# Humanizing the worm: Building PreciseProxies in Epilepsy Genes

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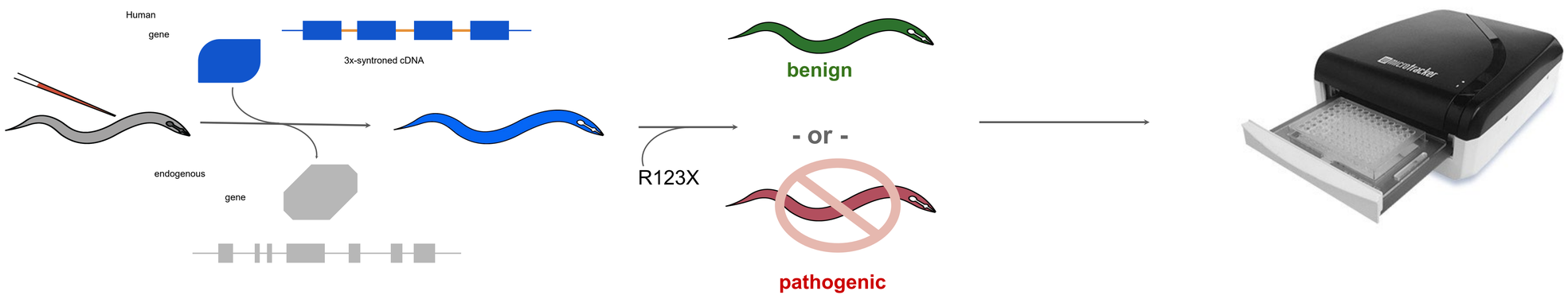
## Synopsis:

Genetic diagnostics of disease have opened the door into a new era of precision medicine. Geneticists are now engaged in trying to understand huge natural genetic variation through the functional analysis of variants. When a variant is identified as pathogenic in a disease state, clinicians and patients are next faced with making care decisions. To address these needs, we are undertaking experimental partnerships with clinicians to model human diseases and disease associated genes in *C. elegans*. First, we create a HumanSwap worm in which we replace a homolog gene with human cDNA and perform phenotypic profiling to ensure the gene is functioning in the worm. Next, we make PreciseProxy worms using CRISPR to introduce point mutations that precisely model clinical variants of interest. We can then use these PreciseProxy worms to screen for the efficacy of available drugs on pathogenic patient variants. Taken together, this platform gives the in vivo experimental evidence needed for predictive personalized medicine.

For 3 epilepsy genes tested, rescue of function was observed with the humanized configurations. PreciseProxy variants have been created to test for pathogenicity and molecular function. Next, we will perform screening on these worms with known drugs to correlate responses with patient populations.

***The ClinPhen Platform***

***HumanSwap*** ***PreciseProxy*** ***Drug Screening***




## 1. STXBP1

**Rescue of function obtained by insertion of STXBP1 into the native *unc-18* locus**

**Phenotypic profiling is used to detect pathogenic variants of STXBP1**

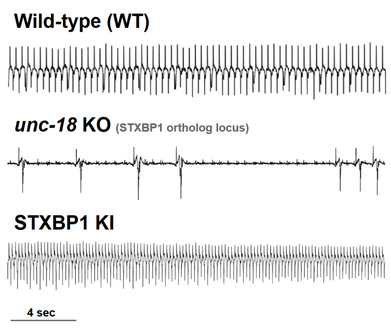
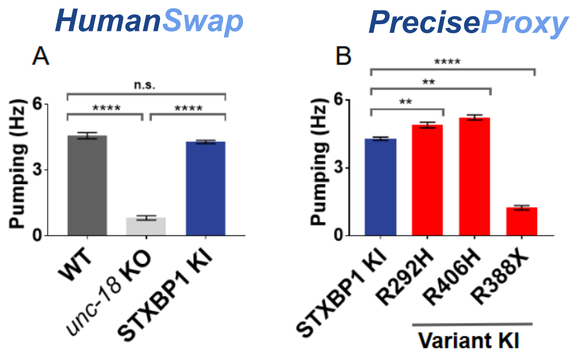



**Figure 1.** EPG traces obtained from the ScreenChip platform for wild-type (WT), *unc-18* knock out (KO) and STXBP1 knock in (KI) individuals

