Figure S1. Site-specific integration of a single copy of EGFP-WT-hTorsin1a or EGFP-ΔE-hTorsin1a cDNA into Flp-In 293 T-REx cells under control of a tetracycline-sensitive transcriptional repressor. *Relates to main text Figure 1.*

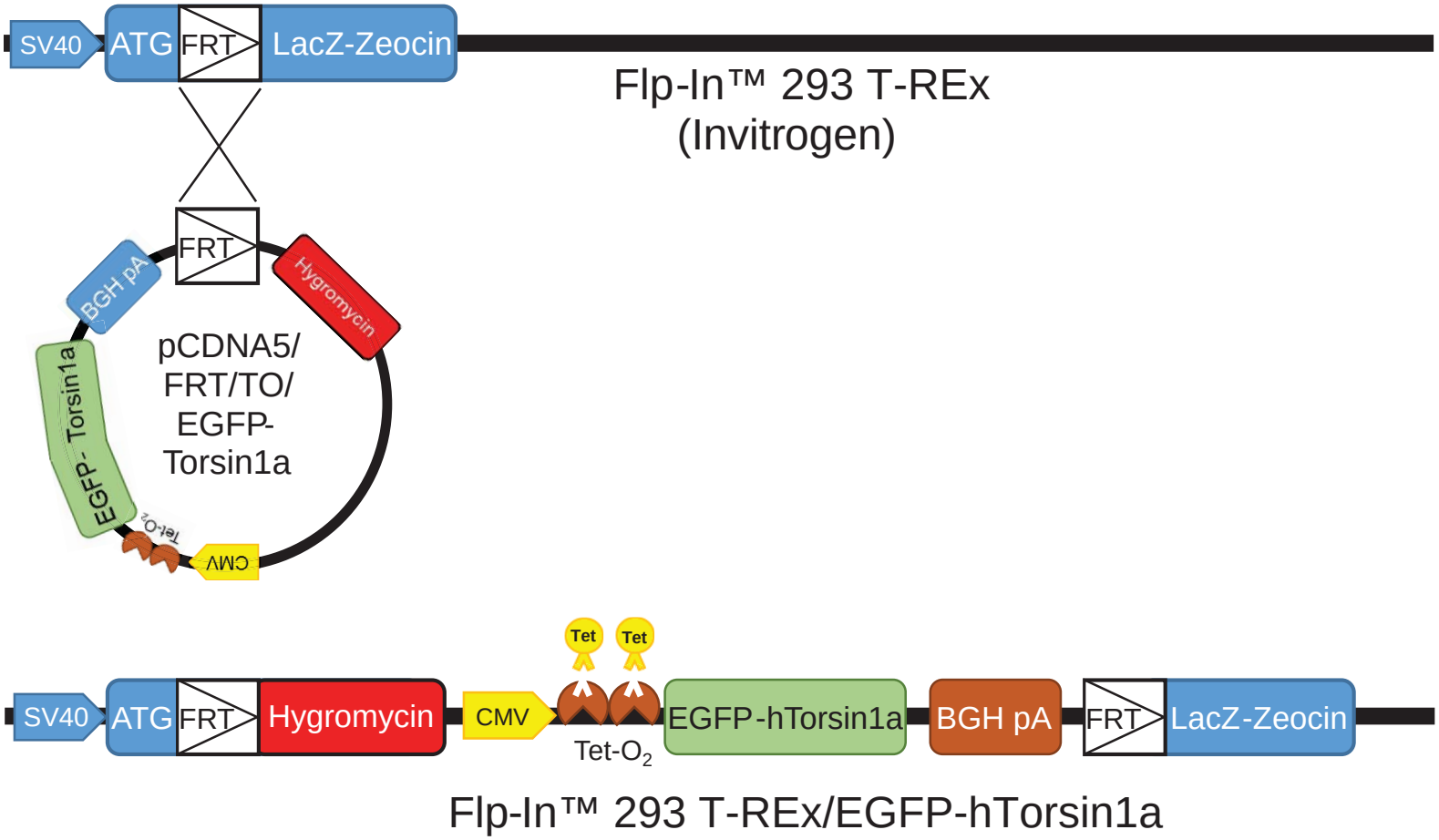


Figure S2. High-content image analysis and assay performance under high-throughput screening conditions.

Relates to main text Figure 1.

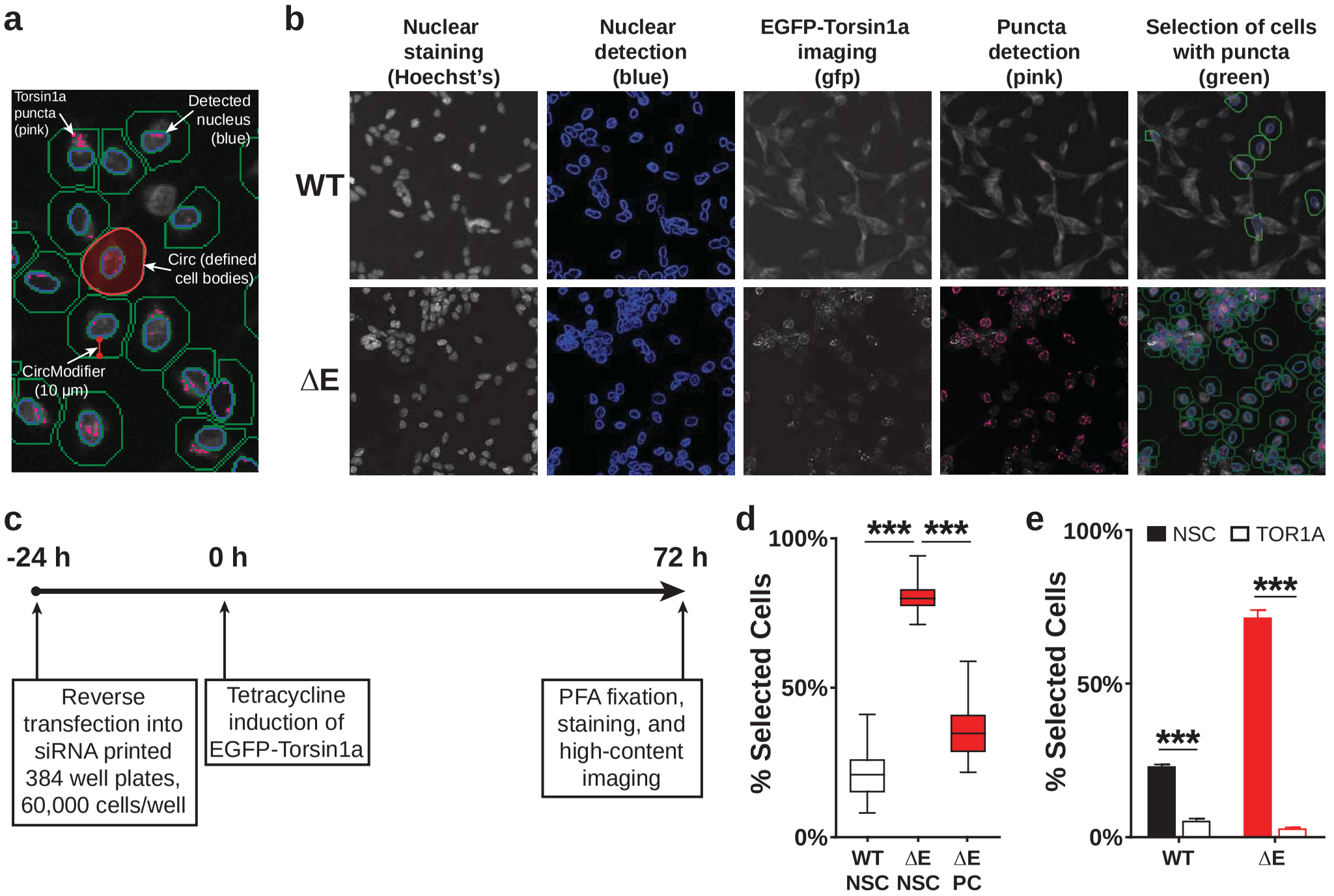
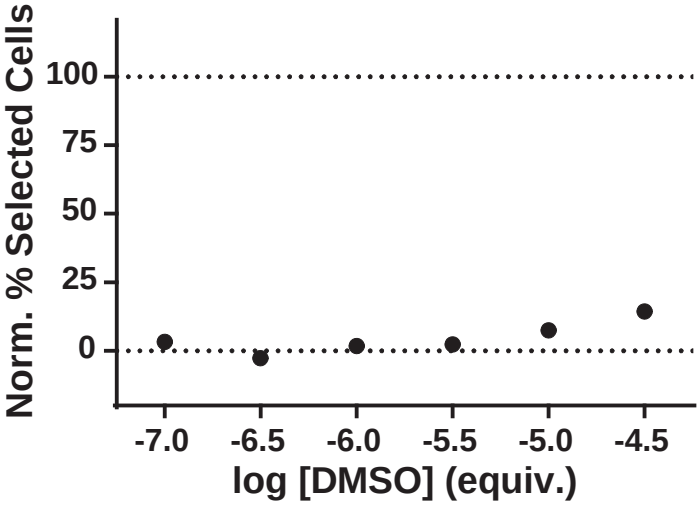


