Supplemental Results

PG/VG as the Solvent

Plasma THC

Plasma THC levels for mice with PG/VG as the solvent are shown in Figure S2A, B. Analysis of plasma THC levels with a 4-way ANOVA (Age, Sex, Dose, and Timepoint) revealed significant 4-way interaction [$F_{2,126}$ = 7.53, p=0.0008]. Tukey's post-hoc analyses indicated that adolescent males exposed to 300 mg/ml cannabis vapor reached higher plasma THC levels at 0 min relative to their female counterparts (post-hoc: p=0.0068). There were also significant time-dependent decreases in plasma THC levels with the highest level measured at the 0 min timepoint (post-hoc: Adolescent F CAN150: 0 min > 30 min, p=0.0051, 0 min > 60 min, p=0.0005; Adolescent M CAN150: 0 min > 30 min, p<0.0001, 0 min > 60 min, p<0.0001; Adolescent M CAN300: 0 min > 30 min, p=0.0190, 0 min > 60 min, p=0.0011; Adult F CAN300: 0 min > 30 min, p=0.0600; 0 min > 60 min, p=0.0029; Adult M CAN150: 0 min > 30 min, p<0.0001, 0 min > 60 min, p<0.0001). It is important to note that in contrast to plasma THC levels when PEG was the solvent, these timepoint effects were not present in all groups for each dose when PG/VG was the solvent. Moreover, at the 0 min timepoint, plasma THC levels were higher in adolescent female (post-hoc: p=0.0141) and adult male mice (post-hoc: p=0.0012) exposed to 150 mg/ml cannabis vapor compared to 300 mg/ml cannabis vapor—a dose-dependent effect in the opposite direction as was expected. These findings may provide further examples of the instability issue of cannabis extract when dissolved in PG/VG.

The large proportion of samples with plasma THC metabolites—11-OH-THC and THC-COOH—with values below the limit of detection when the solvent was PG/VG precluded our analysis of plasma THC metabolites.

Behavior

Linear regression analyses of plasma THC levels (z-scores) for mice with PG/VG as the solvent indicated that plasma THC was not significantly correlated with any of the three behavioral outcomes—body temperature difference [adj r^2=-0.0087, p=0.8185; Supplemental Figure S3A], hot plate withdrawal latency difference [adj r^2=-0.0083, p=0.6388; Supplemental Figure S3C], and total movement [adj r^2=-0.0029, p=0.3973; Supplemental Figure S3E].

Group differences in behavioral measures when PG/VG was the solvent were analyzed with 3-way (age, sex, dose) ANOVAs. A significant Sex x Vapor interaction [$F_{2,159}$ = 3.29, p=0.0398] for body temperature difference scores reflected that male mice exposed to either dose of cannabis vapor had lower body temperature difference scores compared to mice exposed to vehicle vapor, indicating a lower body temperature post-exposure (post-hoc: CAN300 vs. vehicle, p=0.0011, CAN150 vs. vehicle, p=0.0192; Supplemental Figure S3B). Males had higher hot plate difference scores compared to females [main effect of Sex: $F_{1,137}$ = 5.73, p=0.0180]. Exposure to CAN300 vapor produced a higher hot plate difference score in adolescent mice [Age x Vapor interaction: $F_{2,137}$ = 5.73, p=0.0041], indicating greater analgesia after vapor exposure compared to CAN150 (post-hoc: p=0.0048) and vehicle vapor-exposed mice (post-hoc: p=0.0239; Supplemental Figure S3D). For locomotor activity in the open field (Supplemental Figure S3F), there was a significant Age x Sex interaction [$F_{1,142}$ = 4.25, p=0.0410]; however, none of the Tukey's post-hoc comparisons were significant.

Supplemental Tables

Table S1. Group information for mice who met exclusion criteria for behavioral endpoints.

	Solvent	Dose	Adolescent Females	Adolescent Males	Adult Females	Adult Males
Body Temperature	PG/VG	Vehicle	0	0	0	0
		CAN150	0	0	0	0
		CAN300	0	0	0	0
					Total excluded: 0	
	PEG	Vehicle	0	0	0	0
		CAN150	0	0	4	0
		CAN300	0	0	0	0
					Total excluded: 4	
Hot Plate	PG/VG	Vehicle	0	1	1	4
		CAN150	1	2	4	1
		CAN300	1	3	1	3
					Total excluded: 22	
	PEG	Vehicle	0	0	0	0
		CAN150	2	0	1	1
		CAN300	1	0	0	2
					Total excluded: 7	
Locomotion	PG/VG	Vehicle	2	0	0	0
		CAN150	2	0	3	6
		CAN300	0	0	2	2
					Total excluded: 17	
	PEG	Vehicle	0	0	0	0
		CAN150	0	0	6	0
		CAN300	0	0	0	0
					Total excluded: 6	

Table S2. Number of plasma THC samples below the detection limit (<1 ng/ml) per group.

