Supplementary Figure Legends:

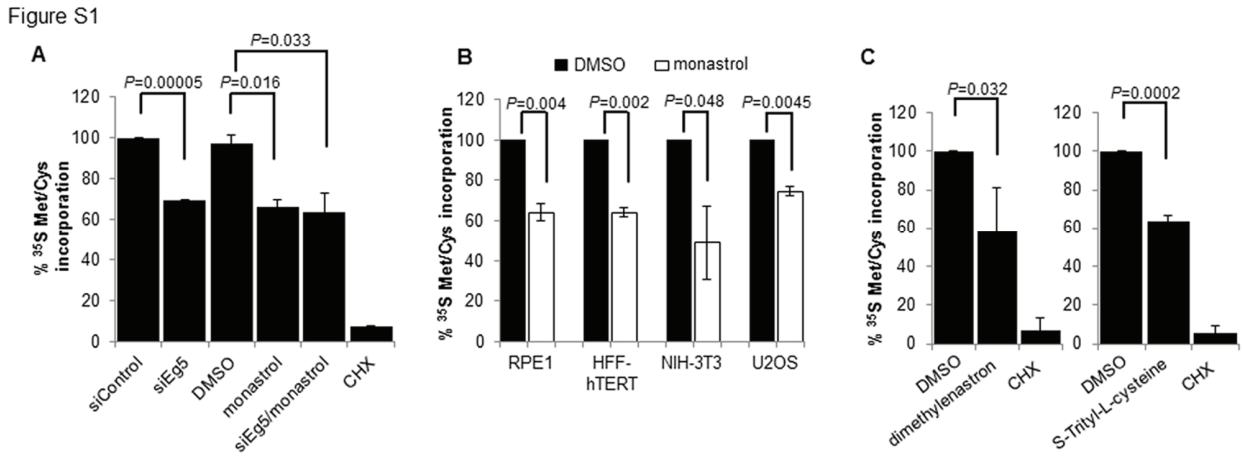

Figure S1. Eg5 is required for optimal protein synthesis. Quantitation of ^{35}S Met/Cys incorporation assays in WCLs from RPE1 cells as indicated (A) after a 24 hr Eg5 knockdown alone (siEg5#1 siRNA), a 4 hr monastrol treatment alone, or a simultaneous treatment of a 24 hr knockdown of Eg5 followed by a 4 hr monastrol treatment, (B) after a 4 hr, 130 μM monastrol treatment in four different cells lines (RPE1, HFF-hTERT, NIH-3T3, and U2OS; black bars represent DMSO and white bars represent monastrol), (C) after a 1 hr treatment with 3 μM dimethylenastron or after a 4 hr treatment with 1.5 μM S-Trityl-L-cysteine in RPE1 cells. Results are represented as means ± s.d., *P* values are derived from Students' t-test (null hypothesis). Each of these experiments was completed at least three independent times.

