

## **Supplementary Figure Legends**

### **Supplementary Figure 1: Immunophenotyping of BMT and tumor cells reveals mesenchymal/osteoblastic identity.**

(A) Flow cytometry analysis (histogram) of directly collected and/or cultured BM from a Cre- mouse (Cre-Direct BM), tumor (Direct DKOT/Cult DKOT) and paired BM cells (Direct DKO BMT/Cult DKO BMT) collected from DKO mouse demonstrating the expression of mesenchymal (Sca-1 and CD29) vs. hematopoietic (CD45.2) markers in tdTomato positive cells; representative experiment. (Bi, Bii) A bar graph demonstrating the percentage of hematopoietic and mesenchymal population in direct and cultured BMT and tumor cells respectively (n=3). (C) mRNA levels of osteoblast markers in DKO BMT and tumor (DKOT) cells vs WT BM (Cre+ WT BM) cells (n=3) (\*\*p-value<0.01, \*\*\*p-value <0.001, \*\*\*\*p-value<0.0001, ns: not significant). (D) Alizarin Red Staining demonstrating calcium deposition during osteoblast differentiation of tumor, BMT and yBM cells. (E) mRNA levels of osteoblast differentiation markers of tumor, BMT and yBM cells. **US**: Unstained, **S**: Stained, **Direct**: Directly collected BM before culturing, **Cult**: Cultured.

### **Supplementary Figure 2: young BM (yBM)-derived cells are of mesenchymal/osteoblastic identity and exhibit metastatic ability.**

(A) Flow cytometry analysis (histogram) of directly collected and/or cultured BM from a Cre negative mouse (Cre-BM-2m), young BM (DKO-Direct BM-2m) demonstrating the expression of mesenchymal (Sca-1 and CD29) vs. hematopoietic (CD45.2) markers in tdTomato positive cells; representative experiment. (B) A bar graph demonstrating the percentage of hematopoietic and mesenchymal population in direct and cultured yBM cells (n=3). (C) mRNA levels of osteoblast markers in yBM and WT BM cells (Cre+WT BM). (\*\* p-value< 0.01, \*\*\*\*p-value<0.0001). (D) Wound healing assay-migration. Time plot representing the Relative Wound Density of yBM, BMT and tumor cells (n=3). (F) Wound healing assay-invasion. Time plot representing the Relative Wound Density of yBM, BMT and tumor cells (n=3). **US**: Unstained, **S**: Stained, **Direct**: Directly collected BM before culturing, **Cult**: Cultured.

### **Supplementary Figure 3: Early Genes Contributing to Osteosarcomagenesis.**

(A) Heat map of the 303 mutual genes between yBM and tumor cells represented in the venn diagram in Fig 5B. (B) Heat map of the DE genes between yBM (n=6) and BMT (n=3) compared to the control BM cells (n=4). (C) Venn diagram analysis of yBM SKO and Tumor DKO (Tum-DKO) representing the common and unique genes between them-upper panel, Bar graph representing the significant pathways (Myc Targets) unique for DKO tumor cells compared to SKO yBM cells-lower panel. (D) Venn diagram analysis of yBM SKO and BMT DKO, representing the common and unique genes between them-upper panel, Bar graph representing the significant pathways (Myc Targets) unique for DKO BMT cells compared to SKO yBM cells-lower panel. (E) Venn diagram analysis of SKO yBM and SKO tumor cells representing the common and unique genes between them-upper panel, Bar graph representing the significant pathways (Myc Targets) unique for SKO tumor cells compared to SKO yBM cells-lower panel. (F) Venn diagram analysis of SKO and DKO tumor cells representing the common and unique genes between them-upper panel, Bar graph representing the significant pathways (Myc Targets) mutual between DKO and SKO tumor cells-lower panel. (G) Heat map of the 40 Myc target genes that are upregulated in yBM, and tumor cells compared to control cells. (H) Heat map of the 39 Myc target genes that are upregulated in yBM and BMT cells compared to control cells.

### **Supplementary Figure 4: Generation of a traceable mouse model of SKO-p53 osteosarcoma cell.**

(A) Schematic illustration of the experimental SKO-p53 OS mouse model. (B) Genomic PCR of Cre negative (Cre-), Cre positive (Cre+ WT) and Single knock out (SKO<sup>TOM</sup>) and Double knock out (DKO<sup>TOM</sup>) mice. Labels in the left panel represent the primer pairs and in the right panel represent the expected band size. (C) Immunoblotting of WWOX, p53 and tdTomato in the SKO BM, DKO BM cells before (0 min) and after (30, 120 min) of ionizing radiation exposure (IR-10Gy). PC-(positive control)-HEPG2 cells treated with nutlin (MDM2-inhibitor). (D) Summary graph of flow cytometry analysis of the percentage of tdTomato positive cells in DKO BM, SKO BM and wild type BM cells (Cre+WT BM) at different ages. (E) A bar graph demonstrating the percentage of hematopoietic and mesenchymal population in direct and cultured

yBM SKO cells (n=3). **(F)** Flow cytometry analysis (histogram) of directly collected and cultured BM from a yBM-SKO demonstrating the expression of mesenchymal (Sca-1 and CD29) vs. hematopoietic (CD45.2) markers in tdTomato positive cells; representative experiment. **WT**: Wildtype, **SKO**: Single Knock Out, **DKO**: Double Knock Out, **BM**: Bone Marrow.

