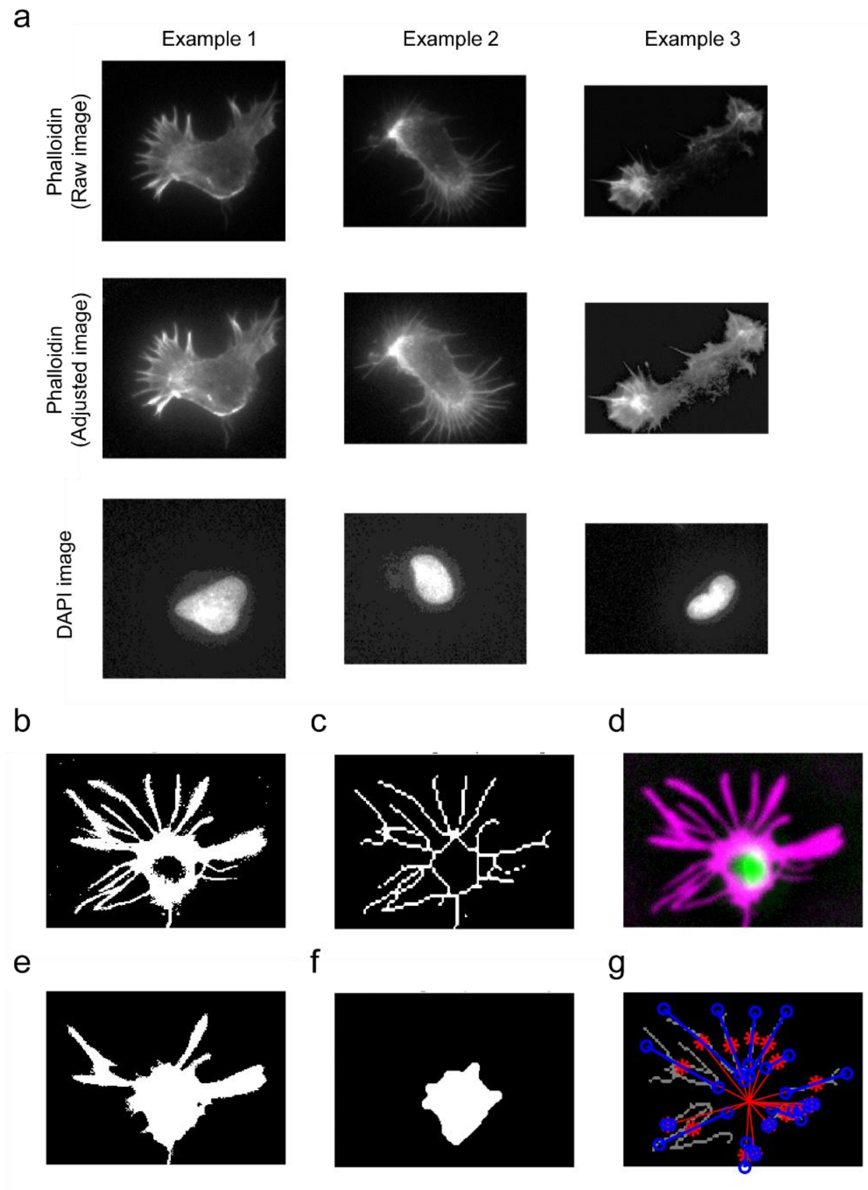
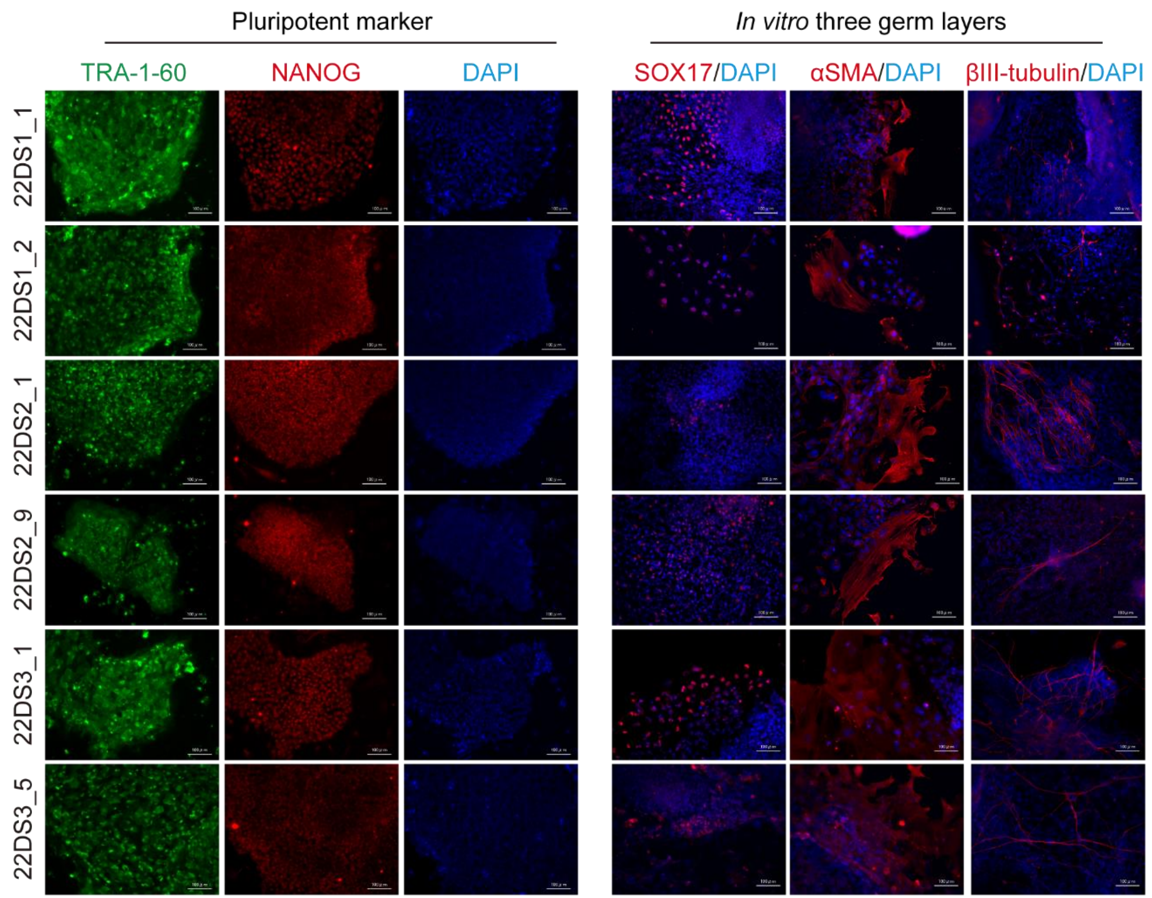
Supplementary Figures