Figure S3. DMSO vehicle control has no effect on WT Torsin1a localization or cell count.
Relates to main text Figure 2.



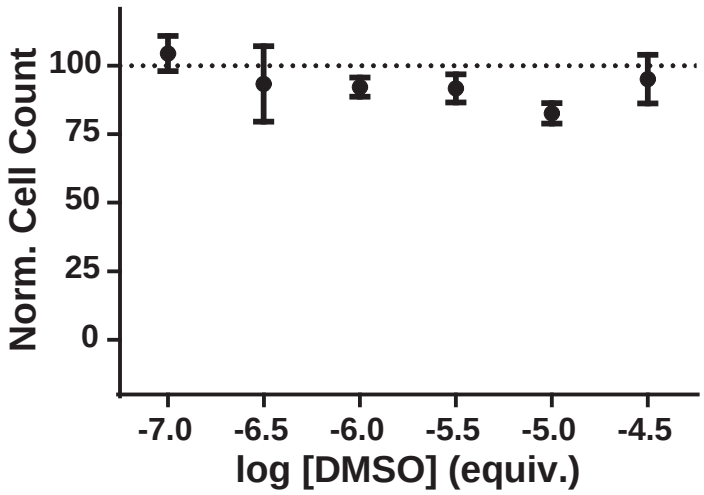
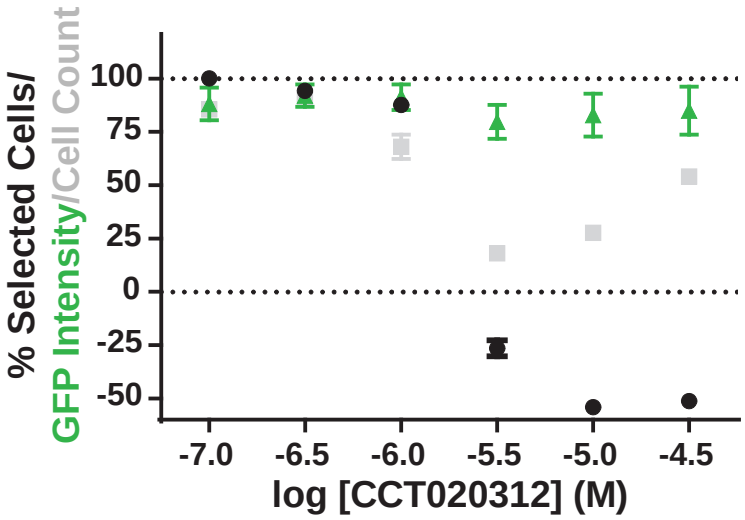
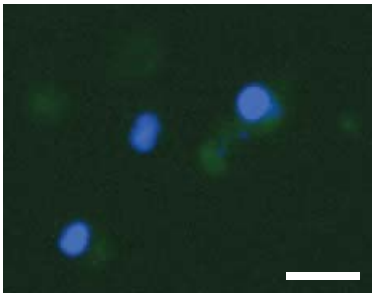


Figure S4. Cytotoxic effects of the direct eIF2α pathway activator CCT020312.

Relates to main text Figure 2.





ΔE Torsin1a +
10 μM CCT020312

Figure S5. Effects of additional compounds targeting the Notch and Glucocorticoid pathways on the ΔE Torsin1a cell line. *Relates to main text Figure 2.*

