Figure S1. Experimental strategy for 3D alginate culture for induction of chondrogenesis of rat MSC. BM-MSC were isolated from the tibia and femurs of Dark Agouti (DA) and Lewis (LEW) rats and expanded in vitro. Chondrogenic differentiation was induced in 3D alginate culture by incubation with TGFβ-3 and BMP-2 for up to 21 days (21d). Differentiated MSC refer to MSC that were cultured in 3D alginate layers and which displayed morphological and gene expression changes consistent with a chondrocyte-like phenotype.

Figure S2. Lewis chondrogenic differentiated MSC do not suppress allogeneic T-cell proliferation. (a) CFSE-labeled allogeneic DA T cells were stimulated with anti-CD3/CD28 beads in the presence of undifferentiated or differentiated Lewis MSC at the indicated ratios. On day 4, cells were harvested and the percentage proliferation was analysed by flow cytometry. (b) Gating strategy used for analysis is shown. $CD4^+$ and $CD8^+$ T cells were identified using anti-CD4-APC and anti-CD8-PE antibodies and analysed by flow cytometry. (c). CFSE dilution was analysed in gated $CD4^+$ and $CD8^+$ cells and the percentage proliferation was calculated, and graphed as shown. Representative results of two independent experiments are shown ± SEM. . *p<0.05, **p<0.01, ***p<0.001

Figure S3. **Chondrogenic differentiated human MSC do not suppress allogeneic T-cell proliferation.** (a) CFSE-labeled allogeneic PBMCs were stimulated with anti-CD3/CD28 soluble antibodies in the presence of undifferentiated or differentiated MSC at indicated ratios for 4 days. (b) The corresponding CFSE dilution histograms are shown to indicate the percentage of $CD4^+$ and $CD4^-$ lymphocyte proliferation. $CD4^+$ and CD4- proliferation was determined similarly to that outlines in the gating strategy in Figure S2b. Data from three independent MSC donors are shown. *p<0.05, **p<0.01, ***p<0.001

Figure S4. **Experimental strategy for the generation of cytotoxic T cells** DA-specific allogeneic cytotoxic T cells (CTLs) were generated in a one-way mixed lymphocyte culture of LEW and DA T cells as outlined. Undifferentiated and differentiated syngeneic and allogeneic MSC were incubated with allo-antigen specific CTLs in an effector to target ratio of 100:1, for 4 hours, following which time the fluorescence of the supernatants were analysed by a fluorescence plate reader. Specific lysis was calculated from mean fluorescence of replicates as follows: (F[sample] – F[spontanous release]) / (F[maximum lysis] – F[spontanous release]) · 100 = % specific lysis.

Figure S5. Subcutaneous Implantation experimental strategy and morphological evidence for chondrogenic differentiation in syngeneic differentiated and allogeneic differentiated alginate layers. (a) The experimental strategy for subcutaneous implantation is outlined here. Syngeneic and allogeneic undifferentiated and differentiated MSC were implanted subcutaneously in LEW rats. Allogeneic splenocytes were implanted as an immunogenicity positive control. 6 weeks later, the area surrounding the implant was removed, formalin fixed and paraffin embedded. The number of $CD3^+$ and $CD68^+$ cells within the implants were analysed by immunohistochemistry (b) Representative histological sections, at 20X magnification (upper row) and 40X magnification (lower row) illustrating H&E staining of syngeneic differentiated, allogeneic undifferentiated and allogeneic differentiated MSC implants. 40X magnification (lower row) clearly illustrates the morphological characteristics of chondrocytes within the syngeneic and allogeneic differentiated alginate layers.

Figure S6. Experimental strategy for the *in vivo* analysis of injection of unencapsulated and implantation of alginate encapsulated differentiated and undifferentiated MSC syngeneic

and allogeneic MSC. The experimental strategy for subcutaneous injection and implantation is outlined here. Syngeneic and allogeneic undifferentiated and differentiated MSC were either injected or implanted subcutaneously in LEW rats. Allogeneic splenocytes were also implanted as an immunogenicity positive control. 6 weeks later, the draining blood, lymph nodes and spleen were removed from each animal. An ex vivo re-stimulation assay was used to analyze the allo-specific and third party memory T cell responses, both locally and systemically, in the recipient animals. Anti-donor antibody responses were analysed by flow cytometry of donor strain (DA) cells incubated with recipient sera from the various groups.

