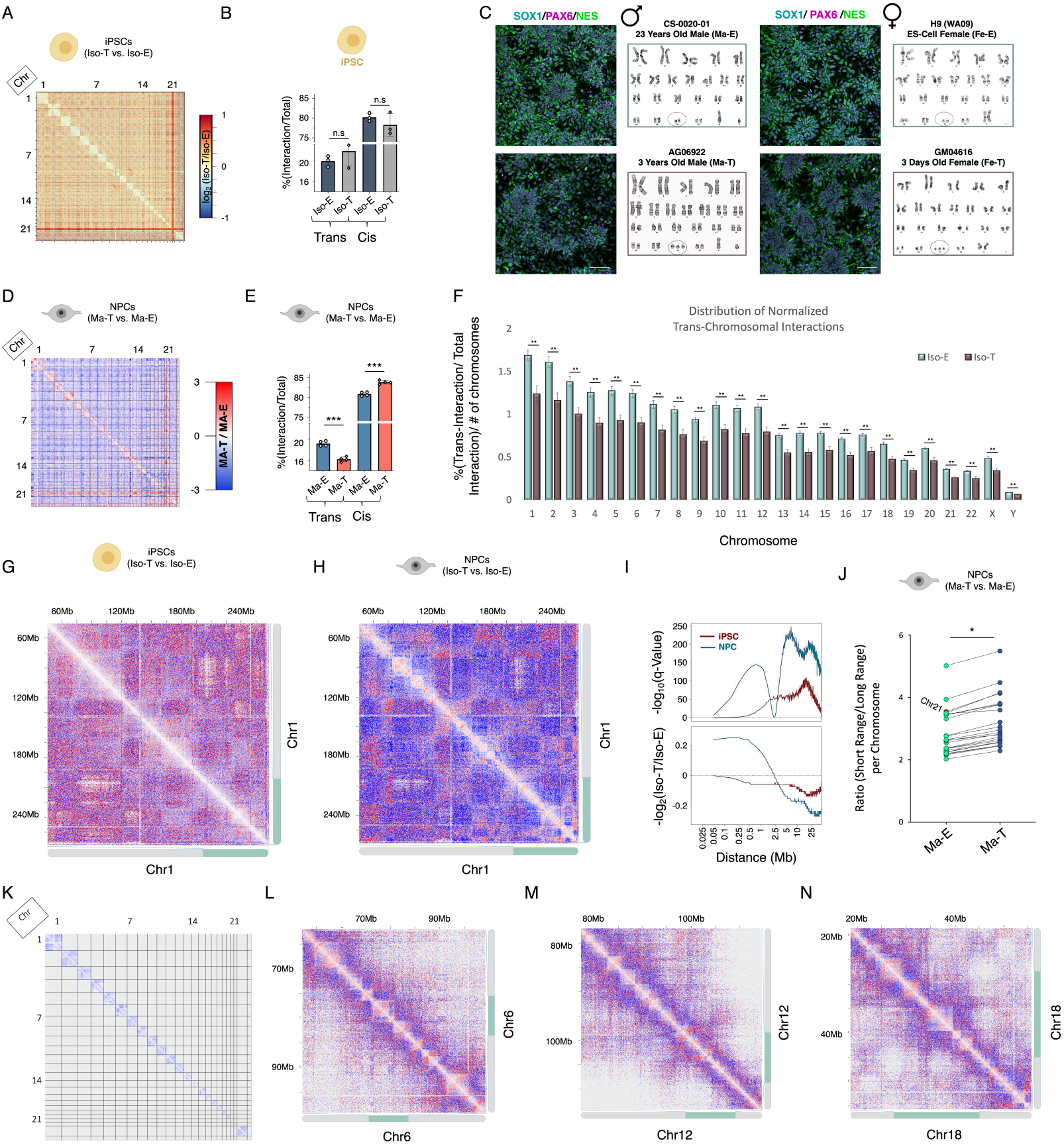
A
iPSCs
(Iso-T vs. Iso-E)
Chr
log2 (Iso-T/Iso-E)
B
iPSC
%(Interaction/Total)
n.s
Iso-E
Iso-T
Trans
Cis
C
SOX1/PAX6/NES
CS-0020-01
23 Years Old Male (Ma-E)
AG06922
3 Years Old Male (Ma-T)
SOX1/ PAX6 /NES
H9 (WA09)
ES-Cell Female (Fe-E)
GM04616
3 Days Old Female (Fe-T)
D
NPCs
(Ma-T vs. Ma-E)
MA-T / MA-E
E
NPCs
(Ma-T vs. Ma-E)
Ma-E
Ma-T
F
Distribution of Normalized
Trans-Chromosomal Interactions
%(Trans-Interaction/ Total
Interaction)/ # of chromosomes
Iso-E
Iso-T
Chromosome
G
iPSCs
(Iso-T vs. Iso-E)
Chr1
H
NPCs
(Iso-T vs. Iso-E)
Chr1
I
iPSC
NPC
-log10(q-Value)
-log2(Iso-T/Iso-E)
Distance (Mb)
J
NPCs
(Ma-T vs. Ma-E)
Ratio (Short Range/Long Range)
per Chromosome
Chr21
Ma-E
Ma-T
K
Chr
L
Chr6
M
Chr12
N
Chr18

Figure S1 – T21 induces genome-wide chromosomal introversion in NPCs, Related to Figure 1. (A) Genome-wide differential (Iso-T/Iso-E) Hi-C interaction maps for iPSCs. (B) Percent distribution of trans- and cis-chromosomal interactions for the isogenic pair of iPSCs. (C) Left panels: Staining of NPCs for SOX1 (cyan), PAX6 (magenta) and Nestin (green) from the euploid male (Ma-E, top left) and trisomic male (Ma-T, bottom left). Right panels: G-band karyotyping of euploid male (Ma-E, top right) and trisomic male (Ma-T, bottom right) NPCs. Right panel: Staining of NPCs (left) from the euploid female (Fe-E, top left) and a trisomic female (Fe-T, bottom left) as well as the respective G-band karyotyping of the female NPCs on the right. (D) Genome-wide differential (Ma-T/Ma-E) Hi-C interaction maps for the male pair of NPCs. (E) Percent distribution of trans- and cis-chromosomal interactions for the male pair (Ma-E and Ma-T) of NPCs. (F) Percent distribution of trans-chromosomal interactions per chromosome for the isogenic pair of NPCs (Iso-E and Iso-T) normalized by the number of chromosomes. (G, H) Representative images of the differential (Iso-T/Iso-E) cis-chromosomal Hi-C interaction maps of chromosome 1 (chr1) for the isogenic pair of iPSCs (G) and NPCs (H). (I) Bottom panel shows the fold change (log_2) of the long-range interaction distribution (y-axis) plotted against the distance of chromatin interactions (x-axis) for the isogenic pair of iPSCs (red) and NPCs (teal) as well as the adjusted p-value (top panel). (J) Dot plots representing the ratio of short-range (<1Mb) to long-range (>1Mb) interactions for the male pair of NPCs (Ma-E and Ma-T). Each dot represents a chromosome and chr21 is represented as a red dot. Wilcoxon rank-sum test shows that trisomic NPCs have significantly increased short range interactions in as compared to the euploid NPCs (p-value=0.043). (L-N) Analysis of genome-wide cis chromosomal interactions without chromosome 21 pre normalization. (K-N) Differential (Iso-T/Iso-E) cis-chromosomal interaction map for the isogenic pair of NPCs, genome-wide (K), chromosome 6 (L), chromosome 12 (M) and chromosome 18 (N).

