# *BDH2* is a poor novel independent maker in CN-AML

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# Cytogenetic normal AML

- approximately 40% to 50% of AML patients are considered to be cytogenetically normal
- molecular analysis of markers that have been incorporated into both the WHO and ELN classifications (such as *NPM1*, *FLT3*, and *CEBPA*) is now routine
- other mutated genes (such as *WT1*, *IDH1/IDH2*, *TET2*, *RUNX1*, and *MLL*) or aberrantly expressed genes (such as *BAALC*, *ERG*, *EVI1*, and *miR-181a*) are likely to be useful for defining molecular risk in CN-AML; Class I, Class II, Class III, etc

*Byrd, et al. Blood 2002；100:4325-4336.*
*Gaidzik, et al. J Clin Oncol 2011, 29:1364-1372.*
*Mithat Gönen, et al. NEJM2012, 366:1079-1089.*

# Iron utility and mammalian cells

- The acquisition of iron by cells is critical for survival, growth, and differentiation.

*Pantopoulos, et al. Biochemistry 2012; 51(29):5705-24*

- Mammalian cells and tissues contain low molecular weight siderophores that bind iron with high affinity.

*Fernandes-Pol et al. Cell 1978; 14(3): 489-99*

- Lipocalin24p3 can bind small molecular weight ligands; involved in multiple biological process including apoptosis, innate immunity and renal development

*Devireddy et al. Cell 2005; 123:1293-305; Li et al. Cytokine 2011; 56:435-41; Flo et al. Nature 2004; 432:917-21.*

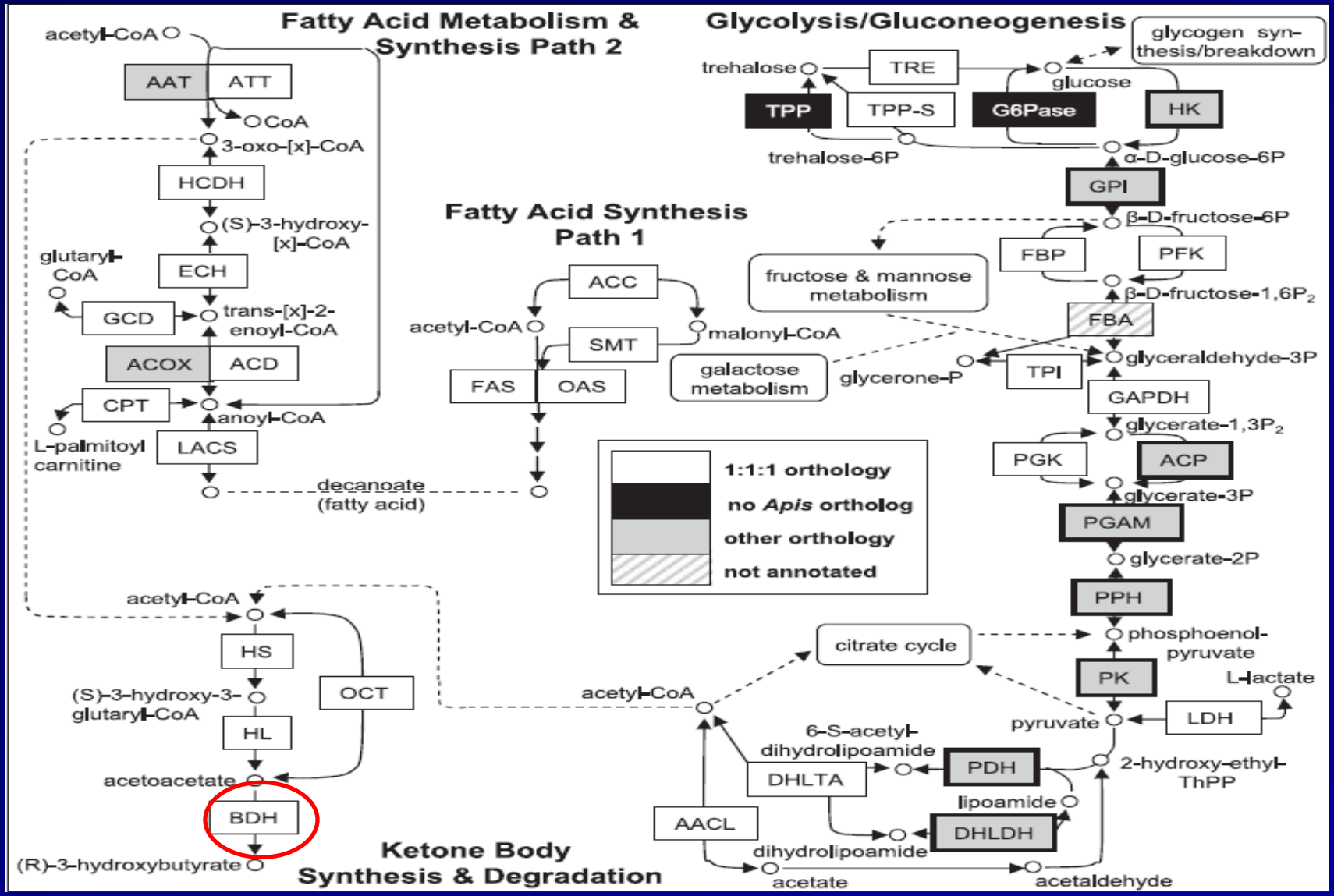
# *BDH2*

- Siderophores are best known small iron binding molecular that facilitate microbial iron transport.
- A member of short chain dehydrogenase family reductases, *BDH2*, catalyzes a rate-limiting step biogenesis of the mammalian siderophore.
- Mitochondrial metabolism
- Depletion of *BDH2* results in abnormal accumulation of cellular iron and mitochondrial iron deficiency.

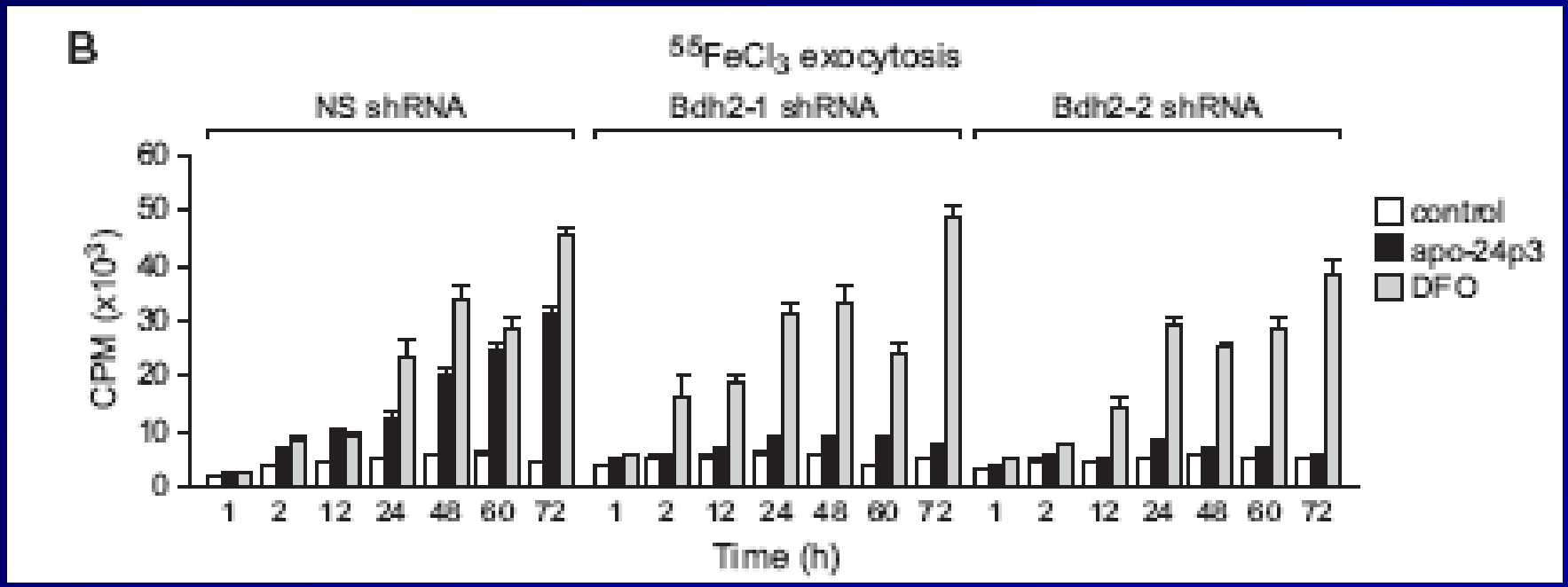
*Devireddy et al, Cell 2010; 141:1006-17*

