

EPIGENETIC MECHANISMS OF NICKEL CARCINOGENESIS

Max Costa

NYU School of Medicine

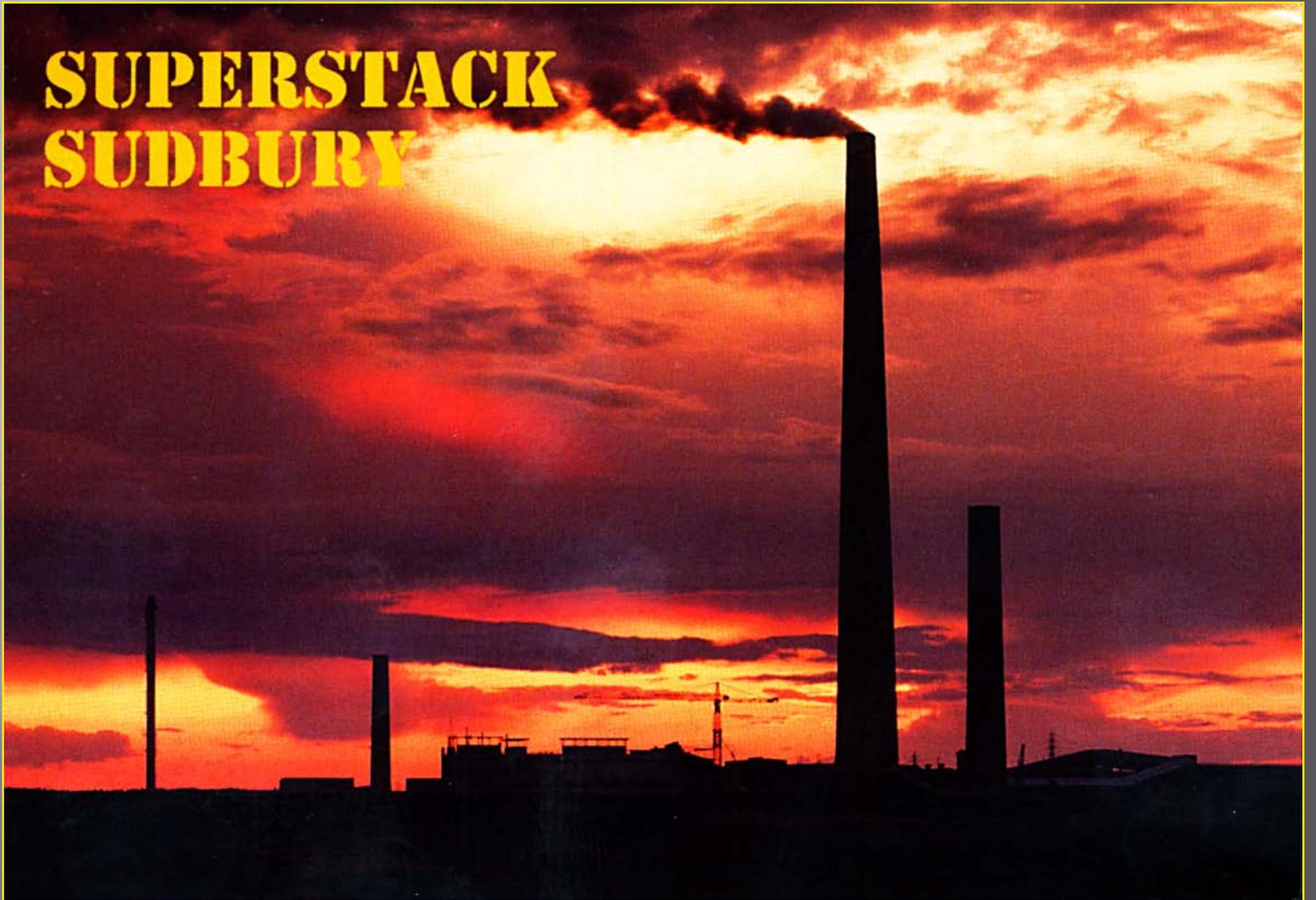
Report Documentation Page

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SUPERSTACK SUDBURY



Nickel Compounds

- Occupational exposures in Nickel refining industry and environmental exposures from oil and coal burning power plants.
- Although Ni ions are required for certain enzymes in bacteria and plants (Ureases, Dehydrogenases), No known function in mammals.
- Certain particulate Ni compounds(Ni₃S₂) that deliver Ni ions into cells, are potently carcinogenic (nasal, lung cancers etc at site of exposure). Not Mutagenic but can induce many diverse types of cancers at the site of exposure and in many different species.
- Ni ions do not induce or bind to Mt or Ferritin, there is little protection in Human cells for these metal ions. However Ni ions are not very toxic to cells which may allow cancer cells to arise with epigenetic and genetic alterations

Facultative
Phagocytosis

+ +
+ +
+ +
+ +
+ +
Amorphous NiS

- -
- -
- -
- -
- -
Crystalline NiS
Ni₃S₂

Ni²⁺
less
Uptake

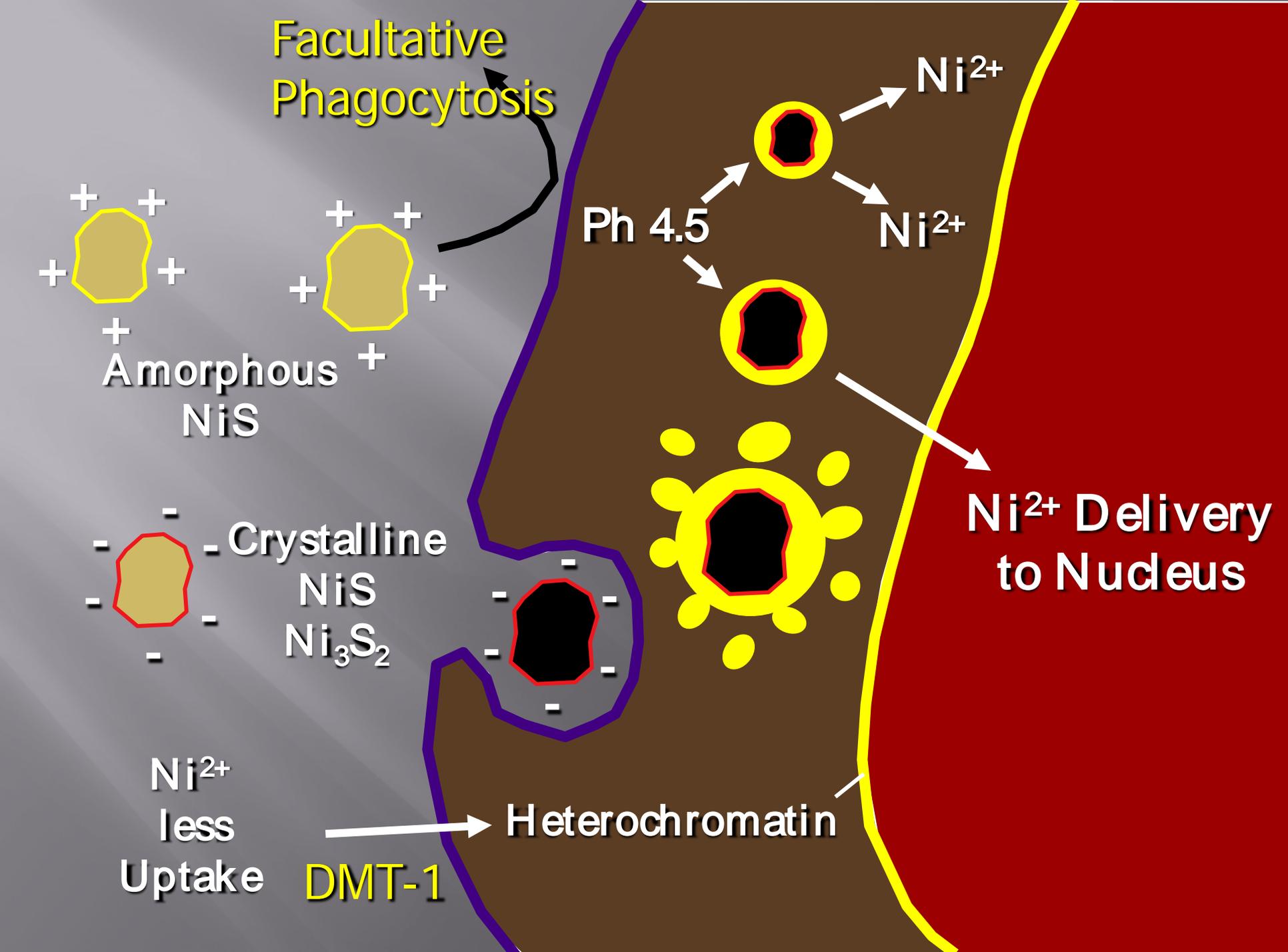
DMT-1

Heterochromatin

Ph 4.5

Ni²⁺
Ni²⁺

Ni²⁺ Delivery
to Nucleus

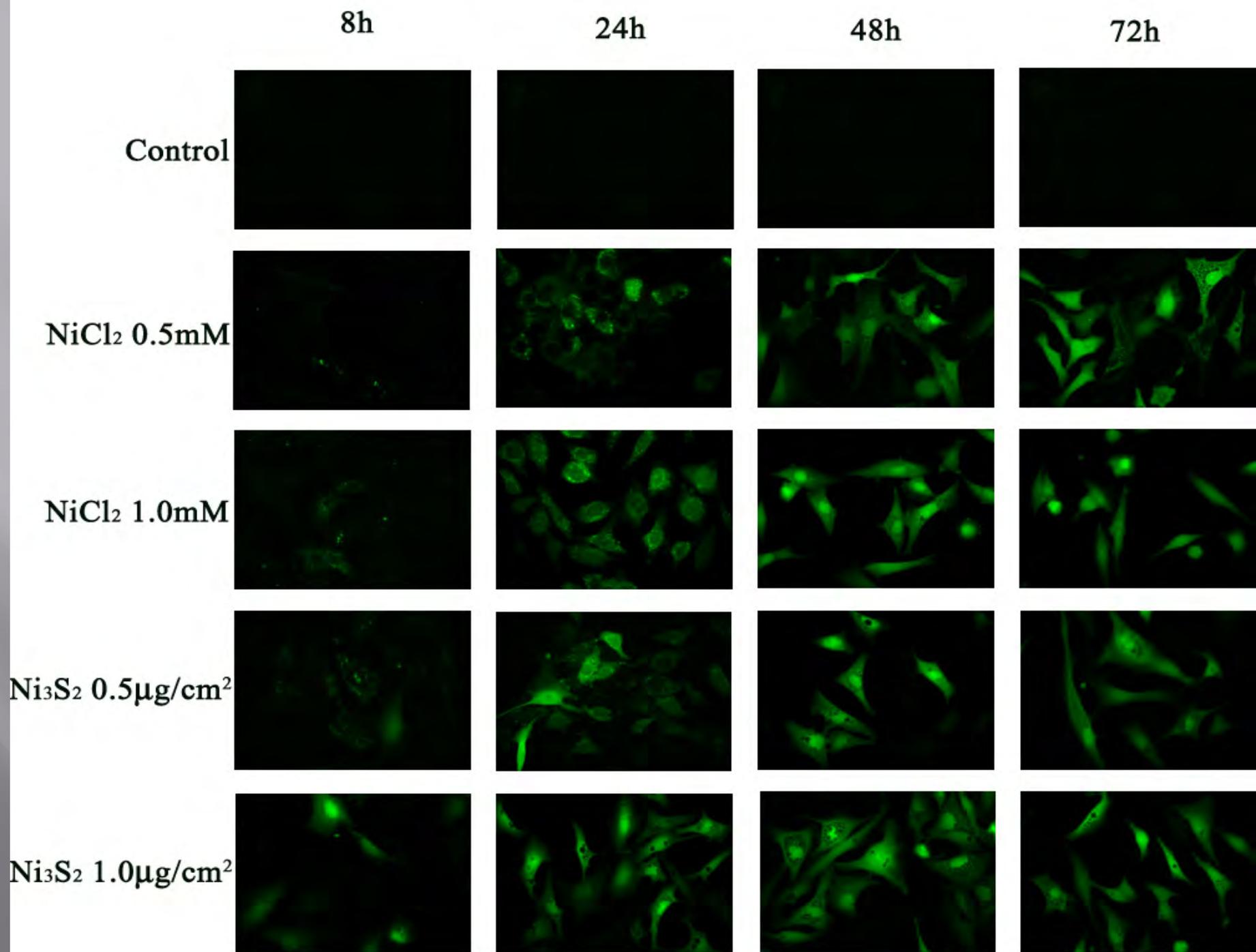


Potential Intracellular Concentration of a Phagocytized Crystalline NiS Particle^a

Mean particle diameter used in calculation (μm)	Approximate NiS cellular concentration ^b (M)
1.45	0.25
4.00	4.75

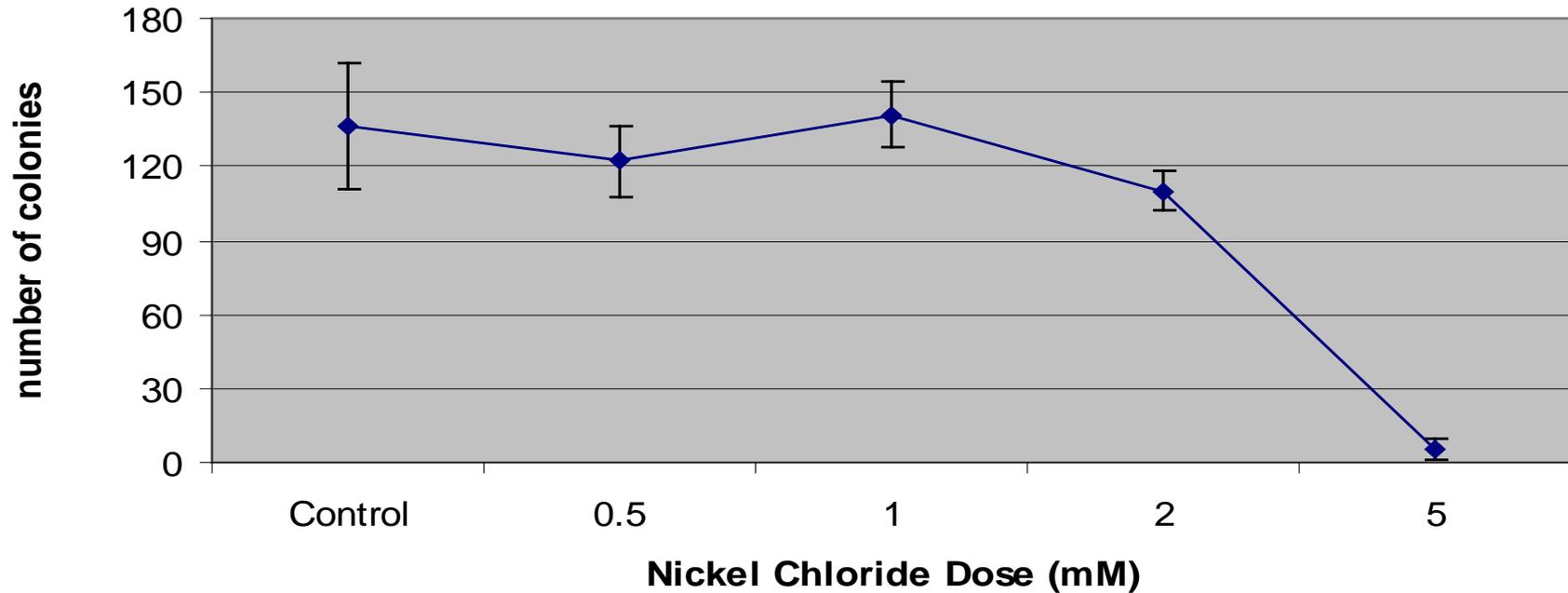
^aCell volume was determined in CHO cells with a Coulter counter-particle size analyzer and log range expander.

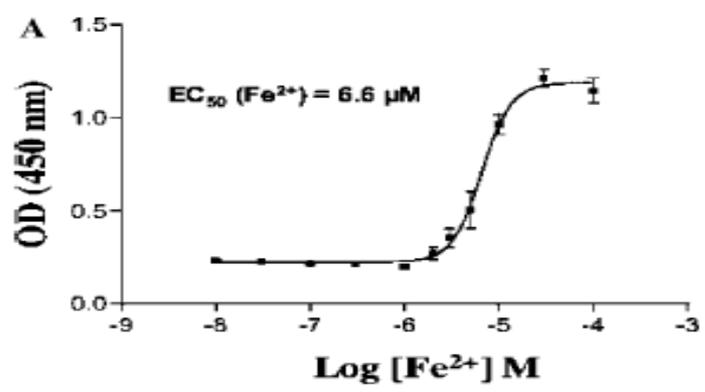
^bCell volume, $393.5, \mu\text{m}^3$; density of NiS, $5.5\text{g}/\text{cm}^3$; particles assumed spherical.



