Title

The expression of cannabinoid-related genes in different cellular models of disease

Authors

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Abstract

The endocannabinoid system comprises a series of ligands and receptors that have putative roles in regulating physiological and cognitive processes such as pre- and post-natal development, appetite, pain sensation, mood, and memory. Besides the endocannabinoids, these endogenous receptors are also capable of mediating the pharmacological effects of phytocannabinoids, which are derived from the cannabis plant. We sought to interrogate a panel of cell lines, representative of multiple human diseases, to characterize their cannabinoid-mediating gene expression. We found all lines expressed one or more gene product that rendered the cell potentially responsive to cannabinoids. Moreover, the expression profiles differed between normal and cancerous cells, as well as between cells derived from the brain, pancreas, and skin. Taken together, given the presence of one or more of these mediating gene products, our findings suggest a potential therapeutic role for phytocannabinoids.

24 ***Introduction***

25 The cannabis plant (*Cannabis sativa* L.) contains a large number of pharmacologically
26 active compounds called phytocannabinoids. The most abundant of these compounds
27 are Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), although the amounts and
28 proportions of the various phytocannabinoids in each plant vary by strain and can be
29 adjusted by breeding.

30

31 Many cannabinoids, including CBD, have been shown to be non-toxic and to possess
32 anti-tumor activity in multiple cancer types (1). Numerous mechanisms have been
33 proposed to explain these anti-tumor effects, including the production of reactive oxygen
34 species (2, 3), promotion of apoptosis (4), a reduction in inflammation (5), activation of
35 receptors that may inhibit drug transport (6) and tumorigenesis (7), and potentiating the
36 activity of multiple types of chemotherapy (8).

37

38 In spite of its wide spectrum of pharmacological activities, little is known about the
39 molecular mechanism of cannabinoid action. Several gene products, however, have been
40 implicated in mediating the effects of cannabinoids. Activation of the endogenous
41 cannabinoid type 1 (CB1) and type 2 (CB2) receptors has been shown to lead to the
42 inhibition of tumor progression (9). CBD does not interact efficiently with CB1R and CB2R,
43 but cannabigerol (CBG), another non-psychotropic cannabinoid that interacts with
44 specific targets involved in carcinogenesis, is a weak partial agonist of both CB1R and
45 CB2R (10). Cannabinoids also target transient receptor potential (TRP) channels (11),
46 including TRPV1 (12) and TRPV2 (13), the cyclooxygenase (COX)-1 and COX-2 (also

47 known as prostaglandin-endoperoxide synthase 2, or PTGS2) enzymes (14), the fatty
48 acid amide hydrolase (FAAH) enzyme (11); the 5-hydroxytryptamine receptor 1A (5-
49 HT1A) and α2-adrenergic receptor (10); the nuclear receptor peroxisome proliferator-
50 activated receptor-gamma (PPAR-γ) (15); and the orphan G-protein-coupled receptor 55
51 (GPR55) (16).
52
53 *CB1R and CB2R.* Many of the effects produced by Δ9-tetrahydrocannabinol (Δ9-THC)
54 and its synthetic cousins depend on their ability to target cannabinoid receptors (17-19),
55 which include the GPCRs CB1R (20) and CB2R (21).
56
57 CB1Rs are located primarily in central and peripheral neurons; CB2Rs, predominantly in
58 immune cells. CB1Rs are also expressed by some non-neuronal cells, including immune
59 cells, and CB2Rs by some neurons both within and outside the brain (reviewed in (22)).
60
61 CBD has very low affinity for either CB1R or CB2R (23). However, there is evidence that
62 despite this low affinity for CB1R and CB2R, CBD can interact with these receptors at
63 reasonably low concentrations (22), yielding such varied effects as GPR55 antagonism
64 (24, 25), activation of TRPV1 (26), and decreased cancer cell proliferation and increased
65 neuroprotection (18, 27).
66
67 *FAAH.* FAAH is an intracellular enzyme that catalyzes the hydrolysis of the endogenous
68 cannabinoid ligand anandamide (arachidonoylethanolamide, AEA) (28, 29). CBD has
69 been reported to inhibit both FAAH-mediated degradation of the endogenous cannabinoid

70 ligand anandamide (30) and anandamide transporter activity (31). These findings suggest
71 that some of the pharmacological actions of CBD might be due to inhibition of AEA
72 degradation.
73
74 *GPR55.* GPR55 is an orphan G protein-coupled receptor (GPCR), meaning it has no
75 known ligand. The *GPR55* gene is expressed in several brain areas including the
76 hippocampus, hypothalamus, frontal cortex, and cerebellum (16). The function of GPR55
77 in the periphery is varied: vasodilatation, cellular proliferation and migration, bone
78 dynamics, energy balance, gastrointestinal processes, and inflammation and pain
79 (reviewed in (16)). In the central nervous system, studies suggest GPR55 could modulate
80 procedural memory, motor coordination, anxiety, and hippocampal release of glutamate
81 (reviewed in (16)).
82
83 GPR55 can be activated by endocannabinoids, though the extent to which the receptor
84 can be fully activated is still unknown (25). Despite poor sequence homology, many
85 studies have reported significant functional interaction between GPR55 and CB1R/CB2R
86 (32-34). As such, GPR55 is being considered as the cannabinoid receptor type 3 (CB3R)
87 by the International Union of Basic and Clinical Pharmacology Committee on Receptor
88 Nomenclature and Drug Classification (32, 35). Given its role in motor coordination,
89 modulation of GPR55 activity has been proposed as a therapeutic target for epilepsy.
90 CBD was recently approved by the US FDA as a treatment for certain childhood forms of
91 epilepsy (Epidiolex®, GW Pharmaceuticals), leading to the hypothesis that one
92 mechanism of action of CBD is GPR55 inhibition (25).

93

94 *Id-1.* CBD has been shown to reduce the expression of the inhibitor of differentiation and
95 DNA binding 1 (Id-1). Id family members negatively regulate the activity of basic helix–
96 loop–helix proteins, which are critical components of developmental processes such as
97 sex determination and neurogenesis (36). Constitutive expression of Id proteins can
98 inhibit the differentiation of various tissues (37, 38). Perhaps most relevant, the Id proteins
99 have been shown to be involved in the pathogenesis of human cancers (39, 40).

