

**Reprogramming of host glutamine metabolism during *Chlamydia trachomatis* infection and its key role in peptidoglycan synthesis**

Karthika Rajeeve<sup>1,§,\*</sup>, Nadine Vollmuth<sup>1</sup>, Sudha Janaki-Raman<sup>2,3</sup>, Thomas F. Wulff<sup>1</sup>, Apoorva Baluapuri<sup>3</sup>, Francesca R. Dejure<sup>3</sup>, Claudia Huber<sup>4</sup>, Julian Fink<sup>6</sup>, Maximilian Schmalhofer<sup>4</sup>, Werner Schmitz<sup>2,3</sup>, Rajeeve Sivadasan<sup>5</sup>, Elmar Wolf<sup>3</sup>, Wolfgang Eisenreich<sup>4</sup>, Almut Schulze<sup>2,#</sup>, Jürgen Seibel<sup>6</sup>, Thomas Rudel<sup>1,7\*</sup>

<sup>1</sup> Department of Microbiology, Biocenter, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany.

<sup>2</sup> Department of Biochemistry and Molecular Biology, Biocenter, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany.

<sup>3</sup> Cancer Systems Biology Group, Department of Biochemistry and Molecular Biology, Biocenter, University of Würzburg, Am Hubland, 97074 Würzburg, Germany.

<sup>4</sup> Lehrstuhl für Biochemie, Technische Universität München, Lichtenbergstr. 4, D-85747 Garching, Germany.

<sup>5</sup> German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, D-69120, Heidelberg, Germany.

<sup>6</sup> Institute of Organic Chemistry, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany.

<sup>7</sup> Helmholtz Institute for RNA-based Infection Research (HIRI), D-97074 Würzburg, Germany.

<sup>§</sup> Department of Biomedicine, The Skou Building, Aarhus University, Høegh-Guldborgs Gade 10, DK-8000, Aarhus C, Denmark.

<sup>#</sup> BioMed X Innovation Center, Im Neuenheimer Feld 583, D-69120 Heidelberg, Germany.

\*For correspondence: karaj@biomed.au.dk, thomas.rudel@biozentrum.uni-wuerzburg.de.

## Abstract

Obligate intracellular bacteria like *Chlamydia trachomatis* undergo a complex developmental cycle between infectious non-replicative (EBs) and non-infectious replicative (RBs) forms. EBs shortly after entering a host cell transform to RBs, a crucial process in infection, initiating chlamydial replication. As *Chlamydia* fail to replicate outside the host cell it is currently unknown how the replicative part of the developmental cycle is initiated. Here we show in a cell-free approach in axenic media that uptake of glutamine by the bacteria is crucial for peptidoglycan synthesis which has a role in *Chlamydia* replication. The increased requirement for glutamine in infected cells is satisfied by reprogramming the glutamine metabolism in a c-Myc-dependent manner. Glutamine is effectively taken up by the glutamine transporter SLC1A5 and metabolized via glutaminase. Interference with this metabolic reprogramming limited growth of *Chlamydia*. Intriguingly, *Chlamydia* failed to produce progeny in SLC1A5 knockout organoids and mice. Thus, we report on the central role of glutamine for the development of an obligate intracellular pathogenic bacterium and the reprogramming of host glutamine metabolism, which may provide a basis for innovative anti-infective strategies.

*Chlamydia trachomatis* is an obligate intracellular bacterium and the most frequent cause of bacterial sexually transmitted diseases <sup>1</sup>. In a biphasic developmental cycle, EBs enter the host cell and convert into RBs, which replicate in a membrane-enclosed structure called “the inclusion”. Later, RBs convert back into EBs, which can readily infect neighbouring cells. For a long time, it was thought that *Chlamydia* do not form peptidoglycan until peptidoglycan ring-like structures were recently discovered exclusively in the septa of dividing RBs <sup>2,3</sup>. Due to its obligate intracellular lifestyle and an evolutionary reduced genome <sup>4</sup>, *Chlamydia* requires multiple metabolites from the host cell. Nevertheless, this pathogen lacks the enzyme hexokinase but encodes the enzymes required to utilize D-glucose-6-phosphate (G6P) and to convert it into pyruvate via glycolysis <sup>5</sup>. Thus, the bacteria depend on G6P from the host cell. In EBs, ATP is generated from G6P either via glycolysis or oxidative phosphorylation, whereas in RBs ATP is provided by the host cell, while G6P is nearly exclusively used for cell wall biosynthesis <sup>6,7</sup>. The TCA cycle of *Chlamydia* is incomplete due to the absence of three key enzymes, citrate synthase, aconitase and isocitrate dehydrogenase. This incomplete metabolic pathway must be complemented by the constant metabolic supply from the host <sup>4</sup>. We have previously shown that *C. trachomatis* takes up host-derived malate to feed the partial TCA cycle <sup>6</sup>. In addition, amino acids have to be acquired from the host cells due to the limited capacity of *Chlamydia* to synthesize amino acids <sup>6</sup>. Despite the advancements in the knowledge about chlamydial metabolism, the processes initiating the transition of EBs to RBs after entry into cells are still unknown.

## Results

### Glutamine metabolism in *Chlamydia*: A pathway to peptidoglycan biosynthesis

In our attempts to further define the metabolic interaction of *Chlamydia* and host cells, we performed metabolite uptake assays in axenic culture. Since glutamine (Gln), glutamate (Glu) and  $\alpha$ -ketoglutarate ( $\alpha$ -KG) were previously predicted to feed the partial chlamydial TCA cycle <sup>4,6,8</sup>, we investigated whether these metabolites are directly taken up by *Chlamydia* (Fig. 1a,b).

87 Whereas the amount of Gln decreased rapidly in the supernatant, neither Glu nor  $\alpha$ -KG were  
 88 significantly consumed (Fig. 1c). Using isotopologue profiling with Gln containing only  $^{13}\text{C}$   
 89 instead of  $^{12}\text{C}$  atoms ( $[\text{U-}^{13}\text{C}_5]\text{Gln}$ ) added to the axenic culture, GC-MS analyses of chlamydial  
 90 extracts revealed that the  $^{13}\text{C}$  label is efficiently transferred to Glu, Asp and intermediates of the  
 91 partial TCA cycle, with the  $^{13}\text{C}$ -labelled fractions ranging from 80% in Gln to less than 3% in  
 92 pyruvate (Fig. 1d). Substantial fractions of  $^{13}\text{C}$ -labelling in Glu (60%), Asp (50%), succinate  
 93 (20%), fumarate (35%), and malate (8%) (Fig. 1d) indicated that exogenous Gln is metabolized  
 94 via glutaminolysis and the TCA cycle to generate Glu,  $\alpha$ -KG, succinate, fumarate, malate,  
 95 oxaloacetate and aspartate. Consistently, the amount of chlamydial NADH probably formed by  
 96 the conversion of  $\alpha$ -KG into succinate and malate into oxaloacetate within the truncated  
 97 chlamydial TCA cycle (Fig. 1a) is significantly increased when exogenous Gln is added to the  
 98 axenic medium (Extended data Fig. 1a).  
 99 Interestingly, significant  $^{13}\text{C}$ -labelling was also detected in Ala (17%) and the peptidoglycan  
 100 precursor, diaminopimelate (DAP) (41%) (Fig. 1d). Whereas Ala contained a high fraction of the  
 101 M+3 isotopologue (*i.e.* a molecule with three  $^{13}\text{C}$ -labelled carbon atoms), DAP contained M+4  
 102 as main compound, accompanied by M+2, M+3, and M+7 isotopologues (Fig. 1d). The detected  
 103 isotopologue composition of Ala can be explained by its formation from pyruvate derived from  
 104 oxaloacetate via PEP catalysed by PEP carboxykinase and pyruvate kinase; an alternative  
 105 could be the decarboxylation of Asp, although no gene with the required enzymatic activity is  
 106 annotated in the genome of *Chlamydia* (scheme in Extended data Fig. 1b). The labelling pattern  
 107 in DAP points at its formation from Asp and pyruvate contributing four and three labelled carbon  
 108 atoms, respectively, and resulting in the detected M+3, M+4 and M+7 isotopologues species  
 109 (Fig. 1d, Extended data Fig. 1b). Minor isotopologues especially M+2 in aspartate and DAP can  
 110 be explained by metabolic cycling *via* gluconeogenesis and the PPP, which both are active in  
 111 *Chlamydia*. To further support the unexpected finding of chlamydial DAP synthesis in axenic  
 112 media, we performed an approximate quantification of DAP (for details see Methods) and could  
 113 estimate a value of about 0.04 mg DAP formation per gram cells of *Chlamydia* in the presence  
 114 of Gln in axenic medium (Extended data Fig. 1c). We also supplied axenic cultures with Gln that

was either labelled in the amino group or the amido group. The  $^{15}\text{N}$ -label was only transferred from the amino group of Gln (Extended data Fig. 1d). Together, these results indicated that Gln serves as a major carbon source for the fuelling of the partial TCA cycle of *Chlamydia* and as a source of amino groups for the generation of amino acids from alpha-keto acids via transamination, e.g. Glu, Asp and Ala, required for peptidoglycan biosynthesis.

Synthesis of DAP in axenic medium was particularly intriguing since peptidoglycan synthesis is restricted to the replicative phase of *Chlamydia* and this has never been observed for *Chlamydia* outside their host cell <sup>2</sup>. We therefore analysed EBs from axenic cultures in the presence or absence of Gln by electron microscopy (EM). Surprisingly, incubation of EBs with Gln, but not Glu led to a gradual time-dependent morphological transition to an intermediate between EBs and RBs, so-called intermediate bodies (IBs) (Fig. 1e; Extended data Fig. 1e). The infectivity of EBs incubated with Gln dropped to 20% (Extended data Fig. 1f), supporting the conclusion that Gln induces the developmental transition of EBs to a non-infectious form.

