Supplementary figure legends

Figure S1: Stepwise tumorigenic cells from epithelial and mesenchymal origin display fractional killing upon TRAIL-treatment. **a-** BJELR cells were treated with the indicated doses of TRAIL during 16 h and apoptosis was analyzed as the percentage of cells displaying positive labeling for APO2.7 apoptosis marker. Histogram representing the mean +/- SD of two independent biological replicates is shown. **b-** HA1ER cells were treated with the indicated doses of TRAIL during 16 h and apoptosis was analyzed as the percentage of cells displaying positive labeling for APO2.7. Histogram representing the mean +/- SD of two independent biological replicates is shown. **c-** Proliferation of HA1ER cells upon sequential treatments with TRAIL. Cells were plated and allowed to proliferate during 20 h before receiving a first dose of TRAIL (100 or 400 ng/ml, as indicated), were maintained under continuous exposure to the cytokine and further challenged with TRAIL at the indicated time points. Untreated cells were grown in parallel as proliferation controls. Images were acquired every hour using INCUCYTE and confluency was evaluated as the percentage of the surface covered by cells. The mean value obtained from two independent wells per time-point is displayed and is representative of 4 independent biological replicates.

Figure S2: Genes showing increased expression along transformation in stepwise models and analysis of PLAU mRNA expression in primary human breast cancer. **a-b-** Heatmaps corresponding to the 35 and 16 genes whose upregulated expression pattern was observed in cells of HEK or BJ stepwise tumorigenesis models. Results from 4 independent transcriptome datasets are displayed ("array1-4"). Gene names and mean fold change differences in their expression between transformed and normal cells from each model ("HA1ER/HEK"; "BJELR/BJ") are indicated. **c-** Phenotype plots representing *PLAU* mRNA levels in breast cancer primary tumors displaying the indicated molecular features. Statistical significance was calculated with two-tailed unpaired student's t-test. *P *value* <0.05; ** P *value*<0.01.

Figure S3: uPA expression supports cell survival upon TRAIL treatment in tumor cells from stepwise tumorigenesis models. **a-** Efficiency of knock down in BJELR cells transfected with pooled siRNAs targeting *PLAU* mRNA ("siPLAU"), *Death Receptor 5* mRNA ("siDR5"), *cFlip* mRNA ("siFlip"), *PLAUR* mRNA ("siPLAUR") and scramble

siRNAs ("scr") was analyzed 48 h post-transfection by Western blot. β–Actin, loading control. **b-** BJELR cells were transfected with pooled siRNAs targeting *PLAU* mRNA ("siPLAU") or non-targeting scramble siRNAs ("scr"). Forty-eight hours post-transfection apoptosis was analyzed by flow cytometry as the percentage of cells displaying positive labeling for the APO2.7 either in untreated populations (white bars) or upon TRAIL treatment (16 h, 1μg/ml of TRAIL, black bars). Results obtained from two independent biological replicates are displayed as histograms (mean +/- SD). Statistical significance was calculated by applying two-tailed, unpaired Student's t-test, **P value*<0.05. **c-** HA1ER cells were transfected with pooled siRNAs targeting *PLAUR* mRNA ("siPLAUR") or non-targeting scramble siRNAs ("scr"). Forty-eight hours post-transfection apoptosis was analyzed by flow cytometry as the percentage cells displaying positive labeling for APO2.7 either in untreated populations (white bars) or upon TRAIL treatment (16 h, 1μg/ml of TRAIL, black bars). Results from two independent biological replicates are displayed as histograms (mean +/- SD). Statistical significance was calculated by applying two-tailed, unpaired Student's t-test, **P value*<0.05. **d-** HA1ER cells were transfected with siRNAs targeting *PLAU* mRNA ("siPLAU") or non-targeting scramble siRNAs ("scr"). Forty-eight hours post-transfection apoptosis rate was analyzed by flow cytometry as percentage of cells displaying positive labeling for cleaved PARP either in untreated populations (white bars) or upon TRAIL treatment (3 h, 1μg/ml of TRAIL, black bars). Results from two independent biological replicates are displayed as histograms (mean +/- SD). Statistical significance was calculated by applying two-tailed, unpaired Student's t-test, ***P value*<0.005. **e-** Efficiency of knock down in HA1ER cells transfected with pooled siRNAs targeting *PLAU* mRNA ("siPLAU"), *PLAUR* mRNA ("siPLAUR") was analyzed 48 h post-transfection by Western blot.

