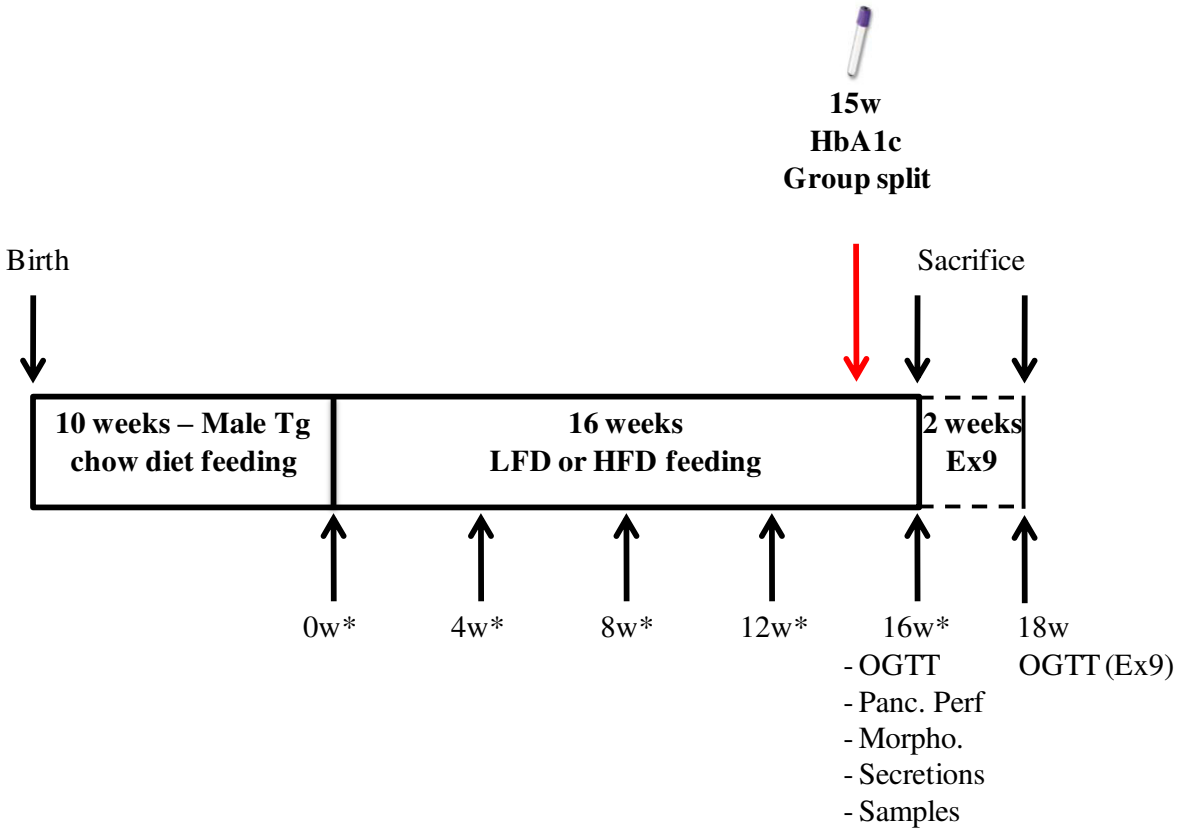
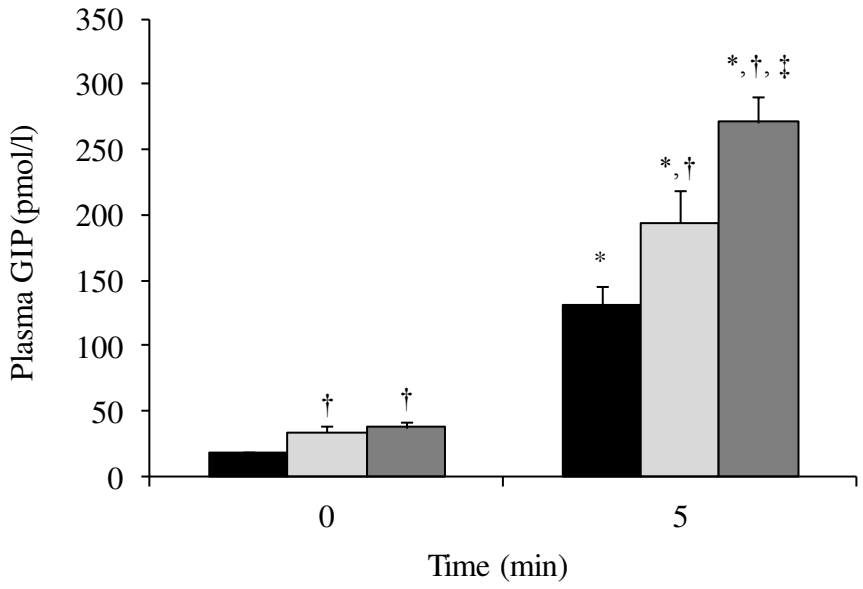
Supplemental Fig.1