**EPG**




**Movement**

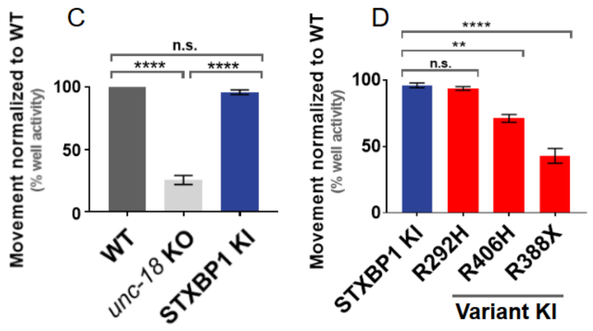
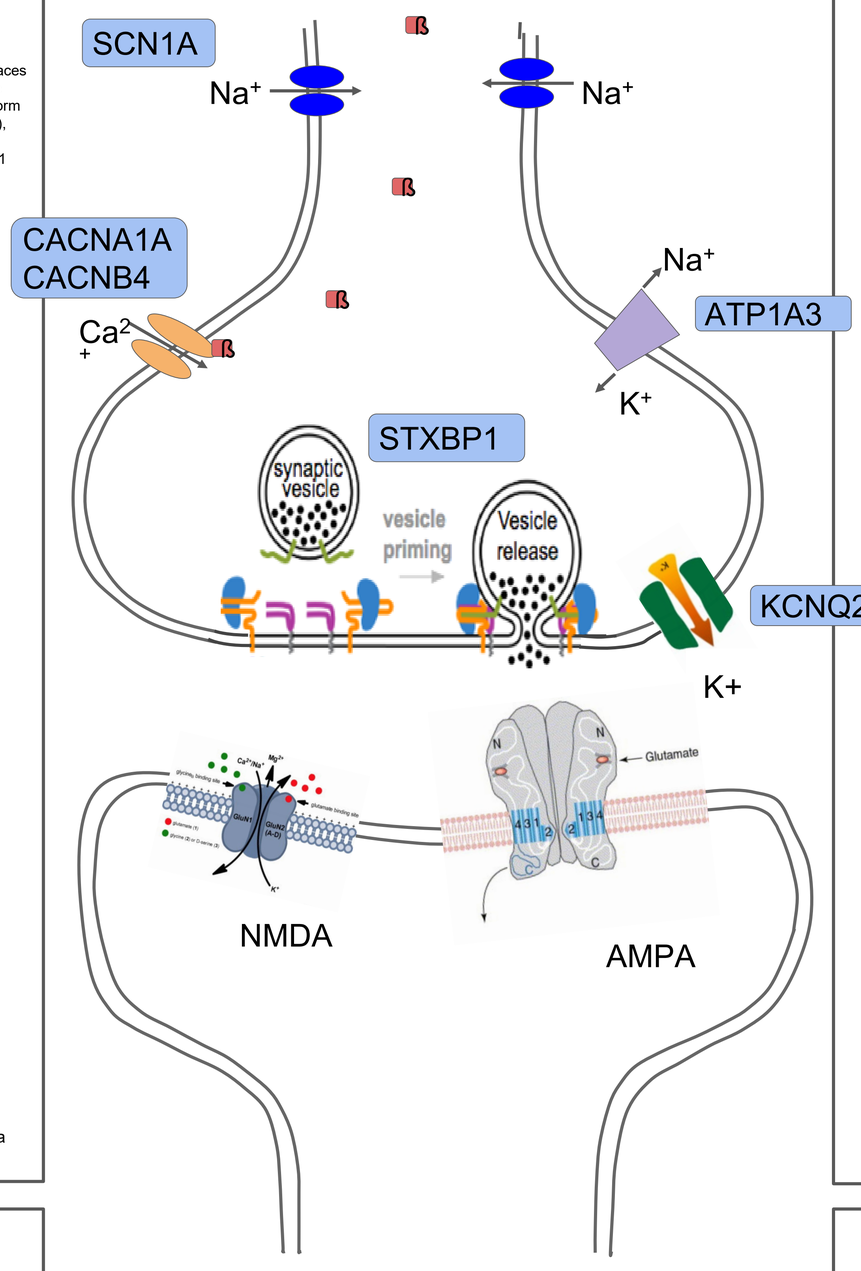



**Figure 2.** A) Comparison of pumping frequency between WT, *unc-18* KO and STXBP1 KI animals. B) Comparison of STXBP1 and STXBP1 genes inserted containing pathogenic variants (red). C) and D) WMicrotracker-based movement assay measuring thrashing in liquid for *unc-18* KO, STXBP1 and pathogenic STXBP1 KI individuals. Data are means ± 1 S.E.M. (**p<0.01, ****p<0.0001, n.s. not significant)

