



(PD42-05)

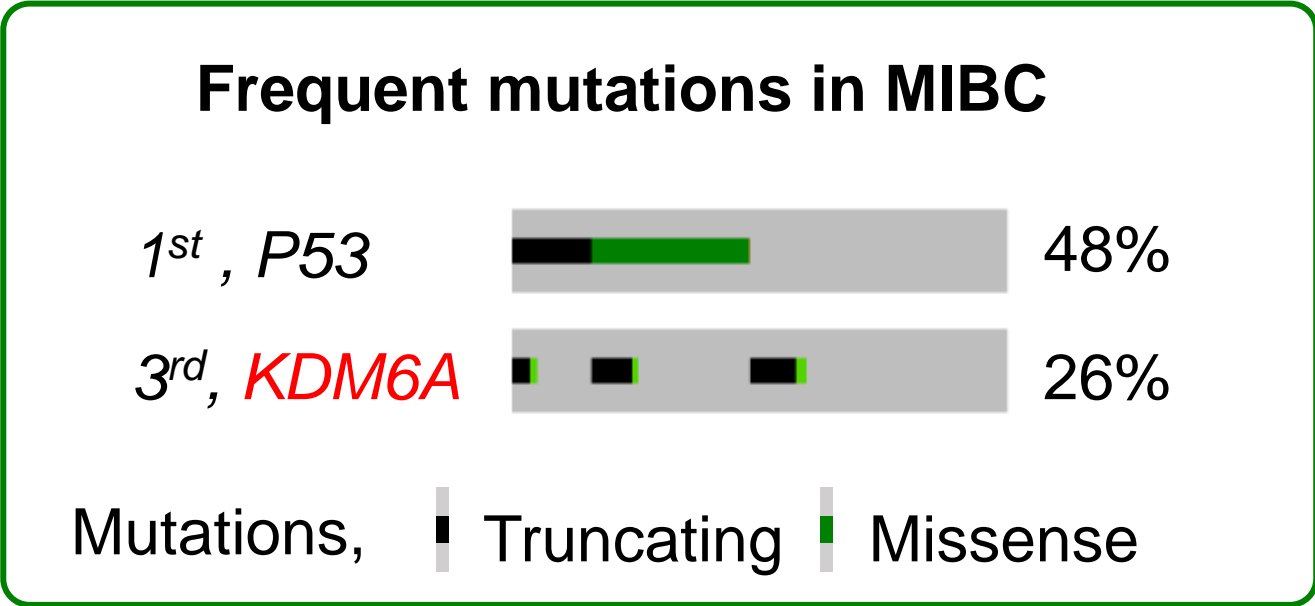
***Kdm6a* deficiency activates inflammatory pathways, promotes M2 macrophage polarization and causes bladder cancer with *p53* dysfunction**

Kohei Kobatake, Kenichiro Ikeda, Yuichiro Nakata, Norimasa Yamasaki, Tetsutaro Hayashi, Kazuhiro Sentani, Wataru Yasui, Osamu Kaminuma, Shigeo Horie, Peter C. Black, Akio Matsubara, and Hiroaki Honda

Podium Presenter

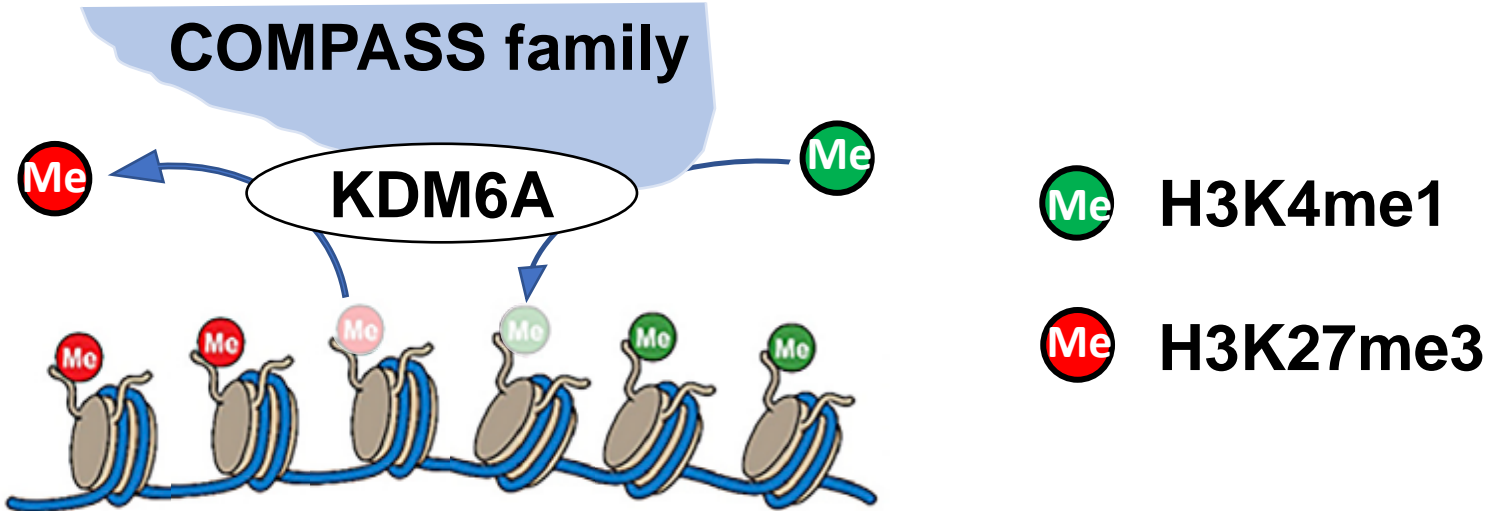
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Introduction



