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OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

Using VBIM technique to identify drug resistance genes in ovarian cancer

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Indiana University School of Medicine

Outline

- Background of Validation-Based Insertional Mutagenesis (VBIM) technique
- Example 1: VBIM technique identifies F-box leucine repeat rich protein (FBXL11) as a novel regulator of NF-κB
- Example 2: VBIM technique and drug resistance gene discovery in ovarian cancer

COLD SPRING HARBOR PERSPECTIVES IN BIOLOGY

NF-κB

A Network Hub Controlling Immunity, Inflammation, and Cancer

Use of Forward Genetics to Discover Novel Regulators of NF-κB

Tao Lu and George R. Stark

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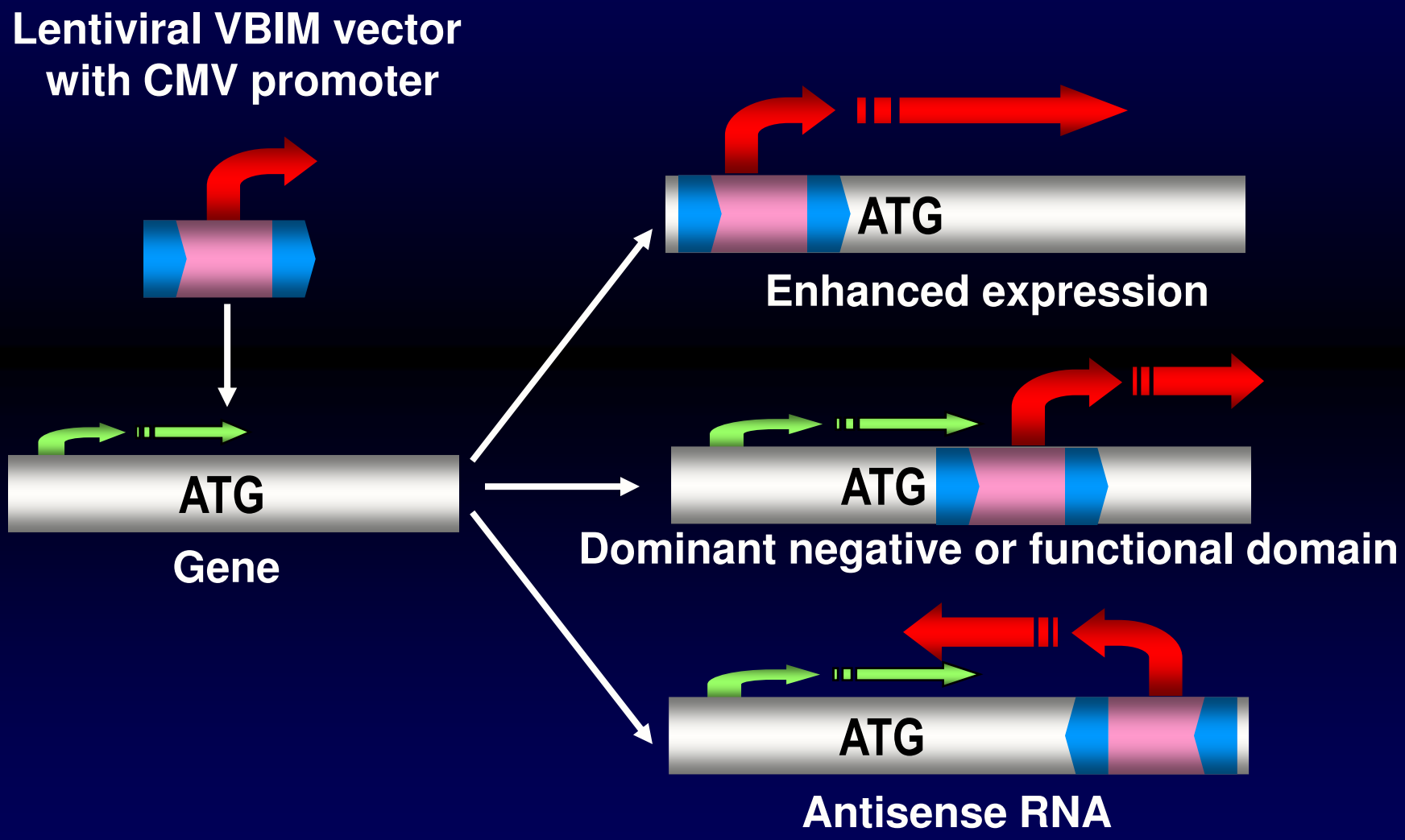
Correspondence: starkg@ccf.org

Book Chapter, p253-264. Cold Spring Harbor Press (2009).