We then synthesized a clickable form of the dipeptide DA-DA to detect peptidoglycan in these bacteria <sup>2</sup> (see Methods). Chlamydial particles incubated in the presence of Gln in this cell-free approach produced detectable peptidoglycan which could be quantified by FACS (Fig. 1f, g, Extended data Fig. 1g). Since peptidoglycan synthesis in *Chlamydia* has been shown to be associated with replication in host cells, we also investigated if Gln could initiate transcription in *Chlamydia* in axenic culture. RNA-seq analysis revealed activation of several genes upon exposure to glutamine, including those previously described as early genes <sup>9</sup> (Fig. 1h, Extended data Fig. 1h). This finding was also confirmed by quantitative PCR (qPCR) of several genes already reported to be expressed at early time points of EB to RB conversion <sup>10</sup> (Extended data Fig. 1i). Interestingly, in the presence of Gln, a significant increase in the copy number of chlamydial DNA was detected in axenic media (Fig. 1i), indicating that Gln access initiates limited replication in *Chlamydia*.

## ***Chlamydia* triggers altered glutamine metabolism in infected cells**

142 Since our data suggested uptake of Gln as a central node for the interaction between host and  
 143 bacterial metabolism, we investigated how *Chlamydia* infection influences the metabolism of  
 144 primary human umbilical vein endothelial cells (HUVECs). *Chlamydia* infection induced an  
 145 increased depletion of most amino acids from the culture medium, indicative of their enhanced  
 146 uptake by infected cells (Fig. 2a). Gln was one of the most depleted amino acids in the medium  
 147 (Fig. 2a) and Gln but not Glu uptake was significantly increased in infected cells (Fig. 2b,c).  
 148 We then performed isotope labelling experiments by adding [U-<sup>13</sup>C<sub>5</sub>]-Gln to the culture medium.  
 149 HUVECs were then infected with *Chlamydia* and incubated in this medium for 36 hours. LC-MS  
 150 analysis revealed the labelling pattern of Gln, Glu, Asp and several TCA cycle metabolites (Fig.  
 151 2d). We also observed evidence for reductive carboxylation of  $\alpha$ -KG by the host cells as there  
 152 was a substantial amount of the M+5 isotopologue of citrate. In contrast, Gln-derived labelling  
 153 of pyruvate was almost absent (Fig. 2d), indicating that the enzymes of gluconeogenic pathway  
 154 responsible for the conversion of oxaloacetate to pyruvate are inactive under these conditions.  
 155 Interestingly, while the labelled fraction of Gln did not change upon infection, the M+5 labelled  
 156 isotopologues of Glu and  $\alpha$ -KG increased, indicating increased glutaminolysis in infected cells  
 157 (Fig. 2d). Moreover, the M+4 isotopologues of succinate, fumarate, malate and aspartate also  
 158 increased following *Chlamydia* infection, most likely due to enhanced entry of Gln-derived  
 159 carbons into the TCA cycle (Fig. 2d).  
 160 To analyse if *Chlamydia* obtain building blocks for DAP biosynthesis from the host cell, we  
 161 performed stable isotope labelling using [U-<sup>13</sup>C<sub>5</sub>]-Gln and extracted bacterial cell wall  
 162 components by acidic hydrolysis. LC-MS detection of DAP confirmed that glutamine-derived  
 163 carbons are indeed incorporated into bacterial biomolecules essential for *Chlamydia*  
 164 proliferation (Fig. 2e,f). Moreover, the high proportion of the M+4 isotopologue in DAP suggests  
 165 that *Chlamydia* either takes up glutamine directly from the host cells and uses it to produce Asp  
 166 via its truncated TCA cycle, or that glutamine is first converted by the host cell into metabolic  
 167 intermediates that contain four <sup>13</sup>C carbon atoms (*i.e.* succinate, fumarate, malate, oxaloacetate  
 168 or Asp), which are then taken up by *Chlamydia* and used for the synthesis of DAP (Fig. 2d-f,  
 169 diagram in Fig. 2e). Moreover, the high abundance of the M+1, M+2 and M+3 isotopologues in

DAP also indicates that *Chlamydia* uses metabolic intermediates that are formed by the complete TCA cycle of the host cell. Production of these isotopologues depends on the second and third round of the TCA cycle, which requires the activity of citrate synthase, aconitase and isocitrate dehydrogenase present only in the host metabolism (see diagram in Extended data Fig. 2). Together, these results clearly demonstrate that *Chlamydia*-infected cells increase uptake and metabolism of several amino acids, particularly Gln which provides the metabolic intermediates required for bacterial peptidoglycan production.

### ***Chlamydia* infection increases c-Myc levels**

We next focused on how infected cells compensate for the increased Gln demand. RNA-seq analysis and Gene Set Enrichment Analysis (GSEA, see Methods) including subsequent validation by qPCR revealed upregulation of MYC target genes (Hallmark\_MYC\_Targets\_V2) in cells infected with *Chlamydia* (Fig. 3a; Extended data Fig. 3a). The proto-oncogene *c-MYC* is also known as a 'master regulator' of cellular metabolism<sup>11</sup>, in particular mitochondrial glutamine metabolism<sup>12,13</sup>. In agreement with previous data<sup>14</sup>, c-Myc levels were strongly increased already 12 hours post infection (hpi) and remained elevated up to 36 hpi in primary epithelial cells from human or mouse fimbriae (Fimb cells) (Extended data Fig. 3b,c), and human osteosarcoma U2OS cells (Extended data Fig. 3d). Similar results were obtained upon infection with different *Chlamydia* species (Extended data Fig. 3e-g).

Further investigation unveiled a transient upregulation of c-Myc mRNA expression in response to *Chlamydia* infection (Extended data Fig. 3h) and the accumulation of c-Myc protein in the nucleus (Extended data Fig. 3i,j). In addition, c-Myc was phosphorylated at the conserved residues serine 62 (S62) and threonine 58 (T58) (Fig. 3b) involved in the control of c-Myc protein stability<sup>15</sup> in response to mitogenic signalling<sup>16</sup> and *Chlamydia* infection<sup>14</sup>. Indeed, we found markedly reduced levels of ubiquitinated c-Myc (Fig. 3c) and stabilized c-Myc in infected cells with inhibited translation (Fig. 3d), demonstrating that c-Myc is really stabilized upon infection.

### **c-Myc is stabilized in *Chlamydia*-infected cells via MAPK and PI3K signalling pathways**

MAPK and PI3K pathways activated during infection are both critical for chlamydial development<sup>17-19</sup>. The same pathways have been demonstrated to function in the stabilization of c-Myc (Fig. 3e)<sup>20</sup>. Inhibition of MAPK or PI3K pathways using specific inhibitors indeed prevented the up-regulation of c-Myc protein levels and attenuated the propagation of the bacteria in infected cells (Fig. 3f,g). Interestingly, the downstream effector GSK3 $\beta$  (Fig. 3e) was phosphorylated at S9 in infected cells (Fig. 3h,i), a modification known to inhibit glycogen synthesis and to increase free glucose in the cell<sup>21</sup>.

Intriguingly, *Chlamydia* primary infection (Fig. 4a-c; AHT control in Extended data Fig. 4a) and infectivity (Extended data Fig. 4b,c) was rescued in U2OS<sup>Tet-On</sup> cells upon anhydrous tetracycline (AHT)-induced expression of c-Myc<sup>22</sup> despite inhibition of the MAPK or PI3K pathway, indicating that the anti-chlamydial activity of U0126 and Ly294002 is mediated by the down-regulation of c-Myc. The central role of c-Myc for chlamydial growth was further supported by experiments with the chemical c-Myc inhibitor 10058-F4 (Fig. 4d, Extended data Fig. 4d) and the silencing of c-Myc expression (Fig. 4e,f; Extended data Fig. 4e,f).

### ***Chlamydia* depends on host cell Gln uptake**

GSEA of the RNA-seq data also revealed a strong influence of *Chlamydia* infection on the host metabolite transporter and cellular amino acid and amine metabolic pathway (Extended data Fig. 5a-c). Gln turned out to be essential for the intracellular growth of *Chlamydia*, since they failed to form inclusions and replicate in host cells cultured in medium without Gln (Fig. 5a,b and Extended data Fig. 5d). Bacteria from these cultures did not initiate infections in fresh cells in the presence of Gln, demonstrating that no infectious progeny were produced in the absence of Gln (Fig. 5c,d, Extended data Fig. 5e). To investigate if Gln plays a role in EB to RB conversion in host cells, we used the EB-RB reporter strain Ct mCh(GroL2) GFP(OmcAL2)<sup>23</sup>. In the absence of Gln, *Chlamydia*, did not convert into the actively replicating RB form, with low GFP expression and constant mCherry fluorescence (Fig. 5e).

c-Myc can function in a glutamine sensing pathway via a mechanism dependent on the 3' UTR of the gene<sup>24</sup>. We used HCT116 cells expressing the MYC-ER fusion protein from a construct



lacking the 3'UTR, which allows the restoration of c-Myc expression in Gln-deprived cells. However, Gln depletion severely attenuated the growth and development of *Chlamydia* (Fig. 5f,g), and even complementing with downstream metabolites like nucleosides did not compensate for the lack of Gln (Extended data Fig. 5f), indicating that c-Myc cannot rescue chlamydial growth in the absence of Gln.

### ***Chlamydia* infection reprograms host cell metabolism by inducing glutamine uptake and catabolism**

The data obtained so far suggested that Gln is also a central host-derived amino acid metabolised by fast replicating *Chlamydia*. We therefore investigated the regulation of genes related to glutamine uptake and catabolism. These analyses revealed SLC1A5/ASCT2<sup>25</sup> among the top upregulated genes during infection (Fig. 6a). SLC1A5/ASCT2 and glutaminase (GLS1), one of the enzymes converting Gln to Glu (Fig. 6b), have been shown to be c-Myc target genes induced to enhance glutamine uptake and catabolism in cancer cells<sup>12,26</sup>.

Western blots of control and *Chlamydia*-infected samples revealed a 5.2-fold up-regulation of SLC1A5 and a 1.8-fold up-regulation of GLS1 (Fig. 6c). Induction of SLC1A5 depended on viable bacteria since heat-killed *Chlamydia* neither induced c-Myc nor SLC1A5 (Extended data Fig. 6a). Furthermore, depletion of SLC1A5 or GLS1 (Fig. 6d,e) or chemical inhibition of SLC1A5 or GLS1 using GPNA and BPTES (Fig. 6f,g) drastically reduced chlamydial replication. Since SLC1A5 and GLS1 are transcriptional targets of c-Myc<sup>12,27</sup>, we knocked down c-Myc with siRNA, which resulted in strong depletion of both proteins and also blocked chlamydial growth (Fig. 6h).

