

VEGF/Flk1 Mechanism Is Involved in Roxarsone Promotion of Rat Endothelial Cells Growth and B16F10 Xenograft Tumor Angiogenesis

Shihao Chen^{1,2†}, Jinge Xu^{1,3†}, Qianhan Wei¹, Zeting Zhao^{1,2}, Xin Chen¹, Hengmi Cui^{2*}, Yumei Zhang^{1,4*}

1 College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu, China;

2 College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China;

3 Guizhou Animal Husbandry and Veterinary Research Institute, Guiyang, Guizhou, China;

4 Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, Jiangsu, China

† Shihao Chen and Jinge Xu should be considered joint first author.

* Hengmi Cui and Yumei Zhang should be considered joint corresponding author. Hengmi Cui, College of Animal Science and Technology, Yangzhou University, No. 12 East Wenhui Road, Yangzhou, Jiangsu 225009, China. Tel.: 86 514 87979032, Fax: 86 514 87350440, E-mail address: hmcui@yzu.edu.cn; Yumei Zhang, College of Veterinary Medicine, Yangzhou University, No. 12 East Wenhui Road, Yangzhou, Jiangsu 225009, China. Tel.: 86 514 87979044, Fax: 86 514 87972218, E-mail address: ymzhnet@sina.com.cn.

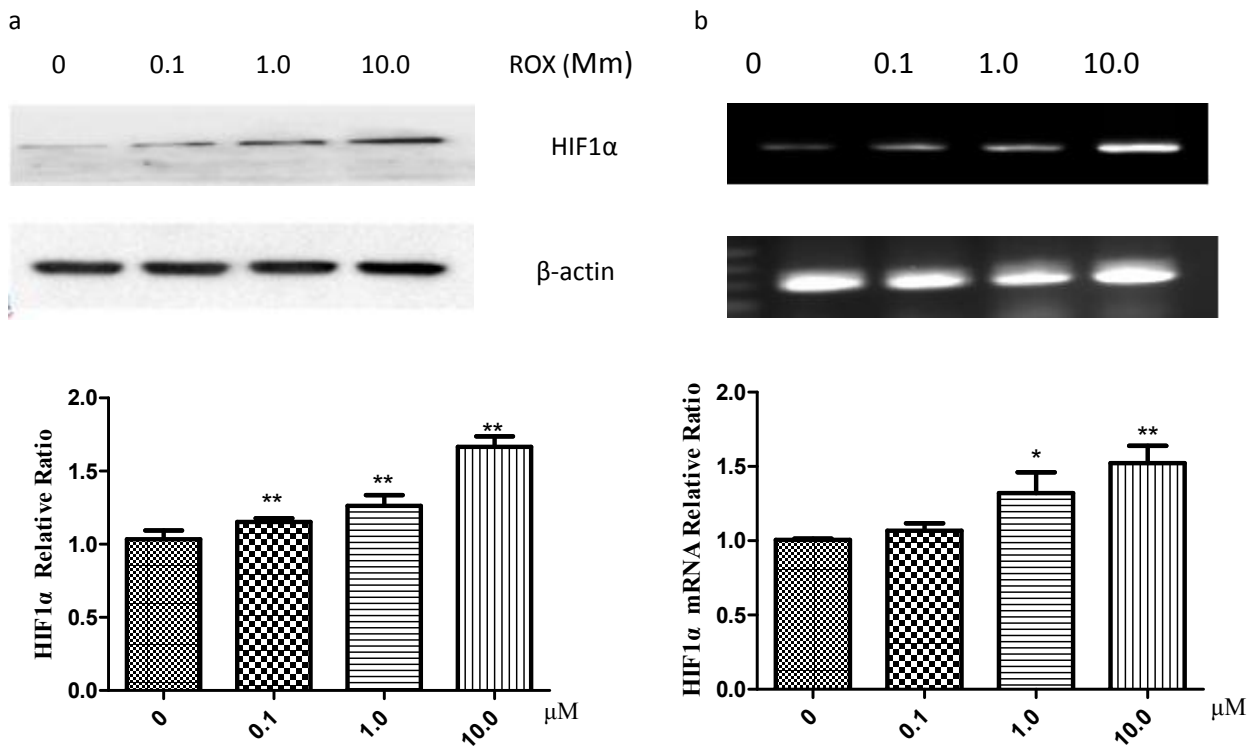
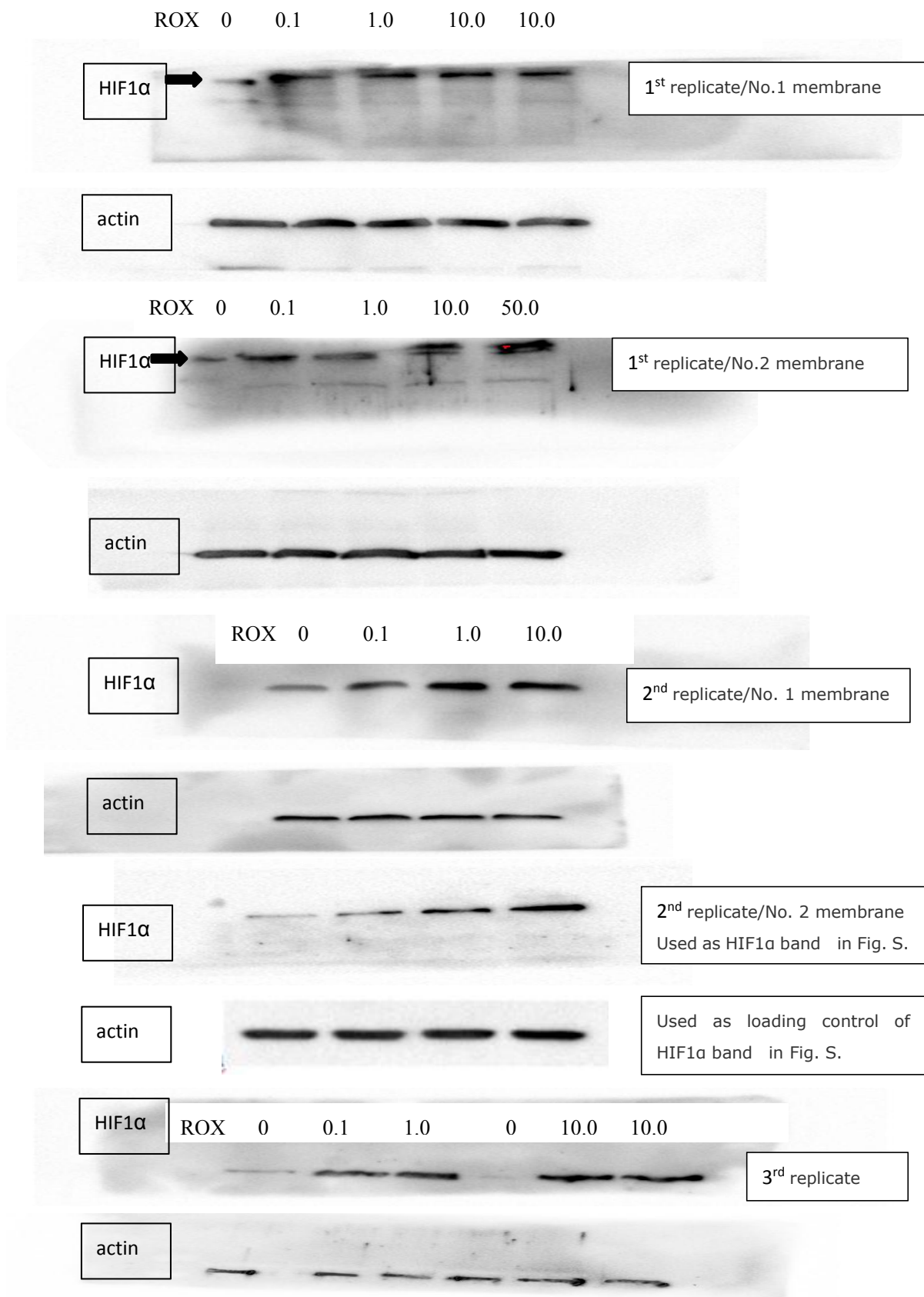


