Supplementary information

Direct interaction of avian cryptochrome 4 with a cone specific G-protein

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Figure S1

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CLUSTAL O(1.2.4) multiple sequence alignment

ErGtα          ------------------------------------------------------------  0
BtGαi1         MLKRPGLVFFRFPTLAALPPAPQGAGPHTSLGVSKREEIITLSSRKPARRTSRERQPAFA  60


ErGtα          ---MGSGASAEDKEMAKRSKELEKKLQEDADKEAKTVKLLLLGAGESGKSTIVKQMKIIH  57
BtGαi1         PGTMGCTLSAEDKAAVERSKMIDRNLREDGEKAAREVKLLLLGAGESGKSTIVKQMKIIH  120
                  **.  *****  .:*** ::::*:**.:* *: ************************

ErGtα          QDGYTKEECMEFKSIIYGNILQSILAIIRAMSTLGIDYAESSCADEGRLLFNLADSIEEG  117
BtGαi1         EAGYSEEECKQYKAVVYSNTIQSIIAIIRAMGRLKIDFGDSARADDARQLFVLAGAAEEG  180
               : **::*** ::*:::*.* :***:******. * **:.:*: **:.* ** **.: ***

ErGtα          TMPPELVNCIKKLWKDGGVQACFDRAAEYQLNDSAAYYLNQLDRITAANYLPNEQDVLRS  177
BtGαi1         FMTAELAGVIKRLWKDSGVQACFNRSREYQLNDSAAYYLNDLDRIAQPNYIPTQQDVLRT  240
                *  **.. **:****.******:*: *************:****:  **:*.:*****:

ErGtα          RVKTTGIIETKFSVKDLNFRMFDVGGQRSERKKWIHCFEGVTCIIFCGALSAYDMVLVED  237
BtGαi1         RVKTTGIVETHFTFKDLHFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSDYDLVLAED  300
               *******:**:*:.***:*:**********************.**** *** **:**.**

ErGtα          DEVNRMHESLHLFNSICNHKFFAATSIILFLNKKDLFEEKIKKVHLSICFPEYDGPNTFE  297
BtGαi1         EEMNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEKIKKSPLTICYPEYAGSNTYE  360
               :*:******::**:****:*:*: ******************  *:**:*** * **:*

ErGtα          DAGNYIKTQFLDLNMRKDVKEIYSHMTCATDTQNVKFVFDAVTDVIIKENLKDCGLF     354
BtGαi1         EAAAYIQCQFEDLNKRKDTKEIYTHFTCATDTKNVQFVFDAVTDVIIKNNLKDCGLF     417
               :*. **: ** *** ***.****:*:******:**:************:********
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Figure S1. Sequence alignment. European robin gene *ErGNAT2* encoding ErGtα and the inhibitory G protein α-subunit type 1 from Bovine taurus (BtGαi1) are compared. In ErGtα amino acids 220 to 298 were replaced with the corresponding Bovine sequence Giα-1 (amino acids 283 to 361). The region is highlighted in yellow.

Figure S2

Figure S2. *Er*GNAT2 cDNA sequence (coding for cone specific G*α*-subunit) reconstruction from *Erithacus rubecula* (blastn). Known GNAT2 sequences from different bird species [*Ficedula albicollis* (collared flycatcher), *Taeniopygia guttata* (zebra finch) and *Gallus gallus* (chicken)] were used to search the robin (*Erithacus rubecula)* genome from the Bird 10.000 genomes (B10K) Project (PRJNA545868) (S Feng S et al. Dense sampling of bird diversity increases power of comparative genomics. Nature 587, 252-257 (2020), ref. [49]. The cDNA was reconstructed from these exons as shown, with overlapping ends highlighted in yellow.

