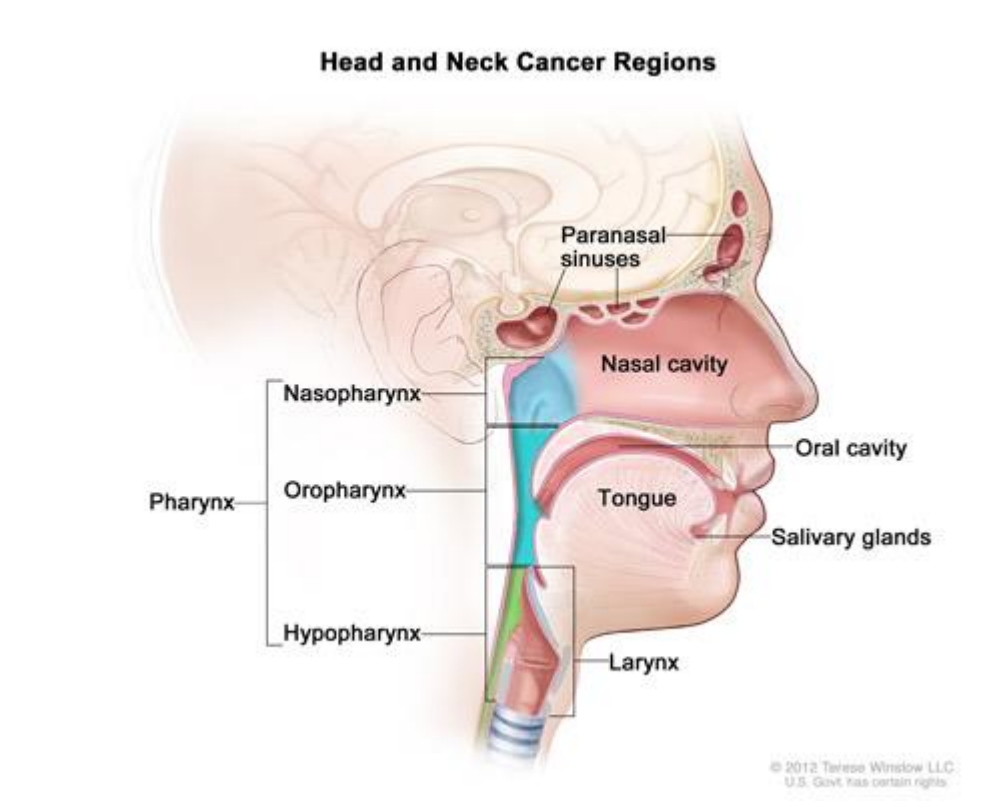
CONTEXT SPECIFIC ROLE OF DEUBIQUITYLASE ENZYME, USP9X, IN HEAD AND NECK CANCER

Devathri Nanayakkara

Eskitis Institute for Drug Discovery

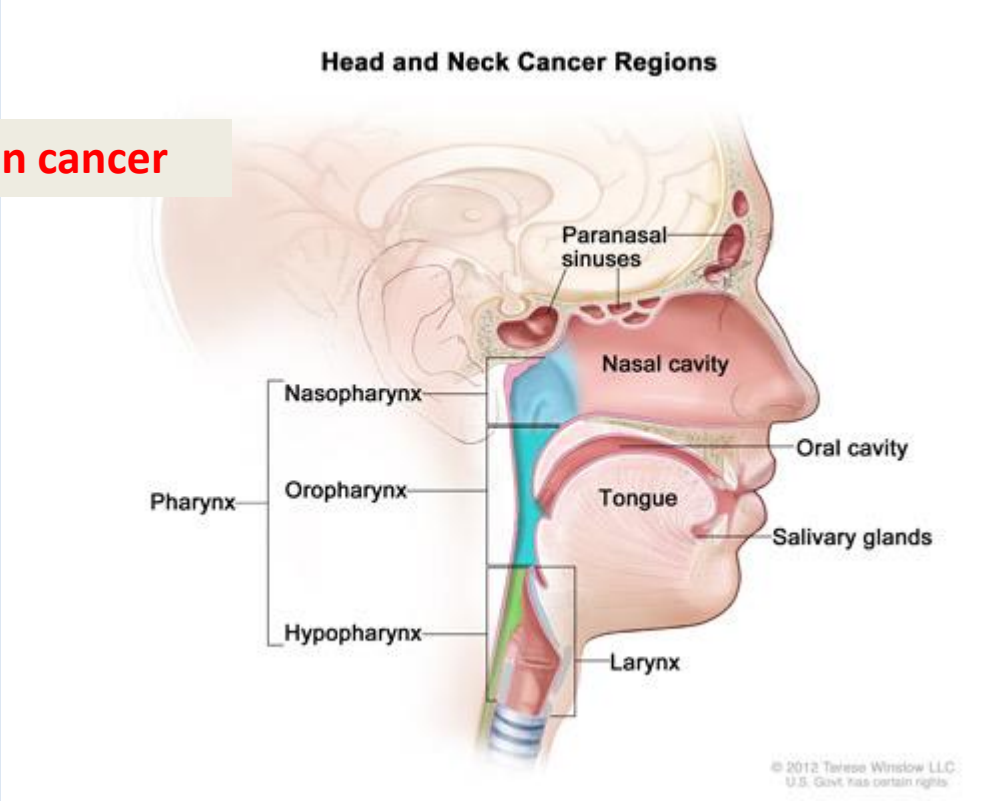
Griffith University

Head and neck cancer



Head and neck cancer

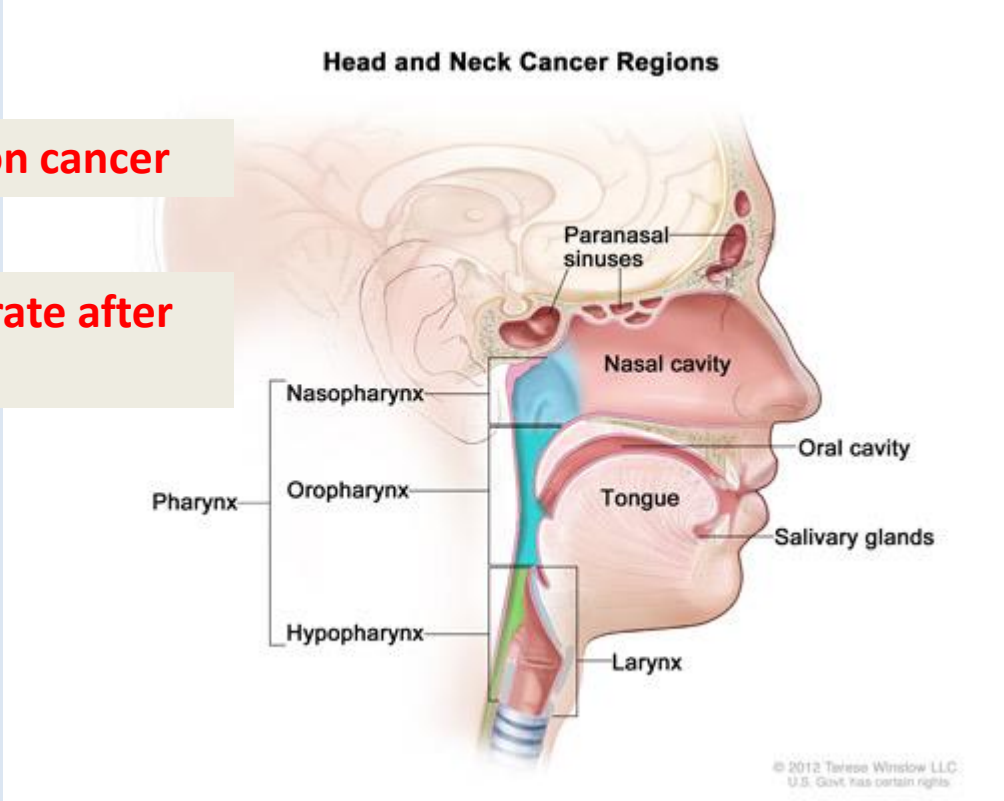
Sixth most common cancer



Head and neck cancer

Sixth most common cancer

Five year survival rate after diagnosis, 50%



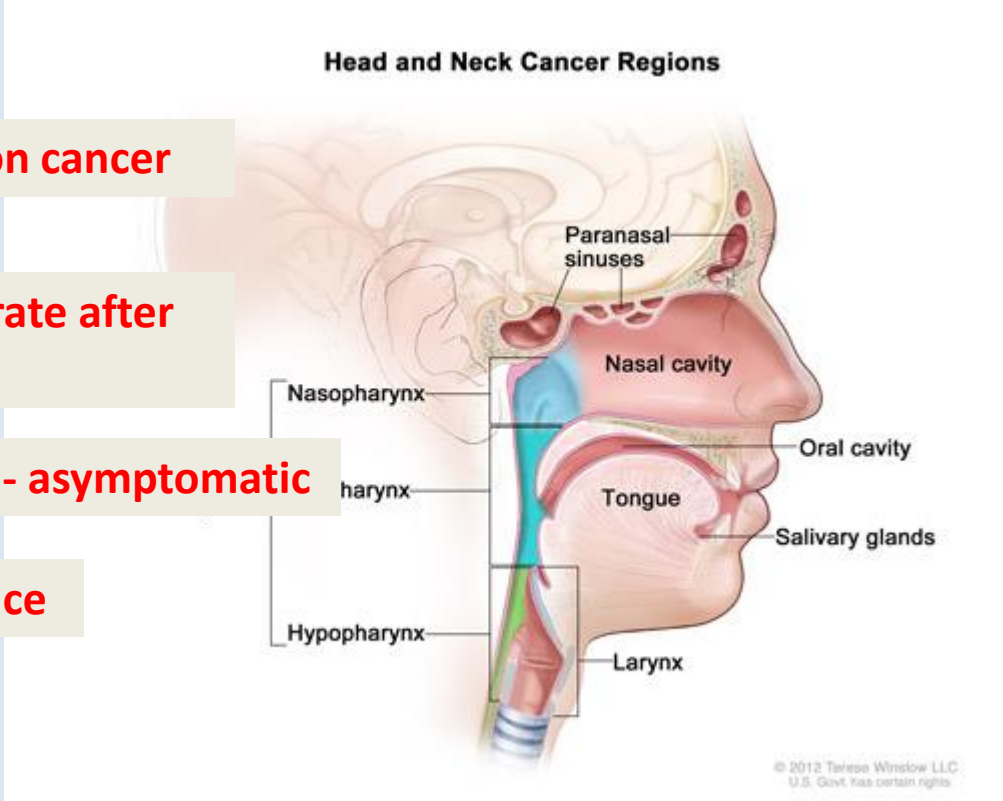
Head and neck cancer

Sixth most common cancer

