Cell Genomics, Volume *4*

Supplemental information

Variant-to-function analysis of the childhood obesity chr12q13 locus implicates rs7132908 as a causal variant within the 3′ UTR of *FAIM2*

Sheridan H. Littleton, Khanh B. Trang, Christina M. Volpe, Kieona Cook, Nicole DeBruyne, Jean Ann Maguire, Mary Ann Weidekamp, Kenyaita M. Hodge, Keith Boehm, Sumei Lu, Alessandra Chesi, Jonathan P. Bradfield, James A. Pippin, Stewart A. Anderson, Andrew D. Wells, Matthew C. Pahl, and Struan F.A. Grant

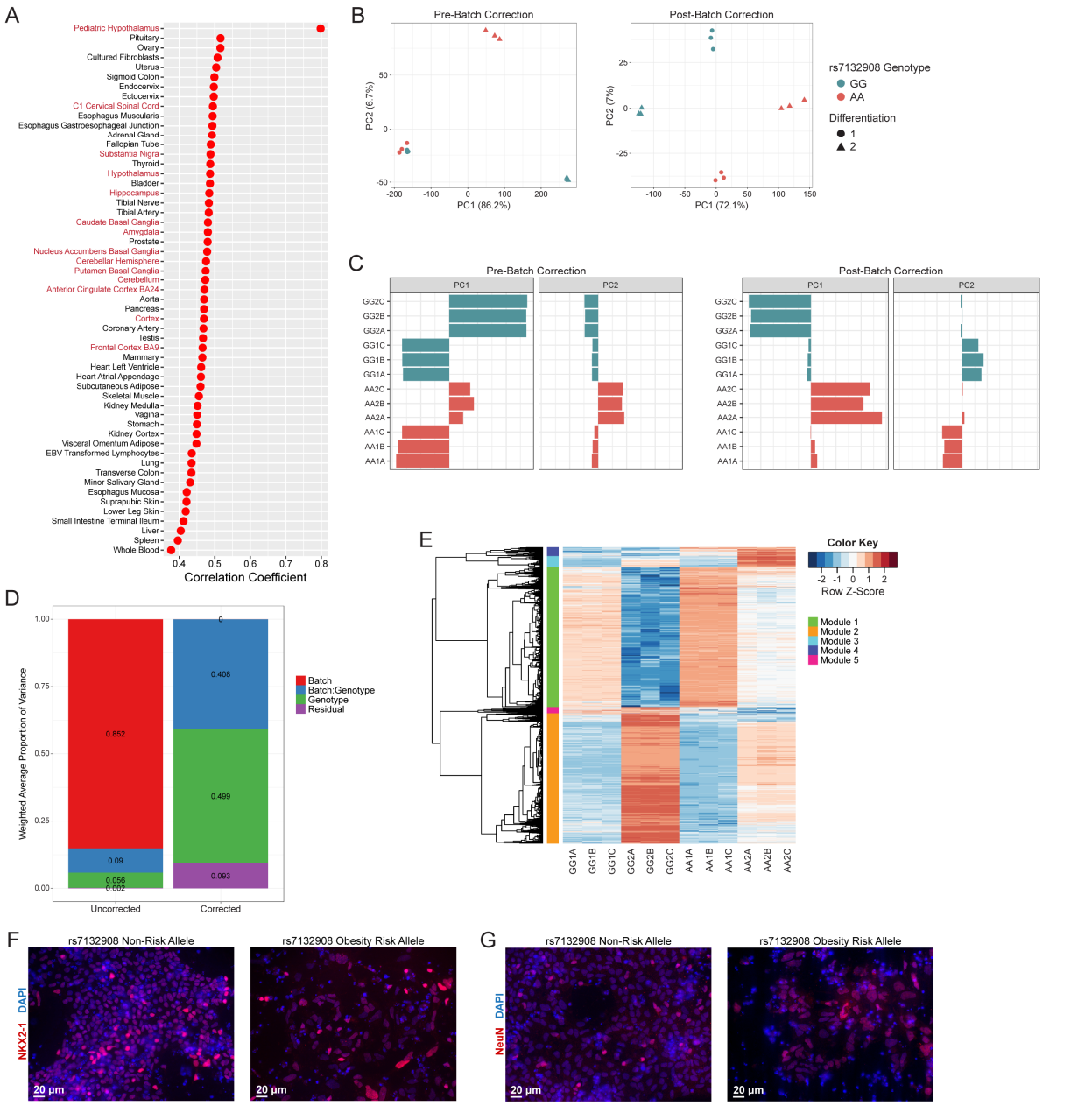

Figure S1 – Hypothalamic neural progenitors, related to Figure 3. A) Dot plot of Spearman's rank correlation coefficients resulting from comparing TPMs of 16,159 genes expressed in rs7132908 non-risk allele homozygous hypothalamic neural progenitors, rs7132908 non-risk allele homozygous human pediatric hypothalamus tissue, and human tissues or cells in the GTEx RNA-seq database. Red dots indicate significant correlations (*P*-value < 0.05). Tissue names in red indicate brain tissues. B) PCA plots of hypothalamic neural progenitor RNA-seq libraries before (left) and after (right) batch correction (GG n = 2 biological replicates with 3 technical replicates each, AA n = 2 biological replicates with 3 technical replicates each). C) Bar plots representing PCA loadings for PC1 and PC2 from hypothalamic neural progenitor RNA-seq libraries before (left) and after (right) batch correction. D) Stacked bar plots of weighted average proportion of variance from principal variance component analysis of hypothalamic neural progenitor RNA-seq libraries before (left) and after (right) batch correction. E) Heatmap depicting significantly differentially expressed genes (adjusted *P*-value < 0.05, |log2 fold change| > 0.58) due to the rs7132908 obesity risk allele in hypothalamic neural progenitors. Genes were clustered into 5 modules using hierarchical clustering (green, orange, light blue, dark blue, pink). F-G) Representative images of hypothalamic neural progenitors on day 14, with immunostaining for a marker of the developing hypothalamus, NKX2-1 (red) (F) and a marker of post-mitotic neurons, NeuN (red) (G) (scale bar = 20 µm). Nuclei were stained with DAPI (blue). Cells were homozygous for either the rs7132908 non-risk allele (left) or obesity risk allele (right).

