SUPPLEMENTAL MATERIAL

Treatment of Autosomal Dominant Hypocalcemia Type 1 with the Calcilytic NPSP795 (SHP635)

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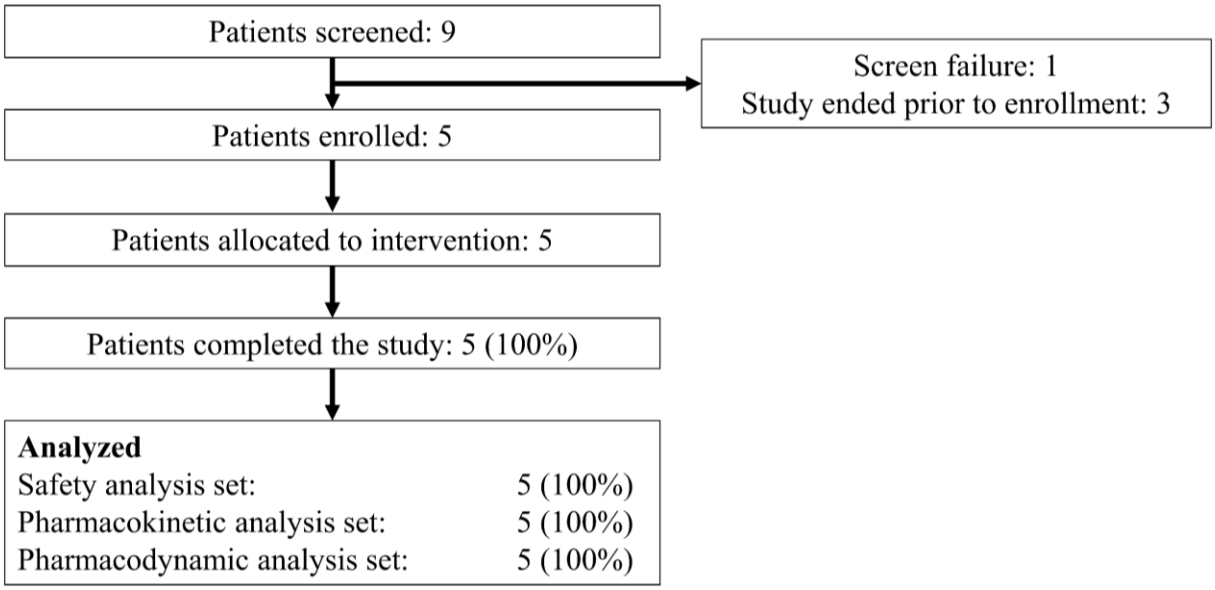
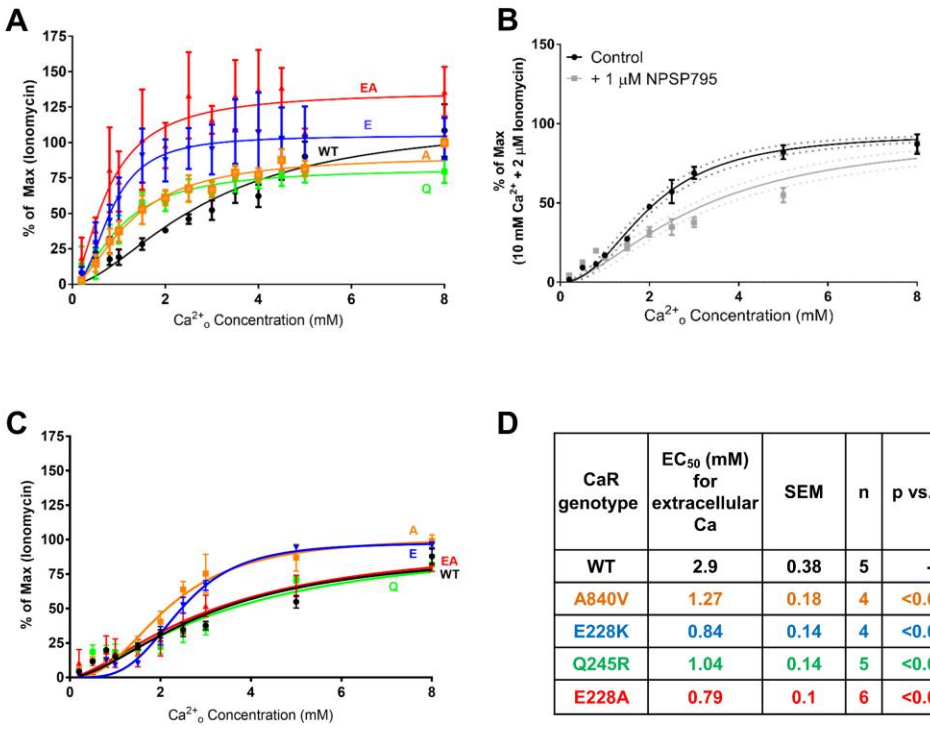
Supplemental Figures

Supplemental Figure 1



Supplemental Figure 1. Consolidated Standards of Reporting Trials (CONSORT) diagram. A total of 9 subjects were screened for the study. Only 5 subjects were enrolled in the study, as 1 subject did not meet the study entry criteria and the study was closed after 5 subjects had been enrolled. Of the 5 subjects, all completed the study as per the protocol. All subjects received the study drug and were included in the safety analysis set, pharmacokinetics (PK) analysis set and pharmacodynamics (PD) analysis set.