Five year survival rate after diagnosis, 50%

Early tumors - asymptomatic

Drug resistance



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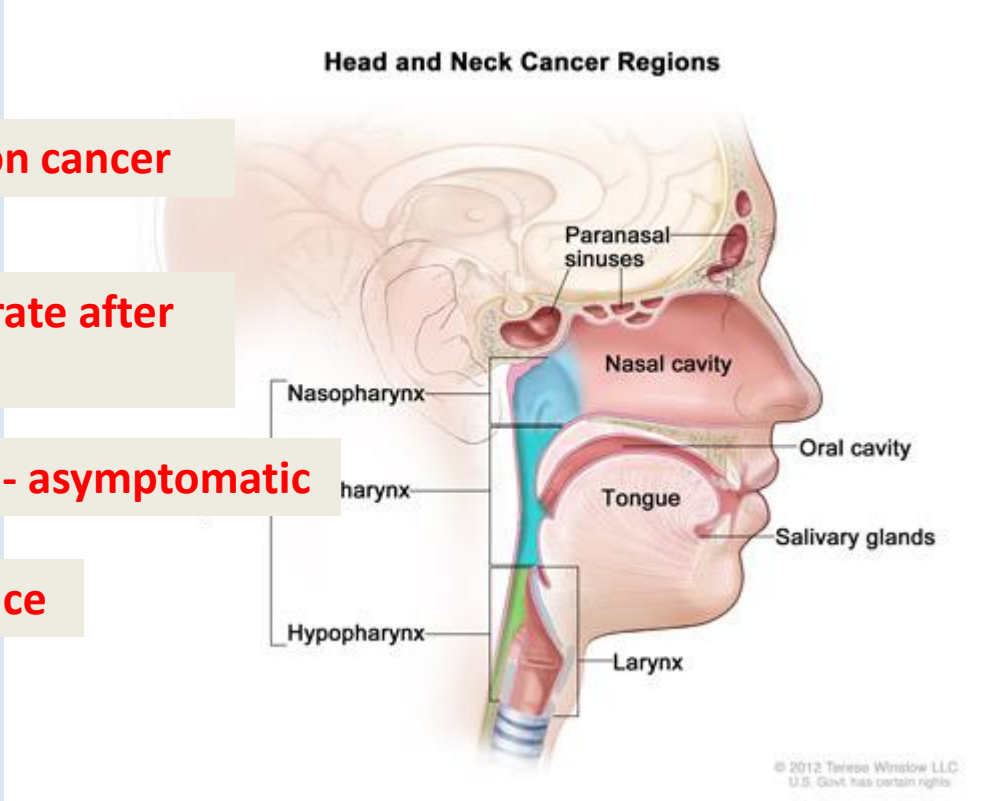
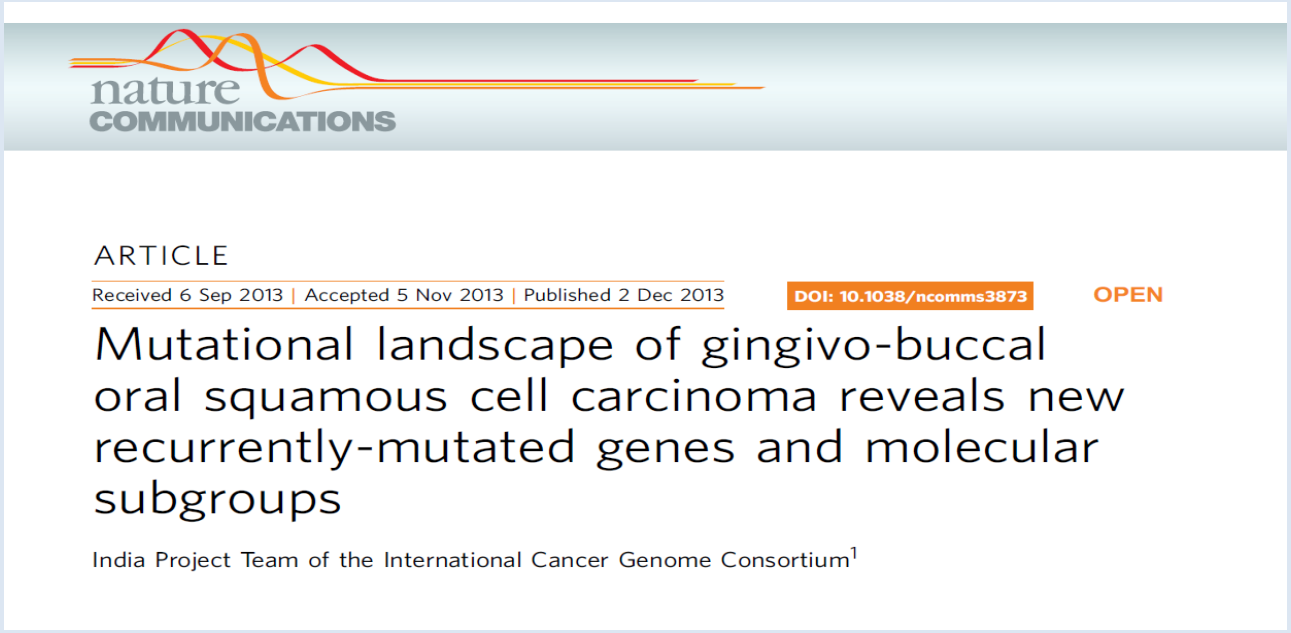


"Need to study the underlying molecular pathways unveiling potential detection markers and drug targets"

In a recent study,



nature COMMUNICATIONS

ARTICLE

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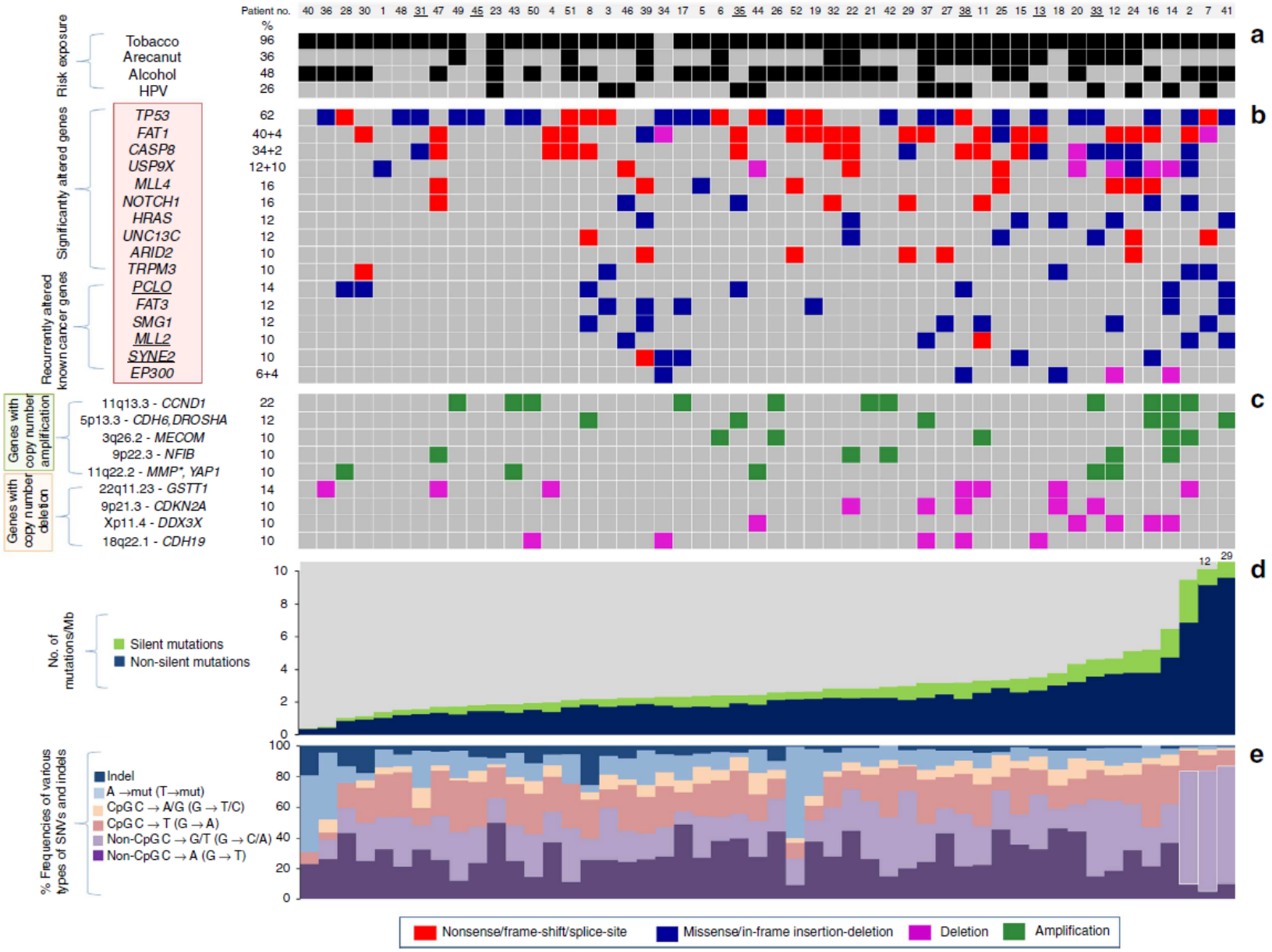
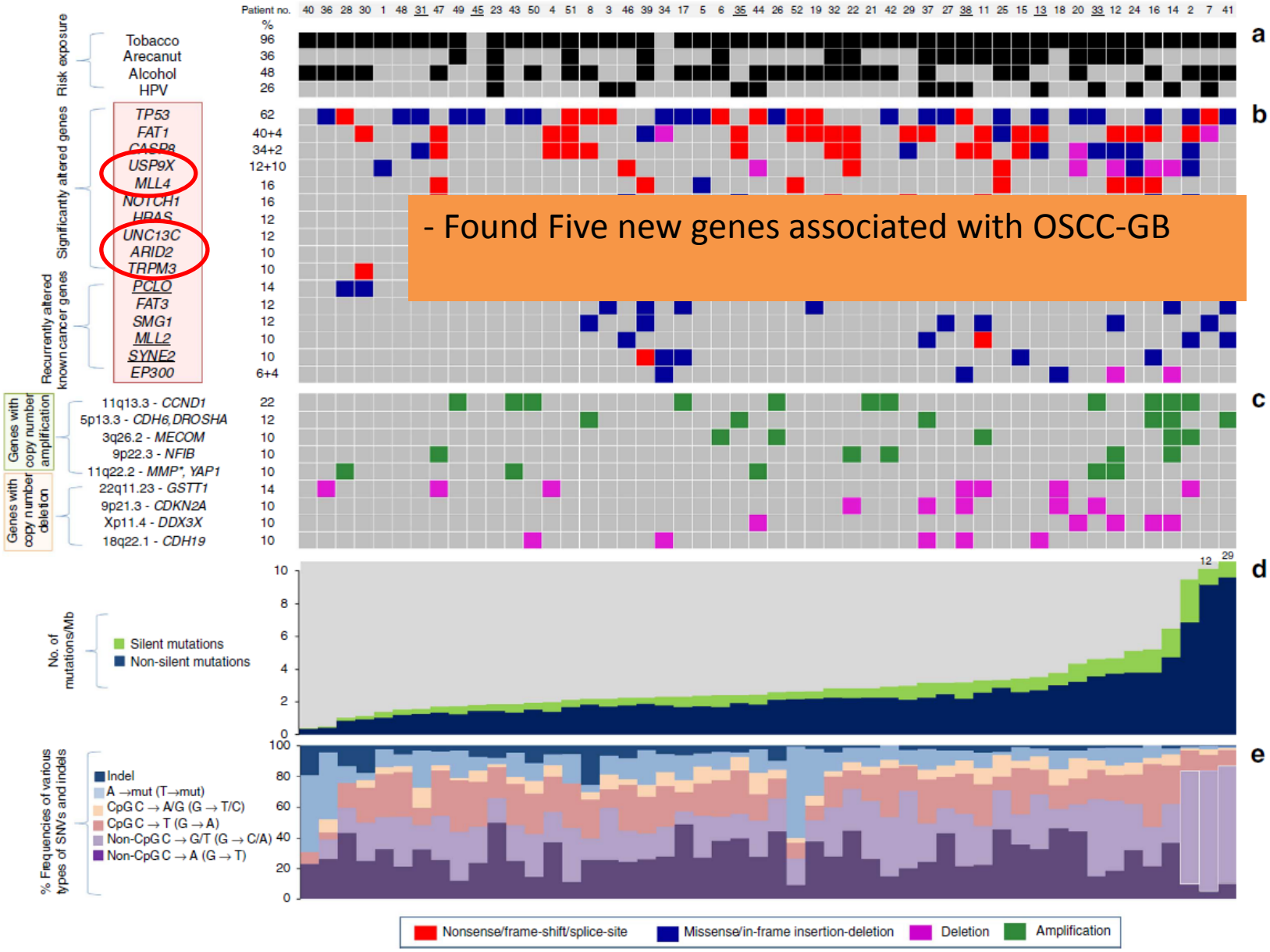
DOI: 10.1038/ncomms3873 OPEN

Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups

India Project Team of the International Cancer Genome Consortium[1]

- Characterized the somatic mutation landscape of OSCC-GB


Patient no.
40 36 28 30 1 48 31 47 49 45 23 43 50 4 51 8 3 46 39 34 17 5 6 35 44 26 52 19 32 22 21 42 29 37 27 38 11 25 15 13 18 20 33 12 24 16 14 2 7 41
%
Risk exposure
Tobacco 96
Arecanut 36
Alcohol 48
HPV 26
a
Significantly altered genes
Recurrently altered known cancer genes
TP53 62
FAT1 40+4
CASP8 34+2
USP9X 12+10
MLL4 16
NOTCH1 16
HRAS 12
UNC13C 12
ARID2 10
TRPM3 10
PCLO 14
FAT3 12
SMG1 12
MLL2 10
SYNE2 10
EP300 6+4
b
Genes with copy number amplification
11q13.3 - CCND1 22
5p13.3 - CDH6,DROSHA 12
3q26.2 - MECOM 10
9p22.3 - NFIB 10
11q22.2 - MMP*, YAP1 10
Genes with copy number deletion
22q11.23 - GSTT1 14
9p21.3 - CDKN2A 10
Xp11.4 - DDX3X 10
18q22.1 - CDH19 10
c
No. of mutations/Mb
Silent mutations
Non-silent mutations
10 8 6 4 2 0
12 29
d
% Frequencies of various types of SNVs and indels
Indel
A →mut (T→mut)
CpG C → A/G (G → T/C)
CpG C → T (G → A)
Non-CpG C → G/T (G → C/A)
Non-CpG C → A (G → T)
100 80 60 40 20 0
e
Nonsense/frame-shift/splice-site
Missense/in-frame insertion-deletion
Deletion
Amplification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60
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Nonsense/frame-shift/splice-site
Missense/in-frame insertion-deletion
Deletion
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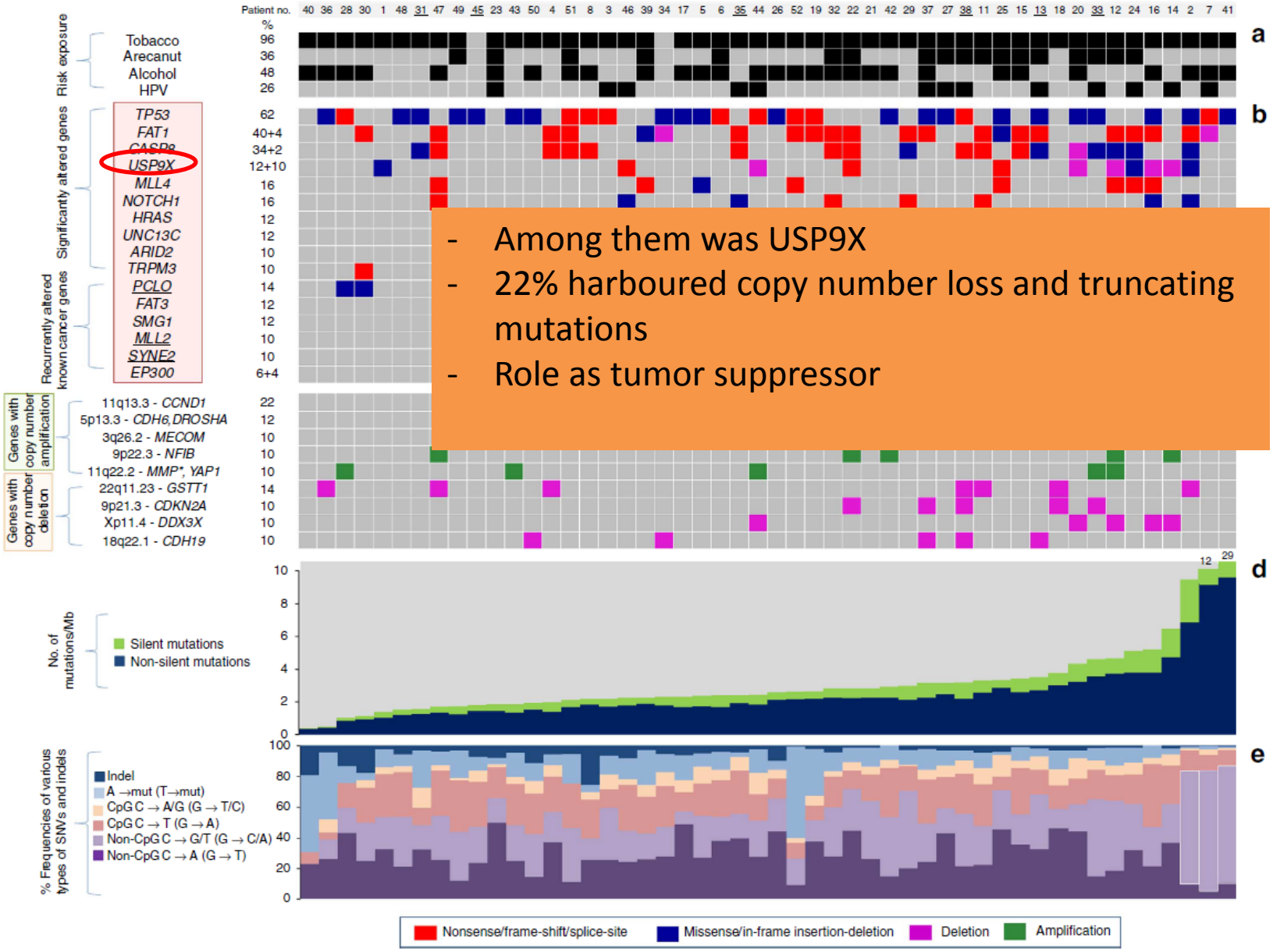


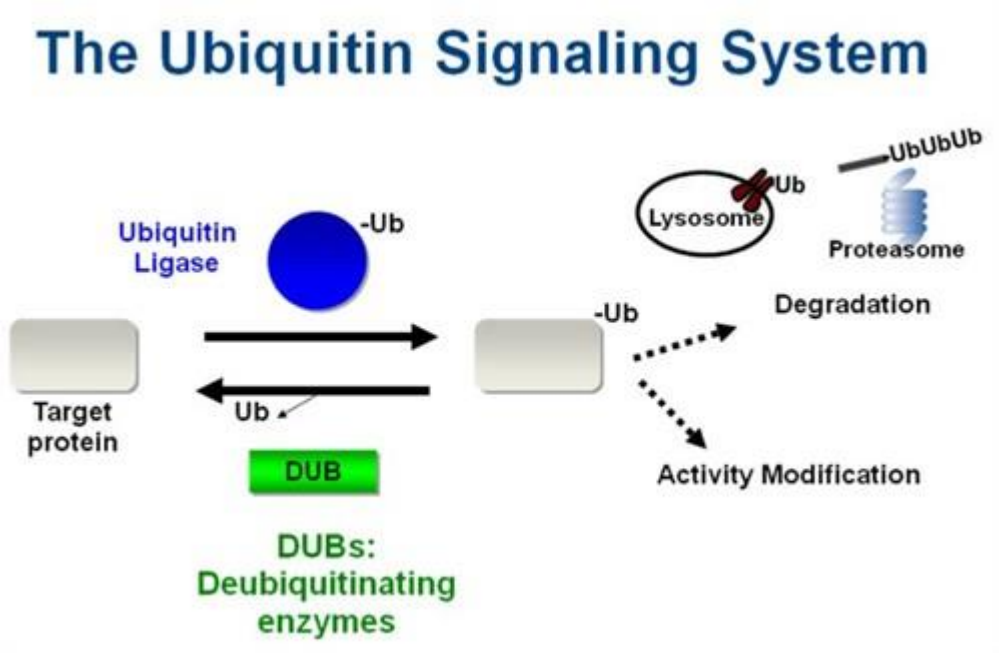
- Among them was USP9X
- 22% harboured copy number loss and truncating mutations
- Role as tumor suppressor