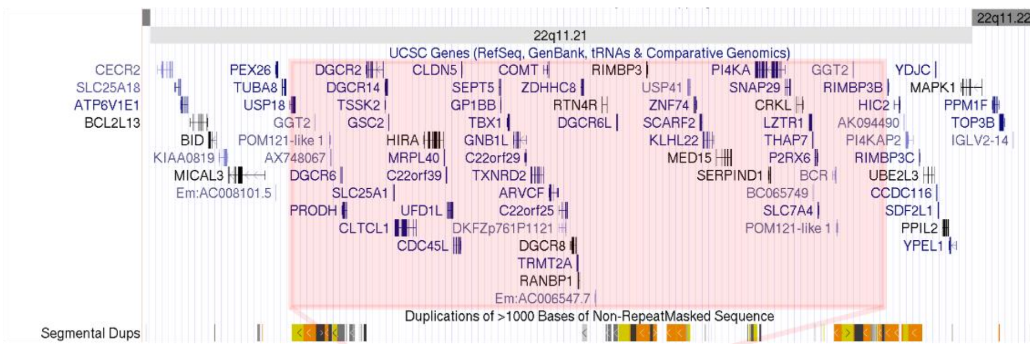
Supplementary Figure 1. Automatic detection of F-actin dynamics.

