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(71) Applicant: **PATH THERAPEUTICS, INC.** [CA/CA];
2212 Uxbridge Drive NW, Calgary, Alberta T2N 3Z4 (CA).

(72) Inventors; and

(71) Applicants: **KURRASCH, Deborah M.** [US/CA]; 2212
Uxbridge Drive NW, Calgary, Alberta T2N 3Z4 (CA). **IB-
HAZEHIEBO, Kingsley** [CA/CA]; 2950 Toronto Cres-
cent, Calgary, Alberta T23 3W5 (CA).(74) Agent: **DAVY, Brian E.**; Bozicevic, Field & Francis LLP,
201 Redwood Shores Pkwy., Suite 200, Redwood City, Cal-
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(54) Title: METHODS OF TREATING EPILEPSY VIA PHOSPHODIESTERASE 4 (PDE4) INHIBITION

(57) Abstract: Provided are methods of treating epilepsy. The methods include adminis-
tering to an individual having epilepsy a therapeutically effective amount of a phospho-
diesterase 4 (PDE4) inhibitor. Also provided are methods of identifying an anti-epileptic
agent. Such methods include contacting a PDE4 polypeptide with a candidate agent in a
PDE4 activity assay, where inhibition of activity of the PDE4 polypeptide by the candi-
date agent identifies the candidate agent as an anti-epileptic agent.

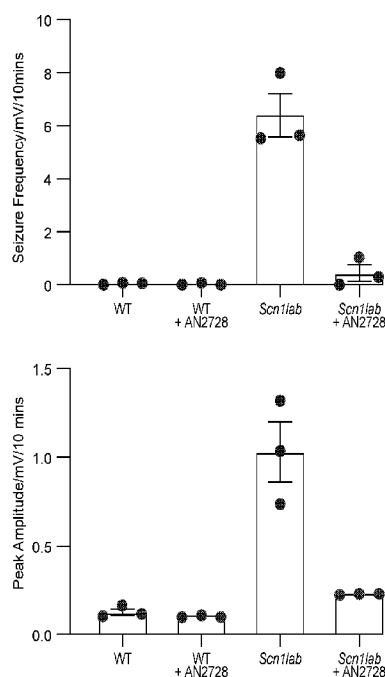


FIG. 9B

METHODS OF TREATING EPILEPSY VIA PHOSPHODIESTERASE 4 (PDE4) INHIBITION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 62/796,002, filed January 23, 2019, which application is incorporated herein by reference
5 in its entirety.

INTRODUCTION

Tens of millions of people suffer from epilepsy. Despite nearly eight decades of research and the advent of many new drugs, the efficacy rates for seizure relief have not significantly changed. Drugs are the mainstay treatment for epilepsy but a stubborn 30-
10 40% of epileptic patients are refractory to current medications and will have unremitting recurrent seizures and attendant life-long health problems. By way of example, Dravet syndrome (DS) is a childhood epilepsy that usually appears in the first year of life in an otherwise healthy baby as a febrile seizure lasting more than five minutes (often longer than 30 minutes). Most cases of DS are due to loss-of-function mutations in the *Scn1a* gene
15 encoding brain voltage-gated sodium channel type-I, Nav1.1. Despite its genetics being understood, DS remains highly pharmacoresistant and thousands of children struggle through numerous ineffective therapies.

Following DS diagnosis, families are shuffled in and out of clinics and ERs with the kids having repeated EEGs, CTs, MRIs, and spinal taps as clinicians try to understand the
20 disease and assess treatment strategies. This means young children (and their families) are put through the gruelling task of trying various primarily adult antiseizure therapies singly and in combination (n=8 drugs and/or surgery and/or the ketogenic diet) in the hopes of finding an efficacious strategy. Given that it can take 8-10 weeks to test one strategy, only 5-6 combinations might be tested in a year and multiple years are often required to
25 identify a treatment strategy – and all the while the children continue to have uncontrolled seizures and visits to the emergency room.

In June 2018, the FDA approved cannabidiol (CBD; Epidiolex) as the first antiseizure medication for DS. While an important step forward, CBD only reduces seizure frequency in 43% of patients and fewer than 5% achieved full seizure freedom (the ultimate goal).
30 Thus, there remains a significant unmet need for efficacious anti-epileptic agents.

SUMMARY

Provided are methods of treating epilepsy. The methods include administering to an individual having epilepsy a therapeutically effective amount of a phosphodiesterase 4 (PDE4) inhibitor. Also provided are methods of identifying an anti-seizure agent. Such methods include contacting a PDE4 polypeptide with a candidate agent in a PDE4 activity assay, where inhibition of activity of the PDE4 polypeptide by the candidate agent identifies the candidate agent as an anti-epileptic agent.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1: An overview of a representative PDE4 gene structure.

FIG. 2: Amino acid sequence alignment of human PDE4A polypeptides that may be inhibited according to embodiments of the present disclosure.

FIG. 3: Amino acid sequence alignment of human PDE4B polypeptides that may be inhibited according to embodiments of the present disclosure.

FIG. 4: Amino acid sequence alignment of human PDE4C polypeptides that may be inhibited according to embodiments of the present disclosure.

FIG. 5: Amino acid sequence alignment of human PDE4D polypeptides that may be inhibited according to embodiments of the present disclosure.

FIG. 6: Panel A: data demonstrating that an example PDE4 inhibitor (AN2728) dose-dependently restores bioenergetics to baseline levels in *scn1lab* mutant zebrafish. Panel B: data demonstrating that a variety of PDE4 inhibitors (in this example, Rolipram, Cilomilast, Roflumilast, Ibudilast, Theophylline, Drotaverine, and Irsogladine) are effective in restoring bioenergetics to baseline levels in *scn1lab* mutant zebrafish.

FIG. 7: Data demonstrating that PDE4B-, 4C- and 4D-morpholinos are effective in restoring bioenergetics to baseline levels in *scn1lab* mutant zebrafish.

FIG. 8: Data demonstrating that two examples of PDE4 inhibitors (Roflumilast and Theophylline) restore bioenergetics in a *kcna1* model of generalizable epilepsy.

FIG. 9: Panels A and B: Data demonstrating that an example PDE4 inhibitor (AN2728) blocks seizure-like hyperexcitability to baseline levels in *scn1lab* zebrafish, including decreases in seizure frequency and peak amplitude.

FIG. 10: Panels A and B: Data demonstrating that a PDE4B morpholino is effective in blocking seizure-like hyperexcitability to baseline levels in *scn1lab* zebrafish.

FIG. 11: Data demonstrating that an example PDE4 inhibitor (AN2728) decreases hyperexcitability in mouse *Scn1a* mutant brain *ex vivo* slices.

FIG. 12: Data demonstrating that an example PDE4 inhibitor (AN2728) protects against induction of seizures in a 6Hz-induction mouse model.

FIG. 13: Data demonstrating that an example PDE4 inhibitor (AN2728) protects against hyperthermia-induced seizures in *Scn1a* mutant mice.

FIG. 14: Data demonstrating that a two-fold selective PDE4B inhibitor (rolipram) is effective in protecting against hyperthermia-induced seizures in *Scn1a* mutant mice.

5 **FIG 15:** Data demonstrating that three examples of PDE4 inhibitors (AN2728, Rolipram, Roflumilast) partially or fully block seizures in *Scn1a* mutant mice.

DETAILED DESCRIPTION

10 Provided are methods of treating epilepsy. The methods include administering to an individual having epilepsy a therapeutically effective amount of a phosphodiesterase 4 (PDE4) inhibitor. Also provided are methods of identifying an anti-epileptic agent. Such methods include contacting a PDE4 polypeptide with a candidate agent in a PDE4 activity assay, where inhibition of activity of the PDE4 polypeptide by the candidate agent identifies the candidate agent as an anti-epileptic agent.

15 Before the methods of the present disclosure are described in greater detail, it is to be understood that the methods are not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the methods will be limited only by the appended claims.

20 Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the methods. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the methods, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either
25 or both of those included limits are also included in the methods.

Certain ranges are presented herein with numerical values being preceded by the term "about." The term "about" is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the
30 term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the methods belong. Although any methods similar or equivalent to those described herein can also be used in the practice or testing of the methods, representative illustrative methods
5 are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the materials and/or methods in connection with which the
10 publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present methods are not entitled to antedate such publication, as the date of publication provided may be different from the actual publication date which may need to be independently confirmed.

It is noted that, as used herein and in the appended claims, the singular forms “a”,
15 “an”, and “the” include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

It is appreciated that certain features of the methods, which are, for clarity, described
20 in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the methods, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments are specifically embraced by the
25 present disclosure and are disclosed herein just as if each and every combination was individually and explicitly disclosed, to the extent that such combinations embrace operable processes and/or compositions. In addition, all sub-combinations listed in the embodiments describing such variables are also specifically embraced by the present methods and are disclosed herein just as if each and every such sub-combination was individually and
30 explicitly disclosed herein.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present
35 methods. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

METHODS OF TREATMENT

As summarized above, the present disclosure provides methods of treating epilepsy. The methods of treating epilepsy include administering to an individual having epilepsy a therapeutically effective amount of a phosphodiesterase 4 (PDE4) inhibitor. The methods are based in part on the unexpected findings described herein that PDE4 inhibition: blocks a bioenergetics and hyperexcitable neuronal phenotype in both zebrafish and mouse models of epilepsy; protects against hyperthermia-induced seizures in a mouse model of epilepsy; and blocks seizures using a 6Hz test – the entry-point model of the NIH-backed Epilepsy Therapy Screening Program.

Cyclic nucleotide phosphodiesterases (PDEs) catalyze the hydrolysis of the cyclic nucleotide second messengers – cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). The PDE4 family is one of three cAMP-specific PDE families. PDE4s have been shown to regulate several cellular physiological processes, including protein phosphorylation via cAMP-dependent protein kinase A (PKA), gene transcription through cAMP response elements, and cyclic nucleotide gated ion channels. These processes have been linked to cognitive function, depression, schizophrenia, hypertension, and cardiomyocyte contractility.

The PDE4 gene family is composed of four gene isoforms: PDE4A, PDE4B, PDE4C, and PDE4D. The isoforms arose via a gene duplication event in a common eukaryotic ancestor before the separation of sponges and eumetazoans. Transcripts from all four PDE4 gene isoforms have been detected in mammalian species. An overview of a representative PDE4 gene structure is provided in FIG. 1. PDE4 genes are composed of multiple exons connected by either a dashed line (facultative exons), or solid line (constitutive exons). The PDE4 long form amino-termini specifying exons (1), are located in the upstream region of the genes, each under different promoter control. The upstream conserved region-1 (UCR1) is composed of three exons (UCR1a, UCR1b, and UCR1c). The PDE4 short form amino termini specifying exon(s) (1a) are located downstream of the linker region 1 (LR1) exon. Upstream conserved region 2 (UCR2) is composed of three exons (UCR2a, UCR2b and UCR2c), which are interrupted by the super-short form amino-terminus specifying exon (1b). The amino-terminus of truncated super-short PDE4 splice variants is found within the UCR2b exon (1c). The enzymatic core of PDE4 is encoded by several constitutive exons (found in all isoforms) located in the farthest downstream regions of the gene. Further details regarding the gene structure and splice variants of the PDE4 isoforms are found, e.g., in Johnson et al. (2010) *BMC Evol Biol.* 10:247, the disclosure of which incorporated herein by reference in its entirety for all purposes. In some embodiments, a PDE4 polypeptide inhibited according to the methods of the present

disclosure is a PDE4 polypeptide provided in FIG. S1 of Johnson et al. (2010) *BMC Evol Biol.* 10:247.

Non-limiting examples of PDE4 polypeptides which may be inhibited (alone or in any combination) according to the methods of the present disclosure are provided in Table 1.

5 **Table 1 – Example Human PDE4 target polypeptides**

PDE4A (Human Isoform 1) UniProtKB – P27815-1) SEQ ID NO:1	MEPPTVPSEERSLSLSPGPREGQATLKPPPQHLWRQPRTPIRIQQRGYS DSAERAERERQPHRPIERADAMDTSDRPLRTTRMSWPSSFHGTGTGS GGAGGGSSRRFEAENGPTSPGGRSPLDSQASPLVLHAGAATSQRRES FLYRSDSDYDMSPKTMSSRNSSVTSEAHAEIDLIVTPFAQVLASLSVRSNF SLLTNVPVPSNKRSPPLGGPTPVCKATLSEETCQQLARETLEELDWCLEQL ETMQTYRSVSEMASHKFKRMLNRELTHLSEMSRSGNQVSEYISTTFLDK QNEVEIPSPTMKEREKQQAPRPRPSQPPPPVPHLQPMSSQITGLKKLMH SNSLNNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTC IMYMIFQERDILLKKFRIPVDTMVTYMLTLEDHYHADVAHYNSLHAADVLO STHVLLATPALDAVFTDLEILAALFAAAIHVDVHPGVSNQFLINTNSELALM YNDESLENHHHLAVGFKLLQEDNCDIFQNLSSKRQRQSLRKMVIDMVLATD MSKHMTLLADLKTMTVETKKVTSSGVLLLDNYSRIQVLRNMVHCADLSN PTKPLELYRQWTDRIAMAEFFQQGDRERERGMESPMCDKHTASVEKSQ VGFDIYVHPLWETWADLVHPDAQEILDITLEDNRDWYYSAIRQSPSPPE EESRGP GHPPLPDKFQFELTEEEEEEEISMAQIPCTAQEALTAQGLSGV EEALDATIAWEASPAQESLEVMAQEASLEAELEAVYLTQQAQSTGSAPV APDEFSSREEFVAVSHSSPSALALQSPLLPAWRTLSVSEHAPGLPGLPS TAAEVEAQREHQAAKRACSACAGTFGEDTSALPAPGGGGSGGDPT
PDE4A (Human Isoform 2) UniProtKB – P27815-2) SEQ ID NO:2	MARPRGLGRIPELQLVAFPVAVAAEAEFLPEPLAPRAPRRRSPSPSSPV FFASPSPTFRRRLRLLRSCQDLGRQAWAGAGFEAENGPTSPGGRSPLD SQASPLVLHAGAATSQRRESFLYRSDSDYDMSPKTMSSRNSSVTSEAH AEDLIVTPFAQVLASLSVRSNFSLLTNVPVPSNKRSPPLGGPTPVCKATLS EETCQQLARETLEELDWCLEQLTETMQTYRSVSEMASHKFKRMLNRELTH LSEMSRSGNQVSEYISTTFLDKQNEVEIPSPTMKEREKQQAPRPRPSQ PPPPVPHLQPMSSQITGLKKLMHSNSLNNSNIPRFGVKTDQEELLAQELEN LNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDILLKKFRIPVDTMVTYMLT LEDHYHADVAHYNSLHAADVLOSTHVLLATPALDAVFTDLEILAALFAAAIH VDVHPGVSNQFLINTNSELALMYNDESLENHHHLAVGFKLLQEDNCDIFQ NLSSKRQRQSLRKMVIDMVLATDMSKHMTLLADLKTMTVETKKVTSSGVLL DNYSRIQVLRNMVHCADLSNPTKPLELYRQWTDRIAMAEFFQQGDRERE RGMEISPMCDKHTASVEKSQVGFDIYVHPLWETWADLVHPDAQEILDIT LEDNRDWYYSAIRQSPSPPEEESRGP GHPPLPDKFQFELTEEEEEEEIS MAQIPCTAQEALTAQGLSGVEEALDATIAWEASPAQESLEVMAQEASLEA ELEAVYLTQQAQSTGSAPVAPDEFSSREEFVAVSHSSPSALALQSPLLP AWRTLSVSEHAPGLPGLPSTAAEVEAQREHQAAKRACSACAGTFGEDT SALPAPGGGGSGGDPT
PDE4A (Human Isoform 3) UniProtKB – P27815-3) SEQ ID NO:3	MCPFPVTTVPLGGPTPVCKATLSEETCQQLARETLEELDWCLEQLTETMQ TYRSVSEMASHKFKRMLNRELTHLSEMSRSGNQVSEYISTTFLDKQNEV EIPSPTMKEREKQQAPRPRPSQPPPPVPHLQPMSSQITGLKKLMHSNSL NNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTCIMYM IFQERDILLKKFRIPVDTMVTYMLTLEDHYHADVAHYNSLHAADVLOSTHV LLATPALDAVFTDLEILAALFAAAIHVDVHPGVSNQFLINTNSELALMYNDE SVLENHHHLAVGFKLLQEDNCDIFQNLSSKRQRQSLRKMVIDMVLATDMSK HMTLLADLKTMTVETKKVTSSGVLLLDNYSRIQVLRNMVHCADLSNPTK PLELYRQWTDRIAMAEFFQQGDRERERGMESPMCDKHTASVEKSQVGFDI YVHPLWETWADLVHPDAQEILDITLEDNRDWYYSAIRQSPSPPEEESR GPGHPPLPDKFQFELTEEEEEEEISMAQIPCTAQEALTAQGLSGVEEAL

	DATIAWEASPAQESLEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDE FSSREEFVAVSHSSPSALALQSPLLPAWRTL SVSEHAPGLPGLPSTAAE VEAQREHQAAKRACSACAGTFGEDTSALPAPGGGGSGGDPT
PDE4A (Human Isoform 4) UniProtKB – P27815-4) SEQ ID NO:4	MPLVDFFCETCSKPWLVGWWDQFKRMLNRELTHLSEMSRSGNQVSEYI STTFLDKQNEVEIPSPMTKEREKQQAPRPRPSQPPPPVPHLQPM SQIT GLKKLMHSNSLNNNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYA GGRSLTCIMYMIFQERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSL HAADVLQSTHVLLATPALDAVFTDLEILAAALFAAAIHVDVHPGVSNQFLINT NSELALMYNDESVLENHHLAVGFKLLQEDNCDIFQNL SKRQRQSLRKMVI DMVLATDMSKHMTLLADLKT MVETKKVTSSGVLLLDNYSDRIQVLRNMV HCADLSNPTKPLELYRQWTDRI MAEFFQQGDRERERGM EISPMCDKHT ASVEKSQVGFIDYIVHPLWETWADLVHPDAQEILD TLEDNRDWYYSAIRQ SPSPPEEEESRGPGHPPLPDKFQFELT EEEEEEEI SMAQIPCTAQEALT AQGLSGVEEALDATIAWEASPAQESLEVMAQEASLEAELEAVYLTQQAQ STGSAPVAPDEFSSREEFVAVSHSSPSALALQSPLLPAWRTL SVSEHAP GLPGLPSTAAEVEAQREHQAAKRACSACAGTFGEDTSALPAPGGGGSG GDPT
PDE4A (Human Isoform 5) UniProtKB – P27815-5) SEQ ID NO:5	MVLPSDQGFKLLGNVLQGPEPYRLLTSGRLRHQELENLNKWGLNIFCVS DYAGGRSLTCIMYMIFQERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYH NSLHAADVLQSTHVLLATPALDAVFTDLEILAAALFAAAIHVDVHPGVSNQF LINTNSELALMYNDESVLENHHLAVGFKLLQEDNCDIFQNL SKRQRQSLR KMVIDMVLATDMSKHMTLLADLKT MVETKKVTSSGVLLLDNYSDRIQVLR NMVHCADLSNPTKPLELYRQWTDRI MAEFFQQGDRERERGM EISPMCD KHTASVEKSQVQARGIDGRAQGGFY
PDE4A (Human Isoform 6) UniProtKB – P27815-6) SEQ ID NO:6	MRSGAAPRARPRPPALALPPTGPESLTHFPFSD EDRRHPPGRSVSFEA ENGPTPSPGRSPLDSQASPLVLHAGAATSQRRESFLYRSDSDYDMSP KTMSRNSSVTSEAHAE DLIVTPFAQVLASLRSVRSNFSLLTNVPVPSNKR SPLGGPTPVCKATLSEETCQQLARETLEELDWCLEQLETMQTYRSVSEM ASHKFKRMLNRELTHLSEMSRSGNQVSEYISTTFLDKQNEVEIPSPMTKE REKQQAPRPRPSQPPPPVPHLQPM SQITGLKKLMHSNSLNNNSNIPRFG VKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLK KFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDA VFTDLEILAAALFAAAIHVDVHPGVSNQFLINTNSELALMYNDESVLENHHL AVGFKLLQEDNCDIFQNL SKRQRQSLRKMVIDMVLATDMSKHMTLLADLK TMVETKKVTSSGVLLLDNYSDRIQVLRNMVHCADLSNPTKPLELYRQWT DRIMAEFFQQGDRERERGM EISPMCDKHTASVEKSQVGFIDYIVHPLWE TWADLVHPDAQEILD TLEDNRDWYYSAIRQSPSPPEEEESRGPGHPPLP DKFQFELT EEEEEEEI SMAQIPCTAQEALTAQGLSGVEEALDATIAWEAS PAQESLEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFV AVSHSSPSALALQSPLLPAWRTL SVSEHAPGLPGLPSTAAEVEAQREHQ AAKRACSACAGTFGEDTSALPAPGGGGSGGDPT
PDE4A (Human Isoform 7) UniProtKB – P27815-7) SEQ ID NO:7	MKRSR SALS VAGTGDERSRETPESDRANMLGADLRRPRRRLSSGPGLG WAQPEPSDPGVPLPPRPTTLLIPPRISITRAENDSFEAENGPTPSPGRS PLDSQASPLVLHAGAATSQRRESFLYRSDSDYDMSPKTMSRNSSVTSE AHAEDLIVTPFAQVLASLRSVRSNFSLLTNVPVPSNKRSP LGGPTPVCKA TLSEETCQQLARETLEELDWCLEQLETMQTYRSVSEMASHKFKRMLNRE LTHLSEMSRSGNQVSEYISTTFLDKQNEVEIPSPMTKEREKQQAPRPRPS QPPPPVPHLQPM SQITGLKKLMHSNSLNNNSNIPRFGVKTDQEELLAQEL ENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIPVDTMVTYML TLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDAVFTDLEILAAALFAA AIHVDVHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEDNCD IFQNL SKRQRQSLRKMVIDMVLATDMSKHMTLLADLKT MVETKKVTSSGV LLLDNYSDRIQVLRNMVHCADLSNPTKPLELYRQWTDRI MAEFFQQGDR ERERGM EISPMCDKHTASVEKSQVGFIDYIVHPLWETWADLVHPDAQEIL DTLEDNRDWYYSAIRQSPSPPEEEESRGPGHPPLPDKFQFELT EEEEE EEISMAQIPCTAQEALTAQGLSGVEEALDATIAWEASPAQESLEVMAQEA

	SLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVAVSHSSPSALALQS PLLPAWRTLSVSEHAPGLPLPSTAAEVEAQREHQAAKRACSACAGTFG EDTSALPAPGGGGSGGDPT
PDE4B (Human Isoform 1) UniProtKB – Q07343-1) SEQ ID NO:8	MKKSRSVMTVMADDNVKDYFECSLSKSYSSSSNTLGIDLWRGRRCCSG NLQLPPLSQRQSERARTPEGDGISRPTTLPLTTLPISIAITTVSQECFDVEN GPSPGRSPLDPQASSSAGLVLHATFPGHSQRRESFLYRSDSDYDLSPKA MSRNSSLPSEQHGDDLIVTPFAQVLASLRSVRNFTILTNLHGTSNKRSP AASQPPVSRVNPQEESYQKLAMETLEELDWCLDQLETIQTYRSVSEMAS NKFKRMLNRELTHLSEMSRSGNQVSEYISNTFLDKQNDVEIPSPTQKDRE KKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNTENEDHLAKELEDLNK WGLNIFNVAGYSHNRPLTCIMYAIFQERDLLKTFRISSDTFITYMMTLEDH YHSDVAYHNSLHAADVAQSTHVLLSTPALDAVFTDLEILAAIFAAAIHDVD HPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEEHCDIFMNL KKQRQTLRKMVIDMVLATDMSKHMSLLADLKTMTVETKKVTSSGVLLLDN YTDRIQVLRNMVHCADLSNPTKSLELYRQWTDRIEMEEFFQQGDKERER MEISPMCDKHTASVEKSQVGFIDYIVHPLWETWADLVQPDADQDILDTLED NRNWKYQSMIPQSPSPPLDEQNRDCQGLMEKFQFELTLDEEDSEGPEKE GEGHSYFSSTKTLCTVIDPENRDSLGETDIDIATEDKSPVDT
PDE4B (Human Isoform 2) UniProtKB – Q07343-2) SEQ ID NO:9	MKEHGGTFSSSTGISGGSGDSAMDSLQPLQPNYMPVCLFAEESYQKLAM ETLEELDWCLDQLETIQTYRSVSEMASNKFKRMLNRELTHLSEMSRSGN QVSEYISNTFLDKQNDVEIPSPTQKDREKKKKQQLMTQISGVKKLMHSS LNNTSISRFGVNTENEDHLAKELEDLNKWGLNIFNVAGYSHNRPLTCIMY AIFQERDLLKTFRISSDTFITYMMTLEDHYHSDVAYHNSLHAADVAQSTHV LLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTNSELALMYNDE SVLENHHLAVGFKLLQEEHCDIFMNLTKKQRQTLRKMVIDMVLATDMSKH MSLLADLKTMTVETKKVTSSGVLLLDNYTDRIQVLRNMVHCADLSNPTKSL ELYRQWTDRIEMEEFFQQGDKERERGMESPMCDKHTASVEKSQVGFIDY IVHPLWETWADLVQPDADQDILDTLEDNRNWKYQSMIPQSPSPPLDEQNRD CQGLMEKFQFELTLDEEDSEGPEKEGEGHSYFSSTKTLCTVIDPENRDSL GETDIDIATEDKSPVDT
PDE4B (Human Isoform 3) UniProtKB – Q07343-3) SEQ ID NO:10	MTAKDSSKELTASEPEVCIKTFKEQMHLELELPRLPGNRPTSPKISPRSSP RNSPCFFRKLNVNKSIRQRRRTVAHTCFDVENGPSPPGRSPLDPQASSS AGLVLHATFPGHSQRRESFLYRSDSDYDLSPKAMSRNSSLPSEQHGDDL IVTPFAQVLASLRSVRNFTILTNLHGTSNKRSPAASQPPVSRVNPQEES YQKLAMETLEELDWCLDQLETIQTYRSVSEMASNKFKRMLNRELTHLSE MSRSGNQVSEYISNTFLDKQNDVEIPSPTQKDREKKKKQQLMTQISGVK KLMHSSSLNNTSISRFGVNTENEDHLAKELEDLNKWGLNIFNVAGYSHNR PLTCIMYAIFQERDLLKTFRISSDTFITYMMTLEDHYHSDVAYHNSLHAAD VAQSTHVLLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTNSEL ALMYNDESVLENHHLAVGFKLLQEEHCDIFMNLTKKQRQTLRKMVIDMVL ATDMSKHMSLLADLKTMTVETKKVTSSGVLLLDNYTDRIQVLRNMVHCADL SNPTKSLELYRQWTDRIEMEEFFQQGDKERERGMESPMCDKHTASVEKS QVGFIDYIVHPLWETWADLVQPDADQDILDTLEDNRNWKYQSMIPQSPSPPL DEQNRDCQGLMEKFQFELTLDEEDSEGPEKEGEGHSYFSSTKTLCTVIDP ENRDSLGETDIDIATEDKSPVDT
PDE4B (Human Isoform 5) UniProtKB – Q07343-4) SEQ ID NO:11	MPEANYLLSVSWGYIKFKRMLNRELTHLSEMSRSGNQVSEYISNTFLDK QNDVEIPSPTQKDREKKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNT ENEDHLAKELEDLNKWGLNIFNVAGYSHNRPLTCIMYAIFQERDLLKTFRI SSDTFITYMMTLEDHYHSDVAYHNSLHAADVAQSTHVLLSTPALDAVFTD LEILAAIFAAAIHDVDHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFK LLQEEHCDIFMNLTKKQRQTLRKMVIDMVLATDMSKHMSLLADLKTMTVET KKVTSSGVLLLDNYTDRIQVLRNMVHCADLSNPTKSLELYRQWTDRIEMEE FFQQGDKERERGMESPMCDKHTASVEKSQVGFIDYIVHPLWETWADLV QPDADQDILDTLEDNRNWKYQSMIPQSPSPPLDEQNRDCQGLMEKFQFELT LDEEDSEGPEKEGEGHSYFSSTKTLCTVIDPENRDSLGETDIDIATEDKSPV DT

PDE4C (Human Isoform 1) UniProtKB – Q08493-1) SEQ ID NO:12	MENLGVGEGAEACSRLSRSRGRHSMTRAPKHLWRQPRRPIRIQQRFYSDPKSAGCRERDLSRPELRKSRLSWPVSSCRRFDLENGLSCGRRALDPQSSPGLGRIMQAPVPHSQRRESFLYRSDSDYELSPKAMSRNSSVASDLHGEDMIVTPFAQVLASLRTVRSNVAALARQQCLGAAKQGPVGNPSSSNQLPPAEDTGQKLALETLDLWCLDQLETQTRHSGEMASNKFKRILNRELTHLSETSRSGNQVSEYISRTFLDQQTEVELPKVTAEEAPQPMRSRISGLHGLCHSASLSSATVPRFGVQTDQEEQLAKELEDTNKWGLDVFKVAELSGNRPLTAIIFSIFQERDLLKTFQIPADTLATYLLMLEGHYHANVAYHNSLHAADVAQSTHVLLATPALEAVFTDLEILAALFASAIHDVDHPGVSQFLINTNSELALMYNDASVLENHHLAVGFKLLQAENCDIFQNLSAKQRLSLRRMVIDMVLATDMSKHMNLLADLKTMTVETKKVTSLSGVLLLDNYSDRIQVLQNLVHCADLSNPTKPLPLYRQWTDRIMAEFFQQGDRERESGLDISPMCDKHTASVEKSQVGFIDYIAHPLWETWADLVHPDAQDLLDTLEDNREWYQSKIPRSPSDLTNPERDGPDRFQFELTLEEAEEDDEEEEEEGEETALAKEALELPDTELLSP EAGPDPGDLPLDNQRT
PDE4C (Human Isoform 2) UniProtKB – Q08493-2) SEQ ID NO:13	MQAPVPHSQRRESFLYRSDSDYELSPKAMSRNSSVASDLHGEDMIVTPFAQVLASLRTVRSNVAALARQQCLGAAKQGPVGNPSSSNQLPPAEDTGQKLALETLDLWCLDQLETQTRHSGEMASNKFKRILNRELTHLSETSRSGNQVSEYISRTFLDQQTEVELPKVTAEEAPQPMRSRISGLHGLCHSASLSATVPRFGVQTDQEEQLAKELEDTNKWGLDVFKVAELSGNRPLTAIIFSIFQERDLLKTFQIPADTLATYLLMLEGHYHANVAYHNSLHAADVAQSTHVLLATPALEAVFTDLEILAALFASAIHDVDHPGVSQFLINTNSELALMYNDASVLENHHLAVGFKLLQAENCDIFQNLSAKQRLSLRRMVIDMVLATDMSKHMNLLADLKTMTVETKKVTSLSGVLLLDNYSDRIQVLQNLVHCADLSNPTKPLPLYRQWTDRIMAEFFQQGDRERESGLDISPMCDKHTASVEKSQVGFIDYIAHPLWETWADLVHPDAQDLLDTLEDNREWYQSKIPRSPSDLTNPERDGPDRFQFELTLEEAEEDDEEEEEEGEETALAKEALELPDTELLSPEAGPDPGDLPLDNQRT
PDE4C (Human Isoform 3) UniProtKB – Q08493-3) SEQ ID NO:14	MQGPPAPAPVPGPGSPRGSPRGSPGLFRKLLVNQSIQLQRRFTVAHPLCFDLENGLSCGRRALDPQSSPGLGRIMQAPVPHSQRRESFLYRSDSDYELSPKAMSRNSSVASDLHGEDMIVTPFAQVLASLRTVRSNVAALARQQCLGAAKQGPVGNPSSSNQLPPAEDTGQKLALETLDLWCLDQLETQTRHSGEMASNKFKRILNRELTHLSETSRSGNQVSEYISRTFLDQQTEVELPKVTAEEAPQPMRSRISGLHGLCHSASLSSATVPRFGVQTDQEEQLAKELEDTNKWGLDVFKVAELSGNRPLTAIIFSIFQERDLLKTFQIPADTLATYLLMLEGHYHANVAYHNSLHAADVAQSTHVLLATPALEAVFTDLEILAALFASAIHDVDHPGVSQFLINTNSELALMYNDASVLENHHLAVGFKLLQAENCDIFQNLSAKQRLSLRRMVIDMVLATDMSKHMNLLADLKTMTVETKKVTSLSGVLLLDNYSDRIQVLQNLVHCADLSNPTKPLPLYRQWTDRIMAEFFQQGDRERESGLDISPMCDKHTASVEKSQVGFIDYIAHPLWETWADLVHPDAQDLLDTLEDNREWYQSKIPRSPSDLTNPERDGPDRFQFELTLEEAEEDDEEEEEEGEETALAKEALELPDTELLSPEAGPDPGDLPLDNQRT
PDE4D (Human Isoform 4) UniProtKB – Q08499-1) SEQ ID NO:15	MEAEGSSAPARAGSGEGSDSAGGATLKAPKHLWRHEQHHQYPLRQPQFRLLHPHHHLPPPPPPSPQPQPQCPLQPPPPPPPLPPPPPPPGAARGRYASSGATGRVVRHRGYS DTERYLYCRAMDRTSYAVETGHRPGLKKSRMSWPSSFQGLRRFDVDNGTSAGRSPLDPMTPSGSGLILQANFVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASDIHGDDLIVTPFAQVLASLRTVRNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQKLASETLEELDWCLDQLETQTRHVSSEMASNKFKRMLNRELTHLSEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKKRPMSQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVLAKELEDVNKWGLHVFRIAEELSGNRPLTVIMHTIFQERDLLKTFKIPVDLITYLMTLEDHYHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAALFASAIHDVDHPGVSQFLINTNSELALMYNDSSVLENHHLAVGFKLLQEE NCDIFQNLTKKQRQSLRKMVIDIVLATDMSKHMNLLADLKTMTVETKKVTS SGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQWTDRIIEEEFFRQ GDRERERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA

	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEEAVGEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 3) UniProtKB – Q08499-2) SEQ ID NO:16	MMHVNNFPFRRHSWICFDVDNGTSAGRSPLDPMTSPGSGLILQANFVHSQRRESFLYRSDSDYDLSPKSMRSRNSSIASDIHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQKLASETLEELDWC CLDQLETLQTRHSVSEMASNKFKRMLNRELTHLSEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKRPMSSQISGVKKLMHSSSLTNSSIPRFGV KTEQEDVLAKELEDVNKWGLHVFRIAEELSGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLMTLEDHYHADVAYHNNIHAADVQSTHVLSTPALEAVFTD LEILAAIFASAIHDVDHDPGVSNQFLINTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRSLRKMVIDIVLATDMSKHMNLLADLKT MVEKKVTSSGVLLLDNYSQDRIQVLQNMVHCADLSNPTKPLQLYRQWTDRIEEMEEFFRQGDREMERGMESPMCDKHNASVEKSQVGFIDYIVHPLWETWAD LVHPDAQDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEE AVGEEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 10) UniProtKB – Q08499-3) SEQ ID NO:17	MSRNSSIASDIHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQKLASETLEELDWCCLDQLETLQTRHSVSEMASN KFKRMLNRELTHLSEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKRPMSSQISGVKKLMHSSSLTNSSIPRFGVKTQEDVLAKELEDVNKWGL HVFRIAEELSGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLMTLEDHYHADVAYHNNIHAADVQSTHVLSTPALEAVFTDLEILAAIFASAIHDVDHDPGV SNQFLINTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRSLRKMVIDIVLATDMSKHMNLLADLKT MVEKKVTSSGVLLLDNYSQDRIQVLQNMVHCADLSNPTKPLQLYRQWTDRIEEMEEFFRQGDREMERGMESPMCDKHNASVEKSQVGFIDYIVHPLWETWAD LVHPDAQDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEE AVGEEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 1) UniProtKB – Q08499-4) SEQ ID NO:18	MKEQPSCAGTGHPMAGYGRMAPFELASGPVKRLRTESPFPCLFAGEEAYQKLASETLEELDWCCLDQLETLQTRHSVSEMASNKFKRMLNRELTHLSEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKRPMSSQISGVKKLMHSSSLTNSSIPRFGVKTQEDVLAKELEDVNKWGLHVFRIAEELSGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLMTLEDHYHADVAYHNNIHAADVQSTHVLSTPALEAVFTDLEILAAIFASAIHDVDHDPGVSNQFLINTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRSLRKMVIDIVLATDMSKHMNLLADLKT MVEKKVTSSGVLLLDNYSQDRIQVLQNMVHCADLSNPTKPLQLYRQWTDRIEEMEEFFRQGDREMERGMESPMCDKHNASVEKSQVGFIDYIVHPLWETWAD LVHPDAQDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEE AVGEEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 2) UniProtKB – Q08499-5) SEQ ID NO:19	MASNKFKRMLNRELTHLSEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKRPMSSQISGVKKLMHSSSLTNSSIPRFGVKTQEDVLAKELEDVNKWGLHVFRIAEELSGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLMTLEDHYHADVAYHNNIHAADVQSTHVLSTPALEAVFTDLEILAAIFASAIHDVDHDPGVSNQFLINTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRSLRKMVIDIVLATDMSKHMNLLADLKT MVEKKVTSSGVLLLDNYSQDRIQVLQNMVHCADLSNPTKPLQLYRQWTDRIEEMEEFFRQGDREMERGMESPMCDKHNASVEKSQVGFIDYIVHPLWETWAD LVHPDAQDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEE AVGEEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 5)	MAQQTSPDTLTVPEVDNPHCPNPWLNEDLVKSLRENLLQHEKSKTARKSVSPKLSPVISPRNSPRLLRRMLLSSNIPKQRRFTVAHTCFDNDNGTSAGRSPLDPMTSPGSGLILQANFVHSQRRESFLYRSDSDYDLSPKSMRSRNSSI

UniProtKB – Q08499-6) SEQ ID NO:20	SDIHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSI NKATITEEAYQKLASETLEELDWCLDQLETLQTRHSVSEMASNKFKRMLN RELTHLSEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKRPMSEQ ISGVKKLMHSSSLTNSSIPRFGVKTEQEDVLAKELEDVNKWGLHVFRIAE SGNRPLTVIMHTIFQERDLLKTFKIPVDLTILITYLMTLEDHYHADVAYHNNIH AADVVQSTHVLLSTPALEAVFTDLEILAAIFASAIHDVDHPGVSQFLINTN SELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQRQSLRKMVIDI VLATDMSKHMNLLADLKTMTVETKKVTSSGVLLLDNYSDRIQVLQNMVHC ADLSNPTKPLQLYRQWTDRIEIEFFRQGDREMERGMEISPMCDKHNAS VEKSQVGFIDYIVHPLWETWADLVHPDAQDILDTLEDNREWYQSTIPQSP SPAPDDPEEGRQGGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCSD SKTLCTQDSESTEIPLDEQVEEEAVGEEESQPEACVIDDRSPDT
PDE4D (Human Isoform N3) UniProtKB – Q08499-7) SEQ ID NO:21	MAQQTSPDTLTVPEVDNPHCPNPWLNEDLVKSLRENLLQHEKSKTARKS VSPKLSPVISPRNSPRLRRMILLSSNIPKQRRFTVAHTCFDNDNGTSAGR SPLDPMTPSGSGLILQANFVHSQRRESFLYRSDSDYDLSPKSMSRNSSIA SDIHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSI NKATITGLYNGIIAFL
PDE4D (Human Isoform 6) UniProtKB – Q08499-8) SEQ ID NO:22	MPEANYLLSVSWGVIKFKRMLNRELTHLSEMSRSGNQVSEFISNTFLDKQ HEVEIPSPTQKEKEKKRPMSEQISGVKKLMHSSSLTNSSIPRFGVKTEQE DVLAKELEDVNKWGLHVFRIAEISGNRPLTVIMHTIFQERDLLKTFKIPVD LITYLMTLEDHYHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAA IFASAIHDVDHPGVSQFLINTNSELALMYNDSSVLENHHLAVGFKLLQEE NCDIFQNLTKKQRQSLRKMVIDIVLATDMSKHMNLLADLKTMTVETKKVT SGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQWTDRIEIEFFRQ GDREMERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGGQTEKFQFELTLEED GESDTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEE EESQPEACVIDDRSPDT
PDE4D (Human Isoform 8) UniProtKB – Q08499-9) SEQ ID NO:23	MAFWWDPLGATVPGPSTRAKSRLRFSKSYSFDVDNGTSAGRSPLDPM TPSGSGLILQANFVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASDIHGDD LIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEE AYQKLASETLEELDWCLDQLETLQTRHSVSEMASNKFKRMLNRELTHLS EMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKRPMSEQISGVKKL MHSSSLTNSSIPRFGVKTEQEDVLAKELEDVNKWGLHVFRIAEISGNRPL TVIMHTIFQERDLLKTFKIPVDLTILITYLMTLEDHYHADVAYHNNIHAADVQ STHVLLSTPALEAVFTDLEILAAIFASAIHDVDHPGVSQFLINTNSELALM YNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQRQSLRKMVIDIVLATD MSKHMNLLADLKTMTVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSN PTKPLQLYRQWTDRIEIEFFRQGDREMERGMEISPMCDKHNASVEKSQ VGFIDYIVHPLWETWADLVHPDAQDILDTLEDNREWYQSTIPQSPSPAPD DPEEGRQGGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCT QDSESTEIPLDEQVEEEAVGEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 9) UniProtKB – Q08499-10) SEQ ID NO:24	MSIIMKPRSRSTSSLRTAEAVCEDVDNGTSAGRSPLDPMTPSGSGLILQA NFVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASDIHGDDLIVTPFAQVL ASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQKLASET LEELDWCLDQLETLQTRHSVSEMASNKFKRMLNRELTHLSEMSRSGNQ VSEFISNTFLDKQHEVEIPSPTQKEKEKKRPMSEQISGVKKLMHSSSLTN SIPRFGVKTEQEDVLAKELEDVNKWGLHVFRIAEISGNRPLTVIMHTIFQE RDLLKTFKIPVDLTILITYLMTLEDHYHADVAYHNNIHAADVQSTHVLLSTPA LEAVFTDLEILAAIFASAIHDVDHPGVSQFLINTNSELALMYNDSSVLENH HLAVGFKLLQEENCDFQNLTKKQRQSLRKMVIDIVLATDMSKHMNLLADL KTMTVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW TDRIEIEFFRQGDREMERGMEISPMCDKHNASVEKSQVGFIDYIVHPLW ETWADLVHPDAQDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGGQTE

	KFQFELTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 7) UniProtKB – Q08499-11) SEQ ID NO:25	MKRNTCDLLSRKSASEETLHSSNEEEDPFRGMPEYLVRRRLSCRNIQLP PLAFRQLEQADLKSESENIQRPTSLPLKILPLIAITSAESSGFDVDNGTSAG RSPLDPMTSPGSGILILQANFVHSQRRESFLYRSDSDYDLSPKSMRNSSI ASDIHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPS INKATITEEAYQKLASETLEELDWCLETLQTRHSVSEMASNKFMRML NRELTHLSEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKRPM QISGVKKLMHSSSLTNSSIPRFGVKTEQEDVLAKLEDVNKWGLHVFRIA ELSGNRPLTVIMHTIFQERDLLKTFKIPVDLTILITYLMTLEDHYHADVAYHNN IHAADVQSTHVLLSTPALEAVFTDLEILAAIFASAIHDVDHPGVSQFLINT NSELALMYNDSSVLENHHLAVGFKLLQEENDIFQNLTKKQRQSLRKMVI DIVLATDMSKHMMNLLADLKTMTVETKKVTSSGVLLLDNYSDRIQVLQNMVH CADLSNPTKPLQLYRQWTDRIEEFFRQGDREERERGMEISPMCDKHNA SVEKSQVGFIDYIVHPLWETWADLVHPDAQDILDLEDNREWYQSTIPQS PSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCS DSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 12) UniProtKB – Q08499-12) SEQ ID NO:26	MAQQTSPDTLTVPEVDNPHCPNPWLNEDLVKSLRENLLQHEKSKTARKS VSPKLSPVISPRNSPRLRRMLSSNIPKQRRFTVAHTCFDNDNGTSAGR SPLDPMTSPGSGILILQANFVHSQRRESFLYRSDSDYDLSPKSMRNSSIA SDIHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSI NKATITGSWMELNPYTLLDM

As used herein, a “PDE4 inhibitor” or an “inhibitor of PDE4” is an agent that inhibits (e.g., reduces or abolishes) phosphodiesterase activity of one or more PDE4 isoforms as compared to the phosphodiesterase activity of the one or more PDE4 isoforms in the absence of the agent. In certain embodiments, the inhibitor results in the phosphodiesterase activity of at least one of the one or more PDE4 isoforms being 80% or less, 75% or less, 70% or less, 65% or less, 60% or less, 55% or less, 50% or less, 45% or less, 40% or less, 35% or less, 30% or less, 25% or less, 20% or less, 15% or less, 10% or less, 5% or less, 4% or less, 3% or less, 2% or less, or 1% or less, as compared to the phosphodiesterase activity of the at least one of the one or more PDE4 isoforms in the absence of the inhibitor.

PDE4A is expressed in the CNS, including in the cortex, hippocampus, and cerebellum. PDE4B is widely expressed in the CNS, including in the striatum, amygdala, thalamus, and hypothalamus. PDE4C is not widely expressed in the CNS, although expression has been observed in the olfactory bulb with limited expression in the cortex. PDE4D is expressed in the CNS, including in the hippocampus and elsewhere.

In some embodiments, the methods include administering a PDE4 inhibitor that inhibits one or more of PDE4A, PDE4B, PDE4C and PDE4D. In certain embodiments, the PDE4 inhibitor inhibits two, three, or each of PDE4A, PDE4B, PDE4C and PDE4D. The methods may include administering a PDE4 inhibitor that exhibits selectivity among PDE4A, PDE4B, PDE4C, and PDE4D. By “selectivity” is meant the PDE4 inhibitor either:

exclusively inhibits PDE4A, PDE4B, PDE4C, or PDE4D; or inhibits two or more of PDE4A, PDE4B, PDE4C, and PDE4D, where the inhibition of at least one of the two or more PDE4 isoforms is greater than the inhibition of a different isoform among the two or more PDE4 isoforms inhibited by the inhibitor. In some embodiments, the PDE4 inhibitor is selective
5 for a PDE4 isoform. As used herein, a PDE4 inhibitor is “selective for” a PDE4 isoform when the inhibitor either: exclusively inhibits the PDE4 isoform (PDE4A, PDE4B, PDE4C, or PDE4D); or inhibits the PDE4 isoform to a greater extent than at least one other PDE4 isoform. For example, in certain embodiments, the PDE4 inhibitor is selective for PDE4B such that the inhibition of PDE4B is greater than the inhibition of a second PDE4 isoform,
10 e.g., greater than the inhibition of PDE4D.

In some embodiments, the PDE4 inhibitor does not inhibit (or does not substantially inhibit) one, two, or three of PDE4A, PDE4B, PDE4C, and PDE4D. As used herein, an inhibitor does not “substantially inhibit” the activity of a PDE4 isoform when contacting the PDE4 isoform with the inhibitor does not inhibit the activity of the isoform by more than 20%,
15 15%, 10%, 5%, 4%, 3%, 2%, or 1%, as compared to the activity of the isoform in the absence of the inhibitor. In certain embodiments, the methods include administering a PDE4B inhibitor that does not inhibit (or does not substantially inhibit) PDE4D.

In some embodiments, the PDE4 inhibitor acts by binding to the catalytic domain (e.g., the active site) of PDE4. In other embodiments, the PDE4 inhibitor acts by
20 allosterically inhibiting PDE4 activity. All eleven PDE superfamily members (PDE1-11) exhibit a high degree of sequence conservation across the catalytic domain, making PDE families distinguished instead by motifs that encode unique regulatory domains. Traditional strategies to target PDEs have largely focused on ligands that bind the catalytic domain, thereby leading to complete inhibition of cAMP hydrolysis and associated toxicities, as well
25 as a narrow therapeutic range due to nonselective binding. PDE4A, PDE4B, PDE4C, and PDE4D each contain three signature regulatory domains: upstream conserved regions 1 (UCR1) and 2 (UCR2) forming a regulatory module, and a control region (CR3) domain at the C-terminus. PDE4B and PDE4D crystal structures show that phosphodiesterase activity is regulated by UCR2 allosteric control of the catalytic active site. Allosteric modulation of
30 PDE4s is therefore an underappreciated approach to selectively interfere with PDE4 activity. Indeed, small molecule PDE4D allosteric modulators are potent in cellular and *in vivo* assays. Further, the UCR2 of PDE4B has been shown to intimately interact in *trans* with the active site in the catalytic domain. In some embodiments, the methods include administering an inhibitor that selectively inhibits PDE4B (e.g., versus PDE4D), e.g., by
35 binding to PDE4B in a manner in which the UCR2 closes over the active site, thereby preventing access by cAMP.

In some embodiments, the PDE4 inhibitor is an allosteric modulator that selectively inhibits PDE4A, PDE4B, and/or PDE4C (e.g., versus PDE4D) by capture of the C-terminal regulatory helix (CR3) across the active site in a conformation that closes access by cAMP. For example, small molecules can interact with different residues along the CR3 helix resulting in multiple “closed” conformations. Fox et al. (2014) *Cell Signal.* 26(3):657-63. The CR3 helix can adopt slightly different orientations across the active site, each with unique helical registries. Examples of allosteric inhibitors that exhibit selectivity for PDE4B versus PDE4D and may be administered according to the methods of the present disclosure are known and include the 2-arylpyrimidine derivative compound A-33 (as described in Hagan et al. (2014) *Bioorg Med Chem Lett.* 24(16):4031-4 and Fox et al. (2014) *Cell Signal.* 26(3):657-63)) and the triazine compounds described in Hagan et al. (2014) *Bioorg Med Chem Lett.* 24(16):4031-4.