USP9X

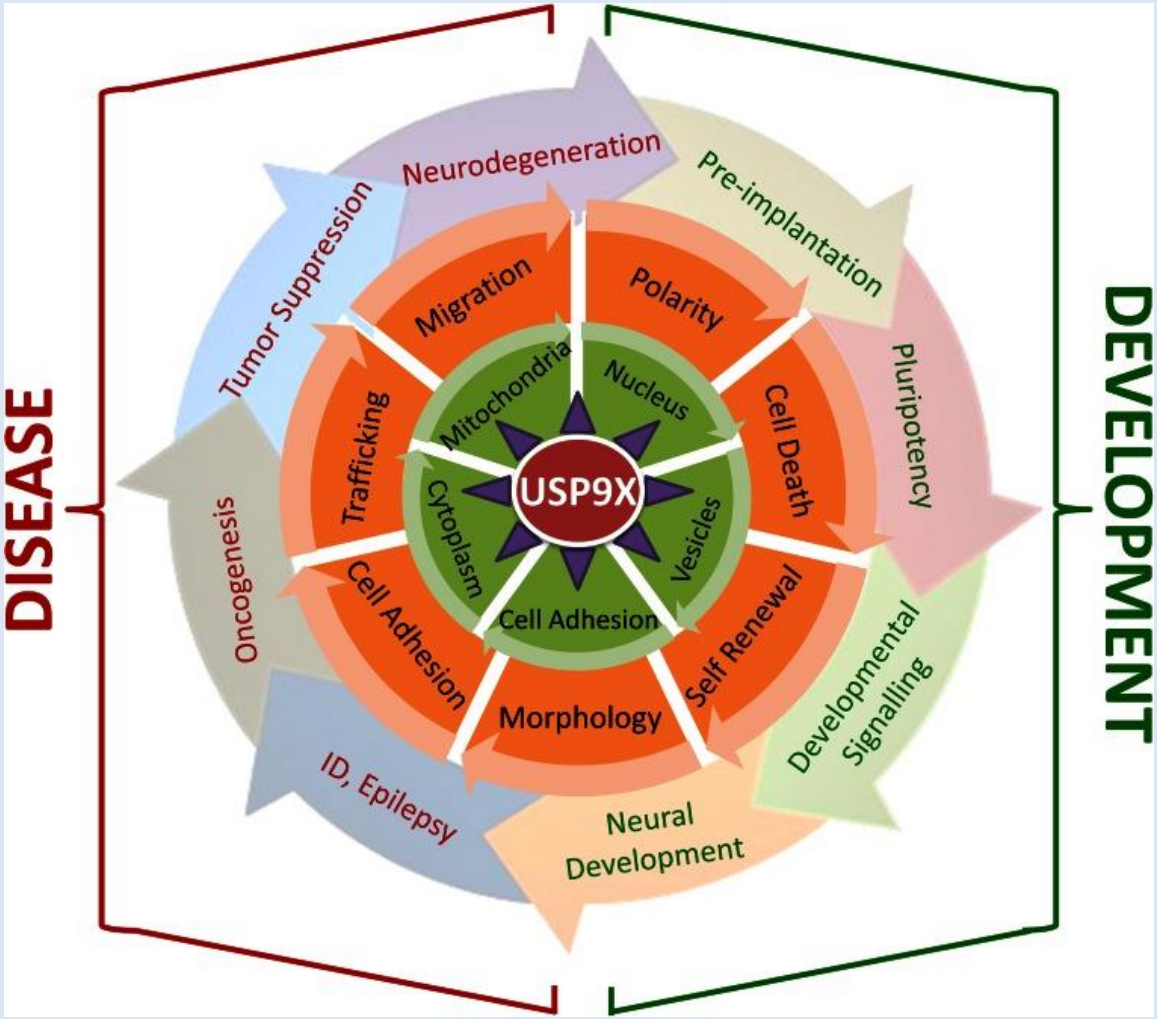
- Deubiquitylating enzyme
  - Family of cysteine and metalloproteases

USP9X

- Deubiquitylating enzyme
  - Family of cysteine and metalloproteases



http://legacy.butler.edu/biology/faculty-staff/research-interests/jkowalski/research-in-the-kowalski-lab/



Murtaza et al, 2015

USP9X in cancer

USP9X in cancer

Therapeutics, Targets, and Chemical Biology

Cancer Research

USP9X Downregulation Renders Breast Cancer Cells Resistant to Tamoxifen

Hendrika M. Oosterkamp[1], E. Marielle Hijmans[1], Thijn R. Brummelkamp[1], Sander Canisius[1], Lodewyk F.A. Wessels[1], Wilbert Zwart[2], and René Bernards[1]

LETTERS

Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival

Martin Schwickart[1]*, XiaoDong Huang[1]*, Jennie R. Lill[2], Jinfeng Liu[3], Ronald Ferrando[4], Dorothy M. French[4], Heather Maecker[5], Karen O'Rourke[1], Fernando Bazan[6], Jeffrey Eastham-Anderson[4], Peng Yue[3], David Dornan[7], David C. S. Huang[8] & Vishva M. Dixit[1]

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RESEARCH PAPER

Cancer Biology & Therapy 13:13, 1319–1324; November 2012; © 2012 Landes Bioscience

Genetic disruption of *USP9X* sensitizes colorectal cancer cells to 5-fluorouracil

Dennis R. Harris,[1] Alexandra Mims[2] and Fred Bunz[1,2,*]

Cui et al. Cancer Cell International (2015) 15:4
DOI 10.1186/s12935-014-0149-x

CANCER CELL INTERNATIONAL

PRIMARY RESEARCH Open Access

EHMT2 inhibitor BIX-01294 induces apoptosis through PMAIP1-USP9X-MCL1 axis in human bladder cancer cells

Jing Cui[1†], Wendong Sun[2†], Xuexi Hao[2], Minli Wei[1], Xiaonan Su[1], Yajing Zhang[1], Ling Su[1] and Xiangguo Liu[1*]

OPEN ACCESS Freely available online

PLOS ONE

The SOX2-Interactome in Brain Cancer Cells Identifies the Requirement of MSI2 and USP9X for the Growth of Brain Tumor Cells

Jesse L. Cox[1☯], Phillip J. Wilder[1☯], Joshua M. Gilmore[2,3], Erin L. Wuebben[1], Michael P. Washburn[2,3], Angie Rizzino[1*]

CrossMark

Ablation of the oncogenic transcription factor ERG by deubiquitinase inhibition in prostate cancer

Shan Wang[a], Rahul K. Kollipara[a], Nishi Srivastava[a], Rui Li[b], Preethi Ravindranathan[b], Elizabeth Hernandez[b], Eva Freeman[b], Caroline G. Humphries[a], Payal Kapur[b,c], Yair Lotan[b], Ladan Fazli[d], Martin E. Gleave[d], Stephen R. Plymate[e], Ganesh V. Raj[b], Jer-Tsong Hsieh[b,f], and Ralf Kittler[a,f,g,h,1]

Original Article

Elevated expression of USP9X correlates with poor prognosis in human non-small cell lung cancer

You Wang[1*], Yu Liu[1*], Bo Yang[1], Hong Cao[2], Chun-Xu Yang[1], Wen Ouyang[1], Shi-Min Zhang[1], Gui-Fang Yang[2], Fu-Xiang Zhou[1,3], Yun-Feng Zhou[1,3], Cong-Hua Xie[1,3]

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Therapeutics, Targets, and Chemical Bi

Breast cancer

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LETTERS

Deubiquitinase USP9X
tumour cell survival

Lymphoma

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OPEN

Mutational landscape of gingivo-buccal
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recurrently-mutated
subgroups

Oral cancer

India Project Team of the International Cancer Genome Consortium[1]

LETTER

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The deubiquitinase USP9X suppresses pancreatic
ductal adenocarcino

Pancreatic cancer

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CANCER CELL INTERNATIONAL

PRIMARY RESEARCH Open Access

EHMT2 inhibitor BIX-
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bladder cancer cells

Bladder cancer

Jing Cui[1†], Wendong Sun[2†], Xuexi Hao[2], Minli Wei[1], Xiaonan Su[1], Yajing Zhang[1], Ling Su[1] and Xiangguo Liu[1*]

OPEN ACCESS Freely available online

PLOS ONE

The SOX2-Interactome in
Requirement of MSI2 an
Tumor Cells

Brain cancer

Brain

Jesse L. Cox[1☯], Phillip J. Wilder[1☯], Joshua M. Gilmore[2,3], Erin L. Wuebben[1], Michael P. Washburn[2,3], Angie Rizzino[1*]

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Lung cancer

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In this study,

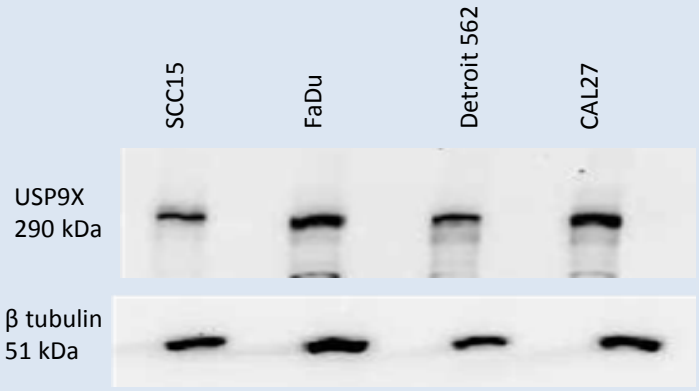
Aims :

- To evaluate the role of USP9X in an *in vitro* system
- To elucidate the molecular mechanisms USP9X is involved in

How?

- *In vitro* cell lines
  - SCC15, CAL27, FaDu and Detroit 562

Immunoblotting to probe for USP9X expression



All 4 cell lines express USP9X

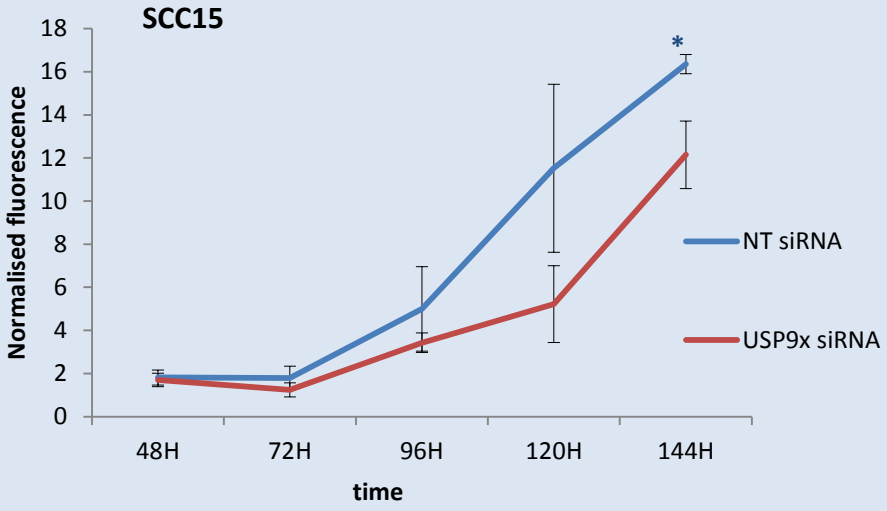
- Knockdown approach
  - siRNA

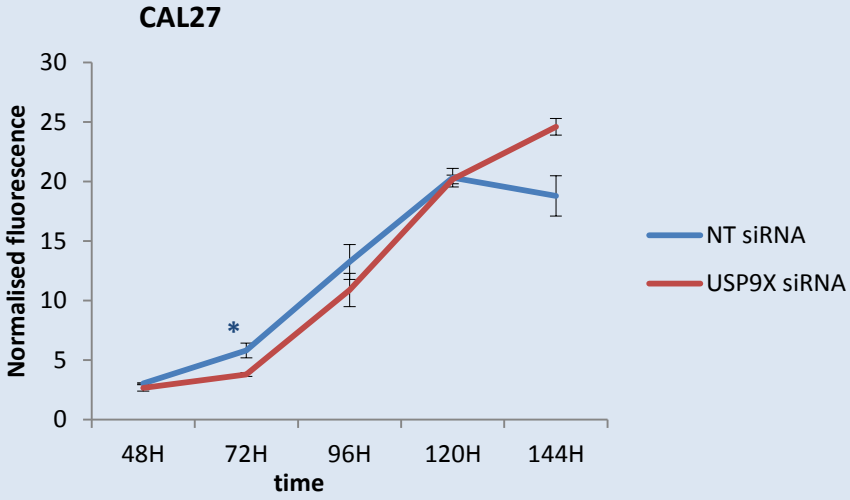
Immunoblotting to probe for USP9X protein levels 72 h after siRNA treatment

