SHORT REPORT

Figures and figure supplements

A kidney-hypothalamus axis promotes compensatory glucose production in response to glycosuria

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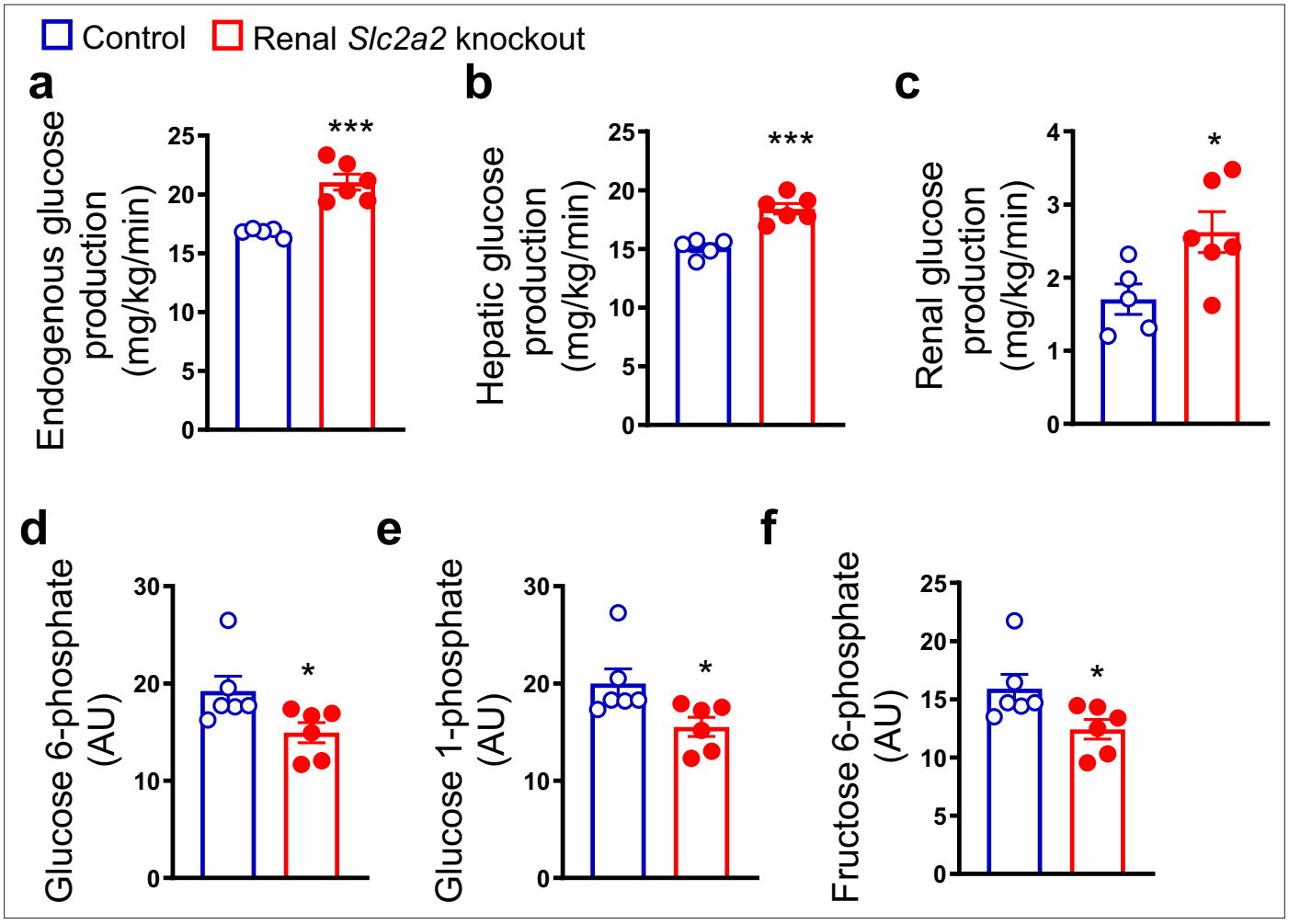

