

**Supplementary Information for:**

**Mitochondrial metabolism and oxidative stress in tropical cockroach  
under fluctuating thermal regimes**

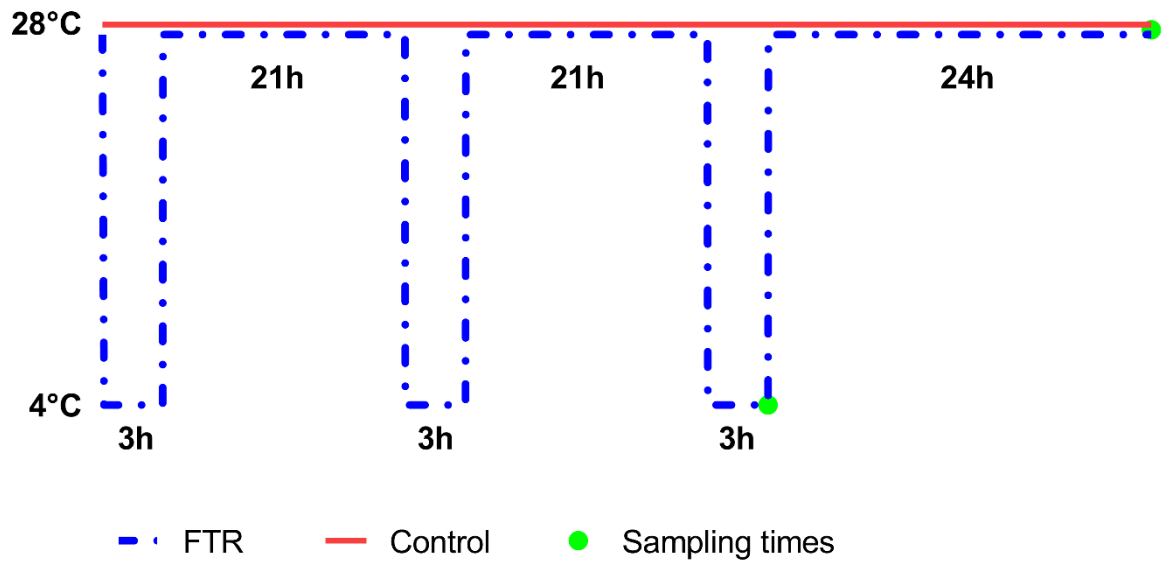