**Supplementary Figure 5: MCM7 upregulation in DKO yBM cells promotes their tumorigenicity.**

**(A)** Representative images of the OS tumors developed in NOD/SCID mice after intratibial (IT) injection of DKO-yBM cells compared to SKO-yBM-upper panel, H&E of the developed tumors-lower panel. Histological validation indicates OS characteristics. **(B)** Bar graph representing the percentage of immunocompromised mice that developed OS after IT injection of SKO and DKO yBM cells. **(C)** Color coded heatmap of normalized expression (TPM) of top 100 genes harbouring the highest Myc enrichment at promoters. **(D)** Color coded heatmap of normalized expression (TPM) of 100 genes depleted in Myc at promoters. **(E)** Gene snapshots of examples of the MYC-enriched promoters. **(F)** Immunoblotting of MCM7 and c-Myc in SKO-yBM and DKO-yBM cells (the numbers indicate biological repeats), quantification of MCM7 and Myc protein levels relative to SKO yBM cells are shown. **(G)** Quantification of colony formation of SKO-yBM and DKO-yBM (n=3). \*\*\*\*p-value<0.0001.

**Supplemental Tabel 1: qPCR primers list and their sequences along with the predicted sizes.**

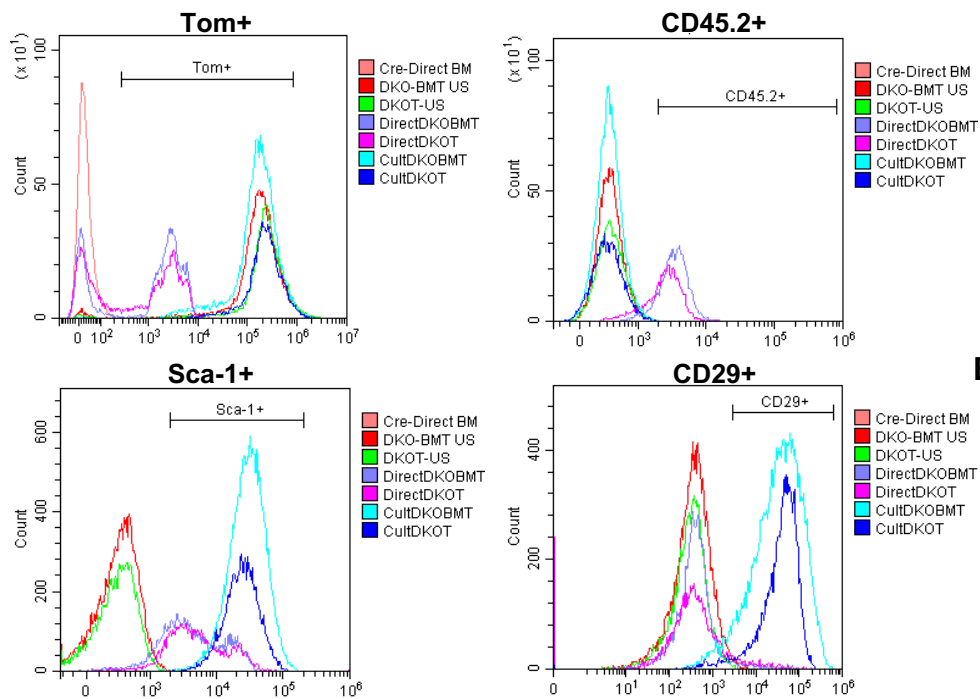
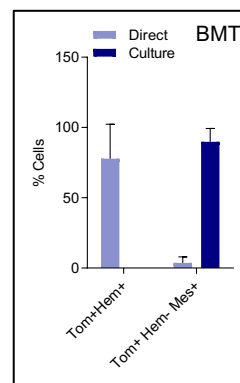
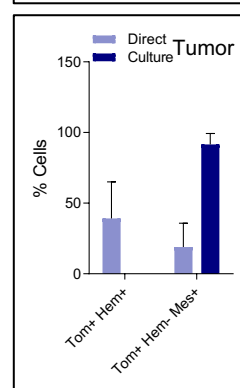
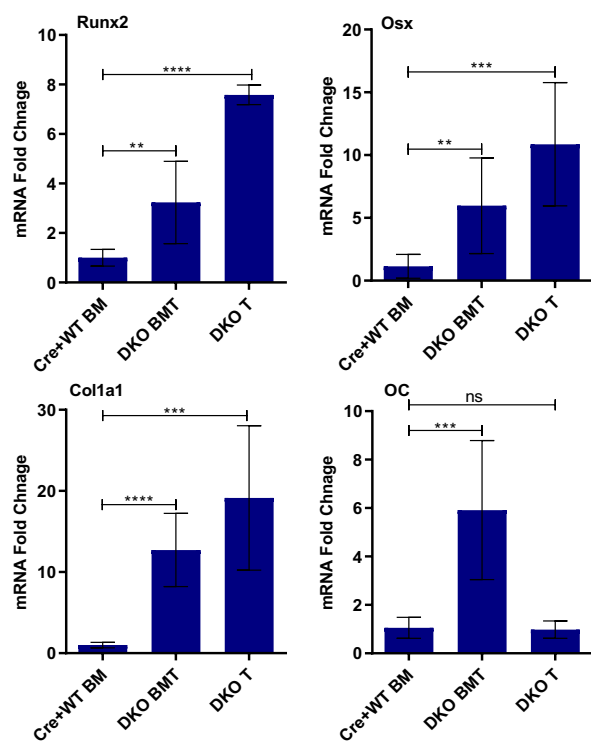
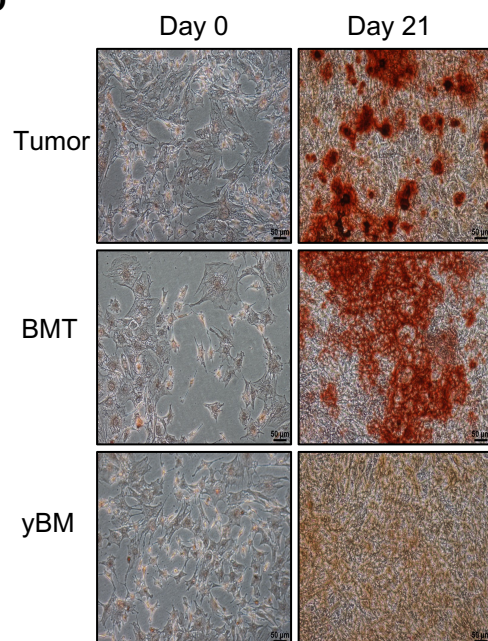
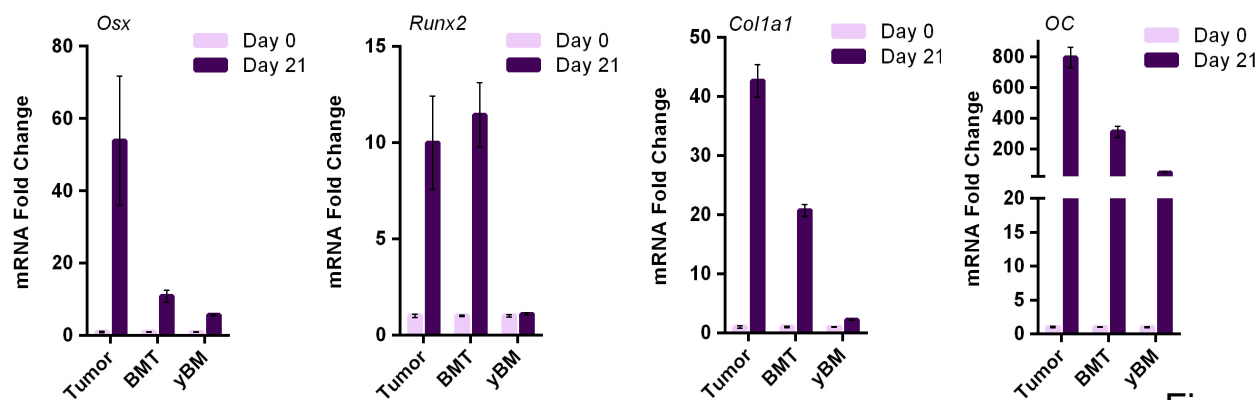
**A****Bi****Bii****C****D****E**

