

The chaperone HSPB1 prepares protein aggregates for resolubilization by HSP70

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Supplementary Figure Legends

Figure S1. Intermolecular luciferase-luciferase and LDH-LDH contacts form in co-aggregates with HSPB1-3D. (a) Cross-linking analysis of co-aggregates composed of 2 μ M luciferase and 20 μ M HSPB1-3D. Samples were incubated at 4 °C or under heat-shock (HS) at 45 °C, in the absence or presence of HSPB1-3D and submitted to cross-linking reactions using the indicated concentrations of DSS and then analyzed by immunoblot (IB). Monomeric species that did not cross-link, as well as cross-linked aggregates or oligomers are indicated. (b, c) Cross-linking analysis of co-aggregates composed of 2 μ M LDH and 20 μ M (b) HSPB1-3D or (c) HSPB1-3D-GxG. Reactions were conducted as in a, except under heat shock (HS) at 55 °C.

Figure S2. HSPB1 remains mainly as a heterogeneous oligomer during disaggregation. (a-c) The elution patterns of (a) HSPB1 before the heat shock (preHS) or in the disaggregation reactions with (b) luciferase or (c) LDH were assessed by immunoblot (IB). (d, e) Quantification of HSPB1 in the reactions with (d) luciferase and (e) LDH from b and c are shown as percentage of the total amount of immunoblot signal. Error bars show standard deviations, $n \geq 3$.

Figure S3. Heat shocked HSPB1-3D forms oligomeric species in the absence of substrate. (a) Elution patterns of HSPB1 in disaggregation reactions in the absence of substrate. Reactions were performed as in Fig. 4f, except with heat shock at 55 °C. The elution patterns before or after heat shock were assessed at the start (0 h) and end (3 h) of disaggregation reactions with or without

chaperones by SEC and immunoblot (IB). **(b)** Quantification of HSPB1 from a in eluted fractions are shown as percentage of the total amount of immunoblot signal. **(c)** Elution patterns of HSPB1-3D in disaggregation reactions in the absence of substrate as in a. **(d)** Quantification of HSPB1-3D from c in eluted fractions are shown as percentage of the total amount of immunoblot signal. Error bars show standard deviations, $n \geq 3$.

Figure S4. Luciferase disaggregation with excess HSPB1-3D. **(a)** Disaggregation of luciferase aggregates with 20 or 80 μM HSPB1-3D. The aggregates were formed at 45 °C for 15 min and transferred to disaggregation reactions containing buffer (- Chap) or the chaperone disaggregation machinery (+ Chap) and ATP. Aggregate resolubilization at the start (0 h) and end (3h) of the disaggregation reactions was analyzed by SEC and immunoblot (IB). **(b-d)** Quantification of **(b)** luciferase and HSPB1-3D at **(c)** 20 μM and **(d)** 80 μM from a in eluted fractions are shown as percentage of the total amount of immunoblot signal. Error bars show standard deviations, $n \geq 3$.

Figure S5. Luciferase refolding is dependent on the HSP70-DJA2 chaperone system while LDH refolding is spontaneous. Guanidine-denatured **(a, b)** luciferase or **(c, d)** LDH was diluted into refolding reactions containing HSP70 and the indicated combinations of co-chaperones. **(a, c)** Kinetics of substrate refolding was determined by measuring substrate enzymatic activity recovery over time. **(c)** Luciferase and **(d)** LDH reactivation as in a and c, after 60 min of refolding reaction with different combinations of chaperones. Native substrate activity was set as maximum activity (100 %). Reactions in the absence of chaperones or HSP70 were used as controls. Error bars show standard deviations, $n \geq 3$.

Figure S6. Co-aggregation with HSPB1-3D-GxG affects LDH and luciferase aggregates differently. **(a, b)** Aggregation of **(a)** luciferase and **(b)** LDH was assessed as in Fig. 1a, b in solutions containing 2 μM substrate with or without 20 μM HSPB1-3D-GxG. Error bars show standard deviations, $n = 3$. **(c, d)** The size distribution of **(c)** luciferase and **(d)** LDH aggregates formed with HSPB1-3D-GxG was estimated by DLS and compared to the size of HSPB1-3D-GxG alone and substrate alone aggregates.

Figure S7. Heat shocked HSPB1-3D-GxG remains as a dimer and does not contribute to luciferase resolubilization. **(a)** Elution pattern of HSPB1-3D-GxG in disaggregation reactions in the absence of substrate. Reactions were performed as in Fig. 4f, except with heat shock at 55 °C. The elution patterns before or after heat shock were assessed at the start (0 h) and end (3 h) of disaggregation reactions with or without chaperones by SEC and immunoblot (IB). **(b)** Quantification of HSPB1-3D-GxG from a in eluted fractions are shown as percentage of the total amount of immunoblot signal. Error bars show standard deviations, $n \geq 3$. **(c)** Disaggregation reactions with luciferase were performed as in Fig. 4 except with HSPB1-3D-GxG. Aggregate solubilization at the start (0 h) and end (3h) of the disaggregation reactions was assessed by SEC and immunoblot (IB).

Figure S8. Original Western blots used in figures.