Most interestingly, when we depleted c-Myc in cells overexpressing SLC1A5 we could retain *Chlamydia* growth during the primary infection, but the progeny was not infectious (Fig. 6i and Extended data Fig. 6b), in line with a role of Gln in establishing the primary infection (Fig. 1e). However, other c-Myc regulated host cell functions, for example glutaminolysis and the production of TCA cycle intermediates, may be essential for the full development of *Chlamydia*.

Taken together, these data demonstrate the importance of an infection-dependent regulation of c-Myc and its targets SLC1A5 and GLS1 in the reprogramming of the Gln metabolism in host cells to promote *Chlamydia* replication and development.

Recently, *Slc1a5* knockout mice have been generated which show no major phenotype<sup>28</sup>. Mass spectrometric analysis revealed that organoids generated from fallopian tube tissue from these mice contain almost 50% lower levels of Gln and the downstream metabolites Glu, Asp and Ala compared to organoids from wild type mice (Extended data Fig. 6c). Interestingly, trans-cervical infection of these mice revealed a significant growth defect of *C. trachomatis* and the mouse pathogenic strain *C. muridarum* in the absence of *Slc1a5* (Fig. 6j,k). Moreover, infection and progeny formation were considerably reduced in fallopian tube organoids developed from *Slc1a5* KO mice (Fig. 6l, Extended data Fig. 6d), demonstrating that cells of *Slc1a5* KO mice have reduced capacity to allow chlamydial replication. Further experiments are required to demonstrate that the strong phenotype observed on chlamydial growth in these organoids depends on disturbed reprogramming of glutamine metabolism. These data suggest that limiting the uptake of the non-essential amino acid glutamine could serve as a therapeutic approach against *Chlamydia* infection.

## Discussion

After the completion of the developmental cycle EBs released from the host cell have to avoid the transition to RBs since only EBs are capable of entering uninfected host cells and initiate a new generation of progeny. The longstanding view that EBs are metabolically inactive has recently been revised, since it was found that EBs kept in axenic medium in the presence of G6P, a metabolite available to *Chlamydia* only inside host cells, are able to generate ATP via glycolysis<sup>7</sup>. However, despite this active metabolism, EBs do not initiate the conversion to RBs under these conditions. Here we provide evidence that Gln is the key metabolite that initiates peptidoglycan synthesis and the EB to RB transition. While the concentration of Gln in uterine fluid is as low as 0.13 mM<sup>29</sup>, in the cytoplasm of mammalian cells Gln can reach levels of 2 to 30 mM<sup>30</sup>. The strong differences in Gln concentrations between the outside and the inside of host cells strongly support a function of Gln as a metabolic trigger for EB to RB transition.

Our finding that L-glutamine serves as a crucial amino acid for the replication of *Chlamydia* corroborates previous findings<sup>31,32</sup>. Many of the glutamine-derived intermediates in *Chlamydia* serve as precursors for peptidoglycan biosynthesis (Fig. 1d and Extended data Fig. 1b) underlining the central role of glutamine for the chlamydial metabolism. Chlamydiae do not form a peptidoglycan sacculus typical for other Gram-negative bacteria but only assemble peptidoglycan rings in the mid-cell of actively dividing RBs. We detected the accumulation of peptidoglycan here also in *Chlamydia* outside of host cells but only in the presence of Gln (Fig. 1f, g), where we also observed a change in the morphology of the EBs (Fig. 1e, Extended data Fig. 1e). Peptidoglycan accumulated in or close to the bacteria and was not assembled as a ring which could indicate that the crosslinking of the peptidoglycan disaccharide pentapeptide did not occur in this setup. The transcriptional activation of early genes and the increase in the copy number of the bacterial genomes (Fig. 1h,i), however, indicated that *Chlamydia* already initiate the start of the replication machinery.

*Chlamydia* and other obligate intracellular bacteria replicate in differentiated cells that run the reduced metabolism of non-dividing cells. Since all metabolites of replicating *Chlamydia* originate from the host cell, the metabolism of the cell must drastically change to meet the requirements of the infection. Glutamine is one of the most abundant amino acids in serum and fast-growing cells take up glutamine to support anabolic metabolism at multiple nodes. We show here that *Chlamydia* depend on the direct uptake of glutamine as well as on glutamine-derived host metabolites and therefore reprograms the host cell by stabilizing the central metabolism regulator c-Myc. The profile of c-Myc regulated genes and the dependence of the replicating *Chlamydia* on glutaminolysis parallels the reprogramming of dormant cells to fast proliferating and in particular tumour cells<sup>33</sup>. c-Myc promotes glutamine uptake and glutaminolysis by increasing the expression of the glutamine transporters ASCT2/SLC1A5 and SNAT5/SN2 and of glutaminase (GLS)<sup>13</sup>. We demonstrate here that bacteria induce host cell glutamine uptake and glutaminolysis for their replication. In addition, chlamydial growth was severely affected in genital infections of ASCT2/SLC1A5 knockout mice and is thereby the demonstration of a dominant role of host cell glutamine reprogramming in an *in vivo* infection. Gln is channelled into

different anabolic and catabolic pathways via glutaminolysis, generating Glu and subsequently  $\alpha$ -KG. While Gln-derived metabolic intermediates have been shown to be essential for the infection and replication of several viruses<sup>34-39</sup>, the relevance of glutamine metabolism for viral infection still has to be demonstrated *in vivo*.

In addition to glutamine transporters and glutaminase, we found other prominent c-Myc-regulated amino acid transporters like SLC43A1, SLC7A11, and SLC7A1 are also upregulated during infection (Fig. 6a). This global reprogramming of amino acid supply in infected host cells by c-Myc stabilization may explain our intriguing finding that overexpression of SLC1A5 can only partially rescue chlamydial infection in c-Myc-depleted cells. While SLC1A5 restores bacterial replication, it fails to support the production of infectious progeny in the absence of c-MYC. Thus, the provision of glutamine is essential, but not sufficient to permit the complex cycle of chlamydial replication and development.

The central addiction of *Chlamydia* to host glutamine is reminiscent of mammalian cell proliferation, malignant transformation and therefore a hallmark of cancer cells which use extracellular glutamine to fulfil the metabolic demands of producing cell mass. Glutamine addicted tumour cells and, as we show here, *Chlamydia*-infected cells are therefore highly sensitive to pharmacological disruption of glutamine metabolism. Current approaches to target c-Myc or glutamine metabolism as targets for innovative cancer therapy may also prove to be efficient in treating *Chlamydia* infection.

## Methods

### *Chlamydia* strains used in the study

*Chlamydia trachomatis* (serovar L2/434/Bu and D) were used in this study. Some experiments were also performed using *Chlamydia muridarum* and *Chlamydia pneumoniae*. *Chlamydia* were prepared as previously published. Briefly, *Chlamydia* were grown in HeLa229 cells (ATCC® CCL2.1™) at an MOI (multiplicity of infection) of 1 for 48 hours (h). The cells were lysed using glass beads (15 mm) for 3 minutes (min) and centrifuged at 2,000 g for 10 min to remove the cell debris. The supernatant containing bacteria was collected and centrifuged at 24,000 g

for 30 min at 4°C. The pellet was washed and resuspended in SPG buffer (0.25 M sucrose/10 mM sodium phosphate/5 mM glutamic acid), aliquoted and stored at -80°C. *Chlamydia* EBs and cell lines used in the study were verified to be free of Mycoplasma contamination via PCR. The bacteria were titrated and were used at an MOI of 1 in all experiments unless other stated. After 1 hpi the media was replaced with fresh RPMI containing 5% FCS, infected cells were cultured at 37°C and 5% CO<sub>2</sub>.

#### **Culture of *C. trachomatis* in axenic medium**

*Chlamydia trachomatis* L2 was propagated in HeLa229 cells, isolated, purified and incubated in different axenic media as previously described <sup>6</sup>. In brief, HeLa229 cells were seeded in T175 flasks and infected at an MOI of one. Forty-eight hours post infection, cells were scraped off, disrupted with glass beads and EBs were purified using 60-20% Renografin gradient (Meglumini diatrizoate (Sigma-Aldrich M5266), Sodium diatrizoate hydrate (Sigma-Aldrich S4506), Sodium citrate hydrate (Applichem A4522), EDTA (Servca 11280) add to 50 ml HBSS (Gibco 14025-050) pH-7.4, sterile filtered in 0.2 µm filter and stored at 4°C). EBs were resuspended in axenic media (basic DMEM (Sigma-Aldrich) supplemented with sodium bicarbonate (44 mM), phenol red (42 µM) and glucose-6-phosphate (0.5 mM) and incubated for respective time points at 37°C. According to the experimental set-up, 1mM of L-glutamine / L-glutamate / pyruvate / α-ketoglutarate / [U-<sup>13</sup>C<sub>5</sub>] glutamate / [U-<sup>13</sup>C<sub>5</sub>] glutamine / [<sup>15</sup>N-amine] glutamine or [<sup>15</sup>N-amide] glutamine was added to the media. After incubation, samples were centrifuged for 30 min at 21,500 g at 4°C and supernatant was transferred to a new tube. Supernatant and pellets were heat-inactivated (10 min at 90°C) and stored at -80°C for further analysis.

#### **Substrate uptake analysis from axenic culture**

##### **a. α-ketoglutarate:**

0.2 ml of the supernatant from the axenic medium was spiked with 20 µl of a 5 mM norvaline solution (internal standard) and dried under N<sub>2</sub> flux. The residue was treated with 50 µl methoxyamine in pyridine (20 mg/ml) at 40°C for 90 min, and subsequently with 50 µl *N*-(*tert*-butyldimethylsilyl)-*N*-methyl trifluoroacetamide containing 1% *tert*-butyldimethylsilyl chloride (MTBSTFA) at 70°C for 30 min. This solution was taken for analysis.

364 **b. Glutamate and glutamine**

365 0.1 ml of the supernatant from the axenic medium containing [U-<sup>13</sup>C<sub>5</sub>] glutamate or [U-<sup>13</sup>C<sub>5</sub>]  
366 glutamine was spiked with 20 µl of a 5 mM non-labelled glutamate or glutamine solution (internal  
367 standard) and dried under N<sub>2</sub> flux. The residue was treated with 50 µl acetonitrile and 50 µl  
368 MTBSTFA at 70°C for 30 min and taken for analysis.