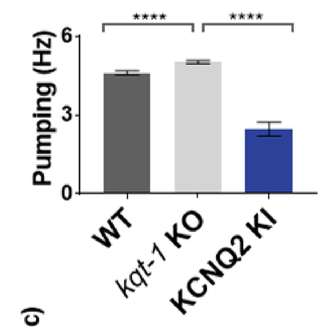



## 3. KCNQ2

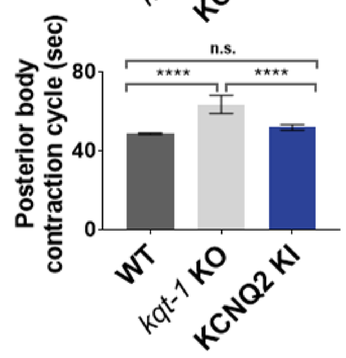
***HumanSwap***

**Multiple assays demonstrate that insertion of human KCNQ2 reverses the effects of *kqt-1* knock out**

**EPG**




**Contractions**




**Size**

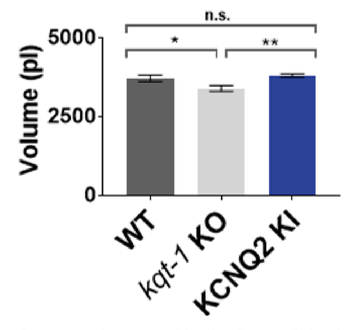



**Figure 4.** Upper: comparison of pumping frequency (measured by the ScreenChip platform) for WT, *ccb-1* KO and KCNQ2 KI individuals. Middle: number of posterior body contractions measured per second for a subset (n=10) of animals. Not all cycles resulted in a defecation event. Lower: worm volume measured using an automated algorithm, NemaSize. Data are means ± 1 S.E.M. (**p<0.01, **p<0.01 ****p<0.0001, n.s. not significant)

## 2. CACNB4

***HumanSwap*: Insertion of CACNB4 reverses lethality induced by *ccb-1* knock out**

***PreciseProxy*: pathogenic and benign variants can be functionally assessed**

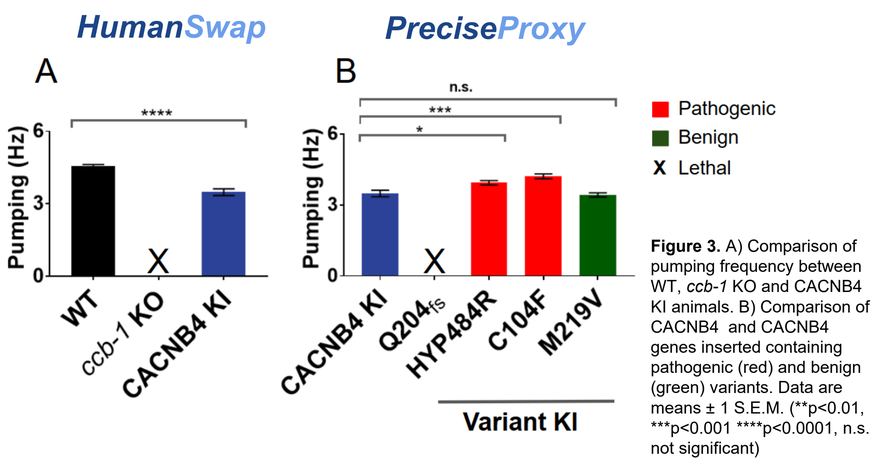



**Figure 3.** A) Comparison of pumping frequency between WT, *ccb-1* KO and CACNB4 KI animals. B) Comparison of CACNB4 and CACNB4 genes inserted containing pathogenic (red) and benign (green) variants. Data are means ± 1 S.E.M. (**p<0.01, ***p<0.001 ****p<0.0001, n.s. not significant)

# Future Partners Wanted for Epilepsy Genes:

**Achieved**

- *STXBP1*
- *KCNQ2*
- *CACNB4*

**Planned**

- *ATP1A3, SCN1A, CACNA1A, CDKL5, MAPT, TARDBP, GRN, PSEN1, APP, LMNA, POLG, DMN1, CHRNA4, GNAO1, PRICKLE2, SLC2A1*

## Methods

***HumanSwap* Methods**: Sequence optimized cDNA inserted at the homologous gene's start codon using CRISPR/Cas9. All endogenous coding is removed and human transgene uses *C. elegans* UTRs.

***PreciseProxy* Methods:** CRISPR/Cas9 is used to insert human clinical variant sequence changes in HumanSwap worms.

**Phenotyping Methods**: Electrophysiological measurements are taken with microfluidic chips. Movement is quantified with IR beam tracker. Defecation periodicity is assessed with video analysis. Size is determined using an automated algorithm.

**Conclusions**: Data suggest human coding sequences function in worm signalling pathways. Clinical variants can be assessed functionally in this model organism.