The landscape of molecular chaperones across human tissues reveals a layered architecture of core and variable chaperones

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The sensitivity of the protein-folding environment to chaperone disruption can be highly tissue-specific. Yet, the organization of the chaperone system across physiological human tissues has received little attention. Through computational analyses of large-scale tissue transcriptomes, we unveil that the chaperone system is composed of core elements that are uniformly expressed across tissues, and variable elements that are differentially expressed to fit with tissue-specific requirements. We demonstrate via a proteomic analysis that the muscle-specific signature is functional and conserved. Core chaperones are significantly more abundant across tissues and more important for cell survival than variable chaperones. Together with variable chaperones, they form tissue-specific functional networks. Analysis of human organ development and aging brain transcriptomes reveals that these functional networks are established in development and decline with age. In this work, we expand the known functional organization of de novo versus stress-inducible eukaryotic chaperones into a layered core-variable architecture in multi-cellular organisms.
Chaperones are highly conserved molecular machines that control cellular protein homeostasis (proteostasis). Across species, they promote de novo protein folding and protein maturation\(^1\), protein translocation\(^2\), protein-complexes assembly and disassembly\(^3\), protein disaggregation and refolding\(^4\), and protein degradation\(^5\). In accordance with their fundamental roles, chaperones are abundant proteins. In human cell lines, for example, they were shown to compose ~10% of the total proteome mass\(^6\).

Chaperones have been grouped into families based on their molecular mass, common domains, protein structure similarity, and common function\(^1\). Families composing the main chaperone machinery, which modulate protein structure without participating in the final protein complex, include prefoldin\(^2\), the small heat shock proteins (sHSP)\(^8\), and the main ATP-hydrolyzing and disassembly\(^3\), protein disaggregation and refolding\(^4\), and fusion and heart tube formation\(^2\). The evolution of the sHSP family is more complex, as members in human, whose distinct functionalities are not totally defined. For example, HSPB1 is required for actin and myosin assembly. Yet, multi-cellular eukaryotes are also composed of distinct members of the same chaperone family. This two-level organization is conserved in multi-cellular eukaryotes, and that this organization illuminates the phenotypic variation of the chaperone system manifests in stress conditions, which lead to extensive upregulation of some chaperones. However, the system also has limitations. Whereas chaperone overexpression typically improves the folding capacity of the chaperone system, overexpression of specific chaperones was shown to disrupt folding\(^19\)-\(^23\). For example, overexpression of the folding enzyme FKBP51 in a tau transgenic mouse model resulted in accumulation of tau and its toxic oligomers\(^21\). Likewise, chaperone downregulation or genetic aberration can cause diseases, such as mutation in the NEF co-chaperone BAG3 that leads to myopathy\(^24\). These observations imply that the quantitative composition of the chaperone system can improve or impair its proteostatic capacity.

The chaperone system has expanded considerably in evolution\(^2\)-\(^4\). The HSP40 family, for example, expanded from three members in \textit{E. coli} and 22 members in budding yeast to 49 members in human, whose distinct functionalities are not totally clear\(^13\). The evolution of the sHSP family is more complex, as plants and some lower eukaryotes have more sHSPs than mammalian and higher eukaryotes\(^27\). Consequently, the chaperone system has been remodeled. Whereas in prokaryotes the same chaperones carry both de novo protein folding and response to stress, unicellular eukaryotes evolved two separately regulated chaperone systems, a basal system and a stress inducible system, each composed of distinct members of the same chaperone families\(^28\). This two-level organization is conserved in multi-cellular eukaryotes. Yet, multi-cellular eukaryotes are also composed of multiple cell types, tissues, and organs, each having different proteomes and thus potentially different folding demands. For example, HSPB1 is required for actin and myofibril assembly and its depletion specifically impairs cardiac progenitor fusion and heart tube formation\(^29\).

Recent years were marked by multiple large-scale mappings of the proteomic and transcriptomic landscapes of tens of human tissues. Major efforts included the Human Protein Atlas (HPA)\(^30\), Fantom\(^31\), and the Genotype Tissue Expression (GTEx) consortium\(^32\). These resources enabled unprecedented quantitative views into the genes and proteins that make up physiological human tissues. These studies and others have revealed tissue-specific regulatory elements, molecular interaction networks, and functional mechanisms underlying traits and diseases\(^32\)-\(^36\). Large-scale studies of the chaperone system were performed in the context of neurodegenerative diseases\(^37\), heat stress\(^38\), and cancer\(^39\) via analysis of samples gathered from patients. These studies revealed different patterns of chaperone network dysregulation. However, a systematic examination of the basal chaperone network in physiological human tissues has been lacking.

In this work, we harness transcriptomic profiles gathered by the GTEx consortium\(^32\), as well as other publicly available omic datasets\(^30\)-\(^37\), to systematically examine the basal chaperone system in various human tissues. We focus on 194 manually curated chaperones, co-chaperones, and folding enzymes of the main chaperone families, henceforth collectively referred to as chaperones. We find that in accordance with the fundamental role of the chaperone system, chaperones are significantly more ubiquitously and highly expressed across all tissues relative to other protein-coding genes, and are also more important for growth. Nevertheless, differential analysis of chaperone expression across tissues shows that most chaperones have tissue-specific behaviors. A proteomic screen of mouse myoblasts cell line and a computational analysis of chaperones with a known aberration that is causal for a Mendelian disease show that these tissue-specific behaviors tend to be conserved and functional. For the community interested in specific chaperones we present a website, https://netbio.bgu.ac.il/chapnet/, for browsing the information on specific chaperones and their relationships across tissues. We further highlight a core set of chaperones that is uniformly expressed across tissues, and show them to be more important for growth and more highly expressed than other chaperones. This core subsystem establishes tissue-specific functional networks, which are enhanced by their tissue-specific relationships with other chaperones. Notably, by analyzing transcriptomic datasets of human organ development\(^42\) and brain aging\(^37\), we find that the core versus variable organization and the tissue-specific functional networks are established throughout development and are challenged in aging. We propose that the combination of core and non-core chaperones constitutes a third-level organization of the chaperone system that is capable of supporting the different proteostatic demands of multi-cellular eukaryotes, and that this organization illuminates the phenotypic outcomes of chaperone aberrations.