Figure S Effect of roxarsone on the expression of HIF1α in EC cultures.

a, Western blotting of total cell lysates from ECs treated with 0, 0.1, 1.0 and 10.0 μM ROX. Values are the mean ± SD of HIF1α expression standardized to β-actin expression in three independent experiments. b, RT-PCR analysis from ECs treated with 0, 0.1, 1.0 and 10.0 μM ROX. Values are the mean ± SD of HIF1α expression standardized to β-actin expression in three independent experiments. * $P < 0.05$, ** $P < 0.01$ relative to PBS by ANOVA.

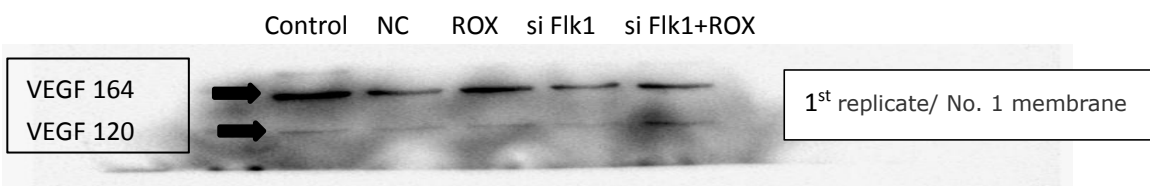
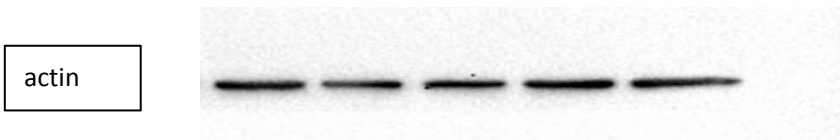
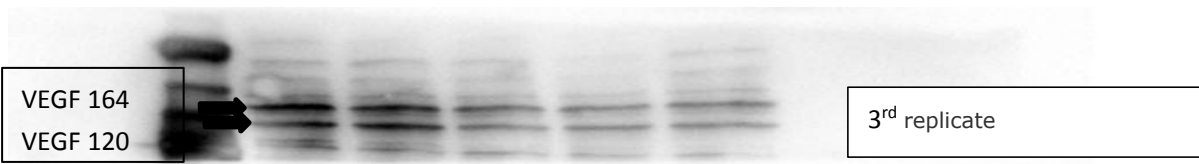
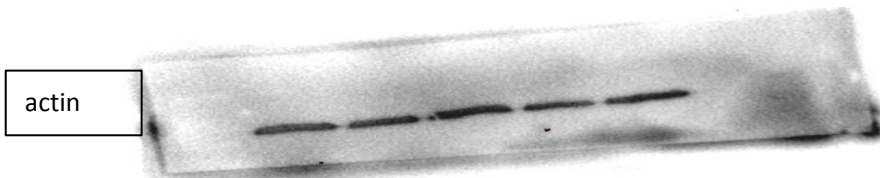
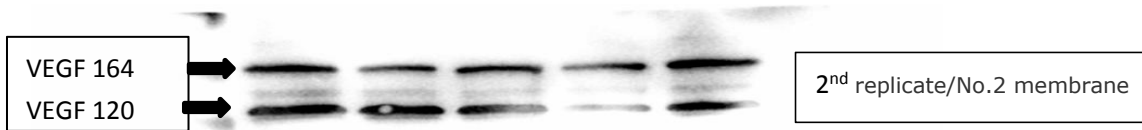
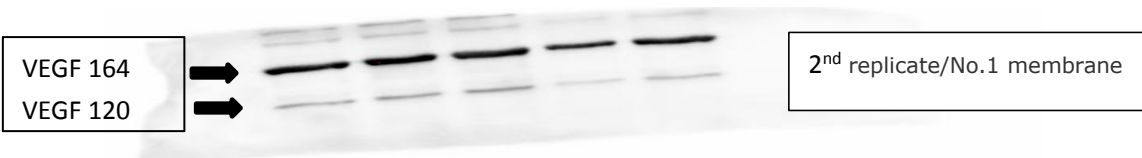
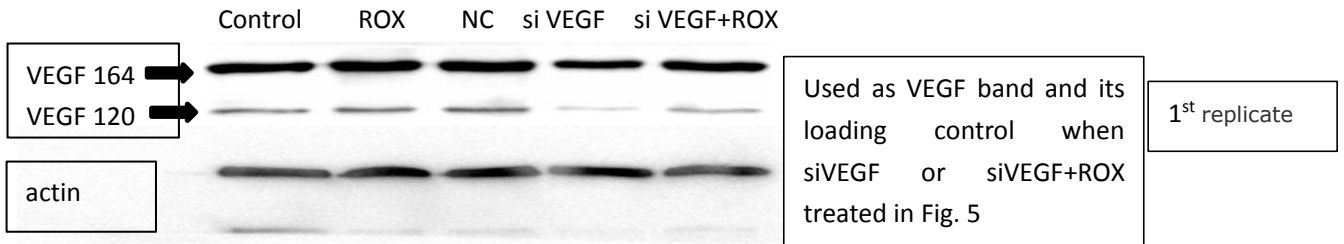
The original WB bands of HIF1 α in Fig. S

