

Supplementary Figure Legends

Supplementary Figure 1. HeLa cells were transfected with siRNAs (siLuc or siJIP4) for overnight and recovered in fresh culture medium for 24 hours, and then transfected with expression plasmids encoding either Flag tagged WT-JIP3 or MT-JIP3 for overnight following 24 hours recovery in fresh culture medium for further experiments. (A) the levels of JIP3, Flag-JIP3 and JIP4 proteins were measured by western blot for validation of transfection. (B) Cell proliferation was measured by MTT assay. Cells transfected with different concentrations of siJIP4. siCtrl was used as controls to normalize the data. $n = 3$, * $P < 0.05$, ** $P < 0.01$. (C) The quantification of western blots for co-IP in Fig.1D. (D) The quantification of the colocalization (120 mins) between Rab7 and Cy3-ASO in Fig. 1E was calculated by Image J using Person's R value. $n = 15$, ** $P < 0.01$. (E) The quantification of the Cy3 integrated fluorescence density in the mTOC area (120 mins) in Fig. 1E was calculated by Image J. $n = 10$, ** $P < 0.01$. (F) Videos were recorded for different siRNA/plasmid treated HeLa cells were incubated with 1 μ M Cy3-labeled PS-ASO 446654 for different time points. The colocalization of Cy3-ASOs and Rab7 (Late endosome marker, LE) were observed by Keyence X800 microscope.

Supplementary Figure 2. (A) The quantification of the colocalization between Rab7 and Cy3-transferrin (120 mins) in Fig. 2D was calculated by Image J using Person's R value. (B) The quantification of the colocalization between Rab5/7 and Cy3-transferrin in Fig. 2E was calculated by Image J using Person's R value. The cell imaging of either patient fibroblasts or healthy control fibroblasts were co-stained with JIP3 and Rab5 (early endosome marker) (C), and JIP3 and Rab7 (late endosome marker) (D). The

colocalization was observed by Keyence BZ-X800 microscope. Colocalization was quantified with Pearson's coefficient by ImageJ. (E) The interaction of JIP3 and JIP4 in control and patient fibroblasts was detected by co-IP. Briefly, Flag antibody was used for pulling down cell lysates and JIP3/4 proteins were measured by western blot.

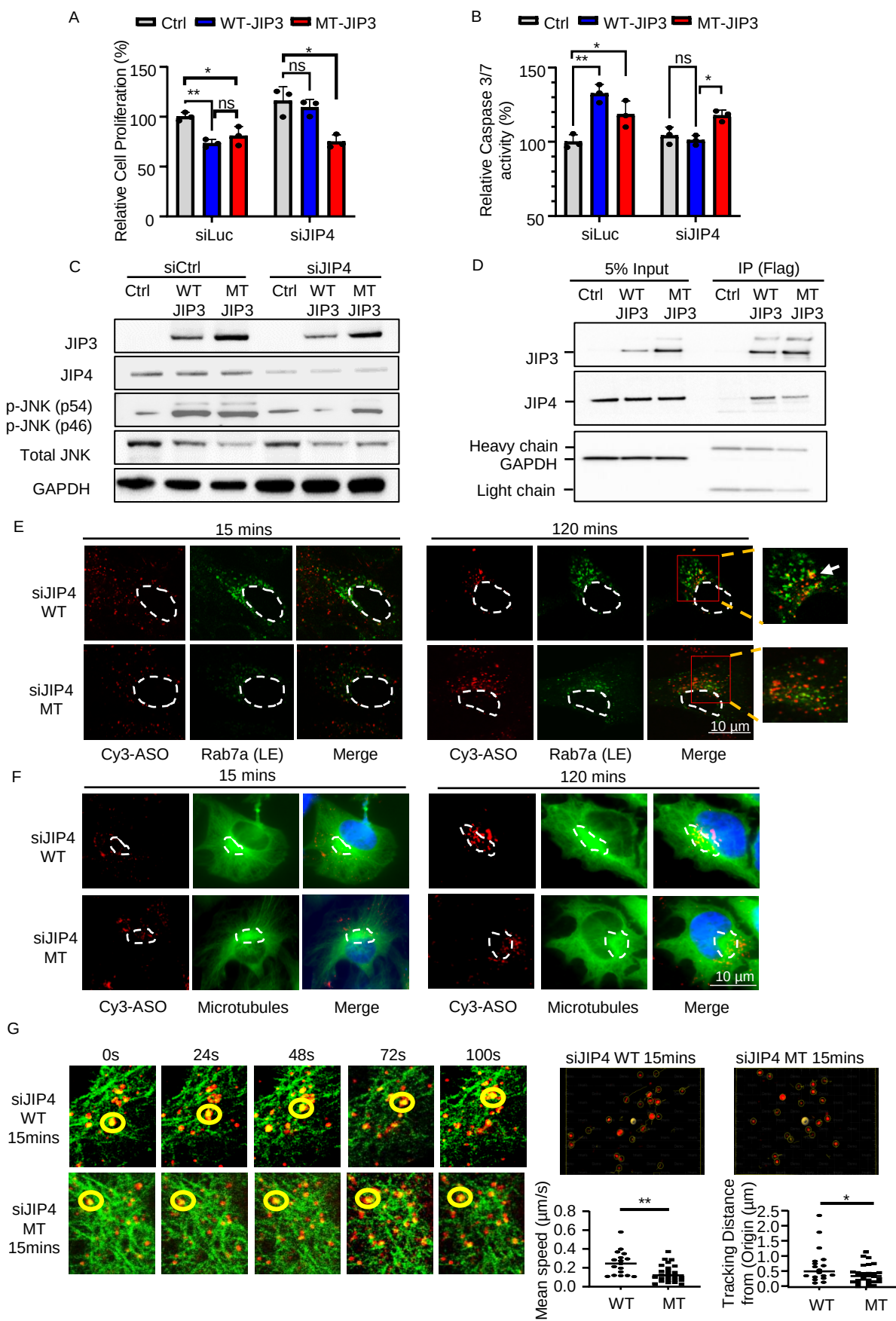
Supplementary Figure 3. (A) The quantification of number of varicosities per sample was analyzed by ImageJ through the function of "analyze particle". Varicosities were represented as particle clusters. ** $P < 0.01$. (B) Crispr control and patient iPSC derived neurons were fixed and stained with anti- β III tubulin antibody. Varicosities in long axons were detected by Keyence BZ-X800 microscope with different magnifications. Representative varicosities were pointed out by white arrows. (C) The cell imaging of either CRISPR control or patient iPSC derived neurons were co-stained with JIP3 and Rab5 (early endosome marker). The colocalization was observed by Keyence BZ-X800 microscope. Colocalization was quantified with Pearson's coefficient by ImageJ. (D) The quantification of the colocalization of Cy3-PS-ASO and Rab7 in iPSC derived neurons in Fig 3E was calculated by Image J using Person's R value. * $P < 0.05$. (E) The quantification of the colocalization of JIP3 and KIF3B in iPSC derived neurons in Fig 3F was calculated by Image J using Person's R value. * $P < 0.05$. (F) The interaction of JIP3 and KIF5b in CRISPR control and patient iPSC derived neuronal cells was detected by co-IP. Briefly, Flag antibody was used for pulling down cell lysates and JIP3 and KIF5b proteins were measured by western blot.

