**Supplementary figure 1:** The effect of *Dicer1* deletion on osteoblasts and bone in vivo. 

a,b frequency of lineage CD45^-CD31^-GFP^+ osteolineage cells in bone marrow (a) or collagenased bone fragments (b) (n=4) 

c-e impaired osteogenic differentiation capacity of OCD^fl/fl^ bone marrow stromal cells as shown by reduced CFU-Alk/CFU-F ratio (c) (n=2, performed in quadruplicate), impaired in vitro osteogenic differentiation upon osteogenic induction (d) and decreased *osteocalcin* gene expression in primary osteolineage cells as shown by RT-PCR on CD45^-Lineage^-CD31^-GFP^+ cells (n=5) (e) 

f, altered texture of the bone matrix g, normal trabecular bone volume (BV) and h, increased cortical bone volume as assessed by histomorphometric analysis (n=8) (TV=total volume; BA=bone area, TA total area). 

i,j reduced osteoblasts (Ob) number per bone surface (Bs) in OCD^fl/fl^ mice as indicated by histomorphometric analysis (n=8) k, the number of osteoclasts (OC) was unaltered in OCD^fl/fl^ mice (n=8). Data are mean ± s.e.m. * p≤0.05, **p≤0.01. CFU-F=colony-forming assay –fibroblast, CFU-ALK=colony forming unit alkaline phosphatase, AP=alkaline phosphatas
Supplementary figure 2: Ineffective hematopoiesis and myelodysplasia in OCD \textsuperscript{fl/fl} mice. \textbf{a}, Leukopenia comprised all leukocyte subsets (n=10). Cytopenias were present despite normal or increased cellularity of the bone marrow (\textbf{b, c}) and normal hematopoietic stem and progenitor cell numbers and function. \textbf{d, e}, frequency of immunophenotypically defined stem (LKS CD150\textsuperscript{+}CD48\textsuperscript{-}) and progenitor (LKS) cells (n=4). \textbf{f}, hematopoietic stem cell function as determined by competitive reconstitution capacity (n=5) \textbf{g}, hematopoietic progenitor cell function as assessed by CFU-C (n=10). \textbf{h}, increased frequency of (CD11b\textsuperscript{+}Gr1\textsuperscript{+}) myeloid cells (n=6) and \textbf{i}, decreased frequency of (CD43\textsuperscript{+}B220\textsuperscript{-}CD19\textsuperscript{+/-}) B-cell progenitors in the bone marrow (n=5) \textbf{j}, increased bone marrow vascularity confirmed by the endothelial cell marker CD31 (PECAM). Inset: CD31 staining of micro-megakaryocytes. Data are mean ± s.e.m. * p<0.05, **p<0.01. LY=lymphocytes, BASO=basophils, NE=neutrophils, MO=monocytes, EO=eosinophils, CFU-C=colony forming unit in culture. LKS= lineage \textsuperscript{−}C-kit\textsuperscript{−} Sca1\textsuperscript{+} cells LKS-SLAM= lineage \textsuperscript{−}C-kit\textsuperscript{−} Sca1\textsuperscript{+} CD150\textsuperscript{+} CD48\textsuperscript{-} cells, H&E=hematoxylin/eosin.
Supplementary figure 3: Extramedullary hematopoiesis rescues peripheral cytopenia in a subfraction of OCD fl/fl mice.

Extramedullary hematopoiesis (EMH) was observed in a subset of 3/10 animals investigated for its presence as indicated by a, histology showing increased hematopoiesis with increased numbers of magakaryocytes. Megakaryocytes display normal morphology b, increased CFU-C spleen, spleen cellularity, relative frequency of erythroid progenitor (CD45+ter119+) cells and primitive hematopoietic (LKS) progenitors (n=3). c,d, significant anemia and thrombocytopenia in OCD fl/fl mice lacking EMH (n=7) in comparison to Dicer fl/+ littermates. Conversely, the occurrence of EMH (n=3) rescued anemia and thrombocytopenia in these mice. Data are mean ± s.e.m. * p≤0.05, **p≤0.01.
Supplementary figure 4: Representative example of flow-cytometric assessment of primitive hematopoietic LKS/CD150/CD48 subpopulations
Supplementary figure 5: Myelodysplastic features of OCD fl/fl mice. a, neutrophilic dysplasia in peripheral blood. Arrow indicating giant platelet. b, micro-megakaryocytes with hyperchromatic nuclei. c, frequency of dysplastic cells (n=12 mice, ≥ 20 cells counted/sample; average ± sem, range 15.0%-89.5% and 6.5%-92.0% for neutrophils and megakaryocytes respectively)
Supplementary figure 6: Apoptosis in hematopoietic progenitor cells in OCD fl/fl mice: representative FACS plots.

LKS = lineage "C-kit" Sca1" cells 
LKS-SLAM = lineage "C-kit" Sca1" CD150" CD48" cells 
L-K+ = lineage "C-kit" cells 
L-K-int = lineage "C-kit intermediate"

OCD fl/+ OCD fl/fl OCD fl/+ OCD fl/fl

Annexin V 7AAD

LKS - SLAM LKS L-K+ L-K-int
Supplementary figure 7: Apoptosis in hematopoietic progenitor subsets in OCD fl/fl mice.

a. Representative FACS plots of progenitor identification and apoptosis rates, b. overall data (n=4). Data are mean ± s.e.m. * p≤0.05, **p≤0.01.
Supplementary figure 8: Increased proliferation of hematopoietic stem and progenitor cells at the endosteal surface in OCD fl/fl mice. 

**a**, representative FACS histogram of BRDU+ cells by in vivo BRDU labeling **b**, confocal microscopy of transplanted DiD-labeled LKS cells. (osteoblasts green, vasculature red, LKS cells (arrows) white) **c**, distance to endosteal surface (n=2, 20±3.6 cells/animal) and **d**, frequency of cell duplets. Data are mean ± s.e.m. * p≤0.05, **p≤0.01.
Supplementary figure 9: Representative example of flow-cytometric assessment of B-cell subpopulations
Supplementary figure 10: Hematological abnormalities in OCD \textsuperscript{fl/fl} mice cannot be propagated in a hematopoietic cells autonomous manner. Bone marrow mononuclear cells of OCD \textsuperscript{fl/fl} or littermate OCD \textsuperscript{fl/+} mice (n=2) were transplanted into lethally irradiated WT (B6.SJL) mice (n=4 per OCD mouse). \textbf{a}, near complete donor chimerism 16 weeks post-transplant with normalization of \textbf{b}, peripheral blood numbers, and \textbf{c}, apoptosis of primitive progenitors. Data are mean ± s.e.m. * p≤0.05, **p≤0.01.
Supplementary figure 11: Hematopoietic cells from OCD \textit{fl/fl} mice do not confer hematological abnormalities: Data of mutant into WT transplantation at 12 months post-transplant. \textbf{a}, bone marrow mononuclear cells of OCD \textit{fl/fl} (n=4) or littermate OCD \textit{fl/+} mice (n=6) were transplanted into lethally irradiated WT (B6.SJL) mice. \textbf{b}, near complete donor chimerism \textbf{c}, peripheral blood numbers, \textbf{d}, granulocyte and megakaryocyte morphology, \textbf{e}, frequency of B cells and B cell progenitors. \textbf{f}, frequency of myeloid cells \textbf{g}, apoptosis of primitive progenitors. Data are mean \pm s.e.m. * \( p \leq 0.05 \), ** \( p \leq 0.01 \).
Supplementary figure 12: Myelodysplasia in OCD \textsuperscript{fl/fl} mice is induced by the bone marrow microenvironment. Wildtype (CD45.1) B6.SJL cells were transplanted into OCD \textsuperscript{fl/+} (n=8) or OCD \textsuperscript{fl/fl} (n=8) mice. a,b, hematopoiesis at 8 weeks was predominantly of WT origin but c, displayed prominent dysplastic features (n=4; 40 neutrophils or megakaryocytes were scored per sample) d,e, anemia and thrombopenia f, increased frequency of myeloid cells in the bone marrow with g, reduced frequency of B-cells and B-cell progenitors and h, increased apoptosis of hematopoietic progenitors. Data are mean ± s.e.m. * p≤0.05, **p≤0.01.
Supplementary figure 13: Co-culture assays reveal direct effects of OCD \textsuperscript{fl/fl} osteolineage cells on hematopoietic progenitor cell proliferation and megakaryocytic differentiation

