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Supplementary Fig. S1.

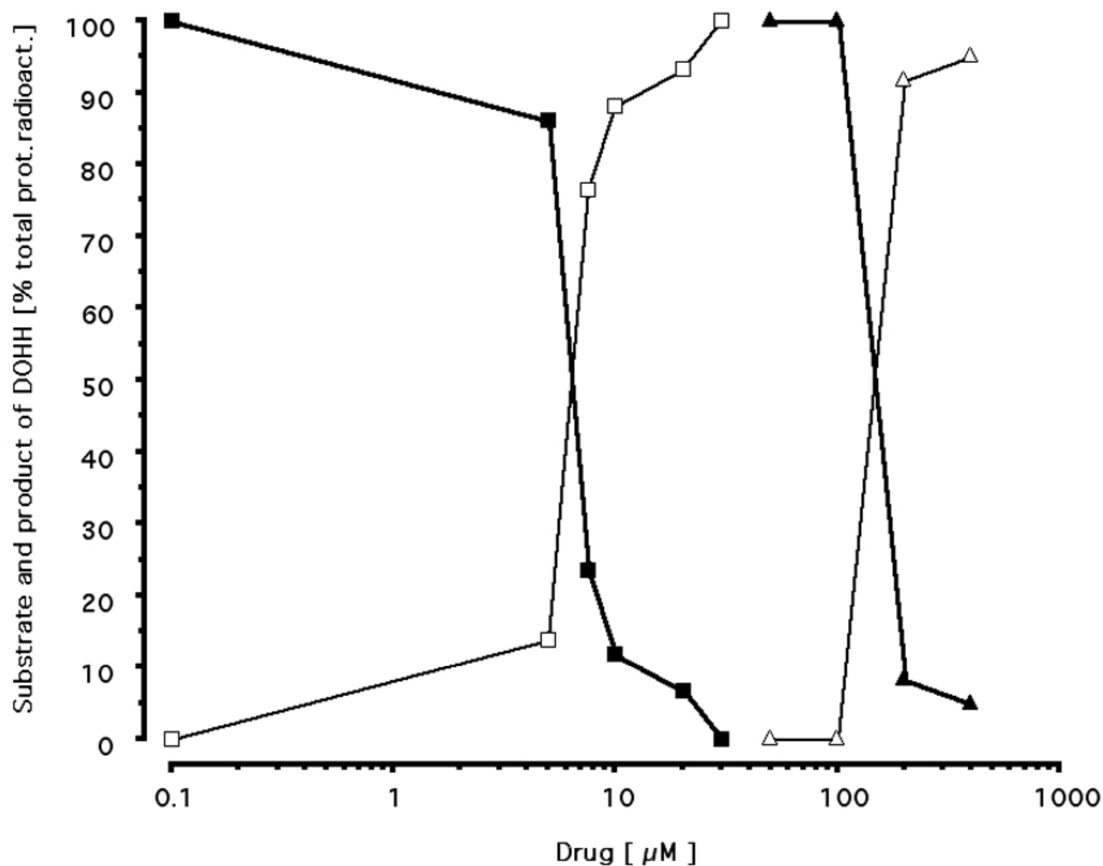

Fig. S1. Inhibition of DOHH by ciclopirox and deferiprone.

HeLa cells were incubated with [^{3}H]-spermidine for 24 hr in the presence of ciclopirox or deferiprone at the concentrations indicated. Cells were harvested and washed, then acid hydrolysates were fractionated and fractions were assayed for radioactivity as described previously (20). Radioactivity recovered as hypusine and as deoxyhypusine are plotted as a percentage of the total in each sample.

Solid symbols: hypusine; *open symbols*: deoxyhypusine.
Squares: ciclopirox; *triangles*: deferiprone

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Supplementary Fig. 2.

