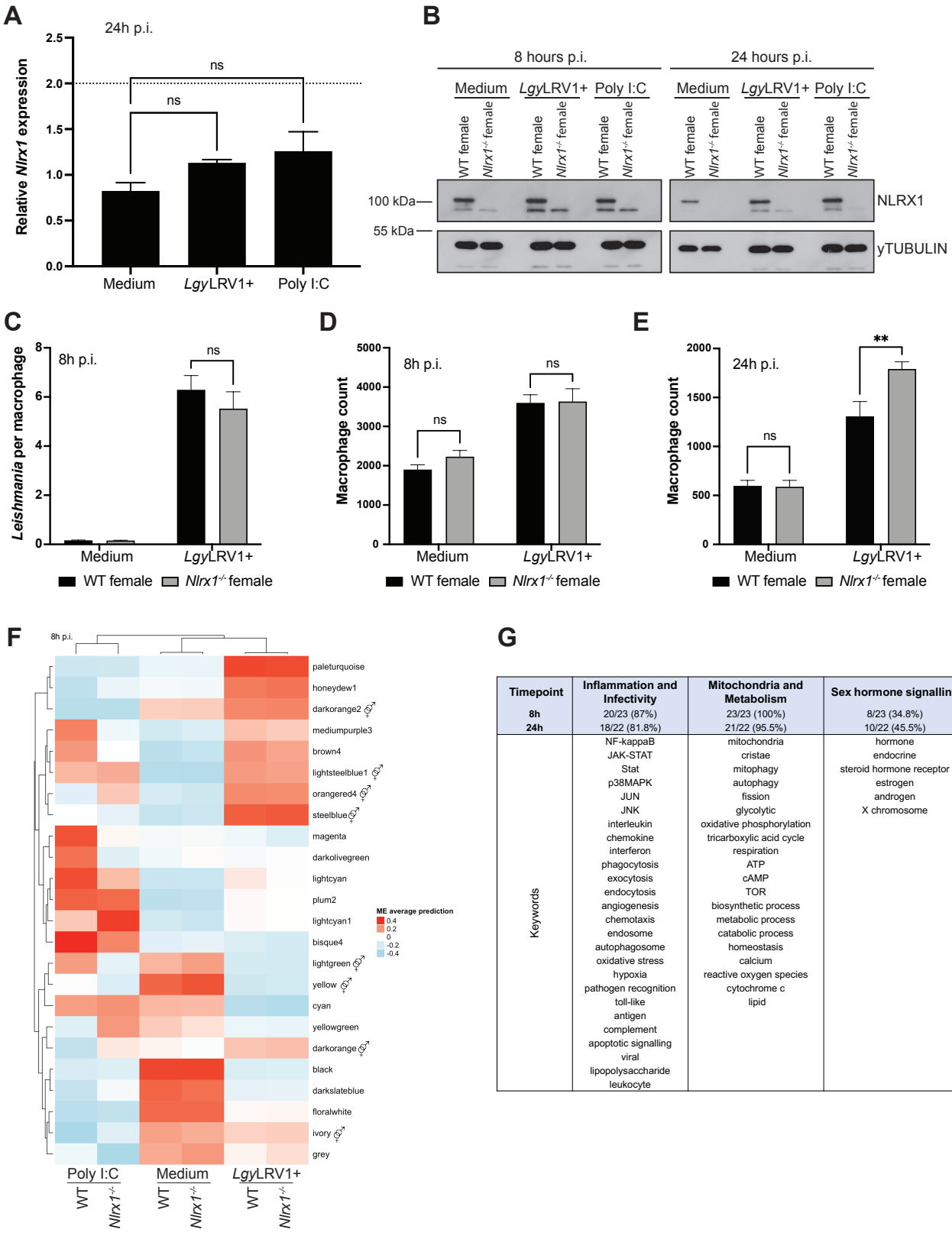
