Description of Supplementary Files

Title: Supplementary Information

Description: Supplementary Figures and Supplementary Tables
Supplementary Figure 1. Reducing ATRX suppresses CDK4i and doxorubicin induced senescence in LS8817 cells. LS8817<sup>scr</sup> and LS8817<sup>shATRX</sup> cells were generated as described in the legend to Figure 1, but this time using a different shRNA targeting ATRX (shX588). The cells were treated with either 1μM PD0332991 or 100nM doxorubicin for seven days. (A) Knockdown was confirmed by immunofluorescence. Representative images are shown (left) and foci were quantified (right). (B-F) The accumulation of cyclin A was measured by immunoblot (B), accumulation of SA-β-gal (C) and SAHF (D) by microscopy and immunofluorescence, and accumulation of SASP (E) and HRAS (F) mRNAs by qPCR. Representative SAHF images are shown in panel D. All data are represented as mean ± SEM from at least 3 independent experiments. * indicates p < 0.05 using a two-sided Students t-test. Scale bars indicate 20 microns.
Supplementary Figure 2. ATRX is required for senescence induced by MDM2 knockdown. The LS8817
scr and LS8817
shATRX cells (left) were described in Figure 1. Similarly generated LS8313
scr and LS8313
shATRX cells (right) were prepared. (A-B) These cells were transduced with an shRNA vector targeting MDM2 and the effect on protein expression (A) and accumulation of SA-β-gal (B) were determined. ATRX was detected with the D-5 antibody. All data are represented as mean ± SEM from at least 3 independent experiments. * indicates p < 0.05 using a two-sided Students t-test.
Supplementary Figure 3. ATRX facilitates senescence in response to CDK4 inhibition in the ATRX-deficient cell line U2OS. U2OS cells were stably transfected with wild type ATRX as described in the legend to Figure 3. (A) The expression of cyclin A and phosphorylated Rb before and after drug treatment in parental U2OS cells and U2OS cells expressing ATRX was determined by immunoblot. (B-C) The accumulation of SA-β-gal (B) and SAHF (C) were measured in each condition. Representative SAHF images are shown in panel C. (D) Clonogenic growth was measured by crystal violet staining three weeks after the removal of the drug and replating the cells at low density in the absence of drug. Representative images are shown (left) and colony number is quantified (right). (E) Telomere restriction fragment length assay was performed on 1μg of genomic DNA in the indicated cell lines. All data are represented as mean ± SEM from at least 3 independent experiments. * indicates $p < 0.05$ using a two-sided Students t-test. Scale bar indicates 20 microns.
Supplementary Figure 4. The number of ATRX foci increases in senescent but not quiescent LS8817 cells. (A) ATRX immunofluorescence was carried out in LS8817 cells in which senescence was induced by MDM2 knockdown as described in the legend to Supplementary Figure 2. Representative images are shown below. (B) LS8817<sup>scr</sup> and LS8817<sup>shATRX</sup> were treated with doxorubicin as described in the legend to Figure 1 and ATRX foci detected by immunofluorescence, counted, and average number plotted. Representative images are shown below. (C) LS8817 cells were serum starved (0.5%) for 5 days. The proliferation of these cells was assessed by BrdU incorporation. Senescence was assessed by the accumulation of SA-β-gal positive cells. The number of ATRX foci as measured as described previously. All data are represented as mean ± SEM from at least 3 independent experiments. * indicates p < 0.05 using a two-sided Students t-test. Scale bars indicate 20 microns.
Supplementary Figure 5. ATRX foci accumulate in different transformed cells that undergo CDK4i induced senescence but not during quiescence. (A) SNB19 glioma cells were transduced with targeting vectors as described in the legend to Figure 1 and subsequently treated with PD0332991 and the accumulation of ATRX foci (left) and SA-β-gal positive cells (right) was measured. Representative images of ATRX foci are shown (bottom). (B) Three lung cancer derived cell lines were treated with PD0332991 for 7 days and the accumulation of ATRX foci (left) and SA-β-gal positive cells (right) was determined. Representative images of ATRX foci are shown (bottom). (C) MCF7 breast cancer cells were treated with PD0332991 for 7 days (left) or serum starved (0.5% serum) for 5 days (right). The accumulation of ATRX foci and SA-β-gal was measured as described in panel A. Representative images of ATRX foci are shown (bottom). All data are represented as mean ± SEM from at least 3 independent experiments. * indicates $p < 0.05$ using a two-sided Students t-test. Scale bars indicate 20 microns.