Figure S1

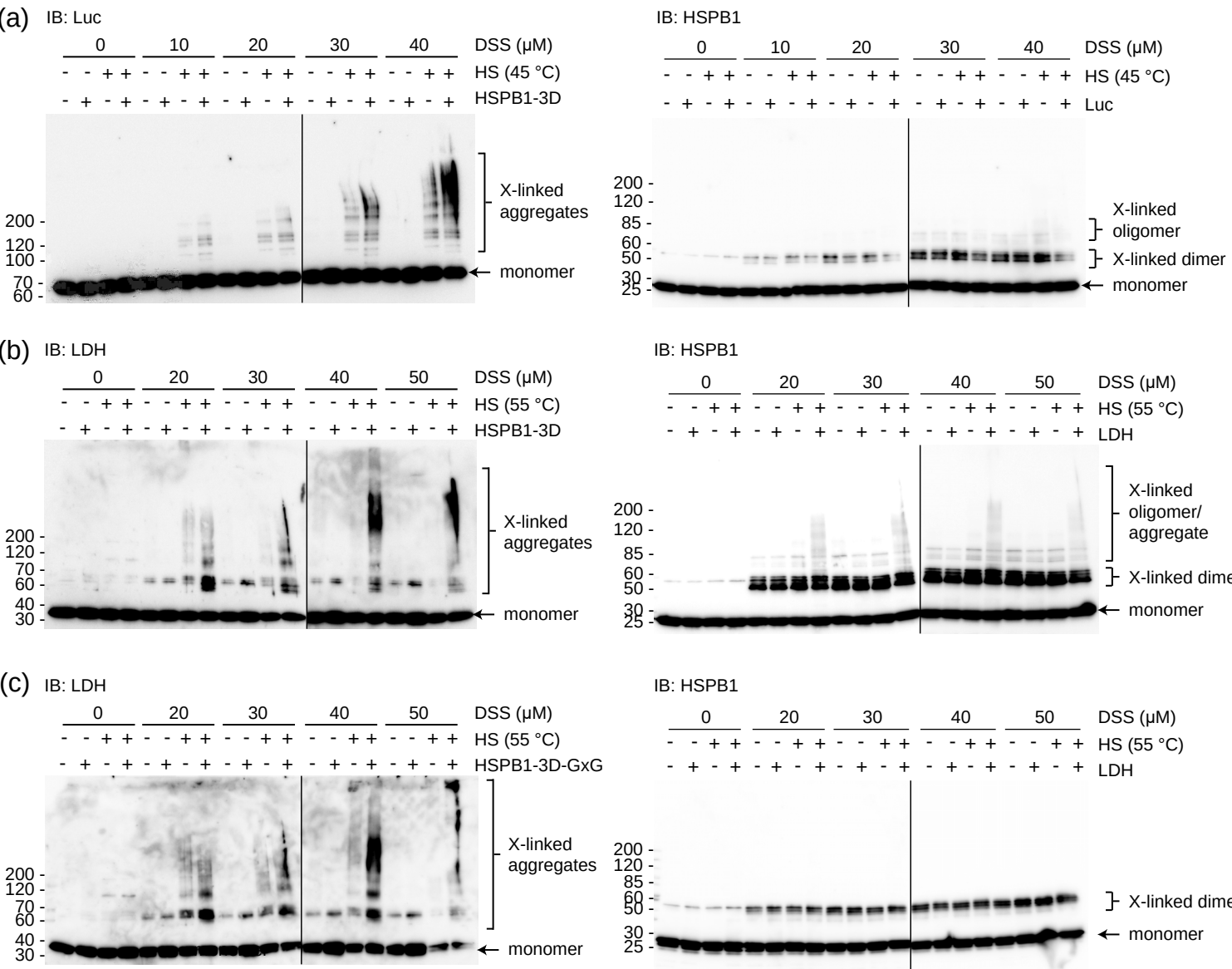


Figure S2

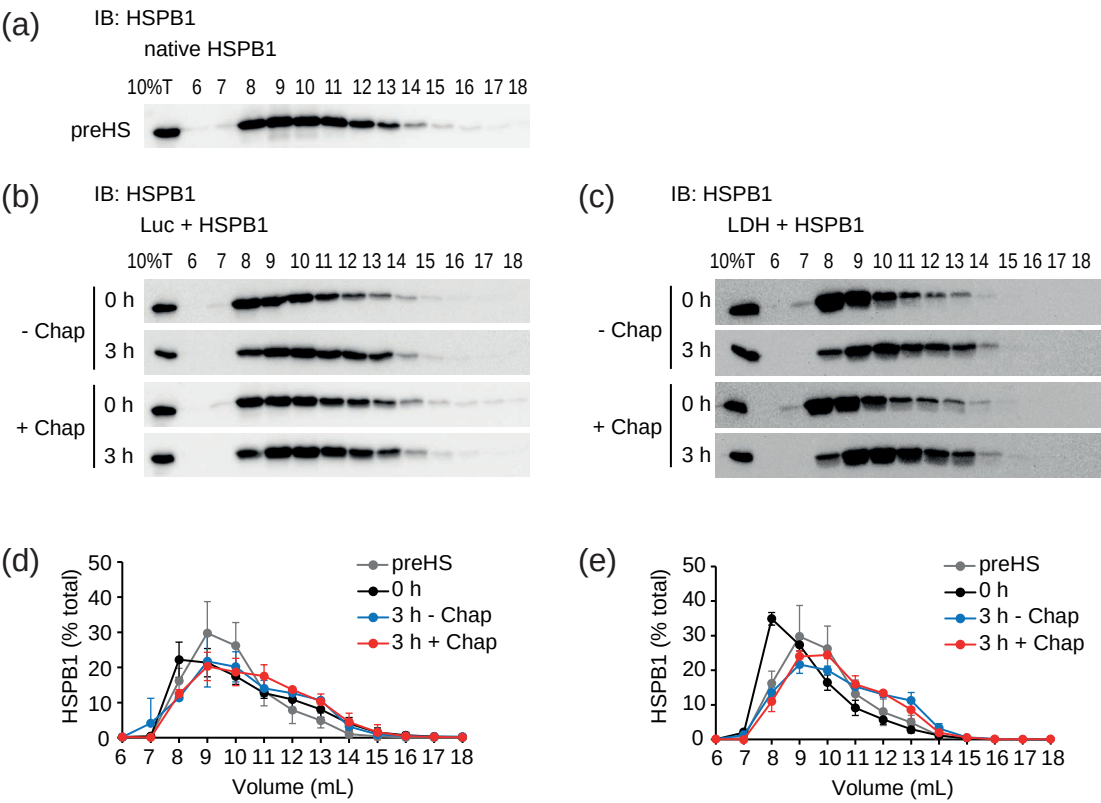


Figure S3

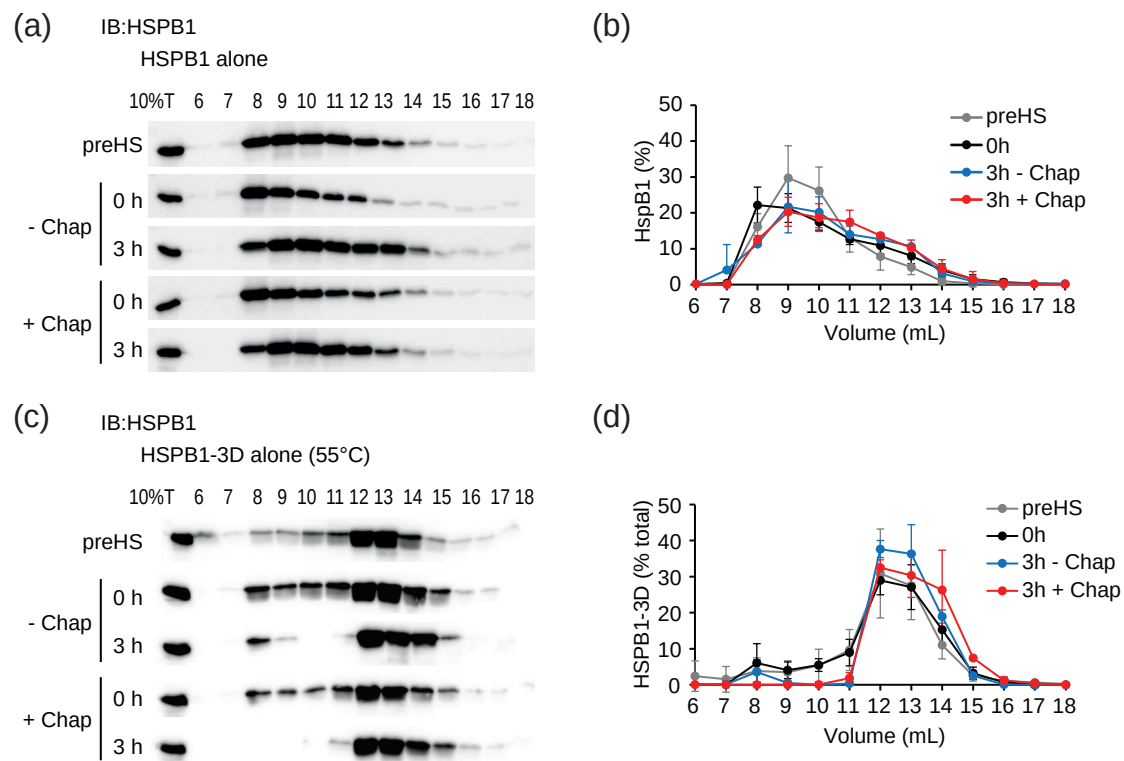


Figure S4

