SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Time course of insulin-mediated eNOS phosphorylation at serine 1177 in HAEC's. HAEC's were treated with 10nM insulin and fixed at time points up to 60minutes. eNOS phosphorylation was quantified using immunofluorescence with 20 cells assessed per time point. There was a transient increase in eNOS phosphorylation at 5 minutes and a sustained increase at 30 minutes.

Supplemental Figure 2. Box and whisker plots showing eNOS activation and insulin resistance in endothelial cells. Top panels show baseline eNOS phosphorylation at serine 1177 and response to insulin comparing diabetic patients and non-diabetic controls. Lower panels show baseline eNOS phosphorylation at threonine 495 and response to insulin comparing diabetic patients and non-diabetic controls.

Supplemental Figure 3. Association of insulin-mediated change in eNOS activation and flow-mediated dilation.

Supplemental Figure 4. Box and whisker plots showing endothelial cell expression of eNOS, Akt, PTEN, and nitrotyrosine in diabetic patients and non-diabetic controls.

Supplemental Figure 5. Box and whisker plots showing endothelial cell expression of PKCβ, p65, and ICAM in diabetic patients and non-diabetic controls. Expression of IκBα in non-diabetic controls, and patients with diabetes before and after treatment with LY379196.

Supplemental Figure 6. Box and whisker plots showing endothelial expression of eNOS phosphorylated at serine 1177 and response to insulin in patients with diabetes before and after treatment with LY379196.

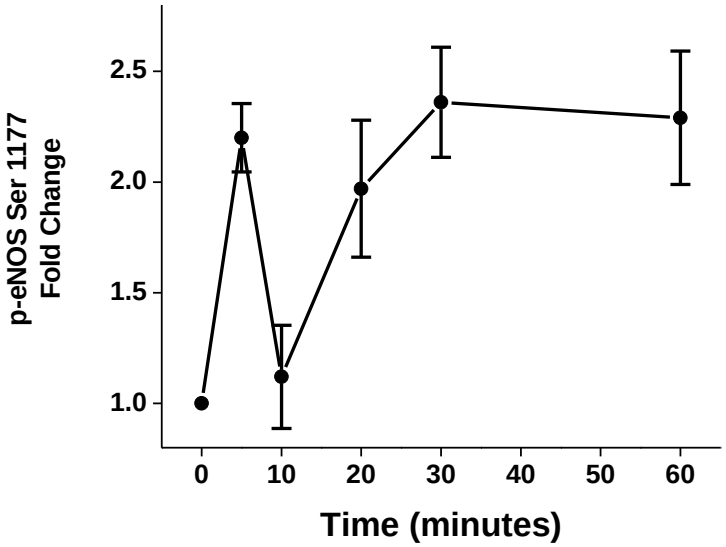
Supplemental Figure 7. Inhibition of PKCβ and inhibitory eNOS phosphorylation. As described in Methods, freshly isolated endothelial cells from patients with diabetes (n=9) were treated with 0 or 30nM LY379196, a pharmacologic inhibitor of PKCβ, along with 0 or 10nM insulin for 30 minutes and eNOS phosphorylation at threonine 495 was measured (20 cells per patient in each condition). **A.** Representative cells from a patient with diabetes treated with LY379196 (right) shows similar basal eNOS phosphorylation at threonine 495 (top, red) compared to control condition (left top, red). Treatment with LY379196 did not change the insulin-mediated increase in eNOS phosphorylation at threonine 495 compared to control condition. **B.** Basal eNOS phosphorylation at threonine 495 was similar in endothelial cells from patients with diabetes treated with LY379196 (P=0.95). **C.** Treatment with LY379196 did not alter eNOS phosphorylation at threonine 495 in response to insulin in endothelial cells from patients with diabetes (P=0.07).

