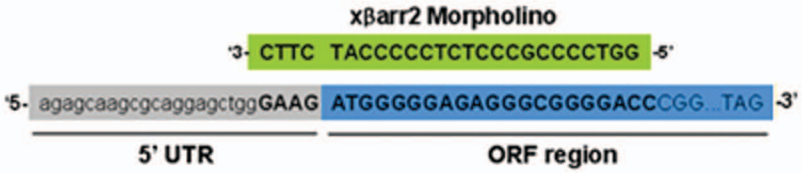
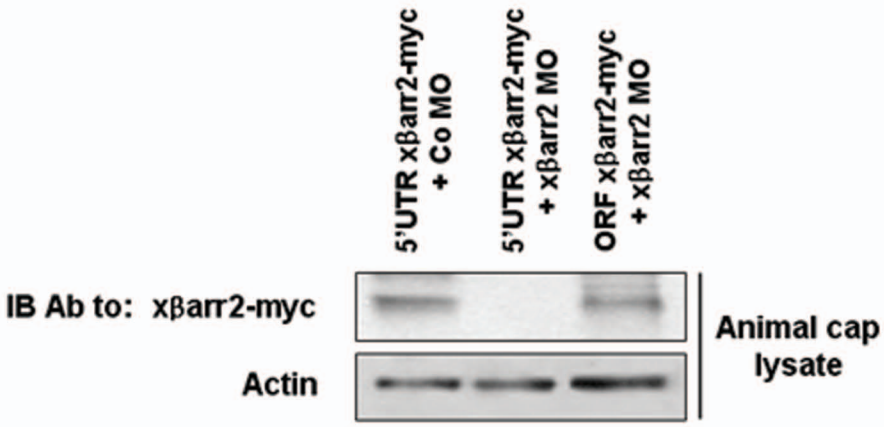
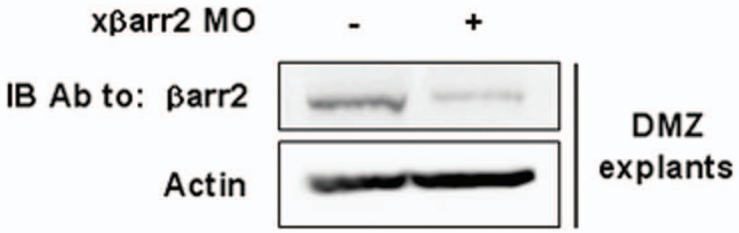
A

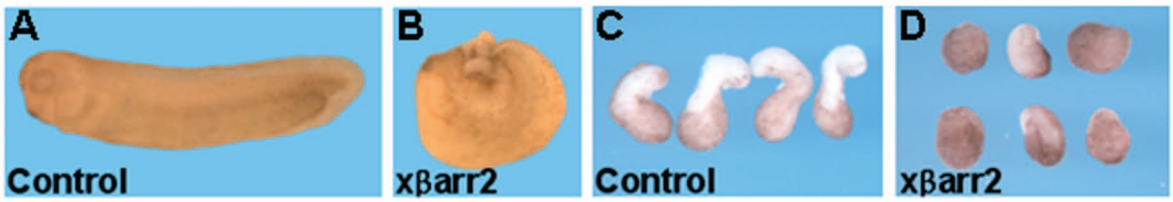

xβarr2 Morpholino
'3- CTTC TACCCCCTCTCCCGCCCCTGG -5'
'5- agagcaagcgcaggagctggGAAG ATGGGGGAGAGGGCGGGGACCCGG...TAG -3'
5' UTR
ORF region

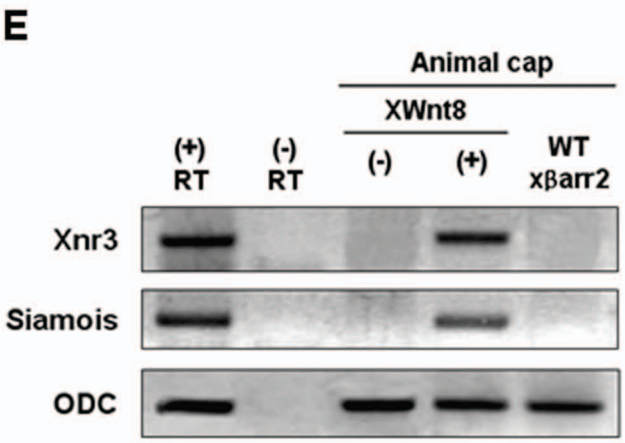
B

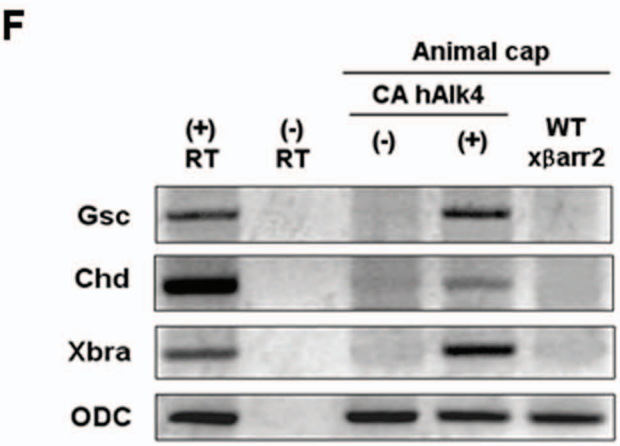

5'UTR xβarr2-myc + Co MO
5'UTR xβarr2-myc + xβarr2 MO
ORF xβarr2-myc + xβarr2 MO
IB Ab to: xβarr2-myc
Actin
Animal cap lysate