Figure S3

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CLUSTAL O(1.2.4) multiple sequence alignment


genome ATGGGGAGCGGGGCCAGTGCTGAGGACAAGGAGATGGCCAAGAGGTCCAAGGAGCTGGAG     60
clone  ATGGGGAGCGGGGCCAGTGCTGAGGACAAGGAGATGGCCAAGAGGTCCAAGGAGCTGGAG     60
       ************************************************************

genome AAGAAGCTCCAGGAAGATGCGGATAAGGAGGCCAAGACAGTCAAGTTGCTGCTGCTTGGG     120
clone  AAGAAGCTCCAGGAAGATGCGGATAAGGAGGCCAAGACAGTCAAGTTGCTGCTGCTTGGG     120
       ************************************************************

genome GCTGGAGAGTCAGGGAAGAGCACCATCGTGAAGCAGATGAAGATCATCCACCAGGACGGT     180
clone  GCTGGAGAGTCAGGGAAGAGCACCATCGTGAAGCAGATGAAGATCATCCACCAGGACGGT     180
       ************************************************************

genome TACACGAAGGAGGAGTGCATGGAGTTCAAGTCCATCATCTATGGCAACATCCTGCAGTCC     240
clone  TACACGAAGGAGGAGTGCATGGAGTTCAAGTCCATCATCTATGGCAACATCCTGCAGTCC     240
       ************************************************************

genome ATCCTGGCCATCATCCGCGCCATGTCCACGCTGGGCATCGACTACGCCGAGTCCTCCTGC     300
clone  ATCCTGGCCATCATCCGCGCCATGTCCACGCTGGGCATCGACTACGCCGAGTCCTCCTGC     300
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       ************************************************************

genome GCGGATGAAGGCCGGCTGCTGTTCAACCTGGCTGACTCCATCGAGGAGGGCACCATGCCC     360
clone  GCGGATGAAGGCCGGCTGCTGTTCAACCTGGCTGACTCCATCGAGGAGGGCACCATGCCC     360
       ************************************************************

genome CCCGAGCTGGTGAACTGCATCAAGAAGCTGTGGAAGGATGGGGGGGTCCAGGCGTGCTTT     420
clone  CCCGAGCTGGTGAACTGCATCAAGAAGCTGTGGAAGGATGGGGGGGTCCAGGCGTGCTTT     420
       ************************************************************

genome GACCGCGCTGCCGAGTATCAGCTCAACGACTCAGCTGCGTATTACCTGAACCAGCTGGAC     480
clone  GACCGCGCTGCCGAGTATCAGCTCAACGACTCAGCTGCGTATTACCTGAACCAGCTGGAC     480
       ************************************************************

genome AGGATCACAGCTGCCAACTACCTCCCCAACGAGCAGGACGTGCTGCGATCCCGAGTGAAG     540
clone  AGGATCACAGCTGCCAACTACCTCCCCAACGAGCAGGACGTGCTGCGATCCCGAGTGAAG     540
       ************************************************************

genome ACCACAGGGATCATCGAGACCAAGTTCTCTGTCAAAGACCTGAATTTCAGGATGTTTGAC     600
clone  ACCACAGGGATCATCGAGACCAAGTTCTCTGTCAAAGACCTGAATTTCAGGATGTTTGAC     600
       ************************************************************

genome GTGGGAGGGCAGCGCTCAGAGAGGAAGAAGTGGATCCACTGCTTCGAGGGGGTGACCTGC     660
clone  GTGGGAGGGCAGCGCTCAGAGAGGAAGAAGTGGATCCACTGCTTCGAGGGGGTGACCTGC     660
       ************************************************************

genome ATCATCTTCTGCGGGGCCCTGAGCGCCTACGACATGGTGCTGGTGGAGGATGATGAAGTG     720
clone  ATCATCTTCTGCGGGGCCCTGAGCGCCTACGACATGGTGCTGGTGGAGGATGATGAAGTG     720
       ************************************************************

genome AACCGGATGCATGAATCCCTGCACCTATTCAACAGTATATGCAACCACAAGTTCTTTGCT     780
clone  AACCGGATGCATGAATCCCTGCACCTATTCAACAGTATATGCAACCACAAGTTCTTTGCT     780
       ************************************************************

