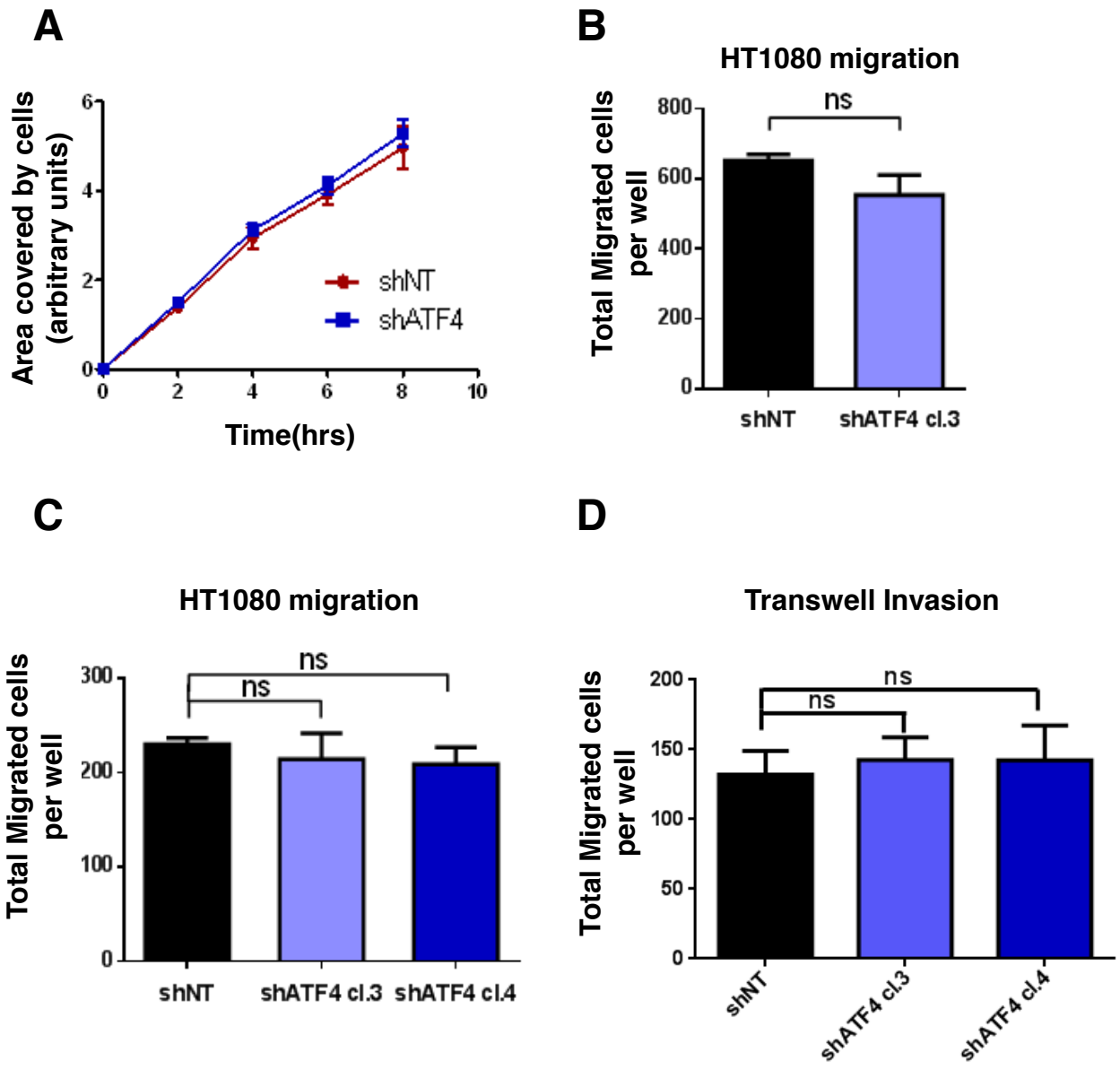


Figure S1. ATF4 expression does not affect cell migration or invasion of fibrosarcoma cells. (A) The migratory ability of shNT.HT1080 and shATF4.HT1080 cells was analyzed with a scratch assay under normoxic conditions. The area of the original scratch covered by the migrated cells is plotted vs. time. Each point represents the average of thee replicates (n=3, mean ± S.D). Statistical significance was calculated by Student's *t test.* (B) shNT.HT1080 and shATF4.HT1080 cells were cultured in transwell migration wells for 10h under hypoxia (0.5% O_2) followed by 10h under normoxia and cells that migrated were stained with DAPI and counted. The average of the total number of migrated cells counted from each of 3 wells is shown (n=3, mean ± S.D). (C) shNT.HT1080 and shATF4.HT1080 (cl.3 and cl.4) cells were subjected to a transwell migration assay for 5h under hypoxic (0.5% O_2) conditions. Each bar represents the mean of the total migrated cells counted from each of 3 wells. (ns- not significant by Student's *t-test).* (D) Transwell invasion assay was performed with shNT.HT1080 and shATF4.HT1080 (cl.3 and cl.4) cells cultured in 24 well transwell matrigel plates 7h under hypoxic (0.5% O_2) conditions. Again each bar represents the mean from each of 3 experiments. (ns- not significant by Student's *t-test).*

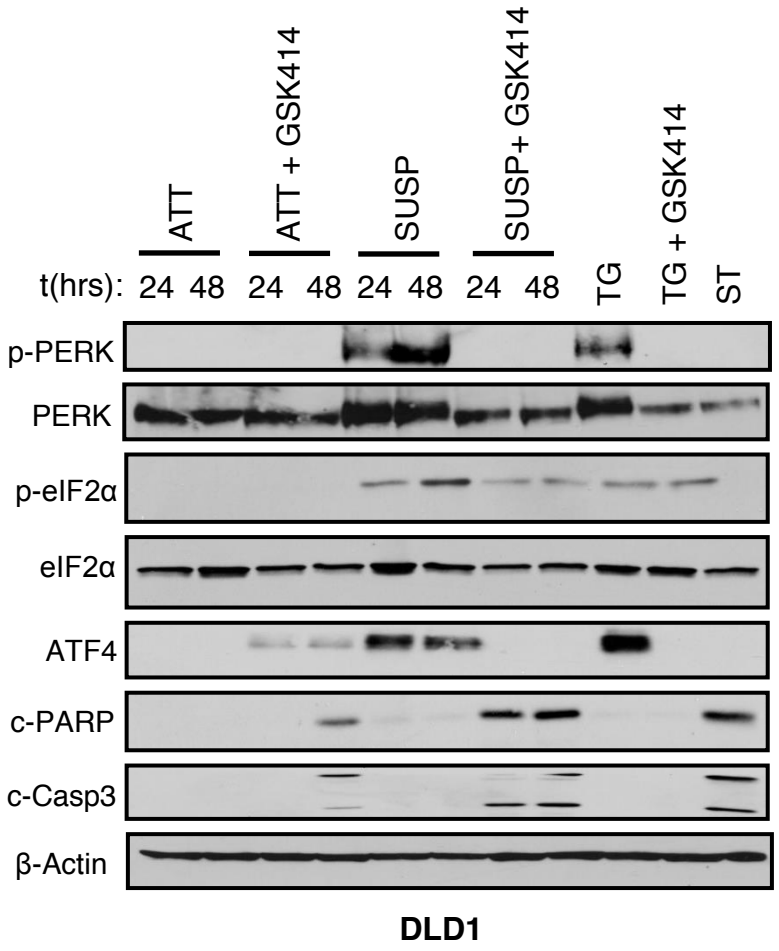


Figure S2. Loss of ISR in DLD1 colorectal adenocarcinoma cells induces anoikis. shNT.DLD1 and shATF4.DLD1 cells were grown in attached (ATT) or suspension conditions (SUSP) for 24h and 48h. 1μM GSK414 was added to attached (ATT+GSK414) or suspension (SUSP+GSK414) conditions. Thapsigargin (TG) and staurosporine (ST) were used as controls to induce ISR and apoptosis respectively. Induction of the ISR and apoptosis was measured by immunoblot analysis for indicated proteins. Immunoblot is a representative of two independent experiments.

