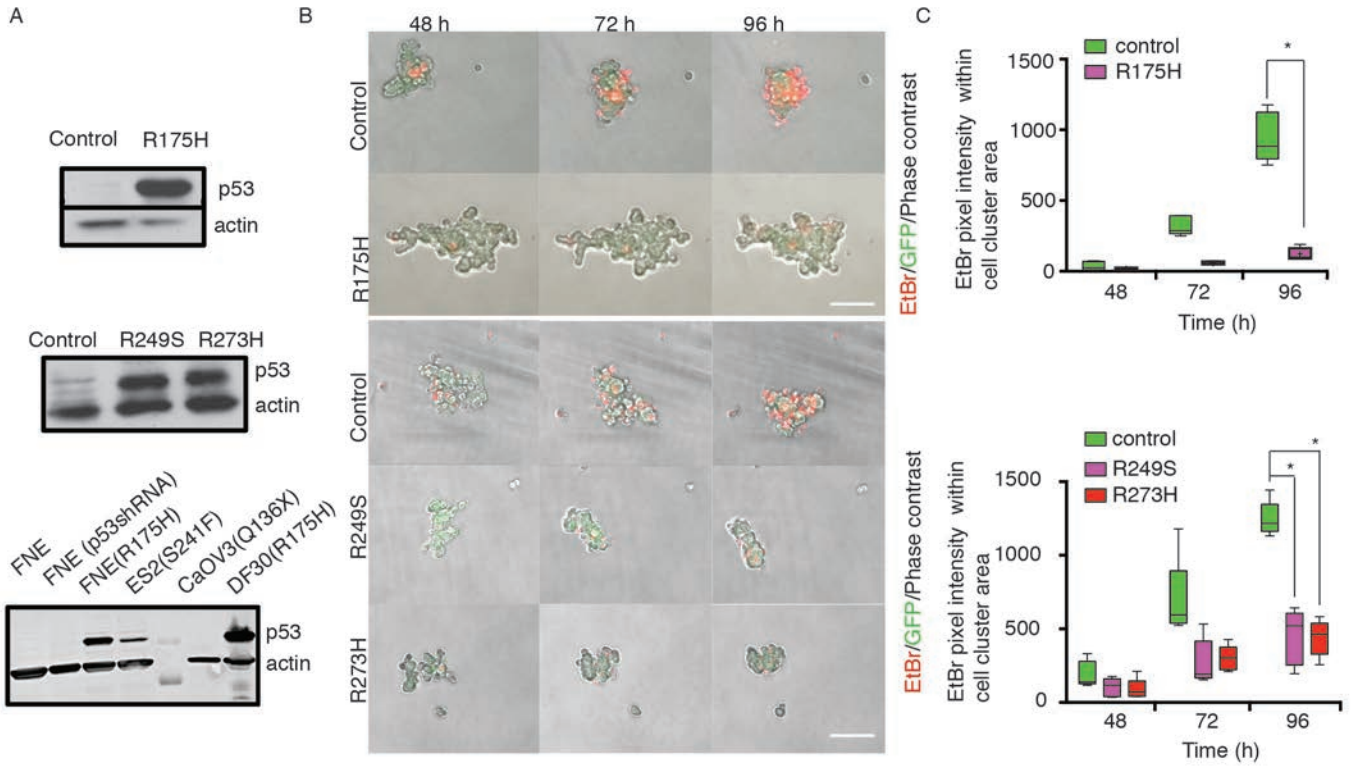
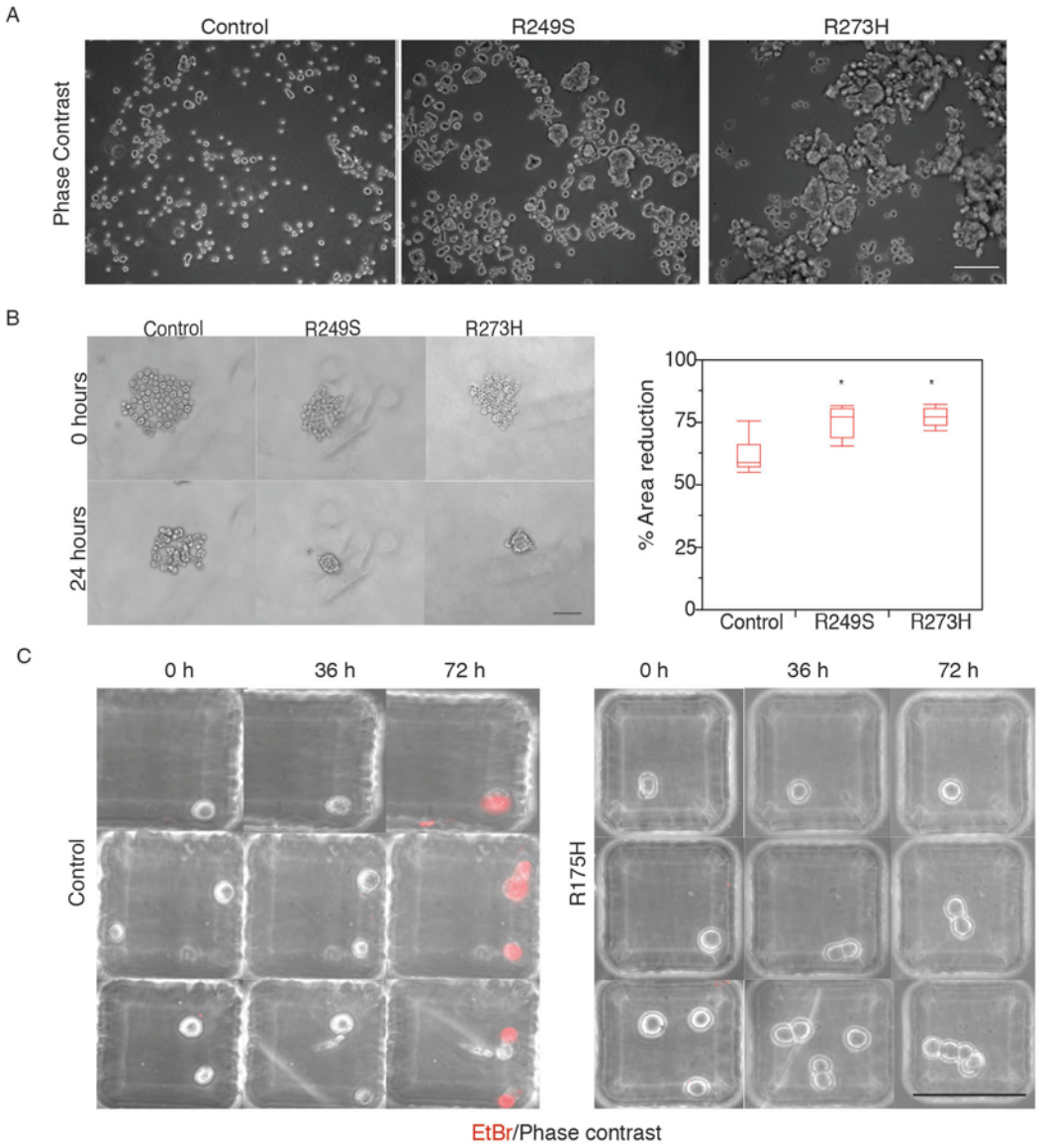
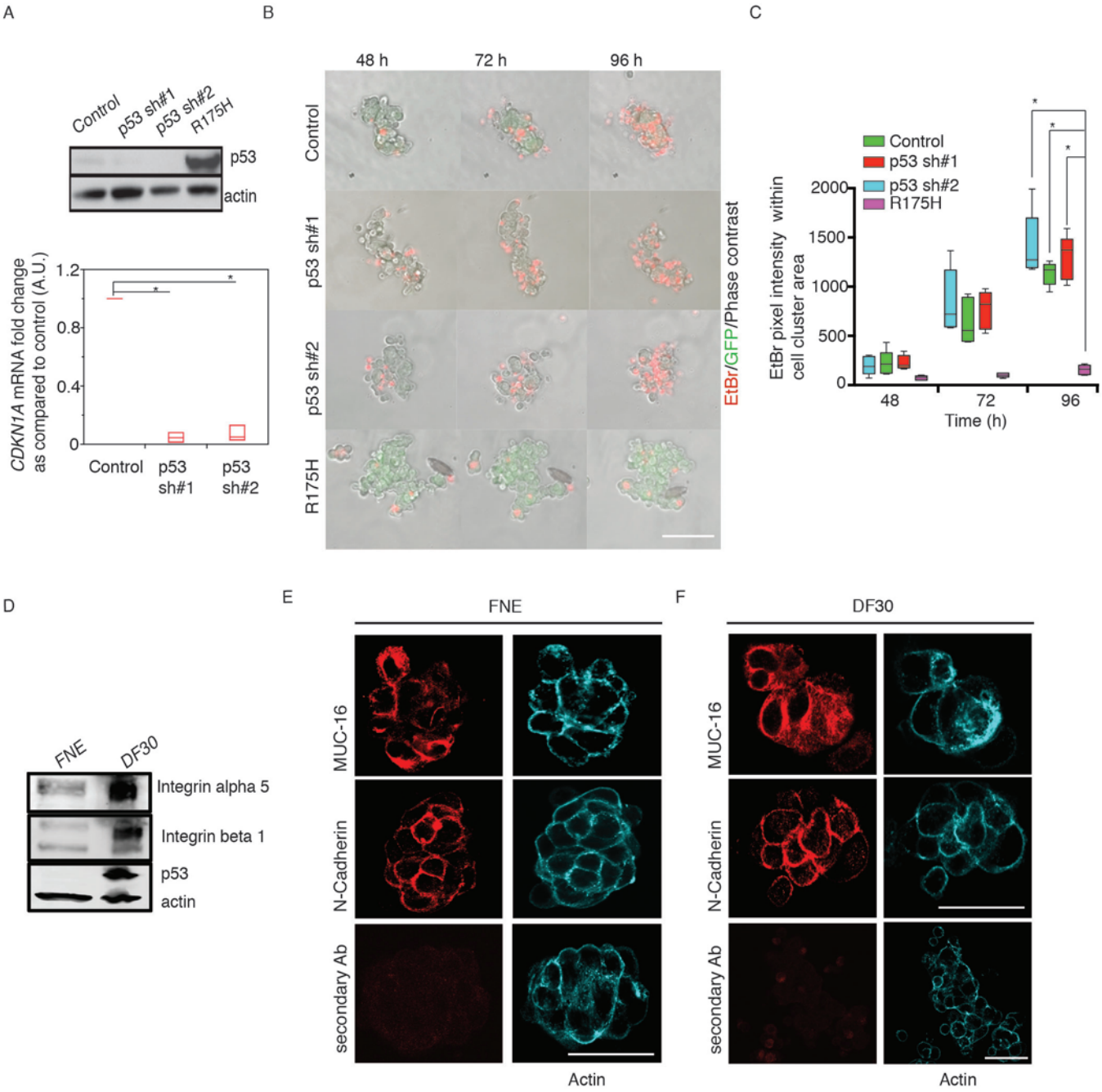


