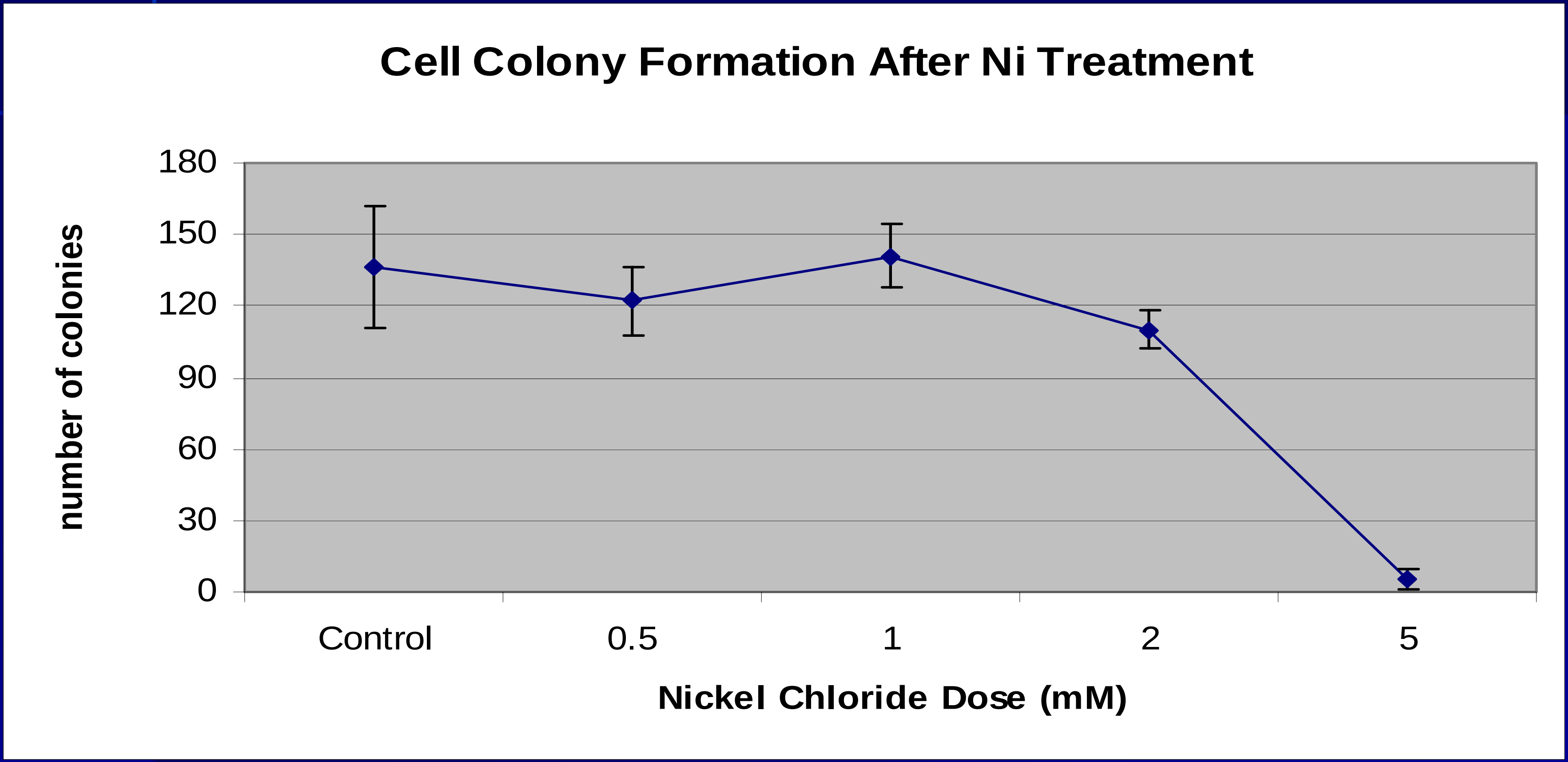
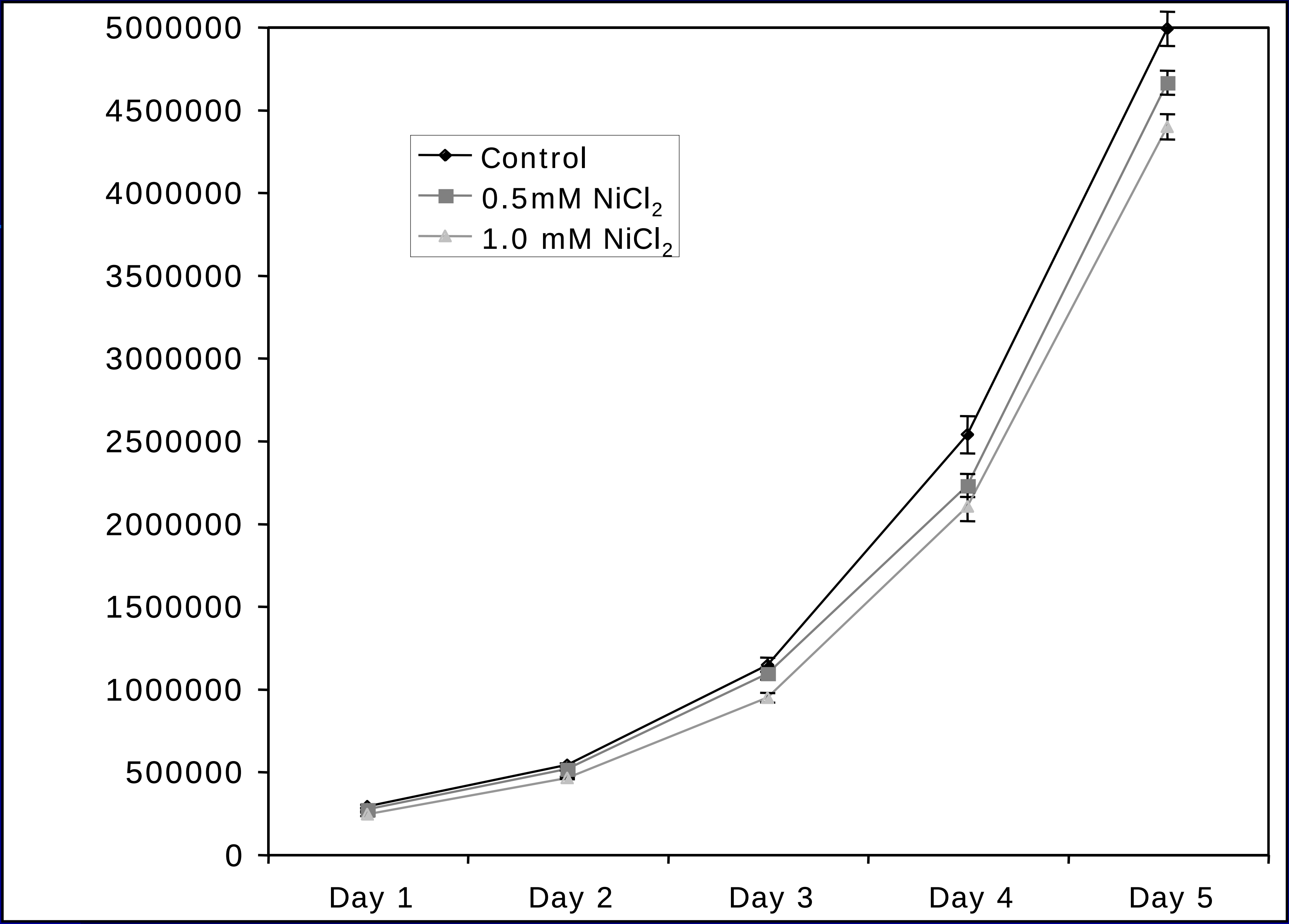
Nickel and Megasites

- Nickel compounds are major contaminants in many superfund sites
- Although Ni ions are required for certain enzymes in bacteria and plants (Ureases, Dehydrogenases), No known function in mammals.
- Certain Ni compounds that deliver Ni ions into cells, are potently carcinogenic (nasal, lung cancers etc at site of exposure)
- Additional toxicities include: contact dermititis, depression of lung function, and cardiovascular effects.

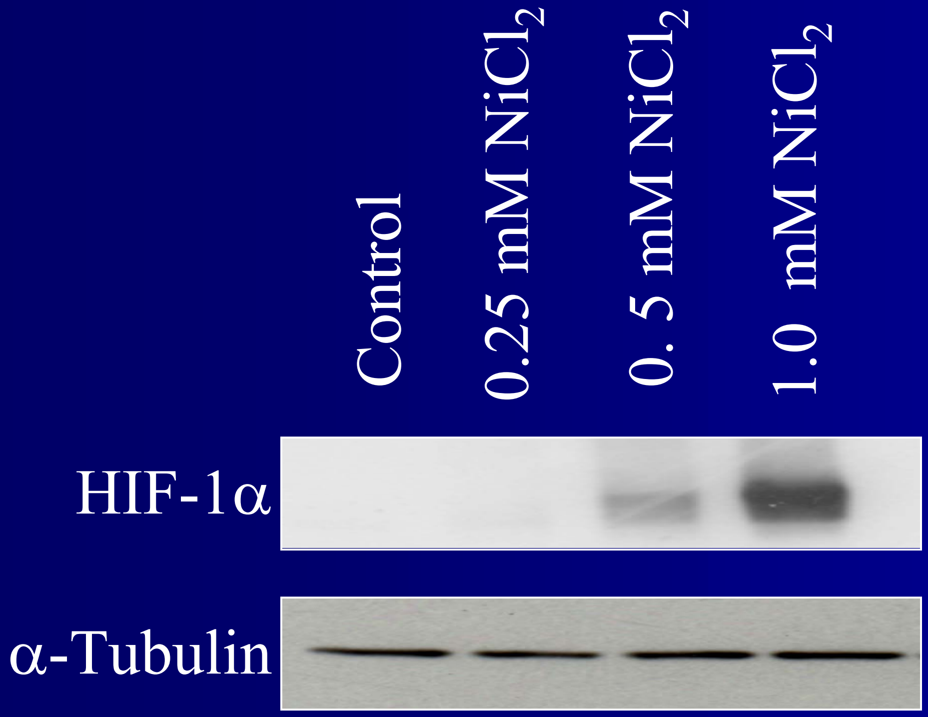
Effect of 24 hr exposure of A549 Cells to $NiCl_2$ On Cell Colony Formation

