Table S1. Cell mask parameters for the analysis of relative fluorescence intensities of EGFP and mCherry using the IncuCyte Zoom basic analyser.

Cell-line	Fluorescent protein	Channel	Exposure (ms)	Background correction	Edge sensitivity	Data presentation
Neuro-2a	EGFP	Green	400	Top-hat (50 μm, 2 GCU)	-15	GCU × μm^2/ image
	mCherry	Red	800	Adaptive (2 RCU)	0	RCU × μm^2/ image

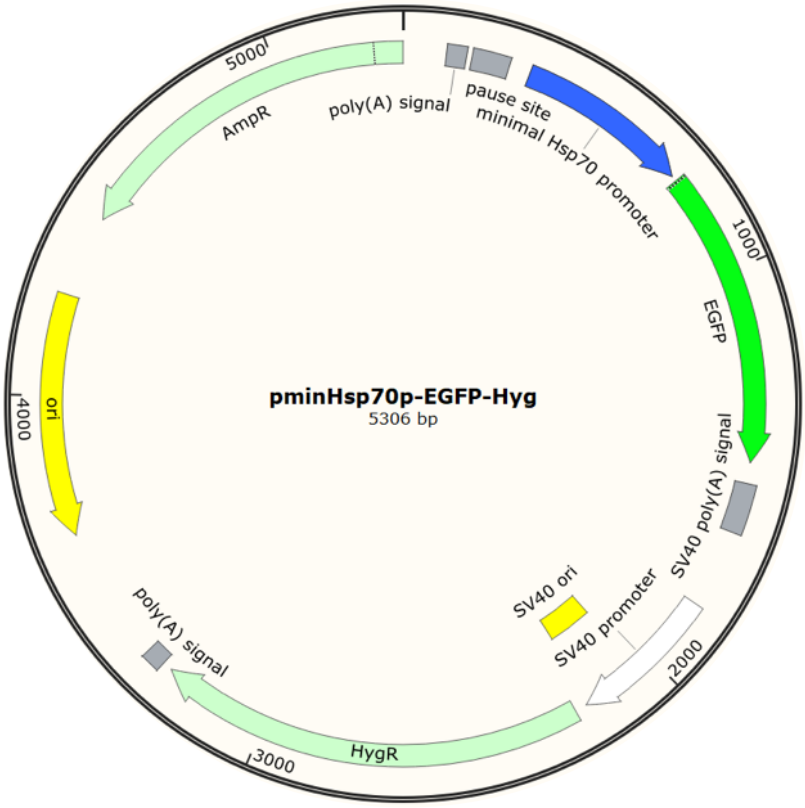
Table S2. Mask parameters for the analysis of relative fluorescence intensities of individual cells in confocal imaging experiments using Cell Profiler.

Object identified	Input image	Diameter (pixel units)	Threshold strategy	Thresholding method	Threshold boundaries	Distinguish clumps	Dividing lines in clumps
Nuclei	Hoechst	20-100	Automatic	-	-	Intensity	Shape
Cells	mCherry	20-65	Global	Otsu	0.01-1.0	Intensity	Propagate
	EGFP	-	Global	Otsu	0.0-1.0	-	-
Inclusions	Cerulean	15-40	Global	Otsu	0.01-1.0	Intensity	Shape

Table S3. Compensation matrix used to compensate samples co-expressing Cerulean and EGFP fluorescent proteins

	[405] 450/50 nm	[488] 525/50 nm
[405] 450/50 nm	100 %	0.17 %
[488] 525/50 nm	1.4 %	100 %

pminHsp70p-EGFP plasmid map:



pminHsp70p-EGFP sequence:

TCGAGGCGCGTCCTCAGAGCCAGCCGGGAGGAGCTAGAACCTTCCCCGCGTTTCTTTCAGCAGCCCTG
AGTCAGAGGCGGGCTGGCCTGGCATAGCCGCCCAGCCTCTCGGCTCACGGCCCGATCCGCCCGAACCT
TCTCCCGGGGTCAGCGCCGCGCTGCGCCGCCCGGCTGACTCAGCCCGGGCGGGCGGGCGGGAGGCTCT
CGACTGGGCGGGAAGGTGCGGGAAGGTTCGCGGCGGCGGGGTCGGGGAGGTGCAAAAGGATGAAAAGC
CCGTGGAAGCGGAGCTGAGCAGATCCGAGCCGGGCTGGCGGCAGAGAAACCGCAGGGAGAGCCTCACT
GCTGAGCGCCCCTCGACGGCGGAGCGGCAGCAGCCTCCGTGGCCTCCAGCATCCGACAAGAAGCTCTC
TAGTCGACGGTATCGATAAGCTTCTTAACATATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGG
TGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAG
GGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTG
GCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGC
AGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGAC
GACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCT
GAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCC
ACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAAC
ATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGT
GCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCG
ATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
TAA

Figure S1. Plasmid map and sequence of the pminHsp70-EGFP-Hyg^r construct used to generate the Neuro-2a (HSE:EGFP) and HEK293 (HSE:EGFP) stable cell line reporters of the HSR. Sequence corresponding to the human minimal Hsp70 promoter (minHsp70p) is shown in blue and the EGFP gene in green. The 8 putative heat shock elements (NGAAN) in the minHsp70p sequence are underlined.

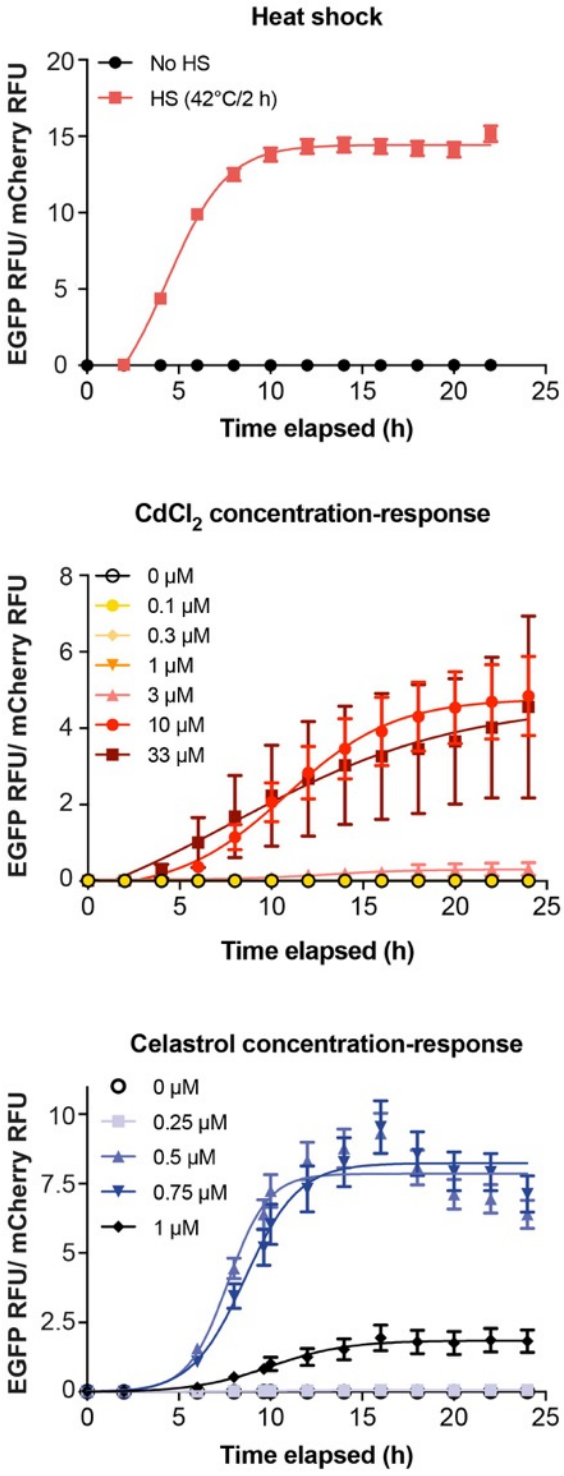


Figure S2. Monitoring induction of the heat shock response in Neuro-2a (HSE:EGFP) cells following heat shock, or treatment with $CdCl_2$ or celastrol . Cells were subjected to heat shock (42 °C/ 2 h), or log and ½ log doses of $CdCl_2$ (0-33 µM) or celastrol (0-1 µM). Following treatment cells were imaged every 2 h using an IncuCyte Live Cell Imaging System to monitor the levels of EGFP as a measure of HSR induction. Data is presented as the fold change in EGFP RFU over time, normalised to mCherry RFU, to account for changes in cell density over the time-course of the experiment. The data shown is the mean ± SEM of three independent repeats.

