Modulation of Cancer Related Molecules in HEK293T Cells Treated with Excretory-Secretory Products of *Clonorchis sinensis* and Dimethylnitrosamine

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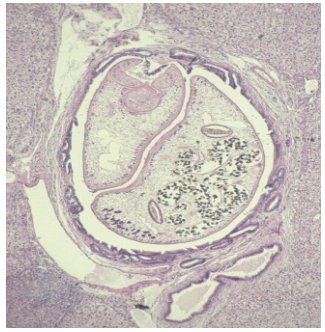
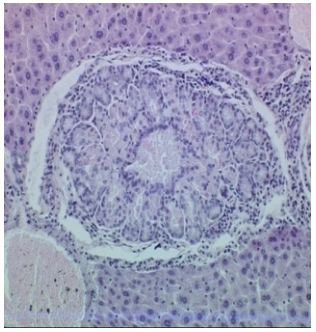
Clonorchis sinensis



X 100



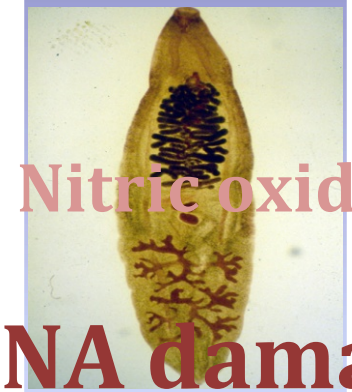
X 400

Periductal fibrosis
Inflammation
Glandular hyperplasia.

Nitrosamines

Food

ES Products



Nitric oxide

Altered cell stage

DNA damage

?

Inhibition of apoptosis

?

Cholangiocarcinoma

Hypotheses

- ***C. sinensis*** **controls the cell proliferation and cell to cell communication via gap junction.**

- ***C. sinensis*** **controls the regulation of cellular proteins including cancer related factors.**

Materials and Methods

- **Materials**
  - HEK293T cells: from ATCC
  - ESP(excretory/secretory proteins of *C. sinensis*)
  - DMN: dimethylnitrosamine

- **Experiment groups**

  Group1: Control

  Group2: DMN 10□/□

  Group3: ESP 10□/□

  Group4: DMN 10□/□+ESP 10□/□

Result

-Cell cycle

-Gap Junction proteins : Connexin43 and 32

-Cancer related proteins

- FACS for cell population by division cycles
- XTT for cell proliferation
- Promoter assay and Western blotting for gene regulation for cancer related molecules
- SiRNA knock down experiment for cancer related genes
- Confocal microscopy

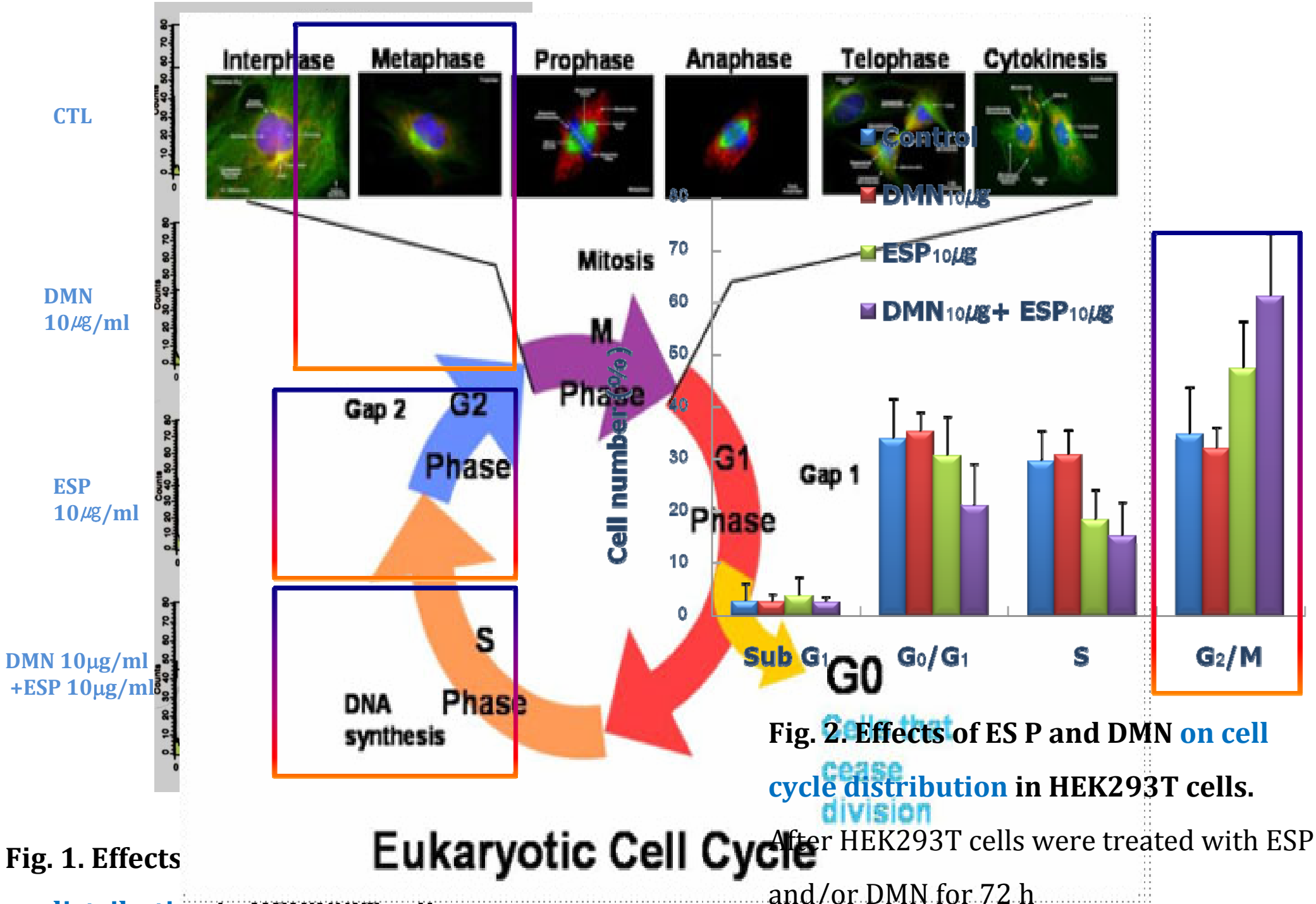


Fig. 1. Effects on cell cycle distribution in HEK293T cells.

Fig. 2. Effects of ES P and DMN on cell cycle distribution in HEK293T cells.

