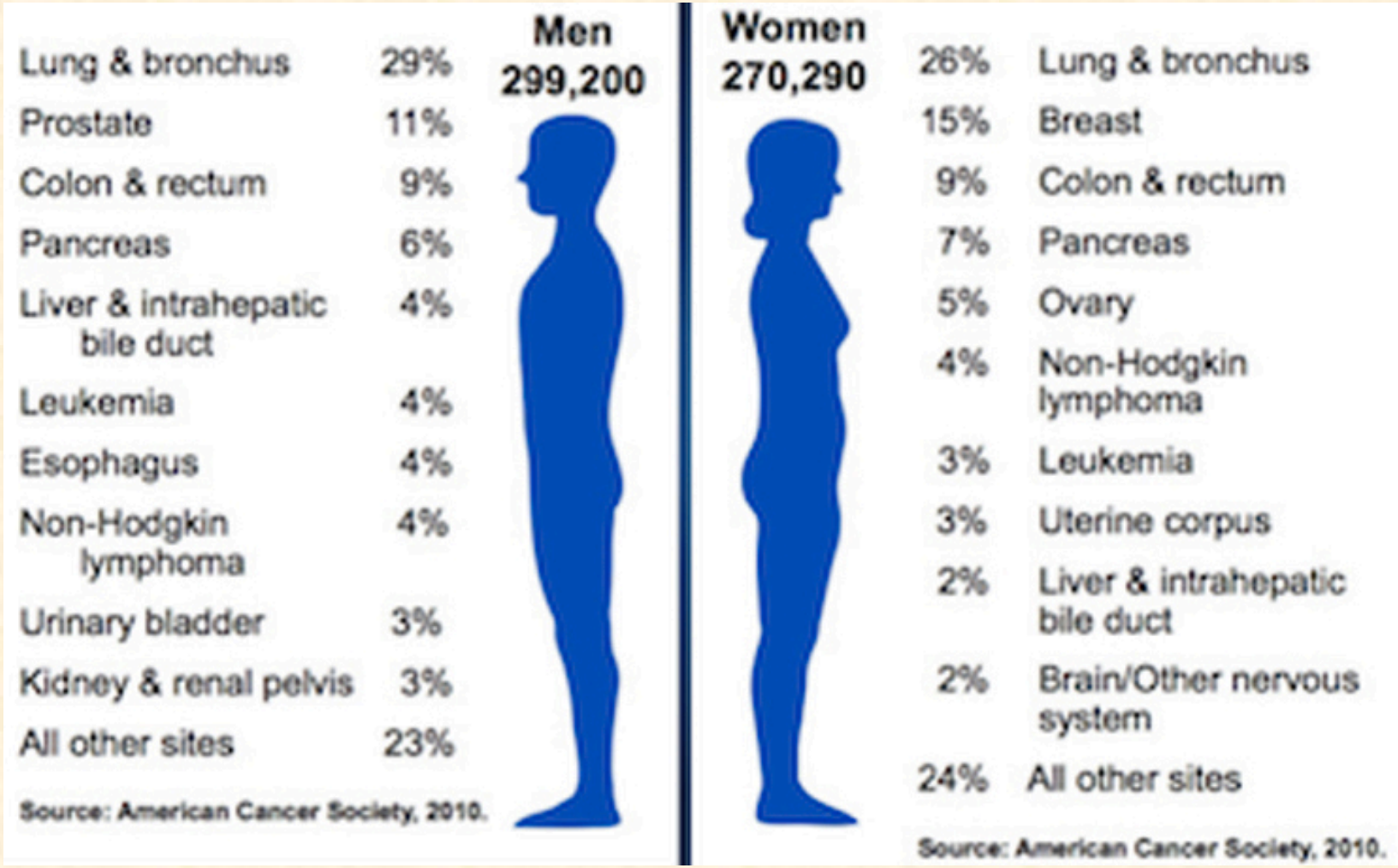
15-lipoxygenases and their metabolites as biomarkers for the early detection of smoking-induced non-small cell lung cancer

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Incidence of lung cancer



Etiology of lung cancer

Smokers

1. Smoking carcinogens: nicotine and its derivative N-nitrosamines, such as nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN). - associated with 70-90% of lung cancer.
2. Genetic factor.
3. Other unidentified factors.

Non-smokers

1. Environmental: environmental tobacco smoke (ETS) or environmental smoke (ES) such as passive/second-hand smoking and emissions from high-temperature frying. - the major factor.
2. Hormonal: estrogen and its receptors.
3. Genetic: family history, racial differences.
4. Viral: human papilloma virus (HPV), jazz siekte sheep retrovirus (JSRV).
5. Dietary.
6. Life style.
7. Previous lung diseases.

Histologic classification of lung cancer

Non-small cell lung carcinoma (NSCLC) (~80%)

Adenocarcinoma (40%)
Large cell carcinoma (25%)
Squamous cell carcinoma (10%)
Others (adenosquamous carcinoma, sarcomatoid carcinoma) (<5%)

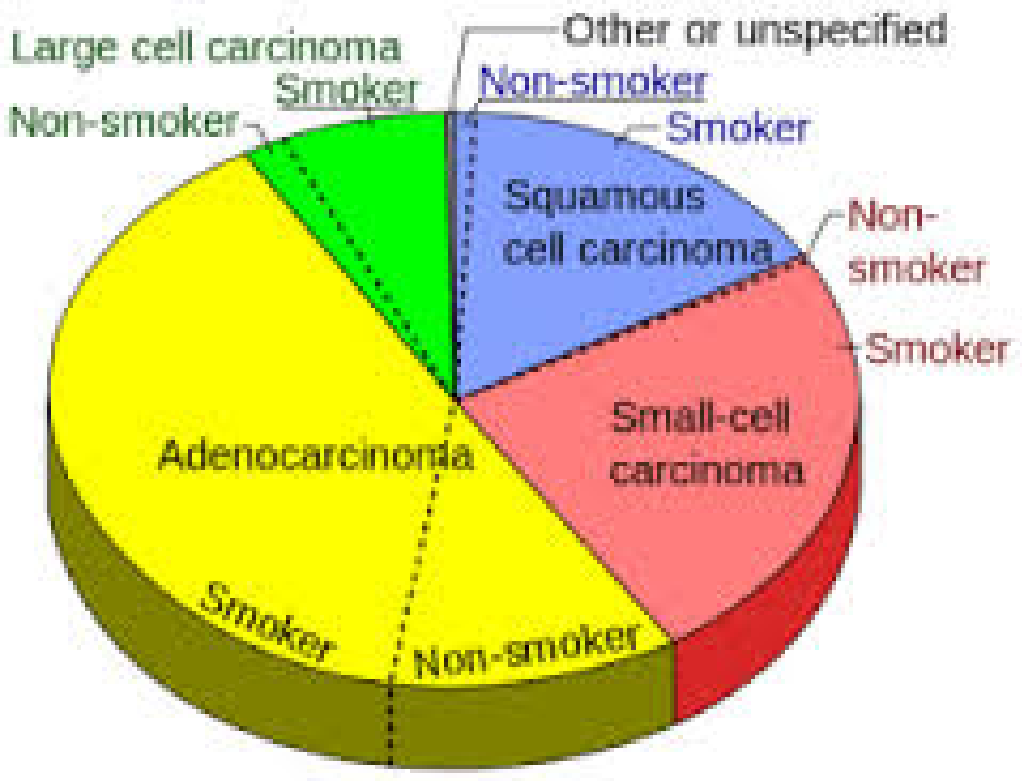
Small cell lung carcinoma (SCLC) (~20%)

Other types of lung cancers (<1%)

Lung carcinoid cancer
adenoid cystic carcinomas
Hamartomas
Lymphomas
sarcomas

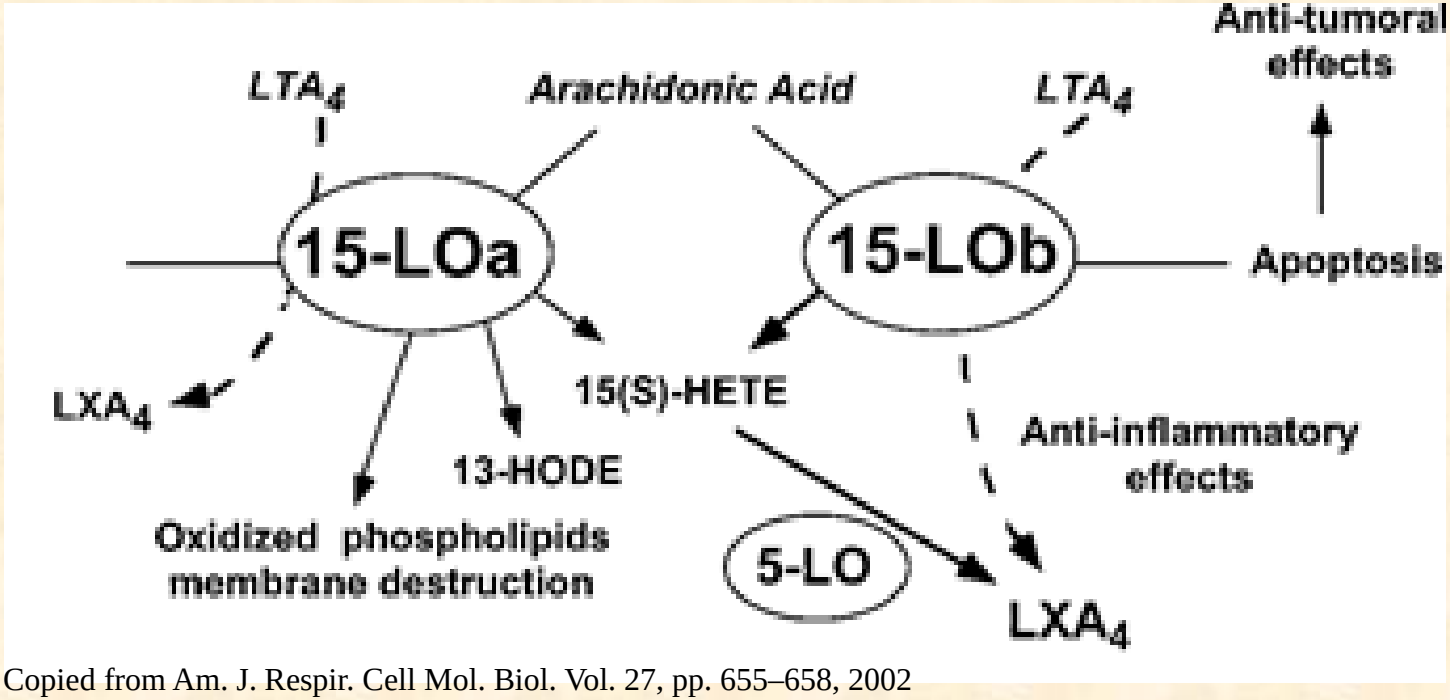
American Family Physician 2007, 75:56-63.