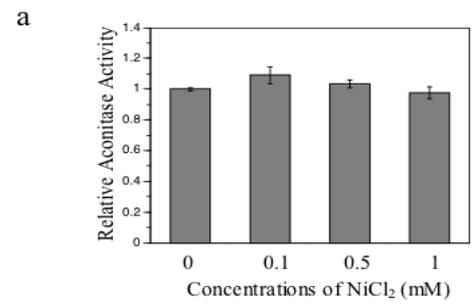
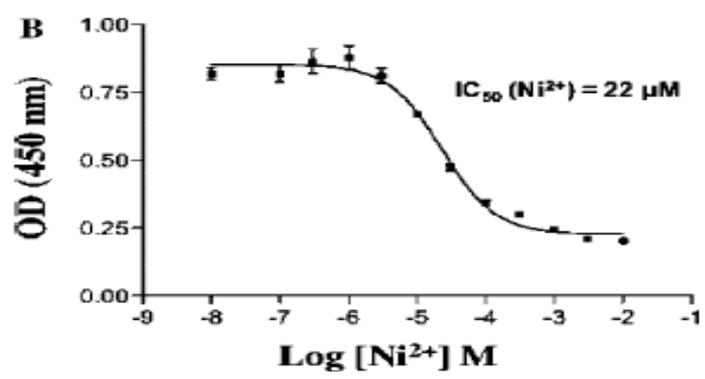
Effect of 24 hr exposure of A549 Cells to NiCl_2 On Cell Colony Formation

Cell Colony Formation After Ni Treatment

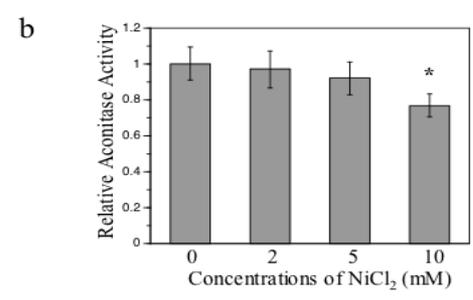




Prolyl
hydrox



aconitase



Periodic Table

12	3 IIIB	4 IVB	5 VB	6 VIB	7 VIIB	8 VIII	9 VIII	10 VIII	11 IB	12 IIB	A
20	Sc 21 44.955910 13 3+ Scandium	Ti 22 47.88 15 4+ Titanium	V 23 50.9415 16 5+ Vanadium	Cr 24 51.9961 16 3+ Chromium	Mn 25 54.93805 15 2+ Manganese	Fe 26 55.847 18 3+ Iron	Co 27 58.9332 18 2+ Cobalt	Ni 28 58.6934 18 2+ Nickel	Cu 29 63.546 19 2+ Copper	Zn 30 65.39 16 2+ Zinc	C
38	Y 39 88.90585 13 3+ Yttrium	Zr 40 91.224 14 4+ Zirconium	Nb 41 92.90638 16 5+ Niobium	Mo 42 95.94 18 6+ Molybdenum	Tc 43 98.9063 19 7+ Technetium	Ru 44 101.57 22 3+4+ Ruthenium	Rh 45 102.9055 22 3+ Rhodium	Pd 46 106.42 22 2+ Palladium	Ag 47 107.8682 19 1+ Silver	Cd 48 112.411 17 2+ Cadmium	I
56	La 57 138.9055 11 3+ Lanthanum	Hf 72 178.49 13 4+ Hafnium	Ta 73 180.9479 15 5+ Tantalum	W 74 183.85 17 6+ Tungsten	Re 75 186.207 19 7+ Rhenium	Os 76 190.2 22 4+ Osmium	Ir 77 192.22 22 4+ Iridium	Pt 78 195.08 22 4+ Platinum	Au 79 196.96654 24 3+ Gold	Hg 80 200.59 19 2+ Mercury	7
88	Ac 89 227.0278 11 3+ Actinium	Rf 104 261.11 - - Rutherfordium	Db 105 262.11 - - Dubnium	Sg 106 263.12 - - Seaborgium	Bh 107 262.12 - - Bohrium	Hs 108 264 - - Hassium	Mt 109 266.1378 - - Meitnerium	Uun 110 269 - - Ununnilium	Uuu 111 272 - - Unununium	Uu 112 277 - - Ununbium	8

Examples of Oxoglutarate Superfamily of Dioxygenases Enzymes in Humans (more than 100 across evol. phyla)

Prolyl Hydroxylases (collagen, HIF-dependent)

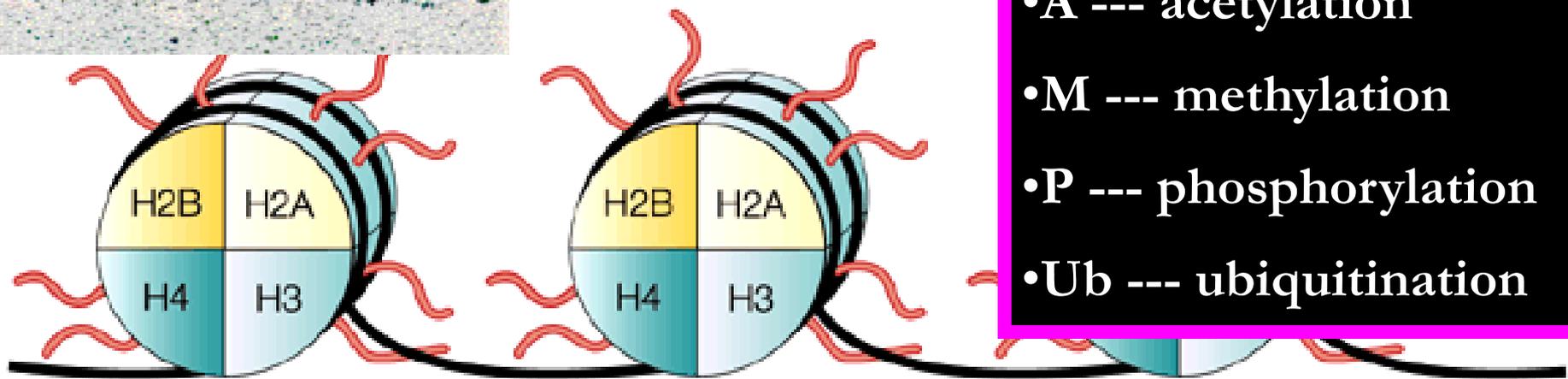
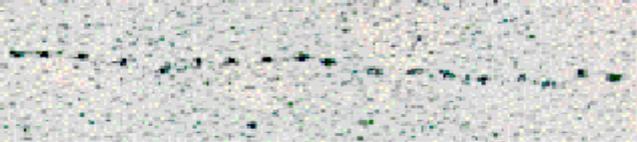
Asparagine Hydroxylases (FIH)

Alk b DNA repair enzymes (1-meA, 3-meC, ABH2 and ABH3)

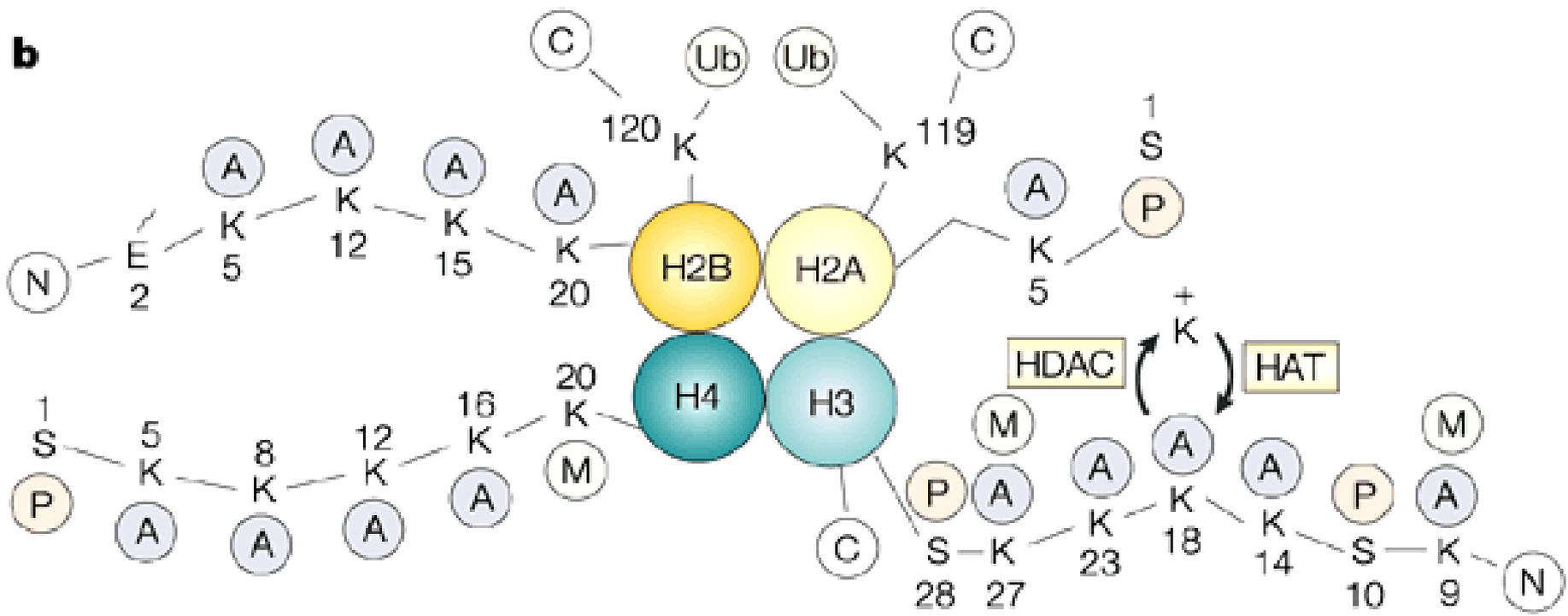
FTO (3-meT, 3meC, overexpression weight gain, Type 2 Diabetes)

Histone Lysine Demethylases

(only use of Ascorbic Acid in our bodies)



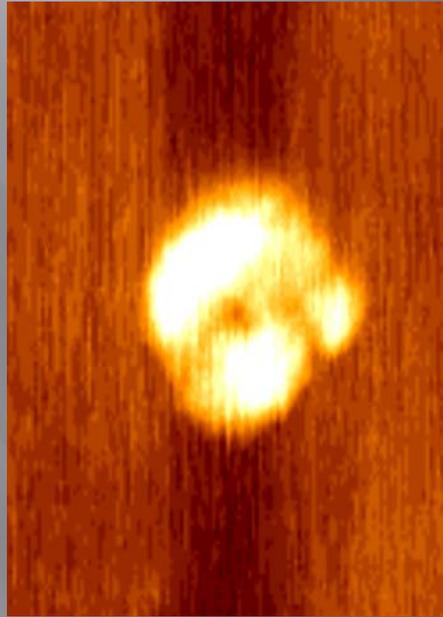
- A --- acetylation
- M --- methylation
- P --- phosphorylation
- Ub --- ubiquitination



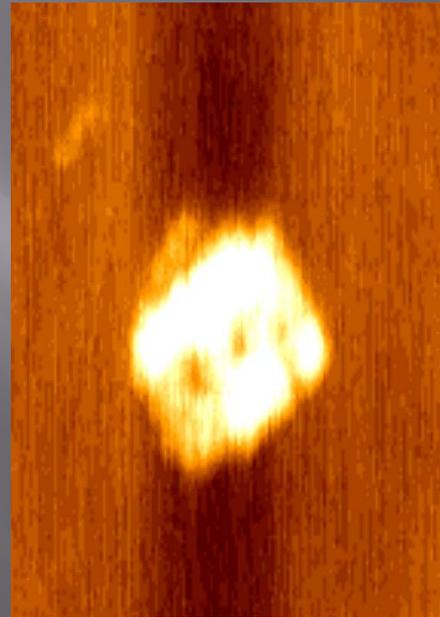
Structure of Nucleosome



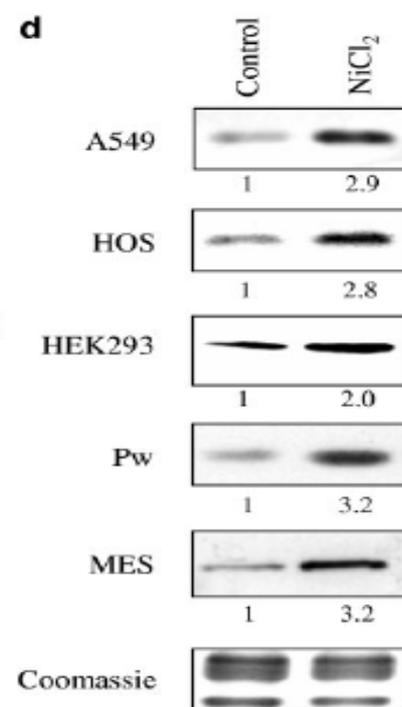
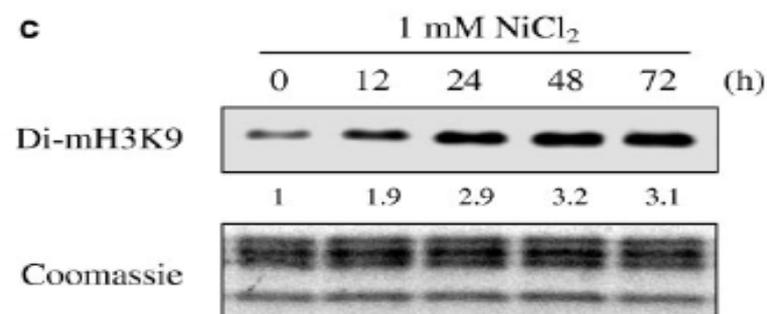
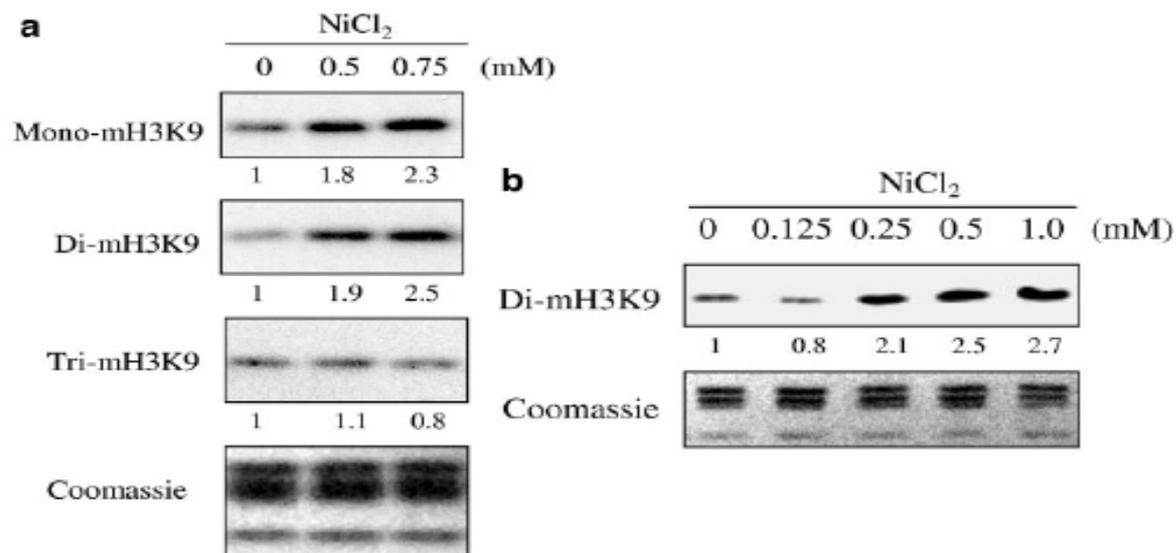
Atomic Force Microscopy View of Chicken Erythrocyte Single Nucleosomes



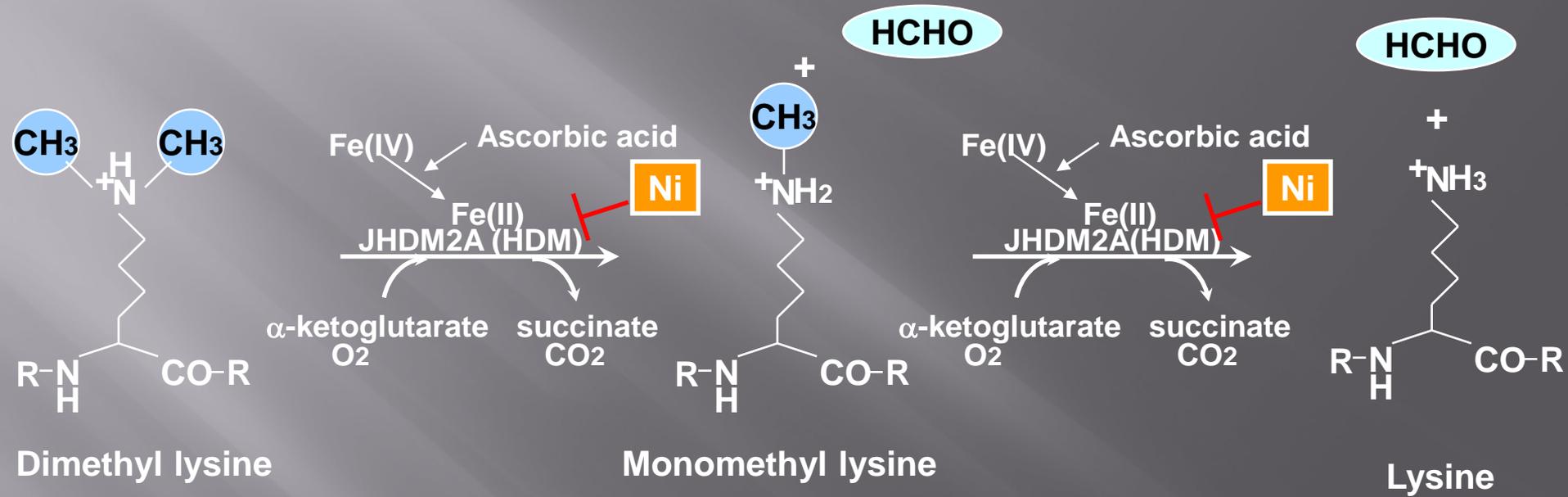
end on
view



face
view



JMJD1A (Histone 3 Lysine 9 Di and Mono Demethylase JHDM2A)



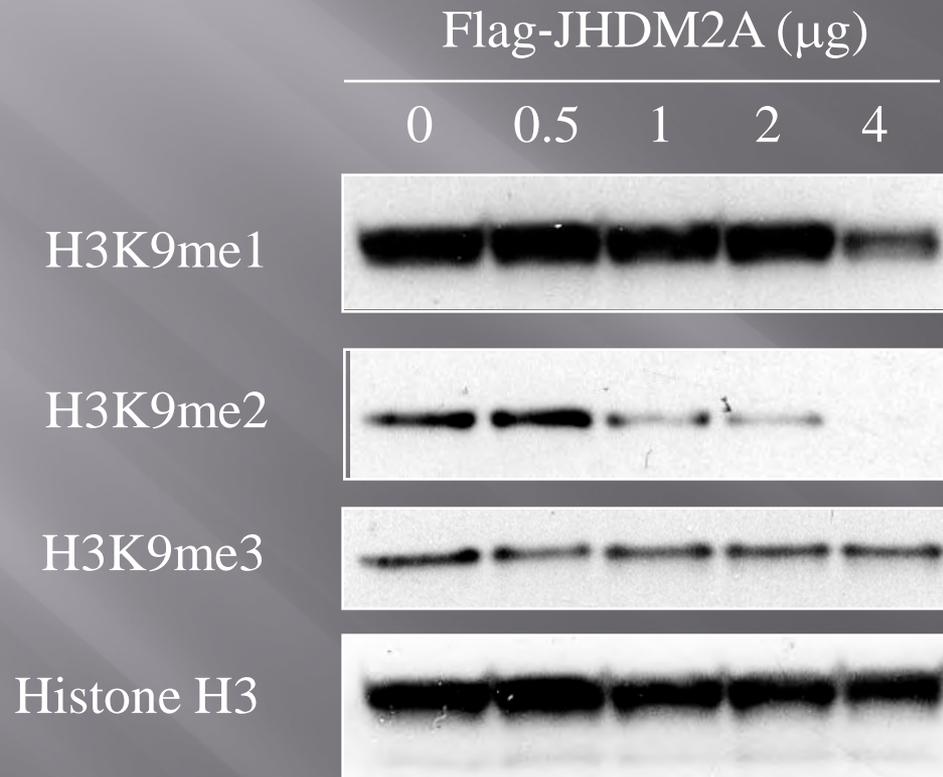
H3K9me₂

Human histone demethylases (HDMs)

