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Supplemental Material to:

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Manipulation of pro-inflammatory cytokine production by the bacterial cell-penetrating effector protein YopM is independent of its interaction with host cell kinases RSK1 and PRK2

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Y. pseudotuberculosis PB1/+   1   MFINPRNVSNTFLQEPLRHSSNLTEMPVEAENVKSKTEYYNAWSEWERNAPPGNGEQREMAVSRLRDCLDRQAHELELNN  80
Y. pestis KIM10+              1   MFINPRNVSNTFLQEPLRHSSNLTEMPVEAENVKSKTEYYNAWSEWERNAPPGNGEQREMAVSRLRDCLDRQAHELELNN  80
Y. enterocolitica 8081        1   MFITPRNVSNTFLQEPLRHSSDLTEMPVEAENVKSKTEYYNAWAVWERNAPPGNGEQREMAVSRLRDCLDRQAHELELNN  80

Y. pseudotuberculosis PB1/+  81   LglsslpelpphleslvascnsltelpelpqslkslqvennNLKALPDLPPSLKKLHVRENDLTDLPELPQSLESLRVDN  160
Y. pestis KIM10+            81   L---------------------------------------GLSSLPELPPHLESLVASCNSLTELPELPQSLKSLLVDN  120
Y. enterocolitica 8081       81   L---------------------------------------GLSSLPELPPHLESLVASCNSLTELPELPQSLKSLQVDN  120

Y. pseudotuberculosis PB1/+ 161   NNLKALSDLPPSLEYLTASSNKLEELPELQNLPFLAAIyadnnlletlpdlppslkklhvrendltdlpelpqsleslQV  240
Y. pestis KIM10+            121   NNLKALSDLPPLLEYLGVSNNQLEKLPELQNSSFLKII---------------------------------------DV  160
Y. enterocolitica 8081       121   NNLKALSDLPPSLEFLAAGNNQLEELPELQNSSFLKII---------------------------------------DV  160

Y. pseudotuberculosis PB1/+ 241   DNNNLKALSDLPPSLEYLTASSNKLEELPELQNLPFLAAIYADNNLLETLPDLPPhleilvasynsltelpelpqslksl  320
Y. pestis KIM10+            161   DNNSLKKLPDLPPSLEFIAAGNNQLEELPELQNLPFLTAIYADNNSLKKLPDLPL------------------------  215
Y. enterocolitica 8081       161   DNNSLKKLPDLPPSLEFLAAGNNQLEELSELQNLPFLTEIHADNNSLKTLPDLPP------------------------  215

Y. pseudotuberculosis PB1/+ 321   rvdnnnlkalsdlppSLEYLTASSNKLEELPELQNLPFLAAIYADNNLLETLPDLPPSLKKLHVRENDLTDLPELPQSLT  400
Y. pestis KIM10+            216   ---------------SLESIVAGNNILEELPELQNLPFLTTIYADNNLLKTLPDLPPSLEALNVRDNYLTDLPELPQSLT  280
Y. enterocolitica 8081       216   ---------------SLKTLNVRENYLTD---------------------LPELPQSLTFLDVSDNIFSGLSELPPNLY  258

Y. pseudotuberculosis PB1/+ 401   FLDVSDNNISGLSELPPNLYYLDASSNEIRSLCDLPPSLVDLNVKSNQLSELPALPPHLERLIASFNYLAEVPELPQNLK  480
Y. pestis KIM10+            281   FLDVSENIFSGLSELPPNLYYLNASSNEIRSLCDLPPSLEELNVSNNKLIELPALPPRLERLIASFNHLAEVPELPQNLK  360
Y. enterocolitica 8081       259   YLDASSNGIRSLCDLPPSLVELDVRDNQLIELPALPPHLERLIASLNHLAEVP--------------------ELPQNLK  318

Y. pseudotuberculosis PB1/+ 481   QLHVEQNALREFPDIPESLEELEMDSERVVDPYEFAHETTDKLEDDVFE  529
Y. pestis KIM10+            361   QLHVEYNPLREFPDIPESVEDLRMNSERVVDPYEFAHETTDKLEDDVFE  409
Y. enterocolitica 8081       319   QLHVEHNALREFPDIPESVEDLRMDSERVTDTYEFAHETTDKLEDDVFE  367
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Figure S1. Amino acid alignment of YopM from *Y. pseudotuberculosis* PB1/+, *Y. pestis* KIM 10+, and *Y. enterocolitica* 8081 performed with the help of the online constraint-based multiple alignment tool (COBALT) from NCBI using standard parameters.