Lung cancer histologic types related to smokers and non-smokers

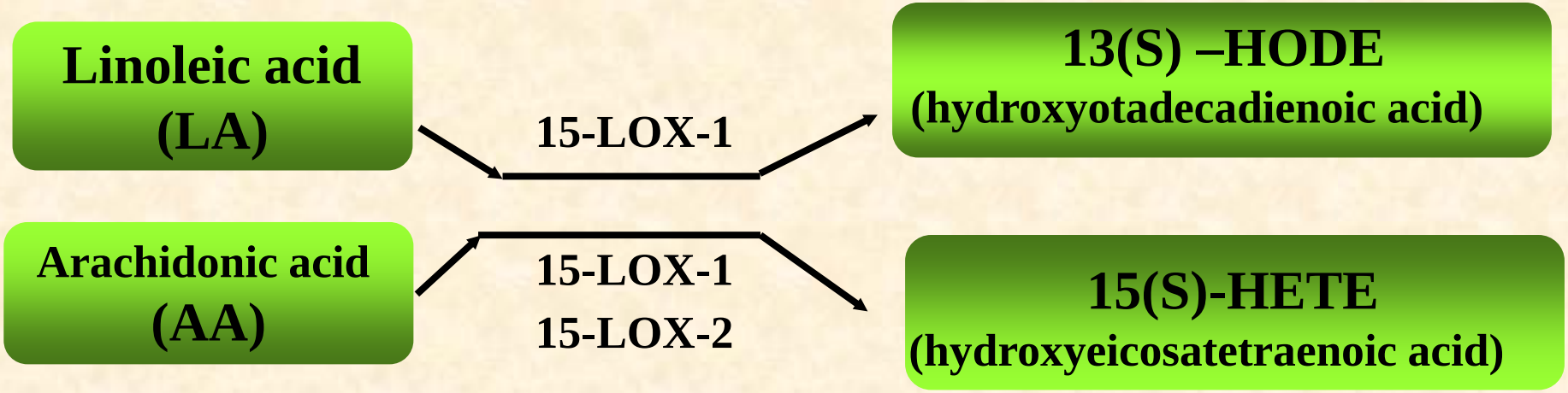


What are 15-lipoxygenases and their metabolites

15-lipoxygenases (LOXs) are members of non-heme iron-containing dioxygenases. In human, 2 isoforms:
15-LOX1 (15-LOa)
15-LOX2 (15-LOb)
In mice, only one form: 12/15-LOX - the murine ortholog to human 15-LOXs.



Copied from Am. J. Respir. Cell Mol. Biol. Vol. 27, pp. 655–658, 2002

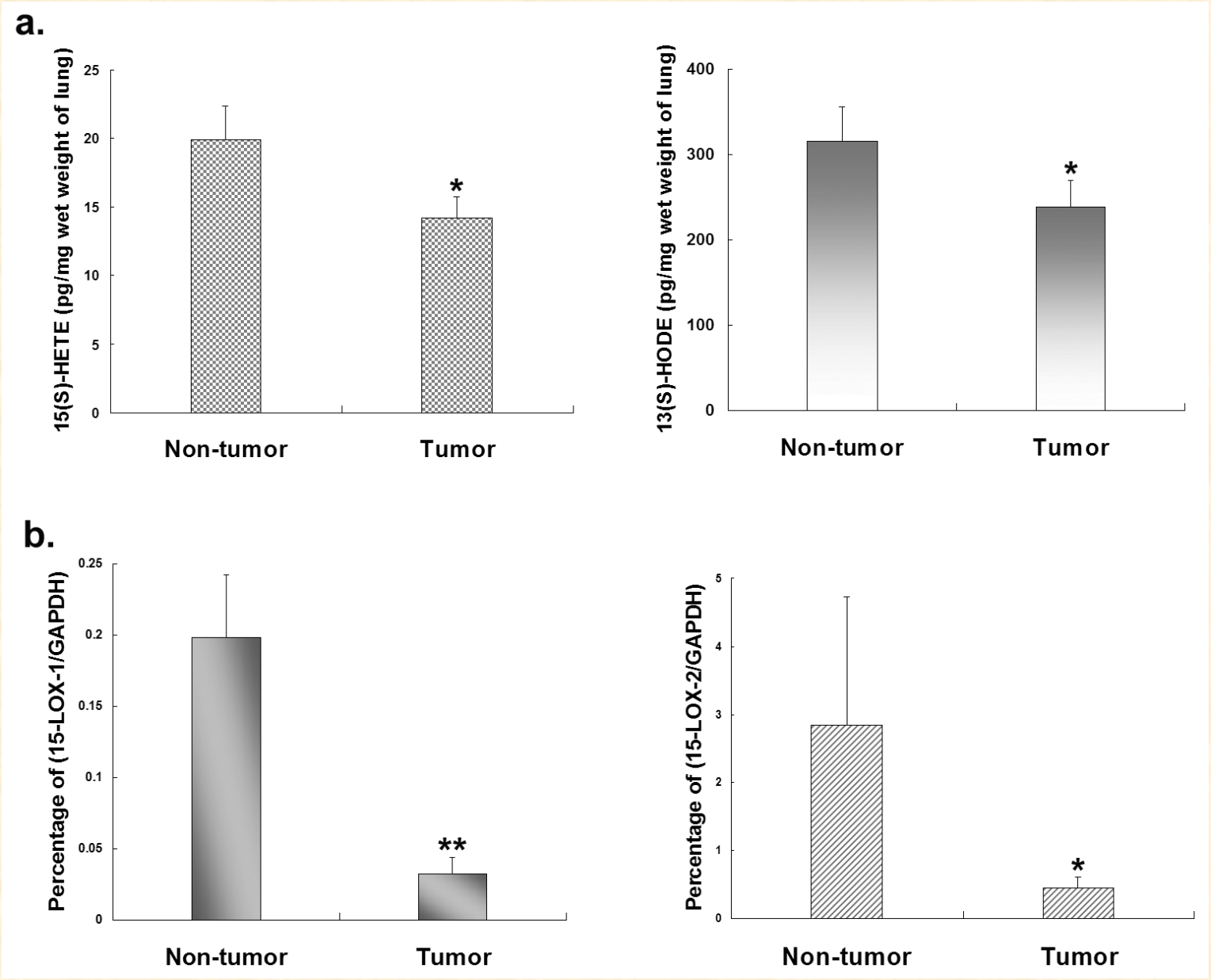

Linoleic acid
(LA)
15-LOX-1
13(S) –HODE
(hydroxyotadecadienoic acid)
Arachidonic acid
(AA)
15-LOX-1
15-LOX-2
15(S)-HETE
(hydroxyeicosatetraenoic acid)

Why were 15-lipoxygenases and their metabolites selected for the study?

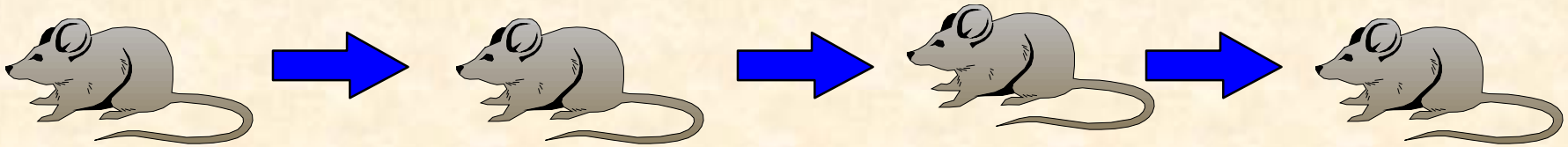
The metabolites of 15-lipoxygenases, 15S-HETE and 13S-HODE, are the endogenous ligands of peroxisome proliferator-activated receptor gamma (PPARg), whose activity is significantly reduced in lung cancer, particularly, smoking-related NSCLC.

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Levels of 15(S)-HETE, 13(S)-HODE, 15-LOX-1 and 15-LOX-2 in human lung tissues



NNK-induced lung tumors in A/J mice



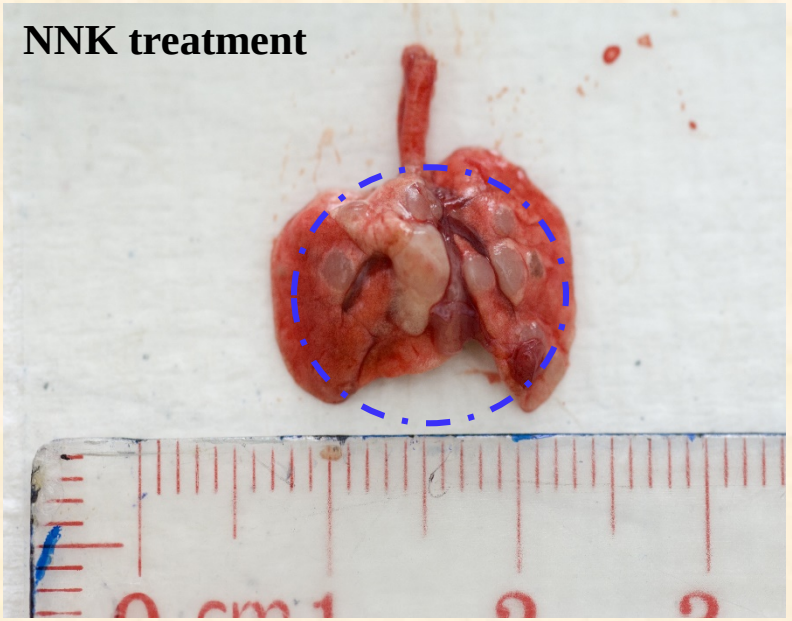
6 weeks old
A single dose of
NNK 100mg/kg
Peritoneal injection (i.p.)

