

Supplementary Material

The Expanding Family of Natural Anion Channelrhodopsins Reveals Large Variations in Kinetics, Conductance, and Spectral Sensitivity

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Supplementary Note

Calling tools from HMMER3:

For the construction of profile HMMs the tool "hmmbuild" was used without additional parameters where "hmmfile" is the name of the produced profile and "msafile" is the name of the used multiple sequence alignment file:

```
hmmbuild hmmfile msafile
```

The profile search was performed using the tool "nhmmer". The used parameters only affect the computation time, the file naming and the line width of the output.

```
nhmmer --cpu 3 -o hmmout -A hmalignment --tblout tableresults --notextw hmmfile  
transcriptomefile
```

Found sequences were aligned to the profile using the tool "hmmalign":

```
hmmalign hmmfile seqfile
```

CrChR1 : --MSRRPWLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLENN--SVICIPNNGO--FCLAWLKSNGTNAEKLA : 91
CrChR2 : -----MDYGGALSAVG-----RELLEVTNPNVVN--SVLVPED--QCYCAGWIESRGTNGAQTAS : 52
iC++ : -----MDYGGALSAVG-----LFQTSYTLENN--SVICIPNNGO--FCLAWLKSNGTNAEKLA : 52
iChLoc : -----MDYGGALSAVG-----RELLEVTNPNVVN--SVLVPED--QCYCAGWIESRGTNGAQTAS : 52
GcACR_457 : -----MSACPPRTPRYGNMSAADKRAEY-----LQKVIDLSPEIY--SYARDNA--E--LAMS SVVTMP--FV : 56
GcACR_145 : -----MLDAQDTGSQAVGFQPEERMVVRG--KAAAYIDGIGPATPYAGMY--EWEI ANP--D--VKNGLHATS-----RE : 63
GcACR_439 : -----MAETGVIRSQEDYYRLVENRETI-----G--TYWDSSSLVY--EWARNG--N--LRQSEVDVQ-----FV : 54
G1ACR_203 : MAARLAKVVHLARLVVLFVAVMLDAQDTGSQAVGFQPEERMVVRG--KAAAYIDGIGPATPYAGMY--EWEI ANP--D--VKNGLHATS-----RE : 83
G1ACR_243 : -----MAS-----ITCDPAIY--EWSRENQ--F--VEKSLITLD-----GI : 32
GtACR1 : -----MAS-----QVYV--EWASTHT--E--EYNMSRIDST-----FV : 28
GtACR2 : -----MTTISE-----VC--VWALDNP--E--IEVSGTN--D-----NV : 28
PsuACR1 : -----MAALHQALEELGGQASRHLLYR-----APEEMVY--KWVEANP--R--IELHDS--D-----YV : 49
PsuACR_433 : -----MAELVD-----EYCLWALENP--E--MEMSYTS--P-----NT : 28
PsuACR_003 : -----MEAG--ATIEQGGLES-----YDHKITY--VWEMQHP--E--FERGIESGD-----AV : 42
R1ACR_477 : -----MALYT-----SQFGIAY--SWEAEND--D--LSMGIASGA-----PV : 33
RsACR_995 : -----MASVAD-----MGIASGA-----PV : 9
R1ACR_367 : -----MSYVED-----PAFGFY--SWEAANQ--D--IERGLSTTN-----NT : 33
R1ACR_877 : -----MSATEN-----PAFGLY--TWEANDHP--Q--VEMGLDTS-----QT : 33
R1ACR_447 : -----MVSHVDE-----PALGPY--SWEANP--D--VALGLTTSK-----NT : 35
R1ACR_741 : -----MSYVED-----PAFGFY--SWEAANQ--D--IERGLSTSN-----NT : 33
R1ACR_799 : -----MAGVQDDWSAVS-----VPAFGQY--TWEANP--E--VEMGLSTSK-----NT : 40
R2ACR_853 : -----MAVAD-----PQGLY--SWEANM--D--AAMGPTITS-----TT : 33
R2ACR_142 : -----MATFNS-----TWTTY--AWEAANP--E--CAEKMHRSSD-----AV : 32
C1ACR_887 : -----MEHHSYDGMRSIQATISGSGSFADIEGHWNQPTSV-----LDNPAVIDGQLY--AYVMFHP--E--HQGEYAP-----IV : 64
C1ACR_023 : -----MTVLEVN-----EAVIIDGVMY--EYIFSHP--Q--IPAEVLV-----VV : 36