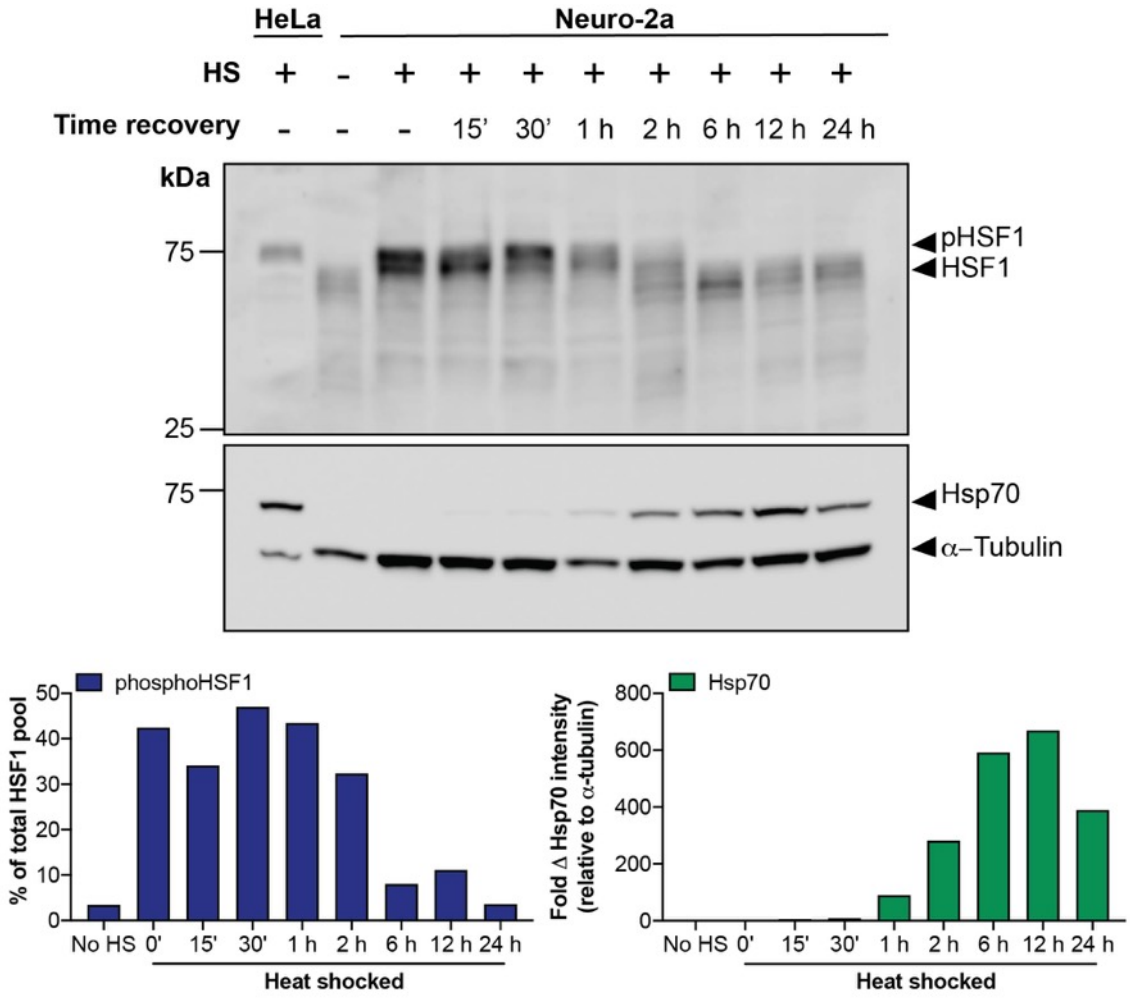


Figure S3. The HSR is induced in Neuro-2a cells after heat shock at 42°C for 2 h. Parental Neuro-2a cells were heat shocked (HS) at 42 °C for 2 h and allowed to recover at 37°C for different time intervals (0,15 and 30 min, and 1, 2, 6, 12, and 24 h). For each time interval, whole cell lysates were prepared for immunoblotting and probed for markers of HSR induction. The HSR markers used were HSF1 activation (phosphorylation; pHSF1, 75 kDa, and HSF1, 70 kDa), and Hsp70 (70 kDa) up-regulation, α-tubulin (50 kDa) as a housekeeping protein to determine relative protein loading. HeLa cells heat shocked at 42°C for 2 h with no recovery were used as a positive control sample for each of the markers of HSR induction. Immunoblot analysis and densitometry showed that HSF1 was activated under these heat shock conditions and displayed hyper-phosphorylated bands after heat shock that persisted for up to 2 h post-heat shock. After 6 h of recovery at 37 °C the phosphorylation status of HSF1 had returned to control (no heat shock) levels. There was a time-dependent increase in Hsp70 levels, such that it was first detected 15 min after heat shock and reached a maximum after 6-12 h. Data shown are from one independent repeat.