Plasma THC						
Solvent	**Dose**	**Timepoint**	**Adolescent Females**	**Adolescent Males**	**Adult Females**	**Adult Males**
PG/VG	CAN150	0 min	0	0	0	0
		30 min	0	0	2	0
		60 min	0	1	3	0
	CAN300	0 min	2	0	0	0
		30 min	0	1	0	0
		60 min	2	1	2	0
					Total (<1 ng/ml): 14	
PEG	CAN150	0 min	0	0	0	0
		30 min	0	0	0	0
		60 min	0	0	1	0
	CAN300	0 min	0	0	0	0
		30 min	0	0	0	0
		60 min	0	0	0	0
					Total (<1 ng/ml): 1	

Table S3. Number of plasma 11-OH-THC samples below the detection limit (<1 ng/ml) per group.

Plasma 11-OH-THC						
Solvent	**Dose**	**Timepoint**	**Adolescent Females**	**Adolescent Males**	**Adult Females**	**Adult Males**
PV/VG	CAN150	0 min	1	1	0	2
		30 min	1	2	3	5
		60 min	4	2	7	3
	CAN300	0 min	5	1	1	3
		30 min	6	3	4	3
		60 min	3	4	6	3
					Total (<1 ng/ml): 73	
PEG	CAN150	0 min	1	0	0	0
		30 min	0	0	0	0
		60 min	0	3	2	0
	CAN300	0 min	0	1	0	0
		30 min	1	0	0	0
		60 min	0	1	2	0
					Total (<1 ng/ml): 11	

Table S4. Number of plasma THC-COOH samples below the detection limit (<1 ng/ml) per group.

Plasma THC-COOH						
Solvent	**Dose**	**Timepoint**	**Adolescent Females**	**Adolescent Males**	**Adult Females**	**Adult Males**
PV/VG	CAN150	0 min	0	0	0	1
		30 min	1	0	2	1
		60 min	0	1	3	1
	CAN300	0 min	5	0	0	1
		30 min	0	2	0	0
		60 min	2	1	2	0
					Total (<1 ng/ml): 23	
PEG	CAN150	0 min	1	0	0	0
		30 min	0	0	0	0
		60 min	1	1	1	0
	CAN300	0 min	0	1	0	0
		30 min	0	0	0	0
		60 min	0	0	1	0
					Total (<1 ng/ml): 6	

Table S5. Statistical effects from a 5-way ANOVA with Solvent (PEG or PG/VG) as a factor for plasma THC levels.

Plasma THC			
Effect	**DF**	**F**	***p***
Solvent	1,251	368.98	***<0.0001**
Dose	1,251	21.66	***<0.0001**
Sex	1,251	2.33	0.1280
Age	1,251	0.54	0.4626
Timepoint	2,251	239.31	***<0.0001**
Age*Sex	1,251	0.69	0.4068
Age*Dose	1,251	3.06	0.0816
Age*Solvent	1,251	1.35	0.2458
Age*Timepoint	2,251	1.24	0.2904
Sex*Dose	1,251	2.29	0.1313
Sex*Solvent	1,251	0.47	0.4957
Sex*Timepoint	2,251	0.14	0.8701
Dose*Solvent	1,251	40.92	***<0.0001**
Dose*Timepoint	2,251	7.96	***0.0004**
Solvent*Timepoint	2,251	103.35	***<0.0001**
Age*Sex*Dose	1,251	0.18	0.6726
Age*Sex*Solvent	1,251	5.13	***0.0243**
Age*Sex*Timepoint	2,251	5.10	***0.0068**
Age*Dose*Solvent	1,251	5.63	***0.0184**
Age*Dose*Timepoint	2,251	0.42	0.6588
Age*Solvent*Timepoint	2,251	1.23	0.2929
Sex*Dose*Solvent	1,251	0.76	0.3853
Sex*Dose*Timepoint	2,251	3.62	***0.0282**
Sex*Solvent*Timepoint	2,251	1.78	0.1702
Dose*Solvent*Timepoint	2,251	17.76	***<0.0001**
Age*Sex*Dose*Solvent	1,251	4.06	***0.0451**
Age*Sex*Dose*Timepoint	2,251	0.22	0.8053
Age*Sex*Solvent*Timepoint	2,251	7.53	***0.0007**
Age*Dose*Solvent*Timepoint	2,251	1.03	0.3572
Sex*Dose*Solvent*Timepoint	2,251	2.22	0.1111
Age*Sex*Dose*Solvent*Timepoint	2,251	4.83	***0.0088**