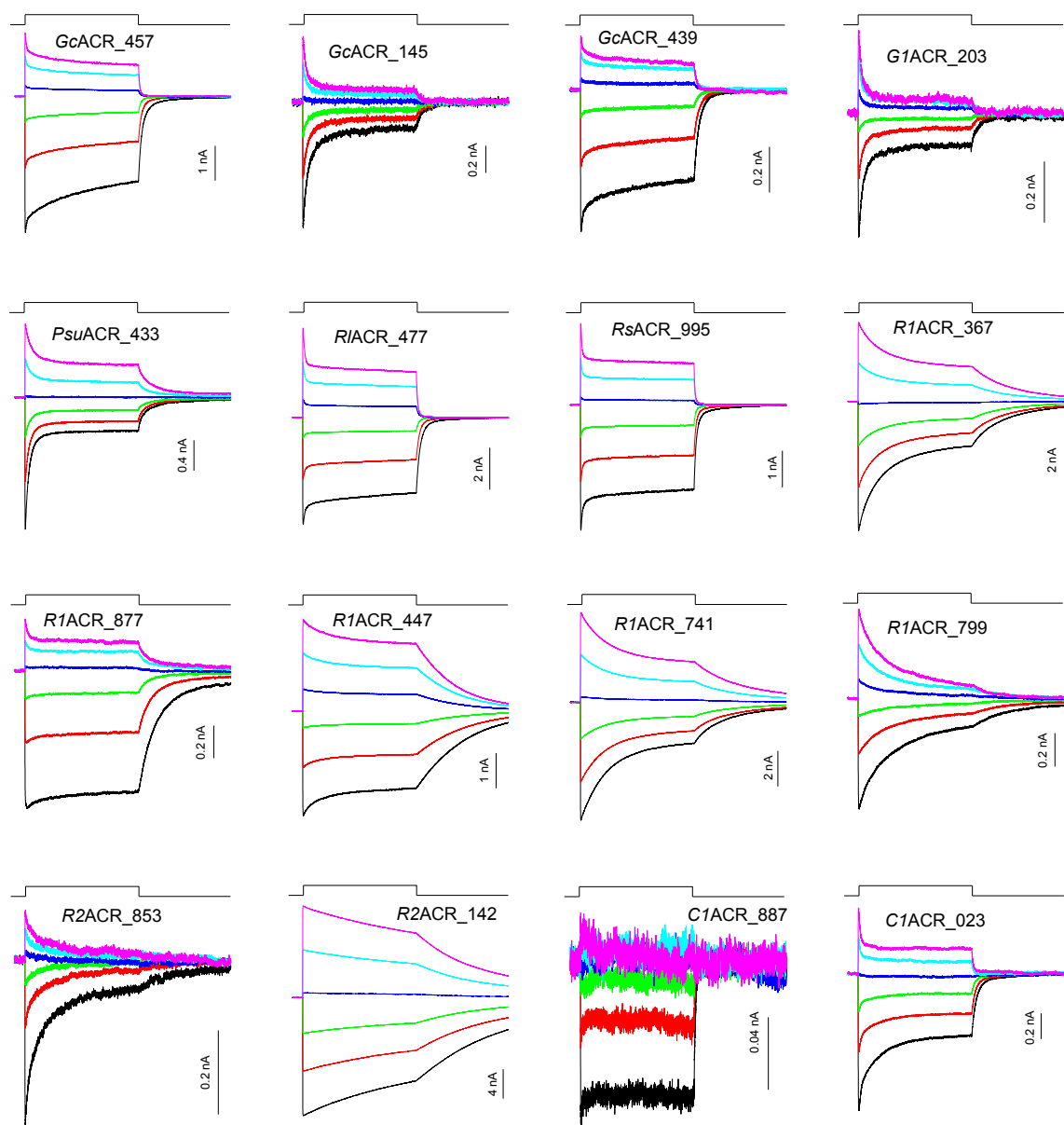
59 63 70 8283 90 97 101 117 123 134
CrChR1 : N L W L T F A L S A L C M F Y G Y Q T W S T C G W E E I Y V A T T E M K I I E Y F F E F D E P A V I Y S S N K N T W L R Y A E W L L T C P V I L I L S N L T G L A N D Y : 184
CrChR2 : N V L W L A G F I L L M F Y A Y Q T W S T C G W E E I Y V C A I E M K V I L E F F F E F K N P S M I Y A T C H R V C W L R Y A E W L L T C P V I L I L S N L T G L S N D Y : 145
iC++ : N L W L S F A S A L C M F Y G Y Q T W S T C G W E I Y V A T T E M K I I E Y F F S F D E P A V I Y S S N K N T W L R Y A E W L L T A P I L I L S N L T G L A N D Y : 145
iChLoc : N V L W L A G F I L L M F Y A Y Q T W S T C G W E I Y V C A I E M K V I L E F F F E F K N P S M I Y A T C H R V C W L R Y A E W L L T C P V I L I L S N L T G L S N D Y : 145
GcACR_457 : K I T O L C M A V M S A C O V L E M F M S R A P R V P W E A Y I L E A T M S I S T A F F C Q Y V R A N G K V L P W C R M A W L C T C P M I G L V S N M A L K Y K S : 143
GcACR_145 : R T I I T L W A V V L C C A L E M M T R A P K V T W E A V Y L E L V C I L C C G A C Y C V L R G D G R M L P L S R M C W L I T C P M I S A F G H H D K L Y G : 150
GcACR_439 : K I O L C M S V V S C O V L E M V S R A P L V P W E A Y I L E M T S I T I G A F T C N Y I R A T G K I L P W A R M A W L C C P M I G L V S N M A L K Y K S : 141
G1ACR_203 : R T I I T L W A V V L C C A L E M M T R A P K V T W E A V Y L E L V C I L C C G A C Y C V L R G D G R M L P L S R M C W L I T C P M I S A F G H H D K L Y G : 170
G1ACR_243 : M I I T L W A V V C M C A L E M M T R A P K V T W E A V Y L E L V C I L C C G A C Y C O L R G D G R M L P L S R M C W L I T C P M I S A F G H H D K L Y G : 86
GtACR1 : K Y V O L M A V V S A C O V E M V T R A P K V P W E A Y I L E T T M I T I T A F T C N Y I R A N G K Y L P W A R M A W L C C P M I G L V S N M A L K Y K S : 119
GtACR2 : S I L O L V W A V S G C O T E M S R A P K V P W E A Y I L E F V S I T I A A S T C N C I L O R G R F F P W A R M A W L C T C P M I G L V S N M A L K Y K S : 115
PsuACR1 : K M A L C F C M V C C O L E M A S Q Y P K V G W E A Y I L E S C C F L T G A S S C N C I O Y D G R L I P W A R Y A W I C T C P S I L L I N T H K C K I S H : 115
ZipACR : Y V F O L C F A V V C A C O V E M F T R A P K V G W E A Y I L E A G V I T I T A A N G E C V L R A D G R I F F A K L A G A V C C P M I G I G G M A Q K Y R T : 136
