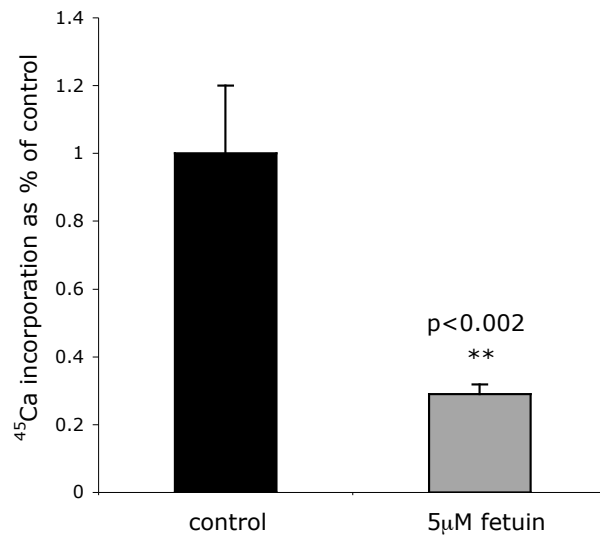


Figure I. Fetuin-A is not expressed in the vessel wall.

A. RT-PCR using primers specific for human fetuin-A was performed on 40 cDNA samples derived from human aortic tissue samples classified as normal, fatty streak (both non-calcified) and atherosclerotic (mildly and heavily calcified). β -microglobulin control RT-PCR reactions confirmed equal loading of cDNA (not shown).

Web supplement Figure I

A.



B.

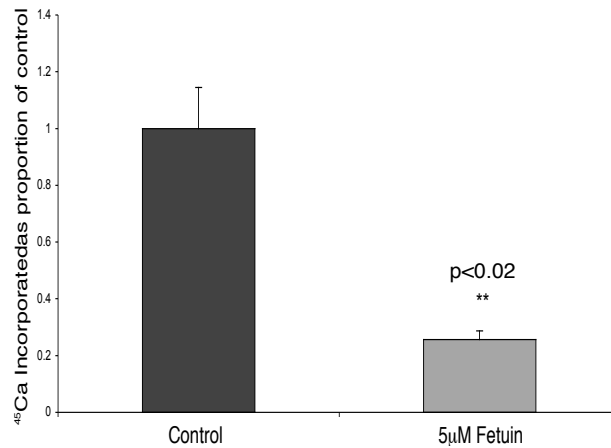


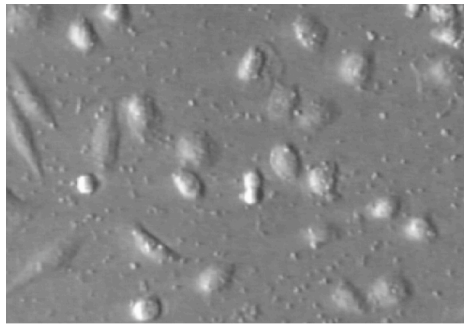
Figure II. Fetuin-A inhibits human VSMC calcification in response to Pi and Cai.

A. Fetuin-A significantly inhibited VSMC calcification induced in response to Pi medium (2.0mM P) that occurred over 10 days.

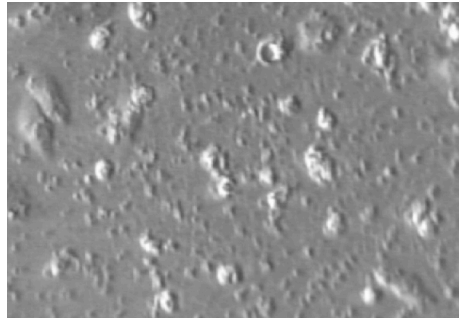
B. Fetuin-A significantly inhibited VSMC calcification induced in response to Cai medium (5.4mM Ca) that occurred over 24 hours.

Mean +/-SD shown, n=3.

A.

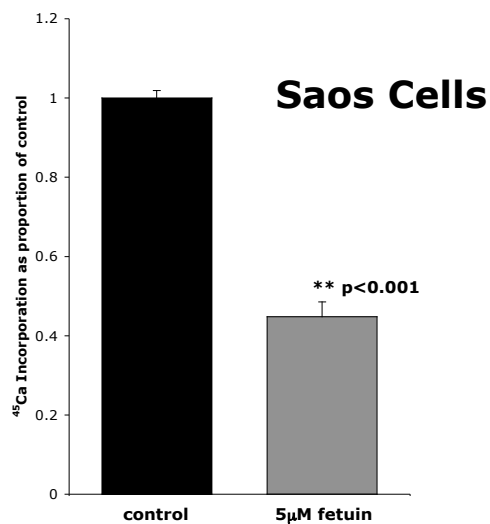


1 hr



20 hrs

B.



C.

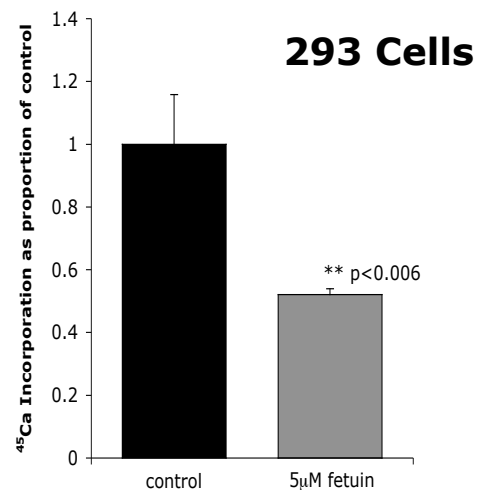


Figure III. Saos2 and 293 cells calcify by a vesicle mediated mechanism in response to extracellular ions. Fetuin-A inhibits calcification.

A. Timelapse still images showing vesicle release and apoptosis (rounded cells) of SAOS cells in response to Cai medium. Vesicle release is evident at 1 hr and the matrix is calcified with a granular pattern at 20 hours. EM identified the nidus for this calcification as extracellular vesicles.

B. Fetuin-A significantly inhibited calcification of Saos2 cells in response to Cai medium and also inhibited apoptosis (not shown). Mean \pm SD, n=3

C. Fetuin-A inhibited calcification of human embryonic kidney epithelial 293 cells in response to Cai and CaPi (shown) media. Mean \pm SD, n=3.