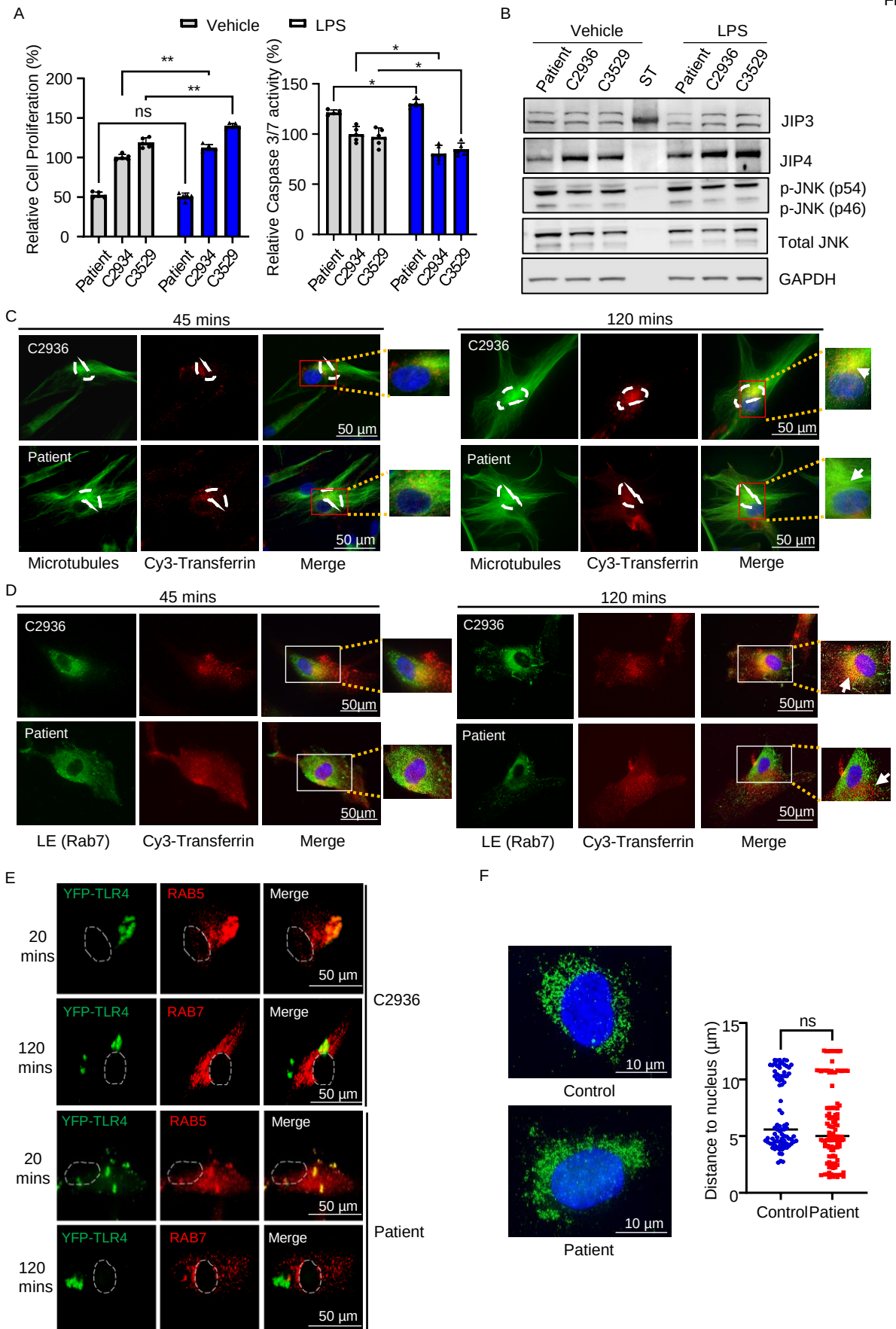
Supplementary Figure 4 (A) mRNA levels of *NLRP2* either in CRISPR control or patient iPSC derived neurons were measured by qPCR. (B) Three healthy subjects derived fibroblasts (2936, 3440 and 3529) or patient fibroblasts were seeded in 96 wells (8000 cells/well). Basal cAMP levels were measured by cAMP-Glo™ Assay (Promega). n = 3.

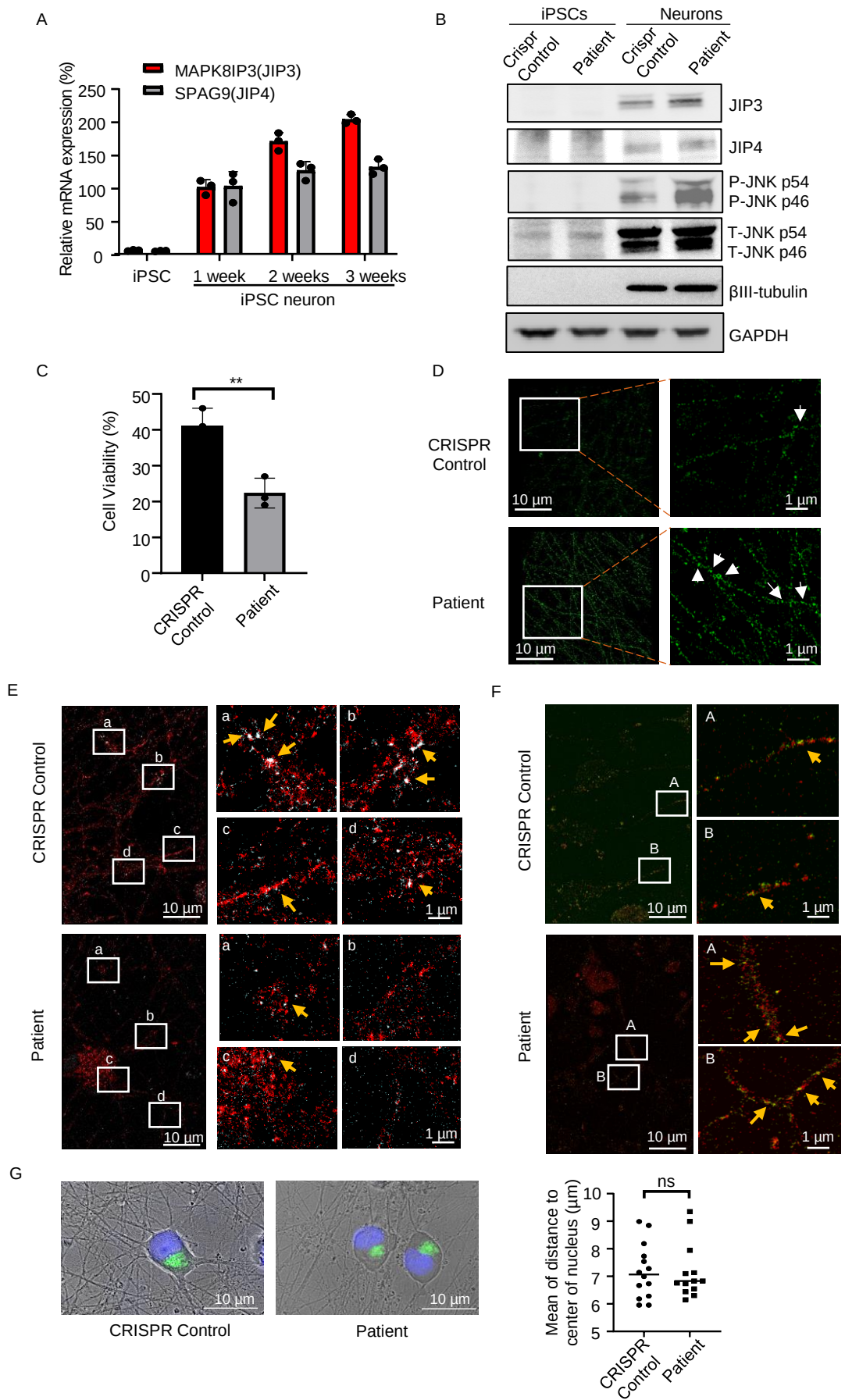
Supplementary Figure 5. Non-allele selective PS-ASOs rescue patient derived fibroblasts from MT-JIP3 induced cell toxicity. Three different health controls (2934, 3529 and 3440) and patient fibroblasts were treated with three different non-allele selective ASO1 (1713794), 687 ASO2 (1713687) and 510 ASO3 (1713510) targeting *MAPK8IP3* (JIP3) for 3 days. (A) After 24 hours incubation, RNA was extracted by GITC assay and *MAPK8IP3* mRNA level was measured q-PCR. Data was normalized to UTC of control cells. n = 3, ** P < 0.01. (B) *SPAG9* (JIP4) mRNA level was measured q-PCR. Data was normalized to UTC of control cells. n = 3, ** P < 0.01. (C) After 72 hours incubation, cell proliferations were measured by MTT assay and normalized to UTC of control cells. n = 3, * P < 0.05, ** P < 0.01. (D) The quantification of the colocalization (2 hrs.) between Rab7 and Cy3-Transferrin in Fig. 6G was calculated by Image J using Person's R value. n= 10, ** P < 0.01.