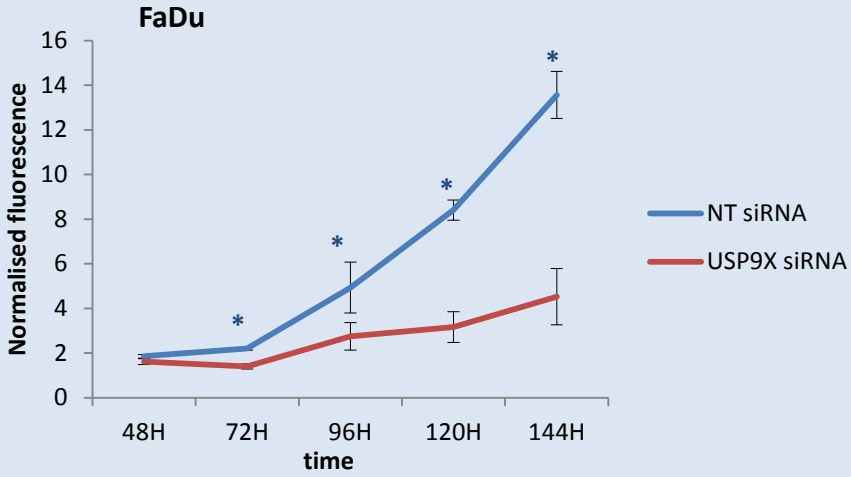
USP9X is efficiently knocked down in all four cell lines

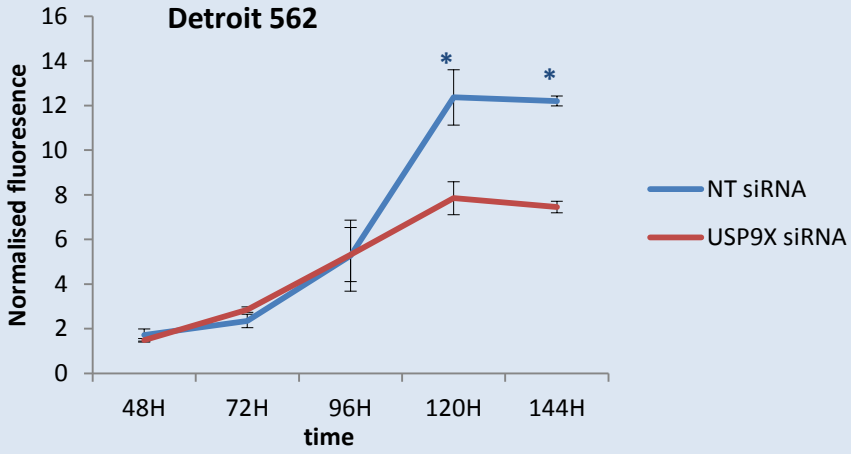
- Effect on cell aspects
  - Cell proliferation
    - CyQUANT Assay

CyQUANT Analysis of cell proliferation following siRNA treatment to knockdown USP9X









* P value < 0.05

In absence of USP9X, a decrease in cell numbers was observed

- Decrease in cell numbers,
  - Apoptosis?

Immunoblotting for cleaved PARP-1

No elevation in apoptosis detected upon depletion of USP9X

Decrease in cell numbers in absence of USP9X prompts a role of a

Decrease in cell numbers in absence of USP9X prompts a role of a **“tumor promoter”**

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- Contradicts predicted oncosupressive role
- Context specific

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  - Pancreatic cancer

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RESEARCH PAPER

Cancer Biology & Therapy 15:8, 1042–1052; August 2014; © 2014 Landes Bioscience

Context-dependent function of the deubiquitinating enzyme USP9X in pancreatic ductal adenocarcinoma

Jesse L Cox, Phillip J Wilder, Erin L Wuebben, Michel M Ouellette, Michael A Hollingsworth, and Angie Rizzino*

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RESEARCH PAPER

Cancer Biology & Thera…

Tumor promotor

Context-dependent … of the deubiquitinating enzyme USP9X in pancreatic ductal adenocarcinoma

Jesse L Cox, Phillip J Wilder, Erin L Wuebben, Michel M Ouellette, Michael A Hollingsworth, and Angie Rizzino*

To further confirm,

- Overexpression of USP9X

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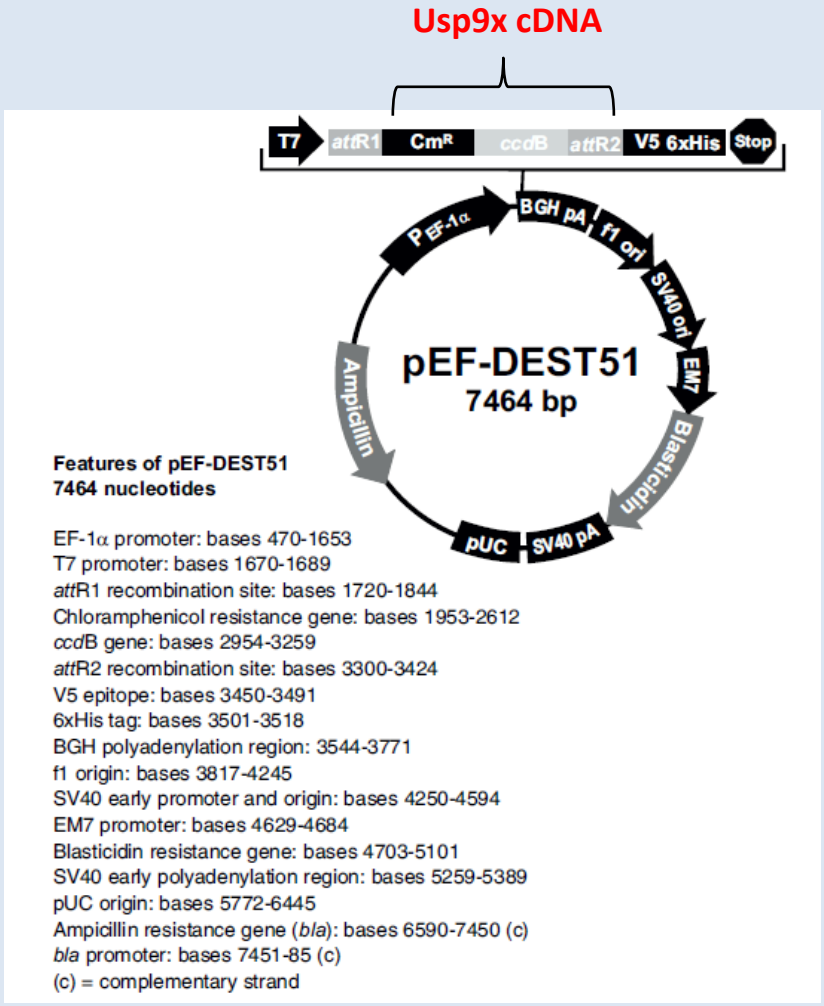
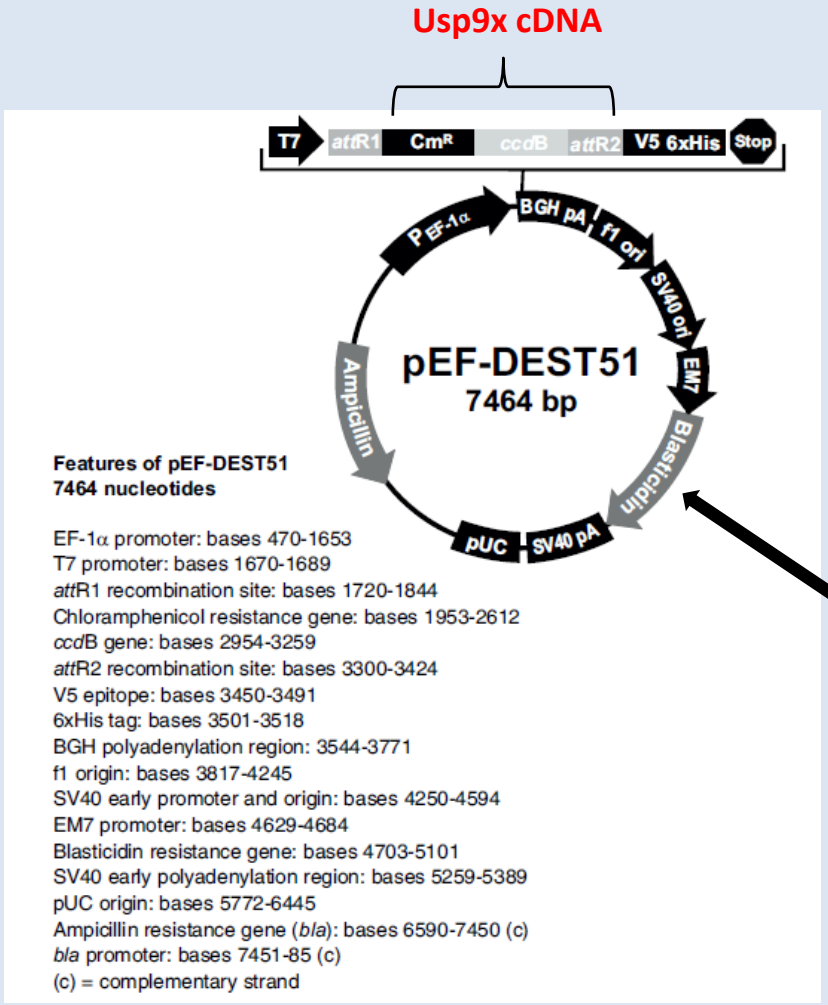


Features of pEF-DEST51
7464 nucleotides

EF-1α promoter: bases 470-1653
T7 promoter: bases 1670-1689
*att*R1 recombination site: bases 1720-1844
Chloramphenicol resistance gene: bases 1953-2612
*ccd*B gene: bases 2954-3259
*att*R2 recombination site: bases 3300-3424
V5 epitope: bases 3450-3491
6xHis tag: bases 3501-3518
BGH polyadenylation region: 3544-3771
f1 origin: bases 3817-4245
SV40 early promoter and origin: bases 4250-4594
EM7 promoter: bases 4629-4684
Blasticidin resistance gene: bases 4703-5101
SV40 early polyadenylation region: bases 5259-5389
pUC origin: bases 5772-6445
Ampicillin resistance gene (*bla*): bases 6590-7450 (c)
bla promoter: bases 7451-85 (c)
(c) = complementary strand