Figure 1S

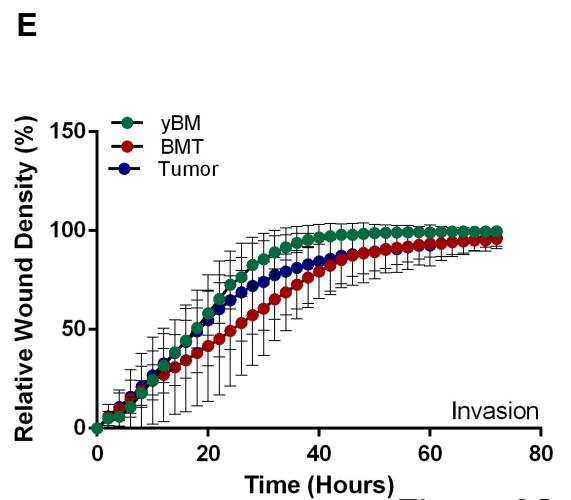
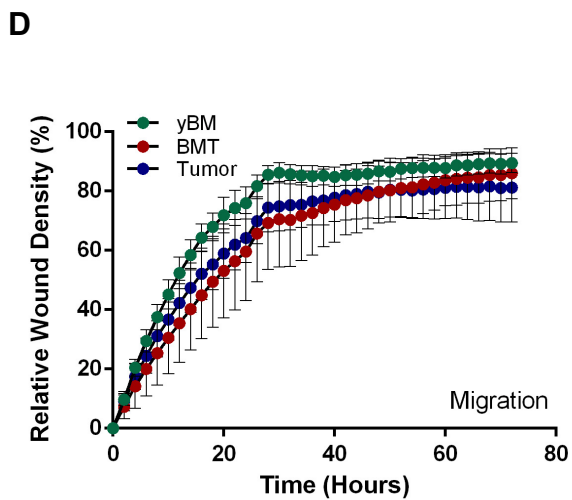
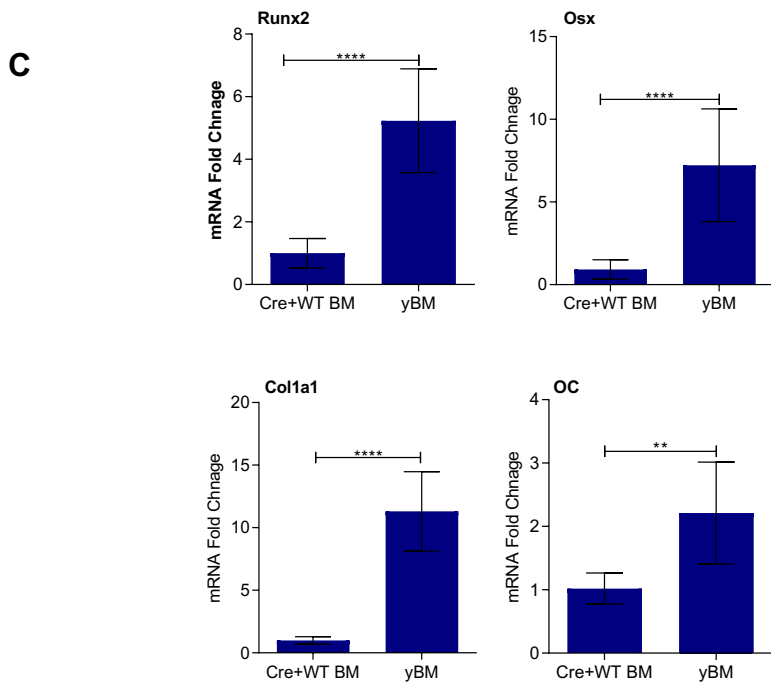
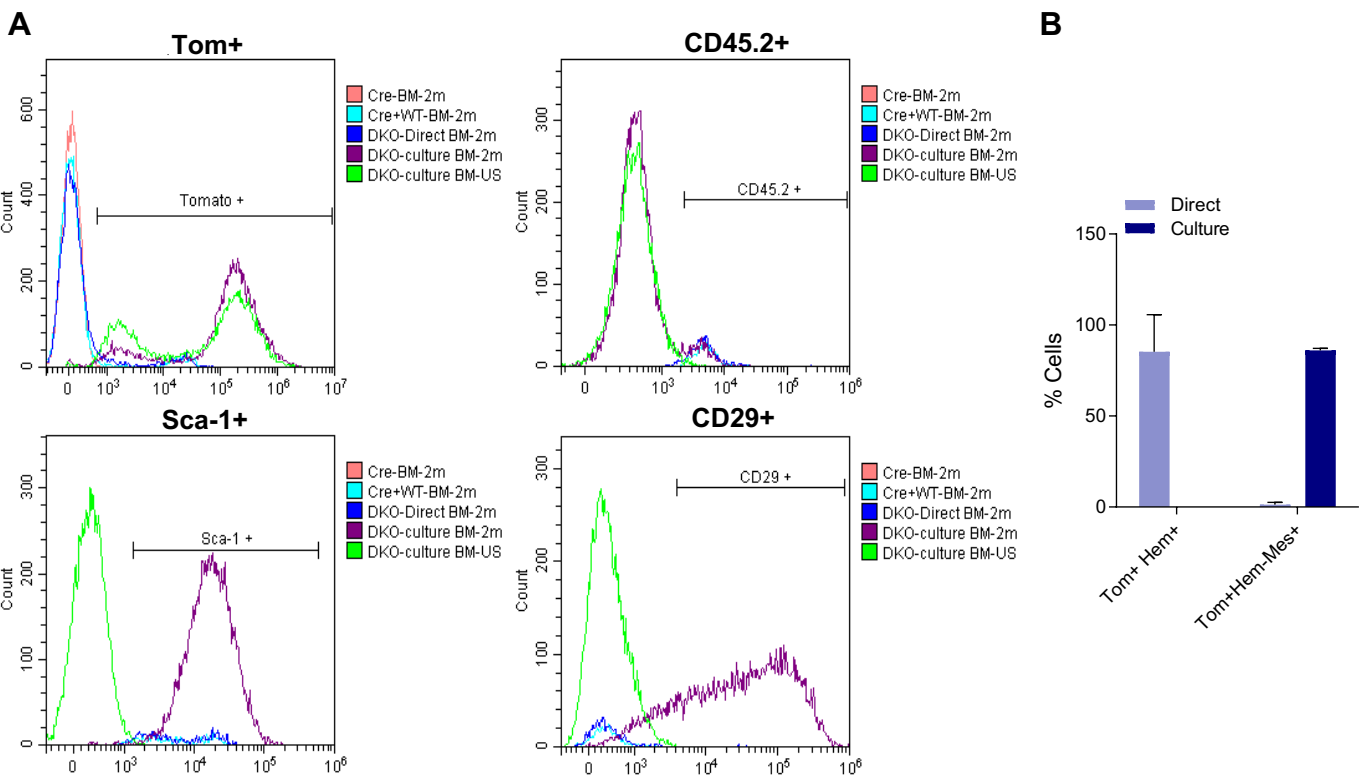


