




HSPB1 -----MTERRVPSLLRGP--SWDPFRDWPHSRLFDQAFGL 35  
 HSPB8 -----MADGQMPFSCHYPSRLRRDPFRDSPLSSRLDDGFGM 37  
 HSPB4 -----MDVTIQHPWFKR-TLGPFI----PSRLFDQFFGE 29  
 HSPB5 -----MDIAIHPWIRR-PFFPFH**S**---PSRLFDQFFGE 30  
 HSPB6 -----MEIPVPQPSWLR--ASAPLPLGLSAPGRLFDQRFGE 35  
 HSPB2 -----MSGRSVPHAHATAE-----YEFANPSRLGQRFGE 31  
 HSPB3 -----MAKII LRHLIEIPVR-----YQEFEFARGLED 27  
 HSPB7 -----MSHRTSSTFRAERSFHSSSSSSSSSTSSSASRALPA 36  
 HSPB9 -----MQRVGNTFSNE-----SRVASRCPSV 21  
 ODF1 MAALSCLLDSVRRDIKKVDRELRLRCIDEFSTRCLCDLYMHPYCCCDLHPYPYCLCYSK 60




HSPB1 PRLPEEWSQWLGGSSWPGYVVRPLPPAAIESPAVAAPAYSRAISRQLSSGVSEIRHTAD-- 93  
 HSPB8 DFPDDELASWPDWALPRLSSAWP-GTLRSGMVPRGPTATARFGVPAEGRTPPPPFGE-- 94  
 HSPB4 GLFEYDLLPFLSSTISPYR--QSLFRT-----VLDSGISEVRSRDRD-- 69  
 HSPB5 HLLSDLFP-TSTSL**S**PFYLRPPSFLRAPS**S**-----WFDTGLSEMRLEKD-- 73  
 HSPB6 GLEAEALALCPTTLAPYYLRAPSVLPE-----VAQVPTDPG-- 72  
 HSPB2 GLLPEEILT--PTLYHGYYVPRAPAGEG-----SRAGASELRLSEG-- 72  
 HSPB3 CRLDHALYALPGPTIVDLRKTRAAQSPVD-----SAEPTPREGKS-- 69  
 HSPB7 QDPPMEKALSMFSDDFGSFMRPHSEPLAFP-----ARPGGAGNIKTLGD-- 80  
 HSPB9 GLAERNRVATMPVRLLRDLP-----AAQEDNDHARD-- 52  
 ODF1 RSRSCGLCDLYPCCLCDYKLYCLRPSLRSLEKRAIRAIEDEKRELAKLRRTTNRILASSC 120



HSPB1 ---RWRVSLDVNHFAPELTVKTKDGVVEITGK**H**EERQDEHGYISR**C**FTRKYT---LPPG 147  
 HSPB8 ---PWKVCNVVHSFKPEELMVKTKDGYVEVSGK**H**EEKQOEGGIVSKNF**T**KKIQ---LPAE 148  
 HSPB4 ---KFVIFLDVKHFSPEDLTKVQDDFVEIHGK**H**NERQDDHGYISREF**H**RRYR---LPSN 123  
 HSPB5 ---RFSVNL DVKHFSPPELKVVLGDVIEVHGK**H**EERQDEHGFISREF**H**RYR---IPAD 127  
 HSPB6 ---HFSVLLDVKHFSPEEIAVKVVGHEVHAR**H**EERPDEHGFVAREF**H**RYR---LPPG 126  
 HSPB2 ---KFQAFLDVSHFTPDEVTVRTVDNLLVESAR**H**PQRLDRHGFSRE**F**CR**T**YV---LPAD 126  
 HSPB3 ---HFQILLDVVQFLPEDIIIQT**F**EGLWLLIK**A**Q**H**GRMDHGFIS**R**S**F**TR**Q**YK---LPDG 123  
 HSPB7 ---AYEFAVDVRDFSPEDIIVTTSNNHIEVR---AEKLAADGTMN**T**FA**H**K**C**Q---LPED 131  
 HSPB9 ---GFQMKLDAHGFAPEELVVQVDGQWLMVTGQQQLDVRDPERSYRMSQ**K**V**H**R**K**MLPSN 109  
 ODF1 CSSNIGSVNVCGFEPDQVKVRVKDGKVCVAERENRYDCLGSKKYSYMNICKFSLPCC 180