PsuACR_433 : K T A L C F C M V C C O L E M G W Q P K V G W E A Y I L E A C M T L S A A S N C I O A D G R I I P W A R M A W I C T C P A L I M G I N G A K C L G H : 115
PsuACR_003 : K I O L C F A V I S I C O V E M F T R A P K V A W E A Y I L E A C T I T I T A F T C Y C V L R S D G R I F F W A R M A W I C T C P M I G L V S A M A L K Y K S : 129
R1ACR_477 : K I O L C W A V V S I C O V E M L T R A P K V A W E A Y I L E L C T I T I T A F S N C Y L R A D G Q I F F W A R M A W L V C P M I G L V S A M A L K Y K S : 120
RsACR_995 : K I O L C W A V V S I C O V E M L T R A P K V A W E A Y I L E L C T I T I T A F T C N Y L R A D G Q I F F W A R M A W L V C P M I G L V S A M A L K Y K S : 96
R1ACR_367 : R M I O L L F A S I C A C O V L E M A S R A P R V T W E A V Y L E V V T V L G A S T C N C S M R A D G R I V P I A R F C W L I T C P M I L F V V G H E L K W R T : 120
R1ACR_877 : N M I H L L F A S I C F C O V L E M A S R A P R V T W E A V Y L E L V T V L G A A S N C N L R S N G R M V P A R F C W L I T C P M I L V V V H D V W F N : 120
R1ACR_447 : K M I I L L F A S I C A C O V L E M A S R A P R V T W E A V Y L E L I E T L M A A S T C N C V L R G D G R V L I A R Y C W L I T C P M I L F V V G H Y D K L W G : 122
R1ACR_741 : R M I O L L F A S I C A C O V L E M A S R A P R V T W E A V Y L E V V T V L G A S T C N C S M R A D G R I V P I A R F C W L I T C P M I L F V V G H E L K W R T : 120
R1ACR_799 : K M I O L L F A S I C A C O V L E M A S R A P R V T W E A V Y L E L V T V L G A S T C N C S M R A D G R M V P I A R F C W L I T C P M I L F V V G H E L K V Y G : 127
R2ACR_853 : K I O L L F A S I C A C O V L E M A S R A P R V T W E A V Y L E L V T L M S A A T C N C N M R S D G R V V I A R F C W L I T C P M I L F V V G H E L K W M G : 120
R2ACR_142 : H V V O L T W A I V S F L C S M E M L S R F P R N A W E A V L E L A E T C L I G A S T C N C Y V T A N G R T L P W G R M A W L A C P I I N G I N A V A T N F H G : 119
C1ACR_887 : K I O L C W A V I C M C O V L E M F S R A P R V P W E A Y I L E G V C V I T I T A F T C N C Y V R V D G R I L T W S R L A W L V C P M I G L I S A H T L K Y Q S : 151
C1ACR_023 : K V F O I C W A V V C M C A E M M T R M P K V P W E A Y I L E M V E A I V V I A Y T C N C Y L K G S G R V L P W A R M A W L V C P M I G L I S G M T L K Y Q S : 123

