Supplementary Figure 1: A robust increase in YPEL3 gene expression in doxorubicin treated Hct116 cells harboring wild-type p53. (A) Hct116+/+ p53 and (B) Hct116-/- p53 were exposed to 0.5 μg/mL of doxorubicin and total RNA extracted at the indicated time point. YPEL3 (black bars) and p21 (grey bars) mRNA levels were assayed by RT-PCR. All gene expression values were normalized to GAPDH expression. Error bars represent 95% confidence intervals. (C) Western blot analysis of p53 protein levels following a treatment with 0.5 μg/mL of doxorubicin for the indicated time points. As expected Hct116+/+ p53 cells show a dramatic induction of p53 protein while Hct116-/-p53 cells show no detectable p53 protein.

Supplementary Figure 2: YPEL3 induction triggers cellular senescence in MCF7 TetR and IMR90 cells. (A) Representative images of MCF7 TetR expressing cells that were mock infected (parental) or infected with YPEL3 or LacZ lentivirus, treated with 1 µg/mL Tetracycline for 6 days and then subjected to SA-beta-galactosidase staining. (B) IMR90 cells transduced with the indicated lentivirus, selected for 7 days and then scored for either SA-beta-galactosidase (left panels) or senescent associated heterochromatin foci (right panels). (C) Histogram of percent positive beta-galactosidase (black bars) or SAHF (grey bars) from at least 100 cells per treatment condition.

Supplementary Figure 3: RNAi targeting of YPEL3 disrupts ras-mediated premature senescence in U2OS osteosarcoma cells. U2OS cells were

transduced with A. H-ras, B. GFP, C. H-ras + GFP, D. YPEL3, E. H-ras + YPEL3, F. H-ras + shp53, G. H-ras + shp53 + YPEL3, H. H-ras + shYPEL3, I. H-ras + shLacZ and incubated under appropriate selection for 7 days. Cells were then subjected to SA-beta-galactosidase staining. SA-beta-galactosidase staining was conducted in two biological replicates. The histogram represents the average percentage of positive cells +/- standard error of the means from at least 100 cells per treatment condition.

Supplemental Figure 4: (A) RT-PCR analysis of YPEL3 gene expression in transduced IMR90 cells (Figure 5). IMR90 cells were transduced with A. H-ras, B. GFP, C. H-ras + GFP, D. YPEL3, E. H-ras + YPEL3, F. H-ras + shp53, G. H-ras + shp53 + YPEL3, H. H-ras + shYPEL3, I. H-ras + shLacZ and incubated under appropriate selection for 7 days. Total RNA was isolated and subjected to RT-PCR to analyze YPEL3 expression using GAPDH as a normalizer. Error bars represent 95% confidence intervals. (B) Total RNA was isolated from H-ras transduced IMR90 cells every day starting at 6 days post infection. YPEL3 and p21 mRNA levels were assayed by RT-PCR and normalized to GAPDH mRNA levels. Error bars represent 95% confidence intervals. Day seven (boxed) represents the day post infection (PI) at which the RNA was isolated from the infected cells in A for RT-PCR analysis.