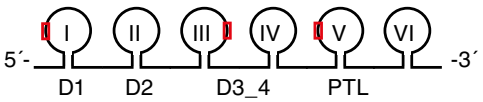
# Expanded View Figures

**A**




**B**

| No | Species | Abbr. | 16S rRNA affiliation nr. | Length [b] | ORN name | 5´-term. [residue] | 3´-term. [residue] |
|---|---|---|---|---|---|---|---|
| 1 | human | Hs | NC_012920.1 | 1557 | *mtPTL | 1045 | 1063 |
| 2 | “ | “ | “ | “ | HsmtD3_4 | 772 | 790 |
| 3 | “ | “ | “ | “ | HsmtD1 | 116 | 134 |
| 4 | cattle | Bt | AF492351.1 | 1570 | *mtPTL | 1057 | 1075 |
| 5 | “ | “ | “ | “ | BtmtD3_4 | 783 | 801 |
| 6 | mouse | Mm | NC_005089.1 | 1582 | *mtPTL | 1059 | 1077 |
| 7 | rat | Rn | KF011917.1 | 1559 | *mtPTL | 1042 | 1060 |

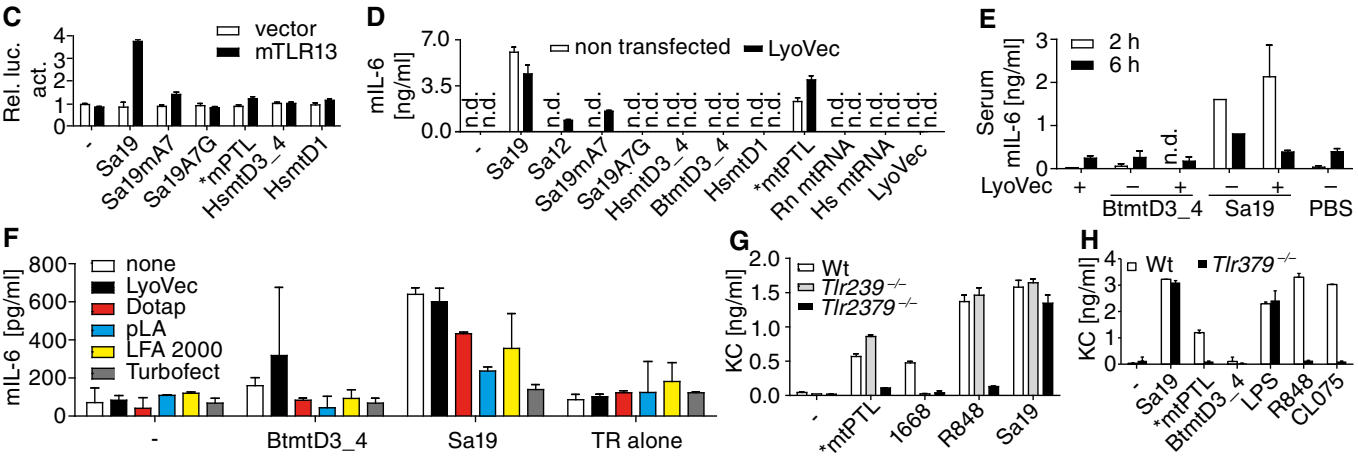



**Figure EV1. Scheme of bacterial and mitochondrial (mt) largest rRNAs, Sa19 “like” segments (ORNs) within the latter, and mostly non-responsiveness of the murine immune system to ORNs and mtRNA.**

A Schematic of both, bacterial 23S and mitochondrial 16S rRNAs (I to VI, domains; red rectangles, Sa19 like segments within 16S mtrRNA; D, domain; PTL, peptidyl transferase loop within V).

B Sa19 “like” 16S rRNA segment information (abbr., abbreviation; term., terminal; *interspecies sequence conservation).

C Luciferase activity (Rel. luc. act.) of transfected and challenged HEK293 cells ($n$ = 2).

D Cytokine release by bone marrow-derived macrophages (BM; LyoVec, transfection reagent; Hs, human; Rn, rat; $n$ = 2).

E Serum cytokine concentrations of mice challenged i.v. with phosphorothioate-stabilized ORNs ($n$ = 3).

