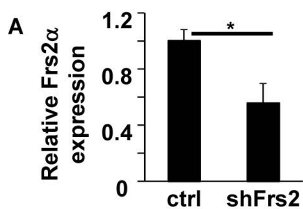
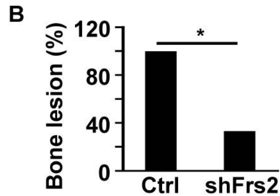
mean ± sd derived from 3 replicate samples. Panels b and d are images of agarose gel electrophoresis showing single band of the PCR products. Ctrl, control siRNA; IP, immunoprecipitation; F/F, $Frs2\alpha^{flox}$; CN, $Frs2\alpha^{CN}$; *, P<0.05.

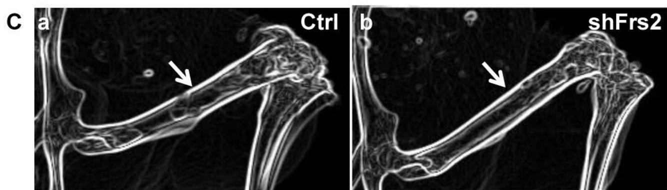
Online Fig. 1. Depletion of FRS2α compromises the tumorigenicity of MDA PCa 118b cells. A. Real-time RT-PCR analyses of MDA PCa 118b cells infected with shFRS2α (n=6) control (n=6) adenoviruses. **B&C**. X-ray analyses of mouse femurs 7 weeks after injection of 1.5 X 10^6 of the indicated cells. Investigators were blinded when assessing the bone lesions and blood vessels. The tumor incidence rates were calculated and expressed as mean ± sd (B). Representative images are shown in (C). **D**. Immunostaining of tumor sections with anti-CD31 antibodies. Panel c is the average percent of CD31 stained areas in randomly viewed areas. Ctrl, control virus infected PC3 cells; *, P<0.05.

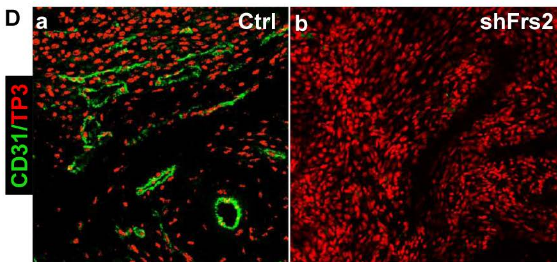
Online Fig. 2. Validation of antibodies used for immunostaining. A&B. Immunostaining of HIF1α, FRS2α, phosphorylated FRS2α, and cJun. Expression of HIF1α in PC3 cells was induced by treating the cells with 150 μM $CoCl_2$ in DEMEM containing 2% serum for 24 hours. Untreated cells were used as a negative control for anti-HIF1α antibody. For anti-FRS2α staining anti-phosphorylated FRS2α, and anti-cJUN, the siRNA depleted cells were used as negative controls. **C**. The human PCa tissue section was co-stained with anti-CD31 antibody and isolectin. The images were captured with a confocal microscope. The data showed that both anti-CD31 and

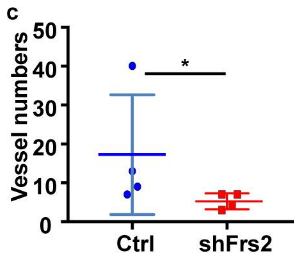
isolectin stained the same areas of the section, indicating that anti-CD31 antibody specifically recognized endothelial cells in the tissue. To-Pro3 (TP3) was used for nuclear counterstaining. Ctrl, control.

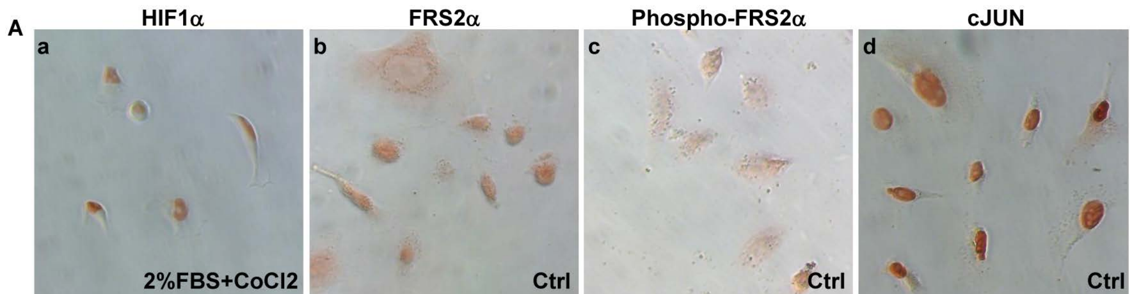

A
Relative Frs2α expression
1.2
0.8
0.4
0
*
ctrl
shFrs2

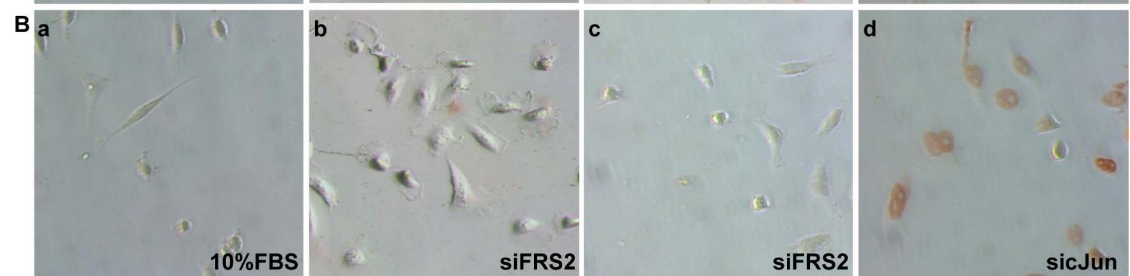

B
Bone lesion (%)
120
80
40
0
*
Ctrl
shFrs2

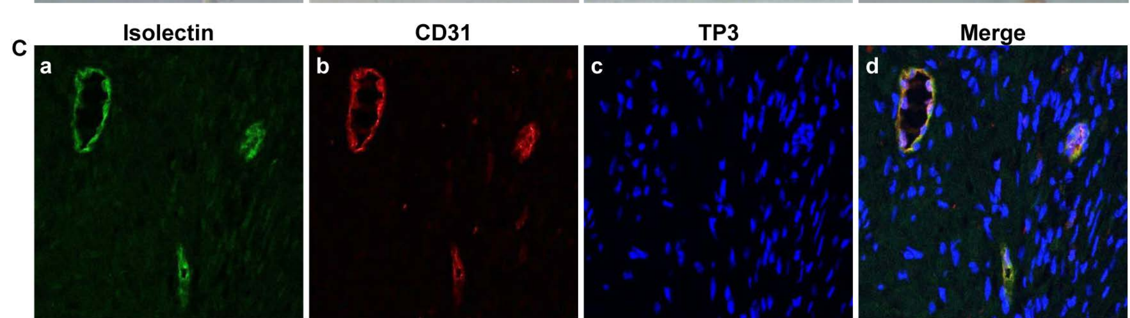

C
a
Ctrl
b
shFrs2


D
a
Ctrl
b
shFrs2
CD31/TP3


c
Vessel numbers
50
40
30
20
10
0
*
Ctrl
shFrs2


HIF1α
FRS2α
Phospho-FRS2α
cJUN
A
a
b
c
d
2%FBS+CoCl2
Ctrl
Ctrl
Ctrl


B
a
b
c
d
10%FBS
siFRS2
siFRS2
sicJun


Isolectin
CD31
TP3
Merge
C
a
b
c
d