Supplement Figure 1.

(A) Genotyping the tail genomic DNA. PCR of Slc6a8 and Cre recombinase in Slc6a8 -/y and control littermates: Agarose gel electrophoresis of PCR products from tail tissue. The PCR product sizes for the wild-type allele is 422 bp, for the *floxed* allele 548 bp, for the Cre recombinase was 350 bp, for the internal control 300 bp. **(B)** RT-PCR of Slc6a8 in Slc6a8 -/y and control littermate brain. β-actin was served as control.

Supplement Figure 2. *Knockout mice have less body fat and are more active.*

Whole body composition measured by Echo magnetic resonance imaging instrument in knockout mice and control littermates at baseline. (A) Body weight, (B) Percent adipose tissue, (C) Percent lean body mass of Slc6a8 knockout mice (Slc6a8 -/y, n=17) and control mice (Slc6a8 flox/y, n=15). Percentage of fat and lean body mass was calculated based on body weight. Data are expressed as means ± SE. *P ≤ 0.05. (D) Locomotor activity of dark and light cycles (12 hours each) at baseline in Slc6a8 knockout mice (Slc6a8 -/y, n=17) and control mice (Slc6a8 flox/y, n=15). Data are expressed as means ± SE. **P ≤ 0.01.

Supplement Figure 3. Representative ^{31}P NMR spectroscopy.

In this figure we show 3 spectra of live mouse brains. The bottom spectrum shows a knockout mouse fed creatine and the middle spectrum is from a knockout mouse fed placebo. Of significance is the profound peak near 0 ppm consistent with the peak position of phosphocreatine, which we attribute to cyclocreatine phosphate.

Supplement Figure 4. *Cyclocreatine was detected in hair of mice fed Cyclocreatine.*

Cyclocreatine content in hair in Slc6a8 brain specific knockout mice (Slc6a8 $^{-/y}$, cCr; n = 7, Cr; n = 5, P; n = 5) and control littermates (Slc6a8 $^{flox/y}$, n = 5 in each group) after 9 weeks of treatment were measured by biochemical assays. Data are expressed as means ± SE. **P ≤ 0.01. cCr = cyclocreatine, Cr = creatine, P = placebo.

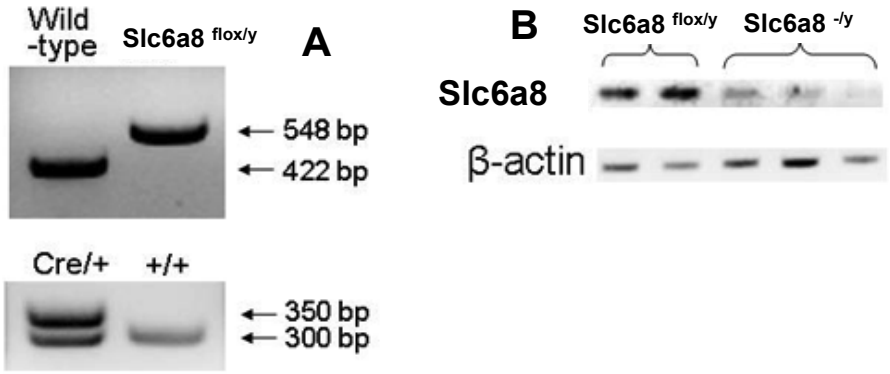
Supplement Figure 5. *Spatial working and reference memory is impaired in knockout mice*.

(A) Spatial working memory error in original baited arms, (B) Spatial working memory error in reversed baited arms, (C) Spatial reference memory error in original baited arms, (D) Spatial reference memory error in reversed baited arms in the radial arm maze. Slc6a8 knockout mice (Slc6a8 -/y, n=17) and control mice (Slc6a8 flox/y, n=15) at 6 months of age were placed in the radial arm maze for baseline measurement. The total number of entries into unbaited arms was tabulated as an index of reference memory error and the number of re-entries into previously baited arms was used as an index of working memory errors. Data are expressed as means ± SE. **P ≤ 0.01, ***P ≤ 0.001.

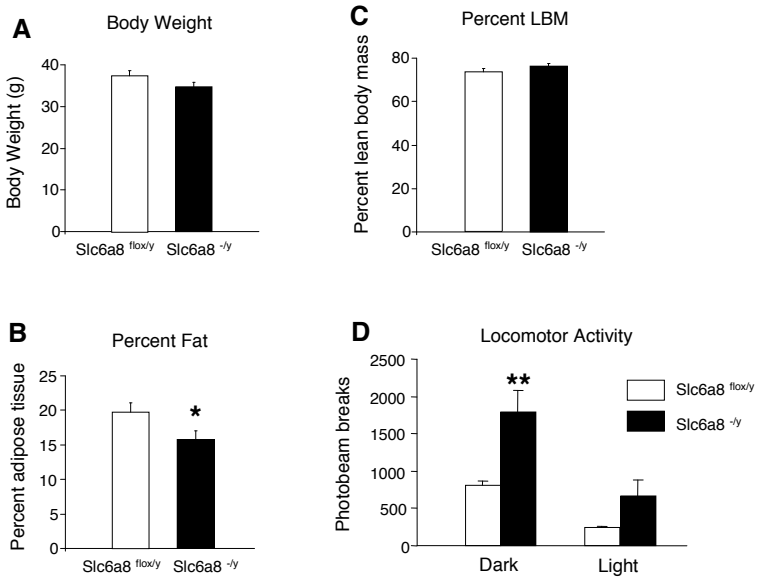
Supplement Figure 6. *Muscle and motor function in knockout mice.*

