

Lysyl oxidase and enhancement of cell proliferation and angiogenesis in oral squamous cell carcinoma

Hani Surianti, DDS, Yin-Hua Shih, MD, Kuo-Wei Chang, PhD, Michael Yuanchien Chen, DDS, MS, Cheng-Chia Yu, PhD, Dan-Jae Lin, PhD, Shih-Min Hsia, PhD, Heng-Li Huang, PhD, Tzong-Ming Shieh, PhD.

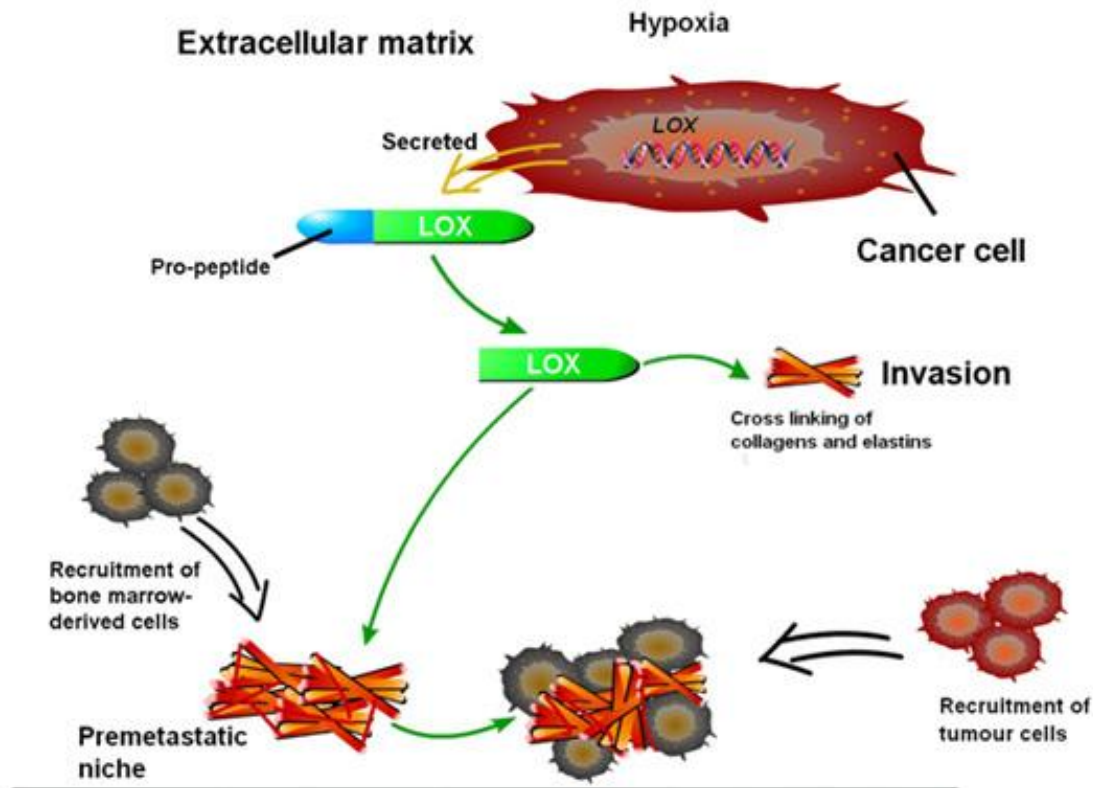


中國醫藥大學附設醫院
China Medical University Hospital

Introduction

- **Lysyl oxidase (LOX)** is a copper-dependent enzyme that cross-links collagen and elastin in extracellular matrix.

Introduction-2



LOX can be produced in response to hypoxia-induced factors, leading to increased invasion and promotion of metastasis to distant sites.

Introduction-3

- Increased soluble **copper** in oral fluids of long-term **betel nut** chewers may relate to pathogenesis and metastasis of oral squamous cell carcinomas (OSCC) through **LOX activation**.

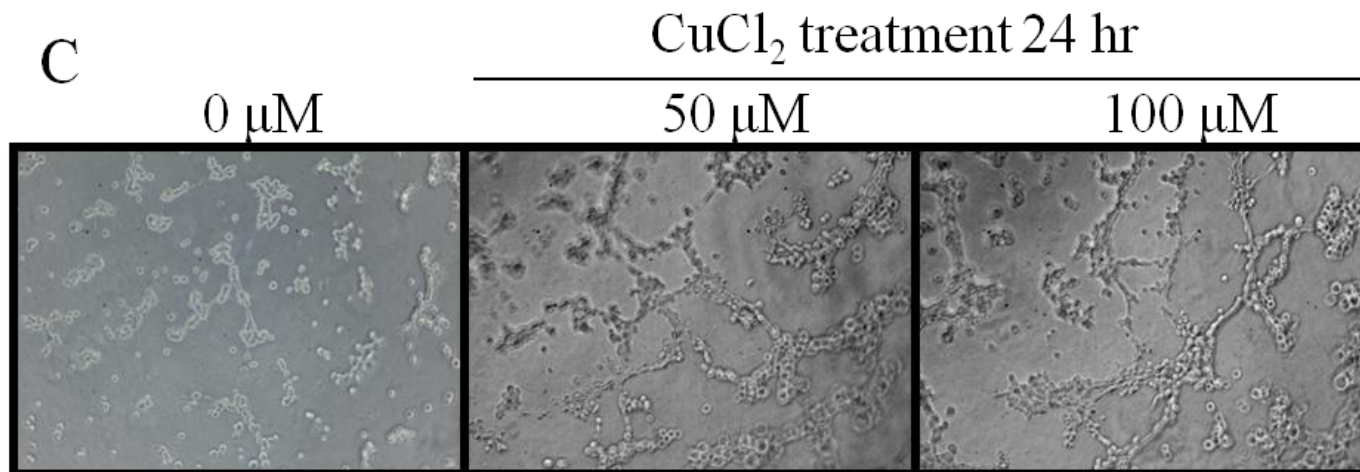
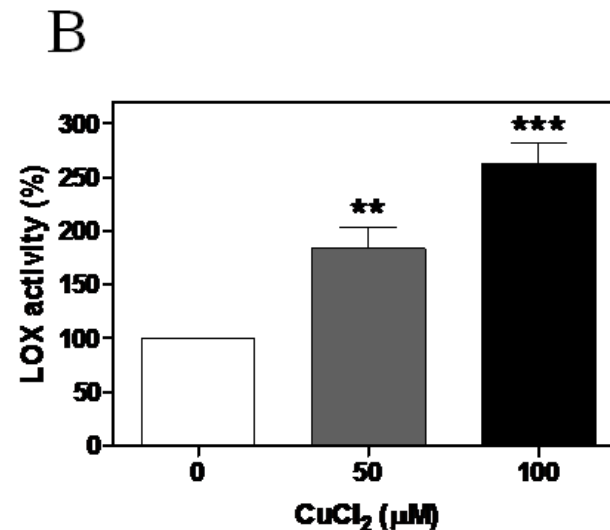
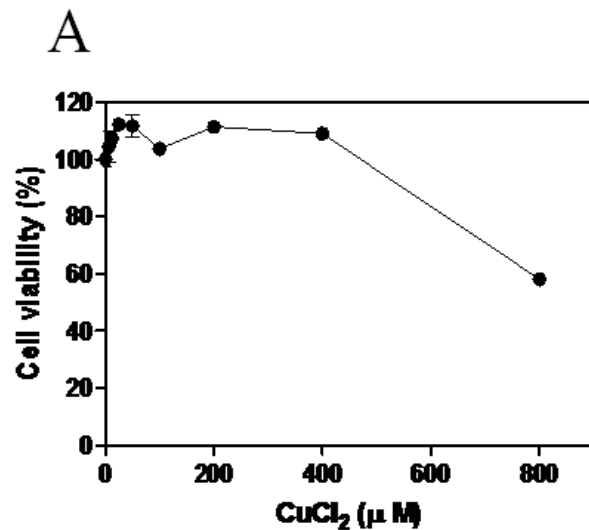
Purpose

