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1 **Impact of membrane lipid polyunsaturation on dopamine D2 receptor ligand binding**  
2 **and signalling**

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44 **Abstract**

45 Increasing evidence supports a relationship between lipid metabolism and mental health. In  
46 particular, the biostatus of polyunsaturated fatty acids (PUFAs) correlates with some  
47 symptoms of psychiatric disorders, as well as the efficacy of pharmacological treatments.  
48 Recent findings highlight a direct association between brain PUFA levels and dopamine  
49 transmission, a major neuromodulatory system implicated in the etiology of psychiatric  
50 symptoms. However, the mechanisms underlying this relationship are still unknown. Here we  
51 demonstrate that membrane enrichment in the n-3 PUFA docosahexaenoic acid (DHA),  
52 potentiates ligand binding to the dopamine D2 receptor (D2R), suggesting that DHA acts as  
53 an allosteric modulator of this receptor. Molecular dynamics simulations confirm that DHA has  
54 a high preference for interaction with the D2R and show that membrane unsaturation  
55 selectively enhances the conformational dynamics of the receptor around its second  
56 intracellular loop. We find that membrane unsaturation spares G protein activity but potentiates  
57 the recruitment of  $\beta$ -arrestin in cells. Furthermore, *in vivo* n-3 PUFA deficiency blunts the  
58 behavioral effects of two D2R ligands, quinpirole and aripiprazole. These results highlight the  
59 importance of membrane unsaturation for D2R activity and provide a putative mechanism for  
60 the ability of PUFAs to enhance antipsychotic efficacy.

## 61 **Introduction**

62 Biological membranes are not homogeneous bilayers but rather composed of different lipid  
63 species with various chemical and biophysical properties that actively modulate protein  
64 localization and function, signaling or vesicular trafficking [1]. However, several aspects of  
65 membrane complexity including the impact of lipid heterogeneity across tissues, cells, and  
66 subcellular compartments on cell signaling and physiology are only starting to emerge.

67

68 This is particularly true for neuronal function for which the impact of membrane lipid  
69 composition has been largely overlooked, despite the brain having the second highest lipid  
70 content after adipose tissue [2]. Yet, convergent findings support some links between lipid  
71 biostatus and mental health. For instance, a decrease in the levels of n-3 polyunsaturated fatty  
72 acids (PUFAs)-containing phospholipids has been consistently described in a subset of  
73 patients suffering from neurodevelopmental psychiatric diseases [3]. Likewise, various studies  
74 suggest alterations in common fatty acid metabolic pathways across several psychiatric  
75 disorders [4, 5]. Moreover, n-3 PUFA deficiency in rodent models has been associated with  
76 putative pathophysiological mechanisms involving alteration in neurogenesis, neuronal  
77 migration, neuromodulation, and neuroinflammatory processes in various brain regions [2, 6,  
78 7]. These findings remain largely correlative and the precise mechanisms by which PUFA  
79 biostatus directly or indirectly accounts for changes in neuronal function remain largely  
80 unknown. Nevertheless, several studies have demonstrated that membrane lipids regulate the  
81 function of key transmembrane proteins [8] including ion channels and G protein-coupled  
82 receptors (GPCRs) [9, 10].

83

84 The activity of the dopamine D2 receptor (D2R) is particularly relevant in this context. Various  
85 psychiatric disorders, including those where levels of brain n-3 PUFAs are low, display altered  
86 patterns of D2R-dependent signaling [11], and this GPCR is, therefore, a key target in several  
87 pharmacological treatments. Similarly, different studies in rodent models show that n-3 PUFA  
88 deficiency affects dopamine transmission and related behaviors [12, 13]. In line with this  
89 evidence, we recently reported a unique vulnerability of D2R-expressing neurons to PUFA  
90 biostatus that directly accounts for the motivational deficits induced by n-3 PUFA deficiency  
91 [14]. Early experimental studies have reported that n-3 PUFAs, namely docosahexaenoic acid  
92 (DHA, 22:6), enhance the function of the prototypical class A GPCR rhodopsin [15–17].  
93 Subsequent studies showed that DHA-containing phospholipids preferentially solvate  
94 rhodopsin [18, 19], which could impact receptor function. In addition, recent *in silico* studies  
95 [20, 21] demonstrate that the D2R also displays a preference for DHA interaction that could  
96 modulate receptor partitioning into specific membrane signaling platforms, as shown by early

97 experiments [22]. While these findings suggest that membrane PUFA composition could  
98 influence the activity of the D2R, there is still no direct evidence for such an effect.

99

100 In this work, using both cell membrane extracts and model membranes, we found that DHA,  
101 but not n-6 PUFA docosapentaenoic acid (DPA, 22:5), enhances D2R ligand binding affinity.  
102 While DPA and DHA only differ in one double bond, molecular dynamics (MD) simulations  
103 show that they have a strikingly different propensity for interacting with D2R. In addition, we  
104 show that membrane unsaturation strongly influences conformational dynamics of the  
105 receptor, notably of the second intracellular loop. Interestingly, membrane enrichment in either  
106 DHA or DPA have no effect on agonist-induced,  $G_{i/o}$  protein-dependent inhibition of cAMP  
107 production. However, both DHA and DPA similarly enhance the maximal efficacy of the D2R  
108 to recruit  $\beta$ -arrestin2 upon agonist stimulation. Finally, we provide *in vivo* evidence that  
109 decreased membrane unsaturation with n-3 PUFA deficiency blunts the behavioral effects of  
110 two D2R ligands, namely quinpirole and aripiprazole. Altogether, these data highlight the  
111 impact of membrane PUFA composition on D2R activity and suggest that DHA acts as an  
112 allosteric modulator of the receptor.

113

## 114 **Material and Methods**

### 115 **Chemicals**

116 Reagents, unless otherwise specified, were from Sigma-Aldrich (St. Louis, MO, USA) PUFAs  
117 (Cis-4,7,10,13,16,19-DHA (DocosaHexaenoic Acid, ref D2534), DPA n-6 (DocosaPentaenoic  
118 Acid, ref 18566) stock solutions (30 mM) were prepared in absolute ethanol under N<sub>2</sub> [23]. For  
119 anisotropy assays, LigandTag Lite D2 (L0002RED) receptor red antagonist was purchased  
120 from Cisbio Bioassays. Quinpirole (ref 1061) and Forskolin (ref 1099) were from Tocris.

121

### 122 **Cell culture and treatment**

123 HEK 293 or CHO stably expressing the human D2R (Flp-in T-rex 293 SF-D2sWT/FRTTO 293)  
124 were used. See supplementary information for details.

### 125 **Membrane preparation**

126 See supplementary information for the description of membrane preparation.

127

### 128 **Plasmon waveguide resonance (PWR)**

157 PWR experiments were performed with a homemade instrument equipped with a He-Ne laser  
158 at 632 nm whose light is linearly polarized at 45°, allowing acquisition of both *p*- (light that is  
159 parallel to the incident light) and *s*-polarization (light that is perpendicular to the incident light)  
160 data within a single angular scan. The technique has been described previously [24, 25] (see  
161 supplementary information for details).

162

### 163 **Formation of a planar lipid bilayer on the PWR sensor**

164 The method used to prepare the lipid bilayer is based on the procedure by Mueller and Rudin  
165 [26] to make black lipid membranes (See supplementary information for details). To ensure  
166 that a proper solid-supported lipid bilayer is formed, the changes in the resonance minimum  
167 position (resulting from changes in mass density, anisotropy and thickness following film  
168 deposition) for both polarizations are measured and compared to values previously established  
169 to correspond to a lipid bilayer [27]. The lipids used for the two membrane systems studied  
170 were 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1,2-didocosahexaenoyl-  
171 sn-glycero-3-phosphocholine (DDPC).

172

### 173 **Immobilisation of cell fragments on the PWR sensor**

174 The protocol for adhesion of cell fragments on silica (glass slides or PWR sensor) was adapted  
175 from reported work from Perez and collaborators [28] (see supplementary information for  
176 details).

177

### 178 **Partial purification and reconstitution of the dopamine D2 receptor in the lipid bilayer**

179 D2R-expressing membranes from *Pichia pastoris* (gift from JL Banères) were used (see  
180 supplementary information for details). After lipid bilayer formation, detergent-solubilized D2  
181 receptor was reconstituted in the lipid bilayer by the detergent-dilution method (see  
182 supplementary information for details).

183

### 184 **Ligand-induced receptor response**

185 Both systems (cell fragments and reconstituted protein in lipid model systems) were tested for  
186 their capacity to respond to ligand to check whether the dopamine D2 receptor is active after  
187 cell fragment immobilization and the reconstitution process in the lipid membrane (see  
188 supplementary information for details). A control experiment was performed that consisted in

189 adding the same ligand concentrations to a lipid bilayer with no receptor reconstituted to  
190 measure non-specific binding of ligand to lipids alone (Fig. S4I). Graphical analysis of the  
191 spectral changes observed upon ligand addition (spectral shifts observed at maximal ligand  
192 concentration) was performed following published method [29].

193

#### 194 **cAMP accumulation assays**

195 D2R stably expressing HEK 293 (or CHO when indicated) cells were used. The production of  
196 cAMP was measured by using a cAMP Enzyme Immunoassay kit (Sigma, CA200) as  
197 described by the manufacturer using Victor3 (Perkin Elmer) plate reader. The curve fit was  
198 obtained by GraphPad Prism 5 (GraphPad Software, Inc.) (see supplementary information for  
199 details).

200

#### 201 **Fluorescence anisotropy assay**

202 See supplementary information for details.

#### 203 **$\beta$ -Arrestin2 recruitment assay**

204  $\beta$ -arrestin2 recruitment was assessed using the PathHunter<sup>®</sup> express DRD2L CHO-K1 Beta  
205 arrestin GPCR Assay (DiscoverX, Fremont, CA) following the manufacturer's instructions (see  
206 supplementary information for details).

#### 207 **Behavioral experiments**

208 Female C57BL6/J mice were fed with isocaloric diets containing 5% fat with a high (n-3 def  
209 diet) or low LA/ALA ratio (Ctrl diet) across gestation and lactation and offspring were  
210 maintained under the same diet after weaning as previously done (see details in [14]). All  
211 experiments were performed at adulthood (see supplementary information for details).

#### 212 **Operant conditioning**

213 The apparatus and behavioral paradigms were previously described [14] (see supplementary  
214 information for details). Aripiprazole (Arip; 7-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butoxy]-  
215 3,4-dihydro-1H-quinolin-2-one; Merck<sup>®</sup>, Darmstadt, Germany) was dissolved in a mixture of  
216 saline (NaCl 0.9%) and cremiphore (2% of cremiphore in saline) at the doses of 0.1 and 0.5  
217 mg/kg and administered intraperitoneally 10 min before the beginning of the PRx2 test  
218 sessions as previously done (Ducrocq et al., in prep.). The ratio of lever presses under drugs

219 over lever presses after vehicle injection were measured for each animal and averaged in order  
220 to evaluate the effect of the drugs on operant responding.

### 221 **Spontaneous locomotion**

222 Quinpirole-induced locomotor response was evaluated as described previously (Akhisaroglu  
223 et al., 2005). Briefly, animals received 7 intraperitoneal injections of 1mg/kg quinpirole (Quin;  
224 Tocris) dissolved in 0.9% saline every 72 hours. Locomotion was immediately measured for 3  
225 hours after the 8th injection of 0.5mg/kg quinpirole (see supplementary information for details).

### 226 **Western blot**

227 Mice were dislocated and the brains were quickly removed, snap-frozen on dry ice and stored.  
228 Nucleus accumbens samples were punched (No.18035-01, Fine Science Tools) from 200  $\mu$ m  
229 frozen slices in a cryostat. Samples were homogenized and denatured and western blot were  
230 performed as previously described [14] (see supplementary information for details).

### 231 **Cell Membrane Microarray and [<sup>35</sup>S]GTP $\gamma$ S autoradiography**

232 Microarrays were composed of a collection of membrane homogenates isolated from the NAc  
233 of adult mice exposed to Ctrl diet (n=17) or n-3 def diet (n=17) and from rat cerebral cortex as  
234 positive control (see supplementary information for details). Microarrays were fabricated by a  
235 non-contact microarrayer (Nano\_plotter NP2.1) placing the cell membrane homogenates (20  
236 drops/spot) into microscope glass slides treated using a proprietary technology, which enables  
237 the immobilization of cell membranes to supports preserving the structure and functionality of  
238 their proteins [30].

239 [<sup>35</sup>S]GTP $\gamma$ S binding studies were carried out using Cell Membrane Microarrays according to  
240 the following protocol. Briefly, Cell Membrane Microarrays were dried 20 min at room  
241 temperature (r.t.), then they were incubated in assay buffer (50 mM Tris-Cl; 1 mM EGTA; 3  
242 mM MgCl<sub>2</sub>; 100 mM NaCl; 0,5% BSA; pH 7,4) in the presence or absence of 50  $\mu$ M GDP  
243 and/or 100  $\mu$ M quinpirole for 15 min at r.t.. Microarrays were transferred into assay buffer  
244 containing 50  $\mu$ M GDP and 0.1 nM [<sup>35</sup>S]GTP $\gamma$ S, with and without the dopamine D2 agonist,  
245 quinpirole, at 100  $\mu$ M and incubated at 30°C for 30 min. Non-specific binding was determined  
246 with GTP $\gamma$ S (10  $\mu$ M). Finally, microarrays, together with [<sup>35</sup>S]-standards, were exposed to films,  
247 developed, scanned and quantified using the Mapix software.

