REGULATION OF VERTEBRATE NEURON DEATH IN DEVELOPMENT AND NEURODEGENERATION:

ROLE OF TRANSCRIPTION

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WHY BRAIN CELLS *REALLY* DIE

THE CELL CYCLE AND JNK PATHWAYS IN NEUROLOGICAL DISORDERS

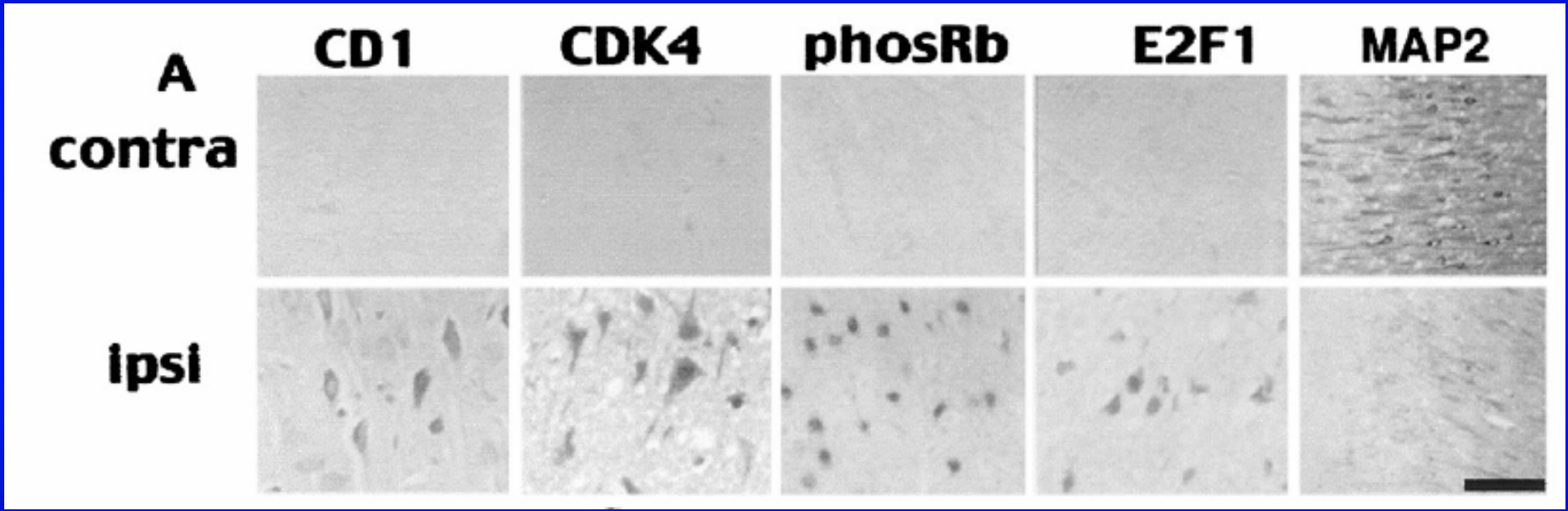
ALZHEIMER DISEASE

PARKINSON DISEASE

STROKE

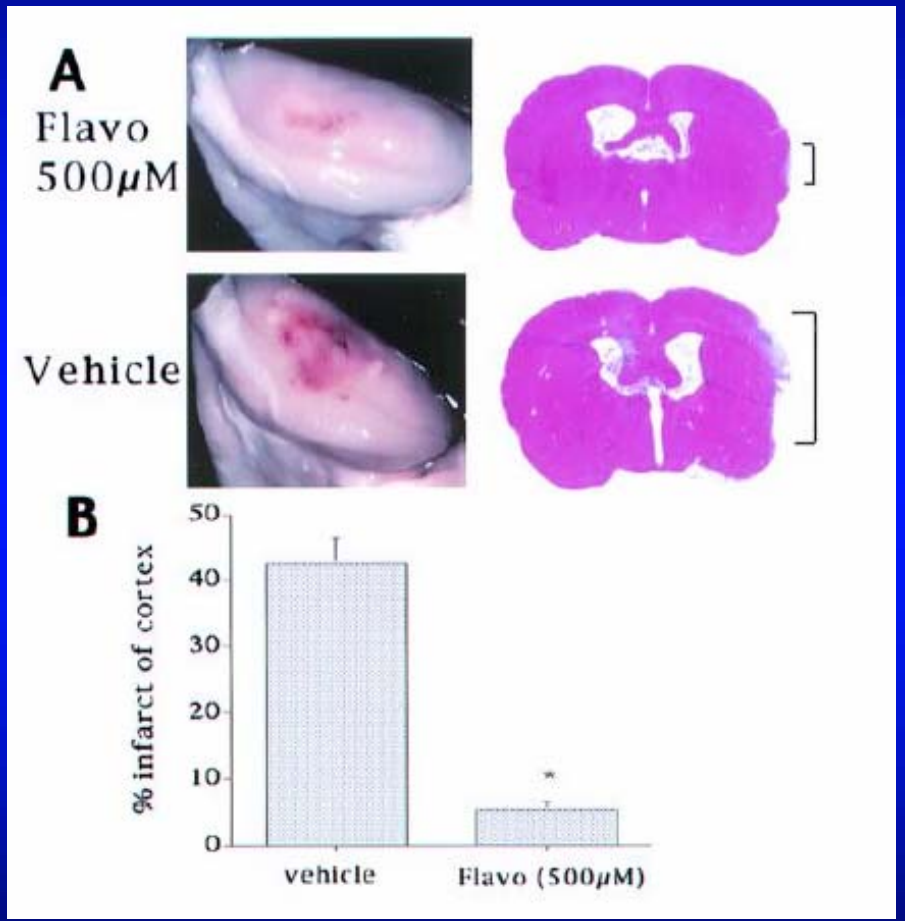
SPINAL CORD INJURY

ELEMENTS OF THE CDK4-E2F PATHWAY ARE UP-REGULATED IN A STROKE MODEL



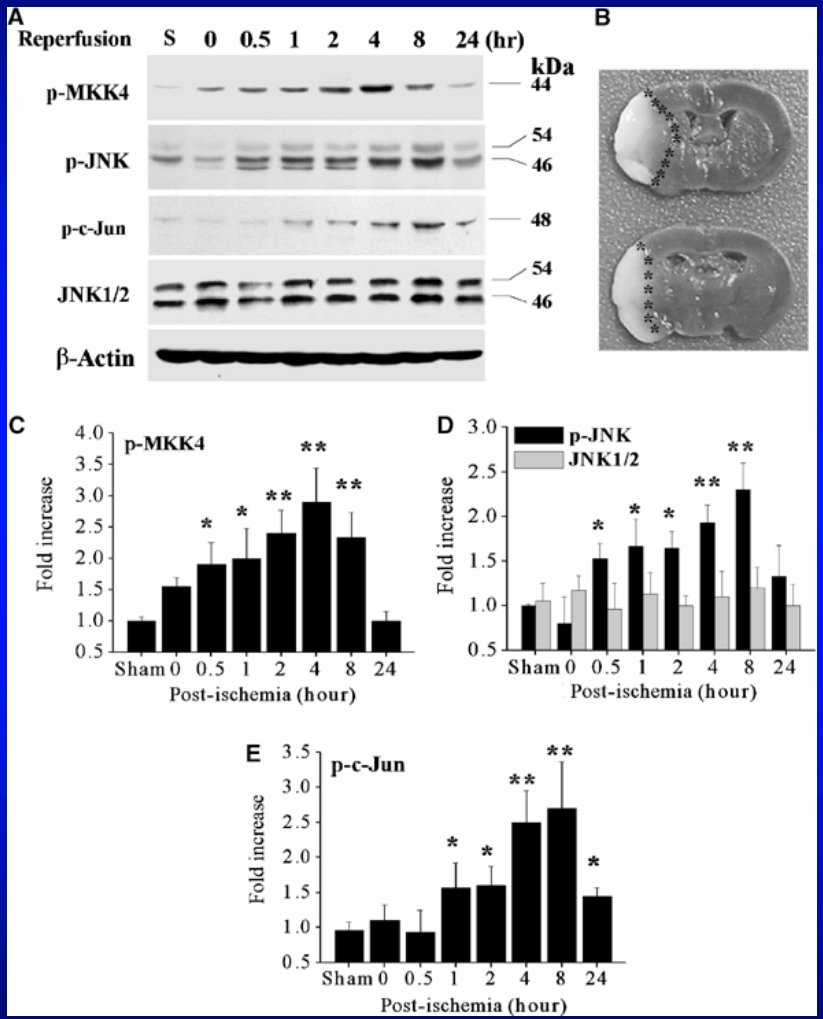
From Osuga et al., PNAS 97: (2000)

BLOCKADE OF THE CDK4-E2F PATHWAY PROTECTS NEURONS IN A STROKE MODEL



From Osuga et al., PNAS 97: (2000)

ACTIVATION OF THE JNK PATHWAY IN A STROKE MODEL

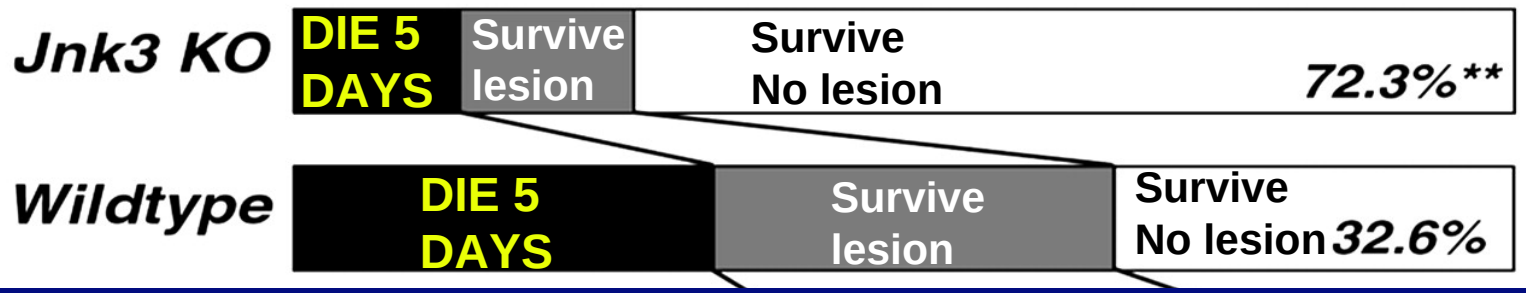


From Gao et al *Journal of Cerebral Blood Flow & Metabolism* (2005)

JNK3 DEFICIENT MICE ARE RESISTANT TO CEREBRAL ISCHEMIA-HYPOXIA

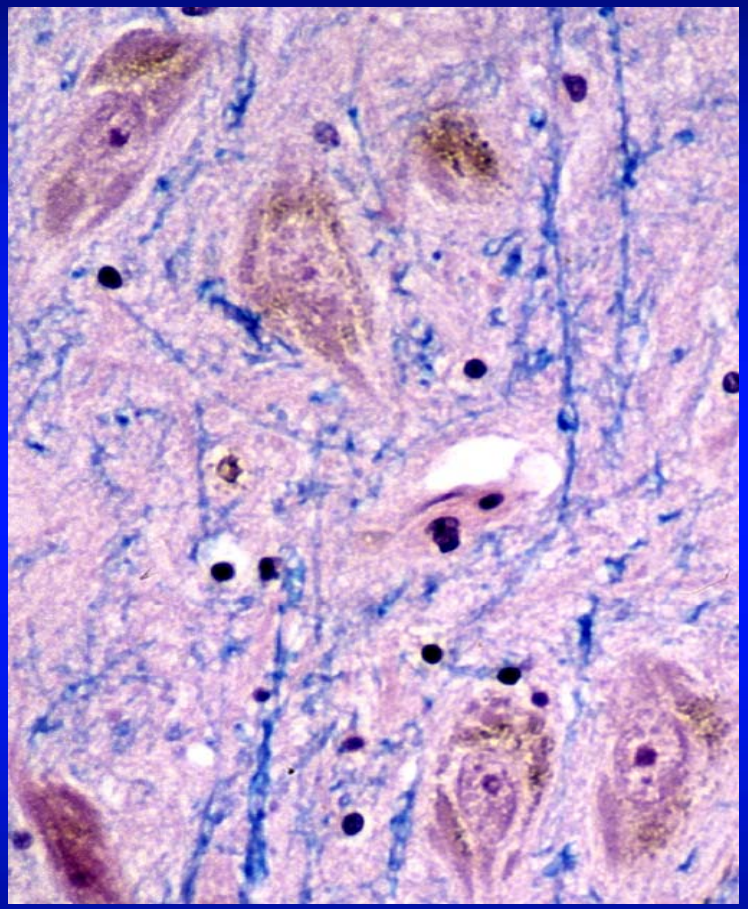
a

Genotype	operated mice	analyzed mice	die within 5 days	survive with lesion*	survive with no lesion*
Jnk3 KO	49	47	12.7%	14.8%	72.3%**
Wildtype	57	49	34.5%	32.6%	32.6%
Jnk2 KO	40	36	41.7%	38.9%	19.4%
Wildtype	40	34	29.4%	35.3%	35.3%

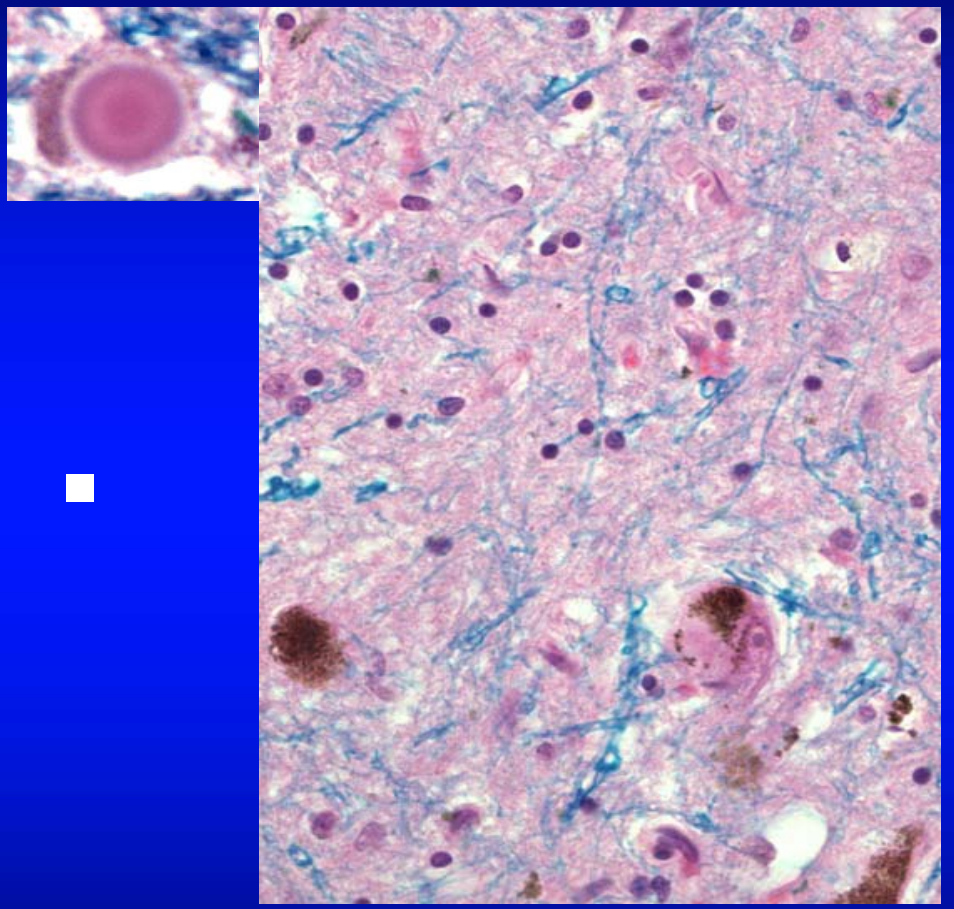


Kuan, Chia-Yi et al. (2003) Proc. Natl. Acad. Sci. USA 100, 15184-15189

Substantia nigra, pars compacta



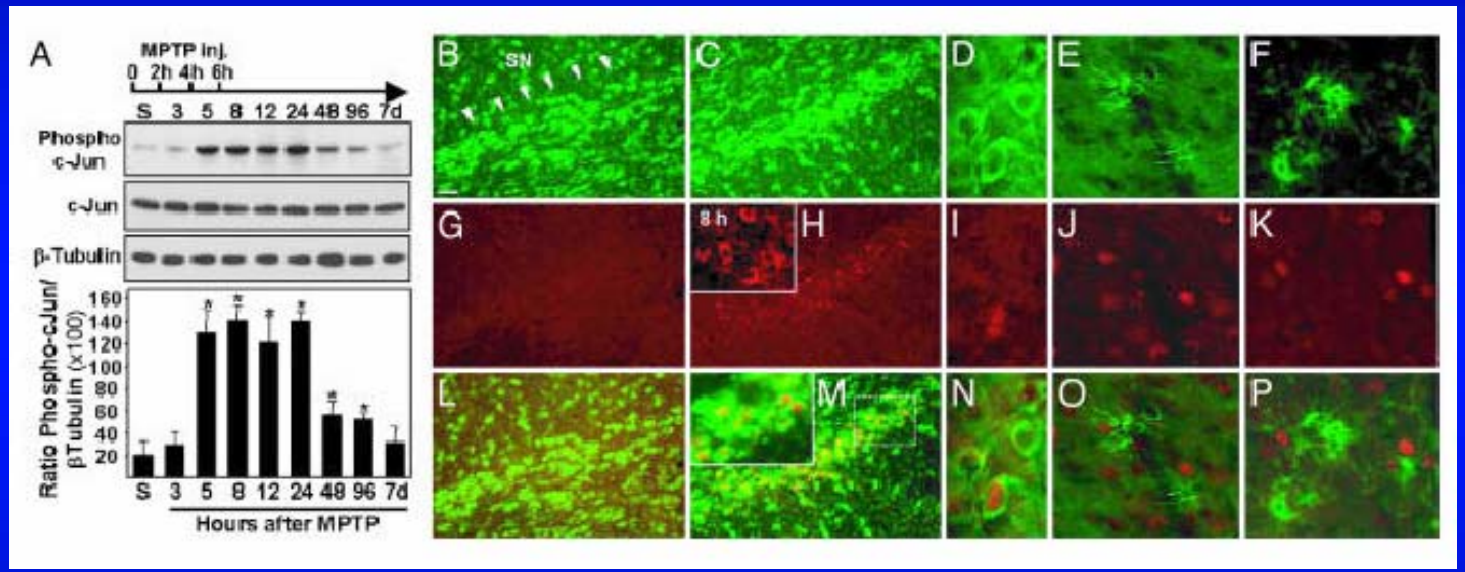
Control



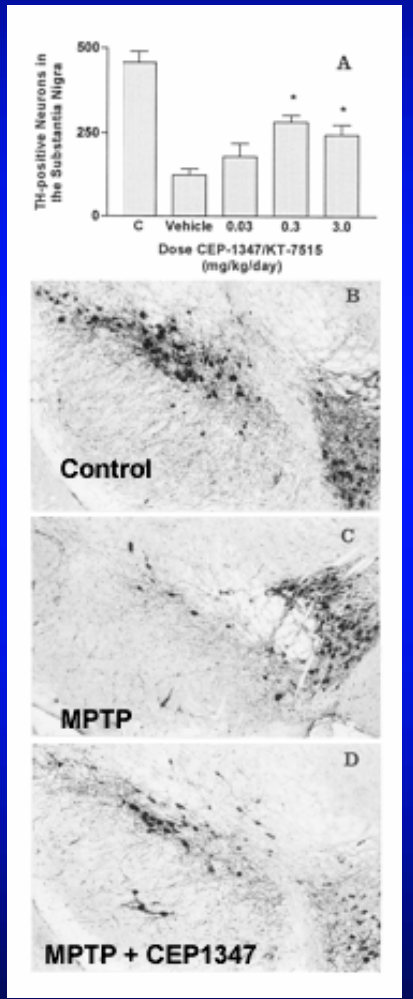
Parkinson disease

LHE, 400x

THE JNK PATHWAY IS ACTIVATED IN A PD MODEL AND A PATHWAY INHIBITOR IS PROTECTIVE



Hunot et al PNAS 2004



Saporito et al J Neurochem 1999

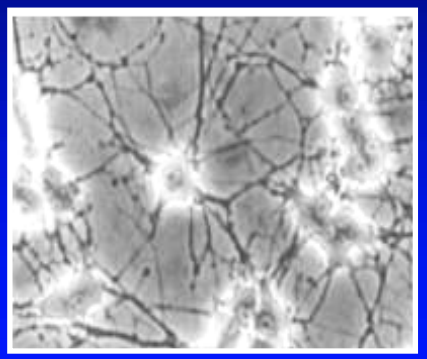
STRATEGIC OVERVIEW

RATIONALE:

- **IN MOST CASES, INCLUDING PD MODELS, NEURON CELL DEATH REQUIRES TRANSCRIPTION-DEPENDENT CHANGES IN GENE EXPRESSION.**

- **IF REGULATED GENES ASSOCIATED WITH PD CAN BE IDENTIFIED, THESE WILL BE POTENTIAL TARGETS FOR THERAPEUTIC INTERVENTION.**

EXPERIMENTAL APPROACH (DISCOVERY)



- MODEL SYSTEM: NEURONALLY-DIFFERENTIATED PC12 CELLS CHALLENGED WITH 6-OHDA FOR 8 HR

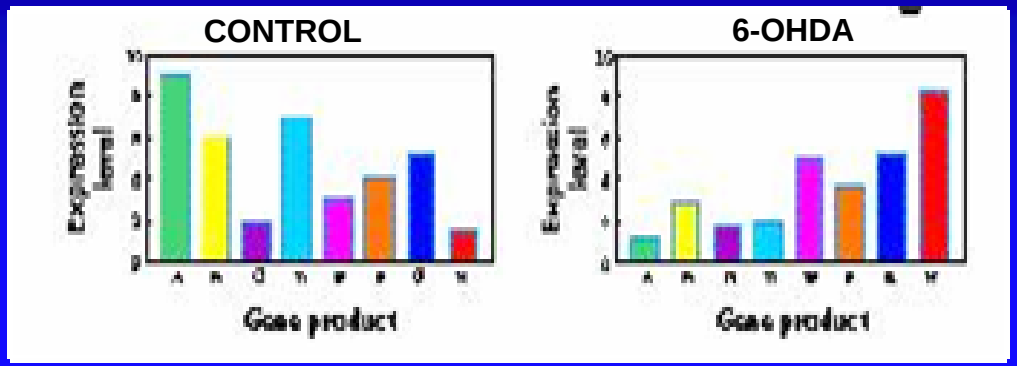
- NEURONAL PC12 CELLS CONVENIENT SYSTEM RESEMBLING SYMPATHETIC NEURONS WHICH AFFECTED IN PD - EASY TO GENETICALLY MODIFY

- 6-OHDA SELECTIVELY TAKEN UP BY DOPAMINERGIC NEURONS IN WHICH CAUSES PD-LIKE DEATH INVOLVING MITOCHONDRIA

- 8 HR ENOUGH TIME FOR TRANSCRIPTIONAL CHANGES, BUT BEFORE OVERT SIGNS OF DEGENERATION AND DEATH

EXPERIMENTAL APPROACH (DISCOVERY)