Any suitable type of PDE4 inhibitor may be employed when practicing the methods of the present disclosure. In some embodiments, the PDE4 inhibitor is a small molecule. By “small molecule” is meant a compound having a molecular weight of 1000 atomic mass units (amu) or less. In some embodiments, the small molecule is 750 amu or less, 500 amu or less, 400 amu or less, 300 amu or less, or 200 amu or less. In certain embodiments, the small molecule is not made of repeating molecular units such as are present in a polymer. Small molecule allosteric modulators of PDE4 activity which may be administered according to the methods of the present disclosure are known and include those described in Gurney et al. (2011) *Handb Exp Pharmacol.* 204:167-92; Burgin et al. (2010) *Nat Biotechnol.* 28(1):63-70; Fox et al. (*supra*); Hagan et al. (*supra*); and elsewhere. In some embodiments, when the PDE4 inhibitor is a small molecule, the PDE4 inhibitor is selected from AN2728 (5-(4-cyanophenoxy)-2,3-dihydro-1-hydroxy-2,1-benzoxaborole), drotaverine, ibudilast, irsogladine, piclamilast, roflumilast, rolipram, theophylline, apremilast, compound A-33 (as described in Hagan et al. (2014) *Bioorg Med Chem Lett.* 24(16):4031-4 and Fox et al. (2014) *Cell Signal.* 26(3):657-63), a triazine compound as described in Hagan et al. (2014) *Bioorg Med Chem Lett.* 24(16):4031-4, and any combination thereof. In certain embodiments, when the PDE4 inhibitor is a small molecule, the PDE4 inhibitor is AN2728. According to some embodiments, the PDE4 inhibitor is not rolipram. In certain embodiments, the PDE4 inhibitor is not rolipram and exhibits a selectivity among PDE4A, PDE4B, PDE4C and PDE4D which is different from the selectivity of rolipram. In some embodiments, the methods employ a PDE4 inhibitor that exhibits less inhibition of PDE4D than rolipram.

According to some embodiments, the PDE4 inhibitor inhibits expression of PDE4. For example, the PDE4 inhibitor may inhibit expression of one, two, three, or each of PDE4A, PDE4B, PDE4C, and PDE4D. In certain embodiments, when the PDE4 inhibitor

inhibits expression of PDE4, the PDE4 inhibitor is a nucleic acid-based inhibitor. By “nucleic acid-based inhibitor” is meant a polymer of two or more linked nucleotides, where the polymer may include naturally occurring nucleotides, non-naturally occurring nucleotides (e.g., nucleotide analogs such as LNA, FANA, 2'-O-Me RNA, 2'-fluoro RNA, and/or the like),
5 or a combination thereof.

In some embodiments, a nucleic acid-based PDE4 inhibitor includes a region complementary to a portion of a messenger RNA (mRNA) that encodes PDE4A, a mRNA that encodes PDE4B, a mRNA that encodes PDE4C, a mRNA that encodes PDE4D, or any combination thereof. The term “complementary” as used herein refers to a nucleotide
10 sequence that base-pairs by non-covalent bonds to all or a region of a target nucleic acid. In the canonical Watson-Crick base pairing, adenine (A) forms a base pair with thymine (T), as does guanine (G) with cytosine (C) in DNA. In RNA, thymine is replaced by uracil (U). As such, A is complementary to T and G is complementary to C. In RNA, A is complementary to U and vice versa. Typically, “complementary” refers to a nucleotide
15 sequence that is at least partially complementary. The term “complementary” may also encompass duplexes that are fully complementary such that every nucleotide in one strand is complementary to every nucleotide in the other strand in corresponding positions. In certain cases, a nucleotide sequence may be partially complementary to a target, in which not all nucleotides are complementary to every nucleotide in the target nucleic acid in all
20 the corresponding positions. For example, a primer may be perfectly (i.e., 100%) complementary to the target nucleic acid, or the primer and the target nucleic acid may share some degree of complementarity which is less than perfect (e.g., 70%, 75%, 85%, 90%, 95%, 99%). The percent identity of two nucleotide sequences can be determined by aligning the sequences for optimal comparison purposes (e.g., gaps can be introduced in
25 the sequence of a first sequence for optimal alignment). The nucleotides at corresponding positions are then compared, and the percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity= # of identical positions/total # of positions×100). When a position in one sequence is occupied by the same nucleotide as the corresponding position in the other sequence, then the
30 molecules are identical at that position. A non-limiting example of such a mathematical algorithm is described in Karlin et al., *Proc. Natl. Acad. Sci. USA* 90:5873-5877 (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) as described in Altschul et al., *Nucleic Acids Res.* 25:389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs
35 (e.g., NBLAST) can be used. In one aspect, parameters for sequence comparison can be set at score=100, wordlength=12, or can be varied (e.g., wordlength=5 or wordlength=20).

According to some embodiments, the nucleotide sequence of a nucleic acid-based PDE4 inhibitor is selected/designed such that the nucleic acid-based PDE4 inhibitor is selective for a PDE4 isoform. For example, the nucleotide sequence of a nucleic acid-based PDE4 inhibitor may be selected/designed such that the inhibitor selectively inhibits expression of PDE4A, PDE4B, and/or PDE4C (e.g., versus PDE4D). In some embodiments, the nucleotide sequence is selected/designed such that the inhibitor selectively inhibits PDE4B expression, e.g., selectively inhibits PDE4B expression versus PDE4D expression.

Non-limiting examples of nucleic acid-based inhibitors that may be employed when practicing the methods of the present disclosure include short interfering RNAs (siRNA), microRNAs (miRNA), morpholinos, and/or the like. Based on the available sequence information for PDE4A (NCBI Gene ID: 5141 for human PDE4A), PDE4B (NCBI Gene ID: 5142 for human PDE4B), PDE4C (NCBI Gene ID: 5143 for human PDE4C) and PDE4D (NCBI Gene ID: 5144 for human PDE4C), and the corresponding transcripts, nucleic acid-based inhibitors such as siRNAs, miRNAs, morpholinos, etc. may be designed using available tools, e.g., siRNA Wizard from Invivogen, siDESIGN Center from Dharmacon, BLOCK-iT™ RNAi Designer from Invitrogen, miR-Synth available at microrna.osumc.edu/mir-synth, WMD3 - Web MicroRNA Designer, a morpholino design tool provided by Gene Tools, etc. Approaches for designing and delivering siRNAs, miRNAs, morpholinos, etc. for targeting a particular mRNA are known and described, e.g., in Chakraborty et al. (2017) *Mol Ther Nucleic Acids* 8:132-143; Ahmadzada et al. (2018) *Biophys Rev.* 10(1):69-86; Zheng et al. (2018) *Trends Biotechnol.* 36(5):562-575; Mohanty et al. (2015) *Curr Pharm Des.* 21(31):4606-13; Gomes et al. (2015) *Ageing Res Rev.* 21:43-54; Gustincich et al. (2017) *Prog Neurobiol.* 155:194-211; Monsoori et al. (2014) *Adv Pharm Bull.* 4(4):313-321; and Xin et al. (2017) *Mol Cancer* 16:134.

As summarized above, aspects of the present disclosure include treating epilepsy (sometimes referred to as "seizure disorder") by administering to an individual having epilepsy (e.g., an individual diagnosed as having epilepsy) a therapeutically effective amount of a PDE4 inhibitor. In some embodiments, the PDE4 inhibitor is any PDE4 inhibitor described elsewhere herein, including any PDE4 inhibitor identified using the methods of identifying anti-epileptic agents of the present disclosure. The older established anti-epileptic drugs (AEDs) phenytoin, carbamazepine, clonazepam, ethosuximide, valproic acid and barbiturates are widely prescribed but suffer from a range of side effects. In addition, there is a significant group of patients (30-40%) that are resistant to the currently available therapeutic agents. Several more recent drugs have been launched, including felbamate, gabapentin, lamotrigine, oxcarbazepine, tiagabine, topiramate, vigabatrin,

zonisamide and levetiracetam. While some such more recent drugs show improved efficacies and side-effect profiles, about 30% of patients with epilepsy remain untreated.

In view of the unexpected findings described herein that PDE4 inhibition blocks a bioenergetics and hyperexcitable neuronal phenotype in both zebrafish and mouse models of epilepsy, protects against hyperthermia-induced seizures in a mouse model of epilepsy, and blocks seizures using a 6Hz test, it will be appreciated that the methods find use in treating a wide variety of epilepsies. In certain embodiments, the individual has an epilepsy selected from benign Rolandic epilepsy, frontal lobe epilepsy, infantile spasms, juvenile myoclonic epilepsy (JME), juvenile absence epilepsy, childhood absence epilepsy, pyknolepsy, febrile seizures, progressive myoclonus epilepsy of Lafora, Lennox-Gastaut syndrome, Landau-Kleffner syndrome, Dravet syndrome (DS), Generalized Epilepsy with Febrile Seizures (GEFS+), Severe Myoclonic Epilepsy of Infancy (SMEI), Benign Neonatal Familial Convulsions (BFNC), West Syndrome, Ohtahara Syndrome, early myoclonic encephalopathy, migrating partial epilepsy, infantile epileptic encephalopathies, Tuberous Sclerosis Complex (TSC), focal cortical dysplasia, Type I Lissencephaly, Miller-Dieker Syndrome, Angelman's syndrome, Fragile X syndrome, epilepsy in autism spectrum disorders, subcortical band heterotopia, Walker-Warburg syndrome, Alzheimer's disease epilepsy, posttraumatic epilepsy, progressive myoclonus epilepsy, reflex epilepsy, Rasmussen's syndrome, temporal lobe epilepsy, limbic epilepsy, status epilepticus, abdominal epilepsy, massive bilateral myoclonus, catamenial epilepsy, Jacksonian seizure disorder, Unverricht-Lundborg disease, and photosensitive epilepsy. According to some embodiments, the individual has Dravet syndrome (DS). In certain embodiments, the individual has an epilepsy caused by a genetic mutation. In some embodiments, the individual has an epilepsy having a non-genetic etiology, non-limiting examples of which include epilepsies caused by concussion, brain injury, and/or the like. According to some embodiments, the individual has been diagnosed as having epilepsy based on the individual having had two or more unprovoked seizures. The individual's epilepsy may include generalized seizures or partial (e.g., focal) seizures.

A variety of individuals are treatable according to the subject methods. Generally such individuals are "mammals" or "mammalian," where these terms are used broadly to describe organisms which are within the class mammalia, including the orders carnivore (e.g., dogs and cats), rodentia (e.g., mice, guinea pigs, and rats), and primates (e.g., humans, chimpanzees, and monkeys). In some embodiments, the individual is a human.

By "treat" or "treatment" is meant at least an amelioration of the symptoms associated with the pathological condition afflicting the individual, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g.,

symptom, associated with the pathological condition being treated, such as disease or disorder associated with PDE4 activity (e.g., epilepsy), where inhibiting PDE4 activity in the individual is beneficial. As such, treatment also includes situations where the pathological condition (e.g., epilepsy), or at least symptoms associated therewith, are completely inhibited, e.g., prevented from happening, or stopped, e.g. terminated, such that the individual no longer suffers from the pathological condition, or at least the symptoms that characterize the pathological condition. In some embodiments, an individual having epilepsy is treated by the present methods when one or more administrations of the PDE4 inhibitor results in the frequency and/or severity of seizures experienced by the individual being 90% or less, 80% or less, 70% or less, 60% or less, 50% or less, 40% or less, 30% or less, 20% or less, 10% or less, 5% or less, or 1% or less, as compared to the frequency and/or severity of seizures experienced by the individual in the absence of the one or more administrations of the PDE4 inhibitor.

Dosing is dependent on severity and responsiveness of the disease state to be treated. Optimal dosing schedules can be calculated from measurements of PDE4 inhibitor accumulation in the body of the individual. The administering physician can determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of the PDE4 inhibitor, and can generally be estimated based on EC₅₀s found to be effective in *in vitro* and *in vivo* animal models, etc. In general, dosage is from 0.01 µg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the PDE4 inhibitor in bodily fluids or tissues. Following successful treatment, it may be desirable to have the subject undergo maintenance therapy to prevent the recurrence of the disease state, where the PDE4 inhibitor is administered in maintenance doses, once or more daily, to once every several months, once every six months, once every year, or at any other suitable frequency.

The therapeutic methods of the present disclosure may include administering a single type of PDE4 inhibitor to an individual, or may include administering two or more types of PDE4 inhibitors to an individual, e.g., a cocktail of different PDE4 inhibitors.

In certain aspects, a PDE4 inhibitor is administered to the individual in combination with a second therapeutic agent as part of a combination therapy. For example, the methods of the present disclosure may include administering to an individual having epilepsy a therapeutically effective amount of a PDE4 inhibitor in combination with a second anti-epileptic drug. Non-limiting examples of a second anti-epileptic drug which may be used in combination with a PDE4 inhibitor include acetazolamide, diazepam, benzodiazepine, cannabadiol, carbamazepine, clobazam, clonazepam, eslicarbazepine

acetate, ethosuximide, ethotoin, felbamate, fenfluramine, fosphenytoin, gabapentin, ganaxolone, huperzine A, lacosamide, lamotrigine, levetiracetam, lorazepam, nitrazepam, oxcarbazepine, perampanel, piracetam, phenobarbital, phenytoin, potassium bromide, pregabalin, primidone, retigabine, rufinamide, sodium valproate, stiripentol, tiagabine, topiramate, valproic acid, vigabatrin, or zonisamide. According to embodiments in which a PDE4 inhibitor and a second therapeutic agent are administered in combination, the PDE4 inhibitor and the second therapeutic agent may be administered concurrently or sequentially.

The PDE4 inhibitor may be administered to the individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration. Conventional and pharmaceutically acceptable routes of administration include intranasal, intramuscular, intra-tracheal, subcutaneous, intradermal, topical application, ocular, intravenous, intra-arterial, nasal, oral, and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the PDE4 inhibitor and/or the desired effect. The PDE4 inhibitor may be administered in a single dose or in multiple doses. In some embodiments, the PDE4 inhibitor is administered by oral, parenteral, intranasal, intrathecal, intracranial, or transdermal administration. In some embodiments, the PDE4 inhibitor is administered orally. In some embodiments, the PDE4 inhibitor is administered locally. In some embodiments, the PDE4 inhibitor is administered ocularly. In some embodiments, the PDE4 inhibitor is administered intracranially. In some embodiments, the PDE4 inhibitor is administered intravenously. In some embodiments, the PDE4 inhibitor is administered by injection, e.g., for systemic delivery (e.g., intravenous infusion) or to a local site. In some embodiments, the PDE4 inhibitor is administered via an inhalational route. In some embodiments, the PDE4 inhibitor is administered intranasally. In some embodiments, the PDE4 inhibitor does not readily cross the blood-brain barrier (BBB), and the PDE4 inhibitor is administered intranasally to bypass the BBB. Further details regarding bypassing the BBB by intranasal administration may be found, e.g., in Mohanty et al. (2015) *Curr Pharm Des.* 21(31):4606-13, and elsewhere.

METHODS OF IDENTIFYING ANTI-EPILEPTIC AGENTS

As summarized above, aspects of the present disclosure include methods of identifying anti-epileptic agents. The methods of identifying an anti-epileptic agent include contacting a PDE4 polypeptide with a candidate agent in a PDE4 activity assay, where inhibition of activity of the PDE4 polypeptide by the candidate agent identifies the candidate agent as an anti-epileptic agent.

The PDE4 polypeptide contacted with the candidate agent may be any PDE4 polypeptide of interest. In some embodiments, the PDE4 polypeptide is PDE4A, PDE4B, PDE4C, or PDE4D, including any PDE4 polypeptide provided in FIGs. 2-5 or Table 1 above, or a functional variant thereof having phosphodiesterase activity. By “functional variant” is meant a PDE4 polypeptide having phosphodiesterase activity that includes one or more amino acid substitutions, deletions, insertions, or combination thereof, relative to the corresponding wild-type PDE4 polypeptide. For example, a functional variant of PDE4B may be a PDE4B polypeptide that includes one or more amino acid substitutions, deletions, insertions, or combination thereof, relative to wild-type PDE4B. A functional variant may include 70% or greater, 75% or greater, 80% or greater, 85% or greater, 90% or greater, 95% or greater, or 99% or greater, amino acid sequence homology or identity across 40% or greater, 50% or greater, 60% or greater, 70% or greater, 80% or greater, 90% or greater, or 95% or greater, of the length of the corresponding wild-type PDE4 polypeptide. The PDE4 polypeptide or functional variant thereof may be fused to a heterologous domain. Non-limiting examples of heterologous domains include linker domains (e.g., a glycine-serine linker, or other suitable linker domain), domains that find use in detecting the PDE4 polypeptide (e.g., a luciferase domain, or other suitable detectable domain), domains that find use in purifying the PDE4 polypeptide (e.g., a HIS tag, or other suitable purification tag), and/or the like.

The PDE4 activity assay may be performed using any suitable reagents (e.g., PDE4 substrates, etc.) and formats for assaying phosphodiesterase activity. In some embodiments, the PDE4 activity assay includes detecting the cleavage of cAMP or cGMP by the PDE4 polypeptide. Detection of PDE4 phosphodiesterase activity may be colorimetric-based, luminescence-based, fluorescence-based, radioactivity-based, and/or the like. In some embodiments, the assay is performed in a single tube, single well, multi-tube, multi-well (e.g., 24-well, 48-well, 96-well, 384-well, or other well format), or other suitable format. PDE4 activity assays are amenable to high-throughput formats such that large numbers of candidate agents (e.g., small molecules of a small molecule library) may be readily contacted with PDE4 polypeptides in parallel, e.g., separate wells.

In some embodiments, in the methods of identifying anti-epileptic agents, the contacting includes combining the PDE4 polypeptide and the candidate agent in a cell-free PDE4 activity assay. Cell-free assay reagents and kits for assessing phosphodiesterase activity and inhibition thereof that may be employed when practicing the methods of the present disclosure are known and include the PDE Activity Assay Kit (96-well colorimetric) available from Abcam, the PDE-Glo™ Phosphodiesterase Assay Kit (1-tube to 1536-well luminescence) available from Promega, the PDEase Kit available from FabGennix, the

Bridge-It® cAMP-Phosphodiesterase Assay Kit available from Mediomix, and the like. Additional approaches for assessing phosphodiesterase activity and inhibition thereof in cell-free format are described, e.g., in Rybalkin et al. (2013) *Methods Mol Biol.* 1020:51-62.

In some embodiments, in the methods of identifying anti-epileptic agents, the contacting includes combining the PDE4 polypeptide and the candidate agent in a cell-based PDE4 activity assay. By “cell-based” is meant the PDE4 polypeptide and the candidate agent are contacted within a cell. In some embodiments, the PDE4 polypeptide is expressed by the cell in which the contacting occurs. Cell-based assay reagents and kits for assessing phosphodiesterase activity and inhibition thereof that may be employed when practicing the methods of the present disclosure are known and include the ACTOne PDE Assay Kit available from eEnzyme, the Cell-Based PDE Assay Kit available from SB Drug Discovery, the K927-Total Phosphodiesterase Activity Assay Kit available from Biovision, and the like. Additional approaches for assessing phosphodiesterase activity and inhibition thereof in cell-free format are described, e.g., in Titus et al. (2008) *J Biomol Screen.* 13(7):609-618 (describing a cell-based PDE4 assay in 1536-well plate format for high throughput screening involving a constitutively active GPCR as a driving force for cAMP production and a cyclic nucleotide gated (CNG) cation channel as a biosensor in 1536-well plates); Allen et al. (1999) *Biochem Pharmacol.* 57(12):1375-82; and Qiu et al. (2003) *Eur J Pharmacol.* 472(1-2):73-80.

In some embodiments, the PDE4 activity assay further includes contacting the PDE4 polypeptide with a positive control agent known to inhibit PDE4 activity. Non-limiting examples of positive control agents that may be employed include AN2728, drotaverine, ibudilast, irsogladine, piclamilast, roflumilast, rolipram, theophylline, apremilast, and any combination thereof.

The candidate agent may be any type of candidate agent of interest. In some embodiments, the candidate agent is a small molecule. In some embodiments, the small molecule was selected as a candidate agent based on an *in silico* screen for PDE4 inhibitors. The *in silico* screen may have been a screen for a PDE4 inhibitor that is selective among PDE4A, PDE4B, PDE4C, and PDE4D. For example, the *in silico* screen may have been an *in silico* screen for a PDE4 inhibitor that selectively inhibits PDE4A, PDE4B, and/or PDE4C (e.g., versus PDE4D). In some embodiments, the small molecule was selected as a candidate agent based on an *in silico* screen for inhibitors that are selective for PDE4B versus PDE4D. Accordingly, in some embodiments, the methods may further include – when the candidate agent is determined to inhibit activity of the PED4 polypeptide – determining whether the candidate agent exhibits selective inhibition among PDE4A, PDE4B, PDE4C, and PDE4D.

In some embodiments, when the candidate agent is a small molecule, the small molecule is part of a library of small molecule candidate agents. In some embodiments, the methods include contacting a library of small molecule candidate agents with PDE4 polypeptides in high-throughput format, e.g., 96-well, 384-well, 1536-well, or other high throughput format.

COMPOSITIONS

Aspects of the present disclosure include compositions. In some embodiments, the compositions find use, e.g., in practicing the methods of the present disclosure.

In some embodiments, a composition of the present disclosure includes any of the PDE4 inhibitors described elsewhere herein, including any anti-epileptic agent identified by the methods of identifying anti-epileptic agents of the present disclosure.

In certain aspects, a composition of the present disclosure includes the PDE4 inhibitor (and optionally, a second anti-epileptic agent) present in a liquid medium. The liquid medium may be an aqueous liquid medium, such as water, a buffered solution, or the like. One or more additives such as a salt (e.g., NaCl, MgCl₂, KCl, MgSO₄), a buffering agent (a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.), a solubilizing agent, a detergent (e.g., a non-ionic detergent such as Tween-20, etc.), a nuclease inhibitor, a protease inhibitor, glycerol, a chelating agent, and the like may be present in such compositions.

Pharmaceutical compositions are also provided. The pharmaceutical compositions of the present disclosure include a PDE4 inhibitor and a pharmaceutically acceptable carrier. Any pharmaceutical composition of the present disclosure may include – in addition to the PDE4 inhibitor – a second anti-epileptic agent. For example, provided are pharmaceutical compositions that include a PDE4 inhibitor and a second anti-epileptic agent. Non-limiting examples of second anti-epileptic agents which may be provided in a pharmaceutical composition of the present disclosure include acetazolamide, diazepam, benzodiazepine, cannabadiol, carbamazepine, clobazam, clonazepam, eslicarbazepine acetate, ethosuximide, ethotoin, felbamate, fenfluramine, fosphenytoin, gabapentin, ganaxolone, huperzine A, lacosamide, lamotrigine, levetiracetam, lorazepam, nitrazepam, oxcarbazepine, perampanel, piracetam, phenobarbital, phenytoin, potassium bromide, pregabalin, primidone, retigabine, rufinamide, sodium valproate, stiripentol, tiagabine, topiramate, valproic acid, vigabatrin, or zonisamide.

The PDE4 inhibitor (and optionally, a second anti-epileptic agent) can be incorporated into a variety of formulations for administration to an individual. More particularly, the PDE4 inhibitor can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable excipients or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, injections, inhalants and aerosols.

Formulations of the PDE4 inhibitor suitable for administration to an individual (e.g., suitable for human administration) are generally sterile and may further be free of detectable pyrogens or other contaminants contraindicated for administration to an individual according to a selected route of administration.

In pharmaceutical dosage forms, the PDE4 inhibitor can be administered alone or in appropriate association, as well as in combination, with a pharmaceutically active compound, e.g., a second anti-epileptic agent. The following methods and carriers/excipients are merely examples and are in no way limiting.

For oral preparations, the PDE4 inhibitor can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

The PDE4 inhibitor can be formulated for parenteral (e.g., intravenous, intra-arterial, intraosseous, intramuscular, intracerebral, intracerebroventricular, intracranial, intrathecal, subcutaneous, etc.) administration. In some embodiments, the PDE4 inhibitor is formulated for oral, parenteral, intranasal, intrathecal, intracranial, intracerebral, intracerebroventricular, or transdermal administration. In some embodiments, the PDE4 inhibitor is formulated for injection by dissolving, suspending or emulsifying the PDE4 inhibitor in an aqueous or non-aqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

Pharmaceutical compositions that include the PDE4 inhibitor may be prepared by mixing the PDE4 inhibitor having the desired degree of purity with optional physiologically acceptable carriers, excipients, stabilizers, surfactants, buffers and/or tonicity agents. Acceptable carriers, excipients and/or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other

organic acids; antioxidants including ascorbic acid, glutathione, cysteine, methionine and citric acid; preservatives (such as ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride, or combinations thereof); amino acids such as arginine, glycine, ornithine, lysine, histidine, glutamic acid, aspartic acid, 5 isoleucine, leucine, alanine, phenylalanine, tyrosine, tryptophan, methionine, serine, proline and combinations thereof; monosaccharides, disaccharides and other carbohydrates; low molecular weight (less than about 10 residues) polypeptides; proteins, such as gelatin or serum albumin; chelating agents such as EDTA; sugars such as trehalose, sucrose, lactose, glucose, mannose, maltose, galactose, fructose, sorbose, raffinose, glucosamine, 10 N-methylglucosamine, galactosamine, and neuraminic acid; and/or non-ionic surfactants such as Tween, Brij Pluronics, Triton-X, or polyethylene glycol (PEG).

