Supplemental Fig. 1 Breeding characteristics in animals used for lipid and cytokine measurements

Maternal weight change over the course of pregnancy in animals fed standard (Stand., grey line), n−3 (blue line) or n−6 (yellow line) enriched diets (**a**). Number of pups per litter (**b**). Weight of pups at P10 (**c**).

Supplemental Fig. 2 Levels of fatty acid and chemokines differently affected by diet in male and female groups.

Levels of fatty acid 20:3 n-9 in blood plasma of male and female pups from mothers fed a standard (Stand.), n−3 enriched or a n−6 enriched diet (**a**). Levels of Eotaxin 2 and CCL22 in brain of males and females born to mothers fed a standard (Stand.), n−3 enriched or a n−6 enriched diet (**b**). Data presented as mean ± SD. One-way ANOVA with post-hoc Tukey's multiple comparison test was performed to compare groups within each sex. Individual p-values are listed within figures for data that are significantly different.

Supplemental Fig. 3 MCAO outcome in male and female pups.

Volume of brain injury 72 hours after tMCAO reperfusion in male and female pups born to mothers fed a standard (grey), n−3 (blue) or n−6 (yellow) enriched diet (**a, b**). Spectrin cleavage 24 hours after reperfusion via caspase-3 -dependent (125KDa band) and calpain-dependent (150KDa band), in injured (ipsi) and non-injured (contra) brain cortex of male and female pups (**c**). Data presented as mean ± SD. Kruskal-Wallis test followed by Dunn's post-hoc test (**a**) or two-way ANOVA followed by Tukey's post-hoc test (**c**) was performed to compare groups. Individual p-values are listed within figures for data that are significantly different.

Supplemental Table 1. Levels of phospholipid fatty acids in blood plasma of the offspring of mothers fed different diets along with p-values of statistical tests between diets (one-way ANOVA, Welch's ANOVA or Kruskal-Wallis as appropriate).

Supplemental Table 2. Total fatty acid lipids in brain of the offspring of mothers fed different diets along with p-values of statistical tests between diets (one-way ANOVA, Welch's ANOVA or Kruskas-Wallis as appropriate).

Supplemental Table 3. Levels of cytokines and chemokines in blood plasma of the offspring of mothers fed different diets along with p-values of statistical tests between diets (one-way ANOVA or Kruskas-Wallis as appropriate).

Supplemental Table 4. Levels of cytokines and chemokines in the brain of the offspring of mothers fed different diets along with p-values of statistical tests between diets (one-way ANOVA or Kruskas-Wallis as appropriate).

Supplemental Table 1. Levels of phospholipid fatty acids in blood plasma of the offspring of mothers fed different diets along with p-values of statistical tests between diets (one-way ANOVA, Welch's ANOVA or Kruskal-Wallis as appropriate).

