SUPPLEMENTARY FIGURE LEGENDS

Figure S1. eIF3h overexpression in CHO-K1 and HeLa cells.
A. Immunoblot analysis of cell extracts of CHO-K1/HA-eIF3h, CHO-K1/eIF3h and CHO-K1/pcDNA3 cells, as well as SK-Br-3 cells known to contain high eIF3h levels. Blots were probed with anti-eIF3h, anti-HA and anti-actin antibodies as described under Experimental Procedures; quantitations of band intensities with anti-eIF3h are reported in Fig. 3A. **B**. Colony formation units (CFU) of CHO-K1 (light grey) and HeLa (dark grey) cells transfected with plasmids expressing HA-eIF3h, eIF3h or empty vector; CFU were measured as described in the legend to Fig. 3C. **C.** Anchorage-independent growth in soft agar of CHO-K1 (light grey) and HeLa (dark grey) cells expressing HA-eIF3h, eIF3h or empty vector; numbers of colonies were determined as described in the legend to Fig. 3D. **D.** eIF3h overexpression in HeLa cells transfected with plasmids expressing HA-eIF3h, eIF3h or empty vector, determined by immunoblotting as described in panel A. eIF3h band intensities were determined, normalized to that of the empty vector and plotted as a bar graph in the lower panel. **E**. Growth curves of HeLa/HA-eIF3h (squares), HeLa/eIF3h (triangles) and HeLa/pcDNA3 (diamonds) were measured as described in the legend to Fig. 3B. **F.** Clonogenecity of HeLa cells transfected with plasmids expressing HA-eIF3h, eIF3h or empty vector. The figure shows photographs of the plates (upper row) and a selected colony at 400x magnification (lower row), as described in the legend to Fig. 3C. The results shown above are a representative of three independent experiments done in triplicate. ** $p < 0.01$ analyzed by student t test for eIF3h-trasnfected cells compared to the vector control.

Figure S2. Inducible expression of HA-eIF3h in NIH3T3 cells.
A. Immunoblot analysis of HA-eIF3h levels in 6 Teton clones (Teton-C1 - C6) with and without induction with Dox for 24 h. The clones are identified at to top of the blot; - and + indicate the presence of Dox. The bar graph below reports the HA-eIF3h band intensities normalized to the actin band intensities in the blot. **B.** Dox dose-response expression of HA-eIF3h in Teton-C1 cells after 24 h induction. Cells were treated with the indicate concentrations of Dox, and lysates were analyzed by immunoblotting (upper panel) with an anti-HA antibody. The eIF3h/actin ratio at different Dox concentration was calculated and graphed below. **C.** Growth of Teton-C1 (squares), Teton-C3 (triangles), Teton-C6 (crosses) and vector (diamonds) was measured as described in the legend to Fig. 3B. **D.** Anchorage-independent growth in soft agar. The upper panel is a photograph of typical colonies for the cell type identified above. The number of colonies formed was measured as described in the legend to Fig. S1C and is reported in the bar graph below. **E.** A Dox dose-response for growth of Teton-C1 in soft agar. Typical colonies induced with 0, 10, 100 and 1000 ng/ml of Dox are shown above. Colony formation efficiencies determined as described in Fig. S1C are graphed below. **F.** Immunofluorescence staining of Teton-C1 cells treated with anti-HA antibodies. Teton-C1 cells growing on cover slips without Dox were treated with anti-HA antibodies (+HA). Similarly, cells were Dox-induced for 24 h, then evaluated without anti-HA (+Dox), with nonimmune IgG (+IgG), and with anti-HA (+HA +Dox). Cells were fixed, treated with antibodies as indicated, then with Alexa Fluor 488-oconjugated anti-mouse antibodies (Molecular Probes). The coverslips were mounted and photographed with a

Leica confocal microscope. The results shown in panels C and C are a representative of three independent experiments. ** $p < 0.01$ analyzed by student t test for Teton-eIF3h cells compared to the vector control or Teton-C1 cells treated with different concentration of Dox compared to those without Dox treatment.