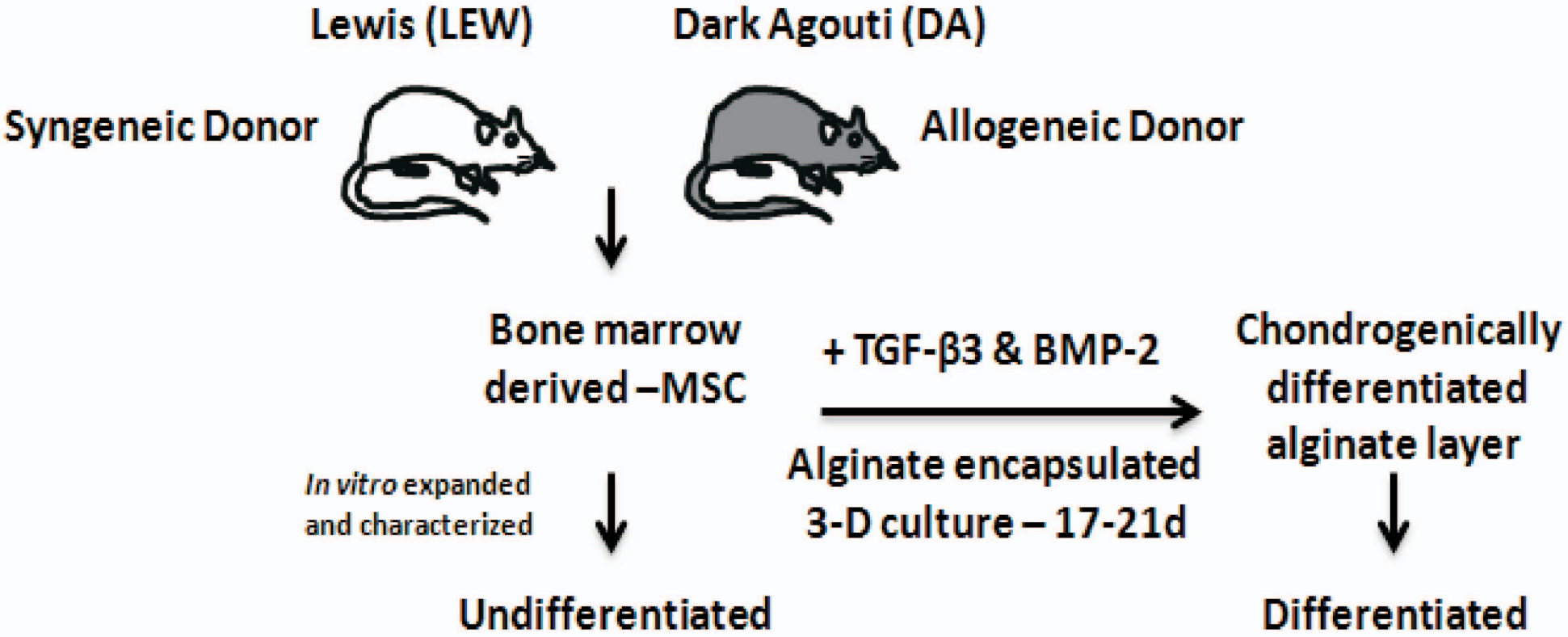
Lewis (LEW)
Dark Agouti (DA)
Syngeneic Donor
Allogeneic Donor
Bone marrow derived –MSC
+ TGF-β3 & BMP-2
Chondrogenically differentiated alginate layer
Alginate encapsulated 3-D culture – 17-21d
In vitro expanded and characterized
Undifferentiated
Differentiated