Supplemental Fig.1: Schematic representation of experimental procedures- 10 weeks male GLU-Venus male mice were subjected to low-fat (LFD, controls) or high-fat (HFD) diets during 16 weeks. After HbA1c measurements (15 weeks), mice were divided into 3 groups (LFD mice, obese HFD mice with HbA1c similar to controls and obese HFD mice exhibiting elevated HbA1c levels compared to controls). Glucose tolerance test, pancreatic perfusion (Panc. Perf.), morphometric analyses (Morpho.), ex vivo secretion assays as well as sample collection were performed at 16 weeks from LFD and HFD mice. Exendin9-39 (Ex9) experiments were performed on a part of HFD mice for 2 additional weeks.

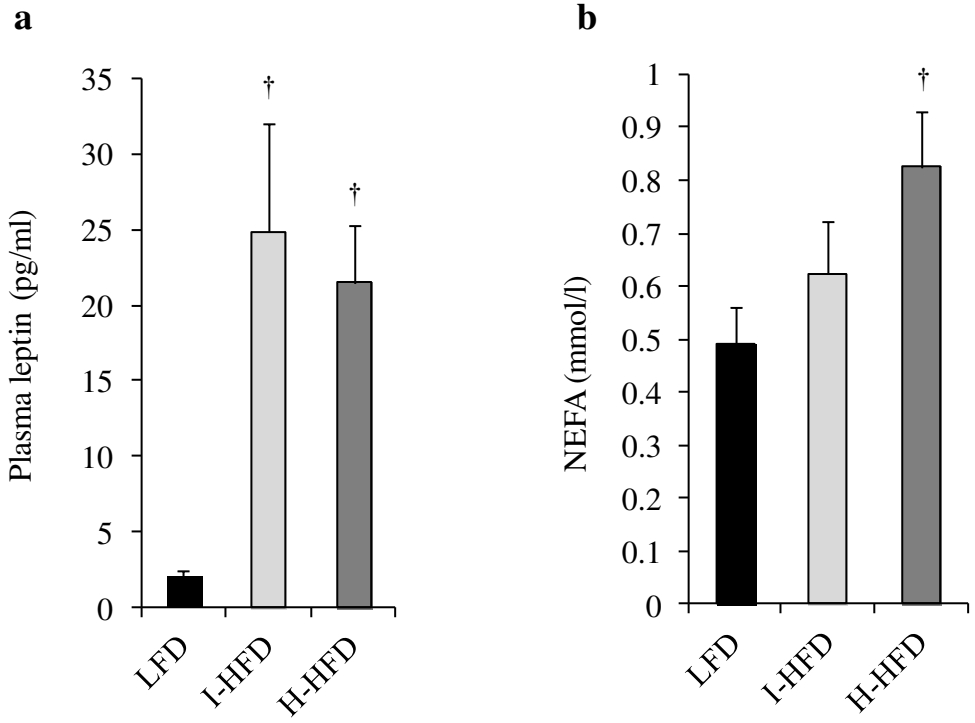
Supplemental Fig.2



Supplemental Fig.2: Plasma GIP of control and obese mice– Plasma GIP levels of LFD (black bars), I-HFD (light-grey bars) and H-HFD (dark-grey bars) mice were evaluated at 16 weeks during OGTT before (T0) and 5 min after glucose gavage (2g/kg) (n=4 mice per group).