*Liu et al , J mol Med 2012; 90:1209-21.*

# Metabolic pathway

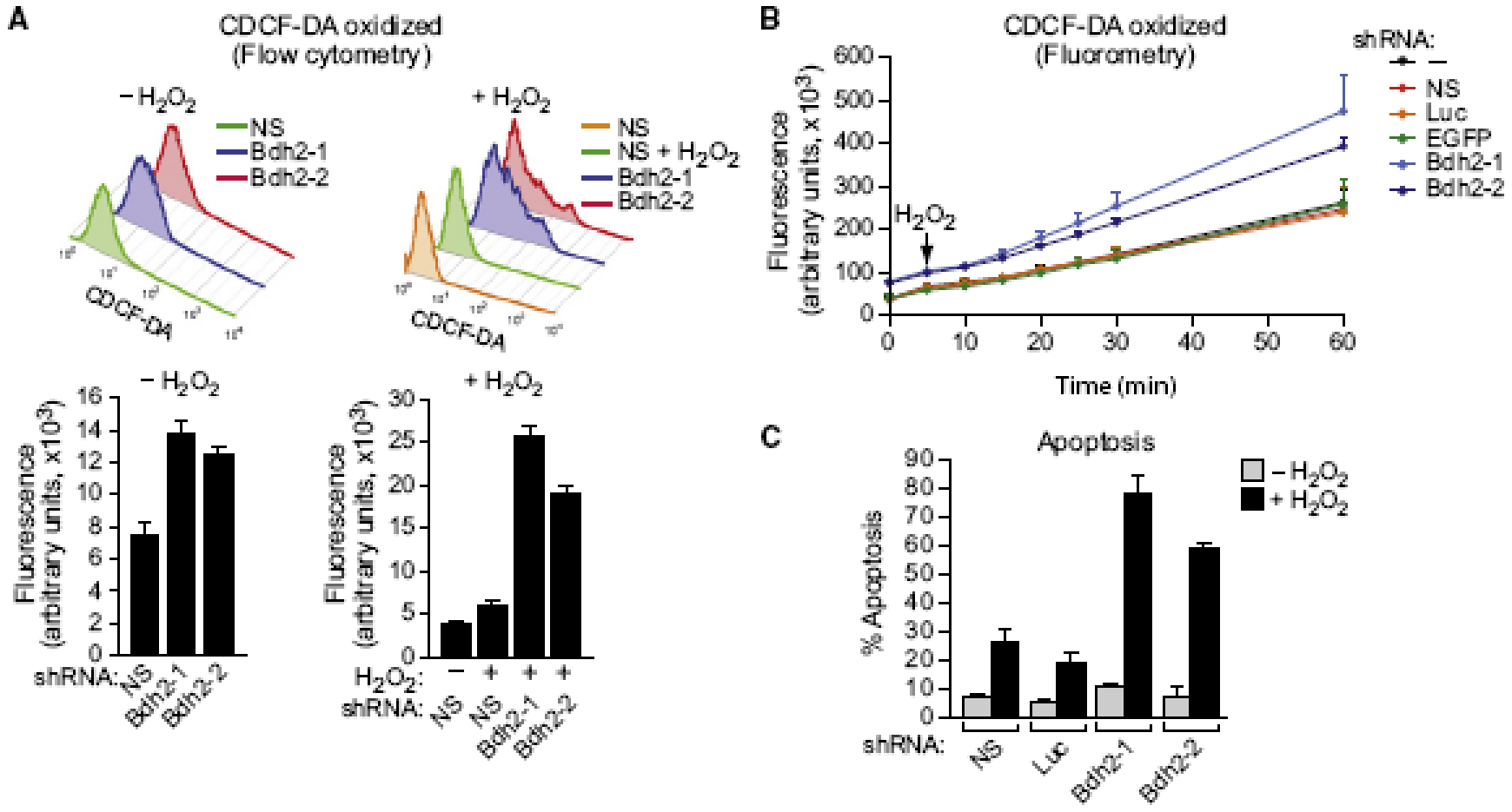


# BDH2 is required for 24p3-mediated iron transport




*Devireddy et al. Cell 2010; 141:1006-17*

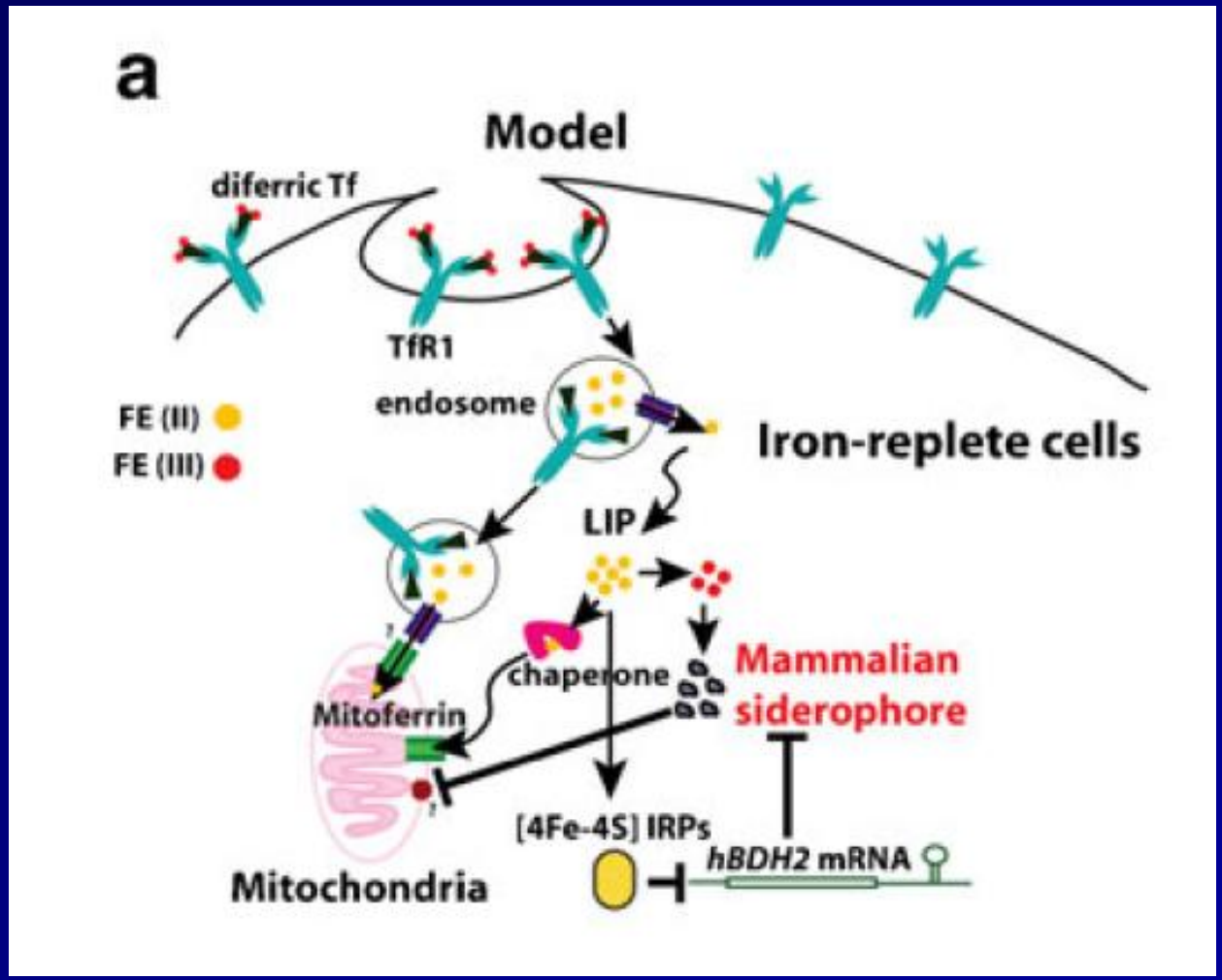
# Siderophore protects cells from oxidative stress




CDDHCF-DA: nonfluorescent probe; CDCF-DA: highly fluorescent

*Devireddy et al. Cell 2010; 141:1006-17*

# Iron regulation of siderophore controls mitochondrial iron



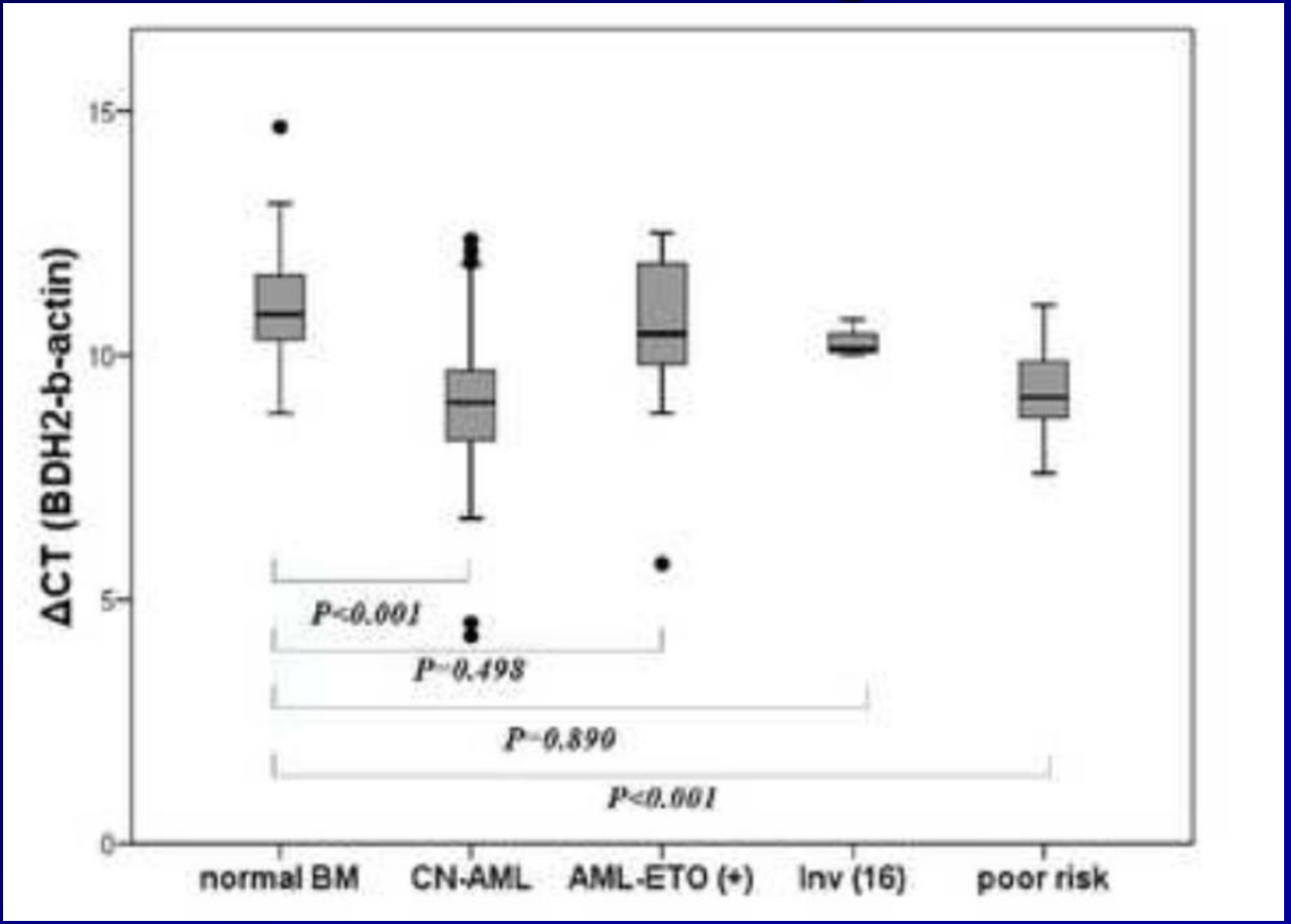

*Zhuomin Liu et al J Mol Med 2012;90:1209-21*

- If *BDH2* expression related to prognosis of CN-AML?
- If the presentation of genetic alternations, like *NPM1* mutation, *FLT3*-ITD, *FLT3*-TKD, *CEBPA*, *IDH1/2*, *and DNMT3A* would be difference in different *BDH2* expression group?