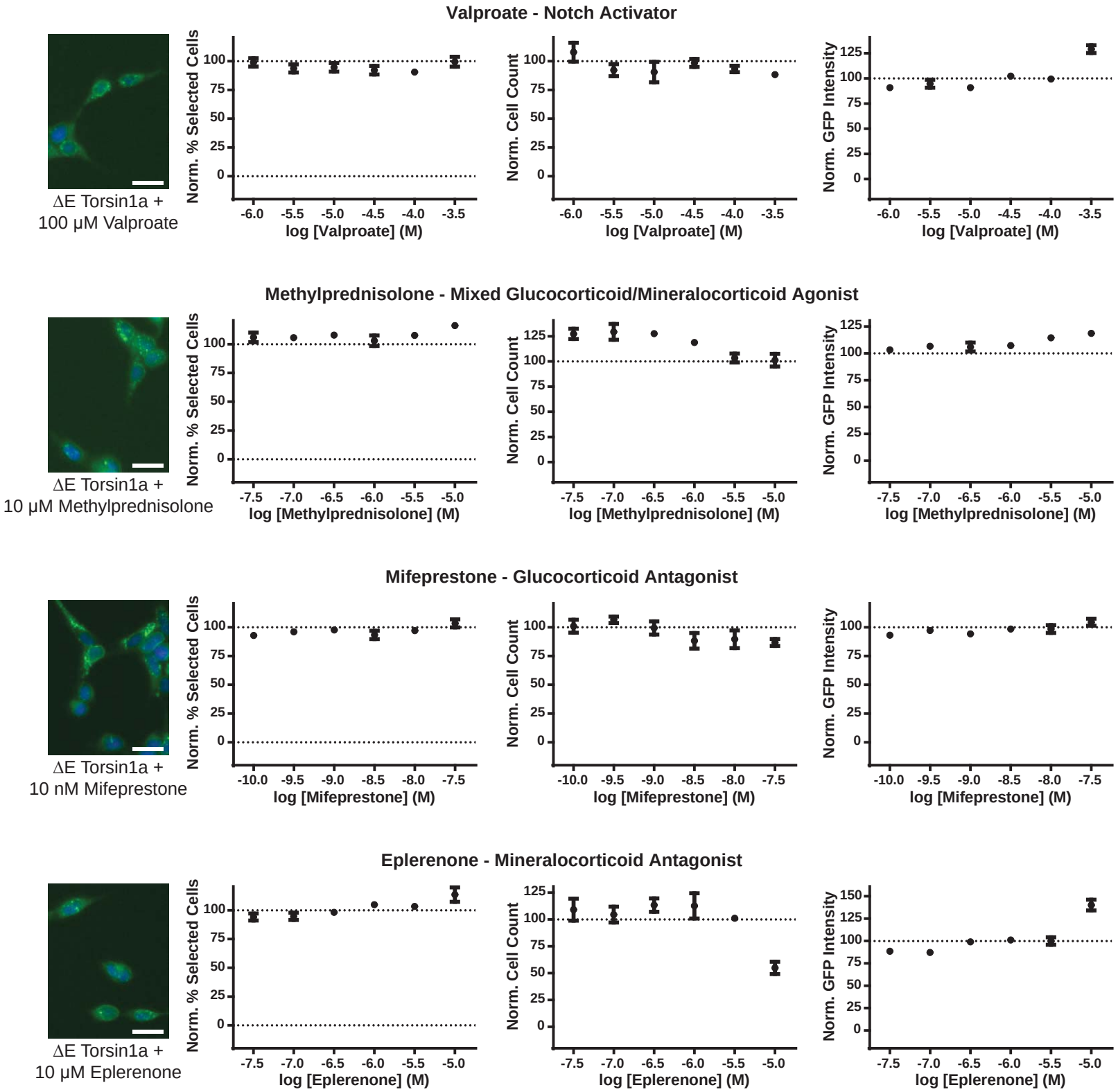


Figure S6. Effects of additional compounds targeting the ATF6 and NF-κB pathways on the ΔE Torsin1a cell line. *Relates to main text Figure 2.*

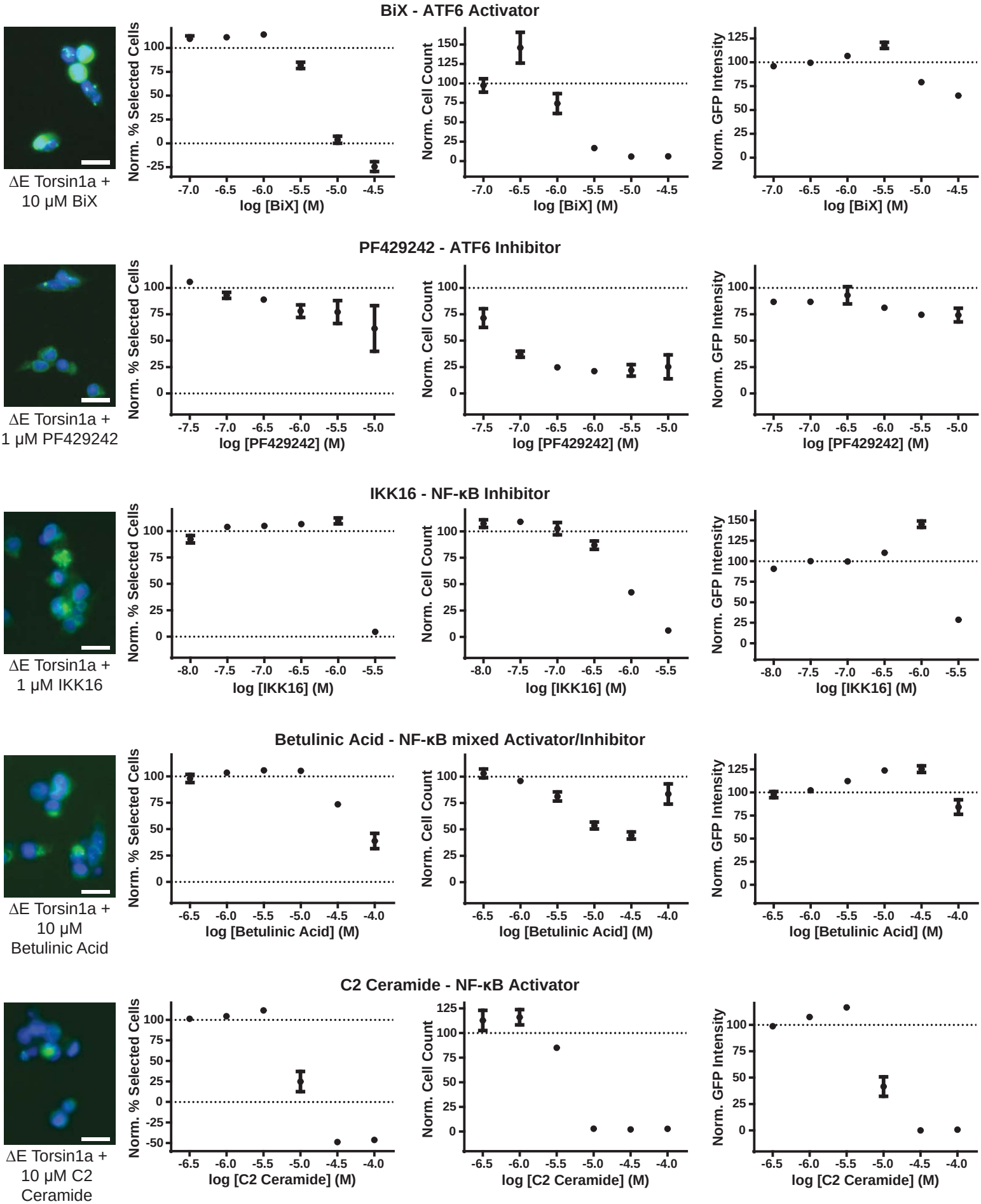


Figure S7. ATF4 residues D35 and Y37 highly are conserved in mammals.

Relates to main text Figure 6.