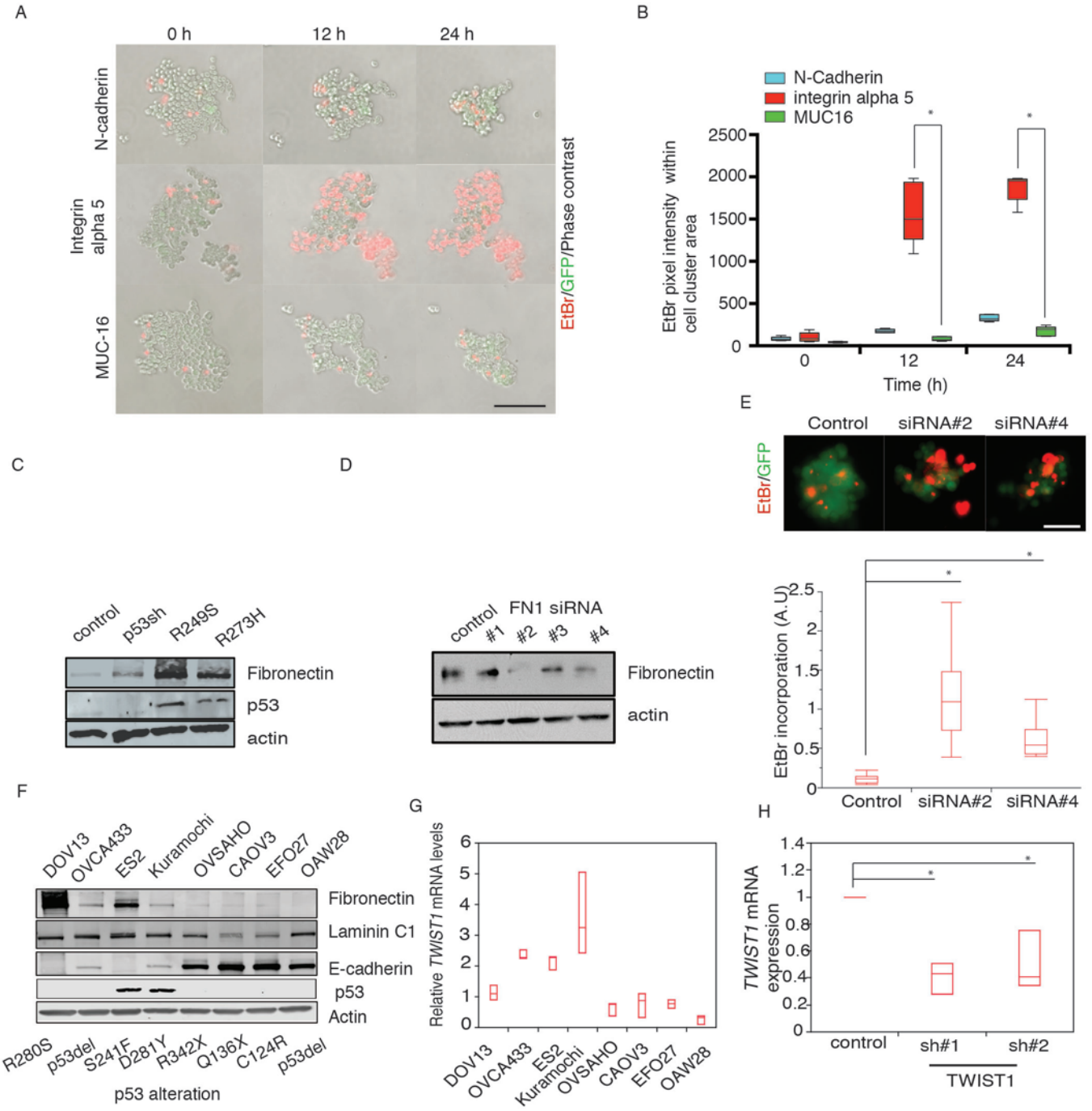
Supplementary Figure 1: Mutant p53 (m-p53) promotes anchorage independent survival of non-ciliated (FNE) cells. (A) Upper and middle panel: Western blots of p53 and actin protein expression in the various FNE cell lines expressing either empty vector or mutant variants of p53. Lower panel: Western blots of p53 and actin protein expression in the various FNE cell lines expressing either empty vector, p53 shRNA or m-p53 (R175H), and in ovarian cancer cells that endogenously express the indicated m-p53 proteins. (B) Representative (n=5 videos/group) video clips of ethidium bromide (EtBr) (red) incorporation into GFP-expressing control and FNE-m-p53 cells (green) cultured in suspension for the indicated time (Supplementary Video 2). Five movies recorded per condition. Scale bar 150 µm. (C) Measurement of EtBr pixel intensity in the various FNE lines from n=5 recorded movies per condition. All movies were acquired during one recording session. All data shown as median (horizontal bar), interquartile range (box), and minimum/maximum values (whiskers). Statistical analysis performed at 96h time point using 2-tailed Student's t test (for control and R175H) and 1-way ANOVA and post hoc Tukey-Kramer test (for R249S, R273H). * indicates P <0.05 comparing each FNE-m-p53 cell line to its control.



