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Supporting Online Material for

An Aneuploid Mouse Strain Carrying Human Chromosome 21 with Down Syndrome Phenotypes

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MATERIALS AND METHODS

Animal Welfare. All experiments were performed in compliance with UK Home Office regulations and the UK Medical Research Council and Wellcome Trust guidelines.

Cell culture. ES cell lines were grown on neo^R embryonic fibroblast feeder layers in DMEM with 15%-20% FCS supplemented with 2 mM L-glutamine and non-essential amino acids (Invitrogen) with β-mercaptoethanol and leukemia inhibitory factor (LIF). The Hsa21 donor cell line, 739, was cultured in DMEM with 10% FCS, 2mM L-glutamine and non-essential amino acids. All cells were maintained in an environment of 5% CO_2 with 95% humidity at 37°C. For the MPI VI ES cell line, karyotyping revealed 14 out of 16 metaphase spreads examined (88%) had a 39, X,0 karyotype which gives rise to fertile female mice.

XMMCT. We carried out XMMCT(*1*) using a recipient female 129S2 mouse ES cell line, MPI VI, kindly provided by Dr Anne Voss (*2*). Hsa21 came from the donor cell line 739, which we had derived from the human cell line HT1080 by targeting a neo^R gene into Hsa21 at *D21S55* (*3*). A total of 1.81 x 10^9 microcells from 739 were γ-irradiated at doses of 3.5, 20 or 50 kRad to induce chromosomal fragmentation, and were then fused to 7.62 x 10^8 MPI VI ES cells in 9 XMMCT experiments. After selection in G418 for the targeted human chromosome (*3*), 18 Hsa21 positive MPI VI cell lines were established. Human chromosomal content was determined by FISH (*3*): 6 (33%) cell lines contained Hsa21 as a freely segregating human chromosome. One cell line (80-6) contained variable numbers of Hsa21 and so was subcloned at limiting dilution to select for a cell line containing a single Hsa21 (80-6c2). Of the 6 Hsa21 positive cell lines established, 5 (74-2c1, 80-2, 80-6c2, 86-1 and 91-1) (28%) of the original 18 Hsa21-positive clones contained Hsa21 as the only human contribution, as determined by reverse-FISH (*3*). Transchromosomic cell line 91-1 came from an experiment in which microcells were irradiated with a dose of 20 kRad.

Estimating Hsa21 gene content in the 91-1 cell line. ENSEMBL (December 2004 archive) estimate the total number of known genes on Hsa21 is 243. From our scoring of Hsa21 markers, in the proximal 91-1 deletion, bounded by *CXADR* and *D21S1922*, some or all of the genes *BTG3*, *Q96PM7*, *C21ORF91*, *CHODL*, *PRSS7* and *PPIA* are deleted – i.e. up to 6 genes. For the distal deletion, bounded by *IFNAR1* and *RUNX1*, some or all of the genes *IFNGR2*, *C21ORF4*, *C21ORF55*, *GART*, *SON*, *DONSON*, *ATP50*, *MRPS6*, *C21ORF82*, *KCNE2*, *KCNE1*, *DSCR1* and *CLIC6* are deleted – i.e. up to 13 genes. Thus 91-1 is missing up to 19 genes, with an estimated 224 present, thus 92% of the Hsa21 genes are present.

Generation of transchromosomic ES cell lines and chimeras. Transchromosomic ES cells, blastocyst injections and chimeric mice were produced as previously described (*3*).

Analysis of cell line and mouse DNA content. Hsa21 loci were amplified by PCR or probed on

Southern blots as previously described (*3*); the markers used are shown in Fig. 1A and table S1. We controlled for possible contamination of the samples by human DNA by amplifying all samples with non-Hsa21 human genes *SOX9* (HsaY) and *DMRT1* (Hsa9). Hsa21-specific, IRS- and COT-1 fluorescence in situ hybridization (FISH) were carried out as previously described (*3*); for whole chromosome painting we used an Hsa21-whole chromosome specific probe (Cambio).

Generation of Tc1 mice strains. Tc1 males and females, were crossed to either BALB/c, C3H/He or C57BL/6J inbred lines, or F1(C57BL/6Jx129S8) animals.

Analysis of Hsa21 retention in mouse tissues. *Quantitative PCR*: Genomic DNA was extracted from tissues from 8 Tc1 and 8 wild-type littermates of 11-13 months old using Chelex (Sigma). Approximately 1-2 mm^3 of tissue was placed into 5% Chelex in molecular biology grade water. Proteinase K was added (0.2 mg/ml), and the tissue was left overnight at 56°C. Samples were then vortexed, incubated in a boiling water bath for 8 mins, vortexed again and centrifuged at >10,000 x g for 2 mins. The supernatant was used for subsequent PCRs. We amplified the DNA using human-specific primers from the *SAMSN1* locus by Taqman PCR and as a control we used a primer pair specific for the mouse *Apob* gene (*4*). The Taqman values were obtained from duplicate samples and were normalised to values ascertained from 91-1 cell line genomic DNA (in which cells were verified by FISH to have 94% Hsa21 retention). This allowed us to calculate the percentage retention in each tissue; no signal for the human primer was detected in genomic DNA from the MPI VI cell line (table S3). *Interphase FISH*: Spleen and brain were harvested from 8 Tc1 mice age 3-5 months and placed in air-buffered IMDM, 5% fetal calf serum, on ice. Cell suspensions were generated by passing organs through a 70 µm cell strainer (BD Falcon), centrifuged at 250 x g for 5 min, and washed in PBS. In the splenocyte suspensions, erythrocytes were lysed by incubating the cells in 0.15M NH_4Cl, 10mM $KHCO_3$, 0.1mM Na_2EDTA, pH7.2-7.4 for 2 min at room temperature, and then washed again in PBS. Brain and spleen cells were resuspended in 0.075 M KCl and left at 37ºC for 2-3 min. Cells were pelleted for 5 min at 100 x g and then slowly fixed in methanol/acetic acid (3:1). Fixative was changed three more times before resuspending the cells in 0.5-1 ml fixative. Cell suspensions were dropped on clean slides and air-dried. Cells were further fixed by dropping 70% acetic acid on each slide and dried again. For staining interphase nuclei we used Hsa21 Starfish chromosome paint, labelled with FITC (Cambio) and a control mouse X Starfish chromosome paint, labelled with Cy3 (Cambio). As a counterstain we used Vectashield mounting medium with DAPI (Vector Laboratories). Hybridisation and detection was carried out according to the manufacturer's protocols (www.cambio.co.uk).