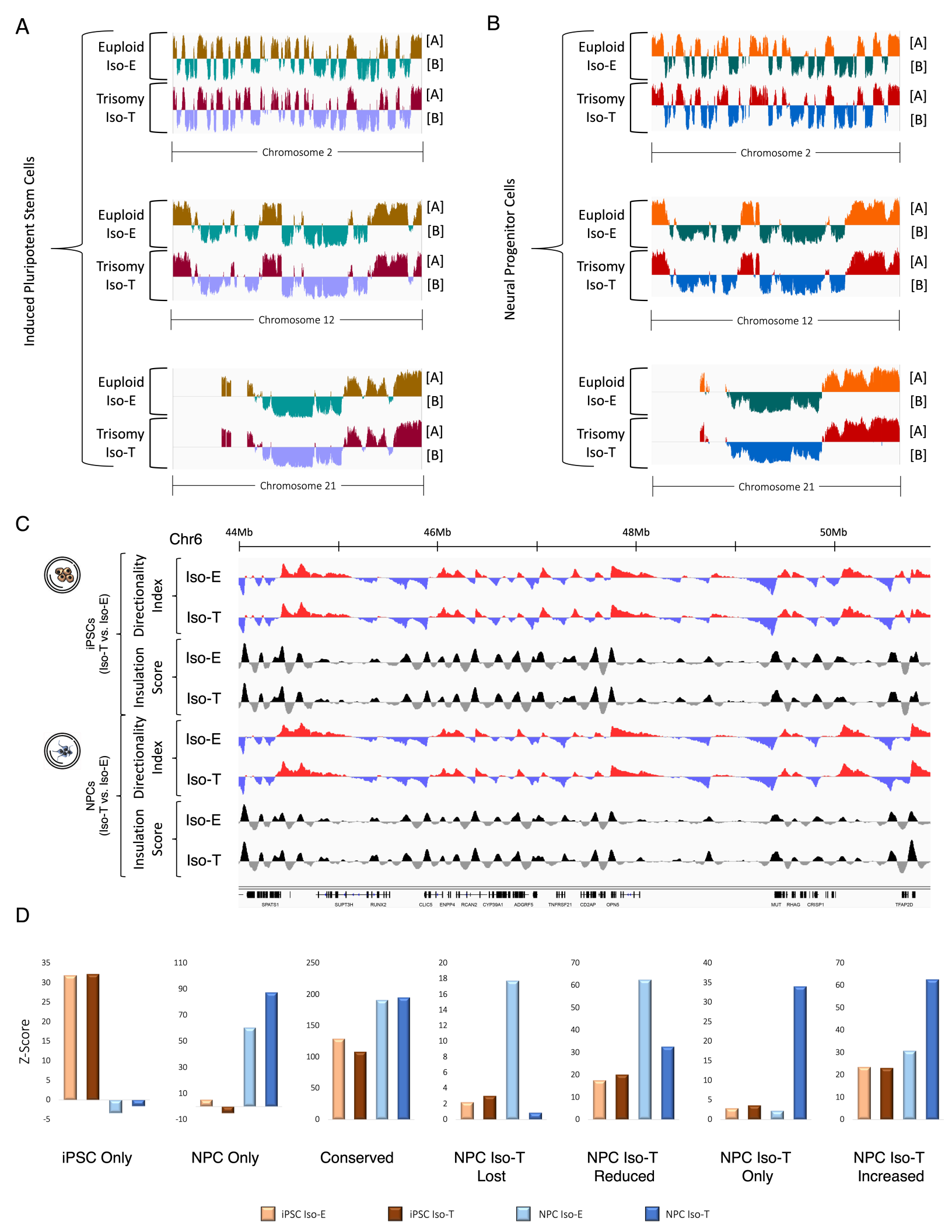
A
Induced Pluripotent Stem Cells
Euploid Iso-E
Trisomy Iso-T
[A]
[B]
Chromosome 2
Chromosome 12
Chromosome 21
B
Neural Progenitor Cells
C
Chr6
44Mb
46Mb
48Mb
50Mb
iPSCs (Iso-T vs. Iso-E)
NPCs (Iso-T vs. Iso-E)
Directionality Index
Insulation Score
Iso-E
Iso-T
SPATS1
SUPT3H
RUNX2
CLIC5
ENPP4
RCAN2
CYP39A1
ADGRF5
TNFRSF21
CD2AP
OPN5
MUT
RHAG
CRISP1
TFAP2D
D
Z-Score
iPSC Only
NPC Only
Conserved
NPC Iso-T Lost
NPC Iso-T Reduced
NPC Iso-T Only
NPC Iso-T Increased
iPSC Iso-E
iPSC Iso-T
NPC Iso-E
NPC Iso-T

Figure S2 – Consequences of T21 on A/B compartments, TADs and chromosomal loops, Related to Figure 2. (A, B) Representative images of A/B compartments identified through principal component analysis from chromosome 2 ,12 and 21 of the isogenic pair (Iso-E and Iso-T) of iPSCs (A) and NPCs (B). (C) Representative IGV plots of TAD directionality index and insulation scores from chromosome 6 for the isogenic pair (Iso-E and Iso-T) of iPSCs and NPCs. (D) Histogram of the z-score of aggregate plot analysis of the genome-wide chromosomal looping identified only in iPSCs or NPCs as well as loops that are conserved in euploid and T21, loops lost/reduced in T21-NPCs and gained/increased in T21-NPCs. Histogram bars are colored beige (Iso-E iPSCs), brown (Iso-T iPSCs), light-blue (Iso-E NPCs) and blue (Iso-T NPCs).