Robertson et al,
Cell. 2017

KDM6A-function



MIBC, Muscle invasive bladder cancer

Purpose

- 1) To clarify the pathogenic mechanisms underlying *Kdm6a*-mutated MIBC
- 2) To develop a new therapeutic strategy for MIBC

Materials and Methods

Mice

Four different genotypes

Control
(*Ctrl*)

Urothelium-specific *Kdm6a*-deficient
(*Kdm6a*^{Δ/Δ})

crossed with *p53*^{+/-} mice

Ctrl p53^{+/-}

Kdm6a^{Δ/Δ} *p53*^{+/-}

Cells

MBT2; Murine BC cell line

Clinical data

Human MIBC data (TCGA, 2017)

*p < 0.05, *p < 0.01, and ***p < 0.001.

BC, bladder cancer

Results

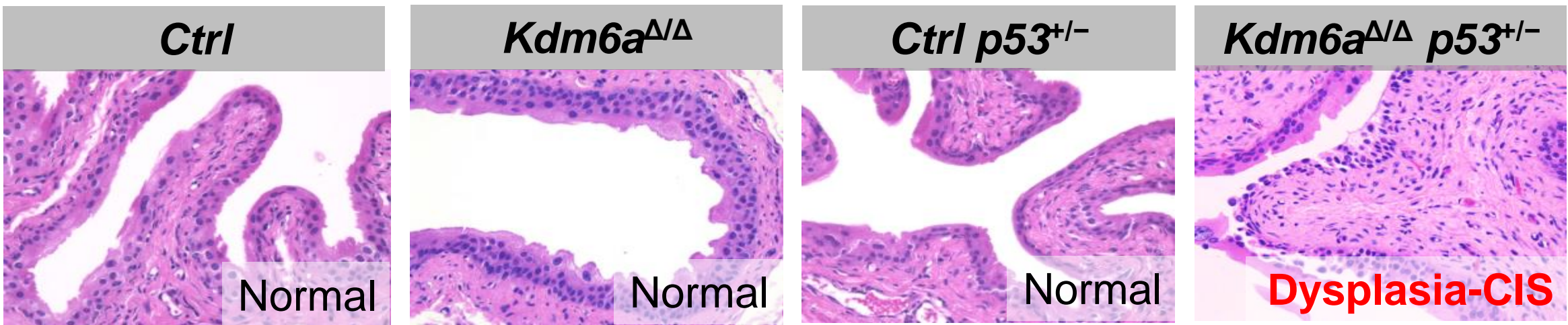
Mice

- ✓ Bladder phenotypes

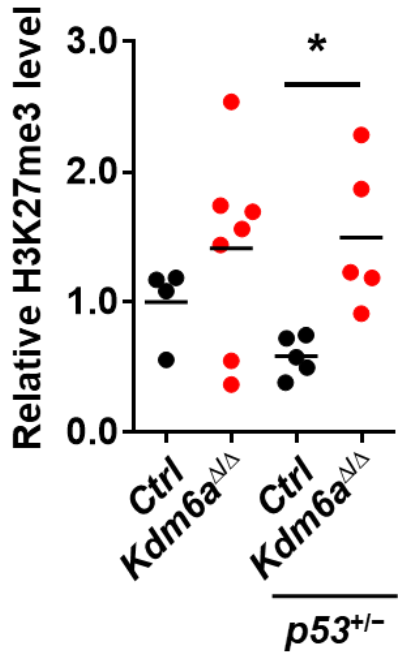
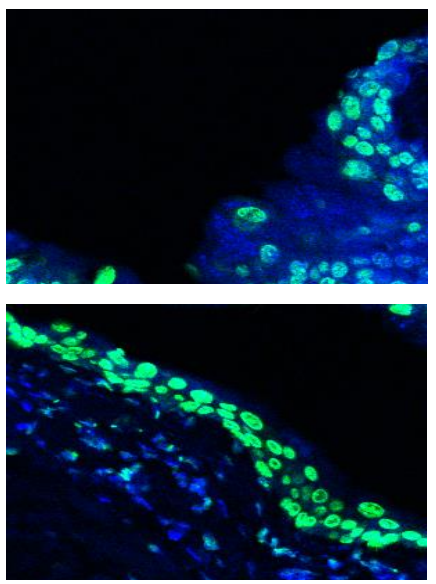
- Spontaneous onset of BC
 - Carcinogen (BBN)-induced onset of BC