(A) Rotarod, (B) Hanging wire, (C) Beam walk in Slc6a8 knockout mice (Slc6a8 -/y, n=17) and control mice (Slc6a8 flox/y, n=15) at baseline. Data are expressed as means ± SE. The groups did not differ (P > 0.05).

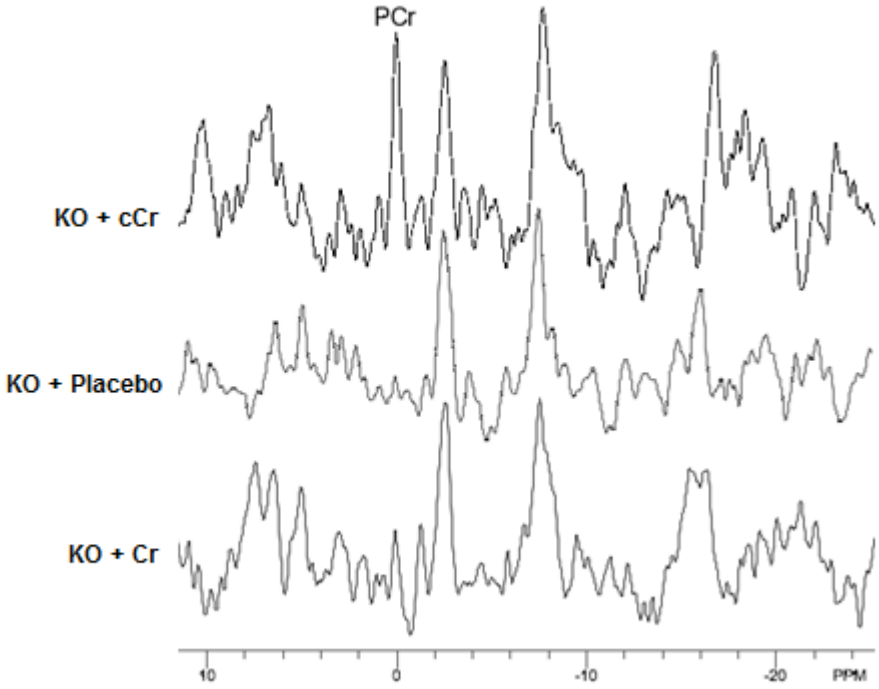
Supplemental Figure 1



Supplement Figure 2



Supplement Figure 3



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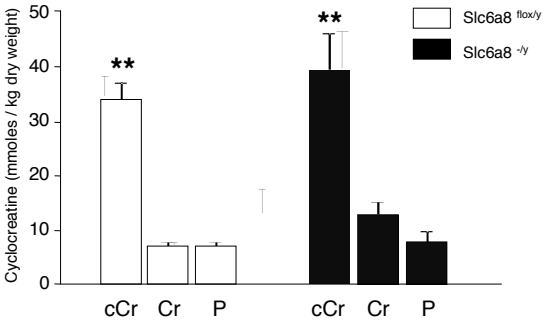
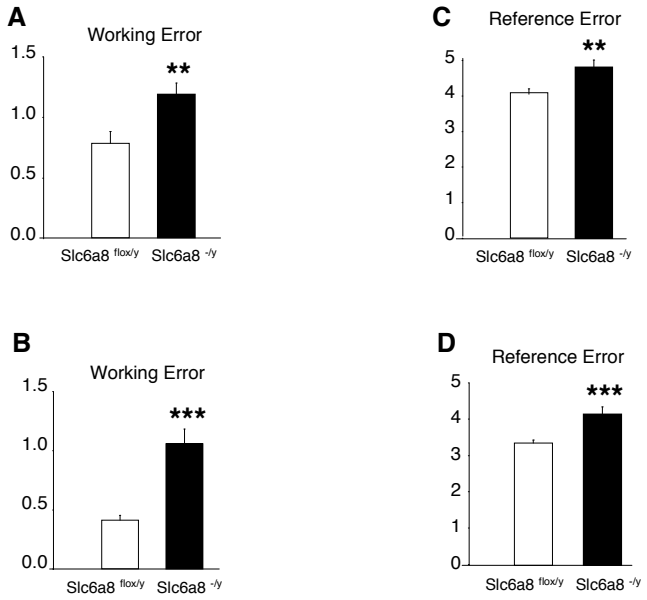


Supplement Figure 5.



Supplement Figure 6.