# Supporting Information

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### SI Materials and Methods

**Materials.** The plasmids (pCMV-Tet3G, pTRE3G, pTRE3G-Luc, pEGFP-C3, and a linear hygromycin marker), Xfect transfection system, tetracycline (Tet) system-approved FBS, and doxycycline (Dox) were purchased from Clontech. PCR primers were synthesized by Integrated DNA Technologies. BamH1 and Sal1 restriction enzymes and Phusion Hot Start DNA polymerase were purchased from New England BioLabs. The Endofree Plasmid Mini Kit and Maxi Kit were obtained from Qiagen. Tissue culture reagents, including MEM, FBS, glutamine, penicillin, and streptomycin, were obtained from Life Technologies. G 418 sulfate was purchased from Calbiochem. Tubulin antibody was purchased from Cell Signaling Technology. Antibodies against late embryogenesis abundant (LEA) proteins AfrLEA2 and AfrLEA3 were raised against the expressed and purified proteins by Aves Labs. Trehalose was obtained from Ferro Pfanstiehl. All other reagents were purchased from Sigma-Aldrich.

**Cell Culture.** Human hepatocellular carcinoma (HepG2) cells were obtained from American Type Culture Collection (catalog no. HB-8065) and cultured in MEM containing 10% FBS (vol/vol), 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were maintained in 25-$cm^2$ T flasks at 37 °C and equilibrated in a humidified atmosphere with 5% $CO_2$ and 95% air (vol/vol). For subculture, cells were detached with 0.25% trypsin-EDTA solution, and $1 \times 10^6$ cells were seeded into new flasks. Cells were counted with a hemocytometer when required during experimental procedures.

**Construction of HepG2 Tet-On Cell Line.** The regulatory plasmid pCMV encoding a Tet transactivator with a neomycin expression gene was transfected into HepG2 cells. In brief, $5 \times 10^5$ HepG2 cells were seeded into single wells of six-well plates, and Clontech Xfect transfection reagent and 2.0 μg of the regulatory plasmid were added into each well containing HepG2 cells following the manufacturer's protocol. After a 24-h incubation, the cells were transiently transfected with pTRE3G encoding with the luciferase reporter gene to test the inducibility of HepG2 Tet-On cells. Selection for stable cell lines was initiated by adding 800 μg/mL G418 into Tet-On culture medium (HepG2 culture medium with Tet-On–approved FBS added instead of the normal FBS). After an approximate 4-wk selection period, single clones appeared, which were cultured and retested for maximum inducibility with the luciferase reporter.

To test the HepG2 Tet-On 3G cells for induction efficiency, Dox, a synthetic Tet derivative, was used to promote the inducible binding of transactivator protein to pTRE3G promotor. The plasmid pTRE3G-Luc, which contains the Tet-responsive element (TRE) and luciferase reporter gene (*Luc*), was transiently transfected into HepG2 Tet-On 3G cells. Various amounts of Dox (0, 1, 10, 100, and 1,000 ng/mL) were added, after which luciferase activity and enzyme expression were analyzed. Luciferase activity was evaluated using the Promega Bright-Glo luciferase assay system and a fluorescence microplate reader. Luciferase protein expression was detected by Western blot analysis using the same dosages for Dox induction as above.

**Gene Transfections and Selection of Stable Cell Lines.** To express *Afrlea2* (~1.1 kb; GenBank accession no. EU477187) and *Afrlea3m* (~0.9 kb; GenBank accession no. FJ592175) under the control of the Tet-On–inducible expression system, and to obtain maximal expression in HepG2 cells, the full-length sequences (1, 2) were revised to reflect human codon bias and synthesized using Gene Oracle software. Sal1 and BamH1, used to insert the LEA genes into the Tet-On expression vector pTRE3G, were added at 5′ and 3′ of each translational sequence. A Kozak sequence was added as well. *AfrLea2* and *AfrLea3m* sequences were then inserted into the precut pTRE3G vector and ligated with T4 ligase. The resulting plasmid was transformed into *Escherichia coli* cells for amplification. The construct was verified by double digestion of the plasmid with Xhol I and EcoR. HepG2 Tet-On cells were cotransfected with the Tet-On expression plasmid and a linear hygromycin marker (20:1) following the procedures described above. After 48 h, Tet-On medium containing 200 μg/mL hygromycin was added to select individual clones that appeared after approximately 3 wk. Single clones were then picked for evaluation of AfrLEA2 and AfrLEA3m protein expression by Western blot analysis.