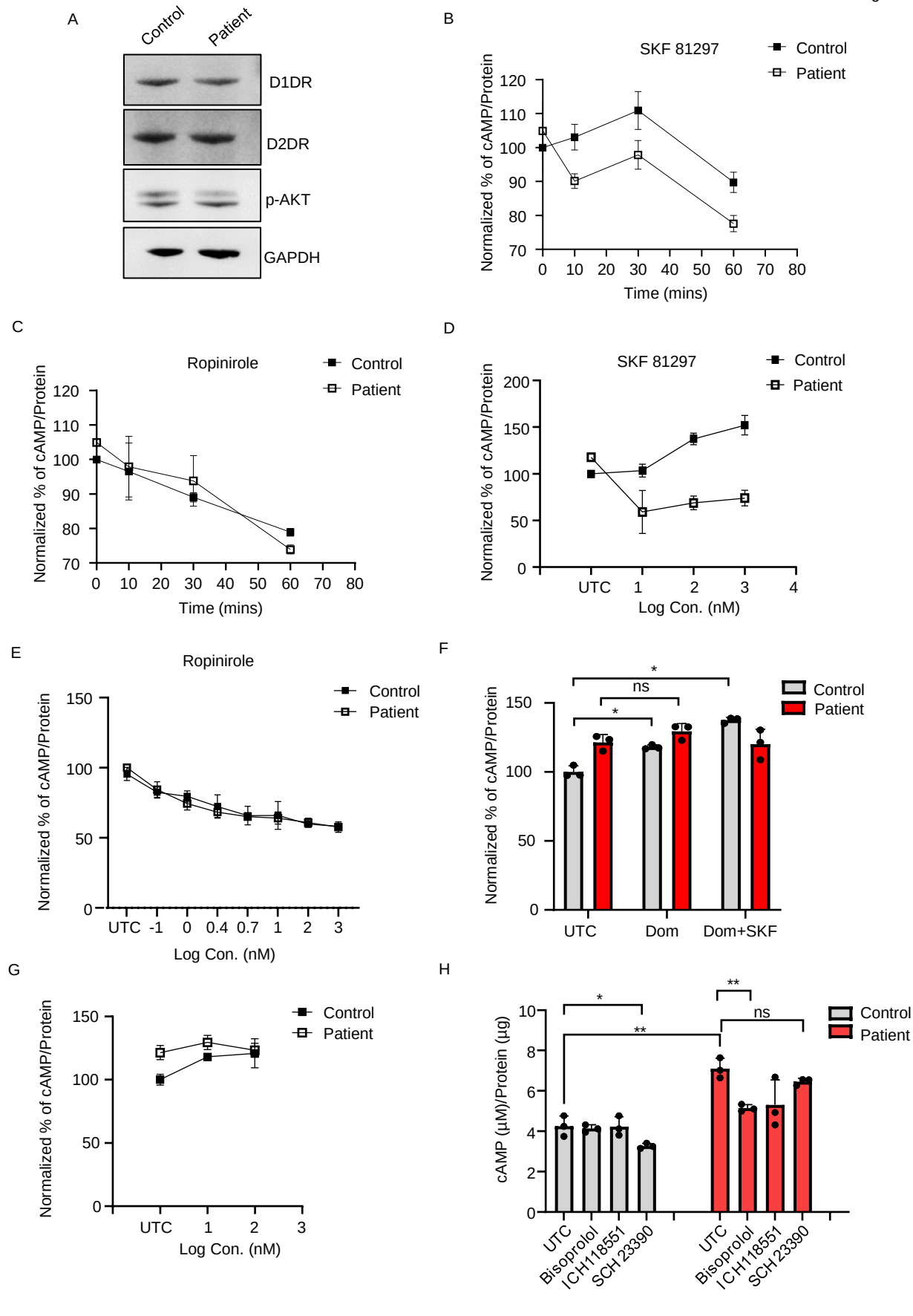
Supplementary Figure 6. Non-allele selective PS-ASOs rescue patient iPSC derived neurons from MT-JIP3 induced cell death (A) Expression of the MT-JIP3 in patient iPSC derived neurons leads to increased risk of death compared with CRISPR Control iPSC derived neurons. A representative bright field images of three iPSC derived neurons,

CRISPR control, Patient and Patient treated with ASO2 (5 μ M) at day 42 are shown (lower-panel). Images were taken by Keyence microscope. Cell viability was measured by trypan blue cell count assay and normalized to UTC of CRISPR control cells (right panel). * $P < 0.05$.

