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(71) Demandeur/Applicant:
PATH THERAPEUTICS, INC., CA

(72) Inventeurs/Inventors:
KURRASCH, DEBORAH M., CA;
IBHAZEHIEBO, KINGSLEY, CA

(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : METHODES DE TRAITEMENT DE L'EPILEPSIE PAR INHIBITION DE LA PHOSPHODIESTERASE 4 (PDE4)
(54) Title: METHODS OF TREATING EPILEPSY VIA PHOSPHODIESTERASE 4 (PDE4) INHIBITION

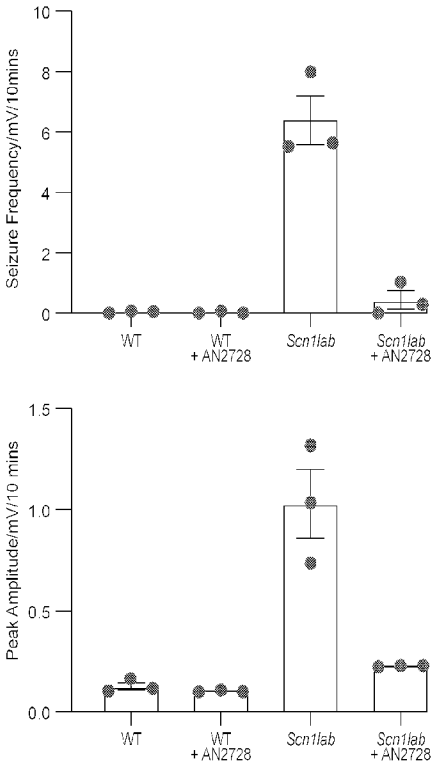

FIG. 9B

(57) Abrégé/Abstract:
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.

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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). **IBHAZEHIEBO, Kingsley** [CA/CA]; 2950 Toronto Crescent, Calgary, Alberta T23 3W5 (CA).

(74) Agent: **DAVY, Brian E.**; Bozicevic, Field & Francis LLP, 201 Redwood Shores Pkwy., Suite 200, Redwood City, California 94065 (US).

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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 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.

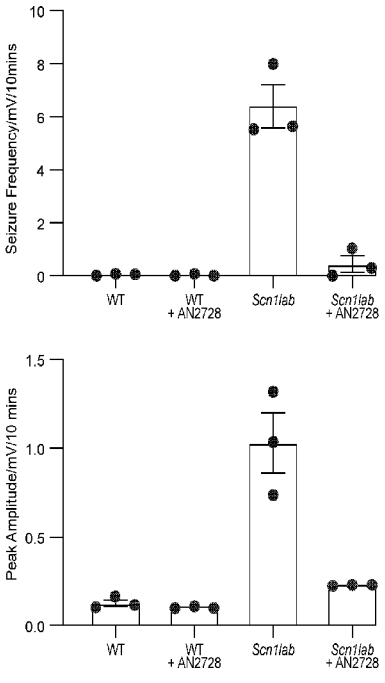

FIG. 9B

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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 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 30-40% 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 encoding brain voltage-gated sodium channel type-I, $Na_V1.1$. 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 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 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). 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.

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

DETAILED DESCRIPTION

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.

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.

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 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 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 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 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”, “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 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 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 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 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.

Table 1 – Example Human PDE4 target polypeptides

PDE4A (Human Isoform 1) UniProtKB – P27815-1) SEQ ID NO:1	MEPPTVPSERSLSLSLPGPREGQATLKPPPQHLWRQPRTPIRIQQRGYS DSAERAERERQPHRPIERADAMDTSDRPGLRTTRMSWPSSFHGTGTGS GGAGGGSSRRFEAENGPTPSPGRSPLDSQASPGLVLHAGAATSQRRES FLYRSDSDYDMSPKTMSRNSSVTSEAHAEDLIVTPFAQVLASLRSVRSNF SLLTNVPVPSNKRSPLGGPTPVCKATLSEETCQQLARETLEELDWCLEQL ETMQTYRSVSEMASHKFKRMLNRELTHLSEMSRSGNQVSEYISTTFLDK QNEVEIPSPTMKEREKQQAPRPRPSQPPPPPVPHLQPMSQITGLKKLMH SNSLNNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTC IMYMIFQERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQ STHVLLATPALDAVFTDLEILAALFAAAIHDVDHPGVSNQFLINTNSELALM YNDESVLENHHLAVGFKLLQEDNCDIFQNLSKRQRQSLRKMVIDMVLATD MSKHMTLLADLKTMVETKKVTSSGVLLLDNYSDRIQVLRNMVHCADLSN PTKPLELYRQWTDRIMAEFFQQGDRERERGMEISPMCDKHTASVEKSQ VGFIDYIVHPLWETWADLVHPDAQEILDTLEDNRDWYYSAIRQSPSPPPE EESRGPGHPPLPDKFQFELTLEEEEEEEISMAQIPCTAQEALTAQGLSGV EEALDATIAWEASPAQESLEVMAQEASLEAELEAVYLTQQAQSTGSAPV APDEFSSREEFVVAVSHSSPSALALQSPLLPAWRTLSVSEHAPGLPGLPS TAAEVEAQREHQAAKRACSACAGTFGEDTSALPAPGGGGSGGDPT
PDE4A (Human Isoform 2) UniProtKB – P27815-2) SEQ ID NO:2	MARPRGLGRIPELQLVAFPVAVAAEDEAFLPEPLAPRAPRRPRSPPSSPV FFASPSPTFRRRLRLLRSCQDLGRQAWAGAGFEAENGPTPSPGRSPLD SQASPGLVLHAGAATSQRRESFLYRSDSDYDMSPKTMSRNSSVTSEAH AEDLIVTPFAQVLASLRSVRSNFSLLTNVPVPSNKRSPLGGPTPVCKATLS EETCQQLARETLEELDWCLEQLETMQTYRSVSEMASHKFKRMLNRELTH LSEMSRSGNQVSEYISTTFLDKQNEVEIPSPTMKEREKQQAPRPRPSQP PPPPVPHLQPMSQITGLKKLMHSNSLNNSNIPRFGVKTDQEELLAQELEN LNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIPVDTMVTYMLTL EDHYHADVAYHNSLHAADVLQSTHVLLATPALDAVFTDLEILAALFAAAIH DVDHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEDNCDIFQ NLSKRQRQSLRKMVIDMVLATDMSKHMTLLADLKTMVETKKVTSSGVLLL DNYSDRIQVLRNMVHCADLSNPTKPLELYRQWTDRIMAEFFQQGDRERE RGMEISPMCDKHTASVEKSQVGFIDYIVHPLWETWADLVHPDAQEILDTL EDNRDWYYSAIRQSPSPPPEEESRGPGHPPLPDKFQFELTLEEEEEEEIS MAQIPCTAQEALTAQGLSGVEEALDATIAWEASPAQESLEVMAQEASLEA ELEAVYLTQQAQSTGSAPVAPDEFSSREEFVVAVSHSSPSALALQSPLLP AWRTLSVSEHAPGLPGLPSTAAEVEAQREHQAAKRACSACAGTFGEDT SALPAPGGGGSGGDPT
PDE4A (Human Isoform 3) UniProtKB – P27815-3) SEQ ID NO:3	MCPFPVTTVPLGGPTPVCKATLSEETCQQLARETLEELDWCLEQLETMQ TYRSVSEMASHKFKRMLNRELTHLSEMSRSGNQVSEYISTTFLDKQNEV EIPSPTMKEREKQQAPRPRPSQPPPPPVPHLQPMSQITGLKKLMHSNSL NNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTCIMYM IFQERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHV LLATPALDAVFTDLEILAALFAAAIHDVDHPGVSNQFLINTNSELALMYNDE SVLENHHLAVGFKLLQEDNCDIFQNLSKRQRQSLRKMVIDMVLATDMSK HMTLLADLKTMVETKKVTSSGVLLLDNYSDRIQVLRNMVHCADLSNPTKP LELYRQWTDRIMAEFFQQGDRERERGMEISPMCDKHTASVEKSQVGFID YIVHPLWETWADLVHPDAQEILDTLEDNRDWYYSAIRQSPSPPPEEESR GPGHPPLPDKFQFELTLEEEEEEEISMAQIPCTAQEALTAQGLSGVEEAL

	DATIAWEASPAQESLEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDE FSSREEFVVAVSHSSPSALALQSPLLPAWRTLSVSEHAPGLPGLPSTAAE VEAQREHQAAKRACSACAGTFGEDTSALPAPGGGGSGGDPT
PDE4A (Human Isoform 4) UniProtKB – P27815-4) SEQ ID NO:4	MPLVDFFCETCSKPWLVGWWDQFKRMLNRELTHLSEMSRSGNQVSEYI STTFLDKQNEVEIPSPTMKEREKQQAPRPRPSQPPPPPVPHLQPMSQIT GLKKLMHSNSLNNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYA GGRSLTCIMYMIFQERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSL HAADVLQSTHVLLATPALDAVFTDLEILAALFAAAIHDVDHPGVSNQFLINT NSELALMYNDESVLENHHLAVGFKLLQEDNCDIFQNLSKRQRQSLRKMVI DMVLATDMSKHMTLLADLKTMVETKKVTSSGVLLLDNYSDRIQVLRNMV HCADLSNPTKPLELYRQWTDRIMAEFFQQGDRERERGMEISPMCDKHT ASVEKSQVGFIDYIVHPLWETWADLVHPDAQEILDTLEDNRDWYYSAIRQ SPSPPPEEESRGPGHPPLPDKFQFELTLEEEEEEEISMAQIPCTAQEALT AQGLSGVEEALDATIAWEASPAQESLEVMAQEASLEAELEAVYLTQQAQ STGSAPVAPDEFSSREEFVVAVSHSSPSALALQSPLLPAWRTLSVSEHAP GLPGLPSTAAEVEAQREHQAAKRACSACAGTFGEDTSALPAPGGGGSG GDPT
PDE4A (Human Isoform 5) UniProtKB – P27815-5) SEQ ID NO:5	MVLPSDQGFKLLGNVLQGPEPYRLLTSGLRLHQELENLNKWGLNIFCVS DYAGGRSLTCIMYMIFQERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYH NSLHAADVLQSTHVLLATPALDAVFTDLEILAALFAAAIHDVDHPGVSNQF LINTNSELALMYNDESVLENHHLAVGFKLLQEDNCDIFQNLSKRQRQSLR KMVIDMVLATDMSKHMTLLADLKTMVETKKVTSSGVLLLDNYSDRIQVLR NMVHCADLSNPTKPLELYRQWTDRIMAEFFQQGDRERERGMEISPMCD KHTASVEKSQVQARGIDGRAQGGFY
PDE4A (Human Isoform 6) UniProtKB – P27815-6) SEQ ID NO:6	MRSGAAPRARPRPPALALPPTGPESLTHFPFSDEDTRRHPPGRSVSFEA ENGPTPSPGRSPLDSQASPGLVLHAGAATSQRRESFLYRSDSDYDMSP KTMSRNSSVTSEAHAEDLIVTPFAQVLASLRSVRSNFSLLTNVPVPSNKR SPLGGPTPVCKATLSEETCQQLARETLEELDWCLEQLETMQTYRSVSEM ASHKFKRMLNRELTHLSEMSRSGNQVSEYISTTFLDKQNEVEIPSPTMKE REKQQAPRPRPSQPPPPPVPHLQPMSQITGLKKLMHSNSLNNSNIPRFG VKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLK KFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDA VFTDLEILAALFAAAIHDVDHPGVSNQFLINTNSELALMYNDESVLENHHL AVGFKLLQEDNCDIFQNLSKRQRQSLRKMVIDMVLATDMSKHMTLLADLK TMVETKKVTSSGVLLLDNYSDRIQVLRNMVHCADLSNPTKPLELYRQWT DRIMAEFFQQGDRERERGMEISPMCDKHTASVEKSQVGFIDYIVHPLWE TWADLVHPDAQEILDTLEDNRDWYYSAIRQSPSPPPEEESRGPGHPPLP DKFQFELTLEEEEEEEISMAQIPCTAQEALTAQGLSGVEEALDATIAWEAS PAQESLEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVV AVSHSSPSALALQSPLLPAWRTLSVSEHAPGLPGLPSTAAEVEAQREHQ AAKRACSACAGTFGEDTSALPAPGGGGSGGDPT
PDE4A (Human Isoform 7) UniProtKB – P27815-7) SEQ ID NO:7	MKRSRSALSVAGTGDERSRETPESDRANMLGADLRRPRRRLSSGPGLG WAQPEPSDPGVPLPPRPTTLPLLIPPRISITRAENDSFEAENGPTPSPGRS PLDSQASPGLVLHAGAATSQRRESFLYRSDSDYDMSPKTMSRNSSVTSE AHAEDLIVTPFAQVLASLRSVRSNFSLLTNVPVPSNKRSPLGGPTPVCKA TLSEETCQQLARETLEELDWCLEQLETMQTYRSVSEMASHKFKRMLNRE LTHLSEMSRSGNQVSEYISTTFLDKQNEVEIPSPTMKEREKQQAPRPRPS QPPPPPVPHLQPMSQITGLKKLMHSNSLNNSNIPRFGVKTDQEELLAQEL ENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIPVDTMVTYM LTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDAVFTDLEILAALFAA AIHDVDHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEDNCD IFQNLSKRQRQSLRKMVIDMVLATDMSKHMTLLADLKTMVETKKVTSSGV LLLDNYSDRIQVLRNMVHCADLSNPTKPLELYRQWTDRIMAEFFQQGDR ERERGMEISPMCDKHTASVEKSQVGFIDYIVHPLWETWADLVHPDAQEIL DTLEDNRDWYYSAIRQSPSPPPEEESRGPGHPPLPDKFQFELTLEEEEE EEISMAQIPCTAQEALTAQGLSGVEEALDATIAWEASPAQESLEVMAQEA

	SLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVVAVSHSSPSALALQS PLLPAWRTLSVSEHAPGLPGLPSTAAEVEAQREHQAAKRACSACAGTFG EDTSALPAPGGGGSGGDPT
PDE4B (Human Isoform 1) UniProtKB – Q07343-1) SEQ ID NO:8	MKKSRSVMTVMADDNVKDYFECSLSKSYSSSSNTLGIDLWRGRRCCSG NLQLPPLSQRQSERARTPEGDGISRPTTLPLTTLPSIAITTVSQECFDVEN GPSPGRSPLDPQASSSAGLVLHATFPGHSQRRESFLYRSDSDYDLSPKA MSRNSSLPSEQHGDDLIVTPFAQVLASLRSVRNNFTILTNLHGTSNKRSP AASQPPVSRVNPQEESYQKLAMETLEELDWCLDQLETIQTYRSVSEMAS NKFKRMLNRELTHLSEMSRSGNQVSEYISNTFLDKQNDVEIPSPTQKDRE KKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNTENEDHLAKELEDLNK WGLNIFNVAGYSHNRPLTCIMYAIFQERDLLKTFRISSDTFITYMMTLEDH YHSDVAYHNSLHAADVAQSTHVLLSTPALDAVFTDLEILAAIFAAAIHDVD HPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLLQEEHCDIFMNLT KKQRQTLRKMVIDMVLATDMSKHMSLLADLKTMVETKKVTSSGVLLLDN YTDRIQVLRNMVHCADLSNPTKSLELYRQWTDRIMEEFFQQGDKERERG MEISPMCDKHTASVEKSQVGFIDYIVHPLWETWADLVQPDAQDILDTLED NRNWYQSMIPQSPSPPLDEQNRDCQGLMEKFQFELTLDEEDSEGPEKE GEGHSYFSSTKTLCVIDPENRDSLGETDIDIATEDKSPVDT
PDE4B (Human Isoform 2) UniProtKB – Q07343-2) SEQ ID NO:9	MKEHGGTFSSTGISGGSGDSAMDSLQPLQPNYMPVCLFAEESYQKLAM ETLEELDWCLDQLETIQTYRSVSEMASNKFKRMLNRELTHLSEMSRSGN QVSEYISNTFLDKQNDVEIPSPTQKDREKKKKQQLMTQISGVKKLMHSSS LNNTSISRFGVNTENEDHLAKELEDLNKWGLNIFNVAGYSHNRPLTCIMY AIFQERDLLKTFRISSDTFITYMMTLEDHYHSDVAYHNSLHAADVAQSTHV LLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTNSELALMYNDE SVLENHHLAVGFKLLQEEHCDIFMNLTKKQRQTLRKMVIDMVLATDMSKH MSLLADLKTMVETKKVTSSGVLLLDNYTDRIQVLRNMVHCADLSNPTKSL ELYRQWTDRIMEEFFQQGDKERERGMEISPMCDKHTASVEKSQVGFIDY IVHPLWETWADLVQPDAQDILDTLEDNRNWYQSMIPQSPSPPLDEQNRD CQGLMEKFQFELTLDEEDSEGPEKEGEGHSYFSSTKTLCVIDPENRDSL GETDIDIATEDKSPVDT
PDE4B (Human Isoform 3) UniProtKB – Q07343-3) SEQ ID NO:10	MTAKDSSKELTASEPEVCIKTFKEQMHLELELPRLPGNRPTSPKISPRSSP RNSPCFFRKLLVNKSIRQRRRFTVAHTCFDVENGPSPGRSPLDPQASSS AGLVLHATFPGHSQRRESFLYRSDSDYDLSPKAMSRNSSLPSEQHGDDL IVTPFAQVLASLRSVRNNFTILTNLHGTSNKRSPAASQPPVSRVNPQEES YQKLAMETLEELDWCLDQLETIQTYRSVSEMASNKFKRMLNRELTHLSE MSRSGNQVSEYISNTFLDKQNDVEIPSPTQKDREKKKKQQLMTQISGVK KLMHSSSLNNTSISRFGVNTENEDHLAKELEDLNKWGLNIFNVAGYSHNR PLTCIMYAIFQERDLLKTFRISSDTFITYMMTLEDHYHSDVAYHNSLHAAD VAQSTHVLLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTNSEL ALMYNDESVLENHHLAVGFKLLQEEHCDIFMNLTKKQRQTLRKMVIDMVL ATDMSKHMSLLADLKTMVETKKVTSSGVLLLDNYTDRIQVLRNMVHCADL SNPTKSLELYRQWTDRIMEEFFQQGDKERERGMEISPMCDKHTASVEKS QVGFIDYIVHPLWETWADLVQPDAQDILDTLEDNRNWYQSMIPQSPSPPL DEQNRDCQGLMEKFQFELTLDEEDSEGPEKEGEGHSYFSSTKTLCVIDP ENRDSLGETDIDIATEDKSPVDT
PDE4B (Human Isoform 5) UniProtKB – Q07343-4) SEQ ID NO:11	MPEANYLLSVSWGYIKFKRMLNRELTHLSEMSRSGNQVSEYISNTFLDK QNDVEIPSPTQKDREKKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNT ENEDHLAKELEDLNKWGLNIFNVAGYSHNRPLTCIMYAIFQERDLLKTFRI SSDTFITYMMTLEDHYHSDVAYHNSLHAADVAQSTHVLLSTPALDAVFTD LEILAAIFAAAIHDVDHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFK LLQEEHCDIFMNLTKKQRQTLRKMVIDMVLATDMSKHMSLLADLKTMVET KKVTSSGVLLLDNYTDRIQVLRNMVHCADLSNPTKSLELYRQWTDRIMEE FFQQGDKERERGMEISPMCDKHTASVEKSQVGFIDYIVHPLWETWADLV QPDAQDILDTLEDNRNWYQSMIPQSPSPPLDEQNRDCQGLMEKFQFELT LDEEDSEGPEKEGEGHSYFSSTKTLCVIDPENRDSLGETDIDIATEDKSPV DT

PDE4C (Human Isoform 1) UniProtKB – Q08493-1) SEQ ID NO:12	MENLGVGEGAEACSRLSRSRGRHSMTRAPKHLWRQPRRPIRIQQRFYS DPDKSAGCRERDLSPRPELRKSRLSWPVSSCRRFDLENGLSCGRRALD PQSSPGLGRIMQAPVPHSQRRESFLYRSDSDYELSPKAMSRNSSVASDL HGEDMIVTPFAQVLASLRTVRSNVAALARQQCLGAAKQGPVGNPSSSNQ LPPAEDTGQKLALETLDELDWCLDQLETLQTRHSVGEMASNKFKRILNRE LTHLSETSRSGNQVSEYISRTFLDQQTEVELPKVTAEEAPQPMSRISGLH GLCHSASLSSATVPRFGVQTDQEEQLAKELEDTNKWGLDVFKVAELSGN RPLTAIIFSIFQERDLLKTFQIPADTLATYLLMLEGHYHANVAYHNSLHAAD VAQSTHVLLATPALEAVFTDLEILAALFASAIHDVDHPGVSNQFLINTNSEL ALMYNDASVLENHHLAVGFKLLQAENCDIFQNLSAKQRLSLRRMVIDMVL ATDMSKHMNLLADLKTMVETKKVTSLGVLLLDNYSDRIQVLQNLVHCADL SNPTKPLPLYRQWTDRIMAEFFQQGDRERESGLDISPMCDKHTASVEKS QVGFIDYIAHPLWETWADLVHPDAQDLLDTLEDNREWYQSKIPRSPSDLT NPERDGPDRFQFELTLEEAEEEDEEEEEEGEETALAKEALELPDTELLSP EAGPDPGDLPLDNQRT
PDE4C (Human Isoform 2) UniProtKB – Q08493-2) SEQ ID NO:13	MQAPVPHSQRRESFLYRSDSDYELSPKAMSRNSSVASDLHGEDMIVTPF AQVLASLRTVRSNVAALARQQCLGAAKQGPVGNPSSSNQLPPAEDTGQ KLALETLDELDWCLDQLETLQTRHSVGEMASNKFKRILNRELTHLSETSR SGNQVSEYISRTFLDQQTEVELPKVTAEEAPQPMSRISGLHGLCHSASLS SATVPRFGVQTDQEEQLAKELEDTNKWGLDVFKVAELSGNRPLTAIIFSIF QERDLLKTFQIPADTLATYLLMLEGHYHANVAYHNSLHAADVAQSTHVLL ATPALEAVFTDLEILAALFASAIHDVDHPGVSNQFLINTNSELALMYNDASV LENHHLAVGFKLLQAENCDIFQNLSAKQRLSLRRMVIDMVLATDMSKHM NLLADLKTMVETKKVTSLGVLLLDNYSDRIQVLQNLVHCADLSNPTKPLPL YRQWTDRIMAEFFQQGDRERESGLDISPMCDKHTASVEKSQVGFIDYIA HPLWETWADLVHPDAQDLLDTLEDNREWYQSKIPRSPSDLTNPERDGP DRFQFELTLEEAEEEDEEEEEEGEETALAKEALELPDTELLSPEAGPDPG DLPLDNQRT
PDE4C (Human Isoform 3) UniProtKB – Q08493-3) SEQ ID NO:14	MQGPPAPAPVPGPGSPRGSPRGSPGLFRKLLVNQSIRLQRRFTVAHPLC FDLENGLSCGRRALDPQSSPGLGRIMQAPVPHSQRRESFLYRSDSDYEL SPKAMSRNSSVASDLHGEDMIVTPFAQVLASLRTVRSNVAALARQQCLG AAKQGPVGNPSSSNQLPPAEDTGQKLALETLDELDWCLDQLETLQTRHS VGEMASNKFKRILNRELTHLSETSRSGNQVSEYISRTFLDQQTEVELPKV TAEEAPQPMSRISGLHGLCHSASLSSATVPRFGVQTDQEEQLAKELEDT NKWGLDVFKVAELSGNRPLTAIIFSIFQERDLLKTFQIPADTLATYLLMLEG HYHANVAYHNSLHAADVAQSTHVLLATPALEAVFTDLEILAALFASAIHDV DHPGVSNQFLINTNSELALMYNDASVLENHHLAVGFKLLQAENCDIFQNL SAKQRLSLRRMVIDMVLATDMSKHMNLLADLKTMVETKKVTSLGVLLLDN YSDRIQVLQNLVHCADLSNPTKPLPLYRQWTDRIMAEFFQQGDRERESG LDISPMCDKHTASVEKSQVGFIDYIAHPLWETWADLVHPDAQDLLDTLED NREWYQSKIPRSPSDLTNPERDGPDRFQFELTLEEAEEEDEEEEEEGEE TALAKEALELPDTELLSPEAGPDPGDLPLDNQRT
PDE4D (Human Isoform 4) UniProtKB – Q08499-1) SEQ ID NO:15	MEAEGSSAPARAGSGEGSDSAGGATLKAPKHLWRHEQHHQYPLRQPQ FRLLHPHHHLPPPPPPSPQPQPQCPLQPPPPPPLPPPPPPPGAARGRYA SSGATGRVRHRGYSDTERYLYCRAMDRTSYAVETGHRPGLKKSRMSW PSSFQGLRRFDVDNGTSAGRSPLDPMTSPGSGLILQANFVHSQRRESFL YRSDSDYDLSPKSMSRNSSIASDIHGDDLIVTPFAQVLASLRTVRNNFAAL TNLQDRAPSKRSPMCNQPSINKATITEEAYQKLASETLEELDWCLDQLET LQTRHSVSEMASNKFKRMLNRELTHLSEMSRSGNQVSEFISNTFLDKQH EVEIPSPTQKEKEKKKRPMSQISGVKKLMHSSSLTNSSIPRFGVKTEQED VLAKELEDVNKWGLHVFRIAELSGNRPLTVIMHTIFQERDLLKTFKIPVDTL ITYLMTLEDHYHADVAYHNNIHAADVVQSTHVLLSTPALEAVFTDLEILAAI FASAIHDVDHPGVSNQFLINTNSELALMYNDSSVLENHHLAVGFKLLQEE NCDIFQNLTKKQRQSLRKMVIDIVLATDMSKHMNLLADLKTMVETKKVTS SGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQWTDRIMEEFFRQ GDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA

	QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEED GESDTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEE EESQPEACVIDDRSPDT
PDE4D (Human Isoform 3) UniProtKB – Q08499-2) SEQ ID NO:16	MMHVNNFPFRRHSWICFDVDNGTSAGRSPLDPMTSPGSGLILQANFVHS QRRESFLYRSDSDYDLSPKSMSRNSSIASDIHGDDLIVTPFAQVLASLRTV RNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQKLASETLEELDW CLDQLETLQTRHSVSEMASNKFKRMLNRELTHLSEMSRSGNQVSEFISN TFLDKQHEVEIPSPTQKEKEKKKRPMSQISGVKKLMHSSSLTNSSIPRFG VKTEQEDVLAKELEDVNKWGLHVFRIAELSGNRPLTVIMHTIFQERDLLKT FKIPVDTLITYLMTLEDHYHADVAYHNNIHAADVVQSTHVLLSTPALEAVFT DLEILAAIFASAIHDVDHPGVSNQFLINTNSELALMYNDSSVLENHHLAVGF KLLQEENCDIFQNLTKKQRQSLRKMVIDIVLATDMSKHMNLLADLKTMVE TKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQWTDRIM EEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWAD LVHPDAQDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFE LTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEE AVGEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 10) UniProtKB – Q08499-3) SEQ ID NO:17	MSRNSSIASDIHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSP MCNQPSINKATITEEAYQKLASETLEELDWCLDQLETLQTRHSVSEMASN KFKRMLNRELTHLSEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEK KKRPMSQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVLAKELEDVNKWG LHVFRIAELSGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLMTLEDHYHAD VAYHNNIHAADVVQSTHVLLSTPALEAVFTDLEILAAIFASAIHDVDHPGVS NQFLINTNSELALMYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQ SLRKMVIDIVLATDMSKHMNLLADLKTMVETKKVTSSGVLLLDNYSDRIQV LQNMVHCADLSNPTKPLQLYRQWTDRIMEEFFRQGDRERERGMEISPM CDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDAQDILDTLEDNREWY QSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGSQVE EDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSP DT
PDE4D (Human Isoform 1) UniProtKB – Q08499-4) SEQ ID NO:18	MKEQPSCAGTGHPMAGYGRMAPFELASGPVKRLRTESPFPCLFAEEAY QKLASETLEELDWCLDQLETLQTRHSVSEMASNKFKRMLNRELTHLSEM SRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKKRPMSQISGVKKLMH SSSLTNSSIPRFGVKTEQEDVLAKELEDVNKWGLHVFRIAELSGNRPLTVI MHTIFQERDLLKTFKIPVDTLITYLMTLEDHYHADVAYHNNIHAADVVQST HVLLSTPALEAVFTDLEILAAIFASAIHDVDHPGVSNQFLINTNSELALMYN DSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKMVIDIVLATDMS KHMNLLADLKTMVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTK PLQLYRQWTDRIMEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFI DYIVHPLWETWADLVHPDAQDILDTLEDNREWYQSTIPQSPSPAPDDPE EGRQGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCTQD SESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 2) UniProtKB – Q08499-5) SEQ ID NO:19	MASNKFKRMLNRELTHLSEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQK EKEKKKRPMSQISGVKKLMHSSSLTNSSIPRFGVKTEQEDVLAKELEDVN KWGLHVFRIAELSGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLMTLEDH YHADVAYHNNIHAADVVQSTHVLLSTPALEAVFTDLEILAAIFASAIHDVDH PGVSNQFLINTNSELALMYNDSSVLENHHLAVGFKLLQEENCDIFQNLTK KQRQSLRKMVIDIVLATDMSKHMNLLADLKTMVETKKVTSSGVLLLDNYS DRIQVLQNMVHCADLSNPTKPLQLYRQWTDRIMEEFFRQGDRERERGM EISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDAQDILDTLEDN REWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDS GSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVI DDRSPDT
PDE4D (Human Isoform 5)	MAQQTSPDTLTVPEVDNPHCPNPWLNEDLVKSLRENLLQHEKSKTARKS VSPKLSPVISPRNSPRLLRRMLLSSNIPKQRRFTVAHTCFDVDNGTSAGR SPLDPMTSPGSGLILQANFVHSQRRESFLYRSDSDYDLSPKSMSRNSSIA

