Supplemental Figure Legends

Supplemental Figure 1. (*A*) Bright-field light microscopy at both 4x and 10x demonstrates that virtually all cells that have been treated with a 4-hydroxytamoxifen-inducible HRasV12 oncogene undergo OIS and uniformly display positive blue staining for senescence-associated β-galactosidase (SA-β-gal) in comparison to vehicle-treated control proliferating cells (CTL). (*B*) Quantification of SA-β-gal staining demonstrates significant increases in staining with oncogenic Ras induction and OIS. (*C*) RNA-seq performed on two biological replicates demonstrates that OIS cells display the expected upregulation of tumor suppressor *CDKN2A*, as well as downregulation of *LMNB1* and cyclin gene, *CDK2*. (*D*) MLL1 KD OIS cells treated with MLL1 shRNA express significantly less MLL1 than SC OIS cells at both the mRNA (*D*) and protein (*E*) levels. (*F*) A second MLL1 shRNA similarly decreases expression of SASP genes. (*G*) Top 6 categories resulting from a Gene Ontology (GO) Analysis of the 224 genes that both increase at least 1.5 fold in OIS from control cells (SC OIS/SC) as well as decrease at least 1.5 fold from OIS to MLL1 KD OIS (-(SC OIS/MLL1 KD OIS)) shows that the categories associated with SASP genes are highly represented (i.e. “cytokine activity”, “cytokine receptor binding”, “chemokine activity”, and “growth factor activity”). (*H*) Examining the top 20 most upregulated SASP genes in BRAF-induced melanocyte OIS by RNA-seq, we found a similar profile of the most highly upregulated SASP genes as compared to the senescent fibroblasts, including *IL1B* which was again the most upregulated of all genes. Log values of the fold change (BRAF/CTL) are reported here so that they may be compared on a similar scale. (*I*) Venn diagram depicting a significant overlap between the top 1% most expressed genes in both fibroblast HRAS-induced OIS

and melanocyte BRAF-induced OIS (P=0.05, Fisher exact test). (*J*) MLL1 shRNA inhibits *MLL1* and SASP gene (*IL1A*, *ILB*, and *IL8*) expression in melanocytes undergoing OIS via a doxycycline-inducible form of *BRAFV600E* (diBRAF).

Supplemental Figure 2. (*A*) Representative images from SC OIS and MLL1 KD OIS (*B*) cells from the conditioned media proliferation assay demonstrating how the images were analyzed in an unbiased fashion by employing image analysis software (see Materials and Methods for details). (*C*) Quantification of these images was used to calculate the fold change between Day 1 and Day 4 for each condition and demonstrates that MLL1 KD OIS conditioned media does not enhance the proliferation of MCF7 cancer cells as compared to SC OIS cells. (*D*) IF (60x) of tumor sections also demonstrates reduced expression of IL1α (green) and IL6 (red) in tumors derived from the MLL1 shRNA-treated MCF7 cells as compared to tumors derived from SC shRNA-treated MCF7 cells. All images were taken with the same exposure time at 60x.

Supplemental Figure 3. (*A*) Examination of 1,215 human breast cancer patient samples from the TCGA demonstrates that the highest 1/3rd of *MLL1*-expressing tumors have significantly higher SASP expression (*IL1A*, *IL8*, *SERPINB2* shown here) than tumors in the lowest 1/3rd of *MLL1* expression. In contrast, housekeeping genes *ACTB*, *GAPDH*, and *LMNA* do not demonstrate the same trends. (*B*) Examination of 515 human prostate cancer patient samples from the TCGA demonstrates that the highest 1/3rd of *MLL1*-expressing tumors have significantly higher SASP expression (*IL1A*, *IL1B*, *IL8*, *SERPINB2* shown here, and *IL6* was not significant) than tumors in the lowest 1/3rd of *MLL1* expression. In contrast, housekeeping genes *ACTB*, *GAPDH*, and *LMNA* do not demonstrate the same trends.

Supplemental Figure 4. (*A*) Large increases in both H3K4me3 and γH2A.X over upregulated SASP gene *IL1B*. (*B*) Plot of the top 1% most highly expressed genes in OIS (as measured by RNA-seq fold change on x-axis) demonstrates that the most highly expressed SASP genes likewise display extensive increases in both gene body enrichment of γH2A.X (y-axis) as well as promoter/TSS increases in H3K4me3 (red color). (*C*) IF demonstrates decreased levels of ATM (phospho S1981) as measured by positively staining nuclear ATM puncta (red) in MLL1 KD OIS cells in comparison to SC OIS cells. All images were taken with the same exposure time at 40x. (*D*) Treatment of IMR90 fibroblasts undergoing OIS demonstrates that treatment with the inhibitor of ATM (phosphor S1981), KU55933 (10uM), decreases expression of the SASP.

Supplemental Figure 5. (*A*) Plot of positive Pearson correlation between *MLL1* (*KMT2A*) and *ATM* co-expression by RNA-seq across 24 human cancers demonstrates positive correlation between *MLL1* and *ATM* across all cancers for which there is RNA-seq expression data from TCGA database. (*B*) MLL1 mRNA expression plotted against ATM mRNA expression as measured by RNA-seq in breast cancer (Pearson correlation 0.77, Spearman correlation 0.81). Blue dots indicate tumors without any *ATM* or *MLL1* mutations, pink dots indicate tumors with *MLL1* mutations, gold dots indicate tumors with *ATM* mutations, and red dots indicate that both *ATM* and *MLL1* are mutated in that tumor. Notably, mutational status appears to have no effect on the expression correlation between the two genes. (*C*) Distribution of pairwise correlation of all genes in the genome across all tested human cancers demonstrates that the level of co-expression of *MLL1* with *ATM* is highly significant amongst all other genes tested.

Supplementary Figure 6. (*A*) GO Analysis of the 500 genes that lose the most H3K4me3 enrichment by ChIP-seq with MLL1 KD in OIS demonstrates numerous categories that contain the same pro-proliferative cell cycle genes. (*B*) H3K4me3 ChIP-seq delta track (MLL KD OIS/SC OIS) displays extensive losses of promoter H3K4me3 over critical pro-proliferative cell cycle genes such as *CDK1* (*B*), *CCNB1* (*C*), *TOP2A* (*D*), *CHEK1* (*E*), PLK1 (*F*), and CDC20 (*G*). (*H*) GO Analysis of the 500 genes that lose the most expression by RNA-seq with MLL1 KD in control proliferating IMR90 cells (MLL1 KD/SC) demonstrates that the similar categories containing pro-proliferative cell cycle regulators and cancer target genes are preferentially enriched as in (*A*). (*I*) GFP transfection into IMR90 fibroblasts in control (*I*) and OIS (*J*) demonstrates that transient transfections can successfully transfect the majority of cells.

Supplementary Table 1. 224 genes that are the most dynamically changing genes in OIS, with and without MLL1 KD. All genes increase at least 1.5-fold as measured by RNA-seq from SC to SC OIS, and then decrease at least 1.5-fold from SC OIS to MLL1 KD OIS.