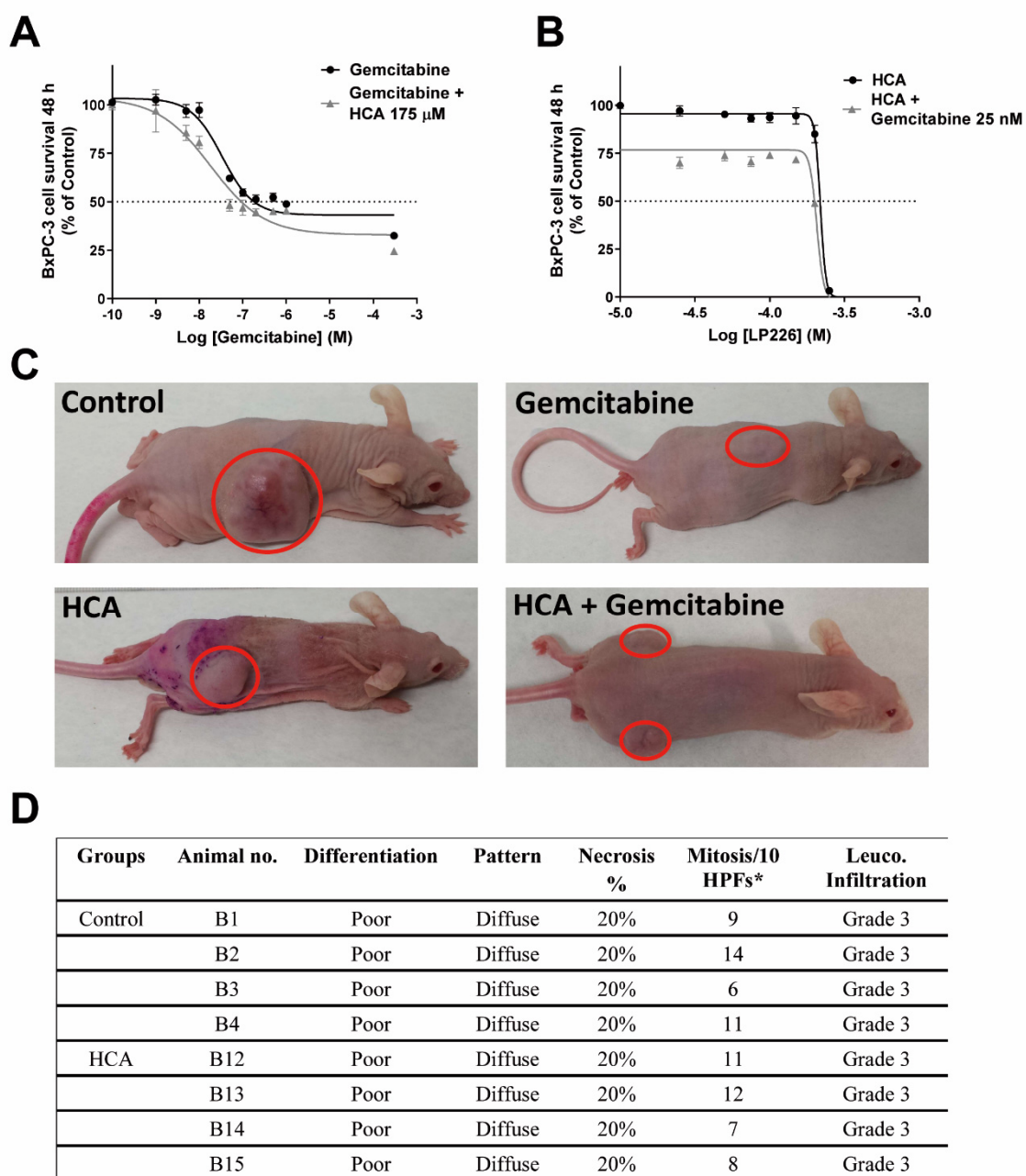


## SUPPLEMENTARY TABLE

**Table S1.** Efficacy of HCA, gemcitabine, and the combination of HCA and gemcitabine against different pancreatic cancer cell lines. The IC<sub>50</sub> values for HCA, gemcitabine, and the combination of HCA and gemcitabine against pancreatic cancer cell proliferation after a 48 h treatment (mean  $\pm$  SEM from 3 independent experiments performed in quadruplet). HCA, 2-hydroxycervonic acid.