F Cytokine release of splenocytes transfected with ORNs with various transfectants (pLA, poly-L-arginine; LFA, Lipofectamine 2000; TR, transfection reagent only; $n$ = 2).

G, H Cytokine release of challenged wild-type (Wt) and k.o. BMs ($n$ = 3).

Data information: (C–H) Graphs show mean ± SD; –, unchallenged; n.d., not detected.

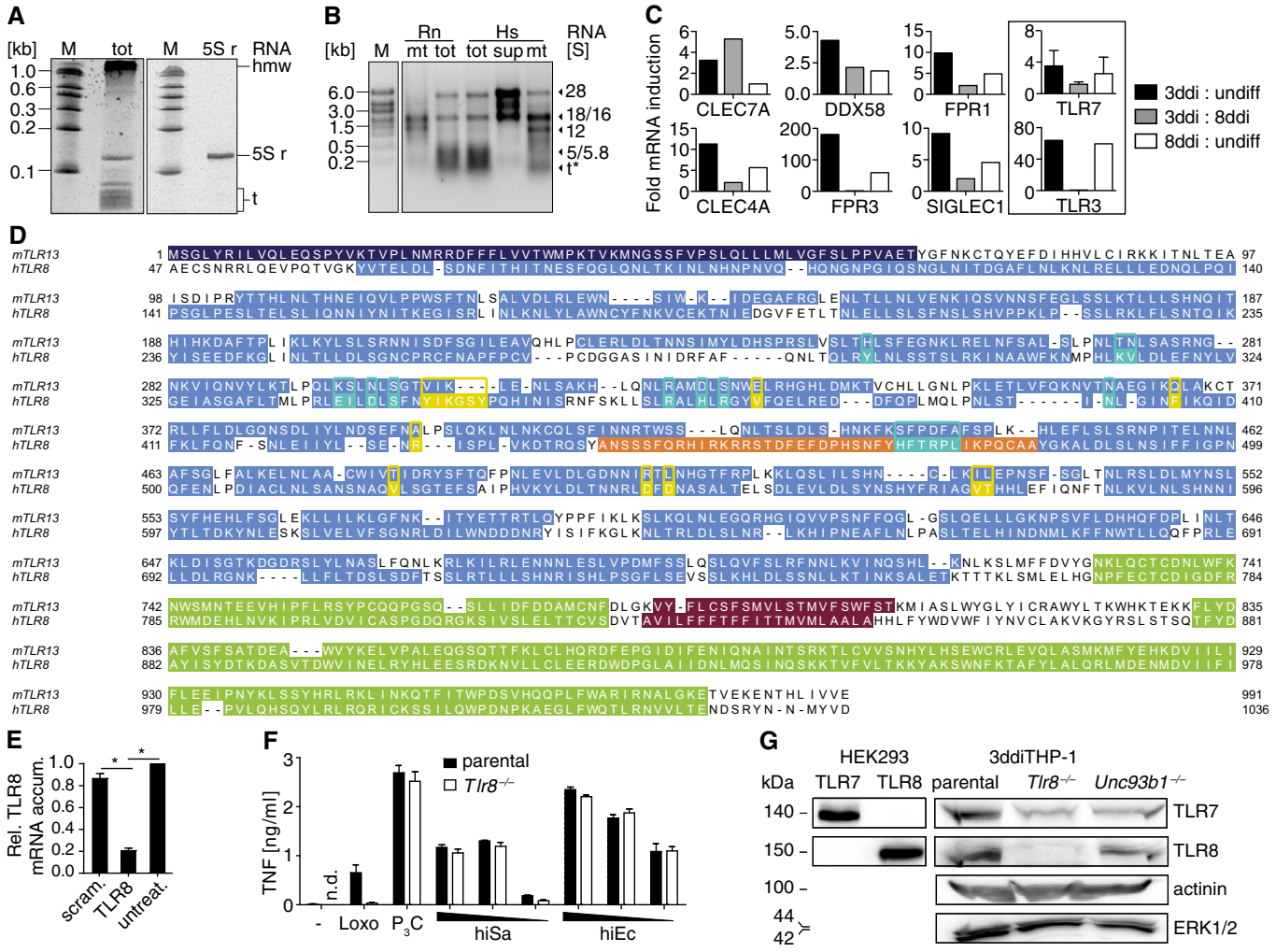



**Figure EV2. RNA subpopulation isolation, candidate mRNAs, mTLR13-hTLR8 alignment, TLR8 mRNA knockdown, and responsiveness as well as TLR7/8 expression of differentiated k.o. THP-1 cells.**

