

**GADD45 induces G2/M arrest in human pharynx and  
nasopharyngeal carcinoma cells by cucurbitacin E**

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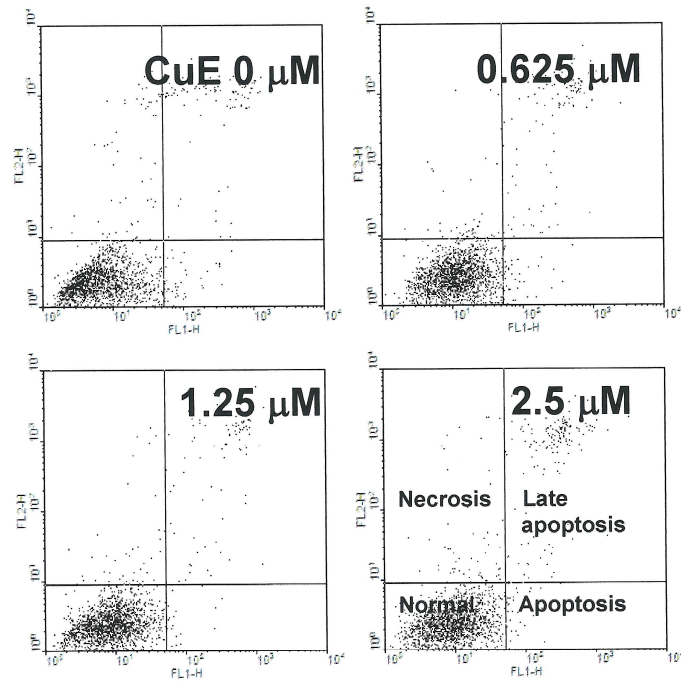
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Supplementary figures and tables

Figure. S1

(A) Detroit 562 cells



(B) Hone-1 cells

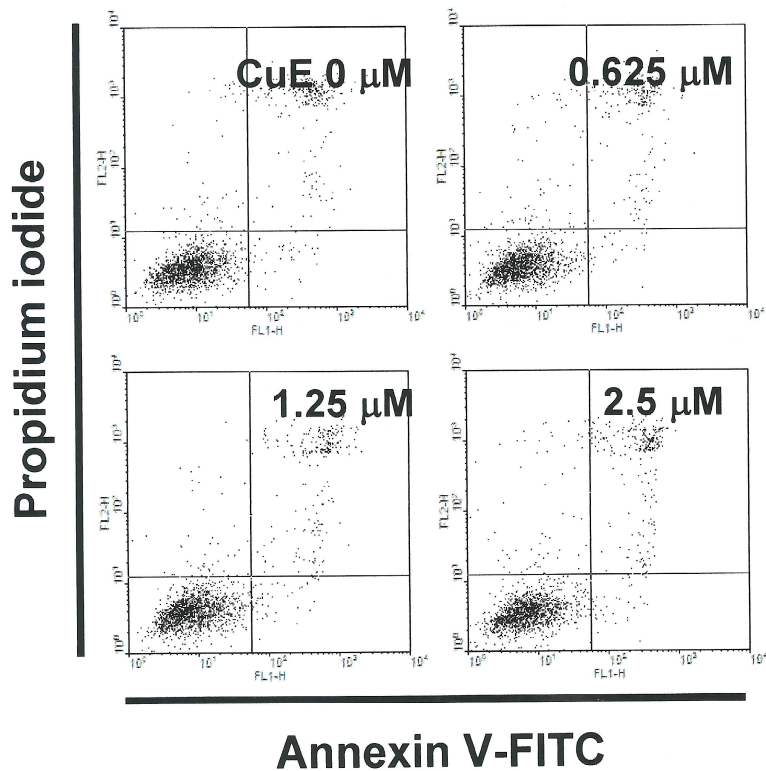
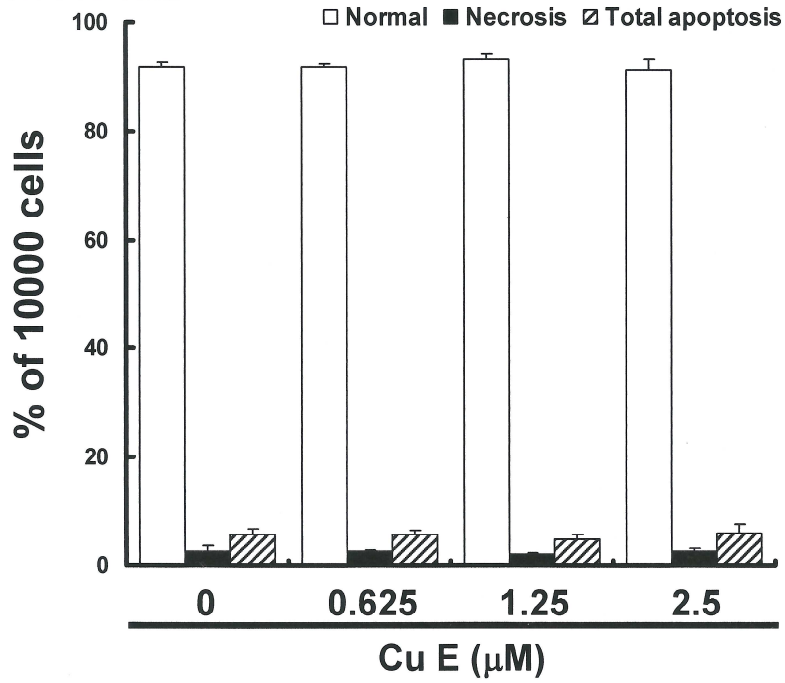


