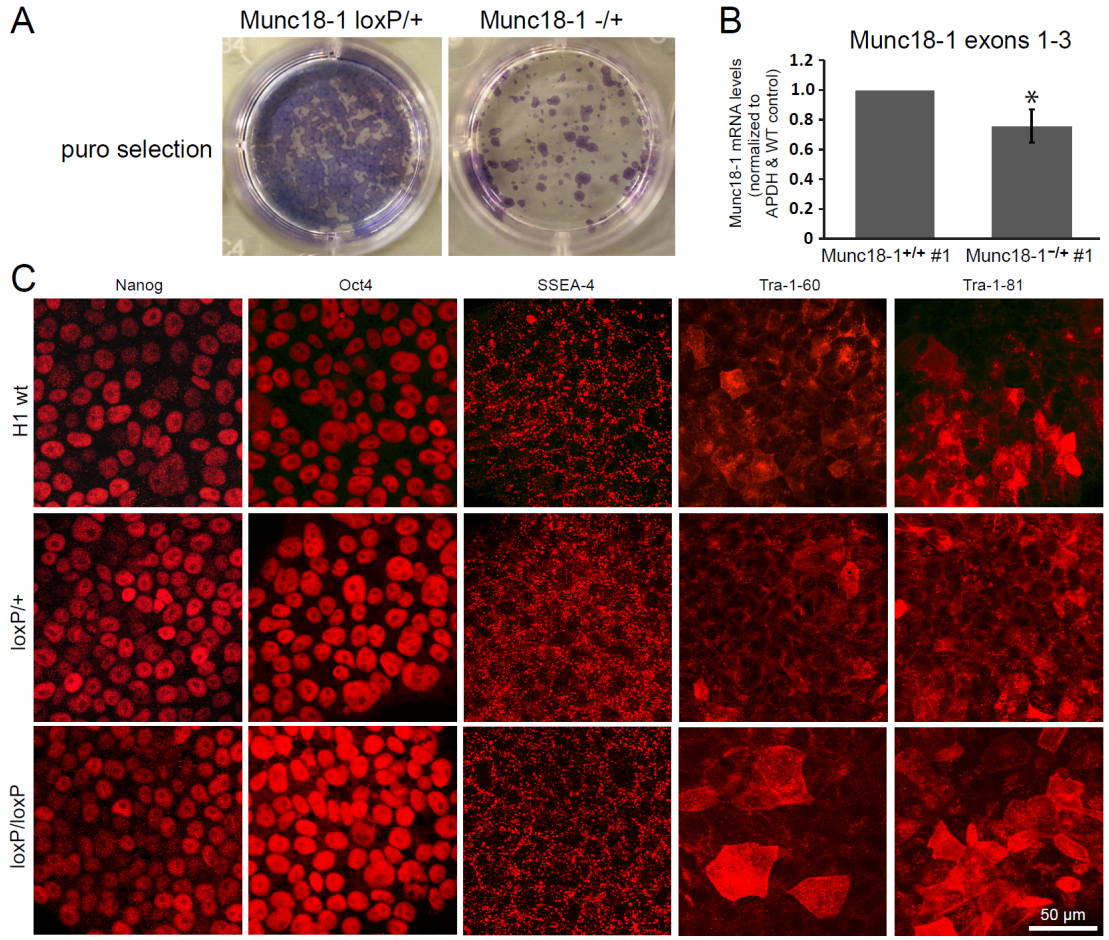
SUPPLEMENTAL MATERIALS for Patzke et al.,

"Analysis of Conditional Heterozygous *STXBP1* Mutations in Human Neurons"