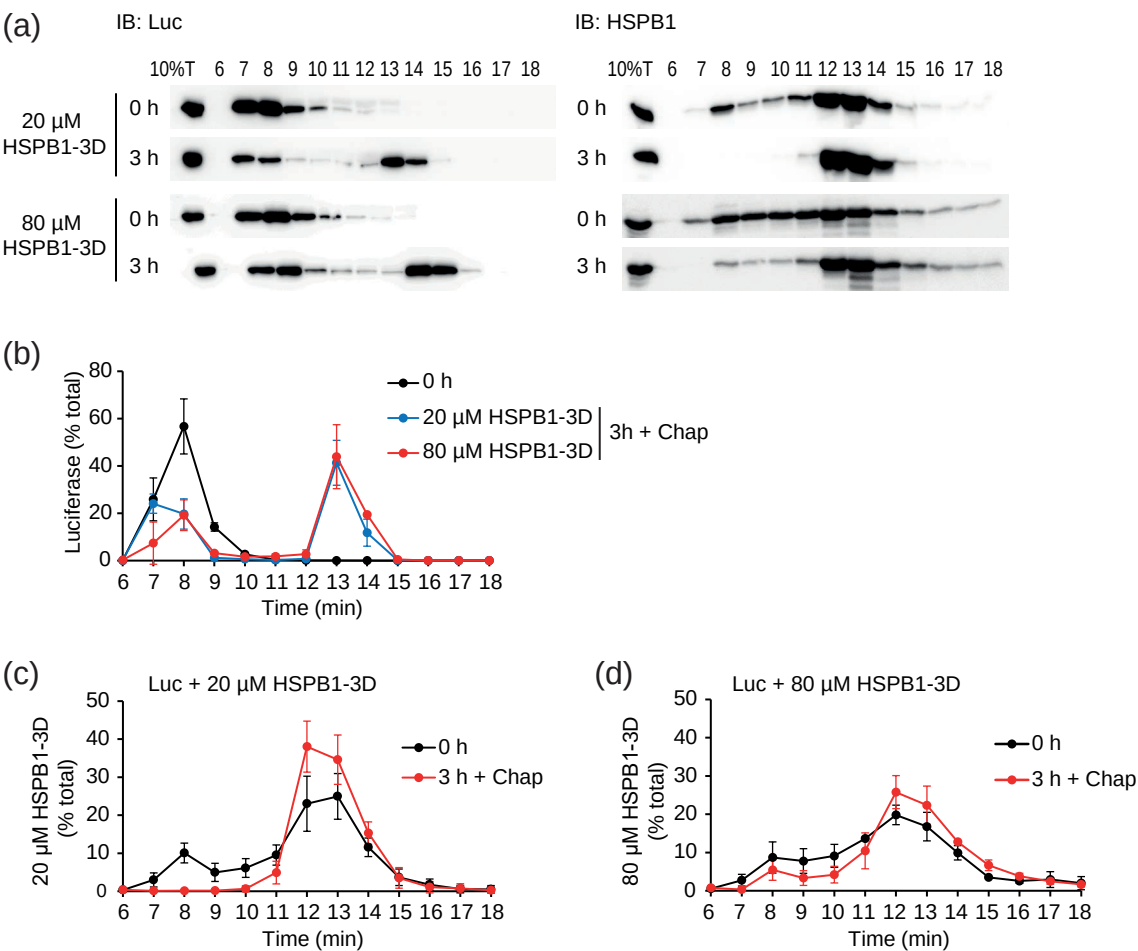


Figure S5

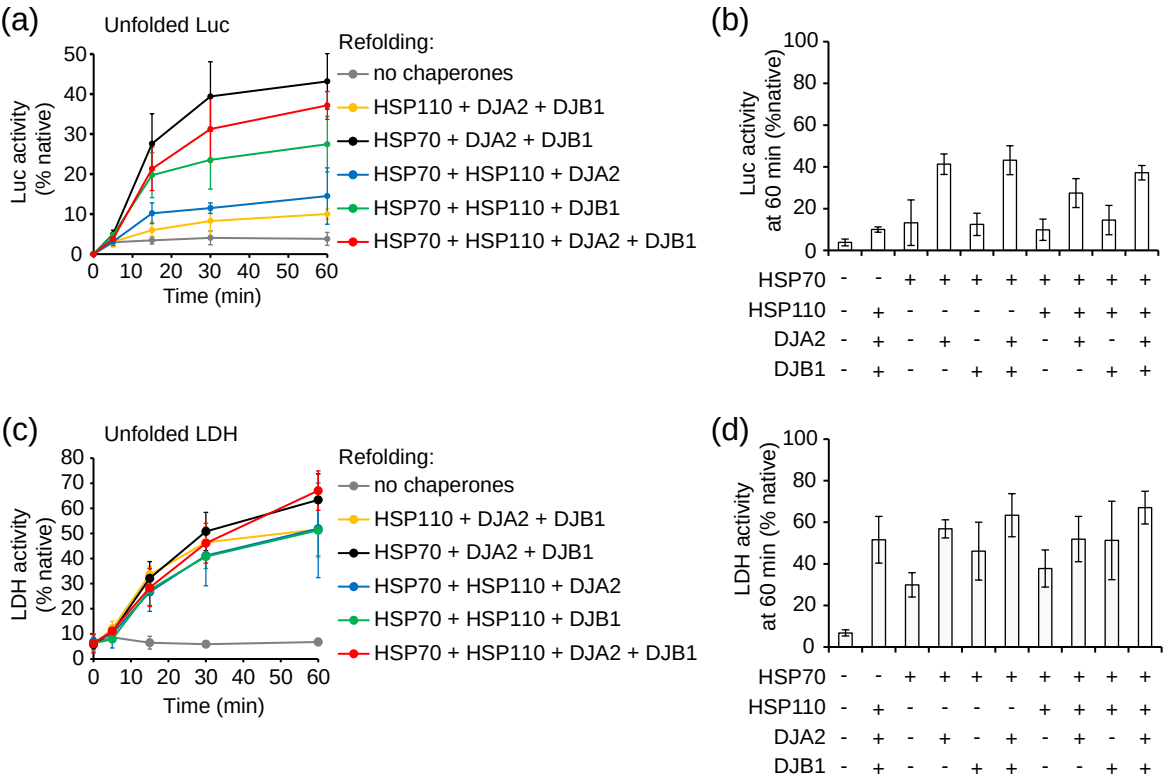


Figure S6

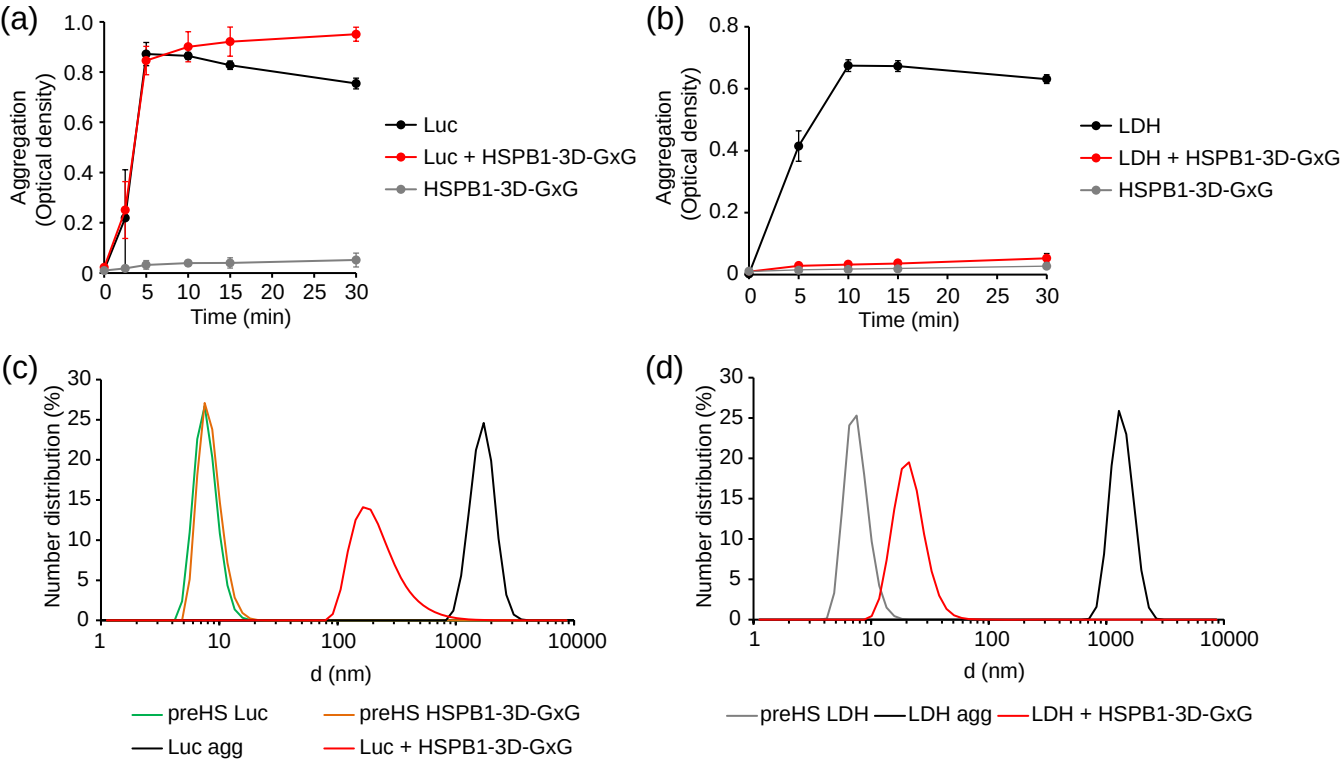


Figure S7

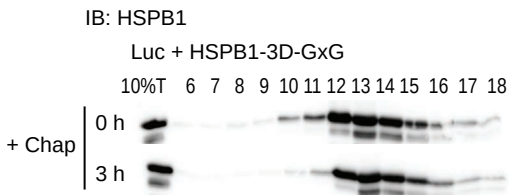
