LETTERS

Trisomy represses *Apc*^{Min}-mediated tumours in mouse models of Down's syndrome

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Epidemiological studies spanning more than 50 yr reach conflicting conclusions as to whether there is a lower incidence of solid tumours in people with trisomy 21 (Down's syndrome)[1,2]. We used mouse models of Down's syndrome and of cancer in a biological approach to investigate the relationship between trisomy and the incidence of intestinal tumours. *Apc*^{Min}-mediated tumour number was determined in aneuploid mouse models Ts65Dn, Ts1Rhr and Ms1Rhr. Trisomy for orthologues of about half of the genes on chromosome 21 (Hsa21) in Ts65Dn mice or just 33 of these genes in Ts1Rhr mice resulted in a significant reduction in the number of intestinal tumours. In Ms1Rhr, segmental monosomy for the same 33 genes that are triplicated in Ts1Rhr resulted in an increased number of tumours. Further studies demonstrated that the *Ets2* gene contributed most of the dosage-sensitive effect on intestinal tumour number. The action of *Ets2* as a repressor when it is overexpressed differs from tumour suppression, which requires normal gene function to prevent cellular transformation. Upregulation of *Ets2* and, potentially, other genes involved in this kind of protective effect may provide a prophylactic effect in all individuals, regardless of ploidy.

The most widely used model of Down's syndrome is the Ts65Dn mouse, which is trisomic for orthologues of about 100 Hsa21 genes and recapitulates in detail several phenotypes of Down's syndrome[3,4] (Supplementary Fig. 1). Mice that are heterozygous for the *Apc*^{Min} mutation accumulate tumours analogous to those in familial adenomatous polyposis along the wall of the small intestine and colon[5]. *APC* is also mutated in a high proportion of spontaneous intestinal cancers in human beings. Although the mouse mutation is completely penetrant, the number of tumours that develop is dependent on both genetic modifier genes and environmental factors[6].

Female Ts65Dn mice were crossed to *Apc*^{Min} males and the number of tumours in the small intestine was determined in their trisomic and euploid *Apc*^{Min} progeny at 120 days of age (Supplementary Fig. 2). Trisomic mice showed a significant 44% reduction in the number of tumours compared to their euploid, *Apc*^{Min} littermates, from 45.4 to 23.8 tumours (Table 1). This establishes a biological basis for the effects of trisomy on tumour formation and shows that trisomy for orthologues of about half of the genes on Hsa21 is sufficient to reduce tumour incidence in this model.

We reanalysed these data considering the inheritance of susceptible or resistant alleles of the modifier of *Min 1* (*Mom1*) locus that result in higher or lower tumour number (*Mom1*^{s} and *Mom1*^{r}, respectively; genetic background of all crosses is shown in Supplementary Fig. 3)[7,8]. The inheritance of a single *Mom1*^{r} allele reduced the average tumour number from 62.6 to 21.3 in euploid mice (66%) as expected, and a similar 59% reduction occurred in Ts65Dn (Table 1). Ts65Dn, *Mom1*$^{s/s}$ mice had a highly significant 50% reduction in small intestine tumour number compared to euploid *Mom1*$^{s/s}$ mice ($P = 0.0028$). Trisomic mice that inherited a *Mom1*^{r} allele (*Mom1*$^{s/r}$) also had substantially reduced tumour numbers relative to euploid mice, although this reduction did not reach a statistically significant level in the small sample of Ts65Dn, *Mom1*$^{s/r}$ mice available for this post-hoc analysis. Thus the *Mom1*^{r} effect seems to be additive with the protective effect of trisomy, suggesting that independent mechanisms are involved.

We analysed Ts1Rhr mice to narrow the candidate region for the gene or genes responsible for reduced tumour number. These mice have segmental trisomy for 33 of the genes that are triplicated in Ts65Dn (Supplementary Fig. 1). These genes represent a 'critical region' of Hsa21, previously thought to be sufficient to cause several phenotypes of Down's syndrome[9]. Ts1Rhr, *Apc*^{Min} mice had a significant 26% reduction in the average number of tumours in the small intestine when compared to euploid, *Apc*^{Min} mice (Table 1).

When *Apc*^{Min} mice were crossed to Ms1Rhr, which have segmental monosomy for the 33 genes that are triplicated in Ts1Rhr, we observed a significant 101% increase in tumour number in the monosomic mice compared to euploid (Table 1). These results demonstrate that a gene (or combination of genes) in this region is dosage sensitive in both directions with respect to the effect on tumour number.

The 33 genes at dosage imbalance in Ts1Rhr and Ms1Rhr mice include several possible candidates for the tumour number effect (Supplementary Table 1), including the *Ets2* 'proto-oncogene'. Although generally considered a 'pro-cancer' gene, *Ets2* has several

Table 1 | Average numbers of intestinal tumours in aneuploid and euploid mice at 120 days of age

	Average no. of tumours	s.d.	No. of mice	*t*-test significance (*P* value)
Either *Mom1* allele				
Euploid	45.4	29.9	24	0.008
Ts65Dn	23.8	14.2	10	
Mom1$^{s/s}$				
Euploid	62.6	26.4	14	0.0028
Ts65Dn	31.2	13.7	6	
Mom1$^{s/r}$				
Euploid	21.3	13.3	10	0.105
Ts65Dn	12.8	5.0	4	
Segmental aneuploidies*				
Euploid (B6)	107.3	45.0	16	0.043
Ts1Rhr (B6)	79.6	29.9	21	
Euploid (B6/C3H)	37.0	16.0	9	0.048
Ms1Rhr (B6/C3H)	74.4	39.7	7	

* Genetic background is shown in parentheses. Ts65Dn and euploid controls are B6/C3H (Supplementary Fig. 3).

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activities consistent with a role in repressing the early stages of transformation[10,11]. We performed a three-way cross to produce mice carrying Apc^{Min} that were either euploid or had the Ts1Rhr segmental trisomy, and which segregated an allele of *Ets2* that deletes exons 3–5 and fails to produce functional Ets2 protein (F.L. and M.C.O., manuscript in preparation). Tumours were counted at 120 days (Fig. 1). This independent cohort of mice replicated the observation (Table 1) that trisomy for three copies of *Ets2* and 32 flanking genes in Ts1Rhr results in a significantly reduced tumour incidence, from a mean of 100.8 to 53.9 ($P = 0.001$). However, when *Ets2* was returned to the normal two copy level in mice that were still trisomic for the 32 flanking genes (Apc^{Min}, $Ets2^{+/-}$, Ts1Rhr), average tumour number increased significantly to 81.2 ($P = 0.012$). Thus, a substantial portion though not all of the tumour repression in Ts1Rhr is accounted for by the extra copy of *Ets2*.