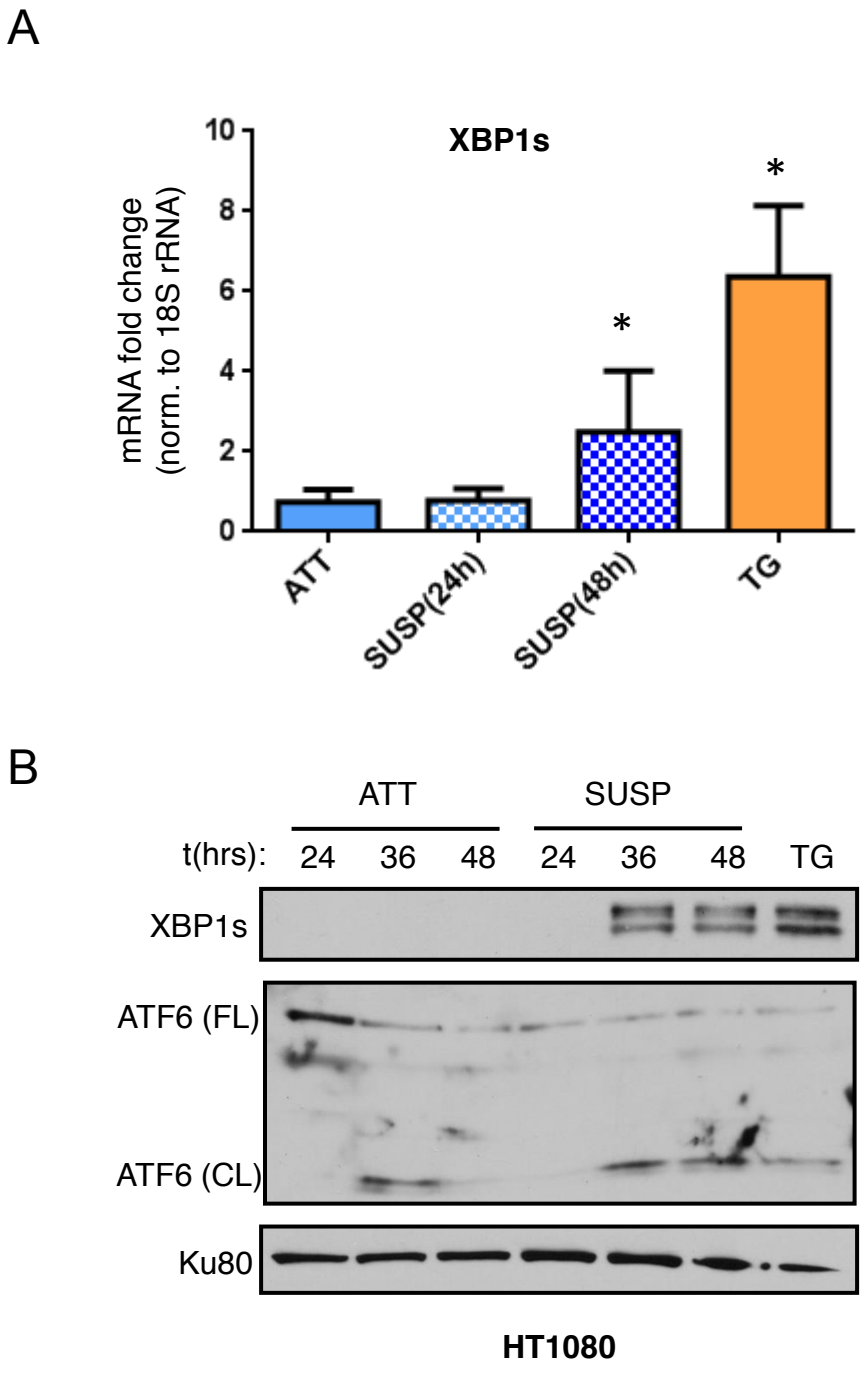


Figure S3. Loss of matrix attachment induces the IRE1α and ATF6 arms of UPR. (A) HT1080 cells were grown in attached (ATT) and suspension (SUSP) cultures for 24h and 48h. RT-PCR analysis for *XBP1* spliced transcript (XBP1s) were performed for three independent experiments (n=3). Data is plotted as mean ± S.D. and normalized to 18S rRNA (*p<0.05). (B) HT1080 cells were grown in attached or suspension conditions for 24h, 36h and 48h. Lysates from treatments were immunoblotted for XBP1s, ATF6 and Ku80. Thapsigargin (TG) was used as positive control for UPR induction. ATF6(FL) and ATF6(CL) stands for full length and processed form of ATF6 respectively. Immunoblots are representative of 2 independent experiments.