- Investigate copper-enhanced LOX activity in endothelial cells and LOX overexpression in OSCC cell lines regarding induction of angiogenesis and tumor development.

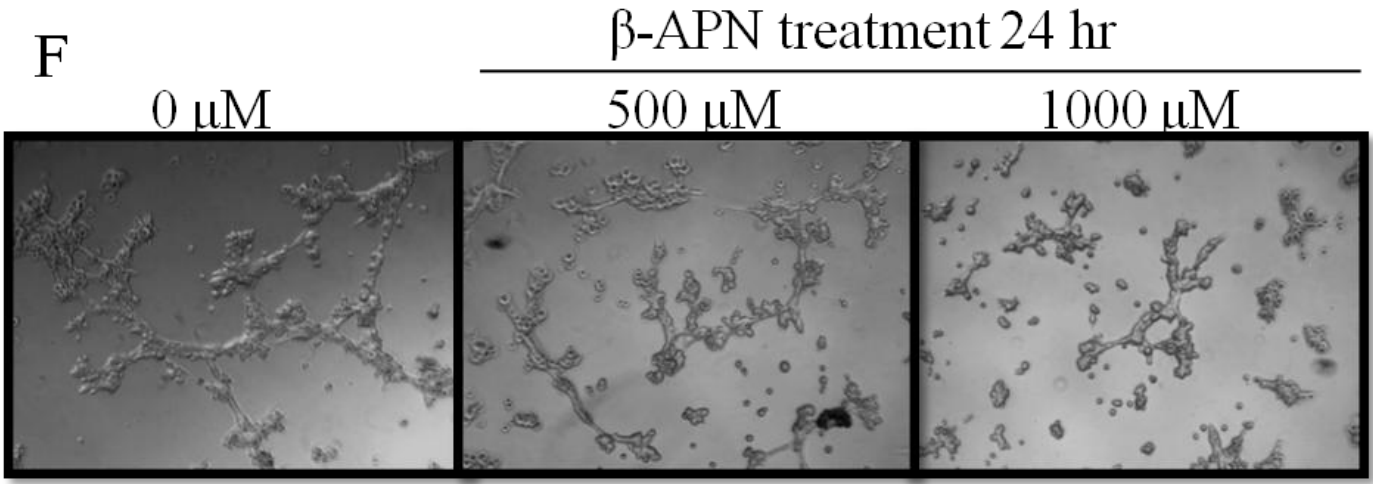
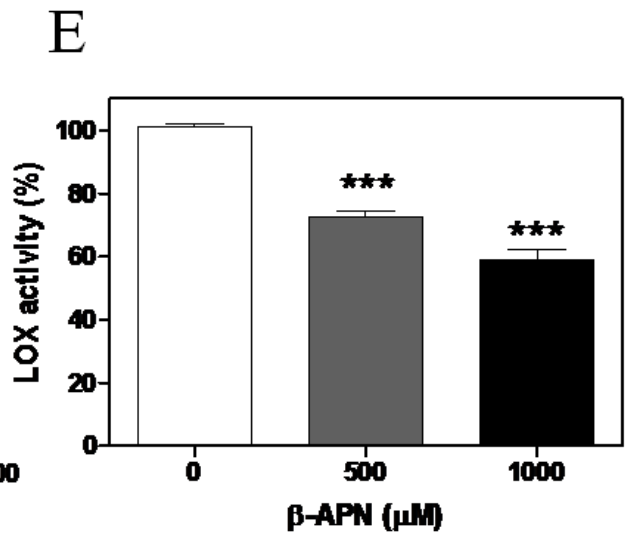
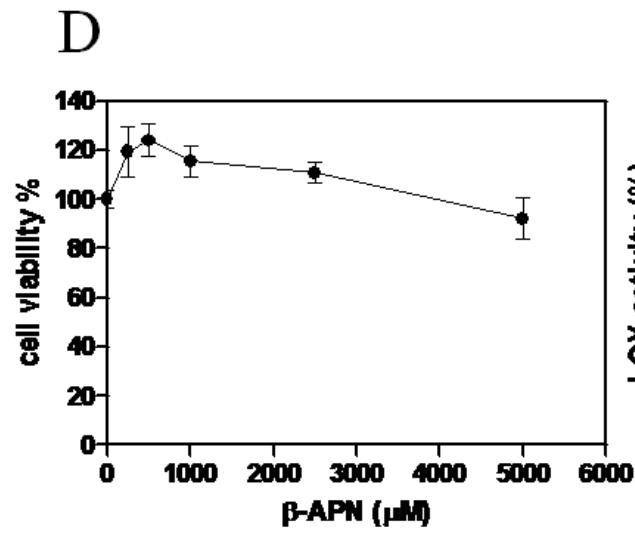
Materials & Methods

- Chemical treatment and gene transfection were used to induce LOX overexpression or inhibition in cell lines SAS and SVEC₄-10.
- LOX mRNA, protein, and activity were confirmed before tube formation assay and tumorigenesis.
- The microvessels were detected by immunostaining CD31- and LOX protein positive.