CrChR1 : N K R T M G L I V S D I G T I V W C T T A L S K G --YV V I F E L M G L C Y G I Y T F F N A A K V Y I E A Y H T V P -----K G I --C R D L V R Y L A W L Y F C S W A M F V : 267
CrChR2 : S R R T M G L I V S D I G T I V W C A T S A A T G --YV V I F E L C L G C Y G A N T F F H A A K A V I E G Y H T V P -----K G R --C R Q V V T G M A W L F V S W G M F E I : 228
iC++ : N K R T M G L I V S D I G T I V W C T T A L S K G --YV V I F E L M G L C Y G I Y T F F N A A K V Y I E A Y H T V P -----K G R --C R Q V V T G M A W L F V S W G M F E I : 228
iChLoc : S R R T M G L I V S D I G T I V W C A T S A A T G --YV V I F E L C L G C Y G A N T F F H A A K A V I E G Y H T V P -----K G R --C R Q V V T G M A W L F V S W G M F E I : 228
GcACR_457 : Q P L N P M M A A S I R T V F G I S A T A E T N -D V I T H E F F A V C F I F E M S C A F A T E A L T I D D E Q -Q I G S P L A M -K V V R I L Y L L M V F F G T W S C F E I : 233
GcACR_145 : I Q R M P I C A A S I R T V C L L A S T T D T K I A K W L F E L G F S F Y T M E I T V W F L F Q Q I A K T P T T D V P E N A -T A L R L Y F M F I F T S S T A F F E I : 242
GcACR_439 : Q P L N P M A A S I R T V F G I S A T M S D D S Q P A K W F E F I A V I F F E L T C V Y A T E G M T I A D E A -Q I O S P L G D -A V V R I N I L R G I E F A S W C F E I : 232
G1ACR_203 : I Q R M P I C A A S I R T V C L L A S T T D T K I A K W L F E L G F S F Y T M E I T V W F L F Q Q I A K T P T T D V P E N A -T A L R L Y F M F I F T S S T A F F E I : 262
G1ACR_243 : I Q R M P I C A A S I R T V C L L A S T T D T K V W K F F E L G F C F F S M E I G S V W F L F Q Q I A K A P T T D V P E N A -T A L R L Y F M F I F T S S T A F F E I : 178
GtACR1 : I P L N P M A A S S I C T V F G I S A T S V L D P -L H V W L Y C F I S I F F E M V V A F A T I T I D E Q -T I G S P M S L -K V V E R I K L M I R I V E V S W M A Y E I : 209
GtACR2 : I P L N P M A A S I R T V F G I S A T S P A E -Y M K W L F E F F A T C L F V E Y S V V T F L F Q V L Y G E -S V D T P L A Q -K V V R I K M L R I E I A M T A F F E I : 205
PsuACR1 : F N L N T F I V Q A D I L N M I M G V T G A L T T N -I A F K W I Y A I G C I L F I F I V L V Y D I M T S A A K E W K -A K G D S K G N -L V T R I L L R W I R I V S W C V Y E L : 205
ZipACR : I P L N N V I A A S I R I I F G A S A T A S D -P A R K W F E F C A W I C Y L T E V G I T L T M A V A I S D E S -K I T E L Q G -W V V R I Q T M R I I L V A T S F E V : 226
PsuACR_433 : F N L N T F I V Q A D I L N M I M G I N T L N H -T Y L K W I Y G I A M V L F G C I M G I T L F S S T A R K K -S V D T P L A Q -K V A V R I M L R I E I A M T A F F E I : 205
PsuACR_003 : V P L N N V M A A S I R V C M G I T A T V T P N D -N L K W M F E F F G V T C L L F E Y T C V F T F Q L T I N D E -E I G S P L G N -F V A G R A R I L R I E P H T A T C F F V : 219
R1ACR_477 : L P L N N V M A A S I R V C M G I S A T I T P N E -Q L K W M F E F F G V S M L T F E Y F C V F T F E S I T I A D E Q -E I D T P R S R -F V V G R I K I L R I V E S A T A F F E I : 210
RsACR_995 : L P L N N V M A A S I R V C M G I S A T I T P N E -Q L K W M F E F F G V S M L T F E Y F C V F T F E S I T I A D E Q -E I D T P R S R -L V V G R I K I L R I V E S A T A F F E I : 186
R1ACR_367 : I S C K N M V M A A S I R T V F G I G A S V T I T -P T M R W V O F L S L S F F L F E L I V V Y O F S A G L K K E G -A V K T S L N N -I V Y S R I M F L R F L F L S W S S F E I : 210
R1ACR_877 : V S T R Y M V I A A S I R T V F G I G A S V T I T -D T M R W V O F L L S C I C A F E M V V A H Q T Y K G A L K K E A -A V K T T L N G -V V Y R I M L R I E L F F S N A F E I : 210
R1ACR_447 : I S C K H M V M A A S I R T V F G I G A S V L I D -D Q M R W V O F L S Y A F F F E L Y C A Y M F E G A A L K Q G -E V R T T L N S -I V Y S R I M F L R F L F L S W S S F E I : 212
R1ACR_741 : I S C K N M V M A A S I R T V F G I G A S V T I T -P T M R W V O F L S L S F F L F E L I V V Y O F S A G L K K E G -A V K T S L N N -I V Y S R I M F L R F L F L S W S S F E I : 210
R1ACR_799 : I S C K N M V M A A S I R T V F G I G A S V I T D -D K M R W I O F M L S C I F L F E L W C V O Y N A G L K K E S -A V K T P L N N -I V Y S R I Q L R L R F L F L S W S S F E I : 217
R2ACR_853 : V S C K N M V A A L I Q T V Y G I G A S V I S N -N F M K W F L F L G C I C L A F D F L I V S S F E A G L K K E G -A F R S K L N N -I V H R I M F M S L E F F A V S F E I : 210
R2ACR_142 : I S L N R I Q C A S I L T I V M G V T A S V T D E -P P M O A F E G L T C L I V E Y V I T T I I S A G V A K Q P Y V E E S E T C R K A I G R I L Y L V R N M I F S T A A F F E I : 211
C1ACR_887 : I P L N P M I A C S I R T V F G V S A G V Q E E -P L K W V E W L G I F F A F E L L G C Y L F A T A I A D E -A C K T E L A N -Q V I G R I K T M R A I I F I C P A T T V : 241
C1ACR_023 : I P I N P M V I A A S I R V M G V T A T I V T T E -D L K W A F E G C G L V C L L F E Y A S V Y T F E A V T I G D E A -D A K T D L G N -Q V V A R I K S I E S I E V A T A F F E I : 213

