Figure legend - Supportive Information

FIG S1. Comparative analysis of httEx1-mRFP versus httEx1-EGFP transgene expression, polyQ aggregation and toxicity. A. Inclusion body (IB) formation is time- and polyQ length and time dependent in HeLa cells expressing HttEx1Q25, 47, 72 or 97mRFP. N=3, Mean ±SD. B. HttEx1Q25mRFP- and EGFP are homogeneously expressed in HeLa cells, whilst some cells expressing httEx1Q97mRFP- or EGFP form cytoplasmic IBs, as indicated by the arrows, and quantified for mRFP in (A). C. Western blots showing similar expression of httEx1Q25/Q72/Q97 fused to either EGFP (top panel) or mRFP (lower panel) over a 3 day time course (number 1-3 indicate days after transfection). A primary antibody detecting httEx1 (S830) and secondary antibody fluorescently labelled with Alexa Fluor 680 was used with subsequent detection and quantification using LICOR (see methods). Quantification of several experiments (N=3) did not show a difference between EGFP versus mRFP transgenes on each day (data not shown). D. There is no difference in inclusion body (IB) formation for httEx1Q97EGFP and httEx1Q97mRFP expressing HeLa cells over time. N= 4-6, ±SD. E. HttEx1Q97EGFP- or mRFP SDS-insoluble material similarly accumulates over time in HeLa cells (detection by an anti-httEx1 antibody, S830), whilst no SDS-insoluble material is detected for httEx1Q25EGFP- or mRFP transfected cells. Quantification between httEx1Q97EGFP versus mRFP showed no statistical significant difference (N=3, data not shown). G. HttEx1Q25/97mRFP expressing cells were fixed and subjected to immunofluorescence analysis using an anti-cytochrome c (cyt c) antibody followed by staining with an Alexa 488 secondary antibody. Cells showing homogenous-or no cyt c staning as opposed to mitochondrially localised staining were counted as cells with cyt c release, N=3, ±SD. H. HttEx1Q25/97mRFP expressing cells were fixed and immunostained with an anti-active caspase-3 antibody followed by Alexa 488 staining. Cells with clearly elevated signals compared to cell not treated with the primary antibody were counted as positive, N=3, ±SD. Student's t-test (unpaired) * $p<0.05$.

FIG S2. Live-cell confocal ROS analysis. A. Example of a confocal image taken sequentially of living HeLa cells transfected with either httEx1Q25mRFP- or Q97mRFP followed by incubation with CM-H2DCF-DA, typically used for analysis (see material and methods). Only mRFP-positive cells were selected for subsequent analysis. B. Image of HeLa cells expressing either httEx1Q25EGFP- or Q97EGFP as in (A), but incubated with dihydroethidium (DHE) as described in method sections. C. Raw data for a representative experiment measuring mean DCF (ROS) production of untransfected HeLa cells versus cells expressing httEx1Q25mRFP or

httEx1Q97mRFP at the 24h time point, Number of cells, N=50-60, Means ±SD, Student's t-test (unpaired) * $p<0.05$.

FIG S3. Early rise in ROS precedes toxicity in PC12 tebufenozide inducible httEx1Q103EGFP cells and antioxidants protect against toxicity. A. Examples of PC12 cells after 48h of induction with tebufenozide expressing either httEx1Q25EGFP (left) or Q103EGFP (right). Inclusion body indicated by the white arrow. B. Quantification of httEx1Q25 (green line)- and Q103EGFP (red line) toxicity over 3 days measured as the percentage of cells showing nuclear abnormalities. Also shown is the percentage of httEx1Q103EGFP cells containing an inclusion body (black line). N=3, Means ±SD. C. Fold change in DHE oxidation in cells expressing httEx1Q103EGFP compared to httEx1Q25EGFP control cells. Expression of expanded httEx1-EGFP induces ROS at 10-24h when no toxicity does occur (see B), but polyQ aggregation (IB formation) is present in some cells. N=3, Means ±SD. D. L-NAC and Trolox treatment protect PC12 cells against Q103EGFP toxicity. Cells were induced with tebufenozide for 3 days in the presence of absence of antioxidants. N=2, Means ±SD, Student's t-test (unpaired) * $p<0.05$, ** $p<0.01$.

FIG S4. PolyQ oligomerisation in the ponasterone A inducible cell system occurs between 3-6 hours after induction. A. SDS-PAGE showing a typical time course of httEx1Q72EGFP transgene induction. Leakage of the transgene under uninduced conditions is detectable (band in 0h lane). No other bands are detectable on the SDS-PAGE gel at any time point, indicating that any oligomeric species are SDS soluble or are too large to enter the gel. B. Native gel shows that after 6h post transgene induction prominent bands appear that are of a higher molecular weight compared to the expected monomeric size of httEx1Q103EGFP (see lane 0h and lane 3h) indicating oligomerisation (small oligomers). After 24h of induction the bands corresponding to small oligomers are no longer present and a band at the top of the gel indicates that larger oligomers are the predominant species. Native gel markers were co-migrated (indicated by the arrows). C. Dot Blot analysis indicates that 3h after induction of the httEx1Q72EGFP transgenes SDS- insoluble material (>200nm) is also detectable that is markedly increased at 24h. Lower lane 0.5 x loading compared to upper lane. Cell lysates for A-C were taken in a parallel experiment and results are directly comparable. Representative images of 2-3 independent experiments with similar findings are shown.

FIG S5. Comparable kinetics of IB formation and toxicity due to httEx1Q97EGFP or Q81-GFP expression. A. IB formation in HeLa cells expressing httEx1Q97EGFP (blue line) or

Q81-GFP (green line) constructs, N=3, Means ±SD. B. Toxicity, measured as cells showing nuclear abnormalities, is similar in cells transfected with httEx1Q25/Q97EGFP constructs versus cells expressing Q19/Q81-GFP constructs, N=3, Means ±SD.

