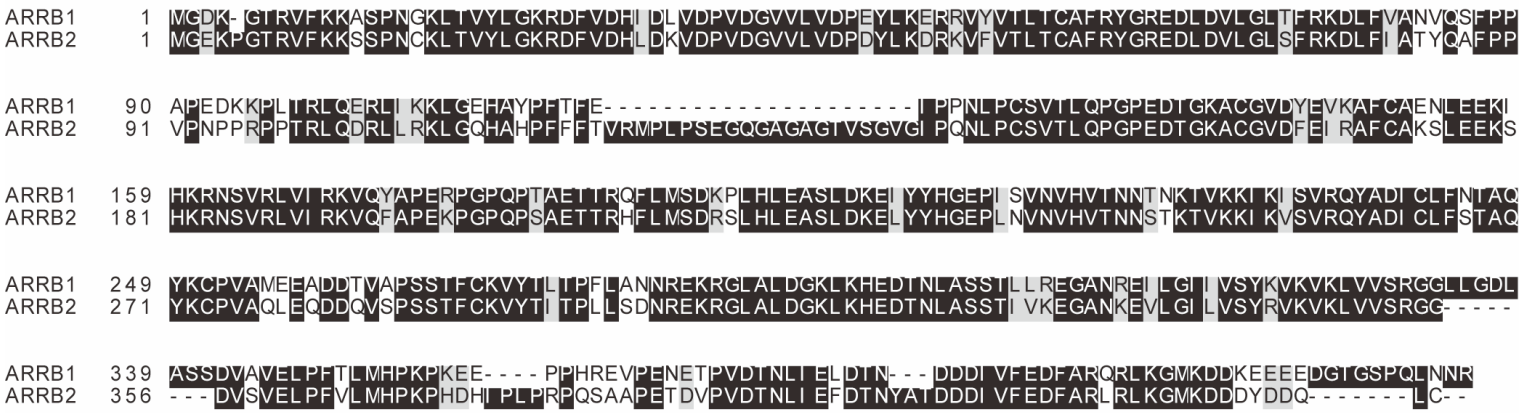
Extended Data Figure 1

a

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



b

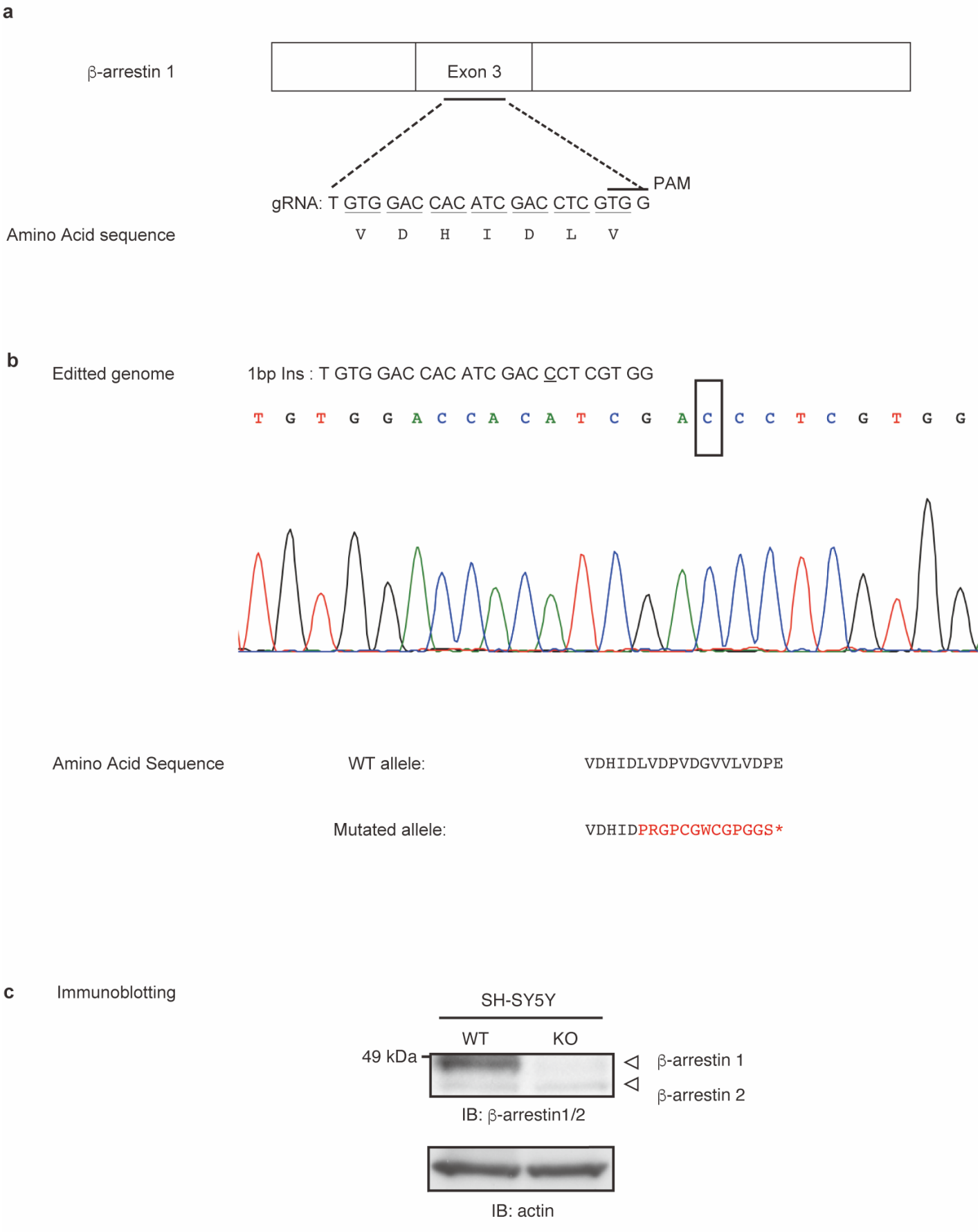
		Percent Identity	E value
beta-arrestin-1 Isoform A	beta-arrestin-2 Isoform 1	76.14%	0
	2	73.25%	0
	3	72.48%	0
	4	80.46%	8.00×10^{-150}
	5	74.00%	0
	6	71.19%	0
	7	73.09%	4.00×10^{-112}

		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.