After HEK293T cells were treated with ESP and/or DMN for 72 h

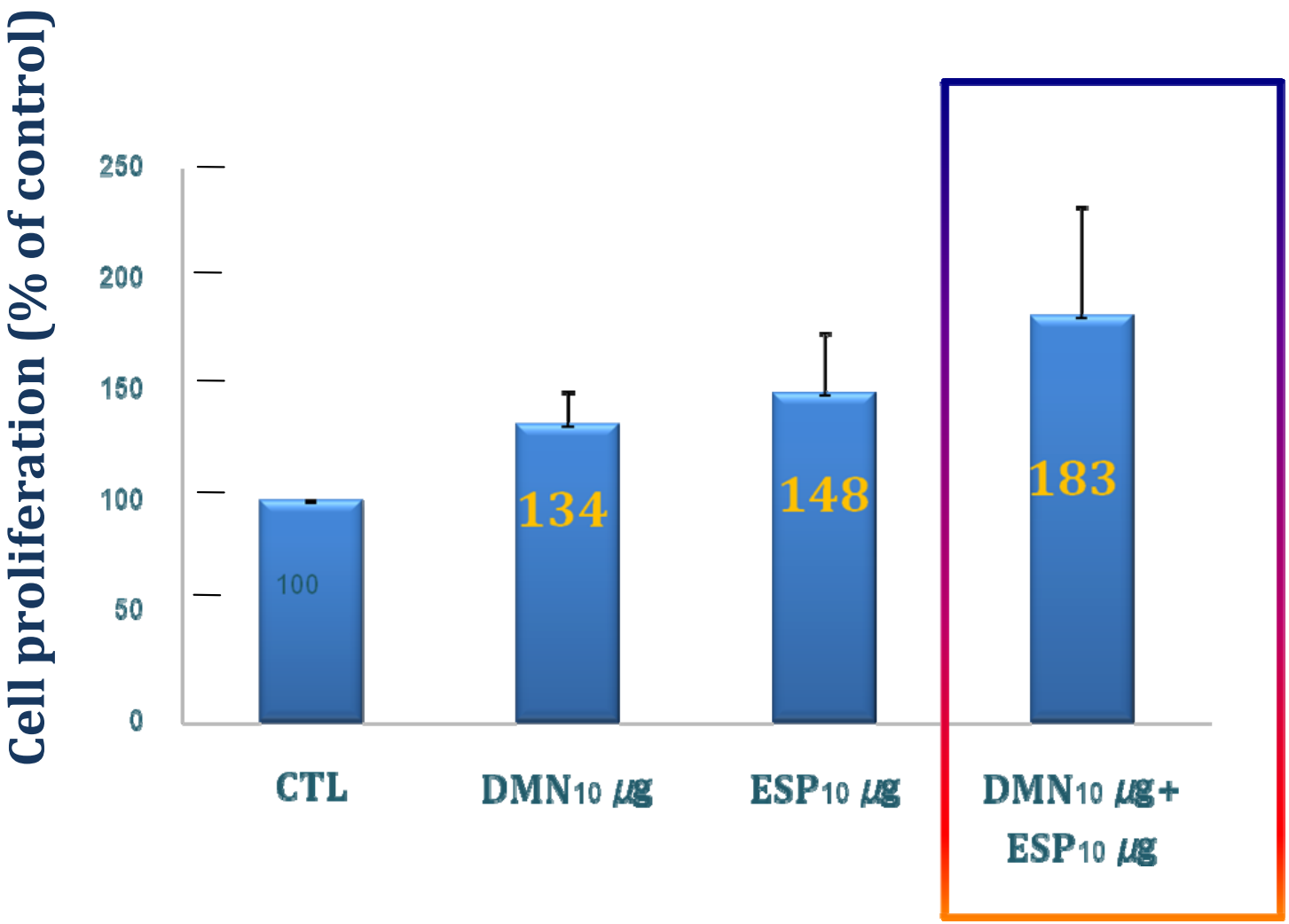


Fig. 3. Effect of ESP and DMN on cell proliferation in HEK293T cells. Cells were plated in 96-well plates (1.5×10^4 cells/well). After a 24-h incubation, the medium was replaced with low serum medium (2% FBS-RPMI1640 without phenol red). The cells were incubated in PBS (vehicle) or ESP and/or DMN for another 24h.

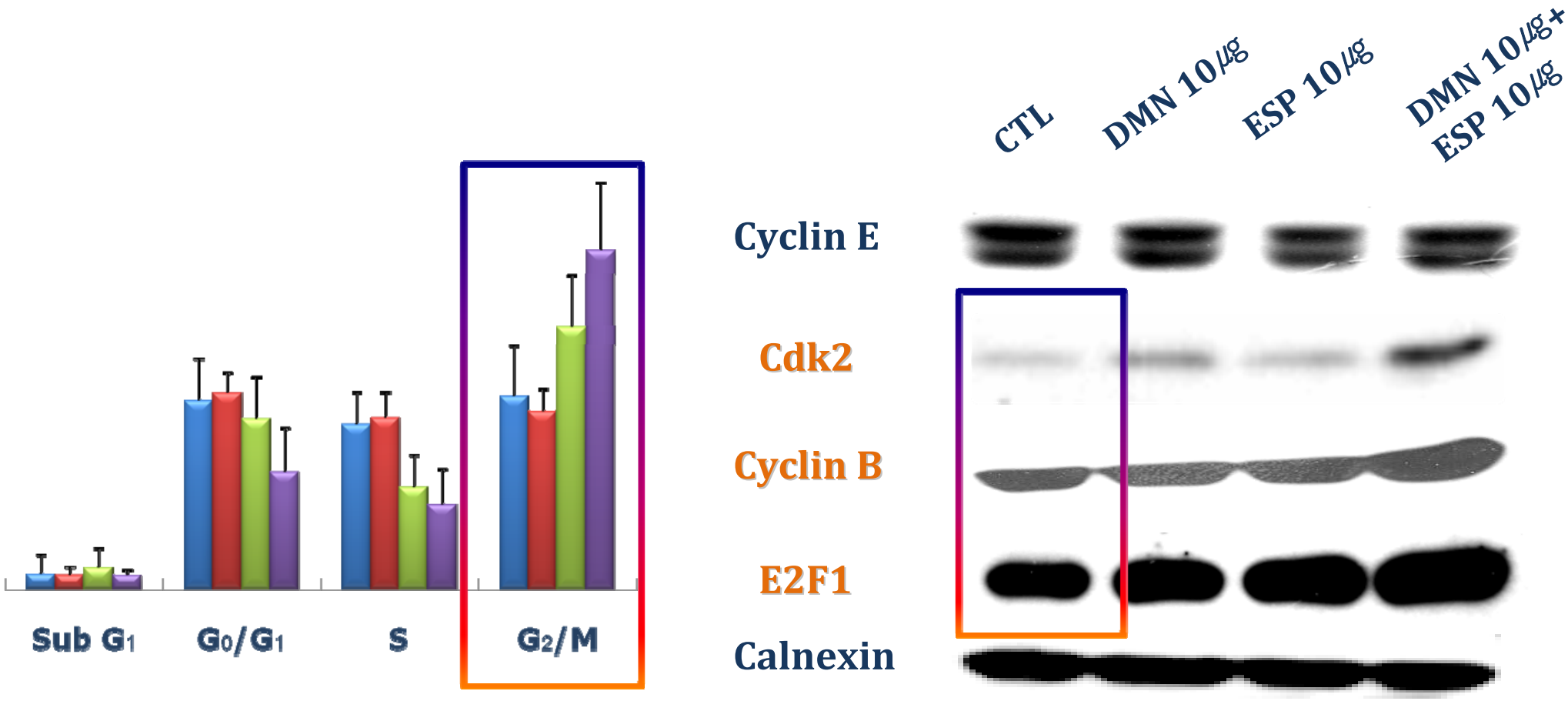
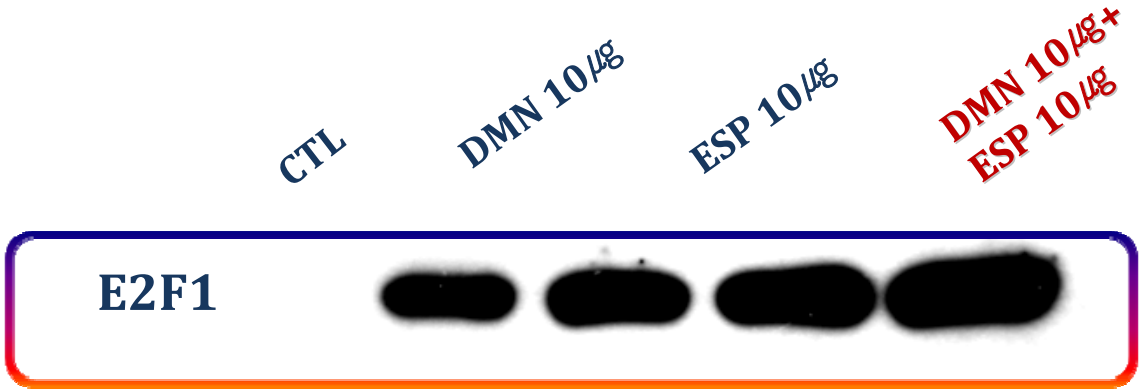