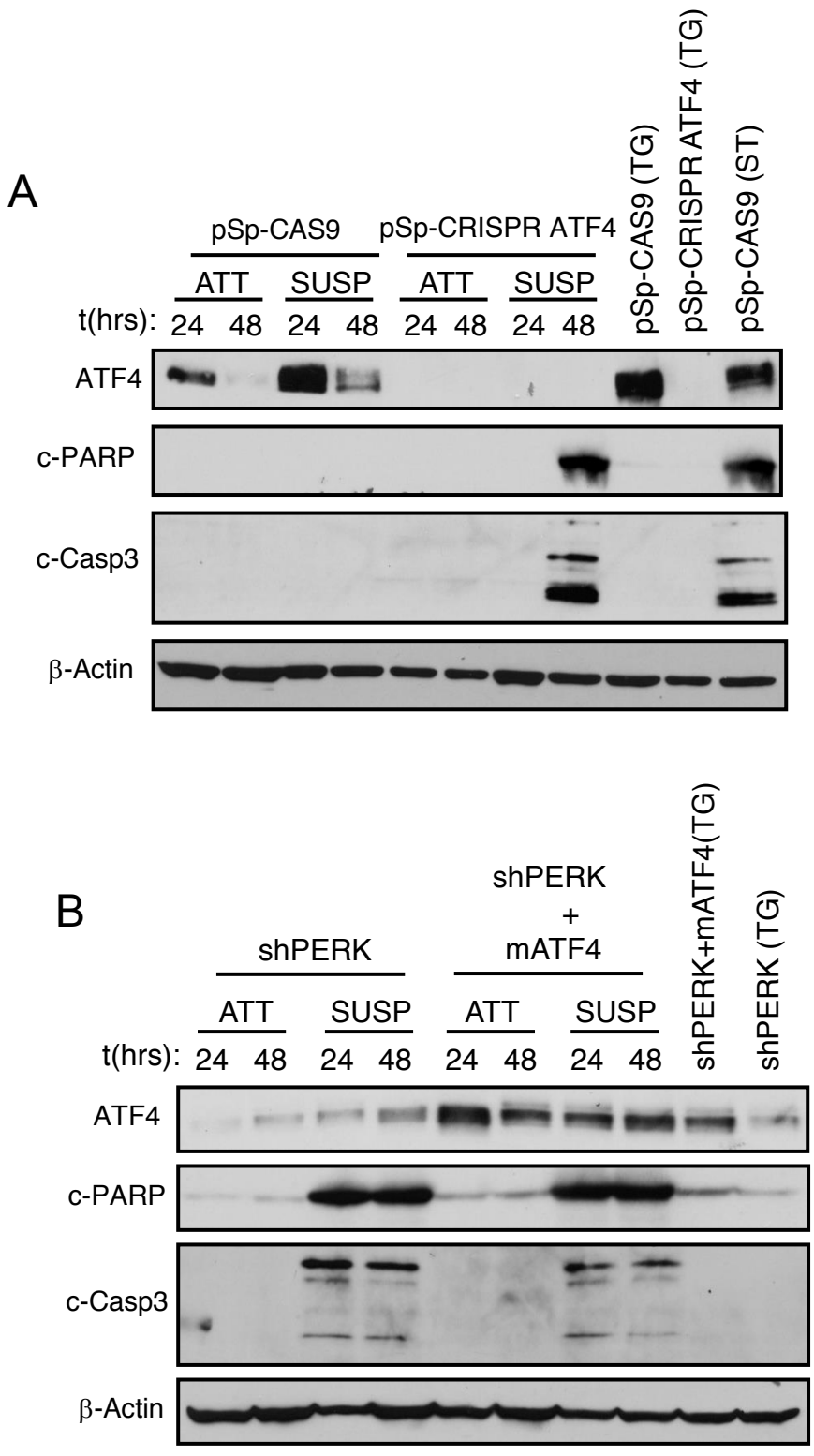


Figure S4. Loss of ATF4 and PERK induces anoikis. (A**)** pSp-CAS9 and pSp-CRISPR ATF4 HT1080 cells were grown in attached (ATT) or suspension conditions (SUSP) for 24h and 48h. Thapsigargin (TG) and staurosporine (ST) were used as controls to induce ISR and apoptosis. Induction of the ISR and apoptosis were measured by immunoblot analysis. (B) shPERK.HT1080 cells were transduced with an empty vector or full length adenoviral-mouse ATF4 (mATF4). Both cells were grown either in attached and suspension culture for up to 48 h and apoptosis markers were probed by immublot analysis. All immunoblots are representative of 2 independent experiments.

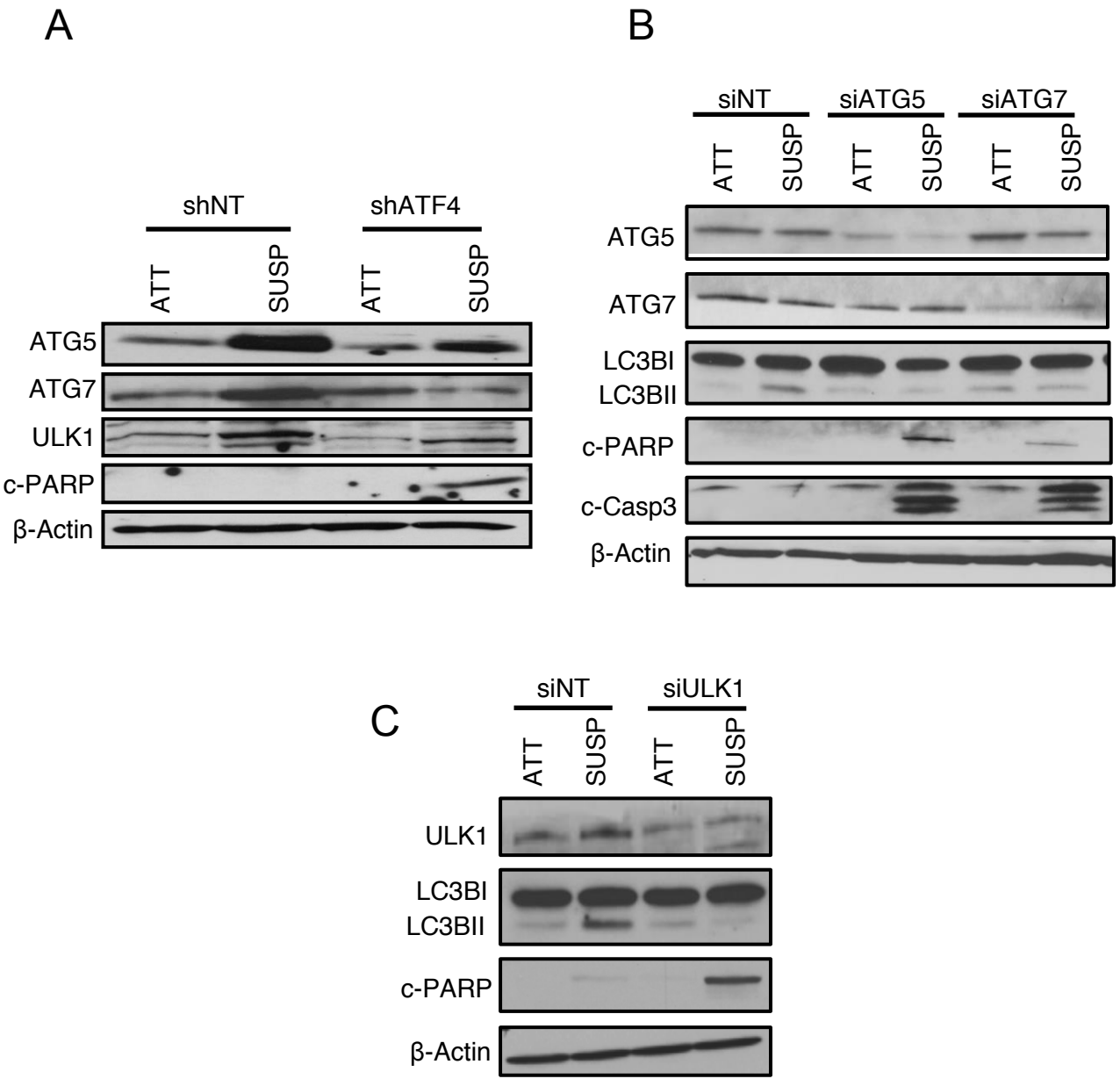


Figure S5. **ATF4 modulates critical regulators of autophagy during matrix detachment.** (A) shNT and shATF4.HT1080 cells were cultured in attached (ATT) and suspension (SUSP) conditions. Immunoblots for ATG5, ATG7, ULK1, c-PARP and β-Actin were performed. HT1080 cells transfected with siRNAs against ATG5 (B), ATG7(B), ULK1 (C) or scrambled control (siNT) and were incubated in attached (ATT) or suspension conditions (SUSP) for 48 h. Confirmation of knockdown of ATG5, ATG7, ULK1 as well as LC3B, c-PARP and c-Casp3 were measured by western blot analysis. All immunoblots are representative of 2 independent experiments.

