Supplementary information 4:

Reagents: Protease inhibitors cocktail was from Calbiochem (Biosciences, Inc. La Jolla, CA). Protein assay and electrophoresis reagents were from Bio-Rad Laboratories (Rishon Le Zion, Israel). β-glycerolphosphate, and dNTP mix were from Sigma (St. Louis, MO). The ELISA kit specific for rat MCP-1 was from Pierce (Pierce Biotechnology, Inc; Rockford, IL, USA), the rat TNF-α and IL-4 ELISA kits were from R&D Systems (Minneapolis, USA), the rat LTC_4/LTB_4 EIA kits and Protein-G Sepharose 4 Fast Flow beads were from Amersham Pharmacia Biotech (Buckinghamshire, UK), Fluo3 and pluronic acid were from Molecular Probes Inc. (Eugene, OR, USA), QuantiTect SYBR Green real time PCR kit and RNeasy mini kit were from Qiagen (Hilden, Germany); Taq "zol" polymerase was from Talron (Rehovot, Israel) and Reverse-iT 1st Strand Synthesis kit from ABgene (Surrey, UK). BSA, fraction V (Sigma, St Louis, MO), was derivatized with 11 molecules of DNP PKC specific inhibitors: Go6976, Go6983 were a generous gift of Prof. Y. Zick (Weizmann Institute, Rehovot, Israel). The following luciferase reporter plasmids were kindly provided by Drs. A. Rao (Harvard Medical School, MA) – NFAT-Luc; S. Watanabe (Teikyo University, Japan) c-Fos-Luc; N.H. Colburn (National Cancer Institute – Frederick, MD) AP-1-Luc. The Cytokine Array was from RayBiotech, Inc. (Norcross, GA).

Antibodies: Mouse polyclonal IgG and its $F(ab')_2$ was purchased from Jackson ImmunoResearch Labs (Pennsylvania). DNP specific mouse mAb A2IgE, MAFA specific mAb G63, its $F(ab')_2$ and the conjugates of average of 3 DNP molecules to $F(ab')_2$ of either mouse polyclonal IgG or mAb G63 were prepared as previously described (Licht *et al.*, 2005). Rabbit monoclonal Abs specific for: pMEK-1/2, pIκB, NF-κB, p-panPKC, were from Cell Signaling Technology Inc, Beverly, MA; Mouse mAbs specific for phosphotyrosine (PY99), p-c-Jun and RasGAP, as well as rabbit polyclonal antibodies specific for PKC-ε and phosphorylated-PKB, were from Santa Cruz Biotechnology, Inc. CA. The mAbs specific for SHIP, Dok-2 and Shc were from Transduction Labs (Lexington, KY). Rabbit polyclonal antibodies specific for Dok-1 and Gab2 were generous gifts of Dr. J. Cambier (National Jewish Center, Denver, CO.), Dr.H.Gu (Harvard Medical School, Boston, MA) respectively. The rabbit polyclonal Abs specific for the FcεRI γ- and mouse monoclonal Abs specific for the

FcεRI β– subunit and PTK Syk were generous gifts of Dr. R. Siraganian (NIH, Bethesda, MD, USA); Abs specific for phosphorylated and total Erk-1/2, phosphorylated-p38 and phosphorylated-JNK were kind gifts of Dr. R. Seger (Weizmann Institute, Rehovot, Israel).

Cell culture and stimulation: IgE-primed cells (incubated overnight with 200ng of A2IgE per ml) were detached by incubation with 10 mM EDTA (in DMEM) for 15 min at 37°C. Cells were counted and seeded in ∅10cm petri dishes ($7x10^6$ cells/dish) and incubated at 37°C in DMEM overnight. Dishes were washed (3x) by Tyrode's buffer (130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 10 mM HEPES, 0.1 % BSA, pH 7.4). Adherent cells were treated with different concentrations of Ag or mAb G63 for indicated time periods. Treatments were stopped by adding ice-cold PBS containing 2mM sodium orthovanade; 10mM β-glycerolphosphate.

Immunoprecipitation and Immunoblotting: Treated cells were scraped from the dishes and lyzed with lysis buffer (1% Triton X-100, 50 mM HEPES, 100 mM NaF, 10 mM EDTA, 2 mM sodium orthovanadate, 10% glycerol, 10 mM sodium pyrophosphate, protease inhibitor cocktail 1:200, pH 7.4, $30x10^6$ cells/ml) on ice for 30 min. Lysates were centrifuged for 15 min at 15,000 x g, 4˚C and the post nuclear supernatants containing equal protein amounts were incubated with Protein-G Sepharose beads coupled to specific antibodies for 3 h with rotation at 4˚C. Beads were washed (3x) by ice-cold lysis buffer, and once by PBS. Bound proteins were eluted by boiling (5 min) in sample buffer, separated by SDS-PAGE, electrotransferred to nitrocellulose membranes and blocked for 30 min with 3% milk solution. This was followed by Western blotting (WB) with the specific antibodies and detection by enhanced chemiluminiscence (ECL).

Affinity isolation by ITIM peptides: MAFA ITIM peptides: non phosphorylated (MADNSIYSTLELC), tyrosine phosphorylated (MADNSIpYSTLELC), tyrosine/serine phosphorylated (MADNSIpYpSTLELC), and Y-2 serine phosphorylated (MADNpSIYSTLELC) were synthesized and conjugated to Sepharose beads as previously described (Xu *et al.*, 2001). RBL-2H3 cell lysates

(30x106cells/ml sample) were pre-cleaned with Sepharose beads and incubated with the peptide-conjugated Sepharose beads (40 μl/ml of lysate) for 2 h at 4°C with shaking. Beads were washed (4x) with ice-cold lysis buffer and once with PBS and the bound proteins were eluted by boiling for 5 min with reducing SDS-sample buffer.