

Supplemental information

Microbial regulation of hexokinase 2 links

mitochondrial metabolism and cell death in colitis

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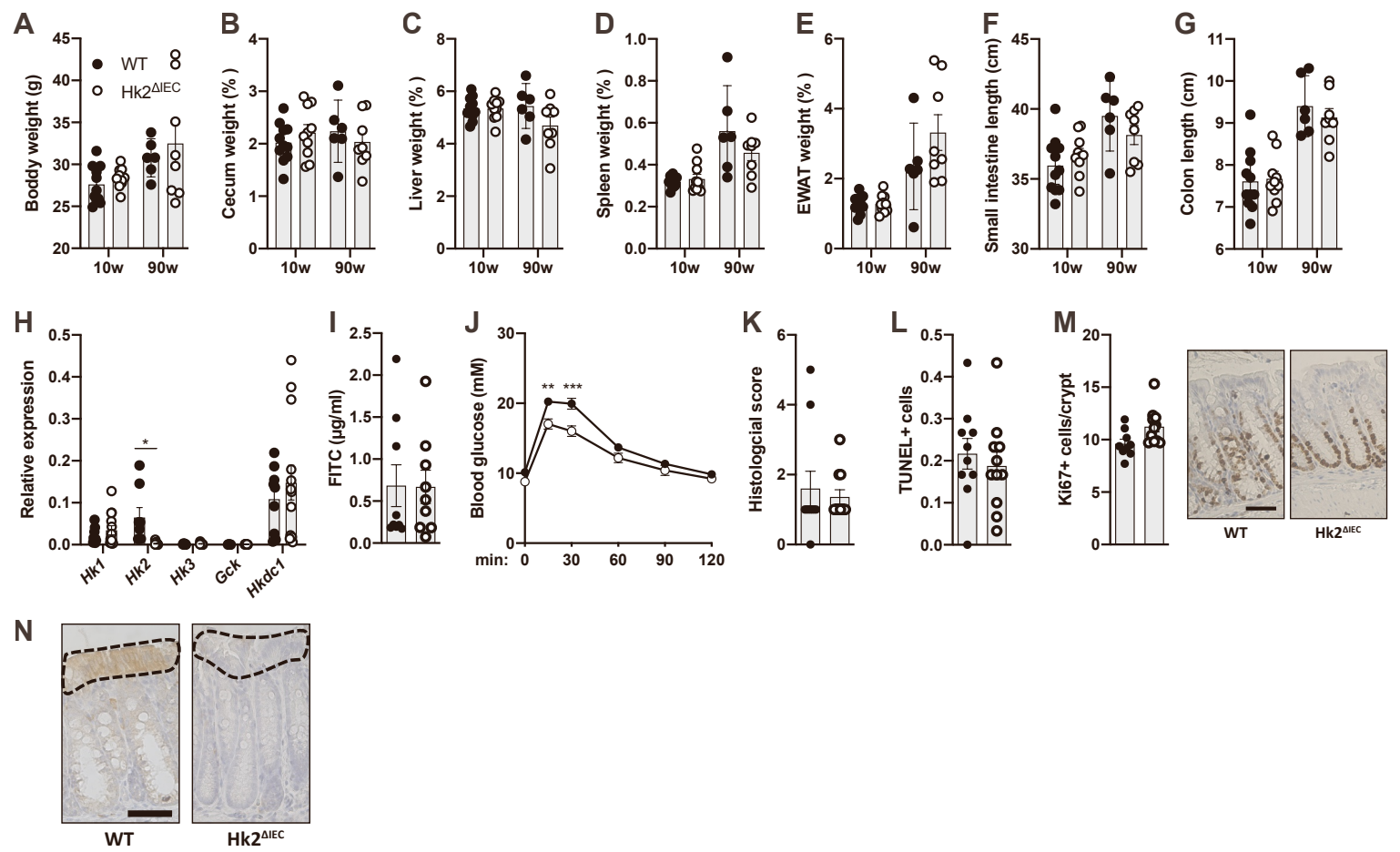