FIG S6. Pgl-135 suppresses polyQ aggregation and does not act as an antioxidant. A. Dot blot analysis detecting SDS-insoluble protein of HeLa cells transfected with httEx1Q97mRFP and either untreated or treated with increasing concentrations of Pgl-135. Note that at a concentration of 50μM Pgl-135 no polyQ protein is detectable using an anti-httEx1 antibody (S830). B. Pgl-135 (50μM) reduces httEx1Q97mRFP inclusion body formation over time, N=3, Means ±SD. C. Pgl-135 (50μM) is unable to rescue HeLa cell from cell death induced by increasing concentrations of hydrogen peroxide, N=3, Means ±SD. D. Pgl-135 (50μM) does not reduce basal levels of ROS production in cells expressing httEx1Q25EGFP as measured by pixel intensity of DHE fluorescence (raw values shown), N=3, Means ±SEM. Student's t-test (unpaired) * $p<0.05$, ** $p<0.01$. E. Pgl-135 dose dependently reduces IB formation in PC12 cells induced with ponasterone for 24h. Level of IBs of untreated, induced cells taken as 100% and percentage reduction of IBs under Pgl-135 treatment is shown, N=2, Means ±SEM. Student's t-test (unpaired) * $p<0.05$.

FIG S7. MW7 intrabody is co-expressed with httEx1 polyQ mRFP constructs and inhibits IB formation. A. HeLa cells transfected with httEx1Q25/97mRFP constructs and subsequent transfection with a plasmid expressing the MW7 intrabody (see methods) were stained with an antibody detecting FLAG-tagged MW7 (anti-FLAG antibody). mRFP-positive cells (arrows) show immunoreactivity against MW7 confirming co-expression of the two plasmids (arrowheads: large inclusion bodies). B. mRFP positive HeLa cells, transfected as described in (A), were analysed and the percentage of mRFP +ve cells containing an inclusion body (IB) quantified over time. MW7 co-expression significantly reduced the formation of IBs, N=2, Means ±SD. Student's t-test (unpaired), ** $p<0.01$.

FIG S8. Details of oligomeric and protofibrilar structures formed by httExQ53 after *in vitro* cleavage for 5hours and AFM analysis.

FIG S9. Mean relative number of oligomers without or with Pgl-135 compound or MW7 antibody incubation. Six images for each condition were chosen randomly and oligomers of an

apparent size of 100nm or smaller were selected and the mean number in each 10 micrometer square was calculated. Means ±SD. Student's t-test (unpaired) * $p<0.05$, ** $p<0.01$.

FIG S10. *In vitro* aggregation of Aβ1-40 and Aβ1-42 is associated with hydrogen peroxide production. A. AFM scans showing Aβ1-40 (upper panel) or Aβ1-42 (lower panel) aggregation over a time course of 3 days. Note the earlier aggregation of Aβ1-42 compared to Aβ1-40 (representative images are shown). B. Production of hydrogen peroxide (H_2O_2) due to incubation of 50μM Aβ1-40 (blue line) or Aβ1-42 (red line), N=2-3, Means ±SEM.

FIG S11. H2A.X Serine-139 phosphorylation is increased in cell expressing httEx1Q97-mRFP compared to httEx1Q25-mRFP at day 1. A. Control experiment showing HeLa cells treated with/without AraC (200μM) for 24h to induce DNA damage as indicated by increased nuclear H2A.X-Ser139 staining**.** B. Examples of cells expressing either httEx1Q25-mRFP (upper panel) or httEx1Q97-mRFP (lower panel, inclusion body containing cells) that were fixed at day 1 after transfection and stained in parallel with H2A.X-Ser139 specific antibody. Increased staining is visible in nuclei of cells expressing httEx1Q97-mRFP. C. Quantification of H2A.X-Ser139 nuclear staining as shown in (B) expressed as pixel intensity ratios of nuclear/cytoplasmic immunoreactivity. The mean pixel intensity ratios of all cells analysed are shown from 2-3 independent experiments (10-30 cells analysed for each experiment), Means ±SD. Student's t-test (unpaired), * $p<0.05$.

FIG S12. Antioxidants do not modulate IB formation. A. HeLa cells were transiently transfected and either untreated (black bars), treated with L-NAC (red bars) or treated with Trolox (blue bars) for 1, 2 or 3 days. B. PC12 tebufenozide inducible cells were treated with the inducer (tebufenozide) for 24h and left untreated or treated with L-NAC or Trolox. C. Ponasterone inducible PC12 cells were induced and left untreated of treated with L-NAC or Trolox for 24h. At the end of each experiment cells were fixed and IB containing cells scored as positive. Exact description of treatments as for other experiments is described in Material and Methods. Means ±SD, N=2-3.

