Supplemental Figure Legends

Fig. 1. Alignment of Human MCRs, Zebrafish MCRs, and Elephant Shark MC2R.   The amino acid sequences of human MC1R (AAD41355.1), MC2R (AA067714.1) , MC3R (AA072726.1), MC4R (AA092061.1), MC5R (NP_005904.1), and zebrafish MC1R (AA162848.1), MC2R (NP_775386.1), MC3R (AA024744.1), MC4R (AAL85494.1), MC5R (NP_775386.1), and *C. milii* MC2R (FAA704.1), and *Lampetra fluviatilis* MCaR (lamprey; ABB36647.1) were aligned as described previously (37). Amino acid positions shaded in gray were identical in 75% of the taxa. N-linked glycosylation sites are underlined. A (*) denotes an amino acid position that is in the proposed HFRW binding site of the receptor (33). Abbreviations: TM – transmembrane spanning domain; EC – extracellular loop; IC – intracellular loop.

Fig. 2. Comparison of Selected Vertebrate Melanocortin-2 Receptor Sequences

The amino acid sequences of several melanocortin-2 receptor orthologs were aligned (36) by inserting four gaps into the alignment. Amino acid positions shaded in gray were identical in at least 75% of the sequences analyzed. Proposed N-linked glycosylation sites are underlined. The following sequences were aligned: mammals - human (hs) AA067714.1, and *Mus musculus* (mm) AA139212.1; bird - *Gallus gallus* (gg) NP_001026686.1; reptile - *Anolis carolinensis* (ac) (   ), amphibian - *Xenopus tropicalis* (xt) XP002936118.1; teleost fishes - *Takifugi rubripes* (tr) AA024748.1, *Oreochromis mossambicus* (om) ( ), and *Danio rereo* (dr) AA024743.1; cartilaginous fish - *Callorhynchus milii* (cm) FAA00704.1.

Fig. 3. Functional expression of the Elephant Shark MC2 receptor co-transfected with Elephant shark MRAP2 and stimulated with hACTH(1-24). CHO cells were transfected with a *elephant shark mc2 receptor* cDNA construct, a *cre/luc* cDNA construct and either with or without a *elephant shark mrap2* cDNA construct. Two days post-transfection, wells containing 1×10^5 cells were stimulated with hACTH(1-24) at concentrations ranging from 10^{-6}M to 10^{-12}M as described

in Methods. Results are expressed as mean$\pm$ S.E.M.; n=3. The results were normalized to the average readings for cells treated with 1µM hACTH(1-24).

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                          N-terminal                    [----------TM1--------
       hMC1R    M------AVQGSQRRLLGSLNST------PTAIPQLGLAANQTGARCLEVSISDGLFLSLGLVSLVENALVV
       zMC1R    M--------NDSSRHHFSMKHMDYMYNADNNITLNSNSTASDINVTGIAQIMIPQELFLMLGLISLVENILVV
       hMC2R    MK--------------------------HIINSYENINNTARNNSDCPRVVLPEEIFFTISIVGVLENLIVL
       zMC2R    M------------------------PSAESPSSIHTDCAEVQVPGQVFLVIAVASLSENLLVL
       hMC3R    MNAS-----CCLPSVQPTLPNGS-------EHLQAPFFSNQSSSAFCEQVFIKPEVFLSLGIVSLLENILVI
       zMC3R    MNDS-----HLQFLKGQKSVNST-------SLPPNGSLADSPAGTLCEQVQIQAEVFLTLGIVSLLENILVI
       hMC4R    MVNS----THRGMHTSLHLWNRS---SYRLHSNASESLGKGYSDGGCYEQLFVSPEVFVTLGVISLLENILVI
       zMC4R    MNTS------HHHGLHHSFRNHSQ----GALPVGKPSHGDRGSASGCYEQLLISTEVFLTLGLVSLLENILVI
       hMC5R    MNSS------FHLHFLDLNLNAT------EGNLS---GPNVKNKSSPCEDMGIAVEVFLTLGVISLLENILVI
       zMC5R    MHVN--------SSPASYILNATETP-------------SHNKPKACEQLNIATEVFLILGIVSLLENILVI
       cmMC2R   MSGA------DTSASPWLANVT----TAVMNTSGF----MNGSGICRQLEIPLEVYLILGGVGMLENLLVI
       LMCaR    MNLSEALFPNPFVGTSGPDDNGT---ASASANRT--------RFSPCHNFSIPTEVFLALGIVSLVENALVI

