Supporting Information

Patra et al. 10.1073/pnas.1304837110

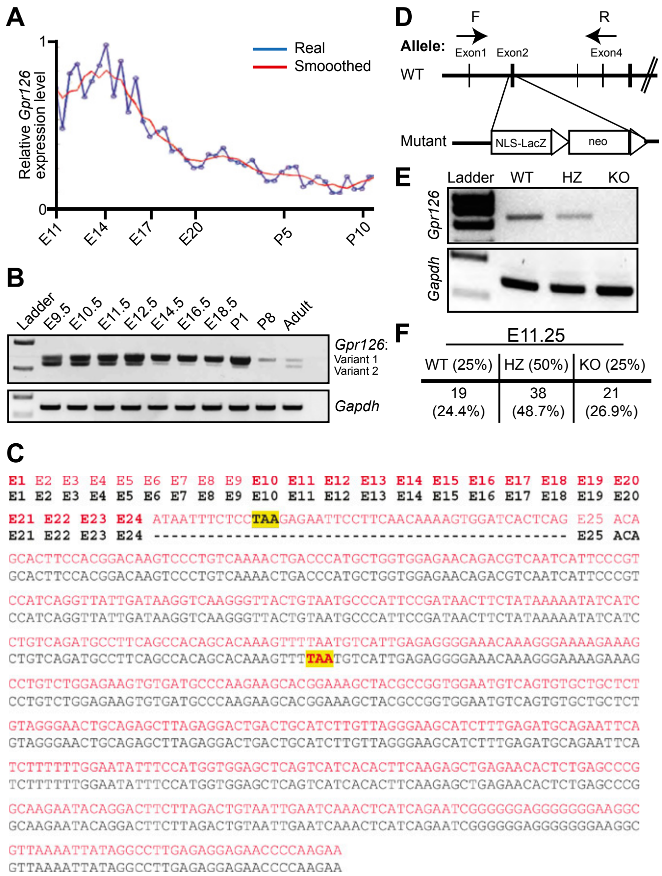

Fig. S1. Generation of R-G protein-coupled receptor 126 (*Gpr126*)$^{-/-}$ mice. (*A*) Microarray analysis (Affymetrix GeneChip Rat Expression Set 230) demonstrating the relative expression of *Gpr126* mRNA in rat heart tissue from E11 to P10.5 in 12-h intervals. (*B*) RT-PCR analysis showing the temporal *Gpr126* mRNA expression profile during mouse heart development. *Gapdh* was used as loading control. (*C*) Mouse *Gpr126* variant analysis. Variant 1 is in red, and variant 2 is in black. TAA, stop codons; E, Exon. (*D*) Scheme of the wild-type *Gpr126* locus and the mutant allele after homologous recombination. Arrows indicate primer binding sites to test the *Gpr126* mRNA transcript levels. F, forward primer; R, reverse primer. (*E*) RT-PCR analysis demonstrating the absence of *Gpr126* mRNA in *Gpr126*$^{-/-}$ mice heart. (*F*) Live offspring at E11.25 from Gpr126$^{+/-}$ matings. E, embryonic day; HZ, heterozygous; KO, knockout; P, postnatal day; WT, wild type.

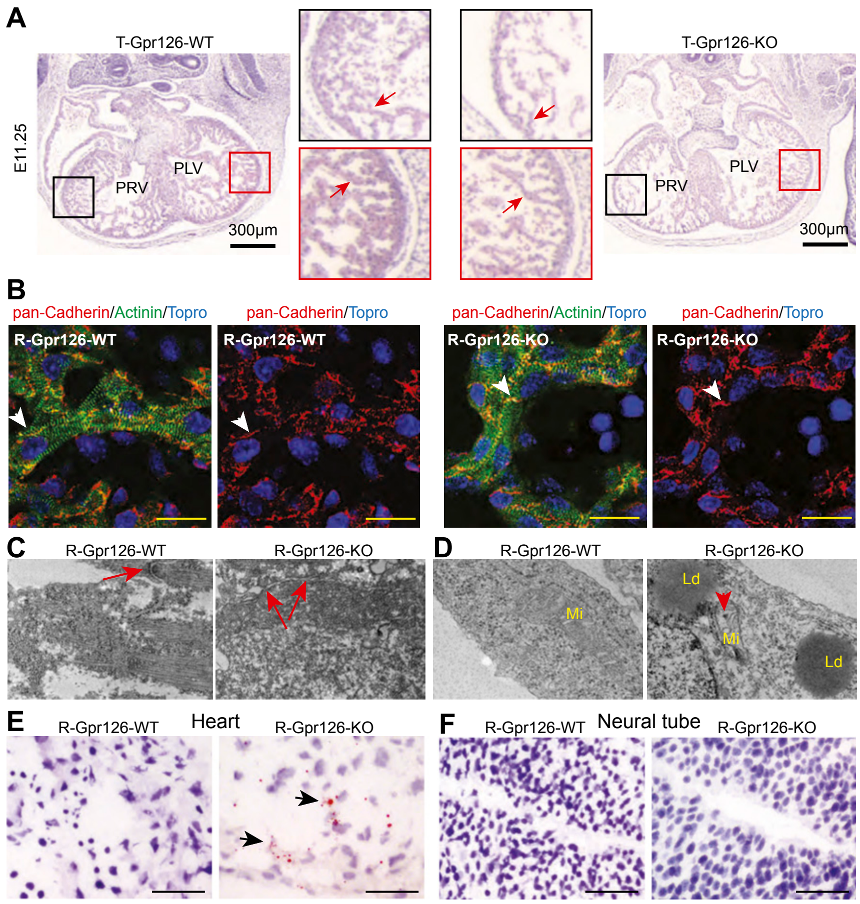

Fig. S2. Morphological and cellular analysis of *Gpr126*$^{-/-}$. (*A*) T-*Gpr126*$^{-/-}$ mice used to study myelination exhibit defects in cardiac trabeculation. Hematoxylin and eosin staining of heart sections reveal hypotrabeculation and thinner trabeculae (arrows) in *Gpr126*$^{-/-}$ hearts ($n = 2$) at E11.25. PLV, primitive left ventricle; PRV, primitive right ventricle. (*B*–*F*) Analysis of R-*Gpr126*$^{-/-}$ mice. (*B*) Confocal images of 5-μm thin heart tissue sections stained for pan-Cadherin (red), cardiomyocyte-specific α-actinin (green), and DNA (Topro; blue). White arrowheads indicate the typical expression pattern of Cadherin proteins at cell–cell adherent junctions in WT and KO hearts. (Scale bar: 25 μm.) (*C* and *D*) Transmission electron micrographs at E11.25 of the myocardium (*C*) and endocardium (*D*). (*C*) Cell–cell contact (arrows) appears normal in KO hearts. (*D*) Endocardial cells from KO mice contain frequently lipid droplets (Ld) and electron-dense precipitates (arrowhead) in their mitochondria (Mi). (*E* and *F*) Cryosections from E11.25 WT and KO tissues stained for lipid (red) and costained with hematoxylin (blue). Lipid droplets (arrows) are visible in Gpr126$^{-/-}$ hearts but are detected neither in WT hearts (*E*) nor in neural tissue from WT and in gpr126$^{-/-}$ mice (*F*). (Scale bar: 65 μm.)