Figure. S1

(C)

Detroit 562 cells



Hone-1 cells

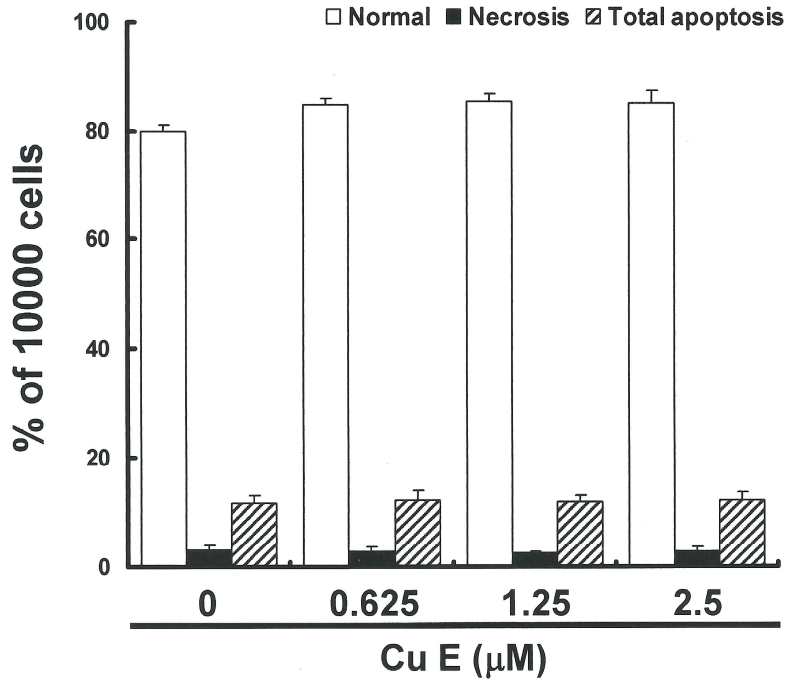


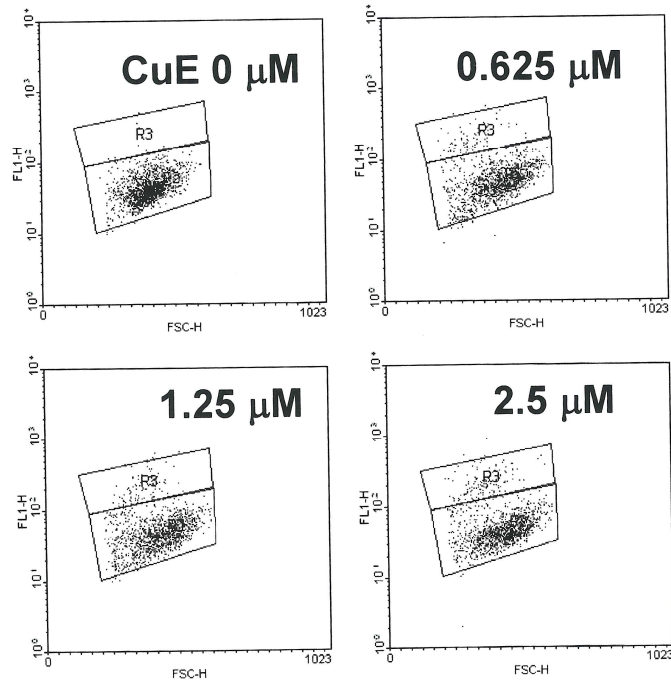
Figure. 1S- (A and B) Influence of CuE on apoptosis/necrosis in NPC cell lines. (C)