# Enrolled patients

- Enroll 113 newly diagnosed CN-AML patients; 2000.2 ~ 2012.2
- 86 patients received conventional induction C/T with I3A7 (followed by 70% I3A7 if not reach CR at 7th~14th days)
- Twenty two patients with secondary CR, one with first CR and five with refractory disease received allogenetic PBSCT
- 43 patient with normal bone marrow (most of them are lymphoma without bone marrow involvement); good risk (11 patients with AML-ETO (+); 3 patients with Inv (16)) and 25 poor risk patients with multiple chromosome abnormality

# *BDH2* mRNA expression




*Yang et al. J Biomedical Sci 2013; 20:58*

**Table 1. Comparison of clinical manifestations and laboratory features between patients with AML in low and high *BDH2* expression groups***

| Variables | Total (n = 113) | Low *BDH2* expression (n = 57) | High *BDH2* expression (n = 56) | *P* |
|---|---|---|---|---|
| Sex† | 113 | 57 | 56 | *0.467* |
| Male | 64 | 23 | 26 | |
| Female | 49 | 34 | 30 | |
| Age (years)‡ | 54.5 (21–86) | 48 (22–86) | 57 (21–85) | *0.193* |
| Laboratory data‡ | | | | |
| WBC, $\mu L^{-1}$ | 30,830 (300–296,300) | 37,615 (300–216,820) | 24,370 (600–243,290) | *0.452* |
| Hb, g/dL | 8.3 (4.2–15.6) | 8.3 (4.4–14.3) | 8.4 (4.2–15.6) | *0.365* |
| Platelet, ×1,000/μL | 42 (3–369) | 44.5 (4–175) | 41 (3–369) | *0.582* |
| Blast, $\mu L^{-1}$ | 20,188.5 (0–287,411) | 13,074.15 (126–145,572.7) | 26,001 (0–287,411) | *0.172* |
| Blast in BM, % | 74.8% (24.8%–97.4%) | 70.8% (24.8%–94.7%) | 79% (27.2%–94.4%) | *0.424* |
| CD34 (+) in BM, % | 31.1% (0%–99.9%) | 29.5% (0%–99.9%) | 33.25% (0%–99.5%) | *0.272* |
| FAB† | | | | |
| M0 | 3 | 1 (1.75%) | 2 (2.44%) | *0.057* |
| M1 | 28 | 8 (13.89%) | 20 (43.90%) | |
| M2 | 47 | 25 (47.23%) | 22 (34.15%) | |
| M3 | 0 | 0 (0%) | 0 (0%) | |
| M4 | 23 | 15 (23.61%) | 9 (14.63%) | |
| M5 | 7 | 6 (9.72%) | 1 (0%) | |
| M6 | 0 | 0 (0%) | 0 (0%) | |
| M7 | 4 | 2 (2.78%) | 2 (4.88%) | |
| Undetermined | 0 | 0 (0%) | 0 (0%) | |
| Induction response†§ | | | | |
| CR | 61 (70.93%) | 37 (80.42%)¶ | 24 (60%)# | *0.032*** |
| PR and Refractory | | 4 | 20 | |
| Induction death | | 1 | 0 | |
| Reach CR time‖ | | | | |
| | 38 ± 38.035 | 40.5 ± 44.3 | 36 ± 29.744 | *0.526* |

* The median value of *BDH2* expression in the total population was used as the cutoff level of 9.006 to define low- and high-expression groups.

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# Difference of genetic alterations

**Table 2** Comparison of other genetic alterations between AML patients in low and high *BDH2* expression groups

| Variant | Number of patients with the gene mutation (percentage) | | | *P* |
|---|---|---|---|---|
| | Whole cohort | Low *BDH2* expression | High *BDH2* expression | |
| *NPM1*$^{mut}$ | 36 (31.85%) | 16 (28.07%) | 20 (35.71%) | *0.271* |
| *FLT3*-ITD | 23 (20.35%) | 7 (12.28%) | 16 (28.57%) | *0.030** |
| *FLT3*-TKD | 8 (7.08%) | 5 (8.77%) | 3 (5.36%) | *0.338* |
| *NPM1*$^{mut}$/*FLT3*-ITD$^{neg}$ | 20 (17.70%) | 11(19.30%) | 9 (16.07%) | *0.368* |
| *CEBPA*[a] | 34 (30.09%) | 16 (28.07%) | 18 (32.14%) | *0.444* |
| *CEBPA*$^{Double\ mutation}$ | 9 (7.96%) | 3 (5.26%) | 6 (10.71%) | *0.593* |
| *IDH1*[b] | 3 (3.75 %) | 1 (2.56 %) | 2 (4.88%) | *0.592* |
| *IDH2*[b] | 8 (10 %) | 3 (7.69%) | 5 (12.20%) | *0.508* |
| *DNMT3A*[b] | 12 (15 %) | 10 (25.641%) | 2 (4.88%) | *0.009** |

Values are number (%) of patients with alteration.

[a] *CEBPA* single and double mutations.

[b] only 80 patients with high quality of DNA to sequence; 39 patients are low BDH2 expression and 41 patients are high expression.

* Statistically significant ($P < 0.05$).

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**Table 3** Univariate analyses of the impact on overall survival in patients with CN-AML who received intensive induction chemotherapy

| Variable | No. of patients | Overall survival | |
|---|---|---|---|
| | | Median[a] | *P* |
| Age, years | | | *0.001** |
| 60 or younger | 66 | 32.2 | |
| Older than 60 | 22 | 4.5 | |
| WBC | | | *0.469* |
| 50000/iL or less | 49 | 31.433 | |
| Greater than 50000/iL | 37 | 13 | |
| *BDH2* | | | *0.007** |
| Lower expression | 46 | 53.667 | |
| Higher expression | 40 | 9 | |
| *CEBPA* | | | *0.755* |
| Double mutation | 9 | 31.433 | |
| Single mutation | 16 | 53.667 | |
| No mutation | 61 | 16.267 | |
| *NPM1* | | | *0.179* |
| Wild | 28 | 10.4 | |
| mutated | 58 | 32.2 | |
| *FLT3*-ITD$^-$ | | | *<0.001** |
| Mutated | 17 | 4.767 | |
| Wild | 70 | 48.633 | |
| *FLT3*-TKD | | | *0.596* |
| Mutated | 7 | 13.00 | |
| Wild | 79 | 18.567 | |
| *NPM1*/*FLT3*-ITD | | | *0.314* |
| *NPM1*$^+$/*FLT3*-ITD$^-$ | 15 | NR | |
| Others | 71 | 15.067 | |

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| | | | |
|---|---|---|---|
| *IDH1*[b] | | | *0.796* |
| Mutated | 3 | 22.21 | |
| Wild | 61 | 27.055 | |
| *IDH2*[b] | | | *0.749* |
| Mutated | 4 | 21.91 | |
| Wild | 60 | 27.16 | |
| *DNMT3A*[b] | | | *0.508* |
| Mutated | 11 | 32.58 | |
| Wild | 53 | 25.63 | |
| $BDH2^{low}$/*FLT3-ITD* | | | *<0.001** |
| $BDH2^{low}$/$FLT3\text{-}ITD^{wild\ type}$ | 40 | 722.16 | |
| $BDH2^{low}$/$FLT3\text{-}ITD^{mutation}$ | 5 | 519.73 | |
| $BDH2^{high}$/$FLT3\text{-}ITD^{wild\ type}$ | 27 | 371.56 | |
| $BDH2^{high}$/$FLT3\text{-}ITD^{mutation}$ | 13 | 182.27 | |

Abbreviation: NR indicates not reached.

[a] Median, months.

[b] only 64 patients who had high quality DNA preserved for sequence received standard intensive induction chemotherapy

* Statistically significant ($P < 0.05$)

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**Table 4.** Multivariate Analyses (Cox regression) of Relapse-free Survival and Overall Survival[a]

| Variables | Relapse-free Survival | | | | Overall Survival | | | |
|---|---|---|---|---|---|---|---|---|
| | | 95% CI | | | | 95% CI | | |
| | HR | Lower | Upper | *P* | HR | Lower | Upper | *P* |
| Whole cohort (n = 86) | | | | | | | | |
| Age[b] | 4.322 | 1.771 | 10.550 | *0.001** | 6.706 | 2.248 | 20.002 | *0.001** |
| WBC[c] | 1.748 | 0.633 | 4.825 | *0.281* | 1.105 | 0.445 | 2.747 | *0.829* |
| *NPM1*[d] | 0.836 | 0.291 | 2.405 | *0.740* | 0.396 | 0.123 | 1.271 | *0.119* |
| *FLT3*-ITD[e] | 1.854 | 0.550 | 6.250 | *0.319* | 8.980 | 2.613 | 30.864 | *<0.001** |
| *FLT3*-TKD | 0.584 | 0.138 | 2.475 | *0.466* | 0.721 | 0.133 | 3.920 | *0.705* |
| *CEBPA*[f] | 0.562 | 0.153 | 1.566 | *0.386* | 0.800 | 0.271 | 2.364 | *0.687* |
| *IDH1*[d] | 0 | 0 | | *0.985* | 0.849 | 0.203 | 3.554 | *0.823* |
| *IDH2*[d] | 0.755 | 0.093 | 6.147 | *0.793* | 2.259 | 0.423 | 12.072 | *0.341* |
| *DNMT3A*[d] | 0.357 | 0.065 | 1.974 | *0.238* | 1.200 | 0.346 | 4.163 | *0.774* |
| *BDH2*[g] | 1.565 | 0.662 | 3.703 | *0.308* | 2.217 | 1.094 | 4.494 | *0.027** |

Abbreviations: HR indicates Hazard ratio; CI, confidence interval.

[a]Only patients with intensive induction chemotherapy enrolled

[b] Age > 60 relative to Age ≤ 60 (the reference).

[c] WBC > 50,000/μL versus < 50,000/μL.

[d] $NPM1^{mut}$ versus $NPM1^{wild\ type}$; $IDH1^{mut}$ versus $IDH1^{wild\ type}$; $IDH2^{mut}$ versus $IDH2^{wild\ type}$; $DNMT3A^{mut}$ versus $DNMT3A^{wild}$ type