genome GCCACCTCCATCATCCTCTTCCTCAACAAGAAGGACCTTTTTGAGGAAAAGATCAAGAAA     840
clone  GCCACCTCCATCATCCTCTTCCTCAACAAGAAGGACCTTTTTGAGGAAAAGATCAAGAAA     840
       ************************************************************

genome GTTCATCTCAGCATCTGCTTCCCAGAGTATGATGGTCCAAACACGTTTGAGGACGCAGGG     900
clone  GTTCATCTCAGCATCTGCTTCCCAGAGTATGATGGTCCAAACACGTTTGAGGACGCAGGG     900
       ************************************************************

genome AATTACATCAAGACCCAGTTCCTGGACCTCAACATGAGGAAGGATGTGAAGGAGATCTAC     960
clone  AATTACATCAAGACCCAGTTCCTGGACCTCAACATGAGGAAGGATGTGAAGGAGATCTAC     960
       ************************************************************

genome AGCCACATGACCTGTGCCACAGACACGCAGAACGTCAAGTTCGTGTTCGACGCCGTCACA    1020
clone  AGCCACATGACCTGTGCCACAGACACGCAGAACGTCAAGTTCGTGTTCGACGCCGTCACA    1020
       ************************************************************

genome GACGTGATCATCAAAGAGAACCTCAAGGACTGTGGCCTCTTCTGA 1065
clone  GACGTGATCATCAAAGAGAACCTCAAGGACTGTGGCCTCTTCTGA 1065
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Figure S3. ErGNAT2 nucleotide sequence alignment. The reconstructed European robin ErGNAT2 sequence (Fig. S6) was aligned to the sequence derived from cloning from European robin retina cDNA (see Materials and Methods).

Figure S4

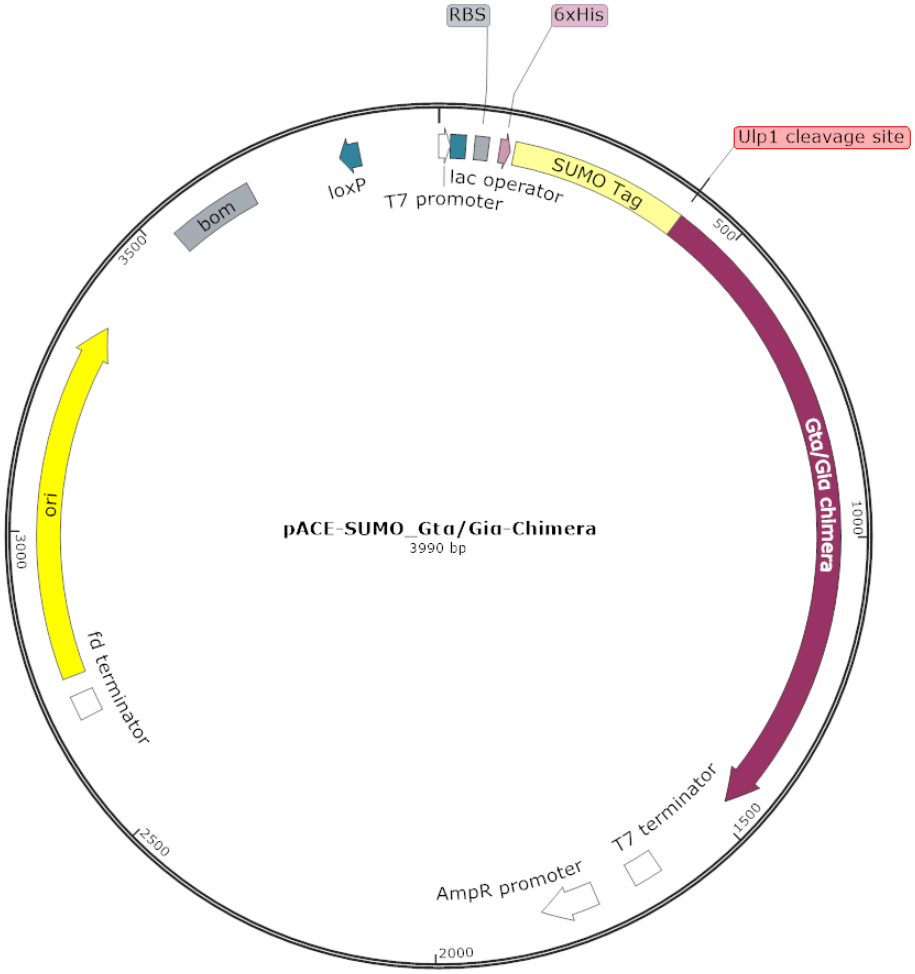
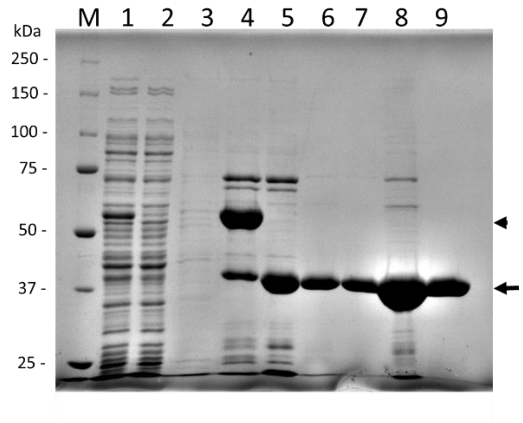