UniProtKB – Q08499-6) SEQ ID NO:20	SDIHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSI NKATITEEAYQKLASETLEELDWCLDQLETLQTRHSVSEMASNKFKRMLN RELTHLSEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKKRPMSQ ISGVKKLMHSSSLTNSSIPRFGVKTEQEDVLAKELEDVNKWGLHVFRIAEL SGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLMTLEDHYHADVAYHNNIH AADVVQSTHVLLSTPALEAVFTDLEILAAIFASAIHDVDHPGVSNQFLINTN SELALMYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKMVIDI VLATDMSKHMNLLADLKTMVETKKVTSSGVLLLDNYSDRIQVLQNMVHC ADLSNPTKPLQLYRQWTDRIMEEFFRQGDRERERGMEISPMCDKHNAS VEKSQVGFIDYIVHPLWETWADLVHPDAQDILDTLEDNREWYQSTIPQSP SPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCSD SKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform N3) UniProtKB – Q08499-7) SEQ ID NO:21	MAQQTSPDTLTVPEVDNPHCPNPWLNEDLVKSLRENLLQHEKSKTARKS VSPKLSPVISPRNSPRLLRRMLLSSNIPKQRRFTVAHTCFDVDNGTSAGR SPLDPMTSPGSGLILQANFVHSQRRESFLYRSDSDYDLSPKSMSRNSSIA SDIHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSI NKATITGLYNGIIAFL
PDE4D (Human Isoform 6) UniProtKB – Q08499-8) SEQ ID NO:22	MPEANYLLSVSWGYIKFKRMLNRELTHLSEMSRSGNQVSEFISNTFLDKQ HEVEIPSPTQKEKEKKKRPMSQISGVKKLMHSSSLTNSSIPRFGVKTEQE DVLAKELEDVNKWGLHVFRIAELSGNRPLTVIMHTIFQERDLLKTFKIPVDT LITYLMTLEDHYHADVAYHNNIHAADVVQSTHVLLSTPALEAVFTDLEILAA IFASAIHDVDHPGVSNQFLINTNSELALMYNDSSVLENHHLAVGFKLLQEE NCDIFQNLTKKQRQSLRKMVIDIVLATDMSKHMNLLADLKTMVETKKVTS SGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQWTDRIMEEFFRQ GDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA QDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTEKFQFELTLEED GESDTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEE EESQPEACVIDDRSPDT
PDE4D (Human Isoform 8) UniProtKB – Q08499-9) SEQ ID NO:23	MAFVWDPLGATVPGPSTRAKSRLRFSKSYSFDVDNGTSAGRSPLDPMT SPGSGLILQANFVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASDIHGDD LIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEE AYQKLASETLEELDWCLDQLETLQTRHSVSEMASNKFKRMLNRELTHLS EMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKKRPMSQISGVKKL MHSSSLTNSSIPRFGVKTEQEDVLAKELEDVNKWGLHVFRIAELSGNRPL TVIMHTIFQERDLLKTFKIPVDTLITYLMTLEDHYHADVAYHNNIHAADVVQ STHVLLSTPALEAVFTDLEILAAIFASAIHDVDHPGVSNQFLINTNSELALM YNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKMVIDIVLATD MSKHMNLLADLKTMVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSN PTKPLQLYRQWTDRIMEEFFRQGDRERERGMEISPMCDKHNASVEKSQ VGFIDYIVHPLWETWADLVHPDAQDILDTLEDNREWYQSTIPQSPSPAPD DPEEGRQGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCT QDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 9) UniProtKB – Q08499-10) SEQ ID NO:24	MSIIMKPRSRSTSSLRTAEAVCEDVDNGTSAGRSPLDPMTSPGSGLILQA NFVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASDIHGDDLIVTPFAQVL ASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITEEAYQKLASET LEELDWCLDQLETLQTRHSVSEMASNKFKRMLNRELTHLSEMSRSGNQ VSEFISNTFLDKQHEVEIPSPTQKEKEKKKRPMSQISGVKKLMHSSSLTNS SIPRFGVKTEQEDVLAKELEDVNKWGLHVFRIAELSGNRPLTVIMHTIFQE RDLLKTFKIPVDTLITYLMTLEDHYHADVAYHNNIHAADVVQSTHVLLSTPA LEAVFTDLEILAAIFASAIHDVDHPGVSNQFLINTNSELALMYNDSSVLENH HLAVGFKLLQEENCDIFQNLTKKQRQSLRKMVIDIVLATDMSKHMNLLADL KTMVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW TDRIMEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLW ETWADLVHPDAQDILDTLEDNREWYQSTIPQSPSPAPDDPEEGRQGQTE

	KFQFELTLEEDGESDTEKDSGSQVEEDTSCSDSKTLCTQDSESTEIPLDE QVEEEAVGEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 7) UniProtKB – Q08499-11) SEQ ID NO:25	MKRNTCDLLSRSKSASEETLHSSNEEEDPFRGMEPYLVRRLSCRNIQLP PLAFRQLEQADLKSESENIQRPTSLPLKILPLIAITSAESSGFDVDNGTSAG RSPLDPMTSPGSGLILQANFVHSQRRESFLYRSDSDYDLSPKSMSRNSSI ASDIHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPS INKATITEEAYQKLASETLEELDWCLDQLETLQTRHSVSEMASNKFKRML NRELTHLSEMSRSGNQVSEFISNTFLDKQHEVEIPSPTQKEKEKKKRPMS QISGVKKLMHSSSLTNSSIPRFGVKTEQEDVLAKELEDVNKWGLHVFRIA ELSGNRPLTVIMHTIFQERDLLKTFKIPVDTLITYLMTLEDHYHADVAYHNN IHAADVVQSTHVLLSTPALEAVFTDLEILAAIFASAIHDVDHPGVSNQFLINT NSELALMYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKMVI DIVLATDMSKHMNLLADLKTMVETKKVTSSGVLLLDNYSDRIQVLQNMVH CADLSNPTKPLQLYRQWTDRIMEEFFRQGDRERERGMEISPMCDKHNA SVEKSQVGFIDYIVHPLWETWADLVHPDAQDILDTLEDNREWYQSTIPQS PSPAPDDPEEGRQGQTEKFQFELTLEEDGESDTEKDSGSQVEEDTSCS DSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT
PDE4D (Human Isoform 12) UniProtKB – Q08499-12) SEQ ID NO:26	MAQQTSPDTLTVPEVDNPHCPNPWLNEDLVKSLRENLLQHEKSKTARKS VSPKLSPVISPRNSPRLLRRMLLSSNIPKQRRFTVAHTCFDVDNGTSAGR SPLDPMTSPGSGLILQANFVHSQRRESFLYRSDSDYDLSPKSMSRNSSIA 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 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, 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%, 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 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 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 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 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), 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 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 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 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 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 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 (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, vigabartrin,

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 EC_{50}s 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 Mediomics, 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, $MgCl_2$, KCl, $MgSO_4$), 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, 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, 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 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 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 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 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 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, 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.

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. 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 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 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, 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 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.

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 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.

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 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 embodiments 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 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 Ibhazehiebo 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 1μM and 10μM fully restoring OCR response to WT levels (FIG. 6, panel A). The 1nM-100nM doses were ineffective and the 100μM dose was toxic as demonstrated by the drop if 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 μM 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 μM) 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 itcal (>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 μM) 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-morpolinos 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 μM). 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

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 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.

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, 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 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 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 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 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 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.

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.

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.

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 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.

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.

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.

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.

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.

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.

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.

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

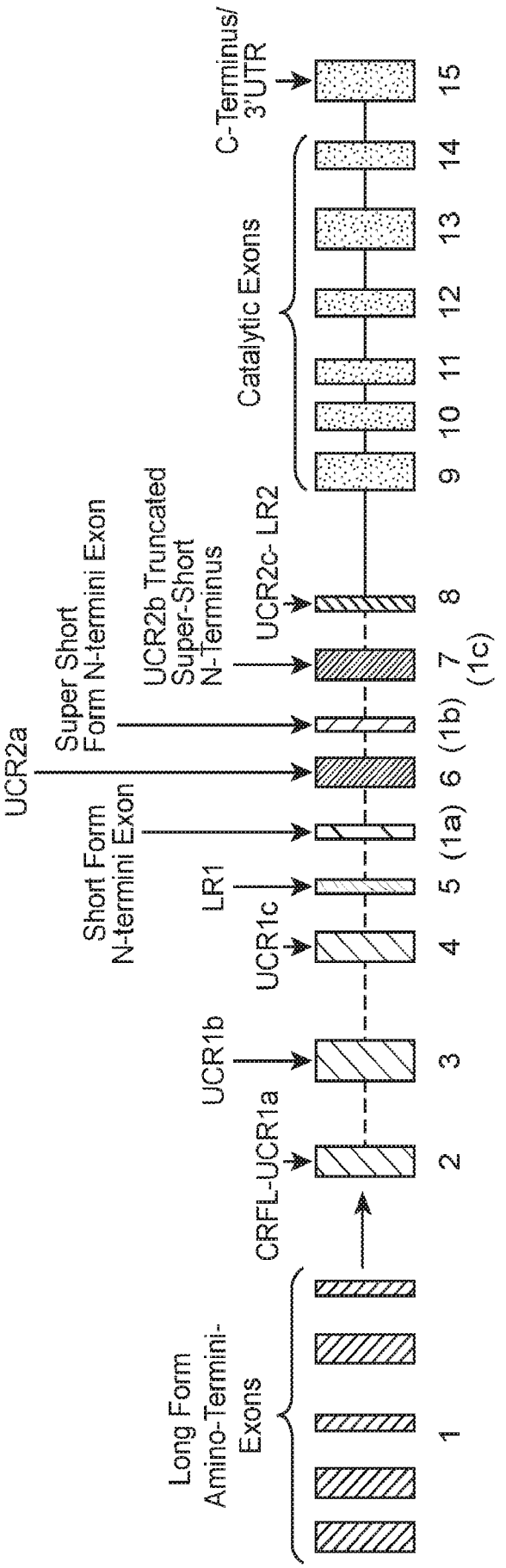
Long Form Amino-Termini-Exons
CRFL-UCR1a
UCR1b
UCR1c
LR1
Short Form N-termini Exon
UCR2a
Super Short Form N-termini Exon
UCR2b Truncated Super-Short N-Terminus
UCR2c- LR2
Catalytic Exons
C-Terminus/ 3'UTR
1
2
3
4
5 (1a)
6 (1b)
7 (1c)
8
9
10
11
12
13
14
15

FIG. 1

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P27815    PDE4A_HUMAN     1 MEPPTVPSERSLSLSLPGPREGQATLKPPPQHLWRQPRTP-----IRI--QQRGYSDSAE   53
P27815-2  PDE4A_HUMAN     1 ------------MA-------------RPRGLGRIPELQLVAFPVAVAAEDEAFLPEPL   34
P27815-3  PDE4A_HUMAN     1 ------------------------------------------------------------    0
P27815-4  PDE4A_HUMAN     1 ------------------------------------------------------------    0
P27815-5  PDE4A_HUMAN     1 ------------------------------------------------------------    0
P27815-6  PDE4A_HUMAN     1 ------------------------------------------------------MRSGA    5
P27815-7  PDE4A_HUMAN     1 ----MKRS--RSALSVAG--------------------------------TGDERSRE   20

P27815    PDE4A_HUMAN    54 RAERE----------RQPHRPTERADAM----DTSDRPGLRTTRMSWPSSFHGTGTGSGGA  100
P27815-2  PDE4A_HUMAN    35 APRAP----------RRPRSPPSS-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-------GPESLTHFPFSDEDTRRH   39
P27815-7  PDE4A_HUMAN    21 TPESDRAWMLGADLRRPRRRLSSGPGLGWAQPEPSDPGVP--LPPRPTTLPLLIPPRISI   78

P27815    PDE4A_HUMAN   101 GGGSSRRFEAEMGPTPSPGRSPLDSQASPGLVLHAGAATSQRRESFLYRSDSDYDMSPKT  160
P27815-2  PDE4A_HUMAN    75 QAWAGAGFEAEMGPTPSPGRSPLDSQASPGLVLHAGAATSQRRESFLYRSDSDYDMSPKT  134
P27815-3  PDE4A_HUMAN     1 ------------------------------------------------------------    0
P27815-4  PDE4A_HUMAN     1 ------------------------------------------------------------    0
P27815-5  PDE4A_HUMAN     1 ------------------------------------------------------------    0
P27815-6  PDE4A_HUMAN    40 PPGRSVSFEAEMGPTPSPGRSPLDSQASPGLVLHAGAATSQRRESFLYRSDSDYDMSPKT   99
P27815-7  PDE4A_HUMAN    79 TRAENDSFEAEMGPTPSPGRSPLDSQASPGLVLHAGAATSQRRESFLYRSDSDYDMSPKT  138

P27815    PDE4A_HUMAN   161 MSRMSSVTSEAHAEDLIVTPFAQVLASLRSVRSNFSLLTNVPVPSMKRSPLGGPTPVCKA  220
P27815-2  PDE4A_HUMAN   135 MSRMSSVTSEAHAEDLIVTPFAQVLASLRSVRSNFSLLTNVPVPSMKRSPLGGPTPVCKA  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 MSRMSSVTSEAHAEDLIVTPFAQVLASLRSVRSNFSLLTNVPVPSMKRSPLGGPTPVCKA  159
P27815-7  PDE4A_HUMAN   139 MSRMSSVTSEAHAEDLIVTPFAQVLASLRSVRSNFSLLTNVPVPSMKRSPLGGPTPVCKA  198
```

FIG. 2

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

P27815     PDE4A_HUMAN  281  NQVSEYISTTFLDKQNEVEIPSPTMKEREKQQAPRPRPSQPPPPPVPHLQPMSQITGLKK  340
P27815-2   PDE4A_HUMAN  255  NQVSEYISTTFLDKQNEVEIPSPTMKEREKQQAPRPRPSQPPPPPVPHLQPMSQITGLKK  314
P27815-3   PDE4A_HUMAN   81  NQVSEYISTTFLDKQNEVEIPSPTMKEREKQQAPRPRPSQPPPPPVPHLQPMSQITGLKK  140
P27815-4   PDE4A_HUMAN   42  NQVSEYISTTFLDKQNEVEIPSPTMKEREKQQAPRPRPSQPPPPPVPHLQPMSQITGLKK  101
P27815-5   PDE4A_HUMAN    1  -----------------------------------------------MVLP--SDQGFKL   11
P27815-6   PDE4A_HUMAN  220  NQVSEYISTTFLDKQNEVEIPSPTMKEREKQQAPRPRPSQPPPPPVPHLQPMSQITGLKK  279
P27815-7   PDE4A_HUMAN  259  NQVSEYISTTFLDKQNEVEIPSPTMKEREKQQAPRPRPSQPPPPPVPHLQPMSQITGLKK  318
                                                                            : *   .   *:* 

P27815     PDE4A_HUMAN  341  LMHSNSLNNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQ  400
P27815-2   PDE4A_HUMAN  315  LMHSNSLNNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQ  374
P27815-3   PDE4A_HUMAN  141  LMHSNSLNNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQ  200
P27815-4   PDE4A_HUMAN  102  LMHSNSLNNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQ  161
P27815-5   PDE4A_HUMAN   12  L--GNVLQGPE--PYR-LLTSGLRLHQELENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQ   66
P27815-6   PDE4A_HUMAN  280  LMHSNSLNNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQ  339
P27815-7   PDE4A_HUMAN  319  LMHSNSLNNSNIPRFGVKTDQEELLAQELENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQ  378
                             *  .* *:.  :   :     .   * *********************************

P27815     PDE4A_HUMAN  401  ERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDAVFTD  460
P27815-2   PDE4A_HUMAN  375  ERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDAVFTD  434
P27815-3   PDE4A_HUMAN  201  ERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDAVFTD  260
P27815-4   PDE4A_HUMAN  162  ERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDAVFTD  221
P27815-5   PDE4A_HUMAN   67  ERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDAVFTD  126
P27815-6   PDE4A_HUMAN  340  ERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDAVFTD  399
P27815-7   PDE4A_HUMAN  379  ERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDAVFTD  438
                             ************************************************************
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FIG. 2 (Cont.)

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

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

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

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