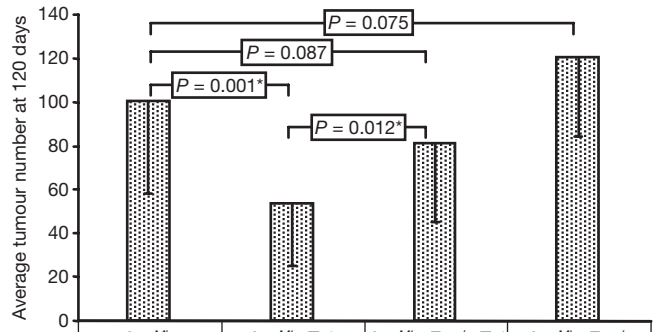
Mice that carried a single copy of *Ets2* in a euploid background showed a substantial, 20% increase in tumour frequency ($P = 0.075$), reminiscent of the increase in tumours in Ms1Rhr mice, which carry a single copy of this gene. These mice developed severe disease much earlier than mice of other genotypes and several did not survive long enough for tumours to be counted. Thus this difference in tumour number is probably under-represented. *Ets2* messenger RNA and protein levels corresponded directly to gene copy number in all of the genotypes (Supplementary Fig. 4).

The size of tumours in a given genetic background provides one indicator of tumour initiation and growth rates. We compared the size of tumours between trisomic and euploid Apc^{Min} mice (Fig. 2a). Ts65Dn, $Mom1^{s/s}$ mice showed a significant 34% reduction in average and median tumour size at 120 days compared to euploid ($P < 0.005$). Note that Ts65Dn mice in this experiment had 48% fewer tumours than did euploid animals, a significantly lower level that replicates in this independent cross the reduction in tumour number reported for the independent cohort of mice represented in Table 1 ($P < 0.04$, $N = 4$ euploid, 5 Ts65Dn).

To determine whether this difference was evident earlier in the course of tumour formation, intestines of trisomic and euploid mice carrying the Apc^{Min} allele were immunostained for β-catenin at 60 days of age (Supplementary Fig. 2)[12]. As at 120 days of age, the number and average size of tumours in Ts65Dn mice was significantly less than in their euploid counterparts (Fig. 2b). No tumours were seen at 30 days of age in two euploid or one trisomic Apc^{Min} mouse after β-catenin staining. Thus the repression of tumour number and size in Ts65Dn mice was evident early in tumour formation.

In contrast to Ts65Dn mice, tumour size was not different from euploid in either Ts1Rhr or Ms1Rhr mice (data not shown). The absence of a tumour size phenotype even though tumour number is reduced in Ts1Rhr mice indicates that multiple genes on Mmu16 (and Hsa21) may contribute to different aspects of tumour repression caused by trisomy.

For 50 yr, epidemiological studies examining rates of solid tumours in individuals with Down's syndrome have reached discrepant conclusions about whether trisomy is protective against cancer[2,13–16] (Supplementary Table 2). Although our demonstration of tumour repression owing to gene dosage applies specifically to the role of trisomy and especially *Ets2* dosage in Apc-induced tumours, it provides biological evidence supporting the protective effect of trisomy. It will be important to determine the range of cancer types



	Apc^{Min}	Apc^{Min}, Ts1	Apc^{Min}, $Ets^{+/-}$, Ts1	Apc^{Min}, $Ets^{+/-}$
Mean	100.8	53.9	81.2	120.3
s.d.	43.18	28.70	36.21	36.20
N	14	15	18	21
Ets2 copy no.	2	3	2	1

Figure 1 | *Ets2* dosage is substantially responsible for tumour number repression or increase. Average tumour number at 120 days is measured for the four genotypes, error bars indicate s.d. Number of mice analysed, *P* value and the gene copy number of *Ets2* in each strain are indicated. *, statistical significance by Student's *t*-test of the designated pair. Although the increased tumour number in euploid $Ets2^{+/-}$ mice at 120 days did not reach a formal level of statistical significance, this result underestimates the impact of reduced *Ets2* dosage, because four Apc^{Min}, $Ets2^{+/-}$ mice became sick and were euthanized before tumours could be counted at 120 days. None of the 47 mice representing the other 3 genotypes died before 120 days.

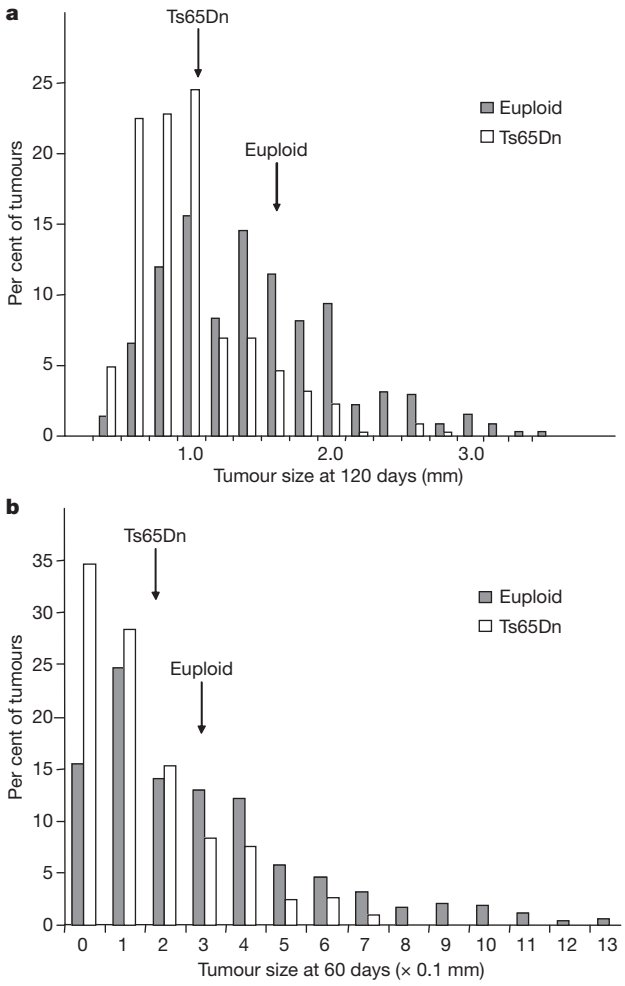


Figure 2 | Tumour growth and number are reduced in Ts65Dn mice. Distribution of tumour sizes for trisomic (open bars) and euploid (closed bars) mice. **a**, At 120 days of age, tumour number is reduced and tumours are significantly smaller in Ts65Dn, $Mom1^{s/s}$ than in euploid mice. Mean tumour size is reduced by 34%; Ts65Dn = 0.91 mm, euploid = 1.38 mm ($P = 0.005$, $N = 347$ and 577 tumours for Ts65Dn and euploid, respectively). **b**, At 60 days, the number of tumours identified after staining with β-catenin is significantly reduced in Ts65Dn ($P = 0.037$) and mean tumour size is reduced 36%; mean = 18 μm in trisomic and 28 μm in euploid mice ($P = 0.029$, $n = 346$ and 636 tumours for Ts65Dn and euploid, respectively). Arrows indicate mean tumour size in each genotype.

and the range of dosage-sensitive genes that contribute to this protective effect in different tissues.