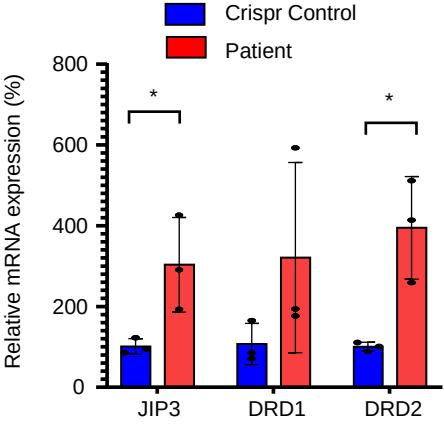




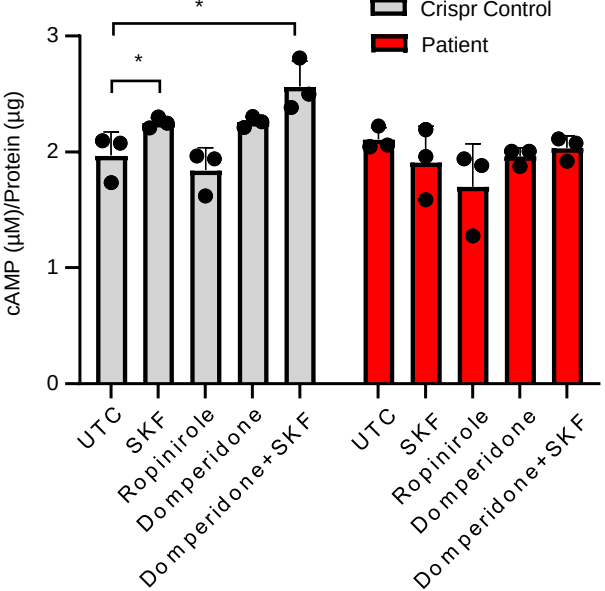




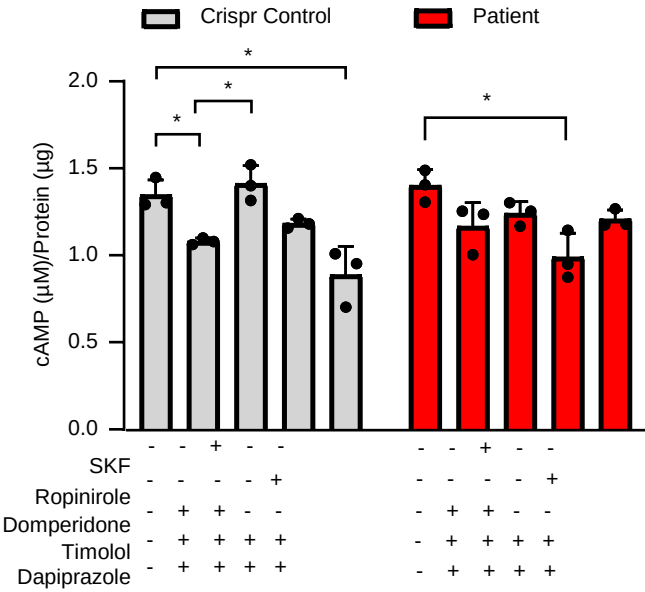
A



B



C



D