Fig.S1

Figure S1, related to Figure 1: *Hk2*^{ΔIEC} mice do not display an evident immunological or metabolic phenotype under baseline conditions. A-G) 10- or 90-week-old WT and *Hk2*^{ΔIEC} mice were sacrificed and organ measures taken. **A)** Body weight. **B)** Cecum weight. **C)** Liver weight. **D)** Spleen weight. **E)** Epididymal white adipose tissue (EWAT) weight. **F)** Small intestine length. **G)** Colon length. **H)** Relative expression of all hexokinase family members in unfractionated colon tissue of 10-week-old WT and *Hk2*^{ΔIEC} mice as per qPCR. **I)** Intestinal permeability as measured by fluorescein isothiocyanate (FITC) levels in blood of 10-week-old WT and *Hk2*^{ΔIEC} mice 1h after oral gavage. **J)** Blood glucose levels during oral glucose tolerance test in 10-week-old WT and *Hk2*^{ΔIEC} mice. **K)** Colonic histological score of 10-week-old WT and *Hk2*^{ΔIEC} mice. **L)** TUNEL-positive cells per colon crypt of 10-week-old WT and *Hk2*^{ΔIEC} mice. **M)** Ki-67-positive cells per colon crypt of 10-week-old WT and *Hk2*^{ΔIEC} mice including representative images. The scale bar represents 50 μm. **N)** Expression pattern of the HK2 protein in the colonic epithelium of untreated 10-week-old WT and *Hk2*^{ΔIEC} mice. The scale bar represents 50 μm. The dotted line indicates the tip epithelium area used for quantification of HK2 protein expression. Note the dominant HK2 expression in the colonic tip epithelium.