Two hundred DS-red LKS or MEP were co-cultured on bone marrow derived stromal cells for 7 days as described in the section 'methods' \textbf{a}, osterix and osteocalcin gene expression in stromal cells in A-MEM indicating osteolineage commitment in the absence of terminally differentiated (osteocalcin\textsuperscript{+}) osteoblasts \textbf{b}. The total number of hematopoietic cells was significantly increased in LKS co-cultured with OCD \textsuperscript{fl/fl} stroma with \textbf{c}, preferential expansion of ckit+lin- cells in the absence of decreased apoptosis in this fraction \textbf{d} consistent with increased proliferation of primitive hematopoietic subsets on OCD \textsuperscript{fl/fl} stroma. Plating of MEPs on OCD \textsuperscript{fl/fl} stromal cells resulted in increased numbers of hematopoietic cells \textbf{e} but significantly impaired differentiation towards (CD41+) megakaryocytes \textbf{f,g}. Apoptosis was not increased in hematopoietic subsets under these conditions \textbf{h} \textbf{i}, representative picture of morphological abnormalities of CD41+ megakaryocytic cells showing small megakaryocytes with condensed, hypolobular nuclei on OCD \textsuperscript{fl/fl} stroma. (Data show representative experiments with n=2 mice, experiments performed at least in triplicate; data are mean \pm s.e.m. * p≤0.05, **p≤0.01). ND=not detectable, AMEM=minimal essential medium alpha
Supplementary figure 14: Deletion of Dicer1 in terminally differentiated osteoblasts does not confer myelodysplasia. a, deletion of Dicer1 in long bones and calvarium of ocn-cre+Dicer fl/+ and fl/fl animals. b, alteration in texture of bone matrix and, c, increased cortical bone volume (n=2). Effects on hematopoiesis were examined in 4-6 weeks old animals showing d, no effect on peripheral blood cell numbers, e, peripheral blood cells morphology, f, bone marrow vasculature or megakaryocyte morphology g, frequency of myeloid cells and h, B-cell progenitors. n=5. Data are mean ± s.e.m.
Supplementary figure 15: Hematopoietic phenotype of OCD fl/fl mice at the age of three weeks. 

- **a,** anemia, 
- **b,** dysplasia of neutrophils and megakaryocytes, 
- **c,** decreased frequency of B-cell progenitors, 
- **d,** increased frequency of myeloid cells, 
- **e,** Increased apoptosis of hematopoietic progenitor cells. (n=5 for all analyses). Data are mean ± s.e.m. *p≤0.05, **p≤0.01.
Supplementary figure 16: Genomic location and probe information on cytogenetic abnormalities in myeloid sarcomas as detected by CGH

a, tumor1  b, tumor2  c, tumor3
Supplementary figure 17: Infiltrative tumor behavior a, infiltration of tumor into muscle (a1) and glandular tissue (a2).
Supplementary figure 18: Targeted deletion of the Sbds gene from osteoprogenitor cells

a, altered texture of the cortical bone similar to OCD \( ^{+/+} \) mice, b, reduced frequency of B cells and pre-B cells in the bone marrow \( (n=8) \) c, increased frequency of myeloid cells in the bone marrow \( (n=8) \). Data are mean ± s.e.m. * \( p \leq 0.05 \), ** \( p \leq 0.01 \).
Supplementary tables

Supplementary table 1: Significantly (p<0.05 (t-test) up (> 2-fold) - and down regulated (>2-fold) genes in OCD fl/fl in comparison to OCD fl/+ osteolineage cells