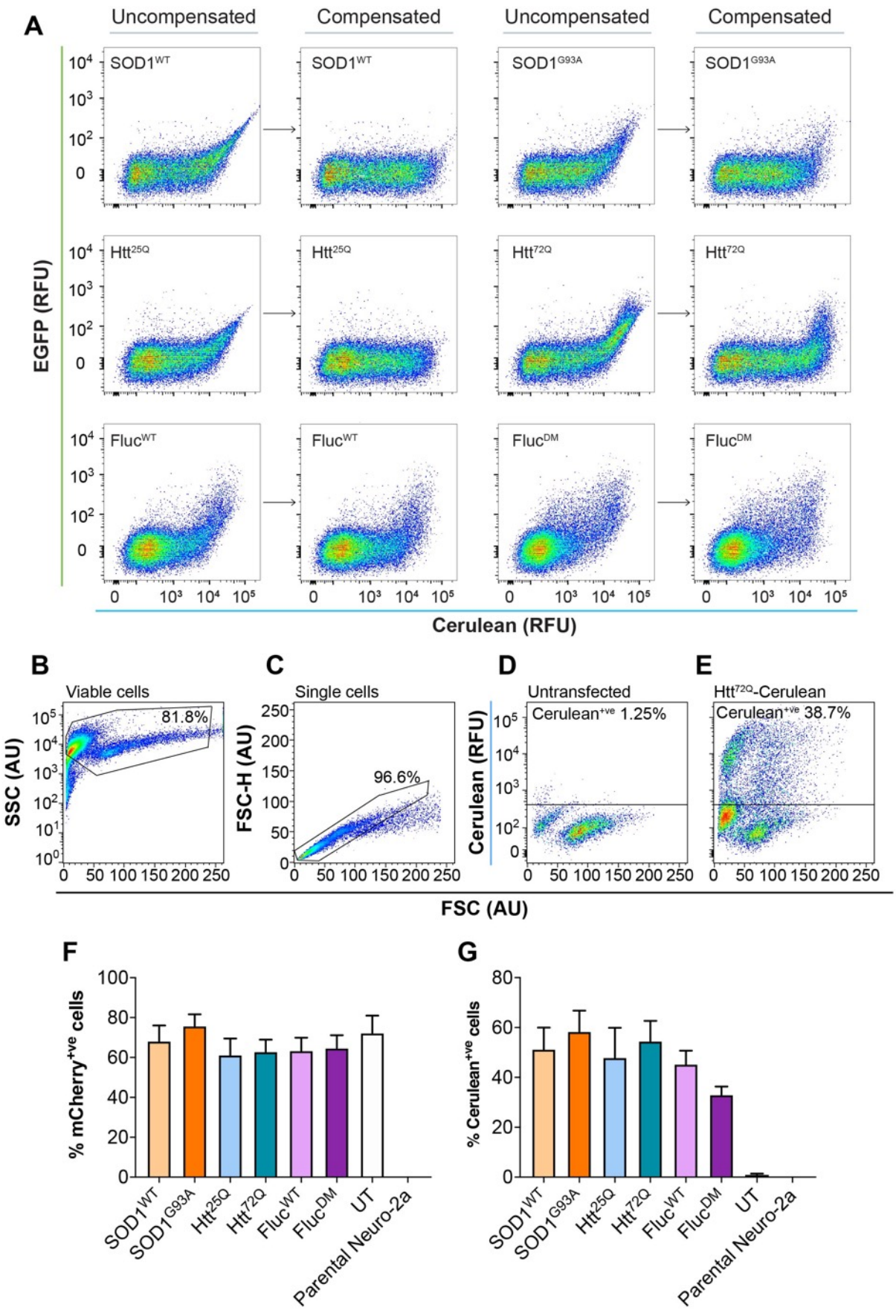


Figure S4. Flow cytometric data from Neuro-2a (HSE:EGFP) expressing EGFP and Cerulean fluorescent proteins were compensated to account for spectral overlap prior to analysis of HSR induction. Neuro-2a cells were transfected to express cerulean tagged $SOD1^{WT}$, $SOD1^{G93A}$, Htt^{25Q}, Htt^{72Q}, $Fluc^{WT}$, or $Fluc^{DM}$ proteins and, 48 h post-transfection, cells were analysed by flow cytometry. (A) Due to the spectral overlap between Cerulean and EGFP fluorescence emissions it was necessary to compensate the flow cytometric data. Bivariate plots of EGFP and Cerulean fluorescence are shown for each of the samples before ("uncompensated") and after ("compensated") spectral compensation. Representative cytograms presented here are the same as in Figure 3 in the main text. (B) Plots of FSC and SSC of cells were used to resolve cellular debris and cell clumps. A polygonal gate was set around cells of interest and all downstream analyses was performed on this population. (C) Plots of FSC-height (FSC-H) and FSC-area (FSC-A) were used to resolve singlet and doublet events and a polygonal gate was used to exclude all doublets from downstream analyses. (D) Untransfected cells were used as a $cerulean^{-ve}$ control to identify $Cerulean^{+ve}$ events. (E) Representative plot of Neuro-2a (HSE:EGFP) cells transfected to express Htt^{72Q}-cerulean are shown and the percent of $Cerulean^{+ve}$ cells in the gate are denoted. Data shown are representative of three independent repeats. (F) The proportion of cells that were mCherry positive in each sample. (G) The transfection efficiency, determined by the proportion of $Cerulean^{+ve}$ cells, in each sample.