**Results**

Chaperones are ubiquitously and highly expressed across human tissues. We manually curated a list of chaperones and co-chaperones of the main eukaryotic chaperone families, which drive basic cellular processes needed in living cells. These include sHSP, HSP40, HSP60/HSP10, HSP70, HSP90, prefoldin, and folding enzymes, as well as chaperones not structurally or functionally associated with a specific chaperone family, such as ER chaperones, which we denoted as “other” (see the “Methods” section, Supplementary Data 1). Most of the chaperones in this list were not substrate-specific. We also included the myosin chaperone UNC45 and the collagen chaperone SERPINH1, since they interact with multiple members of the myosin and collagen protein families, respectively (Supplementary Data 1). To analyze chaperone expression across tissues, we used RNA-sequencing profiles of adult human tissues that were made available by the GTEx consortium\(^32\). We focused on tissues with at least five
available profiles sampled from donors with traumatic injury fitting with sudden death, and avoided other death causes to limit the effect of treatment, inflammation, and stress on chaperone expression44–47. This resulted in a total of 29 tissues, 488 profiles, and 194 expressed chaperones, co-chaperones and folding enzymes, collectively referred to as chaperones (Supplementary Data 1 and 2).

We first compared the distribution across tissues of expressed chaperones to that of other protein-coding genes. We considered a gene as expressed in a tissue if its median expression level in samples of that tissue was above commonly used expression thresholds (1, 5, or 10 transcripts per million (TPM), see the “Methods” section). The number of chaperones expressed in each tissue at a threshold of 1 TPM was similar (176 ± 6, Supplementary Data 2). Next, we associated each gene with the number of tissues in which it was expressed (Fig. 1a, Supplementary Fig. 1A). This resulted in a bi-modal distribution, as previously shown for protein-coding genes48. However, chaperones were significantly more broadly expressed than other protein-coding genes ($p = 2.7E-9$, Kolmogorov–Smirnov (KS) test, Fig. 1a). For example, 74% of the chaperones were expressed in all tissues, relative to 47% of the other protein-coding genes. Similar results were observed with different TPM thresholds (Supplementary Fig. 1A).

Next, we examined the expression levels of chaperones relative to other protein-coding genes. Within each of the 29 tissues that we analyzed, the expression levels of chaperones were significantly higher ($p < 1.7E-7$, Mann–Whitney (MW) test, Fig. 1b), also upon considering other TPM thresholds (Supplementary Fig. 1B). Since some chaperones are known to be upregulated in stressful conditions, they could be driving the high expression that we observed. To test for this, we defined a subset of 64 stress-induced human chaperones, which were determined previously by using a meta-analysis of microarray data38 (see the “Methods” section). Indeed, stress-induced chaperones were more highly

![Image](https://doi.org/10.1038/s41467-021-22369-9)

**Fig. 1 Chaperones are broadly expressed across human tissues.** a The distribution of 194 chaperones, 130 non-stress-induced chaperones and 17,689 other protein-coding genes by the number of tissues expressing them at a level ≥1 transcripts per million (TPM; the first bin represents genes expressed in a single tissue or two tissues, the second bin represents genes expressed in three or four tissues, etc.). Chaperones and non-stress-induced chaperones were significantly more broadly expressed than other protein-coding genes ($p = 2.7E-9$ for both, two-sided Kolmogorov–Smirnov test). b The median expression levels per tissue of chaperones, non-stress-induced chaperones and other protein-coding genes. Only genes expressed at a level ≥1 TPM were considered. Chaperones and non-stress-induced chaperones tend to be significantly more highly expressed across all 29 tissues (adjusted $p$ between 1.7E−7 and 1.4E−19, and adjusted $p$ between 0.026 and 4.9E−8, respectively, two-sided Mann–Whitney test). Chaperones $n = 155$–192; non-stress-induced chaperones $n = 102$–129; other protein-coding genes $n = 10,689$–15,555. c The cumulative distribution of the impact on growth of 186 chaperones and 17,932 other protein-coding genes, measured in 769 cell lines harboring CRISPR-induced gene inactivation (CRISPR score). Each gene was associated with its minimal CRISPR score. Chaperones were significantly more important for growth than other protein-coding genes ($p = 1.5E-5$, one-sided Kolmogorov–Smirnov test). Source data are provided as a Source Data file.
expressed than non-stress-induced chaperones (Supplementary Fig. 1C). Nevertheless, upon excluding stress-induced chaperones from the analysis, the remaining non-stress-induced chaperones were still significantly more broadly expressed \((p = 2.7E-9, \text{Fig. } 1a \text{ and Supplementary Fig. } 1A)\) and more highly expressed than other protein-coding genes \((p < 0.026, \text{Fig. } 1b \text{ and Supplementary Fig. } 1B)\). Finally, to validate the generality of these observations, we repeated the above analyses by using transcriptomic profiles of 37 human tissues that were made available by the HPA\(^{30}\). The results obtained with this dataset were similar to those obtained with the GTEx dataset, reinforcing our conclusions (Supplementary Figs. 2A–C and 3).

To further examine to what extent chaperones are fundamental components of living cells, we utilized large-scale data of gene essentiality from the DepMap project\(^{41}\). The DepMap dataset measured the impact of CRISPR-induced individual gene inactivation on growth rate in 769 human cell lines (see the “Methods” section). We found that relative to inactivation of other protein-coding genes, inactivated chaperones lowered growth rates significantly, and were thus considered more important for growth \((p = 1.5E-5, \text{KS test, Fig. } 1c)\). The relatively high impact of chaperones could be common to other ubiquitously expressed protein-coding genes. However, upon limiting our analysis to the subsets of chaperones and other protein-coding genes that were ubiquitously expressed, chaperones were still more important for growth \((p = 0.0025, \text{KS test, Supplementary Fig. } 1D)\). Similar results were obtained by using gene essentiality scores in 318 cell lines from Project Score\(^{40}\) (Supplementary Fig. 4A, B). Altogether, chaperones appear to constitute a ubiquitous and highly expressed cellular system that is fundamental for cell growth.

**Chaperone expression levels vary across human tissues.** Despite the ‘house-keeping’ functionality of chaperones, different tissues have different folding, assembly, and maintenance demands. For example, the demands of the sarcomere in muscle cells, which contain titin, the largest protein in the human body\(^{49,50}\) likely vary from those of synaptic neurotransmission in neurons, where the membrane protein synaptic vesicle protein 2 (SV2) is amongst the most abundant and conserved components\(^{51,52}\). Such demands become phenotypically apparent in face of mutations. To test whether these demands are met by tissue-specific chaperones, we gathered 62 tissue-selective heritable disorders known to be caused by aberrations in 43 distinct chaperones\(^{44,53,54}\), and analyzed the expression patterns of these chaperones across tissues (see the “Methods” section, Supplementary Data 3). In contrast to the tissue-specific clinical manifestation of these disorders, most of the 43 underlying chaperones were expressed ubiquitously across tissues (Fig. 2a), also when considering other expression thresholds (Supplementary Fig. 1E), in agreement with the behavior of other tissue-selective heritable disorders\(^{55,56}\). Thus, chaperone disruption is harmful only in specific tissues, likely depending on tissue folding demands.