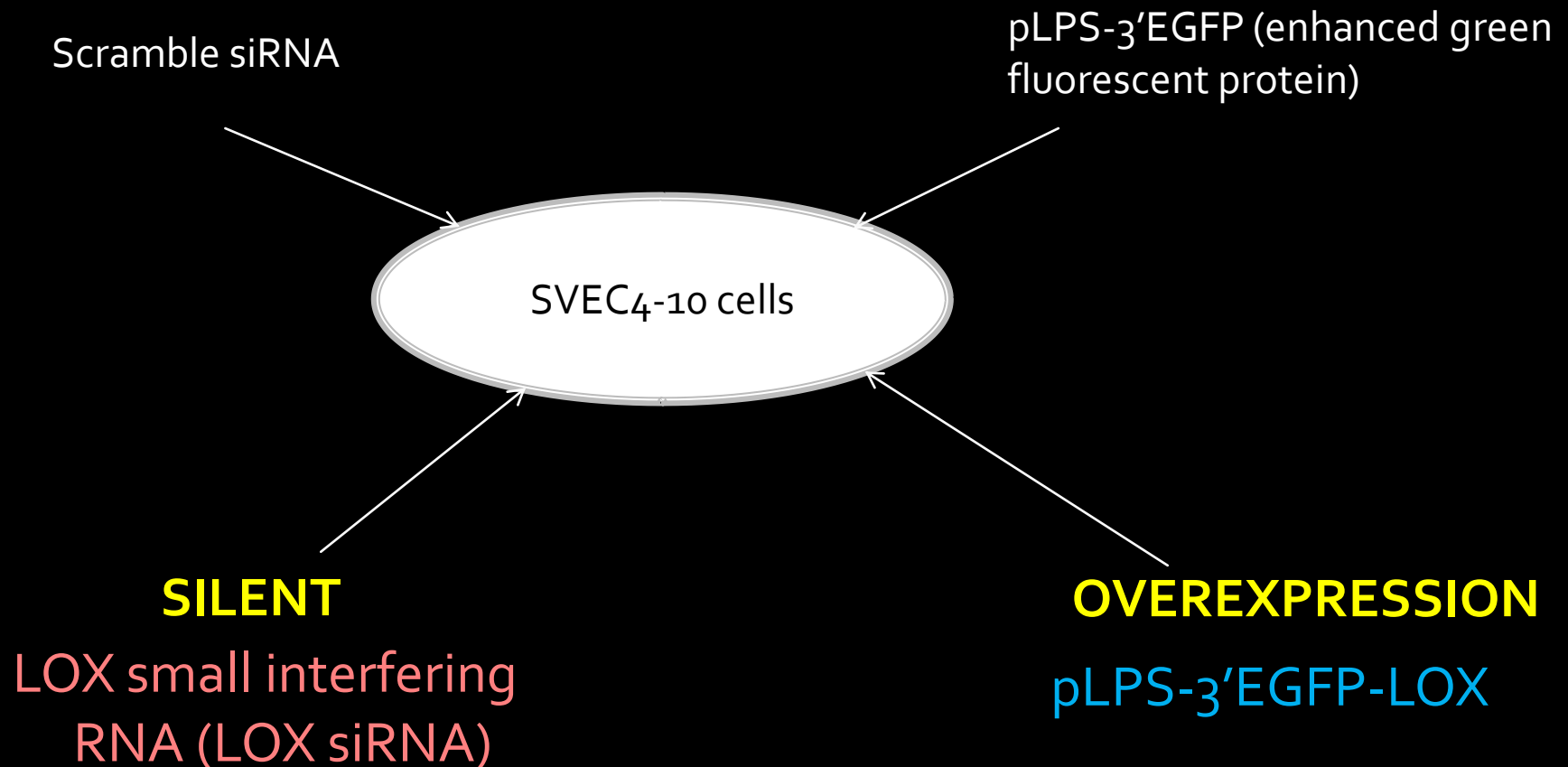
Copper induction enhanced LOX activity and increased SVEC4-10 tube formation



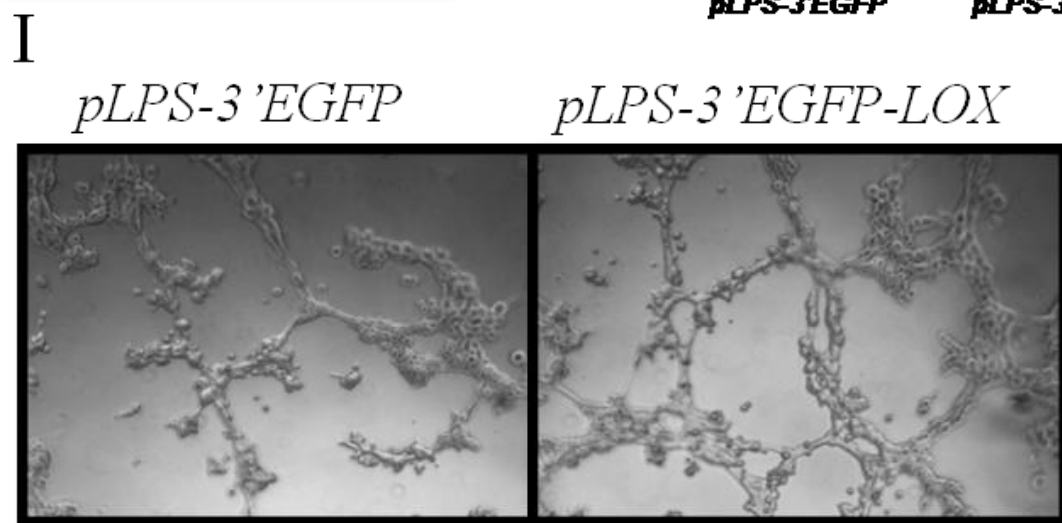
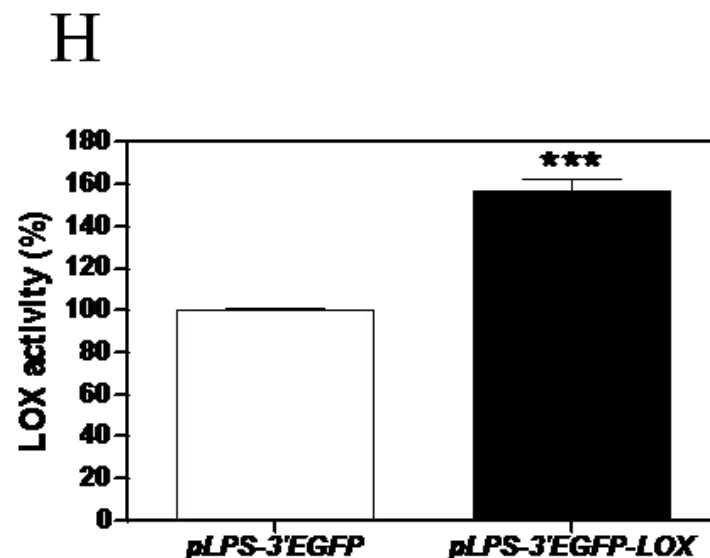
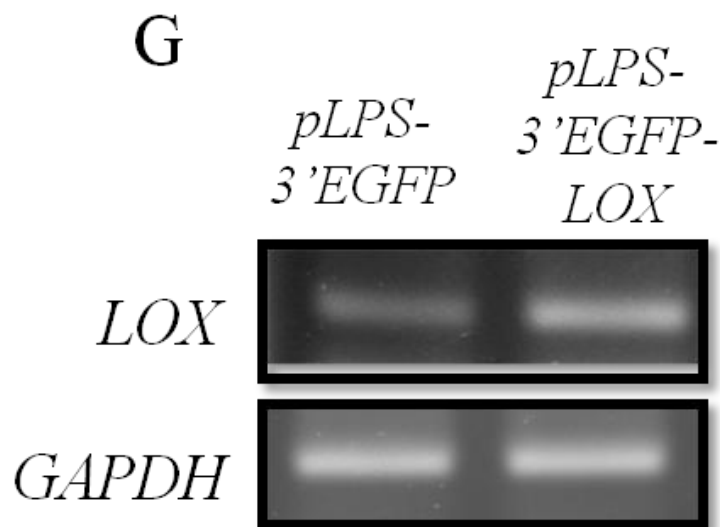
β -aminopropionitrile (β -APN) inhibited LOX activity and decreased SVEC₄-10 tube formation