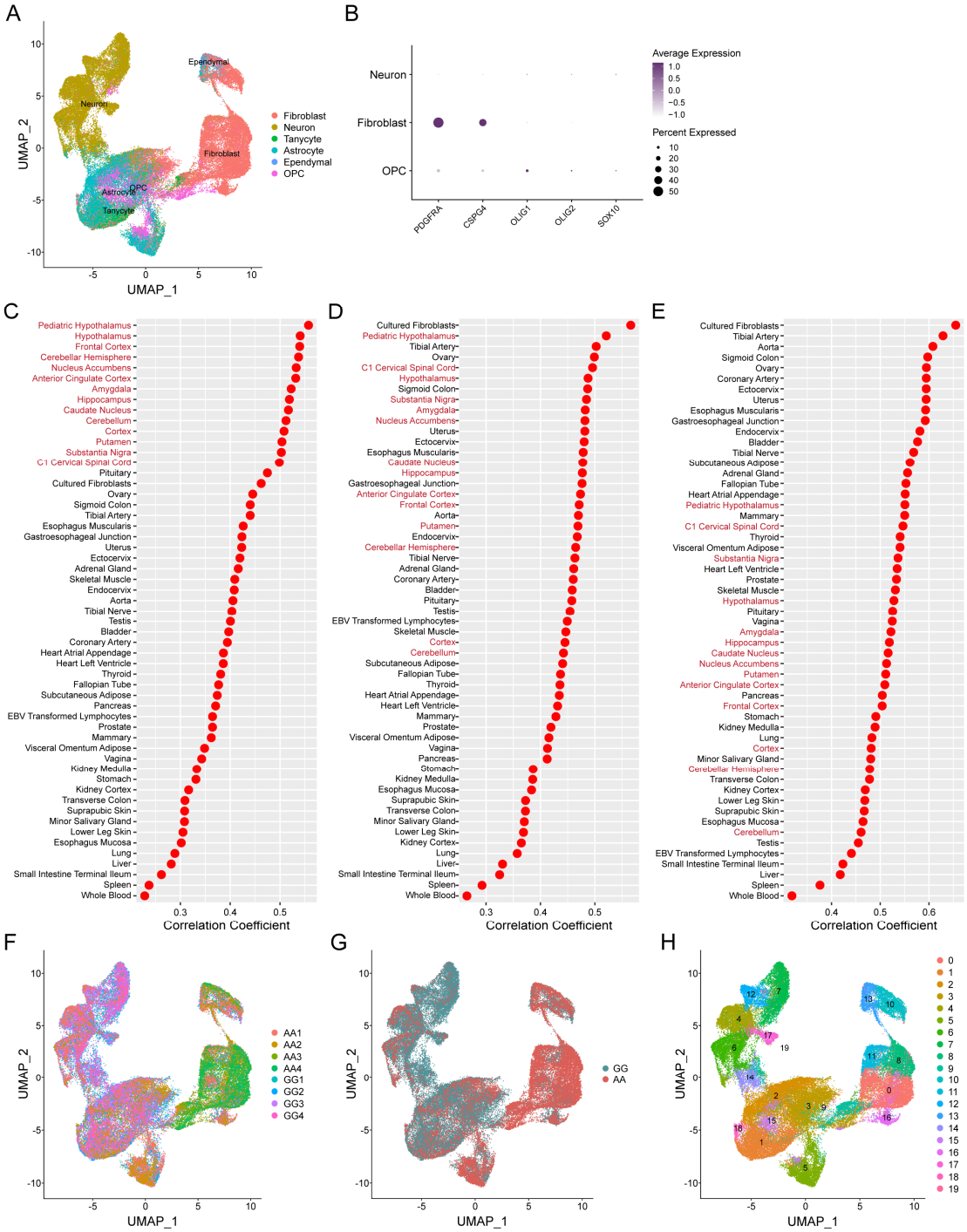

Figure S2 – Hypothalamic single-nucleus RNA-seq analysis, related to Figure 4. A, F-H) UMAP depicting all cells clustered by single-nucleus RNA-seq profile and annotated by predicted cell type annotation before cells with classification scores below the 0.8 threshold were removed (A), replicate sample (F), rs7132908 genotype (G), and cluster identity (H). B) Dot plot depicting average expression (scaled and log2 normalized counts) and percent of cells that expressed canonical OPC marker genes (*PDGFRA*, *CSPG4*, *OLIG1*, *OLIG2*, and *SOX10*), split by cell type. C-E) Dot plots of Spearman's rank correlation coefficients resulting from comparing TPMs of genes expressed in rs7132908 non-risk allele homozygous hypothalamic neurons (C), OPCs (D), and fibroblasts (E) to human pediatric hypothalamus tissue from donors homozygous for the rs7132908 non-risk allele and human tissues or cells in the GTEx RNA-seq database. Red dots indicate significant correlations (*P*-value < 0.05). Tissue names in red indicate brain tissues.