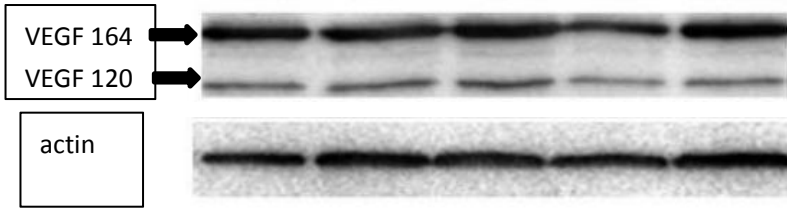
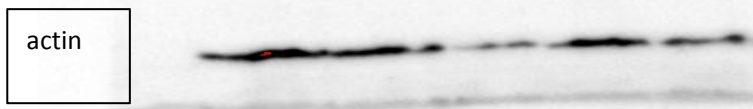


Some original images of WB or box-plot figures as below.

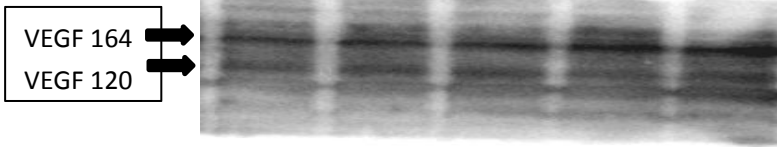
The WB bands for VEGF and Flk1/Flt1 used in Fig. 5

VEGF:



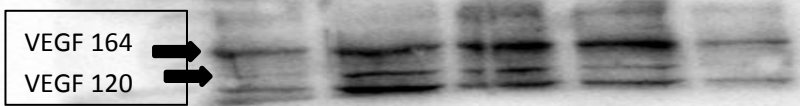


1st replicate/ No. 2 membrane
Used as VEGF band and its loading control when siFlk1 or siFlk1+ROX treated in Fig. 5.

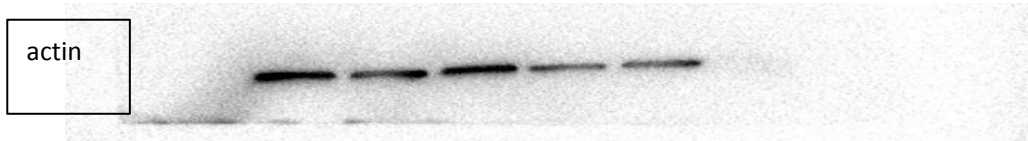


2nd replicate

Control NC ROX si Flk1+ROX si Flk1

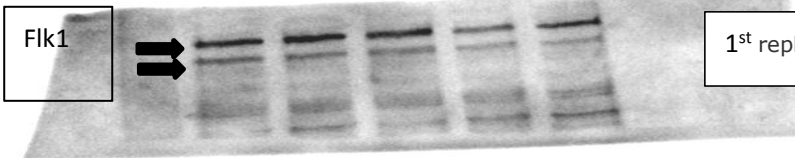


3rd replicate

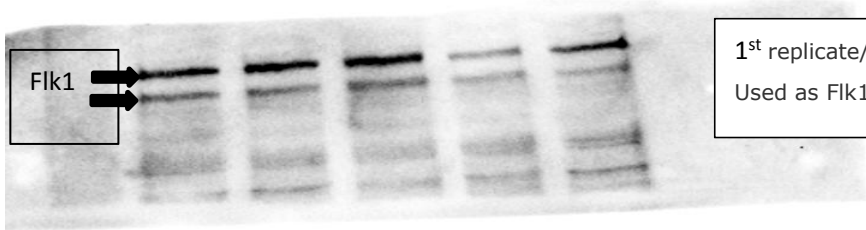


Flk1:

Control ROX NC si Flk1 si Flk1+ROX

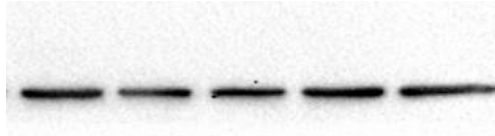


1st replicate/No. 1 membrane



1st replicate/No. 2 membrane
Used as Flk1 band in Fig. 5.

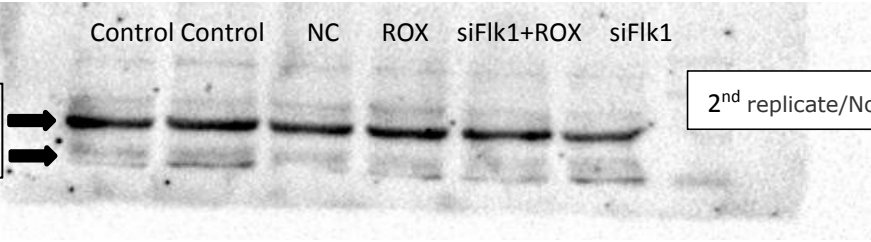
actin



Used as loading control of Flk1
WB in Fig. 5

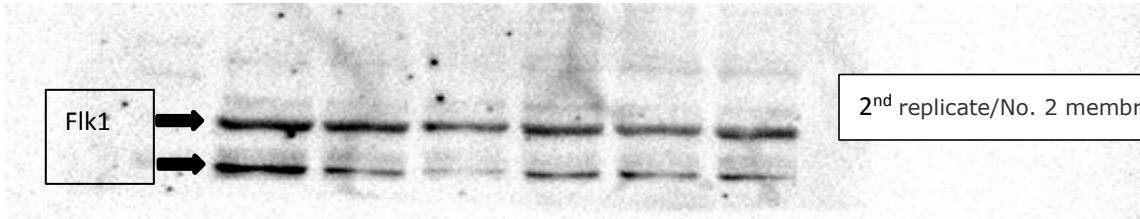
Control Control NC ROX siFlk1+ROX siFlk1