Fig. 4. Expression of cell cycle-related proteins after treatment with ES products. HEK293T cells were incubated with either PBS (vehicle) or various concentrations of ES products for 24 h, and the cells were collected for protein extraction.

E2F1; regulating cell cycle at the G2/M phase
cell cycle progression
DNA replication
key factor in the development of cancer (Huang CL et.,al)



Promoter assay

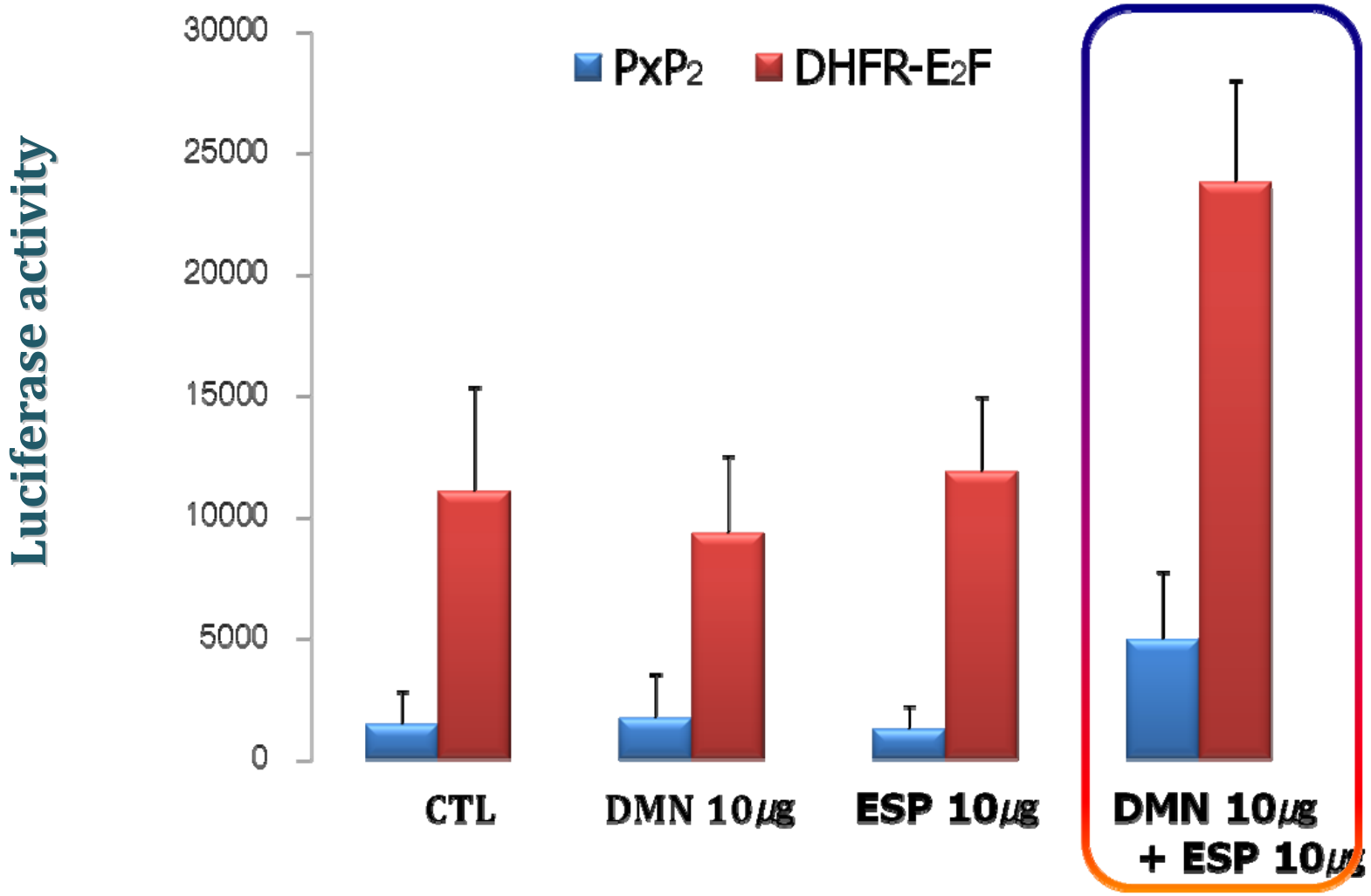


Fig. 5. DHFR promoter-driven luciferase activity in HEK293T cells by stimulation with ESP and DMN. HEK293T cells were transfected with the luciferase reporter gene *dhfr*-luc or *pXP2*-luc (control).

