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Supplementary Materials for

A Monoclonal Antibody Against $\alpha_V\beta_3$ Integrin Inhibits Development of Atherosclerotic Lesions in Diabetic Pigs

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Fig. S1. Effect of C-loop β_3 F(ab)$_2$ on IGF-1 signaling.
Fig. S2. β_3 phosphorylation in lesions.
Fig. S3. Effect of C-loop β_3 F(ab)$_2$ infusion on IGF signaling events in porcine arteries.

Supplemental Material: Figure Legends

Fig S1 Effect of C-loop β3 F(ab)$_2$ on IGF-I signaling events

SMCs were maintained and treated as described for Fig 1.

(A) β3 phosphorylation (left hand panel) was visualized following immunoprecipitation and immunoblotting with an anti-phosphotyrosine antibody (p-Tyr). β3 protein (right hand panel) was visualized by direct immunoblotting with an anti-β3 antibody. The arrow indicates the (phosphorylated) β3 subunit.

(B) Shc phosphorylation was visualized following immunoprecipitation (IP) with an anti-Shc antibody and immunoblotting (IB) with an anti-phosphotyrosine antibody (p-Tyr; left hand panel). Shc association with Grb-2 was determined by immunoprecipitating with an anti-Shc antibody and immunoblotting with an anti-Grb-2 antibody (middle panel). To control for loading, blots were reprobed with an anti-Shc antibody (right hand panel).

(C) Phosphorylated/total ERK1/2 was visualized following immunoblotting (IB) with an anti-phospho ERK1/2 (left hand panel) or anti-ERK antibody (right hand panel).

Fig S2 β3 phosphorylation in lesions. Aortic tissue was obtained from 3 hyperglycemic and 3 normoglycemic pigs that were from the same strain and of similar age. Lysates were prepared as described in methods. The lysates were immunoprecipitated using anti-β3 antibody and immunoblotted for phosphotyrosine (left hand panel) or immunoblotted using an anti-β3 antibody directly (right hand panel), as described in methods. The arrow indicates the (phosphorylated) β3 subunit. The graph shows the extent of the β3 phosphorylation in aortic extracts expressed as scanning units (mean±SEM). The aortic tissue was obtained from 8 normoglycemic and to 8 hyperglycemic animals.

Fig S3: Effect of C-loop β3 F(ab)$_2$ infusion on IGF signaling events in porcine arteries.

Femoral artery lysates were prepared as described for fig 3. Proteins were visualized following western immunoblotting (IB) with the appropriate antibody as indicated above each panel. Lanes 1 & 2 (homogenates from arteries that received control vehicle. Lanes 3 & 4 homogenates from arteries that received the anti C-loop β3 F(ab)$_{2)}$.