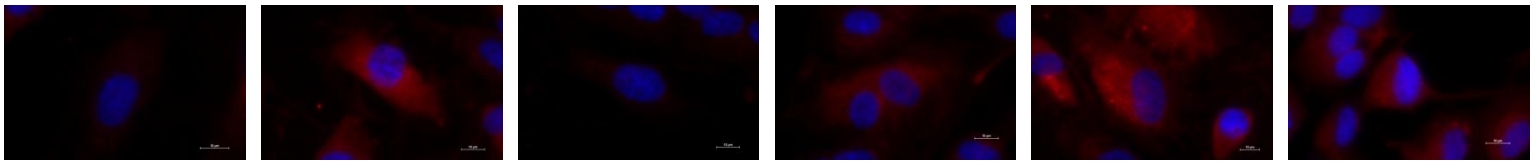
Supplemental Table 1. Medication use and eNOS activation in patients with diabetes

	Statin			ACEI or ARB			Metformin			Insulin		
	No N=5	Yes N=7	P	No N=4	Yes N=8	P	No N=4	Yes N=8	P	No N=6	Yes N=6	P
p-eNOS Ser1177 (au)	49±24	62±23	0.37	41±12	64±24	0.10	53±7	59±29	0.69	51±25	63±22	0.39
Insulin-induced Δp-eNOS Ser1177 (%)	-13±13	-8±6	0.41	-6±4	-12±11	0.36	-12±7	-9±11	0.65	-11±14	-9±4	0.72

mean±SD

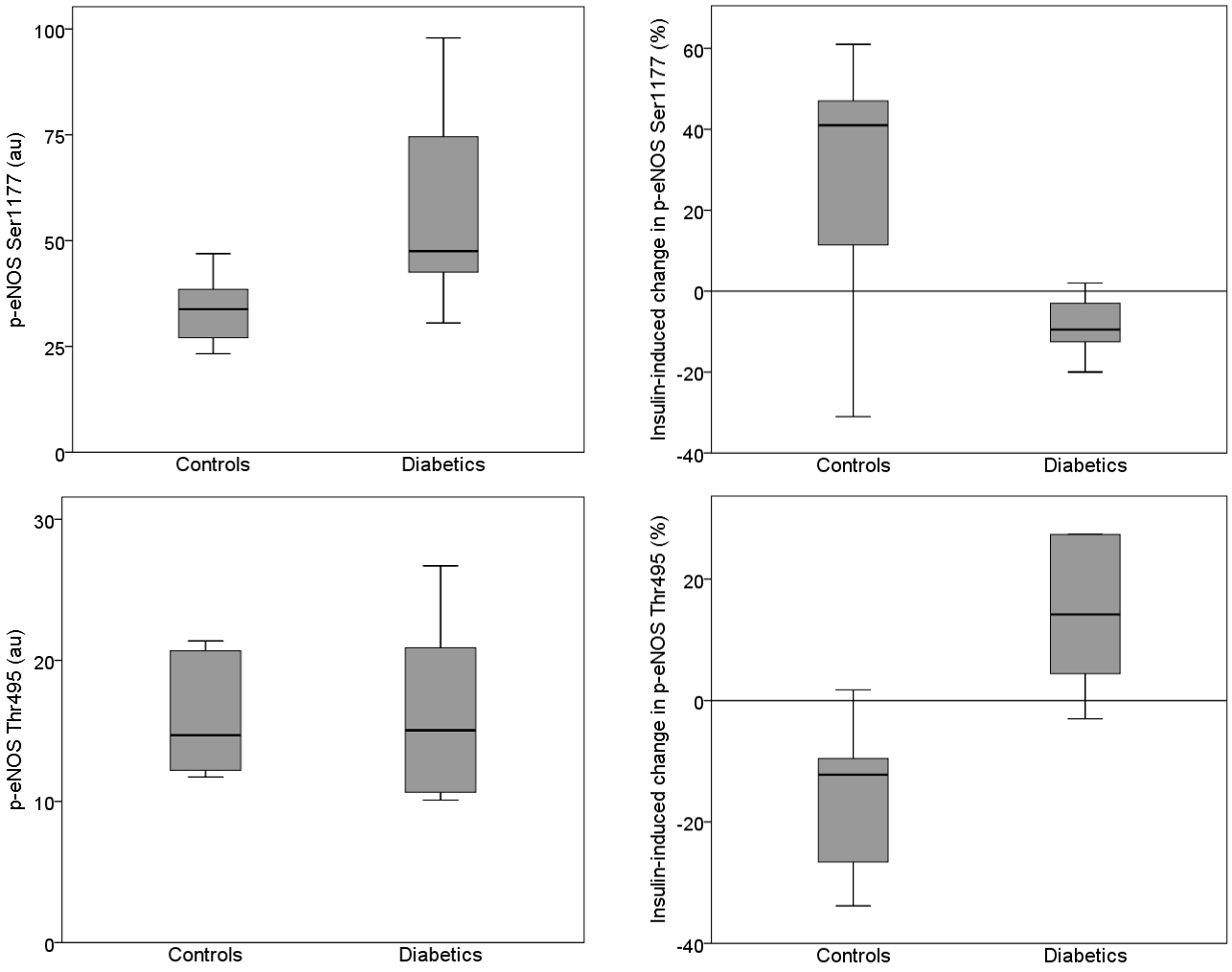
ACEI: Angiotensin converting enzyme inhibitor; ARB: Angiotensin receptor blocker; eNOS: endothelial nitric oxide synthase