Week 20-24
Epithelial/alveolar
hyperplasia

Week 34
Adenocarcinoma

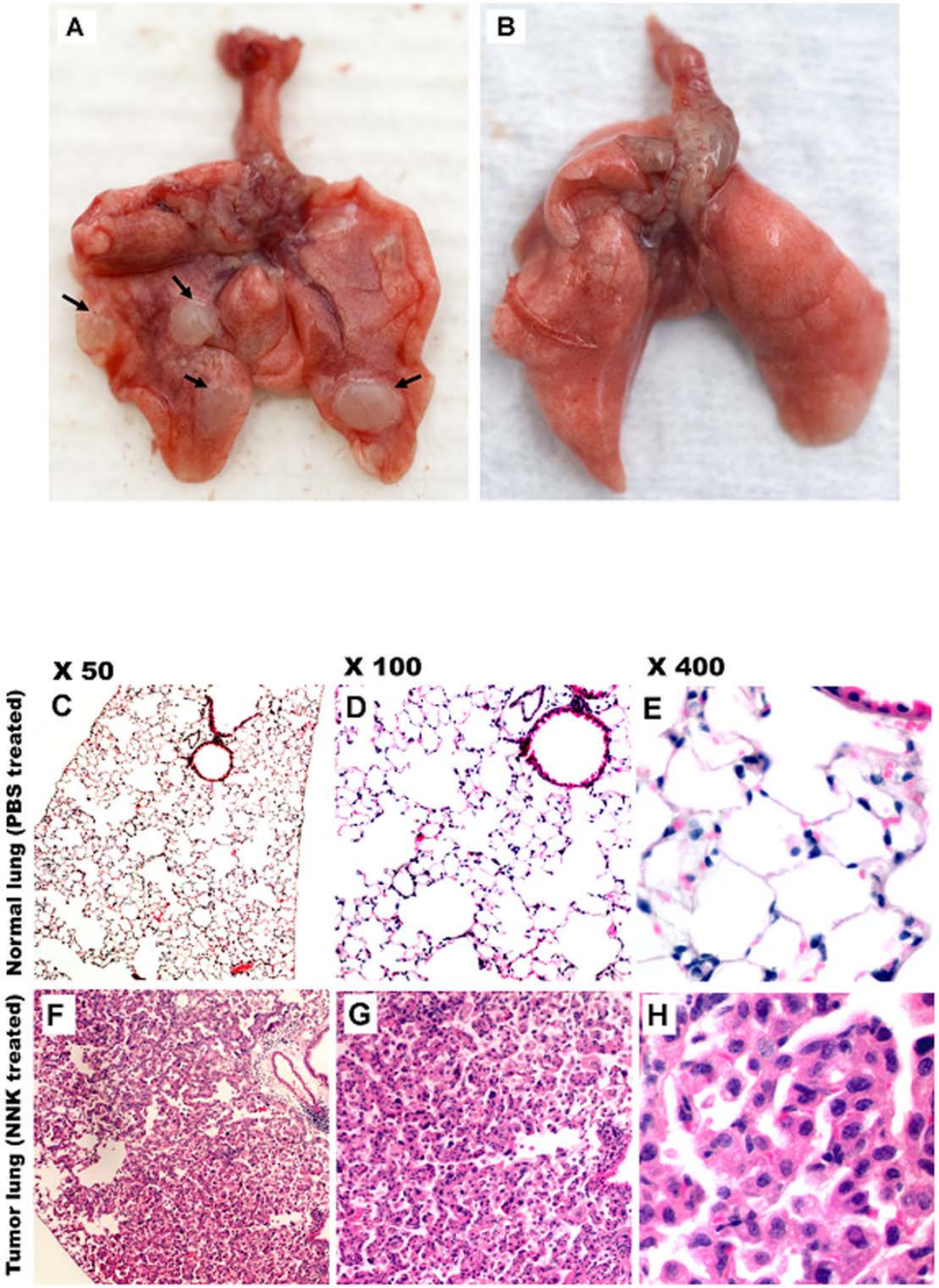
Week 38
End of experiments





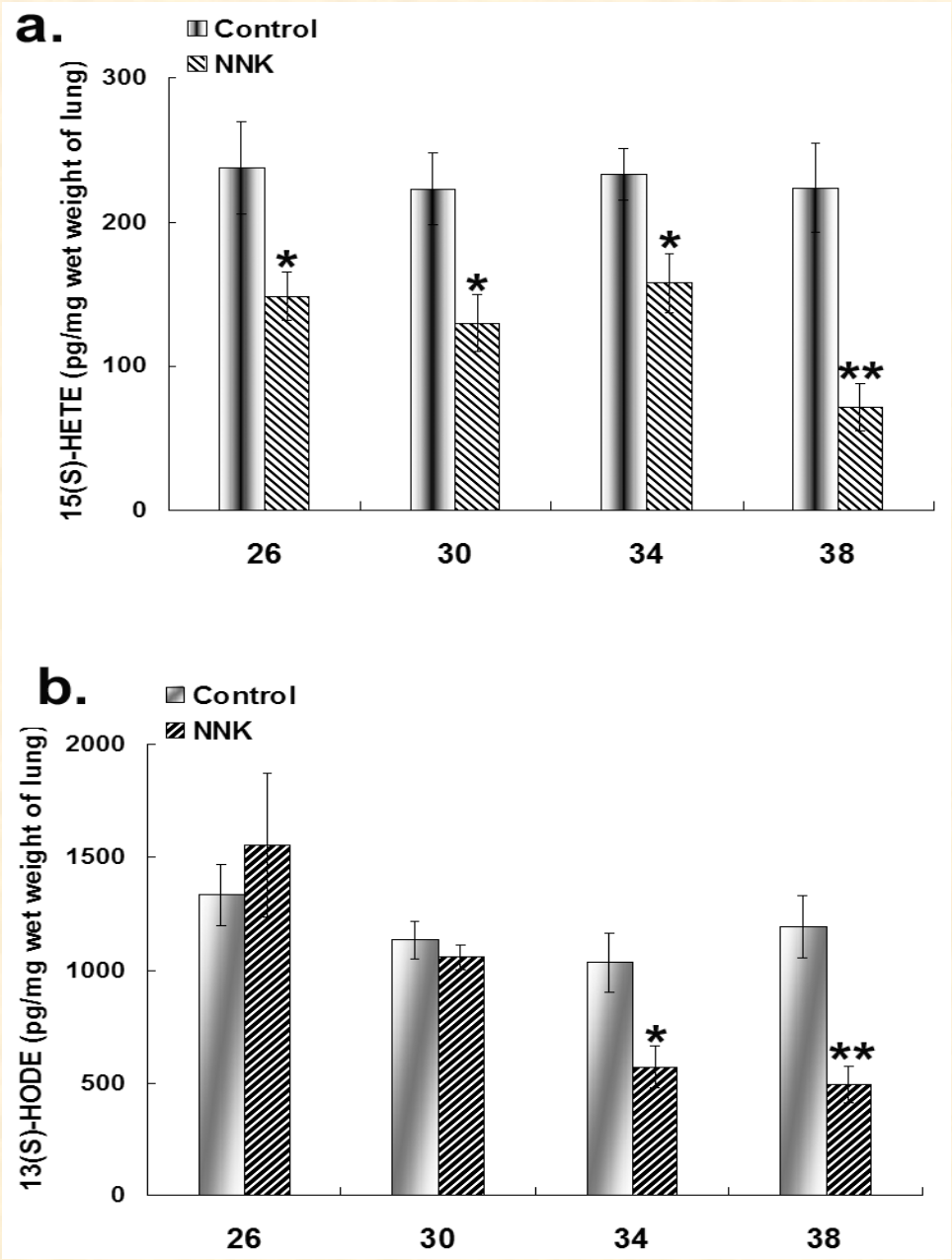
NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a major cigarette smoking carcinogen.

Fig. 2.



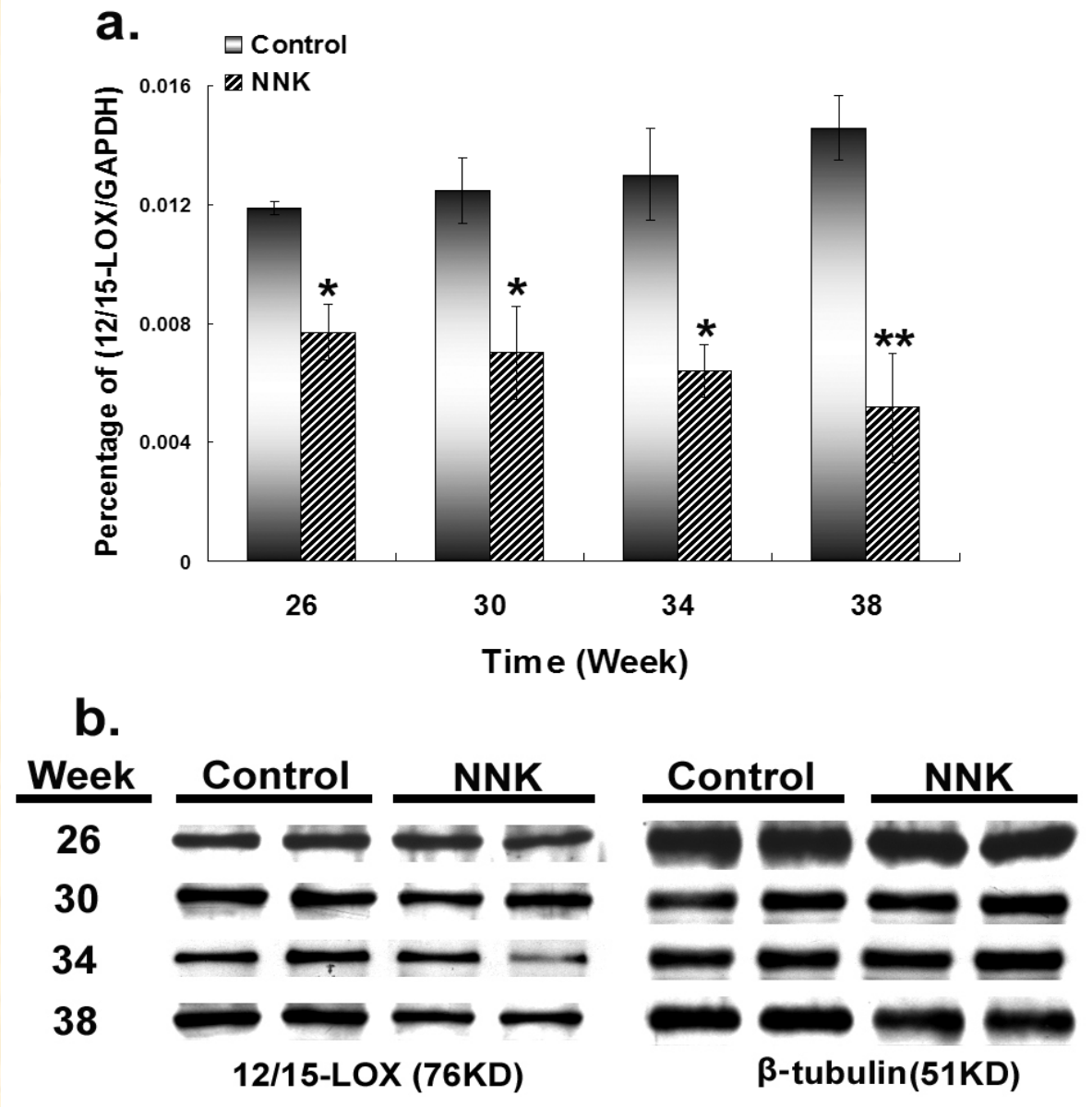
Histopathology of lungs in control and NNK-treated group