| Gene name         | Description                                                                 | T-test p-value | Fold Change | Status |
|-------------------|-----------------------------------------------------------------------------|----------------|-------------|--------|
| ESF1              | 2610101J03RIK                                                               | 0.036          | 5.42682     | up     |
| PRG2              | PRG2:proteoglycan 2, bone marrow (natural killer cell activator, eosinophil granule major basic protein) | 0.04697        | 4.69371     | up     |
| FABP6             | FABP6:fatty acid binding protein 6, ileal (gastrotropin)                    | 0.02693        | 4.16036     | up     |
| SLC26A6           | SLC26A6:solute carrier family 26, member 6                                 | 0.02134        | 3.83104     | up     |
| AA591059          | na                                                                          | 0.04591        | 3.76227     | up     |
| TUBA4             | TUBA4:tubulin, alpha 4                                                      | 0.03639        | 3.27486     | up     |
| C430042M11RIK     | na                                                                          | 0.02185        | 3.23017     | up     |
| CRTAC1            | CRTAC1:cartilage acidic protein 1                                           | 0.0473         | 3.09731     | up     |
| GP49A /// LILRB4  | na                                                                          | 0.04749        | 2.75924     | up     |
| D3ERTD108E        | na                                                                          | 0.03166        | 2.70639     | up     |
| ZFP397            | na                                                                          | 0.0182         | 2.68709     | up     |
| COL4A4            | COL4A4:collagen, type IV, alpha 4                                           | 0.05033        | 2.60923     | up     |
| KIF13B            | KIF13B:kinesin family member 13B                                           | 0.0291         | 2.60515     | up     |
| SLC7A2            | SLC7A2:solute carrier family 7 (cationic amino acid transporter, y+ system), member 2 | 0.04061       | 2.5453      | up     |
| 2310007H09RIK     | na                                                                          | 0.00718        | 2.49706     | up     |
| 4930441O14RIK     | na                                                                          | 0.05163        | 2.49114     | up     |
| CML3              | na                                                                          | 0.0004         | 2.4063      | up     |
| ITSN2             | ITSN2:intersectin 2                                                         | 0.05006        | 2.3858      | up     |
| 4930579C12RIK     | na                                                                          | 0.05379        | 2.35791     | up     |
| WRN               | WRN:Werner syndrome                                                         | 0.03028        | 2.30526     | up     |
| TEX19             | na                                                                          | 0.05461        | 2.30136     | up     |
| ARL6IP2           | ARL6IP2:ADP-ribosylation factor-like 6 interacting protein 2                | 0.04964        | 2.29179     | up     |
| LOC548102         | na                                                                          | 0.05069        | 2.27919     | up     |
| CUZD1             | CUZD1:CUB and zona pellucida-like domains 1                                 | 0.02772        | 2.24676     | up     |
| RABGAP1           | RABGAP1:RAB GTPase activating protein 1                                     | 0.04486        | 2.21999     | up     |
| SMEK1             | 1110034C04RIK                                                               | 0.03913        | 2.19501     | up     |
| MLSTD1            | MLSTD1: male sterility domain containing 1                                 | 0.01118        | 2.18289     | up     |
| SYT1              | SYT1:synaptotagmin I                                                        | 0.03581        | 2.1705      | up     |
| 2700022O18RIK     | na                                                                          | 0.03128        | 2.15625     | up     |
| LSAMP             | LSAMP:limbic system-associated membrane protein                             | 0.0478         | 2.14451     | up     |
| IL2RB             | IL2RB:interleukin 2 receptor, beta                                          | 0.01355        | 2.13979     | up     |
| AI844685          | na                                                                          | 0.01838        | 2.13573     | up     |
| Gene       | Description                                                                 | Log2 Fold Changes | p-value |
|------------|-----------------------------------------------------------------------------|-------------------|---------|
| RAD23B     | RAD23 homolog B (S. cerevisiae)                                              | 0.02611           | 2.1244  |
| STX19      | A030009B12RIK                                                               | 0.01625           | 2.12178 |
| 9430081G06RIK |                                             | 0.0154            | 2.12048 |
| 1810033B17RIK |                                             | 0.004             | 2.1112  |
| DHPS       | DHPS: deoxyhypusine synthase                                                 | 0.03132           | 2.10313 |
| 1700034F02RIK |                                             | 0.01501           | 2.05275 |
| STX19      | A030009B12RIK                                                               | 0.01625           | 2.12178 |
| 9430081G06RIK |                                             | 0.0154            | 2.12048 |
| 1810033B17RIK |                                             | 0.004             | 2.1112  |
| DHPS       | DHPS: deoxyhypusine synthase                                                 | 0.03132           | 2.10313 |
| 1700034F02RIK |                                             | 0.01501           | 2.05275 |
| STX19      | A030009B12RIK                                                               | 0.01625           | 2.12178 |
| 9430081G06RIK |                                             | 0.0154            | 2.12048 |
| 1810033B17RIK |                                             | 0.004             | 2.1112  |
| DHPS       | DHPS: deoxyhypusine synthase                                                 | 0.03132           | 2.10313 |
| 1700034F02RIK |                                             | 0.01501           | 2.05275 |
| STX19      | A030009B12RIK                                                               | 0.01625           | 2.12178 |
| 9430081G06RIK |                                             | 0.0154            | 2.12048 |
| 1810033B17RIK |                                             | 0.004             | 2.1112  |
| DHPS       | DHPS: deoxyhypusine synthase                                                 | 0.03132           | 2.10313 |
| 1700034F02RIK |                                             | 0.01501           | 2.05275 |
| STX19      | A030009B12RIK                                                               | 0.01625           | 2.12178 |
| 9430081G06RIK |                                             | 0.0154            | 2.12048 |
| 1810033B17RIK |                                             | 0.004             | 2.1112  |
| DHPS       | DHPS: deoxyhypusine synthase                                                 | 0.03132           | 2.10313 |
| 1700034F02RIK |                                             | 0.01501           | 2.05275 |
| STX19      | A030009B12RIK                                                               | 0.01625           | 2.12178 |
| 9430081G06RIK |                                             | 0.0154            | 2.12048 |
| 1810033B17RIK |                                             | 0.004             | 2.1112  |
| DHPS       | DHPS: deoxyhypusine synthase                                                 | 0.03132           | 2.10313 |
| 1700034F02RIK |                                             | 0.01501           | 2.05275 |

**Note:** The table above lists genes and their associated fold changes and p-values. The direction of change (up or down) is indicated by 'up' or 'down.'
| Gene        | Description                                                                 | Log2 Fold Change | p Value |
|-------------|------------------------------------------------------------------------------|------------------|---------|
| SHF         | SHF:Src homology 2 domain containing F                                       | 0.004            | 4.09982 |
| GLOD4       | 2010085E05Rik                                                                 | 0.04616          | 4.06067 |
| NR2F6       | NR2F6:nuclear receptor subfamily 2, group F, member 6                        | 0.0285           | 4.03937 |
| RUSC2       | RUSC2:RUN and SH3 domain containing 2                                         | 0.01736          | 4.03404 |
| TIPRL       | TIPRL:TIP41, TOR signalling pathway regulator-like (S. cerevisiae)           | 0.0255           | 4.03344 |
| BTBD5       | BTBD5:BTB (POZ) domain containing 5                                           | 0.0146           | 4.02652 |
| EIF2C2      | EIF2C2:eukaryotic translation initiation factor 2C, 2                         | 0.0503           | 3.89805 |
| NFIA        | NFIA:nuclear factor I/A                                                       | 0.0445           | 3.89446 |
| PROS1       | PROS1:protein S (alpha)                                                      | 0.02681          | 3.83777 |
| MAP4K3      | MAP4K3 LOC675560                                                             | na               |         |
| LDB2        | LDB2:LIM domain binding 2                                                     | 0.002            | 3.79608 |
| RBM19       | RBM19:RNA binding motif protein 19                                            | 0.0466           | 3.78786 |
| MCMDC1      | MCMDC1:minichromosome maintenance deficient domain containing 1              | 0.01879          | 3.74637 |
| SMO         | SMO:smoothened homolog (Drosophila)                                          | 0.001            | 3.72639 |
| DDX24       | DDX24: DEAD (Asp-Glu-Ala-Asp) box polypeptide 24                              | 0.02098          | 3.69234 |
| PLAC9       | PLAC9:placenta-specific 9                                                    | 0.01527          | 3.65417 |
| 2610014I16RIK| na                                                                           | 0.04731          | 3.64898 |
| GLT28D2     | na                                                                           | 0.04701          | 3.61421 |
| NAGLU       | NAGLU:N-acetylglucosaminidase, alpha- (Sanfilippo disease IIIB)              | 0.01324          | 3.60552 |
| SLC10A3     | SLC10A3:solute carrier family 10 (sodium/bile acid cotransporter family), member 3 | 0.02873          | 3.59452 |
| NFATC2IP    | NFATC2IP:nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 interacting protein | 0.05147          | 3.59288 |
| MCOLN1      | MCOLN1:mucolipin 1                                                           | 0.02559          | 3.59093 |
| FBXO18      | FBXO18:F-box protein, helicase, 18                                           | 0.05395          | 3.57977 |
| HOXA10      | HOXA10:homeobox A10                                                          | 0.01077          | 3.57055 |
| DNAJA4      | DNAJA4:DnaJ (Hsp40) homolog, subfamily A, member 4                            | 0.05295          | 3.56585 |
| 9230104K21RIK| na                                                                           | 0.04956          | 3.56259 |
| TMEM16K     | TMEM16K:transmembrane protein 16K                                            | 0.04528          | 3.55635 |
| ENOPH1      | 2310057D15RIK                                                                 | 0.04873          | 3.54497 |
| ABHD14B     | ABHD14B:abhydrolase domain containing 14B                                    | 0.03567          | 3.52646 |
| NINJ1       | NINJ1:ninjurin 1                                                              | 0.004            | 3.5251 |
| SMAD3       | SMAD3:SMAD, mothers against DPP homolog 3 (Drosophila)                       | 0.0405           | 3.50195 |
| TRABD       | 5730502D15RIK                                                                 | 0.03846          | 3.4988 |
| YIPF2       | YIPF2:Yip1 domain family, member 2                                           | 0.0275           | 3.48257 |
| MTIF2       | MTIF2:mitochondrial translational initiation factor 2                         | 0.0486           | 3.46648 |
| METTL7A     | METTL7A /// UBIE ///                                                          | na               |         |
| ISLR        | ISLR:immunoglobulin superfamily containing leucine-rich repeat                | 0.001            | 3.45555 |