RNAi specific; E2F1

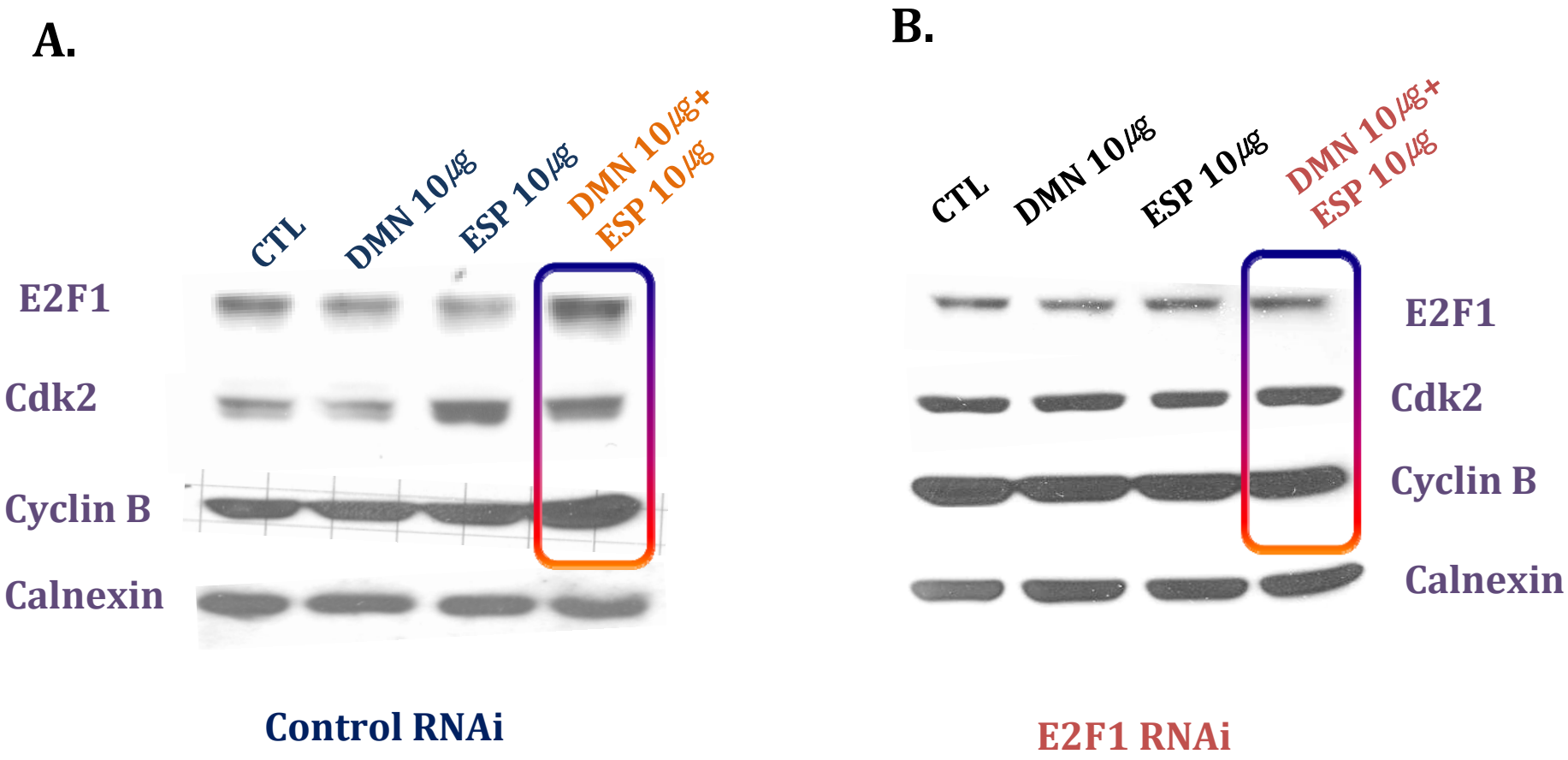


Fig. 6. Expression of cell cycle related proteins after transfection with non-specific control RNAi (A) or RNAi specific for human E2F1 (B).

Result

ES Products

Nitric oxide

Food

Nitrosamines

Altered cell stage

DNA damage

?