Levels of 15(S)-HETE and 13(S)-HODE in lung tissues of A/J mice

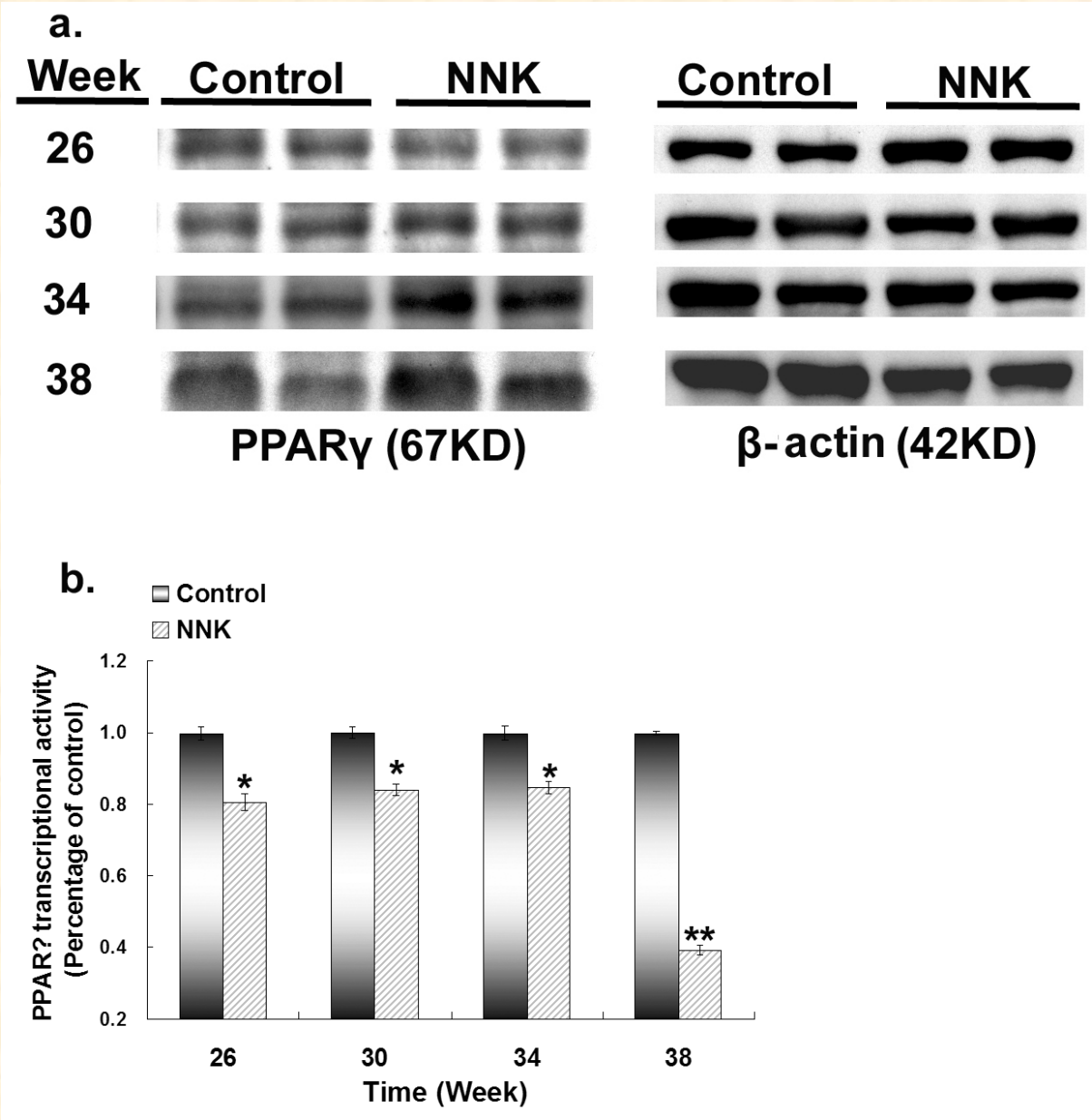


Levels of 12/15-LOX in lung tissues of A/J mice

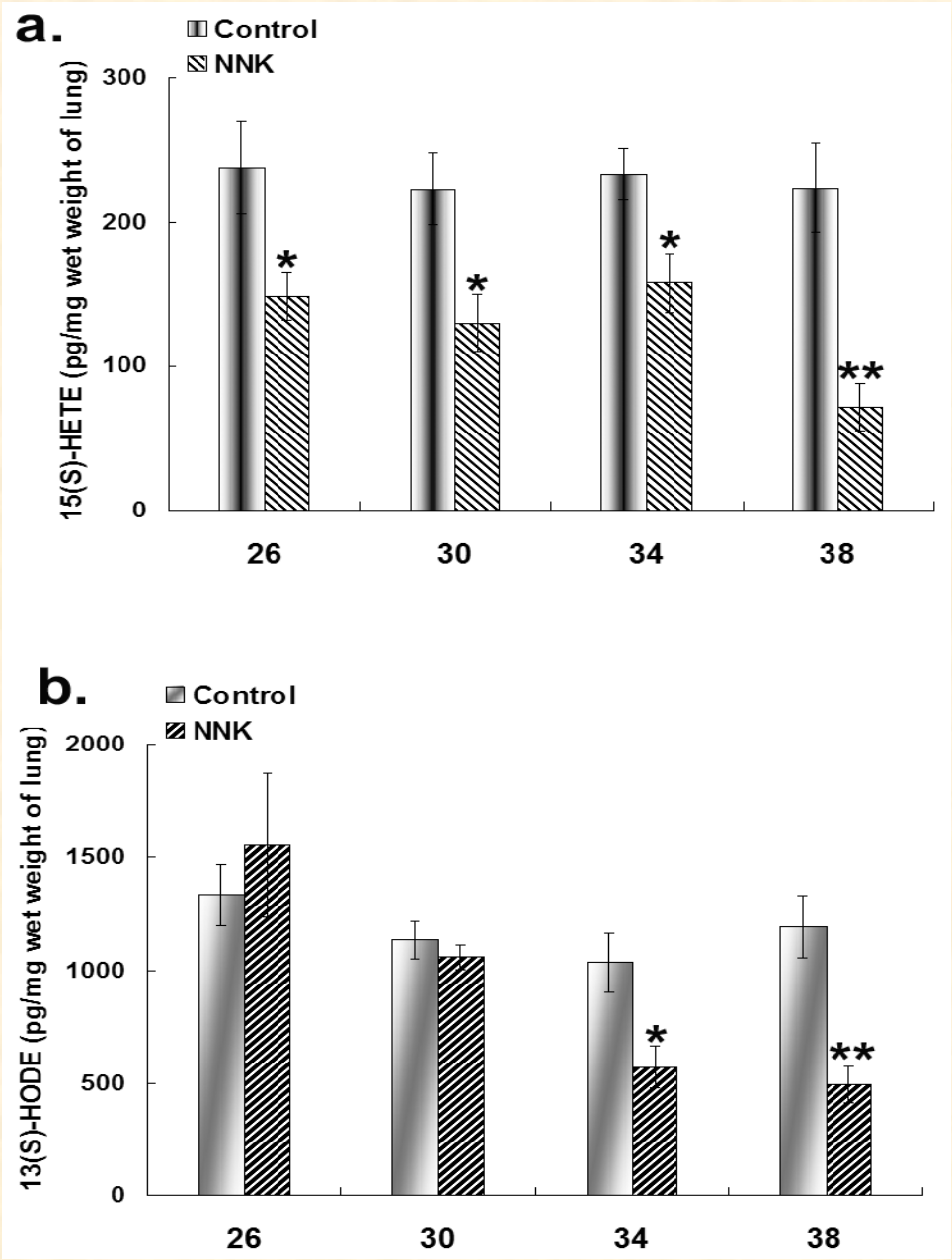
(12/15-LOX is ortholog to human 15-LOXs)



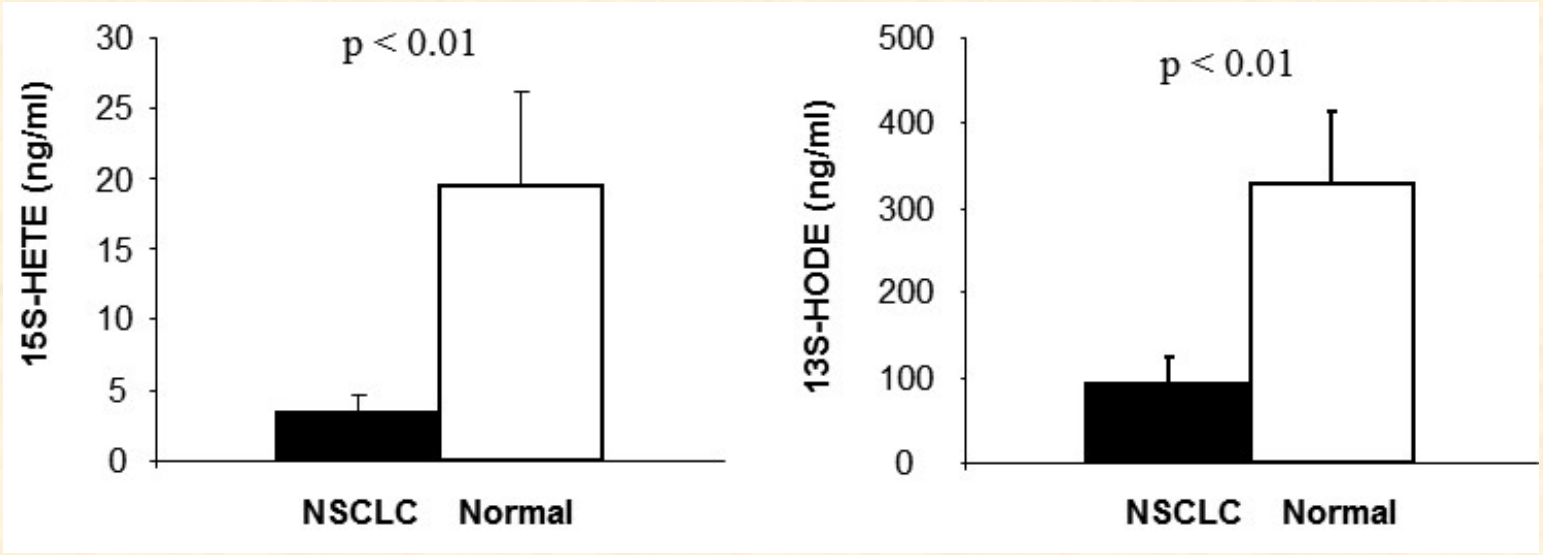
Expression of PPARγ protein and PPARγ transcriptional activity during NNK-mediated lung tumorigenesis in A/J mice