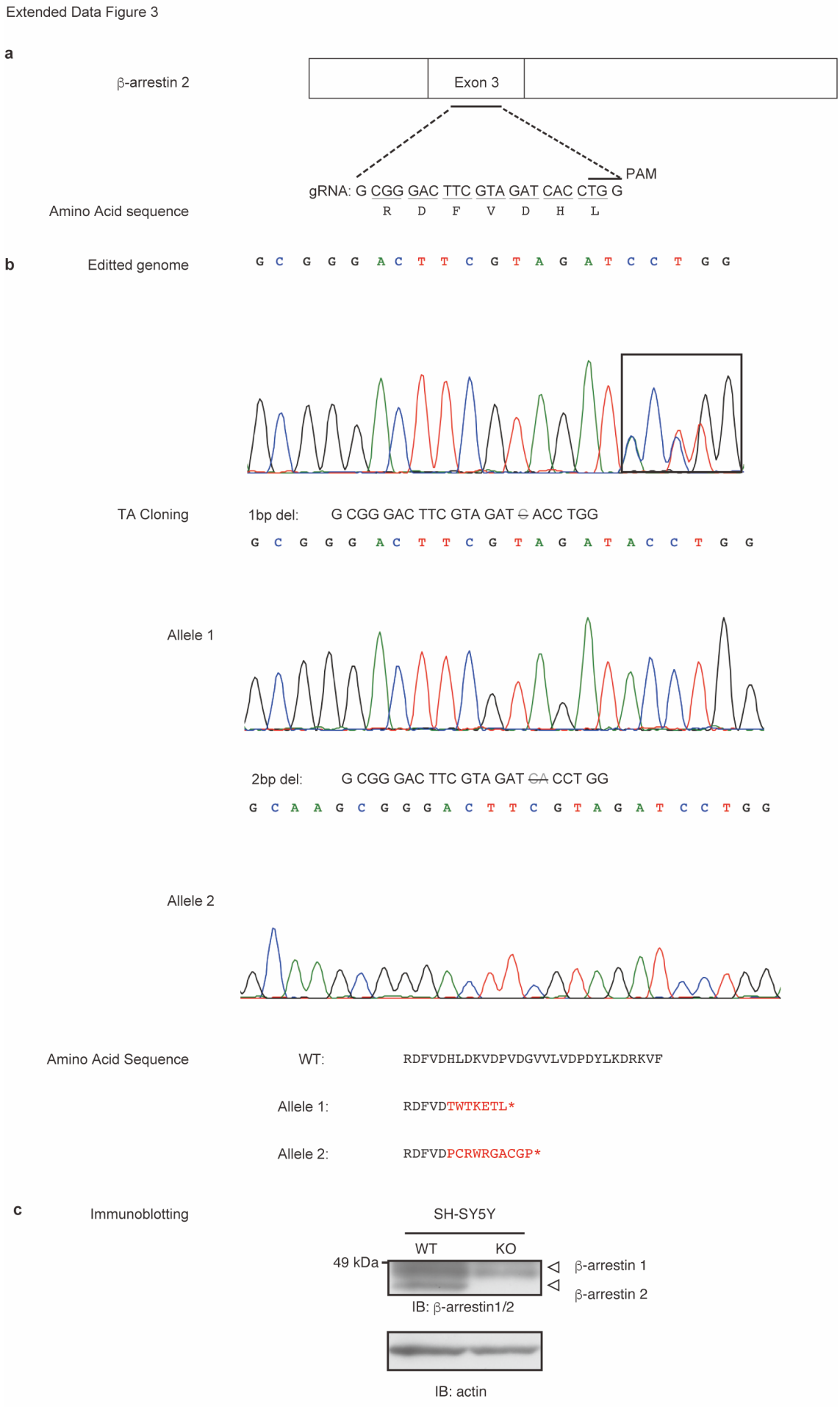
a. Alighment search between β-arrestin 1 isoform A and β-arrestin 2 isoform 3 as a representative diagram. BOXSHADE version 3.21 was used. b. β-arrestin 1 isoform A and B were compared with β-arrestin 2 isoforms.

