



## **Supplemental Material to:**

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**Tumor-suppressive functions of 15-Lipoxygenase-2 and  
RB1CC1 in prostate cancer**

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**<http://www.landesbioscience.com/journals/cc/article/28757>**

## Supplementary Figure legend:

### Figure S1. Prostatic hyperplasia in 15-LOX2 Tg mice does not progress to PIN.

- (A). Representative whole-mount (WM) HE images of 12-week ventral prostates (VP) of the genotypes indicated.
- (B). Quantification of hyperplasia using a point-intersect method. A positive point consisted of the intersection of two major grid lines overlying epithelium. Bars represent mean  $\pm$  S.D (n = 12 for each genotype).
- (C). Representative HE images of 12-week VPs of p53<sup>-/-</sup> and 15-LOX2;p53<sup>-/-</sup> mice (n = 5 for each genotype).

### Figure S2. IHC of 15-LOX2, Myc, and $\alpha$ -SMA in 6-month old Hi-Myc and Myc;LOX prostates.

Shown are representative WM HE images from one 6.4 month-old Hi-Myc (top; a-d) and one 6.7 month-old Myc;LOX dTg (bottom; g-h) prostates. The orientation of the prostate is shown in the lower left panel (U, urethra; DP, dorsal prostate; LP, lateral prostate; VP, ventral prostate). Note the prominent adenocarcinomas in the Hi-Myc LP (a) compared to much less prominent lesions in the Myc;LOX LP (e). Serial WM sections were used in IHC of 15-LOX2, Myc, and  $\alpha$ -SMA. Note that 15-LOX2 was strongly expressed in the VP and LP of the dTg prostates (f).  $\alpha$ -SMA was lacking in the Hi-Myc LP tumors (d) but still observed in some Myc;LOX LP glands (h).

### Figure S3. IHC of 15-LOX2, Myc, and $\alpha$ -SMA in 6-month old Hi-Myc and Myc;LOX prostates.

Shown are representative IHC images of 15-LOX2, Myc, and  $\alpha$ -SMA from one 6.4 month-old Hi-Myc and one 7 month-old Myc;LOX dTg prostates.  $\alpha$ -SMA.  $\alpha$ -SMA was lacking in the Hi-Myc tumor but still observed in the Myc;LOX LP glands (below).

### Figure S4. 15-LOX2, Myc, and RB1CC1 in normal prostatic tissues and HPCa samples.

Shown are high-magnification IHC images (400x) of 15-LOX2, Myc, and RB1CC1 in the benign/normal (N) and tumor (T) regions of two HPCa samples (i.e., HPC11 and HPCa16) presented in Fig. 5A-B. Note the reciprocal expression patterns between 15-LOX2 and Myc. In contrast to 15-LOX2, which is lost HPCaT, RB1CC1 protein is not lost but actually overexpressed in HPCaT.