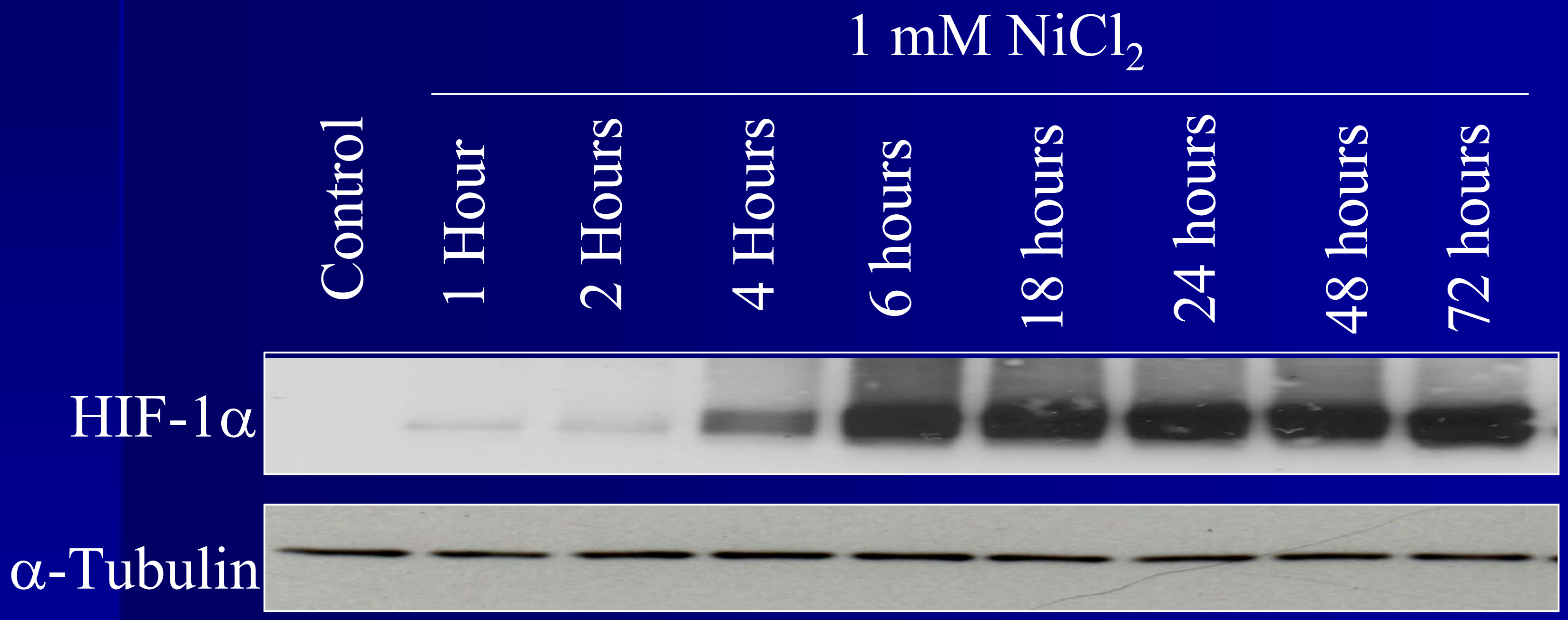




5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
Control
0.5mM $NiCl_2$
1.0 mM $NiCl_2$
Day 1
Day 2
Day 3
Day 4
Day 5

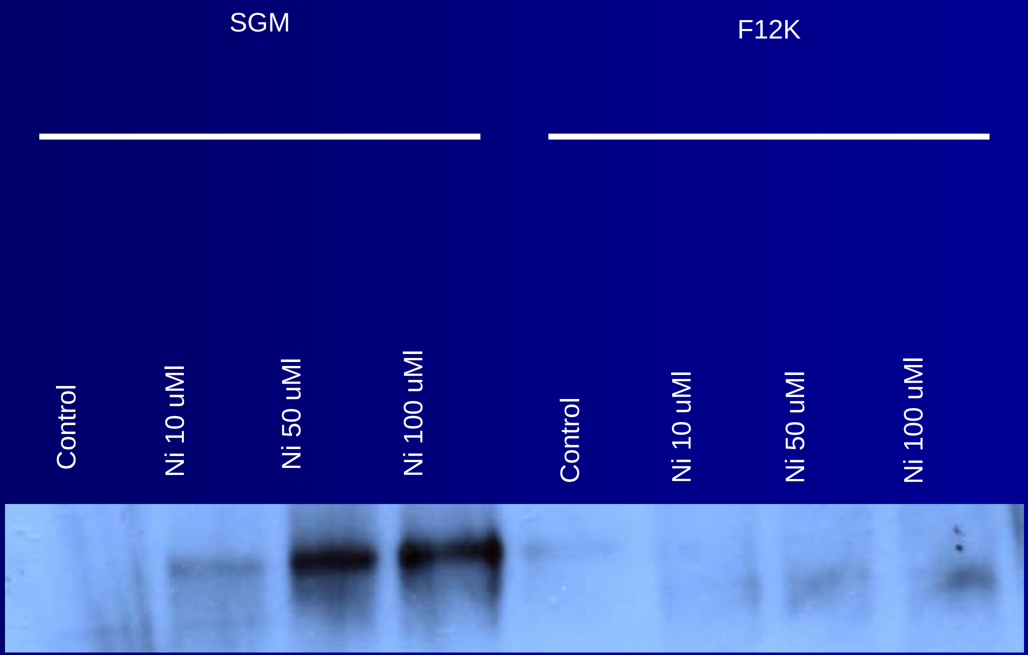
HIF-1 alpha protein levels at 5 hrs



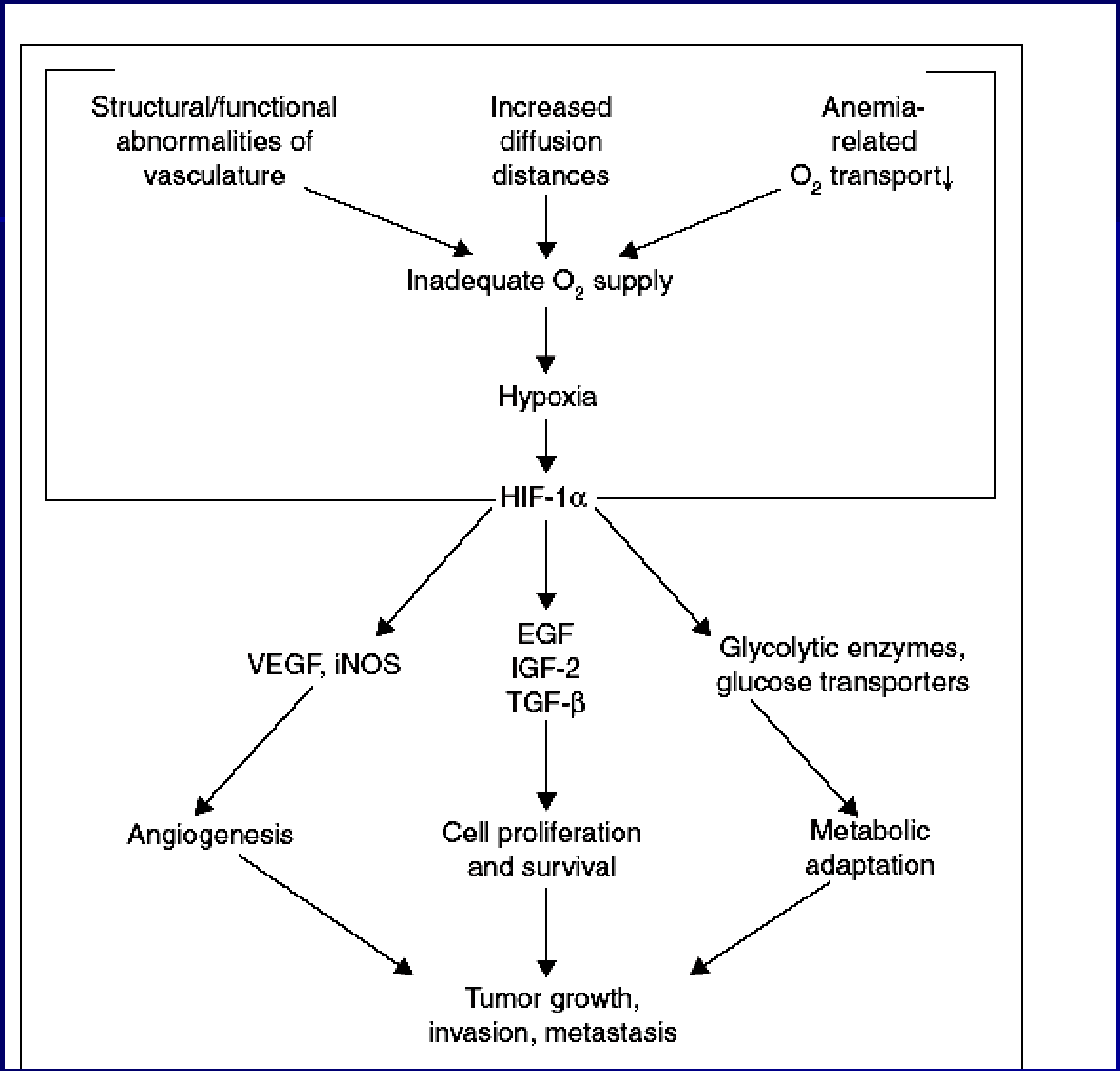

1 mM $NiCl_2$
Control
1 Hour
2 Hours
4 Hours
6 hours
18 hours
24 hours
48 hours
72 hours
HIF-1α
α-Tubulin

A549

5 hr exp.

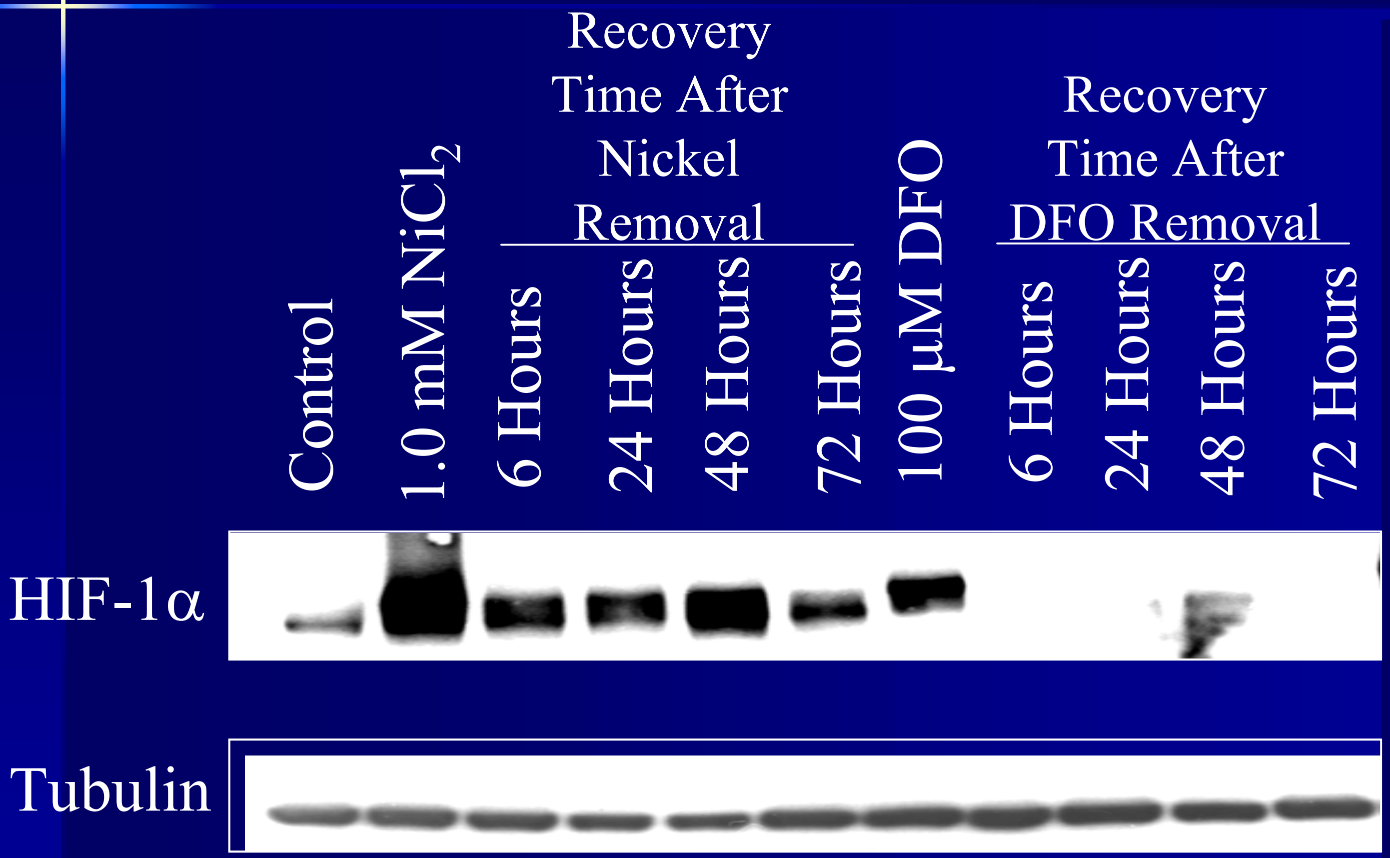


SGM
F12K
Control
Ni 10 uMl
Ni 50 uMl
Ni 100 uMl
Control
Ni 10 uMl
Ni 50 uMl
Ni 100 uMl