SUPPLEMENTARY FIGURES and FIGURE LEGENDS

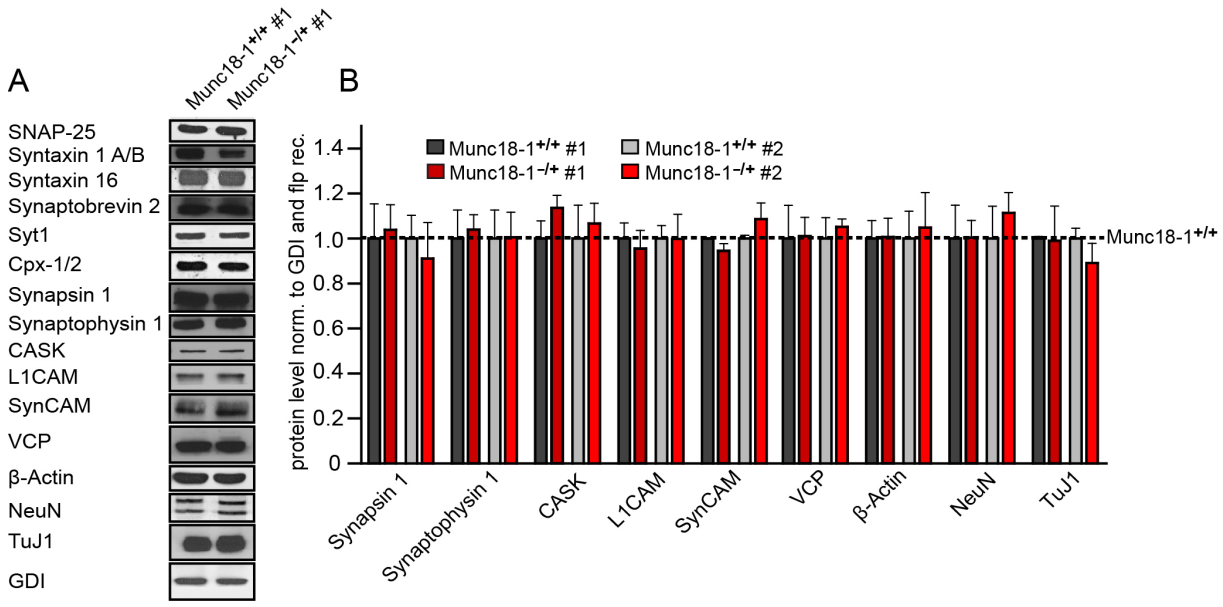


Supplemental Figure 1: **Production of conditionally mutant ES cells targeting the *STXBP1* gene that encodes Munc18-1**

(A), Generated H1 cells mutated for the first allele of the Munc18-1 (*STXBP1*) gene, before and after cre-recombination, (referred to as "Munc18-1 loxP/+" and "Munc18-1 -/+" respectively), removing the resistance cassette for puromycin.