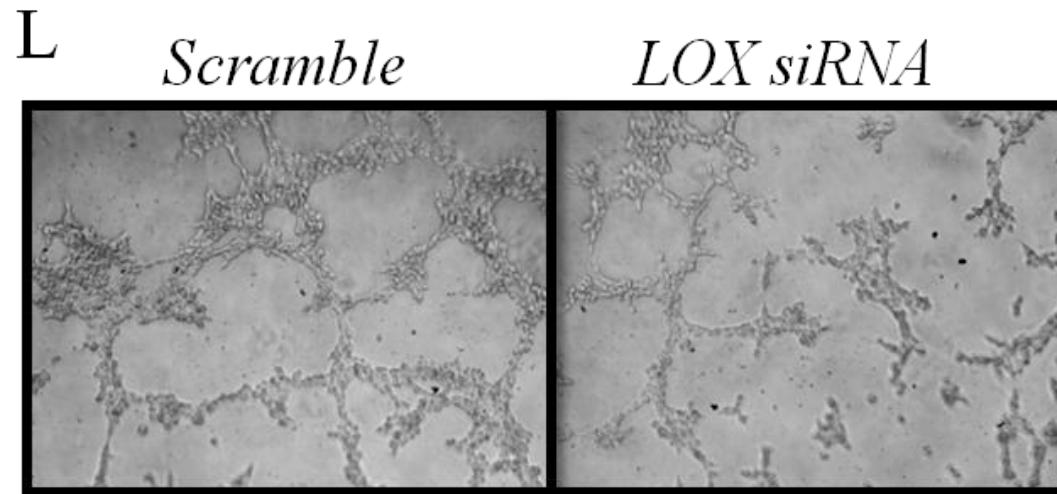
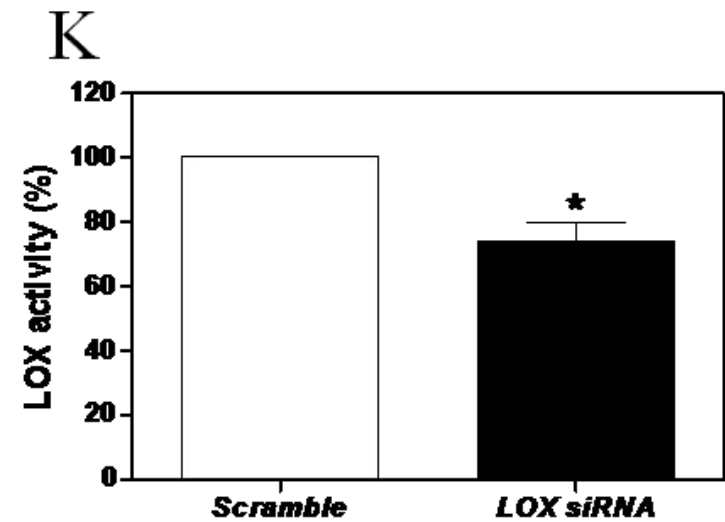
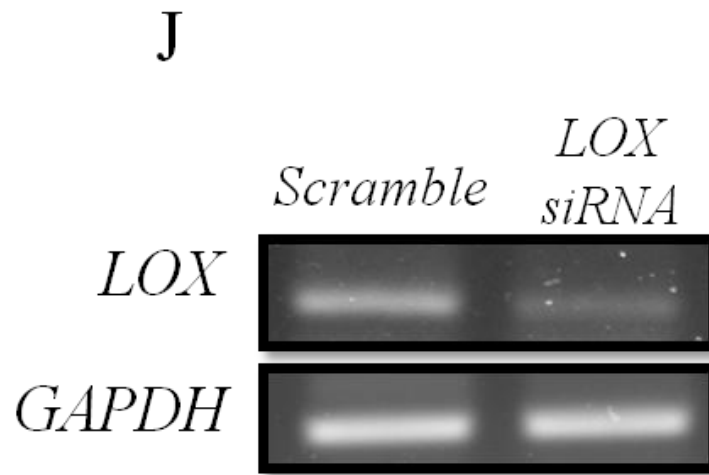
Transient LOX Transfection



Transient **pLPS-3'EGFP-LOX** transfection increased LOX mRNA expression, LOX activity, and tube formation in SVEC4-10 cells.



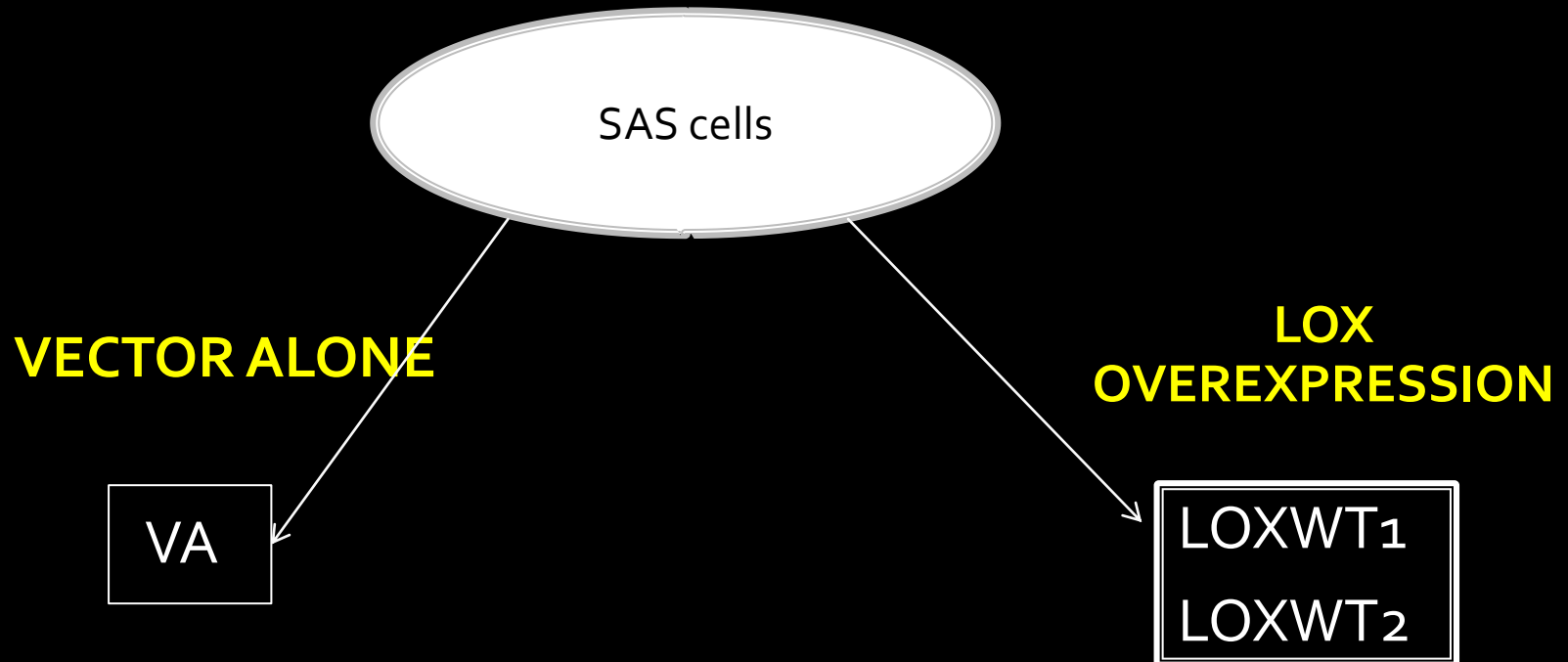
Transient **LOX siRNA** transfection decreased LOX mRNA expression, LOX activity, and tube formation in SVEC₄₋₁₀ cells.



