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Data Article

Data for amino acid alignment of Japanese stingray melanocortin receptors with other gnathostome melanocortin receptor sequences, and the ligand selectivity of Japanese stingray melanocortin receptors

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ABSTRACT

This article contains structure and pharmacological characteristics of melanocortin receptors (MCRs) related to research published in "Characterization of melanocortin receptors from stingray *Dasyatis akajei*, a cartilaginous fish" (Takahashi et al., 2016) [1]. The amino acid sequences of the stingray, *D. akajei*, MC1R, MC2R, MC3R, MC4R, and MC5R were aligned with the corresponding melanocortin receptor sequences from the elephant shark, *Callorhinchus milii*, the dogfish, *Squalus acanthias*, the goldfish, *Carassius auratus*, and the mouse, *Mus musculus*. These alignments provide the basis for phylogenetic analysis of these gnathostome melanocortin receptor sequences. In addition, the Japanese stingray melanocortin receptors were separately expressed in Chinese Hamster Ovary cells, and stimulated with stingray ACTH, α-MSH, β-MSH, γ-MSH, δ-MSH, and β-endorphin. The dose response curves reveal the order of ligand selectivity for each stingray MCR.

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Specifications Table

Subject area	Biology
More specific subject area	Endocrinology
Type of data	Text files, graphs
How data was acquired	Amino acid sequences were aligned using MEGA 6.0. Ligand selectivity assays were done using the CRE/Luciferase reporter assay [2]. Luminescence was measured using a Bio-Tek Synergy HT plate reader (Bio Tek, Winooski, VT, USA), and the data were analyzed and graphed using Kaleidagraph software (Synergy Software, Reading, PA, USA)
Data format	Raw
Experimental factors	Melanocortin DNAs were cloned from stingray genomic DNA or brain mRNA. Cloned DNA were expressed in Chinese Hamster Ovary cells
Experimental features	Sequence alignment was done using MEGA 6.0. The ligand selectivity assays were done as described in reference [3].
Data source location	Kitasato University, Sagamihara, Kanagawa, Japan. University of Denver, Denver, Colorado, USA
Data accessibility	Data is within this article

Value of the data

- These data are valuable for researchers participated in endocrinology of primitive fish and evolution of melanocortin systems.
- These could be used as probes to explore orthologs in other cartilaginous fish such as skates, sharks and chimaeras.
- The data on ligand selectivity could be useful tools for structure–function relationship studies in endocrinology and pharmacology.

1. Data

Data provided in this article show amino acid sequence comparison of melanocortin receptors (MCRs) in vertebrates and ligand selectivity of stingray MC peptides on these receptors. The amino acids sequences of MC1R (Fig. 1), MC2R (Fig. 2), MC3R (Fig. 3), MC4R (Fig. 4), and MC5R (Fig. 5) of stingray (*Squalus acanthias*) which determined by us [1] were compared to corresponding sequences from two species of other cartilaginous fishes (i.e., *Callorhinchus milii*, elephant shark and *S. acanthias*, dogfish), a teleost (*Carassius auratus*, goldfish), and a mammal (*Mus musculus*, mouse). Data are also provided for ligand selectivity include effects of stingray Des-acetyl-α-MSH, β-MSH, γ-MSH, δ-MSH, ACTH(1-24) and β-endorphin on MC1R, MC3R, MC4R, and MC5R (Figs. 6, 8–10) and those of stingray Des-acetyl-α-MSH, ACTH(1-24), human ACTH(1-24) and NDP-MSH on stingray MC2R (Fig. 7).

2. Experimental design, materials and methods

In order to align the amino acid sequences of the melanocortin receptors for the Japanese stingray, *D. akajei*, the dogfish, *S. acanthias,* the elephant shark, *C. milii*, the goldfish, *C. auratus*, and the mouse, *M. musculus*, it was essential to identify putative transmembrane domains in each receptor sequence. To this end, the program "MEMSAT3" (http://bioinf.cs.ucl.ac.uk/psipred/) was used. The amino acid sequences where then aligned using the program MEGA 6.0.

