

## ABSTRACT

Viewing interspecies relationships through the lens of bioenergetics enables a flexible conceptual framework to understand why virulence is context-dependent in arthropod-borne diseases. Here, we engineered a system of metabolic interdependence in *Ixodes scapularis* where nutrients were allocated according to the glycolytic or oxidative phosphorylation cellular state. The rickettsial agent *Anaplasma phagocytophilum* and the Lyme disease spirochete *Borrelia burgdorferi* induced glycolysis during infection and inhibition of oxidative phosphorylation enhanced microbial colonization of tick cells. Through an unbiased metabolomics approach, we discovered that  $\beta$ -aminoisobutyric acid (BAIBA) was an important metabolite for tick-microbe interactions. Whereas distinct levels of BAIBA affected tick weight and survival *in vivo*, disrupting BAIBA levels through genetic manipulation of catabolic enzymes reduced bacterial infection and restores tick fitness. Collectively, the metabolite BAIBA draws antagonistic pleiotropy on seemingly unrelated evolutionary traits in *Ixodes scapularis* ticks. Bioenergetics and resource allocation have yet to be explored as a strategy to constrain the public health burden of arthropod-borne diseases.

## BACKGROUND

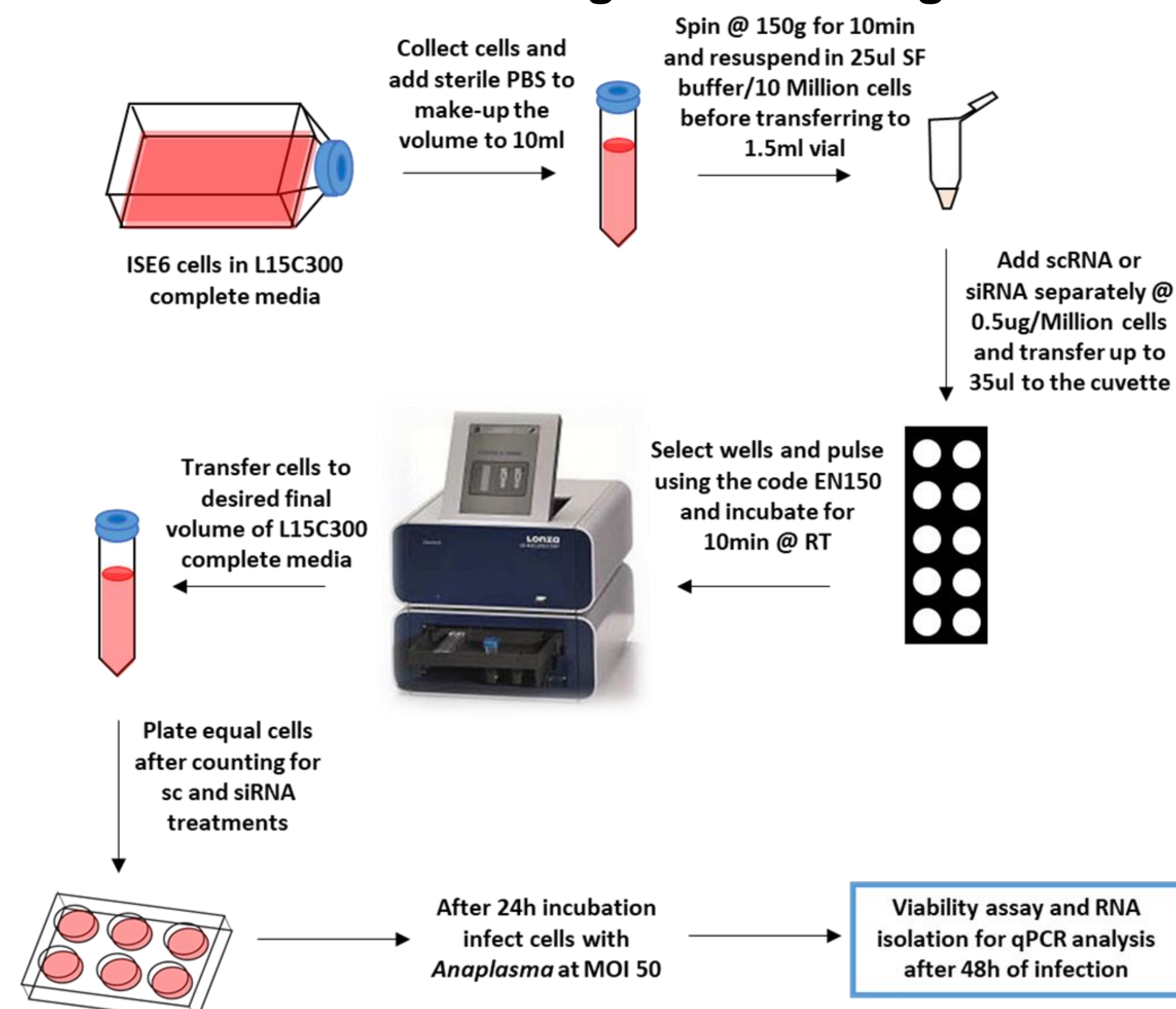
Metabolism plays a key role in maintaining organismal fitness by prioritizing one life history trait over others at a given stage. Such traits include growth, reproduction and maintenance. Finite metabolic resources are channelized for sustaining homeostasis, but in case of a microbial encounter the resources are somewhat reprogrammed. There are evidence showing that based on the resource availability the microbes switch their metabolic lifestyle. Conventional principles of biasing such interactions to be 'pathogenic' or 'mutualistic' is something reconsidered in this study. We build a system to engineer metabolism in tick cells and used tick-transmitted microbes as vector-microbe system to understand the type of interaction from a metabolic perspective.