```
P27815     PDE4A_HUMAN  701 PPLPDKFQFELTLEEEEEEEISMAQIPCTAQEALTAQGLSGVEEALDATIAWEASPAQES 760
P27815-2   PDE4A_HUMAN  675 PPLPDKFQFELTLEEEEEEEISMAQIPCTAQEALTAQGLSGVEEALDATIAWEASPAQES 734
P27815-3   PDE4A_HUMAN  501 PPLPDKFQFELTLEEEEEEEISMAQIPCTAQEALTAQGLSGVEEALDATIAWEASPAQES 560
P27815-4   PDE4A_HUMAN  462 PPLPDKFQFELTLEEEEEEEISMAQIPCTAQEALTAQGLSGVEEALDATIAWEASPAQES 521
P27815-5   PDE4A_HUMAN  324 ---------------------------------------------------------- 323
P27815-6   PDE4A_HUMAN  640 PPLPDKFQFELTLEEEEEEEISMAQIPCTAQEALTAQGLSGVEEALDATIAWEASPAQES 699
P27815-7   PDE4A_HUMAN  679 PPLPDKFQFELTLEEEEEEEISMAQIPCTAQEALTAQGLSGVEEALDATIAWEASPAQES 738

P27815     PDE4A_HUMAN  761 LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVVAVSHSSPSALALQSPL 820
P27815-2   PDE4A_HUMAN  735 LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVVAVSHSSPSALALQSPL 794
P27815-3   PDE4A_HUMAN  561 LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVVAVSHSSPSALALQSPL 620
P27815-4   PDE4A_HUMAN  522 LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVVAVSHSSPSALALQSPL 581
P27815-5   PDE4A_HUMAN  324 ---------------------------------------------------------- 323
P27815-6   PDE4A_HUMAN  700 LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVVAVSHSSPSALALQSPL 759
P27815-7   PDE4A_HUMAN  739 LEVMAQEASLEAELEAVYLTQQAQSTGSAPVAPDEFSSREEFVVAVSHSSPSALALQSPL 798

P27815     PDE4A_HUMAN  821 LPAWRTLSVSEHAPGLPGLPSTAAEVEAQREHQAAKRACSACAGTFGEDTSALPAPGGGG 880
P27815-2   PDE4A_HUMAN  795 LPAWRTLSVSEHAPGLPGLPSTAAEVEAQREHQAAKRACSACAGTFGEDTSALPAPGGGG 854
P27815-3   PDE4A_HUMAN  621 LPAWRTLSVSEHAPGLPGLPSTAAEVEAQREHQAAKRACSACAGTFGEDTSALPAPGGGG 680
P27815-4   PDE4A_HUMAN  582 LPAWRTLSVSEHAPGLPGLPSTAAEVEAQREHQAAKRACSACAGTFGEDTSALPAPGGGG 641
P27815-5   PDE4A_HUMAN  324 ---------------------------------------------------------- 323
P27815-6   PDE4A_HUMAN  760 LPAWRTLSVSEHAPGLPGLPSTAAEVEAQREHQAAKRACSACAGTFGEDTSALPAPGGGG 819
P27815-7   PDE4A_HUMAN  799 LPAWRTLSVSEHAPGLPGLPSTAAEVEAQREHQAAKRACSACAGTFGEDTSALPAPGGGG 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  MKKSRSVMTVMADDNVKDYFECSLSKSYSSSSNTLGIDLWRGRRCCSGNLQLPPLSQRQS  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----------LPLTTLPSIAITTVSQECFDVEWGPSPGRSPLDPQA 112
Q07343-2  PDE4B_HUMAN    1  ------------------------------------------------------------   0
Q07343-3  PDE4B_HUMAN   40  --PTSPKISPRSSPRMSPCFFRKLLVWKSIRQRRRFTVAHICFDVEWGPSPGRSPLDPQA  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  LASLRSVRWNFTILTWLHGTSNKRSPAASQ---PP-VSRVMPQEESYQKLAMETLEELDW 228
Q07343-2  PDE4B_HUMAN    7  ----------TFSS-TGISGGSGDSAMDSLQPLQPNYMPVCLFAEESYQKLAMETLEELDW  56
Q07343-3  PDE4B_HUMAN  158  LASLRSVRWNFTILTWLHGTSNKRSPAASQPP----VSRVMPQEESYQKLAMETLEELDW 213
Q07343-4  PDE4B_HUMAN    1  ------------------------------------------------------------   0
```

FIG. 3

```
Q07343    PDE4B_HUMAN   229 CLDQLETIQTYRSVSEMASNKFKRMLNRELTHLSEMSRSGMQVSEYISNTFLDKQMDVEI 288
Q07343-2  PDE4B_HUMAN    57 CLDQLETIQTYRSVSEMASNKFKRMLNRELTHLSEMSRSGMQVSEYISNTFLDKQMDVEI 116
Q07343-3  PDE4B_HUMAN   214 CLDQLETIQTYRSVSEMASNKFKRMLNRELTHLSEMSRSGMQVSEYISNTFLDKQMDVEI 273
Q07343-4  PDE4B_HUMAN     1 -----MPEANYLLSVSWGYIKFKRMLNRELTHLSEMSRSGMQVSEYISNTFLDKQMDVEI  55
                               .*   . .  ************************************************

Q07343    PDE4B_HUMAN   289 PSPTQKDREKKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNTEMEDHLAKELEDLNKW 348
Q07343-2  PDE4B_HUMAN   117 PSPTQKDREKKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNTEMEDHLAKELEDLNKW 176
Q07343-3  PDE4B_HUMAN   274 PSPTQKDREKKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNTEMEDHLAKELEDLNKW 333
Q07343-4  PDE4B_HUMAN    56 PSPTQKDREKKKKQQLMTQISGVKKLMHSSSLNNTSISRFGVNTEMEDHLAKELEDLNKW 115
                             ************************************************************

Q07343    PDE4B_HUMAN   349 GLNIFMVAGYSHNRPLTCIMYAIFQERDLLKTFRISSDTFITYMMTLEDHYHSDVAYHNS 408
Q07343-2  PDE4B_HUMAN   177 GLNIFMVAGYSHMRPLTCIMYAIFQERDLLKTFRISSDTFITYMMTLEDHYHSDVAYHNS 236
Q07343-3  PDE4B_HUMAN   334 GLNIFMVAGYSHMRPLTCIMYAIFQERDLLKTFRISSDTFITYMMTLEDHYHSDVAYHNS 393
Q07343-4  PDE4B_HUMAN   116 GLNIFMVAGYSHMRPLTCIMYAIFQERDLLKTFRISSDTFITYMMTLEDHYHSDVAYHNS 175
                             ************************************************************

Q07343    PDE4B_HUMAN   409 LHAADVAQSTHVLLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTNSELALM 468
Q07343-2  PDE4B_HUMAN   237 LHAADVAQSTHVLLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTNSELALM 296
Q07343-3  PDE4B_HUMAN   394 LHAADVAQSTHVLLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTNSELALM 453
Q07343-4  PDE4B_HUMAN   176 LHAADVAQSTHVLLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTNSELALM 235
                             ************************************************************
```

FIG. 3 (Cont.)

```
Q07343     PDE4B_HUMAN  469 YNDESVLENHHLAVGFKLLQEEHCDIFMNLTKKQRQTLRKMVIDMVLATDMSKHMSLLAD
Q07343-2   PDE4B_HUMAN  297 YNDESVLENHHLAVGFKLLQEEHCDIFMNLTKKQRQTLRKMVIDMVLATDMSKHMSLLAD
Q07343-3   PDE4B_HUMAN  454 YNDESVLENHHLAVGFKLLQEEHCDIFMNLTKKQRQTLRKMVIDMVLATDMSKHMSLLAD
Q07343-4   PDE4B_HUMAN  236 YNDESVLENHHLAVGFKLLQEEHCDIFMNLTKKQRQTLRKMVIDMVLATDMSKHMSLLAD
                           ************************************************************

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

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

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

Q07343     PDE4B_HUMAN  709 CVIDPENRDSLGETDIDIATEDKSPVDT
Q07343-2   PDE4B_HUMAN  537 CVIDPENRDSLGETDIDIATEDKSPVDT
Q07343-3   PDE4B_HUMAN  694 CVIDPENRDSLGETDIDIATEDKSPVDT
Q07343-4   PDE4B_HUMAN  476 CVIDPENRDSLGETDIDIATEDKSPVDT
                           ****************************
```

FIG. 3 (Cont.)

```
Q08493   PDE4C_HUMAN    1 MENLGVGEGAEACSRLSRSRGRHSMTRAPKHLWRQPRRPIRIQ--QRFYSDPDKSAGCRE  58
Q08493-2 PDE4C_HUMAN    1 ------------------------------------------------------------   0
Q08493-3 PDE4C_HUMAN    1 --------------------------------MQGPPAPAPVPGPGSPRGSPRGSPGLFR  28

Q08493   PDE4C_HUMAN   59 RDLSPRPELRKSRLSWPVSSCRRFDLENGLSCGRRALDPQSSPGLGRIMQAPVPHSQRRE 118
Q08493-2 PDE4C_HUMAN    1 ------------------------------------------------MQAPVPHSQRRE  12
Q08493-3 PDE4C_HUMAN   29 KLLV-NQSI-RLQRRFTVAHPLCFDLENGLSCGRRALDPQSSPGLGRIMQAPVPHSQRRE  86
                                                                         ************

Q08493   PDE4C_HUMAN  119 SFLYRSDSDYELSPKAMSRNSSVASDLHGEDMIVTPFAQVLASLRTVRSNVAALARQQCL 178
Q08493-2 PDE4C_HUMAN   13 SFLYRSDSDYELSPKAMSRNSSVASDLHGEDMIVTPFAQVLASLRTVRSNVAALARQQCL  72
Q08493-3 PDE4C_HUMAN   87 SFLYRSDSDYELSPKAMSRNSSVASDLHGEDMIVTPFAQVLASLRTVRSNVAALARQQCL 146
                          ************************************************************

Q08493   PDE4C_HUMAN  179 GAAKQGPVGNPSSSNQLPPAEDTGQKLALETLDELDWCLDQLETLQTRHSVGEMASNKFK 238
Q08493-2 PDE4C_HUMAN   73 GAAKQGPVGNPSSSNQLPPAEDTGQKLALETLDELDWCLDQLETLQTRHSVGEMASNKFK 132
Q08493-3 PDE4C_HUMAN  147 GAAKQGPVGNPSSSNQLPPAEDTGQKLALETLDELDWCLDQLETLQTRHSVGEMASNKFK 206
                          ************************************************************

Q08493   PDE4C_HUMAN  239 RILNRELTHLSETSRSGNQVSEYISRTFLDQQTEVELPKVTAEEAPQPMSRISGLHGLCH 298
Q08493-2 PDE4C_HUMAN  133 RILNRELTHLSETSRSGNQVSEYISRTFLDQQTEVELPKVTAEEAPQPMSRISGLHGLCH 192
Q08493-3 PDE4C_HUMAN  207 RILNRELTHLSETSRSGNQVSEYISRTFLDQQTEVELPKVTAEEAPQPMSRISGLHGLCH 266
                          ************************************************************
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FIG. 4

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

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

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

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

Q08493    PDE4C_HUMAN 539 QNLVHCADLSNPTKPLPLYRQWTDRIMAEFFQQGDRERESGLDISPMCDKHTASVEKSQV 598
Q08493-2 PDE4C_HUMAN 433 QNLVHCADLSNPTKPLPLYRQWTDRIMAEFFQQGDRERESGLDISPMCDKHTASVEKSQV 492
Q08493-3 PDE4C_HUMAN 507 QNLVHCADLSNPTKPLPLYRQWTDRIMAEFFQQGDRERESGLDISPMCDKHTASVEKSQV 566
                         ************************************************************
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FIG. 4 (Cont.)

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Q08493    PDE4C_HUMAN  599  GFIDYIAHPLWETWADLVHPDAQDLLDTLEDNREWYQSKIPRSPSDLTNPERDGPDRFQF  658
Q08493-2  PDE4C_HUMAN  493  GFIDYIAHPLWETWADLVHPDAQDLLDTLEDNREWYQSKIPRSPSDLTNPERDGPDRFQF  552
Q08493-3  PDE4C_HUMAN  567  GFIDYIAHPLWETWADLVHPDAQDLLDTLEDNREWYQSKIPRSPSDLTNPERDGPDRFQF  626
                             ************************************************************

