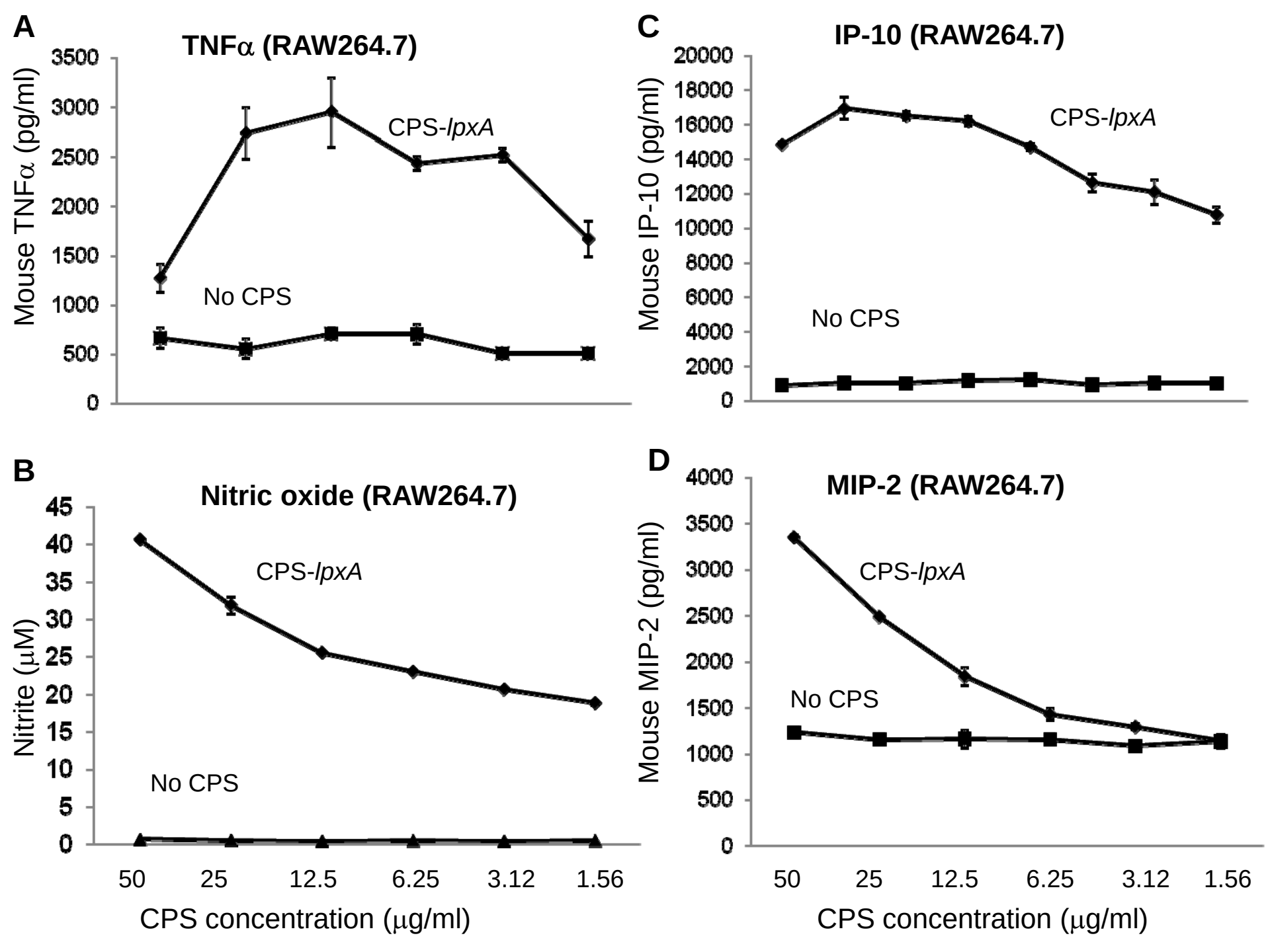
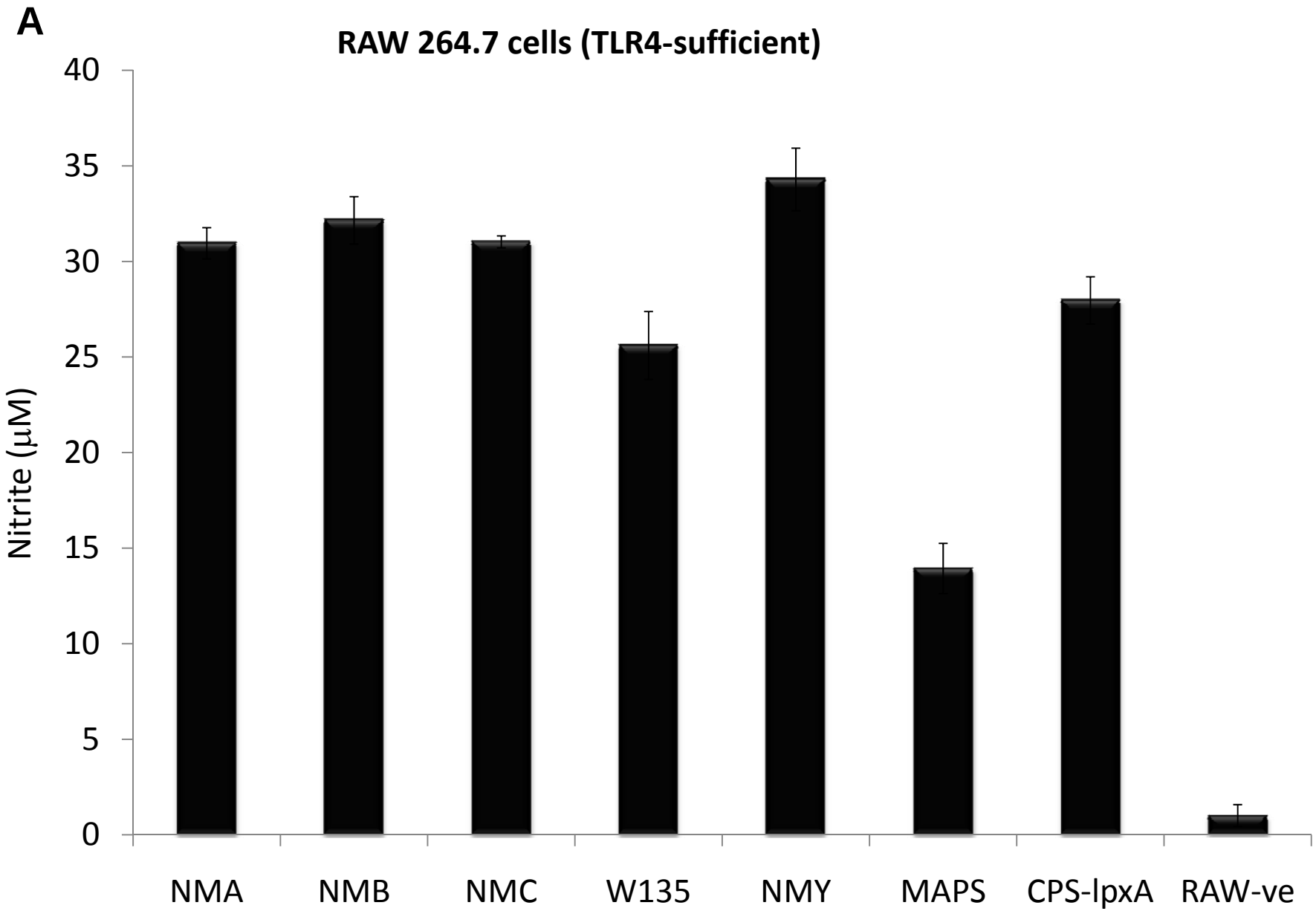