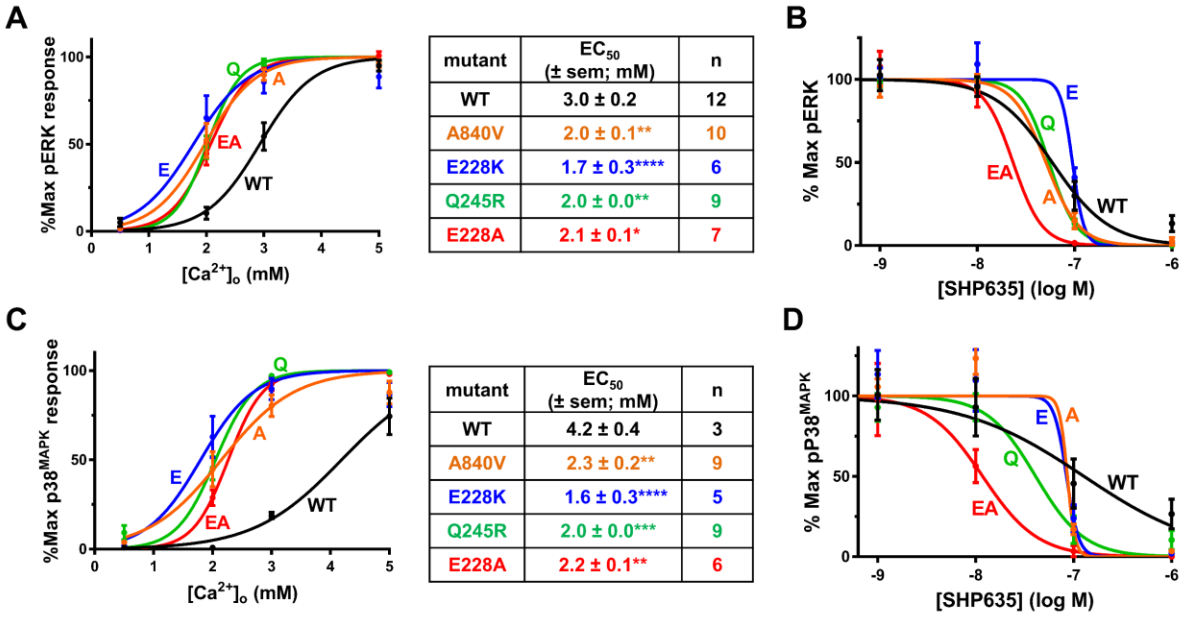
Supplemental Figure 2



CaR genotype	EC_{50} (mM) for extracellular Ca	SEM	n	p vs. WT
WT	2.9	0.38	5	-
A840V	1.27	0.18	4	<0.001
E228K	0.84	0.14	4	<0.001
Q245R	1.04	0.14	5	<0.001
E228A	0.79	0.1	6	<0.001

Supplemental Figure 2. Half-maximal activation (EC_{50}) for extracellular calcium in an *in vitro* model of wild type (WT) and mutant calcium sensing receptors (CaRs) and response to NPSP795. All mutant CaRs had an EC_{50} at lower concentrations of extracellular calcium compared to WT CaR (A). Treatment with NPSP795 increased the EC_{50} for all CaR mutants. A representative concentration-response curve for the A840V mutation (C) shows an increase in the EC_{50} towards that of the WT CaR (B) with NPSP795 treatment. n = number of experiments