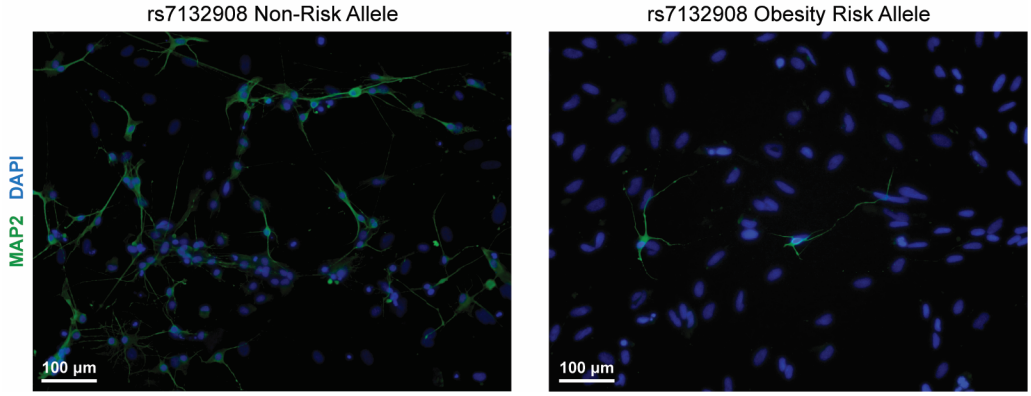

Figure S3 – MAP2 expression, related to Figure 6. Representative composite images of hypothalamic neurons post-differentiation on day 40, with immunostaining for a mature neuron marker, MAP2 (green) (scale bar = 100 µm). Nuclei were stained with DAPI (blue). Cells were homozygous for either the rs7132908 non-risk allele (left) or obesity risk allele (right).