Table S6. Notable Tukey's post-hoc comparisons for plasma THC from the significant 5-way interaction in Table S5. Significant comparisons and their *p*-value are in bold.

Plasma THC - Age*Sex*Dose*Solvent*Timepoint interaction					
Solvent	**Age**	**Sex**	**Dose**	**Timepoint**	***p***
PEG	Adolescent	Female	CAN150	**0 > 30 min**	***0.0020**
				0 > 60 min	***<0.0001**
				30 = 60 min	1.0000
			CAN300	**0 > 30 min**	***<0.0001**
				0 > 60 min	***<0.0001**
				30 = 60 min	1.0000
PEG	Adolescent	Male	CAN150	**0 > 30 min**	***0.0152**
				0 > 60 min	***0.0042**
				30 = 60 min	1.0000
			CAN300	**0 > 30 min**	***<0.0001**
				0 > 60 min	***<0.0001**
				30 = 60 min	0.8552
PEG	Adult	Female	CAN150	**0 > 30 min**	***<0.0001**
				0 > 60 min	***<0.0001**
				30 = 60 min	0.9805
			CAN300	**0 > 30 min**	***<0.0001**
				0 > 60 min	***<0.0001**
				30 = 60 min	1.0000
PEG	Adult	Male	CAN150	**0 > 30 min**	***<0.0001**
				0 > 60 min	***<0.0001**
				30 = 60 min	1.0000
			CAN300	**0 > 30 min**	***<0.0001**
				0 > 60 min	***<0.0001**
				30 = 60 min	1.0000
PG/VG	Adolescent	Female	CAN150	0 = 30 min	0.9990
				0 = 60 min	0.9886
				30 = 60 min	1.0000
			CAN300	0 = 30 min	1.0000
				0 = 60 min	0.9853
				30 = 60 min	1.0000
PG/VG	Adolescent	Male	CAN150	0 = 30 min	0.9583
				0 = 60 min	0.7297
				30 = 60 min	1.0000
			CAN300	0 = 30 min	0.9999
				0 = 60 min	0.9941
				30 = 60 min	1.0000
PG/VG	Adult	Female	CAN150	0 = 30 min	1.0000
				0 = 60 min	1.0000
				30 = 60 min	1.0000
			CAN300	0 = 30 min	1.0000
				0 = 60 min	0.9979
				30 = 60 min	1.0000
PG/VG	Adult	Male	CAN150	0 = 30 min	0.4370
				0 = 60 min	0.4818

				30 = 60 min	1.0000
			CAN300	0 = 30 min	1.0000
				0 = 60 min	1.0000
				30 = 60 min	1.0000
PEG	**Adol > Adult**	Female	CAN300	0 min	***0.0159**
PG/VG	Adol = Adult	Female	CAN300	0 min	1.0000
PEG	Adolescent	**F > M**	CAN300	0 min	***<0.0001**
PG/VG	Adolescent	F = M	CAN300	0 min	0.9993
PEG	Adolescent	Female	**CAN300 > 150**	0 min	***<0.0001**
PG/VG	Adolescent	Female	CAN300 = 150	0 min	0.9998
PEG = PG/VG	Adolescent	Female	CAN150	0 min	0.0564
PEG > PG/VG	Adolescent	Female	CAN300	0 min	***<0.0001**
PEG = PG/VG	Adolescent	Male	CAN150	0 min	0.2880
PEG > PG/VG	Adolescent	Male	CAN300	0 min	***<0.0001**
PEG > PG/VG	Adult	Female	CAN150	0 min	***0.0032**
PEG > PG/VG	Adult	Female	CAN300	0 min	***<0.0001**
PEG > PG/VG	Adult	Male	CAN150	0 min	***<0.0001**
PEG > PG/VG	Adult	Male	CAN300	0 min	***<0.0001**

Table S7. Statistical effects from a 4-way ANOVA with Solvent (PEG or PG/VG) as a factor for body temperature difference scores (post-exposure body temperature – pre-exposure body temperature).