100

101 The level of expression of the *ID1* gene has been shown to be strongly correlated to the
102 invasive and metastatic behavior of breast cancer (41, 42) and glioblastoma (GBM) (36)
103 cells. Higher levels of *ID1* have been documented for a variety of aggressive tumor types
104 including melanoma and cancers of the head and neck (squamous cell carcinoma [SCC]),
105 esophagus (SCC), oral cavity (SCC), liver, colon, pancreas, thyroid, ovary, cervix,
106 endometrium, and prostate (reviewed in (39)), especially when compared to normal cells
107 of the same tissue origin (39, 40).

108

109 *PTGS2.* The *PTGS2* gene encodes the COX-2 enzyme, which is one of two
110 cyclooxygenases in humans. It is involved in the conversion of arachidonic acid to
111 prostaglandin H2, an important precursor of prostacyclin, which is expressed in
112 inflammation. PTGS2 is not expressed in most cells under normal conditions, but elevated
113 levels are found during inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs)
114 inhibit prostaglandin production by both PTGS1 (COX-1) and PTGS2.

115

116 The expression of *PTGS2* is upregulated in many cancers. Inhibiting *PTGS2* expression
117 may therefore be beneficial in preventing and treating certain types of cancer (43).
118
119 COX-2 plays a significant role in intraneuronal CB1R signal transduction and modulation
120 of *PTGS2* and *CNR1* gene expression (44). CBD and Δ9-THC have been found to
121 increase expression and activity of PTGS2, with *PTGS2* being one of the 25 most up-
122 regulated genes upon treatment with either cannabinoid in T98G (but not U-87 MG) cells
123 (45).
124
125 *TRPV1 and TRPV2.* TRP channels are pore-forming transmembrane proteins that allow
126 ions and xenobiotics to permeate biological membranes (46). It has been suggested that
127 TRP channel-expressing cancer cells may allow entry of charged molecules (47-50) and
128 certain anti-proliferative and cytotoxic molecules (47) by permeation.
129
130 CBD has been found to be a full, though weak, agonist of human TRPV1 (26). The TRPV2
131 channel is triggered by agonists such as Δ9-THC and CBD (51, 52).
132
133 Given the importance of each of these genes to normal and pathological processes, we
134 sought to assess the level of each across a panel of human cell lines.
135
136

137 ***Methods***

138 *Cell lines*

139 The GBM lines A-172, T98G, and U-87 MG; pancreatic cancer lines MIA PaCa-2 and
140 PANC-1; actinic keratosis line HT 297.T; melanoma lines A-375 and A101D; and normal
141 skin fibroblasts BJ and Detroit 551 were all obtained from the American Tissue Culture
142 Collection (ATCC) and grown according to the manufacturer's recommendations. All
143 assays were performed using cells that had been passaged fewer than 20 times upon
144 arrival.

145

146 The SCC (skin) line HSC-5 (53) was obtained from the Japanese Collection of Research
147 Biosources Cell Bank (JCRB) and Sekisui XenoTech.

148

149 *RNA extraction*

150 RNA from cell samples was generated using the RNeasy Mini Kit (Qiagen) according to
151 the manufacturer's instructions.

152

153 *Generation of complementary DNA (cDNA)*

154 RNA was converted to cDNA using the RT^2 First Strand Kit (Qiagen) according to the
155 manufacturer's instructions.

156

157 *Quantitative PCR (qPCR)*

158 The expression of *ACTB* (β-actin), *CNR1* (CB1R), *CNR2* (CB2R), *FAAH*, *GAPDH*,
159 *GPR55*, *ID1* (Id-1), *PTGS2*, *TRPV1*, *TRPV2*, was measured by qPCR using validated

160 human primer sets (RT^2 qPCR Primer Assays, Qiagen) and the RT^2 SYBR Green qPCR
161 Mastermix (Qiagen). Values for gene expression were determined using the ΔCt method,
162 where the cycle number threshold (Ct) of either glyceraldehyde 3-phosphate
163 dehydrogenase (GAPDH) or β-actin (as housekeeping genes) is subtracted from the Ct
164 value of each gene. Expression levels for the housekeeping gene transcripts were
165 normalized to one million copies.
166
167

168 ***Results***

169 *Glioblastoma*

170 We compared the level of expression of the set of cannabinoid-related genes across three
171 different GBM lines, A-172, T98G, and U-87 MG. A-172 cells were the only cells of those
172 assessed that had measurable expression of all genes tested. Two of the GBM lines
173 tested, A-172 and U-87 MG, exhibited the highest expression of *ID1* among all the genes
174 analyzed (**Figure 1A** and **Figure 1C**, respectively), as well as having fairly robust
175 expression of *TRPV1*. T98G cells had the highest expression of *GPR55*, followed by *ID1*
176 (**Figure 1B**). A-172 and U-87 MG cells also exhibited robust expression of *PTGS2*, while
177 only U-87 MG cells had robust expression of *TRPV2*.

178

179 **Figure 1. Expression of cannabinoid-related genes in glioblastoma cells.** Expression
180 levels in (**A**) A-172 cells, (**B**) T98G cells, and (**C**) U-87 MG cells were normalized to one
181 million copies of either GAPDH (**A**) or β-actin (**B**, **C**).

182

183 *Pancreatic cancer*

184 The pancreatic cancer line MIA PaCa-2 had observable and robust expression of all
185 cannabinoid-related genes except *TRPV2* (**Figure 2A**). PANC-1 cells, on the other hand,
186 were fairly low expressers of all genes except *FAAH*, *ID1*, and *TRPV1* (**Figure 2B**).

187

188 **Figure 2. Expression of cannabinoid-related genes in pancreatic cancer cells.**
189 Expression levels in (**A**) MIA PaCa-2 cells and (**B**) PANC-1 cells were normalized to one
190 million copies of β-actin.

191

192 *Skin*

193 The malignant melanoma line A-375 had observable expression of all cannabinoid-
194 related genes except *TRPV2* (**Figure 3A**). The genes with the highest levels of
195 expression were *CNR1*, *GPR55*, *ID1*, and *TRPV1*. Another melanoma line, A101D, had
196 observable expression of all genes (**Figure 3B**). It, too, had *ID1* as the highest-expressing
197 gene.

198

199 **Figure 3. Expression of cannabinoid-related genes in melanoma cells.** Expression
200 levels in (**A**) A-375 cells and (**B**) A101D cells were normalized to one million copies of β-
201 actin.

202

203 The SCC line HSC-5 (**Figure 4**) and actinic keratosis line HT 297.T (**Figure 5**) both had
204 observable expression of *FAAH*, *ID1*, *PTGS2*, and *TRPV1;* HSC-5 cells also expressed
205 *CNR1*. Similar to other lines, ID1 expression was the highest of all genes tested.