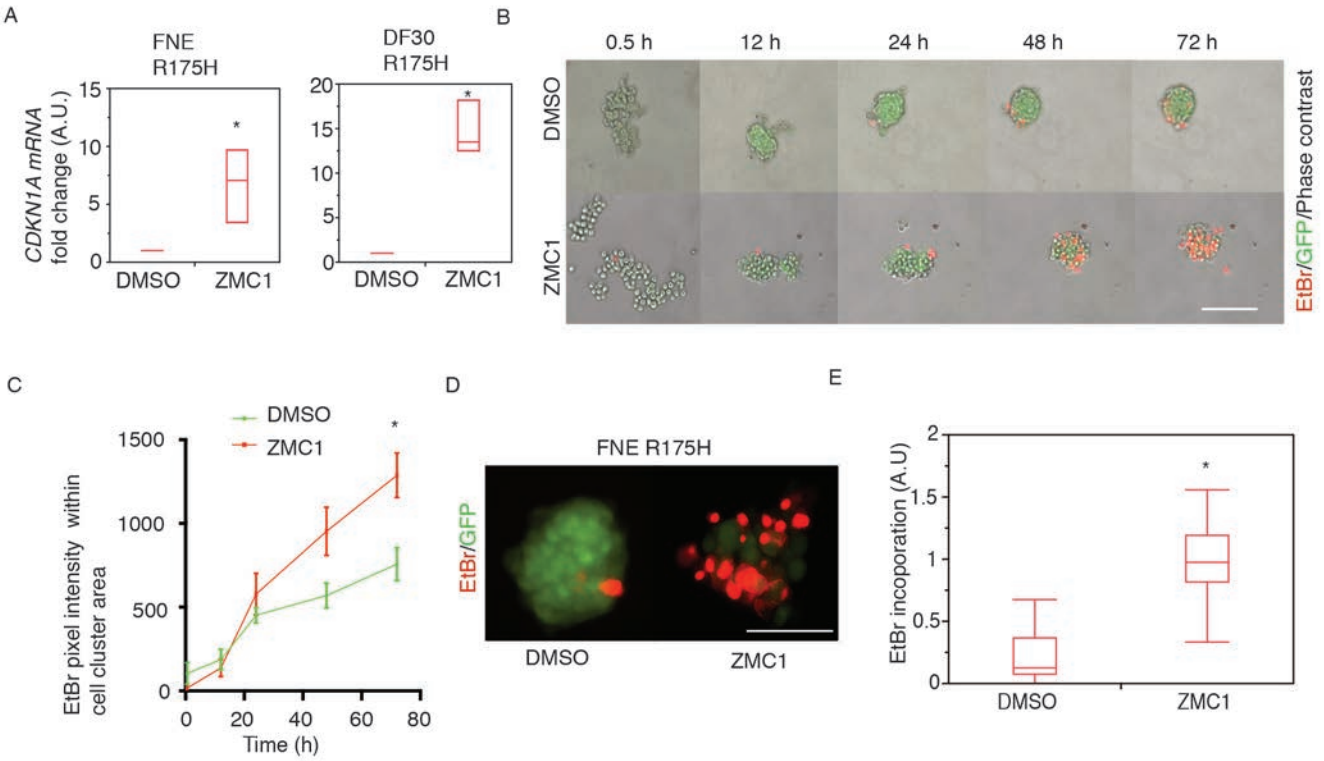
Supplementary Figure 2: Cell aggregation is not required for anchorage independent survival of fallopian tube non-ciliated (FNE) cells expressing mutant p53 (m-p53). (A) Phase contrast images of control and FNE-m-p53R249S or FNE-m-p53R273H cells cultured in suspension for 24 h. Scale bar 300 µm. (B) Upper panel: Representative video clips of control and FNE-m-p53 (R249S, R273H) cells cultured in suspension for the indicated time points. Scale bar 150 µm. 7-10 independent movies were recorded per cell type. Lower panel: Percent area reduction of cell clusters over 24 h from experiment in panel B. n=7-10 cell clusters scored per condition. All data shown as median (horizontal bar), interquartile range (box), and minimum/maximum values (whiskers). Statistical analysis performed using 1-way ANOVA and post hoc Tukey-Kramer test. * indicates P <0.05 for each engineered FNE cell line relative to the vector control line. (C) Overlaid phase contrast and ethidium bromide (EtBr) (red) images of control or FTE-m-p53R175H single cells imaged in suspension for the indicated time. Scale bar 100 µm.