Fatty Acid	Mean±SD (Weight %)			p-values		
	Stand.	n-3	n-6	Stand./n−3	Stand./n−6	n−3/n−6
18:3 n−3 (ALA)	0.07±0.01	0.06±0.02	0.02±0.01	0.085	**<0.001**	**<0.001**
20:5 n−3 (EPA)	0.20±0.05	5.66±1.90	0.03±0.01	**<0.001**	**<0.001**	**<0.001**
22:5 n−3 (DPA)	0.24±0.02	0.72±0.13	0.10±0.03	**<0.001**	**<0.001**	**<0.001**
22:6 n−3 (DHA)	6.77±0.98	11.48±0.93	2.51±0.56	**<0.001**	**<0.001**	**<0.001**
18:2 n−6 (LA)	26.50±2.15	17.00±3.40	23.89±3.43	**<0.001**	0.119	**<0.001**
18:3 n−6	0.15±0.01	0.11±0.01	0.21±0.01	**0.006**	0.1	**<0.001**
20:2 n−6	1.56±0.44	0.57±0.23	1.31±0.26	**<0.001**	0.178	**<0.001**
20:3 n−6	2.84±0.49	2.24±0.60	2.60±0.55	**0.033**	0.557	0.244
20:4 n−6 (AA)	11.07±2.15	5.85±1.03	13.63±1.13	**<0.001**	**0.006**	**<0.001**
22:4 n−6	0.10±0.03	0.04±0.01	0.29±0.09	**0.033**	**<0.001**	**<0.001**
22:5 n−6	0.12±0.03	0.06±0.01	1.68±0.16	**<0.001**	**<0.001**	**<0.001**
14:0	0.49±0.33	0.53±0.28	0.60±0.21	0.939	0.615	0.817
16:0	25.90±5.00	29.33±2.85	28.38±2.00	0.149	0.337	0.730
17:0	0.15±0.02	0.27±0.03	0.15±0.01	**<0.001**	1	**<0.001**
18:0	15.70±1.40	14.83±0.75	14.85±1.04	0.171	0.186	1
20:0	0.11±0.02	0.10±0.01	0.10±0.01	0.080	0.399	0.652
22:0	0.11±0.03	0.10±0.02	0.10±0.02	0.876	0.454	0.746
23:0	0.05±0.02	0.05±0.01	0.04±0.01	0.992	0.162	**0.013**
24:0	0.32±0.13	0.30±0.08	0.25±0.08	0.865	0.265	0.537
16:1 n−7	0.23±0.05	0.61±0.11	0.26±0.03	1.000	1.000	**<0.001**
18:1 n−7	1.15±0.19	1.59±0.20	1.10±0.06	**<0.001**	0.690	**<0.001**
18:1 n−9	3.39±0.40	5.98±1.61	4.48±1.02	0.227	**0.012**	0.394
16:1 n−9	0.08±0.03	0.10±0.02	0.11±0.03	**0.000**	**0.011**	**0.040**
20:1 n−9	0.26±0.07	0.18±0.05	0.28±0.05	**0.004**	0.561	**<0.001**
20:3 n−9	0.05±0.02	0.05±0.01	0.06±0.01	0.985	0.050	**<0.001**
22:1 n−9	0.08±0.05	0.63±0.02	0.07±0.03	1	1	1
24:1 n−9	1.32±0.72	1.04±0.32	1.13±0.38	0.381	0.630	0.918

Supplemental Table 2. Total lipid fatty acids in brain of the offspring of mothers fed different diets along with p-values of statistical tests between diets (one-way ANOVA, Welch's ANOVA or Kruskal-Wallis as appropriate).