The pharmaceutical composition may be in a liquid form, a lyophilized form or a liquid form reconstituted from a lyophilized form, wherein the lyophilized preparation is to be reconstituted with a sterile solution prior to administration. The standard procedure for 15 reconstituting a lyophilized composition is to add back a volume of pure water (typically equivalent to the volume removed during lyophilization); however solutions comprising antibacterial agents may be used for the production of pharmaceutical compositions for parenteral administration.

An aqueous formulation of the PDE4 inhibitor may be prepared in a pH-buffered 20 solution, e.g., at pH ranging from about 4.0 to about 7.0, or from about 5.0 to about 6.0, or alternatively about 5.5. Examples of buffers that are suitable for a pH within this range include phosphate-, histidine-, citrate-, succinate-, acetate-buffers and other organic acid buffers. The buffer concentration can be from about 1 mM to about 100 mM, or from about 5 mM to about 50 mM, depending, e.g., on the buffer and the desired tonicity of the 25 formulation.

A tonicity agent may be included in the formulation to modulate the tonicity of the formulation. Example tonicity agents include sodium chloride, potassium chloride, glycerin and any component from the group of amino acids, sugars as well as combinations thereof. In some embodiments, the aqueous formulation is isotonic, although hypertonic or 30 hypotonic solutions may be suitable. The term "isotonic" denotes a solution having the same tonicity as some other solution with which it is compared, such as physiological salt solution or serum. Tonicity agents may be used in an amount of about 5 mM to about 350 mM, e.g., in an amount of 100 mM to 350 mM.

A surfactant may also be added to the formulation to reduce aggregation and/or 35 minimize the formation of particulates in the formulation and/or reduce adsorption. Example surfactants include polyoxyethylensorbitan fatty acid esters (Tween), polyoxyethylene alkyl

ethers (Brij), alkylphenylpolyoxyethylene ethers (Triton-X), polyoxyethylene-polyoxypropylene copolymer (Poloxamer, Pluronic), and sodium dodecyl sulfate (SDS). Examples of suitable polyoxyethylenesorbitan-fatty acid esters are polysorbate 20, (sold under the trademark Tween 20™) and polysorbate 80 (sold under the trademark Tween 80™). Examples of suitable polyethylene-polypropylene copolymers are those sold under the names Pluronic® F68 or Poloxamer 188™. Examples of suitable Polyoxyethylene alkyl ethers are those sold under the trademark Brij™. Example concentrations of surfactant may range from about 0.001% to about 1% w/v.

A lyoprotectant may also be added in order to protect the PDE4 inhibitor against destabilizing conditions during a lyophilization process. For example, known lyoprotectants include sugars (including glucose and sucrose); polyols (including mannitol, sorbitol and glycerol); and amino acids (including alanine, glycine and glutamic acid). Lyoprotectants can be included in an amount of about 10 mM to 500 nM.

In some embodiments, the pharmaceutical composition includes the PDE4 inhibitor, and one or more of the above-identified agents (e.g., a surfactant, a buffer, a stabilizer, a tonicity agent) and is essentially free of one or more preservatives, such as ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride, and combinations thereof. In other embodiments, a preservative is included in the formulation, e.g., at concentrations ranging from about 0.001 to about 2% (w/v).

KITS

Also provided by the present disclosure are kits. In some embodiments, the kits find use, e.g., in practicing the methods of the present disclosure.

In some embodiments, a kit of the present disclosure includes any of the PDE4 inhibitors described elsewhere herein, including any anti-epileptic agent identified by the methods of identifying anti-epileptic agents of the present disclosure.

In some embodiments, a kit of the present disclosure includes a pharmaceutical composition including a PDE4 inhibitor and a pharmaceutically acceptable carrier. For example, provided are kits that include any of the pharmaceutical compositions of the present disclosure, including any of the pharmaceutical compositions described in the Compositions section hereinabove. In some embodiments, a kit of the present disclosure includes a pharmaceutical composition that – in addition to a PDE4 inhibitor – further includes a second anti-epileptic agent. For example, a kit that finds use in methods of treating epilepsy may include a pharmaceutical composition that includes a PDE4 inhibitor and a second anti-epileptic agent. In some embodiments, a kit of the present disclosure further includes a second anti-epileptic agent provided in a pharmaceutical composition

separate from the pharmaceutical composition comprising the PDE4 inhibitor. Non-limiting examples of second anti-epileptic agents which may be provided in a kit of the present disclosure (in the same or different pharmaceutical composition as the PDE4 inhibitor) include acetazolamide, diazepam, benzodiazepine, cannabidiol, carbamazepine, clobazam, clonazepam, eslicarbazepine acetate, ethosuximide, ethotoin, felbamate, fenfluramine, fosphenytoin, gabapentin, ganaxolone, huperzine A, lacosamide, lamotrigine, levetiracetam, lorazepam, nitrazepam, oxcarbazepine, perampanel, piracetam, phenobarbital, phenytoin, potassium bromide, pregabalin, primidone, retigabine, rufinamide, sodium valproate, stiripentol, tiagabine, topiramate, valproic acid, vigabatrin, or zonisamide.

Kits for practicing the subject methods may include a quantity of the PDE4 inhibitor (and optionally, a second anti-epileptic agent), present in unit dosages, e.g., ampoules, or a multi-dosage format. As such, in certain embodiments, the kits may include one or more (e.g., two or more) unit dosages (e.g., ampoules) of a pharmaceutical composition that includes the PDE4 inhibitor (and optionally, a second anti-epileptic agent) and/or one or more (e.g., two or more) unit dosages (e.g., ampoules) of a pharmaceutical composition that includes a second anti-epileptic agent.

The term "unit dosage", as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of the composition calculated in an amount sufficient to produce the desired effect. The amount of the unit dosage depends on various factors, such as the particular PDE4 inhibitor employed, the effect to be achieved, and the pharmacodynamics associated with the PDE4 inhibitor, in the individual. In yet other embodiments, the kits may include a single multi dosage amount of a composition including the PDE4 inhibitor (and optionally, a second anti-epileptic agent).

Components of the kits may be present in separate containers, or multiple components may be present in a single container. For example, in a kit that includes both a PDE4 inhibitor and a second anti-epileptic agent, the PDE4 inhibitor and the second anti-epileptic agent may be provided in the same composition (e.g., in one or more containers) or may be provided in separate compositions in separate containers. Suitable containers include individual tubes (e.g., vials), ampoules, or the like.

A kit of the present disclosure may further include instructions. For example, a kit that includes a PDE4 inhibitor may include instructions for administering the PDE4 inhibitor to an individual in need thereof. In some embodiments, the instructions include instructions for administering the PDE4 inhibitor to an individual having epilepsy, including one or more of any type of epilepsy described elsewhere herein.

The instructions may be recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or sub-packaging) etc.

5 In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g., portable flash drive, DVD, CD-ROM, diskette, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g. via the internet, are provided. An example of this embodiment is a kit that includes a web address where the
10 instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, the means for obtaining the instructions is recorded on a suitable substrate.

Notwithstanding the appended claims, the present disclosure is also defined by the following embodiments:

1. A method of treating epilepsy, comprising administering to an individual having
15 epilepsy a therapeutically effective amount of a phosphodiesterase 4 (PDE4) inhibitor.
2. The method according to embodiment 1, wherein the PDE4 inhibitor is a small molecule.
3. The method according to embodiment 2, wherein the PDE4 inhibitor is selected from the group consisting of: AN2728, drotaverine, ibudilast, irsogladine, piclamilast, roflumilast,
20 rolipram, theophylline, apremilast, and any combination thereof.
4. The method according to embodiment 2, wherein the PDE4 inhibitor is AN2728.
5. The method according to embodiment 1, wherein the PDE4 inhibitor inhibits one or more of PDE4A, PDE4B, PDE4C, or PDE4D.
6. The method according to embodiment 1, wherein the PDE4 inhibitor exhibits
25 selectivity among PDE4A, PDE4B, PDE4C, and PDE4D.
7. The method according to embodiment 6, wherein the PDE4 inhibitor is selective for PDE4B.
8. The method according to any one of embodiments 1 to 7, wherein the administering is by oral, parenteral, intranasal, intrathecal, intracranial, or transdermal administration.
- 30 9. The method according to embodiment 1, wherein the PDE4 inhibitor inhibits expression of PDE4.
10. The method according to embodiment 9, wherein the PDE4 inhibitor is a nucleic acid-based inhibitor comprising a region complementary to a portion of a messenger RNA (mRNA) that encodes PDE4A, a mRNA that encodes PDE4B, a mRNA that encodes
35 PDE4C, a mRNA that encodes PDE4D, or any combination thereof.

11. The method according to embodiment 10, wherein the nucleic acid-based inhibitor selectively hybridizes to an mRNA that encodes PDE4A, PDE4B, PDE4C, or PDE4D.

12. The method according to embodiment 11, wherein the nucleic acid-based inhibitor selectively hybridizes to an mRNA that encodes PDE4B.

5 13. The method according to any one of embodiments 10 to 12, wherein the nucleic acid-based inhibitor is a morpholino, a short interfering RNA (siRNA), or a microRNA (miRNA).

14. The method according to any one of embodiments 1 to 13, wherein the individual has an epilepsy selected from the group consisting of: benign Rolandic epilepsy, frontal
10 lobe epilepsy, infantile spasms, juvenile myoclonic epilepsy (JME), juvenile absence epilepsy, childhood absence epilepsy, pyknolepsy, febrile seizures, progressive myoclonus epilepsy of Lafora, Lennox-Gastaut syndrome, Landau-Kleffner syndrome, Dravet syndrome (DS), Generalized Epilepsy with Febrile Seizures (GEFS+), Severe Myoclonic Epilepsy of Infancy (SMEI), Benign Neonatal Familial Convulsions (BFNC), West
15 Syndrome, Ohtahara Syndrome, early myoclonic encephalopathy, migrating partial epilepsy, infantile epileptic encephalopathies, Tuberous Sclerosis Complex (TSC), focal cortical dysplasia, Type I Lissencephaly, Miller-Dieker Syndrome, Angelman's syndrome, Fragile X syndrome, epilepsy in autism spectrum disorders, subcortical band heterotopia, Walker-Warburg syndrome, Alzheimer's disease epilepsy, posttraumatic epilepsy,
20 progressive myoclonus epilepsy, reflex epilepsy, Rasmussen's syndrome, temporal lobe epilepsy, limbic epilepsy, status epilepticus, abdominal epilepsy, massive bilateral myoclonus, catamenial epilepsy, Jacksonian seizure disorder, Unverricht-Lundborg disease, and photosensitive epilepsy.

15. The method according to any one of embodiments 1 to 13, wherein the individual
25 has an epilepsy caused by a genetic mutation.

16. A method of identifying an anti-epileptic agent, comprising contacting a phosphodiesterase 4 (PDE4) polypeptide with a candidate agent in a PDE4 activity assay, wherein inhibition of activity of the PDE4 polypeptide by the candidate agent identifies the candidate agent as an anti-epileptic agent.

30 17. The method according to embodiment 16, wherein the contacting comprises combining the PDE4 polypeptide and the candidate agent in a cell-free PDE4 activity assay.

18. The method according to embodiment 16, wherein the contacting comprises combining the PDE4 polypeptide and the candidate agent in a cell-based PDE4 activity assay.

19. The method according to any one of embodiments 16 to 18, wherein the PDE4 activity assay further comprises contacting the PDE4 polypeptide with a positive control agent known to inhibit PDE4 activity.

20. The method according to embodiment 19, wherein the positive control agent is selected from the group consisting of: AN2728, drotaverine, ibudilast, irsogladine, piclamilast, roflumilast, rolipram, theophylline, apremilast, and any combination thereof.

21. The method according to any one of embodiments 16 to 20, wherein the candidate agent is a small molecule.

22. The method according to any one of embodiments 16 to 21, wherein the PDE4 polypeptide is PDE4A, PDE4B, PDE4C, or PDE4D.

23. The method according to embodiment 22, wherein the PDE4 polypeptide is PDE4B.

24. The method according to any one of embodiments 16 to 23, further comprising, when the candidate agent is determined to inhibit activity of the PED4 polypeptide, determining whether the candidate agent exhibits selective inhibition among PDE4A, PDE4B, PDE4C, and PDE4D.

25. A pharmaceutical composition comprising an anti-epileptic agent identified by the method according to any one of embodiments 16 to 24.

26. A method comprising administering to an individual having epilepsy a therapeutically effective amount of anti-epileptic agent identified by the method according to any one of embodiments 16 to 24.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Example 1 – PDE4 inhibition restores bioenergetics to baseline levels in *scn1lab* and *kcna1* mutant zebrafish

Shown in FIG. 6 (panel A) is data demonstrating that an example PDE4 inhibitor (AN2728) dose-dependently restores bioenergetics to baseline levels in *scn1lab* mutant zebrafish (in FIGs. 6-8, “*Scn1a*” mutant zebrafish are *scn1lab* mutant zebrafish). The *scn1lab* mutant zebrafish employed in this Experimental section are homozygous, loss-of-function mutants. To measure bioenergetics in whole, living zebrafish (further details of which may be found in lbhazehiebo et al. (2018) *Brain* 141(3):744–761), zebrafish larvae (5 days post-fertilization, dpf) were seeded into 24-well islet capture microplates (Agilent) and pre-exposed to vehicle or drug for 20 minutes prior to the start of the assay. Oxygen consumption rates (OCR) were measured using the Seahorse Bioanalyzer (Agilent). In this example, *scn1lab* mutant zebrafish larvae displayed depressed OCR relative to wild-type

(WT) control, indicating that mitochondrial respiration is decreased in the *scn1lab* mutant background. In the presence of AN2728, a dose-dependent restoration of bioenergetics was observed, with 1 μ M and 10 μ M fully restoring OCR response to WT levels (FIG. 6, panel A). The 1nM-100nM doses were ineffective and the 100 μ M dose was toxic as demonstrated by the drop in OCR response below that of the mutant levels.

Shown in FIG. 6 (panel B) is data demonstrating that a variety of PDE4 inhibitors (in this example, Rolipram, Cilomilast, Roflumilast, Ibudilast, Theophylline, Drotaverine, and Irsogladine) are effective in restoring bioenergetics to baseline levels in *scn1lab* mutant zebrafish. In a similar assay to that described above, *scn1lab* mutants are exposed to a 40 μ M dose of a range of PDE4 inhibitors. Rolipram, cilomilast, roflumilast, ibudilast all restored OCR response to baseline levels observed in the WT larvae, indicating that inhibition of PDE4 by these drugs reversed the decrease in bioenergetics demonstrated in the *scn1lab* mutant zebrafish when exposed to vehicle.

Shown in FIG. 7 is data demonstrating that PDE4B-, 4C- and 4D-morpholinos are effective in restoring bioenergetics to baseline levels in *scn1lab* mutant zebrafish. *scn1lab* mutant zebrafish embryos were injected at the one cell stage with an ATG- and/or a splice-blocking morpholino (Gene-Tools, Philomath, OR) designed to knockdown specific PDE4 isoforms in the genetic background of an epileptic zebrafish. Due to gene duplication in the zebrafish genome, many genes that have a single ortholog in humans have two isoforms in zebrafish. Morpholinos were designed to block each zebrafish isoform singly. Morpholino-injected *scn1lab* mutant zebrafish were sorted at 24hpf and viable embryos were seeded into islet microplates for bioenergetics assay (Seahorse bioanalyzer) at 5dpf. Morpholinos targeting PDE4Ba, PDE4Bb, PDE4Cb, and PDE4D restored OCR response to WT baseline levels in the epileptic background, indicating that knockdown of PDE4 signaling alleviates the decrease in mitochondrial function observed in the *scn1lab* mutants.

Shown in FIG. 8 is data demonstrating that two example PDE4 inhibitors (Roflumilast and Theophylline) restore bioenergetics in a *kcna1* mutant zebrafish back to baseline levels observed in a wild-type fish. *kcna1* mutants are a model for generalizable epilepsy and also sudden unexpected death in epilepsy (SUDEP), indicating that inhibiting PDE4 is likely to elicit effective anti-seizure properties across epilepsy conditions more broadly.

Example 2 – PDE4 inhibition blocks seizure-like hyperexcitability to baseline levels in *scn1lab* zebrafish

Shown in FIG. 9 is data demonstrating that an example PDE4 inhibitor (AN2728) blocks seizure-like hyperexcitability to baseline levels in *scn1lab* zebrafish. Briefly, 6

dpf zebrafish (WT and *scn1lab* mutants) were paralyzed (α -bungarotoxin, 1mg/ml, Tocris) and embedded in agarose. The dorsal side of the zebrafish was exposed to the agarose gel surface and a glass microelectrode was placed into the tectum opticum of zebrafish and electrophysiological measurements were recorded. After 20 min of baseline recording, drugs (final concentration 20 μ M) were added directly to the embryo media and continued recordings in the same zebrafish were captured over the next 20 min. Care was taken not to disrupt the pipette or move the fish in any manner. Seizure-like activity was defined by high-frequency, large amplitude spikes, as defined elsewhere (Baraban et al, 2013). Both ictal (>1000 ms in duration) and inter-ictal (<300 ms in duration) activity was observed upon zooming in on a section of the EEG trace (data not shown), consistent with epileptiform events. Exposure to PDE inhibitor (AN2728, 40 μ M) blocked this seizure-like activity to baseline levels. Quantification of these hyperexcitable events across multiple *scn1lab* mutant zebrafish (n=6-10) (FIG. 9, panel B) shows a decrease in seizure frequency and peak amplitude in the mutants following treatment with AN2728.

Shown in FIG. 10 is data demonstrating that a PDE4B morpholino is effective in blocking seizure-like hyperexcitability to baseline levels in *scn1lab* zebrafish. As described above, injection of PDE4 isoform-specific morpholinos targeting PDE4Ba and PDE4Bb blocked the hyperexcitable phenotype observed in *scn1lab* mutants. PDE4Ca-, PDE4Cb-, and PDE4D-morpholinos all partially blocked this seizure-like activity, whereas PDE4A-morpholino had no effect and perhaps even exacerbated the hyperexcitable phenotype. Quantification of these hyperexcitable events in *scn1lab* mutant zebrafish (n=6-10) that had been injected with a PDE4 isoform-targeting morpholino shows a decrease in seizure frequency and amplitude for PDE4B-, PDE4C-, and PDE4D-morpholinos when compared to *scn1lab* levels shown in FIG. 9, panel B.

Example 3 – PDE4 inhibition decreases hyperexcitability in mouse *Scn1a* mutant brain *ex vivo* slices

FIG. 11 provides EEG data demonstrating that an example PDE4 inhibitor (AN2728) decreases hyperexcitability in mouse *Scn1a* mutant brain *ex vivo* slices. The mouse *Scn1a* mutants employed in this Experimental section were heterozygous mutants (*Scn1a*^{-/-} mice die early postnatally). As shown, EEG recordings in *Scn1a* mutant mouse *ex vivo* showed that AN2728 decreased a hyperexcitable phenotype (n = 3). In this assay, adult *Scn1a* mutant brains were rapidly removed and sectioned for electrophysiological assays. Here, extracellular field recordings were conducted to measure baseline electrophysiological

activity, prior to the addition of PDE4 inhibitor (AN2728, 40 μ M). A decrease in hyperexcitable amplitude and frequency was measured in the presence of AN2728.

Example 4 – PDE4 inhibition protects against induction of seizures in a 6Hz-induction mouse model

5 Provided in FIG. 12 is data demonstrating that an example PDE4 inhibitor (AN2728) protects against induction of seizures in a 6Hz-induction mouse model. To examine the acute effect of PDE4 inhibition on seizures, the 6Hz psychomotor model that represents therapy-resistant limbic seizures was employed (White *et al.*, 1995; Barton *et al.*, 2001). Briefly, compounds were screened for their ability to block psychomotor seizures induced
10 by a low-frequency (6 Hz), long-duration (3 sec) stimulus delivered through corneal electrodes. These seizures are believed to model partial seizures observed in humans. When the animals were dosed 30 min prior to the 6 Hz test, only 1 animal responded at the highest dose (300 mg/kg). However, when treated with AN2728 two hours prior to the 6 Hz test, three out of four animals responded.

15 Example 5 – PDE4 inhibition protects against hyperthermia-induced seizures in *Scn1a* mutant mice

 As shown in FIG. 13 (panels A and B), an example PDE4 inhibitor (AN2728) protects against hyperthermia-induced seizures in *Scn1a* mutant mice. In this model, a *Scn1a* mutant animal's core temperature is raised using an external heat source and consequently,
20 brain temperature is raised and this leads to hyperthermic seizures. These hyperthermic seizures are thought to represent febrile seizures. In the *Scn1a* mutants, seizures begin around 41°C, whereas in the WT seizures are rarely detected even at temperatures as high as 43°C. Following treatment with a PDE4 inhibitor (AN2728), *Scn1a* mutants are protected against hyperthermia-induced seizures, whereas some animals do not ever experience a
25 seizure (n=3) and the rest only experience a seizure at higher temperatures (e.g., 42°C). FIG12B is the same data graphed differently.

 Shown in FIG. 14 is data demonstrating that a two-fold selective PDE4B inhibitor (rolipram) is effective in protecting against hyperthermia-induced seizures in *Scn1a* mutant mice. Using the hyperthermia-induction assay described above, treatment of *Scn1a* mutant
30 mice with a different PDE4B inhibitor (rolipram, 3 mg/kg) was also neuroprotective against hyperthermia-induced seizures, raising not only the temperature required to induce a seizure (panel A) but also the severity of the seizure from mostly 4-5 level seizure to level 3, as measured using the Racine scale (panel B).

Shown in FIG. 15 is data comparing the effects of three example PDE4 inhibitors (AN2728, Rolipram, Roflumilast) in blocking seizures using the mouse *Scn1a* hyperthermia model described above. A partial block in seizures is noted when the temperature required to induce a seizure is statistically different than wildtype and a full block in seizures occurs
5 when no seizure is elicited following pre-treatment with the PDE4 inhibitor.

Accordingly, the preceding merely illustrates the principles of the present disclosure. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and
10 conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are
15 intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein.

WHAT IS CLAIMED IS:

1. A method of treating epilepsy, comprising administering to an individual having epilepsy a therapeutically effective amount of a phosphodiesterase 4 (PDE4) inhibitor.

5 2. The method according to claim 1, wherein the PDE4 inhibitor is a small molecule.

3. The method according to claim 2, wherein the PDE4 inhibitor is selected from the group consisting of: AN2728, drotaverine, ibudilast, irsogladine, piclamilast, roflumilast, rolipram, theophylline, apremilast, and any combination thereof.

10

4. The method according to claim 2, wherein the PDE4 inhibitor is AN2728.

5. The method according to claim 1, wherein the PDE4 inhibitor inhibits one or more of PDE4A, PDE4B, PDE4C, or PDE4D.

15

6. The method according to claim 1, wherein the PDE4 inhibitor exhibits selectivity among PDE4A, PDE4B, PDE4C, and PDE4D.

7. The method according to claim 6, wherein the PDE4 inhibitor is selective for
20 PDE4B.

8. The method according to any one of claims 1 to 7, wherein the administering is by oral, parenteral, intranasal, intrathecal, intracranial, or transdermal administration.

25 9. The method according to claim 1, wherein the PDE4 inhibitor inhibits expression of PDE4.

10. The method according to claim 9, wherein the PDE4 inhibitor is a nucleic acid-based inhibitor comprising a region complementary to a portion of a messenger RNA (mRNA) that encodes PDE4A, a mRNA that encodes PDE4B, a mRNA that encodes PDE4C, a mRNA that encodes PDE4D, or any combination thereof.

30

11. The method according to claim 10, wherein the nucleic acid-based inhibitor selectively hybridizes to an mRNA that encodes PDE4A, PDE4B, PDE4C, or PDE4D.

35

12. The method according to claim 11, wherein the nucleic acid-based inhibitor selectively hybridizes to an mRNA that encodes PDE4B.

13. The method according to any one of claims 10 to 12, wherein the nucleic acid-based inhibitor is a morpholino, a short interfering RNA (siRNA), or a microRNA (miRNA).

14. The method according to any one of claims 1 to 13, wherein the individual has an epilepsy selected from the group consisting of: benign Rolandic epilepsy, frontal lobe epilepsy, infantile spasms, juvenile myoclonic epilepsy (JME), juvenile absence epilepsy, childhood absence epilepsy, pyknolepsy, febrile seizures, progressive myoclonus epilepsy of Lafora, Lennox-Gastaut syndrome, Landau-Kleffner syndrome, Dravet syndrome (DS), Generalized Epilepsy with Febrile Seizures (GEFS+), Severe Myoclonic Epilepsy of Infancy (SMEI), Benign Neonatal Familial Convulsions (BFNC), West Syndrome, Ohtahara Syndrome, early myoclonic encephalopathy, migrating partial epilepsy, infantile epileptic encephalopathies, Tuberous Sclerosis Complex (TSC), focal cortical dysplasia, Type I Lissencephaly, Miller-Dieker Syndrome, Angelman's syndrome, Fragile X syndrome, epilepsy in autism spectrum disorders, subcortical band heterotopia, Walker-Warburg syndrome, Alzheimer's disease epilepsy, posttraumatic epilepsy, progressive myoclonus epilepsy, reflex epilepsy, Rasmussen's syndrome, temporal lobe epilepsy, limbic epilepsy, status epilepticus, abdominal epilepsy, massive bilateral myoclonus, catamenial epilepsy, Jacksonian seizure disorder, Unverricht-Lundborg disease, and photosensitive epilepsy.