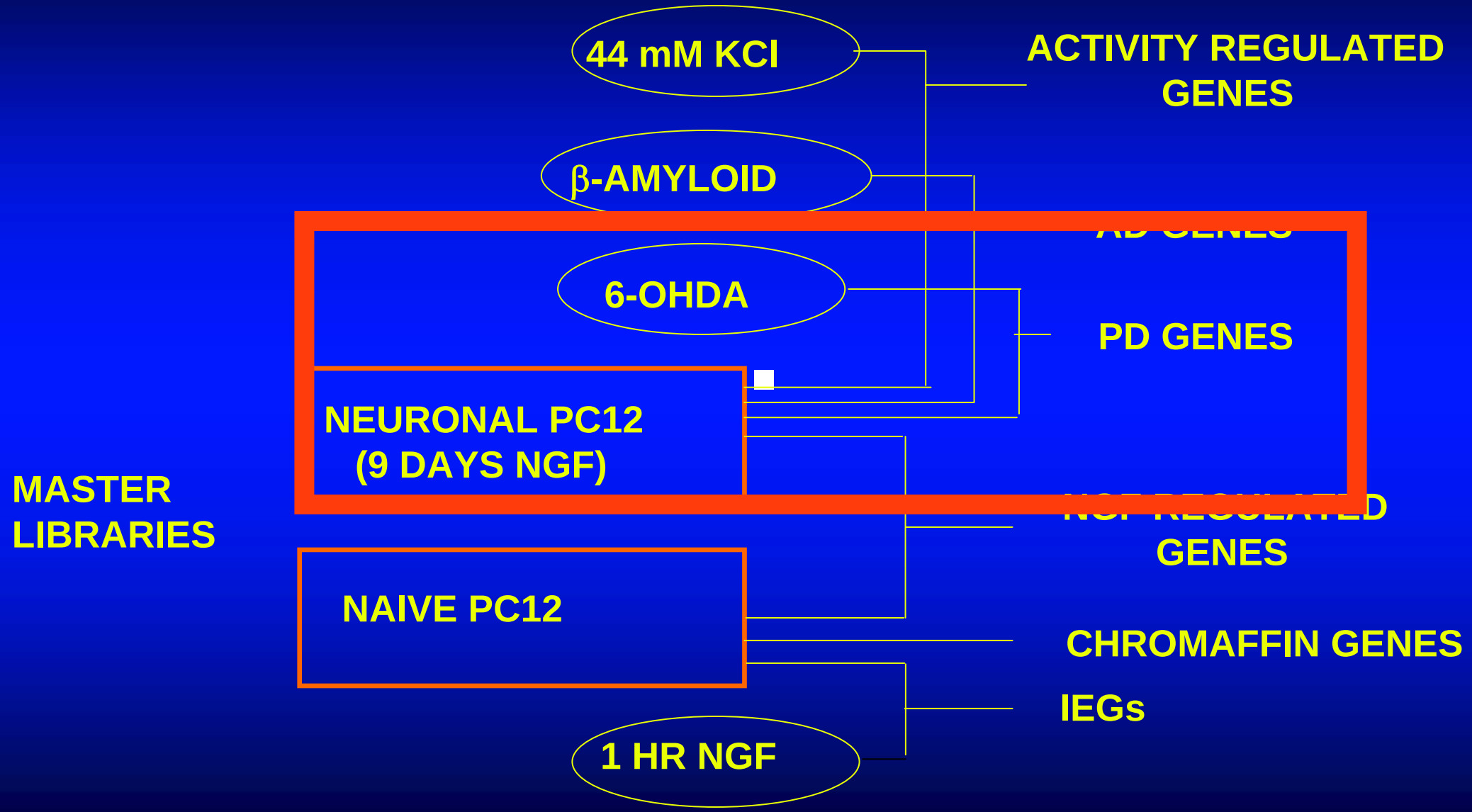
- **ANALYTICAL APPROACH: SERIAL ANALYSIS OF GENE EXPRESSION (SAGE)**



- **SAGE HIGHLY QUANTITATIVE, UN-BIASED**

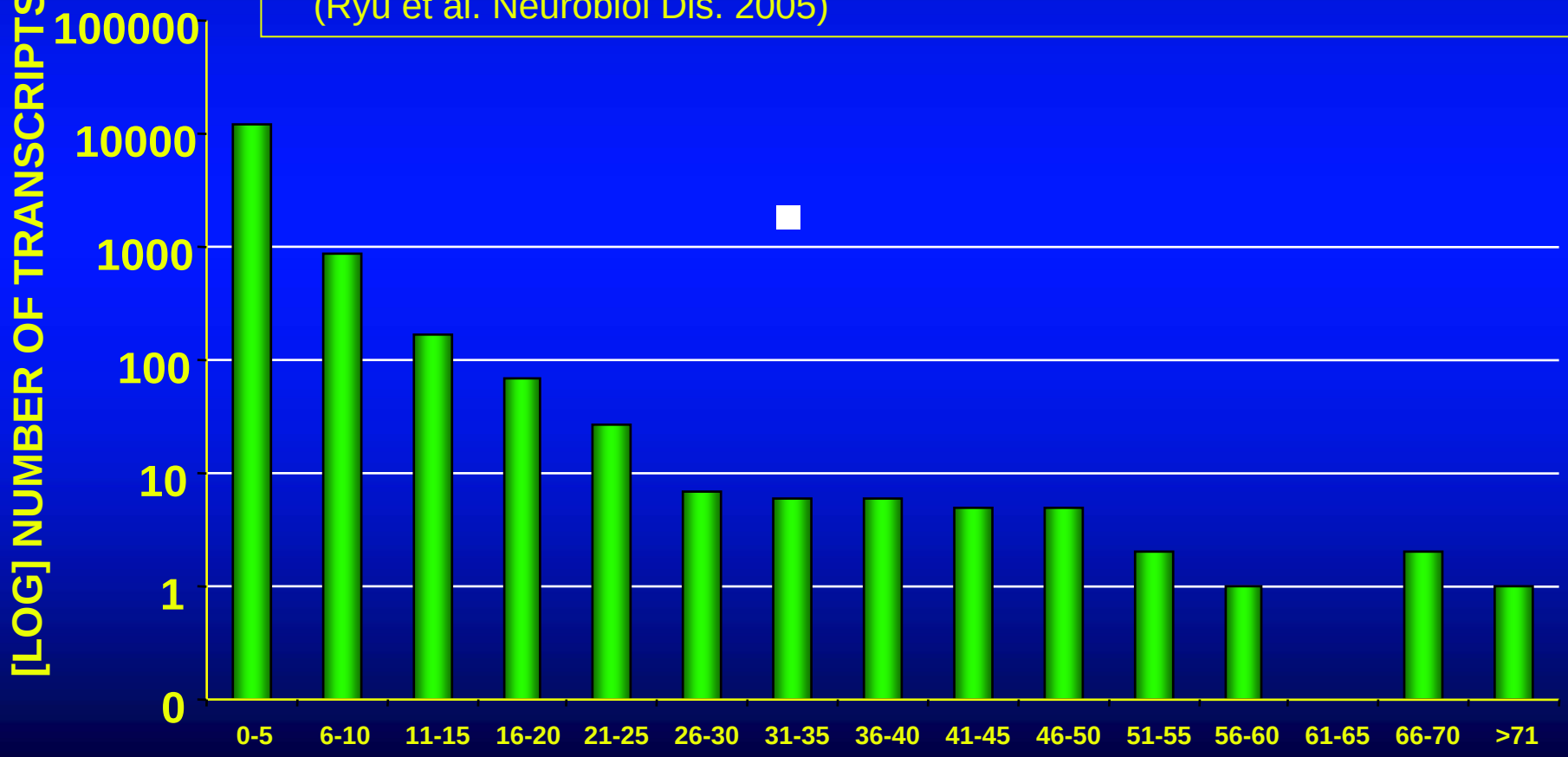
- **WE ALREADY HAVE LARGE DATA BASE FOR CONTROL NEURONAL PC12 CELLS**

CURRENT PC12 CELL SAGE LIBRARIES



6-OHDA-EVOKES CHANGES IN GENE EXPRESSION

- **SAGE OUTCOME. APPROXIMATELY 1,200 (OF A TOTAL OF ABOUT 14,000) SIGNIFICANTLY ELEVATED TRANSCRIPTS DETECTED OF WHICH APPROXIMATELY 500 HAVE BEEN MATCHED WITH KNOWN GENES.**
  (Ryu et al. Neurobiol Dis. 2005)



- **BIG PICTURE: MULTIPLE PRO- AND ANTI-APOPTOTIC GENES ARE REGULATED BY 6-OHDA**

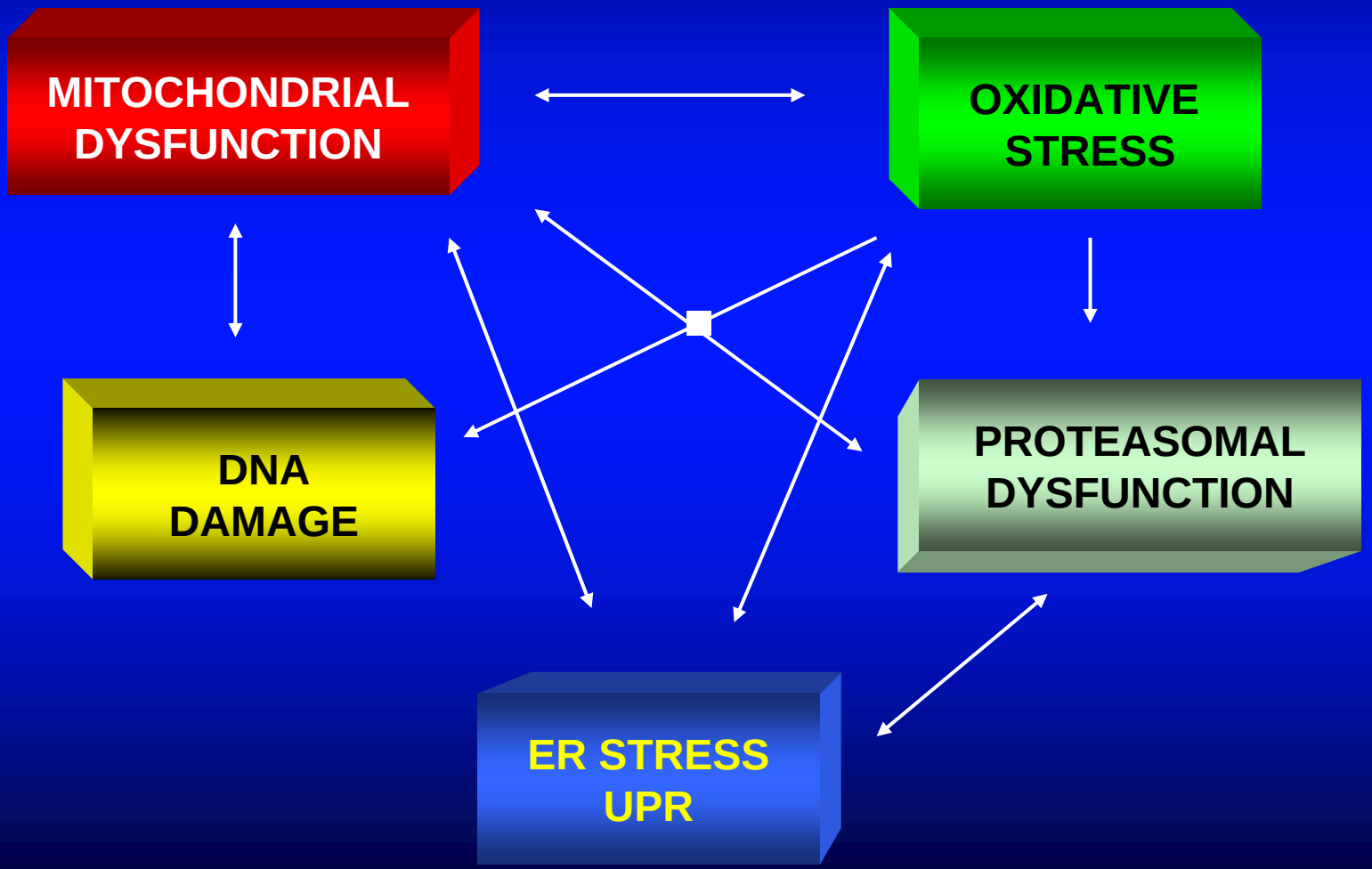
6-OHDA INDUCES EXPRESSION OF PRO-APOPTOTIC GENES

Table 4: 6-OHDA-REGULATED GENES ENCODING APOPTOSIS-RELATED PROTEINS

TAG	TAG # -	TAG # +	FOLD CHANGE	GENE	ACCESS #
TTTGTTAAAAC	0	41	98	RTP801	NM_080906
GGATATGTGGT	1	17	41	NGFI-A: Early growth response 1	NM_012551
TGCCCAATAAA	0	10	24	Neuronal cell death-inducible putative kinase: NIPK	NM_144755
GGAGATGTCGA	0	5	12	Ankrd3: ankyrin repeat domain 3 (RIP4/DIK/PKK)	AF302127
ACGTGCATCAT	1	4	10	Ppm1g: protein phosphatase 1G (formerly 2C)	NM_147209
CAAAGGCTGGC	0	4	10	Ortholog of murine Gadd45b	AK010420
TGGGCTTTGAG	0	4	10	Ortholog of murine DEDD2	XM_133256
GAGACTGGCAG	5	19	9	Gadd45g	XM_237999
TATGACTTAAT	2	7	8	Rac1: RAS-related C3 botulinum substrate 1	AF385833
TGCCTTACTTT	2	6	7	Ortholog of murine programmed cell death 6 (PS2/Alg2)	NM_011051
ATTTTTGTATG	2	6	7	Bbc3: Bcl-2 binding component 3 (PUMA)	NM_17383
ATCAAGGGAAT	2	6	7	Nmt1: N-myristoyltransferase 1	NM_148891
GGGATGGACAT	2	6	7	Ppp1cb: Protein phosphatase 1, catalytic subunit, β isoform	NM_013065
TGACTTTTTAA	1	3	7	rhoB (Arhb)	NM_022542
CAAATAAAGAC	0	3	7	Ortholog of murine lymphotoxin B receptor: Ltbr	NM_010736
TGCCTTCGCCC	0	3	7	Amyloid β (A4) precursor protein-binding family A member3	NM_031781
TGATGCAAATA	0	3	7	Ortholog of murine Psen1: presenilin 1	NM_008943
AACTTAAAAAA	1	3	7	Calcineurin subunit A alpha	NM_017041
TTAATAAAATA	0	3	7	Gspt1: G1 to phase transition 1	XM_220151
TATTCACTATT	0	3	7	Similar to Scotin	XM_217272

Ryu et al. Neurobiol Dis. 2005

MULTIPLE SYSTEM FAILURES MAY UNDERLIE PD



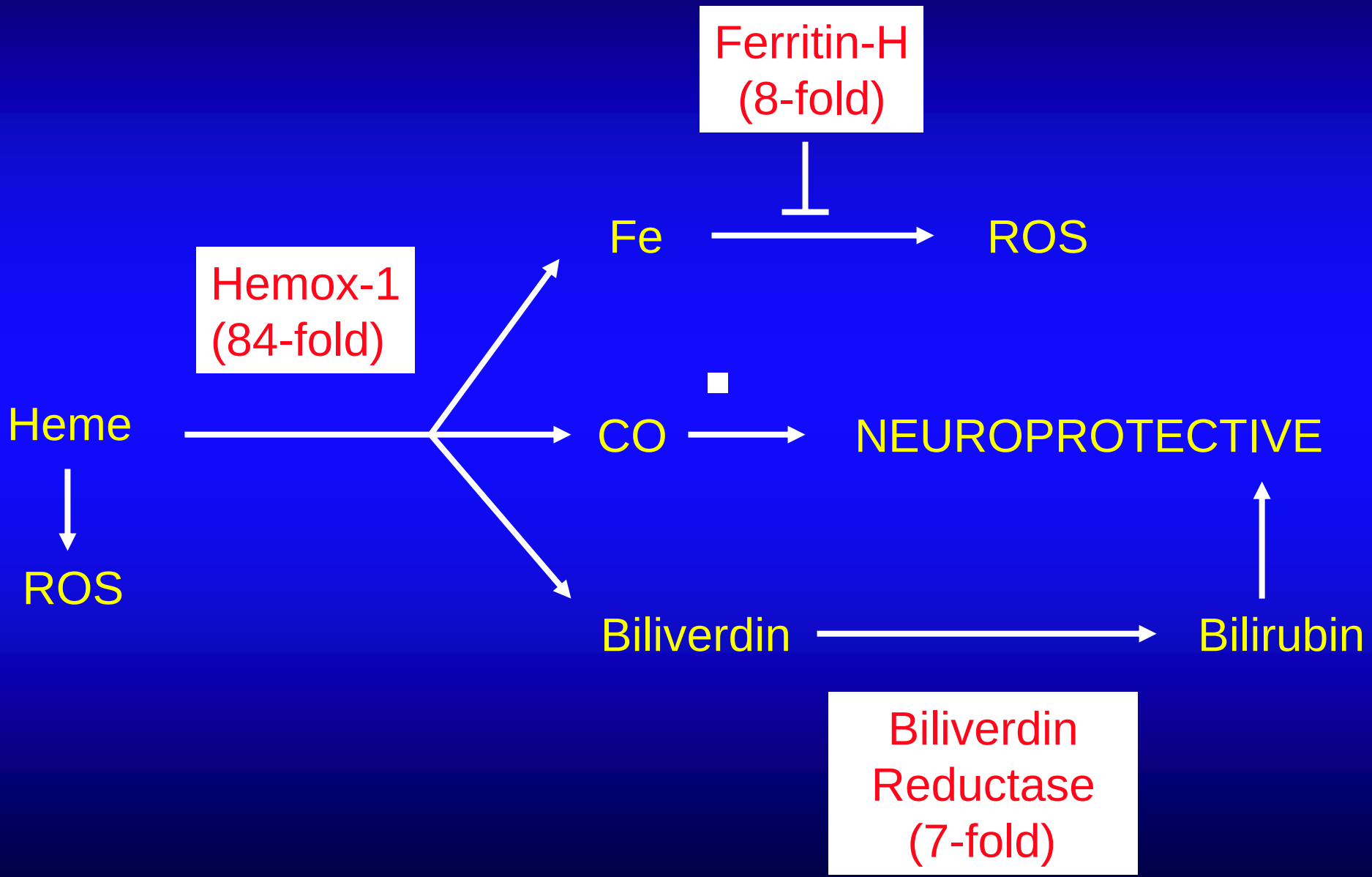
6-OHDA INDUCES EXPRESSION OF ANTI-APOPTOTIC GENES

Table 6: 6-OHDA-REGULATED GENES ENCODING ANTI-APOPTOTIC PROTEINS

TAG	TAG # -	TAG # +	FOLD CHANGE	GENE	ACCESS #
GCCACTTTGAT	5	175	84	Heme oxygenase-1	NM_012580
TGAATAATAAA	0	15	36	Metallothionein 1: MT1	NM_138826
TTCATTATACT	20	95	11	Ptma: Prothymosin alpha	NM_021740
TGATGTTAACT	0	4	10	Hk2: Hexokinase 2	NM_013820
GGTTTTTAAAA	1	4	10	RalA-binding protein 1	NM_032067
TCAGGCTGCCT	62	206	8	Ferritin subunit H	U58829
GATTGTCAGAG	4	14	8	Similar to immediate early response 3	XM_215304
AGGCGCAGACT	0	3	7	Protein phosphatase 1D magnesium-dependent, delta isoform:	XM_213418
TGTAATAAAGT	1	3	7	14-3-3 theta	NM_013053
ACAAAATAAAA	1	3	7	Ortholog of murine Nfe2l1	

Ryu et al. Neurobiol Dis. 2005

POTENTIAL PROTECTIVE ACTIVITIES OF 6-OHDA REGULATED GENES



BIG PICTURE: MULTIPLE PRO- AND ANTI-APOPTOTIC GENES ARE REGULATED BY 6-OHDA

- THE DECISION OF A GIVEN NEURON TO DIE IN RESPONSE TO 6-OHDA OR OTHER PD-LIKE STRESSES MAY REFLECT A BALANCE BETWEEN PRO- AND ANTI-APOPTOTIC INFLUENCES
- BOTH PRO-AND ANTI-APOPTOTIC GENES ARE POTENTIAL TARGETS FOR THERAPEUTIC INTERVENTION IN PD
- NEURON DEATH MAY REFLECT THE SUMMATION OF MULTIPLE SUBTHRESHOLD APOPTOTIC PATHWAYS