**Figure S5. Overexpression of RB1CC1 causes NHP cell growth arrest, senescence, and/or apoptosis.**

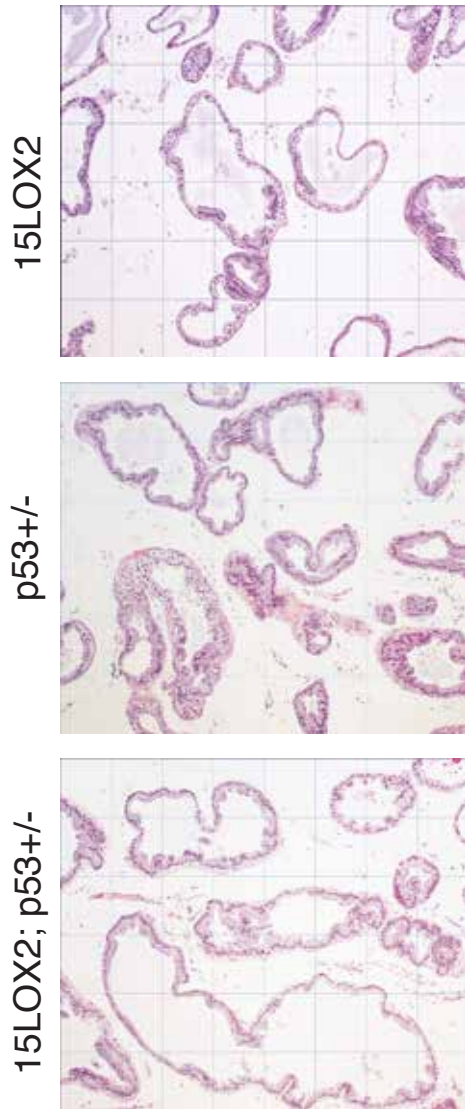
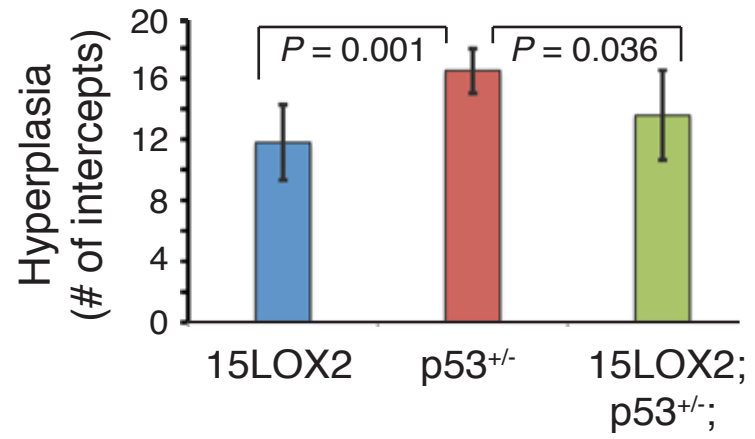
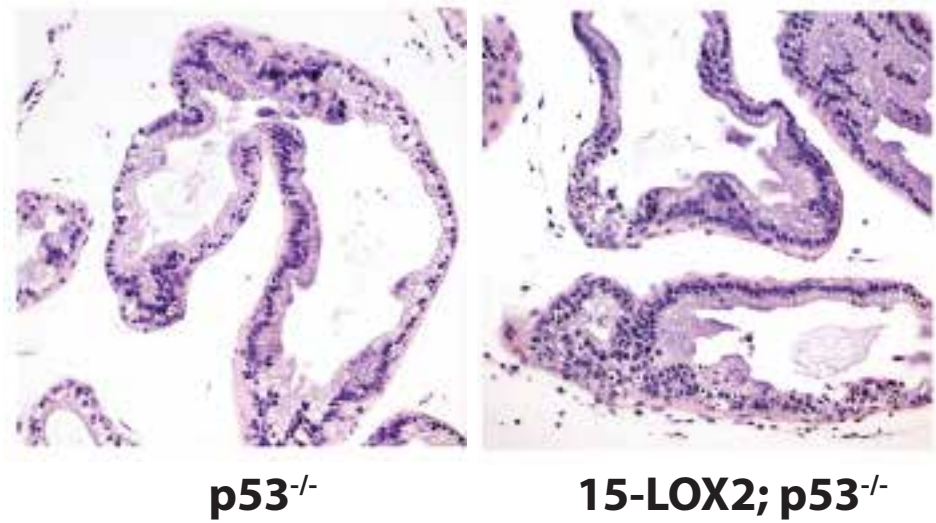
Primary strains of NHP cells of the equal numbers as indicated at different passages (P) were transfected with either the empty vector (p3xFLAG-CVM10) or the RB1CC1-encoding expression vector. Images were taken 72 h after transfection. Note significantly reduced cell numbers and apoptotic and senescent morphologies in RB1CC1-overexpressing cultures. Original magnifications, x200.