TRET1 was cloned from anhydrobiotic larvae of *Polypedilum vanderplanki* (3) and the original vector (a gift from Takahiro Kikawada, National Institute of Agrobiological Sciences, Ibaraki, Japan). The TRET1 gene was then inserted into the expression vector (pcDNA 6.2/V5-DEST Gateway Vector; Invitrogen), and its sequence was verified by presequencing PCR with T7 promoter and V5 reverse primers, followed by sequencing of the cleaned product. Note that the Tet-On vector pTRE3G was not used for this gene, so that TRET1 expression in HepG2 Tet-On cells was constitutive. Transfection into HepG2 Tet-On cell lines (with or without the capacity for AfrLEA2/AfrLEA3m expression) was done using GenePORTER transfection reagent (Genlantis) according to the manufacturer's instructions. Stable cell lines were selected with 5 μg/mL blasticidine for 5–7 d and then maintained in Tet-On medium with 2 μg/mL blasticidine. RT-PCR was used to verify TRET1 expression in the HepG2 cells. Total RNA was extracted using a RNeasy Mini Kit (Qiagen) and reverse-transcribed to cDNA using a DyNAmo cDNA Synthesis Kit (Thermo Scientific). The forward PCR primer was 5′-GTTTGGCAACATCGGCATCCTCAT-3′, and the reverse primer was 5′-AATCTTGCGTCCCAGCCGATCTAT-3′. The PCR used Crimson Taq DNA polymerase (New England BioLabs) and 25 cycles of 95 °C for 30 s, 60 °C for 30 s, and 68 °C for 60 s, followed by a final extension at 72 °C for 5 min. Human β-actin served as the loading control, and the primers were 5′-GGCACCCAGCACAATGAAGATCAA-3′ (forward) and 5′-ACTCGTCATACTCCTGCTTGCTGA-3′ (reverse).

**Western Blot Analysis.** Luciferase and AfrLEA2/AfrLEA3m protein expression were evaluated by Western blot analysis. Transfected cells were harvested with SDS sample buffer containing 2% (wt/vol) SDS, 25% (vol/vol) glycerol, 5% (vol/vol) β-mercaptoethanol, and 0.625 M Tris-HCl (pH 6.8). The protein concentration was determined using Pierce 660-nm protein assay reagent with ionic detergent compatibility reagent (Thermo Scientific) using BSA as the standard. Equal amounts of protein from the whole-cell lysates were electrophoresed on 10% (wt/vol) SDS/PAGE gels, and the proteins were electrotransferred to nitrocellulose membranes. Membranes were incubated with blocking solution containing 5% (wt/vol) nonfat milk prepared in Tris-buffered saline [50 mM Tris base, 150 mM NaCl, 0.05% Tween-20 (pH 7.6) with HCl], and then incubated with the primary antibodies for AfrLEA2 and AfrLEA3m (brine shrimp anti-chick IgY, 1:100,000; Aves Labs, Inc.) and for luciferase (firefly anti-rabbit IgG fraction, 1:1,000 dilution; Sigma-Aldrich) at 4 °C overnight. After three 5-min rinses with Tris-buffered saline,

the membrane was incubated with the secondary antibodies (1:10,000, rabbit anti-IgY for AfrLEA2/AfrLEA3m; 1:10,000, anti-rabbit IgG for luciferase) conjugated with HRP at room temperature for 1 h. The LumiGlo developing system and high-performance chemoluminescence film (GE Healthcare) were used to develop blots.

**Confocal Imaging of AfrLEA2-GFP and Immunofluorescent Staining of AfrLEA3m.** For transient transfection with AfrLEA2-GFP, HepG2 Tet-On cells were detached from culture flasks using 0.25% trypsin-EDTA solution and replated into six-well glass-bottom plates coated with type 1 rat tail collagen. When cells reached 60% confluency, GenePORTER transfection reagent was used to transfect the cells with the expression vector encoding for the AfrLEA2-GFP protein (Clontech pEGFP-C3). Before confocal visualization, cells were stained with 100 nM MitoTracker Red (Life Technologies) for 20 min and then washed three times with PBS. Cellular localization of the chimeric protein was visualized by confocal imaging performed daily across a 3-d period after transfection. Images were acquired with a Leica Microsystems TCS SP2 microscope equipped with a Plan Apo 63× NA 1.4 oil immersion lens. Two lasers, 488 nm at 15% power and 543 nm at 25% power, were used to sequentially excite GFP and MitoTracker Red, respectively. Emission passes of 500–535 nm and 560–650 nm were used to detect the signals.

HepG2 Tet-On cells stably transfected with *Afrlea3m* were induced with Dox for 3 d, and uninduced cells were grown in parallel. Live cells were stained with MitoTracker Red as above, then fixed in 0.4% paraformaldehyde for 20 min at room temperature, washed with PBS, blocked with 5% (vol/vol) normal goat serum and 0.3% Triton X-100 in PBS for 1 h, and incubated with anti-AfrLEA3m chick IgY at 1:200 dilution in 5% normal goat serum and PBS overnight. Control cells without primary antibody were prepared as well. After another washing in PBS, cells were incubated in secondary antibody (goat anti-chick IgY conjugated with FITC; excitation, 488 nm; emission, 515 nm; Jackson ImmunoResearch) at a 1:200 dilution in 5% normal goat serum in PBS for 1 h. Cells were then washed three times in PBS, treated with ProLong Gold antifade solution (Life Technologies), and imaged with confocal microscopy.