Summary-1

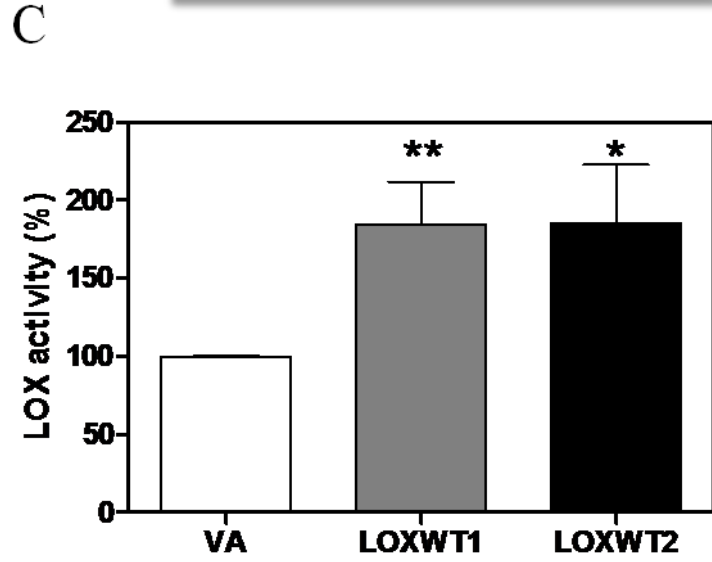
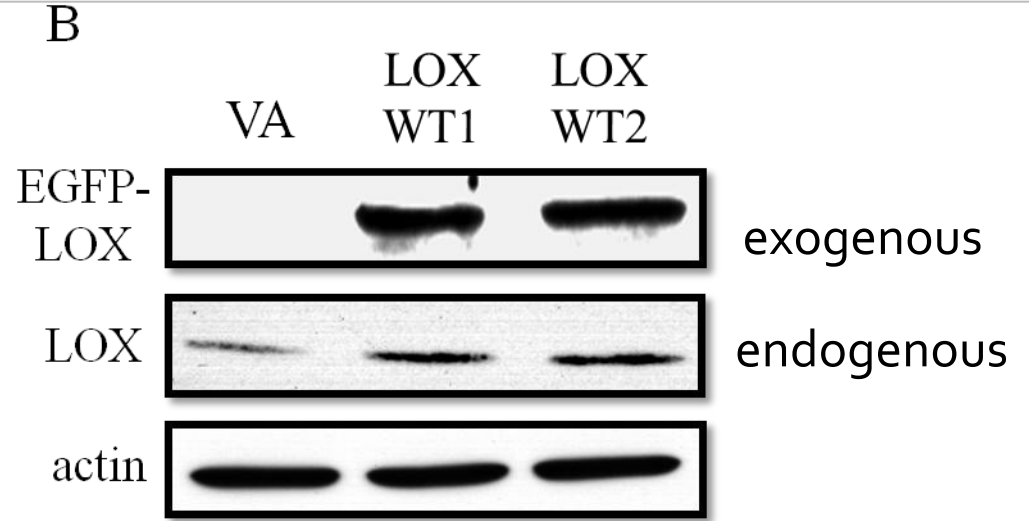
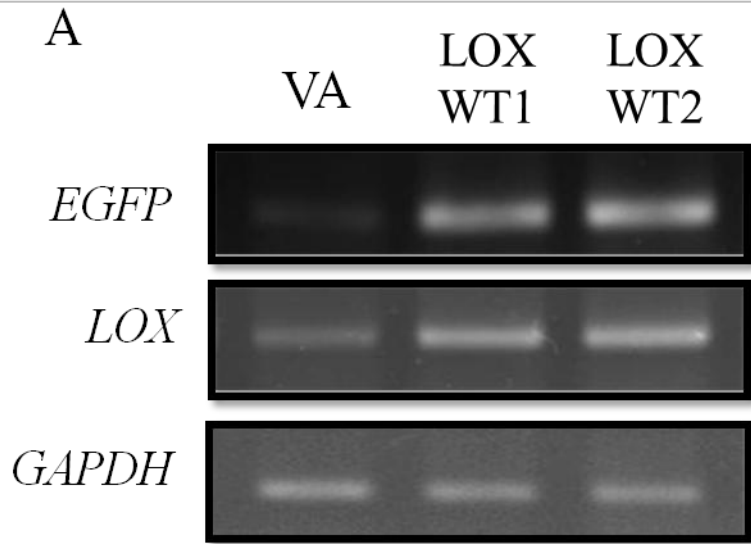
- LOX overexpression and copper induction increased LOX activity and SVEC₄-10 tube formation.
- LOX silencing and β -aminopropionitrile (β -APN) inhibition of LOX activity had opposite effects.

Transient LOX Transfection



Anti-EGFP and anti-LOX antibodies detected LOX proteins

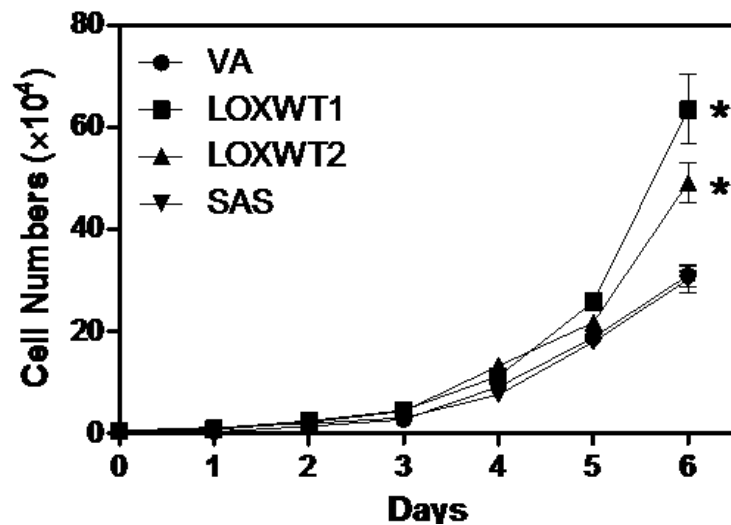
LOX signal included endogenous and exogenous LOX mRNA and LOX.