Cell line	Treatment 48 h	IC <sub>50</sub>
MIA PaCa-2	Gemcitabine	55 $\pm$ 21 nM
	HCA	205 $\pm$ 05 $\mu$ M
	Gemcitabine + HCA 175 $\mu$ M	2 $\pm$ 1.9 nM
	HCA + Gemcitabine 25 nM	145 $\pm$ 10 $\mu$ M
BxPC-3	Gemcitabine	180 $\pm$ 25 nM
	HCA	218 $\pm$ 06 $\mu$ M
	Gemcitabine + HCA 175 $\mu$ M	93 $\pm$ 40 nM
	HCA + Gemcitabine 25 nM	199 $\pm$ 02 $\mu$ M
PANC-1	Gemcitabine	—
	HCA	232 $\pm$ 16 $\mu$ M

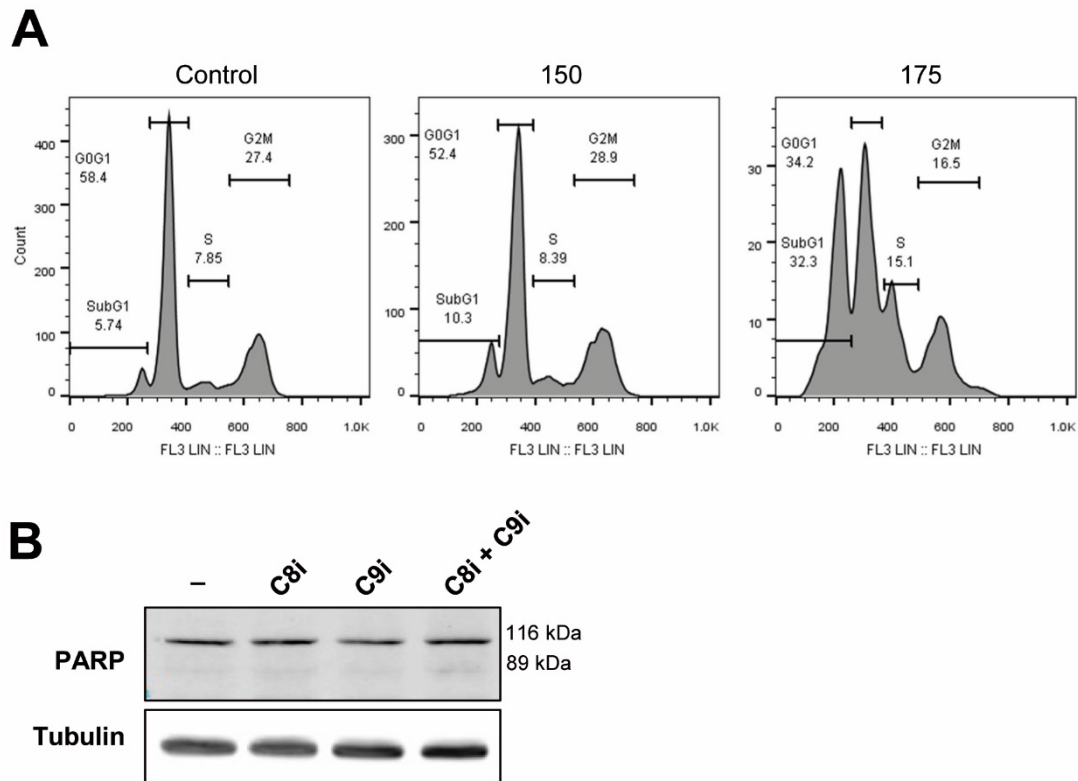
## SUPPLEMENTARY FIGURES



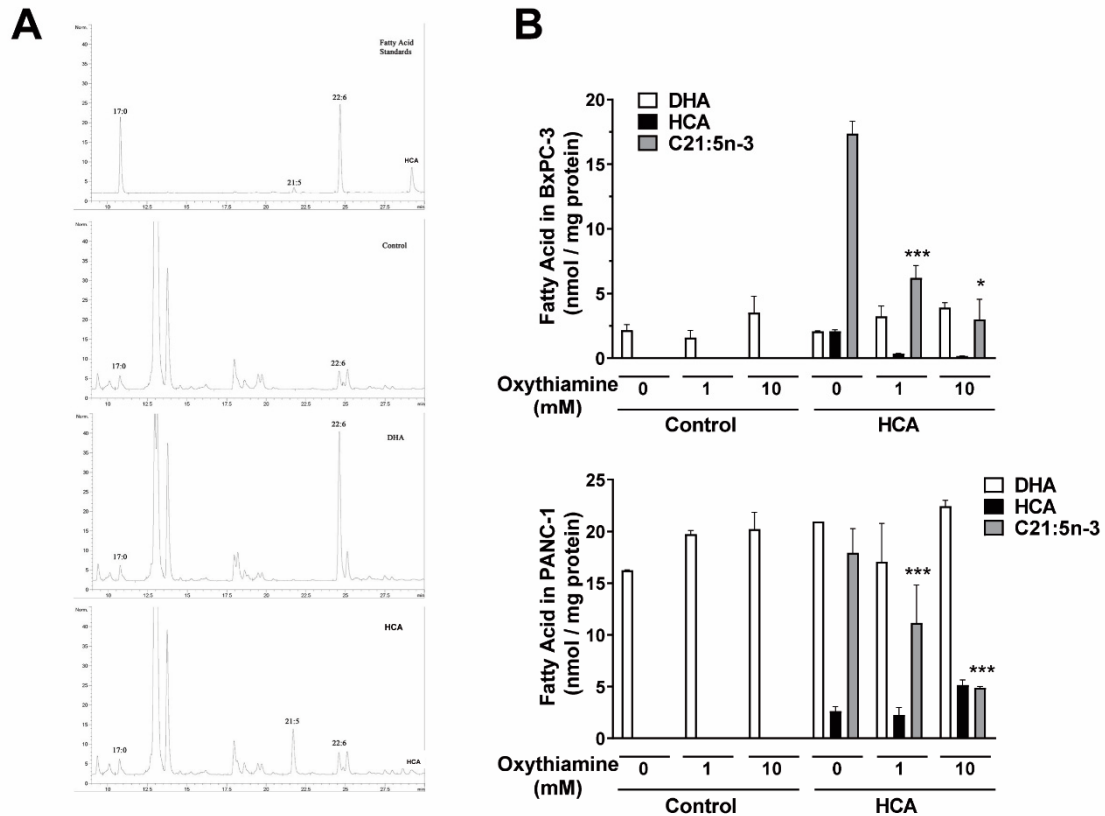
\*Mitotic count per 10 high-power fields.

**Figure S1.** Efficacy of HCA to combat pancreatic cancer. (A) Concentration-dependent inhibition of human pancreatic cell (BxPC-3) cell growth by gemcitabine and gemcitabine in combination with HCA (175  $\mu$ M) for 48 h. (B) Concentration-dependent inhibition of human pancreatic cell (MIA PaCa-2) growth by HCA and HCA in combination with gemcitabine (25 nM) for 48 h (mean  $\pm$  SEM from 3 independent experiments performed in quadruplet). (C) Representative photographs of mice that received a xenograft of MIA PaCa-2 cells and treated with the vehicle alone (Control), gemcitabine (100 