### 248 **Lipid analyses**

249 Cells submitted to different treatments were randomly analyzed according to Joffre et al. [31].  
250 Total lipids were extracted according to the method developed by Folch et al. [32] and were  
251 submitted to fatty acid methylation using 7% boron trifluoride in methanol following Morrison  
252 and Smith protocol [33] (see supplementary information for details).

## 253 **MD simulations**

254 VMD1.9.4 [34] was used to preprocess both inactive crystal (PDB ID: 6CM4) and active  
255 cryoEM structures (PDB ID: 6VMS) of the dopamine D2 to set up the simulations of the apo  
256 and holo states, respectively. Any co-crystallization atoms different than water molecules  
257 closer than 5 Å to the protein were removed. MODELLER [35] was used to: (a) mutate back  
258 to the native sequence any mutation present in the structure, namely A122I, A375L, and A379L  
259 in PDB ID: 6CM4, and I205T, L222R, L374M, Y378V, L381V, and I421V in PDB ID: 6VMS,  
260 and (b) model residue CYS443, which is missing in the 6CM4 structure, to have the D2R  
261 palmitoylation site available. The HomolWat server [36] was used to place internal water  
262 molecules not present in the initial structure, and the sodium ion in the case of apo structures.  
263 An inactive apo structure was then generated by simply removing the risperidone ligand bound  
264 to PDB ID: 6CM4. Likewise, an active apo structure was generated by removing the ligand  
265 bromoergocryptine from PDB ID: 6VMS. This second apo structure was used to dock one  
266 molecule of either dopamine or aripiprazole into the orthosteric binding pocket of the receptor  
267 using AutoDock Vina [37].

268 The CHARMM-GUI builder [38, 39] was used to embed each refined structure into a 90 x 90  
269 Å<sup>2</sup> multicomponent membrane rich in either DHA or DPA, using a specific and realistic lipid  
270 composition [20], which is detailed in Table S2.

271 Each protein-membrane system was placed into a water box made of explicit water molecules,  
272 their charge was neutralized, and the ionic strength of the system adjusted, throughout  
273 CHARMM-GUI builder's pipeline. All titratable residues of the protein were left in their dominant  
274 protonation state at pH 7.0, except for Asp80. Disulfide bridges were inserted between Cys107-  
275 Cys182, and Cys399-Cys401, and a palmitoyl moiety was covalently linked to Cys443.  
276 Systems were first energy minimized and then equilibrated for 50 ns at constant pressure (NPT  
277 ensemble). The harmonic positional restraints initially applied to all C $\alpha$  atoms of the protein  
278 were gradually released throughout the equilibration phase. Production simulations for each  
279 replica were run for 1  $\mu$ s each, at constant volume (NVT ensemble), 1,013 bar and 310 K. The  
280 production simulations of this study yielded an aggregated time of 16  $\mu$ s (4 systems x 4 replicas  
281 x 1  $\mu$ s). All simulations were run using ACEMD [40] in combination with the CHARMM36m  
282 force field [41]. Dopamine and aripiprazole ligand charges were optimized in the ANI-2x mode

283 using the parameterize module of HTMD [42]. Figures from simulations were rendered using  
284 the Tachyon renderer [43] and the R ggplot2 library [44].

285 Lipid-protein contact ratios between lipid species were calculated by dividing the number of  
286 contacts per atom of one lipid group over the other. Atoms closer than 4.2 Å to the center of  
287 mass of the protein were considered in contact. Only lipid tail atoms were used in these  
288 calculations. Each contact value was previously normalized by the total number of atoms in  
289 that particular selection. For example, sn-1 SDP(d)C / SAT ratios were calculated as the  
290 number of atoms of DHA < 4.2 Å of protein's center of mass divided by the total number of  
291 SDP(d)C chain atoms in the system. Likewise, SAT is calculated in the former ratio as the  
292 number of atoms of any DPPC, DSPC, or PSM chain closer than 4.2 Å of protein's center of  
293 mass divided by the total number atoms of these tails.

294 The average lipid occupancy across the simulations was computed using the Python package  
295 PyLipID [45]. The Jensen-Shannon distance [46] was used to compare structural ensembles  
296 from the simulations via the relative entropy analysis module of the PENSA library [47].

## 297 **Statistical analyses**

298 Data are reported as mean  $\pm$  SD (unless otherwise indicated) (see supplementary information  
299 for details).

300

## 301 **Data availability**

302 The datasets generated and/or analyzed during the current study are available from the  
303 corresponding author on reasonable request. All MD simulation trajectories generated in this  
304 study can be visualized and inspected through the GPCRmd online resource [48].

305

## 306 **Results**

### 307 **1. Membrane n-3 PUFAs, but not n-6 PUFAs, modulate the binding affinity of D2R** 308 **ligands**

309 To determine the impact of membrane lipid unsaturation on D2R ligand binding, HEK cells  
310 were incubated with PUFAs, which is known to result in their esterification into the *sn*-2 position  
311 of phospholipids [49]. We used two different PUFAs, the n-3 PUFA DHA (C22:6) and the n-6  
312 PUFA DPA (C22:5), which display the same carbon chain length, but differ in one double bond.  
313 A lipid analysis confirmed the enrichment of phospholipids in DHA or DPA and monitored its  
314 efficient incorporation in cell membranes, leading to a comparable proportion of either PUFA  
315 after treatment (Fig. S1). We measured the binding affinity of D2R ligands on supported cell

316 membranes expressing the D2R using plasmon waveguide resonance (PWR) spectroscopy  
317 [50]. Membrane enrichment in DHA, but not DPA, significantly enhanced ligand binding  
318 affinities, for agonists (quinpirole and dopamine), antagonist (spiperone), and partial agonist  
319 (aripiprazole) (Fig. 1A). Thus, dissociation constants ( $K_D$ ) calculated from resonance position  
320 shifts followed in *p*- and *s*-polarization upon incremental addition of ligands were decreased  
321 across all D2R ligands tested under DHA membrane enrichment (Fig. 1A-B).  $K_D$  values  
322 correspond to the average between *p*- and *s*-data and are comparable to the ones reported in  
323 the literature, which validates our system and confirms that the receptor maintains functionality  
324 in these conditions (Fig. S2) [51, 52]. We further confirmed the potentiating effect of membrane  
325 DHA enrichment on D2R ligand binding affinity for the spiperone-derived fluorescent ligand  
326 NAPS-d2 through fluorescence anisotropy in cell membrane fragments [53, 54] (Fig. S3) and  
327 PWR with partially purified D2R reconstituted in model membranes of pure POPC (16:0 / 18:1)  
328 and pure DDPC (22:6 / 22:6) (Fig. S4). Of note, ligand addition to cell membrane fragments  
329 not expressing the D2R resulted in negligible spectral changes (Fig. S4). Therefore, spectral  
330 changes observed upon ligand binding with D2R expression are mainly due to receptor  
331 conformational changes and accompanying lipid reorganization.

332 Graphical analysis of the spectral changes allowed determination of the contribution of mass  
333 and structural changes. Overall, the data indicate that spectral changes are a result of both  
334 mass and structural changes with mass changes predominating (Fig. S4J). Interestingly, we  
335 observed that both agonist and antagonist lead to different polarization responses that were  
336 positive both for agonists (quinpirole, dopamine) and partial agonist (aripiprazole), and  
337 negative for the antagonist spiperone (Fig. 1B-D and Fig. S2). These results show that different  
338 classes of ligands induce distinct conformational changes upon binding to the D2R, as  
339 previously reported for other GPCRs [24, 55–58]. Importantly, the receptor conformational  
340 changes became less anisotropic for dopamine and more anisotropic for aripiprazole (as  
341 shown by the respective decrease and increase in *p*-polarization shifts; Fig. 1B). Overall, the  
342 presence of membrane PUFAs significantly influence changes in receptor's conformation  
343 induced by ligands along the receptor long axis (*p*-polarization) (Fig. 1C-D).

344

## 345 **2. MD simulations reveal that n-3 and n-6 PUFAs differentially interact with the** 346 **D2R**

347

348 With the aim of gaining structural insights into the differential modulation exerted by DPA and  
349 DHA in PUFA-enriched cell membranes, we used MD simulations to study the interaction  
350 between the D2R and membranes enriched in either DHA or DPA. We first simulated four

351 replicas of the D2R embedded in a multicomponent membrane (see Methods) enriched in  
352 either DHA-containing phospholipids (1-stearoyl-2-docosahexaenoyl-sn-glycero-3-  
353 phosphocholine, SD(h)PC) or DPA-containing phospholipids (1-stearoyl-2-  
354 docosa(p)entaenoyl-sn-glycero-3-phosphocholine, SD(p)PC). In line with previous reports,  
355 [20, 21], SD(h)PC tends to displace saturated phospholipids from the membrane “solvation”  
356 shell that surrounds the D2R, due to the much higher propensity of DHA tails for the interaction  
357 with the receptor (Fig. S5). Strikingly, the loss of just one double bond (i.e. replacing DHA by  
358 DPA) abolishes this propensity, as shown by the much lower interaction ratio of SD(p)PC  
359 molecules with the receptor during the simulation (Fig. S5). Specifically, the propensity for the  
360 interaction with the D2R is three-fold higher for DHA tails (Table 1).

361  
362 Moreover, our simulations show that DHA and DPA display a different pattern of interaction  
363 with the receptor (Fig. 2A). Interestingly, SD(p)PC lipid molecules display a clear preference  
364 over SD(h)PC for interaction with the extracellular segments of transmembrane helix (TM) 1,  
365 TM2, and TM7(Fig. 2B, bottom left).

366  
367 We then simulated the D2R bound to dopamine or aripiprazole, and embedded into a  
368 multicomponent membrane enriched in SD(h)PC (see Methods). Interestingly, while the  
369 aripiprazole-bound D2R simulations also showed an increased level of DHA around the  
370 receptor (Fig. 2C), the DHA solvation effect was absent or even diminished in dopamine-bound  
371 D2R simulations (Fig. 2C). Specifically, the propensity for the interaction between DHA tails  
372 and the protein was approximately two-fold higher in the aripiprazole-bound compared to  
373 unbound D2R simulations (Table 2). Furthermore, as shown in Fig. 2D, the presence of  
374 dopamine or aripiprazole in the binding pocket induced different patterns of interactions  
375 between SD(h)PC and the D2R, including a different interaction signature at the extracellular  
376 segment of the receptor where ligand binds (Fig. 2D, upper left and right panels).

377  
378 Lastly, we investigated the effect of DHA vs DPA on the conformational dynamics of the D2R.  
379 To find D2R structural differences between DHA-rich and DPA-rich simulations, we used a  
380 relative entropy analysis (see Methods) to compare the conformational ensemble of the  
381 receptor across all replicas. As shown in Fig. 2E, DHA- and DPA-rich systems induce very  
382 similar conformational dynamics of the D2R. To investigate whether decreasing the amount of  
383 membrane PUFAs has an effect on the conformational dynamics of the receptor, we simulated  
384 four replicas of the D2R embedded into a multicomponent membrane depleted of SD(h)PC  
385 (see Methods). The relative entropy analysis revealed that a decrease in PUFAs has a strong  
386 effect on D2R’s conformational dynamics. As shown in Fig. 2F-H, most of these differences  
387 are at the level of D2R intra- and extracellular loops, likely due to a different hydrophobic

388 mismatch between the receptor and the lipid bilayer. Interestingly, despite being relatively  
389 short, the intracellular loop 2 (ICL2) of the receptor displays completely different dynamics  
390 under PUFA depletion. A visual inspection of the simulations showed that PUFA-rich  
391 membranes preserve the helicity of the intracellular end of helix 4, whereas this segment of  
392 the receptor is completely unfolded in PUFA-depleted membranes. Specifically, helix 4 losses  
393 more than one helix turn at the level of residues 146 - 150 (Fig. 2I-J). These figures clearly  
394 show that the loss of this turn particularly affects the position of two consecutive serine residues  
395 in helix 4, namely S147 and S148, leading to rearrangement of ICL2.

396

### 397 **3. n-3 and n-6 PUFAs do not influence the D<sub>2</sub>R G<sub>αi/o</sub>-mediated signaling pathway but** 398 **enhance maximal recruitment of β-arrestin2**

399

400 In order to evaluate the downstream effect of PUFAs, we performed dose-response  
401 experiments where we probed the signaling efficacy of the D<sub>2</sub>R in the presence and absence  
402 of PUFAs in heterologous systems. First, we treated cells with 10 μM PUFAs (Fig. S6) and  
403 measured the inhibition of forskolin-induced cAMP production upon treatment with quinpirole,  
404 dopamine and aripiprazole as a proxy for G<sub>i/o</sub> protein activation. As shown in Fig. S7A and  
405 Table S1, both control and PUFA-rich cells display a similar agonist-dependent decrease in  
406 cAMP production, which suggests that PUFAs do not modulate the G<sub>i/o</sub> protein signaling  
407 pathway. Next, we used the same experimental conditions to study the G<sub>i/o</sub> protein-  
408 independent signaling pathway by measuring β-arrestin2 recruitment. In these experiments,  
409 while PUFAs did not affect the potency of β-arrestin2 recruitment (i.e. EC<sub>50</sub>), they slightly  
410 (quinpirole and dopamine) affected its efficacy (i.e. E<sub>max</sub>) (Fig. S7D-F). To confirm this trend,  
411 we treated cells with a higher amount of PUFAs (i.e. 30 μM) (Fig. S6). We did not observe any  
412 impact of PUFAs enrichment on basal cAMP production (Fig. S7B). We then performed dose-  
413 response experiments upon ligand stimulation. Higher PUFA manipulations did not influence  
414 cAMP production in two cell lines (Fig. 3A, C, E and Fig. S7C), or the EC<sub>50</sub> for β-arrestin2  
415 recruitment, but significantly increased the E<sub>max</sub> of β-arrestin2 recruitment for all ligands in both  
416 DHA- and DPA-rich cells (Fig. 3B, D, F).