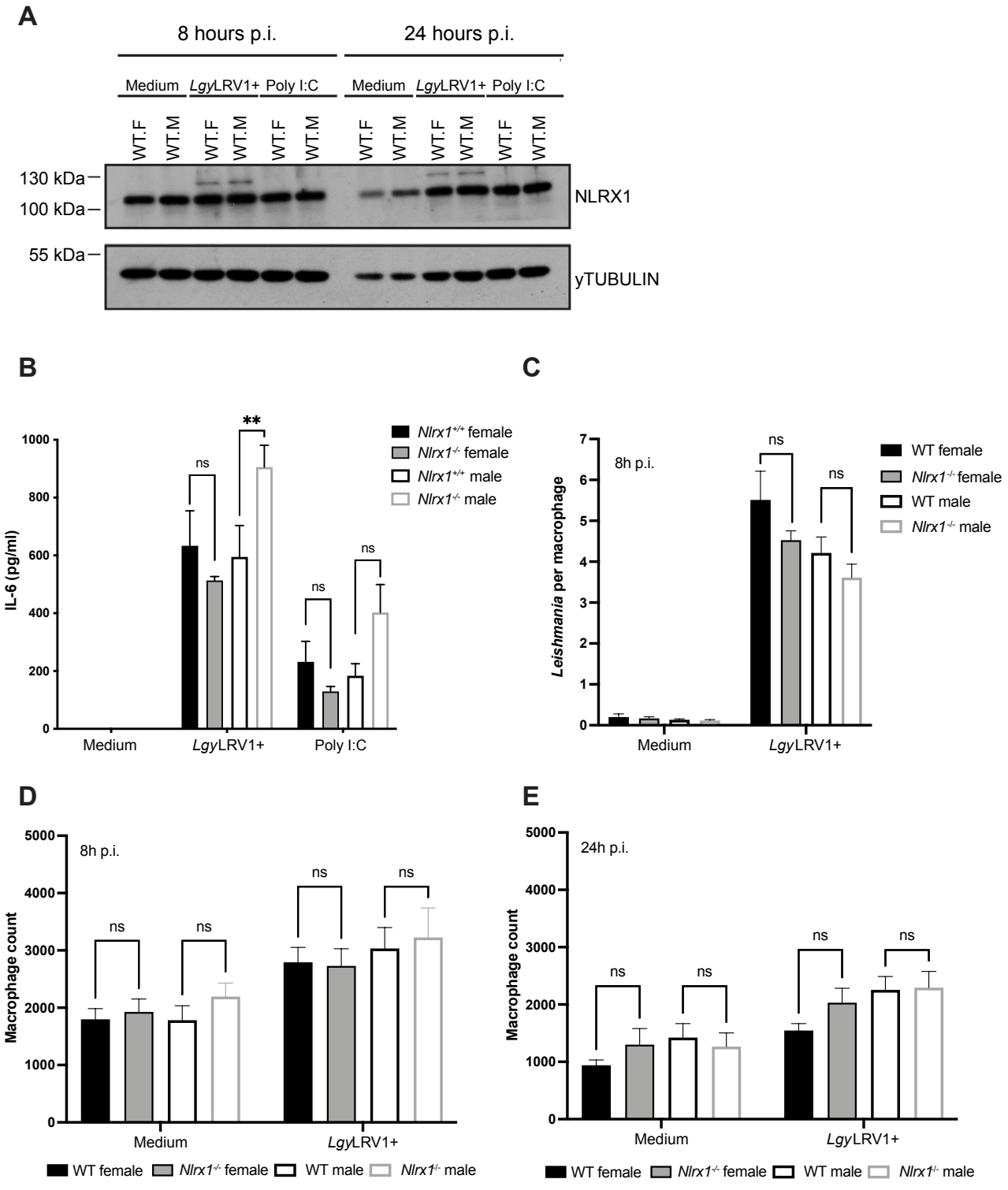
Supplementary Material



