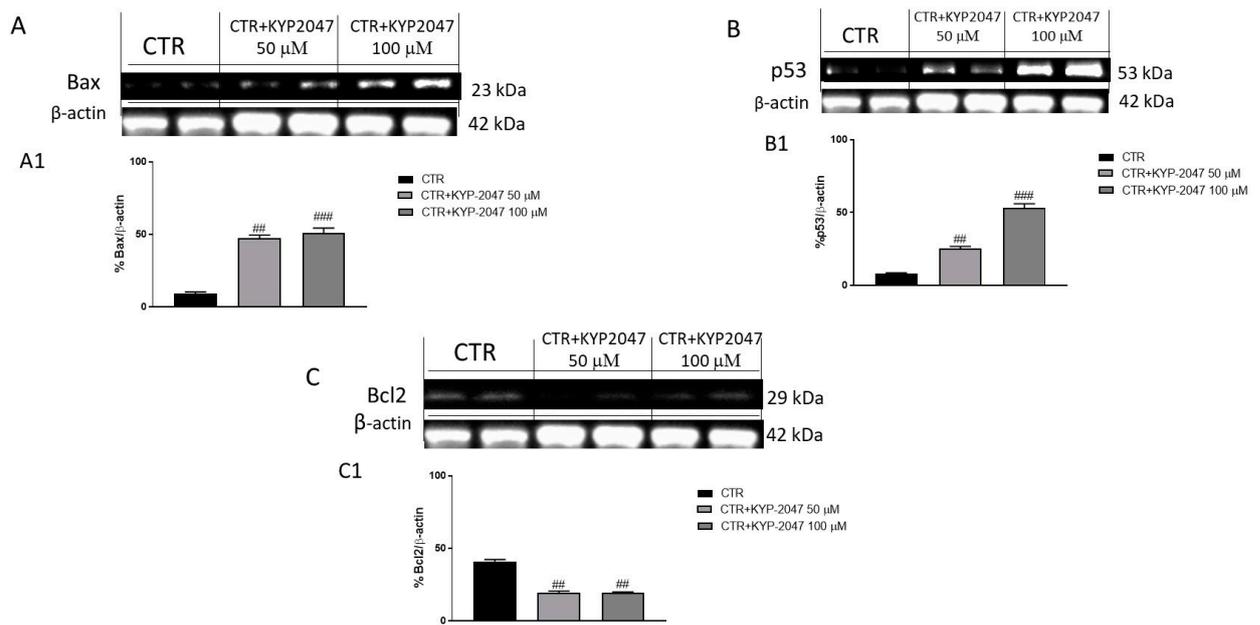


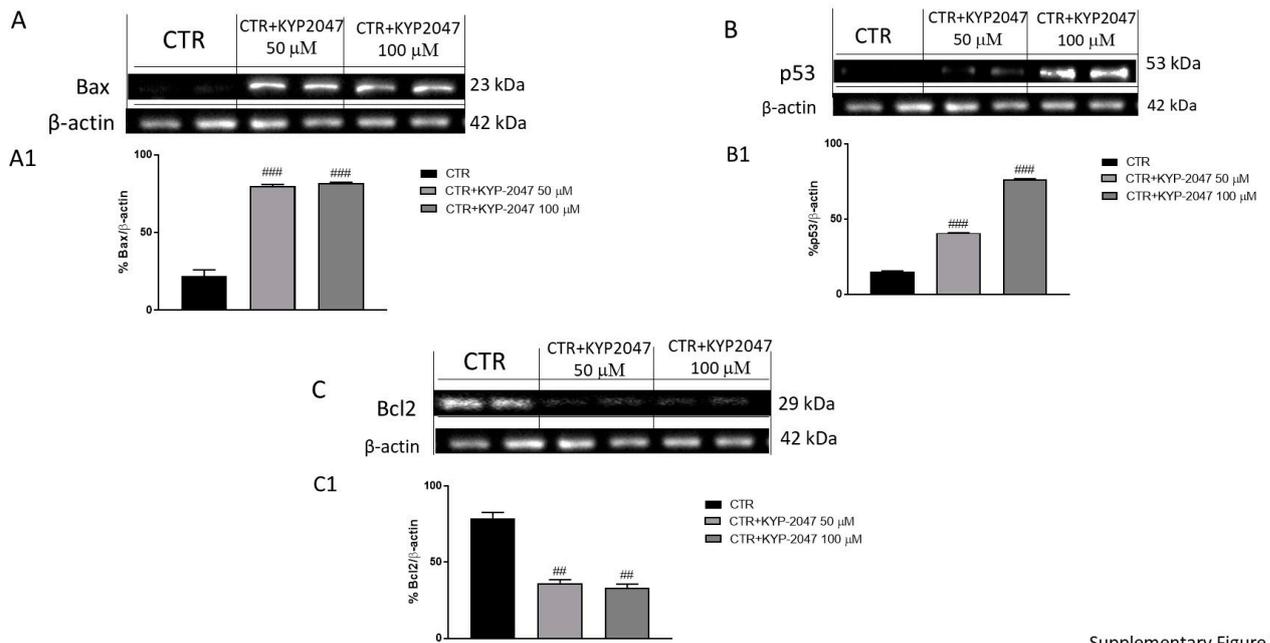
# Supplementary Material: KYP-2047, an Inhibitor of Prolyl-Oligopeptidase, Reduces Glioblastoma Proliferation through Angiogenesis and Apoptosis Modulation