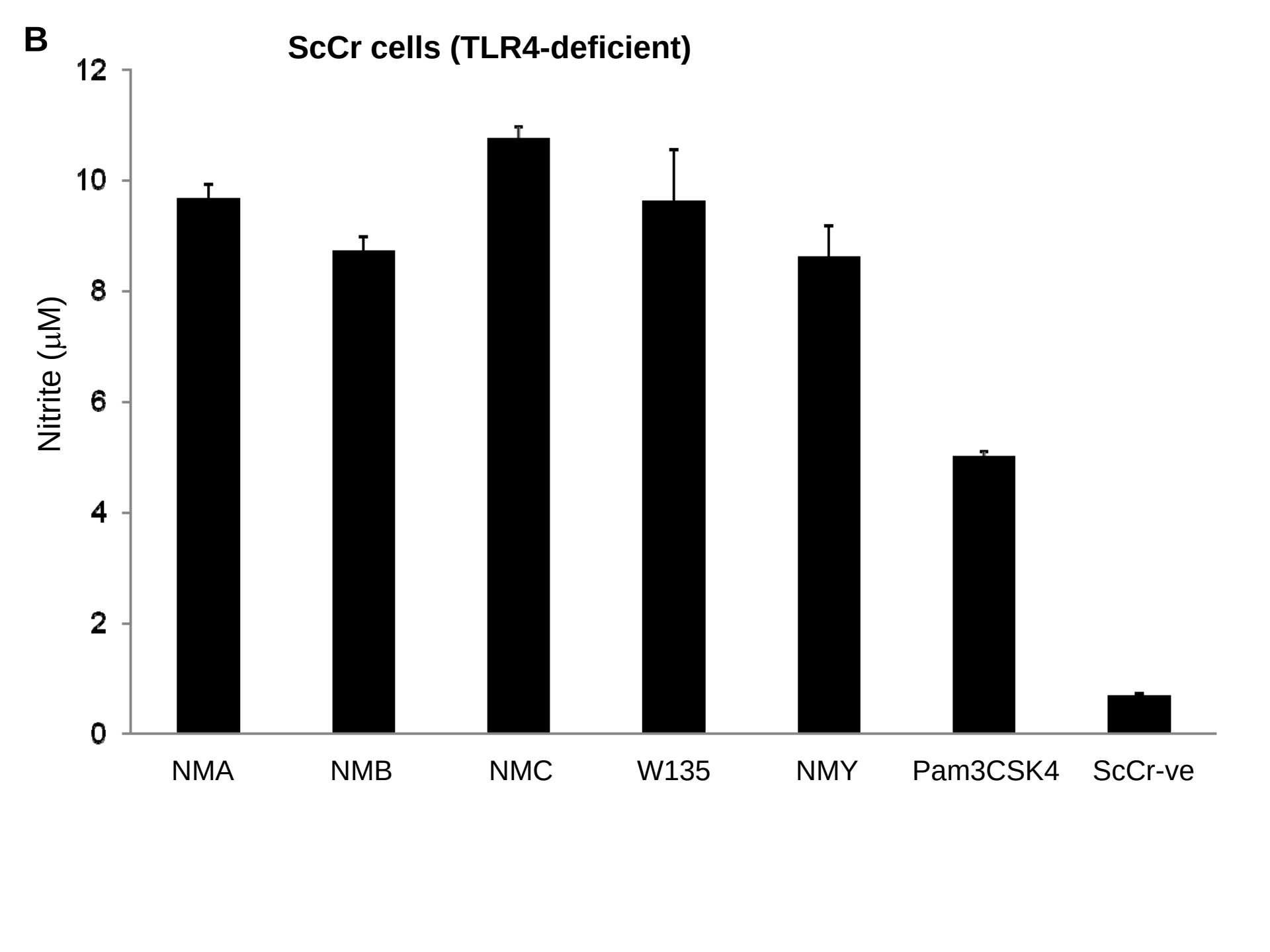

