SUPPLEMENTARY DATA

Supplementary Table 1. Area under the curve (AUC) of perifusion studies on Top: isolated WT and EPAC2A KO islets, First phase: 0-10 min after glucose stimulation (glucose increases from 3 to 10 mM); Second phase: 10-20 min after glucose stimulation.
Bottom: Δprkar1a and Δprkar1a/EAPAC2A KO islets. N=3 in each group. First phase: 0-5 min after glucose stimulation (glucose increases from 3 to 10 mM); Second phase: 5-20 min after glucose stimulation.

Treatment	Phase of insulin secretion	Insulin AUC WT islets pg/islet (mean±SEM)	Insulin AUC EPAC2A KO pg/islet (mean±SEM)	P
-	1st phase	235±21	197±7	NS
-	2nd phase	222±20	200±26	NS
E4 10 nM	1st phase	340±16	238±4	<0.05
	2nd phase	287±15	185±8	<0.05
High fat diet	1st phase	510±10	317±34	<0.05
	2nd phase	373±25	315±41	NS
PMAP 50 nM	1st phase	291±16	186±21	<0.05
	2nd phase	160±37	160±6	NS
6BNZ 10 μM	1st phase	442±27	452±89	NS
	2nd phase	411±49	411±23	NS
ESCA 10 μM	1st phase	288±59	204±17	<0.05
	2nd phase	352±47	194±23	<0.05

	Phase of insulin secretion	Insulin AUC Δprkar1a pg/islet (mean±SEM)	Insulin AUC Δprkar1a/EPAC2A KO pg/islet (mean±SEM)	P
-	1st phase	295±32	219±30	<0.05
-	2nd phase	573±158	554±175	NS

SUPPLEMENTARY DATA

Supplementary Table 2. Antibodies used for co-immunoprecipitation assays and immunoblots.

Detected antigen	Antibody source Catalog number	Band size on immunoblot
EPAC1	Abcam ab109415	105 kD
EPAC2	Santa Cruz sc25633	116 kD
Rim1/2	Santa Cruz sc16677	190 kD
SNAP25	Santa Cruz sc7538	25 kD
Syntaxin1A	Abcam ab41453	33 kD
VAMP2	Santa Cruz sc58309	18 kD
Actin	Millipore MAB1501	40 kD

Supplementary Figure 1. Incretin receptor activation but not GPR40 receptor activation increases Islet cAMP levels similarly in WT EPAC2A KO islets. WT and EPAC2A KO islets have similar glucokinase activity.

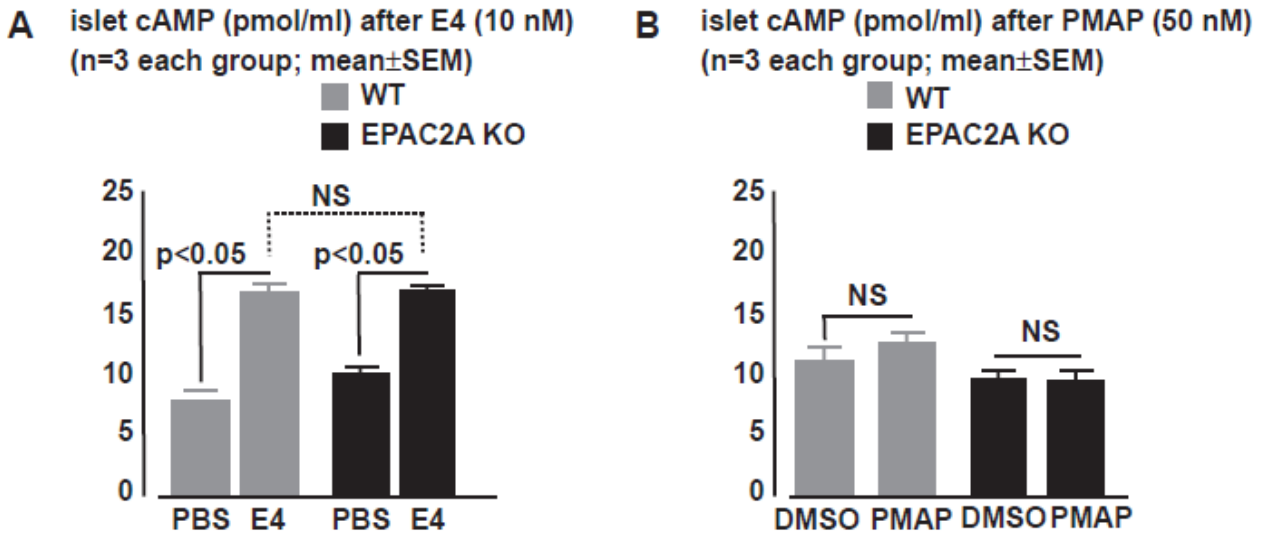
A: E4 stimulation generates cAMP production to similar degrees in both cultured WT (grey bars) and EPAC2A KO (black bars) islets. EPAC2A ablation does not influence E4 stimulated cAMP generation.