Figure S3. Effects of eIF3h abundance on protein synthesis and growth.
A. Polysome profiles of Teton-C1 cells at 0h, 2h, 4h and 8h following exposure to 1 μg/ml Dox. The migration positions of the 40S, 60S and 80S ribosomes and the polysome are labeled in the "No Dox" panel. The ratio of A_{254} in the combined polysome fractions to that in the combined ribosome fractions was calculated and reported as P/M in the upper-right corner of each profile. **B.** [^{35}S]methionine incorporation into protein was measured in Teton-C1 cells treated 24 h with various concentrations of Dox, as described in the legend to Fig. 5D. **C.** Knockdown of eIF3h by siRNA. MDA436 and PC-3 cells were treated with a control siRNA (siRNA) or an siRNA against eIF3h (sieIF3h) as described under Experimental Procedures. eIF3h levels were measured by immunoblotting as described in the legend to Fig. 4A. Efficiencies of colony formation in soft agar of MDA436 and PC-3 cells transfected with a control siRNA (siNC) and an siRNA against eIF3h (sieIF3h) were measured as described in the legend to Fig. S1C. **D.** [^{35}S]methionine incorporation into protein of siNC- and sieIF3h- transfected MDA436 and PC-3 cells at 48h post-transfection. The results shown above are a representative of three independent experiments done in triplicate. ** $p<0.01$ analyzed by student t test for sieIF3h transfected cells compared to siNC-transfected cells.

Figure S4. Exogenous eIF3h expression rescues PrEC cells from Myc-dependent apoptosis.
A. Cell lysates from PrEC, PrEC/Myc and PrEC/eIF3h/Myc cells were analyzed by western blots probed with anti-Myc and anti-actin. The relative Myc levels in the above cells are shown below the blots. **B.** Quantitation of TdT reaction in situ (TUNEL) positive staining cells in PrEC/Myc and PrEC/eIF3h/Myc after treatment with 300nM camptothecin for 24h. **C.** Colony formation efficiencies of 3T3/pcDNA5, 3T3/eIF3hAla, 3T3/eIF3h, 3T3/eIF3hAsp, 3T3/eIF3hGlu on soft agar are graphed in columns. The results shown above are a representative of three independent experiments done in triplicates. **$p<0.01$ analyzed by student t test for PrEC/eIF3h/Myc compared to PrEC/Myc cells, or all the other 3T3 cell lines compared to 3T3/pcDNA5 control.

REFERENCES

1. Sramkoski, R.M., *A new human prostate carcinoma cell line, 22Rv1*. In Vitro Cell Dev. Biol. Anim., 1999. **35**: p. 403-409.
2. Zhang, L., X. Pan, and J.W.B. Hershey, *Individual overexpression of five subunits of human translation initiation factor eIF3 promotes malignant transformation of immortal fibroblast cells.* J. Biol. Chem., 2007. **282**: p. 5790-5800.