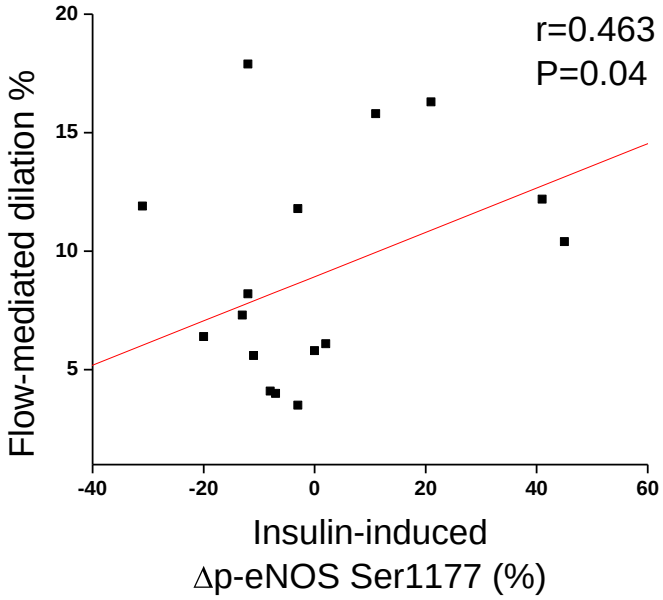


0 min | 5 min | 10 min | 20 min | 30 min | 60 min

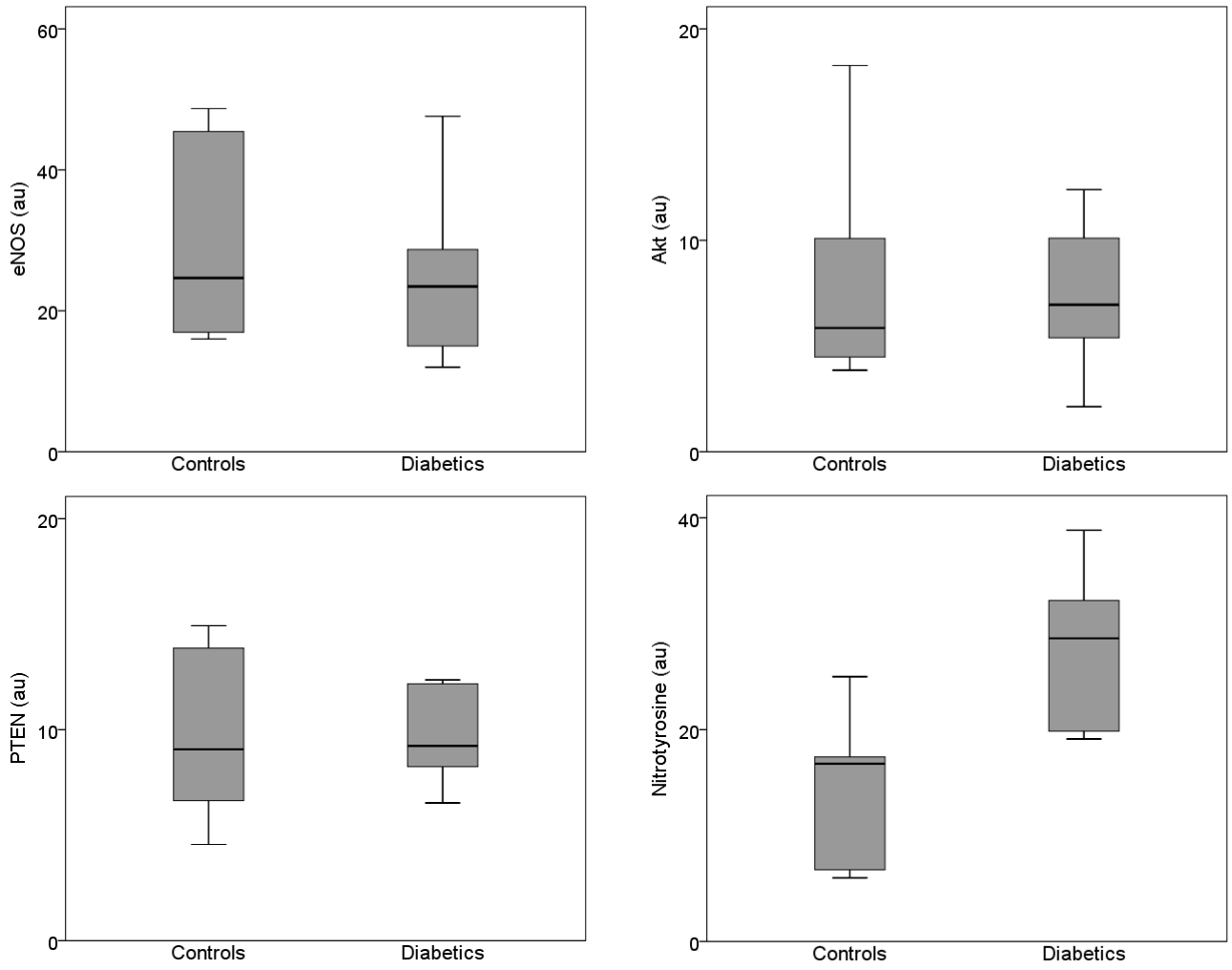