Extended Data Figure 2



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

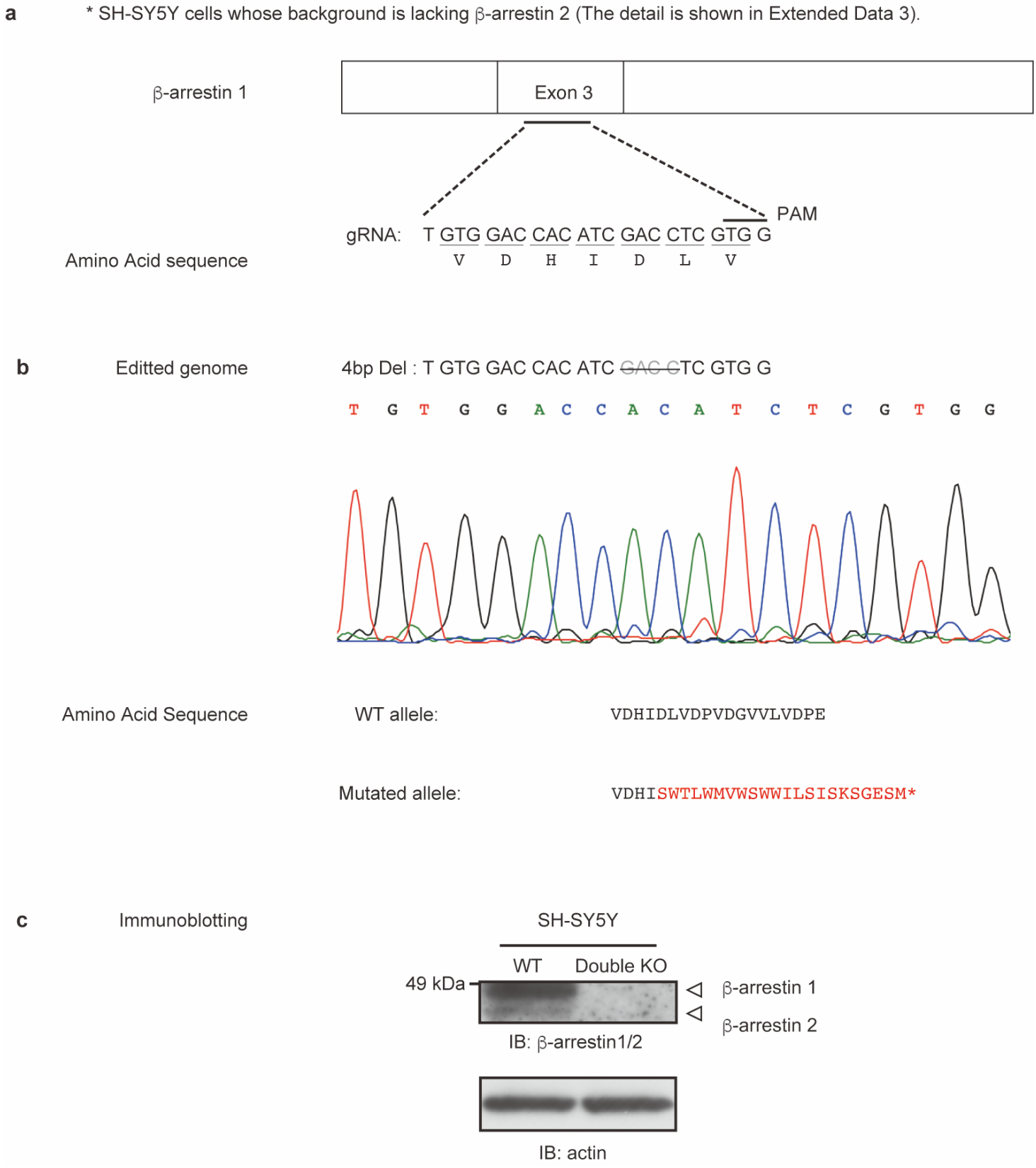
The gRNA design (a) as well as obtained genomic and amino acid sequence (b) for establishing knockout cell lines using CRISPR/Cas9. Western blotting analysis was conducted to confirm the loss of β-arrestin 1 expression.



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.