Next, we asked whether the expression levels of each chaperone remain high and uniform, or whether they vary across tissues. For this, we computed the differential expression of chaperones in any given tissue relative to all other tissues\(^7\) (see the “Methods” section, Supplementary Data 4). Most chaperones \((162, 83.5\%)\) had over 2-fold change in their expression in at least one tissue, henceforth considered as variable chaperones. To display the expression variability of chaperones across tissues, we clustered chaperones and tissues hierarchically according to their differential expression profiles (Fig. 2b). Physiologically related tissues, such as skeletal muscle and heart, or different brain tissues, clustered together, suggesting a biological relevance to the differential expression patterns of chaperones.

To explore the biological relevance of tissue-specific differential expression profiles, we asked whether tissue-specific changes in expression were related to tissue-specific phenotypes. We focused on skeletal muscle, since it showed a distinct differential expression profile relative to other tissues. Notably, this distinct profile was not due to a higher number of expressed chaperones in skeletal muscle relative to other tissues \((170 \text{ versus } 176 \text{ chaperones, Supplementary Data } 2)\), or due to a high total expression of chaperones \((11,604 \text{ TPMs versus average of } 14,283 \text{ TPMs})\).

We found that out of the 10 chaperones in which mutations that cause disorders affecting skeletal muscle were identified (Supplementary Data 3), seven chaperones were expressed at least 2-fold higher in skeletal muscle compared to other tissues \((p = 0.0078, \text{Fisher exact test, Fig. } 2c)\). For example, DNAJB6 \((\log_{2} \text{fold-change} (\log_{2}FC) \text{ of } 2.24)\) and the NEF chaperone BAG3 \((\log_{2}FC \text{ of } 3.13)\) lead to Limb-girdle muscular dystrophy 1E\(^{58}\) and myopathies, respectively\(^{59,61}\). Mutations in the three remaining chaperones \((\text{BCS1L, SIL1, and FKBP14})\) lead to diseases that affect multiple tissues apart from skeletal muscle (Supplementary Data 3). We also examined the remaining chaperones that were upregulated in skeletal muscle for association with muscle disease or function by literature mining. We found evidence for 31/50 chaperones (Supplementary Table 1), including 17 chaperones that were associated with muscle diseases (e.g., HSPB8 with myopathies) and 27 chaperones that were associated with muscle function (e.g., the myosin chaperone UNCA45B). Thus, chaperone expression levels are likely designed to meet tissue-specific demands, and consequently affect disease risks in a tissue-specific manner.

**A conserved chaperone expression pattern in muscle.** Since our analyses relied on transcriptomic datasets, which might not be informative of tissue proteomes, we decided to test chaperone expression patterns at the protein level. We first compared the transcriptomic profile of skeletal muscle to a recently published proteomic profile of the same tissue\(^{43}\). The two profiles correlated significantly for chaperones and protein-coding genes \((r = 0.61 \text{ and } r = 0.37, \text{respectively, } p = 2.2E-16, \text{Pearson correlation, Supplementary Fig. } 5A)\). Next, we asked whether this pattern is conserved. For this, we profiled the proteome of the C2C12 mouse myoblast cell line before differentiation (day 0) and after 8 days of differentiation to myotubes (day 8, myotubes). A subset of undifferentiated muscle cells (reserve cells) was used as a control (day 8, reserve cells; Fig. 3a). We then compared the differential proteomic profile of C2C12 cell line (day 8 myotubes vs. day 0) to the differential transcriptomic profile of human skeletal muscle. In support of the functional relevance and conservation of the GTEx muscle transcriptomic profile, the proteomic and transcriptomic profiles were indeed correlated, both upon considering all protein-coding genes \((r = 0.65, p = 2.2E-16, \text{Pearson correlation, Fig. } 3b)\) and upon considering chaperones alone \((r = 0.47, p = 7.8E-5, \text{Pearson correlation, Fig. } 3c)\). In contrast, no correlation was observed when we compared the differential proteomic profile of reserve cells to the differential transcriptomic profile of human skeletal muscle (Fig. 3d).

To substantiate the experimental results, we also compared the proteomic profile of C2C12 myotubes to the transcriptomic profile of skeletal muscle from the HPA\(^{30}\) (Supplementary Fig. 5B), and to the proteomic profile of human skeletal muscle\(^{43}\) (Supplementary Fig. 5C). The proteomic profile of C2C12 myotubes indeed correlated with both datasets, though to a lesser extent (Supplementary Fig. 5B, C). Lastly, we
analyzed the expression of a subset of chaperones (10 sHSP genes) using qPCR in an alternative muscle cell line model consisting of cycling versus 5 days differentiated human myoblasts (LHCNM2 cells). Six of the seven sHSP that were expressed at least 2-fold higher in skeletal muscle according to the GTEx dataset were also upregulated in differentiated LHCNM2 cells (Supplementary Fig. 5D). The three remaining sHSP chaperones, which were not upregulated in skeletal muscle according to the GTEx dataset, were also not upregulated in differentiated LHCNM2 cells (Supplementary Fig. 5D). These data provide further experimental support for the conservation of the muscle-specific chaperone expression pattern.

We further tested the functional conservation of muscle chaperones by comparing between human and the evolutionary distant multicellular organism *Caenorhabditis elegans*. We used a set of *C. elegans* muscle chaperones, and compared them to human chaperones with *C. elegans* orthologs that were expressed at least 2-fold higher in skeletal muscle relative to other tissues (see the “Methods” section, Supplementary Table 1). We found that the two subsets overlapped significantly (27 muscle chaperones, \( p = 0.0009 \), one-sided Fisher exact test, Fig. 3e).
the HSP40 protein DNAJB6 had over 4-fold change in human skeletal muscle and its
C. elegans homolog, dnj-24, was highly expressed in C. elegans body-wall muscle cells. Moreover, 23/27
orthologous muscle chaperones had a known association with muscle folding, such as the myosin chaperone
UNC45, and the CCT subunits that are linked to actin folding (Supplementary Table 1). Altogether, these observations suggest an inherent
requirement for chaperones in muscle tissue function that is
conserved in evolution.