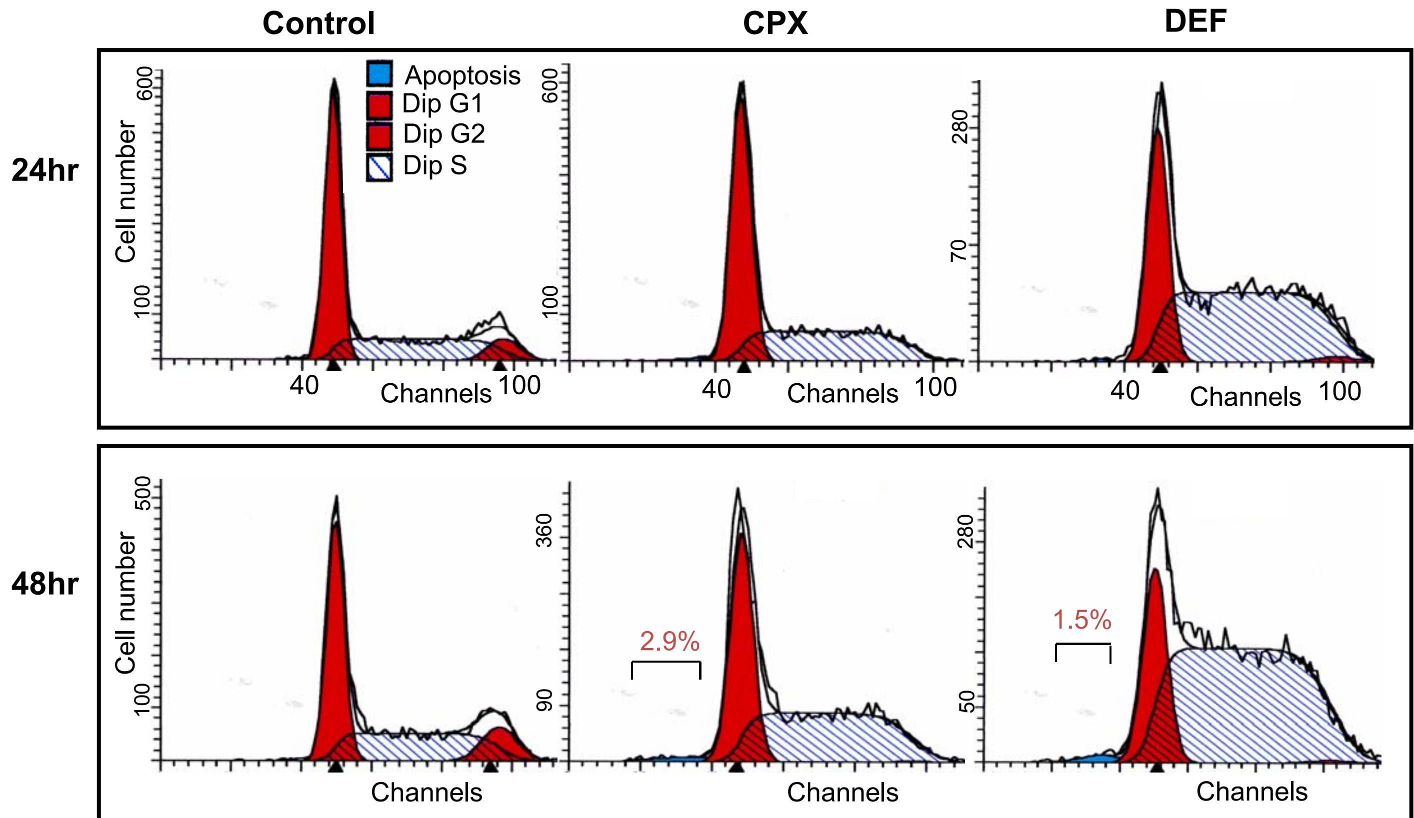

Fig. S2. Cell cycle analysis.

HeLa cells were incubated with CPX (30 µM) or DEF (200 µM) or without drugs for 24 or 48 hr, then trypsinized, resuspended in PBS and fixed in ethanol. After treatment with RNase A (1 mg/ml) and propidium iodide (50 µg/ml), the cells were analyzed for DNA content by flow cytometry (accumulated counts: ~10^5 cells). Apoptotic cells (bracketed) were enumerated.

Supplementary Fig. S3.