15. The method according to any one of claims 1 to 13, wherein the individual has an epilepsy caused by a genetic mutation.

16. A method of identifying an anti-epileptic agent, comprising contacting a phosphodiesterase 4 (PDE4) polypeptide with a candidate agent in a PDE4 activity assay, wherein inhibition of activity of the PDE4 polypeptide by the candidate agent identifies the candidate agent as an anti-epileptic agent.

17. The method according to claim 16, wherein the contacting comprises combining the PDE4 polypeptide and the candidate agent in a cell-free PDE4 activity assay.

18. The method according to claim 16, wherein the contacting comprises combining the PDE4 polypeptide and the candidate agent in a cell-based PDE4 activity assay.

19. The method according to any one of claims 16 to 18, wherein the PDE4 activity assay further comprises contacting the PDE4 polypeptide with a positive control agent known to inhibit PDE4 activity.
- 5 20. The method according to claim 19, wherein the positive control agent is selected from the group consisting of: AN2728, drotaverine, ibudilast, irsogladine, piclamilast, roflumilast, rolipram, theophylline, apremilast, and any combination thereof.
- 10 21. The method according to any one of claims 16 to 20, wherein the candidate agent is a small molecule.
22. The method according to any one of claims 16 to 21, wherein the PDE4 polypeptide is PDE4A, PDE4B, PDE4C, or PDE4D.
- 15 23. The method according to claim 22, wherein the PDE4 polypeptide is PDE4B.
24. The method according to any one of claims 16 to 23, further comprising, when the candidate agent is determined to inhibit activity of the PED4 polypeptide, determining whether the candidate agent exhibits selective inhibition among PDE4A, PDE4B, PDE4C, and PDE4D.
- 20 25. A pharmaceutical composition comprising an anti-epileptic agent identified by the method according to any one of claims 16 to 24.
- 25 26. A method comprising administering to an individual having epilepsy a therapeutically effective amount of anti-epileptic agent identified by the method according to any one of claims 16 to 24.

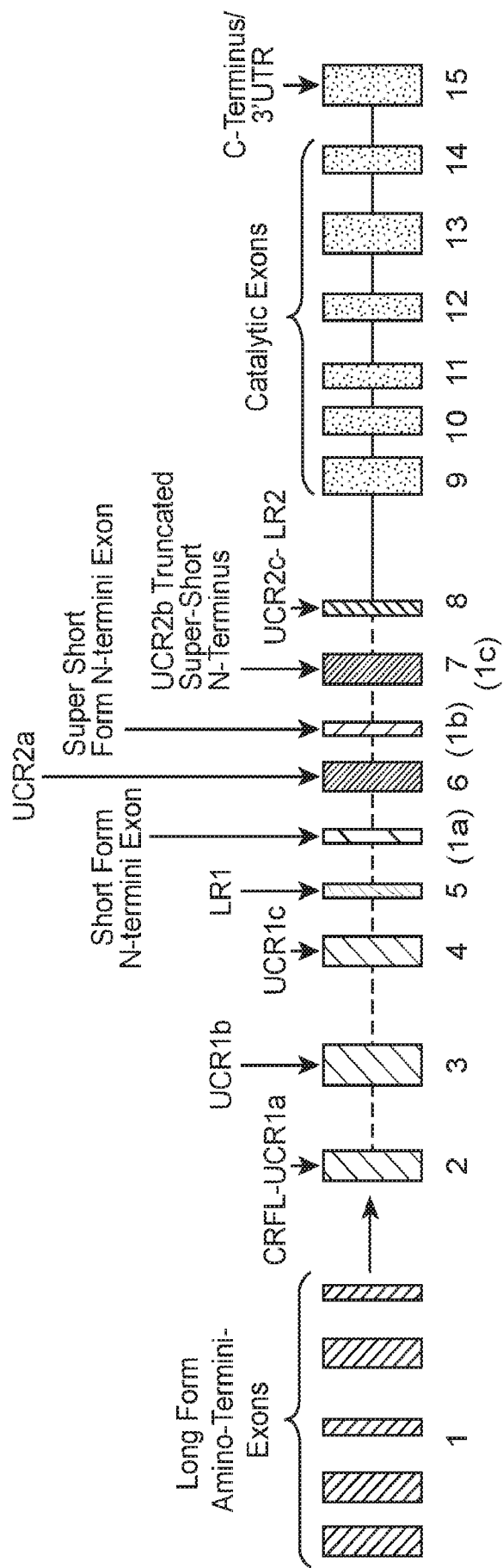


FIG. 1

P27815	PDE4A_HUMAN	1	MEPPTVPSERSLSLPGPREGQATLKPPPHQLWRQPRTP----	IRI--QQRGYSDSAE	53
P27815-2	PDE4A_HUMAN	1	-----MA-----	-----RPRGLGRIPELQLVAFPVAAAEDEAFLPEPL	34
P27815-3	PDE4A_HUMAN	1	-----	-----	0
P27815-4	PDE4A_HUMAN	1	-----	-----	0
P27815-5	PDE4A_HUMAN	1	-----	-----	0
P27815-6	PDE4A_HUMAN	1	-----	-----MRSQA	5
P27815-7	PDE4A_HUMAN	1	-----MKRS--RSALSVAG-----	-----TGDESRSE	20
P27815	PDE4A_HUMAN	54	RAERE-----RQPHRPIERADAM-----	-----DTSDRPGRLTTRMSWPSSFHGTGTGSGGA	100
P27815-2	PDE4A_HUMAN	35	APRAP-----RRPRSPSS-PVF-----	-----FASPSPTFRR-RLRLLRSCQ-----DLGR	74
P27815-3	PDE4A_HUMAN	1	-----	-----	0
P27815-4	PDE4A_HUMAN	1	-----	-----	0
P27815-5	PDE4A_HUMAN	1	-----	-----	0
P27815-6	PDE4A_HUMAN	6	APRA-----RPRPPA-----L-----ALPPT-----	-----GPESLTHFPFSDIEDTRRH	39
P27815-7	PDE4A_HUMAN	21	TPESDRANMLGADLRAPRRRLSSGPGLGMAQPEPSDGPV--	-----LPPRPPTLLPLIPPRISI	78
P27815	PDE4A_HUMAN	101	GGGSSRRFEAENGPTPSPGRSPLDSQASPGVLHAGAATSQRRESFLYRSDSDYDMSPKT		160
P27815-2	PDE4A_HUMAN	75	QAMAGAGFEAENGPTPSPGRSPLDSQASPGVLHAGAATSQRRESFLYRSDSDYDMSPKT		134
P27815-3	PDE4A_HUMAN	1	-----	-----	0
P27815-4	PDE4A_HUMAN	1	-----	-----	0
P27815-5	PDE4A_HUMAN	1	-----	-----	0
P27815-6	PDE4A_HUMAN	40	PPGRSVSFEAENGPTPSPGRSPLDSQASPGVLHAGAATSQRRESFLYRSDSDYDMSPKT		99
P27815-7	PDE4A_HUMAN	79	TRAENDSFEAENGPTPSPGRSPLDSQASPGVLHAGAATSQRRESFLYRSDSDYDMSPKT		138
P27815	PDE4A_HUMAN	161	MSRNSSVTSEAHAEELIVTPFAQVLASLSRVSRSNFSLLTNVPVPSNKRSPLOGGPTPVCKA		220
P27815-2	PDE4A_HUMAN	135	MSRNSSVTSEAHAEELIVTPFAQVLASLSRVSRSNFSLLTNVPVPSNKRSPLOGGPTPVCKA		194
P27815-3	PDE4A_HUMAN	1	-----	-----MCPFP-VTTVPLGGPTPVCKA	20
P27815-4	PDE4A_HUMAN	1	-----	-----M	1
P27815-5	PDE4A_HUMAN	1	-----	-----	0
P27815-6	PDE4A_HUMAN	100	MSRNSSVTSEAHAEELIVTPFAQVLASLSRVSRSNFSLLTNVPVPSNKRSPLOGGPTPVCKA		159
P27815-7	PDE4A_HUMAN	139	MSRNSSVTSEAHAEELIVTPFAQVLASLSRVSRSNFSLLTNVPVPSNKRSPLOGGPTPVCKA		198

FIG. 2

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P27815 PDE4A_HUMAN 221 TLSEETCQQLARETLEELDWCLEQLEETMQTYRSVSEMAASHKFKRMLNRELTHLSEMSRSG 280
P27815-2 PDE4A_HUMAN 195 TLSEETCQQLARETLEELDWCLEQLEETMQTYRSVSEMAASHKFKRMLNRELTHLSEMSRSG 254
P27815-3 PDE4A_HUMAN 21 TLSEETCQQLARETLEELDWCLEQLEETMQTYRSVSEMAASHKFKRMLNRELTHLSEMSRSG 80
P27815-4 PDE4A_HUMAN 2 PLVDFFCETCS-----KPWL-----VGMMDQFKRMLNRELTHLSEMSRSG 41
P27815-5 PDE4A_HUMAN 1 ----- 0
P27815-6 PDE4A_HUMAN 160 TLSEETCQQLARETLEELDWCLEQLEETMQTYRSVSEMAASHKFKRMLNRELTHLSEMSRSG 219
P27815-7 PDE4A_HUMAN 199 TLSEETCQQLARETLEELDWCLEQLEETMQTYRSVSEMAASHKFKRMLNRELTHLSEMSRSG 258

P27815 PDE4A_HUMAN 281 NOVSEYISTTFLDKQNEVEIPSPMTMKEREKQQAAPRPRPSQPPPPVPHLQPMQSQITGLKK 340
P27815-2 PDE4A_HUMAN 255 NOVSEYISTTFLDKQNEVEIPSPMTMKEREKQQAAPRPRPSQPPPPVPHLQPMQSQITGLKK 314
P27815-3 PDE4A_HUMAN 81 NOVSEYISTTFLDKQNEVEIPSPMTMKEREKQQAAPRPRPSQPPPPVPHLQPMQSQITGLKK 140
P27815-4 PDE4A_HUMAN 42 NOVSEYISTTFLDKQNEVEIPSPMTMKEREKQQAAPRPRPSQPPPPVPHLQPMQSQITGLKK 181
P27815-5 PDE4A_HUMAN 1 -----NVLP--SDQGFKL 11
P27815-6 PDE4A_HUMAN 220 NOVSEYISTTFLDKQNEVEIPSPMTMKEREKQQAAPRPRPSQPPPPVPHLQPMQSQITGLKK 279
P27815-7 PDE4A_HUMAN 259 NOVSEYISTTFLDKQNEVEIPSPMTMKEREKQQAAPRPRPSQPPPPVPHLQPMQSQITGLKK 318
      * * * * * ; * * * * *

P27815 PDE4A_HUMAN 341 LMHSNSLNNSNIPRFGVKTDQEEELLAQELENLNKMGLNIFCVSDYAGGRSLTCIMYMIQ 400
P27815-2 PDE4A_HUMAN 315 LMHSNSLNNSNIPRFGVKTDQEEELLAQELENLNKMGLNIFCVSDYAGGRSLTCIMYMIQ 374
P27815-3 PDE4A_HUMAN 141 LMHSNSLNNSNIPRFGVKTDQEEELLAQELENLNKMGLNIFCVSDYAGGRSLTCIMYMIQ 200
P27815-4 PDE4A_HUMAN 102 LMHSNSLNNSNIPRFGVKTDQEEELLAQELENLNKMGLNIFCVSDYAGGRSLTCIMYMIQ 161
P27815-5 PDE4A_HUMAN 12 L--GNVLQGP--PYR--LLTSGRLHQELENLNKMGLNIFCVSDYAGGRSLTCIMYMIQ 66
P27815-6 PDE4A_HUMAN 280 LMHSNSLNNSNIPRFGVKTDQEEELLAQELENLNKMGLNIFCVSDYAGGRSLTCIMYMIQ 339
P27815-7 PDE4A_HUMAN 319 LMHSNSLNNSNIPRFGVKTDQEEELLAQELENLNKMGLNIFCVSDYAGGRSLTCIMYMIQ 378
      * * * * * ; * * * * *

P27815 PDE4A_HUMAN 401 ERDLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLSQTHVLLATPALDAVFTD 460
P27815-2 PDE4A_HUMAN 375 ERDLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLSQTHVLLATPALDAVFTD 434
P27815-3 PDE4A_HUMAN 201 ERDLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLSQTHVLLATPALDAVFTD 260
P27815-4 PDE4A_HUMAN 162 ERDLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLSQTHVLLATPALDAVFTD 221
P27815-5 PDE4A_HUMAN 67 ERDLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLSQTHVLLATPALDAVFTD 126
P27815-6 PDE4A_HUMAN 340 ERDLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLSQTHVLLATPALDAVFTD 399
P27815-7 PDE4A_HUMAN 379 ERDLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLSQTHVLLATPALDAVFTD 438
      * * * * * ; * * * * *

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FIG. 2 (Cont.)

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P27815 PDE4A_HUMAN 461 LEILAAALFAAAIHVDVHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEDNCD 520
P27815-2 PDE4A_HUMAN 435 LEILAAALFAAAIHVDVHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEDNCD 494
P27815-3 PDE4A_HUMAN 261 LEILAAALFAAAIHVDVHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEDNCD 320
P27815-4 PDE4A_HUMAN 222 LEILAAALFAAAIHVDVHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEDNCD 281
P27815-5 PDE4A_HUMAN 127 LEILAAALFAAAIHVDVHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEDNCD 186
P27815-6 PDE4A_HUMAN 400 LEILAAALFAAAIHVDVHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEDNCD 459
P27815-7 PDE4A_HUMAN 439 LEILAAALFAAAIHVDVHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEDNCD 498
*****

P27815 PDE4A_HUMAN 521 IFQNLKRQRQSLRKMVIDMVLATDMSKHMNTLLADLKTIMVETKKVTSSGVLLLDNYSMRI 580
P27815-2 PDE4A_HUMAN 495 IFQNLKRQRQSLRKMVIDMVLATDMSKHMNTLLADLKTIMVETKKVTSSGVLLLDNYSMRI 554
P27815-3 PDE4A_HUMAN 321 IFQNLKRQRQSLRKMVIDMVLATDMSKHMNTLLADLKTIMVETKKVTSSGVLLLDNYSMRI 380
P27815-4 PDE4A_HUMAN 282 IFQNLKRQRQSLRKMVIDMVLATDMSKHMNTLLADLKTIMVETKKVTSSGVLLLDNYSMRI 341
P27815-5 PDE4A_HUMAN 187 IFQNLKRQRQSLRKMVIDMVLATDMSKHMNTLLADLKTIMVETKKVTSSGVLLLDNYSMRI 246
P27815-6 PDE4A_HUMAN 460 IFQNLKRQRQSLRKMVIDMVLATDMSKHMNTLLADLKTIMVETKKVTSSGVLLLDNYSMRI 519
P27815-7 PDE4A_HUMAN 499 IFQNLKRQRQSLRKMVIDMVLATDMSKHMNTLLADLKTIMVETKKVTSSGVLLLDNYSMRI 558
*****

P27815 PDE4A_HUMAN 581 QVLRNMVHCADLSNPTKPLELYRQWTDRIIMAEFFQGGDRERERGERMEISPMCDKHTASVEK 640
P27815-2 PDE4A_HUMAN 555 QVLRNMVHCADLSNPTKPLELYRQWTDRIIMAEFFQGGDRERERGERMEISPMCDKHTASVEK 614
P27815-3 PDE4A_HUMAN 381 QVLRNMVHCADLSNPTKPLELYRQWTDRIIMAEFFQGGDRERERGERMEISPMCDKHTASVEK 440
P27815-4 PDE4A_HUMAN 342 QVLRNMVHCADLSNPTKPLELYRQWTDRIIMAEFFQGGDRERERGERMEISPMCDKHTASVEK 401
P27815-5 PDE4A_HUMAN 247 QVLRNMVHCADLSNPTKPLELYRQWTDRIIMAEFFQGGDRERERGERMEISPMCDKHTASVEK 306
P27815-6 PDE4A_HUMAN 520 QVLRNMVHCADLSNPTKPLELYRQWTDRIIMAEFFQGGDRERERGERMEISPMCDKHTASVEK 579
P27815-7 PDE4A_HUMAN 559 QVLRNMVHCADLSNPTKPLELYRQWTDRIIMAEFFQGGDRERERGERMEISPMCDKHTASVEK 618
*****

P27815 PDE4A_HUMAN 641 SQVGFIDYIVHPLWETWADLVHPDAQEILTLEDNRDWMYSAIRQSPSPPEEESRGPGR 700
P27815-2 PDE4A_HUMAN 615 SQVGFIDYIVHPLWETWADLVHPDAQEILTLEDNRDWMYSAIRQSPSPPEEESRGPGR 674
P27815-3 PDE4A_HUMAN 441 SQVGFIDYIVHPLWETWADLVHPDAQEILTLEDNRDWMYSAIRQSPSPPEEESRGPGR 500
P27815-4 PDE4A_HUMAN 402 SQVGFIDYIVHPLWETWADLVHPDAQEILTLEDNRDWMYSAIRQSPSPPEEESRGPGR 461
P27815-5 PDE4A_HUMAN 307 SQVQARGIDGRAQGGFY----- 323
P27815-6 PDE4A_HUMAN 580 SQVGFIDYIVHPLWETWADLVHPDAQEILTLEDNRDWMYSAIRQSPSPPEEESRGPGR 639
P27815-7 PDE4A_HUMAN 619 SQVGFIDYIVHPLWETWADLVHPDAQEILTLEDNRDWMYSAIRQSPSPPEEESRGPGR 678
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FIG. 2 (Cont.)

P27815	PDE4A_HUMAN	701	PPLPKFQFELTLEEEEEIIISMAQIPCTAQEALTAQGLSGVEEALDATTIAWEASPAQES	760
P27815-2	PDE4A_HUMAN	675	PPLPKFQFELTLEEEEEIIISMAQIPCTAQEALTAQGLSGVEEALDATTIAWEASPAQES	734
P27815-3	PDE4A_HUMAN	501	PPLPKFQFELTLEEEEEIIISMAQIPCTAQEALTAQGLSGVEEALDATTIAWEASPAQES	560
P27815-4	PDE4A_HUMAN	462	PPLPKFQFELTLEEEEEIIISMAQIPCTAQEALTAQGLSGVEEALDATTIAWEASPAQES	521
P27815-5	PDE4A_HUMAN	324	-----	323
P27815-6	PDE4A_HUMAN	640	PPLPKFQFELTLEEEEEIIISMAQIPCTAQEALTAQGLSGVEEALDATTIAWEASPAQES	699
P27815-7	PDE4A_HUMAN	679	PPLPKFQFELTLEEEEEIIISMAQIPCTAQEALTAQGLSGVEEALDATTIAWEASPAQES	738
P27815	PDE4A_HUMAN	761	LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVAVSHSSPSALALQSPL	820
P27815-2	PDE4A_HUMAN	735	LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVAVSHSSPSALALQSPL	794
P27815-3	PDE4A_HUMAN	561	LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVAVSHSSPSALALQSPL	620
P27815-4	PDE4A_HUMAN	522	LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVAVSHSSPSALALQSPL	581
P27815-5	PDE4A_HUMAN	324	-----	323
P27815-6	PDE4A_HUMAN	700	LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVAVSHSSPSALALQSPL	759
P27815-7	PDE4A_HUMAN	739	LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVAVSHSSPSALALQSPL	798
P27815	PDE4A_HUMAN	821	LPAWRTLVSSEHAPGLPGLPSTAAEVEAQREHQAAKRAACSACAGTFFGEDTSALPAPGGGG	880
P27815-2	PDE4A_HUMAN	795	LPAWRTLVSSEHAPGLPGLPSTAAEVEAQREHQAAKRAACSACAGTFFGEDTSALPAPGGGG	854
P27815-3	PDE4A_HUMAN	621	LPAWRTLVSSEHAPGLPGLPSTAAEVEAQREHQAAKRAACSACAGTFFGEDTSALPAPGGGG	680
P27815-4	PDE4A_HUMAN	582	LPAWRTLVSSEHAPGLPGLPSTAAEVEAQREHQAAKRAACSACAGTFFGEDTSALPAPGGGG	641
P27815-5	PDE4A_HUMAN	324	-----	323
P27815-6	PDE4A_HUMAN	760	LPAWRTLVSSEHAPGLPGLPSTAAEVEAQREHQAAKRAACSACAGTFFGEDTSALPAPGGGG	819
P27815-7	PDE4A_HUMAN	799	LPAWRTLVSSEHAPGLPGLPSTAAEVEAQREHQAAKRAACSACAGTFFGEDTSALPAPGGGG	858
P27815	PDE4A_HUMAN	881	SGGDPT	886
P27815-2	PDE4A_HUMAN	855	SGGDPT	860
P27815-3	PDE4A_HUMAN	681	SGGDPT	686
P27815-4	PDE4A_HUMAN	642	SGGDPT	647
P27815-5	PDE4A_HUMAN	324	-----	323
P27815-6	PDE4A_HUMAN	820	SGGDPT	825
P27815-7	PDE4A_HUMAN	859	SGGDPT	864

FIG. 2 (Cont.)

Q07343	PDE4B_HUMAN	1	KKKRSVMTVMADDNVKDYFECSLSKSYSSSNTLGIDLWRGRRCCSGNLQLPPLSQRQS	60
Q07343-2	PDE4B_HUMAN	1	-----	0
Q07343-3	PDE4B_HUMAN	1	-----MTAKDSS-----KELTASEPEVCIKTFKEQM--HLELELPRLPGNR--	39
Q07343-4	PDE4B_HUMAN	1	-----	0
Q07343	PDE4B_HUMAN	61	ERARTPEGDGISRPTT-----LPLITLPSIAITTVSQECFDVENGPSPGRSPLDPQA	112
Q07343-2	PDE4B_HUMAN	1	-----	0
Q07343-3	PDE4B_HUMAN	40	--PTSPKISPRSSPRNSPCFFRKLLVNKSIRQRRRFTVAHTCFDVENGPSPGRSPLDPQA	97
Q07343-4	PDE4B_HUMAN	1	-----	0
Q07343	PDE4B_HUMAN	113	SSSAGLVLHATFPGHSQRRESFLYRSDSDYDLSPKAMSRNSSLPSEQHGDDLIVTPFAQV	172
Q07343-2	PDE4B_HUMAN	1	-----MKEHGG-----	6
Q07343-3	PDE4B_HUMAN	98	SSSAGLVLHATFPGHSQRRESFLYRSDSDYDLSPKAMSRNSSLPSEQHGDDLIVTPFAQV	157
Q07343-4	PDE4B_HUMAN	1	-----	0
Q07343	PDE4B_HUMAN	173	LASLRSVRNNTILTNLHGTSNKRSPAASQ---PP-VSRVNPQEESYQKLAMETLEELD	228
Q07343-2	PDE4B_HUMAN	7	-----TFSS-TGISGGSDSAMDLSQPLQPNYMPVCLFAEESYQKLAMETLEELD	56
Q07343-3	PDE4B_HUMAN	158	LASLRSVRNNTILTNLHGTSNKRSPAASQPP---VSRVNPQEESYQKLAMETLEELD	213
Q07343-4	PDE4B_HUMAN	1	-----	0

FIG. 3

Q07343	PDE4B_HUMAN	229	CLDQLETIQTYRSVSEMASNKFKRMLNRELTHLSEMSRSGNQVSEYISNTFLDKQNDVEI	288
Q07343-2	PDE4B_HUMAN	57	CLDQLETIQTYRSVSEMASNKFKRMLNRELTHLSEMSRSGNQVSEYISNTFLDKQNDVEI	116
Q07343-3	PDE4B_HUMAN	214	CLDQLETIQTYRSVSEMASNKFKRMLNRELTHLSEMSRSGNQVSEYISNTFLDKQNDVEI	273
Q07343-4	PDE4B_HUMAN	1	-----MPEANYLLSVSMGYIKFKRMLNRELTHLSEMSRSGNQVSEYISNTFLDKQNDVEI	55
			* . , *****	
Q07343	PDE4B_HUMAN	289	PSPTQKDREKKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNTENEDHLAKELEDLNKW	348
Q07343-2	PDE4B_HUMAN	117	PSPTQKDREKKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNTENEDHLAKELEDLNKW	176
Q07343-3	PDE4B_HUMAN	274	PSPTQKDREKKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNTENEDHLAKELEDLNKW	333
Q07343-4	PDE4B_HUMAN	56	PSPTQKDREKKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNTENEDHLAKELEDLNKW	115

Q07343	PDE4B_HUMAN	349	GLNIFNVAGYSHNRPLTCIMYAIQERDLLKTRISSDIFITYMMTLEDHYHSDVAYHNS	408
Q07343-2	PDE4B_HUMAN	177	GLNIFNVAGYSHNRPLTCIMYAIQERDLLKTRISSDIFITYMMTLEDHYHSDVAYHNS	236
Q07343-3	PDE4B_HUMAN	334	GLNIFNVAGYSHNRPLTCIMYAIQERDLLKTRISSDIFITYMMTLEDHYHSDVAYHNS	393
Q07343-4	PDE4B_HUMAN	116	GLNIFNVAGYSHNRPLTCIMYAIQERDLLKTRISSDIFITYMMTLEDHYHSDVAYHNS	175

Q07343	PDE4B_HUMAN	409	LHAADVAQSTHVLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTINSELALM	468
Q07343-2	PDE4B_HUMAN	237	LHAADVAQSTHVLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTINSELALM	296
Q07343-3	PDE4B_HUMAN	394	LHAADVAQSTHVLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTINSELALM	453
Q07343-4	PDE4B_HUMAN	176	LHAADVAQSTHVLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTINSELALM	235

FIG. 3 (Cont.)