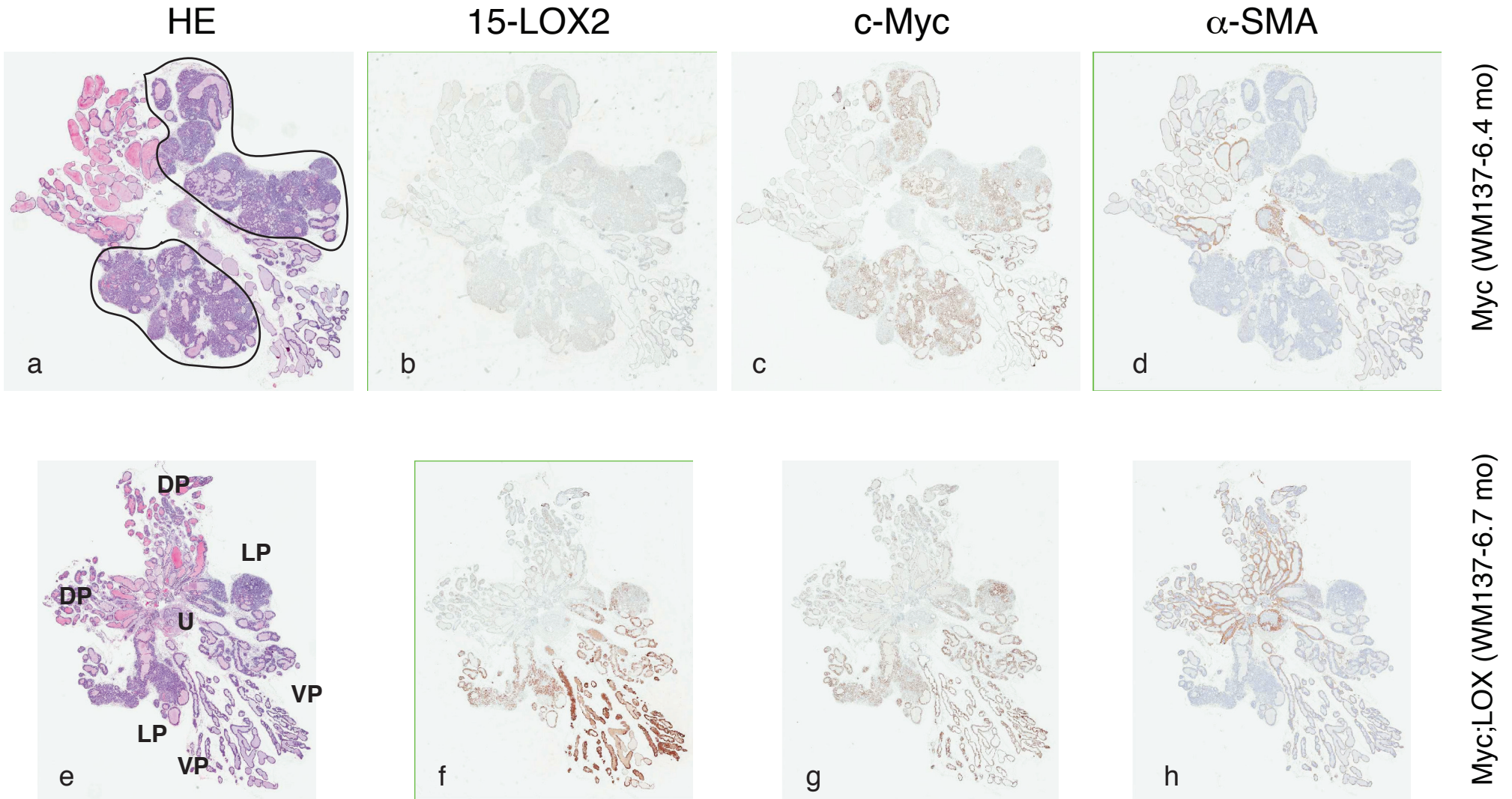
Table S1 . Primary antibodies used in the current study

<b>Antibody</b>	<b>Host</b>	<b>Clonality</b>	<b>Company</b>	<b>Catalog #</b>
$\alpha$ -SMA	mouse	monoclonal	Sigma	A2547
$\beta$ -Actin	mouse	monoclonal	Sigma	A5316
c-Myc	rabbit	monoclonal	Epitomics	1472-1
Lamin	goat	polyclonal	Santa Cruz	sc-6214
Phosphorylated-Rb	goat	polyclonal	Santa Cruz	sc-12901
pRb	rabbit	polyclonal	Santa Cruz	sc-50
p27	mouse	monoclonal	BD	554069
p53	rabbit	polyclonal	Cell Signaling	9287
RB1CC1	rabbit	polyclonal	Novus Biological	NBP1-30010
RB1CC1	rabbit	polyclonal	Sigma	SAB4200135
15LOX-2	rabbit	polyclonal	Oxford Biomed	LX 25