[e] $FLT3\text{-ITD}^{mut}$ versus $FLT3\text{-ITD}^{neg}$

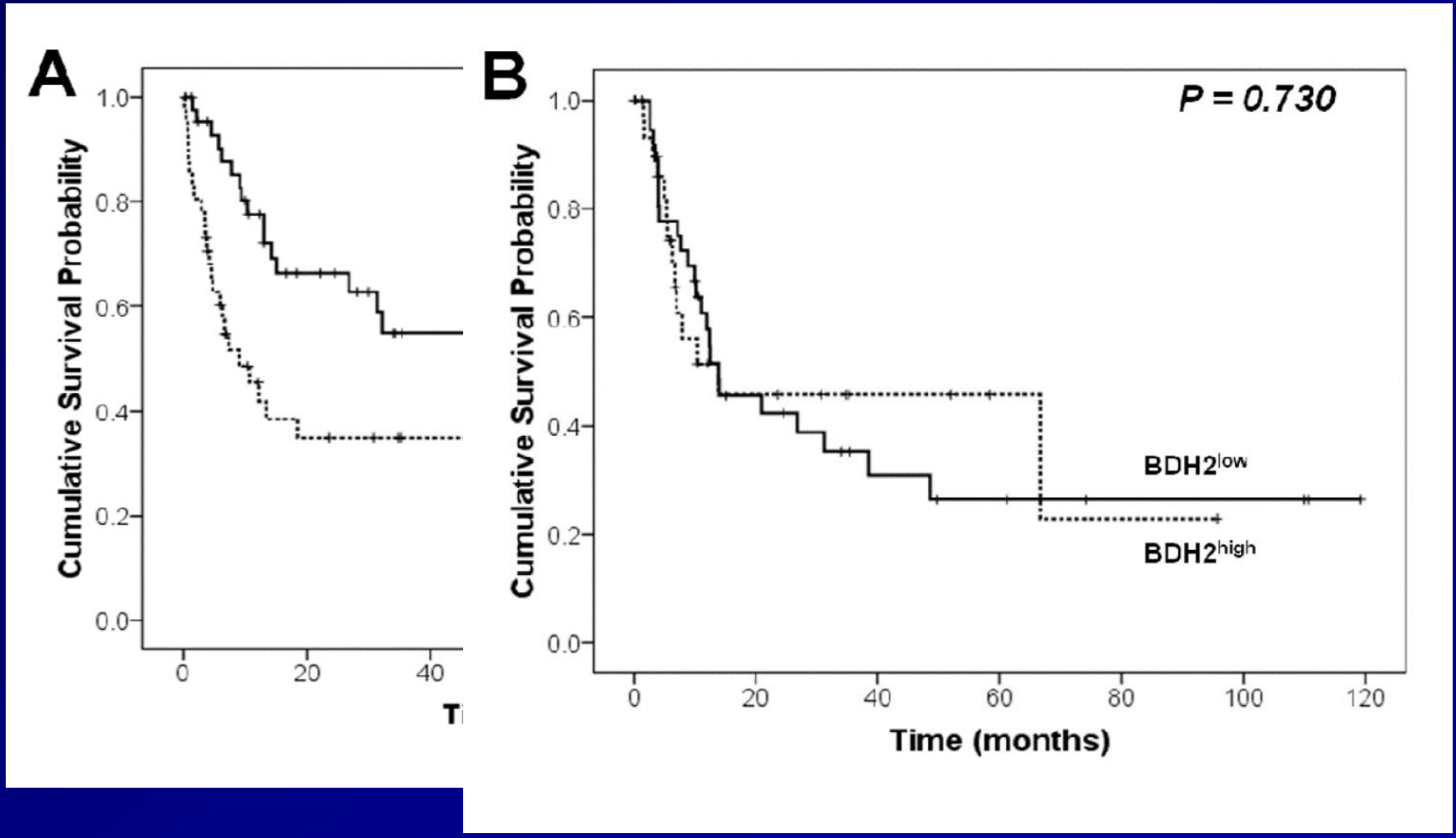
[f] $CEBPA^{double\text{-}mutation}$ versus $CEBPA^{single\ mutation}$ versus $CEBPA^{no\ mutation}$.

[g] $BDH2^{high}$ relative to $BDH2^{low}$ group.

* Statistically significant (P < 0.05).

# Survival between *BDH2* expression

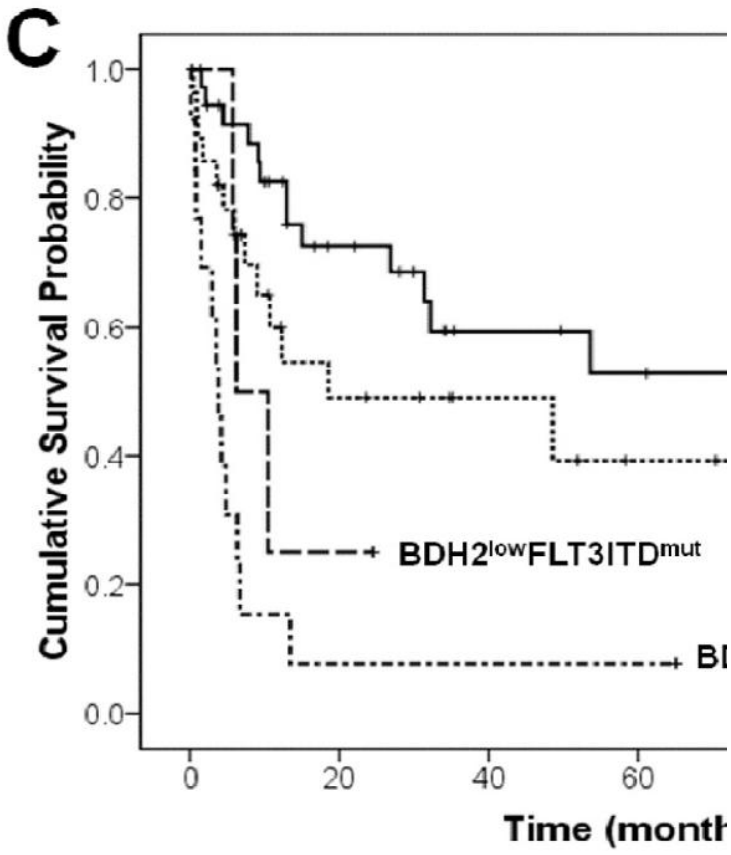
## Overall Survival Progression Free Survival

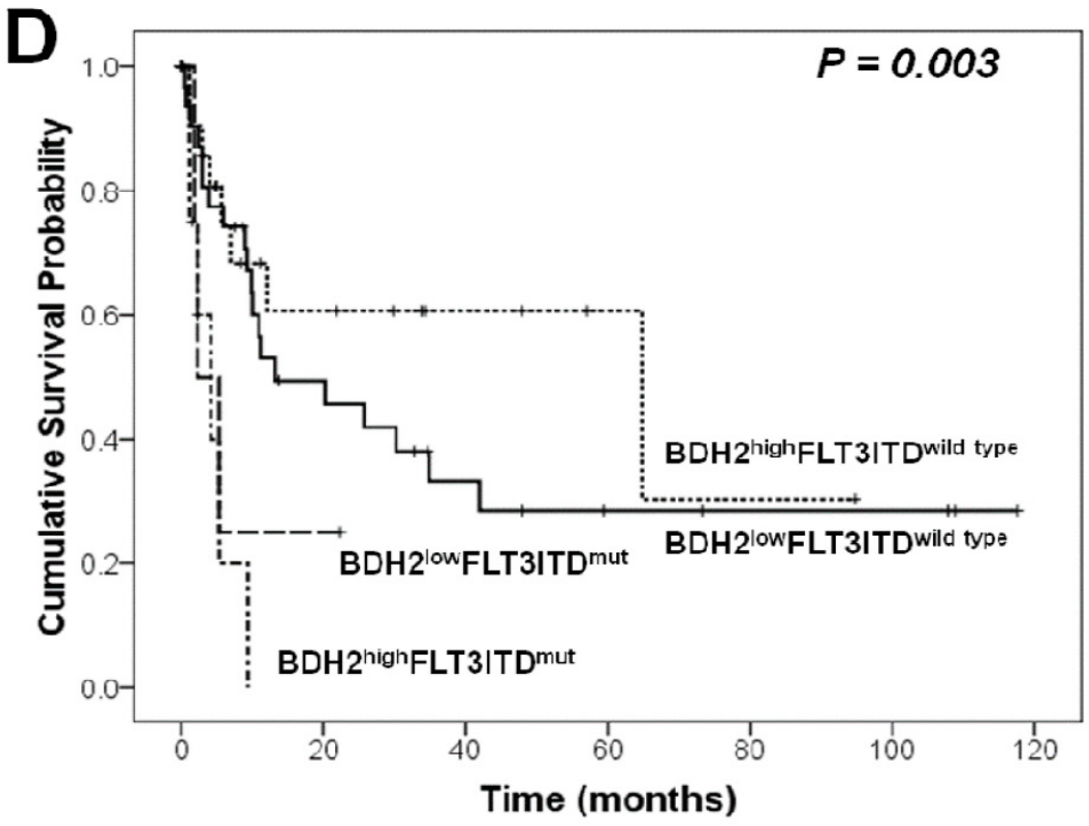



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# Survival between 4 groups

## Overall Survival  Progression Free Survival

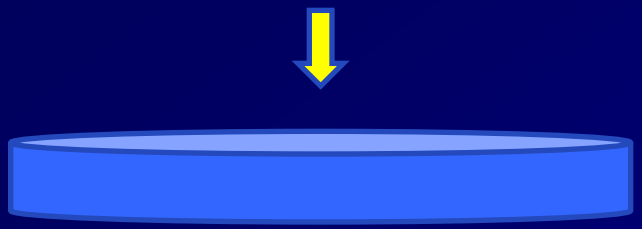


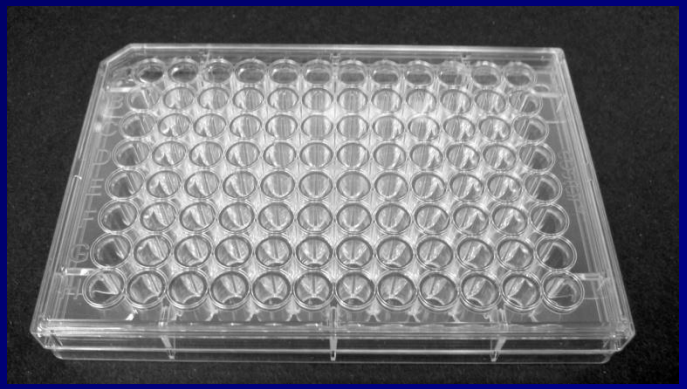
# How does it happen?