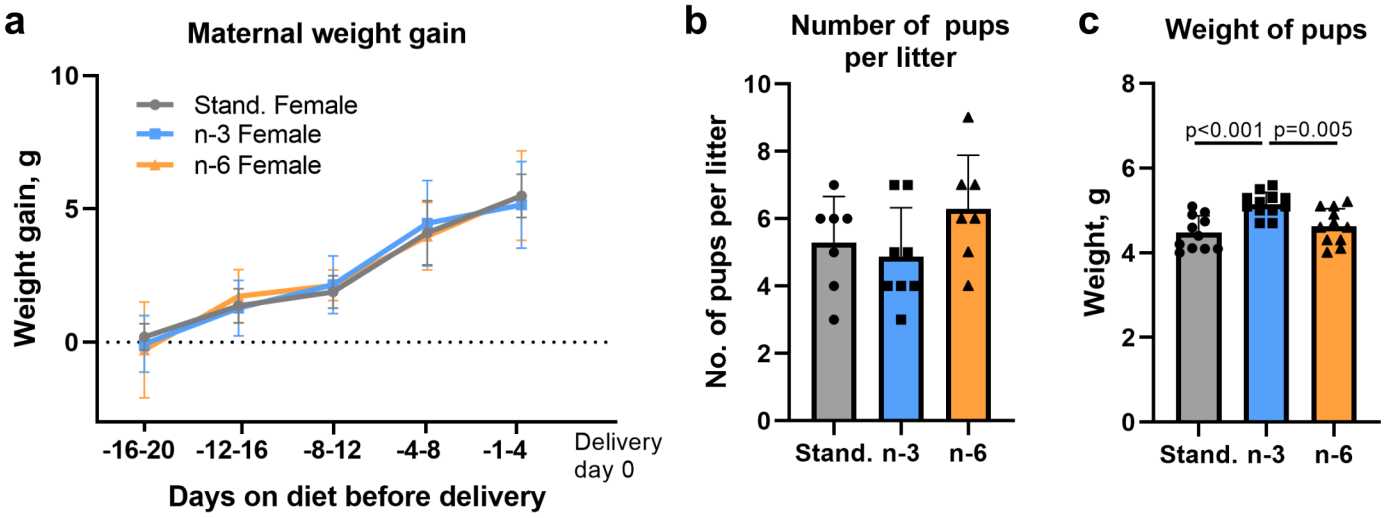
Fatty Acid	Mean±SD (Weight %)			p-values		
	Stand.	n-3	n-6	Stand./n−3	Stand./n−6	n−3/n−6
18:3 n−3 (ALA)	0.02±0	0.017±0	0.016±0	0.081	**0.032**	0.909
20:5 n−3 (EPA)	0.04±0.01	0.51±0.09	0.01±0	**<0.001**	**<0.001**	**<0.001**
22:5 n−3 (DPA)	0.20±0.03	0.76±0.04	0.09±0.01	**0.016**	**0.016**	**<0.001**
22:6 n−3 (DHA)	15.49±1.67	19.46±1.00	8.85±0.69	**<0.001**	**<0.001**	**<0.001**
18:2 n−6 (LA)	0.83±0.04	0.74±0.06	0.76±0.08	**0.005**	**0.019**	0.867
18:3 n−6	0.06±0.01	0.04±0	0.07±0.01	**<0.001**	**0.023**	**<0.001**
20:2 n−6	0.21±0.01	0.13±0.01	0.20±0.02	**<0.001**	0.146	**<0.001**
20:3 n−6	0.63±0.06	0.98±0.05	0.48±0.04	**<0.001**	**<0.001**	**<0.001**
20:4 n−6 (AA)	14.91±1.13	9.59±0.57	16.29±1.61	**<0.001**	**0.019**	**<0.001**
22:4 n−6	1.64±0.36	0.52±0.20	2.00±0.33	**0.002**	0.36	**<0.001**
22:5 n−6	0.62±0.06	0.14±0.03	3.97±0.23	**<0.001**	**<0.001**	**<0.001**
14:0	1.36±0.41	1.62±0.40	1.59±0.52	0.327	0.416	0.984
16:0	28.46±1.04	29.50±1.39	29.60±1.90	0.211	0.160	0.987
17:0	2.22±2.63	2.19±2.57	2.09±2.49	1	0.543	0.243
18:0	17.17±1.01	16.63±0.88	17.13±1.13	0.399	0.994	0.460
20:0	0.08±0.01	0.12±0.10	0.08±0.01	0.930	0.882	0.994
22:0	0.07±0.01	0.07±0.01	0.06±0	0.967	0.601	0.456
23:0	0.01±0	0.01±0	0.01±0	0.542	0.646	0.985
24:0	0.06±0.01	0.07±0.01	0.05±0	**0.031**	0.131	**<0.001**
16:1 n−7	1.42±0.11	1.64±0.18	1.57±0.13	**0.002**	**0.046**	0.421
18:1 n−7	2.04±0.08	2.05±0.09	2.18±0.09	0.998	**0.002**	**0.002**
16:1 n−9	1.45±0.12	1.37±0.11	1.67±0.17	0.395	**<0.001**	**<0.001**
18:1 n−9	10.17±0.39	11.03±0.41	10.36±0.49	**<0.001**	0.539	**0.002**
20:1 n−9	0.21±0.01	0.25±0.01	0.21±0.01	**<0.001**	0.920	**<0.001**
20:3 n−9	0.16±0.02	0.24±0.03	0.17±0.03	**<0.001**	0.314	**<0.001**
22:1 n−9	0.28±0.34	0.22±0.26	0.28±0.33	1	1	1
24:1 n−9	0.09±0.01	0.89±0.01	0.09±0.01	0.949	0.992	0.981

Supplemental Table 3. Levels of cytokines and chemokines in blood plasma of the offspring of mothers fed different diets along with p-values of statistical tests between diets (one-way ANOVA).