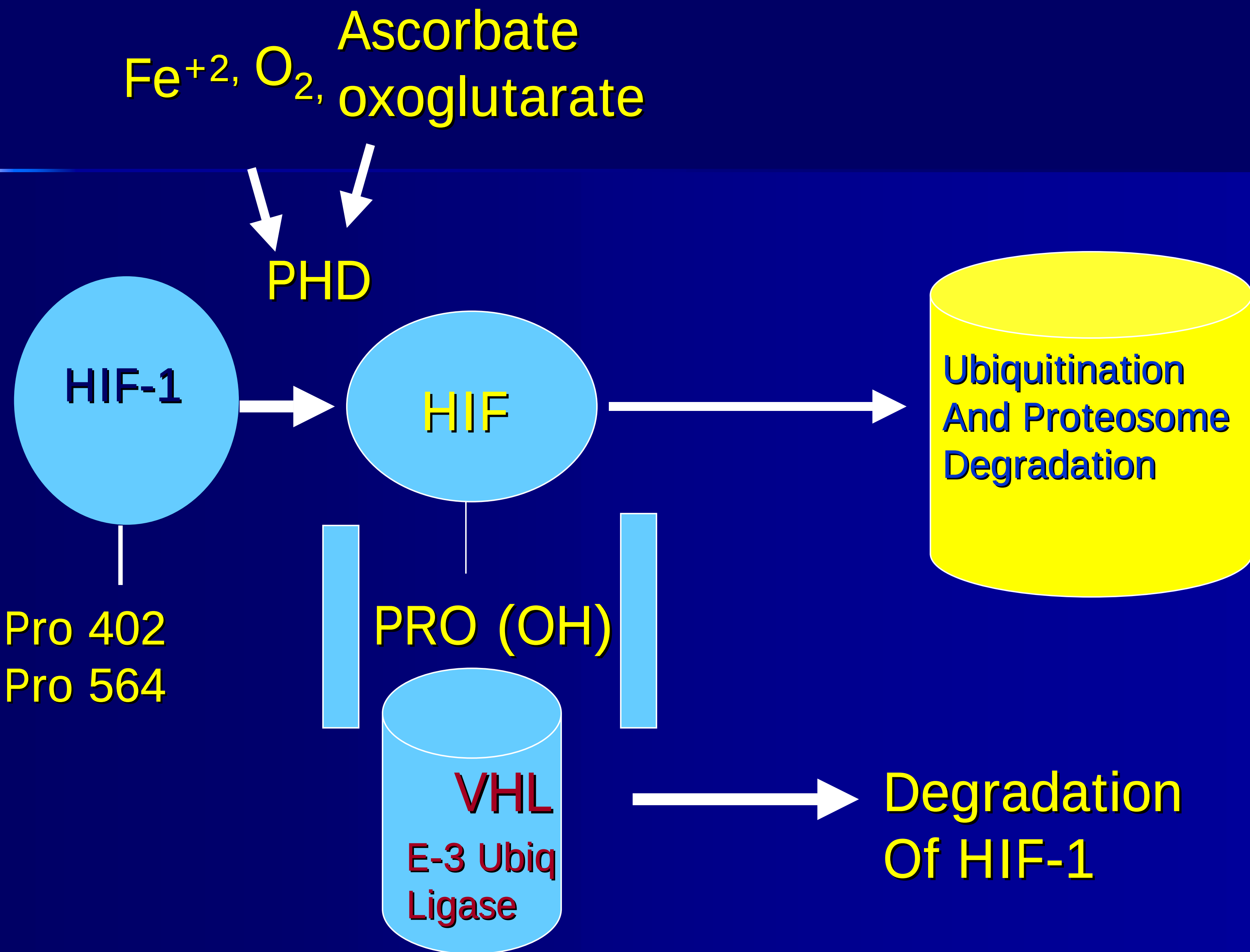


Structural/functional abnormalities of vasculature
Increased diffusion distances
Anemia-related O_2 transport↓
Inadequate O_2 supply
Hypoxia
HIF-1α
VEGF, iNOS
EGF
IGF-2
TGF-β
Glycolytic enzymes, glucose transporters
Angiogenesis
Cell proliferation and survival
Metabolic adaptation
Tumor growth, invasion, metastasis

Persistent Stabilization of HIF-1 alpha by $NiCl_2$

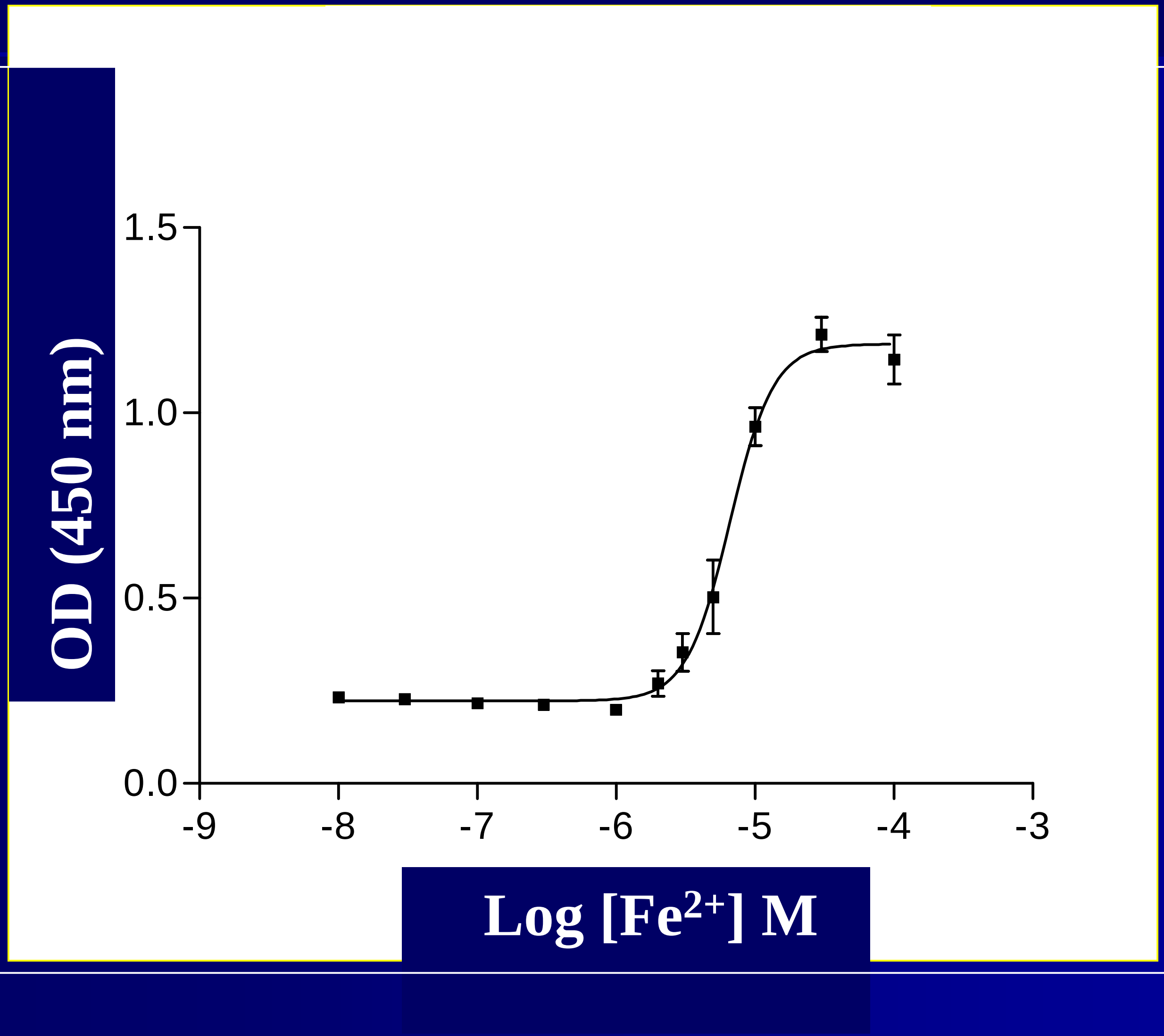


Prolyl Hydroxylase Regulates Hif-1 stability

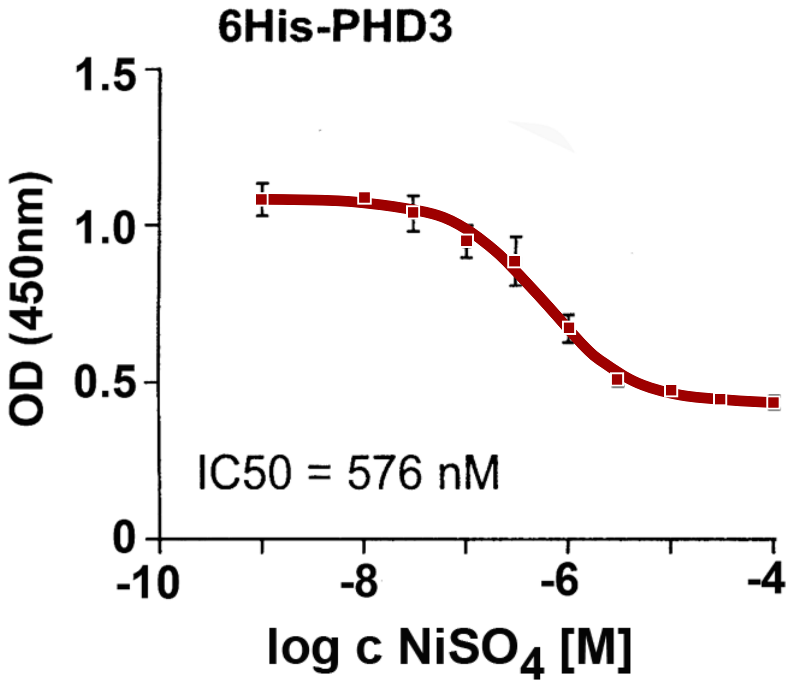


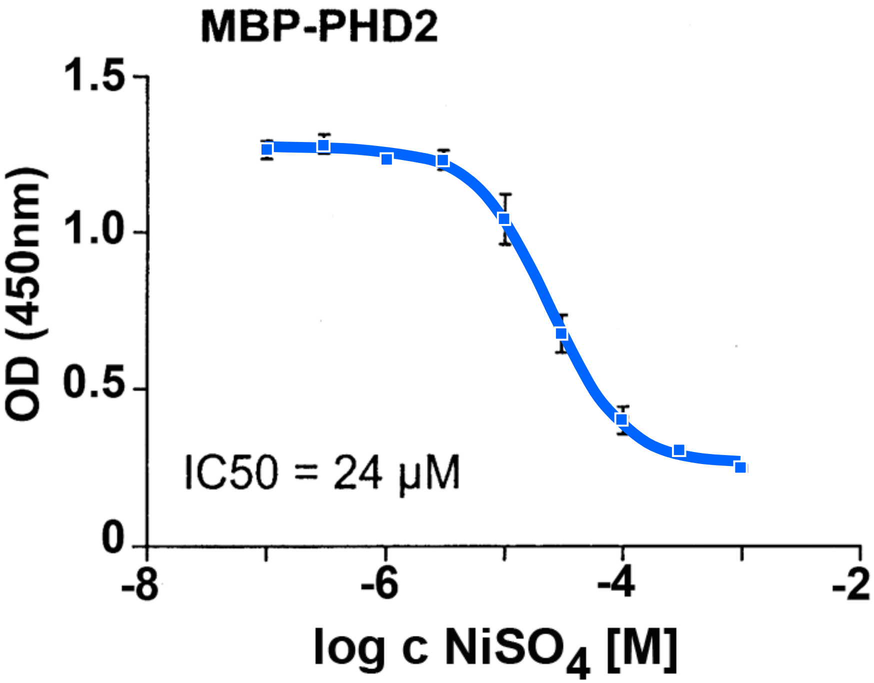
Lack of reversibility of Ni Inhibition of HIF hydroxylation

