

Supplementary Materials for  
**Immune activation is essential for the antitumor activity of EZH2 inhibition  
in urothelial carcinoma**

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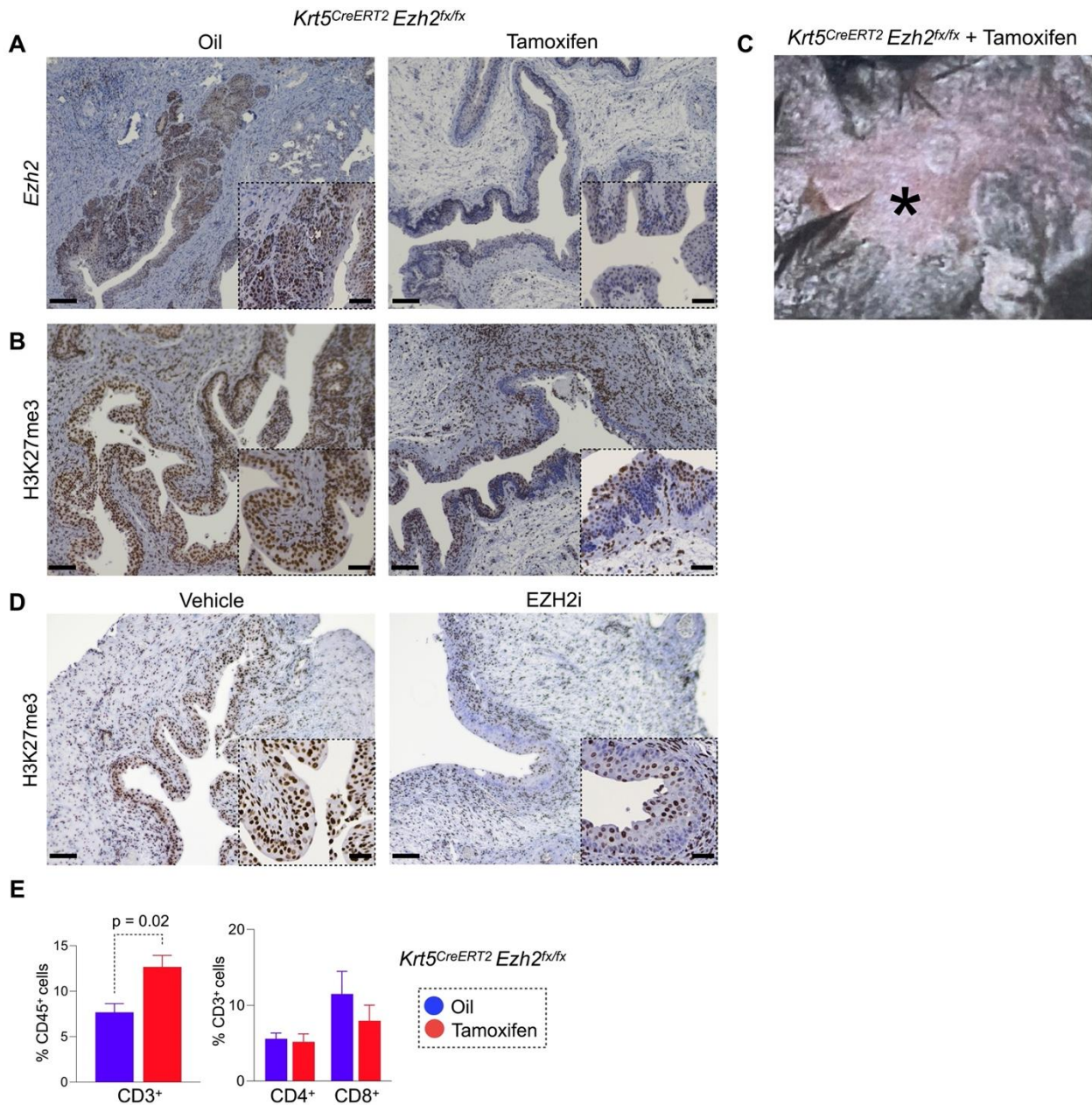
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**This PDF file includes:**

