SUPPLEMENTAL DATA

MATERIALS & METHODS

Cell Culture

Murine MSCs from C57Bl/6 mice were obtained from the lab of Prof. Dr. D Prockop, Tulane University, USA (*1*). Cells were kept in culture at a cell density of 55,000 MSCs per cm² in a 5% CO_2 humidified incubator under normoxic conditions in growth medium containing Iscove's Modified Dulbecco's Medium (IMDM; Gibco, Invitrogen, Carlsbad, CA, USA), 10% fetal bovine serum (FBS; Lonza BioWhittaker, Basel, Switzerland), 10% horse serum (Biochrom, Berlin, Germany), 1% L-glutamine (Gibco), and 1% penicillin/streptomycin (Gibco).

Rat MAPCs isolated from fisher rats were obtained as previously described (*2*). For rat MAPC culture, cells were seeded at a cell density of 2,000 MAPCs per cm³ on fibronectin-coated dishes, and growth media containing 60% low glucose Dulbecco's Modified Eagle Media (DMEM; Gibco), 40% MCDB-201 (Sigma-Aldrich, St Louis, MO, USA), 1x insulin-transferrin-selenium (ITS), 1x linoleic acid bovine serum albumin (LA-BSA), 10^{-9} M dexamethasone, 10^{-4} M ascorbic acid (all from Sigma-Aldrich), 1% penicillin/streptomycin, 2% FBS, 100μM 2-mercaptoethanol, 10 ng/mL human platelet-derived growth factor (PDGF; R&D Systems, Minneapolis, MN, USA), 10 ng/mL mouse epidermal growth factor (EGF; Sigma-Aldrich), and 1000 units/mL mouse leukemia inhibitory factor (LIF; Chemicon, Millipore, Billerica, MA, USA). MAPCs were cultured in a 5% CO_2 humidified incubator under hypoxic conditions (5% O_2).

In Vitro Toxicity Assays

MSC Differentiation. For adipogenic differentiations, cells were plated at a density of 10400 cells/cm² in differentiation medium 1, containing αMEM (Gibco), 10% FBS, 100 units of penicillin and 1000 units of streptomycin, 1μM dexamethasone, 10μg/mL human insulin, 100μM indomethacin and 25μM methyl-isobutylxanthine (all from Sigma-Aldrich). Medium was changed twice a week, and from the second week, cells were placed on differentiation medium 2 containing αMEM, 10% FBS, 1% penicillin/streptomycin, 1nM dexamethasone, 10μg/mL human insulin, 50μM indomethacin and 500nM methyl-isobutylxanthine.

After 14 days, cells were rinsed with PBS, fixed using unifix (Klinipath, Duiven, The Netherlands) for 20 minutes and stained with fresh Oil red O solution (Sigma-Aldrich) for 10 minutes. Images were taken using an inverted Zeiss axiovert microscope (Hertfordshire, UK). For quantification of the lipid droplet staining, the dye was extracted from the cells using 100% Ethanol and aliquots of 200µL were transferred to a 96-well plate in triplicate. Absorbance was measured at 450 nm using a Victor 1420 plate reader (Wallac, Perkin Elmer, Waltham, MA, USA).

To assess the effect on osteogenic differentiation capacity, MSCs were seeded at 8400 cells/cm² in normal growth medium and allowed to become confluent for 48h before adding osteogenic differentiation medium. Differentiation medium contained DMEM, 10% FBS, 1% penicillin/streptomycin, 50 µg/mL L-Ascorbic acid 2-phosphate sequimagnesium salt hydrate (AA-P; Sigma-Aldrich), 100nM dexamethasone and 10mM glycerol-2-phosphate disodium salt hydrate (bGP). Medium was changed twice a week for 3 weeks.

At day 21, cells were rinsed with PBS, fixed with ice-cold 70% ethanol for 1h and stained with Alizarin red S solution (Sigma) for 30 minutes. Images were taken using an inverted Zeiss axiovert microscope. Afterwards, the dye was extracted from the cells using a 10% cetylpiridinium chloride solution for 1h. Aliquots of 200µL were transferred to a 96-well plate in triplicate, and absorbance was measured at 560 nm.

Furthermore, cells were lysed using a 0.05% Triton X-100 in PBS solution and alkaline phosphatase (ALP) activity was assessed using the BluePhos microwell substrate kit (KPL, Gaithersburg, MD, USA). DNA content was measured using the Quant-iT^{TM} dsDNA HS Assay (Invitrogen) for normalization of the ALP values.

