

## Supplementary information for

### **A biosensor of protein foldedness identifies increased “holdase” activity of chaperones in the nucleus following increased cytosolic protein aggregation**

Candice B. Raeburn, Angelique R. Ormsby, Dezerae Cox, Chloe A. Gerak, Christian Makhoul, Nagaraj S. Moily, Simon Ebbinghaus, Alex Dickson, Gawain McColl and Danny M. Hatters

#### **This document contains:**

**Fig S1. Linker control to assess impact of cellular localization on FRET.**

**Fig S2. Assessment of aggregation and derivation of the  $A_{50\%}$  values.**

**Fig S3. Upregulation of HSPA1A and DNAJB1 upon over expression.**

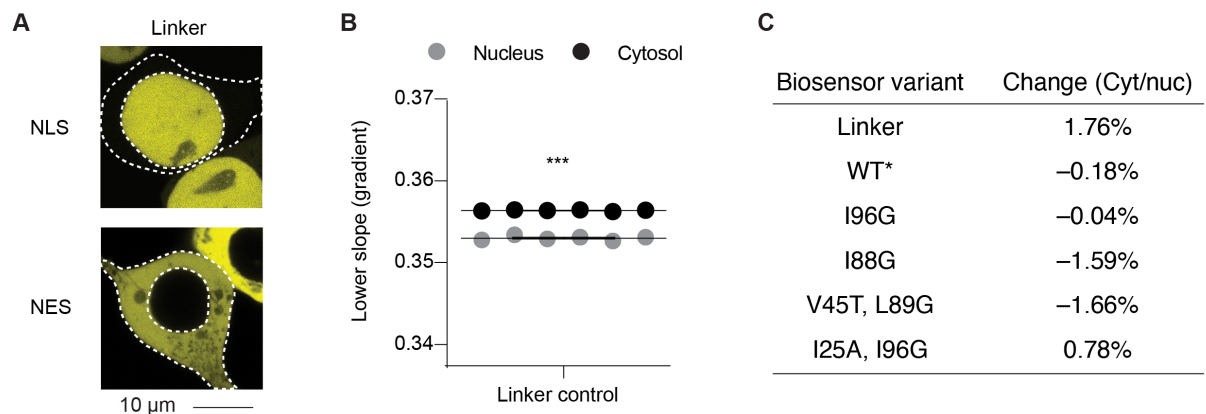
**Fig S4. Pelleting assay showing affect of chaperone overexpression on aggregation.**

**Fig S5. VER-155008 affects cell viability.**

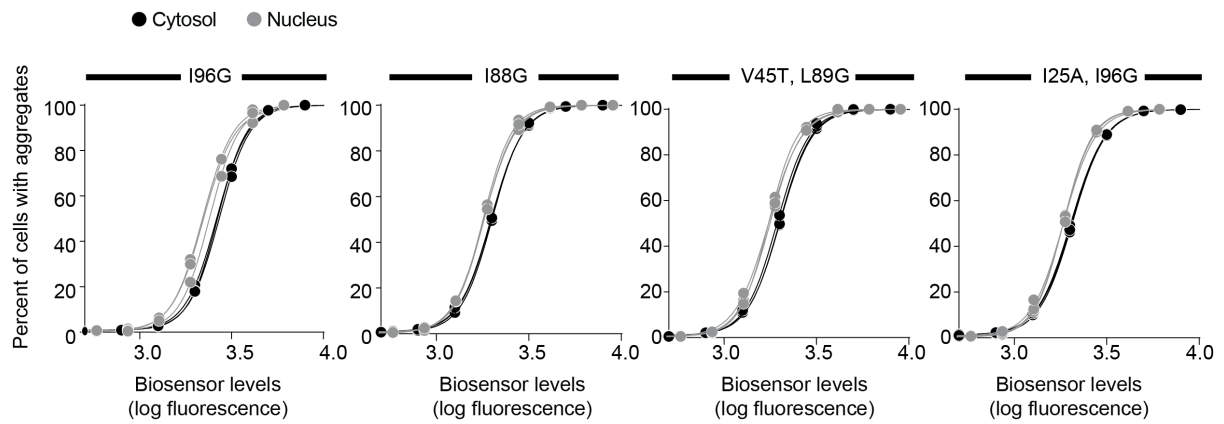
**Fig S6. Co-localization patterns of mTFP1 and Venus with Httex1 inclusions.**

