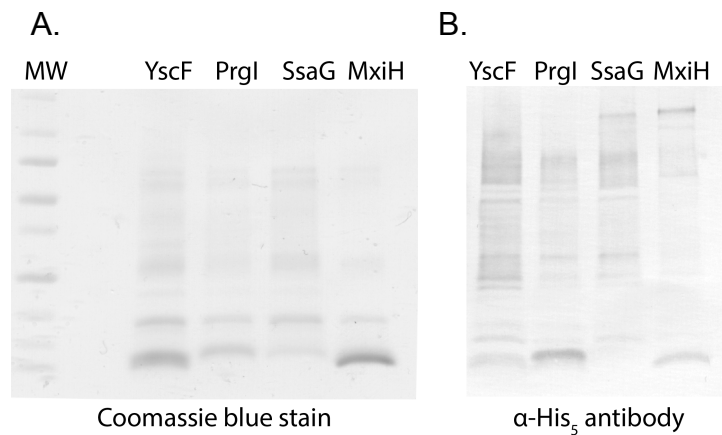


73 **Supplemental Figure 1.** (A) Coomassie Blue stained SDS-PAGE gel of needle-protein
74 purifications and (B) Immunoblot of needle proteins probed with Anti-His₅ antibody.

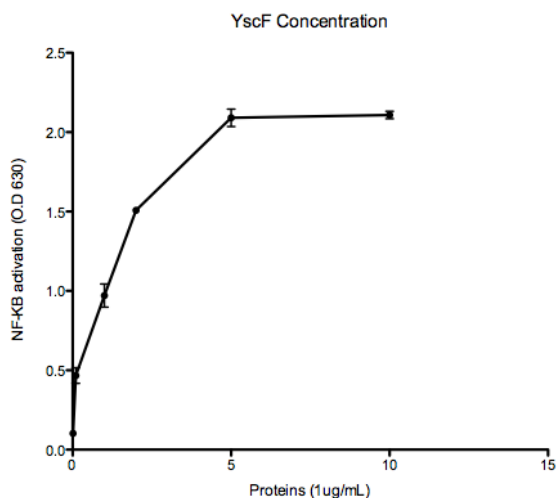


75 **Supplemental Figure 2. YscF activation of NF-kB/AP-1 is concentration dependent.**

76 THP1-X-Blue cells were seeded in wells and treated with PBS, or YscF dissolved in
77 PBS at indicated concentrations. SEAP activity was measured as a representation of
78 NF-kB/AP-1 activation.

79 A.

80

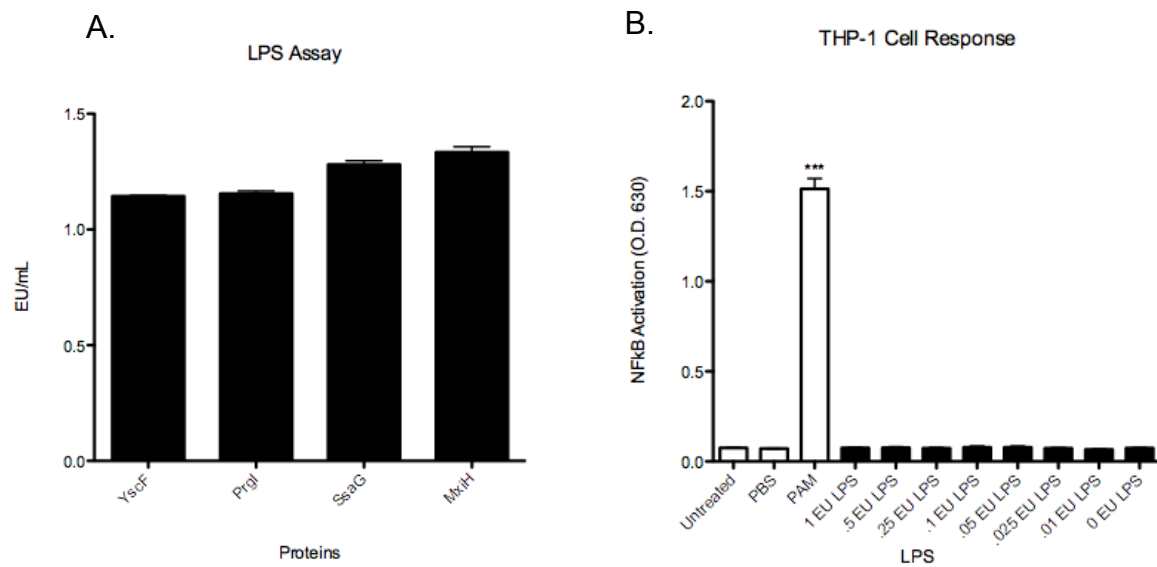


81 **Supplemental Figure 3. Activation of NF- κ B/AP-1 in THP1-XBlue cells by needle**
82 **proteins is not due to LPS contamination.**

83 (A) An LPS kit was used to analyze the level of LPS contamination in the needle protein
84 preparations. (B) The LPS standards were used to treat THP1-XBlue cells. For both (A)
85 and (B) SEAP activity was measured as a representation of NF- κ B/AP-1 activation.