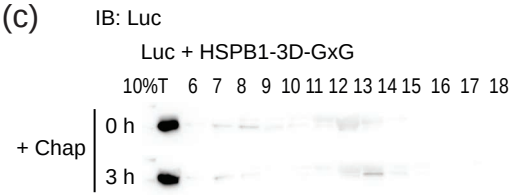
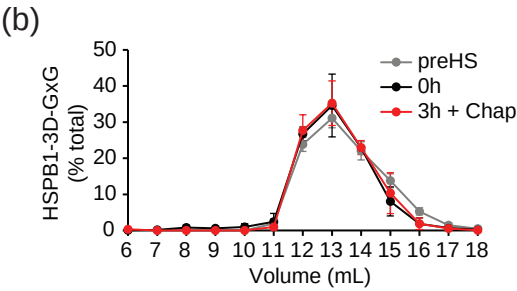


Figure S8

Original Western blots

Figure 3a

IB: Luc

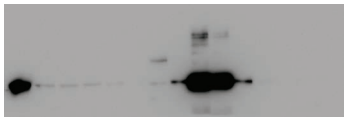


Figure 3b

IB: LDH

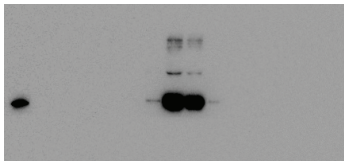


Figure 3c

IB: Luc

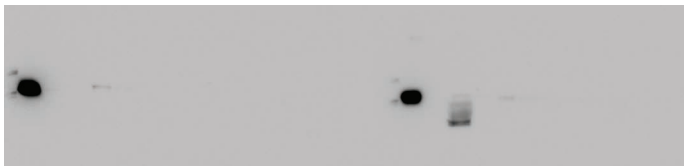
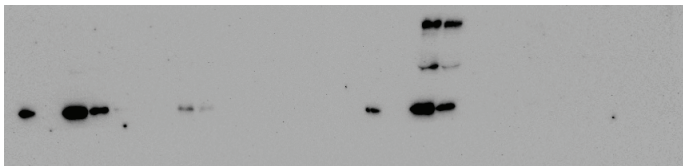