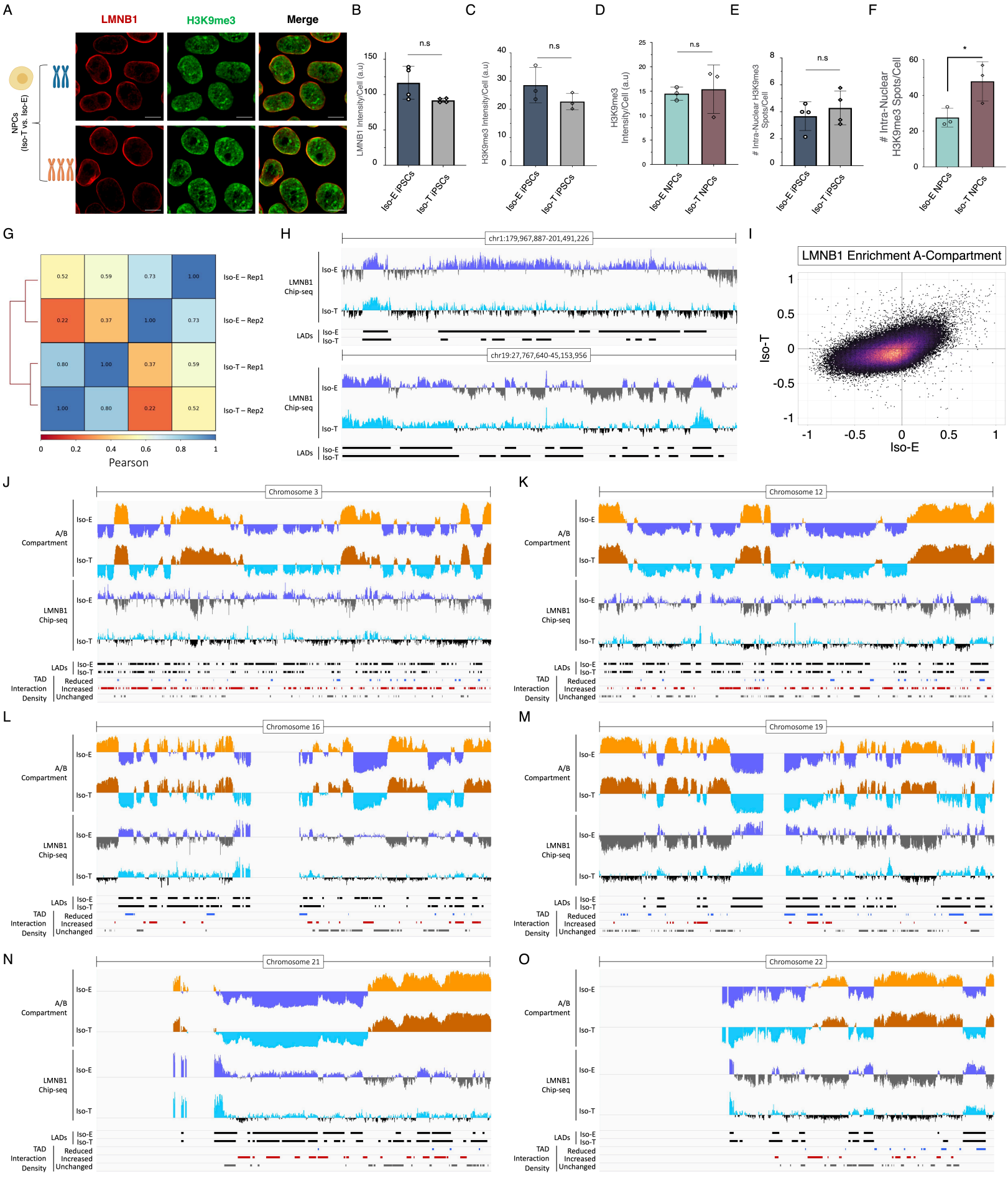
A
LMNB1
H3K9me3
Merge
NPCs
(Iso-T vs. Iso-E)
B
LMNB1 Intensity/Cell (a.u)
n.s
Iso-E iPSCs
Iso-T iPSCs
C
H3K9me3 Intensity/Cell (a.u)
n.s
Iso-E iPSCs
Iso-T iPSCs
D
H3K9me3 Intensity/Cell (a.u)
n.s
Iso-E NPCs
Iso-T NPCs
E
Intra-Nuclear H3K9me3 Spots/Cell
n.s
Iso-E iPSCs
Iso-T iPSCs
F
Intra-Nuclear H3K9me3 Spots/Cell
*
Iso-E NPCs
Iso-T NPCs
G
Iso-E – Rep1
Iso-E – Rep2
Iso-T – Rep1
Iso-T – Rep2
Pearson
H
chr1:179,967,887-201,491,226
chr19:27,767,640-45,153,956
LMNB1 Chip-seq
LADs
Iso-E
Iso-T
I
LMNB1 Enrichment A-Compartment
Iso-T
Iso-E
J
Chromosome 3
K
Chromosome 12
L
Chromosome 16
M
Chromosome 19
N
Chromosome 21
O
Chromosome 22
A/B Compartment
LMNB1 Chip-seq
LADs
TAD Interaction Density
Reduced
Increased
Unchanged

Figure S3 – T21 induced disruption of the nuclear lamina in NPCs, Related to Figure 3. (A) Immunofluorescence of H3K9me3 (green) and LMNB1 (red) in Iso-E and Iso-T iPSCs. (B) Quantification of LMNB1 staining intensity in the isogenic pair of NPCs. Each dot on the histogram represents a replicate experiment of ~2,500 total nuclei analyzed for the isogenic pair of iPSCs. (C) Quantification of H3K9me3 staining intensity in the isogenic pair of NPCs. (D) Quantification of H3K9me3 staining intensity in the isogenic pair of NPCs. Each dot on the histogram represents a replicate experiment of ~440 total nuclei analyzed for the isogenic pair of NPCs. (E, F) Quantification of the number of H3K9me3 spots that are not colocalized with LMNB1 in Iso-E and Iso-T iPSCs (E) and NPCs (F). (G) Heatmap and histogram of the Pearson correlation of the replicates for LMNB1 ChIP-seq experiments from Iso-E and Iso-T NPCs. (H) IGV plot of LMNB1 ChIP-seq of Iso-E (blue) and Iso-T (light-blue) of region of chromosome 1 (top) and chromosome 19 (bottom). (I) Scatter plot of LMNB1 enrichment over input in Iso-E (x-axis) and Iso-T (y-axis) of the genomic region in the B-compartment. (J-O) IGV plot of A-compartment (Iso-E (yellow) and Iso-T (orange)) and B-compartment (Iso-E (blue) and Iso-T (light-blue)), LMNB1 ChIP-seq of Iso-E (blue) and Iso-T (light-blue), as well as the location of LADs (black) and the differentially interacting TADs (reduced-ID (blue), increased-ID (red) and unchanged-ID (gray)) an 87Mb region of chromosome 3 (J), chromosome 12 (K), chromosome 16 (L), chromosome 19 (M), chromosome 21 (N) and chromosome 22 (O).

