mask image (green) where IgE aggregates or Syk clusters were isolated from background signal. (C) Overlay of the raw data and BAMF reconstruction. (D) Using the IgE BAMF reconstructed image (green), the borders of the IgE aggregates were defined (red outlines). (E) The Syk clusters determined using BAMF were overlaid with the defined IgE borders to correlate the intensity between each Syk cluster and its respective IgE aggregate. Scale bars, 5 μm.




**Supplemental Figure S5**. Syk recruitment requires FcεRIγ ITAM phosphorylation. RBL-Lyn/γ $^{dKO}$ cells expressing Syk-mNG (green) and either (A) γ-WT or (B) a mutant lacking both tryosines, γ-FF. Cells were incubated overnight with IgE-AF647 (magenta). After 8 min of receptor crosslinking with 0.1 μg/mL $DNP_{25}$-BSA, Syk-mNG is recruited to γ-WT(n=77) but not γ-FF (n=65). See also Supplemental Figure S1. Scale bars are 5 μm.




**Supplemental Figure S6.** Degranulation of RBL-Lyn$^{KO}$ cells is delayed. (A) Degranulation was measured by relative β-hexosaminidase released after incubation with 0.1 μg/mL $DNP_{25}$-BSA at the respective time points. Average values from 3 replicate experiments; mean +/- SD.

## Supplemental Video Legend

**Supplemental Video 1**: Example image series of Syk-mNG SPT and corresponding trajectories after 1 min of 0.1 μg/mL $DNP_{25}$-BSA addition in RBL-WT cells. Image (greyscale) overlaid with identified single particle trajectories as they were identified across time. Video played in real time at 10 frames/sec. Scale displayed on axes. Pixel size is 106 nm.