Figure 1. Renal *Slc2a2* knockout mice exhibit increased glucose production. In vivo increase in total (**a**), hepatic (**b**), and renal (**c**) glucose production through gluconeogenesis with pyruvate as a substrate in 28-week-old male renal *Slc2a2* knockout mice. Decreased hepatic glucose 6-phosphate (**d**), glucose 1-phosphate (**e**), and fructose 6-phosphate (**f**) in renal *Slc2a2* knockout mice 12 weeks after inducing the *Slc2a2* deficiency. *p<0.05, ***p<0.001, unpaired two-tailed Student's t-test. Data are presented as mean ± SEM.

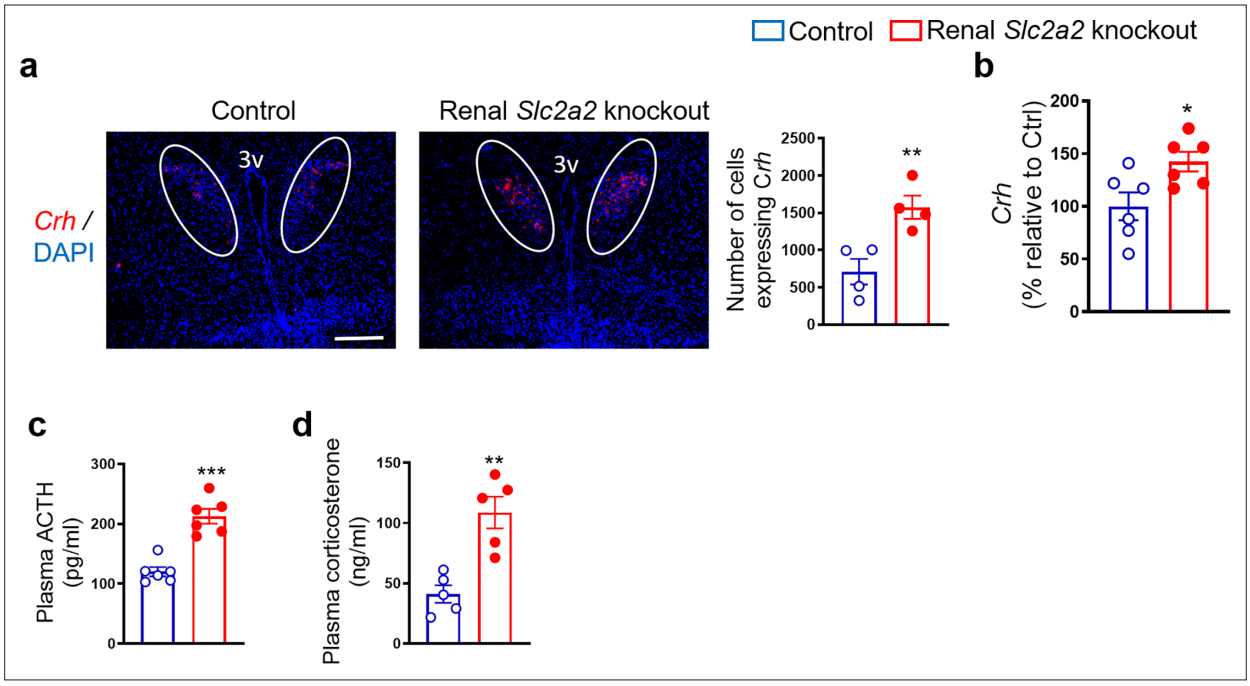

Figure 2. Enhanced activity of hypothalamic-pituitary-adrenal axis in renal *Slc2a2* knockout mice. Representative images from fluorescence RNA in situ hybridization showing an increase in expression of corticotropin-releasing hormone (Crh) in the paraventricular nucleus of the hypothalamus (which is identified here using a white oval shape) in 28 weeks old male renal *Slc2a2* knockout mice (**a**). Scale, 100 µm. For the quantification shown next to the images, four sections per mouse and three areas of interest per section were analyzed in four mice. qRT-PCR analysis showing an increase in hypothalamic *Crh* (**b**), data from ELISA demonstrating an increase in plasma adrenocorticotropic hormone (ACTH) (**c**) and corticosterone (**d**) in 12 weeks old male renal *Slc2a2* knockout mice 12 weeks after inducing the *Slc2a2* deficiency. *p<0.05, **p<0.01, ***p<0.001, unpaired two-tailed Student's t-test. Data are presented as mean ± SEM.