Figure 2S

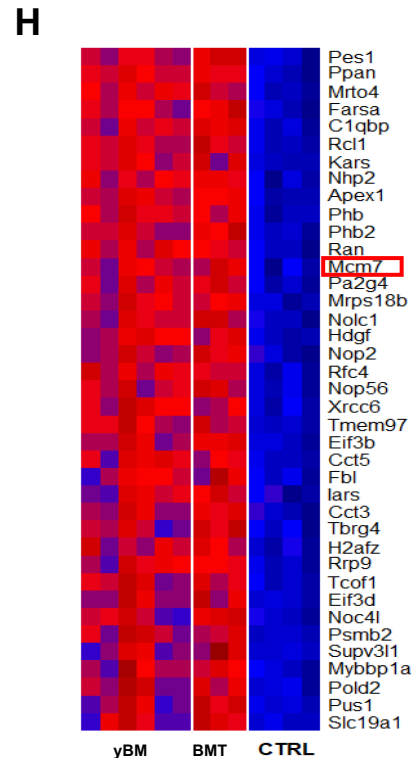
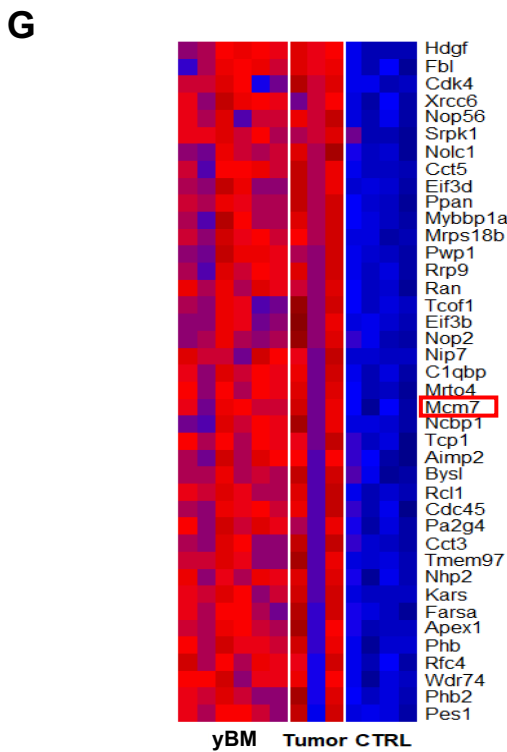
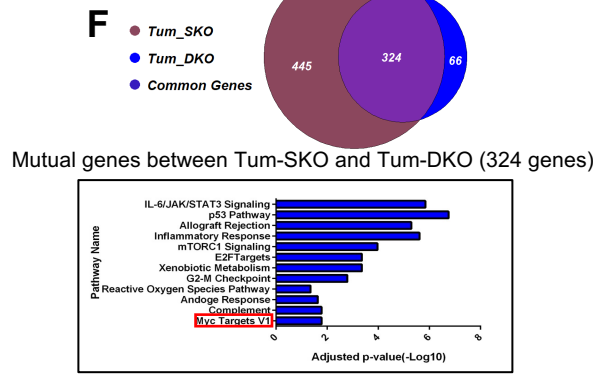
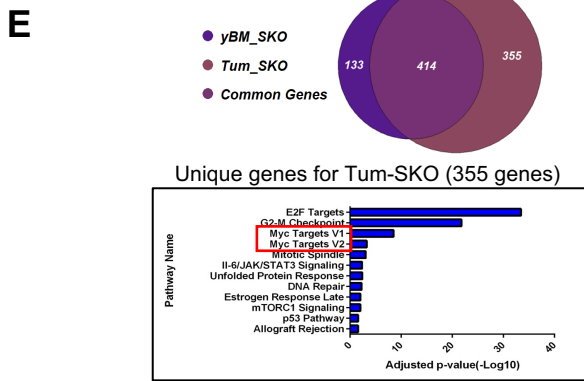
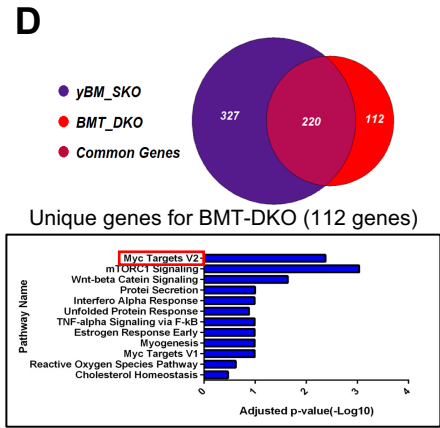
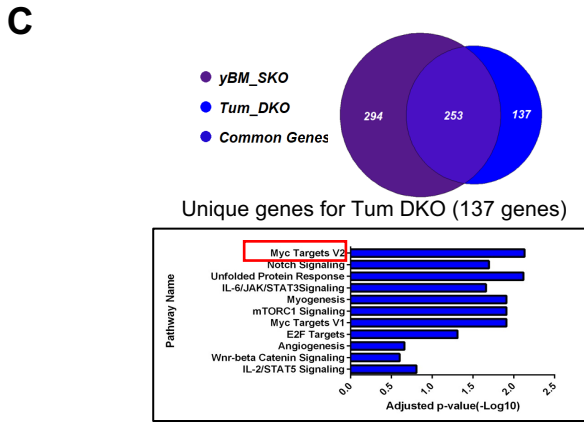
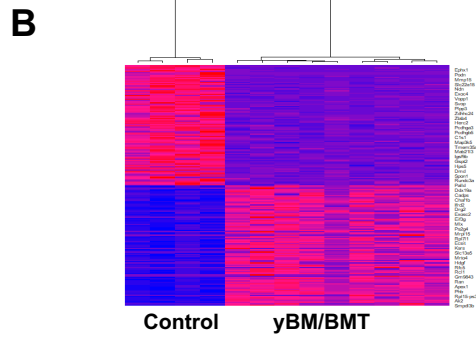
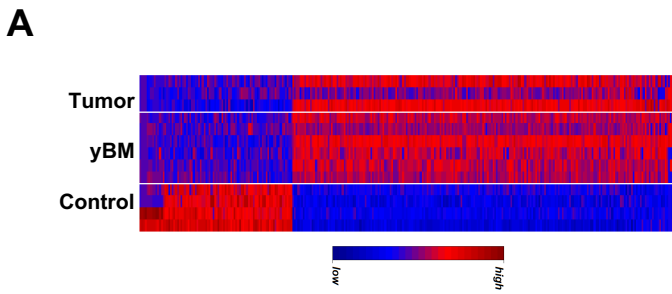