http://cshperspectives.cshlp.org/cgi/collection/nf-kb

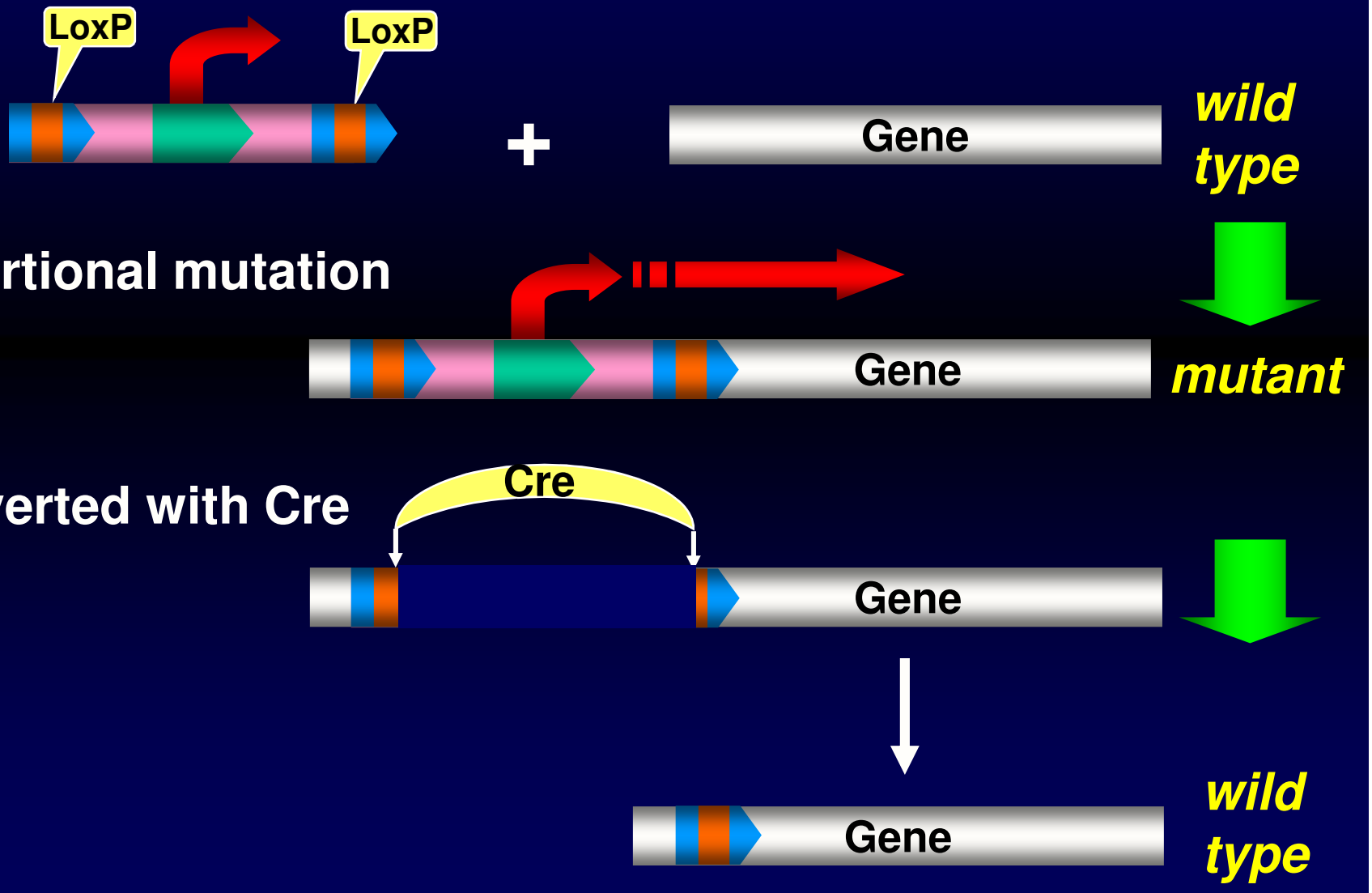
EDITED BY Michael Karin
Louis M. Staudt

Lentiviral VBIM mutagenesis method



Reversible insertional mutation

Lentiviral vector with loxP sites (defective promoter in LTR)



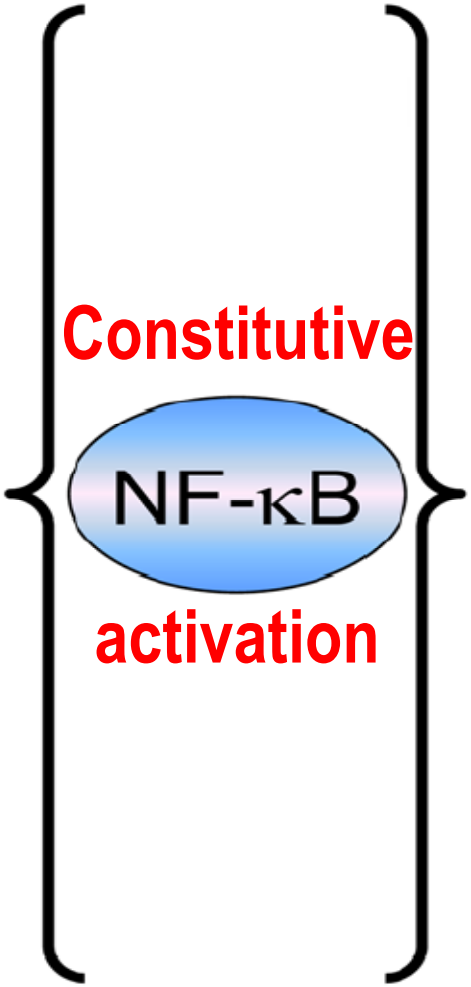
Part I

VBIM technique identifies F-box leucine repeat rich protein (FBXL11) as a novel regulator of NF-κB

Human cancers

HEMATOLOGICAL MALIGNANCIES

- Multiple Myeloma
- Mantle Cell Lymphoma
- MALT Lymphoma
- Diffuse Large B-cell Lymphoma
- Hodgkin's Lymphoma
- Myelodysplasic Syndrome
- Adult T-cell Leukemia (HTLV-1)
- Acute Lymphocytic Leukemia
- Acute Myeloid Leukemia
- Chronic Lymphocytic Leukemia
- Chronic Myeloid Leukemia