Q08493    PDE4C_HUMAN  659  ELTLEEAEEEDEEEEEGEETALAKEALELPDTELLSPEAGPDPGDLPLDWQRT  712
Q08493-2  PDE4C_HUMAN  553  ELTLEEAEEEDEEEEEGEETALAKEALELPDTELLSPEAGPDPGDLPLDWQRT  606
Q08493-3  PDE4C_HUMAN  627  ELTLEEAEEEDEEEEEGEETALAKEALELPDTELLSPEAGPDPGDLPLDWQRT  680
                             **********************************************************
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FIG. 4 (Cont.)

```
Q08499    PDE4D_HUMAN   1 MEAEGSSAPARAGSGEGSDSAGGATLKAPKHLWRHEQHHQYPLRQPQFRLLHPHHHLPPP  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 ---------------------------------------------------MAQQTSPDT   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 PPPSPQPQPQCPLQPPPPPPLPPPPPPPGAARGRYASSGATGRVRHRGYSDTER----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 -----MAQQTSPDTLTVPEVDNPHCPNPWLNED----------------------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 -----MKRNTCDLLSRSKSA----------SEETLHS----SNEEEDPFRGNEPYLVRRL  41
Q08499-12 PDE4D_HUMAN   1 -----MAQQTSPDTLTVPEVDNPHCPNPWLNED----------------------LVKSL  33

Q08499    PDE4D_HUMAN 117 YCRANDRTS-YAV----------ET--GHRPELKK---SRMSMP---SSFQGLRRFDVDN 157
Q08499-2  PDE4D_HUMAN   1 ------------------------------MM---HVNNFP---FRRHSMICFDVDN  21
Q08499-3  PDE4D_HUMAN   1 ------------------------------------------------------------   0
Q08499-4  PDE4D_HUMAN   1 ------------------------------------------------------------   0
Q08499-5  PDE4D_HUMAN   1 ------------------------------------------------------------   0
Q08499-6  PDE4D_HUMAN  58 VS---PKLS-PVI----------SP--RNSPRLLRRNLLSSNIPKQRRFTVAHTCFDVDN  93
Q08499-7  PDE4D_HUMAN  34 RENLLQHEKSKTARKSVSPKLSPVISPRNSPRLLRRNLLSSNIPKQRRFTVAHTCFDVDN  93
Q08499-8  PDE4D_HUMAN   1 ------------------------------------------------------------   0
Q08499-9  PDE4D_HUMAN   1 -------MA-FVN----------DPLGATVPG-------PSTRAKSRLRFSKSYSFDVDN  35
Q08499-10 PDE4D_HUMAN   1 ------------------------------MSIINKPRSRSTSSLRTAEAVCFDVDN  27
Q08499-11 PDE4D_HUMAN  42 SCRNIQLPP-LAFRQLEQADLKSESENIQRPTSLPLK----ILPLIAITSAESSGFDVDN  96
Q08499-12 PDE4D_HUMAN  34 RENLLQHEKSKTARKSVSPKLSPVISPRNSPRLLRRNLLSSNIPKQRRFTVAHTCFDVDN  93
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FIG. 5

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Q08499    PDE4D_HUMAN 158 GTSAGRSPLDPMTSPGSGLILQANPVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASD-- 215
Q08499-2  PDE4D_HUMAN  22 GTSAGRSPLDPMTSPGSGLILQANPVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASD--  79
Q08499-3  PDE4D_HUMAN   1 ----------------------------------------------MSRNSSIASD--  10
Q08499-4  PDE4D_HUMAN   1 ----------------------------------------------MKEQPSCAGTGH  12
Q08499-5  PDE4D_HUMAN   1 ------------------------------------------------------------   0
Q08499-6  PDE4D_HUMAN  94 GTSAGRSPLDPMTSPGSGLILQANPVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASD-- 151
Q08499-7  PDE4D_HUMAN  94 GTSAGRSPLDPMTSPGSGLILQANPVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASD-- 151
Q08499-8  PDE4D_HUMAN   1 ------------------------------------------------------------   0
Q08499-9  PDE4D_HUMAN  36 GTSAGRSPLDPMTSPGSGLILQANPVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASD--  93
Q08499-10 PDE4D_HUMAN  28 GTSAGRSPLDPMTSPGSGLILQANPVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASD--  85
Q08499-11 PDE4D_HUMAN  97 GTSAGRSPLDPMTSPGSGLILQANPVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASD-- 154
Q08499-12 PDE4D_HUMAN  94 GTSAGRSPLDPMTSPGSGLILQANPVHSQRRESFLYRSDSDYDLSPKSMSRNSSIASD-- 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 PNAGY-GRNAPFELAS------------------GPVKR---LRTESPFPCLFAEEAYQ  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 -IHGDDLIVTPFAQVLASLRTVRNNFAALTNLQDRAPSKRSPMCNQPSINKATITGSWME 210

Q08499    PDE4D_HUMAN 275 KLASETLEELDWCLDQLETLQTRHSVSENASNKFKRMLNRELTHLSEMSRSGNQVSEFIS 334
Q08499-2  PDE4D_HUMAN 139 KLASETLEELDWCLDQLETLQTRHSVSENASNKFKRMLNRELTHLSEMSRSGNQVSEFIS 198
Q08499-3  PDE4D_HUMAN  70 KLASETLEELDWCLDQLETLQTRHSVSENASNKFKRMLNRELTHLSEMSRSGNQVSEFIS 129
Q08499-4  PDE4D_HUMAN  50 KLASETLEELDWCLDQLETLQTRHSVSENASNKFKRMLNRELTHLSEMSRSGNQVSEFIS 109
Q08499-5  PDE4D_HUMAN   1 --------------------------NASNKFKRMLNRELTHLSEMSRSGNQVSEFIS  32
Q08499-6  PDE4D_HUMAN 211 KLASETLEELDWCLDQLETLQTRHSVSENASNKFKRMLNRELTHLSEMSRSGNQVSEFIS 270
Q08499-7  PDE4D_HUMAN 211 IIAFL------------------------------------------------------ 215
Q08499-8  PDE4D_HUMAN   1 ------NPEANYL----------LSVSWGYIKFKRMLNRELTHLSEMSRSGNQVSEFIS  43
Q08499-9  PDE4D_HUMAN 153 KLASETLEELDWCLDQLETLQTRHSVSENASNKFKRMLNRELTHLSEMSRSGNQVSEFIS 212
Q08499-10 PDE4D_HUMAN 145 KLASETLEELDWCLDQLETLQTRHSVSENASNKFKRMLNRELTHLSEMSRSGNQVSEFIS 204
Q08499-11 PDE4D_HUMAN 214 KLASETLEELDWCLDQLETLQTRHSVSENASNKFKRMLNRELTHLSEMSRSGNQVSEFIS 273
Q08499-12 PDE4D_HUMAN 211 LNPYTLLDM---------------------------------------------------- 219
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FIG. 5 (Cont.)

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

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

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

```
Q08499     PDE4D_HUMAN 515 INTNSELALNYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKNVIDIVLATD 574
Q08499-2   PDE4D_HUMAN 379 INTNSELALNYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKNVIDIVLATD 438
Q08499-3   PDE4D_HUMAN 310 INTNSELALNYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKNVIDIVLATD 369
Q08499-4   PDE4D_HUMAN 290 INTNSELALNYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKNVIDIVLATD 349
Q08499-5   PDE4D_HUMAN 213 INTNSELALNYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKNVIDIVLATD 272
Q08499-6   PDE4D_HUMAN 451 INTNSELALNYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKNVIDIVLATD 510
Q08499-7   PDE4D_HUMAN 216 ------------------------------------------------------------ 215
Q08499-8   PDE4D_HUMAN 224 INTNSELALNYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKNVIDIVLATD 283
Q08499-9   PDE4D_HUMAN 393 INTNSELALNYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKNVIDIVLATD 452
Q08499-10  PDE4D_HUMAN 385 INTNSELALNYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKNVIDIVLATD 444
Q08499-11  PDE4D_HUMAN 454 INTNSELALNYNDSSVLENHHLAVGFKLLQEENCDIFQNLTKKQRQSLRKNVIDIVLATD 513
Q08499-12  PDE4D_HUMAN 220 ------------------------------------------------------------ 219

Q08499     PDE4D_HUMAN 575 MSKHMNLLADLKTNVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW 634
Q08499-2   PDE4D_HUMAN 439 MSKHMNLLADLKTNVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW 498
Q08499-3   PDE4D_HUMAN 370 MSKHMNLLADLKTNVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW 429
Q08499-4   PDE4D_HUMAN 350 MSKHMNLLADLKTNVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW 409
Q08499-5   PDE4D_HUMAN 273 MSKHMNLLADLKTNVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW 332
Q08499-6   PDE4D_HUMAN 511 MSKHMNLLADLKTNVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW 570
Q08499-7   PDE4D_HUMAN 216 ------------------------------------------------------------ 215
Q08499-8   PDE4D_HUMAN 284 MSKHMNLLADLKTNVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW 343
Q08499-9   PDE4D_HUMAN 453 MSKHMNLLADLKTNVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW 512
Q08499-10  PDE4D_HUMAN 445 MSKHMNLLADLKTNVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW 504
Q08499-11  PDE4D_HUMAN 514 MSKHMNLLADLKTNVETKKVTSSGVLLLDNYSDRIQVLQNMVHCADLSNPTKPLQLYRQW 573
Q08499-12  PDE4D_HUMAN 220 ------------------------------------------------------------ 219

Q08499     PDE4D_HUMAN 635 TDRINEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA 694
Q08499-2   PDE4D_HUMAN 499 TDRINEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA 558
Q08499-3   PDE4D_HUMAN 430 TDRINEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA 489
Q08499-4   PDE4D_HUMAN 410 TDRINEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA 469
Q08499-5   PDE4D_HUMAN 333 TDRINEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA 392
Q08499-6   PDE4D_HUMAN 571 TDRINEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA 630
Q08499-7   PDE4D_HUMAN 216 ------------------------------------------------------------ 215
Q08499-8   PDE4D_HUMAN 344 TDRINEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA 403
Q08499-9   PDE4D_HUMAN 513 TDRINEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA 572
Q08499-10  PDE4D_HUMAN 505 TDRINEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA 564
Q08499-11  PDE4D_HUMAN 574 TDRINEEFFRQGDRERERGMEISPMCDKHNASVEKSQVGFIDYIVHPLWETWADLVHPDA 633
Q08499-12  PDE4D_HUMAN 220 ------------------------------------------------------------ 219
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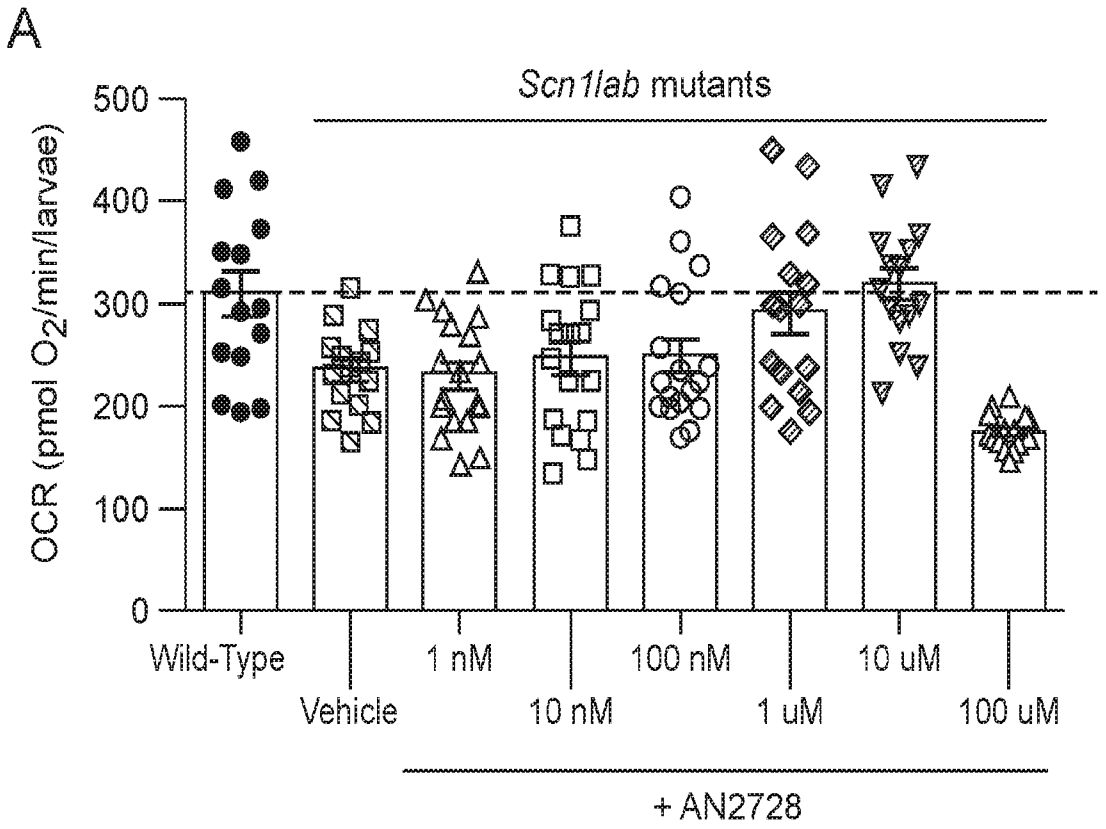
FIG. 5 (Cont.)

```
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 QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT 809
Q08499-2   PDE4D_HUMAN 619 QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT 673
Q08499-3   PDE4D_HUMAN 550 QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT 604
Q08499-4   PDE4D_HUMAN 530 QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT 584
Q08499-5   PDE4D_HUMAN 453 QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT 507
Q08499-6   PDE4D_HUMAN 691 QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT 745
Q08499-7   PDE4D_HUMAN 216 ---------------------------------------------------------- 215
Q08499-8   PDE4D_HUMAN 464 QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT 518
Q08499-9   PDE4D_HUMAN 633 QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT 687
Q08499-10  PDE4D_HUMAN 625 QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT 679
Q08499-11  PDE4D_HUMAN 694 QVEEDTSCSDSKTLCTQDSESTEIPLDEQVEEEAVGEEEESQPEACVIDDRSPDT 748
Q08499-12  PDE4D_HUMAN 220 ---------------------------------------------------------- 219
```

FIG. 5 (Cont.)


A
Scn1lab mutants
OCR (pmol O2/min/larvae)
500
400
300
200
100
0
Wild-Type
Vehicle
1 nM
10 nM
100 nM
1 uM
10 uM
100 uM
+ AN2728

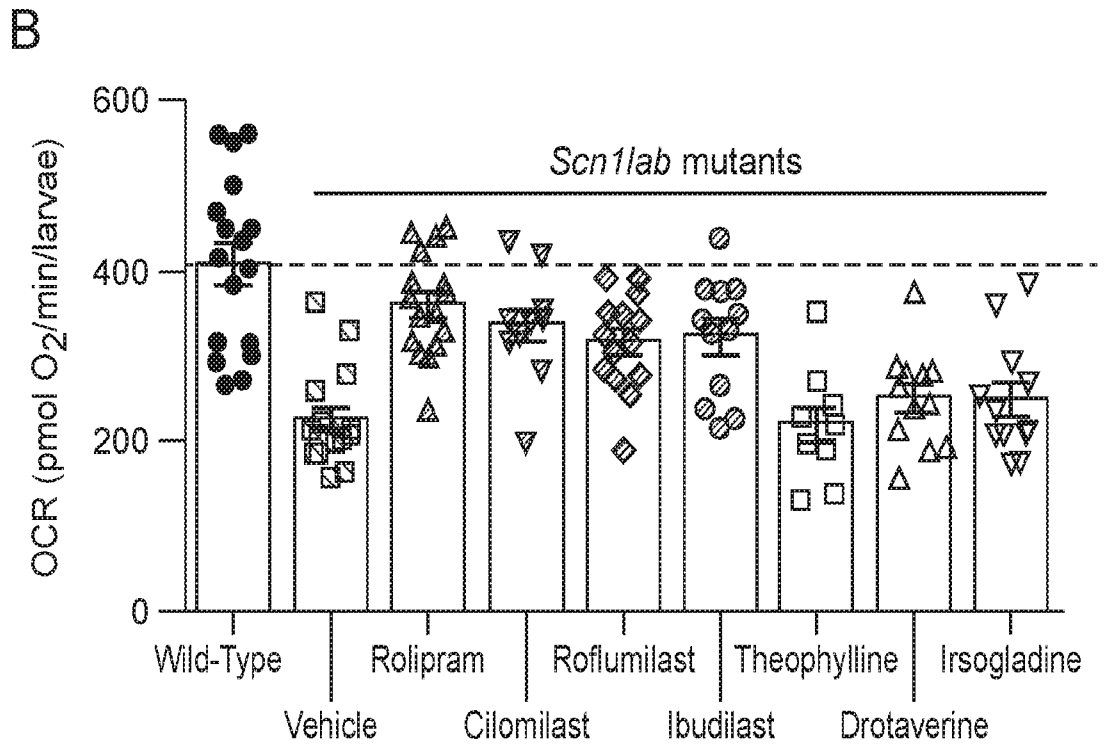

B
Scn1lab mutants
OCR (pmol O2/min/larvae)
600
400
200
0
Wild-Type
Vehicle
Rolipram
Cilomilast
Roflumilast
Ibudilast
Theophylline
Drotaverine
Irsogladine