C

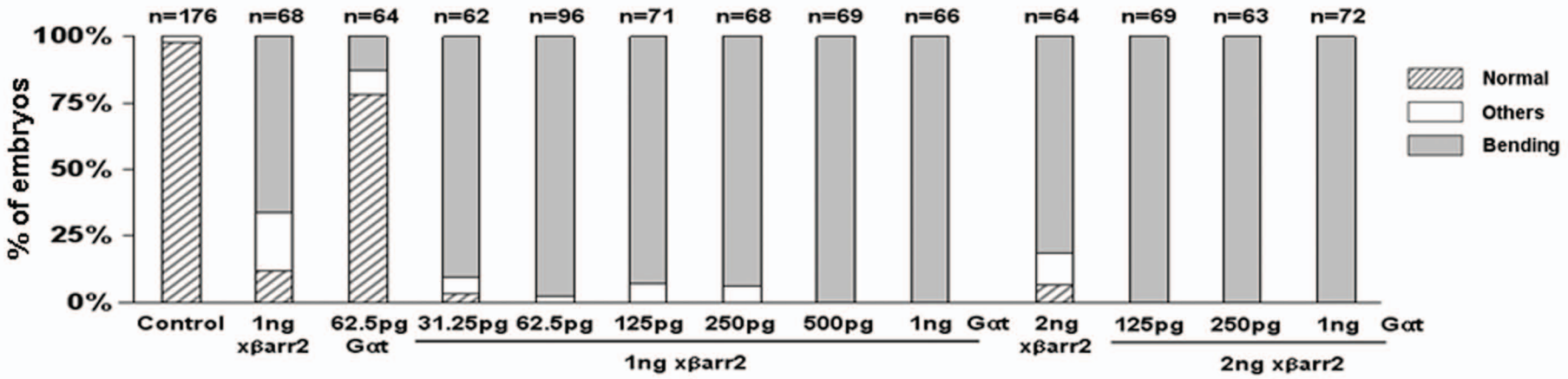

xβarr2 MO - +
IB Ab to: βarr2
Actin
DMZ explants


A
Control
B
xβarr2
C
Control
D
xβarr2

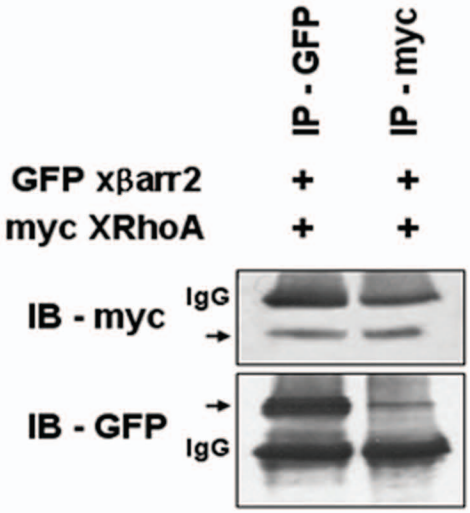

E
Animal cap
XWnt8
(+) RT
(-) RT
(-)
(+)
WT xβarr2
Xnr3
Siamois
ODC


F
Animal cap
CA hAlk4
(+) RT
(-) RT
(-)
(+)
WT xβarr2
Gsc
Chd
Xbra
ODC

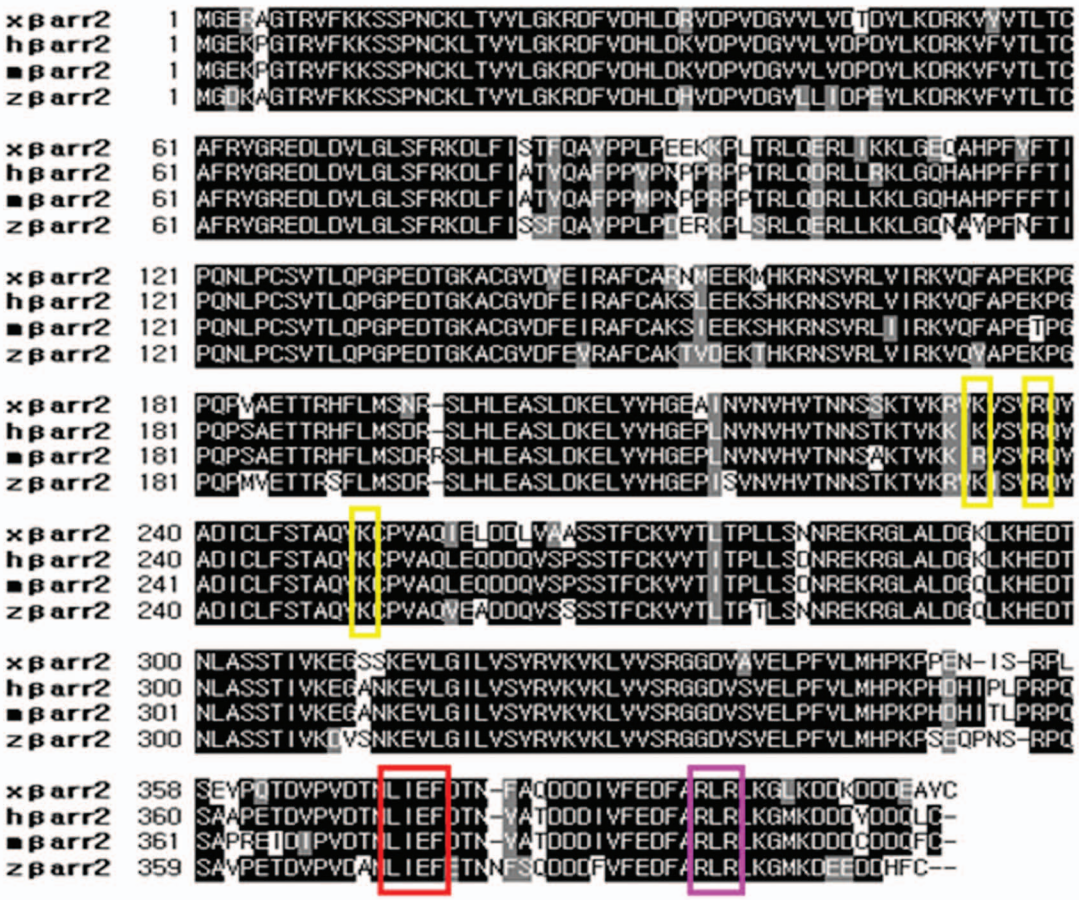
Kim_Fig S2


n=176
n=68
n=64
n=62
n=96
n=71
n=68
n=69
n=66
n=64
n=69
n=63
n=72
100%
75%
50%
25%
0%
% of embryos
Control
1ng xβarr2
62.5pg Gαt
31.25pg
62.5pg
125pg
250pg
500pg
1ng Gαt
1ng xβarr2
2ng xβarr2
125pg
250pg
1ng Gαt
2ng xβarr2
Normal
Others
Bending

Kim_Fig S3



Kim_Fig S4



Yellow (KRK→Q)
: Phosphoinositide-binding site

Red (LIEF →AAEA)
: Clathrin-binding site

Pink (RLR →ALA)
: AP2-binding site

Figure S1. Antisense morpholino oligonucleotides of xβarr2. (A) Scheme of the morpholino sequence targeting the xβarr2 5' sequences. (B) xβarr2 MO specifically inhibited the translation of its cognate mRNA. 5'UTR (500pg) and ORF (500pg) xβarr2-myc mRNA were injected with xβarr2 MO (10ng) into the animal regions of embryos at the four-cell stage. Animal cap explants isolated at early gastrula stages were then subjected to IB analysis with anti-myc antibodies. Actin served as a specificity control. (C) xβarr2 MO reduced the endogenous levels of βarr2 protein. DMZ explants were blotted with anti-βarr2 antibodies.

