Cells of the osteoblast lineage affect the homing and the number of long-term repopulating haematopoietic stem cells, haematopoietic stem cell mobilization and lineage determination and B cell lymphopoiesis. Osteoblasts were recently implicated in pre-leukaemic conditions in mice. However, a single genetic change in osteoblasts that can induce leukaemogenesis has not been shown. Here we show that an activating mutation of β-catenin in mouse osteoblasts alters the differentiation potential of myeloid and lymphoid progenitors leading to development of acute myeloid leukaemia with common chromosomal aberrations and cell autonomous progression. Activated β-catenin stimulates expression of the Notch ligand jagged 1 in osteoblasts. Subsequent activation of Notch signalling in haematopoietic stem cell progenitors induces the malignant changes. Genetic or pharmacological inhibition of Notch signalling ameliorates the pathogenesis of acute myeloid leukaemia and demonstrates the pathogenic role of the Notch pathway. In 38% of patients with myelodysplastic syndromes or acute myeloid leukaemia, increased β-catenin signalling and nuclear accumulation was identified in osteoblasts and these patients showed increased Notch signalling in haematopoietic cells. These findings demonstrate that genetic alterations in osteoblasts can induce acute myeloid leukaemia, identify molecular signals leading to this transformation and suggest a potential novel pharmacotherapeutic approach to acute myeloid leukaemia.

Mice expressing a constitutively active β-catenin allele in osteoblasts, referred to here as Ctnnb1CAosb (CA, constitutively active; osb, osteoblast specific constitutive activity) are osteopetrotic-14, and die before 6 weeks of age (Fig. 1a) for unknown reasons. Upon further examination, Ctnnb1CAosb mice were anaemic at 2 weeks of age with peripheral blood monocytes, neutrophilia, lymphocytopenia and thrombocytopenia (Extended Data Fig. 1a). Erythroid cells were decreased in the marrow and extramedullary haematopoiesis was observed in the liver (Fig. 1c and Extended Data Fig. 1b, i, l). Although the number of myeloid (CD11b+ /Gr1+) cells decreased due to osteopetrosis, their relative percentage increased, indicating a shift in the differentiation of HSCs to the myeloid lineage (Fig. 1d and Extended Data Fig. 1c, d). The haematopoietic stem and progenitor cell (HSPC) population in the bone marrow (Lin-Sca-c-Kit, LSK) cells decreased twofold in Ctnnb1CAosb mice, but their percentage was twofold greater in wild-type littermates (Fig. 1e and Extended Data Fig. 1e, f). The long-term repopulating HSC progenitors (LT-HSCs) increased in numbers and percentages, whereas the lymphoid-biased multipotent progenitors, LSK- /FLT3+, and the granulocyte/monocyte progenitors (GMP) (Extended Data Fig. 1g-j) decreased. The GMP percentage increased (Fig. 1f). Identiﬁed abnormalities were observed in the spleen of Ctnnb1CAosb mice (Extended Data Fig. 1n-p). The mutation was introduced in osteoblasts but not in any cells of the haematopoietic compartment (Extended Data Fig. 1q-t) of Ctnnb1CAosb mice.

Blasts (12–90%) and dysplastic neutrophils (13–81%) were noted in the blood and there was dense and diffuse infiltration with myeloid and monocytic cells, blasts (30–53% for n = 12 mice) and dysplastic neutrophils in the marrow and spleen of Ctnnb1CAosb mice (Fig. 1g–k, Extended Data Fig. 2a–c). In the liver, clusters of immature cells with atypical nuclear appearance were seen (Fig. 1l). The increase in immature myeloid cells was conﬁrmed by staining with myeloid markers in bones, spleen and liver (Extended Data Fig. 2d–h). Reduced B-cell lymphopoiesis without changes in T-cell populations was observed in Ctnnb1CAosb mice (Extended Data Fig. 2i–t). Differentiation blockade was demonstrated by the presence of immature myeloid progenitors in Ctnnb1CAosb marrow and differentiation cultures (Fig. 1m, n and Extended Data Fig. 2u–x). These cellular abnormalities fulﬁl the criteria of AML diagnosis in mice11 with principle features of human AML12,13.

A clonal abnormality involving a Robertsonian translocation Rb(1;19) was identiﬁed in myeloid cells of the spleen of a Ctnnb1CAosb mouse (Extended Data Fig. 2y). Recurrent numerical and structural chromosomal alterations were also detected in myeloid cells of the spleen of all mutant mice examined (Fig. 2a and Extended Data Table 1). Frequent abnormalities were detected in chromosome 5, the mouse orthologue of human chromosome 7q associated with common cytogenetic abnormalities in patients with myelodysplastic syndromes (MDS) or acute myeloid leukaemia (AML)14. Whole-exome sequencing identiﬁed 4 non-silent somatic mutations in myeloid cells from 3 Ctnnb1CAosb mice (Fig. 2b and Extended Data Fig. 2z), including a recurrent one in Tnfrsf21 and a single somatic mutation in Cyrb1 previously reported in human AML15, but sample size has insufﬁcient statistical power to determine if it is a driver or passenger mutation. Hence, constitutive activation of β-catenin in osteoblasts facilitates clonal progression and is associated with somatic mutations in myeloid progenitors.

Transplantation of bone marrow cells from Ctnnb1CAosb leukaemic mice into lethally irradiated wild-type recipients induced all features of haematopoietic dysfunction and AML observed in Ctnnb1CAosb mice including blasts (15–80%) and dysplastic neutrophils (15–75%) in the blood and blasts (30–40%) and abnormal megakaryocytes in the marrow and early lethality (Extended Data Fig. 3a–i). Transplantation of wild-type bone marrow cells to lethally irradiated Ctnnb1CAosb mice also resulted in AML with early lethality (Extended Data Fig. 3j–r). Transplantation of LT-HSCs, but not other haematopoietic populations, from Ctnnb1CAosb mice to sub-lethally irradiated wild-type recipients resulted in AML with early lethality (Fig. 2c, d and Extended...
Data Fig. 3a–z) indicating that LT-HSCs are the leukaemia-initiating cells (LIGs). These results demonstrate that osteoblasts are the cells responsible for AML development in this model. Remarkably, HSCs of Ctnnb1<sup>CAosb</sup> mice have acquired a permanent self-perpetuating genetic alteration that becomes independent of the initial mutation in osteoblasts.