Total apoptosis and necrosis in NPC cell lines after 4 h of incubation with CuE.

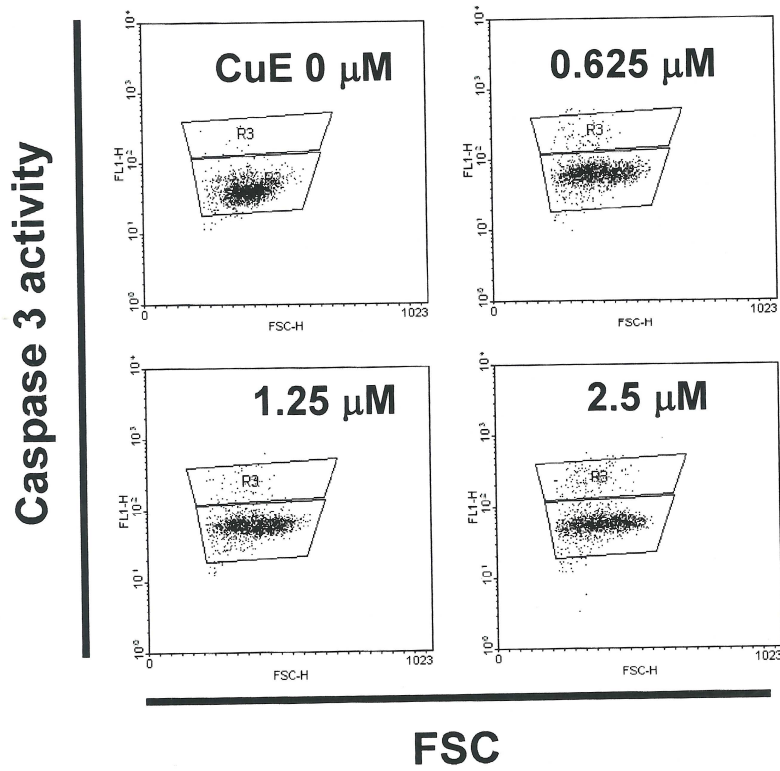
Results were expressed as a percentage of the total number of apoptotic cells  
(early and late apoptosis)

**Figure. S2**

**(A) Detroit 562 cells**

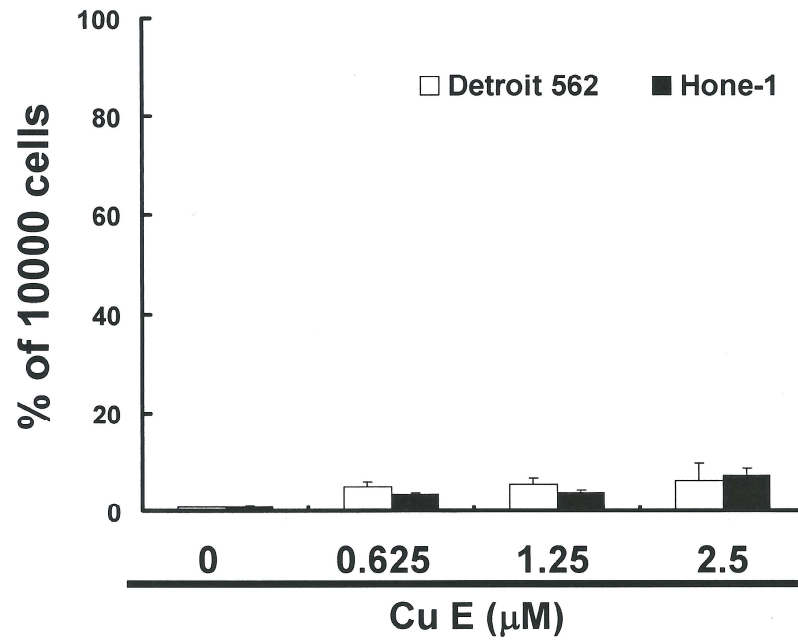


**(B) Hone-1 cells**



**Figure. S2**

(C)