Figure 3S

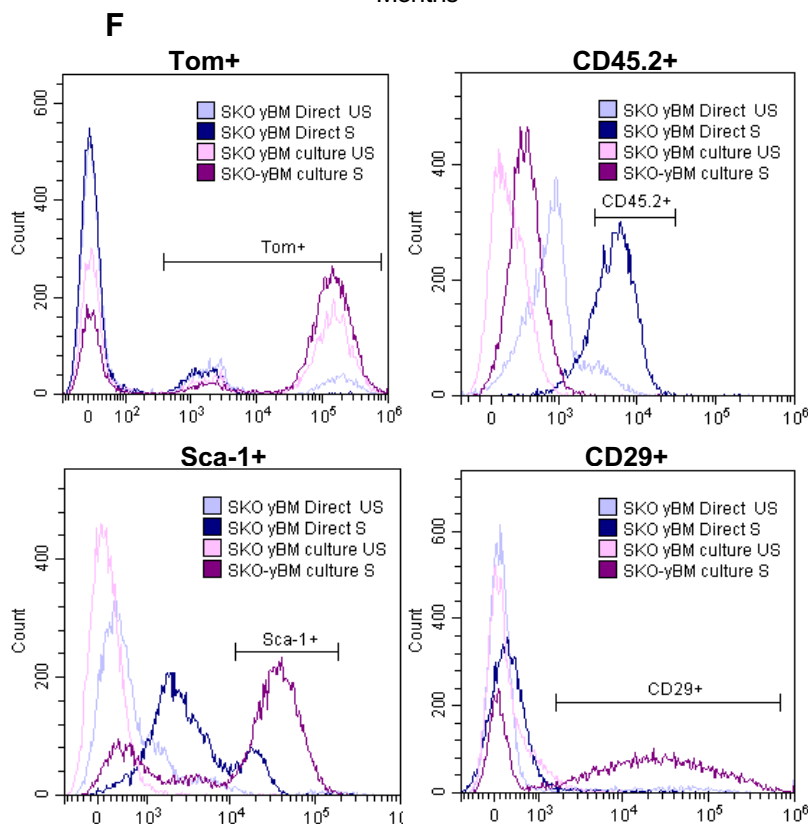
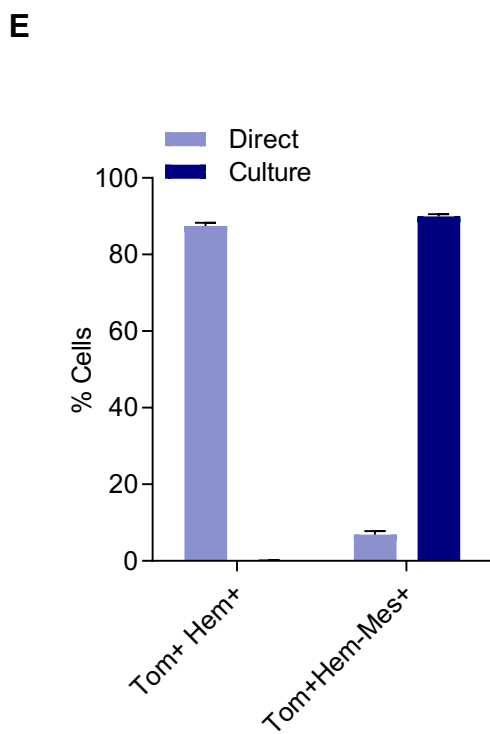
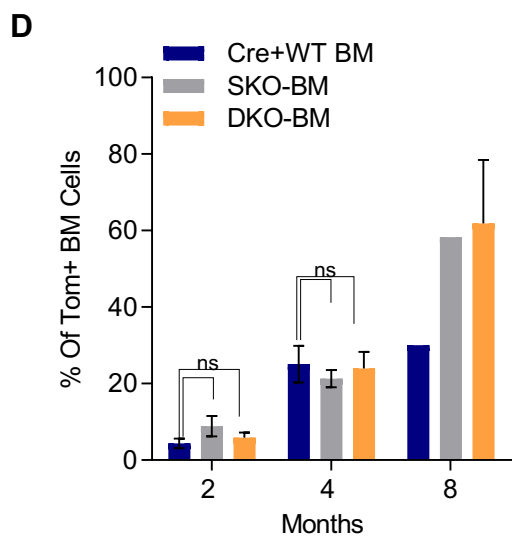
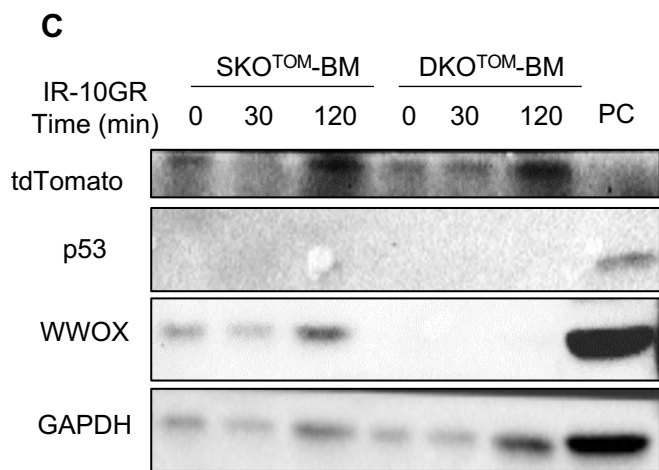