* indicates statistical significance compared to T0. † and ‡ indicate statistical significance compared to LFD and to I-HFD mice respectively.

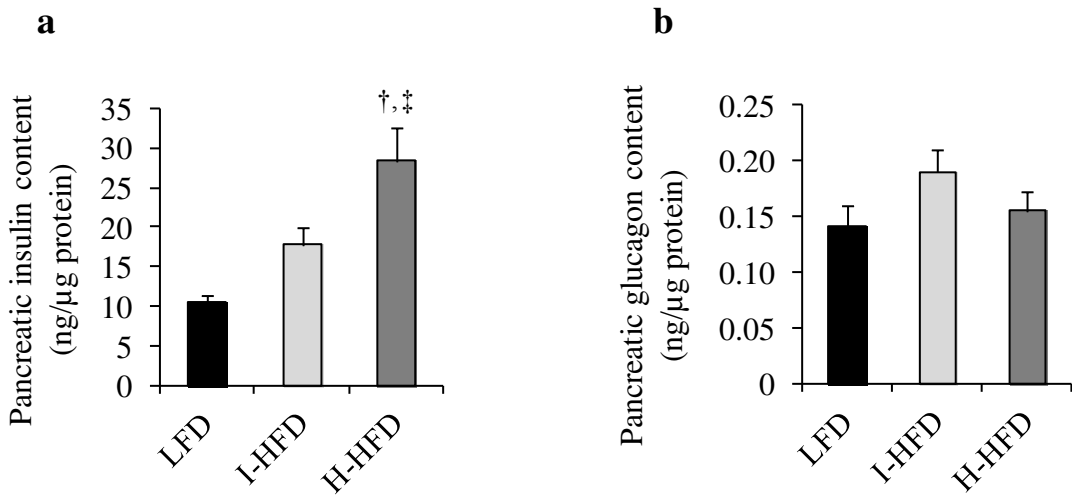
Supplemental Fig.3



Supplemental Fig.3: Plama leptin and NEFA of control and obese mice– Leptinemia (a) and circulating NEFA (b) of LFD (black bars), I-HFD (light-grey bars) and H-HFD (dark-grey bars) mice were evaluated at 16 weeks (n=8 mice per group).

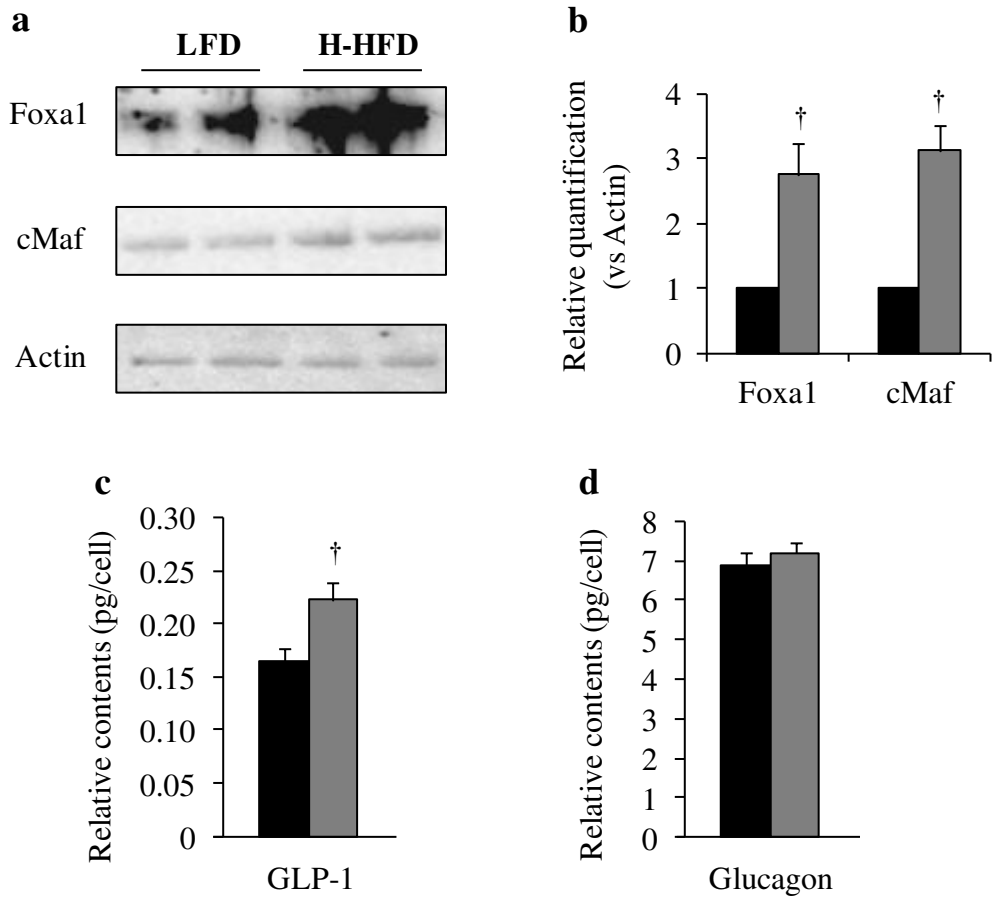
† indicates statistical significance compared to LFD mice.

