Supplemental figure legends

Supplemental Figure S1. The strategies for selecting potential GLP-1R interactors of interest. From 99 potential GLP-1R interactors, we narrowed the list down to 17 interactors that were commonly identified in both CHO and MIN6 cell lines. Then, 11 interactors were selected because they were localized to the cell membrane. Furthermore, we selected 4 of these 11 interactors because they were associated with GPCR functions and not G-proteins. Last, we narrowed the list down from 4 to 3 potential interactors (PGRMC1, Rab5b and Rab5c) that showed significant association with liganded GLP-1R (**p<0.01, *p<0.05, n= 8-11) based on spectral counting (emPAI) data.

Supplemental Figure S2. A) siRNA mediated PGRMC1 knock-down effect on GLP-1 induced cAMP response in INS1 832/3 cells (*p<0.05, n=3 per group). B) Total insulin content from PGRMC1 overexpressed INS1 832/3 cells (p>0.05, n=3 per group).

Supplemental Figure S3. Effect of progesterone (P4) on GSIS and GIIS in pancreatic beta cells (A, B) and isolated mouse islets (C) (p>0.05).

Supplemental Figure S4. A) Effects of EGFR and PKG inhibitor on insulin secretion from PGRMC1 overexpressed INS1 cells (*p<0.05, n=3). 100nM AG1478 and 30μM RP8 were used to inhibit EGFR and PKG respectively. B) EGF effect on insulin secretion from INS1 832/3 cells (*p<0.05, n=3). 15nM EGF was used for treatment.

Supplemental Figure S5. PAQR6 effect on GIIS from INS1 832/3 cells (*p<0.05, n=3 per group).

Supplemental Figure S6. FRET assay control studies. A) Competition analysis demonstrates the specificity of the FRET assay. Transfecting increasing amounts of untagged GLP-1R competes with the Flag-tagged counterpart to bind SLC15A4-HA (a GLP-1R interactor revealed from MYTH(17)) (**p<0.01, *p<0.05 compared to SLC15A4-

HA + GLP-1R-Flag group). B) FRET experimentation demonstrates that co-expression of GLP-1R-Flag and interactor-HA is absolutely required to produce FRET signal. Mixing of detergent solubilized lysates from individually expressing GLP-1R-Flag or interactor-HA cells show no FRET signal (**$p<0.01$, compared to pcDNA3.1 control). * Represents the mixing of lysates from separate cells.

Supplemental Figure S7. PGRMC1 overexpression enhanced GLP-1-induced intracellular calcium flux in INS1 832/3 cells (*$p<0.05$, n=3 per group).

Supplemental Figure S8. MS/MS view of single peptide identified proteins in CHO cells (Separate PDF file).

Supplemental Figure S9. MS/MS view of single peptide identified proteins in MIN6 cells (Separate PDF file).