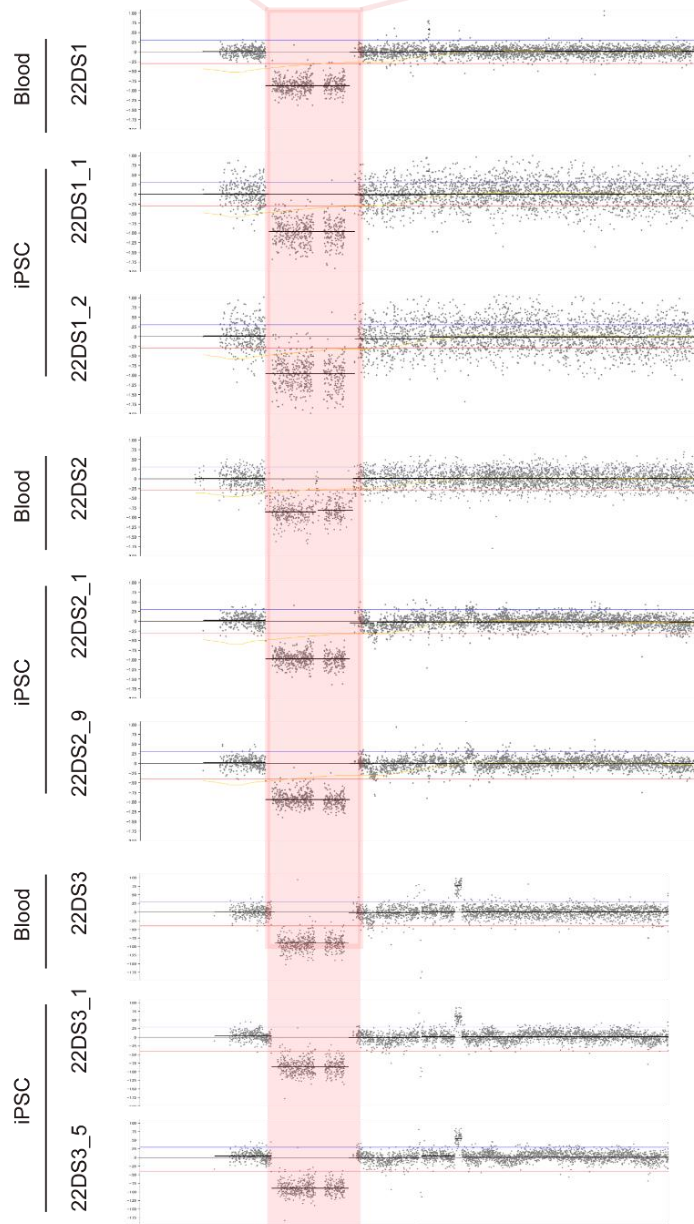
A series of automatically detected filopodia-like protuberances.



Supplementary Figure 2. Generation of iPSCs.

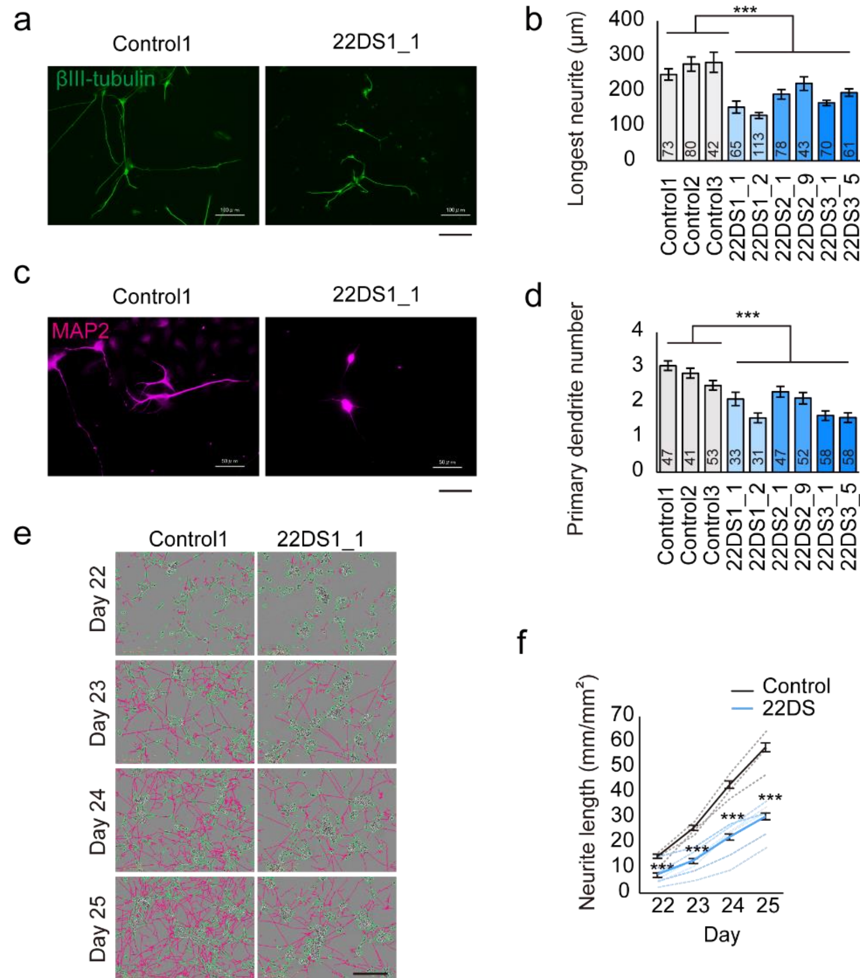
Left panel: Immunostaining for TRA-1-60 and NANOG in iPSC lines. *Right panel*: Evaluation of the capacity to differentiate into all three germ layers by SOX17 staining (endodermal marker), αSMA staining (mesodermal marker), and βIII-tubulin staining (ectodermal marker). The black scale bar under the images represents 100 μm.





