Supplementary Materials

Scalable Generation of Mesenchymal Stem Cells from Human Embryonic Stem Cell in 3D

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Table S1. Percentage of cells positive for four typical MSC markers

	MSC markers				
MSCs	CD90	CD44	CD105	CD73	Negative control markers
$EMSC_{Sp\text{-}ML}$ from CT2	94.1	100.0	83.8	99.6	0.9
$EMSC_{Sp\text{-}ML}$ from CT3	91.7	100.0	73.7	99.9	0.1
$EMSC_{Sp\text{-}ML}$ from H9	99.4	99.1	76.2	98.7	0.3
$EMSC_{Sp\text{-}ML}$ from Envy	ND	99.6	64.5	99.0	8.9
$EMSC_{Sp\text{-}ML}$ from PBY4	78.1	100.0	22.8	99.8	13.1
BMSC from donor #3	94.5	99.9	95.6	98.0	22.3
BMSC from donor #4	88.9	100.0	98.8	98.0	27.1

Note: $EMSC_{Sp\text{-}ML}$ were derived from four hESC lines and determined for percentage of cells positive for four typical MSC markers and a mixture of multiple negative control markers. ND: not done

Table S2. Sequences of primers used for RT-PCR and RT-qPCR

Gene	Forward primer	Reverse primer
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA
IDO1	ATGCAGACTGTGTCTTGGCA	AGCTATTTCCAACAGCGCCT
PDL1	CTGGCATTTGCTGAACGCAT	GGGAGAGCTGGTCCTTCAAC
CCL2	AAACTGAAGCTCGCACTCTCGC	ATTCTTGGGTTGTTGAGTGAGT
CXCL10	ACCTCCAGTCTCAGCACCATG	TGGGAGGATGGCAGTGGAAG
IL6	CCTTCGGTCCAGTTGCCTTC	CACAGCTCTGGCTTGTTCCTC
IL8	CACCGGAAGGAACCATCTCACT	TCAGCCCTCTTCAAAAACTTCTCC

TGS6	AGATGACCCAGGTTGCTTGG	CCTTGACTGGATTTGGATACAGGA
CGA	CAACCGCCCTGAACACATCC	CAGCAAGTGGACTCTGAGGTG
CDX2	TGGACACGGACCACCAG	GCTCTGGGACACTTCTCAGAGG
CD9	TTCCTCTTGGTGATATTCGCCA	AGTTCAACGCATAGTGGATGG
CGB	TGAGATCACTTCACCGTGGTCTCC	TTTATACCTCGGGGTTGTGGG G
KRT7	AGG ATG TGG ATG CTG CCT AC	CACCACAGATGTGTCGGAGA

Supplemental figure legend

Fig. S1. Expression of pluripotency markers and size of hESC spheroids.

(A, B) hESC in spheres remained positive for the pluripotency markers as analyzed per immunostaining (A) and flow cytometry (B). Scale bar is 50 μm.

(C) The range of sizes of hESC spheres on day 2 following spheroid formation (n = 115).

Fig. S2. Further characterization of EMSC spheres.

(A) Expression of trophoblast-associated genes was detected per RT-qPCR, and the levels are calculated as fold change to those in $hESC_{ML}$ and $hESC_{Sp}$. Two independent experiments were performed in duplicate. Data are normalized to the expression of the house-keeping gene GAPDH and presented as mean ± SE.

(B) Normal karyotypes identified on $EMSC_{Sp\text{-}ML}$ derived from H9 and CT3 hESC spheres.

Fig. S3. Alleviation of DSS-induced mouse colitis by IFNγ-treated $EMSC_{Sp\text{-}ML}$

(A). The mice of DSS induced colitis were sacrificed on day 15 and their colons were isolated and aligned together for photography.

(B) The mice of TNBS induced colitis were sacrificed on day 5 and their colons were isolated for photography.

(C) H&E staining of mouse colons in DSS induced colitis. Scale bar is 100 μm.

(D) H&E staining of mouse colons in TNBS induced colitis. Scale bar is 100 μm.

Fig. S4. Microarray analysis of $EMSC_{ML}$ and $EMSC_{Sp\text{-}ML}$ at p3 and p8, and treated with or without IFNγ for 24 h, in comparison with the parental hESC

(A) Correlation coefficient between the seven microarray samples.

(B) Global gene expression profile of the seven microarray samples.