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                    Signal Peptide                           Cub Domain
vari1 MISFISGRWWRWKFQNTLAVFLLLICLSTSVAQSCQSSTSCNVVLTDSQGSFTSPCYPND 60
Vari2 MISFISGRWWRWKFQNTLAVFLLLICLSTS------------------------------ 30
Vari3 MISFISGRWWRWKFQNTLAVFLLLICLSTS------------------------------ 30
Vari4 MISFISGRWWRWKFQNTLAVFLLLICLSTSVAQSCQSSTSCNVVLTDSQGSFTSPCYPND 60

vari1 YPPSQSCNWTIQAPAGFIVQITFLDFELEEAQGCIYDRVVVKTGTSDAKFCGLTANGLTL 120
Vari2 ------------------------------------------------------------
Vari3 ------------------------------------------------------------
Vari4 YPPSQSCNWTIQAPAGFIVQITFLDFELEEAQGCIYDRVVVKTGTSDAKFCGLTANGLTL 120

                                                  PTX Domain
vari1 NSTGNVMEVFFNSDFSVQKKGFHISYKQVAVTLRNQKVTMPKSSKTILRVSNSISIPVLT 180
Vari2 ----------------------------VAVTLRNQKVTMPKSSKTILRVSNSISIPVLT 62
Vari3 ----------------------------VAVTLRNQKVTMPKSSKTILRVSNSISIPVLT 62
Vari4 NSTGNVMEVFFNSDFSVQKKGFHISYKQVAVTLRNQKVTMPKSSKTILRVSNSISIPVLT 180

vari1 AFTVCFEIARTAQKATETIFTLSDAAGTSILAFEKTSNGMELFIGASYCSVDNLLTSSDI 240
Vari2 AFTVCFEIARTAQKA--TIFTLSDAAGTSILAFEKTSNGMELFIGASYCSVDNLLTSSDI 120
Vari3 AFTVCFEIARTAQKATETIFTLSDAAGTSILAFEKTSNGMELFIGASYCSVDNLLTSSDI 122
Vari4 AFTVCFEIARTAQKATETIFTLSDAAGTSILAFEKTSNGMELFIGASYCSVDNLLTSSDI 240

vari1 TATMKPLCLTWTKSSGLIGVYFGGHYFSSICSASQIYTLQSGGLLQIAGKGSSSVSVDDQ 300
Vari2 TATMKPLCLTWTKSSGLIGVYFGGHYFSSICSASQIYTLQSGGLLQIAGKGSSSVSVDDQ 180
Vari3 TATMKPLCLTWTKSSGLIGVYFGGHYFSSICSASQIYTLQSGGLLQIAGKGSSSVSVDDQ 182
Vari4 TATMKPLCLTWTKSSGLIGVYFGGHYFSSICSASQIYTLQSGGLLQIAGKGSSSVSVDDQ 300

vari1 NLDGFIYNFRLWDHAMLSSELSALTCDTVGNVVDWDHSYWTIPGSSTQTDSTLSCS---- 356
Vari2 NLDGFIYNFRLWDHAMLSSELSALTCDTVGNVVDWDHSYWTIPGSSTQTDSTLSCS---- 236
Vari3 NLDGFIYNFRLWDHAMLSSELSALTCDTVGNVVDWDHSYWTIPGSSTQTDSTLSCICFPN 242
Vari4 NLDGFIYNFRLWDHAMLSSELSALTCDTVGNVVDWDHSYWTIPGSSTQTDSTLSCICFPN 360

vari1 ----------TAITTLSPGTAGCASGLGCPATLTVTI----------------------- 383
Vari2 ----------TAITTLSPGTAGCASGLGCP------------------------------ 256
Vari3 CIHLKASTSGTAITTLSPGTAGCASGLGCPATLTVTI----------------------- 279
Vari4 CIHLKASTSGTAITTLSPGTAGCASGLGCPALTTTTISHPSTFSMPLTTTALLTPTTTVK 420

vari1 ------------TSIATTNIIPTNA------------------TTHEDIFYRSTLVVTDE 413
Vari2 ----------------------------------------------EDIFYRSTLVVTDE 270
Vari3 ------------TSIATTNIIPTNA------------------TTHEDIFYRSTLVVTDE 309
Vari4 TTTSAHTETPTATSAKTTTTVPTTSNLPPIQPAAATNSPLSIRKTNEDIFYRSTLVVTDE 480

vari1 QTPDRDATAIISQWLNQTFQNWMYRVYVDGISLQLITVLSRITTTRQTYLALLVYKNTTD 473
Vari2 QTPDRDATAIISQWLNQTFQNWMYRVYVDGI---------RITTTRQTYLALLVYKNTTD 321
Vari3 QTPDRDATAIISQWLNQTFQNWMYRVYVDGISLQLITVLSRITTTRQTYLALLVYKNTTD 369
Vari4 QTPDRDATAIISQWLNQTFQNWMYRVYVDGISLQLITVLSRITTTRQTYLALLVYKNTTD 540

                                                   HormR Domain
vari1 VNLAEVEIESMLRSAPAIGNGLTLDSVTVNLMENCQADEFPVHYRWPESRPTVTQYVPCF 533
Vari2 VNLAEVEIESMLRSAPAIGNGLTLDSVTVNLMENCQADEFPVHYRWPESRPTVTQYVPCF 381
Vari3 VNLAEVEIESMLRSAPAIGNGLTLDSVTVNLMENCQADEFPVHYRWPESRPTVTQYVPCF 429
Vari4 VNLAEVEIESMLRSAPAIGNGLTLDSVTVNLMENCQADEFPVHYRWPESRPTVTQYVPCF 600

vari1 PYKDRNASRTCMINRDNYTSFWALPDRGNCTNITSITVSQENAMDVAVQLADISNNGLSK 593
Vari2 PYKDRNASRTCMINRDNYTSFWALPDRGNCTNITSITVSQENAMDVAVQLADISNNGLSK 441
Vari3 PYKDRNASRTCMINRDNYTSFWALPDRGNCTNITSITVSQENAMDVAVQLADISNNGLSK 489
Vari4 PYKDRNASRTCMINRDNYTSFWALPDRGNCTNITSITVSQENAMDVAVQLADISNNGLSK 660
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Fig. S3. (Continued)