Figure S1

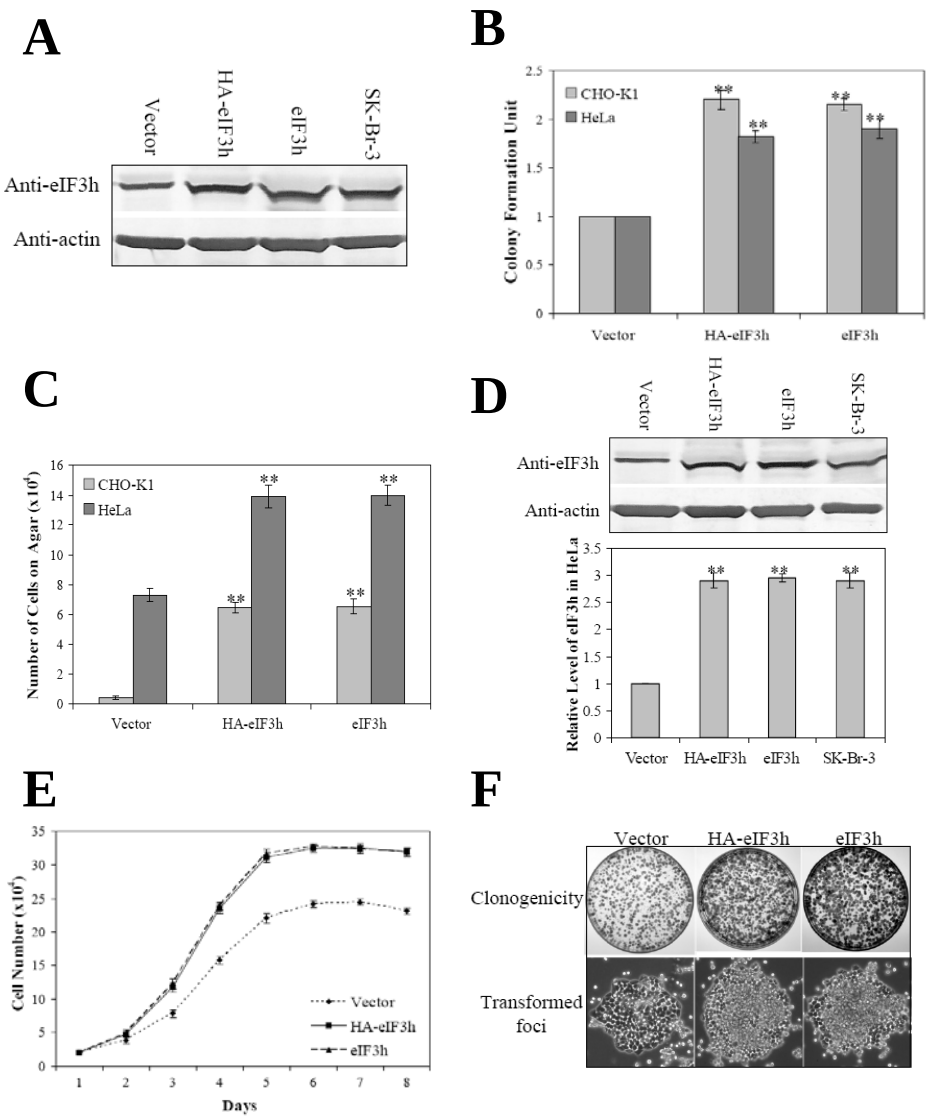

A
Vector
HA-eIF3h
eIF3h
SK-Br-3
Anti-eIF3h
Anti-actin
B
CHO-K1
HeLa
Colony Formation Unit
Vector
HA-eIF3h
eIF3h
C
CHO-K1
HeLa
Number of Cells on Agar (x10⁴)
Vector
HA-eIF3h
eIF3h
D
Vector
HA-eIF3h
eIF3h
SK-Br-3
Anti-eIF3h
Anti-actin
Relative Level of eIF3h in HeLa
Vector
HA-eIF3h
eIF3h
SK-Br-3
E
Cell Number (x10⁴)
Vector
HA-eIF3h
eIF3h
Days
F
Vector
HA-eIF3h
eIF3h
Clonogenicity
Transformed foci