- ✓ Gene expression profiles

Mice Bladder phenotypes (spontaneous onset of BC)

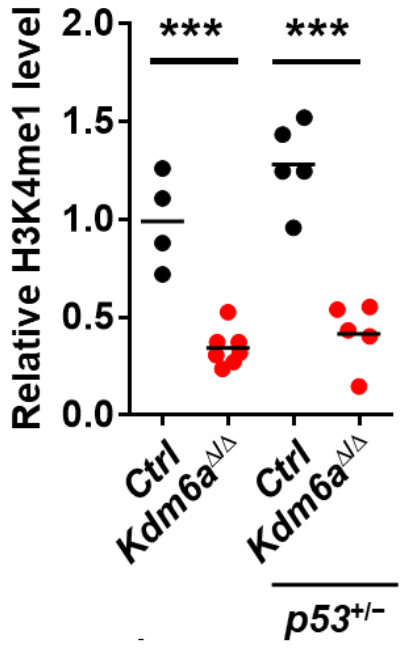
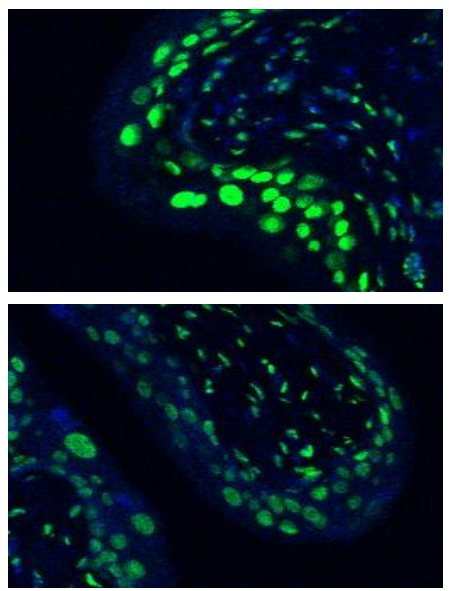


H3K27me3



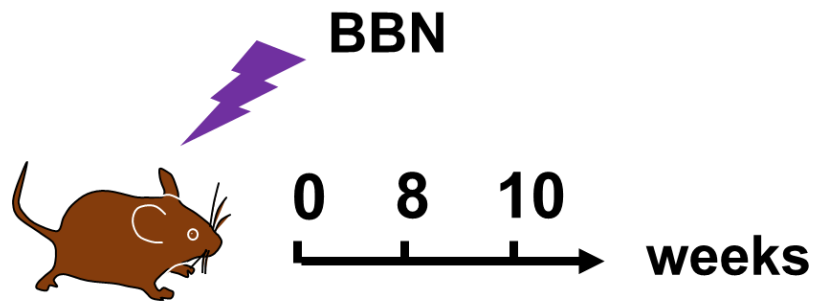
H3K4me1

Ctrl



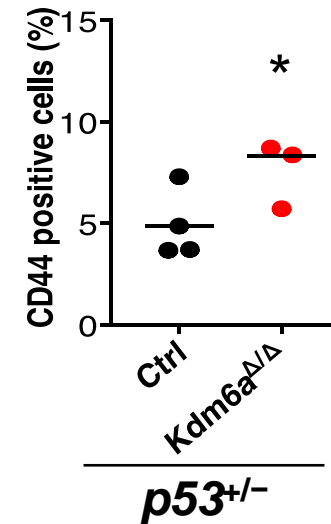
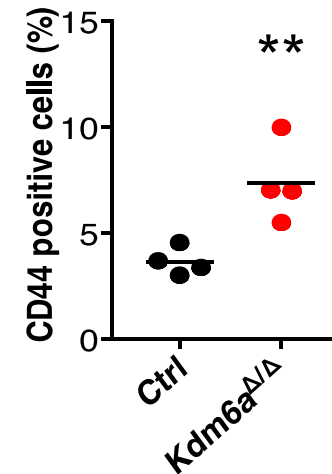
Kdm6a^{Δ/Δ} developed BC in cooperation with *p53*^{+/-}.

Mice Bladder phenotypes (BBN-induced onset of BC)

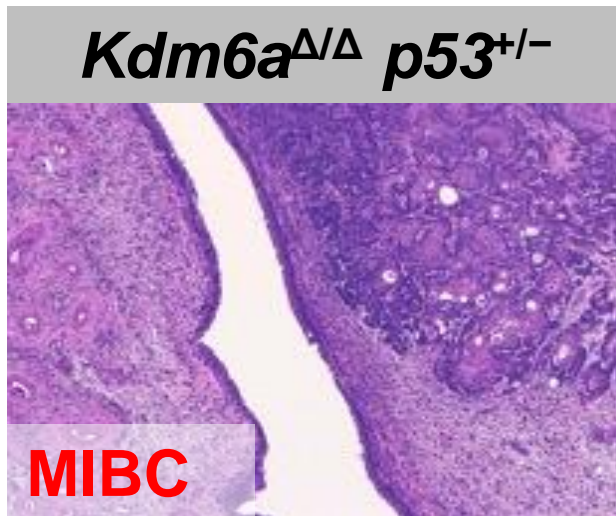


(At 8 weeks)