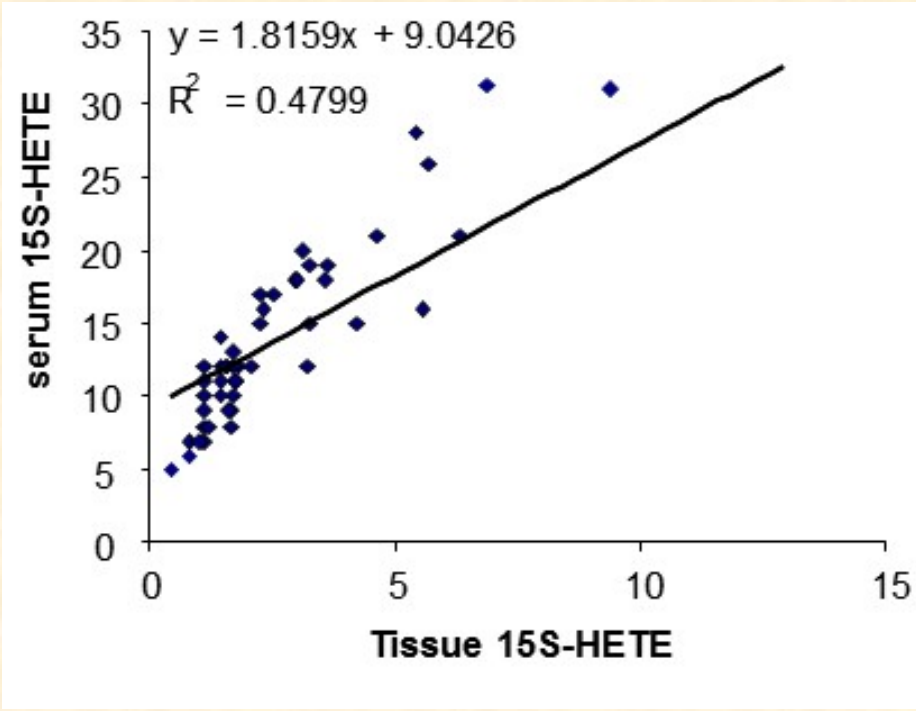
Levels of 15(S)-HETE and 13(S)-HODE in lung tissues of A/J mice

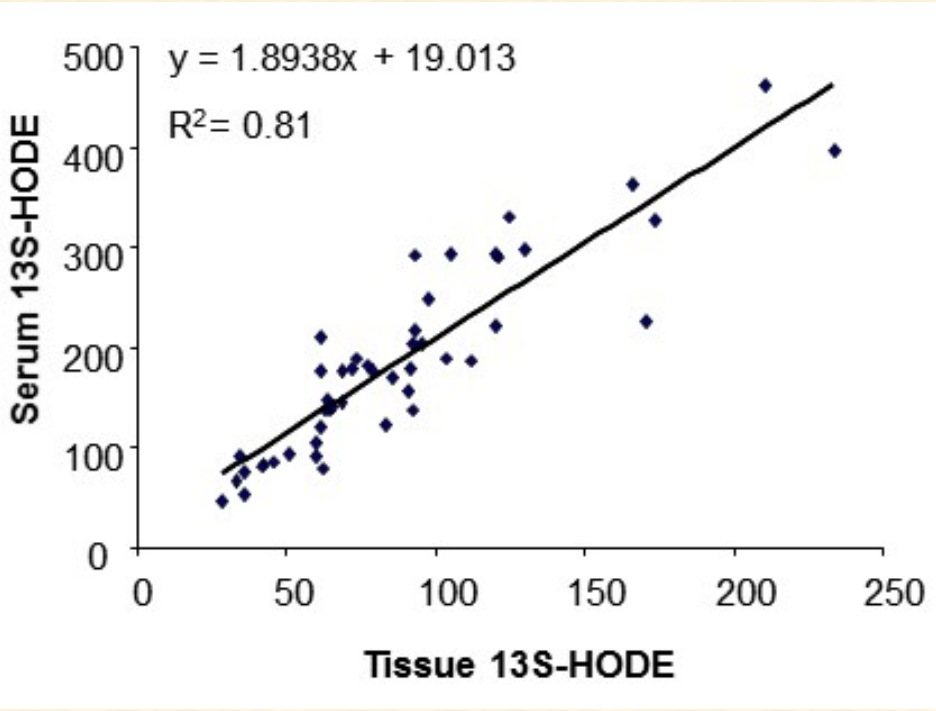


Serum 15S-HETE and 13S-HODE in patients with NSCLC

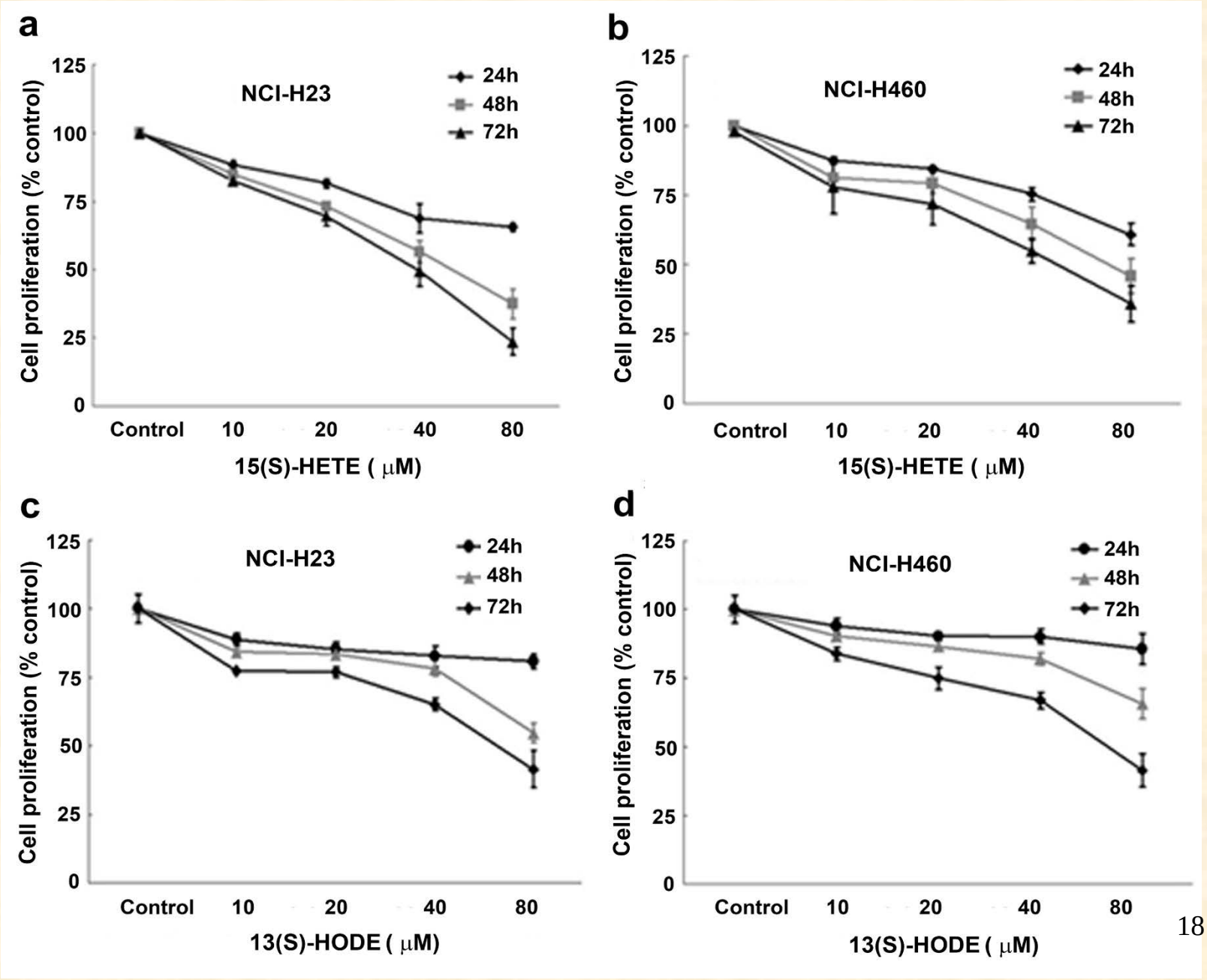


Correlation between Serum 15S-HETE/13S-HODE and tissue15S-HETE/13S-HODE in patients with NSCLC

