**Supplemental information**

Gut HIF2α signaling is increased after

VSG, and gut activation of HIF2α decreases

weight, improves glucose, and increases GLP-1 secretion

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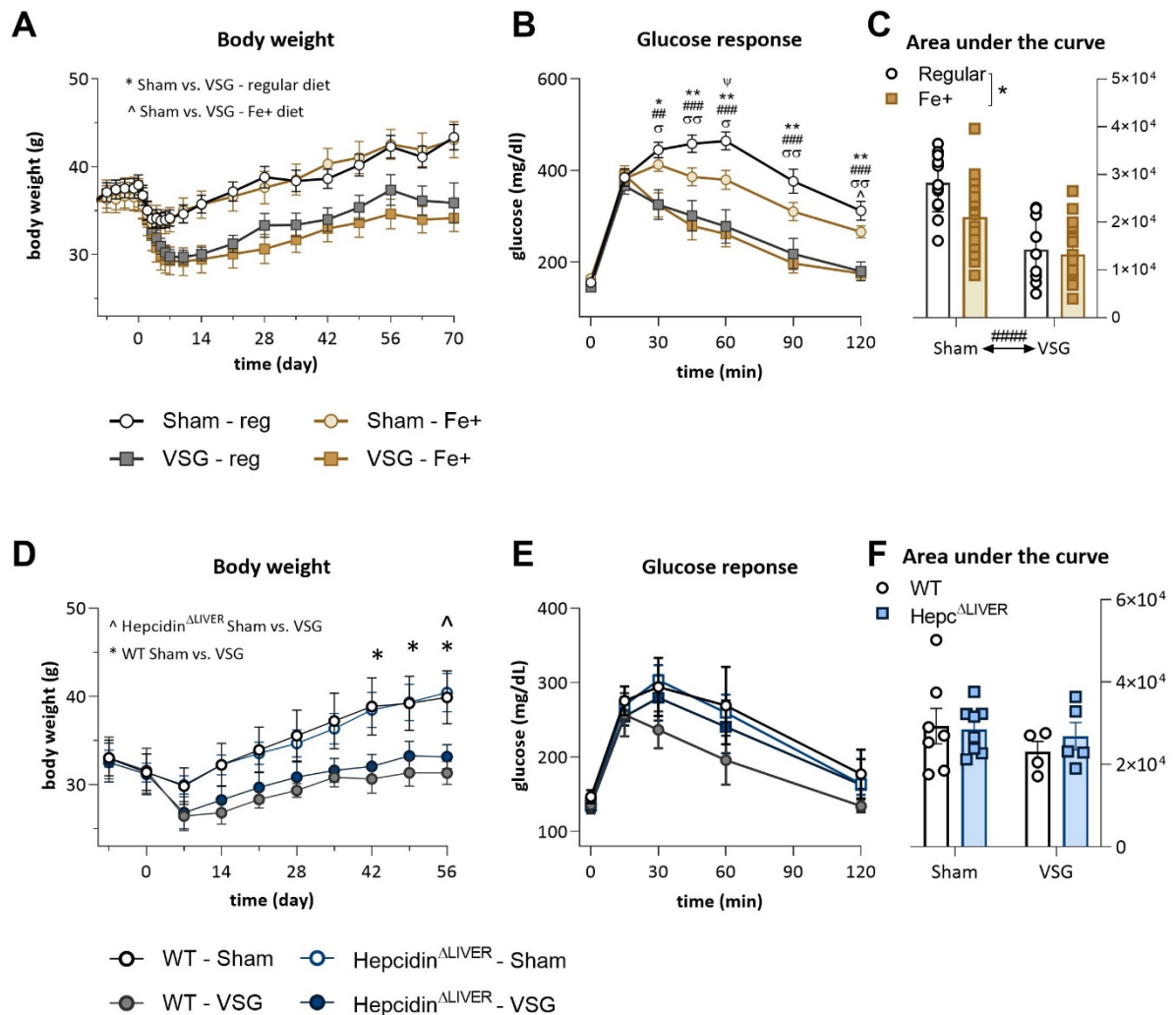
Supplement