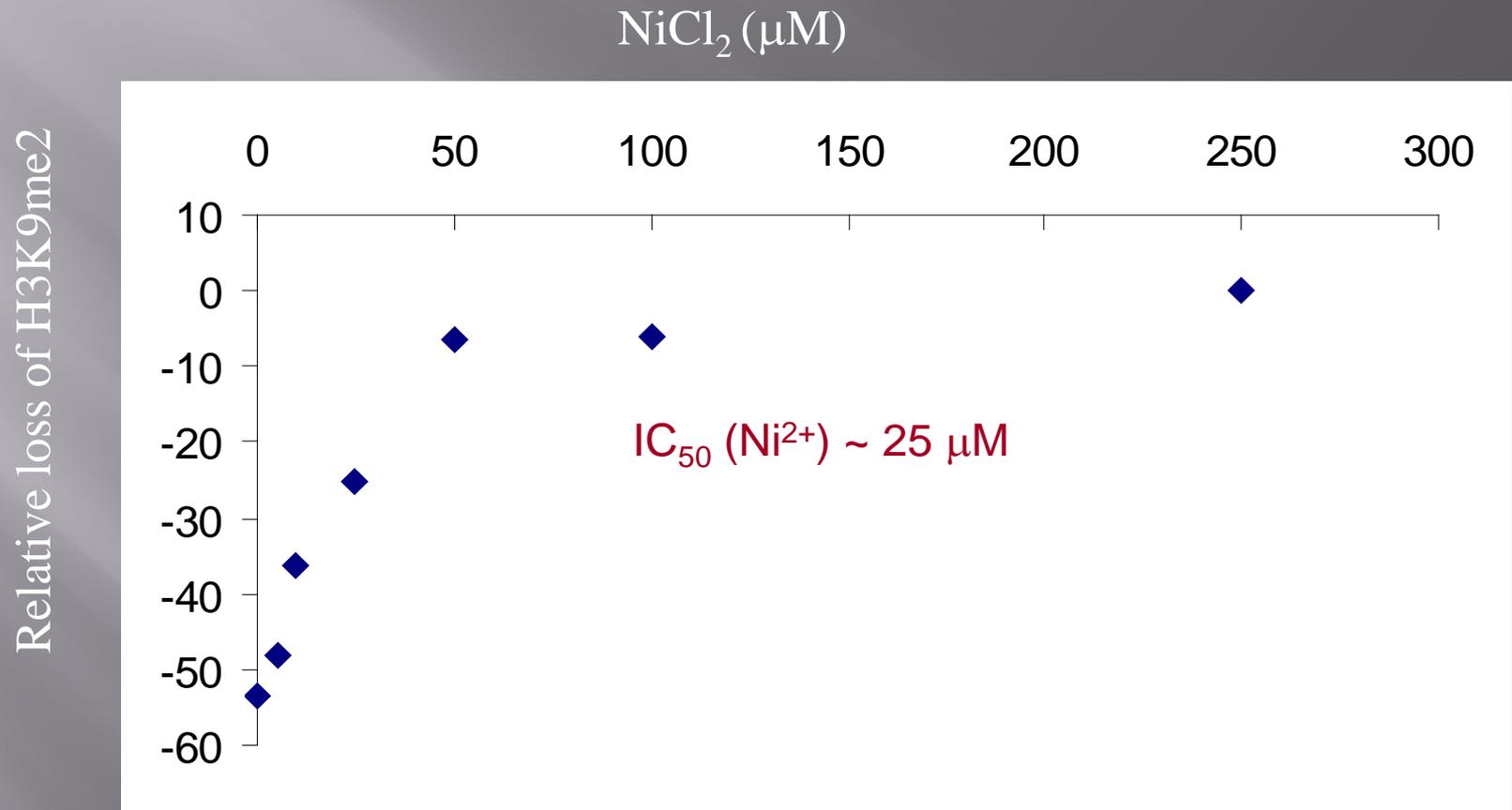
name	substrate
PADI4	R on H3, H4 (No Fe)
LSD1	H3K4 (No Fe)
JHDM1	H3K9, H3K36 (di-), Fe dep
JHDM2A /JMJD1A	H3K9 (mono-, di-)
JHDM3A /JMJD2A	H3K9 (tri-), H3K36 (tri-)
GASC1/JMJD2C	H3K9 (di-, tri-)
JARID-1 family (4 members Jmjcdom)	H3K4 (Tri)



In vitro H3K9 Demethylation by Flag-JHDM2A recombinant protein

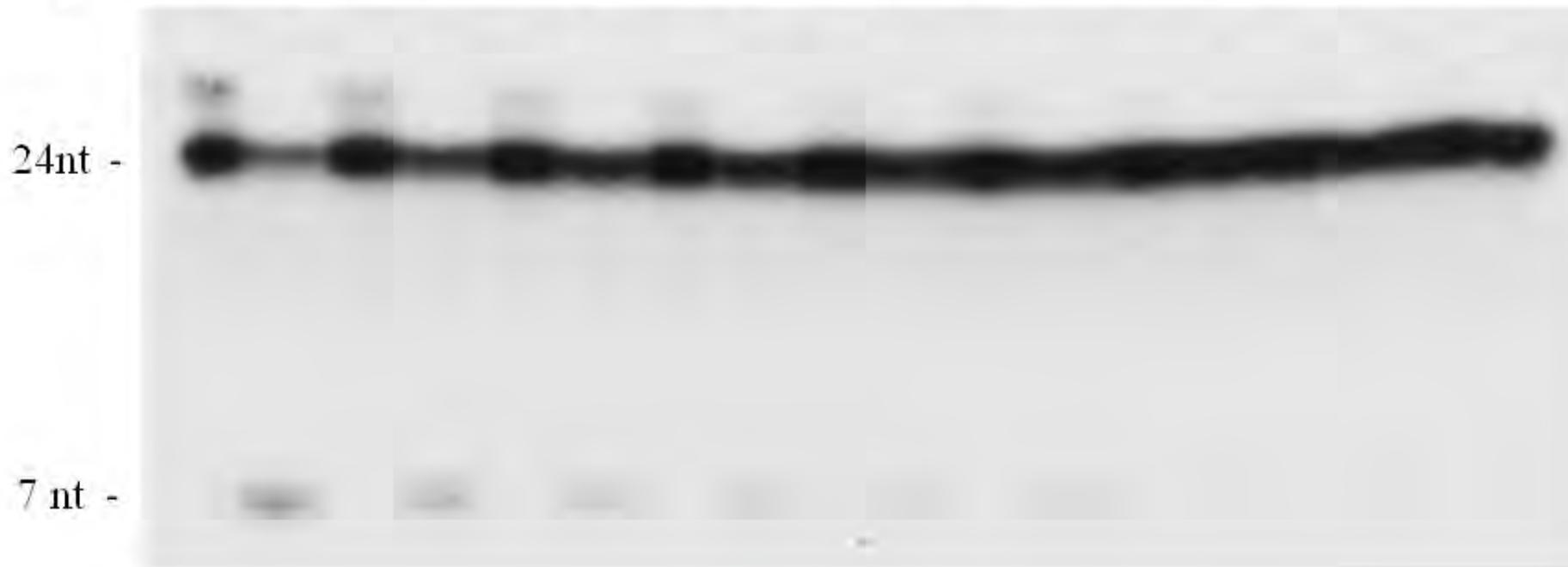


Inactivation of JHDM2A by Ni ions



Dose-dependent inactivation of ABH2 by Ni ions

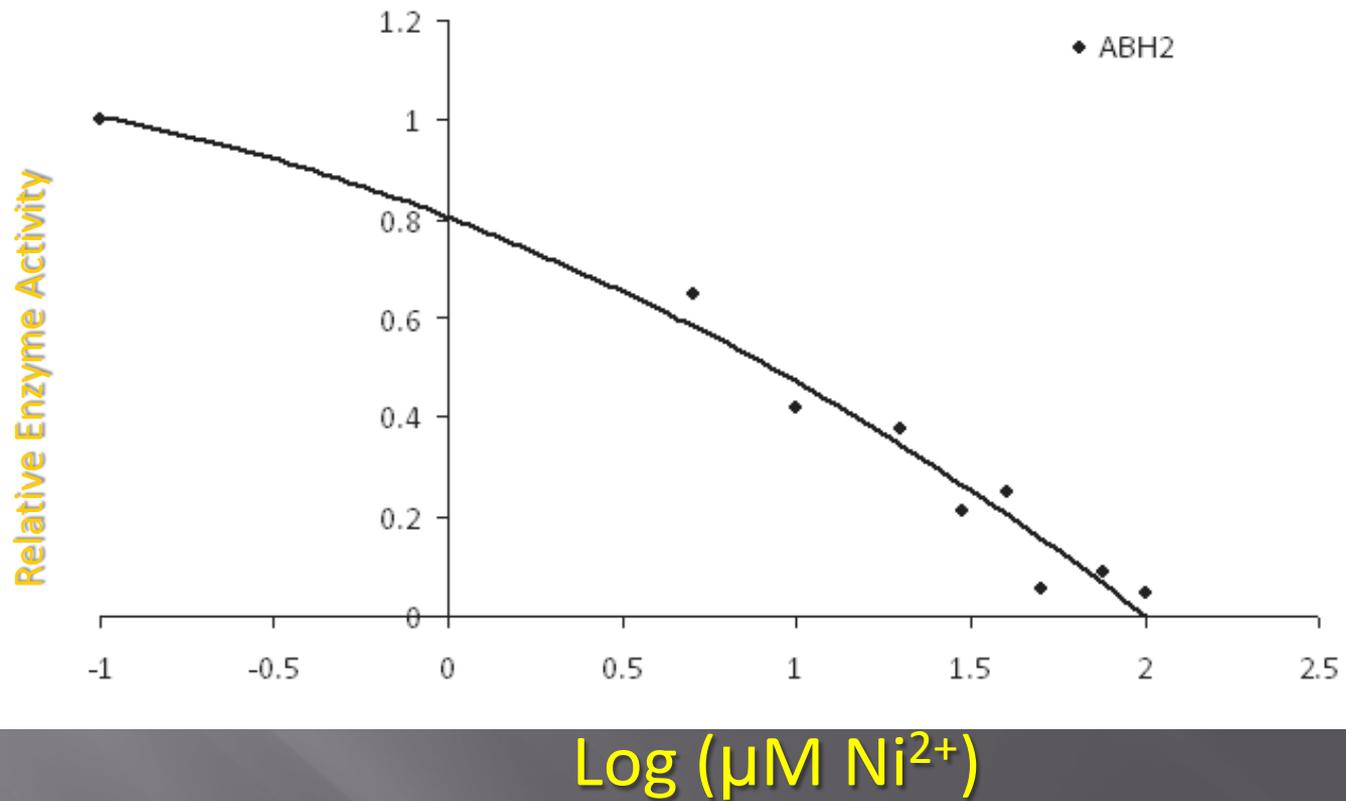
NiCl ₂ (μM)	0		5		10		20		30		40		50		75		100	
HpaII	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+



Oligo 3-meC: 5'-³²PAAA GCA ^{3-me}CCG GTC GAA AAA GCG AAA-3'

30 min 1 μg ABH2 demethylation → Annealing → 1 h HpaII cutting

Data Quantification

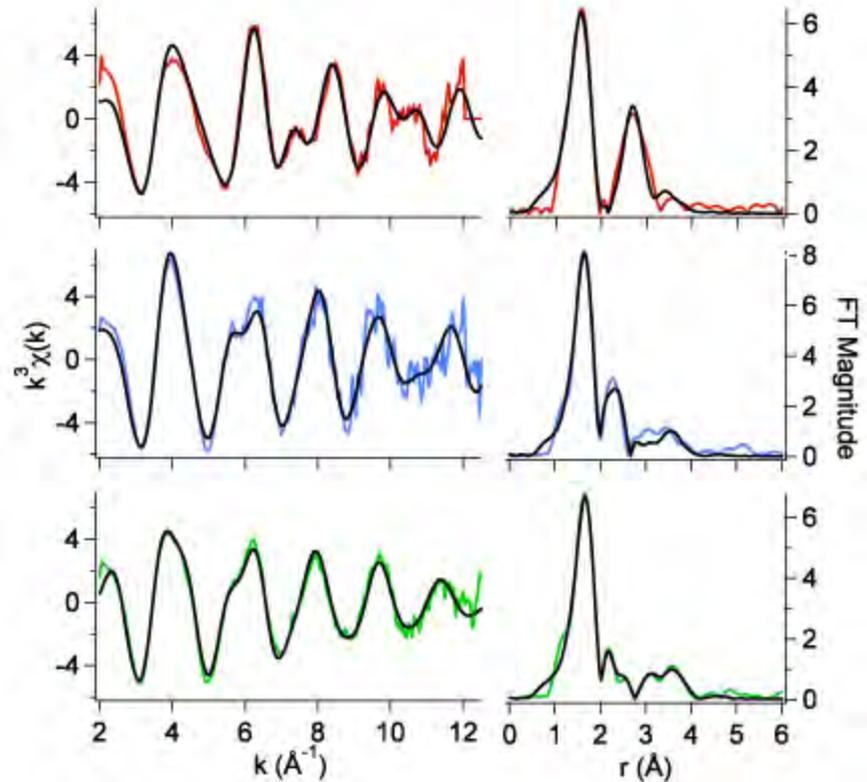
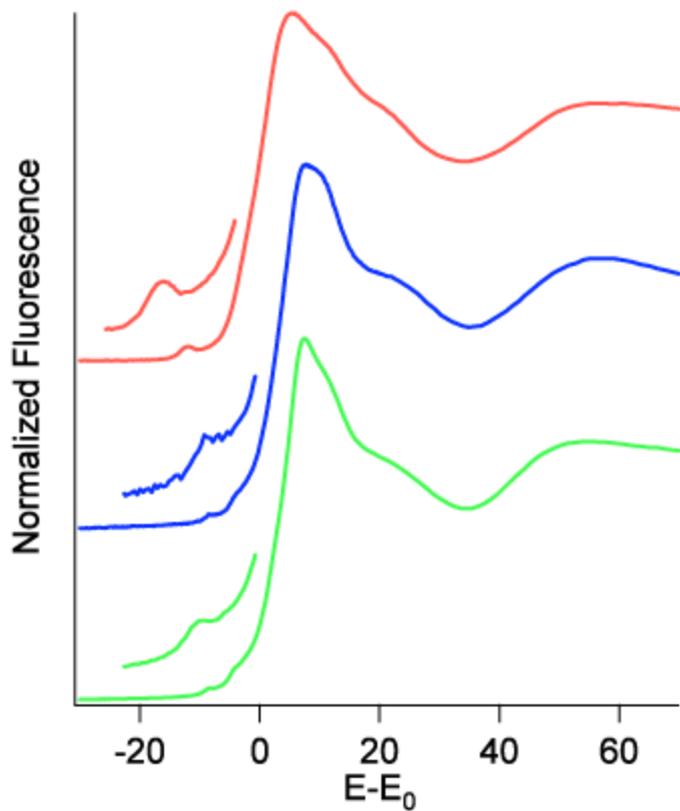


Kd for Ni and Fe binding to ABH2 (DNA Demethyl)

Fe=4.5uM

Ni=1.7uM

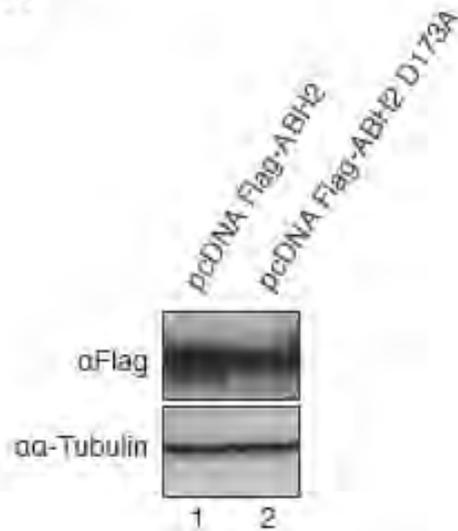
Giri and Maroney, Dept Chem, Univ of Mass



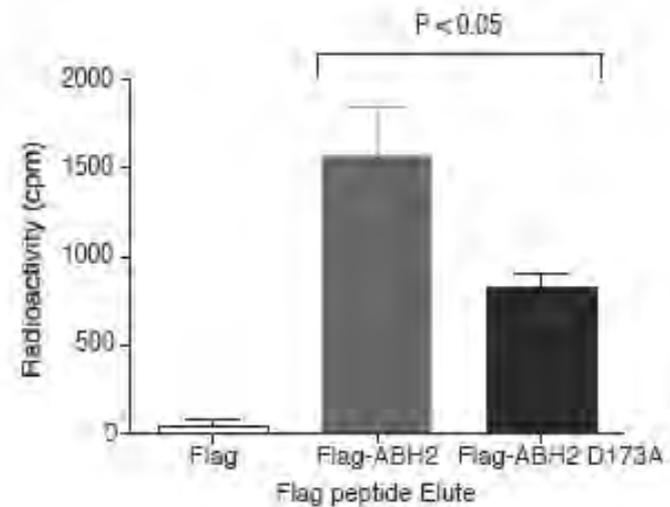
XAS analysis of nickel binding to ABH2. A). *K*-Edge XANES spectra for Fe-ABH2 (red), Ni-ABH2 (blue) and Ni-ABH2 + 2-oxoglutarate (green). Inserts: Expansions of the preedge XANES region showing peaks associated with $1s \rightarrow 3d$ electronic transitions. B). Unfiltered, k^3 -weighted EXAFS spectra (colored lines, red = Fe-ABH2, blue = Ni-ABH2 and green = Ni-ABH2 + 2-oxoglutarate) and best fits from Table 1 (black lines). Left: k -space spectra and fits. Right: FT-data and fits.

Ni ion binding to ABH2 In Intact Cells

A



B



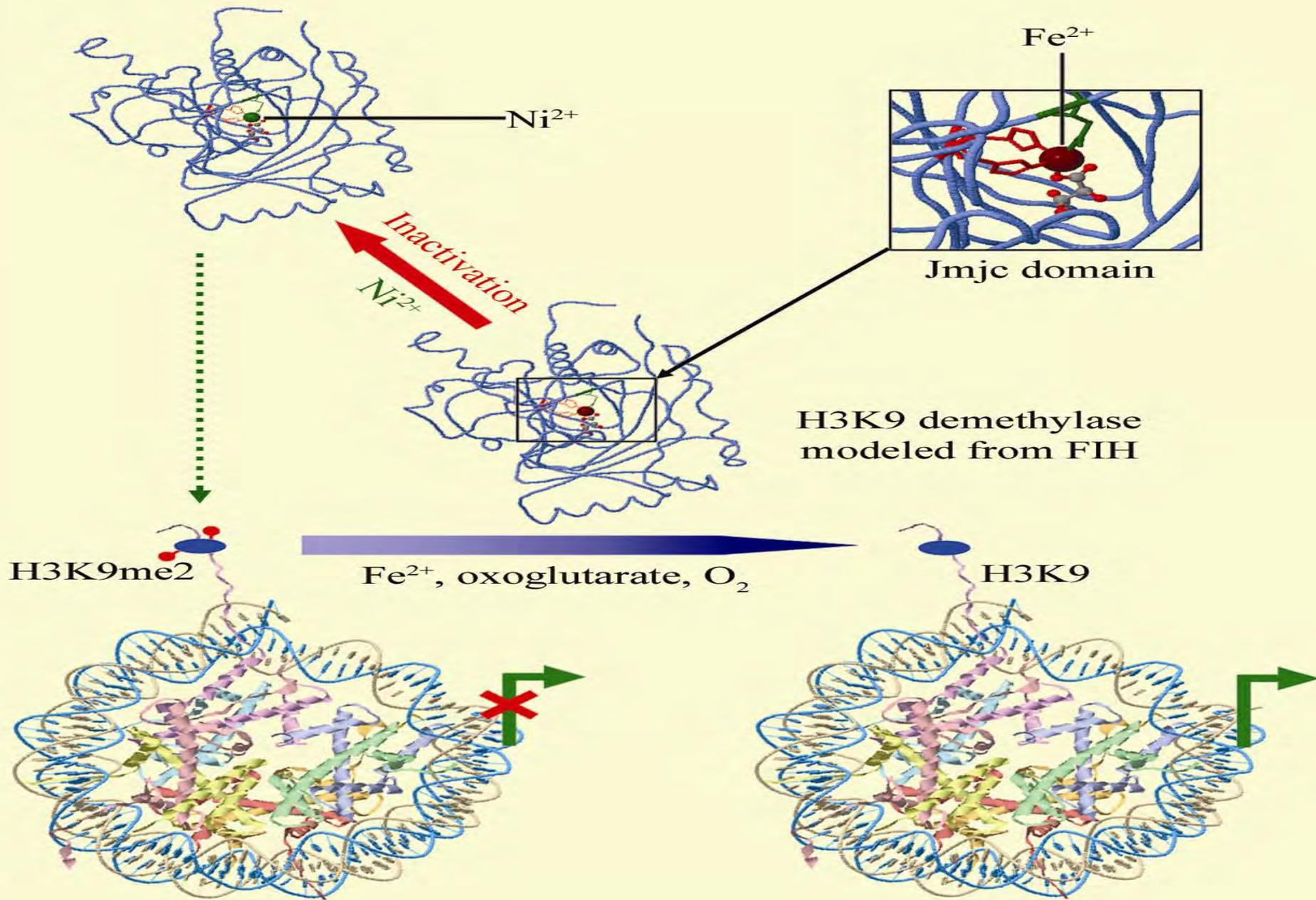
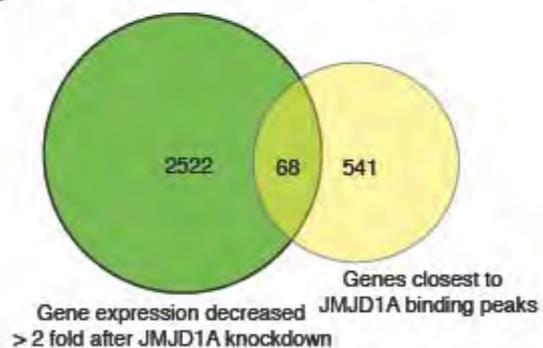


