Periostin Promotes Atrioventricular Mesenchyme Matrix Invasion and Remodeling Mediated by Integrin Signaling through Rho/PI 3-Kinase

Jonathan T. Butcher, Russel A. Norris, Stanley Hoffman, Corey Mjaatvedt, and Roger R. Markwald

Supplemental Figure Legends

Supplemental Fig. 1: Adenovirus construct to which cassettes of full length periostin overexpressing (sense, PN OX), antisense (PN αS), or LacZ control were added. All viruses were driven by the constitutively active cytomegalovirus (CMV) promoter. To facilitate detection of virally produced periostin, a hemagluttinin tag was incorporated into the C-terminus of the original parent clone by PCR (asterisk).

Supplemental Fig. 2: Quantification of HH25 AV cushion mesenchyme invasion through type I collagen gels after 3 days culture. The numbers of invaded cells decreased monotonically with depth. Comparison between virus infection determined that PNOX > LacZ > PNαS in terms of cell numbers over almost all of the depths analyzed.

Supplemental Fig. 3: No expression of cardiac sarcomeric myosin MF20 (red color) was detected in either the invaded hanging drop aggregate cultures or in the collagen gels populated with isolated cushion mesenchymal cells, indicating no contamination by cardiomyocytes. Cells counterstained for cell nuclei (Draq-5, 1:1000).

Supplemental Fig. 4: Surface area measurements of migrating hanging drop aggregates placed on collagen I gels. No significant differences in migration area were noted between PN αS, PN OX, and LacZ controls.

Supplemental Fig. 5: Phase contrast images of surface (0 μm) and deep (-80 μm) planes of invaded hanging drop aggregates incubated with either the PI 3-kinase inhibitor wortmannin or the Rho kinase inhibitor Y-27632. While minimal differences in cell morphology were noted at the surface of the gels, invaded cells of wortmannin treated cultures were noticeably more rounded with fewer extensions in comparison to the Y-27632 cultures (and controls as seen in Fig. 4). This suggests that PI 3-kinase may be involved in filipodia extension in invading cushion mesenchyme.