                --]     IC1    [----------TM2---------]     EC1    [----------TM3-----
       hMC1R    ATIAKNRNLHSPMYCFICCLALSDLLVSGSNVLETAVILLLEAGALVARAAVLQQLDNVIDVITCSSMLSSLC
       zMC1R    VAIIKNRNLHSPMYYFICCLAVADMLVSVSNVVETLFMLLTEHGLLLVTAKMLQHLDNVIDIMICSSVVSSLS
       hMC2R    LAVFKNKNLQAPMYFFICSLAISDMLGSLYKILENILIILRNMGYLKPRGSFETTADDIIDSLFVLSLLGSI-
       zMC2R    VAVIKNKNLHSPMYCFICNLAVFNTISSFSKALENILLLFKDAGRLNLRGPFELKIDDIMDSLLCMCFLSSIF
       hMC3R    LAVVRNGNLHSPMYFFLCSLAVADMLVSVSNALETIMIAIVHSDYLTFEDQFIQHMDNIFDSMICISLVASIF
       zMC3R    SAVVKNKNLHSPMYFFLCSLAAADMLVSVSNSLETIVIAVLNSRLLVASDQLCRLMHNVCDSMICISLVASIC
       hMC4R    VAIAKNKNLHSPMYFFICSLAVADMLVSVSNGSETIVITLLNSTD-TDAQSFTVNIDNVIDSVICSSLLASLC
       zMC4R    AAIVKNKNLHSPMYFFICSLAVADLLVSVSNASETVVMALITGGNLTNRESIIKNMDNVFDSMICSSLLASIW
       hMC5R    GAIVKNKNLHSPMYFFVCSLAVADMLVSMSSAWETITIYLLNNKHLVIADAFVRHIDNVFDSMICISVVASMC
       zMC5R    CAIVKNKNLHSPMYFFVCSLAVADMLVSVSNAWETIVIYLLTNRQLVVEDHFIRQMDNVFDSMICISVVASMC
       cmMC2R   IAVVNNRNLHSPMYLFICSLAMADMLVSVGKASEAVI-IFLDQNSHLLTETLIDHLDYLFDSLICISLIASIL
       LMCaR    AAIARNRNMHSPMYCFICSLAVADMLVSVSNAWETIIMALLQNGSLAMQEDTLKQMDNIMDSMICTSVVASMC

                ---]      IC2          [--------TM4---------]    EC2    [-------TM5-----
       hMC1R    FLGAIAVDRYISIFYALRYHSIVTLPRARRAVAAIWVASVVFSTLFIAYYDHVAVLLCLVVFFLAMLVLMAV-
       zMC1R    FLCTIAADRYITIFYALRYHSIMTTQRAVGIILVVWLASITSSSLFIVYHTDNAVIACLVTFFGVTLVFTAV-
       hMC2R    SLSVIAADRYITIFHALRYHMLMTMRVTFCTGTGITMVIFSHHVPTVITFTSLFPLMLVFIL-
       zMC2R    SILAIAVDRYISIFHALRYHMLMTMRRVLIILFTIWVLCGTSGALMVGFFEAATVTIFFIVLFFTALLLILL-
       hMC3R    NLLAIAVDRYVTIFYALRYHSIMTVRKALTLIVAIWVCCGVCGVVFIVYSESKMVIVCLITMFFAMMLLMGT-
       zMC3R    NLLAIAVDRYVTIFYALRYHSIVTVRRALVAIAVIWLVCVVCGIVFIVYSESKTVIVCLITMFFAMLVLMAT-
       hMC4R    SLLSIAVDRYFTIFYALQYHNIMTVKRVGIIISCIWAACTVSGILFIIYSDSSAVIICLITMFFTMLALMAS-
       zMC4R    SLLAIAVDRYVTIFYALRYHNIMTQRRAGTIITCIWTFCTVSGVLFIVYSESTTVLICLISMFFTMLALMAS-
       hMC5R    SLLAIAVDRYVTIFYALRYHHIMTARRSGAIIAGIWAFCTGCGIVFILYSESTYVILCLISMFFAMLFLLVS-
       zMC5R    SLLAIAVDRYITIFYALRYHNIMTQRRAGTIITCIWTFCTGCGIVFIIYSDNTSVIVCLVSMFFIMLALMAS-
       cmMC2R   SLGAIATDRYLTIFHALRYHQIMTVKRAALIISALWTFCTFSGSFIIKFNRRKNAFPGSLITMYFTTLFVIVS
       LMCaR    SLLAIAVDRYVTIFYALRYHNIMTVRRAASIIGAIWGACVVSGTLFITYWDHRTVIVCLIALFVTMLVLMAS-