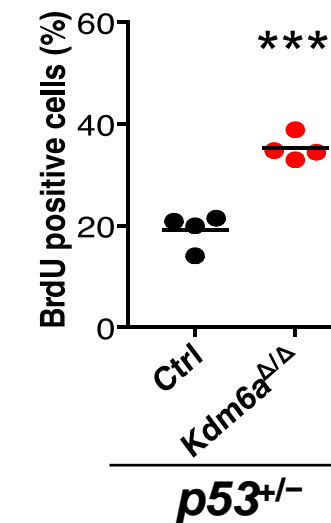
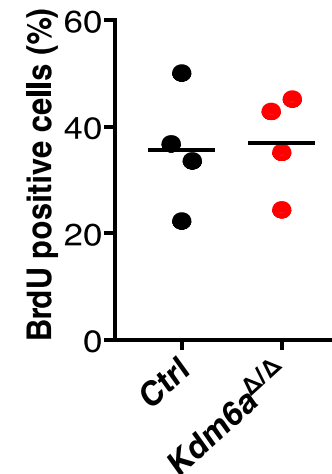
CD44+ cells
(CSC marker)



(At 10 weeks)



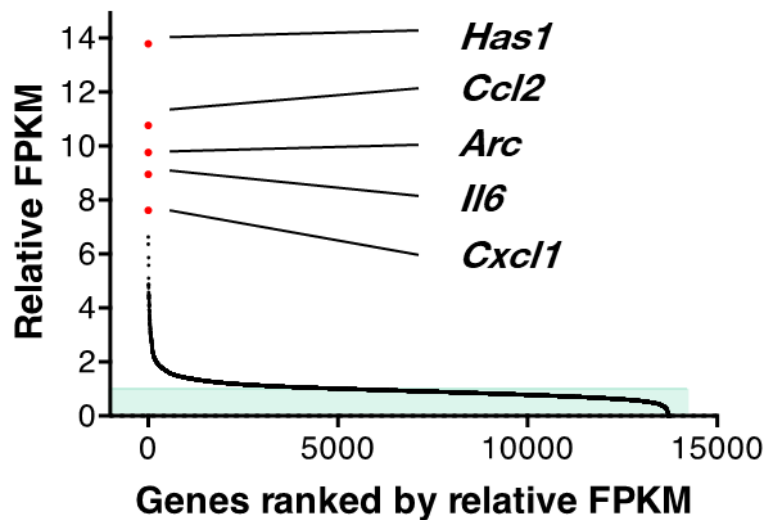
BrdU+ cells
(Cell cycle marker)



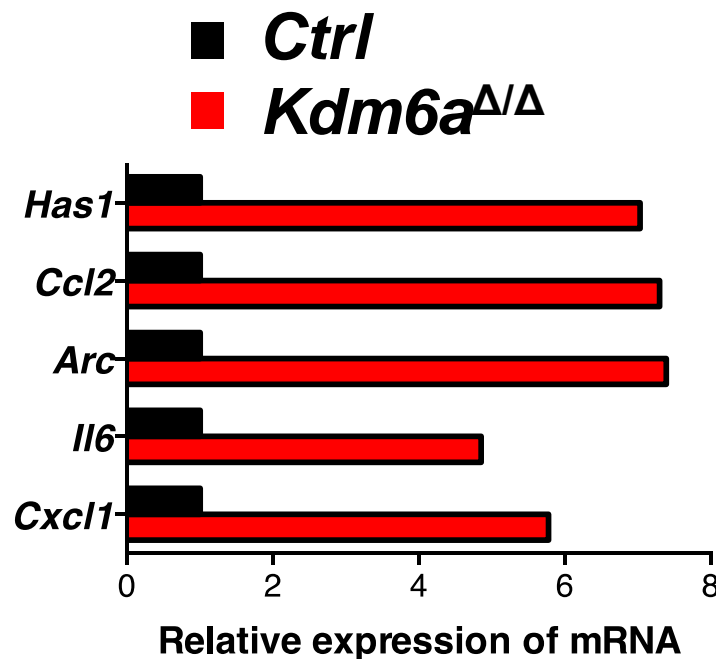
Kdm6a Δ/Δ promotes CSCs and accelerates cell cycle in cooperation with p53^{+/-}.