Sarah Adriana Scuderi, Giovanna Casili, Alessio Ardizzone, Stefano Forte, Lorenzo Colarossi, Serena Sava, Irene Paterniti, Emanuela Esposito, Salvatore Cuzzocrea and Michela Campolo



Supplementary Figure 1

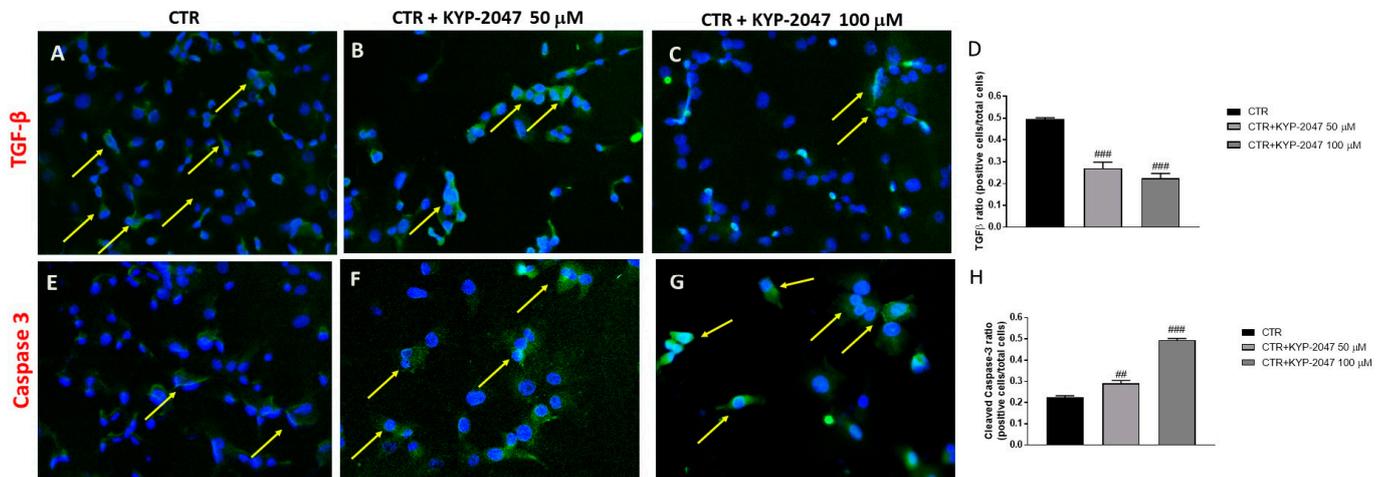
**Figure S1.** Effect of KYP-2047 on apoptosis pathway in A-172 cell lysates. The blots on A172 cell lysates showed an increase of Bax and p53 expression following KYP-2047 treatment at the concentrations of 50  $\mu$ M and 100  $\mu$ M compared to control group (A,B). Additionally, KYP-2047 at the concentrations of 50  $\mu$ M and 100  $\mu$ M reduced significantly Bcl2 expression compared to control group (C). Data are representative of at least three independent experiments. (A) ##  $p < 0.01$  vs CTR; ###  $p < 0.001$  vs. CTR; (B) ##  $p < 0.01$  vs. CTR; ###  $p < 0.001$  vs. CTR; (C) ##  $p < 0.01$  vs. CTR.



Supplementary Figure 2

**Figure S2.** Effect of KYP-2047 on apoptosis pathway in U-138 cell lysates. The blots on U-138 cell lysates showed an increase of pro-apoptotic Bax and p53 expression after KYP-2047 treatment at the concentrations of 50  $\mu$ M and 100  $\mu$ M compared to control group (A,B). Whereas, KYP-2047 at the concentrations of 50  $\mu$ M and 100  $\mu$ M reduced significantly Bcl2 expression compared to control group (C). Data are representative of at least three independent experiments. (A) <sup>###</sup>  $p < 0.001$  vs CTR; (B) <sup>###</sup>  $p < 0.001$  vs. CTR; (C) <sup>##</sup>  $p < 0.01$  vs. CTR.