206

207 **Figure 4. Expression of cannabinoid-related genes in HSC-5 cells, a squamous cell**
208 **carcinoma line derived from skin.** Expression levels were normalized to one million
209 copies of β-actin.

210

211 **Figure 5. Expression of cannabinoid-related genes in HT 297.T cells, an actinic**
212 **keratosis line.** Expression levels were normalized to one million copies of β-actin.

213

214 We also examined gene expression in normal skin fibroblasts. The two lines studied, BJ
215 and Detroit 551, exhibited different patterns of expression. BJ cells had high levels of
216 *PTGS2* and *TRPV2* expression (**Figure 6A**), while Detroit 551 cells had high levels of *ID1*
217 and *PTGS2* (**Figure 6B**). interestingly, BJ cells had completely undetectable levels of *ID1*.
218
219 **Figure 6. Expression of cannabinoid-related genes in normal skin fibroblasts.**
220 Expression levels in (**A**) BJ cells and (**B**) Detroit 551 cells were normalized to one million
221 copies of β-actin.
222
223 A summary of the expression levels of the cannabinoid-related genes can be found in
224 **Table 1**. A heatmap visualization of the same data can be found in **Figure 7**.
225

226

Table 1. Expression of cannabinoid-related genes across multiple cell lines.

Cell line	*CNR1*	*CNR2*	*FAAH*	*GPR55*	*ID1*	*PTGS2*	*TRPV1*	*TRPV2*
Glioblastoma								
A-172	+	+	+	+	++	+	+	+
T98G	+	+	+	++	+	ND	+	+
U-87 MG	ND	+	+	ND	++	++	+	+
Pancreatic cancer								
MIA PaCa-2	++	++	++	++	++	++	++	ND
PANC-1	+	ND	+	ND	++	ND	+	ND
Skin – Squamous cell carcinoma								
HSC-5	+	ND	++	ND	++	+++	+	ND
Skin – Actinic keratosis								
HT 297.T	ND	ND	+	ND	++	+	+	ND
Skin – Melanoma								
A-375	++	+	+	++	+++	+	++	ND
A101D	+	+	+	+	++	++	+	++
Skin – Normal								
BJ	+	+	+	+	ND	++	+	++
Detroit 551	+	+	+	ND	++	++	+	+

Expression levels: +++, < 1 log difference of β-actin expression
++, > 1 and < 3 logs difference of β-actin expression
+, > 3 logs difference of β-actin expression
ND, not detected

227

228 **Figure 7. Heatmap depicting the expression of cannabinoid-related genes across**
229 **multiple cell lines.** The colors indicate levels of gene expression and range from white
230 (nadir of expression) to dark green (zenith of expression).

231

232 When visualized in the aggregate, all cell types expressed cannabinoid-related genes,
233 though to varying degrees (**Figure 8**). The highest total gene-expressing cell line was
234 MIA PaCa-2, while the highest gene-expressing cell type was melanoma.

235

236 **Figure 8. Expression of cannabinoid-related genes in all cell lines.** The total
237 expression levels of all cannabinoid-related genes in this study are aggregated for ease
238 of comparison.

239

240 *ID1 expression*

241 Because of the special role Id-1 appears to play in tumorigenesis, and the fact that CBD
242 has been shown to down-regulate *ID1* gene expression and associated glioma cell
243 invasiveness and self-renewal (36), we paid particular attention to its expression levels in
244 the cell lines assessed in this study. We found that expression of *ID1* was consistently
245 high in cancerous cell lines (**Figure 9**). The only other diseased line available, actinic
246 keratosis, had the lowest levels of *ID1* expression. Two normal skin fibroblast lines, BJ
247 and Detroit 551, showed different levels of *ID1* expression, with BJ having undetectable
248 *ID1* levels.

249

250 **Figure 9. ID1 mRNA expression in different cell lines.** Expression levels were
251 normalized to one million copies of β-actin.

252

253

254 ***Discussion***

255 Cannabinoids, and CBD in particular, have been proposed as therapeutic agents for a
256 wide variety of diseases. Although the precise mechanism(s) of action by which
257 cannabinoids exert their effects remain(s) unknown, it has been suggested that the
258 endogenous expression of enzymes, receptors, signaling molecules, and other mediators
259 that comprise the endocannabinoid system render the human body capable of functional
260 responses to this class of compounds. To our knowledge, this study is the first systematic
261 assessment of cannabinoid-related genes across multiple cell lines that span human
262 diseases.

263

264 Virtually all of the cell lines we tested exhibited robust expression of *ID1*, a gene that has
265 been implicated in the pathogenesis of human cancers (39, 40). (U-87 MG cells were
266 previously shown to be negative for *ID1* expression (54).) As CBD has been shown to
267 reduce the expression of *ID1*, thereby reducing either the likelihood of cellular
268 transformation or the aggressiveness of existing tumors, these results suggest
269 therapeutic targeting of these diseases with CBD may improve outcomes.

270

271 The expression of *PTGS2* was second only to *ID1* across the cell lines tested. Given its
272 role in mediating inflammation, it was not surprising to observe fairly high levels in
273 transformed cell lines (even if some are characterized as “normal”). This observation is
274 consistent with the finding that *PTGS2* expression is upregulated in many cancers. What
275 is counter-intuitive, however, is the observation that CBD was found to up-regulate
276 expression of *PTGS2*, as well as increase the activity of the COX-2 enzyme (45).

277 Increasing the activity of a pro-inflammatory mediator such as COX-2 contradicts the
278 narrative that CBD and other cannabinoids are potent anti-inflammatory drugs, exerting
279 their effects through induction of apoptosis, inhibition of cell proliferation, suppression of
280 cytokine production, and induction of regulatory T cells (T_{regs}) (5). Further investigation
281 into the effect cannabinoid treatment of these cell lines has on *PTGS2* expression is
282 warranted.
283
284 CBD has very low affinity for either CB1R or CB2R (23). In addition, CBD has been
285 reported to inhibit both FAAH-mediated degradation of the endogenous cannabinoid
286 ligand, anandamide (30) and the RBL-2H3 cell anandamide transporter activity (31).
287 These findings raised the possibility that some of CBD's pharmacological actions might
288 be due to inhibiting the degradation of anandamide. Anandamide has been identified as
289 an endogenous CB1R agonist and as a ligand for CB2R (despite low affinity).
290
291 Another important site at which anandamide acts is TRPV1 (55), which acts as a
292 molecular integrator of noxious stimuli and is important for thermal nociception and
293 inflammatory hyperalgesia (56, 57). CBD has been shown to be a full, although weak,
294 agonist of human TRPV1 (26). Thus, CBD could activate either cannabinoid receptors
295 indirectly through anandamide or vanilloid receptors directly or indirectly via anandamide.
296
297 In conclusion, the cell types assessed in this study – diseased and normal cell types,
298 cancer and non-cancerous cell types – all exhibited expression of one or more gene
299 products that have been shown to mediate the pharmacological effects of cannabinoids.