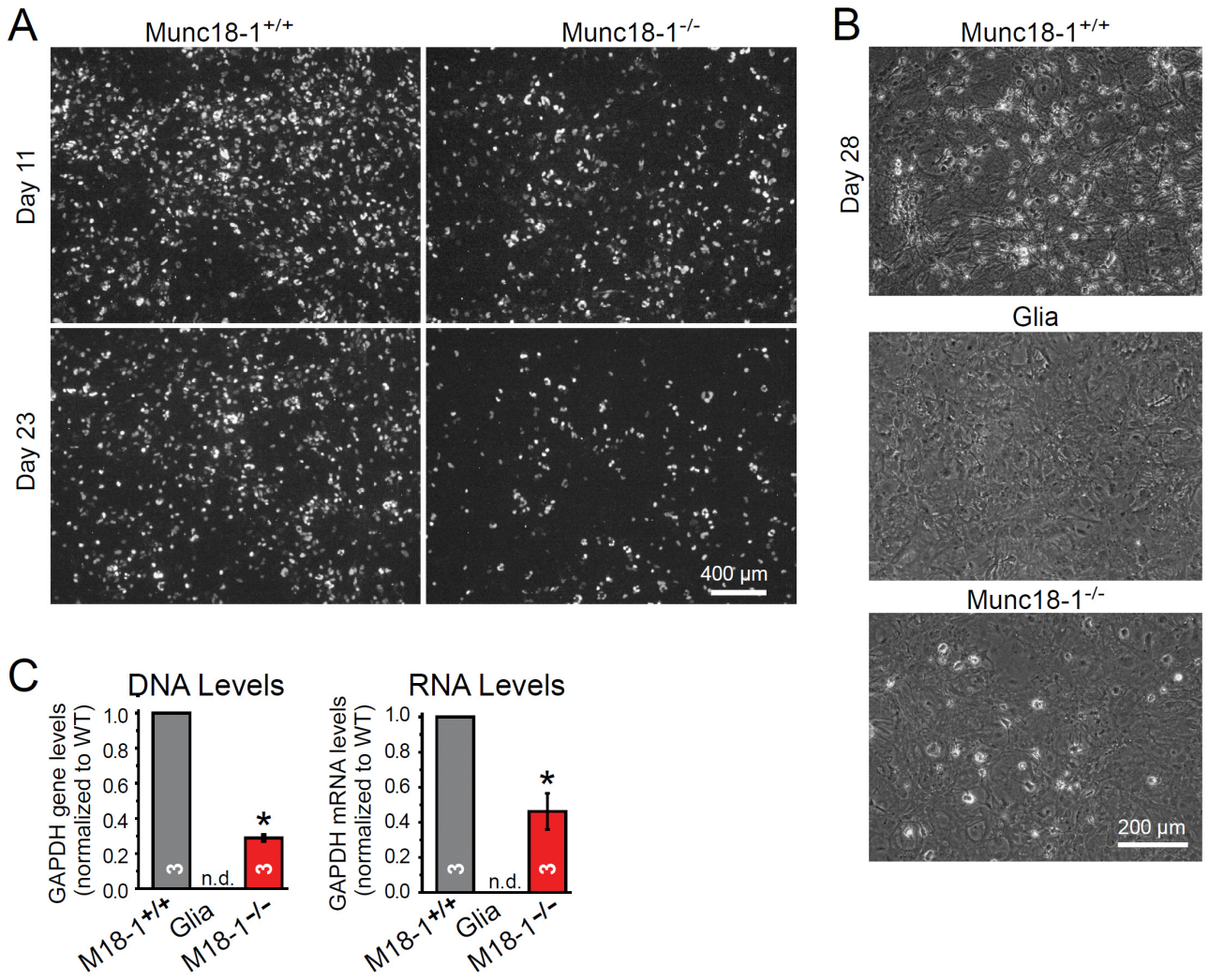
(B), Summary graph of quantitative RT-PCR data obtained with a probe for exons 1-3 of Munc18-1 showing an approximately 25% reduction of Munc18-1 mRNA in heterozygous mutant iN cells. Data shown are means +/- SEM (n=3). Statistical significance was assessed using Student's t-test (* = $p<0.05$).

(C) Immunofluorescence images of H1 ESCs, mutated for one or both alleles of Munc18-1 before cre-or flp-recombination and untargeted H1 ESCs (referred to as "loxP/+", "loxP/loxP" or "H1 wt" respectively). All cell lines are positive for the stem cell markers Nanog, Oct4, SSEA-4, Tra-1-60, and Tra-1-81.



Supplemental Figure 2: **Immunoblotting analyses of heterozygous Munc18-1 (*STXBP1*) mutant iN cells.**

(A) and (**B)**, Representative immunoblots (A) and quantifications of proteins levels (B; normalized to those obtained for wild-type controls analyzed in the same experiments). These experiments represent the complete analysis that is shown in Figure 2B. Data in B are means ± SEM; no statistically significant differences among samples were observed.



Supplemental Figure 3: **imaging (A) and qRT-PCR assessed quantification of neuronal cell death in homozygous *STXBP1*-mutant iN cells (B) and (C)**

(A) Representative fluorescence images of iN cells that contain either the wild-type or heterozygous mutant Munc18-1 alleles at two different cultures times. For quantifications of neuronal survival at multiple time points, see Figure 2C. Cells were visualized by mCherry preceded by a nuclear localization sequence (under the control of neuron specific synapsin-promotor.

(**B**) Representative images of iN cells or glia cells only after four weeks of cultivation.

(**C**) Summary graphs of quantitative PCR measurements of the relative amounts of human GAPDH gene DNA (left) and of GAPDH mRNA (right) in human iN cells that contain wild-type *STXBP1* alleles and were cultured on mouse glia (M18-1$^{+/+}$), mouse glia alone, and human iN cells that contain homozygous mutant *STXBP1* alleles, and were also cultured on mouse glia (M18-1$^{-/-}$). DNA and RNA levels were normalized to Munc18-1$^{+/+}$. Cells were analyzed after 35 days in culture (n.d.: not detectable). Summary graphs exhibit means ± SEM n=3 independent cultures). Statistical significance levels were assessed by unpaired, one-tailed Student's t-test (*, $p<0.05$) for the comparisons of the means.