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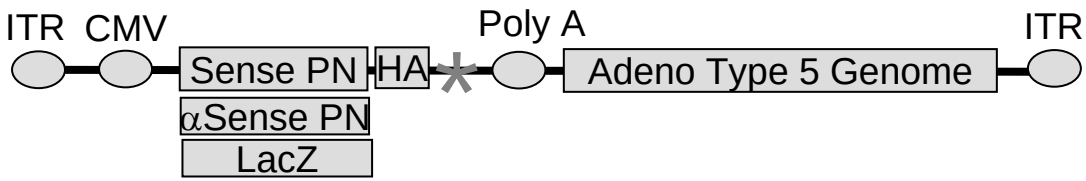
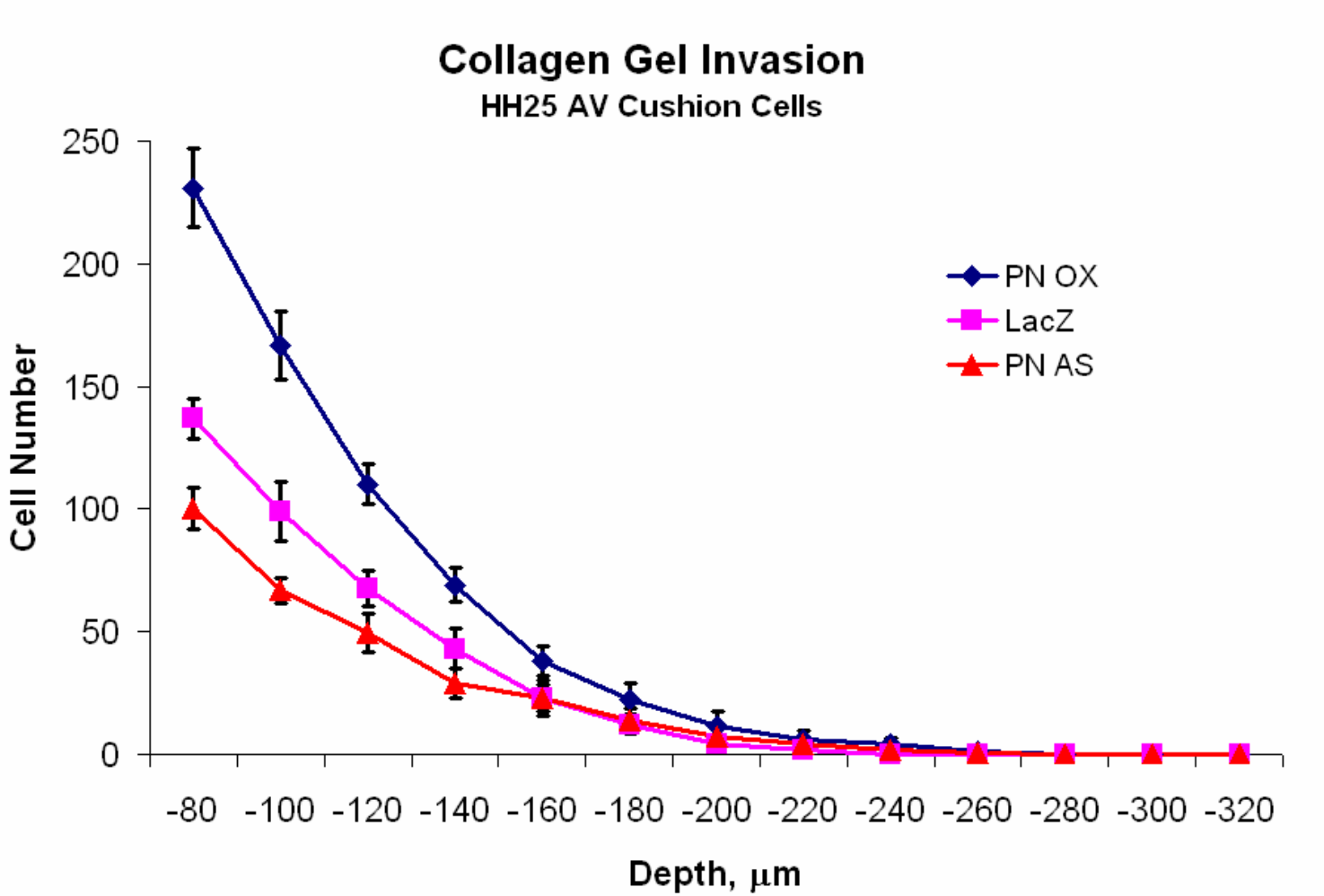
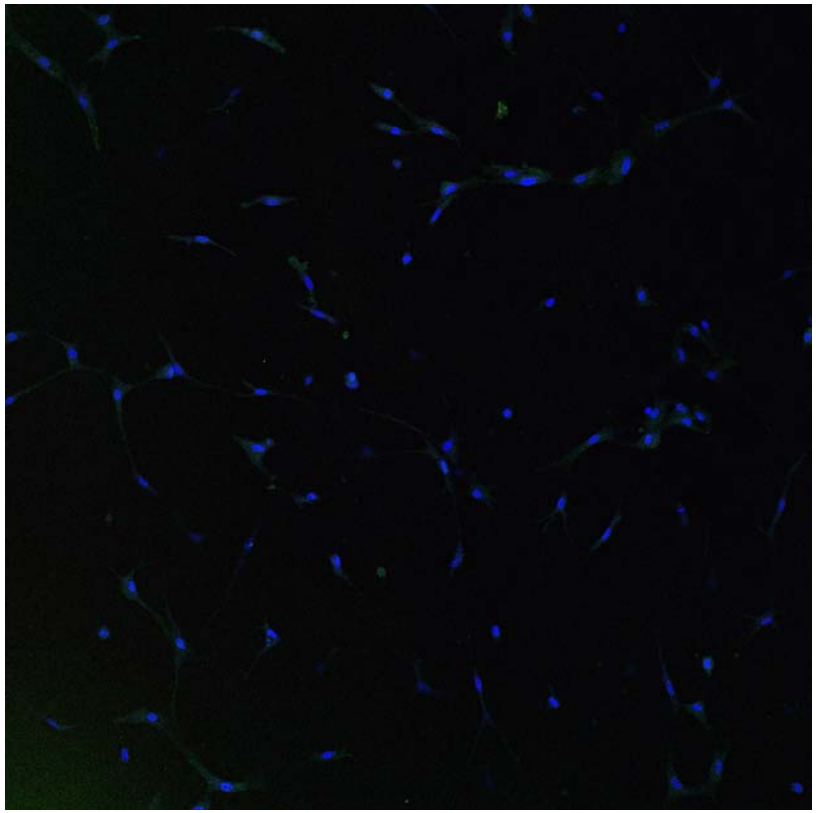
Supplemental Figures