Figure 1

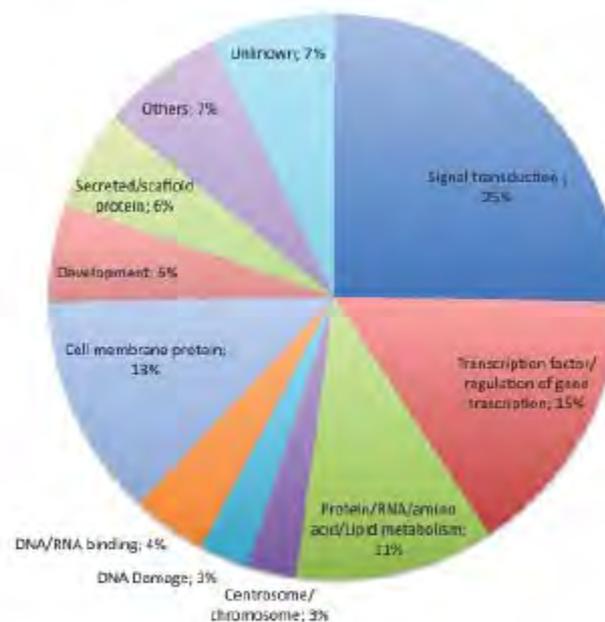
A



B

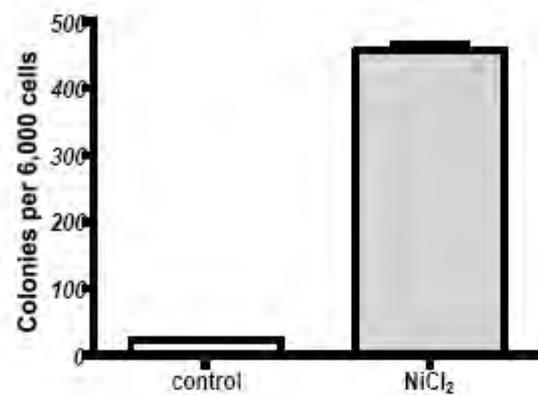
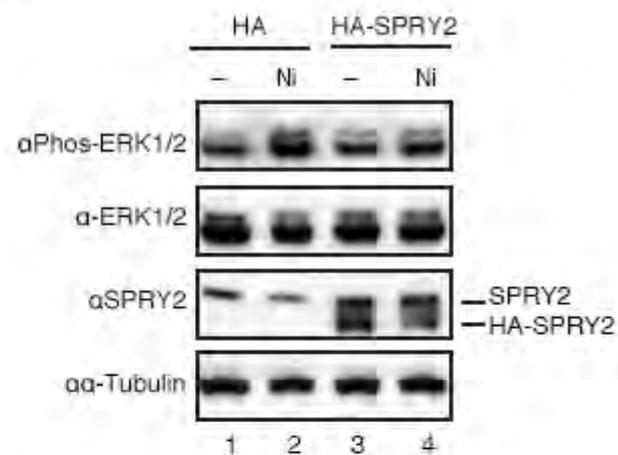
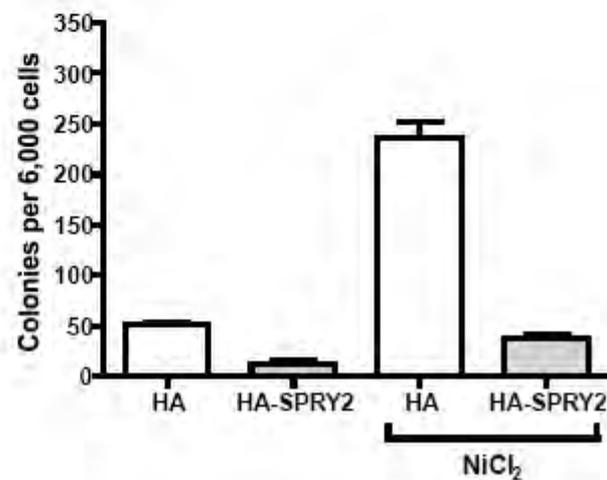
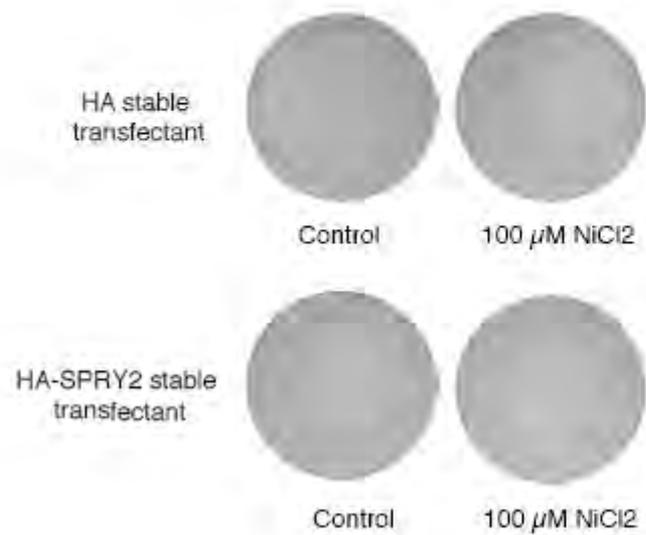


C



SPRY2 and JHMD2A

- ▣ JHMD2A binds and activates SPRY2 transcription.
- ▣ SPRY2 is epigenetically silenced with H3K9 dimethylation marks in its promoter in Nickel treated and transformed BEAS2B cells.
- ▣ SPRY2 inhibits growth factor signaling in cells and is a tumor suppressor.

D**E****F**

Heat Maps of Genes Changed in Nickel-transformed Cells

High express

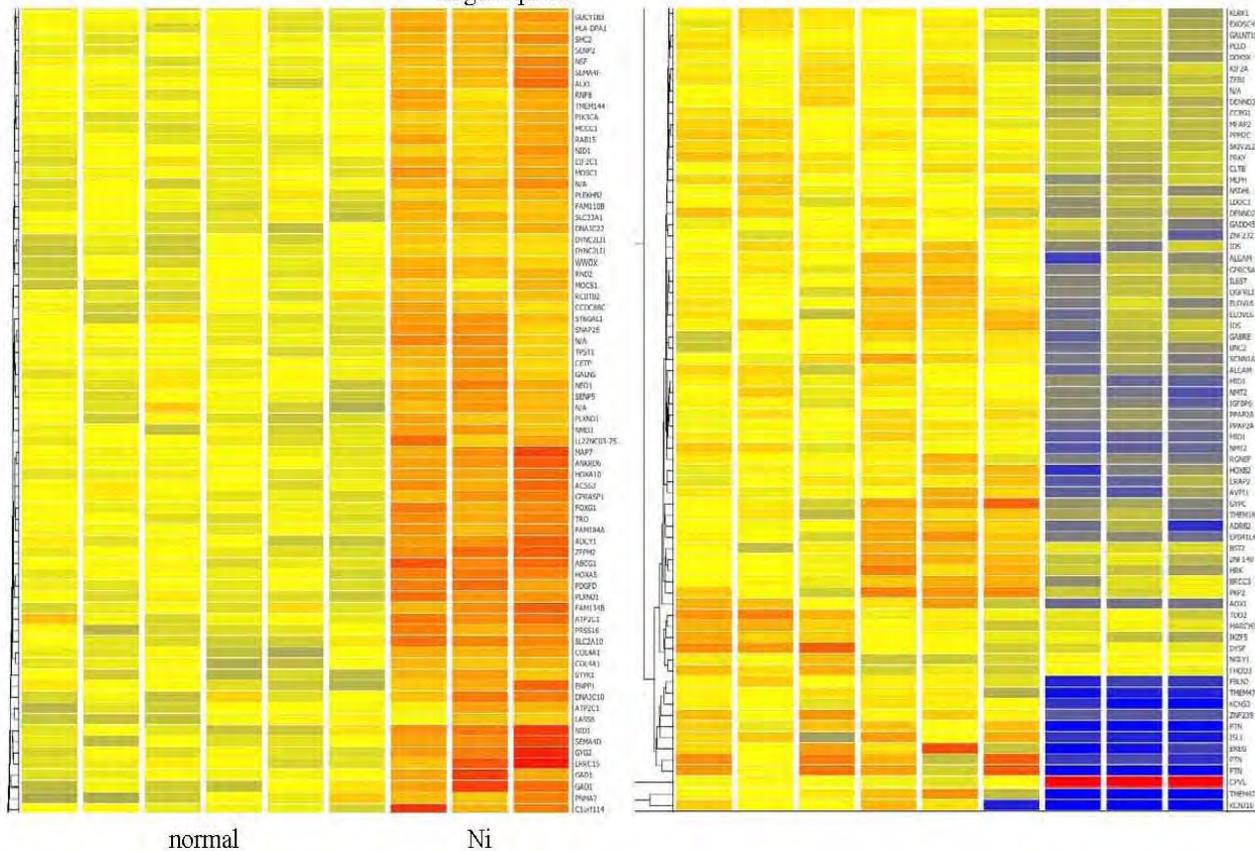


Figure 12. Affymetrix gene expression array heat maps from non-tumorigenic normal BEAS2B cell clones and NiCl₂ transformed BEAS-2B cells. BEAS2B cells were exposed to NiCl₂ at 100uM concentration for 4 weeks and then either unexposed or exposed BEAS2B were plated to proliferate in soft agar. Shown in the figure are Affymetrix arrays from spontaneously derived small agar colonies from untreated cells (first 6 lanes) or large agar colonies from nickel-treated cells. Colonies were picked from the agar and Affymetrix gene expression was determined directly from these agar colonies. The left panel compares the most unregulated genes while the right panels compares the most down regulated genes based upon the nickel transformed clones.

W

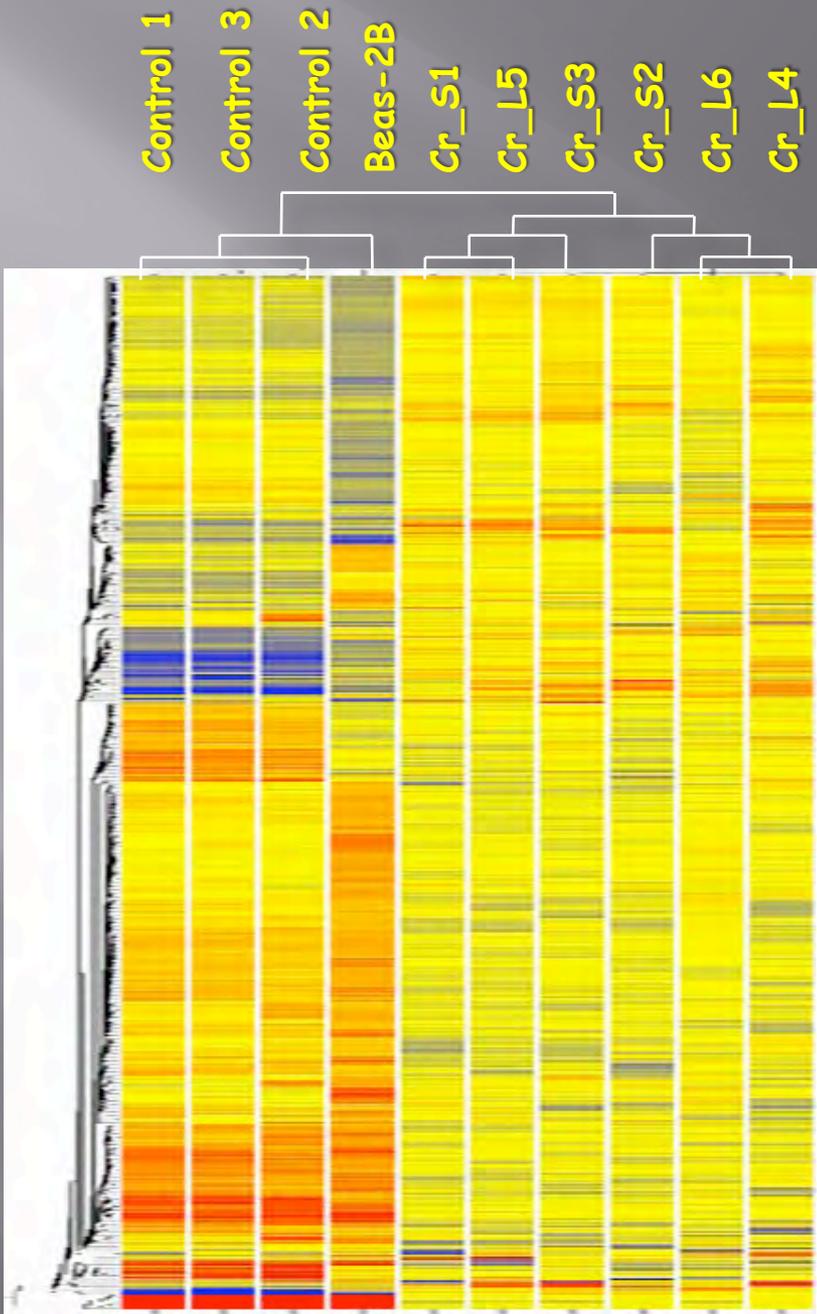
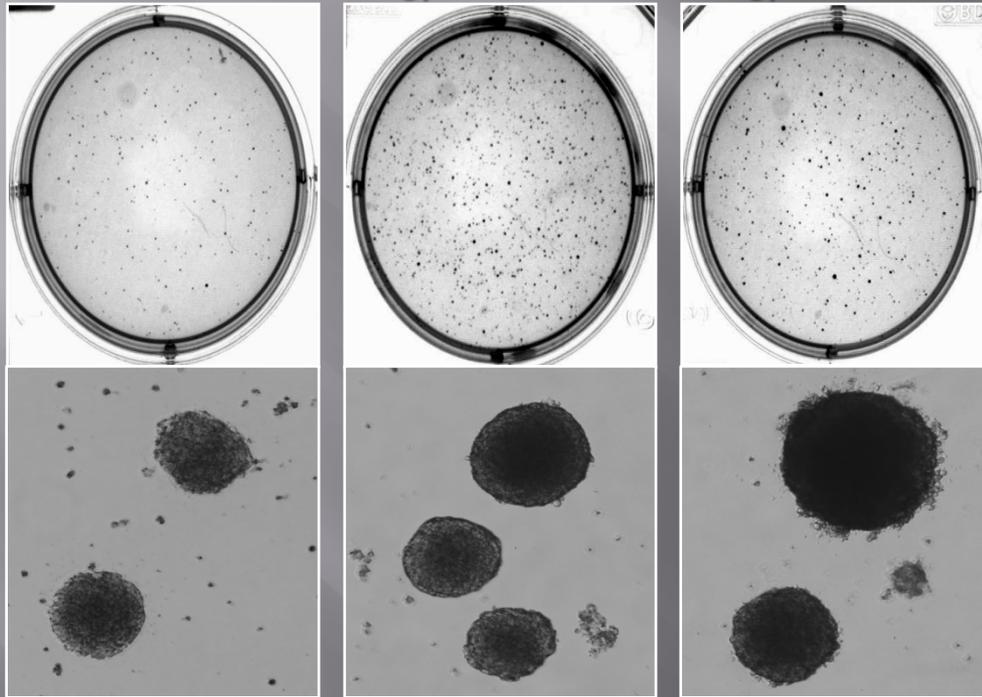
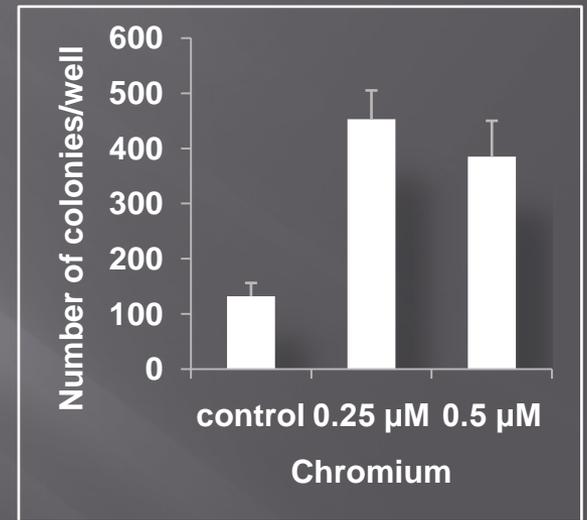
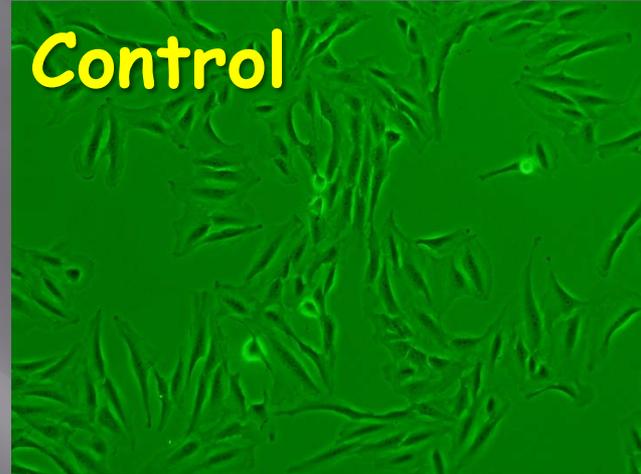
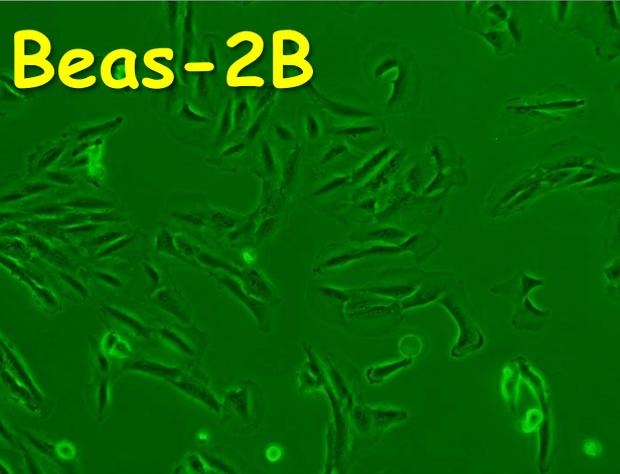