To functionally express and determine the ligand selectivity of the stingray (sr) MC1R, srMC2R, srMC3R, srMC4R, and srMC5R paralogs, the nucleotide sequences for the *srmcrs* were separately

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Dasyatis akajei          MMNITTLAPR GNEQKDISFH WLPRN---VN NSY-NASSMQ CKHINIPEEV FLTLGILSFV  56
Callorhinchus milii                 E.LRL. ...HG---A. S.S-.M.AVA .Q.VSV.... ..S...V.L.  42
Carassius auratus          MNDSSRH YFSM.HMDYI YNID.NITL. TTLGEMNATG IAQ.M..Q.L ..M..LI.L.  57
Mus musculus               MS.QE.- ---..SLLGS LNSNATSHLG LAT-.Q.EPW .LYVS..DGL ..S..LV.L.  52

Dasyatis akajei          ENILVIIAII KNQNLHSPMY YLICCLAMAD TLVSMSNTIE TIVLILMEKE VLTVQNHILK 116
Callorhinchus milii      .....MT... ..R......H .F.....A.. M...V..MV. ........RG .MV...YL.. 102
Carassius auratus        .....VA... ..R..... .F.....VS. M...V..VV. .LFML.K.HG L.L.TAKM.Q 117
Mus musculus             ..V..V...T ..R..... .F.....LS. LM..V.IVL. .TIIL.L.AG I.VARVALVQ 112

Dasyatis akajei          QIDNIIDLMI CTSMVSSLSF LAAIAADRYI TIFYALRYHV IMTTRKAVII IVGIWIVSCT 176
Callorhinchus milii      ....L..M.. .S........ .G..... ........T .....R..GV M....V..I.  162
Carassius auratus        HL..V..I.. .S.V..... .CT..... .........S ....QR..A. .AVV.LT.I. 177
Mus musculus             .L..L..VL. .G......C. .GI..I.... S........S .V.LPR.RRA V....M..IV 172

Dasyatis akajei          SSIMFIVYSE SSAVIICLIS FFFMMLVIMG GLYFHMFMLA QMHTKKIMAQ RKKRP-THQA 235
Callorhinchus milii      ..AI..... N...V..... ...V.VIF.. A..L...T.. RI.A.R.... H...T-L... 221
Carassius auratus        ..SL....HT DN...A..VT ..GLT..FTA V..L...I.. HV.SRR...L H.S.---R.. 234
Mus musculus             ..TL..T.YK HT..LL..VT ..LA..AL.A I..A...TR. CQ.AQG.AQL H.R.RSIR.G 232

Dasyatis akajei          ANMKGAITLT ILLGLFLICW SPFFLHLLLI ISCPKNPYCL CFNSHFNMFL ILIICNSVFD 295
Callorhinchus milii      TS........ ....V..... .......I.. .L..T....Q ..T..... ........I. 281
Carassius auratus        TS........ ....V.V... G......I.. LI..T....K .YF....L.. .......LI. 294
Mus musculus             FCL...A... ....I.FL.. G......... VL..QH.T.S .IFKN..L.. L..VLS.TV. 292

Dasyatis akajei          PIIYAFRSQE LRKTLKEFIP CSW     318
Callorhinchus milii      ...                           284
Carassius auratus        .L...Y.... .......M.F ...LFAM 321
Mus musculus             .L........ ..M....VLL ...     315
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Fig. 1. Amino acid sequence comparison of MC1R used for phylogenetic analysis. Species names are *Dasyatis akajei* for stingray, *Callorhinchus milii* for elephant shark, *Carassius auratus* for goldfish, and *Mus musculus* for mouse. Dot shows identical amino acid to stingray sequence. Hyphen shows gap. Accession numbers: LC108746 (*Dasyatis akajei*), BR000855 (*Callorhinchus milii*), AB618067 (*Carassius auratus*), and BC119296 (*Mus musculus*). The percent identify for the MC1R orthologs was 33%.

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Dasyatis akajei          MPDMMIPGYG TLLDSNGILP MPPDATISPH SHPTISPWLP YGTEVVIDTI NQTNMNATEE  60
Callorhinchus milii                                 MSG ADTSA....A NV.TA.MN.S GFM.G-SGGI  32
Carassius auratus                                                    MNS--S.E ALSTH--PTD  14
Mus musculus                                                      MK HIINSYEH.N DTARN--NSD  20

Dasyatis akajei          CSQIEIPTEV YLILGLVSLL ENLLVVIAVL KNKKLHFPMY FFICSLAVSD ILLCLSKAWE 120
Callorhinchus milii      .R.L...L.. .....G.GM. .....I...V N.RN..S... L......MA. M.VSVG..S.  92
Carassius auratus        .AEVQV.SQ. FMAIAVA..S ..I..IL..I ..RN..S... C...N...FN TISS.C.SL.  74
Mus musculus             .PDVVL.E.I FFTISVIGI. ...I.LL..I ...N.QS... .......I.. M.GS.Y.IL.  80