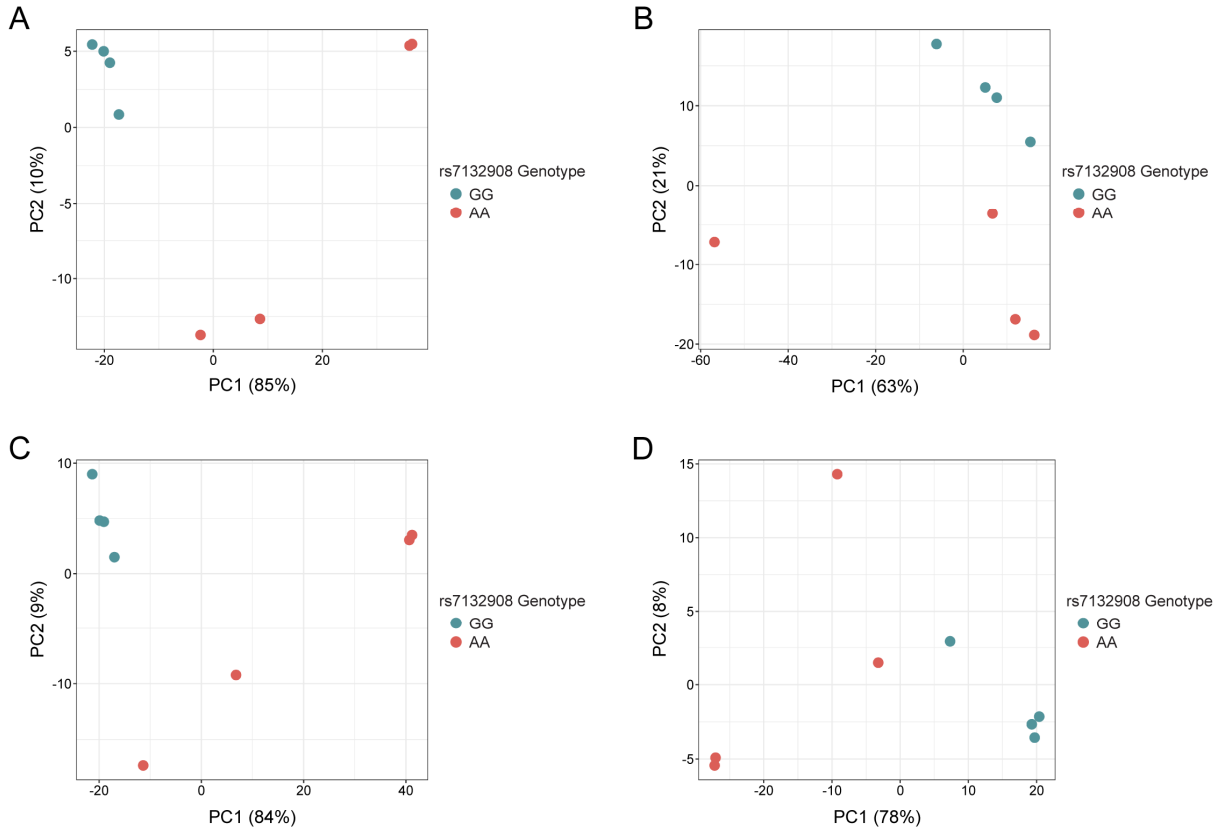

Figure S4 – Hypothalamic single-nucleus RNA-seq differential expression analysis, related to Figure 7. A-D) PCA plots of single-nucleus RNA-seq libraries (GG n = 4, AA n = 4) when considering all cells (A), neurons (B), OPCs (C), and fibroblasts (D).