Mice Gene expression profiles (RNA-sequencing)

Relative FPKM in *Kdm6a*^{Δ/Δ}

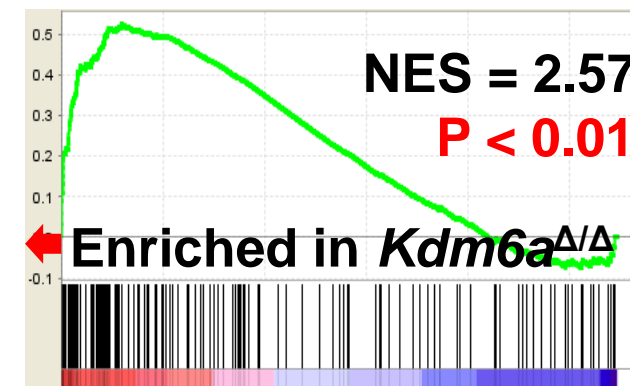


Real-time PCR



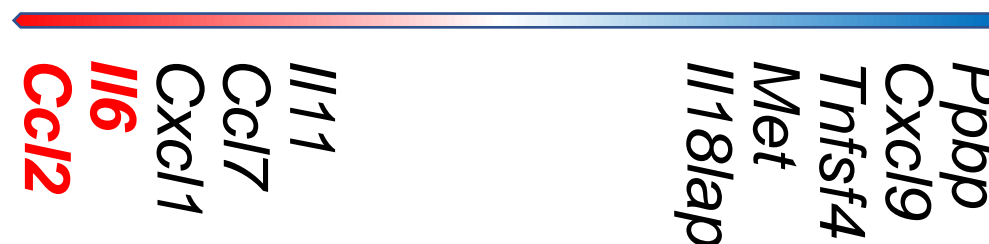
GSEA

Cytokine_Cytokine_Receptor_Interaction



Increased Top5

Decreased Top5



Kdm6a^{Δ/Δ} activates proinflammatory signaling to develop BC.

Results

Cells

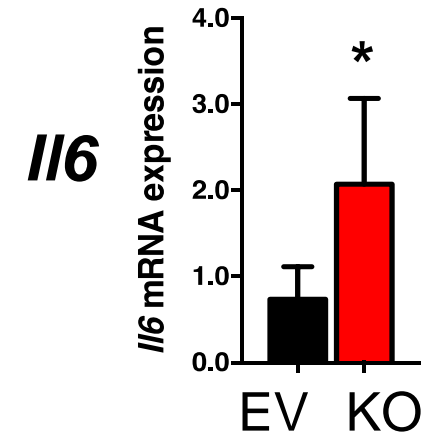
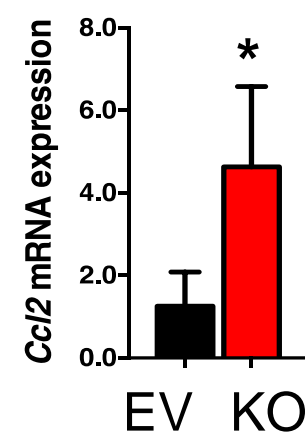
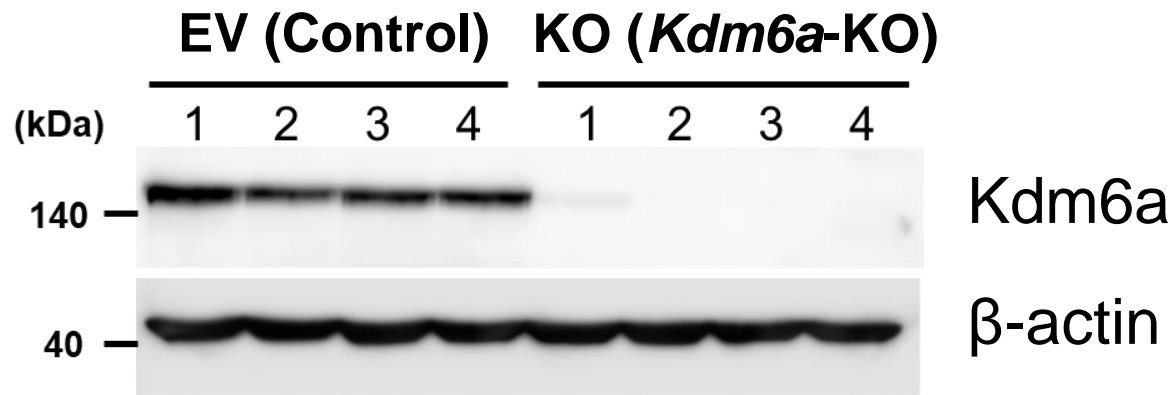
Kdm6a-KO MBT2 (by CRISPR-Cas9 system)