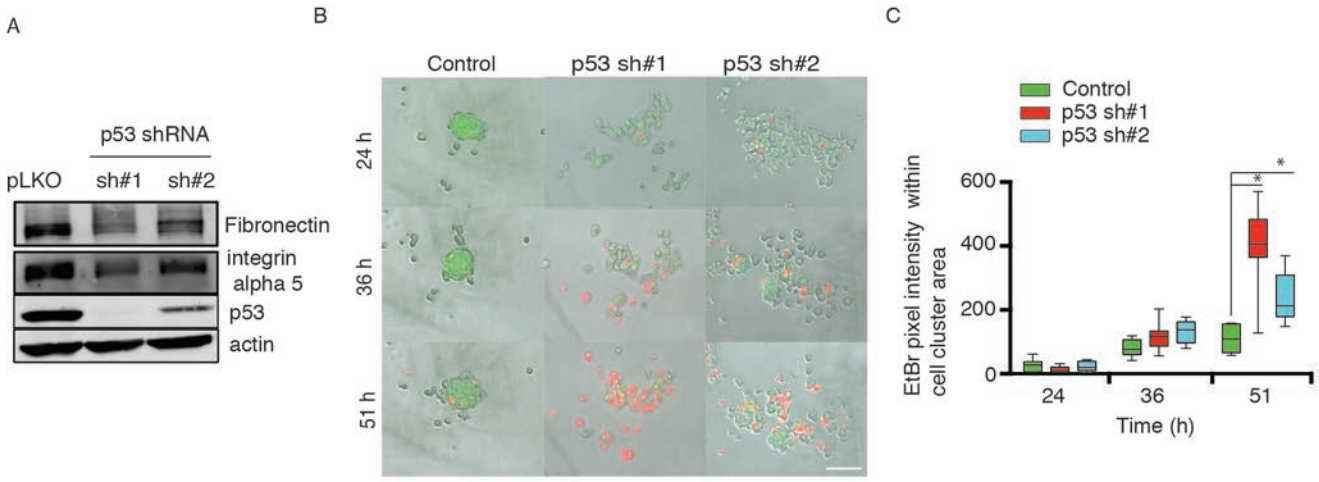
Supplementary Figure 3: shRNA-mediated attenuation of wild-type p53 expression does not promote anchorage independent survival.
(A) Upper panel: Western blot analysis of p53 and actin protein expression in the various fallopian tube non-ciliated epithelial (FNE) cell lines. Lower panel: CDKN1A mRNA fold change in FNE cells expressing p53 shRNA hairpins. Values were normalized to control FNE cells expressing empty vector. Three independent experiments performed with three technical replicates per condition. (B) Representative video clips of ethidium bromide (EtBr) (red) incorporation into GFP-expressing control, FNE-p53shRNA (#1 or 2) and FNE-m-p53R175H cells (green) cultured in suspension for the indicated time (Supplementary Video 8). Scale bar 150 µm. Five movies were acquired per condition in one recording. (C) Measurement of EtBr pixel intensity in the various FNE lines (n=5 wells/condition) from experiment in panel B. (D) Western blots of integrin α5, integrin β1, p53 and actin expression in FNE and DF30 cells. This experiment was performed twice. (E-F) Laser scanning confocal images of Mucin 16 (MUC16), N-cadherin and actin immunofluorescence staining in FNE and DF30 cells. Secondary Ab alone was used as a control. Scale bar 50 µm. Data shown as median (horizontal bar), interquartile range (box), and minimum/maximum values (whiskers). Statistical analysis performed using 1-way ANOVA and post hoc Tukey-Kramer test. In (A) * indicates P<0.05, comparing FNE-control to FNE-p53shRNA (#1 or #2). In (C) * indicates P <0.05, comparing FNE-m-p53R175H to either FNE control or FNE-p53shRNA (#1 or #2) at the 96h time point.