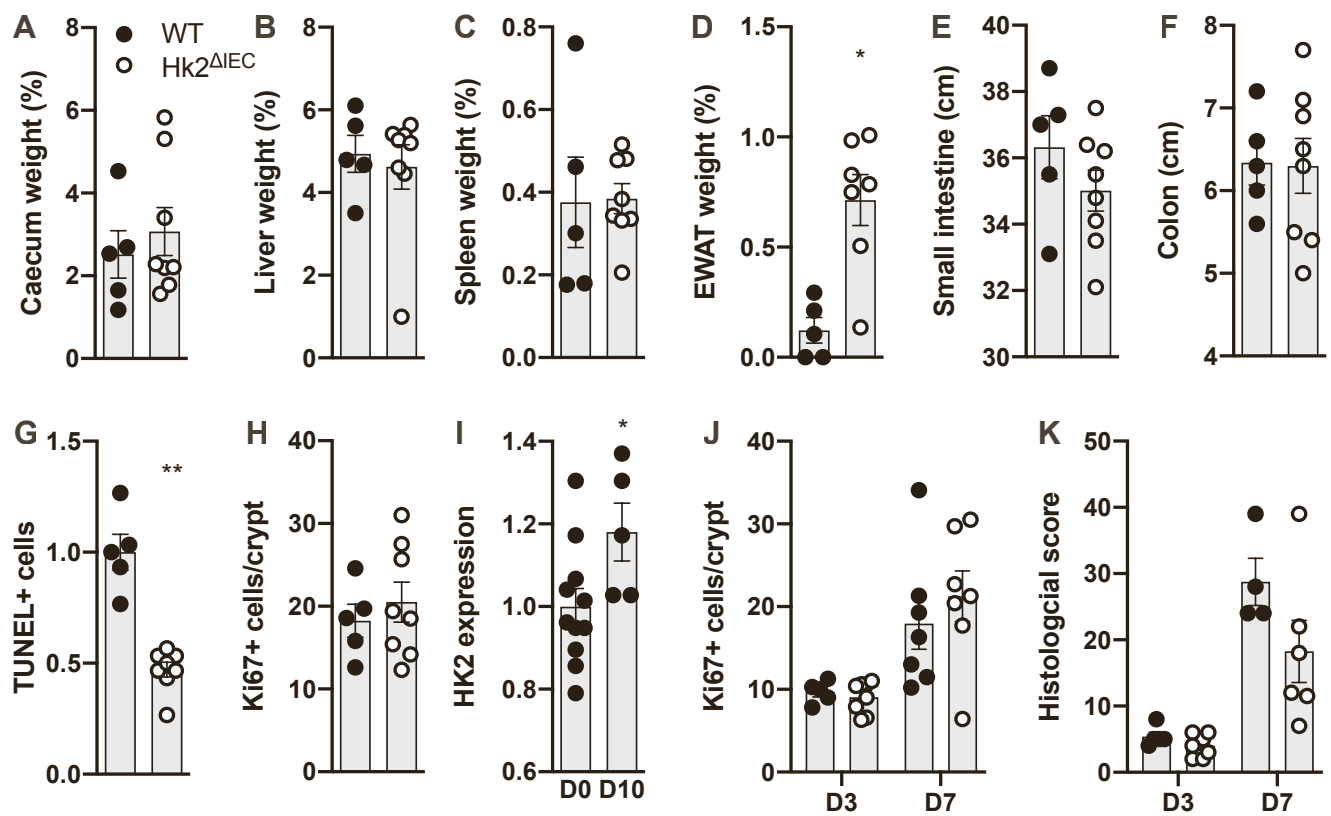


Fig.S2

Figure S2, related to Figure 1: Phenotyping of WT and *Hk2*^{ΔIEC} mice during DSS-induced colitis. A-H) Organ and histological data from WT and *Hk2*^{ΔIEC} mice sacrificed at the end (day 10) of DSS colitis. **A)** Cecum weight. **B)** Liver weight. **A)** Spleen weight. **D)** Epididymal white adipose tissue (EWAT) weight. **E)** Small intestine length. **F)** Colon length. **G)** TUNEL-positive cells per colon crypt. **h)** Ki-67-positive cells per colon crypt. **I)** HK2 protein levels in colon epithelium of WT mice at baseline (day 0) and at day 10 during DSS colitis as determined per immunohistochemistry. **J)** Ki-67-positive cells per colon crypt and **K)** histological score of WT and *Hk2*^{ΔIEC} mice at day 3 and 7 during DSS colitis.

Figure S3, related to Figure 1: Heatmap of genes differentially expressed in colon of WT and *Hk2*^{ΔIEC} mice at days 0, 3 and 7 during DSS colitis. WT and *Hk2*^{ΔIEC} mice were given 2% DSS in drinking water and analyzed on day 0, 3 and 7 of treatment. RNA was isolated from unfractionated colon and sequenced to identify transcripts with differential expression dependent on the loss of epithelial HK2 during the onset of inflammation.

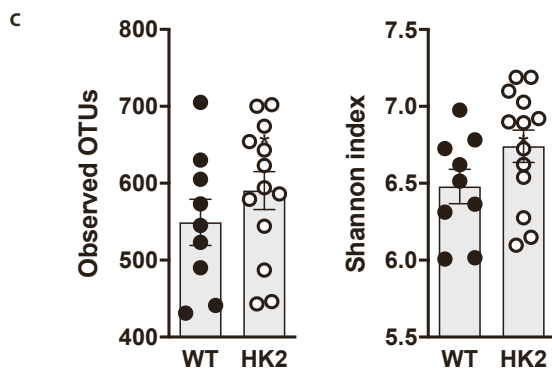
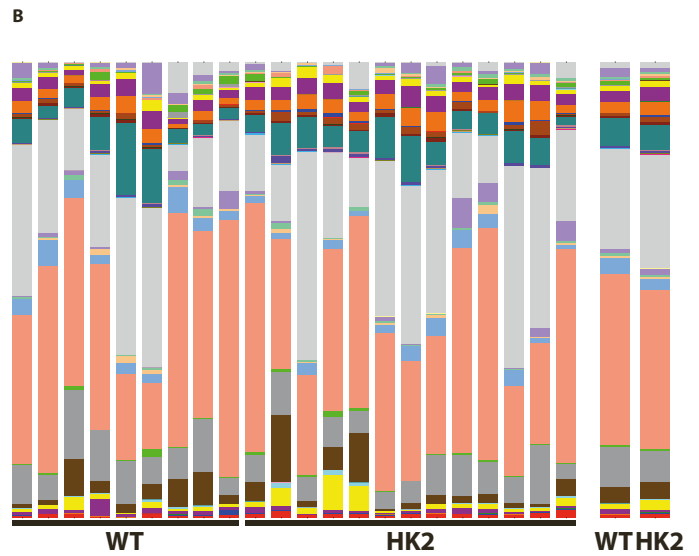
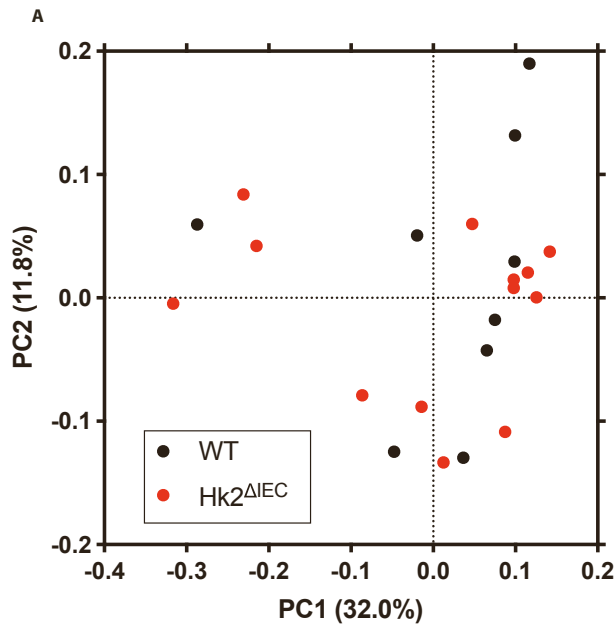