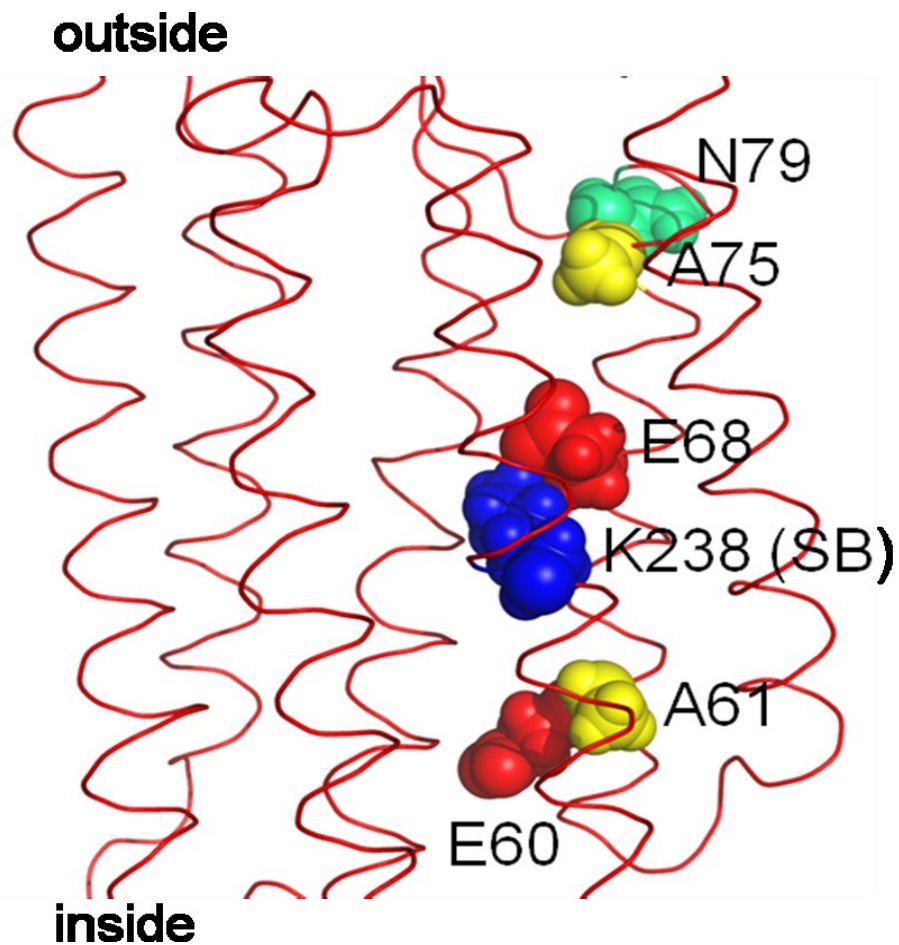
242 246 258 265268 273
CrChR1 : L F I L G P E F G H I N F N S A T A H A L D L A S K N A S M G H F L R V K I E H I L L Y G D I R K K Q V N -----V A G Q E M E V E T M V H E E D D E ---- : 345
CrChR2 : L F I L G P E F G V L S Y G S I V G H T I I D L M S K N C N G L I G H Y L R V L I E H I L I H G D I R K T T K L N -----I G G T E I E V T L V E D E A E A G A V N : 310
iC++ : L F I L G P E F G V L S Y G S I V G H T I I D L M S K N C N G L I G H Y L R V L I E H I L I H G D I R K T T K L N -----I G G T E I E V T L V E D E A E A G A V N : 309
iChLoc : L F I L G P E F G V L S Y G S I V G H T I I D L M S K N C N G L I G H Y L R V L I E H I L I H G D I R K T T K L N -----I G G T E I E V T L V E D E A E A G A V N : 310
GcACR_457 : L W I I S S T Y M C V I D E N A S A L L Y L V A D I S C K N C Y G L L W S T I G L N -C K D R E Y A R N R D E A G M L -----M ----E T D E K Q V I E P P K E N F D V K L -- : 316
GcACR_145 : B W I S S T L C V I S E Q T S A G Y L C A D A L C K N Y G V I Q T N V G L G -C M T P K K P S K D D E A P T N -S H N K E E D T F A A P H G R F N S A ----- : 326
GcACR_439 : L W I V S S T S A C L L D E N I S A V L Y L A D A L C K N S Y G V I L W S T I G L N -C K D R E Y P R N R D I D G L M -----M D -I -D P A K E K Q L E E M P N Q F I K I M G : 319
G1ACR_203 : B W I S S T L C V I S E Q T S A G Y L C A D A L C K N Y G V I Q T N V G L G -C M T P K K P S K D D E A P T N -S H N K E E D T F A A P H G R F N S A K E I D - : 351
G1ACR_243 : B W I S S T L C V I S E Q T S A G Y L C A D A F C K N Y G V I Q T N V G L G -C M T P K K P S K D D E A P T N -S H N K E E D T F A P H G R F N S A K E I Q D T Y : 269
GtACR1 : L W S F S S T C A C I M S E N T S V L Y L L G A L C K N Y G I I L W A T I G L N -C K D R D Y V K R N V D G T L -----M P E Y -E Q D L E K G N T E R Y D A R A G E T -- : 295
GtACR2 : L W I I S P T Q C V I H E N V A I L L Y L A D G L C K N Y G V I L W N T I G H N -C K D P A C L P G Q E K P E A D -----D P F G -L N H E K N -----A P P N D E N I R M F G : 290
PsuACR1 : L W I I S P Q A T C A S E D V I S A H F L C D A F A K N M G F I M W R L R D L D -C H D I S R H Y P -----Q S S Y A R D G K E E E Q M T A M S Q T D D T E K P H : 287
ZipACR : L W V I G Y T F C V I H E D I A L L Y L A D L L S K N Y G V I M W H T I V K N -C K D R E F A A A G G H E A L K -----K A -L E Q D V E I G A G E N Q N Q L T A R Q -- : 311
PsuACR_433 : L W I I S P Q A T C S U R E D V I S C L H F V S D F F A K N Y G I L W N T I G H N -C K D R E F A K N A D K D G E M -----K A -----I E E G T A G P A A D A K D E I K L F G : 296
PsuACR_003 : L W V I S S T C T C M L D E N T S A T L F M A D A A C K N M G L I L W N T I G H N -C K D R D Y V R S Q I E A K E E -----K P ----A D K E M S -Q V P P A A Q D D G V K V F G : 304
R1ACR_477 : L W V I S S T L C V I S E N I S V C Y M A D A A C K N Y G V I L W N T I G H N -C K D R E F A K N A D K D G E M -----K A -----I E E G A A G P A A D A K D E I K L F G : 296
RsACR_995 : L W V I S S T L C V I S E N I S V C Y M A D A A C K N Y G I L W N T I G H N -C K D R E F A K N A D K D G E M -----K A -----I E E G T A G P A A D A K D E I K L F G : 292
R1ACR_367 : L W I I S S T F C L V N D E D V S L A Y L V A D M I C K N Y G I T N A N T L R V I N -C K T P T E T E E A Q -P K D N E -H A H V G E T Y F E Q M L S G L R G S R H ----- : 294
R1ACR_877 : I W A I S S T L C L M I S E D V S L A Y L A D M I C K N Y G I T N A N T L R V I N -C A V P T E T E E H A Q -----A K D H L T Y L E M M N N L G T G R H H D E L : 293
R1ACR_447 : L W I I S S T L C L M I S E D V S L A Y L A D M I C K N Y G I T N A N T L R V I D -C K T P T E T E E A S G V K K A N -P N H V G E T Y L E M M N N L G T G R H H D E L : 300
R1ACR_741 : L W I I S S T F C L V N D E D V S L A Y L V A D M I C K N Y G I T N A N T L R V I N -C K T P T E T E E A M L -P K D H E -H A H V G E T Y F E Q M L S G L R G S R R R D H ----- : 297
R1ACR_799 : V W I I S S T L C L M I S E D G S L A Y L A D M I C K N Y G I T N A N T L R V I D -C K T P T E T E E A A -----N -T -Y G I I N A N T L F R V L N G W P E T E E E A K N : 303
R2ACR_853 : I W T A P T C I M I N E N A V L A Y L A D M I C K N Y G I I N A N T L Q V N -C S V P T P K L E S K L P Q K -----N E V H E T Y I D M A L D S W R G K A S E T E A - : 296
R2ACR_142 : V W I I S P Q C A C V F G E D V V P L Y L A D A L C K N Y G I L W N T I G H N -C K T P T E T E E A A Q -----A P A L Q E M T A N T S V I M A P A V I Q E T P C : 295
C1ACR_887 : T W V I S S A T C L V D N Y T I G E V I D A V C K N M G V I W S T I G L N -C K D R D Y A R T F Q E E E Q -----Q E E A P D F A K G G A M P A N D N V E W K M F : 329
C1ACR_023 : L W I I S S T C T C V I D E N I S A V A Y L I S A F C K N F N G I L W S T I G L N -C K D R D F V R A P P A A E S -----E K -----D V Q D A P G -----K S D V E V K M F G : 294

