SUPPLEMENTARY INFORMATION

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

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]

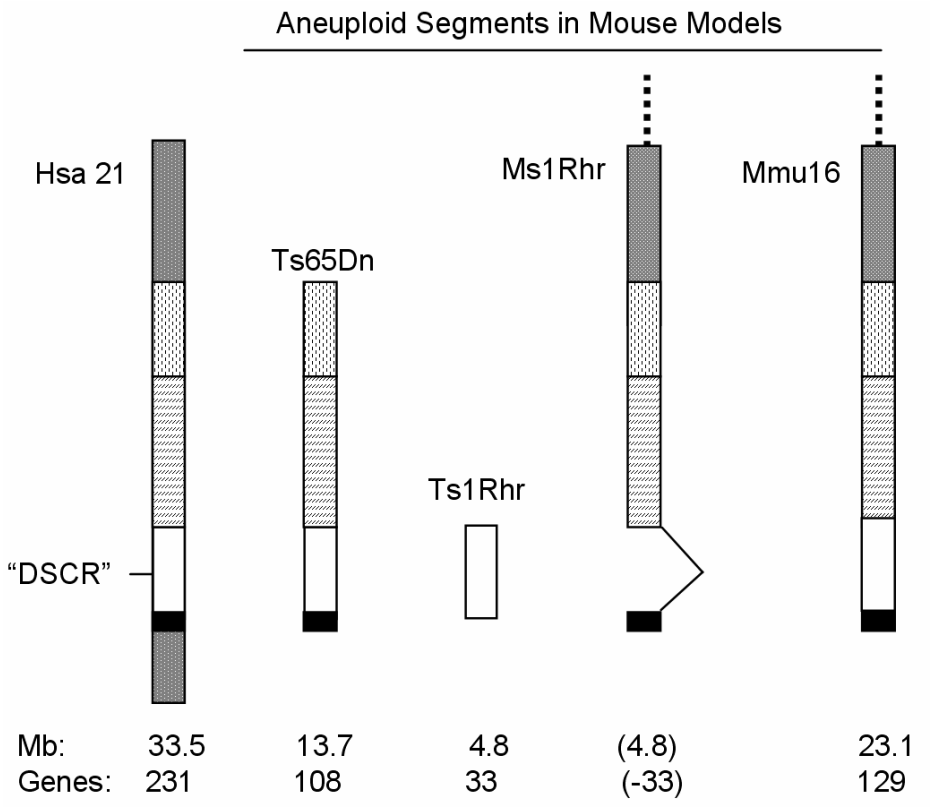
Note on Down syndrome and cancer.

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].

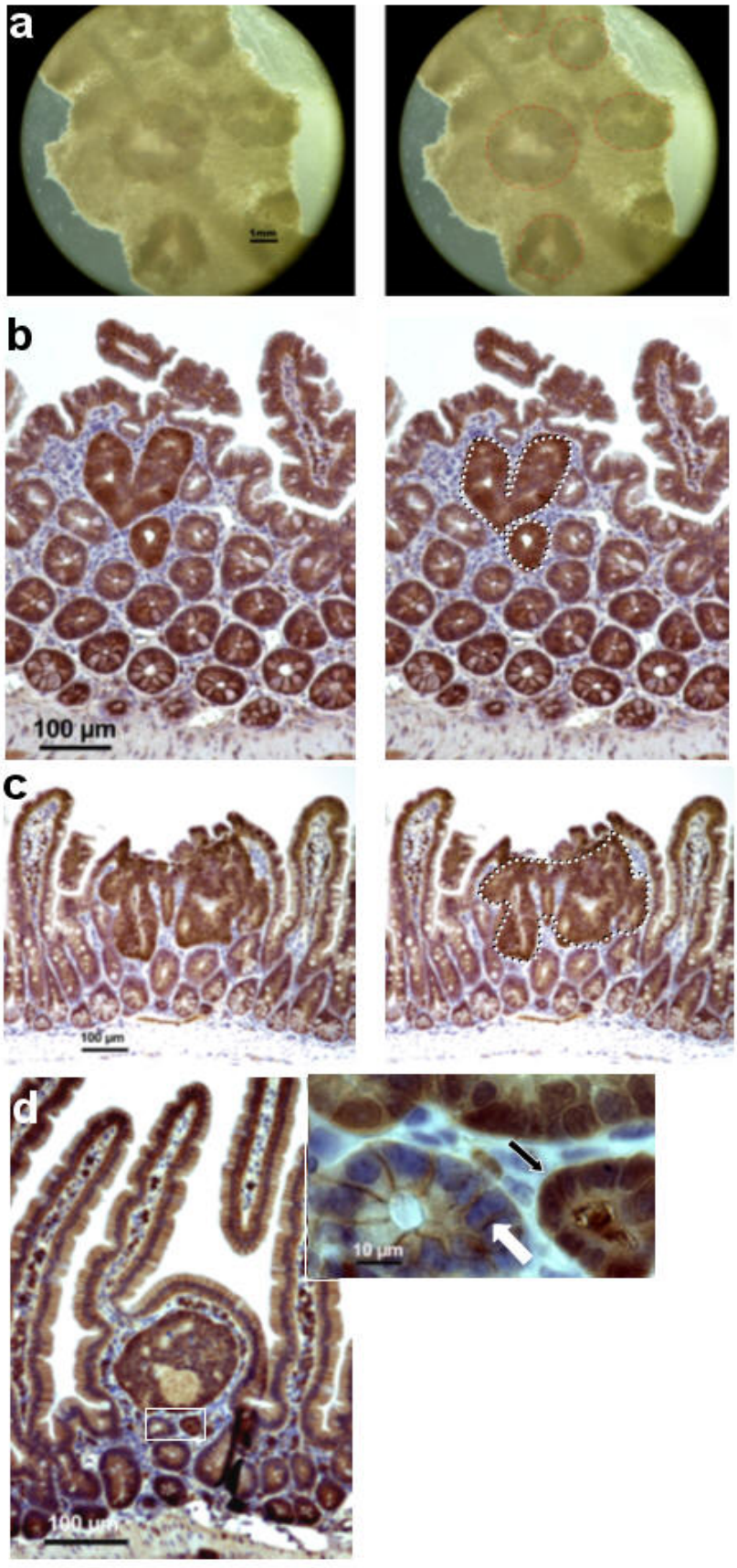
Paradoxically, several epidemiological studies provide evidence supporting a decades old suggestion that people with DS have a reduced incidence of solid tumors. Suppl. Table 2 summarizes results from some of the larger studies reported in the last 25 years. Early studies of the causes of death among 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.