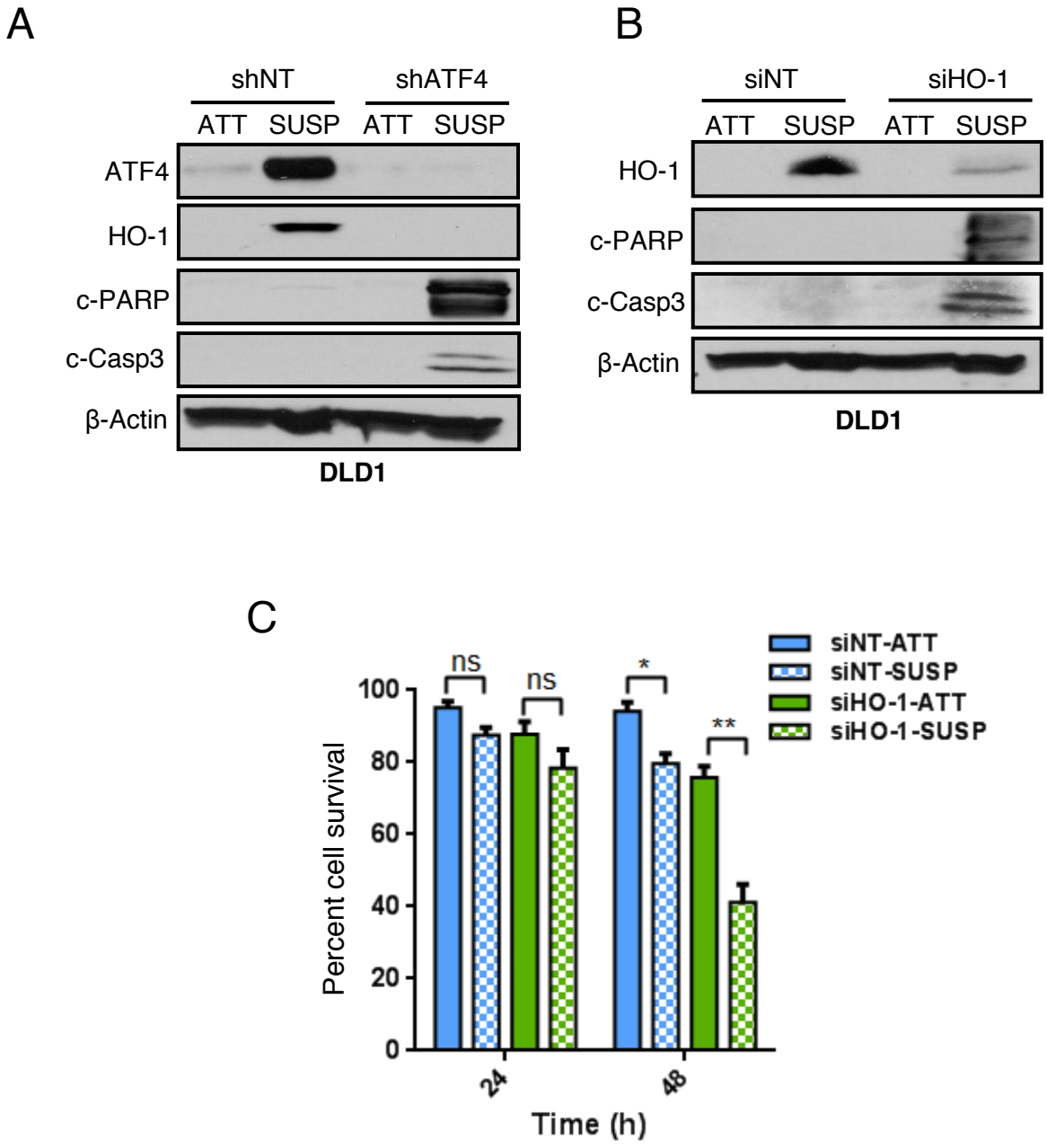


Figure S6. HO-1 is required for DLD1 cell survival following loss of matrix attachment. (A) shNT and shATF4. DLD1 cells were grown in suspension for 48 h. Immunoblots for ATF4, HO-1, c-PARP, c-caspase 3 and β-Actin were performed. (B) DLD1 cells were transfected with non-targeting siRNA (siNT) or siHO-1 and grown in suspension for 48 h. Immunoblots for indicated proteins were performed. All immunoblots are representative of 2 independent experiments.(C) Percentage cell survival in DLD1 cells from three independent experiments (n=3) expressed as mean ± S.D. Statistical significance was calculated by Student's *t test* (*p<0.05; **p<0.001, ns-not significant).