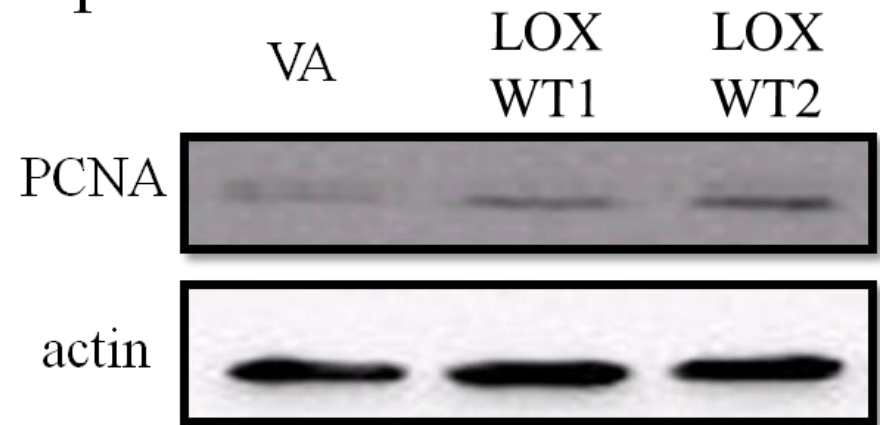
LOX overexpression OSCC cells showed higher and equivalent numbers of LOX activity in both clones.

LOX overexpression clones exhibited higher growth rates and PCNA expression

E



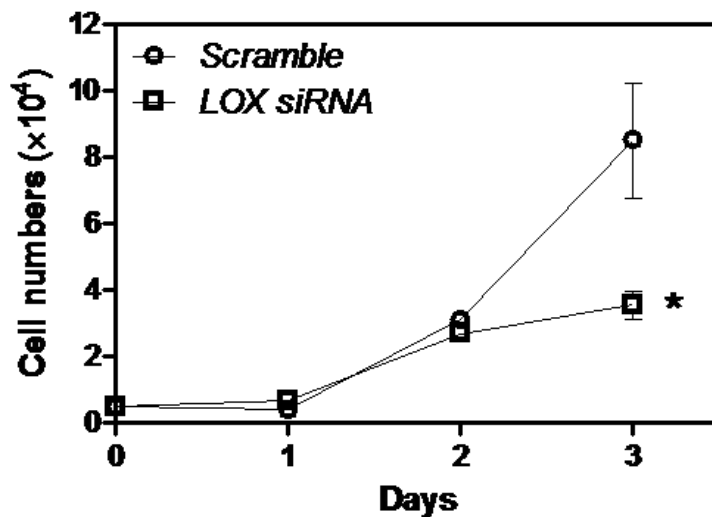
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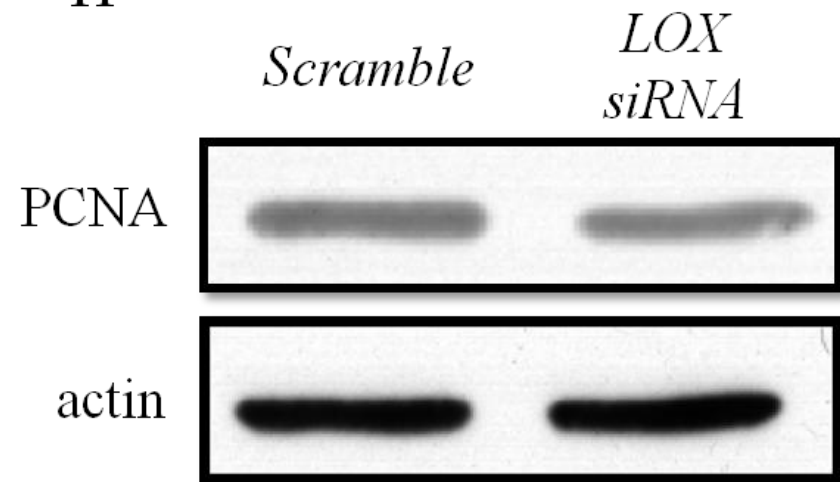
Proliferating cell nuclear antigen (PCNA)

Transient LOX siRNA transfection decreased cell growth rate and PCNA expression

G

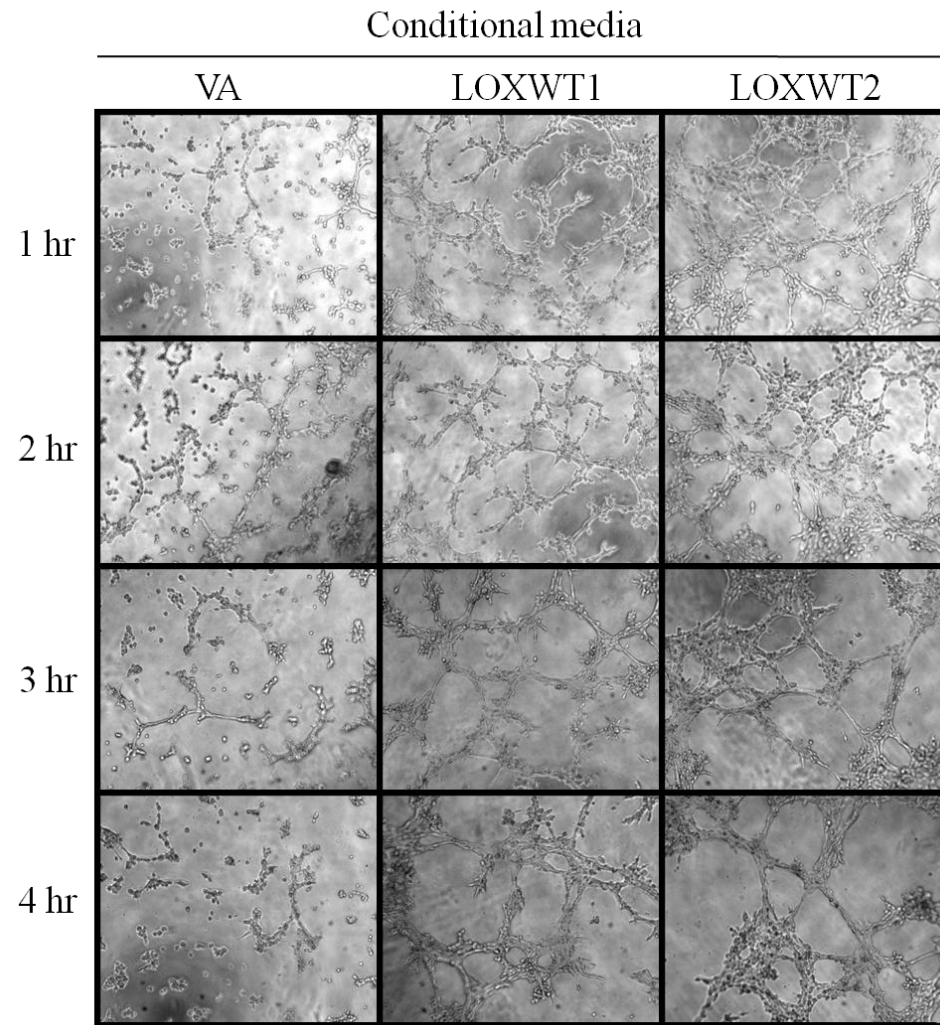


H



Proliferating cell nuclear antigen (PCNA)