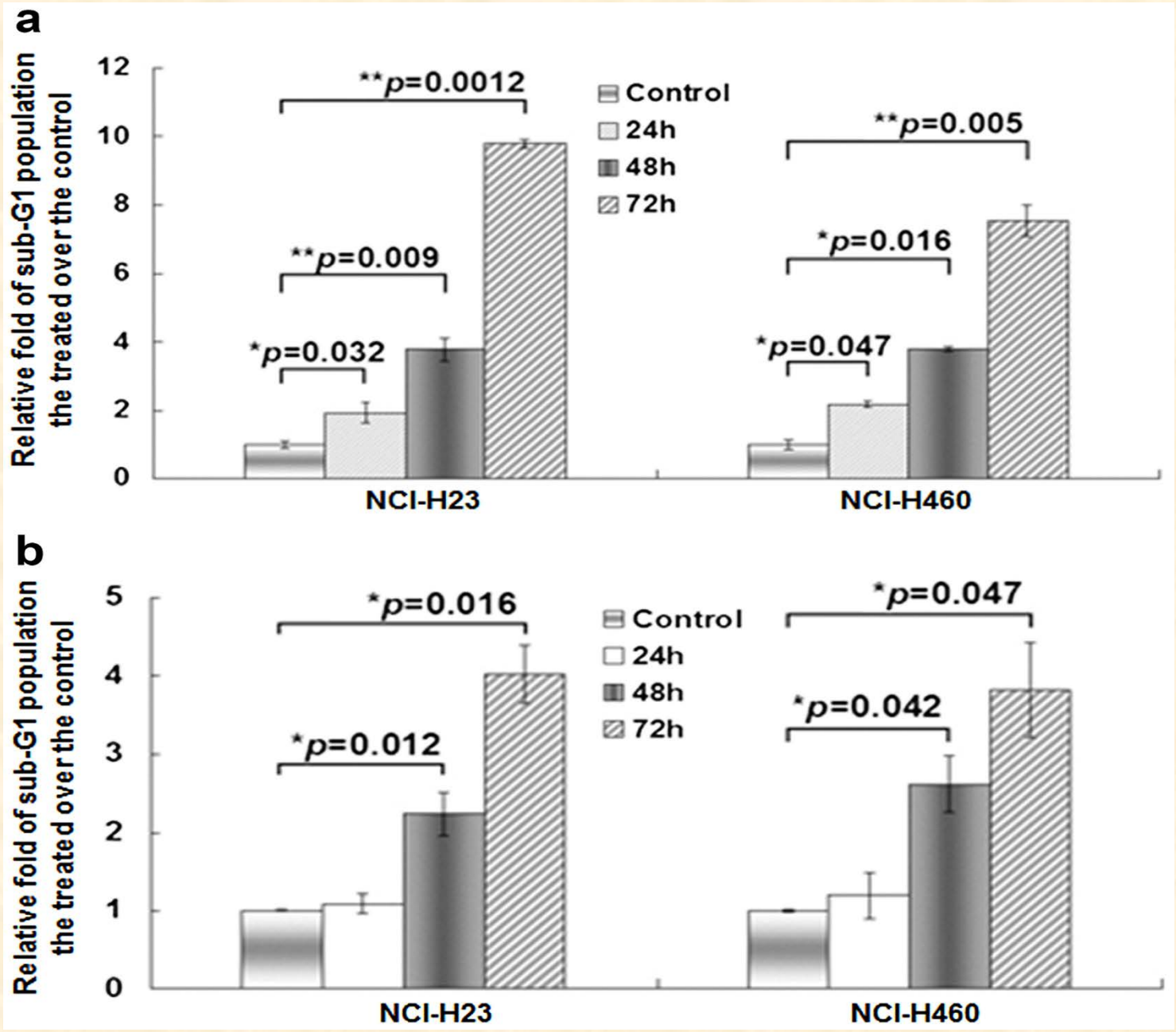




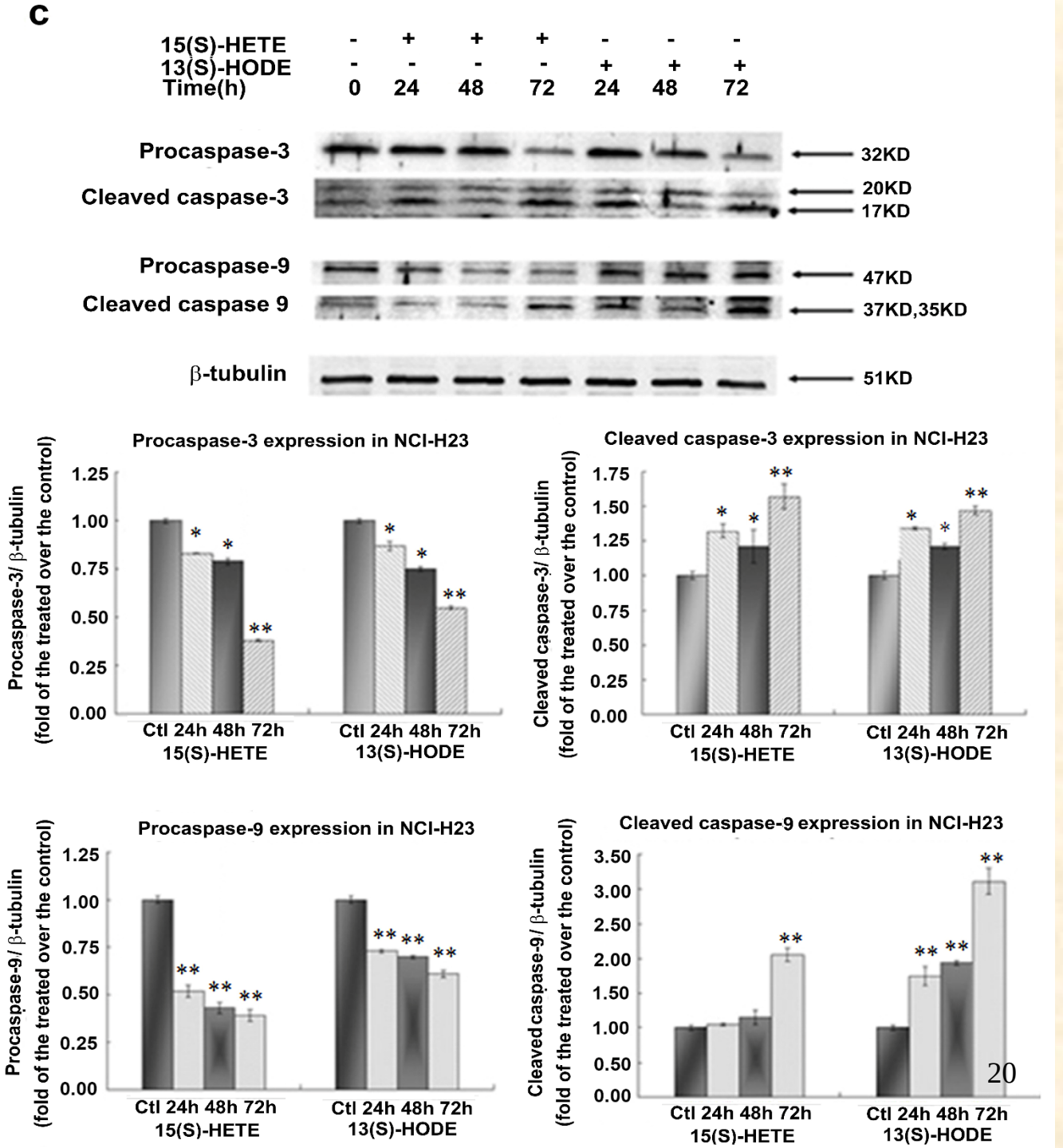
Effects of 15(S)-HETE and 13(S)-HODE on the proliferation of human NSCLC cells



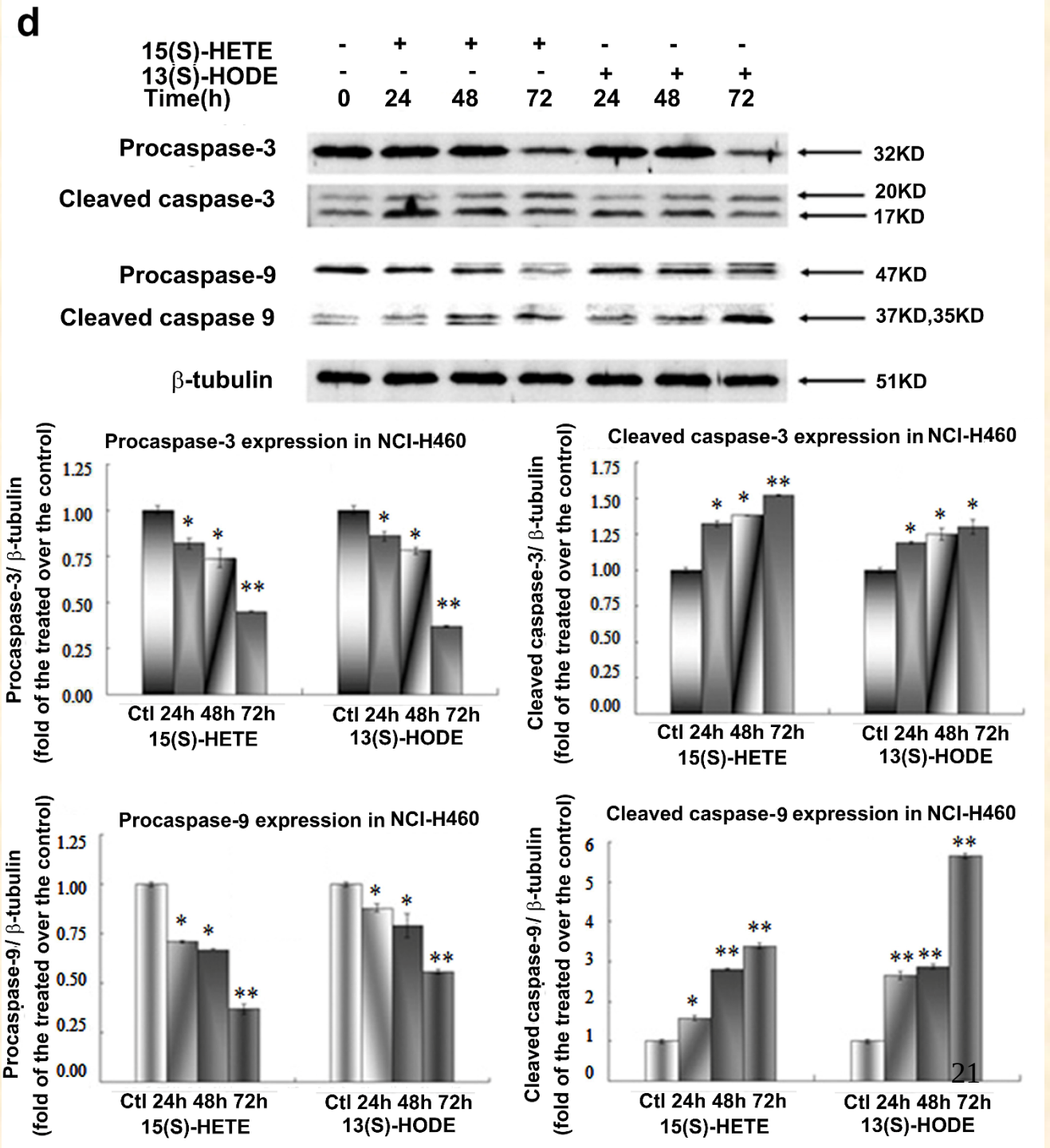
Induction of apoptosis by 15(S)-HETE and 13(S)-HODE – sub-G1 population