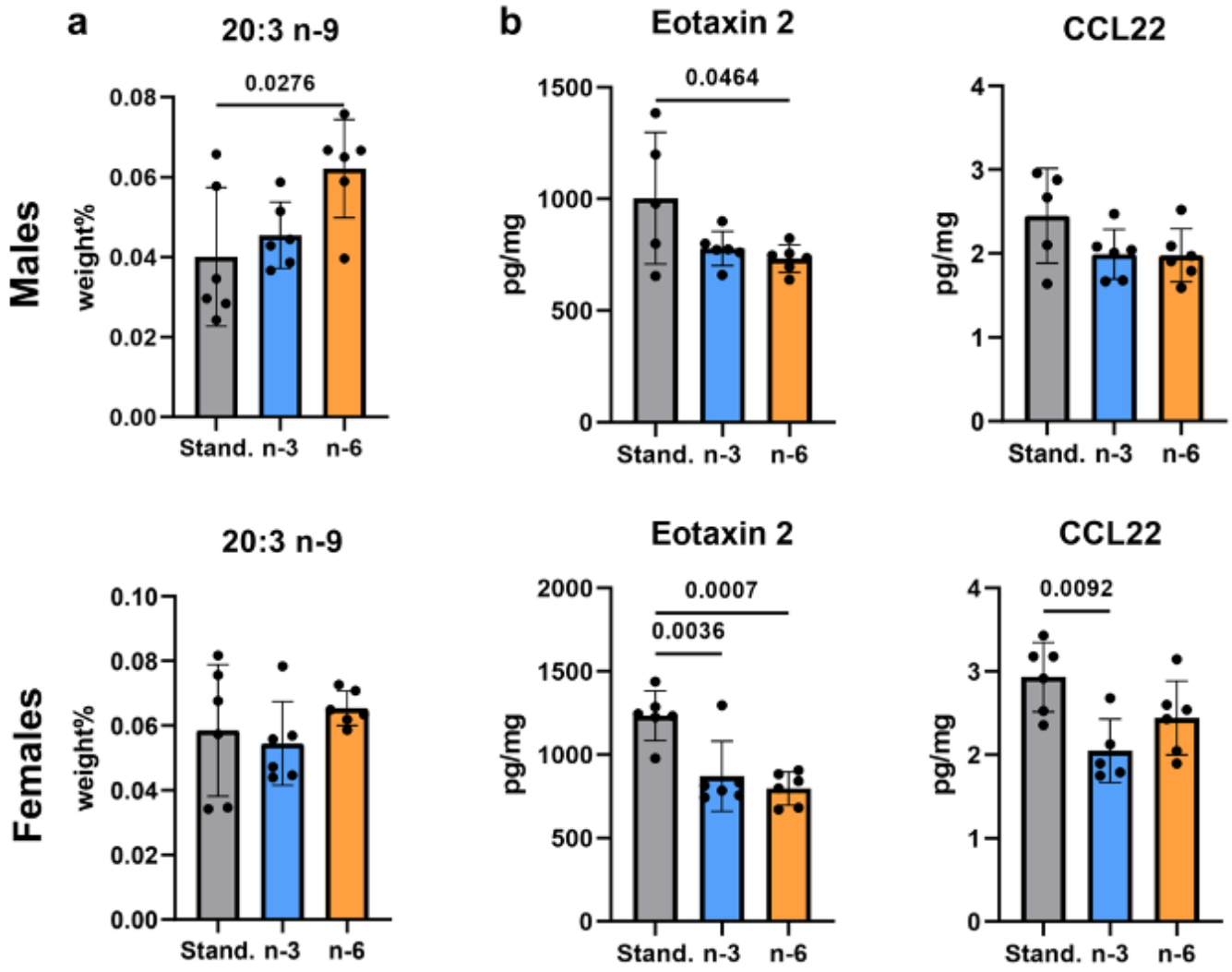
Cytokine/ chemokine	Mean±SD (pg/ml)			p-values		
	Stand.	n-3	n-6	Stand./ n−3	Stand./ n−6	n−3/ n−6
IL1b	296.90±110.40	302.10±114.50	276.20±56.87	0.991	0.863	0.794
IL2	11.79±6.34	12.81±6.67	11.35±4.05	0.914	0.980	0.832
IL4	51.40±11.10	49.57±19.04	53.83±13.66	0.954	0.921	0.769
IL6	68.28±29.47	72.43±44.81	65.09±15.3	0.956	0.977	0.875
IL10	650.80±292.50	768.10±497.50	553.50±247.10	0.897	0.836	0.548
IL16	636.70±152.10	722.40±200.60	647.80±104.20	0.437	0.986	0.516
INFg	119.20±50.75	136.00±78.81	124.90±49.92	0.803	0.975	0.901
GM-CSF	3.65±2.32	3.76±2.02	4.01±1.57	0.989	0.894	0.949
TNFa	111.50±50.61	112.80±64.12	98.54±35.38	0.112	0.996	0.122
CCL1	59.75±14.50	48.65±9.83	59.28±13.87	0.952	0.463	0.640
CCL2/MCP-1	368.20±150.20	351.90±157.20	301.00±76.23	0.952	0.463	0.640
CCL3/MIP1a	10.77±5.01	12.09±7.74	9.18±3.20	0.854	0.803	0.454
CCL4/MIP1b	113.50±41.01	117.40±50.38	109.70±33.74	0.973	0.975	0.897
CCL5/RANTES	45.59±13.64	55.08±20.64	52.82±11.55	0.362	0.562	0.939
CCL7/MCP-3	130.30±46.16	135.30±20.77	134.70±39.27	0.951	0.959	0.999