Supplementary Figure S1. ClustalW protein alignment of seven-helix transmembrane domains of ACR homologs tested in this study. Also included are two CCRs (lines 1 & 2), two engineered Cl⁻-conducting CCR mutants (lines 3 & 4) and three previously characterized ACRs (lines 10-12). On the right are shown the numbers of the last residue in each line. The residue numbers on top of the alignment correspond to the sequence of *CrChR2*. The red lines show transmembrane helices according to 3ug9 structure.

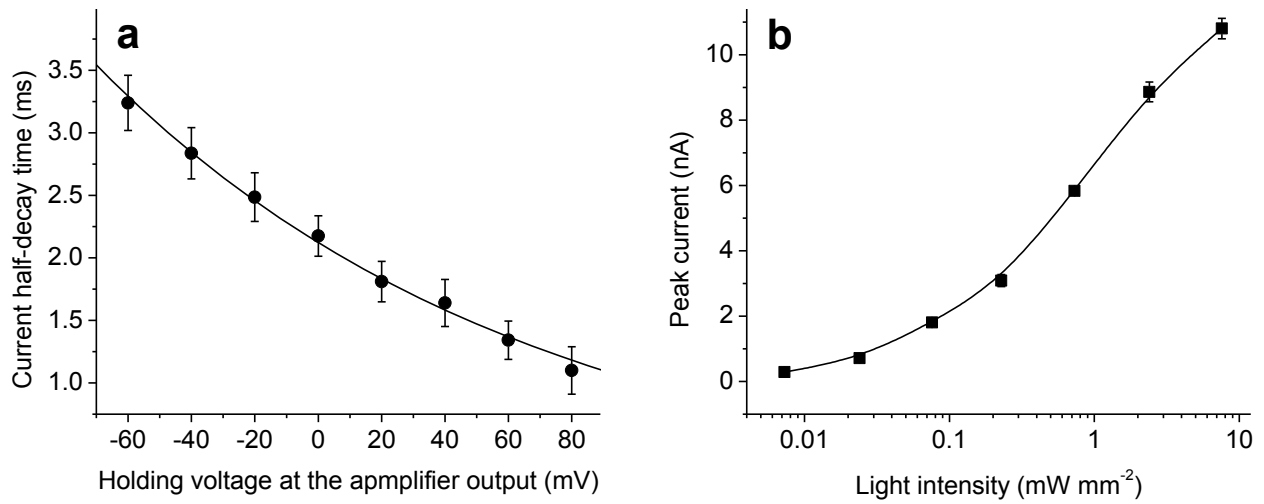
The four levels of gray shading reflect the degree of conservation (from the darkest to the lightest): 100% conserved, $\geq 80\%$ conserved, 60%-80% conserved, and $< 60\%$ conserved. Colored residues are positions in which mutations have been introduced to confer Cl⁻ selectivity to CCRs mutants and those corresponding to the residues that contribute to the central and inner gates in the high-resolution crystal structure 3ug9 of a hybrid CCR. The color code is: blue, positively charged residues; red, negatively charged residues; green, polar residues; yellow, non-polar residues.



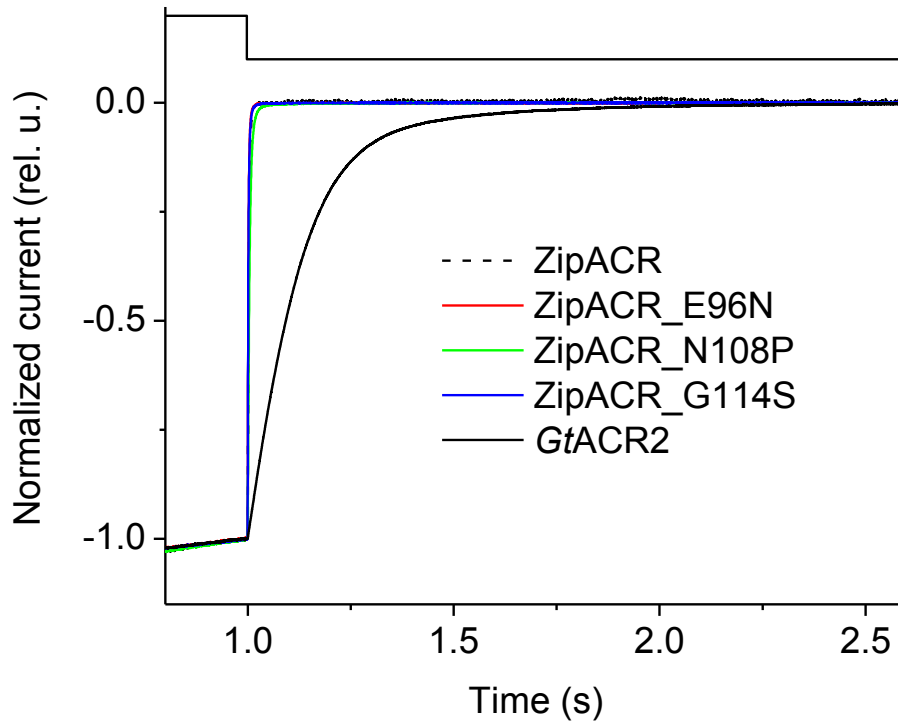
Supplementary Figure S2. Representative series of photocurrents generated by ACR homologs expressed in HEK293 cells. The traces were recorded in the standard bath in response to a 1-s light pulse (shown on top; also serves as a time scale bar). Note the different scaling of the Y axis in different panels. The holding voltage was changed in 20-mV steps from -60 mV at the amplifier output (bottom trace). Abbreviated protein names are shown in each panel (see Table 1 in the main text for their accession numbers and source organisms).