Dasyatis akajei          AFTISLVNNH EDLFIQTFLL SLDNVFDTLI CISFLASIFN LAAITTDRYI SIFHALRYHN 180
Callorhinchus milii      .VI.F.DQ.- SH.LTE.LID H..YL..S.. ...LI...LS .G..A....L T........Q 151
Carassius auratus        TILLLFKEAG HLN--GR.E. NI.DIM.S.L .MC..G...S ILT.AV.... .........T 132
Mus musculus             NIL.MFR.MG YLKPRGS.ES TA.DII.CMF IL.L.G...S .SV.AA.... T.....Q..S 140

Dasyatis akajei          IMTGKRVAFA IAGIWVFCTA TGILMINFHN SQGIISFYII FFLLSVVLIV SLYIYMFLLA 240
Callorhinchus milii      ...V..A.LI .SAL.T...F S.SFI.K.NR KNAFPGSL.T MYFTTLFV.. ...V..... 211
Carassius auratus        L..MR..VVT LST.....GT S.V...G.S. AATVKISSLC SSSTALL..L L..VH..... 192
Mus musculus             .V.MR.TIIT LTI..M...G S..T.VI.SH HIPTVLTFTS L.P.ML.F.L C...H..... 200

Dasyatis akajei          QMHARKIRIL PG-HTAHQGI NFKGAFTVTV LLGVFIFCWA PLSLHFILFL LCPSDPYCAC 299
Callorhinchus milii      RR..QC..S. ..-QRV...T SL...I.L.I ...I..I... .FF..L..V. A...N...T. 270
Carassius auratus        RH..NR.ASM ..L.ARQRQS GLR..L.L.I .I...VA... .F...LLISM I..EN...E. 252
Mus musculus             RS.....ST. .-------RT .M...M.L.I .......... .FV..VL.MT F..NN...V. 253

Dasyatis akajei          FMSLFQIDLI FIMCHSIIDP LIYAFRDPEL SNTFKKMMFC HKKQWYFHAS PSFLNI 355
Callorhinchus milii      Y.....V... L...N..... ..F...S... R......CI. FN..L.           316
Carassius auratus        YR....LHVL LLVS.AV.E. A.....ST.. R..Y..VFL. SASRIFKECV       302
Mus musculus             Y.....VNGM L...NAV... F.....S... RDA..R.L.. NRY              296
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Fig. 2. Amino acid sequence comparison of MC2R used for phylogenetic analysis. Species names are *Dasyatis akajei* for stingray, *Callorhinchus milii* for elephant shark, *Carassius auratus* for goldfish, and *Mus musculus* for mouse. Dot shows identical amino acid to stingray sequence. Hyphen shows gap. Accession numbers: LC108747 (*Dasyatis akajei*), BR000856 (*Callorhinchus milii*), AB618068 (*Carassius auratus*), and NM_008560 (*Mus musculus*). The percent identity for the MC2R orthologs was 24%.

synthesized with a V-5 epitope tag at the N-terminal of the receptor, and inserted into a pcDNA3.1 expression vector (GenScript; Picataway, NJ, USA). Each *srmcr* cDNA was separately transiently transfected into Chinese Hamster Ovary (CHO) cells. The CHO cells were grown at 37 °C in a humidified 5%

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Dasyatis akajei      MNSTPSISF FHPAMRNGTE DLNESSILNN RNGTGFCEQV PIKAELFFCL GIISFLENVL  59
Callorhinchus milii  ....H---. LFDLQL..SG ...K.....- .SNP...... ...S.V.LT. ....L...I.  55
Squalus acanthias   M....H..I. LQLPTM.S.. .....NV... .SSA...... .....V.LI. ..L.L...I.  60
Carassius auratus    ..DSYLQFL KGQKPA.S.S LPPNG.TVD- PPAGAL.... Q.Q..V.LT. ..V.L...I.  58
Mus musculus         ...SCCL.S VS.MLP.LS. HPAAPPAS.- .S.S...... F..P.V.LA. ..V.LM..I.  58

Dasyatis akajei     VILAVAKNKN LHSPMYLFLC SLAVADMLVS VSNALETIVM AFLKNGFLIA NDQLIQQMDN  119
Callorhinchus milii ....IL.... ......F... .......... .......... .L.N..Y.V. ...F..I...  115
Squalus acanthias   ...SIL.... ......F... .......... .......... ...N..Y.V. ...F......  120
Carassius auratus   .....V.... ......F... ...A...... ...S.....I .V.NSRL.V. S.HFVRL...  118
Mus musculus        .....VR.G. ......F... ...A...... L..S....MI .VINSDS.TL E..F..H...  118