417 Altogether, our data reveal that membrane unsaturation with either DPA or DHA enrichment  
418 does not affect G<sub>i/o</sub> protein activation but enhances β-arrestin2 recruitment efficacy.

419

### 420 **4. Decreased unsaturation by n-3 PUFA deficiency blunts D<sub>2</sub>R ligand-induced** 421 **alterations in locomotion and motivation**

422

423 To translate our *in vitro* findings to *in vivo* settings, we used a validated model of n-3 PUFA  
424 deficiency in mice in which we demonstrated a dysfunction of D2R-expressing neurons [14].  
425 We previously showed that, in the brain of n-3 PUFA deficient mice, DHA is replaced by DPA,  
426 mostly in phosphoethanolamine (PE) species [14]. We calculated an unsaturation index [59,  
427 60] and found that n-3 PUFA deficiency leads to decreased unsaturation within PEs (Fig S8A-  
428 C).

429 Using striatal extracts – in which D2R is highly expressed – we found that i) Gi protein  
430 recruitment in response to quinpirole application is unaffected (Fig. 4A) and ii) phosphorylation  
431 of the glycogen synthase kinase  $\beta$  (GSK3 $\beta$ ), a key signaling enzyme downstream of  $\beta$ -arrestin2  
432 [61], is decreased at basal states in n-3 PUFA deficient animals (Fig. 4B and Fig. S9). These  
433 latter findings are consistent with our *in vitro* data showing that membrane PUFA enrichment  
434 potentiates the recruitment of  $\beta$ -arrestin2 at the D2R. Next, we used n-3 PUFA deficient  
435 animals to study behavioral responses to D2R ligands. Our results show that n-3 PUFA  
436 deficient animals display decreased locomotor response under quinpirole administration (Fig.  
437 4C). Finally, we found that peripheral administration of aripiprazole blunted performance in an  
438 operant conditioning-based motivational task in control animals, while n-3 PUFA deficient mice  
439 were partially insensitive to aripiprazole (Fig. 4D).

440

## 441 **Discussion**

442 While most early reports on the preferential interaction between PUFAs and GPCRs focused  
443 on the interaction of rhodopsin with DHA [15–19], recent studies have confirmed that PUFAs  
444 can also modulate the activity and function of other GPCRs including the cannabinoid CB1  
445 receptor [62], the D2R [20, 21] and the adenosine A2A receptor [20, 21, 63]. Here, we focus  
446 on the interplay of PUFAs with the D2R, considering the relevance of this receptor to the  
447 pathophysiology of neuropsychiatric disorders and their pharmacological treatments. We found  
448 that DHA- but not DPA-rich phospholipids potentiate the binding affinity of both agonists and  
449 antagonists for the D2R. In line with this finding, MD simulations show a preferential interaction  
450 of the D2R with DHA tails compared to DPA tails. Both DHA and DPA seem to induce very  
451 similar conformational dynamics on the D2R and relative entropy analyses showed that the  
452 presence of PUFAs in the membrane selectively affects the conformation of ICL2. At the  
453 signaling level, both DPA and DHA enhance  $\beta$ -arrestin2 recruitment efficacy without affecting  
454  $G_{i/o}$  protein activity. Finally, we show that n-3 deficient animal models, in which membrane  
455 unsaturation is lower, display altered behavioral responses upon treatment with D2R ligands.

456

457 Our findings clearly show that DHA, but not DPA, enhances D2R ligand binding, suggesting  
458 that DHA acts as an allosteric modulator of the receptor. Various studies demonstrate that

459 PUFAs tend to preferentially interact with GPCRs [18, 19, 64, 65] and our MD simulations  
460 confirm that DHA – but not DPA – tails preferentially interact with the D2R when compared to  
461 saturated ones, as previously reported [20, 21]. In addition, we found that DHA- and DPA-  
462 containing phospholipids differentially interact with extracellular segments of transmembrane  
463 helix (TM) 1, TM2, and TM7, which define entry crevices for water and phospholipid  
464 headgroups into the binding pocket [66]. These findings suggest that direct interactions with  
465 the receptor could partly mediate the effect of DHA on D2R ligand binding. PUFA-containing  
466 phospholipids can also exert a strong influence on the physical and mechanical properties of  
467 biological membranes including thickness, bending or rigidity [67] and, hence, subtle changes  
468 in these properties can indirectly modulate the function of transmembrane proteins [67–69].  
469 Therefore, PUFA-rich membranes could potentially favor specific conformations of the D2R to  
470 modulate its ligand binding affinity. In particular, n-3 PUFAs provide transmembrane proteins  
471 with a flexible environment that enables such modulation [69–71]. Despite differing in just one  
472 double bond, n-6 PUFAs do not seem to increase membrane elasticity to the same extent [72],  
473 which could also account for the differential effects of DHA and DPA on D2R ligand-binding  
474 affinity. Furthermore, by increasing membrane fluidity and/or packing defects [8], n-3 PUFAs  
475 could alter the accessibility of ligand into the D2R or its exit from the binding site, thereby  
476 modulating ligand binding affinity. In fact, most antipsychotics (i.e. D2R ligands) display high  
477 membrane partitioning properties [73, 74]. Of note, Lolicato et al. recently suggested that the  
478 entry pathway of dopamine into the D2R likely requires this neurotransmitter to first partition  
479 into the membrane [75].

480  
481 Intriguingly, our simulations show that the preferential interaction of DHA with the D2R is lower  
482 or completely absent in the presence of ligands in the binding pocket. This result suggests a  
483 scenario where the conformational state induced by the ligand could also modulate the  
484 interaction propensity between the membrane and the receptor, as recently suggested for  
485 cholesterol and the oxytocin receptor [76]. While further investigation will be needed to unravel  
486 the precise molecular mechanisms behind the effect of DHA on ligand binding affinity, our work  
487 suggests that DHA can act as an allosteric modulator of the D2R, both by influencing the bulk  
488 membrane properties and by establishing direct interactions with the receptor.

489  
490 Our dose-response experiments in cells show that the selective enhancing effect of DHA on  
491 D2R ligand binding affinity does not correlate with enhanced potency of relevant downstream  
492 protein effectors. Membrane PUFA enrichment does not alter the potency or efficacy of D2R  
493 agonists inhibiting cAMP production supporting a lack of effect on  $G_{i/o}$  protein signaling,  
494 consistent with previous reports [77–79]. Similarly, there was no effect of DPA or DHA on the  
495 potency ( $EC_{50}$ ) of agonist-induced  $\beta$ -arrestin recruitment, despite an enhancement of maximal

496 efficacy ( $E_{max}$ ). These data suggest that the effects of PUFAs on D2R signaling are at least  
497 partially uncoupled from the changes in receptor binding properties. One possibility is that the  
498 relatively small changes in ligand binding affinity induced by PUFAs on ligand binding affinity  
499 might be difficult to detect using signalling assays in heterologous system. Alternatively, while  
500 highly speculative, our findings are in line with the emerging notion of “loose allosteric  
501 coupling”, which implies that conformational changes in the binding pocket region facilitate, but  
502 do not necessarily dictate specific conformations at the intracellular end of the receptor [80,  
503 81].

504  
505 Our findings consistently show that both DHA and DPA enhance the maximal efficacy of  $\beta$ -  
506 arrestin2 recruitment. This is compatible with the fact that PUFAs can alter the lateral  
507 organization of membranes [49, 82–84] and change the domain partitioning properties of  
508 GPCRs including the D2R [20, 21]. As previously suggested [85–90], altering receptor  
509 partitioning could modulate recruitment of GRKs and receptor phosphorylation and, in turn,  
510 influence  $\beta$ -arrestin coupling. It is also worth speculating that PUFA-induced membrane  
511 packing defects [8] could alter  $\beta$ -arrestin recruitment by modulating the membrane anchoring  
512 of its C-edge loops [91]. Interestingly, our MD simulations show that the presence of membrane  
513 PUFAs influence the conformational dynamics of the D2R, especially within a protein segment  
514 of the ICL2 that encompasses two consecutive serine residues – S147 and S148. These  
515 residues are involved in D2R phosphorylation [92] and located just upstream of Lysine 149, an  
516 important residue for  $\beta$ -arrestin binding at the D2R [93]. Considering the important role of ICL2  
517 in the recruitment of  $\beta$ -arrestin by the D2R [94], the effect of membrane unsaturation on the  
518 structure of this loop could therefore be one key mechanism underlying enhancement of  $\beta$ -  
519 arrestin2 recruitment by PUFAs.

520  
521 Overall, our *in vitro* and *in silico* findings show that membrane unsaturation can influence the  
522 activity of the D2R in at least two partially distinct ways, i) changes in ligand binding affinity  
523 which seems to be specific to DHA, and ii) recruitment of  $\beta$ -arrestin signaling. These effects  
524 may have important physiological impact in the context of chronic alterations in PUFA levels,  
525 in particular when occurring during brain development. This hypothesis is supported by our *in*  
526 *vivo* findings in a model of chronic deficiency in n-3 PUFAs [14] that results in a decrease of  
527 overall membrane unsaturation. In fact, in accordance with our *in vitro* results, while n-3 PUFA  
528 deficiency does not seem to affect D2R-mediated activation of the  $G_{i/o}$  protein in the striatum,  
529 the decrease in phospho-GSK3 expression is consistent with an impairment of  $\beta$ -arrestin2-  
530 dependent signaling, since GSK3 phosphorylation is reduced by  $\beta$ -arrestin2 recruitment to the  
531 D2R [61]. Notably, D2R-mediated locomotor response has recently been shown to also

532 depend on  $\beta$ -arrestin2-, but not  $G_{i/o}$  protein-, mediated signalling [95]. In line with this, we find  
533 that D2R agonist (quinpirole)-induced increase in locomotion is blunted in n-3 PUFA deficient  
534 animals, consistent with an impairment in  $\beta$ -arrestin2 recruitment and downstream signalling.  
535 However, we find that aripiprazole-induced decrease in motivation – which might result from  
536 an antagonistic activity of aripiprazole at the D2R - is also blunted in n-3 PUFA deficient  
537 animals. D2R-mediated modulation of motivation has been shown to be independent of  $\beta$ -  
538 arrestin2-dependent signalling [95], but rather to rely on  $G_{i/o}$  protein-mediated transmission at  
539 synaptic terminals in the ventral pallidum [96]. This raises the intriguing hypothesis that  
540 membrane PUFAs could differentially modulate D2R signalling activity in distinct neuronal  
541 subcompartments. Even though further work will be needed to disentangle the precise  
542 mechanisms by which PUFAs modulate D2R activity *in vivo*, these latter data are in line with  
543 the recent demonstration that motivational deficits in n-3 PUFA deficient animals directly  
544 relates to a dysfunction of D2R-expressing neurons [14].

545

546 Uncovering the precise mechanisms by which brain PUFA biostatus influences D2R-  
547 dependent signalling and downstream-related behaviors will require further work. Nonetheless,  
548 taken together, our results show that n-3 PUFAs can act as allosteric modulators of the D2R  
549 and potentiate its signalling activity, in particular the  $\beta$ -arrestin2 component. This raises the  
550 intriguing hypothesis that n-3 PUFA supplementation could alter the effects of D2R ligands,  
551 including antipsychotics. In accordance with this idea, several clinical trials have shown that n-  
552 3 PUFA supplementation accelerates treatment response, improves the tolerability of  
553 antipsychotics in first-episode psychoses [97, 98], and reduces prescription rate of  
554 antipsychotics [99]. Our data suggest that direct action of PUFA membrane composition on  
555 D2R activity could mediate such effects.

556

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574

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576 M-L.J, V.D.P, R.G-G, I.D.A. and P.T. conceived and supervised the study. J.S., G.B-G., E.M.,  
577 T.D. and J.A.J. provided expertise, reagents and supervised specific experiments. M-L.J.,  
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579 performed experiments and analyzed the data. M-L.J., V.D.P., R.G.-G., I.D.A. and P.T. wrote  
580 the original version of the manuscript. All authors discussed the results and reviewed the  
581 manuscript.

