Human 343delT HSPB5 Chaperone associated with Early-onset Skeletal Myopathy causes Defects in Protein Solubility

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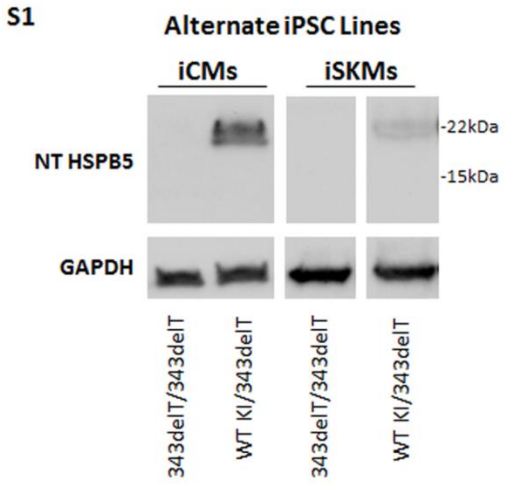
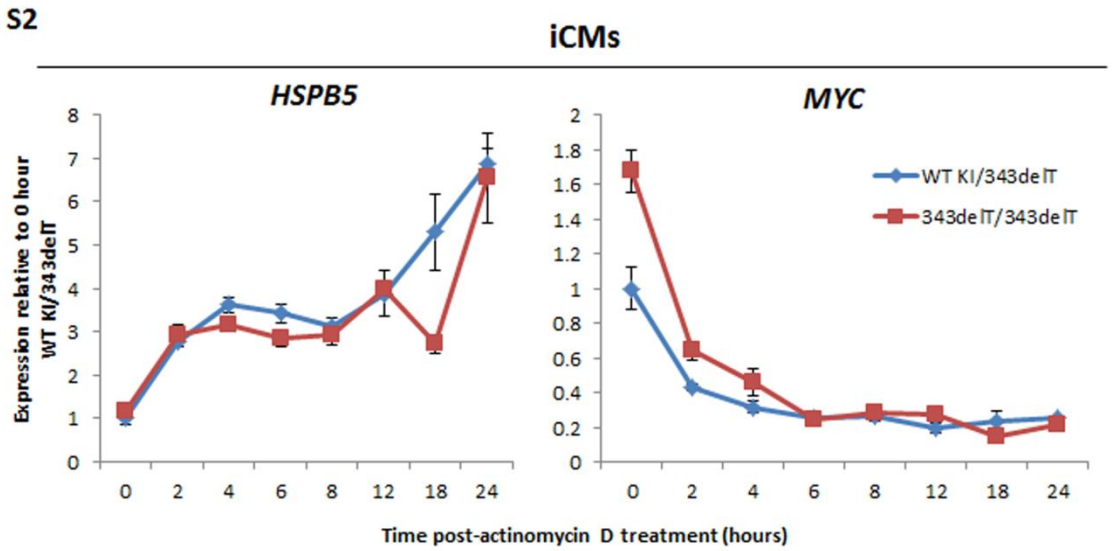
SUPPLEMENTARY DATA

Supplementary Figure S1: Lack of 343delT Protein in an Alternate iPSC Line
An alternate iPSC line derived from 343delT/343delT patient dermal fibroblasts was gene-corrected using the same approach as described previously. Both lines were differentiated to iCMs and iSKMs and a western blot was performed and probed with antibodies that recognize NT HSPB5 and GAPDH as a loading control.