Supplementary Figure 6. Loss of ATRX does not affect PML foci. (A) PML foci were stained in LS8817 cells treated with CDK4 inhibitor and the number of foci was plotted as described in the legend to Figure 2A. (B) The presence of PML foci was assessed in LS8817<sup>scr</sup> and LS8817<sup>shATRX</sup> cells after treatment with PD0332991 for seven days. Graph represents the fold-change in the number of foci per cell in PD0332991 treated cells vs. untreated controls. (C) LS8817 cells were treated as described in the diagram in Figure 4A. PML foci were detected and quantified as described previously. Representative images are shown (left) and the number of PML foci per cell is quantified (right). All data are represented as mean ± SEM from at least 3 independent experiments. * indicates p < 0.05 using a two-sided Students t-test. Scale bar indicates 20 microns.
Supplementary Figure 7. *HRAS* is repressed in a variety of different transformed cell lines undergoing senescence. The cells were treated as indicated and expression of *HRAS*, *ZNF107*, and *β-ACTIN* were measured as described. The outcome of treatment, vis a vis quiescence or senescence is indicated in the graph.
Supplementary Figure 8. Altering the level of HRAS expression regulates the transition from quiescence to senescence. LS8107 cells were treated with PD0332991 for seven days and then infected with lentiviral vectors targeting HRAS. PD treatment was maintained as viral selection occurred. (A) The efficacy of knockdown was assessed by qPCR. (B-E) The effect of reducing HRAS on the accumulation of SA-β-gal (B), SAHF (C), or ATRX foci (D) and the induction of SASP mRNAs as measured by qPCR (E) was determined as described. Representative SAHF images are shown in panel C. All data are represented as mean ± SEM from at least 3 independent experiments. * indicates p < 0.05 using a two-sided Students t-test. Scale bar indicates 20 microns.
Supplementary Figure 9. Reducing ATRX suppresses the SASP in response to CDK4 inhibition in LS8817 cells. ATRX was reduced in LS8817 cells as described in the legend to Figure 1 and cells were treated with PD0332991 for seven days (7D PD). (A) Conditioned media was collected and cytokine expression measured as described in the methods. Representative images are shown (left) and induction of secreted SASP factors is shown (right). (B) mRNA was harvested from the indicated LS8817 cells as described in the methods and the induction of expression of the indicated SASP factors was measured.
Supplementary Figure 10. Uncropped immunoblots. Blots from the corresponding figures were probed for the protein as indicated above.
Supplementary Table 1. Intergenic loci of senescence-specific ATRX peaks.

| Chrom | Start (chromosome) | End (chromosome) | Annotation |
|-------|--------------------|------------------|------------|
| 1     | 91925938           | 91925939         |            |
|       | 102018219          | 102018404        |            |
|       | 105412514          | 105412523        |            |
|       | 209498416          | 209498486        |            |
|       | 23938803           | 239389186        |            |
|       | 249240657          | 249240661        |            |
| 2     | 13501361           | 13501428         |            |
|       | 18476404           | 18476451         |            |
|       | 68677257           | 68677357         |            |
|       | 89830349           | 89830498         | Satellite repeat |
|       | 89842097           | 89842579         | Satellite repeat |
|       | 90372669           | 90373039         | Satellite repeat |
|       | 170324275          | 170324747        |            |