ER STRESS AND THE UNFOLDED PROTEIN RESPONSE IN PD

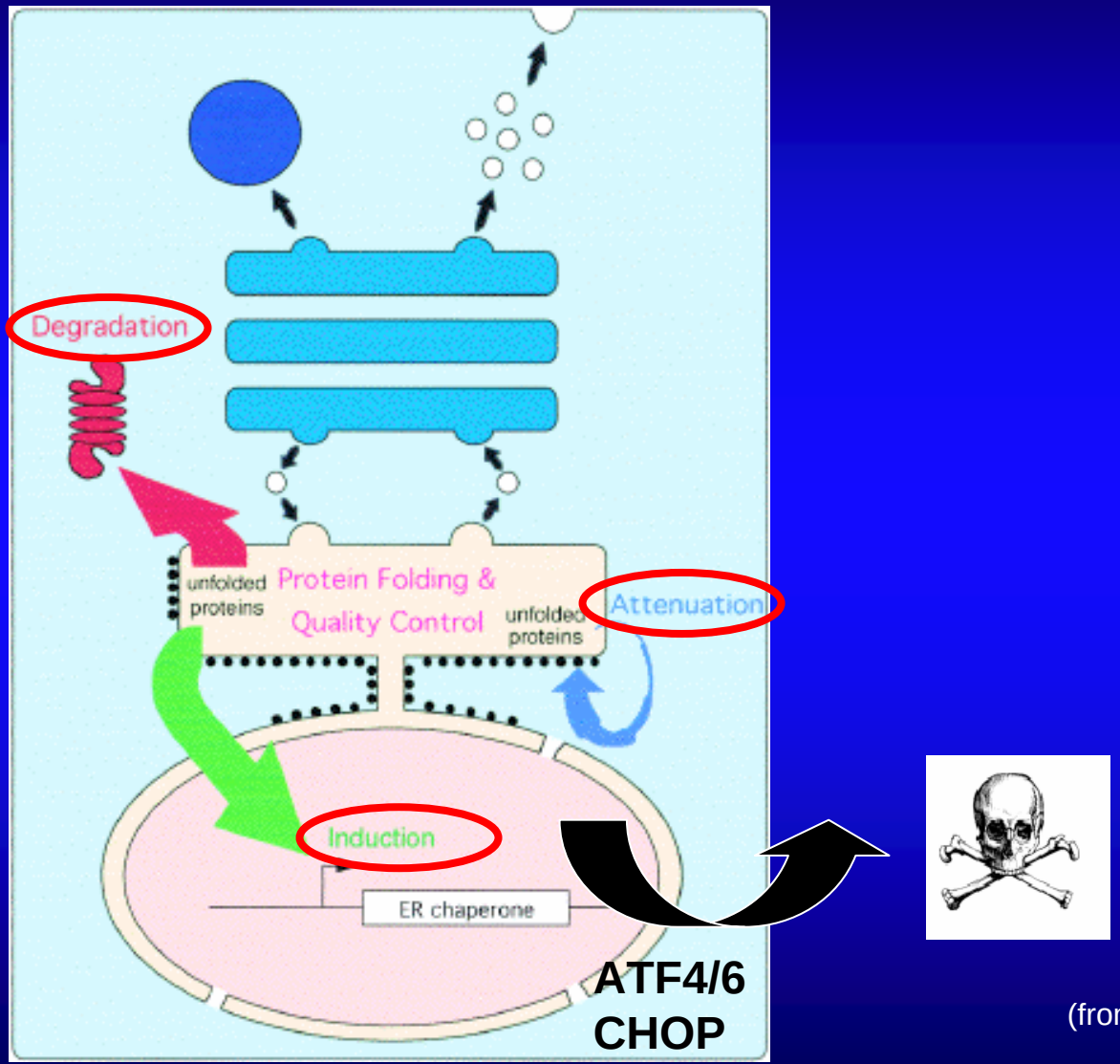
6-OHDA INDUCES ER STRESS/UNFOLDED PROTEIN RESPONSE

Table 7: 6-OHDA-REGULATED GENES ENCODING ER STRESS AND PROTEIN-FOLDING PROTEINS

TAG	TAG # -	TAG # +	FOLD CHANGE	GENE	ACCESS #
TTAGCAGGACT	0	25	60	HERP	BC061849
GCGGCCGGCTT	1	22	53	C/EBP-β	NM_024125
TTCTTTGCGTT	0	20	48	Heat shock protein 27: Hsp27	M86389
CACATCTGGTG	9	162	43	BiP (Heat shock 70kD protein 5)	M14050
GATGTGGTACG	1	43	43	Ccylophiliin B	AF071225
ACAAATAAACC	0	18	43	Homolog of human Sec61 alpha 1 subunit	NM_013336
TTATACTCCAT	1	16	38	DnaJ (Hsp40) homolog, subfamily B, member 9 (Mdg1)	NM_012699
GACACGCCTTA	0	16	38	GADD34 (Myd116)	AF020618
TGTATAAAAAT	2	24	29	similar to Endoplasmin precursor (ER protein 99/GRP94)	XM_343192
GCCTGTTCCAT	1	10	24	TERA: Transitional ER ATPase	U11760
TGTTCTCATTG	1	10	24	Ortholog murine P63 transmembrane protein	AK03070
ACAGTCTCGCC	0	9	22	Microsomal signal peptidase 25 kDA subunit	Q15005
TTGCCATCCTC	1	7	17	Ortholog of mUbiquitin-conjugating enzyme E2G (UBC7)	NM_019803
TCCGTGCTAAG	4	28	17	Growth response protein (CL-6/INSIG1)	L13619
GTGGCTCACCT	1	7	17	Heat shock protein 70.2	Z75029
TCATCTTTAAC	1	7	17	Calreticulin	AA859713
TCTCAAGTACC	0	7	17	SDF2L1: Stromal cell-derived factor 2-like 1	AB043006
TTGTAAAAGGA	0	7	17	Valosin containing protein (Vcp)	BC060518
CTGAGGAGGGG	10	61	16	CHOP (Gadd153)	U36994
GTTGTAAAGTT	1	6	14	DnaJ homolog 2/HSP40	U95727
CCTAGAGTGCC	0	6	14	Oxygen regulated protein (150kDa) (ORP150)	BC065310
TTTGCCTTCCT	1	5	12	SRPR: Signal recognition particle receptor	XP_006257
CAATTAAAAAA	1	5	12	Similar Hepatocarcinogenesis-related transcription factor	XM_214067
TGATCTTTTTG	2	10	12	Activating factor 4: ATF4	NM_024403
CAGCTGCTAAG	2	9	11	14-3-3 protein beta-subtype	NM_019377
AGAAGCAGTTC	2	9	11	Spc21: Signal peptidase 21kDa subunit	AB022714
GGTACGGTGAT	2	9	11	Similar to BAG-family molecular chaperone regulator-3	XM_215054
TATGCTGGATG	2	8	10	Grp75: 75 kDa glucose regulated protein	NM_010481
CCTCTGTTTGG	2	8	10	Similar to murine DnaJ homolog, subfamily C, member 5	NM_016775
AGTAAAAGAAA	2	8	10	Prss15: protease, serine, 15 (Lon)	NM_133404
GTATTTAACAT	1	4	10	Ortholog of murine Vapa	NM_013933
GTGTCTATAGC	1	4	10	Similar to murine DnaJ homolog, subfamily B member 11	NM_026400
AGCAGTGTCAC	1	4	10	Similar to signal recognition particle 68kDa	XM_343986
AGACAAAGTTT	1	4	10	Heat shock 70 kDa protein 4 (IRP94/Hsp110)	AF077354
CAATAAAACAA	1	4	10	Similar Sar1a protein promoting vesicle budding from ER	XM_213283
GGCCTAAGGCA	1	4	10	Ortholog of murine Heat shock protein, A (Hsp74/Grp75)	AK030299
TGTTTTAATAT	0	4	10	Similar to Ab2-292	XM_215528
TCTACAAGAAT	20	63	8	Similar to heat shock protein 84 - mouse	XM_217339
CAGGAGGAGTT	6	20	8	Grp58: glucose regulated protein, 58 kDa	NM_017319
TGGCCTGTGTG	4	14	8	Protein disulfide isomerase	NM_012998
TGTGAAAAGAT	5	17	8	Olfm1: Olfactomedin related ER localized protein	NM_053573
ACCTATATTGT	3	10	8	KDELR2: KDEL ER protein retention receptor 2	BC007146
TGATCATTGTG	3	10	8	Ortholog of murine Hsp105: heat shock protein	NM_013559
GCAGCCATCAA	2	7	8	Ortholog of mThioredoxin domain containing 7 (CaBP5)	BC006865
ACCTTGCCCTC	3	9	7	Ssr2: signal sequence receptor, beta	XM_215619
GCTCTCAAACC	3	9	7	Orthoolog of murine Cryac: crystallin, alpha C (HSP22)	NM_030704
AGTCATCCAGC	2	6	7	Ortholog of murine Thioredoxin domain containing 1	BC021533
GTAGGCCGCCT	1	3	7	Ortholog of human Heat shock protein 75	BC023585
TACATTATACT	1	3	7	Similar to dolichol-phosphate-mannose synthase	XM_215949
GTGCCAGCACT	1	3	7	Similar to FK506 binding protein 11	XM_217041
TATCATAGGGA	1	3	7	Similar to ORM1-like 3	XM_340893
TCTTACTGGCA	1	3	7	Ortholog of murine Hrd1-pending: HRD1 protein	NM_028769
TGCAATAAGAA	1	3	7	Ortholog of mDnaJ homolog, subfamily B, member 12	AK089427
TCTATAGAGTG	1	3	7	Rcn: reticulocalbin	NM_022535
CAAACAATCGT	0	3	7	Ortholog of human SEC24A	AJ131244
GACTTTACATA	0	3	7	Ortholog of mAkap1: A kinase (PRKA) anchor protein 1	XM_147636
TTATTAAAATC	0	3	7	Ortholog of murine Pfdn4: prefoldin 4	XM_355370
AATGAAATAAA	1	3	7	St13	NM_031122
TATTCACTATT	0	3	7	Similar to Scotin	XM_217272
GGTGGGTGGTT	0	3	7	Ortholog of murine Crebl1 (ATF6-beta)	NM_017406
GAATAATAAAA	18	47	6	Heat shock protein 8 (Heat shock cognate protein 70)	BC061547
TAATTTGCCTA	8	14	4	SERP1	AB018546
CCTCCCTTTTA	12	22	4	Heat shock 10 kDa protein 1	NM_012966
TCCACACTGCT	4	7	4	Hsp70 binding protein	AF187880

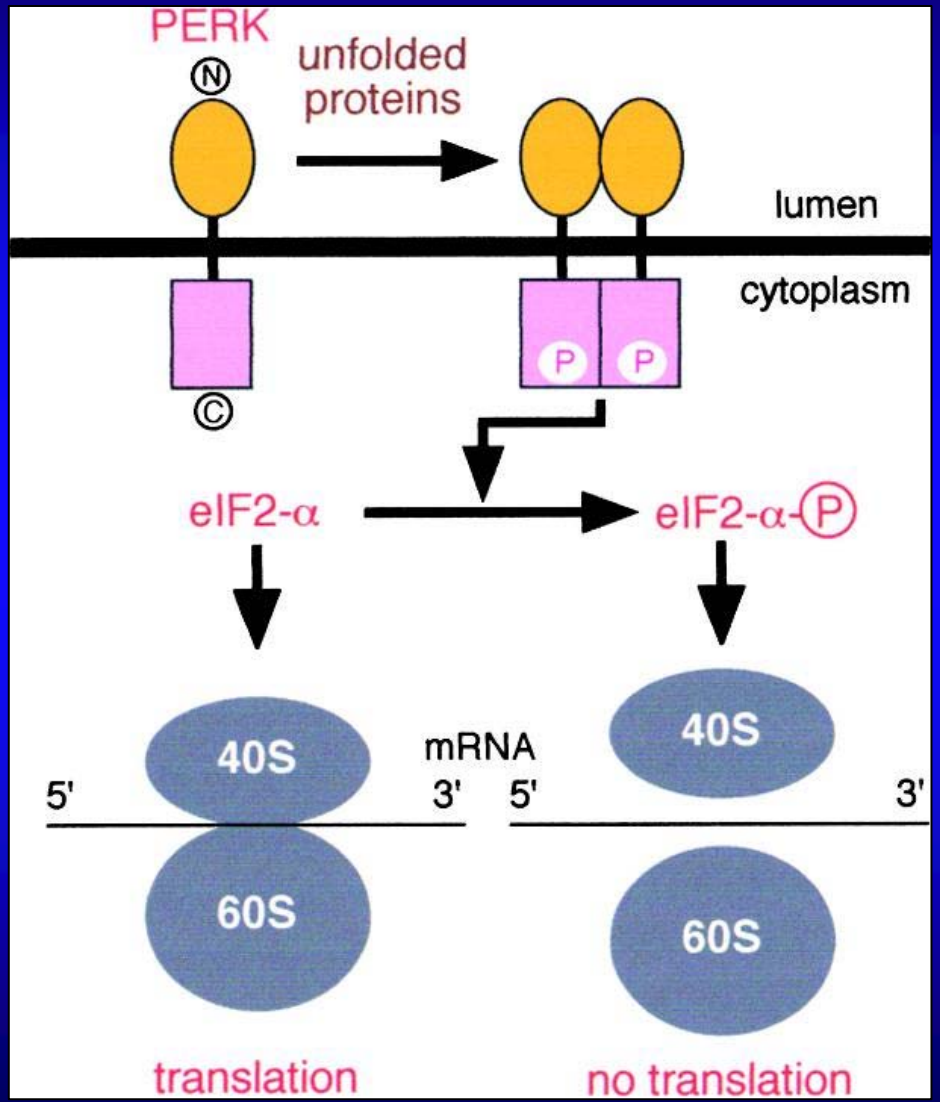
Ryu et al. J Neurosci. 2003
Ryu et al. Neurobiol Dis. 2005

Components of the Unfolded Protein Response

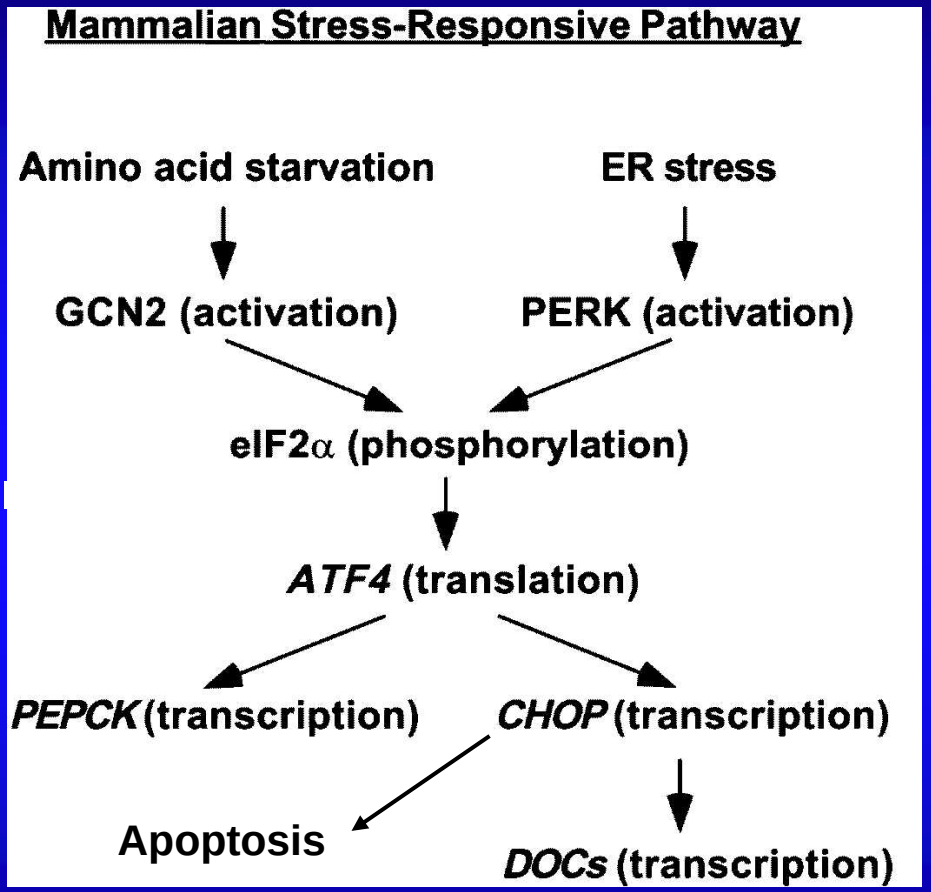


(from Mori, Cell 2000)

SENSORS AND EXECUTORS OF THE ER STRESS RESPONSE

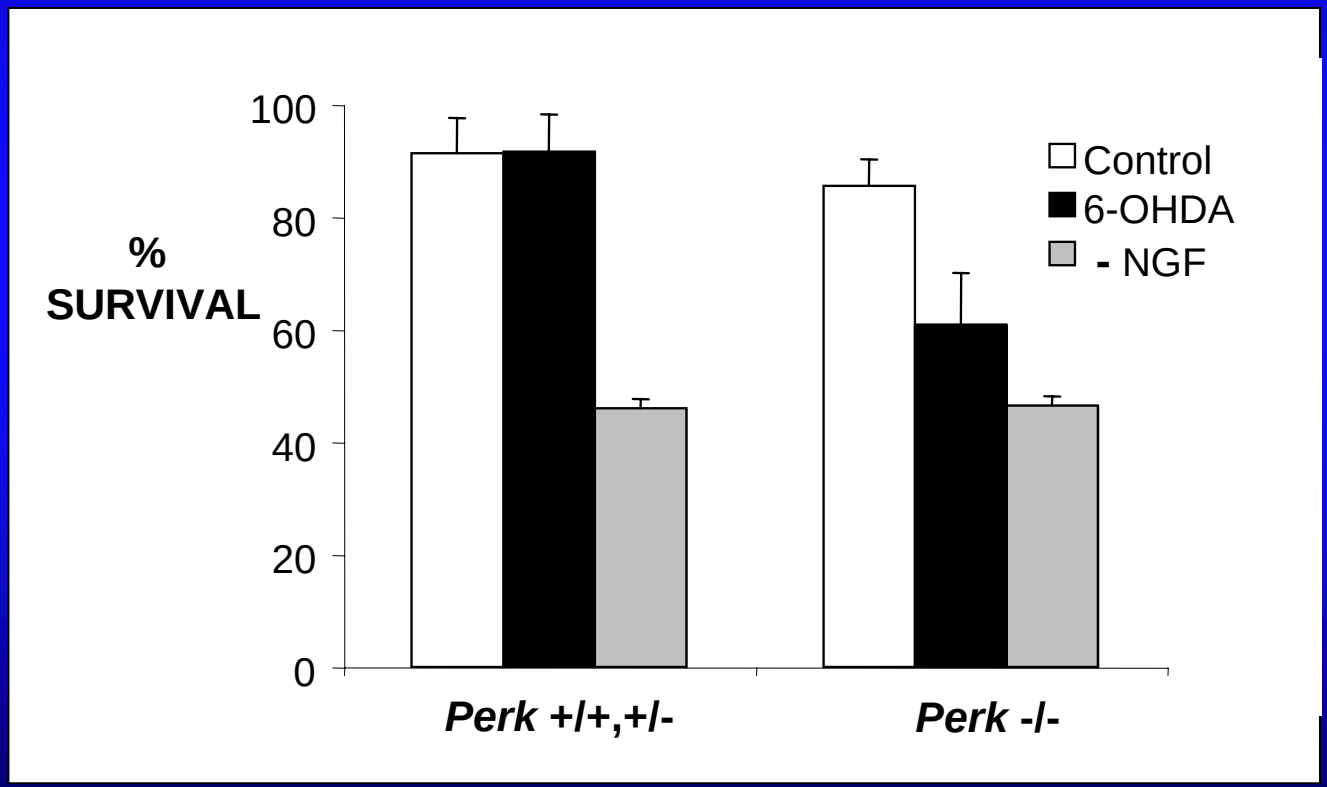


(Mori, Cell 2000)



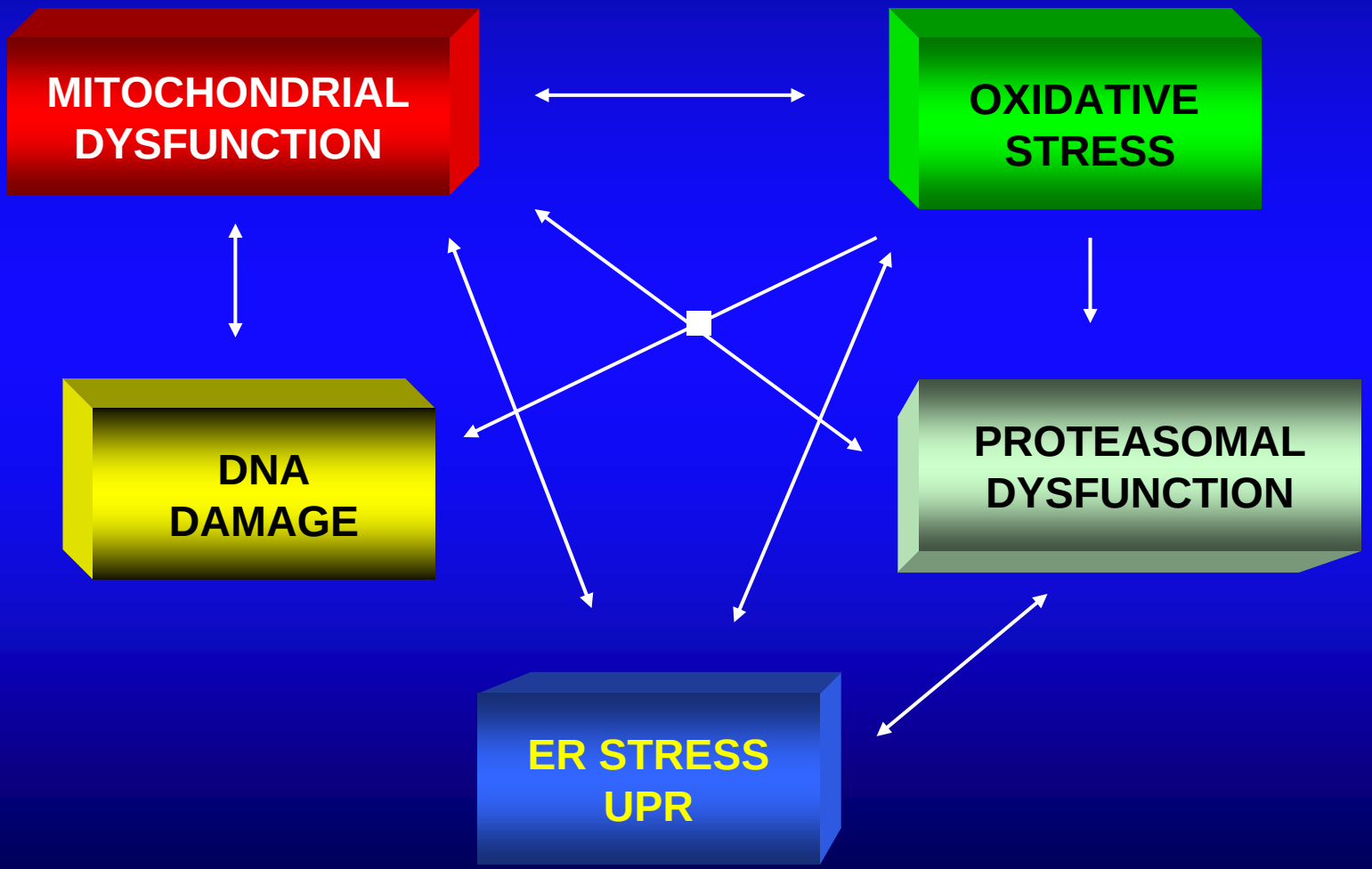
(Harding et al., Mol Cell 2000)