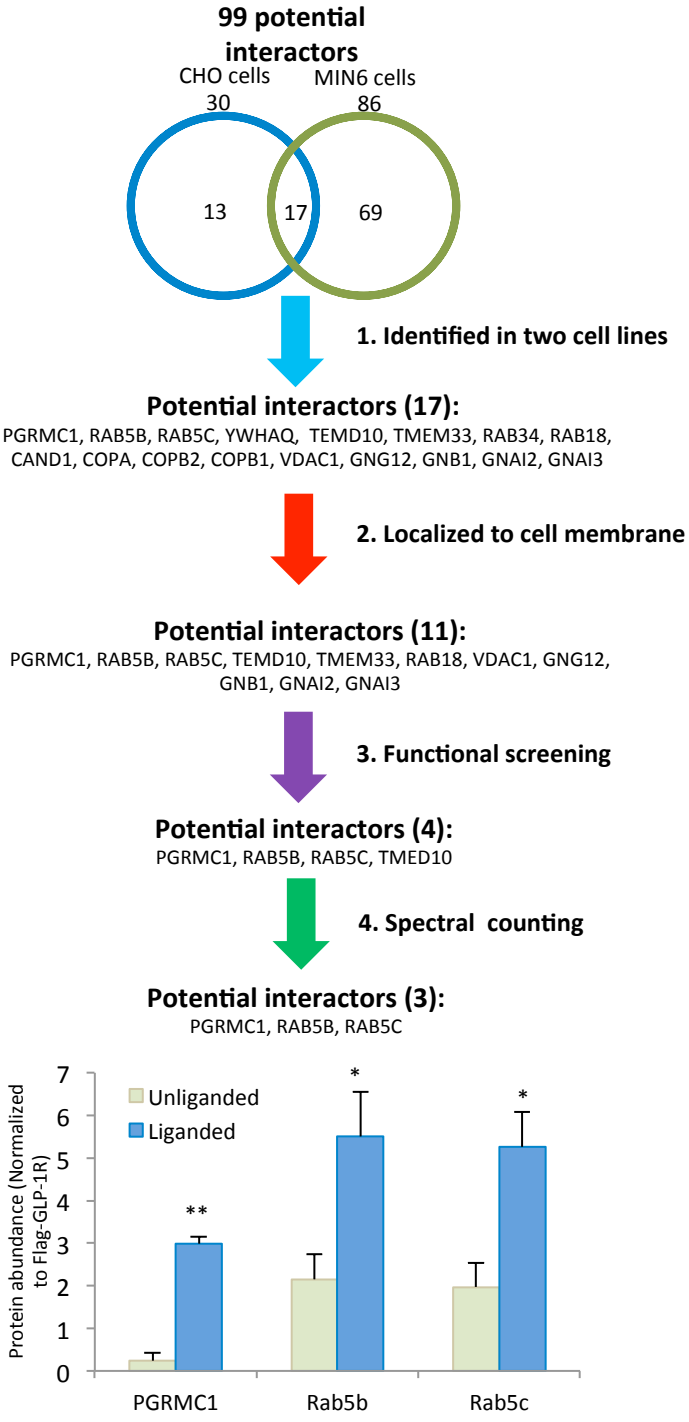
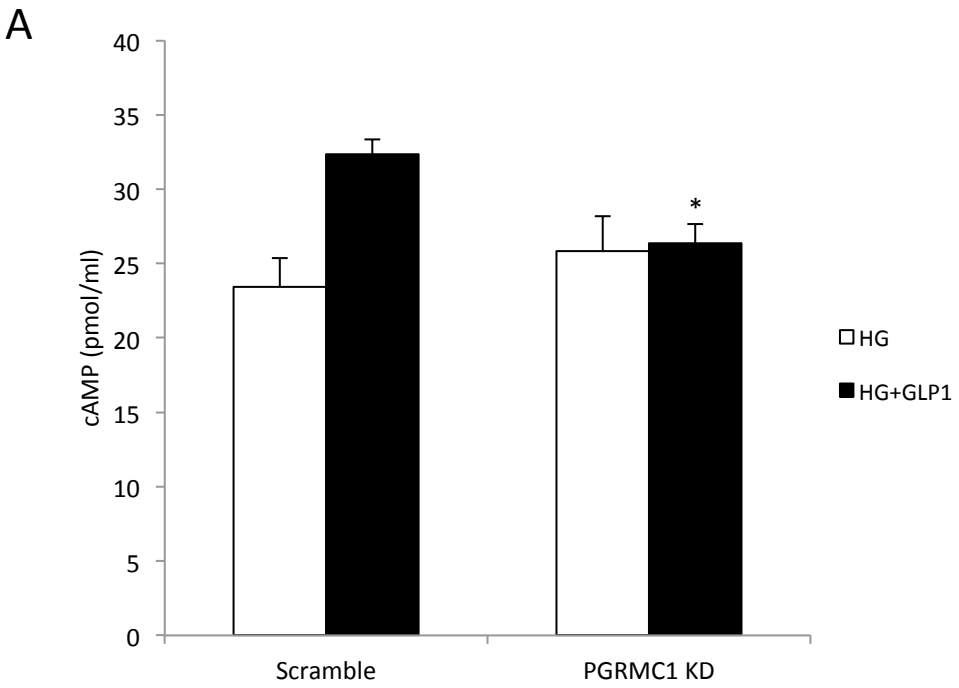