|       | 234696198          | 234696415        |            |
| 3     | 5280825            | 5281398          |            |
|       | 176015569          | 176015636        |            |
| 4     | 3783531            | 3783536          |            |
|       | 12668659           | 12668821         |            |
|       | 32841093           | 32841720         |            |
|       | 49093261           | 49093513         | Centromeric repeat |
|       | 49101046           | 49101397         | Centromeric repeat |
|       | 49152326           | 49152682         | Centromeric repeat |
|       | 49644961           | 49645328         | Centromeric repeat |
|       | 72550758           | 72550863         |            |
|       | 174406163          | 174406321        |            |
| 5     | 1871481            | 1871550          |            |
|       | 18762886           | 18763065         |            |
|       | 10347767           | 10347851         |            |
|       | 10347928           | 10347944         |            |
|       | 10861184           | 10861241         |            |
|       | 49941286           | 49941585         |            |
|       | 55302722           | 55303293         |            |
|       | 97567854           | 97567952         |            |
|       | 123085501          | 123085540        |            |
|       | 152076921          | 152077079        |            |
|       | 152077755          | 152077644        |            |
| 6     | 12167595           | 12167896         |            |
|       | 28557300           | 28557702         |            |
|       | 95950978           | 95950985         |            |
|       | 115389236          | 115389304        |            |
|       | 115389604          | 115389811        |            |
|       | 156028585          | 156028662        |            |
| 7     | 303448             | 303662           |            |
|       | 13410402           | 13410825         |            |
|       | 1368078            | 1368166          |            |
|       | 24470293           | 24470403         |            |
|       | 61753537           | 61753692         | Centromeric repeat |
|       | 61754739           | 61754758         | Centromeric repeat |
|       | 61755180           | 61755181         | Centromeric repeat |
|       | 61785764           | 61785943         | Centromeric repeat |
|       | 61976292           | 61976485         | Centromeric repeat |
|       | 64686706           | 64686764         |            |
|       | 64687462           | 64687553         |            |
|       | 64688394           | 64688449         |            |
|       | 67957852           | 67957855         |            |
|       | 89587945           | 89588409         |            |
|       | 109256914          | 109256962        |            |
|       | 112436507          | 112436597        |            |
|       | 112437364          | 112437777        |            |
|       | 152595074          | 152595085        |            |
| 8     | 580611             | 581274           |            |
|       | 123511634          | 123511942        |            |
|       | 144830890          | 144831040        |            |
| 10    | 4538086            | 45380839         |            |
|       | 35212938           | 35213434         |            |
|       | 35273735           | 35274491         |            |
| Chromosome | Start Position | End Position | Repeat Type |
|------------|----------------|--------------|-------------|
| Chromosome 12 | 28802780 | 28803520 | Satellite repeat |
| Chromosome 13 | 45998983 | 45999069 | Satellite repeat |