A
TNFα (RAW264.7)
Mouse TNFα (pg/ml)
3500
3000
2500
2000
1500
1000
500
0
CPS-lpxA
No CPS
C
IP-10 (RAW264.7)
Mouse IP-10 (pg/ml)
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
CPS-lpxA
No CPS
B
Nitric oxide (RAW264.7)
Nitrite (μM)
45
40
35
30
25
20
15
10
5
0
CPS-lpxA
No CPS
50
25
12.5
6.25
3.12
1.56
CPS concentration (μg/ml)
D
MIP-2 (RAW264.7)
Mouse MIP-2 (pg/ml)
4000
3500
3000
2500
2000
1500
1000
500
0
CPS-lpxA
No CPS
50
25
12.5
6.25
3.12
1.56
CPS concentration (μg/ml)

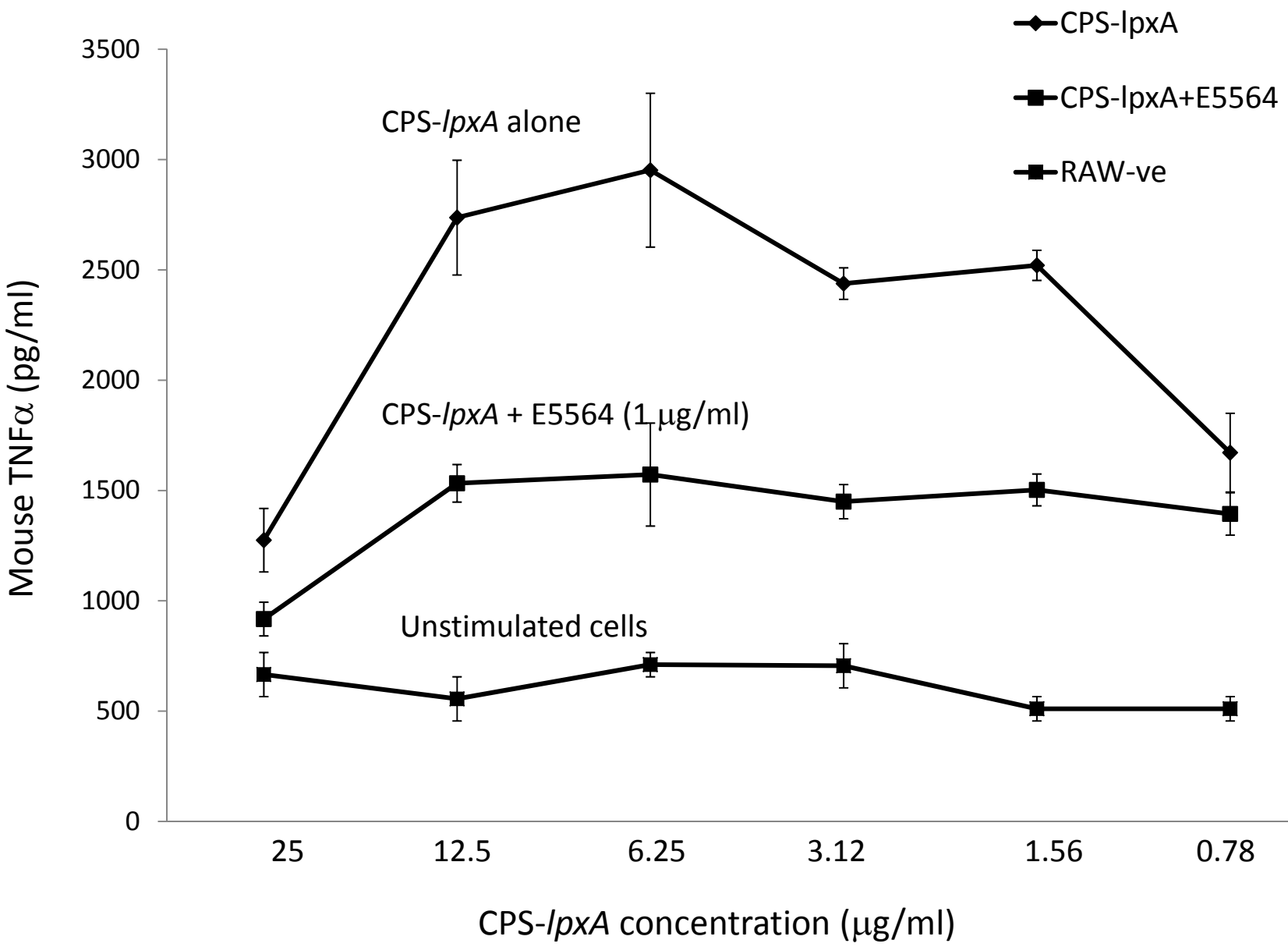
Supplemental Figure 1: Meningococcal serogroup B CPS polymers from an LOS-deficient (*lpxA*) mutant (CPS-*lpxA*) induced inflammatory cytokines, chemokines and nitric oxide release from murine macrophages. RAW264 cells (250×10^3/well) in 96-well plates induced with CPS-*lpxA* polymers overnight. Cytokine release was quantified by ELISA and nitric oxide release was quantified as nitrite accumulation by the Greiss method. **A**: TNFα; **B**: Nitric oxide; **C**: IP-10 (CXCL10) and **D**: MIP-2 release. Unstimulated macrophages incubated simultaneously were used as the control. Error bars represent SD from the mean of quadruplicate readouts. The results are representative of 3 independent experiments.