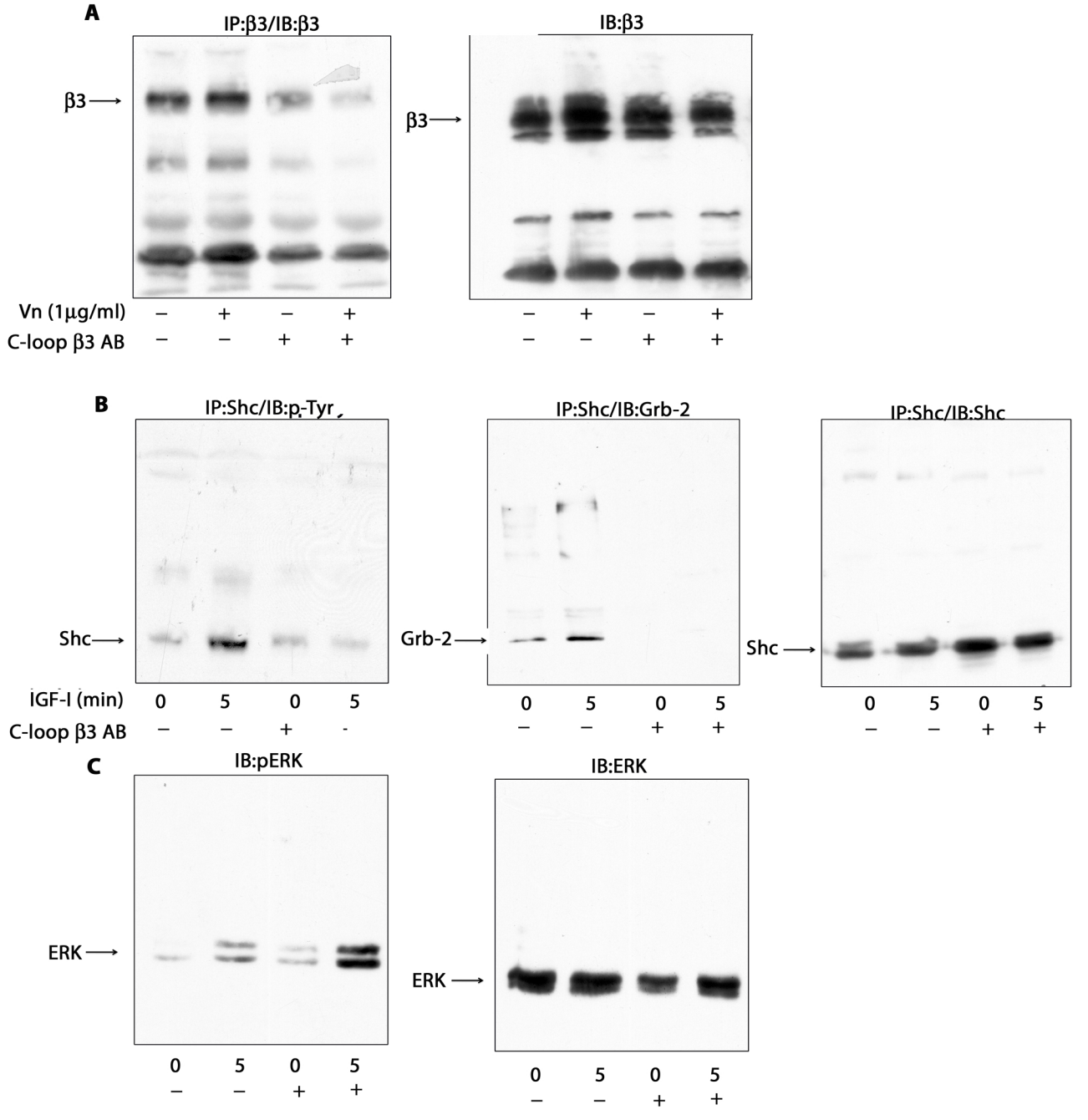

A
IP:β3/IB:β3
IB:β3
β3
β3
Vn (1μg/ml) – + – +
C-loop β3 AB – – + +
– + – +
– – + +
B
IP:Shc/IB:p-Tyr
IP:Shc/IB:Grb-2
IP:Shc/IB:Shc
Shc
Grb-2
Shc
IGF-I (min) 0 5 0 5
C-loop β3 AB – – + -
0 5 0 5
– – + +
0 5 0 5
– – + +
C
IB:pERK
IB:ERK
ERK
ERK
0 5 0 5
– – + +
0 5 0 5
– – + +