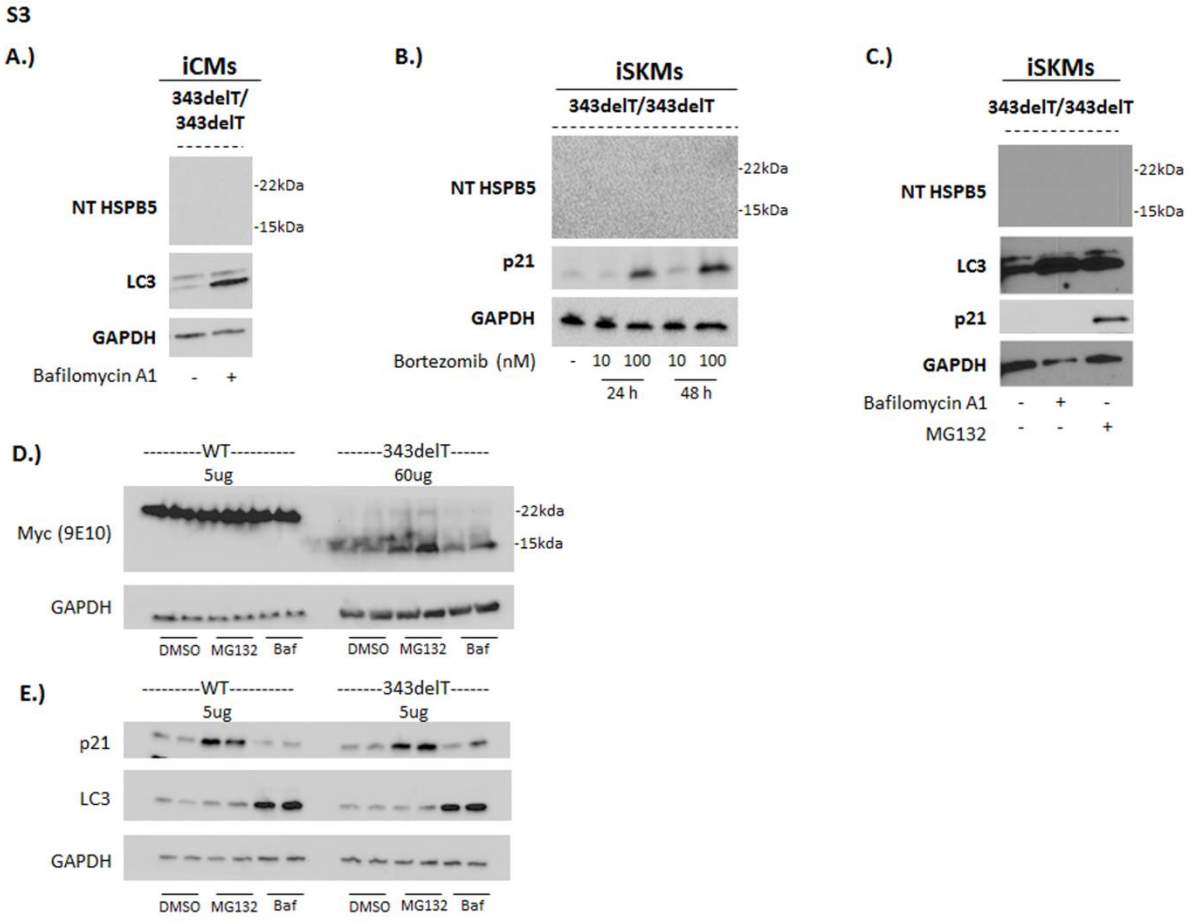
Supplementary Figure S2: 343delT Transcript Stability

To measure *HSPB5* transcript stability, iCMs were re-plated on day 27 as described in Experimental Procedures and seeded into a 12 well 0.1% gelatin coated plate at 80% confluency. The following day, cells were treated with 1μM actinomycin D (Sigma) to inhibit transcription and harvested at indicated time points for qRT-PCR. *HSPB5* mRNA and *MYC* (an unstable control mRNA) levels were normalized to *18s rRNA* and plotted as mean ± s.e.m. expression relative to 0 hour WT KI/343delT sample. Data is representative of two independent experiments performed. Decrease in *MYC* transcript levels from 0-4 hours indicates the actinomycin D treatment successfully inhibited transcription. *HSPB5* levels did not decline over 24 hours, indicating transcript stability. The unexpected increase in *HSPB5* mRNA levels may be explained by incomplete inhibition of transcription by actinomycin D, which acts as a cellular stress and upregulates stress response genes including *HSPB5*.

