

**Table S1 Sample sizes in experiments.**

Experiment	Sampling point	Pathway	Temperature	Development stage	Sex	Sample size		
SCP	6 days	Diapause	20°C	Endogenous diapause	Female	11		
					Male	13		
		Direct	20°C	Direct development	Female	11		
					Male	7		
	24 days	Cold diapause	2°C	Endogenous diapause	Female	8		
					Male	8		
		Warm diapause	20°C	Endogenous diapause	Female	4		
					Male	4		
	114 days	Cold diapause	2°C	Post-termination quiescence	Female	8		
					Female	8		
		Warm diapause	20°C	Endogenous diapause	Male	4		
					Male	4		
	158 days	Cold diapause	20°C	Post-termination development	Female	8		
					Male	16		
Warm diapause		20°C	Endogenous diapause	Female	8			
				Male	8			
Cold shock tolerance	6 days	Diapause	-5°C	Endogenous diapause	Female	12		
					Male	12		
					-10°C		Female	12
							Male	12
					-15°C		Female	12
							Male	12
					-20°C		Female	12
							Male	12
				Direct	-5°C	Direct development	Female	12
							Male	12
						-10°C	Female	12
							Male	12

			-15°C		Female	12
					Male	12
			-20°C		Female	12
					Male	12
	24 days	Cold diapause	-5°C	Endogenous diapause	Female	12
					Male	12
			-10°C		Female	12
					Male	12
			-15°C		Female	12
					Male	12
			-20°C		Female	12
					Male	12
		Warm diapause	-5°C	Direct development	Female	12
					Male	12
			-10°C		Female	12
					Male	12
			-15°C		Female	12
					Male	12
			-20°C		Female	12
					Male	12
	114 days	Cold diapause	-5°C	Post-termination quiescence	Female	12
					Male	12
			-10°C		Female	12
					Male	12
			-15°C		Female	12
					Male	12
			-20°C		Female	12
					Male	12
	158 days	Cold diapause	-5°C	Post-termination development	Female	12
					Male	12
			-10°C		Female	7

					Male	8
			-15°C		Female	12
					Male	12
			-20°C		Female	7
					Male	7
Metabolome	0 days	Diapause	20°C	Endogenous diapause	Female	6
					Male	6
		Direct	20°C	Direct development	Female	6
					Male	6
	3 days	Diapause	20°C	Endogenous diapause	Female	6
					Male	6
		Direct	20°C	Direct development	Female	6
					Male	6
	6 days	Diapause	20°C	Endogenous diapause	Female	6
					Male	6
		Direct	20°C	Direct development	Female	6
					Male	6
	9 days	Diapause	20°C	Endogenous diapause	Female	6
					Male	6
		Direct	20°C	Direct development	Female	6
					Male	6
	24 days	Diapause	2°C	Endogenous diapause	Female	6
					Male	6
		Warm diapause	20°C	Endogenous diapause	Female	6
					Male	6
	54 days	Diapause	2°C	Endogenous diapause	Female	6
					Male	6
		Warm diapause	20°C	Endogenous diapause	Female	6
					Male	6
	84 days	Diapause	2°C	Around diapause termination	Female	6
					Male	6

		Warm diapause	20°C	Endogenous diapause	Female	6
					Male	6
	114 days	Diapause	2°C	Post-termination quiescence	Female	6
					Male	6
		Warm diapause	20°C	Endogenous diapause	Female	6
					Male	6
	144 days	Diapause	2°C	Post-termination quiescence	Female	6
					Male	6
		Warm diapause	20°C	Endogenous diapause	Female	6
					Male	6
	148 days	Diapause	10°C	Post-termination development	Female	6
					Male	6
	154 days	Diapause	20°C	Post-termination development	Female	6
					Male	6

**Table S2 Generalized linear models explaining supercooling point.** Results are shown split per time point of *Pieris napi* pupae undergoing direct development or diapause at low or high temperature.