                ----]       IC3                   [----------TM6----------]     EC3
       hMC1R    LYVHMLARACQHAQGIARLHK-----RQRPVHQGFGLKGAVTLTILLGIFFLCWGPFFLHLTLIVLCPEHPTC
       zMC1R    LYLHMFILAHVHSRRITALHK--------SRRQTTSMKGAITLTILLGVFILCWGPFFLHLILILTCPTNPYC
       hMC2R    LYVHMFLLARSHTRKISTLP------------RANMKGAITLTILLGVFIFCWAPFVLHVLLMTFCPSNPYC
       zMC2R    LYVHMFLLARHHANRIASMP------GAQAQHRKSGLRGALTLTIKKGVFVACWAPFSLHLLIMMICPENQYC
       hMC3R    LYVHMFLFARLHVKRIAALP----PADGVAPQQHSCMKGAVTITILLGVFIFCWAPFFLHLVLIITCPTNPYC
       zMC3R    LYVHMFLLARLHVQRIAALPPAAPGAGNPAPRQRSCMKGAVTISILLGVFVCCWAPFFLHLILLVSCPHHPLC
       hMC4R    LYVHMFLMARLHIKRIAVLPG------TGAIRQGANMKGAITLTILIGVFVVCWAPFFLHLIFYISCPQNPYC
       zMC4R    LYVHMFLLARLHMKRIAALPG------NGPIWQAANMKGAITITILLGVFVVCWAPFFLHLIIMISCPRNPYC
       hMC5R    LYIHMFLLARTHVKRIAALPGASS------ARQRTSMQGAVTVTMLLGVFIVCWAPFFLHLTLMLSCPQNLYC
       zMC5R    LYSHMFMLARSHVKRIAALPG------YNSIHQRASMKAAVTLTILGIFIVCWAPFFLHLIMISCPRNLYC
       cmMC2R   LYVYMFLLARRHAQCIRS-------LPGQRVHQGTSLKGAITLTILLGIFII-WAPFFLHLILVLACPSNPYC
       LMCaR    LYAHMFALARSHAQRISAQP---RSSRQGQQNGAASLKGAVTLSILLGVFVFCWAPFFLHLTFIISCPANPYC

