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Kim_Fig S2





xBarr2	1	MGERAGTRVFKKSSPNCKLTVYLGKRDFVDHLDRVDPVDGVVLVDTDYLKDRKVVVTLTC
hBarr2	1	MGERFGTRVFKKSSPNCKLTVYLGKRDFVDHLDRVDPVDGVVLVDPDVLKDRKVFVTLTC
■Barr2	1	MGEKEGTRVFKKSSPNCKLTVYLGKRDFYDHLDKYDPYDGYYLYDPDYLKDRKVFYTLTC
ZBarr2	1	MGDKAGTRVFKKSSPNCKLTVYLGKRDFVDHLDHVDPVDGVLLIDPEVLKDRKVFVTLTC
xBarr2	61	AFRYGREDLDVLGLSFRKOLF ISTFOAVPPLPEKKPLTRLOERLIKKLGEOAHPFYFT I
hBarr2	61	AFRYGREOLDVLGLSFRKOLF I ATVOAFPPVPN-PRPSTRLOORLLRKLGQHAHPFFFT I
Barr2	61	AFRYGREDLDVLGLSFRKOLF I ATYQAFPPMPNEPRPETRLODRLLKKLGQHAHPFFFT I
ZBarr2	61	AFRYGREDLDVLGLSFRKDLF ISSEQAYPPLPDESKPLSRLOERLLKKLGQNAMPFNFT I
xBarr2	121	PONLPCSVTL0P6PEDT6KAC6VDVE1RAFCAENMEEKMHKRNSVRLV1RKV0FAPEKP6
hBarr2	121	PONLPCSVTLOPGPEDTGKACGVDFE I RAFCAKSLEEKSHKRNSVRLV I RKVOFAPEKPG
Barr2	121	PONLPCSVTLOPGPEDTGKACGVDFE I RAFCAKSTEEKSHKRNSVRL I I RKVOFAPETPG
ZBarr2	121	PONLPCSVTLOPGPEDTGKACGVDFEVRAFCAKTVDEKTHKRNSVRLVIRKVOVAPEKPG
xBarr2	181	POPWAETTRHFLMSNF-SLHLEASLOKELYVHGEALNVNVHVTNNSSKTVKR KVS RUV
hBarr2	181	POPSAETTRHFLMSDE-SLHLEASLOKELVYHGEPLNYNYHYTNNSTKTYKK KVSYROV
Barr2	181	POPSAETTRHFLMSDBSSLHLEASLOKELVYHGEPLNYNYHYTNNS%KTYKK RVSYROV
ZBarr2	181	POPMMETTER FLMSDE SLHLEASLOKELVYHGEPINSYNYHYTNNSTKTYKRYK ISYROV
xBarr2	240	AD I CLESTAON KOPVAQUELODU WAASSTECKVVTUTPLUSNNEKRGLALOGKLKHEDT
hBarr2	240	AD LCL ESTADYKOPVADLEDDDDVSPSSTECKVVTILTPL LSTNREKRGLALDG&LKHEDT
Barr2	241	AD LCL ESTADY KOPVAGLEODDOVSPSSTECKVYTI TPL LSTNREKRGLALDGTLKHEDT
ZBarr2	240	AD LCLESTADYKOPVADVE2000VSSSSTECKVVTI TETIL SANREKRGLALDGU KHEDT
	210	
xBarr2	300	NLASST LVKEGSSKEVLG I LVSVRVKVKLVVSRGGDVAVELPEVLMHPKPEN- IS-REL
hBarr2	300	NLASST LYKEGANKEVLG LLVSVRVKVKLVVSRGGDVSVELPEVLMHPKPHDH IPLPRPD
Barr2	301	NLASST LYKEGANKEVLG LLVSVRVKVKLVVSRGGDVSVELPEVLMHPKPHDH I TLPRPD
ZBarr2	300	NLASST LYKTYSNKEVLG I LYSYRYKYKLYYSRGGDYSVELPEVLMHPKESEDPNS-RPD
xBarr2	358	SEVEDTOVEVOTAL LEEDTN-EACODD LVEEDFARLELKGEKODKODDEAVC
hBarr?	360	
Barr2	361	SAPSEIDTPYDTNL LEFDTN-VATIODD LYFEDFARLR KGMKDDDODDOFC-
ZBarr?	359	SAVPETOVPYDANLIEF TNNESODDOFVFEDFARLR KOMKDEEDOHEC

Yellow (KRK→Q) : Phosphoinositide-binding site Red (LIEF →AAEA) : Clathrin-binding site Pink (RLR →ALA) : AP2-binding site Figure S1. Antisense morpholino oligonucleotides of x β arr2. (A) Scheme of the morpholino sequence targeting the x β arr2 5' sequences. (B) x β arr2 MO specifically inhibited the translation of its cognate mRNA. 5'UTR (500pg) and ORF (500pg) x β arr2-myc mRNA were injected with x β arr2 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) x β arr2 MO reduced the endogenous levels of β arr2 protein. DMZ explants were blotted with anti- β arr2 antibodies.

Figure S2. Gain-of-function of $x\beta arr2$ disrupts CE movements. (A-D) Overexpression of $x\beta arr2$ 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 $x\beta arr2$ mRNA (1 ng). (E-F) $x\beta arr2$ 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 $x\beta arr2$ (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 (Gat). The CE-defective phenotypes

caused by x β arr2 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 x β arr2 and XRhoA. HEK293FT cells were transfected with GFP x β arr2 and myc XRhoA. HEK293TF Cell lysates were immunoprecipitated with anti-GFP antibodies for x β arr2 (lane 1) and anti-myc antibodies for Hrs (lane 2), respectively. Immunocomplexes were blotted with specific antibodies.

Figure S5. A site-directed mutagenesis of x β arr2. β arr2 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 \rightarrow 3Q; Red, LIEF \rightarrow AAEA; Pink, RLR \rightarrow ALA).