Body Temperature Difference Score			
Effect	**DF**	**F**	***p***
Solvent	1,293	90.46	***<0.0001**
Vapor	2,293	31.58	***<0.0001**
Sex	1,293	0.93	0.3364
Age	1,293	3.62	0.0580
Age*Sex	1,293	2.56	0.1106
Age*Vapor	2,293	2.02	0.1339
Age*Solvent	1,293	0.02	0.9005
Sex*Vapor	2,293	3.18	***0.0430**
Sex*Solvent	1,293	0.95	0.3293
Vapor*Solvent	2,293	9.91	***<0.0001**
Age*Sex*Vapor	2,293	0.19	0.8268
Age*Sex*Solvent	2,293	0.02	0.8858
Age*Vapor*Solvent	2,293	0.54	0.5856
Sex*Vapor*Solvent	2,293	0.31	0.7325
Age*Sex*Vapor*Solvent	2,293	1.16	0.3152

Table S8. Statistical effects from a 4-way ANOVA with Solvent (PEG or PG/VG) as a factor for withdrawal latency difference scores (post-exposure latency – pre-exposure latency) in the hot plate test.

Withdrawal Latency Difference Score			
Effect	**DF**	**F**	***p***
Solvent	1,268	19.96	***<0.0001**
Vapor	2,268	17.48	***<0.0001**
Sex	1,268	3.86	0.0505
Age	1,268	0.01	0.9372
Age*Sex	1,268	0.00	0.9972
Age*Vapor	2,268	3.37	***0.0360**
Age*Solvent	1,268	0.02	0.8953
Sex*Vapor	2,268	0.44	0.6462
Sex*Solvent	1,268	2.13	0.1455
Vapor*Solvent	2,268	3.61	***0.0284**
Age*Sex*Vapor	2,268	0.05	0.9553
Age*Sex*Solvent	2,268	4.21	***0.0412**
Age*Vapor*Solvent	2,268	2.62	0.0747
Sex*Vapor*Solvent	2,268	1.03	0.3582
Age*Sex*Vapor*Solvent	2,268	0.49	0.6114

Table S9. Statistical effects from a 4-way ANOVA with Solvent (PEG or PG/VG) as a factor for total movement (cm) in the open field.

Total Movement (cm)			
Effect	**DF**	**F**	***p***
Solvent	1,274	26.59	***<0.0001**
Vapor	2,274	13.23	***<0.0001**
Sex	1,274	3.91	***0.0490**
Age	1,274	0.65	0.4199
Age*Sex	1,274	2.39	0.1236
Age*Vapor	2,274	0.25	0.7773
Age*Solvent	1,274	0.17	0.6782
Sex*Vapor	2,274	4.55	***0.0114**
Sex*Solvent	1,274	0.54	0.4633
Vapor*Solvent	2,274	11.96	***<0.0001**
Age*Sex*Vapor	2,274	1.43	0.2417
Age*Sex*Solvent	2,274	2.40	0.1222
Age*Vapor*Solvent	2,274	4.89	***0.0082**
Sex*Vapor*Solvent	2,274	0.10	0.9013
Age*Sex*Vapor*Solvent	2,274	1.41	0.2467