All Ctnnb1<sup>CAosb</sup> mice examined develop AML between 2 (40%) and 3.5 (60%) weeks of age. Livers of Ctnnb1<sup>CAosb</sup> newborn mice show increased LSK cells and cells of the myeloid lineage, and a decrease in erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j). Micro-hypolobated megakaryocytes, Pelger Huet neutrophils, seen in MDS and other congenital entities, and nuclear cytoplasmic asynchrony in the erythroid and B-lymphoid cells (Extended Data Fig. 4a–j).
These characteristics indicate deregulated haematopoiesis with neutrophil dyspoiesis at birth. Less than 20% blasts were seen in the marrow, consistent with a diagnosis of MDS with excess blasts (RAEB1/2). Differentiation blockade was not observed in newborn animals and fetal HSCs did not transfer the disease (Extended Data Fig. 4n–w) due to lack of HSC–osteoblast interaction in the fetal liver. These results confirm that AML is induced by defective niche signals that are restricted to the bone marrow osteoblasts.

β-catenin target genes in osteoblasts that may regulate HSC fate were identified by microarray analysis. One gene, the Notch ligand jagged 1, fulfilled 4 criteria: (1) acts on adjacent cells; (2) activates a pathway of which many targets are increased in the array; (3) has been implicated in haematopoiesis; and (4) is regulated transcriptionally by β-catenin (Extended Data Fig. 5a–d and ref. 16). Accordingly, jagged 1 expression was increased in Ctnnb1CAosb bone cells and expression of the Notch targets Hes1, Hey5, Hey1, Hey2 increased and Hes1 targets Cebpa (also known as Cebp) and Spil (also known as Pu.1) decreased in Ctnnb1CAosb LSK cells of Ctnnb1CAosb mice, indicating increased Notch signalling in this population (Fig. 3a, b and Extended Data Fig. 5a, b, f, g). Notch1 and Notch2 expression was not affected (Extended Data Fig. 5e). Increased Notch signalling occurred specifically in the leukaemia-initiating LT-HSCs without changes in the other LSK compartments (Extended Data Fig. 5f, g).

To determine if jagged 1 in osteoblasts contributes to AML development in Ctnnb1CAosb mice, we removed one allele of jagged 1 in osteoblasts (Ctnnb1CAosbJag1osb+/– mice). These genetic manipulations decreased Notch signalling is LSK cells, rescued anaemia and deregulation of HSC lineage differentiation and prevented AML development (Fig. 3d–f, Extended Data Fig. 6a–j). Ctnnb1CAosbJag1osb+/– mice survived and were healthy for the entire time they were observed, even though they remained osteoprotic (Fig. 3g and Extended Data Fig. 6k). Similarly, pharmacological inhibition of Notch signalling with a γ-secretase inhibitor reversed haematopoietic deregulation and myeloid expansion in blood, marrow and spleen and reversed AML in Ctnnb1CAosb mice without affecting osteoporosis (Extended Data Figs 5h–s and 7), indicating that osteopetrosis is not enough to drive AML. These observations suggest that Notch signalling is required for AML development in Ctnnb1CAosb mice and that chromosomal alterations may result from increased Notch signalling. Alternatively, healthy HSCs in the endothelial and perivascular niche can multiply and outgrow leukemic HSCs in DBZ-treated Ctnnb1CAosb mice. The gene jagged 1 is required for leukemia induction; whether it is involved in leukemia maintenance with a therapeutic benefit remains to be examined.

To assess the relevance of these findings to humans we examined activation of β-catenin signalling in bone marrow biopsies from MDS or AML patients. Forty-one out of 107 patients examined with all MDS subtypes, AML, or MDS that had transformed to AML (38.3%) showed nuclear localization of β-catenin in osteoblasts (Fig. 4a, b, Extended Data Figs 8a–h and 9 and Supplementary Table 1) but in none of the 56 healthy controls examined (Fig. 4c and Extended Data Fig. 9a–g, i, j). Myeloid and erythroid cells and megakaryocytes in all patients and healthy control subjects showed membrane staining for β-catenin. Notch signalling was specifically activated only in patients with nuclear accumulation of β-catenin as indicated by HEY1 nuclear staining in their haematopoietic cells (Fig. 4d and Extended Data Fig. 8a–f). Expression of all examined β-catenin target genes and JAG1 and DLL1 was upregulated over twofold in osteoblasts from MDS/AML patients with β-catenin nuclear accumulation in osteoblasts (Fig. 4h, i) but not in healthy controls. Notch activity was increased in haematopoietic cells from the same patients, but not healthy controls, as indicated by twofold increase in the expression of Notch transcriptional targets (Fig. 4i). It is possible that aberrant β-catenin signalling in osteoblasts of these patients may be the consequence of haematopoietic clones altering expansion or functionality of different stromal cell lineages, as recently reported. During screening of assumed healthy controls, two individuals had nuclear β-catenin in osteoblasts. Re-evaluation showed that one patient developed MDS and the second an underlying MPN/MDS, a pre-AML condition with features of both a myeloproliferative neoplasm (MPN) and MDS (Extended Data Fig. 8g, h) suggesting a potential prognostic value.

Notch activation promotes expansion of myeloid cells and acute megakaryoblastic leukaemia-like disease in mice. Other studies show that the Notch pathway may act as tumour suppressor in AML. However, in these models, LICs are found in GMPs, whereas in our model LICs are in LT-HSCs, suggesting that different LICs can have distinct consequences. Additionally, increased mammalian jagged 1 expression may not elicit identical outcomes as increased Notch signalling by all Notch receptors and Ctnnb1CAosb osteoblasts may stimulate additional signals that act in combination with Notch to induce mutations contributing to AML. Notch also has a role in T-cell acute lymphoblastic leukaemia (T-ALL) pathogenesis, but T-cell–specific cooperative signals seem to be required to induce transformation.