369 **Isotopologue profiling with *Chlamydia* from axenic culture:**

370 Bacterial pellets were suspended in 1 ml of methanol and were mechanically disrupted using a  
371 ribolyser (3 x 20 sec 6.5 m/s). Afterwards the solution was centrifuged (10,000 g for 20 min,  
372 4°C). This procedure was performed twice. The supernatants were combined and then dried  
373 under N<sub>2</sub> flux. The residue was treated with 50 µl of MTBSTFA and 50 µl of water free acetonitrile  
374 at 70°C for 30 min. The *tert*-butyldimethylsilyl (TBDMS)-derivatives of amino acids and other  
375 metabolites were then analysed by GC/MS. The residual cell debris after centrifugation was  
376 subjected to acidic hydrolysis as described earlier <sup>40</sup> and protein bound amino acids as well as  
377 diaminopimelate (DAP; retention time, 24.48 min; m/z 589) were analysed as TBDMS  
378 derivatives.

379 **GC/MS conditions**

380 All derivatives mentioned above were analysed by GC-MS using a GCMS-QP 2010 Ultra  
381 spectrometer (Shimadzu, Duisburg, Germany) equipped with a Equity<sup>TM</sup>-5, fused silica capillary  
382 column, 30 m x 0.25 mm x 0.25 µm film thickness. All data were collected using LabSolution  
383 software (Shimadzu). The samples were analysed three times as technical replicates. The  
384 overall <sup>13</sup>C excess (mol-%) and the relative contributions of isotopomers (%) were computed by  
385 an Excel-based in-house software package according to published procedures <sup>41</sup>.

386 **TBDMS-derivatives of polar metabolite mixtures**

387 The column was first developed at 100°C for 2 min, then using a gradient of 3°C min<sup>-1</sup> to 234°C,  
388 followed by 1°C min<sup>-1</sup> to 237°C and 3°C min<sup>-1</sup> to 260°C. Finally, the column was heated at a  
389 gradient of 10°C min<sup>-1</sup> to a final temperature of 320°C where it was hold for 2 min.

390 **Analysis of TBDMS-amino acids and DAP**

391 The column was first developed at 150°C for 3 min, then using a gradient of 7°C min<sup>-1</sup> to 280°C  
392 where it was hold for 5 min. For quantitative DAP analysis in *Chlamydia* DAP DL-2,6-  
393 Diaminopimelic acid (Sigma Aldrich No. 92591) was used as standard for the calibration.  
394 Chlamydial DAP synthesis in axenic media was performed by an approximate quantification of  
395 DAP on the basis of peak integrals of the samples used for isotopologue profiling with <sup>13</sup>C<sub>5</sub>-Gln  
396 (Fig. 1d). This analysis revealed that the preparation contained approximately 0.1 mg DAP per  
397 gram wet weight of *Chlamydia*. The newly built DAP is highly labelled and the overall <sup>13</sup>C excess  
398 of 40% shown as a mixture of newly built <sup>13</sup>C labelled DAP and residual unlabelled DAP from  
399 the inoculum. On the basis of 40% newly formed DAP, we could estimate a value of about 0.04  
400 mg DAP formation per gram cells of *Chlamydia* in the presence of Gln in axenic medium  
401 (Extended data Fig. 1c).

#### 402 **Transmission electron microscopy**

403 Chlamydial EBs were incubated with the axenic medium with G6P and with or without Gln. The  
404 bacteria pellet was fixed with 2.5% glutaraldehyde (50 mM sodium cacodylate (pH 7.2), 50 mM  
405 KCl, 2.5 mM MgCl<sub>2</sub>) at room temperature. The cells were incubated for 2 h at 4°C with 2%  
406 OsO<sub>4</sub> buffered with 50 mM sodium cacodylate (pH 7.2), washed with distilled H<sub>2</sub>O and  
407 incubated overnight at 4°C with 0.5% uranyl acetate (in distilled H<sub>2</sub>O). The cells were  
408 dehydrated, embedded in Epon812 and ultrathin-sectioned at 50 nm. Sections were stained  
409 with 2% uranyl acetate in ethanol followed by staining with lead citrate and analysed in a  
410 Zeiss EM10 microscope (Zeiss). Electron micrographs were processed using ImageJ (Fiji).

#### 411 **Click chemistry and FACS analysis of chlamydial EBs**

412 Click chemistry was performed as described in <sup>2</sup>. Axenic culture with purified EBs from  
413 *Chlamydia* was fed with ADA-DA (10 µM). Clickable Alexa Fluor 532-azide and Click-iT® Cell  
414 Reaction Buffer Kit were purchased from Invitrogen and fixed with 4% PFA. The bacteria were  
415 stained with cHsp60 antibody and subjected to microscopy or further analysed by FACS  
416 (Accuri).

#### 417 **Copy number of *Chlamydia* genomes in axenic culture**

418 *Chlamydia* were grown in HeLa229 for 48 h. The cells were lysed and the EBs were purified by  
419 renografin gradient separation as explained above. The bacteria were pooled by centrifugation  
420 and re-suspended in axenic medium without Gln. The re-suspended *Chlamydia* were split into  
421 two aliquots and Gln was added into one of them and incubated at 35°C for 24 h. The bacteria  
422 were further pelleted and DNA was isolated using DNAzol reagent (Thermo Fisher Scientific).  
423 Quantitative PCR was used to enumerate *Chlamydia* genome copy number. The following  
424 primers were used for amplifying the *C. trachomatis* *lytA* gene that was cloned into the vector:  
425 forward primer 5'-TCTAAAGCGTCTGGTGAAAGCT-3' and reverse primer 5'-  
426 GAAATAGCGTAGTAATAATACCCG-3'. Data were analysed by using the Step One Plus  
427 software package (Applied Biosystems). GraphPad Prism 7 was used to generate the graph.

#### 428 **Cell culture and transfection**

429 HeLa229 were used for propagating bacteria and for basic experiments. Epithelial cells isolated  
430 from human fimbriae (Fimb cells), U<sub>2</sub>OS (ATCC® HTB-96™), HCT116 (ATCC® CRL-247™) and  
431 HUVECs (ATCC® CRL-1730™) were also used in the study. HUVECs were used in the high  
432 throughput RNA sequencing. All cell lines were tested negative for mycoplasma contamination  
433 via PCR. HeLa229 and human Fimb cells were grown in RPMI1640 + GlutaMAX™ (Gibco™  
434 72400-054) with 10% heat inactivated FCS (Sigma-Aldrich F7524). U<sub>2</sub>OS and HCT116 cells  
435 were cultured in DMEM (Sigma-Aldrich D6429) with 10% heat inactivated FCS. HUVECs were  
436 cultured in Medium 200 (Gibco™ M200500) containing 1x LSGS (Gibco™ S00310). For  
437 glutamine deprivation experiments, the cells were first seeded in RPMI1640 + GlutaMAX™ or  
438 DMEM, high glucose (Sigma-Aldrich D6429). The following day the medium was changed to the  
439 basic formulation of DMEM (Sigma-Aldrich D5030) supplemented with 5% dialyzed FCS  
440 (Sigma-Aldrich F0392), 1 or 4.5 g/l D-glucose (for HeLa229/ U<sub>2</sub>OS or human Fimb respectively)  
441 and varying concentrations of L-glutamine (according to experimental setup). All cell lines  
442 obtained from ATCC were authenticated by the company.

443 Cells were transfected with plasmid DNA at a confluency of 60% with Polyethylenimine (PEI) or  
444 X-treme GENE™ HP DNA transfection reagent (Roche) and OptiMEM transfection medium  
445 (Gibco) in 5% FCS medium. After 5 h, transfection medium was replaced by fresh RPMI



supplemented with 5% FCS medium. The plasmids used in the study are described in the Extended data file. siRNA against SLC1A5 (sc-60210) and c-Myc (sc-29226) was obtained from Santa cruz Biotech.

### **Metabolic profiling**

For this study HUVECs were seeded in triplicates, either uninfected or infected with *C. trachomatis* serovar L2 for 36 hours. After the respective time medium was collected, snap frozen in liquid nitrogen, and the cells were washed with ice cold 154 mM ammonium acetate (Sigma) and snap frozen in liquid nitrogen. The cells were harvested after adding 480  $\mu$ l cold MeOH/H<sub>2</sub>O (80/20, v/v) (Merck) to each sample containing Lamivudine (Sigma) standard (10  $\mu$ M). The cell suspension was collected by centrifugation and transferred to an activated (by elution of 1 ml CH<sub>3</sub>CN (Merck)) and equilibrated (by elution of 1 ml MeOH/H<sub>2</sub>O (80/20, v/v)) RP18 SPE-column (Phenomenex). The eluate was collected and evaporated in a SpeedVac concentrator. The residue was dissolved in 50  $\mu$ L of 5mM NH<sub>4</sub>OAc in CH<sub>3</sub>CN/H<sub>2</sub>O (25/75). Each sample was diluted 1:2 (cells) or 1:5 (medium) in CH<sub>3</sub>CN. 5  $\mu$ l of sample was applied to HILIC column (Acclaim Mixed-Mode HILIC- 1, 3  $\mu$ m, 2.1 \* 150 mm). Metabolites were separated at 30°C by LC using a DIONEX Ultimate 3000 UPLC system (Solvent A: 5 mM NH<sub>4</sub>OAc in CH<sub>3</sub>CN/H<sub>2</sub>O (5/95), Solvent B: 5 mM NH<sub>4</sub>OAc in CH<sub>3</sub>CN/H<sub>2</sub>O (95/5); Gradient: linear from 100% B to 50% B in 6 min, followed by 15 min const. 40% B). MS-Analysis was done on a Thermo Scientific QExactive instrument in alternating positive and negative mode. Peak determination and semi-quantitation were performed using TraceFinder™ Software. For determination of protein content for the data normalization, BCA assay (Thermo Fisher Scientific) was performed. The pellet of the cell samples was dried, resuspended in 0.2 M sodium hydroxide (Roth), boiled for 20 min at 95°C and absorbance was measured at 550 nm. Prism GraphPad was used for statistical analysis.