Transcription analysis. Total RNA was isolated from two E14.5 Tc1 whole embryos and one wild-type littermate by mechanical homogenisation of the sample in Trizol (Invitrogen) and precipitation of RNA as per manufacturer's instructions followed by resuspension in Tris-EDTA. RNA was processed for hybridisation to HG-U133A GeneChips according to the standard

Affymetrix protocol (www.affymetrix.com). Labelled cRNA samples were first hybridised to Affymetrix Test3 arrays and scaled to an overall target intensity of 500 to check sample quality. All samples passed the quality criteria (>20% of probe sets were called present, and the 3'/5' *GAPDH* and β-actin ratios were less than 3). The samples were hybridised to Affymetrix Human Genome U133A GeneChips, scanned (Agilent Technologies UK Ltd, Stockport, UK) and analysed in MicroArray Suite v5 (Affymetrix UK Ltd, High Wycombe, UK).

Microarray analysis: HG-U133A arrays were scaled to an overall target intensity of 500 to allow comparisons to be made. Each Tc1 sample was compared to the wild-type sample, and probe sets called "Increased" by the software (P <0.01) in both comparisons were identified. Additional increased probe sets were found by changing the threshold P-value for a significant increase to 0.05, and by searching for genes which were detectable on both arrays and showed a >1.5-fold difference in signal intensity, regardless of the change call assigned by the software.

Assessing Hsa21 gene expression in Tc1 tissues by RT-PCR and immunoblotting. We assayed for the expression of selected genes in Tc1 tissues (compared to wild-type littermate mouse and to human brain) by RT-PCR and/or immunoblotting.

RT-PCR: Total RNA was extracted from E14.5 wild-type and Tc1 embryo littermates, from Tc1 and wild-type adult mice (10 weeks old) and from euploid human brain (65 year old normal female), by Trizol as described earlier and subjected to RT-PCR (OneStep RT-PCR kit; Qiagen) using human primer pairs for *APP* (f: 5′-tgccatctttgaccgaaacg-3′, r: 5′-cgggcatcaacaggctcaa-3′), *SOD1* (f: 5′-tggccgatgtgtctattgaa-3′, r: 5′-acctttgcccaagtcatctg-3′), *SIM2* (f: 5′-aaaaatcctccagagccacc-3′, r: 5′-gtggtagttgagcagcacga-3′), *DYRK1A* (f: 5′-gttggtcttggtcatttgcc-3′, r: 5′-aaaaacccaaatttcccacc-3′), *BACE2* (f: 5′- cctagcttatgccacacttg-3′, r: 5′-gctccacacatctgcatgg-3′) and with *GdX* as internal control (f: 5′-ggcagctgatctccaaagtcctgg-3′, r: 5′-aacgttcgatgtcatccagtgtta-3′). Samples were analysed by electrophoresis on a 2% agarose gel. *APP* and *BACE2* were analysed by SYBR-Green quantitative RT-PCR, using *GAPDH* as a normalising control (f: 5'-tcaccaccatggagaaggc-3', r: 5'-gctaagcagttggtggtgca-3').

Immunoblotting: Protein from mouse organs and human brain was extracted from Trizol-treated samples, after the extraction of RNA, as per manufacturer's instructions (Invitrogen). Five to 10 mg each extract was subjected to electrophoresis under reducing conditions on 10-15% SDS-PAGE gels and transferred to nitrocellulose membrane as per standard techniques. Membranes were probed with an appropriate primary α-SOD1 antibody (1/500, Chemicon) and detection was performed using an HRP-conjugated secondary antibody followed by enhanced chemiluminescence (ECL; Amersham) and exposure to X-ray film. Membranes were stripped and re-probed with an anti-beta tubulin primary antibody (1/2000, Sigma) to demonstrate equal loading of protein.

We found that human *APP* (RT-PCR, whole embryos, E14.5), human *SOD1* (RT-PCR of brain, age 10 weeks; western blot, heart, lung, liver, muscle, brain, kidney, spleen, testis, age 10

weeks), human *SIM2* (RT-PCR brain, heart, muscle, age 10 weeks), human *DYRK1A* (RT-PCR of brain, age 10 weeks) and human *BACE2* (RT-PCR, whole embryos, E14.5) were expressed in Tc1 mice (fig. S2).