**Table S2. Real-time PCR primers used in gene expression studies and PCR primers used for genotyping**

Gene	Forward Primer (5'--3')	Reverse Primer (5'--3')	Mouse/Human
C-Fos	CTCCAGTGCCAACTTCATTCCCAC	GCAGCCATCTTATTCCTTTCCCTTCG	human
C-Jun	AACGACCTTCTATGACGATGCCCT C	CCCGTTGCTGGACTGGATTATCAG	human
RB1	CATGCTGTTCAAGGAGACATTCAAACG	ACACGGTCGCTGTTACATACCATCTG	human
RB1CC1	TCACCAGTAATGCCACTCAGTTGCC	TTGGATTTGTGTCCGTCCTCCAGC	mouse
<b>Primers used for genotyping</b>			
	Forward Primer (5'--3')	Reverse Primer (5'--3')	
β-Casein	GATGTGCTCCAGGCTAAAGTT	AGAAACGGAATGTTGTGGAGT	
β-Globin	GTGTTGTTTAGAATGGGAAGATGT	TAAAGAGAAAGGCAGGATGATGA	
C-Myc	CAATGTCTGTGTACAACCTGCCAACTGGGATGC	TTACGCACAAGAGTTCCGTAGCTGTTC	
P-53 WT	GTGTTTCATTAGTTCCCCACCTTGAC	AGAGCAAGAATAAGTCAGAAGCCG	
P-53-NULL	TTTACGGAGCCCTGGCGCTCGATGT	GTGGGAGGGACAAAAGTTCGAGGCC	

**A****B****C**



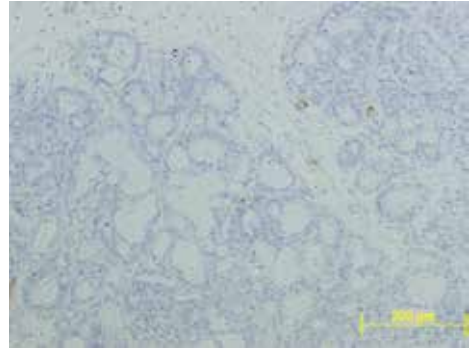
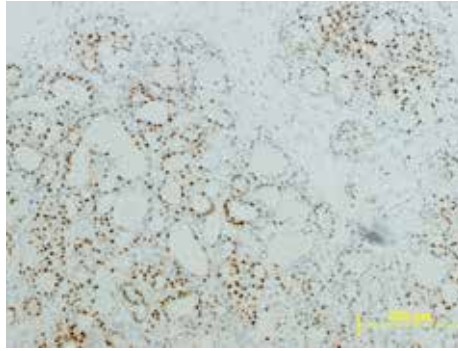
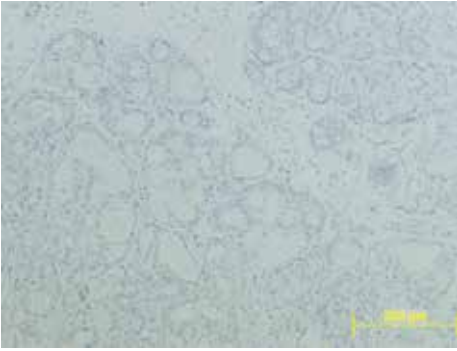


15-LOX2

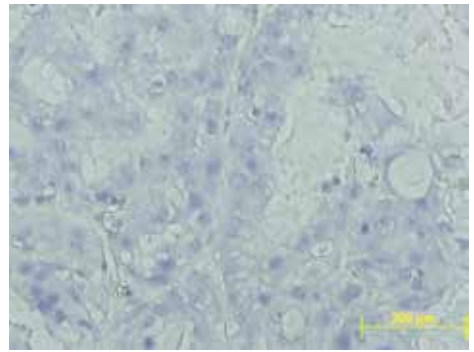
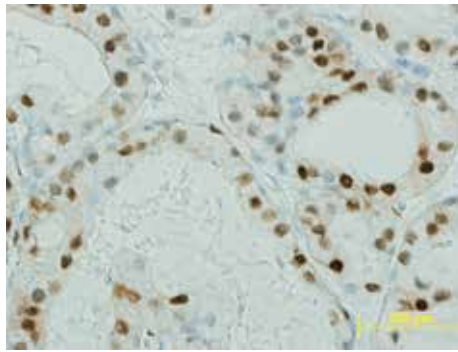
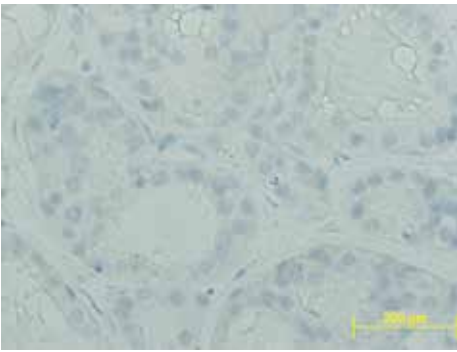
Myc

