Cell Host & Microbe, Volume *23*

Supplemental Information

Combined Human Genome-wide RNAi and Metabolite Analyses Identify IMPDH as a Host-Directed Target against *Chlamydia* Infection

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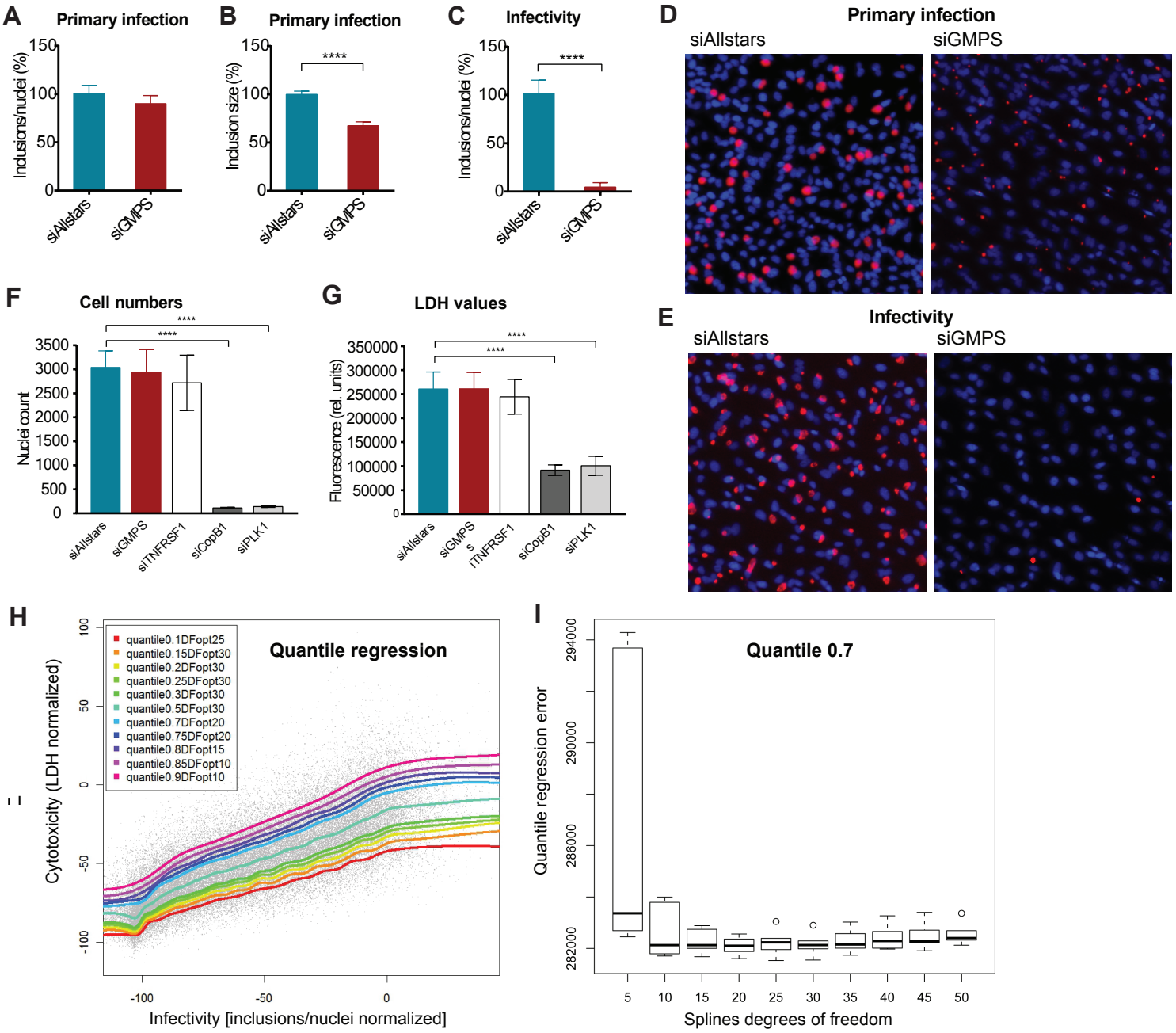