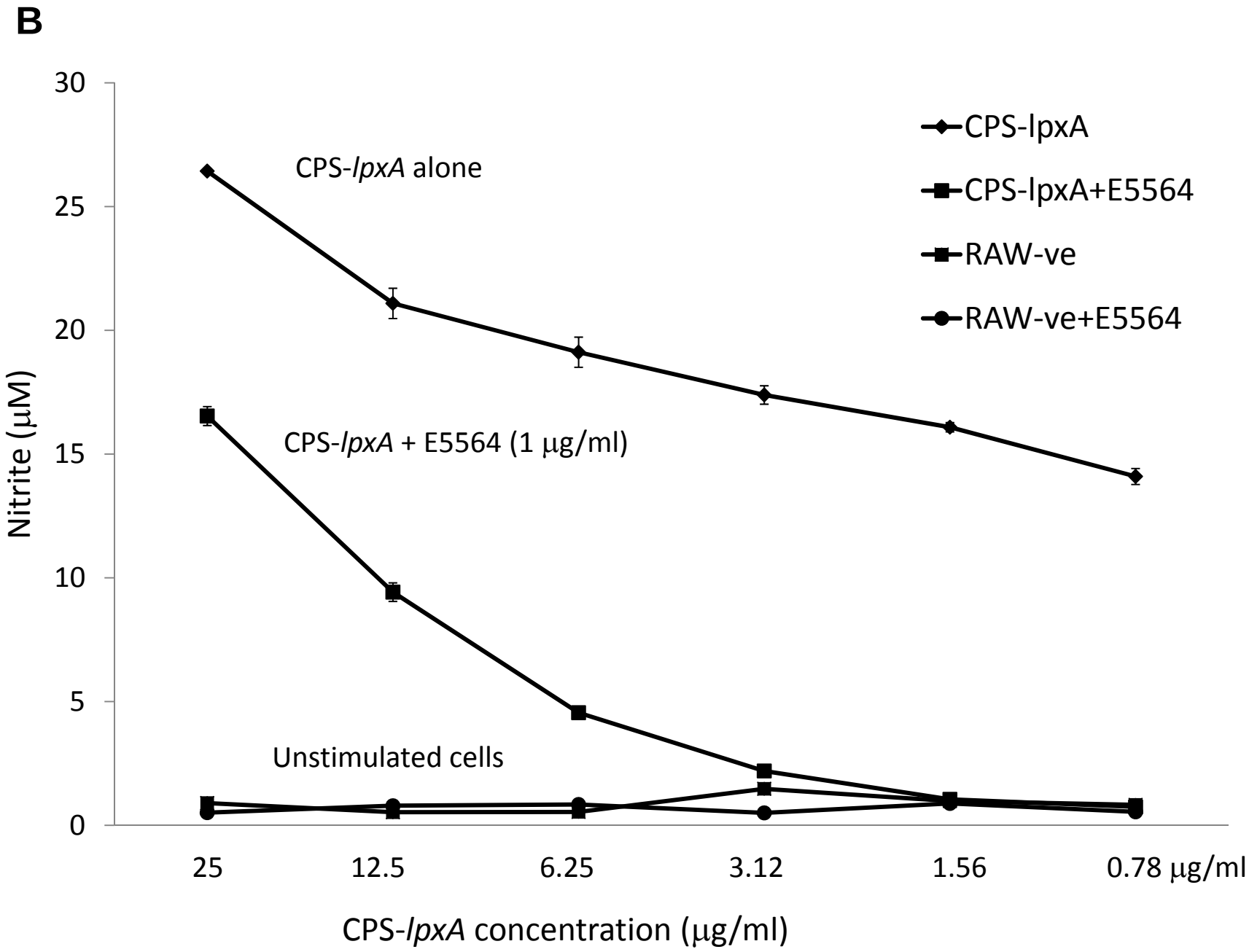

A
RAW 264.7 cells (TLR4-sufficient)
Nitrite (µM)
40
35
30
25
20
15
10
5
0
NMA
NMB
NMC
W135
NMY
MAPS
CPS-lpxA
RAW-ve


B
ScCr cells (TLR4-deficient)
Nitrite (μM)
0
2
4
6
8
10
12
NMA
NMB
NMC
W135
NMY
Pam3CSK4
ScCr-ve

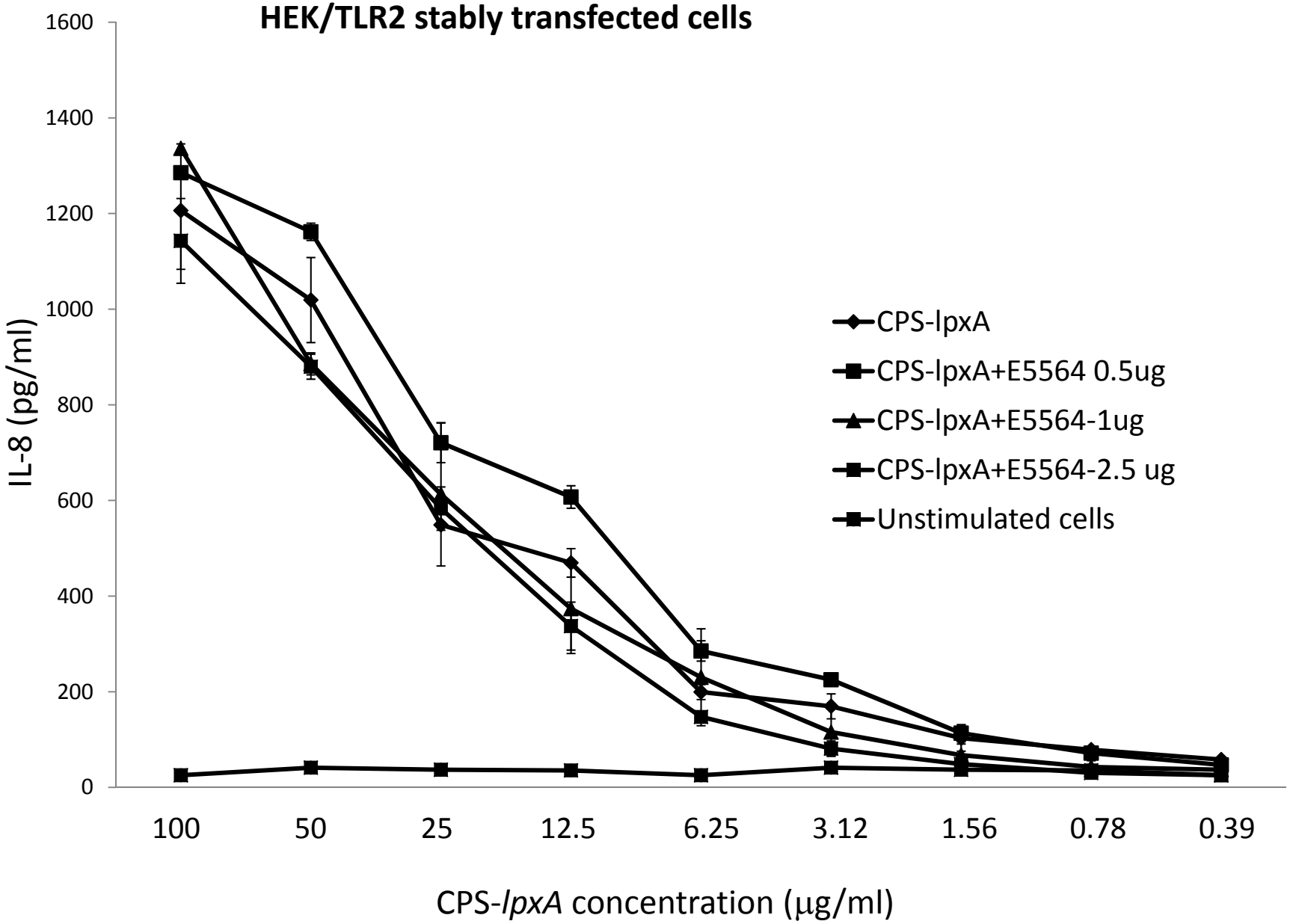
Supplemental Figure 2: Meningococcal capsular polysaccharides induce nitric oxide release from TLR4-sufficient and TLR4-deficient murine macrophages. **A**: Dose-dependent release of nitric oxide from RAW264 (TLR4-sufficient) induced with meningococcal CPS from serogroups A, B, C, W135, Y, the vaccine grade serogroup A CPS (MAPS) and from the serogroup B CPS-*lpxA* deficient strain. **B**: ScCr (TLR4-deficient) cells induced with 50 μg of meningococcal CPS polymers. Pam3CSK4, a synthetic TLR2 ligand, was used as a control. Stimulated cells were incubated overnight and nitrite accumulation was measured by the Greiss method. Unstimulated cells were used as controls. The results are representative of 3 independent determinations.