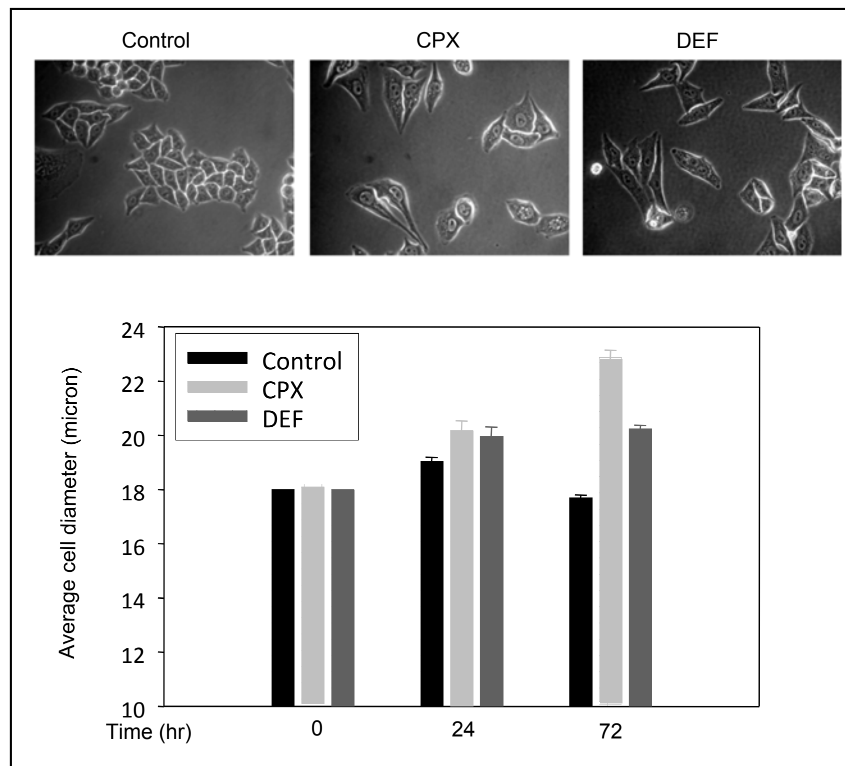

Fig. S3. Effects of CPX and DEF on cell size and morphology.

HeLa cells were treated for 24 or 72 hr with CPX (30 µM) or DEF (200 µM) as indicated, or left untreated. Cells were photographed at 24 hr using phase microscopy with a Nikon inverted microscope (40x objective; *top*). After trypsinization and washing with PBS, average cell diameter (with SD) was measured at the times indicated (*bottom*) using a Vi-Cell Analyzer (Beckman). The morphological changes observed may relate to the observations that Hsp27 binds actin and inhibits its polymerization (1), NM23 forms complexes with β-tubulin (2), DJ-1 knockdown leads to cytoskeleton disruption (3), and eIF5A hypusination is associated with cell differentiation (4).

1. Miron T, Vancompernolle K, Vandekerckhove J, Wilchek M, Geiger B. A 25-kD inhibitor of actin polymerization is a low molecular mass heat shock protein. J Cell Biol. 1991;114:255-61.
2. Lombardi D, Sacchi A, D'Agostino G, Tibursi G. The association of the Nm23-M1 protein and beta-tubulin correlates with cell differentiation. Exp Cell Res. 1995;217:267-71.
3. He X, Zheng Z, Li J, Ben Q, Liu J, Zhang J, et al. DJ-1 promotes invasion and metastasis of pancreatic cancer cells by activating SRC/ERK/uPA. Carcinogenesis. 2012;33:555-62.
4. Luchessi AD, Cambiaghi TD, Hirabara SM, Lambertucci RH, Silveira LR, Baptista IL, et al. Involvement of eukaryotic translation initiation factor 5A (eIF5A) in skeletal muscle stem cell differentiation. J Cell Physiol. 2009;218:480-9.

Supplementary Fig. S4.

