

Article

The Function of *Drosophila* USP14 in Endoplasmic Reticulum Stress and Retinal Degeneration in a Model for Autosomal Dominant Retinitis Pigmentosa

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Supplementary Materials

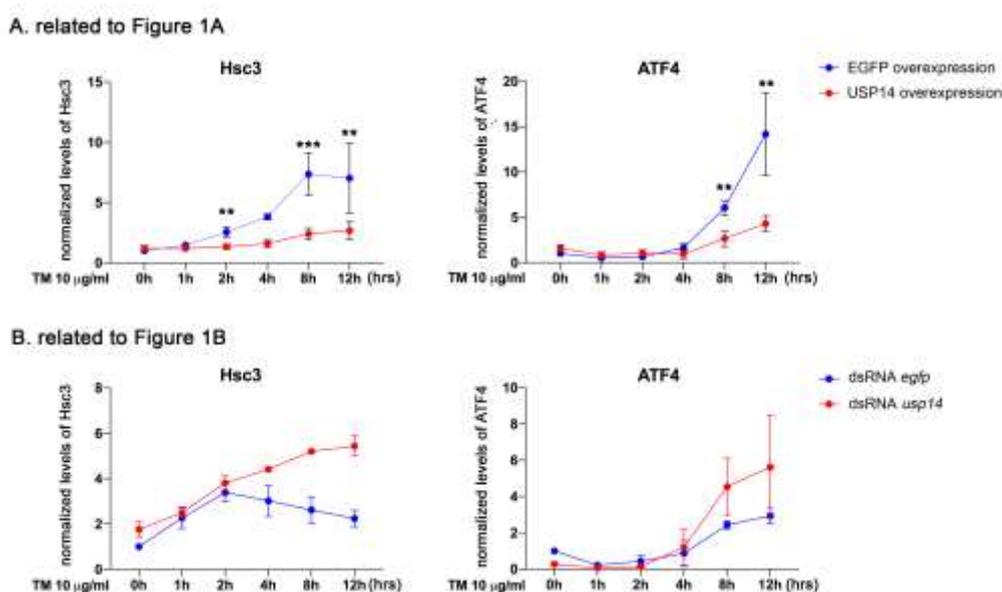


Figure S1. Quantification of the normalized Hsc3 or ATF4 band intensity, using ImageJ software. Error bars, \pm SEM. (a) $n > 3$. The p values were calculated using Student's t test: ** $P < 0.05$, *** $P < 0.001$. (b) $n = 2$.

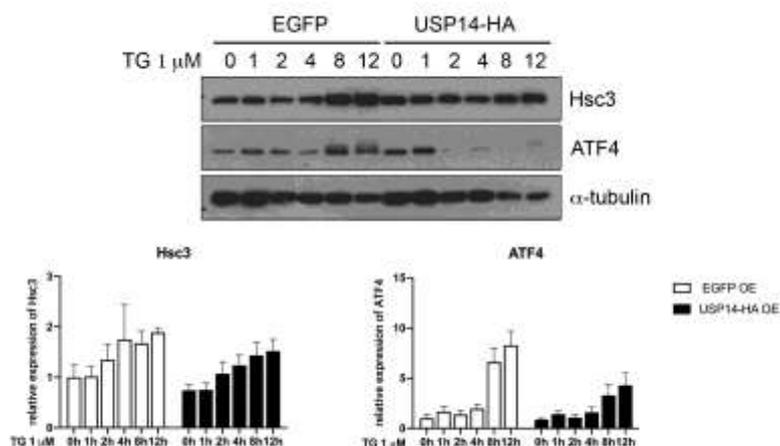


Figure S2.

Overexpression of USP14 decreased the levels of thapsigargin-induced ER stress. (a) Control cells (EGFP overexpressing) and USP14 overexpressing S2 cells were treated with 1 μM thapsigargin (TG) for the indicated intervals, and the extracts were analyzed for the expression of hsc3, ATF4, and α-tubulin using immunoblotting. (b) Quantification of the average normalized hsc3 or ATF4 band intensity using ImageJ. Error bars, \pm SEM.

