

a

Alignment search: beta-arrestin-1 isoformA (ARRB1) and beta-arrestin-2 isoform3 (ARRB2)

ARRB1 1 MGDK- GTRVFKKASPN GKLTVYLGKRDFVDHI DLVDPV DGVVLVDPEYLKERRRVYVTLC AFRYGREDL DVLGLTFRKDLFVANVQS FPP
 ARRB2 1 MGEKPGTRVFKKSSPNCKLTVYLGKRDFVDHLDK VDPV DGVVLVDPDYLKDRKVFTLTC AFRYGREDL DVLGLSFRKDLFI ATYQAF FPP

ARRB1 90 APIEDKKPPLTRLQERLIIKKLGEHAYPFTIE- - - - - I PPNL PCSVTLQPGPEDT GKA CGVDYEVKA FCAENL EEKI
 ARRB2 91 VPNPPRPPTRLQDRLLRKLGQHAPFFFTRMPLPSEGQGAGATVSGVGI PQNL PCSVTLQPGPEDT GKA CGVD FEA RFCAKS LEEKS

ARRB1 159 HKRNSVRLVI RKVQYAPE R PGPQPTAETTRQFLMSDKPLHL EASL DKEI YYHGEPI SVNVHVNNTNKTVKKI KI SVRQYADI CLFNTAQ
 ARRB2 181 HKRNSVRLVI RKVQFAPEK PGPQPSAETTRHFLMSDRSLHL EASL DKEI YYHGEPL NVNVHVTNNSTKTVKKI KVSVRQYADI CLFSTAQ

ARRB1 249 YKCPVAMEEADDTVAPSSTFCKVYITTPFLAN NREKRL AL DGKL KHEDT NLASSTI L REGANREI L GIV VSYKVKVKL VVS RGG L GDL
 ARRB2 271 YKCPV AQLEQDDQVSPSSTFCKVYTTPLLSDNREKRL AL DGKL KHEDT NLASSTI VKEGANKEVL GIV VSYRVKVKL VVS RGG - - -

ARRB1 339 ASSDVIA VELPFTL MHPKPKI EEE- - - PPHREVIPENETPVDTNLIEIDTN- - - DDDI VFEDFARQRLKG MKDDKEEEE DGTGSPQLNNR
 ARRB2 356 - - - DVSVELPFTL MHPKPHDHI PLPRPQSAAPETDVPVDTNLIEFDTNYAIDDDI VFEDFARLRLKG MKDDDYDDQ- - - - - LC -

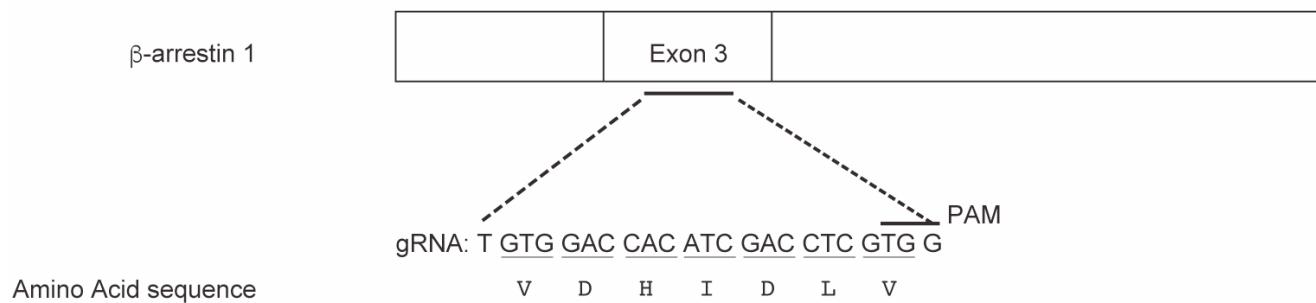
b

		Percent Identity	E value
beta-arrestin-1 Isoform A	beta-arrestin-2 Isoform 1	76.14%	0
	2	73.25%	0
	3	72.48%	0
	4	80.46%	8.00×10^{-150}
	5	74.00%	0
	6	71.19%	0
	7	73.09%	4.00×10^{-112}

		Percent Identity	E value
beta-arrestin-1 Isoform B	beta-arrestin-2 Isoform 1	77.64%	0
	2	74.69%	0
	3	73.83%	0
	4	80.46%	9.00×10^{-153}
	5	75.42%	0
	6	72.55%	0
	7	75.81%	4.00×10^{-115}

Extended data Figure 1. Homology in amino acid sequence between β -arrestin 1 and 2, as searched by BLAST in the NCBI database.