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vari1 EELTQVVTKVMELVNIAKINATLASTVVTIISNVMVSSEDAQKDASETALKAVDELVQKI 653
Vari2 EELTQVVTKVMELVNIAKINATLASTVVTIISNVMVSSEDAQKDAS--ALKAVDELVQKI 499
Vari3 EELTQVVTKVMELVNIAKINATLASTVVTIISNVMVSSEDAQKDASETALKAVDELVQKI 549
Vari4 EELTQVVTKVMELVNIAKINATLASTVVTIISNVMVSSEDAQKDASETALKAVDELVQKI 720

vari1 EFDGPSLTISSKNLVVGVSALDTTNFNGSTLSAFIATNTTDPQIDFDSEAHNALAVVTLP 713
Vari2 EFDGPSLTISSKNLVVGVSALDTTNFNGSTLSAFIATNTTDPQIDFDSEAHNALAVVTLP 559
Vari3 EFDGPSLTISSKNLVVGVSALDTTNFNGSTLSAFIATNTTDPQIDFDSEAHNALAVVTLP 609
Vari4 EFDGPSLTISSKNLVVGVSALDTTNFNGSTLSAFIATNTTDPQIDFDSEAHNALAVVTLP 780

vari1 PTLLQNLSLSQIEKVSRINFMFFGRTGLFQDHQNNGLTLNSYVVASSVGNFTIKNLQDPV 773
Vari2 PTLLQNLSLSQIEKVSRINFMFFGRTGLFQDHQNNGLTLNSYVVASSVGNFTIKNLQDPV 619
Vari3 PTLLQNLSLSQIEKVSRINFMFFGRTGLFQDHQNNGLTLNSYVVASSVGNFTIKNLQDPV 669
Vari4 PTLLQNLSLSQIEKVSRINFMFFGRTGLFQDHQNNGLTLNSYVVASSVGNFTIKNLQDPV 840
                                          GPS Motif
vari1 RIEIAHLEYQKDPNPQCVFWDFNLQNYSGGCNSDGCKVGSDSNSNRTVCLCNHLTHFGIL 833
Vari2 RIEIAHLEYQKDPNPQCVFWDFNLQNYSGGCNSDGCKVGSDSNSNRTVCLCNHLTHFGIL 679
Vari3 RIEIAHLEYQKDPNPQCVFWDFNLQNYSGGCNSDGCKVGSDSNSNRTVCLCNHLTHFGIL 729
Vari4 RIEIAHLEYQKDPNPQCVFWDFNLQNYSGGCNSDGCKVGSDSNSNRTVCLCNHLTHFGIL 900
                                    TM1
Vari1 MDVSRAAELIDEKNNRVLTFITYIGCGISAIFSAATLLTYIAFEKLRRDYPSKILMNLST 893
Vari2 MDVSRAAELIDEKNNRVLTFITYIGCGISAIFSAATLLTYIAFEKLRRDYPSKILMNLST 739
Vari3 MDVSRAAELIDEKNNRVLTFITYIGCGISAIFSAATLLTYIAFEKLRRDYPSKILMNLST 789
Vari4 MDVSRAAELIDEKNNRVLTFITYIGCGISAIFSAATLLTYIAFEKLRRDYPSKILMNLST 960
           TM2                                   TM3
vari1 SLLFLNMVFLLDGWLASYEIKELCVTVAVFLHFFLLTSFTWMGLESIHMYIALVKVFNTY 953
Vari2 SLLFLNMVFLLDGWLASYEIKELCVTVAVFLHFFLLTSFTWMGLESIHMYIALVKVFNTY 799
Vari3 SLLFLNMVFLLDGWLASYEIKELCVTVAVFLHFFLLTSFTWMGLESIHMYIALVKVFNTY 849
Vari4 SLLFLNMVFLLDGWLASYEIKELCVTVAVFLHFFLLTSFTWMGLESIHMYIALVKVFNTY1020
                  TM4
vari1 IRRYILKFCIVGWGVPAAIVGIVLAVSKDSYGKNYYGKGKDGQGTSEFCWILNPVVFYVT1013
Vari2 IRRYILKFCIVGWGVPAAIVGIVLAVSKDSYGKNYYGKGKDGQGTSEFCWILNPVVFYVT 859
Vari3 IRRYILKFCIVGWGVPAAIVGIVLAVSKDSYGKNYYGKGKDGQGTSEFCWILNPVVFYVT 909
Vari4 IRRYILKFCIVGWGVPAAIVGIVLAVSKDSYGKNYYGKGKDGQGTSEFCWILNPVVFYVT1080
               TM5                                       TM6
vari1 CVAYFSIIFLMNVAMFIVVMIQICGRNGKRSNRTLREDILRNLRSVVSLTFLLGMTWGFA1073
Vari2 CVAYFSIIFLMNVAMFIVVMIQICGRNGKRSNRTLREDILRNLRSVVSLTFLLGMTWGFA 919
Vari3 CVAYFSIIFLMNVAMFIVVMIQICGRNGKRSNRTLREDILRNLRSVVSLTFLLGMTWGFA 969
Vari4 CVAYFSIIFLMNVAMFIVVMIQICGRNGKRSNRTLREDILRNLRSVVSLTFLLGMTWGFA1140
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vari1 FFAWGPVSLAFMYLFTIFNSLQGLFIFVFHCALKENVQKQWRRYLCCGKLRLADNSDWSK1133
Vari2 FFAWGPVSLAFMYLFTIFNSLQGLFIFVFHCALKENVQKQWRRYLCCGKLRLADNSDWSK 979
Vari3 FFAWGPVSLAFMYLFTIFNSLQGLFIFVFHCALKENVQKQWRRYLCCGKLRLADNSDWSK1029
Vari4 FFAWGPVSLAFMYLFTIFNSLQGLFIFVFHCALKENVQKQWRRYLCCGKLRLADNSDWSK1200

vari1 TATNNTKKVSSDNLGKSLSSSSFGSTTANWTSKAKATLNPFARHSNADSTLQ 1185
Vari2 TATNNTKKVSSDNLGKSLSSSSFGSTTANWTSKAKATLNPFARHSNADSTLQ 1031
Vari3 TATNNTKKVSSDNLGKSLSSSSFGSTTANWTSKAKATLNPFARHSNADSTLQ 1081
Vari4 TATNNTKKVSSDNLGKSLSSSSFGSTTANWTSKAKATLNPFARHSNADSTLQ 1252
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Fig. S3. Multiple sequence analysis of four zebrafish *gpr126* splice variants. Sequence alignment had been done by CLUSTAL 2.1 multiple sequence alignment and domain analysis by Simple Modular Architecture Research Tool.