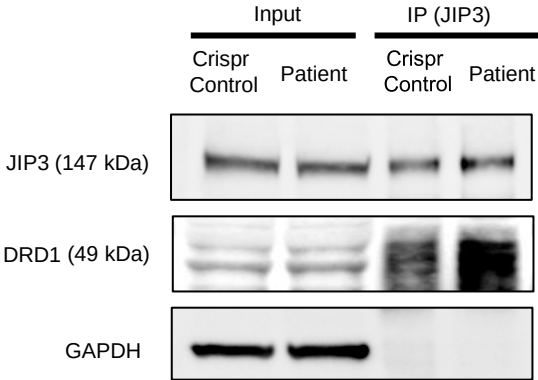
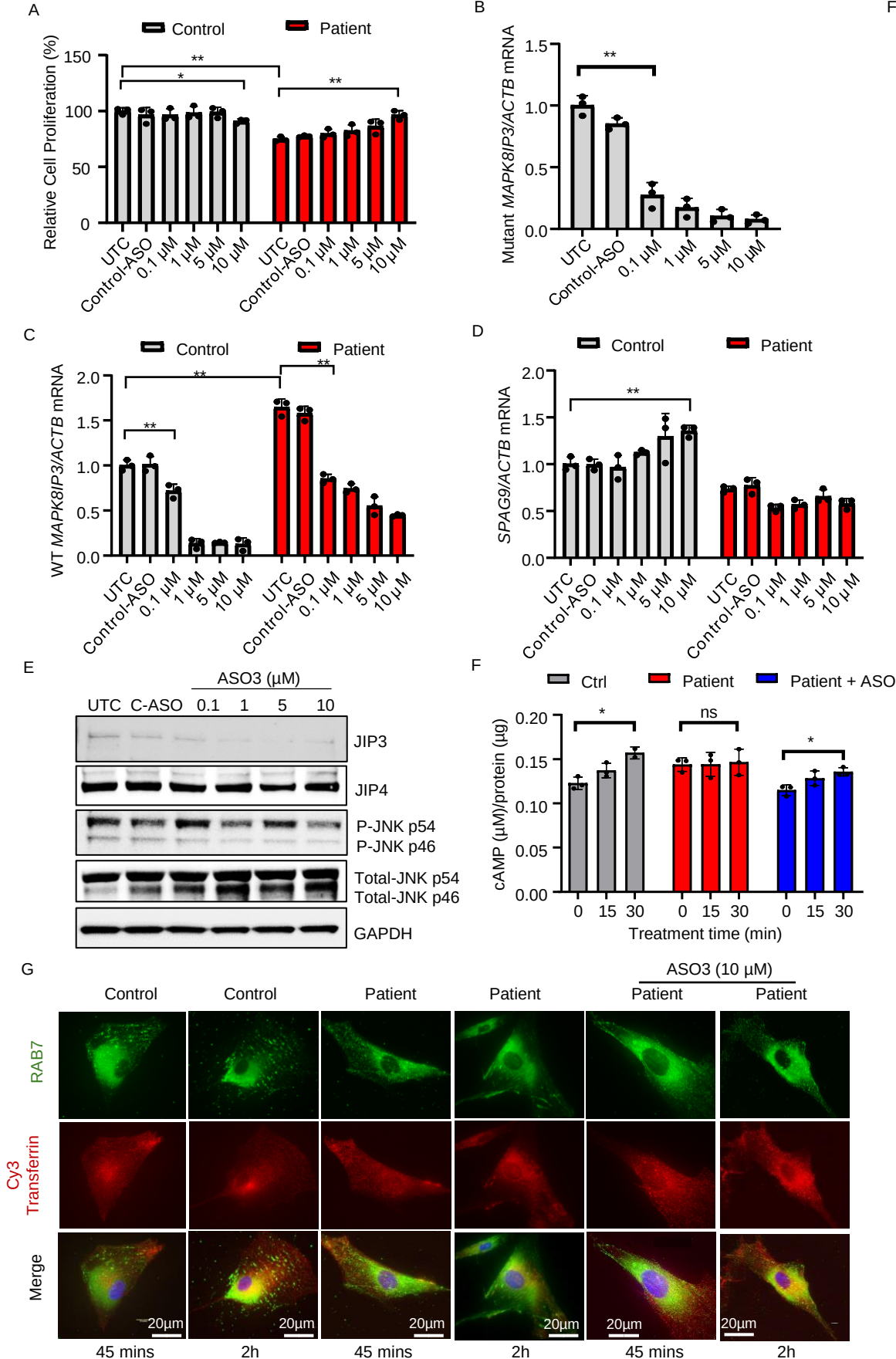
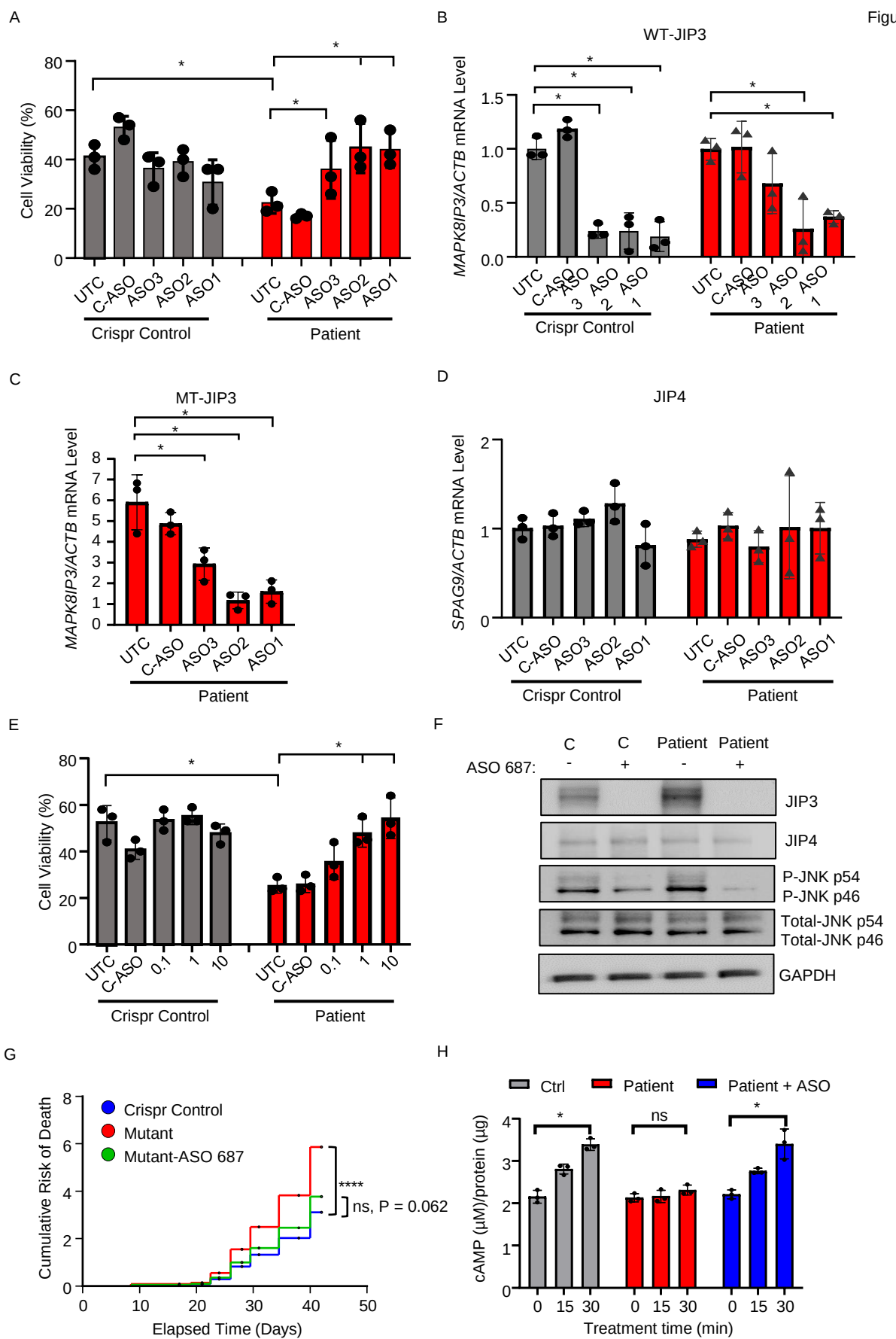
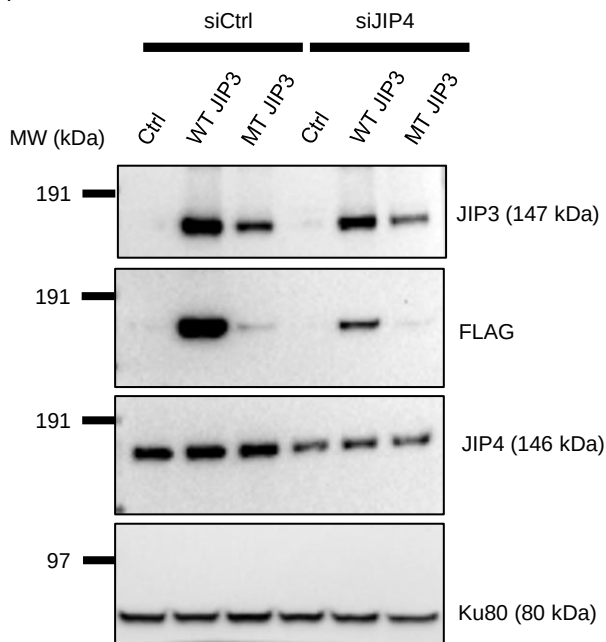