Figure S4, related to Figure 1: Ablation of HK2 in IECs does not alter the composition of the intestinal microbiota. A) Principal coordinate analysis of fecal microbiota from WT and *Hk2*^{ΔIEC} mice. **B)** Taxonomic overview on genus level. **C)** Alpha diversity (the variation of microorganisms in a single sample).

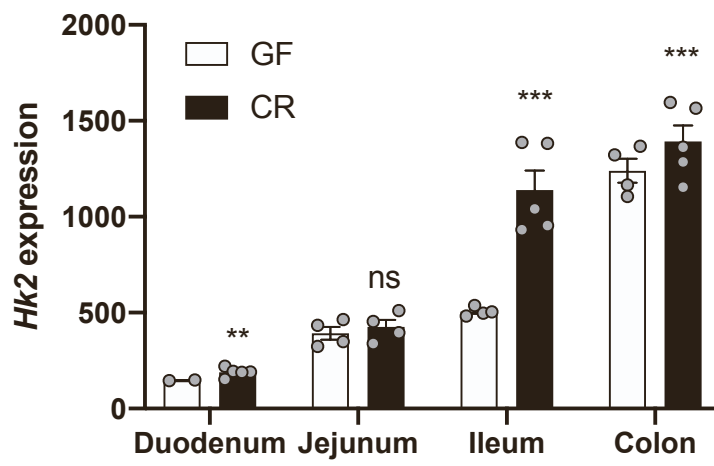


Figure S5, related to Figure 3: *Hk2* expression increases along the intestinal tract. *Hk2* expression was determined in whole tissue specimen collected along the length of the intestinal tract of adult C57BL6/J GF and CR mice. Data from (Larsson et al., 2012). n=4-5 per group. ** p<0.01 and *** p<0.001.