Figure S1

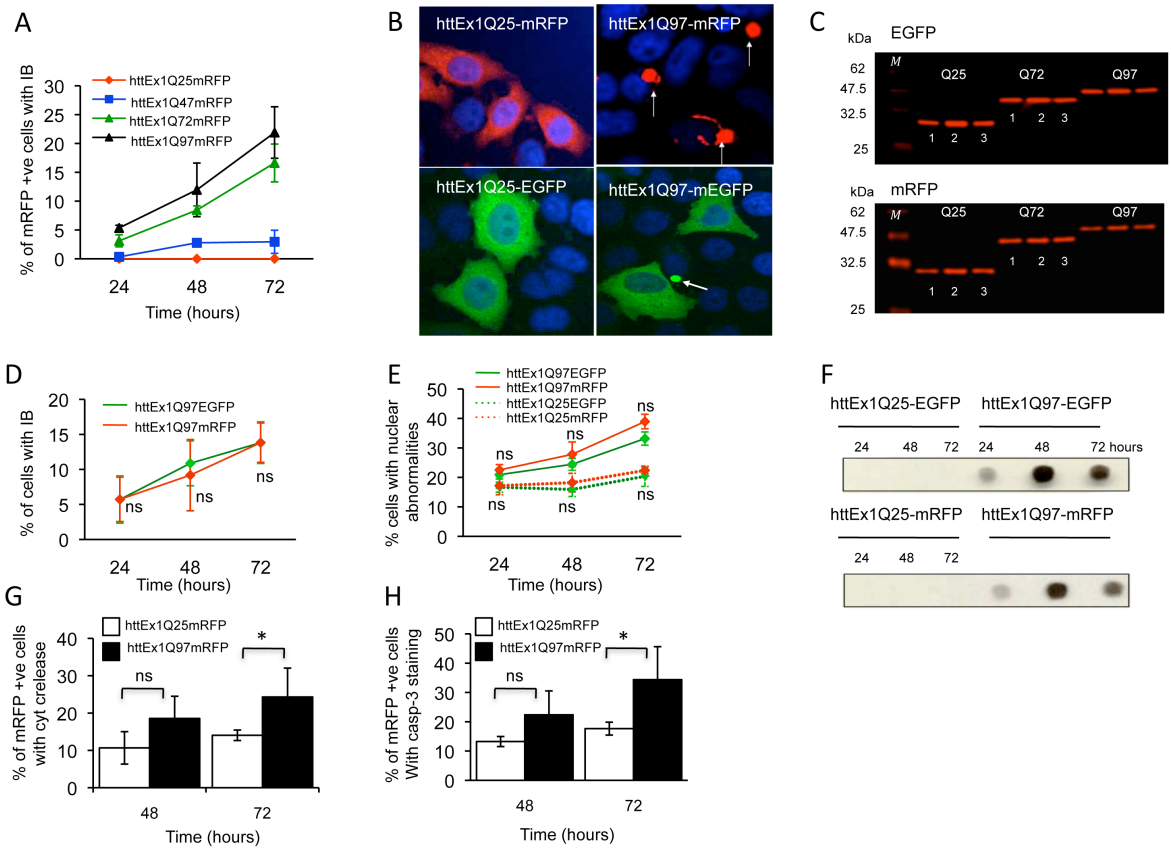

A
% of mRFP +ve cells with IB
httEx1Q25mRFP
httEx1Q47mRFP
httEx1Q72mRFP
httEx1Q97mRFP
Time (hours)
B
httEx1Q25-mRFP
httEx1Q97-mRFP
httEx1Q25-EGFP
httEx1Q97-mEGFP
C
kDa
EGFP
M
Q25
Q72
Q97
mRFP
D
% of cells with IB
httEx1Q97EGFP
httEx1Q97mRFP
ns
E
% cells with nuclear abnormalities
httEx1Q25EGFP
httEx1Q25mRFP
F
httEx1Q25-EGFP
httEx1Q97-EGFP
72 hours
httEx1Q25-mRFP
httEx1Q97-mRFP
G
% of mRFP +ve cells with cyt crelease
*
H
% of mRFP +ve cells With casp-3 staining

Figure 2

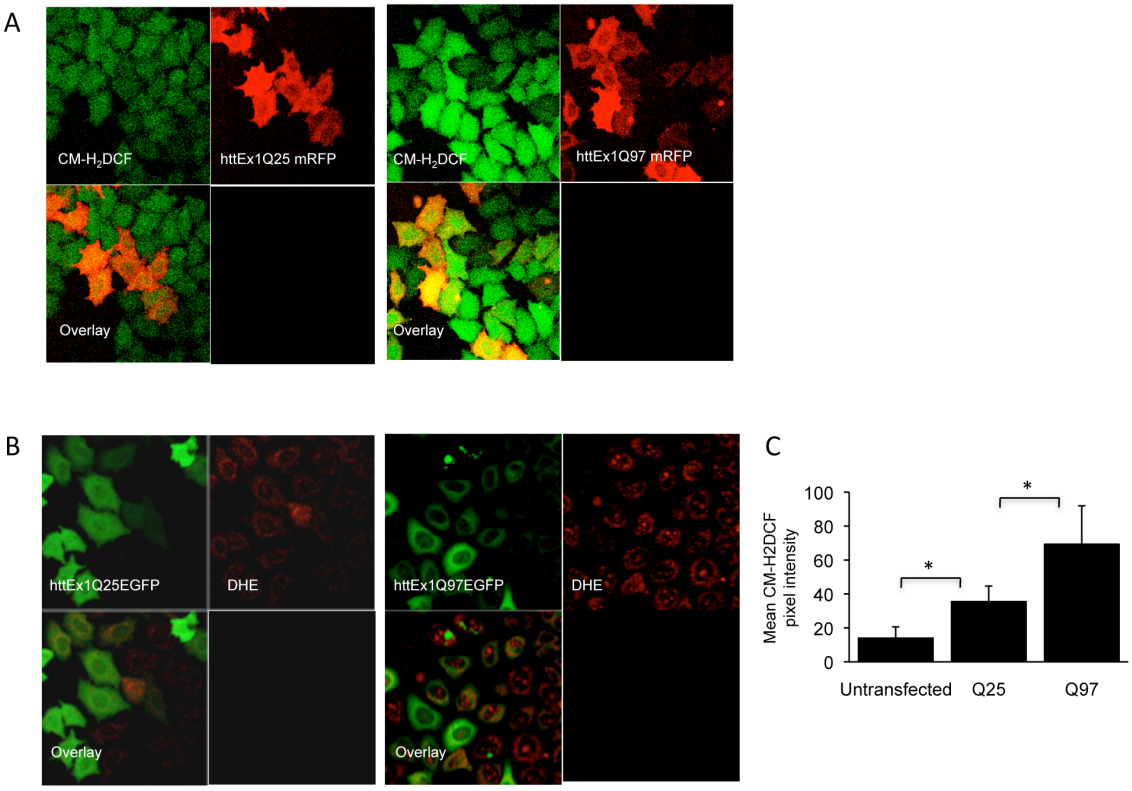

A
CM-H2DCF
httEx1Q25 mRFP
Overlay
CM-H2DCF
httEx1Q97 mRFP
Overlay
B
httEx1Q25EGFP
DHE
Overlay
httEx1Q97EGFP
DHE
Overlay
C
Mean CM-H2DCF pixel intensity
100
80
60
40
20
0
*
*
Untransfected
Q25
Q97

