

## **Supplementary Legends:**

**Table S1. List of primers used for real-time PCR in this study.**

**Table S2. Detailed cytogenetic abnormalities of the cells in Figure 1A.**

**Table S3. Tumorigenic properties of MSCs.** Table summarizing the incidence (also shown in supplementary tables 4 and 5), average onset, and average tumor doubling time (calculation detailed in materials and methods) after administration of  $1 \times 10^6$  cells subcutaneously (s.c.) into NOD/SCID mice. Non-tumorigenic cells (Clone1 and MSC20) were also injected with higher amounts ( $3 \times 10^6$  and  $5 \times 10^6$  cells/mouse), and through a different route of administration (i.v. – intravenous), and no tumors were formed. The tumorigenicity of MSC20 and Clone1 was examined at passages 10 and 40. Tumorigenic cells (MSCs 1, 7, 2, and 8) were also injected with a higher dose ( $3 \times 10^6$  cells/mouse) and compared to the lower dose ( $1 \times 10^6$  cells/mouse), no significant increase in tumorigenicity was found. Cells with high tumorigenic potential (MSCs 2, 9, 5) observed at the low dose injections ( $1 \times 10^6$  cells/mouse) were also examined in i.v. administration and in syngeneic animals, and none formed tumors, except for syngeneic mice injected with MSC9. \*NOD/SCID $\beta$ 2 mice were also used.

**Table S4. List of MSC populations used in the study, their origin, ploidy and tumorigenic incidence.** The tumor incidence of each cell was calculated by dividing the "number of mice with tumors" with "total mice injected". MSCs 1 and 7 are also designated as MBA-13, MSC2 and MSC8 as MBA-15 and MSC20 as MBA-1 in our previous publications<sup>28</sup>.

**Table S5. List of MSC clones used in the study, their origin, ploidy and tumorigenic incidence.** The tumor incidence of each cell was calculated by dividing the "number of mice with tumors" with "total mice injected". Clone1 is also designated as 14F1.1 in our previous publications<sup>28</sup>.

**Fig. S1. Spectral karyotype analysis of tumor cells originating from MSC injections.** Representative images of spectral karyotype analysis of tumors derived from injections of MSC7, MSC8 and MSC9. Beneath each image, the full karyotype of the tumor cells originating from the indicated MSC is listed. Only common aberrations are listed, as the different cells in the tumor also harbored unique non-clonal aberrations (as indicated by the cp[#], # = number of cells with unique aberrations).

**Fig. S2. Gene expression analysis comparing diploid and tetraploid MSCs.** A heatmap generated using Expander (**A**, red – higher expression) summarizing the 136 genes which are differentially expressed between diploid and tetraploid MSCs ( $p\text{-val} < 0.05$ ,  $\text{fold-change} > 2$ ). Top ten genes expressed in diploid MSCs (**B**), and in tetraploid MSCs (**C**). Differentially active transcription regulator networks as revealed by Ingenuity Pathway Analysis is shown in **D**. The top two transcription regulators, MYC (more active in diploid MSCs) and TP53 (more active in tetraploid MSCs), are shown in **E** and **F**, respectively (red – higher expression in diploid MSCs, green – higher expression in tetraploid MSCs). (**G**) Gene ontology analysis using GSEA and Leading edge, showing that cell cycle related genes are overexpressed in diploid MSCs (red).

**Fig. S3. Gating of diploid and polyploid hepatocytes for sorting.** **a**, Hepatocytes were selected based on their forward and side scatter. **b**, Identification of diploid and tetraploid fractions was made based on area and width of Hoechst stained cell. A single representative experiment (out of seven) is shown.

**Fig. S4. Centrosome numbers in 2N, 2N+4N and 4N MSCs.** **a**, The proportion of cells in each ploidy group with more than 2 centrosomes is shown. Total number of cells inspected is shown on top of each bar. Two different 2N cells, seven different 2N+4N cells, and seven different 4N cells were used. **b**, An example of an individual cell within MSC20 population with more than two centrosomes. **c**, At the beginning of mitosis (prophase), centrosomes seemed to cluster together.

**d**, At the final stages of mitosis (anaphase), only bi-polar spindles were observed, and centrosomes were clustered at either side of the pole. Blue: DAPI, red:  $\gamma$ -tubulin.

**Fig. S5. Diploid and tetraploid MSCs express higher H19 levels upon reaching confluence.** **a,b**, H19 expression in dilute and dense cultures of diploid (**b**) and tetraploid (**c**) mesenchymal cells. Cells were seeded at dilute conditions (day 1) and allowed to reach confluence (days 7-9). Experiment was done in triplactes. Bars indicate mean  $\pm$ S.D.

**Fig. S6. Diploid MSCs express higher c-Myc protein levels.** **A.** The protein levels of c-Myc, p53 and AuroraB were measured in diploid and tetraploid MSCs (**i**), as well as in diploid MSCs subjected to H19 knock down (**ii**). GAPDH levels served as a loading control, and densitometry of bands is presented in panels **Bi** and **Bii**, respectively. **C.** Immunofluorescence of AuroraB in representative diploid and tetraploid cells (**i**) and in diploid MSCs subjected to H19 knock down (**ii**) during metaphase (1) and telophase (2) is shown (Blue: DAPI, red: AuroraB). RNA levels of c-Myc, p21 and p53 were measured using real time PCR and are shown in **Di** and **Dii**.

**Fig. S7. Molecular analysis of tumors derived from MSC injections.** **(A)** Protein levels of c-Myc and p53 in different tumorigenic MSCs, before injection (culture), and in the different tumors formed (numbered). GAPDH served as a loading control. **(B)** RNA levels of c-Myc, p53 and p21 in two tumorigenic MSCs (MSC3 and MSC9), before injection (culture) and in the different tumors formed (numbered).

**Fig. S8. Artificial tetraploidization of diploid MSCs.** Diploid MSCs 4 and 5 (**a** and **c** respectively), were subjected to artificial tetraploidization, which resulted in the formation of a 4N sub-population observed in flow cytometry (**b** and **d** respectively). Clones were derived from both MSCs after the procedure, resulting in 3/11 and 2/8 4N clones from MSC4 and MSC5, respectively. None of the 4N clones were found to be tumorigenic (at least 5 mice were injected with each cell).