Figure 6

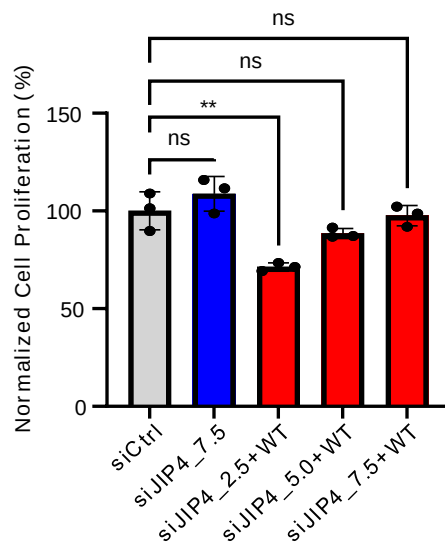




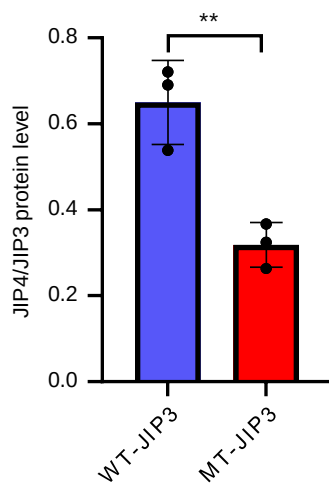
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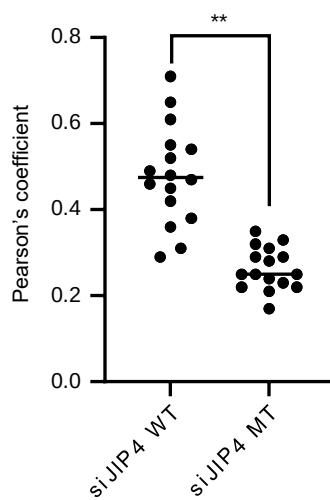
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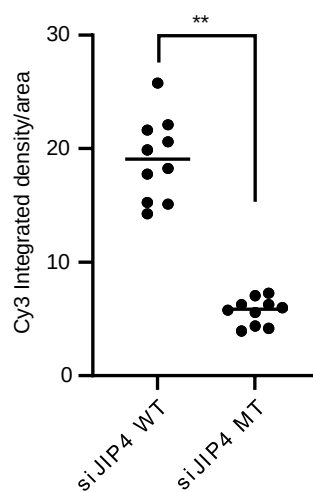
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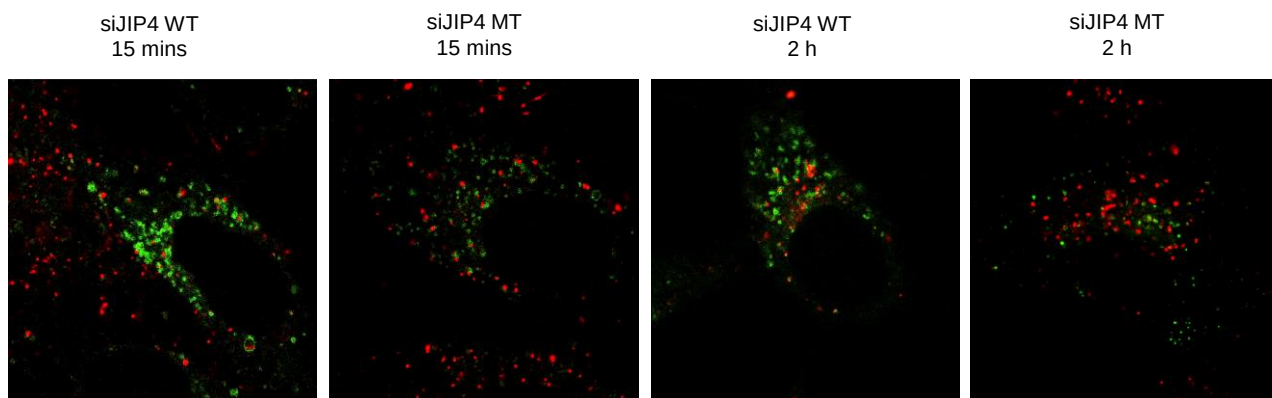
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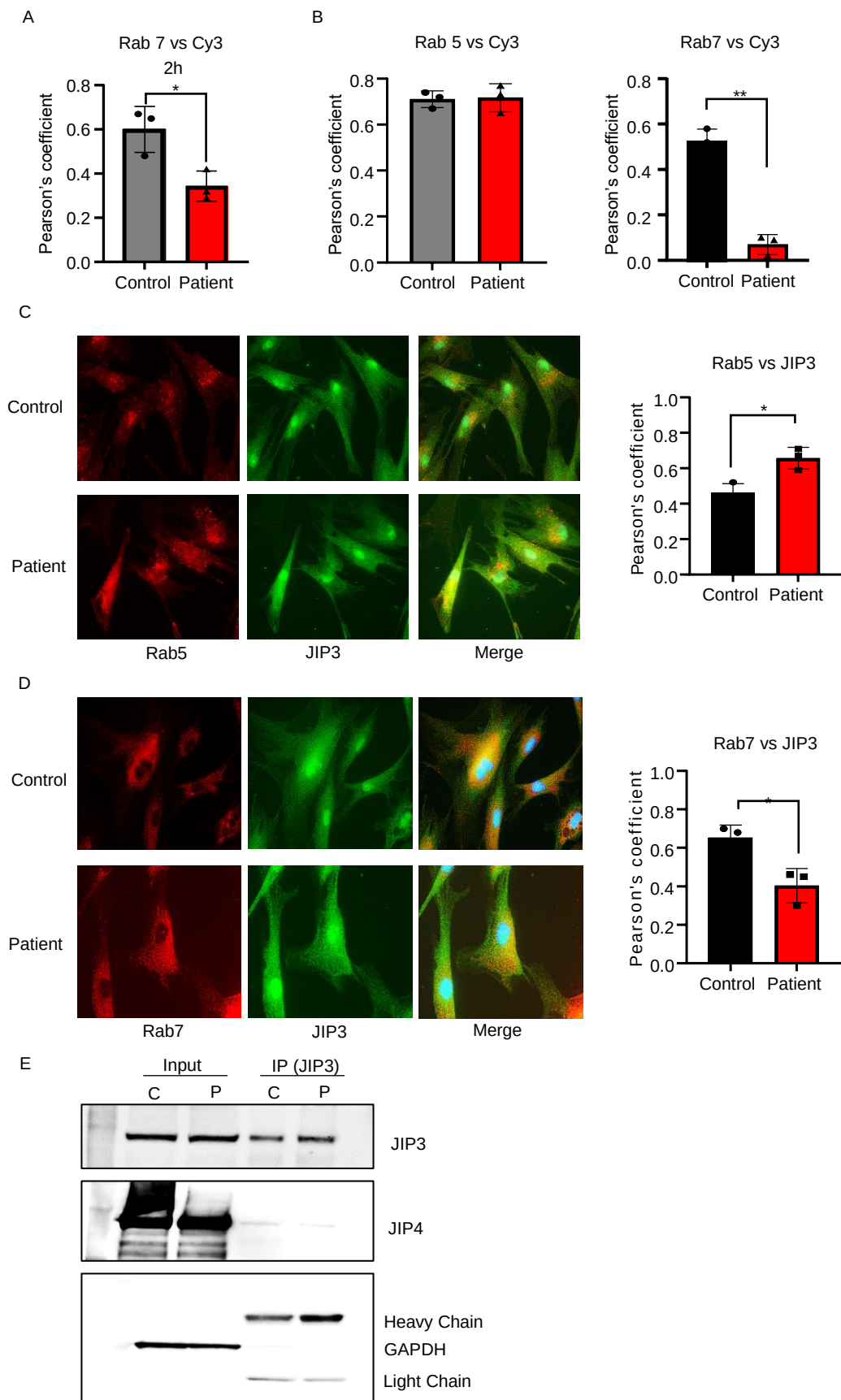