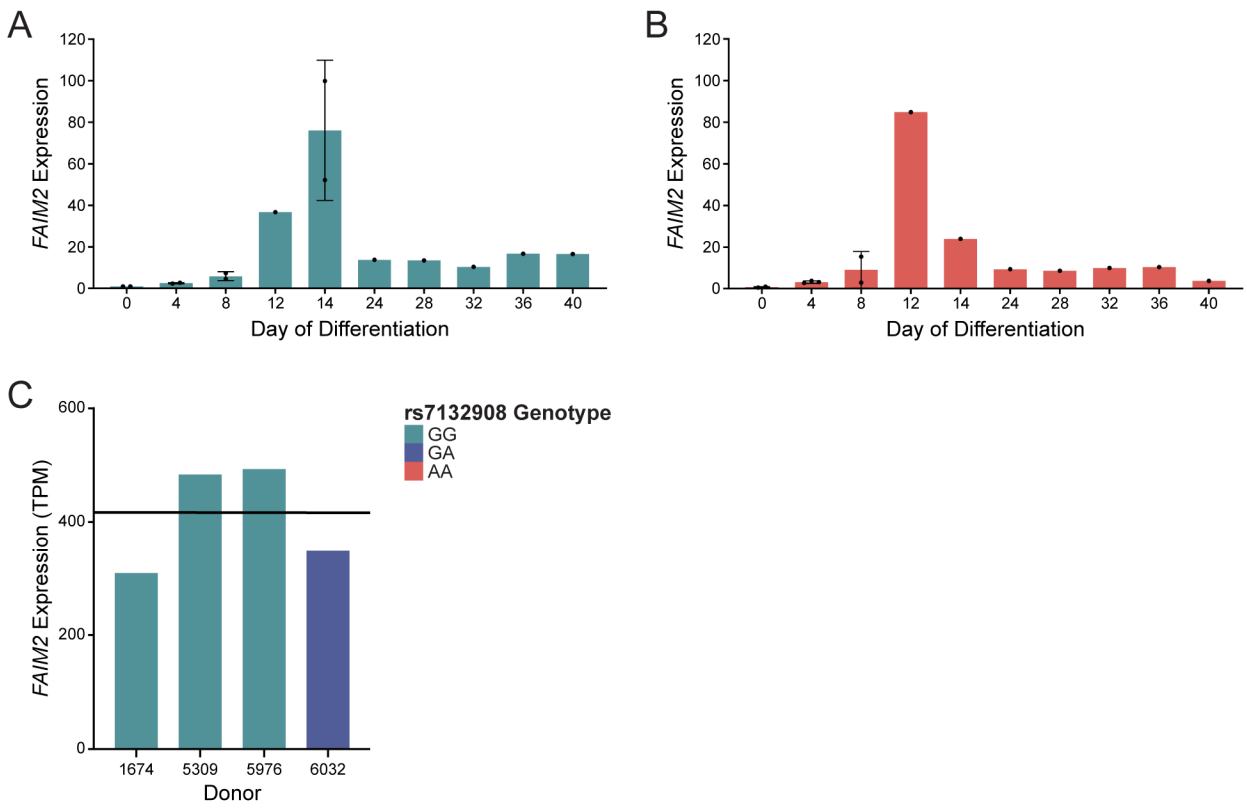

Figure S5 – *FAIM2* expression, related to Figure 7. A-B) Relative normalized *FAIM2* mRNA expression in cells homozygous for the rs7132908 non-risk allele (A) and obesity risk allele (B) measured by RT-qPCR throughout ESC differentiation to hypothalamic neurons. *FAIM2* expression was normalized to *18S* ribosomal RNA expression. Relative *FAIM2* expression was calculated relative to non-risk allele cells on day 0. Data are represented as mean ± SD when n > 1. C) *FAIM2* expression (TPM) in primary human pediatric hypothalamus tissue. Black horizontal line indicates median expression (n=4). Blue bars indicate donors homozygous for the rs7132908 non-risk G allele and the indigo bar indicates a donor heterozygous at rs7132908.