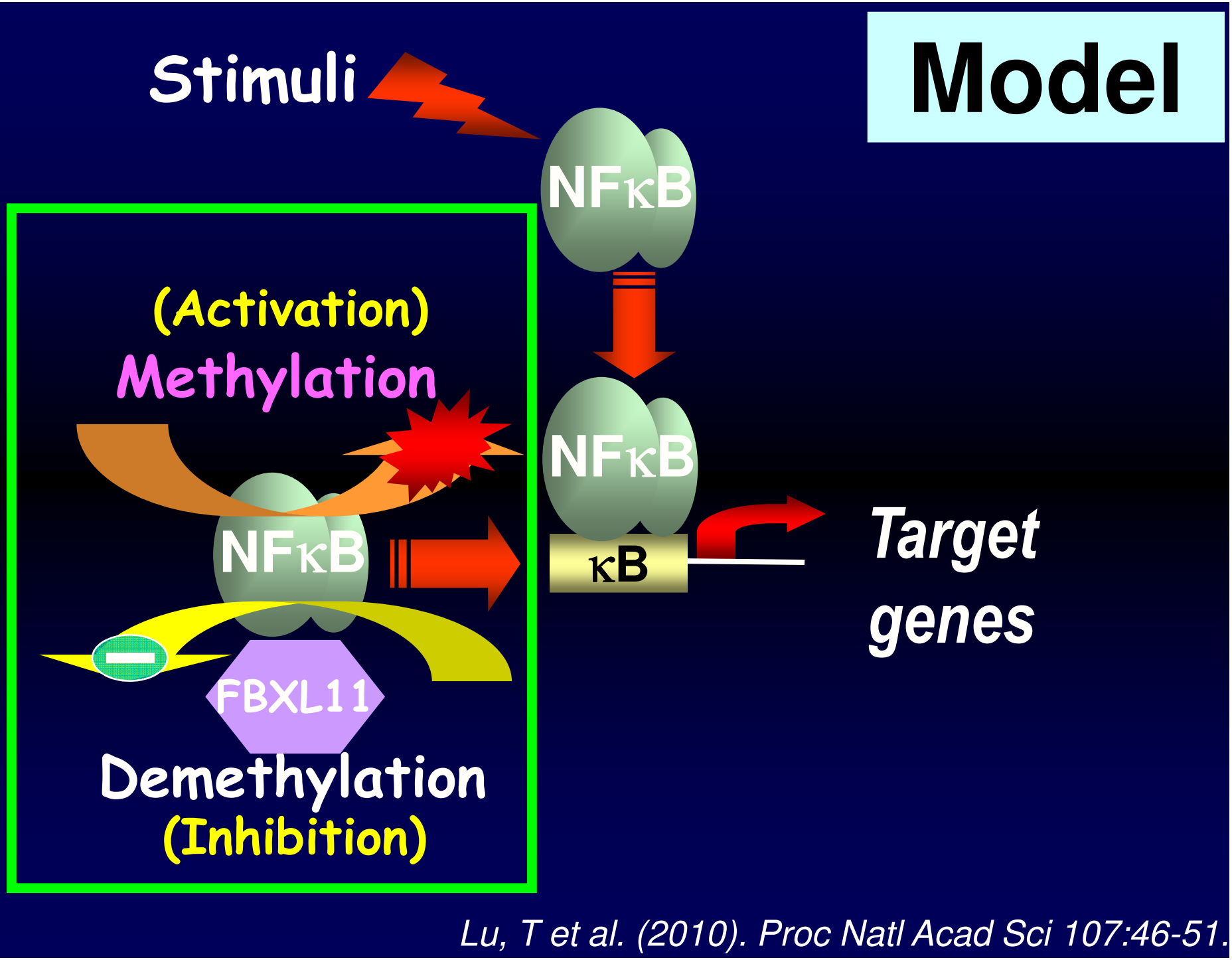


SOLID TUMORS

- Breast Cancer
- Cervical Cancer
- Prostate Cancer
- Renal Cancer
- Lung Cancer
- Colon Cancer
- Liver Cancer
- Pancreatic Cancer
- Esophageal Cancer
- Gastric Cancer
- Laryngeal Cancer
- Thyroid Cancer
- Parathyroid Cancer
- Melanoma
- Bladder Cancer
- Cylindroma
- Squamous Cell Carcinoma (Skin)
- Squamous Cell Carcinoma (Head and Neck)
- Oral Carcinoma
- Endometrial Carcinoma
- Ovarian Cancer
- Retinoblastoma
- Astrocytoma/Glioblastoma

Baud V and Karin M. (2009).Nat Rev Drug Discov 8, 33-40.

Model



Lu, T et al. (2010). Proc Natl Acad Sci 107:46-51.

Take-home message

- We successfully developed the lentiviral VBIM technique, which has broad application in a variety of signaling systems.

- Using VBIM technique we identified and confirmed that FBXL11 is a novel negative regulator of NF-κB.

Lu, T et al. (2009). Proc Natl Acad Sci 106:16339-44.