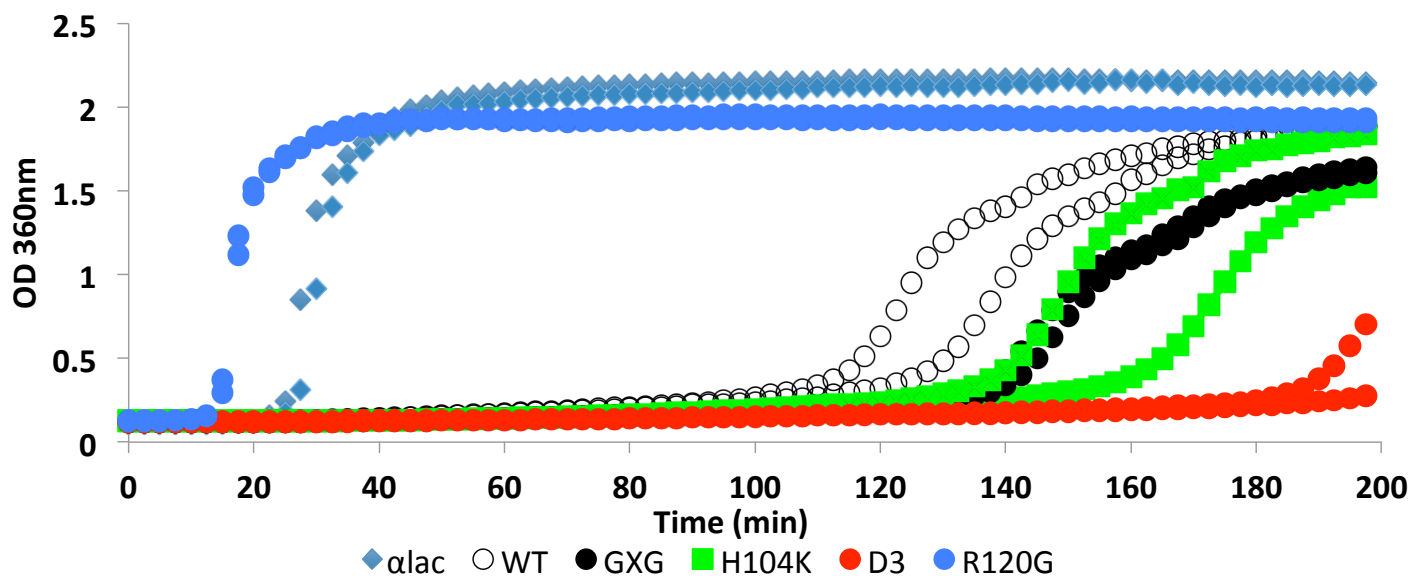


HSPB1 VDP**T**QVSSLSPEGTLTVEAPMKLATQ**S**NEIT**T**IPVTFESRAQLGGPEAAKSDETAAK-- 205  
 HSPB8 VDPVTVFASLSPEGLLIEAPQVPPYST**F**GES----S**F**N**N**ELPQDSQEV**T**CT----- 196  
 HSPB4 V**D**Q**S**ALCSLSADGMLTFCGPKI**Q**TGLDATHAERAIPVS**R**EEKP--TSAPSS----- 173  
 HSPB5 VDPLTITSSLSDGLTVNGPR**Q**---VSGPERT**I****P**T**R**E**E**KPAV**T**AAP**K**K----- 175  
 HSPB6 VDPAAVTSALSPEGVLSI-----QAAPAS**A**QAPP--PAA**K**----- 160  
 HSPB2 VDPWRVRAALSHDGILNLEAPRGGRHLDT**E**VNEVYISLLPAPPDPEEEEE**A**IVEP---- 182  
 HSPB3 VEIKDLSAVLCHDGILVVEVKD-----PV**G**TK----- 150  
 HSPB7 VDPTS**V**TSALRE**D**GS**L**TIRARR-----HP**H**TE**H**VQ**Q**TFR**T**E**I**K**I**----- 170  
 HSPB9 LSPTAMTCCLTP**S**Q**L**WVRGQCVALALPE**A**Q**T**GPS**R**LGSLGSKAN**L**TR----- 159  
 ODF1 VDEKDV**T**YSYGLGSCVKIESPC**P**CT**S**PC**S**PC**S**PC**N**PC**S**PC**N**PC**S**YD**P**C**N**PC**P**CG 240



HSPB1 -----  
 HSPB8 -----  
 HSPB4 -----  
 HSPB5 -----  
 HSPB6 -----  
 HSPB2 -----  
 HSPB3 -----  
 HSPB7 -----  
 HSPB9 -----  
 ODF1 SRFSCRKMI**L** 250