Notable among the Hsa21 genes that have been implicated in pro- or anti-tumorigenesis is endostatin, an inhibitor of angiogenesis that has been shown to be a potent inhibitor of tumour growth in model systems[17]. Elevated expression of another Hsa21 gene, *RCAN1*, can reduce endothelial cell proliferation and angiogenesis, affecting size and vascularity of xenografted tumours[18]. However, *Rcan1* is not trisomic in Ts1Rhr, and the *Col18a1* gene (which encodes endostatin) is not triplicated in either Ts65Dn or Ts1Rhr. Therefore, these genes do not account for the reduction in tumour number seen here.

Two general implications that stem from the observation that trisomy and specifically *Ets2* dosage can repress or promote tumour growth are worth special note. First, repression of tumorigenesis when *Ets2* expression is elevated may in fact be a characteristic of many genes identified previously as oncogenes or tumour suppressor genes. Natural variation in average expression levels of ETS family (or other) repressor genes may exist in tumour-prone families without a known molecular basis for a high cancer frequency (reduced expression of *Ets2*) or in cancer-resistant families (elevated expression). This phenomenon might be exploited to identify a pharmacological-based approach to tumour protection.

Second, previous observations about the role of the *ETS2* proto-oncogene in cancer could not have predicted that elevation of expression beyond euploid levels would provide a natural repression of tumour formation and growth. If trisomy for Hsa21 was not viable, the correlation of increased gene expression with lower solid tumour frequency would not occur in a systematic manner and may not have been observed for some time. The implication for promoting tumour resistance in all people on the basis of gene dosage of 'oncogenes' is thus a product of the genetic heritage of those with Down's syndrome.

METHODS SUMMARY

C57BL/6J-Apc^{Min} mice (herein Apc^{Min}) and B6EiC3Sn *a*/*A*-Ts(17^{16})65Dn (herein Ts65Dn) mice were purchased from the Jackson Laboratory and genotyped as described[19]. B6.Dup(Cbr1-ORF9)1Rhr mice (herein Ts1Rhr)[9] were backcrossed eight or more generations onto C57Bl/6J (B6). B6C.3Del(16Cbr1-ORF9)1Rhr (herein Ms1Rhr) and Ts65Dn mice were maintained as an advanced intercross between B6 and C3H. For tumour analysis, mice were euthanized at 120 ± 2 days, intestines were placed in fresh PBS, and tumours counted under $20\times$ magnification. Tumour size was determined for the longest axis, using an eyepiece reticule. Statistical significance was determined using a Student's *t*-test. Detailed methods are in Supplementary Information and Methods.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions T.E.S. and R.H.R. designed the experiments. T.E.S. and A.Y. managed husbandry and collected tumour data, which were analysed by T.E.S., A.Y. and R.H.R.; F.L. and M.C.O. designed the *Ets2* conditional knockout mice; and A.Y., F.L. and M.C.O. analysed *Ets2* expression. R.H.R. wrote the paper with substantial input from all authors.

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METHODS

Mice. C57BL/6J-*Apc*^{Min} mice (herein *Apc*^{Min}) were purchased from the Jackson Laboratory (Bar Harbor, ME) and maintained by repeated backcrossing to C57Bl/6J (B6) mice. B6EiC3Sn *a*/*A*-Ts(17^{16})65Dn (herein Ts65Dn) mice were purchased from the Jackson Laboratory. B6C3Del(16Cbr1-ORF9)1Rhr (herein Ms1Rhr)[9] were maintained in our colony where both Ms1Rhr and Ts65Dn mice were maintained as an advanced intercross by crossing to (B6 × C3H/HeJ)F_1 mice. B6.Dup(Cbr1-ORF9)1Rhr mice (herein Ts1Rhr)[9] were backcrossed eight or more generations onto C57Bl/6J. Mice carrying a null allele of *Ets2* (herein *Ets2*$^{+/-}$ mice, F.L. and M.C.O., in preparation) were backcrossed for more than nine generations onto B6 before being used in these experiments. The genetic backgrounds of all mice produced for this study are shown in Supplementary Fig. 3. In general, groups of euploid and trisomic littermates from related mothers were used in crosses that generated aneuploid mice to minimize genetic variation.

Genotyping. *Apc*^{Min}, Ts1Rhr and Ms1Rhr mice were genotyped by PCR as described[9,20]. Ts65Dn mice were identified by fluorescent *in situ* hybridization (FISH) as described[19].

For *Mom1*, PCR primers were designed to amplify the wild-type (*Mom1*^{r}) (Mom Common-TGGGGAAATGATTTGGCTTA, MomWT-TGGCATCCTT-GGGGGAT) and mutant (*Mom1*^{s}) (Mom Common, Mom MUT-TGGCA-TCCTTGGGGGAA) alleles. These primers were used with the LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics Corporation), with conditions: 95 °C 10 min, (95 °C 10 s, 58 °C 5 s, 72 °C 20 s) × 55. Presence of an allele resulted in a five-cycle shift in the amplification curve. This result was confirmed by melting curve analysis which yielded distinct profiles for *Mom1*^{r} and *Mom1*^{s} .

PCR was used to type *Ets2*$^{+/-}$ mice. The wild-type allele was detected using primers Ets2I2P10 (CGCTTGCTAGGCAAGTGCTCTACC) and Ets2I2P9 (GCTGACACAGGGTTTTGGTGTCATGC), and the *Ets2* deleted allele was detected using primers Ets2I2P10 and Est2I25P3 (CTAAGCCAGCCT-GGCTACAGAACC), under the following cycling conditions: 95 °C 2 min, (94 °C 45 s, 55 °C 45 s, 72 °C 1 min) for 35 cycles, 72 °C 10 min. The wild-type band was 300 bp and the deleted band was 600 bp.

Tumour analysis. All animals were assessed blind to genotype in all assays. Groups of littermate mice from closely related mothers (and inbred fathers) were euthanized at 120 ± 2 days of age. Intestines were removed and rinsed then cut longitudinally and placed in fresh PBS. Tumours were counted under 20× magnification across the entire length of the small intestine. For Table 1, tumours were scored if they were ≥0.4 mm in diameter; small tumours that did not involve multiple crypts were excluded. Because the process of identifying tumours is disruptive and tumour tissue rapidly degrades under dissection conditions, multiple observers are not used for the same mice in the *Apc*^{Min} tumour assay. Rather, independent crosses were assessed by independent observers to confirm the effects of aneuploidy on tumorigenesis. The Ts65Dn *Apc*^{Min} tumour analysis was done three times by two observers (T.E.S. and A.Y.)(data in Table 1, Fig. 2a and Fig. 2b) and the Ts1Rhr × *Apc*^{Min} analysis was performed twice by two observers (Table 1 and Fig. 1). A summary of the crosses and data collection process is in Supplementary Fig. 3.