                [----------TM7-------]    C-terminal
       hMC1R    GCIFKNFNLFLALIICNAIIDPLIYAFHSQELRRTLKEVLTCSW
       zMC1R    KCYFSHFNLFLILIICNSLIDPLIYAYRSQELRKTLKELIFCSWCFAV
       hMC2R    ACYMSLFQVNGMLIMCNAVIDPFIYAFRSPELRDAFKKMIFCSRYW
       zMC2R    ECYRSLFQLHVVLLVSHAVIDPAINAFRSVELRNTYKKQLLSSASRICKRCA
       hMC3R    ICYTAHFNTYLVLIMCNSVIDPLIYAFRSLELRNTFREILCGCNGMNLG
       zMC3R    LCYMSHFTTYLVLIMCNSVIDPLIYAFRSLEMRKTFKEI-LCCFGCQPAL
       hMC4R    VCFMSHFNLYLILIMCNSIIDPLIYALRSQELRKTFKEI-ICCYPLGGLCDLSSRY
       zMC4R    VCFMSHFNMYLILIMCNSVIDPLIYAFRSQEMRKTFKEI-CCCWYGLASLCV
       hMC5R    SRFMSHFNMYLILIMCNSVMDPLIYAFRSQEMRKTFKEI-ICCRGFRIACSFPRRD
       zMC5R    MCFMSHFNMYLILIMCNSVIDPLIYAFRSQEMRKTLKEI-ICCYSLRNVFGMSR
       cmMC2R   TCYMSLFQVDLILIMCNSIIDPLIFAFRSPELRNTFKKMCICFNKQLY
       LMCaR    CAYIAYFPLYLLLIMINSVIDPLIYAFRSPELRVIIRDTLRKCGRGRGRGANGTRGSSCCCVQVR
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                        N-terminal                      [---------TM1----------]        IC1
hsMC2R MK---------------HIINSYENINNTARNNSDCPRVVLPEEIFFTISIVGVLENLIVLLAVFKNKNLQAPM                  74
mmMC2R MK---------------HIINSYEHTNDTARNNSDCPDVVLPEEIFFTISVIGILENLIVLLAVIKNKNLQSPM
ggMC2R MSTEKPFNLILSAHAGQTSIPSLENITDFSLNITDCNQVVVPEEVFFTVAAAGILENLLVLVAVIRNKNLHLPM
acMC2R MS--------------------NAVNTSDCAPVVVPEEIFFIIATLGLKENLLVLIAVGRNKNLHSPM
xtMC2R MK-------------------NVTILSVNSTKCSSVHVPEVVYLTVSAIGLLENLLVLLAVIKNKNLHLPM
trMC2R M--------------------NATTVNRSDCPEVNVPIHVFFTIGFVSLLENLLVIGAISWNRNLHSPM
omMC2R M--------------------NDVSALPSNHTDCQVVKVPHLVFLVLGMVSLSENLLVVVAVVRNKNLHSPM
daMC2R M--------------------PSAESPSSIHTDCAEVQVPGQVFLVIAVASLSENLLVIVAVIKNKNLHSPM
cmMC2R MS---GADTSASPWLANVTTAVMNTSGFMNGSGGICRQLEIPLEVYLILGGVGMLENLLVIIAVVNNRNLHSPM

              [----------TM2----------]       EC1      [----------TM3------------]        IC2
hsMC2R YFFICSLAISDMLGSLYKILENIL-IILRNMGYLKPRGSFETTADDIIDSLFVLSLLGSIFSLSVIAADRYITI                  148
mmMC2R YFFICSLAISDMLGSLYKILENIL-IMFRNMGYLKPRGSFESTADDIIDCMFILSLLGSIFSLSVIAADRYITI
ggMC2R YFFICSLAISDMLGSLYKTLENIF-IILCKMGYLTRRGDFEKKLDDAMDSMFILSLLGSIFSLLAIAADRYITI
acMC2R YIFICSLAVSDMLGSLYKAVENIFYIIFCKMQYVKCRGKLAKTMDDILDFMFILSLLSAIAADRYITI
xtMC2R YFFICSLAVSDMLFSLYKILETII-IILANIGFLDRNGPFEKKMDDVMDWIFVLSLLGSIFSISAIAADRYITV
trMC2R YCFIGSLAAFNTVASVTKTWENLM-ITFAEVGHLRKVGFSERKADDVVDSLLCMSFLGSIFSFLAIAVDRYITI
omMC2R YMFICSLATFNTISSLSKTWETLM-MEFSDVGQLDSRGDSVRRVDDIIDALLCMSFIGCICSFLAIAVDRYVTI
drMC2R YCFICNLAVFNTISSFSKALENIL-LLFKDAGRLNLRGPFELKIDDIMDSLLCMFLSSIFSILAIAVDRYISI
cmMC2R YLFICSLAMADMLVSVGKASEAVI--IFLDQNSHLLTETLIDHLDYLFDSLICISLIASILSLGAIATDRYLTI