582

#### 583 **Conflict of Interest**

584 The authors declare no competing financial interests.

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851

## 852 **Tables**

853

<b>Ratio</b>	<b>0 - 250 ns</b>	<b>250 - 500 ns</b>	<b>500 – 750 ns</b>	<b>750 ns - 1 <math>\mu</math>s</b>
SD(h)PC <sub>SN-1</sub>	0.97	1.69	2.95	2.42

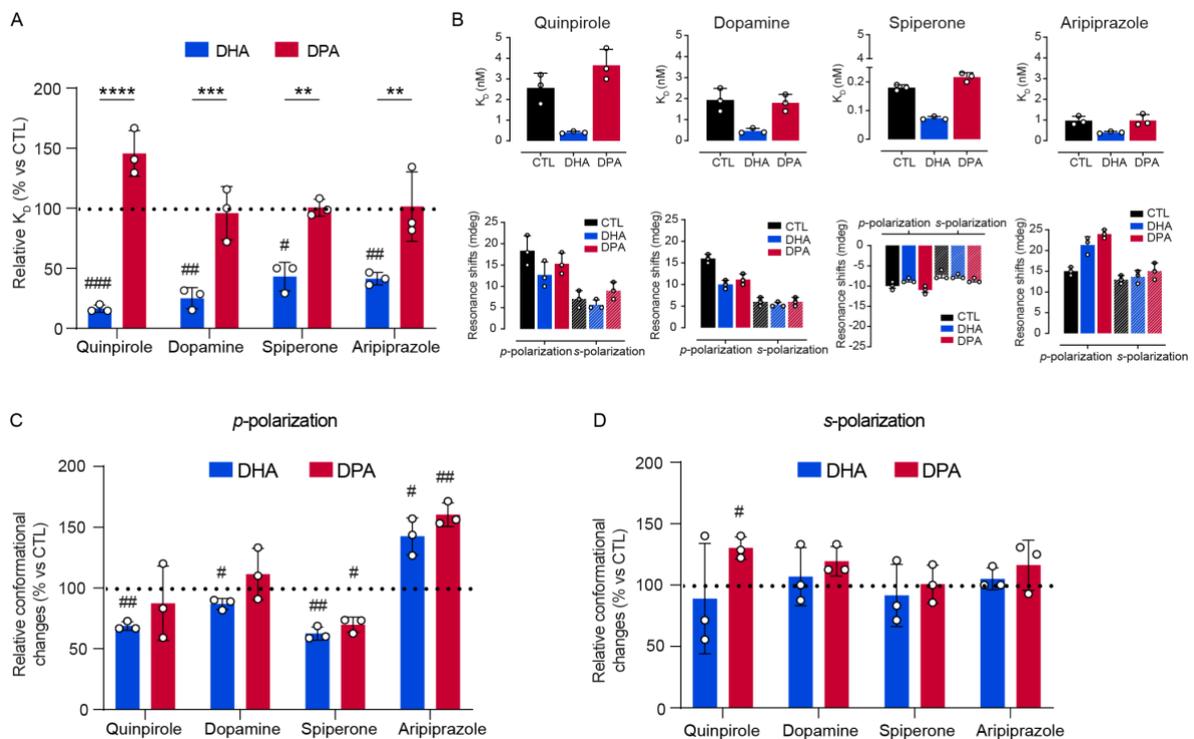
<b>SD(h)PC</b> <i>sn-2</i> (DHA)	2.01	3.05	4.61	4.08
SD(p)PC <i>sn-1</i>	1.49	1.48	1.48	1.63
<b>SD(p)PC</b> <i>sn-2</i> (DPA)	1.47	1.95	2.28	2.33

854 **Table 1. Evolution of lipid-protein contacts during MD simulations.** The table shows the average  
855 relative proportion of lipid-protein contacts at four stages of the simulation. Contact ratios represent the  
856 number of atomic contacts between each SD(h)PC or SD(p)PC chain (i.e. sn-1 or sn-2) and the D2R,  
857 with respect to the number of contacts between saturated phospholipids (i.e. DPPC, DSPC, and PSM)  
858 and the protein (see Methods for more details).  
859  
860

	Ratio	0 - 250 ns	250 - 500 ns	500 - 750 ns	750 ns - 1 $\mu$ s
DA	SD(h)PC <i>sn-1</i>	1.16	0.98	1.00	0.81
	<b>SD(h)PC</b> <i>sn-2</i> (DHA)	<b>1.78</b>	<b>1.63</b>	<b>1.62</b>	<b>1.73</b>
ARI	SD(h)PC <i>sn-1</i>	0.97	1.46	1.65	1.67
	<b>SD(h)PC</b> <i>sn-2</i> (DHA)	<b>1.75</b>	<b>2.87</b>	<b>3.12</b>	<b>3.07</b>

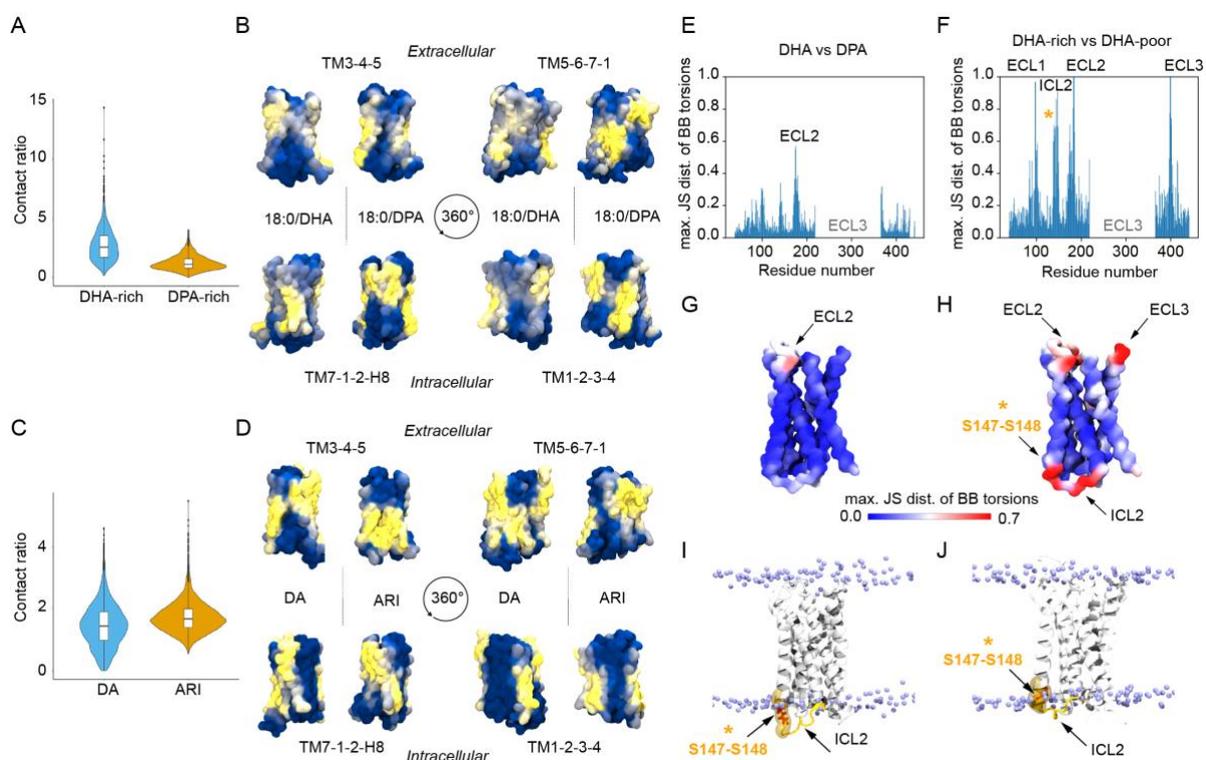
861 **Table 2. Evolution of lipid-protein contacts during ligand-bound D2R simulations.** The table shows  
862 the average relative proportion of lipid-protein contacts at four stages of the simulation. Contact ratios  
863 represent the number of atomic contacts between each SD(h)PC chain (i.e. sn-1 or sn-2) and the  
864 dopamine (DA)- or aripiprazole (ARI)-bound D2R, with respect to all saturated lipids (i.e. DPPC, DSPC  
865 and PSM) (see Methods for exact membrane composition and details on the calculation of these ratios).  
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869

## Figure Legends



870

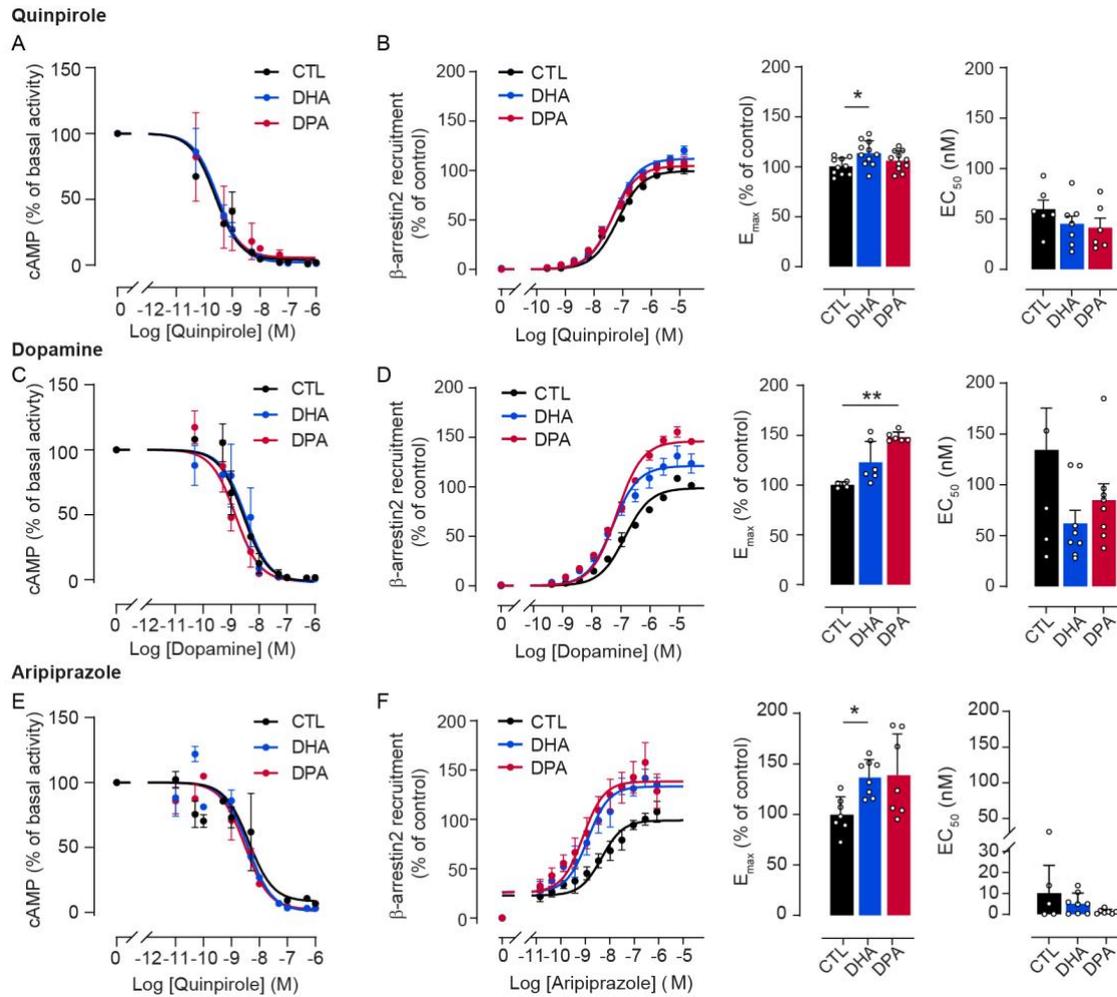
871 **Figure 1.** D2R binding affinity in PUFA-enriched cell membranes shown by PWR. (A) PUFA  
 872 effect on relative binding affinity of all ligands under DHA or DPA membrane enrichment. (B)  
 873 Dissociation constants ( $K_D$ ) and maximum resonance shifts measured for each ligand. (C, D)  
 874 Relative receptor conformational changes, for  $p$ -polarization (C) and  $s$ -polarization (D). Data  
 875 are mean  $\pm$  SD values from 3 independent experiments. Multiple comparisons were evaluated  
 876 by two-way ANOVA and Šídák's post hoc tests with \*\*\*\*  $p < 0.0001$ , \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  
 877  $p < 0.05$ . Comparisons to the control were evaluated by a one-sample t-test with ###  $p < 0.001$ ,  
 878 ##  $p < 0.01$ , #  $p < 0.05$ .