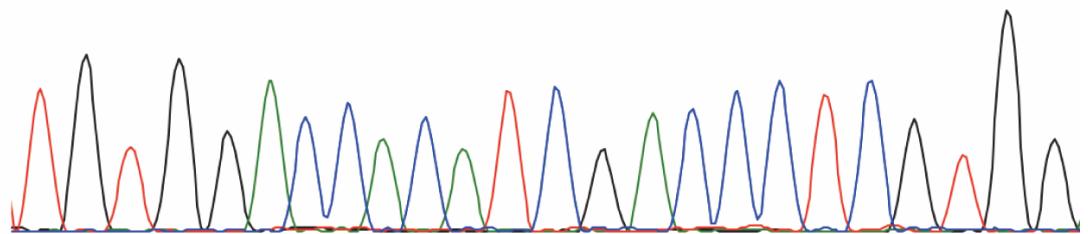
a. Alignment search between β -arrestin 1 isoform A and β -arrestin 2 isoform 3 as a representative diagram. BOXSHADE version 3.21 was used. b. β -arrestin 1 isoform A and B were compared with β -arrestin 2 isoforms.

a**b**

Edited genome

1bp Ins : T GTG GAC CAC ATC GAC CCT CGT GG

T G T G G A C C A C A T C G A C C T C G T G G



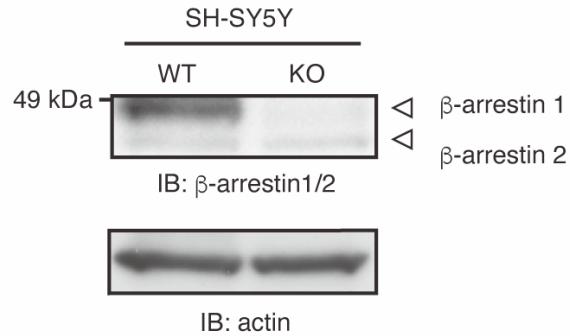
Amino Acid Sequence

WT allele:

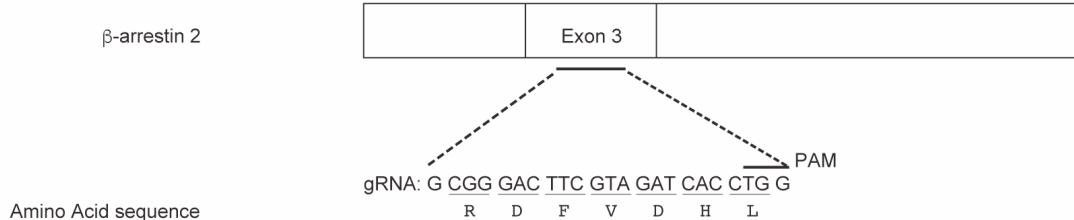
VDHIDLVDPVDGVVLVDPE

Mutated allele:

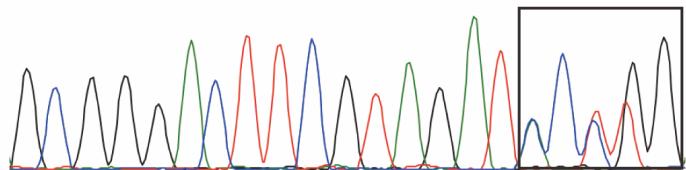
VDHIDPRGPGCGWCGPGGS*

c Immunoblotting**Extended data Figure 2. Genetic depletion of β-arrestin 1 in SH-SY5Y cells with use of CRISPR/Cas9.**

The gRNA design (a) as well as obtained genomic and amino acid sequence (b) for establishing knockout cell lines using CRISPR/Cas9. Western blotting analysis was conducted to confirm the loss of β-arrestin 1 expression.