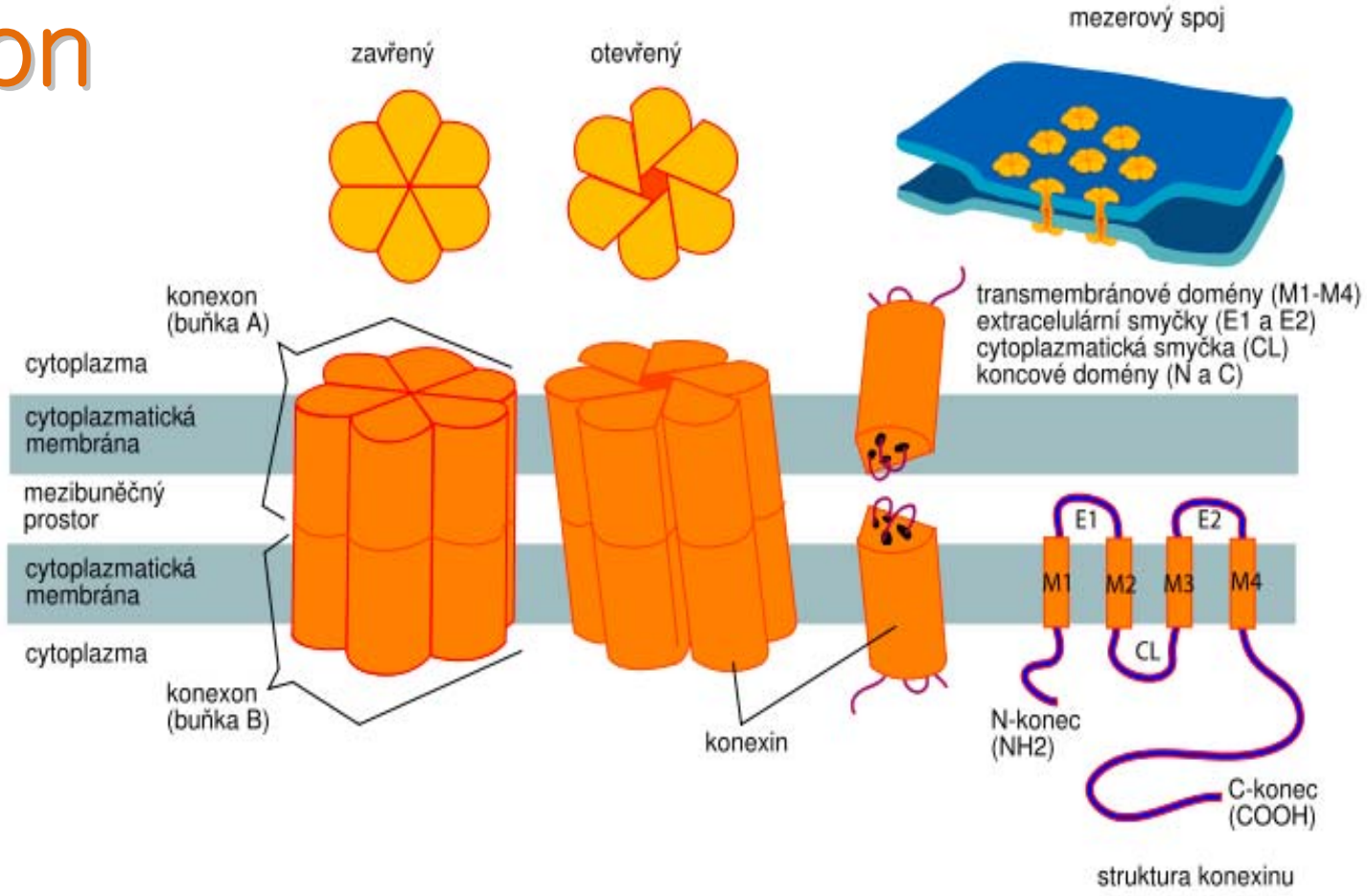
E2F1 is a key factor

Inhibition of apoptosis

?

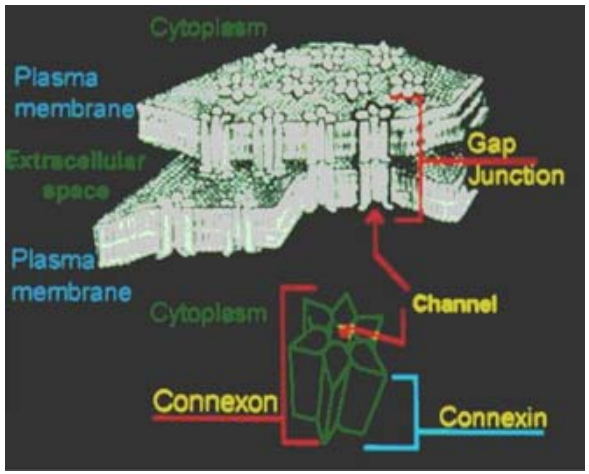
Cholangiocarcinoma

Gap Junction



- Gap junctions are cylindrical channels between animal cells that allow small m olecules and ions to pass from the inside of one cell to the inside of the adjacen t cell
- Gap junctions help cells to communicate chemical and electrical signals quickly and achieve homeostasis, or physiological balance.
- Connexins are regarded as the major gap junction protein

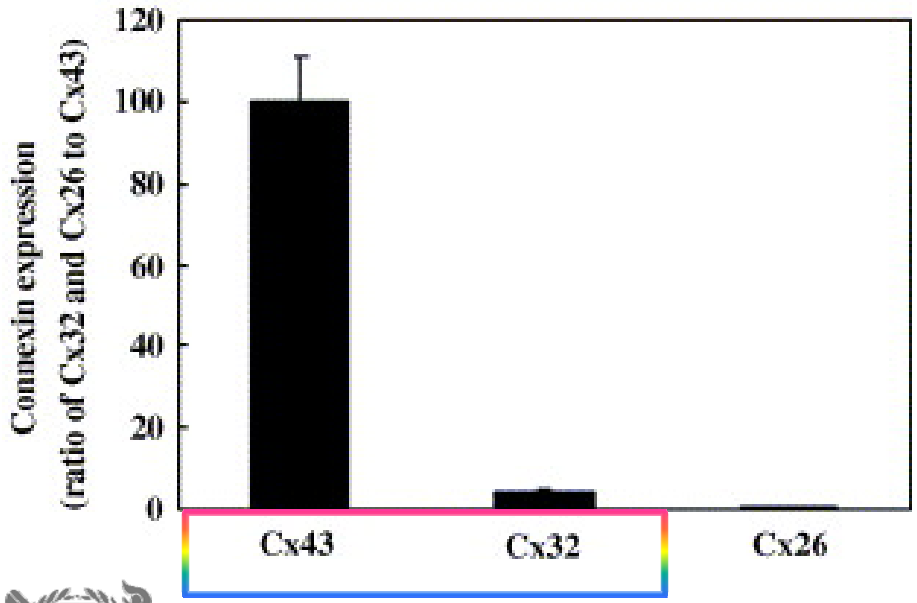
Gap Junctions



Cx genes suppress the proliferation or malignancy of human cancer …

On the other hand

Some subtypes of Cx genes are up-regulated in lung cancer, hepatocellular carcinoma(HCC)



Cx43 expression promotes malignancy of HuH7 hepatocellular carcinoma cells via the inhibition of GJIC composed of Cx32 (Cancer Letters , 2007)