Electrophysiology. Tc1 and wild-type littermate mice, aged 4-8 months, were anaesthetized with an intraperitoneal injection of urethane (1.8 g/kg). LTP was investigated in the dentate gyrus using a concentric bipolar stimulating electrode positioned in the perforant path (AP-level with Lamda, ML-3 mm from midline, DV-1.5 mm) and a glass micropipette recording electrode positioned in the hilus of the ipsilateral dentate gyrus (AP-2 mm posterior to bregma, ML-1.6 mm from midline, DV-1.5 mm, with minor variation judged by shape of response). Test responses were evoked using monophasic stimuli (0.033 Hz; 70-300 μA, 60 μs). Using a wide range of stimulus intensities input-output curves were generated plotting the average of three sampled responses for each intensity. Prior to assessment of LTP pairs of pulses (inter-pulse intervals, 10-1000 ms) were used to study paired-pulse facilitation of EPSP and population spike. EPSP slope facilitation was studied using a stimulus intensity sufficient to drive a pure EPSP response of around 1 mV. Population spike facilitation was studied using a higher stimulus intensity sufficient to evoke a spike of approximately 1 mV. Three responses were also averaged for each inter-pulse interval. To study LTP a baseline stimulus intensity was selected sufficient to drive a spike of approximately 1 mV and an EPSP slope approximately 40% of maximum. 40 baseline recordings were made over 20 min prior to applying a tetanic stimulation, consisting of 6 series of 6 trains of 6 stimuli at 400 Hz, 200 ms between trains, 20 s between series. During the tetanus pulse width was doubled. 120 further evoked recordings were then made at baseline frequency and intensity over the course of the following hour. The slope of the field excitatory postsynaptic potential (fEPSP) was used as an index of LTP and expressed as a percentage change relative to the mean response in the final 10 min before tetanus. Data are given as mean ± SEM.

Behavioural analysis.

*Habituation***:** Mice were exposed to the behavioural room for 30 mins and handled by the experimenter for 5 min on each of three days prior to behavioural testing in order to habituate them to the surroundings.

*Open field exploration***:** Locomotor activity was assessed over a 5-min period in an open walled (15 cm) arena. The floor of the field (45 x 33 cm) was marked into six square compartments of equal size (2 by 3). Tc1 mice (n=10) and wild-type littermate controls (n=12) were placed in the corner of one compartment facing the wall at the beginning of the session and subsequent exploration was recorded and scored. The number of crossings from one compartment to another (all four paws criterion) was used as an index of activity.

Novel object recognition: Tc1 mice (n=10) and littermate controls (n=12) were tested for learning and memory deficits in the novel object recognition task. The apparatus consisted of a dark circular arena (65 cm in diameter). Mice were given an extra habituation session in this arena for

10 min on a single day before exposure to objects. Training commenced the following day with two 10-min trials. In each of the trials, mice were placed at the centre of the arena and left to explore three differently shaped objects (made of Lego). Objects were cleaned with 70% ethanol after each trial. No significant difference in exploration levels was observed between the three objects. This training procedure was then repeated the following day. Memory of these objects was then tested 10 min later. Mice were placed back into the same arena, but one of the objects was replaced by a novel object of a different shape to any of the training objects. Time spent exploring each object was scored and the discrimination ratio (time spent exploring novel object/mean time exploring familiar object) was calculated.

Spontaneous alternation in a T-maze: The T-maze was made of plastic and consisted of two arms (60 cm in length) perpendicular to a central stem (60 cm in length). The floor of the maze was 8 cm in width and opaque, and covered with loose saw-dust mouse bedding, and the walls were 13 cm in height and transparent. Two opaque removable sliding gates were positioned so as to partition the two arms from the main stem and a third to create a home arena at the base of the stem from which the mice commenced each trial. The maze was positioned in the middle of a room with surrounding visual cues. The protocol consisted of a forced trial, in which the mouse was presented with only one open arm, which it could freely enter and explore. The sliding door to this arm was then closed and the mouse remained in the arm for 30s before being returned to the start arena. Saw-dust bedding was quickly re-scattered to disperse any odour cues in a further 30s period (total delay 1 min), and a free choice trial then followed in which both arms were left open for exploration. Exploration of the opposing arm to that presented in the forced trial was scored as alternation. This protocol was repeated nine further times with a 30 min gap between trials and the alternation rate scored as a percentage. 5 Tc1 mutants and 7 wild-type littermate controls were tested using this procedure.

Static rod paradigm: Coordination of movement was assessed using a wooden rod 60 cm in length and 2.8 cm in diameter. This rod protruded from a table elevated 80 cm above a padded floor. Mice were placed on the rod facing away from the home table and allowed a maximum period of 5 min to turn 180^0 and return to the table. The time taken to achieve this was scored for 5 Tc1 mutants and 7 wild-type littermates. Animals falling off were allocated a maximum score of 5 min. After reaching the end of the rod mice were retrieved and returned to the home cage. Scores were averaged over 10 trials each.

Elevated plus maze: Mice were allowed to freely explore a plastic plus-shaped maze, consisting of two opposing open un-walled arms 90 cm in length and 6.5 cm in width, two opposing enclosed arms of the same size, but with 10 cm high walls and lids, and a central 6.5 cm^2 open area. The maze was elevated 80 cm above the floor. Again saw-dust bedding was scattered on the floor of the maze and re-scattered after each of 5 Tc1 mutants and 7 wild-type controls were allowed to explore the maze. Time spent in the open and enclosed arms was scored and the

percentage of time spent in the open arms ((open time/total time) x 100) was calculated and used as an index of anxiety.

Statistical analysis: Non-parametric tests were used for all analyses as the data rarely fulfilled the requisite criterion of homogeneity of variance. All comparisons used unpaired two-tailed Mann-Whitney t-tests except the novel object recognition; here a repeated measures Friedman ranks ANOVA was used with a post-hoc Dunn's test to evaluate different comparisons between the three objects in the test phase.

Brain histopathology and cerebellar neuron counts. Brains from 7 wild-type and 7 Tc1 age-matched littermates, aged between and 9 and 21 months were formalin fixed, paraffin embedded and paraffin sections were cut at 3 μm nominal thickness. Sections were stained with haematoxylin and eosin or immunostained for MAP2, neurofilament 200, synaptophysin, glial fibrillary acidic protein (GFAP), Iba-1 (microglia), Tau AT270, Tau AT8, and β A4 amyloid. For determination of cell counts, cerebellar granule neurons in four areas were counted in areas of 0.01-0.02 mm^2 and analysed using Student's two-tailed t-test.