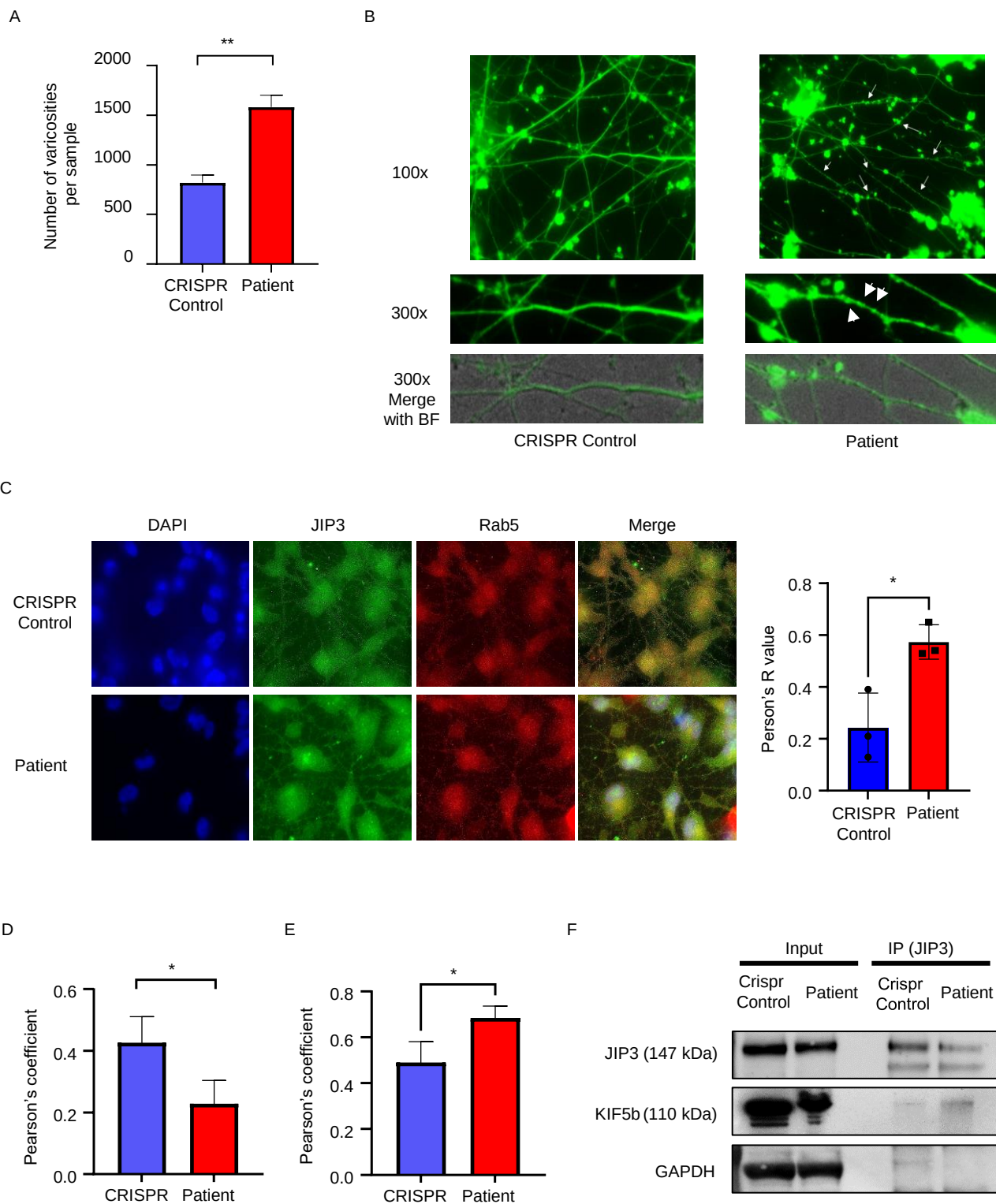
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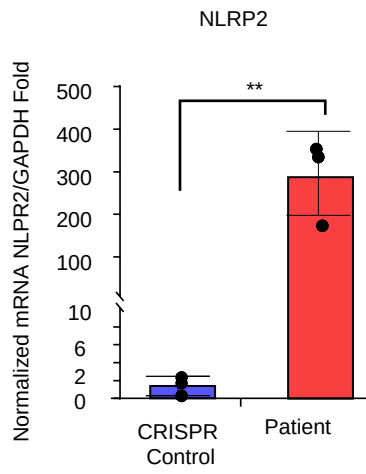
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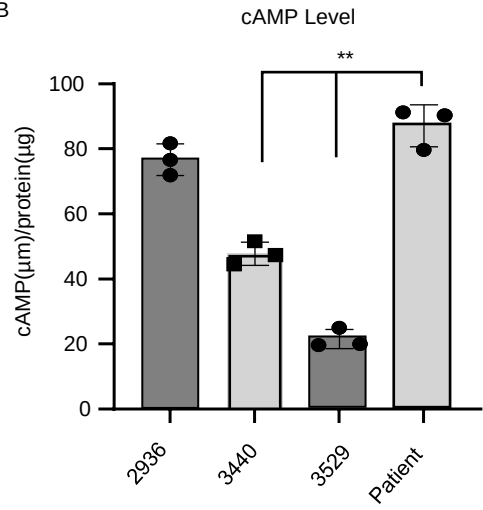




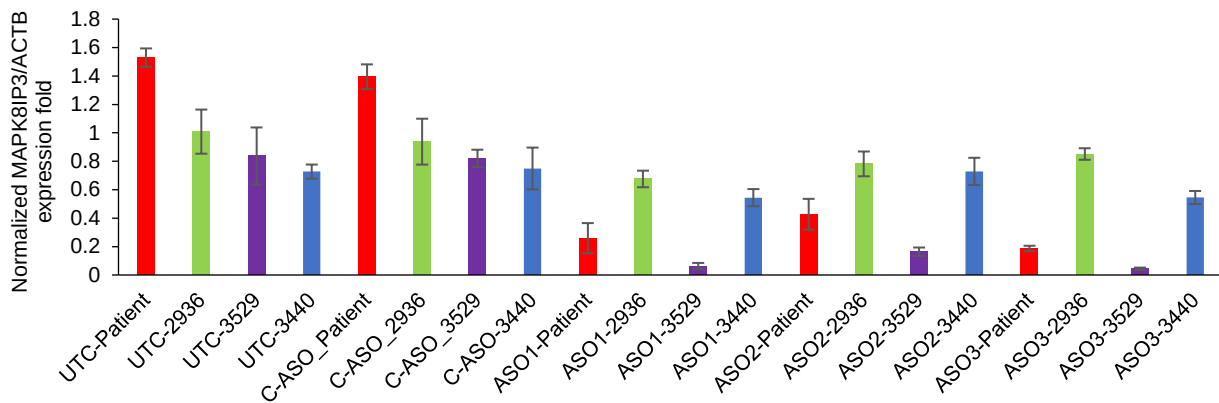
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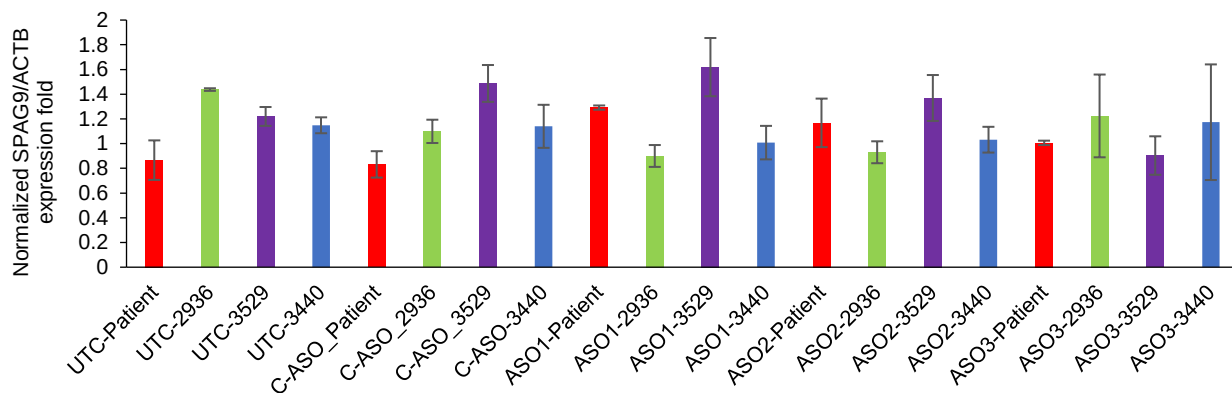
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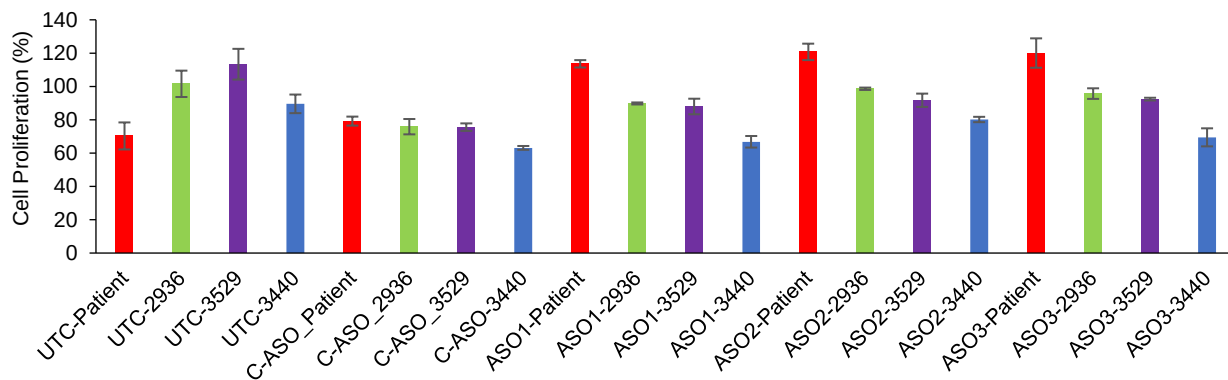
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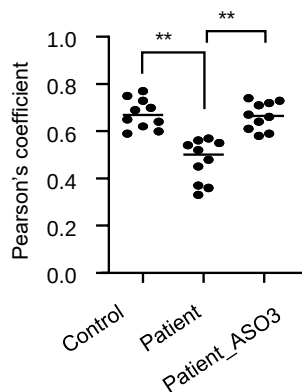
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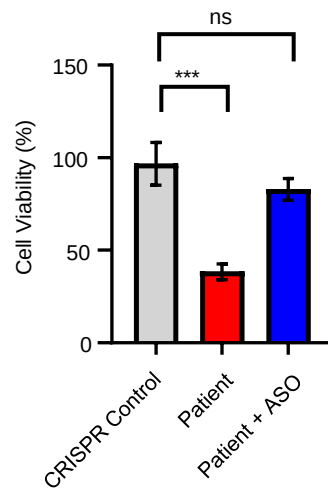
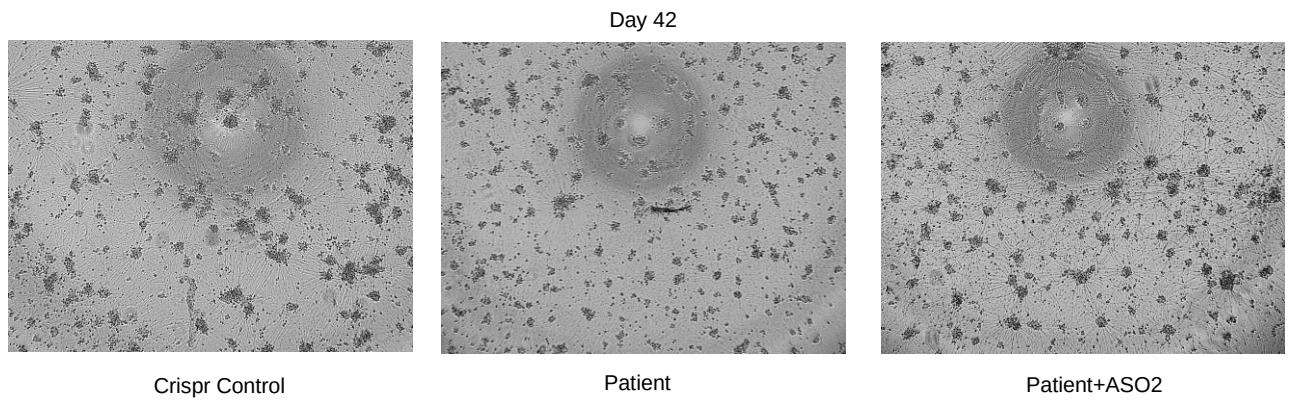
C