For chondrogenic differentiations, micromasses were generated by seeding 20µL droplets of cell suspension each containing 20000 cells into separate wells of a 24-well plate. After attachment for 3 hours expansion medium was added. The next day, chondrogenic differentiation medium was added containing DMEM, 5% FBS, 100nM dexamethasone, 1x ITS, 100µM AA-P and 10ng/mL Transforming Growth Factor-β (TGF-β). Every 2 days, medium was changed for 21 days.

At day 21, cells were fixed using unifix and stained overnight with Alcian Blue. The dye was extracted using 6M guanidine HCL. The optical density of the extracted dye was measured at 595 nm

in triplicates. Additionally, cell pellets formed were embedded in paraffin and sections were stained with toluidine blue and alcian blue. Furthermore, an immunohistochemical staining was performed on sections for Aggrecan using the peroxidase-based EnVision System (Dako, Glostrup,Denmark). Nonspecific binding sites were blocked with 3% normal goat serum, and slides were incubated for 1h at room temperature with the primary anti-mouse-Aggrecan antibody (1:500; Abcam, Cambridge, UK). Following washing, the tissue was incubated for 30 min with a goat anti-mouse horseradish peroxidase-conjugated secondary antibody. Subsequently, peroxidase was visualized using diaminobenzidine, and tissue was counterstained using Mayer's hematoxylin (Sigma). Control sections were subjected to the same staining protocol, with exclusion of the primary antibody. Sections were visualized using a photomicroscope equipped with an automated camera (Nikon Eclipse 80i, Nikon).

MAPC Differentiation. All MAPC differentiations were performed in a 5% CO_2 humidified incubator under normal oxygen conditions, unless stated otherwise.

Cells were seeded on 2% matrigel-coated (BD, Erembodegen, Belgium) surfaces at a density of 52600 cells per cm² in expansion medium for hepatogenic differentiations. On day 1, differentiation medium was added consisting of 60% low-glucose DMEM, 40% MCDB-201, 1% penicillin/streptomycin, 0.25x ITS, 0.25x LA-BSA, 10^{-6} M dexamethasone, 10^{-4} M ascorbic acid 3-phosphate, 100µM 2-mercaptoethanol. Medium was changed every 2 days for 20 days. Cytokines (all from R&D systems) were added sequentially as follows: Activin-A (100 ng/ml) and Wnt3a (50 ng/ml) from day 0 to day 6, bone morphogenetic protein (BMP)4 (50 ng/ml) FGF2 (10 ng/ml) from day 7 to day 10, acidic (a)FGF (50 ng/ml), FGF4 (10 ng/ml) and FGF8b (25 ng/ml) from day 11 to 14, and from day 15 to 28, hepatocyte growth factor (HGF) (20 ng/ml) and follistatin (100 ng/ml). RNA samples were collected every 7 days. Furthermore, on day 20, cells were fixed and stained for Hnf4a and Albumin using immunofluorescent antibodies.

To assess the effect on smooth muscle differentiation capacity, MAPCs were seeded on a fibronectin-coated surface at a density of 1500 cells per cm² in expansion medium. After 24h, differentiation medium was added, containing 60% low glucose-DMEM (Gibco), 40% MCDB-201, 1x ITS, 1x LA-BSA, 10^{-4} M ascorbic acid 3-phosphate, 10^{-9} M dexamethasone, 1% penicillin/streptomycin, 5ng/mL PDGF and 2,5ng/mL TFG-β; both from R&D systems. Cells were kept under hypoxic conditions (5% O_2) and medium was changed every 2 days for 6 days. RNA samples

were collected every 2 days. On day 6, samples were also fixed and stained for Sm22α, H1-calponin and SMαActin.

For neuroectodermal differentiation, cells were plated at a density of 1500 cells/cm² on 0,1% gelatin-coated flasks in N2B27 differentiation medium containing Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12 (DMEM/F12):Neurobasal A medium (1:1; both from Gibco), 0.5x N2Plus supplement (R&D systems), 0.5x B27 supplement (Gibco), 1% penicillin/streptomycin, 0.1mM 2-mercaptoethanol, and 200µM L-glutamine. After 2 days, cells were put on NSE differentiation medium containing Euromed medium (Euroclone, Siziano, Italy), 1x N2plus supplement (R&D systems), 2mM L-glutamine, 10 ng/mL basic fibroblast growth factor (bFGF) and 10ng/mL epidermal growth factor (EGF; both from R&D Systems). Every 2 days, bFGF and EGF (each 10ng/mL) were added. At day 6, RNA samples were taken and cells were transferred to low attachment surfaces in BRFF differentiation medium, consisting of BRFF P4-8F (AthenaES, Baltimore, MD, USA), 20 ng/mL bFGF, 10 ng/mL EGF, 2mM L-glutamine and 1% penicillin/streptomycin. Every two days, 20 ng/mL bFGF and 10 ng/mL EGF were added. At day 14, differentiation was terminated and RNA samples were collected.