300 Moreover, the diversity of these cell types is consistent with the notion that our bodies,
301 whether healthy or sick, are innately capable of responding to cannabinoids. Our findings
302 strongly suggest there is a potential therapeutic role for phytocannabinoids in the context
303 of disease.
304
305

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309

310

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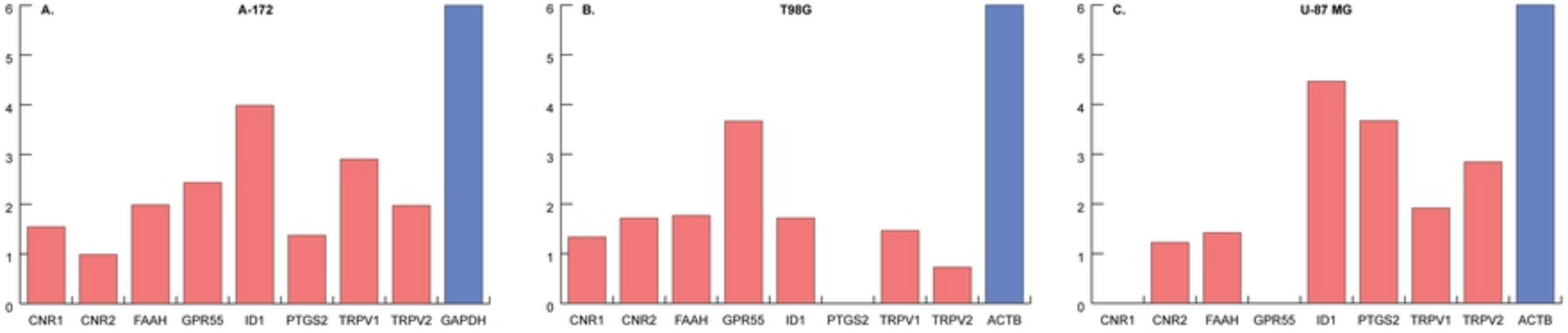
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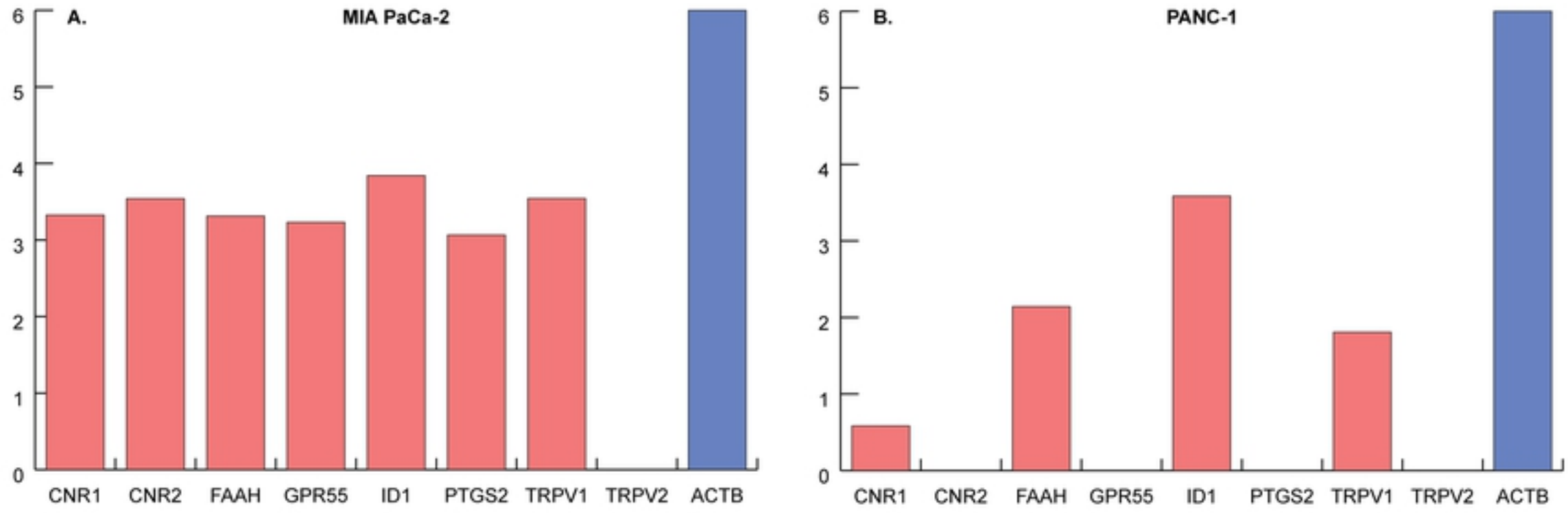
A-172
B.
T98G
C.
U-87 MG
0
1
2
3
4
5
6
CNR1
CNR2
FAAH
GPR55
ID1
PTGS2
TRPV1
TRPV2
GAPDH
ACTB