A, B Electrophoresis gels of total *S. aureus* RNA with tRNA and 5S rRNA (left panel) or isolated 5S rRNA (right panel; PAGE) and total or purified mammalian mtRNA (agarose), respectively (kb, kilobases; M, RNA size marker; tot, total; S, Svedberg; r, ribosomal; hmw, high molecular weight; t, transfer; mt, mitochondrial RNA; sup, supernatant at initial mitochondria separation step; Rn, rat; Hs, human; *S not applicable).

C Receptor mRNA amounts increased ≥ 2-fold in 3-day differentiated (ddi) as compared to undifferentiated (undiff) and 8ddiTHP-1 cells, except for the data marked by a frame (depicted for comparison; dual TLR7 probe set; CLEC7A, C-type lectin domain family 7A; FPR3, formyl peptide receptor 3; DDX58, DEAD box polypeptide; CLEC4A, C-type lectin domain; FPR1, formyl peptide receptor; SIGLEC1, sialic acid binding Ig-like lectin; $n$ = 1), according to the comparative gene array-based transcriptome profiling.

D Alignment of murine (m) TLR13 and human (h) TLR8 sequences (46 residue signal and leucine-rich repeat N-terminal domain, LRRNT, of the latter is not depicted for clarity; dark blue, signal peptide and LRRNT; light blue, LRRs; violet, transmembrane domains; green, LRR C-terminal and cytoplasmic Toll-IL1 receptor resistance gene domains; orange, z-loop; yellow and turquoise, residues known to contribute to hTLR8 ligand recognition site one and two, respectively).

E Relative (Rel.) mRNA accumulation (accum.) in 3ddiTHP-1 cells upon transfection of siRNAs (scram., scramble control; untreat., untreated; *$P \leq 0.01$; unpaired $t$-test; $n$ = 3).

F Activity of 3ddiTHP-1 cells challenged with different amounts of heat-inactivated (hi) *S. aureus* and *E. coli* (Sa and Ec, respectively; –, unchallenged; n.d., not detected; Loxo, loxoribine; $P_3C$, $Pam_3CSK_4$; corresponding to Fig 3C; $n$ = 3).

G Immunoblot analysis of R848 overnight-primed 3ddiTHP-1 cell lysates (genotypes indicated, TLR7$^+$ or TLR8$^+$ HEK293 cell lysates for positive and cytoplasmic protein detection as loading controls; $n$ = 3).

Data information: Graphs show mean ± SD.