Dasyatis akajei     VFDSMICISL VASICNLLVI AIDRYITIFY ALRYHSIMTV KRAIILIVVI WIFCIFCGII  179
Callorhinchus milii .I..L..... .......... .......... .......... ...LL..I.. ..A.......  175
Squalus acanthias   .......... .......... .......... .......... ...L..DRSY LDCLY.....  180
Carassius auratus   .......... ........A. .V...V.... .......V.. R..LVA.AG. .LV.VV...V  178
Mus musculus        I......... ........A. .....V.... .......... RK.LT..G.. .VC.GI..VM  178

Dasyatis akajei     FIIYSESQTV IICLITMFFV MLFLMTTLYV HMFMLARLHI KRIATLPVPG VVH------Q  233
Callorhinchus milii .....N.K.. .........T ..V....... .......... .K..A...D. I.R------P  229
Squalus acanthias   .....D.K.A .........T ..SS...... .......... ........N. M.R------.  234
Carassius auratus   ..V....K.. .V.......A ..V..A.... ...L.....V Q...A..PAA AAAGNPAPR.  238
Mus musculus        .......KM. .V.......A .VL..G...I ...LF....V Q...V..PA. ..A----PQ.  234

Dasyatis akajei     RTCMKGAITI TILLGIFIIC WAPFFLHLIM IISCPKNPYC ICYTSHFNTY LILIMCNSVI  293
Callorhinchus milii .......... .......... .........L .......A.. .......... ..........  289
Squalus acanthias   .......... ........V. .........L .......... .......... ..........  294
Carassius auratus   .S..E..V.. S..I.V.VC. .........L LV...HH.L. L..M...T.. .V........  298
Mus musculus        HS.....V.. .....V..F. ........VL ..T..T.... ....A..... .V........  294

Dasyatis akajei     DPIIYAFRSQ EMRKTFKEIL CCYCMNFNFR CK  325
Callorhinchus milii ..M....... .........A ...G..L.S. ..Y 322
Squalus acanthias   .......... .........I ......L.L. ..  326
Carassius auratus   ..L...C..L .......... ..FGCQPPL      327
Mus musculus        ..L......L .L.N...... .GCNSMNLG      323
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Fig. 3. Amino acid sequence comparison of MC3R used for phylogenetic analysis. Species names are *Dasyatis akajei* for stingray, *Callorhinchus milii* for elephant shark, *Squalus acanthias* for dogfish, *Carassius auratus* for goldfish, and *Mus musculus* for mouse. Dot shows identical amino acid to stingray sequence. Hyphen shows gap. Accession numbers: LC108748 (*Dasyatis akajei*), BR000857 (*Callorhinchus milii*), AY560605 (*Squalus acanthias*), AB618069 (*Carassius auratus*), and NM_008561 (*Mus musculus*). The percent identity for the MC3R orthologs was 52%.

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Dasyatis akajei     MDLSYTRGPA DTTQNRNQSV SGFTG-ANIL HSN-GSSSGC NEQLWISTEV FVMLGIVSLL   58
Squalus acanthias   .NS.FHHRLP E.P.L..H.. AR.AS-..GS R.D-.F.... Y......... .LT...F...   58
Carassius auratus   .NT.HHH..H HS--Y..H.Q GALPV-GKPD QGER..T... Y...L..... .LT..L....   57
Mus musculus        .NSTHHH.MY TSLHLW.R.S Y.LH.N.SES LGKGHPDG.. Y...FV.P.. ..T..VI...   60

Dasyatis akajei     ANILVVAAIV KNKNLHSPMY FFICSLAVAD MLISVSNAWE TITIAMLKSR HLLAQDKLIK  118
Squalus acanthias   .....I.... .......... .......... ..V....... ..F....... ..T.PEN...  118
Carassius auratus   E....I...I .......... .......... L.V.....S. .VVM.LITGG N.TYRESI..  117
Mus musculus        E....IV..A .......... .......... ..V....GS. ..V.TL.N.T DTD..S-FTV  119

Dasyatis akajei     SIDNVFDSVI CSSLLASICS LLAVAVDRYI TIFYALRYHN IMTVRRALTV IAGIWAVCIG  178
Squalus acanthias   NM......M. .......... ...I.I.... .......... .V......MI ..A...A.T.  178
Carassius auratus   NM..I...M. ........W. ...I...... .......... ...Q...G.I .TC..TL.TV  177
Mus musculus        N....I.... .......... ..SI.....F ......Q... ......VGII .SC...A.TV  179