#### **An additional file includes:**

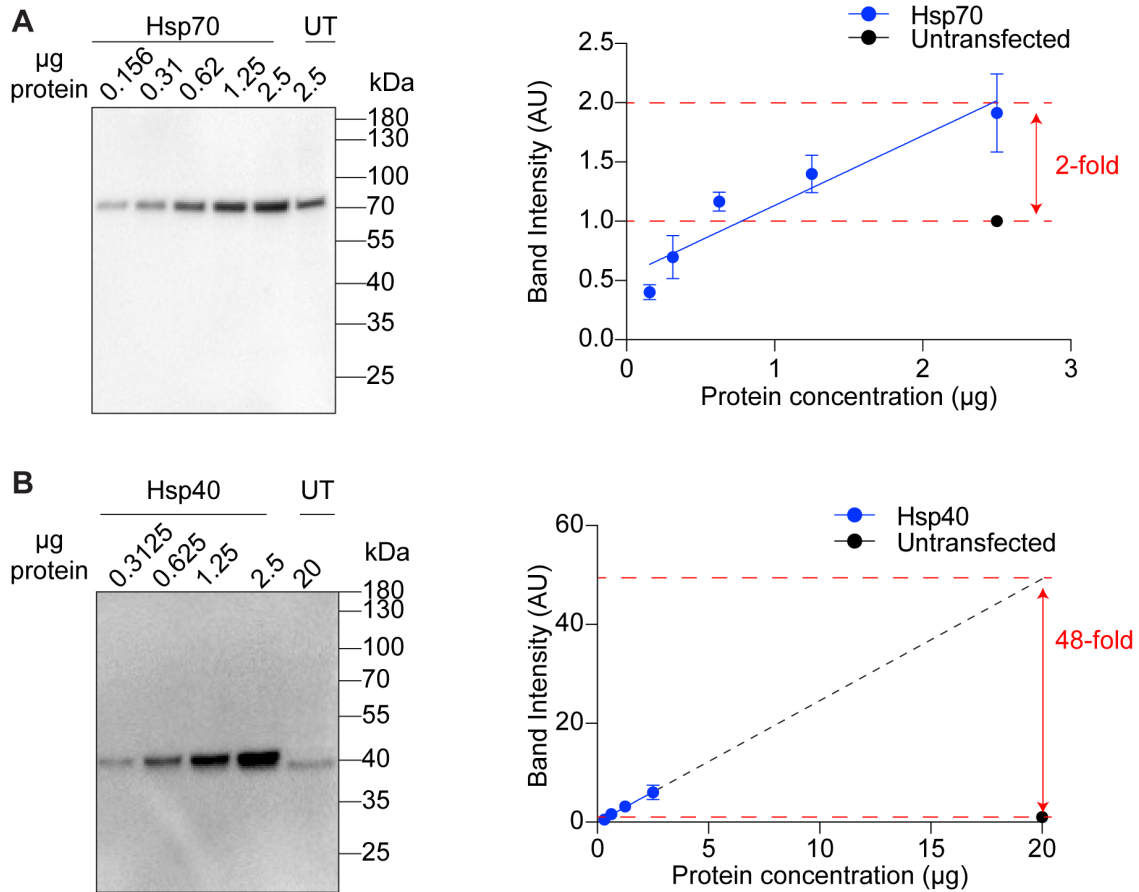
**Dataset S1. Raw flow cytometry data used to calculate data in Figure 2.**