mg/kg, ip, twice weekly), HCA (200 mg/kg, p.o., daily), or HCA + gemcitabine (100 mg/kg, p.o., daily + 100 mg/kg, i.p., once weekly) over a 40-day treatment. (D) Analysis of histopathological images of tumors. Grading used at finding level: Grade 1 (minimal/very few/very

small), grade 2 (slight/few/small), grade 3 (moderate/moderate number/moderate size), grade 4 (marked/many/large), grade 5 (severe/massive). HCA, 2-hydroxycervonic acid.



**Figure S2.** Figure 2. HCA induces intrinsic and extrinsic apoptosis in pancreatic cancer cells. (A) Representative histogram of the cell cycle phases at different phases of the cell cycle: distribution of MIA PaCa-2 cells incubated with or without HCA (150 or 175  $\mu$ M) for 48 h. (B) Representative immunoblot of the effects of C8i (inhibitor zIETD-fmk, 50  $\mu$ M), C9i (inhibitor zLEHD-fmk, 50  $\mu$ M) and C8i+C9i (zIETD-fmk+zLEHD-fmk, 50 + 50  $\mu$ M) on PARP proteolysis in MIA PaCa-2 cells for 48 h. C8i, caspase 8 inhibitor (zIETD-fmk); C9i, caspase 9 inhibitor (zLEHD-fmk); C8i+C9i, caspase 8 inhibitor + caspase 9 inhibitor (zIETD-fmk+zLEHD-fmk); PARP, Poly (ADP-Ribose) Polymerase.