For visible tumours (at 20× magnification), tumour size was determined for the longest axis of the tumour using an eyepiece reticule. Statistical significance was determined using a Student's *t*-test. For microscopic tumours, intestines were recovered from *Apc*^{Min} and Ts65Dn, *Apc*^{Min} littermates 60 days of age. Intestines were removed, washed in 4 °C PBS several times and then cut into three sections (proximal to distal). Each section was cut open longitudinally and fixed overnight in 10% formalin. The next day the intestine was rolled up and embedded in paraffin as a 'Swiss roll', and ten slides each containing 3 sections of 6 microns thick were recovered at an interval of 50 microns. Slides were deparaffinized, stained with β-catenin antibody (BD Biosciences Clone 14, Vector M.O.M immunodetection Kit) and co-stained with haematoxylin, and tumours from ten slides per mouse were measured under a light microscope with an eye piece reticule. Tumour size and number were counted and results compiled (Supplementary Fig. 2).

RNA and protein analysis. Mouse embryo fibroblasts (MEFs) were established from fetuses at E13.5. Fetuses were removed and the visceral tissue separated. Remaining tissue was minced in Trypsin/EDTA and incubated at 37 °C for an hour. Trypsin was neutralized by addition of medium (DMEM plus 10% serum and antibiotics) and cells collected and plated, taking care to avoid transfer of larger pieces of tissue. The next day, cells were re-fed, then passaged as they reached confluence. For these experiments, cells were used between 6–8 passages.

Total RNA was isolated from mouse small intestine or MEFs with TRIzol reagent (invitrogen) and RNeasy Mini Kit (Qiagen), including a DNase I treatment step. RNA concentration was determined by UV spectrophotometry and 1 μg was reverse transcribed with GeneAmp RNA PCR kit (Applied Biosystems). After dilution, 10 ng of complementary DNA was amplified by real-time PCR with SYBR Green PCR master mix (Applied Biosystems) using specific primers for *Ets2* (Forward, AGAGAAGGGAGCACAGCAAA; Reverse, AAGAACA-TGGACCAAGTGGC) (http://mouseprimerdepot.nci.nih.gov/) and β-actin (Forward, AGTGTGACGTTGACATCCGTA; Reverse, GCCAGAGCAGTAA-TCTCCTTCT). Real-time PCR was carried out under the following conditions: 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C (Applied Biosystems 7500 System). C_t values were determined by subtracting the average β-actin C_t value from the average *Ets2* C_t value. The s.d. of the difference was calculated from the s.d. s of *Ets2* and β-actin values. After each real-time RT–PCR, a melting profile was done to rule out non-specific contributions from PCR products and primer dimers.

For western blots, whole cell lysates were prepared by lysing MEFs with RIPA buffer, and 100 μg of protein from each sample was separated by 8% SDS PAGE. The membrane was blotted overnight with anti-Ets2 (ref. 21) 1:1,000, 5% milk in 0.05% TBST (TBST is 0.05% Tween-20, 20mM Tris-HCl pH7.6 and 150mM NaCl), probed with anti-Rabbit HRP and developed for ECL. Blots were stripped and reprobed with anti-tubulin (1:1,000 in 5% milk in 0.05% TBST) antibody. Scanned images of each blot were inverted by NIH Image J and the density calculated for Ets2 and tubulin in each sample. The background was measured and subtracted and the ratio of Ets2/tubulin density was used to compare protein expression level of Ets2 in MEFs of different genotypes. The average level of Ets2:tubulin in euploid mice was arbitrarily set at 1.0 and Ets2 levels in other genotypes were calculated in proportion.

SUPPLEMENTARY INFORMATION

Supplemental materials, T.S. Sussan et al., Trisomy represses *Apc*^{Min}-mediated tumors in mouse models of Down syndrome.

Suppl. Table 1. **Gene list for Ts1Rhr.**
Suppl. Table 2. **Conflicting epidemiological evidence for cancer rates in DS** and note on Down syndrome and cancer.

Suppl. Fig. 1. **Comparative maps of mouse models**.
Suppl. Fig. 2. **Visualizing intestinal tumors in *Apc*^{Min} mice**.
Suppl. Fig. 3. **All crosses used in this study** and note on genetic backgrounds.
Suppl. Fig. 4. **Ets2 RNA and protein levels reflect gene copy number**.

Bibliography

Suppl. Table 1. Genes conserved with Hsa21 that are triplicated in Ts1Rhr mouse and monosomic in Ms1Rhr [1].

Symbol	Status[1]	Gene name
CBR3	C	Carbonyl reductase
C21orf5	C	
AK009785	MC	
KIAA0136	C	ATP-binding domains
CHAF1B	C	Chromatin assembly factor
CLDN14	C	Cell adhesion protein in tight junctions
SIM2	C	Transcription factor; HLH, 2 PAS, 1 PAC domain
HLCS	C	Holocarboxylase synthase
DSCR6	MC	
DSCR5	C	2 transmembrane domains; Down syndrome critical region protein 5
TTC3	C	Tetratricopeptide repeats
DSCR3	C	Vacuolar protein sorting-associated protein (Vps) 26 motif
DYRK1A	C	serine-threonine protein kinase; tyrosine phosphorylation regulated
as-DYRK1	C	
KCNJ6	C	Potassium inwardly-rectifying channel, subfamily J, member 6
KCNJ15	C	Potassium inwardly-rectifying channel, subfamily J, member 15
as-KCNJ15	MC	
ERG	C	ETS-related; SAM/Pointed and ETS domains; transcription factor
ETS2	C	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian); transcription factor
DSCR2	C	Leucine rich
WDR9	C	8 Trp-Aps domains; 2 bromo (DNA binding) domains
HMG14	C	high-mobility group (nonhistone chromosomal) protein 14
WRB	C	Signal sequence; 2 transmembrane domains; trp-rich C terminus
C21orf13	C	
SH3BGR	C	Signal sequence; Pro-rich putative SH3 domain; Glu-rich C-terminus
B3GALT5	C	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 5
IGSF5	C	immunoglobulin superfamily, member 5
PCP4	C	PEP19; brain specific peptide
DSCAM	C	Down syndrome cell adhesion molecule
as-DSCAM	MC	
BACE2	C	Asparty protease; b-site APP cleavage
MX1	C	Interferon-induced cellular resistance mediator protein; Dynamin and Dynamin GTPase effector domains
C21orf11	C	

[1] C, conserved, MC, moderately conserved as per Gardiner [2].