Figure 1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B.
PANC-1
0
1
2
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4
5
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CNR2
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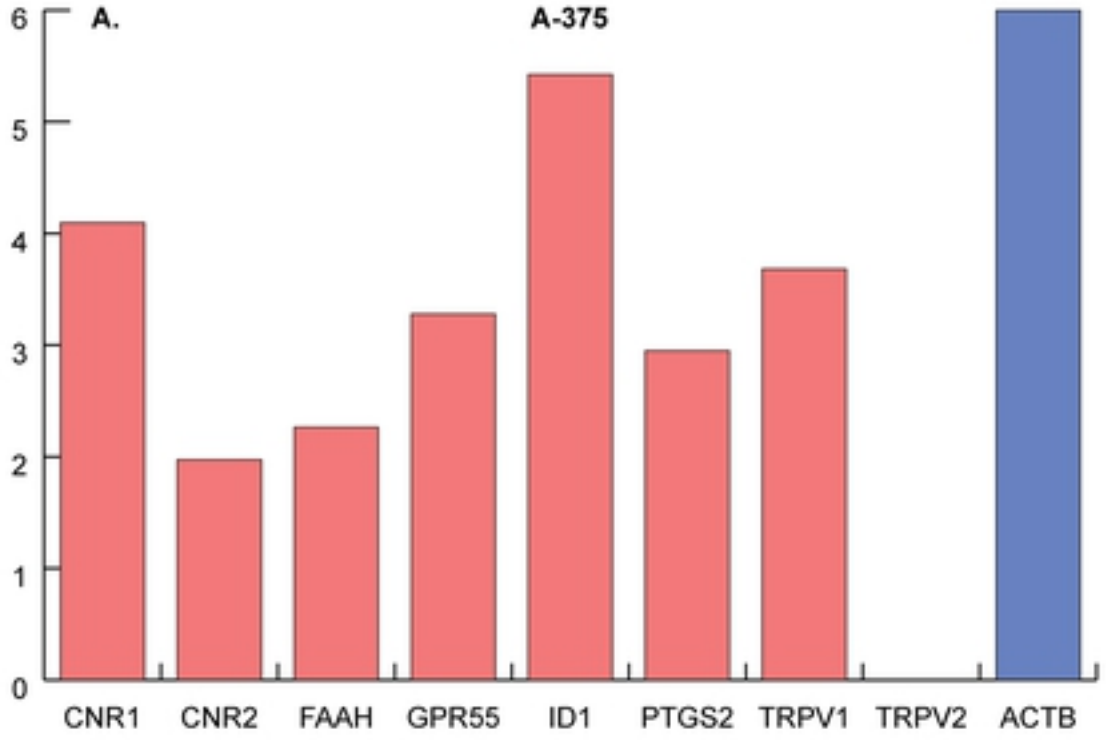
GPR55
ID1
PTGS2
TRPV1
TRPV2
ACTB

Figure 2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A-375
6
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CNR2
FAAH
GPR55
ID1
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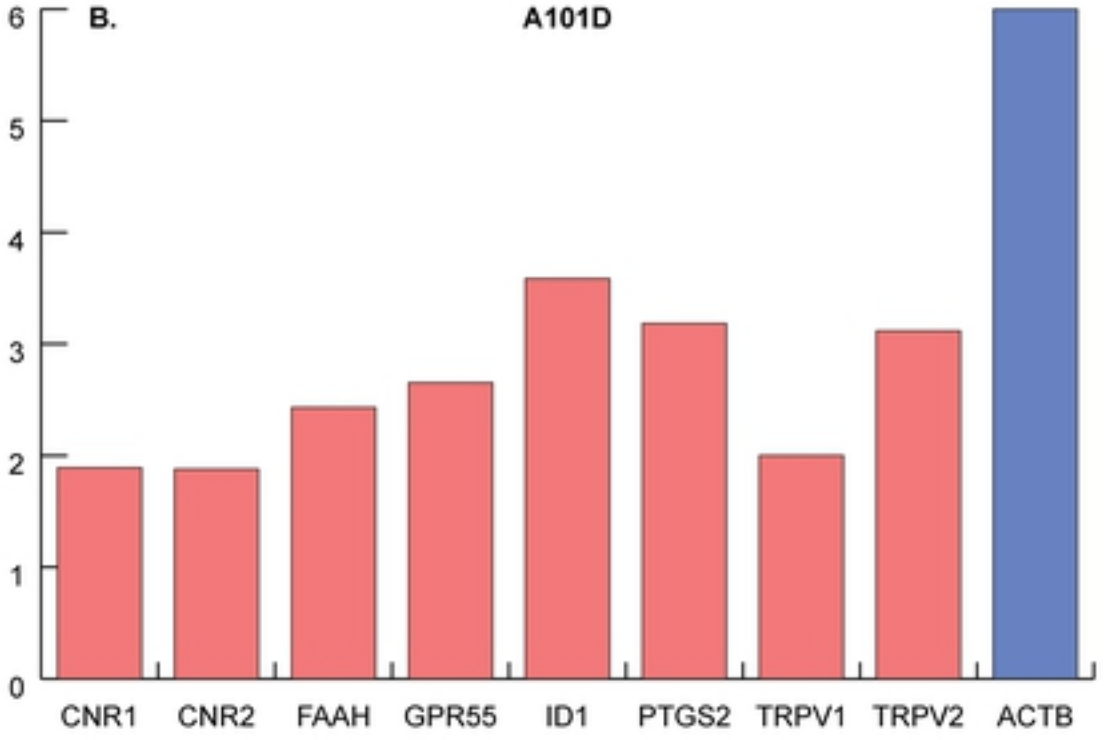
TRPV1
TRPV2
ACTB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5
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CNR2
FAAH
GPR55
ID1
PTGS2
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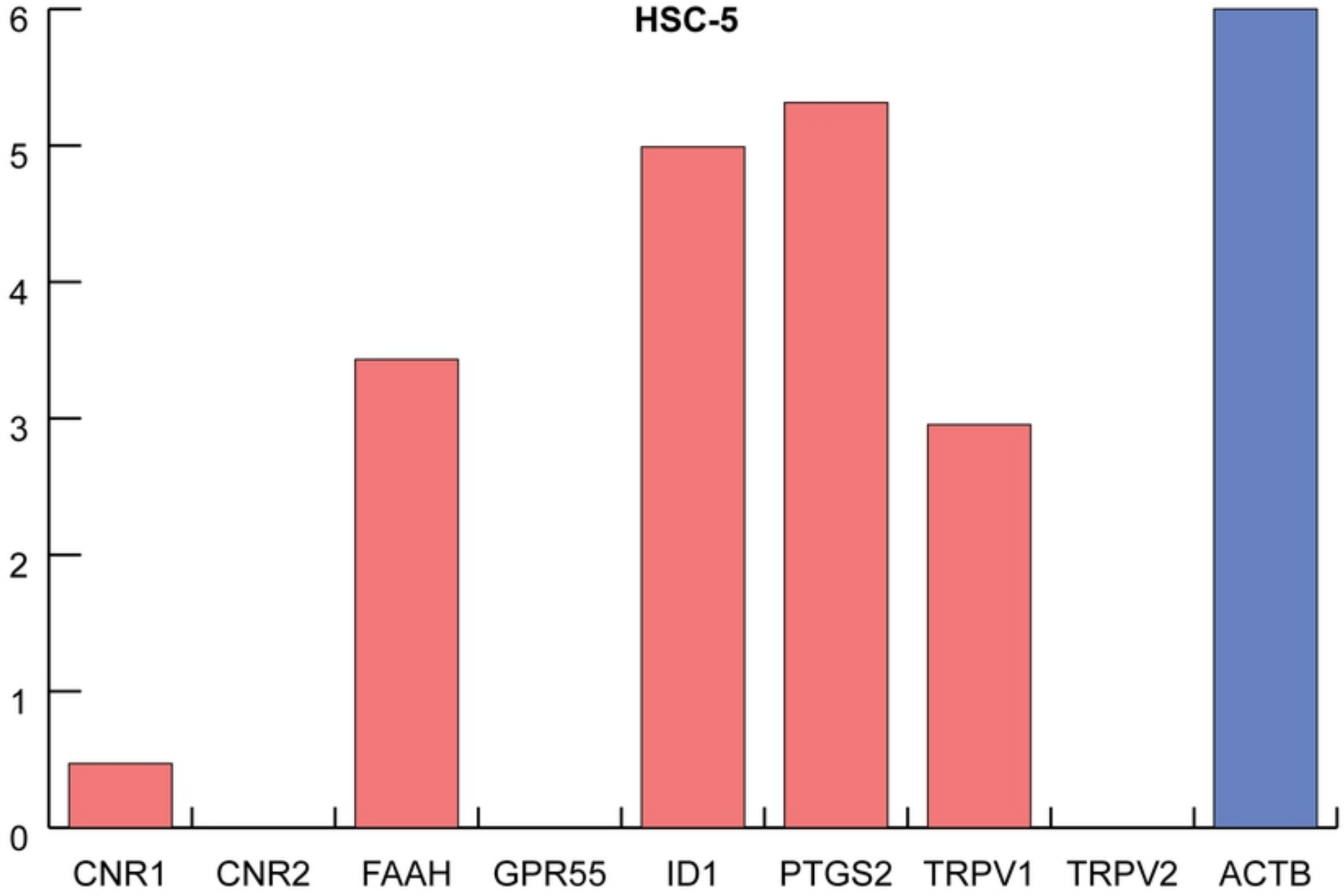
TRPV2
ACTB