**Note:** Log2 Fold Change and p Value columns represent the statistical significance of the changes in gene expression.
| Gene        | Description                                                                 | Log2 Fold Change | Significance |
|------------|------------------------------------------------------------------------------|-----------------|--------------|
| ACYP1      | acylphosphatase 1, erythrocyte (common) type                                 | 0.03783         | down         |
| FBXL3      | F-box and leucine-rich repeat protein 3                                       | 0.05226         | down         |
| HNRPH3     |                                                                | na              | down         |
| LOC669773  |                                                                | na              | down         |
| PQLC3      | PQLC3:PQ loop repeat containing 3                                            | 0.0431          | down         |
| PALLD      | PALLD:palladin, cytoskeletal associated protein                              | 0.04649         | down         |
| 5330431N19RIK |                                                                | 0.03947         | down         |
| ALCAM      | ALCAM:activated leukocyte cell adhesion molecule                             | 0.01269         | down         |
| RG9MTD2    | RG9MTD2:RNA (guanine-9-) methyltransferase domain containing 2               | 0.04216         | down         |
| LRRC17     | LRRC17:leucine rich repeat containing 17                                     | 0.02977         | down         |
| EIF2AK1    | EIF2AK1:eukaryotic translation initiation factor 2-alpha kinase 1            | 0.01791         | down         |
| EXTL2      | EXTL2:exostoses (multiple)-like 2                                            | 0.03894         | down         |
| CRTC3      | CRTC3:CREB regulated transcription coactivator 3                             | 0.0159          | down         |
| PAOX       | PAOX:polyamine oxidase (exo-N4-amino)                                        | 0.00757         | down         |
| HMBOX1     | HMBOX1:homeobox containing 1                                                 | 0.046           | down         |
| GTF3C2     | GTF3C2:general transcription factor IIIC, polypeptide 2, beta 110kDa        | 0.02639         | down         |
| NEK8       | NEK8:NIMA (never in mitosis gene a)- related kinase 8                        | 0.02814         | down         |
| BNIP1      | BNIP1:BCL2/adenovirus E1B 19kDa interacting protein 1                         | 0.04001         | down         |
| RFX5       | RFX5:regulatory factor X, 5 (influences HLA class II expression)             | 0.005           | down         |
| KNS2       | KNS2:kinesin 2                                                               | 0.01367         | down         |
| SORBS2     | SORBS2:sorbin and SH3 domain containing 2                                    | 0.01624         | down         |
| COL5A3     | COL5A3:collagen, type V, alpha 3                                             | 0.03875         | down         |
| EXT1       | EXT1:exostoses (multiple) 1                                                  | 0.04278         | down         |
| 1600002K03RIK |                                                                | 0.02141         | down         |
| CLIP3      | 1500005P14RIK /// AL                                                         | 0.05159         | down         |
| POLR3C     | POLR3C:polymerase (RNA) III (DNA directed) polypeptide C (62kD)              | 0.0366          | down         |
| TBCC       | TBCC:ubilin-specific chaperone c                                             | 0.03781         | down         |
| ERC1       | ERC1:ELKS/RAB6-interacting/CAST family member 1                             | 0.04342         | down         |
| TRIP6      | TRIP6:thyroid hormone receptor interactor 6                                 | 0.04633         | down         |
| HSBP1      | HSBP1:heat shock factor binding protein 1                                   | 0.04248         | down         |
| PPP2CB     | PPP2CB:protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform  | 0.0088          | down         |
| PANK3      | PANK3:parothenate kinase 3                                                   | 0.01014         | down         |
| TMEM50A    | TMEM50A:transmembrane protein 50A                                            | 0.03696         | down         |
| AV025504   |                                                                | 0.00837         | down         |
| WDFY2      | WDFY2:WD repeat and FYVE domain containing 2                                | 0.03629         | down         |
| DDR2       | DDR2:discoidin domain receptor family, member 2                              | 0.03946         | down         |
| ZFP618 /// LOC667396 |                                                                | 0.00761         | down         |
| Gene Symbol | Description | log2FoldChange | P.Value  |
|-------------|-------------|----------------|----------|
| PTX3        | PTX3:pentraxin-related gene, rapidly induced by IL-1 beta | 0.03079 | 2.92064 | down |
| BC017612    | na          | 0.05424        | 2.89922  | down |
| ATG4C       | ATG4C:ATG4 autophagy related 4 homolog C (S. cerevisiae) | 0.00837 | 2.89476 | down |
| RBM43       | 0610033I05RIK | 0.04051 | 2.87449  | down |
| RBM42       | 3100004P22RIK /// 23 | 0.03254 | 2.87222  | down |
| ALOX12      | ALOX12:arachidonate 12-lipoxygenase | 0.01951 | 2.86592 | down |
| NCOA5       | NCOA5:nuclear receptor coactivator 5 | 0.01723 | 2.86006 | down |
| WNK4        | WNK4:WNK lysine deficient protein kinase 4 | 0.04324 | 2.85777 | down |
| PGP         | 1700012G19RIK | 0.02712 | 2.84566  | down |
| MYO18A      | MYO18A:myosin XVIIIA | 0.02934 | 2.84086 | down |
| CYB5R3      | CYB5R3:cytochrome b5 reductase 3 | 0.02474 | 2.83949 | down |
| AA536749    | na          | 0.03247        | 2.8289   | down |
| BC005624    | na          | 0.03948        | 2.82785  | down |
| IPO9        | IPO9:importin 9 | 0.03469 | 2.81781  | down |
| EXOSC7      | EXOSC7:exosome component 7 | 0.03156 | 2.81313 | down |
| AP3S1       | AP3S1:adaptor-related protein complex 3, sigma 1 subunit | 0.05259 | 2.8084  | down |
| OXNAD1      | OXNAD1:oxidoreductase NAD-binding domain containing 1 | 0.05392 | 2.80498 | down |
| RRAS        | RRAS:related RAS viral (r-ras) oncogene homolog | 0.03705 | 2.79071 | down |
| 2610002F03RIK | na          | 0.04775        | 2.78366  | down |
| ZCCHC14     | ZCCHC14:zinc finger, CCHC domain containing 14 | 0.0329 | 2.77777 | down |
| STS         | STS:steroid sulfatase (microsomal), arylsulfatase C, isozyme S | 0.00883 | 2.76881 | down |
| RCOR3       | RCOR3:REST corepressor 3 | 0.02713 | 2.74565 | down |
| ST3GAL2     | ST3GAL2:ST3 beta-galactoside alpha-2,3-sialyltransferase 2 | 0.01937 | 2.7313  | down |
| HPS4        | HPS4:Hermansky-Pudlak syndrome 4 | 0.04004 | 2.68525 | down |
| 1110005A03RIK | na          | 0.01849        | 2.68201  | down |
| D930050J11  | na          | 0.01927        | 2.67306  | down |