$\alpha$ -SMA

100x

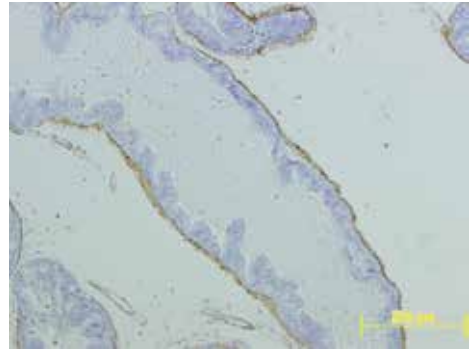
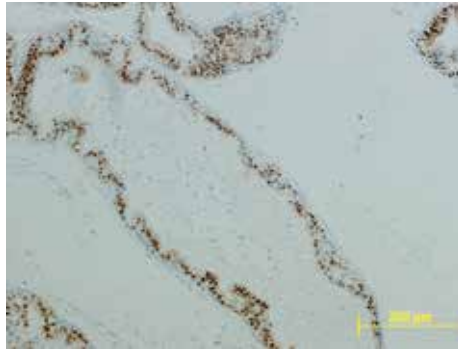
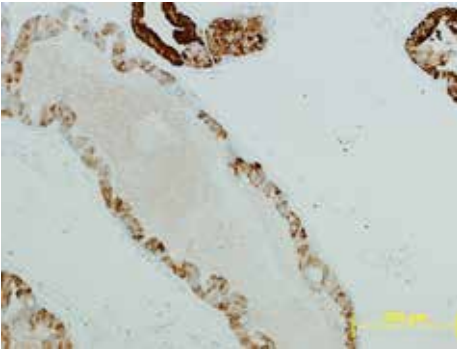


400x

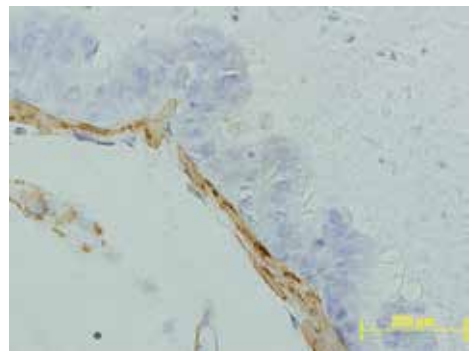
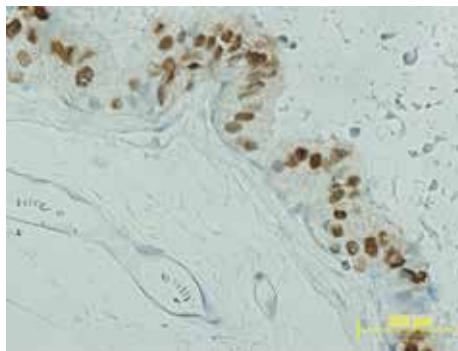
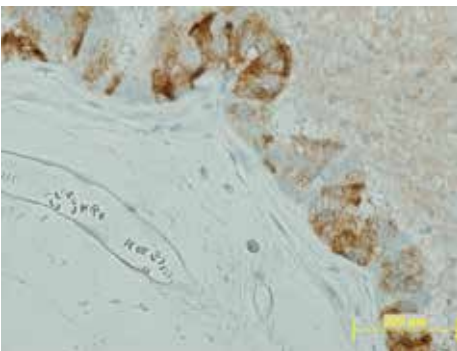


Myc (WM24-6.4 mo)

100x



400x



Myc;LOX (WM128-7 mo)