- ✓ Growth advantage
- ✓ Inhibition of proinflammatory signaling

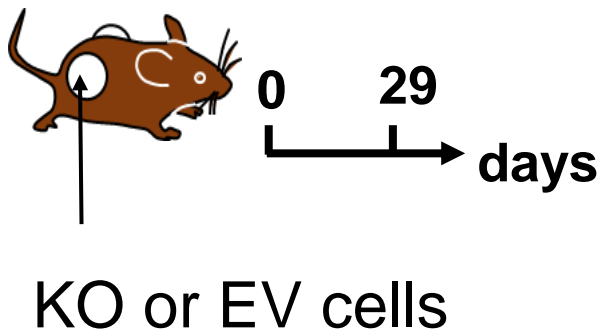
MΦ; Macrophages

Cells

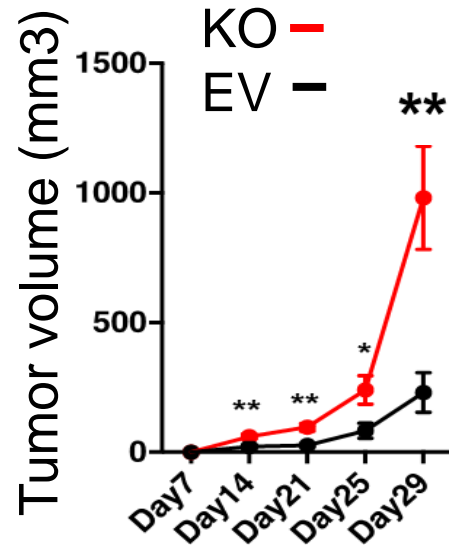
Growth advantage of *Kdm6a*-KO cells



(Allograft)

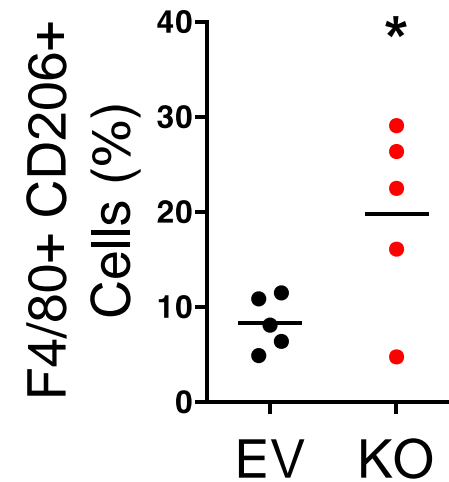


Tumor growth



EV KO

M2-M Φ



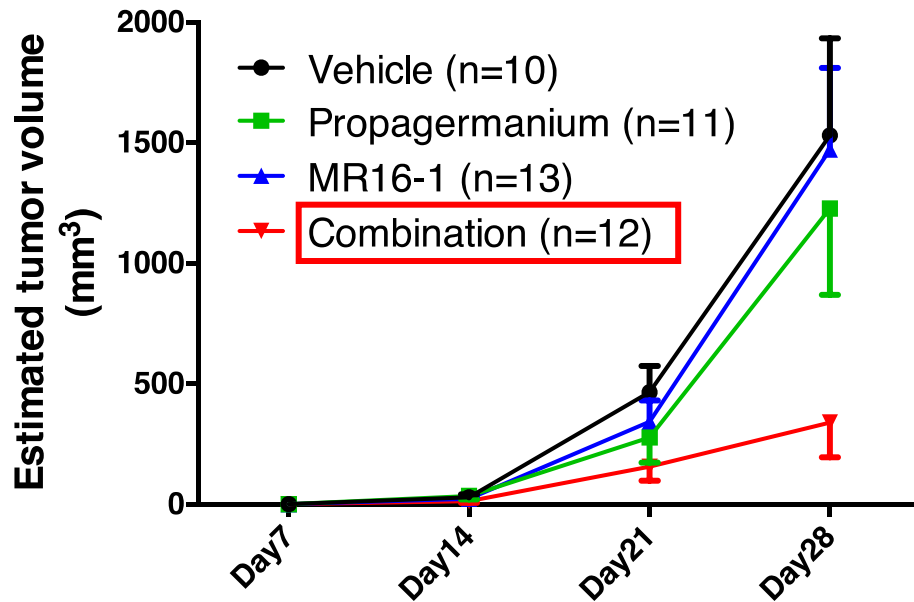
***Kdm6a* deficiency conferred a growth advantage on BC.**

- **Vehicle**, an IgG control
- **Propagermanium**, an inhibitor of Ccr2 (a receptor for Ccl2)
- ▲ **MR16-1**, a neutralizing antibody against the mouse Il6 receptor
- ▼ **Combination**, MR16-1 and Propagermanium