## KEY METHODS

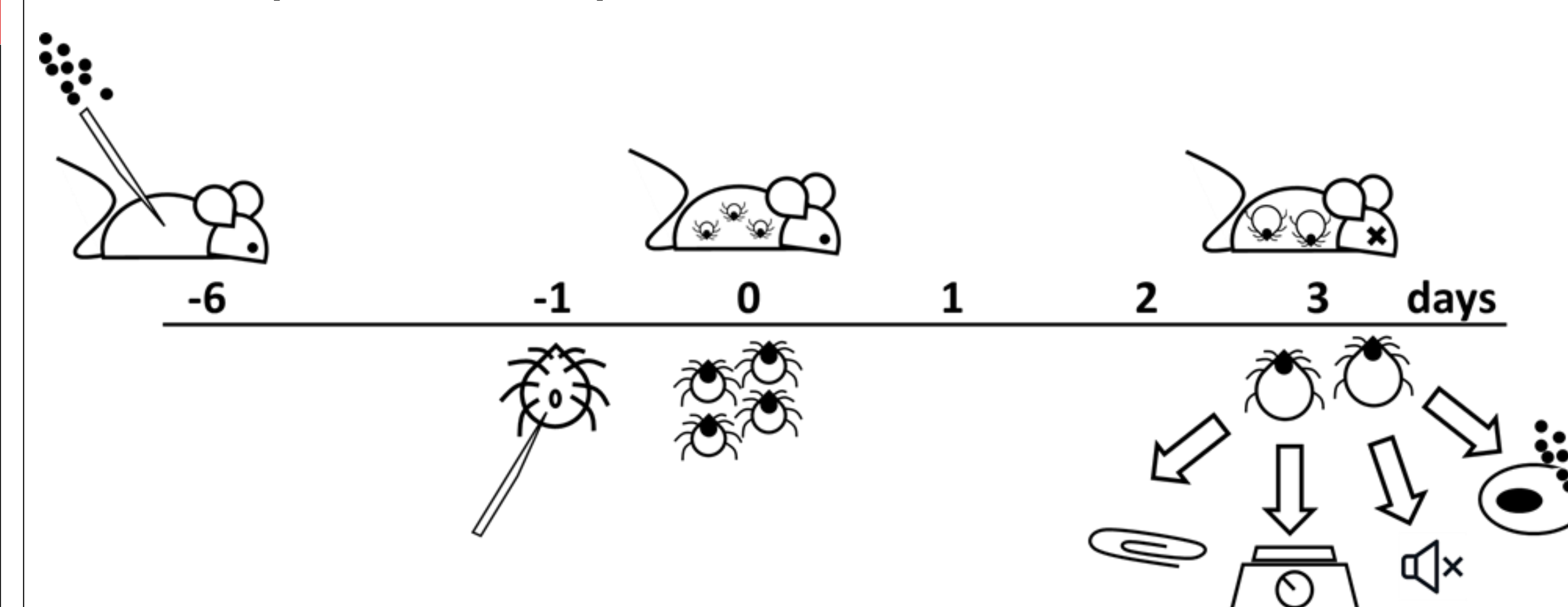
### 1. Modified L15C media for measuring metabolic changes:

Seahorse DMEM	Modified L15C (mL15C)	L15C300 complete
10mM glucose	10mM glucose	100mM glucose
2mM glutamine	2mM glutamine	5.4mM glutamine
1mM sodium pyruvate	5mM sodium pyruvate	5mM sodium pyruvate
No D-galactose	5mM D-galactose	5mM D-galactose
No bicarbonate	No bicarbonate	No bicarbonate
No $\alpha$ -ketoglutarate	3.0mM $\alpha$ -ketoglutarate	3.0mM $\alpha$ -ketoglutarate
No glutamic acid	3.3mM glutamic acid	3.3mM glutamic acid
No proline	3.9mM proline	3.9mM proline
No fetal bovine serum (FBS)	No fetal bovine serum (FBS)	10% fetal bovine serum (FBS)
No tryptose phosphate broth (TPB)	No tryptose phosphate broth (TPB)	10% tryptose phosphate broth (TPB)
No lipoprotein-cholesterol concentrate (LPPC)	No lipoprotein-cholesterol concentrate (LPPC)	0.01% lipoprotein-cholesterol concentrate (LPPC)
pH = 7.4	pH = 7.4	pH = 6.0-7.0

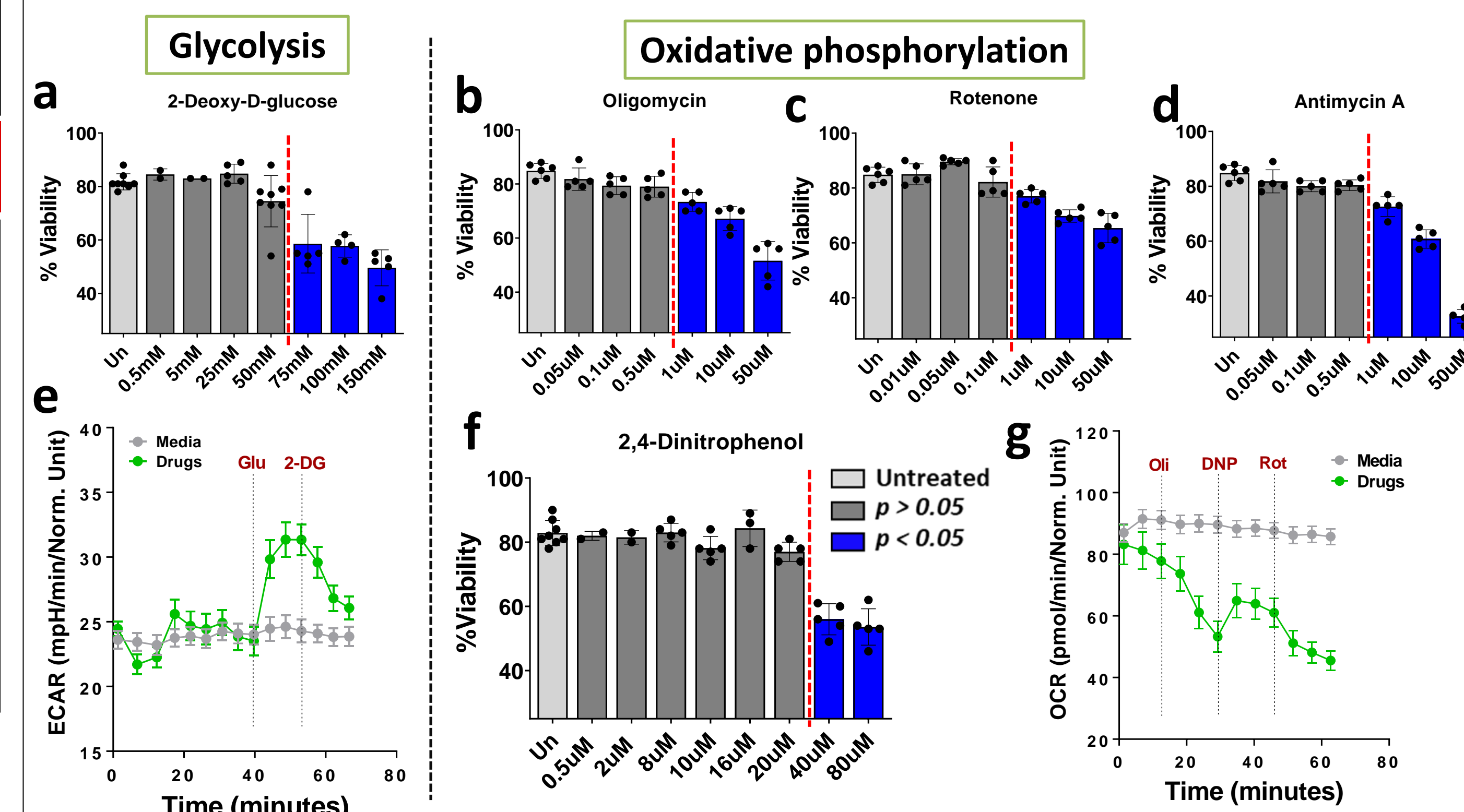
### 2. Nucleofection to silence target metabolic genes:



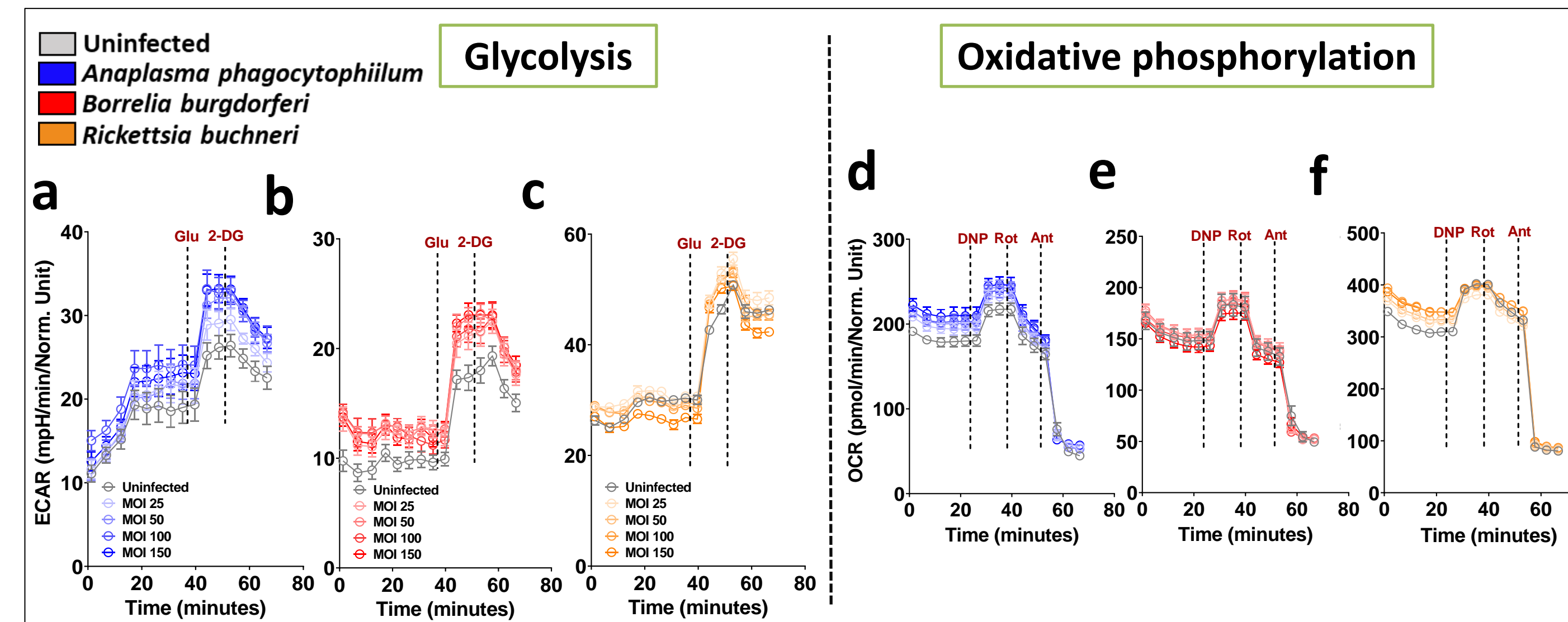
### 3. Mouse experimental set-up to measure infection and fitness:



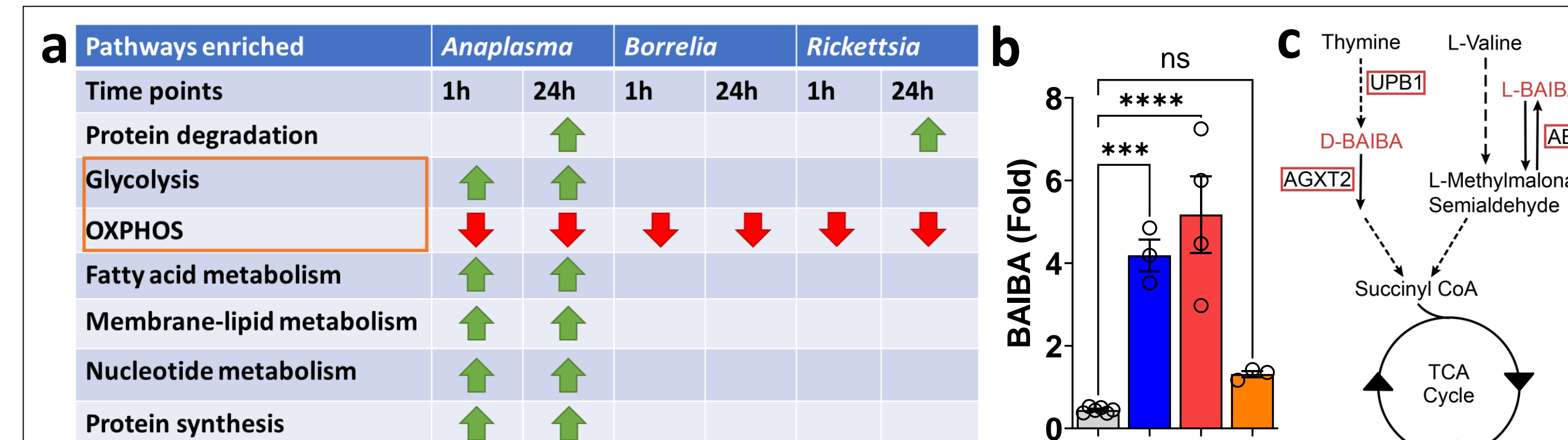
## RESULTS



**Figure 1: Measuring bioenergetics in tick cells.** ISE6 cells were treated with drugs that either blocks glycolysis (2-Deoxy-D-glucose) (a) or oxidative phosphorylation (oligomycin, rotenone, 2,4-DNP and antimycin A) (b-d, f). Seahorse analysis of glycolysis and oxidative phosphorylation (e,g).