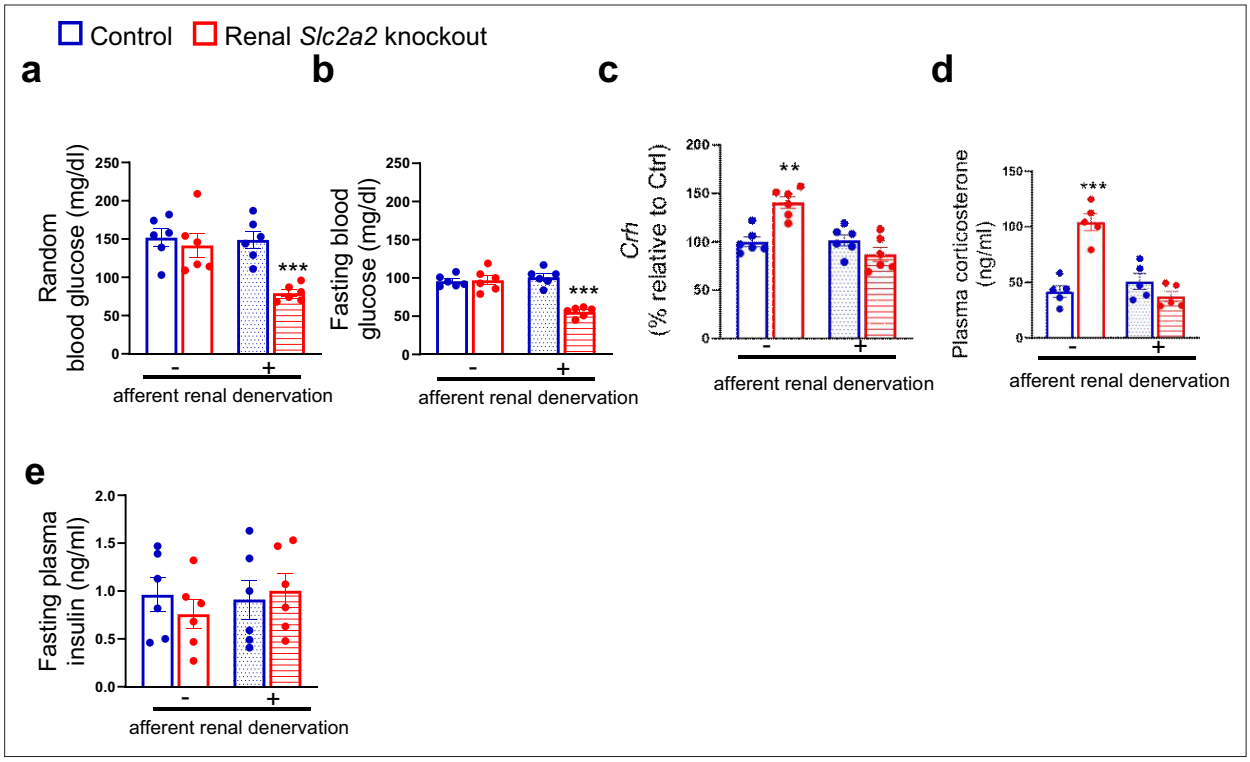

Figure 3. Effects of afferent renal denervation on blood glucose levels and hypothalamic-pituitary-adrenal axis in renal *Slc2a2* knockout female mice. Afferent renal denervation decreases fed (random) and fasting (overnight, 6:00 pm – 9:00 am) blood glucose levels (**a, b**), restores expression of hypothalamic corticotropin-releasing hormone (Crh) (**c**) measured using RT-qPCR, and plasma corticosterone (**d**) without affecting plasma insulin levels (**e**) in 30 weeks old female renal *Slc2a2* knockout mice 16 weeks after inducing the *Slc2a2* deficiency. **p0.01, ***p<0.001, two-way ANOVA followed by a Tukey's post hoc multiple comparison test. Data are presented as mean ± SEM.