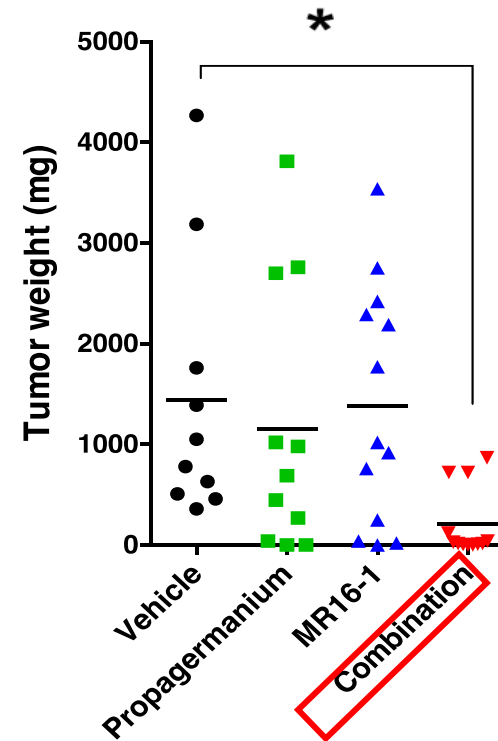


KO cells

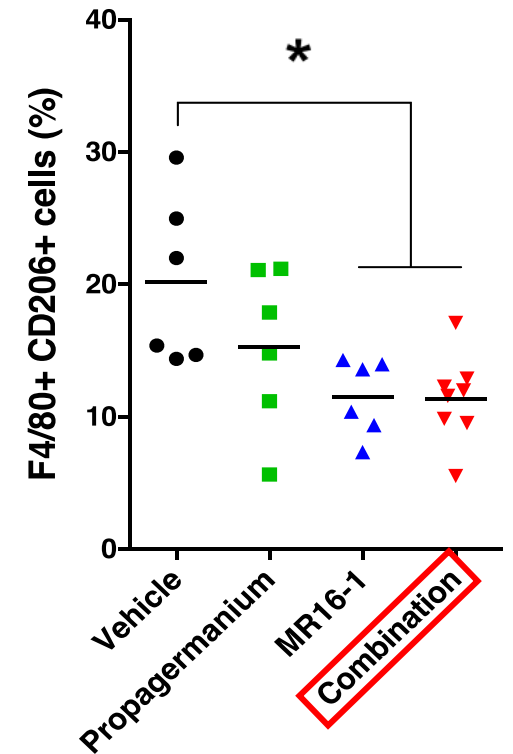
Tumor growth



Tumor weight



M2-MΦ



The combined inhibition of Il6 and Ccl2 signaling can be a novel therapeutic approach for KDM6A-deficient BC.

Results

Clinical data

Human MIBC data (n=413) †

✓ Effects of *KDM6A*-alterations in human MIBC

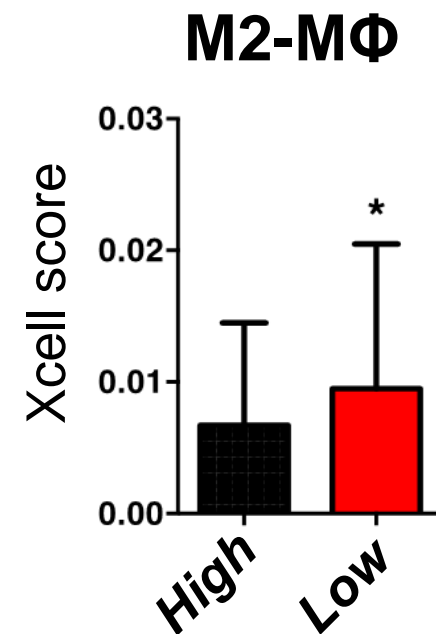
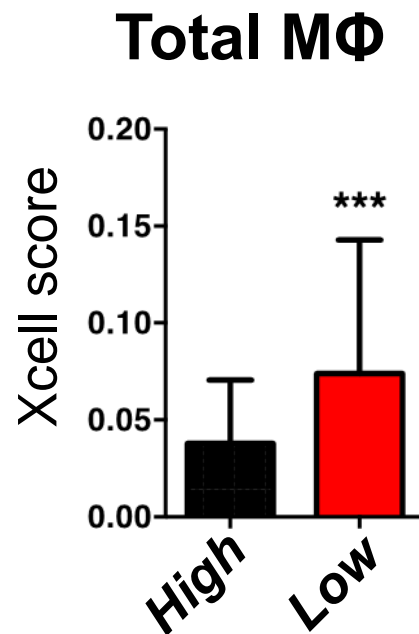
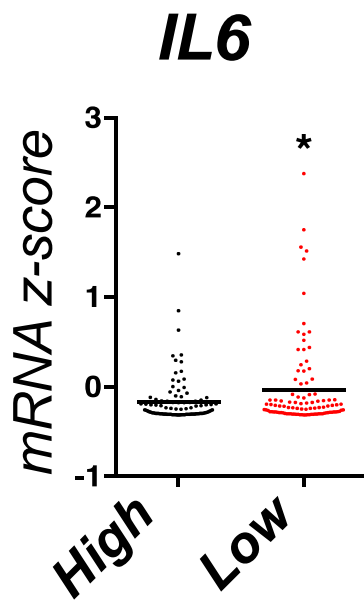
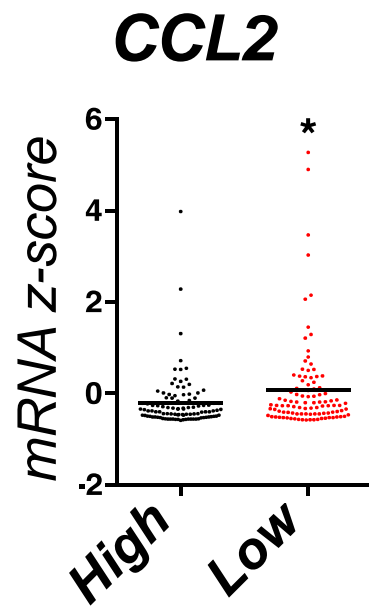
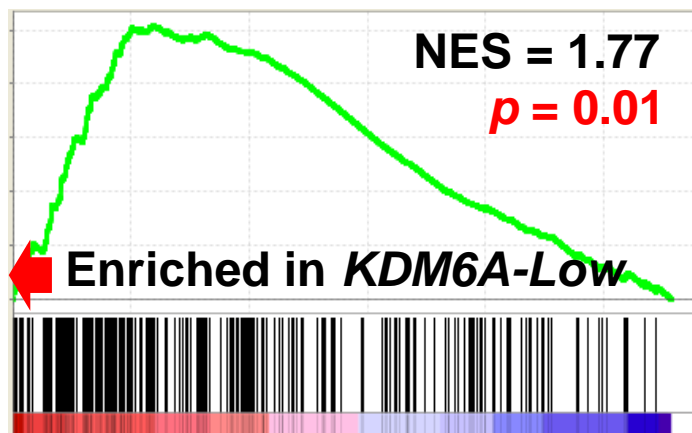
***KDM6A*-expression**