Supplemental Figure Legends

Figure S1. Site-specific integration of a single copy of EGFP-WT-hTorsin1a or EGFP-ΔE-hTorsin1a cDNA into Flp-In 293 T-REx cells under control of a tetracycline-sensitive transcriptional repressor. Schematic displaying the targeting vector and site-specific mediated integration of either WT or ΔE EGFP-hTOR1A cDNA into Flp-In 293 T-REx cells.

Figure S2. High-content image analysis and assay performance under high-throughput screening conditions. (a, b) Automated image analysis and determination of EGFP-Torsin1a localization using Cellomics compartmentalization protocol software. (a) Representative image of algorithm parameters. *Blue circles* – nucleus as detected by Hoechst's staining of genomic DNA. *Red bar* – CircModifier; the arbitrary distance from the nucleus utilized to define the outer perimeter of cell bodies. *Pink* – EGFP-Torsin1a puncta recognized according to punctate object parameters. *Green circles* – assigned cell body perimeter. (b) Representative images of algorithm function on WT and ΔE assay cell lines. Steps 1 and 2 – Hoechst's nuclear staining determines viable cells by masking intact nuclei. Steps 3 and 4 – EGFP signal is used to identify Torsin1a localized to puncta (pink overlay). Step 5 – Cells containing at least 1 EGFP-Torsin1a puncta are selected (green circles). Assay output (% selected cells) is the percentage of cells that are identified with puncta. (c) Time course of the high-throughput high-content RNAi screening assay. (d) The percentage of selected cells was robustly higher in the ΔE compared to WT Torsin1a assay cells (Z' = 0.614 ± 0.077), and treatment with a Positive Control (PC) siRNA reliably led to a marked reduction in the percent of selected cells. n = 96 independent wells per condition. ***, $p < 0.0005$ by unpaired t test. (e) As predicted, TOR1A siRNA mediated silencing of assay readout protein (EGFP-Torsin1a) reduces the percent of selected cells to near zero. n = 16 independent wells treated with non-silencing siRNA control and 32 independent wells treated with *TOR1A* siRNA (Qiagen #s SI00002198, SI00002212, SI00002205, SI03114342). ***, $p < 0.0005$ by unpaired t test. Data in (d) presented as box-and-whisker plot displaying 90% confidence interval, data in (e) presented as means ± S.E.M.

Figure S3. DMSO vehicle control has no effect on WT Torsin1a localization or cell count. Effects of DMSO on Torsin1a localization and cell count in the WT assay cell line. Range of Torsin1a localization effect was normalized to the percentage of cells with puncta in vehicle-treated ΔE cell line as the maximum and that in WT cell line as the minimum. Cell count was normalized to the vehicle-treated WT cell line as the maximum. All dose response data are the average of 4 independent experiments per dose. Untreated control data used for normalization are the average of 24 independent experiments. All data are presented as means ± S.E.M.

Figure S4. Cytotoxic effects of the direct eIF2α pathway activator CCT020312. *Left* – Effects of CCT020312 on Torsin1a localization (*black*), cell count (*grey*), and EGFP-Torsin1a expression (*green*) in the ΔE assay cell line. Range of Torsin1a localization effect was normalized to the percentage of cells with puncta in vehicle-treated ΔE cell line as the maximum and that in WT cell line as the minimum. Cell count and EGFP-Torsin1a expression were normalized to their respective values in the vehicle-treated ΔE cell line as the maximum. *Right* – Representative image from CCT020312 treatment. Scale bar = 20 μm. All dose response data are the average of 4 independent experiments per dose. Untreated control data used for normalization are the average of 24 independent experiments. All data are presented as means ± S.E.M.

Figure S5. Effects of additional compounds targeting the Notch and Glucocorticoid pathways on the ΔE Torsin1a cell line. Effects of the indicated compounds on Torsin1a localization (% Selected Cells), cell count, and EGFP-Torsin1a expression (GFP Intensity) in the ΔE assay cell line. Range of Torsin1a localization effect was normalized to the percentage of cells with puncta in vehicle-treated ΔE cell line as the maximum and that in WT cell line as the minimum. Cell count and EGFP-Torsin1a expression were normalized to their respective values in the vehicle-treated ΔE cell line as the maximum. *Left* – Representative images from the respective treatments. Scale bars = 20 μm. All dose response data are the average of 4 independent experiments per dose. Untreated control data used for normalization are the average of 24 independent experiments. All data are presented as means ± S.E.M.

Figure S6. Effects of compounds targeting the ATF6 and NF-κB pathways on the ΔE Torsin1a cell line. Effects of the indicated compounds on Torsin1a localization (% Selected Cells), cell count, and EGFP-Torsin1a expression (GFP Intensity) in the ΔE assay cell line. Range of Torsin1a localization effect was normalized to the percentage of cells with puncta in vehicle-treated ΔE cell line as the maximum and that in WT cell line as the minimum. Cell count and EGFP-Torsin1a expression were normalized to their respective values in the vehicle-treated ΔE cell line as the maximum. *Left* – Representative images from the respective treatments. Scale bars = 20 μm. All dose response data are the average of 4 independent experiments per dose. Untreated control data used for normalization are the average of 24 independent experiments. All data are presented as means ± S.E.M.

Figure S7. ATF4 residues D35 and Y37 are highly conserved in mammals. ATF4 sequence alignment displaying the high rate of conservation of residues D35 and Y37 among mammalian species. Residue 35 is an aspartic acid in all annotated mammalian species. Residue 37 is a tyrosine in all annotated mammalian species except *Bos Taurus*.

Table S1. Gene hits used for pathway analysis. *Relates to main text Table 1.*

HUGO Gene Symbol	***Entrez Gene ID***
AASS	10157
ACOT13	55856
AGGF1	55109
ANXA3	306
ARHGEF11	9826
ATG9A	79065
ATP13A1	57130
ATP5A1	498
BDP1	55814
BOP1	23246
C16orf58	64755
C5orf44	80006
CCDC86	79080
CCZ1	51622
CDCA4	55038
CEBPD	1052
CEP78	84131
CLEC4A	50856
CNOT6L	246175
COASY	80347
CTRB1	1504
DDX24	57062
DHX15	1665
DIXDC1	85458
DLL3	10683
DNM1	1759
DUSP5	1847
EPDR1	54749
ERMP1	79956
ERO1LB	56605
EXOSC1	51013
FAM92B	339145
FLRT2	23768

HUGO Gene Symbol	***Entrez Gene ID***
GOLGA1	2800
GP1BB	2812
HERC2P9	440248
HHEX	3087
HIGD2B	123346
HNRPUL1	11100
JPH1	56704
KIAA1257	57501
KMT2A	4297
LOC285033	285033
LOC286186	286186
LOC441204	442519
MAML2	84441
MERTK	10461
MRC2	9902
NGLY1	55768
NTPCR	84284
P4HTM	54681
PBX2	5089
PCDHGB3	56102
PHACTR3	116154
POLR2E	5434
POLR2K	5440
PPP1R16A	84988
PRRG1	5638
PSMD4	5710
PTF1A	256297
PVT1	5820
RBM3	5935
RPAP3	79657
RPL10A	4736
RPL10L	140801
RPL11	6135