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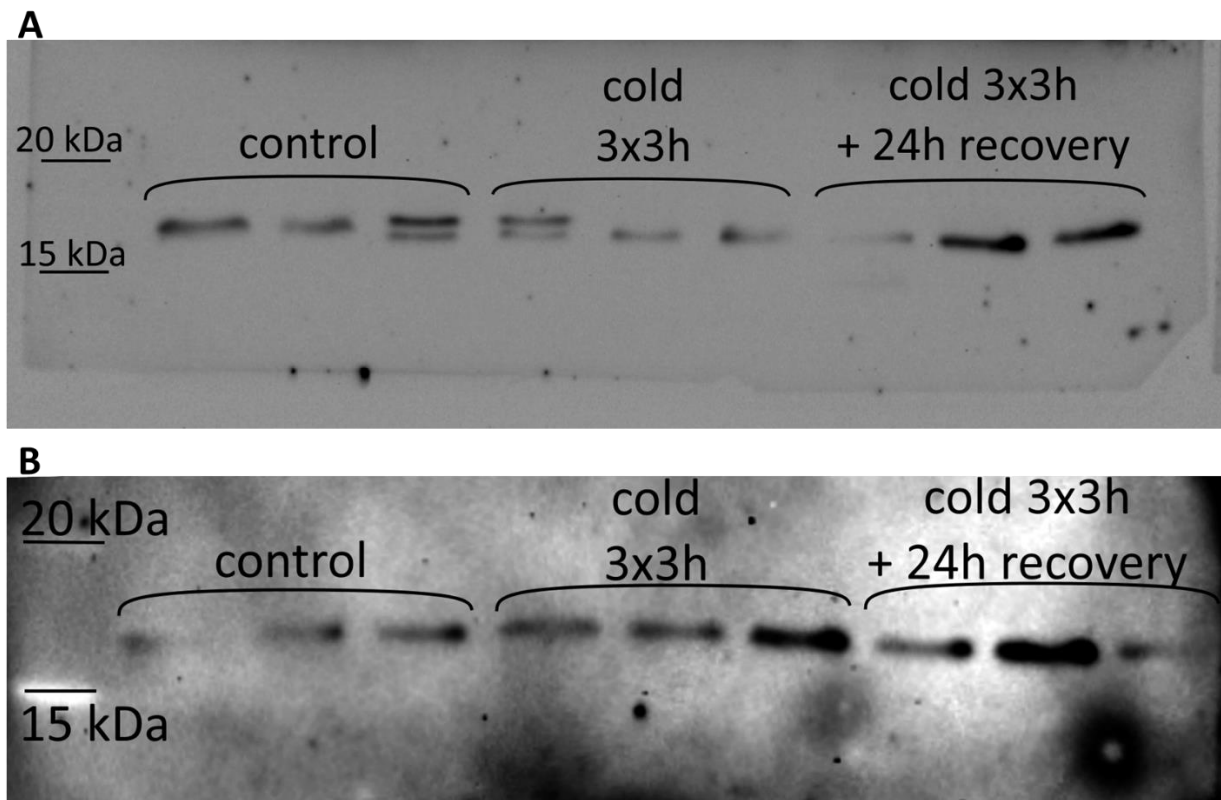
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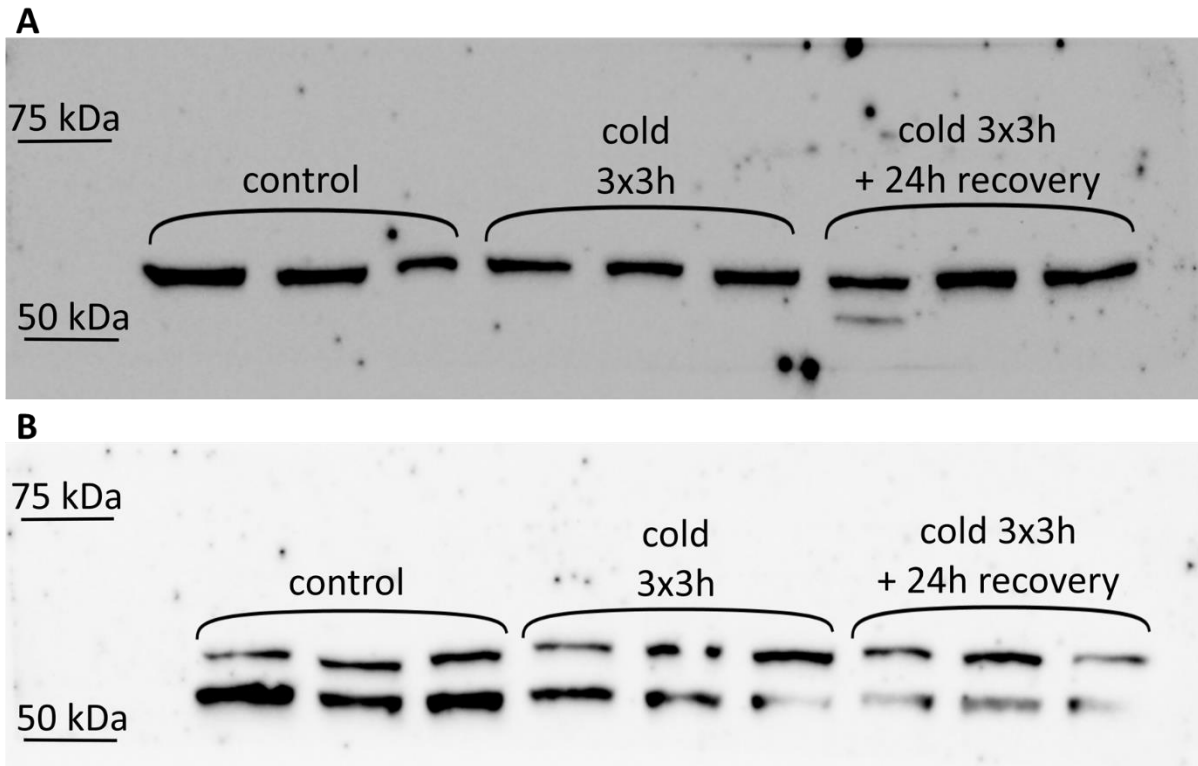