- Increase of *BDH2* expression may cause leukemia cells resistant to stress, like ROS and chemotherapy, induced death, that is related to poor response rate.

shRNA-BDH2
(retrovirus)

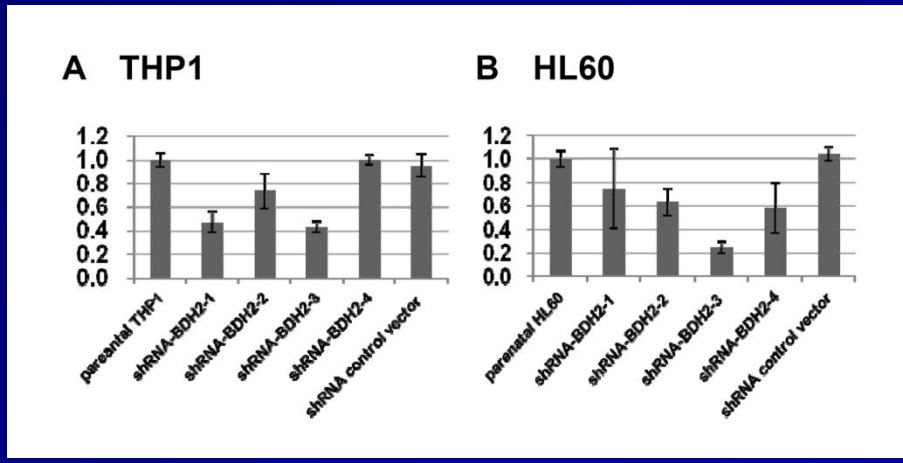


THP1
HL60

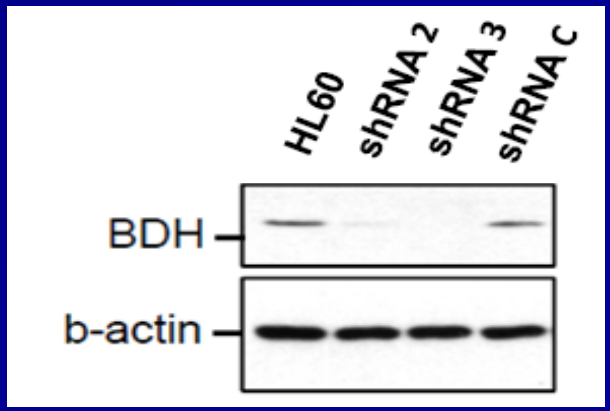


Selected by
puromycin

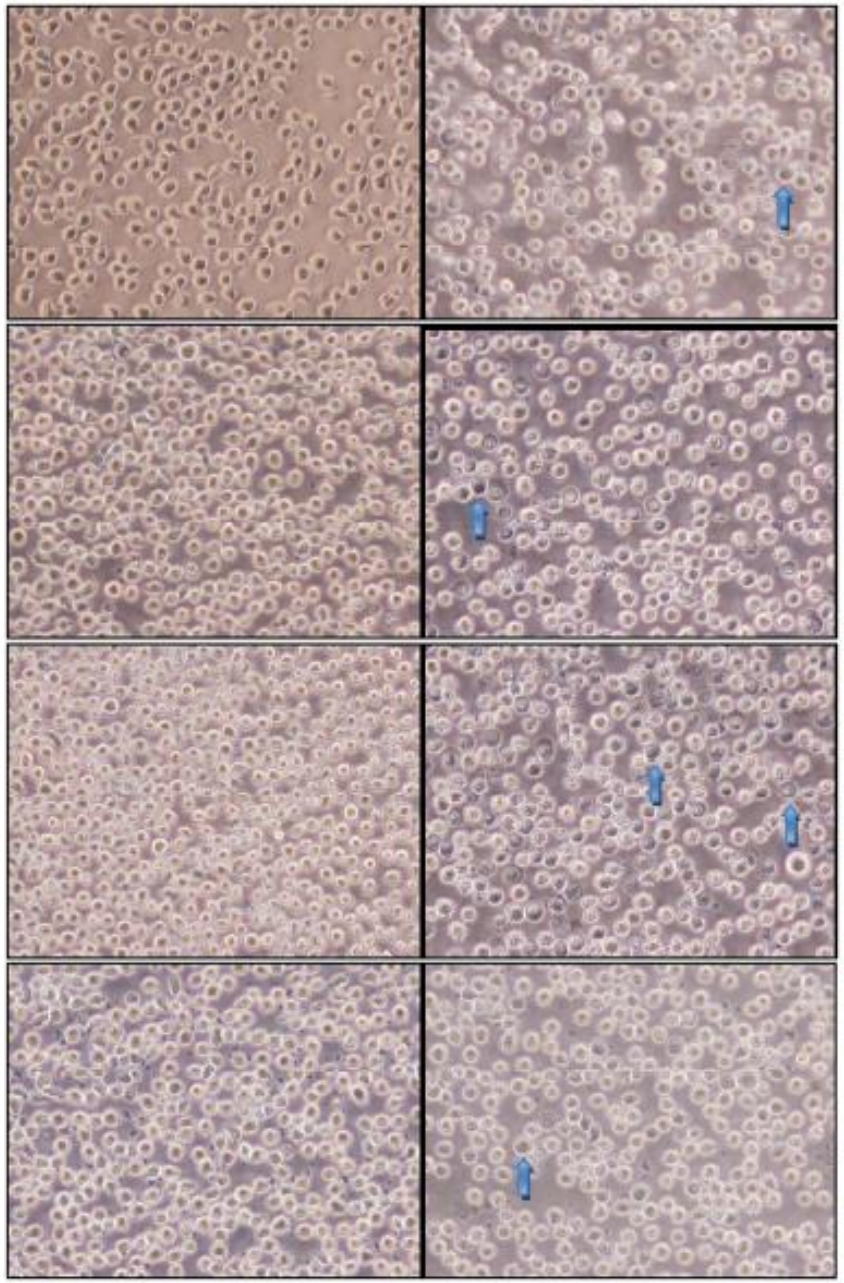
BDH2 mRNA




Western Blot

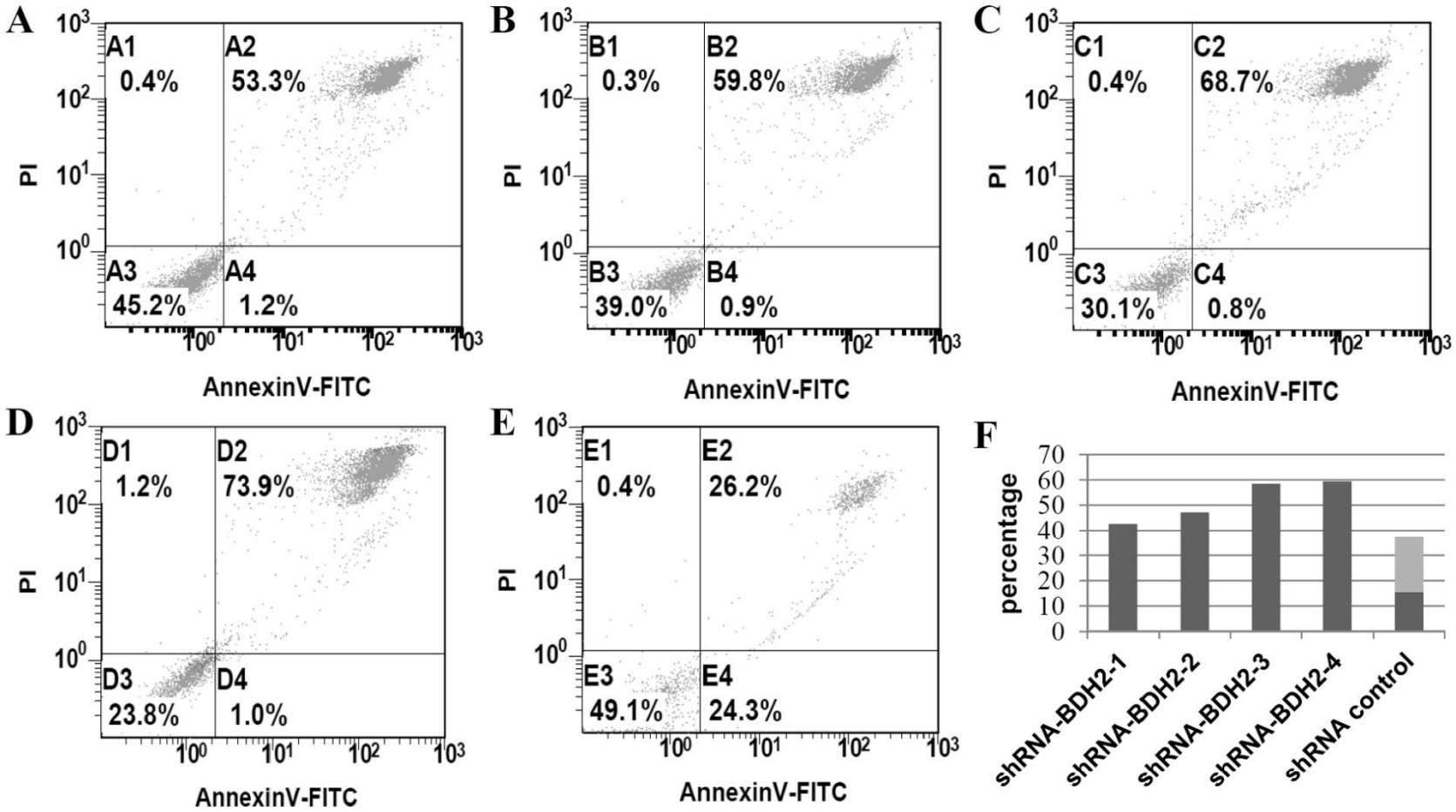


$H_2O_2$ 50uM
2hours
200x

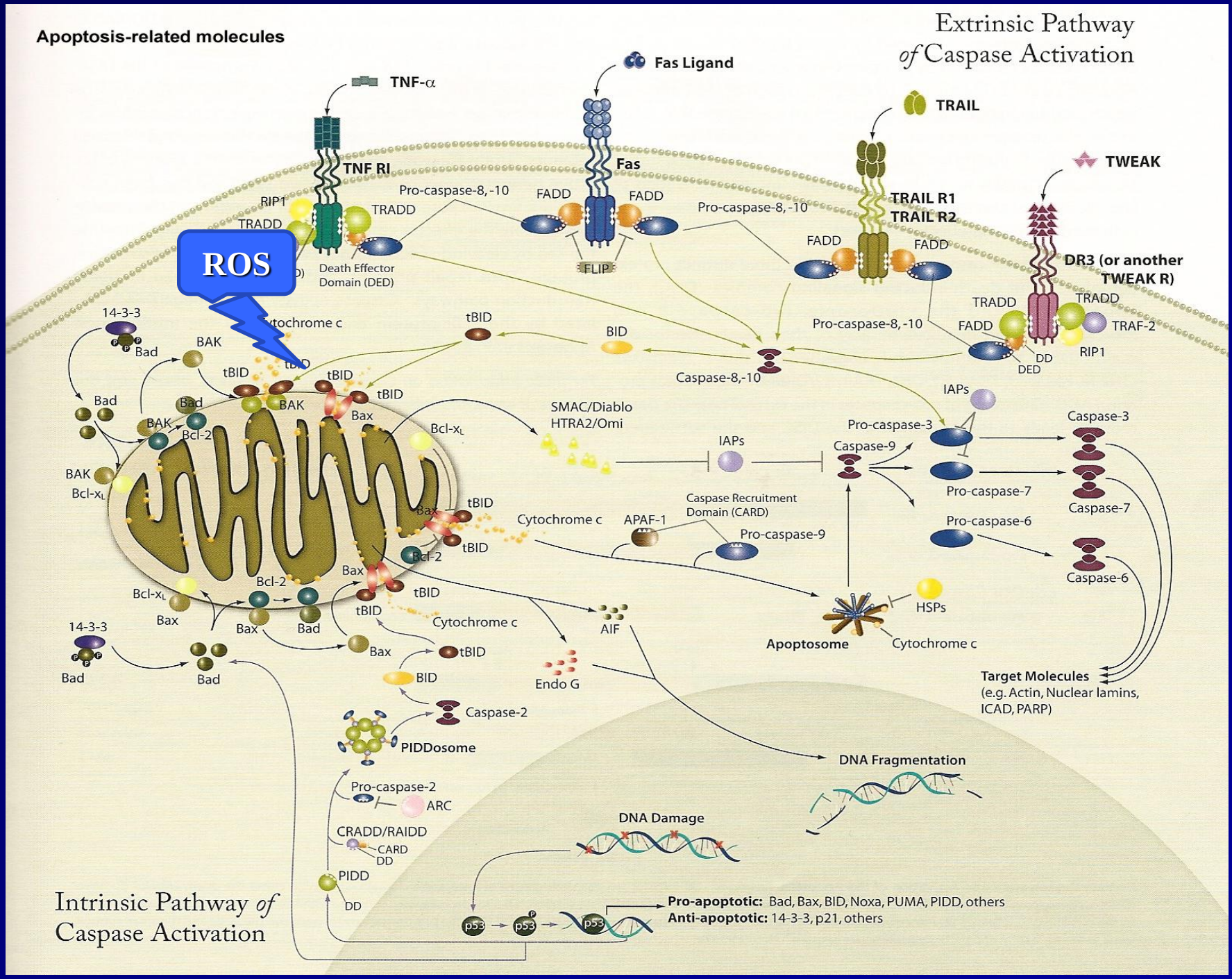



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# Apoptosis assay with 50uM $H_2O_2$, 2hours in HL60