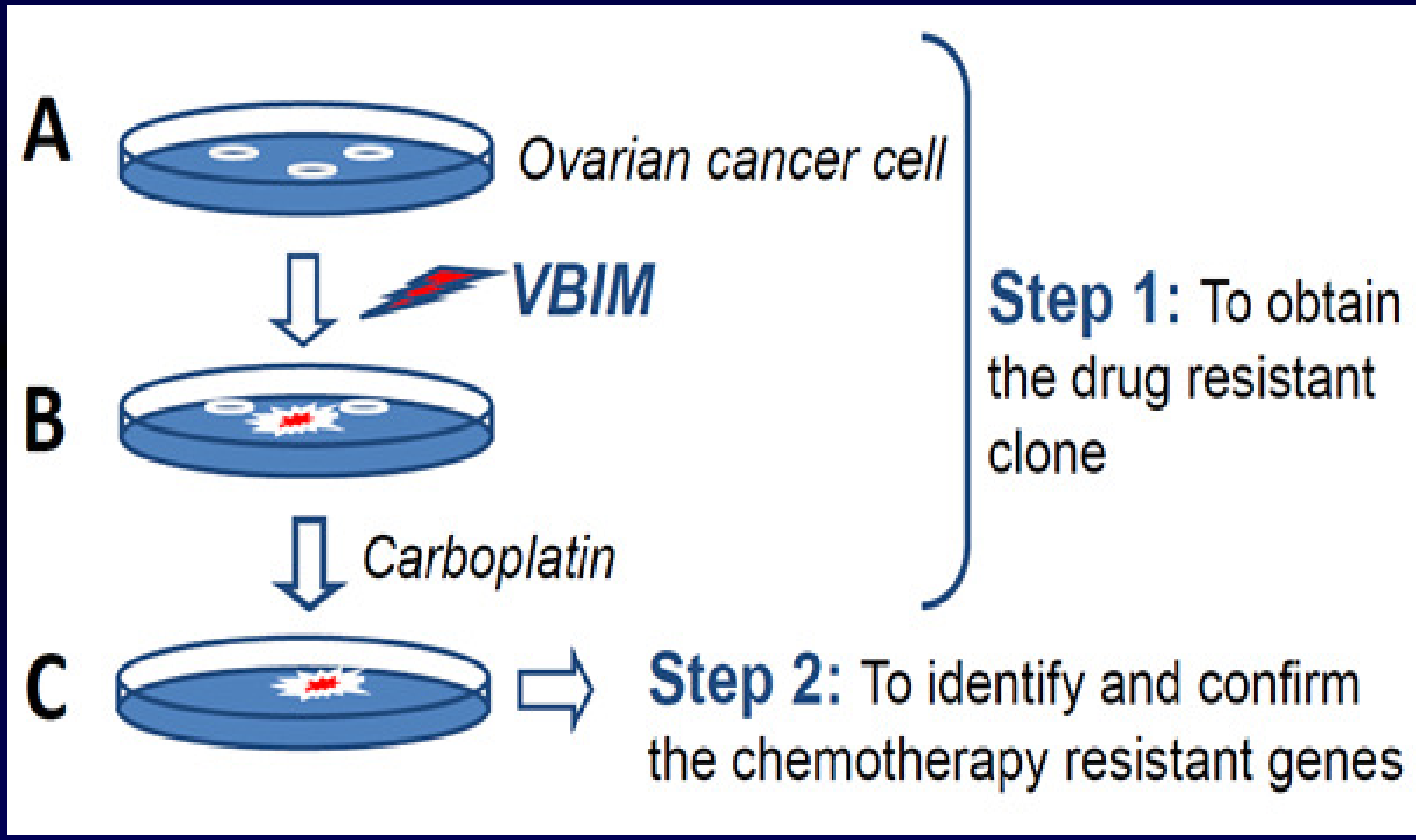
Part II

Target discovery: Using VBIM to identify carboplatin resistance gene in ovarian cancer (OC) cells

Background

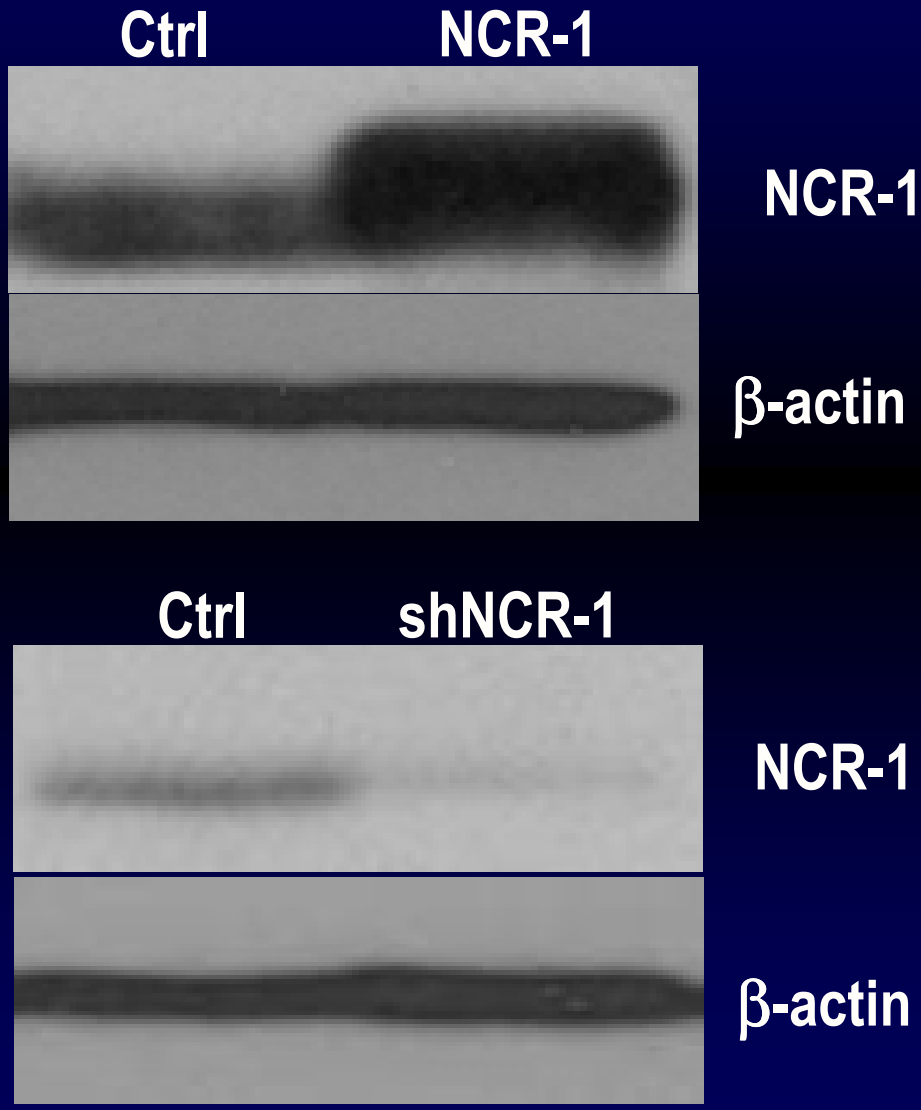
1. Ovarian cancer is the sixth most common cause of cancer in women globally with over 200,000 cases diagnosed annually.
2. Chemotherapy resistance is a complex process using different mechanisms and pathways. However, the mechanism is NOT fully understood.