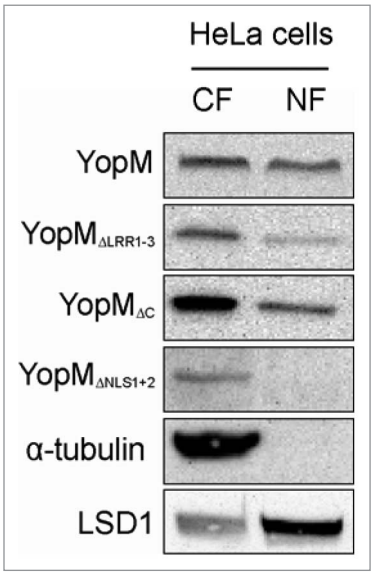

Figure S2. Immunoblot of subcellular fractionations of HeLa cells incubated with different truncated versions of rYopM for 3 h at 37 °C. In order to assess their purity, fractions were analyzed using antibodies against α-tubulin (cytosol) and LSD1 (nucleus). rYopM partition was assessed using a YopM-specific polyclonal antibody. CF, cytosolic fraction; NF, nuclear fraction.

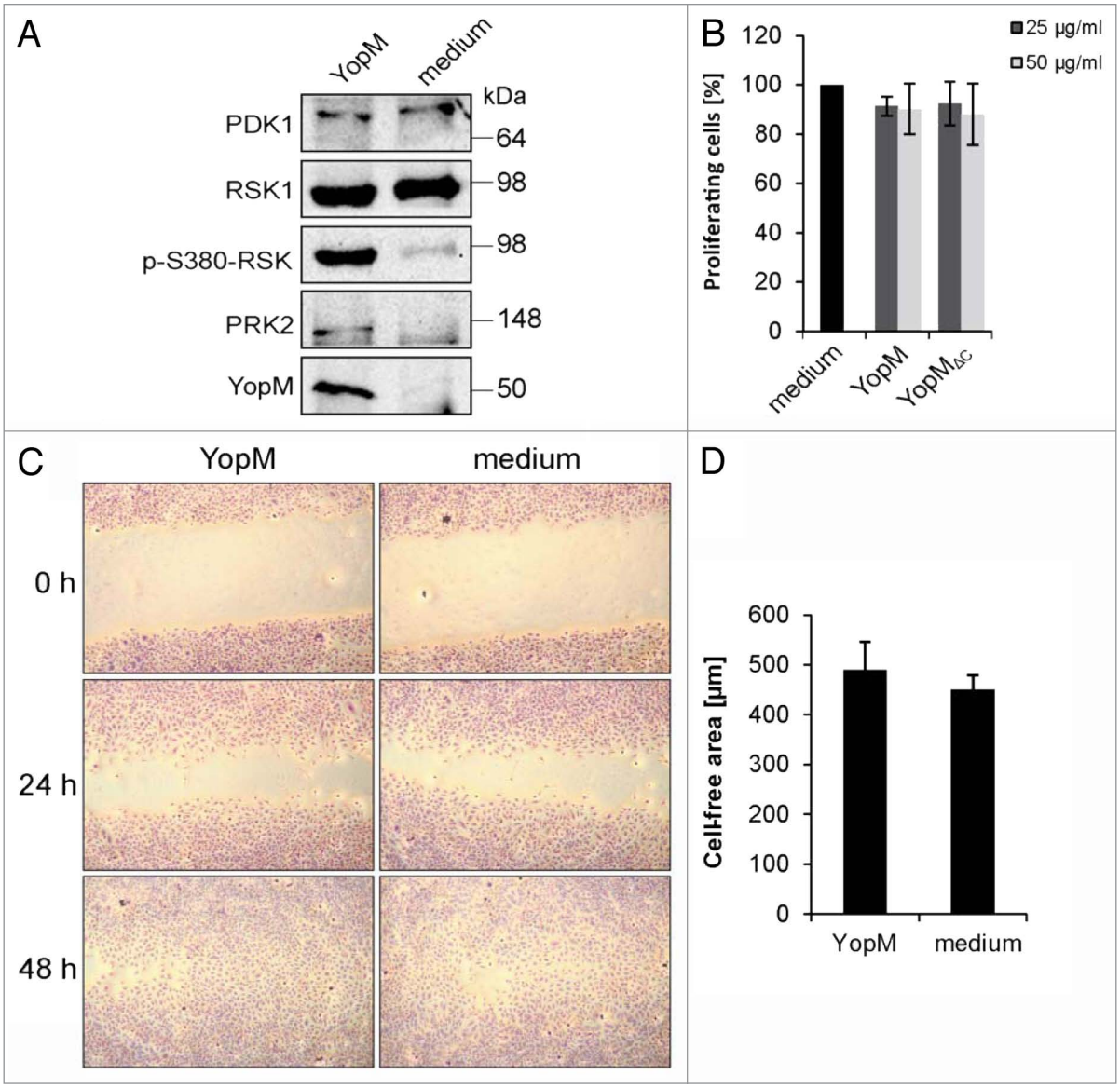

Figure S3. (**A**) rYopM does not interfere with recruitment of PDK1 to RSK1. HeLa cells were starved and incubated with or without 25 µg/ml rYopM for 16 h at 37 °C. RSK1 was immunoprecipitated by a specific antibody. PDK1 is recruited to RSK with or without rYopM to the same extent. (**B**) Cell proliferation assay of HeLa cells after incubation for 48 h with the indicated recombinant proteins (25 µg/ml and 50 µg/ml, respectively), performing each condition in triplicate. Proliferation of cells was analyzed using a colorimetric BrdU-Assay. Values were correlated to cells grown in absence of recombinant protein and expressed as mean percentage of proliferating cells. Error bars indicate SD. $n = 3$ independent experiments. The Student t test revealed no significant difference between the means. (**C**) rYopM does not affect the migration rate of HeLa cells. Migration assay of rYopM-incubated HeLa cells at a cell-free gap. For the assay, 1×10^5 cells