FIG. 6

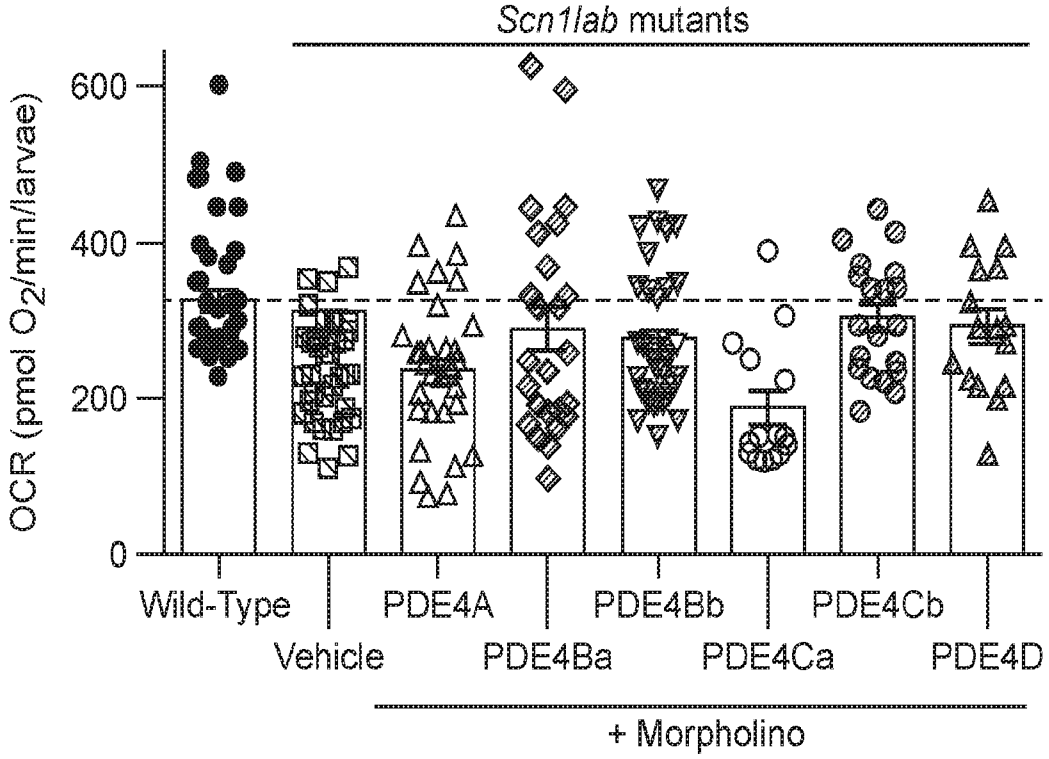

Scn1lab mutants
OCR (pmol O2/min/larvae)
600
400
200
0
Wild-Type
Vehicle
PDE4A
PDE4Ba
PDE4Bb
PDE4Ca
PDE4Cb
PDE4D
+ Morpholino

FIG. 7

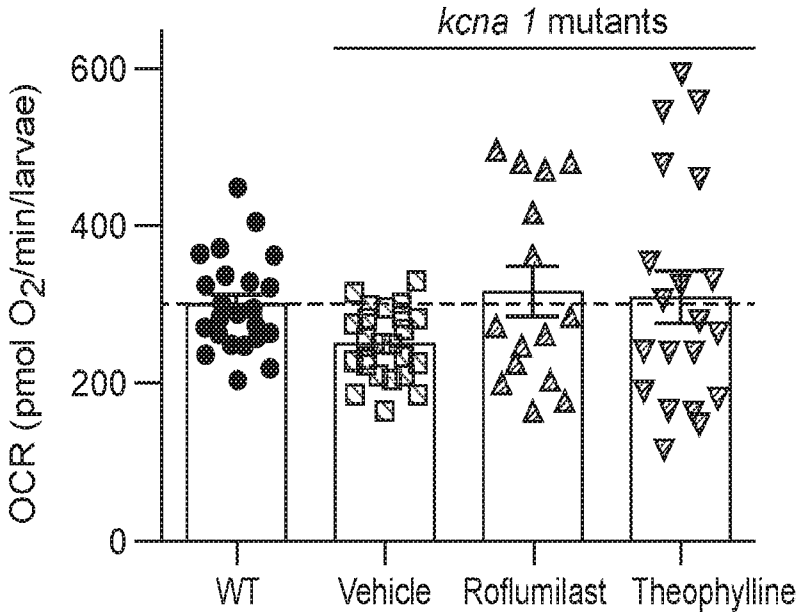
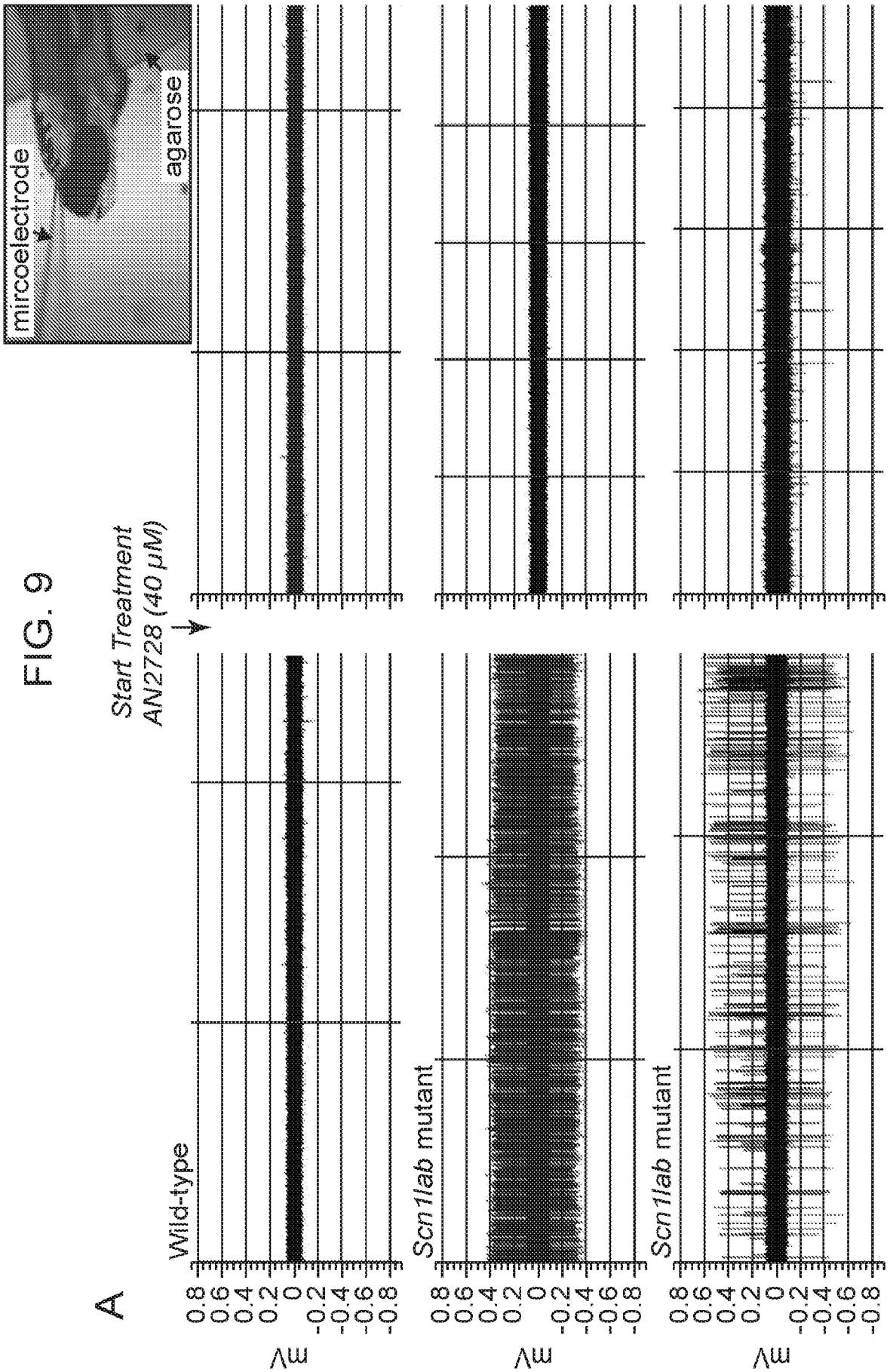

kcna 1 mutants
OCR (pmol O2/min/larvae)
600
400
200
0
WT
Vehicle
Roflumilast
Theophylline

FIG. 8

FIG. 9


A
mircoelectrode
agarose
Start Treatment
AN2728 (40 μM)
Wild-type
Scn1lab mutant
Scn1lab mutant
mV
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8

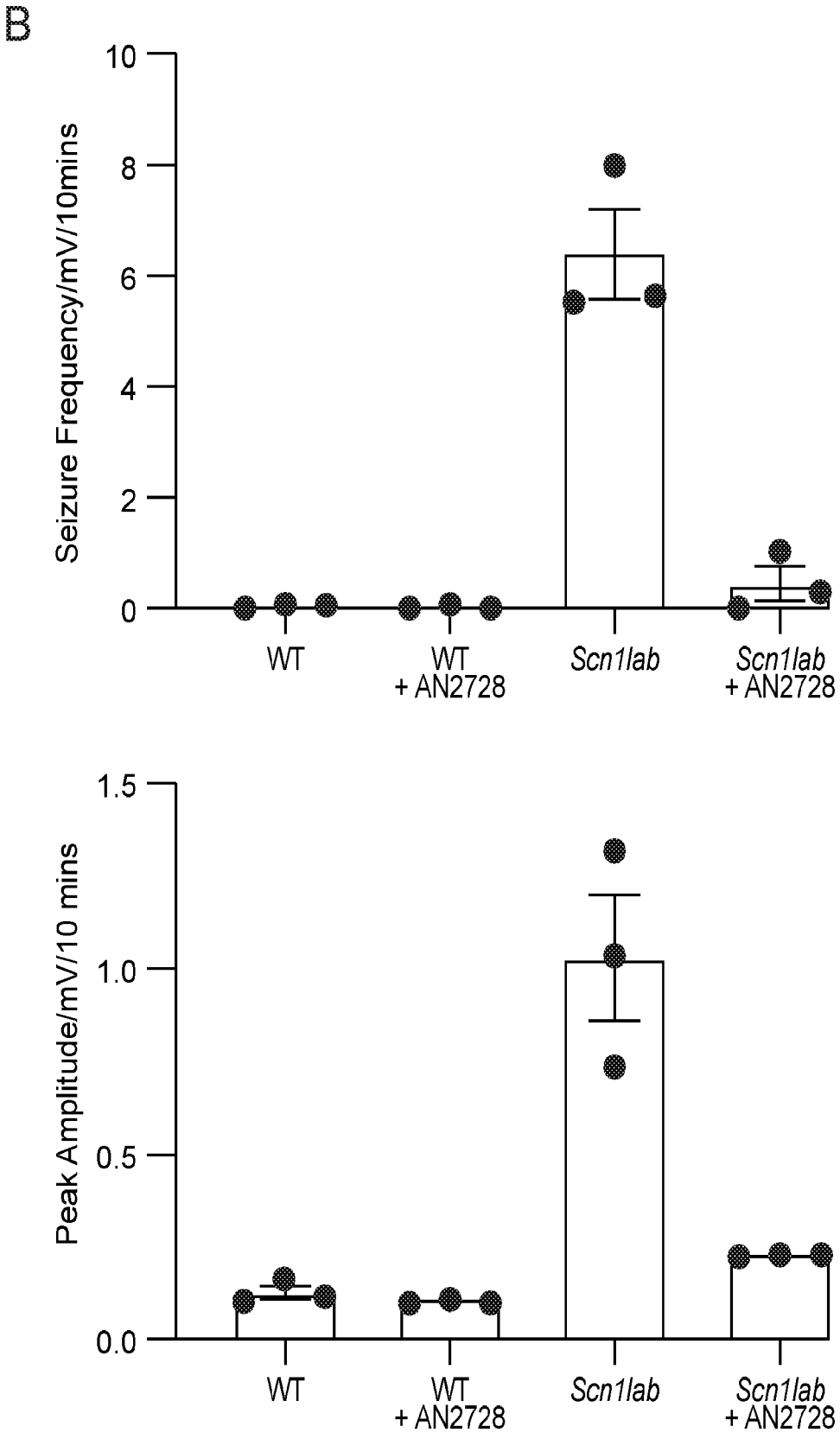

B
Seizure Frequency/mV/10mins
10
8
6
4
2
0
WT
WT + AN2728
Scn1lab
Scn1lab + AN2728
Peak Amplitude/mV/10 mins
1.5
1.0
0.5
0.0
WT
WT + AN2728
Scn1lab
Scn1lab + AN2728

FIG. 9 (Cont.)

A

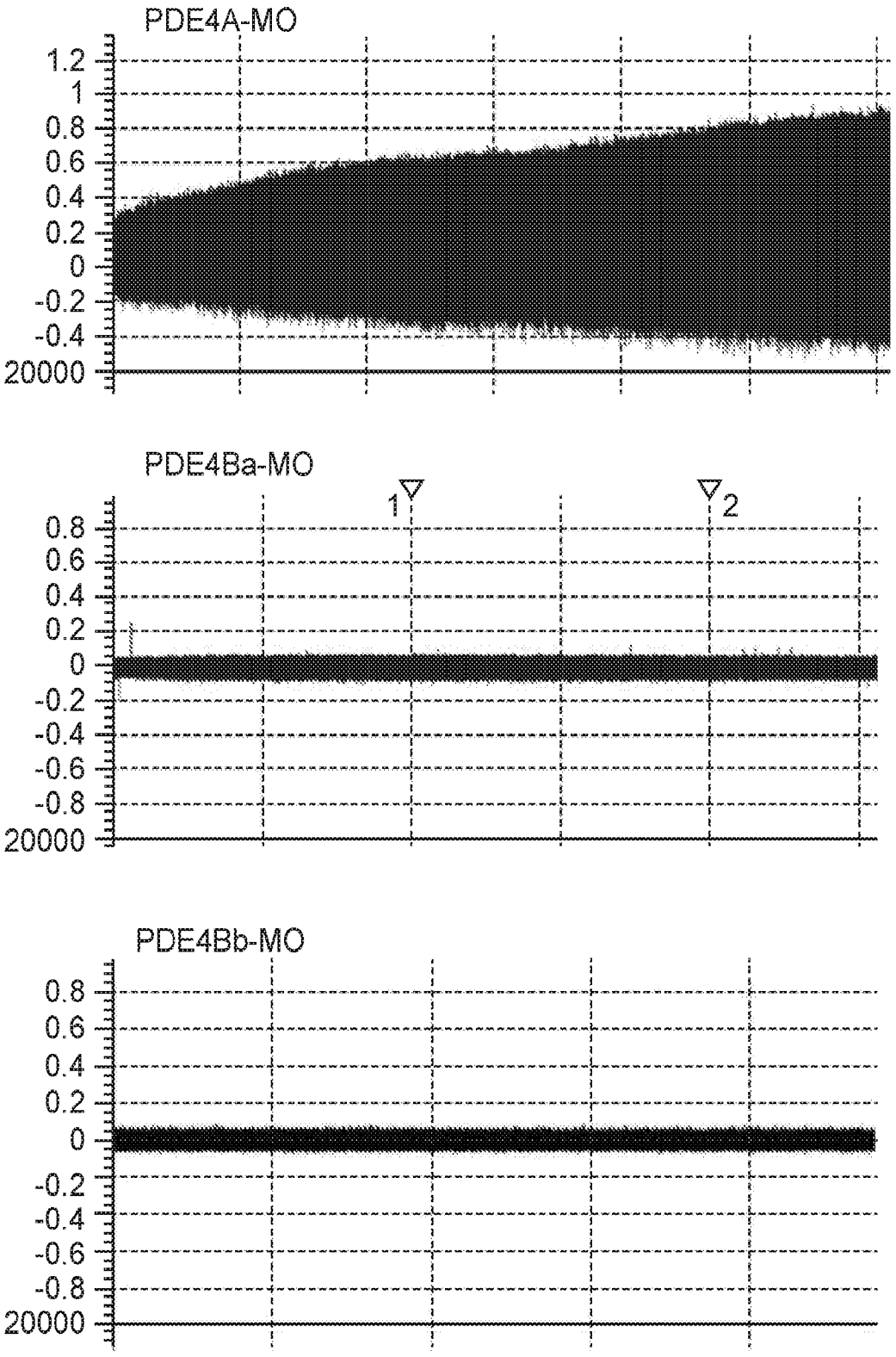

PDE4A-MO
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
20000
PDE4Ba-MO
1
2
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
20000
PDE4Bb-MO
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
20000

FIG. 10

A (Cont.)