Experimental Design



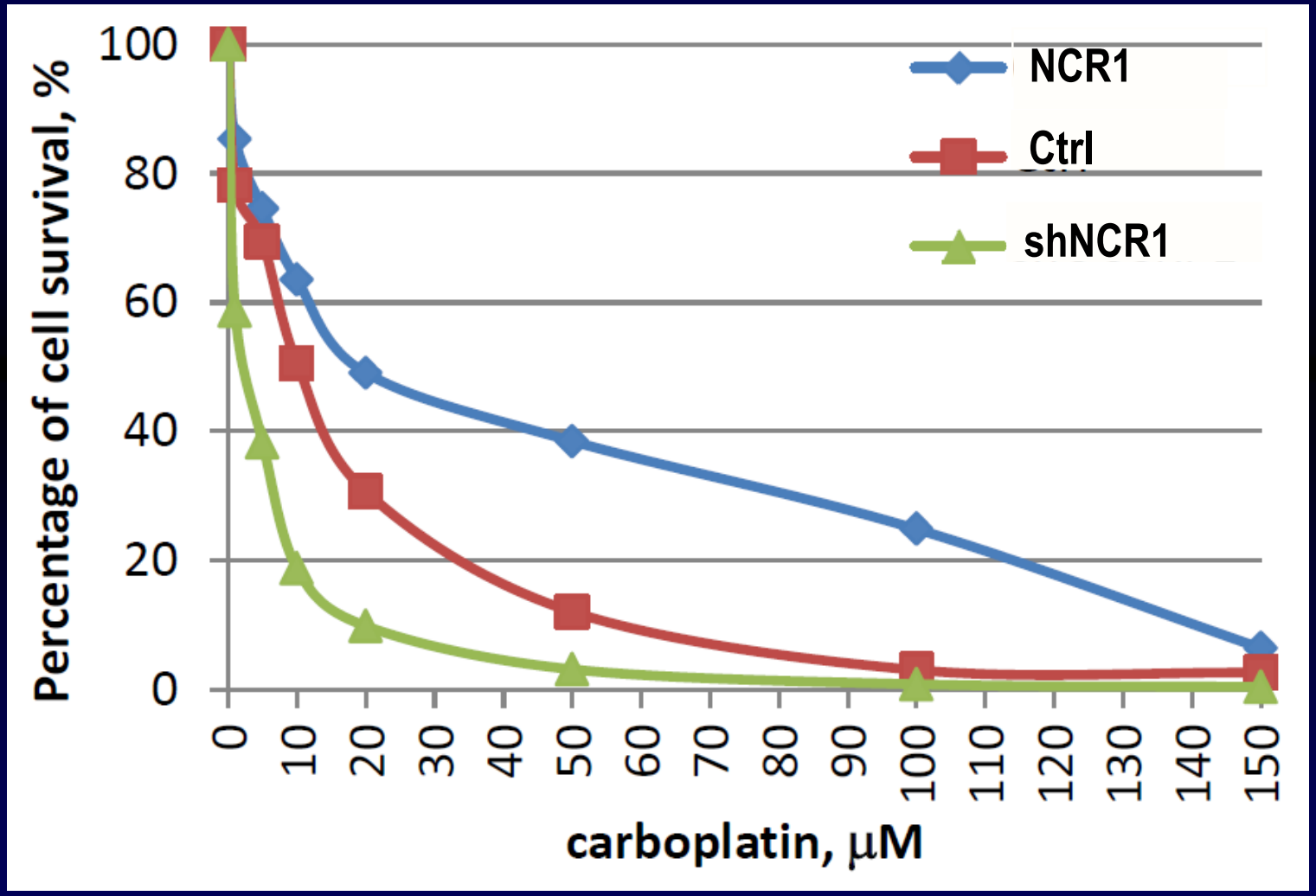
In our ongoing carboplatin resistance gene identification study, NCR-1, 2, 3 (Novel carboplatin resistance protein) have been identified in A2780 OC cells