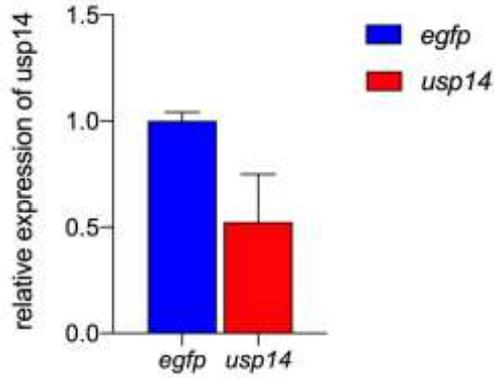


Figure S3. Knockdown efficiency of *usp14* in *Drosophila* S2 cells. As a control, RNAi against *egfp* was used. Error bars, \pm SEM.

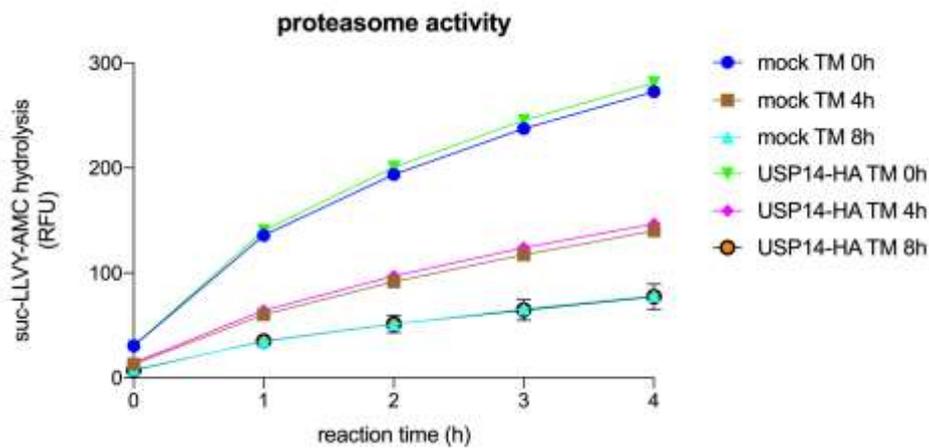


Figure S4. Kinetic measurement of proteasome activity. (a and b) *Drosophila* S2 cells expressing enhanced green fluorescent protein (EGFP, control) or USP14-HA were treated with 10 μ g/ml tunicamycin (TM) for the indicated intervals. Proteasome activity was measured based on the hydrolysis of the Suc-LLVY-AMC fluorogenic substrate at the indicated time points in tunicamycin-treated *Drosophila* S2 cells. The values were normalized to basal activity, which was determined upon treatment with 20 μ M MG132. RFU, relative fluorescence units.