879  
 880 **Figure 2.** Lipid-protein contacts during MD simulations and effect on the structure of the D2  
 881 receptor. (A, C) Relative proportion of atomic lipid-protein contacts for the simulations of the  
 882 apo state of the D2R embedded in DHA- versus DPA-rich membranes (A), and the simulations  
 883 of dopamine- (DA) versus aripiprazole (ARI)-bound D2R embedded in DHA-rich membranes  
 884 (C). Specifically, figures depict the lipid-protein contact ratio of each SD(h)PC or SD(p)PC  
 885 chain versus all saturated lipids (i.e. DPPC, DSPC, and PSM) (see Methods for a detailed  
 886 description of these ratios). (B, D) Average lipid occupancy map for the simulations of the apo

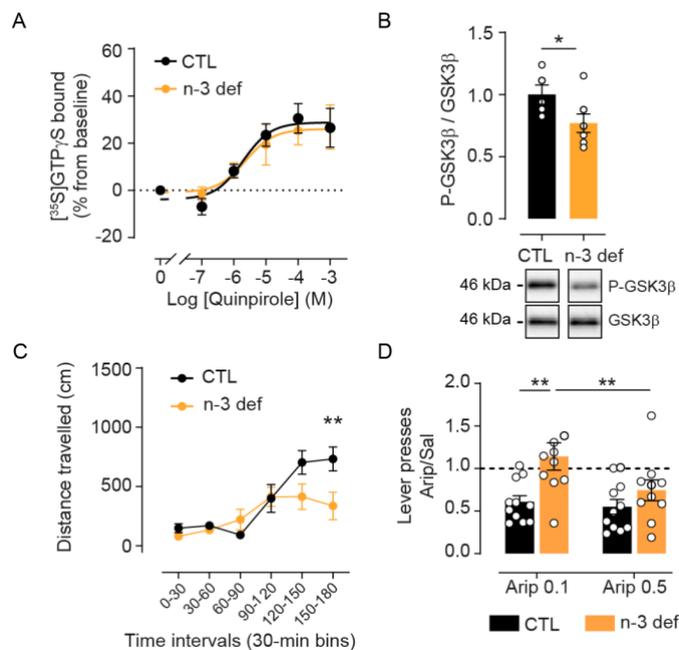
887 state of the D2R embedded in DPA- versus DHA-containing phospholipids (B), and the  
888 simulations of dopamine- (DA) versus aripiprazole (ARI)-bound D2R embedded in DHA-rich  
889 membranes (D). The percentage of frames where SD(h)PC or SD(p)PC lipid molecules  
890 contacted the D2R is depicted in a blue (low) to yellow (high) color gradient mapped to the  
891 surface of the receptor. (E, F) Relative entropy analysis of the D2R depicted as the maximum  
892 value of the Jensen-Shannon (JS) distance of the protein backbone (BB) torsion angles (y-  
893 axis) for each residue (x-axis) across all MD simulation replicas. The JS distance is a way of  
894 measuring how different two probability distributions, in this case, ensembles of BB torsion  
895 angles, are across two systems. Thus, the higher the JS distance, the higher the difference  
896 between the two ensembles. The plot shows a relative entropy analysis for DHA- versus DPA-  
897 rich (E), and DHA-rich versus DHA-poor (F) systems. The peak highlighted by the orange  
898 asterisk corresponds to the region of helix 4 that follows intracellular loop 2 (ICL2), namely  
899 residues 146 to 150. Subpanels (G) and (H) display an averaged D2R structure with the former  
900 relative entropy values depicted in a blue to red color gradient mapped to the surface of the  
901 receptor. To facilitate the comparison between both sets of results, the maximum red color of  
902 the gradient encompasses all values  $\geq 0.7$ . (I) and (J) show a representative snapshot of the  
903 DHA-poor and DHA-rich simulations, respectively. The D2R receptor and ICL2 are shown as  
904 white and orange cartoons, respectively. Serine residues S147 and S148 are shown as red  
905 sticks, within the 146 to 150 protein segment, which is highlighted by an orange transparent  
906 surface. Phospholipid phosphorous atoms are shown as purple beads to highlight the  
907 membrane boundaries.

908



909

910 **Figure 3.** Forskolin-stimulated cAMP production and β-arrestin2 recruitment upon ligand  
 911 stimulation and with different membrane PUFAs enrichment. (A, C, E) Dose-response  
 912 experiments of cAMP production with the D2R ligands quinpirole (A), dopamine (C) and  
 913 aripiprazole (E), on forskolin-stimulated cells incubated in the presence of 0.03% ethanol as  
 914 control, 30 μM DHA and 30 μM DPA n-6. Values are expressed as the percentage of cAMP in  
 915 the absence of agonist in n=3 independent experiments. (B, D, F) Quinpirole (B), Dopamine  
 916 (D) and aripiprazole (F) activity on D2R-mediated β-arrestin2 recruitment in CHO-K1 cells  
 917 expressing the DRD2L (left panel), and associated E<sub>max</sub> (middle) and EC<sub>50</sub> (right panel) under  
 918 30 μM PUFAs enrichment. Data are mean ± SD values from three independent experiments  
 919 with \*\*\*\* p<0.0001, \*\*\* p <0.001, \* p<0.05. cAMP assay and β-arrestin2 recruitment curves are  
 920 mean ± SEM. Comparisons to the control were evaluated by a Kruskal-Wallis and Dunn's post-  
 921 hoc tests.



922

923 **Figure 4.** Effect of n-3 PUFA deficiency *in vivo* on D2R signalling and associated behaviors.

924 (A) [<sup>35</sup>S]GTPγS assay on nucleus accumbens extracts upon increasing concentrations of

925 quinpirole from control (CTL) or n-3 PUFA deficient animals (n-3 def). CTL : logEC<sub>50</sub> = -5.22 ±

926 0.28 %, E<sub>max</sub> = 30.06 ± 3.47 % ; n-3 def : logEC<sub>50</sub> = -5.06 ± 0.29; E<sub>max</sub> = 28.15 ± 3.51 % (B)

927 Western blots measuring the phosphorylation of GSK3β (P-GSK3β) relative to GSK3β levels.

928 Bars represent the mean of 5 and 7 subjects for CTL and n-3 def respectively and error bars

929 the SEM. \*p<0.05 by Mann Whitney test. (C) Locomotor response to 1 mg/kg quinpirole

930 administration is represented as the distance travelled in cm divided in 30 min intervals in

931 control (CTL, n=10) or n-3 def (n=10) animals. \*\* p< 0.01 by two-way (RM) ANOVA test with a

932 post-hoc Bonferroni test (D) Ratio of lever presses performed by animals under aripiprazole

933 (0.1 or 0.5 mg/kg) over saline administration in a progressive ratio task in CTL (n=11) or n-3

934 def (n=10) animals. Data are mean ± SEM values. \*\* p< 0.01 by two-way (RM) ANOVA test

935 with a post-hoc Bonferroni test.

## 1 **Supplementary Methods :**

### 2 **Cell culture and treatment**

3 Cells were maintained at 37°C under 5% CO<sub>2</sub> in the following culture medium: DMEM  
4 (Dulbecco's modified Eagle's medium) Glutamax (InVitroGen) containing 10% of heat  
5 inactivated fetal bovine serum, 100 UI/ml penicillin and 0.1 mg/ml streptomycin and  
6 supplemented with 100 µg/ml Hygromycin (InVitrogen), 15 µg/ml Blastidin (Cayla Invivogen)  
7 and 1 µg/ml Tetracycline (Sigma Aldrich). Upon reaching 80-90% confluence, in the same  
8 culture medium, cells were treated with 10 µM and 30 µM DHA or DPA or 0.03% and 0.01%  
9 ethanol (vehicle) respectively as control for 24h. After treatment, cells were dissociated by  
10 using an Enzyme-Free Cell Dissociation Solution (S-014-B, Merck Millipore) and washed  
11 twice in wash buffer (50 mM Tris-HCl, 50 mM NaCl, 2 mM EDTA, pH 7.5 at 20°C) by  
12 centrifugation at 500 × g for 8 min at 20°C. Cell pellets were used either for Western blot  
13 analyses or for membrane preparation.

### 14 **Membrane preparation**

15 The cell pellets of HEK cells were resuspended in 10 ml of lysis buffer (10 mM Tris-HCl, 1 mM  
16 EDTA, pH 7.5 at 4°C) supplemented with anti-protease inhibitor cocktail (PIC; 1 µg/mL)  
17 (P8340, Sigma Aldrich) and incubated for 30 min in ice. The cell lysate was homogenized using  
18 a 10 ml glass-Teflon homogenizer with 30 gentle strokes. The resulting suspension was  
19 centrifuged at 183 × g for 10 min at 4°C. The supernatant was collected and the residual pellet  
20 was washed twice by centrifugation successively in 5 and 2.5 ml of lysis buffer. The resulting  
21 supernatants were pooled and centrifuged at 48000 × g for 30 min at 4°C. The pellet containing  
22 the membrane proteins was resuspended in binding buffer (50 mM Tris-HCl, 50 mM NaCl, 2  
23 mM EDTA, 2 mM CaCl<sub>2</sub>, pH 7.5 at 4°C) supplemented with PIC. Protein concentration was  
24 determined by using Bicinchoninic Acid (BCA) protein assay (Uptima, Montluçon, France) and  
25 membranes were diluted to 1 µg/µl in binding buffer.

26

### 27 **Plasmon waveguide resonance (PWR)**

28 Experiments were carried out at controlled room temperature, e.g. 23°C. The sensor consists  
29 in a 90° angle prism whose hypotenuse is coated with a silver layer (50 nm) and overcoated  
30 with silica (460 nm) and is in contact with the cell sample Teflon block, with an aperture of  
31 approximately 3 mm diameter through which the lipid bilayer is formed. This is placed on a  
32 rotating table mounted on a corresponding motion controller (Newport, Motion controller XPS;  
33 ≤ 1 mdeg resolution).

34

### 35 **Formation of a planar lipid bilayer on the PWR sensor**

36 The planar lipid bilayer is formed across the small aperture (~ 3 mm) in the Teflon PWR cell  
37 by fusing freshly sonicated small unilamellar vesicles (SUV, 3mg/ml) with the silica surface.  
38 SUVs were prepared by initially dissolving the appropriate amount of phospholipids in  
39 chloroform, to obtain the desired final concentration. A lipid film was then formed by  
40 removing the solvent using a stream of N<sub>2</sub> (g) followed by 3 h vacuum. The lipid film was  
41 dispersed in PBS and thoroughly vortexed to form multi-lamellar vesicles (MLVs). To form  
42 SUVs, the MLVs dispersion was sonicated 5 times during 10 minutes at 40Hz frequency just  
43 before use.

### 44 **Immobilisation of cell fragments on the PWR sensor**

45 The protocol for adhesion of cell fragments on silica (glass slides or PWR sensor) was adapted  
46 from reported work from Perez and collaborators [28]. The silica surface was washed with  
47 ethanol, cleaned and activated by Plasma cleaner for 2 min (Diener, Bielefeld, Germany). The  
48 silica surfaces were then incubated with a solution of poly-L-lysine (PLL, 0.1 mg/mL) for 40  
49 minutes following wash with PBS buffer. Cells grown to less than 50% confluence were washed  
50 with PBS and covered with water to induce osmotic swelling of the cells. Immediately, the glass  
51 coverslip of the sensor was placed directly on top of cells. Pressure was applied for about 2  
52 min on the glass slide or prism to induce cell rupture and caption of cell fragments. Then they  
53 were removed ripping off cell fragments containing specially the upper membrane. The glass  
54 slide or sensor was washed with PBS to remove cell debris and kept with buffer to prevent  
55 drying and loss of membrane protein activity. PWR measurements were performed right away.  
56 The PWR cell sample (volume capacity of 250 µL) was placed in contact with the prism and  
57 filled with PBS.

### 58 **Partial purification and reconstitution of the dopamine D2 receptor in the lipid bilayer**

59 D2R-expressing membranes from *Pichia pastoris* (gift from JL Banères) were used. All steps  
60 were performed at 4°C or on ice. Isolated membranes were diluted to 5 mg/ml with 50 mM  
61 Tris-HCl, pH 8, 500 mM NaCl, 5% glycerol, 1 mM PMSF, 0.1 mM TCEP, 10 mM imidazole. N-  
62 Dodecyl-β-D-maltoside (DDM) mixed with cholesteryl hemisuccinate (CHS) was added to a  
63 final concentration of 1%/0.2% DDM/CHS (w/v) and incubated for 15 min at 4°C. The  
64 suspension was centrifuged for 20 min at 120,000 x g. Solubilized receptor was purified by  
65 immobilized metal ion affinity chromatography (IMAC) on a Talon column (1 ml). The column  
66 was equilibrated in purification buffer (50 mM Tris-HCl, 500 mM NaCl, 5% glycerol, 1 mM  
67 PMSF, 0.1 mM TCEP, with 10 mM imidazole, 0.1%/0.02% DDM/CHS (w/v), pH 8). Solubilized  
68 proteins were loaded on the column (0.1 mL/min flow-rate) and washed in purification buffer  
69 until baseline was reached (UV absorbance 280 nm). After a washing step (50 mM Tris-HCl,

70 500 mM NaCl, 5% glycerol, 1 mM PMSF, 0.1 mM TCEP, 20 mM imidazole 0.1%/0.02%  
71 DDM/CHS (w/v), pH 8), proteins were eluted by elution buffer (50 mM Tris-HCl, 500 mM NaCl,  
72 5% glycerol, 1 mM PMSF, 0.1 mM TCEP, 250 mM imidazole, 0.1%/0.02% DDM/CHS (w/v),  
73 pH 8) and eluted fractions containing protein were pooled. Enrichment of the D2R in the eluates  
74 was verified by western blot (Fig. S4D) by assessing relative D2R (anti-D2R: Millipore Cat. No.  
75 ABN462, 1/500) amounts as compared to total membrane extract with equal amounts of  
76 protein loaded (2.5  $\mu$ g).

77 After lipid bilayer formation, detergent-solubilized D2 receptor was reconstituted in the lipid  
78 bilayer by the detergent-dilution method (see supplementary information for details). Briefly,  
79 the receptor was purified in a DDM/CHS mixture at concentrations that are about 10 fold over  
80 the critical micelle concentration (cmc) of DDM. Part of the detergent was then removed from  
81 the sample by use of centricons (Merck Millipore) with a cutoff of 50 KDa. This consisted in  
82 concentrating and reducing the initial volume of the solubilized protein by a factor of 5. The  
83 insertion of a small volume (about 20  $\mu$ L) of DDM/CHS solubilized protein into the PWR  
84 chamber, leads to drastic and quick drop in the detergent concentration. If the detergent  
85 concentration drops below the cmc during this step, this results in immediate positive PWR  
86 shifts for both *p*- and *s*-polarisations with small changes in the Total Internal Reflection (TIR)  
87 angle. In order to compare data among different experiments, data were normalized relative  
88 to the amount of reconstituted protein in the membrane.