Effect	Wald $\chi^2$	d.f.	Sig.
<i>6 (direct vs diapause)</i>			
Intercept	49.609	1	< 0.001
Weight	0.559	1	0.455
Pathway	86.324	1	< 0.001
<i>24 (diapause vs warm diapause)</i>			
Intercept	13.246	1	< 0.001
Weight	4.545	1	0.033
Pathway	11.445	1	0.001
<i>114 (diapause vs warm diapause)</i>			
Intercept	36.421	1	< 0.001
Weight	0.238	1	0.626
Pathway	0.466	1	0.495
<i>158 (diapause vs warm diapause)</i>			
Intercept	35.650	1	< 0.001
Weight	1.734	1	0.188
Pathway	23.602	1	< 0.001

**Table S3 Generalized linear models of cold shock tolerance.** Results for *Pieris napi* pupae undergoing direct development or diapause maintenance or termination development. In A) the data is split by pathway and in B) by time point.

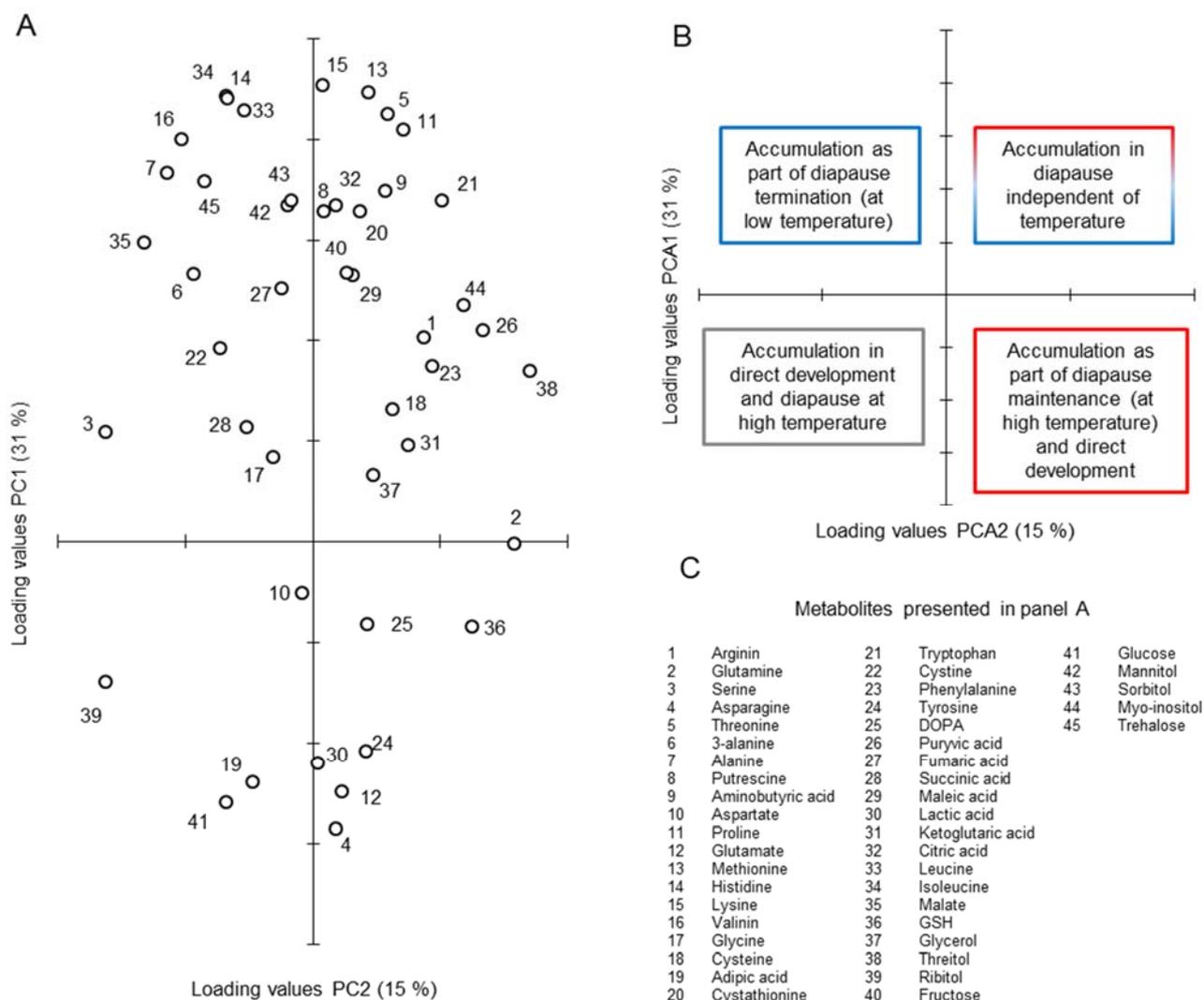
Effect	Wald $\chi^2$	d.f.	Sig.
A) Data split by pathway			
<i>Direct</i>			
Intercept	18.506	1	< 0.001
Temperature	22.567	1	< 0.001
Time point	NA	NA	NA
<i>Diapause termination</i>			
Intercept	47.680	1	< 0.001
Temperature	36.543	1	< 0.001
Time point	26.391	3	< 0.001
<i>Diapause maintenance</i>			
Intercept	0.029	1	0.865
Temperature	0.412	1	0.521
Time point	NA	NA	NA
B) Data split by time point			
<i>6 (Direct vs diapause termination)</i>			
Intercept	35.487	1	< 0.001
Temperature	39.212	1	< 0.001
Pathway	36.967	1	< 0.001
<i>24 (Diapause maintenance vs termination)</i>			
Intercept	2.754	1	0.097
Temperature	2.439	1	0.118
Pathway	4.110	1	0.043
<i>114 (Only diapause maintenance)</i>			
Intercept	14.032	1	< 0.001
Temperature	7.398	1	0.007
Pathway	NA	NA	NA
<i>158 (Only diapause maintenance)</i>			
Intercept	12.556	1	< 0.001
Temperature	18.893	1	< 0.001
Pathway	NA	NA	NA