**Figure. 2S-** Caspase-3 activity in NPC cell lines by CuE 24 h treatment. (A and B)

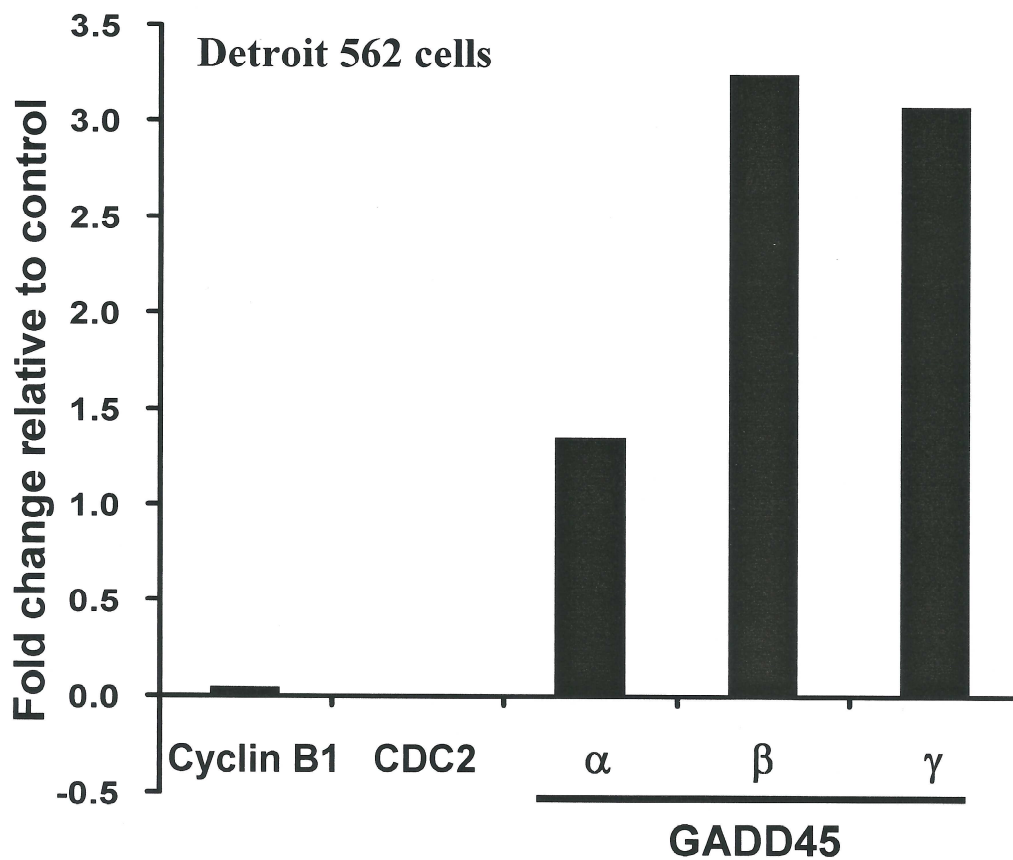
The cells were treated with CuE (0, 0.625, 1.25 and 2.5  $\mu\text{M}$ ) for 24 h.

Following treatment, the cells were harvested and labeled using FITC rabbit anti-active caspase-3. Activation was quantified by flow cytometry. (C)

Quantification by flow cytometry. All data was reported as the mean ( $\pm\text{SEM}$ )

