Supplementary Figure S1. Cell death at high insulin concentrations is specific to human NSCs. Cell viability was assessed by MTT assays over a range of insulin concentrations (0-4.3 µM) in differentiating human NSCs derived from hESCs (HSF-6, A), hiPSCs (IMR90-4, B), the human cortical (hCtx, C) and ventral midbrain (VM, D) tissues, and rat NSCs derived from the ventral midbrain (E13 VM, E) and ganglionic eminence (E13 GE, F) tissues at rat embryonic day 13, and mouse E12 VM (G) and E12 GE (H) tissues. The NSCs were proliferated in the presence of bFGF (or bFGF+EGF) and then induced to differentiate by withdrawal of mitogen. The differentiating NSC cultures were exposed to the different concentrations of insulin for 5 days, beginning on the third day of differentiation. MTT assays were performed on the last day of differentiation. Data represent the values (n=8 per group) relative to their respective cultures at 0 µM of insulin.

Supplementary Figure S2. Different insulin products exhibit similar hNSC cell death patterns. Cell viability was assessed by MTT assays over a range of insulin concentrations (0-4.3 µM). The insulin products tested were Gibco 12585-014 (A), Sigma I1882 (B), and Sigma I6634 (C). Values are relative to the MTT value of 0.04 µM insulin of each group. NSC death/viability were further assessed with 0.86 µM or 4.3 µM insulin made from Gibco 12585-014 (Gibco), Sigma I1882 (Sigma1), or Sigma I6634 (Sigma 2) using LDH (D) and MTT (E). Values are relative to the values of the Gibco sample in their respective groups. *Significant difference from their respective Gibco values ($P < 0.001$, n = 8 per group).

Supplementary Figure S3. Insulin concentration effects on proliferation and survival of hNSC at cell proliferation stage. H9 hESC-derived NSCs were plated at 8 x $10^4/cm^2$ and induced to proliferate with bFGF for 4 days in media supplemented with 0, 0.22, and 4.3 µM of insulin. Viable cell numbers were counted on phase-contrast microscope during 4 days of proliferation (A and B). At proliferation day 4, cells were stained with the proliferating cell-specific Ki67 (C and D) and M-phase marker pHH3 (E and F). *Significant difference from their respective 0.22 µM insulin samples ($P < 0.01$, n = 3 coverslips per group) Scale bars = 40 µm

Supplementary Figure S4. High insulin concentrations did not affect apoptotic cell death of mouse Ctx primary neurons. Mouse neurons derived from embryonic cortices at E12 were subject to the same culture conditions and exposure to insulin as in Figure 3. Cultures were then labeled with cleaved caspase-3 (A) and co-labeled with the neuron-specific markers Tuj1 and MAP2 (B). *Significant difference from their respective 0.22 µM+X (X=insulin concentration included in B27) insulin samples ($P < 0.001$, n = 3 coverslips per group). Scale bars = 30 µm.

Supplementary Figure S5. Microarray analysis of genes differentially expressed in hNSCs cultured in high insulin concentrations. H9 hES-derived hNSCs were cultured in optimal (0.22 uM) and high (4.3 uM) insulin concentrations for 2 days and subjecte to microarray analysis. Data is presented as expression values in high versus optimal insulin dosage. Out of 47,231 analyzed genes, those related to insulin resistance and intracellular signaling were selected and categorized based on gene ontology (GO). A, Scatterplot representing expressions of the genes associated with insulin resistance and intracellular signaling. The colored dots in the scatterplot represent the expressions of insulin resistance-related genes, which significantly up- or down-regulated in high insulin concentration. The expression changes are further summarized in (B) and table (C). D, Heat map for expression changes of

the gene associated with intracellular signaling. (GO:0033554, cellular response to stress; GO:0045787, positive regulation of cell cycle; GO:0031418, L-ascorbic acid binding; GO:0012501, programmed cell death; GO:0006915, apoptosis; GO:0009890, negative regulation of biosynthetic process; GO:0010558, negative regulation of macromolecule biosynthetic process; GO:0045934, negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; GO:0010605, negative regulation of macromolecule metabolic process; GO:0010629, negative regulation of gene expression; GO:0065004, protein-DNA complex assembly; GO:0042981, regulation of apoptosis; GO:0043067, regulation of programmed cell death; GO:0034599, cellular response to oxidative stress; GO:0006979, response to oxidative stress; GO:0042326, negative regulation of phosphorylation; GO:0001933, negative regulation of protein amino acid phosphorylation; GO:0050767, regulation of neurogenesis; GO:0050727, regulation of inflammatory response; GO:0046627, negative regulation of insulin receptor signaling pathway) The expression data were calculated as a ratio relative to the median and then converted to a logarithmic scale. The figure shows a typical output where a green signal represents a gene whose expression is below the median, while a red signal indicates a gene whose expression is above the median.