Dasyatis akajei     SGILFIIYSE STTAVICLIA MFFAMLAIMA SLYVHMFMLA RLHLKRIAAL PSSGAICQAA   38
Squalus acanthias   ......V... ..AVI....T .......L.. .......... ...V...... .GN..VR...  238
Carassius auratus   ..V...V... ...VL....S ...T...L.. .......L.. ...M...... .GN.P.W...  237
Mus musculus        ..V......D .SAVI....S ...T..VL.. .......LM. ...I....V. .GT.T.R.GT  239

Dasyatis akajei     NMKGAITLTI LLGVFVVCWA PFFLHLILMI SCPRNPYCIC FMSHFNMYLI LILCNSIIDP  298
Squalus acanthias   .......... .......... ...M...... ...Q....V. .......... ..M...V...  298
Carassius auratus   .......I.. .......... .......... .......... .......... ..M...V...  297
Mus musculus        .......... .I........ ......LFY. ...Q....V. ......L... ..M..AV...  299

Dasyatis akajei     LIYAFRSQEM RKTFKEIICC YS-LRGSCDL LSNINTH 334
Squalus acanthias   .......... .......... ..-.P.L... T.EY    331
Carassius auratus   .......... .......C.. WYG.ASL.V          326
Mus musculus        ....L....L .........F .P-.G.I.E. S.RY    332
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Fig. 4. Amino acid sequence comparison of MC4R used for phylogenetic analysis. Species names are *Dasyatis akajei* for stingray, *Squalus acanthias* for dogfish, *Carassius auratus* for goldfish, and *Mus musculus* for mouse. Dot shows identical amino acid to stingray sequence. Hyphen shows gap. Accession numbers: LC108749 (*Dasyatis akajei*), AY169401 (*Squalus acanthias*), AJ534337 (*Carassius auratus*), and BC116959 (*Mus musculus*). The percent identity for the MC4R orthologs was 55%.

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Dasyatis akajei                                MNLTKL S--------- ------LMSV TLAEAMVNGT   21
Squalus acanthias                               ....G. QS-------- --REPWPKNL .P.NDIT.R.   26
Carassius auratus                             M..TSEA TLS------- --LWAISANS SPVLDLL.T.   28
Mus musculus       MQDQSPVNRR FNSQKPPGTR EESCLP.RGA EQNGKSDAKK WGHSLPA.NS SSTLTVL.L.   60

Dasyatis akajei    RSST------ -------ALC EQVSVAVEVF LILGIISLLE NILVIAAIIK NRNLHSPMYF   68
Squalus acanthias  K.TS------ -------G.. ....I..... .T...M.... .....T.... .K.......   73
Carassius auratus  ETPSHA---- -----KPKA. ..LNI.T... .......... .....C..V. .K.......   79
Mus musculus       LNASEDGILG SNVKNKSLA. .EMGI..... .T..LV.... .....G..V. .K.......  120

Dasyatis akajei    FVCSLAVADM LVSVSNAWET IVITLLHSRH LVVKDSFVKH VDNVFDSMIC TSVVASMCSL  128
Squalus acanthias  .......... ...A...... ...A..N... .I.E.....Q .......... I.........  133
Carassius auratus  .......... .......... ...Y..TN.Q ...E.H.IRQ M.........  I.........  139
Mus musculus       Y.G....... ...M...... VT.Y..NNK. ..IA.T..R. I.........  I.........  180

Dasyatis akajei    LAIAVDRYIT IFYALRYHHI MSMKRAAFII AGIWALCIGC GIIFIIYSES PTVIICLVTM  188
Squalus acanthias  ........V. .......... .TV...T... ....TF.... .......... .......IA.  193
Carassius auratus  ........V. ........N. .TVR...... G...TF.TS. ..V.....DN TS..V...S.  199
Mus musculus       .......... .......... .TAR.SGV.. .C..TF..S. ..V....Y.. KY.....IS.  240

Dasyatis akajei    FFIMLLIMAS LYSHMFLLAR SHAKRIAAMS SSNSIHQQAS MKGAITLTIL LGIFIVCWAP  248
Squalus acanthias  .....VL... .......... ........L. .Y.....R.. .......... ..........  253
Carassius auratus  .....AL... ......M... ..V.....LP GY.....R.. ..A.V..... ..........  259
Mus musculus       ..T..FF.V. ..I....... N.V.....SP RY..VR.RT. ........M. ........S.  300

Dasyatis akajei    LFLHLILMIS CPRNLYCTCF MSHFNLYLIL IMCNSIIDPL IYSFRSQEMR KTLKEIICCH  308
Squalus acanthias  F.......... ..G....V.. .......... .......... ..A......W ..F......Y  313
Carassius auratus  F.......... .......M.. .....M.... .....V.... ..A....... .........Y  319
Mus musculus       F.......... ..Q.V..S.. ..Y..M.... .....V.... ..AL...... R.F...V...  360