A2780 NCR-1 overexpressing and shRNA knockdown cells



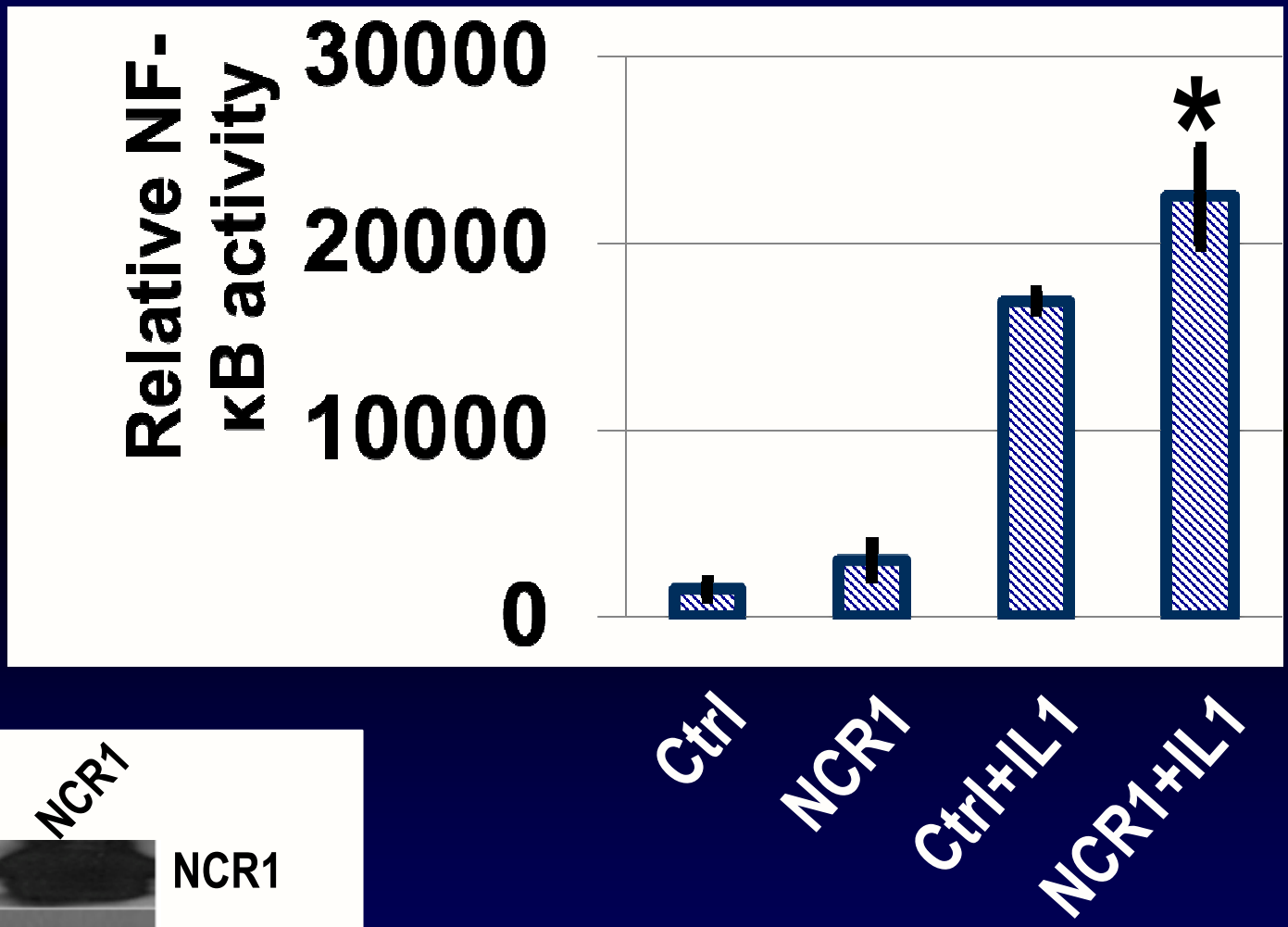
Wei H et al, unpublished data

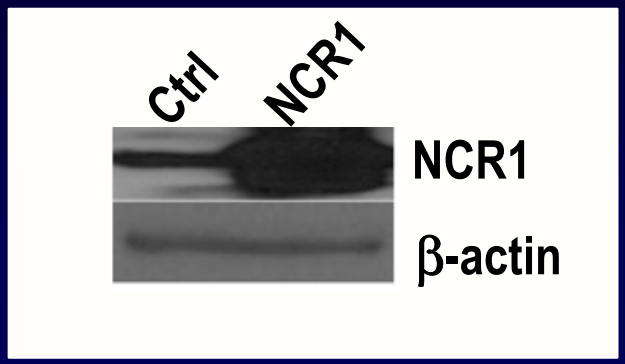
Effect of NCR-1 on carboplatin resistance in A2780 OC cells