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Q07343    PDE4B_HUMAN    469  YNDESVLNHHHLAVGFKLLQEEHCDIFMNLTKKQRTLRKMVIDMVLATDMSKHMSSLAD
Q07343-2  PDE4B_HUMAN    297  YNDESVLNHHHLAVGFKLLQEEHCDIFMNLTKKQRTLRKMVIDMVLATDMSKHMSSLAD
Q07343-3  PDE4B_HUMAN    454  YNDESVLNHHHLAVGFKLLQEEHCDIFMNLTKKQRTLRKMVIDMVLATDMSKHMSSLAD
Q07343-4  PDE4B_HUMAN    236  YNDESVLNHHHLAVGFKLLQEEHCDIFMNLTKKQRTLRKMVIDMVLATDMSKHMSSLAD
*****

Q07343    PDE4B_HUMAN    529  LKTMVETKKVTSSGVLLLDNYTDRIQVLRNMVHCADLSNPTKSLEYRQWTDRIIMEEFFQ
Q07343-2  PDE4B_HUMAN    357  LKTMVETKKVTSSGVLLLDNYTDRIQVLRNMVHCADLSNPTKSLEYRQWTDRIIMEEFFQ
Q07343-3  PDE4B_HUMAN    514  LKTMVETKKVTSSGVLLLDNYTDRIQVLRNMVHCADLSNPTKSLEYRQWTDRIIMEEFFQ
Q07343-4  PDE4B_HUMAN    296  LKTMVETKKVTSSGVLLLDNYTDRIQVLRNMVHCADLSNPTKSLEYRQWTDRIIMEEFFQ
*****

Q07343    PDE4B_HUMAN    589  QGDKERERGMIEISPMCDKHTASVEKSQVGFIDYIVHPLWETWADLVQPDAAQDILDITLEDN
Q07343-2  PDE4B_HUMAN    417  QGDKERERGMIEISPMCDKHTASVEKSQVGFIDYIVHPLWETWADLVQPDAAQDILDITLEDN
Q07343-3  PDE4B_HUMAN    574  QGDKERERGMIEISPMCDKHTASVEKSQVGFIDYIVHPLWETWADLVQPDAAQDILDITLEDN
Q07343-4  PDE4B_HUMAN    356  QGDKERERGMIEISPMCDKHTASVEKSQVGFIDYIVHPLWETWADLVQPDAAQDILDITLEDN
*****

Q07343    PDE4B_HUMAN    649  RNWYQSMIPQSPSPPLDEQNRDCQGLMEKFQFELTLDDEEDSEGPEKEGEGHSYFSSIKTL
Q07343-2  PDE4B_HUMAN    477  RNWYQSMIPQSPSPPLDEQNRDCQGLMEKFQFELTLDDEEDSEGPEKEGEGHSYFSSIKTL
Q07343-3  PDE4B_HUMAN    634  RNWYQSMIPQSPSPPLDEQNRDCQGLMEKFQFELTLDDEEDSEGPEKEGEGHSYFSSIKTL
Q07343-4  PDE4B_HUMAN    416  RNWYQSMIPQSPSPPLDEQNRDCQGLMEKFQFELTLDDEEDSEGPEKEGEGHSYFSSIKTL
*****

Q07343    PDE4B_HUMAN    709  CVIDPENRDSLGETIDIDIATEDKSPVDI
Q07343-2  PDE4B_HUMAN    537  CVIDPENRDSLGETIDIDIATEDKSPVDI
Q07343-3  PDE4B_HUMAN    694  CVIDPENRDSLGETIDIDIATEDKSPVDI
Q07343-4  PDE4B_HUMAN    476  CVIDPENRDSLGETIDIDIATEDKSPVDI
*****

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FIG. 3 (Cont.)

Q08493	PDE4C_HUMAN	1	MENLGVGEAEACSLRSRGRHSMTRAPKHLWRQRRP	IRIQ--QRFYSDPKSAGCRE	58
Q08493-2	PDE4C_HUMAN	1	-----	-----	0
Q08493-3	PDE4C_HUMAN	1	-----	-----MOGPPAPAPVPGSGSPRGSPGLFR	28
Q08493	PDE4C_HUMAN	59	RDLSPRPELRKSLSWPVSSCRRFDLENGLSGRRALDPQSSPGLGRIMQAPVPHSQRRRE		118
Q08493-2	PDE4C_HUMAN	1	-----	-----MQAPVPHSQRRRE	12
Q08493-3	PDE4C_HUMAN	29	KLLV-NQSI-RLQRRFTVAHPLCFDLENGLSGRRALDPQSSPGLGRIMQAPVPHSQRRRE		86

Q08493	PDE4C_HUMAN	119	SFLYRSDSDYELSPKAMSRNSSVASDLHGEDI	MIVTPFAQVLASLRTVRSNVAALARQQCL	178
Q08493-2	PDE4C_HUMAN	13	SFLYRSDSDYELSPKAMSRNSSVASDLHGEDI	MIVTPFAQVLASLRTVRSNVAALARQQCL	72
Q08493-3	PDE4C_HUMAN	87	SFLYRSDSDYELSPKAMSRNSSVASDLHGEDI	MIVTPFAQVLASLRTVRSNVAALARQQCL	146

Q08493	PDE4C_HUMAN	179	GAAKQGPVGNPSSSNQLPPAEDTGQKLALETLD	ELDWCLDQLETLQTRHSGEMASNKFK	238
Q08493-2	PDE4C_HUMAN	73	GAAKQGPVGNPSSSNQLPPAEDTGQKLALETLD	ELDWCLDQLETLQTRHSGEMASNKFK	132
Q08493-3	PDE4C_HUMAN	147	GAAKQGPVGNPSSSNQLPPAEDTGQKLALETLD	ELDWCLDQLETLQTRHSGEMASNKFK	206

Q08493	PDE4C_HUMAN	239	RIILNRELTHLSETSRSGNQVSEYISRTFLDQQTE	VELPKVTAEAPQPMRSISGLHGLCH	298
Q08493-2	PDE4C_HUMAN	133	RIILNRELTHLSETSRSGNQVSEYISRTFLDQQTE	VELPKVTAEAPQPMRSISGLHGLCH	192
Q08493-3	PDE4C_HUMAN	207	RIILNRELTHLSETSRSGNQVSEYISRTFLDQQTE	VELPKVTAEAPQPMRSISGLHGLCH	266

FIG. 4

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Q08493   PDE4C_HUMAN   299   SASLSSATVPRFGVQIDQEEQLAKELEDITNKWGLDVFKVAELSGNRPLTAIIFSIFQERD   358
Q08493-2 PDE4C_HUMAN   193   SASLSSATVPRFGVQIDQEEQLAKELEDITNKWGLDVFKVAELSGNRPLTAIIFSIFQERD   252
Q08493-3 PDE4C_HUMAN   267   SASLSSATVPRFGVQIDQEEQLAKELEDITNKWGLDVFKVAELSGNRPLTAIIFSIFQERD   326
*****

Q08493   PDE4C_HUMAN   359   LLKTFQIPADTILATYLLMLEGHYHANVAYHNSLHAADVAQSTHVLLATPALEAVFTDLEI   418
Q08493-2 PDE4C_HUMAN   253   LLKTFQIPADTILATYLLMLEGHYHANVAYHNSLHAADVAQSTHVLLATPALEAVFTDLEI   312
Q08493-3 PDE4C_HUMAN   327   LLKTFQIPADTILATYLLMLEGHYHANVAYHNSLHAADVAQSTHVLLATPALEAVFTDLEI   386
*****

Q08493   PDE4C_HUMAN   419   LAALFASAIHDVDHPGVSNQFLINTNSELALMYNDASVLENHHLAGVFKLLQAENCDIFQ   478
Q08493-2 PDE4C_HUMAN   313   LAALFASAIHDVDHPGVSNQFLINTNSELALMYNDASVLENHHLAGVFKLLQAENCDIFQ   372
Q08493-3 PDE4C_HUMAN   387   LAALFASAIHDVDHPGVSNQFLINTNSELALMYNDASVLENHHLAGVFKLLQAENCDIFQ   446
*****

Q08493   PDE4C_HUMAN   479   NLSAKQRLSLRRMVIDMVLATDMSKHMNILLADLKTMTVETKKVTSLGVLILDNYSRIQVL   538
Q08493-2 PDE4C_HUMAN   373   NLSAKQRLSLRRMVIDMVLATDMSKHMNILLADLKTMTVETKKVTSLGVLILDNYSRIQVL   432
Q08493-3 PDE4C_HUMAN   447   NLSAKQRLSLRRMVIDMVLATDMSKHMNILLADLKTMTVETKKVTSLGVLILDNYSRIQVL   506
*****

Q08493   PDE4C_HUMAN   539   QNLVHCADLSNPTKPLPLYRQWTDRIIMAEFFQQGDREERESGLDISPMCDKHTASVEKSQV   598
Q08493-2 PDE4C_HUMAN   433   QNLVHCADLSNPTKPLPLYRQWTDRIIMAEFFQQGDREERESGLDISPMCDKHTASVEKSQV   492
Q08493-3 PDE4C_HUMAN   507   QNLVHCADLSNPTKPLPLYRQWTDRIIMAEFFQQGDREERESGLDISPMCDKHTASVEKSQV   566
*****

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FIG. 4 (Cont.)

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Q08493	PDE4C_HUMAN	599	GFIDYIAHPLWETWADLVHPDAQDLDLTLEDNREWYQSKI PRSPDL TNPERDGPDRFQF	658
Q08493-2	PDE4C_HUMAN	493	GFIDYIAHPLWETWADLVHPDAQDLDLTLEDNREWYQSKI PRSPDL TNPERDGPDRFQF	552
Q08493-3	PDE4C_HUMAN	567	GFIDYIAHPLWETWADLVHPDAQDLDLTLEDNREWYQSKI PRSPDL TNPERDGPDRFQF	626

Q08493	PDE4C_HUMAN	659	ELTLEEAEDEEEEEEEGETALAKEALELPDTELLSPEAGDPGDLPLDNQRT	712
Q08493-2	PDE4C_HUMAN	553	ELTLEEAEDEEEEEEEGETALAKEALELPDTELLSPEAGDPGDLPLDNQRT	606
Q08493-3	PDE4C_HUMAN	627	ELTLEEAEDEEEEEEEGETALAKEALELPDTELLSPEAGDPGDLPLDNQRT	680

FIG. 4 (Cont.)

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Q08499	PDE4D_HUMAN	1	NEAEGSSAPARAGSGEGSDSAGGATLKAPKHLWRHEQHQPPLRQPQFRLPHPHHLPPP	60
Q08499-2	PDE4D_HUMAN	1	-----	0
Q08499-3	PDE4D_HUMAN	1	-----	0
Q08499-4	PDE4D_HUMAN	1	-----	0
Q08499-5	PDE4D_HUMAN	1	-----	0
Q08499-6	PDE4D_HUMAN	1	-----NAQQTSPDT	9
Q08499-7	PDE4D_HUMAN	1	-----	0
Q08499-8	PDE4D_HUMAN	1	-----	0
Q08499-9	PDE4D_HUMAN	1	-----	0
Q08499-10	PDE4D_HUMAN	1	-----	0
Q08499-11	PDE4D_HUMAN	1	-----	0
Q08499-12	PDE4D_HUMAN	1	-----	0
Q08499	PDE4D_HUMAN	61	PPSPQPQPQCPLQPPPPPLPPPPPPGAARGRYASSGATGRVVRHGYSDTER----YL	116
Q08499-2	PDE4D_HUMAN	1	-----	0
Q08499-3	PDE4D_HUMAN	1	-----	0
Q08499-4	PDE4D_HUMAN	1	-----	0
Q08499-5	PDE4D_HUMAN	1	-----	0
Q08499-6	PDE4D_HUMAN	10	LTVPEVDNPHCPN-----PWLNE-DLVKSLRENLLQHEK-SKTAR----KS	49
Q08499-7	PDE4D_HUMAN	1	----NAQQTSPDTLTVPEVDNPHCPNPWLNE-----LVKSL	33
Q08499-8	PDE4D_HUMAN	1	-----	0
Q08499-9	PDE4D_HUMAN	1	-----	0
Q08499-10	PDE4D_HUMAN	1	-----	0
Q08499-11	PDE4D_HUMAN	1	----MKRNTCOLLSRSKSA-----SEETLHS----SNEEDPFRGNEPVLVRL	41
Q08499-12	PDE4D_HUMAN	1	----NAQQTSPDTLTVPEVDNPHCPNPWLNE-----LVKSL	33
Q08499	PDE4D_HUMAN	117	YCRANDRTS-YAV-----ET--GHRPGLKK---SRNSMP---SSFQGLRRFDVDN	157
Q08499-2	PDE4D_HUMAN	1	-----NM--HVNNFP---FRHSHWICFDVDN	21
Q08499-3	PDE4D_HUMAN	1	-----	0
Q08499-4	PDE4D_HUMAN	1	-----	0
Q08499-5	PDE4D_HUMAN	1	-----	0
Q08499-6	PDE4D_HUMAN	50	VS---PKLS-PVI-----SP--RNSPRLLRMLLSSNIPKQRRFTVAHTCFVDN	93
Q08499-7	PDE4D_HUMAN	34	RENLLQHEKSKTARKSVSPKLSPVISPRNSPRLLRMLLSSNIPKQRRFTVAHTCFVDN	93
Q08499-8	PDE4D_HUMAN	1	-----	0
Q08499-9	PDE4D_HUMAN	1	-----NA-FVW-----DPLGATVPG-----PSTRAKSRLRFKSYSFVDN	35
Q08499-10	PDE4D_HUMAN	1	-----MSIMKPRSRSTSSLRTAECFVDN	27
Q08499-11	PDE4D_HUMAN	42	SCRNIQLPP-LAFRQLEQADLKSESENIQPTSLPLK----ILPLIAITSAESSGFVDN	96
Q08499-12	PDE4D_HUMAN	34	RENLLQHEKSKTARKSVSPKLSPVISPRNSPRLLRMLLSSNIPKQRRFTVAHTCFVDN	93

FIG. 5

SUBSTITUTE SHEET (RULE 26)

Q08499	PDE4D_HUMAN	158	GTSAGRSPLDPMTSPGSGILIQANFVHSQRRESFLYRSDSDYDLSPKMSRNSSIASD--	215
Q08499-2	PDE4D_HUMAN	22	GTSAGRSPLDPMTSPGSGILIQANFVHSQRRESFLYRSDSDYDLSPKMSRNSSIASD--	79
Q08499-3	PDE4D_HUMAN	1	-----NSRNSSIASD--	10
Q08499-4	PDE4D_HUMAN	1	-----MKEQPSCAGTGH	12
Q08499-5	PDE4D_HUMAN	1	-----	0
Q08499-6	PDE4D_HUMAN	94	GTSAGRSPLDPMTSPGSGILIQANFVHSQRRESFLYRSDSDYDLSPKMSRNSSIASD--	151
Q08499-7	PDE4D_HUMAN	94	GTSAGRSPLDPMTSPGSGILIQANFVHSQRRESFLYRSDSDYDLSPKMSRNSSIASD--	151
Q08499-8	PDE4D_HUMAN	1	-----	0
Q08499-9	PDE4D_HUMAN	36	GTSAGRSPLDPMTSPGSGILIQANFVHSQRRESFLYRSDSDYDLSPKMSRNSSIASD--	93
Q08499-10	PDE4D_HUMAN	28	GTSAGRSPLDPMTSPGSGILIQANFVHSQRRESFLYRSDSDYDLSPKMSRNSSIASD--	85
Q08499-11	PDE4D_HUMAN	97	GTSAGRSPLDPMTSPGSGILIQANFVHSQRRESFLYRSDSDYDLSPKMSRNSSIASD--	154
Q08499-12	PDE4D_HUMAN	94	GTSAGRSPLDPMTSPGSGILIQANFVHSQRRESFLYRSDSDYDLSPKMSRNSSIASD--	151
Q08499	PDE4D_HUMAN	216	-IHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQ	274
Q08499-2	PDE4D_HUMAN	80	-IHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQ	138
Q08499-3	PDE4D_HUMAN	11	-IHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQ	69
Q08499-4	PDE4D_HUMAN	13	PMAGY-GRMAPFELAS-----GPVKR---LRTSEPFPCLFAEEAYQ	49
Q08499-5	PDE4D_HUMAN	1	-----	0
Q08499-6	PDE4D_HUMAN	152	-IHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQ	210
Q08499-7	PDE4D_HUMAN	152	-IHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITGLYNG	210
Q08499-8	PDE4D_HUMAN	1	-----	0
Q08499-9	PDE4D_HUMAN	94	-IHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQ	152
Q08499-10	PDE4D_HUMAN	86	-IHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQ	144
Q08499-11	PDE4D_HUMAN	155	-IHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQ	213
Q08499-12	PDE4D_HUMAN	152	-IHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITGSWNE	210
Q08499	PDE4D_HUMAN	275	KLASETLEELDNCLDQLETQTRHSVSENASNFKRMLNRELTHLSEMSRSGNQVSEFIS	334
Q08499-2	PDE4D_HUMAN	139	KLASETLEELDNCLDQLETQTRHSVSENASNFKRMLNRELTHLSEMSRSGNQVSEFIS	198
Q08499-3	PDE4D_HUMAN	70	KLASETLEELDNCLDQLETQTRHSVSENASNFKRMLNRELTHLSEMSRSGNQVSEFIS	129
Q08499-4	PDE4D_HUMAN	50	KLASETLEELDNCLDQLETQTRHSVSENASNFKRMLNRELTHLSEMSRSGNQVSEFIS	109
Q08499-5	PDE4D_HUMAN	1	-----NASNFKRMLNRELTHLSEMSRSGNQVSEFIS	32
Q08499-6	PDE4D_HUMAN	211	KLASETLEELDNCLDQLETQTRHSVSENASNFKRMLNRELTHLSEMSRSGNQVSEFIS	270
Q08499-7	PDE4D_HUMAN	211	IIAFL-----	215
Q08499-8	PDE4D_HUMAN	1	-----MPEANYL-----LSVSWGYIKFKRMLNRELTHLSEMSRSGNQVSEFIS	43
Q08499-9	PDE4D_HUMAN	153	KLASETLEELDNCLDQLETQTRHSVSENASNFKRMLNRELTHLSEMSRSGNQVSEFIS	212
Q08499-10	PDE4D_HUMAN	145	KLASETLEELDNCLDQLETQTRHSVSENASNFKRMLNRELTHLSEMSRSGNQVSEFIS	204
Q08499-11	PDE4D_HUMAN	214	KLASETLEELDNCLDQLETQTRHSVSENASNFKRMLNRELTHLSEMSRSGNQVSEFIS	273
Q08499-12	PDE4D_HUMAN	211	LNPHYLLDM-----	219

FIG. 5 (Cont.)

Q08499	PDE4D_HUMAN	335	NTFLDKQHEVEIPSPTQKEKEKKRPMISQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVL	394
Q08499-2	PDE4D_HUMAN	199	NTFLDKQHEVEIPSPTQKEKEKKRPMISQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVL	258
Q08499-3	PDE4D_HUMAN	130	NTFLDKQHEVEIPSPTQKEKEKKRPMISQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVL	189
Q08499-4	PDE4D_HUMAN	110	NTFLDKQHEVEIPSPTQKEKEKKRPMISQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVL	169
Q08499-5	PDE4D_HUMAN	33	NTFLDKQHEVEIPSPTQKEKEKKRPMISQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVL	92
Q08499-6	PDE4D_HUMAN	271	NTFLDKQHEVEIPSPTQKEKEKKRPMISQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVL	330
Q08499-7	PDE4D_HUMAN	216	-----	215
Q08499-8	PDE4D_HUMAN	44	NTFLDKQHEVEIPSPTQKEKEKKRPMISQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVL	103
Q08499-9	PDE4D_HUMAN	213	NTFLDKQHEVEIPSPTQKEKEKKRPMISQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVL	272
Q08499-10	PDE4D_HUMAN	205	NTFLDKQHEVEIPSPTQKEKEKKRPMISQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVL	264
Q08499-11	PDE4D_HUMAN	274	NTFLDKQHEVEIPSPTQKEKEKKRPMISQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVL	333
Q08499-12	PDE4D_HUMAN	220	-----	219

Q08499	PDE4D_HUMAN	395	AKELEDVKNWGLHVFRIAEISGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLNTLEDH	454
Q08499-2	PDE4D_HUMAN	259	AKELEDVKNWGLHVFRIAEISGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLNTLEDH	318
Q08499-3	PDE4D_HUMAN	190	AKELEDVKNWGLHVFRIAEISGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLNTLEDH	249
Q08499-4	PDE4D_HUMAN	170	AKELEDVKNWGLHVFRIAEISGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLNTLEDH	229
Q08499-5	PDE4D_HUMAN	93	AKELEDVKNWGLHVFRIAEISGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLNTLEDH	152
Q08499-6	PDE4D_HUMAN	331	AKELEDVKNWGLHVFRIAEISGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLNTLEDH	390
Q08499-7	PDE4D_HUMAN	216	-----	215
Q08499-8	PDE4D_HUMAN	104	AKELEDVKNWGLHVFRIAEISGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLNTLEDH	163
Q08499-9	PDE4D_HUMAN	273	AKELEDVKNWGLHVFRIAEISGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLNTLEDH	332
Q08499-10	PDE4D_HUMAN	265	AKELEDVKNWGLHVFRIAEISGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLNTLEDH	324
Q08499-11	PDE4D_HUMAN	334	AKELEDVKNWGLHVFRIAEISGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLNTLEDH	393
Q08499-12	PDE4D_HUMAN	220	-----	219

Q08499	PDE4D_HUMAN	455	YHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAAIFASAIHVDHDPGVSNQFL	514
Q08499-2	PDE4D_HUMAN	319	YHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAAIFASAIHVDHDPGVSNQFL	378
Q08499-3	PDE4D_HUMAN	250	YHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAAIFASAIHVDHDPGVSNQFL	309
Q08499-4	PDE4D_HUMAN	230	YHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAAIFASAIHVDHDPGVSNQFL	289
Q08499-5	PDE4D_HUMAN	153	YHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAAIFASAIHVDHDPGVSNQFL	212
Q08499-6	PDE4D_HUMAN	391	YHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAAIFASAIHVDHDPGVSNQFL	450
Q08499-7	PDE4D_HUMAN	216	-----	215
Q08499-8	PDE4D_HUMAN	164	YHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAAIFASAIHVDHDPGVSNQFL	223
Q08499-9	PDE4D_HUMAN	333	YHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAAIFASAIHVDHDPGVSNQFL	392
Q08499-10	PDE4D_HUMAN	325	YHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAAIFASAIHVDHDPGVSNQFL	384
Q08499-11	PDE4D_HUMAN	394	YHADVAYHNNIHAADVQSTHVLLSTPALEAVFTDLEILAAIFASAIHVDHDPGVSNQFL	453
Q08499-12	PDE4D_HUMAN	220	-----	219

FIG. 5 (Cont.)