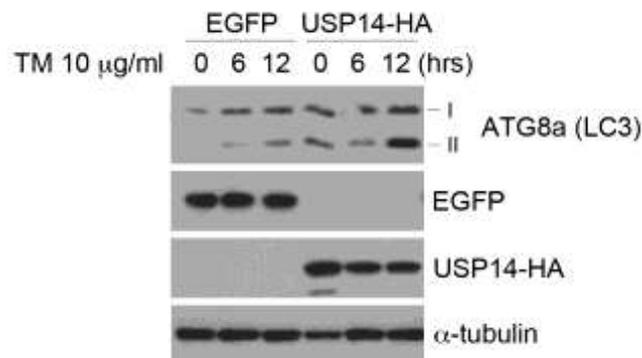
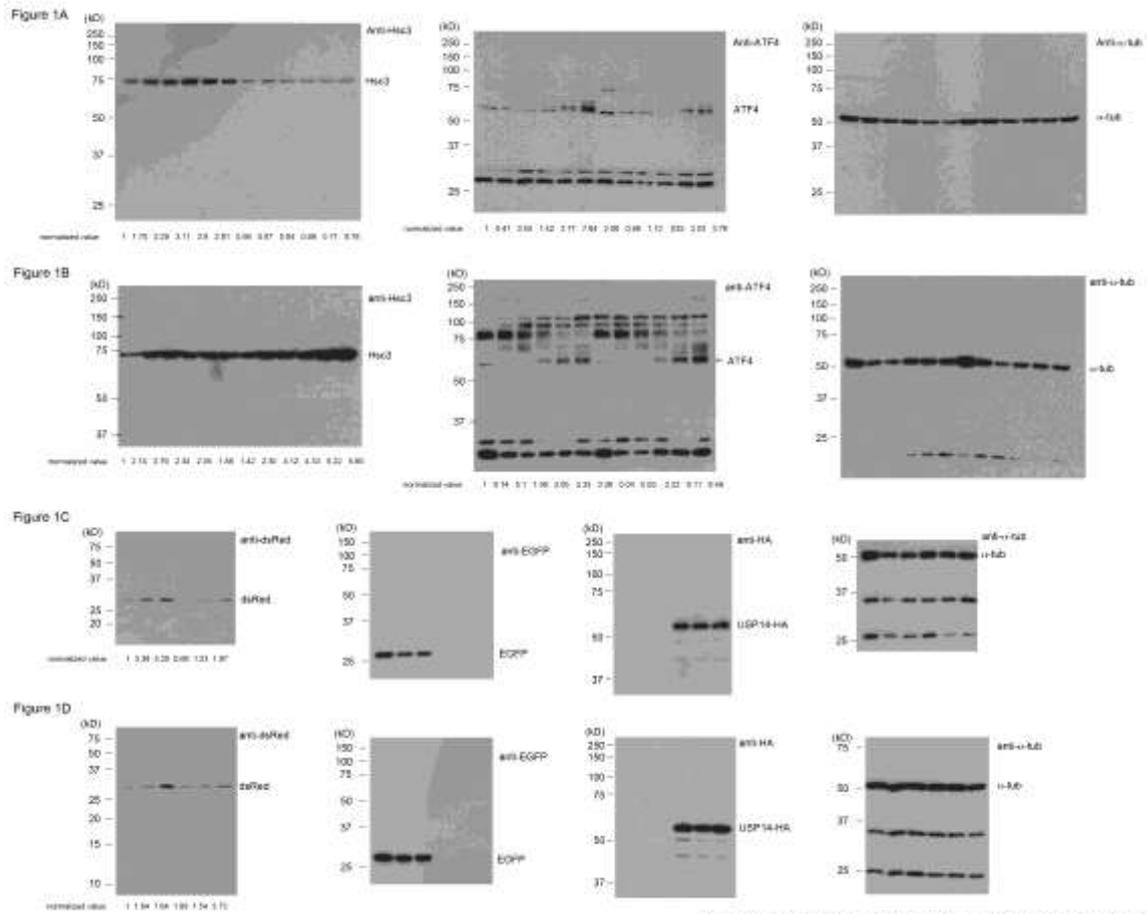


Figure S5. USP14 overexpression resulted in increased levels of Atg8a-II. *Drosophila* S2 cells were transfected with either EGFP (lanes 1–3) or USP14 (lanes 4–6) and treated with 10 μ g/ml tunicamycin (TM) for the indicated intervals. The induction of autophagy was detected using the anti-Atg8 antibody. The increase in Atg8a-II levels was enhanced in USP14-overexpressing S2 cells.



