Supporting Information

A novel corrector for variants of SLC6A8: a therapeutic opportunity for Creatine Transporter Deficiency

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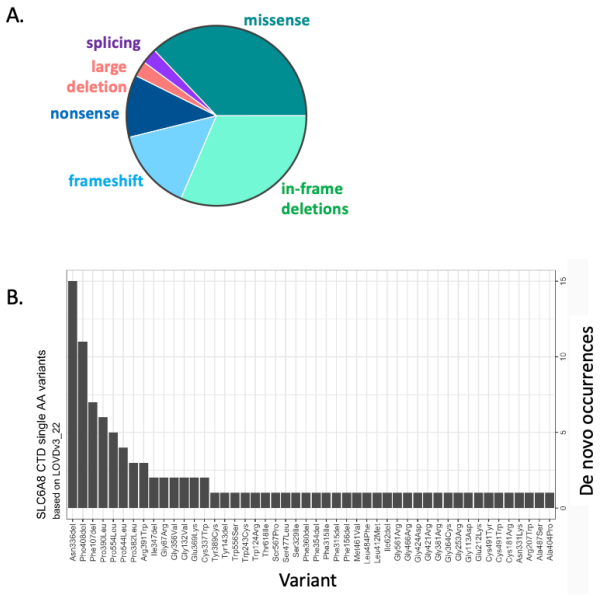

Figure S1. Pathogenic genetic aberrations reported in SLC6A8. (A) Genetic aberrations categorized by number of independent mutations reported in each category. (B) De novo occurrences of each single amino acid variant. All reported SLC6A8 variants were previously documented[10-13].

