

# **A genetic platform to model sarcomagenesis from primary adult mesenchymal stem cells**

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Supplementary information includes figures showing candidate genes involved in the transformation process of MSCs, and the role of Lrf in MSCs differentiation and sarcomagenesis through Dlk1 and Sox9.

## **SUPPLEMENTARY FIGURE LEGENDS**

### **Supplementary Figure S1.**

#### **Candidate genes that couple with p53 loss to transform MSCs.**

(A) I.F. staining for  $\gamma$ H2AX and DAPI in MSCs cultured for 2 months at 20% of oxygen or 1% of oxygen. Treatment with doxorubicin was used as positive control. Representative pictures are shown on the left, while quantification is shown on the right. n=30 fields counted for each condition. (B) Protein expression levels of c-myc in p53<sup>KO</sup> MSCs transduced with viral vector of control (CTR) or over-expressing c-myc. (C) Protein expression levels of k-Ras<sup>G12V</sup> in p53<sup>KO</sup> MSCs transduced with viral vector of control (CTR) or over-expressing k-Ras<sup>G12V</sup>. (D) Protein expression levels of IDH2<sup>R172K</sup> in p53<sup>KO</sup>

MSCs transduced with viral vector of control (CTR) or over-expressing IDH2<sup>R172K</sup>. (E) Protein expression levels of Pten in p53<sup>KO</sup> MSCs transduced with a scramble viral vector of control (CTR) or silenced for Pten expression (shPten). (F) Protein expression levels of Pml in p53<sup>KO</sup> MSCs transduced with a scramble viral vector of control (CTR) or silenced for Pml expression (shPml). (G) Protein expression levels of Lrf in p53<sup>KO</sup> MSCs transduced with a scramble viral vector of control (CTR) or silenced for Lrf expression (shLrf).

## Supplementary Figure S2.

### Lrf plays as oncosuppressor triggering tumorigenesis in MSCs.

(A) Schematic overview of the experimental design to obtain *p53<sup>KO</sup>Zbtb7a<sup>F/F</sup>-CTR* or *p53<sup>KO</sup>Zbtb7a<sup>F/F</sup>-CRE* cells, and Lrf protein expression after transduction of *p53<sup>KO</sup>Zbtb7a<sup>F/F</sup>* MSCs with lentiviral vectors containing CRE or CTR. (B) Growth curve of *p53<sup>KO</sup>Zbtb7a<sup>F/F</sup>-CTR* and *p53<sup>KO</sup>Zbtb7a<sup>F/F</sup>-CRE* cells. Data show one representative experiment out of 3 independent experiments. (C) Focus formation assay with *p53<sup>KO</sup>Zbtb7a<sup>+/+</sup>* MSCs transduced with CRE or CTR lentiviral vectors. (D) Lrf protein expression levels in *p53<sup>KO</sup>Zbtb7a<sup>F/F</sup>-CTR* or *p53<sup>KO</sup>Zbtb7a<sup>F/F</sup>-CRE* cells before transplantation into mice, or recovered from scaffolds transplanted into mice. (E) Growth curve of *p53<sup>KO</sup>Zbtb7a<sup>F/F</sup>-CTR* or *p53<sup>KO</sup>Zbtb7a<sup>F/F</sup>-CRE* cells recovered from scaffolds transplanted into mice (1<sup>st</sup> recipients). (F) Relative expression of LRF mRNA (left chart) and LRF protein (panel in the middle) in human bone marrow-derived MSCs, transduced with shRNA against LRF compared to cells transduced with a scramble shRNA, and LRF protein expression in uncommitted MSCs or during their differentiation process toward adipocytes (right panel).

### **Supplementary Figure S3.**

**Lrf expression does not affect the CFU-F capacities of MSCs nor it triggers MSCs to senescence or apoptosis.**

(A) Schematic overview of experimental design is shown on the left, while Lrf expression in CTR-cells or CRE-cells 3 days after transduction with lentiviral vectors is shown on the right. (One representative western blot is shown, while the quantifications of Lrf protein levels (bottom chart) is shown as average of 5 independent experiments,  $\pm$  SEM. (B) CFU-F colonies derived from CTR-cells or CRE-cells. A representative colony for each condition is shown at higher magnification, while all the colonies of a representative well are shown stained with crystal violet. The quantification of colonies *per* well is shown on the right as average of 3 independent experiments  $\pm$  SEM. (C) Senescence assay for SA- $\beta$ -galactosidase detection. Representative pictures on the left and quantification of cells positive for SA- $\beta$ -galactosidase expression on the right (Results are shown as average of 3 biological independent replicates  $\pm$  SEM). (D) Annexin-V detection in CTR-cells and CRE-cells. (E) cleaved PARP (cPARP) expression in CTR-cells and CRE-cells.

### **Supplementary Figure S4.**

**Lrf regulates the differentiation process of MSCs through the repression of Dlk1.**

(A) Relative expression of Lrf, Sox9, Mia and Col2 mRNA in CTR-shSCR, CRE-shSCR and CRE-shSox9 MSCs is shown on the left, while western blot detection of Sox9 protein in CTR-shSCR, CRE-shSCR and CRE-shSox9 MSCs is shown on the right. (B) Alternative shRNA against Sox9 [shSox9(2) and shSox9(3)]. Relative expression of Lrf

and Sox9 upon transduction with shRNAs is shown on the left, while the differentiation assay toward adipocytes is shown in the middle panel and on the right. (C) Fabp4 mRNA expression levels in cells silenced with shSox9(2) and shSox9(3) and undergoing differentiation toward adipocytes. (D) Luciferase activity of Dlk1 promoter mutagenized in the various Lrf putative binding sites. Schematic representation of the binding sites mutagenesis is shown on the top panel while the luciferase assay is shown on the bottom. Results are shown as average of 3 biological independent replicates  $\pm$  SEM. (E) Alternative shRNA against Dlk1 [shDlk1(2) and shDlk1(3)]. Relative expression of Lrf and Sox9 upon transduction with shRNAs is shown on the left, while the differentiation assay toward adipocytes is shown in the middle panel and on the right. (F) Fabp4 mRNA expression levels in cells silenced with shDlk1(2) and shDlk1(3) and undergoing differentiation toward adipocytes.

### **Supplementary Figure S5.**

#### **Lrf plays a role as oncosuppressor in triggering the tumorigenesis of MSCs through Sox9 and Dlk1.**

(A) Sox9 and Lrf protein expression in  $p53^{KO}Zbtb7a^{F/F}$  cells transduced with CTR-shSCR, CTR-shSox9, CTR-shDlk1, CRE-shSCR, CRE-shSox9 and CRE-shDlk1. (B) Relative expression of Dlk1 mRNA expression in  $p53^{KO}Zbtb7a^{F/F}$  cells transduced with *CTR-shSCR*, *CRE-shSCR* and *CRE-shDlk1*. (C) Focus formation assay with alternative shRNAs against Sox9 [shSox9(2) and shSox9(3)], and alternative shRNAs against Dlk1 [shDlk1(2) and shDlk1(3)]. mRNA expression levels of Dlk1 and Sox9 are shown on the left, while representative pictures of foci and their quantification is shown on the left.