Supplemental Figures

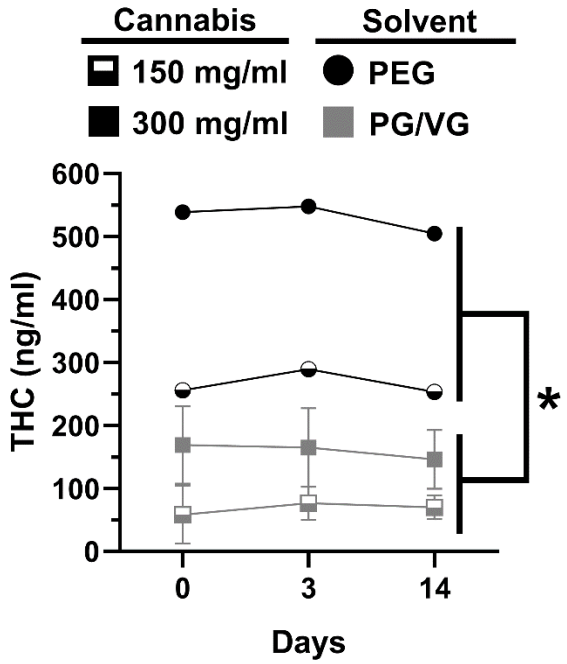

Figure S1. Time course of THC levels (ng/ml) in cannabis extract solutions (150 mg/ml, 300 mg/ml) made with different solvents (PEG, PG/VG). THC levels were significantly higher in cannabis extract solutions dissolved in PEG compared to PG/VG (main effect of Solvent: *p<0.05).

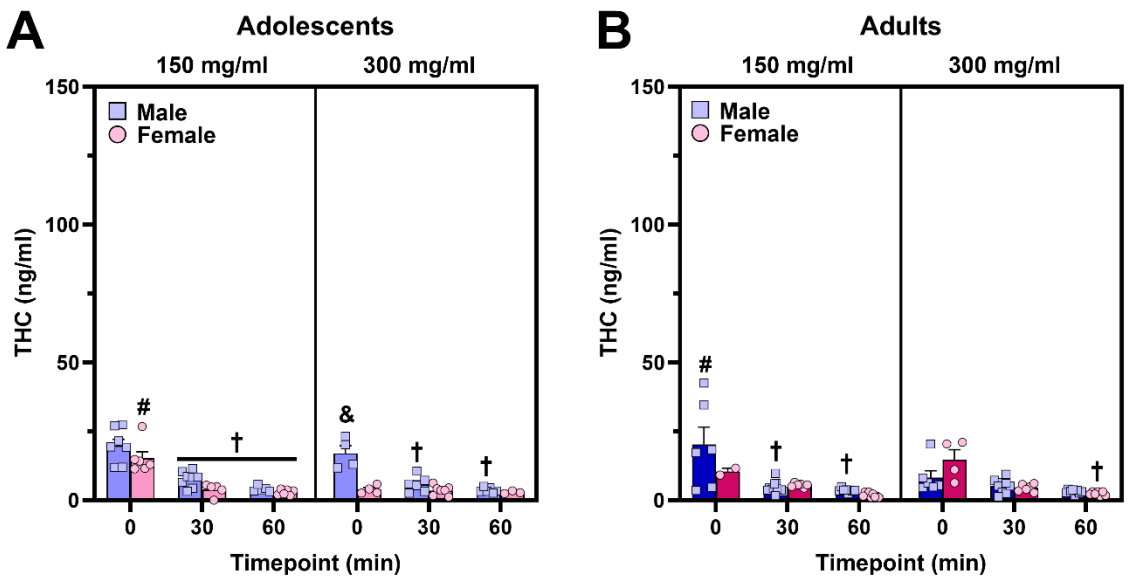

Figure S2. Time course of plasma THC and metabolites following 30-min vapor exposure session with PG/VG as the solvent. (**A,B**) Plasma THC levels when mice were exposed to cannabis dissolved in PG/VG showed time-dependent decreases (*post-hoc*: $^{\dagger}p<0.05$, vs. 0 min timepoint within age, sex, and dose), although this was not present in all groups at both doses. There were unexpected dose-dependent *decreases* in plasma THC when comparing CAN150 and CAN300 groups (*post-hoc*: $^{\#}p<0.05$, vs. CAN300 within sex, age, and timepoint). Adolescent male mice exposed to 300 mg/ml cannabis vapor reached higher plasma THC levels relative to their female counterparts at the 0 min timepoint (*post-hoc*: $^{\&}p<0.01$, vs. adolescent females at the 0 min timepoint).