Heart analysis techniques. Studies were carried out on 11 E14.5 Tc1 fetuses and 10 wild-type littermates, blind to genotype. Genotypes were decoded after phenotypic analysis. Fetuses were fixed in paraformaldehyde and processed for wax embedding. Transverse sections were cut and stained with haematoxylin and eosin using standard techniques, and were photographed using a Zeiss Axiophot microscope and a digital camera.

Assessment of facial bone morphology. For the preliminary analyses, adult heads were skinned, fixed in 95% ethanol and stored at room temperature. As much flesh was removed from the heads as possible prior to bleaching them in a 3% H_2O_2 solution and placing them on a shaker at room temperature overnight. For the next 5 days further flesh was removed from the heads, without damaging the underlying bones and the heads were replaced into fresh 3% H_2O_2 solution. The heads were then placed into 1% H_2O_2 for 2 days prior to washing in water and placing into fresh 1% sodium hypochlorite solution for 6 hours at room temperature on a shaker. The heads were then washed in water and stored in water overnight at 4°C. Heads were then placed into a 1% sodium hypochlorite solution for 3 hours before being washed and stored in water overnight at 4°C. All remaining tissues were removed from the skulls and they were placed in a 1% sodium hypochlorite for 1 hour before being left to air dry. Tc1 head skeletons were compared with littermate controls of the same age using conventional light microscopy. For the high-resolution investigations, 10 wild-type and 10 Tc1 littermate heads were scanned using an eXplore Locus micro-CT (GE Healthcare, London, Canada). Volumes produced after reconstruction consisted of cubic voxels (47 x 47 x 47 μm^3). In order to establish any differences in size and shape between the wild-type and Tc1 littermates morphometric analysis was performed. Based on work performed by Reeves, Richtsmeier and colleagues (*5-7*), 15 points located at anatomical landmarks on the skulls and mandibles were identified (fig. S5). Each point

was given a number and its co-ordinates recorded. On each skull, points were placed at the following locations: the nasale (1), the nasion (2), the bregma (3), the intersection of parietal and interparietal bones (4), the intersection of the interparietal and occipital bones at the midline (5), the frontal squamosal intersection at the temporal crest (6, 7) and the intersection of the parietal, temporal and occipital bones (8, 9) (fig.S5). On each mandible points were placed at the coronoid process (1, 3), the mandibular angle (2, 4), and the superior-most point on the incisor alveolar rim (5, 6). Euclidean distances were then calculated between selected points in order to establish any variation between the two populations. All data were then analysed using Microsoft Excel and distances between points in wild-type and Tc1 mice compared using T-tests (table S6).

T lymphocyte activation. To evaluate T cell activation, we stimulated cells from the spleen or lymph node with anti-CD3ε (2C11, 8μg/ml) in the presence or absence of anti-CD28 (37-51,10μg/ml) for 16 hr at 37^{o}C and used flow cytometry to monitor for the upregulation of the activation markers CD25 or CD69 on $CD4^{+}$ and $CD8^{+}$ cells (fig. S6).

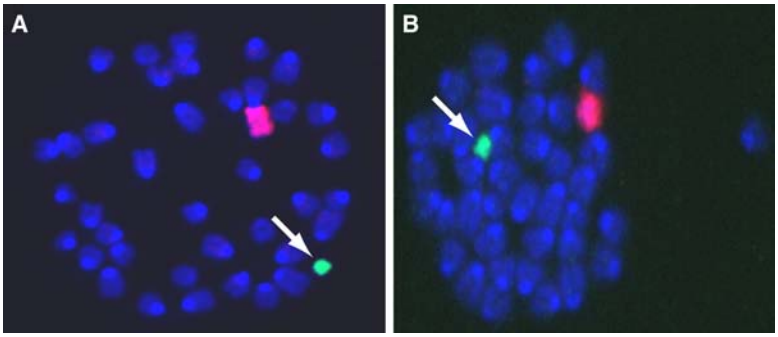

FIGURE S1. Fluorescence in situ hybridisation on metaphase spreads from the 91-1 cell line and Tc1 mouse.

(A) FISH analysis of metaphase spreads from transchromosomic ES cell line 91-1. Metaphase spreads were prepared from the 91-1 ES cell line and FISH was performed with a FITC-labelled paint specific for Hsa21 (green; arrowed) and a Cy3-labelled paint specific for MmuX (red) with a DAPI counterstain (blue). **(B)** FISH analysis of metaphase spreads from transchromosomic Tc1 mouse. Metaphase spreads were prepared from blood taken from the tail of a male Tc1 mouse backcrossed to C57BL/6 (N2 generation). FISH was performed with a FITC-labelled paint specific for Hsa21 (green; arrowed) and a Cy3-labelled chromosome paint specific for MmuY (red) with a DAPI counterstain (blue). Hsa21 is visible on the normal mouse karyotype. The same results were found in FISH of metaphase spreads from spleens of male and female mice backcrossed to C57BL/6 (N4 generation) (data not shown).