Figure S1: Body weight progression and composition following RYGB and VSG surgery in rats
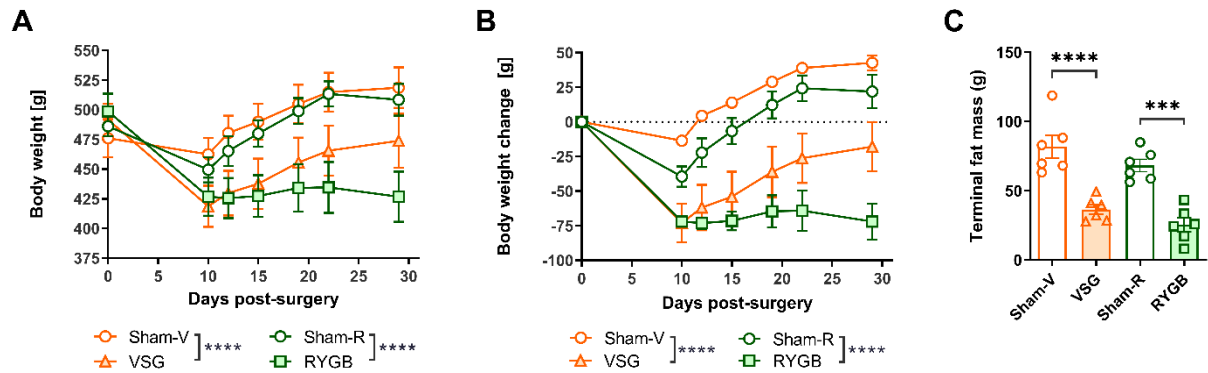
Fig S1) In vivo data from the rat VSG and RYGB cohort used for RNA sequencing analyses of duodenum and ileum. A) Average body weight from time of surgery (day 0) until termination (day 30). Both VSG and RYGB induce weight loss of the course of the study (****P<0.0001, RM-ANOVA). B) Relative body weight from time of surgery (day 0) until termination (day 30). Both VSG and RYGB induce relative weight loss of the course of the study (****P<0.0001, RM-ANOVA). C) Fat mass at termination shows both VSG and RYGB result in a reduction of total body adiposity as measured by EchoMRI (***P<0.001, ****P<0.0001 one-way ANOVA). Data is presented as average±sem (n=6/group). Data is related to methods section: Rat cohort for RNA sequencing study; and manuscript figure 1A-C.

Figure S2: Body weight progression and glucose responses following VSG surgery in high dietary iron fed mice and Hepcidin$^{\Delta LIVER}$ mice.

Fig. S2) High dietary iron supplementation or Hepcidin$^{\Delta LIVER}$ do not affect the metabolic response to VSG. A) Mice fed a 45% HFD containing 350ppm supplemented iron responded equally to VSG surgery at the level of body weight maintenance as mice fed a 45% HFD containing 35ppm iron (Two-way ANOVA: $F_{(66, 814)}$=13.19, P<0.0001, main effect post hoc Tukey ****P<0.0001 Sham-reg>VSG-reg, ####P<0.0001 Sham-reg>VSG-Fe+, ^^^^P<0.0001 Sham-Fe+>VSG-reg, $^{\sigma\sigma\sigma\sigma}$P<0.001 Sham-Fe>VSG-Fe+. B) Glucose levels following ip glucose administration (ip 2g/kg glucose) are reduced in VSG compared to Sham groups and lower in Sham-Fe+ compared to Sham-reg (rm-ANOVA: $F_{(18,312)}$=6.714, simple effects per row post hoc Tukey, *P<0.05, **P<0.01 Sham-Reg>VSG-reg, ##P<0.01, ###P<0.001 Sham-reg>VSG-Fe$^+$; $^{\sigma}$P<0.05, $^{\sigma\sigma}$P<0.01 Sham-Fe+>VSG-Fe$^+$, ^P<0.05 Sham-Fe$^+$>VSG-reg; $^{\Psi}$P<0.05 Sham-reg>Sham-Fe$^+$. C) The area under curve of the glucose response is lowered by VSG (Two-way ANOVA, main effects: ####P<0.0001 Surgery effect), but to a lesser extent so did dietary iron ($^{\sigma}$P<0.05 dietary iron effect). Data presented in 2S A-C is related to methods section:

Response to VSG in mice fed a high iron containing diet; and manuscript figure 2A-C. D) Hepcidin$^{\Delta Liver}$ mice respond similar to VSG surgery as WT mice at the level of body weight *P<0.05: WT Sham > VSG ^P<0.05: Hepcidin$^{\Delta Liver}$ Sham > VSG, post hoc Tukey; E) glucose response, and F) area under the curve of glucose response, E) fasting glucose levels, $^{\#}$P<0.05 two-way ANOVA main effect of surgery. Data is presented as average±sem. Data presented in 2S D-F is related to method section: Response to VSG in a mouse model of hereditary hemochromatosis; and manuscript figure figure 2D-F.