Supplementary Figure 4: Integrin α5 mediates anchorage independent survival of fallopian tube non-ciliated epithelial cells expressing mutant p53R175H (FNE-m-p53R175H cells). (A) Representative images of overlaid phase contrast, GFP (green) and ethidium bromide (EtBr) (red) images of FNE-m-p53R175H cells treated with 4 µg/ml of functional blocking Ab to N-cadherin or Mucin 16 (MUC16), or 2.7 µg/ml of functional blocking Ab to integrin α5 (Supplementary Video 10). Five movies were recorded per condition. Scale bar 150 µm. (B) Measurement of EtBr pixel intensity from the experiment described in (A). * designates P <0.05 relative to the samples treated with MUC16 as determined by 1-way ANOVA and post hoc Tukey-Kramer test. (C) Western blots of fibronectin, p53 and actin expression in the various FNE cells cultured in suspension for 24 h. Anti-fibronectin and anti-actin blots were repeated three times. (D) Western blots of fibronectin and actin expression in FNE-m-p53R175H cells treated with control or various FN1 (Fibronectin) siRNA oligonucleotides. (E) Upper panel: Representative (n=19-20/group) pseudo-colored fluorescence images documenting the level of EtBr incorporation into GFP-labeled m-p53R175H cells treated with control or FN1 siRNA. Scale bar 50 µm Lower panel: Quantification of the EtBr incorporation distribution of m-p53R175H cell clusters treated with control or FN1 siRNA (n=19-20/group). * designates p<0.05 comparing siRNA#2 or siRNA#4 to control as determined by 1-way ANOVA and post hoc Tukey-Kramer test. (F) Western blots of fibronectin, laminin C1, E-cadherin, p53 and actin expression in various ovarian cancer cells lines. This experiment was repeated twice. (G) TWIST family BHLH Transcription Factor 1 (TWIST1) mRNA expression among various ovarian cancer cell lines. Three independent PCR reactions were performed. 1-way ANOVA was used to determine significant differences between the means of unrelated groups P<0.05). (H) TWIST1 mRNA expression levels in FNE-m-p53R175H cells transduced with empty pLKO or TWIST1 shRNA #1 or 2. Three independent PCR reactions were performed. * designates P <0.05 for each condition relative to control sample as determined by 1-way ANOVA and post hoc Tukey-Kramer test. Data shown as median (horizontal bar), interquartile range (box), and minimum/maximum values (whiskers).