**Table S4 GLM results for total relative A) sugar and B) free amino acid content.** Results for *Pieris napi* pupae undergoing direct development or diapause maintenance or termination development, full data.

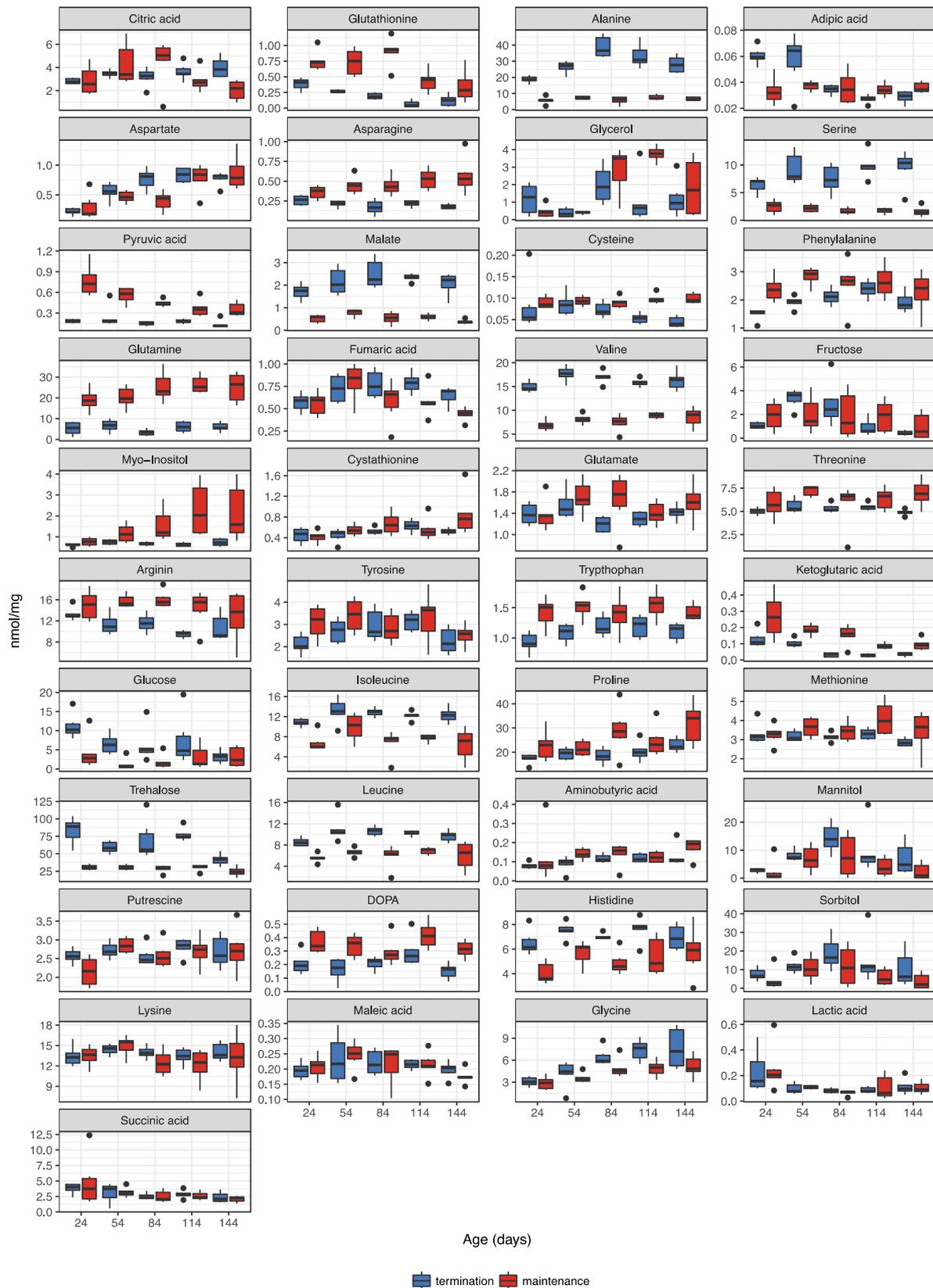
Effect	Wald $\chi^2$	d.f.	Sig.
A) Total sugars			
Intercept	1149.619	1	< 0.001
Path	254.164	2	< 0.001
Age	465.205	11	< 0.001
Path*Age	46.947	8	< 0.001
B) Total free amino acids			
Intercept	14004.079	1	< 0.001
Path	156.634	2	< 0.001
Age	602.515	11	< 0.001
Path*Age	142.880	8	< 0.001