a**b**

Edited genome

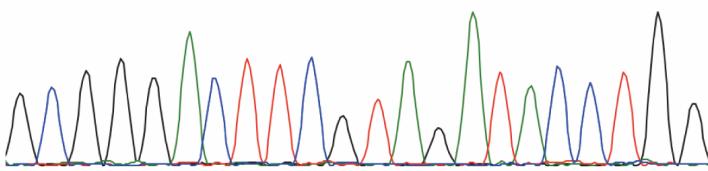


TA Cloning

1bp del: G CGG GAC TTC GTA GAT ⊕ ACC TGG

G C G G A C T T C G T A G A T A C C T G G

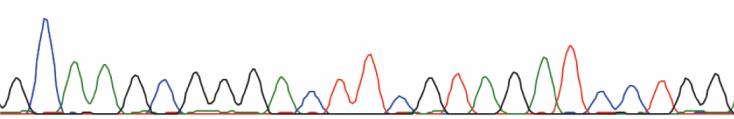
Allele 1



2bp del: G CGG GAC TTC GTA GAT ⊕ A CCT GG

G C A A G C G G G A C T T C G T A G A T C C T G G

Allele 2



Amino Acid Sequence

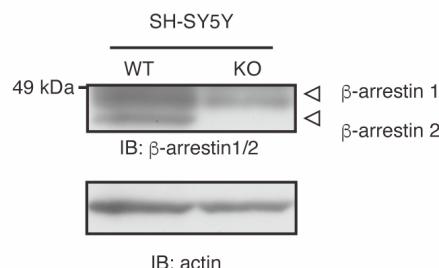
WT: RDFVDHLDKVDPVGVVLVDPDYLKDRKVF

Allele 1: RDFVDWTKEETL*

Allele 2: RDFVDPCRWRGACGP*

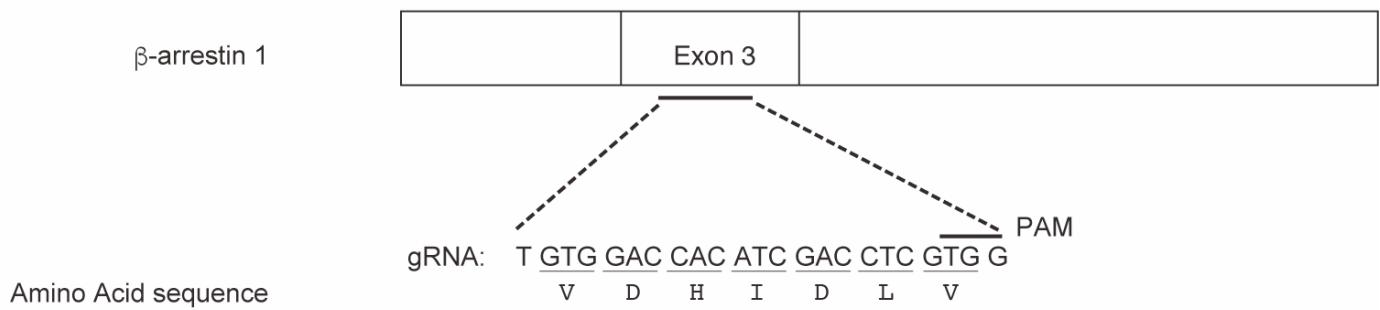
c

Immunoblotting

**Extended data Figure 3. Genetic depletion of β -arrestin 2 in SH-SY5Y cell with use of CRISPR/Cas9.**

The gRNA design (a) as well as obtained genomic and amino acid sequence (b) for establishing knockout cell lines using CRISPR/Cas9. Western blotting analysis was performed to confirm the loss of β -arrestin 2 expression.

a * SH-SY5Y cells whose background is lacking β -arrestin 2 (The detail is shown in Extended Data 3).