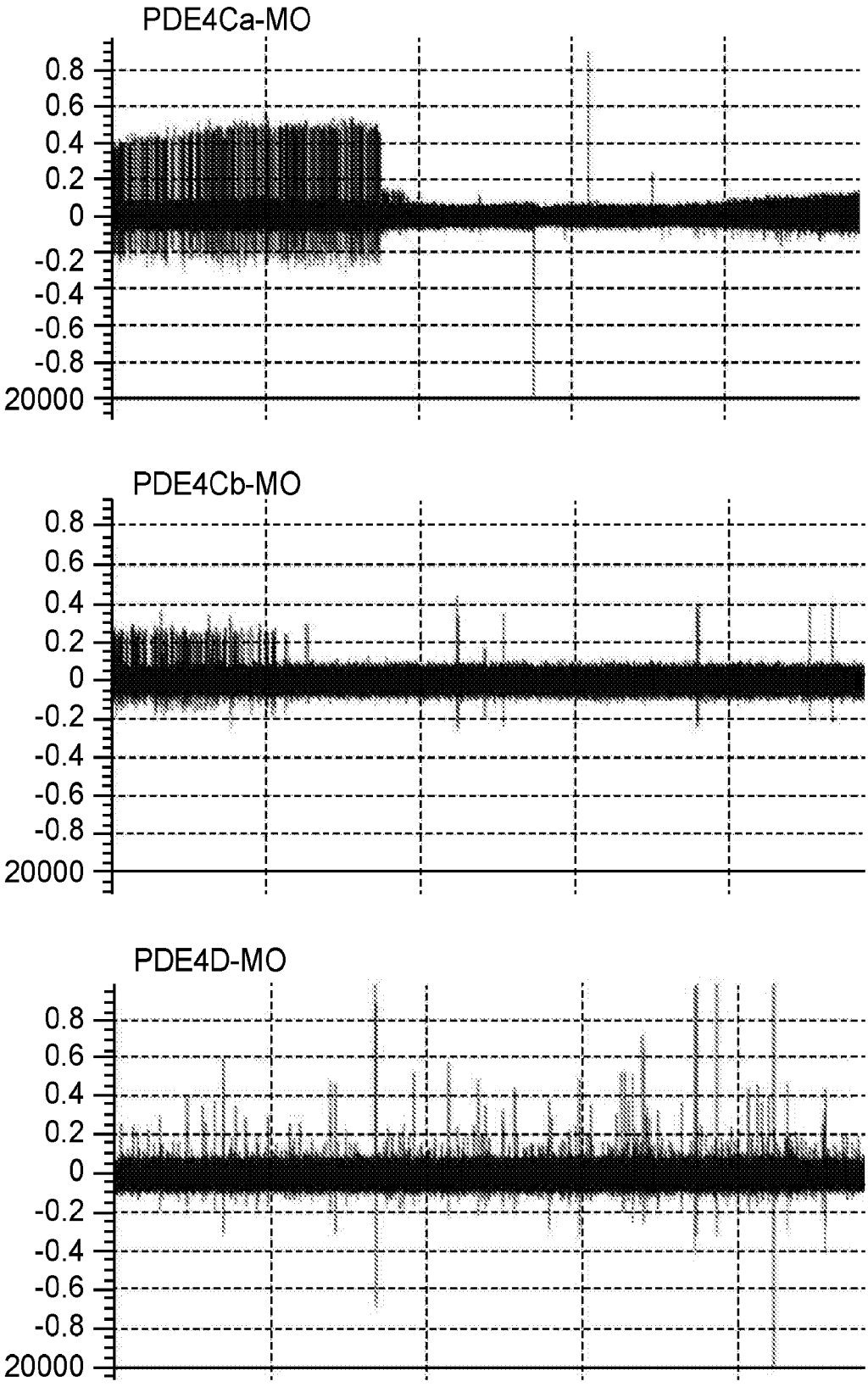


FIG. 10 (Cont.)

B

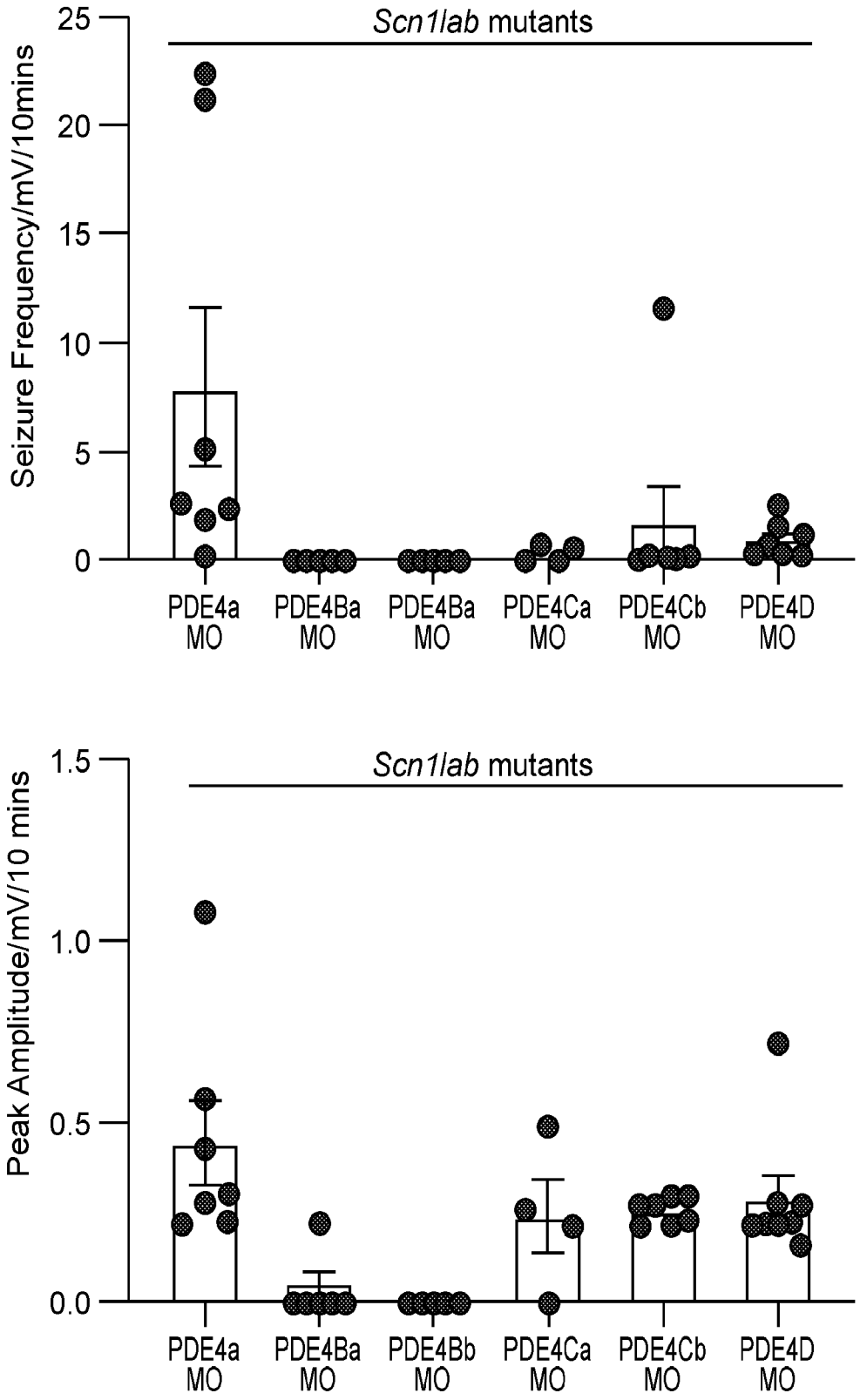

Scn1lab mutants
Seizure Frequency/mV/10mins
25
20
15
10
5
0
PDE4a MO
PDE4Ba MO
PDE4Ba MO
PDE4Ca MO
PDE4Cb MO
PDE4D MO
Scn1lab mutants
Peak Amplitude/mV/10 mins
1.5
1.0
0.5
0.0
PDE4a MO
PDE4Ba MO
PDE4Bb MO
PDE4Ca MO
PDE4Cb MO
PDE4D MO

FIG. 10 (Cont.)