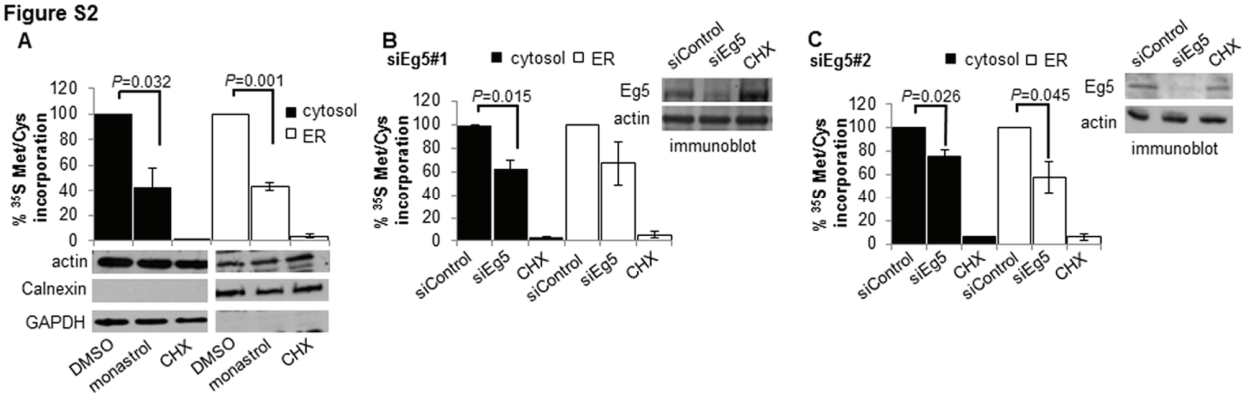

Figure S2. Eg5 is required for efficient synthesis of proteins translated in both the cytosol and the ER compartments. ^{35}S Met/Cys incorporation assays were completed and RPE1 cells were lysed and separated into ER and cytosolic fractions, as described in Materials and Methods. Quantitation is shown in (A) after a 4 hr, 130 μM monastrol treatment, or (B,C) 24 hrs after one of two different nonoverlapping siRNAs; siEg5#1 was used in (B) and siEg5#2 was used in (C). Representative immunoblots in (A) are shown to demonstrate complete fractionation of cytosolic and ER compartments. Calnexin was used as an ER marker, GAPDH was used as the cytosolic marker, and actin was used as a loading control. Immunoblots in (B) and (C) are representative of the experiments and are shown to demonstrate similar knockdown by each of the Eg5 siRNAs. Black bars represent cytosolic fractions, and white bars represent ER fractions. Results are represented as means ± s.d., *P* values are derived from Students' t-test (null hypothesis*)*. Each of these experiments was completed at least three independent times.