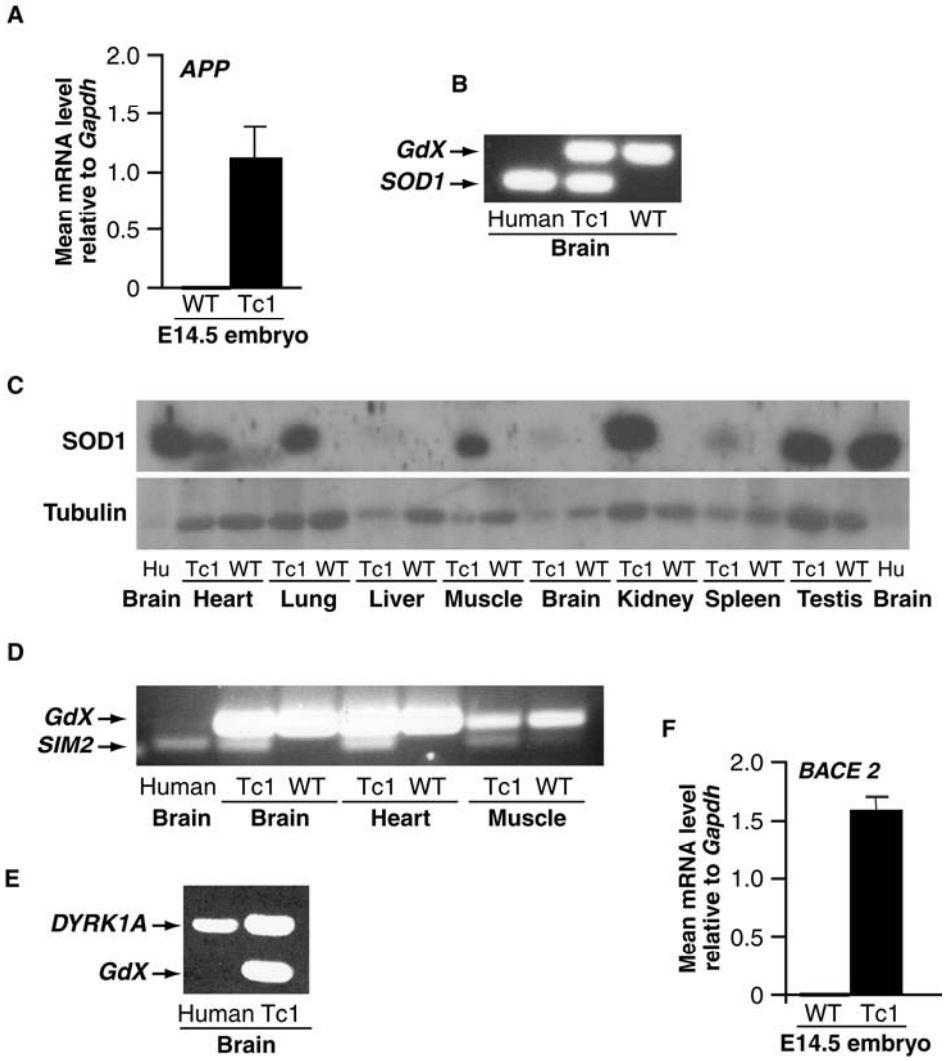

FIGURE S2. Expression of selected human genes previously implicated in various DS neuronal phenotypes, in Tc1 tissues by RT-PCR and/or immunoblotting.

(A) Quantitative RT-PCR for human *APP* in wild-type (WT) and Tc1 embryo littermates. RNA was extracted from two wild-type and two Tc1 whole embryos (E14.5) and analysed for expression of human *APP* by SYBR-Green RT-PCR using human-specific primers. Mouse *Gapdh* was used as a normalising control and samples were measured relative to a standard curve of transchromosomic embryo cDNA. Means and standard errors are shown. No human *APP* expression was detected in wild-type littermates. **(B)** Gel showing amplified products generated by RT-PCR of mRNA from the human *SOD1* and mouse *GdX* genes in RNA samples derived from human brain, and wild-type (WT) and Tc1 adult mouse brains. Mouse *GdX* gene expression served as a control. **(C)** Immunoblot of cell extracts from human brain (Hu), and wild-type (WT) and Tc1 adult mouse brains probed with antibodies against human SOD1 and mouse

β-tubulin. The latter served as a loading control. **(D)** RT-PCR for human *SIM2* in human brain, wild-type (WT) and Tc1 adult mouse brain, heart and muscle. Mouse *GdX* gene expression was again used as a control. **(E**) RT-PCR for human *DYRK1A* in human brain and Tc1 adult mouse brain. Mouse *GdX* gene expression was used as a control. **(F)** Quantitative RT-PCR for human *BACE2* in wild-type and Tc1 embryo littermates was carried out as in (A). No expression of human *BACE2* was detected in wild-type littermates.

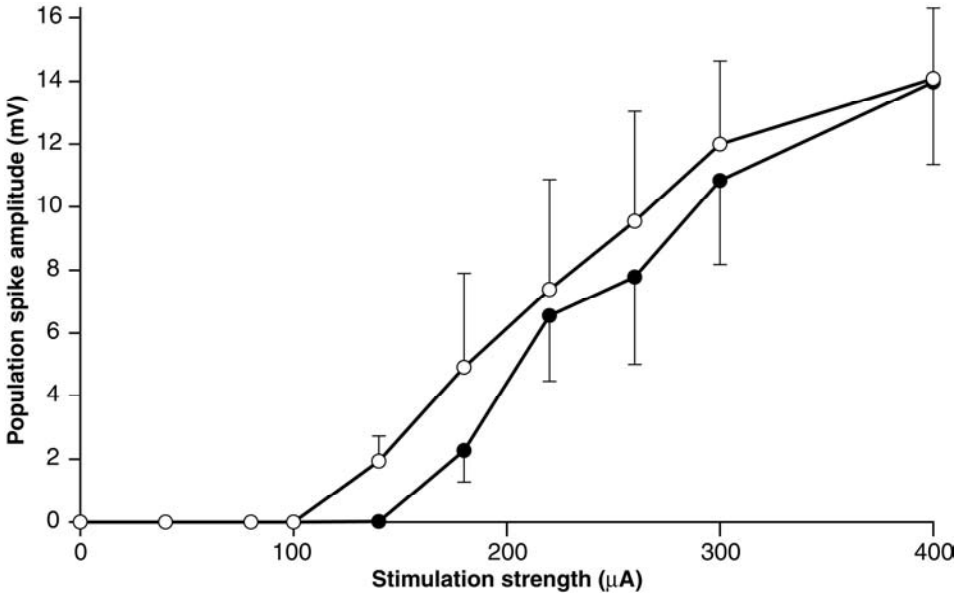

FIGURE S3. Input-output curve for population spike.

