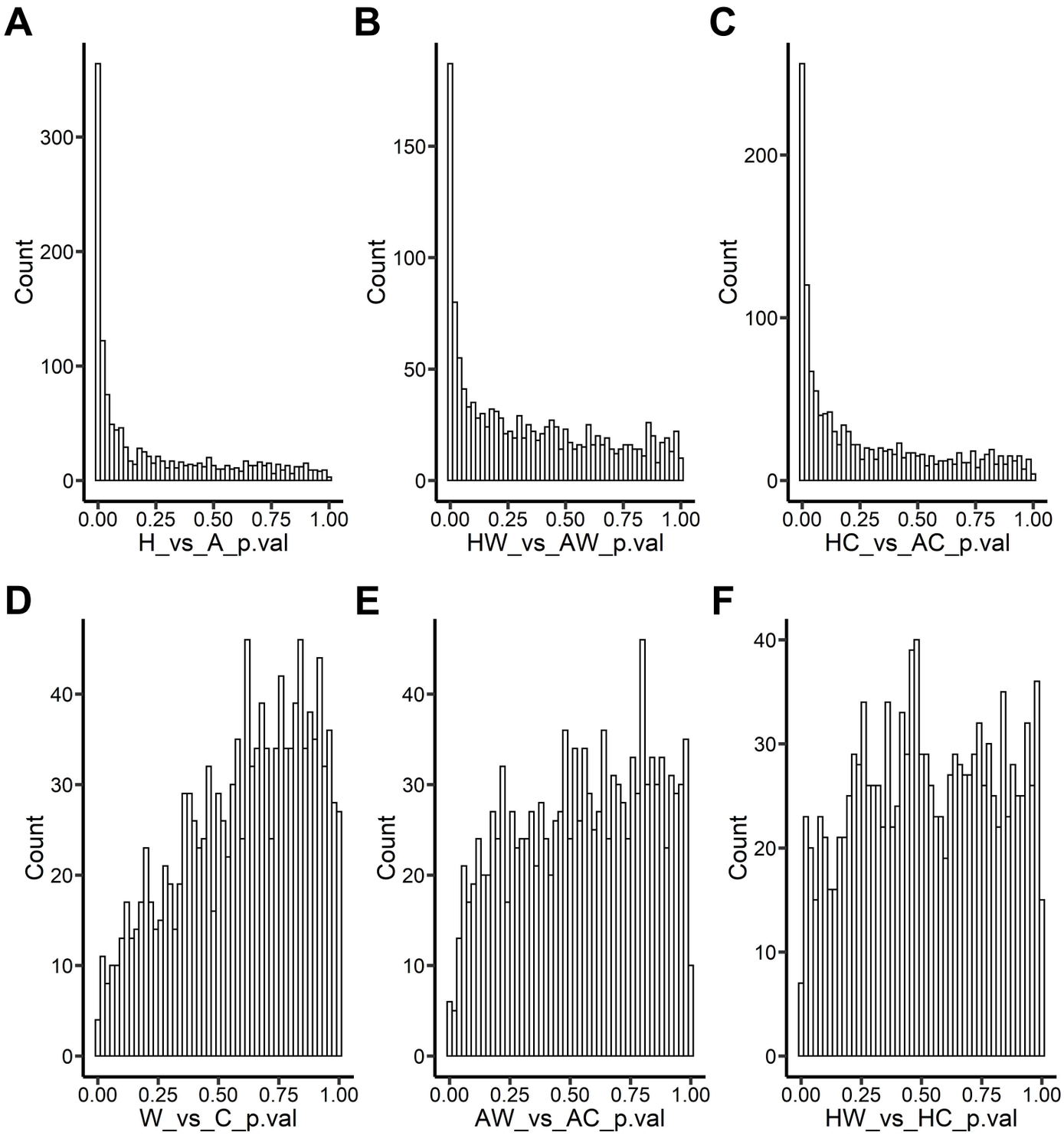

Fig. S1. Histograms of p-values resulting from pairwise comparisons of the abundances of 1316 proteins in the painted turtle cardiac proteome. The top panel shows results from the differential expression analysis for the main effect of developmental *stage* (A) and its interaction with *temperature* in warm-acclimated turtles (B) and cold-acclimated turtles (C). The bottom panel shows results for the main effect of *temperature* (D) and its interaction with developmental *stage* in adult (E) and hatchling (F) turtles. The strong peak and left-skewed histograms indicate that a non-negligible proportion of true positives exist in the dataset, whereas the uniformly distributed p-values suggest no true effects (Pascovici et al. 2016). These results were used to define differentially abundant proteins for *stage* effects, and to reject further consideration of discrete *temperature* effects.

Supplementary Materials and Methods 1. Setting specifications for LC/MS/MS provided by SPARC BioCenter Molecular Analysis (The Hospital for Sick Children, Toronto, ON).