Figure S2. Gain-of-function of xβarr2 disrupts CE movements. (A-D) Overexpression of xβarr2 blocked CE movements in intact embryos and dorsal marginal zone (DMZ) tissues. Two blastomeres of four-cell stage embryos were injected at the dorsal equatorial region with xβarr2 mRNA (1 ng). (E-F) xβarr2 did not induce the dorsal mesodermal genes expressed by Wnt and Activin signaling. Four-cell stage embryos were microinjected into the animal regions of all blastomeres with xβarr2 (1 ng), Wnt8 (20 pg), or CA hAlk4 (2 ng). Animal caps were dissected at stage 8.5, cultured until stage 10.5 and analyzed by RT-PCR. *ODC*, a loading control. (–) RT, minus reverse transcription control sample.

Figure S3. Rescue assays using βarr2 and α-transducin (Gαt). The CE-defective phenotypes

caused by xβarr2 were not rescued by α-transducin. Quantitative rescue assays were performed more than three times. n, total number of embryos. Others indicate a truncated and mild kinked axis.

Figure S4. Reciprocal immunoprecipitation analysis of xβarr2 and XRhoA. HEK293FT cells were transfected with GFP xβarr2 and myc XRhoA. HEK293TF Cell lysates were immunoprecipitated with anti-GFP antibodies for xβarr2 (lane 1) and anti-myc antibodies for Hrs (lane 2), respectively. Immunocomplexes were blotted with specific antibodies.

Figure S5. A site-directed mutagenesis of xβarr2. βarr2 sequences, which are important to target receptors to clathrin-coated pits for endocytosis, from other species is conserved with *Xenopus* orthologue. Mutation of these sequences was indicated by the color boxes (Yellow, KRK→3Q; Red, LIEF→AAEA; Pink, RLR→ALA).