Supplemental Fig.4



Supplemental Fig.4: Insulin and glucagon pancreatic contents in control and obese mice- Insulin (a) and glucagon (b) contents were evaluated from pancreases of LFD (black bars), I-HFD (light-grey bars) and H-HFD (dark-grey bars). Pancreases were collected at the end of protocol and homogenized in acid-ethanol solution. Insulin and glucagon contents were normalized to total protein amounts (ng/µg of total protein) (n=10 mice for LFD; n=5 for I-HFD and H-HFD). † and ‡ indicate statistical significance compared to LFD and to I-HFD mice respectively.

Supplemental Fig.5



Supplemental Fig.5: FOXA1, cMAF and GLP-1 measurements from Venus+ alpha-cells of control and obese H-HFD mice- Total protein extracts were isolated and resolved from 40,000 sorted Venus+ alpha-cells from LFD and H-HFD mice after 16 weeks of protocol. Immunoblottings were performed from cell extracts using rabbit anti-Foxa1 (gift by Pr. Whitsett JA, Cincinnati Children's Hospital Medical Center, OH), anti-cMaf (Bethyl) and anti-Actin (Sigma). FOXA1 and cMAF protein levels were quantified from 30 µg of proteins of 3 independent samples of LFD and H-HFD mice (corresponding each to sorted alpha-cells from 3 mice). Illustrative western-blot analyses of FOXA1 and cMAF as well as ACTIN protein levels are shown (a) and quantified by semi-quantitative analysis relative to Actin levels using ChemiDoc MP Imaging System software (b). GLP-1 (c) and glucagon (d) cellular contents were evaluated from 1,000 sorted alpha-cells of at least 4 LFD and H-HFD mice. Hormonal contents were expressed in pg per cell. † indicates statistical significance compared to LFD mice.