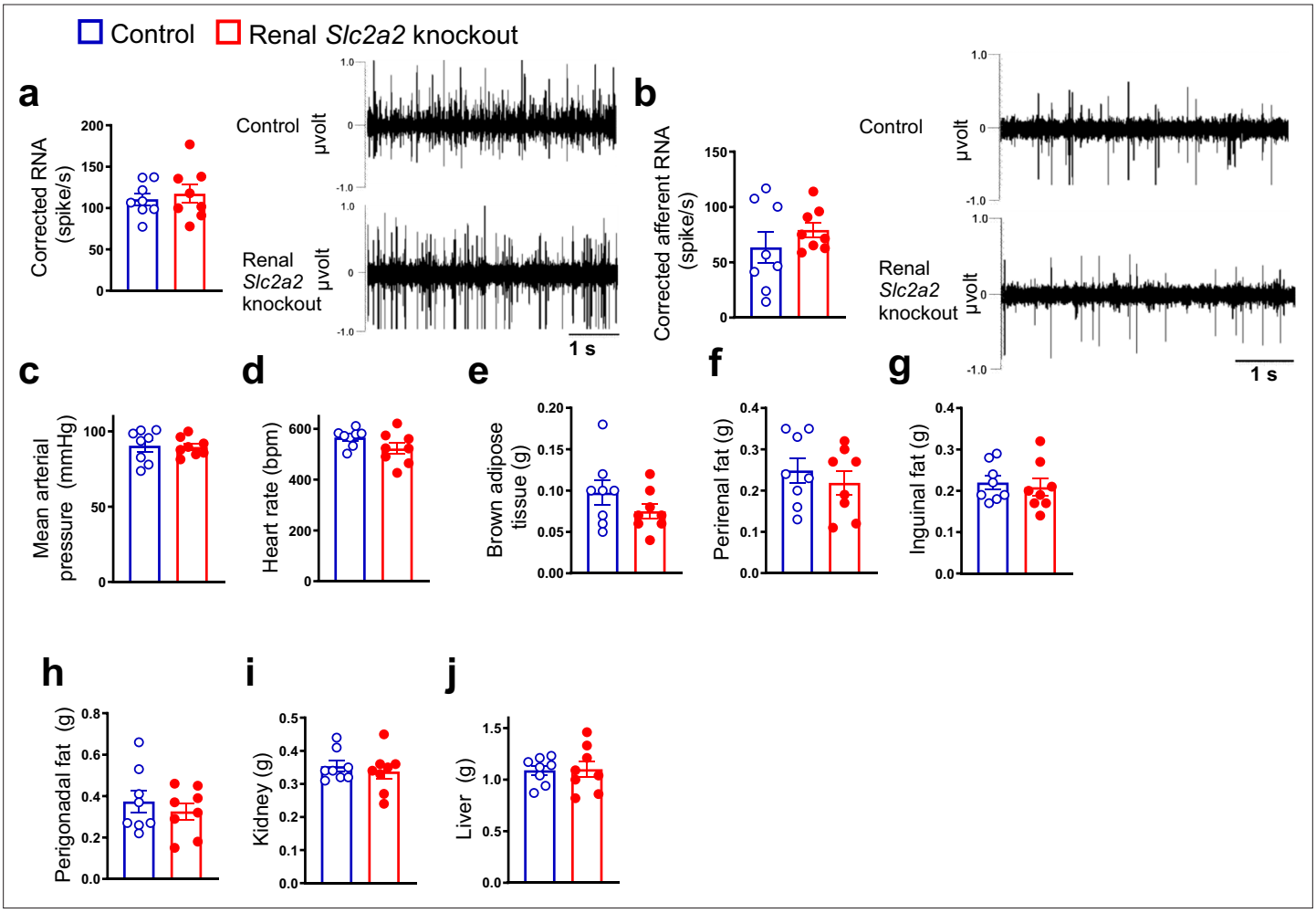

Figure 3—figure supplement 1. No change in renal nerve activities and tissue weights between renal Slc2a2 knockout mice and their littermate controls. Total and afferent renal nerve activity including the representative traces (**a, b**), mean arterial pressure and heart rate (**c, d**), weights of brown (**e**) and regional white (**f–h**) adipose tissues, kidney (**i**), and liver (**j**), in 30 weeks old female renal *Slc2a2* knockout and their littermate control mice 16 weeks after inducing the *Slc2a2* deficiency.