Figs. S1 to S4

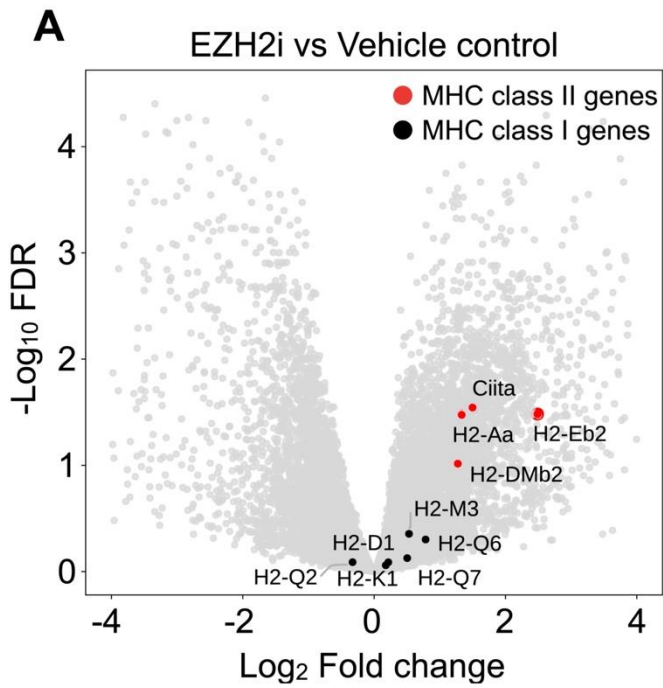
Supplementary Figures:

Fig. S1.



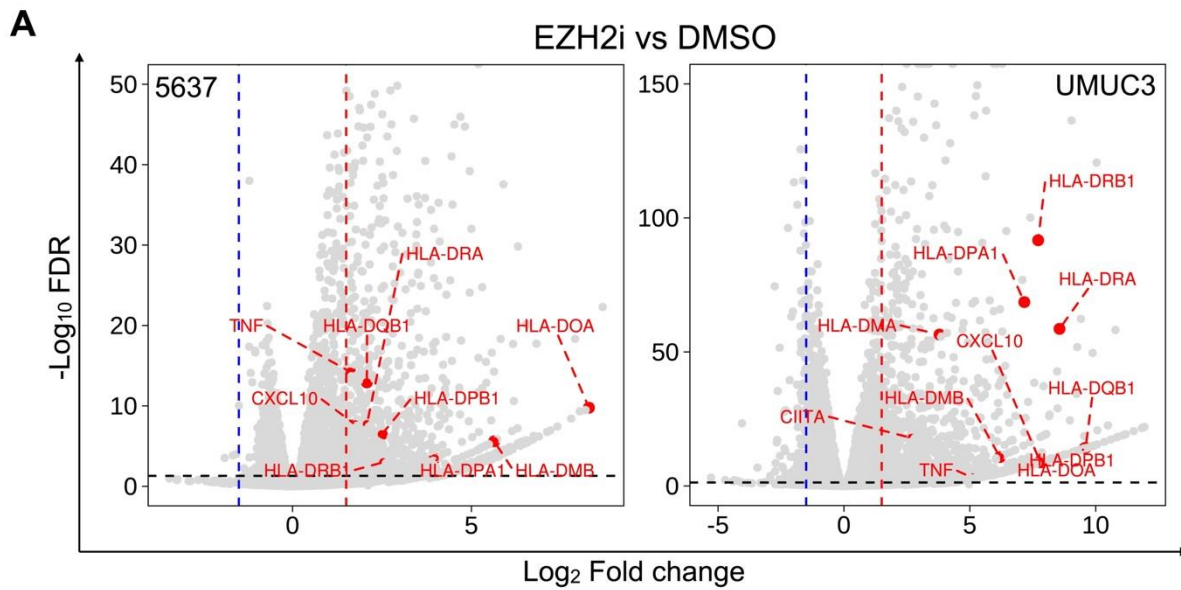
**Fig. S1: Characterization of *Krt5Cre-ER<sup>T2</sup>; Ezh2<sup>fx/fx</sup>* mice.** (A-B) Immunohistochemistry for A) EZH2 and B) H3K27me3 in *Krt5Cre-ER<sup>T2</sup>; Ezh2<sup>fx/fx</sup>* mice administered tamoxifen or Oil (control) post BBN (Scale bar: primary image 100μM, inset image 50 μM). C) Image of a skin section from a *Krt5Cre-ER<sup>T2</sup>; Ezh2<sup>fx/fx</sup>* mouse administered tamoxifen. [\*] highlights skin section with loss of hair follicles. D) Immunohistochemistry for H3K27me3 in BBN tumors from mice treated with EZH2i or vehicle (Scale bar: primary image 100μM, inset image 50 μM). E) Immune cell fractions in *Krt5Cre-ER<sup>T2</sup>; Ezh2<sup>fx/fx</sup>* mice administered tamoxifen or Oil (control) post BBN. Left: CD3<sup>+</sup> cells as a fraction of total CD45<sup>+</sup> immune cells. Right: CD4<sup>+</sup> and CD8<sup>+</sup> cells as a fraction of total CD3<sup>+</sup> cells. There is no statistical difference in the distribution of the CD4<sup>+</sup> or CD8<sup>+</sup> in the two groups.