| SLC45A4     | SLC45A4:solute carrier family 45, member 4 | 0.04853 | 2.67167 | down |
| PANK1       | PANK1:pantothenate kinase 1 | 0.04199 | 2.67069 | down |
| CCDC84      | CCDC84:coiled-coil domain containing 84 | 0.03089 | 2.66777 | down |
| SAPS1       | SAPS1:SAPS domain family, member 1 | 0.04673 | 2.64874 | down |
| POMT1       | POMT1:protein-O-mannosyltransferase 1 | 0.04783 | 2.64598 | down |
| PSD3        | 4931420C21RIK | 0.03173 | 2.64312 | down |
| REXO1       | REXO1:REX1, RNA exonuclease 1 homolog (S. cerevisiae) | 0.02204 | 2.63142 | down |
| MLLT1       | MLLT1:myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 1 | 0.05544 | 2.62691 | down |
| CARM1       | CARM1:coactivator-associated arginine methyltransferase 1 | 0.04902 | 2.60999 | down |
| PBXIP1      | PBXIP1:pre-B-cell leukemia transcription factor interacting protein 1 | 0.04893 | 2.60147 | down |
| Gene Symbol | Description | Fold Change | Status |
|-------------|-------------|-------------|--------|
| ANTXR2      | anthrax toxin receptor 2 | 0.04345     | down   |
| PARG        | poly (ADP-ribose) glycohydrolase | 0.004       | down   |
| 31100821I7RIK |          | 0.03931     | down   |
| 2700097O09RIK |          | 0.003       | down   |
| 2510003E04RIK |          | 0.04721     | down   |
| MED25       | mediator of RNA polymerase II transcription, subunit 25 homolog (S. cerevisiae) | 0.0162 | down |
| FKB1B       | FK506 binding protein 1B, 12.6 kDa | 0.04954     | down   |
| C030013C21RIK |          | 0.01692     | down   |
| METAP1      |          | 0.04655     | down   |
| PDPK1       | 3-phosphoinositide dependent protein kinase-1 | 0.05226 | down |
| A130038J17RIK |          | 0.04504     | down   |
| PXMP3       | peroxisomal membrane protein 3, 35kDa (Zellweger syndrome) | 0.03348 | down |
| ZFAND2B     | zinc finger, AN1-type domain 2B | 0.045459    | down   |
| 5230400M03RIK |          | 0.034336    | down   |
| 2410006H16RIK |          | 0.034442    | down   |
| B230216N24RIK |          | 0.008545    | down   |
| ENO3        | enolase 3 (beta, muscle) | 0.010466    | down   |
| CNNM2       | cyclin M2 | 0.023387    | down   |
| WHSC2       | Wolf-Hirschhorn syndrome candidate 2 | 0.052867 | down |
| 2610507B11RIK |          | 0.042471    | down   |
| 4930471M23RIK |          | 0.045363    | down   |
| PREPL       | prolyl endopeptidase-like | 0.040585    | down   |
| COQ6        | coenzyme Q6 homolog, monooxygenase (S. cerevisiae) | 0.036103 | down |
| SBDS        | Shwachman-Bodian-Diamond syndrome | 0.027655 | down |
| C920006C10RIK |          | 0.040843    | down   |
| ZFAND3      | zinc finger, AN1-type domain 3 | 0.051068 | down |
| 5830472M02RIK |          | 0.022833    | down   |
| ACADVL      | acyl-Coenzyme A dehydrogenase, very long chain | 0.051178    | down   |
| 5330421F07RIK |          | 0.031995    | down   |
| TCF7L2      | transcription factor 7-like 2 (T-cell specific, HMG-box) | 0.017742    | down   |
| C1R         | complement component 1, r subcomponent | 0.034414 | down |
| PVRL2       | poliovirus receptor-related 2 (herpesvirus entry mediator B) | 0.016279 | down |
| UBE2G1      | ubiquitin-conjugating enzyme E2G 1 (UBC7 homolog, yeast) | 0.052156 | down |
| 0610037M15RIK |          | 0.030055    | down   |
| TOM1L2      | target of myb1-like 2 (chicken) | 0.042417 | down |
| Gene ID       | Description                                              | Fold Change | p-Value  |
|--------------|-----------------------------------------------------------|-------------|----------|
| 4930453N24RIK| SKI:v-ski sarcoma viral oncogene homolog (avian)          | 0.012838    | 2.333928 |
| BC048355     | na                                                        | 0.021395    | 2.328441 |
| PPCDC        | PPCDC:phosphopantothenoylcysteine decarboxylase           | 0.002518    | 2.31653  |
| SKAP2        | SKAP2:src kinase associated phosphoprotein 2              | 0.048548    | 2.316329 |
| LOC619719    | na                                                        | 0.031426    | 2.303352 |
| TPD52L1      | TPD52L1:tumor protein D52-like 1                          | 0.052966    | 2.303352 |
| 1810048P08RIK| na                                                        | 0.038413    | 2.295201 |
| COMMD4       | COMMD4:COMM domain containing 4                           | 0.043682    | 2.292841 |
| HEXA         | HEXA:hexosaminidase A (alpha polypeptide)                 | 0.041359    | 2.285667 |
| SLC44A1      | SLC44A1:solute carrier family 44, member 1                | 0.020211    | 2.259007 |
| 3010001K23RIK| na                                                        | 0.038181    | 2.242436 |
| FLI1         | FLI1:Friend leukemia virus integration 1                  | 0.006129    | 2.224242 |
| DENND1A      | DENND1A:DENN/MADD domain containing 1A                    | 0.010247    | 2.210609 |
| TRIP10       | TRIP10:thyroid hormone receptor interactor 10             | 0.022136    | 2.206315 |
| RBL1         | RBL1:retinoblastoma-like 1 (p107)                         | 0.029564    | 2.190662 |
| PHF2         | PHF2:PHD finger protein 2                                 | 0.04758     | 2.187651 |
| FZD1         | FZD1:frizzled homolog 1 (Drosophila)                      | 0.035345    | 2.181633 |
| TMEM141      | TMEM141:transmembrane protein 141                        | 0.019349    | 2.166158 |
| C030040A22RIK| na                                                        | 0.041693    | 2.147752 |
| 2810439F02RIK| na                                                        | 0.041809    | 2.136875 |
| NME7         | NME7:non-metastatic cells 7, protein expressed in (nucleoside-diphosphate kinase) | 0.037948    | 2.135189 |
| INPP5A       | INPP5A:inositol polyphosphate-5-phosphatase, 40kDa        | 0.050672    | 2.131062 |
| IFT172       | IFT172:intraflagellar transport 172 homolog (Chlamydomonas) | 0.025341    | 2.111549 |
| RNF19        | RNF19:ring finger protein 19                              | 0.042536    | 2.09939  |
| E130303B06RIK| na                                                        | 0.034074    | 2.098219 |
| SGTB         | SGTB:small glutamine-rich tetratricopeptide repeat (TPR)-containing, beta | 0.040253    | 2.09804  |
| MUM1         | MUM1:melanoma associated antigen (mutated) 1              | 0.026382    | 2.092568 |
| NCBP2        | NCBP2:nuclear cap binding protein subunit 2, 20kDa        | 0.032454    | 2.089116 |
| ARM CX1      | ARM CX1:armadillo repeat containing, X-linked 1          | 0.037973    | 2.081989 |
| SPHK1        | SPHK1:sphingosine kinase 1                                | 0.007448    | 2.076194 |
| MTA3         | MTA3:metastasis associated 1 family, member 3             | 0.008551    | 2.073348 |
| C530028I08RIK| na                                                        | 0.015106    | 2.062652 |
| 6330403L08RIK| na                                                        | 0.047984    | 2.062485 |
| IFI47        | na                                                        | 0.030068    | 2.056686 |
| ARHGAP5      | ARHGAP5:Rho GTPase activating protein 5                   | 0.02651     | 2.05092  |