Figure S1

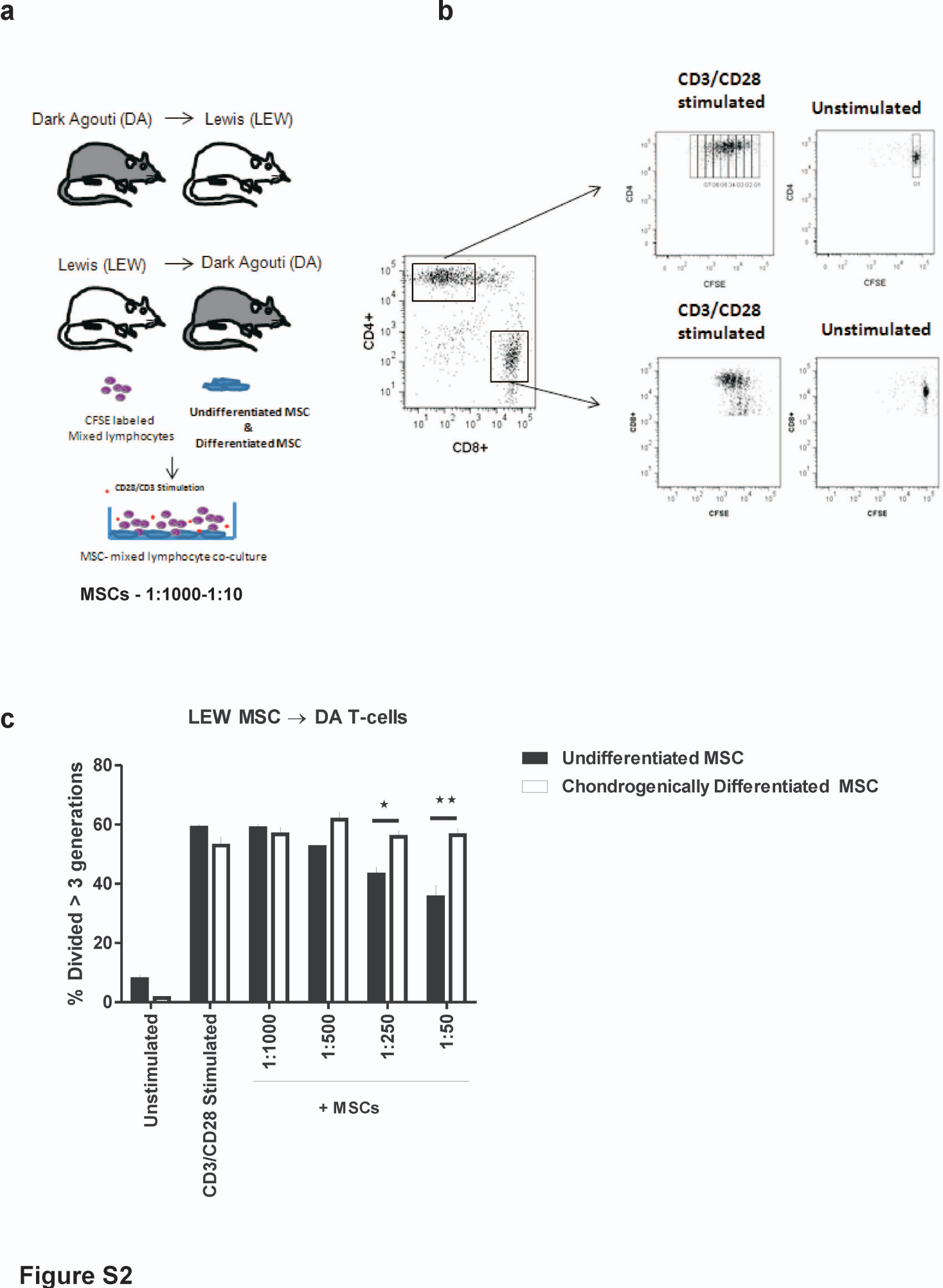
a
Dark Agouti (DA) → Lewis (LEW)
Lewis (LEW) → Dark Agouti (DA)
CFSE labeled
Mixed lymphocytes
Undifferentiated MSC
&
Differentiated MSC
CD28/CD3 Stimulation
MSC- mixed lymphocyte co-culture
MSCs - 1:1000-1:10
b
CD3/CD28
stimulated
Unstimulated
CD4+
CD8+
CFSE
c
LEW MSC → DA T-cells
Undifferentiated MSC
Chondrogenically Differentiated MSC
% Divided > 3 generations
0
20
40
60
80
Unstimulated
CD3/CD28 Stimulated
1:1000
1:500
1:250
1:50
+ MSCs
★
★★