ITR
CMV
Sense PN
HA
Poly A
Adeno Type 5 Genome
ITR
αSense PN
LacZ

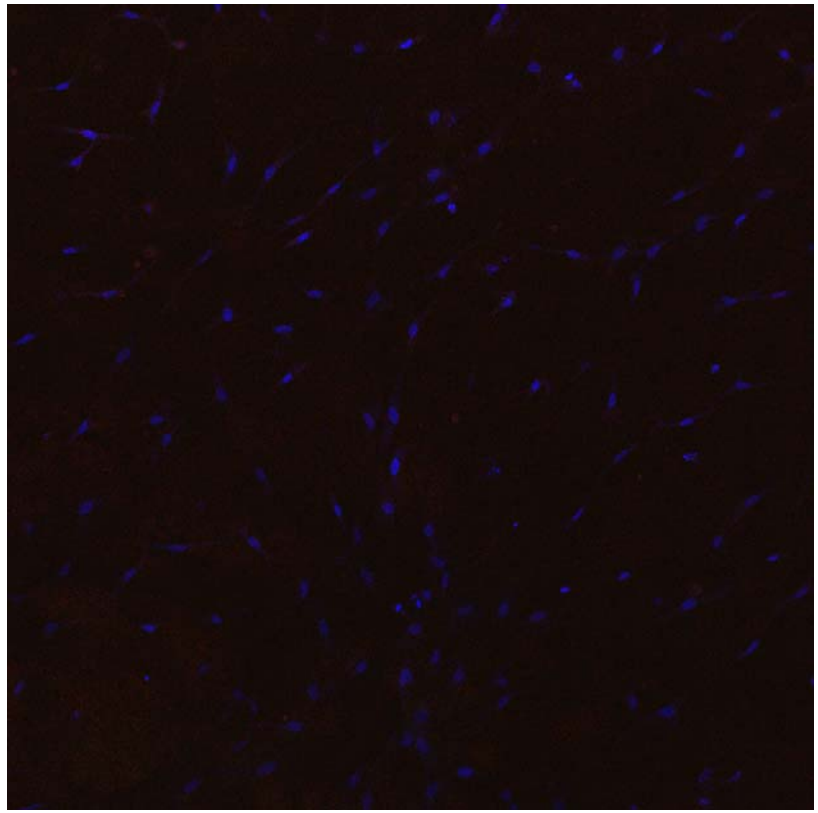
Supp. Fig. 1


Collagen Gel Invasion
HH25 AV Cushion Cells
Cell Number
250
200
150
100
50
0
-80
-100
-120
-140
-160
-180
-200
-220
-240
-260
-280
-300
-320
Depth, μm
PN OX
LacZ
PN AS