Figure S2

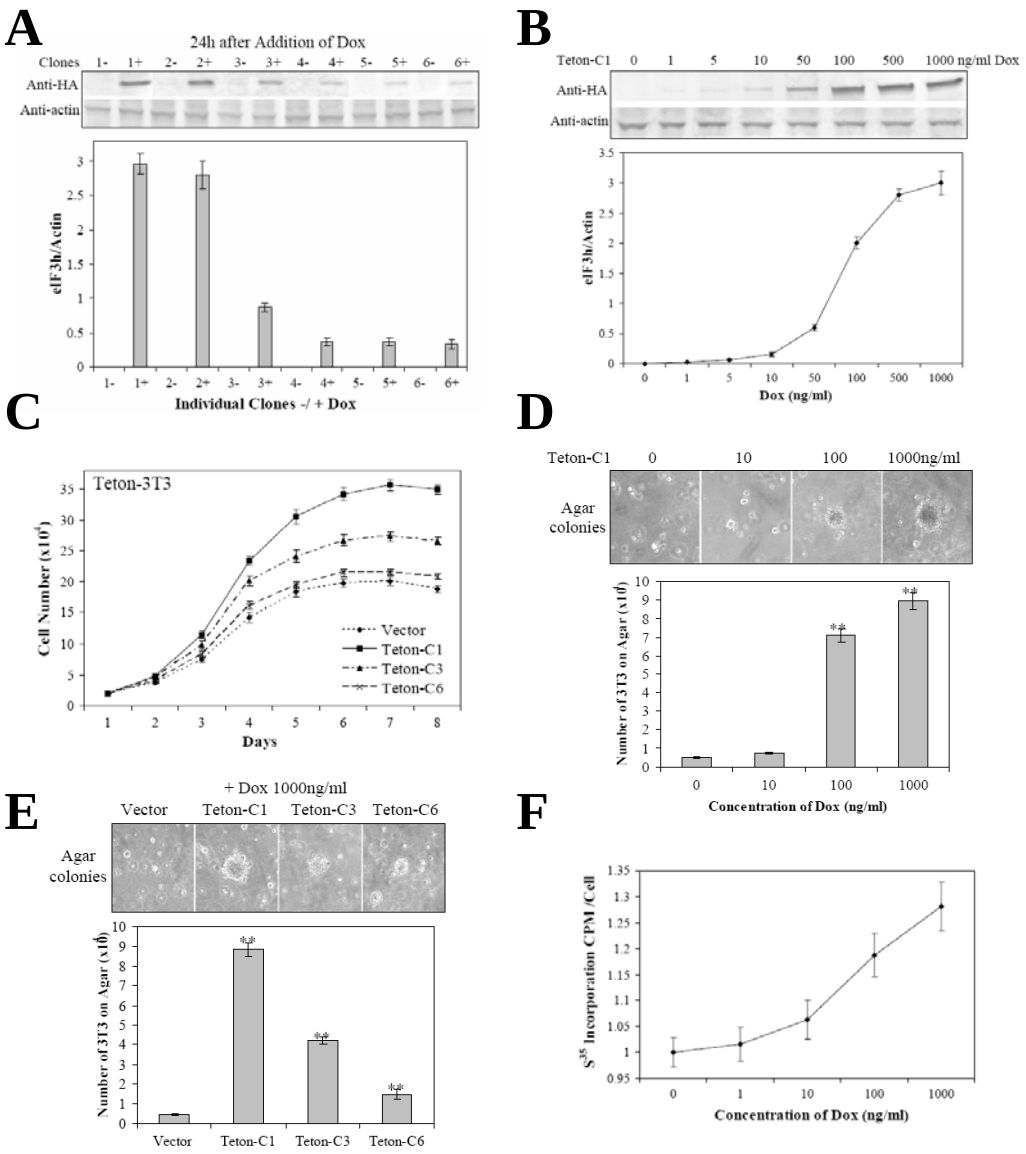
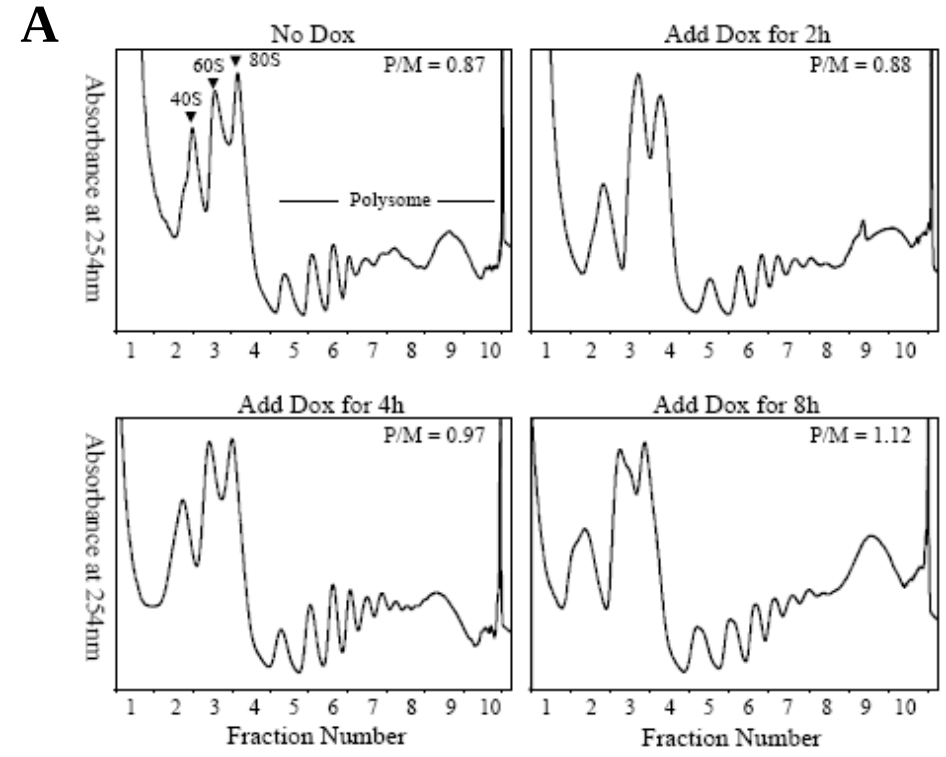
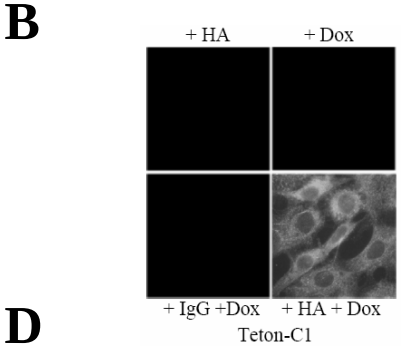
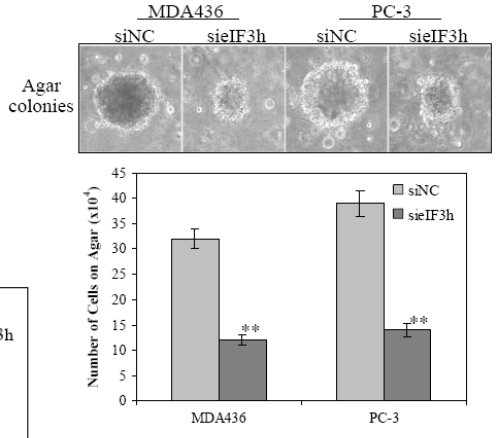