Fig. S2.



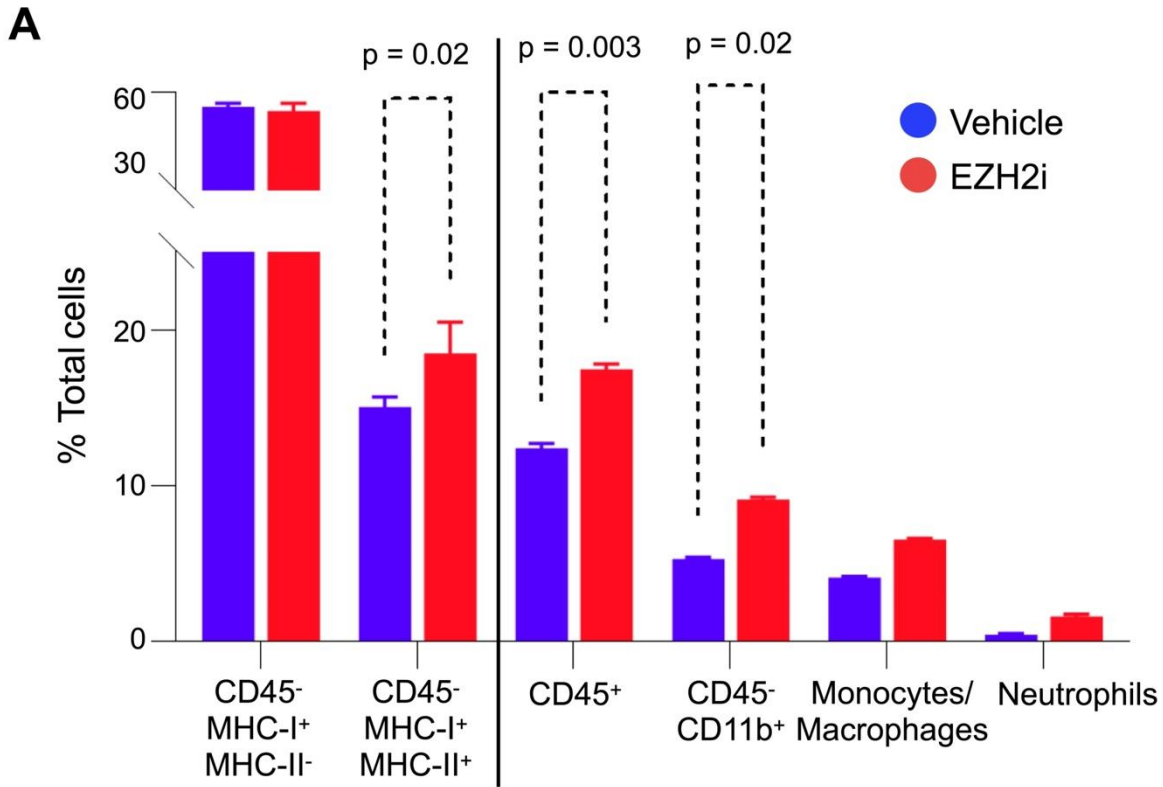
**Fig. S2: EZH2 inhibition upregulates MHC class II genes in BBN tumors.** (A) Volcano plot highlighting MHC-class I and MHC-class II gene expression changes in EZH2i treated BBN tumors relative to vehicle controls. Red dots represent MHC-II genes and Ciita expression while the grey dots represent MHC-I genes expression.

Fig. S3.



**Fig. S3: EZH2 inhibition upregulates MHC class II genes in human bladder cancer cell-lines.** (A) Volcano plot highlighting differential gene expression in two bladder cancer cell-lines 5637 and UMUC3 post EZH2i treatment.

Fig. S4.



**Fig. S4: EZH2 inhibition increases immune infiltration into bladder tumors.** (A) Percentages of indicated immune cell populations as a fraction of total cells in BBN tumors treated with EZH2i or vehicle. Macrophage/monocytes and neutrophils percentages show no statistically significant difference.