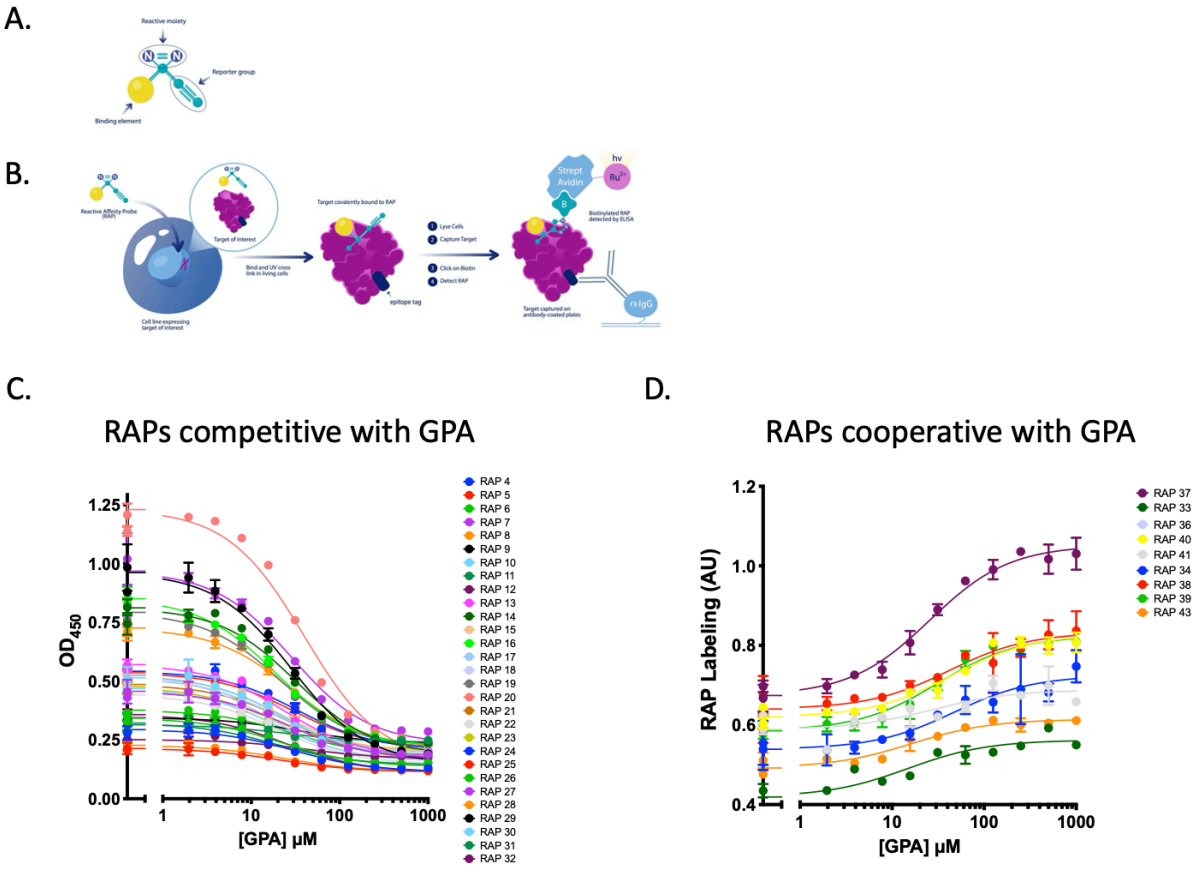

Figure S2. Validated GPA-competitive and cooperative RAPs that label SLC6A8. (A, B) RAPID schematic. (A) A reactive affinity probe (RAP) consists of a binding element, a reactive moiety and a reporter group. (B) A cell line expressing the epitope-tagged target of interest is incubated with a RAP that binds to the target of interest via its binding element. The RAP is covalently bound to the target following UV crosslinking of its reactive moiety. Cells are lysed and the target of interest is captured on antibody-coated plates via the epitope tag. Biotin (B) is conjugated to the RAP's reporter group in a click reaction and the biotinylated RAP was detected by an HRP-tagged streptavidin conjugate in a chemiluminescent reaction. In a RAPID high-throughput screen, cells expressing the target of interest are co-incubated with a RAP and a library of small molecules. Small molecules with affinity to the target of interest may compete or cooperate with RAP binding. (C) Validated RAPs that label SLC6A8 which is competitive with GPA. SLC6A8 labeling by 20 µM RAP (where each line is a different RAP) in the presence of indicated concentrations of GPA (values represent mean signal with n=2 technical replicates with standard deviation). (D) As described in (C) with the RAPs that label SLC6A8 cooperatively with GPA.