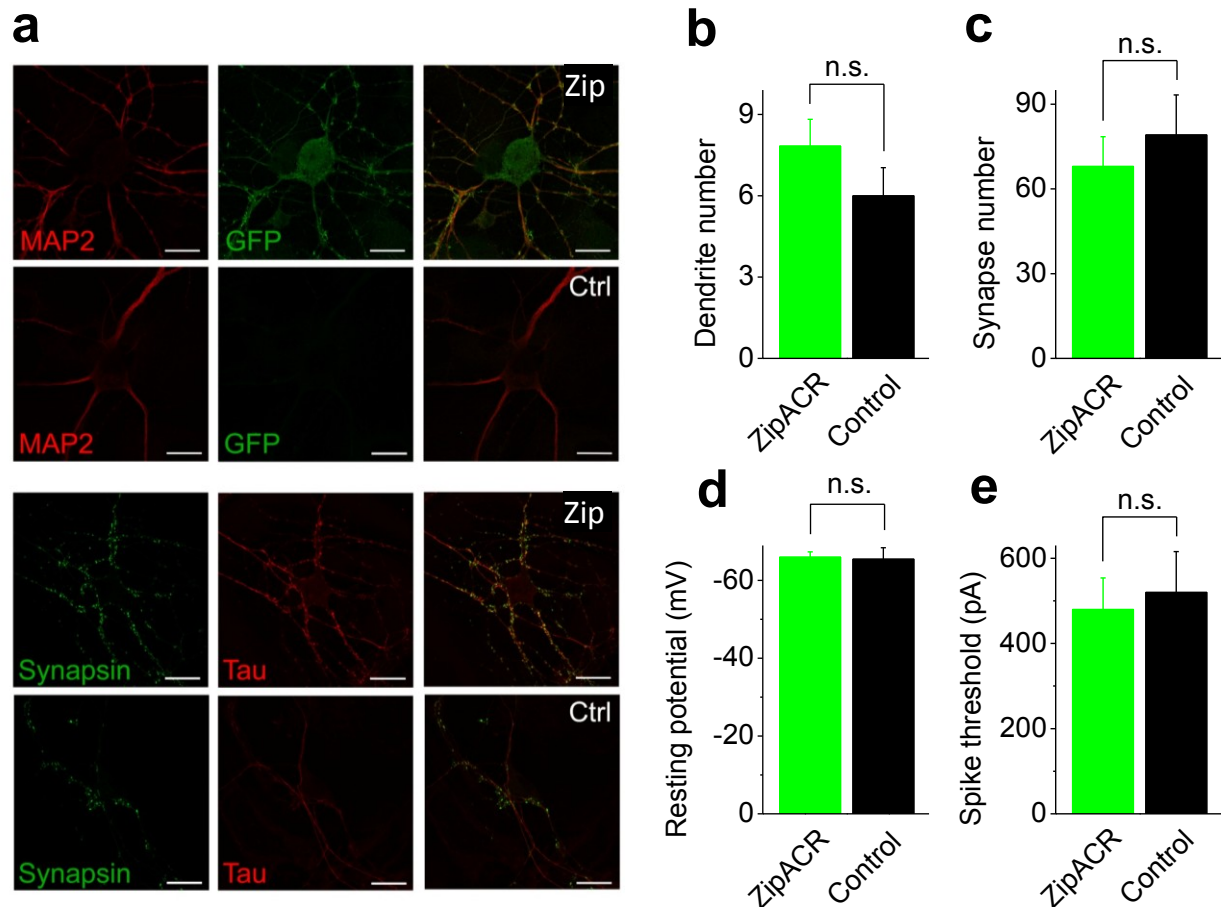
Supplementary Figure S3. A homology model of *GtACR1* built on Robetta server using the **3ug9 template.** Shown are the side chains of the residues that were replaced with glutamates for testing in this study (Ala61, Ala75 and Asn79), conserved glutamates in helix 2 (Glu60 and Glu68) and the Schiff base (Lys238).



Supplementary Figure S4. Properties of ZipACR photocurrents in HEK293 cells. (a) The dependence of ZipACR photocurrent half-decay time on the holding voltage. The data points are the mean values $\pm$ sem ($n = 6$ cells). (b) The dependence of ZipACR photocurrent amplitude on the stimulus intensity. The data points are the mean values $\pm$ sem ($n = 3$ cells).



Supplementary Figure S5. Photocurrent decay rate in ZipACR is not affected by the E96N, N108P and G114S mutations. The currents traces were measured in HEK293 cells in standard buffer at -60 mV and normalized at the end of a 1-s light pulse the time course of which is schematically shown on top. A trace recorded from *GtACR2* is shown for comparison.



Supplementary Figure S6. ZipACR expression does not affect neuronal morphology and physiology. (a) Representative images of cultured mouse hippocampal neurons expressing ZipACR (Zip) or control non-transduced neurons (Ctrl). The neurons were stained with antibodies against MAP2 (a dendritic marker), GFP, Synapsin (a synaptic marker) and Tau (an axonal marker). Scale bar 20 μ m. (b) The number of primary dendrites per neuron at a 25 μ m radius around the center of the soma. (c) The number of synapses per neuron in a 25 μ m circle around the center of the soma. (d) The resting membrane potential in the dark. (e) The spike threshold in the dark (the injected current at the time of first spike induced by ramp stimulation). The data in panels b-c are the mean values $\pm$ sem ($n = 5-12$ cells). There was no significant difference between transduced and non-transduced neurons (p values by Mann-Whitney test were >0.05).

Supplementary Table S1. Cryptophyte transcriptomes searched in this study for ACR homologs.