Figure S2

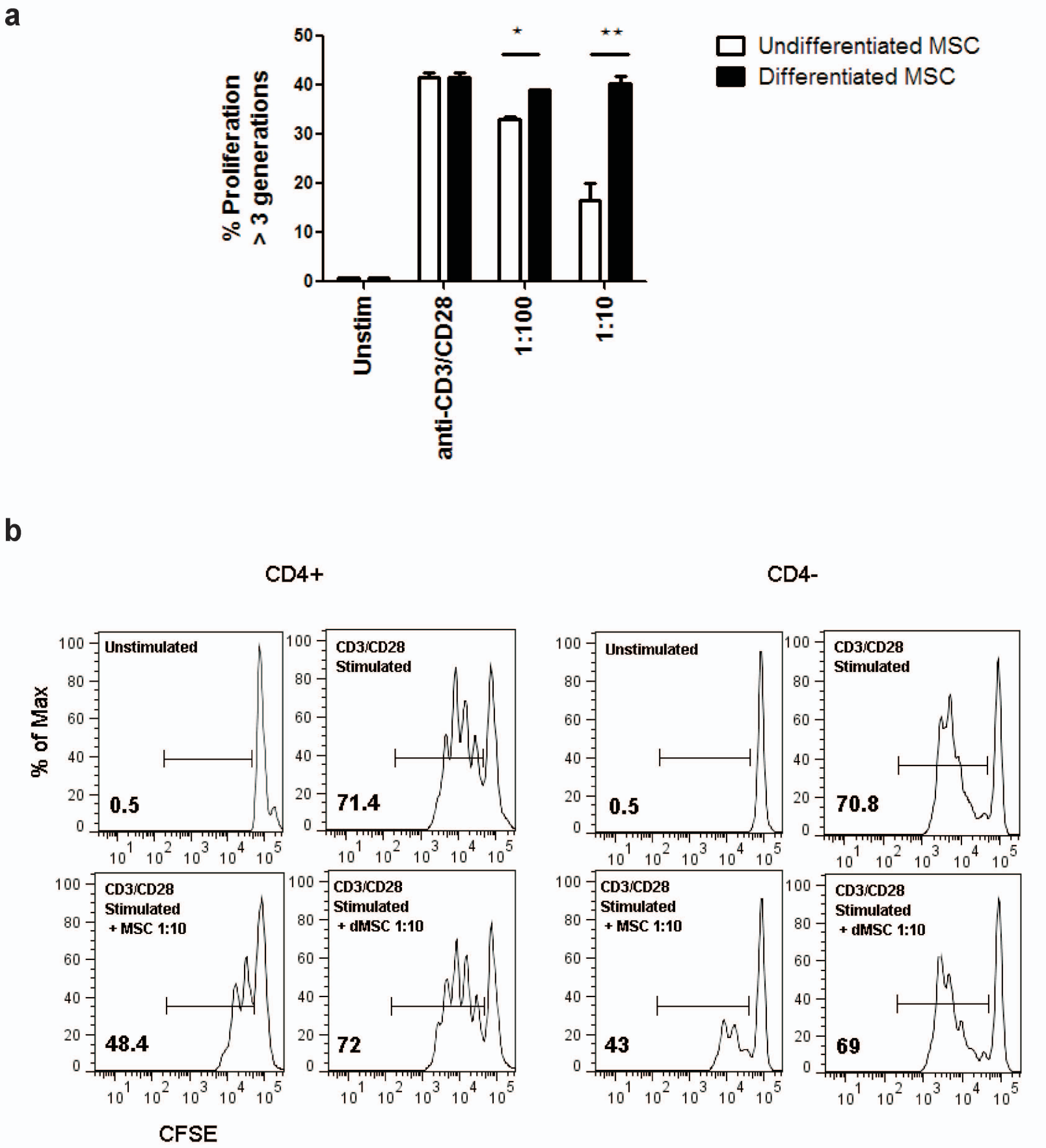
a
% Proliferation > 3 generations
Undifferentiated MSC
Differentiated MSC
Unstim
anti-CD3/CD28
1:100
1:10
b
CD4+
CD4-
% of Max
CFSE
Unstimulated
CD3/CD28 Stimulated
CD3/CD28 Stimulated + MSC 1:10
CD3/CD28 Stimulated + dMSC 1:10
0.5
71.4
48.4
72
0.5
70.8
43
69