Supplemental Table 1

	LFD	SEM LFD	I-HFD	SEM I-HFD	H-HFD	SEM H-HFD
Gcg	**1**	0.04	**1.04**	0.06	**1.33** †, ‡	0.12
Pcsk2	**1**	0.05	**0.85**	0.07	**1.06**	0.09
Pcsk1/3	**1**	0.04	**1.72**	0.46	**5.02** †, ‡	1.03
Pax6	**1**	0.08	**1.10**	0.07	**1.24**	0.08
Foxa1	**1**	0.04	**1.97**	0.46	**2.91** †	0.60
Foxa2	**1**	0.10	**0.97**	0.11	**1.30**	0.23
Foxa3	**1**	0.05	**0.93**	0.05	**0.91**	0.04
Arx	**1**	0.03	**0.98**	0.07	**0.95**	0.07
MafB	**1**	0.10	**0.91**	0.07	**0.87**	0.07
cMaf	**1**	0.09	**1.42**	0.36	**3.58** †	0.94
Pou3f4	**1**	0.15	**0.70**	0.06	**0.65** †	0.10
HNF-1alpha	**1**	0.07	**1.09**	0.11	**0.99**	0.12
HNF-4alpha	**1**	0.05	**1.09**	0.09	**0.90**	0.13
Tcf7l2	**1**	0.09	**0.95**	0.09	**1.10**	0.11
Nkx2.2	**1**	0.09	**0.91**	0.11	**0.92**	0.14
Isl1	**1**	0.07	**0.93**	0.04	**0.85**	0.05
NeuroD1	**1**	0.05	**0.94**	0.06	**0.81**	0.06
Gck	**1**	0.03	**1.10**	0.07	**1.13**	0.08
Slc2a1	**1**	0.05	**0.84**	0.11	**1.04**	0.10
Slc5a1	**1**	0.22	**4.09** †	1.21	**1.54**	0.49
Slc5a2	**1**	0.08	**1.72**	0.49	**1.73**	0.37
Ffar1	**1**	0.06	**0.84**	0.12	**1.05**	0.10
Gpr119	**1**	0.08	**0.86**	0.10	**0.88**	0.12
Ffar4	**1**	0.06	**1.15**	0.07	**1.07**	0.08
Ucp2	**1**	0.02	**1.52**	0.23	**1.46**	0.20
Kcnj11	**1**	0.04	**0.89**	0.08	**1.07**	0.10
Abcc8	**1**	0.05	**1.09**	0.10	**1.15**	0.10
Cacna1C	**1**	0.08	**1.20**	0.15	**1.53** †	0.12
Cacna1A	**1**	0.08	**1.42**	0.19	**1.38**	0.14
Cacna1B	**1**	0.11	**1.21**	0.15	**1.07**	0.11
Cacna1E	**1**	0.06	**1.16**	0.19	**1.09**	0.21
Scn3a	**1**	0.10	**1.13**	0.07	**1.70** †	0.26
Scn9a	**1**	0.08	**1.19**	0.12	**1.05**	0.15
Stx1A	**1**	0.08	**1.02**	0.08	**0.83**	0.11
Snap25	**1**	0.06	**1.18**	0.09	**1.12**	0.10
Syt7	**1**	0.06	**1.07**	0.08	**1.09**	0.14
InsR	**1**	0.04	**1.05**	0.09	**1.06**	0.08
Irs1	**1**	0.05	**1.05**	0.10	**0.97**	0.10
Irs2	**1**	0.06	**1.23**	0.15	**1.22**	0.13
PIk3cd	**1**	0.06	**1.21**	0.13	**0.97**	0.12
PIk3r1	**1**	0.04	**1.08**	0.07	**1.17**	0.11
Mapk8	**1**	0.05	**1.17**	0.10	**0.98**	0.09
Mapk14	**1**	0.07	**0.97**	0.04	**0.94**	0.06
Ptpn1	**1**	0.04	**1.04**	0.10	**1.03**	0.06
Pten	**1**	0.08	**1.07**	0.04	**1.02**	0.07
GipR	**1**	0.09	**1.20**	0.17	**1.04**	0.09
Il6R	**1**	0.08	**0.84**	0.07	**0.92**	0.12
Il6st	**1**	0.07	**0.92**	0.06	**0.85**	0.13

Supplemental Table 1: Molecular analyses of Venus+ alpha-cells from obese mice- FACS-purified Venus+ alpha-cells from LFD, I-HFD and H-HFD mice were collected at the end of protocol and analyzed for relative mRNA quantification of specific genes coding for proteins involved in alpha-cell identity, glucagon biosynthesis and secretion. Specific mRNA levels were evaluated from amplified cDNA and relative to *RPS9*, *HPRT*, and *Cyclophilin* values. Data are analysed and presented as the means (Fold of littermate controls) ± SEM.

† and ‡ indicate statistical significance compared to LFD and to I-HFD mice respectively.