Input-output curves for the population spike are similar in wild-type (open circles) and Tc1 littermates (filled circles). Error bars are S.E.M.s.

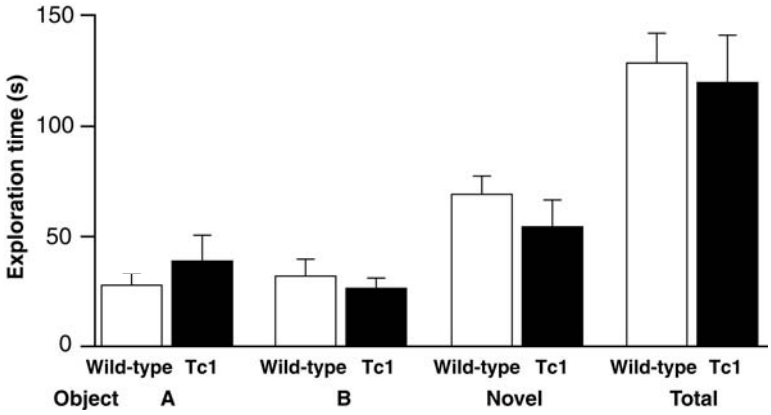

FIGURE S4. Absolute exploration times of familiar and novel objects.

Exploration of familiar objects A and B, the novel object and total time exploring all objects by wild-type (open bars) and Tc1 littermates (filled bars). Error bars are S.E.M.s.

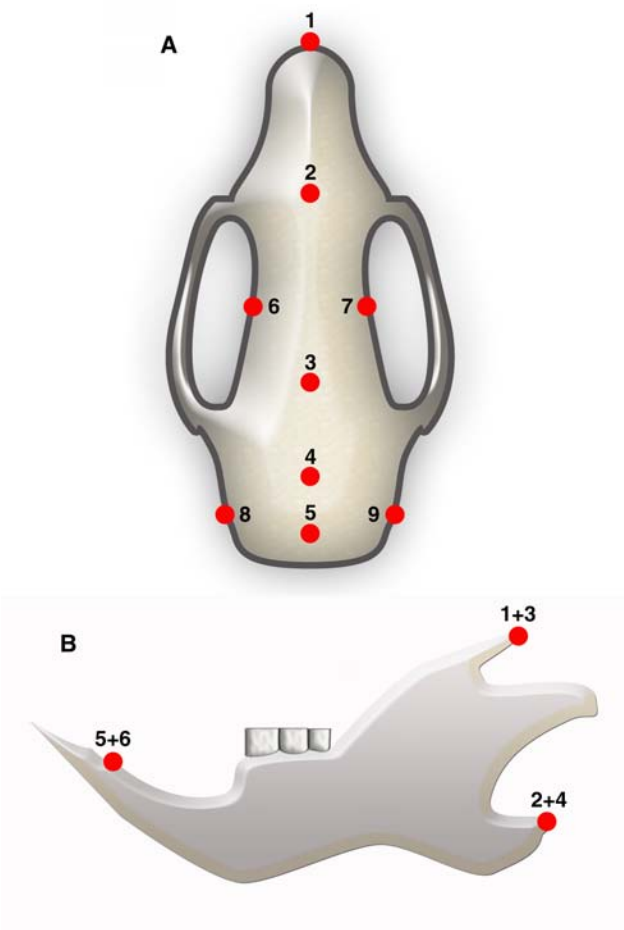

FIGURE S5. Diagrammatic representation the cranium and mandible showing the positions of 15 anatomical landmarks selected for morphometric analysis.

(A) The locations of 9 anatomical landmarks on the cranium are indicated: the nasale (1), the nasion (2), the bregma (3), the intersection of parietal and interparietal bones (4), the intersection of the interparietal and occipital bones of the midline (5), the frontal squamosal intersection of the temporal crest (6 and 7) and the intersection of the parietal, temporal and occipital bones (8 and 9). **(B)** The locations of 6 anatomical landmarks on the mandible are indicated: the coronoid process (1 and 3), the mandibular angle (2 and 4), the superior-most point on the incisor alveolar rim (5 and 6). Where two numbers are indicated for a single landmark on the mandible, the higher number refers to the same point on the right hand side of the head.

