

## **Supplementary File**

### **The $\beta$ -secretase substrate Seizure 6-like protein (SEZ6L) controls motor functions in mice**

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Supplementary Table 1 is provided as a separate Microsoft Excel file

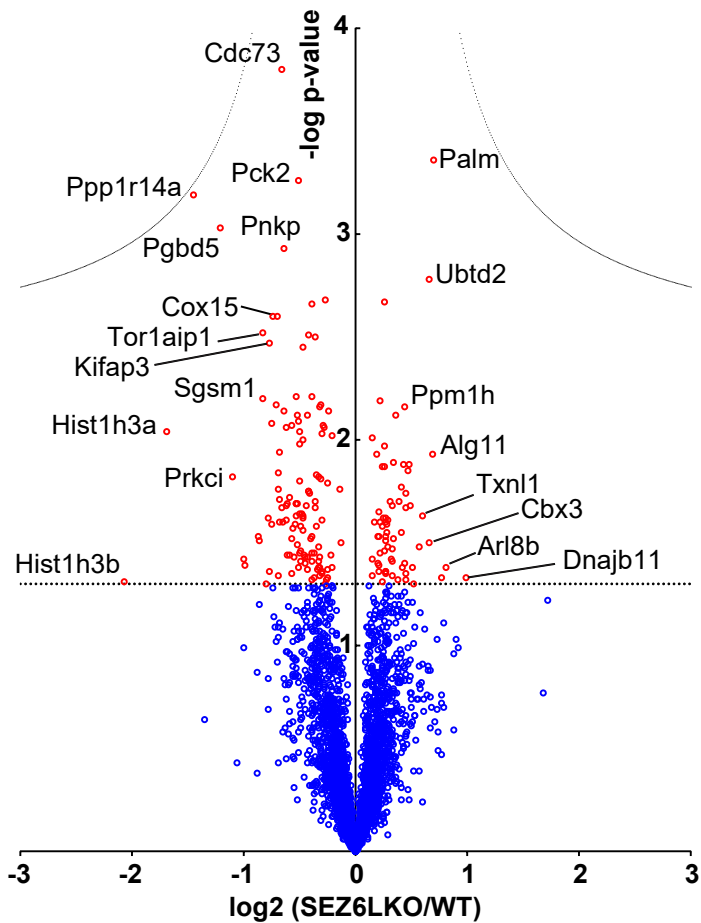
**Supplementary Table 2: DigiGait indices analysed with 2-way ANOVA with genotype and sex as factors to identify parameter changes that may be sex-specific.** 15cm/s: WT n = 24 (9 male, 15 female), SEZ6L het n = 29 (14 male, 15 female) and SEZ6L KO n = 23 (15 male, 8 female). 25cm/s: WT n = 19 (6 male, 13 female), SEZ6L het n = 28 (13 male, 15 female) and SEZ6L KO n = 20 (13 male, 7 female). n.s. = not significant (p>0.05).

<b>DigiGait indices</b>	<b>Treadmill speed</b>	<b>Examination of forelimb/paw or hindlimb/paw</b>	<b>Effect of genotype: p-value</b>	<b>Effect of sex: p-value</b>	<b>Interaction: p-value</b>
Stance width (cm)	15 cm/s	Between forelimbs	n.s.	n.s.	n.s.
		Between hindlimbs	n.s.	n.s.	n.s.
	25 cm/s	Between forelimbs	n.s.	n.s.	n.s.
		Between hindlimbs	n.s.	n.s.	n.s.
Stride length (cm)	15 cm/s	Forelimb average	n.s.	n.s.	n.s.
		Hindlimb average	n.s. (0.0509)	n.s.	n.s.
	25 cm/s	Forelimb average	0.0133	n.s.	n.s.
		Hindlimb average	0.0061	n.s.	n.s.
Stride frequency (steps/s)	15 cm/s	Forelimb average	n.s.	n.s.	n.s.
		Hindlimb average	0.0200	n.s.	n.s.
	25 cm/s	Forelimb average	0.0197	n.s.	n.s.
		Hindlimb average	0.0059	n.s.	n.s.
Stride time (s)	15 cm/s	Forelimb average	n.s.	n.s.	n.s.
		Hindlimb average	0.0085	n.s.	n.s.
	25 cm/s	Forelimb average	0.0129	n.s.	n.s.
		Hindlimb average	0.0041	n.s.	n.s.
Stance duration (s)	15 cm/s	Forelimb average	0.0356	n.s.	n.s.
		Hindlimb average	0.0324	n.s.	n.s.
	25 cm/s	Forelimb average	0.0040	n.s.	n.s.
		Hindlimb average	0.0319	0.0198	n.s.
Swing duration (s)	15 cm/s	Forelimb average	n.s.	n.s.	n.s.
		Hindlimb average	0.0047	n.s.	n.s.
	25 cm/s	Forelimb average	n.s.	n.s.	n.s.
		Hindlimb average	0.0092	n.s.	n.s.
Propulsion phase (s)	15 cm/s	Forelimb average	n.s.	n.s.	n.s.
		Hindlimb average	n.s.	n.s.	n.s.
	25 cm/s	Forelimb average	n.s.	0.0054	n.s.
		Hindlimb average	n.s.	0.0017	n.s.
Brake duration (s)	15 cm/s	Forelimb average	0.0299	n.s.	n.s.
		Hindlimb average	0.0243	n.s.	n.s.
	25 cm/s	Forelimb average	n.s.	n.s.	n.s.
		Hindlimb average	n.s.	n.s.	n.s.