Supplemental Table 2

	LFD	SEM LFD	I-HFD	SEM I-HFD	H-HFD	SEM H-HFD
Gcg	**1**	0.09	**2.40** †	0.23	**1.19** ‡	0.15
Pcsk1/3	**1**	0.07	**1.31**	0.09	**0.64** †, ‡	0.11
Arx	**1**	0.05	**1.89** †	0.18	**0.69** ‡	0.10
Foxa1	**1**	0.12	**1.24**	0.24	**0.85**	0.07
Foxa2	**1**	0.12	**2.11** †	0.31	**1.07** ‡	0.29
Foxa3	**1**	0.09	**1.04**	0.11	**0.84**	0.14
Isl1	**1**	0.09	**1.62** †	0.14	**0.74** ‡	0.10
Nkx2.2	**1**	0.09	**0.93**	0.05	**0.94**	0.09
NeuroD1	**1**	0.10	**1.13**	0.08	**0.79**	0.11
Tcf7l2	**1**	0.06	**2.31** †	0.31	**1.11** ‡	0.16
Cdx2/3	**1**	0.06	**1.58** †	0.18	**0.91** ‡	0.09
Pax6	**1**	0.08	**1.72** †	0.22	**0.62** ‡	0.09
Pax4	**1**	0.17	**2.61**	0.65	**1.05**	0.33
MafB	**1**	0.15	**1.17**	0.25	**0.99**	0.11
cMaf	**1**	0.09	**0.85**	0.10	**0.92**	0.17
Slc5a1	**1**	0.10	**0.51** †	0.06	**0.62**	0.06
Slc2a2	**1**	0.18	**0.60**	0.13	**0.56**	0.13
Slc2a5	**1**	0.16	**0.63**	0.11	**0.63**	0.07
Gck	**1**	0.11	**1.84** †	0.18	**0.77** ‡	0.11
Mlxipl	**1**	0.13	**1.31**	0.22	**0.59** ‡	0.14
Gpbar1	**1**	0.24	**1.42**	0.21	**0.81**	0.08
Ffar1	**1**	0.15	**1.61** †	0.21	**0.62** ‡	0.10
Ffar3	**1**	0.18	**2.06**	0.53	**1.50**	0.37
Ffar2	**1**	0.12	**1.60**	0.21	**0.73** ‡	0.17
Gpr119	**1**	0.12	**1.42**	0.19	**0.94**	0.15
Ffar4	**1**	0.07	**1.58** †	0.23	**0.67** ‡	0.11
InsR	**1**	0.06	**1.05**	0.07	**0.59** †, ‡	0.05
Irs1	**1**	0.11	**1.04**	0.13	**0.57**	0.13
Irs2	**1**	0.12	**1.21**	0.24	**1.28**	0.24
GipR	**1**	0.13	**2.23** †	0.33	**1.64**	0.26
Il6R	**1**	0.16	**1.34**	0.22	**0.83**	0.12
Il6st	**1**	0.11	**0.94**	0.16	**0.73**	0.08
Kcnj11	**1**	0.06	**1.77** †	0.17	**0.70** ‡	0.13
Abcc8	**1**	0.07	**1.63** †	0.16	**0.72** ‡	0.11
Cacna1C	**1**	0.10	**1.24**	0.14	**0.63** ‡	0.07
Cacna1A	**1**	0.13	**2.00** †	0.34	**0.86** ‡	0.17
Kcnq1	**1**	0.07	**0.94**	0.05	**0.79**	0.10
Scn3a	**1**	0.21	**1.42**	0.27	**0.95**	0.14
Stx1A	**1**	0.09	**1.96** †	0.26	**0.97** ‡	0.20
Snap25	**1**	0.16	**1.60**	0.22	**0.96**	0.16
Syt7	**1**	0.13	**1.26**	0.18	**0.57** ‡	0.12

Supplemental Table 2: Molecular analyses of Venus+ L-cells from small intestine of obese mice- After isolation, cell dissociation and FACS-sorting, specific mRNA levels were evaluated from amplified cDNA of intestinal Venus+ L-cells from small intestine (jejunum and ileum) of LFD, I-HFD and H-HFD mice. Data are presented as the means (Fold of littermates LFD) ± SEM and analyzed.

† and ‡ indicate statistical significance compared to LFD and to I-HFD mice respectively.

