Supplemental figure S1

A. 

B. 

C. 

D. 

E. 

F. 

Supplemental information
Figure S1: Effect of cytoskeleton inhibitors on some gene expression parameters. (A and B) Cytoskeleton inhibitors do not interfere with transcription or with expression of the CFTR gene. 6CFSMEo- (A upper panels) and IB3 (A lower panels) cells were incubated with DMSO, CytoD, JPK, COL, TAX, or amlexanox for 48 h. The level of CFTR pre-mRNA was measured by RT-PCR and normalized to the level of GAPDH mRNA. (B) 16HBE14o- cells were incubated with DMSO, CytoD, JPK, COL or TAX for 48 h. The level of CFTR mRNA was measured by RT-PCR and normalized to the level of GAPDH mRNA. The five leftmost lanes represent two-fold serial dilutions of RNA from Calu3 cells overexpressing CFTR mRNA. These results combine two independent experiments. (C and D) Cytoskeleton inhibitors do not induce cell death or apoptosis in IB3 or 6CFSMEo- cells. IB3 and 6CFSMEo- cells were incubated with DMSO, CytoD, JPK, COL, or TAX for 48 h and then stained with Annexin V and propidium iodide. Cell viability (C) and the apoptosis rate (D) were then measured. These results are representative of three independent experiments. (E) Cytoskeleton inhibitors do not affect natural NMD substrates. 6CFSMEo- cells were incubated with DMSO, CytoD, JPK, COL, or TAX for 48 h. RT-PCR was performed to measure the levels of SC35 mRNA, NAT9 mRNA, and TBL2 mRNA. The mRNA levels were normalized to the level of GAPDH mRNA. The three leftmost lanes represent two-fold serial dilutions of RNA from 6CFSMEo- cells. These results are representative of three independent experiments. (F) Cytoskeleton inhibitors do not affect translation. 6CFSMEo- cells were incubated with DMSO, CytoD, JPK, COL, or TAX for 48 h or with 200 µg/ml cycloheximide (CHX) (as positive control) for 4 h. One hour before harvest, 25 µM L-azidohomoalanine (L-AHA) was added to the cell culture medium, to be incorporated into newly synthesized proteins. Then cells were collected and the Click-iT Protein Analysis Detection Kit (Lifetechnologies) was used to measure the translation efficiency. These results are representative of three independent experiments.
Supplemental figure S2A