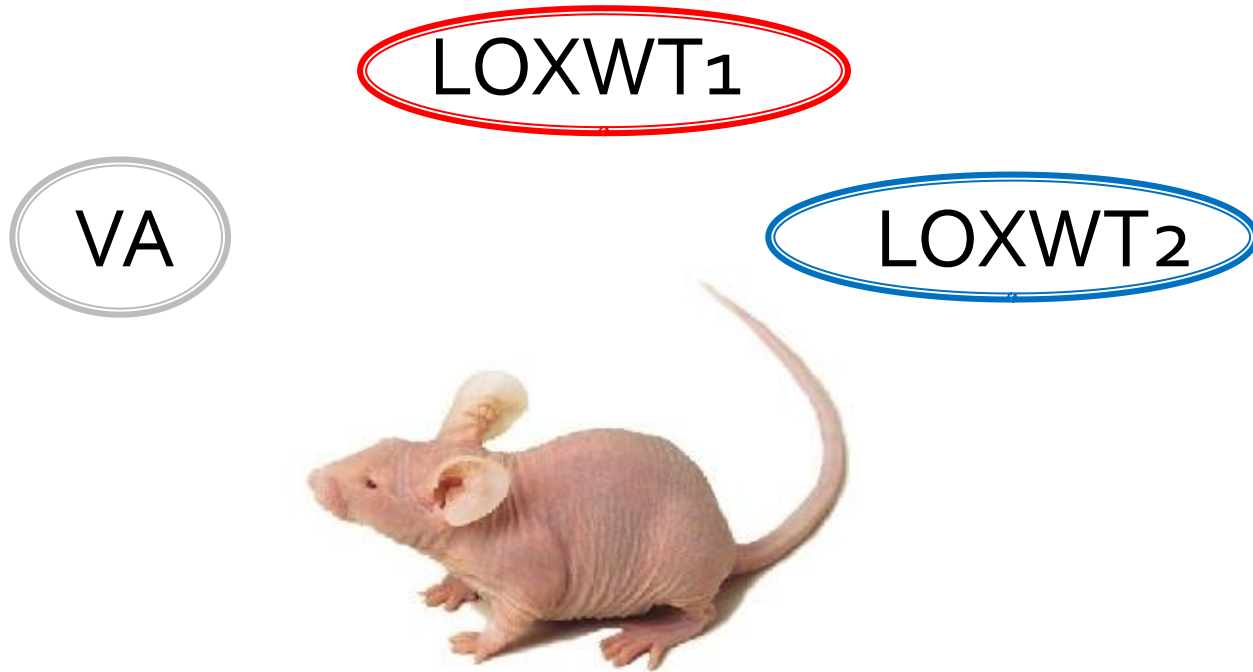
LOX expression clones showed higher LOX activity, therefore induced profound tube formation in SVEC₄-10 cells.



Summary-2

- High LOX expression induced cell proliferation and growth.