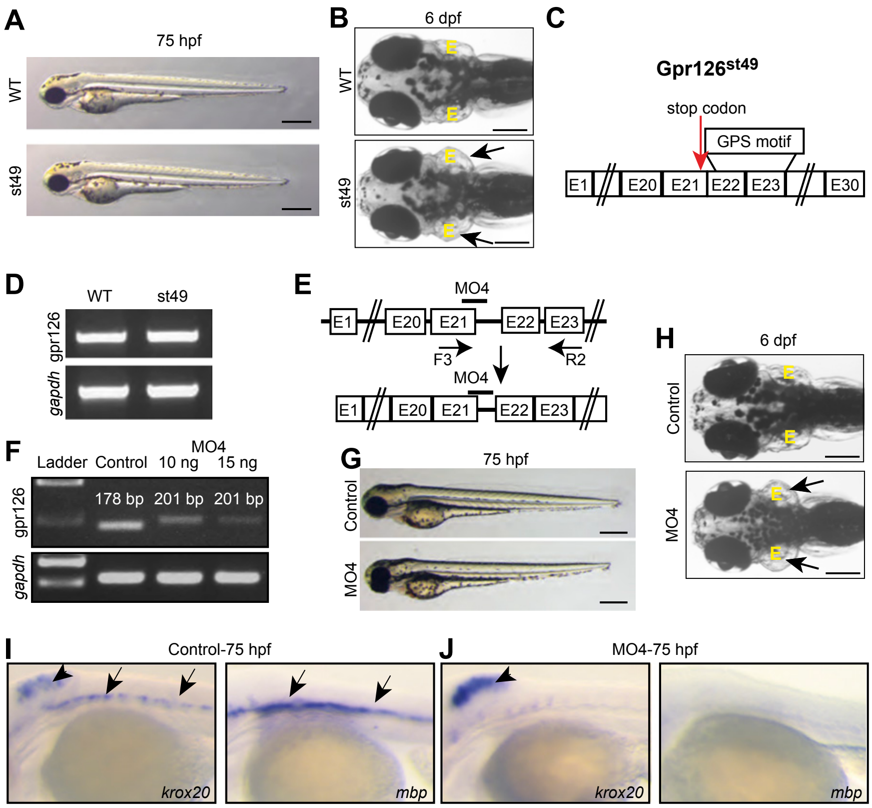

Fig. S4. Inhibition of the 7TM domain of zebrafish Gpr126 does not disrupt heart morphogenesis. (*A*) No pericardial edema is observed at 75 h postfertilization (hpf) in *gpr126*^{st49} zebrafish mutant embryos. (Scale bar: 400 μm.) (*B*) Dorsal view of a WT and a *gpr126*^{st49} mutant larva at 6 d postfertilization (dpf). *gpr126*^{st49} mutant larva showing enlarged ears (arrows). (Scale bar: 200 μm.) (*C*) Schematic depiction of zebrafish *gpr126*^{st49} mutant mRNA. The mutation introduces a premature stop codon before the GPS motif (red arrow). (*D*) RT-PCR analysis showing that *gpr126* mRNA transcripts could be detected in *gpr126*^{st49} mutants. *gapdh* was used as loading control. (*E*) Schematic depiction of zebrafish prespliced *gpr126* mRNA. Exons are represented by boxes, and introns are represented by lines. Bar represents the MO4 binding site. Arrows below the respective Exons indicate primer binding sites; these are used to analyze the fate of prespliced *gpr126* mRNA after MO4 injection. (*F*) RT-PCR analysis of mRNAs from WT and MO4-injected 3-dpf zebrafish embryos using primers as indicated in *E*. *gapdh* was used as loading control. MO4 injection resulted in partial intron insertion. (*G*) MO4 injection did not cause pericardial edema at 75 hpf. (Scale bar: 400 μm.) (*H*) Dorsal view of a phenol red-injected WT larva and a MO4-injected larva at 6 dpf. MO4-injected larva showing enlarged ears (arrows). (Scale bar: 200 μm.) (*I* and *J*) Whole-mount in situ hybridization demonstrating *krox20/egr2* [hindbrain, arrowhead; posterior lateral line nerve (PLLn) Schwann cells, arrows] and *mbp* (PLLn Schwann cells, arrows) expression at 75 hpf. MO4 morphants, which express NTF$^{\Delta GPS}$ but not CTF, exhibit normal expression of *krox20/egr2* in the hindbrain (arrowhead), but both *krox20/egr2* and *mbp* expression are down-regulated in the PLLn at 75 hpf. E, ear.