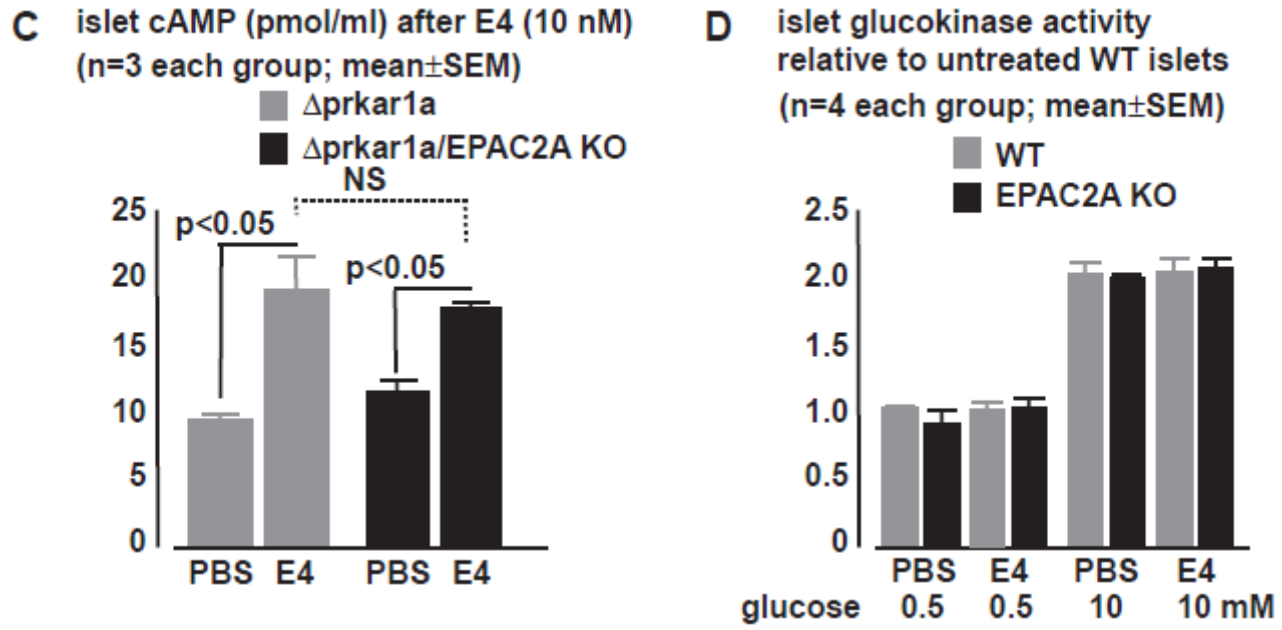
B: PMAP does not stimulate cAMP production in WT (grey bars) or EPAC2A (black bars) islets.

C: E4 stimulation generates cAMP production to similar degrees in Δprkar1a (grey bars) and Δprkar1a/EPAC2A KO (black bars) islets. Δprkar1a islets do not exhibit higher E4 stimulated cAMP production as compared to WT islets (shown panel A).

D: Glucokinase (GK) activity is similar in WT (grey bars) and EPAC2A KO (black bars) islets and is not changed by acute (30 min) E4 treatment Values are normalized to WT islets

Results are shown as mean±SEM of studies performed at least in triplicate. * indicates $p<0.05$. For details see text.

