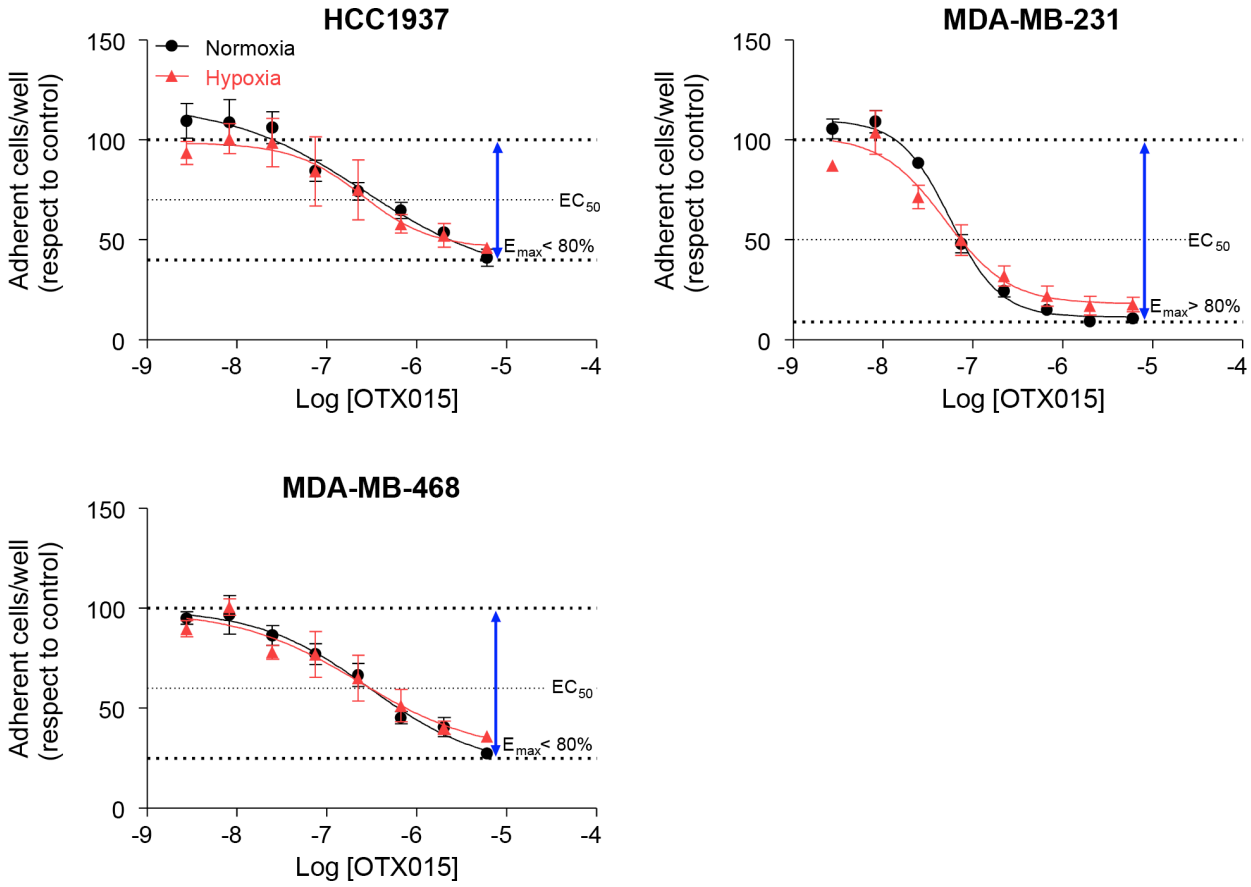
The bromodomain inhibitor OTX015 (MK-8628) exerts anti-tumor activity in triple-negative breast cancer models as single agent and in combination with everolimus