D



A



Supplementary Table 1. (PPSets) Taqman assays

Name	Target	Manufacturer	Cat#
MAPKrs2076431	MAPK8IP3 Wt/Mt	ThermoFisher Scientific	4351379
Hs00187715_m1	SPAG9	ThermoFisher Scientific	4331182
Mm01301203_m1	MAPK8I3 (Ms)	ThermoFisher Scientific	4331182
Hs01049862_m1	MAPK8IP3 (Human)	ThermoFisher Scientific	4331182
Hs00265245_s1	DRD1	ThermoFisher Scientific	4331182
Hs00241436_m1	DRD2	ThermoFisher Scientific	4331182
Hs.PT.39a.22214847	ACTB	Integrated DNA technologies	-
siJIP4	JIP4	Santa Cruz Biotechnology	sc-62514
Control siRNA-A	N/A	Santa Cruz Biotechnology	sc-37007

Table S1. List of Primer probe sets and siRNAs used in the study, their manufacturer and catalogue number

Supplementary Table 2. (Antibodies)

Antibody	Host	Manufacturer	Cat#
JIP3 Polyclonal antibody	Rb	Proteintech	25212-1-AP
Phospho-c-Jun (Ser63) (54B3)	Rb	Cell Signaling Technology	2361S
SAPK/JNK	Rb	Cell Signaling Technology	9252S
Phospho-c-Jun (Ser73) (D47G9)	Rb	Cell Signaling Technology	3270S
Phospho-SAPK/JNK (Thr183/Tyr185) (81E11)	Rb	Cell Signaling Technology	4668S
c-Jun (60A8)	Rb	Cell Signaling Technology	9165S
Rab5 (C8B1)	Rb	Cell Signaling Technology	3547S
Cleaved Caspase-3 (Asp175) (5A1E)	Rb	Cell Signaling Technology	9664S
Caspase-3 (D3R6Y)	Rb	Cell Signaling Technology	14220S
D1DR/Dopamine Receptor D1	Ms	Santa Cruz Biotechnology	sc-33660
D2DR/Dopamine D2 Receptor	Ms	Santa Cruz Biotechnology	sc-5303
JIP-4 Antibody (H-8)	Ms	Santa Cruz Biotechnology	sc-271492
Rab 7 Antibody (B-3)	Ms	Santa Cruz Biotechnology	sc-376362
JIP-3 Antibody (F-6):	Ms	Santa Cruz Biotechnology	sc-46663
GAPDH Antibody (G-9)	Ms	Santa Cruz Biotechnology	sc-365062
Ku80 Antibody (EPR3468)	Rb	Abcam	ab80592
Secondary Ab	Host	Manufacturer	Cat#
Alexa Fluor™ 647	Rb	Invitrogen	A21245
Alexa Fluor™ 488	Ms	Invitrogen	A11001
Alexa Fluor™ 488	Ms	Invitrogen	A21042
Goat Anti-Rabbit	Rb	BioRad	1706515
Goat Anti-Mouse	Ms	BioRad	1706516

light chain specific Goat Anti-Mouse	Ms	Jackson Immuno Research Lab	115-035-174
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Table S2. List of primary and secondary antibodies, host species, manufacturers and respective catalogue numbers.

Supplementary Table 3. (ASO)

ASO name	Target	Manufacturer	Sequence
1	MAPK8IP3	IDT	ACoAoGoAoTATATTTTAGCoGoAGT
2	MAPK8IP3	IDT	CCoAoGoToTTTTTCAGTAToToCCA
3	MAPK8IP3	IDT	ACoGoGoToCACATGGATGCoToCAC
Control	Non-targeting	IDT	CCoToAoTAGGACTATCCAoGoGoAA

Table S3. List of PS-ASOs and their respective targets and manufacturer details. Orange indicate 2'-MOE (2'-methoxyethyl) modifications; black represents a 2'-deoxyribosyl sugar moiety; 'o' means phosphodiester linkage; all other linkages are phosphorothioate.