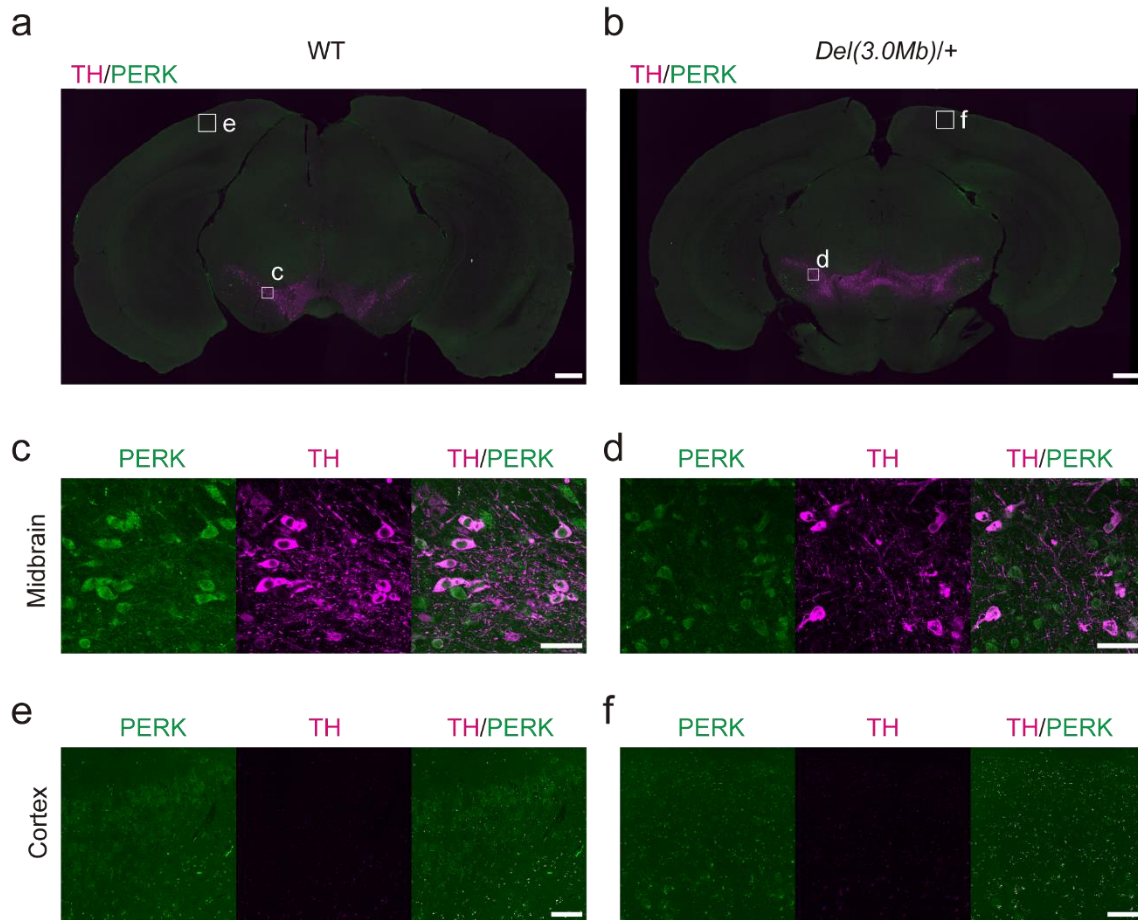
Supplementary Figure 3. Confirmation of the 22q11.2 deletion in patient-derived iPSCs.