Figure 5

A

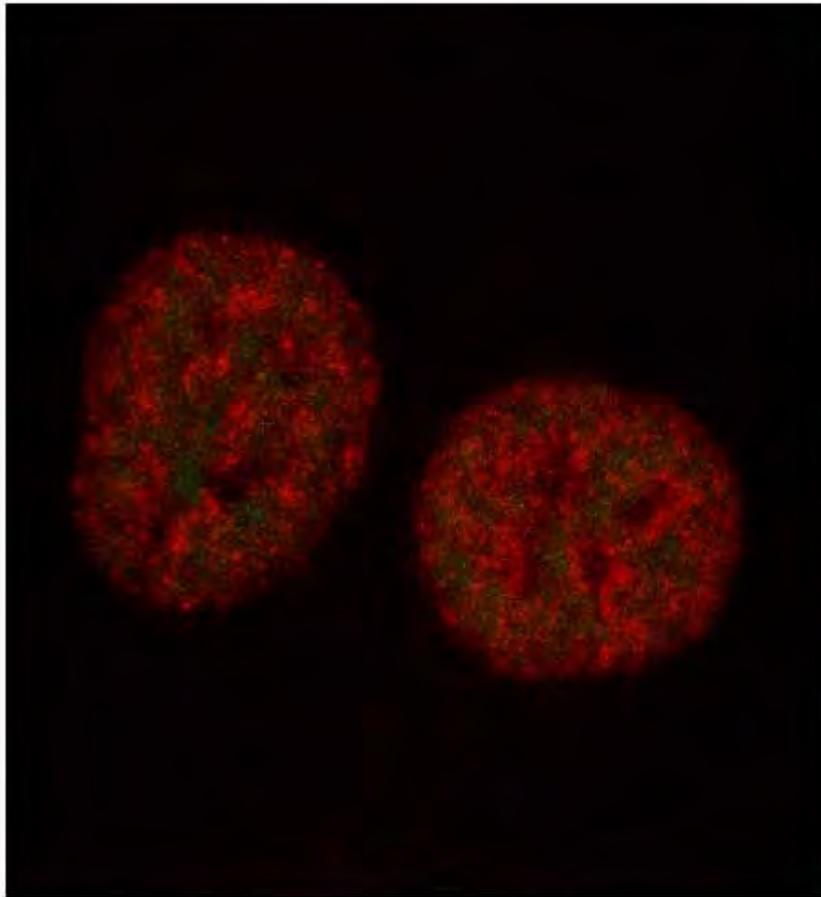
Control

0.25 μM
Cr0.5 μM
Cr**B****Figure 2**



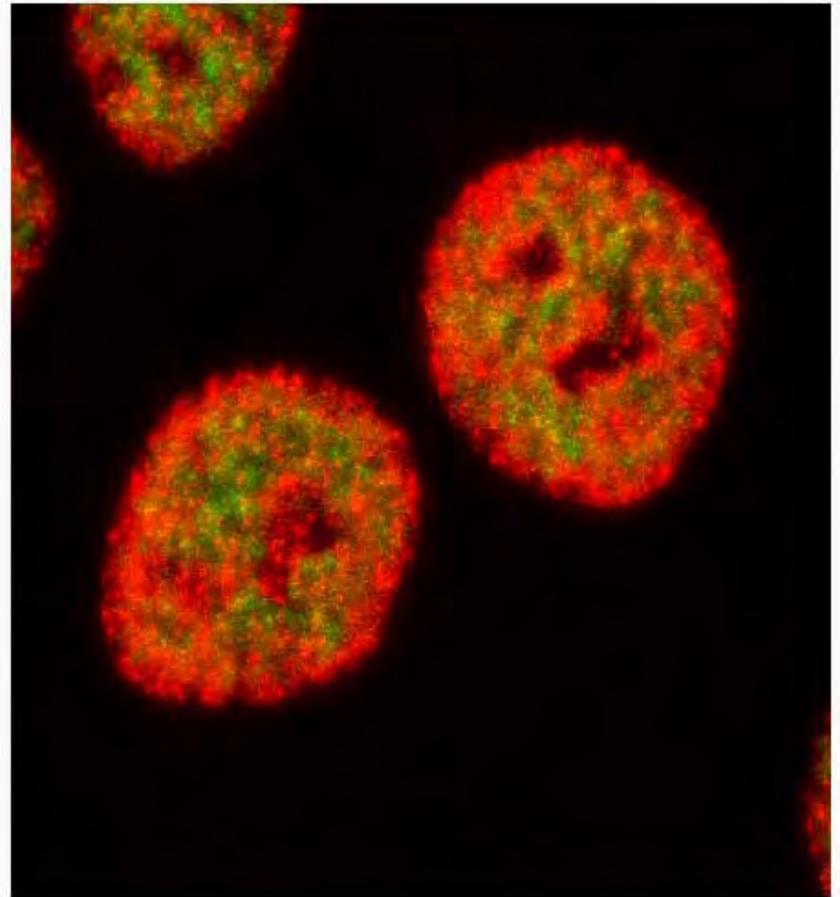
No
tumors

tumors
in nude
mice



Control

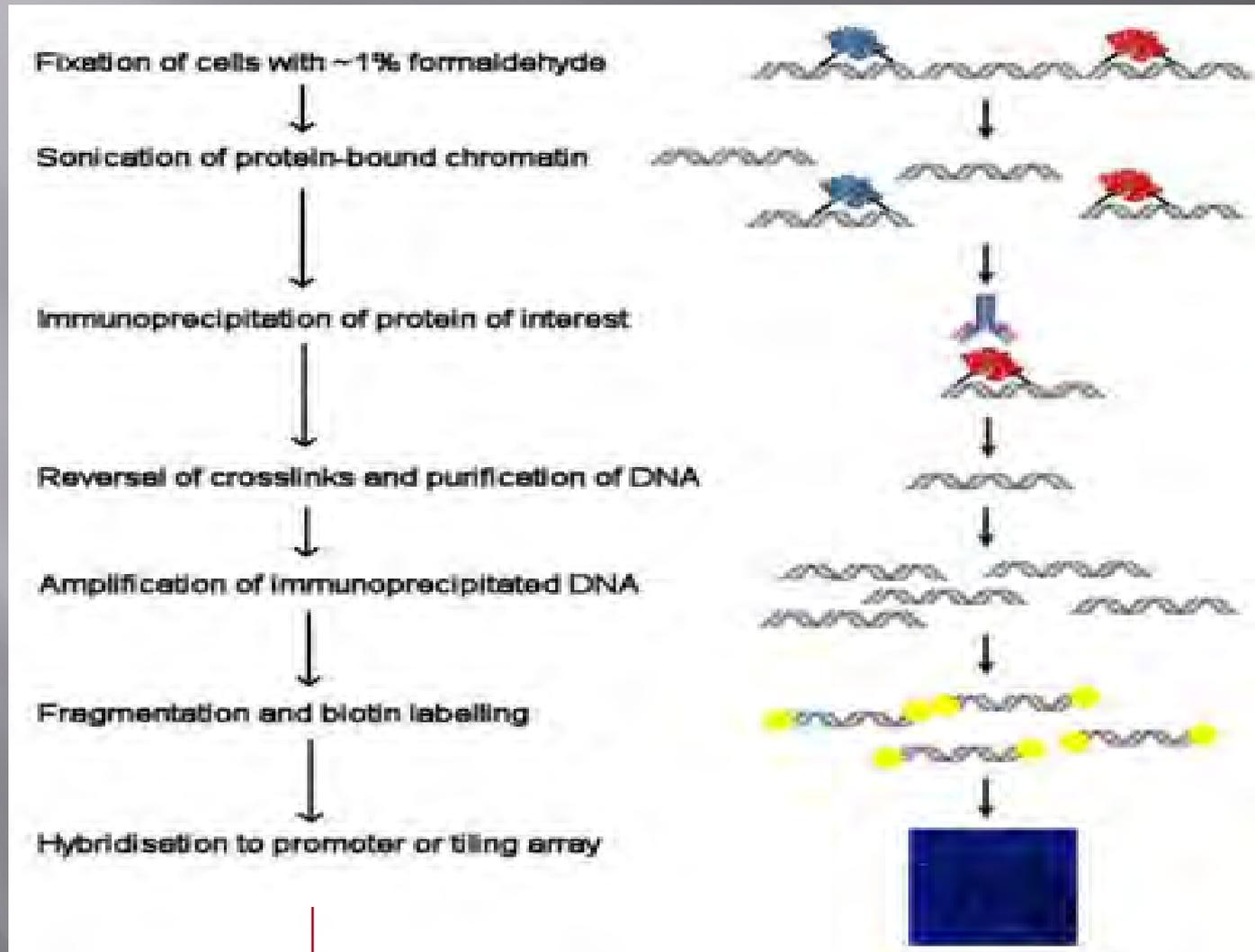
Red=H3K9me2



1 mM Ni

Green=H3K4me3

Chip-on-chip and Chip-Seq



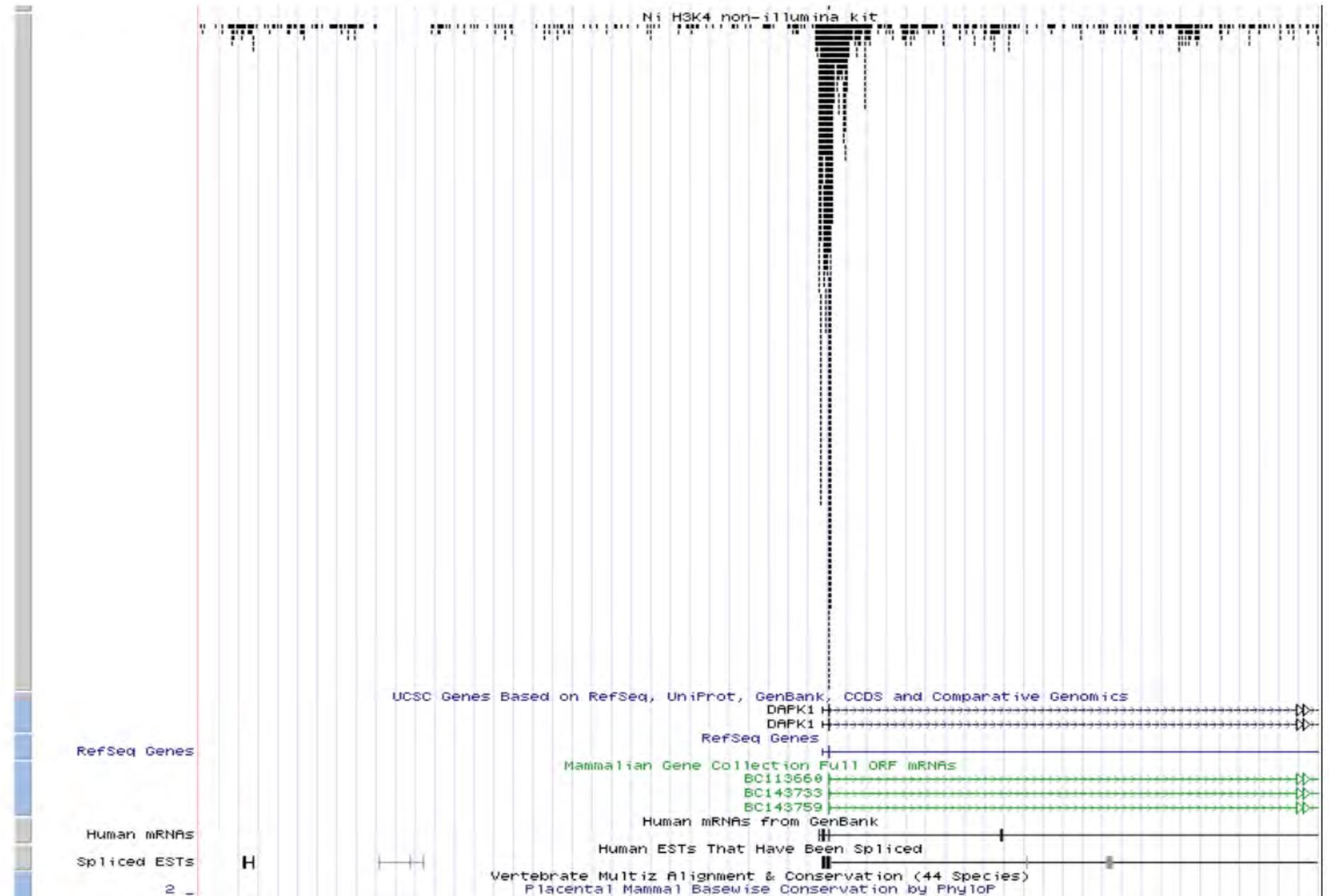
Chip-Seq sequence 36 BP DNA

Correlation of increases of H3K9me2 in gene promoter and Gene expression

strand	annotation	Symbol	Chromosome	Start	End	expression
-	IL12B	NM_002187	5	158694368	158694540	↓ 8.4
-	C10orf86	NM_01761	10	123730785	123730944	↓ 3.3
+	CD3EAP	NM_01209	5	96056126	96056327	↓ 3.5
+	CCT7	NM_01590	7	100312376	100312519	↓ 3.0

Correlation of increases of H3K4me3 in gene promoter and Gene expression

strand	annotation	Symbol	Chromosome	Start	End	Gene expres sion
+	NDRG1	NM_006096	8	134,376,200	134,377,500	↑ 21.2
+	CA9	NM_001216	9	35663914	35671151	↑ 6.5
+	STC2	NM_003714	5	172,685,450	172,685,600	↑ 3.5
+	EGLN3	NM_022073	14	33463173	33490036	↑ 3.3



Ni H3K4 non-illumina kit

UCSC Genes Based on RefSeq, UniProt, GenBank, CCDS and Comparative Genomics

DAPK1

DAPK1

RefSeq Genes

Mammalian Gene Collection Full ORF mRNAs

BC113660

BC143733

BC143759

Human mRNAs from GenBank

Human ESTs That Have Been Spliced

Vertebrate Multiz Alignment & Conservation (44 Species)
Placental Mammal Basewise Conservation by PhyloP