were seeded in each well. The cell gap was generated by a stainless steel cylinder. The incubation with 25 µg/ml rYopM was carried out for 0, 24, and 48 h. Control cells (–YopM) were incubated for the same period with culture medium. Subsequently, cells were fixed with Wright solution, and documentation was performed with a digital SLR camera and an inverted microscope (Axiovert 100, Carl Zeiss). (**D**) Densitometric quantification of the migration assay. The cell-free area after 24 h was quantified using ImageJ 1.44p (NIH). Error bars indicate SD; $n = 3$. The Student t test revealed no significant difference between the means.

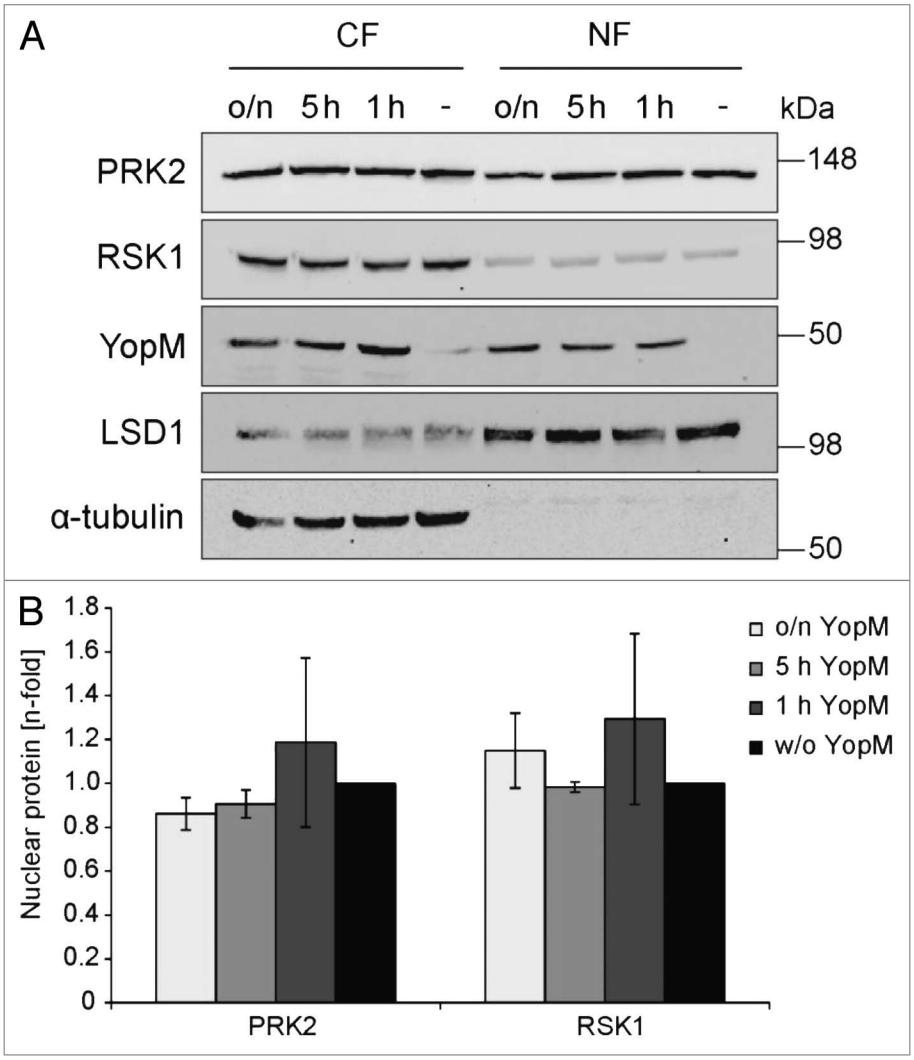

Figure S4. (**A**) Western blot analysis of cytosolic and nuclear distribution of RSK1 and PRK2 in the presence of rYopM. Immunoblot of subcellular fractionations of HeLa cells incubated with rYopM for 1 or 3 h, or overnight at 37 °C. In order to assess their purity, fractions were analyzed using antibodies against α-tubulin (cytosol) and LSD1 (nucleus). (**B**) Comparison of the nuclear PRK2 and RSK1 ratio at 37 °C. Immunoblots were densitometrically analyzed using Lumi-Imager T1 and Lumi Analyst software (Roche Diagnostics), normalized by the LSD1 signals and the nuclear PRK2 and RSK1 ratio was calculated. Error bars indicate SD. $n = 2$ independent experiments. The Student t test revealed no significant difference between the means.