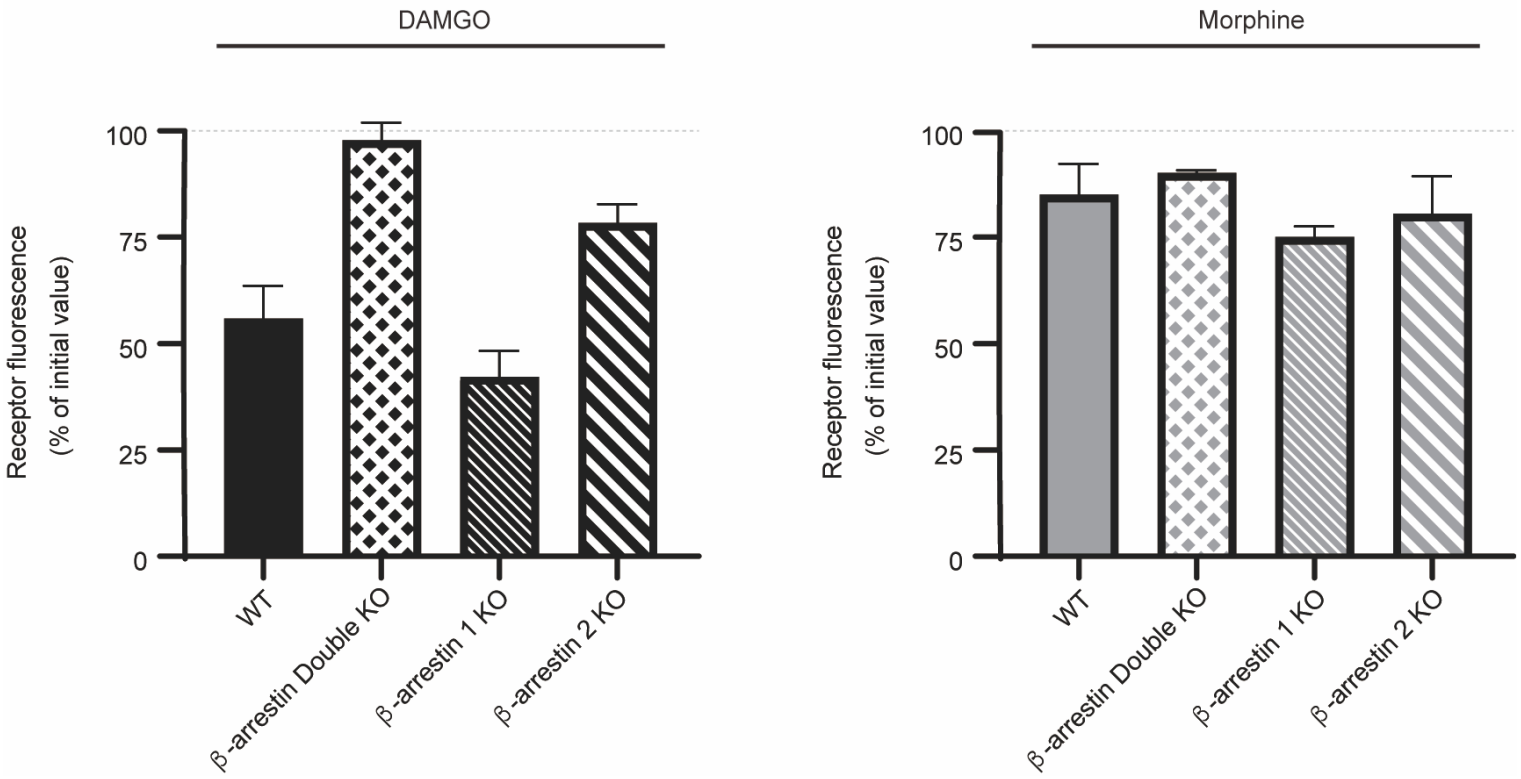
Extended Data Figure 4



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.

