Supplementary Figure Legends

Supplementary Figure 1. **A.** Apg1 and Apg2 are potent NEFs for human Hsp70 and Hsc70. Dissociation rates of MABA-ADP from Hsp70 or Hsc70 were determined upon addition of the human Hsp110 homologs Apg1 and Apg2. Data for Apg2 represent averages of at least 3 experiments with s.e.; for Apg1, one representative dataset out of two is shown. **B.** Bag-1 and Snl1ΔN are NEFs for the human Hsp70 chaperones. Dissociation rates of MABA-ADP from Hsc70 were determined upon addition of 0.5 µM of Apg2 or Snl1ΔN, dissociation from Hsp70 was monitored upon addition of 0.5 µM Bag-1. Stimulation of nucleotide release by Bag-1 or Snl1ΔN was ca. 7-fold over the respective basal release rates. Data for Apg2 represent the averages of 5 experiments with s.e.; for Bag-1 and Snl1ΔN, one representative dataset out of two is shown. **C.** NEF-mediated effects on luciferase disaggregation are concentration-dependent. The employed NEFs were titrated with their cognate Hsp70 system (Apg1, Apg2, Hsp105 and Bag-1 with Hsc70 + Hdj1; Sse1 and Snl1ΔN with Ssa1 + Ydj1) to concentrations between 0.1 and 2 µM. The yield of reactivated luciferase after chemical aggregation followed by 90 minutes of disaggregation in presence of the respective NEF is shown. Not all possible combinations of NEF and concentration were tested, such that the absence of a column indicates that disaggregation under the respective condition was not determined. Experiments were performed once except for combinations with Apg1, Apg2 and Hsp105 where one representative dataset is shown.

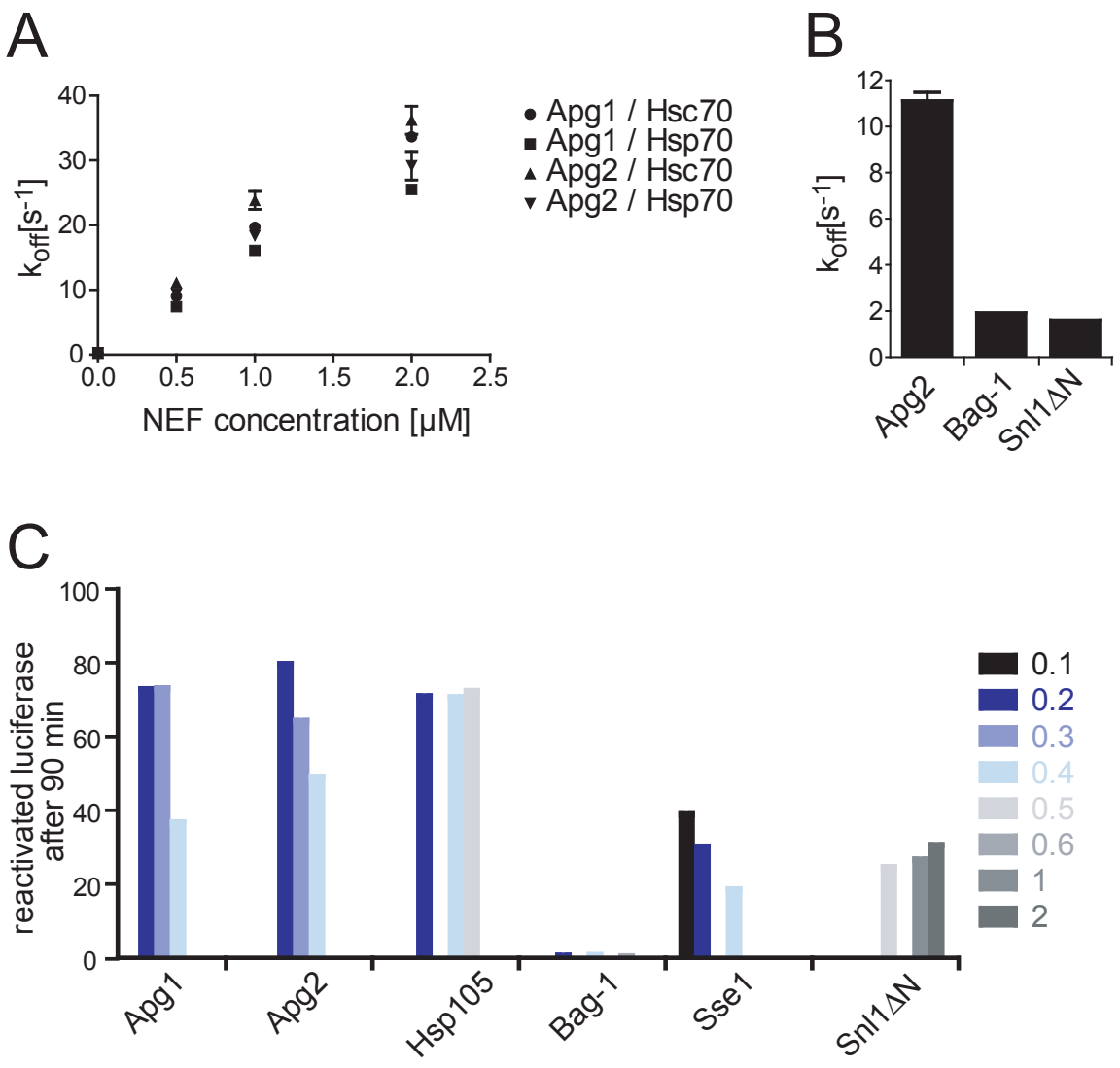
