Response to Luca L Fava and colleagues

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Despite the discrepancies with our results, we are confident in our data for several reasons:

1) A second siRNA (called siRNA Beclin-1 (#2)) in our article gave similar results to those obtained with siRNA Beclin1-ER (see Supplementary Fig S1 in our paper by Frémont et al [1]).

2) A HeLa cell line stably expressing an (HA)-Beclin-1 construct resistant to the siRNA partially rescued the Beclin-1 depletion phenotype with respect to mitotic progression (the rescue of the other phenotypes ascribed to Beclin-1 was not assessed), arguing against an off-target effect of the siRNA. In examining this experiment in our paper, Fava et al remark: “Upon Beclin-1 knockdown, [however], both cell lines show a more than two-fold increase in G2/M cells when compared to the control. This actually documents that the Becn1-ER siRNA triggers the same relative increase in G2/M cells in both cell lines investigated and that this effect is not prevented by the expression of RNAi-resistant HA-Beclin 1.” However, we analyzed our results differently. We stated that ectopic expression of siRNA-resistant (HA)-Beclin-1 partially rescues (35%) the accumulation of 4n DNA content cells. Our conclusions were based on the calculation of the ratio of cells with 4n DNA content (G2/M cells) to cells with 2n DNA content cells (G0/G1 cells) in each experimental condition. Ratios obtained in cells depleted for Beclin-1 were compared to those obtained for siRNA control cells in each cell line. The effects of Beclin-1 depletion were thus compared between the two cell lines.

3) Most importantly, both our article and Fava’s report agree that treatment with siRNA Beclin1-ER leads to an increase in the time elapsed between nuclear envelope breakdown (NEBD) and anaphase. The severity of this phenotype depends on the concentration of transfected siRNA Beclin1-ER in both studies (see Fig 2H in our article and Fig 1G in Fava’s report). However, because this phenotype did not correlate with the level of Beclin-1 detected by Western blot in Fava et al, they conclude that the siRNA Beclin1-ER-induced phenotype is an off-target effect (Fig 1F). In contrast, in our paper, the concentration of transfected siRNA is indeed inversely proportional to the level of Beclin-1 detected by Western blot and to the number of correct mitotic events observed. Although knockdown of Beclin-1 with siRNA Becn1-II seems as efficient as the depletion induced by siRNA Becn1-ER transfection (see Supplementary Fig S1B), quantitative comparison of the efficiency of Beclin-1 depletion with these siRNAs (Becn1-II and Becn1-ER) at protein level should be performed head-to-head to reconcile these conflicting data.

4) Despite the research in databases (Blastn and RNA plex) and transcriptomic experiments, Fava et al could not identify a “smoking gun” off-target candidate that might explain the discrepancies between our paper and their report.

5) In addition to the mitotic progression phenotype of the siRNA treatment, we also showed that Beclin-1 associates with kinetochore microtubules, and forms discrete foci at the kinetochores of attached chromosomes. Moreover, we also identified a direct interaction between Beclin-1 and the outer kinetochore protein Zwint-1 (previously reported by Behrends and colleagues [2]), a component of KMN (KNL-1/Mis12/Ndc80) complex which is essential for kinetochore–microtubule interactions and chromosomes congression. These results are coherent with

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the phenotype of our siRNA studies and argue for a genuine role of Beclin-1 in chromosome congression.

Taken together, the collection of data and controls in our paper support a role for Beclin-1 in mitosis progression. The report from Fava et al. does reveal how siRNA treatment may have a widespread effect on cellular transcripts, but it does not address Beclin-1’s function in kinetochore assembly.

References