Reviewer 1

1. The discussion of obligate substrates in the introduction and how they were classified a such is helpful, but a table within the manuscript that clearly lists the substrates that are shown in Fig. 1 is needed, preferably one that also provides details on expression changes in the different backgrounds. These are currently just shown as unlabeled red dots.

We agree and added a table (Table 1) to clearly lists the GroE substrates, which are shown in Fig. 1.

2. The discussion of the proteomic data would benefit from an introductory description that summarizes the range of expression changes in the ~1300 proteins examined and clearly states the number of GroE substrates (obligate or otherwise) that were in the dataset. As it is, the supplementary data provides more information on the proteins that were NOT in the dataset ~~then~~ than those that were in the dataset (Supp. Table 1).

According to the suggestion, we summarized the proteome changes including the GroE-substrates in the revised manuscript (lines 83-84 in main text and the legend of Supplementary Figure S1A). Regarding the dataset, we apologize for not uploading the supplemental dataset at the initial submission. We properly prepared and upload the tables, supplementary tables, and supplementary datasets in this revision. We hope now that the information about the proteomes including the GroE-substrates meets the requirement raised by the reviewer.

3. There are two cases where the authors say something but no citation is provided: " As reported previously, the deletion of dnaKJ caused drastic proteome changes (Figure 2A and Supplementary Dataset S2) " and As reported previously, the MS analysis confirmed the GroE depletion-induced large proteome alteration, including the overexpressions of MetE and several heat shock proteins such as ClpB and DnaK (Supplementary Dataset S1). Moreover, if results from these datasets are already published, it would be helpful to clearly distinguish what is different between the current study and the prior study.

Thank you for pointing out this. We had carelessly failed to cite the reference, and thus cited the appropriate references.
Regarding the comparison between the prior study and the current study, the *E. coli* strains used in the previous data are only a small subset of the strains used in the current study,

so direct detailed comparisons seem to be not important. However, we would like to note that the proteome data in the cited references were obtained by shotgun proteomics 10 years ago and that the current MS data are much better in quality and quantity.

4. Also, since the authors are vague about the identity of the GroE substrates that are examined, it is not clear whether the same substrates are red dots in both Figs. 1 and 2.

According to the suggestion, we included tables (Tables 1 and 2) that show the identity of the GroE substrates in the revision.

5. Since the authors did not find a role for DnaJ/K for the obligate GroE substrates it would be interesting if they did see a role of the other classes of GroE substrates that were discussed in the introduction.

According to the suggestion, we also included additional proteome data for other classes of GroE substrates (Supplementary Figures S1 and S2) to show that there are no notable biases for GroE substrates other than Class IV, *i.e. in vivo* obligate GroE substrates (lines 123-125).