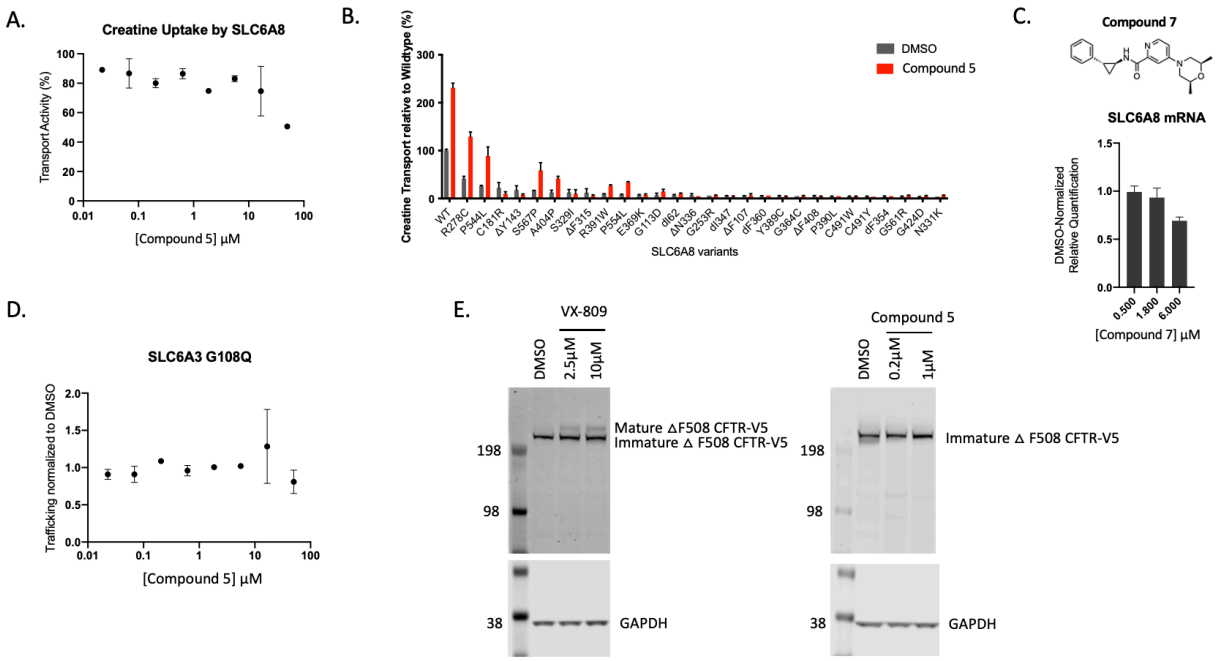

Figure S3. Characterization of the activity and selectivity of Compound 5. (A) Transport Inhibition Assay: 30 minute compound incubation followed by creatine uptake in U-2 OS cells overexpressing SLC6A8 variant P544L. (B) Transport Correction Assay measuring creatine transport of SLC6A8 variants following 24 hours treatment with DMSO or Compound 5 (values represent means normalized to wildtype SLC6A8 from n=2 technical replicates with standard deviation). (C) SLC6A8 mRNA was quantified with a closely related analog to Compound 5, Compound 7. (D) Trafficking Assay where surface localization of SLC6A3 G108Q was measured following 24 hour treatment with Compound 5. (E) Immunoblot of the mature glycoform and immature ΔF508 mutant CFTR glycoform transiently expressed in Flp-In 293 cells detected by the V5 tag following 24 hours of treatment with VX-809 or Compound 5.

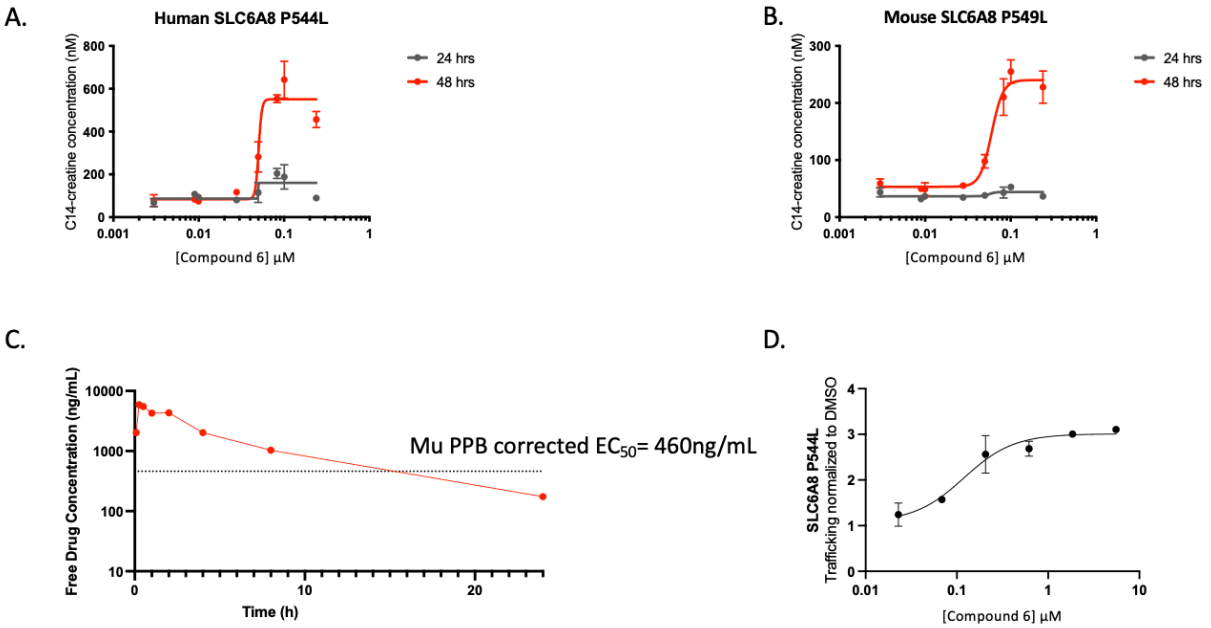

Figure S4. in vitro characterization of Compound 6 as a corrector for SLC6A8 P544L. (A) Radiometric creatine transport correction assay in SLC6A8 knock-out U-2 OS cells reconstituted with human SLC6A8 P544L following 24 and 48 hours of treatment with Compound 6 (values represent means normalized to DMSO from n=2 technical replicates with standard deviation). (B) As described in (A) with mouse SLC6A8 P549L. (C) Compound 6 plasma pharmacokinetics. Animals were dosed at 30mpk in a single oral dose by gavage and values represent means from N=3 mice at each timepoint indicated. (D) Trafficking assay in stable SLC6A8 P544L U-2 OS MEM-EA cell line (values represent means normalized to DMSO from n=2 technical replicates with standard deviation).

Figure S5. Genetic confirmation of SLC6A8 P549L knock-in mice. (A) Sanger sequencing trace from genomic DNA in P549L knock-in mouse.