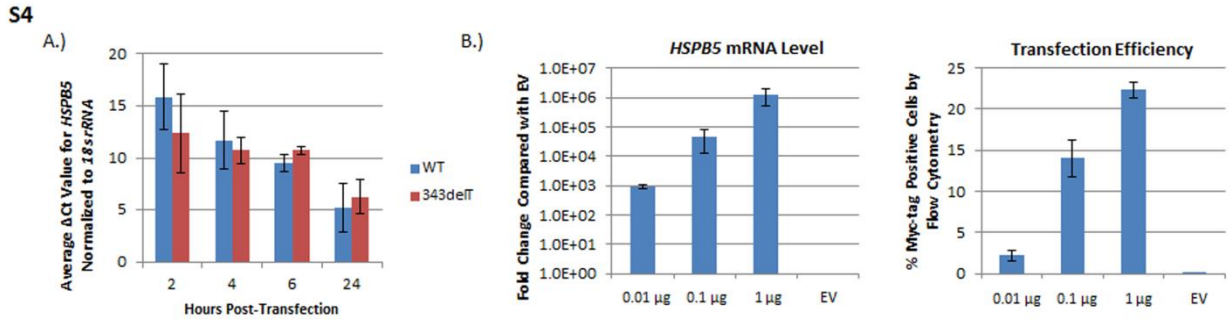


Supplementary Figure S3: Overexpressed 343delT Protein is Degraded by Both the Proteasome and Autophagy

A.) 343delT/343delT iCMs were treated with DMSO or 80 nM bafilomycin A1 to inhibit autophagy for 12 hours. Western blots of WCL were probed with antibodies recognizing NT HSPB5, LC3, and GAPDH. Results are representative of two independent experiments. B.) 343delT/343delT iSKMs were treated with DMSO (-) or 10 or 100nM bortezomib for 24 or 48 hours to inhibit the proteasome and WCLs were probed by western blot with antibodies recognizing NT HSPB5, p21, and GAPDH. C.) 343delT/343delT iSKMs were treated with DMSO (-), 80nM bafilomycin A1, or 10μM MG132 for 12 hours and WCL were run on western blots probed with antibodies recognizing NT HSPB5, LC3, p21, and GAPDH. MCF7 cells were transfected with pCMV-MYC-WT or 343delT constructs for 24 hours followed by treatment with DMSO, 5 μM MG132, or 80 nM bafilomycin (Baf) for 12 hours. D.) Western blots with 5 μg (WT) or 60 μg (343delT) of WCL were stained with antibodies against MYC (9E10) and GAPDH as a loading control. Samples are shown in duplicates. Results are representative of two independent experiments. E.) 5 μg of protein for each of the same samples in (D) was run on a gel and stained with antibodies against p21, LC3, and GAPDH. Both inhibition of the proteasome (MG132) and inhibition of autophagosome maturation (Baf) resulted in slightly elevated levels of 343delT.



Supplementary Figure S4: Transfection and RNA levels are Equal between WT and 343delT
A.) MCF7 cells transfected with pCMV-MYC-WT or 343delT HSPB5 constructs were harvested 2, 4, 6, or 24 hour after transfection for qRT-PCR. Graph shows mean ± s.e.m. ΔCt values for *HSPB5* normalized to *18s rRNA* from n=3. B.) MCF7 cells were transfected with empty vector (EV) (1 µg) or pCMV-MYC-WT in increasing amounts (0.01 µg, 0.1 µg, 1 µg). Samples (n=4 for each group) were harvested 24 hours after transfection and divided using half for qRT-PCR analysis of gene expression (left graph) and the other half fixed and analyzed by flow cytometry (right graph). *HSPB5* gene expression is normalized to *18s rRNA*, with the graph expressing mean ± s.e.m. fold change relative to EV. Transfection efficiency was analyzed by flow cytometry using anti-MYC-tag (Cell Signaling 2278, 1:500) with the graph showing mean ± s.d. percentage of MYC-tag positive cells. Results indicate similar transfection efficiencies between WT and 343delT.



Supplementary Figure S5: Effects of 343delT Expression on Proliferation and Cell Toxicity
HEK 293FT cells transfected with EV, myc-tagged WT, or myc-tagged 343delT were harvested after 24 hours and A.) total cell number as well as B.) percent viable cells by trypan blue staining were counted using a Countess Automated Cell Counter (Life Technologies). Graphs depict mean ± s.d. from n=4 for each group. Results indicate no impact on cell proliferation or viability with transfection of WT or 343delT constructs.