G

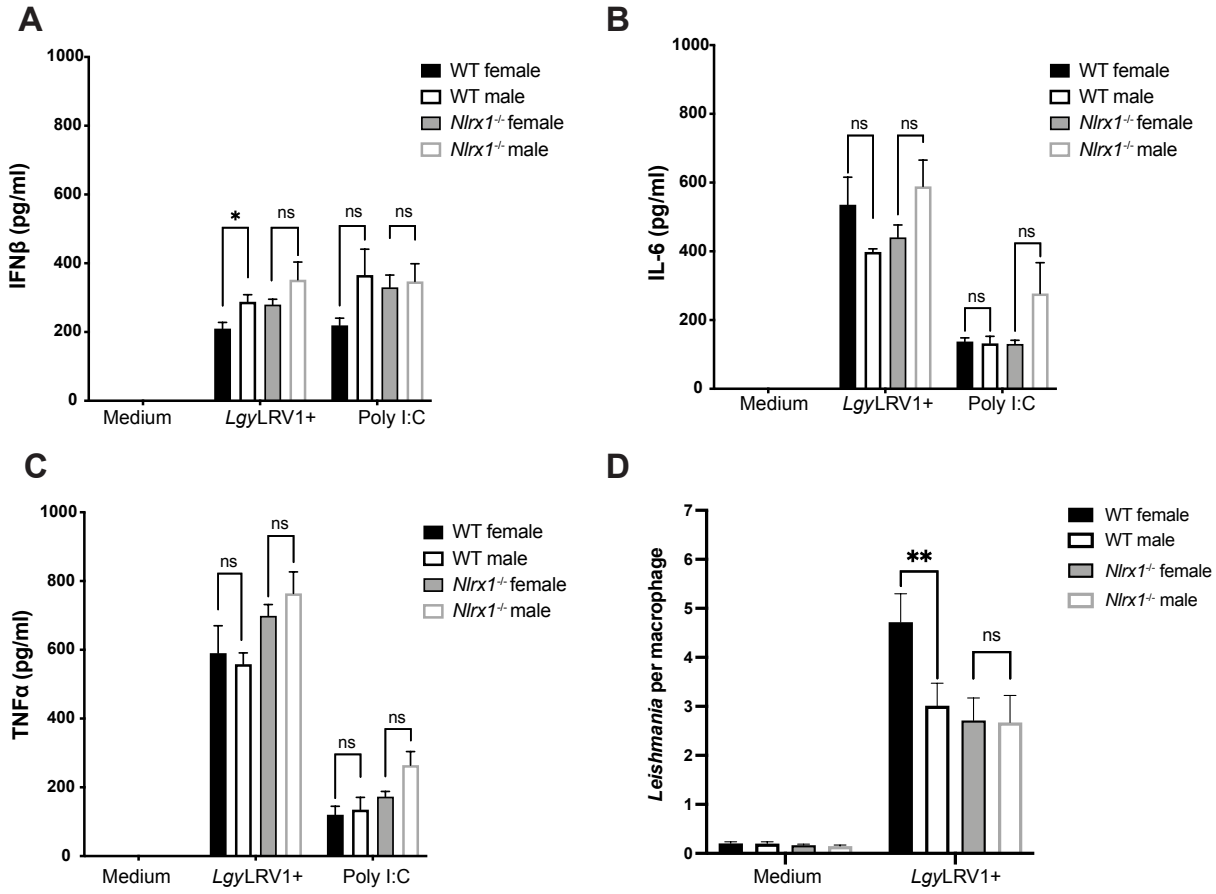
Timepoint	Inflammation and Infectivity	Mitochondria and Metabolism	Sex hormone signalling
8h	20/23 (87%)	23/23 (100%)	8/23 (34.8%)
24h	18/22 (81.8%)	21/22 (95.5%)	10/22 (45.5%)
Keywords	NF-kappaB JAK-STAT Stat p38MAPK JUN JNK interleukin chemokine interferon phagocytosis exocytosis endocytosis angiogenesis chemotaxis endosome autophagosome oxidative stress hypoxia pathogen recognition toll-like antigen complement apoptotic signalling viral lipopolysaccharide leukocyte	mitochondria cristae mitophagy autophagy fission glycolytic oxidative phosphorylation tricarboxylic acid cycle respiration ATP cAMP TOR biosynthetic process metabolic process catabolic process homeostasis calcium reactive oxygen species cytochrome c lipid	hormone endocrine steroid hormone receptor estrogen androgen X chromosome