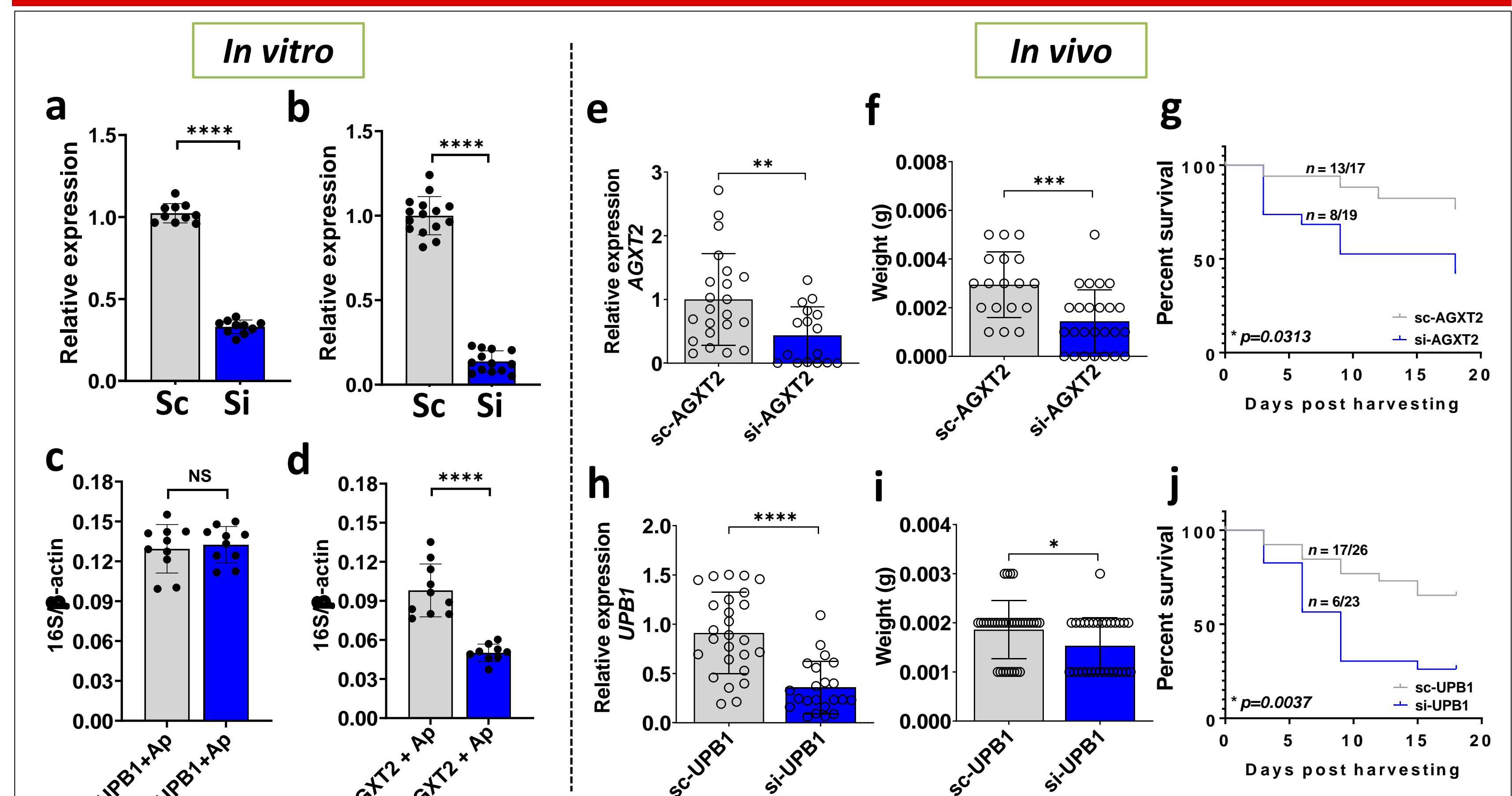


**Figure 2: Comparing bioenergetics between pathogen and commensal.** ISE6 cells were treated with *A. phagocytophilum* (a,d), *B. burgdorferi* (b,e) and *R. buchneri* (c,f) at different multiplicity of infection (MOI). Glycolysis and Oxphos were measured using seahorse analyzer.