HUGO Gene Symbol	***Entrez Gene ID***
RPL12	6136
RPL39	6170
RPL5	6125
RPL6	6128
RPUSD4	84881
SCD	6319
SH2D3C	10044
SPANXC	728712
SPDYE4	388333
SPPL2A	84888
SPTLC1	10558
SRGAP3	9901
SRP19	6728
SRP68	6730
SRPR	6734
SSBP2	23635
TAF15	8148
TGFB1	7040
TMEM258	746
TRAPPC3	27095
UBE2I	7329
UGT8	7368
UNG	7374
WBSCR22	114049
WIBG	84305
XAGE1D	9503
YIPF3	25844

Table S2. Bioinformatic analysis of ≥ 2 S.D. rescuers. *Relates to main text Table 1.*

	Ingenuity Canonical Pathways	***p-value***
1	EIF2 Signaling (10)	0.000033
2	Notch Signaling (3)	0.0078
3	Assembly of RNA Polymerase III Complex (2)	0.0083
4	Coenzyme A Biosynthesis (2)	0.0096
5	Assembly of RNA Polymerase II Complex (3)	0.016
6	Protein Ubiquitination Pathway (7)	0.022
7	Glucocorticoid Receptor Signaling (7)	0.025
8	Cell Cycle: G1/S Checkpoint Regulation (3)	0.029
9	Gαi Signaling (4)	0.046
10	Estrogen Receptor Signaling (4)	0.048

Table S3. Whole exome sequencing cohort patient characteristics. *Relates to main text Figure 6.*

Gender	
Men	3
Women	17
Age of onset	
Mean (SD)	47.4 (±9.6)
Site of onset	
Arm	6
Leg	1
Cranial	3
Cervical	13
Site at examination	
Arm	6
Leg	1
Cranial	3
Cervical	13
Distribution	
Focal	6
Segmental	11
Multifocal	3
Family History	
Negative	16
Positive	2
Possible	2
Not Informed	0

Table S4. *ATF4* validation cohort patient characteristics. *Relates to main text Figure 6.*

Variable	MGH[a]	Coriell[b]
Race		
Caucasian	101	126
Other	1	8
Unknown		3
Gender		
M	28	37
F	74	100
Age of Onset		
Mean (SD)	42.1 (±15.9)	48.6 (±11.3)
Median (Range)	44 (3-73)	50 (21-75)

[a]From Mt. Sinai/MGH collections.
[b]From the NINDS Human Genetics DNA Repository

Table S5. Enrichment of rare ATF4 variants in human sporadic cervical dystonia patients. *Relates to main text Figure 6.*

ATF4 Variant	Impact	SNP	Variant Carriers		Variant Allele Freq.		Fold Enrichment	SIFT		Polyphen2	
			MGH[a]	Coriell[b]	Dystonia	Control[c]		Score	Prediction	Score	Prediction
Rare											
c.1A>G	p.Met1Val	rs201201452	0/102	1/137	0.21%	0.0032%	65.6	0.01	deleterious	0.958	probably damaging
c.103G>A	p.Asp35Asn	rs780640405	2/102	1/137	0.63%	0%	N/A	0.03	deleterious	0.972	probably damaging
c.103G>T	p.Asp35Tyr	N/A	1/102	0/137	0.21%	Novel	N/A	N/A		0.994	probably damaging
c.137C>T	p.Pro46Leu	rs111719524	2/102	1/137	0.63%	0.2%	3.2	0.01	deleterious	0.511	possibly damaging
Common											
c.65A>C	p.Gln22Pro	rs4894	53/102	71/137	31.20%	30.70%	1.02	0.25	tolerated	0.004	benign

[a]From Mt. Sinai/MGH collections.
[b]From the NINDS Human Genetics DNA Repository.
[c]From the ExAC Exome Aggregation Consortium (exac.broadinstitute.org), cases of European ancestry only.

Supplemental Experimental Procedures

Cell lines and cell culture

To limit variation in expression levels associated with random integration or transient expression, single copies of either WT or ΔE human *TOR1A* cDNAs with N-terminal EGFP fusions (courtesy of Dr. W. Dauer, U. Michigan) were inserted at the FRT site in Flp-In™ T-REx™ 293 cells (Thermo Fisher #R780-07, RRID:CVCL_U427) via flippase recombinase-mediated cassette exchange according to the manufacturer's recommended protocols (Figure S1). This system includes an inducible expression feature (TetON) that avoids selective pressure for potential toxic effects of chronic expression of mutant proteins. Flp-In T-REx 293 cells inducibly expressing either WT or ΔE Torsin1a were maintained in selective media [DMEM-HG (Thermo Fisher Scientific #11965) + 1x GlutaMax (Thermo Fisher Scientific #35050-061) + 75 µg/mL hygromycin (Thermo Fisher Scientific #10687-010) + 15 µg/mL blasticidin S (Thermo Fisher Scientific #R210-01) + 10% tetracycline screened FBS (Hyclone #SH30070.03T) + 1% penicillin/streptomycin/amphotericin (Mediatech Inc. #30-004-Cl)]. The cells were maintained at 37°C/5% CO_2. All experiments were performed on cells with fewer than 5 passages. HEK 293T cells (ATCC #CRL-3216, RRID:CVCL_0063) were maintained in HEK-T media [DMEM (Thermo Fisher #11995) + 10% FBS (Hyclone #SH30070.03) + 1x GlutaMAX + 1% penicillin/streptomycin/amphotericin] at 37°C/5% CO_2. Human dermal fibroblast lines were maintained in FGM media [MEM (Thermo Fisher #11095-080) + 15% FBS (Hyclone #SH30070.03) + 1% Non-Essential Amino Acids (Lonza #13-114E) + 1% penicillin/streptomycin/amphotericin] at 37°C/5% CO_2.

Torsin1a localization assay

Whole-genome RNAi Screen

The genome-wide RNAi screen on Flp-In T-REx 293 cells inducibly expressing either EGFP-WT or EGFP-ΔE Torsin1a was performed at the Duke University RNAi screening facility (Durham, NC). Pairs of library or control siRNAs (see Supplemental Experimental Procedures) were reverse transfected as earlier described (Barrows et al. 2010). Flp-In T-REx 293 WT or ΔE Torsin1a cells were dispensed at 3,000 cells/well in 50 µL assay media [DMEM (Thermo Fisher) + 1% tetracycline-screened FBS (Hyclone) + 1x GlutaMAX (Thermo Fisher) + 1% penicillin/streptomycin/amphotericin (Corning)] using a Matrix WellMate., and incubated at 37°C/5% CO_2 overnight. The following day, 15 µL assay media + 26.7 µg/mL tetracycline (final tetracycline concentration: 5µg/mL) was added to all wells except the uninduced control. Seventy-two hours after induction, cells were fixed with 4% PFA (Sigma) in PBS, permeabilized in PBS + 0.5% Triton X-100 (Sigma), and stained with Hoechst 33342 nuclear dye (Sigma; 13.3 µg/mL in PBS). Plates were then sealed and imaged on a Cellomics ArrayScan automated high-content imaging system.