Figure S4. pACE-SUMO vector for Gtα/Giα chimera expression. Plasmid map was generated using SnapGene viewer. A commercial pACE vector (https://geneva-biotech.com/product_category/e-coli-cell-expression/multicoli/) was modified to contain a T7-lac expression cassette with a strong ribosome binding site and an N-terminal 6x histidine and Small Ubiquitin-like Modifier (SUMO) tag. Additional modifications include addition and removal of various restriction enzyme recognition sites.

Figure S5

A



B

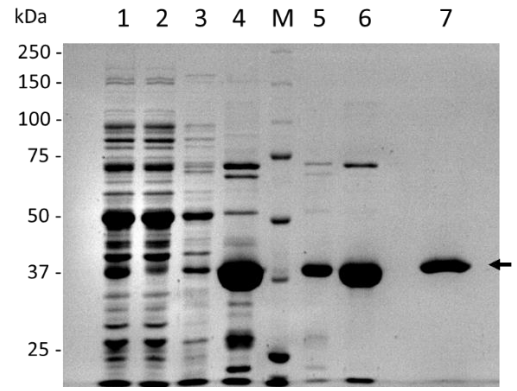


Figure S5. Expression and purification of the G_tα/Giα chimera. Different steps yielding purified non-myristoylated (**A**) and myristoylated (**B**) G_tα/Giα were analysed by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). (**A**) M, molecular mass marker; 1, soluble fraction of protein expression in E.coli that is applied on the Ni-NTA column; 2, nonbound fraction of Ni-NTA chromatography; 3, wash fraction; 4, elution form Ni-NTA column, arrow head at position of SUMO fusion construct; 5, digestion to remove the SUMO tag; 6, second Ni-NTA chromatography of G_tα/Giα after digestion, nonbound fraction; 7, wash fraction; 8, fraction applied on the SEC column; 9, purified G_tα/Giα after SEC, arrow.
(**B**) M, molecular mass marker; 1, soluble fraction of protein expression in *E.coli*.; 2, nonbound fraction of Ni-NTA chromatography; 3, Ni-NTA wash fraction; 4, Ni-NTA elution; 5, input fraction to IEC; 6, pooled fractions from IEC and applied to SEC; 7, representative collected SEC Fraction, arrow indicates purified Gtα/Giα.