Suppl. Table 2. Conflicting epidemiological evidence for cancer rates in DS.

Author	Scholl	Hasle	Hermon	Boker	Yang	Hill	Hill	Goldacre	Patja	Day
Year	1982	2000	2001	2001	2002	2003	2003	2004	2006	2005
Incidence (I)/Mortality (M)	M	I	M	I	M	I	M	I	I	M
All Solid Tumors		0.5			0.07	0.8		1.2	0.6	1.0
Gastric	0.32			11.9					1.3	
Stomach		1.1	1.53		0.13	3.5	6.4		1.5	
Small Intestine						8.3	3.3		0	
Colon		0.89			0.08	2.1	7.2	3.1	1.5	
Peritoneum		67.77								
Lung	0	0.24			0.02				0	
Liver					0.41	6			2.4	
Breast	0.09	0	0.62	0.38	0.04	0.5			0.4	
Endometrial (Uterus)		0.83			0.22	2.2			0.4	
Ovary		1.97	4.05		0.07				0.5	
Testis		1.86	8.4		3.23	3.7	25.2	12	4.8	
Other Male Genital	0.11					45.5			9.8	
Prostate					0.08				0	
Kidney		0.84			0.08	0.6			0.5	
Bladder		1.69			0.2				0	
Skin		0.25			0.06				0.2	
Brain		0.3			0.09	0.7			0.4	
Eye		3.68							0.3	
Oral					0.05					
Pancreas					0.14		1.4		0.9	
Gall Bladder							8.2		6	
Bone									2.1	
Endocrine						1.4			0.3	
Unspecified	0.13	3.27				0.6	0.6		0	
Total Tumors Observed	10	24	5	13	217	28	22	5	32	18
Total DS Individuals	793	2814	346	789	17897	4872	742	1453	3581	600

[a] M is mortality, I is incidence. Green indicates fewer than expected cases in DS, yellow represents no difference or over-representation in DS. Numbers correspond to relative frequency of tumors in DS compared to expected frequency. Statistical methods and the consideration or not of age of mortality/ incidence vary between studies. [3-12]

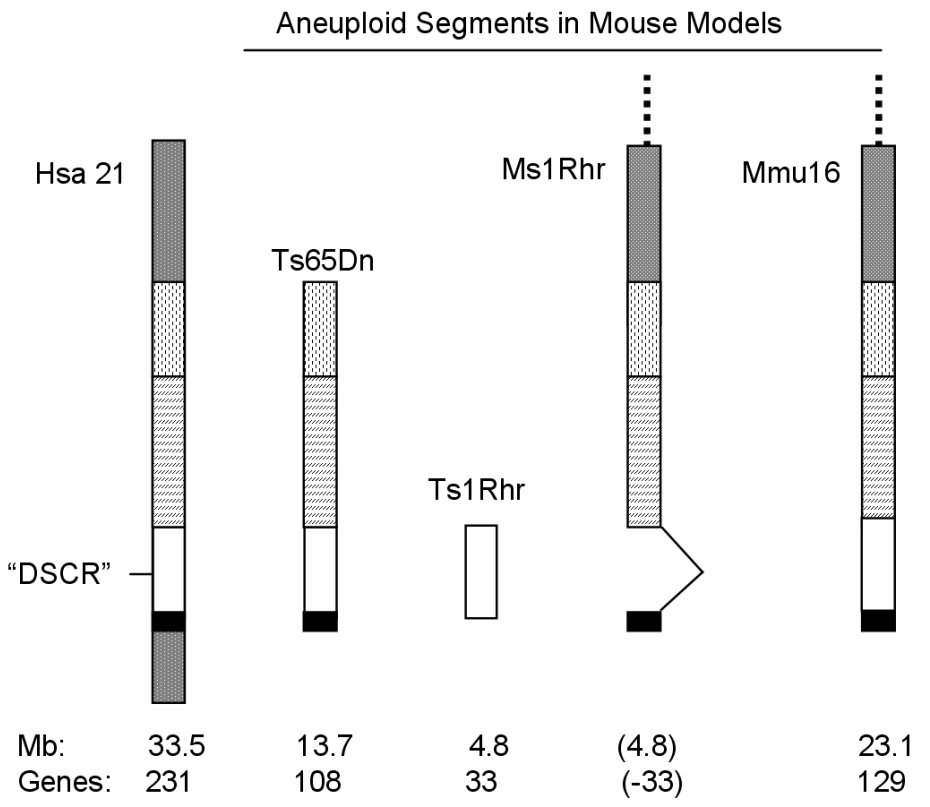
Note on Down syndrome and cancer.

Down syndrome (DS) is associated with two contrary cancer-related phenotypes. Children with DS have a significantly increased risk for leukemia, especially the acute megakaryoblastic leukemia sub-type (AMKL) [13]. AMKL occurs approximately 500-fold more frequently in DS than in the general population and the risk is elevated further in children born with transient myeloid disease (TMD) [14]. In DS but not in euploid children, AMKL almost always occurs in concert with a somatic mutation in the *GATA1* transcription factor [15].