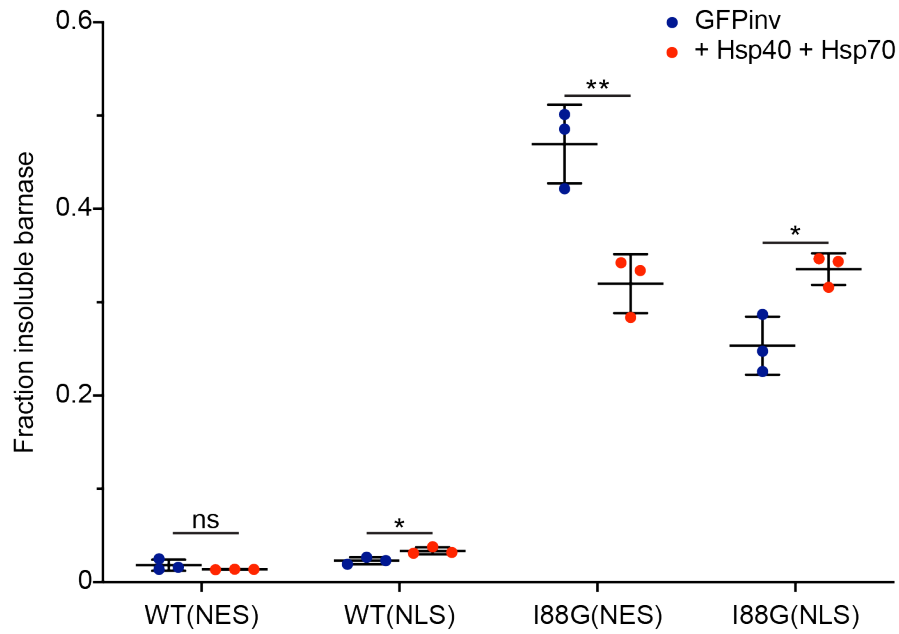
**Fig S1. Linker control to assess impact of cellular localization on FRET.** **A.** Confocal images of HEK293T cells transfected with the linker constructs. The dashed lines show the boundaries of the nucleus and cell. **B.** Lower slope values of the linker construct. Individual biological replicates shown with means  $\pm$  S.D. and with differences (nucleus v cytosol) assessed by Student's t-test (2-tailed); \*\*\*  $p < 0.001$ , ns  $\Rightarrow 0.05$ . **C.** Table of the difference in lower slope values for the different biosensor variants. Values indicate change (in %) between the NLS and NES biosensor forms for each variant shown for the data in Fig S1B (for linker) and for Fig 2B for the other variants.



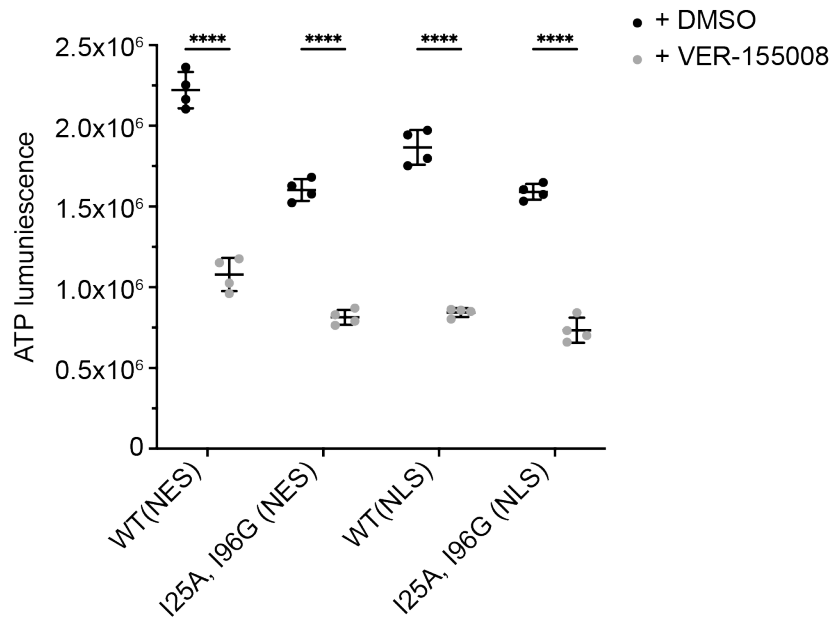
**Fig S2. Assessment of aggregation and derivation of the  $A_{50\%}$  values.** The raw flow cytometry data for each mutant is shown in **Dataset S1**. Shown here are example histograms extracted from that data showing percent cells in the upper slope binned into different biosensor levels (based on Venus “donor” fluorescence). The percents are derived from the number of cells in the upper slope of a bin, divided by the sum of the cells in the upper and lower slopes in a bin. Curves are fits to Hill equation. Data were fitted independently within each replicate dataset (n=3 biological replicates).