Supplementary Figure S1. NLRX1 modulated inflammation, infection, and metabolism in female BMDMs. BMDMs from WT and *Nlrx1*$^{-/-}$ female mice were infected with *Lgy*LRV1+ parasites or stimulated with poly I:C. 2 μg/ml). **(A)** *Nlrx1* mRNA levels were quantified by qRT-PCR at 24 hours p.i. (n=3 independent experiments). **(B)** After 8 and 24 hours, proteins were extracted blotted for NLRX1 and γ-TUBULIN. Representative western blot of 3 independent experiments is shown. **(C)** Intracellular parasite load (8 hours p.i.) and **(D-E)** macrophage count (8 and 24 hours p.i.) were quantified (n=2 independent experiments). Transcriptomics analysis of WT and *Nlrx1*$^{-/-}$ female BMDMs (n=3 mice per group) infected with *Lgy*LRV1+ parasites or stimulated with poly I:C (2 μg/ml) for 8 and 24 hours. **(F)** The heatmap represents the global weighted correlation network analysis (wgcna) at 8 hours pi. and module names are represented by a color. **(G)** A gene ontology (GO) enrichment analysis for each module was performed to identify the biological processes associated to each module and terms were grouped into 3 categories. ⚥ represents modules enriched in GO terms associated to sex hormone signaling. Graphs are presented as mean +- SEM and significance was tested by two-way ANOVA with multiple comparisons (B-D). ns = non-significant, **p ≤ 0.01.