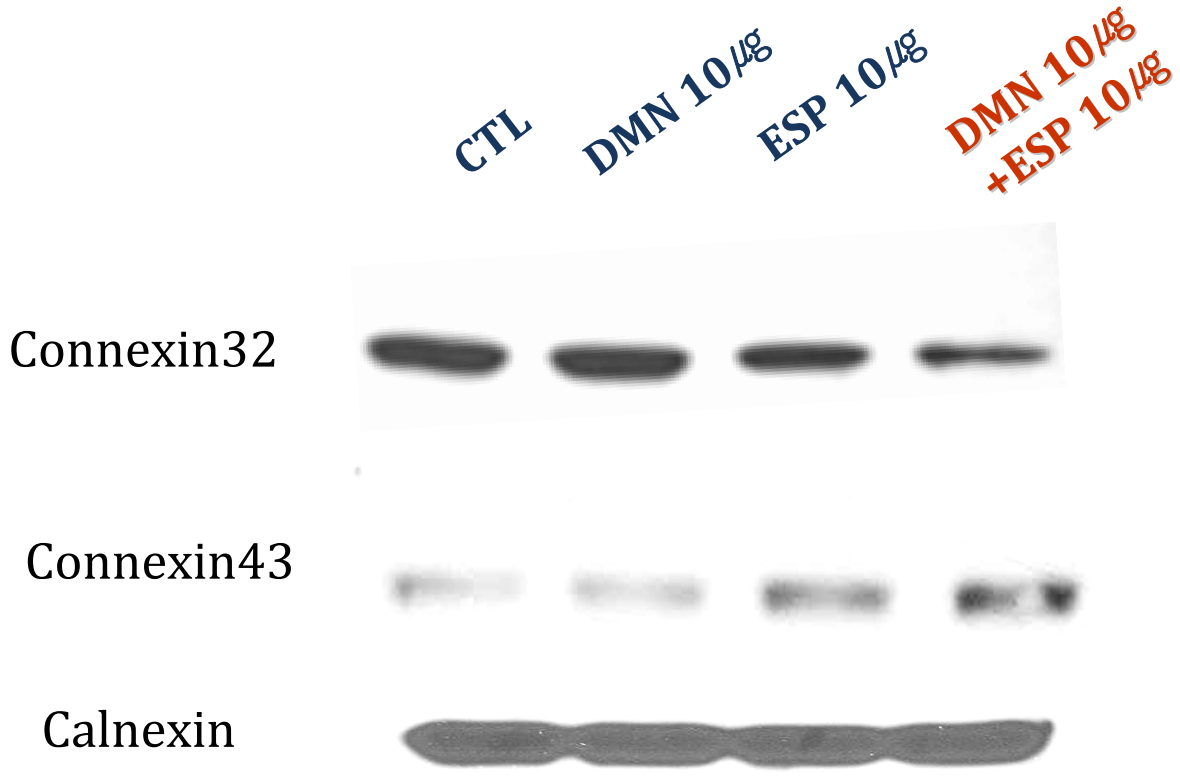


Fig. 7. Expression of gap-junction proteins, connexin 32 and 43, in four groups. HEK293T cells were treated with ESP and/ or DMN for 72 h and the cells were collected for protein extraction.

Laser scanning microscopy

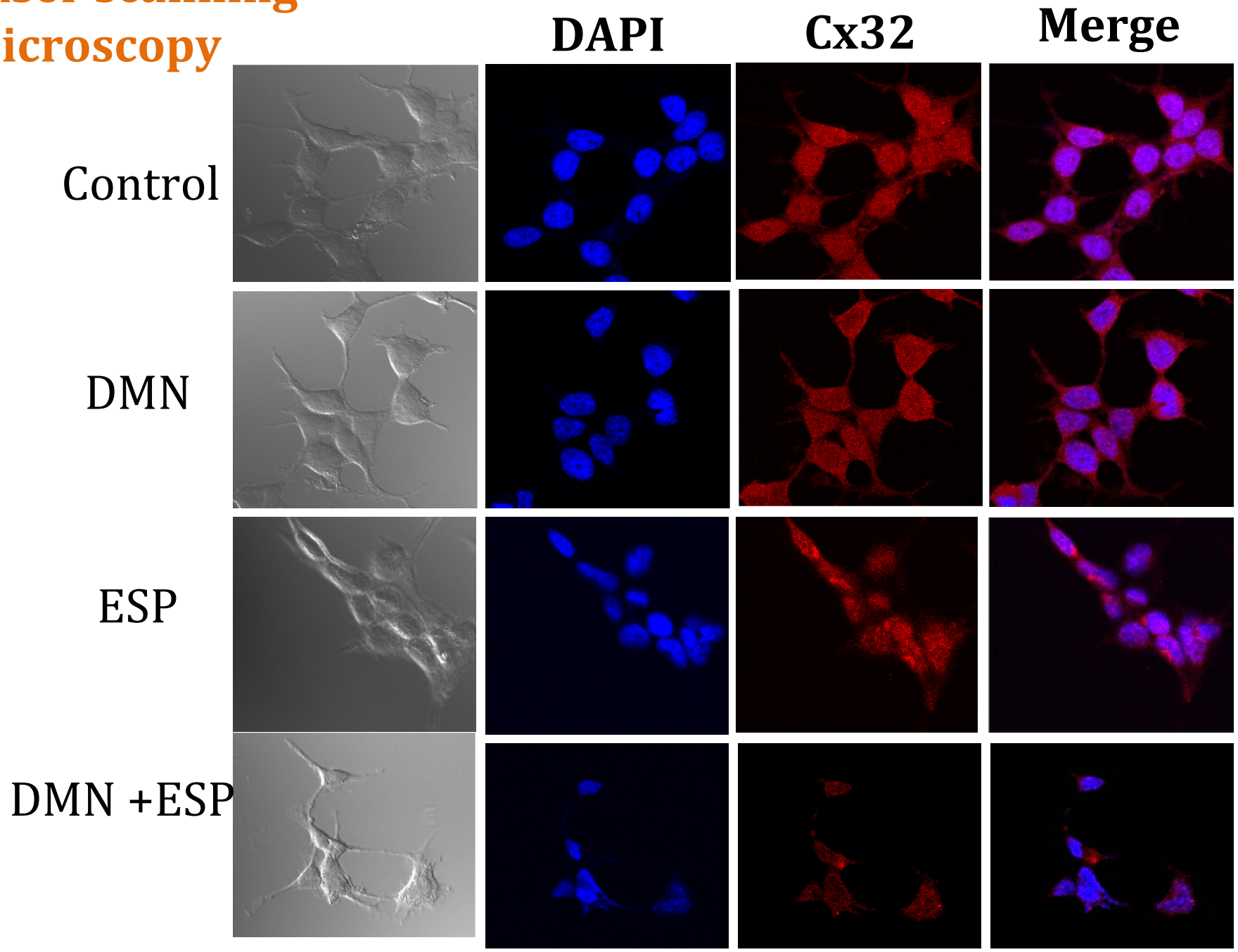


Fig 8. Concentration of intracellular connexin 32 measured by laser scanning microscopy , LSM PASCAL (Carl Zeiss)