Dasyatis akajei    SLRAVCRLTV K  319
Squalus acanthias  ....A.G.SG .  324
Carassius auratus  ...N.FGMSR    329
Mus musculus       GF.RP...LG GY 372
```

Fig. 5. Amino acid sequence comparison of MC5R used for phylogenetic analysis. Species names are *Dasyatis akajei* for stingray, *Squalus acanthias* for dogfish, *Carassius auratus* for goldfish, and *Mus musculus* for mouse. Dot shows identical amino acid to stingray sequence. Hyphen shows gap. Accession numbers: LC108750 (*Dasyatis akajei*), AY562212 (*Squalus acanthias*), AJ576322 (*Carassius auratus*), and BC100720 (*Mus musculus*). The percent identity for the MC5R orthologs was 61%.

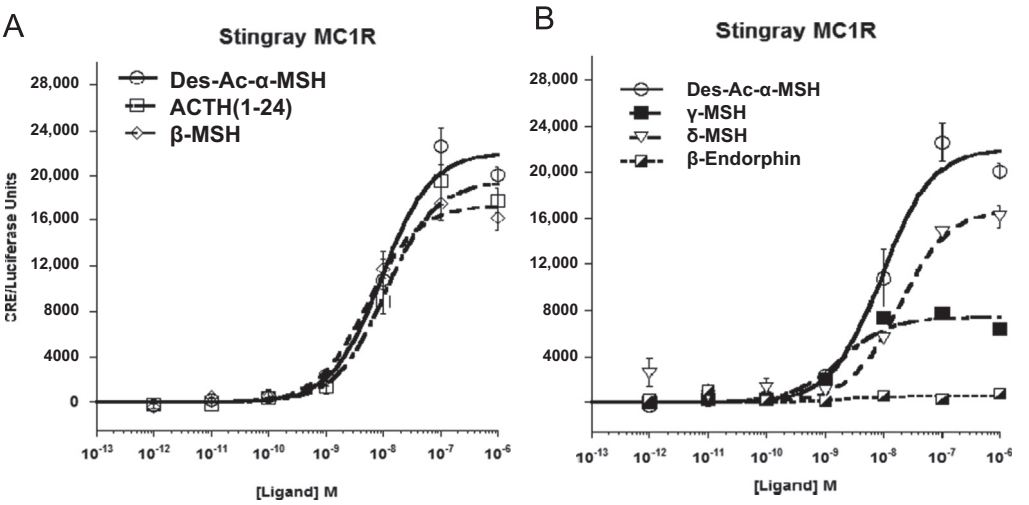

Fig. 6. Ligand selectivity of stingray MC1R. (A) Functional activation of the stingray MC1R after stimulation with the following stingray melanocortins: Des-acetyl-α-MSH (Des-Ac-α-MSH), ACTH(1-24), or β-MSH. (B) Functional activation of stingray MC1R after stimulation with the following stingray melanocortins: Des-Ac-α-MSH, γ-MSH, δ-MSH or β-endorphin(1-20). As described in methods, CHO cells were transiently transfected with a stingray *mc1r* cDNA construct and a *cre*/*luc* cDNA construct. Two days post-transfection, wells containing 1×10^5 cells were stimulated with the stingray melanocortin ligands at concentrations ranging from 10^{-6} M to 10^{-12} M. Results are expressed as mean ± S.E.M.; $n=3$.

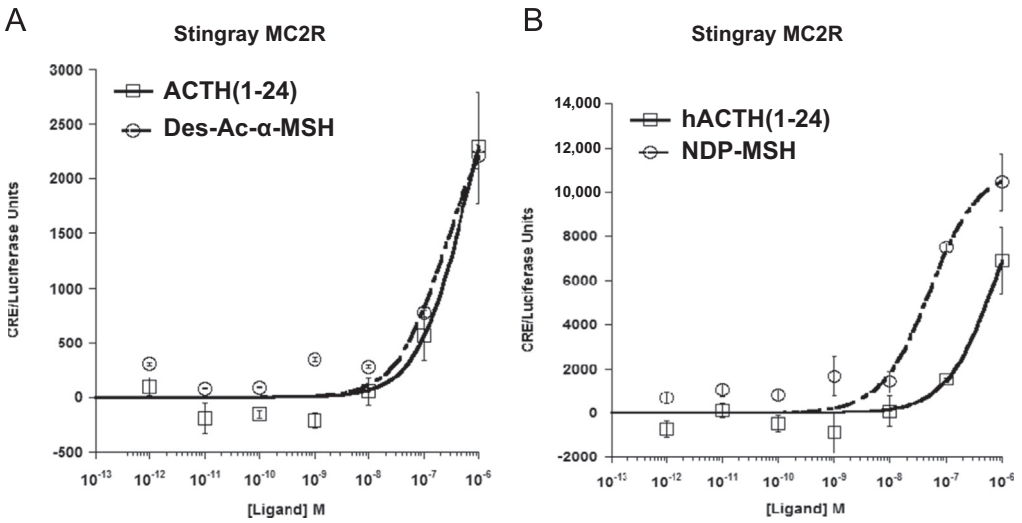