**Table S5 GLM results with data split by time point for total relative A) sugar and B) free amino acid content.** Results for *Pieris napi* pupae undergoing direct development or diapause at high or low temperature.

Age	Effect	A) Total sugars			B) Total free amino acids		
		Wald $\chi^2$	d.f.	Sig.	Wald $\chi^2$	d.f.	Sig.
0	Intercept	169.298	1	< 0.001	902.632	1	< 0.001
	Path	6.908	1	0.009	2.766	1	0.096
3	Intercept	467.491	1	< 0.001	1908.603	1	< 0.001
	Path	23.575	1	< 0.001	35.047	1	< 0.001
6	Intercept	106.532	1	< 0.001	2644.558	1	< 0.001
	Path	16.393	1	< 0.001	111.082	1	< 0.001
9	Intercept	285.903	1	< 0.001	3861.165	1	< 0.001
	Path	123.584	1	< 0.001	843.535	1	< 0.001
24	Intercept	382.828	1	< 0.001	3471.786	1	< 0.001
	Path	71.253	1	< 0.001	0.516	1	0.473
54	Intercept	681.66	1	< 0.001	2553.472	1	< 0.001
	Path	53.433	1	< 0.001	2.652	1	0.103
84	Intercept	104.474	1	< 0.001	1017.160	1	< 0.001
	Path	14.437	1	< 0.001	1.467	1	0.226
114	Intercept	727.55	1	< 0.001	4052.373	1	< 0.001
	Path	117.381	1	< 0.001	2.804	1	0.094
144	Intercept	167.337	1	< 0.001	1900.477	1	< 0.001
	Path	10.727	1	0.001	0.574	1	0.449



**Figure S1** PC analysis of overall metabolomics of direct and diapause development in *Pieris napi*. (A) Loading values for the overall PCA analysis (Fig. 3). (B) Graphical interpretation of loading values according to the three compared groups. (C) Key for the metabolites shown in (A).



**Figure S2 Non-normalized amounts of the 41 analyzed metabolites.** Each panel is the concentration ( $\text{nmol mg}^{-1}$ ) of a single metabolite plotted as a function of time (age in days). Diapause termination is shown as blue and diapause maintenance as red.