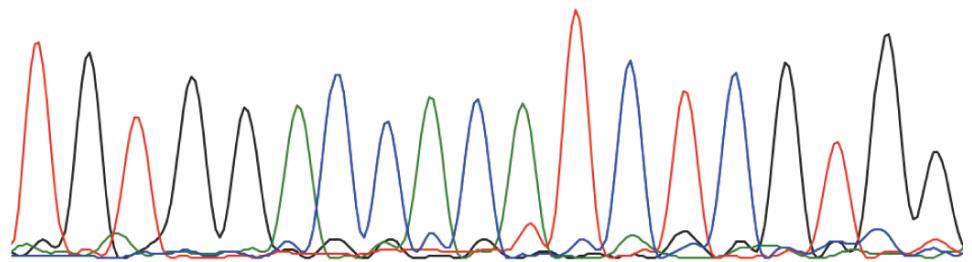


b

Edited genome

4bp Del : T GTG GAC CAC ATC GAC CTC GTG G

T G T G G A C C A C A T C T C G T G G



Amino Acid Sequence

WT allele:

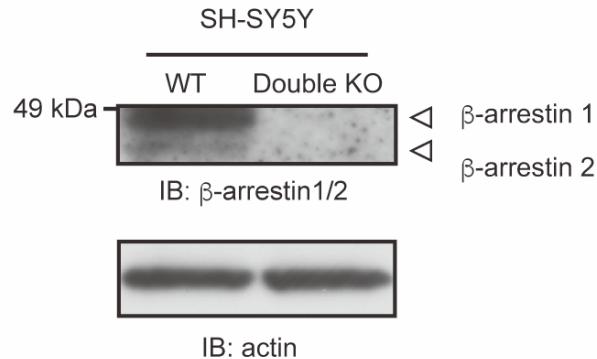
VDHIDLVDVDPVDGVVLVDPE

Mutated allele:

VDHISWTLWMVWSWWILISISKSGESM*

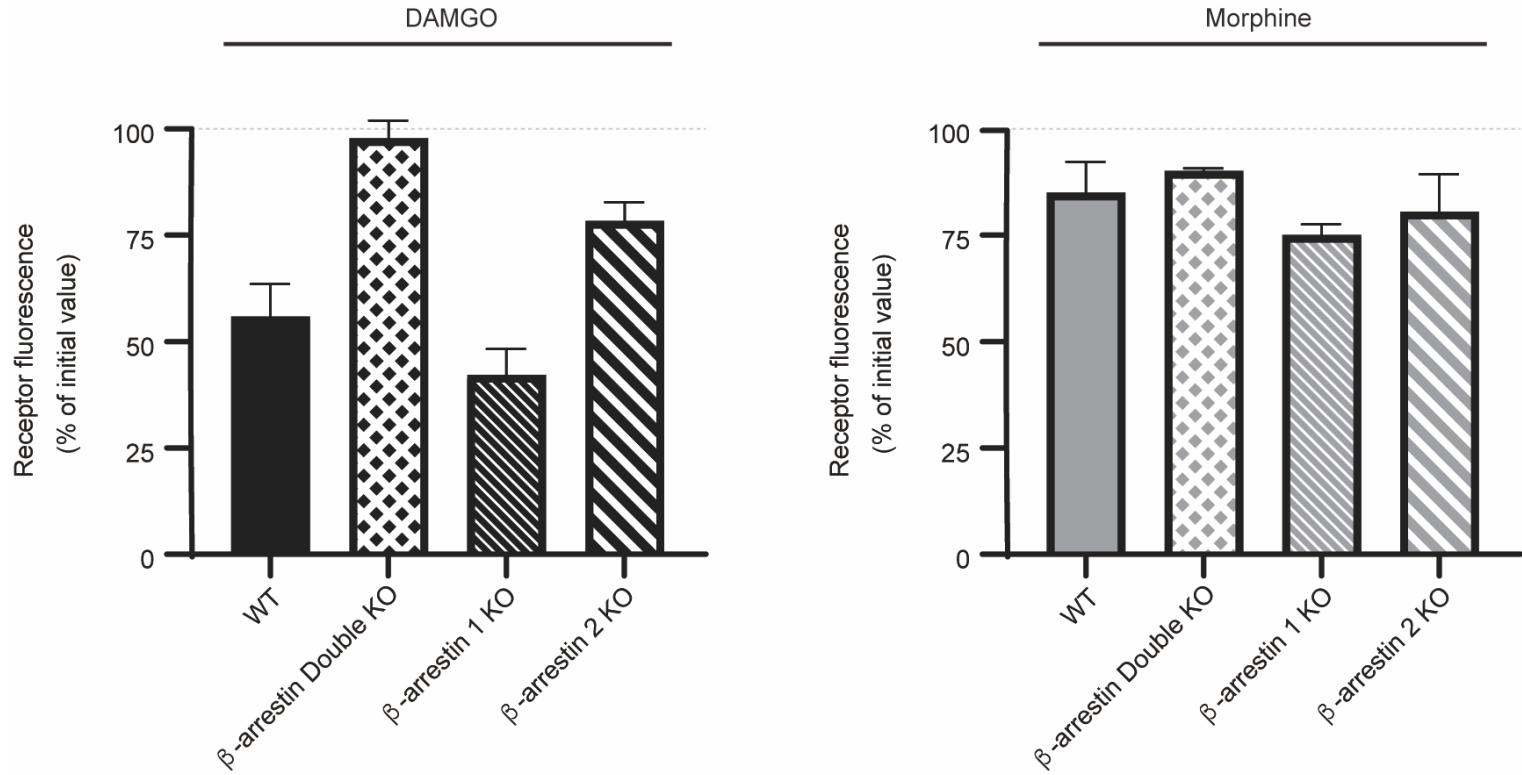
c

Immunoblotting



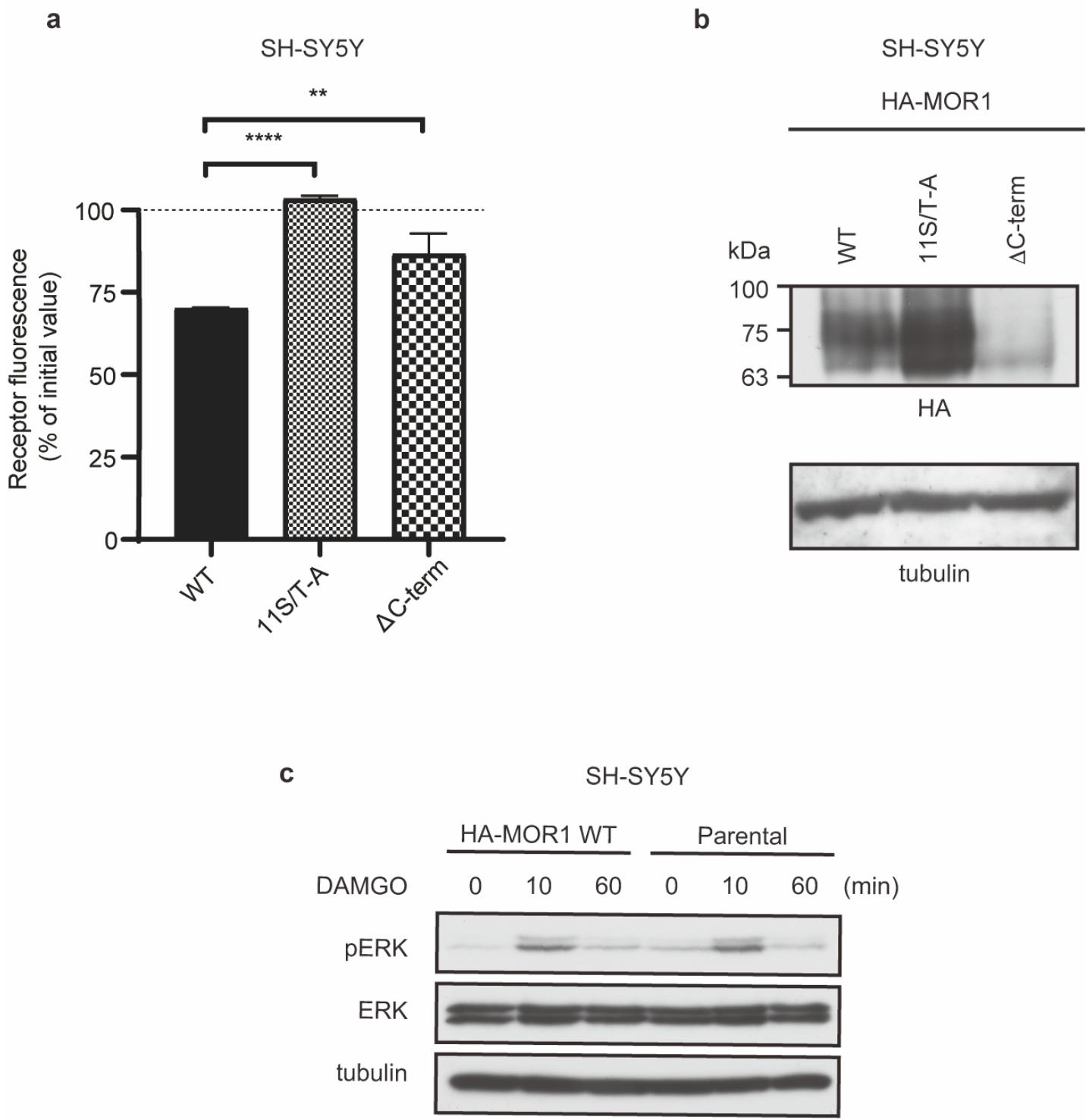
Extended data Figure 4 Genetic depletion of both β -arrestin 1 and 2 simultaneously in SH-SY5Y cell with use of CRISPR/Cas9.