RefSeq Genes

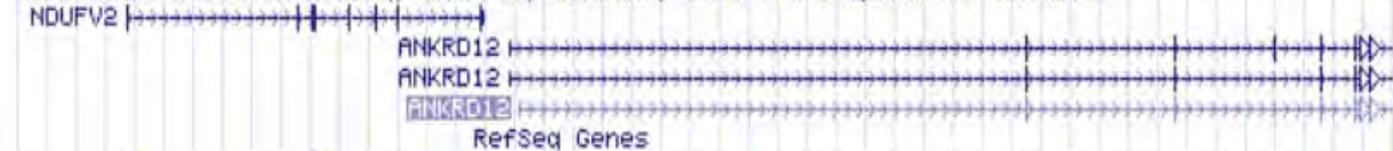
Human mRNAs

Spliced ESTs

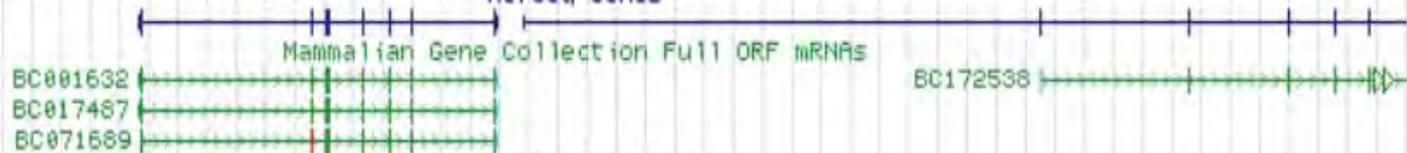
H

Ni H3K4 non-illumina kit

UCSC Genes Based on RefSeq, UniProt, GenBank, CCDS and Comparative Genomics

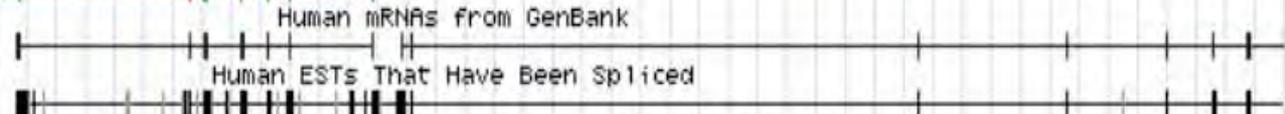


RefSeq Genes



Human mRNAs

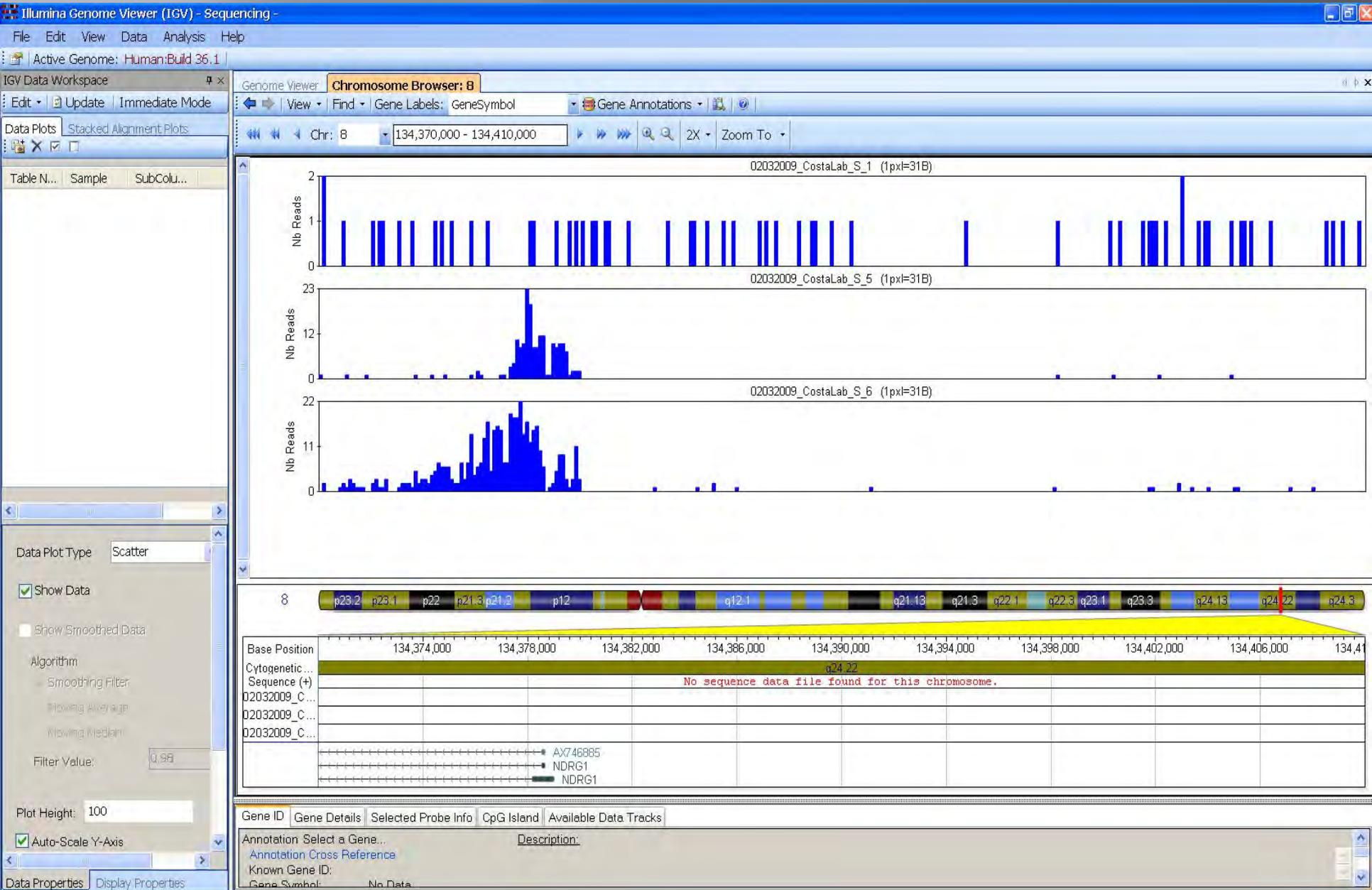
Spliced ESTs



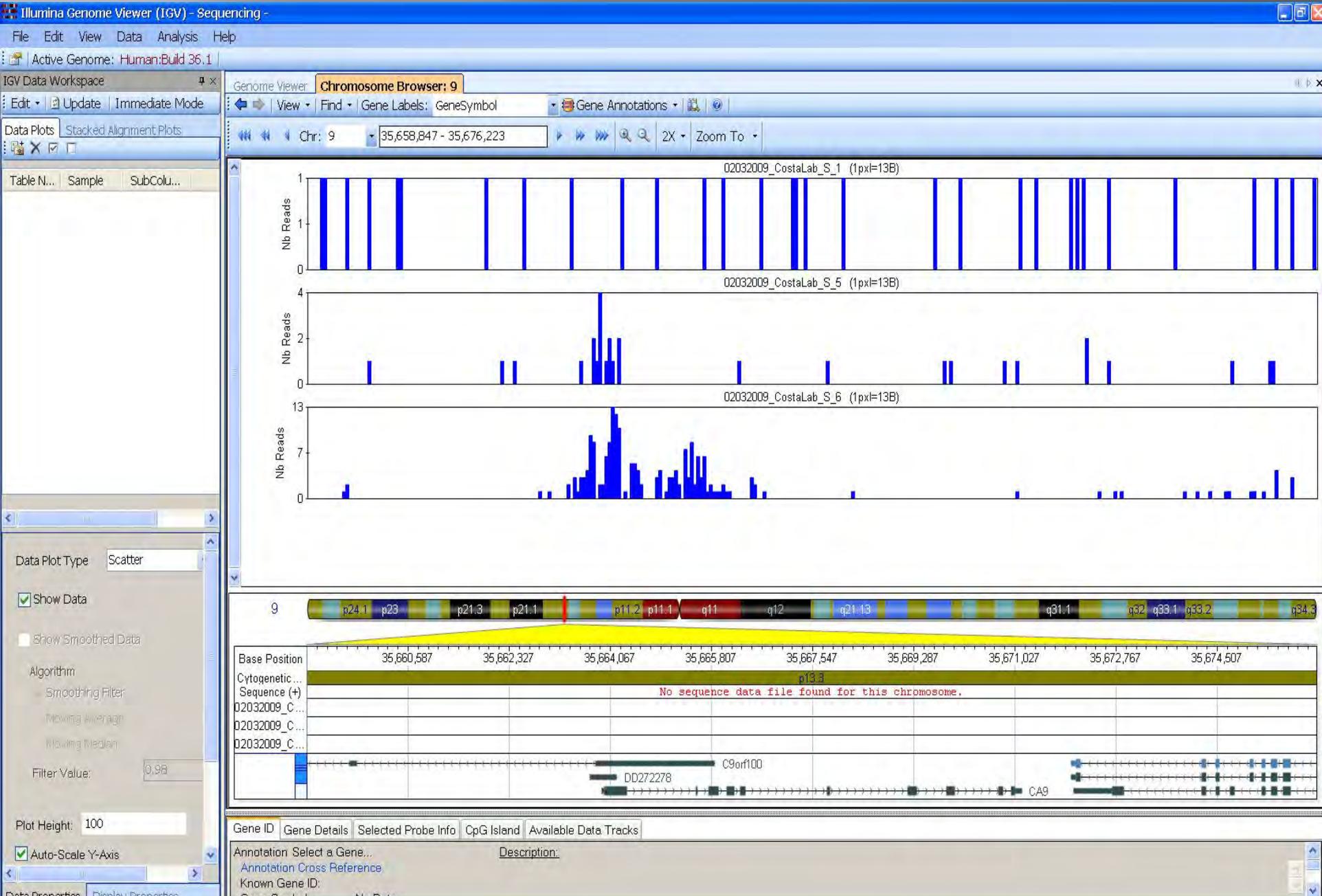
Vertebrate Multiz Alignment & Conservation (44 Species)

Placental Mammal Basewise Conservation by PhyloP

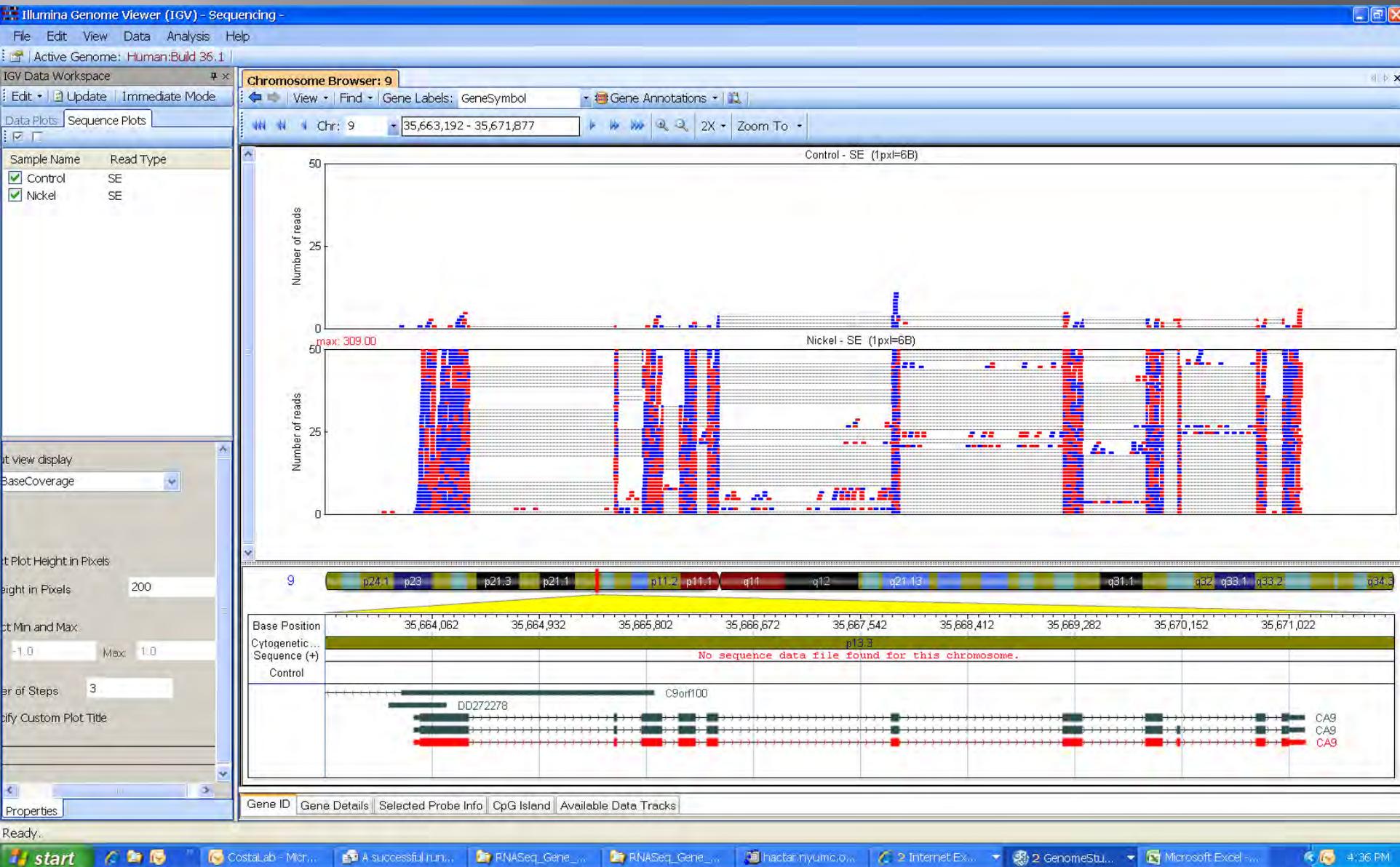
Chip-Seq H3K4 trime NDRG1/Cap43



Chip-Seq H3K4 trimeth CA-9



RNA-Seq +/- Nickel A549 Cells CA-9



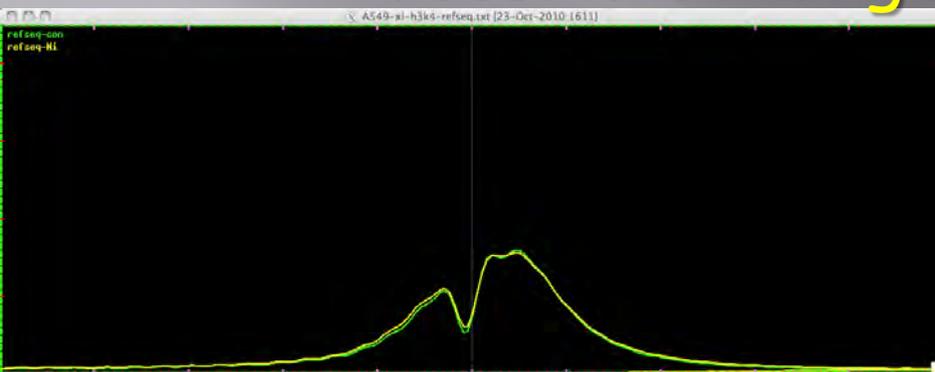
RNA-Seq +/- Nickel A549 Cells NDRG1/Cap43



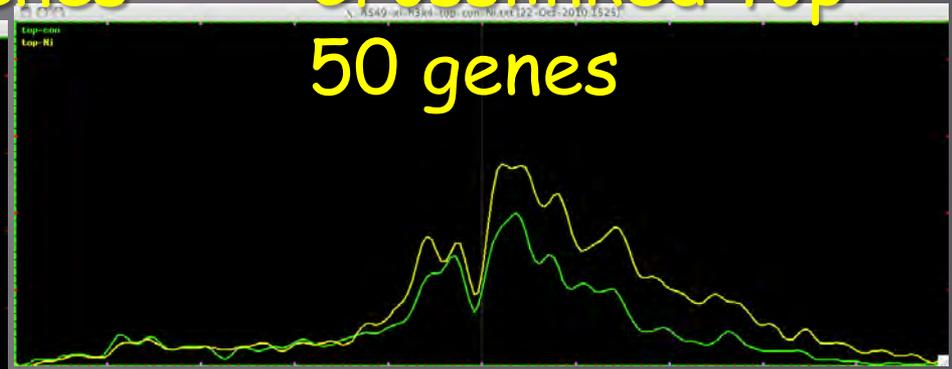
RNA-Seq +/- Nickel A549 Cells ANGPTL4



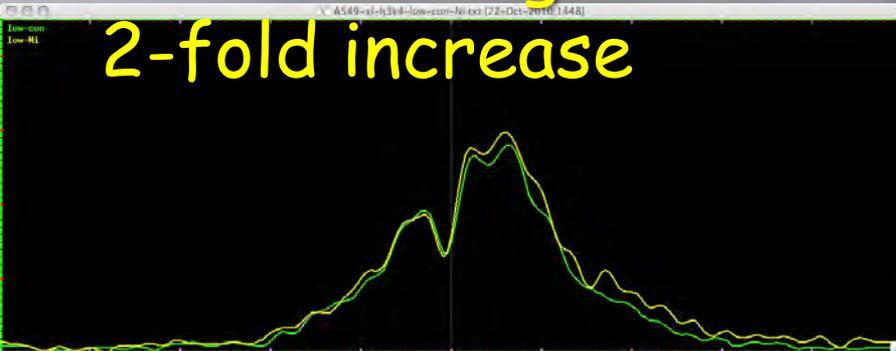
Crosslinked all genes



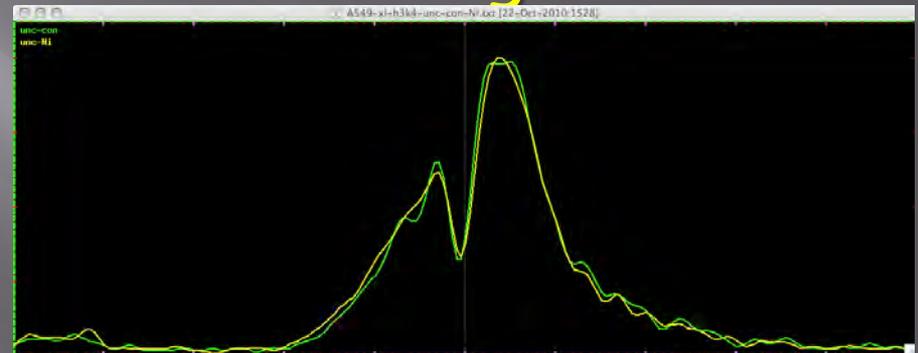
Crosslinked top 50 genes



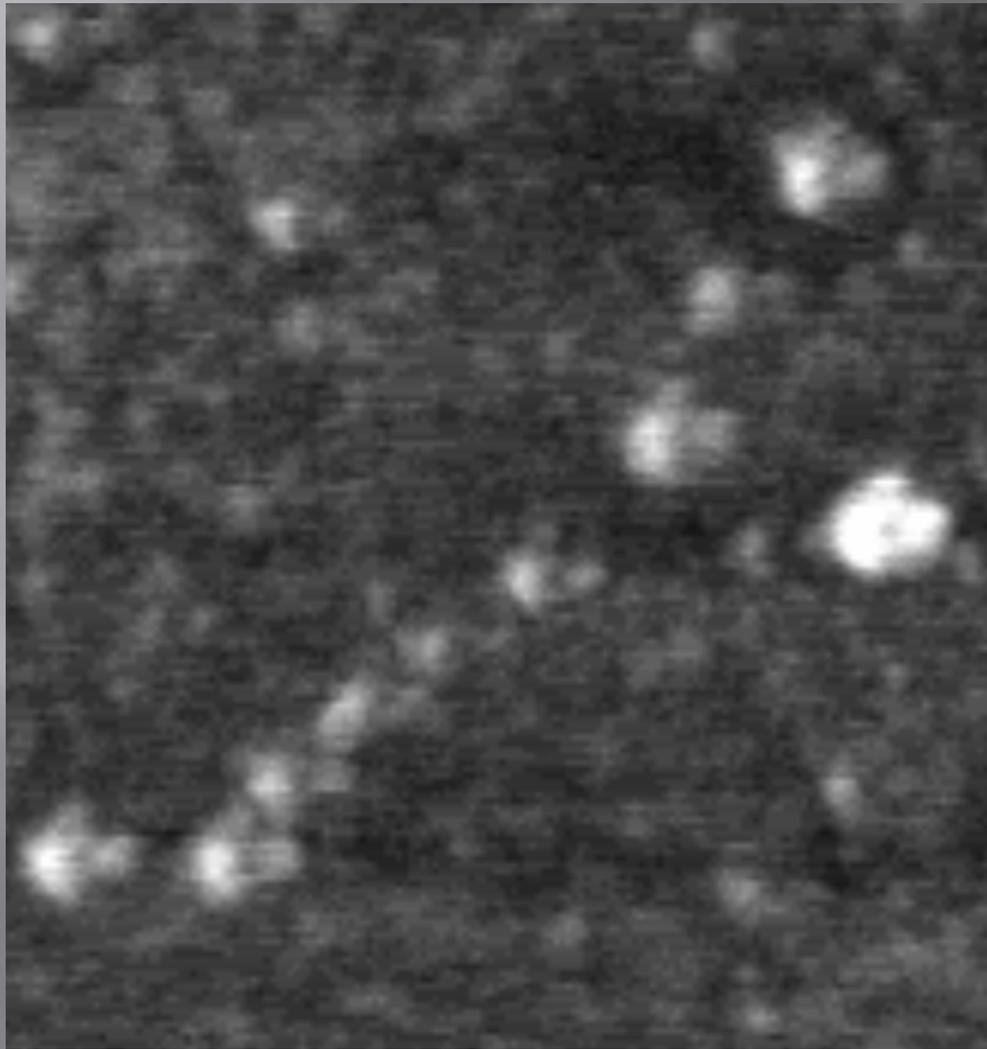
Crosslinked genes with 2-fold increase



Crosslinked genes no

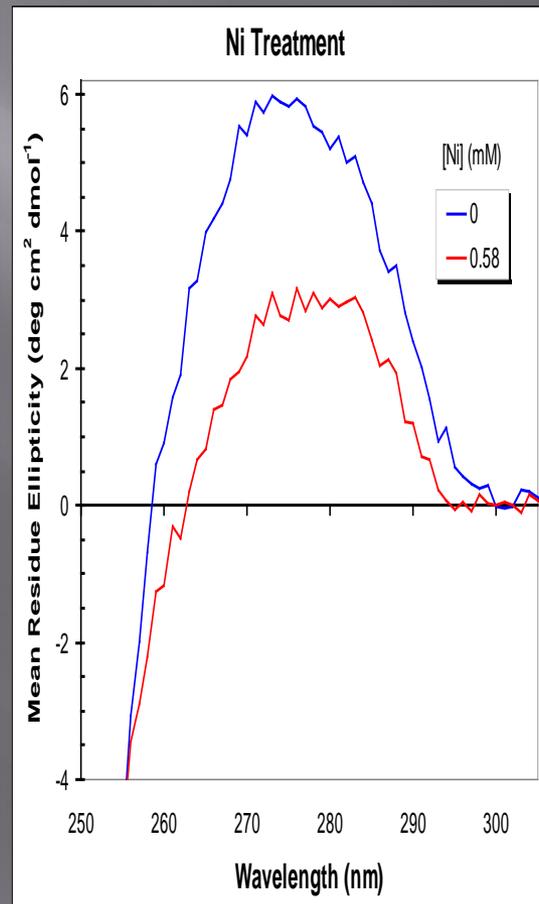
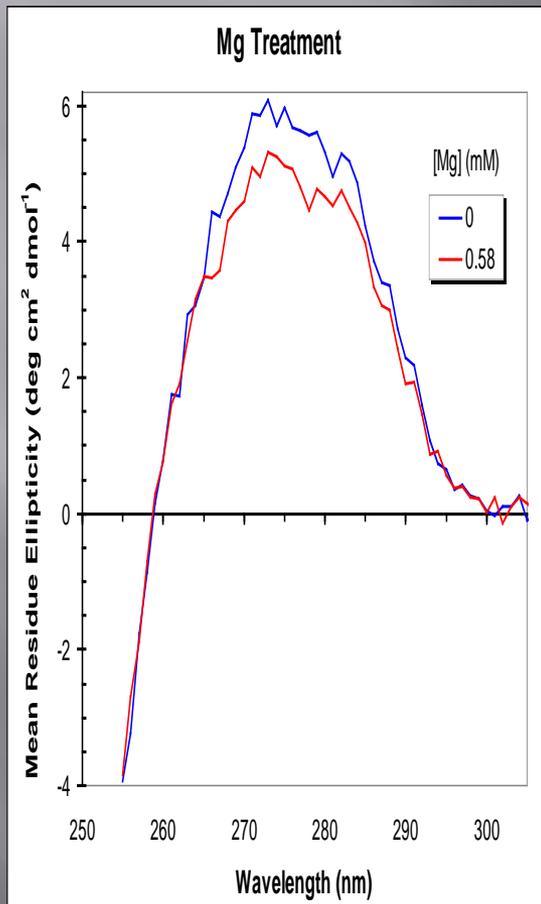