Laser scanning microscopy

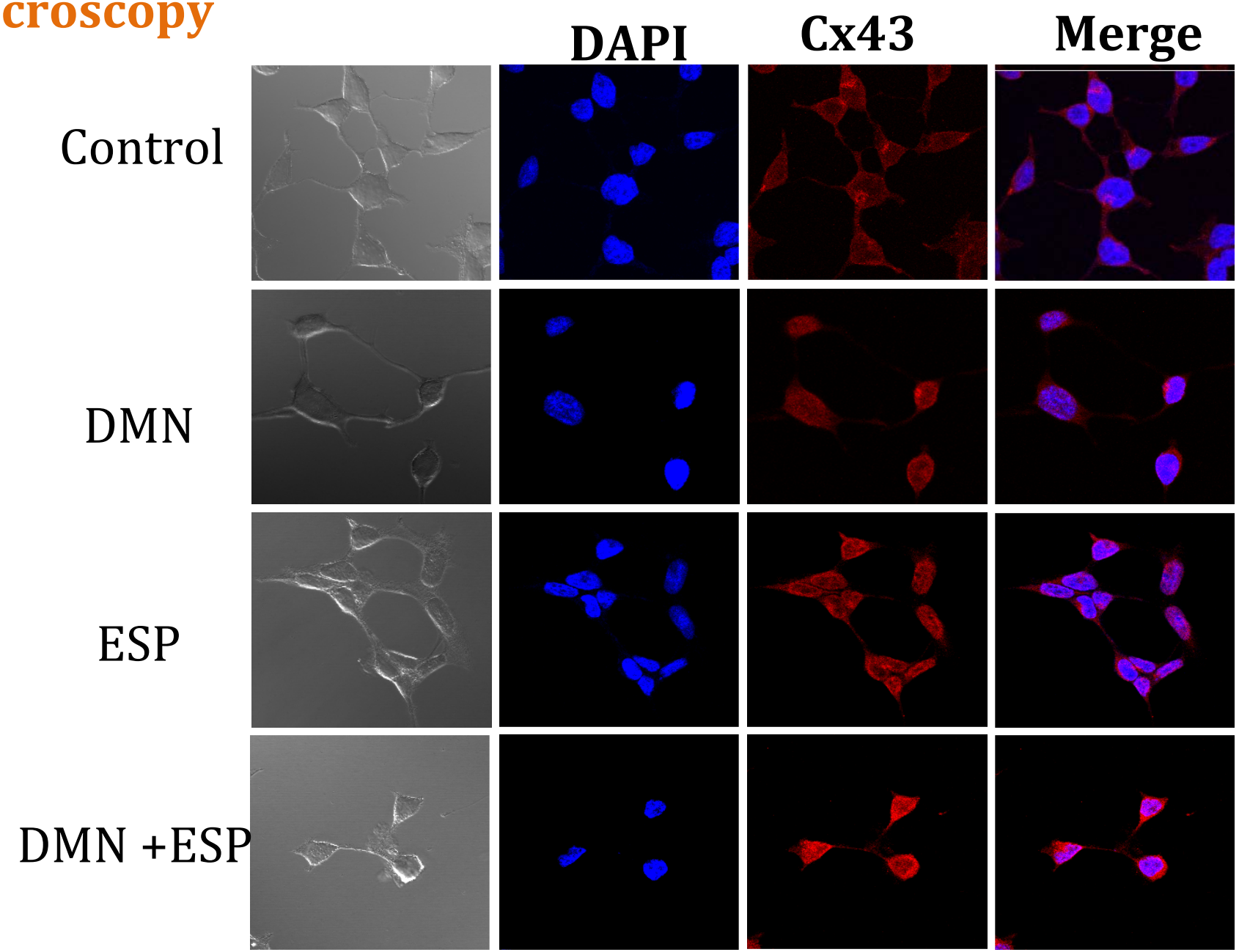


Fig. 9. Concentration of intracellular connexin 43 measured by laser scanning microscopy LSM PASCAL (Carl Zeiss)