The idea that osteolineage cells can induce myeloid malignancies has been previously put forward. Our observations that osteoblasts determine the appearance of cell-autonomous AML with 100% penetrance, and the molecular and genetic dissection of how this occurs in mice and humans, demonstrate the role of the marrow niche as a determinant of haematological disorders. This finding may also be informative

**Figure 3** Inactivation of jagged 1 in osteoblasts prevents AML in Ctnnb1CAosb mice. a, b, Expression of jagged 1 in bone (a) and Notch targets in LSK+ cells (b) (n = 4). c–e, The bone marrow of Ctnnb1CAosbJag1osb+/– mice shows rescue of anaemia (c) and of proportions of myeloid (d) and CD11b+/Gr1−/CD11c+ (e) cells. f, Blood histology. g, Survival of Ctnnb1CAosbJag1osb+/– mice. *P < 0.05 versus wild type and †P < 0.05. h, Jagged 1+ versus Jagged 1−/− mice. n = 8. Results show a representative of two independent experiments. Results are mean ± s.d.
mice was βcat(ex3)xob. Catnb−/−;Lin+/− mice express a β-catenin mutant allele in which exon 3, encoding all serine and threonine residues phosphorylated by glycosynthase kinase 3β, is flanked by loxP sites. All the protocols and experiments were conducted according to the guidelines of the Institute of Comparative Medicine, Columbia University.

**Patient samples.** Bone marrow biopsies from patients with AML and MDS were consecutively obtained from 2000 to 2008 and reviewed under a research exempt waiver approved by the institutional review boards (IRB) of Memorial Sloan-Kettering Hospital and Columbia University and Human Biospecimen Utilization Committee.

**Online Content** Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions A.K. and S.K. initiated the study and designed the experiments. A.K., J.S.M. and S.K. analysed the data. A.K. carried out most of the experimental work with the help of J.S.M., I.M. and N.L. J.S.M. performed the flow cytometry analysis. H.K. A.L. and R.R. performed whole-exome sequencing analysis. I.M. confirmed exome mutations. N.L. performed immunofluorescence. C.V.R. reviewed and discussed haematopoiesis data and bone marrow transplantations. G.B. D.P. and J.T.-F. performed histology in mouse samples. J.T.-F. and D.P. performed histology in human samples. A.R., S.M., N.G., J.T.-F. and E.B. provided human AML and MDS samples and reviewed and discussed human bone marrow and bone biopsy data. M.V. performed G-banding karyotype analysis. R.F. analysed microarray data. A.B. assisted with mouse flow cytometry experiments. A.K. and S.K. wrote the manuscript. S.K. directed the research. All authors discussed and commented on the manuscript.

Author Information Microarray and aCGH data were deposited in Gene Expression Omnibus (accession numbers GSE43242, GSE51690) and exome sequencing data were deposited in Sequence Read Archive (accession number SRP031981). Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.K. (sk2836@columbia.edu).
Animals. Generation of z1(I)Collagen-Cre and Catnb-flox(ex3) mice has previously been reported32–34. Catnb-flox(ex3) mice express a β-catenin mutant allele in which exon 3, encoding all serine and threonine residues phosphorylated by glycogen synthase kinase 3β (GSK-3β)35, is flanked by loxP sites. Mice with osteoblast-specific constitutive activation of β-catenin were generated by crossing Catnb-flox(ex3) mice with z1(I)Col-Cre mice expressing Cre under the control of 2.3 kb of the proximal promoter of the mouse pro-z1(I) collagen gene. The transgene is expressed at high levels in osteoblasts specifically36. There is no expression in chondrocytes, condensed mesenchymal cells, perichondrial or periosteal fibroblasts, or any other type I collagen-producing cells, or other fibroblast-rich tissues such as muscle, heart or tendons. The resulting offspring, termed Catnb-Cαosb (original nomenclature lacft(ex3)lac), express a constitutively active β-catenin allele in osteoblasts. Mice with osteoblast-specific deletion of jag1 were generated by crossing previously described Jag1flox mice37 with z1(I)Col-Cre mice. Genotyping was performed at weaning stage by PCR analysis of genomic DNA. In each experiment the mice used were all littermates of the same genetic background. One-month-old female mice were used for the bone histomorphometric analysis. Ctnnb1-flox mice lack teeth due to osteopetrosis and therefore were fed a normal powdered diet that contained all the required nutrients (20% protein, 3.0 p.p.m. folacin, 51 μg per kg vitamin B12 from PicoLab Rodent Diet 20). Folate and vitamins B12 levels in B12 levels were confirmed to be adequate by measuring the intake of critical nutrients. Folate and vitamin B12 levels were measured by Antech Diagnostics using a chemiluminescence based kit (Siemens). All the protocols and experiments were conducted according to the guidelines of the Institute of Comparative Medicine, Columbia University. Randomization was done according to genotype and gender was blinded as approved by the institutional review board (IRB) for the IRB for the use of samples from the Tissue Repository. Patient samples. Bone marrow biopsies from patients with AML and MDS were consecutively obtained from 2000 to 2008 and reviewed under a research exempt waiver approved by the institutional review board (IRB) of Memorial Sloan-Kettering Hospital and Human Biospecimen Utilization Committee. Bone marrow biopsies and aspirates obtained from Columbia University from patients with MDS and AML were stored in the IRB-approved Tissue Repository at Columbia University Medical Center after informed consent. This study was conducted under protocol approval from the IRB for the use of samples from the Tissue Repository.