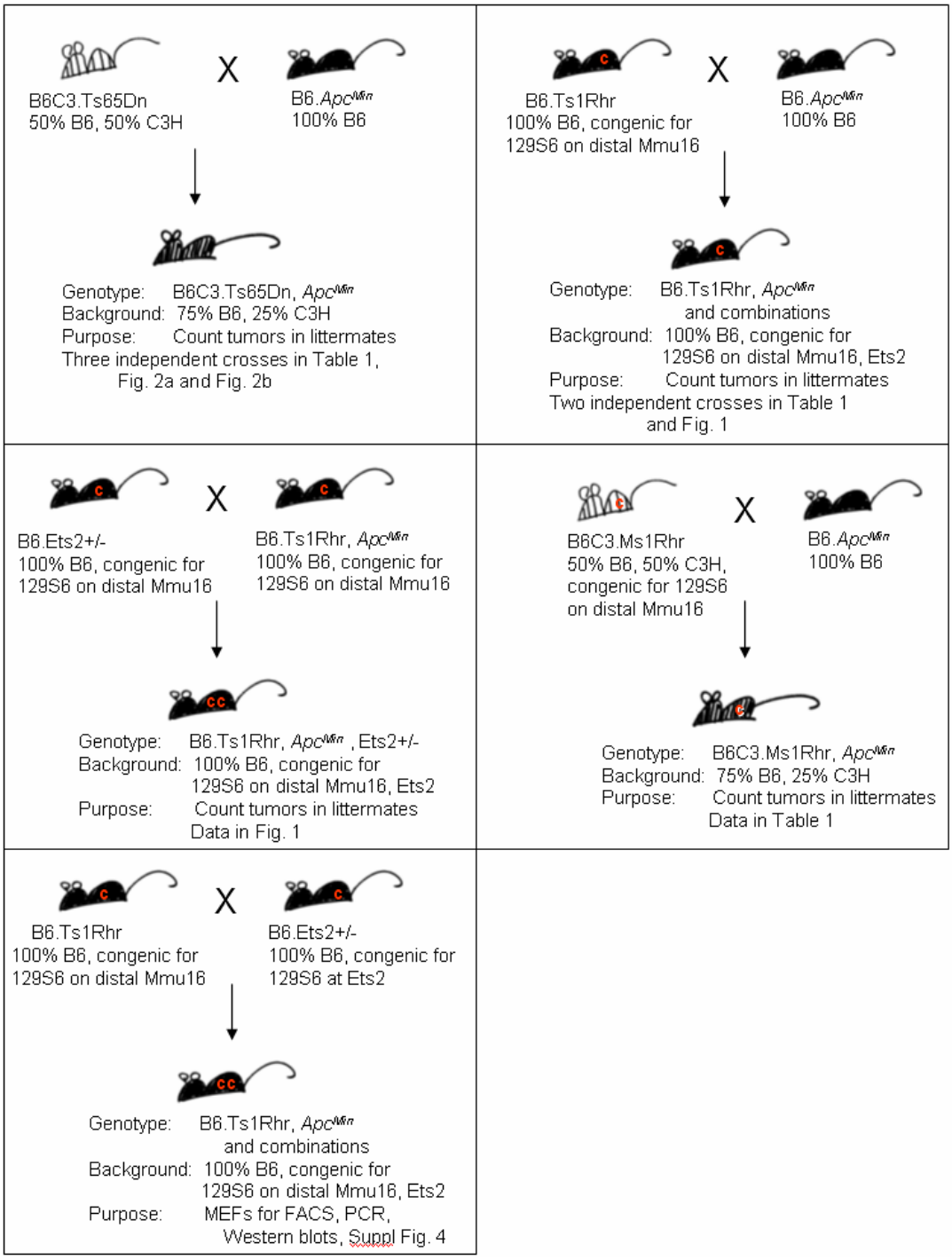


Suppl. Fig. 2. Visualizing intestinal tumors in *Apc*^{Min} mice.



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.

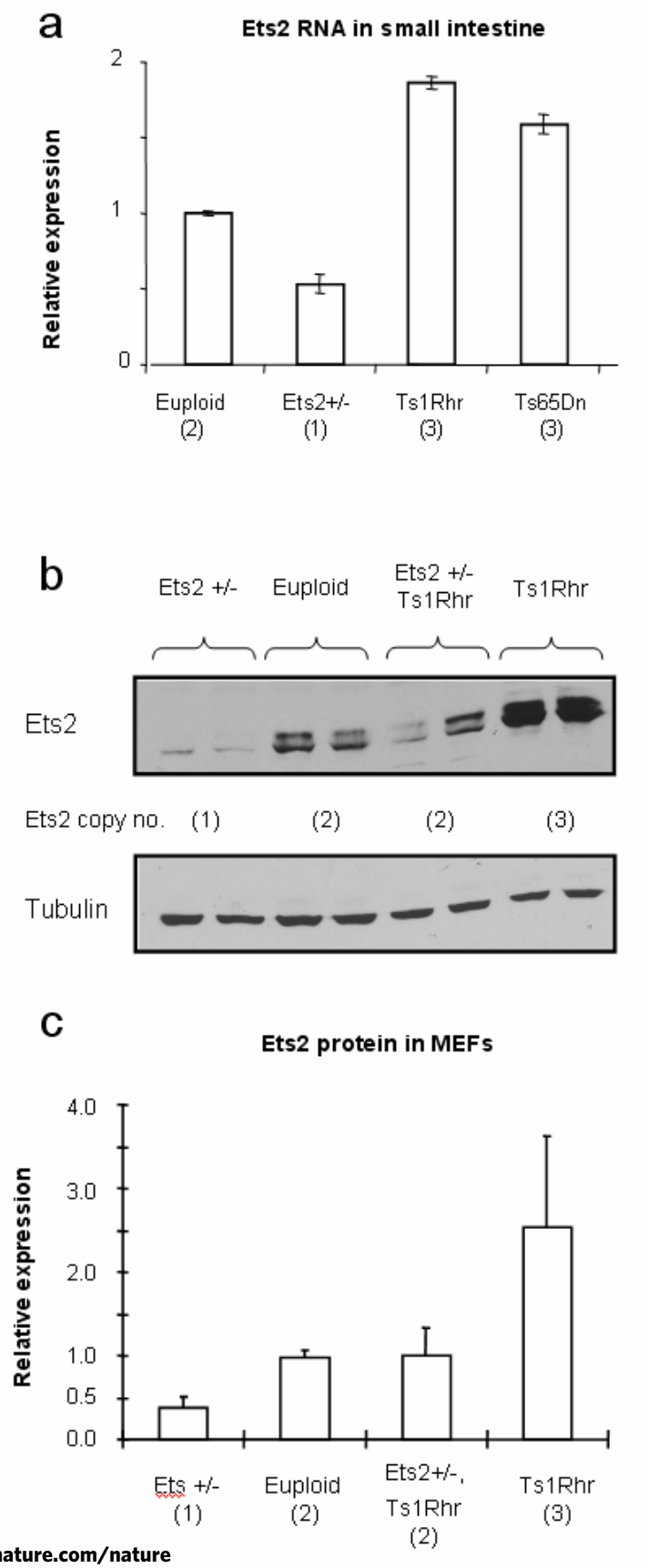


Note on genetic backgrounds: For both Ts65Dn and Ts1Rhr mice, independent cohorts of mice were assessed for effects on tumor frequency of backgrounds to assure repeatability between experiments (three times for Ts65Dn, Table 1, Fig. 2a and Fig. 2b; and twice for

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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