	Project code	Organism	Habitat	Search output
1.	MMETSP0038	<i>Cryptomonas paramaecium</i>	freshwater	No ACR homologs identified, low quality
2.	MMETSP0043	<i>Hemiselmis andersenii</i>	marine	ACR homologs identified
3.	MMETSP0046	<i>Guillardia theta</i>	marine	ACR homologs identified & confirmed previously ⁹
4.	MMETSP0047	<i>Chroomonas cf. mesostigmatica</i>	marine	No ACR homologs identified, low quality
5.	MMETSP0484	<i>Rhodomonas lens</i>	ubiquitous	ACR homologs identified & confirmed
6.	MMETSP0799	<i>Geminigera cryophila</i>	marine	ACR homologs identified & confirmed
7.	MMETSP0986	Unidentified eukaryote sp.	marine	ACR homologs identified & confirmed
8.	MMETSP0987	Unidentified eukaryote sp.	marine	ACR homologs identified & confirmed
9.	MMETSP0988	Unidentified eukaryote sp.	marine	ACR homologs identified
10.	MMETSP0989	Unidentified eukaryote sp.	marine	ACR homologs identified
11.	MMETSP1041	<i>Hemiselmis andersenii</i>	marine	ACR homologs identified
12.	MMETSP1042	<i>Hemiselmis andersenii</i>	marine	ACR homologs identified
13.	MMETSP1043	<i>Hemiselmis andersenii</i>	marine	ACR homologs identified
14.	MMETSP1047	<i>Rhodomonas salina</i>	marine	ACR homologs identified & confirmed
15.	MMETSP1048	<i>Hanusia phi</i>	marine	ACR homologs identified
16.	MMETSP1049	<i>Proteomonas sulcata</i>	marine	ACR homologs identified & confirmed previously ¹²⁻¹³
17.	MMETSP1050	<i>Cryptomonas curvata</i>	freshwater	No ACR homologs identified
18.	MMETSP1091	<i>Rhodomonas</i> sp.	marine	ACR homologs identified & confirmed
19.	MMETSP1101	<i>Rhodomonas abbreviata</i>	marine	ACR homologs identified
20.	MMETSP1102	<i>Geminigera</i> sp.	marine	ACR homologs identified & confirmed
21.	MMETSP1355	<i>Hemiselmis tepida</i>	marine	No ACR homologs identified
22.	MMETSP1356	<i>Hemiselmis virescens</i>	marine	ACR homologs identified
23.	MMETSP1357	<i>Hemiselmis rufescens</i>	marine	ACR homologs identified
24.	MMETSP1389	<i>Rhodomonas</i> sp.	marine	ACR homologs identified & confirmed

25.	BAKF	<i>Cryptomonas curvata</i>	freshwater	No ACR homologs identified, low quality
26.	IAYV	<i>Rhodomonas</i> sp.	marine	ACR homologs identified & confirmed
27.	IRZA	<i>Proteomonas sulcata</i>	marine	ACR homologs identified & confirmed previously ¹²⁻¹³
28.	MJMQ	<i>Hemiselmis virescens</i>	marine	No ACR homologs identified
29.	ROZZ	<i>Chroomonas</i> sp.	freshwater	No ACR homologs identified

Supplementary Table S2. Numerical data on ACR diversity for Fig. 4 in the main text. The mean and sem values of the peak current, inactivation (measured as the difference between the peak value and the value at the end of the 1-s light pulse divided by the peak value, per cent), half-decay time (the time till the current reached 50% amplitude after the light-off) and the number of sampled cells for each ACR homolog tested in this study. The data for the previously characterized *Gt*ACR1, *Gt*ACR2 and *Psu*ACR1 are also included for comparison.

#	Protein name abbreviation	Mean peak current (nA)	Sem peak current (nA)	Mean inactivation (%)	Sem inactivation (%)	Mean half-decay time (ms)	Sem half-decay time (ms)	Number of cells
1	<i>Gc</i> ACR_457	4.8	0.4	41.7	1.3	20.4	1.3	5
2	<i>Gc</i> ACR_145	1.2	0.3	82.9	0.8	25.0	1.0	3
3	<i>Gc</i> ACR_439	1.2	0.5	31.7	3.8	37.7	7.9	3
4	<i>G1</i> ACR_203	0.8	0.4	76.1	3.0	38.0	2.5	3
5	<i>G1</i> ACR_243							
6	<i>Gt</i> ACR1	7.5	0.9	12.0	1.2	128.4	15.8	12
7	<i>Gt</i> ACR2	7.4	1.0	20.7	1.6	75.7	8.1	8
8	<i>Psu</i> ACR1	0.7	0.2	43.8	1.6	21.3	0.9	16
9	<i>Psu</i> ACR_973 /ZipACR	10.7	1.3	17.0	2.3	3.2	0.2	6
10	<i>Psu</i> ACR_433	1.2	0.1	79.5	2.2	26.0	1.5	3
11	<i>Psu</i> ACR_003							
12	<i>R1</i> ACR_477	5.0	0.5	27.7	1.7	29.7	3.2	7
13	<i>Rs</i> ACR_995	3.9	1.1	32.2	3.9	14.7	3.2	3
14	<i>R1</i> ACR_367	6.0	0.4	74.0	0.5	145.5	38.5	3
15	<i>R1</i> ACR_877	1.0	0.1	12.5	1.1	115.0	4.0	3
16	<i>R1</i> ACR_447	2.3	0.5	34.1	7.2	182.3	67.1	3

17	<i>R1ACR_741</i>	8.8	0.9	73.6	1.3	160.4	11.7	5
18	<i>R1ACR_799</i>	0.6	0.6	73.8	5.0	257.5	15.5	3
19	<i>R2ACR_853</i>	0.2	0.1	83.6	1.4	233.0	83.0	4
20	<i>R2ACR_142</i>	15.0	1.6	43.5	2.7	305.8	36.0	12
21	<i>C1ACR_887</i>	0.05	0.04	27.5	9.3	8.0	2.5	3
22	<i>C1ACR_023</i>	0.6	0.5	51.5	8.9	38.7	9.8	3