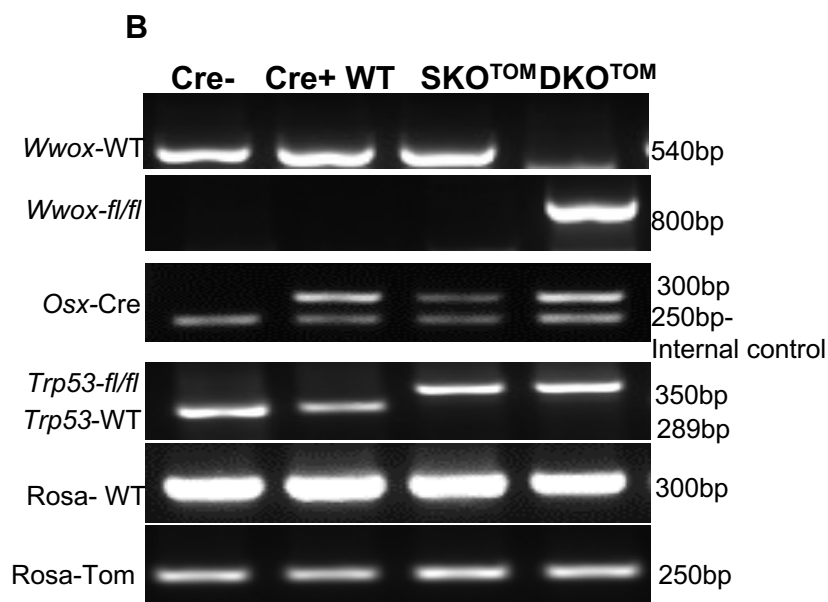
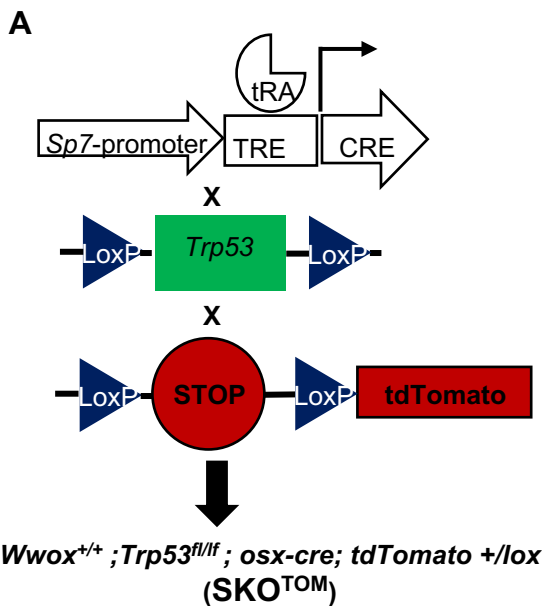
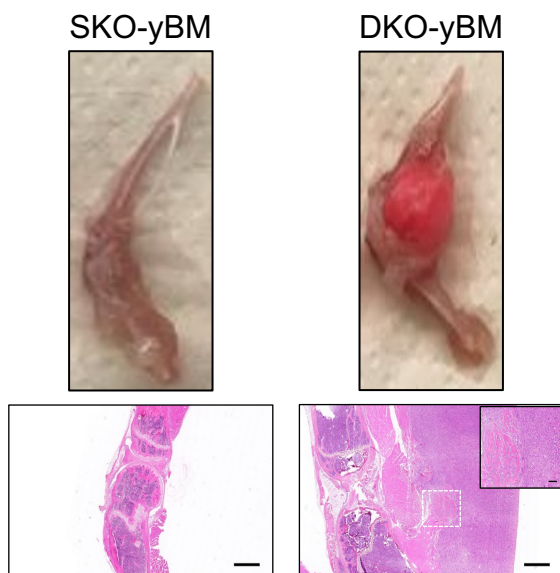
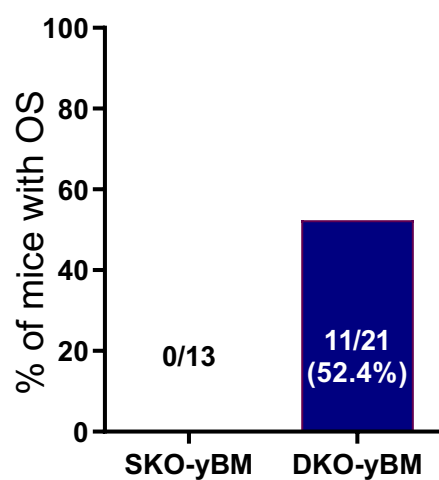
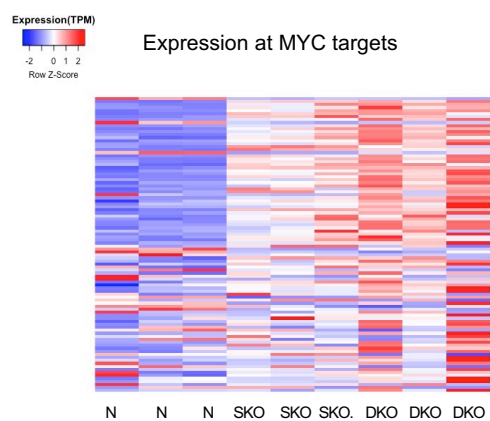