CCL11/Eotaxin	329.60±152.80	285.20±110.00	297.80±78.01	0.663	0.800	0.968
CCL12	28.53±7.29	33.55±7.32	32.08±7.96	0.277	0.518	0.892
CCL17	108.40±32.06	137.10±81.33	120.80±43.94	1	1	1
CCL19/MIP3b	1360.00±343.30	1376.00±545.50	1321.00±201.40	0.995	0.976	0.950
CCL20/MIP3a	78.94±30.36	65.04±16.24	86.26±26.30	0.372	0.755	0.110
CCL22	200.80±55.11	170.60±37.46	230.90±73.90	0.503	0.451	0.071
CCL24/Eotaxin2	3891.00±1065.00	3777.00±1875.00	3763.00±985.20	0.977	0.972	1.000
CCL27	3487.00±1762.00	3397.00±1547.00	5223.00±2529.00	0.994	0.115	0.085
CXCL1/KC	143.20±58.45	153.70±88.44	126.10±32.95	0.925	0.821	0.574
CXCL5	2455.00±2064.00	4121.00±3853.00	699.20±191.80	1	0.156	**0.019**
CXCL10	768.50±201.20	808.00±245.30	781.10±127.20	0.883	0.988	0.944
CXCL11	580.30±151.10	594.60±271.00	593.50±173.00	0.986	0.989	1
CXCL12	722.80±153.80	898.70±330.20	797.90±240.70	0.268	0.771	0.614
CXCL16	534.20±222.60	533.90±150.30	668.00±211.00	1	0.235	0.234
CXCL13/BCA1	674.40±244.70	759.80±350.60	739.80±219.90	0.759	0.843	0.985
CX3CL1/ Fraktalkine	334.80±48.07	329.60±56.44	344.30±33.26	0.963	0.898	0.767