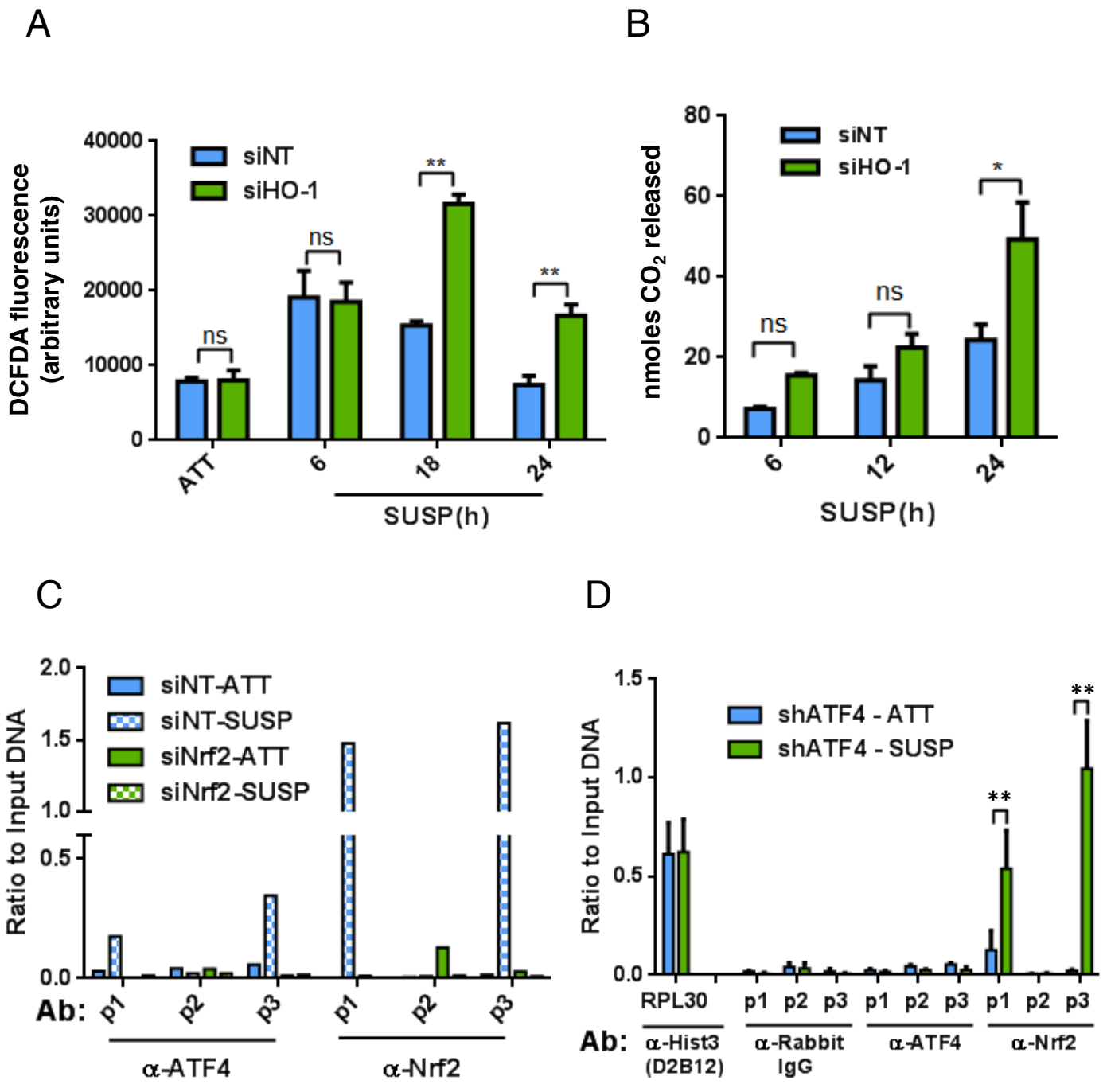
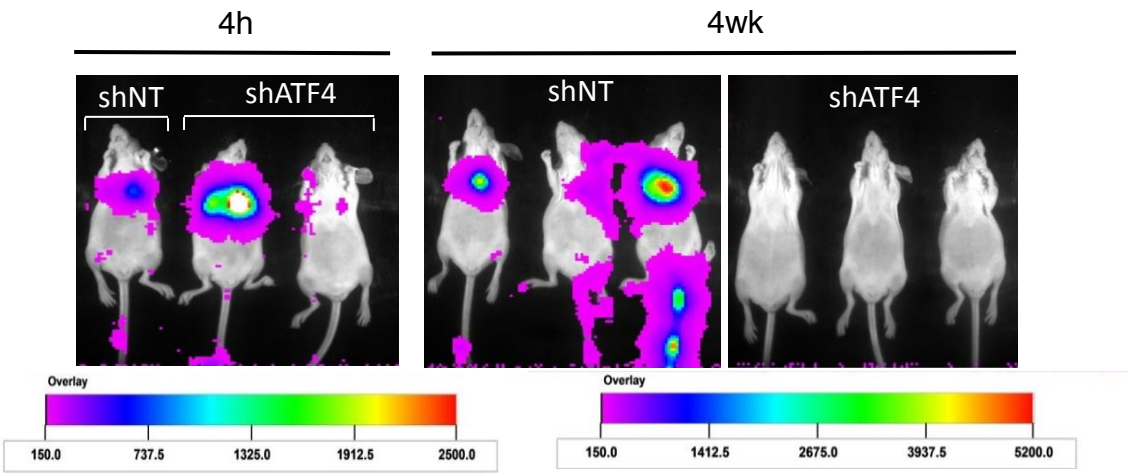
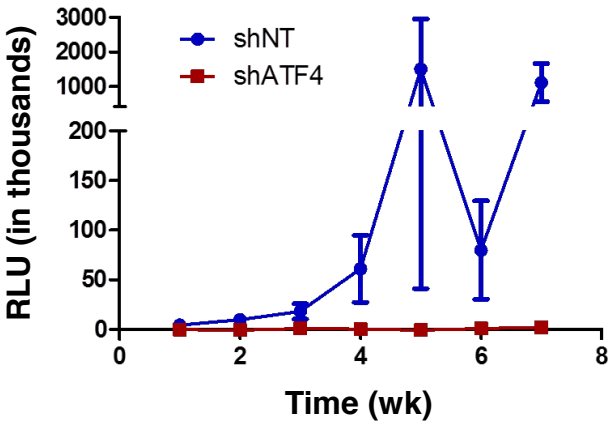


Figure S7. HO-1 ameliorates oxidative stress during matrix detachment and its promoter is bound by ATF4 and Nrf2. (A) DCFDA fluorescence levels were measured in siNT and siNrf2 .HT1080 cells at indicated time points. (B) OPPC measurements in suspended HT1080 cells transfected with either non-targeting siRNA (siNT) or siHO-1. Reactions were stopped at 4, 12 and 24 h and nmoles CO_2 were measured. Results of (A) and (B) are represented as mean of three independent experiments (n=3, mean ± S.D). Statistical significance is calculated by Student's *t test* (* $p<0.05$, ** $p<0.001$, ns-not significant). (C) ChIP analysis in HT1080 cells transfected with siRNA against Nrf2 (siNrf2) or non targeting control (siNT) grown in suspension conditions or attached. (D) ChIP analysis in shATF4.HT1080 cells grown in attached and suspension conditions. Immunoprecipitations in both (C) and (D) were performed with ATF4 and Nrf2 antibodies and fold enrichment was measured by primer sets *p1, p2, p3* encompassing the AREs. (**p<.001). Result in (D) is represented as mean of 2 independent experiment (n=2, mean ± S.D). Statistical significance was calculated by Student's *t* test (** p<0.001)

A



B



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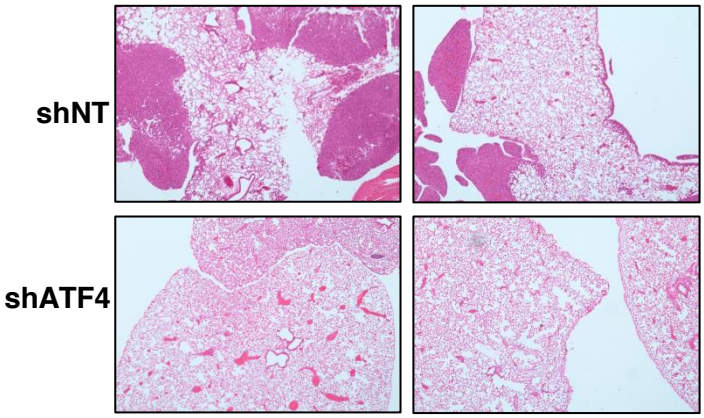
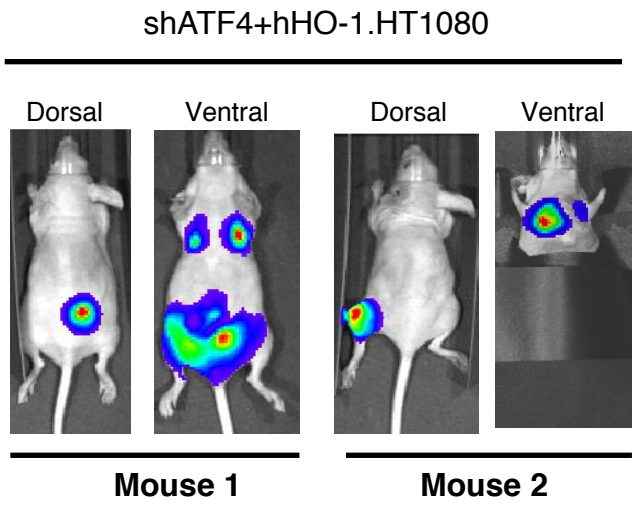