A

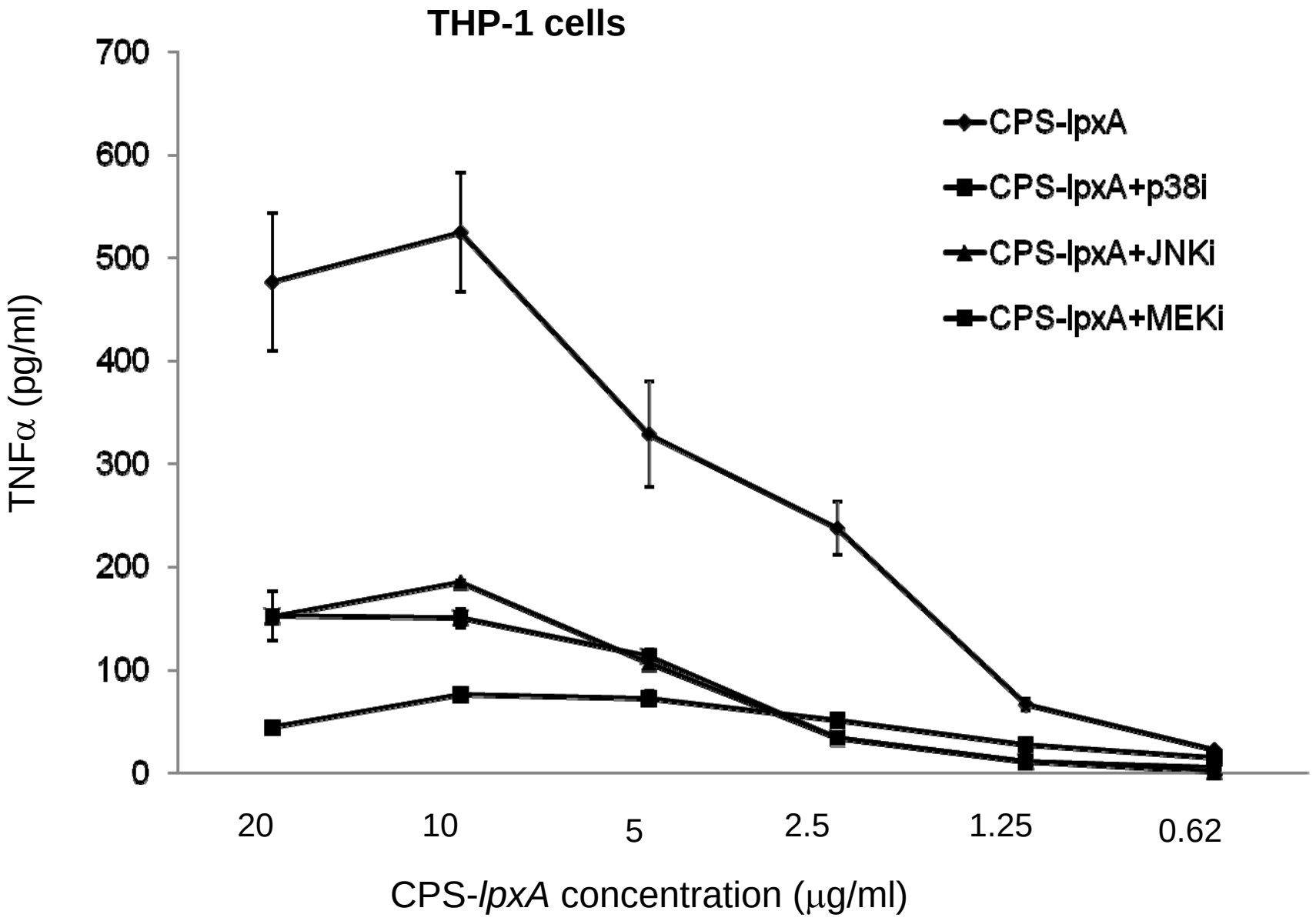

CPS-lpxA
CPS-lpxA+E5564
RAW-ve
CPS-*lpxA* alone
CPS-*lpxA* + E5564 (1 μg/ml)
Unstimulated cells
Mouse TNFα (pg/ml)
3500
3000
2500
2000
1500
1000
500
0
25
12.5
6.25
3.12
1.56
0.78
CPS-*lpxA* concentration (μg/ml)


B
30
25
20
15
10
5
0
Nitrite (μM)
CPS-lpxA
CPS-lpxA+E5564
RAW-ve
RAW-ve+E5564
CPS-lpxA alone
CPS-lpxA + E5564 (1 μg/ml)
Unstimulated cells
25
12.5
6.25
3.12
1.56
0.78 μg/ml
CPS-lpxA concentration (μg/ml)

C


HEK/TLR2 stably transfected cells
IL-8 (pg/ml)
1600
1400
1200
1000
800
600
400
200
0
100
50
25
12.5
6.25
3.12
1.56
0.78
0.39
CPS-lpxA
CPS-lpxA+E5564 0.5ug
CPS-lpxA+E5564-1ug
CPS-lpxA+E5564-2.5 ug
Unstimulated cells
CPS-*lpxA* concentration (μg/ml)

Supplemental Figure 3: The lipid A antagonist E5564 exerts inhibitor effects only in TLR4-expressing cells. **A:** Mouse TNFα release from RAW264 macrophages stimulated with meningococcal CPS-*lpxA* doses in presence of E5564 (1μg/ml). **B:** Nitrite release quantified in the same supernatants of RAW264 induced as in panel A using the Griess method. **C:** E5564 does not inhibit TLR2 mediated recognition of meningococcal