Results of the aCGH analysis performed using 22q11.2DS patient-derived blood and iPSCs.



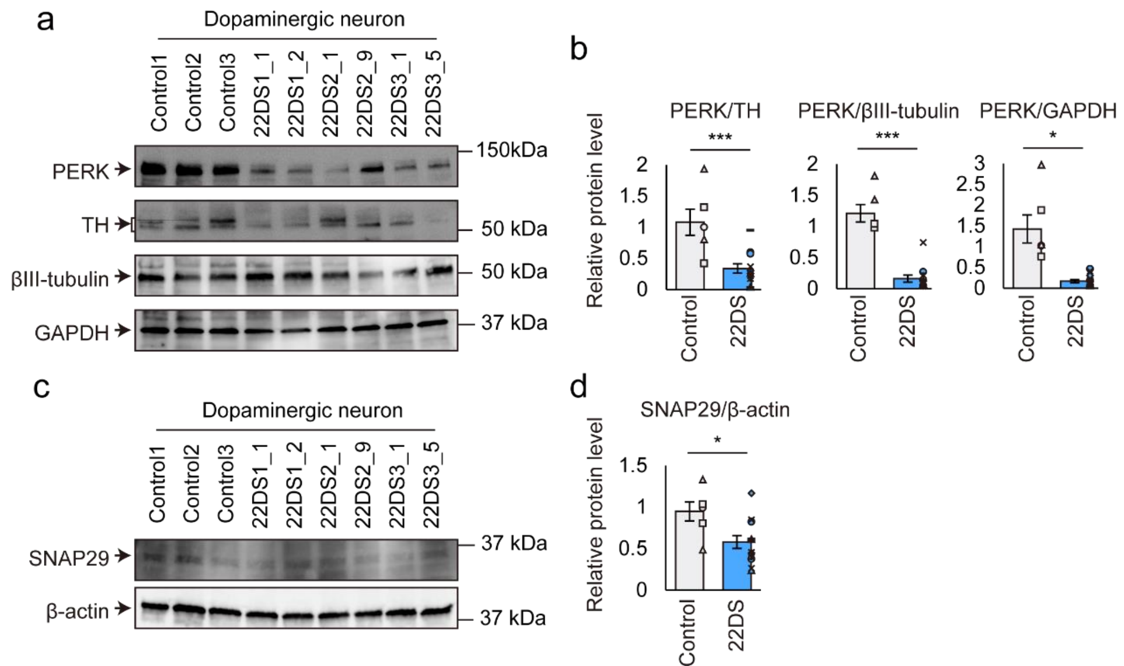
Supplementary Figure 4. Characterisation of 22q11.2DS iPSC-derived dopaminergic neuron development.

(a) Representative images of dopaminergic neurons (Day 24) immunostained for βIII-tubulin. The black scale bar under the images represents 100 µm. (b) Measurements of the longest neurites. The numbers indicated in the bars represent the number of counted cells. The bars represent means ± SEs. ***$P < 0{\cdot}001$. (c) Representative images of dopaminergic neurons (Day 28) immunostained for MAP2. The black scale bar under the images represents 50 µm. (d) Measurement of the number of MAP2 positive primary dendrites. The numbers indicated in bars represent the number of counted cells. The bars represent means ± SEs. ***$P < 0{\cdot}001$. (e) Results of the automatic detection of cell bodies and neurites using time-lapse analysis. Pink = neurites. Green = cell bodies. Images were merged with phase-contrast images. The black scale bar represents 200 µm. (f) Neurite length at each time point assessed using automatic detectors. The number of fields that were used was as follows: Control, n = 58 (Control1, n = 14; Control2, n = 24; Control3, n = 20), and 22DS, n = 90 (22DS1_1, n = 16; 22DS1_2, n = 18; 22DS2_1, n = 13; 22DS2_9, n = 13; 22DS3_1, n = 12; 22DS3_5, n = 18). Plots represent means ± SE. ***$P < 0{\cdot}001$ (Control group vs. 22DS group). Each dashed line represents the value of each line (Grey = Control, Pale blue = 22DS). The solid line represents the average of the group (Black = Control, Blue = 22DS).



