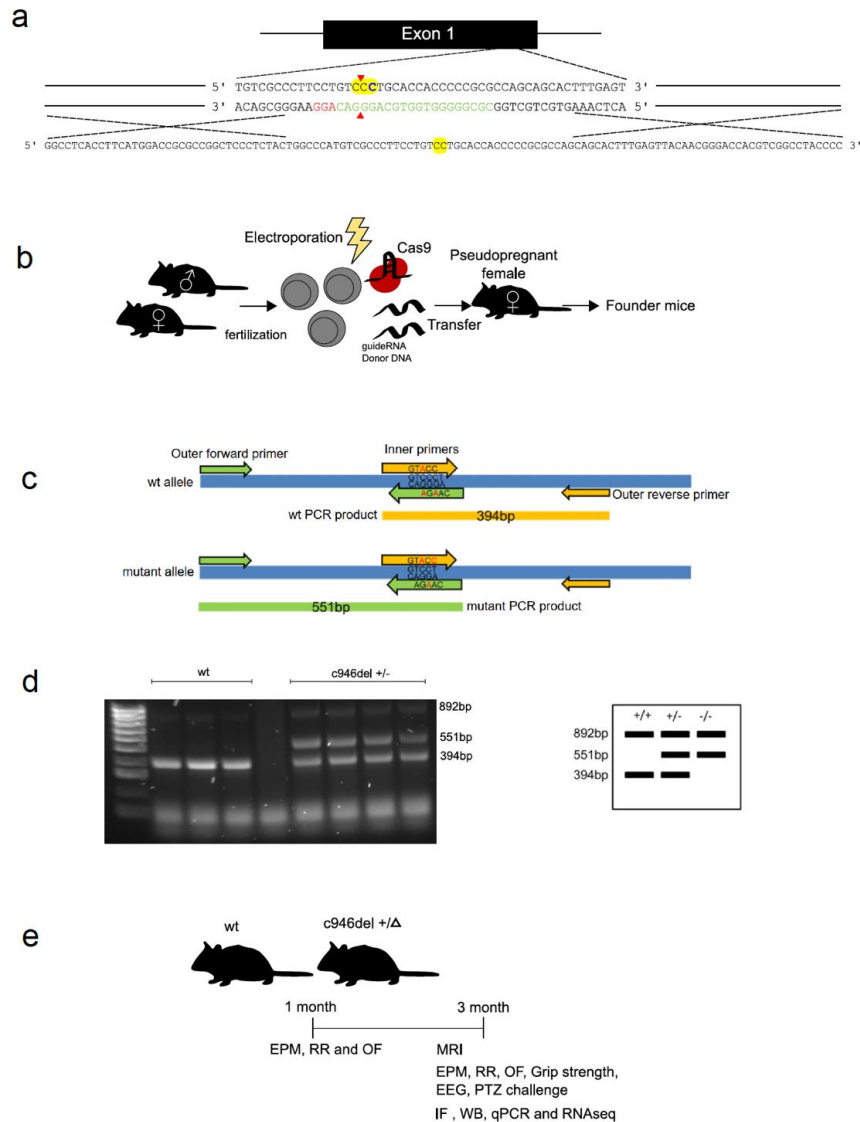
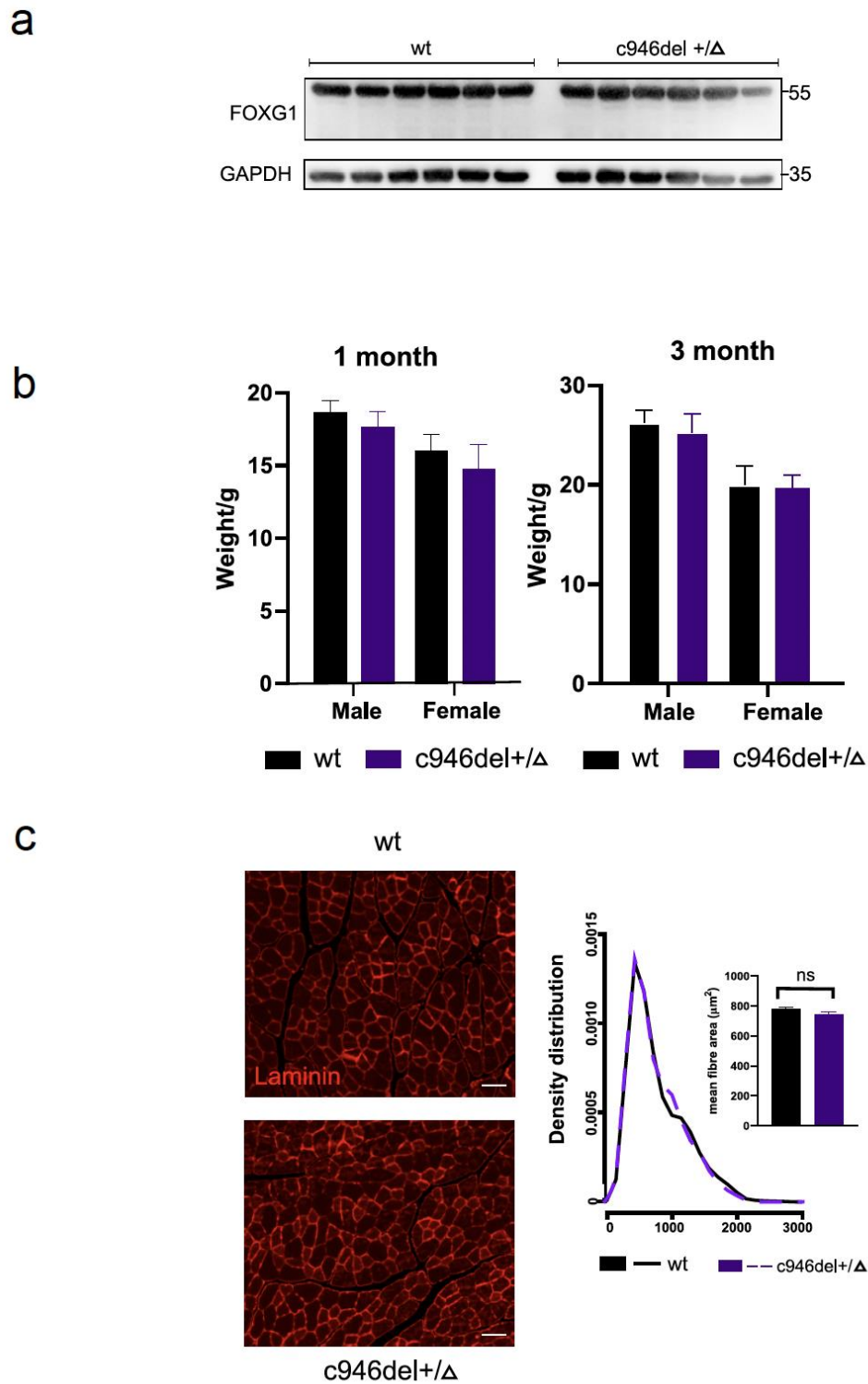


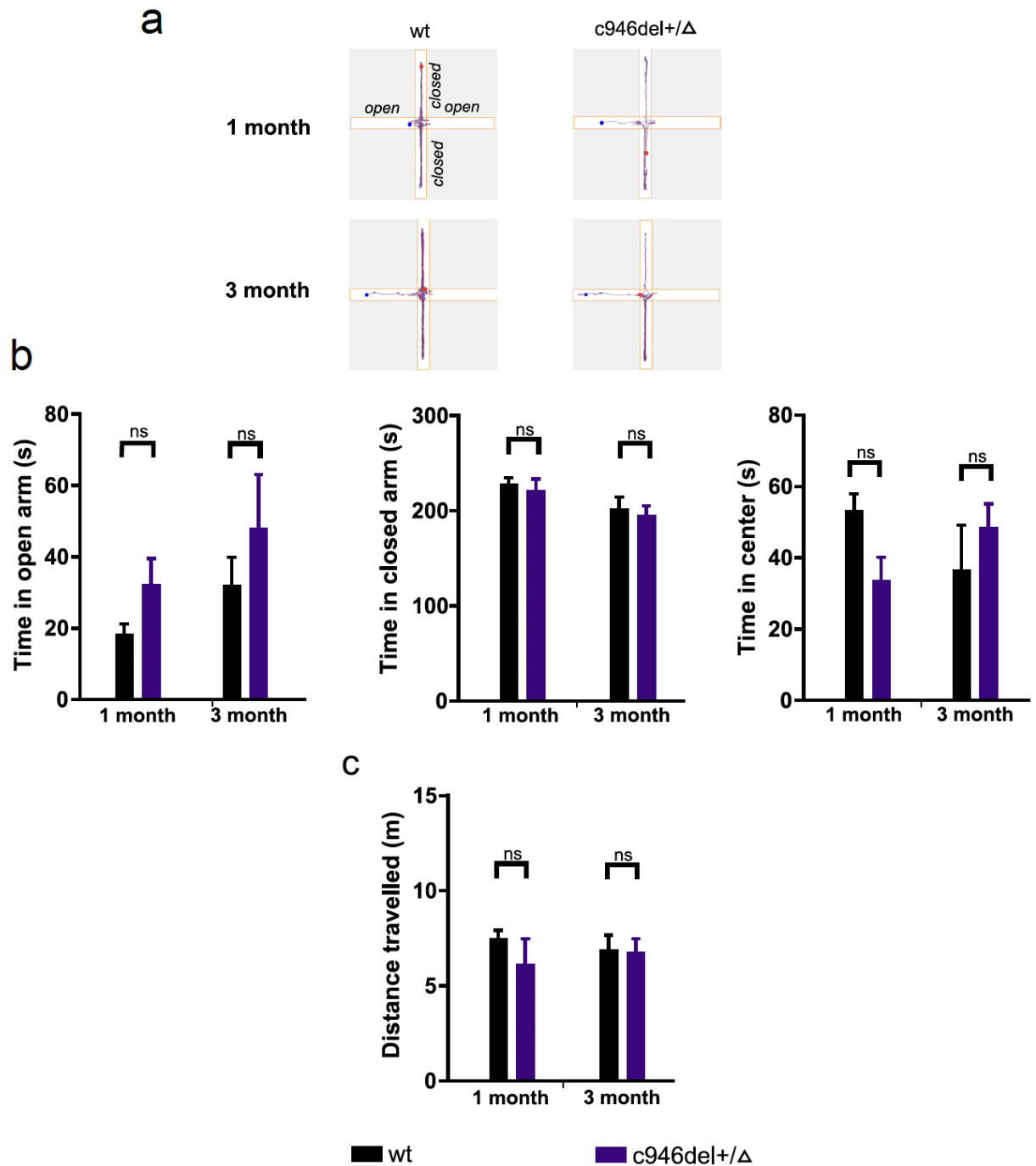
Tan et al. Supplementary Figures



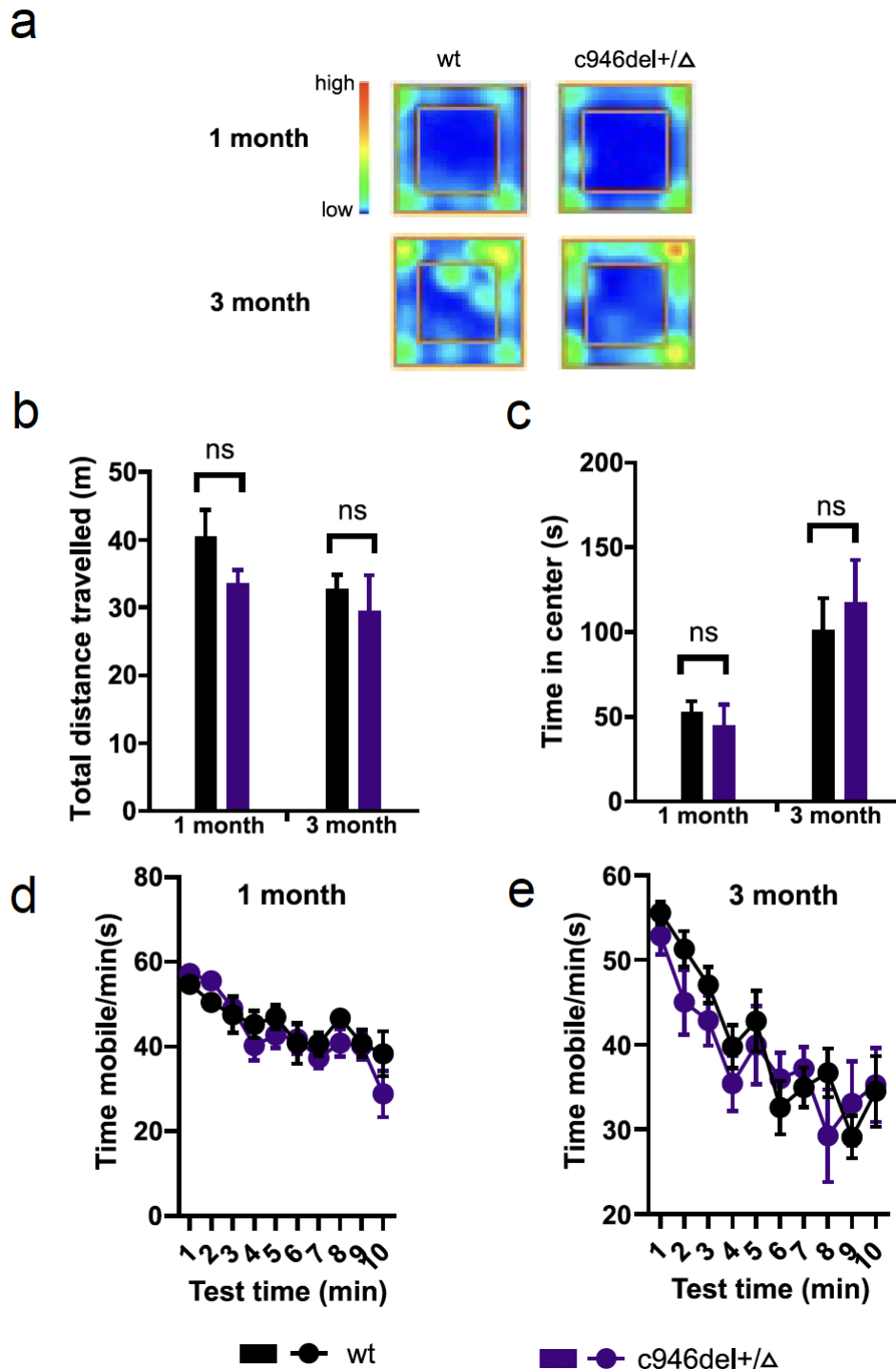
Supplementary Figure 1. Genome editing, genotyping and phenotyping strategies to characterize the c946del mice; (a) Schematic of the genome editing strategy: the Cas9-sgRNA RNPs (green, protospacer adjacent motif in red) induce double-strand breaks induced by Cas9 (red arrows). Homologous Directed Repair (HDR) using a ssOligo (bottom) results in the deletion of a cytosine at position 922 (blue). (b) Workflow to generate the mutant mice: C57BL/6J zygotes were electroporated in medium containing the Cas9 RNP complexes and repair template before reimplantation into pseudo-pregnant females. (c) Schematic of the primers used for the Tetra-ARMS PCR and resulting WT (394bp) and mutant (551bp) bands. (d) Illustrative electrophoresis gel for WT and Heterozygous c946 mice. (e) Phenotypic characterization: mice underwent elevated plus maze (EPM), Rotarod (RR) and Open field (OF) tests at 1 and 3 months of age. Grip strength, electroencephalogram (EEG) and pentylene-tetrazole (PTZ) challenges together with immunofluorescence (IF), western blot (WB), quantitative PCR (qPCR) and RNA sequencing (RNAseq) were carried out on 3-month-old mice.