Figure 3

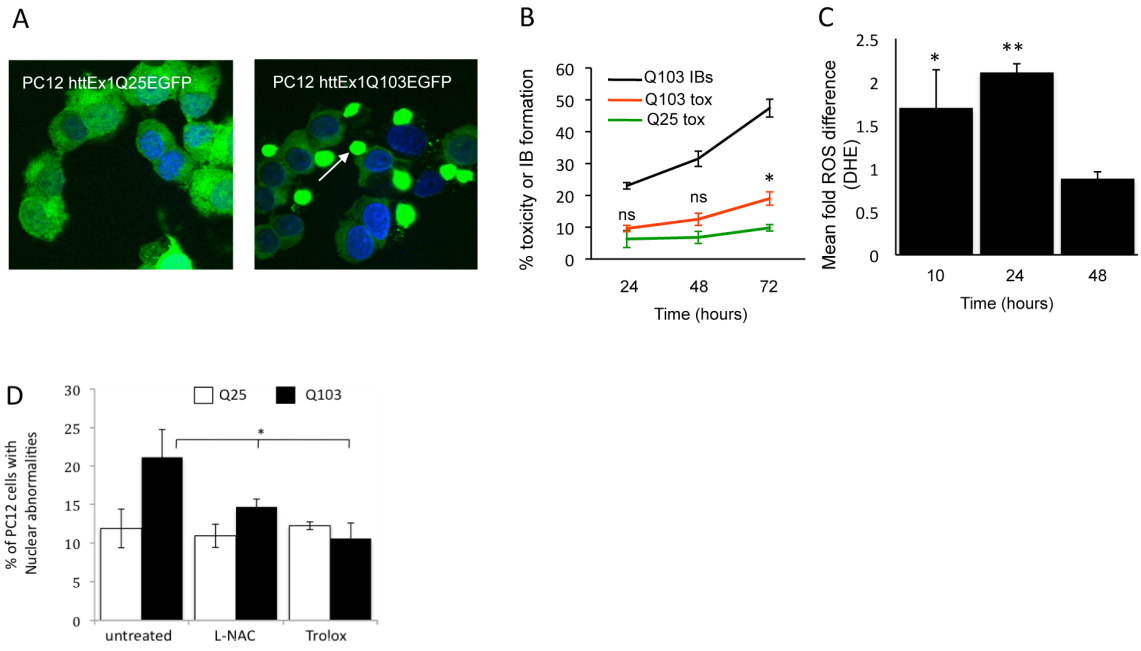

A
PC12 httEx1Q25EGFP
PC12 httEx1Q103EGFP
B
% toxicity or IB formation
Q103 IBs
Q103 tox
Q25 tox
ns
ns
*
Time (hours)
C
Mean fold ROS difference (DHE)
*
**
Time (hours)
D
% of PC12 cells with Nuclear abnormalities
Q25
Q103
*
untreated
L-NAC
Trolox

Figure 4

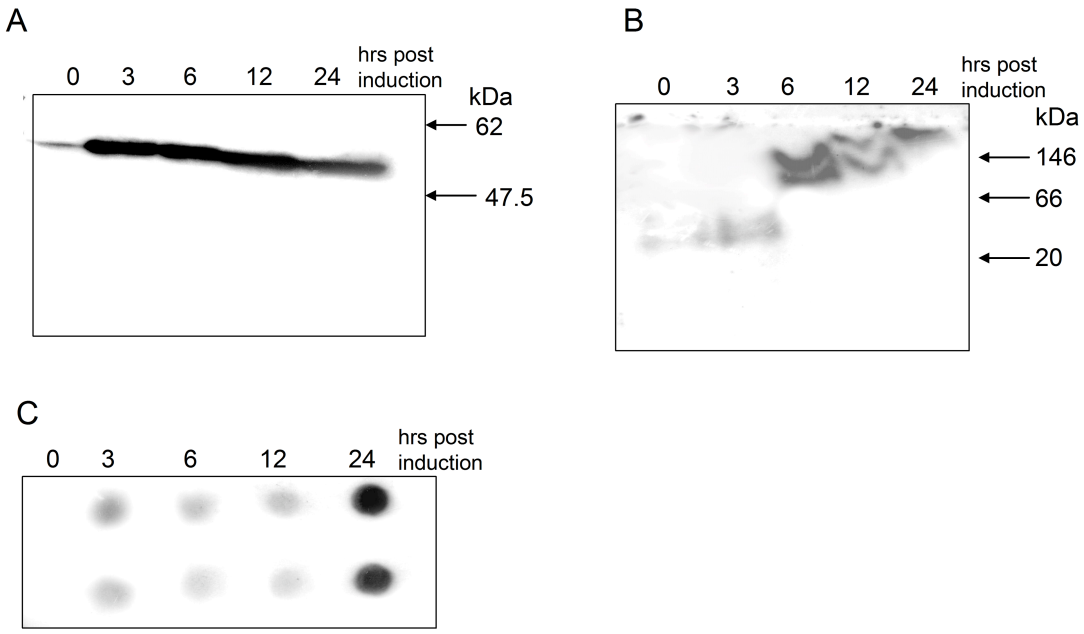

A
0 3 6 12 24
hrs post induction
kDa
62
47.5
B
0 3 6 12 24
hrs post induction
kDa
146
66
20
C
0 3 6 12 24
hrs post induction