Figure S6

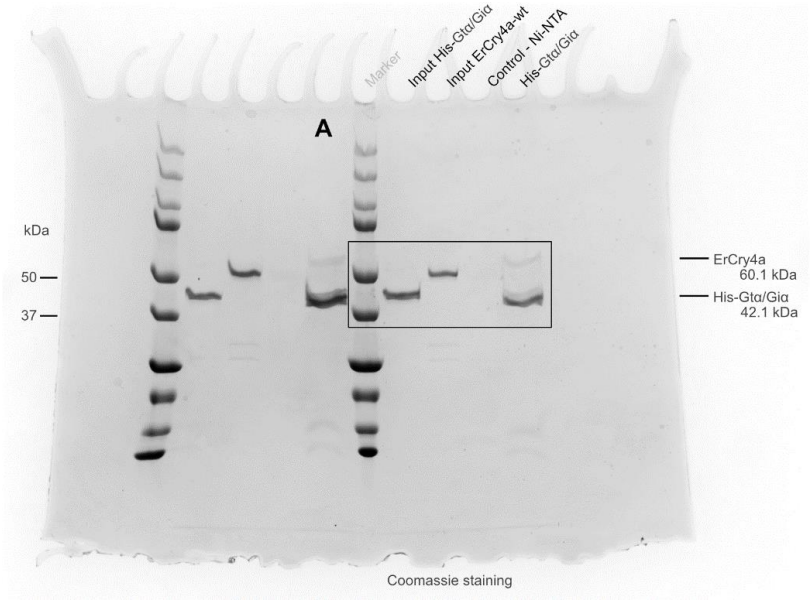


Figure S6. Full size image of pulldown experiment shown in Fig.2 of the main text. Input lanes show the proteins used for the pulldown experiment. As a negative control only *Er*Cry4a, but no His-tagged G_tα/Giα chimera, was incubated with a Ni-NTA affinity matrix. For the experiment, a pre-incubated mixture of both proteins was incubated with the matrix.

Figure S7

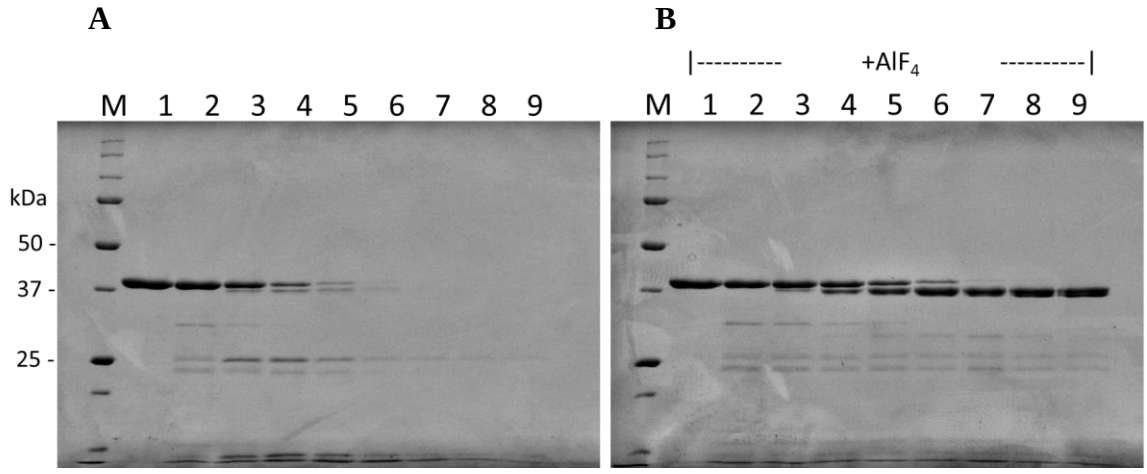


Figure S7. Limited proteolysis of Gtα/Giα in the absence or presence of AlF_4^-. (A) AlF_4^- absent (B) AlF_4^- Present. Analysis by SDS-PAGE. M – molecular mass marker in kDa, 1 – Sample before protease addition, 2 – Sample at 30s, 3 – Sample at 2 minutes, 4 – Sample at 5 minutes, 5 – Sample at 10 min, 6 – Sample at 20 min, 7 – Sample at 40 min, 8 – Sample at 60 min, 9 – Sample at 90 min.