Q08499	PDE4D_HUMAN	515	INTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRQSLRKNVIDIVLATD	574
Q08499-2	PDE4D_HUMAN	379	INTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRQSLRKNVIDIVLATD	438
Q08499-3	PDE4D_HUMAN	310	INTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRQSLRKNVIDIVLATD	369
Q08499-4	PDE4D_HUMAN	290	INTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRQSLRKNVIDIVLATD	349
Q08499-5	PDE4D_HUMAN	213	INTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRQSLRKNVIDIVLATD	272
Q08499-6	PDE4D_HUMAN	451	INTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRQSLRKNVIDIVLATD	510
Q08499-7	PDE4D_HUMAN	216	-----	215
Q08499-8	PDE4D_HUMAN	224	INTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRQSLRKNVIDIVLATD	283
Q08499-9	PDE4D_HUMAN	393	INTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRQSLRKNVIDIVLATD	452
Q08499-10	PDE4D_HUMAN	385	INTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRQSLRKNVIDIVLATD	444
Q08499-11	PDE4D_HUMAN	454	INTNSELALMYNDSSVLENHHLAVGFKLLQEENCDFQNLTKKQQRQSLRKNVIDIVLATD	513
Q08499-12	PDE4D_HUMAN	220	-----	219
Q08499	PDE4D_HUMAN	575	MSKHMNLLADLKTIVETKKVTSSGVLLLDNYSRIQVLQNMVHCADLSNPTKPLQLYRQW	634
Q08499-2	PDE4D_HUMAN	439	MSKHMNLLADLKTIVETKKVTSSGVLLLDNYSRIQVLQNMVHCADLSNPTKPLQLYRQW	498
Q08499-3	PDE4D_HUMAN	370	MSKHMNLLADLKTIVETKKVTSSGVLLLDNYSRIQVLQNMVHCADLSNPTKPLQLYRQW	429
Q08499-4	PDE4D_HUMAN	350	MSKHMNLLADLKTIVETKKVTSSGVLLLDNYSRIQVLQNMVHCADLSNPTKPLQLYRQW	409
Q08499-5	PDE4D_HUMAN	273	MSKHMNLLADLKTIVETKKVTSSGVLLLDNYSRIQVLQNMVHCADLSNPTKPLQLYRQW	332
Q08499-6	PDE4D_HUMAN	511	MSKHMNLLADLKTIVETKKVTSSGVLLLDNYSRIQVLQNMVHCADLSNPTKPLQLYRQW	570
Q08499-7	PDE4D_HUMAN	216	-----	215
Q08499-8	PDE4D_HUMAN	284	MSKHMNLLADLKTIVETKKVTSSGVLLLDNYSRIQVLQNMVHCADLSNPTKPLQLYRQW	343
Q08499-9	PDE4D_HUMAN	453	MSKHMNLLADLKTIVETKKVTSSGVLLLDNYSRIQVLQNMVHCADLSNPTKPLQLYRQW	512
Q08499-10	PDE4D_HUMAN	445	MSKHMNLLADLKTIVETKKVTSSGVLLLDNYSRIQVLQNMVHCADLSNPTKPLQLYRQW	504
Q08499-11	PDE4D_HUMAN	514	MSKHMNLLADLKTIVETKKVTSSGVLLLDNYSRIQVLQNMVHCADLSNPTKPLQLYRQW	573
Q08499-12	PDE4D_HUMAN	220	-----	219
Q08499	PDE4D_HUMAN	635	TDRINEEFFRQGDREERERGNEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA	694
Q08499-2	PDE4D_HUMAN	499	TDRINEEFFRQGDREERERGNEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA	558
Q08499-3	PDE4D_HUMAN	430	TDRINEEFFRQGDREERERGNEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA	489
Q08499-4	PDE4D_HUMAN	410	TDRINEEFFRQGDREERERGNEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA	469
Q08499-5	PDE4D_HUMAN	333	TDRINEEFFRQGDREERERGNEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA	392
Q08499-6	PDE4D_HUMAN	571	TDRINEEFFRQGDREERERGNEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA	630
Q08499-7	PDE4D_HUMAN	216	-----	215
Q08499-8	PDE4D_HUMAN	344	TDRINEEFFRQGDREERERGNEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA	403
Q08499-9	PDE4D_HUMAN	513	TDRINEEFFRQGDREERERGNEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA	572
Q08499-10	PDE4D_HUMAN	505	TDRINEEFFRQGDREERERGNEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA	564
Q08499-11	PDE4D_HUMAN	574	TDRINEEFFRQGDREERERGNEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA	633
Q08499-12	PDE4D_HUMAN	220	-----	219

FIG. 5 (Cont.)

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Q08499	PDE4D_HUMAN	695	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGS	754
Q08499-2	PDE4D_HUMAN	559	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGS	618
Q08499-3	PDE4D_HUMAN	490	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGS	549
Q08499-4	PDE4D_HUMAN	470	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGS	529
Q08499-5	PDE4D_HUMAN	393	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGS	452
Q08499-6	PDE4D_HUMAN	631	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGS	690
Q08499-7	PDE4D_HUMAN	216	-----	215
Q08499-8	PDE4D_HUMAN	404	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGS	463
Q08499-9	PDE4D_HUMAN	573	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGS	632
Q08499-10	PDE4D_HUMAN	565	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGS	624
Q08499-11	PDE4D_HUMAN	634	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGS	693
Q08499-12	PDE4D_HUMAN	220	-----	219
Q08499	PDE4D_HUMAN	755	QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT	809
Q08499-2	PDE4D_HUMAN	619	QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT	673
Q08499-3	PDE4D_HUMAN	550	QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT	604
Q08499-4	PDE4D_HUMAN	530	QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT	584
Q08499-5	PDE4D_HUMAN	453	QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT	507
Q08499-6	PDE4D_HUMAN	691	QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT	745
Q08499-7	PDE4D_HUMAN	216	-----	215
Q08499-8	PDE4D_HUMAN	464	QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT	518
Q08499-9	PDE4D_HUMAN	633	QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT	687
Q08499-10	PDE4D_HUMAN	625	QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT	679
Q08499-11	PDE4D_HUMAN	694	QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEEESQPEACVIDDRSPDT	748
Q08499-12	PDE4D_HUMAN	220	-----	219

FIG. 5 (Cont.)

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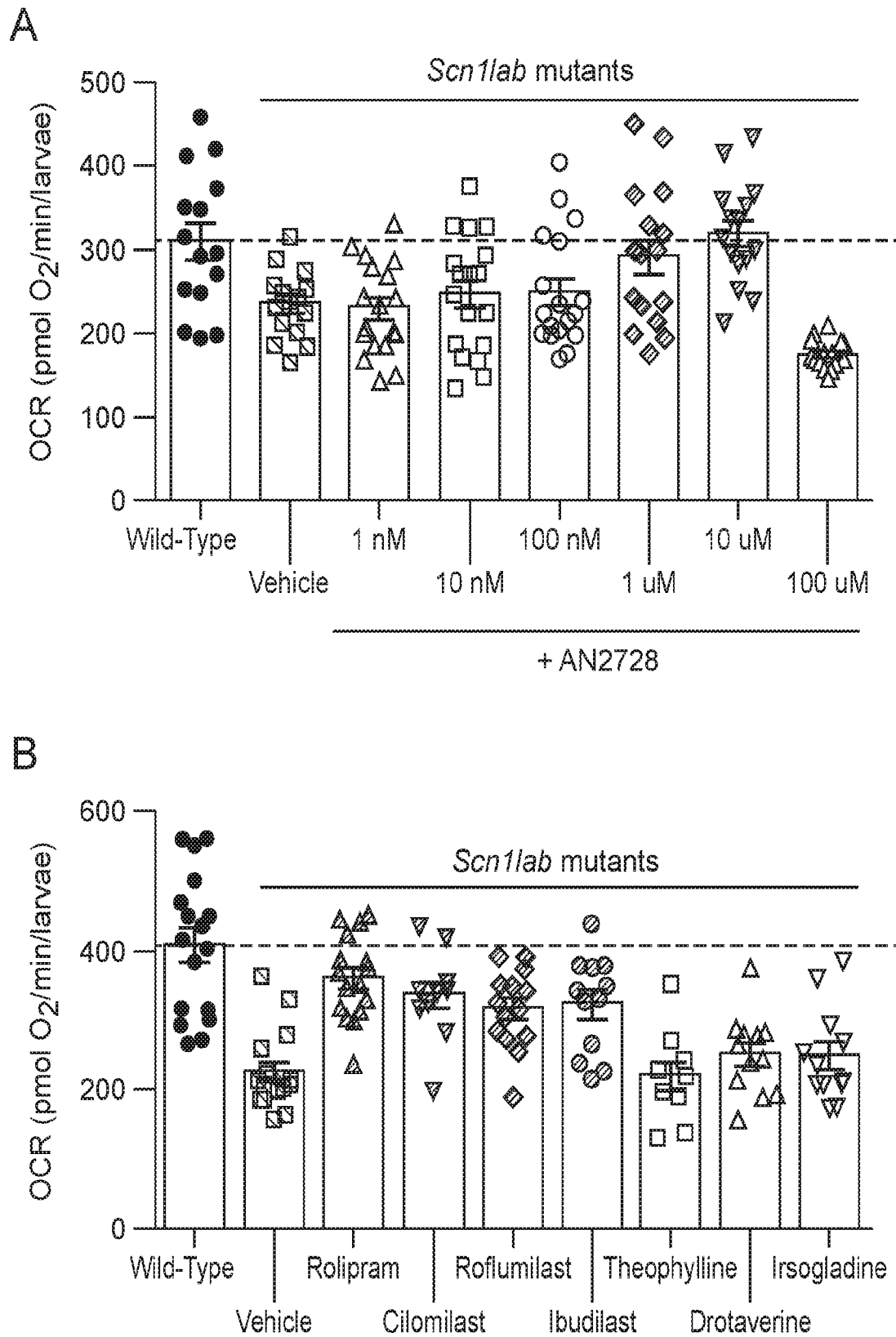


FIG. 6

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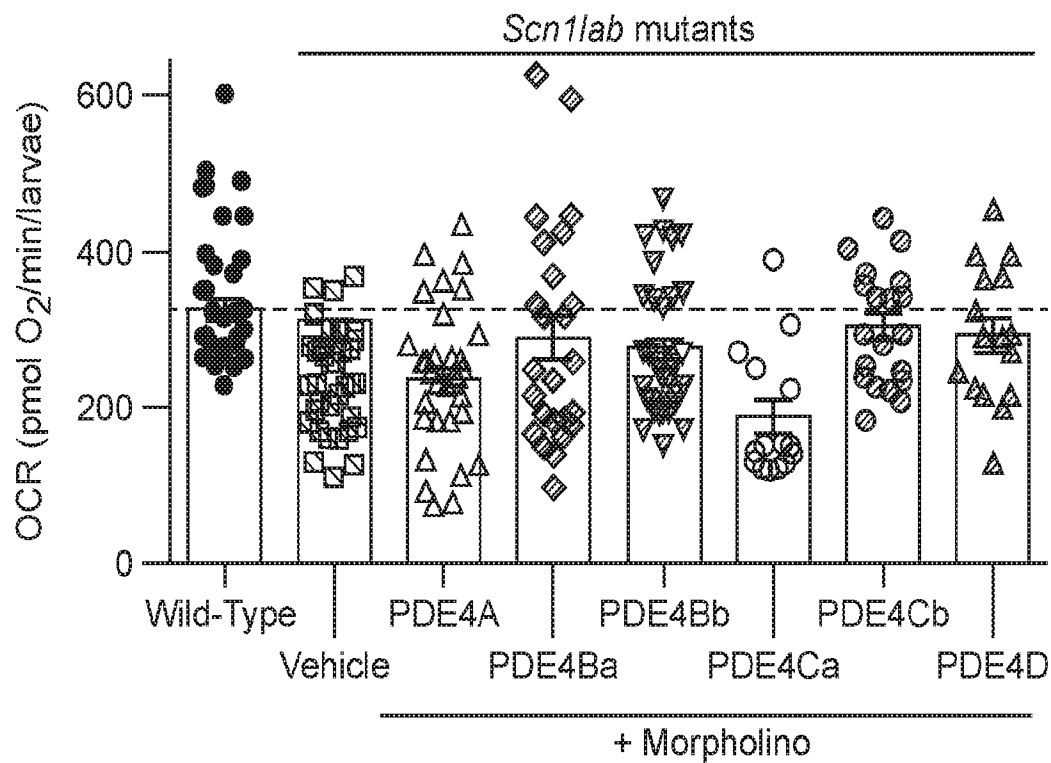


FIG. 7

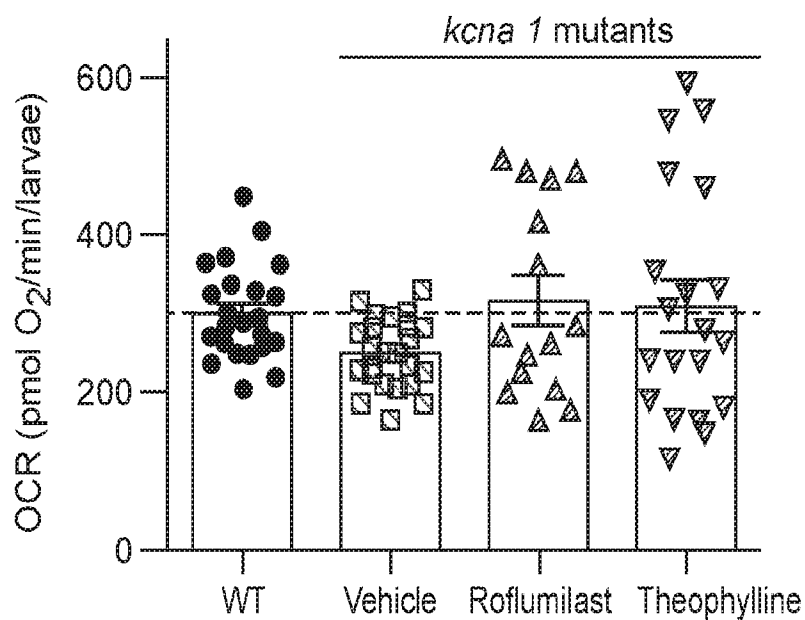
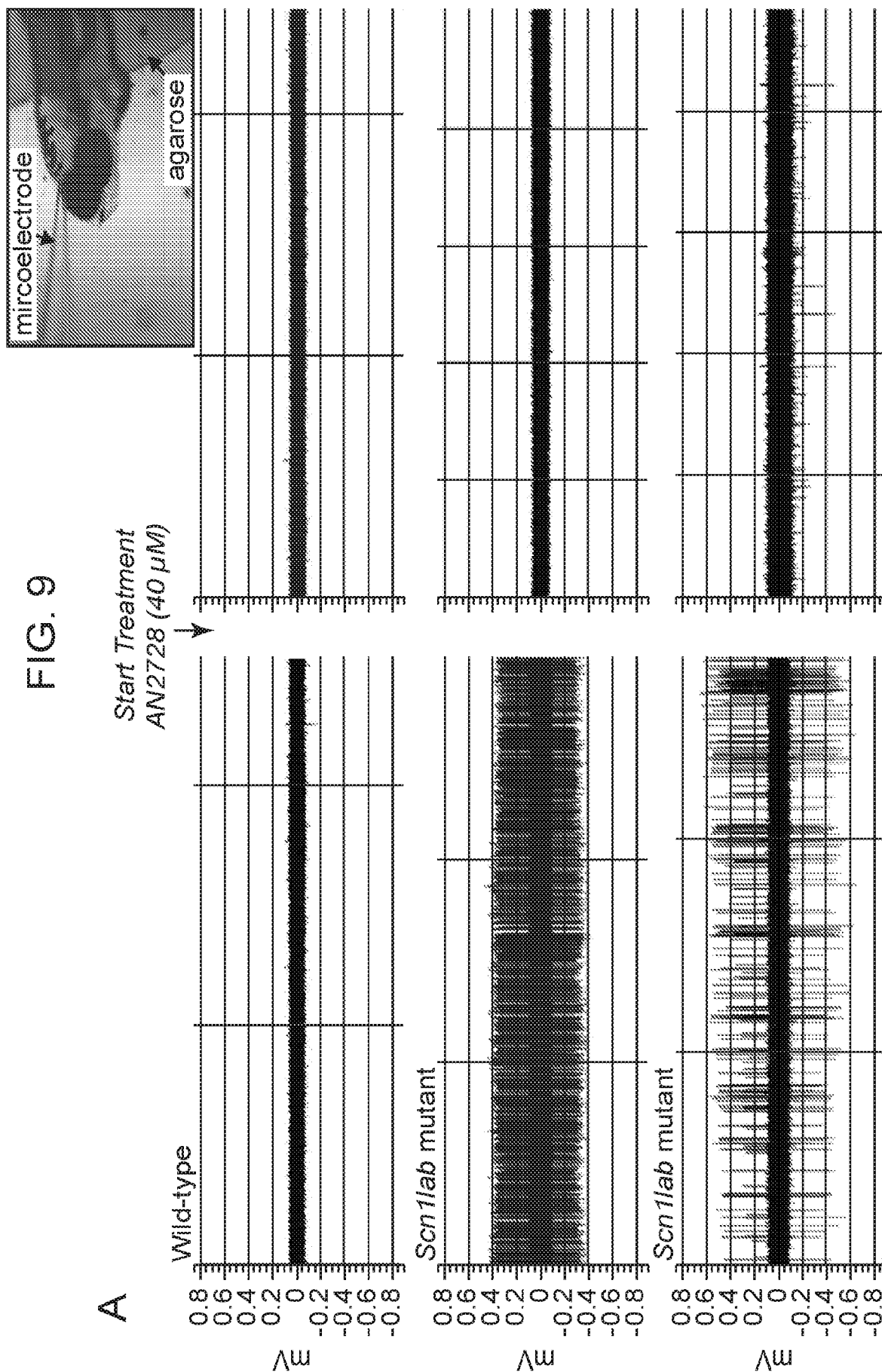


FIG. 8



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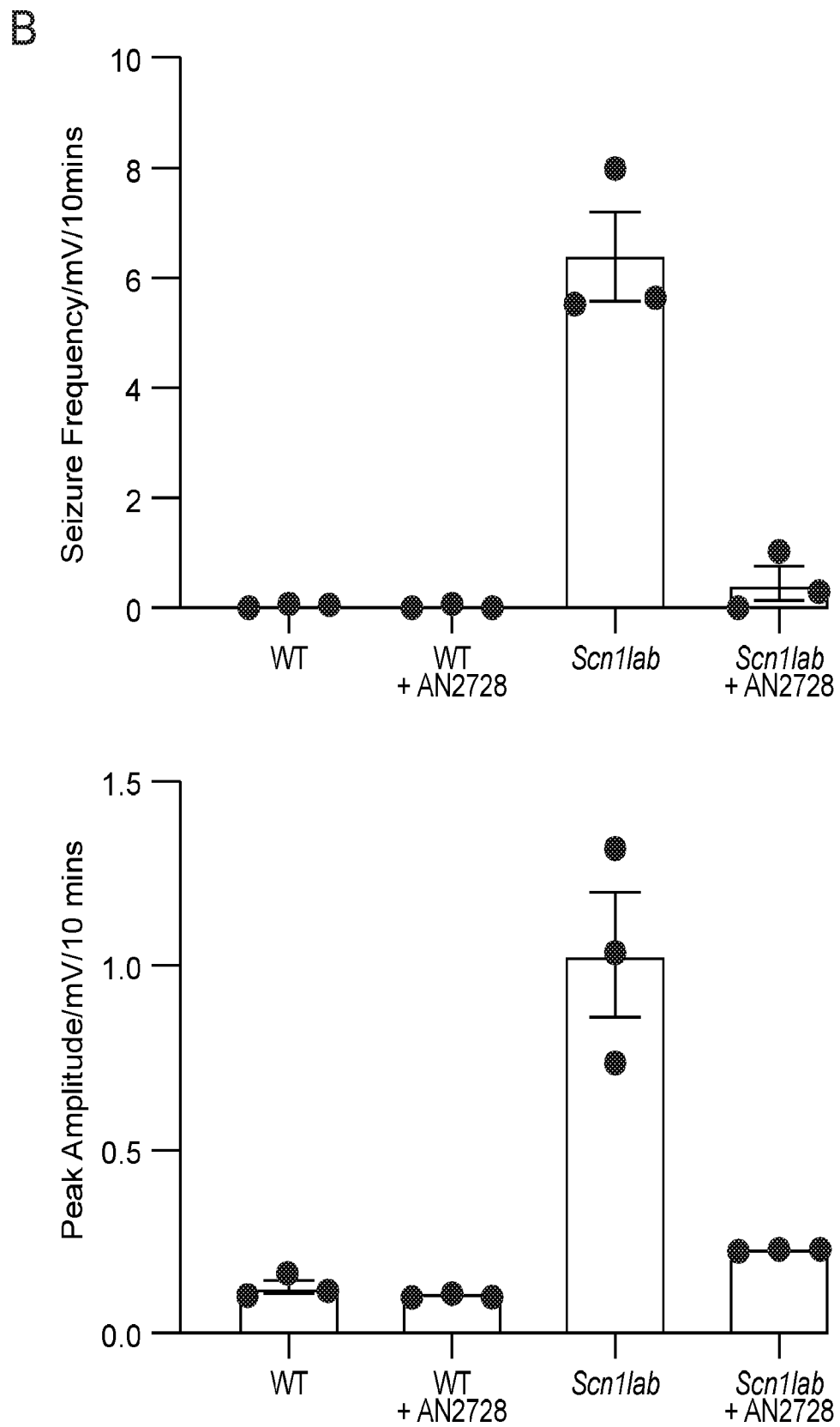


FIG. 9 (Cont.)

A

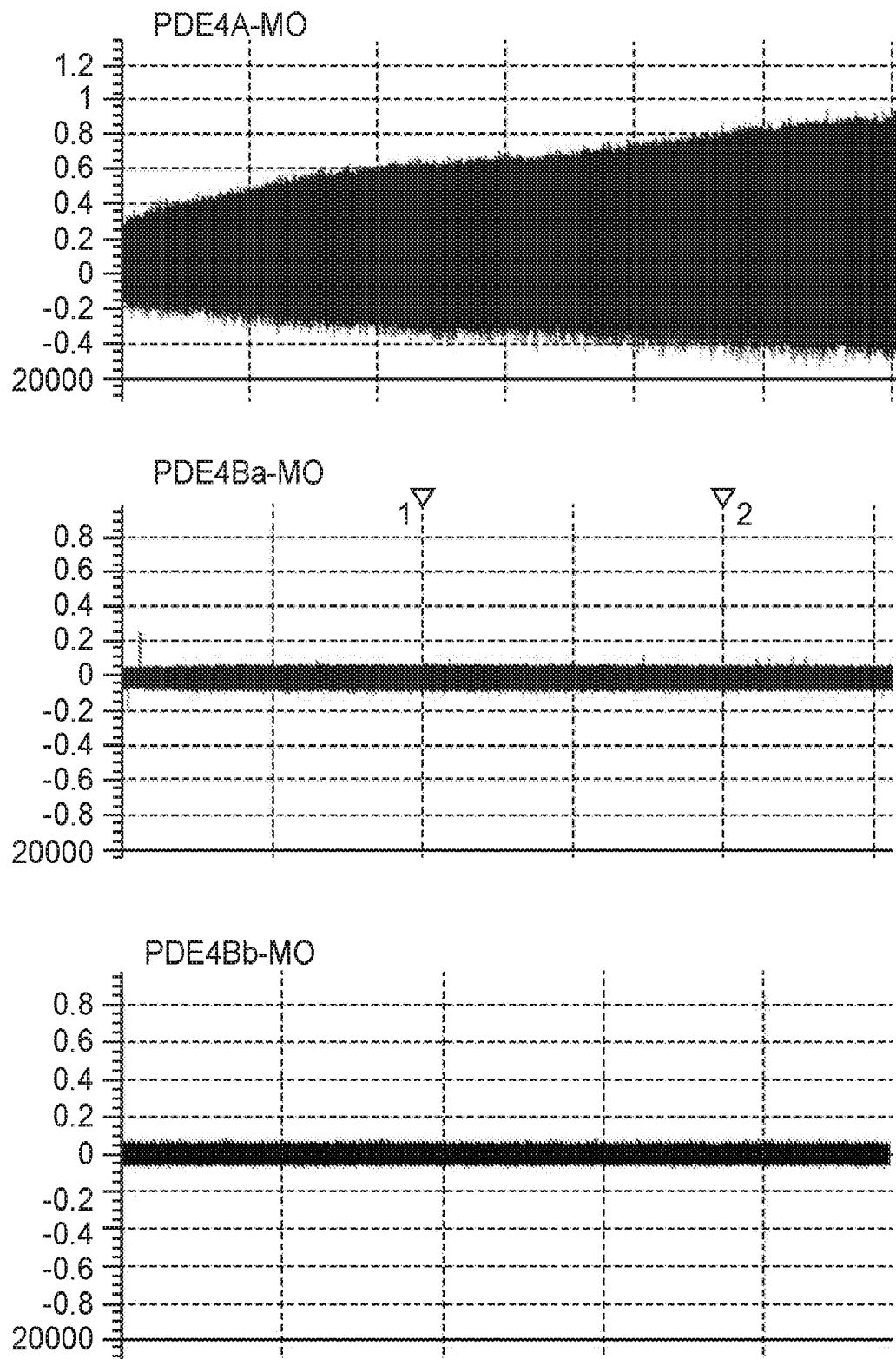


FIG. 10

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A (Cont.)