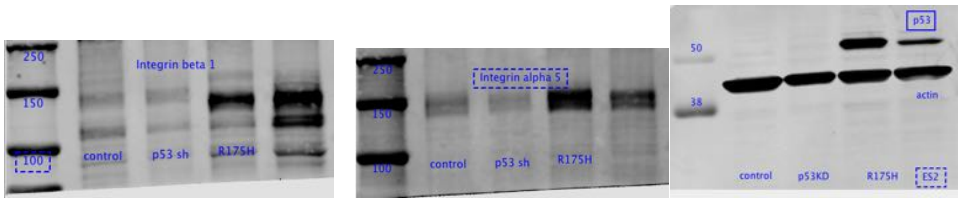
Supplementary Figure 5: Restoration of mutant p53R175H (m-p53R175H) conformation promotes anoikis. (A) Cyclin-dependent kinase inhibitor 1A (CDKN1) mRNA fold change in fallopian tube non-ciliated epithelial (FNE) cells expressing m-p53R175H (FNE-mp53R175H cells) or DF30 cells treated with DMSO or 250 nM Zinc Metallochaperone 1 (ZMC1). Values were normalized to DMSO control. (B) Overlaid phase contrast, GFP and ethidium bromide (EtBr) video clips of DF30 cells treated with DMSO or 250 nM ZMC1 and cultured in suspension for the indicated time (Supplementary Video 12). Five movies were acquired per condition during one recording session. Scale bar 150 µm. (C) Measurement of EtBr pixel intensity in DF30 cell clusters (n=5/condition) cultured in suspension over the indicated time. Data shown as mean +/- SEM. Statistical analysis performed on values for the 72h time point. * indicates P<0.05 comparing ZMC1 to DMSO. (D) Pseudo-colored fluorescence images of EtBr incorporation (red) by FTE-m-p53R175H clusters (green) treated with DMSO or 250 nM ZMC1. (E) Distribution of EtBr incorporation by FTE-m-p53R175H clusters from two independent experiments with n=25 (DMSO) and n=26 (ZMC1) clusters scored. Each cluster consisted of 100-150 cells. In A and E, data shown as median (horizontal bar), interquartile range (box), and minimum/maximum values (whiskers).
Statistical analysis performed using 2-tailed Student's t test. *P <0.05



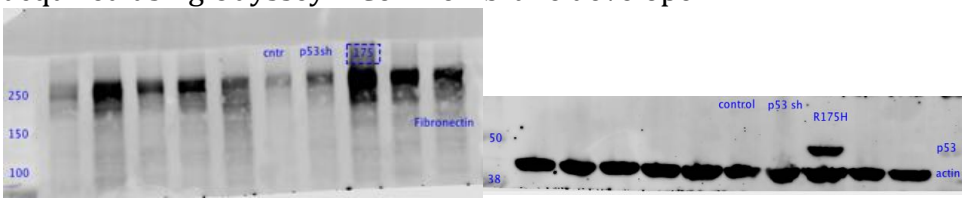
Supplementary Figure 6: Knockdown of mutant p53 reduces anchorage independent survival of DF30 high-grade serous ovarian carcinoma cells. (A) Western blots of fibronectin, integrin α5, p53 and actin in DF30 cells transduced with control shRNA (pLKO) or p53 shRNAs (#1 or #2). This experiment was performed twice. (B) Overlaid phase contrast, GFP (green) and ethidium bromide (EtBr) (red) images of DF30 cells expressing control or p53 shRNA #1 or #2 (Supplementary Video 16). Nine movies were recorded in control group, eight movies in p53 shRNA#1 group and nine movies in p53 shRNA#2 group. Scale bar 150 µm. (C) Quantification of EtBr pixel intensity in DF30 lines (n=8-9 recordings/condition) cultured in suspension over the indicated time. This experiment was performed three times. All data shown as median (horizontal bar), interquartile range (box), and minimum/maximum values (whiskers). Statistical analysis performed using 1-way ANOVA and post hoc Tukey-Kramer test. * indicated P <0.05 comparing control FNE cell line relative to p53shRNA (#1 or #2) at 51 hour time point.

Supplemental Figure 7

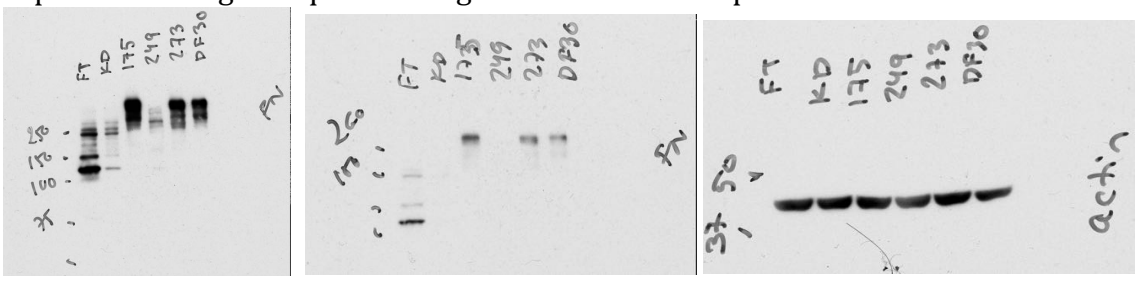
Full unedited gel for Figure 5A. First three lanes after molecular weight marker (control, p53sh and R175H) of this blot are shown in figure. First scan shows Integrin β1 expression. Second scan shows Integrin α5 expression. Third scan shows p53 and actin. Images acquired using Odyssey LiCor membrane developer.