Figure 3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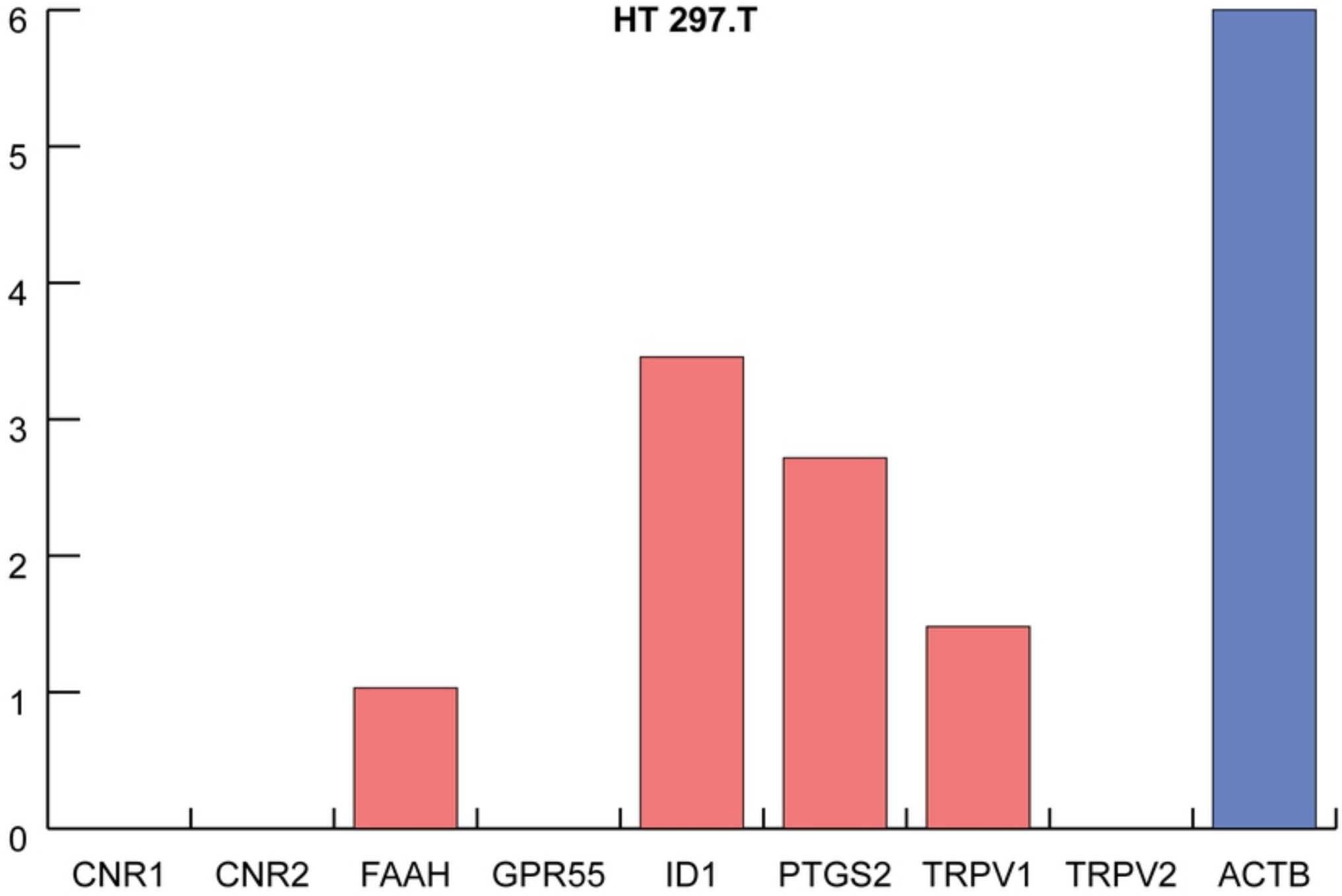
CNR2
FAAH
GPR55
ID1
PTGS2
TRPV1
TRPV2
ACTB