Figure 5

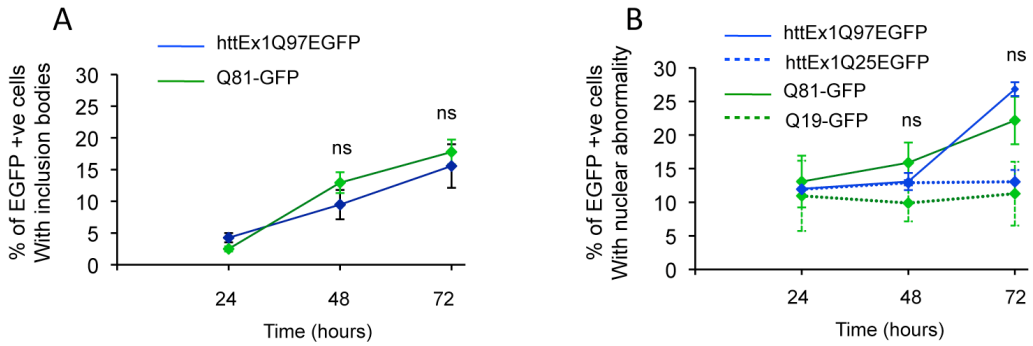

A
httEx1Q97EGFP
Q81-GFP
% of EGFP +ve cells
With inclusion bodies
30
25
20
15
10
5
0
ns
ns
24
48
72
Time (hours)
B
httEx1Q97EGFP
httEx1Q25EGFP
Q81-GFP
Q19-GFP
% of EGFP +ve cells
With nuclear abnormality
ns
ns
24
48
72
Time (hours)

Figure 6

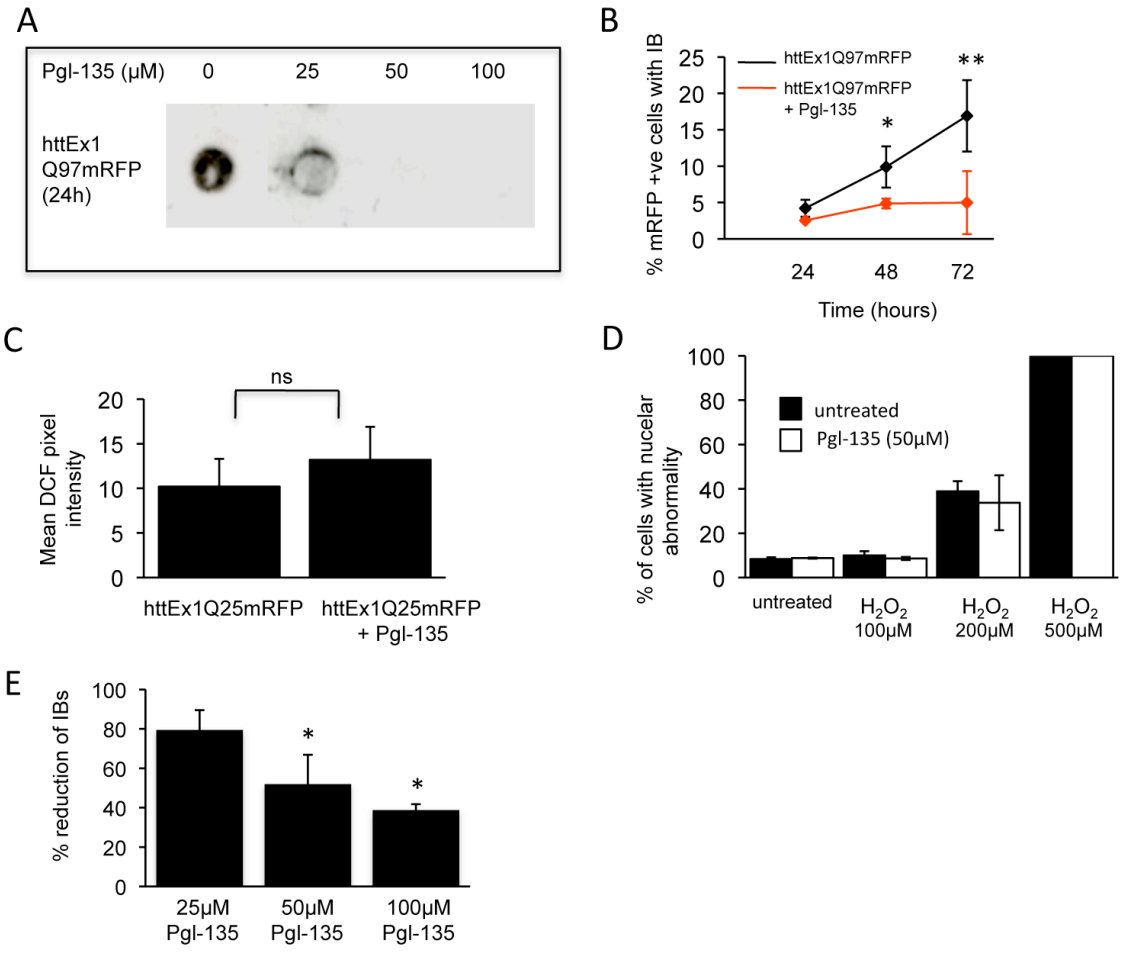

A
Pgl-135 (μM) 0 25 50 100
httEx1 Q97mRFP (24h)
B
% mRFP +ve cells with IB
httEx1Q97mRFP
httEx1Q97mRFP + Pgl-135
**
*
Time (hours)
C
Mean DCF pixel intensity
ns
httEx1Q25mRFP
httEx1Q25mRFP + Pgl-135
D
% of cells with nucelar abnormality
untreated
Pgl-135 (50μM)
untreated
H2O2 100μM
H2O2 200μM
H2O2 500μM
E
% reduction of IBs
25μM Pgl-135
50μM Pgl-135
100μM Pgl-135