D

Cells	Fraction of mice with lung colonies
shNT	6/7
shATF4	0/11

Figure S8. Ablation of ATF4 prevents HT1080 fibrosarcoma lung colonization. (A) Bioluminescent images at 4h and 4 weeks post-injection of nude female mice injected intravenously with shNT or shATF4.HT1080 cells. Representative mouse from each group is shown. Signal intensity scale applies to all mice. (B) The average bioluminescent signal of the chest/lung area of mice injected intravenously with shNT or shATF4.HT1080 is plotted against time. Lines represent the mean signal from mice injected with shNT (n=6) or shATF4 (n=11) cells (mean ± S.E.M)(C) H&E staining of 2 lungs obtained from either shNT or shATF4.HT1080 cells injected mice. (D) Table summarizing the results from all tail vein injection experiments with the shNT and shATF4.HT1080 cells. The fractions represent the number of mice with bioluminescent signal in the chest over the total number of mice injected with that cell type.

A



B

Cells	Fraction of mice with lung colonies
shNT	4/5
shATF4	0/5
shATF4+hHO-1	7/10

C

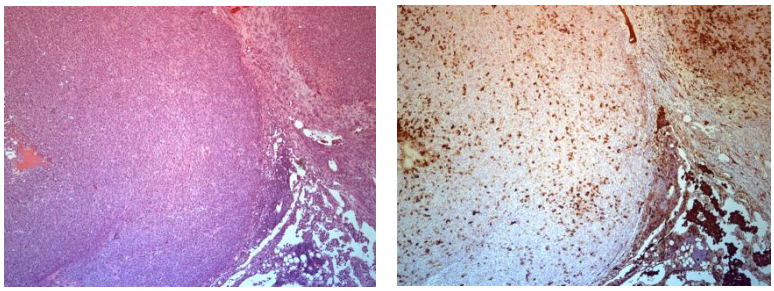


Figure S9. Expression of hHO-1 in ATF4 deficient cells partially rescues lung colonization and promotes metastasis. (A) Bioluminescence analysis from lungs (Ventral view) as well as secondary tumor sites (Dorsal view) in two representative shATF4+hHO-1.HT1080 cells injected mice. The tumor in the hindleg of the mouse 2 (with higher intensity) is covered to prevent masking of the signal from the lungs as well as to determine the signal only from thoracic region. The mean intensity obtained from the lungs is plotted in Figure 6D. (B) Fraction of mice treated with either shNT, shATF4 or shATF4+hHO-1.HT1080 cells that had lung colonies. (C) H&E staining and HO-1 immunohistochemistry staining of the secondary metastatic tumors from mouse injected with shATF4+hHO-1.HT1080 cells (10X magnification) .

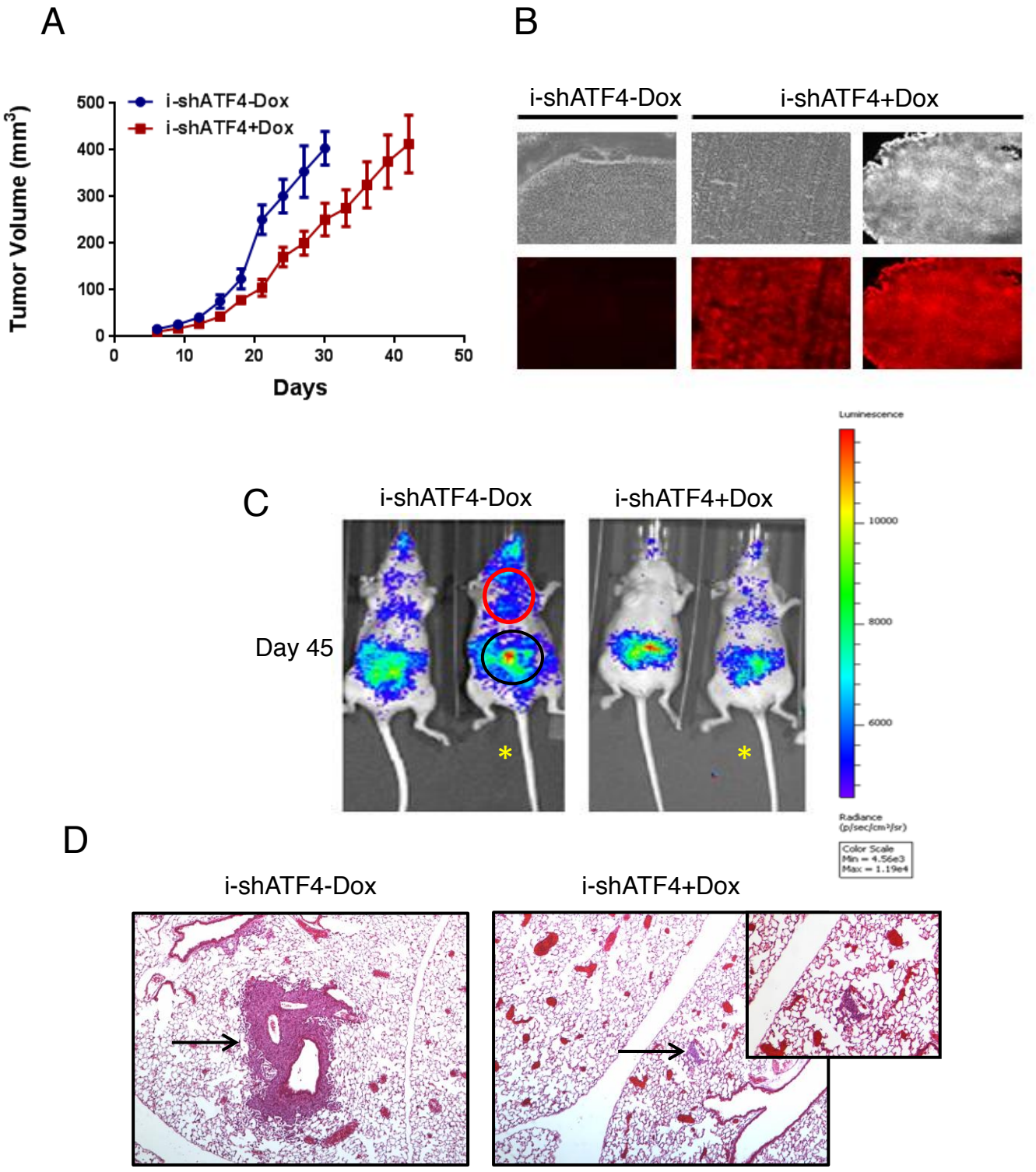


Figure S10. Effect of ATF4 on primary and metastatic tumor growth. (A) Mice were injected subcutaneously with i-shATF4.HT1080 cells. 48 h after tumor cell injection, mice were provided drinking water supplemented with1mg/ml doxycycline (+Dox) (n=8) or without (-Dox) (n=7) and mean tumor volumes were measured and plotted against time (days) (mean ± S.E.M). At about 400 mm^3 volume- tumors were excised to allow for metastasis to occur. (B) Representative phase contrast and fluorescence images of the tumors from mice from +Dox and –Dox group. Images are depicted at 10X magnification. (C) Bioluminescence images from +Dox and –Dox group of mice at day 45 after primary tumor removal. The red circle and the black circle signify the chest area and primary tumor injection site respectively. Signal intensity scale applies to all mice. (*) denotes the representative mouse from each group whose H&E stain of lung is shown in (D). Arrow denotes visible metastatic sites (4X magnification). Inset represents 10X magnification of the region indicated by the arrow.