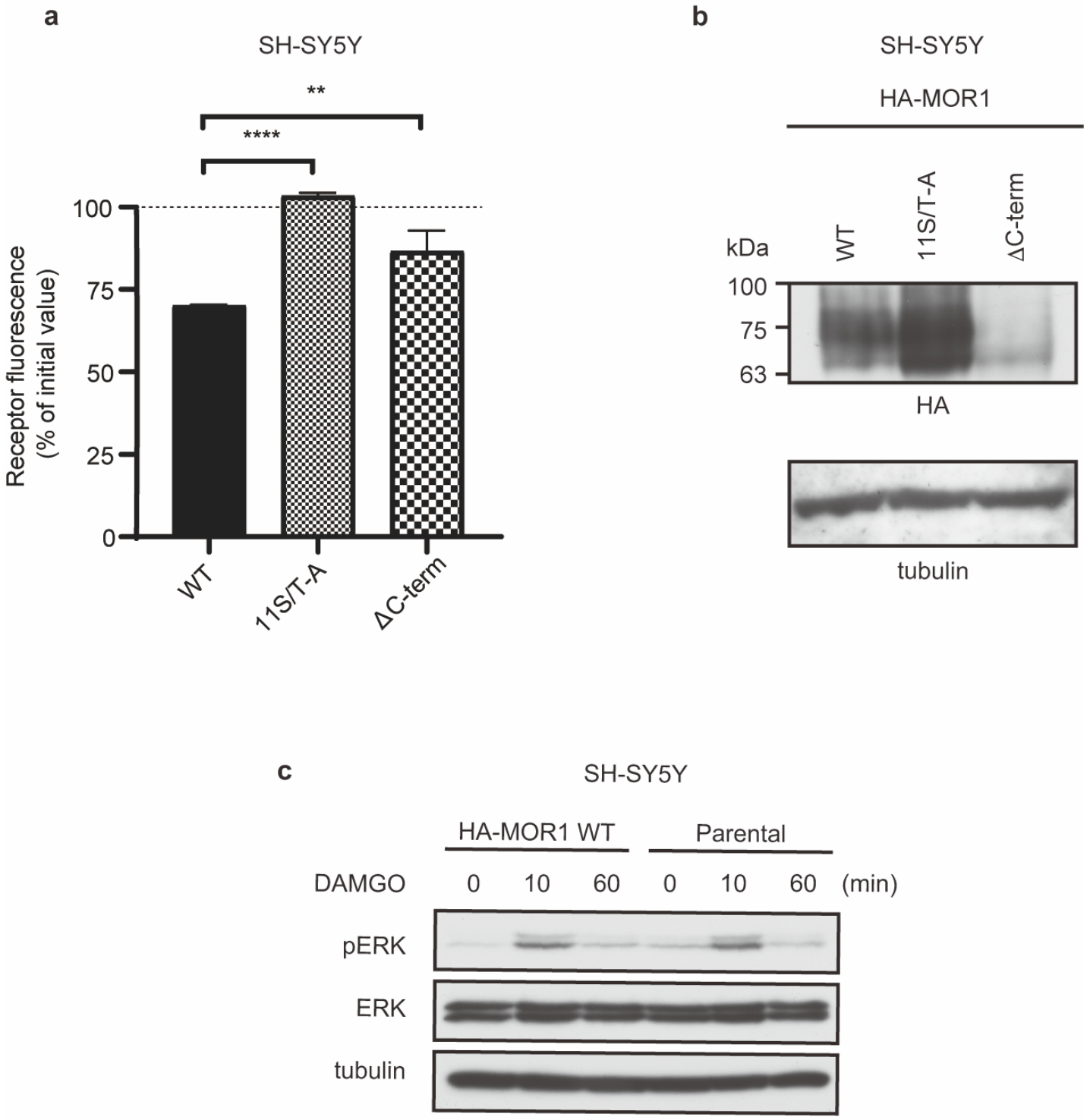
Extended Data Figure 5



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

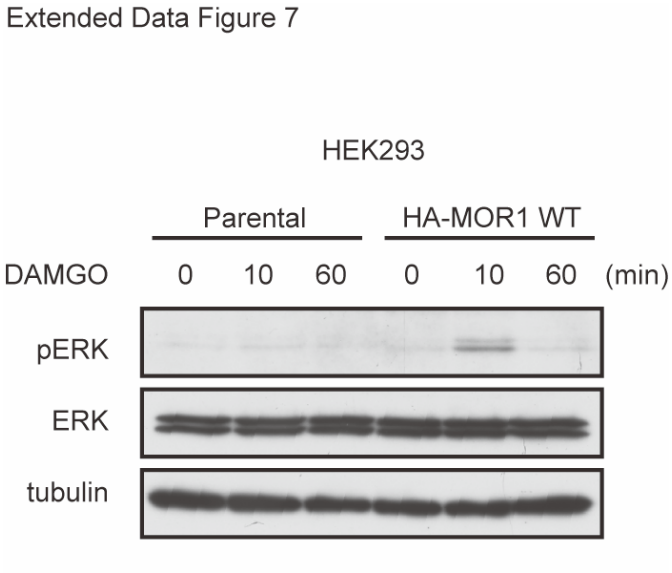
MOP internalisation under the simultaneous loss of both β-arrestin 1 and 2, or either one of the β-arrestins in SH-SY5Y cells overexpressing HA-tagged MOR1 upon stimulation with 10 μM of DAMGO (left panel) or morphine (right panel) as indicated for 10 min.