Supplementary Materials

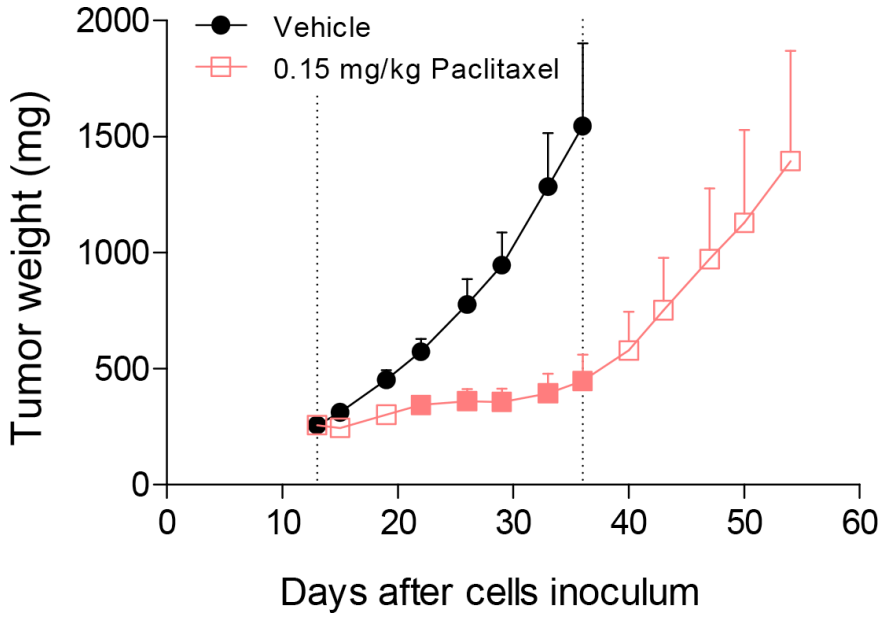


Supplementary Figure S1: Cell growth inhibition curves under normoxic and hypoxic conditions, by cell count. Cells were treated with increasing concentrations of OTX015 (2.7–6000 nM) or 0.1% (v/v) DMSO (vehicle control group, not shown) in normoxia and hypoxia. The effects on cell proliferation were evaluated at 72 h employing a Beckman Coulter cell counter. The dotted lines indicate the E_{max} values. The EC_{50} values were calculated with the equation for sigmoidal dose response using Prism 5.00 for MS Windows software. Each point and vertical line represents the mean ± SEM ($n \geq 3$).

Evaluation of paclitaxel in MDA-MB-231 cell line-derived xenografts

6-week-old female nude Foxn1 mice (≈25 g, Harlan Laboratories, Italy) were maintained at a constant temperature and humidity, according to institutional guidelines. Protocols were approved by the Ethics Committee of the IRCCS-Istituto di Ricerche Farmacologiche Mario Negri (Italy), in compliance with national (D.lgs 26/2014; Authorisation n.19/2008-A issued March 6, 2008 by Ministry of Health) and international laws (EU Directive 2010/63/EU).

Mice were subcutaneously injected in the right flank with 10×10^6 MDA-MB-231 cells and 12 days after randomized (eight animals/group) into vehicle (cremophor:ethanol 1:1 was used to dissolve paclitaxel, then diluted 1:5 with saline; once weekly, intravenous) or 0.15 mg/kg paclitaxel, once weekly, intravenous. Tumor weight and treatment efficacy were evaluated as described in the Material and Methods section. Tolerability was determined on the basis of body weight loss, clinical observation and mortality. Mice were sacrificed at the first sign of severe distress.



Supplementary Figure S2: Anti-tumor effect of paclitaxel in MDA-MB-231 xenografts. Tumor weight was compared between drug regimens at each time point using a two-way ANOVA test ($p < 0.001$) followed by Bonferroni a posteriori test on log-transformed data. Color-filled symbols indicate significant ($p < 0.01$) differences between treated vs. vehicle mice. Dotted vertical lines indicate the treatment period ($n = 8$).

Supplementary Table S1: LIMMA results in MDA-MB-231 cells. See Supplementary_Table_S1

Supplementary Table S2: LIMMA results in MDA-MB-468 cells. See Supplementary_Table_S2

Supplementary Table S3: Common up-regulated genes in MDA-MB-231 and MDA-MB-468 cell lines. See Supplementary_Table_S3

Supplementary Table S4: Common down-regulated in MDA-MB-231 and MDA-MB-468 cell lines

Symbol	Definition	Synonyms
GPR56	Homo sapiens G protein-coupled receptor 56 (GPR56), transcript variant 3, mRNA.	BFPP; DKFZp781L1398; TM7LN4; TM7XN1
FJX1	Homo sapiens four jointed box 1 (Drosophila) (FJX1), mRNA.	FLJ25593; FLJ22416
PHF15	Homo sapiens PHD finger protein 15 (PHF15), mRNA.	JADE2; KIAA0239
SPDEF	Homo sapiens SAM pointed domain containing ets transcription factor (SPDEF), mRNA.	bA375E1.3; PDEF; RP11-375E1__A.3
CCDC86	Homo sapiens coiled-coil domain containing 86 (CCDC86), mRNA.	FLJ22321; MGC2574
TRIP6	Homo sapiens thyroid hormone receptor interactor 6 (TRIP6), mRNA.	MGC29959; MGC3837; MGC10556; OIP1; ZRP-1; MGC4423; MGC10558