                  [---------TM4---------]    EC2     [--------TM5----------]
hsMC2R FHALRYHSIVTMRRTVVVLTVIWTFCTGTGITMVIFSHHVPTVITFTSLFPIMLVFILC-LYVHMFLLARSHTR                  222
mmMC2R FHALQYHSIVTMRRTIITLTIIWMFCTGSGITMVIFSHHIPTVLTFTSLFPIMLVFILC-LYIHMFLLARSHAR
ggMC2R FYALRYHNIMTLQRALVILAIIWTFCAGSSIAIALFSHEVATVIPFTILFPLMMIFILC-LYIHMFLLARSHAK
acMC2R FYALRYHNIMTLKRALVMLGVIWAFCTGSGIAMVLFSYEAVTVVSFTVLFCFMLILILC-LYIHMFLLARSHAK
xtMC2R FHALHYHNIMTVKRASVILAVIWTFCGGSGIAIIMLFHDTAMIICLTVMFLLLLVLIVC-LYIHMFLLARSHAK
trMC2R FHALRYHNIMTMQRTGAILGLIWTTCGVSAMLMVRFFDSNLIMSCFVVFFIISLAIIYI-LYVYMFILARVHAR
omMC2R FHALRYHNIMTTRRAAAALAGIWALCGVAGAVMVAFCDATVIKIFFIVLFLISLLILF-LYVHMFLLARSHAR
drMC2R FHALRYHMLMTMRRVLIILFTIWVLCGTSGALMVGFFEAATVTIFFIVLFFTALLLILL-LYVYMFLLARHHAN
cmMC2R FHALRYHQIMTVKRAALIISALWTFCTFSGSFIIKFNRRKNAFPGSLITMYFTTLFVIVSLYVYMFLLARRHAQ

            IC3                 [-----------TM6-----------]      EC3      [----TM7----
hsMC2R KISTLP------------RANMKGAITLTILLGVFIFCWAPFVLHVLLMTFCPSNPYCACYMSLFQVNGMLIMC                  296
mmMC2R KISTLP------------RTNMKGAMTLTILLGVFIFCWAPFVLHVLLMTFCPNNPYCVCYMSLFQVNGMLIMC
ggMC2R KIASLP------TSAVHQRTNMKGAITLTIFLGVFLCCWAPFVLHILLARFCPHNPYCACYMSIFHVNGTLIMC
acMC2R KIA------LMTTSSVHQGANMKGAITLTVLVAVFLCCWSPFVLHMLLMTFCPQNPYCICYGSIFHVHGMLIMC
xtMC2R KIASL----SGQWNSVQQRANINGAITLTILLGLFICCWSPFVLHLLLYVLCRYNPYCACYLSMLNVNGTLILF
trMC2R KIAALPNGSGKHQHQRRWGHGMRGILTLTILFGAFMVCWAPFFLHLIFLMACPMNPYCECYRSMFQLHLVLLMS
omMC2R KIAALP-------GSAMPHRSLRGALTLTMLFGVFVVCWAPFFLHLLLIMVCVENPYCECYRSLFQLNLVLLMS
drMC2R RIASMP-----GAQAQHRKSGLRGALTLTIKKGVFVACWAPFSLHLLIMMICPENQYCECYRSLFQLHVVLLVS
cmMC2R CIRSLP------GQRVHQGTSLKGAITLTILLGIFII-WAPFFLHLILVLACPSNPYCTCYMSLFQVDLILIMC

          ----------]        C-terminal
hsMC2R NAVIDPFIYAFRSPELRDAFKKMIFCSRYW                                                             355
mmMC2R NAVIDPFIYAFRSPELRDAFKRMLFCNRY
ggMC2R NAIIDPMIFAFRSPELRSTFKKMFCCARYNWNWWKLNEGEYYRSTPMQHHFAELKILT
acMC2R NAVIDPMIYAFRSPELRSTFRRLFCCHKPNWT
xtMC2R SSVIDPLIYAFRSPELRNTFKKMLCCWMTM
trMC2R HALIDPVIYAFRIPELRHTFRRMLPCLNWRWR
omMC2R HAVVDPAIYAFRSAELRNTFRKMLFCSDSPLCYKVKALFH
drMC2R HAVIDPAINAFRSVELRNTYKKQLLSSASRICKRCA
cmMC2R NSIIDPLIFAFRSPELRNTFKKMCICFNKQLY
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