Furthermore, gene expression analysis of MAPC differentiation was performed using Real-Time quantitative PCR. Herefore, total RNA was extracted and treated with DNase using the RNeasy micro-kit (Qiagen, Hilden, Germany). 1 µg of RNA was used to synthesize cDNA with the Superscript III First-strand synthesis system (Invitrogen). Real-time quantitative PCR (qRT-PCR) was performed with 2µL of cDNA, primers for the gene of interest and Platinum SYBR Green qPCR Supermix-UDG (Invitrogen), where cDNA underwent 40 rounds of amplification (Effendorf Realplex, Hamburg, Germany) as follows: initial UDG incubation and denaturation (50°C-2min, 95°C-2min) followed by 40 cycles of a 2-step PCR (95°C-15s, 60°C-45s). Primers used are listed in Table 1. GAPDH was used as a housekeeping gene for normalization of mRNA levels. Results are analyzed and expressed as ΔCt-values.

To further confirm MAPC differentiation capacity, immunofluorescent stainings were performed. Cells were fixed using unifix for 20 min, permeabilized with PBS-T and blocked with 5% normal goat or donkey serum (Dako, or Jackson ImmunoResearch Laboratories, Suffolk, UK). Cells were incubated overnight at 4 °C with the primary antibody or isotype antibody diluted with DAKO diluents (DAKO,

Glostrup, Denmark). Fluorescently labeled secondary antibodies (1:500, Alexa Fluor®, Invitrogen) and Hoechst dye (1:2000, Sigma) were incubated for one hour at room temperature in the dark. Specimens were analyzed with an inverted microscope (Zeiss Axiovert).

SUPPLEMENTAL TABLE 1. Primers used for qRT-PCR.

Gene	Forward primer (5'-3')	Reverse primer (3'-5')
Housekeeping gene		
Gapdh	AAGGGCTCATGACCACAGTC	GGATGCAGGGATGATGTTCT
Pluripotency genes		
Oct4	CCAATCAGCTTGGGCTAGAG	CCTGGGAAAGGTGTCCTGTA
Sox2	AACCCCAAGATGCACAACTC	CCGGGAAGCGTGTACTTATC
Hepatocyte differentiation		
Gsc	GAGAACCTCTTCCAGGAGAC	TTCTTAAACCAGACCTCCACC
Hnf4α	GGTCAAGCTACGAGGACAGC	ATGTACTTGGCCCACTCGAC
Afp	GAGTGCTTCCAGACAAAGAG	ATAATGGTTGTTGCCTGGAG
Alb	AGACTGCCTTGTGTGGAAGACT	TCAACTGTCAGAGCAGAGAAGC
G6P	TCTTCAAGTGGATTCTGTTTGG	GACAGGGAACTGCTTTATTATAGG
Mrp2	TAGTCTTCGCCGTACACTGAGC	ACATTCACATTTTTAAGGAGTT
Smooth muscle differentiation		
MyoCD	CTGTGTGGAGTCCTCAGGACAAACC	GATGTGTTGCGGGCTCTTCAG
Sm22α	CCACAAACGACCAAGCCTTTT	CGGCTCATGCCATAGGATG
Smtn	CCAGAGGCTCCTCTAACACTAAGAG	TTGCTCTTGATTTTGGGTTGGCTG
SMαA	CGCCATCAGGAACCTCGAGA	CAAAGCCCGCCTTACAGA
Cnn1	ACATCATTGGCCTACAGATG	CAAAGATCTGCCGCTGGTG
SM-MHC	CAAGAGTTCCGGCAACGCTA	TCCATCCATGAAGCCTTTGG
Neuroectodermal differentiation	GTCCATCTTTGCTTGGGAAA	TAGCCAGGTTGCGAAGAACT
Pax6		

Gapdh: glyceraldehyde 3-phosphate dehydrogenase,Oct4: octamer-binding transcription factor 4, Sox2: (sex determining region Y)-box 2, Gsc: homeobox protein goosecoid, Hnf4α: hepatocyte nuclear factor 4 alpha,Afp: alpha-fetoprotein, Alb: albumin, G6P: glucose-6-phosphate, Mrp2: multidrug resistance-associated protein 2, MyoCD: myocardin, Smtn: smoothelin, SMαA: smooth muscle α actin, Cnn1: calponin-1, SM-MHC: smooth muscle-myosin heavy chain, Pax6: paired box protein Pax-6