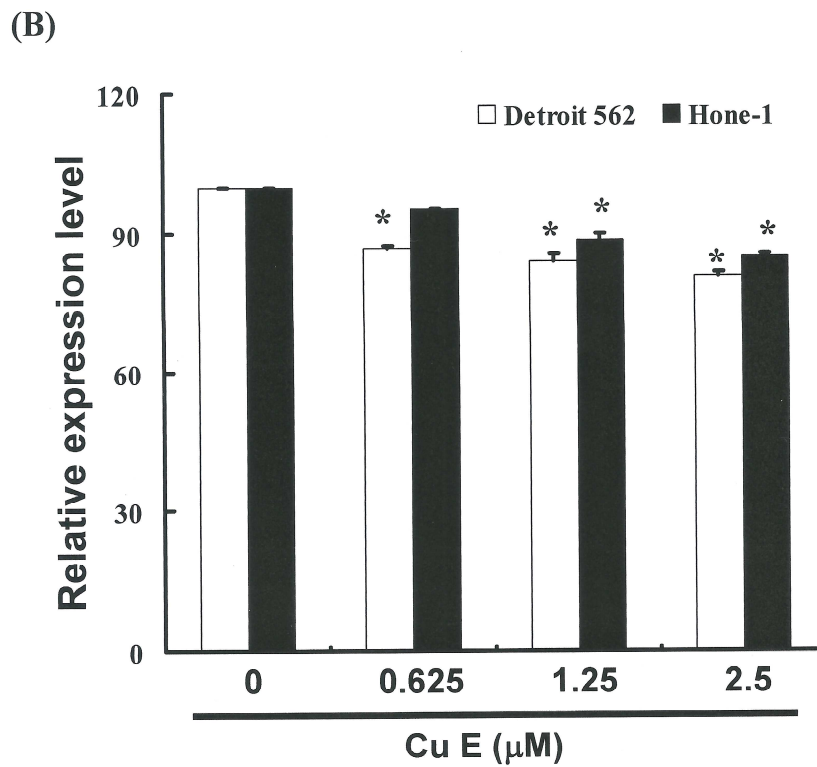
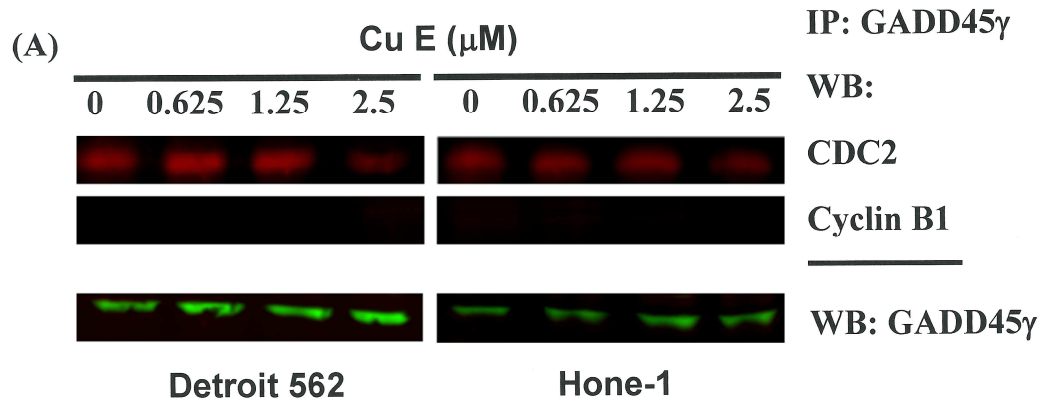
of at least three separate experiments.

Figure. S3



**Figure. 3S-** The genes downregulated or upregulated in Detroit 562 cells following exposure to CuE. The cyclin B1, CDC2 and GADD45- $\alpha$ , - $\beta$ , - $\gamma$  gene expression profile was studied in Detroit 562 cells exposed for 4 hours to the vehicle (DMSO) or to the CuE 5 $\mu$ M.

Figure. S4



**Figure. 4S-** CuE induced the GADD45 $\gamma$ /CDC2 complex in Detroit 562 and HONE-1

cells. The cells were treated with CuE (0, 0.625, 1.25 and 2.5  $\mu$ M) for 24 h and GADD45 $\gamma$ /cyclin B1 and GADD45 $\gamma$ /CDC2 complex were subsequently detected by Co-IP analysis. (A) Co-IP and western blotting representative blot



from 3 independent experiments. (B) Quantification of band intensities. All data was reported as the mean ( $\pm$ SEM) of at least three separate experiments. Statistical analysis was performed using a t-test, with significant differences determined at the level of  $*P < 0.05$  versus 0  $\mu$ M control group.