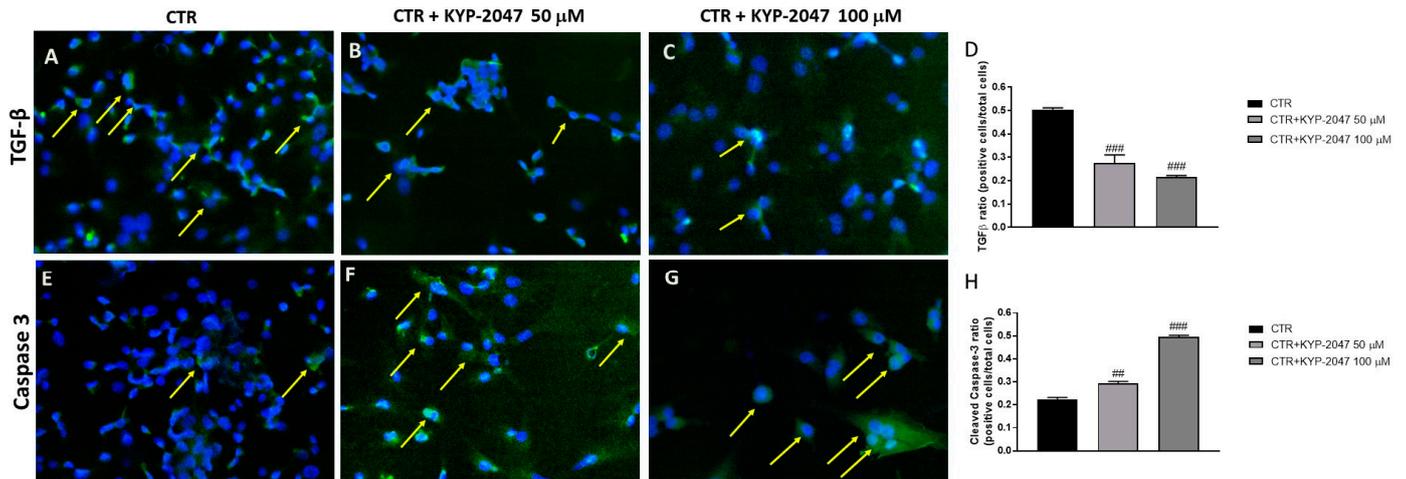
#### A172



Supplementary Figure 3

**Figure S3.** Effect of KYP-2047 on TGF- $\beta$  and Caspase3 expression in A-172 cells. Immunofluorescence assay on A-172 cells revealed an increased expression of TGF- $\beta$  in the control group (A), while the treatment with KYP-2047 at the concentrations of 50  $\mu$ M and 100  $\mu$ M reduced significantly TGF- $\beta$  expression (B,C). Moreover, immunofluorescence staining showed an increase of Caspase-3 levels in the groups treated with KYP-2047 at the concentrations of 50  $\mu$ M and 100  $\mu$ M (E,G) compared to control group (E). Data are representative of at least three independent experiments. (D) <sup>###</sup>  $p < 0.001$  vs. CTR; (H) <sup>##</sup>  $p < 0.01$  vs. CTR; <sup>###</sup>  $p < 0.001$  vs. CTR.