Figure S3: Duodenal mRNA expression in Hif2α$^{\Delta Gut}$ mice following VSG surgery
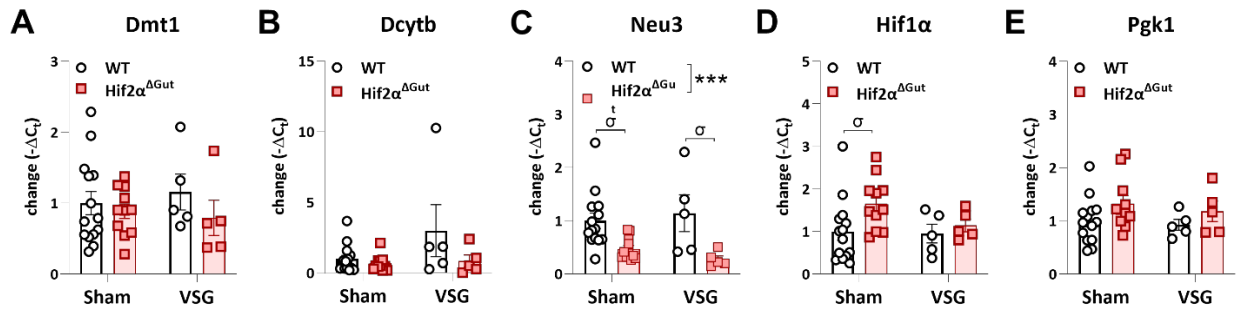
Fig. S3) Non-fasting HIF2α target genes expressions in duodenal tissue from Hif2α$^{\Delta Gut}$ mice that had either sham or VSG surgery show no down regulation of iron transporters A) *Dmt1*, and B) *Dcytb*. C) *Neu3* is down regulated in duodenal tissue of Hif2α$^{\Delta Gut}$ mice (***P<0.001, two-way ANOVA main effect of genotype, $^{\sigma}$P<0.05 post hoc Tukey. D) An increase of duodenal *Hif1α* was observed in Sham operated rats ($^{\sigma}$P<0.05, two-way ANOVA post hoc Tukey). E) No changes in expression of HIF1α target gene Pgk1 was observed in duodenal tissue of Hif2α$^{\Delta Gut}$ mice independent of surgery. Data is presented as average±sem. Data is related to method section: Response to VSG in intestinal Hif2α knockout mice; and manuscript figure 3. Average Ct data are presented in Table S4.

Supplemental Tables:

Table S1: Cycle threshold (Ct) of gene expression in the duodenum and ileum.

Gene of interest:	Vhl		Phd2		Hif2a		Dmt1		Dcytb		Neu3		Hif1a		Pgk1		Glut1	
	Avg	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*
Duodenum (Ct, n=8)	32.33	0.24	29.35	0.25	28.84	0.26	27.76	0.24	30.18	0.30	33.66	0.37	28.36	0.25	26.50	0.27	32.65	0.33
Ileum (Ct, n=8)	31.90	0.16	29.06	0.16	30.32	0.16	29.18	0.18	35.55	0.35	37.07	0.23	28.98	0.21	26.57	0.18	32.33	0.30

Numbers are presented as average (Avg) ± standard error of the mean (sem) referring to ΔCt gene expression levels in figure 1F.

Table S2: Cycle threshold (Ct) of gene expression in the duodenum of VSG and Sham operated mice.

Gene of interest:	Vhl		Phd2		Hif2a		Dmt1		Dcytb		Neu3		Hif1a		Pgk1		Glut1	
	Avg	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*
VSG (Ct, n=16)	32.03	0.09	28.75	0.13	28.97	0.10	27.68	0.20	29.50	0.29	33.70	0.15	27.73	0.17	25.50	0.15	32.24	0.20
Sham (Ct, n=8)	32.33	0.41	29.08	0.45	28.82	0.29	28.52	0.41	31.10	0.64	34.14	0.40	27.61	0.36	25.74	0.44	32.58	0.35

Numbers are presented as average (Avg) ± standard error of the mean (sem) referring to ΔCt gene expression levels in figure 1G.

Table S3: Cycle threshold (Ct) of gene expression in the duodenum of WT and Hif2α^ΔGut mice.

Gene of interest:	Hif2a-Exon2-3				Cre			
Surgery group:	Sham		VSG		Sham		VSG	
	Avg	sem	Avg	sem	Avg	sem	Avg	sem
WT	31.59	1.06	32.28	1.38	39.63	2.69	43.82	2.89
Hif2α^ΔGut	34.74	0.30	35.16	1.01	30.47	0.21	30.28	0.49

Groups sizes: Wt-Sham: n=14, Wt-VSG: n=6, Hifα^ΔGut-Sham: n=13, Hifα^ΔGut-VSG: n=6.
Numbers are presented as average (Avg) ± standard error of the mean (sem) referring to ΔCt gene expression levels in figure 3A/B.

Table S4: Cycle threshold (Ct) of gene expression in the duodenum of WT and Hif2α^ΔGut mice.