99 potential interactors
CHO cells
30
MIN6 cells
86
13
17
69
1. Identified in two cell lines
Potential interactors (17):
PGRMC1, RAB5B, RAB5C, YWHAQ, TEMD10, TMEM33, RAB34, RAB18, CAND1, COPA, COPB2, COPB1, VDAC1, GNG12, GNB1, GNAI2, GNAI3
2. Localized to cell membrane
Potential interactors (11):
PGRMC1, RAB5B, RAB5C, TEMD10, TMEM33, RAB18, VDAC1, GNG12, GNB1, GNAI2, GNAI3
3. Functional screening
Potential interactors (4):
PGRMC1, RAB5B, RAB5C, TMED10
4. Spectral counting
Potential interactors (3):
PGRMC1, RAB5B, RAB5C
Protein abundance (Normalized to Flag-GLP-1R)
Unliganded
Liganded
0
1
2
3
4
5
6
7
**
*
*
PGRMC1
Rab5b
Rab5c

Figure S1


A
40
35
30
25
20
15
10
5
0
cAMP (pmol/ml)
*
HG
HG+GLP1
Scramble
PGRMC1 KD

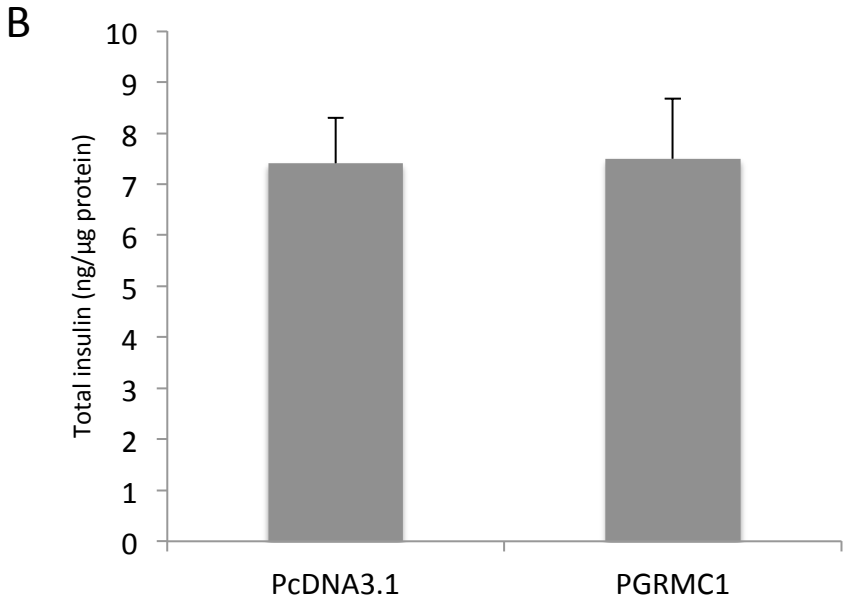

B
10
9
8
7
6
5
4
3
2
1
0
Total insulin (ng/µg protein)
PcDNA3.1
PGRMC1

Figure S2

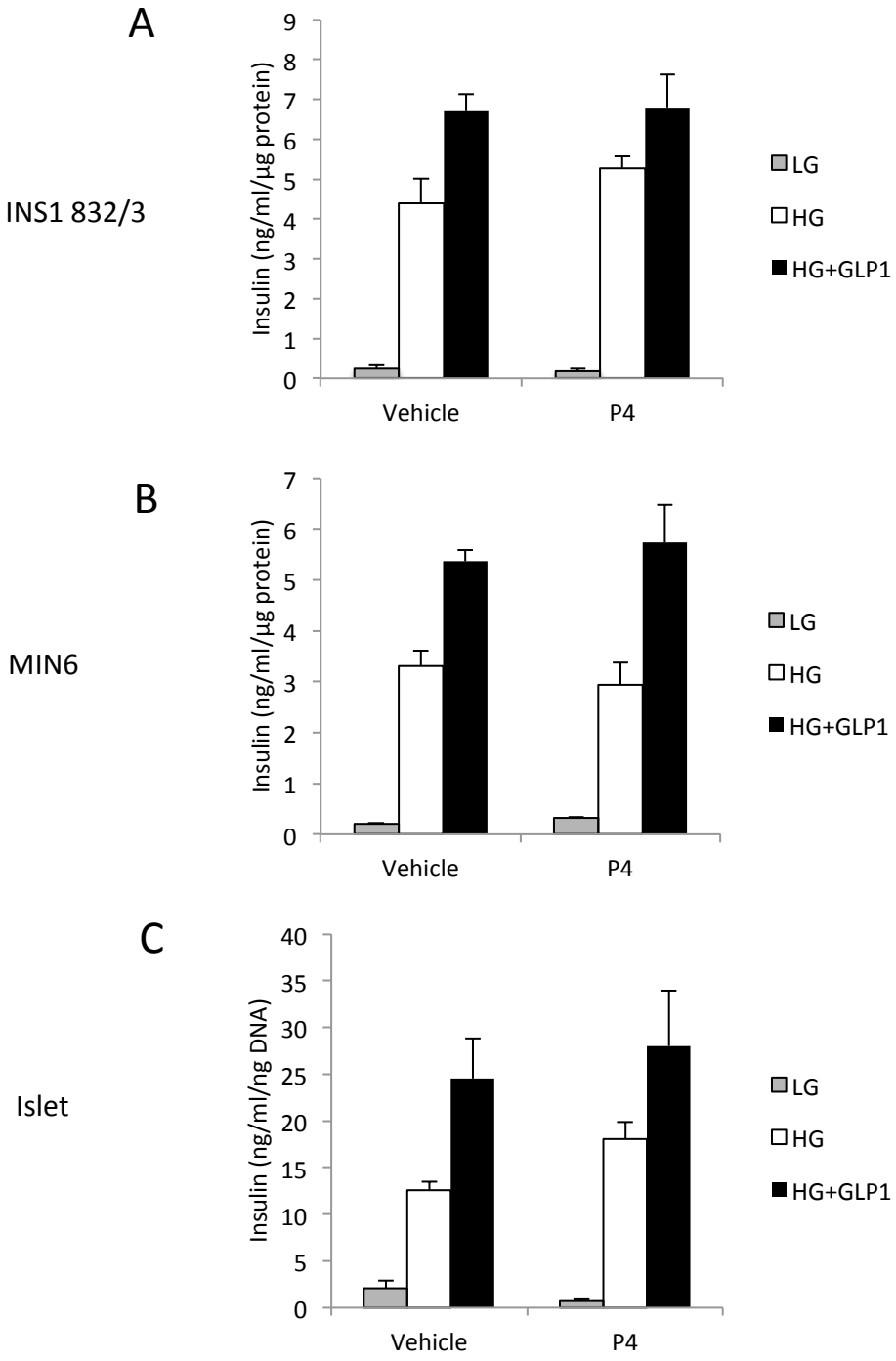

A
INS1 832/3
Insulin (ng/ml/µg protein)
9
8
7
6
5
4
3
2
1
0
Vehicle
P4
LG
HG
HG+GLP1
B
MIN6
Insulin (ng/ml/µg protein)
7
6
5
4
3
2
1
0
Vehicle
P4
LG
HG
HG+GLP1
C
Islet
Insulin (ng/ml/ng DNA)
40
35
30
25
20
15
10
5
0
Vehicle
P4
LG
HG
HG+GLP1