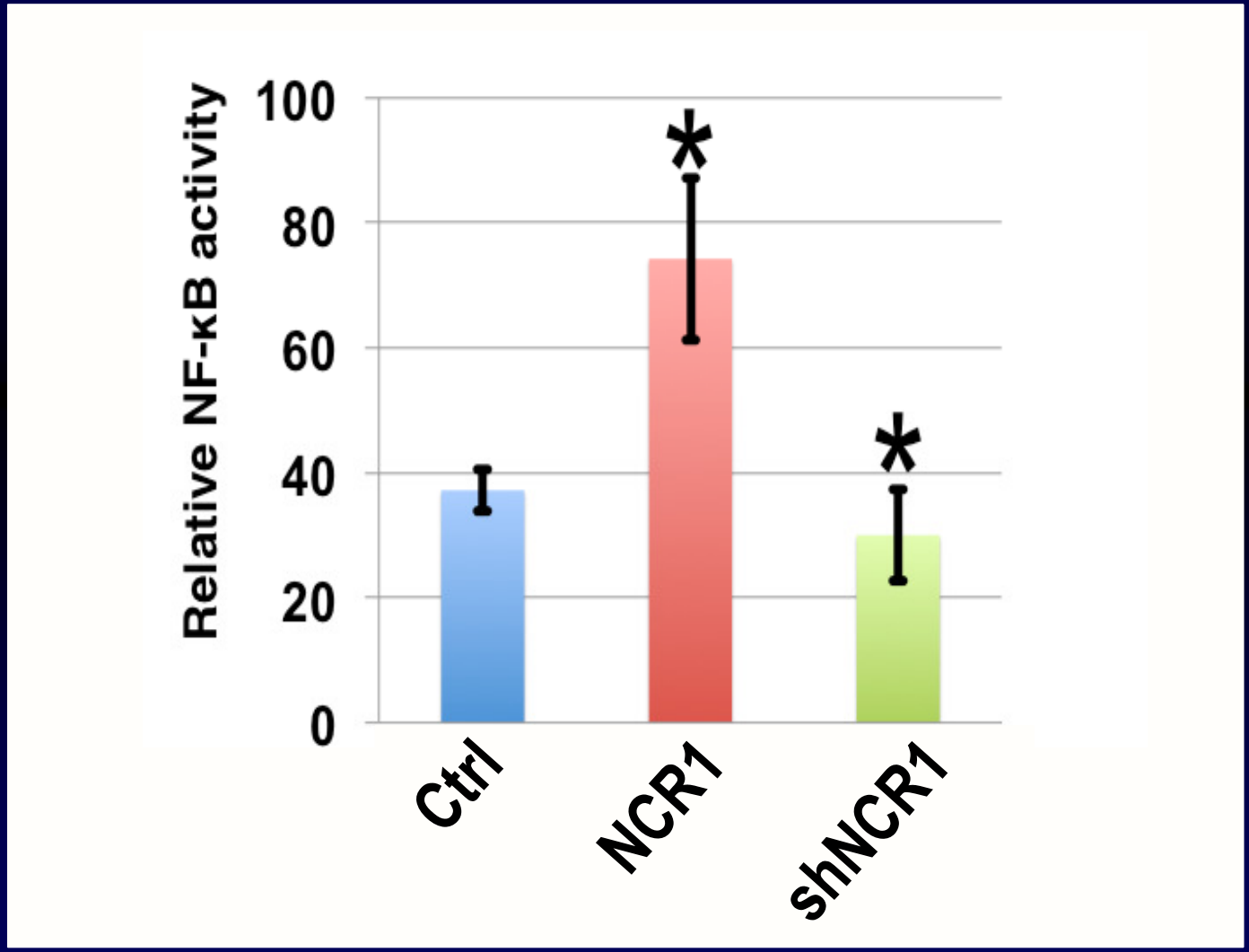
Wei et al, unpublished data

NCR1 is an NF-κB activator in 293 cells



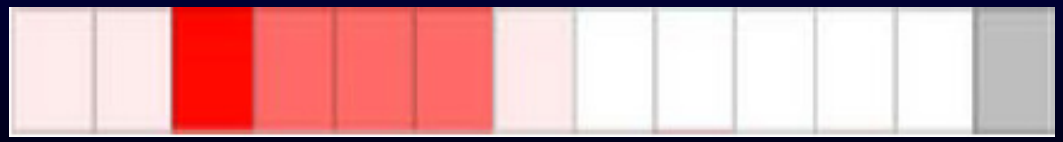


NCR1 is an NF-κB activator in A2780 OC cells



Expression of NCR-1 in cancer

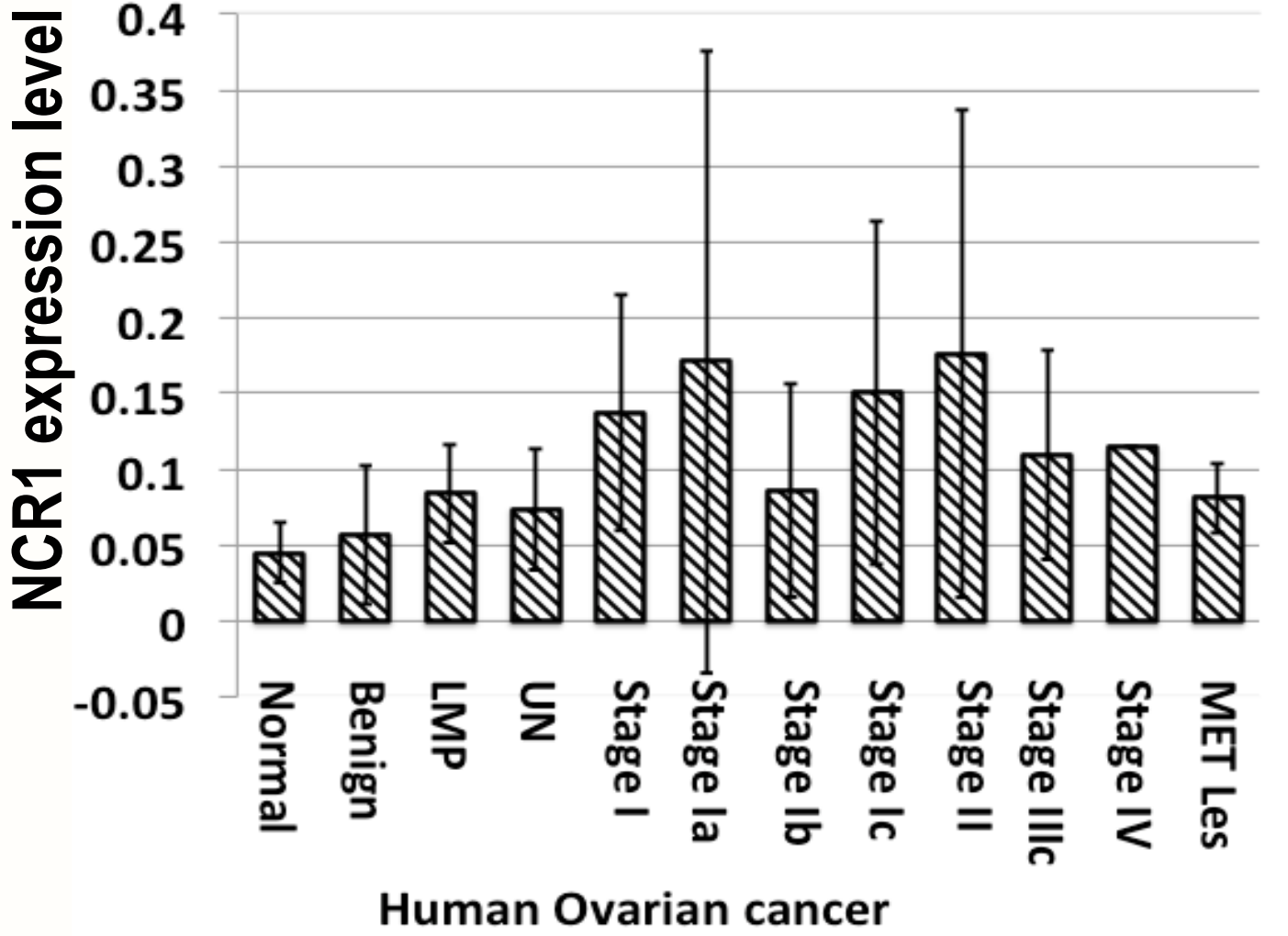
Ovarian cancer



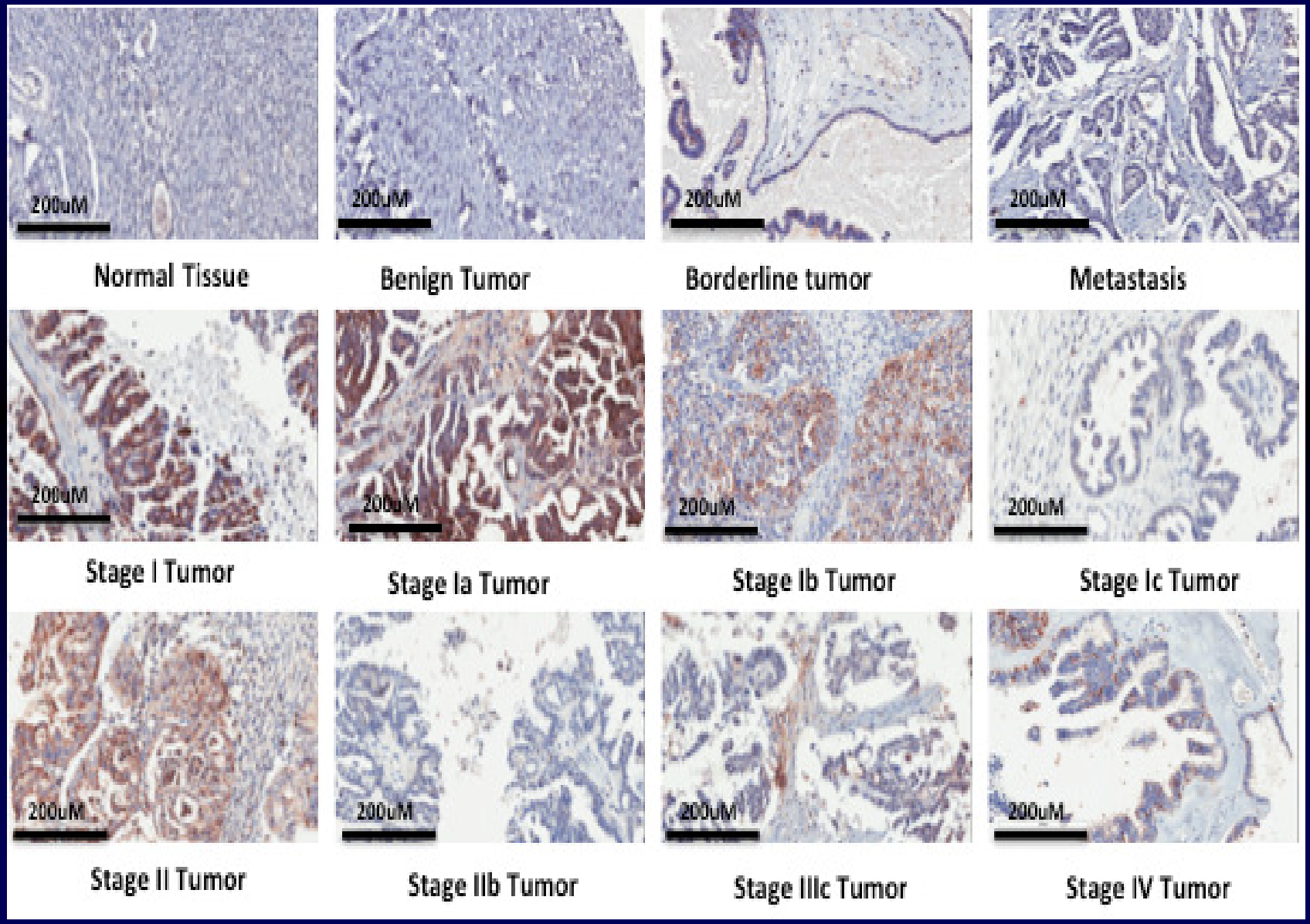
Breast cancer

Oncomine data

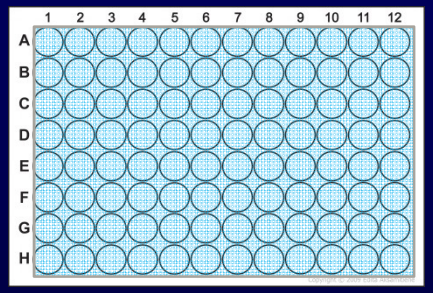
Correlation between NCR1 expression and OC (Tumor microarray)



NCR is overexpressed in OC

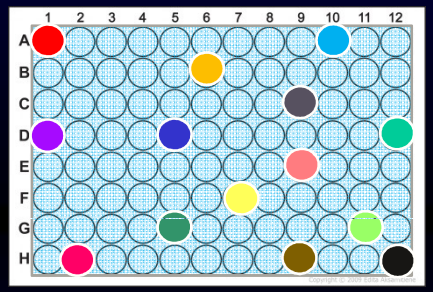


VBIM and high throughput screen (HTS) of drug resistance gene in OC cells

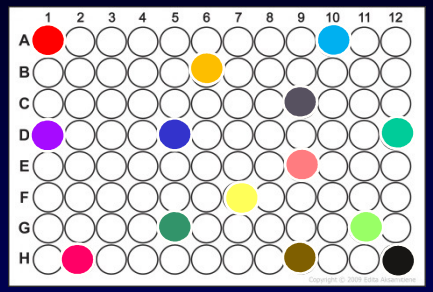


Plated A2780 ovarian cells

VBIM virus



carboplatin



Drug resistant mutant cells

Options:

1. Multiple plates
2. Multiple drugs

whole genome RNAseq

PCR and targeted RNAseq experiment

data

Bioinformatics analysis

- Oncomine
- TCGA
- ICGC

Expression profile in ovarian cancer cells & tissues

Significance

1. Lead to the discovery of novel carboplatin resistant genes in ovarian cancer.

2. Yield mechanisms of gene-mediated carboplatin resistance, so that reversal or bypass of this resistance can be achieved by developing small chemical inhibitors in ovarian cancer.

3. In a broader scope, the findings in ovarian cancer would further shed light on mechanisms of carboplatin resistance in other cancers as well.

Successful applications of VBIM technology

1. Lu T*, Jackson MW, Singhi AD, Kandel ES, Yang MJ, Zhang Y, Gudkov AV, and Stark GR*. (2009). Validation-based insertional mutagenesis identifies lysine demethylase FBXL11 as a negative regulator of NF-kB. *Proc Natl Acad Sci USA. 106, 16339-16344. (*corresponding authors).*