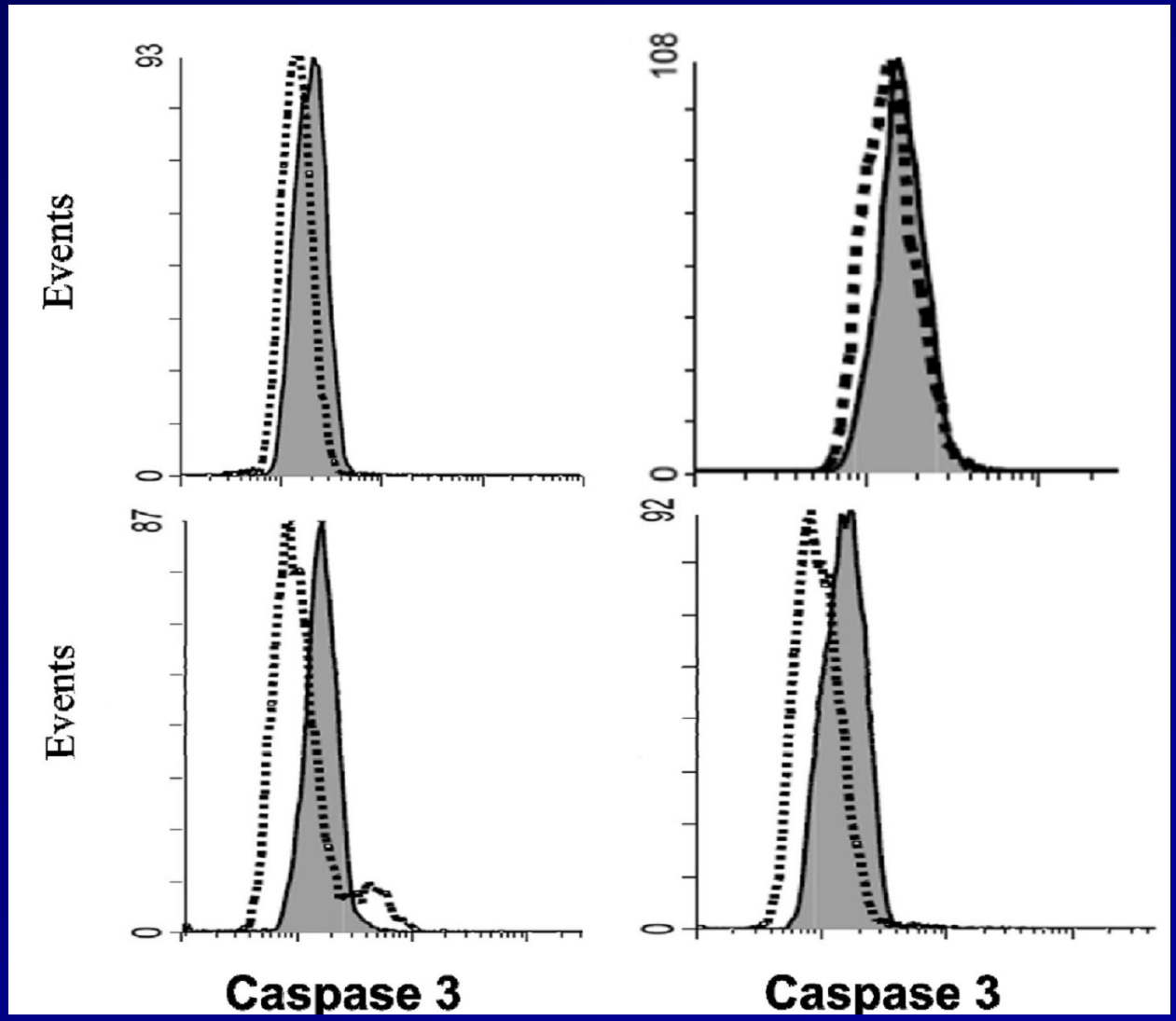

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Apoptosis-related molecules
Extrinsic Pathway of Caspase Activation
TNF-α
Fas Ligand
TRAIL
TWEAK
TNF RI
Fas
TRAIL R1
TRAIL R2
DR3 (or another TWEAK R)
RIP1
TRADD
FADD
Pro-caspase-8, -10
Death Effector Domain (DED)
FLIP
TRAF-2
DD
DED
ROS
14-3-3
Bad
BAK
Bcl-2
Bcl-xL
Bax
tBID
BID
Cytochrome c
Caspase-8,-10
SMAC/Diablo
HTRA2/Omi
IAPs
Caspase-3
Caspase-9
Caspase-7
Caspase-6
Pro-caspase-3
Pro-caspase-7
Pro-caspase-6
Caspase Recruitment Domain (CARD)
APAF-1
Pro-caspase-9
Apoptosome
HSPs
AIF
Endo G
Caspase-2
PIDDosome
Pro-caspase-2
ARC
CRADD/RAIDD
CARD
DD
PIDD
DNA Damage
DNA Fragmentation
Target Molecules
(e.g. Actin, Nuclear lamins, ICAD, PARP)
p53
Pro-apoptotic: Bad, Bax, BID, Noxa, PUMA, PIDD, others
Anti-apoptotic: 14-3-3, p21, others
Intrinsic Pathway of Caspase Activation