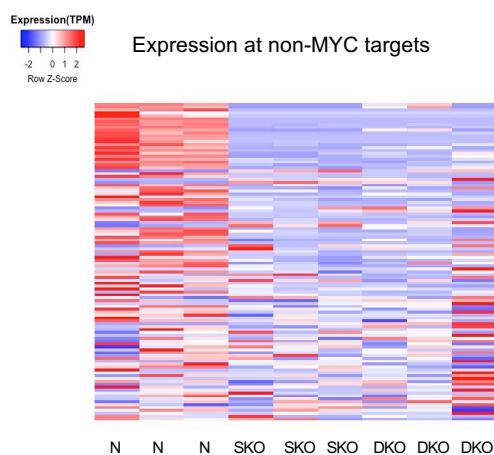
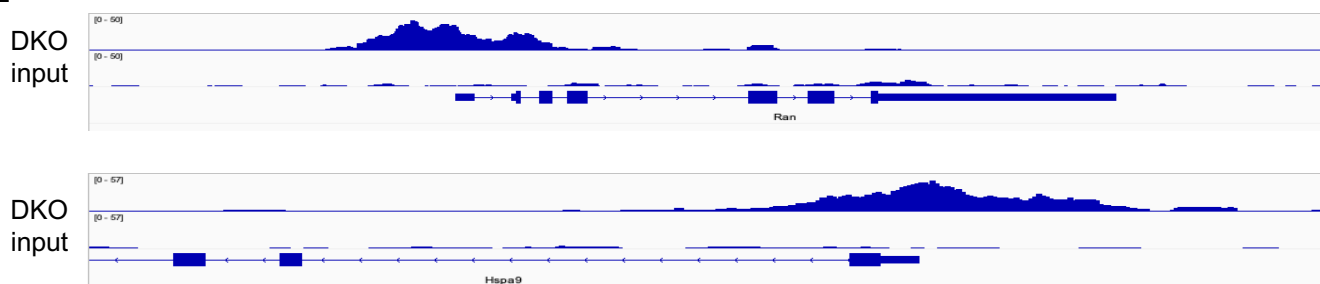
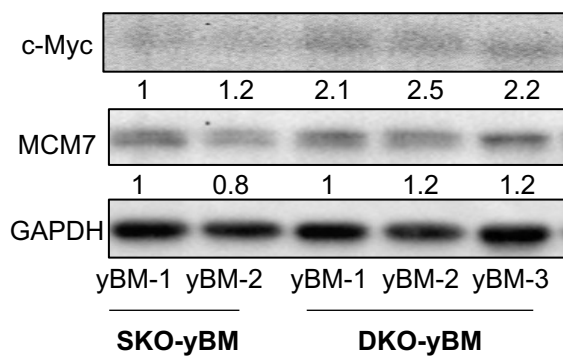
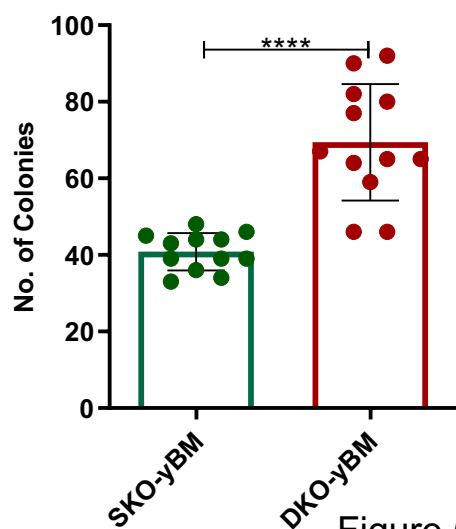