**Chaperone families show variable behavior across tissues.** Since
members of each chaperone family share functional and struc-
tural attributes, we examined whether they also share expression
patterns. We focused on the tendency of chaperones for
ubiquitous expression, for variable expression across tissues, and
their impact on growth. To assess the ubiquitous expression of
each chaperone family, we recorded the number of tissues in
which chaperones were expressed (as in Fig. 1a, Supplementary
Data 1). Apart from sHSPs, all families were more ubiquitously
expressed than the rest of the protein-coding genes (adjusted
\( p < 0.029 \), MW test). Members of the Prefoldin family and the ATP-
hydrolyzing chaperone families were ubiquitously expressed,
except for the HSP60 chaperonin that encodes a CCT subunit-
like protein, CCT8L2, which was expressed speci-
fically in testis (Supplementary Fig. 6A). Members of co-chaperone families
showed a more variable pattern, with both tissue-specific and
ubiquitously expressed members, except for NEFs (Supplemen-
tary Fig. 6A). These included, for example, the skeletal muscle-
specific coHSP90 chaperone UNC45B. sHSPs were more tissue-specific than other chaperone families (adjusted \( p = 0.027 \), MW test). In fact, sHSPs were also more commonly causal for tissue-specific and neuro-muscular diseases\(^63-68\) (5/10 sHSPs, Supplementary Data 3).

To assess the tendency of chaperone families for uniform vs. variable expression across tissues, we associated each chaperone with the number of tissues in which it was variably expressed (Supplementary Fig. 6B, Supplementary Data 1). All families, except HSP70s and sHSPs, were expressed more uniformly across tissues than the rest of the protein-coding genes (adjusted \( p < 0.018 \), MW test). HSP60s and coHSP90s were also expressed more uniformly than other chaperone families (adjusted \( p < 0.019 \), MW test). In contrast, sHSPs and HSP70s were more variably expressed than other families (adjusted \( p < 0.033 \), MW test), with sHSPs also more variably expressed than other protein-coding genes (adjusted \( p = 0.015 \), MW test). Notably, HSP70 essential members, including the main cytosolic member HSPA8, the ribosome-associated member HSPA14, the ER member HSPA5 and the mitochondrial member HSPA9, were relatively uniformly expressed (Supplementary Fig. 6B, Supplementary Data 1).

Lastly, we assessed the impact on growth of each family (Supplementary Fig. 6C, Supplementary Data 1). Prefoldins, HSP60s, HSP70s, and coHSP90s were more important for growth than the rest of the protein-coding genes (adjusted \( p < 0.05 \), MW test), and prefoldins and HSP60s were also more important for growth than other families (adjusted \( p < 0.045 \), MW test). However, the impact of individual chaperones on growth was highly diverse in most chaperone families. For example, most CCT subunits were important for growth, yet CCT subunits such as the testis-specific CCT8L2 and the variably expressed CCT6B were less important. sHSPs were less important for growth than other chaperone families (adjusted \( p < 0.066 \), MW test), fitting with them having tissue-specific expression. In summary, whereas certain features were family-based, members of most chaperone families showed distinct features hinting to distinct functionalities across tissues.

### Core chaperones versus variable chaperones

We next focused on the small subset of 32 (16%) chaperones that were expressed uniformly across tissues, henceforth denoted as core chaperones. Literature mining of the cellular processes in which these core chaperones participate showed that they covered a variety of basic processes, including de novo protein folding maturation and assembly; ER-associated targeting, transport, folding, and degradation; and translocation into the mitochondria (Fig. 4a). Core chaperones were also slightly enriched for Prefoldins, CCTs, and coHSP90s, and were depleted for sHSPs.

Next, we asked whether core chaperones included chaperones with known heritable disease-causing aberrations (see the “Methods” section). Only 3/32 core chaperones had such aberrations, in contrast to variable chaperones, which were significantly enriched for them (\( p = 0.04 \), Fisher exact test, Fig. 4b). This suggests that core chaperones do not cause diseases, either because core chaperones are functionally redundant, or, inversely, because their aberration is embryonically lethal. One such example is the CCT5 CRISPR knockout (Cct5\(^{−/−}\)(Impc\(^{−/−}\)(Ctcp)) mutant mice that showed preweaning lethality. To quantitatively distinguish between the two alternatives, we compared between the impact on growth of mutations in core and variable chaperones\(^69\). We found that core chaperones were significantly more important for growth than variable chaperones (\( p = 1.9E−6 \), KS test, Fig. 4c, Supplementary Fig. 4c), also upon limiting variable chaperone to those that were expressed ubiquitously (\( p = 2.7E−12 \). Lastly, we compared the median expression levels of core versus variable chaperones across tissues (Fig. 4d, Supplementary Fig. 2D). Core chaperones were significantly more highly expressed than variable chaperones (\( p < 2.2E−16 \), MW test). Taken together, we propose that core chaperones establish an essential subset of chaperones with stringent, uniform demand across tissues.

### Chaperone networks differ between tissues

Chaperones act as part of a functional network. Previous studies analyzed the physical interactions of a subset of chaperones in tissue culture\(^29\) and cancer cell lines\(^70\), or studied chaperones that were co-regulated in cancer samples\(^39\) or in aging\(^37\). Yet, the functional network of chaperones in physiological human tissues was rarely analyzed.

To examine this network, we focused on the co-expression of chaperone pairs across tissues. For each pair of chaperones and for each tissue, we computed the pairwise correlation between chaperone expression levels across samples of that tissue (Supplementary Data 5). We then constructed a heatmap presenting these correlations for pairs composed of core chaperones (Fig. 5a) and for mixed pairs composed of core and variable chaperones (Supplementary Fig. 7A). Though core chaperones were uniformly expressed, their expression levels were not uniformly correlated across tissues (Fig. 5a). In some tissues, such as heart and brain, pairs showed similar and relatively high correlations. However, in most other tissues, and more so among mixed pairs, the correlations varied, suggesting that chaperones create tissue-specific networks with distinct folding capacities.

To demonstrate this, we focused on the CCT complex, which is composed of core and variable subunits (Fig. 5b). Again, we found that in a subset of the tissues, a subset of CCT subunits were highly correlated. Strikingly, CCT pairs that showed low or anti-correlated expression were all mixed pairs (e.g., the variable chaperones CCT6B or CCT8L2 with core chaperones). These co-expression changes hint toward the functional plasticity of the chaperone network across tissues. For example, the core chaperone CCT5 was highly correlated with the variable chaperone CCT6B specifically in testis (\( r = 0.89 \)), whereas in other tissues CCT5 was highly correlated with the core chaperone CCT6A, the paralog of CCT6B (median correlation 0.79; Fig. 5b). In agreement, CCT6B high levels are specifically associated with testicular cancer, whereas high levels of CCT6A are associated with a broad range of cancers\(^51\).

To quantify the tissue-specificity of functional relationship between core–core versus mixed chaperone pairs at large-scale, we associated each chaperone pair with the number of tissues in which the pair was highly correlated (see the “Methods” section). We found that the mixed relationships were significantly more tissue-specific than core–core relationships (\( p = 6.2E−5 \), KS test, Fig. 5c and Supplementary Fig. 7B), thereby giving rise to distinct, tissue-specific functional networks.