SYMPATHETIC NEURONS DEFICIENT IN PERK AND THE ER STRESS RESPONSE ARE MORE SENSITIVE TO 6-OHDA

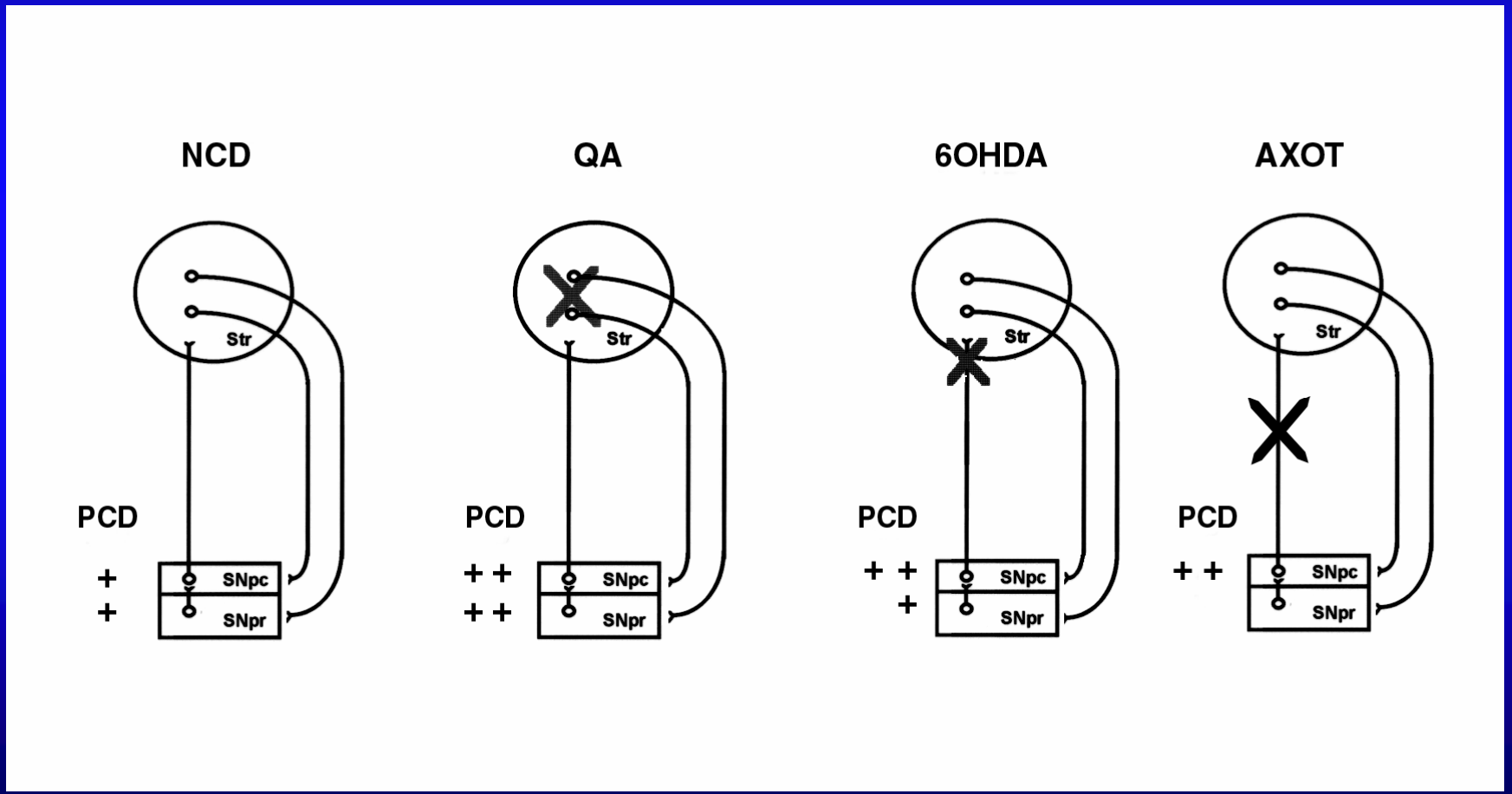


Ryu et al, J Neurosci 2003

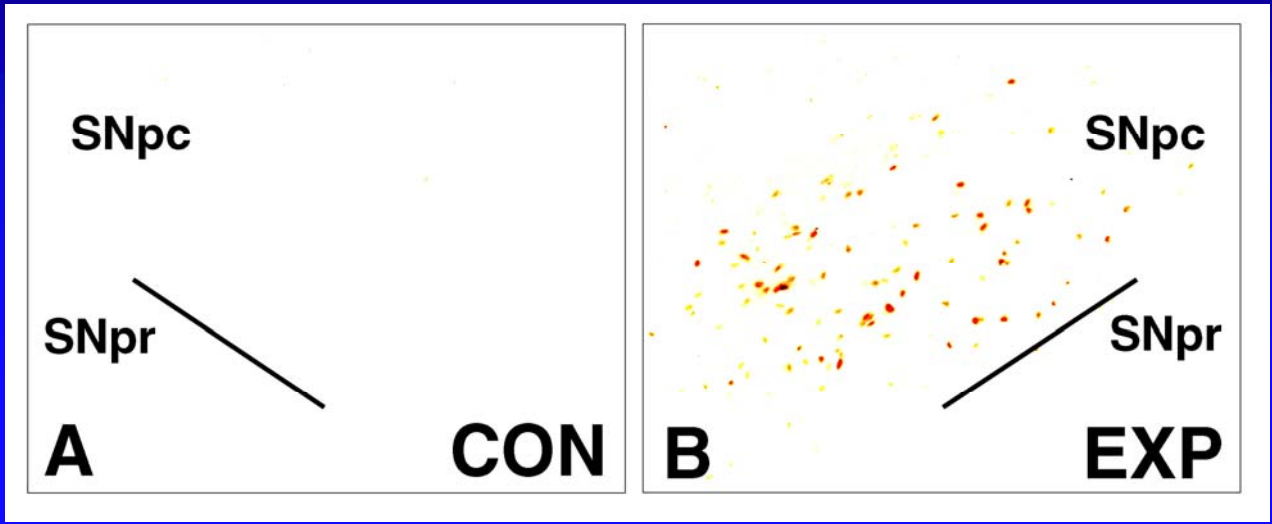
MULTIPLE SYSTEM FAILURES MAY UNDERLIE PD

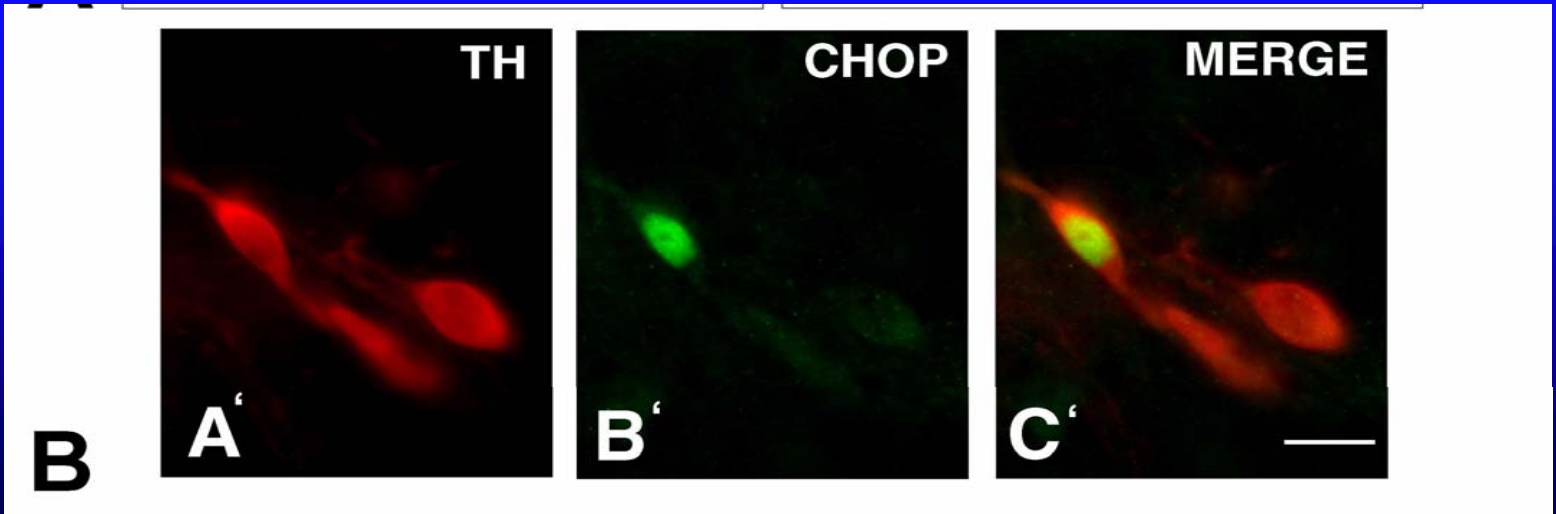


APOPTOSIS IN SN DOPAMINE NEURONS IN VIVO: MODELS



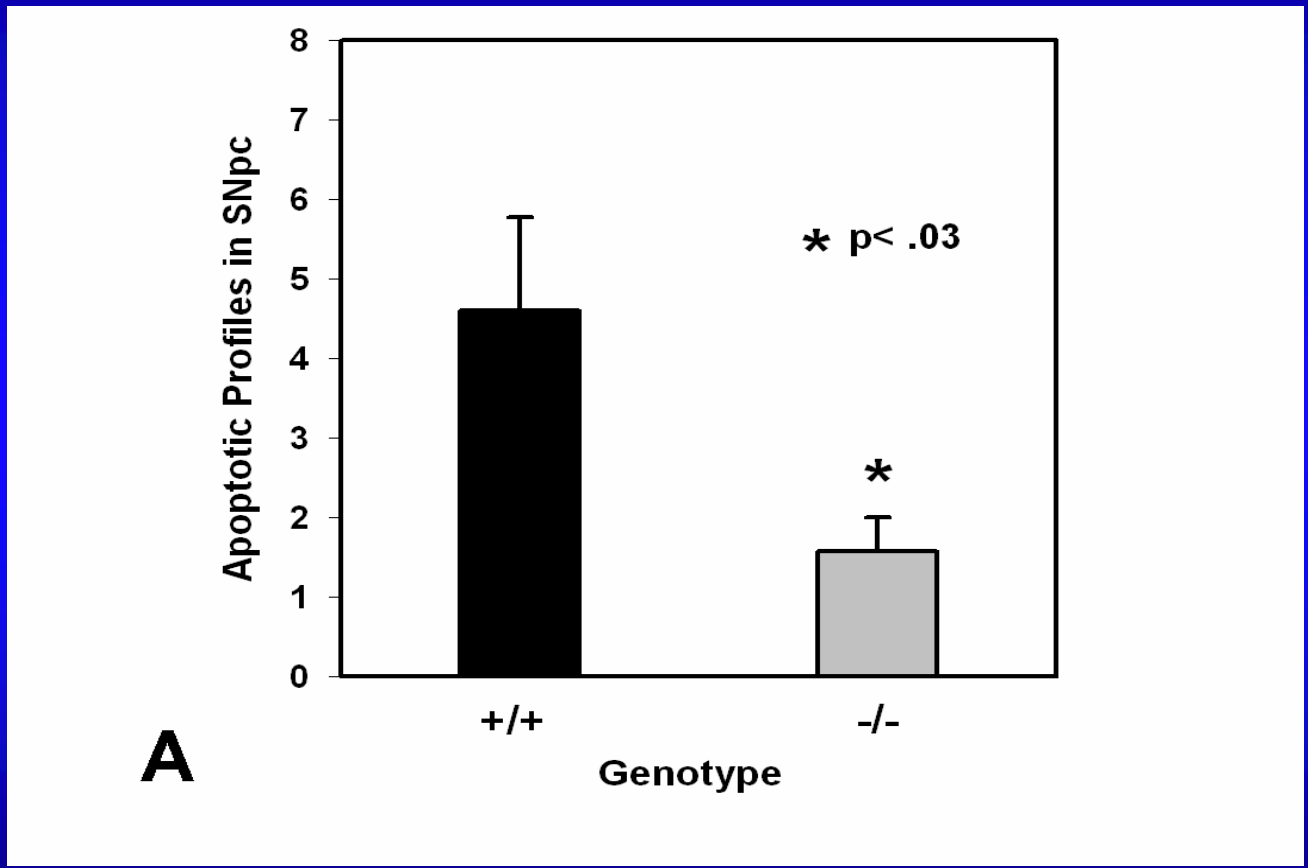
6-OHDA INDUCES CHOP EXPRESSION IN SNpc NEURONS *IN VIVO*





SILVA ET AL UNPUBLISHED

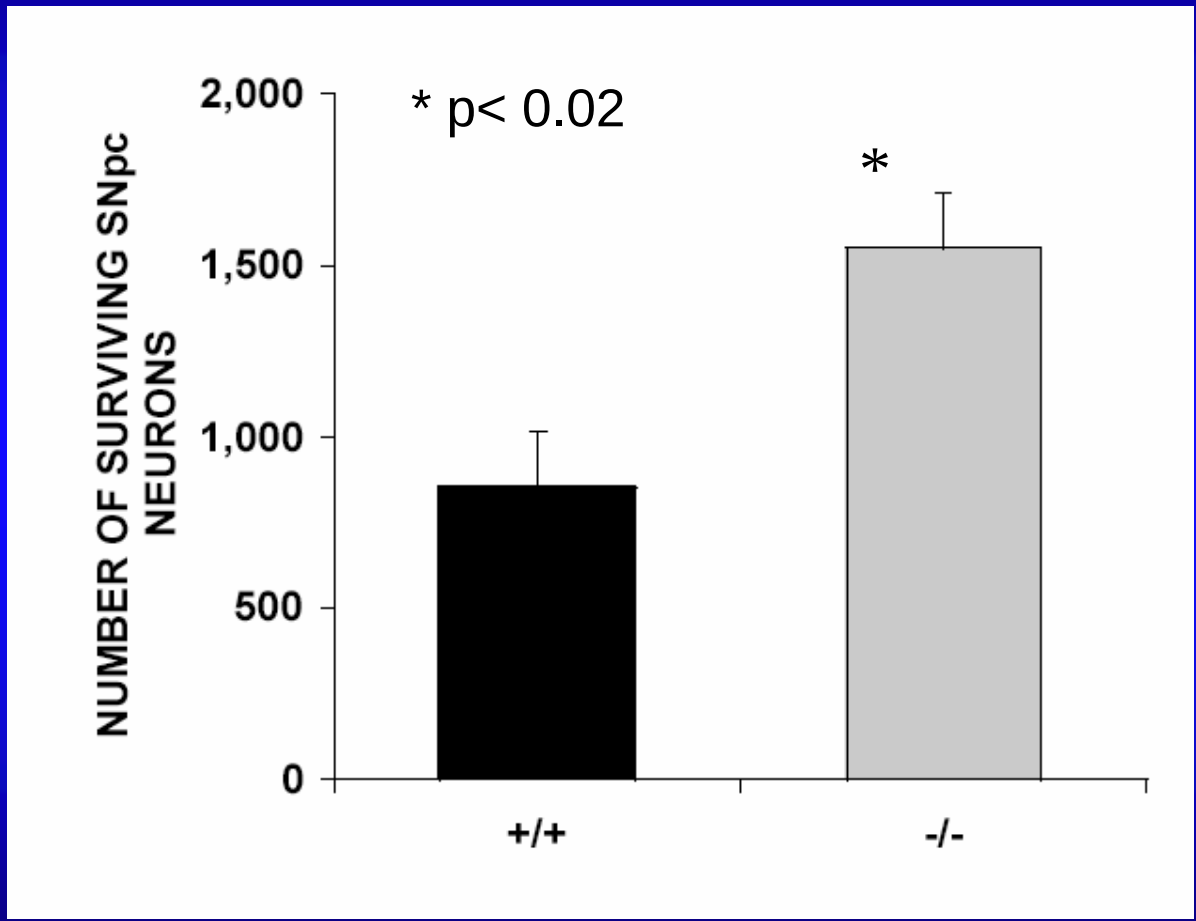
SNpc NEURONS IN CHOP-NULL MICE ARE RESISTANT TO DEATH PROMOTED BY 6-OHDA *IN VIVO*



POST LESION DAY 6

SILVA ET AL UNPUBLISHED

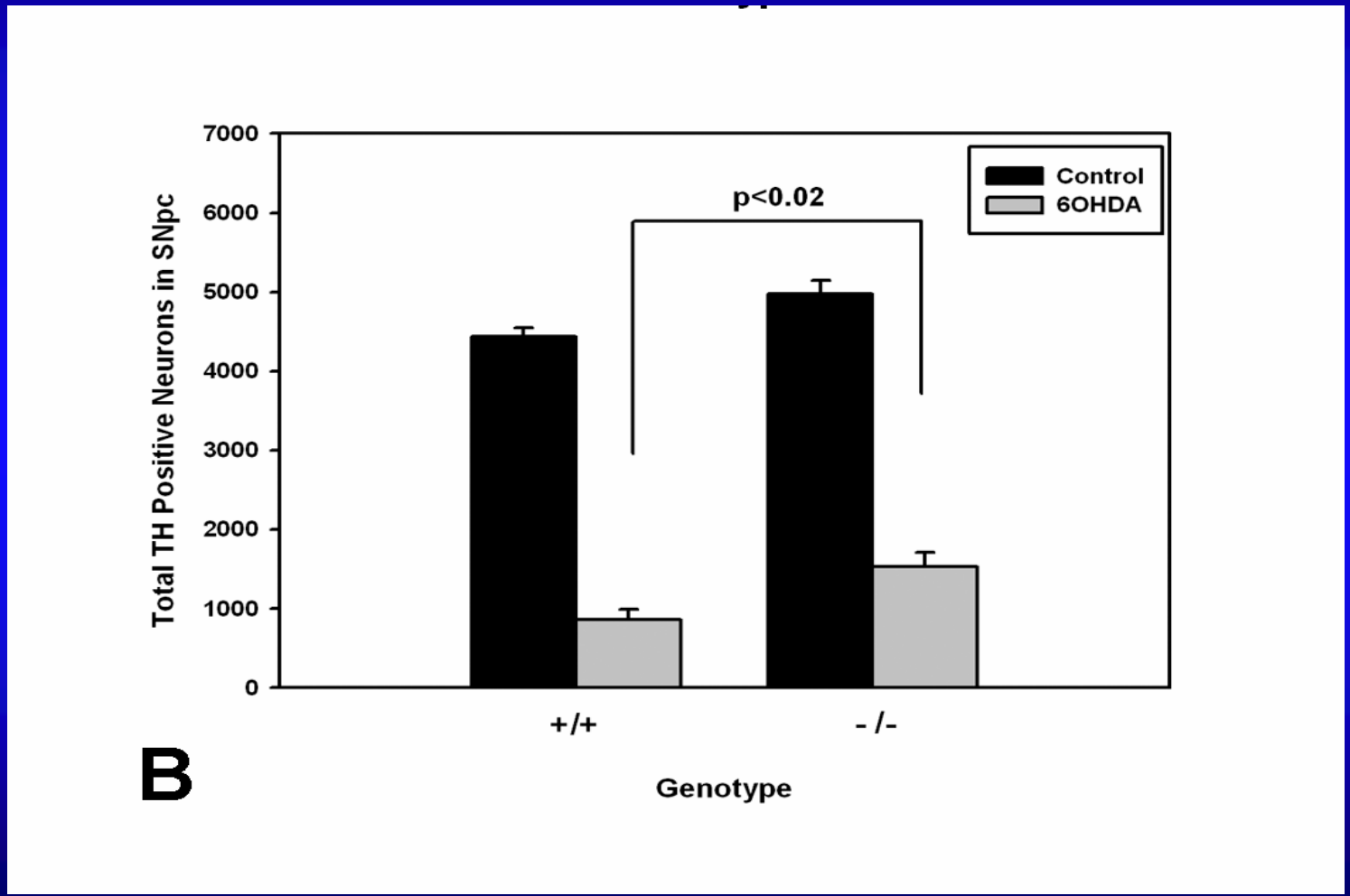
SNpc NEURONS IN CHOP-NULL MICE ARE RESISTANT TO DEATH PROMOTED BY 6-OHDA *IN VIVO*