Figure S1 Related to Figure 1.
(A-C) Establishment and performance of screening controls. Cells were seeded in 384 wells and reversely transfected with neutral (siAllstars) and inhibiting siRNA control (siGMPS). After 3 d, cells were infected with CTL2 at MOI 0.3. Primary infection cells were fixed 24 h p.i. For quantification of infectious progeny cells were lysed 48 h p.i., lysate was titered onto fresh cells, which were fixed 24 h later. Inhibiting control siGMPS led to smaller inclusion size in primary infection and strong reduction in formation of infectious progeny, while inclusion number was not significantly altered in primary infection. Knockdown thus disturbs intracellular growth but not invasion of CTL2. Data show mean ± SD of three independent experiments;**** $P < 0.0001$, two-tailed Mann-Whitney test. (D) Corresponding representative IF images of siAllstars- and siGMPS-transfected cell 24 h after infection (primary infection) and (E) resulting infectivity. Nuclei, blue. CTL2 inclusions, red. Upon knockdown with the inhibitory screening control siGMPS inclusions in primary infection are smaller compared to neutral control siAllstars and resulting infectivity is strongly reduced. (F) Quantification of transfected cells. Cells were seeded in 384 wells and reversely transfected with siAllstars, siGMPS and cytotoxic (siCopB1 and siPLK1) siRNA controls, as described above. Nuclei count in primary infection was determined using automated microscopy and resulting LDH activity from lysates prepared 48 h p.i. was assessed in parallel (G), demonstrating high correlation between original cell count and resulting total LDH activity. Data show mean percentage of LDH activity ± SD of three independent experiments; ***$P < 0.0001$, one-way ANOVA with Bonferroni post-hoc test. (H) The optimal splines are displayed for the quantiles 0.1, 0.15, 0.2, 0.25, 0.3, 0.5, 0.7, 0.75, 0.8, 0.85, 0.9 and the optimal DF for each reported in the legend. For every quantile value, the “extreme” genes were determined, i.e. the genes whose LDH values lie below the quantile curves for quantiles 0.1, 0.15, 0.2, 0.25, 0.3 and above those for 0.7, 0.75, 0.8, 0.85, 0.9. (I) Boxplots derived from the five test errors for quantile 0.7. Quantile regression errors calculated for quantile 0.7 are displayed for degrees of freedom from 5 to 50 with intermitting steps of 5. The minimal median error is obtained for DF=20 and is very small. The number of training data is high with respect to the DF, so overfitting is small for all tested DFs. For DF=5 underfitting can be observed, i.e. the approximating function is too rigid to account for the variation in the data. See also Table S1. Abbreviations: siGMPS – siRNA targeting guanosine monophosphate synthase; siTNFRSF1 – siRNA targeting tumor necrosis factor receptor superfamily, member 1A; siCopB1 – siRNA targeting coatomer protein complex subunit beta 1; siPLK1 – siRNA targeting polo-like kinase 1.