Requirement for Fe of PH2 (2ug protein)



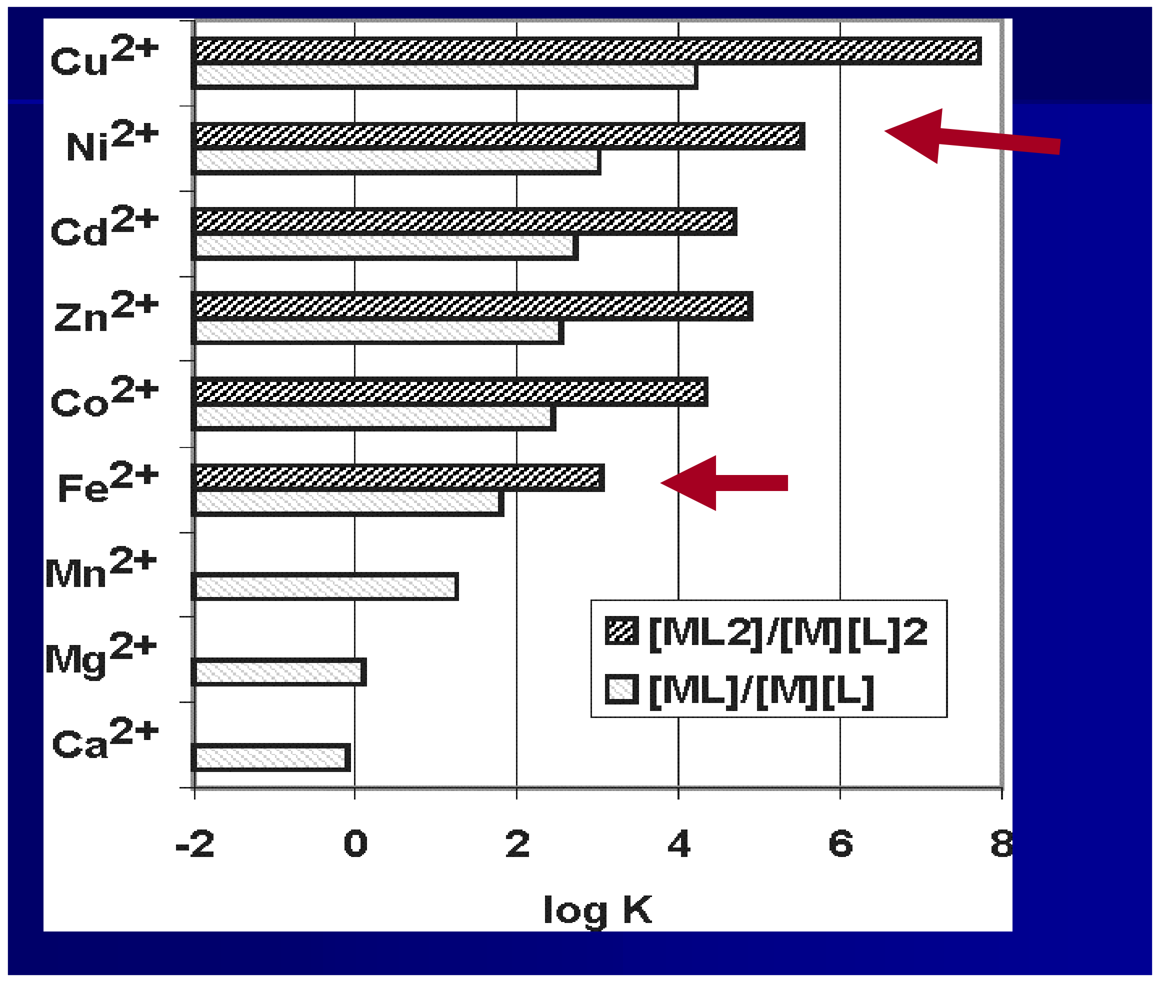
Inhibition of HIF-PHD2 and HIF-PHD3 by $NiSO_4$





Biochemical assay performed according to Oehme et al. (2004) Anal. Biochem. **330**, 74 - 80
6His-PHD3: expressed in insect cells, initially purified by incubation with DEAE-Sepharose
MBP-PHD2: expressed in E. coli, purified by affinity chromatography with Amylose Resin
MBP-PHD2: 2 µg 6His-PHD3: 20 ng (estimated)
Measurements were performed in triplicate and are show as mean values ± SEM

Binding Constants of Metal Ions to Immidazole



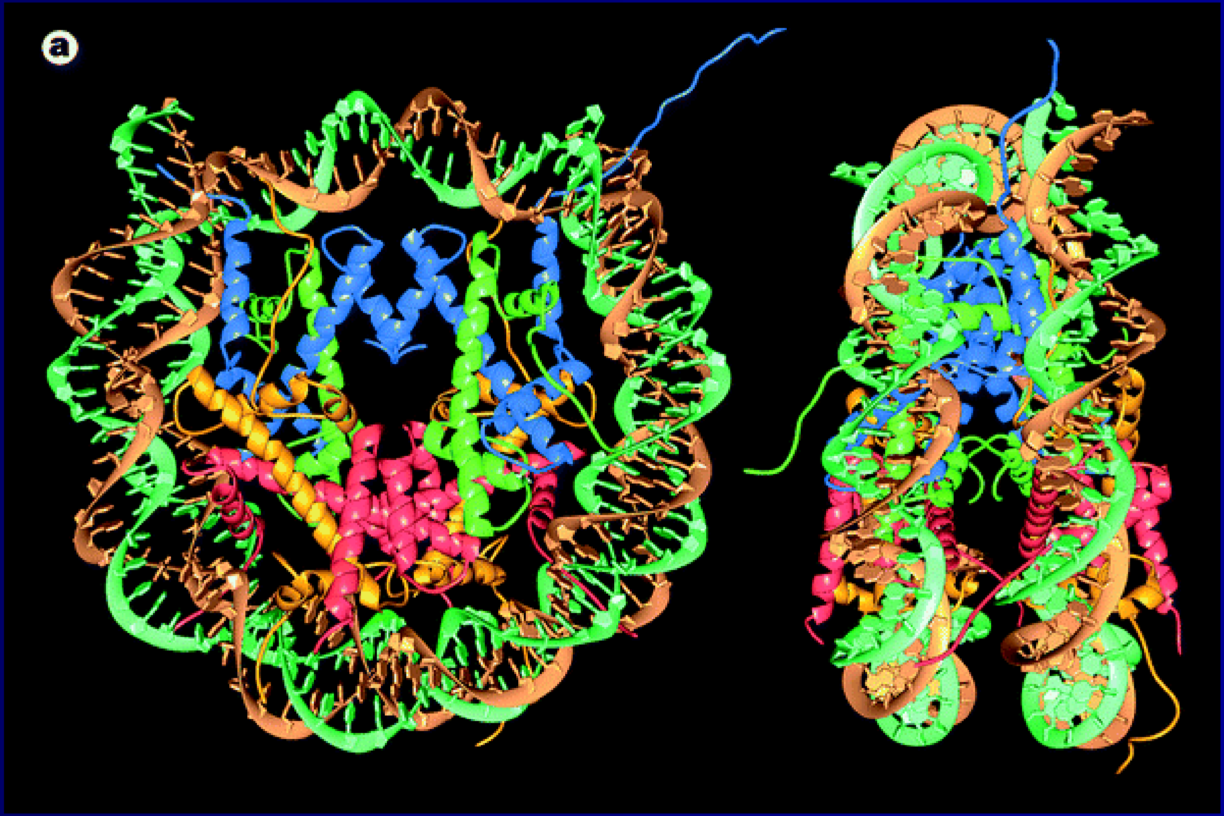
Periodic Table

3 IIIB	4 IVB	5 VB	6 VIB	7 VIIB	8	9 VIII	10	11 IB	12 IIB
Sc 21 44.955910 1.3 3+ Scandium	Ti 22 47.88 1.5 4+ Titanium	V 23 50.9415 1.6 5+ Vanadium	Cr 24 51.9961 1.6 3+ Chromium	Mn 25 54.93805 1.5 2+ Manganese	Fe 26 55.847 1.8 3+ Iron	Co 27 58.9332 1.8 2+ Cobalt	Ni 28 58.6934 1.8 2+ Nickel	Cu 29 63.546 1.9 2+ Copper	Zn 30 65.39 1.6 2+ Zinc
Y 39 88.90585 1.3 3+ Yttrium	Zr 40 91.224 1.4 4+ Zirconium	Nb 41 92.90638 1.6 5+ Niobium	Mo 42 95.94 1.8 6+ Molybdenum	Tc 43 98.9063 1.9 7+ Technetium	Ru 44 101.57 2.2 3+,4+ Ruthenium	Rh 45 102.9055 2.2 3+ Rhodium	Pd 46 106.42 2.2 2+ Palladium	Ag 47 107.8682 1.9 1+ Silver	Cd 48 112.411 1.7 2+ Cadmium
La 57 138.9055 1.1 3+ Lanthanum	Hf 72 178.49 1.3 4+ Hafnium	Ta 73 180.9479 1.5 5+ Tantalum	W 74 183.85 1.7 6+ Tungsten	Re 75 186.207 1.9 7+ Rhenium	Os 76 190.2 2.2 4+ Osmium	Ir 77 192.22 2.2 4+ Iridium	Pt 78 195.08 2.2 4+ Platinum	Au 79 196.96654 2.4 3+ Gold	Hg 80 200.59 1.9 2+ Mercury
Ac 89 227.0278 1.1 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

Summary of Findings

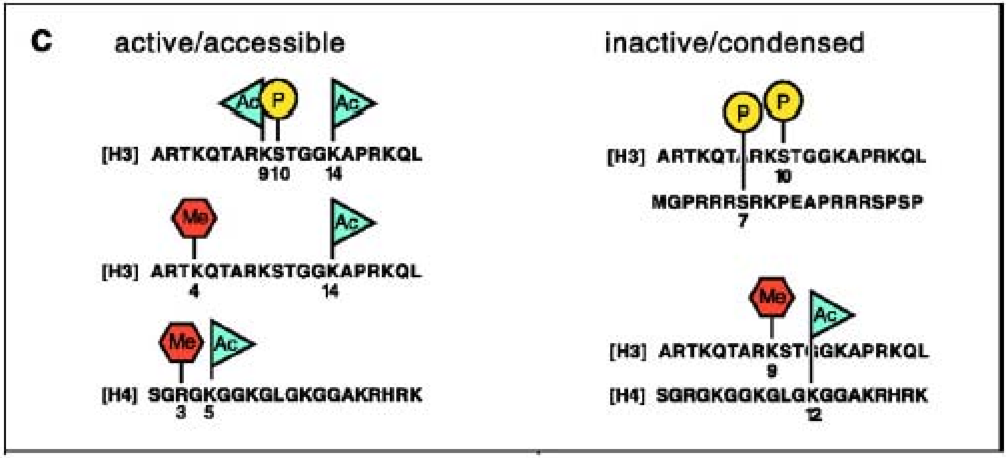
- Transformation of Chinese hamster primary embryo fibroblast by Nickel Compounds inactivated tumor suppressor gene by DNA Methylation (*Science* **251**:796-799 (1991)
- Carcinogenic Nickel compounds Induce trangene silencing based upon the location of the trangene near Heterochromatin (mammalian cells) or a telomere silencing in yeast. (Lee et al MCB 15 2547 1995)

Structure of Nucleosome



Luger K et al. Nature, **389:**251

Blue: H3; green: H4; yellow: H2A; red: H2B



Jenuwein and Allis, Science, August 2001.