Supplemental Table 4. Levels of cytokines and chemokines in the brain of the offspring of mothers fed different diets along with p-values of statistical tests between diets (one-way ANOVA).

Cytokine/ chemokine	Mean±SD (pg/mg)			p-values		
	Stand.	n-3	n-6	Stand./n−3	Stand./n−6	n−3/n−6
IL1b	52.60±10.74	39.50±5.43	41.20±9.10	**0.003**	**0.009**	0.886
IL2	6.32±2.33	4.46±0.95	4.15±1.28	**0.022**	**0.007**	0.892
IL4	11.22±2.23	8.15±1.18	8.89±2.11	**0.001**	**0.013**	0.610
IL6	5.66±1.40	3.96±0.65	4.05±1.10	**0.002**	**0.003**	0.974
IL10	39.15±20.22	19.73±8.39	27.83±15.17	**0.022**	0.346	0.317
IL16	312.10±62.31	269.00±64.35	267.70±56.54	0.210	0.193	0.999
INFg	16.90±3.65	11.89±1.87	13.56±3.30	**0.001**	**0.032**	0.375
GM-CSF	0.26±0.08	0.20±0.05	0.20±0.05	0.054	0.074	0.988
TNFa	31.28±6.23	24.30±3.01	26.02±5.37	**0.006**	**0.044**	0.682
CCL1	5.01±1.24	3.55±0.54	3.86±0.89	**0.002**	**0.013**	0.699
CCL2/MCP-1	30.10±5.12	17.96±6.00	21.01±7.36	**<0.001**	**0.01**	0.538
CCL3/MIP1a	5.86±1.15	5.35±0.81	5.99±1.08	0.466	0.944	0.281
CCL4/MIP1b	10.42±2.47	7.48±1.69	7.75±2.56	**0.014**	**0.023**	0.958
CCL5/RANTES	3.99±0.90	2.70±1.13	2.64±0.73	**0.008**	**0.004**	0.981
CCL7/MCP-3	8.10±1.41	6.68±1.17	7.19±0.90	**0.020**	0.169	0.546
CCL11/Eotaxin	9.29± 2.04	7.95±1.68	7.45±1.29	0.154	**0.035**	0.747
CCL12	3.76±1.26	3.01±0.42	3.83±1.05	0.195	0.981	0.129
CCL17	15.66±3.21	12.67±2.30	14.26±2.22	**0.025**	0.410	0.301
CCL19/MIP3b	300.10±57.07	245.20±28.61	240.80±38.61	**0.011**	**0.006**	0.965
CCL20/MIP3a	7.06±1.70	5.26±1.01	5.22±0.88	**0.004**	**0.003**	0.997
CCL22	2.71±0.53	2.02±0.32	2.21±0.44	**0.008**	0.087	1
CCL24/Eotaxin2	1130.00±245.50	825.60±158.90	765.80±86.28	**0.041**	**0.003**	1
CCL27	558.80±128.30	420.70±65.93	455.00±80.05	**0.003**	**0.031**	0.656
CXCL1/KC	6.69±1.76	4.59±1.09	5.14±1.28	**0.002**	**0.028**	0.608
CXCL5	64.25±14.68	51.83±10.96	52±11.33	0.051	0.055	0.999
CXCL10	213.70±48.62	167.50±25.77	177.60±28.51	**0.009**	**0.048**	0.770
CXCL11	202.40±33.30	167.40±48.46	170.60±40.77	0.111	0.159	0.980
CXCL12	181.60±39.85	140.60±21.53	151.50±27.22	**0.007**	0.054	0.658
CXCL16	8.73±1.48	7.08±0.88	7.23±0.85	**0.003**	**0.007**	0.941
CXCL13/BCA1	158.20±32.80	119.90±14.33	123.90±17.14	**0.001**	**0.003**	0.901
CX3CL1/ Fraktalkine	381.40±68.79	315.00±47.50	329.40±39.64	**0.012**	0.058	0.789


a
Maternal weight gain
Stand. Female
n-3 Female
n-6 Female
Weight gain, g
10
5
0
-16-20
-12-16
-8-12
-4-8
-1-4
Delivery day 0
Days on diet before delivery
b
Number of pups per litter
No. of pups per litter
10
8
6
4
2
0
Stand.
n-3
n-6
c
Weight of pups
p<0.001
p=0.005
Weight, g
8
6
4
2
0
Stand.
n-3
n-6