To further confirm,

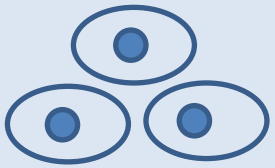
- Overexpression of USP9X



To establish **stable cell lines**,

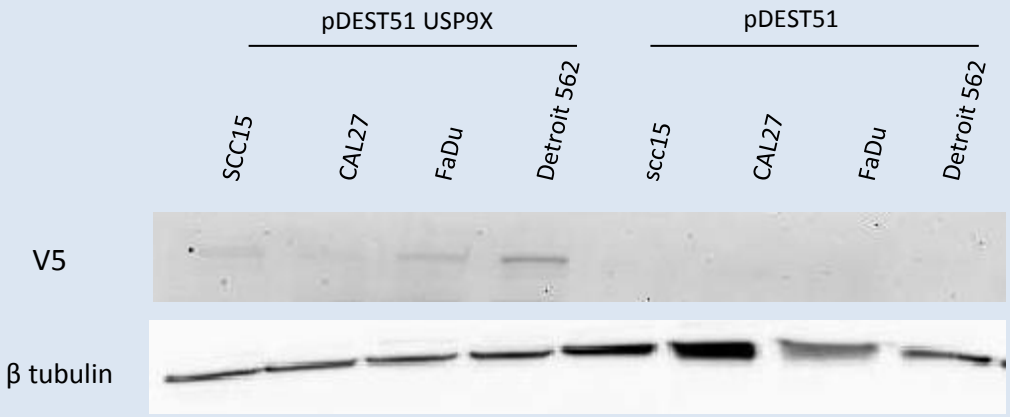
Linearized the plasmid

Lipofectamine 2000



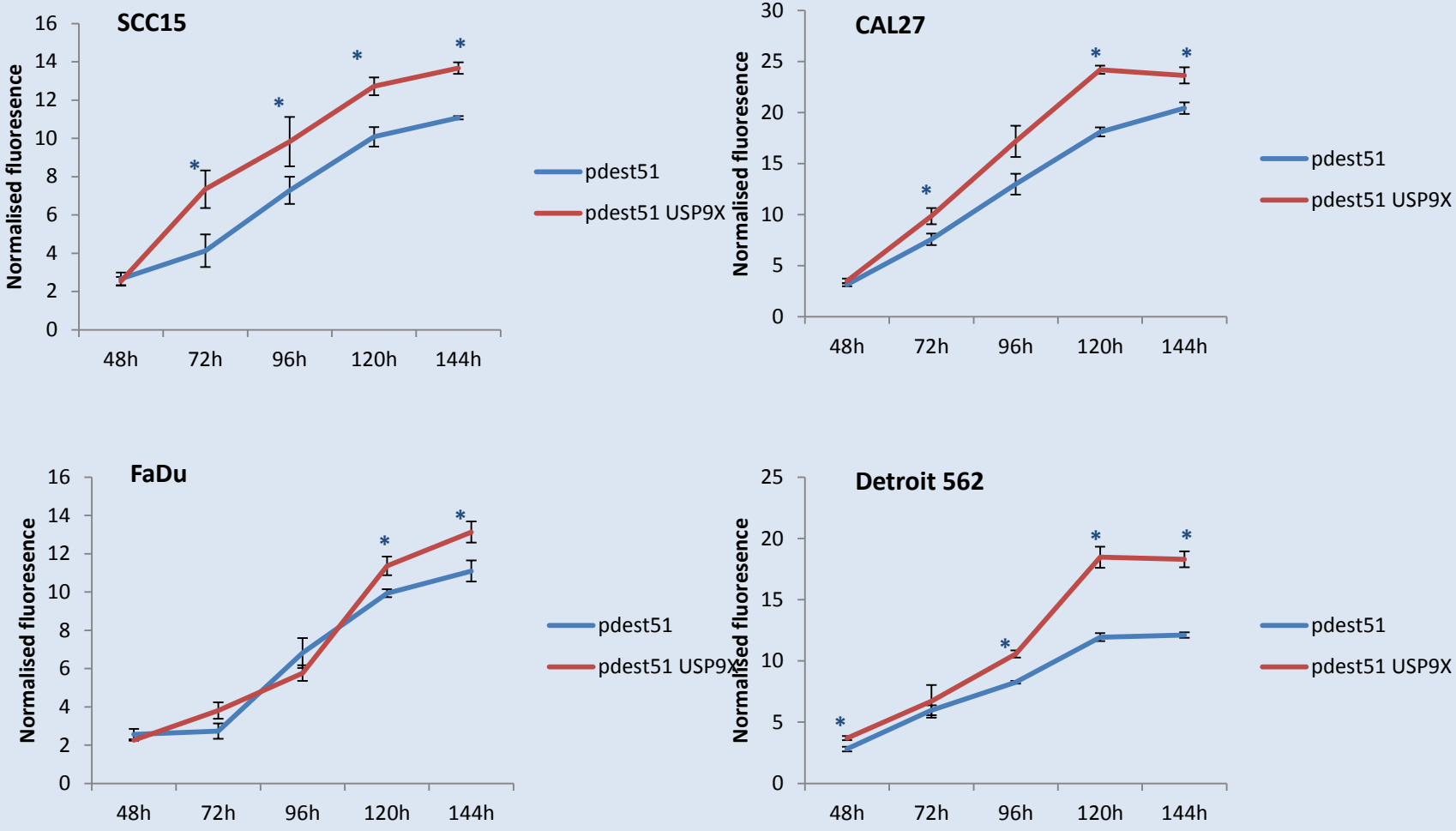
After 3 days:
antibiotic selection
(12 days)

Immunoblotting to detect ectopic expression of USP9X



USP9X is ectopically expressed in all four cell lines

CyQUANT Analysis of cell proliferation following ectopic expression of USP9X



* P value < 0.05

Ectopic USP9X protein expression increased cell proliferation

Cell numbers are directly proportional to level of USP9X protein

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has an **oncogenic role**

Molecular mechanism regulated by USP9X

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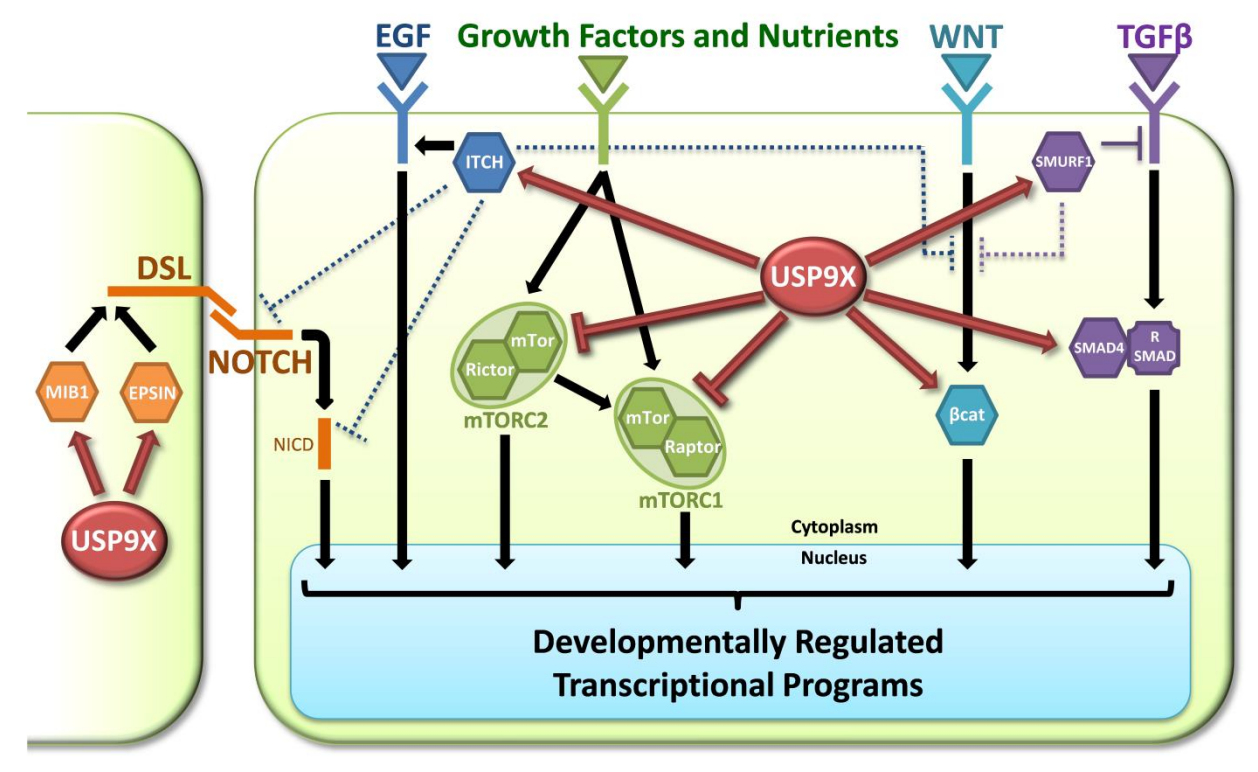

Murtaza et al, 2015

Molecular mechanism regulated by USP9X

- mTOR
- Wnt
- Notch

- Regulates cell proliferation
- Known USP9X substrates

Molecular mechanism regulated by USP9X?

- mTOR
- Wnt
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CyclinD1, c-MYC, HES1

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Quantitate the RNA levels by qPCR