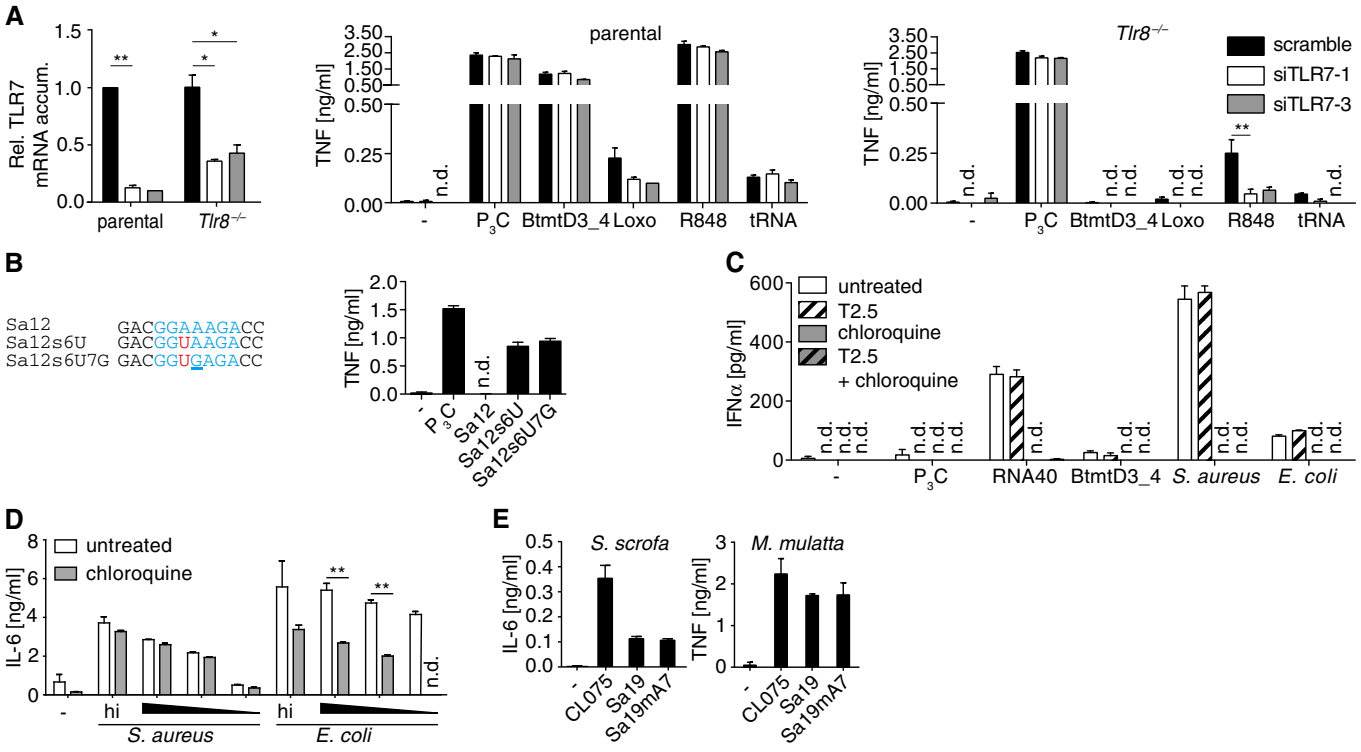



**Figure EV3. TLR7 mRNA knockdown in k.o. THP-1 cells and consequent responsiveness, Sa12 derivative sensitivity, bacterial infection-driven type I IFN or IL-6 production and inhibition of PBMCs or whole blood, respectively, and further species PBMC responsiveness.**

A Knockdown upon transfection of control (scramble) or two different TLR7 mRNA-specific siRNAs (siTLR7-1/3) in and cytokine release of respective 3-day differentiated THP-1 cells upon challenge (Rel., relative; accum., accumulation; Loxo, loxoribine; R848, small molecule, 50 μg/ml; tRNA, 200 ng/well of 96-well plate *E. coli* transfer RNA; $n = 3$).

B Sequence alignments of Sa12 and Sa12s6U with another Sa12 variant carrying in addition, as compared to the latter, ORN and, reminiscent of the respective motif in HsmtD1, a UGA motif (blue, core sequence; red, A6U; underlined, A7G). Diagram depicts PBMC activities upon challenges with Sa12 and derivatives ($n = 3$).

C PBMC type I IFN production upon pretreatments (T2.5, TLR2-neutralizing mAb; chloroquine, lysososmal function inhibitor) and challenge with TLR ligands or infection ($5 \times 10^4$ cfu/ml; $n = 2$; corresponding to Fig 4B).

D Cytokine release of whole blood culture upon lysosome inhibition and challenge with heat-inactivated (hi) and viable bacteria (triangle, decreasing dose of infection; $n = 3$; corresponding to Fig 4C).

E Cytokine release of *Sus* (*S.*) *scrofa* and *Macaca* (*M.*) *mulatta* PBMCs upon challenge with ORN variants ($n = 3$).

Data information: Graphs show mean ± SD; $*P \leq 0.05$; $**P \leq 0.01$; unpaired *t*-test; –, unchallenged; n.d., not detected; $P_3C$, $Pam_3CSK_4$.