Supplementary Figure 6a, related to Figure 1

Figure 2A

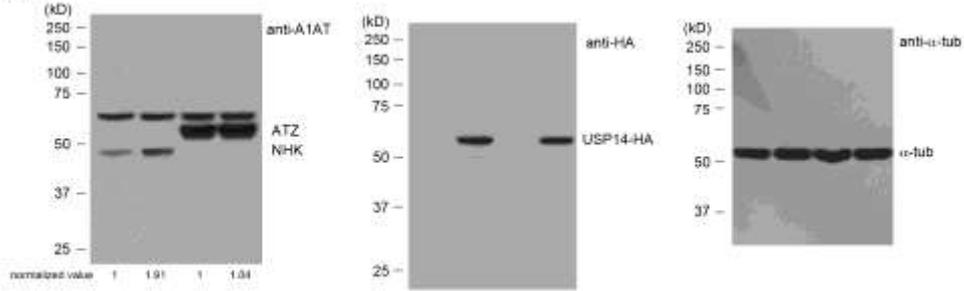


Figure 2B

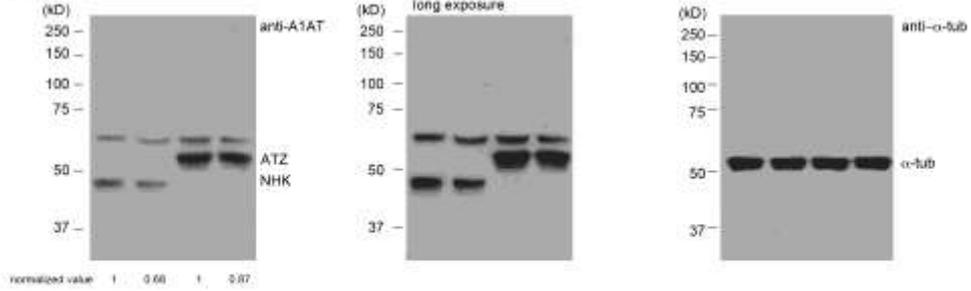
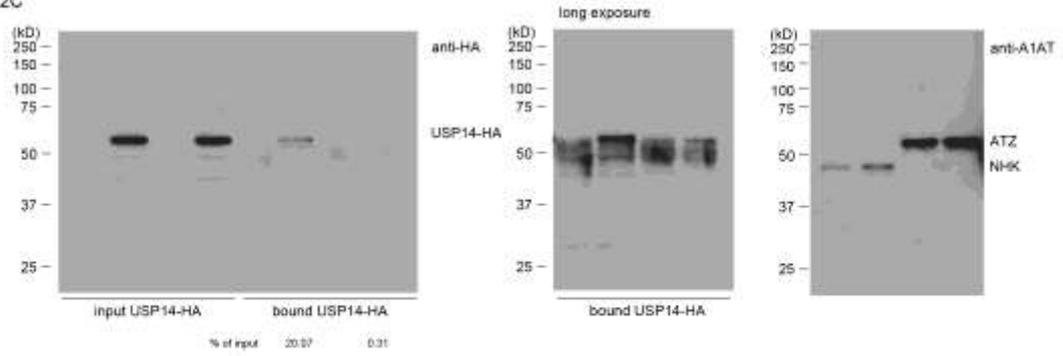


Figure 2C



Supplementary Figure 6b, related to Figure 2

Figure 3A

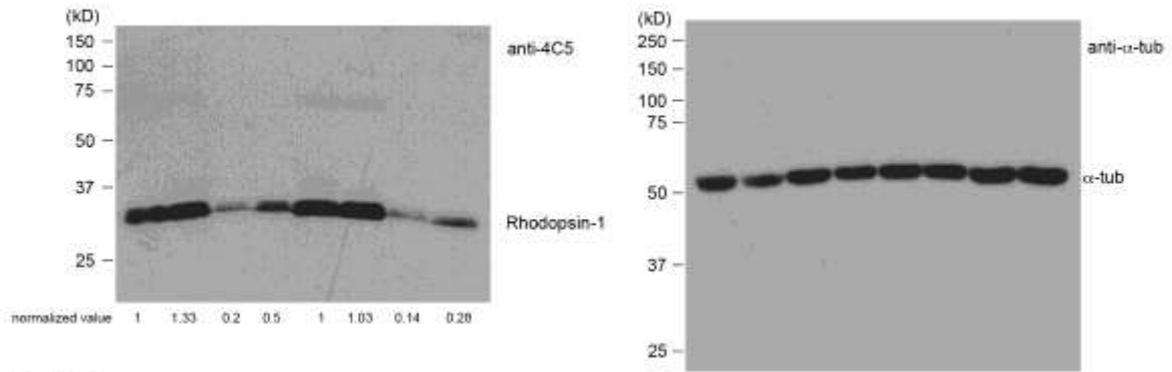
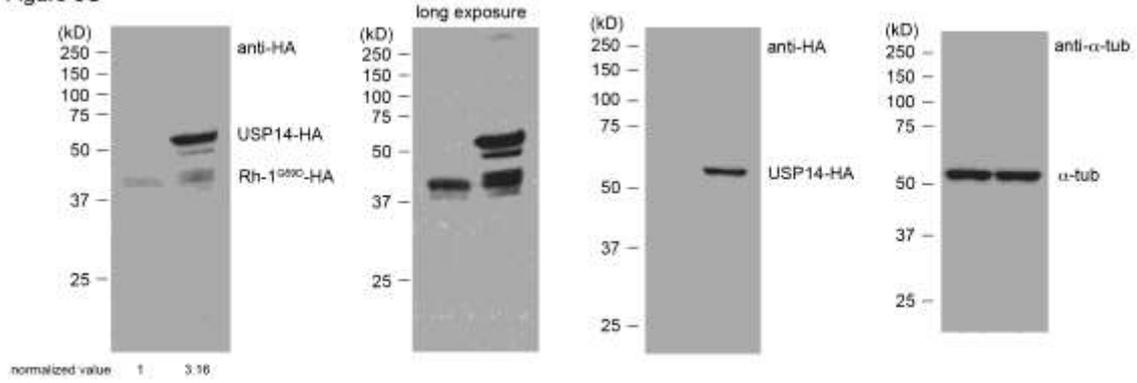
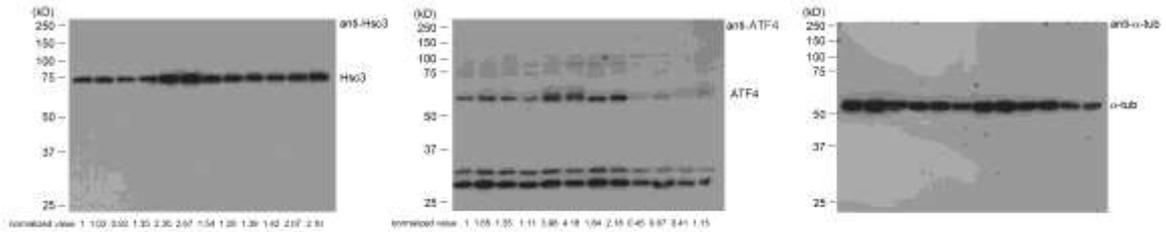