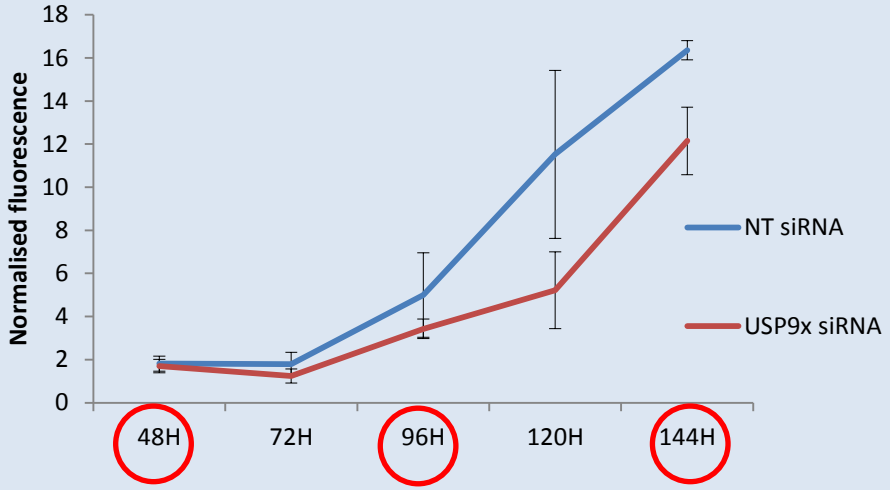
RNA extraction

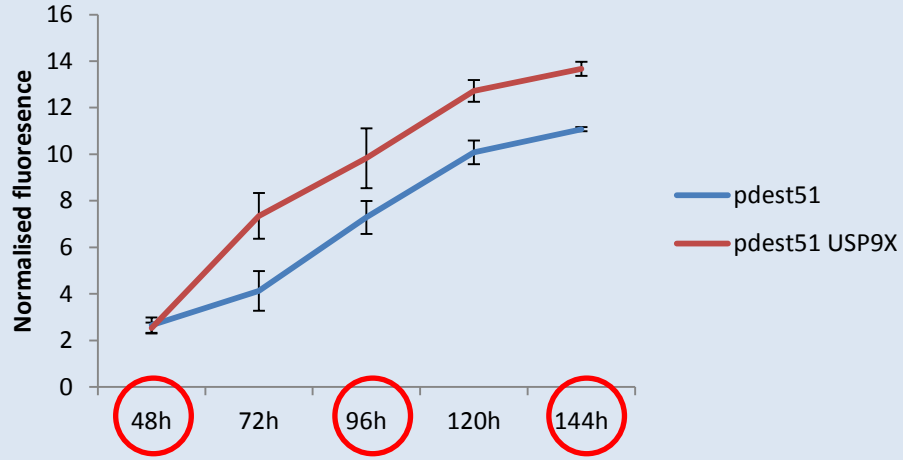
↓

cDNA

↓

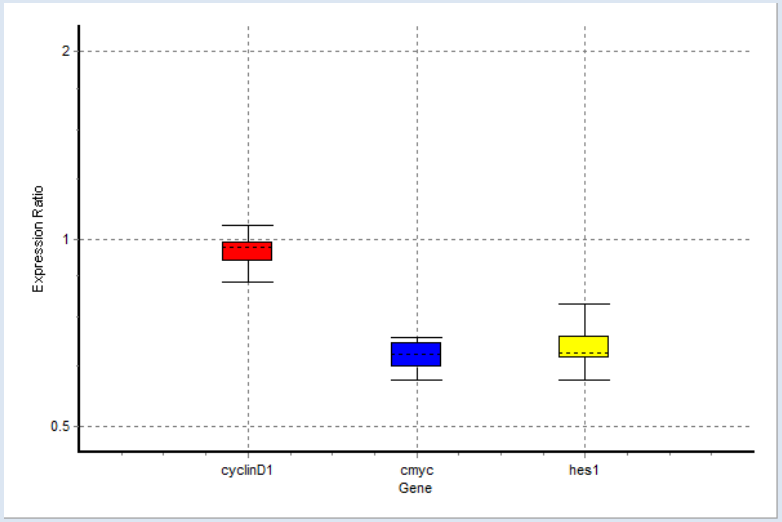
qPCR



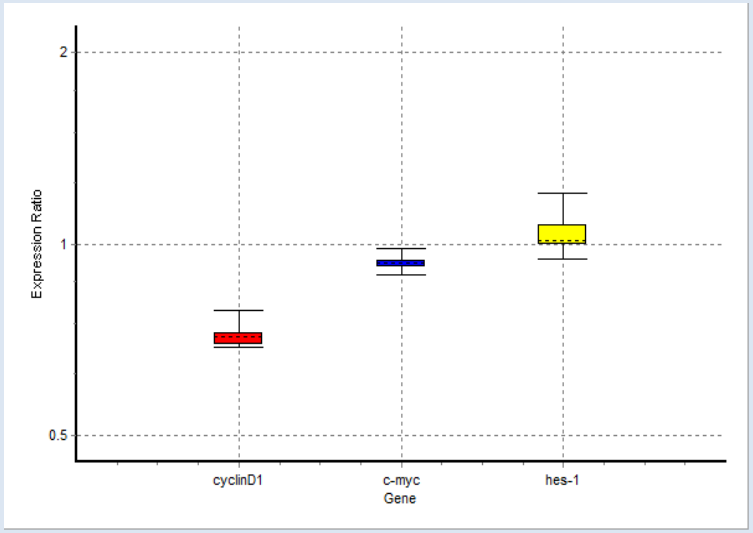


Fold change of target genes 144 h after knockdown of USP9X

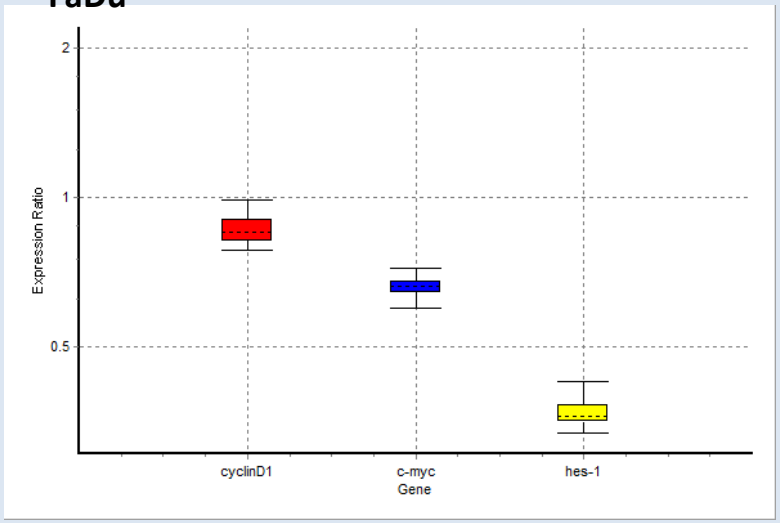
SCC15



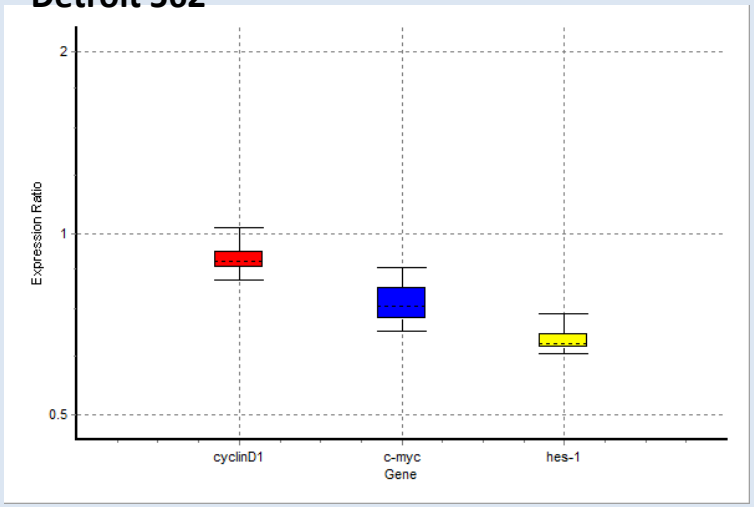
CAL27



FaDu



Detroit 562



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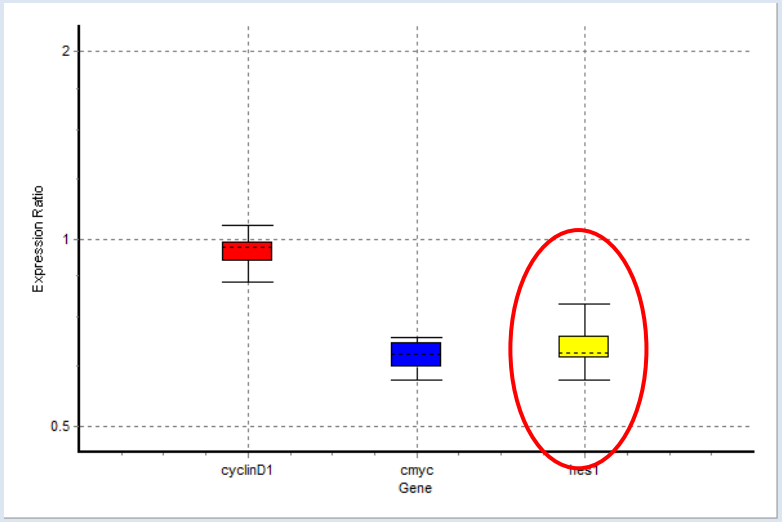

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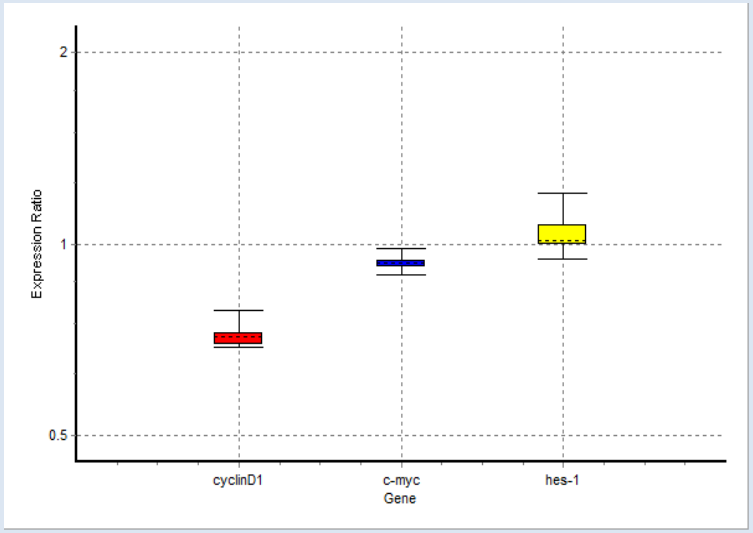

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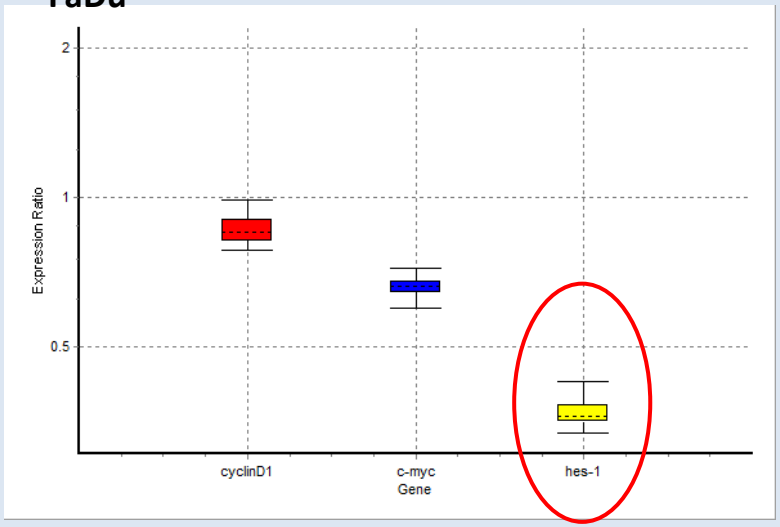

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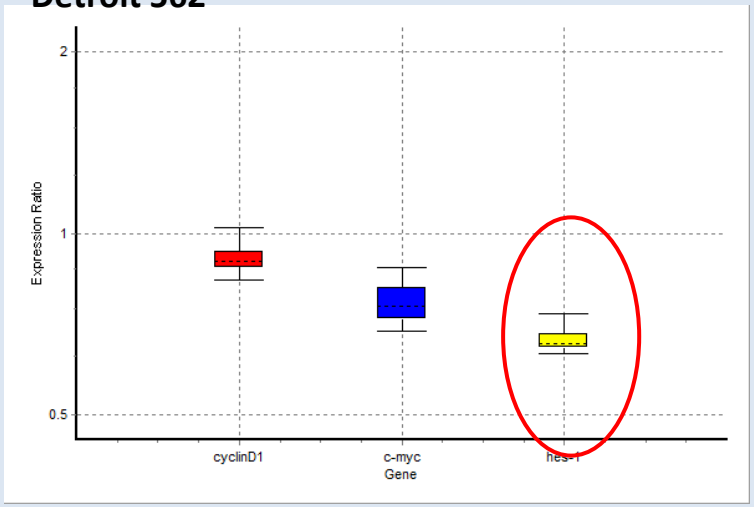