**Table S1. KEGG pathways of the genes up- or down regulated in Detroit 562 cells following exposure to CuE (1.25  $\mu$  M)**

Rank	Pathway	PValue
1	hsa04010:MAPK signaling pathway	0.000
2	hsa05200:Pathways in cancer	0.000
3	hsa05217:Basal cell carcinoma	0.000
4	hsa04530:Tight junction	0.001
5	hsa04115:p53 signaling pathway	0.002
6	hsa04340:Hedgehog signaling pathway	0.002
7	hsa04670:Leukocyte transendothelial migration	0.010
8	hsa04012:ErbB signaling pathway	0.010

The genome-wide gene expression profile was studied on whole human genome SurePrint G3 arrays array in Detroit 562 cells following a 4 hour-exposure to vehicle (DMSO) or to the CuE. By importing the complete list of Entrez Gene IDs or down- or up-regulated genes into KEGG pathways related to the set of imported showing  $p < 0.01$  by a modified Fisher Exact test were identified. The pathways are listed with the category and p-value.

**Table S2. Top 10 genes down-regulated in Detroit 562 cells following exposure to CuE (1.25  $\mu$  M)**

Down-regulated Genes (n=431)

Gene Name	Fold Change	Gene bank	Description
LOC644578	-160.256	XM_001715474	PREDICTED: Homo sapiens similar to hCG2040199
A2ML1	-58.431	NM_144670	Homo sapiens alpha-2-macroglobulin-like 1
ZNF610	-50.664	NM_173530	Homo sapiens zinc finger protein 610 (ZNF610)
OLFM1	-49.048	NM_006334	Homo sapiens olfactomedin 1 (OLFM1)
LOC441687	-35.381	XM_001717077	PREDICTED: Homo sapiens similar to testis expressed gene 21 (LOC441687)
ERC2	-23.038	NM_015576	Homo sapiens ELKS/RAB6-interacting/CAST family member 2 (ERC2)
UGT2A3	-21.452	NM_024743	Homo sapiens UDP glucuronosyltransferase 2 family, polypeptide A3 (UGT2A3)
AOX2P	-18.980	NR_001557	Homo sapiens aldehyde oxidase 2 pseudogene (AOX2P)
LOC100128354	-18.234	NR_001557	Homo sapiens aldehyde oxidase 2 pseudogene (AOX2P)
NRARP	-15.898	NM_001004354	Homo sapiens NOTCH-regulated ankyrin repeat protein (NRARP)

**Table S3. Top 10 genes up-regulated in Detroit 562 cells following exposure to CuE (1.25  $\mu$  M)**

Up-regulated Genes (n=941)

Gene Name	Fold Change	Gene bank	Description
KIAA1239	85.405	NM_001144990	Homo sapiens KIAA1239 (KIAA1239)
LOC285556	77.931	XM_373030	Homo sapiens hypothetical protein LOC285556
ATF3	45.129	NM_001040619	Homo sapiens activating transcription factor 3 (ATF3)
BACH2	35.593	NM_021813	Homo sapiens BTB and CNC homology 1, basic leucine zipper transcription factor 2 (BACH2)
MYLK	35.561	NM_053025	Homo sapiens myosin light chain kinase (MYLK)
CHAC1	33.970	NM_024111	Homo sapiens ChaC, cation transport regulator homolog 1(E. coli) (CHAC1)
C8orf4	29.718	NM_020130	Homo sapiens chromosome 8 open reading frame 4 (C8orf4)
LOC728114	27.240	XR_040708	PREDICTED: Homo sapiens hypothetical protein LOC728114 (LOC728114)
DNAI2	24.667	NM_023036	Homo sapiens dynein, axonemal, intermediate chain 2 (DNAI2)
GBP2	22.082	NM_004120	Homo sapiens guanylate binding protein 2, interferon-inducible (GBP2)

The genome-wide gene expression profile was studied on whole human genome SurePrint G3 arrays array in Detroit 562 cells following a 4 hour-exposure to vehicle (DMSO) or to the CuE 1.25  $\mu$  M. The fold changes greater than 2 or smaller than 0.5 calculated by the expression levels in the CuE treated cells divided by those in the vehicle-treated cells, were considered a substantial up- or down- regulation. Top ten of them are listed with gene symbol, gene name, and fold change.