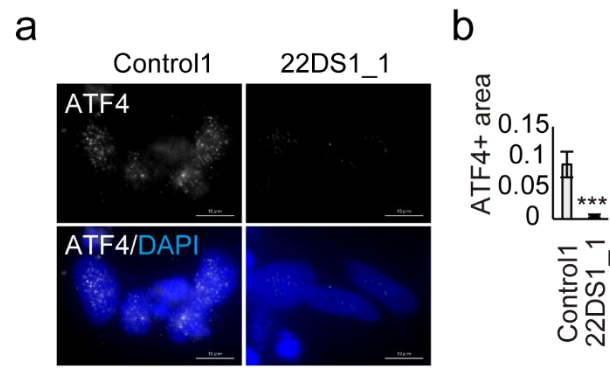
Supplementary Figure 5. PERK protein is expressed in TH-positive neurons of the *Del(3·0Mb)*/+ mouse brain.

(a–b) Low-magnification image of a brain slice that includes the TH-positive region (substantia nigra and ventral tegmental area) from a WT-littermate (a) and *Del(3·0Mb)*/+ mouse (b). Scale bars in images indicate 500 µm. (c–f) Magnification image of substantia nigra from a WT-littermate (c) and *Del(3·0Mb)*/+ mouse (d) or cerebral cortex from a WT-littermate (e) and *Del(3·0Mb)*/+ mouse (f). Scale bar in images indicate 50 µm.



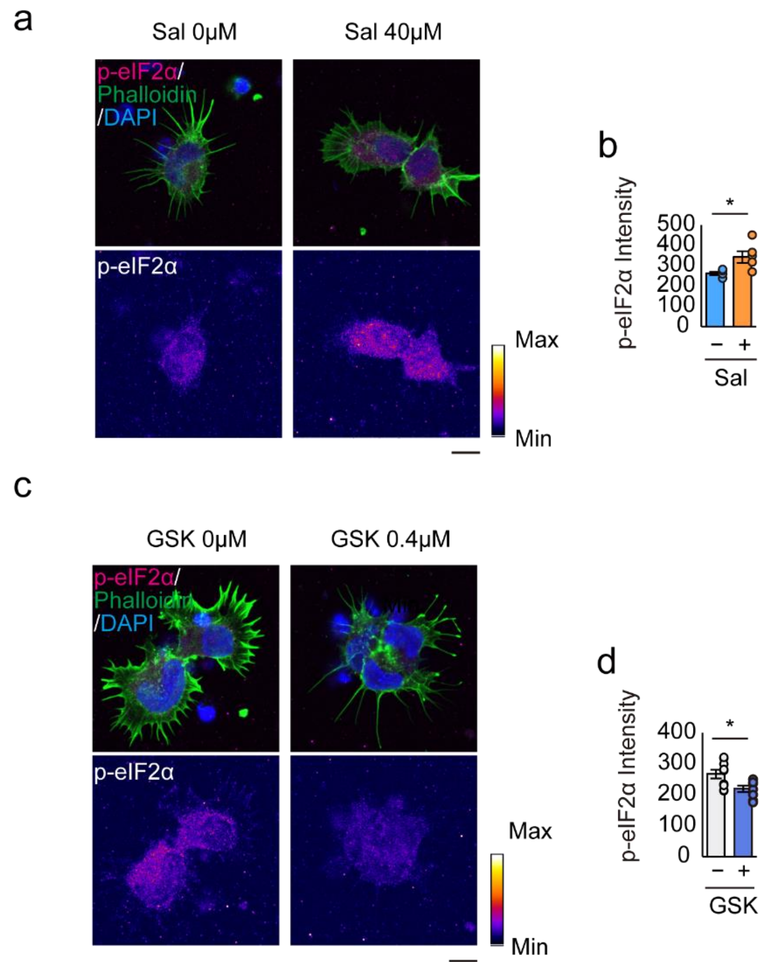
Supplementary Figure 6. Comparison of the results of 22DS model mouse brain with iPSC-derived dopaminergic neurons.