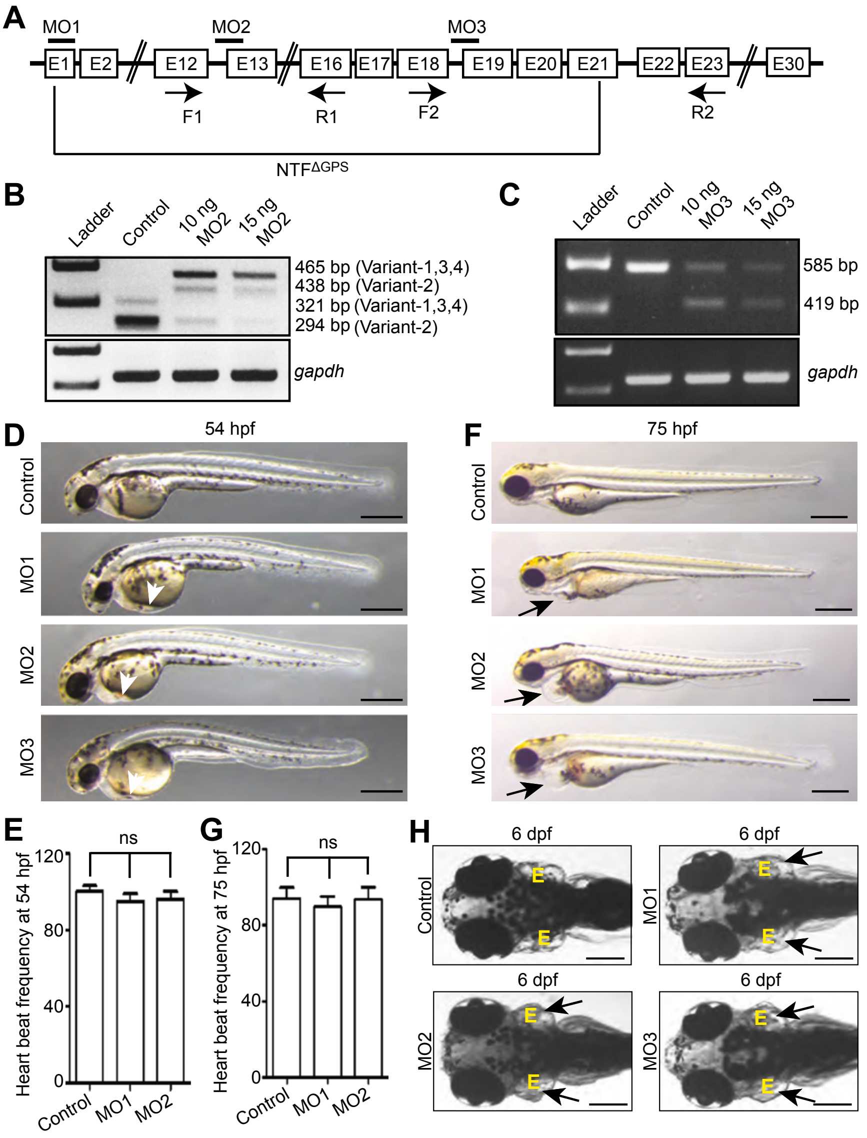

Fig. S5. Full-length *gpr126* knockdown causes pericardial edema. (*A*) Schematic depiction of zebrafish prespliced *gpr126* mRNA. Exons are represented by boxes, and introns are represented by lines. Bars represent the different morpholino binding sites. Arrows below the respective exons indicate primer binding sites; these are used to analyze the efficiency of gene knockdown by RT-PCR. (*B* and *C*) RT-PCR analysis of cDNA made from RNA isolated from 2-dpf control and MO2- or MO3-injected embryos using F1 and R1 primers for MO2 (*B*) and F2 and R2 primer for MO3 (*C*) as indicated in *A*. *gapdh* was used as loading control. MO2 binding resulted in 144-bp-long intron insertions (*B*). MO3 binding caused 166-bp-long exon deletions (*C*) causing a frame shift. (*D* and *F*) Bright-field images of control- or morpholino-injected embryos at 54 hpf (*D*) and 75 hpf (*F*). *gpr126* knockdown resulted in mild accumulation of blood at the sinus venosus (arrowheads) at 54 hpf (*D*). Prominent pericardial edema (arrows) was observed at 75 hpf in Gpr126-depleted embryos (*F*). (Scale bar: 400 μm.) (*E* and *G*) Quantification of cardiac contraction frequency of phenol red (control)- or morpholino-injected embryos at 54 hpf (*E*) or 75 hpf (*G*). Cardiac contraction frequency of the phenol red-injected embryos was set to 100%. (*H*) Dorsal view of a phenol red-injected WT larva and MO1-, MO2-, or MO3-injected larva at 6 dpf. MO1, MO2, and MO3 injection all caused enlarged ears (arrows). (Scale bar: 200 μm.) ns, not significant; $NTF^{\Delta GPS}$, N-terminal fragment up to the st49 mutation site.

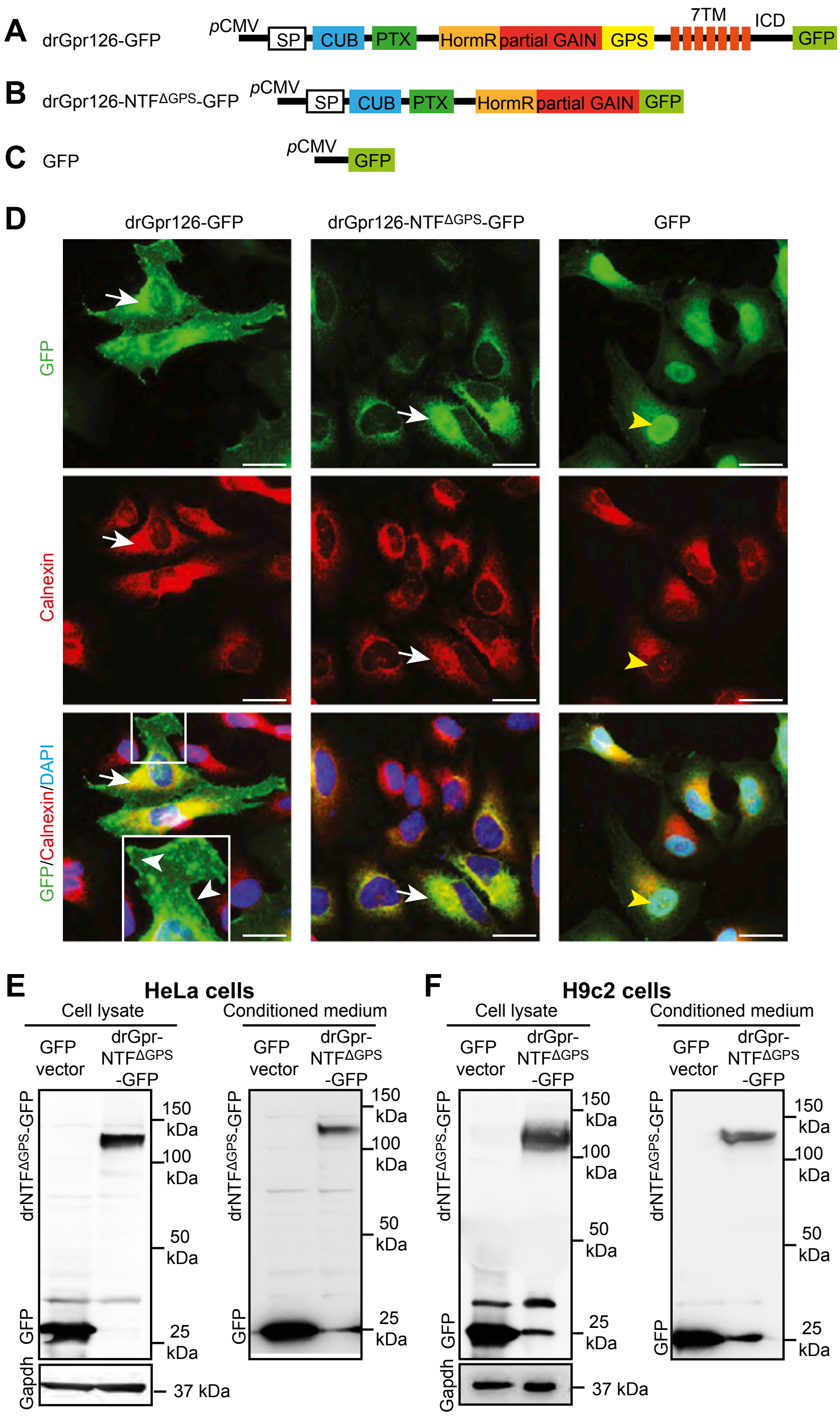

Fig. S6. The NTF part of the st49 mutant form of zebrafish Gpr126 is an independent, stable, secreted protein. (*A* and *B*) Schematic depiction of the C-terminal GFP-tagged drGpr126 (*A*) and drGpr126-NTF$^{\Delta GPS}$ (*B*). ICD, intracellular domain. (*C*) GFP vector used as control. (*D*) Immunofluorescence analysis of HeLa cells stained for GFP (green) and calnexin (red) after transfection with constructs described in *A*–*C*. Nuclei were stained with DAPI (blue). drGpr126-GFP localized to the plasma membrane (white arrowheads) and colocalized with calnexin (arrows), an endoplasmic reticulum marker protein. drGpr126-NTF$^{\Delta GPS}$-GFP was not detected at the plasma membrane but colocalized with calnexin (arrows). GFP was predominantly expressed in the nucleus (yellow arrowheads). (*E* and *F*) Western blot analysis of cell lysates and conditioned medium from HeLa (*E*) and H9c2 cells (*F*) after transfection with C-terminal GFP-tagged drGpr126-NTF$^{\Delta GPS}$ or GFP. Note that GFP-tagged NTF$^{\Delta GPS}$ (predicted band size: 113 kDa) and GFP (predicted band size: 27 kDa) were detected in both lysate and conditioned medium. Gapdh was used as loading control for cell lysates.

