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.