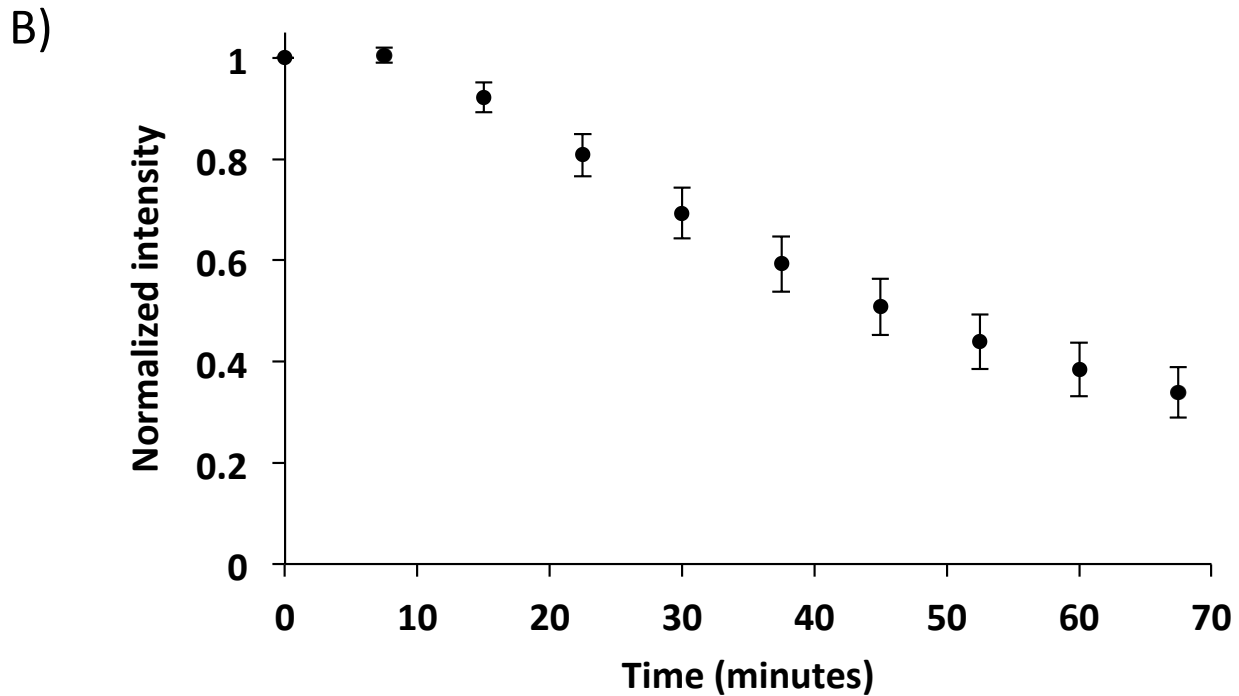
Supplemental Figure 1. Multiple sequence alignment of the ten human sHSPs, with regions highlighted: NTR (blue), ACD (green), and CTR (red). Positions mutated in one of the HSPB5 variants studied here are highlighted in colored bold font: S19, S45 and S59 (red); H104 and R120 (purple); I159 and I161 (magenta).