Karyotype analysis. Metaphase chromosome preparations were prepared from cells obtained from spleen specimens from Ctnnb-Cαosb mice after overnight culture in complete RPMI medium using standard methods. Giemsa banding was performed and the images were captured using Cytovision Imaging system (Applied Imaging) attached to a Nikon Eclipse 600 microscope. Twenty to thirty karyotypes were formed and the images were captured using Cytovision Imaging system (Applied Imaging). Twenty to thirty karyotypes were formed and the images were captured using Cytovision Imaging system (Applied Imaging). Twenty to thirty karyotypes were formed and the images were captured using Cytovision Imaging system (Applied Imaging). Twenty to thirty karyotypes were formed and the images were captured using Cytovision Imaging system (Applied Imaging). Twenty to thirty karyotypes were formed and the images were captured using Cytovision Imaging system (Applied Imaging). Twenty to thirty karyotypes were formed and the images were captured using Cytovision Imaging system (Applied Imaging). Twenty to thirty karyotypes were formed and the images were captured using Cytovision Imaging system (Applied Imaging). Twenty to thirty karyotypes were formed and the images were captured using Cytovision Imaging system (Applied Imaging). Twenty to thirty karyotypes were formed and the images were captured using Cytovision Imaging system (Applied Imaging).

Array comparative genomic hybridization (aCGH). aCGH analysis was performed in the spleen of Ctnnb-Cαosb mice using the mouse genome CGH 244A platform (Agilent Technologies) according to the manufacturer’s instructions. In brief, spleen DNA from wild-type littermates was used as reference DNA. Genomic DNA was subjected to restriction digestion before labelling and purification (SureTag DNA labelling kit, Agilent Technologies). For each 244 K array, 2 μg of labelled DNA and 2 μg of gnomeline reference DNA were labelled with Cy5 and Cy3, respectively. Differentially labelled test (tumour) DNA and normal reference DNA were hybridized simultaneously to normal chromosome spreads. Data extraction was conducted using the Agilent feature extraction software. Data files were analysed using the Agilent DNA analytics software. Data were deposited in Gene Expression Omnibus (accession number GSE51690).

Whole-exome capture and massively parallel sequencing. We focused only on substitution mutations. Finally, in the tumour samples, we removed all variants found present in any of the normal samples. The mutations were subjected to validation (present in tumour, absent in normal) by conventional Sanger-based resequencing analysis of PCR products obtained from tumour DNA using primers specific for the exon encompassing the variant. Data were deposited in the Sequence Read Archive (accession number SRP031981).

Microarray analysis. Total RNA was extracted from primary osteoblasts isolated from mouse calvaria using TRIzol reagent (Invitrogen). Microarray analysis was performed using the GeneChip 3′ IVT Express kit and mouse genome 430 2.0 array gene chips (Affymetrix) according to the manufacturer’s instructions. In brief, antisense RNA was synthesized from 500 ng of RNA and was biotinylated followed by purification and fragmentation using the GeneChip 3′ IVT Express kit. Fragmented aRNA was hybridized to Affymetrix mouse genome 430 2.0 array gene chips. Following hybridization, chips were scanned with a GeneChip Scanner 3000 7G (Affymetrix). Data were normalized using the Mas5 method38, and then log transformed. Data were deposited in Gene Expression Omnibus (accession number GSE43242)39. Differential expression was analysed using LIMMA40. We focused on 20 genes which we selected in advance of the analysis. Genes were considered when either are active in AML, are amplified according to our CGH results, activate Notch, or whose transcription is induced by Notch. A significance cutoff of a raw P < 0.05 was used, as is appropriate for small previously determined gene sets41. Representative probe sets of genes whose expression changed greater than ± 20% in at least one of the two mutants relative to wild type appear in Supplementary Table 1.

Bone marrow transplantation. For bone marrow transplantation studies, adult wild-type B6.SJL (CD45.1) recipient mice (8 weeks of age) were lethally irradiated (10 Gy, split dose) and were then transplanted with 1 × 105 of total bone marrow cells from Ctnnb-Cαosb (CD45.2) or wild-type B6.SJL (CD45.2) mice (4 weeks of age) by retro-orbital venous plexus injection. Engraftment efficiency in recipients was monitored by donor contribution of CD45.2+ cells using FACS analysis. For the reverse experiment, because of the early lethality of Ctnnb-Cαosb mice, 1 × 105 of total bone marrow cells from wild-type B6.SJL (CD45.1) mice were transplanted into lethally irradiated (600 rads, split dose) newborn (P1) Ctnnb-Cαosb mice or wild-type littermates by liver injections. Engraftment efficiency in recipients was monitored by donor contribution of CD45.1+ cells using FACS analysis. For HSC and progenitor transplantation studies, sublethally (5.5 Gy) irradiated wild-type B6.SJL (CD45.1) recipient mice (8 weeks of age) were injected with fractionated donor bone marrow subsets isolated from Ctnnb-Cαosb (CD45.2) or wild-type B6.SJL (CD45.2) mice (4 weeks of age). Engraftment efficiency in recipients was monitored by donor contribution of CD45.2+ cells using FACS analysis.

Treatment of animals with γ-secretase inhibitor. Two-week-old Ctnnb-Cαosb mice or their wild-type littermates were treated with vehicle, the γ-secretase inhibitor DBZ (2S,2′-[2-(3,5-dihydroxyphenyl)-acetyl]amino)-N-(5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl)-propionamide, 2 μmol kg−1 daily by intraperitoneal injection for 10 days. DBZ is cell-permeable, selective, non-translocation and non-competitive inhibitor of the γ-secretase complex. DBZ was synthesized to our validated procedures42 and assessed using a liquid chromatography/mass spectrometry (LC/MS) (Syncom) and suspended in a 0.5% methylcellulose (w/v, Colorcon) and 0.1% (v/v) Tween-80 (Sigma) solution43. Immediately before intraperitoneal injection, DBZ was sonicated for 2 min to achieve a homogenous suspension.

Haematological measurements and peripheral blood morphology. Haematological measurements, blood was collected by cardiac puncture. Peripheral blood cell counts were performed on a FORCYTE haematology analyser (Oxford Science). For morphological assessment, peripheral blood smears were stained with Wright-Giemsa stain (Sigma-Aldrich) for 10 min followed by rinsing in DIH2O for 3 min. Images were taken using a 60× objective on a Leica microscope outfitted with a color camera.