Flk1



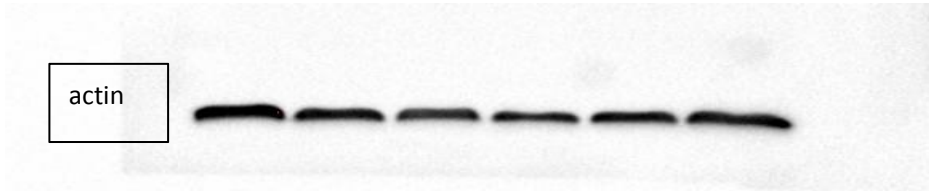
2nd replicate/No. 1 membrane

Flk1

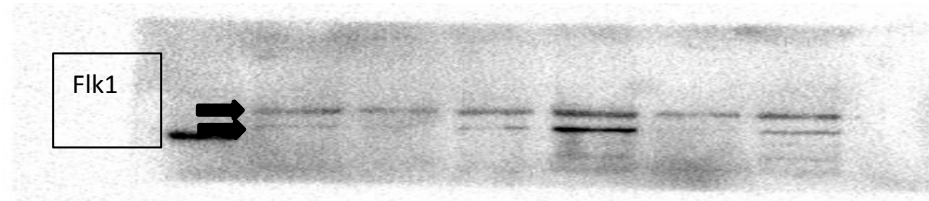


2nd replicate/No. 2 membrane

actin

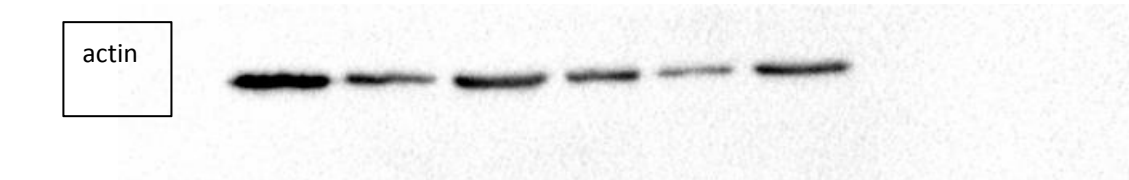


Flk1



3rd replicate

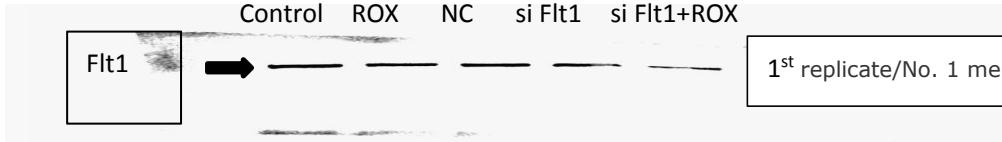
actin



Flt1:

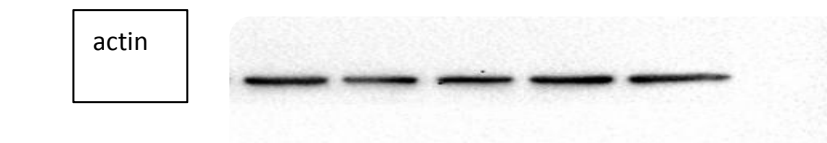
Control ROX NC si Flt1 si Flt1+ROX

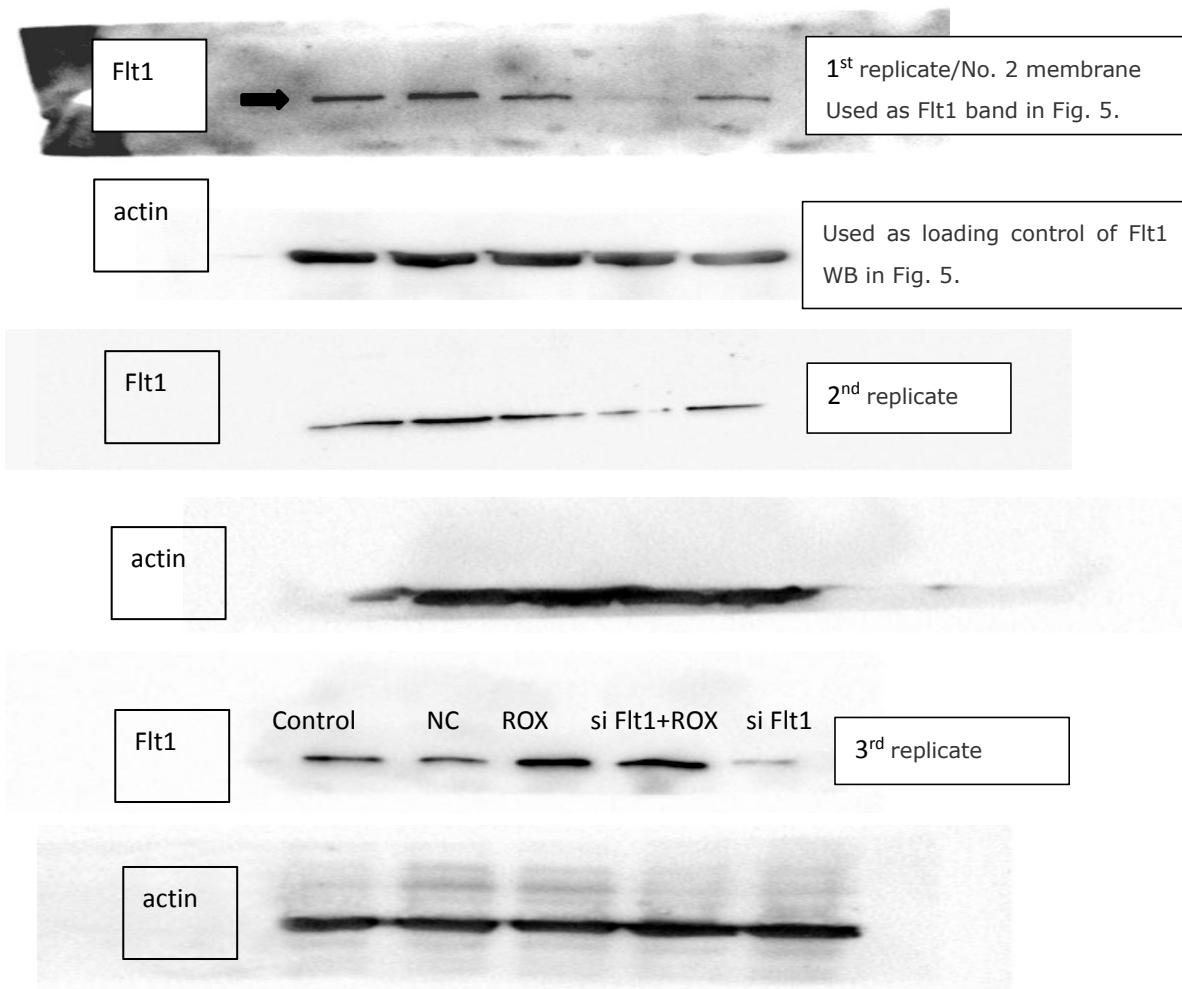
Flt1



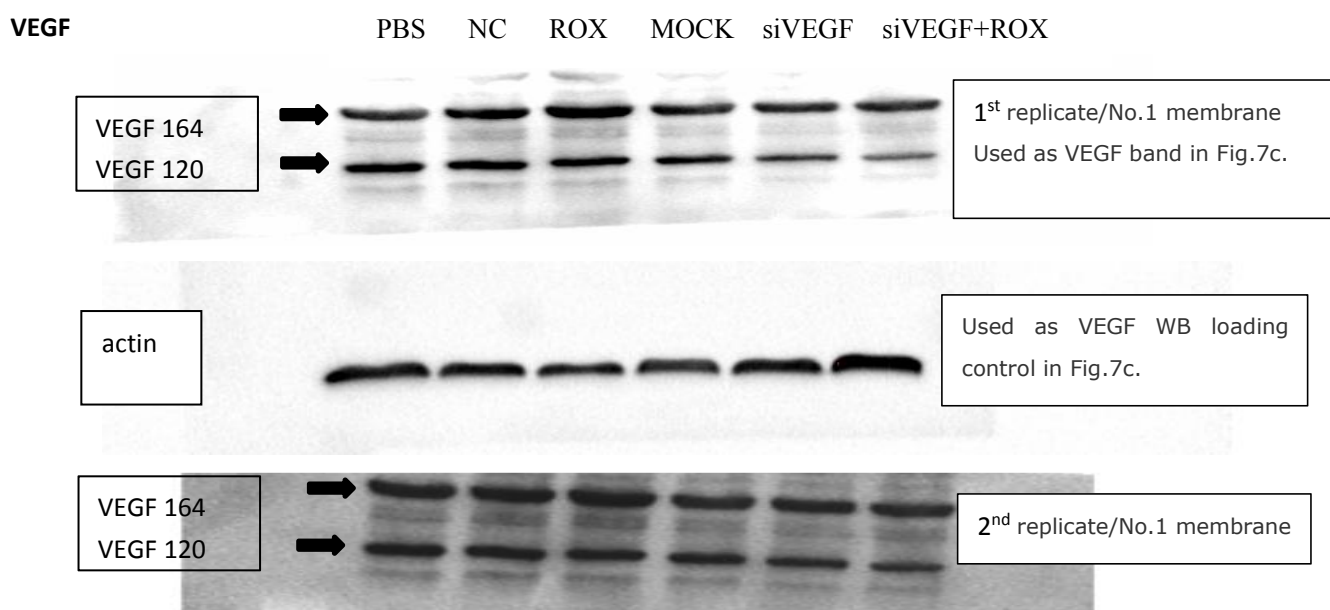
1st replicate/No. 1 membrane

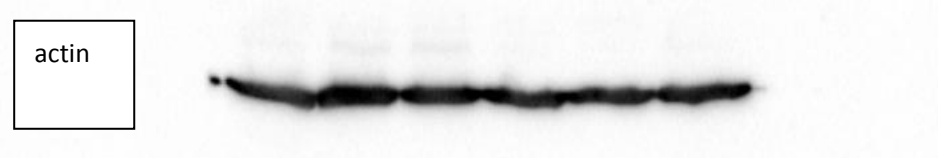
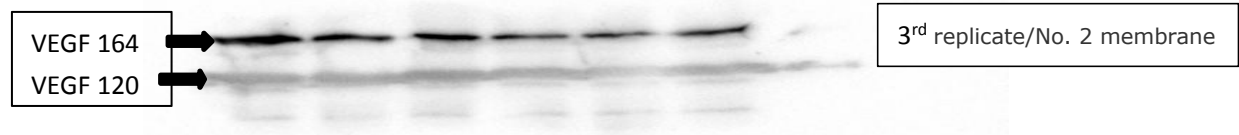
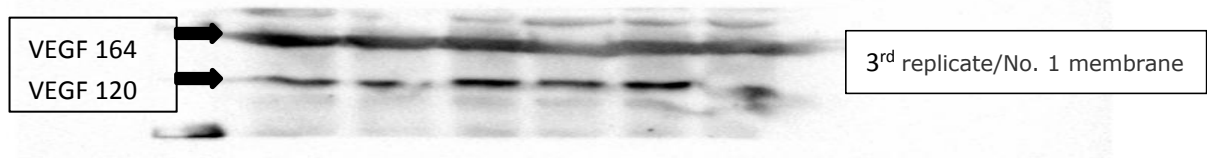
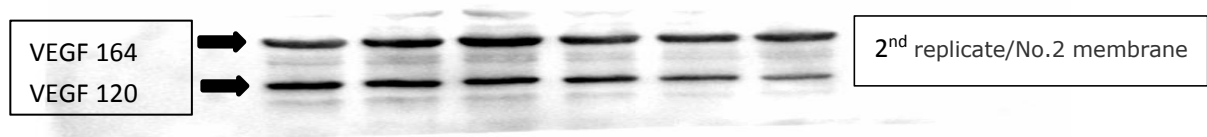
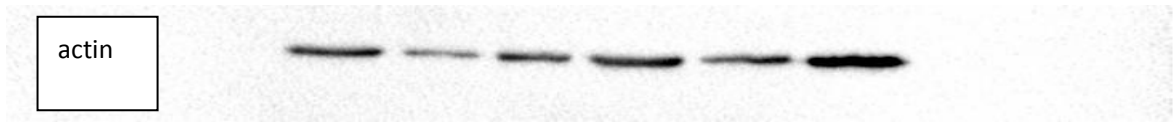
actin