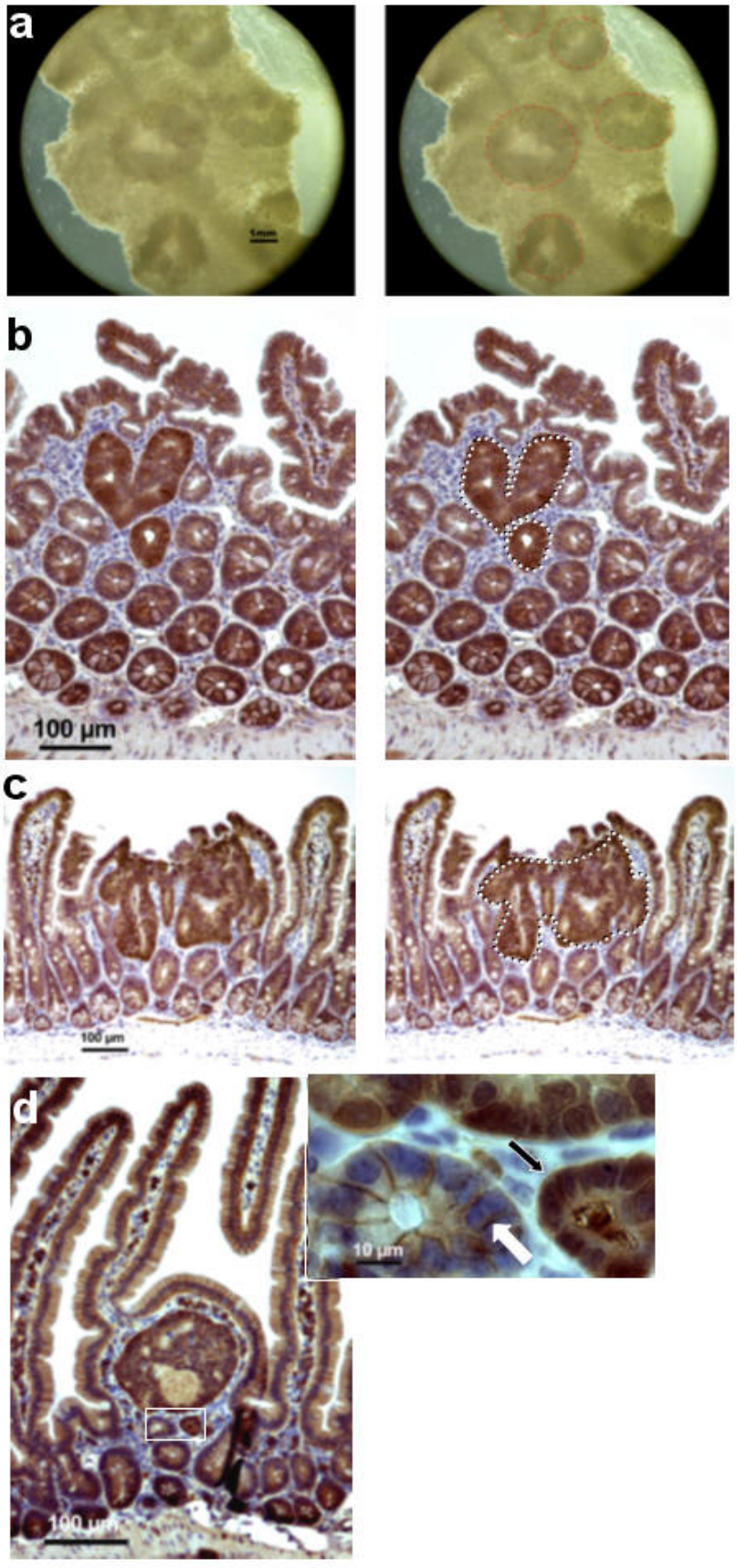
Paradoxically, several epidemiological studies provide evidence supporting a decades old suggestion that people with DS have a reduced incidence of solid tumors. Suppl. Table 2 summarizes results from some of the larger studies reported in the last 25 years. Early studies of the causes of death among children with DS found none attributed to solid tumors out of a total of 187 DS cases [16, 17]. However, Holland et al. reported that non-leukemia cancer deaths were increased by 2.6-fold in mortality associated with DS [18]. In all cases, it is necessary to account for a shorter life span in DS, which was not always considered. These early studies especially were limited by small sample sizes and by the low average age of death and concomitant small number of expected cases of cancer. Combined with variable approaches to analysis, the conflicting conclusions regarding cancer incidence in DS are not surprising.

Improvements in healthcare over the last 20 years have greatly increased life expectancy in DS, thereby increasing the lifetime window for developing cancer [7]. Yang et al. at the CDC tested the hypothesis that overall risk of solid tumors is significantly lower in DS than in the general population using a survey of more than 17,000 death records of people with DS [7]. This study showed the expected increase in leukemia and in testicular cancer (the latter is believed to be secondary to undescended testes and not a direct consequence of gene dosage in germ cells). In contrast, the age-corrected odds ratio for mortality from all solid tumors in the 17,000+ Down syndrome cohort was just 7% of the frequency expected in a euploid cohort, a highly significant reduction (Suppl. Table 2). Even with this large DS population, the degree to which DS is observed to reduce tumor incidence in specific cancers relies on relatively small subsets of individuals with imprecisely defined disease and frequencies predicted across studies vary by more than an order of magnitude. These retrospective analyses of hospital and death records do not provide insight into whether an altered tumor profile in DS is the result of genetic or environmental conditions. An epidemiological approach cannot define the genetic mechanisms by which gene dosage reduces tumor formation. Conflicting results about a protective effect of trisomy 21 continue to be reported [5, 8, 9].

Suppl. Fig. 1. Comparative maps of mouse models. Comparative maps of aneuploid segments in mouse models. Numbers of Mmu16 genes conserved with Hsa21 and sizes in megabases (Mb) adapted from Gardiner et al. [2]. All crosses used in this study are shown in Suppl. Fig. 3.