**Fold Change** and **p-Value** indicate the relative decrease or increase in expression compared to the reference.
| Gene     | Description                                               | Fold Change | Status |
|----------|-----------------------------------------------------------|-------------|--------|
| NUDCD3   | NudC domain containing 3                                  | 0.047587    | down   |
| GAG      | na                                                        | 0.040636    | down   |
| D8BWG1414E |                                        | 0.04943     | down   |
| PPP1R12B | PPP1R12B:protein phosphatase 1, regulatory (inhibitor) subunit 12B | 0.026326    | down   |
| D15WSU169E |                                        | 0.028883    | down   |
| ODZ4     | ODZ4:odz, odd Oz/ten-m homolog 4 (Drosophila)             | 0.043972    | down   |
Supplementary table 2: GSEA: Gene sets significantly enriched in OCDflo+ osteolineage cells

| PATHWAY                          | NAME                          | SIZE | NES     | NOM p-value | FDR q-value |
|----------------------------------|-------------------------------|------|---------|-------------|-------------|
| Osteogenic differentiation¹      | UP_GENES_OSTEOGENIC          | 43   | 1.60    | 0           | 0.000       |
| TGF-β                            | TGFBETA_EARLY_UP              | 45   | 2.578963| 0           | 0.000       |
|                                 | TGF_BETA_SIGNALING_PATHWAY    | 46   | 2.433784| 0           | 0.008223    |
|                                 | TGFBETA_ALL_UP               | 75   | 2.432784| 0           | 0.006167    |
| Wnt/βcatenin                     | AMBROSETTI_UP-WNT ACTIVATION  | 54   | 1.797488| 0           | 0.001945    |
|                                 | KENNY_WNT_DN                 | 39   | 1.757821| 0           | 0.032086    |
|                                 | ST_WNT_BETA_CATENIN_PATHWAY   | 30   | 1.699135| 0           | 0.041116    |
| UV                               | UVC_LOW_ALL_DN               | 49   | 2.407943| 0           | 0.004112    |
|                                 | UVB_NHEK3_C5                 | 33   | 2.405828| 0           | 0.003524    |
|                                 | UVB_NHEK1_DN                 | 233  | 2.254912| 0           | 0.005181    |
|                                 | UVC_TTD_ALL_DN               | 319  | 2.227531| 0           | 0.009852    |

Gene Sets from:

¹ Schilling, T. et al., Microarray analyses of transdifferentiated mesenchymal stem cells, J. Cell Biochem. 103(2), 413 (2008).

² Ambrosetti, D. et al., Fibroblast Growth Factor Signaling Uses Multiple Mechanisms To Inhibit Wnt-Induced Transcription in Osteoblasts. Molecular and Cellular Biology, 28 (15), 4759 (2008)

Details of other gene sets can be found at [http://www.broad.mit.edu/gsea/](http://www.broad.mit.edu/gsea/)
### Supplementary Table 3: Cytokines, interleukins, growth factors and other secreted factors differentially expressed in OCD \(^{fl/fl}\) osteolineage cells (\(>1.5\)-fold; \(t\)-test\(<0.05\))

Factors upregulated in OCD \(^{fl/fl}\) osteolineage cells

| Symbol | Name                                      | \(t\)-test | Fold-difference |
|--------|-------------------------------------------|-------------|----------------|
| PF4    | platelet factor 4                         | 0.0006      | 1.58           |
| NPTX2  | neuronal pentraxin II                     | 0.004       | 1.61           |
| IL31   | interleukin 31                            | 0.005       | 1.78           |
| CCK    | cholecystokinin                           | 0.039       | 1.71           |
| OLFM4  | olfactomedin 4                            | 0.039       | 1.94           |
| PRG2   | proteoglycan 2, bone marrow               | 0.046       | 4.69           |
| CRTAC1 | cartilage acidic protein 1                | 0.047       | 3.10           |
| STC1   | stanniocalcin 1                           | 0.048       | 1.54           |
| COL4A4 | collagen, type IV, alpha 4                | 0.050       | 2.61           |

Factors downregulated in OCD \(^{fl/fl}\) osteolineage cells

| Symbol | Name                                      | \(t\)-test | Fold-difference |
|--------|-------------------------------------------|-------------|----------------|
| PLAC9  | placenta-specific 9                       | 0.015       | 3.65           |
| PROS1  | protein S (alpha)                         | 0.027       | 3.84           |
| JAG1   | jagged 1 (Alagille syndrome)              | 0.034       | 4.58           |
| COL5A3 | collagen, type V, alpha 3                 | 0.039       | 3.12           |
| BAI1   | brain-specific angiogenesis inhibitor 1    | 0.045       | 1.61           |
| ADM    | adrenomedullin                            | 0.050       | 4.57           |
Supplementary methods and materials

Mice and genotyping

Osx-Cre transgenic mice⁹, Ocn-Cre transgenic mice²⁰ and floxed Dicer1 mice¹⁰, have been described. B6.SJL-Ptprc⁸ Pep3⁰/BoyJ mice were purchased from the Jackson Laboratory. Floxed Dicer1 mice were on a mixed C57/B6/J129 background. Other mice strains were on a C57/B6 background. Genotyping of Cre transgenic mice was performed by PCR using primers detecting the Cre sequence³⁶. The floxed and wild-type Dicer1 alleles were detected by using primers, P1: 5'-AGTGTAGCCTTAGCCATTTG-3' and P2: 5'-CTGGTGGCTTGAGGACAGAC-3'. These primers amplify the region spanning the downstream loxP sequence. Deletion of the floxed sequence from the Dicer1 gene was demonstrated by using primers: P1: 28290: 5'-AGTAATGTGAGCAATAGTCCCAG-3' and P2: 32050AS: 5'-CTGGTGGCTTGAGGACAGAC-3'. OCD fl/fl animals were compared to OCD fl/+ littermates for the studies described in this paper. The Subcommittee on Research Animal Care of the Massachusetts General Hospital approved all animal work according to federal and institutional policies and regulations.