A
24h after Addition of Dox
Clones 1- 1+ 2- 2+ 3- 3+ 4- 4+ 5- 5+ 6- 6+
Anti-HA
Anti-actin
eIF3h/Actin
Individual Clones -/ + Dox
B
Teton-C1 0 1 5 10 50 100 500 1000 ng/ml Dox
Anti-HA
Anti-actin
eIF3h/Actin
Dox (ng/ml)
C
Teton-3T3
Cell Number (x10⁴)
Days
Vector
Teton-C1
Teton-C3
Teton-C6
D
Teton-C1 0 10 100 1000ng/ml
Agar colonies
Number of 3T3 on Agar (x10⁴)
Concentration of Dox (ng/ml)
E
+ Dox 1000ng/ml
Vector Teton-C1 Teton-C3 Teton-C6
Agar colonies
Number of 3T3 on Agar (x10⁴)
F
S35 Incorporation CPM /Cell
Concentration of Dox (ng/ml)

Figure S3


A
No Dox
Add Dox for 2h
Add Dox for 4h
Add Dox for 8h
P/M = 0.87
P/M = 0.88
P/M = 0.97
P/M = 1.12
40S
60S
80S
Polysome
Absorbance at 254nm
Fraction Number
1 2 3 4 5 6 7 8 9 10


B
+ HA
+ Dox
+ IgG +Dox
+ HA + Dox
Teton-C1


C
MDA436
PC-3
siNC
sieIF3h
siNC
sieIF3h
Agar colonies
Number of Cells on Agar (x10⁴)
siNC
sieIF3h
**
**
MDA436
PC-3

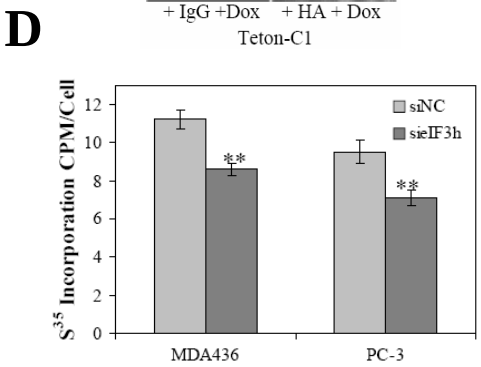
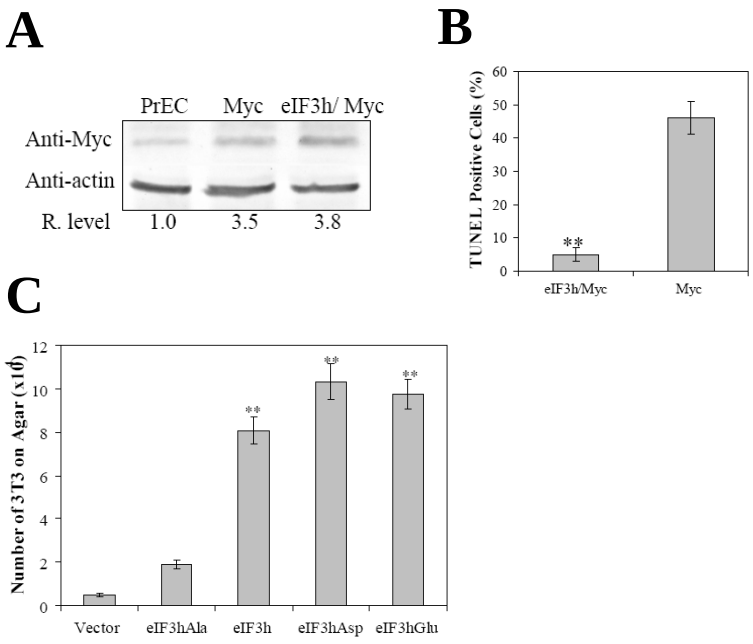

D
S35 Incorporation CPM/Cell
siNC
sieIF3h
**
**
MDA436
PC-3

Figure S4


A
PrEC
Myc
eIF3h/ Myc
Anti-Myc
Anti-actin
R. level
1.0
3.5
3.8
B
TUNEL Positive Cells (%)
60
50
40
30
20
10
0
**
eIF3h/Myc
Myc
C
Number of 3T3 on Agar (x10)
12
10
8
6
4
2
0
**
**
**
Vector
eIF3hAla
eIF3h
eIF3hAsp
eIF3hGlu