Real-time PCR. Total RNA was isolated from LSK or haematopoietic cells using RNeasy Micro Plus kit (Qiagen). Total RNA from bone marrow-free long bones was isolated using TRIzol reagent after removal of the periosteal layer. Quantitative real-time PCR was performed using the SYBR Green Master Mix (Bio-Rad) as previously described37. β-actin was used as endogenous control. Gene expression in LT-HSCs, ST-HSCs and MPPs was performed using the Power SYBR Green Cells-to-Ct kit (Ambion Life Technologies).

Reporter constructs and luciferase assays. The Jag1 promoter region carries multiple potential TCF/LEF binding sites (CTCTGTTG) located up to nucleotide −4075, −2626, −2578, −2343, −1962, −1857, −1566, −1221, −782). The mouse reporter constructs −4112/+130 and −2100/+130 for Jag1-luc were generated by PCR amplification of the corresponding fragments using mouse genomic DNA as template and subsequent subcloning into the BgII and Kpn1/BgII sites of the pGL3Basic vector (Promega), respectively. Transient transfection
assays were performed in HEK293T using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Cells were seeded in 24-well plates at a density of 0.3 × 10^6 cells per well. Then 24 h later, cells were transfected with a total amount of 350 ng of DNA containing 150 ng reporter plasmid and 50 ng β-catenin and TCF-1 expression vectors. A total of 5 ng of PRL-CMV Renilla (Promega) was used as an internal control to normalize for transfection efficiency and equivalent amounts of DNA were achieved with pcDNA3 vector. Forty hours after transfection luciferase activity was determined using the dual luciferase reporter assay system (Promega) and quantified using Fluostar Omega (BMG Labtech). Luciferase activity is presented as fold induction over basal conditions normalized to empty luciferase vector for identical experimental conditions.

**Chromatin immunoprecipitation (ChIP) assay.** Primary osteoblasts were seeded in 10-cm dishes at a density of 5 × 10^6. Cells were crosslinked with 1% formaldehyde for 10 min. Following Dounce homogenization, nuclei were collected and sonicated on ice to an average length of 0.5 kb. The samples were centrifuged and precleared with protein G in the presence of sonicated J. DNA and bovine serum albumin for 2 h at 4 °C. One-tenth of the volume of supernatant was used as input, and the remaining volume was immunoprecipitated with β-catenin antibody and the immune complexes were collected by absorption to protein G-sepharose, washed, eluted from the beads and incubated for 5 h at 65 °C to reverse crosslinks. After treatment with proteinase K, DNA was extracted with phenol-chloroform and precipitated with ethanol. Immunoprecipitated chromatin was analysed by PCR using primers corresponding to TCF/LEF binding sites on the tagged 1 promoter (−4075, −3072, −2626, −2578, −2434, −1992, −1957, −1566, −1221, −782). Putative binding elements were identified by using the TRANSFAC database. The products of the PCR amplifications were analysed by agarose gel electrophoresis.

**Antibodies and flow cytometry analysis.** Freshly isolated bone marrow cells and spleen cells were resuspended in flow-staining buffer (PBS plus 2% FBS) and the primary conjugated antibodies were added. After 30 min incubation at 4 °C, the cells were then washed twice before flow cytometry analysis. The following monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC), Allophycocyanin (APC) phycoerythrin (PE), PE-Cy7, APC-CY7, Per-CPCY5.5, Pacific Blue, and clonal antibodies conjugated with fluorescein isothiocyanate (FITC), Allophycocyanin were used. After 30 min incubation, cells were washed twice using flow buffer. Flow sorting analysis, osteoblasts were identified from the digested bone samples as a population of CD34+ Lin–Ocn– cells, in which Ocn (osteocalcin) is an osteoblast-specific, non-nuclear protein commonly used for isolation of live osteoblastic cells44–46. For microarray analysis and for experiments in mice, all gene expression studies were repeated using calvaria-derived cells a population rich in committed osteoblast progenitors and routinely used as osteoblast representative. Primary murine osteoblasts were prepared from calvaria of 2-day-old pups as previously described47–49. Mice calvaria were sequentially digested for 20, 40 and 90 min at 37 °C in a modified minimal essential medium (Gibco); 10% FBS containing 0.25% trypsin (Gibco). Cells of the first two digests were discarded, whereas cells released from the third digestion were plated in a minimal essential medium–10% FBS.

Osteoblasts were counted in each human bone biopsy as defined by standard histomorphometry guidelines50–52. The number of osteoblasts per mm of bone surface was calculated. The number of osteoblasts counted depends on the size of the sample and the bio-/pathophysiological characteristics of the individual and for this study, the size of the biopsy (1 cm) allowed for counting of 30 osteoblasts per biopsy.

**Histological analysis of human biopsies and murine bone, spleen and liver.** Bone marrow biopsies were fixed overnight in 10% neutral formalin solution, embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin (H&E) for immunohistochemistry, specimens were incubated with CD-117 (C-kit; Abcam), CD13 (Santa Cruz) or Myeloperoxidase (MPO) antibodies after an antigen retrieval step and blocking of endogenous peroxidase with 3% H2O2. Sections were then incubated with biotinylated secondary antibody and immune complexes formed were detected using standard avidin-biotin complex method.