Extended Data Figure 6



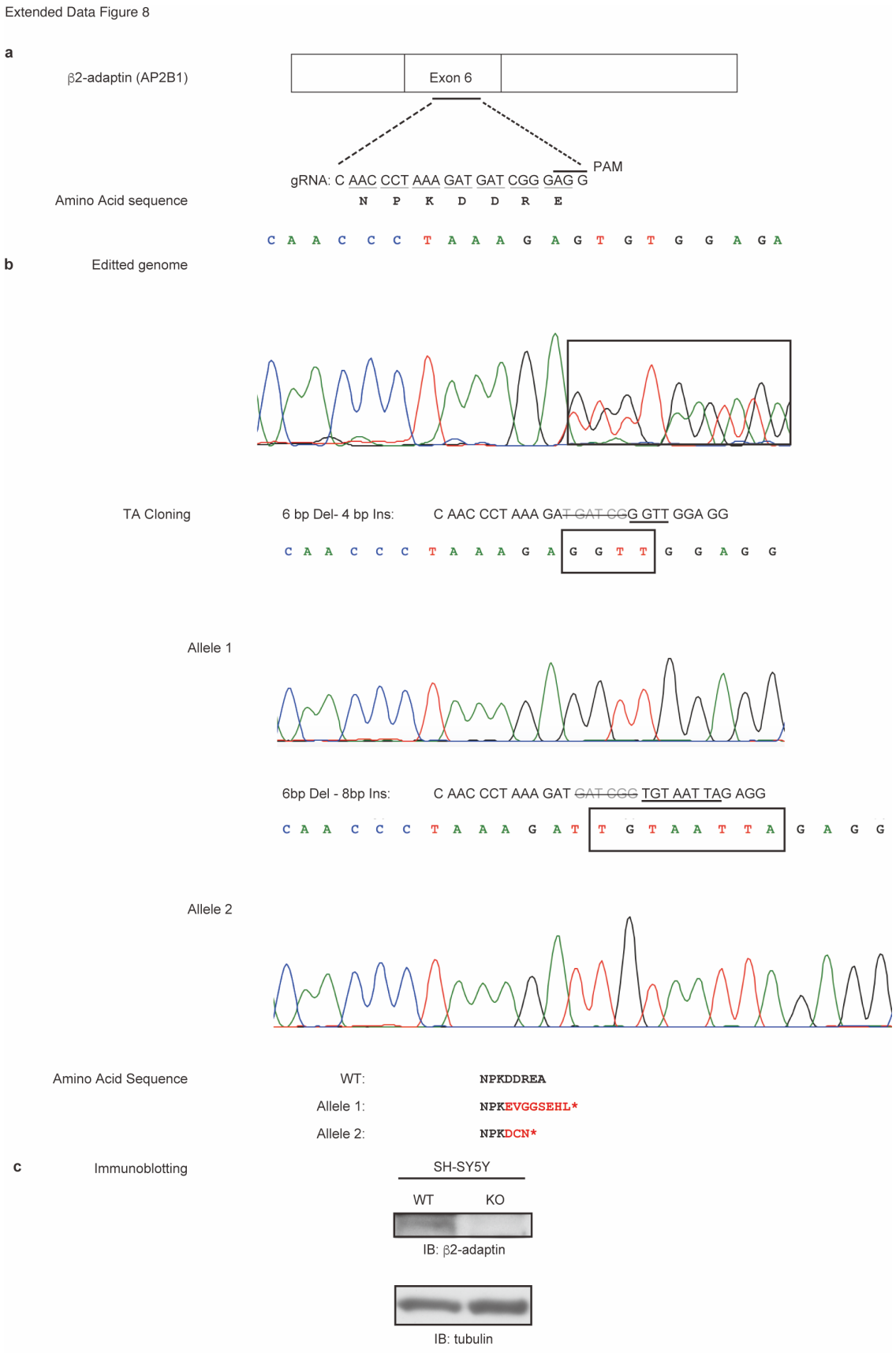
Extended data Figure 6. Endogenous MOP drives MAPK signalling almost comparably to ectopically overexpressed MOP in SH-SY5Y cells.

a. Receptor internalisation when wild-type or mutated MOP tagged with HA sequence were transfected into SH-SY5Y cells and stimulated with 10 μM 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).