(a) Immunoblotting for PERK, TH, βIII-tubulin and GAPDH using proteins extracted from dopaminergic neurons at Day 24. (b) Quantification of the PERK/TH, PERK/βIII-tubulin and PERK/GAPDH ratios. Two independent experiments were performed (Control: n = 6; 22DS: n = 12). The value of Control1 is 1. Each plot represents the value of each line. Bars represent means ± SEs. $*P < 0·05$, $***P < 0·001$. (c) Immunoblotting for SNAP29 and β-actin using proteins extracted from dopaminergic neurons at Day 24. (d) Quantification of the SNAP29/β-actin ratio. Two independent experiments were performed (Control: n = 6; 22DS: n = 12). The value of Control1 is 1. Each plot represents the value of each line. The bars represent means ± SEs. $*P < 0·05$



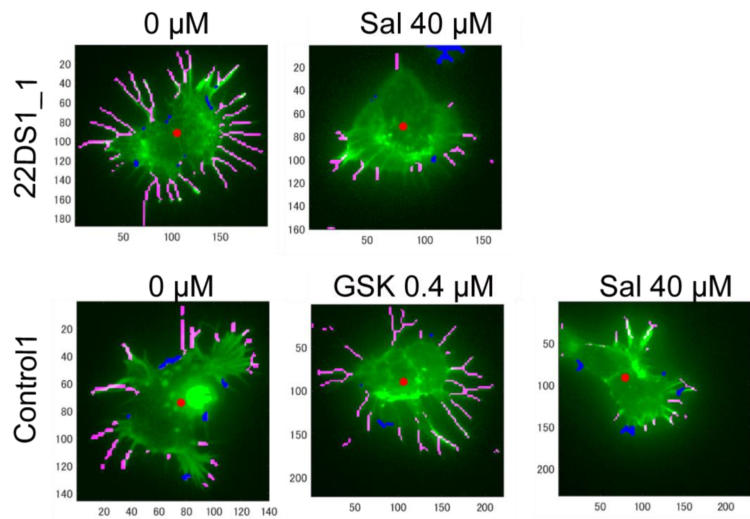
Supplementary Figure 7. Low activity of PERK pathway in the 22DS group under ER stress.

(a) Representative images of dopaminergic neurons immunostained for ATF4 at Day 24 after TCM treatment (1 μg/ml). The black scale bar under the images represents 10 μm. (b) Quantification of the ATF4-positive area/DAPI area ratio. Control1, n = 9; 22DS1_1, n = 5. The bars represent means ± SEs. ****P* < 0·01

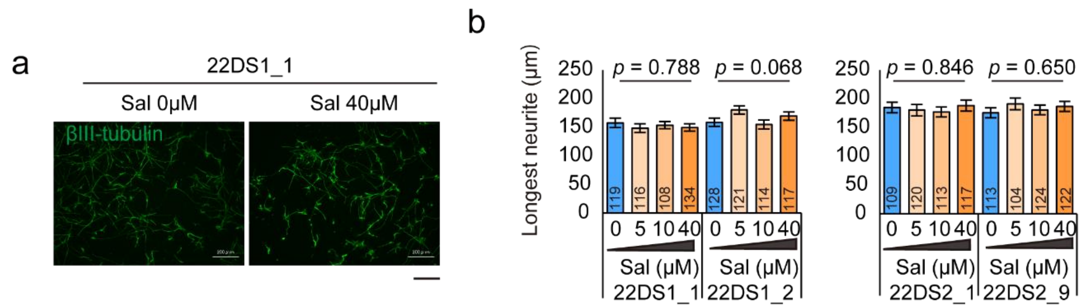


Supplementary Figure 8. Pharmacological manipulation of the PERK pathway.

(a) *Upper panel*: Representative images of cells at Day 22 stained with phalloidin and immunostained for p-eIF2α, with or without salubrinal (Sal) in the 22DS group. *Lower panel*: Fluorescence intensity of p-eIF2α. The black scale bar under the images represents 10 μm. (b) Quantification of the intensity of the p-eIF2α signal. Each plot represents the value of each line. The bars represent means ± SEs. $*P < 0{\cdot}05$. Five fields were used. (c) *Upper panel*: Representative images of cells at Day 22 stained with phalloidin and immunostained for p-eIF2α, with or without GSK2656157 (GSK) in the control group. The black scale bar under the images represents 10 μm. *Lower panel*: Fluorescence intensity of p-eIF2α. (d) Quantification of the intensity of the p-eIF2α signal. Each plot represents the value of each line. The bars represent means ± SEs. $*P < 0{\cdot}05$. The number of fields used was as follows: GSK 0 μM, n = 9; and GSK 0·4 μM, n = 8.