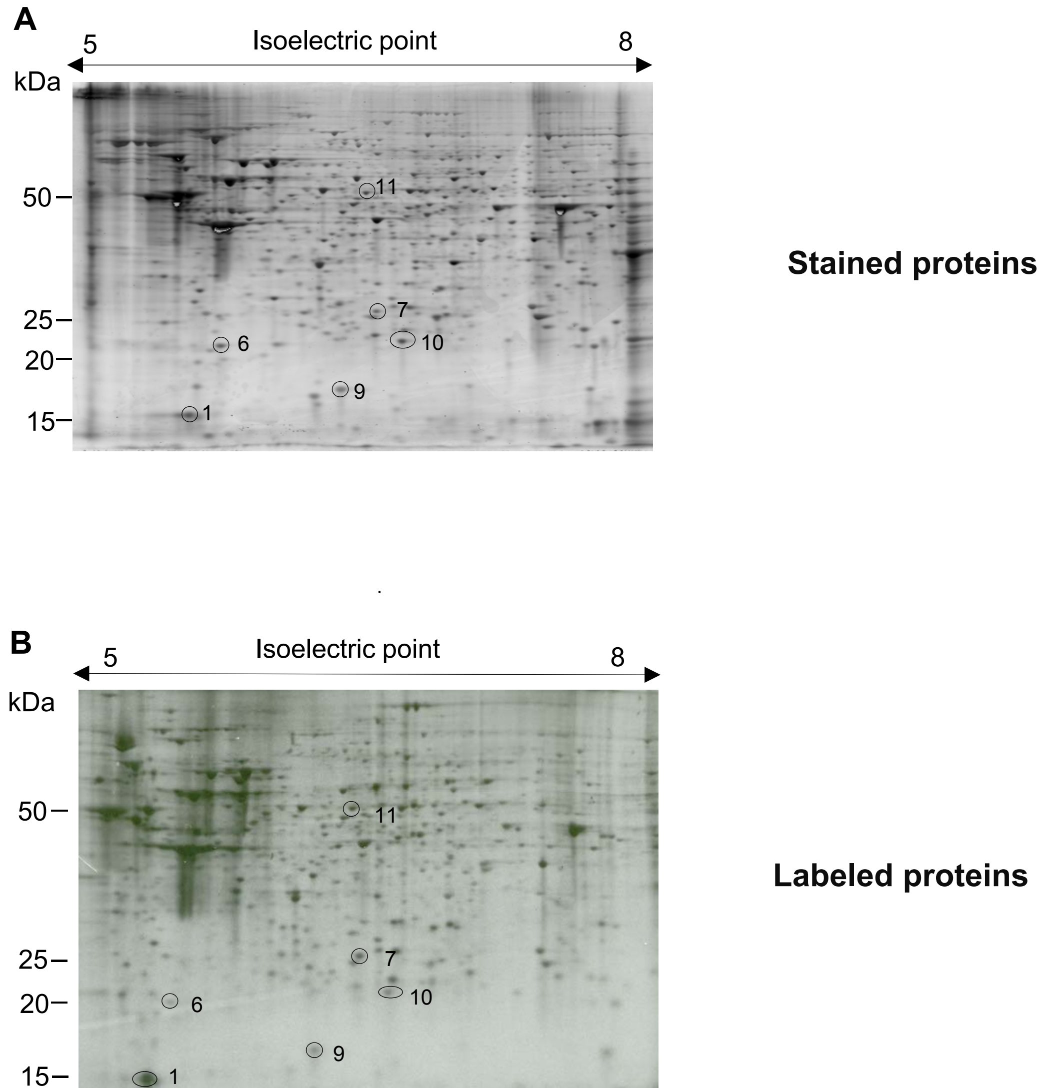

Fig. S4. Effect of CPX and DEF on HeLa cell proteins analyzed by 2D gel electrophoresis.

A: Extracts of HeLa cells treated with or without CPX (30 µM) or DEF (200 µM) for 24 hr were resolved in 2D gels and stained with SYPRO® Ruby. The pH gradient for the first dimension is shown on the top, and migration of molecular mass markers in the second dimension is shown on the left. Numbered circles show spots picked for mass spectrometry. **B:** As A, except that the cells were labeled with [^{35}S]-methionine and [^{35}S]-cysteine for 3 hr before extraction and analysis by autoradiography.