Supplementary Figure 2. **A.** Luciferase thermally aggregated at 2 µM and diluted to 20 nM (2 µM) migrates more deeply into a glycerol gradient than luciferase aggregated at 20 nM . Left panel: Native or thermally aggregated luciferase was centrifuged in a 3-23% glycerol gradient, fractions were collected from top to bottom and analyzed by α-luciferase and α-Hsp26 Western blot. Right panels: Quantitation of native (above) and 20 nM luciferase aggregates (below) in gradient fractions. Data represent averages from at least 7 experiments with s.d. **B.** Luciferase thermally aggregated at 2 µM in the absence of Hsp26 cannot be reactivated by the yeast bichaperone system. Reactivation of luciferase from aggregates induced at 45°C in the absence of Hsp26 was monitored after dilution to 50 nM and addition of the yeast chaperones (2 µM Ssa1 and 1 µM Ydj1 +/- 0.1 µM Sse1 +/- 1 µM Hsp104). One representative dataset out of two is shown. **C.** Luciferase was thermally aggregated at 20 nM in the absence of Hsp26 and reactivation was monitored upon addition of the human Hsp70 system (2 µM Hsc70, 1 µM Hdj1 +/- 0.2 µM Apg2). **D.** Reactivation of luciferase (treated as

in (C) was monitored upon addition of the yeast Hsp70 or bichaperone system (2 µM Ssa1, 1 µM Ydj1 +/- 0.2 µM Sse1, +/- 1 µM Hsp104). Reactivation data in (C) and (D) represent the average of 3 experiments +/- s.e. **E.** Reactivation of thermally aggregated MDH by the yeast chaperones requires the disaggregase Hsp104. Reactivation of MDH was monitored upon addition of 2 µM Ssa1 and 1 µM Ydj1 alone or in presence of 0.2 µM Sse1 and 1 µM Hsp104. Reactivation data represent the average of at least 4 experiments +/- s.e.