Supp. Fig. 2

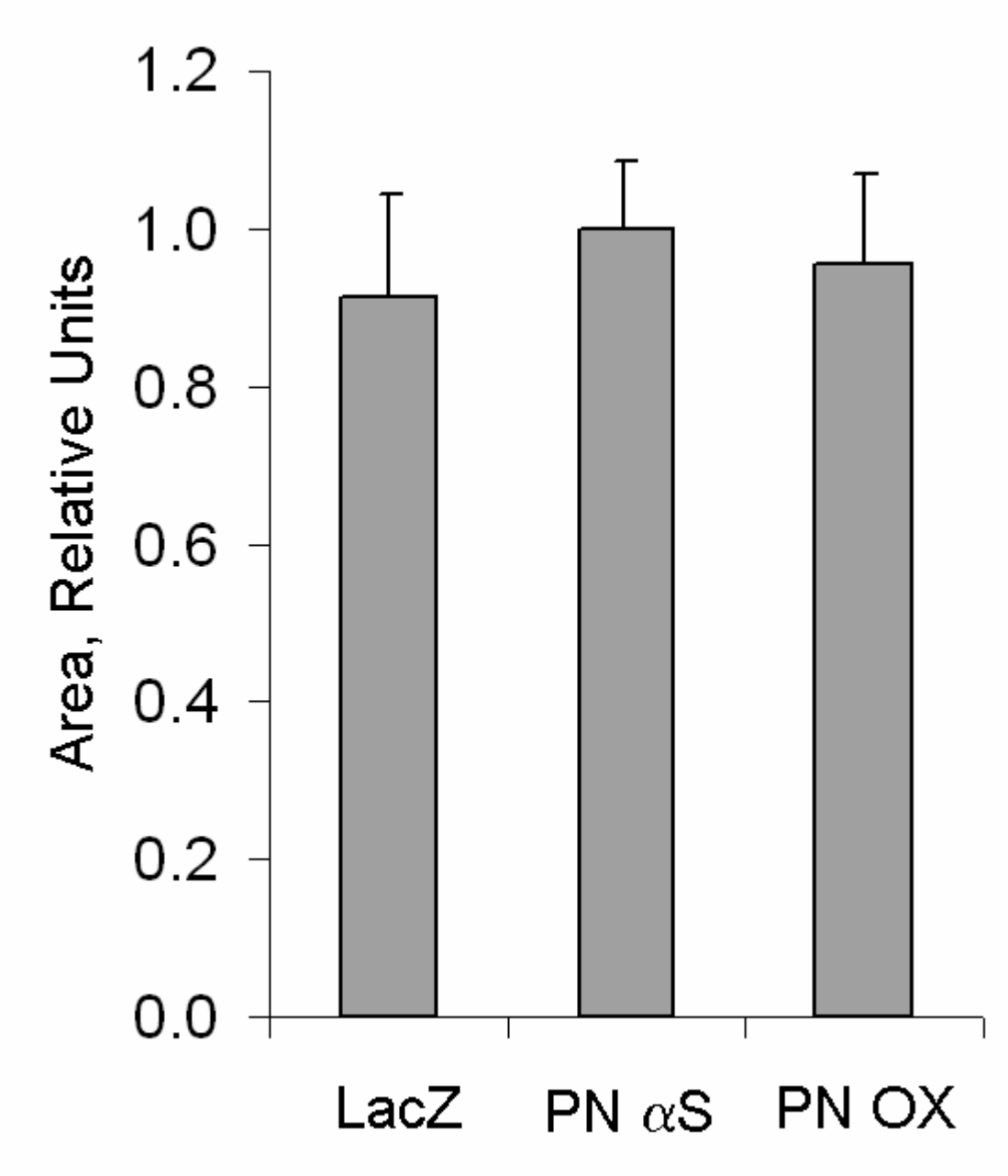


Disk Gels

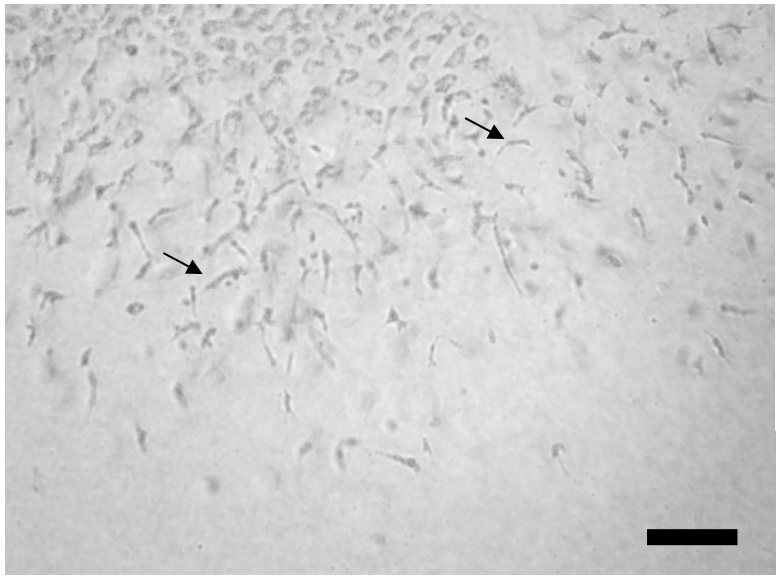


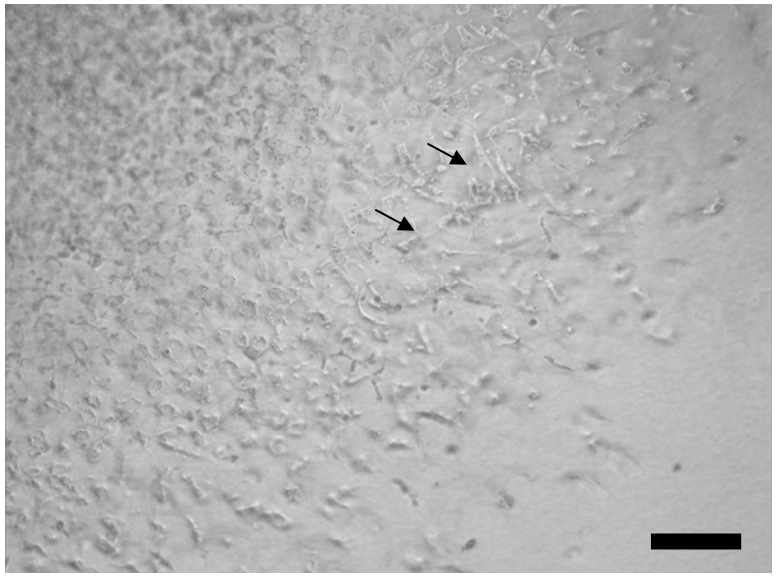
Invaded Aggregates