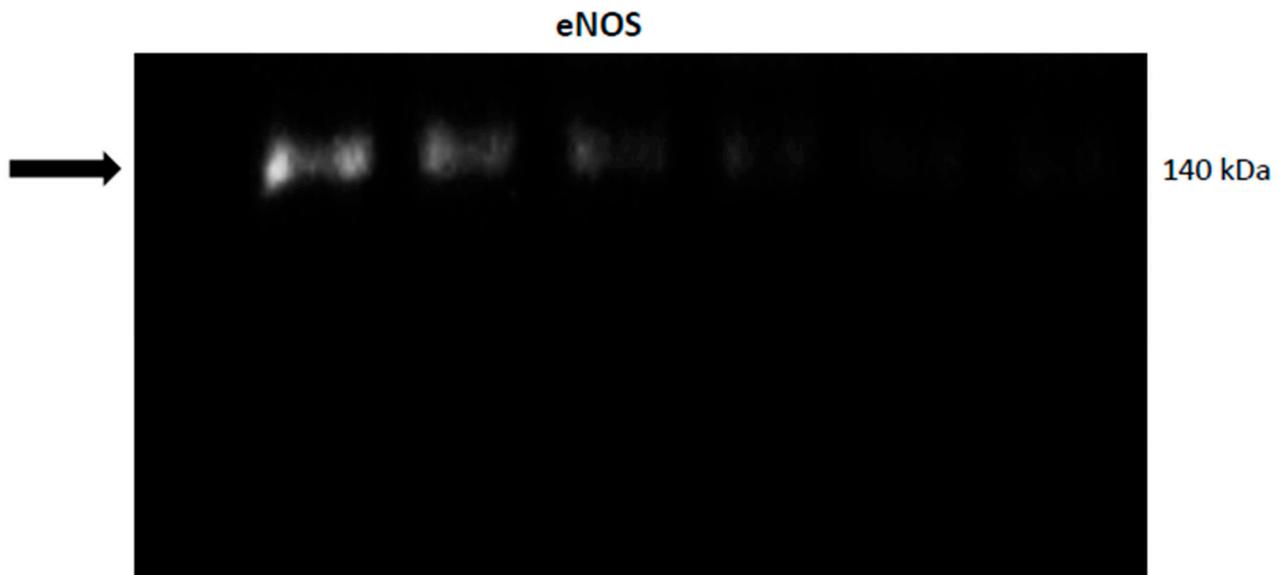
U138



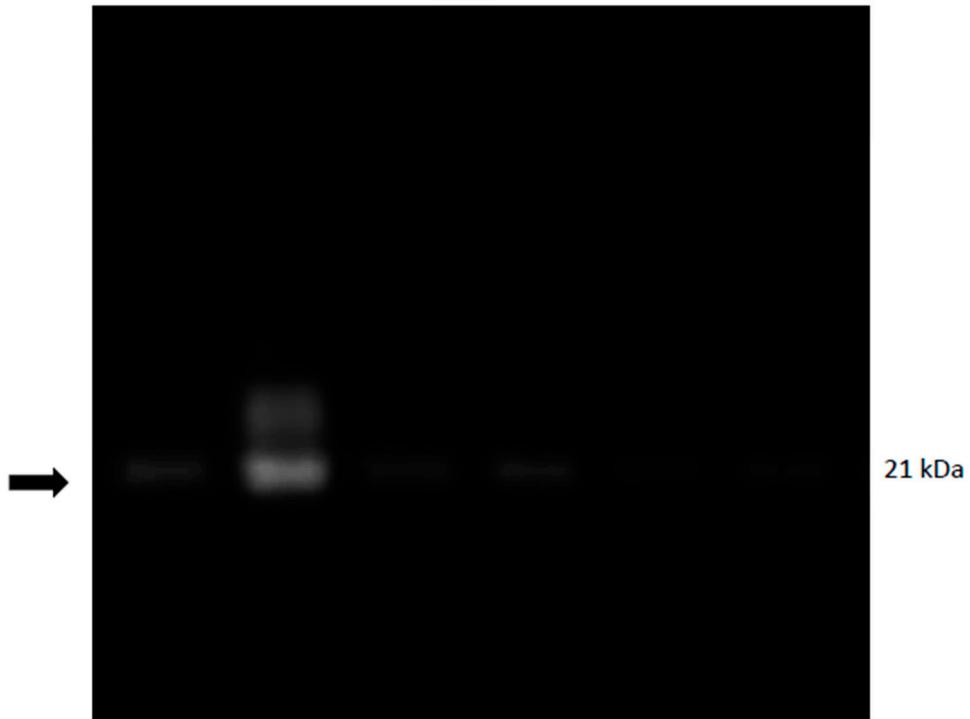
Supplementary Figure 4

**Figure S4.** Effect of KYP-2047 on TGF-β and Caspase3 expression in U-138 cells. Immunofluorescence assay on U-138 cells showed a marked expression of TGF-β in the control group (A), while the treatment with KYP-2047 at the concentrations of 50 μM and 100 μM reduced significantly TGF-β expression (B,C). Immunofluorescence assay showed an increase of Caspase-3 expression in the groups treated with KYP-2047 at the concentrations of 50 μM and 100 μM (F,G) compared to control group (E). Data are representative of at least three independent experiments. (D) ###  $p < 0.001$  vs. CTR; (H) ##  $p < 0.01$  vs. CTR; ###  $p < 0.001$  vs. CTR.

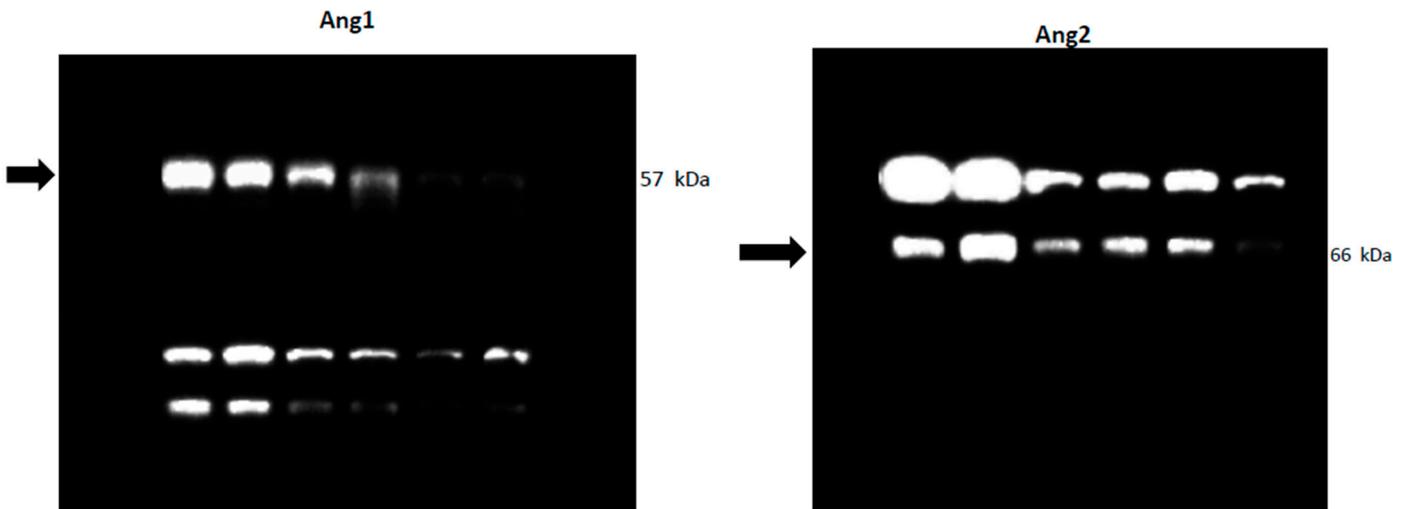
Original Western Blot  
Tumor samples U87-xenograft  
model