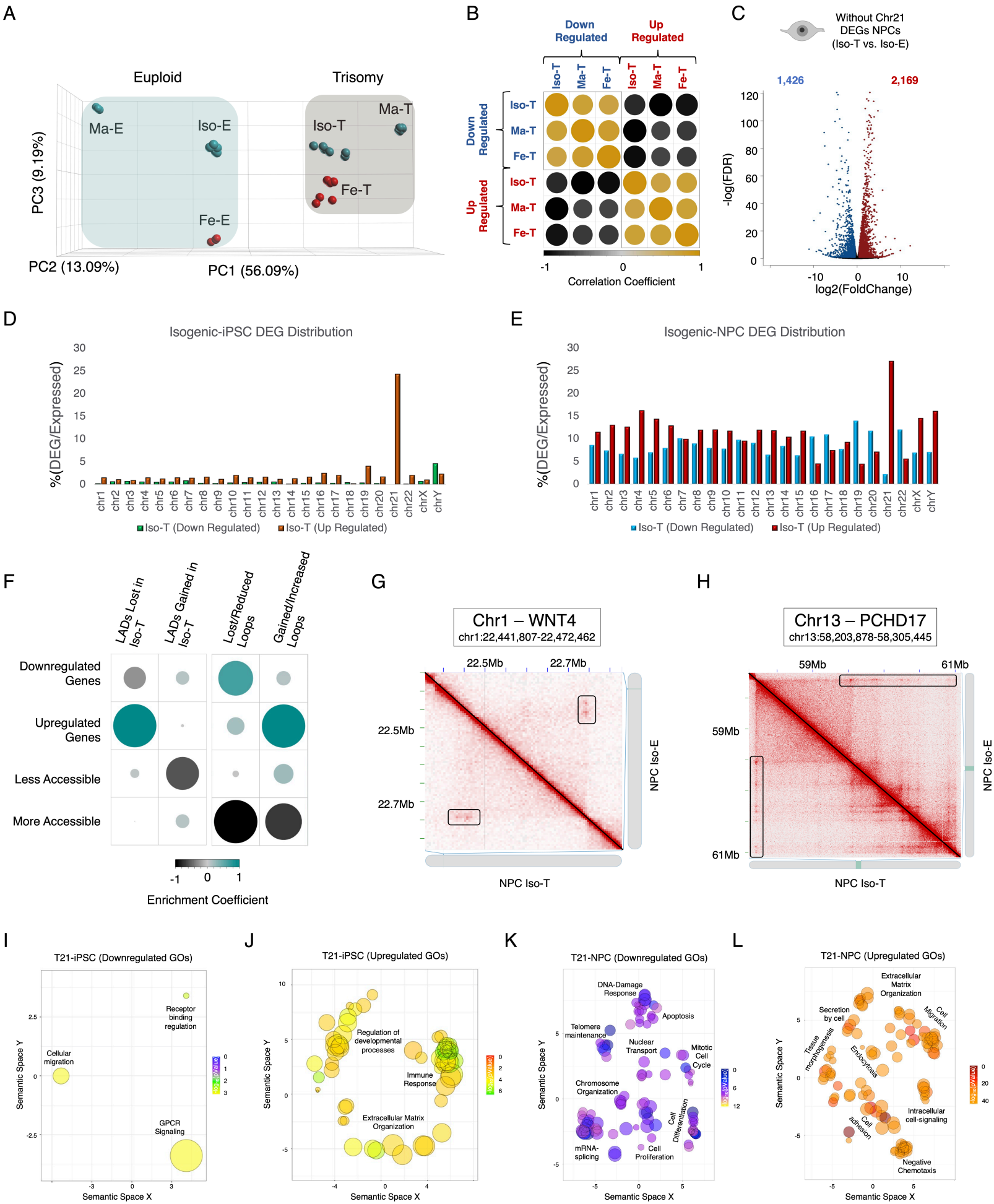
A
Euploid
Trisomy
Ma-E
Iso-E
Iso-T
Ma-T
Fe-T
Fe-E
PC3 (9.19%)
PC2 (13.09%)
PC1 (56.09%)
B
Down Regulated
Up Regulated
Iso-T
Ma-T
Fe-T
Correlation Coefficient
C
Without Chr21 DEGs NPCs (Iso-T vs. Iso-E)
1,426
2,169
-log(FDR)
log2(FoldChange)
D
Isogenic-iPSC DEG Distribution
%(DEG/Expressed)
Iso-T (Down Regulated)
Iso-T (Up Regulated)
E
Isogenic-NPC DEG Distribution
F
LADs Lost in Iso-T
LADs Gained in Iso-T
Lost/Reduced Loops
Gained/Increased Loops
Downregulated Genes
Upregulated Genes
Less Accessible
More Accessible
Enrichment Coefficient
G
Chr1 – WNT4
chr1:22,441,807-22,472,462
NPC Iso-E
NPC Iso-T
H
Chr13 – PCHD17
chr13:58,203,878-58,305,445
I
T21-iPSC (Downregulated GOs)
Receptor binding regulation
Cellular migration
GPCR Signaling
Semantic Space X
Semantic Space Y
J
T21-iPSC (Upregulated GOs)
Regulation of developmental processes
Immune Response
Extracellular Matrix Organization
K
T21-NPC (Downregulated GOs)
DNA-Damage Response
Apoptosis
Telomere maintenance
Nuclear Transport
Mitotic Cell Cycle
Chromosome Organization
Cell Differentiation
mRNA-splicing
Cell Proliferation
L
T21-NPC (Upregulated GOs)
Extracellular Matrix Organization
Secretion by cell
Cell Migration
Tissue morphogenesis
Endocytosis
Intracellular cell-signaling
Cell adhesion
Negative Chemotaxis

Figure S4 – T21 induced differential TAD-interactions are correlated with DEGs in NPCs, Related to Figure 4. (A) Principal component analysis (PCA) identified separation by karyotype for the male (teal) and female (red) euploid and trisomic NPCs. (B) Randomized permutation test represented as a correlation heatmap of downregulated (blue) and upregulated (red) genes identified in T21 NPCs. Color intensity (positive correlations are displayed in yellow and negative correlations in black) and the size of the circle are proportional to the correlation coefficients of the DEGs induced by T21 in the NPCs identified through pairwise comparisons (Iso-T vs. Iso-E, Ma-T vs. Ma-E and Fe-T vs Fe-E). (C) Volcano plots of the differentially expressed genes (DEGs) identified genome-wide in the isogenic pair of NPCs where the data was normalized and processed in the absence of genes from chromosome 21. (D, E) Histogram of the percent DEG distribution per chromosome for the isogenic pair of iPSCs (D) and NPCs (E). (F) Enrichment plot of the randomized permutation test between DEGs and DARs with LADs that are lost or gained in NPCs harboring T21 as well as with differentially looped