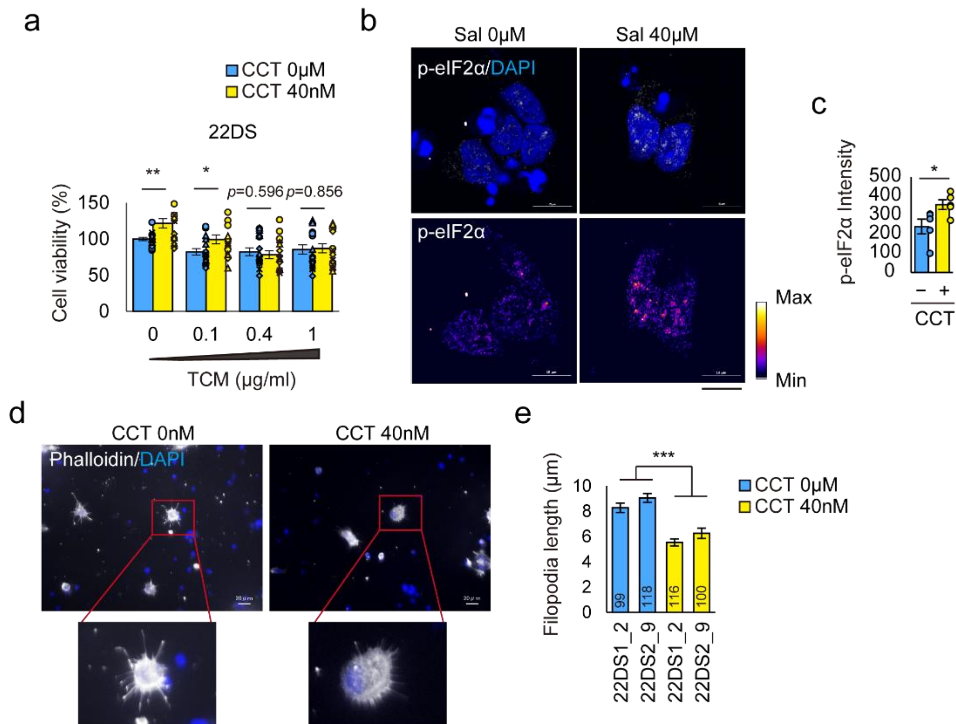


Supplementary Figure 9. Effects of salubrinal or GSK2656157 treatment on F-actin dynamics.
Representative images of F-actin dynamics assessed using an automatic detection system. Sal, salubrinal (40 μM); GSK, GSK2656157 (0·4 μM). The blue lines were out of evaluation.



Supplementary Figure 10. Effects of salubrinal treatment on neurite length.

(A) Representative images of 22DS1_1-derived dopaminergic neurons (Day 24) with or without Sal, immunostained for βIII-tubulin. The black scale bar under the images represents 200 μm. (B) Measurement of the neurite length. The numbers indicated in bars represent the number of counted cells. The bars represent means ± SEs.



Supplementary Figure 11. Effects of PERK activator, CCT020312, treatment on cell viability to ER stress and filopodia length in the 22DS group.

(a) Cell viability with or without CCT020312 (CCT) in the presence of TCM. n = 16 (22DS1_1, n = 4; 22DS1_2, n = 4; 22DS2_1, n = 4; 22DS2_9, n = 4). Each plot represents the value of each line. Bars represent means ± SEs. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$. (b) *Upper panel*: Representative images of cells at Day 22 immunostained for p-eIF2α, with or without CCT 40 nM in the 22DS group. *Lower panel*: Fluorescence intensity of p-eIF2α. The black scale bar under the images = 10 μm. (c) Quantification of the intensity of the p-eIF2α signal. Each plot represents the value of each field. Bars represent means ± SEs. *$P < 0{\cdot}05$. Five fields were used. (d) Representative images of cells stained with phalloidin at Day 22 with or without CCT (40 nM) in the 22DS group. The scale bar in the images = 20 μm. (e) Measurement of filopodium length. The numbers indicated in the bars represent the number of counted filopodia. The bars represent means ± SEs. ***$P < 0{\cdot}001$.