Figure 4S

**A****B****C****D****E****F****G**



| Gene        |         | Sequence                   | Product size |
|-------------|---------|----------------------------|--------------|
| Osterix     | Osx     | F:CCCTTCTCAAGCACCAATGG     | 85 bp        |
|             |         | R:AGGGTGGGTAGTCATTTGCATAG  |              |
| RUNX2       | RUNX2   | F:CGGCCCTCCCTGAACTCT       | 75 bp        |
|             |         | R:TGCCTGCCTGGGATCTGTA      |              |
| Colagen1A1  | Col1A1  | F:CTTGGTGGTTTTGTATTCGATGAC | 101 bp       |
|             |         | R:GCGAAGGCAACAGTCGCT       |              |
| Osteocalcin | OC      | F:CTGACAAAGCCTTCATGTCCAA   | 59 bp        |
|             |         | R:GCGCCGGAGTCTGTTCACTA     |              |
| MCM7        | MCM7    | F:AGTATGGGACCCAGTTGGTTC    | 115 bp       |
|             |         | R:GCATTCTCGCAAATTGAGTCG    |              |
| c-Myc       | c-Myc   | F:ATGCCCTCAACGTGAACTTC     | 228 bp       |
|             |         | R:CGCAACATAGGATGGAGAGCA    |              |
| HPRT        | HPRT    | F:TCAGTCAACGGGGACATAAA     | 142 bp       |
|             |         | R:GGGGCTGTA CTGCTTAACCAG   |              |
| Supv311     | Supv311 | F:CTGGCAGATTCAGCTCACAC     | 223 bp       |
|             |         | R:TGCCCATCAACTTGTGCAAA     |              |
| Cad         | Cad     | F:GCAAGTGGTTTGAATCCTCGG    | 246 bp       |
|             |         | R:CATTGGGGTCCACGAATGG      |              |
| Mrto4       | Mrto4   | F:GCCAACATGAGGAACAGCAA     | 170 bp       |
|             |         | R:AGCCCAACTTCACCTCTCAA     |              |