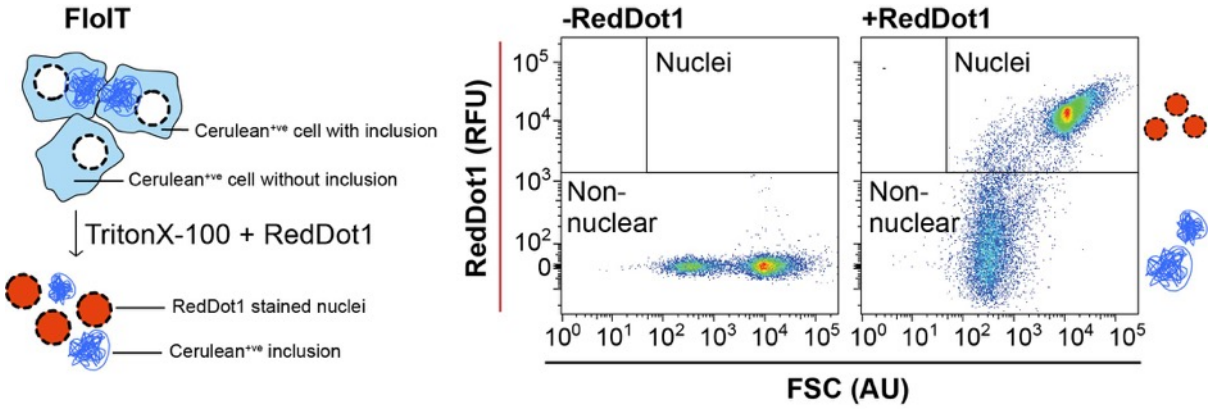
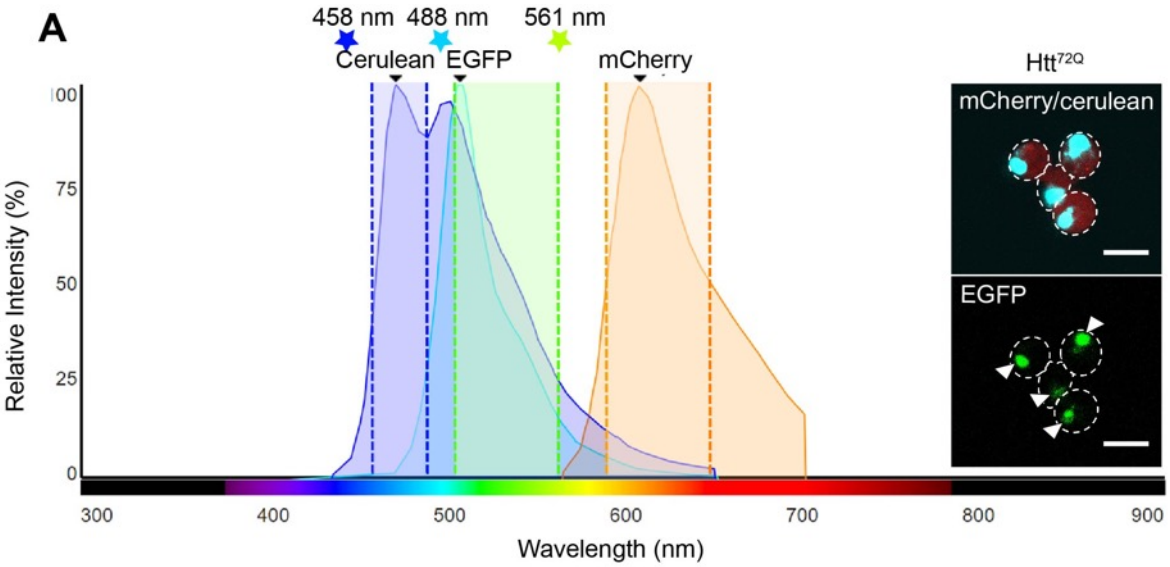