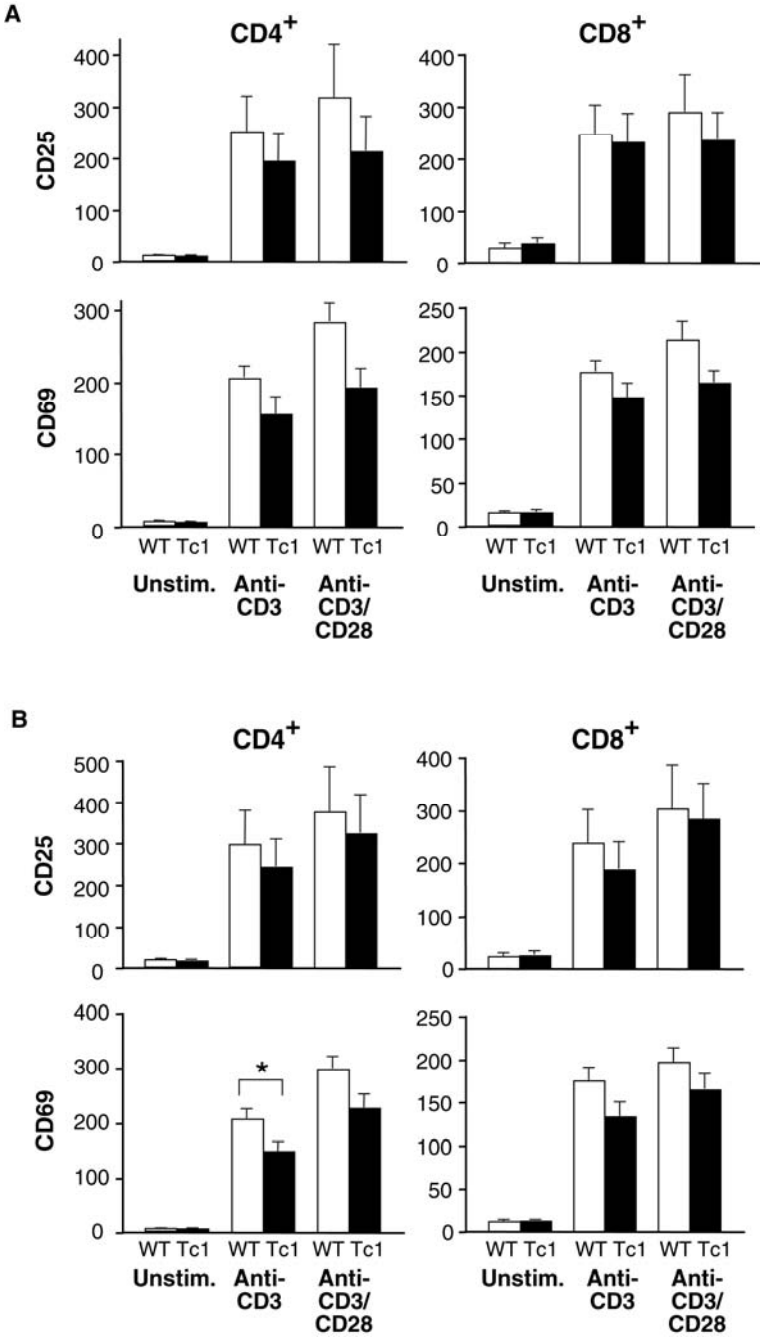

FIGURE S6. T cell activation.

Graphs showing mean (± S.E.M.) cell surface levels of CD25 or CD69 (as measured by mean fluorescence intensity of staining with antibodies) on $CD4^+$ or $CD8^+$ T cells from the **(A)** spleen or **(B)** lymph nodes of wild-type (white bars) or Tc1 mice (black bars) (n=7) either unstimulated

(Unstim.) or stimulated *in vitro* with anti-CD3ε antibodies alone or with anti-CD3ε and anti-CD28 antibodies. We consistently found that both $CD4^+$ and $CD8^+$ T cells from both spleen and lymph nodes of Tc1 mice upregulated CD25 and CD69 to a lesser extent compared to wild-type cells. This was observed with cells stimulated either with anti-CD3ε alone, or together with anti-CD28. While we saw a consistent reduction in the levels of CD25 and CD69 to 70-90% of the levels in wild-type cells, only one of the reductions was statistically significant (*, $P < 0.05$).

TABLE S1. Human markers used to assess the chromosome 21 content of transchromosomic cell lines and mice.

Gene or marker	Position
D21S5	36868 Hsa21p BAC probe (*3*)
D21Z1	centromeric repeat BAC probe (*3*)
STCH	14667638
D21S16	14752492
D21S13	15353747
CXADR	17807195
RH46998	17866133
BTG3, SHGC-172493	17899136
D21S385	18113719
PRSS7, D21S406	18602486
D21S11	19476134
D21S1922	21220691
D21S417	21290208
D21S414	21350649
D21S214	21637618
D21S278	21888794
D21S367	24500930
APP	26175498
D21S82	29137533
D21S304	31341896
D21S389	31848426
SOD1	31961507
IFNAR1	33649973
IFNGR2, RH80707	33726679
IFNGR2, SHGC-12906	33731272
D21S1254	33997382
KCNE1	34743378
DSCR1, D21S323	34891243
CLIC6, SHGC-170123	34983739
RH120345	35028355
RUNX1	35115486
CBR1	36366876
SIM2	37043696
DSCR5	37359773
D21S336	37580180
D21S394	37660274
DYRK1A	37780639
KCNJ6	37919062
D21S55	38006303
ERG	38675652
ETS2	39117007
PCP4	40223166
MX1	41752620
ABCG1	42589223
D21S49	42772994
SLC37A1	42868574
PWP2H	44315536
PFKL	44543467
PTTG1IP	45095560
ITGB2	45130615
D21S403	46198352
COL6A1	46248374

Positions according to the most proximal base pair of the primer pairs for PCR markers, ENSEMBL BLASTView, December 2004

TABLE S2. Breeding profile of Tc1 mice and transmission of Hsa21 on different genetic backgrounds.

Transchromosomic male mouse Tc1-01 on a F1(C57BL/6x129S2) background was crossed with female F1(C57BL/6Jx129S8) mice and females from three different inbred lines, BALB/c, C3H/He and C57BL/6J, and produced a total of 363 progeny of which 78 (22%) carried Hsa21. These progeny were crossed to their maternal mouse strain and subsequent transmission of Hsa21 is shown below.