High-content screening image analysis

The image analysis algorithm was developed using the Cellomics ArrayScan V CompartmentAnalysisV2 protocol. Hoechst's nuclear staining (Ch1: excitation 350 nm; emission 461 nm) was used for focusing and for cell identification by nuclear size, shape, and intensity. Automated exposure times for each plate were determined by setting a target saturation of 25% in channel 1 in wells containing tetracycline-induced ΔE cells. Detected cells were gated according to nuclear area, length-to-width ratio, and perimeter-to-area ratio. For the purpose of analysis, cell bodies were defined by a radius of 10 µm from the outer edge of the defined nucleus. Overlapping cells were automatically segmented according to the Cellomics ArrayScanV CompartmentAnalysisV2 object segmentation feature. EGFP fluorescence (Ch2: excitation 488 nm; emission 509 nm) was used for puncta detection. Automated exposure times for each plate were determined by setting a target saturation of 35% in channel 2 in wells containing induced ΔE cells. Torsin1a puncta were identified using the spot detection feature of the Compartmental AnalysisV2 protocol. The percentage of cells containing ≥ 1 puncta was used as the primary metric in this assay. As a technical control for the specificity of the signal, we confirmed that RNAi targeting Torsin1a eliminated inclusions (Figure S2E).

Pharmacological experiments

WT or ΔE Torsin1a Flp-In T-REx 293 cells were plated in clear-bottom 384-well plates at 3,000 cells/well in 30 µL assay media and incubated at 37°C/5% CO_2 overnight. The following day, serial drug dilutions were prepared at 2x final concentration in assay media containing 10 µg/mL tetracycline and 30 µL of the drug/tetracycline mixture was added to the appropriate wells, while control wells received 30 µL of assay media alone or assay media + 10 µg/mL

tetracycline (final tetracycline concentration for all wells except negative controls: 5 µg/mL). Cells were then incubated at 37°C/5% CO_2 for 48 hours and fixed/imaged as described above.

ATF4 overexpression

WT or ΔE Torsin1a Flp-In T-REx 293 cells were plated as described above. The following day, cells were transfected with empty vector or pRK/FLAG-ATF4 using OptiMEM/Lipofectamine2000 according to the manufacturer's instructions (56 ng DNA/well, 10 µL total volume/well). 4 hours later, 20 µL of assay media with 15 µg/mL tetracycline was added to each well (5 µg/mL final concentration), and cells were incubated at 37°C/5% CO_2 for 48 hours. Cells were then fixed and imaged as described above. After initial imaging, cells were stained for FLAG-ATF4: 50 µL blocking solution [10% normal goat serum (Thermo Fisher) in PBS] was added to each well and the cells were incubated for 20' at RT. Excess blocking solution was then aspirated and 50 µL primary antibody solution [mouse anti-FLAG M2 (Sigma), 1:1000 in blocking solution] was added to each well for 30' at RT. Cells were then washed twice with PBS and 50 µL secondary antibody solution [Alexa594-conjugated goat anti-mouse (Thermo Fisher), 1:1000 in blocking solution] was added to each well for 30' at RT. Lastly, plates were washed 4 times with PBS, sealed, and re-imaged**.**

DNA/RNA constructs and mutagenesis

Whole-genome screen siRNAs: The following siRNA constructs were obtained from Qiagen: Genomic siRNA library v1.0, consisting of four distinct siRNAs (A, B, C, and D) targeting each of 22,909 known and putative human genes, non-silencing siRNA control (#SI03650325), *TOR1B* siRNAs (#s SI00749574, SI04135404, SI04221728, SI04270672), and a positive control siRNA pair (targeting sequences ACGGTGGAAACTGGTATCCGA and CAGCAAGTATCTATAATCTAT). Library siRNAs were utilized as a 1:1 mixture of orthogonal pairs (either AB or CD). *Expression constructs:* N-terminally FLAG tagged human ATF4 expression construct (pRK/FLAG-ATF4) was acquired from Addgene. Amino acid response element-sensitive *Renillia* luciferase (AARE-RLuc) was obtained from SwitchGear Genomics. Constitutively expressed *Cyperidina* luciferase (pCMV-CLuc 2) was obtained from New England Biolabs. All ATF4 mutations were introduced into pRK/FLAG-ATF4 using a QuikChange Lightning site-directed mutagenesis kit (Agilent) according to the manufacturer's instructions. pAARE-RLuc, pCMV-CLuc 2, and all wildtype and mutant pRK/FLAG-ATF4-derived constructs were confirmed by Sanger sequencing.

Messenger RNA expression profiling of assay cell line

Flp-In T-REx 293 cells inducibly expressing ΔE Torsin1a were seeded at $2x10^6$ cells per 10 cm^2 dish. Cells were treated with tetracycline (5 µg/mL, Sigma #87128) or PBS (uninduced control) 24 h after initial plating. Cells were harvested 72 h after tetracycline induction. RNA was extracted using the RNeasy RNA extraction kit (Qiagen #74104) as per manufacturer's protocol. Microarray expression profiling was performed at the Duke Center for Genomic and Computational Biology (Durham, NC) using standard protocols and the GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix). The Affymetrix Gene Chip microarray data underwent strict quality control processing using the *simpleaffy* package in Bioconductor. Log-scale Robust Multiarray Analysis (RMA) from the affy package in Bioconductor was used for normalization to eliminate systematic differences across the arrays. The mas5 algorithm from the affy package was used to make present/absent calls. Bioinformatics analysis of RNAi screen hits was performed exclusively on probesets found to be present. Gene expression data has been uploaded to the Gene Expression Omnibus (GEO) repository, submission ID GSE89180.

Assay for luciferase secretion in human dermal fibroblasts

Normal control and DYT1 patient human dermal fibroblasts (listed below) were plated in 6-well plates at 25,000 cells/well in FGM media (2 ml/well) and incubated 37°C/5% CO_2 overnight. The following day, cells were co-infected with lentivirus delivering CMV-Cypridina luciferase (CLuc) and EF1a-β-galactosidase (LacZ) in Opti-MEM containing 2 µg/mL polybrene (Santa Cruz Biotechnology #sc-134220) with centrifugation at 500 x g for 1 h. Media was replenished with FGM and incubated at 37°C/5% CO_2 for 3 days. Cells were then transferred to 96-well plates at 5,000 cells/well in 100µL of FGM media. The following day, cells were infected with shRNA lentivirus, as indicated (listed below), in Opti-MEM containing 2 µg/mL polybrene with centrifugation at 500 x g for 1 h. Media was replenished with FGM, cells incubated at 37°C/5% CO_2 for 3 days, media replenished again, and incubated 48 h. CLuc activity in the conditioned media samples and LacZ activity in the clarified lysates was measured using Cypridina Luciferin (Nanolight Technology #305) and 2-Nitrophenyl β-D-galactopyranoside (Sigma #73660).