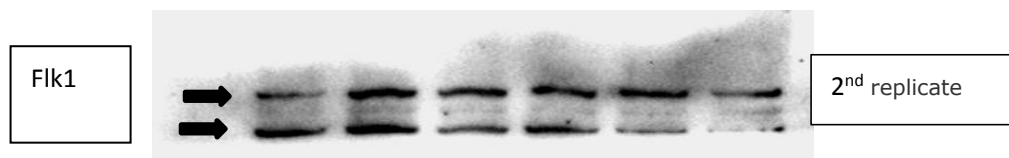
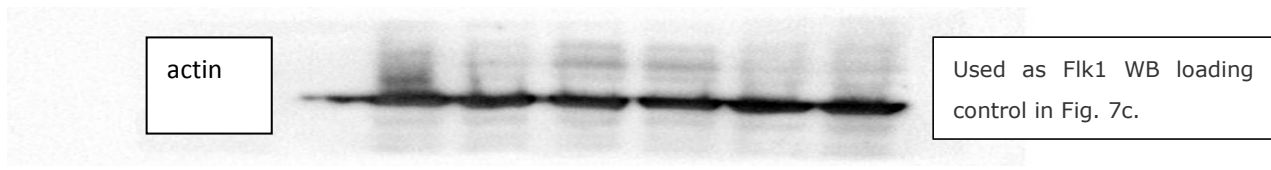
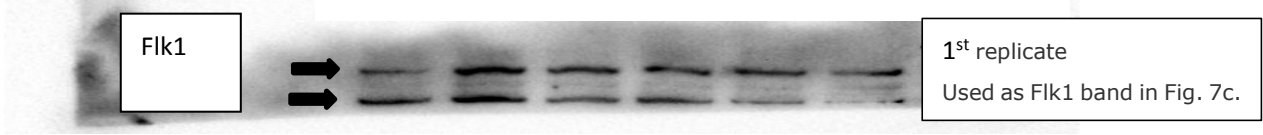
The WB bands of VEGF and Flk1 in B16F10 tumors used in Fig. 7c

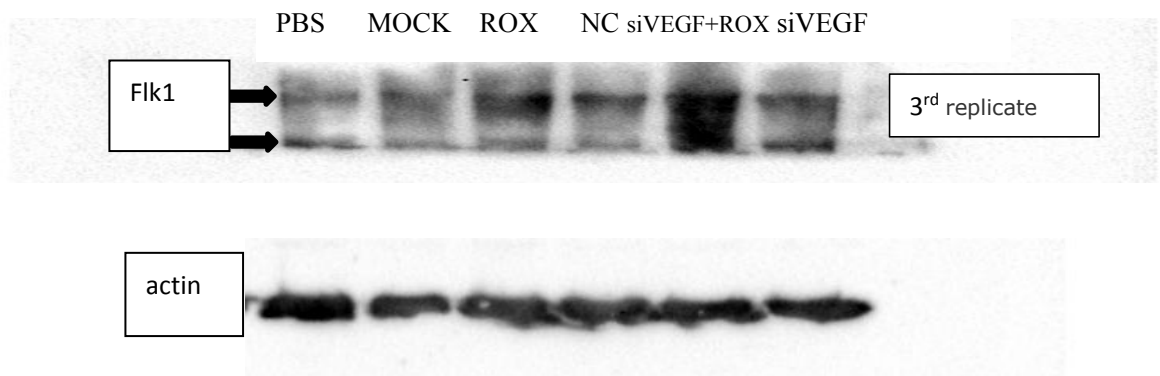




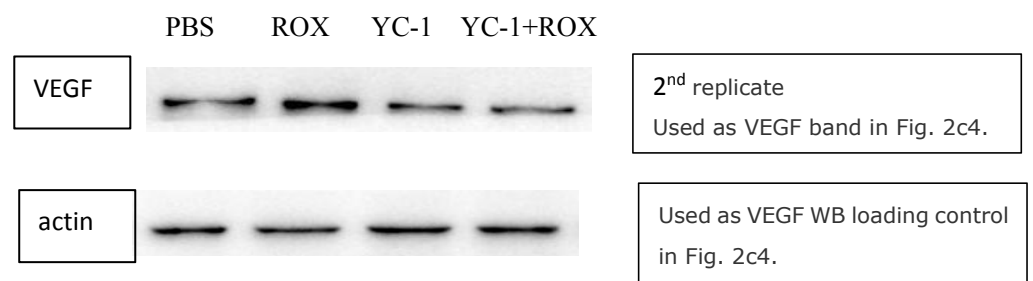
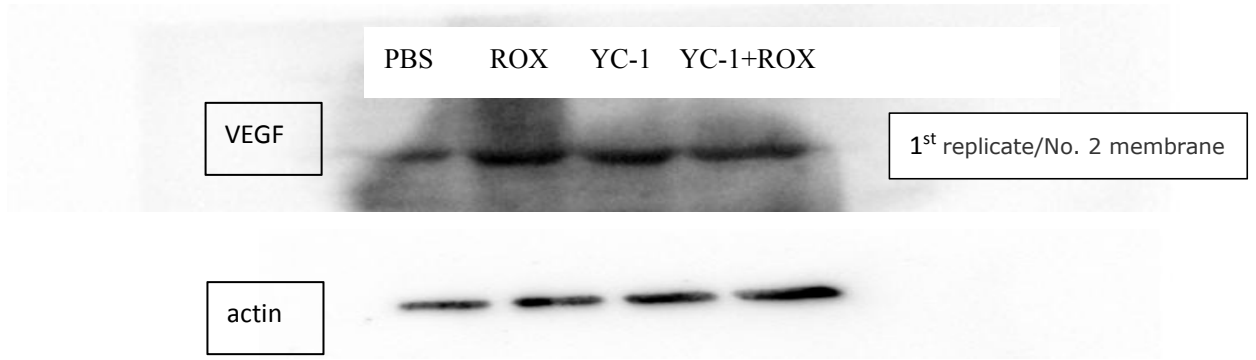
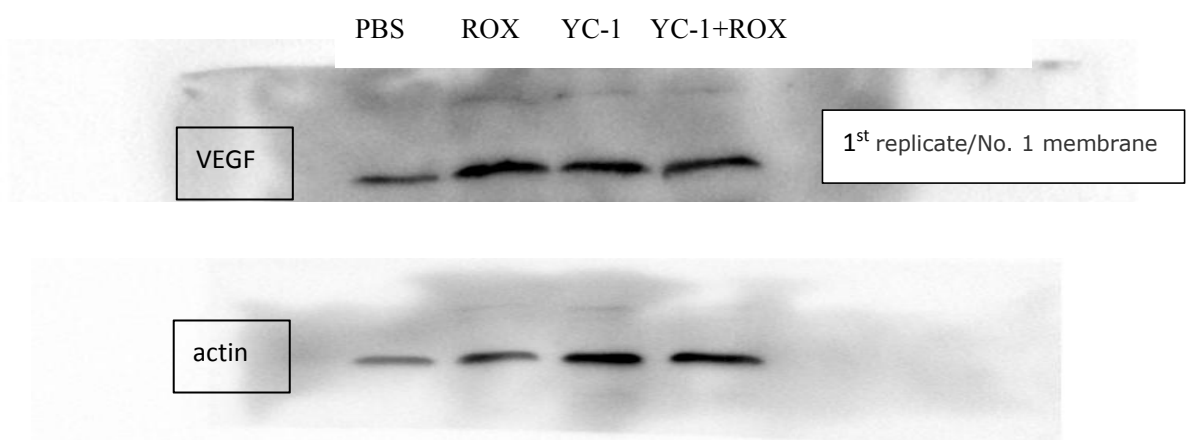
Flk1:

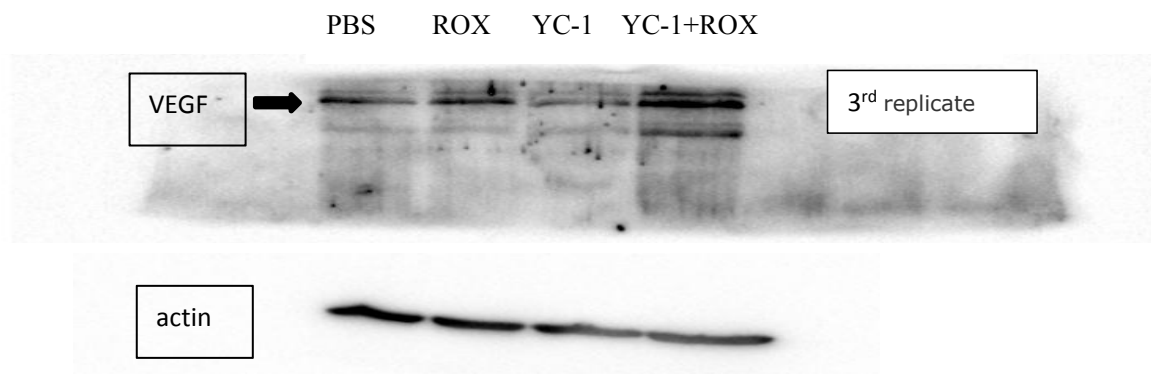
PBS ROX MOCK NC siVEGF siVEGF+ROX





The bands of HIF by WB used in Fig.2c4





The Relative VEGF data by WB in Fig.2c3

Fig.2c3 VEGF level by WB

	PBS	ROX 1.0	ROX 1.0+anti-Flt1	ROX 1.0+anti-Flk1	anti-Flt1	anti-Flk1	anti-VEGF
1	1.0021	1.1414	1.0849	1.0364	0.9895	0.9529	0.981
2	1.0111	1.1492	1.0928	1.0411	1.0013	0.9502	0.9768
3	0.9935	1.1339	1.0771	1.0316	0.9781	0.9611	0.9921

The Relative VEGF data by WB in Fig.1d

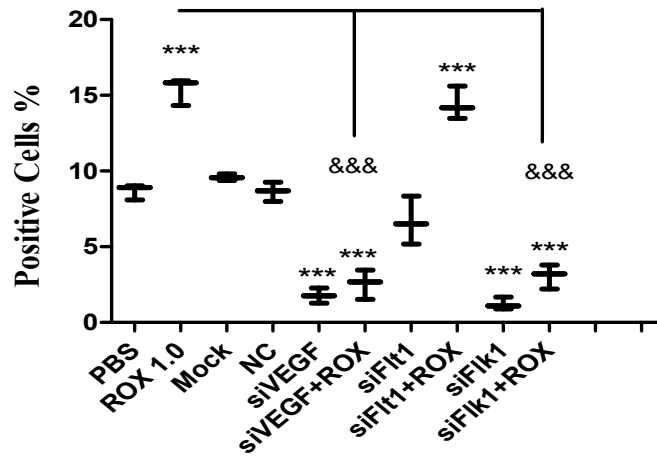
Relative VEGF

	Control	VEGF	Rox 0.1	Rox 1.0	Rox 10.0	Rox 50.0
1	1	1.2054	1.0317	1.1414	1.0369	1.0202
2	1	1.2097	1.0437	1.145	1.0483	1.021
3	1	1.1843	1.0426	1.1377	1.0403	1.0225

Some box-plot figures as below:

Figure 3

b



d

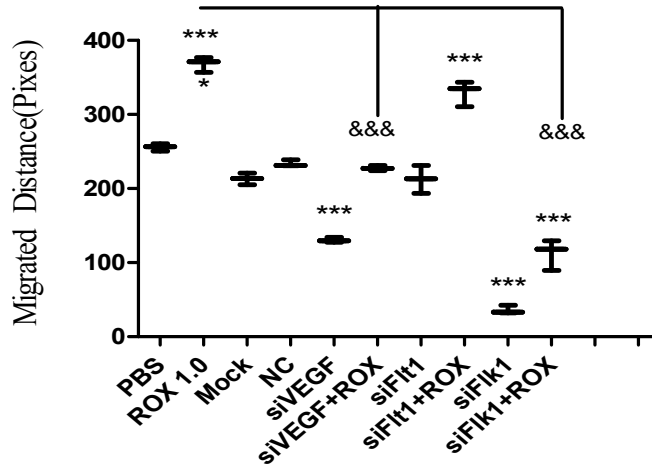


Figure 4b

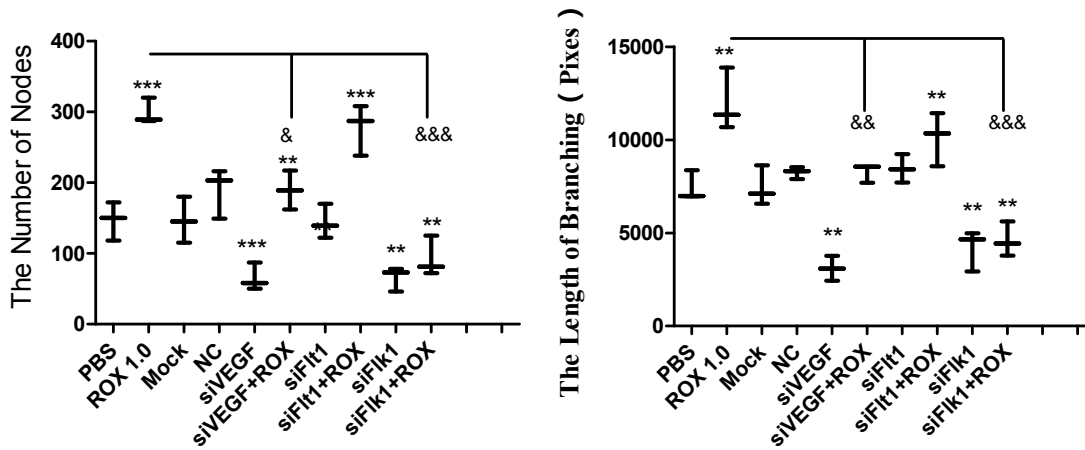


Figure 5b

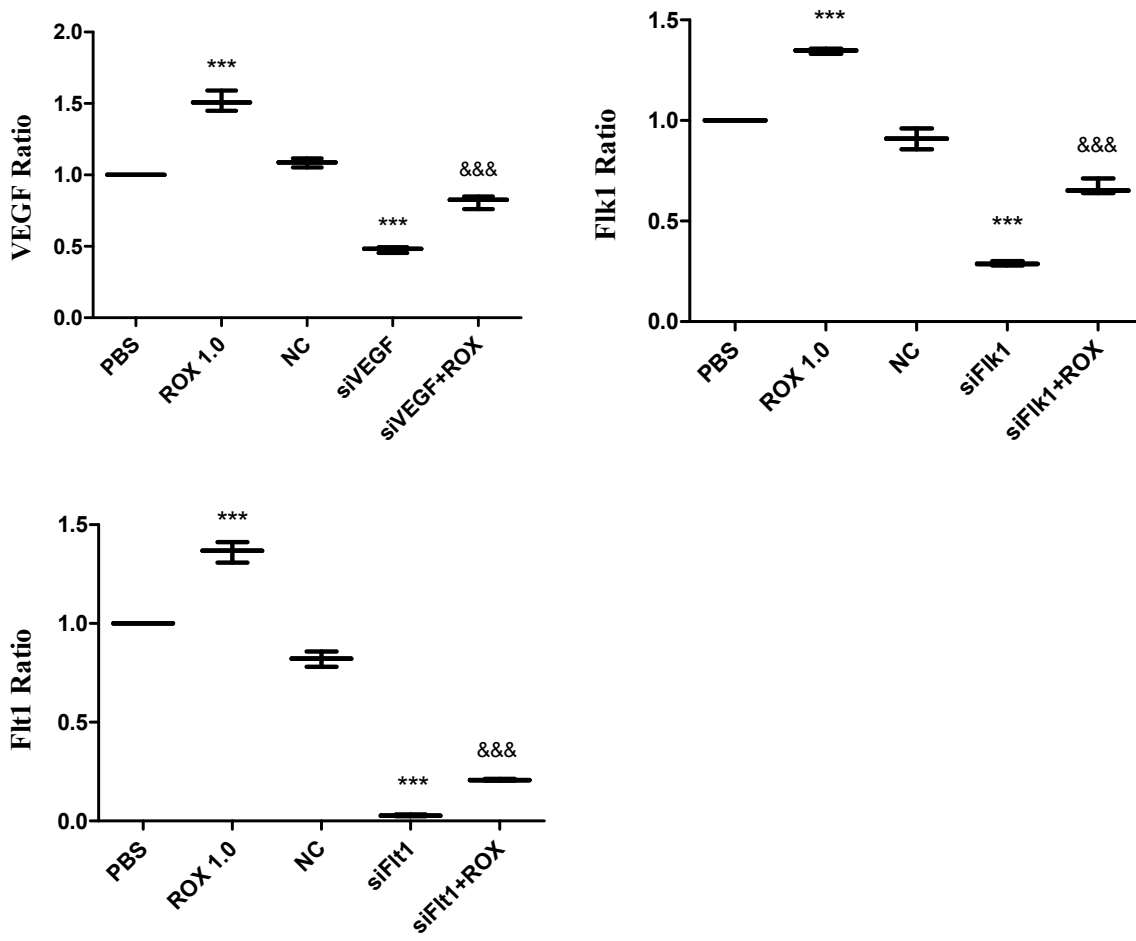


Figure 6b

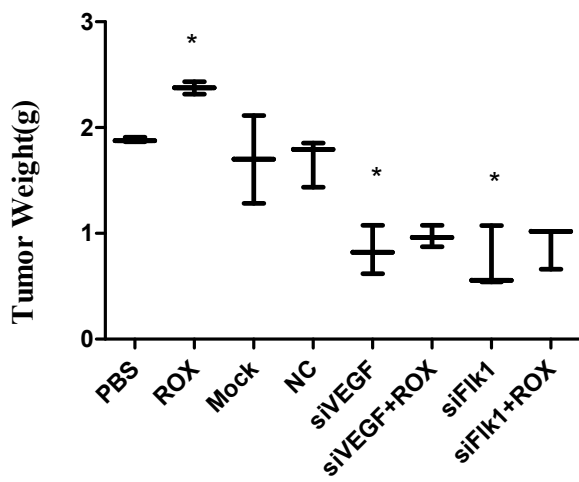


Figure 7b