No Cation

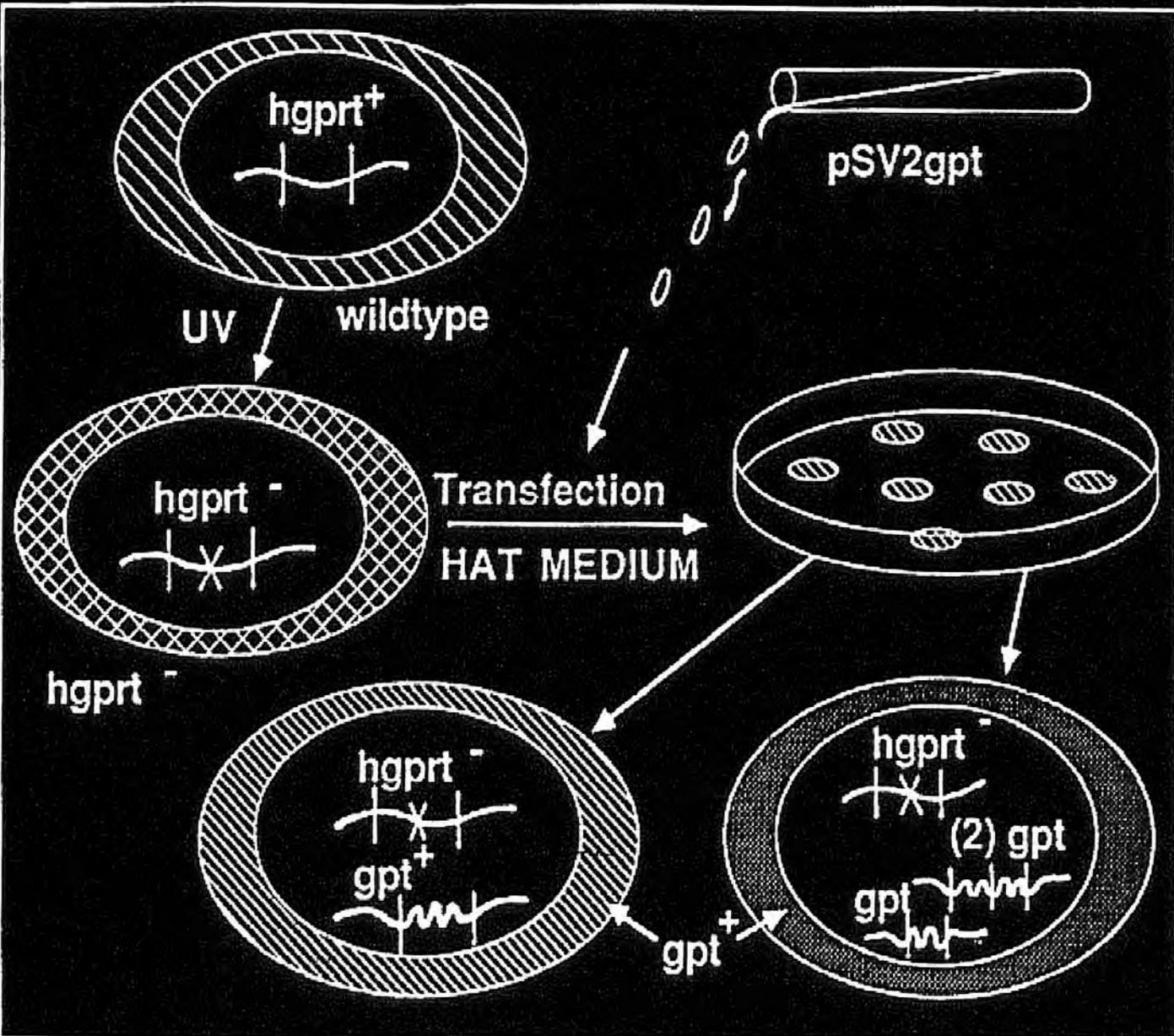


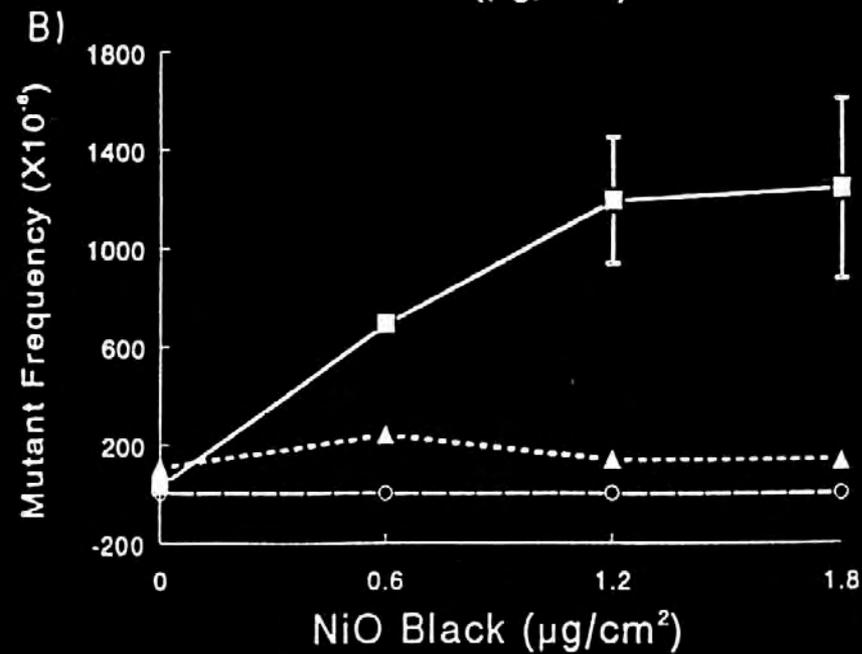
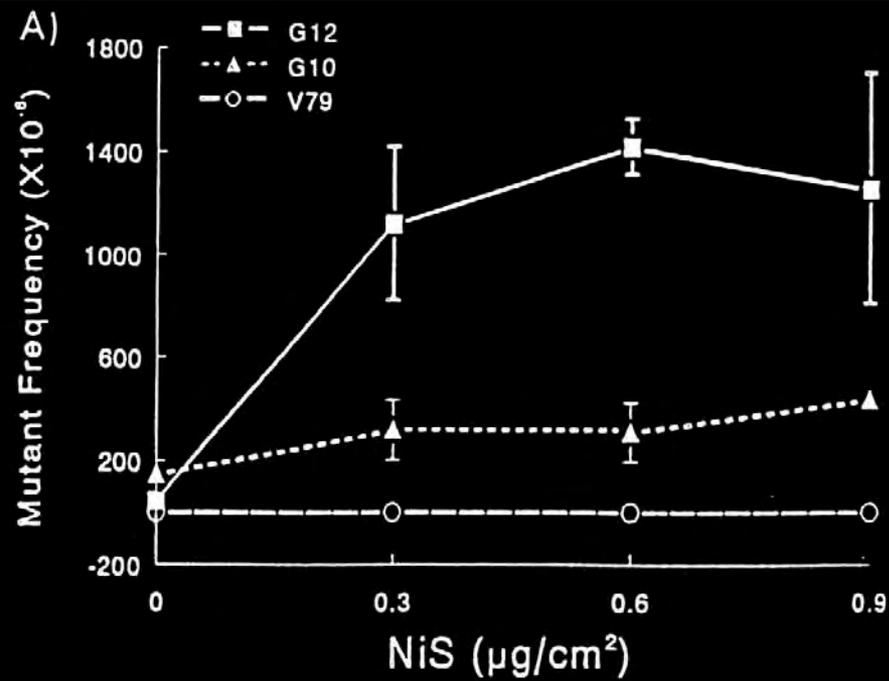
1.1mM Mg⁺²

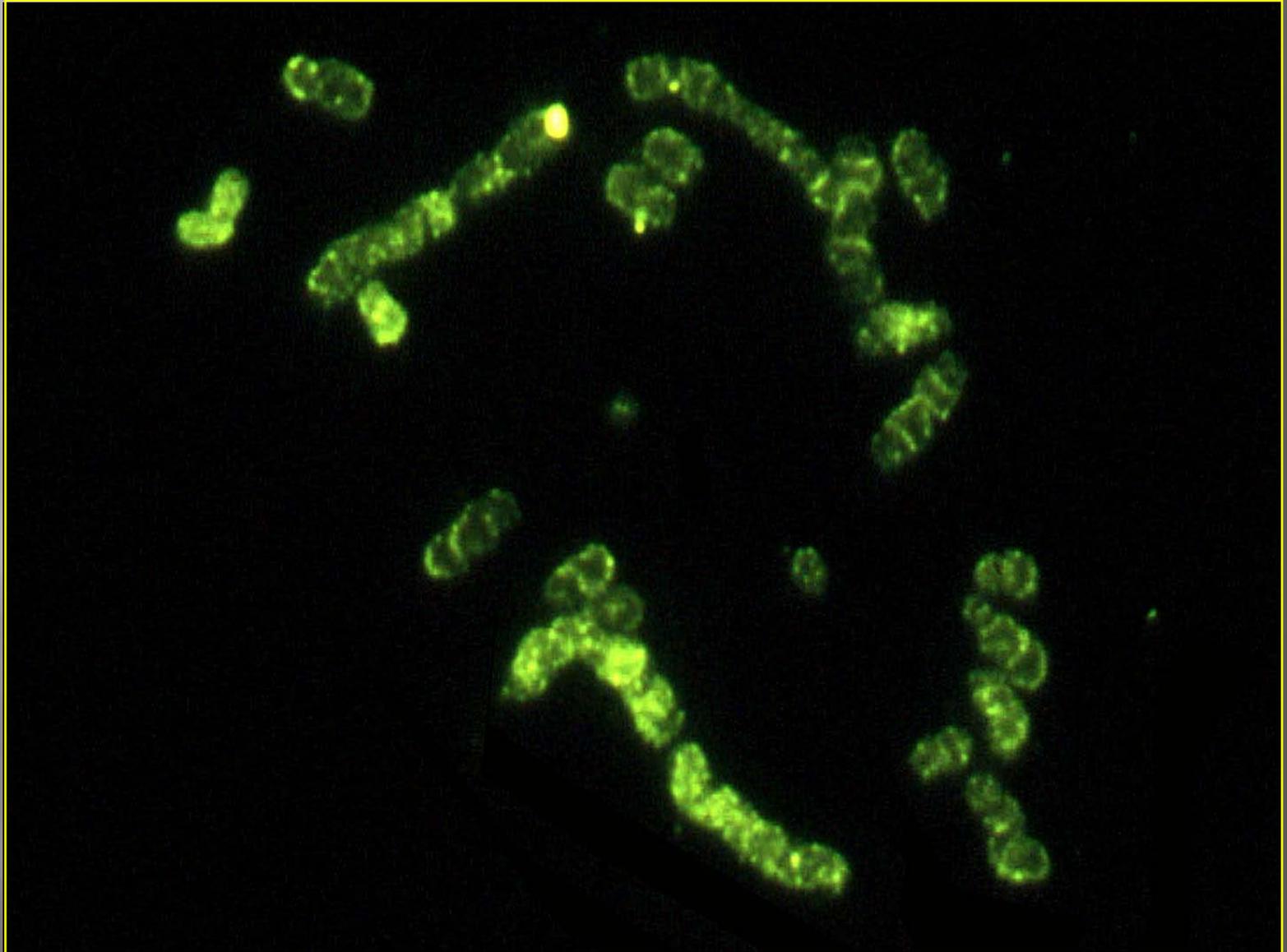




CD spectral difference of dodecanucleosome (12-mer) samples in 0 or 0.58 mM divalent cation. The left panel shows Mg²⁺-treated and the right panel shows Ni²⁺-treated oligonucleosomes. In each case the top curve is the untreated oligonucleosomes and the lower curve is divalentcation-treated oligonucleosomes.







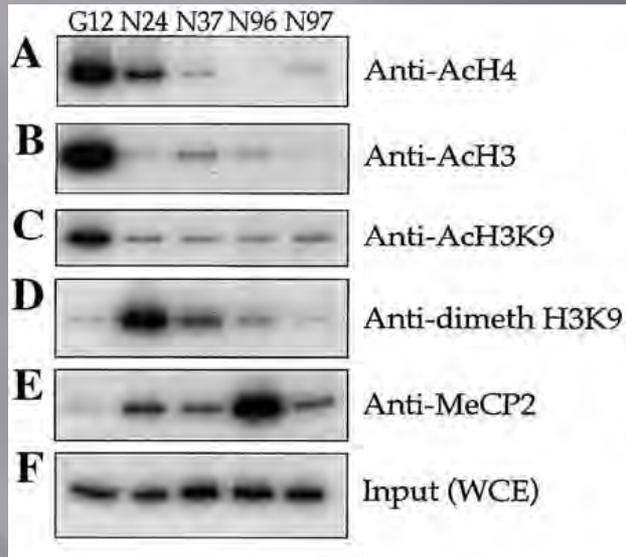
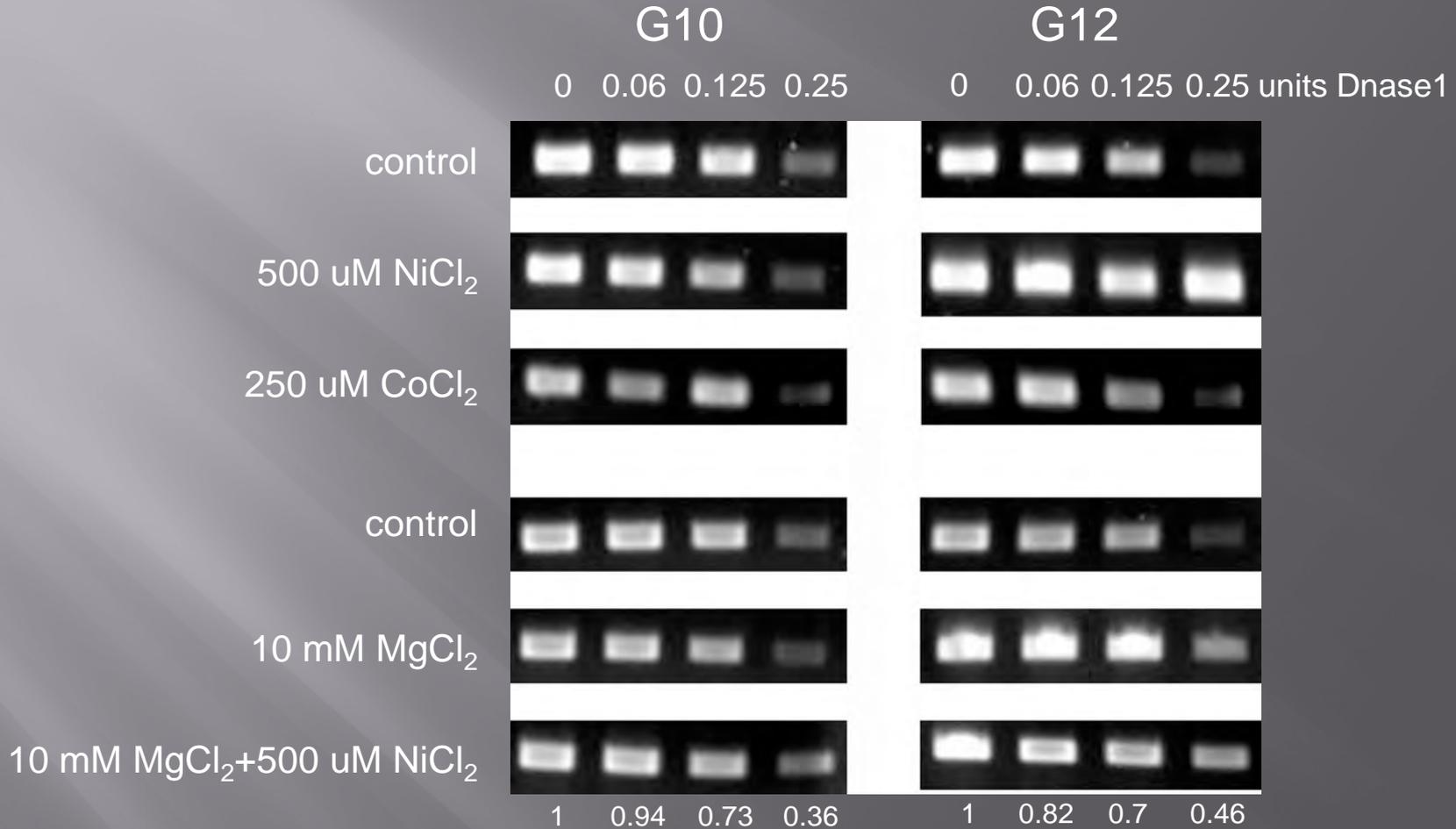


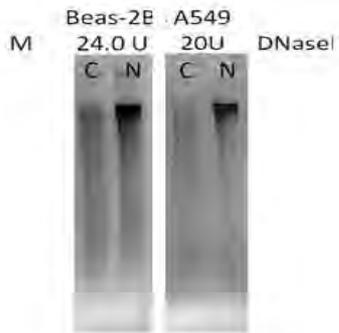
Figure 4. Analysis of chromatin proteins associated with Ni-silenced *gpt* gene using the chromosome immunoprecipitation assay. The ChIP assay was performed with anti-acetylated H4 (A), anti-acetylated H3 (B), anti-acetylated H3K9 (C), anti-dimethyl-H3K9 (D), and anti-MeCP2 (E). Input DNA fractions were amplified by PCR to adjust for chromatin loading (F). A representative gel is shown but similar results were observed in three replicate experiments. G12 indicates the wild-type clone with *gpt* expression while the N24, N37, N96, and N97 are nickel induced *gpt*-silenced clones.

GPT Dnase1 Protection Assay



* 0.125 units Dnase1

** 0.06 units Dnase1



C control
 N-0.25mM NiCl₂ 75 min on ice

•DNaseI 20min 37C buffer with 200uM CaCl₂

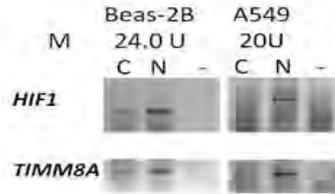


Figure 10. Effect of nickel chloride on the condensation of chromatin in BEAS-2B Cells and A549 cells.