86 Error bars represent SEM. n=3. Shown experiments are representative of at least three
87 experiments. *** represents p of <0.001

88

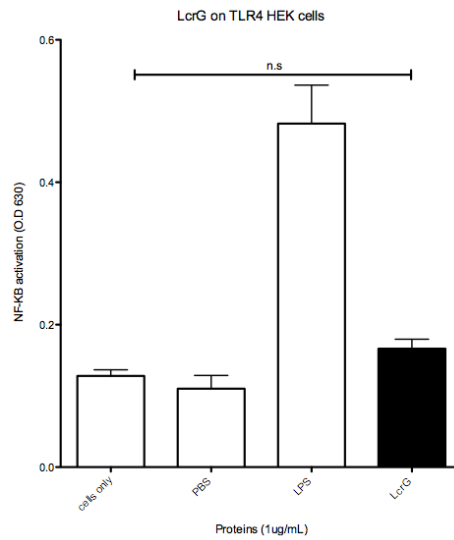
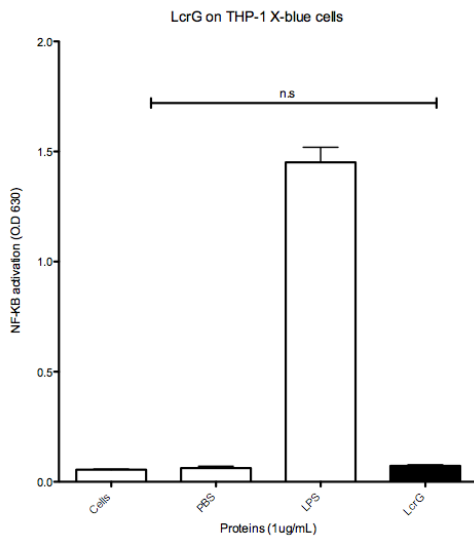


90 **Supplemental Figure 4. His₆-tagged LcrG does not activate NF-κB/AP-1 in THP1-**
91 **XBlue cells.**
92 (A) THP1-Xblue cells (B) HEK-Blue hTLR2 cells or (C) HEK-Blue hTLR4 cells were
93 tested with 1 μg/ml of His-tagged LcrG proteins dissolved in PBS. LPS, 1 μg/ml, was the
94 positive control for THP-1 cells and HEK cells expressing TLR4. HKLM, 1 μg/ml, was the
95 positive control for HEK-Blue hTLR2 cells. SEAP activity was measured as a
96 representation of NF-κB/AP-1 activation. Error bars represent SEM. n=3. Shown
97 experiments are representative of at least three experiments. *** represents p of <0.001.

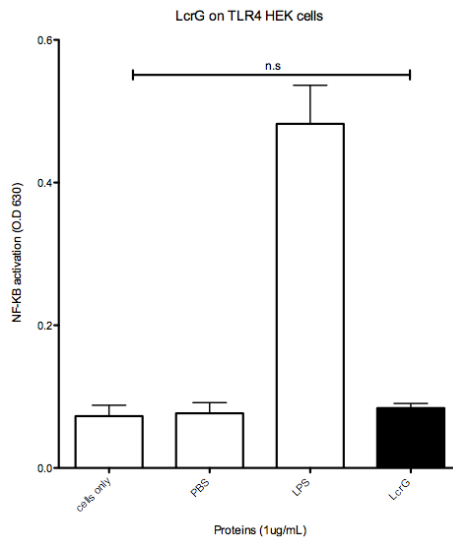
98
99 A.

B.

100



C.



101 **Supplemental Figure 5. Purified Needles treated with lipoprotein lipase and**
 102 **polymyxin B indicate needles cause NF-kB/AP-1 activation.** Purified Needles (1
 103 $\mu\text{g/ml}$), PBS, LPS (1 $\mu\text{g/ml}$) or Pam3 (1 $\mu\text{g/ml}$) were incubated with (A) lipoprotein lipase
 104 (LPL; 50 $\mu\text{g/ml}$), (B) polymyxin B (PMB; 20 $\mu\text{g/ml}$) before addition to THP1-XBlue cells.
 105 SEAP activity was measured as a representation of NF-kB/AP-1 activation. Error bars
 106 represent SEM. $n=3$. Shown experiments are representative of at least three
 107 experiments. *** represents p of <0.001 .
 108
 109