**Trehalose Uptake Kinetics.** HepG2 cells with or without TRET1 were incubated in cell culture medium containing 50 mM trehalose for specified time periods (0, 8, 18, and 24 h) to load the sugar. The trehalose-containing medium was then completely removed, and the attached cells were rinsed twice with PBS. Cells were harvested by incubation for 5 min in 0.25% trypsin-EDTA, dilution with cell culture medium, and centrifugation. Cells were then resuspended in deionized water, subjected to three freeze–thaw cycles in liquid nitrogen, and then centrifuged at 16,000 × *g* for 15 min. Trehalose in the supernatant was analyzed by HPLC as described previously (4). The trehalose content per million cells was converted to an approximate intracellular concentration using a measured diameter of 15.1 μm for HepG2 cells and assuming a spherical form and 70% osmotically active water.

**Spin-Drying of Cells.** Monolayers of HepG2 cells expressing various combinations of AfrLEA2, AfrLEA3m, and TRET1 were allowed to attach to round glass coverslips (22-mm diameter, collagen-coated; BD BioCoat catalog no. 354089; BD Biosciences) during an 18-h incubation in cell culture medium. For all cells expressing TRET1, the medium also contained 50 mM trehalose. Cells were prevented from attaching to the edge of the coverslips using 20-mm-diameter Press-to-Seal silicone isolators (Life Technologies). Immediately before spin-drying, the cell culture media was completely removed using a Pasteur pipette and replaced with the spin-drying solution (1.8 M trehalose, 10 mM KCl, 5 mM glucose, 20 mM Hepes, and 120 mM choline chloride; pH 7.4). This trehalose-containing solution was essential for a successful outcome regardless of the intracellular variables tested. Spin-drying was performed using a commercially available spinning machine (Brewer Science model Cee 200) as described previously (4).

FTIR spectromicroscopy was performed with a Nicolet Continuum FTIR spectromicroscope (Thermo Scientific) equipped with a mercury cadmium telluride detector, to examine the uniformity of water distribution in spin-dried trehalose films prepared with the aforementioned drying solution and determine the average moisture content of these noncellular samples (4). Residual water was estimated by interpolation using the trehalose-water binary phase diagram (5) derived from the glass-transition temperature ($T_g$) measured by FTIR spectromicroscopy. The FTIR results were then compared with values obtained by bulk gravimetric analysis of cell monolayers overlain with drying solution and spin-dried (4). The two datasets were statistically identical. After drying, the samples were quickly removed from the spin-dryer and placed into six-well (35 mm) culture plates (Corning). Cells were immediately rehydrated in 0.5 mL of cell culture medium, and membrane integrity and long-term viability were evaluated.

**Assessment of Membrane Integrity and Long-Term Viability.** Membrane integrity after spin-drying was assessed using Syto-13/ethydium bromide membrane integrity assays (Molecular Probes). The stock solution for Syto-13/ethydium bromide staining was prepared by adding 10 mL of 1 mg/mL Syto-13 solution (aq.) and 5 mL of 1.0 mg/mL ethydium bromide solution (aq.) to 8 mL of MEM without phenol red or serum (Life Technologies). After rehydration, 500 μL of Syto-13/ethydium bromide solution was added to the cells attached on coverslips, and the samples were incubated at 37 °C for 5 min. These samples were then imaged using an inverted microscope (Carl Zeiss) with FITC and PI filters. Cell viability was determined immediately after rehydration using this technique by counting the live (green) and dead (red) cells in seven representative images obtained at different locations on the coverslip.

Long-term viability and growth profiles were determined by incubating the rehydrated cell samples in fully complemented medium for 7 d in six-well (35 mm) culture plates. Parallel samples were used for the various experimental treatments, and the viability of cells after dehydration was measured by counting cells on days 1, 3, and 7. To ensure that only viable cells were counted, the membrane integrity of these cells was determined by trypan blue exclusion.


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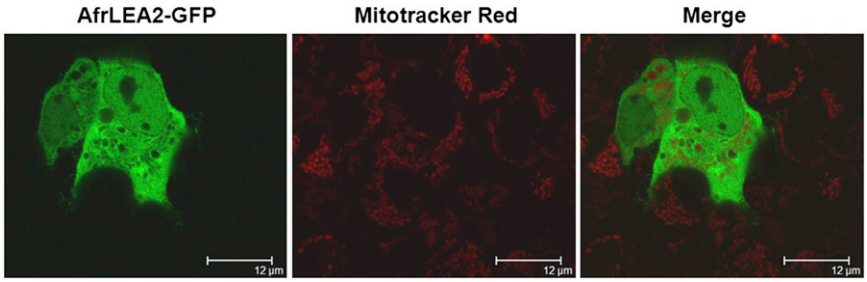


**Fig. S1.** AfrLEA2-GFP exhibits a predominately cytoplasmic distribution, but at 72 h after the initial transfection event, some of the chimeric protein has entered the nucleus, as demonstrated by the increased green staining of the organelle. The nuclear staining is far more prevalent than that seen after 24 h (Fig. 3). (*Left*) Distribution of green fluorescence from AfrLEA2-GFP. (*Center*) Distribution of mitochondria based on MitoTracker Red labeling. (*Right*) Merged image.