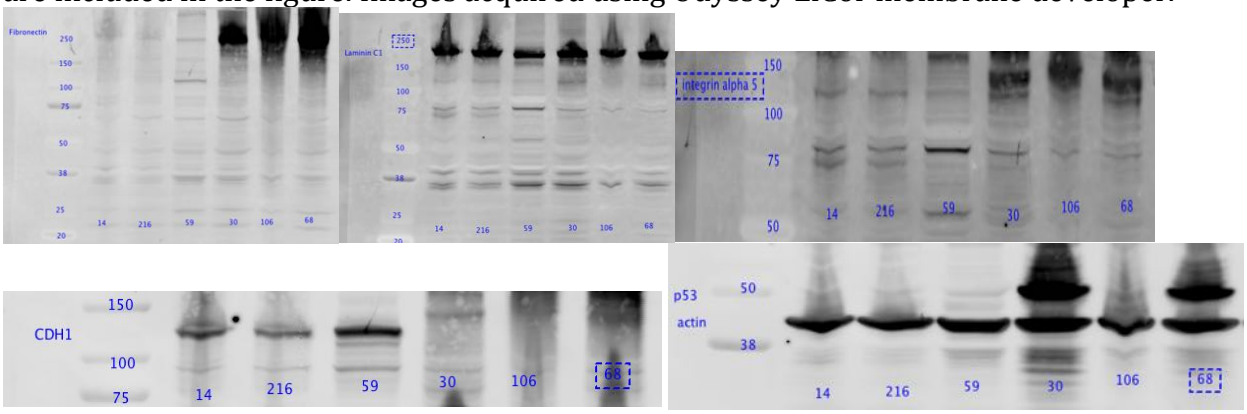
Full unedited gel for Figure 6A. ***Upper panel***: Lanes 6-8 (cntr, p53sh, 175) of this blot are shown in figure. First scan shows fibronectin expression and second scan shows actin and p53 expression. Images acquired using Odyssey LiCor membrane developer.



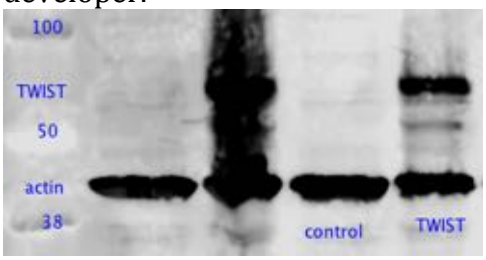
<u>Full unedited gel for Figure 6A</u>. ***Lower panel***: First 3 lanes (FT, KD, 175) of second and third blot are shown in the figure. First scan shows longer exposure of TCA precipitated fibronectin from culture media of indicated samples. Second scan shows low exposure of fibronectin expression. Third scan shows actin expression. Images acquired using Kodak Film developer.



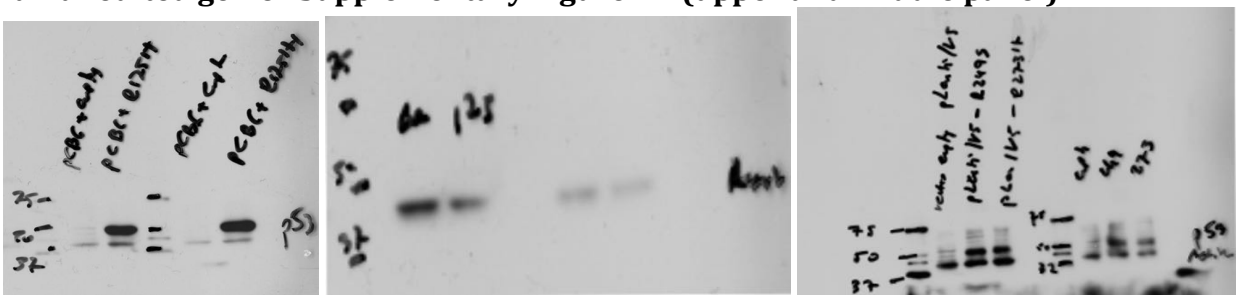
<u>Full unedited gel for Figure 6D.</u> First scan shows fibronectin. Second scan shows Laminin C1. Third scan shows integrin α5. Fourth scan shows E-cadherin (CDH1). Fifth scan shows p53 and actin. All lines are included in the figure. Images acquired using Odyssey LiCor membrane developer.



Full unedited gel for Figure 7A. Last two lanes are depicted in figure. Upper band corresponds to TWIST1-ER, lower band corresponds to actin. Images acquired using Odyssey LiCor membrane developer.

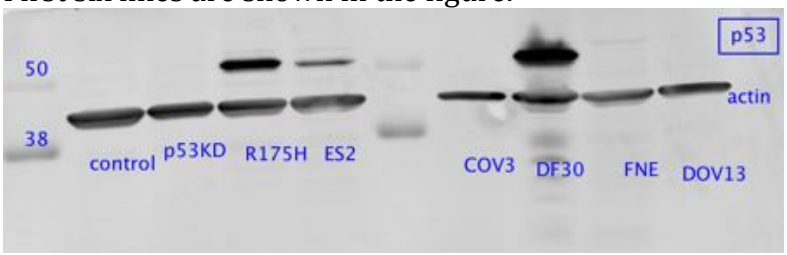


Full unedited gel for Supplementary Figure 1A (upper and middle panel).

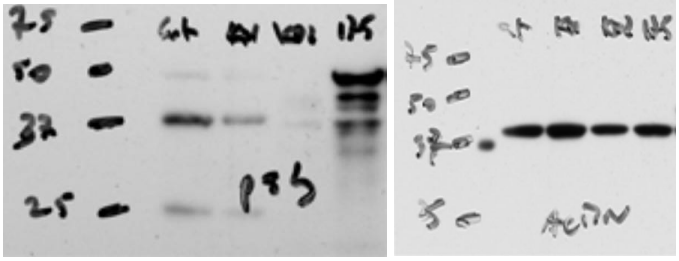


Upper panel blot shows p53 (1st scan) and actin (2nd Scan). First 2 lanes are shown in figure.
Lower panel blot shows p53 and actin (3rd scan). First three lanes are shown in figure. Images acquired using Kodak Film developer.