The following human dermal fibroblast lines were used; all fibroblast lines were acquired from the NIGMS Human Genetic Cell Repository (Coriell). *Normal control lines*: #GM01948 – 27 y, F, RRID:CVCL_9W83; #GM04504B – 31 y, F, RRID:CVCL_7411; #GM23971A – 33 y, M, RRID:CVCL_BW40. *DYT1 patient lines*:

#GM02306 – 13 y, M, RRID:CVCL_4N23; #GM02551 – 31 y, F, RRID:CVCL_4N26; #GM03208 – 29 y, M, RRID:CVCL_4N41; #GM03211 – 30 y, M, RRID:CVCL_1U24.

The following lentiviral shRNA constructs and targeting sequences were used; all shRNA lentiviral constructs were acquired from Sigma Aldrich. *ATP5A1*: #TRCN0000043425 – CGTTTCAATGATGGATCTGAT, #TRCN0000043427 – CCTCTAACACTCATCTTCAAA; *PPP1R16A*: #TRCN0000002675 – CATCTATACTCCAAGCGACTA, #TRCN0000002676 – GCCCGAAATGACCTGGAAGAA; *RPL10A*: #TRCN0000117478 –GTCCACAATCAAGTTCCAAAT, #TRCN0000117479 – GCAGATCAGCTTGAAGAACTA; *SCD*: #TRCN0000056613 – CTACGGCTCTTTCTGATCATT, #TRCN0000056614 – CGTCCTTATGACAAGAACATT; *Non-targeting control*: #SHC016 – GCGCGATAGCGCTAATAATTT.

ER stress assay in human dermal fibroblasts

Normal control and DYT1 patient human dermal fibroblasts were plated in 6-well plates at 25,000 cells/well in FGM media (2 mL/well) and incubated 37°C/5% CO_2 for 4 days. Media was replenished with FGM (1 mL/well) and incubated at 37°C/5% CO_2 overnight. Opti-MEM (1 mL/well) containing 2 µg/mL thapsigargin (Santa Cruz Biotechnology #sc-24017) was added to cells and incubated as indicated (final concentration: 1 µg/mL).

Immunoblotting

Cells were harvested in RIPA buffer [150 mM NaCl/50 mM NaH_2PO_4/2 mM EDTA/1% Triton X-100/0.5% SDS/0.5% deoxycholic acid/50 mM NaF/10 mM $Na_4P_2O_7$/1 mM Na_3VO_4/1x phosphatase inhibitor cocktail (Sigma #P5726)/1x cOmplete Mini EDTA-free protease inhibitor cocktail (Roche #04693159001)]. Total protein concentrations were assessed by BCA assay (Thermo Fisher Scientific #23225). Proteins were resolved on 4-15% TGX gels (Bio-Rad #5671085), transferred to nitrocellulose membrane, blocked in TBS-T + 5% non-fat dry milk, and probed as indicated. The following primary antibodies and dilution ratios were used for immunoblotting experiments: anti-β-Actin – 1:1000, Millipore #MAB1501, RRID:AB_2223041; anti-FLAG – 1:1000, Sigma #F3165, RRID:AB_259529; anti-ATF4 – 1:500, Santa Cruz #SC-200, RRID:AB_2058752; anti-CReP – 1:500, Proteintech #14634-1-AP, RRID:AB_2300036; anti-GAPDH – 1:1000, Abcam #ab9485, RRID:AB_307275.

ATF4 activity assay

HEK293T cells were plated in 6-well plates at 500,000 cells/well in 2 mL HEK-T media [DMEM (Thermo Fisher #11995) + 10% FBS + 1x GlutaMAX + 1x penicillin/streptomycin/amphotericin], or in 96-well plates at 50,000 cells/well in 150 µL HEK-T, and incubated at 37°C/5% CO_2 overnight. The following day, cells were transfected with pAARE-RLuc, pCMV-CLuc2, and empty vector or FLAG-tagged WT/mutant ATF4 as appropriate, using OptiMEM/Lipofectamine2000 as above (2.5 µg total DNA/well, 500 µL total volume/well in 6-well plates; equivalent values in 96-well plates). After 4 hours, transfection media was aspirated and replaced with fresh HEK-T media and cells were incubated at 37°C/5% CO_2. 24 hours after transfection, conditioned media was transferred to a separate plate, and cells were washed once with ice-cold PBS and lysed in ice-cold lysis buffer (25 mM Tris-HCl/12.5 mM NaH_2PO_4/2 mM EGTA/1% Triton X-100/1x cOmplete Mini EDTA-free protease inhibitor; 6-well plates - 250 µL/well, 96-well plates - 50 µL/well). Conditioned media and lysates were stored O/N at -80°C. The following day, CLuc activity in the conditioned media samples and RLuc activity in the clarified lysates was measured using BioLux *Cyperidina* Luciferase (NEB) and Pierce *Renilla* Luciferase (Thermo Fisher) assay kits, respectively, according to the manufacturer's instructions.

Electrophysiological recordings

Animals and slice preparation:

Heterozygous delGAG Torsin1a knockin mice (RRID:IMSR_JAX:025637; courtesy of Dr. W. Dauer, University of Michigan) were crossed with homozygous *Drd1a*-tdTomato mice (Jackson Labs) and the progeny was used at postnatal day 15-21 for experiments. Mice were anesthetized and intracardially perfused with high-sucrose solution (194 mM sucrose, 30 mM NaCl, 4.5 mM KCl, 2 mM $MgCl_2$, 200 µM CaCl2, 1.2 mM NaH2PO4, 26 mM NaHCO3, and 10 mM glucose, saturated with 95% O_2 and 5% CO_2). Animals were then decapitated, their brains dissected, and 300 µm horizontal slices were cut on a Leica VT1200S vibratome. Slices were transferred to artificial cerebrospinal fluid (ACSF; 124 mM NaCl, 2.5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM NaHCO3, 1.2 mM NaH_2PO_4, and 10 mM glucose, saturated with 95% O_2 and 5% CO_2, pH 7.4, 300 mOsm/l) to equilibrate for at least 1 h.