| Chromosome 14 | 19349311 | 19349576 | Satellite repeat |
| Chromosome 15 | 68132281 | 68132735 | Satellite repeat |
| Chromosome 16 | 14379872 | 14379886 | Satellite repeat |
| Chromosome 17 | 25265249 | 25265420 | Satellite repeat |
| Chromosome 18 | 25339273 | 25339486 | Satellite repeat |
| Chromosome 19 | 246216 | 246233 | TAR1 |
| Chromosome 22 | 18715549 | 18715619 | Satellite repeat |
| Chromosome X | 26102926 | 26102957 | Centromeric repeat |

The senescence-specific summits (of the 166) that were not associated with either gene bodies or promoter regions are listed by chromosomal location. Annotated ENCODE blacklist regions within the locus are noted.
**Supplementary Table 2.** List of primers used for DNA sequencing and qPCR

| Gene Symbol | Forward Primer | Reverse Primer |
|-------------|----------------|----------------|
| ARHGAP18    | 5'-ATCAAGAGGTGTTGTGTTGCAA-3' | 5'-ACAATGTCTTCTCAGTTGATCTC-3' |
| ATAD2       | 5'-GGAACACTCGTCACACAGG-3' | 5'-CGCGTGGTATCTCAGTTGATCTC-3' |
| CCNY        | 5'-CAGAGCAGGTGAGAGAAGAAA-3' | 5'-TGTTGAGCTGACTGCTATCA-3' |
| DDX9        | 5'-GCACAGTGGTGAACATCTCTAG-3' | 5'-ACTCAATGCAACGCTGTAG-3' |
| HRAS        | 5'-CAGATCAAACGGGTGTAAGGAC-3' | 5'-GCGTGAGTACGTTCCACAG-3' |
| KIF15       | 5'-CTGAAGGCTTACAGTTGCTGT-3' | 5'-CAGGAGGCTCAGATTTCAACAT-3' |
| PARD3       | 5'-GCCTTTTGCAGCTTACCAAGCA-3' | 5'-GGTCGACTGTCGCTCA-3' |
| SORCS3      | 5'-GAATGCGGACACTAAGAGAAA-3' | 5'-AGCAGCTGATGACTGCTGCT-3' |
| ZNF107      | 5'-TTTTTGGTGGCCCAAGACC-3' | 5'-GGTTGGGTTACTCAGTCAA-3' |
| NRAS        | 5'-TGATTTGCCCAACAGGAGGAC-3' | 5'-CCCTAGGTGCCCATCATCAT-3' |
| KRAS        | 5'-TACACTCTGCTGTAGGGGACCA-3' | 5'-TCCTAGGCTGTGTTGCT-3' |
| CTNN2       | 5'-CCGGAGTGGTACCTCAGATG-3' | 5'-CTACAGTGAGGACTGCTTAC-3' |
| HCN1        | 5'-CCAGCTGATATGGGCTGAA-3' | 5'-GGCATGAGCCACACAGTTT-3' |
| PCLB1       | 5'-CCTGCCGACACTTCCCCATT-3' | 5'-GGCTCCGACATCTGCAA-3' |
| RNF128      | 5'-ATGATCGGCAATCAGTTGAA-3' | 5'-GGTTTGAAGCTTCTCAGTCC-3' |
| ATRX        | 5'-GTAACAGAACCCACACAGG-3' | 5'-CATGAGGTGACCCACATGGG-3' |
| C20orf195   | 5'-CCCTGGTGTAGTCTCAGA-3' | 5'-CCCTGATGTAGAGACACTTA-3' |
| COL26A1     | 5'-GGCACTCTGCTTCACCAAGCA-3' | 5'-TGCGGAGAGGACACCTTTATTG-3' |
| DGK5        | 5'-GTGGCACATACCTCTCTCTCTG-3' | 5'-CCAGACAGGCAACAGCT-3' |
| GAS6-AS1    | 5'-CTGAGATGGTGCATTGACG-3' | 5'-CAGGAGGATTAGCTGTAAGAC-3' |
| HIBADH      | 5'-TGCTGCCACGATTATACAGATG-3' | 5'-GCGAGATGAATGCTGGAATC-3' |
| HRH1        | 5'-TGATGGTACCTCTGTGTTGC-3' | 5'-AGGATGATCTGAGCATGAGCA-3' |
| SIN3B       | 5'-AAGACGGCTCCTACCTATCTTG-3' | 5'-GGGAGAAAGGAGTGTGAACTC-3' |
| TRPS1       | 5'-GACTGCAATAACCCGACAG-3' | 5'-CCTGACATGAGATGTTCCATT-3' |
| IL6         | 5'-ACTCACCTTCCTACAGGAAAT-3' | 5'-CCATCTTTGGAAGGTTCCAGTTG-3' |
| IL8         | 5'-ACTGAGATGATGAGATGAGGAC-3' | 5'-AACCTCTGACACACAGTGGTTCC-3' |
| GMCSF       | 5'-CCTGAACTGAGTAGAGACAC-3' | 5'-TGCTGCTTGATGAGCTGTTG-3' |
| CXCL1       | 5'-AGGGATTTCCACCCCAAGAC-3' | 5'-TGTTCAAGATCTTTCTGATGA-3' |
| CEBPa       | 5'-CACCTGACCACTCAGCATTG-3' | 5'-CCCGTACTGGTCTGTTCT-3' |
| PPARγ       | 5'-ACCAAGAAGTCGAACTCAAGTG-3' | 5'-ATGAGGGAGTGGAAAGGCTCT-3' |
| FABP4       | 5'-ACTCGGTCAGAATTGACAGC-3' | 5'-CTCGGGAAGGCTTCCATT-3' |
| ATRX ADD DOMAIN | 5'-CAGAGCTTGGCCCAAAAGTGG-3' | 5'-CAATGCAAGATGACCTTC-3' |
| ATRX HP1 BINDING DOMAIN | 5'-CCAGCTGGTGAACATCTCAAC-3' | 5'-TTTGTGGTGCTGCGCTC-3' |
| ATRX HELICASE 1 DOMAIN | 5'-AAGATGCTTCACCCCAAGAC-3' | 5'-GCTGCAACACACAGGATGA-3' |
| ATRX HELICASE 2 DOMAIN | 5'-CAGATGCTGATGCTGAGTT-3' | 5'-TGTTCCAGATTGGTATGTTG-3' |
| HRAS        | 5'-TGCTCCAAATAATGTTCTG-3' | 5'-TGAGGAGGATTAGGCGGATA-3' |
| ZNF107      | 5'-CCAACACTCAAACTTAATACCA-3' | 5'-GGTTTGAAGACTGGCTAAAAGC-3' |

**mRNA expression**
**sequence confirmation**
**ChiP-qPCR**