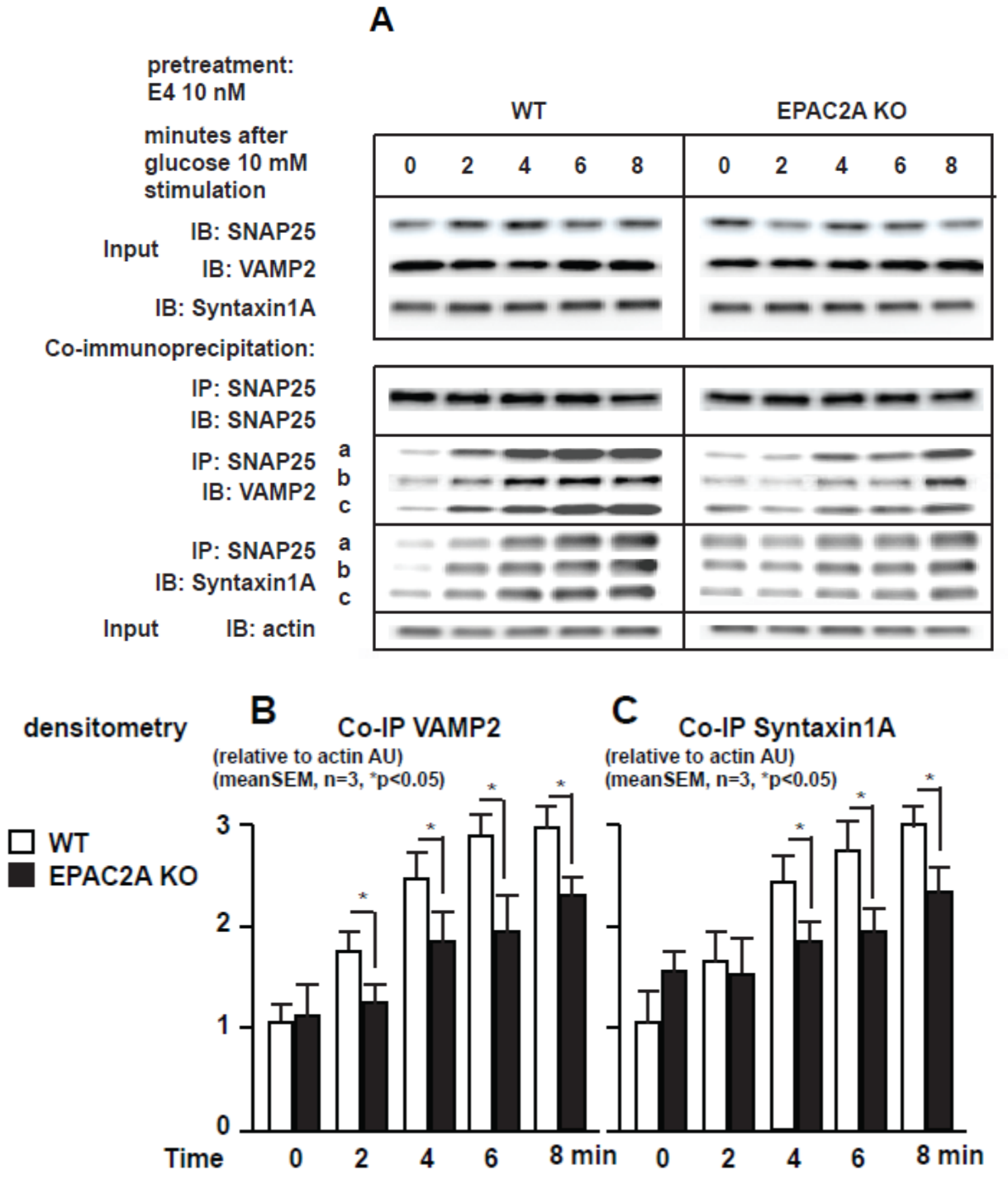




Supplementary Figure 2. Co-immunoprecipitation studies of WT and EPAC2A KO islets. Delayed interaction of SNARE protein components in EPAC2A KO islets stimulated with E4.