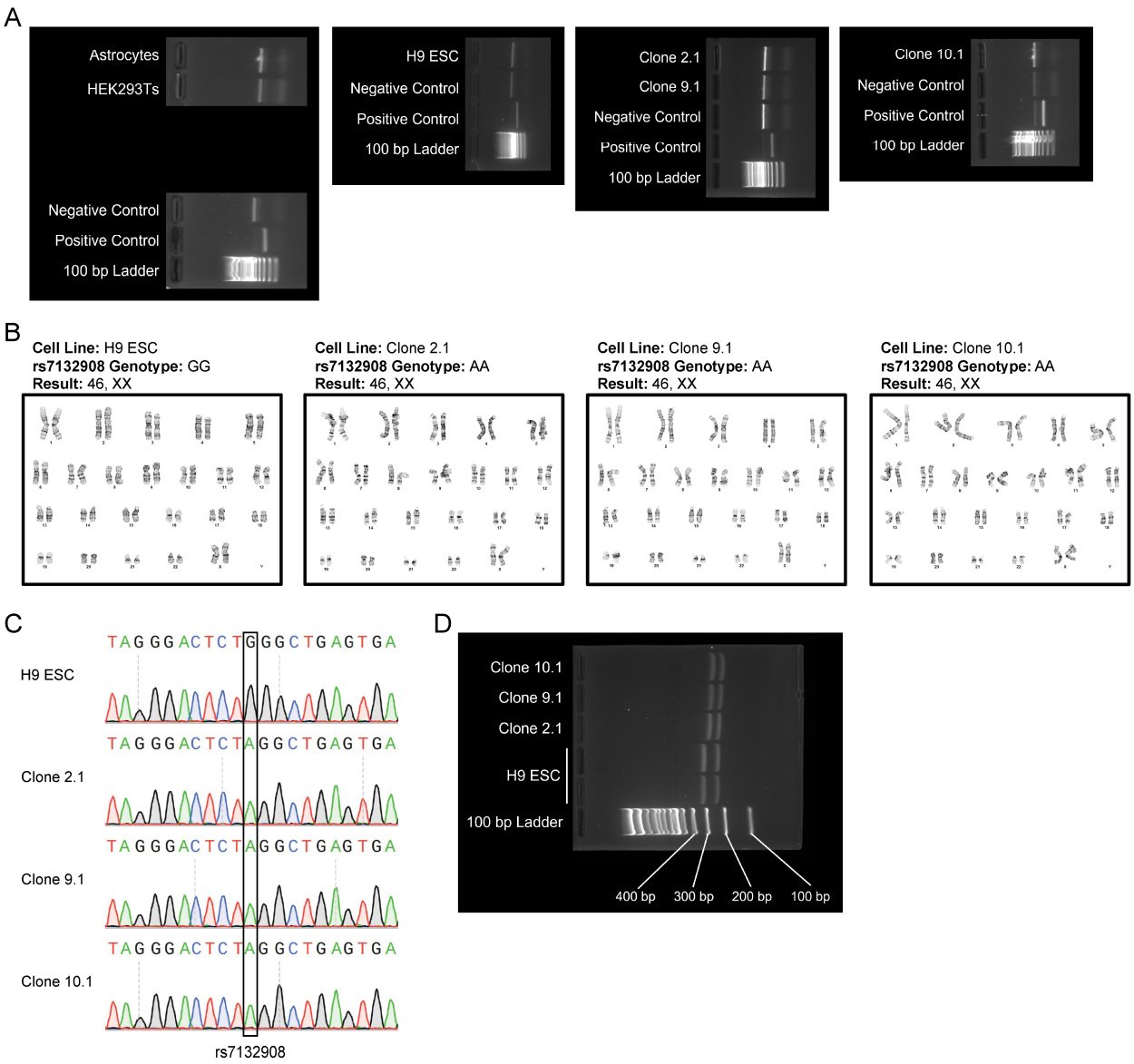

Figure S6 – Validation of experimental models, related to STAR Methods. A) Mycoplasma PCR detection results for all experimental models. Cell lines with bands matching the size of the negative control are not contaminated with mycoplasma. Irrelevant lanes were removed from the farthest left gel image. B) G-band karyotyping reports for ESC lines. C) Electropherograms produced by Sanger sequencing around rs7132908 in ESC lines. D) BfaI restriction enzyme digestion screening in ESC lines. H9 ESCs have one BfaI restriction site in the PCR product around rs7132908, where digestion should produce two bands of 320 bp and 248 bp. After