Generation	Male Tc1 parent		Female Tc1 parent	
	Total number pups produced	Number Hsa21+ pups (% of total)	Total number pups produced	Number Hsa21+ pups (% of total)
F1(C57BL/6Jx129S8) cross				
G2	39	1 (3%)	91	23 (25%)
G3	87	2 (2%)	68	29 (43%)
G4	23	1 (4%)	154	63 (41%)
G5	Not done		97	41 (42%)
G6	Not done		41	19 (46%)
BALB/c cross				
N1	97	20 (21%)	Not done	
N2	108	1 (1%)	127	8 (6%)
N3	90	4 (4%)	31	0 (0%)
N4	27	3 (11%)	2	0 (0%)
C3H/He cross				
N1	100	24 (24%)	Not done	
N2	103	18 (18%)	11	5 (46%)
N3	81	11 (14%)	32	13 (41%)
N4	66	0 (0%)	23	8 (35%)
N5	8	0 (0%)	0	0 (0%)*
C57BL/6J cross				
N2	137	2 (1%)	88	39 (44%)
N3	38	1 (3%)	99	23 (23%)
N4	25	0 (0%)	3	0 (0%)

*C3H/He cross: three female Tc1 N5 generation mice on this background were mated to C3H/He males but no pups were produced.

TABLE S3. Hsa21 retention in tissues from 8 adult Tc1 mice as determined by quantitative PCR.

Percentage retention of Hsa21 in tissues from 8 adult Tc1 mice (A-H). S.E.M = standard error of mean.

Mouse	Brain	Heart	Spinal Cord	Kidney	Liver	Spleen	Testis
A	77	41	63	52	43	20	43
B	63	52	53	64	39	32	64
C	49	33	52	54	28	29	74
D	47	21	47	48	38	33	36
E	59	46	74	52	35	20	44
F	63	51	67	61	41	18	Not done
G	58	46	67	28	37	32	66
H	7	19	14	21	8	7	3
Mean	**53**	**39**	**55**	**47**	**34**	**24**	**47**
S.E.M	**7**	**4**	**6**	**5**	**4**	**3**	**8**

TABLE S4. Hsa21 retention in tissues from 8 adult Tc1 mice as determined by interphase FISH.

Percentage retention of Hsa21 in tissues from 8 adult Tc1 mice (I-P). S.E.M = standard error of mean. A minimum of 50 cells were counted for each data point.

Mouse	Brain	Spleen
I	94	52
J	74	55
K	54	50
L	57	65
M	80	42
N	36	20
0	74	58
P	56	46
Mean	**66**	**49**
S.E.M	**7**	**5**

TABLE S5. Cerebellar granule neuron counts in four different cerebellar areas (each area 0.01-0.02 mm^2) of four wild-type and four Tc1 mice. *this area is cerebellar lobe VIII.

Mouse	Area number	mm^2	Neuron count	Calculated neuron count per mm^2
Wild-type 1	1	0.0210	398	18952
	2*	0.0211	391	18531
	3	0.0149	221	14832
	4	0.0136	243	17868
	Total	**0.0706**	**1253**	**17748**
Wild-type 2	1*	0.0193	321	16632
	2	0.0210	307	14619
	3	0.0253	330	13043
	4	0.0196	254	12959
	Total	**0.0852**	**1212**	**14225**
Wild-type 3	1	0.0190	305	16053
	2*	0.0186	297	15968
	3	0.0190	233	12263
	4	0.0120	193	16083
	Total	**0.0686**	**1028**	**14985**
Wild-type 4	1	0.0144	202	14028
	2	0.0199	359	18040
	3*	0.0209	312	14928
	4	0.0167	250	14970
	Total	**0.0719**	**1123**	**15619**
Tc1 1	1	0.0149	136	9128
	2*	0.0210	304	14476
	3	0.0210	213	10143
	4	0.0162	203	12531
	Total	**0.0731**	**856**	**11710**
Tc1 2	1	0.0176	258	14659
	2	0.0211	315	14929
	3	0.0195	336	17231
	4*	0.0136	171	12574
	Total	**0.0718**	**1080**	**15042**
Tc1 3	1	0.0158	230	14557
	2	0.0171	200	11696
	3	0.0209	301	14402
	4*	0.0210	315	15000
	Total	**0.0748**	**1046**	**13984**
Tc1 4	1*	0.0190	257	13526
	2	0.0209	202	9665
	3	0.0209	260	12440
	4	0.0209	294	14067
	Total	**0.0817**	**1013**	**12399**

TABLE S6. **Distances between anatomical landmarks on the cranium and mandible, indicated by their assigned numbers (Fig. S5).**

Mean values are given for distances between landmarks in ten wild-type and ten Tc1 mice, P values have been calculated using a T-test. P≤0.05 indicates a significant difference between the two populations (shown in bold). Where measurements for the cranium or mandible were made bilaterally, data are presented pooled for both sides.

Cranium	**Wild-type**	**Tc1**	**T-test**	**Mandible**	**Wild-type**	**Tc1**	**T-test**
Anatomical Landmarks	Mean distance between landmarks (mm)	Mean distance between landmarks (mm)	P values	Anatomical Landmarks	Mean distance between landmarks (mm)	Mean distance between landmarks (mm)	P values
1 - 2	8.50594	7.97985	0.05855	**1 - 2**	6.9547	6.4608	**0.026562**
2 - 3	8.08008	7.91647	0.51685	3 - 4	6.6577	6.7965	0.619016
2 - 4	12.5634	12.2881	0.24027	1-2 & 3-4	6.8062	6.6287	0.314692
2 - 5	15.655082	15.695606	0.938003	2 - 5	12.647	11.933	0.118795
6 - 7	4.05299	4.058	0.93252	4 - 6	12.663	12.441	0.479018
8 - 9	10.142353	9.8132797	0.584798	2-5 & 4-6	12.655	12.187	0.086484
6 - 8	11.644134	12.089804	0.140912	**1 - 5**	9.796	8.9484	**0.005472**
7 - 9	11.660563	12.0912	0.267674	**3 - 6**	9.9462	9.1165	**0.010724**
6-8 & 7–9	11.652348	12.090502	0.065584	**1-5 & 3-6**	9.8711	9.0324	**0.000112**
				5 - 6	1.1017	1.1441	0.288044

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