### 89 **Ligand-induced receptor response**

90 The method consisted in incrementally adding a specific dopamine D2 ligand to the cell and  
91 monitor the PWR spectral changes. The first concentration point was chosen to be  
92 approximately 1 order of magnitude lower than the published dissociation constant ( $K_D$ ) value  
93 for that ligand. Before each incremental concentration of ligand added, the system was left to  
94 equilibrate.  $K_D$  values were obtained from plotting the resonance minimum position of the PWR  
95 spectra (this reflects the receptor-ligand complex) as a function of ligand concentration in the  
96 PWR cell and fitting to the hyperbolic function that describes the 1:1 binding of a ligand to a  
97 receptor using GraphPad Prism (GraphPad Software). This was performed separately for data  
98 obtained with the *p*- and *s*-polarization. The reported  $K_D$  is an average of the two values. The  
99 spectral changes (equivalent to  $B_{max}$ ) observed at saturating ligand concentrations for each  
100 experimental condition were plotted. Multiple comparisons were evaluated by two-way ANOVA  
101 and Šídák's post hoc tests. Comparisons to the control were evaluated by a one-sample t-test.

102

### 103 **cAMP accumulation assays**

104 D2R stably expressing HEK 293 (or CHO when indicated) cells were grown on PLL treated  
105 12-well plates as described above. Upon reaching 80-90% confluence, cells were treated with  
106 10  $\mu$ M or 30  $\mu$ M DHA or DPA n-6 or 0.03 % to 0.1 % ethanol (vehicle) as control for 24 h in the  
107 culture medium. The medium was removed and cells were rinsed in DMEM before  
108 pretreatment with 1 mM IsoButylMethylXanthine (IBMX, Sigma I5879) for 15min. Cells were  
109 then stimulated for 30 min with the indicated concentrations of agonists Quinpirole (Tocris  
110 1061), Dopamine (Sigma H8502) and Aripiprazole (Sigma SML0935) in the presence of 1 mM  
111 IBMX and 10  $\mu$ M Forskolin (Tocris 1099). Endogenous phosphodiesterase activity was  
112 stopped by removing the medium and the addition of 0.1 M HCL (300  $\mu$ l/well). After  
113 centrifugation at 600 x g during 10 min, protein concentration of supernatants was quantified  
114 by BCA. Cyclic AMP levels were determined in samples containing 10  $\mu$ g of protein. The  
115 production of cAMP was measured by using a cAMP Enzyme Immunoassay kit (Sigma,  
116 CA200) as described by the manufacturer using Victor3 (Perkin Elmer) plate reader. The curve  
117 fit was obtained by GraphPad Prism 5 (GraphPad Software, Inc.).

118

### 119 **Fluorescence anisotropy assay**

120 Fluorescence anisotropy (FA) was used to measure receptor-ligand binding reaction into  
121 individual wells of black, 96-well Greiner Bio-One microplates in a final volume of 100 $\mu$ l. To  
122 establish a saturation binding curve, the range of protein membrane concentration used was  
123 from 1 to 25  $\mu$ g in the presence of 10 nM of the antagonist NAPS-d2 ligand (L0002 red, Cisbio  
124 Bioassays). The FA was measured on a Tecan Infinite M1000 Pro microplate reader  
125 (Männedorf, Switzerland). Excitation was set at 590  $\pm$  5 nm and emission was collected at 665  
126  $\pm$  5 nm bandpass filters for the Texas Red.

127 The non-specific binding was obtained from membranes that did not express the receptor and  
128 the total binding was measured with D2R-expressing membranes. The binding curve was  
129 performed on the total amount of proteins measured in the sample. The non-specific binding  
130 curve was fitted with a Simple-linear regression in GraphPad Prism (GraphPad Software, San  
131 Diego, CA) and the total binding curve was fitted with a One site – total binding curve in  
132 GraphPad Prism. The specific binding was determined by subtracting the non-specific curve  
133 to the total binding curve and the final specific binding curve was fitted with a One site – specific  
134 binding model in GraphPad Prism. The latter was used to determine relative  $K_D$  values via  
135 separated saturation binding curves in at least three independent experiments and are  
136 reported as mean  $\pm$  SEM. The lower the value, the higher the affinity.

137

### 138 **$\beta$ -Arrestin2 recruitment assay**

139  $\beta$ -arrestin2 recruitment was assessed using the PathHunter<sup>®</sup> express DRD2L CHO-K1 Beta  
140 arrestin GPCR Assay (DiscoverX, Fremont, CA). In brief, cells were plated into 96-well white-  
141 walled assay plates in a volume of 90  $\mu$ l of Cell Plating Reagent (DiscoverX). They were  
142 incubated 24 h at 37 °C, 5 % CO<sub>2</sub>. The next day, PUFAs (Ethanol 0.01% as control vehicle,  
143 DHA, and DPA n-6) were prepared at 10X concentration (300  $\mu$ M) in Cell Plating Reagent and  
144 10  $\mu$ L was added to the cells for 24h at 37 °C, 5 % CO<sub>2</sub>. Serial dilutions (11x) ranging from 154  
145 to 0.0026  $\mu$ M, 281 to 0.0046  $\mu$ M and 9.35 to 0.000157  $\mu$ M, of Quinpirole, Dopamine and  
146 Aripiprazole respectively, were prepared and 10  $\mu$ l of each concentration was added for 90  
147 minutes. Luminescence was measured at 1 h post PathHunter<sup>®</sup> detection reagent addition  
148 using Victor3 plate reader (Perkin Elmer 0.5-s/well integration time). Data were normalized to  
149 control treated with the highest concentration of ligand (100%). This control value corresponds  
150 to Top best fit value determined by non-linear fit of standard slope. Data were fitted to a three-  
151 parameter logistic curve to generate EC<sub>50</sub> and E<sub>max</sub> values (Prism, version 5.0, GraphPad  
152 Software, Inc., San Diego, CA). EC<sub>50</sub> and % E<sub>max</sub> values are the result of three independent  
153 experiments performed in duplicate.

154

## 155 **Behavioral experiments**

156 All animal care and experimental procedures were in accordance with the INRAE Quality  
157 Reference System and to French legislations (Directive 87/148, Ministère de l'Agriculture et  
158 de la Pêche) and European (Directive 86/609/EEC). They followed ethical protocols approved  
159 by the Region Aquitaine Veterinary Services (Direction Départementale de la Protection des  
160 Animaux, approval ID: B33-063-920) and by the animal ethic committee of Bordeaux CEEA50.  
161 Every effort was made to minimize suffering and reduce the number of animals used.  
162 C57BL/6J mouse lines from Janvier Laboratories (Robert Janvier, Le Genest St-Isle France)  
163 were used in this study. Mice were housed in groups of 5-10 animals in standard polypropylene  
164 cages and maintained in a temperature and humidity-controlled facility under a 12:12 light-dark  
165 cycle (8:00 on) with *ad libitum* access to water and food.

### 166 **Operant conditioning:**

167 Animals were food-restricted in order to maintain them at 85-90% of their *ad libitum* weight and  
168 exposed to one session (1 hour) each day, 5-7 days per week. A pavlovian training followed  
169 by fixed and random ratio training were performed in order to make the animals reach an  
170 acquisition criterion (i.e. the number of lever presses and rewards earned) before performing  
171 the motivational task per se as previously described [14]. In the motivational task, namely the  
172 progressive ratio times 2 (PRx2) schedule, the number of lever presses required to earn a

173 reward was doubled respective to the previous one obtained. Mice were tested multiple times  
174 in PRx2 with RR20 sessions intercalated between each PR tasks.

#### 175 **Spontaneous locomotion:**

176 Animals were transferred individually to small Plexiglas cages (10 cm wide, 20 cm deep, 12  
177 cm tall) equipped with a video tracking system (Smart, Panlab, Barcelona, Spain) allowing the  
178 recording of total distance travelled (cm) as a measure of spontaneous locomotor activity in  
179 basal condition for one hour.

#### 180 **Western blot**

181 The following primary antibodies were used: 1:700 rabbit anti- $\beta$ Arr2 (Cell signaling Cat. No.  
182 3857); 1:1000 rabbit anti-GSK3 $\beta$  (Cell signaling Cat. No. 12456); 1:1000 rabbit anti-P-GSK3 $\beta$   
183 (Cell signaling Cat. No. 5558); 1:10000 mouse anti-tubulin (Merck Cat. No. T5168).

184

#### 185 **Cell Membrane preparation for Microarray and [<sup>35</sup>S]GTP $\gamma$ S autoradiography**

186 Tissue samples were homogenized using a Teflon-glass grinder (Heidolph RZR 2020) and a  
187 disperser (Ultra-Turrax® T10 basic, IKA) in 20 volumes of homogenized buffer (1 mM EGTA,  
188 3 mM MgCl<sub>2</sub>, and 50 mM Tris-HCl, pH 7.4) supplemented with 250 mM sucrose. The crude  
189 homogenate was subjected to a 3,000 rpm centrifugation (Allegra™ X 22R centrifuge,  
190 Beckman Coulter) for 5 min at 4°C, and the resultant supernatant was centrifuged at 14,000  
191 rpm (Microfuge® 22R centrifuge, Beckman Coulter) for 15 min (4 °C). The pellet was washed  
192 in 20 volumes of homogenized buffer and re-centrifuged under the same conditions. The  
193 homogenate aliquots were stored at -80 °C until they were used. Protein concentration was  
194 measured by the Bradford method and adjusted to the required concentrations.

#### 195 **Lipid analyses**

196 Gas chromatography on a HewlettPackard Model 5890 gas chromatograph (Palo Alto, CA,  
197 USA) was employed to analyze fatty acid methylesters (FAMES) using aCPSIL-88 column (100  
198 m $\times$ 0.25 mm internal diameter; film thickness 0.20  $\mu$ m; Varian, Les Ulis, France). Hydrogen  
199 was used as a carrier gas (inlet pressure, 210 kPa). The oven temperature was maintained at  
200 60 °C for 5 min, then increased to 165 °C at 15 °C/min and held for 1 min, and then to 225 °C  
201 at 2 °C/min and finally held at 225 °C for 17 min. The injector and the detector were maintained  
202 at 250 °C and 280 °C, respectively. FAMES were identified by comparison with commercial  
203 and synthetic standards and the data were computed using the Galaxie software (Varian). The

204 proportion of each fatty acid was expressed as a percentage of total fatty acids to allow the  
205 comparison of lipid composition in different cell culture conditions.

## 206 **Statistical analyses**

207 Statistical analyses were conducted using Prism 6 software (GraphPad Software, La Jolla, CA,  
208 USA). Two-tailed unpaired Student's t-test or Mann Whitney test were used to assess  
209 differences between two groups. Multiple comparisons in Fig.1 were evaluated by two-way  
210 ANOVA and Šídák's post hoc tests and comparisons to the control were evaluated by a one-  
211 sample t-test. Two-way RM ANOVA followed by post-hoc Bonferroni tests were used for  
212 repeated measures. Differences were considered significant for  $p < 0.05$ .

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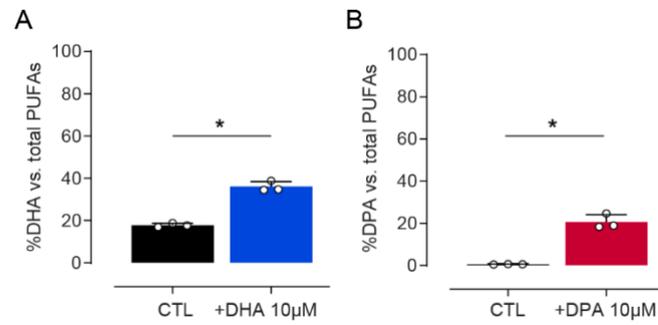
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232 **Supplementary Figures :**

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234 Figures S1 - S8

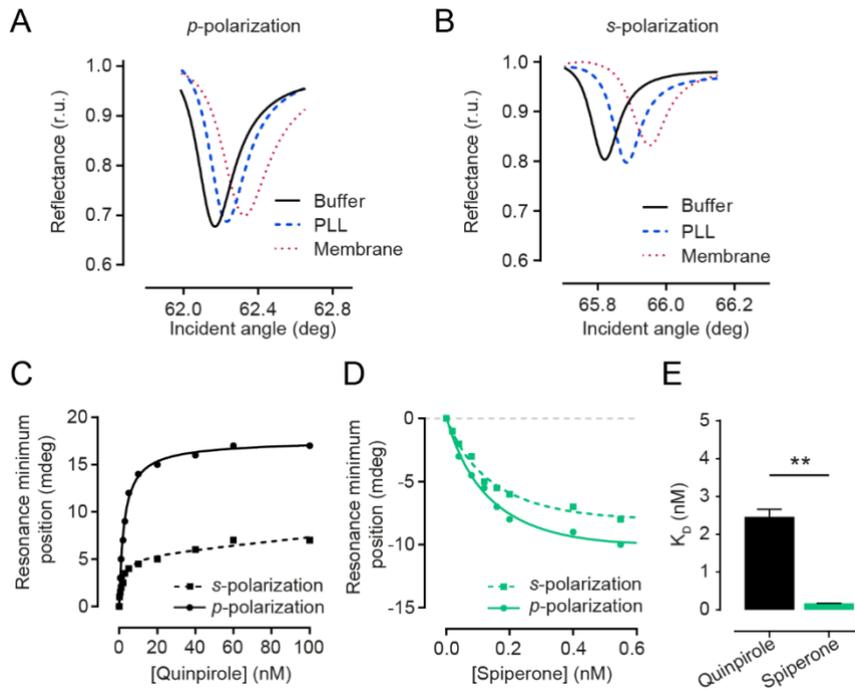
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238 **Supplementary Figure S1. PUFAs enrichment in HEK cells.** HEK cells were incubated with DHA (A)  
239 or DPA (B) at 10 µM concentration. Each PUFA enrichment is compared to control cells incubated with  
240 vehicle only (0.03 % ethanol). Bars represent the mean average of three independent experiments and  
241 error bars represent the standard deviation (SD). \* p < 0.05 by one-tailed Mann-Whitney test.