Supplemental Figure 3

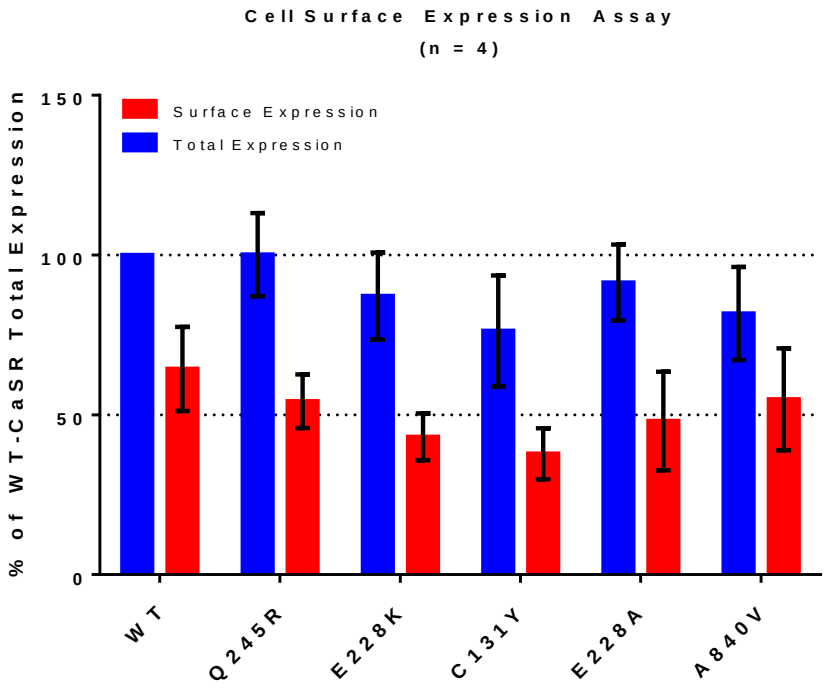


mutant	EC_{50} (± sem; mM)	n
WT	3.0 ± 0.2	12
A840V	2.0 ± 0.1**	10
E228K	1.7 ± 0.3****	6
Q245R	2.0 ± 0.0**	9
E228A	2.1 ± 0.1*	7

mutant	EC_{50} (± sem; mM)	n
WT	4.2 ± 0.4	3
A840V	2.3 ± 0.2**	9
E228K	1.6 ± 0.3****	5
Q245R	2.0 ± 0.0***	9
E228A	2.2 ± 0.1**	6

Supplemental Figure 3. Effects of NPSP795 (SHP635) on extracellular calcium-induced ERK and p38^{MAPK} phosphorylation in an *in vitro* model of wild type (WT) and mutant calcium-sensing receptors (CaRs). Extracellular calcium-induced ERK phosphorylation was enhanced in CaR mutants (A). All mutant CaRs had an EC_{50} at lower concentrations of extracellular calcium compared to WT CaR. NPSP795 treatment inhibited the enhanced ERK phosphorylation present in mutant CaRs (B). Extracellular calcium-induced p38^{MAPK} phosphorylation was enhanced in CaR mutants (C). All mutant CaRs had an EC_{50} at lower concentrations of extracellular calcium compared to WT CaR. NPSP795 treatment inhibited the enhanced p38^{MAPK} phosphorylation present in mutant CaRs (D). Statistical analysis was performed using one-way ANOVA and Dunnett's post-test between log EC_{50} of mutant CaRs vs. WT. n = number of experiments. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Supplemental Figure 4



Supplemental Figure 4. Effect of ADH mutations on CaSR total and surface expression. HEK293 cells were transiently transfected with FLAG-tagged wt or mutant construct before being fixed and probed with anti-FLAG antibody to measure total (blue) or cell surface expression (red). Results were obtained in Flp-In293 cells transfected with vector alone were used for background subtraction and mutants were normalised to the level of total wt CaR expression. In the transient overexpression system, there are no significant differences between wt CaR and the 5 ADH1 mutants at either the total or surface expression level (2-way ANOVA, comparison between rows, $p > 0.05$). Data shown as mean ± SEM.

Supplemental Tables

Supplemental Table 1. Primers used for generation of ADH mutations.

Mutation	Nucleotide change	Direction	Sequence
Q245R	734A>G	Fwd	GAA CTC ATC TCC CGG TAC TCT GAT GAG G
		Rev	CCT CAT CAG AGT ACC GGG AGA TGA GTT C
E228K	682G>A	Fwd	GAT TGA GAA ATT CCG AAA GGA AGC TGA GGA AAG
		Rev	CTT TCC TCA GCT TCC TTT CGG AAT TTC TCA ATC
A840V	2519C>T	Fwd	GTA GAG GTG ATT GTC ATC CTG GCA GCC
		Rev	GGC TGC CAG GAT GAC AAT CAC CTC TAC
E228A	683A>C	Fwd	GAT TGA GAA ATT CCG AGC GGA AGC TGA GGA AAG G
		Rev	CCT TTC CTC AGC TTC CGC TCG GAA TTT CTC AAT C

Supplemental Table 2. Elemental calcium administered to subjects as supplement/rescue (mg)

Patient #	1	2	3	4	5
CaR Mutation	A840V	Q245R	E228K	A840V	E228A
Supplement					
Day 1			1200	250	
Day 2		1200	1200	250	600
Day 3	600	1200	1200	250	600
Day 4	1200	1200	1200	250	600
Rescue					
Day 1					
Day 2		600	1800		
Day 3					
Day 4					