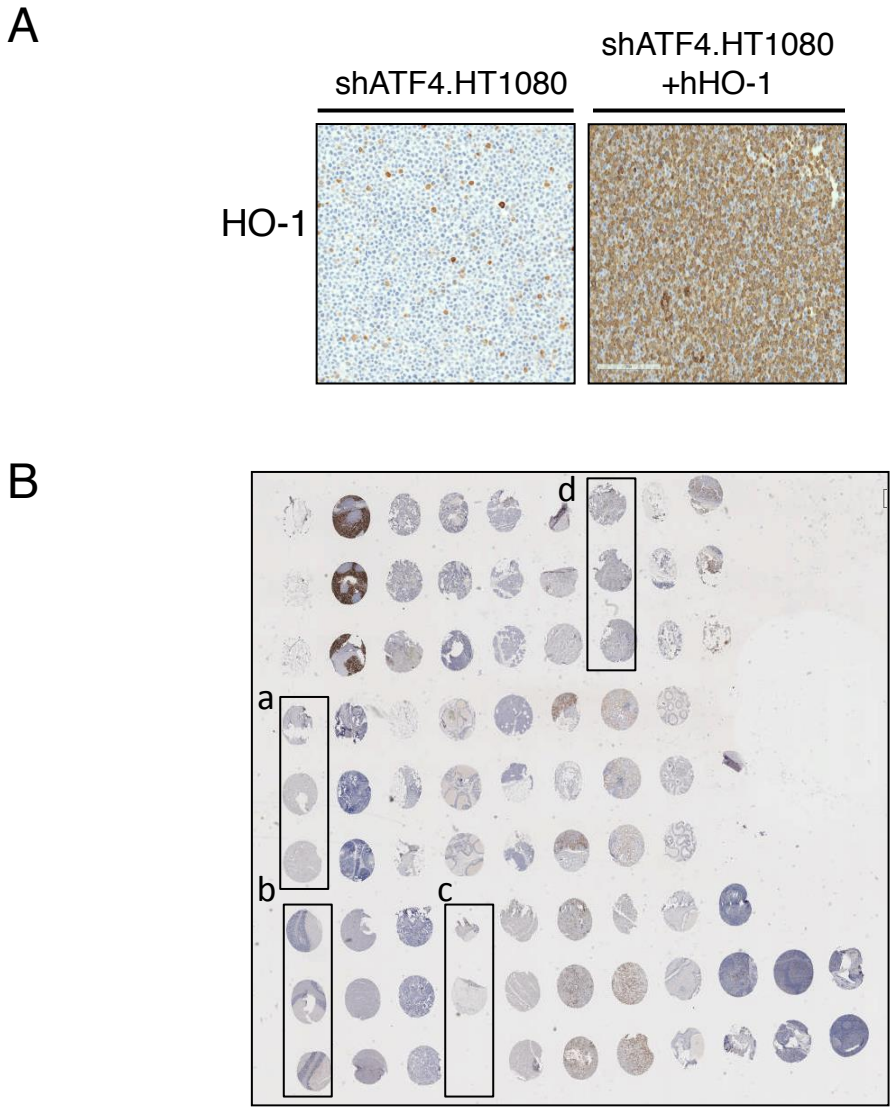


Figure S11. Immunohistochemical detection of HO-1 expression in human tissue samples. (A) Testing the specificity of the HO-1 antibody. shATF4.HT1080 cells expressing an empty vector (left) or human HO-1were collected, fixed and stained with the anti-hHO-1. The same antibody was used for staining human tissue samples shown in Fig. 7D, E. (B) Normal tissue microarray for analysis of HO-1 levels in various tissues including muscle (a), brain (b), breast (c) and lung (d). Positions of stained tissues are represented in tabular format in TMA (Table SII).