YFP-UPF1  DMSO  CytoD  JPK

Alexa 594-ACTIN

Hoechst

Merge
Figure S2: Cytoskeleton inhibitors affect the cellular location of NMD factors. 6CFSMEo- cells transfected with expression constructs encoding YFP-UPF1 (A and B) or YFP-UPF3X (C and D) and GPx1 46Ter were incubated with DMSO, CytoD, JPK, COL, or TAX. After 48 h, the cells were fixed, permeabilized, and incubated sequentially with primary antibodies (actin (A, C) or tubulin (B, D)) and Alexa Fluor 594 secondary antibody (red) before staining of nuclei with Hoechst stain (blue). Green arrows indicate UPF1 or UPF3X cytoplasmic foci; Red arrows indicate concentration of actin or tubulin.
Figure S3: UPF3X concentrates partially in P-bodies under JPK treatment in 6CFSMeo- cells. Cells transfected with an expression construct encoding YFP-UPF3X or GPx1 46Ter were incubated with DMSO or JPK for 48 h. After fixation and permeabilization, the cells were incubated sequentially with anti-DCP1a primary antibody and Alexa Fluor 594 secondary antibody (red). Finally, the cells were incubated with Hoechst stain (blue) to visualize their nuclei. Green arrows indicate UPF3X cytoplasmic foci; red arrows indicate the P-body marker DCP1a; orange arrows indicate colocalization between UPF3X and DCP1a. The histogram represents the percentage of colocalization between UPF3X foci and DCP1a foci. Cells (N=10) were counted from three different experiments.
Figure S4: In 6CFSMEo- cells, endogenous UPF1 protein concentrates mainly in P-bodies upon COL or TAX treatment and only partially upon CytoD or JPK treatment. Cells were treated with DMSO, CytoD, JPK, COL, or TAX for 48 h. After fixation and permeabilization, the cells were incubated first with the anti-DCP1a or anti-UPF1 primary antibody and then with the Alexa Fluor 594 secondary antibody (red) or Alexa Fluor 488 secondary antibody (green). Finally, the nuclei were visualized in blue by Hoechst staining. Green arrows indicate UPF1 cytoplasmic foci; red arrows indicate the P-body marker DCP1a; orange arrows indicate colocalization of UPF1 and DCP1a. The histogram represents the percentage of colocalization between UPF1 foci and DCP1a foci. Cells (N=10) from three different experiments were counted for each condition.
Figure S5: NMD factor UPF3X colocalizes with NMD substrates under cytoskeleton disruptor treatment in 6CFSMEo- cells. 6CFSMEo- cells were transfected with constructs expressing GPx1 46Ter and YFP-UPF3X and then incubated with DMSO, CytoD, JPK, COL, or TAX for 48 h. A FISH assay was performed to detect the cellular localization of GPx1 46Ter mRNA. Nuclei are stained in blue with Hoechst stain. Green arrows indicate UPF3X cytoplasmic foci; red arrows indicate GPx1 mRNA cytoplasmic foci; orange arrows indicate colocalization of the UPF3X factor and NMD substrates.
Figure S6: Cytoskeleton disruptors do not affect the cellular location of wild-type mRNAs in 6CFSMEo- cells. 6CFSMEo- cells were transiently transfected to express GPx1 Norm and YFP-UPF1 (A) or YFP-UPF3X (B) and then incubated with DMSO, CytoD, JPK, COL, or TAX for 48 h. After fixation and permeabilization, they were incubated overnight at 37°C with Texas-red-labeled probes. Nuclei were stained with Hoechst solution (blue).
Figure S7: NMD substrates partially or totally concentrate in P-bodies under cytoskeleton disruptor treatment. 6CFSMEo- cells were transfected with constructs expressing Flag-UPF1 and GPx1 46Ter. The cells were then incubated with DMSO, CytoD, JPK, COL, or TAX for 48 h. The location of GPx1 46Ter mRNA was monitored by FISH (red). The cells were then incubated sequentially with anti-DCP1a primary antibody and Alexa Fluor 488 secondary antibody (green). Nuclei were stained in blue with Hoechst solution. Green arrows indicate P-bodies; red arrows indicate GPx1-mRNA-containing cytoplasmic foci; orange arrows indicate colocalization of DCP1a and NMD substrates. The histogram represents the percentage of colocalization between GPx1 Ter mRNA and DCP1a. Cells (N=10) from three different experiments were counted for each condition.
Figure S8: In 6CFSMEo-cells, NMD factors do not colocalize with stress granules upon cytoskeleton inhibitor treatment. 6CFSMEo-cells transfected with a construct expressing YFP-UPF1 (A) or YFP-UPF3X (B) and the construct pCMV-GPx1 46Ter were incubated with DMSO, 1μM CytoD, 1μM JPK, 10 μM COL, or 1 μM Taxol for 48h. 6CFSMEo-cells treated with 1.5 mM H2O2 for 4 h were used as positive control. Cells were fixed and permeabilized prior to incubation for 1 h at 4°C with primary antibody against eIF3β (a marker of stress granules), washed three times with PBS, and incubated with goat anti-rabbit antibody labeled with Alexa Fluor 594 (red). Cells were washed three times with PBS and incubated with Hoechst stain (blue) for 2 min at room temperature.
Figure S9: NMD factors do not colocalize with autophagosomes in 6CFSMEo-cells treated with cytoskeleton inhibitors. 6CFSMEo-cells transfected with a construct expressing YFP-UPF1 (A) or YFP-UPF3X (B) and the construct pCMV-GPx1 Ter were incubated respectively with DMSO, 1 μM CytoD, 1 μM JPK, 10 μM COL or 1 μM Taxol for 48 h. As positive control, 6CFSMEo-cells were incubated in serum-free medium for 24 h. Cells were then fixed and permeabilized prior to incubation for 1 h at 4°C with primary antibody (anti-LC3B antibody), washed three times with PBS, and incubated with goat anti-rabbit antibody labeled with Alexa Fluor 594 (red). Cells were washed three times with PBS and incubated with Hoechst stain (blue) for 2 min at room temperature.
Figure S10: Amlexanox causes UPF1 to localize to cytoplasmic foci distinct from P-bodies. 6CFSMEo- cells were transfected with constructs expressing YFP-UPF1 before treatment with DMSO (left column) or 25µM amlexanox (right column) for 48 h. Cells were incubated sequentially with DCP1a antibody and Alexa Fluor 594 labeled secondary antibody (red). The percentage of colocalization between UPF1 and DCP1a is presented in a histogram at the bottom of the figure. Cells (N=10) from three different experiments were counted for each condition. Nuclei were stained with Hoechst solution (blue). Green arrows indicate UPF1 cytoplasmic foci. Red arrows indicate P-bodies; orange arrows indicate colocalization foci.
Figure S11: Wild-type GPx1 mRNA does not concentrate in readthrough bodies under G418 treatment. 6CFSMEo- cells were transfected with pCMV-GPx1 Norm and a construct expressing YFP-DCP1a (left panels) or YFP-UPF1 (right panels) before treatment of cells for 48 hours with G418.
Figure S12: Characterization of readthrough bodies. (A) Measure of the distance between P-bodies and readthrough bodies. Using ImageJ software, distances of P-bodies (DCP1a staining) from a readthrough body were measured in concentric areas centered on that readthrough body (no overlap between UPF1 staining and DCP1a staining) and represented on a boxplot. The measurement was performed with from 5 to 76 readthrough bodies from 6CFSMEo- cells treated with colchicine (COL), Taxotere (TAX), G418 or cytochalasin D (CytoD). (B) Dynamics of readthrough bodies (R-bodies). 6CFSMEo- cells were transfected with YFP-UPF1 and RFP-DCP1a constructs before exposure to cytochalasin D (CytoD) or G418 to induce readthrough body formation. A FRAP assay was then performed to measure the dynamics of readthrough bodies as compared to P-bodies. Upon cytochalasin D treatment, no fluorescence recovery was observed for P-bodies (purple line) or readthrough bodies (blue line). Upon G418 treatment fluorescence recovery was observed and measured for two P-bodies (red lines) and for two readthrough bodies (green lines).
Supplemental figure S13A