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drGpr126      -MISFISGRWWRWKFQNTLAVFLLLICLSTSVAQSCQSSTSCNVVLTDSQGSFTSPCYPN
hGpr126       MMFRSDRMWSCHWKWKPSPLLFLFALYIMC-VPHSVWGCANCRVVLSNPSGTFTSPCYPN
mGpr126       MMFDTLGKRCCPWRLKPSALLFLFVLCVTC-VPLSVCGCGSCRLVLSNPSGTFTSPCYPN
               *:          *: : :  :**: : :    *  *   .. .*.:**:: .*:********

drGpr126      DYPPSQSCNWTIQAPAGFIVQITFLDFELEEAQGCIYDRVVVKTGTSDAKFCGLTANGLT
hGpr126       DYPNSQACMWTLRAPTGYIIQITFNDFDIEEAPNCIYDSLSLDNGESQTKFCGATAKGLS
mGpr126       DYPNTQSCSWTLRAPAGYIIQITFNDFDIEEAPNCIYDSLSLDNGESQTKFCGATAKGLS
              ***  :*:* **::**:*:*:****  **::***  **** : :..* *::**** **:**:

drGpr126      LNSTGNVMEVFFNSDFSVQKKGFHISYKQVAVTLRNQKVTMPKSSKT-ILRVSNSISIPV
hGpr126       FNSSANEMHVSFSSDFSIQKKGFNASYIRVAVSLRNQKVILPQTSDAYQVSVAKSISIPE
mGpr126       FNSSVNEMHVSFSSDFSIQKKGFNASYIRVAVSLRNQKVILPQTLDAYQVSVAKSISIPE
              :**: * *.* *.****:*****. ** :***:****** :*:: .:  : *::*****

drGpr126      LTAFTVCFEIARTAQKA-TETIFTLSDAAGTSILAFEKTSNGMELFIGASYCSVDNLLTS
hGpr126       LSAFTLCFEATKVGHEDSDWTAFSYSNASFTQLLSFGKAKSGYFLSISDSKCLLNNALPV
mGpr126       LKAFTLCFEASKVGNEGGDWTAFSYSDESLTQLLSLEKASNGYFLSISGSRCLLNNALPV
              *.***:*** ::...:      * *: *: : *.:*:: *:..*  * *. * * ::* *

drGpr126      SD----ITATMKPLCLTWTKSSGLIGVYFGGHYFSSICSASQIYTLQSGGLLQIAGKGSS
hGpr126       KEKEDIFAESFEQLCLVWNNSLGSIGVNFKRNYETVPCDSTISKVIPGNGKLLLGSNQ--
mGpr126       KDKEDIFTENLEQLCLVWNNSWGSIGINFKKNYETVPCDSTISAVVPGDGTLLLGSDR--
              .:     :: .:: ***.*.:* * **: *   .* :  *.::   .: . * * :...

drGpr126      SVSVDDQNLDGFIYNFRLWDHAMLSSELSALTCDTVGNVVDWDHSYWTIPGSSTQTDSTL
hGpr126       ---NEIVSLKGDIYNFRLWNFTMNAKILSNLSCNVKGNVVDWQNDFWNIPNLALKAESNL
mGpr126       ---DEVASLRGSIYNFRLWNFTMDLKALSNLSCSVSGNVIDWHNDFWSISTQALKAEGNL
                 :   .* * *******:.:*   . ** *:*.. ***:**...:*.*    : :::..*

drGpr126      SCSTAITTLSPGTAGCASGLGCPATLTVTITSIATTNIIPTNATTHEDIFYRSTLVV-TD
hGpr126       SCGSYLIPLPAAELASCADLGT--------------------LCQDGIIYRISVVIQNI
mGpr126       SCGSYLIQLPAAELTNCSELGT--------------------LCQDGIMYRISVVIHND
              **.: :   *   .     .: **                    :: *:** ::*: .

                                                              ↓ S2-Cleavage site
drGpr126      EQTPDRDATAIISQWLNQTFQNWMYRVYVDGISLQLITVLSRITT--------TRQTYLA
hGpr126       LRHPEVKVQSKVAEWLNSTFQNWNYTVYVVNISFHLSAGEDKIKVKRSL-EDEPRLVLWA
mGpr126       FNHPEVKVQTKVAEWLNSTFQNWNYTVYVVNISFHQKVGEDRMKVKRDIMDDDKRLVLWA
               . *: .. : :::***.***** * ***  **::  .   .::..         * .  *

drGpr126      LLVYKNTTDVNLAEVEIE-SMLRSAPAIGNGLTLDSVTVNLMENCQADEFPVHYRWPESR
hGpr126       LLVYNATNNTNLEGKIIQQKLLKNNESLDEGLRLHTVNVRQLGHCLAMEEPKGYYWPSIQ
mGpr126       LLVYNATNNVSLNEEKIKQKLMTNNASLEDGLRLCEVDVNQLGMCSALEDPDGFSWPATL
              ****: *.:..*      *: .:: .  :: :** *  * *. :   * * * *   : **

drGpr126      PTVTQYVPCFPYKDRNASRTCMINRDN-YTSFWALPDRGNCTNITSITVSQENAMDVAVQ
hGpr126       PSEY-VLPCPDKPGFSASRICFYNATNPLVTYWGPVDISNCLKEANEVA---------NQ
mGpr126       PSVY-KQPCPNKPGFFMTRACLSNGT---STFWGPVDTSNCSRQSNEVA---------NE
              *:     **         :* *: *       ::*.  * .** . :. ..          :

drGpr126      LADISN--NGLSKEELTQVVTKVMELVN-IAKINATLASTVVTIISNVMVSSEDAQKDAS
hGpr126       ILNLTADGQNLTSANITNIVEQVKRIVNKEENIDITLGSTLMNIFSNILSSSDSDLLESS
mGpr126       ILNQTGDGQNLTSANINSIVEKVKRIVNKEENIDITLGSTLMNIFSNILSSSDSDLLESS
              : : :    : *:. ::..:* :* .:**    :*: **.**::.*:**:: **:.    ::*

drGpr126      ETALKAVDELVQKIEFDG-PSLTISSKNLVVGVSALDTTNFNGSTLS-AFIATNTTDPQI
hGpr126       SEALKTIDELAFKIDLNSTSHVNITTRNLALSVSSLLPGTNAISNFSIGLPSNNESYFQM
mGpr126       TEALKTIDELAFKIDLNSTPHVNIETQNLALGVSSLIPGTNAPSNFSIGLPSNNESYFQM
                ***::***. **:::.    :.* ::**.:.**:*      .     *.:* .: :.* :  *:

drGpr126      DFDSEAHNALAVVTLPPTLLQNLSLSQIEKVSRINFMFFGRTGLFQDHQNNGLTLNSYVV
hGpr126       DFESGQVDPLASVILPPNLLENLSPEDSVLVRRAQFTFFNKTGLFQDVGPQRKTLVSYVM
mGpr126       DFGNGQTDPLASVILPPNLLENLSPEDSVLVRRAQFTFFNKTGLFQDVGSQRKVLVSYVM
              **  .   : ** * ***.**:*** .:   .:    * * :* ** :******    :   .* ***:
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Fig. S7. (Continued)