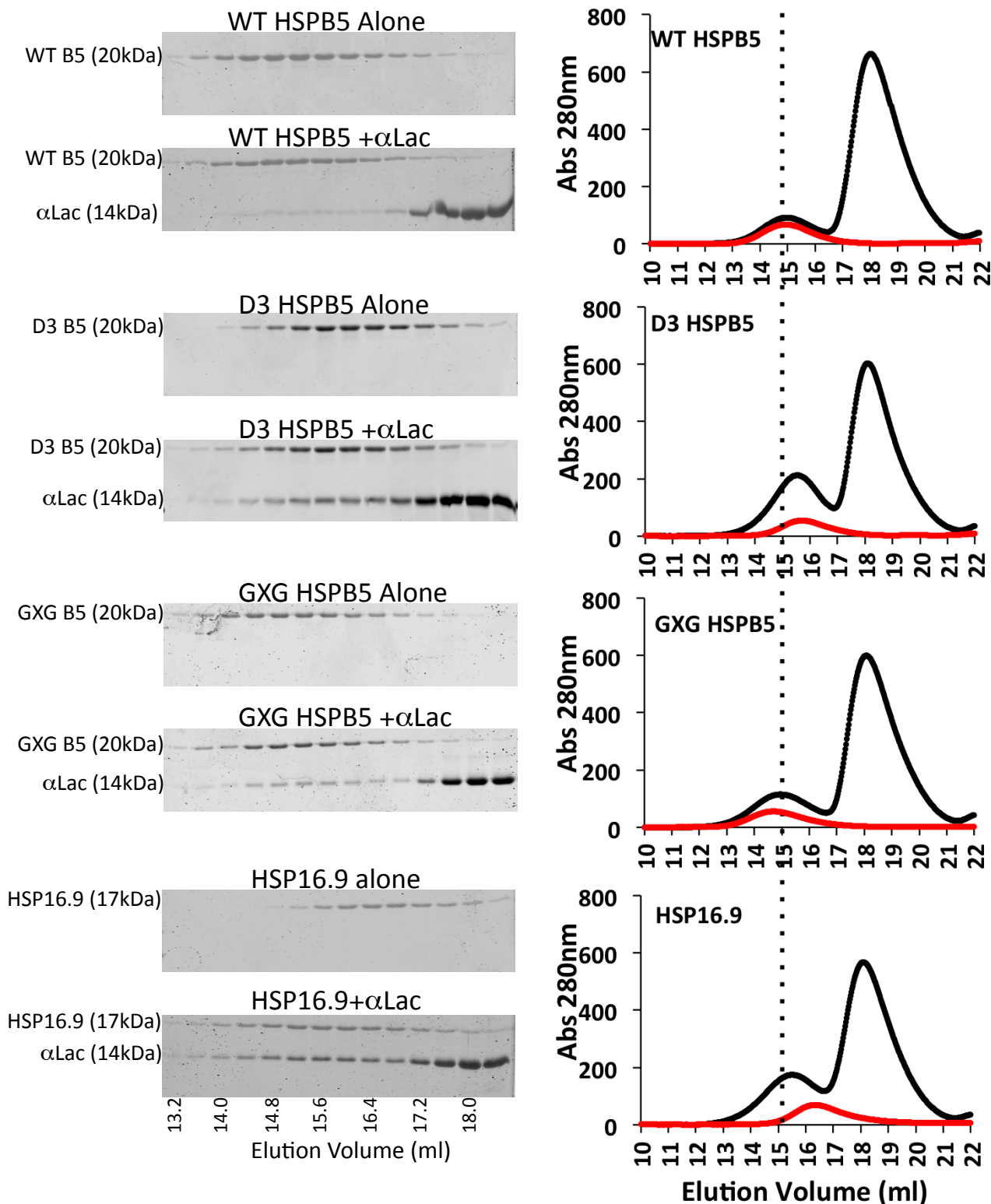
*Supplemental Figure 2. Chaperone activity of HSPB5 and HSPB5 mutants with destabilized  $\alpha$ Lac by light scattering.* The aggregation of 600  $\mu$ M  $\alpha$ Lac, destabilized by the addition of DTT was monitored by the increase in light scattering as detected by  $OD_{360\text{ nm}}$  as a function of time. The presence of either 100  $\mu$ M HSPB5 or 100  $\mu$ M HSPB5 mutants delays the onset of aggregation. Duplicates within the experiment are shown for each condition presented in Figure 2 and are representative of 2 independent assays. The variability observed in the H104K curves is the largest observed across all assays present.

A)

WT B5	D3 B5	R120G B5	H104K B5	GXG B5	HSP16.9
3.7%	5.3%	0.1%	0.1%	0.1%	9.6%



*Supplemental Figure 3. A) Comparison of intensities of the peak at 0.860 ppm in 1D NMR spectra of isolated sHSPs at 100 $\mu$ M sHSP and isolated 400  $\mu$ M DTT-destabilized  $\alpha$ Lac at time 0 min. sHSP intensities are presented as a percentage of the initial  $\alpha$ Lac intensity ( $I_{\text{sHSP}}/I_{\alpha\text{Lac}} \times 100$ ). B) *Reproducibility of NMR 1D  $\alpha$ Lac curves.* Normalized peak intensities at 0.860 ppm of 400  $\mu$ M DTT-destabilized  $\alpha$ Lac measured as a function of time in 5 independent experiments. The average curve for the 5 experiments is plotted and the variability in the curves is shown as +/- one standard deviation for each timepoint. The intensity is constant across the first two timepoints (lag phase) where the association with sHSPs was estimated.*



Supplemental Figure 4. (left) SDS-PAGE gels of fractions collected from SEC analyses of sHSPs (100  $\mu$ M) and mixtures with DTT-destabilized  $\alpha$ lac (Figure 5, 7). 0.2 ml fractions were collected, starting at 13.2 mL and every other fraction was run on a gel, ending at 18.4 mL for each condition. Corresponding elution volumes are shown. (Right) SEC profiles of sHSP/Client mixtures (replicates for Figure 4). SEC elution profiles of HSPB5 and HSPB5 mutants alone (red curves) and incubated with destabilized  $\alpha$ Lac for 30 minutes at 42  $^{\circ}$ C prior to SEC (black curves). A vertical line shown through all elution profiles denotes the peak elution volume of WT HSPB5 for reference. Gels and replicates of H104K SEC traces were not collected as this interaction has been previously reported in detail (Ref. 16).