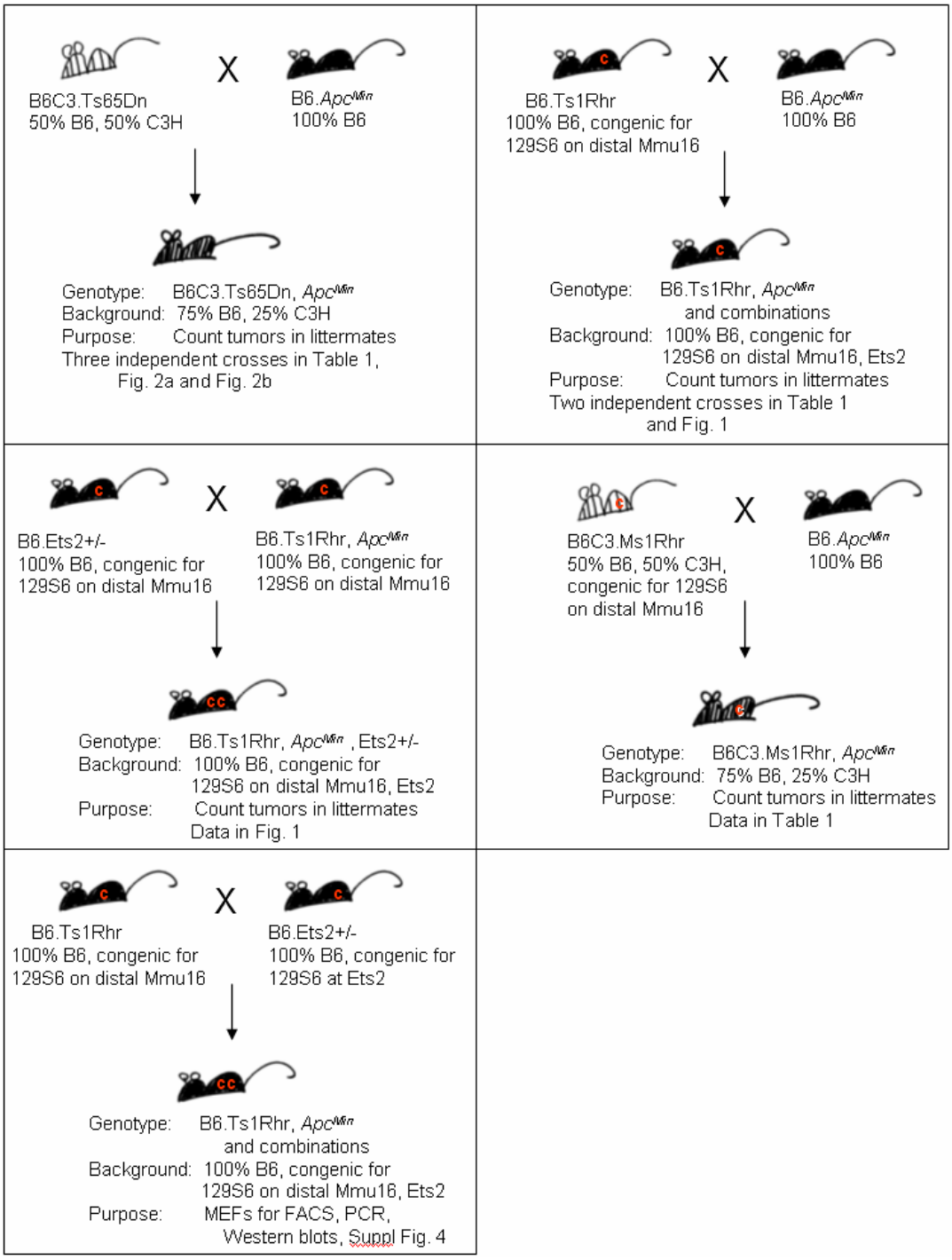


Suppl. Fig. 2. Visualizing intestinal tumors in *Apc*^{Min} mice.



a) Section of small intestine from a mouse carrying the *Apc*^{Min} mutation at 120 days of age, 20x magnification. Tumors are outlined in the right panel.
b and c) Microtumors at 60 days of age detected by immunostaining with β-catenin. Microtumors varied in size, sometimes restricted to a single crypt (b) or involving adjacent transformed crypts (c).
d) Untransformed crypts show β-catenin accumulated on membranes (inset, white arrow) while tumors show high levels of nuclear and cytoplasmic staining (black arrow).

Suppl. Fig. 3. All crosses used in this study.

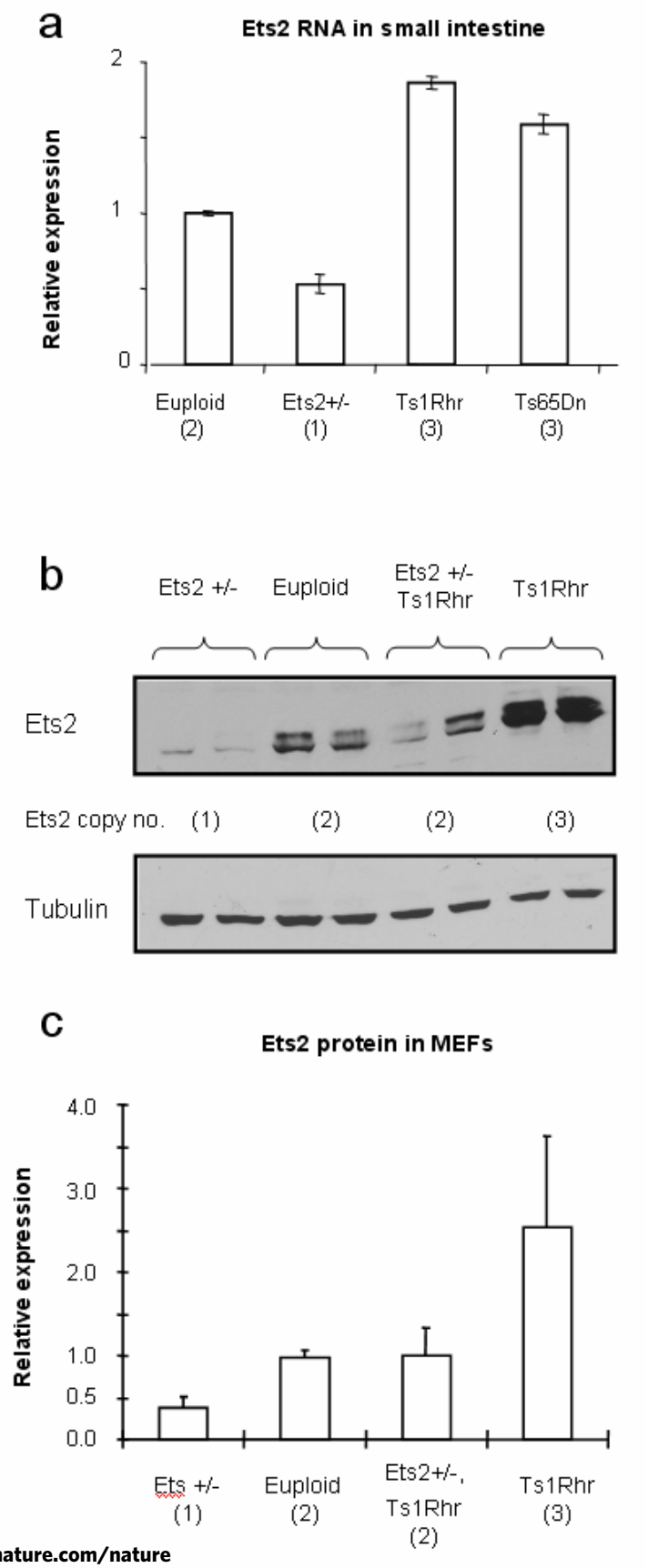


Note on genetic backgrounds: For both Ts65Dn and Ts1Rhr mice, independent cohorts of mice were assessed for effects on tumor frequency of backgrounds to assure repeatability between experiments (three times for Ts65Dn, Table 1, Fig. 2a and Fig. 2b; and twice for

Ts1Rhr, Table 1 and Fig. 1). All showed a significant reduction in tumor number in trisomic vs. euploid mice. The *Apc*^{Min} mutation was derived by ENU mutagenesis on a B6 strain background and is maintained on that background, thus there is no issue of a congenic contribution from these mice.

The advanced backcross generations of Ts1Rhr (≥N8) and Ets2+/- (≥N9) onto B6 assure minimal background variation in these strains. Both of these and the genetically engineered segment in Ms1Rhr were derived in ES cells from the 129S6 strain and will retain this background as a congenic segment in the vicinity of the engineered regions. The fact that Ts65Dn, which has never had any 129S6 strain contribution, has the same repressor phenomenon as Ts1Rhr argues against an exclusive effect of a tightly linked 129S6-derived tumor number modifier in Ts1Rhr. Further, Ets2+/- mice, congenic for the region surrounding *Ets2*, have more tumors while Ts1Rhr mice, which are congenic for a region that includes *Ets2*, have less. A 129S6-derived repressor flanking the Ts1Rhr site is unlikely because similar flanking congenic regions from 129S6 occur in Ts1Rhr, which shows fewer tumors, and in Ms1Rhr, which has more tumors. Finally, extensive studies have mapped more than a dozen loci that modify tumor number in *Apc*^{Min} mice, but no modifier of Min has been mapped in any strain to mouse chromosome 16 and no tumor suppressor activity maps to distal mouse chromosome 16.