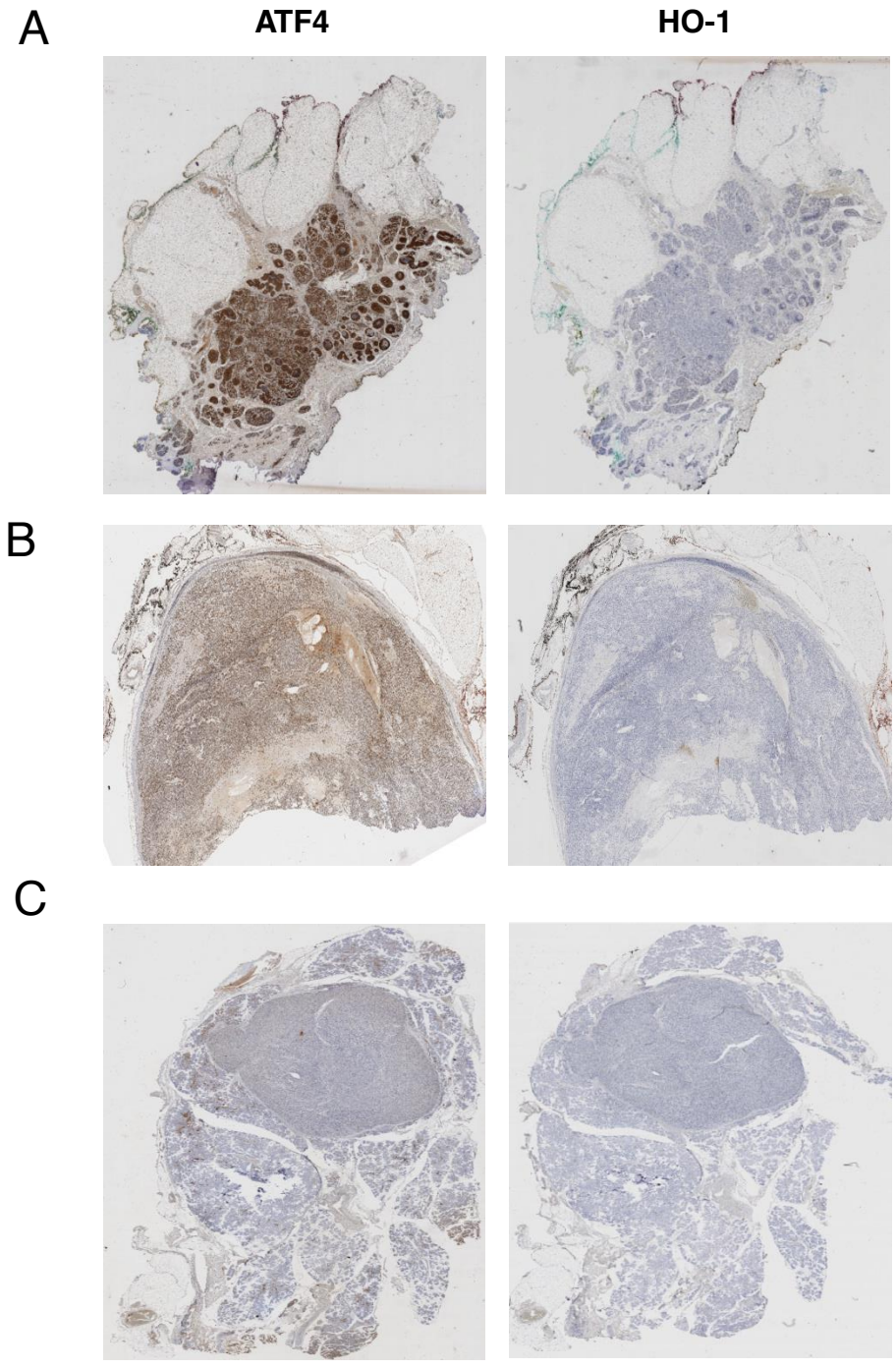


Figure S12. Immunohistochemical detection of ATF4 and HO-1 expression in human tumor samples. Immunohistochemical detection of ATF4 and HO-1 in serial tissue sections from (A) human primary breast tumor with no known positive metastatic lesion at the time of surgical removal, (B) metastatic undifferentiated sarcoma in brain and (C) leiomyosarcoma with no confirmed metastasis at time of surgical resection.

Table SI: List of RT-PCR primers

Transcript	Primer sequences (Forward and Reverse)
18S rRNA	5'-CAATTACAGGGCCTCGAAAG-3' 5'-AAACGGCTACCACATCCAAG-3'
ATF3	5'-TAGGCTGGAAGAGCCAAAGA-3' 5'-TTCTCACAGCTGCAAACACC-3'
CHOP	5'-GCGCATGAAGGAGAAAGAAC-3' 5'-CCAATTGTTCATGCTTGGTG-3'
ASNS	5'-TACAACCACAAGGCGCTACA-3' 5'- AAGGGCCTGACTCCSATAGGT-3'
ATG5	5'-AAAGATGTGCTTCGAGATGTGT-3' 5'-CACTTTGTCAGTTACCAACGTCA-3'
ATG7	5'-CAGTTTGCCCCTTTTAGTAGTGC-3' 5'- CCAGCCGATACTCGTTCAGC-3'
ULK1	5'-AGCACGATTTGGAGGTCGC-3' 5'-GCCACGATGTTTTCATGTTTCA-3'
HO-1	5'-GCAGAGAATGCTGAGTTCATG-3' 5'-CACATCTATGTGGCCCTGGAGGAGG-3'
XBP1s	5'- CCGCAGCAGGTGCAGG-3' 5'-GAGTCAATACCGCCAGAATCCA-3'
SOD-2	5'-GGAAGCCATCAAACGTGACTT-3' 5'-CCCGTTCCTTATTGAAACCAAGC-3'
GPX1	5'-CAGTCGGTGTATGCCTTCTCG-3' 5'-GAGGGACGCCACATTCTCG-3'
p1	5'-GCTGAGTCGCGATTTCCTCAT-3' 5'-GAGGCTTCTGCCGTTTTCTA-3'
p2	5'-GCAGGAAGTTGAAGCCAAAA-3' 5'-CAAGGAAACAGAGCCAAACA-3'
p3	5'-CCCTGCTGAGTAATCCTTTCC-3' 5'-TTAAACCTGGAGCAGCTGGA-3'

Table SII: Normal tissue microarray used for determining HO-1 levels

	1	2	3	4	5	6	7	8	9	10	11
1	Adipose	Spleen	Placenta	Parathyroid	Salivary	Esophagus	Lung	Colon	Stomach		
2	Adipose	Spleen	Placenta	Parathyroid	Salivary	Esophagus	Lung	Colon	Stomach		
3	Adipose	Spleen	Placenta	Parathyroid	Salivary	Esophagus	Lung	Colon	Stomach		
4	Muscle	Thymus	Endometrium	Thyroid	Pancreas	Appendix	Kidney	Testes	Cervix		
5	Muscle	Thymus	Endometrium	Thyroid	Pancreas	Appendix	Kidney	Testes	Cervix		
6	Muscle	Thymus	Endometrium	Thyroid	Pancreas	Appendix	Kidney	Testes	Cervix		
7	Cerebellum	Spinal	Ovary	Breast	Heart	Liver	Adrenal	Prostate	Lymph		
8	Cerebellum	Spinal	Ovary	Breast	Heart	Liver	Adrenal	Prostate	Lymph	Tonsil	Tonsil
9	Cerebellum	Spinal	Ovary	Breast	Heart	Liver	Adrenal	Prostate	Lymph	Tonsil	Tonsil
	1	2	3	4	5	6	7	8	9	10	11

Supplementary Methods and materials:

Scratch assay

shNT and shATF4 HT1080 cells were seeded onto 6-well plates that had previously been coated with fibronectin (10µg/ml for 2h at 37°C). Once cells were confluent, two scratches per well were made and then phase images of each scratch using the 10x objective were immediately captured on a Nikon Eclipse TE2000-U (Tokyo, Japan) with Image-Pro Plus 6.0 software (MediaCybernetics, Rockville, MD). Images were taken every 2h at the reference etching until the scratches were closed. To analyze the migration, the area of the open scratch (not covered by cells) was determined for each time point using Image J software (National Institutes of Health, Bethesda, MD). The scratch area at each time was subtracted from the original scratch area to determine the change in area covered by the cells with time.

Transwell migration assay

BD Falcon™ FluoroBlok™ individual transwell cell culture inserts with an 8µm pore size were used for this assay. Cells were seeded into the upper wells in media without FBS. Media with 10% FBS was added to the lower chamber as the chemoattractant. Transwell plates were incubated at 37°C for the indicated length of time, and then migrated cells were fixed with methanol and stained with DAPI. For each well, the 20x objective of the Nikon Eclipse TE2000-U and Image-Pro plus 6.0software were used to take six random images of the migrated cells. The number of nuclei in each image was counted and the total number of cells from all six images was determined for each well.

Transwell invasion assay

Cell Invasion was measured by BD BioCoat™ FluoroBlok™ Invasion System (BD#354165) as per manufacturer's protocol. HT1080 cells (shNT and shATF4 cl3, cl4) were plated at a density of $5X10^5$ cells in each of 24 well Transwell matrigel plate and were incubated at 37°C for 8 h at O_2 concentration of 0.5%. At the end of the experiment cells were fixed and stained with DAPI. Similarly the number of nuclei in each image was counted and the total number of cells from all six images was determined for each well.

Hypoxia treatments

Cells were subjected to hypoxia (0.5%) using the $InVivo_2$ 400® (Russkin Technologies, Bridgend, UK) chamber.