**Statistical analysis.** All data are represented as mean ± standard deviation. Statistical analyses were performed using a one-way ANOVA followed by Student–Newton–Keuls test and a P value less than 0.05 was considered significant. Time-to-event analysis was used to assess median survival time to death. Kaplan–Meier curves were generated to illustrate time to death, stratified by group status. Statistical significance of the between-group difference in the median time-to-endpoint was assessed by the log-rank test. Statistical analyses were performed using XLSTAT (2012.6.02, Addinsoft) and SAS (version 9.2, SAS Institute). A P value less than 0.05 was considered statistically significant.
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Extended Data Figure 1 | Anaemia, peripheral blood leukocytosis and monocytosis and deregulated haematopoiesis specific activation of β-catenin in osteoblasts of Ctnnb1CAosb mice. a. Haematopoietic parameters. White blood cells (WBC), red blood cells (RBC), haemoglobin (HB), haematocrit (HCT), platelets, lymphocytes (LY), neutrophils (NE) and monocytes (MO) in 2-week-old mice. b–k, Bone marrow samples. b, Erythroid cell numbers. c, Representative flow cytometry image showing monocytic/granulocytic (CD11b/Gr1) subset. d, Numbers of the CD11b/Gr1 subset. e, Distribution of LSK (Lineage-2Sca-1C-kit+) population. f, LSK numbers. g, h, Frequency (g) and percentage (h) of LT-HSCs and ST-HSCs. i, Numbers of LSK+/FLT3+ cells. j, Myeloid progenitor profile by CD34 versus FcγRII/IIIA analysis of electronically gated Lin- Sca-1 c-Kit+ bone marrow cells. k, Numbers of myeloid progenitor populations. l, Spleen weight. m, Extramedullar haematopoiesis in the liver of 3-weeks-old Ctnnb1CAosb mice indicated by megakaryocytes (black arrow), myeloid (white arrow) and rare erythroid precursors (dotted arrow). n–p, Percentage of Ter119+ (n), CD11b+/Gr1+ (o) and myeloid progenitor (p) populations in the spleen. q, PCR analysis of genomic DNA from osteoblasts and indicated haematopoietic populations from wild-type and Ctnnb1CAosb mice. r–t, Real-time PCR analysis of β-catenin targets in bone marrow CD45+CD34+CD31+ cells (r), spleen (s) and bones (t). In a, n = 6; b–k, n–p, n = 8; l, m, n = 5 and r–t, n = 4 mice per group. Results are mean ± s.d. and show a representative from five (a–p) or 2 (q–t) independent experiments. *P < 0.05 versus wild type. MNC, mononuclear cells.
Extended Data Figure 2 | Multi-organ infiltration with blasts and dysplastic cells and myeloid differentiation block in Ctnnb1CAosb mice. a, Blast infiltration (solid arrows) and neutrophil hypersegmentation (open arrow and magnified panels) in the blood of Ctnnb1CAosb mice. Images at ×40 or ×100 magnification. b, Blast infiltration (solid arrows) and micro-megakaryocytes with hyperchromatic nuclei (white arrows) in the bone marrow of Ctnnb1CAosb mice. Images at ×60 magnification. c, Blast infiltration (solid arrows and magnified panel) and presence of dysplastic megakaryocytes (yellow arrows and magnified panel) in the spleen of Ctnnb1CAosb mice. Image at ×400 magnification. d–f, Myeloperoxidase (MPO) staining of long bone (d), spleen (e), and liver (f) showing massive invasion of myeloid cells. g, CD117 (c-kit) staining of bone sections showing CD117+ blasts in Ctnnb1CAosb mice. h, CD13 staining of bone sections showing myeloid/monocytic infiltration in Ctnnb1CAosb mice. In d–h images at ×60 magnification. i–l, B-cell progenitors numbers in the bone marrow (i, j), spleen (k) and lymph nodes (l) of mice. m–t, Proportion of T cells. u, Lack of myeloid cell differentiation in Ctnnb1CAosb bone marrow cells following treatment with cytokines. v–x, Percentage of immature myeloid cells in ex vivo bone marrow cultures treated with cytokines. y, Robertsonian translocation between chromosomes 1 and 19 in 2 of 30 metaphases of the spleen of an 18-day-old Ctnnb1CAosb mouse. Inset shows the same abnormality in another cell. z, Whole-exome sequencing of myeloid malignancies (CD11b+/Gr1+) from 3 Ctnnb1CAosb mice and 3 germline normal (tail) samples. In i–x, n = 6 mice per group. *P < 0.05 versus wild type. Results are mean ± s.d. and show a representative of five (i–t) or three (u–x) independent experiments.
Extended Data Figure 3 | Cell autonomous AML development by bone marrow and LT-HSCs cells of Ctnnb1CAosb mice. a, Engraftment efficiency of CD45.2 Ctnnb1CAosb bone marrow cells in lethally irradiated CD45.1 wild-type mice 7 weeks following transplantation. b–f, Percentage of indicated populations in bone marrow of transplanted mice. g, Blasts in blood (15–80%, solid arrows) of lethally irradiated CD45.1 wild-type mice transplanted with CD45.2 Ctnnb1CAosb marrow cells 7 weeks following transplantation. h, Blasts (solid arrows) and dysplastic megakaryocytes (open arrow) in bone marrow of transplanted mice. Images at ×60 magnification. i, Lethality curves. j, Engraftment efficiency of CD45.2 Ctnnb1CAosb bone marrow cells in lethally irradiated CD45.1 wild-type mice 7 weeks following transplantation. k–o, Increased percentage of LSK cells (k), myeloid progenitors (l), and CD11b+/Gr1+ (m) cells and decreases in erythroid cells (n) and B-lymphopoiesis (o) in the bone marrow of transplanted mice. p, q, Blasts in the blood (p) and bone marrow (q) (black arrows) of transplanted mice. Images were taken at ×100 (o) and ×60 (p) magnification. r, Lethality curves. s, Engraftment efficiency of indicated bone marrow haematopoietic populations from 4-week-old CD45.2 Ctnnb1CAosb or wild-type mice in sublethally irradiated CD45.1 wild-type mice after 4 weeks (for LT-HSCs) and 8 weeks (for other populations) of transplantation. t, Blood counts in wild type mice transplanted with indicated haematopoietic cells from Ctnnb1CAosb mice. u–w, Lack of blasts in the blood of wild-type mice transplanted with indicated haematopoietic cells from Ctnnb1CAosb mice. x, Disease development in wild-type mice transplanted with indicated haematopoietic cells from Ctnnb1CAosb mice. y, Splenomegaly in wild-type mice transplanted with LT-HSCs from Ctnnb1CAosb. z, Spleen size and weight in wild-type mice transplanted with indicated haematopoietic populations from WT or Ctnnb1CAosb mice. n = 6 mice per group. Results are mean ± s.d. and show a representative of two independent experiments. *P < 0.05 wild type–wild type versus wild type–Ctnnb1CAosb or versus Ctnnb1CAosb–wild type transplanted group.