Figure S3

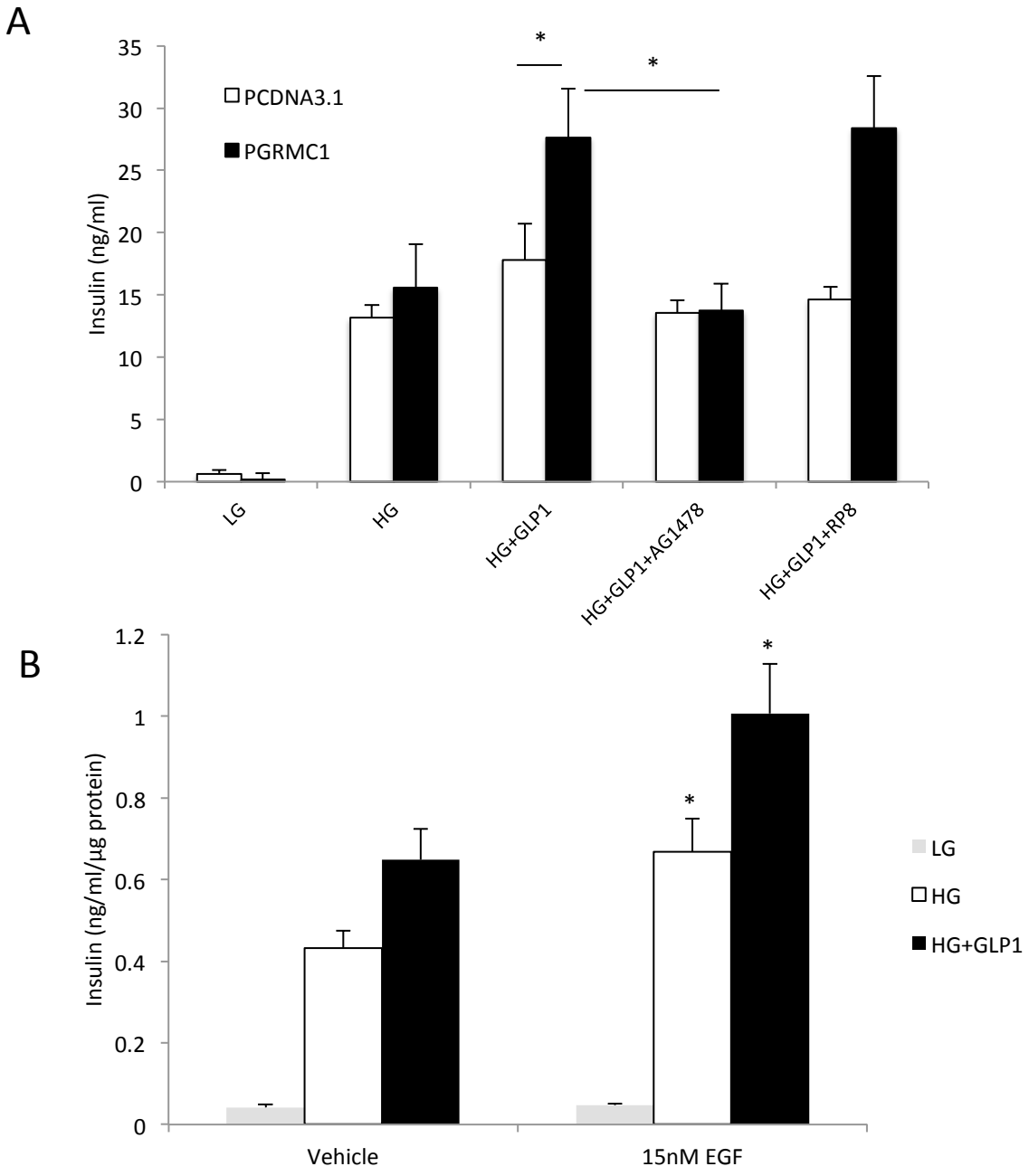

A
35
30
25
20
15
10
5
0
Insulin (ng/ml)
PCDNA3.1
PGRMC1
*
*
LG
HG
HG+GLP1
HG+GLP1+AG1478
HG+GLP1+RP8
B
1.2
1
0.8
0.6
0.4
0.2
0
Insulin (ng/ml/μg protein)
*
*
LG
HG
HG+GLP1
Vehicle
15nM EGF