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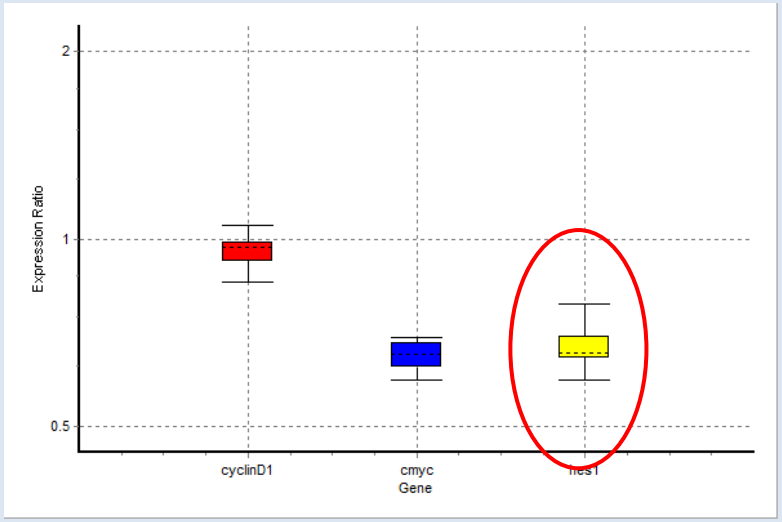

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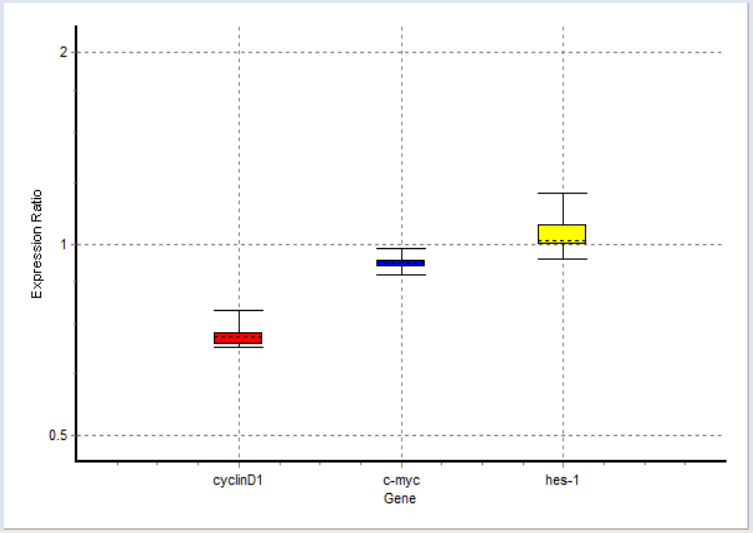

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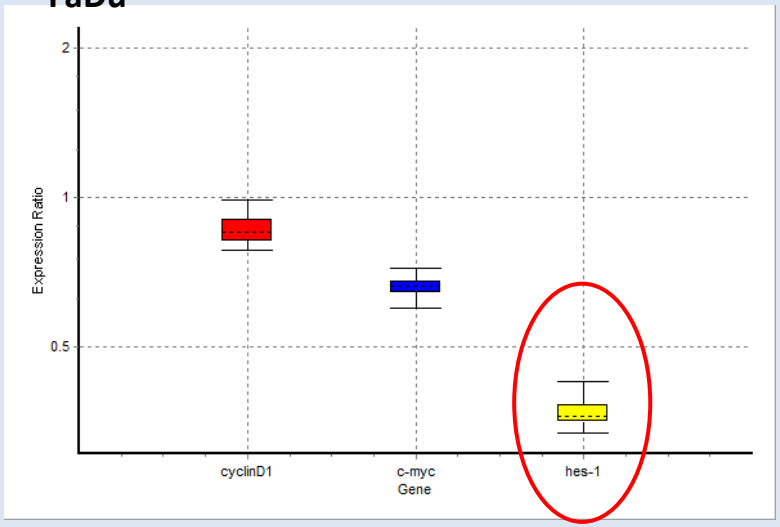

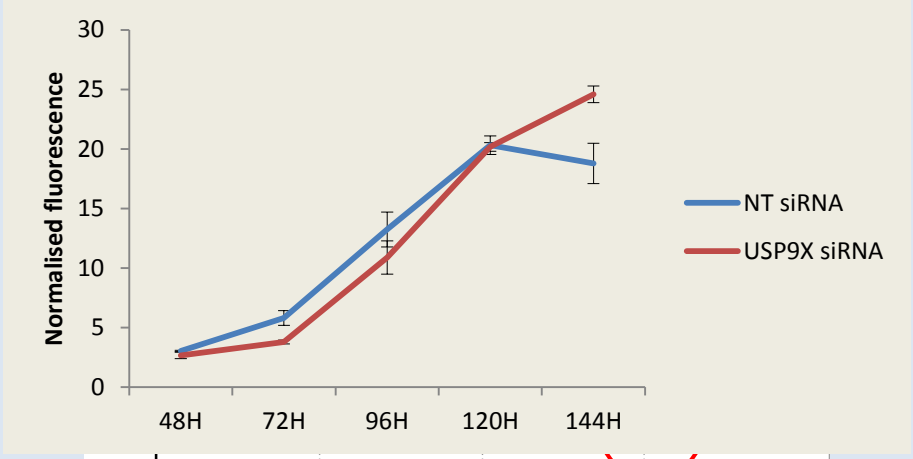

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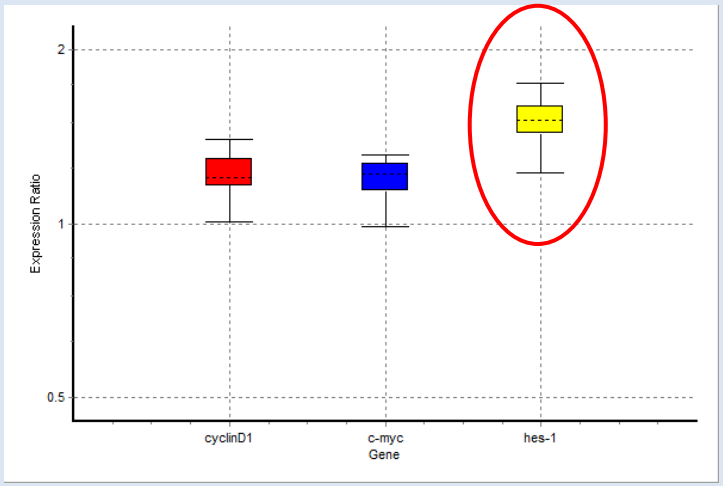

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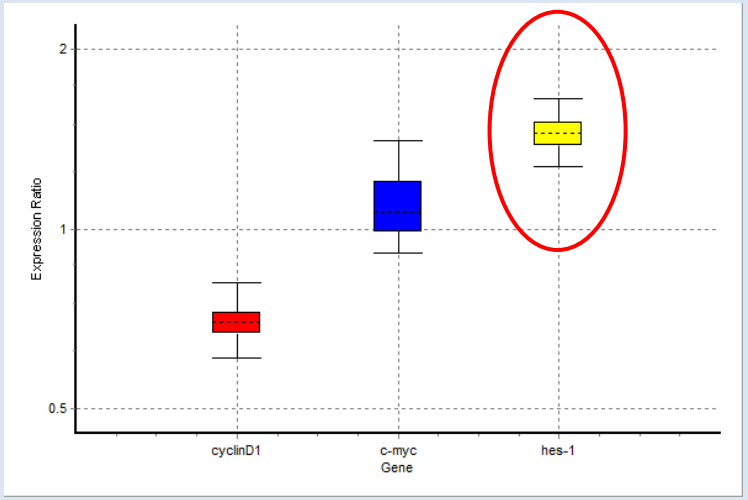

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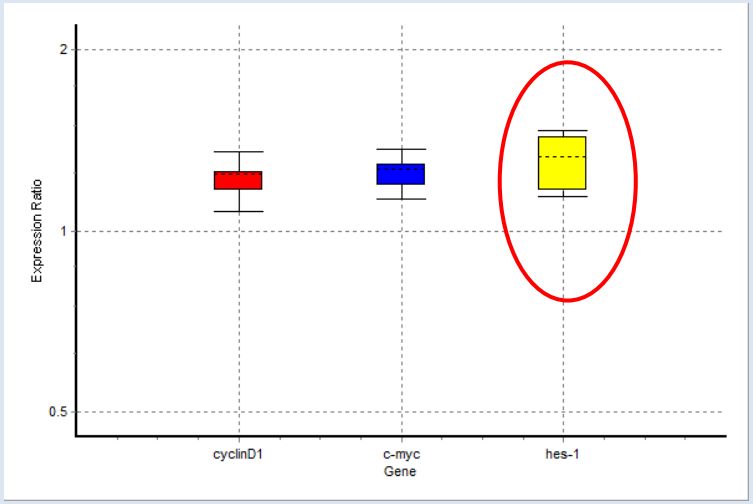

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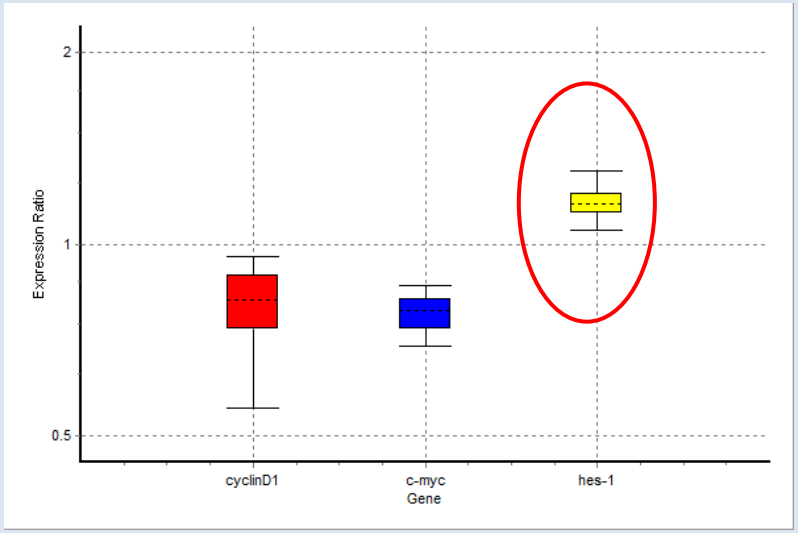

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- USP9X seems to positively regulate notch pathway

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Hypothesis: USP9X regulates proliferation of head and neck cancer cells through notch pathway

Conclusions

- USP9X depletion caused a decrease in cell proliferation
- Ectopic expression of USP9X led to increase in cell proliferation
- USP9X positively regulates Notch pathway

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George
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THANK YOU!