Figure 4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6
5
4
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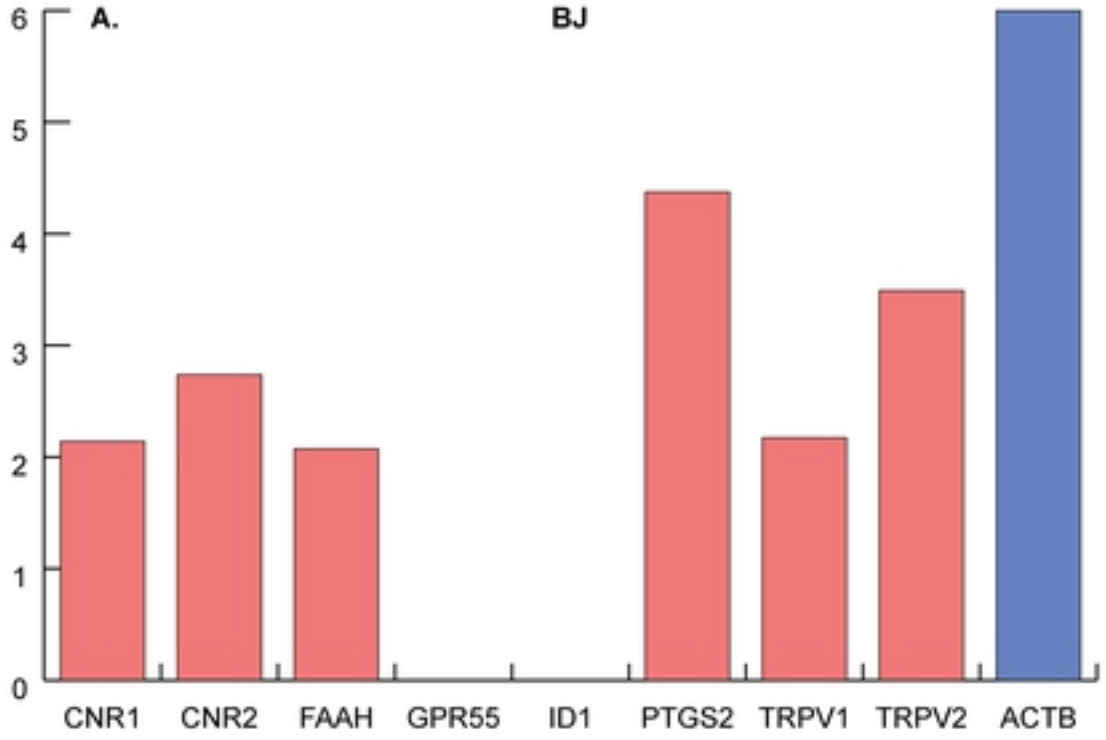
CNR2
FAAH
GPR55
ID1
PTGS2
TRPV1
TRPV2
ACTB

Figure 5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CNR2
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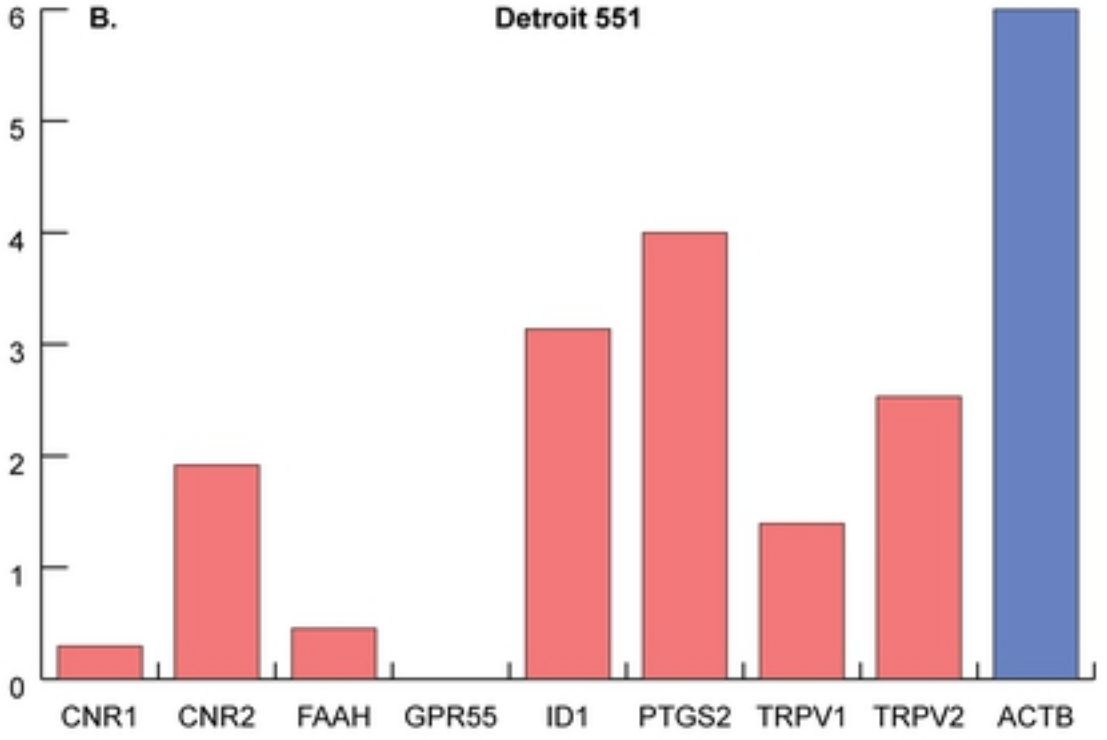
ID1
PTGS2
TRPV1
TRPV2
ACTB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Detroit 551
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5
4
3
2
1
0
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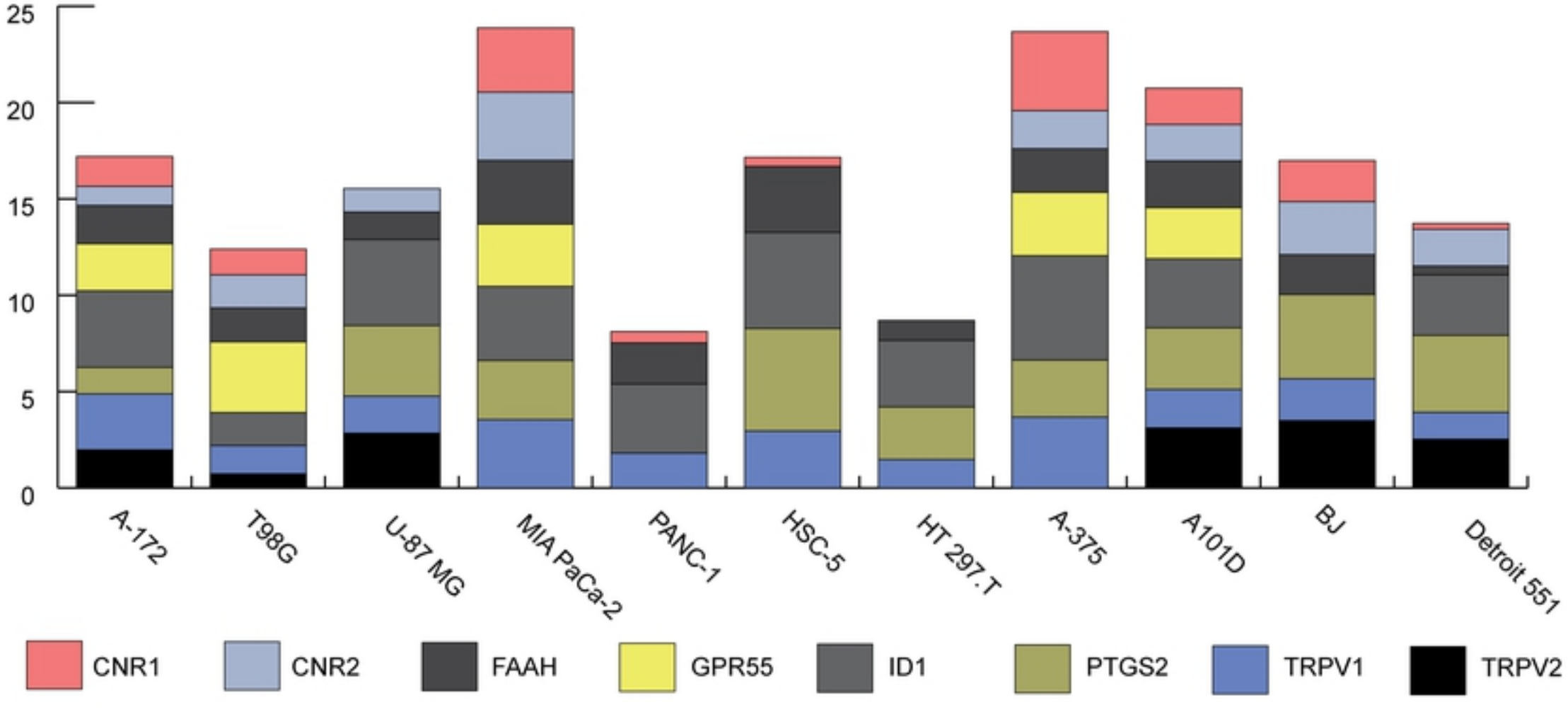
CNR2
FAAH
GPR55
ID1
PTGS2
TRPV1
TRPV2
ACTB