To facilitate interrogation of the functional relationships between chaperones across tissues, we created the ChaperoneNet webtool (https://netbio.bgu.ac.il/chapnet/). Users can query ChaperoneNet by chaperone and tissue, and obtain a graphical network representation of the functional relationships of the query chaperone in that tissue. The network highlights core chaperones, marks chaperone families, and reports known disease associations.

### Consistent chaperone organization across development and aging

To understand how fundamental is the chaperone organization that we observed in adult human tissues, we went on to test whether it is maintained during human organ development. To answer this question, we relied on a recent transcriptomic...
analysis of seven human organs, including brain (cerebrum), cerebellum, heart, kidney, liver, ovary, and testis. These organs were profiled at 23 time points that ranged from early organogenesis (14 time points in weeks 4–20 post conception) to adulthood. The study then associated each gene with measures that represented its organ specificity, and with its time point specificity in each organ (0 non-specific, 1 most specific). We first compared between the organ and time point specificities of chaperones versus other protein-coding genes. In accordance with their ubiquitous expression in adult tissues, chaperones were significantly more broadly expressed (i.e., non-specific) across developing organs and across developmental time points in each organ (p = 1.9E–6, one-sided Kolmogorov–Smirnov test). Core chaperones were significantly more highly expressed (p = 2.2E–16, one-sided Mann–Whitney test). In the boxplot representation, center line, median; box limits, upper and lower quartiles; whiskers, 1.5× interquartile range. Source data are provided as a Source Data file.

It is commonly acknowledged that the deterioration observed in aging, particularly of the brain, involves reduced capacity for proper protein folding, thereby leading to increased risk for protein-misfolding neurodegenerative diseases, such as Alzheimer’s and Parkinson’s diseases. To illuminate the relationship

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**Fig. 4** The fundamental roles and impact of core chaperones. **a** The functional roles of the 32 core chaperones. Chaperones with several functions appear in all categories that apply. Core chaperones performed basic function required by different cell types. **b** The overlap between core chaperones (top) or variable chaperones (bottom), and chaperones with known aberrations that are causal for Mendelian diseases. Core chaperones tend to be depleted of such chaperones, whereas variable chaperones tend to be enriched for them (p = 0.04, one-sided Fisher exact test). **c** The cumulative distribution of the impact on growth of core (blue) versus variable chaperones (pink) measured in 769 cell lines harboring CRISPR-induced gene inactivation. 31 core and 155 variable chaperones for which CRISPR scores were available were considered. Each gene was associated with its minimal CRISPR score. Core chaperones were significantly more important for growth (p = 1.9E–6, one-sided Kolmogorov–Smirnov test). **d** The median expression levels per tissue of core versus variable chaperones (based on n = 928 and n = 4173 values, respectively). Core chaperones were significantly more highly expressed (p = 2.2E–16, one-sided Mann–Whitney test). In the boxplot representation, center line, median; box limits, upper and lower quartiles; whiskers, 1.5× interquartile range. Source data are provided as a Source Data file.
between aging and the brain-specific chaperone network, we relied on a large-scale analysis of chaperone expression in aging brains\(^\text{37}\). There, pairs of chaperones with highly correlated expression in aging brain were identified, and the expression of each chaperone was tested for its correlation with age. To examine whether the functional relationships between chaperones were conserved in aging, we compared between highly correlated pairs observed in adult brain and in aging brain. We found a highly significant overlap between them (265 pairs, \(p = 1.4E\text{–}32\), Fisher exact test, Fig. 6c). Most of the overlapping pairs were highly correlated only in brain and not in other tissues (167/265 pairs, Fig. 6c). Moreover, overlapping pairs belonged primarily to modules that were repressed in aging brain\(^\text{37}\) (258/265). This suggests that a brain-specific chaperone network is conserved across time and is repressed with age.

Next, we focused on the main components of the brain-specific chaperone network, namely core chaperones and variable chaperones that were upregulated in adult brain, and examined the change in their expression with age. We found that the expression levels of both core chaperones and brain-upregulated variable chaperones were generally reduced with age (Fig. 6d, Supplementary Fig. 8B). In contrast, the expression levels of variable chaperones that were not upregulated in brain did not change in brain aging. This further suggests that the brain-required chaperone system declines with age. Further decline in the brain-required chaperone system was observed when we examined the relative expression levels of chaperones in Alzheimer’s disease patients versus age-matched controls\(^\text{37}\) (Fig. 6E). Taken together, these results suggest a reduced protein-folding capacity of a brain-specific chaperone system in aging that could contribute to age-dependent neurodegeneration.

**Discussion**

Chaperones are basic components of all living organisms, and are thus commonly regarded as house-keeping genes\(^1\). But, are all folding environments similar to each other? In a multicellular organism, owing to the specialized structures, functions, and environments of the different cell types, folding requirements could differ greatly. This raises the question of whether the chaperone system is organized as a one size fits all, or whether the requirements of each tissue are met by a tailored system. To address this question systematically, we set out to examine the expression landscape of molecular chaperones across physiological human tissues.

We first examined the expression of chaperones across adult human tissues, as measured via RNA-sequencing by the GTEx consortium\(^\text{32}\). We determined that most chaperones were expressed in all tissues, were highly expressed in most of them, and were more important for growth than other protein-coding genes, suggesting that they act as major building blocks across all tissues (Fig. 1). Nevertheless, chaperones are also associated with tissue-specific phenotypes, since germline aberrations in ubiquitous chaperones or in ubiquitous aggregation-prone proteins can lead to tissue-specific pathologies (Fig. 2a), such as muscle disorders (Fig. 2c) or neurodegeneration\(^\text{63,73–75}\) (Fig. 6e). Indeed, we found that most chaperones were variably expressed across tissues, suggesting that the chaperone system is not simply common and consistent across tissues (Fig. 2b). We did not consider substrate-specific chaperones, whose expression likely correlates with the expression of their target (for example, SERPINH1 expression is correlated with collagen,
its client, in collagen-synthesizing cells\textsuperscript{76}). The results of our analyses are available as a webserver https://netbio.bgu.ac.il/chapnet/ that can be queried by chaperone and tissue.