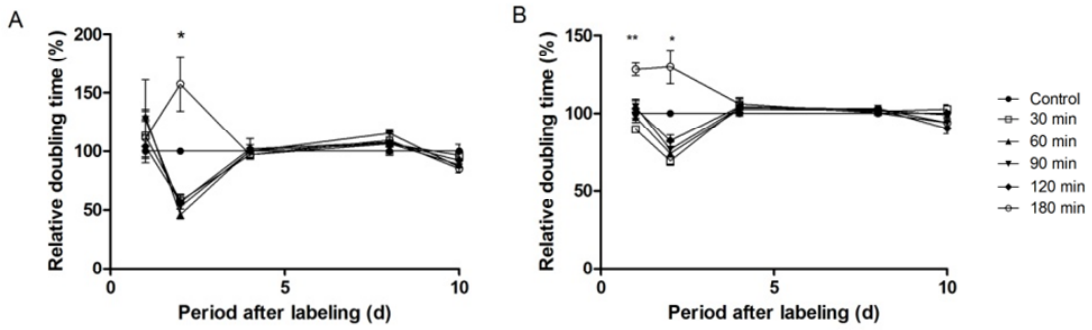
SUPPLEMENTAL TABLE 2. Primary antibodies used for Immunocytochemical stainings

Primary antibody	Dilution
Mouse anti-Hnf4α	1:200
Rabbit anti-Albumin	1:8000
Goat anti-SM22α	1:500
Rabbit anti-IgG (isotype antibody)	1:400
Mouse anti-IgG2a (isotype antibody)	1:1000

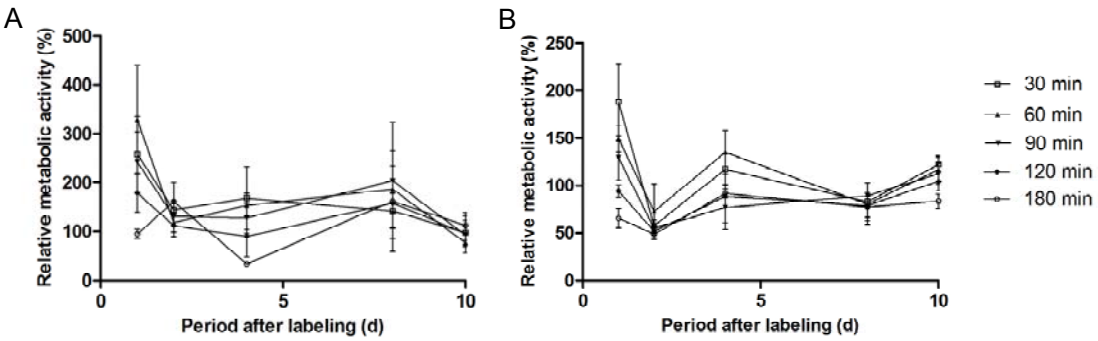
SUPPLEMENTAL REFERENCES

1. Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, Prockop DJ. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. *Blood.* Mar 1 2004;103(5):1662-1668.

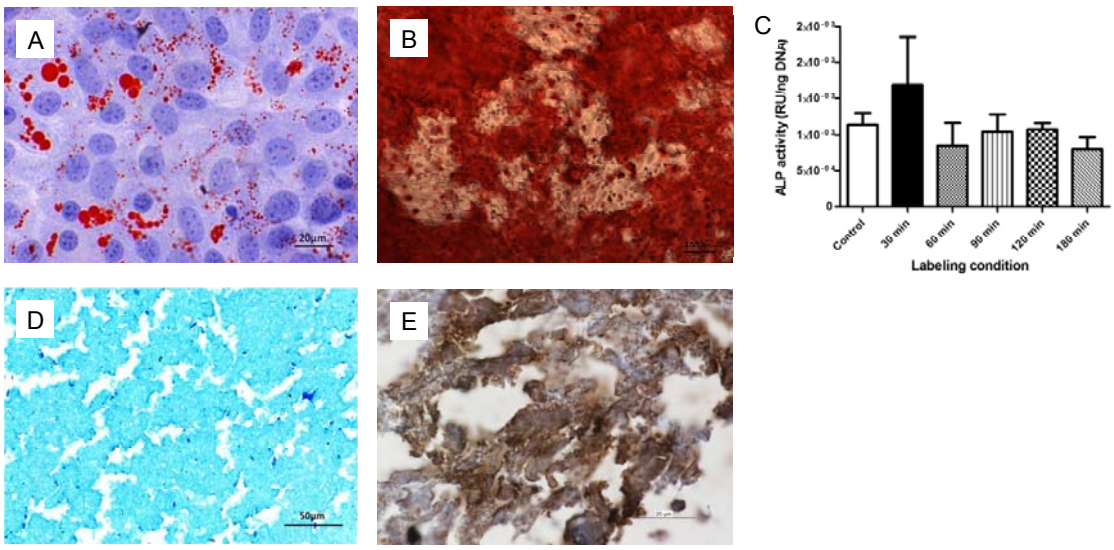
2. Breyer A, Estharabadi N, Oki M, et al. Multipotent adult progenitor cell isolation and culture procedures. *Exp Hematol.* Nov 2006;34(11):1596-1601.