### **Western blotting and antibodies**

Lysates for Western blot analysis were prepared by directly lysing cells in SDS sample buffer (62.5 mM Tris, pH 6.8, 2% SDS, 20% glycerol and 5%  $\beta$ -mercaptoethanol) in ice. Western blot analysis was performed as described<sup>42</sup>. Briefly protein samples were separated in the 6-12%

474 SDS-PAGE (Peqlab) and transferred to a PVDF membrane (Roche) in a semidry electroblotter  
475 (Thermo Fisher Scientific). The membrane was further blocked in tris buffer saline containing  
476 0.05% Tween20 and 5% bovine serum albumin or dry milk powder. The primary antibody  
477 against c-Myc (Y69: ab-32072), pc-Myc Thr58 (ab-28842) and pc-Myc Ser62 (ab-51156)  
478 SLC1A5 (ab-84903) glutaminase (ab-156876) was purchased from Abcam. The T-ERK (cs-  
479 9180), pERK (cs-9106), T-AKT (cs-9272), pAKT Ser473 (cs-9271), were obtained from Cell  
480 Signaling. Chlamydial HSP60 (sc-57840) and anti-ubiquitin (sc-8017) antibody was purchased  
481 from Santa Cruz Bioscience and  $\beta$  Actin antibody from Sigma (A5441). Proteins were detected  
482 with secondary antibodies coupled with HRP (Santa Cruz Bioscience) using ECL system  
483 (Pierce) and Intas Chem HR 16-3200 reader. Quantification of blots was done by FIJI (ImageJ)  
484 software.

#### 485 **Immunoprecipitation**

486 Uninfected and *Chlamydia*-infected (MOI 1) HeLa229 cells were lysed using denaturing buffer  
487 (RIPA lysis buffer: 50mM Tris-HCl pH-7.5, 150mM NaCl, 1% Triton-X100, 1% NP-40, 0.1% SDS,  
488 10% glycerol containing Complete protease inhibitor cocktail (Roche) and MG-132, proteasome  
489 inhibitor) to prevent co-precipitation of interacting partners of c-Myc. Lysates from  $7 \times 10^6$  cells  
490 were prepared as described before and incubated with 3  $\mu$ g anti-c-Myc antibody for 1 h at 4°C  
491 followed by incubation with protein G magnetic beads (Dynabeads, Thermo Fisher Scientific)  
492 for 2 h at 4°C. The samples were washed several times and eluted by addition of 2x SDS-sample  
493 buffer and heating to 94°C. Samples were separated with SDS-PAGE and visualized by  
494 immunoblotting after probing against anti ubiquitin antibody.

#### 495 **Nuclear-cytoplasmic isolation**

496 HeLa229 cells were plated in 150 mm dishes and either left uninfected or infected with  
497 *Chlamydia* (MOI 1) for the mentioned period of time. The cells were washed with ice cold PBS.  
498 The cells were scraped into a falcon. The cells were centrifuged and resuspended in buffer  
499 containing 10 mM Hepes-KOH pH 7.9, 10 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.5 mM DTT, 0.05% NP-40,  
500 protease inhibitors and incubated for 20 min in ice. The cells were then homogenized with a  
501 dounce homogenizer (10 strokes). The cells were centrifuged at 4,000 RPM for 5 min at 4°C,

the supernatant containing cytoplasmic proteins was collected and lysed with 2x SDS-sample buffer. The pellet was resuspended in buffer containing 20 mM Hepes-KOH pH 7.9, 400 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 15% glycerol, 0.5 mM DTT, protease inhibitors and incubated for another 20 min and vortexed. Further the sample was centrifuged at 14000 RPM for 10 min at 4°C, the pellet containing the nuclear extract was lysed in 2x SDS-sample buffer and analysed using Western blot.

### **Immunofluorescence analysis**

The immunostaining was performed as described earlier. Briefly, HeLa229 and HUVEC cells were grown on cover slips and infected with the indicated *C. trachomatis* strain at an MOI 1 for indicated time points. The cells were washed with PBS, fixed with 4% PFA/Sucrose and permeabilized with 0.2% Triton-X-100/PBS for 30 min. Samples were blocked with 2% FCS/PBS for 1 h. All primary antibodies were incubated for 1 h at room temperature. Primary antibodies were used in the following dilutions in 2% FCS/PBS: anti-Hsp60 (1:500), c-Myc (1:200). Samples were washed three times and incubated with a Cy2-/Cy3-/Cy5-conjugated secondary antibody for 1 h in the dark. The cells were mounted on microscopic slide using Mowiol. Slides were air dried for at least 24 h and examined using Leica DM2500 fluorescence microscope, the images were analysed using LAS AF and Image J software.

### **Inhibitor studies**

HeLa229 cells were grown in the RPMI1640 +GlutaMAX™ or in the basic DMEM medium supplemented with 5% dialysed FCS and 1 g/l of D-Glucose and 4 mM L-glutamine. The cells were treated with 30 μM MAPK inhibitor UO126 (Cell Signaling Technology), 30 μM PI3K inhibitor LY297004 (Cell Signaling Technology), the cell-permeable thiazolidinone c-Myc inhibitor 10058-F4 (Sigma-Aldrich), 5 μM glutaminase inhibitor BPTES (Sigma-Aldrich) or the 5 mM SLC1A5 inhibitor GPNA (Sigma-Aldrich) for the respective period of time. In general, cells were infected with *Chlamydia* at an MOI 1 for different time points. The vehicle used for each inhibitor was used in the appropriate concentration. The cells were lysed and analysed using Western blotting.

### **RNA Sequencing and NGS Data Analysis**

530 **Differential gene expression from infected host cells:** Total RNA was isolated from  
531 uninfected or infected cells (12, 24 and 36 h post infection) and was used for RNA sequencing  
532 (RNA seq). Libraries for RNA Seq were generated using NEBNext® Ultra™ RNA Library Prep  
533 Kit for Illumina® with 12 PCR cycles for amplification and sequenced on Illumina NextSeq500  
534 platform. FASTQ generation was carried out using CASAVA and the quality check was  
535 performed using FastQC. Reads from FASTQ files were aligned to hg19 genome using Bowtie2  
536 and differential gene regulation calculation was carried out based on edgeR algorithm. The gene  
537 expression changes between infected and uninfected cells were normalized to library size were  
538 used for carrying out Gene Set Enrichment Analysis (GSEA) using weighted parameters on C2,  
539 C5 and Hallmark gene sets downloaded from MSigDB database <sup>43</sup>. In this analysis, the nominal  
540 p-value is calculated using an empirical phenotype-based permutation test procedure. The  
541 permutation-based false-discovery rate (FDR) Q value is generated by correcting for gene set  
542 size and multiple hypothesis testing. Heat map for Fig 6a was generated using heat map  
543 package in R with default settings.

544 **Differential gene expression of *Chlamydia* in axenic culture:** Renografin purified *Chlamydia*  
545 were cultured either in DMEM media (without Glucose/G6P without Gln) or in DMEM with only  
546 G6P or with DMEM with G6P and Gln for 12 h at 35°C. The bacteria were pelleted by  
547 centrifugation at 12,000 g for 10 min at 4°C. Total RNA was isolated from the bacteria and was  
548 used for RNA sequencing (RNA-seq). Libraries for RNA-seq were generated using  
549 NEBNext® Ultra™ RNA Library Prep Kit for Illumina® with 12 PCR cycles for amplification and  
550 sequenced on IlluminaNextSeq500 platform. FASTQ generation was carried out using GALAXY  
551 platform (DKFZ, Heidelberg) and the quality check was performed using FastQC. Reads from  
552 FASTQ files were aligned to the *C. trachomatis* D serovar 434/Bu genome using alignment with  
553 HISAT2. Differential gene regulation calculation was carried out based on feature Counts  
554 algorithm. The differential gene expression heat map was created with Graph pad 7.

#### 555 **Real Time PCR**

556 RNA was isolated from uninfected and *Chlamydia*-infected HeLa229 cells using RNA easy kit  
557 (Qiagen, Germany). RNA was reverse transcribed using a Revert Aid First Strand synthesis Kit

558 (Fermentas) according to the manufacturer's instructions and diluted 1:10 with RNase free  
559 water. qPCR was performed as previously described <sup>42</sup>. Briefly, qPCR reactions were prepared  
560 with Quanta SYBR (Quanta Bio) and PCR was performed on a Step One Plus device (Applied  
561 Biosystems). Data was analysed using  $\Delta$ Ct method, Step One Plus software package (Applied  
562 Biosystems) and Excel (Microsoft). Endogenous control was GAPDH. Primers were designed  
563 by q Primer Depot. The details of the primer are listed in the table in Extended data file.

#### 564 **Transcervical mouse infections and determination of bacterial burden**

565 ASCT2/SLC1A5 KO mice generated in a C57BL/6 background were obtained from the  
566 Australian National University (ANU) <sup>28</sup>. All animal experiments were performed in accordance  
567 with protocols approved by animal care and experimentation of German Animal Protection  
568 Law approved under the Animal (Scientific Procedures) Act 1986 (project license 55.2-2532-  
569 2-762). The mice used for experiment were between 10-14 weeks old. Five days before  
570 transcervical infection, mice were treated subcutaneously with 2.5 mg of DepoProvera  
571 (medroxy-progesterone acetate). The mice were transcervically infected with either  
572  $1 \times 10^7$  infection-forming units (IFU) of *C. trachomatis* or  $1 \times 10^4$  IFU of *C. muridarum* using a  
573 non-surgical embryo transfer device (ParaTechs Corp.). The mice were euthanized 7 days  
574 post-infection and the uterine horns were taken for further analysis. The uterine horns were  
575 homogenized in SPG buffer and DNA was isolated using DNeasy blood and tissue kit  
576 (Qiagen). Quantitative PCR was used to enumerate *Chlamydia* and host genome copy  
577 number. The following primers were used for amplifying the *C. trachomatis* *lytA* gene that  
578 was cloned into the vector: fwd, 5'-TCTAAAGCGTCTGGTGAAAGCT-3' and rev, 5'-  
579 GAAATAGCGTAGTAATAATACCCG-3'. Normalization of bacterial genome to that of the host  
580 was performed using mouse synectin primers: fwd, 5'-ACTAATGTCAAGGAGCTGTACG-3'  
581 and rev, 5'CCTCCGACTTGAACACTTCC-3'. Quantitative PCR with reverse transcription  
582 (RT-PCR) was performed as described below. Data were analysed using Step One Plus  
583 software package (Applied Biosystems) and expressed as the ratio of chlamydial genome to  
584 host genome (*lytA*/synectin). GraphPad Prism 7 was used to generate a scatter column chart

585 and perform statistical analysis. One-way analysis of variance (ANOVA) with Newman–Keuls  
586 multiple-comparison tests was performed with the significance level set to less than 0.01.  
587 Statistical analysis was performed to decide the sample size used in mouse infection by the  
588 Institute of Mathematics, University of Würzburg under the allowance A2 55.5-2531.01-49/12.  
589 All mouse experiments were carried out with 9 or 10 female mice per treatment group. Mice  
590 in each experiment were age-matched and cage mates were randomly distributed into  
591 different treatment groups to avoid cage effects.