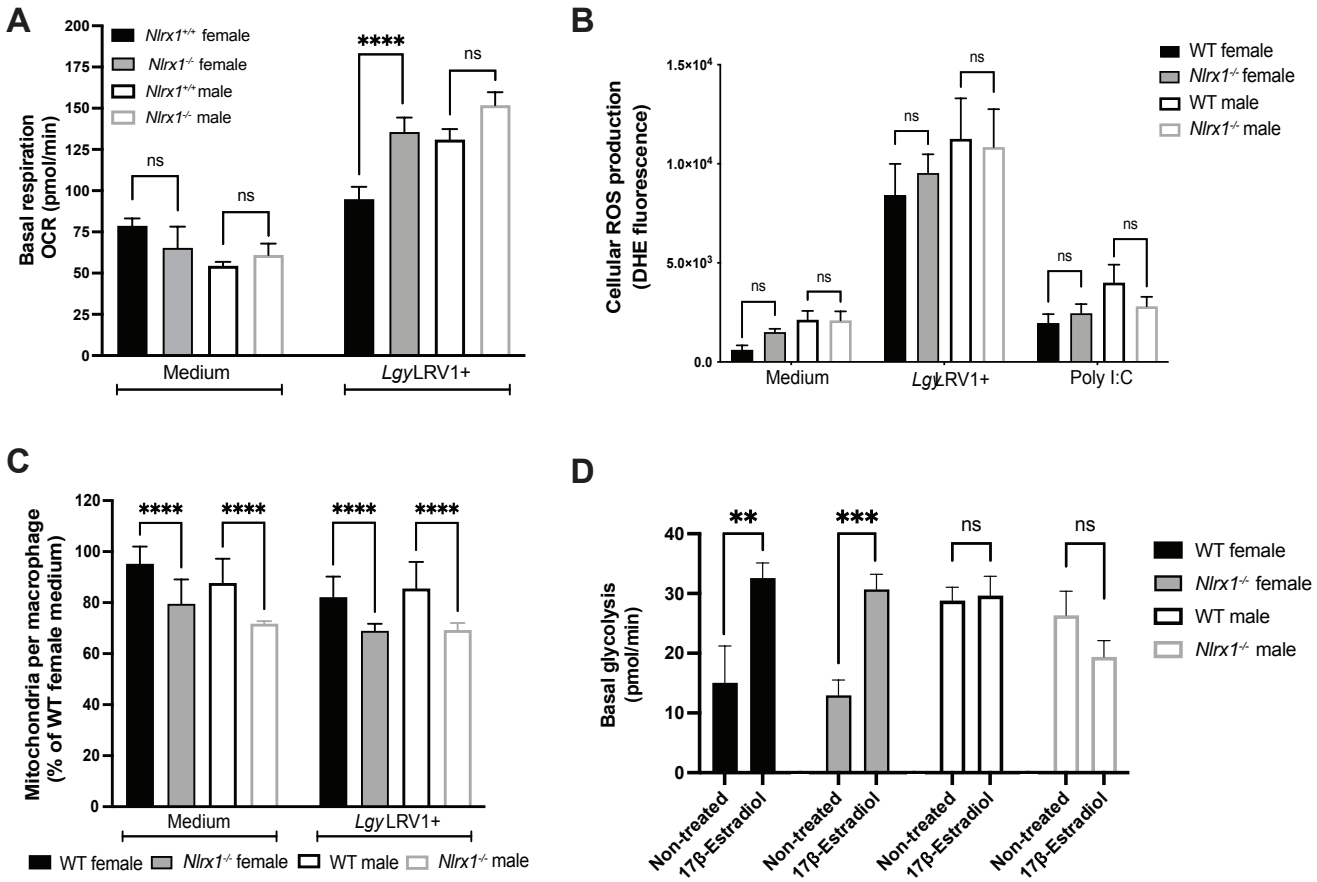


Supplementary Figure S2. Inflammation and infection in female and male macrophages. BMDMs from WT and *Nlrx1-/-* female mice were isolated and infected with *Lgy*LRV1+ parasites

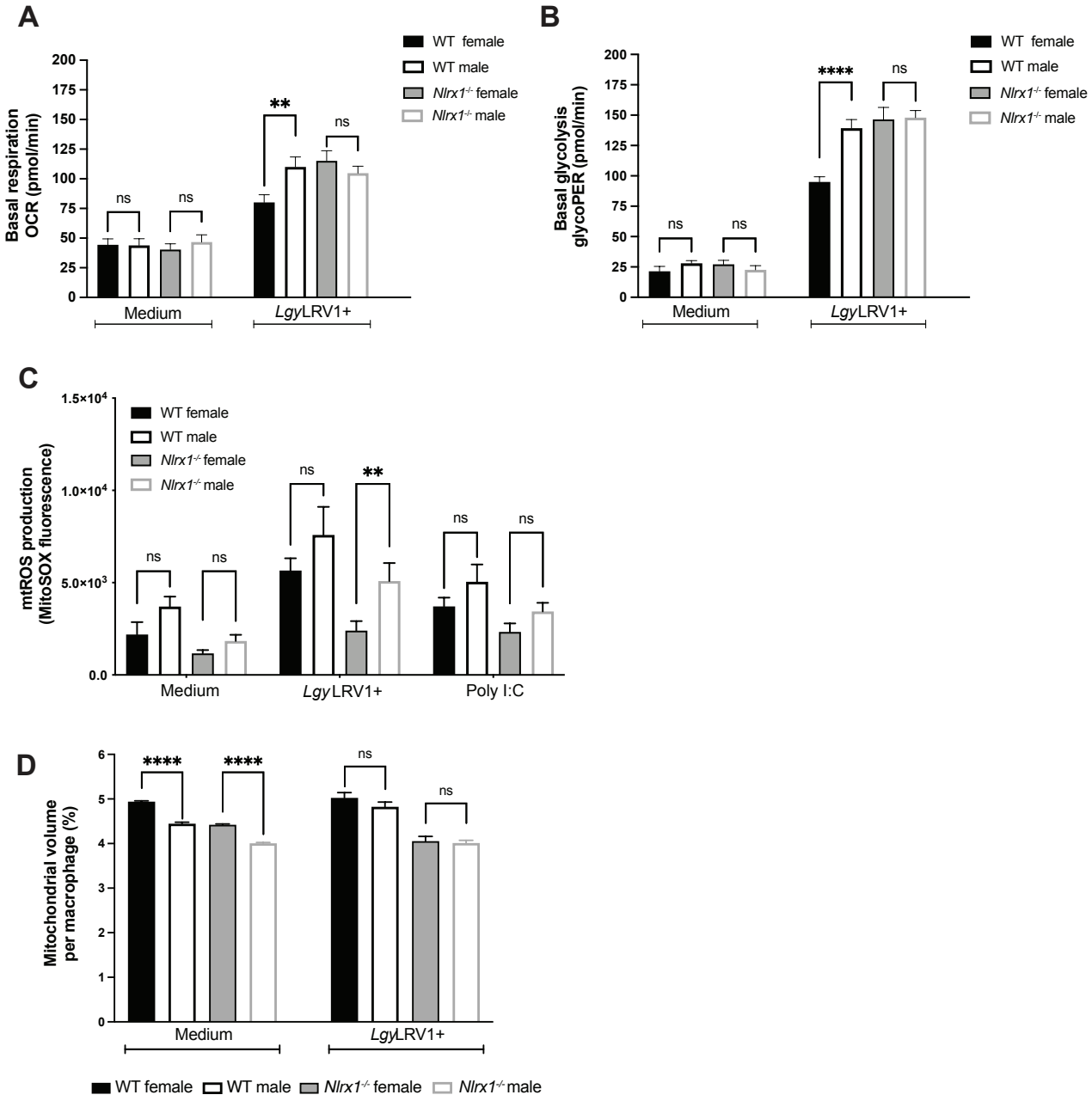
or stimulated with the TLR3 agonist poly I:C (2 μg/ml). **(A)** After 8 and 24 hours, proteins were extracted from cell lysates and blotted for NLRX1 and γ-TUBULIN. WT.F, WT female. WT.M, WT male. Representative western blot of three independent experiments is shown. **(B)** As a control, after 24 hours, IL-6 was measured in in cell-free supernatants by ELISA in *Lgy*LRV1+ infected or poly I:C stimulated BMDMs isolated from littermate controls (representative experiment shown, n=3). At 8 and 24 hours p.i., BMDMs were fixed with 4% PFA and stained with DAPI and phalloidin. Cells were visualized with a high content microscope (40x) and **(C)** intracellular parasite load (8 hours p.i.) and **(D-E)** macrophage count (8 and 24 hours p.i.) were quantified using a MetaXpress software (n=3 independent experiments). Graphs are presented as mean +- SEM and significance was tested by two-way ANOVA with multiple comparisons (B-D). ns = non-significant.