genes. Color intensity (positive correlations are displayed in green and negative correlations in black) and the size of the circle are proportional to the enrichment coefficients. (G, H) Representative Hi-C interaction maps depicting chromosomal loops that are lost (G) or gained (H) in T21-NPCs (Iso-T, bottom half over diagonal line) as compared to euploid NPCs (Iso-E, top half over diagonal line). (I-L) Gene ontology visualized in semantic similarity-based scatterplots for downregulated and upregulated genes identified in iPSCs (I, J) and NPCs (K, L).

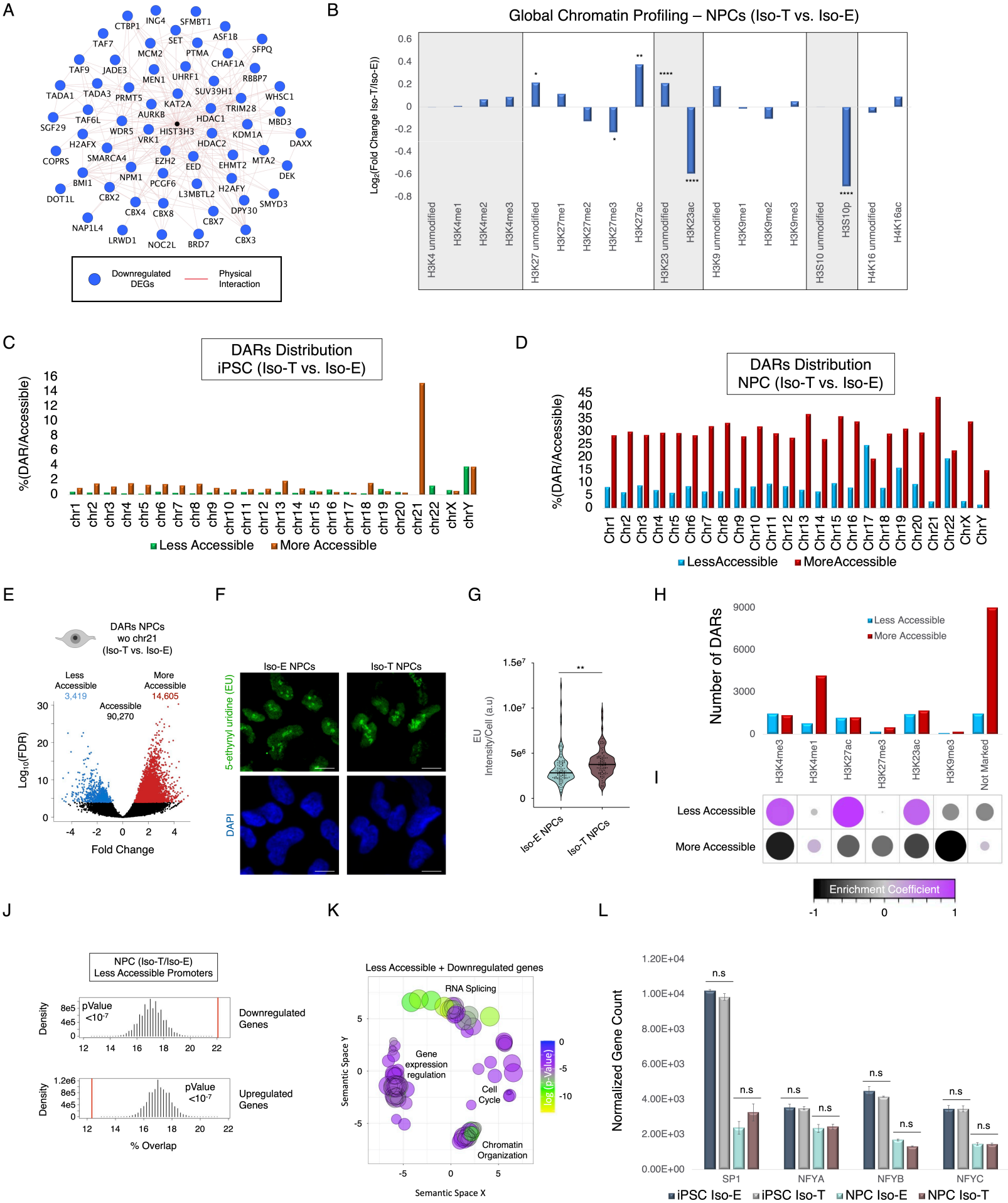
A
ING4
SFMBT1
CTBP1
ASF1B
SET
TAF7
SFPQ
MCM2
PTMA
CHAF1A
TAF9
JADE3
MEN1
UHRF1
RBBP7
TADA1
TADA3
PRMT5
SUV39H1
WHSC1
KAT2A
TRIM28
TAF6L
AURKB
MBD3
SGF29
HDAC1
WDR5
HIST3H3
KDM1A
DAXX
VRK1
H2AFX
HDAC2
COPRS
SMARCA4
EZH2
EED
EHMT2
MTA2
DEK
NPM1
BMI1
PCGF6
H2AFY
DOT1L
CBX2
L3MBTL2
SMYD3
CBX4
CBX8
DPY30
CBX7
NAP1L4
LRWD1
NOC2L
BRD7
CBX3
Downregulated DEGs
Physical Interaction
B
Global Chromatin Profiling – NPCs (Iso-T vs. Iso-E)
Log2(Fold Change Iso-T/Iso-E))
H3K4 unmodified
H3K4me1
H3K4me2
H3K4me3
H3K27 unmodified
H3K27me1
H3K27me2
H3K27me3
H3K27ac
H3K23 unmodified
H3K23ac
H3K9 unmodified
H3K9me1
H3K9me2
H3K9me3
H3S10 unmodified
H3S10p
H4K16 unmodified
H4K16ac
C
DARs Distribution
iPSC (Iso-T vs. Iso-E)
%(DAR/Accessible)
Less Accessible
More Accessible
D
DARs Distribution
NPC (Iso-T vs. Iso-E)
%(DAR/Accessible)
LessAccessible
MoreAccessible
E
DARs NPCs
wo chr21
(Iso-T vs. Iso-E)
Less Accessible
3,419
More Accessible
14,605
Accessible
90,270
Log10(FDR)
Fold Change
F
Iso-E NPCs
Iso-T NPCs
5-ethynyl uridine (EU)
DAPI
G
EU
Intensity/Cell (a.u)
Iso-E NPCs
Iso-T NPCs
H
Number of DARs
Less Accessible
More Accessible
I
Less Accessible
More Accessible
Enrichment Coefficient
J
NPC (Iso-T/Iso-E)
Less Accessible Promoters
Density
pValue
<10-7
Downregulated Genes
Upregulated Genes
% Overlap
K
Less Accessible + Downregulated genes
RNA Splicing
Gene expression regulation
Cell Cycle
Chromatin Organization
Semantic Space X
Semantic Space Y
log (p-Value)
L
Normalized Gene Count
n.s
SP1
NFYA
NFYB
NFYC
iPSC Iso-E
iPSC Iso-T
NPC Iso-E
NPC Iso-T