The gRNA design (a) as well as obtained genomic and amino acid sequence (b) for establishing knockout cell lines using CRISPR/Cas9. Western blotting analysis was used to confirm the simultaneous loss of expression of both β -arrestin 1 and 2.



Extended data Figure 5 MOP internalisation in β -arrestin deficient cell line at an earlier time point on stimulation with DAMGO or Morphine.

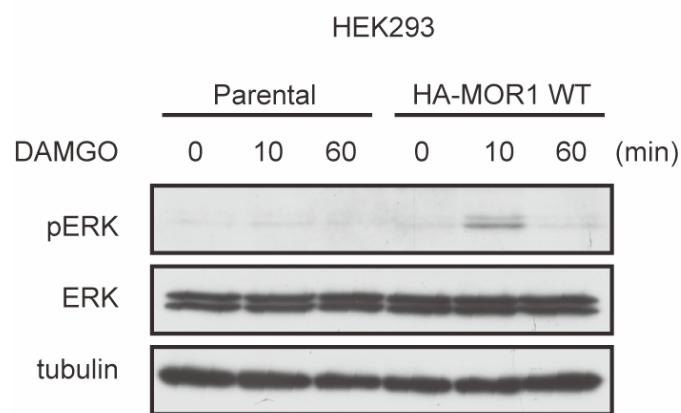
MOP internalisation under the simultaneous loss of both β -arrestin 1 and 2, or either one of the β -arrestins in SH-SY5Y cells overexpressing HA-tagged MOR1 upon stimulation with 10 μ M of DAMGO (left panel) or morphine (right panel) as indicated for 10 min.



Extended data Figure 6. Endogenous MOP drives MAPK signalling almost comparably to ectopically overexpressed MOP in SH-SY5Y cells.