Supplemental Table 3

	LFD	SEM LFD	I-HFD	SEM I-HFD	H-HFD	SEM H-HFD
Gcg	**1**	0.11	**1.63** †	0.24	**1.05**	0.17
Pcsk1/3	**1**	0.04	**1.54** †	0.21	**0.97** ‡	0.17
Arx	**1**	0.08	**1.85** †	0.24	**1.09** ‡	0.20
Foxa1	**1**	0.16	**1.28**	0.16	**1.10**	0.05
Foxa2	**1**	0.08	**1.66** †	0.19	**0.86** ‡	0.06
Foxa3	**1**	0.18	**1.10**	0.09	**0.92**	0.02
Isl1	**1**	0.10	**1.48**	0.18	**1.03**	0.21
Nkx2.2	**1**	0.06	**1.64** †	0.18	**1.11** ‡	0.17
NeuroD1	**1**	0.07	**1.66**	0.25	**1.03**	0.22
Tcf7l2	**1**	0.30	**0.99**	0.06	**1.09**	0.02
Cdx2/3	**1**	0.28	**0.83**	0.07	**0.83**	0.12
Pax6	**1**	0.06	**1.61**	0.31	**1.00**	0.17
Pax4	**1**	0.11	**2.26** †	0.42	**1.36**	0.21
MafB	**1**	0.25	**1.12**	0.11	**1.12**	0.08
cMaf	**1**	0.34	**0.80**	0.07	**1.03**	0.21
Slc5a1	**1**	0.15	**1.09**	0.11	**0.99**	0.07
Slc5a2	**1**	0.17	**1.35**	0.48	**1.54**	0.63
Slc5a5	**1**	0.10	**1.43**	0.24	**1.14**	0.26
Gck	**1**	0.08	**1.22**	0.14	**0.98**	0.10
Mlxipl	**1**	0.11	**2.08** †	0.36	**1.09** ‡	0.24
Gpbar1	**1**	0.12	**2.06** †	0.28	**1.20** ‡	0.17
Ffar1	**1**	0.10	**1.45**	0.30	**1.11**	0.19
Ffar3	**1**	0.18	**1.73** †	0.17	**1.05**	0.16
Ffar2	**1**	0.09	**1.96** †	0.29	**1.17** ‡	0.30
GPR119	**1**	0.11	**1.78**	0.33	**1.20**	0.21
Ffar4	**1**	0.18	**1.37**	0.17	**0.96**	0.10
InsR	**1**	0.22	**1.03**	0.09	**0.95**	0.05
Irs1	**1**	0.09	**1.22**	0.07	**0.93**	0.13
Irs2	**1**	0.21	**1.17**	0.11	**1.02**	0.06
GipR	**1**	0.13	**3.25** †	0.74	**2.00**	0.45
IL6R	**1**	0.10	**1.75** †	0.23	**1.09**	0.15
IL6st	**1**	0.20	**1.15**	0.07	**1.00**	0.10
Kcnj11	**1**	0.07	**1.93** †	0.35	**1.18** ‡	0.27
Abcc8	**1**	0.05	**1.69**	0.28	**1.11**	0.28
Cacna1C	**1**	0.08	**1.61**	0.27	**1.14**	0.19
Cacna1A	**1**	0.06	**1.91**	0.39	**1.11**	0.21
Kcnq1	**1**	0.21	**1.03**	0.12	**0.99**	0.03
Scn3a	**1**	0.26	**1.55**	0.30	**1.41**	0.33
Stx1A	**1**	0.08	**1.82** †	0.25	**1.24** ‡	0.21
Snap25	**1**	0.11	**1.92** †	0.35	**1.17**	0.18
Syt7	**1**	0.04	**1.72**	0.29	**1.16**	0.22

Supplemental Table 3: Molecular analyses of Venus+ L-cells from colon of obese mice- After isolation, cell dissociation and FACS-sorting, specific mRNA levels were evaluated from amplified cDNA of intestinal Venus+ L-cells from colon of LFD, I-HFD and H-HFD mice. Data are presented as the means (Fold of littermates LFD) ± SEM and analyzed.

† and ‡ indicate statistical significance compared to LFD and to I-HFD mice respectively.