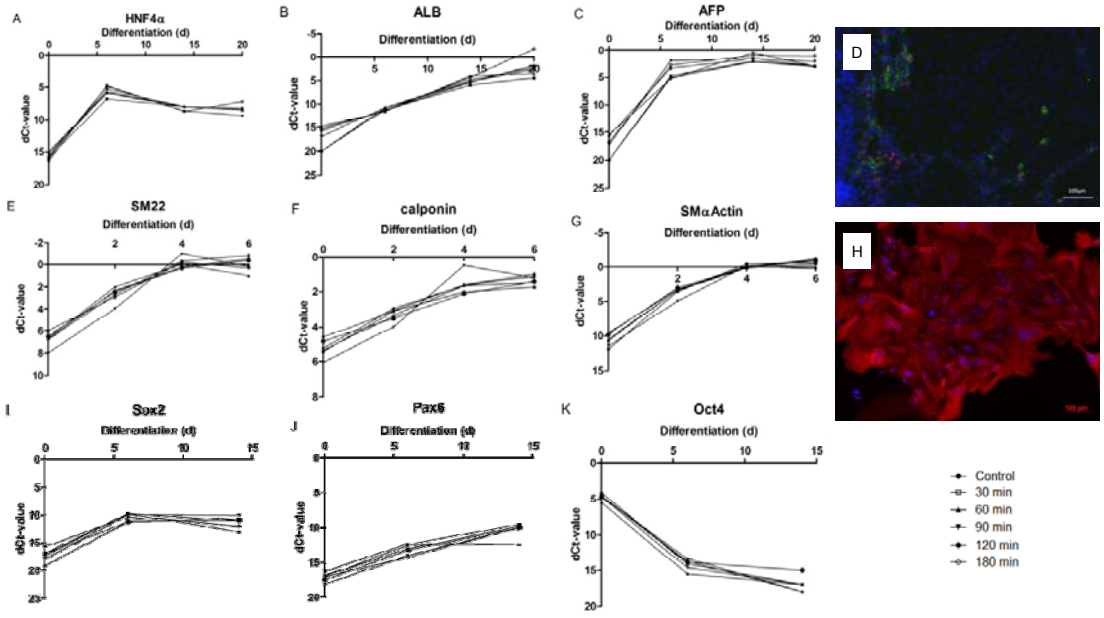
SUPPLEMENTAL FIGURE 1. Doubling times follow-up for 10 days in MSCs (A) and MAPCs (B) after ^{18}F-FDG labeling for different periods.



SUPPLEMENTAL FIGURE 2. Relative metabolic activity in MSCs (A) and MAPCs (B) following ^{18}F-FDG labeling, relative to unlabeled control cells.



SUPPLEMENTAL FIGURE 3. Adipogenic, osteogenic and chondrogenic differentiations of MSCs labeled with ^{18}F-FDG. Oil red O staining of fat droplets in MSCs differentiated to adipoblasts (A). Alizarin red S staining of osteogenic differentiated cells (B). Assessment of ALP activity corrected for DNA content in all osteogenic conditions (C); RU: Absorbance measured at 620nm). Alcian Blue staining of differentiated chondrocytes (D). Anti-aggrecan staining of MSCs differentiated towards chondrogenic lineage (E).



SUPPLEMENTAL FIGURE 4. Hepatic, smooth muscle and neuroectodermal differentiations of MAPCs labeled with ^{18}F-FDG. qRT-PCR results of hepatic MAPC differentiation following ^{18}F-FDG labeling (A-C). Immunofluorescent staining against HNF4α (red), Albumin (Green) and DAPI (blue) confirming hepatic differentiation (D). qRT-PCR results of smooth muscle differentiation for SM22α (E), Calponin (F) and SMαActin(G). Immunostaining for SM22 (red) and DAPI (blue) (H). qRT-PCR results of neuroectodermal differentiation experiment, with Sox2 (I), Pax6 (J) and Oct4 (K) as examined genes.