Figure S5 – T21 induced alterations of chromatin-accessibility is correlated with DEGs in NPCs, Related to Figure 5. (A) Interactome of downregulated genes (blue circles) associated with the histone H3 pathway. (B) Mass spectrometry-based global chromatin profiling (GCP) analysis of various histone modifications (x-axis) represented as a $\log_2$ fold change (y-axis) of the isogenic pair of NPCs (Iso-T/Iso-E). (C, D) Histogram of the distribution of the percent DARs per chromosome for the isogenic pair (Iso-T vs. Iso-E) of iPSCs (C) and NPCs (D). (E) Volcano plots of the differentially accessible regions (DARs) identified genome-wide in the isogenic pair of NPCs where the data was normalized and processed in the absence of chromosome 21. (F, G) Nascent RNA stained for 5-ethynyl uridine (EU, green) (F) and quantification represented as a violin plot where each dot represents a single cell, three replicates for both Iso-E and Iso-T were analyzed and ~300 cells were analyzed for the isogenic pair of NPCs (G). (H) Histogram of the number of less accessible (blue) or more accessible (red) regions associated with previously published histone modifications in H1-NPCs. (I) Enrichment analysis (10 million randomized permutations) of less and more accessible regions with previously published histone modification ChIP-seq datasets from H1-NPCs. Color intensity (positive correlations are displayed in purple and negative correlations in black) and the size of the circle are proportional to the enrichment coefficients. (J) Randomized permutation correlation analysis between the downregulated (top) and upregulated (bottom) DEGs identified as a consequence of T21 in the isogenic pair (Iso-T vs. Iso-E) of NPCs with differentially accessible promoters. Histogram represents the range of expected overlap percentage and the red lines represent the observed percentage overlap. (K) Gene ontology visualized in semantic similarity-based scatterplots for downregulated genes with less accessible promoters. (L) Histogram of normalized transcript counts for SP1 and the different isoforms of NFY (NFYA, NFYB and NFYC).

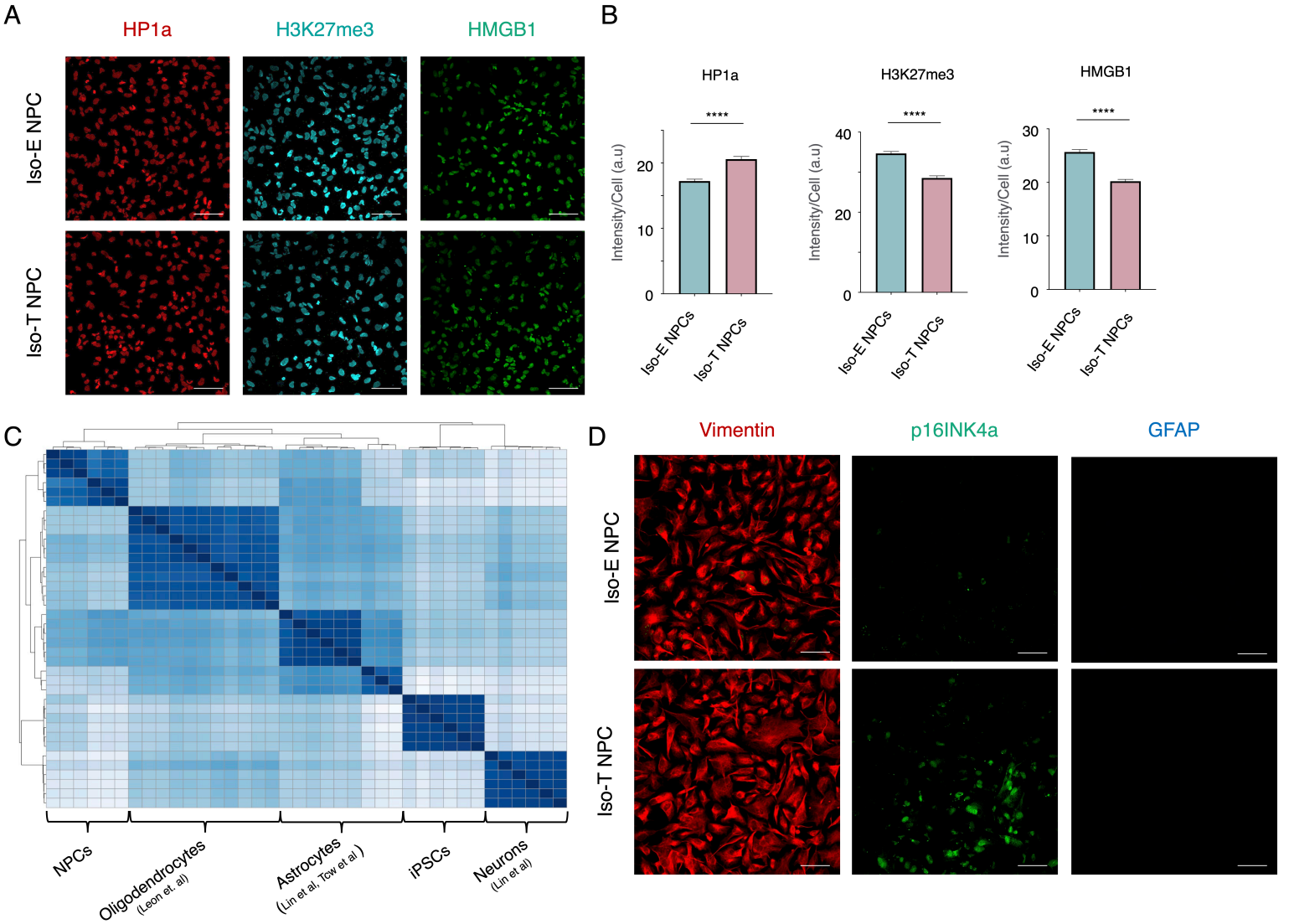
A
HP1a
H3K27me3
HMGB1
Iso-E NPC
Iso-T NPC
B
HP1a
H3K27me3
HMGB1
****
Intensity/Cell (a.u)
Iso-E NPCs
Iso-T NPCs
C
NPCs
Oligodendrocytes
(Leon et. al)
Astrocytes
(Lin et al, Tcw et al)
iPSCs
Neurons
(Lin et al)
D
Vimentin
p16INK4a
GFAP
Iso-E NPC
Iso-T NPC
E
Iso-E Astrocyte
GFAP
Vimentin
GFAP/Vimentin/DAPI
G
1,443 Downregulated T21 only Genes
Transcriptional Regulation
Embryonic Morphogenesis
Cell Signaling
Semantic Space X
Semantic Space Y
log (p-Value)
H
1,391 Downregulated OSIS only Genes
Axon Guidance
Cell Division
Lipid Response
Cell Adhesion
Signal Transduction
Chromosome Organization
I
2,140 Upregulated T21 only Genes
Chemotaxis
Regulation of Cell Differentiation
Cell Adhesion
Cell Morphogenesis