**Supplementary Table 3: SEZ6L KO mice exhibit gait differences as assessed by treadmill walking at 15cm/s.** p-values generated from 1-way ANOVA with male and female data pooled unless otherwise indicated. Tukey's multiple comparisons test significant differences between WT vs. SEZ6L KO indicated with an asterisk (\*), SEZ6L het vs. SEZ6L KO indicated with a hash (#), and WT vs. SEZ6L het with a \$. \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$  (or equivalent symbol). Data presented as mean  $\pm$  SEM. WT n = 24 (9 male, 15 female), SEZ6L het n = 29 (14 male, 15 female) and SEZ6L KO n = 24 (15 male, 9 female). n.s. = not significant ( $p > 0.05$ ).

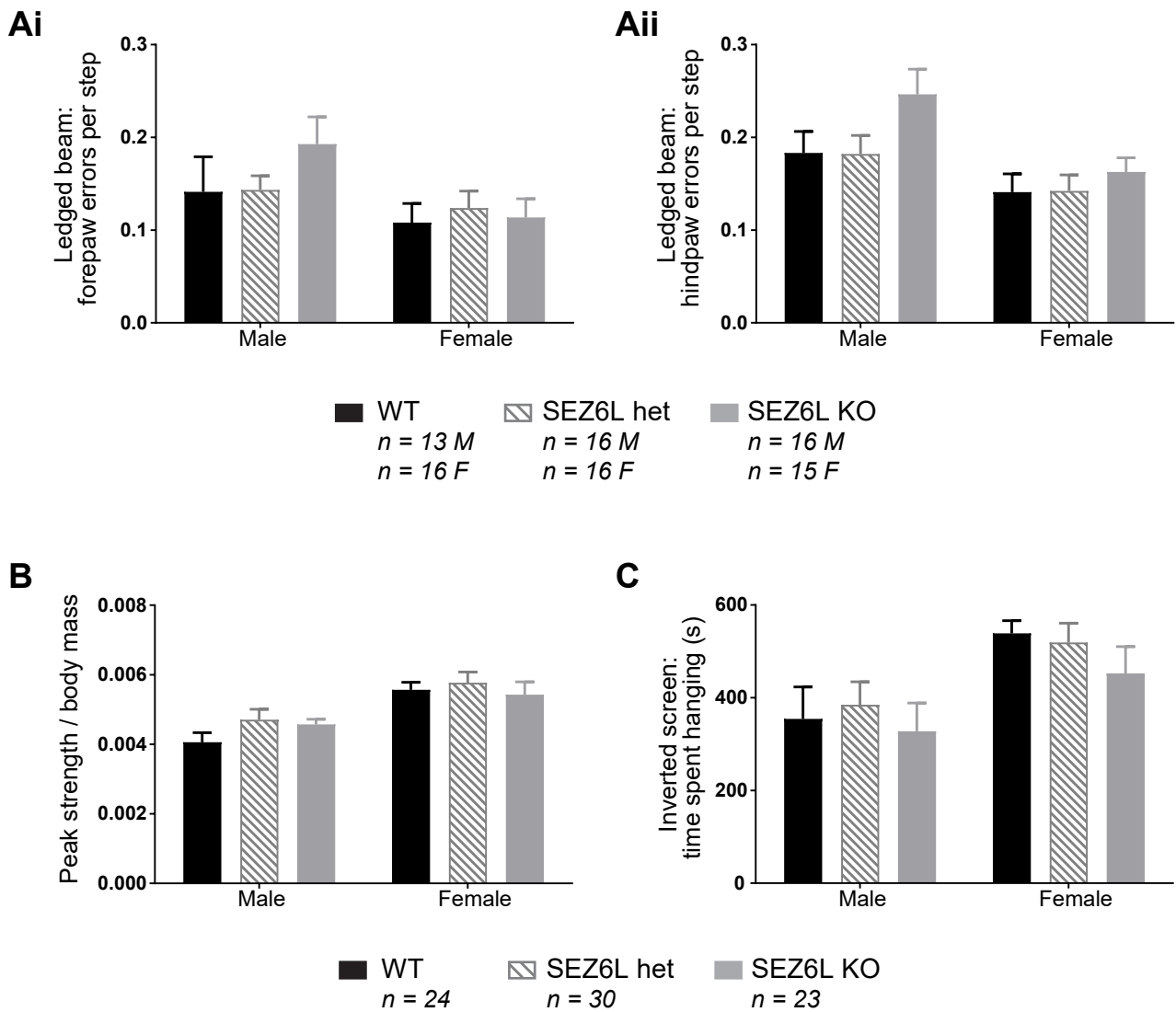
DigiGait indices	Treadmill speed	Examination of forelimb/paw or hindlimb/paw	p-value	WT	SEZ6L het	SEZ6L KO
Stance width (cm)	15 cm/s	Between forelimbs	n.s.	1.713 $\pm$ 0.045	1.734 $\pm$ 0.032	1.733 $\pm$ 0.069
		Between hindlimbs	n.s.	3.208 $\pm$ 0.061	3.255 $\pm$ 0.048	3.075 $\pm$ 0.056
Stride length (cm)	15 cm/s	Forelimb average	n.s.	5.13 $\pm$ 0.212	5.07 $\pm$ 0.072	5.43 $\pm$ 0.166
		Hindlimb average	0.0404	5.14 $\pm$ 0.177	5.08 $\pm$ 0.086	5.55 $\pm$ 0.153 #
Stride frequency (steps/s)	15 cm/s	Forelimb average	n.s.	3.11 $\pm$ 0.080	3.02 $\pm$ 0.044	2.87 $\pm$ 0.091
		Hindlimb average	0.0109	3.10 $\pm$ 0.073	3.04 $\pm$ 0.052	2.81 $\pm$ 0.080 * and #
Stride time (s)	15 cm/s	Forelimb average	0.0399	0.332 $\pm$ 0.008	0.339 $\pm$ 0.005	0.361 $\pm$ 0.011 *
		Hindlimb average	0.0044	0.333 $\pm$ 0.008	0.339 $\pm$ 0.006	0.370 $\pm$ 0.010 ** and #
Stance duration (s)	15 cm/s	Forelimb average	0.0188	0.221 $\pm$ 0.006	0.228 $\pm$ 0.003	0.244 $\pm$ 0.007 *
		Hindlimb average	0.0160	0.241 $\pm$ 0.006	0.246 $\pm$ 0.004	0.263 $\pm$ 0.006 *
Swing duration (s)	15 cm/s	Forelimb average	n.s.	0.111 $\pm$ 0.004	0.110 $\pm$ 0.003	0.118 $\pm$ 0.004
		Hindlimb average	0.0036	0.092 $\pm$ 0.003	0.093 $\pm$ 0.003	0.107 $\pm$ 0.004 ** and ##
Propulsion phase (s)	15 cm/s	Forelimb average	n.s.	0.151 $\pm$ 0.005	0.149 $\pm$ 0.003	0.157 $\pm$ 0.007
		Hindlimb average	n.s.	0.192 $\pm$ 0.006	0.182 $\pm$ 0.005	0.201 $\pm$ 0.008
Brake duration (s)	15 cm/s	Forelimb average	0.0385	0.071 $\pm$ 0.003	0.0795 $\pm$ 0.003	0.087 $\pm$ 0.006 *
		Hindlimb average	0.0250	0.049 $\pm$ 0.002	0.063 $\pm$ 0.004 \$	0.061 $\pm$ 0.005