Figure S4

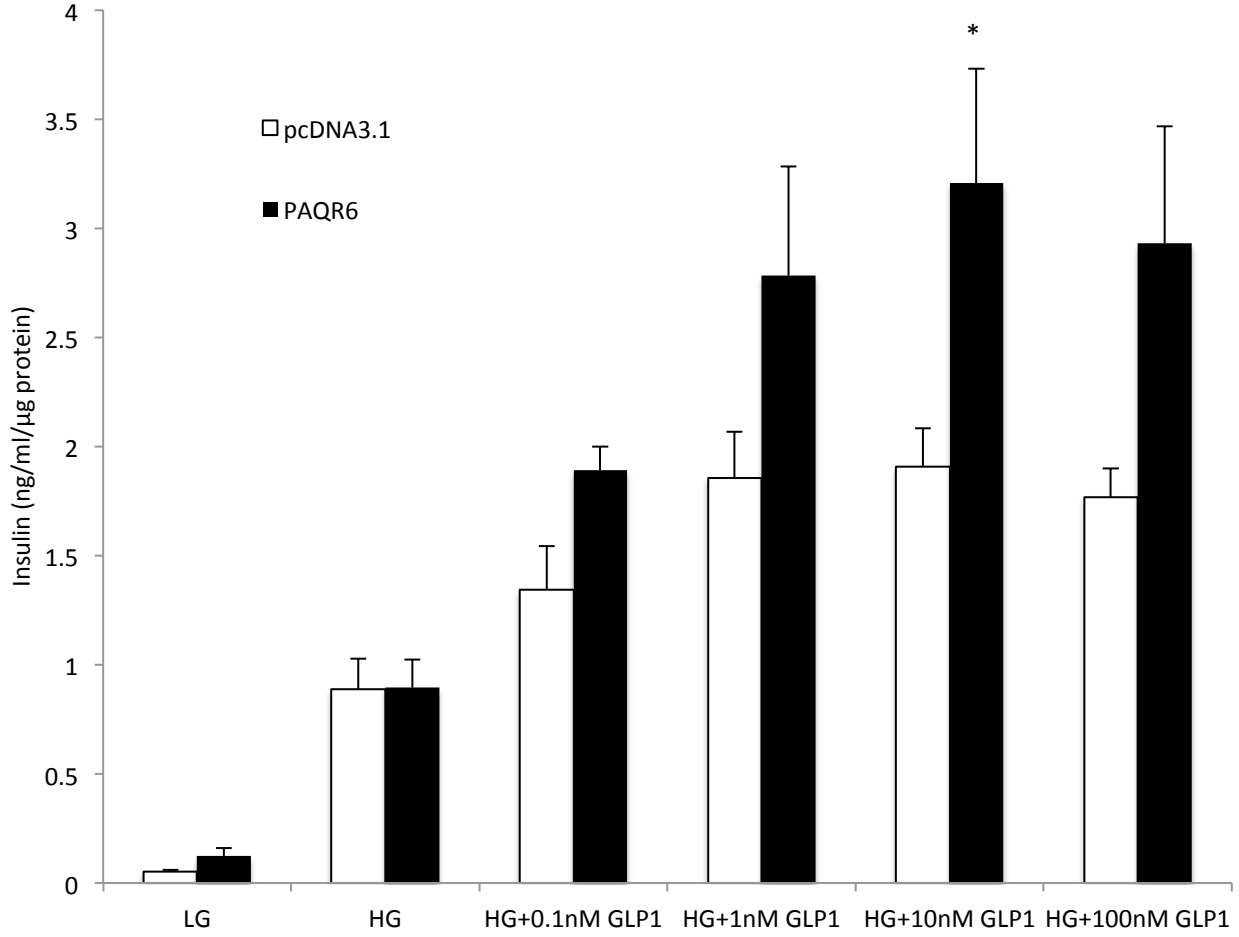

4
3.5
3
2.5
2
1.5
1
0.5
0
Insulin (ng/ml/μg protein)
pcDNA3.1
PAQR6
*
LG
HG
HG+0.1nM GLP1
HG+1nM GLP1
HG+10nM GLP1
HG+100nM GLP1