Supplementary Table S5: MSIGDB GSEA report. See Supplementary_Table_S5

Supplementary Table S6: CMAP GSEA report. See Supplementary_Table_S6

Supplementary Table S7: OTX015 and Everolimus EC_{50} values at 72 h determined by the MTT assay

Cell line	OTX015		Everolimus	
	EC_{50} (nM)	E_{max}%	EC_{50} (μM)	E_{max}%
HCC1937	81.3 (47.0–140.5)	51	13.2 (12.0–14.5)	> 95
MDA-MB-231	81.7 (67.2–99.4)	83	14.8 (12.2–18)	> 95
MDA-MB-468	448.3 (269.2–746.5)	42	17.5 (12.8–19.6)	> 95

Results are expressed as the mean concentration that that gives half-maximal response (EC_{50}) with 95% confidence intervals. E_{max} % indicates the maximum inhibitory effect induced by OTX015 on cell proliferation (with respect to control untreated cells). In all cases, $n \geq 3$.

Supplementary Table S8: Primer sequences (forward and reverse) used for real-time PCR

Gene	Primer	Sequence	Primer length	T_m (°C)	Amplicon length	Comments
c-MYC ***(NM_002467)***	Fw Rv	cgactctgaggaggaacaagaa ggatagtccttccgagtgga	22 20	60.9 59.1	210	Primer not spanning an exon-exon junction
BRD2 ***4 isoforms***	Fw Rv	ccacctcaacctaagaagtcca acttcctccagaaggtccaaag	22 22	60.5 61.0	93	Specific to 4 isoforms Not spanning an exon-exon junction
BRD3 ***(NM_005378.4)***	Fw Rv	gtgcacatcatccaatctcg cttgctgagaacggtttcct	20 20	60.1 59.5	158	Primer spanning an exon-exon junction
BRD4 ***2 isoforms***	Fw Rv	tctacaacaagcctggagatga tctcggtttcttctgtgggtag	22 22	59.9 60.7	99	Specific to 2 isoforms Primer spanning an exon-exon junction
n-MYC ***(NM_005378.4)***	Fw Rv	tgagcgattcagatgatgaaga gcatcgtttgaggatcagc	22 19	60.9 59.3	191	Primer spanning an exon-exon junction
p21 ***2 isoforms***	Fw Rv	gaccagcatgacagatttctacc aagatgtagagcgggcctttt	23 20	60.0 60.2	127	Specific to 2 isoforms Not spanning an exon-exon junction
GAPDH ***2 isoforms***	Fw Rv	gatccctccaaaatcaagtgg ggaggcattgctgatgatct	21 20	60.7 60.2	213	Specific to 2 isoforms Primer spanning an exon-exon junction
HPRT1 ***(NM_0000194.2)***	Fw Rv	tgaatacttcagggatttgaatcat ctcatcttaggctttgtattttgc	25 24	60.0 60.0	76	Primer spanning an exon-exon junction
β-actin ***(NM_001101.3)***	Fw Rv	cagagcctcgcctttgc tcatcatccatggtgagctg	17 20	60.1 58.6	76	Primer spanning an exon-exon junction
EpCAM ***(NM_002354.2)***	Fw Rv	ttgtggttgtggtgatagcag cacccatctcctttatctcagc	21 22	59.6 60.1	105	Primer spanning an exon-exon junction
Musashi-1 ***(NM_002442.3)***	Fw Rv	tgttcatcgggggactca ggtcaattgttttggagtcgag	18 22	61 60.8	210	Primer spanning an exon-exon junction
NANOG ***2 isoforms***	Fw Rv	gccttgctttgaagcatcc gaggaaggaagaggagagacagt	19 23	59.1 60.0	138	Specific to 2 isoforms Primer spanning an exon-exon junction
OCT4 ***5 isoforms***	Fw Rv	cacacaaagcactttatccattct tcacccccagtttaaggatgt	24 21	59.6 60.6	220	Specific to 4 isoforms Not spanning an exon-exon junction
CD24 ***4 isoforms***	Fw Rv	tcctcccagagtacttccaact gagtgagaccacgaagagactg	22 22	59.2 59.1	111	Specific to 4 isoforms Not spanning an exon-exon junction
CD133 ***7 isoforms***	Fw Rv	cagctacttggctcagactgg gtgcatctcttttcagggagtt	21 22	60.2 59.8	128	Specific to 7 isoforms Not spanning an exon-exon junction
CD44 ***8 isoforms***	Fw Rv	ccctccgtcttaggtcactg cggcaggttatattcaaatcg	20 21	59.7 59.5	282	Specific to 8 isoforms Not spanning an exon-exon junction