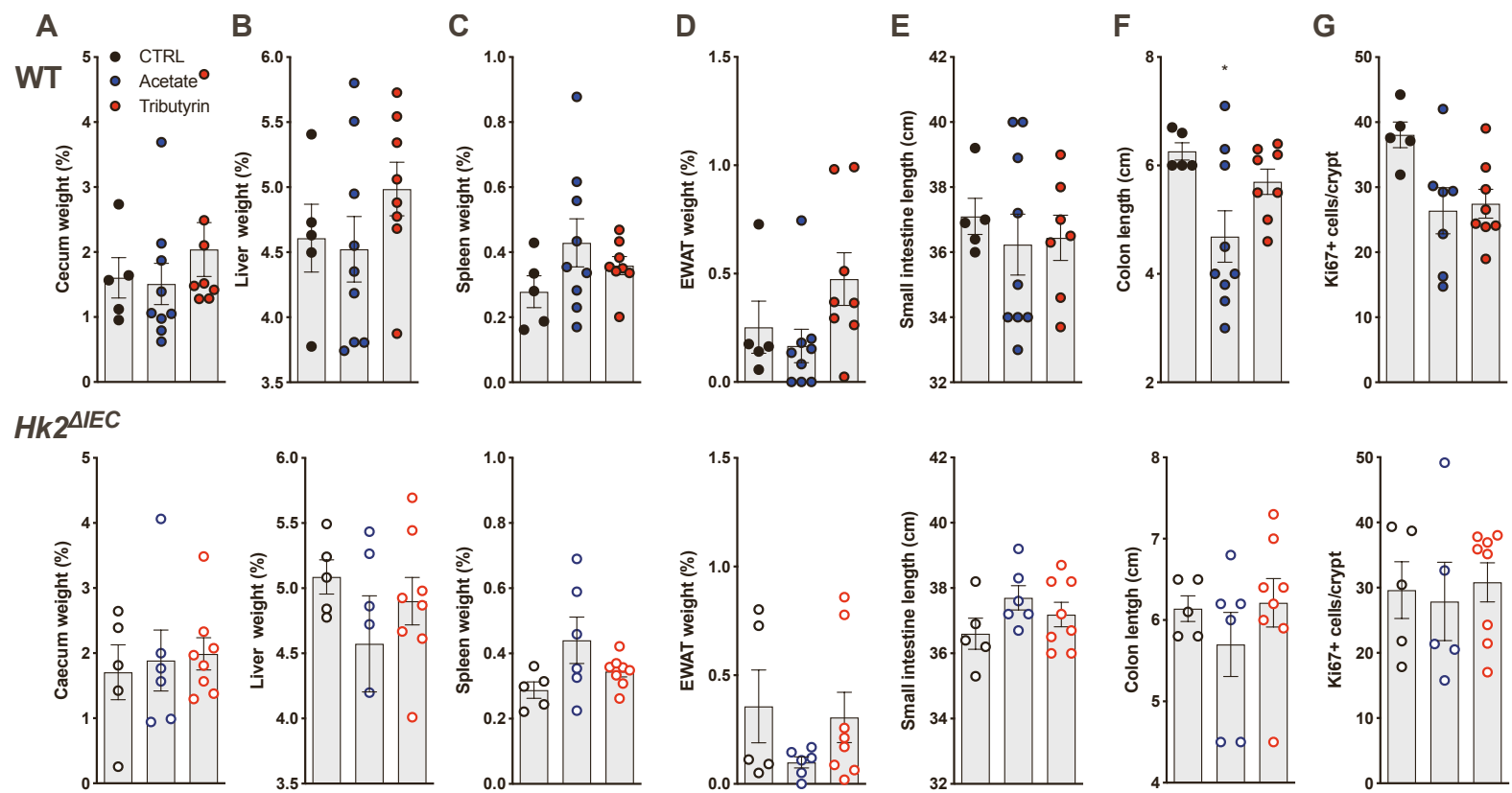


Fig.S6

Figure S6, related to Figure 4: Organ measures and histological data of WT and *Hk2*^{ΔIEC} mice at the end of the dietary SCFA supplementation and DSS-induced colitis experiment. A) Cecum weight. B) Liver weight. C) Spleen weight. D) Epididymal white adipose tissue (EWAT) weight. E) Small intestine length. F) Colon length. G) Ki-67-positive cells per colon crypt.

Table S2, related to Figure 3: Statistics of linear modelling of *Hk2* expression induced by metabolites produced by single OMM bacteria grown *in vitro*. AIC = Akaike Information Criteria. Sigma = standard deviation of the residuals. R² = coefficient of determination, referring to the proportion of variance in the dependent variable that is predictable from independent variables. R = Pearson correlation coefficient of predicted metabolite production with *Hk2* expression data.

Formula	AIC	sigma	R2	R	pval
Acetate	-5.1809804	0.16092295	0.19156729	0.43768401	0.17820082
Butyrate	-5.5801726	0.15802932	0.22037951	0.46944596	0.14517284
Propionate	-2.8925022	0.17856407	0.00460373	0.06785081	0.84287465
Acetate & butyrate	-8.0040261	0.13381316	0.56522852	0.75181681	0.00762065

Table S3, related to STAR Methods: List of primers used in this study.