# Caspase 3 cleavage

HL60

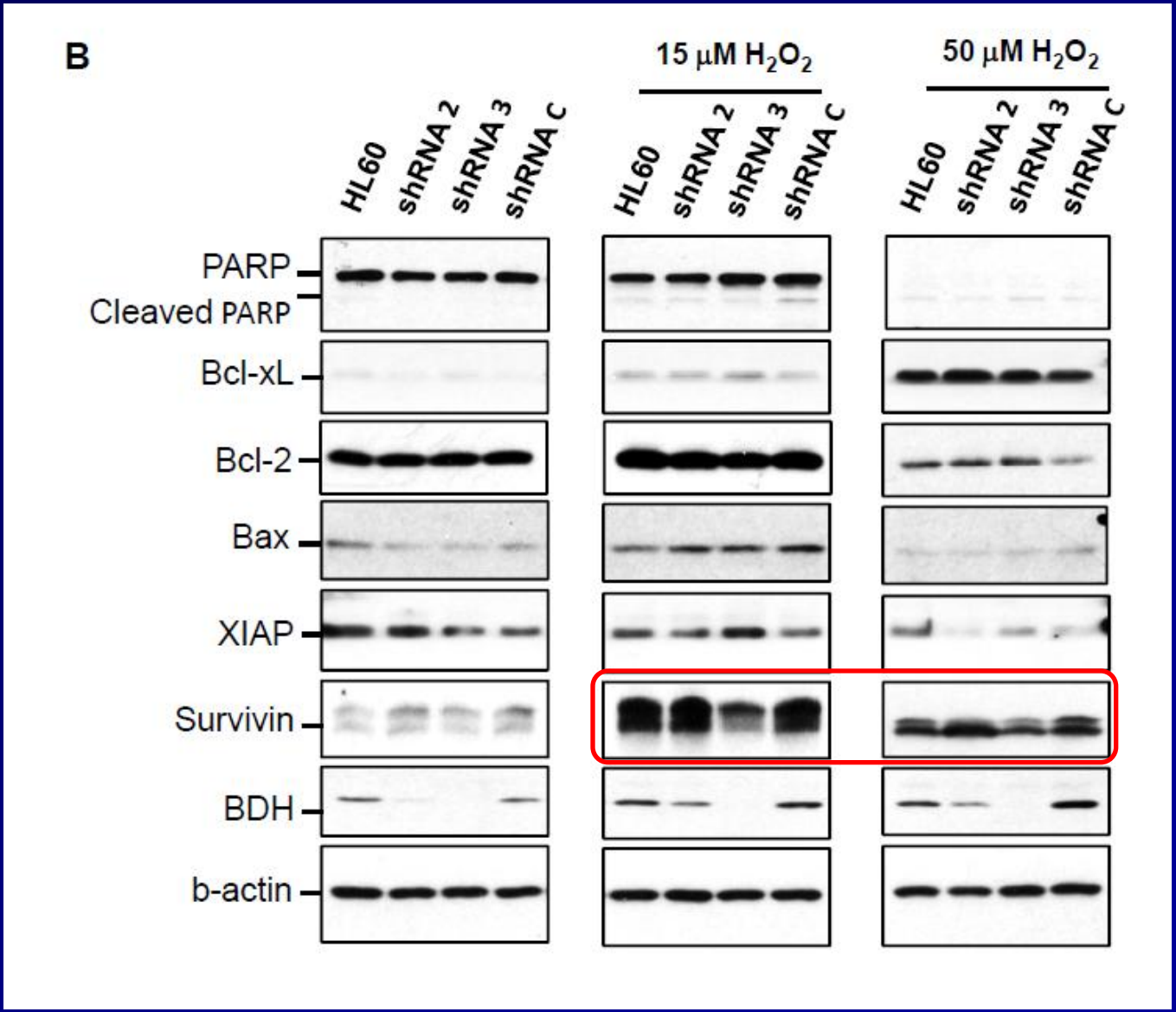
shRNA-*BDH2*-3 HL60




shRNA-*BDH2*-2 HL60

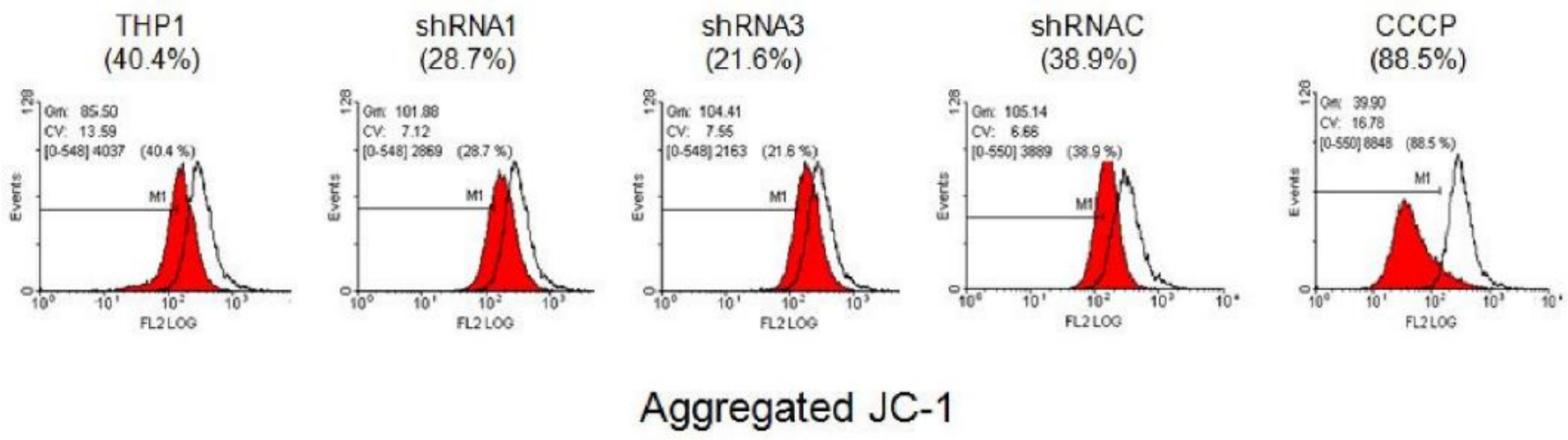
shRNAc HL60

# Apoptosis related protein expression




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# The mitochondrial membrane potential change

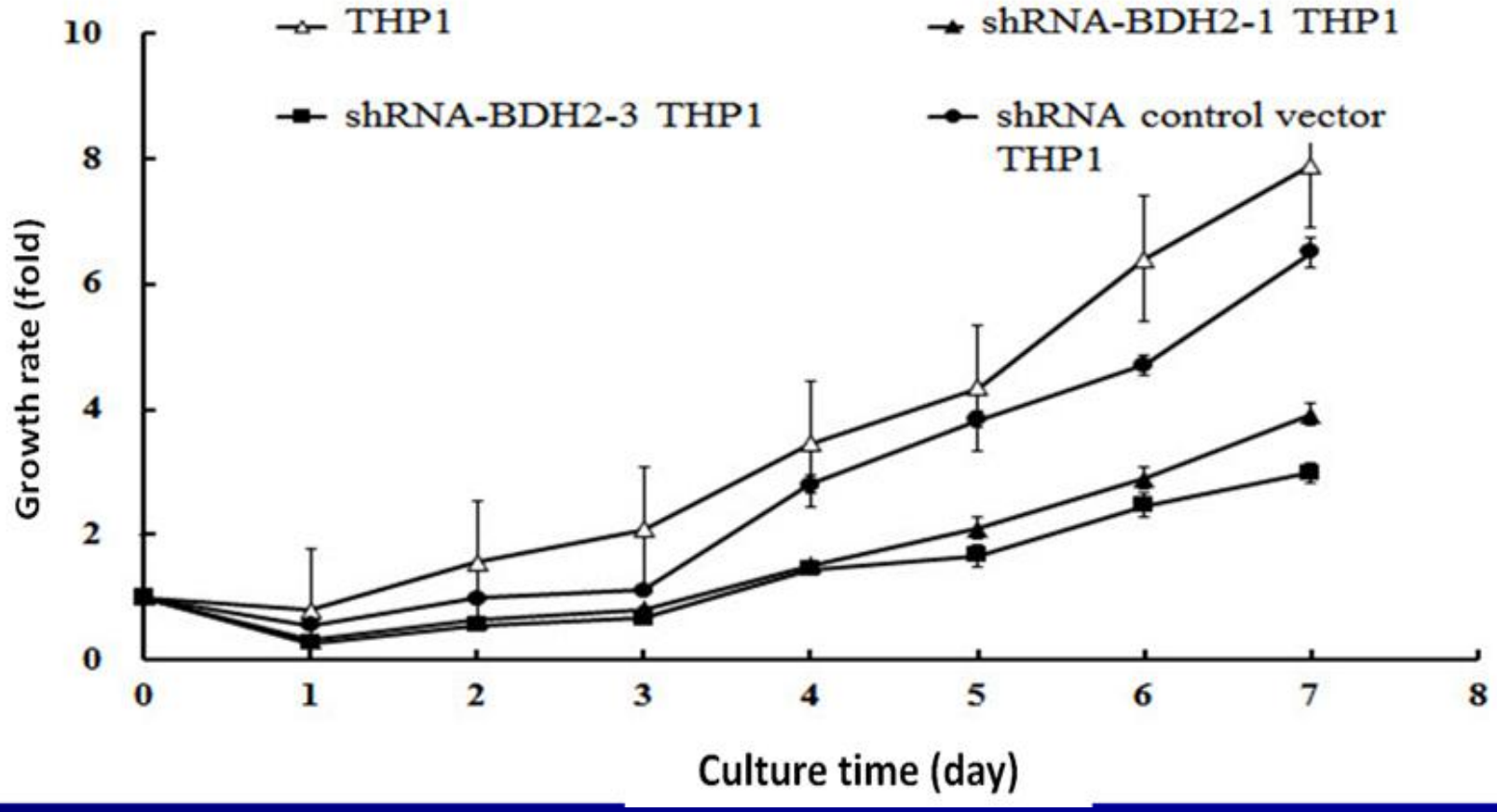



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# Others?

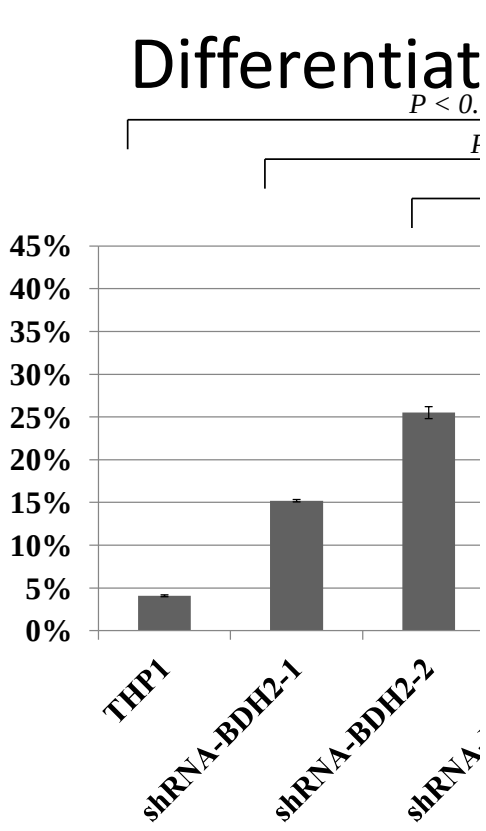
- If higher *BDH2* expression related to poor differentiation and higher proliferation rate ?