RT-PCR

RNA extraction, real-time quantitative RT-PCR and relative gene expression quantitation was performed on sorted cells (GFP+CD45-CD31-Lineage -) as described previously³⁷ using the following primers: Dicer1-F, 5'-AATTGGCTCTCCTCTGGTTAT-3' and Dicer1-R, GTCAGGTCCTCCTCCTCCTC-3'; Osteocalcin-F, 5'-CTGACCTCACAGATCCCAAGC -3' and Osteocalcin-R, 5'-TGGCTGTAGCTGCTCACAAATG -3'; GAPDH-F, 5'-AGGTCAAGGATTTTG -3' GAPDH-R, 5'-TGTAGACCATGTAGTTGAGGTCA -3'
Isolation and osteogenic differentiation of bone marrow derived stromal cells

Mice were sacrificed; tibiae, femurs, and spine were removed and excess soft tissue was eliminated. Using a pestle and mortar, the bones were crushed and washed in PBS with 0.5% FBS and passed through a 40-μm filter into a collection tube. Cells were spun at 1500rpm for 5 minutes; the supernatant was removed, and cells were resuspended in a minimal volume of ACK lysing buffer (Cambrex) for 4 minutes on ice and washed once with PBS. After pelleting once again, the cells were resuspended and plated in αMEM, 20% fetal bovine serum (HyClone), and penicillin and streptomycin solution (CellGro) - henceforth referred to as αMEM20%- and incubated at 33°C with 5% CO₂. After 3 weeks of culture and expansion, plastic adherent cells were CD45 depleted by magnetic isolation (Invitrogen; Dynabeads M-280 Streptavidin, 112-06D) using an anti-mouse CD45 biotin antibody (BD Bioscience; 550539). The plastic adherent CD45 negative cells were then maintained in αMEM20% as before. To assess osteogenic differentiation, bone marrow derived stromal cells (passage 3) were plated at 10 × 10³ cells/well in a 96-well plate (BD Biosciences) at 33° in osteogenic induction medium: α20% modified with glycerol 2-phosphate (2.16 mg/ml), 2-phospho-L-ascorbic acid (0.05 mg/ml), and dexamethasone (10 nM) (Sigma-Aldrich, G6251, 49752 and D1756, respectively ). After 7 days of differentiation, alkaline phosphatase staining was carried out with BCIP/NBT solution (Sigma-Aldrich) per the manufacturer’s instructions. For the von Kossa assay and staining, cells were fixed and washed in water, and a 5% silver nitrate solution was added to the well under incandescent light for 20–45 minutes. After granules developed, the silver nitrate was removed and wells were washed with water to stop the reaction.

CFU-F and CFU-ALK

0.5 × 10⁶ primary bone marrow cells were plated in 12-well plates in αMEM20% for CFU-F assay or osteogenic medium for CFU-Alk assay. Medium was changed at 24 hours to eliminate nonadherent cells. After 7 days, colonies were
assessed by methylene blue staining for the CFU-F assay or BCIP staining (alkaline phosphatase) for CFU-Alk.

**Histomorphometric analysis**

Bones were fixed in 4% paraformaldehyde and undecalcified sections embedded in methyl methacrylate resin. Five-micrometer sections were stained with Masson Trichrome or coverslipped unstained, and histomorphometric analysis was performed with the Osteomeasure system (Osteometrics Inc., Atlanta, GA) using standard procedures. Tibial sections were measured in the proximal metaphysis beginning 340 µm below the chondro-osseous junction. Osteoblasts were identified as mononuclear cells directly abutting either mineralized bone or osteoid and restricted to the endosteal surface.

**In Situ Hybridization.**

*In situ* hybridization was carried out as described\(^3^6\). Complementary \(^{35}\)S-labeled riboprobes were transcribed from the plasmids encoding mouse osteocalcin (OC) using Riboprobe systems from Promega (Madison, WI). Probes for *Osteocalcin* were described\(^3^6\).

**Hematological Measurements**

Peripheral blood samples were obtained by lateral tail vein bleeding. Peripheral blood cell counts were performed on a HEMAVET Multispecies Hematology Analyzer (CDC Technologies).

**Methylcellulose colony formation assay**

Bone marrow or spleen cells (10X10\(^3\)) were plated into methylcellulose M3434 (StemCell Technologies) in a 6-well plate and grown for 10 days before being scored.

**FACS analysis**
Hematopoietic progenitors were identified based on their expression of lineage markers as well as c-Kit, Sca-1, CD48 and CD150 expression. Lineage staining used a cocktail of biotinylated antimouse antibodies to Mac-1α (CD11b), Gr-1(Ly-6G and Ly-6C), Ter119 (Ly-76), CD3, CD4, CD8α (Ly-2), and B220 (CD45R; BD Biosciences). For detection we used lineage-streptavidin conjugated with PERCP, c-Kit-APC (CD117), CD48-Pacific blue (CD135), CD150-PE-Cy7 (all from BD Biosciences) and Sca1-PE-Cy5.5 (Ly 6A/E; Caltag Laboratories). For congeneric strain discrimination, anti-CD45.1-PE and anti-CD45.2 FITC antibodies (BD Biosciences) were used. For the apoptosis assay we used 7-AAD and AnnexinV-APC (BD Biosciences) in combination with lineage-streptavidin-PE and c-kit-FITC (both Biolegend). For the intracellular detection of BRDU-FITC, bone marrow cells were fixed and permeabilized using BD Cytofix/Cytoperm Fixation/Permeabilization Solution Kit (BD Biosciences) according to the manufacturer's recommendations. Compensation and data analysis were performed using Flowjo 8.5.3

**FACS- sorting of osteolineage cells**

Whole bone-marrow and bone cells were collected by crushing tibias and femurs of mice, stained with biotin-conjugated lineage cocktail antibodies and subjected to lineage depletion using magnetic isolation (Invitrogen; Dynabeads M-280 Streptavidin, 112-06D). The resulting lineage-depleted fraction was stained with lineage and CD31-biotin-streptavidin APC-Cy7 (BD Biosciences) and CD45-APC (eBioscience) and sorted using FACS DiVa or FACS ARIA (Becton Dickinson). A small fraction of the collected cells was re-run through the sorter and over 95% purity was consistently confirmed.

**Collagenase treatment of bone for PCR**

Collagenase digestion was performed on the bone fragments left in the mortar and 70-μm filter after crushing long bones. A solution of DMEM (Cellgro; 10-013-CV), 0.2% collagenase (WAKO; 034-10533) and 10mM HEPES (Fisher; BP299-100) was warmed to 37°C. In a centrifuge tube, bone fragments were added to
the collagenase solution and kept at 37°C for 90 minutes, vortexing every 15-30 minutes. Excess PBS was added to the slurry which was then filtered through a 40-μm filter. The flow-through was then pelleted.

**Bone marrow histology and peripheral blood morphology**

For histological analysis, long bones were dissected, fixed in paraformaldehyde 4%, decalcified in 10% EDTA, paraffin-processed, cut, and subjected to hematoxylin/eosin staining. Peripheral blood smears were formalin fixed for 5 minutes, stained with May-Grunwald (Sigma-Aldrich) for 5 minutes, rinsed in distilled water with PBS and in Giemsa stain (Sigma-Aldrich) for another 30 minutes. Permount (Fisher Scientific) was used to mount the sections. Images were acquired with a Nikon Eclipse 80i epifluorescence microscope equipped with a Qimaging Micropublisher digital CCD colour camera. Bone and bone marrow histology was assessed by two independent investigators blinded to mice genotypes.