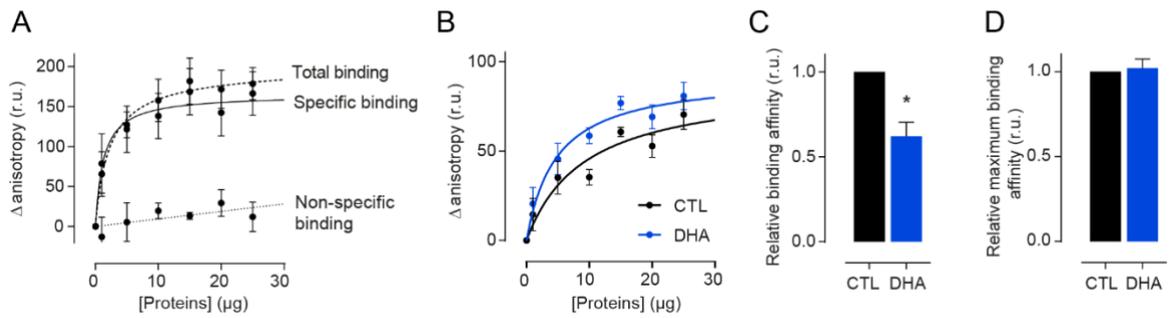


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243 **Supplementary Figure S2. Ligand binding on cell membrane fragments on the PWR sensor.**  
 244 Capture of cell membrane fragments containing the D2R following PLL treatment of the PWR sensor  
 245 followed by PWR using  $p$ - and  $s$ -polarization. **(A and B)** Representative PWR spectra following sensor  
 246 coating with polylysine (PLL; blue) and cell membrane fragment capture (red) obtained with  $p$ - (A) and  
 247  $s$ - (B) polarized light, respectively. **(C and D)** Quinpirole (C) and spiperone (D) were incrementally added  
 248 to the proteolipid membrane and the shifts in the resonance minimum position followed. The data were  
 249 fitted with a hyperbolic binding equation that describes total binding to a single site in the receptor (more  
 250 details in Materials and Methods). **(E)** Affinity binding of quinpirole and spiperone calculated from (C)  
 251 and (D) respectively. \*\*  $p < 0.01$  by two-tailed unpaired t-test.

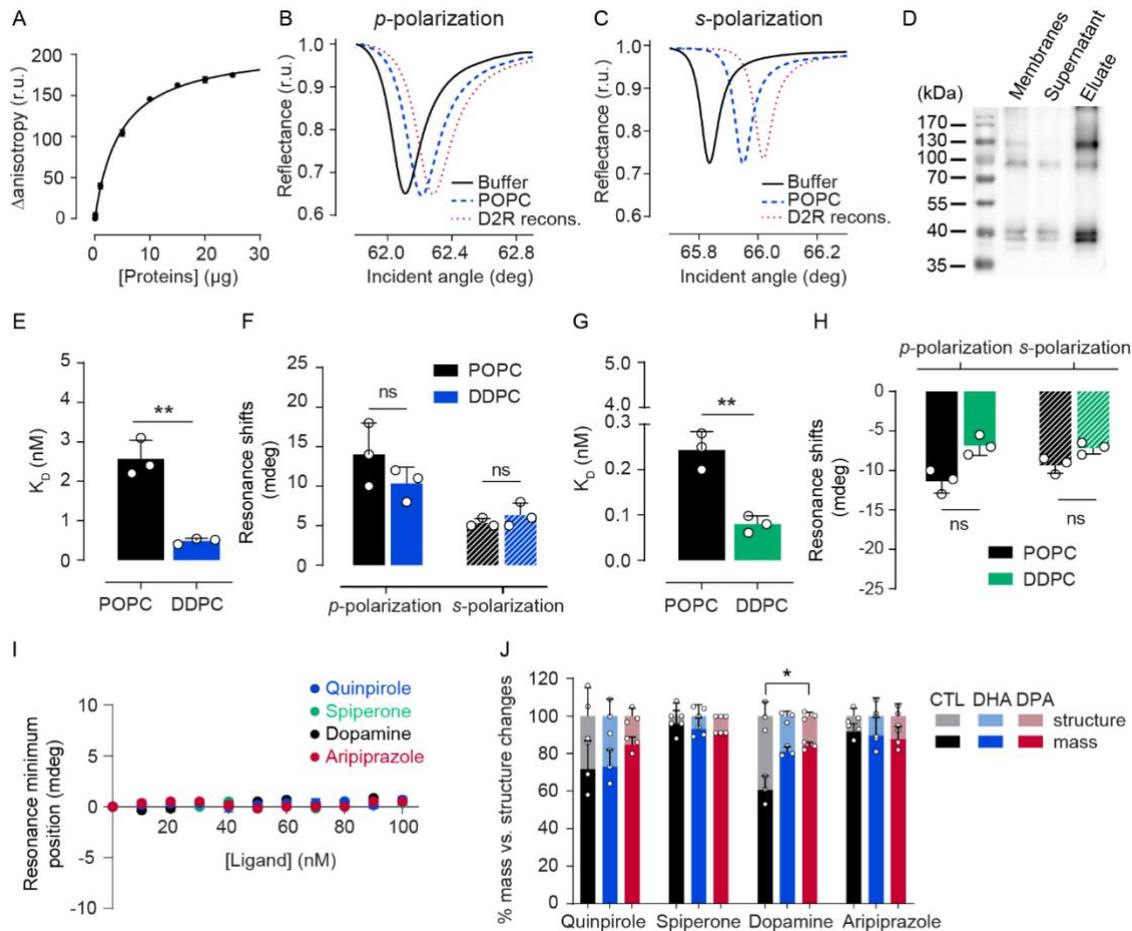
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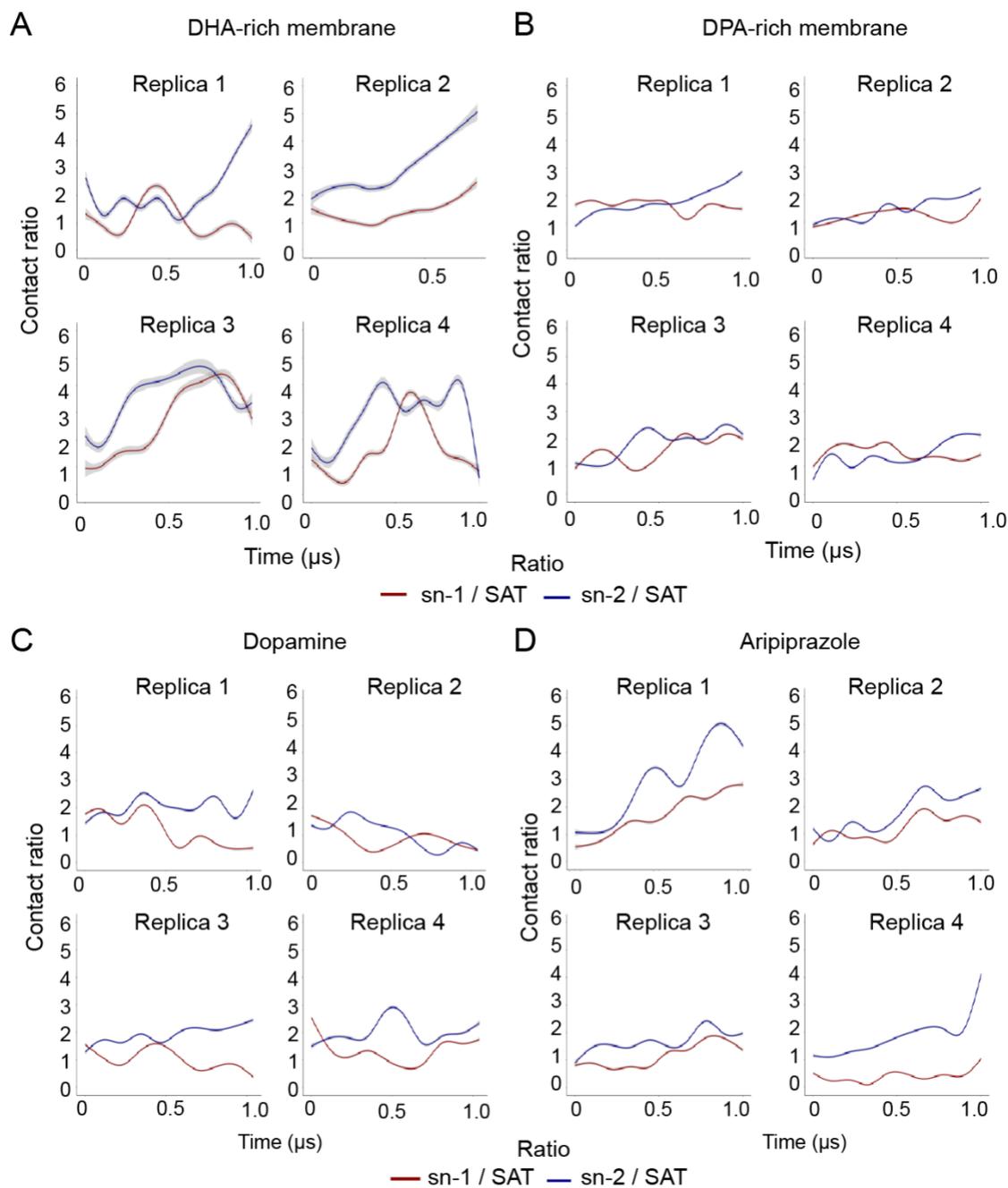
**Supplementary Figure S3. Impact of membrane DHA on D2R ligand binding measured by fluorescence anisotropy. (A)** Fluorescence anisotropy measurement of NAPS-d2 binding on D2R-expressing membranes (total binding) or non-expressing membranes (non-specific binding). **(B)** Antagonist (NAPS-d2) binding to control and DHA-enriched membranes. **(C)** Fold change of ligand affinity to D2R in DHA-enriched membranes compared to control membranes. **(D)** Maximum relative binding affinity to D2R in DHA-enriched membranes. Data are mean  $\pm$  SEM from three independent experiments with \*  $p < 0.05$  by two-tailed unpaired t-test.



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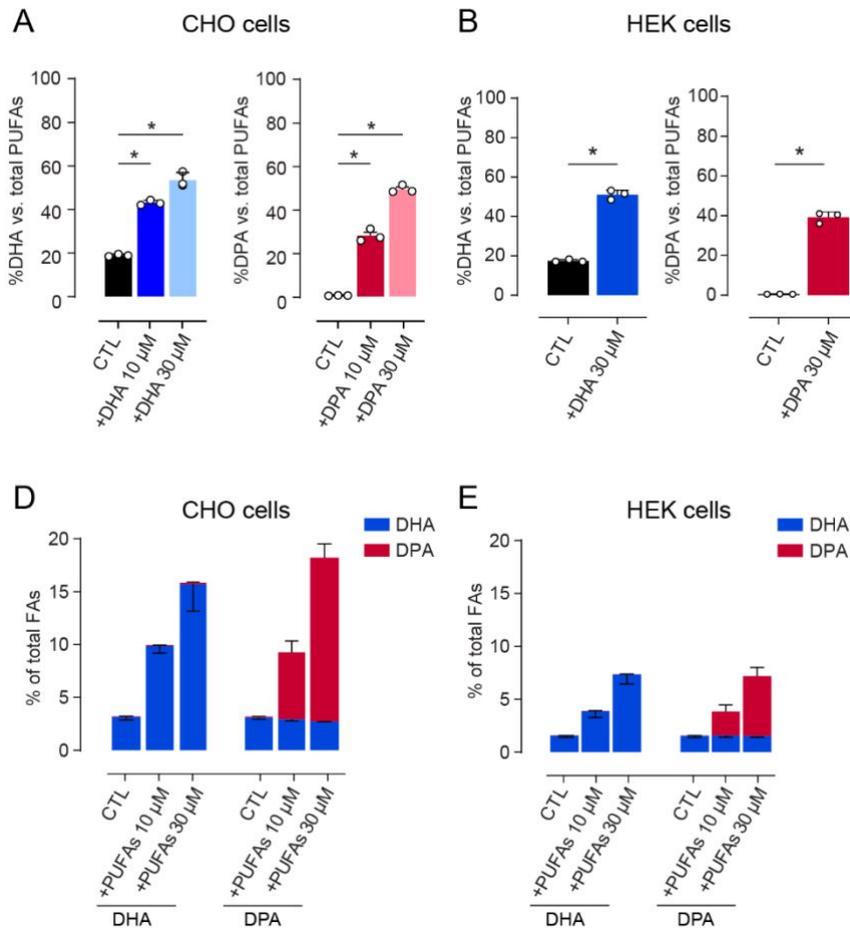
264 **Supplementary Figure S4. Impact of DHA on ligand binding to D2R partially purified and**  
 265 **reconstituted in model membranes shown by PWR. (A)** Fluorescence anisotropy measurement of  
 266 NAPS-d2 binding to partially purified D2R (total binding). **(B and C)** Representative PWR spectra  
 267 following supported POPC lipid membrane formation (blue) and D2R membrane reconstitution (red) for  
 268 *p*- (B) and *s*-polarized (C) light, respectively. **(D)** The D2R was partially purified from membrane extracts  
 269 and the enrichment of the D2R in the eluates was verified by western blot with an antibody anti-D2R. **(E**  
 270 **and F)** Effect of DHA on quinpirole affinity to D2R **(E)** and quinpirole-induced receptor conformational  
 271 changes **(F)** in reconstituted lipid model systems composed of POPC and double chain DHA-PL. **(G and**  
 272 **H)** Effect of DHA on spiperone affinity to D2R **(G)** and spiperone-induced receptor conformational  
 273 changes **(H)** in reconstituted lipid model systems. **(I)** Quinpirole, spiperone, dopamine and aripiprazole  
 274 were incrementally added to cell membrane fragments without D2R. **(J)** Mass and structural changes  
 275 contributions of the PWR spectral shifts observed upon ligand binding to the D2R in cell membrane  
 276 fragments enriched or not with PUFAs (at saturating ligand concentrations) determined by a graphical  
 277 analysis. Data are mean  $\pm$  SD values from at least three independent experiments with \*\*  $p < 0.01$  by  
 278 two-tailed unpaired t-test (E and G) and \*  $< 0.05$  by Kruskal-Wallis and Dunn's post hoc test (J).