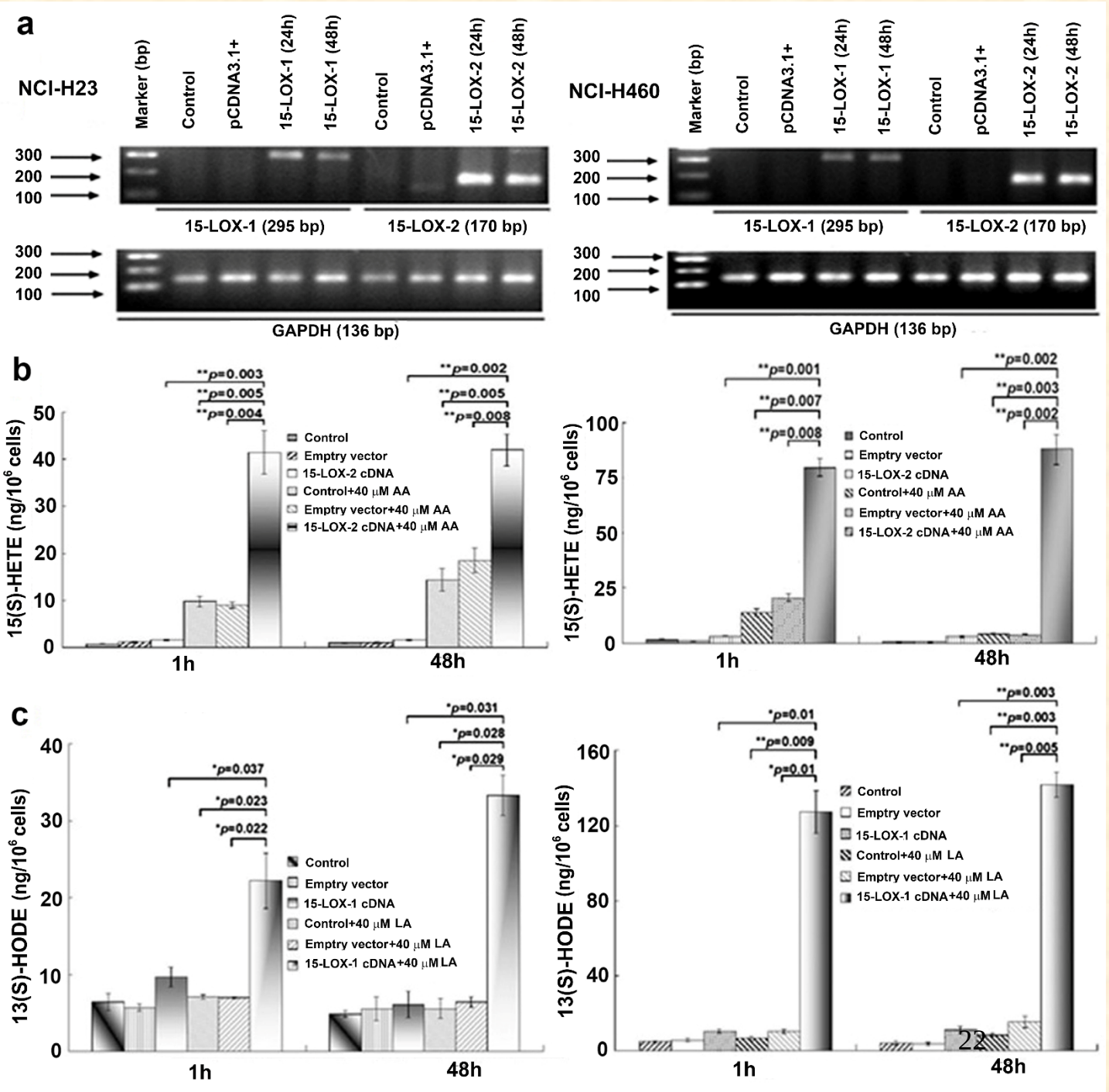
Induction of apoptosis by 15(S)-HETE and 13(S)-HODE – caspases in NCI-23



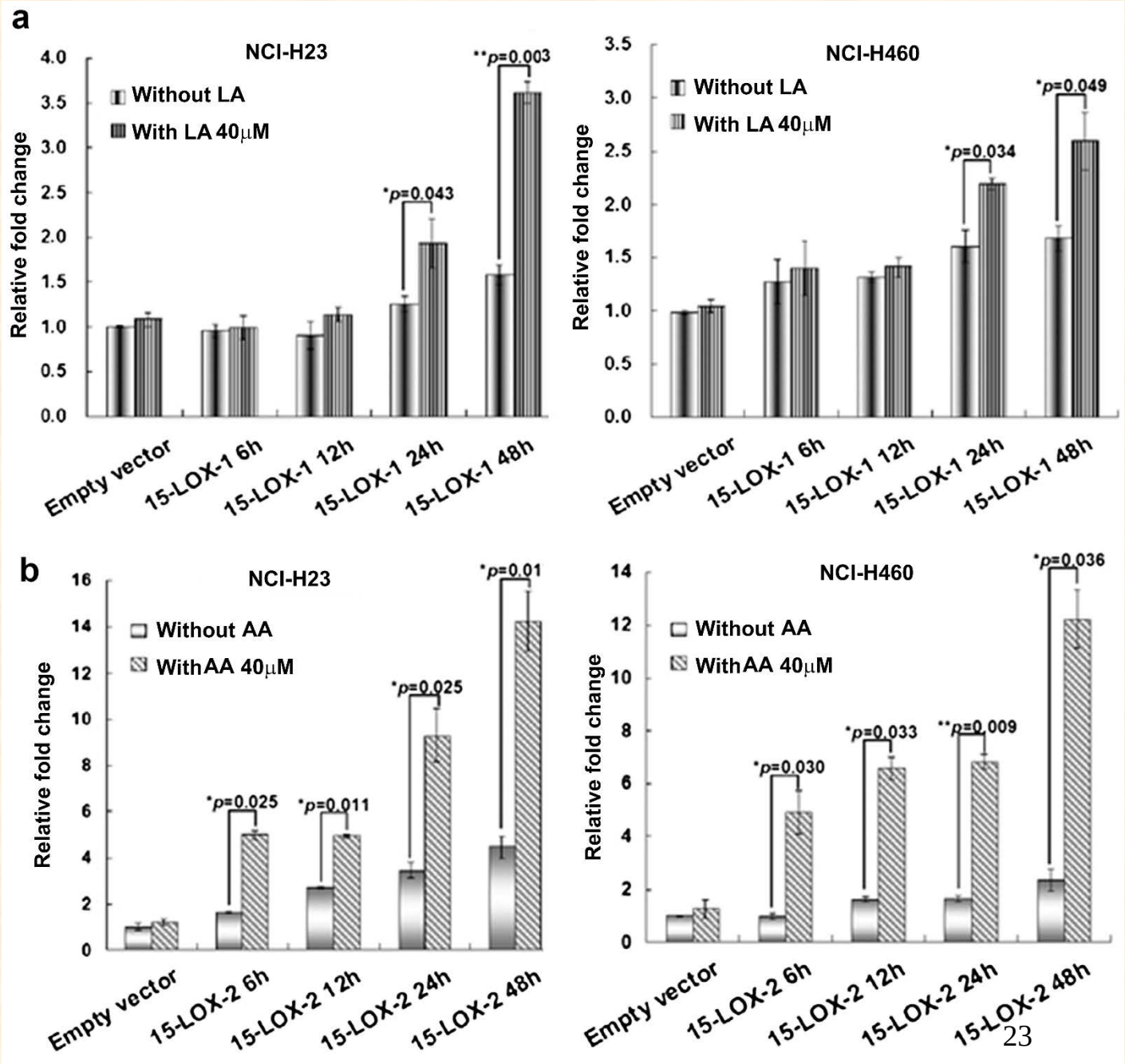
Induction of apoptosis by 15(S)-HETE and 13(S)-HODE – caspases in NCI-460



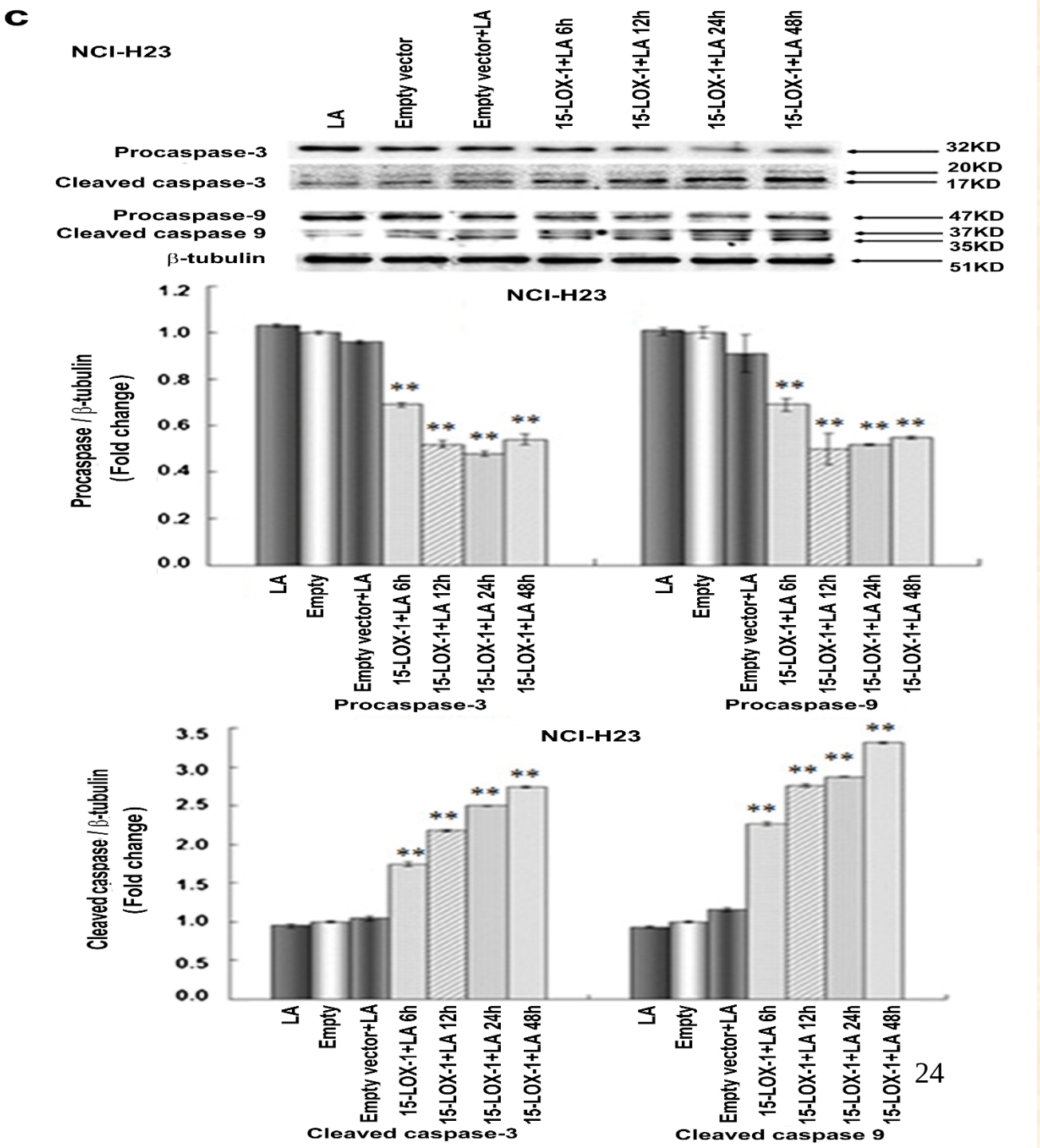
Restoration of 15-LOX-1 and 15-LOX-2 increases the levels of 15(S)-HETE and 13(S)-HODE.