Figure S5

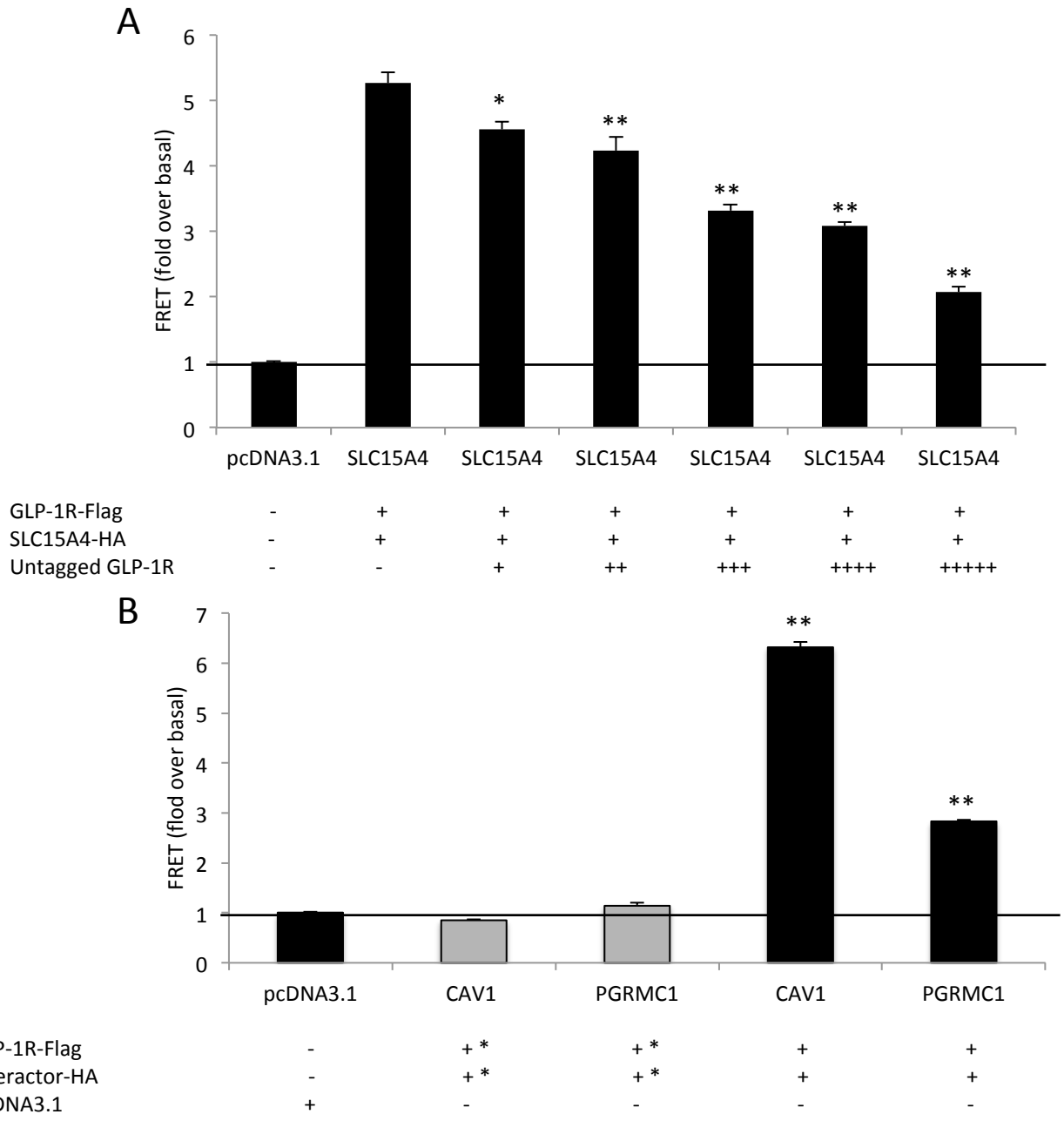

A
FRET (fold over basal)
0
1
2
3
4
5
6
*
**
**
**
**
pcDNA3.1
SLC15A4
SLC15A4
SLC15A4
SLC15A4
SLC15A4
SLC15A4
GLP-1R-Flag - + + + + + +
SLC15A4-HA - + + + + + +
Untagged GLP-1R - - + ++ +++ ++++ +++++
B
FRET (flod over basal)