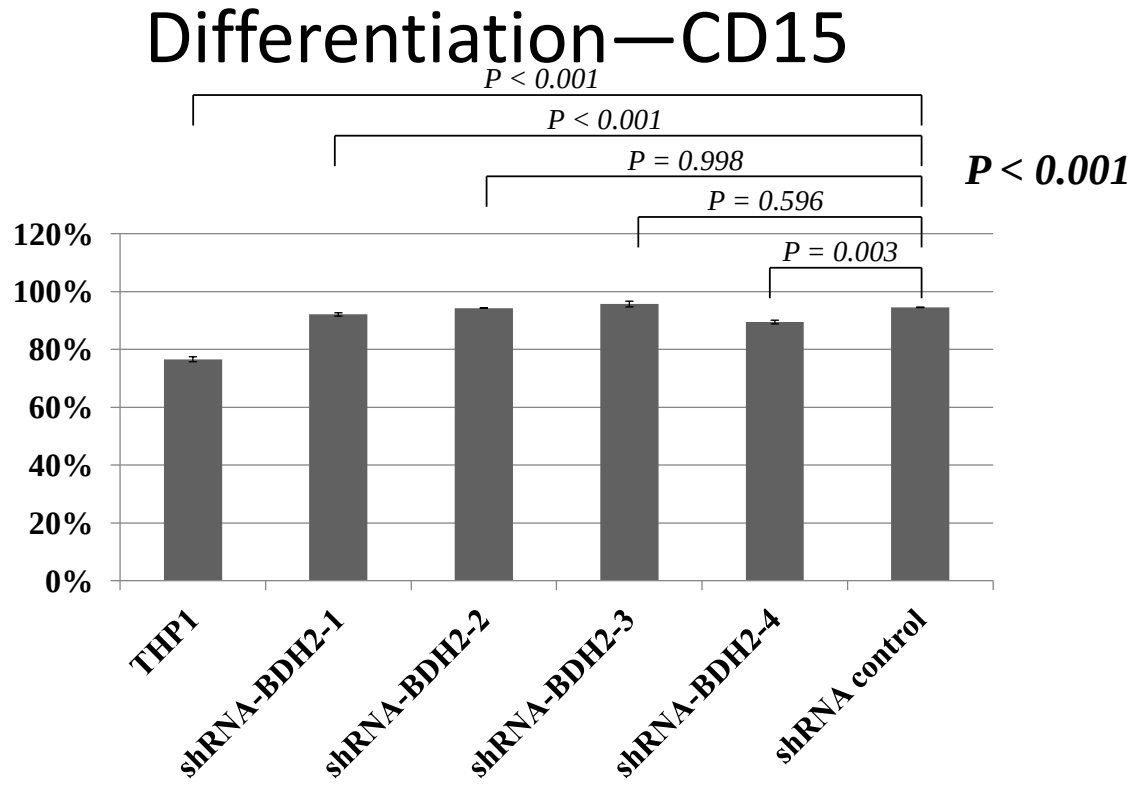
# Growth rate




*Unpublished data*

# Differentiation

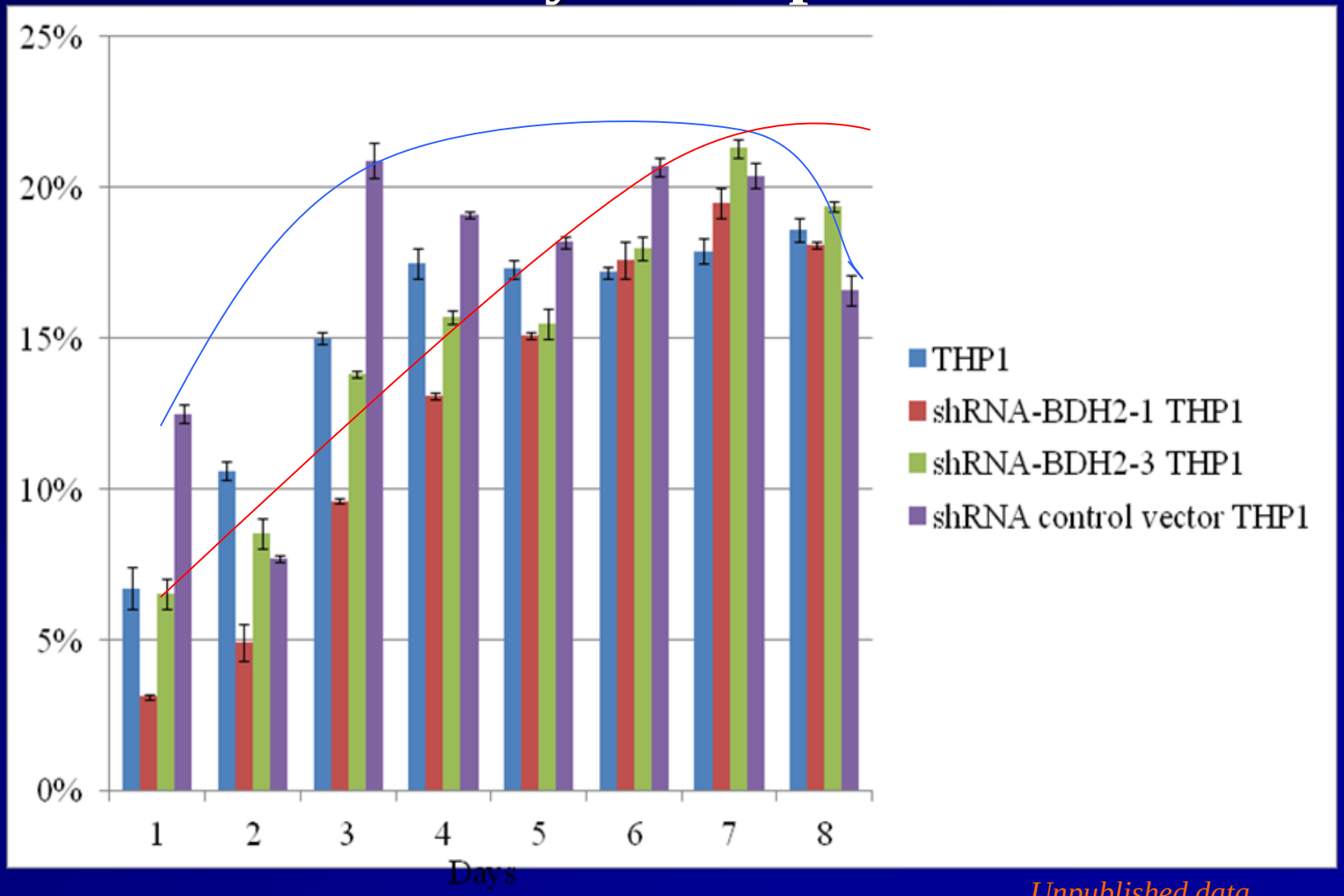


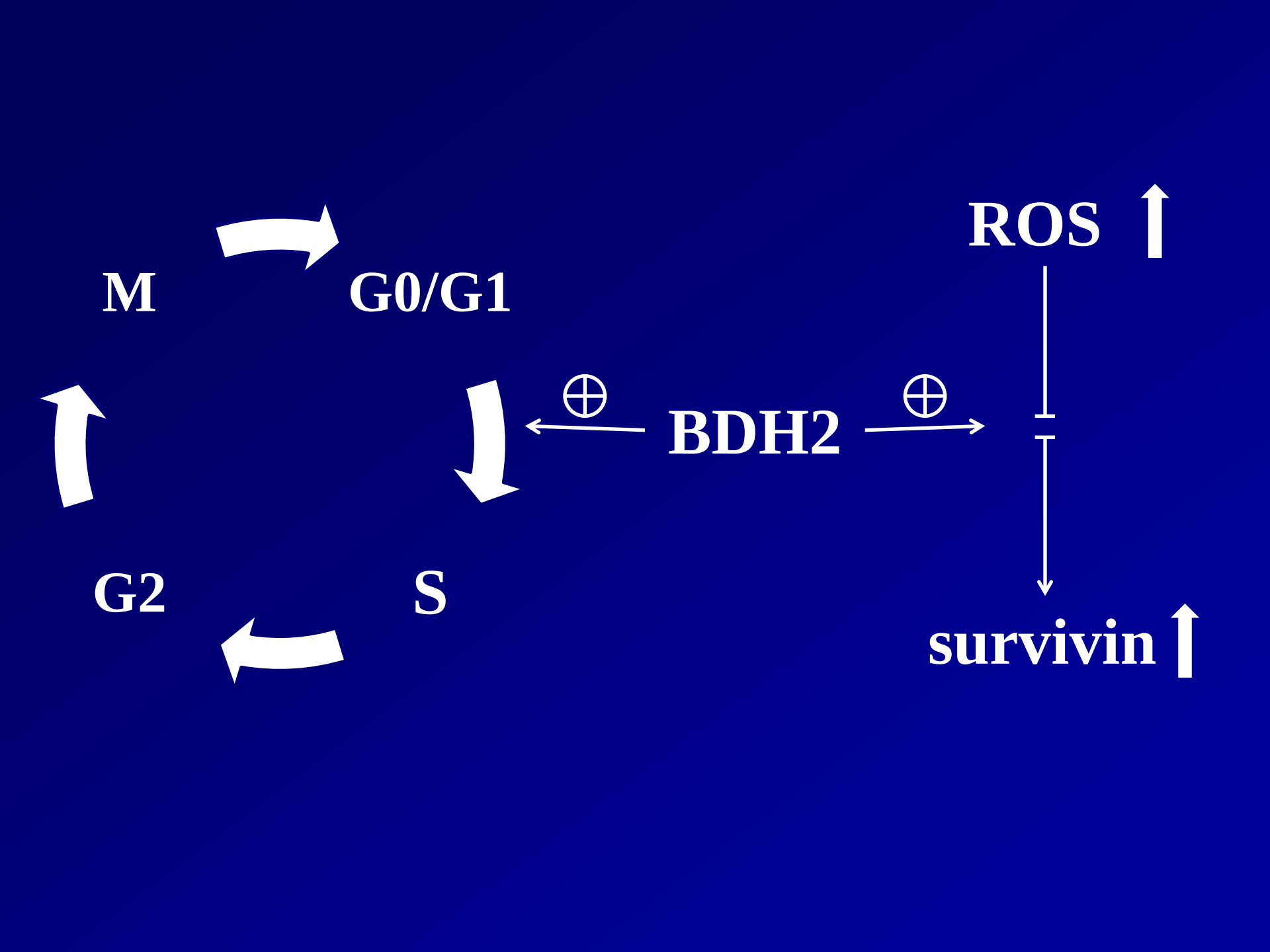

*Unpublished data*

# Cell cycle retardant

## delay into S phase




*Unpublished data*


M
G0/G1
S
G2
BDH2
ROS
survivin

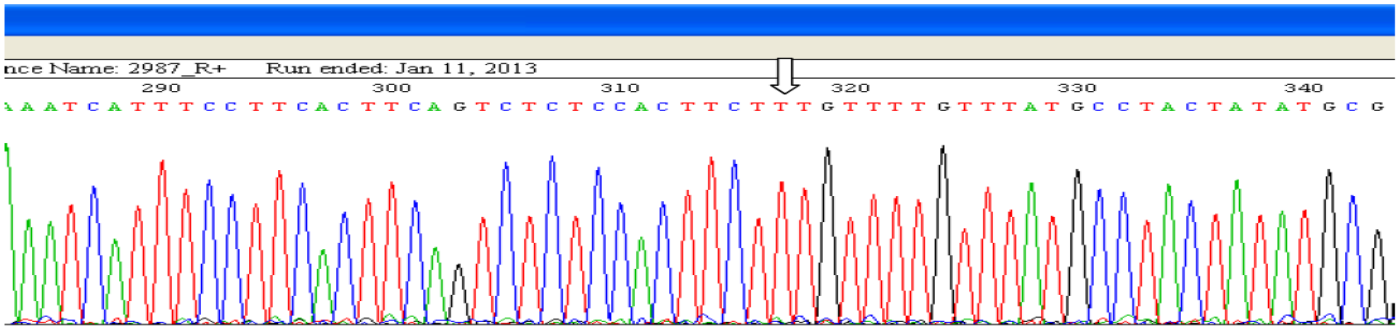
# Conclusion

- Higher *BDH2* expression is a poor prognostic predictor for CN-AML, with lower CR rate and shorter OS.
- *BDH2* works as a anti-apoptotic factor, mediated by survivin.
- *BDH2* knock down THP1 cells showed higher differentiation rate and lower proliferation rate with cell cycle retardant.
- Higher *BDH2* => chemo-resistant => consider allogenic hematological stem cell transplantation

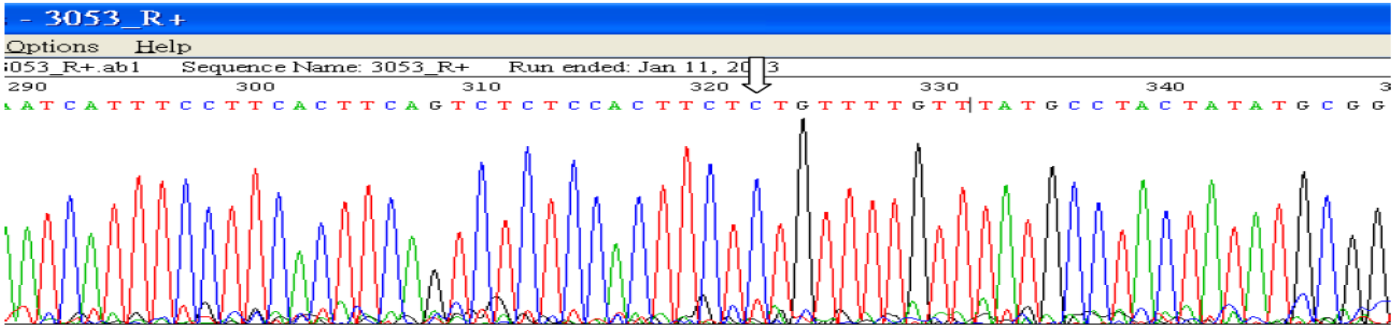
*Thank you for your attention !!*



# The mechanism of *BDH2* increasing in CN-AML patients—preliminary data




(by using reverse primer for sequencing; arrow: the variation area)




(by using reverse primer for sequencing; arrow: the same as promoter sequence)

# Overall survival

# Leukemia free survival