Figure 6

Cell line
CNR1
CNR2
FAAH
GPR55
ID1
PTGS2
TRPV1
TRPV2
ACTB
A-172
T98G
U-87 MG
MIA PaCa-2
PANC-1
HSC-5
HT 297.T
A-375
A101D
BJ
Detroit 551

Figure 7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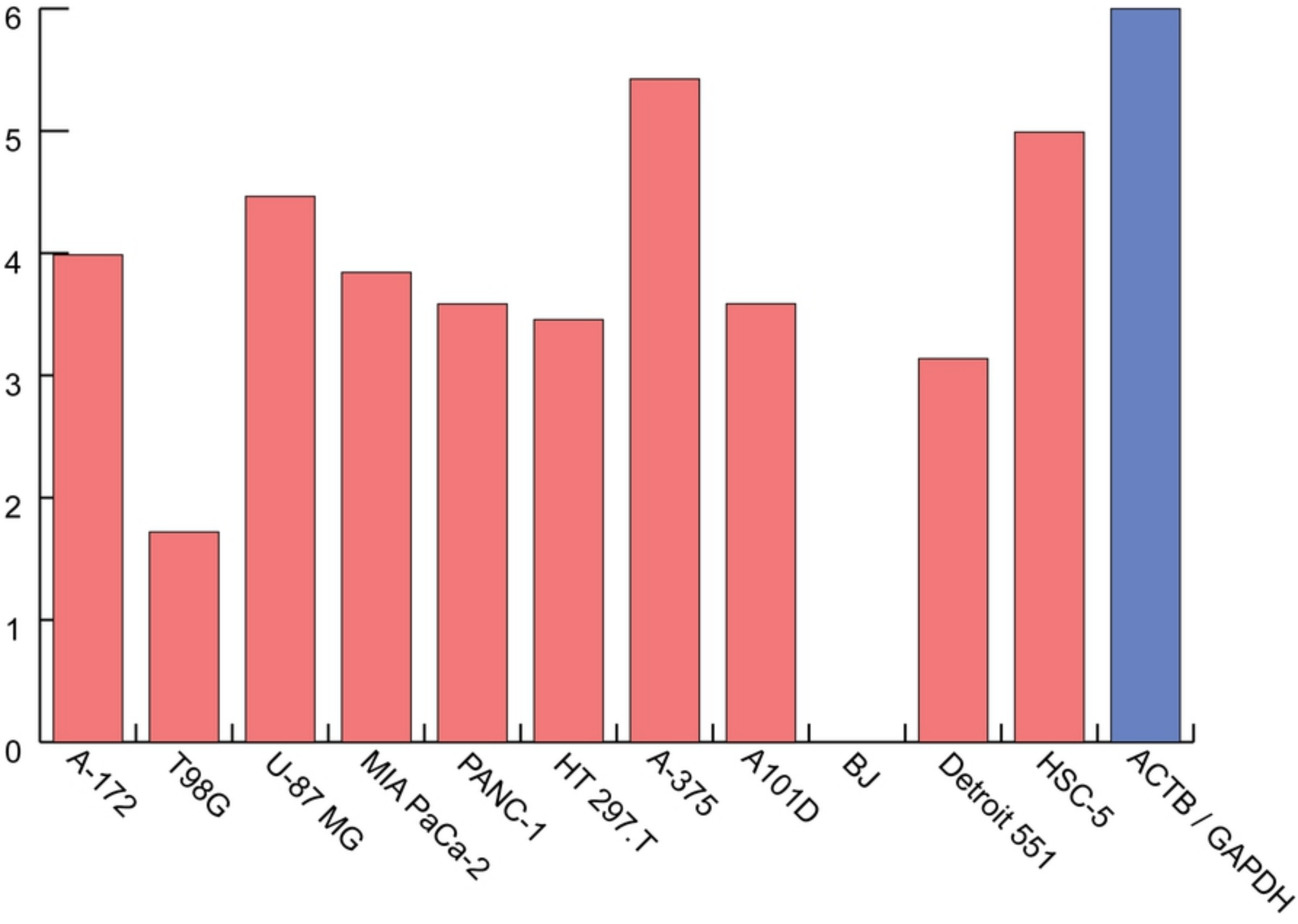
HSC-5
HT 297.T
A-375
A101D
BJ
Detroit 551
CNR1
CNR2
FAAH
GPR55
ID1
PTGS2
TRPV1
TRPV2

Figure 8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PANC-1
HT 297.T
A-375
A101D
BJ
Detroit 551
HSC-5
ACTB / GAPDH

Figure 9