## 592 **Generation of mouse organoids**

593 Generation of mouse organoids was adapted from <sup>44</sup>. Mice were sacrificed and fallopian tube  
594 tissue was prepared and processed within 2 h. Briefly, tissue samples were washed with DPBS  
595 (Gibco) and placed into a sterile Petri dish (Corning) where they were cut into small pieces.  
596 Then, on the top of the minced tissue, a glass slide (VWR) was placed and strongly pressed  
597 down to obtain smaller pieces. The cells were washed with DPBS, placed into a 15 ml falcon  
598 tube and centrifuged at 1,000 g for 10 minutes. The supernatant was removed, the pellet was  
599 resuspended in Matrigel (Corning) and plated in 50 µl drop in wells in a 24-well plate. The plate  
600 was carefully transferred to 37°C incubator to allow the Matrigel to get solidified for 20 min  
601 following the addition 500 µl/well of pre-warmed media (DMEM advanced (Sigma), Wnt (25%),  
602 R-Spondin (25%), Noggin (10%), B27 (2%; Thermo Scientific), Nicotinamide (1 mM; Sigma),  
603 human EGF (50 ng/ml; Thermo Scientific), FGF (100 ng/ml; Thermo Scientific), TGF-β inhibitor  
604 (0.5 mM; Tocris), Rock inhibitor (10 mM; Abmole Bioscience).

605 **Splitting organoids:** Approximately in 7 days later, the drop was carefully resuspended in cold  
606 DMEM medium and centrifuged at 1000 g at 4°C for 5 min. The supernatant was discarded and  
607 50 µl Matrigel was added and further processed as explained above.

608 **Infectivity assay in organoids:** For infection with *C. trachomatis* mature organoids were  
609 released from a confluent Matrigel drop by resuspending it with ice-cold DPBS (Gibco). The  
610 suspension was collected in a low-binding Eppendorf tube and 2 µl of a frozen stock of *C.*  
611 *trachomatis* L2 expressing GFP was added ( $5 \times 10^5$  IFU). The suspension was mixed and placed  
612 on ice for 30 min following centrifugation. 50 µl of Matrigel was added to each tube and seeded

into a 24-well plate (Corning) with following 20-minute incubation at 37°C to allow the Matrigel drop solidify. 6 days post infection the organoids were fixed with 4% PFA and used for immunostaining. In addition, infected organoids were lysed with glass beads and different dilutions were used to infect freshly plated HeLa cells to analyse the infectivity of the progeny.

### **Statistical analysis**

In all experiments, a minimum of three technical replicates was used and the *n* number refers to the number of independent experiments performed. The data are presented as box plots with the mean and s.e.m. Statistical analyses were performed with the Prism 7.2 package (GraphPad Software). ExactTest () function as a part of edgeR module in R 3.3.4 was used to carry out pairwise comparison and calculate p-values. False discovery rates were calculated as q-values using Benjamini-Höschberg algorithm implemented in edgeR module.

### **Author contribution**

KR and TR designed the experiments. The experiments were performed by KR, NV and TW. Next generation sequencing on Illumina platform was performed by AB, EW and resulting RNA-Seq data was analysed by RS and KR. Samples for Mass Spectrometry were prepared by KR and SJ. NV, CH, MS and WE performed the metabolic flux analysis of axenic culture and data interpretation. SJ, WS and AS performed metabolic flux analysis in host cell culture. FD provided plasmids and cell lines. Click reagents were synthesized by JF and JS. KR and TR wrote the manuscript.

### **Acknowledgment**

We kindly acknowledge Stefan Broer and Anselm Enders, Australian National University for providing the SLC1A5 KO mice. We thank Naziia Kurmasheva for supporting the organoid experiment. We acknowledge Claudia Gehring and Daniela Bunsen for processing the TEM samples. We thank Isabelle Derre for the EB-RB reporter strain Ct mCh(GroL2) GFP(OmcAL2), Vera Kozjak-Pavlovic for the cell line expressing shRNA against GLS1 and Andreas Demuth and Werner Goebel for critically reading the manuscript. KR was partially funded by the Frauenbüro in the frame of the “Qualification for junior scientist for professorship programme”

and by the Department of Biomedicine, Aarhus University. This research work was supported by the German Research Foundation (DFG) grant WO 2108/1-1 to WE and the GRK 2157 “3D-Infect” and the European Research Council (ERC-2018-ADG/NCI-CAD) to TR. AB was supported by grants of the German Excellence Initiative to the Graduate School of Life Sciences, University of Würzburg. SJ was supported by the DFG grant SCHU2670/1-1.

#### **Data availability**

Microarray data have been deposited in the Gene Expression Omnibus (GEO; [www.ncbi.nlm.nih.gov/geo/](http://www.ncbi.nlm.nih.gov/geo/)) of the National Center for Biotechnology Information and can be accessed with the GEO accession numbers GSE147538 and GSE147539. Any other data that support the findings of this study are available from the corresponding author upon request.

#### **Code availability**

Code described here has been integrated into the Galaxy codebase and released under the Academic Free License (AFL) v. 3.0 (<https://github.com/galaxyproject/galaxy> <<https://github.com/galaxyproject/galaxy>>). A free public instance of Galaxy can be accessed at <https://usegalaxy.org> <<https://usegalaxy.org>>. For the transcriptomics analysis on sequencing data deposited at gene expression omnibus (GSE147538), open access code implemented in bowtie, samtools, edgeR and GSEA tool and packages was utilized.

#### **Declaration of interests**

There is no conflict of interest



## Figure legends

### Fig 1: Cell wall synthesis of *Chlamydia* grown in axenic medium is induced by glutamine.

**a.** Central metabolic pathways of the chlamydial and host TCA cycle. **b.** Diagram showing the setup of the experiment. **c.** The relative amounts of the indicated metabolites compared to time point 0 were determined in the supernatant of axenic cultures with *Chlamydia* at different time points by GC/MS using internal standards (see Methods). The stability of Gln is shown as the green line where the supernatant with only Gln was analysed at 0 and 24 h. Number of independent experiments to confirm uptake: Gln: n=6, Glu: n=5, KG: n=4. **d.** <sup>13</sup>C-enrichments and isotopologue distributions of selected metabolites in *Chlamydia* cultured in axenic medium with [U-<sup>13</sup>C<sub>5</sub>] Gln for 24 h. n=3. **e.** Purified EBs (Input) were incubated in axenic medium with only G6P or G6P together with Gln or Glu. After 24 h, the bacteria were pelleted and analysed by EM. Representative images from three independent experiments are shown. Scale bar = 2 µm. **f.** Purified EBs were incubated with G6P or G6P and Gln. Both samples were treated with DA-DA for 12 h. Click chemistry was used to detect DA-DA in peptidoglycan (green). Bacteria were stained for cHsp60 (red). The samples were analysed using Structured Illumination Microscopy. Representative images from two independent experiments. The insets show magnifications of the peptidoglycan signal (lower panel). Scale bar = 0.5 µm. **g.** FACS analysis of the bacteria from (f). The fluorescence is presented as bar plot overlaid with data points. Data were averaged (± s.e.m.) from three independent experiments (n=3). Statistical significance was calculated using an unpaired t test (two tailed) (t=5.138, Df=4, p value =0.0068). \*\* Indicates p value <0.01. **h.** EBs were incubated in axenic medium containing only G6P or G6P together with Gln. After 12 h the bacteria were pelleted and total RNA was isolated for RNA-seq (see Methods). The heat map shows expression levels of known early genes. **i.** Genome copy number of *Chlamydia* was determined at the indicated times. Shown is the mean from three independent experiments (± s.e.m.) n=3. Statistical significance was calculated using two-way ANOVA with Dunnett's multiple comparison test (Df=24). \* p value=0.034; \*\* p value =0.002; ns, non-significant.

**Fig. 2: Chlamydial infection triggers altered glutamine metabolism in infected cells.**

HUVECs were either left uninfected or infected with *Chlamydia* at an MOI of 1 for 36 h. Cells or medium samples were extracted and metabolites were analysed by LC-MS (see Methods). **a.** Depletion of amino acids from the culture medium from HUVECs with and without infection (only significant results are shown). Data were averaged ( $\pm$  s.e.m.) from three independent experiments. n=3. **b/c.** Content of Gln and Glu in control and infected cells. Data from three independent experiments are depicted as bar plot overlaid with data points with the mean ( $\pm$  s.e.m.). Paired two tailed t-test was used to calculate statistical significance (**b**)  $t=10.75$ ,  $Df=2$ ,  $p$  value=0.0085; (**c**)  $t=1,664$ ,  $Df=2$ )  $p$  value= 0.2379. \*\*  $p$  value <0.01, ns non-significant. Error bar was defined as mean with SEM **d.** Control or *Chlamydia* infected HUVECs were cultured in the presence of [U- $^{13}\text{C}_5$ ] Gln for 36 h.  $^{13}\text{C}$ -enrichments and isotopologue distributions of selected metabolites were determined by LC-MS. Data present the mean of three independent samples. **e.** Diagram outlining the synthesis of the D-aminopimelic acid (DAP) in *Chlamydia* from host or chlamydial derived metabolites. **f.** Mass isotopologue distribution of DAP extracted from infected host cells using acid hydrolysis. Data present the mean of three independent samples.