**Figure S3.** Effect of HCA on the fatty acid composition of pancreatic cancer cells. (A) Amplified regions of representative GC-FID chromatograms showing the fatty acid composition following different treatments of MIA PaCa-2 cells: fatty acid standards, control (untreated, 48 h), DHA (100  $\mu$ M, 48 h) and HCA (150  $\mu$ M, 48 h). The peaks of interest were identified with standards based on their retention time. (B) BxPC-3 and PANC-1 cells were pretreated with oxythiamine (OT, 1 or 10 mM) for 1.5 h before exposure to HCA (150  $\mu$ M) for 48 h, and the cell's lipids were extracted and quantified by GC (bars correspond to the mean  $\pm$  SEM values of 3 independent experiments). HCA, 2-hydroxycervonic acid; DHA, docosahexaenoic acid; C21:5n-3, heneicosapentaenoic acid. \*  $p < 0.05$ , \*\*\*  $p < 0.001$

		Control	C21:5n-3					
			50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg	1500 mg/kg
Physical factors and gross appearance								
Body weight (g) 1 h		31.7 ± 5.6	38.4 ± 0.4	38.8 ± 3.5	38.2 ± 3.3	41.3 ± 5.4	42.4 ± 4.6	39.2 ± 9.3
Body weight (g) 24 h		31.2 ± 5.3	36.5 ± 1.2	37.5 ± 3.5	37.5 ± 3.3	41.9 ± 5.5	38.4 ± 4.1	37.0 ± 9.6
Presence of whiskers		3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
Appearance of fur		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Patches of missing fur on face		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Patches of missing fur on body		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Wounds		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Observation of behavior in a novel environment								
Transfer behavior		5.0 ± 0.0	4.7 ± 0.6	5.0 ± 0.0	4.3 ± 0.6	4.7 ± 0.6	5.0 ± 0.0	4.8 ± 0.5
Body position		3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
Spontaneous activity		2.0 ± 0.0	1.3 ± 0.6	1.3 ± 0.6	1.0 ± 0.0	1.3 ± 0.6	1.3 ± 0.6	1.3 ± 0.5
Respiration rate		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Tremor		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5
Palpebral closure		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Piloerection		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Gait		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Tail elevation		1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Urination		0.0 ± 0.0	0.3 ± 0.6	0.0 ± 0.0	0.3 ± 0.6	0.3 ± 0.6	0.7 ± 0.6	0.5 ± 0.6
Defecation		2.5 ± 0.7	1.3 ± 1.5	0.3 ± 0.6	0.7 ± 1.2	1.0 ± 1.7	0.3 ± 0.6	0.0 ± 0.0
Reflexes and reactions to simple stimuli								
Touch escape		3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
Positional passivity		1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.7 ± 0.6	0.3 ± 0.6	0.5 ± 0.6
Trunk curl		1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Reaching reflex		3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
Body tone		1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Measures recorded during supine restraint								
Plantar surface skin color		1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Heart rate		1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Limb tone		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Abdominal tone		1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Righting reflex		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Air Righting reflex		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Salivation		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Provoked Biting		1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.7 ± 0.6	1.0 ± 0.0
Motor coordination								
Grip strength		3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
Wire maneuver		0.0 ± 0.0	0.3 ± 0.6	0.0 ± 0.0	0.3 ± 0.6	0.3 ± 0.6	0.7 ± 0.6	1.0 ± 0.0
Inverted screen (s)		60.0 ± 0.0	53.3 ± 11.5	58.3 ± 2.9	60.0 ± 0.0	60.0 ± 0.0	58.3 ± 2.9	49.0 ± 12.2
Pole climb		1.5 ± 0.7	0.3 ± 0.6	0.3 ± 0.6	1.0 ± 1.7	0.7 ± 1.2	0.0 ± 0.0	0.8 ± 1.5

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**Physical factors and gross appearance**

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Body weight	Animal weight (g)
Presence of whiskers	0 = None; 1 = A few; 2 = Most, but not a full set; 3 = A full set
Appearance of fur	0 = Ungroomed and disheveled; 1 = Somewhat disheveled; 2 = Well-groomed (normal)
Piloerection	0 = None; 1 = Most hairs standing on end
Patches of missing fur on face	0 = None; 1 = Some; 2 = Extensive Patches
Patches of missing fur on body	0 = None; 1 = Some; 2 = Extensive
Wounds	0 = None; 1 = Signs of previous wounding; 2 = Slight wounds present; 3 = Moderate wounds present; 4 = Extensive wounds present