High, the highest 25%
or
Low, the lowest 25%

† Robertson, et al., Cell, 2017

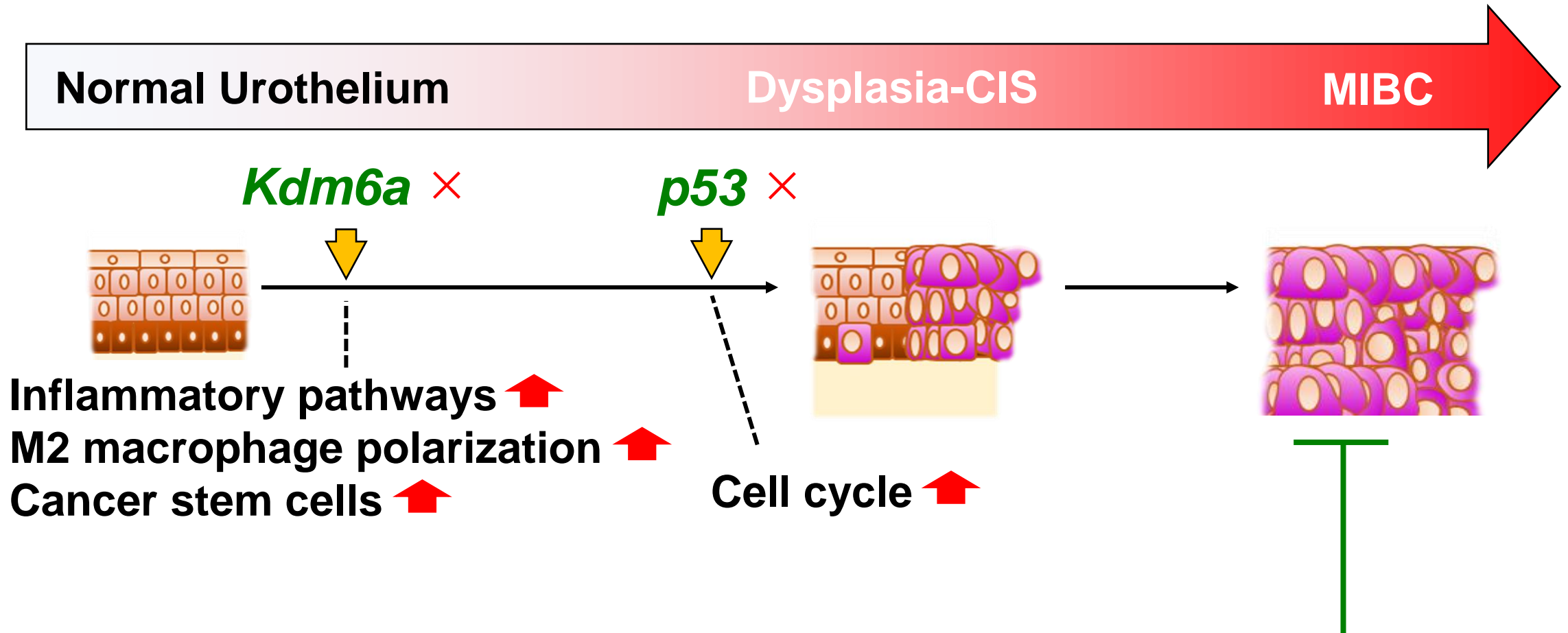
(GSEA)

Cytokine_Cytokine_Interaction



The phenotypes in human MIBC completely correspond to our model.

Conclusions



Normal Urothelium

Dysplasia-CIS

MIBC

Kdm6a ×

p53 ×

Inflammatory pathways ↑
M2 macrophage polarization ↑
Cancer stem cells ↑

Cell cycle ↑

Possible therapeutic strategy for *Kdm6a*-mutated MIBC
Anti-IL6R antibody + CCR2 inhibitor