CRISPR to introduce the rs7132908 obesity risk A allele, a second BfaI restriction site is introduced, where digestion should produce three bands of 294 bp, 248 bp, and 26 bp (not pictured).

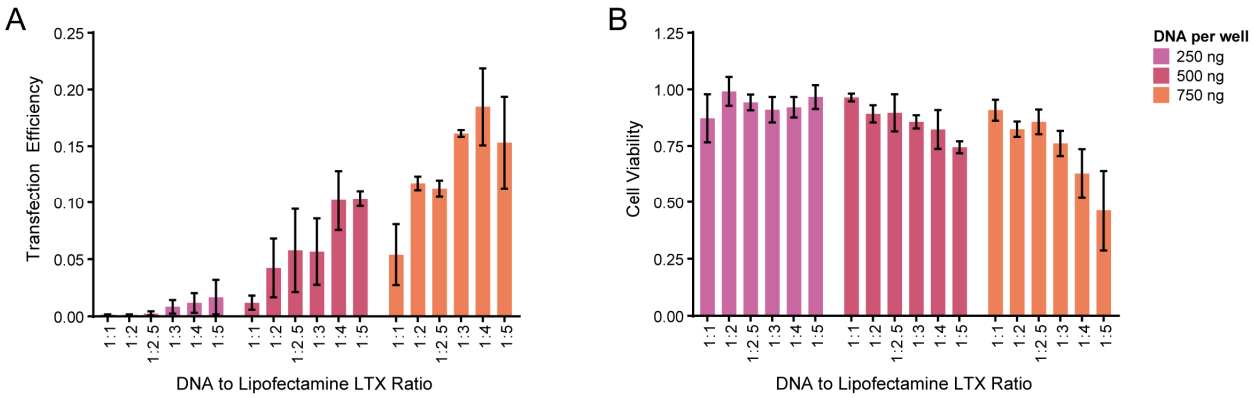

Figure S7 – Primary astrocyte transfection optimization, related to STAR Methods. A) Transfection efficiency resulting from transfecting with 250, 500, or 700 ng DNA per well and varying DNA to Lipofectamine LTX ratios (n = 2 biological replicates). B) Cell viability resulting from transfecting with 250, 500, or 700 ng DNA per well and varying DNA to Lipofectamine LTX ratios (n = 2 biological replicates). Data are represented as mean ± SD.