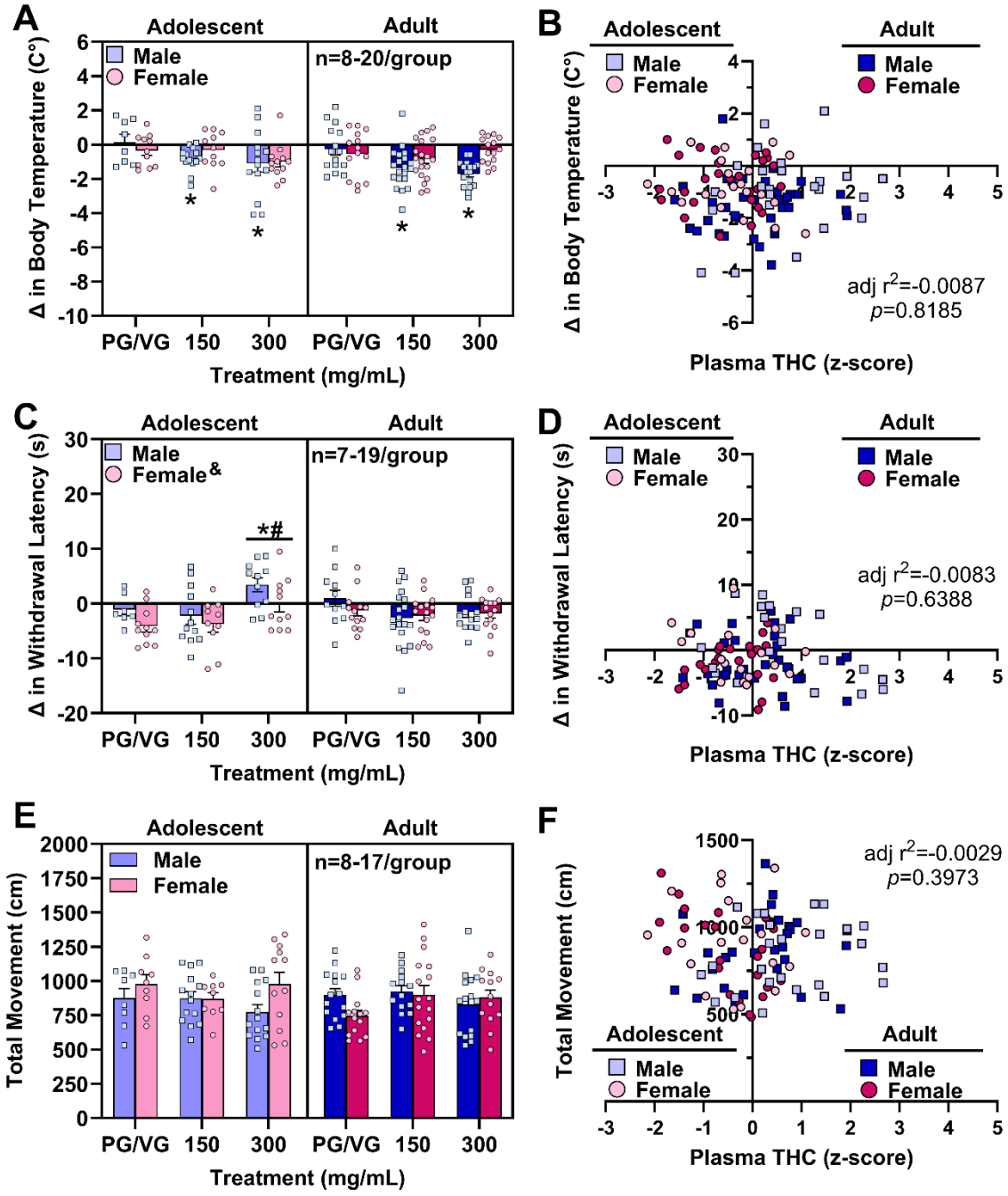

Figure S3. Vaporized cannabis extract dissolved in PG/VG induced hypothermia in male mice and antinociception in adolescent mice; however, plasma THC levels were not significantly correlated with behavioral endpoints. (**A**) Male mice exposed to 150 and 300 mg/ml cannabis vapor exhibited hypothermia relative to mice exposed to vehicle vapor (*post-hoc:* **p's*<0.05), regardless of age. (**B**) Plasma THC levels did not significantly predict changes in body temperature. (**C**) Adolescent mice exposed to 300 mg/ml cannabis vapor displayed greater antinociception relative to mice exposed to the lower dose of cannabis vapor (*post-hoc:* $^{\#}p$<0.01) or vehicle vapor (*post-hoc:* **p*<0.05), regardless of sex. Males had higher withdrawal latency

difference scores than females (main effect of Sex: $^{\&}p<0.05$). (**D**) Plasma THC levels did not significantly predict changes in withdrawal latency in the hot plate test. (**E**) Cannabis vapor at either dose did not significantly impact locomotor activity. (**F**) Plasma THC levels did not significantly predict locomotor activity.