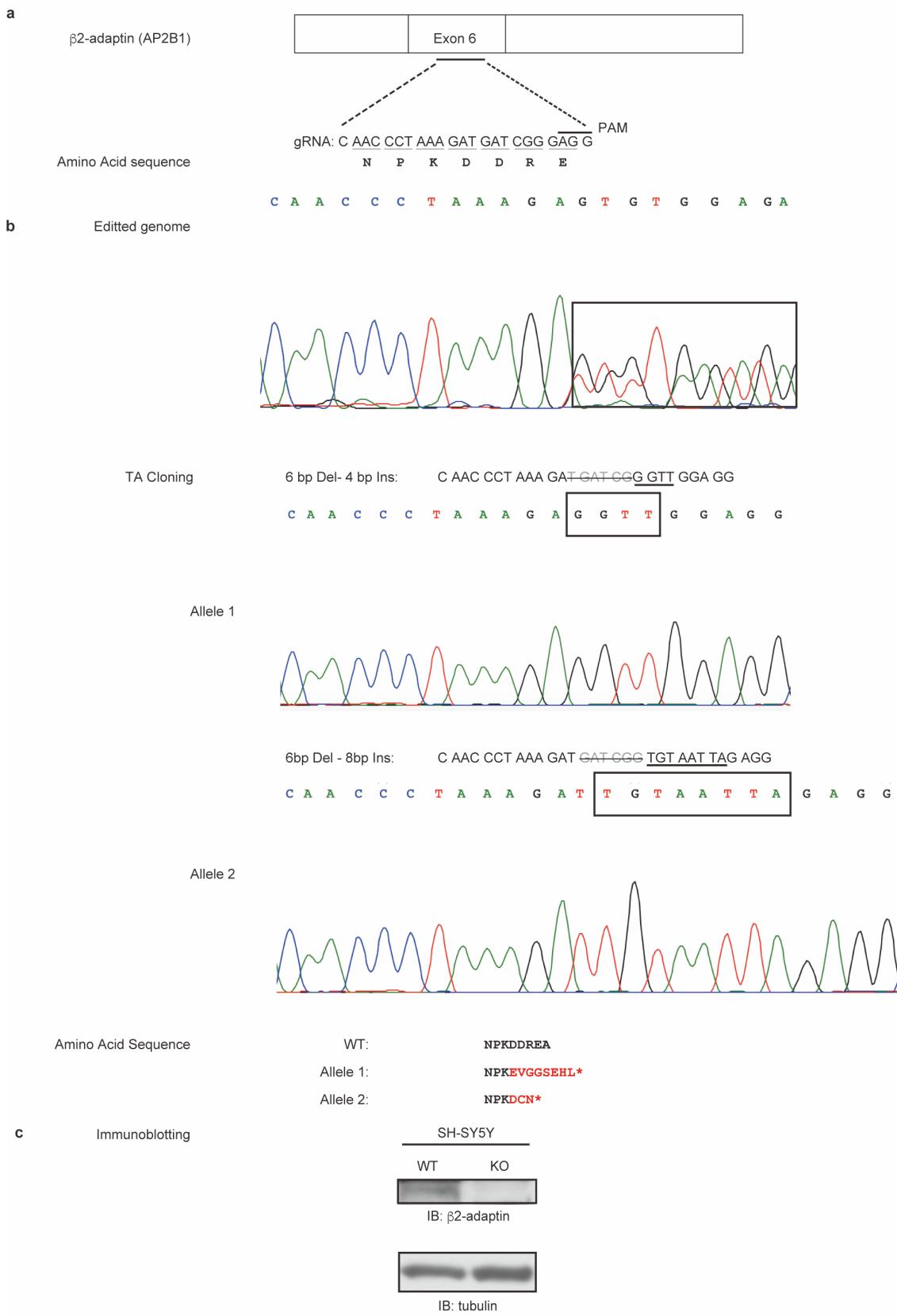
a. Receptor internalisation when wild-type or mutated MOP tagged with HA sequence were transfected into SH-SY5Y cells and stimulated with 10 μ M of DAMGO for 10 min ($**P<0.01$, $****P<0.0001$). b. The expression level of wild-type or mutated MOP expressed in SH-SY5Y cells was analysed by immunoblotting. c. Immunoblot analysis of MAPK activation in whole-cell lysates from SH-SY5Y cells stably expressing wild-type MOP (left) in comparison with the parental cells (right).