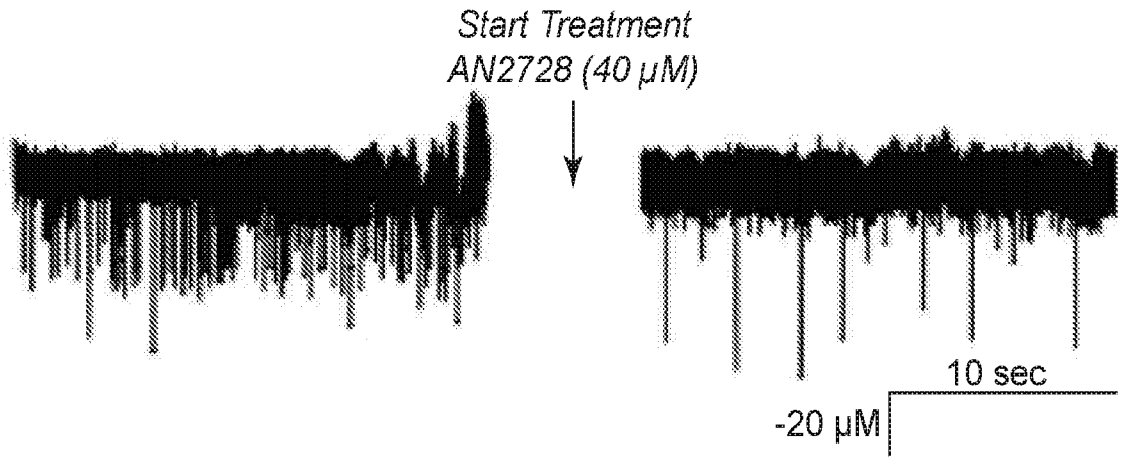

Start Treatment
AN2728 (40 µM)
10 sec
-20 µM

FIG. 11

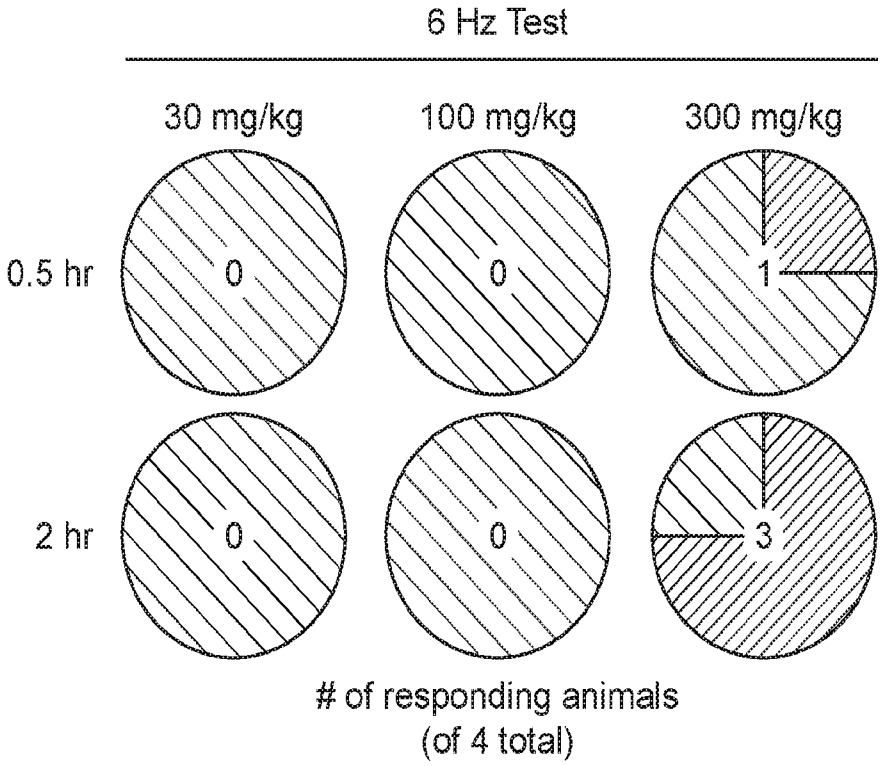

6 Hz Test
30 mg/kg
100 mg/kg
300 mg/kg
0.5 hr
2 hr
0
0
1
0
0
3
# of responding animals
(of 4 total)

FIG. 12

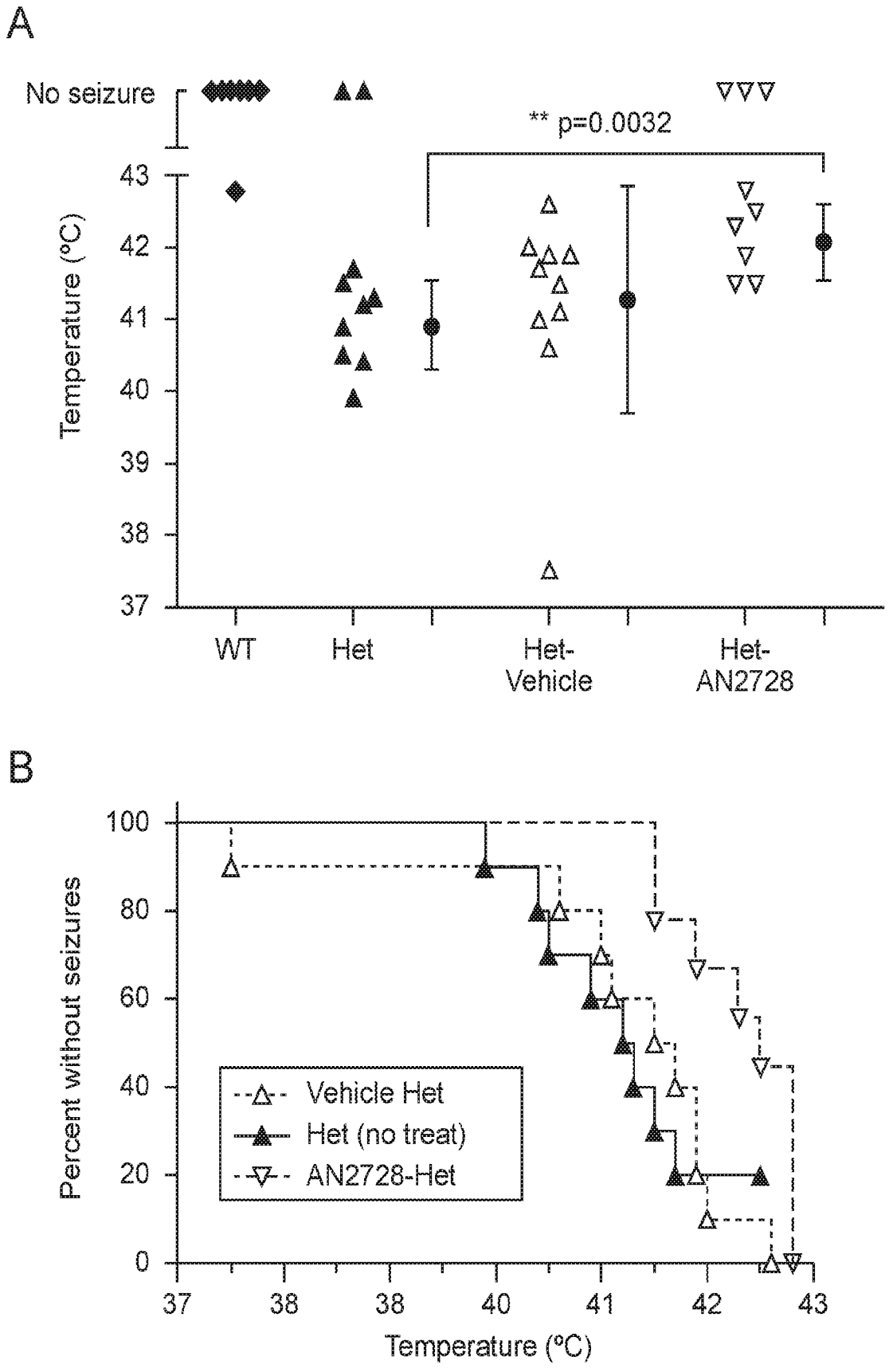
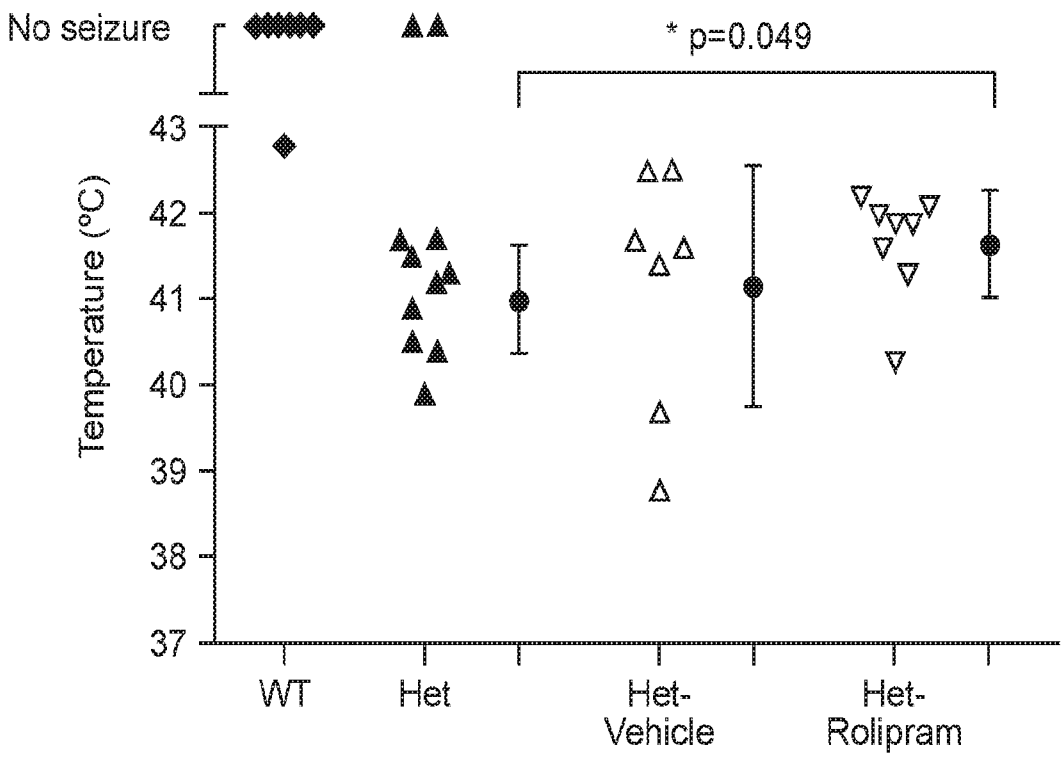

A
No seizure
** p=0.0032
Temperature (°C)
43
42
41
40
39
38
37
WT
Het
Het-Vehicle
Het-AN2728
B
Percent without seizures
100
80
60
40
20
0
Vehicle Het
Het (no treat)
AN2728-Het
37
38
38
40
41
42
43
Temperature (°C)

FIG. 13


No seizure
* p=0.049
Temperature (°C)
43
42
41
40
39
38
37
WT
Het
Het-
Vehicle
Het-
Rolipram

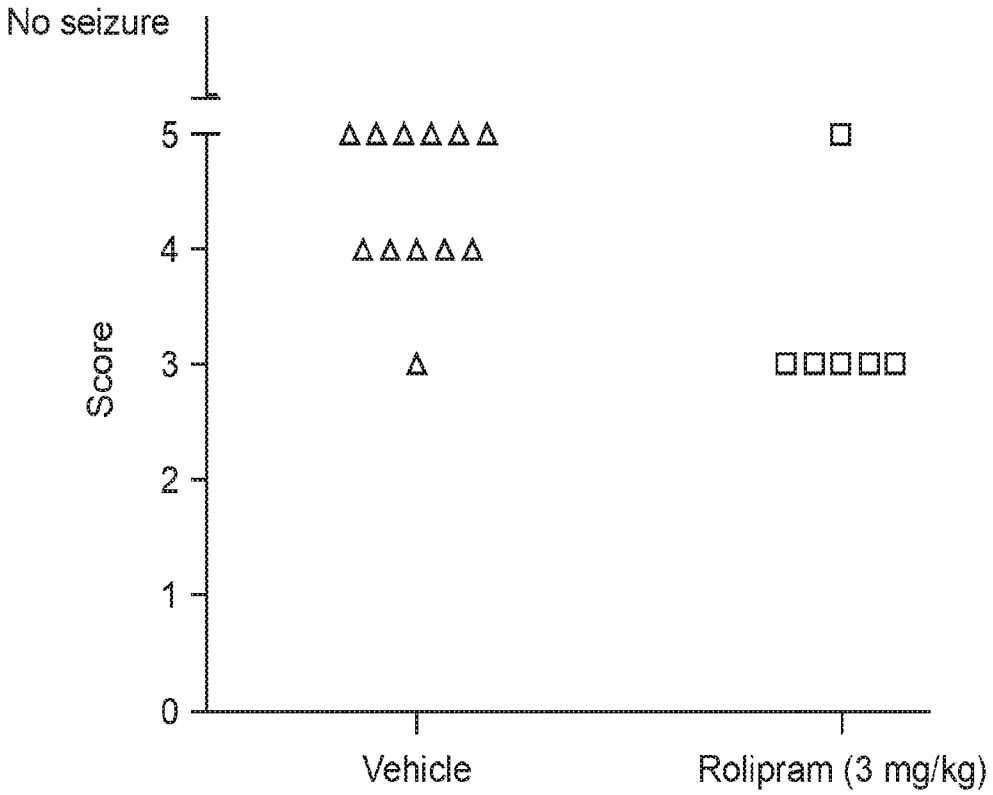

No seizure
Score
5
4
3
2
1
0
Vehicle
Rolipram (3 mg/kg)

FIG. 14

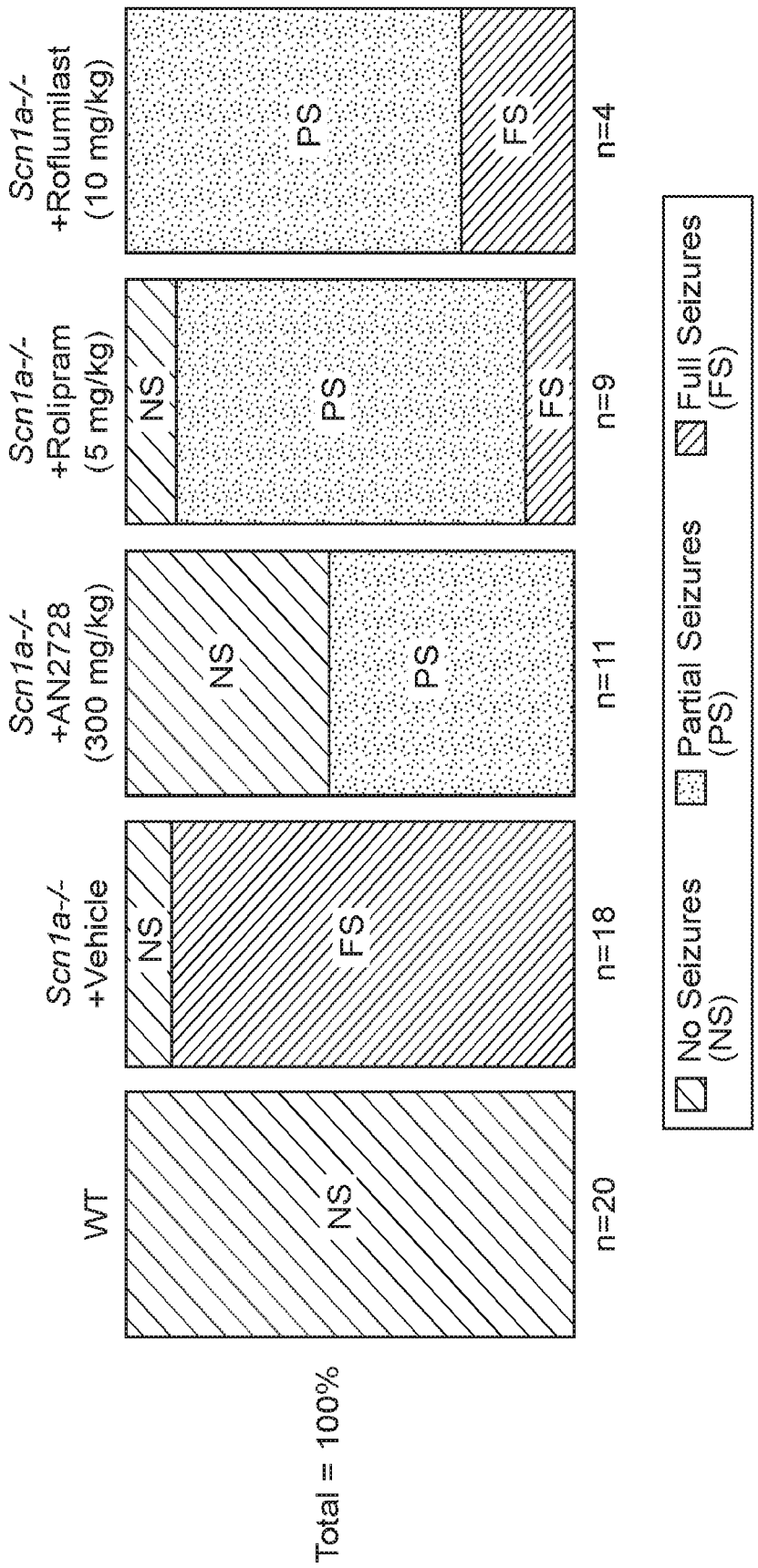

WT
Scn1a-/- +Vehicle
Scn1a-/- +AN2728 (300 mg/kg)
Scn1a-/- +Rolipram (5 mg/kg)
Scn1a-/- +Roflumilast (10 mg/kg)
Total = 100%
NS
FS
PS
n=20
n=18
n=11
n=9
n=4
No Seizures (NS)
Partial Seizures (PS)
Full Seizures (FS)

FIG. 15

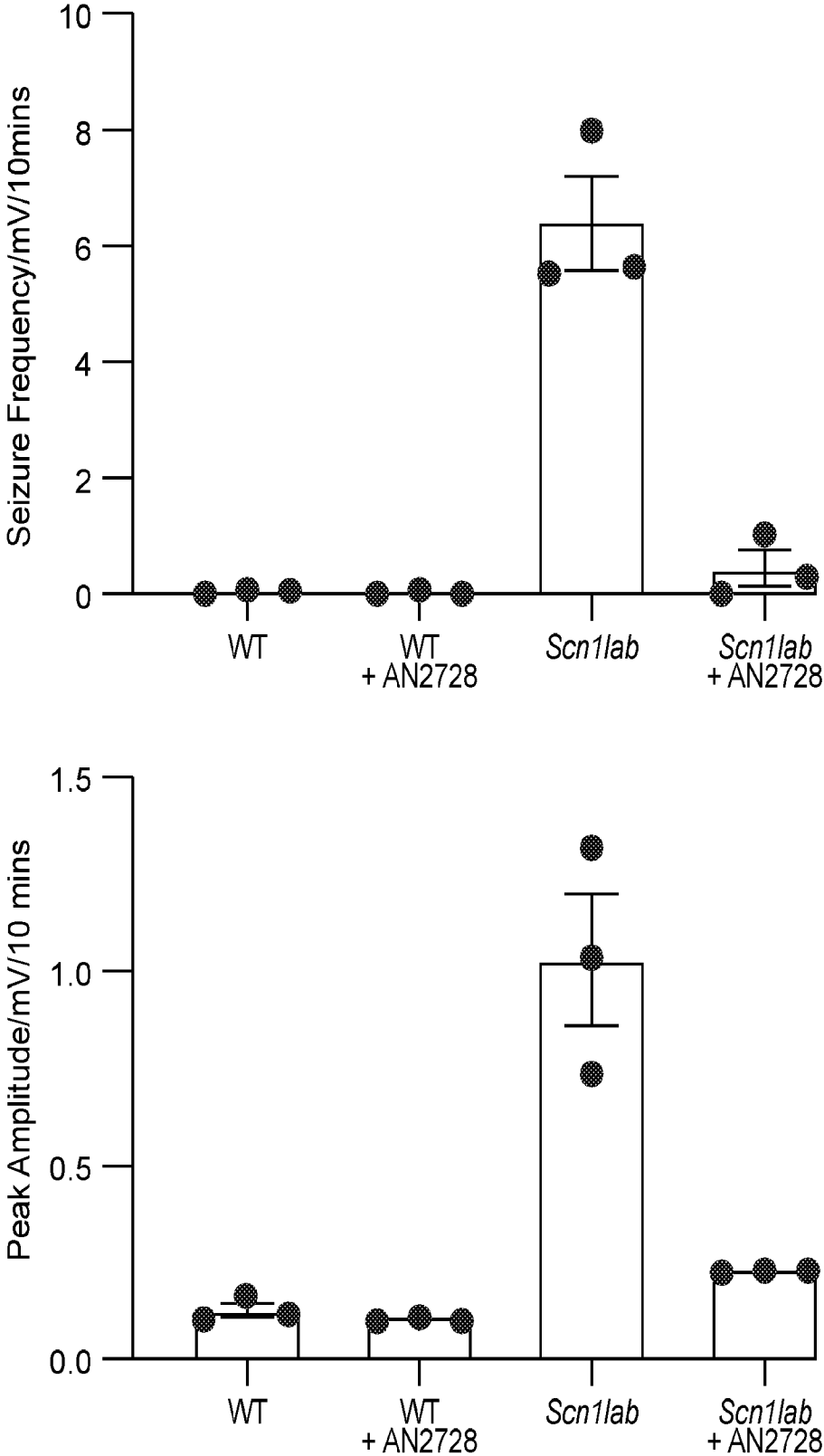

Seizure Frequency/mV/10mins
10
8
6
4
2
0
WT
WT + AN2728
Scn1lab
Scn1lab + AN2728
Peak Amplitude/mV/10 mins
1.5
1.0
0.5
0.0
WT
WT + AN2728
Scn1lab
Scn1lab + AN2728

FIG. 9B