POST LESION DAY 28

SILVA ET AL UNPUBLISHED

SNpc NEURONS IN CHOP-NULL MICE ARE RESISTANT TO DEATH PROMOTED BY 6-OHDA *IN VIVO*

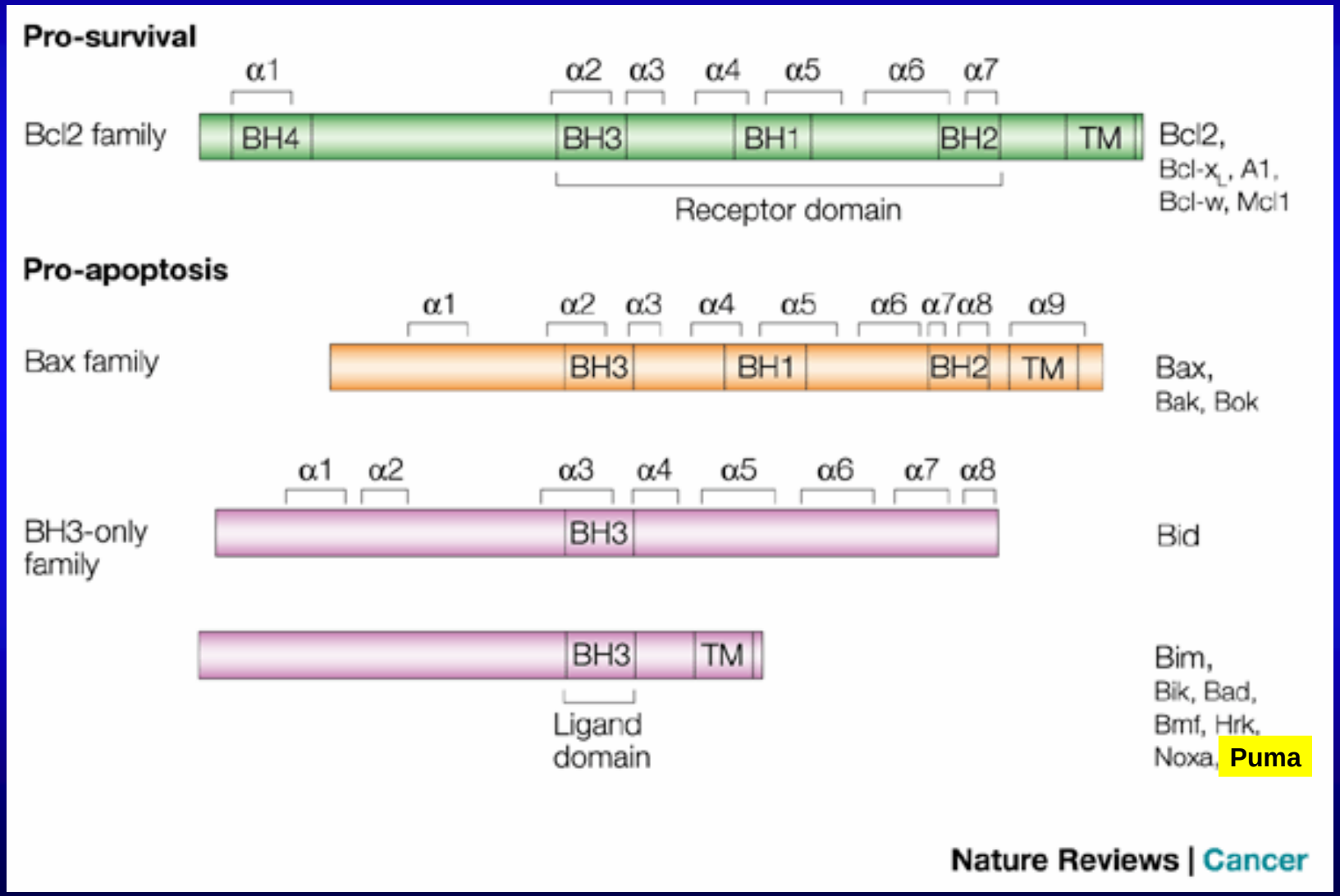


POST LESION DAY 28

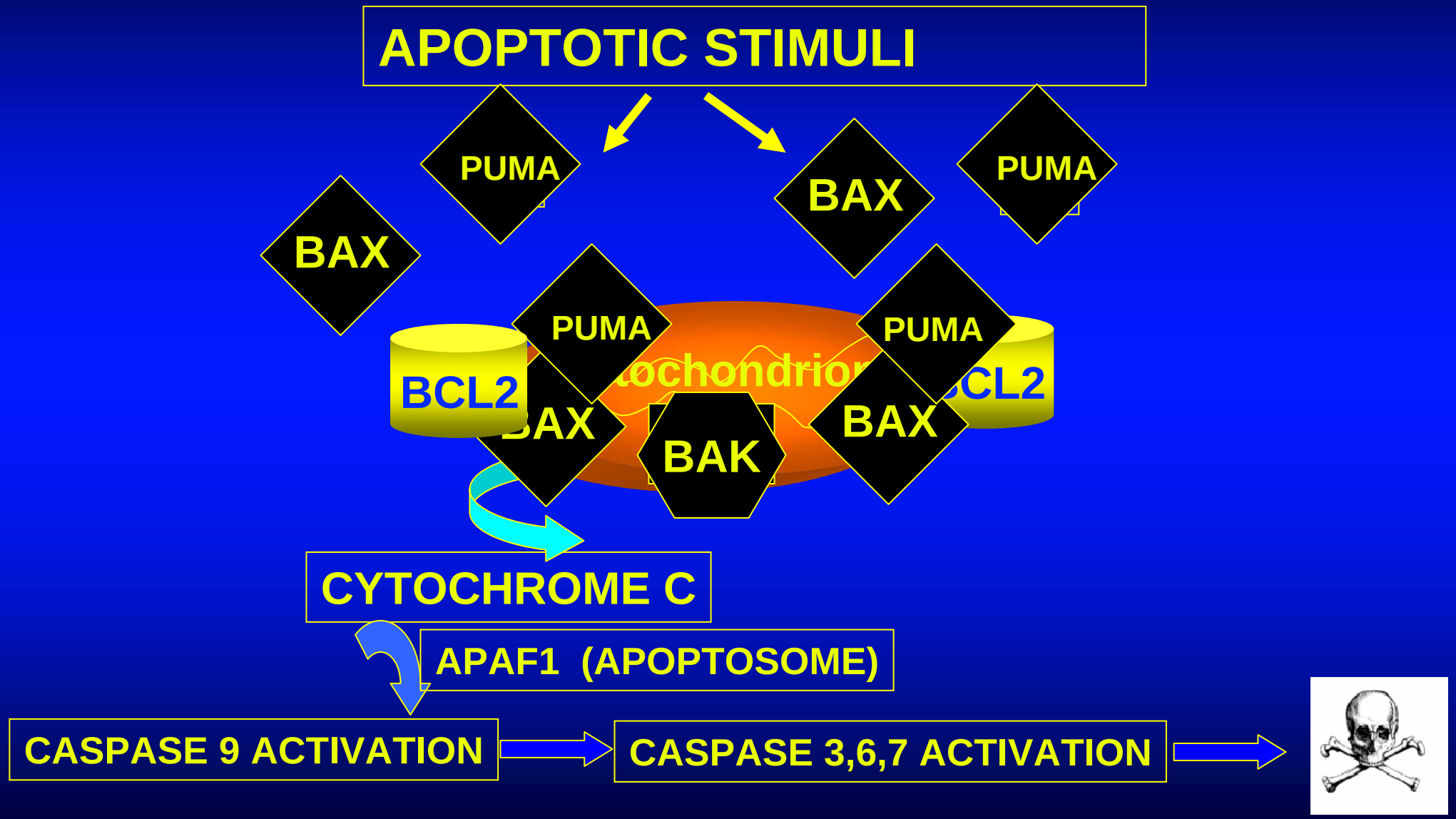
SILVA ET AL UNPUBLISHED

PUMA AND PD

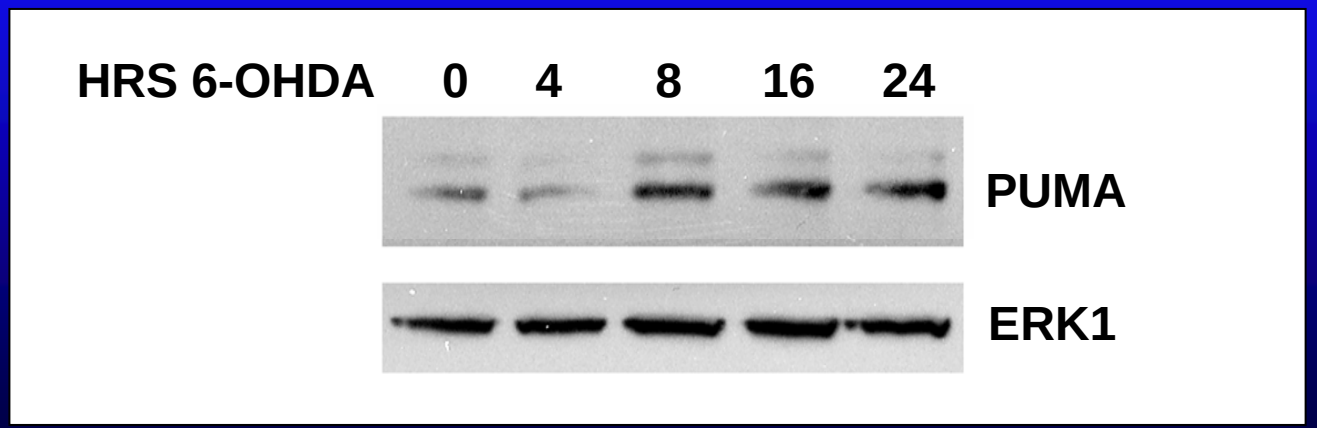
PUMA IS A PRO-APOPTOTIC MEMBER OF THE BCL2 FAMILY



MITOCHONDRIA AND APOPTOTIC DEATH -2



6-OHDA INDUCES PUMA MESSAGE AND PROTEIN



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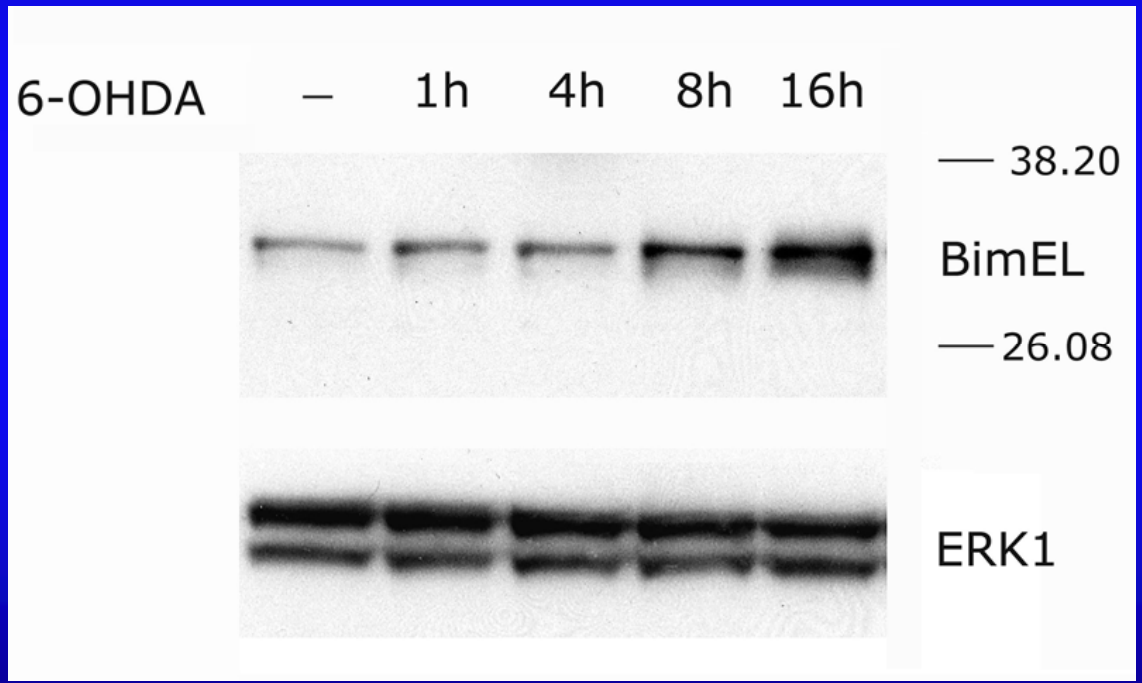
6-OHDA INDUCES BIM PROTEIN

Figure 2.



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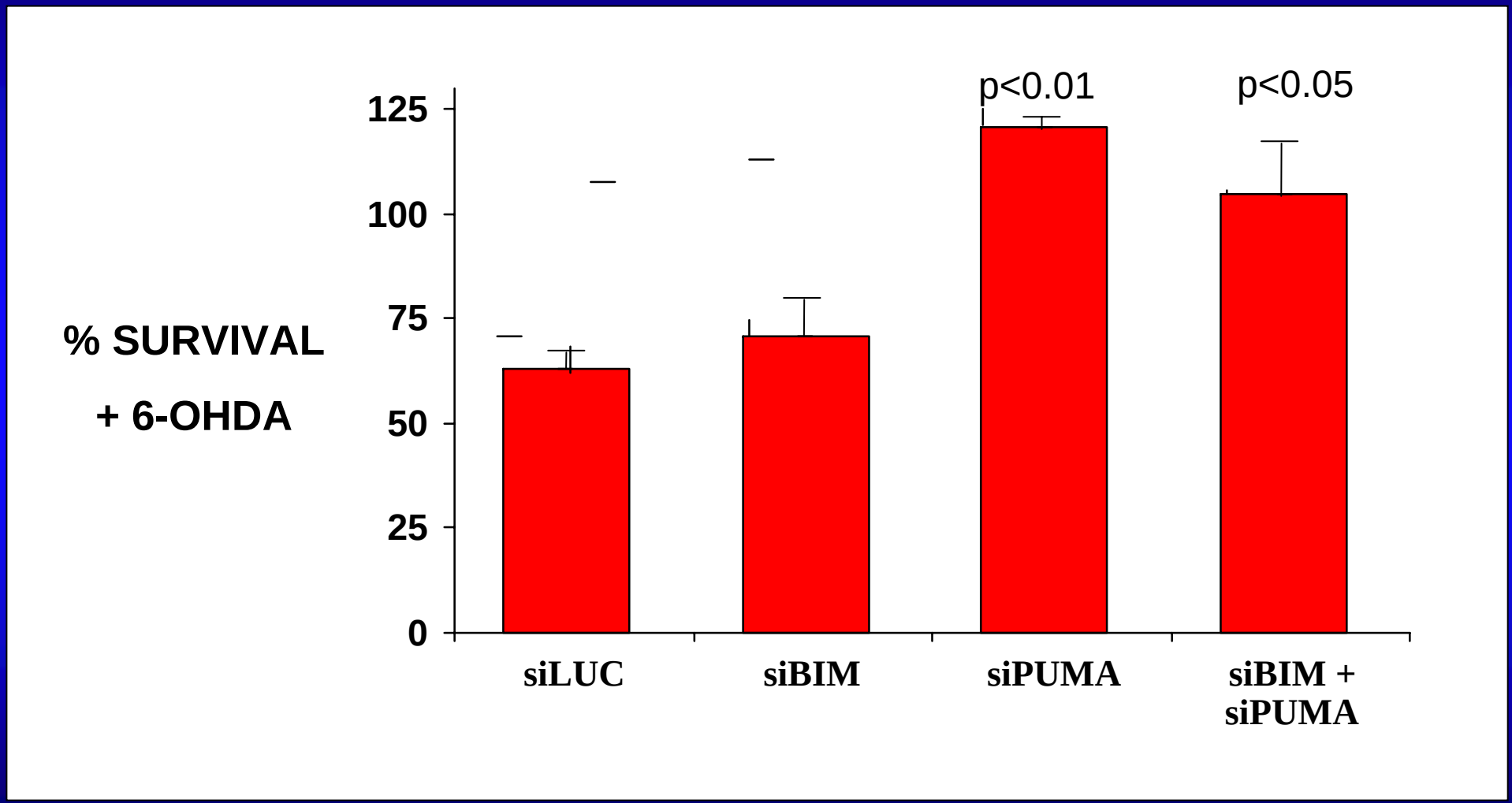
siPUMA BUT NOT siBIM PROTECTS AGAINST 6-OHDA



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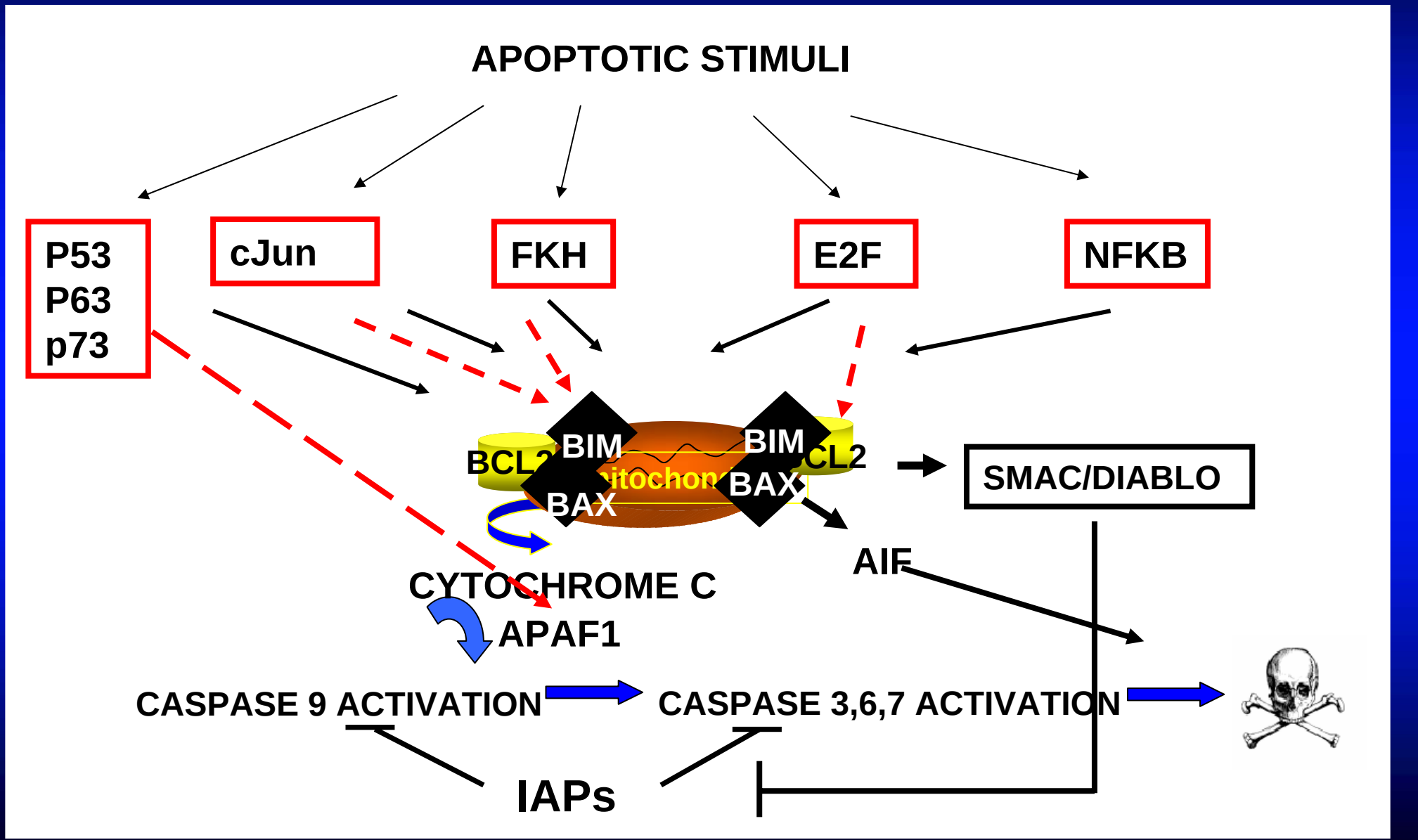
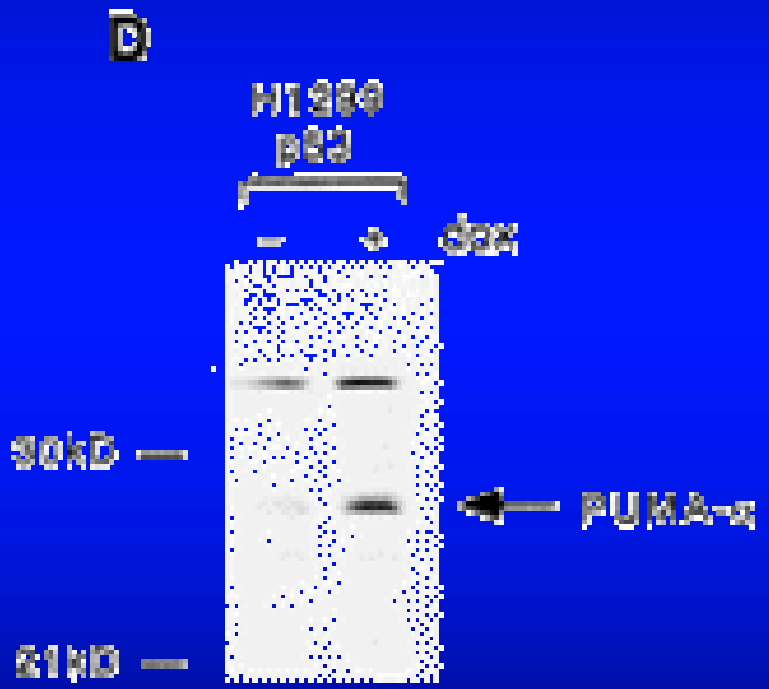
WHO CONTROLS PUMA?



WHO CONTROLS PUMA? PUMA IS A DIRECT TRANSCRIPTIONAL TARGET OF p53

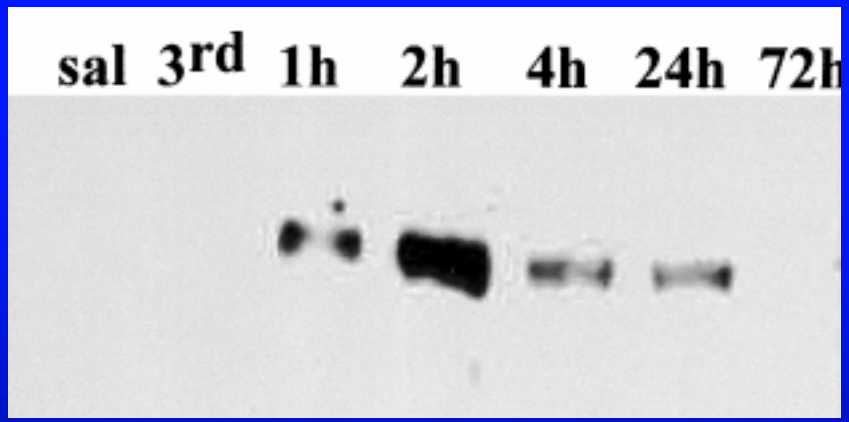


NAKANO AND VOUSEN MOL CELL 2001

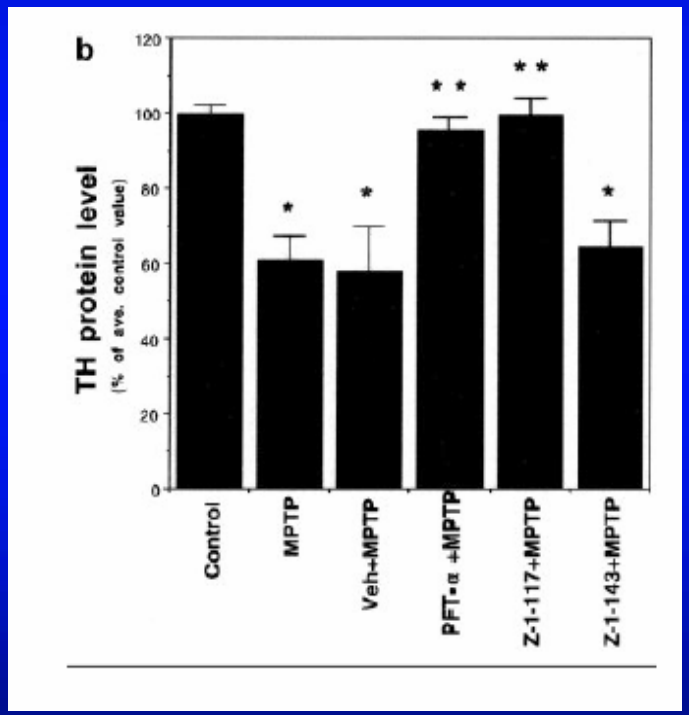
p53 AND PARKINSON DISEASE: P53 IS UPREGULATED IN A PD ANIMAL MODEL AND BLOCK OF p53 ACTIVITY PRESERVES DA NEURONS