**Bone marrow transplantation**

All bone marrow transplantations were performed by retro-orbital venous plexus injection. For competitive transplantation, 5 × 10^5 whole bone-marrow cells from 6-week-old OCD fl/+ or OCD fl/fl (CD45.2) littermates were mixed with 5 × 10^5 CD45.1+ (competitor) WT cells and injected into lethally irradiated (9 Gy, split dose on the day of transplant) recipient BL6-SJL (CD45.1+) mice. Engraftment efficiency in recipients was monitored by donor contribution of CD45.2+ cells using FACS analysis. For limiting dilution assays, 2 × 10^5, 5 × 10^4 1 × 10^4 and 0.5 X 10^4 OCD fl/+ or OCD fl/fl mononuclear bone marrow cells were mixed with 2 × 10^5 wild type bone marrow and injected into lethally irradiated recipients (9 mice per cell dose per genotype). Engraftment efficiency in recipients was monitored by donor contribution of cells using FACS analysis. The frequencies of competitive repopulating units were calculated using the L-Calc software. Greater than or equal to 1% donor cells in both myeloid and lymphoid lineages was used
to determine whether an animal had a positive engraftment. For “wt into mutant” experiments, wildtype congenic BL6/SJL (CD45.1+) bone marrow cells (1 x 10^6 cells/recipient) were transplanted into lethally irradiated 4 week old OCD fl/+ and OCD fl/fl (CD45.2+) recipients. Complete donor cell engraftment by wildtype CD45.1+ cells was confirmed by FACS. Conversely, for “mutant into wt” experiments OCD fl/+ or OCD fl/fl (CD45.2+) were transplanted into lethally irradiated 4 week old BL6/SJL (CD45.1+) animals. Complete donor cell engraftment by CD45.1/CD45.2+ cells was confirmed by FACS.

**Immunohistochemistry**

For immunohistochemistry, antigen retrieval was carried out with proteinase K (20 mg/ml, Roche), followed by 3% H2O2 treatment to block endogenous peroxidase. The TSA Biotin system (PerkinElmer) was used according to the manufacturer's instructions. Specimens were incubated with mouse anti-CD31 antibody (BD Biosciences) or anti-CD13 antibody (Santa Cruz Biotechnology) for 1 hr at room temperature.

**BrdU Labeling and Detection.**

Mice received 150 l BrdU solution (10 mg ml^-1) via intraperitoneal injection. After 15 hrs bone marrow was harvested for flow cytometric detection of BrdU-FITC uptake according to or the manufacture’s instructions (FITC-BrdU Flow Kit (BD Biosciences)).

**In vivo imaging**

In vivo imaging has been extensively described elsewhere. Briefly, 1–5 10^5 wild-type (Bl6/SJL) LKS cells were stained with 5 M DiD in PBS without serum for 10 min at 37 °C, washed once in PBS and immediately injected into the tail vein of recipient mice. Mice were anaesthetized and prepared for in vivo imaging as described. Immediately before imaging 20 l of non-targeted Qdot 800 or 655 (Invitrogen) diluted in 130 l sterile PBS was injected retro-orbitally to allow vasculature visualization. All mice were imaged with a custom-built confocal two-
A photon hybrid microscope specifically designed for live animal imaging. Microscopy and image processing have been described. Images were colored and merged using Adobe Photoshop and LKS-microenvironment distance measures were obtained using Adobe Illustrator and Microsoft Excel. A two-tailed type 2 $t$-test was applied to all data. $P$ values 0.05 were considered statistically significant.

**Co-culture studies**

Bone marrow stromal cells were isolated and CD45 depleted by magnetic isolation upon confluence and expanded for an additional week. The expanded cells were then plated at 1750 cells/well in 384-well tissue culture plates coated with fibronectin (Millipore; FC010) in either αMEM20% or osteogenic induction media. After four days of culture, 200 LKS or MEP (megakaryocyte-erythroid progenitor, lineage−, CD 127−, Sca−,kit+, CD34−,CD16/32− cells.) cells from 8-12 week old Actin-DsRed positive mice (Jackson Laboratory; 005441) were added to each well. Co-culture was performed without any cytokines. After 7 days of co-culture the number of DS-red cells was assessed by automated microscopy. Megakaryocytes were quantified morphologically as large cells with prominent multinucleated megakaryons (the identity of these cells was additionally confirmed by CD41 staining).

**Comparative genomic hybrydization**

Direct amplification of DNA from paraformaldehyde fixed paraffin-embedded (FFPE) tissue samples was performed using a REPLI-g FFPE kit (Qiagen) following the manufactory instruction. Briefly, FFPE samples were incubated at 95C for 10 min followed by lysis at 60C for 60 min and ligation at 24C for 30 min. Amplification took place at 30C for 2h. Agilent genomic DNA labeling kit was used for the amplified FFPE DNA labeling and purification. For each 244K array, 2 ug of FFPE DNA and 2 ug of germline reference DNA were labeled with Cy5 and Cy3 respectively. Labeled FFPE DNA and reference DNA were combined and mixed with Cot-1 DNA, blocking agent and hybridization buffer. After
denaturation at 95°C for 3 min and incubation at 37°C for 30 min, the hybridization mix was loaded onto a gasket slide in an Agilent SureHyb chamber. Array slide was placed on top the gasket slide. The SureHyb chamber was covered, clamped and incubated in a rotator rack in 65°C oven for 40 hour. In an ozone-controlled environment, hybridized arrays were disassembled, and washed in Agilent Oligo aCGH wash buffer 1 for 5 min, in wash buffer 2 for 1 min at 37°C, and immediately scanned using an Agilent DNA microarray scanner. Data extraction was conducted using the feature extraction software. Finally FE data files were analyzed using the Agilent DNA analytics software.

**Oligonucleotide microarrays**

RNA was isolated from sorted GFP+CD45-CD31-Lineage- cells by Trizol extraction (Invitrogen) according to the manufacturer’s protocol. Up to 4 mice were pooled per sample. Linear amplification of 20 ng of total RNA was performed using the Ovation Biotin RNA Amplification and Labeling System (Nugen). The biotinylated cRNA was hybridized to the Affymetrix Mouse430 v2 chip. Signal normalization was performed by RMA method. Data of three samples of OCD fl/fl cells vs. three samples of OCD fl/+ cells was analyzed using GEPAS package. A t-test was carried to identify probes differentially expressed between OCD fl/+ and OCD fl/fl samples. Gene set enrichment analysis was performed using GSEA. The signal-to-noise metric and permutation of gene sets was used to rank the genes and calculate significance and false discovery rate. Analysis was performed by collapsing probe sets to unique gene symbols and used to interrogate an established collection of curated gene sets provided by the Molecular Signatures Database (MsigDB, http://broad.mit.edu/gsea/msigdb). The osteogenic gene expression signature was collected from the literature and used to interrogate the gene expression dataset comparing OCD fl/+ and OCD fl/fl samples for gene set enrichment.

**Statistical analysis**
In all cases, analysis was performed by a standard unpaired, 2-tailed Student’s t test. All data have been plotted as average ± SEM. Statistical significance is indicated by * (P≤0.05) or ** (p≤0.01). The number of experiments is indicated in the figure legends.

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