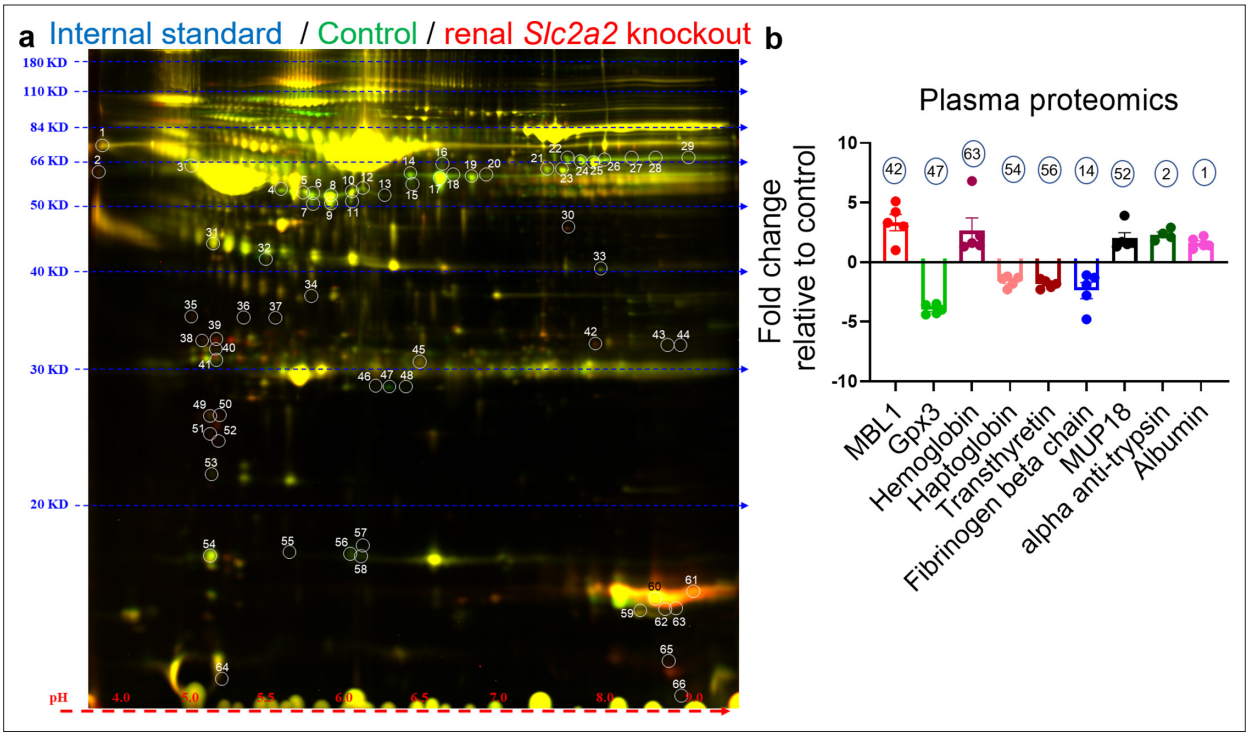

Figure 4. Changes in levels of plasma proteins in renal *Slc2a2* knockout male mice 12 weeks after inducing the *Slc2a2* deficiency. Representative image of two-dimensional difference gel electrophoresis with numbered protein spots of interest is shown in (**a**). Internal standard was prepared using equal amounts of protein of each plasma sample as a quality control (Cy2 labeled, pseudo blue), plasma proteins from control group were labeled using Cy3 dye (shown in pseudo green), and plasma proteins from renal *Slc2a2* knockout mice were labeled using Cy5 dye (shown in pseudo red). The identified proteins and their fold change in 28-week-old male renal *Slc2a2* knockout mice compared to the control