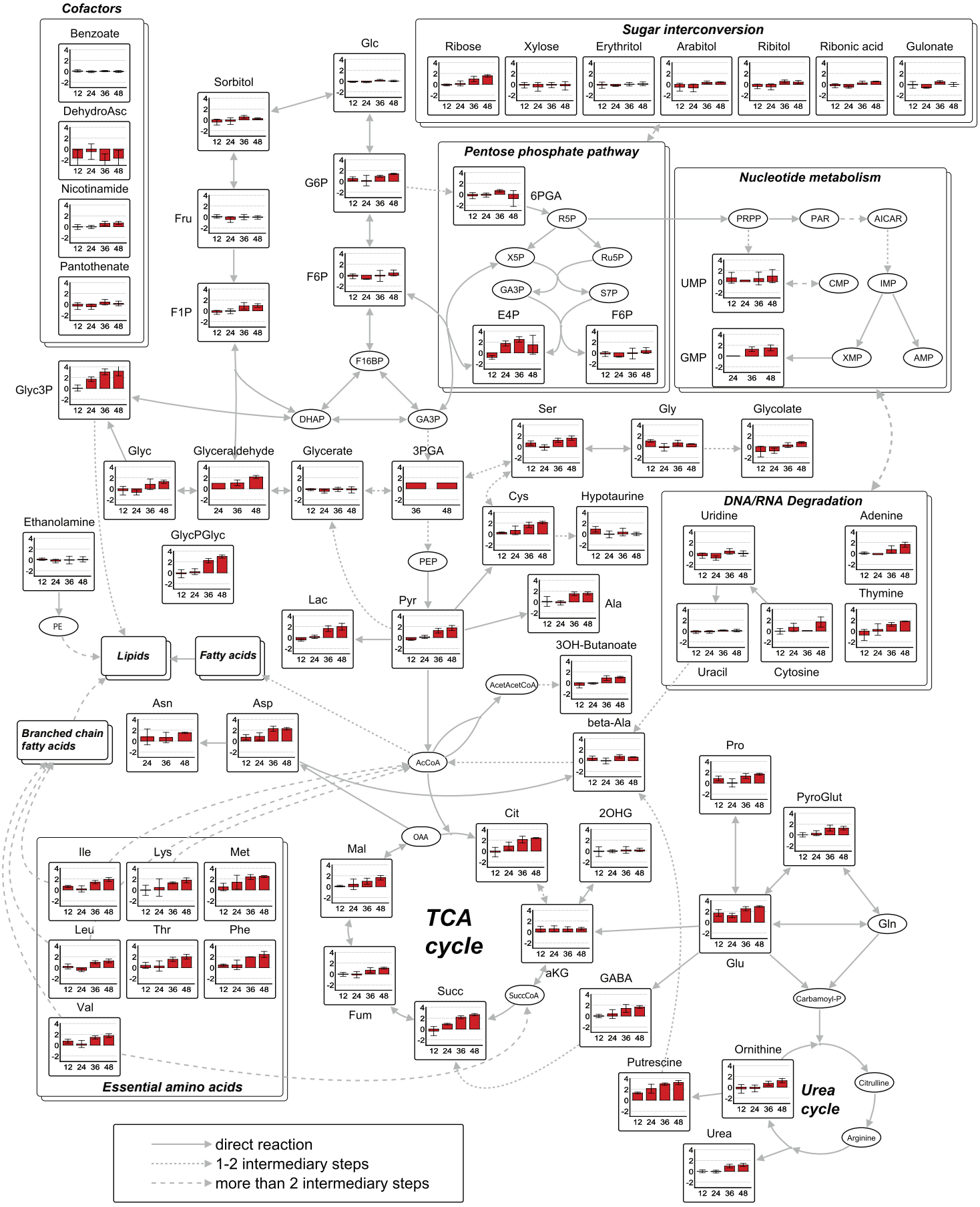

Figure S2 Time course data of metabolite regulation in *Chlamydia*-infected cells. Related to Figure 2.
HeLa cells were infected with CTL2 at MOI 1 or left uninfected for 12, 24, 36 and 48 h in three biological replicates. Diagrams represent the log2 values of the ratios of the fold changes between infected and non-infected samples (mean ± SD). For single time-point data see Table S5. A detailed description of data processing and normalization is provided in the methods section. Abbreviations of metabolites: 2OHG: 2-Hydroxyglutarate; 3OH-Butanoate: (D)-3-Hydroxybutyrate; 3PGA: 3-Phosphoglyceric acid; AcCoA: Acetyl Coenzyme A; AcetAcetCoA: Acetoacetyl-CoA; aKg: alpha-Ketoglutaric acid; Ala: Alanine; Asn: Asparagine; Asp: Aspartate; Beta-Ala: beta-Alanine; Carbamoyl-P: Carbamoyl-phosphate; Cit: Citrate; Cys: Cysteine; DehydroAsc: dehydroascorbic acid; DHAP: Dihydroxyacetone phosphate; E4P: Erythrose 4-phosphate; F16BP: Fructose-1-6-bisphosphate; F1P: Fructose-1-phosphate; F6P: Fructose-6-phosphate; Fru: Fructose; Fum: Fumarate; G6P: Glucose-6-phosphate; GA3P: Glyceraldehyde 3-phosphate; GA3P: Glyceraldehyde 3-phosphate; GABA: gamma-Aminobutyric acid; Glc: Glucose; Gln: Glutamine; Glu: Glutamate; Gly: Glycine; Glyc: Glycerol; Glyc3P: Glycerol-3-phosphate; GlycPGlyc: Glycerophosphoglycerol; Ile: Isoleucine; Lac: Lactate; Leu: Leucine; Lys: Lysine; Mal: Malate; MP:Main product detected by GC-MS; Met: Methionine; OAA: Oxaloacetic acid; PE: Phosphatidylethanolamine; PEP: Phosphoenolpyruvate; PG6: Phosphogluconolactone; Phe: Phenyl-alanine; Pro: Proline; Pyr: Pyruvate; PyroGlut: Pyro-glutamate; R5P: Ribose-5-phosphate; Ru5P: Ribulose-5-phosphate; S7P: D-Sedo Heptulose 7-P; Ser: Serine; Succ: Succinate; SuccCoA: Succinyl Coenzyme A; Thr: Threonine; Val: Valine; X5P: Xylulose 5-phosphate. See also Table S5.

A

MPA parameter	2 mg/kg IV	40 mg/kg PO
t1/2 (h)	4.57	6.99
Tmax (h)		0.08
Cmax [ng/mL]		7982.30
C0 [ng/mL]	15176.20	
AUC 0-inf [h*ng/mL]	8765.60	17486.70
Vss [l/kg]	0.72	
Cl [l/h/kg]	0.17	
F (%)		9.40