Original Western Blot  
Tumor samples U87-xenograft  
model  
VEGF



Original Western Blot  
Tumor samples U87-xenograft  
model



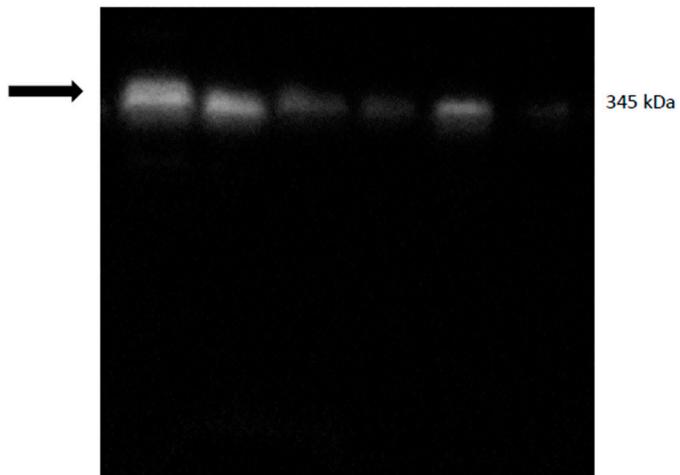
Original Western Blot  
Tumor samples U87-xenograft  
model

$\beta$ -actin for VEGF, eNOS, Ang1  
and Ang2

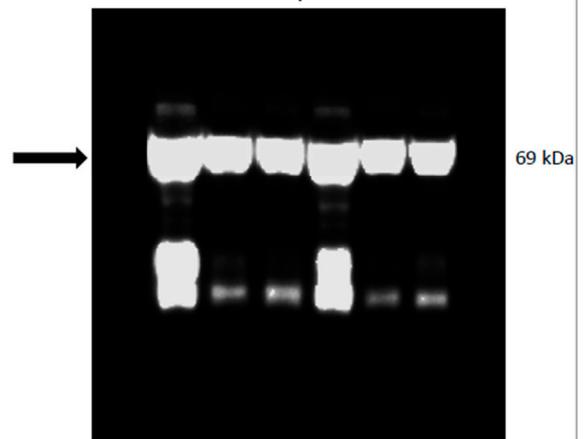


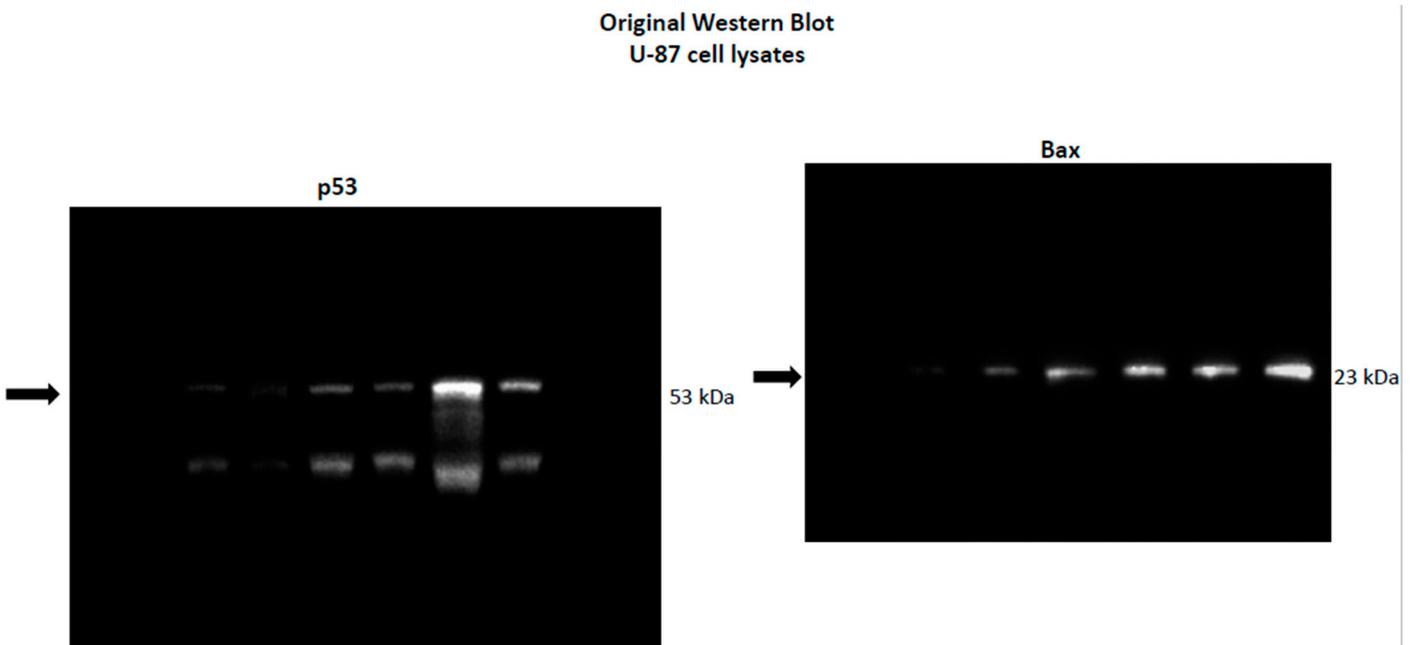
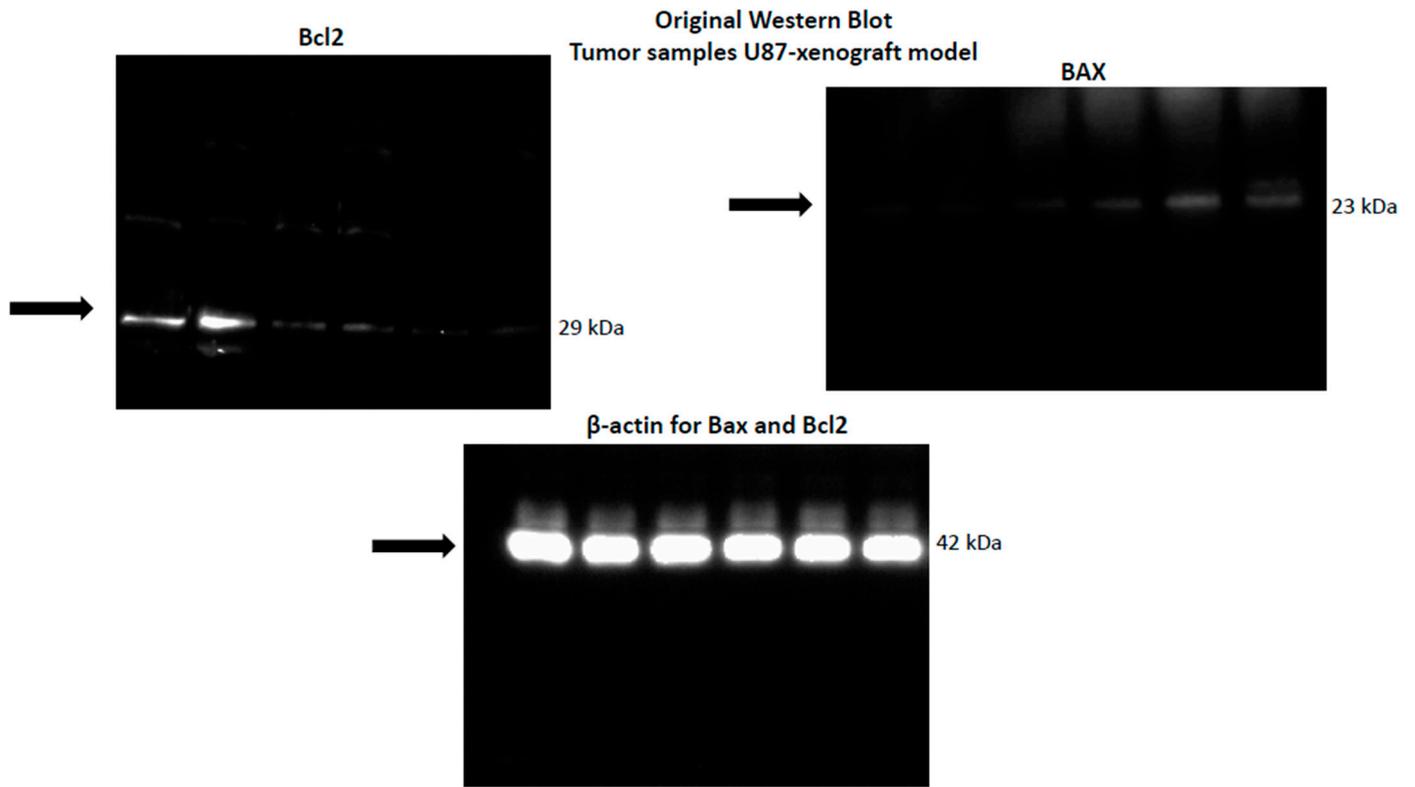
Original Western Blot  
Tumor samples U87-xenograft  
model