Figure 7

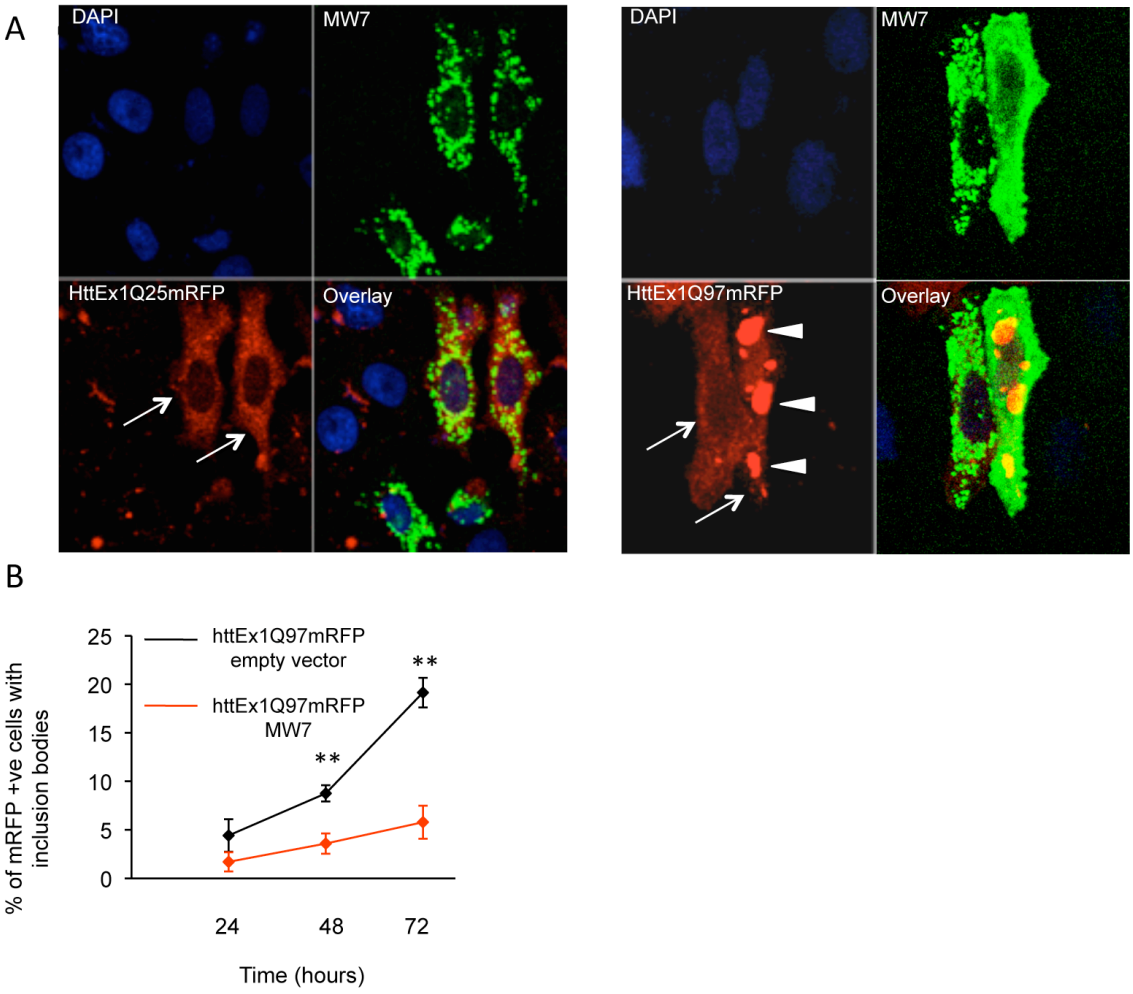

A
DAPI
MW7
HttEx1Q25mRFP
Overlay
DAPI
MW7
HttEx1Q97mRFP
Overlay
B
% of mRFP +ve cells with inclusion bodies
25
20
15
10
5
0
httEx1Q97mRFP
empty vector
httEx1Q97mRFP
MW7
**
**
24
48
72
Time (hours)

Figure 8

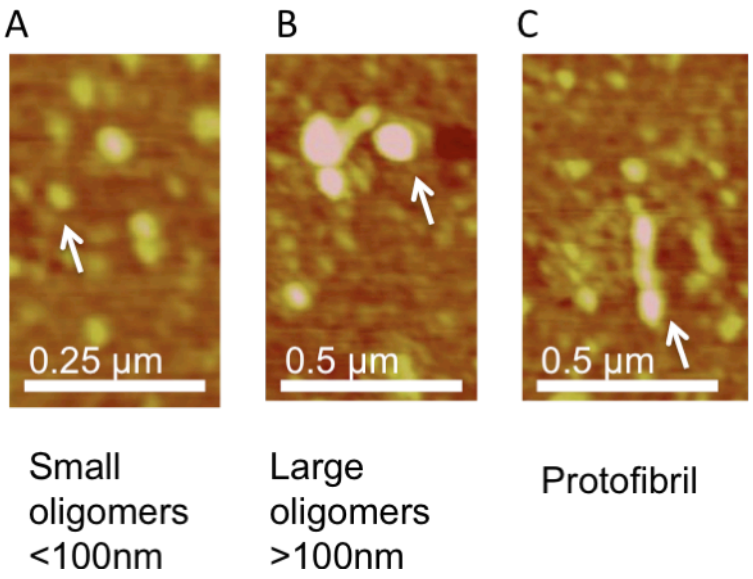

A
B
C
0.25 µm
0.5 µm
0.5 µm
Small oligomers <100nm
Large oligomers >100nm
Protofibril