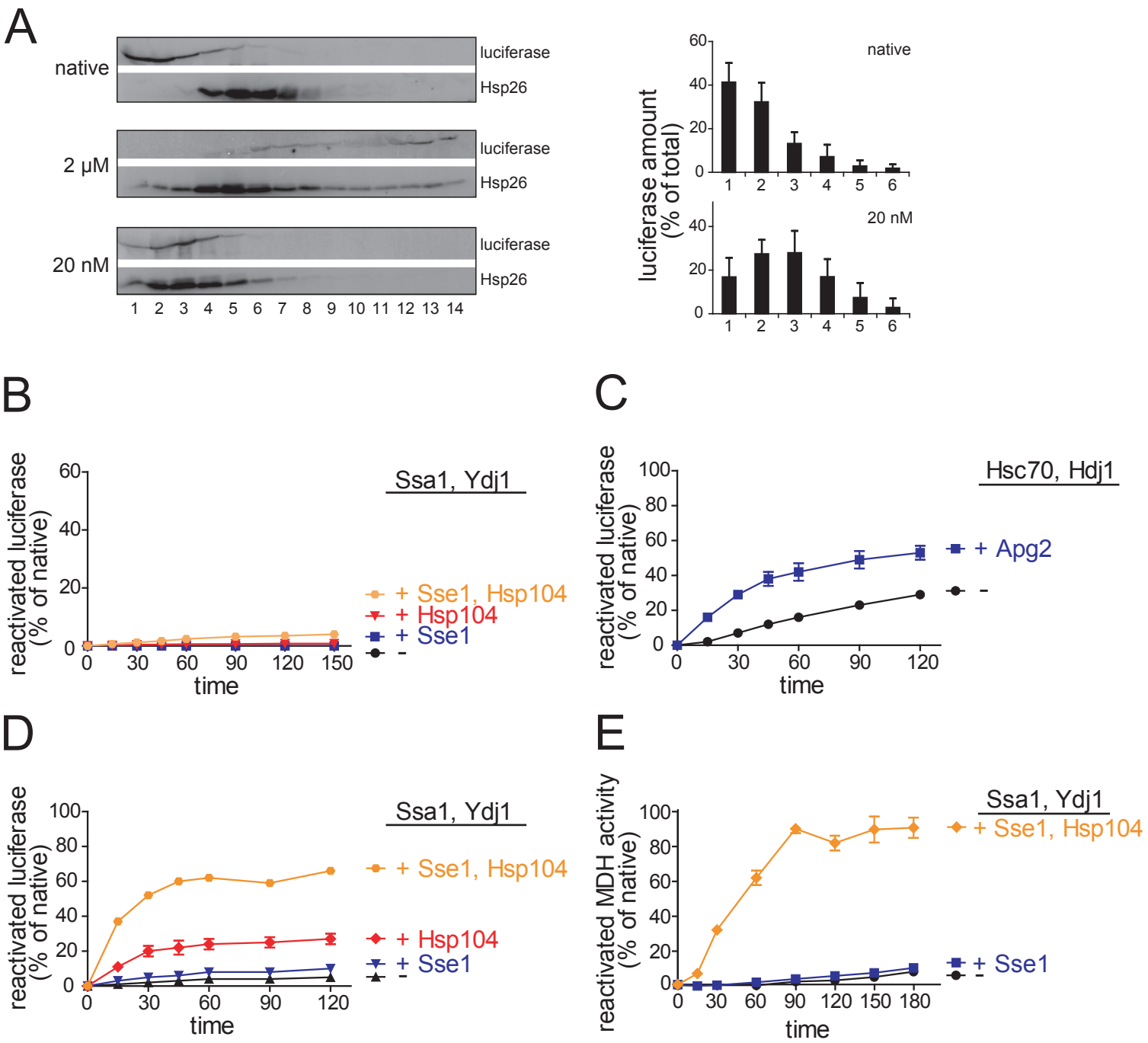
Supplementary Figure 3. **A.** Detail of the sequence alignment of human and yeast Hsp110 proteins. Highlighted in red are the residues N572 and E575 in Sse1 whose mutation causes NEF-deficiency (Polier *et al.*, 2008) as well as the corresponding residues in the human Hsp110s. **B.** Apg2 interacts with luciferase and partially prevents its thermal aggregation. Luciferase or Apg2 alone or combinations thereof were shifted to 42°C and light scattering was monitored at 600 nm. The total protein concentration in all samples was adjusted to 2 µM with BSA. Data shown represent the average of at least 3 experiments +/- s.d. **C.** The Apg2 mutant Apg2-D7S is deficient in ATPase activity. The steady-state ATPase rates of Apg2 and Apg2-D7S were determined by a coupled enzymatic test and used to calculate the k_{cat} of the reactions. Data represent the average from 7 experiments +/- s.e. **D.** Reactivation of luciferase (treated as in Supp. Fig. 2B) was monitored upon addition of the yeast Hsp70 system (2 µM Ssa1, 1 µM Ydj1 or Sis1, 0.2 µM Sse1). One representative dataset out of two is shown. **E.** Reactivation of endogenously expressed EGFP-luciferase from heat-shocked lysates after 4 hours at 30°C in the absence or presence of the indicated chaperones (2 µM Hsc70, 1 µM Hdj1 +/- 0.2 µM Apg2 or 0.4 µM Bag-1). Reactivation data represent the average of at least 4 experiments +/- s.e.