Histone Code Hypothesis:

Different combinations of histone modifications, especially located near or within a gene's promoter, may be VERY SPECIFIC to the transcriptional state of that gene.

Associated with active/accessible chromatin

H3K9 Acetylated
H3K14 Acetylated
H3K4 (di-)Methylated
H4 Acetylated

ADD IN DNA METHYLATION, AND THE TRANSCRIPTIONAL REGULATION OF A GENE CAN BECOME VERY COMPLEX!!!

Associated with inactive/condensed chromatin

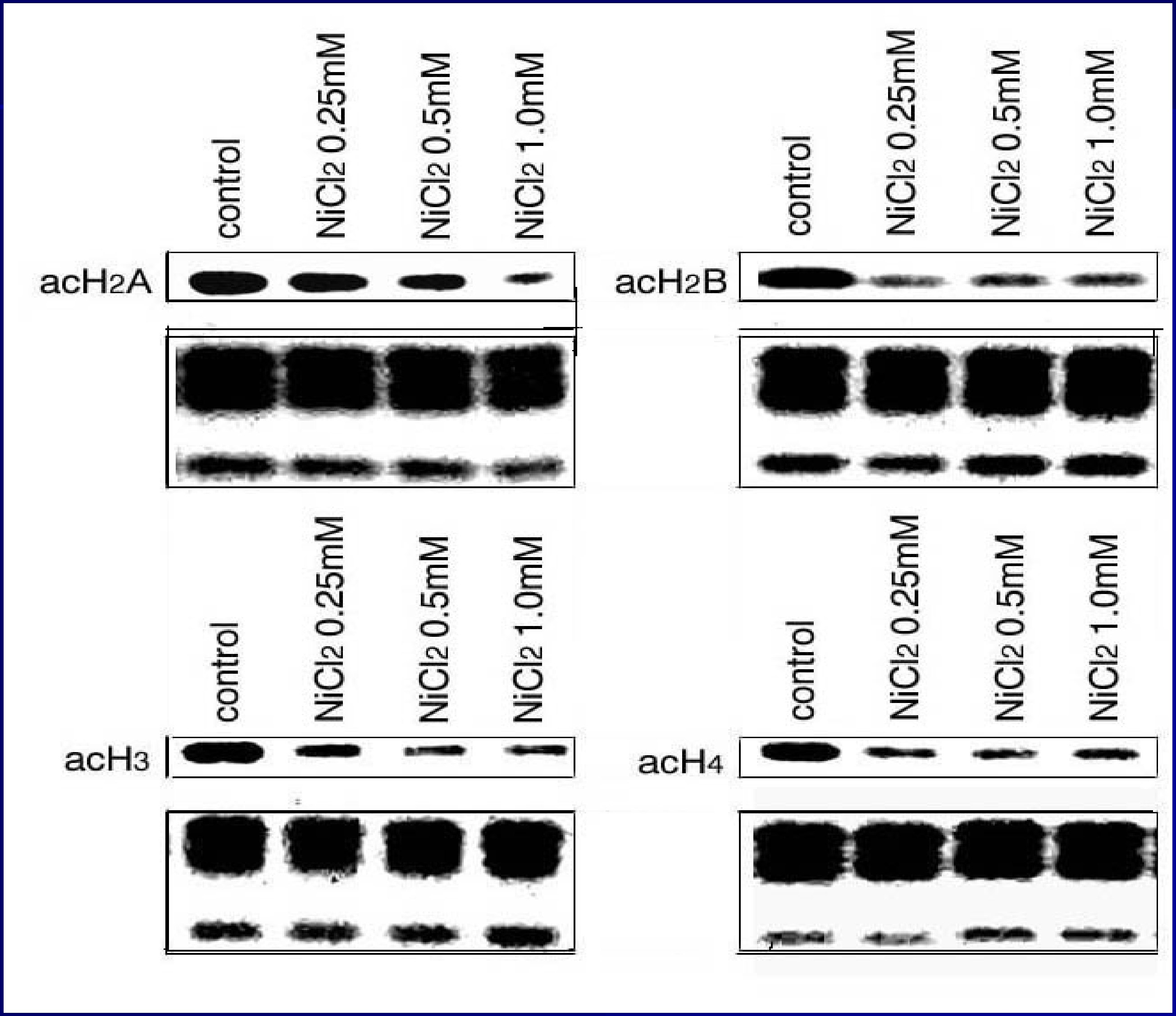
H3K9 Methylated
H3K9 di-Methylated (inactive X-chromosome)
H3K27 tri-Methylated (inactive X-chromosome)
H3K9 tri-Methylated (pericentromeric heterochromatin)
H3K27 mono-Methylated (pericentromeric heterochromatin)

Aim1

Fig.1A. Ni decreases histone acetylation in A549 cells.

A549
24h

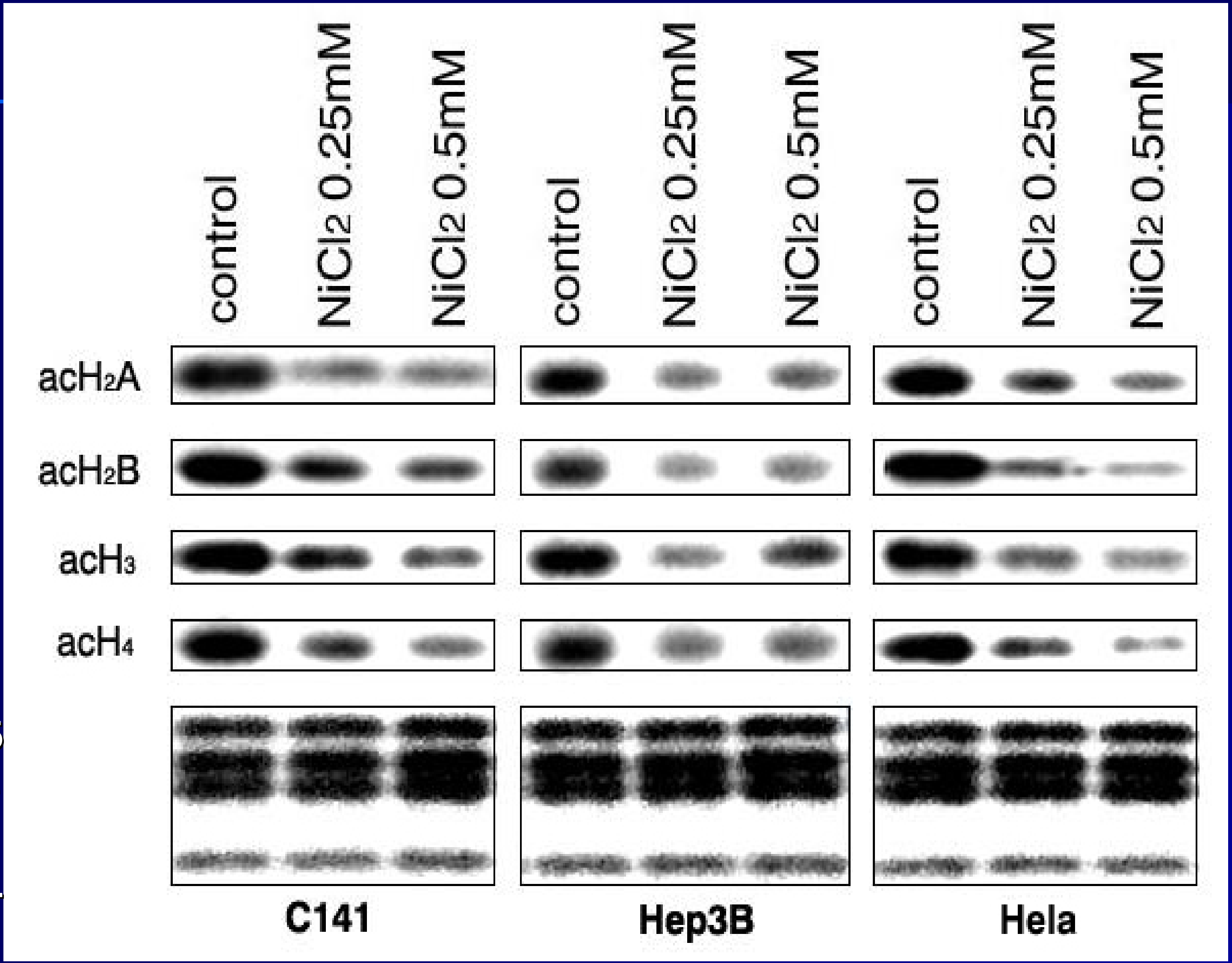
H3 15
H2A 14
H2B 13
H4 11



Aim1

Fig.1B. Ni decreases histone acetylation in other cell lines.

24h



H3 15
H2A 14
H2B 13
H4 11

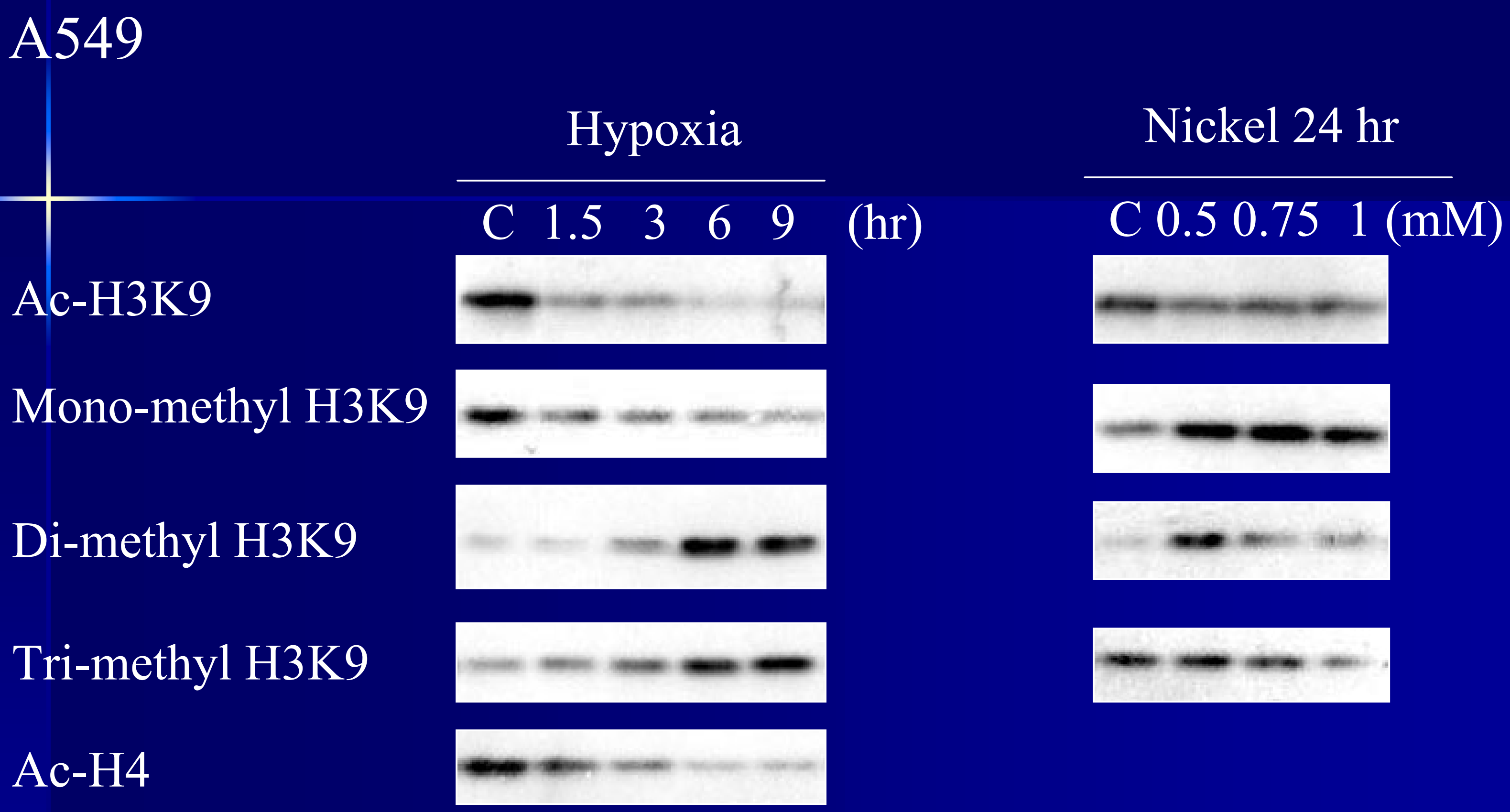
Table 1
Exposure to TSA reverted the ability of Ni-transformed cells to grow in soft agar