Fig. 7. Ligand selectivity of stingray MC2R. (A) Functional activation of the stingray MC2R after stimulation with stingray Des-acetyl-α-MSH (Des-Ac-α-MSH) or stingray ACTH(1-24). (B) Functional activation of stingray MC2R after stimulation with human ACTH(1-24) (hACTH(1-24)) or NDP-MSH. The activation assays were performed as described in the figure legend for Fig. 6. Results are expressed as mean ± S.E.M.; $n=3$.

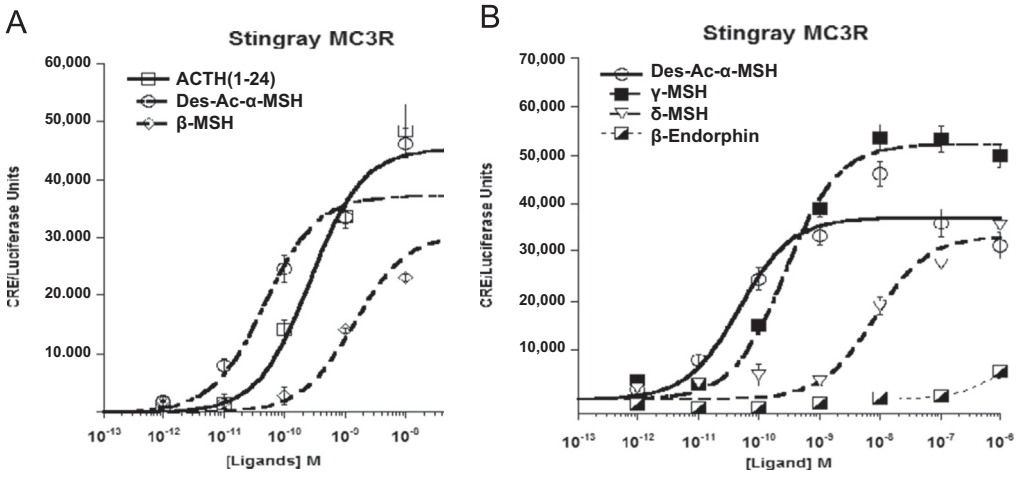

Fig. 8. Ligand selectivity of stingray MC3R. (A) Functional activation of the stingray MC3R after stimulation with the following stingray melanocortins: Des-acetyl-α-MSH (Des-Ac-α-MSH), ACTH(1-24), or β-MSH. (B) Functional activation of the stingray MC3R after stimulation with the following stingray melanocortins: Des-acetyl-α-MSH (Des-Ac-α-MSH), γ-MSH, δ-MSH or β-endorphin(1-20). The activation assays were performed as described in the figure legend for Fig. 6. Results are expressed as mean ± S.E.M.; $n=3$.

CO_2 incubator in DMEM/F12 with 5% fetal calf serum. Each sr cDNA was co-expressed with a CRE/Luciferase reporter plasmid [2] using the Solution T Cell Line Nucleofector Kit (Amaxa Inc., Gaithersburg, MD, USA) and program U-23 [4]. The transiently transfected cells were seeded on a 96-well plate at a density of 1×10^{-5} cells/well. After 48 h in culture, the transfected cells were stimulated with either synthetic srACTH(1-24), srDes-acetyl-α-MSH, srβ-MSH, srγ-MSH, srδ-MSH, srβ-endorphin or hACTH (1-24), or NDP-MSH at concentrations ranging from 10^{-6} M to 10^{-12} M, in serum-free CHO media for four hours at 37 °C. At the end of the incubation period, 100 μl of Bright-Glo luciferase assay reagent