**Fig. 3: Signalling pathways involved in the stabilization of c-Myc in infected cells.** **a.** Plot of gene set enrichment analysis (GSEA) performed with RNA-seq data of the gene set „Positive regulation of c-myc targets“. Gene expression profiles were generated as described in the Methods section and a selected gene set is shown. Vertical black bars indicate the position of genes in the „Positive regulation of c-myc targets“ gene set and the enrichment score is shown as a green line. This analysis demonstrates that genes induced upon infection are known as target genes of c-Myc (NES: normalized enrichment score). Three independent experiments were performed. n=3. See the methods section for statistical analysis. **b.** HeLa229 cells were infected with *Chlamydia* for different time points and the samples were analysed using Western blotting for phosphorylated c-Myc (pc-Myc serine 62), pc-Myc (threonine 58), cHsp60 and  $\beta$  Actin. **c.** c-Myc was immunoprecipitated from lysates of HeLa229 cells infected with *Chlamydia* for different time points. Ubiquitin was detected in the precipitate by immunoblotting. The input

718 from the same experiment was probed against c-Myc, cHsp60 and  $\beta$  Actin. **d.** HeLa229 cells  
719 were either left uninfected or infected with *Chlamydia* for 24 or 36 hpi. The cells were treated  
720 with the translation inhibitor cycloheximide (CHX) for 10, 30 or 60 min. The cells were then lysed  
721 and analysed by Western blotting for c-Myc, cHsp60 and  $\beta$  Actin. For quantification, values were  
722 normalized for each condition to the 0 time point of CHX-treated sample. **e.** Diagram showing  
723 the signalling pathway leading to c-Myc stabilization. **f/g.** HeLa229 cells were treated with the  
724 MAPK inhibitor, (U0126) (**f**) or the PI3K inhibitor (LY294002) (**g**) for 4 h and infected with  
725 *Chlamydia* for 24 h. The cells were lysed and analysed by Western blotting. n=3. **h.** HeLa229  
726 cells were infected with *Chlamydia* for different time points. The cells were harvested for Western  
727 Blot analysis. n=3. **i.** HeLa229 cells were either left uninfected or infected with *Chlamydia* and  
728 treated with the PI3K inhibitor. The cells were lysed and analysed by Western. In all Western  
729 blots, detection of cHsp60 served as an infection and of  $\beta$  Actin as a loading control. The  
730 immunoblots shown in this figure are representative of at least three independent experiments.

731

732 **Fig. 4: c-Myc is downstream of PI3K/MAPK pathway and critical for chlamydial growth.**

733 **a/b.** U2OS cells harboring an inducible c-Myc gene under the control of a Tet-inducible promoter  
734 were induced with AHT for 12 h and either treated with MAPK inhibitor U0126 (**a**), or the PI3K  
735 inhibitor LY294002. n=3. (**b**) or left untreated as indicated. The cells were further infected with  
736 *Chlamydia* and analysed by Western blotting for bacterial infection. n=3. **c.** The cells from the  
737 experiment shown in (a) and (b) were fixed and immunostained to detect chlamydial inclusions  
738 (green) and actin (phalloidin, red). Images are representative for three independent  
739 experiments. UI, uninfected control; U0: treated with U0126; LY, treated with LY294002; AHT,  
740 induced. n=3. Scale bar = 15  $\mu$ m. **d.** HeLa229 cells were treated with the chemical c-Myc  
741 inhibitor 10058-F4. The cells were infected with *Chlamydia* for 24 or 36 hpi and then analysed  
742 for *Chlamydia* infection by Western blotting (cHsp60). n=3. **e.** HCT116 cells engineered to  
743 express a shRNA to silence c-Myc expression under the control of an inducible Tet<sup>on</sup> promoter  
744 were treated with AHT (1  $\mu$ g/ml) for 24 h and then infected with *Chlamydia* for another 24 h. The  
745 cells were lysed and analysed for *Chlamydia* infection by probing for cHsp60. n=3. **f.** An

unrelated control siRNA or a siRNA pool directed against c-Myc was used to knock down c-Myc in HeLa cells. After 48 h of transfection, the cells were infected with *Chlamydia* for 24 h. The cells were harvested and analysed by Western blotting for chlamydial infection. The immunoblots shown in the figures are representative of at least three independent experiments.

**Fig. 5: Glutamine is a limiting metabolite for chlamydial intracellular growth.** **a.** Cells isolated from human fimbriae (Human Fimb) were grown in basic formulation of DMEM containing 1 g/l D-glucose and different concentrations of glutamine. The cells were infected with *Chlamydia* (Ct) for 24 h, lysed and analysed via Western blotting to detect chlamydial Hsp60, c-Myc and  $\beta$  Actin. n=3. **b.** HeLa229 cells were grown in basic formulation of DMEM containing 1 g/l D-glucose. The cells were treated with or without glutamine (2 mM) and infected with *Chlamydia* for different time points. The cells were lysed and analysed for chlamydial growth using Western blotting to detect chlamydial Hsp60, c-Myc and  $\beta$  Actin. n=3. **c/d.** The cells from (b) were lysed and used to infect freshly plated HeLa cells. After 24 h the cells were either fixed and immunostained for chlamydial inclusions (green) and Actin (phalloidin, red), or used for Western blot analysis as shown in panel (d). The scale bar is indicated in the figure. n=3. Scale bar = 10  $\mu$ m. **e.** HeLa cells were infected with the EB/RB reporter strain for 24 h and grown in basic formulation of DMEM containing 1 g/l D-glucose either without (-Gln) or with 2 mM Gln (+Gln). In one set of experiments, Gln-free medium was replaced after 24 h for medium containing 2 mM Gln (lowest panel). The cells were fixed *Chlamydia* (Red/Green) and Actin (phalloidin, grey) was detected. Loss of the green fluorescence is indicative for RBs. n=3. Scale bar = 10  $\mu$ m. **f/g.** HCT116 were grown in basic formulation of DMEM containing 1 g/l D-glucose, with or without L-glutamine. HCT116 expressing 4-hydroxytamoxifen (OHT)-inducible Myc-ER or the vector control were treated overnight with OHT (100 nM) (**f**) to activate Myc-ER or ethanol (1  $\mu$ l) as control (**g**). The cells were then infected with *Chlamydia* at an MOI of 1 for 24 h and analysed for bacterial load by Western blotting. n=3. The immunoblots shown in the figures are representative of at least three independent experiments.

**Fig. 6: *Chlamydia* metabolically reprograms the host cell.** **a.** HUVEC cells infected with *Chlamydia* were subjected to RNA-seq. Shown is the hierarchically clustered heat map depicting gene regulation (normalized log<sub>2</sub>FC) of gene set “Amino acid transport across the plasma membrane” which is a part of Reactome gene sets in C2 Molecular Signature Database. Three independent experiments were performed. n=3. **b.** Diagram of Gln uptake pathways and the targets of the inhibitors. **c.** HeLa229 cells were infected with *Chlamydia* and then subjected to Western blot analysis. **d.** Control siRNA or an siRNA pool against SLC1A5 were transfected and 48 h later infected with *Chlamydia* for 24 h as indicated. The cells were lysed and further analysed by Western blot. **e.** HeLa229 cells expressing a shRNA to silence GLS1 expression under the control of a Tet<sup>on</sup> promoter were induced with AHT for 7 days to deplete GLS1 and then subjected to Western blot analysis. **f.** HeLa229 cells were treated with the SLC1A5 specific inhibitor GPNA or **(g)** the glutaminase inhibitor BPTES for 4 h and infected with *Chlamydia*. The cells were lysed and analysed by Western blotting. **h.** HeLa229 cells were transfected with siRNA against c-Myc and 48 hrs later analysed by Western blotting as indicated. **i.** HCT116 Tet<sup>on</sup> sh-c-Myc cells were either left untreated or treated with AHT for 24 h followed by overexpression of SLC1A5. The cells were then infected with *Chlamydia* for another 24 h and analysed by Western blotting. **j/k.** SLC1A5 <sup>+/+</sup> or SLC1A5 <sup>-/-</sup> mice were infected with *C. trachomatis* (n=10) or *C. muridarum* (n=9) **(k)**. The mice were sacrificed seven days post infection and the copy number of *Chlamydia* was calculated using qPCR. The data are presented as box and whisker plot overlaid with data points with the mean ( $\pm$  s.e.m.). Unpaired t test (two tailed) was used to calculate the significance (**j**) (t=3.714, df=16) and (**k**) (t=3.712, df=10). \*\* Indicates p value <0.01. n=9. **l.** Organoids were derived from SLC1A5 <sup>+/+</sup> or SLC1A5 <sup>-/-</sup> mice and infected with *Chlamydia* (Ct) (see Methods). The infected organoids were fixed and used for immunostaining to detect *Chlamydia* (green), nuclei (blue) and actin (phalloidin, violet). Scale bar = 30  $\mu$ m. The immunoblots shown in the figures are representative of at least three independent experiments.