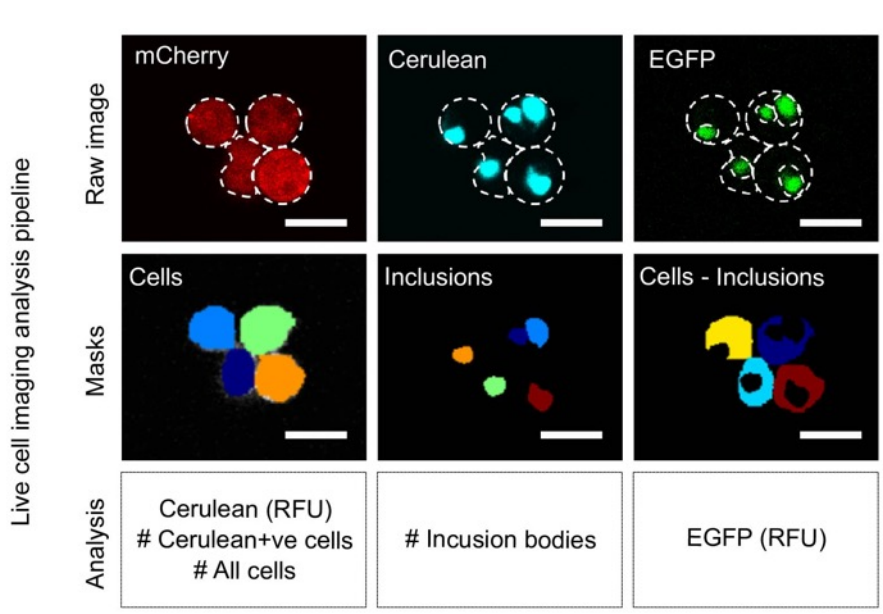
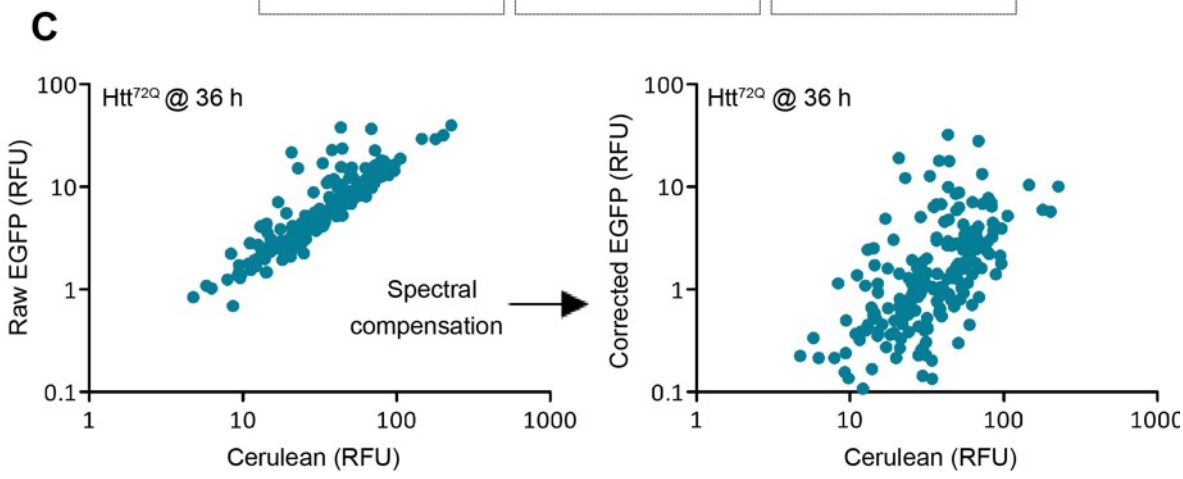