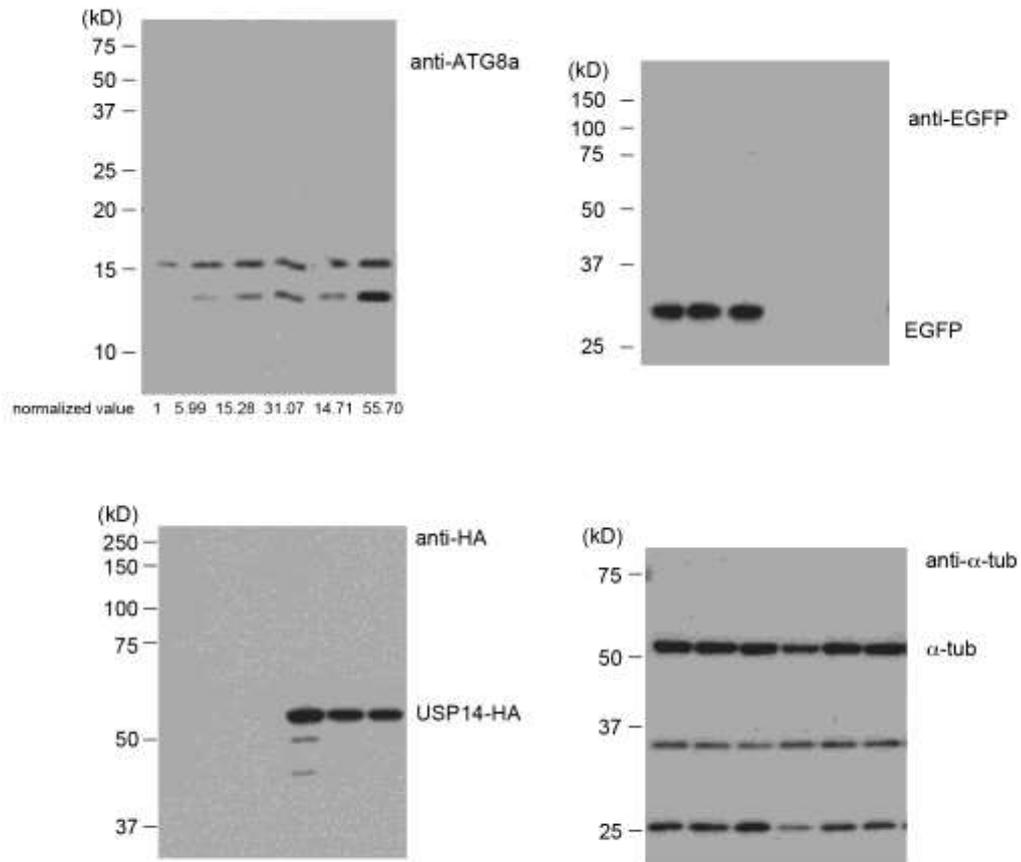
Figure 3C



Supplementary Figure 6c, related to Figure 3



Supplementary Figure 6d, related to Figure S2



Supplementary Figure 6e, related to Figure S5

Figure S6. Densitometric analyses and whole images of representative membranes for all of the Western blots.