HRS MPP+ TREATMENT

p53

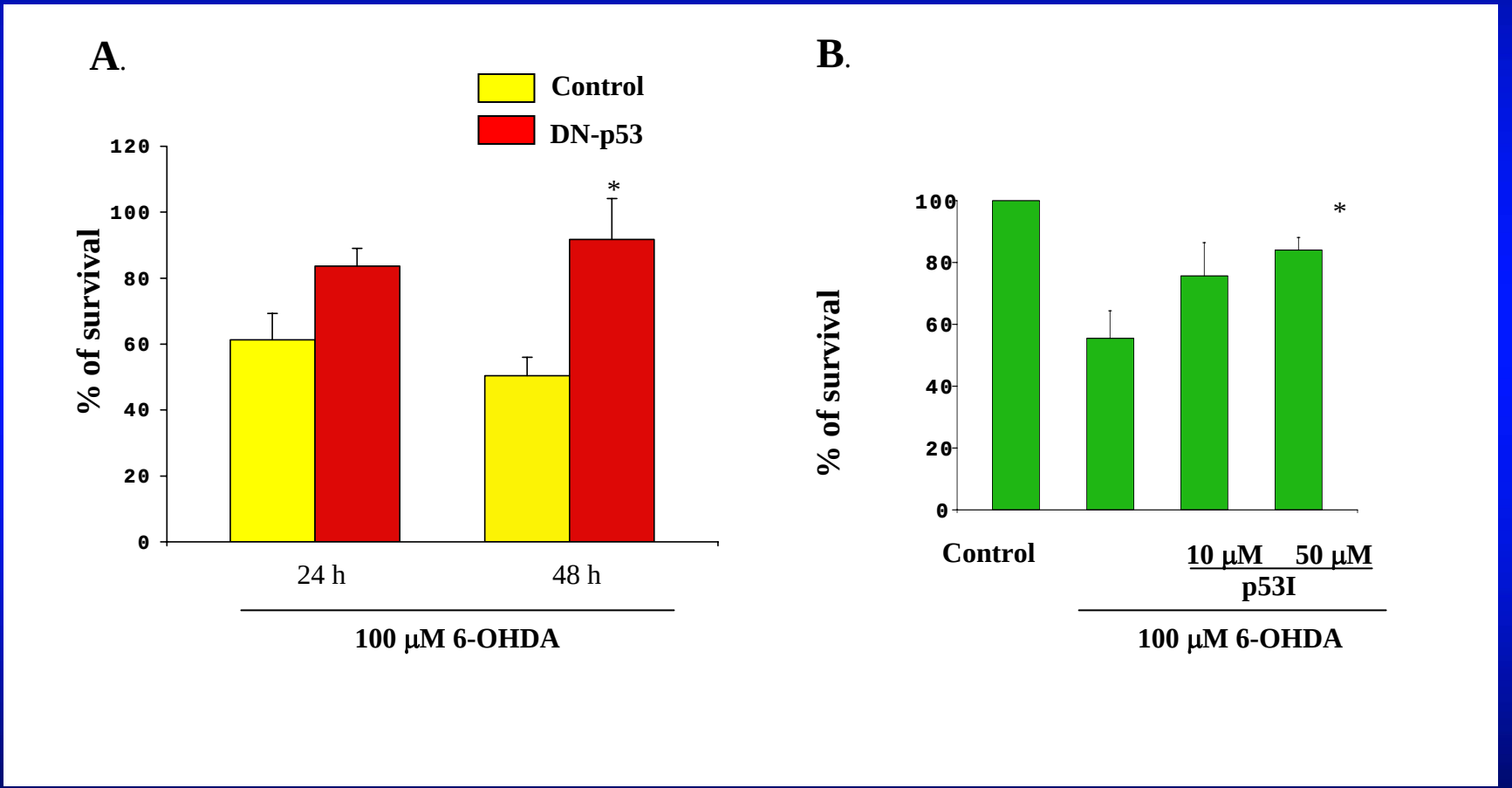


MANDIR ET AL J NEUROCHEM 2002



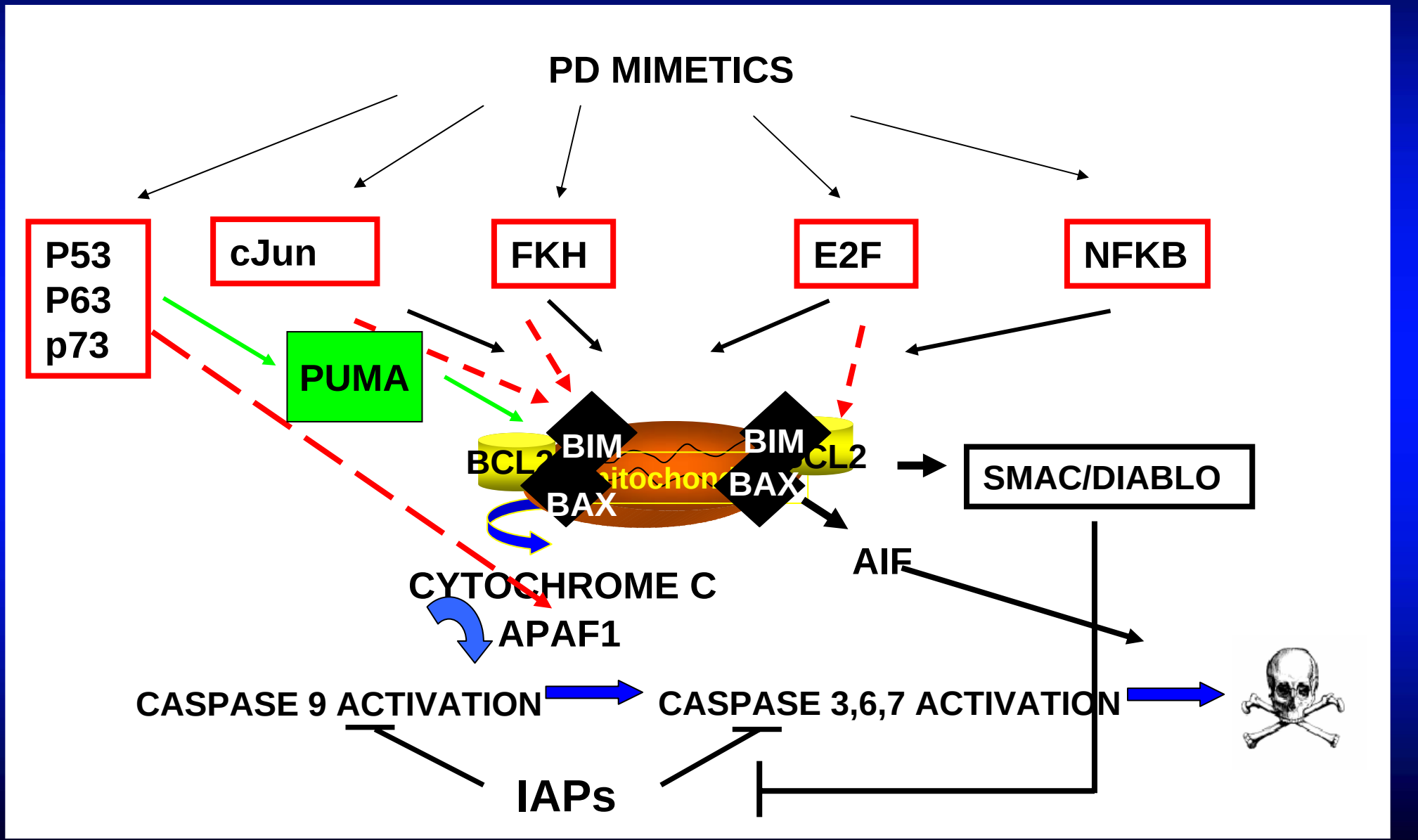
DUAN ET AL ANN NEUROL 2002

INTERFERENCE WITH p53 PROTECTS NEURONAL CELLS IN THE 6-OHDA CELLULAR MODEL OF PD



BISWAS ET AL IN PRESS

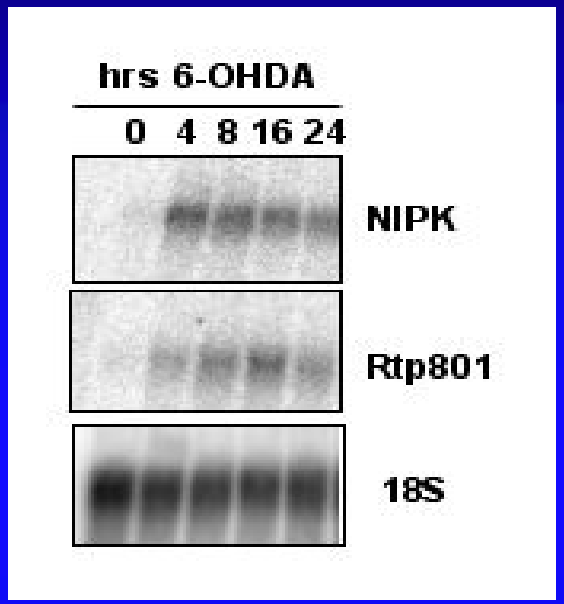
ROLES FOR P53 AND PUMA IN PD?



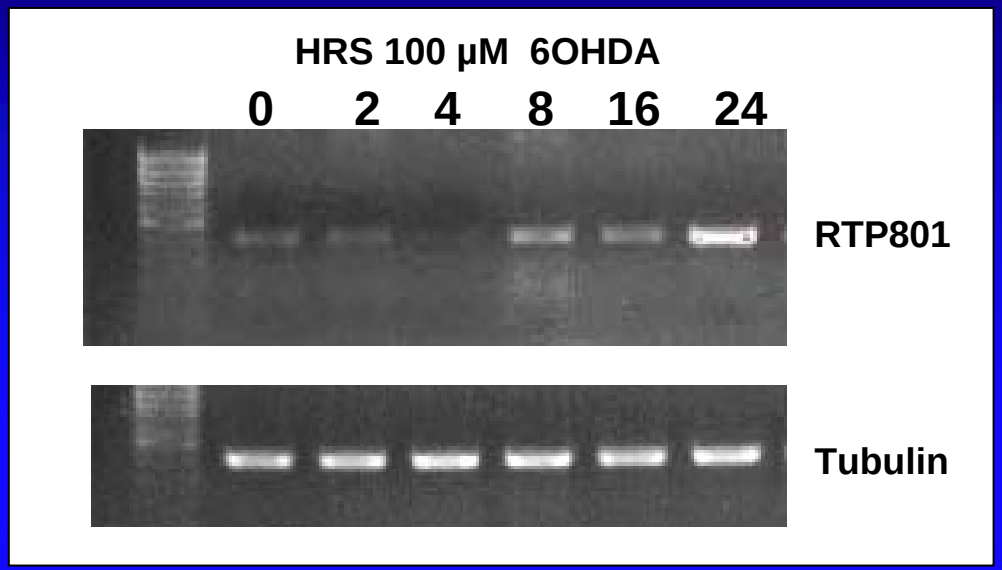
RTP-801/REDD1 AND PD

- STRESS RESPONSIVE GENE
- ANTI- OR PRO-APOPTOTIC DEPENDING ON CELL TYPE AND STIMULUS
- ANTI-APOPTOTIC FOR NAÏVE PC12 CELLS, BUT PRO-APOPTOTIC FOR NEURONAL PC12 CELLS (Shoshani et al. MCB 2002)
- KNOCKDOWN OR LOSS PROTECTIVE FROM BETA AMYLOID IN VITRO (Kim et al. Exp Mol Med 2003) AND OXYGEN-INDUCED RETINOPATHY (Brafman et al., Invest Opthamol Vis Sci 2004)

RTP801 IS INDUCED IN RESPONSE TO 6-OHDA

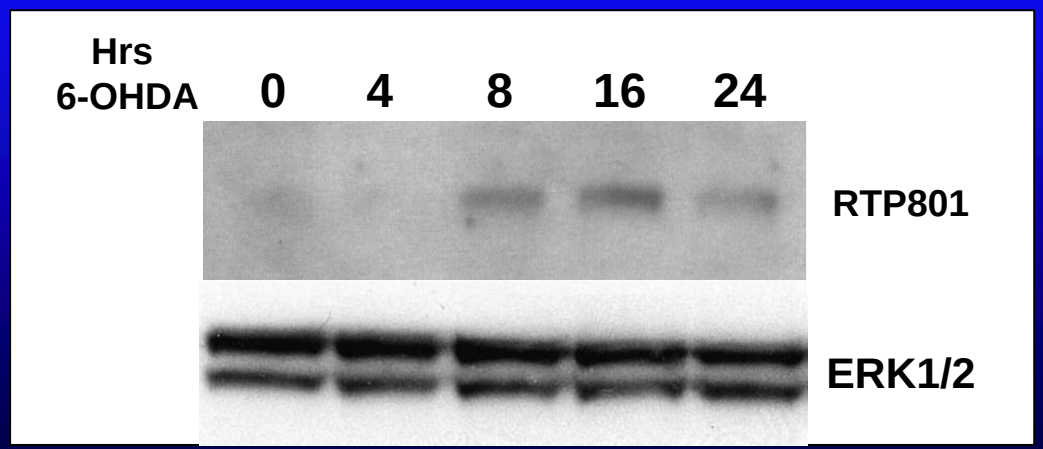


NORTHERN BLOT



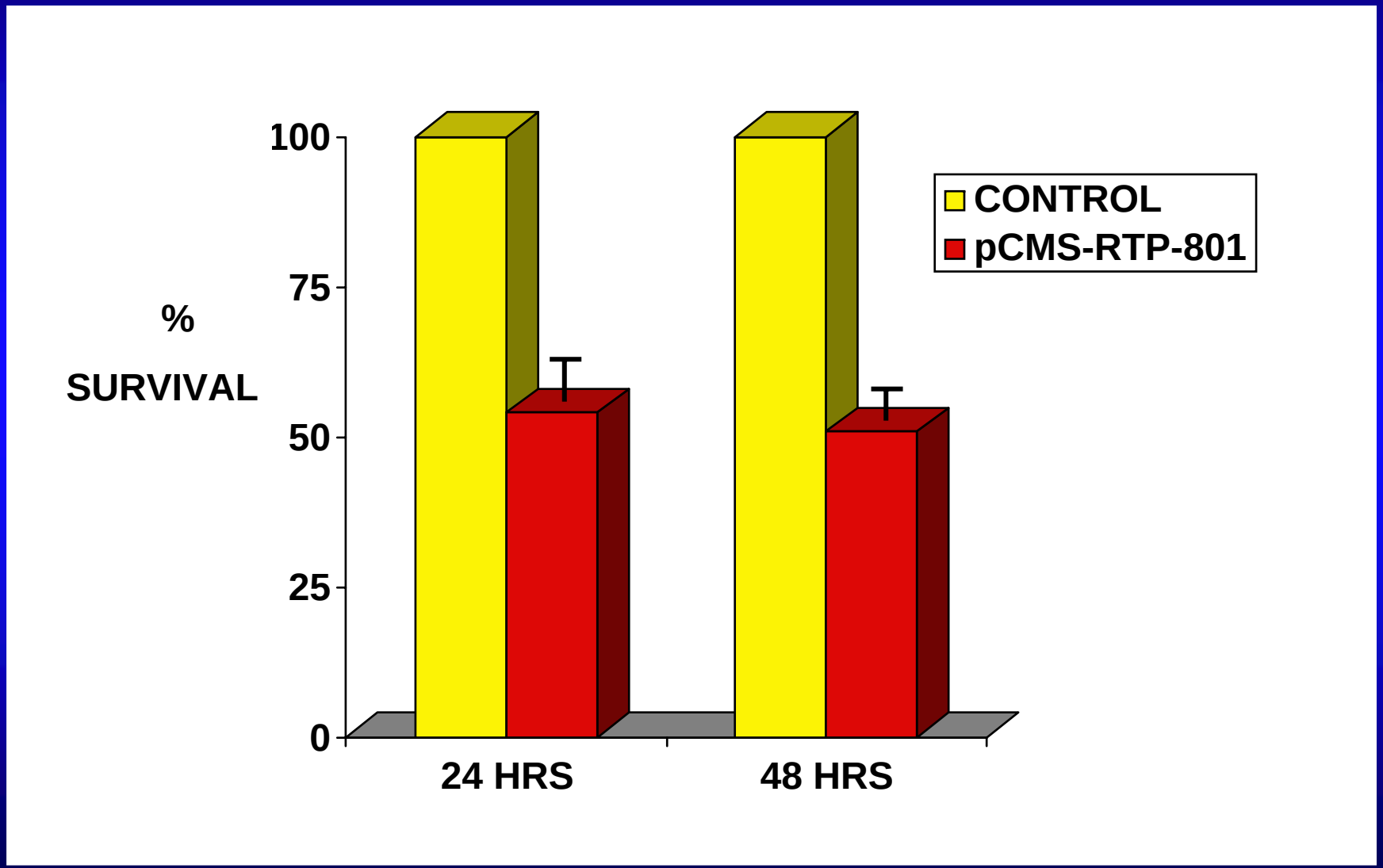
PCR

WESTERN BLOT



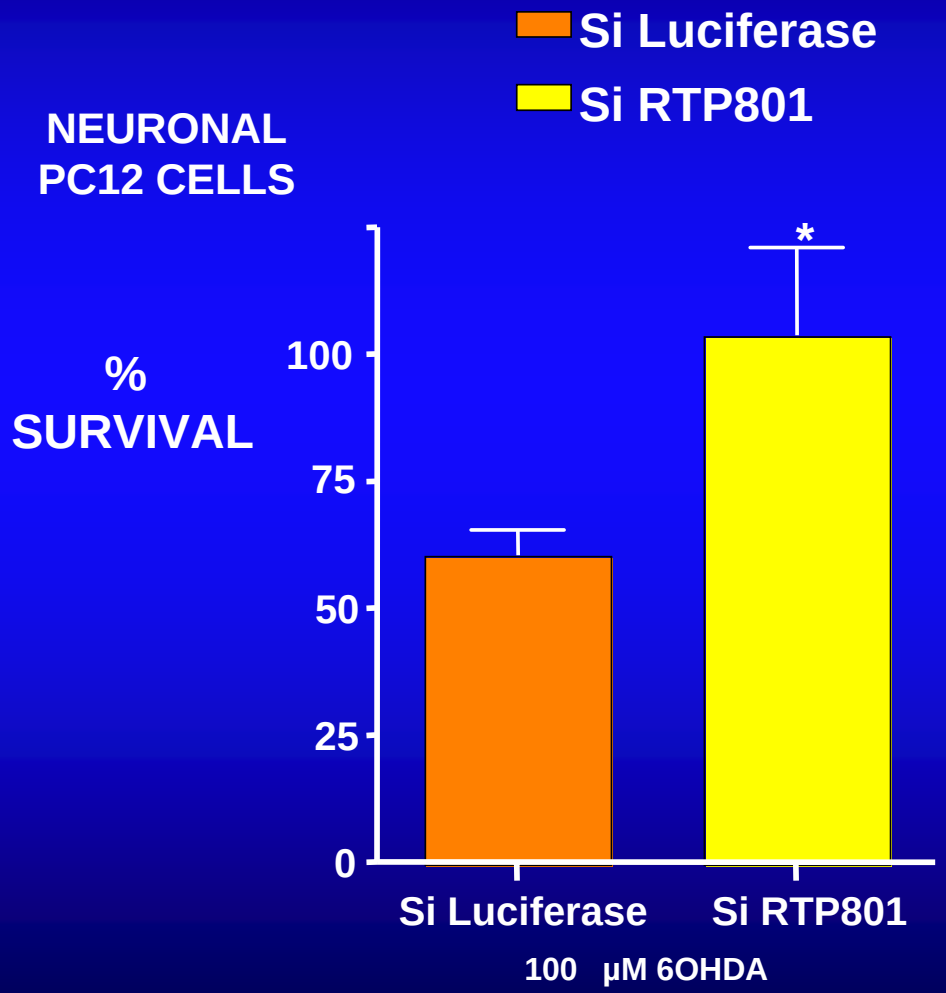
MALAGELADA ET AL UNPUBISHED

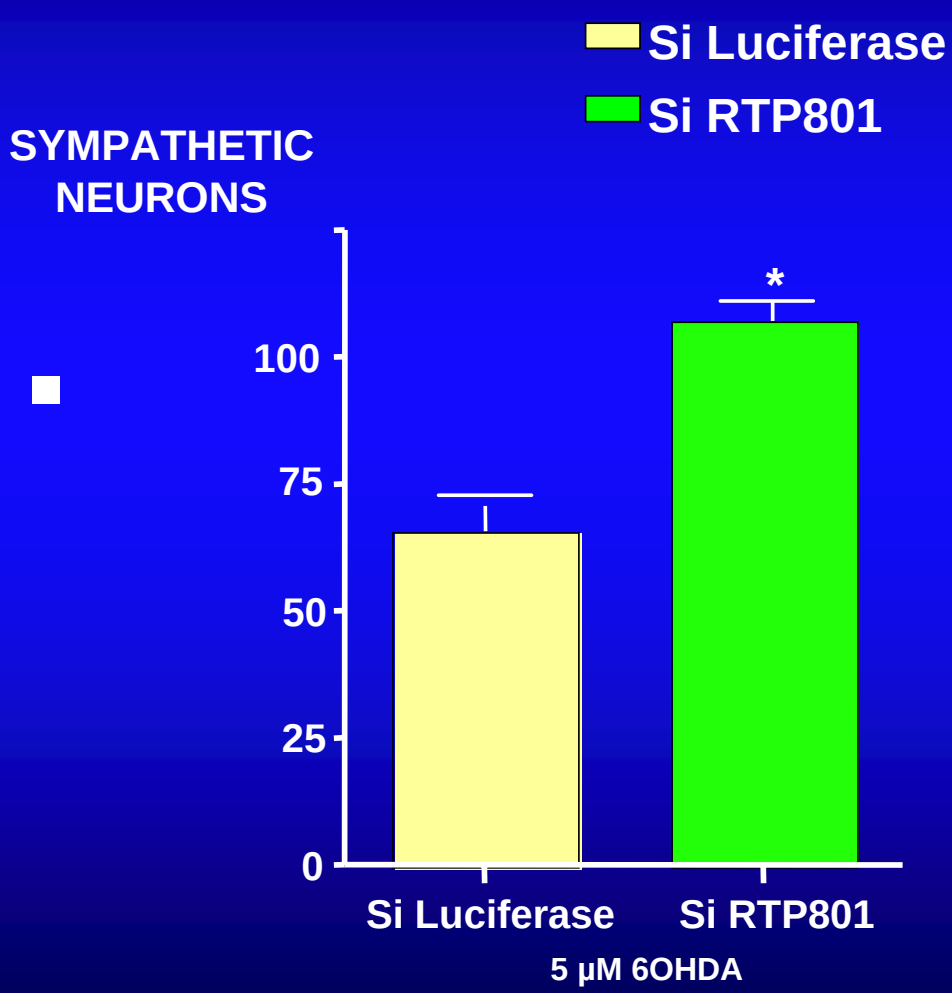
RTP801 OVEREXPRESSION KILLS NEURONAL PC12 CELLS



MALAGELADA ET AL UNPUBISHED

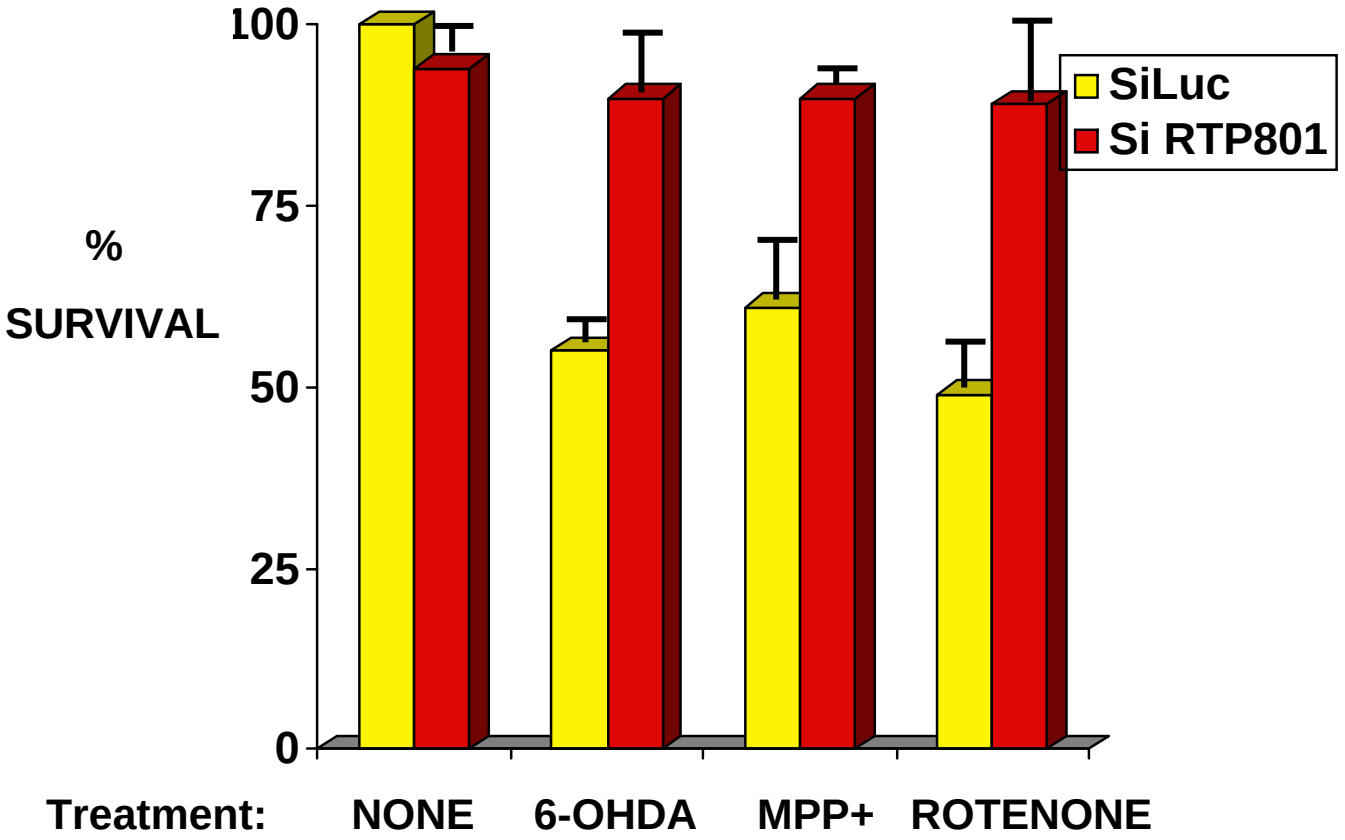
KNOCK DOWN OF RTP801 PROTECTS AGAINST 6-OHDA