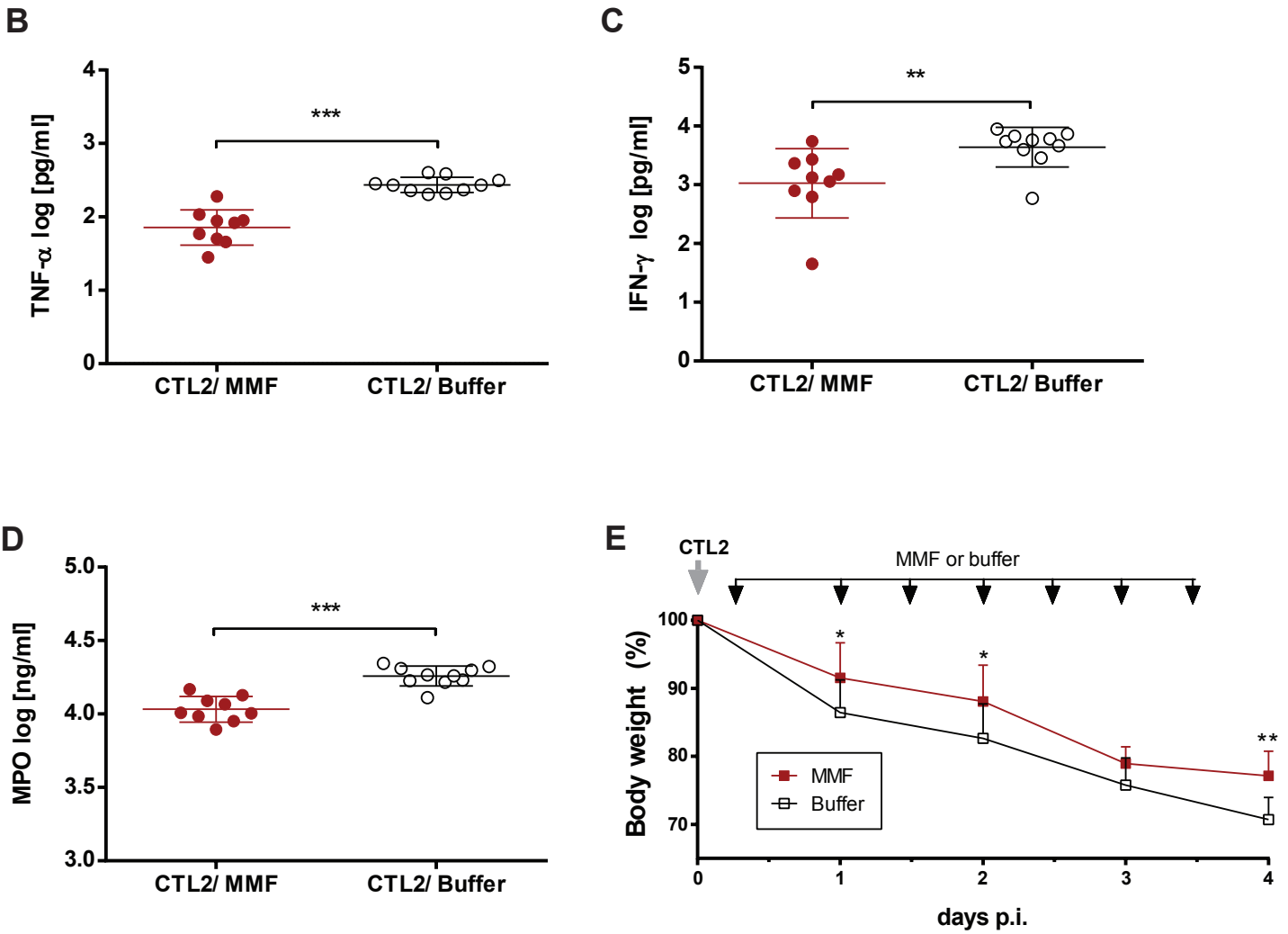

Figure S3 Related to Figure 4.
Pharmacokinetic parameters of the active metabolite mycophenolic acid (MPA) after administration of single doses of 2 mg/kg mycophenolate mofetil (MMF) IV and 40 mg/kg MMF PO to male C57BL/6J mice (**A**). t1/2 [h] - half-life; Tmax [h] - time at which Cmax is observed; Cmax [ng/ml] - maximum plasma drug concentration; C0 [ng/ml] - estimated initial (zero-time) plasma drug concentration; AUC 0-inf [h*ng/ml] - area under the plasma drug concentration-time curve (from zero to infinity); Vss [l/kg] - steady-state volume of distribution; CL [l/h/kg] – clearance; F [%] – bioavailability.
Ten to 12-week old male C57BL/6J mice were intranasally infected with a CTL2 IFU of 8 × 105; 200 mg/kg of MMF was orally applied bidaily starting 6 h p.i., whereas infected control mice received buffer only (arrows in panel E). All mice were sacrificed on day 4 p.i., and in homogenates from right lung lobes levels of TNF-α (**B**) and INF-γ (**C**) and the granulocyte marker enzyme myeloperoxidase (MPO) (**D**) were determined. Mice were monitored daily and body weight was documented (**E**). Mean and standard deviation of n = 10 mice in each group are depicted, *P < 0.05; **P < 0.01; ***P < 0.001.