Supporting Information

de Zoete et al. 10.1073/pnas.1018135108

```
SIGNAL  MRILIGSLYFYFISFLFSKVNG

LRR-NT  FLTQRTSPVSSFPFYNYSYLNLSSVSQAQAPKT

LRR1    ARALNFSYNAIEKITKRD-FEG-FHV------------------------------------  24
LRR2    LEVLDLSHNHIKDIEPGA-FENLLS-------------------------------------  24
LRR3    LVSVDLSFNDKNLLVSG--LAPHLKLIPTSGASGPSQIYMYFQKSAEAALEPSAPAELLPHLE
                                DPPNPGNVNPRFRQRRTEENKTSPPAATLRPDLCGAPI  99
LRR4    NGLLDLSRTKLSNEE----LTAKLDADLCQAQLGT---------------------------  31
LRR5    VLEFNISHSDLEMDLLSL-FILFLPMKD----------------------------------  27
LRR6    IQSVDASYNRITINNIDVEAICHFPFSN----------------------------------  28
LRR7    FSFLNISNNPINSLE----TVC-LPAS-----------------------------------  22
LRR8    ITVIDLSFTNISTIPAN--FAKKLSK------------------------------------  24
LRR9    LERMYVQGNQLIYTVRP--ENPSATPRPPPGTVQ----------------------------  32
LRR10   ISAISLVRNQAGTP-----IES-LPES-----------------------------------  21
LRR11   VKHLKVSNCSIVELP-EW-FANRMQE------------------------------------  24
LRR12   LLFLDLSSNRISM-------LPDLPIS-----------------------------------  20
LRR13   LQQLDISNSDIKIIPPR--FKS-LSN------------------------------------  23
LRR14   VTVFNIQNNKLTEMH-----PEYFPST-----------------------------------  22
LRR15   LTTCDISKNKLKVLS----LTKALEN------------------------------------  22
LRR16   LESLNVSGNLITRLE----PACQLPS------------------------------------  22
LRR17   LTNLDSSHN-LISELPDH-LGQSLLM------------------------------------  24
LRR18   LKHFNLSGNKISFLQ----RGS-LPAS-----------------------------------  22
LRR19   LEELDISDNAITTIVQDT-FGQLTS-------------------------------------  24

LRR-CT  LSVLTVQGKHFFCNCDLYWFVNIYIRNPHLQINGKDDLRCSFPPDRRGSLVKSSNLTLLHCSL

TM      GIQMAITACMAILVVLVLTGLCW

TIR     RFDGLWYVRMGWYWCMAKRRQYKKRPENKPFDAFISYSEHDADWTKEHLL
        KKLETDGFKICYHERDFKPGHPVLGNIFYCIENSHKVLFVLSPSFVNSCW
        CQYELYFAEHRVLDENQDSLIMVVLEDLPPDSVPQKFSKLRKLLKRKTYL
        KWSPEEHKQKIFWHQLAAVLKTTNEPLVRAENGPNEDVIEME
```

Fig. S1. Schematic overview of TLR15. Depicted are the predicted signal sequence (SIGNAL), the N-terminal LRR (LRR-NT), 19 LRRs (LRR1-19), the C-terminal LRR (LRR-CT), the transmembrane domain (TM), and the TIR domain (TIR). The proline-rich loop (aa 352–363) is boxed.

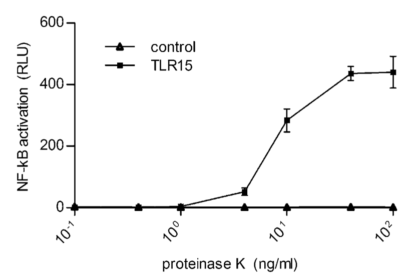

Fig. S2. Dose-dependent activation of TLR15 by proteinase K. NF-κB activation of TLR15- or control-transfected HeLa 57A cells were stimulated for 5 h with increasing amounts of proteinase K. Data are presented as relative luciferase units (RLU) and are the mean ± SEM of three independent experiments.

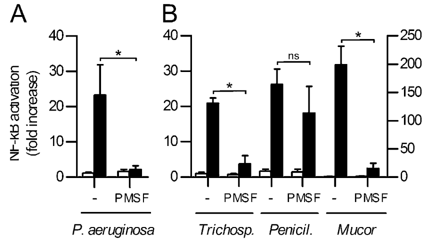

Fig. S3. Inhibition of *P. aeruginosa* and fungi-induced TLR15 activation by PMSF. TLR15-induced NF-κB activation in HeLa 57A cells by culture supernatant of (*A*) *P. aeruginosa* or (*B*) *Trichosporon* spp. (left *y* axis) and *Mucor* spp. (right *y* axis) is inhibited by specific inhibition of proteases by PMSF. TLR15-activation by *Aspergillus* spp. (*B*, left *y* axis) is not affected by PMSF. Proteases were inhibited by PMSF treatment (1 mM, 15 min) before stimulation (5 h). Data are presented as mean ± SEM of stimulated versus unstimulated cells from three independent experiments (*$P < 0.05$; ns, not significant).

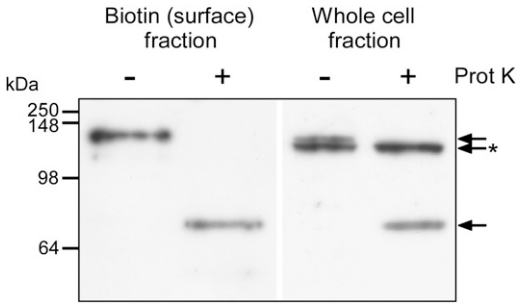

Fig. S4. Only surface-localized TLR15 is subject to proteinase K cleavage. Surface membrane proteins of COS-7 cells expressing C-terminal Flag-tagged TLR15 were labeled with sulfo-NHS-LC-biotin and then treated with 0 or 100 ng mL^{-1} proteinase K for 45 min. The biotin (surface) fraction was separated from the whole-cell fraction by streptavidin-coupled magnetic beads. Surface-localized full-length and cleaved TLR15 (arrows) and intracellular TLR15 (arrow with asterisk) were detected by immunoblot analysis by using M2-α-Flag antibodies.

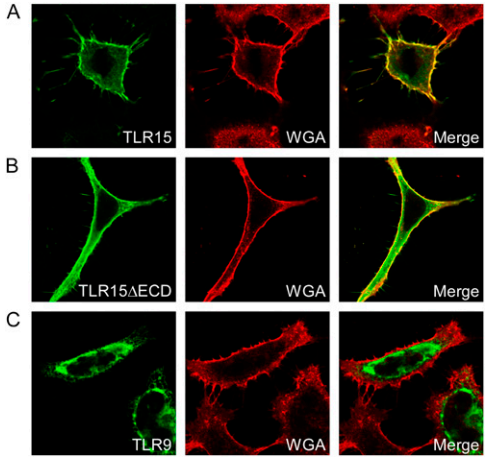

Fig. S5. TLR15ΔECD is localized on the cell surface. (*A*–*C*) Confocal microscopy of HeLa 57A cells transfected with flag-tagged TLR15, TLR15ΔECD, or TLR9. Both TLR15 (*A*, green) and TLR15ΔECD (*B*, green) colocalized with the cell surface marker WGA (red), in contrast to TLR9 (*C*, green).