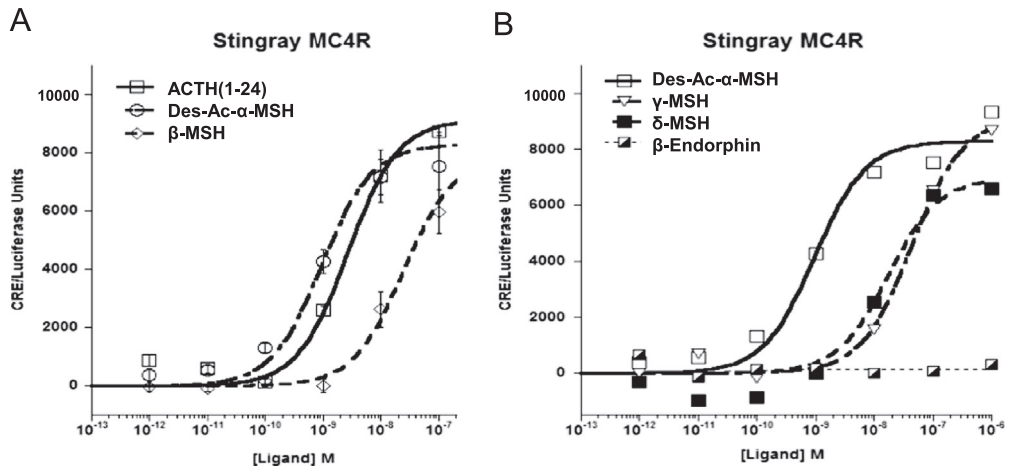

Fig. 9. Ligand selectivity of stingray MC4R. (A) Functional activation of the stingray MC4R after stimulation with the following stingray melanocortins: Des-acetyl-α-MSH (Des-Ac-α-MSH), ACTH(1-24), or β-MSH. (B) Functional activation of stingray MC4R after stimulation with the following stingray melanocortins: Des-acetyl-α-MSH (Des-Ac-α-MSH), γ-MSH, δ-MSH or β-endorphin(1-20). The activation assays were performed as described in the figure legend for Fig. 6. Results are expressed as mean ± S.E.M.; $n=3$.

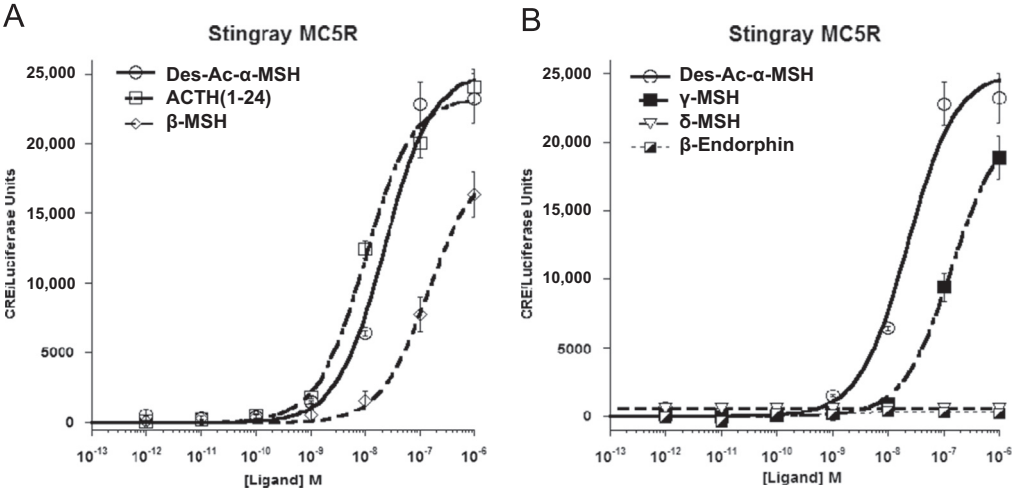

Fig. 10. Ligand selectivity of stingray MC5R. (A) Functional activation of the stingray MC5R after stimulation with the following stingray melanocortins: Des-acetyl-α-MSH (Des-Ac-α-MSH), ACTH(1-24), or β-MSH. (B) Functional activation of stingray MC5R after stimulation with the following stingray melanocortins: Des-acetyl-α-MSH (Des-Ac-α-MSH), γ-MSH, δ-MSH or β-endorphin(1-20). The activation assays were performed as described in the figure legend for Fig. 6. Results are expressed as mean ± S.E.M.; $n=3$.

(Promega Inc., Madison, WI, USA) was added to each well, and incubated for 5 min at room temperature. Luminescence was measured with a Bio-Tek Synergy HT plate reader (Bio Tek, Winooski, VT, USA), and the dose response curves were analyzed by using Kaleidagraph software (Synergy Software, Reading, PA, USA). All experimental treatments were performed in triplicate.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.04.050.

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