Supplementary Figure S3. Statistical analysis of inflammation and infection profiles by sex. To complement Figure 4, the statistical analysis for figures 4C-F was also performed by sex. Graphs are presented as mean +- SEM and significance was tested by two-way ANOVA with multiple comparisons (A-F). ns = non-significant, *$p \leq 0.05$, **$p \leq 0.01$.

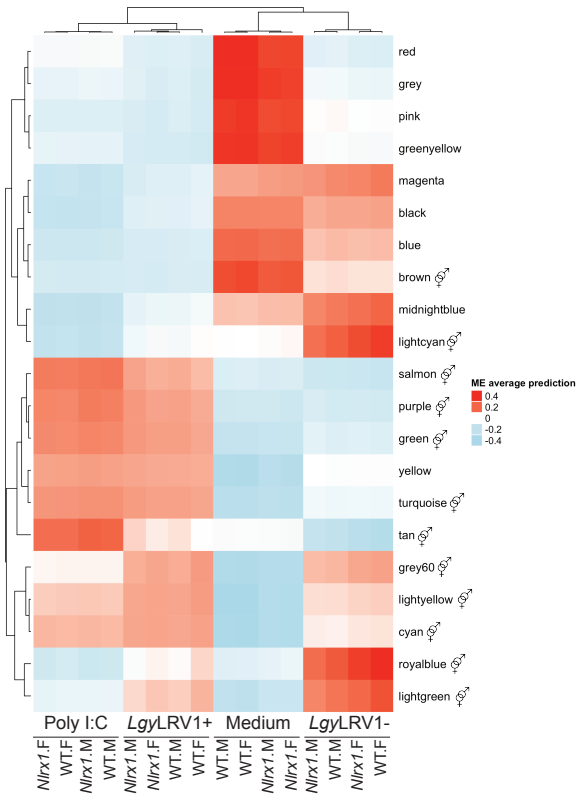

A
Nlrx1+/+ female
Nlrx1-/- female
Nlrx1+/+ male
Nlrx1-/- male
Basal respiration OCR (pmol/min)
200
175
150
125
100
75
50
25
0
ns
ns
****
ns
Medium
LgyLRV1+
B
WT female
Nlrx1-/- female
WT male
Nlrx1-/- male
Cellular ROS production (DHE fluorescence)
1.5×10^4
1.0×10^4
5.0×10^3
0.0
ns
ns
ns
ns
ns
ns
Medium
LgyLRV1+
Poly I:C
C
Mitochondria per macrophage (% of WT female medium)
120
100
80
60
40
20
0
****
****
****
****
Medium
LgyLRV1+
WT female
Nlrx1-/- female
WT male
Nlrx1-/- male
D
Basal glycolysis (pmol/min)
40
30
20
10
0
**
***
ns
ns
Non-treated
17β-Estradiol
Non-treated
17β-Estradiol
Non-treated
17β-Estradiol
Non-treated
17β-Estradiol
WT female
Nlrx1-/- female
WT male
Nlrx1-/- male

Supplementary Figure S4. Genotype- or sex-dependent effects on ROS, mitochondria, and metabolism. (A) As a control, after 8 hours, basal mitochondrial respiration rate was assessed in BMDMs isolated from littermates (representative experiment shown, n=3). **(B)** Cellular ROS (ROS) production was quantified in female and male BMDMs infected with *Lgy*LRV1+ parasites or treated with poly I:C (2 μg/ml) for 8 hours. Cells were stained with DHE (5 μM) for 20 min at 37°C. Fluorescence was measured using a Spectramax i3 plate reader and adjusted to protein concentration per well. (n=3 independent experiments). **(C)** Number of mitochondria per female and male BMDMs infected with *Lgy*LRV1+ parasites for 8 hours were counted in images acquired by transmission electron microscopy (n=2 independent experiments, total of minimum 60 cells were analyzed per group). **(D)** BMDMs from female and male WT and *Nlrx1*$^{-/-}$ mice were pre-treated with 17β-estradiol (200 pg/ml) for 2 hours and estradiol was kept in the assay medium for the duration of the assay. Basal glycolytic rate was assessed by Seahorse XFe96 analyzer and adjusted to protein concentration per well (n=4 independent experiments). Graphs are presented as mean +- SEM and significance was assessed by two-way ANOVA with multiple comparisons (A) or unpaired, parametric t-test (B-C). ns: non-significant, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.



Supplementary Figure S5. Statistical analysis of metabolic profiles by sex. To complement Figure 5, the statistical analysis for figures 5A-C and figure 5E was also performed by sex. Graphs are presented as mean +- SEM and significance was assessed by two-way ANOVA with multiple comparisons (A-C) or unpaired, parametric t-test (D). ns = non-significant, **p ≤ 0.01, ****p ≤ 0.0001.