J
1,195 Upregulated OSIS only Genes
Vesicle Mediated Transport
Phosphorylation
Cell Migration
Immune Response
Cytokine Mediated Signaling
ER Stress

Figure S6 – T21 induces senescence in NPCs, Related to Figure 6. (A) Immunofluorescence of HP1a (red, left), H3K27me3 (cyan, middle) and HMGB1 (green, right) in Iso-E (top) and Iso-T (bottom) NPCs. (B) Quantification of HP1a (left), H3K27me3 (middle) and HMGB1 (right) staining mean intensity in the isogenic pair of NPCs. Each dot on the histogram represents a replicate experiment of ~2,000 total nuclei analyzed for the isogenic pair of NPCs. (C) Clustering of genome-wide transcriptomic data of the isogenic pair of iPSCs and NPCs with previously published iPSC derived neurons, astrocytes and oligodendrocytes. (D) Staining to ensure senescent NPCs ($p16INK^{4a}$, green) do not undergo premature astrocytic differentiation (GFAP, blue). Cells were stained for a pan neural marker vimentin (red). (E) Astrocytes derived from euploid NPCs stained for GFAP (green), vimentin (red) and DAPI (bue). (G-J) Gene ontology visualized in semantic similarity-based scatterplots associated with downregulated genes uniquely identified in T21-NPCs (G) and OSIS cells (H), and upregulated genes uniquely identified in T21-NPCs (I) and OSIS cells (J).

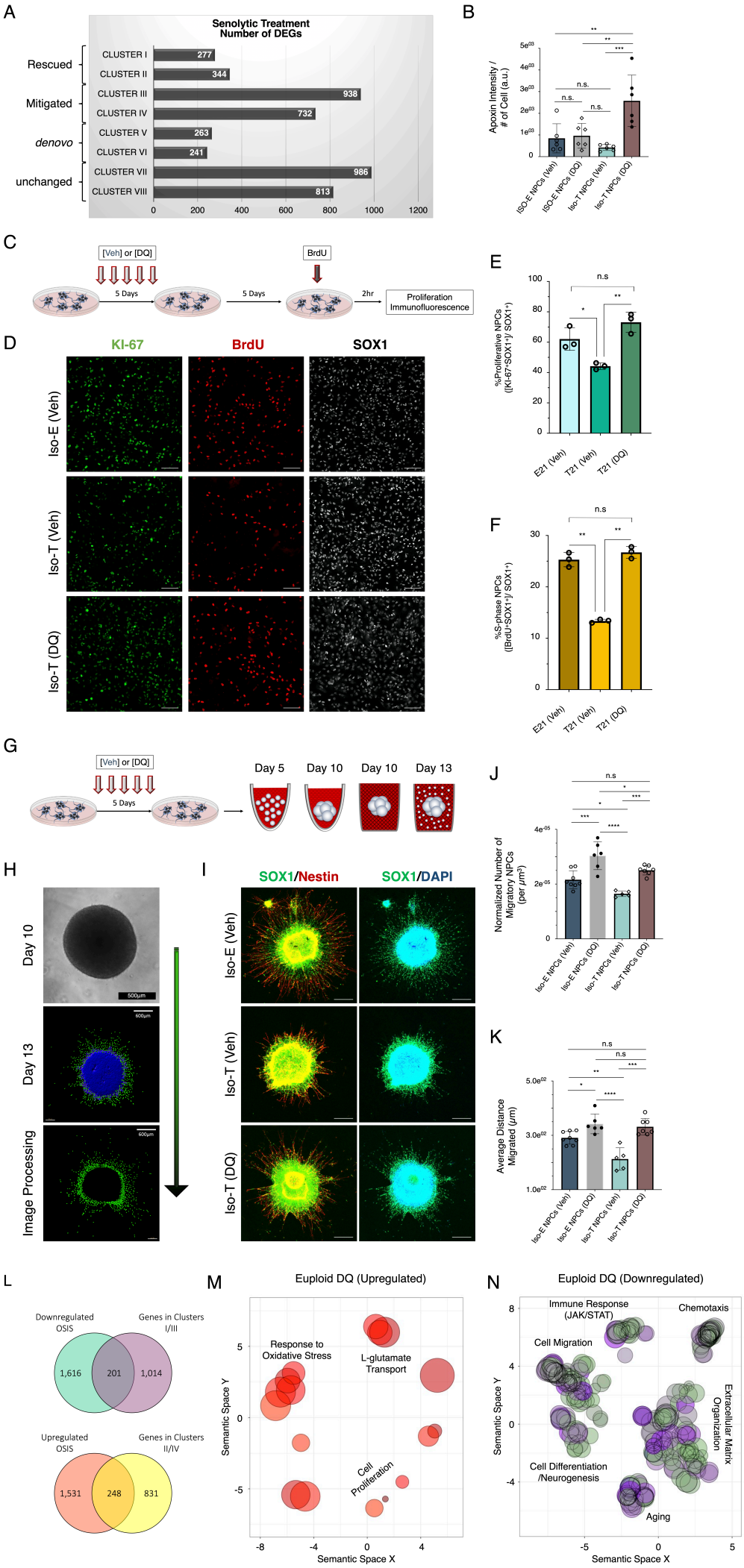
A
Senolytic Treatment
Number of DEGs
Rescued
Mitigated
denovo
unchanged
CLUSTER I
CLUSTER II
CLUSTER III
CLUSTER IV
CLUSTER V
CLUSTER VI
CLUSTER VII
CLUSTER VIII
277
344
938
732
263
241
986
813
0
200
400
600
800
1000
1200
B
Apoxin Intensity / # of Cell (a.u.)
n.s.
ISO-E NPCs (Veh)
ISO-E NPCs (DQ)
Iso-T NPCs (Veh)
Iso-T NPCs (DQ)
C
[Veh] or [DQ]
5 Days
5 Days
BrdU
2hr
Proliferation Immunofluorescence
D
KI-67
BrdU
SOX1
Iso-E (Veh)
Iso-T (Veh)
Iso-T (DQ)
E
n.s
%Proliferative NPCs ([KI-67+SOX1+]/ SOX1+)
E21 (Veh)
T21 (Veh)
T21 (DQ)
F
n.s
%S-phase NPCs ([BrdU+SOX1+]/ SOX1+)
E21 (Veh)
T21 (Veh)
T21 (DQ)
G
[Veh] or [DQ]
5 Days
Day 5
Day 10
Day 10
Day 13
H
Day 10
Day 13
Image Processing
500μm
600μm
I
SOX1/Nestin
SOX1/DAPI
Iso-E (Veh)
Iso-T (Veh)
Iso-T (DQ)
J
n.s
Normalized Number of Migratory NPCs (per μm²)
Iso-E NPCs (Veh)
Iso-E NPCs (DQ)
Iso-T NPCs (Veh)
Iso-T NPCs (DQ)
K
n.s
Average Distance Migrated (μm)
Iso-E NPCs (Veh)
Iso-E NPCs (DQ)
Iso-T NPCs (Veh)
Iso-T NPCs (DQ)
L
Downregulated OSIS
Genes in Clusters I/III
1,616
201
1,014
Upregulated OSIS
Genes in Clusters II/IV
1,531
248
831
M
Euploid DQ (Upregulated)
Response to Oxidative Stress
L-glutamate Transport