Figure S3

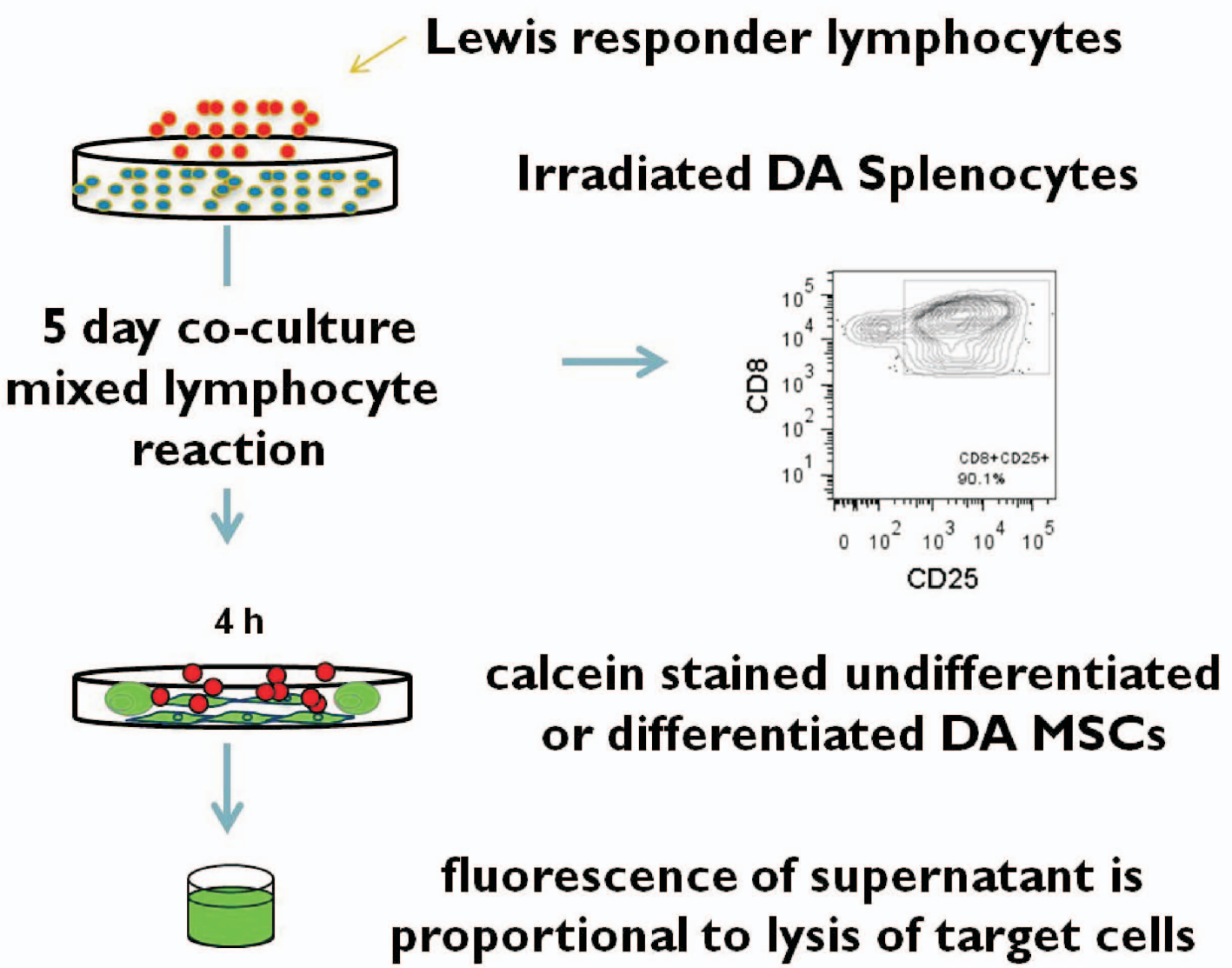
Lewis responder lymphocytes
Irradiated DA Splenocytes
5 day co-culture
mixed lymphocyte
reaction
CD8
CD25
CD8+CD25+
90.1%
4 h
calcein stained undifferentiated
or differentiated DA MSCs
fluorescence of supernatant is
proportional to lysis of target cells

Figure S4

a

Subcutaneous implantation
(alginate encapsulated cells)

