**Supplementary figure legends**

**Supp. Fig. 1.** Disruption of molecular subtype after KDM6A deletion. **(A)** Response of urothelial terminal differentiation marker expression in WT and *Kdm6a*- organoids after treatment with 100 nM ATRA. **(B)** Functional enrichment analysis of genes in *Kdm6a*- organoids that did not respond to 100 nM ATRA as expected. In WT organoids, these genes showed >1.5-fold change after treatment with 100 nM ATRA. **(C)** Basal marker expression changes after KDM6A deletion in luminal (RT4/K2 – left) and basal (SCaBER/B7 – right) subtype bladder cancer cells.

**Supp. Fig. 2.** Chromatin accessibility alterations after *KDM6A* deletion in luminal and basal subtype bladder cancer cells. **(A)** Venn diagram showing shared and private peaks among the three parental bladder cancer cell lines. **(B)** Motif analysis of accessible chromatin peaks enriched in SW780 parental (blue) and S5 knockout (red) cells. **(C)** Motif analysis of accessible chromatin peaks enriched in SCaBER parental (blue) and B7 knockout (red) cells. **(D)** Footprinting analysis at accessible chromatin peaks containing transcription factor motifs enriched in SW780 parental (blue) and S5 knockout (red) cells. Blue and red lines indicate Tn5 bias-corrected insertions in parental and knockout cells, respectively. **(E)** Footprinting analysis at accessible chromatin peaks containing transcription factor motifs enriched in SCaBER parental (blue) and B7 knockout (red) cells.

**Supp. Fig. 3**. Cell proliferation and chromatin accessibility in additional *KDM6A* knockout subclones. **(A)** Cell proliferation of parental (blue) and *KDM6A* knockout (red) bladder cancer cells *in vitro*. **(B)** Principal component analysis of ATAC-seq peaks demonstrating relationship among parental cells (blue) and *KDM6A* knockout subclones (red). **(C)** Motif analysis of accessible chromatin peaks enriched common to *KDM6A* knockout subclones.

**Supp. Fig. 4.** **(A)** Intersection between parental cell and *KDM6A*-wt patient ATAC-seq peaks (left) and between knockout cell and *KDM6A*-mut patient ATAC-seq peaks (right) **(B)** ATAC-seq signal of parental RT4, SW780, and SCaBER cells at *KDM6A*-wt patient peaks (left) and ATAC-seq signal of knockout K2, S5, and B7 cells at *KDM6A*-mut patient peaks (right).

**Supp. Fig. 5.** **(A)** Venn diagram showing shared and private ATAC-seq, H3K27ac ChIP-seq, and H3K4me3 ChIP-seq peaks in RT4 parental and K2 knockout cells. **(B)** Motif analysis of H3K27ac peaks enriched in RT4 parental (blue) and K2 knockout (red) cells.

**Supp. Fig. 6.** **(A)** Profiles and heatmaps showing ATAC signal (top) and H3K27ac ChIP-seq signal (bottom) at FOXA1 ChIP-seq private peaks in RT4 (left) and K2 (right) cells. **(B)** RT4 (blue) and K2 (red) cell proliferation after transduction with non-targeting (solid line) and FOXA1-targeting (dashed line) shRNA constructs, \* indicates p < 0.05.

**Supp. Fig. 7.** AP-1 activity assay in luminal subtype bladder cancer cells shows consistent ATF3 activation after KDM6A deletion. **(A)** AP-1 activity in RT4 parental and K2 knockout cells. **(B)** AP-1 activity in SW780 parental and S5 knockout cells.

**Supp. Fig. 8.** Relationship between genes that are activated by FOXA1 in RT4 parental cells and downregulated by ATF3 in K2 knockout cells. **(A)** Profiles and heatmaps showing ATAC signal (left) and H3K27ac signal (right) at K2 ATF3 private peaks in RT4 and K2 cells. **(B)** Diagram showing three epigenetic contexts for downregulated ATF3-bound genes in K2. Context 1 shows downregulated ATF3-bound genes in K2 that have complete loss of FOXA1 binding. Context 2 shows downregulated ATF3-bound genes in K2 that have partial loss of FOXA1 binding. Context 3 shows downregulated ATF3-bound genes in K2 that have no associated FOXA1 peaks in either RT4 parental or K2 knockout cells. **(C)** Heatmap showing FOXA1 ChIP-seq signal at Context 1 and 2 genes in RT4 parental and K2 knockout cells. **(D)** Genome browser view of ATAC-seq, H3K27ac, FOXA1, KDM6A, and ATF3 signals at *WNT5A* (non-canonical Wnt signaling, context 1), *CTSH* (lysosomal protein degradation, context 2), and *CDKN2C* (control of cell cycle progression, context 3) loci.