CPS-*lpxA* polymer. IL-8 release from stably transfected HEK/TLR2 cells induced with serogroup B CPS-*lpxA* with or without E5564 doses 0.5, 1 or 2.5 μg/ml. **D:** Eritoran inhibits IL-6 release from THP-1 cells induced with meningococcal CPS polymers purified from serogroup A (CPS-A) or from wild type serogroup B (CPS-B) in presence of E5564 at 1 μg/ml. TNFα, IL-8 and IL-6 release was measured by ELISA. Error bars represent SD from the mean of quadruplicate readouts. The results are representative of 2 independent experiments.

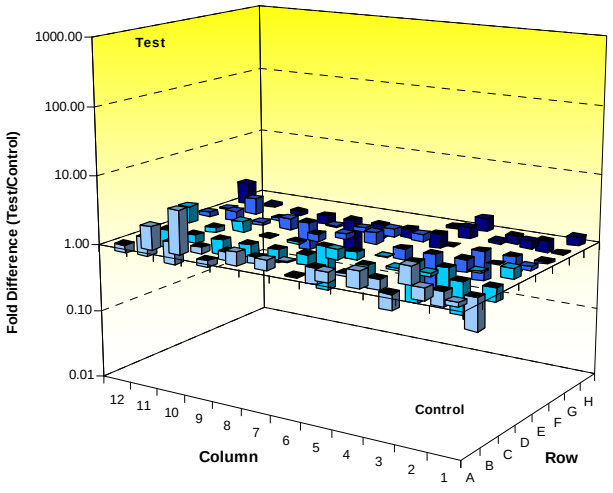

THP-1 cells
700
600
500
400
300
200
100
0
TNFα (pg/ml)
CPS-lpxA
CPS-lpxA+p38i
CPS-lpxA+JNKi
CPS-lpxA+MEKi
20
10
5
2.5
1.25
0.62
CPS-lpxA concentration (μg/ml)

Supplemental Figure 4: MAP kinases mediated TNFa release induced by meningococcal CPS polymers. Signaling pathways was inhibited with specific MAP kinase chemical inhibitors that target p38, JNK and MEK/ERK signaling and used at 10mM in THP-1 cells for 30 min prior to stimulation with CPS polymers. TNFa release was quantified by ELISA and error bars represent ±SD from the mean of 4 independent wells.