Gene of interest:	Dmt1				Dcytb				Neu3				Hif1a				Pgk1			
Surgery group:	Sham		VSG		Sham		VSG		Sham		VSG		Sham		VSG		Sham		VSG	
	Avg	sem	Avg	sem	Avg	sem	Avg	sem	Avg	sem	Avg	sem	Avg	sem	Avg	sem	Avg	sem	Avg	sem
WT	32.09	1.15	32.21	1.80	36.36	1.41	35.51	2.67	35.27	1.16	35.91	1.33	30.63	1.42	31.26	1.37	30.12	0.94	30.66	0.79
Hif2a^ΔGut	32.43	0.34	32.00	1.25	36.77	0.24	36.73	2.37	36.29	0.21	36.80	0.90	29.87	0.32	29.85	0.69	29.78	0.18	29.55	0.63

Groups sizes: Wt-Sham: n=14, Wt-VSG: n=6, Hifα^ΔGut-Sham: n=13, Hifα^ΔGut-VSG: n=6..
Numbers are presented as average (Avg) ± standard error of the mean (sem) referring to ΔCt gene expression levels in supplement figure S3.

Table S5: Cycle threshold (Ct) of gene expression in the duodenum of WT and Vhl$^{\Delta Gut}$ mice.

Gene of interest:	Vhl		Phd2		Hif2a		Dmt1		Dcytb		Neu3		Hif1a		Glut1		Pgk1		Pdk1	
	Avg	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*
WT (Ct, n=8)	33.60	0.20	31.57	0.20	29.93	0.22	28.91	0.38	32.52	0.61	34.88	0.48	29.79	0.19	34.47	0.32	28.40	0.30	32.94	0.23
Vhl$^{\Delta Gut}$ (Ct, n=8)	NA	NA	28.86	0.28	29.25	0.18	25.49	0.30	25.71	0.29	29.41	0.24	32.46	0.24	32.67	0.38	27.35	0.32	30.89	0.30

Numbers are presented as average (Avg) ± standard error of the mean (sem) referring to ΔCt gene expression levels in figure 4A.

Table S6: Cycle threshold (Ct) of gene expression in the ileum of WT and Vhl$^{\Delta Gut}$ mice.

Gene of interest:	Vhl		Phd2		Hif2a		Dmt1		Dcytb		Neu3		Hif1a		Glut1		Pgk1		Pdk1	
	Avg	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*	*Avg*	*sem*
WT (Ct, n=8)	32.50	0.20	28.98	0.20	29.89	0.30	29.19	0.22	37.19	0.36	36.94	0.14	28.10	0.23	31.49	0.26	25.53	0.23	30.83	0.19
Vhl$^{\Delta Gut}$ (Ct, n=8)	NA	NA	27.56	0.25	29.53	0.10	25.52	0.21	33.52	0.38	35.99	0.22	29.41	0.28	29.85	0.26	23.87	0.38	27.56	0.25

Numbers are presented as average (Avg) ± standard error of the mean (sem) referring to ΔCt gene expression levels in figure 4B.

Table S7: Oligonucleotides section of Key Resource Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Vhl - F1 (5'-CTG GTA CCC ACG AAA CTG TC-3')	Integrated DNA technologies	N/A
Vhl - F2 (5'-CTA GGC ACC GAG CTT AGA GGT TTG CG-3')	Integrated DNA technologies	N/A
Vhl - R1 (5'-CTG ACT TCA CTG ATG CTT GTC ACA G-3')	Integrated DNA technologies	N/A
Cre - F (5'-AGT GCG TTC GAA CGC TAG AGC CTG T-3')	Integrated DNA technologies	N/A
Cre - R (5'-GAA CCT GAT GGA CAT GTT CAG G-3')	Integrated DNA technologies	N/A
Gcg - F1 (5'-CCT TCA GAA AAG CTG TCA GA-3')	Integrated DNA technologies	N/A
Gcg - F2 (5'-GCA TTC TAG TTG TGG TTT GTC C-3')	Integrated DNA technologies	N/A
Gcg - RA (5'-TCC TAT GTA ACT GTT TGG CAT G-3')	Integrated DNA technologies	N/A
Glp1r - F1 (5'- TGA GAG CTG ATG GAA GGT GTT G-3')	Integrated DNA technologies	N/A
Glp1r - Mutant F2 (5'-CTG CAT TCT AGT TGT GGT TTG TCC-3')	Integrated DNA technologies	N/A
Glp1r - Common R1(5'-CCT TCA GAT GGG GAA ACA AAG C-3')	Integrated DNA technologies	N/A
Hamp - F (5-TAG GCT GCT TAC CTC TCT TTC TT-3')	Integrated DNA technologies	N/A
Hamp - R (5'-AAT TCC AAG ACT TAG AAG GCA AA-3')	Integrated DNA technologies	N/A

This table is related to the Key Resource Table in the STAR methods section of the manuscript.