Supplementary information

Supplementary Methods

Epifluorescence and confocal fluorescence microscopy

Macrophages were grown overnight on glass coverslips. Cells were stimulated and fixed with 3% paraformaldehyde at room temperature for 15 min. Hoechst (Molecular Probes) staining of nuclei was carried out by adding a 1:10 000 dilution in PBS. Confocal microscopy images were collected using the LSM 710 Laser Scanning Microscopes, Carl Zeiss (Jena, Germany). FITC and YFP were excited and detected by spectral scanning and careful wavelength selection, following acqizition images were used for emission fingerprinting to separate overlapping emission signals by spectral unmixing using Zeiss ZEN 2009 LE software.

Immunoprecipitation assay

Macrophages were grown overnight in 12-well plates. After stimulation with biotinylated GBS RNA, the cells were washed with PBS and then lysed with lysis buffer (200 mM NaCl, 1% Triton, 0.05% CHAPS, 1 mM NaF, 1 mM Na3VO4, 50 mM Tris–HCl, pH 7.5) and a protease inhibitor mix (completemini from Roche Applied Science). The lysates were cleared by centrifugation at 8000 g for 15 min (4°C). Protein concentrations were quantified with the Bradford assay (BioRad) and loaded onto Avidin agarose beads (invitrogene), and incubated for 1 h at 4°C. After washing (3 × in lysis buffer), proteins were eluted (2 × SDS-loading buffer) and separated by electrophoresis on SDS 12% polyacrylamide gels (NuSep, Sydney, Australia) and transferred to a PVDF membrane (BioRad). The membrane was blocked for 1 h in 5% milk in TBST (20 mM Tris–HCl, pH 7.6, 0.15 M NaCl and 0.1% Tween20), then it was incubated overnight at 4°C with primary antibody and subsequently with peroxidase-conjugated secondary antibody for 2 h. Immunoreactive proteins were detected by using ECL detection reagents (Amersham Pharmacia) and and analysed.

Arabidopsis thaliana PR gene expression

Whole leaves of 5-6 weeks grown *A. thaliana* Columbia-0 (Col-0) plants were placed in 1 ml of 50 mM sodium phosphate, pH 7, 0.5 mM potassium ferrocyanide, 0.5 mM potassium ferricyanide, 10 mM EDTA, pH 8, 0.1% Triton X-100. After vacuum infiltration of bacteria, the leaves were incubated at 37°C overnight, and PR gene expression was measured by real time PCR.