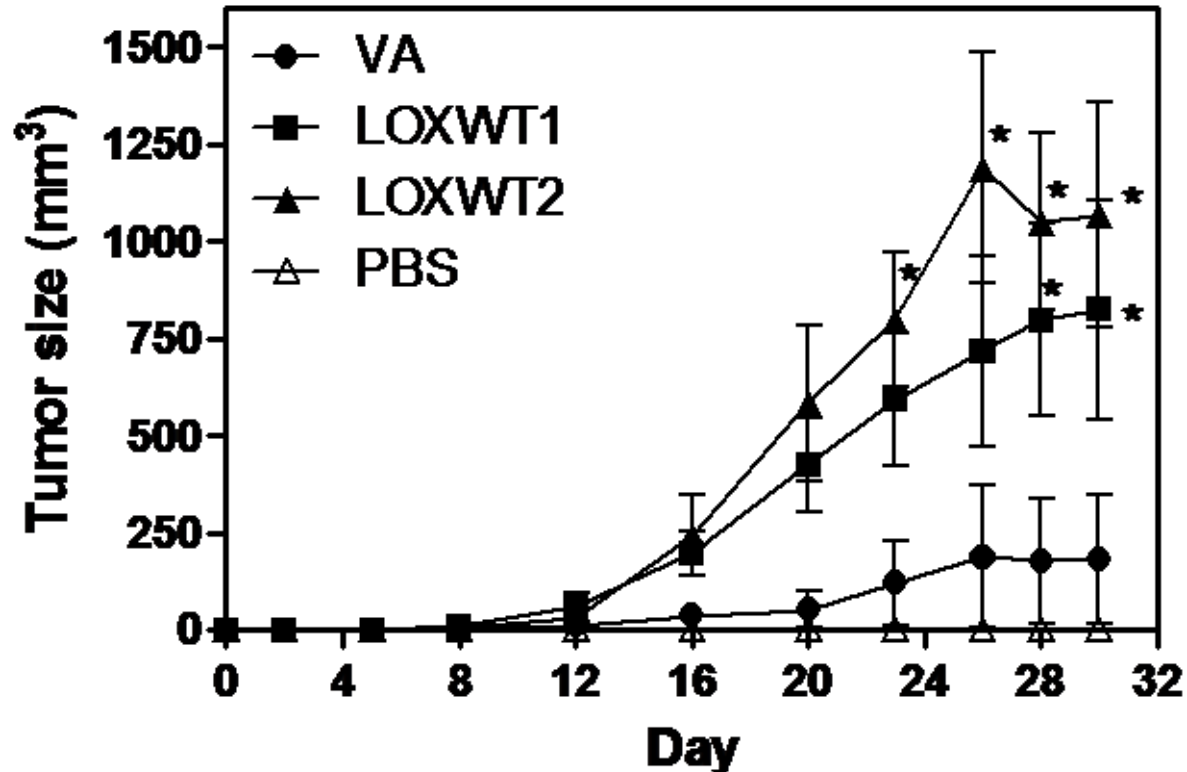
Xenographic Tumorigenesis



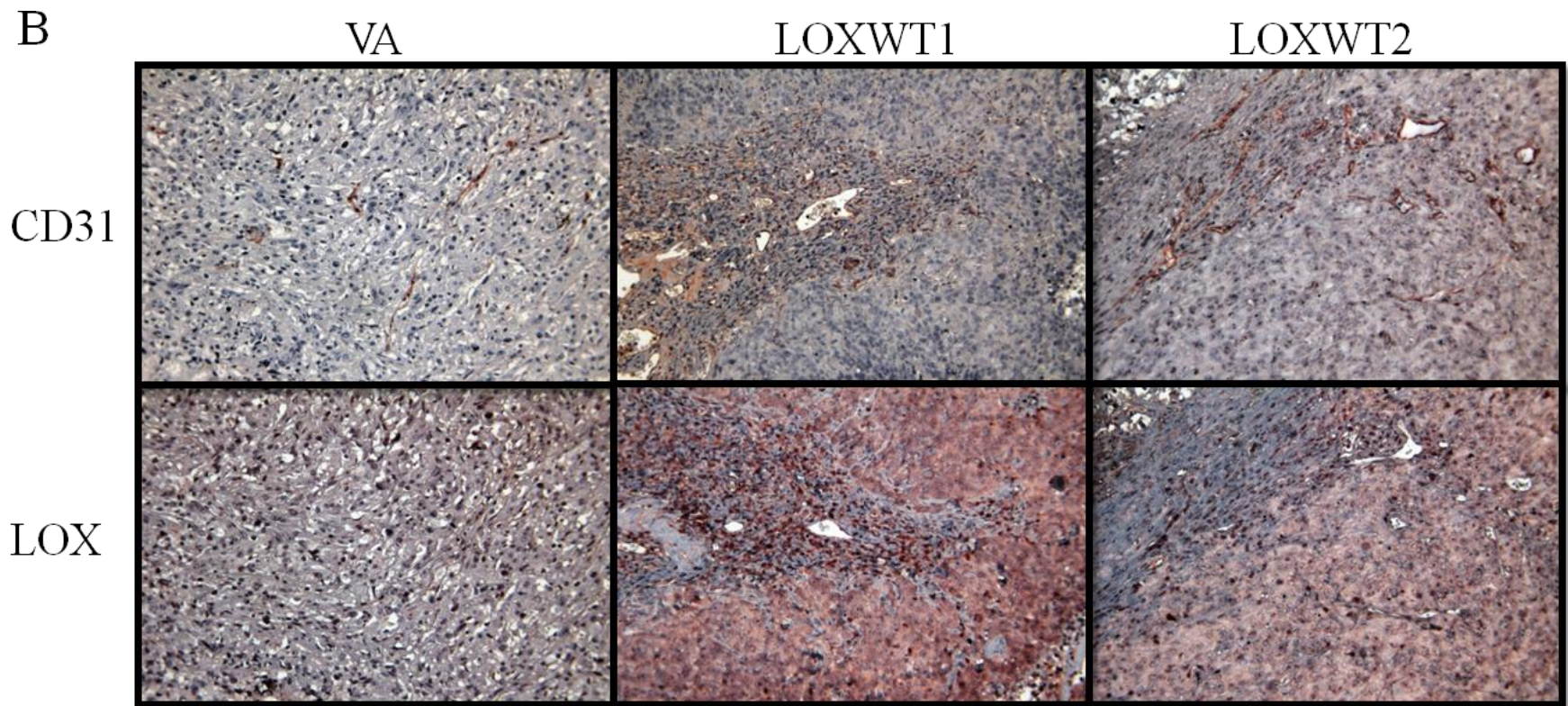
Tumor volumes = $0.5 \text{ (longest diameter)}^2 \text{ (shortest diameter)}$.

LOXWT₁ and LOXWT₂ induced larger xenografic tumors than VA in nude mice

A



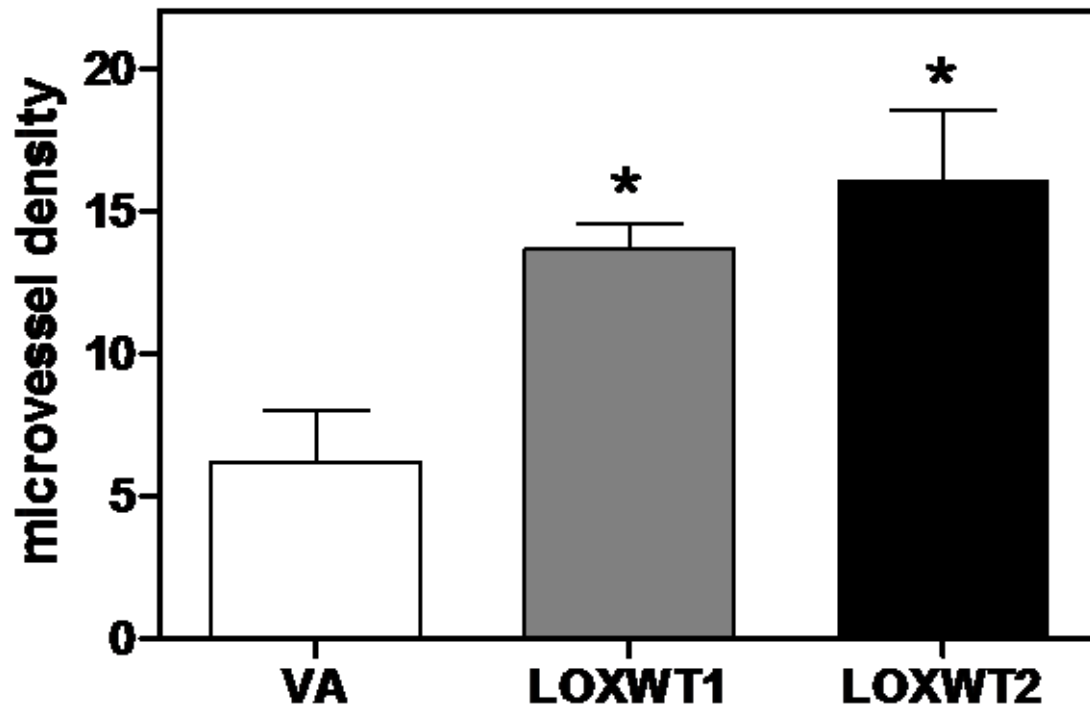
CD 31 and LOX signals were enhanced in LOX overexpression clones than in VA.



Original magnificationx200

Quantification of microvessel densities in tumor sections of LOXWT, LOXWT₂, and VA

C



Summary - 3

- High LOX expression clones **increased tumor size** in a tumorigenesis model.
- The **microvascular** numbers were higher in LOX overexpression tumors than in control tumors.

Conclusion

- High LOX activity or expression can increase cell proliferation and angiogenesis leading to tumor development in vitro and in vivo.
- LOX could potentially provide a target for future anti-cancer or anti-angiogenesis therapies.



THANKS FOR YOUR ATTENTION