RT² PCR Array

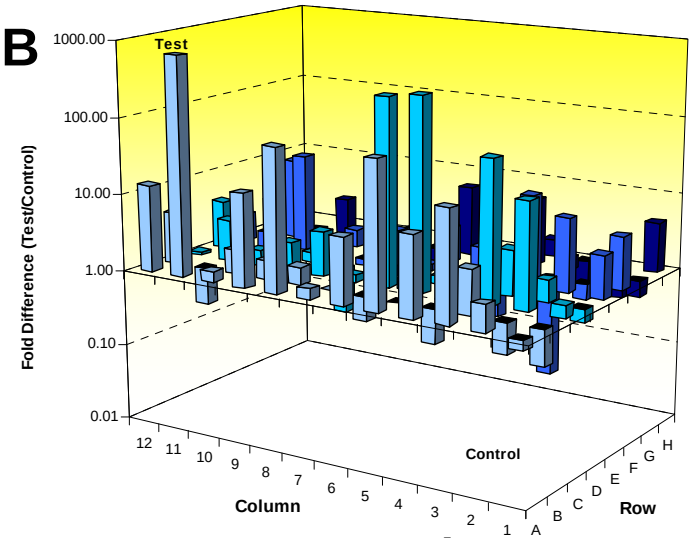
Gene expression profiles in THP-1 cells induced with TLR ligand

D



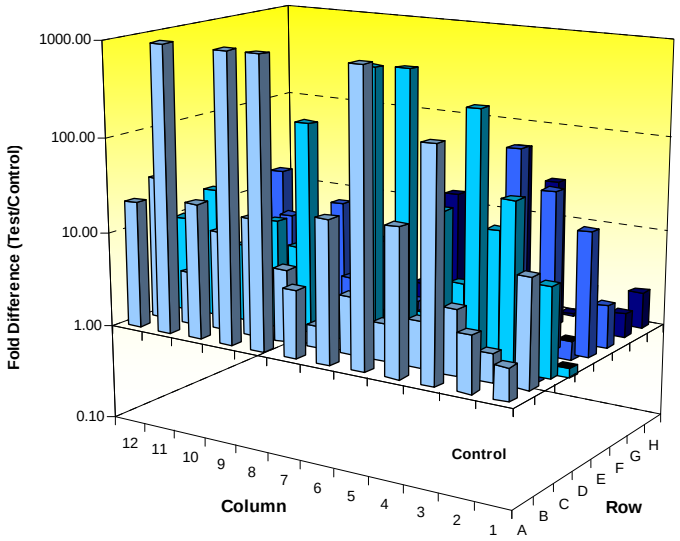
TLR4 antagonist
Rhizobium LPS

B



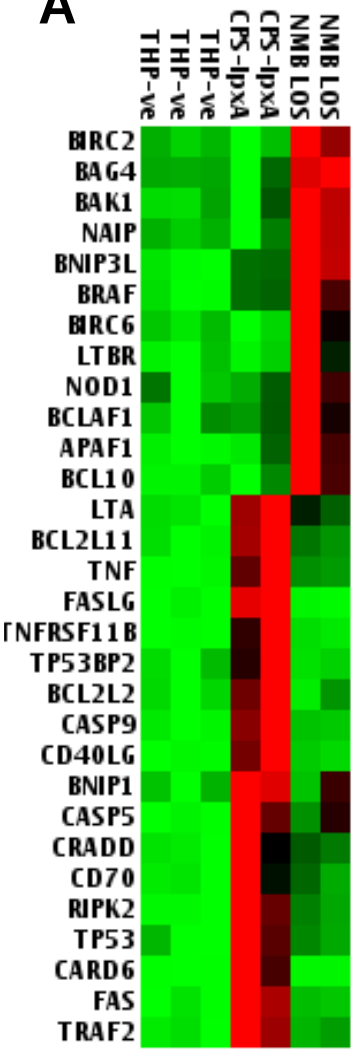
TLR4 agonist
Meningococcal LPS

C



TLR4-MD-2/TLR2 agonist
Meningococcal capsule (CPS)

A



Supplemental Figure 5: Differential gene induction in THP-1 cells induced by CPS-*lpxA*. **A:** Real-Time PCR microarrays (RT^2 profiler™ PCR Array, SABiosciences) gene induction profiles of THP-1 cells induced with meningococcal NMB LOS (2 pmole/ml~4ng/ml) or with CPS-*lpxA* (10μg/ml) or with the TLR4 antagonist Rhizobium LPS (5 μg/ml) for 18 hr (see Methods section). **B**, **C** and **D**: Schematic 3-D presentation of genes induction magnitude and profiles induced by meningococcal LOS, CPS and Rhizobium LPS respectively.