Extended Data Figure 7



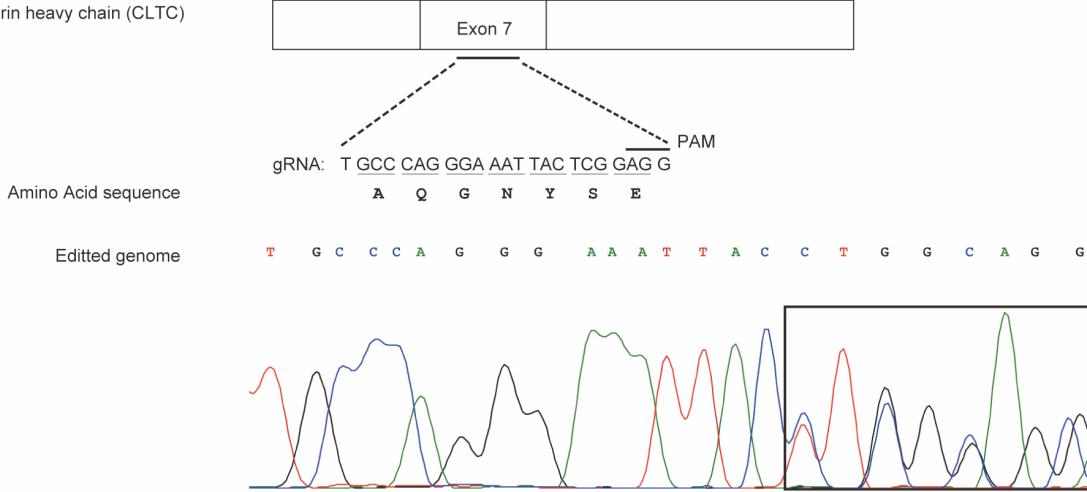
Extended data Figure 7 Ectopic expression system discerns the signals from stably expressed MOP in HEK293 cells.

Immunoblot analysis of MAPK activation in whole-cell lysates from HEK293 cells stably expressing wild-type MOP (right) in comparison with the parental cells (left).



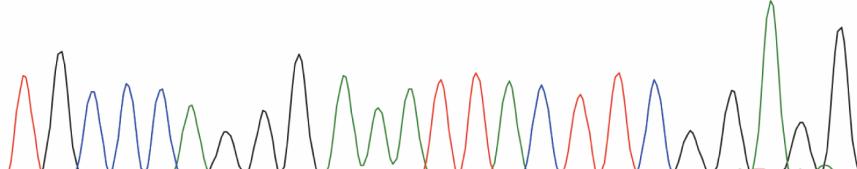
Extended data Figure 8. Genetic depleting of β2-adaptin with CRISPR/Cas9 in SH-SY5Y cells.

The gRNA design (a) as well as obtained genomic and amino acid sequence (b) for establishing knockout cell lines using CRISPR/Cas9. c. Western blotting analysis was conducted to confirm the loss of expression of the β2-adaptin subunit of the AP2 adaptor complex.

a Clathrin heavy chain (CLTC)**b** TA Cloning

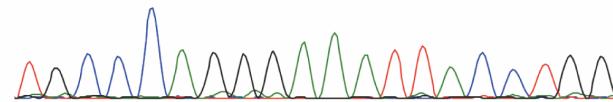
1 bp Ins: T GCC CAG GGA AAT TAC TTC GGA GG
 T G C C C A G G G A A A T T A C T T C G G A G G

Allele 1



5bp Del - 2bp Ins: T GCC CAG GGA AAT TAC TCC GACTG G
 T G C C C A G G G A A A T T A C C T G G

Allele 2

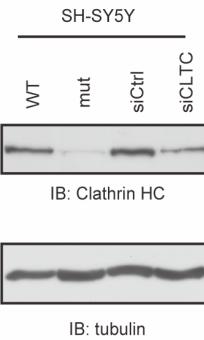


Amino Acid Sequence

WT: **AQGNYS**EAAKVAANAPKVKS**Y**ENEVVMTL**I**H**C***

Allele1: **AQGNYF**GGSKGGC*

Allele2: **AQGNYLA**AKVAANAPKVKS**Y**ENEVVMTL**I**H**C***

c Immunoblotting**Extended data Figure 9. Introducing mutation on clathrin heavy chain with CRISPR/Cas9.**

The gRNA design (a) as well as obtained genomic and amino acid sequence (b) for establishing knockout cell lines using CRISPR/Cas9. c. Western blotting analysis was performed to confirm the reduction of the expression level of clathrin heavy chains. Sample of cell cultures obtained by the knockdown method were used as references.