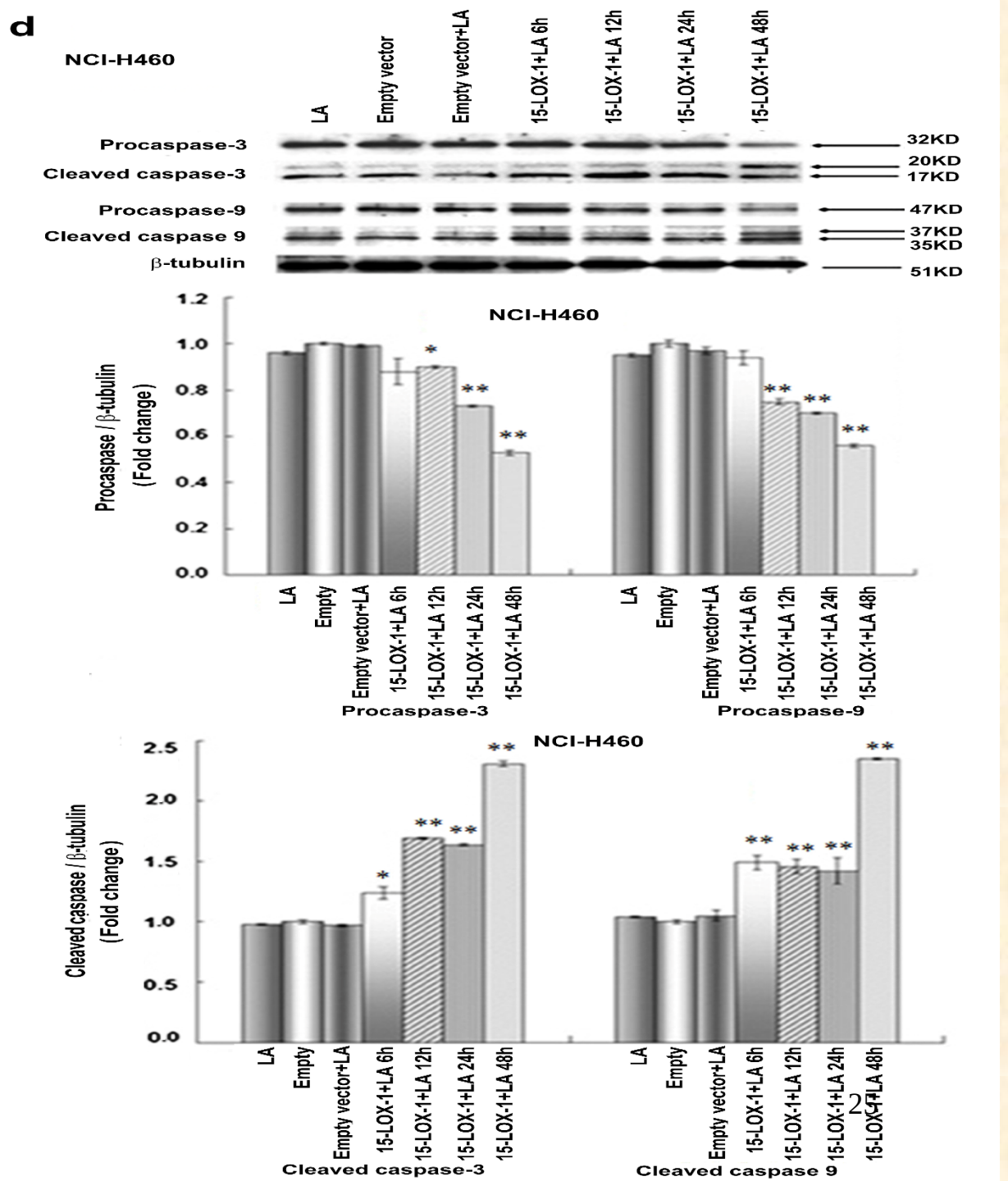
Restoration of 15-LOX-1 and 15-LOX-2 induces apoptosis – sub-G1 population.



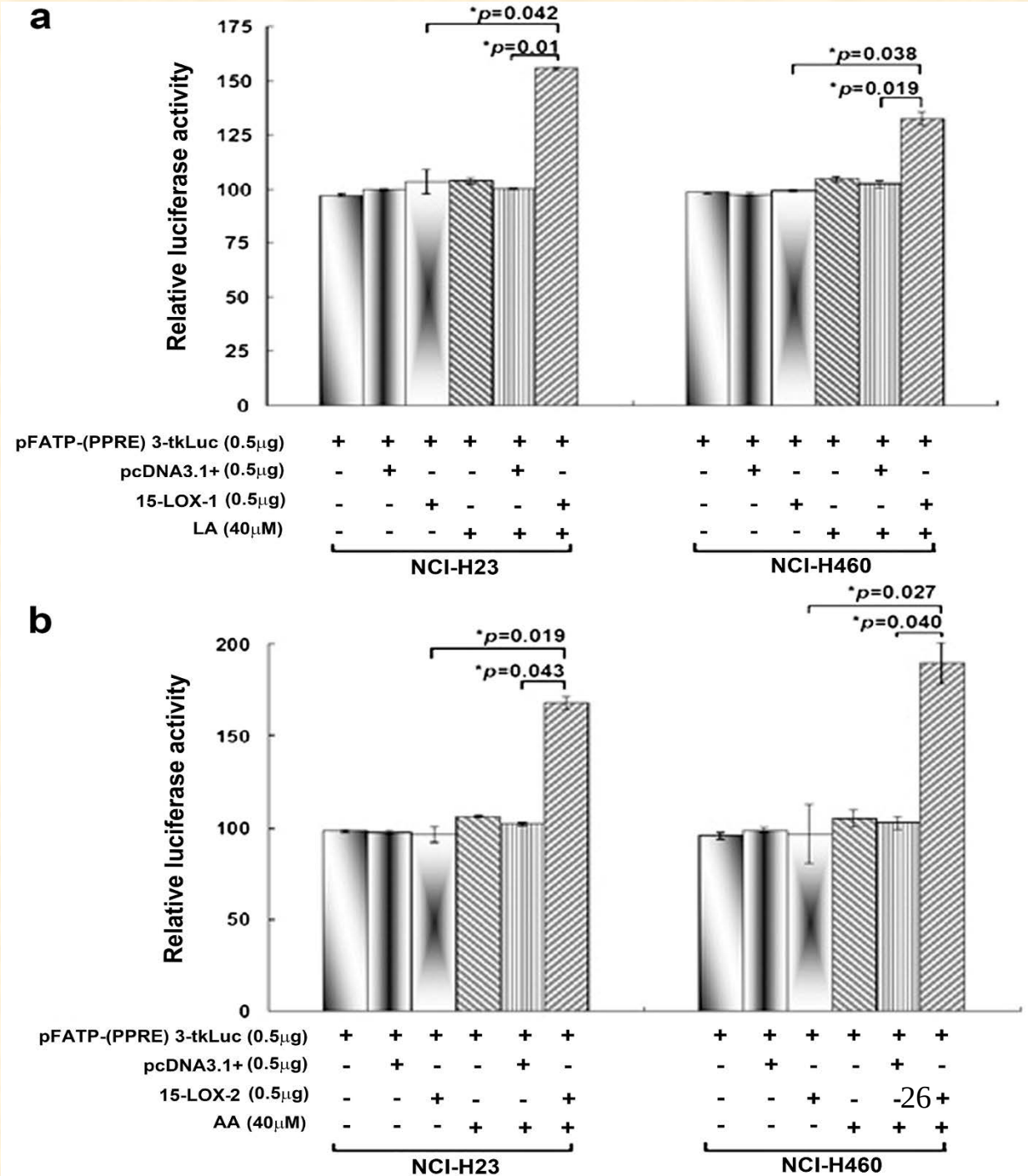
Restoration of 15-LOX-1 and 15-LOX-2 induces apoptosis – caspases in NCI-23.



Restoration of 15-LOX-1 and 15-LOX-2 induces apoptosis – caspases in NCI-H460.



Restoration of 15-LOX-1 and 15-LOX-2 enhances PPARγ activity - luciferase assay.



Conclusions

1. **The levels of 15-LOX-1 and -2 were significantly decreased in lung tissues of human NSCLC compared with the matched non-tumor lung tissues.**
2. **The levels of 15S-HETE and 13S-HODE, the metabolites of 15-LOX-1 and -2, were reduced in the blood of NSCLC patients compared with normal subjects.**
3. **The reduction of 15-LOX, 15S-HETE and 13S-HODE predated the appearance of mouse lung tumor induced by tobacco smoking.**
4. **15(S)-HETE and 13(S)-HODE or 15-LOX-1 and 15-LOX-2 can inhibit the proliferation and growth of human NSCLC cells.**
5. **Strategies to restore 15-LOXs activities and increase the production of endogenous 15(S)-HETE and 13(S)-HODE may offer a novel research direction for the molecular targeting treatment and prevention for smoking-related NSCLC.**

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Thank you!