Supplementary Figure 1



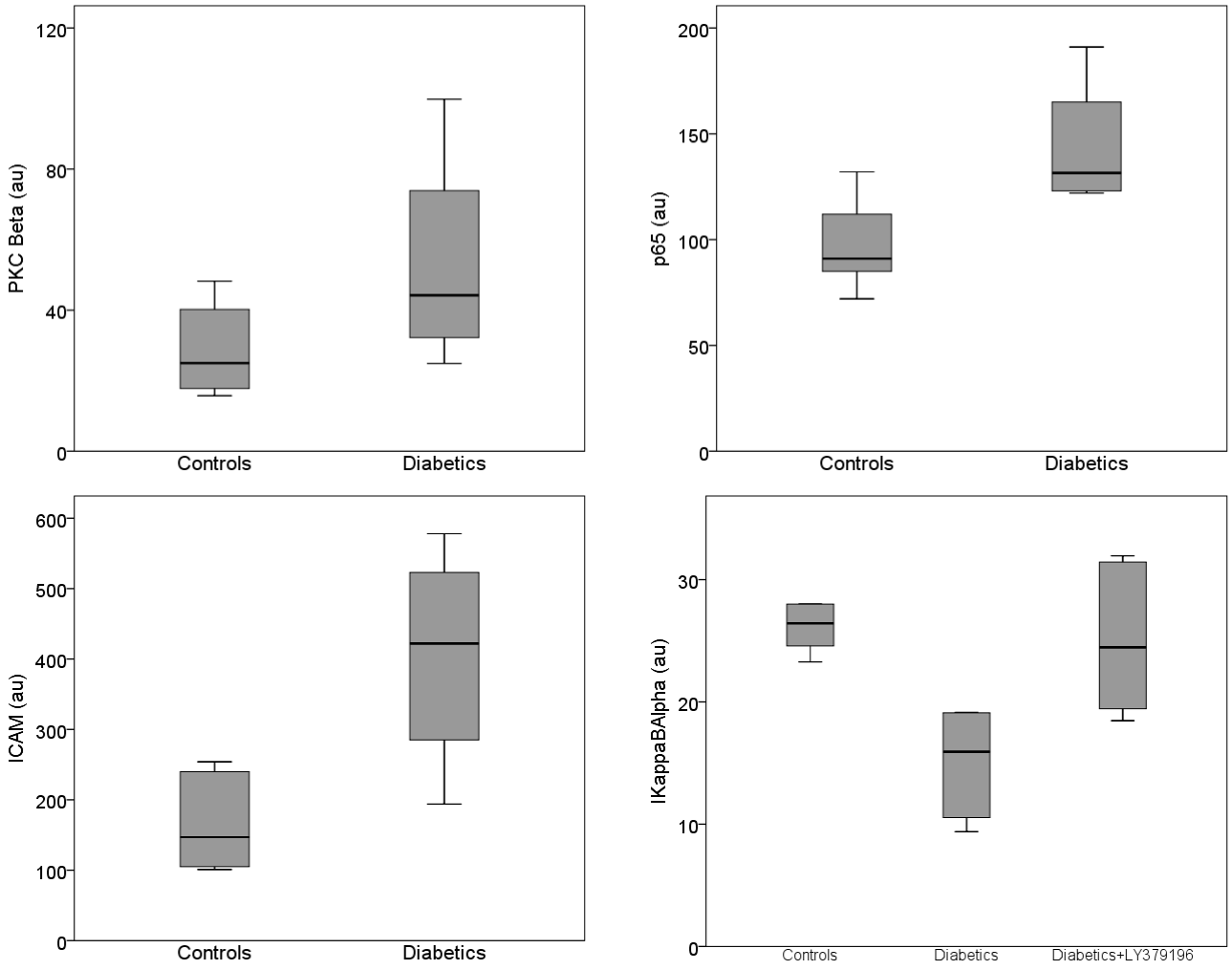
Supplementary Figure 2