2. Lu T*, Jackson MW, Wang B, Yang M, Chance M, Miyagi M, Gudkov AV, and Stark GR*. (2010). Regulation of NF-kB by NSD1/FBXL11-dependent reversible lysine methylation of p65. *Proc Natl Acad Sci USA. 107, 46-51. (*corresponding authors).*

3. Lu T, Stark GR. (2010). Use of forward genetics to discover novel regulators of NF-kB. *Cold Spring Harb Perspect Biol. a001966.*

4. De S, et al. (2009). Overexpression of kinesins mediates docetaxel resistance in breast cancer cells. *Cancer Res.* 69(20):8035-8042.

5. Guo C, et al. (2011). FER tyrosine kinase (FER) overexpression mediates resistance to quinacrine through EGF-dependent activation of NF-kB. *Proc Natl Acad Sci U S A. 108(19):7968-7973.*

6. Tan MH et al. (2012). Specific kinesin expression profiles associated with taxane resistance in basal-like breast cancer. *Breast Cancer Res Treat.* 131(3):849-58.

7. Cipriano R, et al. (2012).FAM83B mediates EGFR- and RAS-driven oncogenic transformation. *J Clin Invest.* 122(9):3197-210.

8. Wang B, Zhang X, Zhao Z. (2013). Validation-based insertional mutagenesis for identification of Nup214 as a host factor for EV71 replication in RD cells. *Biochem Biophys Res Commun*. 437(3):452-6.

9. Cipriano R et al. (2013). FAM83B-mediated activation of PI3K/AKT and MAPK signaling cooperates to promote epithelial cell transformation and resistance to targeted therapies. *Oncotarget 4(5):729-38.*

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