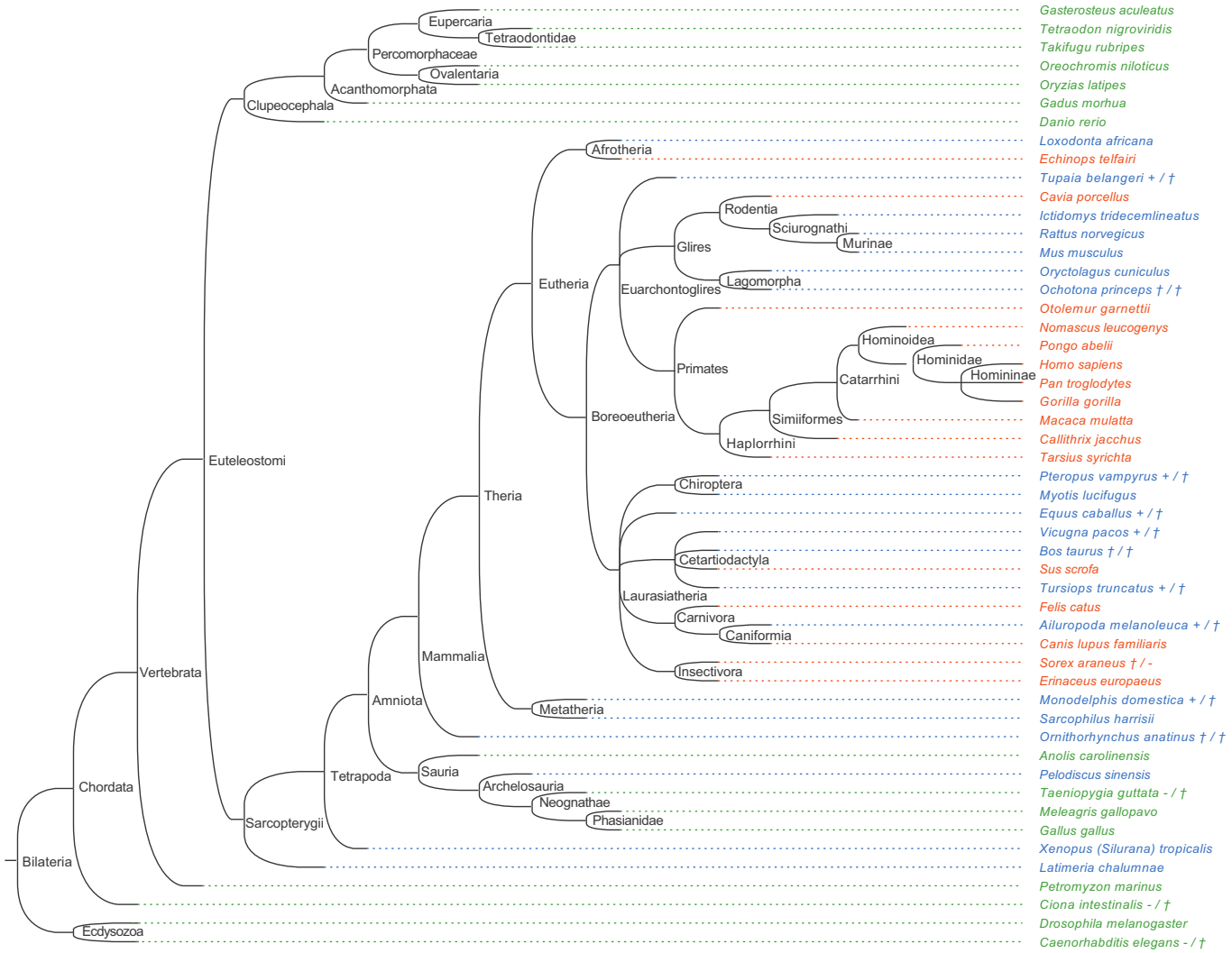



**Figure EV4. Phylogenetic tree illustrating TLR13 and/or TLR8 expression by Coelomata species including human and mouse.**
Species-specific expression of either TLR13 exclusively (green species name), both TLR13 and TLR8 (blue), or TLR8 exclusively (orange). Additional symbols behind the species names indicate if expression of at least one of the two TLRs is uncertain and specific information on gene appearance and protein sequence annotation as found. Separated by a slash, the first mark specifies TLR8 expression, while the second one specifies TLR13 expression (+, expression certain: annotation as protein alone or additional gene annotation in genome sequence; †, expression of functional protein due to potential pseudogene carriage not obligatory: no protein annotation while gene is being annotated in genome sequence; –, expression unlikely: no protein annotation and not annotated in genome or the latter not analyzed due to unavailability of assembled genome).