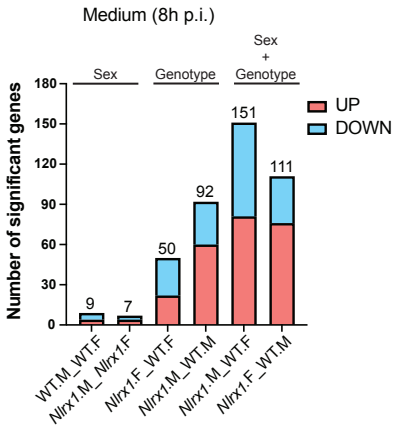
A



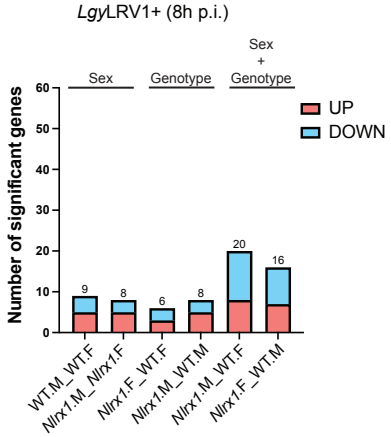
B

Timepoint	Inflammation and Infectivity	Mitochondria and Metabolism	Sex hormone signalling
8h	20/20 (100%)	19/20 (95%)	12/20 (60%)
24h	6/6 (100%)	5/6 (83.3%)	5/6 (83.3%)
Keywords	immunity	mitochondria	hormone
	immune response	fission	steroid hormone receptor
	NF-kappaB	mitophagy	estradiol
	JAK-STAT	autophagy	estrogen
	p38MAPK	glycolytic	androgen
	JNK	oxidative phosphorylation	testosterone
	interleukin	tricarboxylic acid cycle	endocrine
	chemokine	ATP	
	interferon	biosynthetic process	
	phagocytosis	metabolic process	
	exocytosis	catabolic process	
	endocytosis	homeostasis	
	angiogenesis	calcium	
	chemotaxis	reactive oxygen species	
	xenophagy	lipid	
	endosome		
	autophagosome		
	oxidative stress		
	hypoxia		
	toll-like		
	antigen		
	complement		
	apoptotic signalling		
	viral		
	lipopolysaccharide		
	leukocyte		

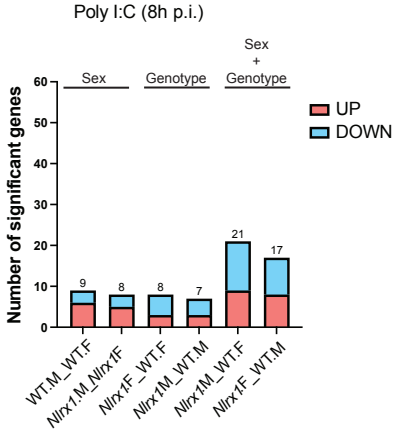
C



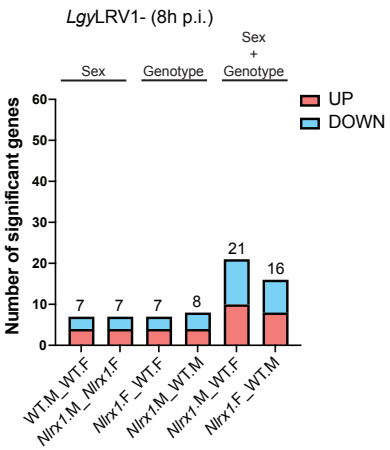
D



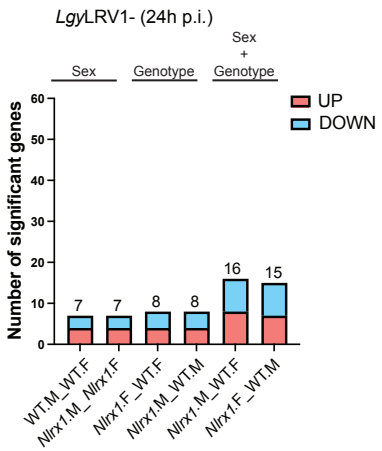
E



F



G



Supplementary Figure S6. Transcriptomics analysis of female and male BMDMs. Transcriptomics analysis of both female and male WT and *Nlrx1*$^{-/-}$ BMDMs (n=3 mice per group) infected with *Lgy*LRV1+ parasites or stimulated with poly I:C (2 μg/ml) for 8 hours. **(A)** The heatmap represents the global weighted correlation network analysis (wgcna) of 8 hours pi. and module names are represented by a color. A gene ontology (GO) enrichment analysis for each module was performed to identify the biological processes associated to each module and **(B)** terms were grouped into 3 categories. ⚥ represents modules enriches in GO terms associated to sex hormone signaling. At 8 hours, the number of differentially expressed genes between groups in **(C)** non-infected, **(D)** *Lgy*LRV1+ infected and **(E)** poly I:C treated conditions, and **(F-G)** at 8 and 24 hours, in LgyLRV1- infected conditions the number of differentially expressed genes between groups is plotted. On each barplot the number represents the total number of differentially upregulated (in red) and downregulated (in blue) genes. Pairwise comparisons are done by "sex" (same genotype, different sex, "WT.F._WT.M" and "*Nlrx1*.F_*Nlrx1.M"),* by "genotype" (same sex, different genotype ("*Nlrx1*.F_WT.F" and "*Nlrx1*.M_WT.M"), or by combining both "sex and genotype" ("*Nlrx1*.M_WT.F" and "*Nlrx1*.F_WT.M"). See **Supplementary tables 12-16** for the list of significantly differentially expressed genes.