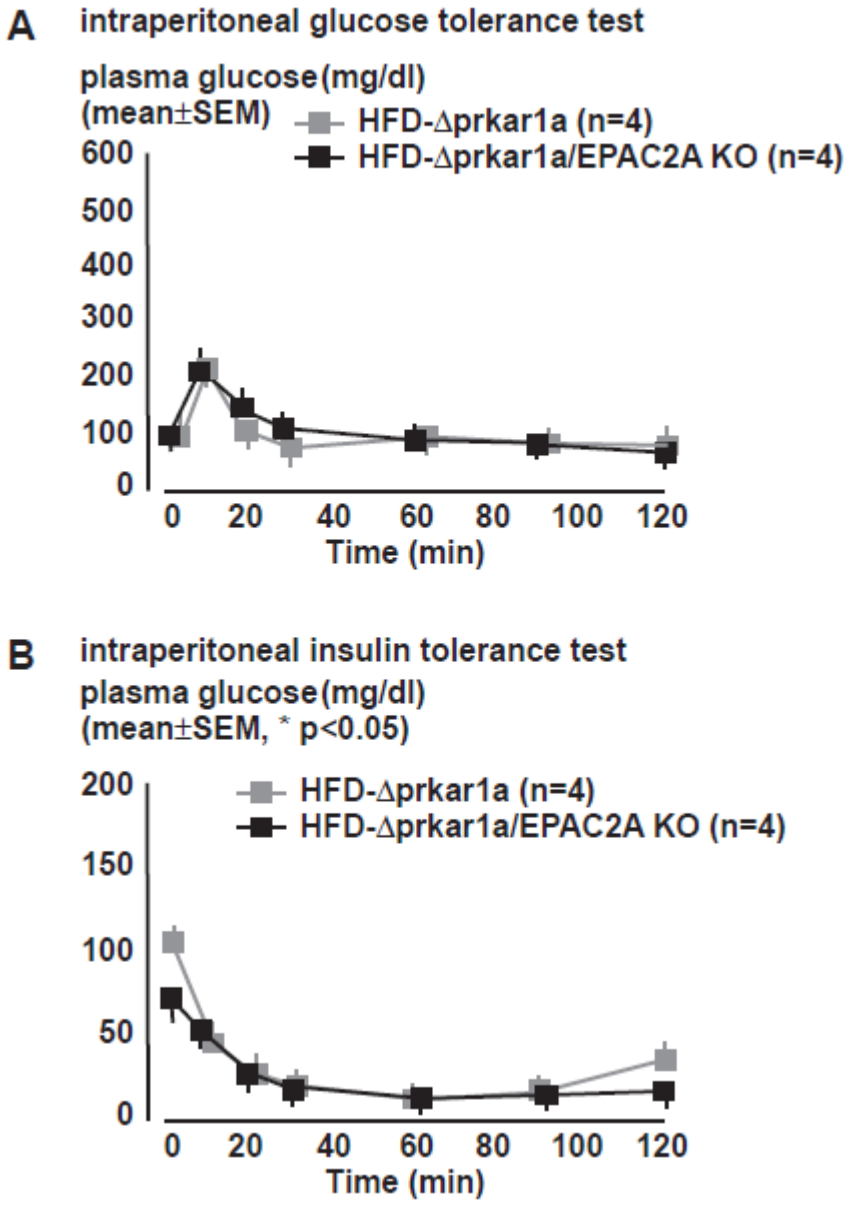
A. WT and EPAC2A KO islets exposed to E4 for 30 minutes at 3 mM glucose were stimulated with 10 mM glucose. Protein extracts were obtained at indicated time points after glucose simulation for Co-IP/IB studies to assess SNAP25-VAMP2 and SNAP25-Syntaxin1A SNARE protein interaction. Input into IP studies (10% of protein extract) and SNAP25-specific IP indicate similar amounts of proteins were used at each time point during the time-course. Representative blots from 3 separate Co-IP studies are shown.
B. Densitometric analysis of VAMP2 Co-IP. Band intensity is normalized to actin of respective time point. Time = 0 min was defined as 1 arbitrary units
C. Densitometric analysis of Syntaxin1A Co-IP. Band intensity is normalized to actin of respective time point. Time = 0 min was defined as 1 arbitrary units.