The GTEx transcriptomic resource allowed for high-resolution tissue-specific analyses, yet was based on samples that were collected from recently deceased donors. Consequently, the observed chaperone levels might represent stressful conditions. To test for this, we repeated analyses while excluding known stress-induced chaperones (Fig. 1). We identified similar trends, suggesting that the impact of stress is partial. Repeated analyses by using the HPA transcriptomic dataset that covered 37 tissues resulted in similar trends (Supplementary Figs. 2 and 3). A limitation of these datasets is that transcript levels might not be indicative of protein levels, as the general correlation between them is not high\textsuperscript{43,77}. Nonetheless, chaperone transcript levels correlated with their protein levels in matched skeletal muscle samples\textsuperscript{43} (Supplementary Fig. 5A). To further assess the relevance of the transcriptomic profiles, we profiled the proteome of a differentiating mouse myoblast cell line (Fig. 3a). Despite the difference in measured molecules (transcripts versus proteins) and species, we observed a strong agreement between the differential transcriptomic profile of human skeletal muscle and the proteomic profile of differentiated C2C12 mouse myotubes, but not reserve cells (Fig. 3b–d). Agreement was also observed upon comparing the proteomic profile of differentiated C2C12 mouse myotubes to a proteomic profile of human skeletal muscle\textsuperscript{43} (Supplementary Fig. 5). Moreover, human chaperone genes that were at least 2-fold differentially expressed in skeletal muscle by GTEx were also enriched for \textit{C. elegans} muscle chaperones (Fig. 3e), and for chaperones causal for muscle disorders (Fig. 2c), supporting the biological relevance of differential tissue transcriptomic profiles.
A small subset of chaperones, which we denoted as core, did act as consistent building blocks across all tissues. Belonging to fundamental cellular processes (Fig. 4a) this core subset was more highly expressed relative to variable chaperones (Fig. 4d). Core chaperones were also significantly more important for growth than other chaperones (Fig. 4c), and ~2.5-fold less likely to be causal for heritable disorders (9% versus 25%, Fig. 4b), suggesting that they could be lethal when mutated in the germline. Despite their consistent expression, core chaperones had tissue-specific functional relationships both with other core chaperones and with variable chaperones (Fig. 5a, b, Supplementary Fig. 7A).

The existence of ubiquitously expressed chaperones was previously suggested but not quantitatively demonstrated. An aging-related subnetwork of chaperones was defined by the impact of chaperone down-regulation on age-dependent neurodegenerative models. A comparison between our core subset and the aging-related subset revealed an overlap of 10 chaperones (p = 8E–5, hypergeometric test, see the “Methods” section) all of which were associated with de novo protein folding or protein maturation. A second study identified an epichaperone, which under stress conditions forms a network of stable complexes that facilitate tumor survival. A comparison between our core subset and this epichaperone revealed an overlap of nine chaperones (p = 0.024, see the “Methods” section), six of which were associated with signaling. Taken together, the core set of chaperones that we defined is multifaceted, and its dysregulation has pathological implications in neurodegeneration and cancer.

We tested the generalizability of the tissue-specific chaperone organization across the time axis, focusing on human organ development and brain aging. Across developing organs and time points, core chaperones were more ubiquitously expressed than variable chaperones. Adjusted p-values for organ specificity and time point specificity in brain, cerebellum, heart, kidney, liver, ovary, and testis, in respective order: chaperones versus protein-coding genes: p = 4.8E–16, 3.24E–18, 7.26E–17, 8.16E–20, 1.48E–14, 3.2E–21, 2.37E–23, 1.9E–20; core versus variable chaperones: p = 5.39E–10, 4.5E–5, 1.4E–4, 2.5E–4, 1.4E–4, 6.44E–5, 3.53E–5, 1.23E–7 (one-sided Mann-Whitney test, Benjamini-Hochberg correction). Chaperones n = 187, 180, 171, 178, 176, 176, 177, 184; protein-coding genes n = 16,955, 14,517, 14,017, 13,828, 14,136, 13,854, 13,988, 15,712; core chaperones n = 31; variable chaperones n = 156, 149, 140, 147, 145, 145, 153. Expression correlation values in adult tissues for chaperone pairs whose pair-mates share, or do not share, a developmental module. Except for ovary, pairs belonging to the same developmental module were more highly correlated than other pairs. Adjusted p-values for brain, cerebellum, heart, liver, and tests, in respective order: p = 3.14E–14, 6.33E–8, 4.7E–9, 8.6E–7, 2.35E–23 (one sided Mann-Whitney test). Pairs in same module n = 916, 1204, 862, 694, 1818; pairs in other modules n = 16,851, 16,563, 16,904, 17,072, 15,948. Consistency between chaperones that were highly correlated (r > 0.8) in adult brain (n = 2014 pairs) and in aging brain (n = 1193 pairs) is shown by their significant overlap (265 pairs, p = 1.4E–32, one-sided Fisher exact test). The distribution of the 265 consistent chaperone pairs by the number of non-brain tissues in which they were highly correlated reveals that most of them were brain-specific, i.e., were not highly correlated in any other tissue. The correlation between chaperone expression levels in prefrontal cortex and age is shown for core chaperones (n = 26), variable chaperones that were upregulated in adult brain (n = 78), and variable chaperones that were not upregulated in brain (n = 73, other). Expression levels of core and brain-upregulated chaperones decreased with age. The expression levels of other variable chaperones did not change (see Supplementary Fig. 8B for additional brain regions). The difference between the two subsets of variable chaperones was statistically significant (p = 6.9E–6, one-sided Mann-Whitney test).

Relative expression levels of chaperones in patients with Alzheimer’s disease and controls is shown for core chaperones (n = 26), variable chaperones that were upregulated in adult brain (n = 78), and remaining variable chaperones (n = 73, other). Expression levels of core and brain-upregulated chaperones decreased in patients, whereas expression levels of remaining variable chaperones did not change. The difference between the two subsets of variable chaperones was statistically significant (p = 4.12E–6, one-sided Mann-Whitney test). In the boxplot representation, center line, median; box limits, upper and lower quartiles; whiskers, 1.5× interquartile range; points, outliers. Source data are provided as a Source Data file.
propose that it could also stem from chaperones collaborating or competing with each other over substrates, thereby leading to a proteostatic environment that is limiting to other proteins83–87. Since the levels of chaperons and substrates are altered between tissues, and since these alterations are functional, the proteostatic environment could both contribute to tissue-specific functionality and elicite tissue-specific phenotypes when perturbed, thereby giving rise to tissue-specific protein misfolding diseases. Targeting ubiquitously expressed chaperones can give rise to non-specific effects and has therapeutic limits88. Our results can help direct efforts toward chaperons that are more sensitive to cell-specific modulation of their expression, and thus might be better suited as potential therapeutic targets for cell-specific diseases. Formulating and quantifying the function of the chaperone system at large scale and within single cells is therefore one of the main challenges in proteostasis research.