Figure 9

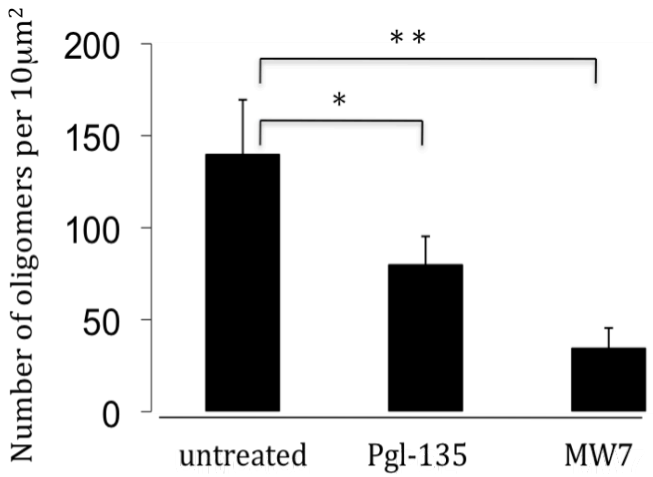
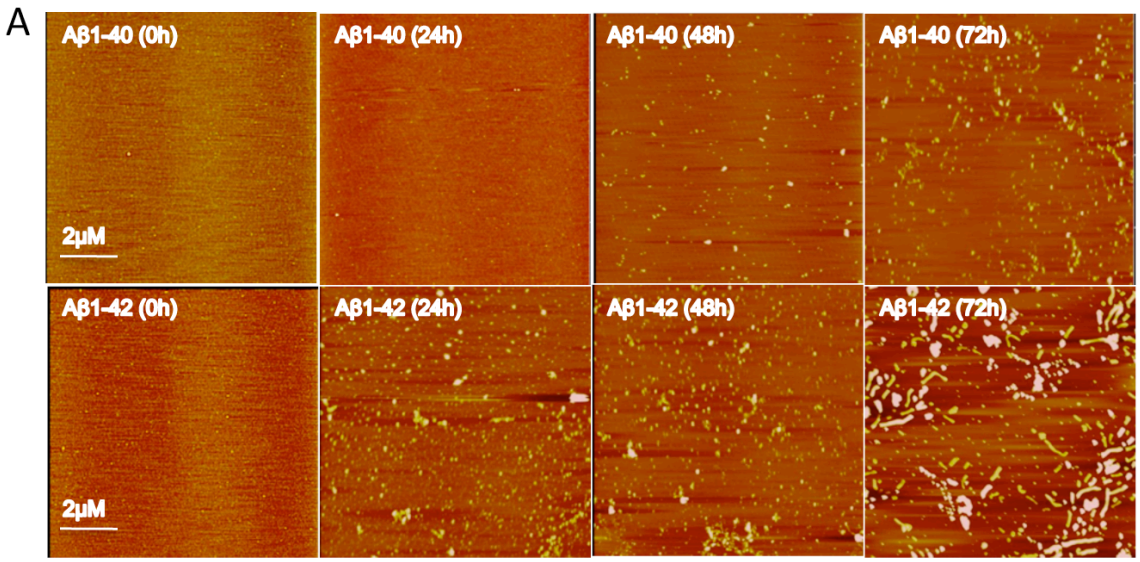

Number of oligomers per 10μm²
200
150
100
50
0
* *
*
untreated
Pgl-135
MW7

Figure 10


A
Aβ1-40 (0h)
Aβ1-40 (24h)
Aβ1-40 (48h)
Aβ1-40 (72h)
2μM
Aβ1-42 (0h)
Aβ1-42 (24h)
Aβ1-42 (48h)
Aβ1-42 (72h)
2μM

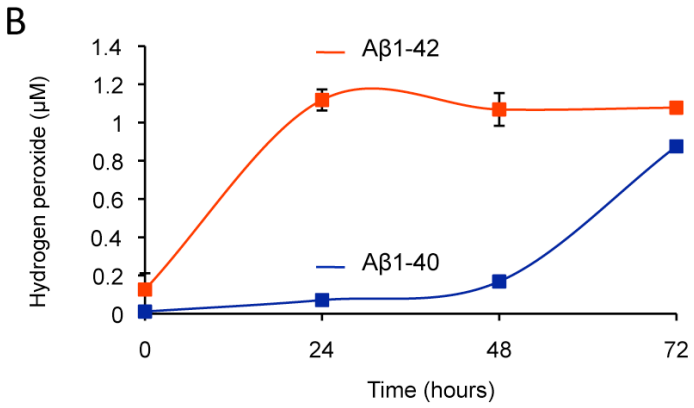

B
Hydrogen peroxide (μM)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Aβ1-42
Aβ1-40
0
24
48
72
Time (hours)