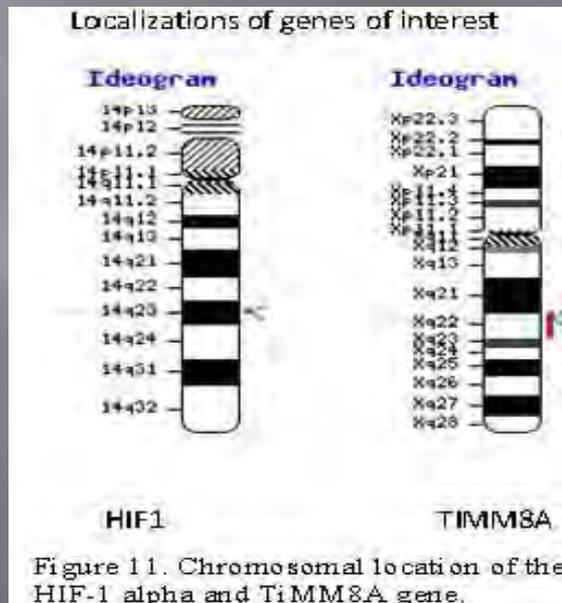
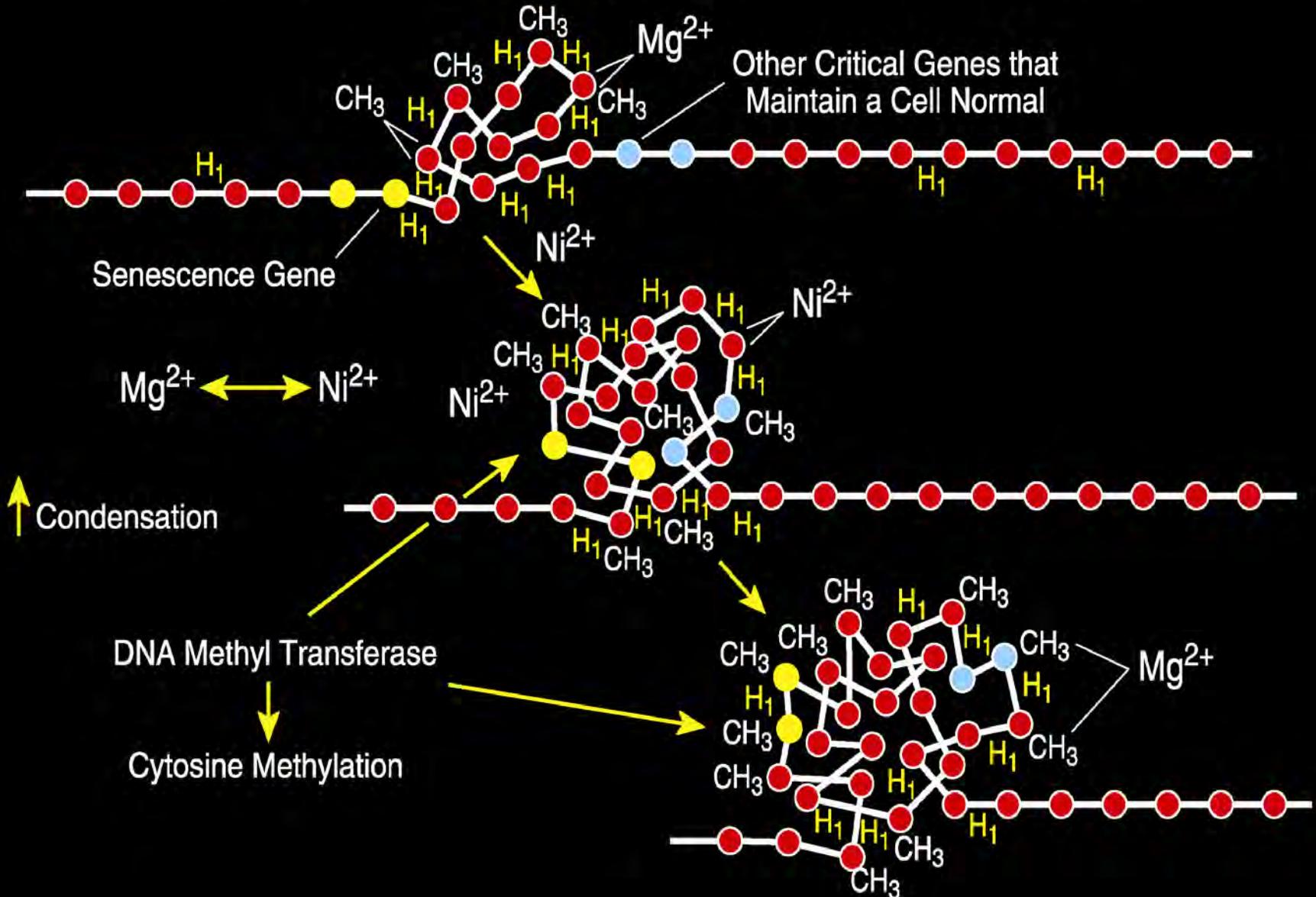


Figure 11. Chromosomal location of the HIF-1 alpha and TiMM8A gene.

Model for Transcriptional Inactivation by Ni²⁺



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Arsenite alters global histone H3 methylation

**Xue Zhou[†], Hong Sun[†], Thomas P. Ellen, Haobin Chen and
Max Costa***

Modulation of histone methylation and *MLH1* gene silencing by
hexavalent chromium *Toxicology and Applied Pharmacology* 237 (2009) 258–266

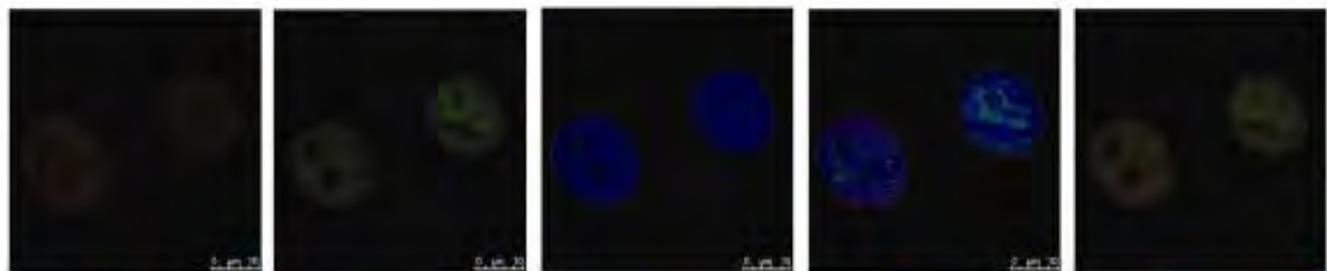
Hong Sun¹, Xue Zhou¹, Haobin Chen, Qin Li, Max Costa*

Effects of nickel, chromate, and arsenite on histone 3 lysine methylation

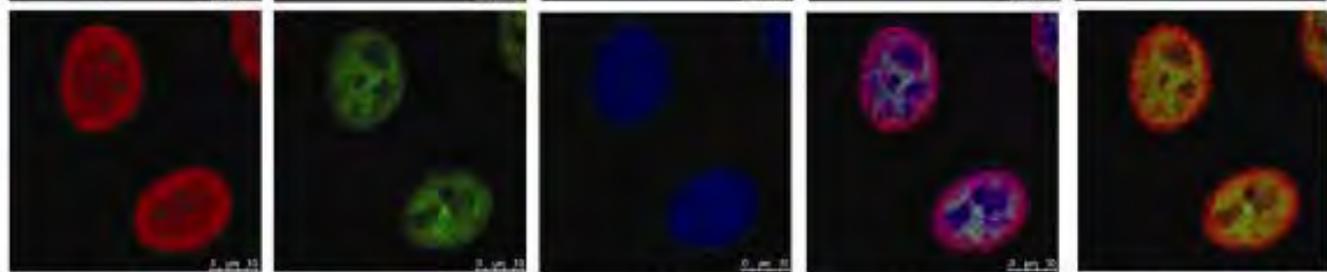
Xue Zhou, Qin Li, Adriana Arita, Hong Sun, Max Costa*

Toxicology and Applied Pharmacology 236 (2009) 78–84

Control



5 μM Cr



H3K9me2

H3K4me3

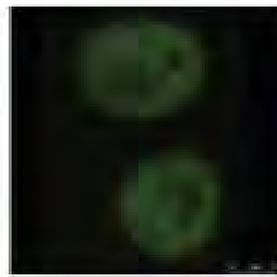
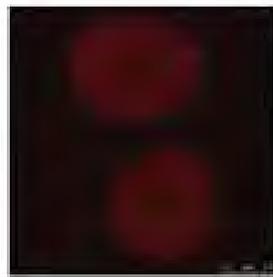
DAPI

Merge

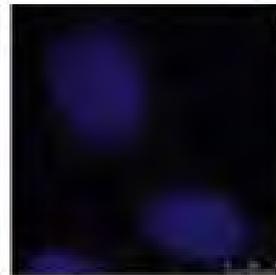
H3K9me2+H3K4me3

C

Control



5 μ M As



H3K9me2

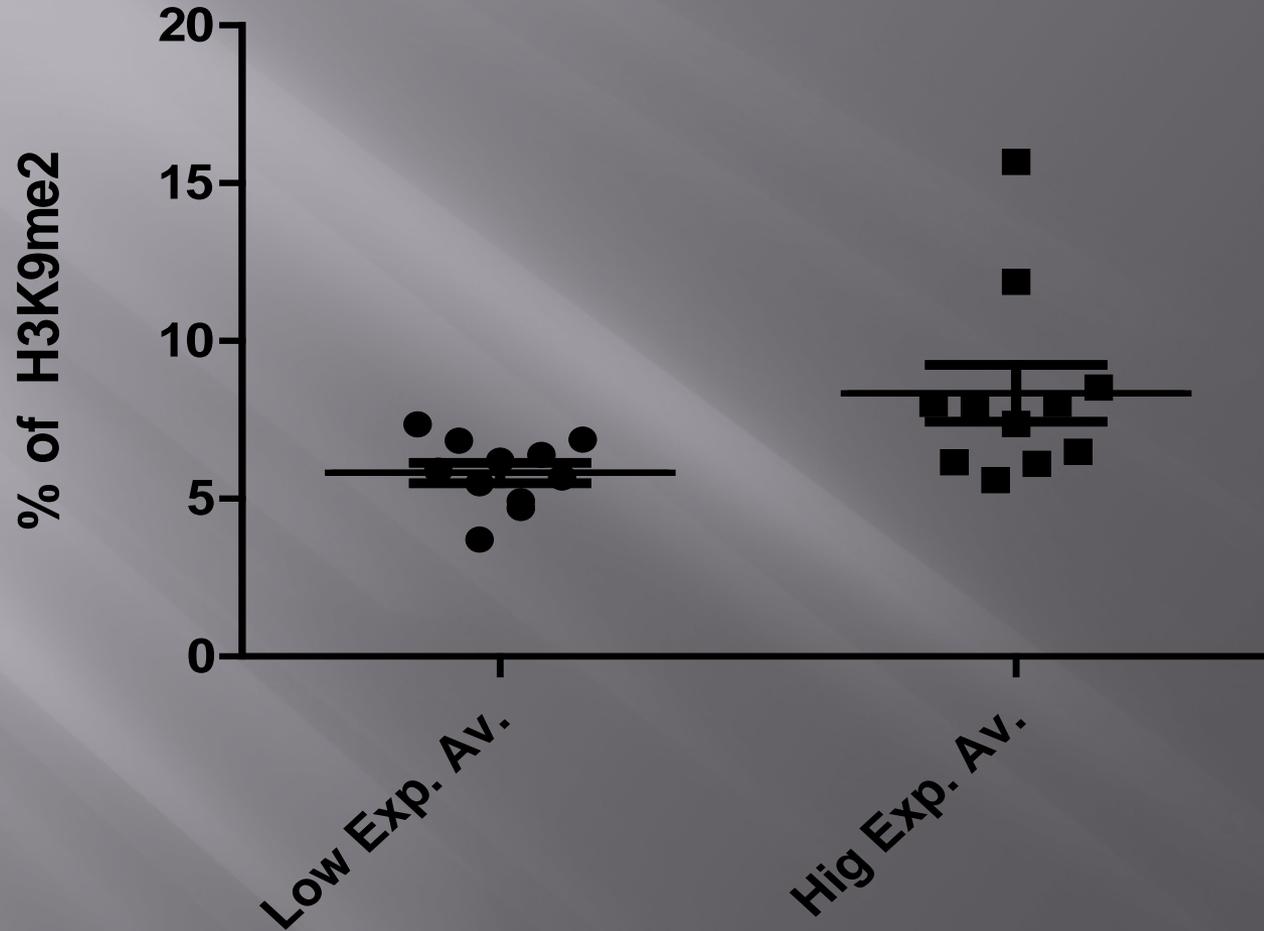
H3K4me3

DAPI

Merge

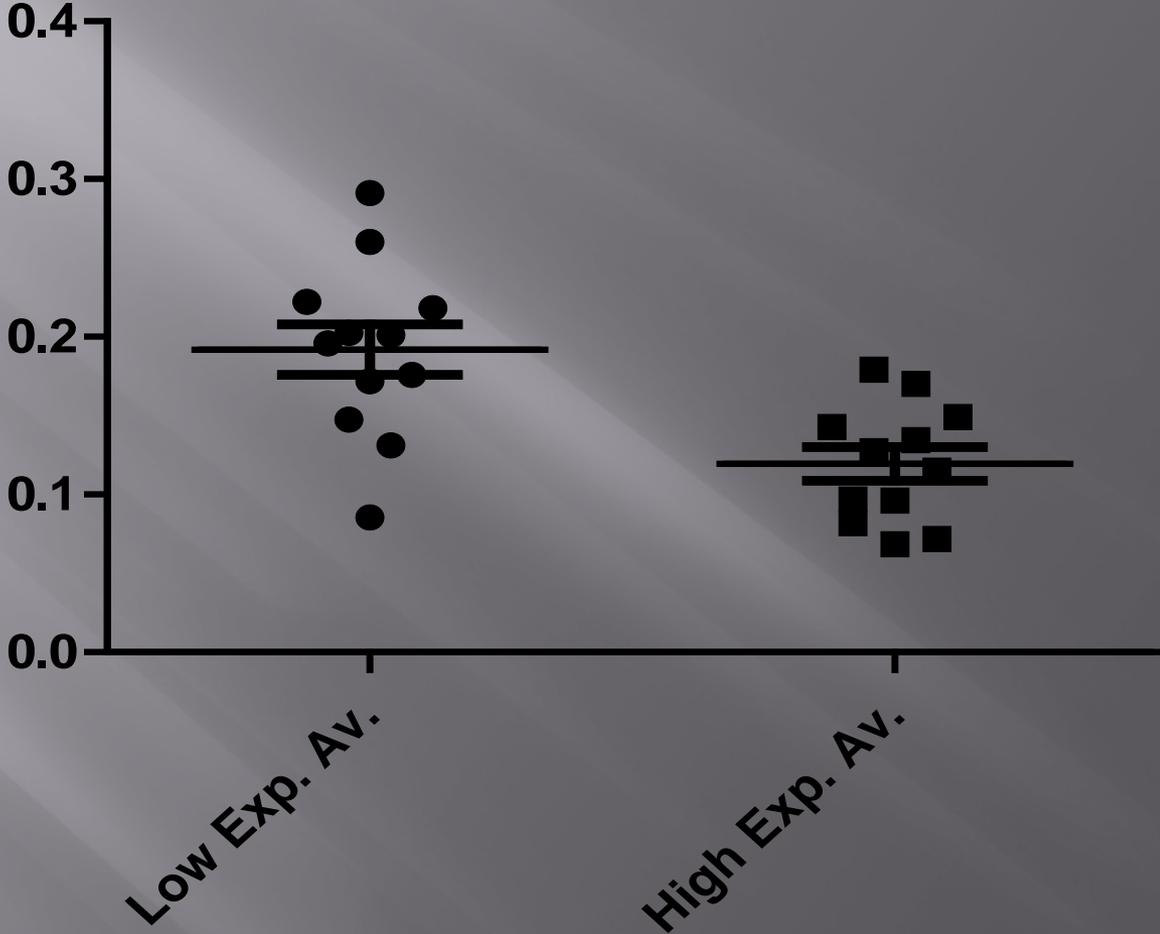
H3K9me2 +
H3K4me3

H3K9me2 All runs Av. with Std.



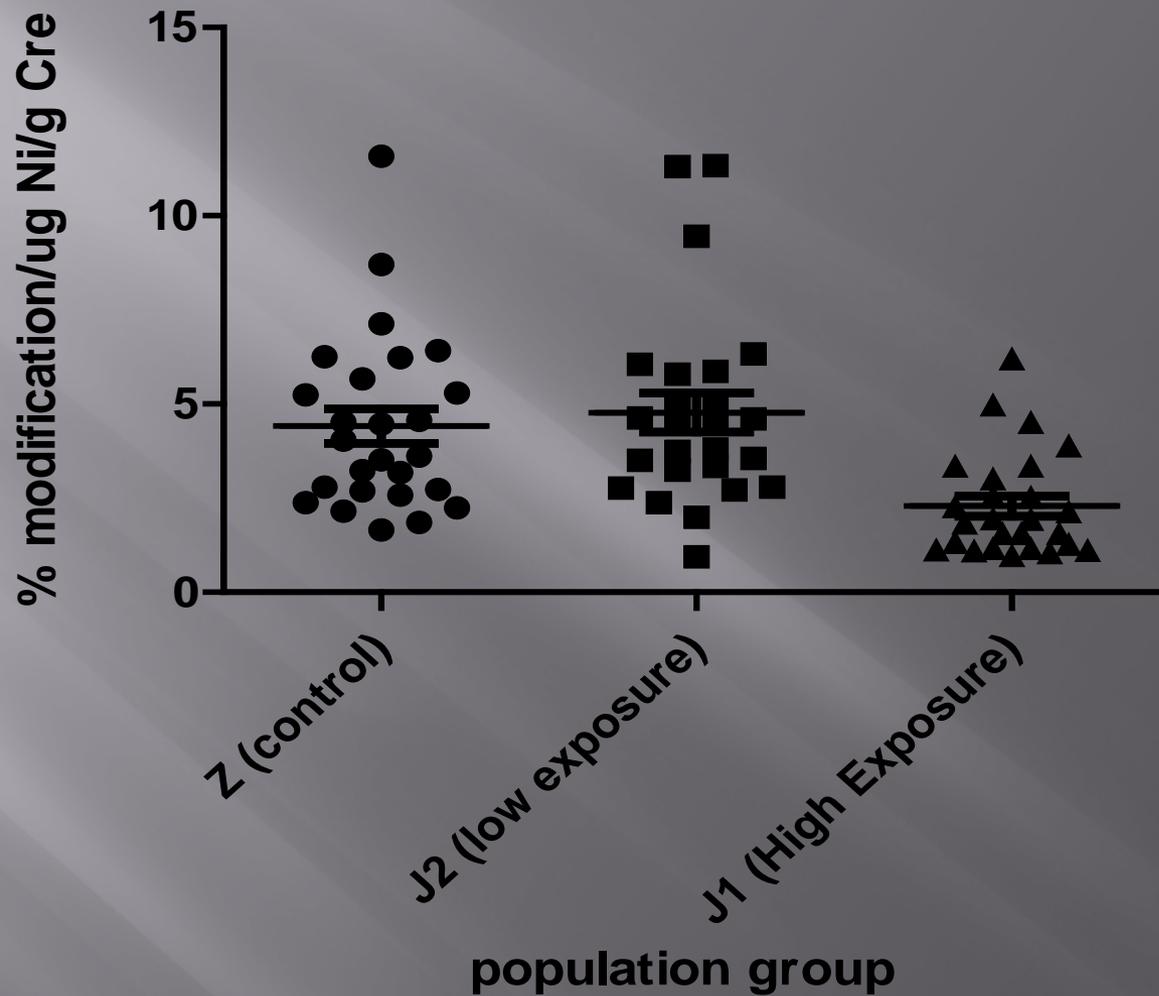
p=0.0157 (CI: 95%)

H3K9acetyl Av. of all runs



p=0.0011 (CI: 95%)

H3K9me2



$p^* = 0.6056$

$p^{**} = 0.0002$

$p^* = t \text{ test (Z vs J2)}$ $p^{**} = t \text{ test (Z vs J1)}$

SUMMARY

- Nickel ions inhibit the dioxygenase histone demethylases leading to increased H3K4 tri and H3K9 di which increases or decreases the expression of specific genes, respectively
- Nickel ions bind and displace the Fe in the His-His Glu facial triad at the active site of dioxygenases such as ABH2
- Mapped genomic positions of H3K4 tri and H3K9 di changes induced by Ni using Chip-on-chip and Chip-Seq technology (correlates with gene expression changes)
- SPRY2 which inhibits ERK signaling is a direct target of histone demethylase JMJD2A
- SPRY2 is epigenetically suppressed in Nickel induced transformed BEAS2B cells (By chronic inhibition of JMJD2A?) and overexpression of SPRY2 reversed the transformed phenotype
- Gene expression changes are very metal specific in Normal Human Bronchial Epithelial cells transformed by Nickel and Chromate
- Nickel induces condensation of chromatin and silencing of genes near or in heterochromatin

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Sterling Forest**



**NYU Cancer Center
Manhattan**

