Tak et al. Supplementary Material

Supplementary Figure Legends

Additional File 1. Guide RNA sequences. Shown are the sizes of the deleted regions, the genomic locations of the enhancers, and the target DNA for the guide RNAs. Two different sets of guide RNAs were used to delete E7 in HCT116 vs. HEK293 cells.

Additional File 2. Confirmation of enhancer deletions. The regions corresponding to the deleted sequences for each enhancer are indicated as bars; the different colors refer to different pairs of guide RNAs used. There are 3 copies of E7 in HCT116 cells and thus there are 3 bars shown for each clone; the other enhancers are in diploid regions of the genome. **(Panel A)** deletion of all E7 alleles was confirmed by PCR (data not shown) and the loss of the enhancer was confirmed through ChIP-seq; (**Panels B and C**) deletion was confirmed for 18qE and 18qNE by PCR using primers flanking each enhancer and internal to each enhancer; **(Panel D)** deletion was confirmed for E7 in HEK293 by PCR.

Additional File 3. List of datasets. Shown is information relevant to the RNA-seq, ChIP-seq, and 4C-seq experiments performed for this study.

Additional File 4. PCA plots of RNA-seq data. Shown are principal components PC1 (x-axis) and PC2 (y-axis) for RNA-seq datasets for **(A)** control vs E7-deleted HCT116 cells**, (B)** 18qNE-deleted HCT116 cells**, (C)** 18qE-deleted HCT116 cells**,** and **(D)** E7-deleted HEK293 cells.

Additional File 5. Genes deregulated upon enhancer deletion. The different worksheets show the genes upregulated or downregulated upon enhancer deletion, as compared to expression levels in control clones (selected after transfection with Cas9-GFP plus gRNA empty vector).

Additional File 6. H3K27Ac and RNA-seq profiles for genes deregulated in E7-deleted cells. Shown is an analysis of genes downregulated upon E7 deletion (A) or upregulated upon E7 deletion (B). For each set of analyses panel 1 shows which genes downregulated or upregulated greater than 1.5-fold have decreased or increased peaks within +/- 100Kb from their TSS; panels 2 and 3 show the H3K27Ac tracks and RNA-seq data in the control and deleted cells for specific genes.

Additional File 7. H3K27Ac peak analysis in control vs. E7- and E24-deleted cells. A list of statistically significant decreased or increased H3K27Ac peaks in the E7- or E24-deleted cells is provided.

Additional File 8. Cell culture assays (A) Replicates of colony forming assays in control and E7-deleted HCT116 cells. **(B)** Photographs of HCT116 vs E7-deleted cells showing that cells was gained a more fibroblast-like morphology upon deletion of E7 in HCT116 cells. **(C)** Replicates of colony forming assays in control and E7-deleted HEK293 cells.

Additional File 9. The MYC pathway is affected by deletion of enhancer 7. The MYC pathway network diagram was created using IPA (QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity). Downregulated genes in the E7-deleted cells with fold changes greater than 1.5 were used for the analysis. A core analysis was run with parameters considering downstream direct relationships with MYC which have been previously experimentally observed**.** The cellular location in which the proteins are located and the function of the genes are also shown; the intensity of color for each molecule is correlated with the fold change. A) 61 genes that are downregulated at least 1.5 fold in E7-deleted HCT116 cells were identified as downstream of MYC B) 39 genes that are downregulated at least 1.5 fold in E7-deleted HEK293 cells were identified as downstream of MYC.