Figure S1

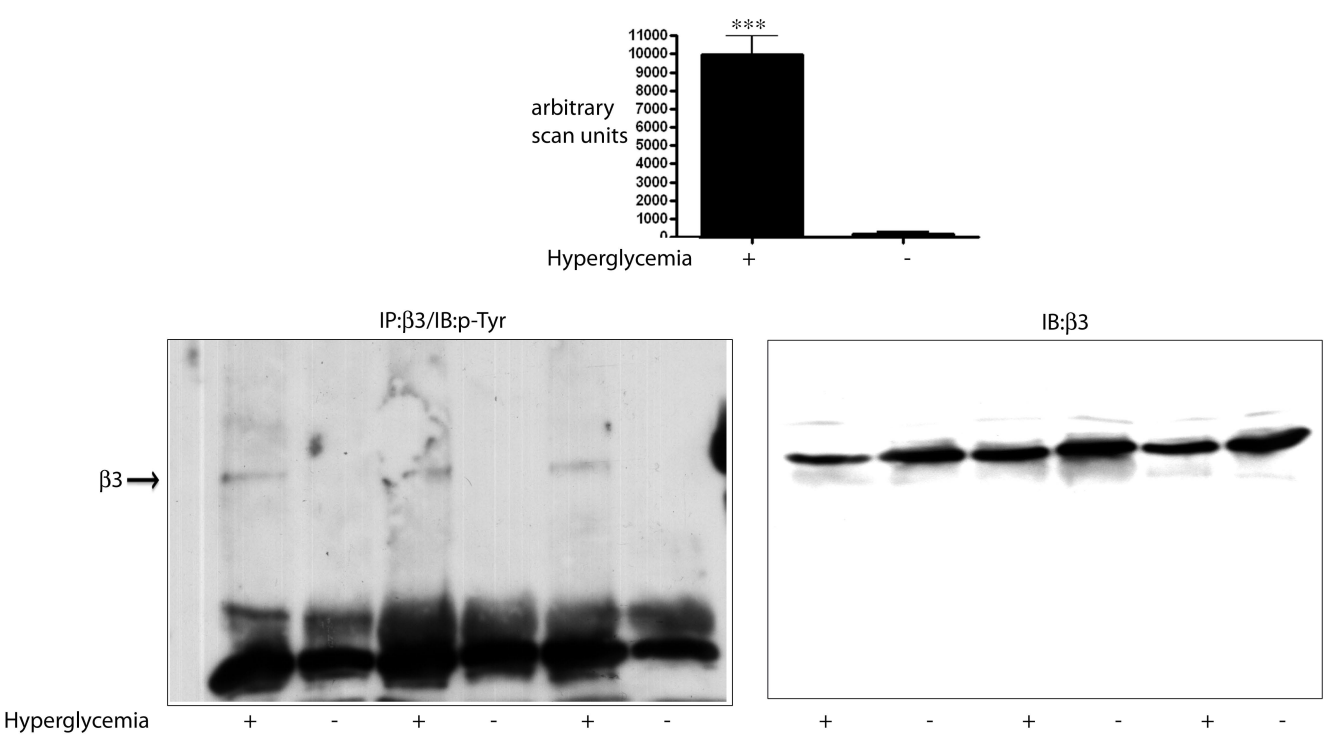
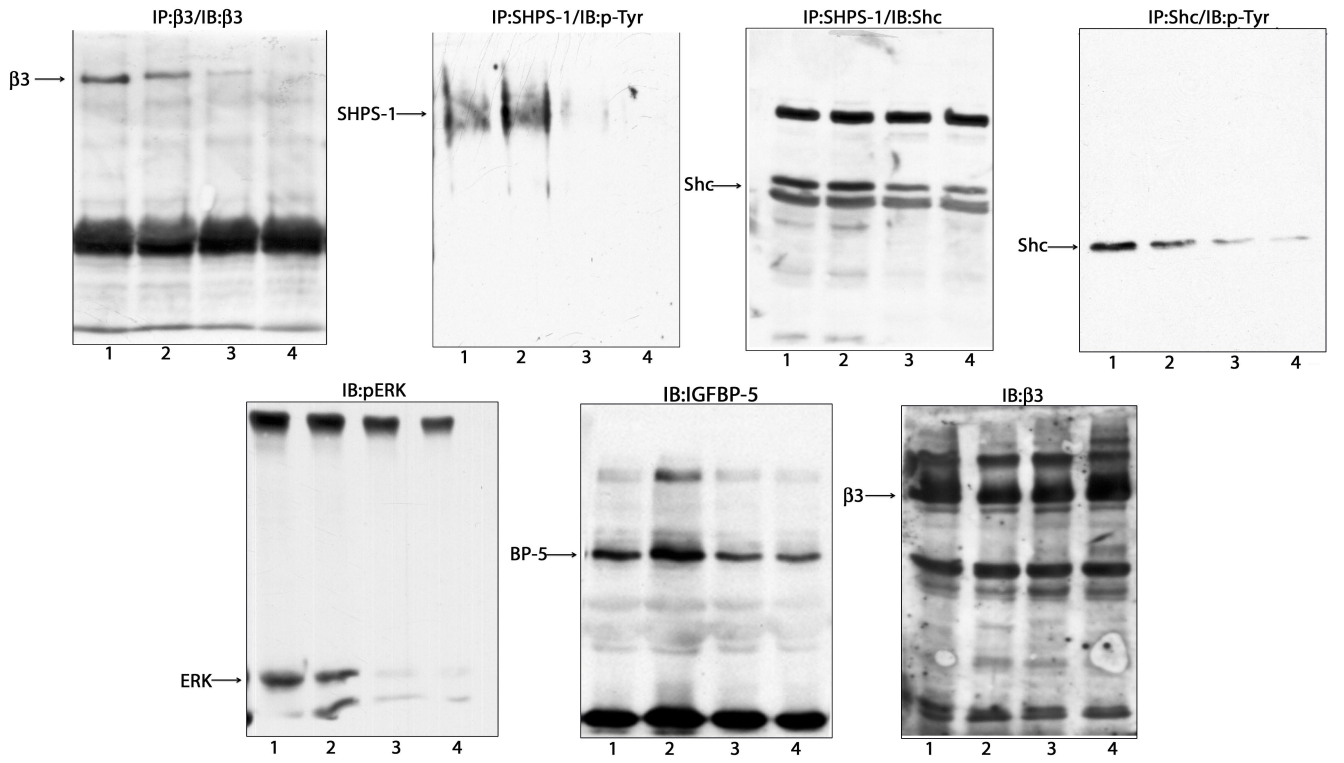

***
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
arbitrary
scan units
Hyperglycemia
+
-
IP:β3/IB:p-Tyr
IB:β3
β3
Hyperglycemia
+
-
+
-
+
-
+
-
+
-
+
-

Figure S2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β3
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SHPS-1
IP:SHPS-1/IB:Shc
Shc
IP:Shc/IB:p-Tyr
Shc
1 2 3 4
IB:pERK
ERK
IB:IGFBP-5
BP-5
IB:β3
β3

Figure S3