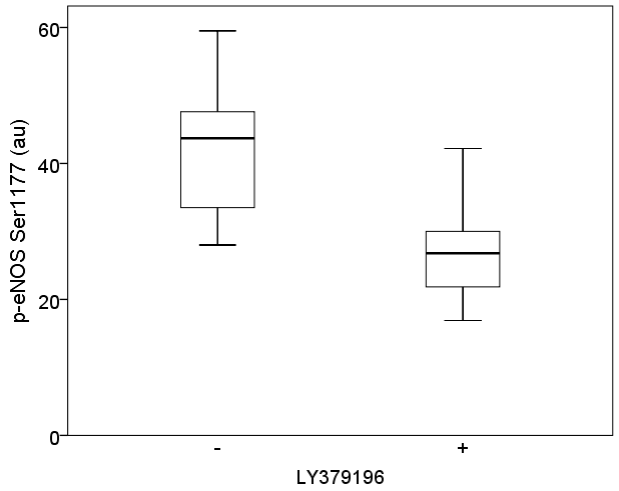
Supplementary Figure 3

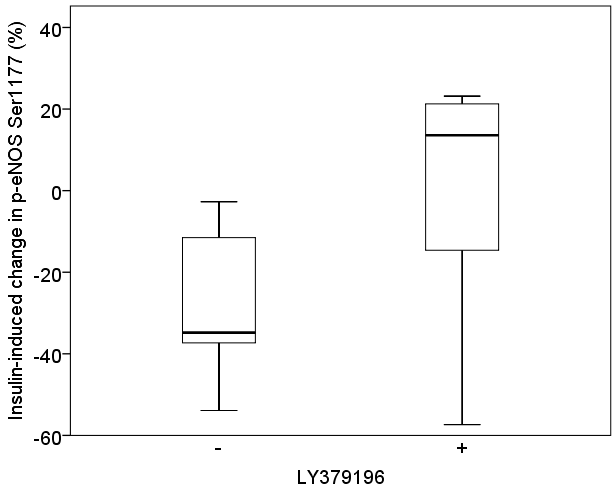


Supplementary Figure 4