Extended Data Figure 4 | Newborn Ctnnb1CAosb mice show MDS but fetal HSCs from Ctnnb1CAosb mice do not transfer AML. a–e. Increased percentage of LSK cells (a), GMPs (b), and CD11b\(^+\)/Gr1\(^+\) (c) cells and decreases in erythroid (d) cells and B-lymphopoiesis (e) in the liver of newborn (P1) Ctnnb1CAosb mice. f–j. Increased percentage of LSK cells (f), GMPs (g), and CD11b\(^+\)/Gr1\(^+\) (h) cells and decreases in erythroid cells (i) and B-lymphopoiesis (j) in the bone marrow of newborn (P1) Ctnnb1CAosb mice. k–m, Liver (k), bone marrow (l) and spleen (m) of newborn (P1) Ctnnb1CAosb mice showing microhypolobated megakaryocytes (open arrows), Pelger Huet neutrophils (yellow arrows) or blasts (solid arrows). Images at \(\times 100\) magnification. n, Percentage of immature myeloid cells in the bone marrow of newborn mice. o–t, Flow cytometry and Giemsa-stained cytospins showing lack of changes in the percentage of immature myeloid cells in \textit{ex vivo} cultures of bone marrow cells from P1 stage Ctnnb1CAosb mice and treated with indicated cytokines. u. Engraftment efficiency of CD45.2 Ctnnb1CAosb LSK cells obtained from the liver of embryonic day 18.5 embryos in sublethally irradiated CD45.1 wild-type mice. v. Normal peripheral blood measurements in transplanted mice. w. Lack of blasts in the blood of transplanted wild-type mice. Images at \(\times 100\) magnification. n = 6 mice per group. Results are mean \pm s.d. and represent at least two independent experiments. *\(P < 0.05\) versus wild type.
Extended Data Figure 5 | Inhibition of increased Notch signalling normalizes blood counts and rescues haematopoietic defects in Ctnnb1CAosb mice. a, Microarray analysis of calvaria-derived osteoblasts from Ctnnb1CAosb mice. AML and Notch-related genes in Ctnnb1CAosb osteoblasts and with P < 0.05 and fold change of ≥20% in one comparison. Genes that are up- or downregulated relative to wild type are shown. b, Flow cytometry analysis of Jagged1 expression in osteoblasts (MFI: mean fluorescent intensity). c, Luciferase activity in HEK293T cells co-transfected with β-catenin, Lef1 and Jag1-Luc reporter constructs (2.4112/1.130) and (2.2100/1.130). Results show fold induction over respective Jag1-Luc reporter constructs. *P < 0.05 versus respective Jag1-Luc. Results are mean ± s.d. d, ChIP in primary osteoblasts using anti-β-catenin antibody. Primers spanned the putative TCF/LEF binding sites (indicated) on the Jag1 promoter. e, Expression of Notch1 and Notch2 in LSK cells. f–g, Expression of Notch targets in LSK subpopulations. h, i, Normal intestinal architecture (h) and PAS staining (i) showing lack of goblet cell (arrows) metaplasia in DBZ-treated mice. Images at ×60 magnification. j, Peripheral blood counts and bone marrow cellularity in wild-type and Ctnnb1CAosb mice treated daily with vehicle or DBZ (2 μmol per kg body weight) for 10 days. k–p, Percentage of LSK cells (k), LSK-Sca1+ subpopulations (l), myeloid progenitors (m), CD11b+Gr1− population (n), erythroid cells (o) and LSK−/FLT3− population (p) in the bone marrow. m, Percentage of erythroid cells and p, LSK−/FLT3− population in the bone marrow. n–s, Percentage of myeloid progenitor populations (q), CD11b+Gr1− (r), cells and erythroid (s), cells in the spleen. In n = 3 mice per group and in b, n = 4 mice per group. In c, d, results represent two independent experiments. In e–g N = 4 mice per group, and *P < 0.05 versus wild type. In h–s n = 8 mice per group and *P < 0.05 versus wild type and #P < 0.05 Ctnnb1CAosb vehicle versus DBZ-treated Ctnnb1CAosb group. Results are mean ± s.d. and show a representative of two independent experiments.
Extended Data Figure 6 | Jag1 inactivation in osteoblasts prevents AML of Ctnnb1CAosb mice. (a–d) Expression of Notch transcriptional targets in bone marrow LSK subpopulations. Rescue of changes in the proportions of LSK (e) and erythroid (f) cells in the bone marrow of Ctnnb1CAosb/Jag1osb<sup>+/−</sup> mice. (g) Improvement of B-lymphopoiesis in Ctnnb1CAosb;Jag1osb<sup>+/−</sup> mice. (h) Bone marrow, spleen (i) and liver (j) histology in Ctnnb1CAosb;Jag1osb<sup>+/−</sup> mice. (k) Long bone sections. Images at ×4 magnification. In (a–d), n = 4 and in (e–k) n = 8 mice per group. *P < 0.05 versus wild type and #P < 0.05 versus Ctnnb1CAosb;Jag1osb<sup>+/−</sup> mice. Results are mean ± s.d. and show a representative of three (a–d) and two (e–k) independent experiments.
Extended Data Figure 7 | Inhibition of Notch signalling reverses AML in Ctnnb1CAosb mice. a, Lack of blasts (solid arrows) and, normal neutrophils (right panel) in blood of DBZ-treated Ctnnb1CAosb mice. b–c, Normal megakaryocytes in the bone marrow (b) and spleen (c). d, Normal spleen histology in DBZ-treated Ctnnb1CAosb mice. Yellow arrows indicate abnormal cells with large nucleoli and dotted arrow indicates abnormal megakaryocytes in Ctnnb1CAosb mice; white arrow indicates normal megakaryocytes in DBZ-treated Ctnnb1CAosb mice. e, Lack of monocyte infiltration in the liver of DBZ-treated Ctnnb1CAosb mice. Arrow indicates cluster of mononuclear cells. f–h, MPO staining of bone marrow (f), spleen (g) and liver (h). i, Per cent of cells staining with MPO in the indicated tissues. j, Increased survival in DBZ-treated Ctnnb1CAosb mice. In a, b, images taken at ×100 magnification. In c–g images taken at ×60 magnification. k–l, Proportion of B-cell populations in the bone marrow (k) and spleen (l). m, Long bone sections. Images at ×4 magnification. n = 6 mice per group. *P < 0.05 versus wild type and #P < 0.05 Ctnnb1CAosb vehicle versus DBZ-treated Ctnnb1CAosb group. Results are mean ± s.d. and show a representative from two independent experiments.
Extended Data Figure 8 | Nuclear accumulation of β-catenin in osteoblasts and increased Notch signalling in 38.3% of patients with MDS/AML and identification of underlying pre-AML conditions by nuclear localization of β-catenin in osteoblasts. a–f, Double immunofluorescence staining with β-catenin and RUNX2 in osteoblasts from bone marrow biopsies from 6 MDS/AML patients harbouring nuclear accumulation of β-catenin in osteoblasts and showing nuclear accumulation of HEY1 in the corresponding patients (×60 magnification). g, h, During screening assumed healthy controls, 2 individuals were identified with nuclear β-catenin in their osteoblasts. Re-evaluation showed underlying hematologic disorder, Case 1: MDS RAEB-1, Case 2: Jak2 positive myelofibrosis. g, Double immunofluorescence staining with β-catenin and RUNX2 in osteoblasts from bone marrow biopsies of the 2 cases (×60 magnification). h, β-catenin cellular localization in cases 1 and 2 with associated cytogenetic abnormalities. NL: normal cytogenetics. In the fourth column percentages indicate osteoblasts with nuclear localization of β-catenin.
Membrane accumulation of β-catenin in osteoblasts in 61.7% of patients with MDS/AML and in healthy subjects and nuclear accumulation of β-catenin in osteoblasts in 38.3% of patients with MDS/AML identified by flow cytometry.