Supp. Fig. 3

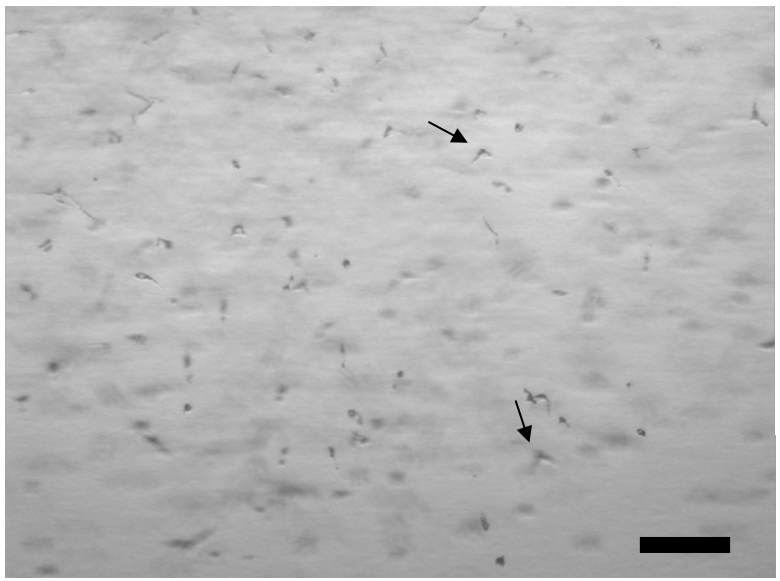


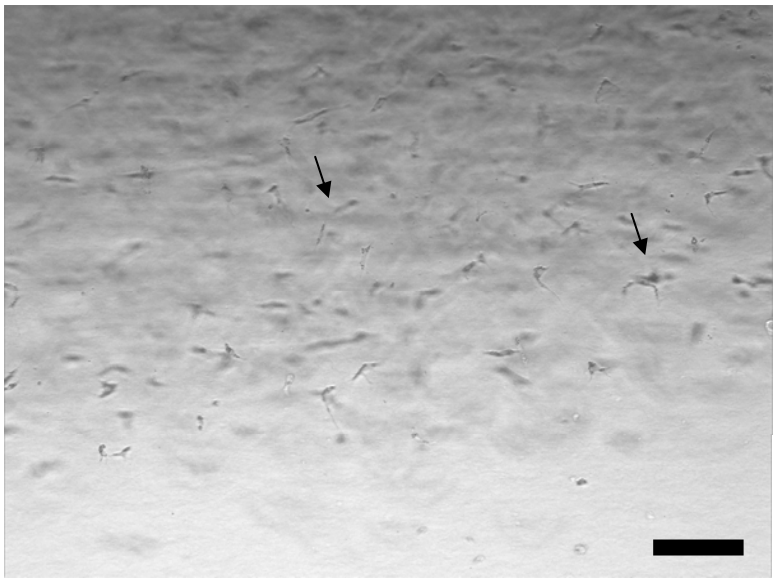
Supp. Fig. 5





Surface





Deep

Wortmannin 1 μM

Y-27632 5 μM

Supp. Fig. 5