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**Observation of behavior in a novel environment**

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Transfer behavior	0 = Coma; 1 = Prolonged freeze (>10 sec.), then slight movement = Extended freeze, then moderate movement; 3 = Brief freeze (a few seconds), then active movement; 4 = Momentary freeze, then swift movement; 5 = No freeze, immediate movement; 6 = Extremely excited ("manic")
Body position	0 = Completely flat (on stomach); 1 = Lying on side; 2 = Lying on back; 3 = Sitting or standing; 4 = Rearing on hind legs; 5 = Repeated vertical leaping
Spontaneous activity	0 = None, resting; 1 = Casual scratch, groom, slow movement; 2 = Vigorous scratch, groom, moderate movement; 3 = Vigorous, rapid/dart movement; 4 = Extremely vigorous, rapid/dart movement
Respiration rate	0 = Gasping, irregular; 1 = Slow, shallow; 2 = Normal; Hyperventilation
Tremor	0 = None; 1 = Mild; 2 = Marked
Palpebral closure	0 = Eyes wide open; 1 = Eyes 1/2 closed; 2 = Eyes closed
Piloerection	0 = None; 1 = Coat stood on end
Gait	0 = Normal; 1 = Fluid but abnormal; 2 = Limited movement only; 3 = Incapacity
Pelvic elevation	0 = Markedly flattened; 1 = Barely touches; 2 = Normal (3 mm elevation); 3 = Elevated (more than 3 mm elevation)
Tail elevation	0 = Dragging; 1 = Horizontally extended; 2 = Elevated (Straub tail)
Urination	0 = None; 1 = Little; 2 = Moderate amount; 3 = Extensive
Defecation	Count the number of fecal boli emitted during the 3-min. period

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**Reflexes and reactions to simple stimuli**

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Touch escape	0 = No response; 1 = Mild (escape response to firm stroke); 2 = Moderate (rapid response to light stroke); 3 = Vigorous (escape response to approach)
Positional passivity	0 = Struggles when restrained by tail; 1 = Struggles when restrained by neck (finger grip, not scruffed); 2 = Struggles when held supine (on back); 3 = Struggles when restrained by hind legs; 4 = Does not struggle
Trunk curl	0 = Absent; 1 = Present
Reaching reflex	0 = None; 1 = Upon nose contact; 2 = Upon vibrassee contact; 3 = Before vibrassee contact (18 mm); 4 = Early vigorous extension (25 mm)
Body tone	0 = Flaccid, no return of cavity to normal; 1 = Slight resistance; 2 = Extreme resistance, board like

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**Measures recorded during supine restraint**

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Plantar surface skin color	0 = Blanched; 1 = Pink; 2 = Bright, deep red flush
Heart Rate	0 = Slow, bradycardia; 1 = Normal; 2 = Fast, tachycardia
Limb Tone	0 = No resistance; 1 = Slight resistance; 2 = Moderate resistance; 3 = Marked resistance; 4 = Extreme resistance
Abdominal Tone	0 = Flaccid, no return of cavity to normal; 1 = Slight resistance; 2 = Extreme resistance, board like
Righting Reflex	0 = No impairment; 1-10 = Number of seconds required to right
Air Righting Reflex	0 = No impairment; 1-10 = Number of seconds required to right
Salivation	0 = None; 1 = Slight margin of sub-maxillary area; 2 = Wet zone entire sub-maxillary area
Provoked Biting	0 = Absent; 1 = Present



#### **Motor coordination**

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Grip strength	0 = None; 1 = Slight grip, semi-effective; 2 = Moderate grip, effective; 3 = Active grip, effective; 4 = Unusually effective
Wire maneuver	0 = Active grip with hindlegs; 1 = Difficulty to grasp with hindlegs; 2 = Unable to grasp with hindlegs; 3 = Unable to lift hindlegs, falls within seconds; 4 = Falls immediately
Inverted screen (s)	0-60 = Number of seconds before falling
Pole climb	0 = Climbs down within 30 sec; 1 = Turns and climbs up the pole; 2 = Turns but the freezes; 3 = Does not move within 30 seconds or climbs down but not off the pole; 4 = Falls off

**Figure S4.** Toxicity study based on the Irwin test in C21:5n-3 treated mice. The Irwin test was assessed as described previously and according to the procedure table (parameters and scores). The Irwin test was evaluated after an acute dose of C21:5n-3 (or vehicle, controls) that did not produce any evidence of toxicity.