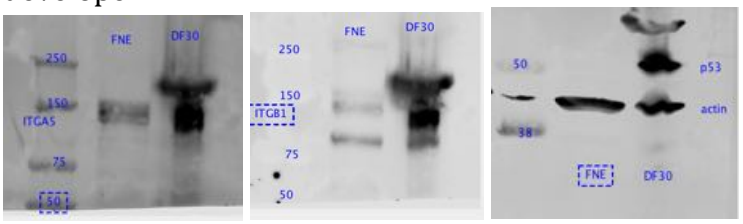
Full unedited gel for Supplementary Figure 1A (lower panel)
First six lines are shown in the figure.



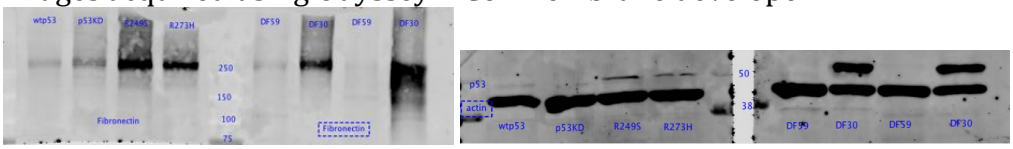
Full unedited gel for Supplementary figure 3A. First scan shows p53 expression. Second scan shows actin expression. All lanes are shown in the figure. Images acquired using Kodak Film developer.



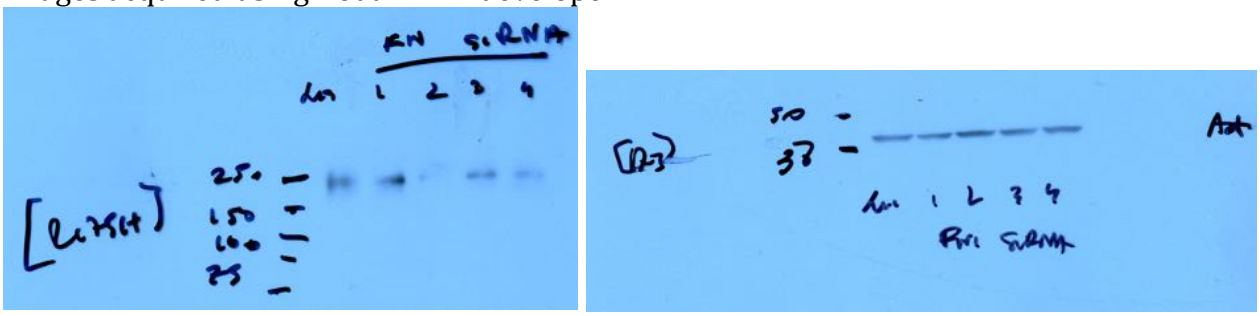
Full unedited gel for Supplementary Figure 3D. First scan shows integrin α5. Second scan shows integrin β1 and third scan shows actin and p53. Images acquired using Odyssey LiCor membrane developer.



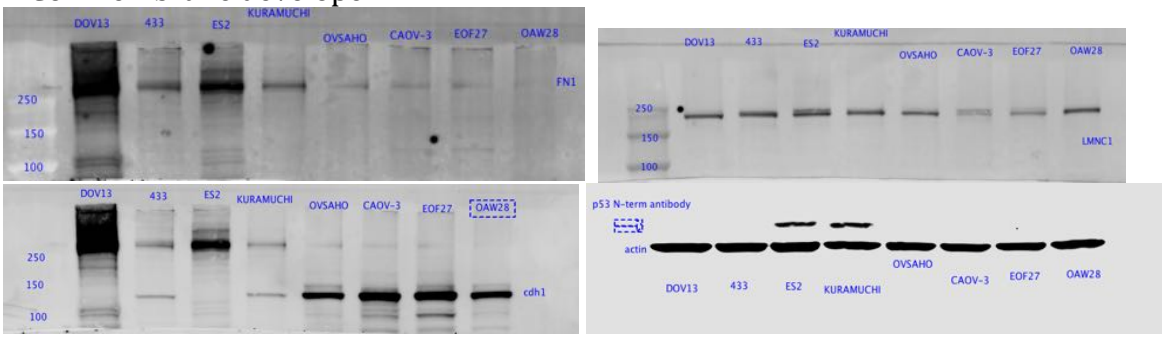
Full unedited gel for Supplementary Figure 4C (upper panel). First scan shows Fibronectin. Second scan shows actin and p53 expression. First four lanes of first and second scan are included in figure. Images acquired using Odyssey LiCor membrane developer.



Full unedited gel for Supplementary Figure 4C (lower panel). All lines are shown in the figure Images acquired using Kodak Film developer



Full unedited gel for Supplementary Figure 4E. First Scan shows fibronectin. Second scan shows laminin C1. Third Scan shows fibronectin (above 250 kDa) and E-cadherin (between 100 and 150 kDa). Fourth scan shows p53 and actin. All lanes are depicted in the figure. Images acquired using Odyssey LiCor membrane developer.



Full unedited gel for Supplementary Figure 6A: First scan shows p53 and actin expression. Second scan shows fibronectin expression. Third scan shows integrin α5 expression. First 3 lanes are shown in figure. Images acquired using Odyssey LiCor membrane developer.