Suppl. Fig. 4. Ets2 mRNA and protein levels correlate with gene copy number.



a) Ets2 mRNA levels reflect gene dosage in small intestine as determined by qPCR. Each open bar is the average from two mice, each of the two samples was run 6 times, error bars indicate standard error. b) Protein was extracted from MEFs of the indicated genotypes and analyzed by Western blotting. The ratio of Ets2: tubulin was calculated for genotype pairs of samples. Independent lysates were assessed on a duplicate gel and the four measurements were averaged +/- S.D. c) Relative Ets2 protein levels reflect RNA levels and gene copy number in MEFs. The bar in Suppl. Fig. 4 represents the mean for each group and the error bar designates standard deviation of each genotype (n=4 replicates per genotype except Ts65Dn where reliable measurements were obtained from three, not four samples). In all panels, Ets2 copy number is shown in parentheses.

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Abstractions

FIRST AUTHOR

Astronomers believe that planets form in 'protoplanetary disks' — swirling masses of gas, dust and other particles that surround newborn stars. But direct proof of this theory has been lacking, and the timescales over which planets form, as well as the process by which they do so, are still up for debate. On page 38, Johny Setiawan and his colleagues at the Max Planck Institute for Astronomy in Heidelberg, Germany, reveal their discovery of a giant planet orbiting a star young enough to still be surrounded by a protoplanetary disk. This is a key piece of evidence in the endeavour to understand planet formation.

Were you determined to prove that the protoplanetary disk is deservedly named?

Yes. By studying planet formation we hope to understand the origin of planetary systems and put our solar system in a universal context. To do so, we have to look among the more than 100 young stars with documented circumstellar disks, in which we believe planets are born. Previous work drew attention to TW Hydrae, an 8 million to 10 million-year-old star. There was speculation that variations in its disk structure could be due to a planet forming. So we decided to take a closer look.

Why has no one found this evidence before?

Previous work focused on the quickest way to discover extrasolar planets — using radial velocity, which measures changes in an object's velocity along the line of sight over time. Most researchers excluded young stars from such surveys because they are rife with noisy data resulting from stellar activity. Now that more than 270 extrasolar planets have been found, attention is turning to the physics of young stars to help us understand the birth of planetary systems. We used radial velocity to search young stars one by one and extracted information carefully. We were lucky that the planet we found is big enough for us to detect around a young star.

Do your findings change our understanding of planet formation?

Our work gives an observational upper limit for the timescale of giant planet formation. Statistical studies of young stars suggested that disk lifetime can be a few tens of millions of years. More recent studies put a typical disk lifetime at about 10 million years. Our work indicates that planet formation should be complete within 8 million years.

Do you intend to search for other planet-forming protoplanetary disks?

Yes. But we are also continuing to observe TW Hydrae. A companion planet could be forming in the disk around it. ■

MAKING THE PAPER

Roger Reeves

Down's syndrome holds genetic clue to cancer prevention.

Scientists have struggled for more than 50 years to resolve the controversial claim that individuals with Down's syndrome are less likely to develop solid tumours. Although the idea has become accepted dogma in recent years, studies hoping to prove or disprove the theory have been less than definitive. Reports of research showing cancer rates in people with Down's syndrome to be equal to or greater than those in the general population appear in the literature just as frequently as those concluding that rates are lower.

The difficulty of searching for low-frequency cancers in an already small sample size (only 1 in 700 people have the extra copy — known as 'trisomy' — of chromosome 21 that leads to Down's syndrome), confounds epidemiological studies. "Looking for lower incidence of an already very rare event makes it difficult to obtain an adequate sample size, which is the Achilles' heel in these studies," says Roger Reeves, a geneticist at the Johns Hopkins University School of Medicine in Baltimore, Maryland. In addition, he says, some studies make no adjustments for the generally shorter lifespan seen in Down's syndrome.

About five years ago, Reeves made what he calls a "leap of faith" after taking a good look at the conflicting epidemiological data. He decided that the statistics had reached an impasse and opted to take a biological approach based on mouse models of Down's syndrome. By studying mice with three copies of a group of mouse genes that correspond to a subset of genes found on human chromosome 21, Reeves and his colleagues have pin-pointed a dosage-dependent tumour 'repressor' gene that may hold promise for cancer prevention (see page 73).

Early in the study, the team showed that a genetic cross between trisomic mice and mice carrying a gene associated with a high proportion of intestinal cancers reduced tumour formation by almost half. Then, Reeves' doctoral student, Thomas Sussan, narrowed the search for the responsible genes by using a mutant mouse with fewer triplicate genes — just 33.

Having found that this also lowered tumour incidence, the team looked more closely at the subset of 33 genes. They found that, despite being known to cause cancer when mutated, in triplicate the transcription factor Ets2 decreases tumour incidence.

As he became more involved with individuals with Down's syndrome, Reeves uncovered much misinformation about their quality of life. He cites published studies indicating that 80–90% of pregnant mothers who are told they will give birth to a child with Down's syndrome are likely to terminate the pregnancy. Yet, "they have little idea of what it means to have a child with Down's syndrome or to be a person with Down's syndrome," says Reeves. He notes that people with Down's syndrome have become actors, authors and musicians — feats many of us only aspire to. And just in the past two years, he says, several studies have made breakthroughs in developing pharmacological approaches to address cognitive deficits that will allow those with Down's syndrome to live even fuller lives.

Reeves sees a great irony in the fact that although their quality of life is often disavowed, it is the genomes of those with three copies of chromosome 21 that may ultimately yield a key to cancer prevention. "If trisomy 21 weren't compatible with a full life, it is unlikely that a study such as this would have been undertaken, let alone funded," he says. "Who would be foolish enough to randomly overexpress genes thought to cause cancer in order to prevent it?" ■

FROM THE BLOGOSPHERE

For those concerned about the effects of conference air travel on the environment, Second Nature, NPG's archipelago in Second Life (www.secondlife.com), was the virtual venue for a series of talks coinciding with the United Nations climate-change conference held in Bali in December (see Joanna Scott's blog for details: http://network.nature.com/blogs/user/joannascott).

Tara LaForce from Imperial College London spoke about whether and how we might capture carbon dioxide from power plants, compress it, and store it long-term in various geological structures such as oil reservoirs and deep saline aquifers. And, in another lecture, Euan Nisbet of Royal Holloway University in Surrey, UK, talked about the necessity for accurate monitoring of the climate, greenhouse gases and 'top producers' to have any realistic hope of tackling global warming. Both of these talks, and their associated slides, are available through Scott's blog.

If you are interested in giving your own research talk in this global environment-friendly format, please contact Joanna via her blog, or find her in Second Life, where she is known as Joanna Wombat. ■

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