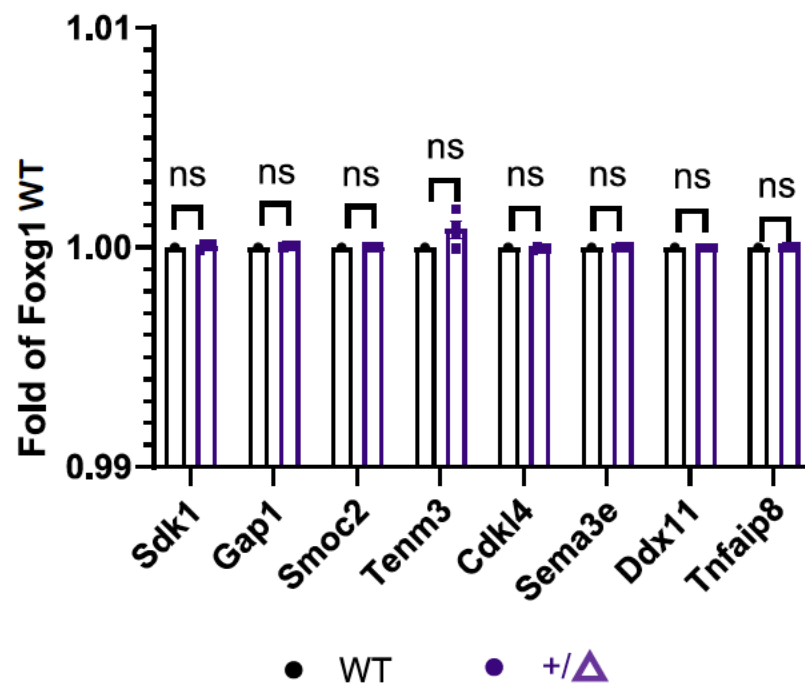
Supplementary Figure 2. Functional deficits in c946del mice are not correlated with muscle changes; (a) Immunoblotting of mouse WT controls and c946del+/D brain lysates using an N-terminal FOXG1 specific antibody. (b) Body weight of c946del+/D mice and WT littermates at 1 and 3 months of age. (c) Laminin-stained cross sections of the soleus muscle showed similar distribution and no muscular atrophy in c946del+/D mice. (Scale bar 50 μm).



Supplementary Figure 3. No anxiety or disinhibition-like behaviour in c946del mice; (a) Representative traces of 1-month-old and 3-month-old WT and c946del+/D mice; in the elevated-plus-maze (EPM) which comprises two open and two closed arms. **(b)** Quantification of time spent in the open arms, closed arms, and centre. **(c)** Quantification of total distance travelled in the EPM. *ns*, not significant; student *t*-test; 1-month-old mice (n = 10 WT, n = 9 c946del+/D), 3-month-old mice (n = 11 WT, n = 8 c946del+/D).



Supplementary Figure 4. No changes in locomotor activity in c946del mice; (a) Representative heat maps of 1-month-old and 3-month-old WT and c946del+/D mice in the open field. Trace intensity represents the amount of time spent in the region of the open field. (b) Quantification of total distance travelled over 10 min. (c) Quantification of time spent in the centre zone. (d,e) Locomotor activity for each minute over a 10min period; No significant differences were observed across all mouse groups. *ns*, not significant; student *t*-test; 1-month-old mice (n = 10 WT, n = 9 c946del+/D), 3-month-old mice (n = 11 WT, n = 9 c946del+/D).



Supplementary Figure 5. qPCR analysis of selected downregulated genes in c946del mice; Quantitative PCR analysis of cortices for selected downregulated genes in c946del+/Δ (n = 4) relative to WT controls (n = 4).