**Methods**

**Curation of an integrated list of human chaperones.** We compiled a curated list of 195 known human chaperone, co-chaperone, and folding enzyme genes, collectively named chaperones (Supplementary Data 1). For that, we compared two curated lists of human chaperones: (1) a list of 332 chaperones curated by considering their biochemical properties and protein domains87; and (2) a list of 194 chaperones compiled based on sequence homology to conserved canonical chaperones and supplemented by a list of known co-HSP90 chaperones48. These lists included 114 genes belonging to well-conserved chaperone families, including Hsp (10 genes), Hsp60/Hsp10 (15 genes), Hsp70 (13 genes), Hsp90 (4 genes), Prefoldin (9 genes), and co-chaperones Hsp40 (49 genes) and Nefs (14 genes). 106 of these chaperones appeared in both lists (~93% overlap). Less agreement between the lists was observed for the co-Hsp90 family, where only 24 (19%) chaperones out of 127 putative co-Hsp90 chaperones appeared in both lists. This inconsistency was due to the lack of a conserved domain shared by all members of the co-Hsp90 family, and uniquely by them. For example, many known co-Hsp90 chaperones have a tetratricopeptide repeat (TPR) motif, but not all TPR-containing proteins are chaperones48. We therefore included 34 co-Hsp90 chaperones that were previously shown to interact or function with Hsp9033,39,42, three of which did not appear in any list and 22 appeared in both lists (~71% overlap). The remaining genes in the initial lists47,48, denoted as ‘others’, included members such as ER chaperones, mitochondrial chaperones, AAA+ proteins and folding enzymes. For each member, we mined the literature to determine whether it functions as a chaperone that transiently assists the folding, unfolding, assembly or disassembly of proteins and protein complexes. For example, we excluded eight nuclear cyclinophilins that appeared in both lists, as these were recently shown to constitute an integral part of spliceosomal complexes and were renamed as spliceophilin50. Of the 47 chaperones that we included as ‘others’, 30 appeared in both lists (~64% overlap). Finally, we excluded genes that were annotated as pseudogenes or as non-catalytic proteins. For example, of the six Hsp90 family genes, we excluded two pseudogenes (Hsp90aa2A and Hsp90b2P). Out of the 195 chaperones, only Ppiaa1A did not meet the expression threshold of 1 TPM in any tissue and was therefore omitted from further analyses.

**Additional chaperone subsets.** We annotated the functionality of core chaperones by manual curation of their Uniprot entry39 and by literature search. Data of curated, muscle chaperones in *C. elegans* were obtained from ref. 19. Data of orthologous chaperones in *C. elegans* were obtained via the OrthoList2 tool for comparative genomic analysis between *C. elegans* and humans49, and each human chaperone was fitted with the *C. elegans* gene(s) that was identified as its ortholog by the maximal number of programs. Data of orthologous chaperones in mouse were obtained from Mouse Genome Informatics (MGI) website46.

Genes with a known genetic aberration that is causal for heritable diseases were obtained from OMIM44, and limited to genes with a known genetic basis regardless of their mode of inheritance. Manually curated data of the tissues that clinically manifest a Mendelian disease were obtained from ref. 37. We additionally mined the literature for chaperones that are ideal for or associated with muscle disorders or muscle function (Supplementary Data 3 and Supplementary Table 1).

Chaperones that are induced by stress were obtained from a meta-analysis study of several microarray datasets of stress-induced genes50.

**Comparison to additional functional subsets.** An aging-related subnetwork of chaperones was defined previously by the impact of chaperone down-regulation on age-dependent neurodegenerative models37. The study included 187 chaperones, of which 20 were core chaperones. 28/187 were considered as an aging-related subset, including 10 core chaperones (p = 8e−3, hypergeometric test). The 10 core chaperones included 6 CCT subunits, HspA14, DNAJ1A1, Hsp90AA1 and its co-chaperone STIP1. A cancer-related study identified an epichaperone, which under stress conditions forms a network of stable complexes that facilitate tumor survival51. The study included 63 chaperones, of which 13 were core chaperones. 26/63 chaperones formed the epichaperome, including 9 core chaperones (p = 0.024, hypergeometric test). These included Hsp90aa1 and its co-chaperones (DNAJ/C7, AHSAI, NUDC, SGTA, STIP1, SUGT1), as well PFDN2, DNAJ1A1. The core chaperones DNAJ1A1, Hsp90AA1, and its co-chaperone STIP1, were part of both the aging-related and the epichaperone subsets.

**Human gene expression dataset.** Data of transcriptomic profiles of human tissues measured via RNA-seqencing were downloaded from the GTEx portal (version 7). We analyzed samples from donors with traumatic injury fitting with a survival end point and, avoided other end points to limit the effect of tissue death causes to limit the effect of treatment and stress on chaperone expression44–47. We considered only physiological tissues (i.e., no transformed cells) with ≥5 samples. Brain sub-tissues were collapsed into three main regions including brain basal ganglia, largely cortex, and brain other, according to Paulson et al. 52. Altogether, we analyzed 488 transcriptomic profiles and 29 tissues (Supplementary Data 2). Genes were mapped to their Ensembl gene identifiers using BioMart48, and filtered to include only protein-coding genes. Data of gene expression pattern during organ development was extracted from ref. 42, and included organ and timepoint specificity scores per gene, and module association per gene and organ. Data of chaperone expression in aging brain were extracted from ref. 87 and included, per chaperone, a correlation between its expression and age, a ratio between its expression in patients with a neurodegenerative disease and age-matched controls, its module association, and a list of highly correlated chaperone pairs. To verify the trends observed with the GTEx dataset, we used transcriptomic profiles of 37 human tissues measured via RNA-sequencing by the HPA50. Expression values in protein TPM (pTPMs) units were downloaded from the HPA portal.