Ki-67

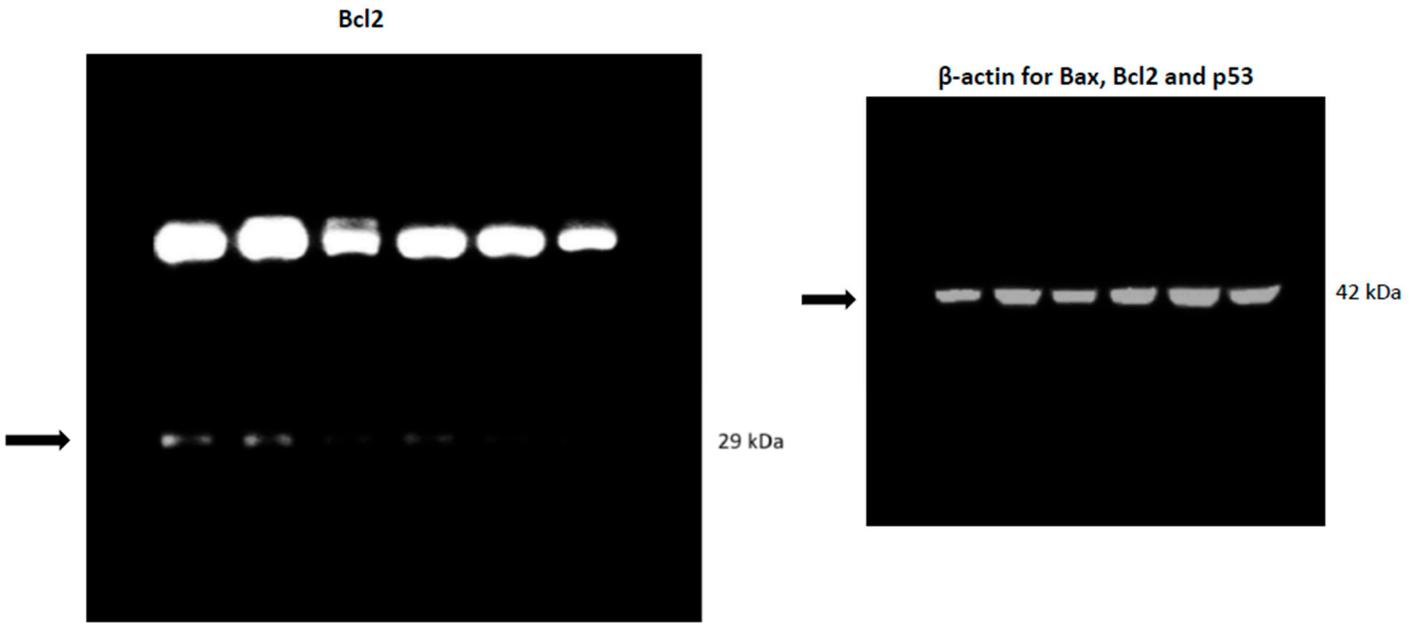


Lamin A/C for Ki-67

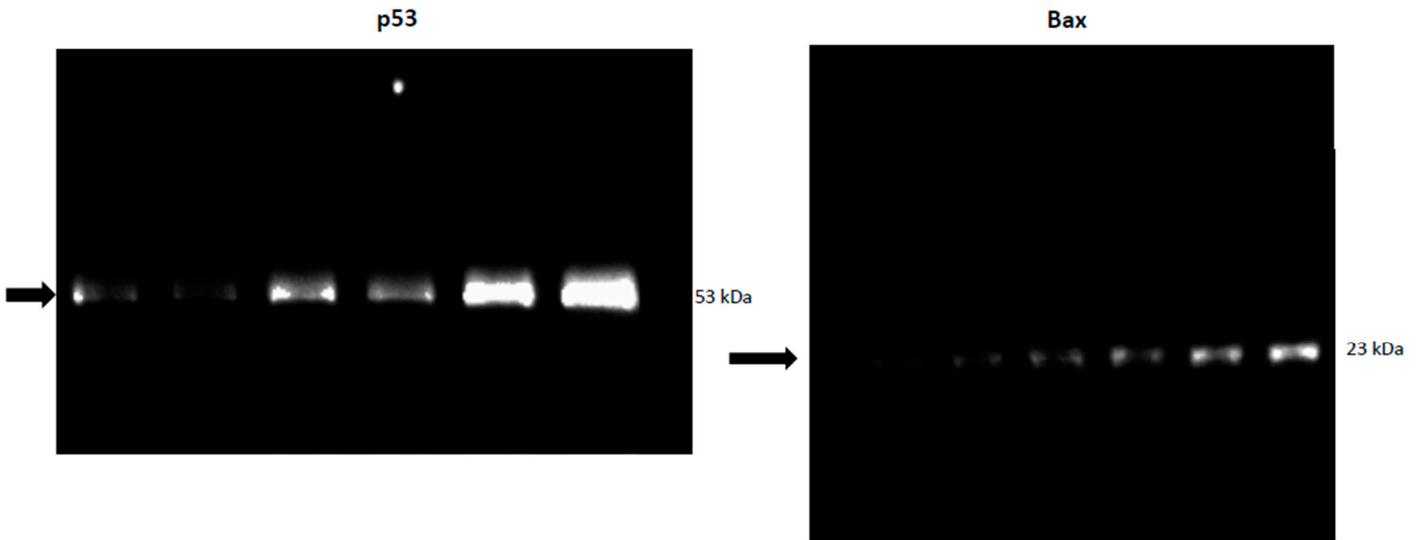




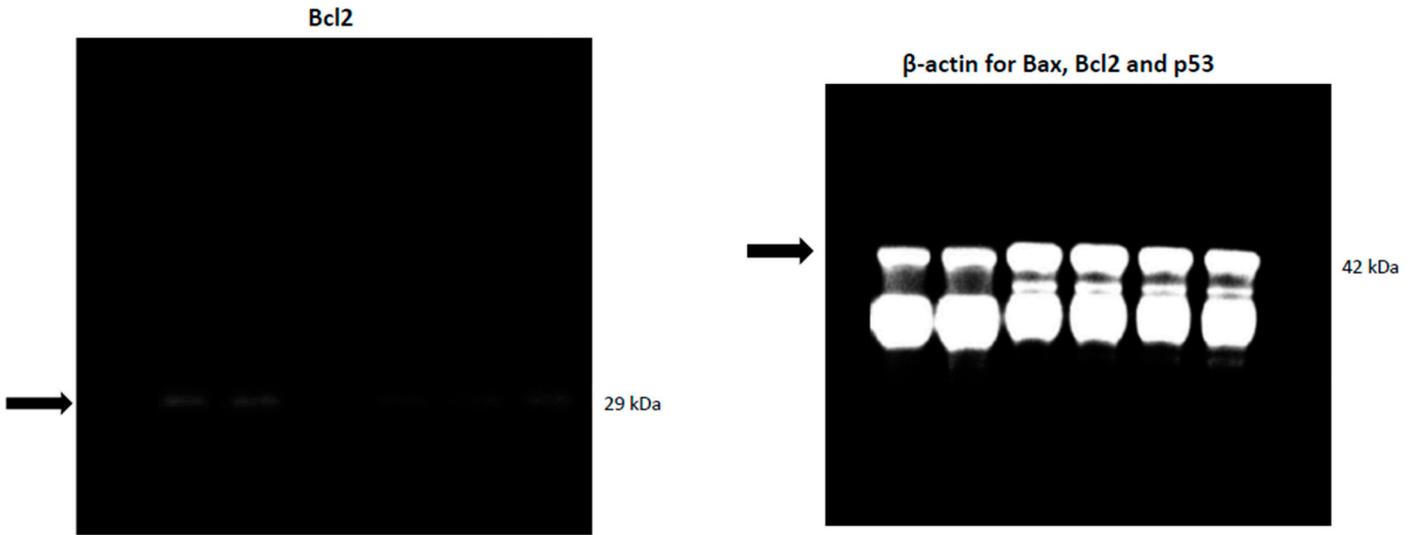
Original Western Blot  
U-87 cell lysates



