

## **Supplementary Data Legends**

### **Supplementary Data S1:**

**Supplementary Data S1A.** Results of EV2 and Royal chromatin regulatory domain CRISPR screens in RMS and non-RMS cells. Depletion values represent the  $\log_2(\text{Fold Change})$  in guide RNA abundance at Day 12 vs. Day 3 for each RMS cell line as depicted in **Figure 1B**. Depletion values are averaged across multiple guides for each targeted domain. Depletion values in non-RMS cell lines represent the average across multiple control cell lines included in this study.

**Supplementary Data S1B.** Results of transcription factor CRISPR screen in RMS and non-RMS cells. Depletion values represent the  $\log_2(\text{Fold Change})$  in guide RNA abundance at Day 12 vs. Day 3 for each RMS cell line as depicted in **Figure 1B**. Depletion values are averaged across multiple guides for each targeted protein. Depletion values in non-RMS cell lines represent the average across multiple control cell lines included in this study.

**Supplementary Data S1C.** Raw read counts for EV2 and Royal chromatin regulatory domain CRISPR screens in RMS cells.

### **Supplementary Data S2:**

**Supplementary Data S2A.** Results of N-terminal PAX3-FOXO1 BioID experiments conducted in HEK293T cells. Results of MaxQuant analysis applying moderated t-test (for detailed information refer to methods section) are shown with key parameters including p-values and  $\log_2$  FC values (complementing Figure 2a). Mass spectrometry was performed on Strep-IP samples after N-terminal BirA tagged PAX3-FOXO1 and BirA only overexpression of 4 biological replicates.

**Supplementary Data S2B.** Results of C-terminal PAX3-FOXO1 BioID experiments conducted in HEK293T cells. Results of MaxQuant analysis applying moderated t-test (for detailed information refer to methods section).are shown with key parameters including p-values and  $\log_2$  FC values (complementing Figure 2b). Mass spectrometry was performed on Strep-IP samples after N-terminal BirA tagged PAX3-FOXO1 and BirA only overexpression of 4 biological replicates.

### **Supplementary Data S3:**

**Supplementary Data S3A.** Proteomic characterization of fraction 16 collected from size-exclusion chromatography (SEC) of ammonium sulfate precipitated nuclear extracts from RH30 cells prior to immunoprecipitation. Peptide-spectrum match (PSM) values are provided for each identified protein.

**Supplementary Data S3B.** Proteomic characterization of fraction 17 collected from size exclusion chromatography (SEC) of ammonium sulfate precipitated nuclear extracts from RH30 cells prior to immunoprecipitation.

**Supplementary Data S3C.** Proteomic characterization of fraction 18 collected from size exclusion chromatography (SEC) of ammonium sulfate precipitated nuclear extracts from RH30 cells prior to immunoprecipitation.

**Supplementary Data S3D.** Proteomic characterization of fraction 16 collected from size exclusion chromatography (SEC) of ammonium sulfate precipitated nuclear extracts from RH4-P3F-FLAG cells prior to immunoprecipitation.

**Supplementary Data S3E.** Proteomic characterization of fraction 17 collected from size exclusion chromatography (SEC) of ammonium sulfate precipitated nuclear extracts from RH4-P3F-FLAG cells prior to immunoprecipitation.

**Supplementary Data S3F.** Proteomic characterization of fraction 18 collected from size exclusion chromatography (SEC) of ammonium sulfate precipitated nuclear extracts from RH4-P3F-FLAG cells prior to immunoprecipitation.

**Supplementary Data S4:**

**Supplementary Data S4A.** Proteins enriched by BRG1 immunoprecipitation of size-exclusion chromatography (SEC) fractions 16-18 from RH30 nuclear extracts. First biological replicate.

**Supplementary Data S4B.** Proteins enriched by BRG1 immunoprecipitation of SEC fractions 16-18 from RH30 nuclear extracts. Second biological replicate.

**Supplementary Data S4C.** Proteins enriched by BRG1 immunoprecipitation of SEC fractions 16-18 from RH4-P3F-FLAG nuclear extracts.

**Supplementary Data S4D.** Proteins enriched by IgG immunoprecipitation of SEC fractions 16-18 from RH30 nuclear extracts.

**Supplementary Data S4E.** Proteins enriched by BRM immunoprecipitation of SEC fractions 16-18 from RH30 nuclear extracts.

**Supplementary Data S4F.** Proteins enriched by PBRM1 immunoprecipitation of SEC fractions 16-18 from RH30 nuclear extracts.

**Supplementary Data S4G.** Proteins enriched by PBRM1 immunoprecipitation of SEC fractions 16-18 from RH4-P3F-FLAG nuclear extracts.

**Supplementary Data S5:**

**Supplementary Data S5A.** Plasmids and guide RNA sequences used in this study.

**Supplementary Data S5B.** Antibodies used in this study.

**Supplementary Data S5C.** TaqMan gene expression reagents used in this study.

**Supplementary Data S5D.** ChIP-qPCR and BioID cloning primer sequences.

**Supplementary Data S5E.** Compounds used in this study.

**Supplementary Data S5F.** Buffers and reagents used in this study.

**Supplementary Data S5G.** Software and algorithms used in this study.