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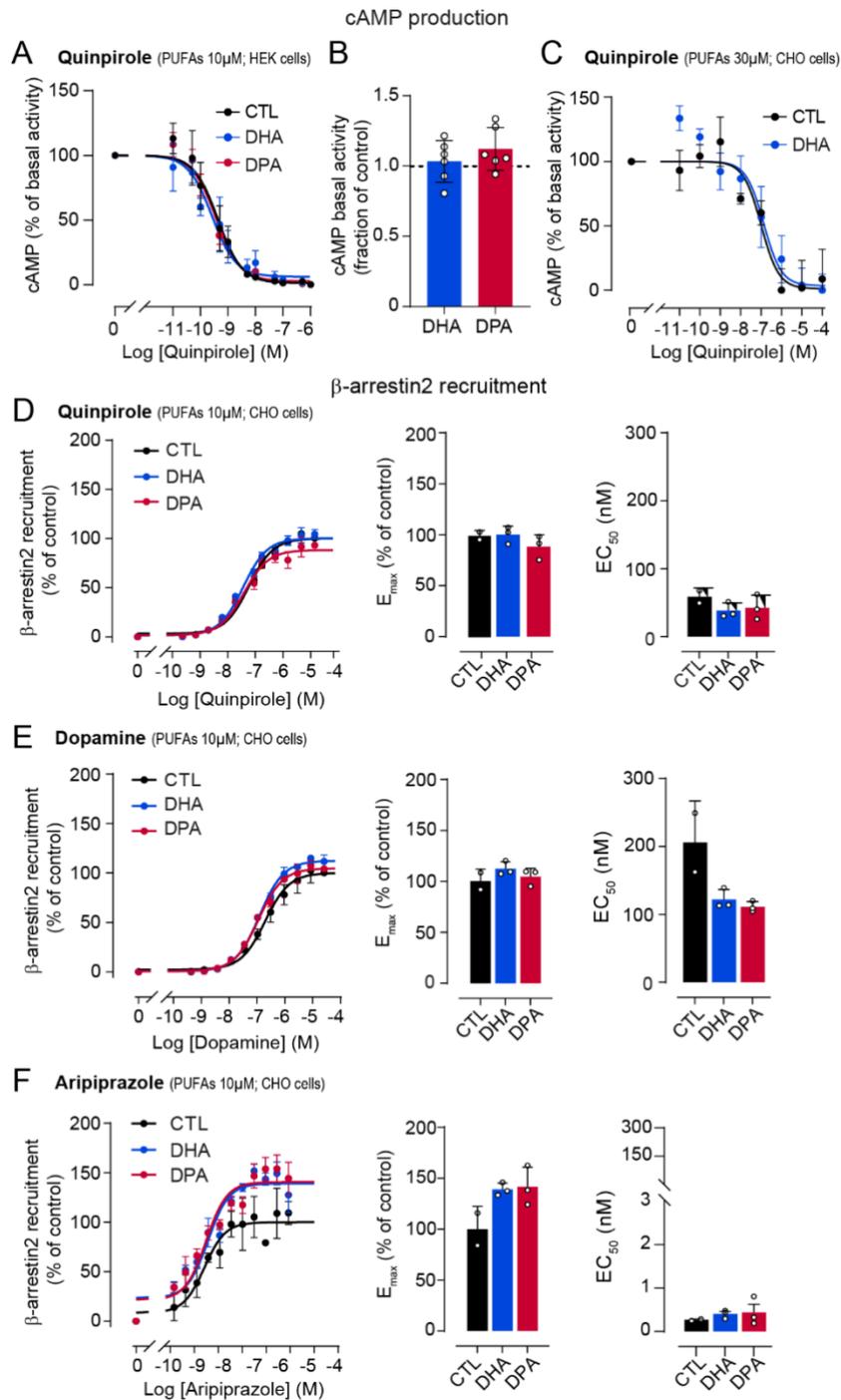
280 **Supplementary Figure S5. Lipid-protein contacts during MD simulations.** Relative proportion of  
 281 atomic lipid-protein contacts (y-axis) over time (x-axis) for the simulations of the apo state of the D2R  
 282 embedded in DHA- (A) versus DPA-rich (B) membranes, and the simulations of dopamine- (C) versus  
 283 aripiprazole-bound D2R (E) embedded in DHA-rich membranes. Specifically, figures depict the contact  
 284 ratio of each SD(h)PC or SD(p)PC chain versus all saturated lipids (i.e. DPPC, DSPC, and PSM) in the  
 285 system (i.e. sn-1 / SAT and sn-2 / SAT) is depicted (see Methods for a detail description of these ratios).

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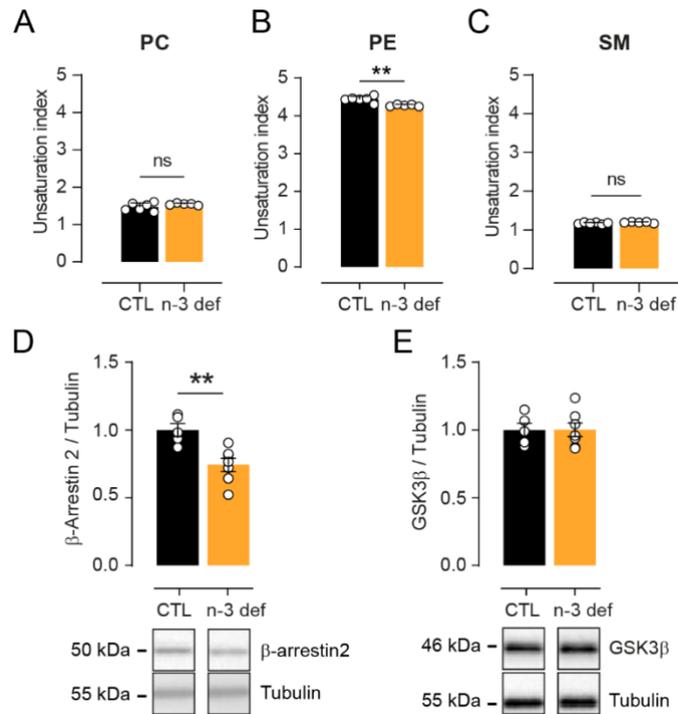
288 **Supplementary Figure S6. PUFAs enrichment in CHO and HEK cells. (A)** DHA and DPA  
 289 incorporation in CHO cells through incubations at 10 $\mu$ M or 30 $\mu$ M. **(B)** DHA and DPA incorporation in  
 290 HEK cells through incubations at 30 $\mu$ M. **(C)** Cumulative PUFA incorporation in CHO cells through  
 291 incubations at 10 $\mu$ M or 30 $\mu$ M. **(D)** Cumulative PUFA incorporation in HEK cells through incubations at  
 292 10 $\mu$ M or 30 $\mu$ M. Bars represent the mean average of three independent experiments and error bars  
 293 represent the standard error of the mean (SEM). \*  $p < 0.05$  by one-tailed Mann-Whitney test.



294

295 **Supplementary Figure S7. cAMP inhibition and  $\beta$ -arrestin2 recruitment by the D2R upon**  
 296 **stimulation by different ligands in cells. (A)** Dose-response experiments of cAMP production with  
 297 the D2R ligand quinpirole on forskolin-stimulated HEK cells incubated in the presence of 0.03% ethanol  
 298 as control, 10  $\mu$ M DHA or 10  $\mu$ M DPA. **(B)** Effect of 30  $\mu$ M PUFA enrichment on basal forskolin-induced  
 299 cAMP production. **(C)** Dose-response experiments of cAMP production with the D2R ligand quinpirole  
 300 on forskolin-stimulated CHO cells incubated in the presence of 0.03% ethanol as control or 30  $\mu$ M DHA  
 301 **(D)** Quinpirole activity on  $\beta$ -arrestin2 recruitment at the D2R in CHO-K1 cells expressing the DRD2L  
 302 (left), and associated E<sub>max</sub> (middle) and EC<sub>50</sub> (right) under 10  $\mu$ M incubation of either PUFA. **(E)**  
 303 Dopamine activity on  $\beta$ -arrestin2 recruitment at the D2R in CHO-K1 cells expressing the DRD2L (left),  
 304 and associated E<sub>max</sub> (middle) and EC<sub>50</sub> (right) under 10  $\mu$ M incubation of either PUFA. **(F)** Aripiprazole  
 305 activity on D2R mediated  $\beta$ -arrestin2 assay in CHO-K1 cells expressing the DRD2L (left), and  
 306 associated E<sub>max</sub> (middle) and EC<sub>50</sub> (right) under 10  $\mu$ M incubation of either PUFA. \*  $p < 0.05$ , \*\*  $p < 0.01$   
 307 by two-tailed unpaired t-test. Data are mean  $\pm$  SD values from three independent experiments with \*\*\*\*

308 p<0.0001, \*\*\* p <0.001, \* p<0.05. cAMP assay and  $\beta$ -arrestin2 recruitment curves are mean  $\pm$  SEM.  
309 Comparisons to the control were evaluated by a Kruskal-Wallis and Dunn's post-hoc tests.



310

311

312 **Supplementary Figure S8. Expression of  $\beta$ -arrestin-2 and GSK3 $\beta$  in n-3 deficient mice (n-3 def).**  
 313 **(A, B, C)** Unsaturation index in mice corresponding to the average number of double bonds per fatty  
 314 acid present in total PUFAs ([percentage of each lipid] $\times$ [number of double bonds/number of fatty acid  
 315 species per lipid]). **(D)** and **(E)** Expression of  $\beta$ -arrestin2 (A) and GSK3 $\beta$  (B) by western blot normalized  
 316 to the intensity of tubulin expression. Bars represent the mean of five and seven subjects for CTL and  
 317 n-3 def respectively and error bars the SEM. \*\* $p < 0.01$  by Mann Whitney test.  
 318

	<i>CTL</i>	<i>DHA 10μM</i>	<i>DPA 10μM</i>	<i>CTL</i>	<i>DHA 30μM</i>	<i>DPA 30μM</i>
<b>Quinpirole</b>	9.50 ± 0.12	9.39 ± 0.25	9.48 ± 0.20	9.40 ± 0.18	9.51 ± 0.08	9.62 ± 0.20
<b>Dopamine</b>	-	-	-	8.70 ± 0.12	8.54 ± 0.20	8.85 ± 0.21
<b>Aripiprazole</b>	-	-	-	8.08 ± 0.25	8.36 ± 0.16	8.46 ± 0.10

319

320 **Supplementary Table S1.** pIC<sub>50</sub> values (as mean ± SEM) from dose-response experiments of cAMP  
 321 production on forskolin-stimulated cells incubated in the presence of 0.03% ethanol as control (CTL),  
 322 10 μM DHA and 10 μM DPA or 30 μM DHA and 30 μM DPA upon quinpirole, dopamine and aripiprazole  
 323 stimulation of the D2R.

324

325

	<i>Percentage (%)</i>						
<b>Model membrane</b>	CHL1	DPPC (diC16:0)	DSPC (diC18:0)	DOPC (diC18:1)	SD(h)PC (C18:0 / C22:6)	SD(p)PC (C18:0 / C22:5)	PSM (C18:1 / C16:0)
<b>DHA-rich</b>	33	14	5	11	13	0	24
<b>DPA-rich</b>	33	14	5	11	0	13	24

326

327 **Supplementary Table S2. Lipid composition of the model membranes used in MD simulations.**  
 328 CHL1: cholesterol, DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DSPC: 1,2-distearoyl-sn-  
 329 glycero-3-phosphocholine, DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine, SD(h)PC: 1-stearoyl-2-  
 330 docosahexaenoyl-sn-glycero-3-phosphocholine, SD(p)PC: 1-stearoyl-2- docosa(p)entaenoyl -sn-  
 331 glycero-3-phosphocholine, PSM: sphingomyelin.