Recording and analysis:
Single slices were transferred to a recording chamber and superfused continuously with ACSF containing 50 µM picrotoxin at 32°C and 3-4 mL/min. Neurons were visualized using infrared differential interference microscopy. Micropipettes were pulled (Narishige) from borosilicate glass tubes (King Precision Glass) for a final resistance of 2.5 – 4.5 MΩ when filled with internal solution (130 mM KSO_4CH_4, 5 mM KCl, 5 mM NaCl, 100 µM EGTA, 10 mM HEPES, 2 mM $MgCl_2$, 50 µM $CaCl_2$, 2 mM ATP-Mg, 400 µM GTP-Na, pH 7.3, 290 mOsm/l). Evoked excitatory postsynaptic potentials (EPSPs) were recorded in the dorsolateral striatum, while stimulating every 30 seconds with a concentric bipolar electrode (FHC) in cortical layer V (see Trusel et al., *Cell Reports*, 2015). To induce long-term depression, 4 trains of 100 Hz stimulation (every 10 s) were applied while the postsynaptic cell was depolarized to -50 mV. Baseline EPSPs were recorded for at least 10 min or until a stable baseline was reached. Data were acquired by pClamp v10 and analyzed using Clampfit v10.4, Origin v8.0, and GraphPad Prism v6. One >2SD outlier result was removed from the DYT1-vehicle group (mean LTD 298.8%); the statistical conclusion was unaffected by the removal of this outlier.

Perinatal viability assay
Breeding and viability determination: Pairs of heterozygous ΔE Torsin1a knockin breeders (courtesy of Dr. W. Dauer, University of Michigan) were randomly assigned to vehicle or salubrinal treatment groups. Starting 10 days after breeding cages were established, each female mouse was given a daily subcutaneous injection of vehicle or salubrinal at approximately 6pm. Cages were checked for new pups three times per day: morning (~8am), early afternoon (~2pm), and during injections (~6pm). In order to minimize stress and the possibility of abandonment, mice with newborn pups were not given injections. Newborn pups were identified by marking limbs with a permanent laboratory marker, and tail samples were taken for genotyping at P0. At approximately P0.5, cages were checked again, and the status of each pup was recorded. If pups were first observed at the 8am check, the 2pm check was considered P0.5. If pups were first observed at the 2pm or 6pm checks, an additional check was performed at approximately midnight, and considered P0.5. Mortality was also tracked the following day, after which time identifying marks were predominately washed off or obscured by fur. Approximately 8 days after birth all remaining pups were sacrificed, and 10 days after birth daily injections resumed for the subsequent litter. All genotyping and, whenever possible, all pup identification and mortality checks were performed blinded to treatment group.

Drug administration: Salubrinal (R&D Systems #2347) stocks were prepared at 26 mM (10x final concentration) in DMSO and stored in single-use aliquots at -80°C. Each day immediately before injections, a single salubrinal aliquot was thawed to room temperature and diluted 1:10 in injection vehicle (final vehicle composition: 1x PBS/10% DMSO/0.1% BSA). Empty injection vehicle was stored at room temperature in single-use aliquots. Mice were given a single subcutaneous injection of 3.3 mg/kg salubrinal (approximately 80 µL for a 30 g mouse) or an equivalent volume of empty injection vehicle. All animal procedures and other handling was performed in accordance with the NIH Guide for Care and Use of Laboratory Animals and approved by the Duke University Institutional Animal Care and Use Committee.

Whole-exome DNA sequencing
Sequencing of DNA was performed at Duke University. Case samples were exome sequenced using the Agilent All Exon 37MB or 50MB kit, and controls using the Agilent All Exon (37MB, 50MB or 65MB) or the Nimblegen SeqCap EZ V2.0 or 3.0 Exome Enrichment kit or whole-genome sequenced, using Illumina GAIIx or HiSeq 2000 or 2500 sequencers according to standard protocols. All samples were processed using the same methods, as follows. The Illumina lane-level fastq files were aligned to the Human Reference Genome (NCBI Build 37) using the Burrows-Wheeler Alignment Tool (BWA). We then used Picard software (picard.sourceforge.net) to remove duplicate reads and process these lane-level SAM files, resulting in a sample-level BAM file that is used for variant calling. We used GATK to recalibrate base quality scores, realign around indels, and call variants. Variants were required to have a quality score (QUAL) of at least 20, a genotype quality (GQ) score of at least 20, at least 10x coverage, a quality by depth (QD) score of at least 2 and a mapping quality (MQ) score of at least 40. Indels were required to have a maximum strand bias (FS) of 200 and a minimum read position rank sum (RPRS) of -20. SNVs were restricted according to VQSR tranche (calculated using the known SNV sites from HapMap v3.3, dbSNP, and the Omni chip array from the 1000 Genomes Project): the cutoffs were a tranche of 99.9%. Variants were excluded if marked by EVS as being failures. Variants were annotated to Ensembl 73 using SnpEff. Only genetically European ethnicity samples were included in the analysis. Samples were screened with KING to remove second-degree or higher relatives; samples with incorrect sexes according to X:Y coverage ratios were removed, as were contaminated samples according to VerifyBamID. We used Analysis Tools for Annotated Variants (ATAV; https://redmine.igm.cumc.columbia.edu/projects/atav/wiki) to identify exons containing coding variants which were

found in at least 2 cases and 0 of 571 controls who were matched to the cases in terms of ethnicity and capture kit. The presence of mutations in *ATF4* causing the P46L amino acid substitution were confirmed by Sanger sequencing.

ATF4 exon 1 Sanger sequencing

Anonymized genomic DNA samples from cervical dystonia patients without family history were obtained and Sanger sequenced between nucleotide 1-225 following PCR amplification (Phusion high-fidelity polymerase, NEB) of genomic DNA using the following primers: TGGTCTTTGCTTGGGTGTC, AGGTCATCTGGCATGGTTTCC. Sequence at identified variant locations was visually confirmed for all subjects.

Human dystonia patients

Whole-exome sequencing subjects:

We collected 20 unrelated patients diagnosed with adult onset, sporadic dystonia (3 men and 17 women) with a mean age of dystonia onset of 47.4 +/- 9.59 years. A diagnostic workup was conducted by a Movement Disorders specialist to confirm the symptoms of dystonia with muscle involvement classified as focal, segmental, multifocal, or generalized. Only presumptive primary cases were recruited. Secondary dystonias associated with conditions such as Parkinson's disease or other neurodegenerative diseases were excluded. Cases suggestive of Mendelian inheritance were also excluded. A complete family and medical history was collected including common toxic exposures and medical comorbidities. Control samples were sequenced as part of other studies at Duke University Medical Center and were not enriched for (but not specifically screened for) dystonia or other neurological disorders.

ATF4 exon 1 sequencing subjects:

Genomic DNA samples were acquired from subjects recruited at Movement Disorder Centers in New York (Mount Sinai Beth Israel) and Boston (Massachusetts General Hospital) as well as additional subject genomic DNA samples and corresponding clinical data that were acquired through the NINDS Human Genetics Resource Center DNA and Cell Line Repository (http://ccr.coriell.org/ninds). Videotaped examinations and determination of affected status was undertaken for those samples recruited through Movement Disorder Centers in New York (Mount Sinai Beth Israel) and Boston (Massachusetts General Hospital) as previously published (Bressman et al., 1989), taking into account recently updated criteria (Albanese et al., 2013). The local institutional review boards approved the studies and all participating individuals gave informed consent prior to participation. NINDS Repository sample numbers corresponding to the samples used are listed in Supplemental Experimental Procedures.

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