Cell Proliferation
Semantic Space Y
Semantic Space X
N
Euploid DQ (Downregulated)
Immune Response (JAK/STAT)
Chemotaxis
Cell Migration
Extracellular Matrix Organization
Cell Differentiation /Neurogenesis
Aging
Semantic Space Y
Semantic Space X

Figure S7 – Senolytic drug combination of dasatinib and quercetin alleviates the cell migration and proliferation deficits induced by T21 in NPCs, Related to Figure 7. (A) Number of genes identified in each transcriptional cluster after senolytic drug treatment. (B) Quantification of apoptotic NPCs (Iso-E and Iso-T) after 6hrs of treatment with vehicle or DQ, measured as the average apoxin intensity. (C) Schematic cartoon representation of experimental design to asses cell cycle and proliferation of NPCs before and after senolytic drug treatment. (D) Representative immunofluorescence images of KI-67 (green) and BrdU (red) of euploid (Iso-E) and trisomic (Iso-T), before and after senolytic treatment of NPCs (SOX1 (white)) used to calculate the proliferative and cycling NPCs after senolytic drug treatment. (E) Quantification of the percentage of proliferative NPCs ([KI-$67^{+}SOX1^{+}$]/ $SOX1^{+}$) and (F) NPCs that have traversed through S phase within 2hrs pre assay termination [SOX+,KI-67+,BrdU+/ SOX1+, KI-67+, BrdU+]. Each dot on the histogram represents a replicate and ~2,000 NPCs were analyzed per condition. (G) Schematic representation of the 3D cell-migration assay performed on euploid (Iso-E) and trisomic (Iso-T) NPCs treated with vehicle or DQ. (H) Image processing pipeline utilizing Imaris to assess the number and distance of migratory NPCs after senolytic drug treatment. (I) Immunofluorescence of SOX1 (green) and Nestin (red) in DQ-treated trisomic (Iso-T (DQ) bottom), vehicle-treated euploid (Iso-E (veh) top) and trisomic (Iso-T (veh) middle) neurospheres (imaged at 5x objective). (J, K) Quantification of the total number of migratory cells normalized to the total volume of DAPI (J) and average distance traveled by NPCs out of the neuroshphere (K). Each dot on the histogram represents 5 neurospheres analyzed per condition and 5,000 -7,000 NPCs were analyzed per condition. (L) Venn diagram of overlapping SA-downregulated (top) and SA-upregulated (bottom) identified in NPCs harboring T21 and compared to genes in clusters ameliorated after DQ treatment. (M, N) Gene ontology visualized in semantic similarity-based scatterplots for upregulated (M) and downregulated (N) genes identified in euploid NPCs after DQ treatment.