

Supplementary information 3:

SPR analysis of the interactions between pITIM-containing peptides derived from the intracellular domain of MAFA and the SH2/PTB domains of SHIP, Shc, Dok-1 and RasGAP.

Synthetic MAFA peptides (LDNSIYSTLEL, LDNSIpYSTLEL; both biotinylated at their N-termini) were immobilized on streptavidin-coated sensor chips (Sensor Chip SA, Biacore AB, Uppsala, Sweden) at 25°C, at a flow rate of 5 µl/min in HEPES-buffered saline (HBS) (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005% surfactant P20, pH 7.4). Ligand binding was monitored following injection (250 µl/sample) of a respective GST-SH2/PTB domain fusion protein (previously dialyzed against HBS) at different concentrations with a flow rate of 20 µl/min in HBS running buffer. Dissociation was monitored during subsequent washing of the chip with HBS running buffer for 3600 s with a flow rate of 20 µl/min. Chip regeneration was performed by injection of 10 µl of HBS running buffer supplemented with 0.01% SDS. SPR measurements employed the BIAcore 2000 apparatus (Pharmacia Biosensor AB, Uppsala, Sweden). All real time curves were best fitted by two-state reaction model and all kinetic and affinity parameters were calculated using the BIAevaluation 3.0.2 software and plotted into the table.

pY-ITIM+PAAP					
Protein	K _d (nM)	k _{on1} (10 ⁴ M ⁻¹ s ⁻¹)	k _{off1} (10 ⁻³ .s ⁻¹)	k _{on2} (10 ³ .s ⁻¹)	k _{off2} (10 ⁻⁵ .s ⁻¹)
GST-SHIP .SH2	1.02 +/- 0.02	7.4 +/- 1.1	1.9 +/- 0.3	1.4 +/- 0.2	5.2 +/- 0.3
GST-Dok-1 .PTB	not detectable				
GST-RasGAP .SH2	not detectable				
GST-Shc .SH2.PTB	not detectable				