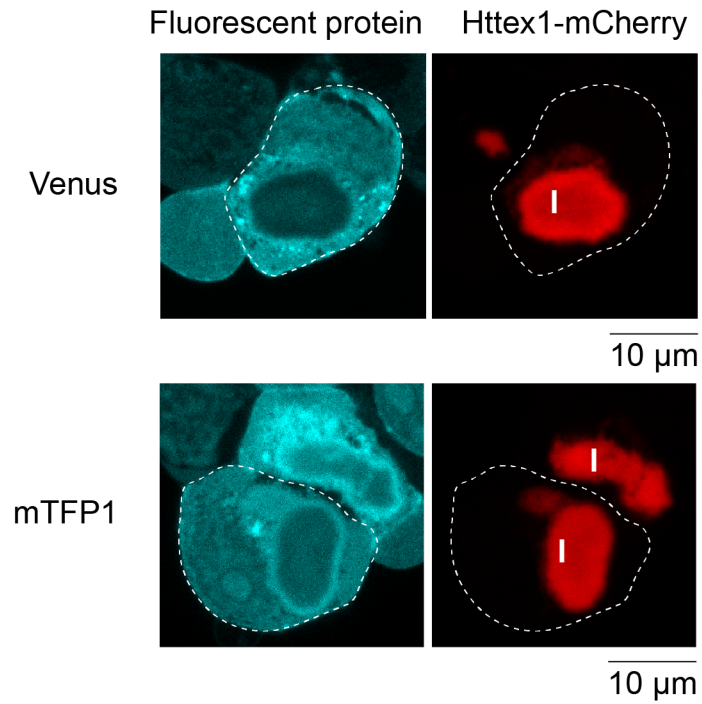
**Fig S3. Upregulation of HSPA1A and DNAJB1 upon over expression.** **A.** Representative Western Blot (left panel) comparing endogenous Hsp70 (untransfected (UT) HEK293T cells) to different amounts of total protein in cells transfected with Hsp70. Blots were probed with anti-Hsp70 antibody. The graph shows the Western Blot band intensities normalised to untransfected control (right panel) with linear regression analysis shown (blue line). N=3 biological replicates and Blots. Means  $\pm$  S.D. shown. **B.** Representative Western blot (left panel) comparing endogeneous Hsp40 to cells transfected with Hsp40. Blots were probed with anti-Hsp40 antibody. Graph plotting western blot band intensities normalised to untransfected control (right panel) with linear regression analysis shown (blue line) and extrapolation out to 20  $\mu$ g protein (black dotted). N=3 biological replicates and Blots. Means  $\pm$  S.D. shown.



**Fig S4. Pelleting assay showing affect of chaperone overexpression on aggregation.** Comparison of cell lysate from HEK293T cells co-transfected with the biosensors, DNAJB1 and HSPA1A or control (a non-fluorescent derivative of GFP [Y66L Emerald; GFPinv] 48 hours post-transfection. Differences (means and SD shown of three biological replicates) assessed by t-test with significance defined as  $p < 0.05$  (and \*\*\*\* for  $p < 0.0001$ , \*\*\* for  $p < 0.001$ ).



**Fig S5. VER-155008 affects cell viability.** Data shows a measure of cell viability (CellTitre Glo assay) of HEK293T cells transfected with barnase biosensor treated with 20  $\mu$ M VER-155008 or DMSO vehicle control. Differences (means and SD shown of three biological replicates) assessed by 2-way ANOVA with significance defined as  $p < 0.05$  (and \*\*\*\* for  $p < 0.0001$ , \*\*\* for  $p < 0.001$ ).



**Fig S6. Co-localization patterns of mTFP1 and Venus with Httex1 inclusions.** Shown are two fluorescence channels of confocal micrographs of HEK293T cells co-transfected with mTFP1 or Venus with Httex1<sub>97Q</sub>-mCherry and which contain an inclusion of Httex1 (marked as I on the figure). The outline of representative cells are shown (white dashed lines).