Table S1. Primers for cloning steps

Primers for cloning into pFast:

ErCRY1a, ErCry1b (for)	5'GGGCGCCATGGGATCCATGGGGGTGAACGCCGTG3'
ErCRY1a (rev)	5'TACCGCATGCCTCGAGTTAATTTGTGCTCTGTCGCTGGAC3'
ErCRY1b (rev)	5'TACCGCATGCCTCGAGCTATTTTGATGTTTTGTCTGG3'
ErGNAT2chimera (for)	5'GGGCGCCATGGGATCCATGGGGAGCGGGGCCAGT3'
ErGNAT2chimera (rev)	5'TACCGCATGCCTCGAGTCAGAAGAGGCCACAGTCC3'

Primers for cloning of ErCry4a-497

Forward	5' – CTCGAGGATCCGAATTCAAG – 3'
Reverse	5' – TTAGCGAGTCAGTTGTGCGG – 3'

Primers for cloning of ErCry variants and constructs for FRET measurements

Primer Nr.	Sequence (5'→3')
1 (for)	CTGGAAAGCGGCGGCGAAG
2 (rev)	GGCGGCGGTCACGAACTC
3 (for)	TTCGTGACCGCCGCCGGGATTACACATGGCATGGACGAGCTGTACAAGAGTGGATC CTCGGGATCATCAGGCGCGCCTATGCTGCATCGCACC
4 (rev)	GCCGCCGCTTTCCAGCTCGAGTTATTCTGTTGTTCGGGCCA
5 (for)	GTAAGGGCCCTATTCTATAGTGTCACC
6 (rev)	TCGAGAGGCGCGCCTGATGATCC
7 (for)	GGATCATCAGGCGCGCCTATGCTGCATCGCACCAT
8 (rev)	AGGGCCCTTACTCGAGCTCGAGTTATCTCGTGAGCTGG
9 (for)	TCTCGAGGTGTCTAAGGGCGAAGAG
10 (rev)	GGCGCGCCGTGGTGATGGTGATGATG
11 (for)	CTGGAAAGCGGCGGCGAAG
12 (rev)	GTGGTGATGGTGATGATGCATGGTGG
13 (for)	CATCACCATCACCACGGCGCGCCTATGGGGAGCGGGGC
14 (rev)	GCCGCCGCTTTCCAGCTCGAGGAAGAGGCCACAGTCCTTGAGG

Primers for cloning of Cone Transducin alpha and the Gtα/Giα chimera

ErGnat into pCold (for)	5'-TTCCACTAGTATGGGGAGCGGGGC-3'
ErGnat into pCold (rev)	5'-AAGGCTCGAGTCAGAAGAGCCGCAGTC-3'
Deletion 220-298 (for)	5'-GCAGGGAATTACATCAAGACCC-3'
Deletion 220-298 (rev)	5' -CCAGTGGGGGAGCTTCGTC- 3'
Bovine 220-298 (for)	5' -TTCGAGGGGGTGACCGCCATCATCTTCTGTGTGGCG- 3'
Bovine 220-298 (rev)	5' -CTACATTAAGGGACGGAGAAGTATACACAAACTCGGAC-3'
Chimera into pET21a (for)	5' -GGAGATATACATATGGGGAGCGGGGCCAGTG- 3'
Chimera into pET21a (rev)	5' -GTGGTGGTGCTCGAGGAAGAGCCCGCAGTCCTTGAG- 3'
Chimera into SUMO (for)	5' – CGTCGGATCCGGCGCCGGGAGCGGGGCCAGTGC – 3'
Chimera into SUMO (rev)	5' – GACTGGGAAAACCCTGGCGAGAATTCAGAAGAGCCCGCAGTCCTTG – 3'
6AA deletion (for)	5' – GGGAGCGGGGCCAG – 3'
6AA deletion (rev)	5' – ACCACCGATCTGTTCGC – 3'