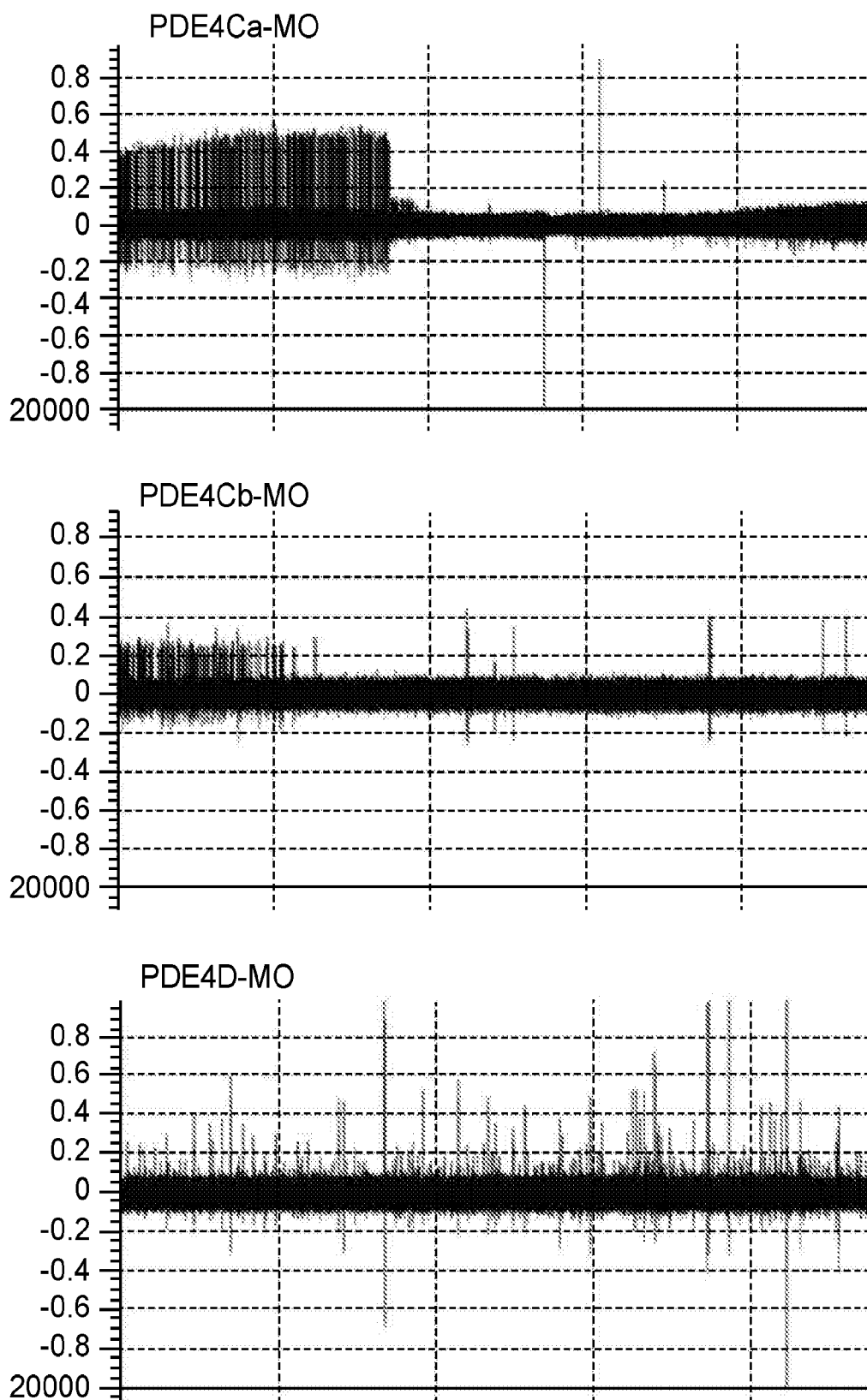


FIG. 10 (Cont.)

SUBSTITUTE SHEET (RULE 26)

B

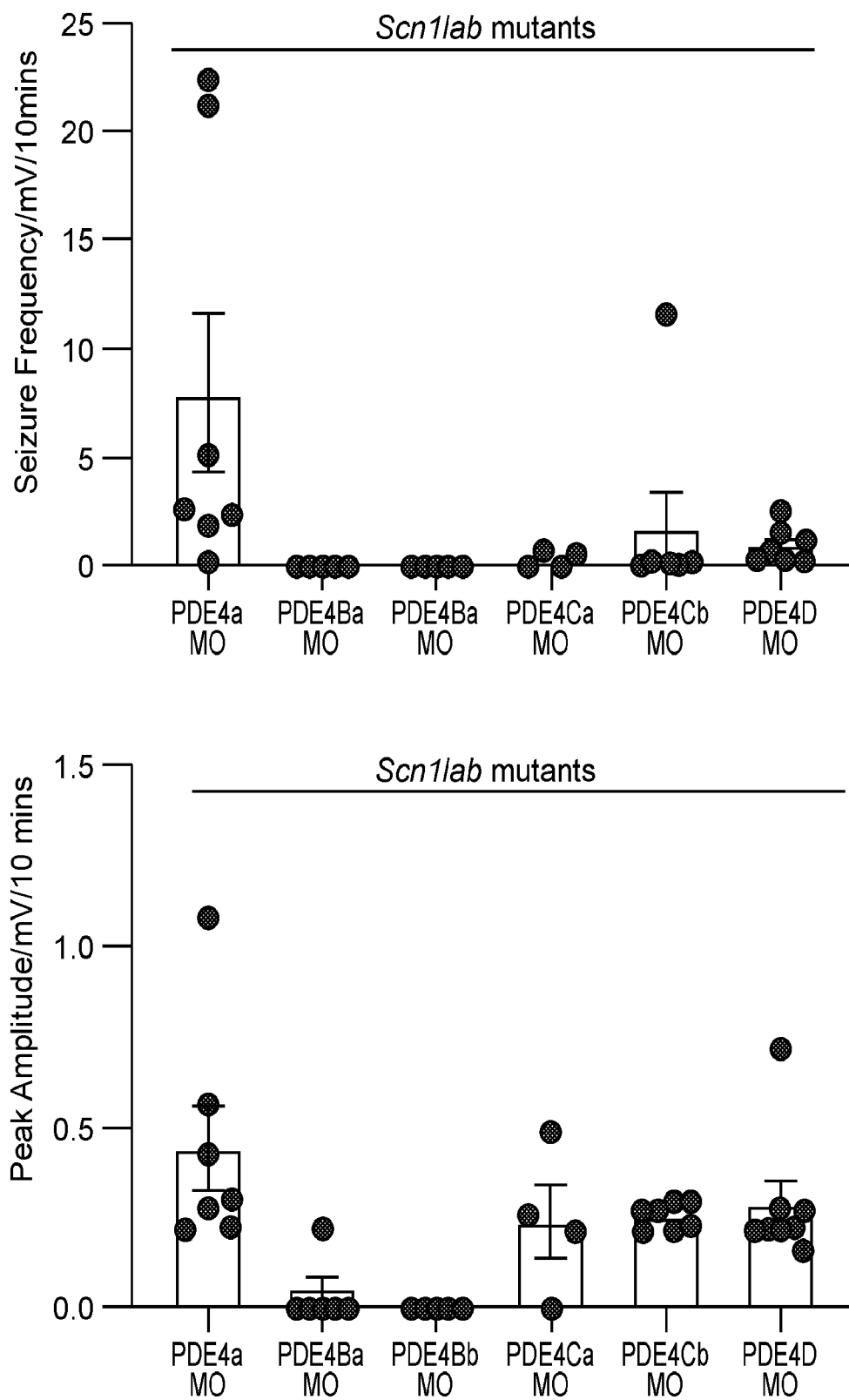


FIG. 10 (Cont.)

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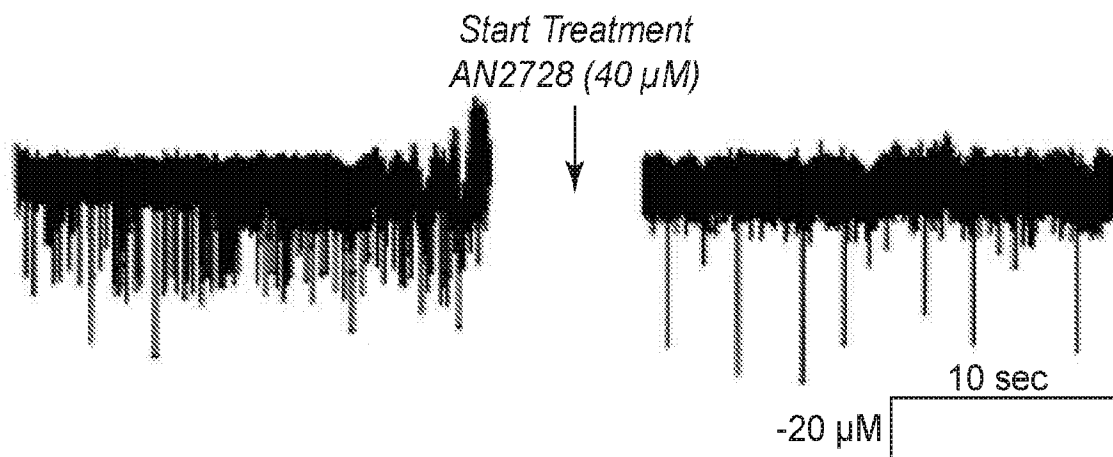


FIG. 11

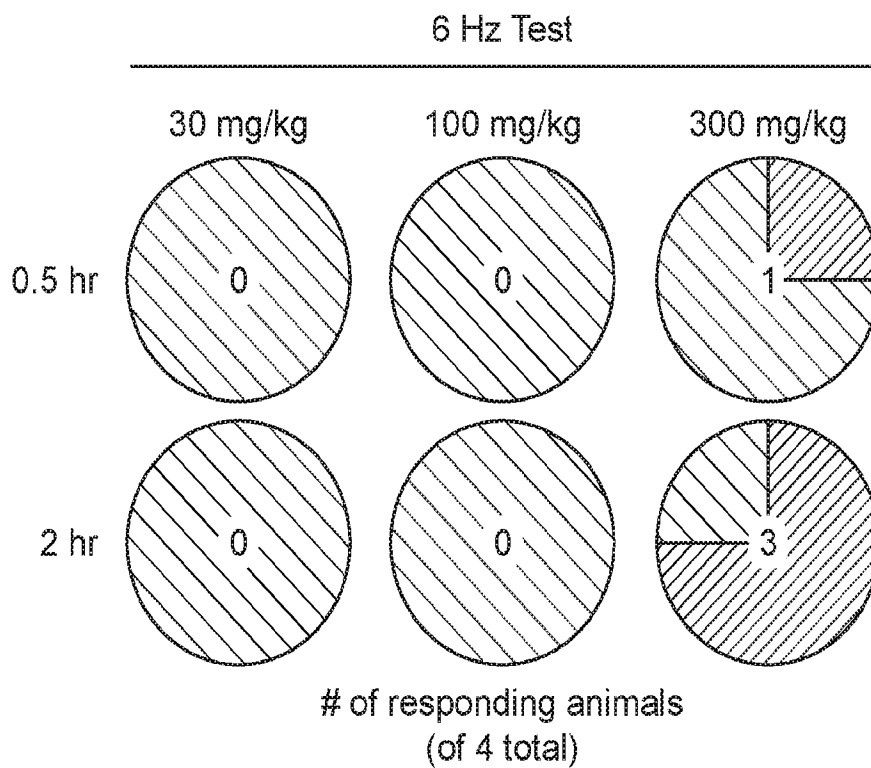
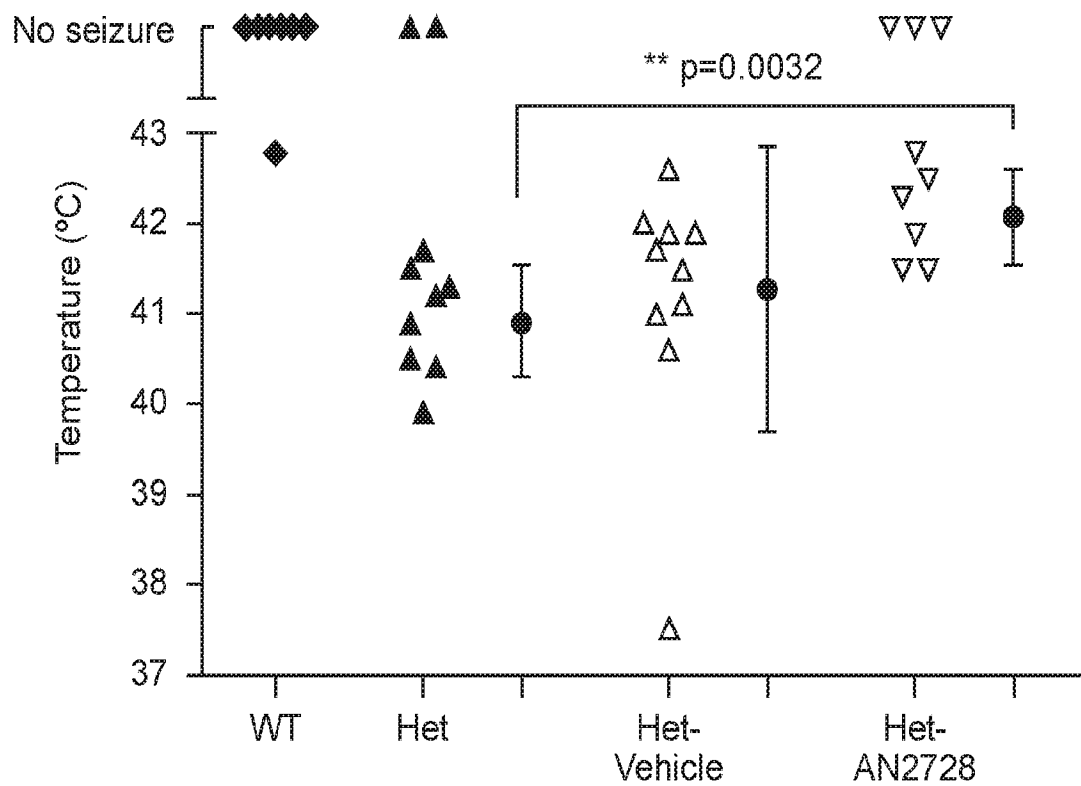


FIG. 12

A



B

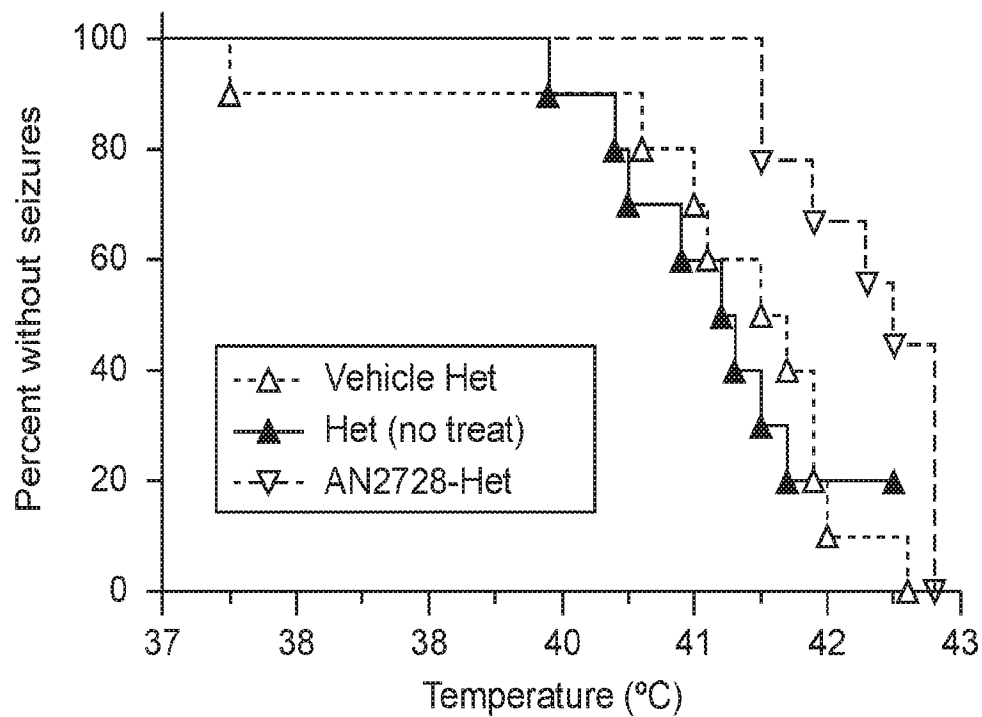


FIG. 13

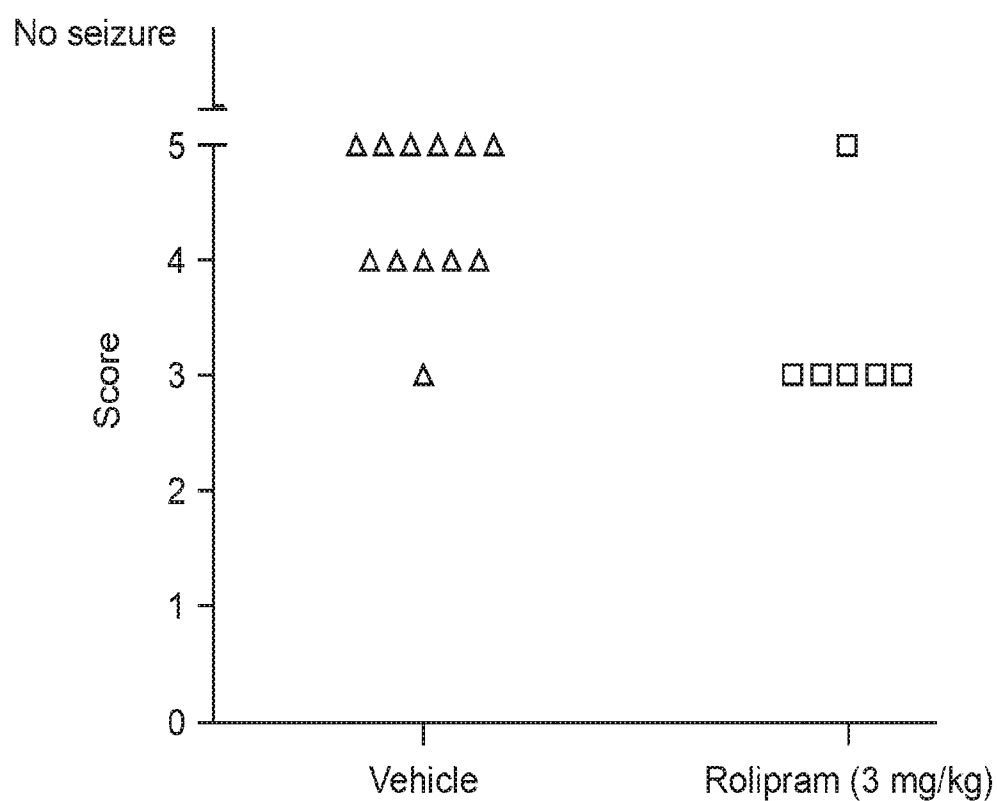
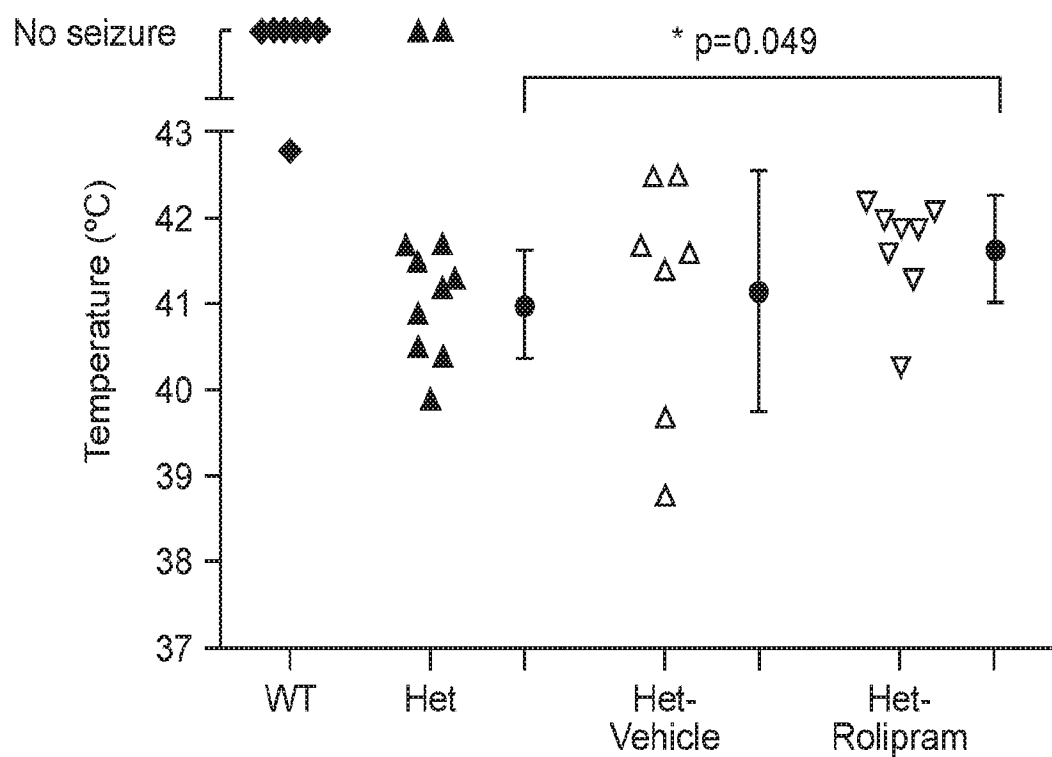


FIG. 14

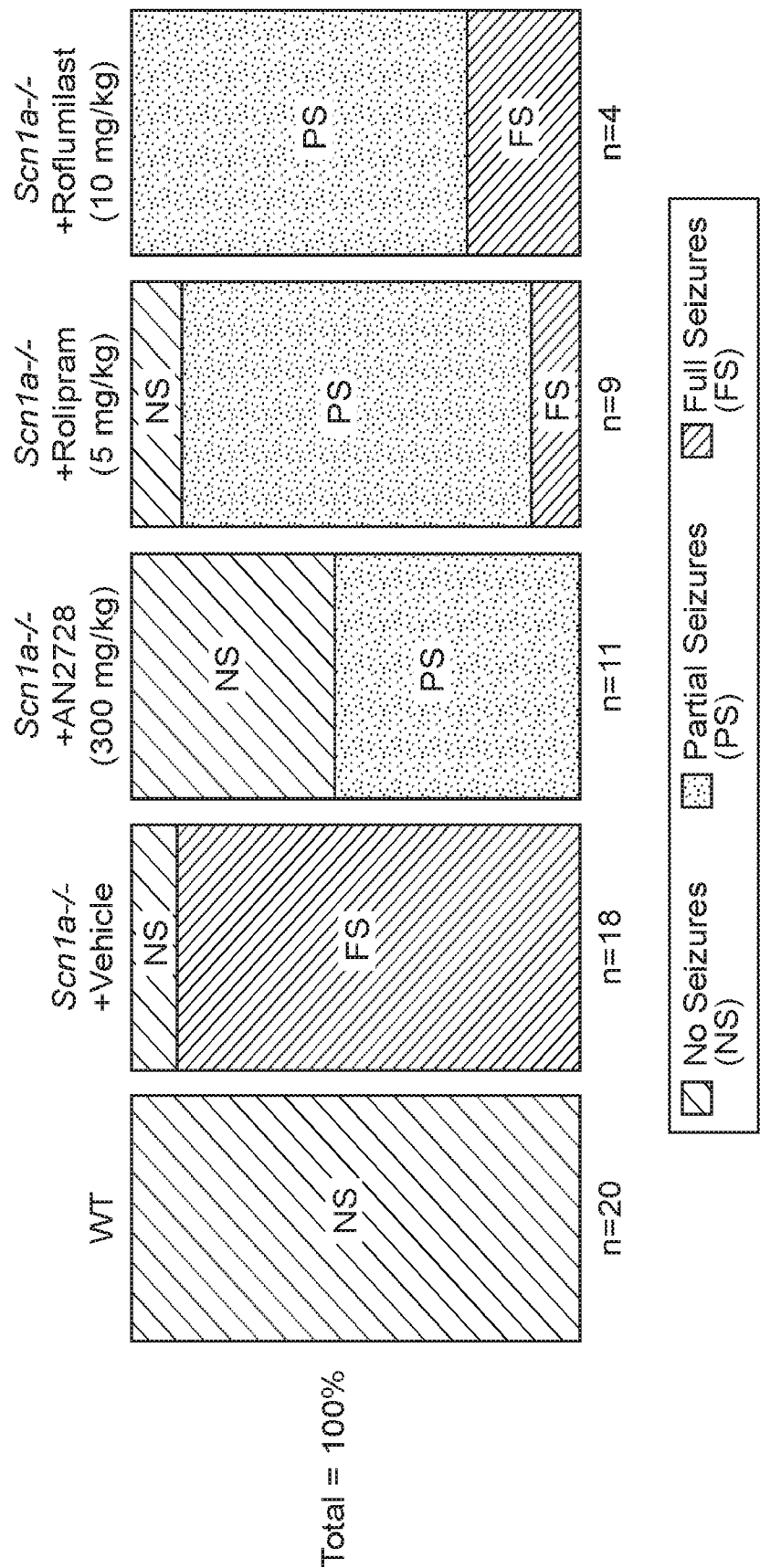


FIG. 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/014819

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 9/16; G01N 33/50 (2020.01)

CPC - C12Q 2600/136; C12Q 2600/178; C12Y 301/04053; G01N 2500/10; G01N 2500/20 (2020.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 435/196; 514/17.7 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/0349969 A1 (AKRON MOLECULES AG) 27 November 2014 (27.11.2014) entire document	1-13
X	US 2013/0178498 A1 (GOVEK et al) 11 July 2013 (11.07.2013) entire document	16-20
A	US 2015/0232857 A1 (LAKEWOOD-AMEDEX, INC.) 20 August 2015 (20.08.2015) entire document	1-13, 16-20
A	WO 2017/145013 A1 (PFIZER INC.) 31 August 2017 (31.08.2017) entire document	1-13, 16-20
A	US 2005/0164275 A1 (THORNTON et al) 28 July 2005 (28.07.2005) entire document	1-13, 16-20
A	US 2006/0106085 A1 (ZELDIS et al) 18 May 2006 (18.05.2006) entire document	1-13, 16-20

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 March 2020

Date of mailing of the international search report

06 APR 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/014819

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 14, 15, 21-26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.