group are shown in (**b**). The number on each bar graph in (**b**) represents the corresponding protein spot on the gel shown in (**a**). MBL1, mannose binding lectin 1; Gpx3, glutathione peroxidase 3; MUP18, major urinary protein 18.

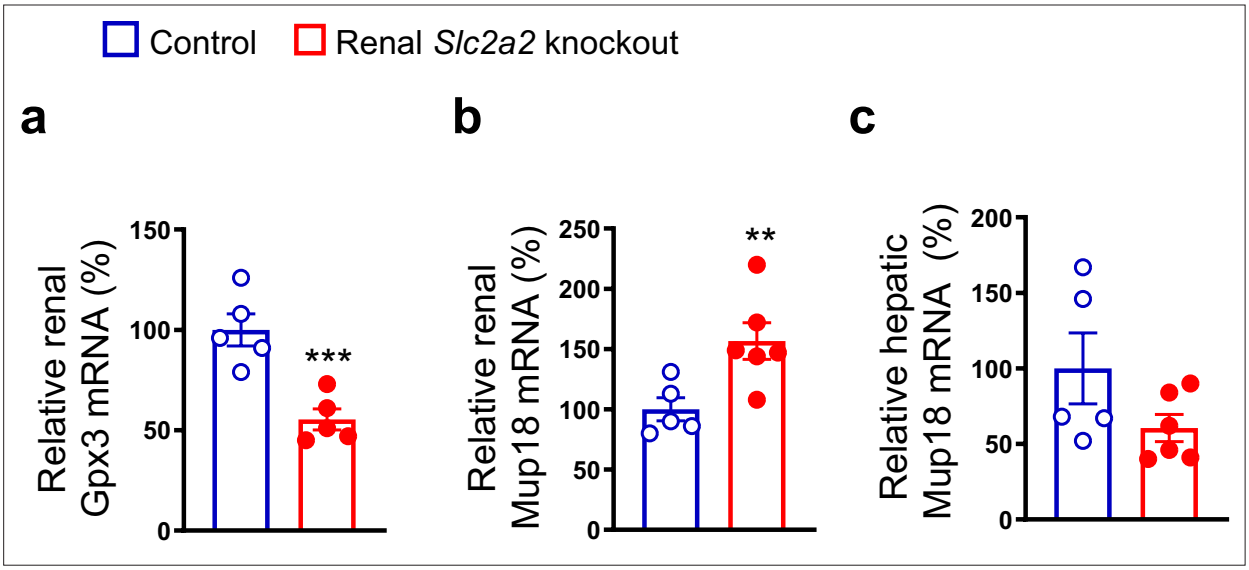

Figure 4—figure supplement 1. Gene expression analyses in renal Slc2a2 knockout mice. Renal *Slc2a2* knockout male mice (28 weeks old) have reduced expression of glutathione peroxidase 3 (Gpx3) (**a**) and major urinary protein 18 (Mup18) (**b**) in the kidneys without affecting hepatic Mup18 (**c**) measured by RT-qPCR 12 weeks after inducing the *Slc2a2* deficiency. **p<0.01, ***p<0.001, unpaired two-tailed Student's t-test. Data are presented as mean ± SEM.