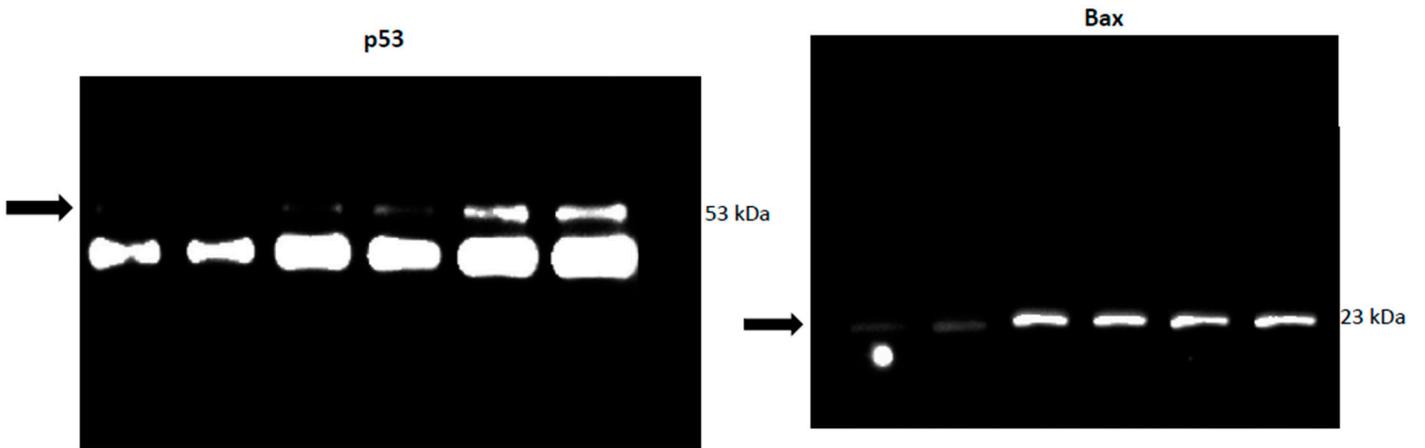
Original Western Blot  
A-172 cell lysates



Original Western Blot  
A-172 cell lysates



Original Western Blot  
U-138 cell lysates



Original Western Blot  
U-138 cell lysates

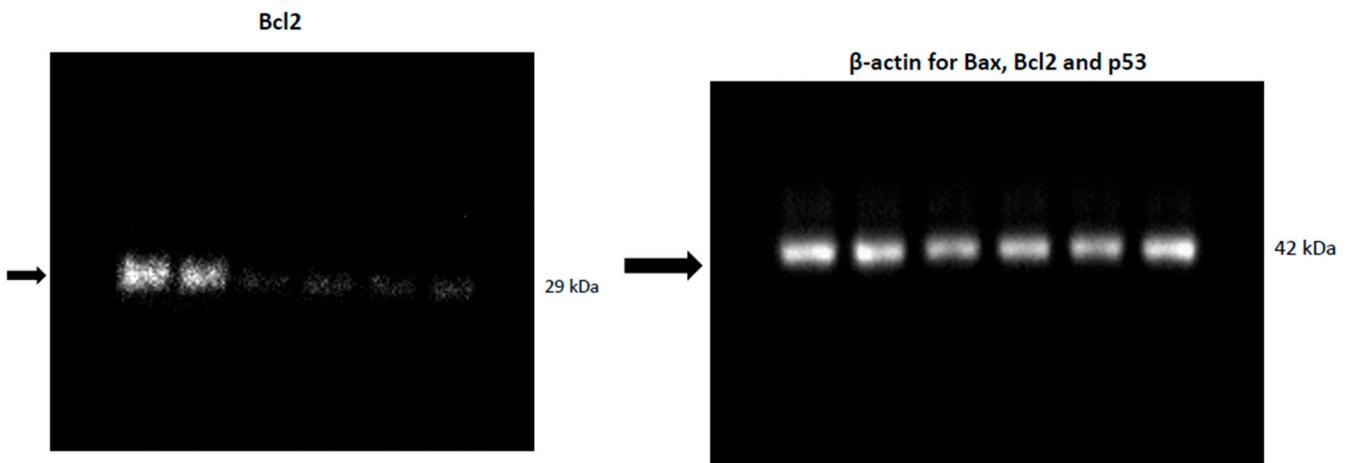


Figure S5. Original Western Blot.