**Figure S1.** Scheme of the experimental design

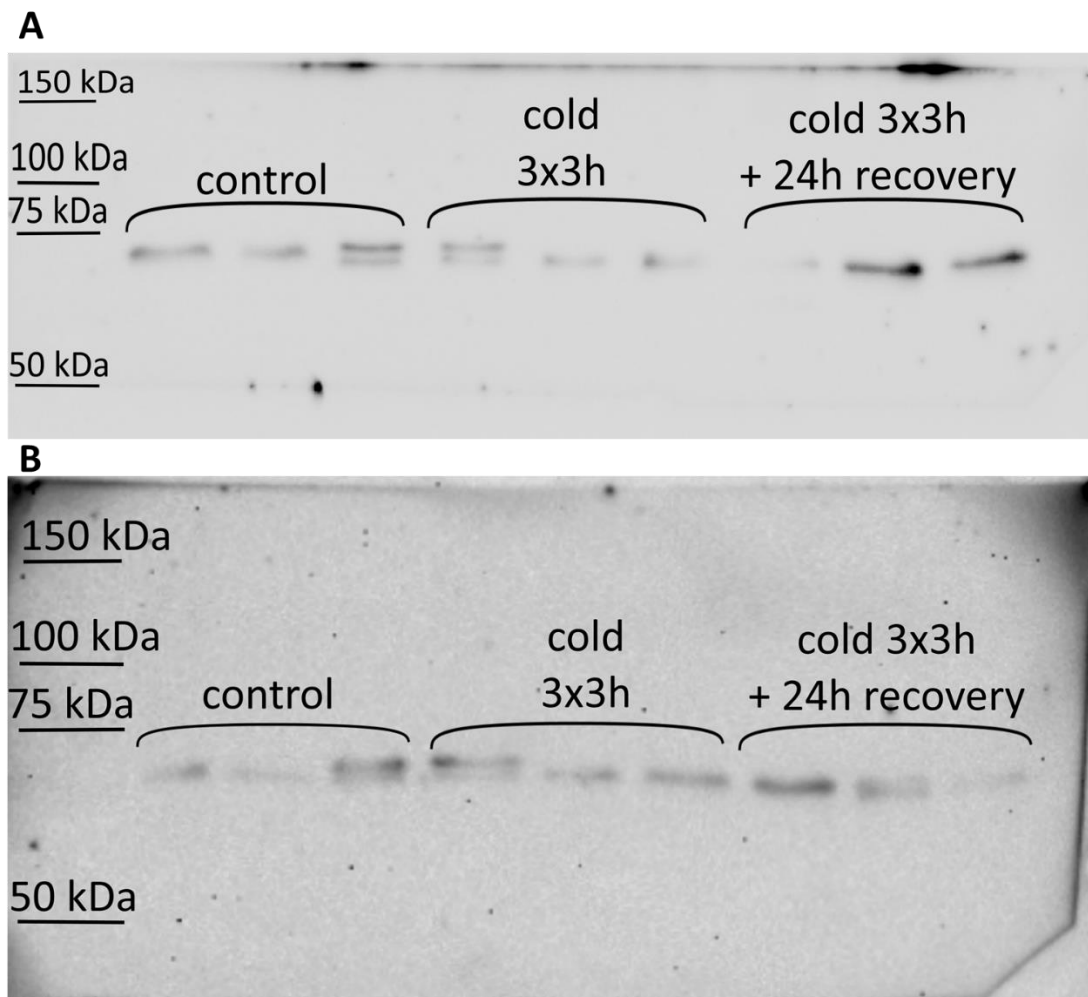


**Figure S2.** Representative western blot immunodetection showing changes in superoxide dismutase (SOD) level in muscle (A) and fat body (B) of *G. coquereliana* after 3x3h cold stress, 3x3h cold stress + 24h