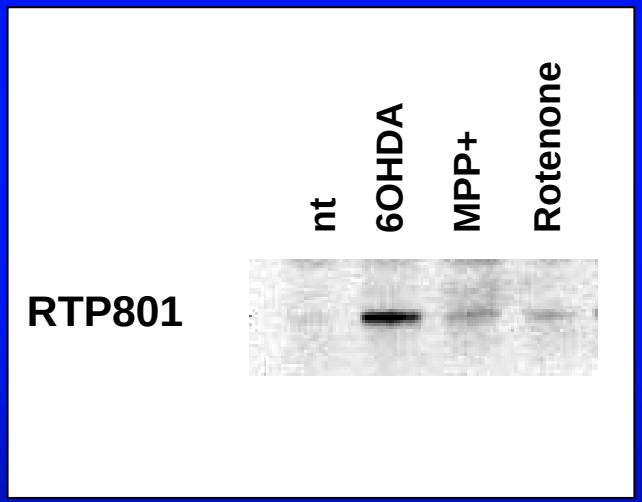




MALAGELADA ET AL UNPUBISHED

siRTP801 PROTECTS NEURONAL PC12 CELLS FROM MULTIPLE PD MIMETICS



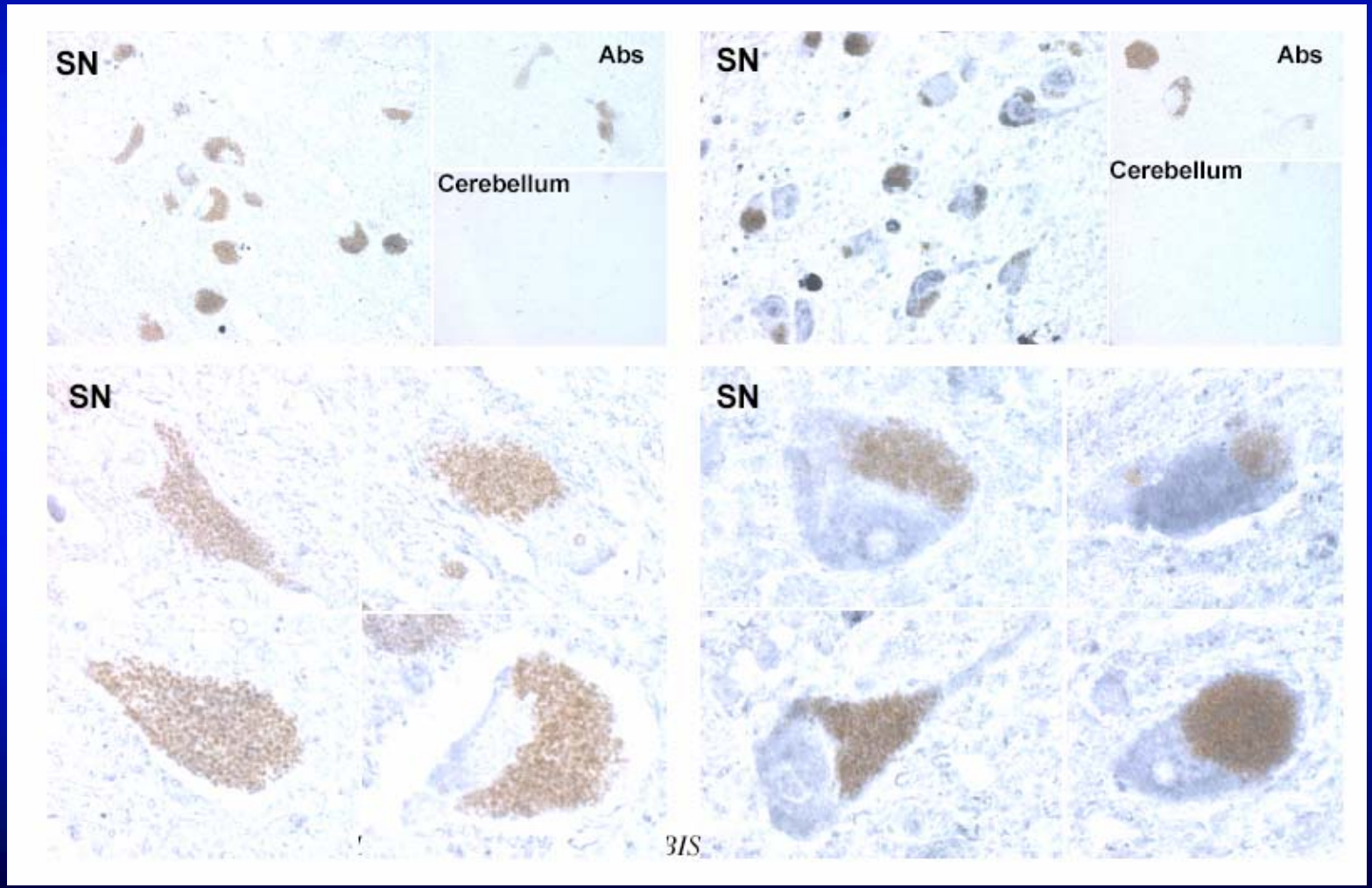


MALAGELADA ET AL UNPUBISHED

RTP801 EXPRESSION IS ELEVATED IN PD SN

Control

PD



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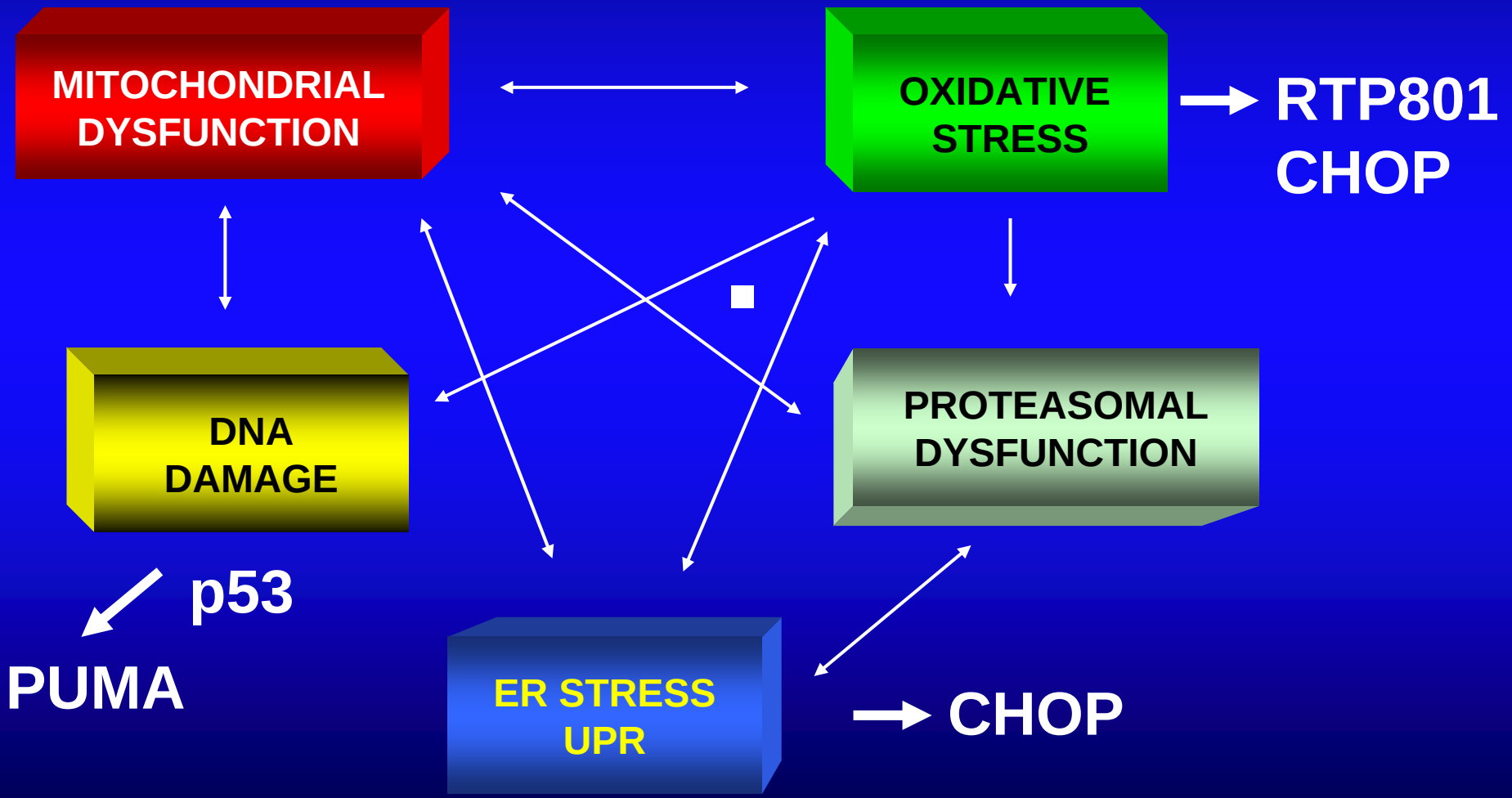
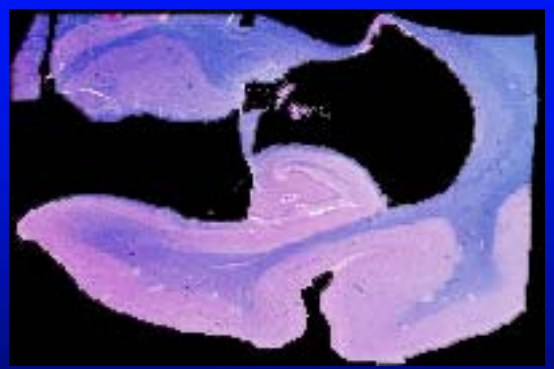
MALAGELADA ET AL UNPUBISHED