**Gene expression analyses.** To analyze the expression pattern of genes across tissues, we considered genes as expressed in a tissue if their median expression level was ≥1 TPM. Results pertaining to expression thresholds of 5 or 10 TPM are presented in Supplementary Fig. 1 and Supplementary Fig. 3. The differential expression of genes per tissue was calculated as in ref. 7. Raw reads were normalized to obtain the same library size for every sample by using the trimmed mean of M-values (TMM) method by the edgeR package59. Genes with ≤10 raw counts per sample across all samples were removed before normalization. In
each sample, we transformed the normalized counts using the volcano method\(^\text{100}\). To compute the differential expression of genes in a given tissue relative to another tissue, we compared all transformed profiles of that tissue to a background set containing transformed profiles of all other tissues. Differential expression of genes in a tissue relative to other tissues was then calculated using the Limma linear model\(^\text{100}\). The differential expression of a gene in a given tissue was then set to its log-fold-change in that tissue, and the p-values were adjusted for multiple testing using the Benjamini–Hochberg correction. We considered genes with an absolute log-fold-change \(\geq 1\) and adjusted \(p\)-value \(\leq 0.05\) as differentially or variably expressed. We defined core chaperones as chaperones that across all tissues had less than a 2-fold change in their expression. In the comparison between organ development and adult tissues, cerebrum and cerebellum were compared to brain 1 and brain 2, respectively. In the analyses of chaperone expression in different regions of the aging brain and in patients with neurodegenerative diseases versus controls, brain regions were matched with brain 1 for calling the expression of several gene markers, including myosins, MYH3, MYOM1, and chaperones. We determined the expression pattern using GenElow TriRNA Pure Kit (Geneaid) and DNase I DNA-free DNA removal Kit (Invitrogen). First-strand cDNA was generated using Iscript cDNA Synthesis Kit (BioRad) for real-time PCR (qPCR). For each reaction 1 \(\mu\)g of total RNA was used. The cDNA was stored at \(-20^\circ\text{C}\) or used immediately. cDNA was amplified in CFX96 Q-OptiGene QPCR Fast Kit (Bio-Rad) using KAPA SYBR FAST Kit (Sigma). For each reaction 20 ng of cDNA was used. The real-time PCR was performed as follows: one cycle of denaturation (95 °C for 3 min) followed by 40 cycles of amplification (95 °C for 3 s, 60 °C for 30 s). Each reaction was monitored by the use of the \(2^{\Delta\Delta C_{T}}\) method, normalizing to the housekeeping gene Hprt1. MW test was used for comparisons between myotubes or reserve cells to control (Supplementary Fig. 5E). A list of primers used is detailed in Supplementary Table 2.

### C2C12 mouse myoblast cell line mass-spectrometric analysis

The proteome of C2C12 mouse myoblast cell line before differentiation (day 0); after 8 days of differentiation to myotubes (day 8, myotubes); and of undifferentiated muscle cells (day 8 reserve cells) were analyzed using LC–MS/MS (\(n = 3\) biological repeats for each treatment; Technion, Israel). Cells were suspended with 9 M urea and 10 mM DTT solution and sonicated with a Cup-Horn sonicator for 2 min with 10 s on and off cycles. After centrifugation to remove cell debris, samples were digested by trypsin (Promega) at a 1:50 enzyme-to-substrate ratio, overnight at 37 °C with an additional 30 min at 65 °C. Trypsin solution and the dry peptide samples were desalted using C18 tips (Top tip, Glygen) dried and re-suspended in 0.1% formic acid. The resulted peptides were analyzed by LC–MS/MS using a Q-Exactive plus mass spectrometer (Thermo) fitted with a capillary HPLC (easy nLC 1000, Thermo-Fisher). The peptides were loaded onto a C18 trap column (0.3 × 5 mm, LC-Packings) connected on-line to a homemade capillary column (20 cm, 75 micron ID) packed with Reprosil C18-Aqua (Dr. Maisch GmbH, Germany) in solvent A (0.1% formic acid in water). The peptides mixture was resolved with a 5–28% linear gradient of solvent B (95% acetonitrile with 0.1% formic acid) for 180 min followed by gradient of 15 min gradient of 28–95% and 95% acetonitrile with 0.1% formic acid, at a flow rate of 0.15 μL/min. Mass spectrometry was performed in a positive ion mode (at mass range of m/z 350–1800 AMU and resolution 70,000) using repetitively full MS scan followed by collision induces dissociation (HCD, at 35 normalized collision energy) of the 10 most dominant ions (+1 charges) selected from the first full MS scan.

The mass spectrometry data was analyzed using the MaxQuant software 1.5.28. (www.maxquant.org) for peak picking identification and quantitation using the Andromeda search engine, searching against Mus musculus part of the Uniprot database (June 2015) with mass tolerance of 20 ppm for the precursor masses and 20 ppm for the fragment ions. Trypsin was set as the protease. Methionine oxidation and acetylation of protein N-term were accepted as variable modifications and carbamidomethyl on cysteine was accepted as static modification. Minimal peptide length was set to six amino acids and a maximum of two misscleavages was allowed. Peptide-level and protein-level false discovery rates (FDR) were calculated to 1% using the target-decoy database. The dataset was filtered to eliminate the identifications from the reverse database, and common contaminants. The data was quantified by label free analysis using the MaxQuant software, based on extracted ion current (XICs) of peptides enabling quantitation from each LC/MS run for each peptide identified in any of experiments. Welch’s t-test was performed on the log2 of the LFQ Intensity values using Perseus software. We used only mouse proteins whose expression was measured at day 0 and day 8 that showed significant differences, and their human homologs. In the comparison to protein abundance in human skeletal muscle\(^\text{16}\), human proteins were compared with their median raw protein abundance across skeletal muscle samples.

### Cell cultivation of LHCN-M2 human myoblast cell line and RT-qPCR analyses

LHCN-M2 (cycling human myoblasts) cells were described elsewhere\(^\text{169}\). The cells were maintained in Ham-F12 supplemented with 100 U/mL penicillin/streptomycin, 20% FBS, and 25 ng/mL of rhFGF-b/FGF-2. For induction of myogenic differentiation, LHCN-M2 cells were cultured in DMEM supplemented with 100 U/mL penicillin/streptomycin, and 30 μg/mL human insulin solution for 5 days. Cells were routinely treated for mycoplasma contamination.

For gene expression analysis in LHCN-M2 cells, total RNA was isolated using Trizol, followed by purification using RNA Clean & Concentrator (Zymo Research, USA) and DNase I Set (Zymo Research, USA). First-strand cDNA was generated using Maxima First Strand cDNA Synthesis Kit for RT-qPCR (ThermoFisher). For each reaction 1 \(\mu\)g of total RNA was used. The samples were incubated at 25 °C for 10 min followed by a step of 50 °C for 15 min, and then 85 °C for 5 min. The cDNA was stored at \(-20^\circ\text{C}\) or used immediately for RT-qPCR. cDNA was amplified in CFX96 Touch Thermal cycler (Bio-Rad) using TAQ SYBR Green qPCR SYBR (ThermoFisher). For each reaction 50 ng of cDNA was used. RT-qPCR was performed as follows: one cycle of denaturation (95 °C for 3 min) followed by 40 cycles of amplification (95 °C for 10 s, 60 °C for 30 s). Each reaction was monitored by the use of a negative control (no template). DNA amounts were quantified using
the ΔΔCt method, normalizing to RPL0and and the cycling condition was set to 1. A list of primers used is detailed in Supplementary Table 2. Student’s t-test was used for comparisons between two groups.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

### Code availability

Data of chaperone families and interactions of chaperones across tissues is available as Supplementary Data 4. Data of pairwise tissues analyzed in the study appear in Supplementary Data 2. Data of disease-related ΔΔCtΔΔCtARTICLE NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-021-22369-9 | www.nature.com/naturecommunications

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Competing interests
The authors declare no competing interests.

Additional information
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