Sequence 5'-->3'	Gene	Organism	For/Rev	Purpose	Primer name
CATGTACGTTGCTATCCAGGC	Actb	Human	F	qPCR	807_hActin_(s)qPCR_2_F
CTCCTTAATGTCACGCACGAT	Actb	Human	R	qPCR	808_hActin_(s)qPCR_2_R
CCCCTTGTAAGCCCAAGATC	Rpl32	Human	F	qPCR	3334_Hs-L32_F
TCTGGGTTTCCGCCAGTTA	Rpl32	Human	R	qPCR	3335-Hs_L32-R
AAGGCTTCAAGGCATCTG	Hk2	Human	F	qPCR	3491_hs_Hk2_F
CCACAGGTCATCATAGTTCC	Hk2	Human	R	qPCR	3492_hs_Hk2_R
CTGCTGGTGAAAATCCGTAGTGG	Hk1	Human	F	qPCR	3730_Hs_HK1_F
GTCCAAGAAGTCAGAGATGCAGG	Hk1	Human	R	qPCR	3731_Hs_HK1_R
GTGAGGTTGGGCTAGTTGTAGA	Hk3	Human	F	qPCR	3732_Hs_HK3_F
GTCCAGGGTATGGTCAAGGT	Hk3	Human	R	qPCR	3733_Hs_HK3_R
GAATGACACGGTGGCCACGATG	Gck	Human	F	qPCR	3734_Hs_GCK_F
CACTCGGTATTGACGCACATGCG	Gck	Human	R	qPCR	3735_Hs_GCK_R
GGCTTACATTCTCATTTCC	Hkdc1	Human	F	qPCR	3512_hs_Hkdc1_F
TGTTGCTGCCTGTTCTG	Hkdc1	Human	R	qPCR	3513_hs_Hkdc1_R
TTCGGCTACAAAGGCTCCAC	Ppif	Human	F	qPCR	3740_Hs_Ppif_F
TGTGCCATTGTGGTTGGTGA	Ppif	Human	R	qPCR	3741_Hs_Ppif_R
GTAGCTAACACAAGTCCAGTCCT	Ffar2	Human	F	qPCR	3896_Ffar2_F
CTAGGTGTTGCTTTGAAGCTTGT	Ffar2	Human	R	qPCR	3897_Ffar2_R
CTAAGGGTATGCGCGCTAAAG	Ffar3	Human	F	qPCR	3898_Ffar3_F
AGCCCAATCCATAGTGTGTGG	Ffar3	Human	R	qPCR	3899_Ffar3_R
ATGTTGGCTATGAACCGCCAG	Hcar2	Human	F	qPCR	3900_Hcar2_F
GCTGCTGTCCGATTGGAGA	Hcar2	Human	R	qPCR	3901_Hcar2_R
TAAGCGTTCCGCAAGGAGAGTTTT	Hk2	Human	F	CRISPR	3608_Hk2_CRIS_F
TTCCTTGCGGAACCGCTTACGGTG	Hk2	Human	R	CRISPR	3609_Hk2_CRIS_R
GGCTGTATCCCCTCCATCG	Actb	Mouse	F	qPCR	3172_b-actin_F
CCAGTTGGTAACAATGCCATGT	Actb	Mouse	R	qPCR	3173_b-actin_R
CCTCTGGTGAAGCCCAAGATC	Rpl32	Mouse	F	qPCR	2664_L32-F
TCTGGGTTTCCGCCAGTTT	Rpl32	Mouse	R	qPCR	2665_L32-R
CCCTGTGAAGATGTTGCCACT	Hk2	Mouse	F	qPCR	3477_Hk2_F
CCTTCGTTGCCATTACGCACG	Hk2	Mouse	R	qPCR	3478_Hk2_R
CGGAATGGGGAGCCTTTGG	Hk1	Mouse	F	qPCR	3479_Hk1_F1
GCCTTCCTTATCCGTTTCAATGG	Hk1	Mouse	R	qPCR	3480_Hk1_R1
TGCTGCCACATACGTGAG	Hk3	Mouse	F	qPCR	3483_Hk3_F
GCCTGTCAGTGTACCACAA	Hk3	Mouse	R	qPCR	3484_Hk3_R
GAGATGGATGTGGTGGCAAT	Gck	Mouse	F	qPCR	3485_Gck(Hk4)_F
ACCAGCTCCACATTCTGCAT	Gck	Mouse	R	qPCR	3486_Gck(Hk4)_R
ATGTTTGCAGTACACTTGGTGG	Hkdc1	Mouse	F	qPCR	3489_Hkdc1_F
AGGGTCTCATCCGAGAGCC	Hkdc1	Mouse	R	qPCR	3490_Hkdc1_R
TGGCTCTCAGTTCTTTATCTGC	Ppif	Mouse	F	qPCR	3738_Mm_Ppif_F
ACATCCATGCCCTCTTTGAC	Ppif	Mouse	R	qPCR	3739_Mm_Ppif_R