(C, D) Differentially expressed genes between $EMSC_{ML}$ and $EMSC_{Sp\text{-}ML}$ at p3 and p8.

Fig. S1

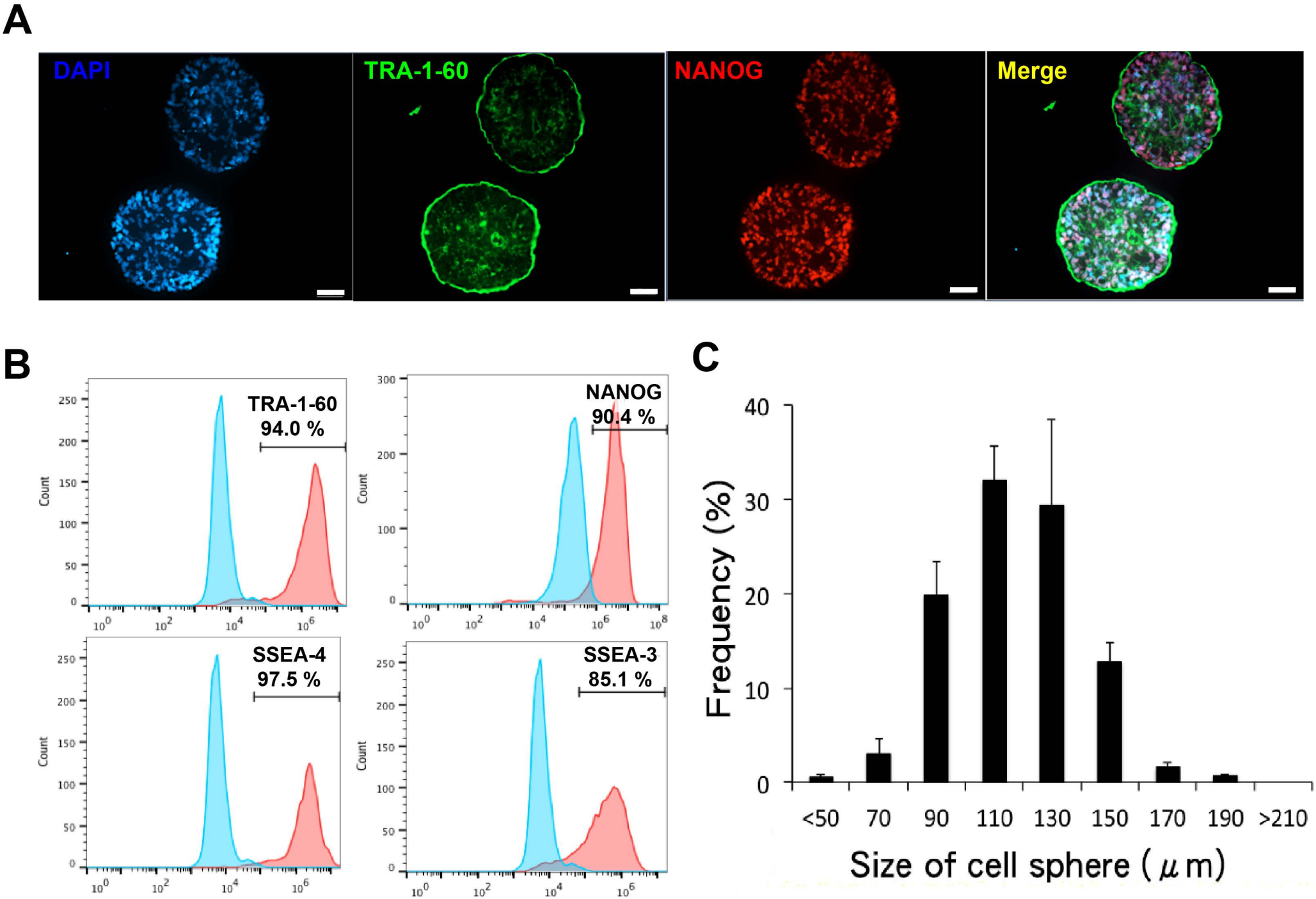

A
DAPI
TRA-1-60
NANOG
Merge
B
TRA-1-60
94.0 %
NANOG
90.4 %
SSEA-4
97.5 %
SSEA-3
85.1 %
Count
C
Frequency (%)
0
10
20
30
40
<50
70
90
110
130
150
170
190
>210
Size of cell sphere (μm)

