Supplemental Materials and Methods

Delivery of fl-tRNAs with chemical based-transfection

5 µL of each fl-tRNAs (1 mg/mL) were added to 90 µL serum-free media (RPMI-1640). Then, 8 µL of INTERFERin (Polyplus, USA) was added, followed by 10 min incubation at room temperature. In the meantime $2x10^5$ U266 cells were spun down at 300g for 5 min and re-suspended in the tRNA-INTERFERin mixture. Then the cells were transferred to a humidified cell culture incubator (5% CO_2) and incubated for 6-7 hrs. Subsequently, the cells were either analyzed by FACS to quantify transfection efficiency, or imaged with confocal microscopy. Alternatively, transfected cells were fixed with 4% PFA, cytospun onto microscope slides, permeabilized with 0.5% triton-x100. Cells were then blocked with 5% goat serum for 30 min followed by incubation with calnexin antibody (Abcam, USA) at 4 ℃ overnight. Confocal imaging was performed following visualization with secondary antibody AF 488 rabbit for 1 hour at room temperature.

Estimation of delivered fl-tRNAs

To estimate the amount of fl-tRNAs delivered into each cells, U266 cells were transfected with AF555-tRNAs using 30-6x1 chip at 30 psi. The sample was first analyzed by FACS to compute the number of successfully transfected cells. Then the fluorescence of the sample was read by a Cytation3 plate reader (Biotek, USA), and normalized by subtracting the fluorescence of a control (untransfected sample). A standard curve was obtained by reading the fluorescence of various concentrations of AF555-tRNAs in the same media. Subsequently, the concentration of the fl-tRNAs in the sample could be computed based on the standard curve. With the number of transfected cell being known, the average amount of fl-tRNAs in each cell was subsequently calculated.

Treatment of U266 cells with Bortezomib

$2.5X10^5$ U266 cells/mL were co-incubated with 10 nM of bortezomib (BZ, EMD Millipore, USA) and various concentrations (0 nM, 250 nM and 500 nM) of cycloheximide (CHX, Sigma-Aldrich, USA) for 48 hrs and then FACS analyzed for viability using 7-AAD (eBioscience, USA).

Figure S2 Delivery of AF555-tRNAs to U266 cells with INTERFERin transfection. (A) Live cell imaging confirmed similar clustering pattern formation outside nucleus (indicated by arrows), suggesting the clustering pattern formation is independent of delivery mechanisms; (B) quantification of delivery efficiency using FACS shows that <10% cells were transfected (blue line) as normalized with control cells (red line) using the INTERFERin transfection, n=3.

	sample 1	sample 2	sample 3	Average
Fluorescence of Transfected cells	401	442	389	410.6667
Fluorescence of Control cells	262	290	314	288.6667
Normalized fluorecence				122
Fluorophore concentration	0.002029	μM		
volume	200	μL		
# of transfected cells	13500			
AF555-tRNAs per cell	18.10293	million		

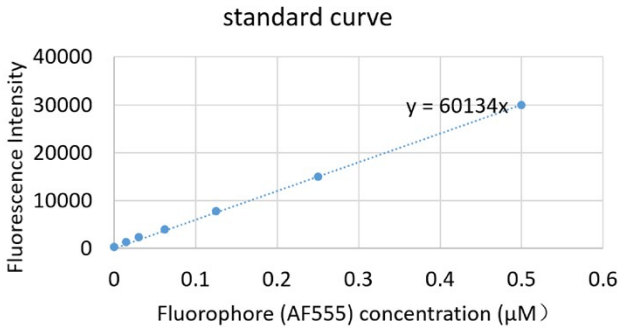

Figure S3 Estimation of fl-tRNAs delivered to each U266 cell on average. About 18 million copies of AF555-tRNAs were delivered to each cell, whereas endogenous tRNAs amount to ~10^8.

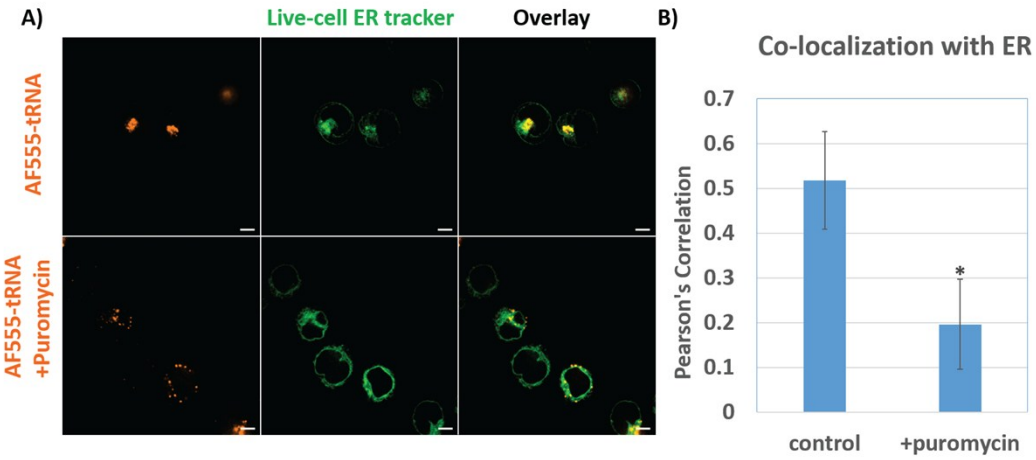

Figure S4. Effect of puromycin treatment on co-localization of AF555-tRNA with ER tracker. (A) U266 cells transfected with AF555-tRNA were stained with live cell ER tracker (A, upper panel). A short 30 minutes treatment of 2 mM puromycin disrupts co-localization of AF555-tRNA with ER tracker (A, lower panel); (B) co-localization with ER tracker staining was quantified using ZEN software. For each group, 20 cells were used to calculate Pearson's correlation efficient. *p<0.01. Scale bar: 5 μm.

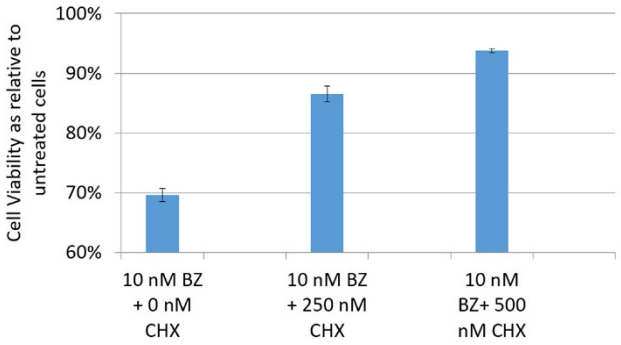

Figure S5 U266 cells sensitivity to bortezomib treatment reduced by cycloheximide in a dose-dependent manner. U266 cell viability was increased by the protein synthesis inhibitor (CHX) treatment in a dose dependent manner, suggesting the importance of protein synthesis rate in determining drug response, n=2.