Figure 3d

IB: LDH



IB: Luc



IB: LDH

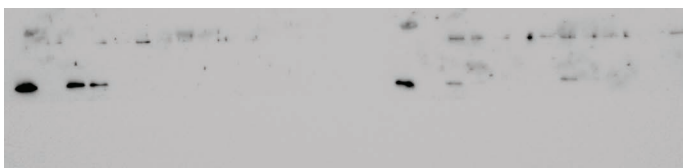


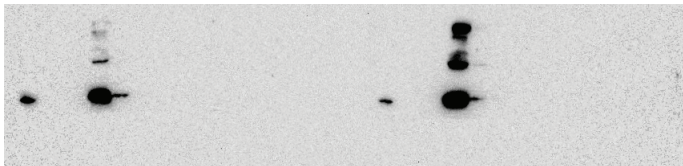
Figure 3e

IB: Luc



Figure 3f

IB: LDH



IB: Luc



IB: LDH

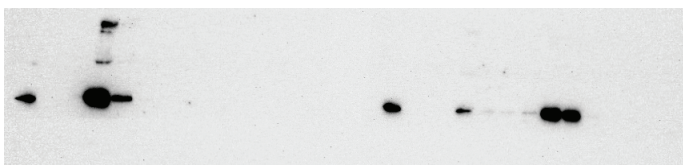


Figure 4a

IB: HSPB1

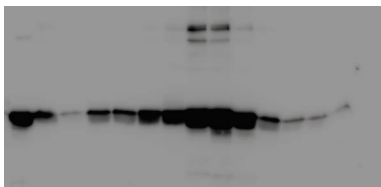


Figure 4b

IB: HSPB1

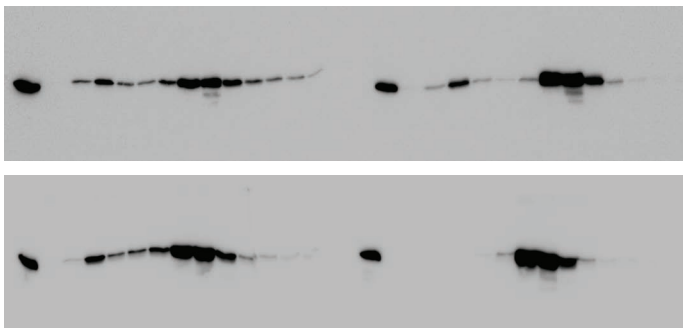


Figure S8 (cont.)

Original Western blots

Figure 4c
IB: HSPB1

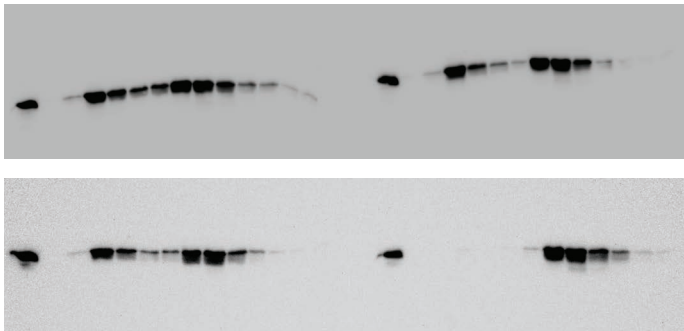


Figure 4f
IB: HSPB1

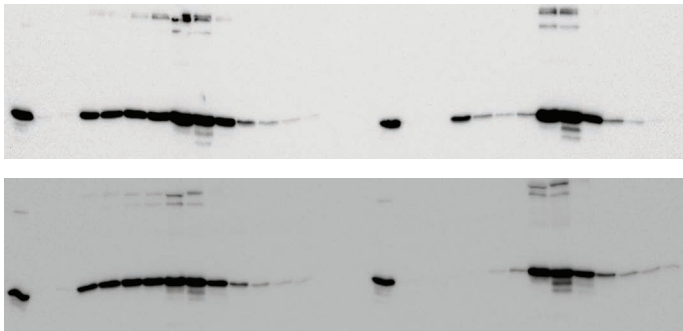


Figure 6c
IB: LDH

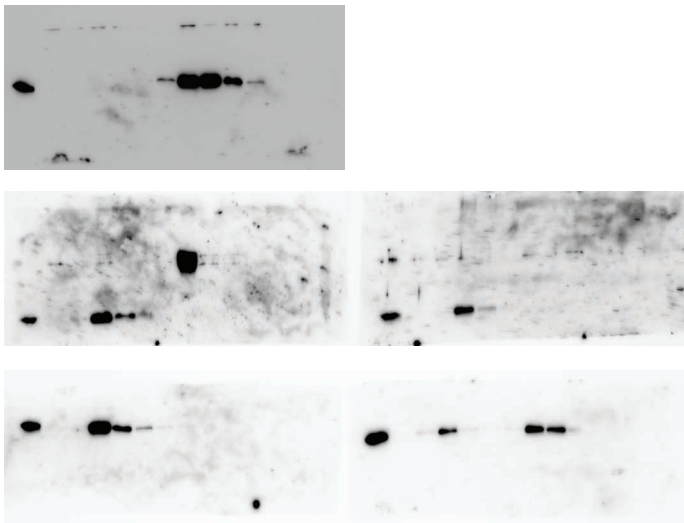


Figure 6d
IB: HSPB1

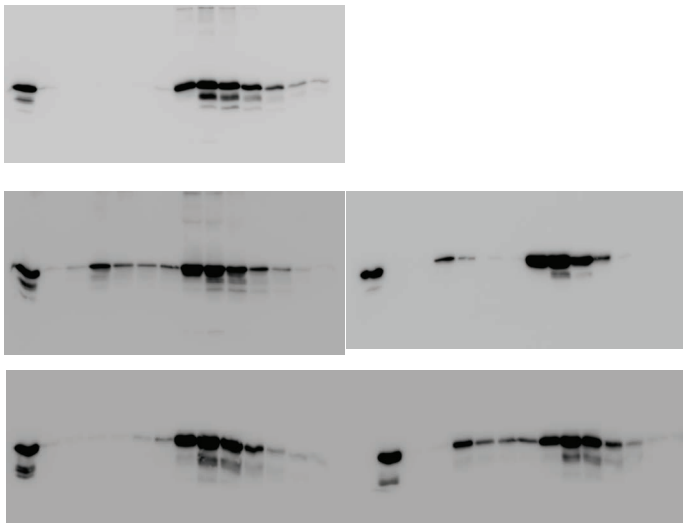
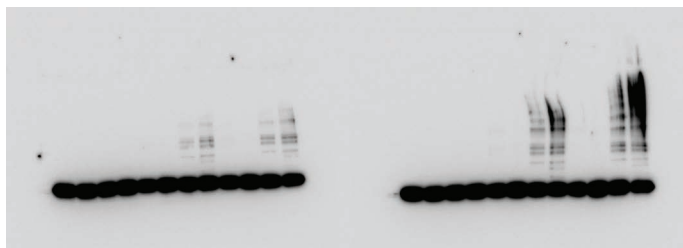


Figure S8 (cont.)