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drGpr126        ASSVGNFTIKNLQDPVRIEIAHLEYQKDPNPQCVFWDFNLQNYSGGCNSDGCKVGSDSNS
hGpr126         ACSIGNITIQNLKDPVQIKIKHTRTQEVHHPICAFWDLNKNKSFGGWNTSGCVAHRDSDA
mGpr126         ACSIGNITIQNLKDPVQIKIKHTRTQEVHHPICAFWDMNKNKSFGGWNTSGCVAHSDLDA
                *.*:**:**:**:***:*:* * .  *:  .* *.***:* ::  ** *:.** .  * ::
                         ↓ GPS-Cleavage site
drGpr126        NRTVCLCNHLTHFGILMDVSRAAELIDEKNNRVLTFITYIGCGISAIFSAATLLTYIAFE
hGpr126         SETVCLCNHFTHFGVLMDLPRSASQLDARNTKVLTFISYIGCGISAIFSAATLLTYVAFE
mGpr126         GETICLCSHFTHFGVLMDLPRSASQIDGRNTKVLTFITYIGCGISAIFSAATLLTYVAFE
                 .*:***.*:****:***: *:*. :* :*.:*****:******************:***

drGpr126        KLRRDYPSKILMNLSTSLLFLNMVFLLDGWLASYEIKELCVTVAVFLHFFLLTSFTWMGL
hGpr126         KLRRDYPSKILMNLSTALLFLNLLFLLDGWITSFNVDGLCIAVAVLLHFFLLATFTWMGL
mGpr126         KLRRDYPSKILMNLSSALLFLNLIFLLDGWVTSFGVAGLCTAVAALLHFFLLATFTWMGL
                ***************::*****::******::*: :  ** :**.:******::******

drGpr126        ESIHMYIALVKVFNTYIRRYILKFCIVGWGVPAAIVGIVLAVS--KDSYGKNYYGKGKDG
hGpr126         EAIHMYIALVKVFNTYIRRYILKFCIIGWGLPALVVSVVLASRNNNEVYGKESYGKEK--
mGpr126         EAIHMYIALVKVFNTYIHRYILKFCIIGWGLPALVVSIILVSRRQNEVYGKESYGKDQ--
                *:***************:********:***:** :*.::*.    :: ***: *** :

drGpr126        QGTSEFCWILNPVVFYVTCVAYFSIIFLMNVAMFIVVMIQICGRNGKRSNRTLREDILRN
hGpr126         --GDEFCWIQDPVIFYVTCAGYFGVMFFLNIAMFIVVMVQICGRNGKRSNRTLREEVLRN
mGpr126         --DDEFCWIQDPVVFYVSCAGYFGVMFFLNVAMFIVVMVQICGRNGKRSNRTLREEVLRN
                   .***** :**:***:*..**.::*::*:*******:****************::***

drGpr126        LRSVVSLTFLLGMTWGFAFFAWGPVSLAFMYLFTIFNSLQGLFIFVFHCALKENVQKQWR
hGpr126         LRSVVSLTFLLGMTWGFAFFAWGPLNIPFMYLFSIFNSLQGLFIFIFHCAMKENVQKQWR
mGpr126         LRSVVSLTFLLGMTWGFAFFAWGPLNIPFMYLFSIFNSLQGLFIFIFHCAMKENVQKQWR
                ************************:.: *****:***********:****:*********

drGpr126        RYLCCGKLRLADNSDWSKTATNNTKKVSSDNLGKSLSSSSFGSTTANWTSKAKATLNPFA
hGpr126         RHLCCGRFRLADNSDWSKTATNIIK-KSSDNLGKSLSSSSIGSNSTYLTSKSKSSSTTYF
mGpr126         RHLCCGRFRLADNSDWSKTATNIIK-KSSDNLGKSLSSSSIGSNSTYLTSKSKSSSTTYF
                *:****::**************  *  *************:**.::  ***:*:: . :

drGpr126        RHSNADSTLQ--------------------------
hGpr126         KRNSHTDNVSYEHSFNKSGSLRQCFHGQVLVKTGPC
mGpr126         KRNSHSDNFS--------------------------
                ::..  ....
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Fig. S7. Amino acid sequence alignment analysis of zebrafish, human, and mouse GPR126 proteins using CLUSTAL 2.1 revealed that zebrafish Gpr126 lacks eight amino acids at the S2 cleavage site.

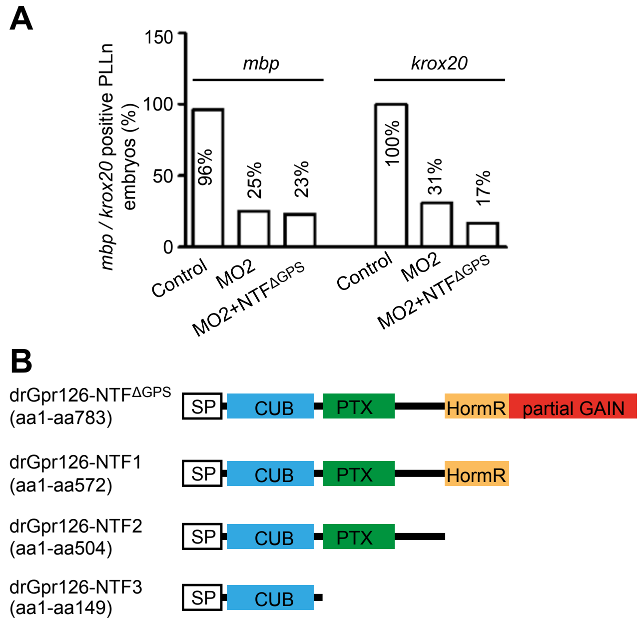


Fig. S8. Rescue experiments. (*A*) Quantitative analysis of control-, MO2-, or MO2+NTF$^{\Delta GPS}$ mRNA-injected embryos at 75 hpf positive for *krox20/egr2* and *mbp* at the PLLn. (*B*) Schematic representation of zebrafish truncated Gpr126-NTF proteins encoded by RNAs used in rescue experiments.