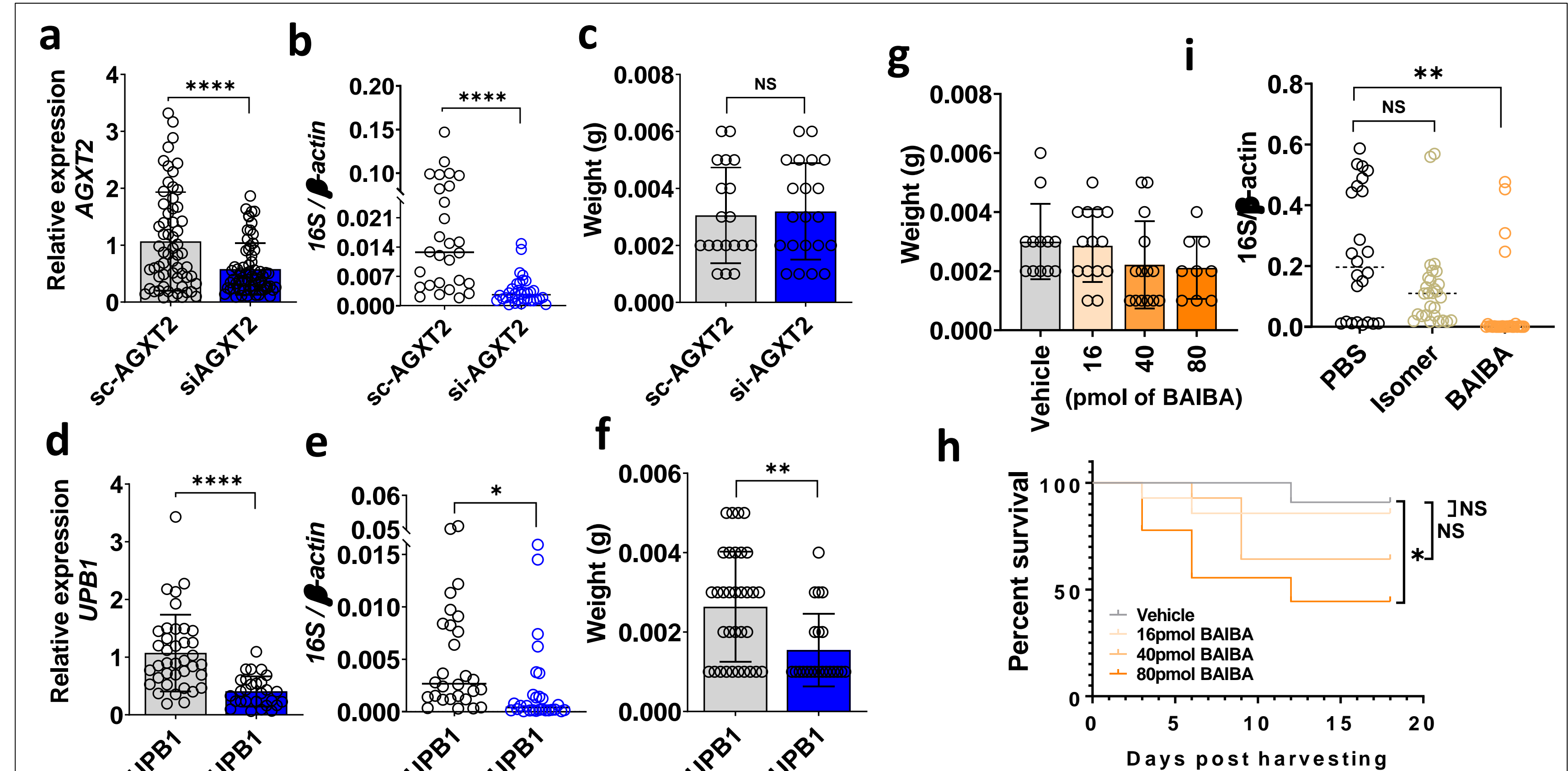


**Figure 3: Metabolomics identifies BAIBA as metabolite in pathogenic infection.** ISE6 cells were treated with all three microbes and samples were send to Metabolon Inc. Several pathways were affected by *A. phagocytophilum* (a) and BAIBA is the enriched metabolite in pathogenic infection(b). BAIBA is an intermediate metabolite of thymine and valine catabolism (c).

## RESULTS



**FIGURE 4: BAIBA metabolism genes affect infection *in vitro* and fitness *in vivo*.** *UPB1* and *AGXT2* were silenced using nucleofection protocol (a,b) and *A. phagocytophilum* (c,d) burden was measured after 48 h in ISE6 cells. *In vivo* ticks were microinjected with *UPB1* and *AGXT2* siRNA and silencing (e,h), weight (f,i) and survival (g,j).



**FIGURE 5: BAIBA restore tick fitness after infection.** Silencing BAIBA degradation (*AGXT2*) (a) causes reduced *A. phagocytophilum* acquisition (b) and restore tick fitness (c), whereas silencing BAIBA production (*UPB1*) (d) reduces acquisition (e) at the cost of tick fitness(g). Dose response to affects tick survival (h) significantly but not feeding (g). However, the negative effect on bacterial acquisition is specific to BAIBA as compared to its isomer (2-aminoisobutyric acid) at the same dosage (i).

## CONCLUSIONS

- Systematic strategy was developed to measure metabolism in tick cells and ticks *in vivo*
- Glycolysis is upregulated in 'human pathogen' infection whereas endosymbionts does not have an effect
- BAIBA ( $\beta$ -aminoisobutyric acid) affect vector-microbe interaction both *in vitro* and *in vivo* and tick fitness

## ACKNOWLEDGEMENTS

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