Original Western blots

Figure S1a

IB: Luc



IB: HSPB1

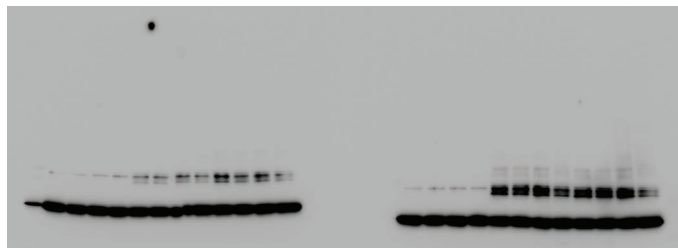
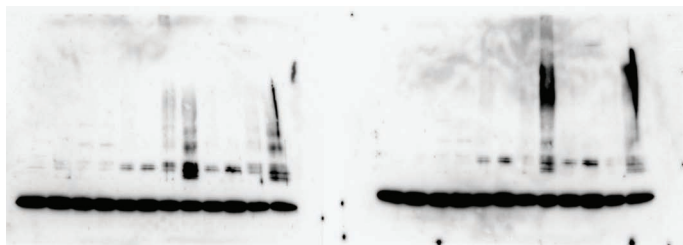


Figure S1b

IB: LDH



IB: HSPB1

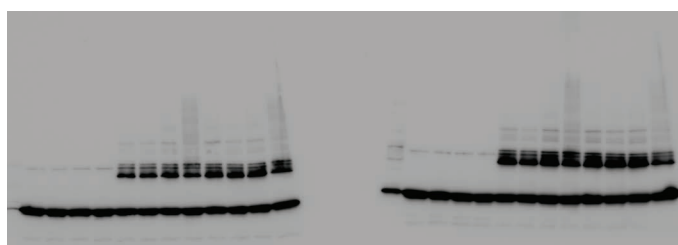
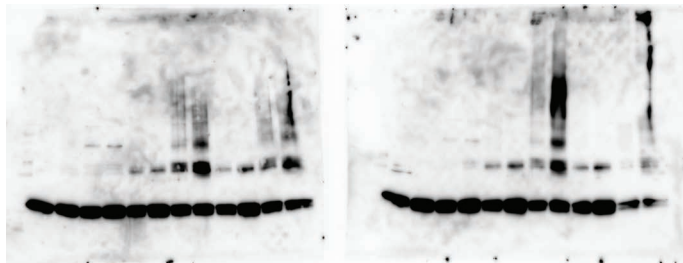


Figure S1c

IB: LDH



IB: HSPB1

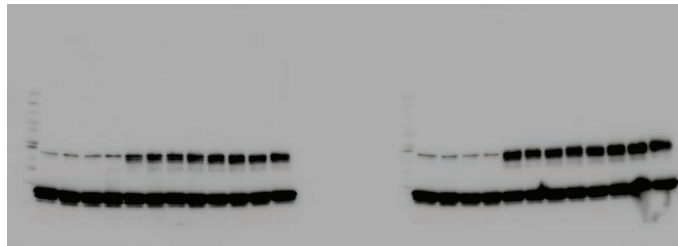


Figure S2a

IB: HSPB1

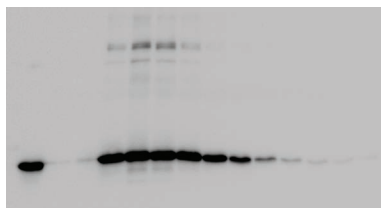


Figure S8 (cont.)