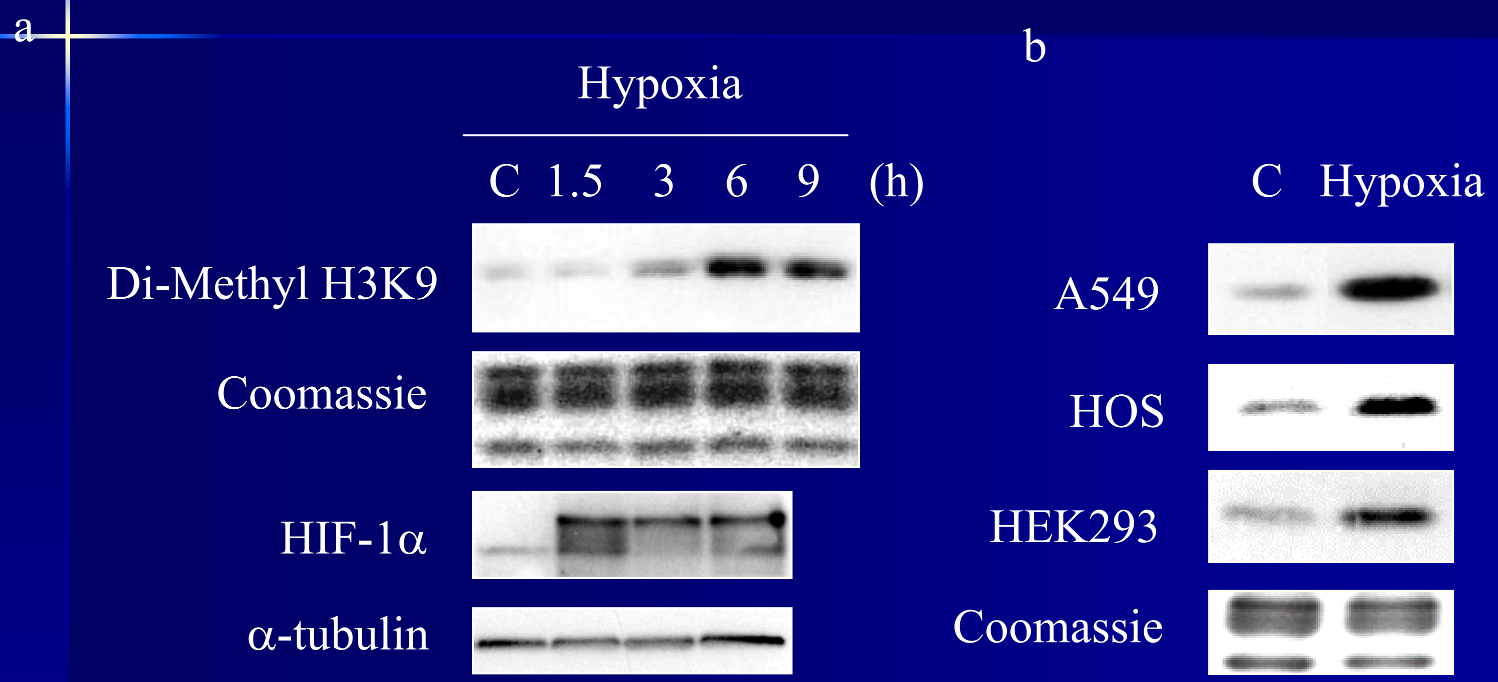
Ni-transformed clones	0 ng/ml TSA	5 ng/ml TSA	25 ng/ml TSA
1	100.0 ± 2.7	38.5 ± 5.2**	16.0 ± 1.3**
2	100.0 ± 12.4	65.7 ± 11.0*	34.2 ± 1.4**
3	100.0 ± 6.4	54.1 ± 6.9**	22.3 ± 0.3**
4	100.0 ± 13.0	48.7 ± 5.5**	36.5 ± 5.9**
5	100.0 ± 0.85	33.3 ± 1.1**	21.6 ± 4.1**

Note. One million of the Ni-transformed cells were seeded into flasks and exposed to 0, 5, or 25 ng/ml TSA for 24 h. The TSA containing medium was removed and cells were rinsed three times with saline A. Fresh medium was added and the cells were allowed to grow to ~80% confluence. The cultures were split and the cells were seeded at a density of 1 million cells again. And cells were treated with TSA from a second time. After third round of TSA exposure, cells were allowed to repopulate the culture prior to test for the ability of anchorage-independent growth in soft agar. Results are expressed as a percentage of control (0 ng/ml TSA treatment group). Values are mean ± SE. Asterisk indicates significant differences from that of control.

* $P < 0.05$.

**$P < 0.005$, $N = 3$.


A549
Hypoxia
Nickel 24 hr
C 1.5 3 6 9 (hr)
C 0.5 0.75 1 (mM)
Ac-H3K9
Mono-methyl H3K9
Di-methyl H3K9
Tri-methyl H3K9
Ac-H4


a
Hypoxia
C
1.5
3
6
9
(h)
Di-Methyl H3K9
Coomassie
HIF-1α
α-tubulin
b
C
Hypoxia
A549
HOS
HEK293
Coomassie