Cancer related Molecules

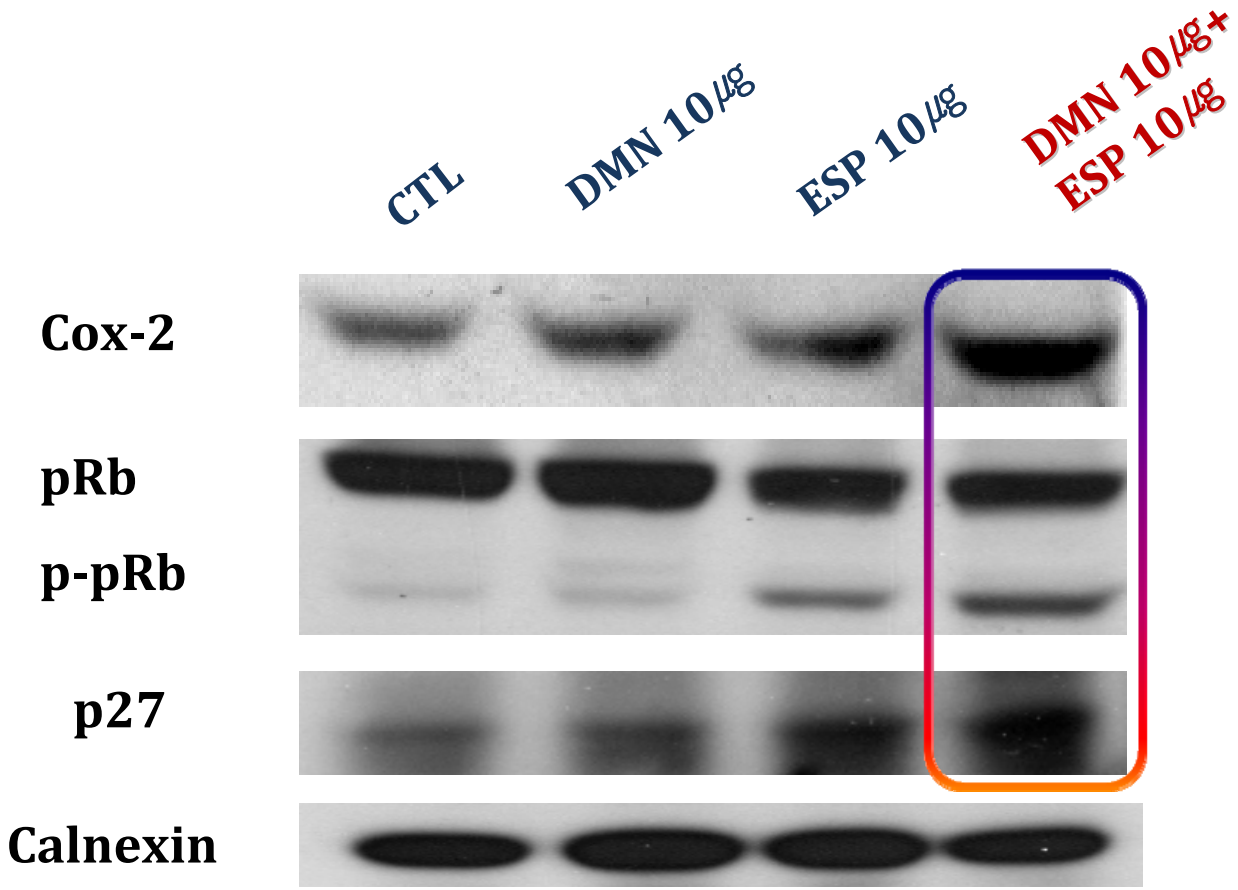


Fig 10. Expression of cancer related proteins in four groups

Cox-2 - cancer related factor

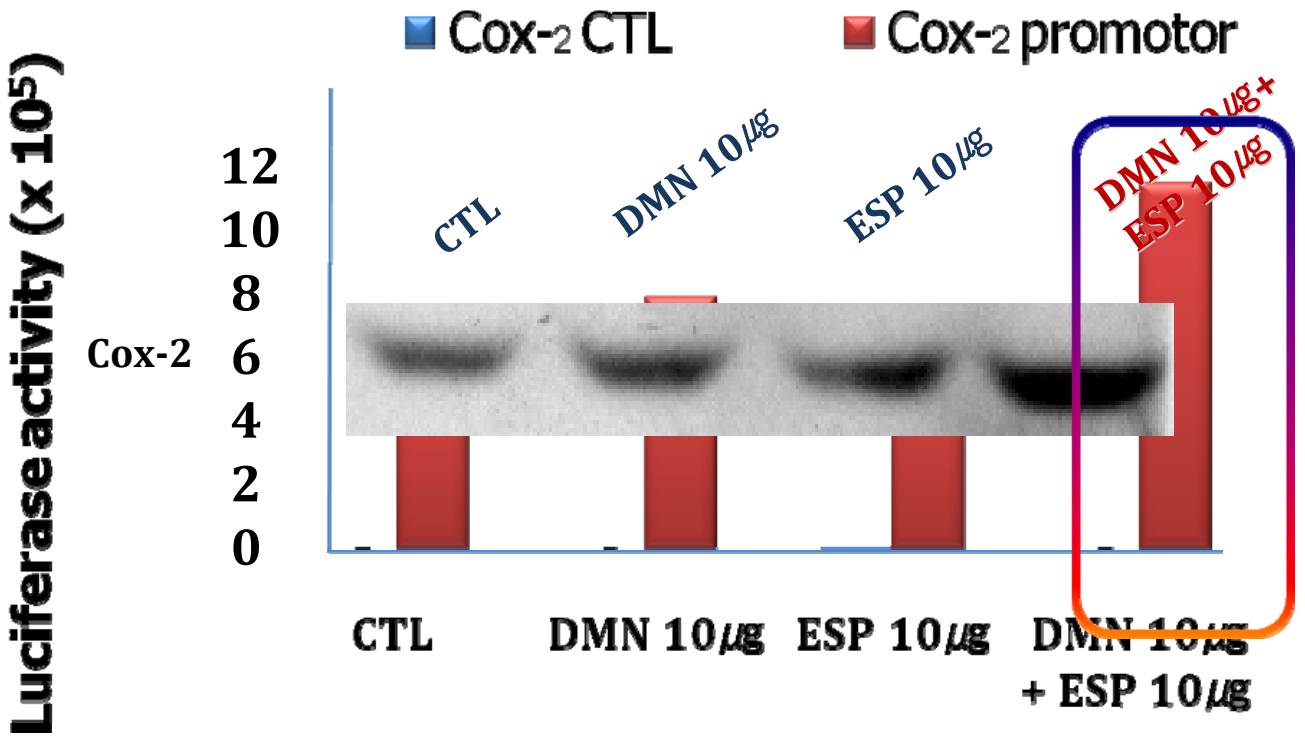


Fig 11. Treatment of ESP and DMN regulates COX-2 expression at transcriptional level.

In Vivo Experiments

- Group1. FVB mice
- Group2. FVB mice given DMN for six months
- Group3. FVB mice infected with 30 C.s MC for six months
- Group4. FVB mice infected with 30 C.s MC and given DMN for six months

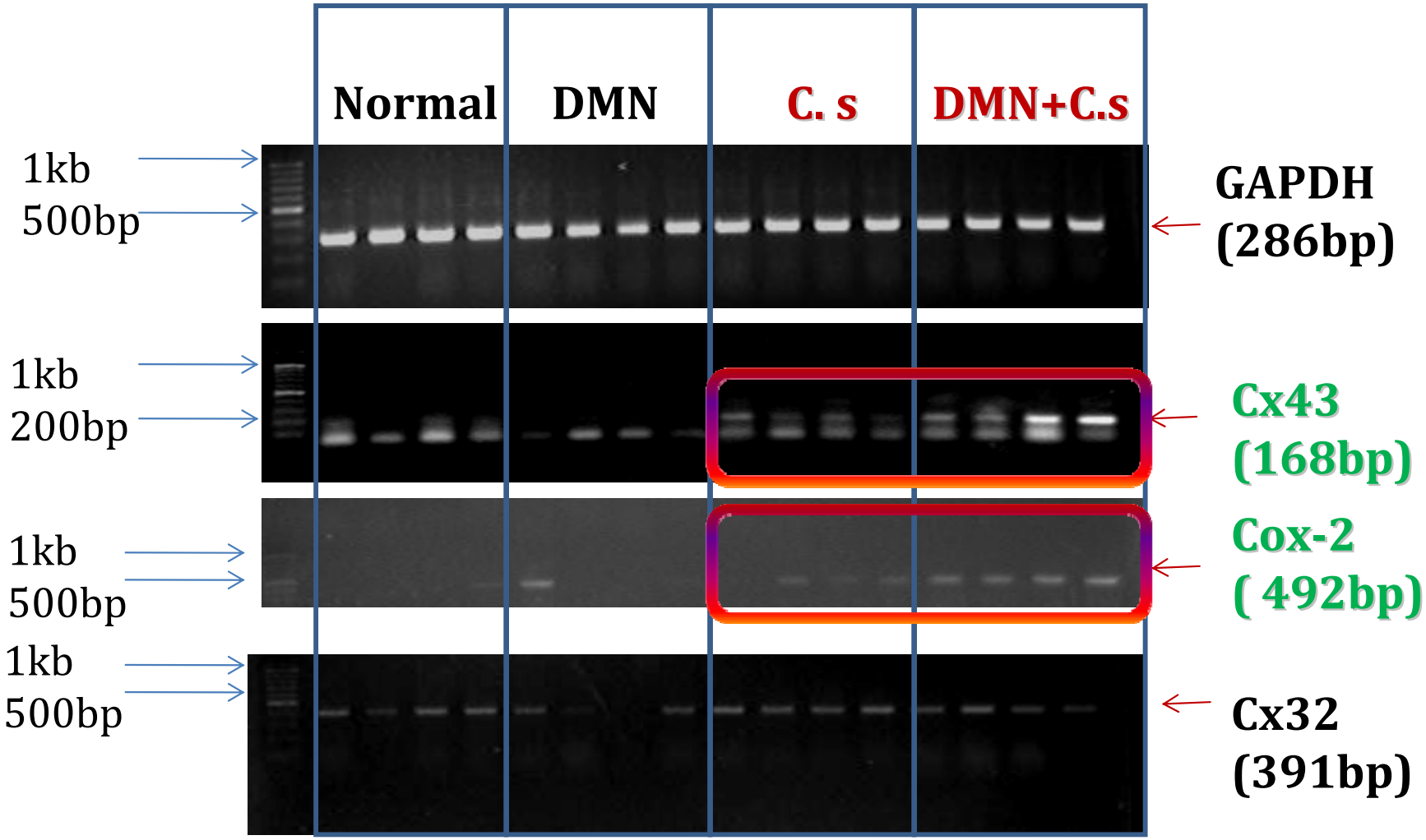


Fig 12. The expression of cancer related proteins in the liver of FVB mice

Conclusion

ES Products

Inflamation

Nitric oxide

Food

Nitrosamines

DNA damage

?

Inhibition of apoptosis

?

Cholangiocarcinoma

Uncontrolled cellular proliferation

E2F1 is a key factor

Malfunction of Gap junction

- Cx43↑ and Cx32↓

Cox-2, , p-pRb and p27

ขอบคุณค่ะ

Thank you