## 801    **References**

- 802    1        Newman, L. *et al.* Global Estimates of the Prevalence and Incidence of Four Curable  
803               Sexually Transmitted Infections in 2012 Based on Systematic Review and Global  
804               Reporting. *PLoS One* **10**, e0143304, doi:10.1371/journal.pone.0143304 (2015).
- 805    2        Liechti, G. W. *et al.* A new metabolic cell-wall labelling method reveals  
806               peptidoglycan in Chlamydia trachomatis. *Nature* **506**, 507-510,  
807               doi:10.1038/nature12892 (2014).
- 808    3        Liechti, G. *et al.* Pathogenic Chlamydia Lack a Classical Sacculus but Synthesize a  
809               Narrow, Mid-cell Peptidoglycan Ring, Regulated by MreB, for Cell Division. *PLoS*  
810               *Pathog* **12**, e1005590, doi:10.1371/journal.ppat.1005590 (2016).
- 811    4        Stephens, R. S. *et al.* Genome sequence of an obligate intracellular pathogen of  
812               humans: Chlamydia trachomatis. *Science* **282**, 754-759 (1998).
- 813    5        Iliffe-Lee, E. R. & McClarty, G. Glucose metabolism in Chlamydia trachomatis: the  
814               'energy parasite' hypothesis revisited. *Mol Microbiol* **33**, 177-187 (1999).
- 815    6        Mehltitz, A. *et al.* Metabolic adaptation of Chlamydia trachomatis to mammalian  
816               host cells. *Mol Microbiol* **103**, 1004-1019, doi:10.1111/mmi.13603 (2017).
- 817    7        Omsland, A., Sager, J., Nair, V., Sturdevant, D. E. & Hackstadt, T. Developmental  
818               stage-specific metabolic and transcriptional activity of Chlamydia trachomatis in  
819               an axenic medium. *Proc Natl Acad Sci U S A* **109**, 19781-19785,  
820               doi:10.1073/pnas.1212831109 (2012).
- 821    8        Kubo, A. & Stephens, R. S. Substrate-specific diffusion of select dicarboxylates  
822               through Chlamydia trachomatis PorB. *Microbiology* **147**, 3135-3140,  
823               doi:10.1099/00221287-147-11-3135 (2001).
- 824    9        Belland, R. J. *et al.* Genomic transcriptional profiling of the developmental cycle of  
825               Chlamydia trachomatis. *Proc Natl Acad Sci U S A* **100**, 8478-8483,  
826               doi:10.1073/pnas.1331135100 (2003).
- 827    10       Belland, R. J. *et al.* Transcriptome analysis of chlamydial growth during IFN-  
828               gamma-mediated persistence and reactivation. *Proc Natl Acad Sci U S A* **100**,  
829               15971-15976, doi:10.1073/pnas.2535394100 (2003).
- 830    11       Miller, D. M., Thomas, S. D., Islam, A., Muench, D. & Sedoris, K. c-Myc and cancer  
831               metabolism. *Clin Cancer Res* **18**, 5546-5553, doi:10.1158/1078-0432.CCR-12-0977  
832               (2012).
- 833    12       Gao, P. *et al.* c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase  
834               expression and glutamine metabolism. *Nature* **458**, 762-765,  
835               doi:10.1038/nature07823 (2009).
- 836    13       Wise, D. R. *et al.* Myc regulates a transcriptional program that stimulates  
837               mitochondrial glutaminolysis and leads to glutamine addiction. *Proc Natl Acad Sci*  
838               *U S A* **105**, 18782-18787, doi:10.1073/pnas.0810199105 (2008).
- 839    14       Al-Zeer, M. A. *et al.* Chlamydia trachomatis Prevents Apoptosis Via Activation of  
840               PDPK1-MYC and Enhanced Mitochondrial Binding of Hexokinase II. *EBioMedicine*  
841               **23**, 100-110, doi:10.1016/j.ebiom.2017.08.005 (2017).
- 842    15       Welcker, M. *et al.* The Fbw7 tumor suppressor regulates glycogen synthase kinase  
843               3 phosphorylation-dependent c-Myc protein degradation. *Proc Natl Acad Sci U S A*  
844               **101**, 9085-9090, doi:10.1073/pnas.0402770101 (2004).
- 845    16       Sears, R. C. The life cycle of C-myc: from synthesis to degradation. *Cell Cycle* **3**,  
846               1133-1137 (2004).

847 17 Rajalingam, K. *et al.* Mcl-1 is a key regulator of apoptosis resistance in Chlamydia  
848 trachomatis-infected cells. *PLoS One* **3**, e3102, doi:10.1371/journal.pone.0003102  
849 (2008).

850 18 Subbarayal, P. *et al.* EphrinA2 receptor (EphA2) is an invasion and intracellular  
851 signaling receptor for Chlamydia trachomatis. *PLoS Pathog* **11**, e1004846,  
852 doi:10.1371/journal.ppat.1004846 (2015).

853 19 Patel, A. L. *et al.* Activation of epidermal growth factor receptor is required for  
854 Chlamydia trachomatis development. *BMC Microbiol* **14**, 277, doi:10.1186/s12866-  
855 014-0277-4 (2014).

856 20 Adhikary, S. & Eilers, M. Transcriptional regulation and transformation by Myc  
857 proteins. *Nat Rev Mol Cell Biol* **6**, 635-645, doi:10.1038/nrm1703 (2005).

858 21 Frame, S., Cohen, P. & Biondi, R. M. A common phosphate binding site explains the  
859 unique substrate specificity of GSK3 and its inactivation by phosphorylation. *Mol*  
860 *Cell* **7**, 1321-1327 (2001).

861 22 Lorenzin, F. *et al.* Different promoter affinities account for specificity in MYC-  
862 dependent gene regulation. *Elife* **5**, doi:10.7554/eLife.15161 (2016).

863 23 Cortina, M. E., Ende, R. J., Bishop, R. C., Bayne, C. & Derre, I. Chlamydia trachomatis  
864 and Chlamydia muridarum spectinomycin resistant vectors and a transcriptional  
865 fluorescent reporter to monitor conversion from replicative to infectious bacteria.  
866 *PLoS One* **14**, e0217753, doi:10.1371/journal.pone.0217753 (2019).

867 24 Dejure, F. R. *et al.* The MYC mRNA 3'-UTR couples RNA polymerase II function to  
868 glutamine and ribonucleotide levels. *The EMBO journal* **36**, 1854-1868,  
869 doi:10.15252/embj.201796662 (2017).

870 25 Kekuda, R. *et al.* Cloning of the sodium-dependent, broad-scope, neutral amino acid  
871 transporter Bo from a human placental choriocarcinoma cell line. *J Biol Chem* **271**,  
872 18657-18661 (1996).

873 26 van Geldermalsen, M. *et al.* ASCT2/SLC1A5 controls glutamine uptake and tumour  
874 growth in triple-negative basal-like breast cancer. *Oncogene* **35**, 3201-3208,  
875 doi:10.1038/onc.2015.381 (2016).

876 27 Wise, D. R. *et al.* Myc regulates a transcriptional program that stimulates  
877 mitochondrial glutaminolysis and leads to glutamine addiction. *P Natl Acad Sci USA*  
878 **105**, 18782-18787, doi:10.1073/pnas.0810199105 (2008).

879 28 Masle-Farquhar, E., Broer, A., Yabas, M., Enders, A. & Broer, S. ASCT2 (SLC1A5)-  
880 Deficient Mice Have Normal B-Cell Development, Proliferation, and Antibody  
881 Production. *Front Immunol* **8**, 549, doi:10.3389/fimmu.2017.00549 (2017).

882 29 Kermack, A. J. *et al.* Amino acid composition of human uterine fluid: association  
883 with age, lifestyle and gynaecological pathology. *Hum Reprod* **30**, 917-924,  
884 doi:10.1093/humrep/dev008 (2015).

885 30 Behjousiar, A., Kontoravdi, C. & Polizzi, K. M. In situ monitoring of intracellular  
886 glucose and glutamine in CHO cell culture. *PLoS One* **7**, e34512,  
887 doi:10.1371/journal.pone.0034512 (2012).

888 31 Allan, I. & Pearce, J. H. Differential amino acid utilization by Chlamydia psittaci  
889 (strain guinea pig inclusion conjunctivitis) and its regulatory effect on chlamydial  
890 growth. *J Gen Microbiol* **129**, 1991-2000, doi:10.1099/00221287-129-7-1991  
891 (1983).

892 32 Allan, I. & Pearce, J. H. Amino acid requirements of strains of Chlamydia  
893 trachomatis and C. psittaci growing in McCoy cells: relationship with clinical  
894 syndrome and host origin. *J Gen Microbiol* **129**, 2001-2007,  
895 doi:10.1099/00221287-129-7-2001 (1983).

896 33 Jin, L., Alesi, G. N. & Kang, S. Glutaminolysis as a target for cancer therapy. *Oncogene*  
897 **35**, 3619-3625, doi:10.1038/onc.2015.447 (2016).

898 34 Porcheray, F. *et al.* Glutamate metabolism in HIV-infected macrophages:  
899 implications for the CNS. *Am J Physiol Cell Physiol* **291**, C618-626,  
900 doi:10.1152/ajpcell.00021.2006 (2006).

901 35 Chambers, J. W., Maguire, T. G. & Alwine, J. C. Glutamine metabolism is essential for  
902 human cytomegalovirus infection. *J Virol* **84**, 1867-1873, doi:10.1128/JVI.02123-  
903 09 (2010).

904 36 Fontaine, K. A., Camarda, R. & Lagunoff, M. Vaccinia virus requires glutamine but  
905 not glucose for efficient replication. *J Virol* **88**, 4366-4374, doi:10.1128/JVI.03134-  
906 13 (2014).

907 37 Thai, M. *et al.* Adenovirus E4ORF1-induced MYC activation promotes host cell  
908 anabolic glucose metabolism and virus replication. *Cell Metab* **19**, 694-701,  
909 doi:10.1016/j.cmet.2014.03.009 (2014).

910 38 Thai, M. *et al.* MYC-induced reprogramming of glutamine catabolism supports  
911 optimal virus replication. *Nat Commun* **6**, 8873, doi:10.1038/ncomms9873 (2015).

912 39 Sanchez, E. L., Carroll, P. A., Thalhofer, A. B. & Lagunoff, M. Latent KSHV Infected  
913 Endothelial Cells Are Glutamine Addicted and Require Glutaminolysis for Survival.  
914 *PLoS Pathog* **11**, e1005052, doi:10.1371/journal.ppat.1005052 (2015).

915 40 Mall, A. *et al.* Reversibility of citrate synthase allows autotrophic growth of a  
916 thermophilic bacterium. *Science* **359**, 563-567, doi:10.1126/science.aao2410  
917 (2018).

918 41 Lee, W. N., Byerley, L. O., Bergner, E. A. & Edmond, J. Mass isotopomer analysis:  
919 theoretical and practical considerations. *Biol Mass Spectrom* **20**, 451-458,  
920 doi:10.1002/bms.1200200804 (1991).

921 42 Karunakaran, K., Subbarayal, P., Vollmuth, N. & Rudel, T. Chlamydia-infected cells  
922 shed Gp96 to prevent chlamydial re-infection. *Mol Microbiol* **98**, 694-711,  
923 doi:10.1111/mmi.13151 (2015).

924 43 Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach  
925 for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* **102**,  
926 15545-15550, doi:10.1073/pnas.0506580102 (2005).

927 44 Kessler, M. *et al.* Chronic Chlamydia infection in human organoids increases  
928 stemness and promotes age-dependent CpG methylation. *Nat Commun* **10**, 1194,  
929 doi:10.1038/s41467-019-09144-7 (2019).

930