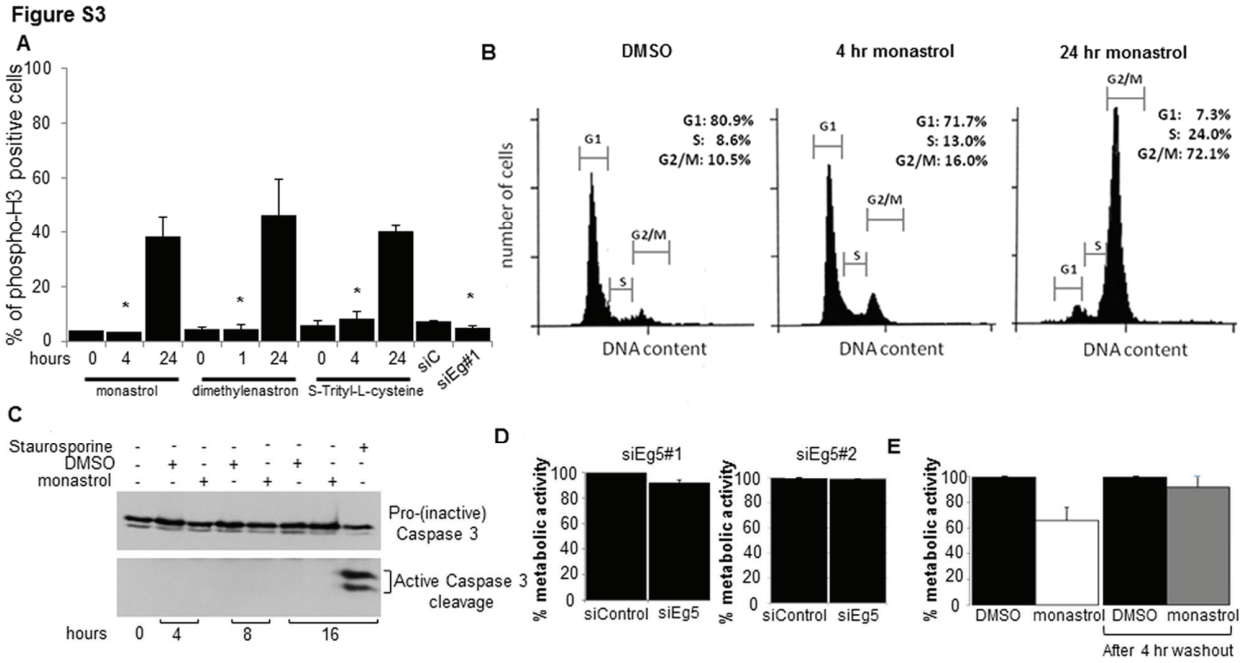

Figure S3. Decrease in translation after Eg5 inhibition was not due to mitotic arrest or cell death. (A) Quantitation of phospho-H3 immuno-positive cells. RPE1 cells were treated with 130 μM monastrol (for 4 or 24 hrs), 3 μM dimethylenastron (for 1 hr or 24 hrs), 1.5 μM S-Trityl-L-cysteine (for 4 or 24 hrs) or knockdown of Eg5 by siEg5#1 (24 hrs) prior to fixation and immunofluorescence analysis with antibodies to phospho-H3. Asterisks represent the timepoint or the siRNA at which all experiments were completed (except where indicated). In each experiment, a minimum of 300 cells were counted and at least three independent experiments were completed. Results are shown as means ± s.d. (B) Propidium iodide stained RPE1 cells were subjected to cell cycle analysis by flow cytometry, 4 or 24 hrs after a 130 μM monastrol treatment. The experiment was completed at least two different times with similar results. (C) Apoptosis in RPE1 cells after a 130 μM monastrol treatment at various time points was investigated. Staurosporine was used as a positive control for caspase-3 cleavage (last lane). Each of these experiments was completed at least two independent times. (D,E) Promegas' CellTiter 96® AQueous One Solution Cell Proliferation Assay was used to investigate metabolic activity after (D) a 24 hr knockdown of Eg5 by two different siRNAs (siEg5#1 or siEg5#2) or (E) after a 4 hr, 130 μM monastrol treatment (white bar), or after a 4 hr monastrol treatment followed by a 4 hr washout (gray bar). Results are shown as means ± s.d. and represent at least three independent experiments. *P* values are derived from Students' t-test (null hypothesis).

Figure S4

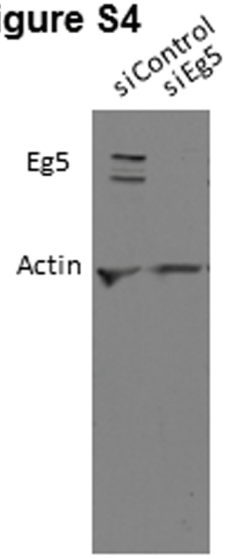

Figure S4: The doublet bands recognized by the Eg5 antibody are both derived from Eg5. WCL's before and after knockdown of Eg5. The doublet recognized by the Eg5 antibody in the siControl sample is Eg5 and the lower band is likely a proteolytic fragment, as after knockdown of Eg5 both bands are lost.

Figure S5

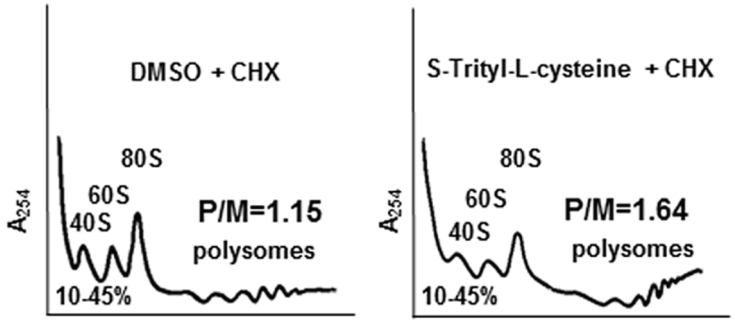

Figure S5. Eg5 inhibition results in a post-initiation defect phenotype. RPE1 cells were treated with 1.5 μM S-Trityl-L-cysteine for 4 hrs prior to CHX addition and polysome profiling. Assay was completed four times with similar results.

Figure S6

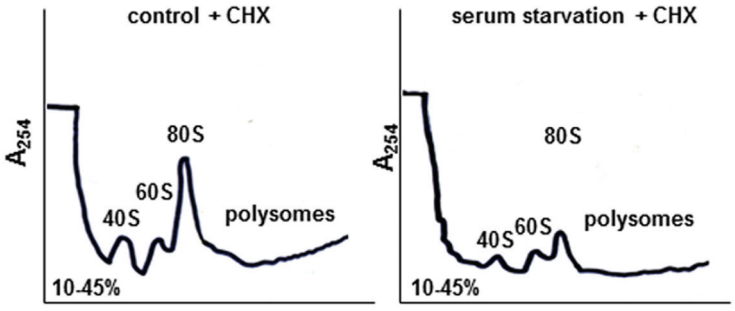

Figure S6. Polysome profiling of senescent cells does not resemble the profiles after Eg5 inhibition. RPE1 cells were incubated for 32 hrs in serum-free medium prior to CHX addition and polysome profiling of equal concentrations of proteins. Serum starvation leads to a decrease in both the 80S ribosomes and polysomes.