0
1
2
3
4
5
6
7
**
**
pcDNA3.1
CAV1
PGRMC1
CAV1
PGRMC1
GLP-1R-Flag - + * + * + +
Interactor-HA - + * + * + +
pcDNA3.1 + - - - -

Figure S6

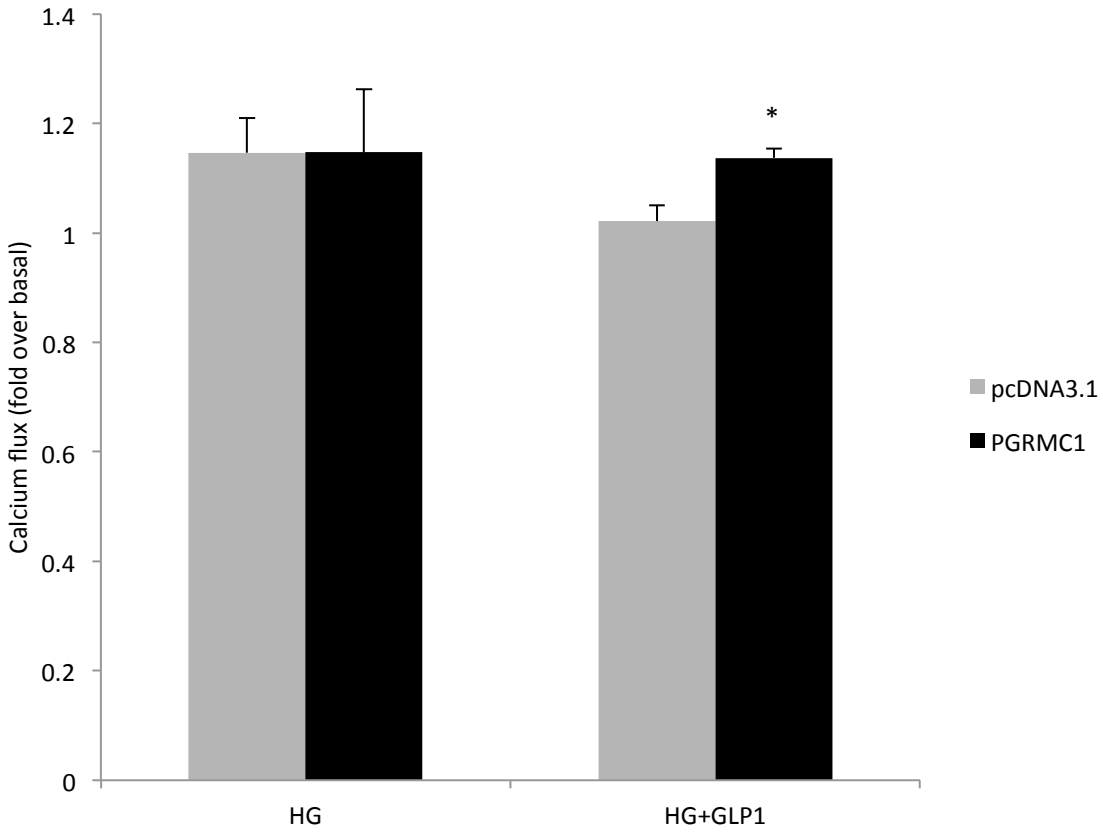

1.4
1.2
1
0.8
0.6
0.4
0.2
0
Calcium flux (fold over basal)
*
HG
HG+GLP1
pcDNA3.1
PGRMC1

Figure S7