1 x 10^6 cells
in alginate layer

Lewis (LEW)
Recipient

- Syngeneic undifferentiated MSC
- Syngeneic Differentiated MSC
- Allogeneic undifferentiated MSC
- Allogeneic Differentiated MSC
- Allogeneic Splenocytes00

d42 (6 weeks)

Isolation of subcutaneous implant site

Histological and Immunohistochemical analysis
(H&E, CD68, CD3)

b

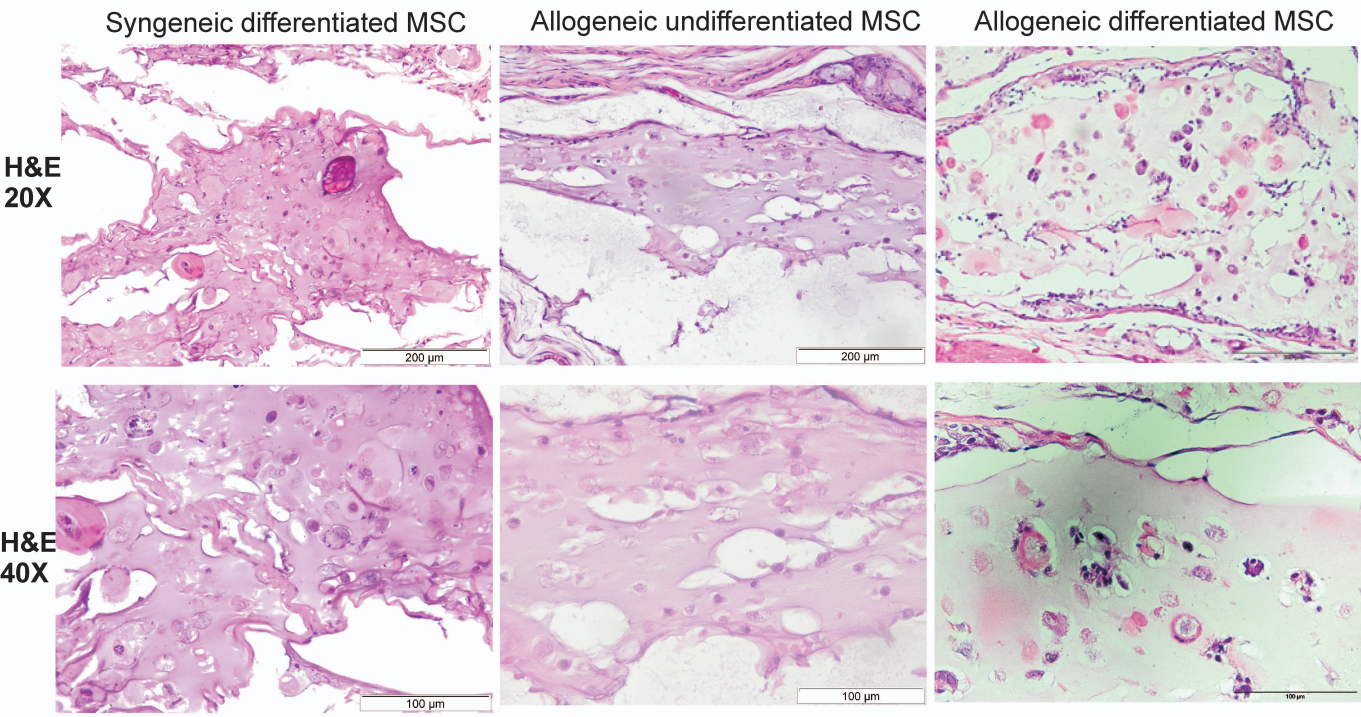

Figure S5

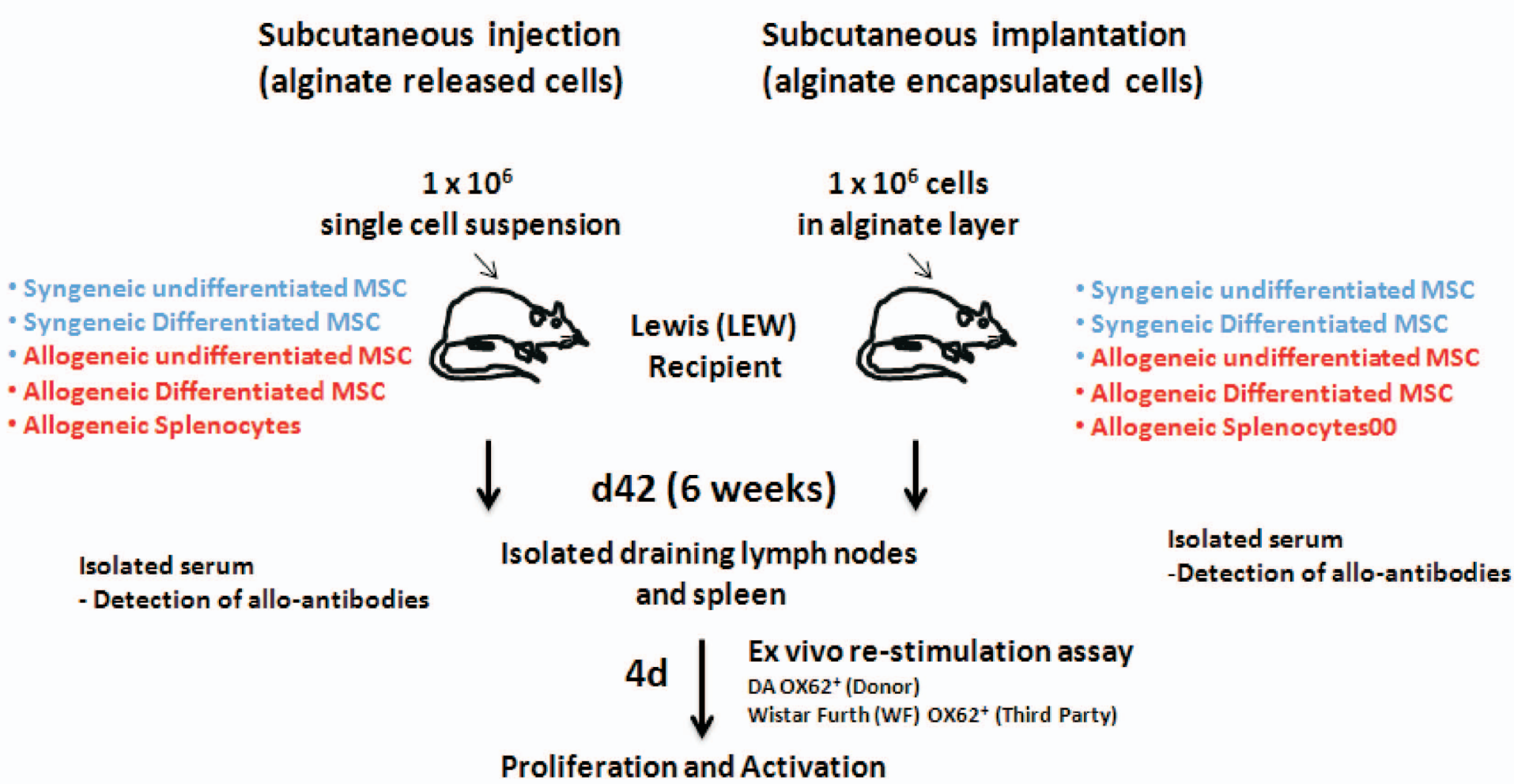
Subcutaneous injection
(alginate released cells)
Subcutaneous implantation
(alginate encapsulated cells)
1 x 10^6
single cell suspension
1 x 10^6 cells
in alginate layer
• Syngeneic undifferentiated MSC
• Syngeneic Differentiated MSC
• Allogeneic undifferentiated MSC
• Allogeneic Differentiated MSC
• Allogeneic Splenocytes
Lewis (LEW)
Recipient
• Syngeneic undifferentiated MSC
• Syngeneic Differentiated MSC
• Allogeneic undifferentiated MSC
• Allogeneic Differentiated MSC
• Allogeneic Splenocytes00
d42 (6 weeks)
Isolated serum
- Detection of allo-antibodies
Isolated draining lymph nodes
and spleen
Isolated serum
-Detection of allo-antibodies
4d
Ex vivo re-stimulation assay
DA OX62+ (Donor)
Wistar Furth (WF) OX62+ (Third Party)
Proliferation and Activation

Figure S6