MULTIPLE SYSTEM FAILURES MAY UNDERLIE PD

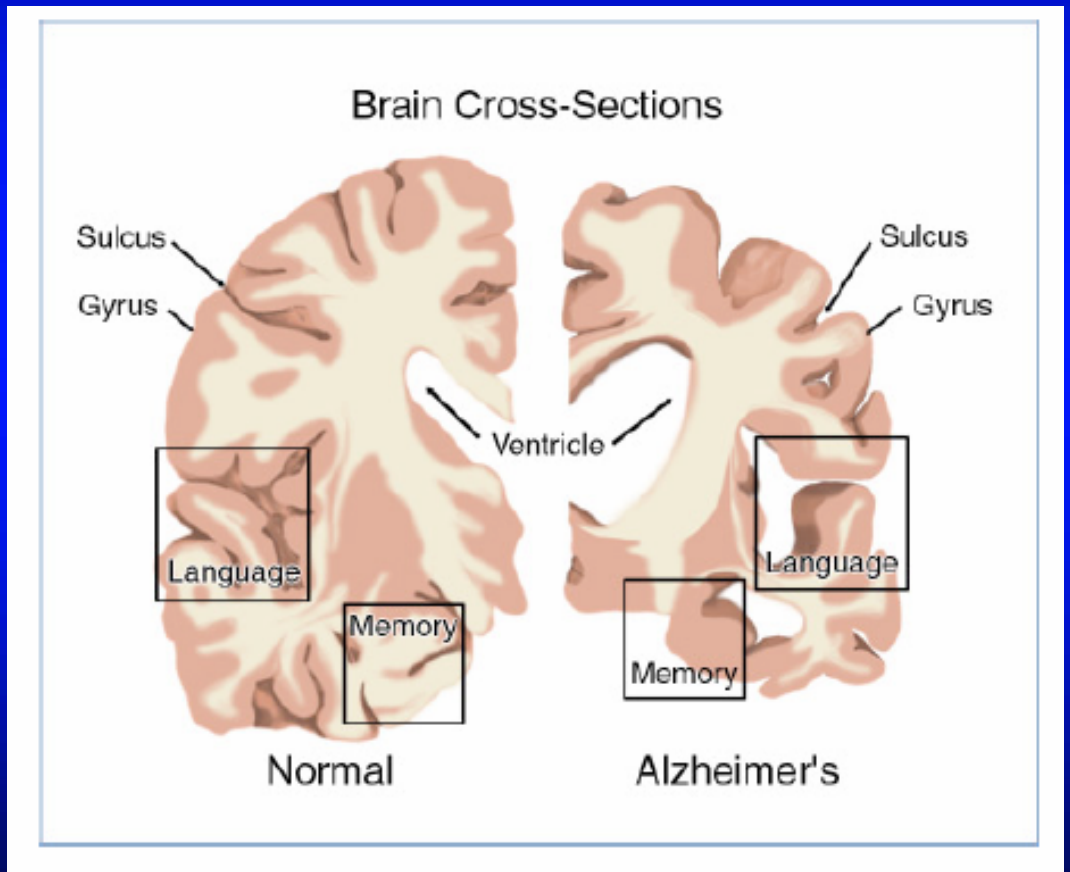


ALZHEIMER DISEASE

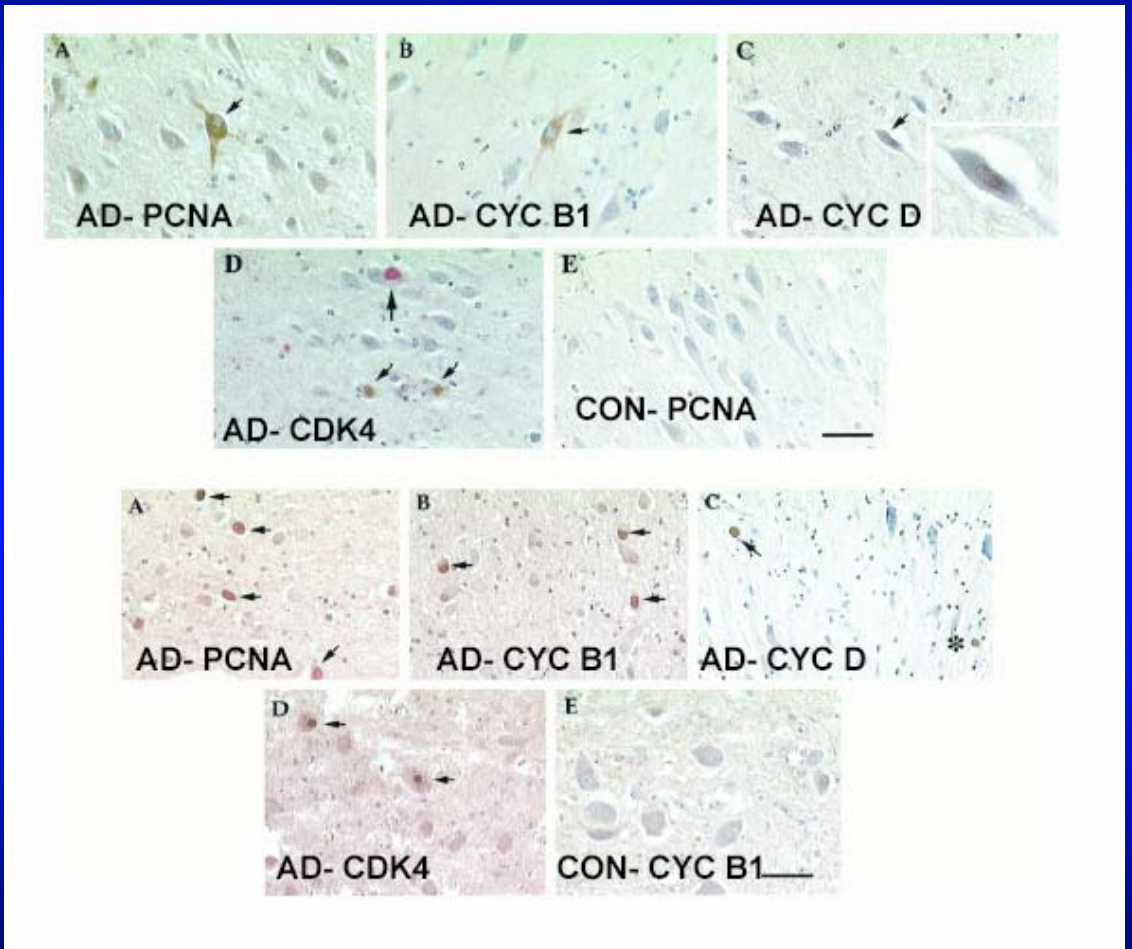
Control



Alzheimer disease

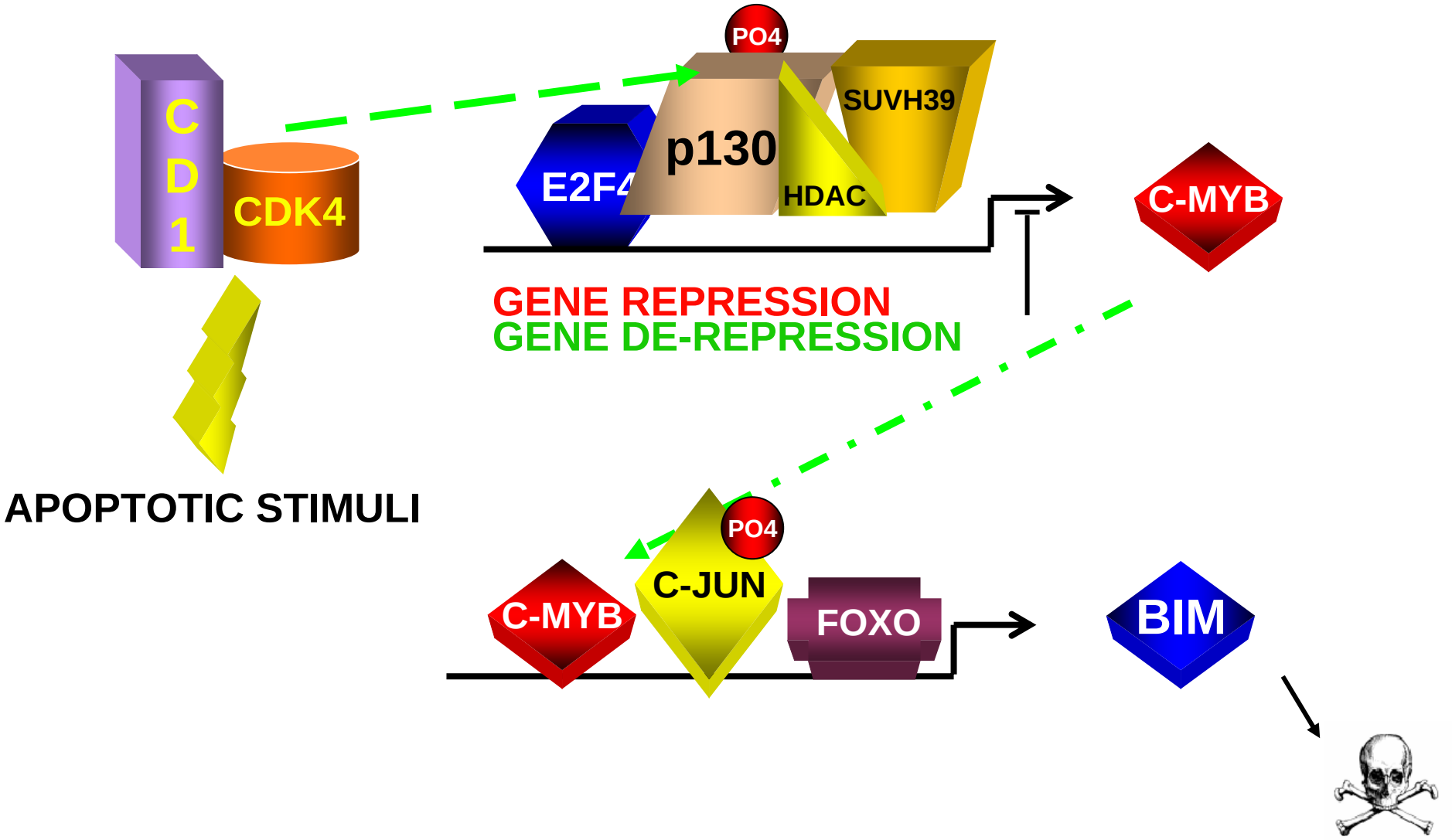


CELL CYCLE MOLECULES ARE UPREGULATED IN NEURONS AT RISK TO DIE IN ALZHEIMER DISEASE

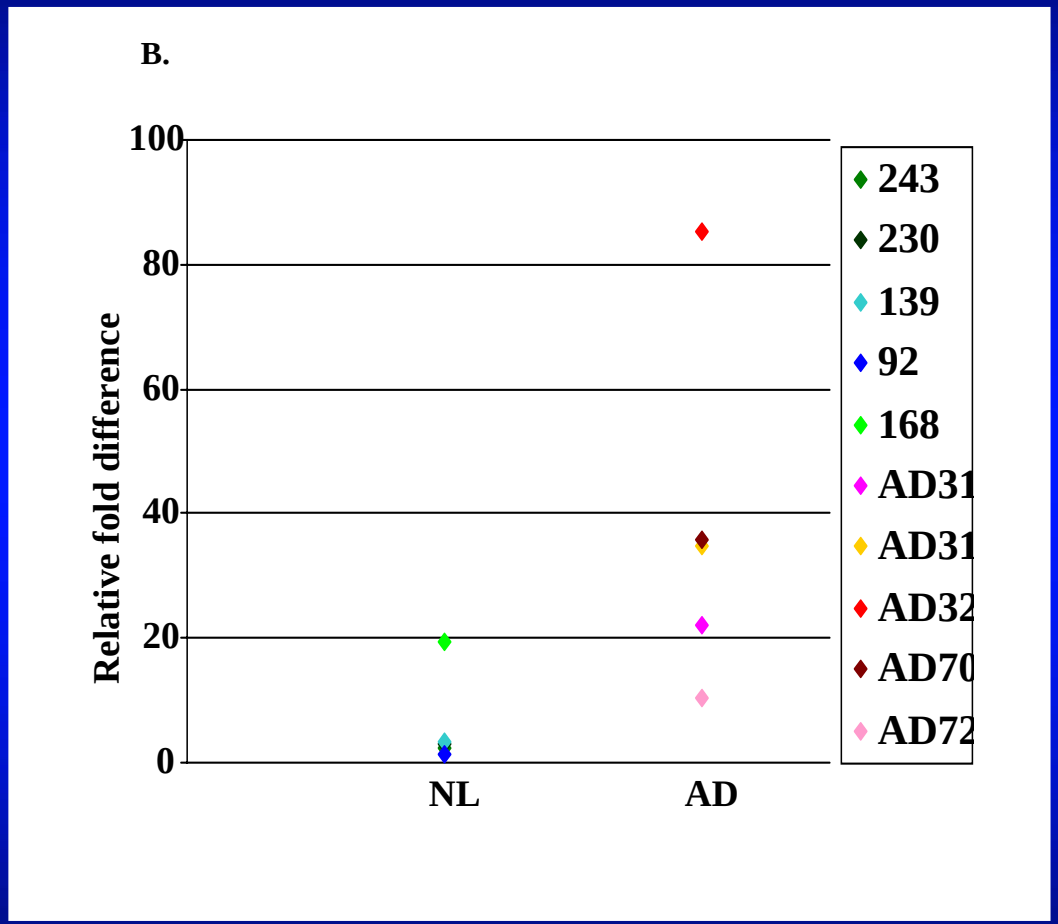


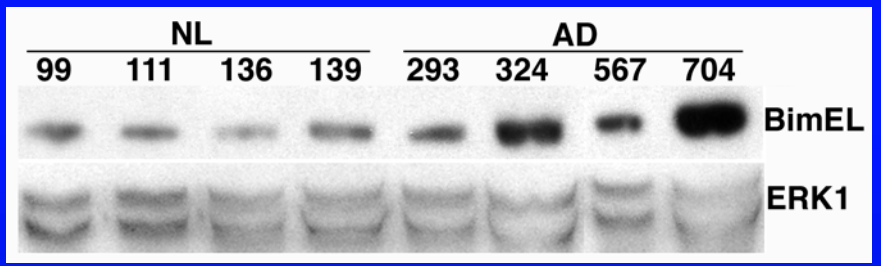
BUSSER ET AL J NEUROSCI 1998

E2F AND NEURON CELL DEATH



BIM IS UPREGULATED IN ALZHEIMER BRAIN





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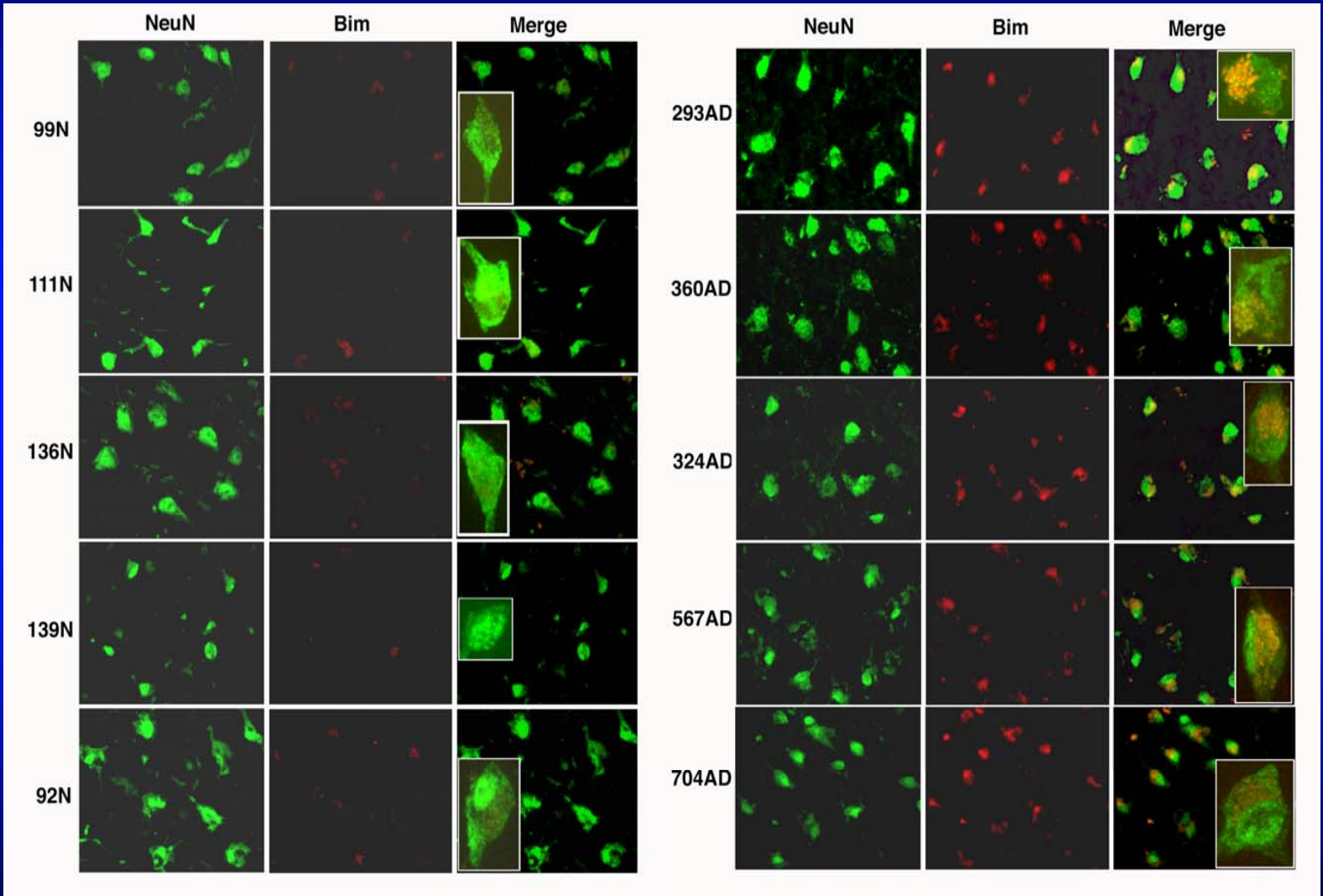
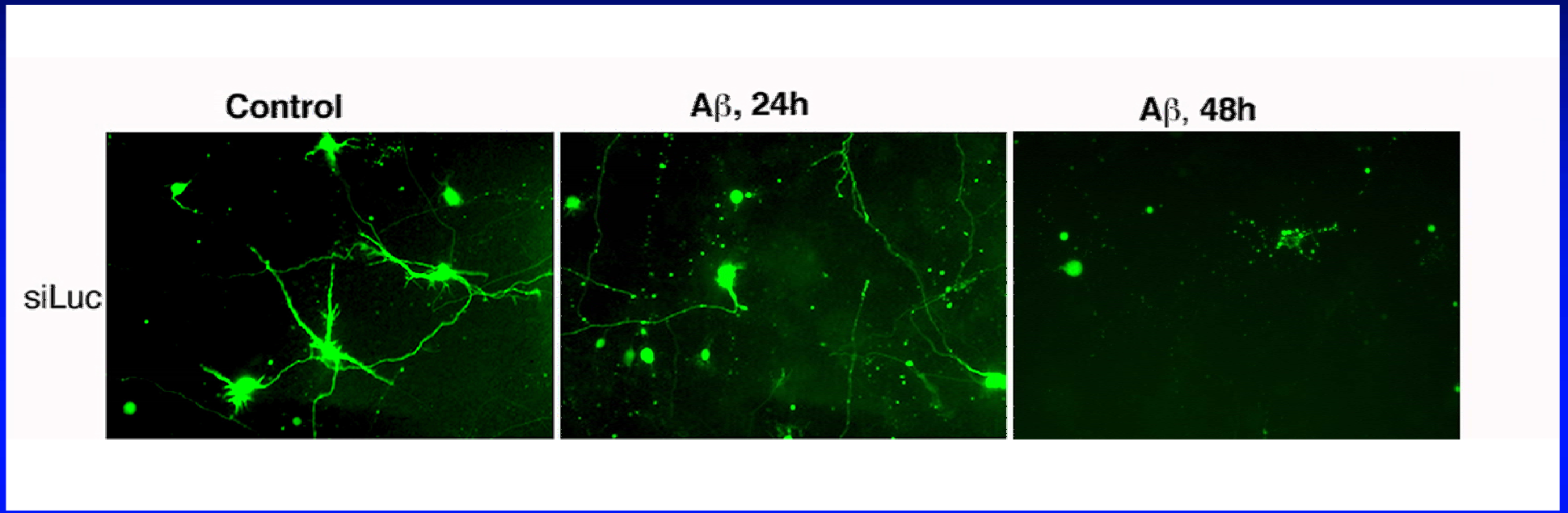
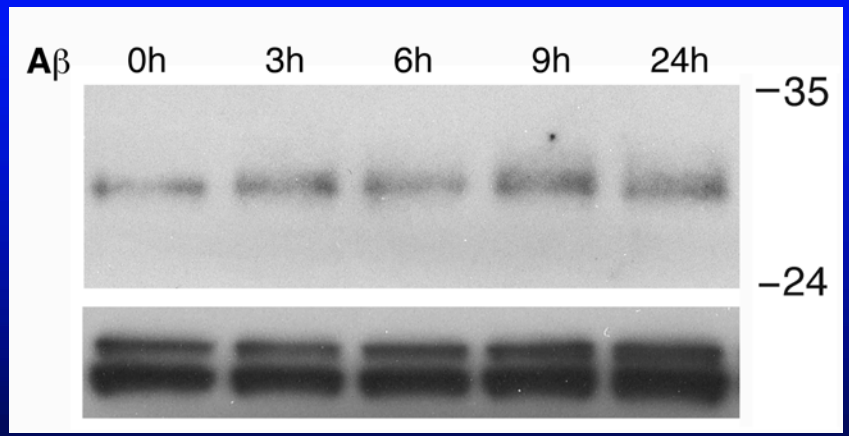
BIM IS UPREGULATED IN ALZHEIMER NEURONS



BISWAS ET AL UNPUBLISHED

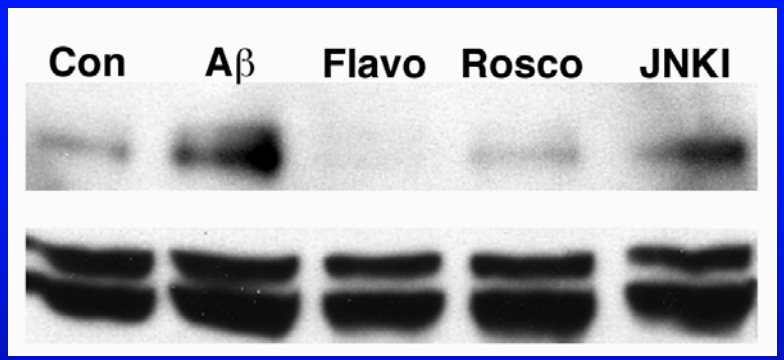
BIM IS UPREGULATED IN A CELLULAR MODEL OF AD



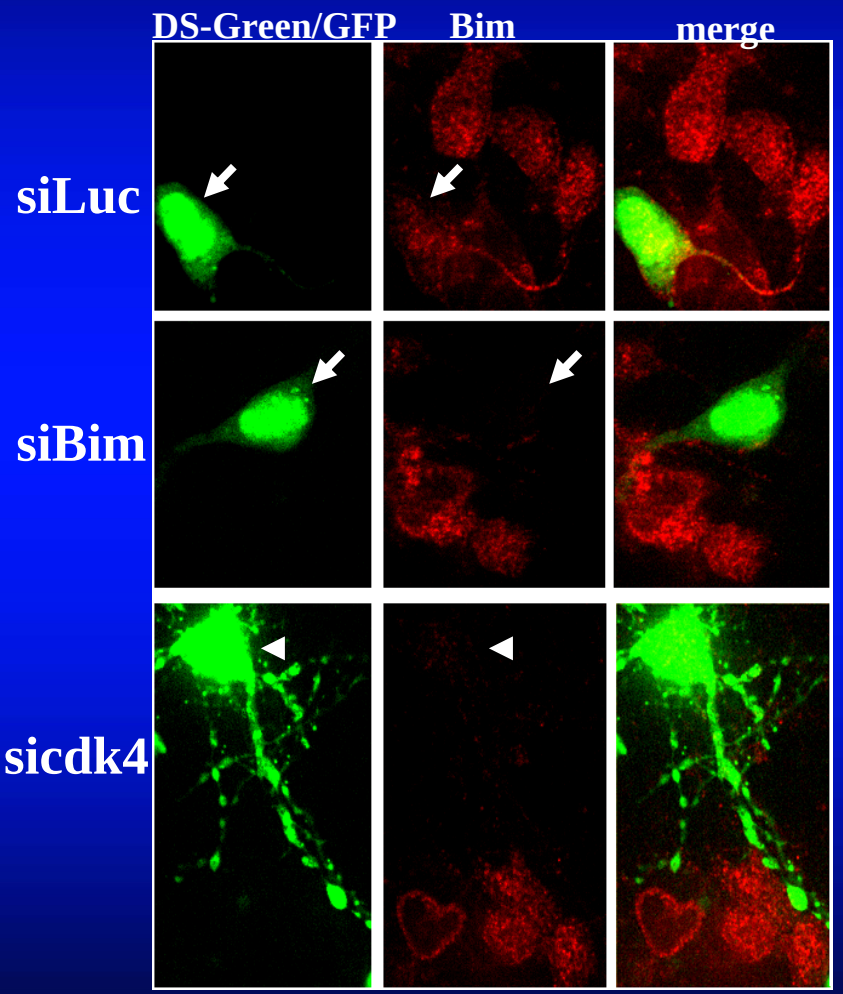


BISWAS ET AL UNPUBLISHED

CDK4 MEDIATES UP-REGULATION OF BIM IN NEURONS BY Aβ

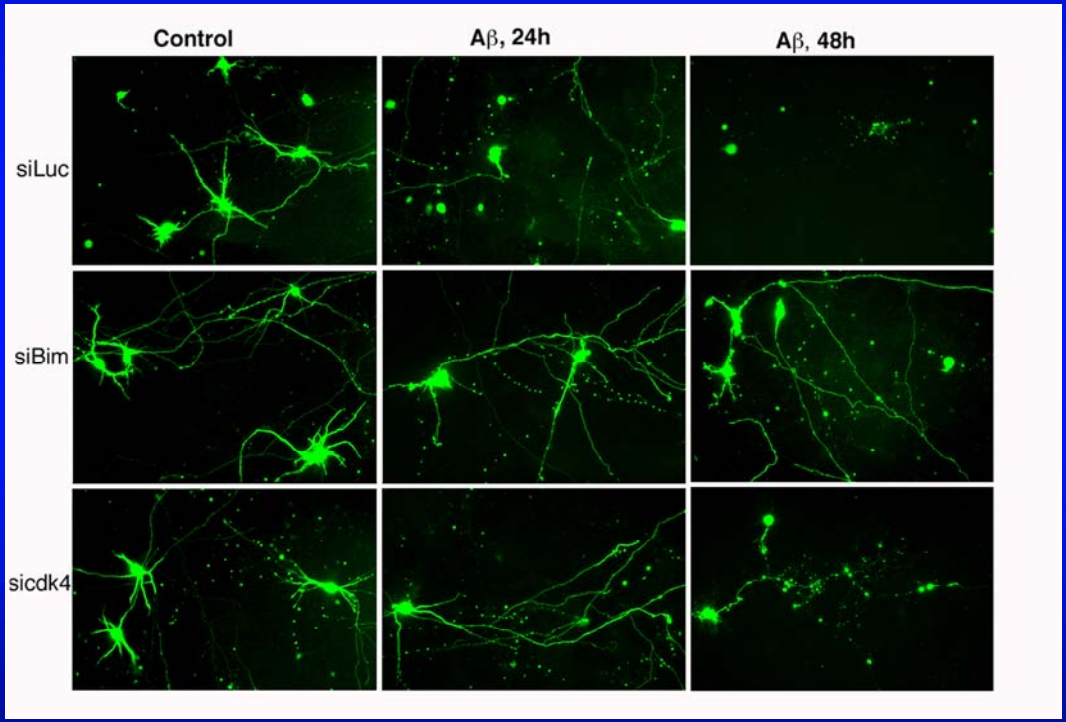


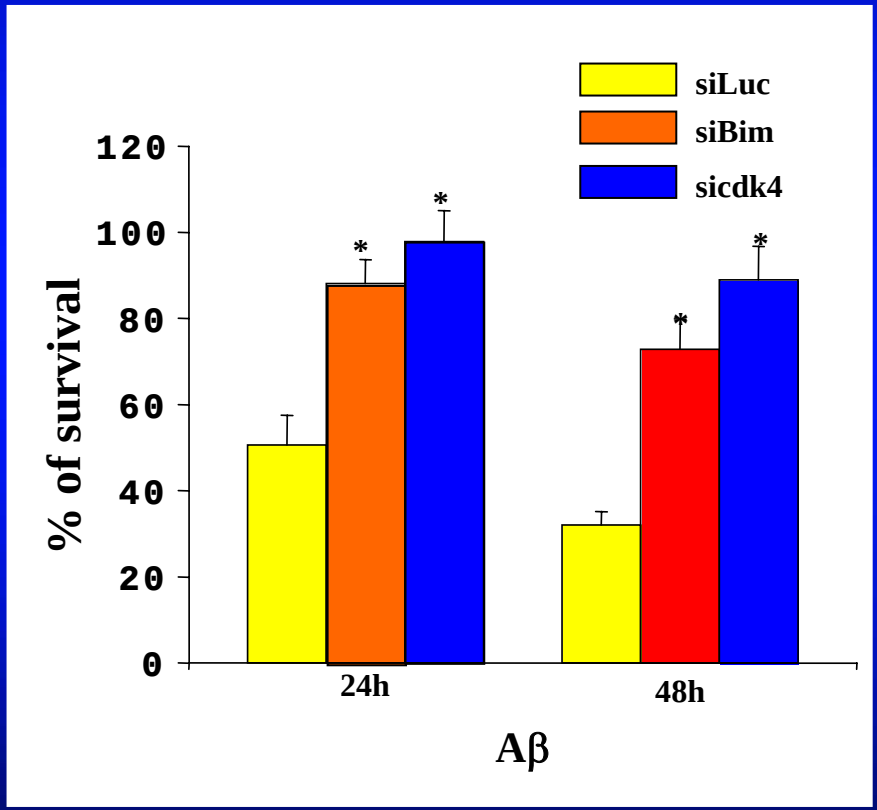
A. Cortical Neurons



BISWAS ET AL UNPUBLISHED

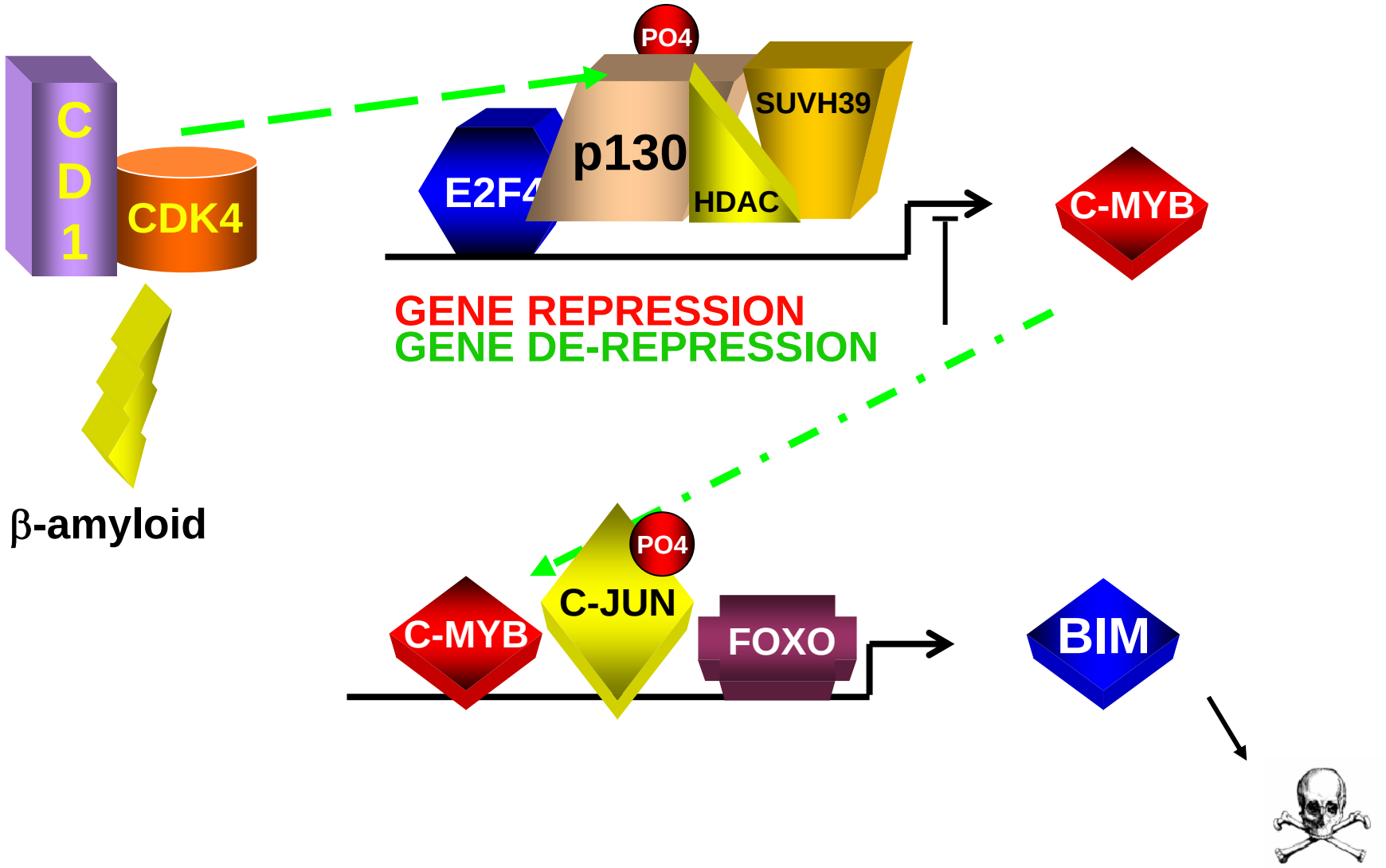
BIM AND CDK4 MEDIATE Aβ NEURON DEATH IN CULTURE





BISWAS ET AL UNPUBLISHED

E2F AND NEURON CELL DEATH



- NEURON DEATH IN DISEASE, AS IN DEVELOPMENTAL DEATH, IS OFTEN DEPENDENT ON TRANSCRIPTIONAL EVENTS

- SOME OF THE SAME PATHWAYS AND MOLECULES INVOLVED IN NORMAL DEVELOPMENTAL DEATH ARE INVOVLED IN DISEASE-RELATED DEATH

- ADDITIONAL TRANSCRIPTIONAL PATHWAYS AND TARGETS MAY BE ACTIVATED IN DISEASE-RELATED DEATH

- NEURON DEATH IN DISEASE MAY REFLECT THE INTEGRATION OF MULTIPLE PRO- AND ANTI-APOPTOTIC PATHWAYS

- KNOWLDEGE ABOUT THE MULTIPLE MOLECULES INVOLVED NEURON DEATH IN DISEASE PROVIDES POTENTIAL THERAPEUTIC TARGETS

Ευχαριστω!

Καλοτύχια!

Χαιρετε!