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SUPPLEMENTARY FIGURES

Nutritional omega-3 modulates neuronal morphology in prefrontal cortex along with depression-related behaviour through corticosterone secretion

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Supplementary Fig. 1 Morphometric analysis in 3D of Golgi Cox-stained pyramidal neurons. Representative brain regions, microphotography (x20) of pyramidal neurons in layer II/III of the dlPFC (top) and dmPFC (bottom) and representative tracing by the Neurolucida software of apical dendritic tree of pyramidal neurons.

Supplementary Fig. 2 Complexity of basal dendritic pyramidal neurons is not impaired in the PFC of n-3 deficient or defeated mice. Analysis of basal dendritic intersections was performed on total dendritic tree in the dorsal m dlPFC of control diet and n-3 deficient mice. There was no significant difference in basal dendritic arborisation in any of the groups. (interaction: F_{1,57} = 0.4507, p > 0.05, diet effect: F_{1,57} = 1.729, p > 0.05, stress effect: F_{1,57} = 0.2305, p > 0.05, Two-way ANOVA, n = 15 neurons per group). Data are expressed as mean ± s.e.m.

Supplementary Fig. 3 Dendritic arborisation correlates with the correspondent corticosterone plasma level. Pearson’s correlations were calculated considering corticosterone plasma levels x number of total apical intersections in the PFC of control diet and n-3 deficient mice under conditions of no stress (undefeated) or following 10 days of social defeat (defeated). Negative correlations were observed between plasma corticosterone levels and dendritic complexity in both (a) the dlPFC (r = -0.6821; p = 0.01) and (b) the dmPFC (r = -0.5968; p < 0.05).

Supplementary Fig. 4 No effect of adrenalectomy in control diet mice. (a) Experimental timeline. Control diet mice (2 month-old) were bilaterally adrenalectomised (Adx) or sham-operated. Adx mice were provided low dose (25µg/ml) of corticosterone for 4 weeks before behavioural measurements. (b) Adx treatment had no effect on social behaviour (t_{9} = 1.294, p > 0.05, unpaired t test, n =
5-6 per group), (c) on anxiety-like behaviour ($t_9 = 1.829$, $p > 0.05$, unpaired $t$ test, $n =$ 5-6 per group), on (d,e) neuronal arborisation in both the PFC (dIPFC: $t_{36} = 1.571$, $p > 0.05$, unpaired $t$ test, $n =$ 19 neurons per group; dmPFC: $t_{49} = 0.9961$, $p > 0.05$, unpaired $t$ test, $n =$ 25 neurons per group), and (f) on corticosterone levels ($t_9 = 1.437$, $p > 0.05$, unpaired $t$ test, $n =$ 5-6 per group). Data are displayed as mean ± s.e.m.