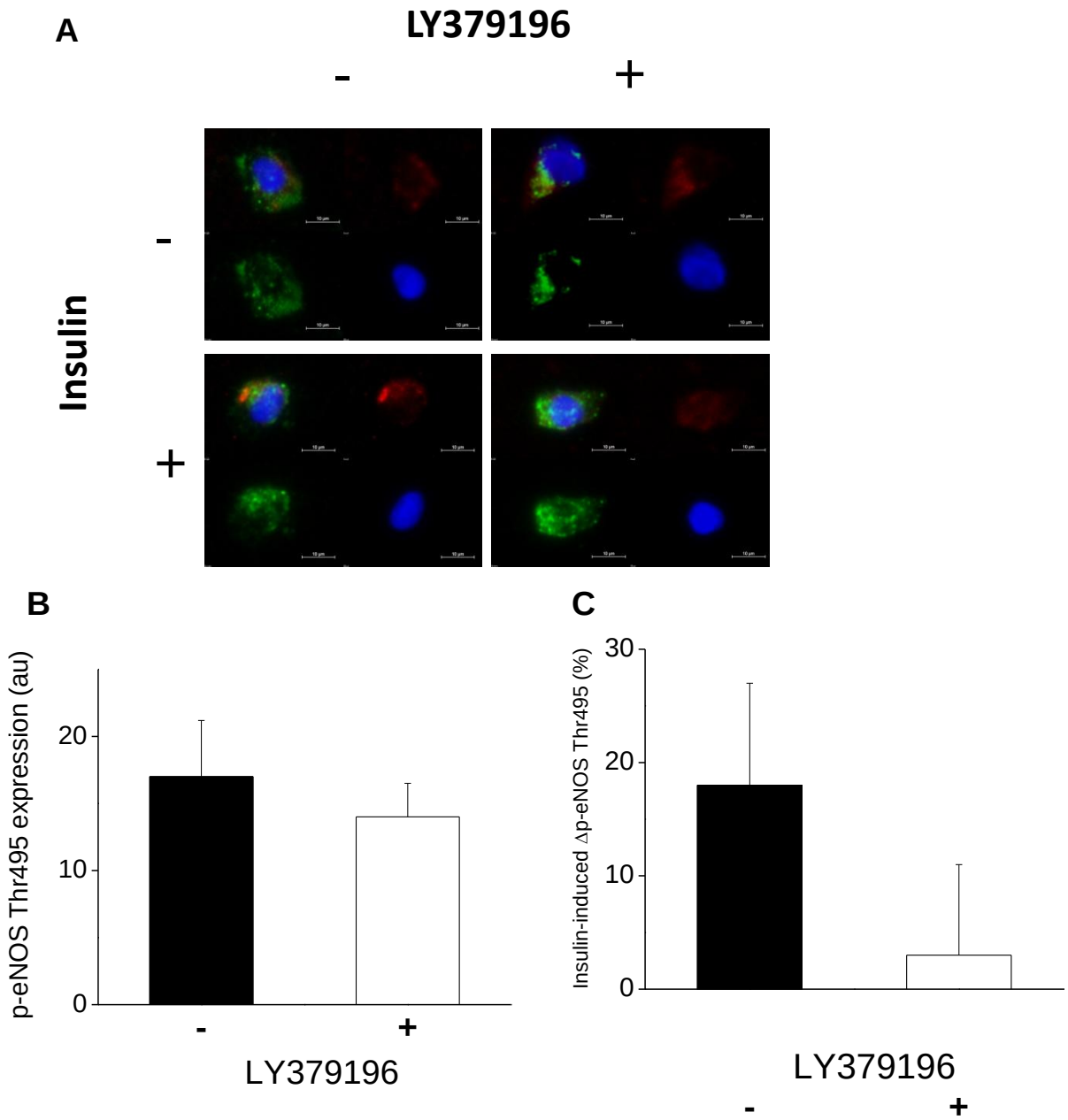


Supplementary Figure 5





Supplementary Figure 6



Supplementary Figure 7