Figure S4: a- Time course analysis of PARP cleavage. BJELR cells were transfected either with pooled siRNAs targeting *PLAU* mRNA ("siPLAU"), *PLAUR* mRNA ("siPLAUR") or non-targeting scramble siRNAs ("scramble") and 48 h post-transfection cell populations were either left untreated or challenged with 1μg/mll TRAIL during 1, 3 or 7 h. The percentage of cells displaying cleaved PARP was determined by flow cytometry. Images from one representative experiment out of three independent replicates are shown. Percentage of cleaved PARP positive cells is indicated. **b-** Efficiency of uPA knock down by individual siRNAs targeting *PLAU* mRNA was analyzed. BJELR cells were transfected with the indicated individiual siRNAs targeting different regions of *PLAU* mRNA (#7,

#8, #9 and #10) or non-targeting scramble siRNA as control. Forty-eight hours post-transfection protein extracts were collected and the level of uPA was determined by Western blot. β–Actin, loading control. **c-** Efficiency of knock down was analyzed by Western blot. BJELR cells were transfected with the indicated siRNAs or non-targeting scramble siRNA as control. Forty-eight hours post-transfection protein extracts were collected and the level of the indicated proteins was determined. Images displayed correspond to one of at least two biological replicates. α–Tubulin, loading control. **d-** Western blot assays of total ERK1/2 (ERK1/2) and phopshorylated ERK1/2 (pERK1/2; Thr202/Tyr204) in BJELR cells treated with 20μM of U0126 MEK1/2 inhibitor or vehicle (DMSO) during 1 or 5 h. α–Tubulin, loading control.

Figure S5: uPA knock down does not affect total protein levels of key components of the TRAIL pathway. **a-** Total protein levels of components of the TRAIL cascade upon uPA knock down. BJELR cells were transfected either with pooled siRNAs targeting *PLAU* mRNA ("siPLAU") or non-targeting siRNAs ("scr"). Forty-eight hours post-transfection the basal protein levels of Death Receptor 5 ("DR5"), Death Receptor 4 ("DR4"), Decoy Receptor 1 ("DcR1"), Decoy Receptor 2 ("DcR2"), Fas-associated via death domain ("FADD"), procaspase-8 ("Casp-8") and cellular Flice inhibitory protein ("cFlip"), and efficiency of uPA knock down were analyzed by Western blot. Images from one representative experiment are displayed. α–Tubulin, loading control. **b-** Efficiency of knock down in HA1ER cells transfected with pooled siRNAs targeting *Death Receptor 5* mRNA ("siDR5"), *Decoy Receptor 2* mRNA ("DcR2") was analyzed 48 h post-transfection by Western blot. α–Tubulin was used as loading control. **c-** The role of DR5 and DcR2 in TRAIL-apoptotic signaling in HA1ER cells. Cells were transfected either with pooled siRNAs targeting *Death Receptor 5* mRNA ("siDR5"), *Decoy Receptor 2* mRNA ("siDcR2") or scramble siRNAs ("scr") and 48 h post-transfection cell populations were either left untreated (control, white bars) or challenged with 1μg/ml TRAIL during 3 h (black bars). Apoptosis was determined as percentage of cells with positive labeling for cleaved PARP by flow cytometry. Histograms represent the mean +/- SD obtained from three independent biological replicates. Statistical significance between either knock down relative to scramble transfected cells was calculated by applying two-tailed, unpaired Student's t-test, **P value*<0.05; ***P value*<0.005.