- CFTR mRNA
- GAPDH mRNA
- CFTR/GAPDH mRNA relative to siRNA control

- CFTR
- ACTIN
- CBP80
siRNA UPF1

**GPx1 Ter mRNA**

**DMSO**

**CytoD**

**G418**

**Supplemental figure S13D**

**Alexa 488-DCP1a**

**Hoechst**

**Merge**
Figure S13: Identification of factors affecting readthrough. (A and B) Actin downregulation inhibits NMD and activates readthrough in 6CFSMEo- cells. (A) The level of CFTR mRNA increased after downregulation of actin, as compared to the level measured in cells transfected with a control siRNA (upper panel). Concomitantly with the inhibition of NMD, readthrough is activated when actin is downregulated as observed by Western-blotting to detect CFTR protein (lower panel). (B) Cytoplasmic foci containing UPF1 or GPx1 Ter mRNA and excluding DCP1a are observed after downregulation of actin. (C-F) The novel cytoplasmic foci are not detected when UPF proteins are downregulated. 6CFSMEo- cells were transfected with pCMV-GPx1 46Ter and (C) siRNA control or siRNA UPF1 (D) or siRNA UPF2 (E). Twenty-four hours after transfection, they were incubated with DMSO, 1 µM CytoD, or 400 µg/ml G418 for 48 h. After fixation and permeabilization, cells were incubated overnight at 37°C with Cy3-red-labeled probes. They were then incubated with anti-DCP1a primary antibody followed by Alexa Fluor 488 labeled secondary antibody (green). Nuclei are stained with Hoechst solution (blue). Green arrows indicate P-bodies; red arrows indicate GPx1 mRNA cytoplasmic foci; orange arrows indicate colocalization of P-bodies and GPx1 mRNA. (F) Twenty-four hours after transfection with siRNA, cells were divided and transfected with a plasmid encoding MUP mRNA and a plasmid encoding YFP-GPx1 Norm or YFP-GPx1 46Ter. After 48 h, the cells were collected and their RNA was extracted. The levels of GPx1 and MUP mRNA were quantified by RT-PCR. The level of GPx1 mRNA was normalized to the level of MUP mRNA. Error bar=SD, Student’s t-test: *p<0.1.
Movie 1: Readthrough occurs in cytoplasmic foci containing UPF1 but devoid of DCP1a. The experimental procedure is described in the legend of Figure 8. The cellular localization of YFP-UPF1 is in white/green, that of RFP-DCP1a is in white/red and that of Gpx1-Neptune is in white/blue. Blue arrows indicate positions of newly synthesized readthrough Gpx1-Neptune, green arrows indicate UPF1-containing cytoplasmic foci, and red arrows show DCP1a-containing cytoplasmic foci (P-bodies).
Movie 2: Translation of wild-type mRNA is not confined to readthrough bodies. The experiment was performed as for Video S1 except for the use of an expression construct encoding wild-type GPx1-Neptune mRNA without any PTC. No Neptune signal is found in UPF1 (green) or DCP1a (red) foci under cytochalasin D treatment.
Movie 3: Readthrough does not occur when translation is inhibited. The experiment was performed as for Video S1 except for cells were treated with cycloheximide for 4 hours before to initiate the transcription of Neptune-GPx1 Ter RNA by doxycycline.