Supplementary methods

Antibodies and reagents

CD3 mAbs, OKT3 and 289 and CD28 mAbs, CD28.2 were reported previously (26). Anti-Myc epitope mAb, 9E10 and anti-phosphotyrosine mAb, 4G10 were purchased from Upstate Biotechnology (Lake Placid, NY). Polyclonal anti-GFP was purchased from Abcam Limited (Cambridge, UK). Monoclonal anti-HA 3F10 and anti-GFP were purchased from Roche (Meylan, France). Monoclonal anti-HA.11 was purchased from Babco (Richmond, CA). The polyclonal antibodies Phospho-p56Dok-2 (Tyr351) (# 3911), Akt (# 9272) and monoclonal antibodies Phospho-Akt (Ser473) (# 4058) were purchased from Cell signaling technology, Inc. GST mAb ND2.1 was a kind gift of Dr. J. L. Teillaud (Inserm U 872, Centre de Recherche des Cordeliers, Paris, France).

Immunoprecipitation and western blotting.

For the immunoprecipitations, lysates were clarified and incubated with anti-GFP coupled to protein A-Sepharose or with anti-HA 3F10 monoclonal antibody coupled to protein-G sepharose, for 2 h at 4 °C. Immunoprecipitates were washed two times in 1 ml of lysis buffer and then boiled in reducing SDS gel sample buffer for 5 min. Samples were resolved by SDS-polyacrylamide gels.

For immunoblotting, membranes were blocked and probed with specific antibodies. Blots were then incubated with the appropriate second antibodies, anti-rabbit IgG or anti-mouse IgG, both conjugated with horseradish peroxidase (DAKO Denmark A/S). Immunoreactive bands were visualized by enhanced chemiluminescence (Amersham Biosciences Limited, England, UK).

Plasmid constructs.

HA-Dok1 and HA-Dok2 expressed in βDNA4 vector have been described previously (1). The mutant lacking the PH domain was obtained by PCR amplification, using the primers: for βDNA4 HA-□PHDok1 (aa 124 to 482) sens $^{5'}$atgtacccatacgacgtcccagactacgctggctgggctttggcgcag$^{3'}$ (HA epitope) and anti-sens $^{5'}$ctatcaggtggaaccctcagacttgac$^{3'}$, and for βDNA4 HA-ΔPHDok2 (aa 118 to 412) sens $^{5'}$atgtacccatacgacgtcccagactacgctaggaaggagctctcggggc$^{3'}$ (HA epitope) and anti-sens $^{5'}$ctatcatttgggcctttctttagtac$^{3'}$. PCR were inserted in pGEMT-Easy vector, sequenced and subcloned in the βDNA4 vector using the restriction site NotI.

The proteins tagged GFP were generated by PCR amplification using the following primers: for pDok1-GFP, sens $^{5'}$agatctcgagctatggacggggctgtgatggag$^{3'}$ and anti-sens $^{5'}$ggtcaagtctgagggttccaccgtcgacggtaccgcg$^{3'}$, and for pDok2-GFP sens $^{5'}$gagctaagcttatgggagacggggcag$^{3'}$ and anti-sens $^{5'}$gttgtactaaagaaaggcccaaagccgggatccatcgcc$^{3'}$. The PCR were sequenced and cloned in pEGFP-N1 (Clontech, Palo Alto, CA) The mutant lacking the PH domain (ΔPH) tagged GFP were obtained using the same strategy: for ΔPHDok1-GFP (aa 124 to 482) sens $^{5'}$tcagatctcgagctcatgggctgggctttggcgcag$^{3'}$ and anti-sens $^{5'}$ggtcaagtctgagggttccaccgtcgacggtaccgcg$^{3'}$, and for ΔPHDok2-GFP (aa 118 to 412), sens $^{5'}$aagcttcgaattctgatggaggaaaatgaattgtacagc$^{3'}$ and anti-sens $^{5'}$gttgtactaaagaaaggcccaaagccgggatccatcgcc$^{3'}$. The PH domain tagged GFP were obtained using the same strategy: for pPHDok1-GFP (aa 1 to 122) sens $^{5'}$tcagatctcgagctcatggacggggctgtgatggag$^{3'}$ and anti-sens $^{5'}$agaaccgcctttccgaaaggcaagcttcgataa$^{3'}$, and for pPHDok2-GFP (aa 1 to 132) sens $^{5'}$aagcttcgaattctgatgggagacggggcagtgaaac$^{3'}$ and anti-sens $^{5'}$ggaaagcagagccggccctgccgggcccgggatccaccg$^{3'}$.

The PH domains fused to the GST protein were obtained by PCR using the primers: for GST-PHDok1 (aa 1 to 117) sens $^{5'}$gcatggatccagaaccgcctttccgaaa$^{3'}$ and anti-sens

$^{5'}$gcatgaattcggcggttctgcataaagtct$^{3'}$ and for GST-PHDok2 (aa 1 to 122) sens $^{5'}$gcatggatccatgggagacggggcagtgaa$^{3'}$ and anti-sens $^{5'}$gcatgaattcccgagagctccttcctctg$^{3'}$. The PCR were sequenced and cloned in pGEX-2T (Amersham Biosciences Limited, England, UK).

Several vectors were described previously, including the pRK5-myc-IpgD WT and pRK5-myc-IpgD-C438S mutant, pEGFP-PHD3X ING2 (2), the pcDNA3-GFP-Lyn-Inp54 vector (3) and pcDNA3-PI5P4KIIβ construct (4). The promoter assay plasmids pIL-2-Luc composed of IL-2 promoter, fused to firefly luciferase reporter gene and pβ-actin-Rluc composed of β-actin promoter, fused with *Renilla* luciferase gene were previously reported (5).

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