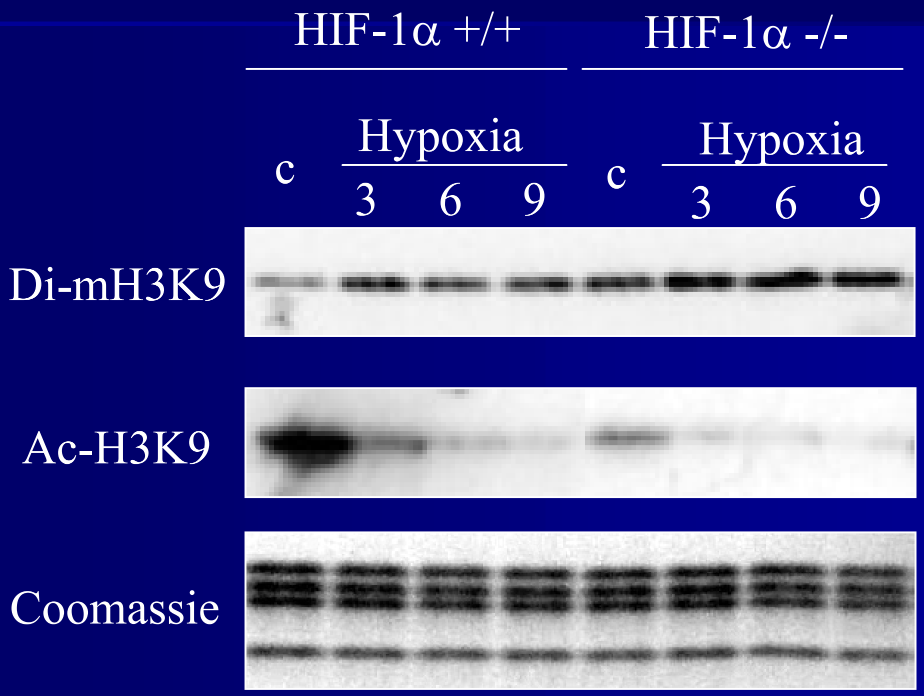
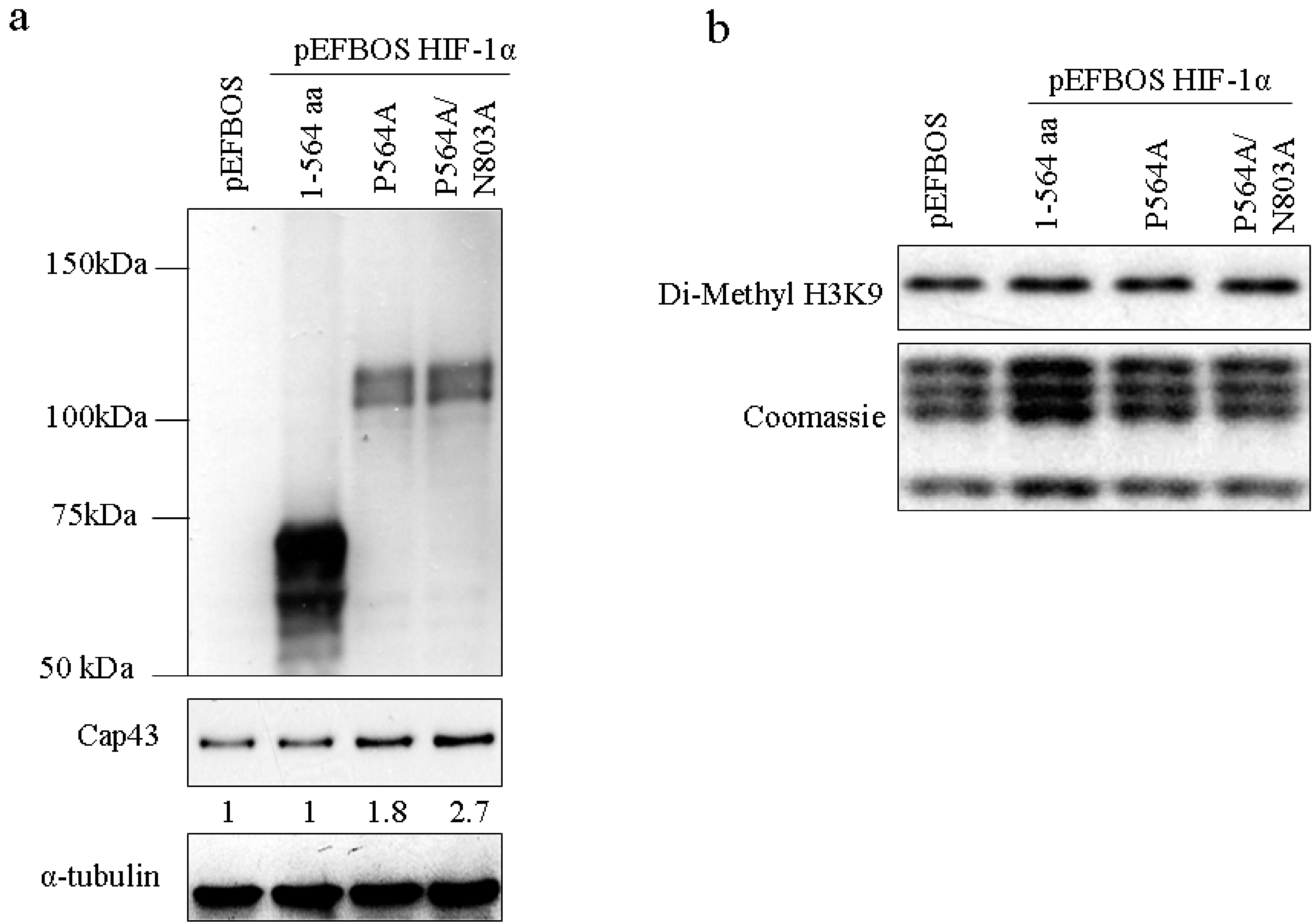
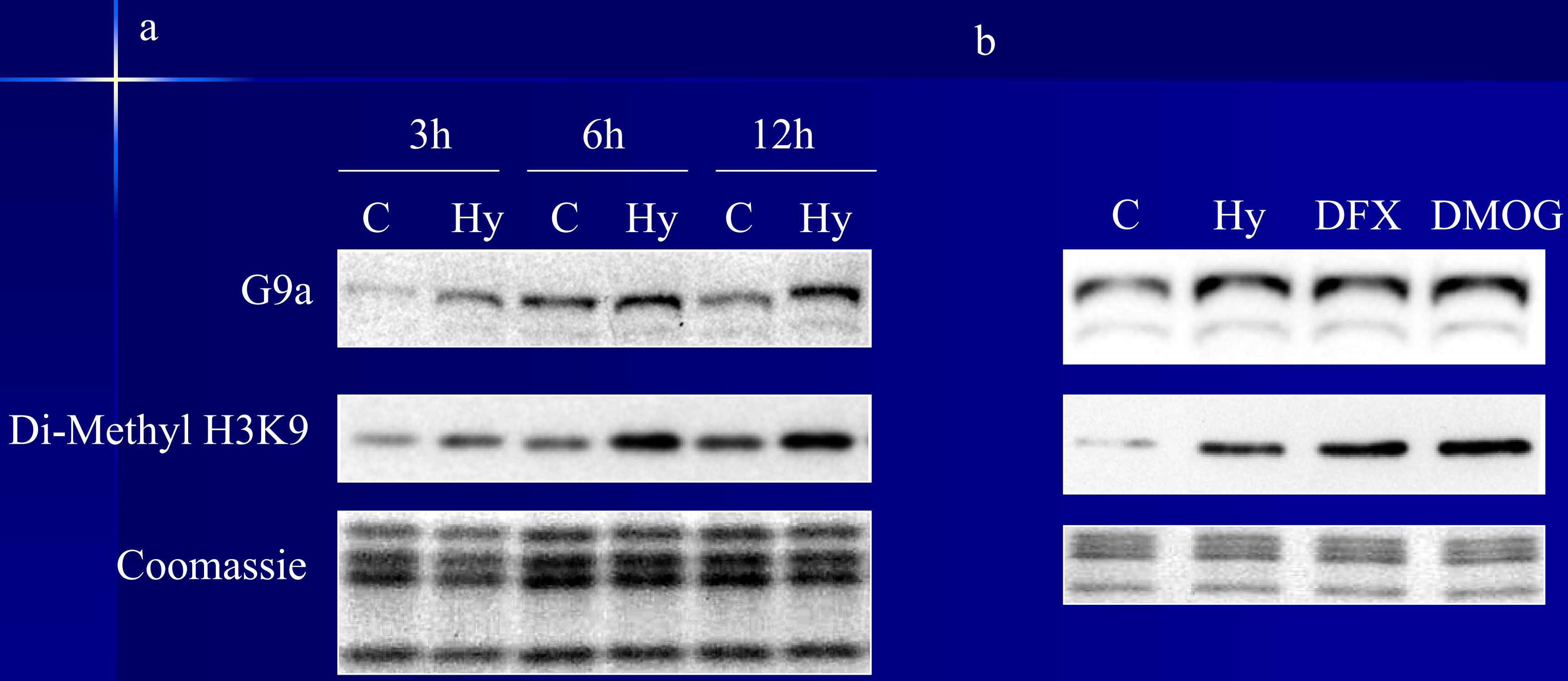
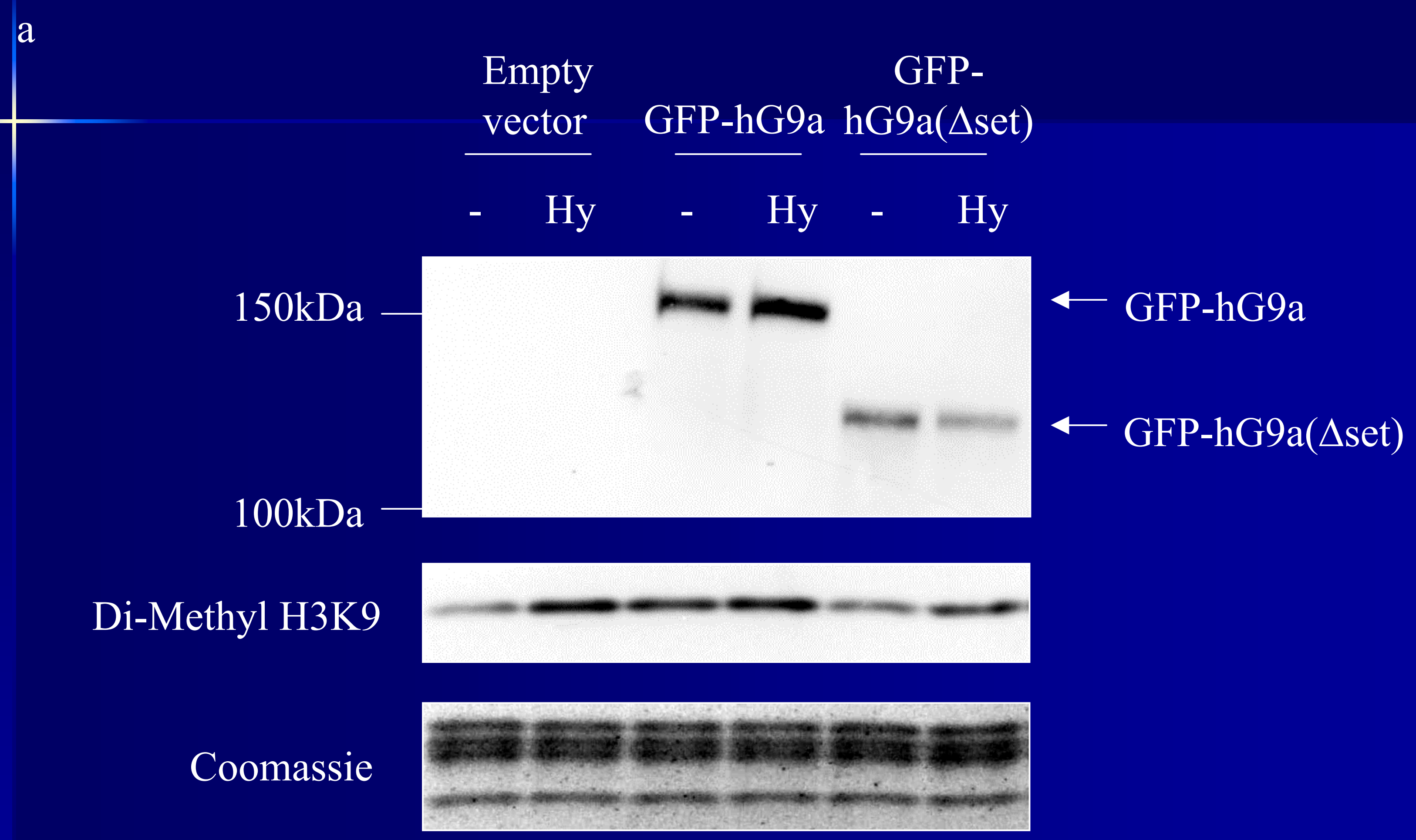