Figure S5. Flow cytometric analysis of inclusions and trafficking (FloIT) was used to determine the relative propensity of each protein to form inclusions in cells. Transfected cells were lysed with 0.1% TritonX-100 supplemented with RedDot1 nuclear stain and analysis by flow cytometry. Cells were first lysed in the absence of RedDot1 to set square gates to capture RedDot1^{+ve} events and RedDot^{-ve} non-nuclear events. Plots of forward scatter (FSC; size) and RedDot1 nuclear dye fluorescence used to enumerate the nuclei in the cell lysates.







D

$$Raw\ EGFP - \left(\frac{Raw\ Cerulean}{1} \times \frac{13}{100}\right) = Corrected\ EGFP$$

Figure F6. Experimental design and analysis of live cell imaging experiments. Neuro-2a (HSE:EGFP) cells were transfected to express Htt^{72Q} or $Fluc^{DM}$ and imaged over time by confocal microscopy. (A) Fluorescence emission spectra of cerulean, EGFP and mCherry fluorescent proteins. The windows for emission collection were set at 462-492 nm (blue dotted lines), 506-563 nm (green dotted lines), and 600-657 nm (orange dotted lines), for cerulean, EGFP and mCherry, respectively. The lasers used to excite each fluorescent protein are depicted by the coloured stars. Inset: A representative image of Neuro-2a (HSE:EGFP) cells transfected to express Htt^{72Q}, demonstrating spectral overlap between the cerulean and EGFP channels (white arrowheads). (B) Analysis pipeline used in Cell Profiler of confocal images acquired during the live cell imaging experiment. The mCherry channel (*left*) was used to define the "cells", which are masked in different colours, and this region was used to measure Cerulean fluorescence intensity, the number of $Cerulean^{+ve}$ cells, and the total number of all cells in each image over the time-course. The cerulean channel (*middle*) was used to identify "inclusions" and this region was used to count the number of inclusions formed in each image over the time-course. The "inclusions" were subtracted from the "cells" to generate a third region for measurement defined as "cells – inclusions", and this region was used to determine the EGFP fluorescence intensity in this channel. All scale bars = 20 µm. (C) Bivariate plots of cerulean and EGFP fluorescence intensities from the "cell – inclusions" region in Htt^{72Q} transfected cells at 36 h, before (left) and after (right) spectral compensation. (D) Equation used to apply spectral compensation on the EGFP data.