## P21 cerebellar proteome (n=7)



### Supplementary Fig. 1

SEZ6L KO cerebellar proteome of the young mice. SEZ6L KO and WT cerebella at postnatal day 21 (P21) with seven biological replicates. The mean protein log<sub>2</sub>-transformed fold changes of each protein is plotted against the negative log<sub>10</sub>-transformed p-value. Proteins with a t-test p-value < 0.05 are shown as red circles, while the proteins with the p-value > 0.05 are shown as blue circles. Hyperbolic curve represents permutation-based FDR correction (FDR < 0.05, s<sub>0</sub> = 0.1). The straight dotted line that crosses the y-axis at the 1.3 represents the p-value of 0.05.



### Supplementary Fig. 2

SEZ6L KO mice exhibit normal locomotor precision and muscular strength. WT, SEZ6L het and SEZ6L KO mice behave similarly in the ledged beam task (Ai, ii: 1-way ANOVA within sex), forelimb grip strength test (B: 1-way ANOVA within sex) and the four limb hanging inverted screen test (C: 1-way ANOVA and Kruskal-Wallis test within sex). Data shown as mean  $\pm$  SEM. Mice tested on the ledged beam: WT n = 29 (13 male, 16 female), 32 SEZ6L hets (16 male, 16 female) and 31 SEZ6L KOs (16 male, 15 female). Mice tested on grip strength / inverted screen test: 24 WTs (9 male, 15 female), 30 SEZ6L hets (15 male, 15 female), 23 SEZ6L KOs (15 male, 8 female)