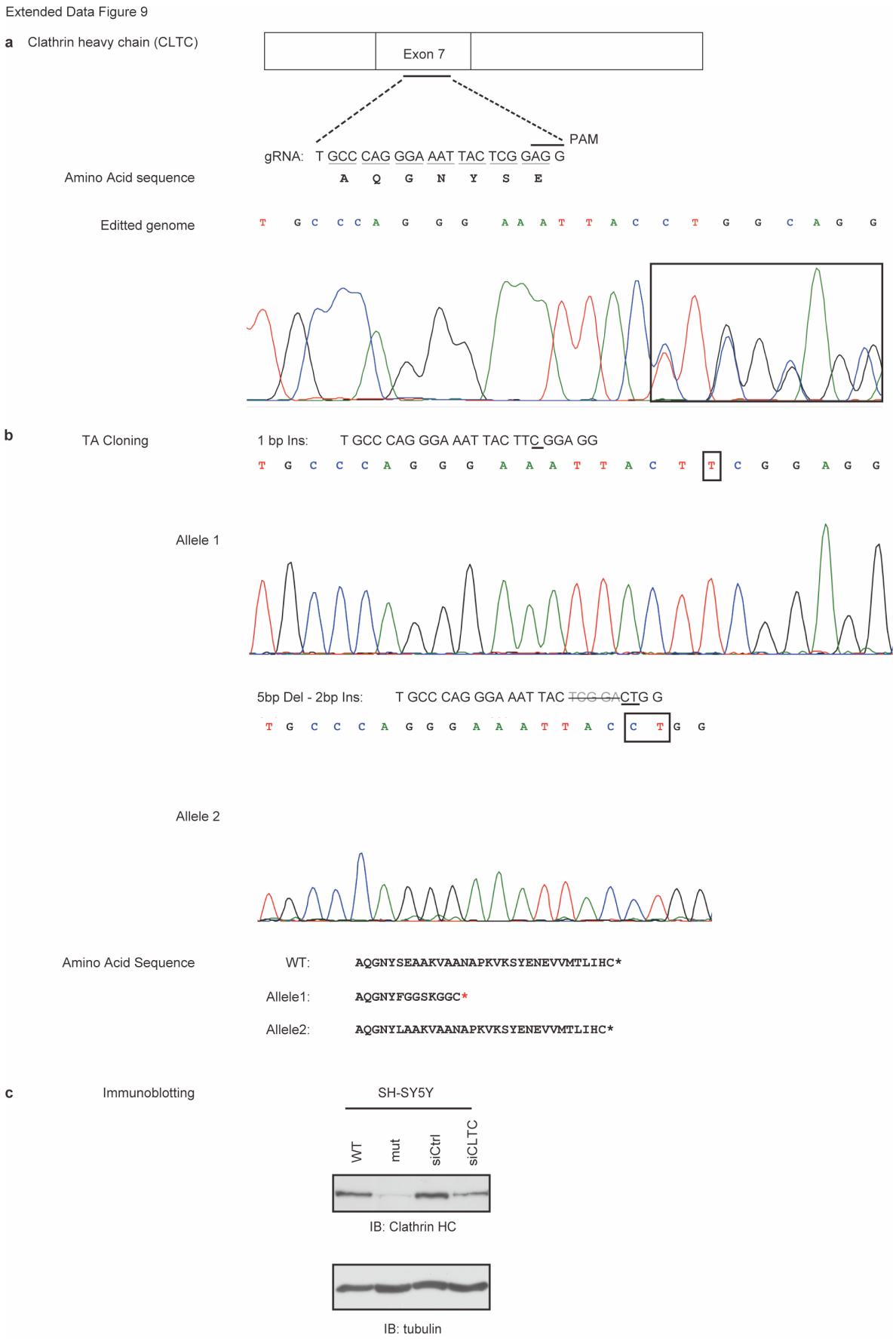
Extended data Figure 7 Ectopical expression system discerns the signals from stably expressed MOP in HEK293 cells.

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.



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.