Suppl. Fig 2


a
b
Males
Females
20:3 n-9
Eotaxin 2
CCL22
weight%
pg/mg
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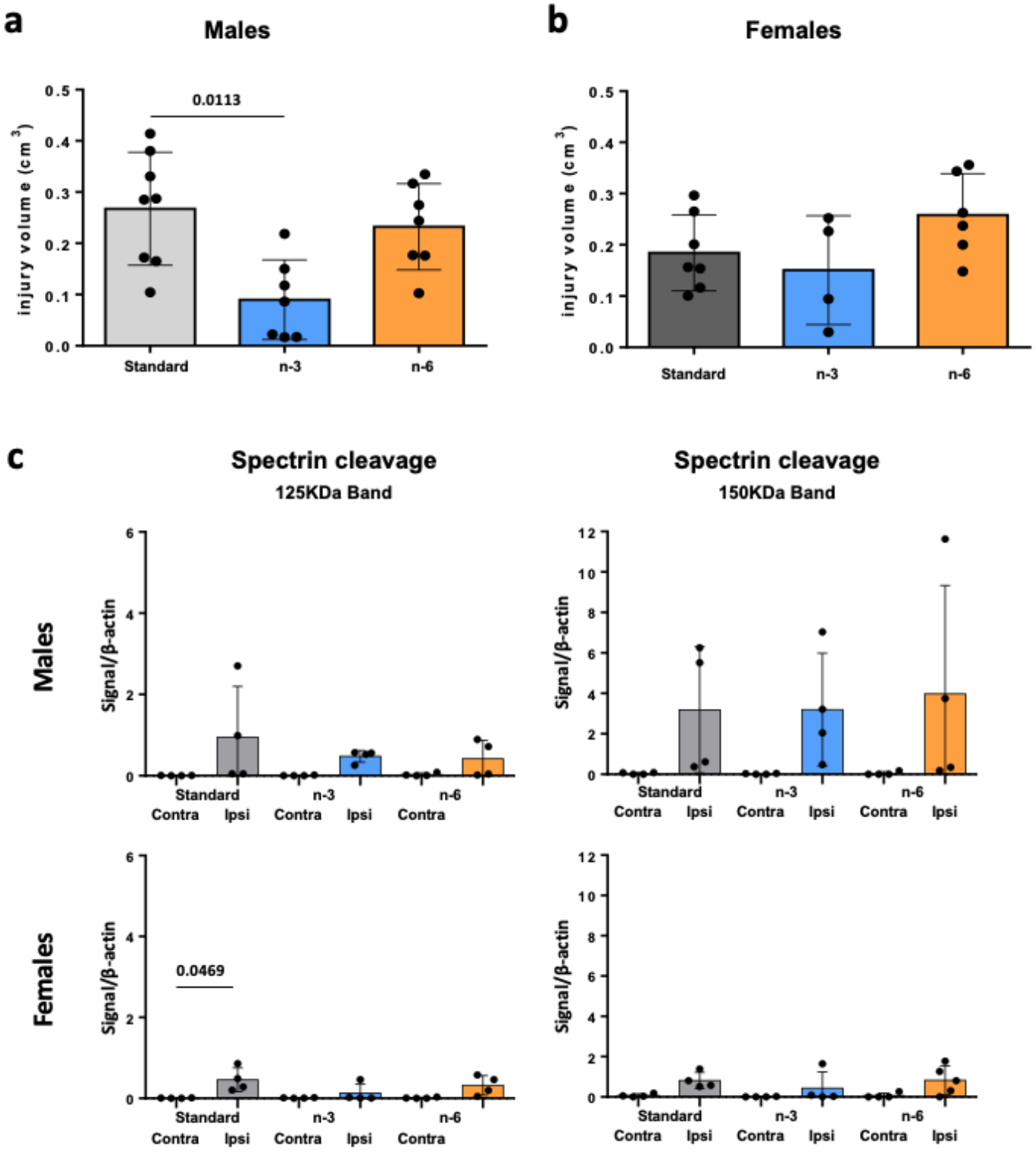
0.0007
0.0036
0.0092
Stand.
n-3
n-6

Suppl. Fig 3


a
Males
injury volume (cm3)
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Standard
n-3
n-6
b
Females
c
Spectrin cleavage
125KDa Band
150KDa Band
Males
Females
Signal/β-actin
0.0469
Contra
Ipsi