Original Western blots

Figure S2b

IB: HSPB1

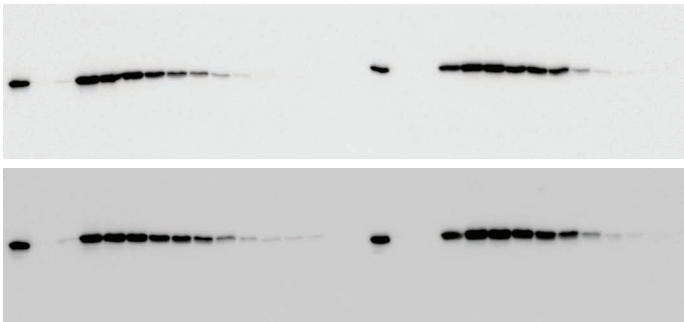


Figure S2c

IB: HSPB1

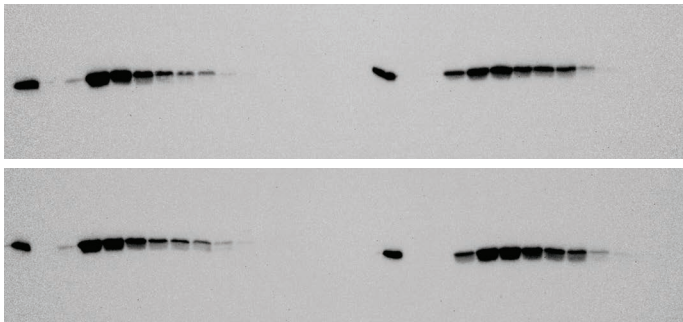


Figure S3a

IB: HSPB1

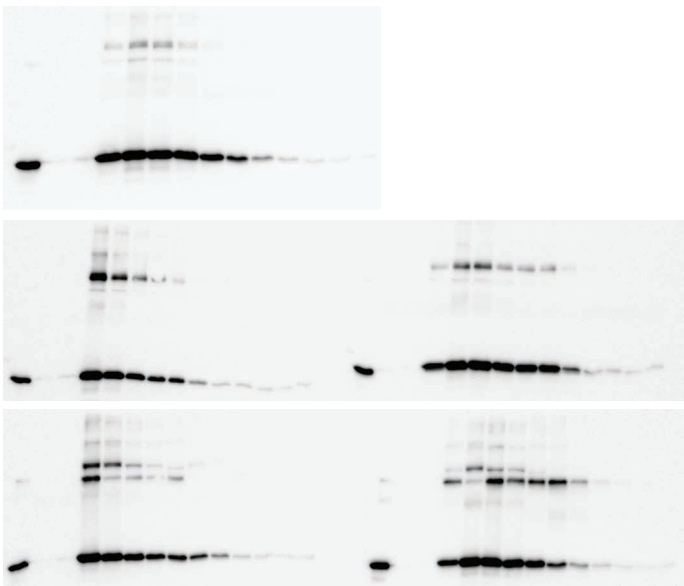


Figure S3c

IB: HSPB1

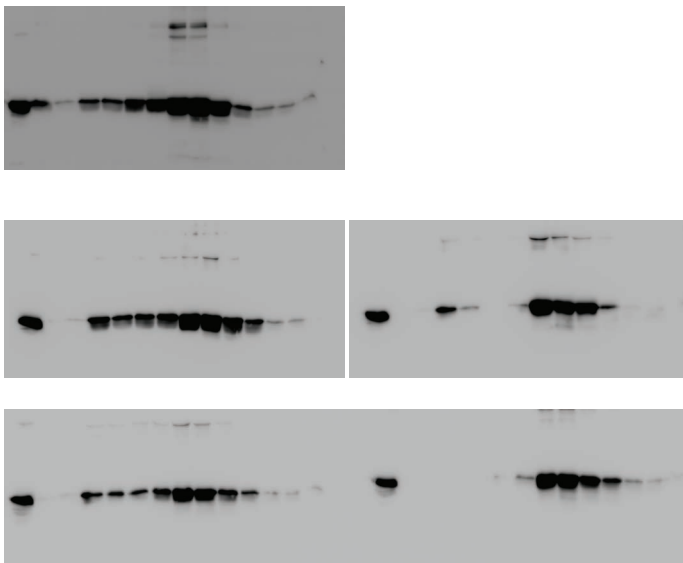
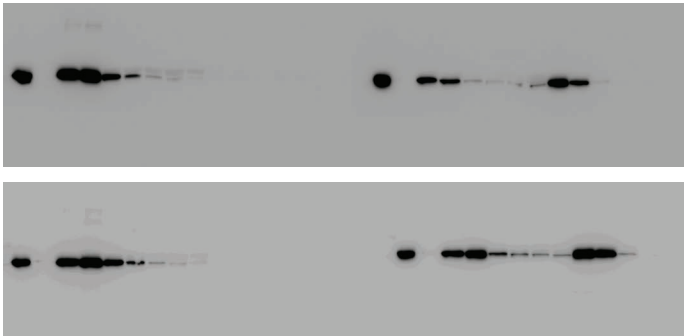


Figure S8 (cont.)

Original Western blots

Figure S4a

IB: Luc



IB: HSPB1

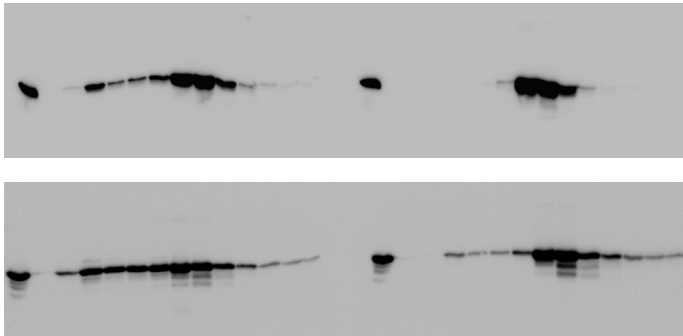


Figure S7a

IB: HSPB1

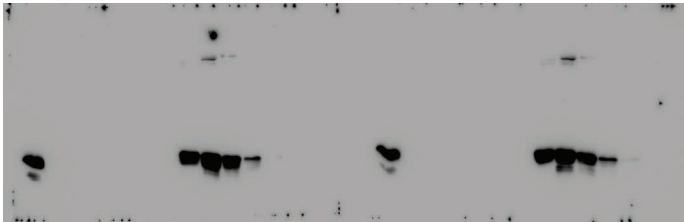
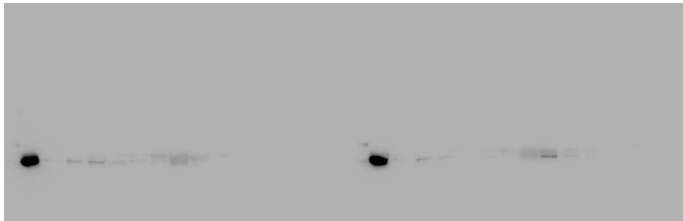


Figure S7c

IB: Luc



IB: HSPB1