Double immunofluorescence staining with β-catenin and Runx2 in osteoblasts from bone marrow biopsies from a–c, 3 MDS/AML patients and d–g, 4 healthy subjects harbouring membrane localization of β-catenin in osteoblasts. h–j, Flow cytometry using a non-phospho β-catenin antibody detecting nuclear/activated β-catenin. Representative plots showing nuclear (h), versus non-nuclear (i), localization of β-catenin in osteoblasts from individual MDS/AML patients and, non-nuclear localization of β-catenin in osteoblasts from 5 healthy subjects as CD34−/Lin−OCN+ cells. (OCN, osteocalcin an osteoblast-specific protein used for isolation of live osteoblastic cells).
Extended Data Table 1 | Structural chromosomal alterations detected by aCGH in Ctnnb1CAsb1 mice

| Mouse Chromosome | Location (Mb) | Size (kb) | Genes | Copy number change | Mouse ID | Human chromosome | Location (Mb) |
|------------------|---------------|-----------|-------|-------------------|---------|------------------|---------------|
| 2                | 54.7          | 114       | Ctnnb1 | Deletion of 2/3 copies | 1       | No ortholog      |               |
| 3                | 63.4          | 608       | Topo2a, Topo1, Topo4, Topo2, Cen10697, Cen10425, Cen10696 | Deletion of 1/3 copies | 1       | No ortholog      |               |
| 4                | 69.3          | 245       | Rspo4, Hhe2, Nbs1 | Deletion of 1/3 copies | 3       | 9q32             | 115.10        |
| 5                | 56.9          | 580       | Spen1, IRX3, DPC1, 4925, 1558, 1560 | Deletion of 1/4 copies | 2, 3    | No ortholog      | 115.16        |
| 6                | 26.3          | 238       | PFeST, E2F2, 452, 240, DAX1, 196 | Deletion of 1/3 copies | 5       |                |               |
| 7                | 15.7          | 506       | Nkx2-5 | Deletion of 3/4 copies | 1, 2, 3, 4 | Tpl121          | 23.9          |
| 8                | 13.2          | 41        | Gpr56  | Deletion of 1/2 copies | 5       |                 | 16.2          |
| 9                | 13.2          | 43        | Nkx2.5 | Deletion of 1/2 copies | 3       |                 |               |
| 10               | 15.7          | 44        | Sall4, E2F1, 196 | Deletion of 1/2 copies | 3       | No ortholog      |               |
| 11               | 30.2          | 494       | Sall4, Mdx2, Dax1, Dax1f, Dax1l, Dax1c, Dax1d, Dax1e, Dax1f, Dax1g, Dax1h | Gain of <1 copies | 3       | 10p13.1          | 16.5          |
| 12               | 51.9          | 86        | Ppp2ca | Deletion of 2/3 copies | 5       |                 | 133.5         |
| 13               | 66.1          | 32        | No genes | Deletion of 1/2 copies | 4       |                  |               |
| 14               | 88.8          | 59        | No genes | Deletion of 2/3 copies | 4       | No ortholog      |               |
| 15               | 108           | 390       | No genes | Heterozygous deletion | 5       |                 |               |
| 16               | 108           | 190       | No genes | Deletion of 1/2 copies | 3       | No ortholog      |               |
| 17               | 20.4          | 70        | ApoE10a, ApoE7c | Gain of <1 copy | 1       | 10q21.31         | 38.4          |
| 18               | 35.4          | 40        | Ctnnb1, Tcf21, 70 | Gain of <1 copy | 3, 4     | 14q32.33         | 105.5         |
| 19               | 36.4          | 10         | Ctnnb1, Tcf21, 70 | Gain of <1 copy | 3, 4     | 10q21.31         | 105.1         |

Mouse ID denotes mouse number (1, mouse 1; 2, mouse 2 and so on).