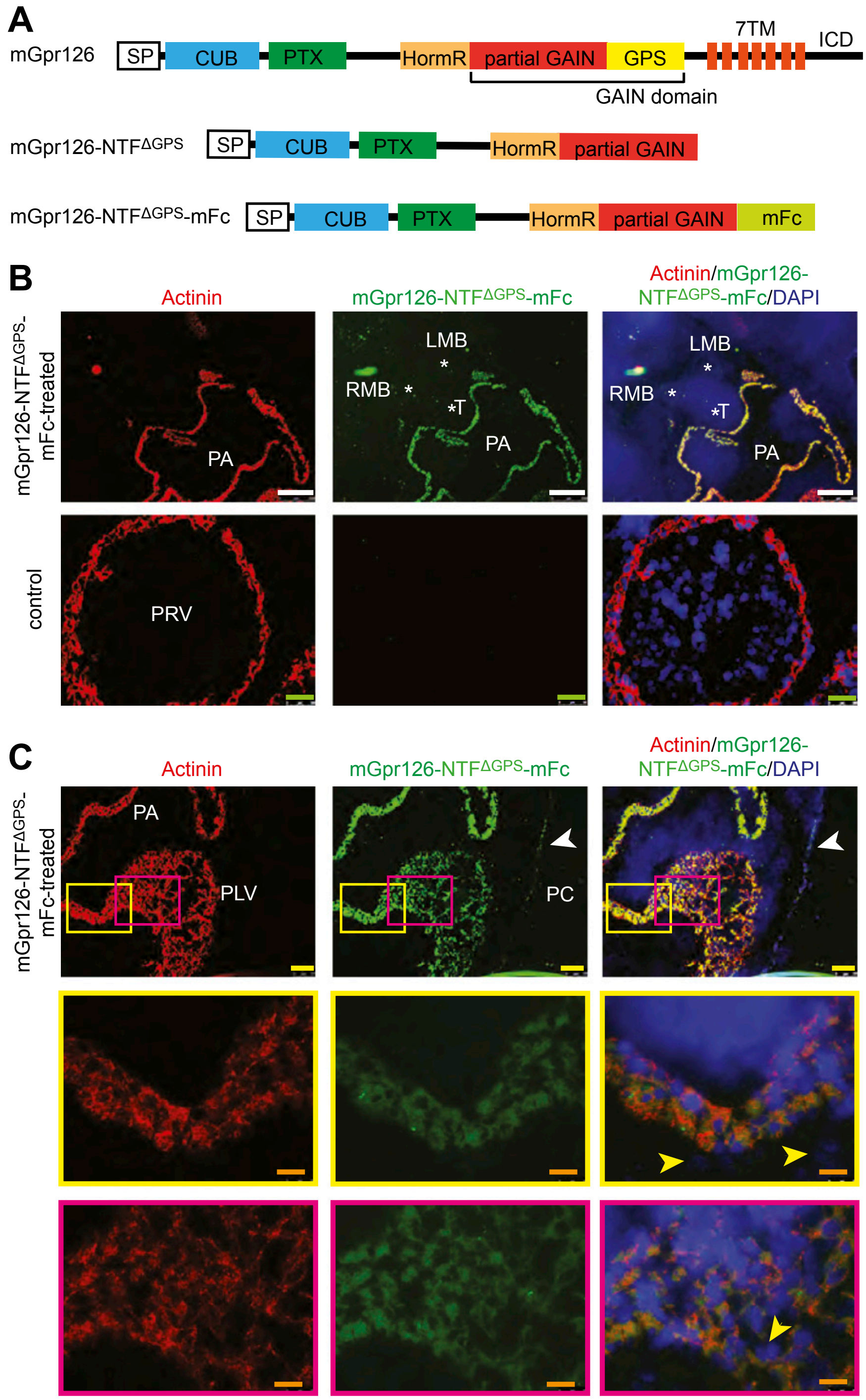
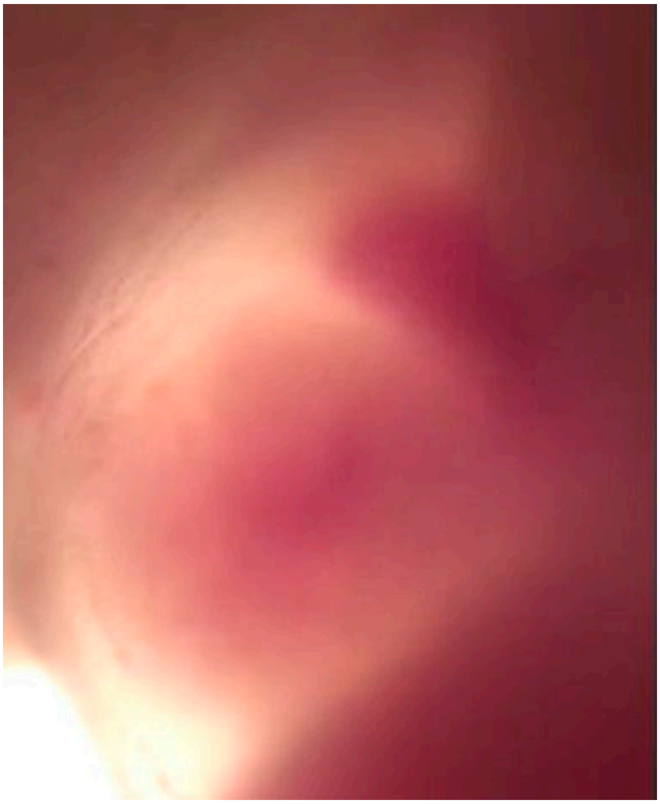


Fig. S9. Mouse Gpr126-NTF$^{\Delta GPS}$ binds to cardiomyocytes and pericardial cells. (*A*) Schematic representation of mouse full-length Gpr126; Gpr126-NTF$^{\Delta GPS}$; and mGpr126-NTF$^{\Delta GPS}$-mFc, a fusion protein of Gpr126-NTF$^{\Delta GPS}$ and the Fc fragment of murine IgG2b subclass (mFc) used for in situ binding studies. (*B* and *C*) Immunofluorescence analysis of 10-μm thin E11.5 mouse embryo cryosections (at the level of the heart) incubated with mGpr126-NTF$^{\Delta GPS}$-mFc protein or concentrated conditioned medium of mock-transfected HEK-293 cells (control). Sections were stained for mGpr126-NTF$^{\Delta GPS}$-mFc (green), cardiomyocyte-specific α-Actinin (red), and DNA (DAPI; blue). Note that mGpr126-NTF$^{\Delta GPS}$-mFc binds to cardiac tissue and the pericardium (white arrowhead in *C*) but not to other embryonic tissues. (*C*) mGpr126-NTF$^{\Delta GPS}$-mFc binds to Actinin-positive cardiomyocytes. In contrast, higher magnifications suggest that it does not bind in the heart to nonmyocytes (yellow arrowheads). LMB, left main bronchus; PA, primitive atrium; PC, pericardium; PLV, primitive left ventricle; RMB, right main bronchus; T, trachea. (Scale bars: white, 250 μm; green, 50 μm; yellow, 75 μm; and orange, 25 μm.)



Movie S1. Representative movie of a beating heart from a E11.25 wild-type mouse embryo.

Movie S1

Movie S2. Representative movie of a beating heart from a E11.25 *Gpr126*$^{-/-}$ mouse embryo exhibiting bradycardia along with cardiac arrhythmia.

Movie S2