Figure 11

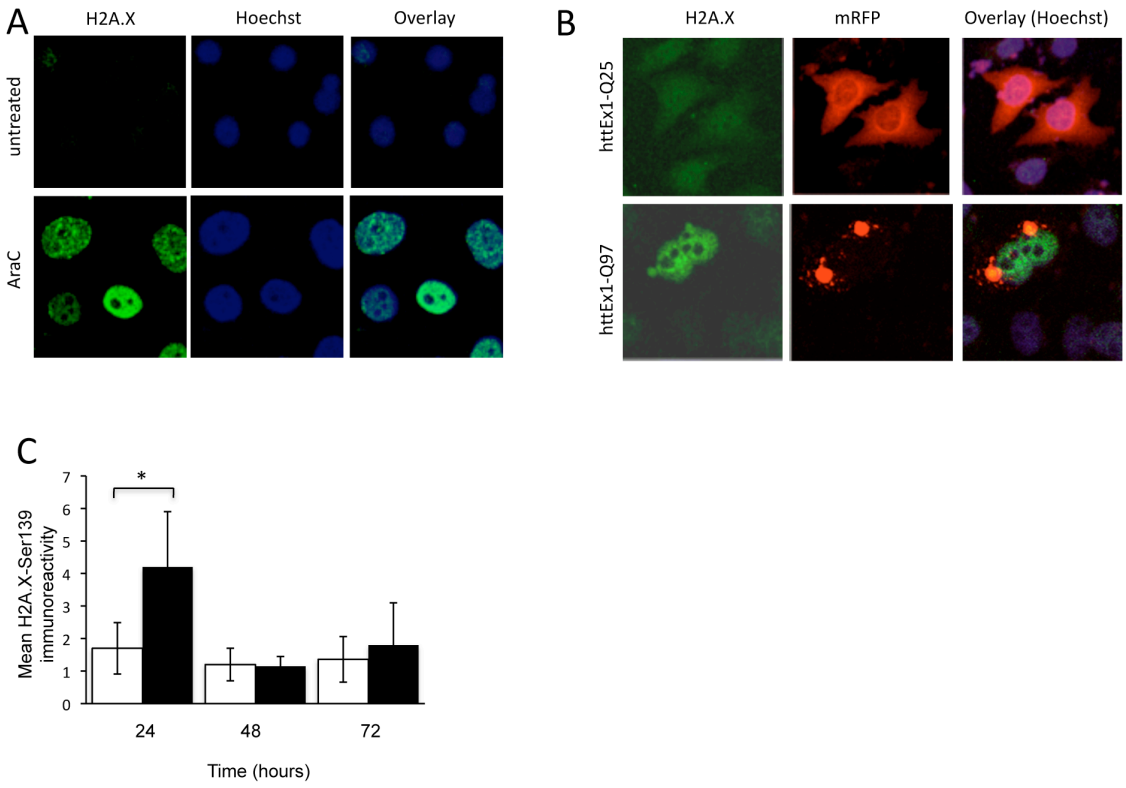
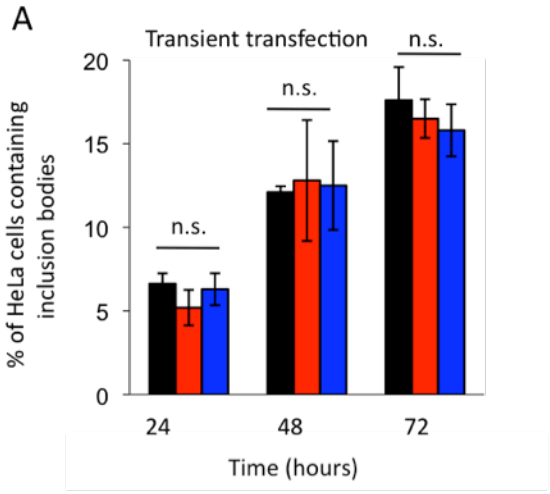
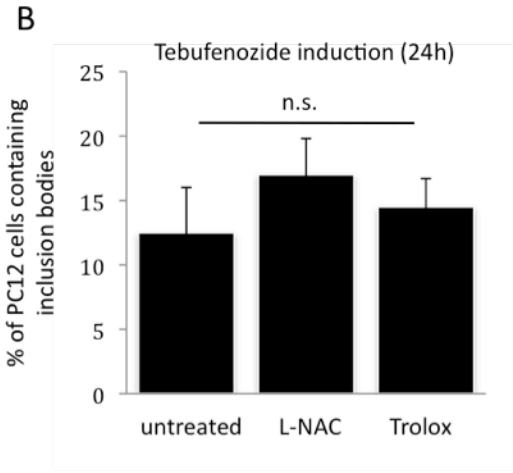
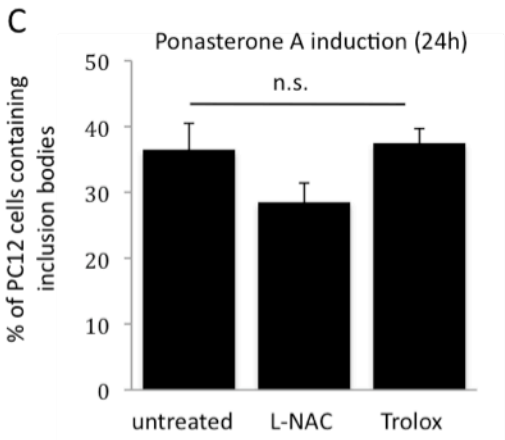

A
H2A.X
Hoechst
Overlay
untreated
AraC
B
H2A.X
mRFP
Overlay (Hoechst)
httEx1-Q25
httEx1-Q97
C
Mean H2A.X-Ser139 immunoreactivity
0
1
2
3
4
5
6
7
*
24
48
72
Time (hours)

Figure 12


A
Transient transfection
n.s.
n.s.
n.s.
% of HeLa cells containing inclusion bodies
20
15
10
5
0
24
48
72
Time (hours)


B
Tebufenozide induction (24h)
n.s.
% of PC12 cells containing inclusion bodies
25
20
15
10
5
0
untreated
L-NAC
Trolox


C
Ponasterone A induction (24h)
n.s.
% of PC12 cells containing inclusion bodies
50
40
30
20
10
0
untreated
L-NAC
Trolox