Orbitrap Fusion Lumos Method Summary

Creator: FUSION-LUMOS\Thermo Scientific
Last Modified: 5/30/2019 3:27:40 PM by FUSION-LUMOS\Thermo Scientific

Global Settings

Use Ion Source Settings from Tune = False
Method Duration (min)= 180
Ion Source Type = NSI
Spray Voltage: Positive Ion (V) = 1900
Spray Voltage: Negative Ion (V) = 600
Sweep Gas (Arb) = 0
Ion Transfer Tube Temp (C) = 275
APPI Lamp = Not in use
Pressure Mode = Standard
Default Charge State = 2
Advanced Precursor Determination = False

Experiment 1

Start Time (min) = 0
End Time (min) = 180
Cycle Time (sec) = 3

Scan MasterScan

MSn Level = 1
Use Wide Quad Isolation = False
Detector Type = Orbitrap
Orbitrap Resolution = 120K
Mass Range = Normal
Scan Range (m/z) = 550-1800
Maximum Injection Time (ms) = 50
AGC Target = 400000
Microscans = 1
RF Lens (%) = 30
Use ETD Internal Calibration = False
DataType = Profile
Polarity = Positive

Source Fragmentation = False
Scan Description =

Filter MIPS
MIPS Mode = Peptide

Filter ChargeState
Include undetermined charge states = False
Include charge state(s) = 2-7
Include charge states 25 and higher = False

Filter DynamicExclusion
Exclude after n times = 1
Exclusion duration (s) = 25
Mass Tolerance = ppm
Mass tolerance low = 10
Mass tolerance high = 10
Exclude isotopes = True
Perform dependent scan on single charge state per precursor only = False

Filter IntensityThreshold
Intensity Filter Type = IntensityThreshold
Maximum Intensity = 1E+20
Minimum Intensity = 20000
Relative Intensity Threshold = 0

Data Dependent Properties
Data Dependent Mode= Cycle Time

Scan Event 1

Scan ddMSnScan
MSn Level = 2
Isolation Mode = Quadrupole
Isolation Offset = Off
Isolation Window = 0.7
Reported Mass = Offset Mass
Multi-notch Isolation = False
Scan Range Mode = Auto Normal
FirstMass = 110
Scan Priority= 1
ActivationType = HCD
Is Stepped Collision Energy On = False
Stepped Collision Energy (%) = 5
Collision Energy (%) = 35

Detector Type = Orbitrap
Orbitrap Resolution = 15K
Maximum Injection Time (ms) = 22
AGC Target = 50000
Inject ions for all available parallelizable time = False
Microscans = 1
Use ETD Internal Calibration = False
DataType = Centroid
Polarity = Positive
Source Fragmentation = False
Scan Description =

Sample pickup:
Volume [l] : 5.00
Flow [l / min] : 40.00

Sample loading:
Volume [l] : 12.00
Flow [l / min] : (unspecified)
Max. pressure [Bar] : 900.00

Gradient:

Time [mm:ss]	Duration [mm:ss]	Flow [nl/min]	Mixture [%B]
00:00	00:00	250	2
68:00	68:00	250	20
170:00	102:00	250	38
172:00	02:00	250	100
180:00	08:00	250	100

Pre-column equilibration:
Volume [l] : 10.00
Flow [l / min] : (unspecified)
Max. pressure [Bar] : 800.00

Analytical column equilibration:
Volume [l] : 3.00
Flow [l / min] : (unspecified)
Max. pressure [Bar] : 900.00

Autosampler wash:
Flush volume [l] : 100.00

Table S1. The cardiac proteome of *C. picta bellii*.

The cardiac proteome was described by a total of 1316 high-quality non-redundant protein identifications. The full list of proteins is provided in 3 ways:

1. Ranked by mean abundance across all 15 individual samples (Columns B-D)
2. Ranked by mean abundance in adult turtles only (Columns F-H)
3. Ranked by mean abundance in hatchling turtles only (Columns J-L)

Click here to download Table S1

Table S2. Differentially abundant proteins for the hatchling and adult painted turtle cardiac proteome. The data is organized into:

1. Main effect – stage: Complete list of differentially abundant proteins significant for the main effect stage
2. Interaction - stage × temperature (warm): Complete list of differentially abundant proteins between warm-acclimated adult and hatchling turtles
3. Interaction - stage × temp (cold): Complete list of differentially abundant proteins between cold-acclimated adult and hatchling turtles

Click here to download Table S2

Table S3. GO term enrichment represented by differentially abundant (DA) proteins in the turtle cardiac proteome. The data is organized into:

1. Biological Process: Complete list of enriched GO terms for Biological Process, for up regulated (top) and down regulated (bottom) proteins in adult relative to hatchling turtle hearts
2. Cellular Component: Complete list of enriched GO terms for Cellular Component, for up regulated (top) and down regulated (bottom) proteins in adult relative to hatchling turtle hearts
3. Molecular Function: Complete list of enriched GO terms for Molecular Function, for up regulated (top) and down regulated (bottom) proteins in adult relative to hatchling turtle hearts

Click here to download Table S3