Supplementary Figure 4. Endogenous Apg2 co-localizes with luciferase aggregates in heat-shocked human U2OS cells expressing EGFP-luciferase. U2OS cells were either grown at 37°C (upper panel) or heat-shocked at 45°C for 30 minutes without recovery period (middle panel) or with 30 minutes of recovery at 37°C (lower panel). Endogenous Apg2 was visualized by immunofluorescence (left image column, channel shown in red in the merge images), nuclei were stained with DAPI (shown in blue in the merge images) and EGFP-luciferase was visualized by the GFP fluorescence (shown in green in the merge images). Scale bar = 10 µm.

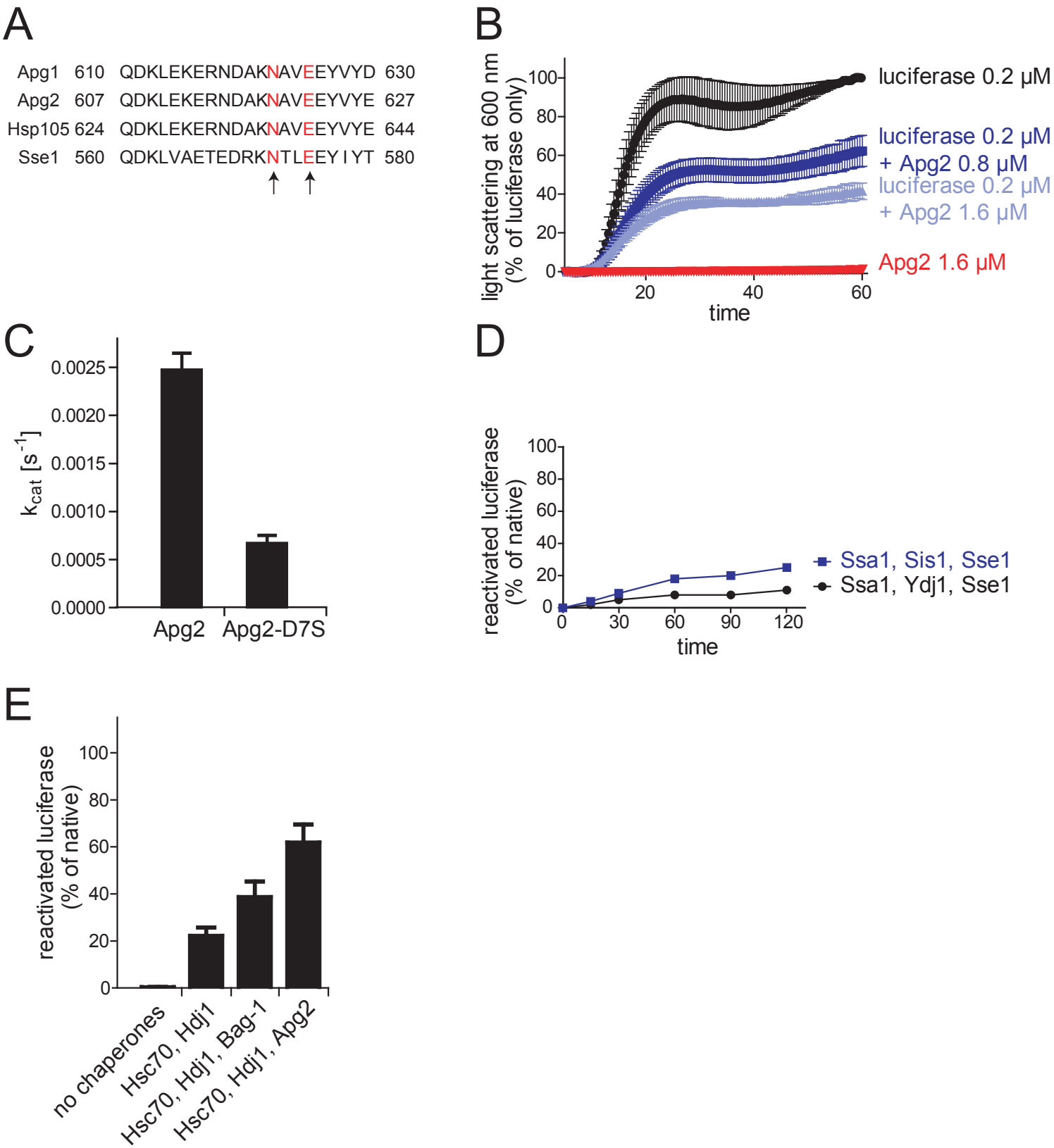
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4