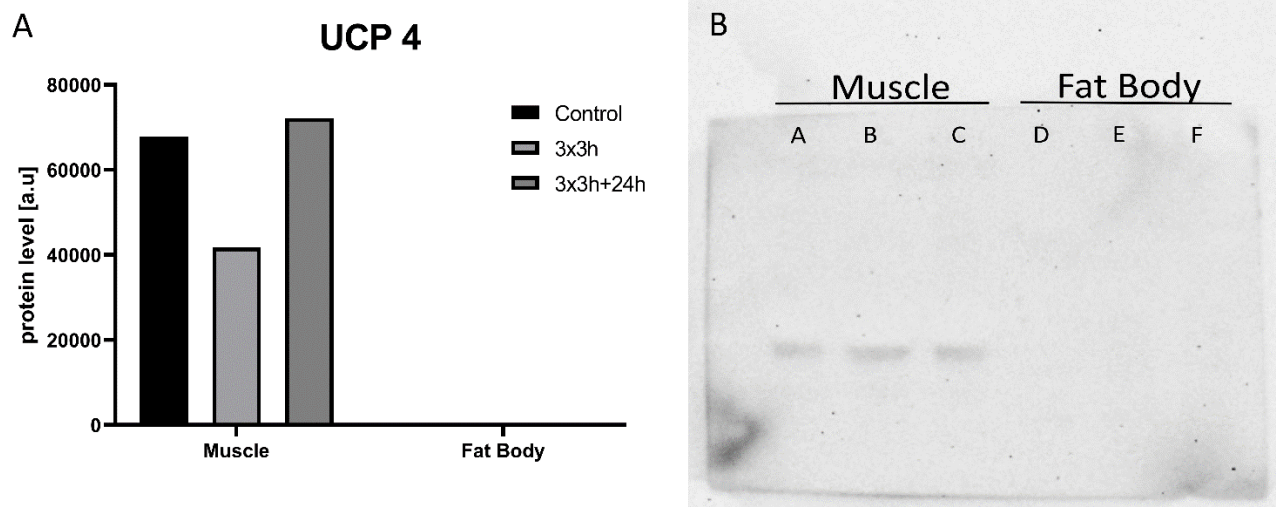
recovery and control. The protein bands were digitally quantified using ImageLab software (BioRad, ver. 6.0.1).



**Figure S3.** Representative western blot immunodetection showing changes in catalase (CAT) level in muscle (A) and fat body (B) of *G. coquereliana* after 3x3h cold stress, 3x3h cold stress + 24h recovery and control. The protein bands were digitally quantified using ImageLab software (BioRad, ver. 6.0.1).



**Figure S4.** Representative western blot immunodetection showing changes in heat shock protein 70 (HSP70) level in muscle (A) and fat body (B) of *G. coquereliana* after 3x3h cold stress, 3x3h cold stress + 24h recovery and control. The protein bands were digitally quantified using ImageLab software (BioRad, ver. 6.0.1).



**Figure S5.** The UCP4 protein level in muscle and fat body tissues of *G. coquereliana* (A) and representative western blot immunodetection showing changes in UCP4 level in muscle and fat body of *G. coquereliana* (B). after 3 x 3 h cold stress, 3 x 3 h cold stress + 24 h recovery and control. The protein bands were digitally quantified using ImageLab software (BioRad, ver. 6.0.1). On the panel B following lines represent: A – muscles after 3 x 3 h cold stress + 24 h recovery, B – muscles after 3 x 3 h cold stress, C – muscles control, D – fat body after 3 x 3 h cold stress + 24 h recovery, E – fat body after 3 x 3 h cold stress and F – fat body control. Despite many attempts, we did not obtain bands of sufficient intensity for the fat body to be quantified. This is probably due to the use of antibodies other than those originally used to identify UCP4 protein in the fat body and muscle tissues of the cockroach by Slocinska et al. (2011). These antibodies are no longer commercially available.

		Muscle		Fat Body	
		3x3h	3x3h+24h	3x3h	3x3h+24h
State 3 respiration					
State 4 respiration					
RCR					
ADP/O ratio					
UCP	Expression				
	Activity				
COX activity					
ATP level					
Superoxide generation					
Lipid peroxidation					
SOD	Expression				
	Activity				
	Level				
CAT	Expression				
	Activity				
	Level				
HSP70	Expression				
	Level				

**Figure S6.** Heat map summarizing the effects of FTR (3 x 3h) and recovery after cold stress (3 x 3h + 24h) on parameters tested in this research. The colors correspond respectively: gray - no change, red - decrease relative to control, green - increase relative to control