HIF-1α +/+
HIF-1α -/-
c
Hypoxia
3
6
9
c
Hypoxia
3
6
9
Di-mH3K9
Ac-H3K9
Coomassie

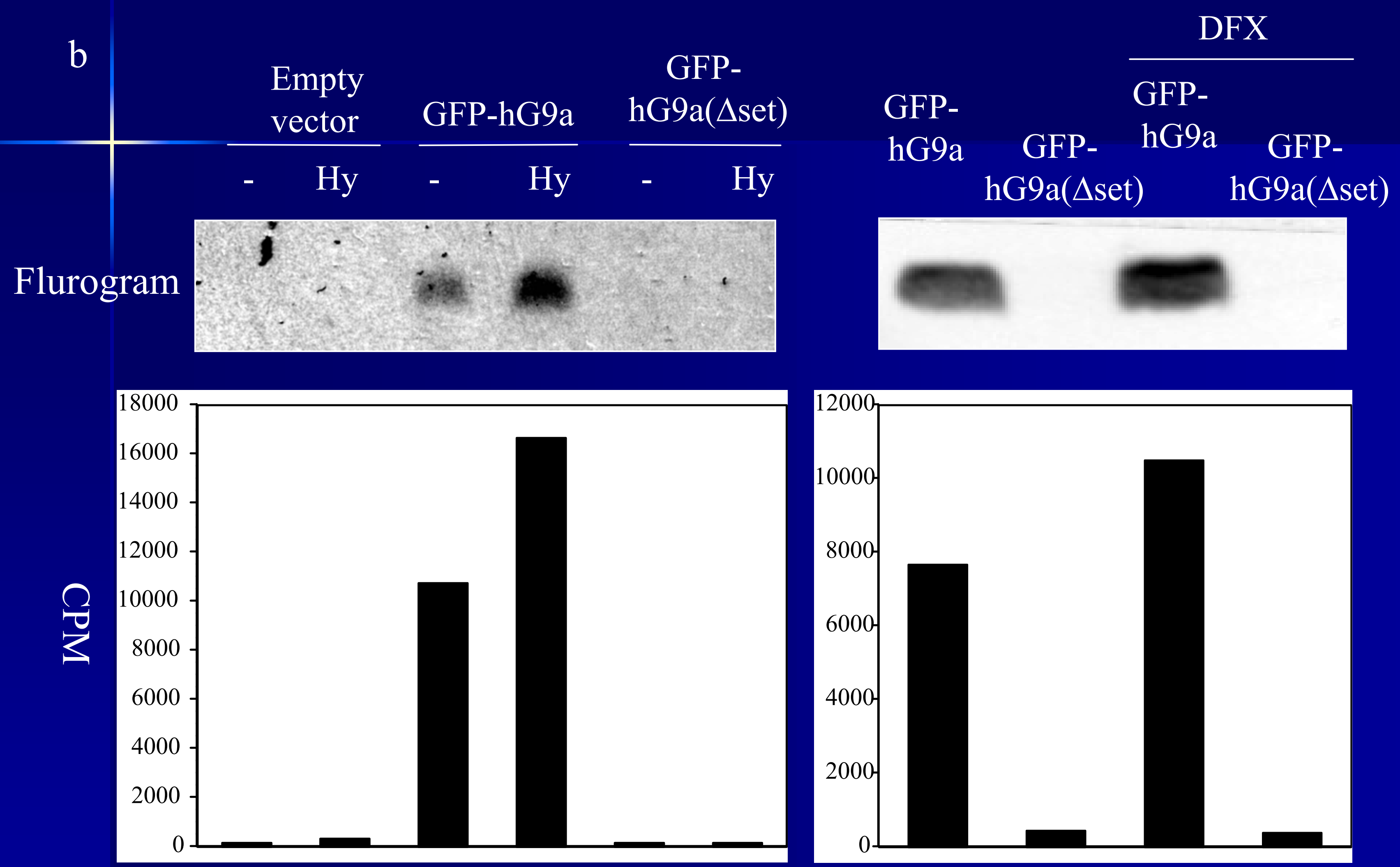
Figure 4

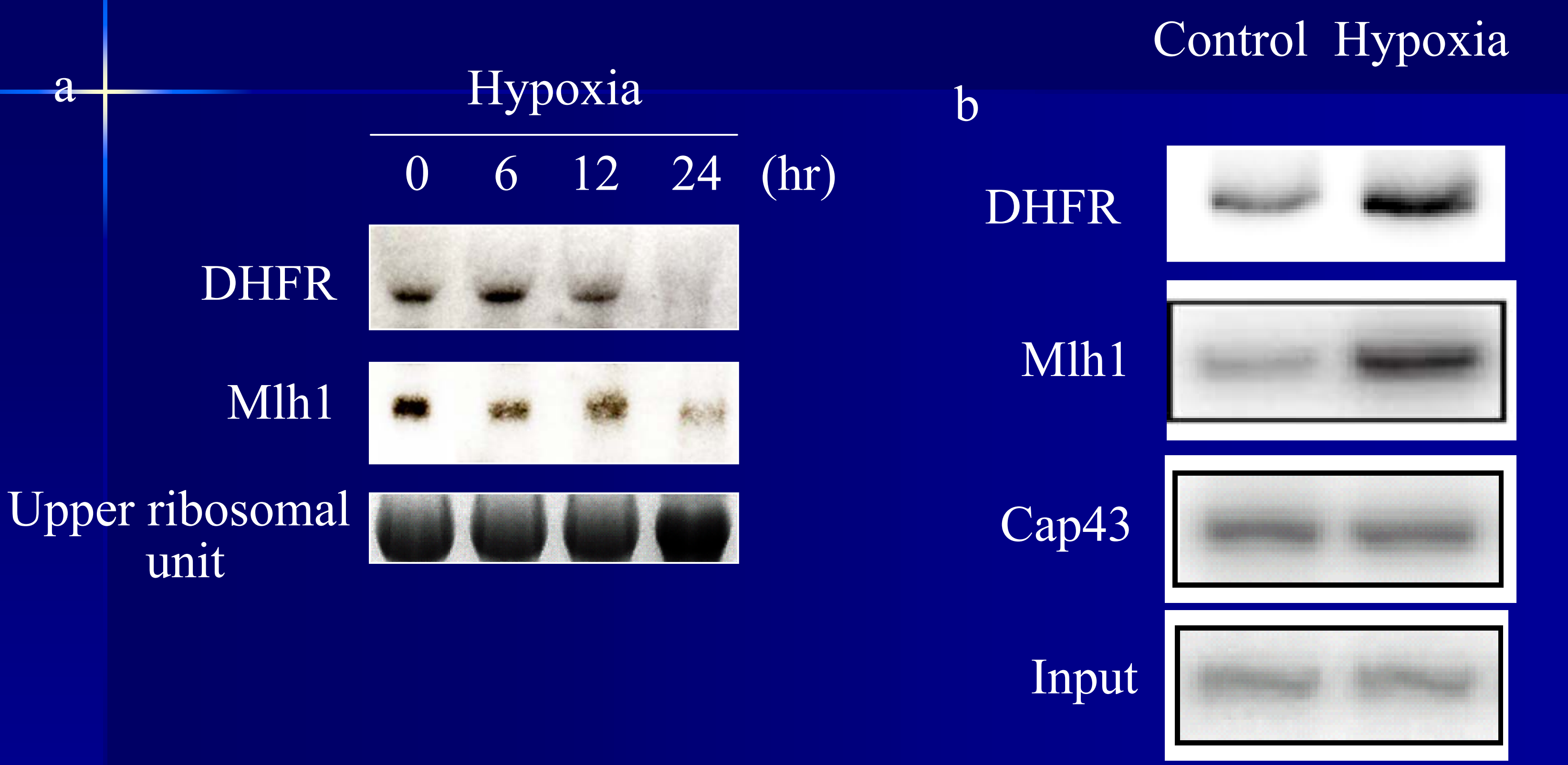

a
pEFBOS HIF-1α
pEFBOS
1-564 aa
P564A
P564A/
N803A
150kDa
100kDa
75kDa
50 kDa
Cap43
1
1
1.8
2.7
α-tubulin
b
pEFBOS HIF-1α
pEFBOS
1-564 aa
P564A
P564A/
N803A
Di-Methyl H3K9
Coomassie



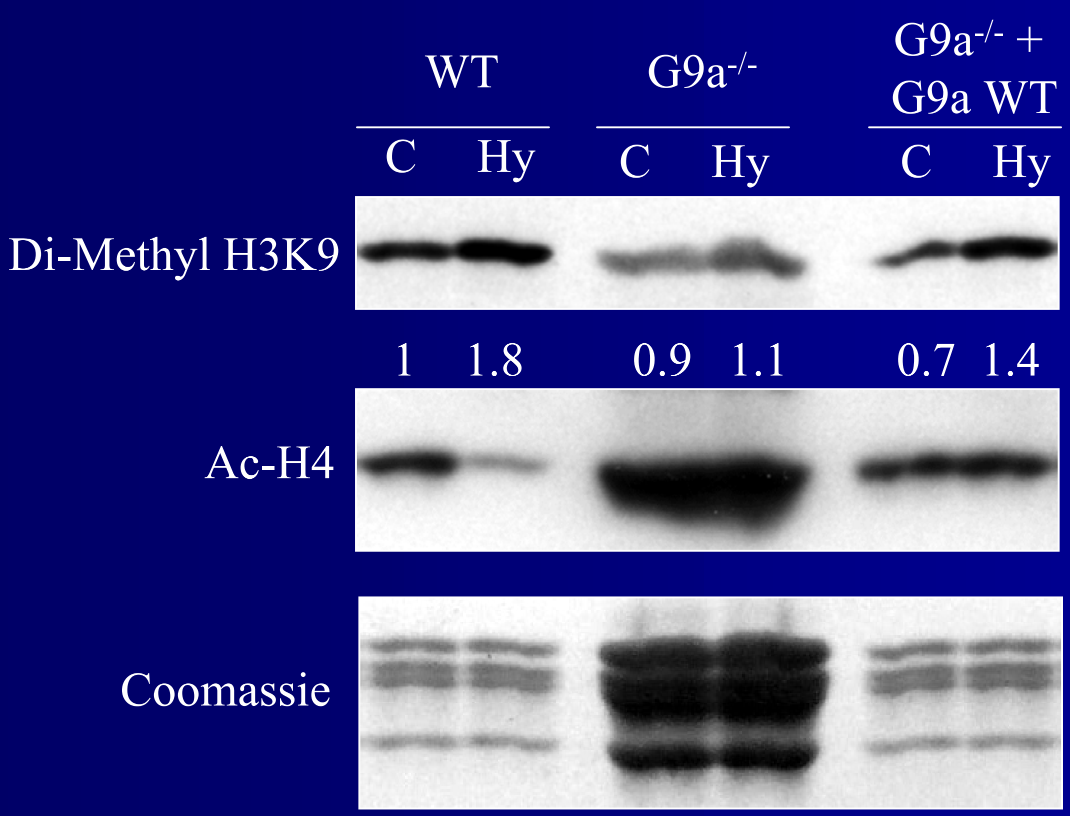
a
3h
6h
12h
C
Hy
C
Hy
C
Hy
G9a
Di-Methyl H3K9
Coomassie
b
C
Hy
DFX
DMOG


a
Empty vector
GFP-hG9a
GFP-hG9a(Δset)
- Hy - Hy - Hy
150kDa
100kDa
GFP-hG9a
GFP-hG9a(Δset)
Di-Methyl H3K9
Coomassie


b
Empty vector
GFP-hG9a
GFP-hG9a(Δset)
-
Hy
-
Hy
-
Hy
DFX
GFP-hG9a
GFP-hG9a(Δset)
GFP-hG9a
GFP-hG9a(Δset)
Flurogram
CPM
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
12000
10000
8000
6000
4000
2000
0

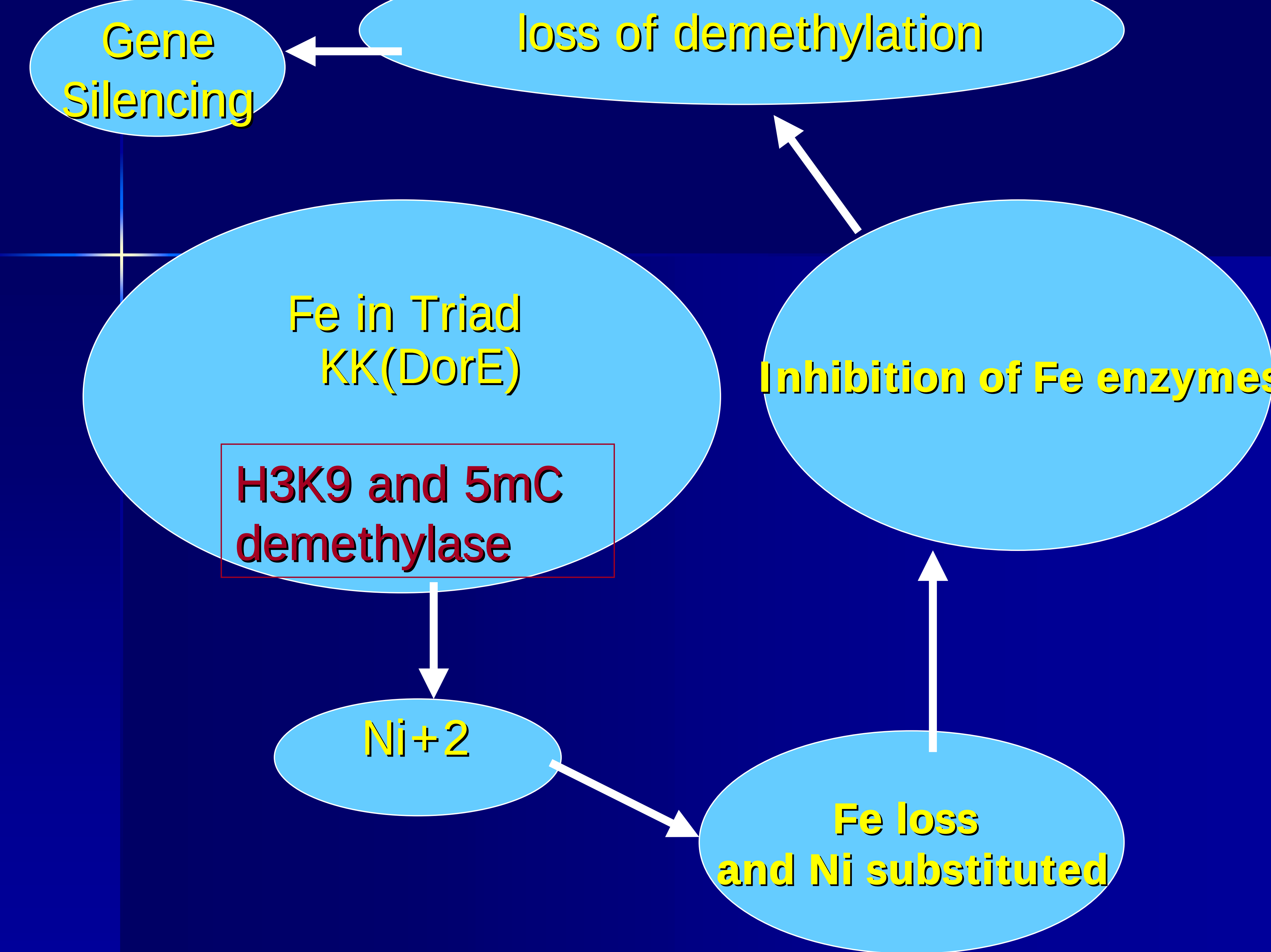

a
Hypoxia
0
6
12
24
(hr)
DHFR
Mlh1
Upper ribosomal unit
b
Control
Hypoxia
DHFR
Mlh1
Cap43
Input

a


WT
G9a-/-
G9a-/- +
G9a WT
C
Hy
C
Hy
C
Hy
Di-Methyl H3K9
1
1.8
0.9
1.1
0.7
1.4
Ac-H4
Coomassie

Summary of the Effect of Ni compounds on G9a and H3K9 methylation

- Ni ions inhibited G9a activity and decreased its presence in the nucleus (see poster by H. Chen)
- We have been working on Fe, Oxoglutarate, ascorbic acid dependent H3K9 demethylase and 5-methylctyosine- demethylase and have found these activities in crude cell extracts. Ni ions are effective inhibitors of these enzymes.
- These are new enzymes and further work on their identification, purification and characterization is required.



Gene Silencing
loss of demethylation
Fe in Triad KK(DorE)
H3K9 and 5mC demethylase
Inhibition of Fe enzymes
Ni+2
Fe loss and Ni substituted

Ackowledgements

Todd Davison
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Qin Li
Yan Yan

Randy Johnson
Yoichi Shinkai