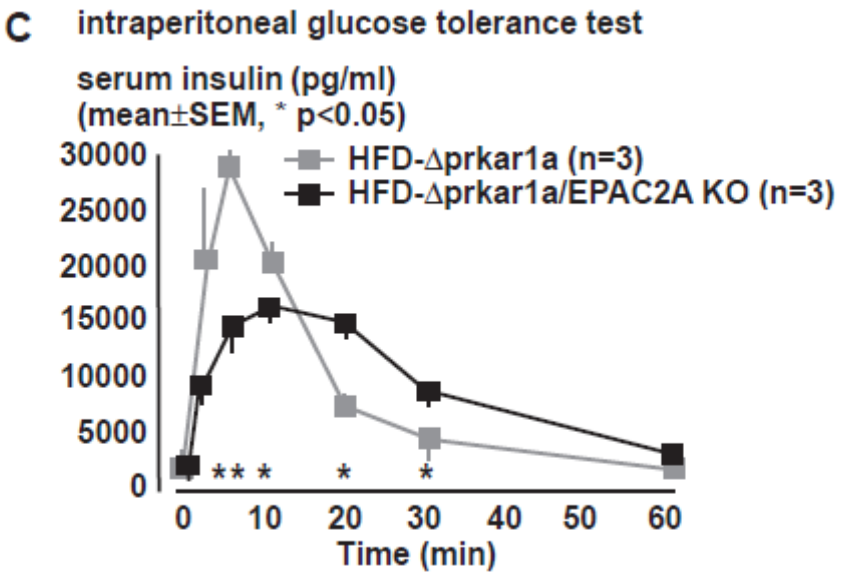
SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 3. EPAC2A ablation impairs dynamic insulin response in HFD fed mice of model of ß-cell autonomous GSIS augmentation (Δprkar1a mice)
A: During ipGTT, HFD- Δprkar1a and HFD-Δprkar1a/EPAC2A KO mice exhibit similar glucose excursions.
B: During ITT, HFD-Δprkar1a and HFD-Δprkar1a/EPAC2A KO show similar insulin sensitivity
C: ipGTT, HFD-□prkar1a exhibit dramatically increased GSIS. HFD-Δprkar1a/EPAC2A KO show increased GSIS, with a blunted first-phase insulin secretory response and a relatively increased second phase insulin secretory response.
Δprkar1a is represented in gray squares; HFD-Δprkar1a/EPAC2A KO is represented in black squares. Results are shown as mean±SEM of studies performed at least in triplicate. * indicates $p<0.05$. For details see text.





Supplementary Figure 4. Perifusion studies of PKA, EPAC2A and GPR40 interplay.
A: EPAC2A is required for full effect of PKA mediated GSIS stimulation. Stimulation with the PKA-selective cAMP agonist 6BZN augments GSIS in WT islets. EPAC2A KO islets also exhibit augmented GSIS under 6BZN stimulation. However, the immediate early (40-42 min) insulin secretory response is diminished in EPAC2A KO islets.
B: Stimulation with the EPAC2-selective cAMP analog (ESCA) augments GSIS in WT islets. ESCA mediated GSIS augmentation is abolished in EPAC2A KO islets, which exhibit GSIS similar to WT islets without any pharmacologic secretagogue stimulation (see Figure 2E)
C: PKA inhibition with PKI abolishes GSIS in WT and EPAC2A KO islets. A small burst of insulin secretion is detectable early (40-45 min) after stimulation with 10 mM glucose. KCL depolarization at completion of perifusion results in insulin release.
D: PKA inhibition with PKI abolishes GSIS in E4 treated WT and EPAC2A KO islets. A small burst of insulin secretion is detectable early (40-45 min) after stimulation with 10 mM glucose. KCL depolarization at completion of perifusion results in insulin release.
E: PKA inhibition with PKI abolishes GSIS in ESCA treated WT and EPAC2A KO islets. A small burst of insulin secretion is detectable early (40-45 min) after stimulation with 10 mM glucose. KCL depolarization at completion of perifusion results in insulin release.
F: PKA inhibition with PKI does not affect GSIS in (GPR40 activated) PMAP treated islets (see Figure 6A). PKA inhibition in EPAC2A KO islets treated with PMAP show blunted GSIS. KCL depolarization at completion of perifusion results in insulin release.
Results are shown as mean±SEM of studies performed at least in triplicate. * indicates $p<0.05$. For details see text.

SUPPLEMENTARY DATA