Fig. S2

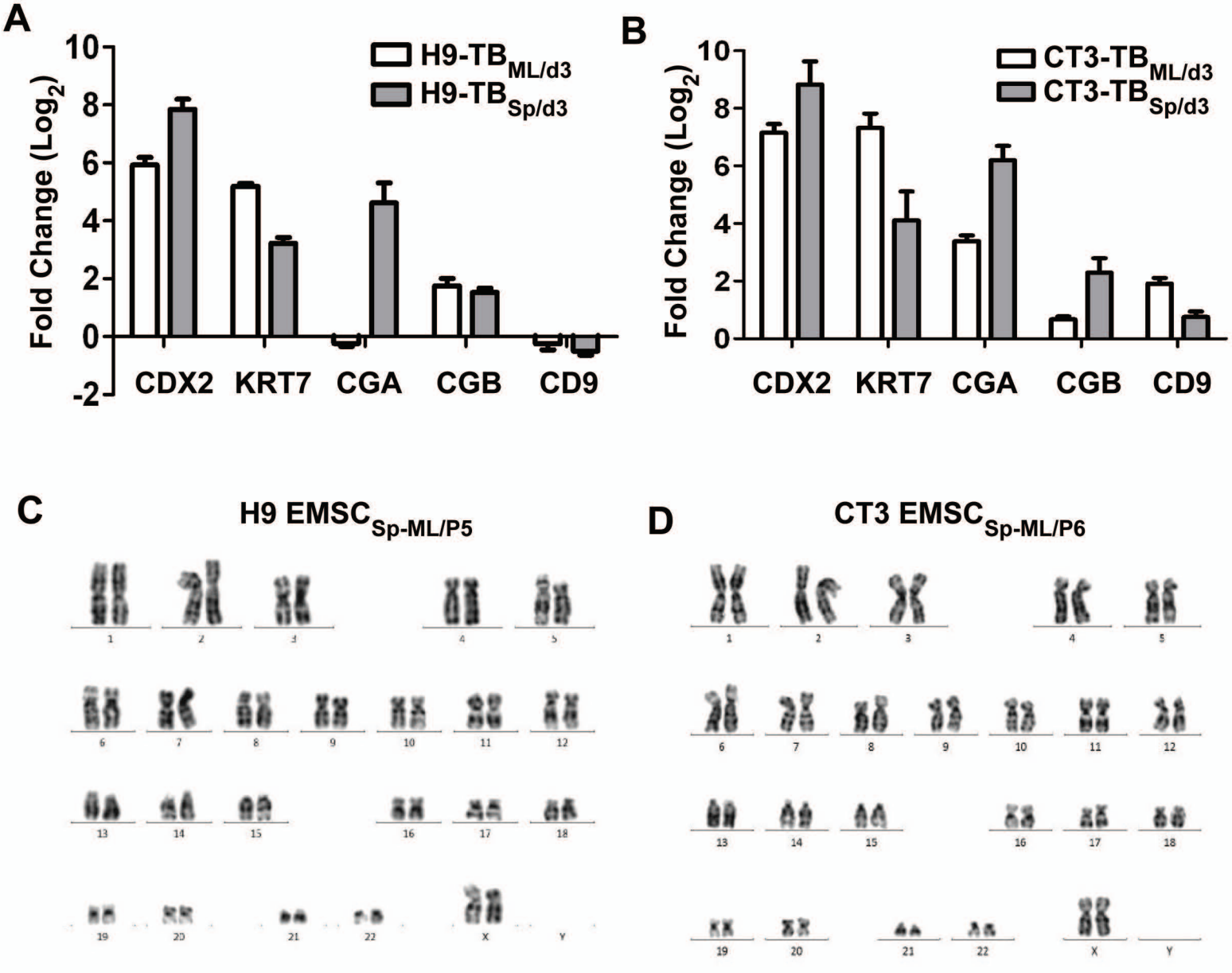

A
Fold Change (Log2)
10
8
6
4
2
0
-2
H9-TBML/d3
H9-TBSp/d3
CDX2
KRT7
CGA
CGB
CD9
B
Fold Change (Log2)
10
8
6
4
2
0
CT3-TBML/d3
CT3-TBSp/d3
CDX2
KRT7
CGA
CGB
CD9
C
H9 EMSCSp-ML/P5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
D
CT3 EMSCSp-ML/P6
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Fig. S3

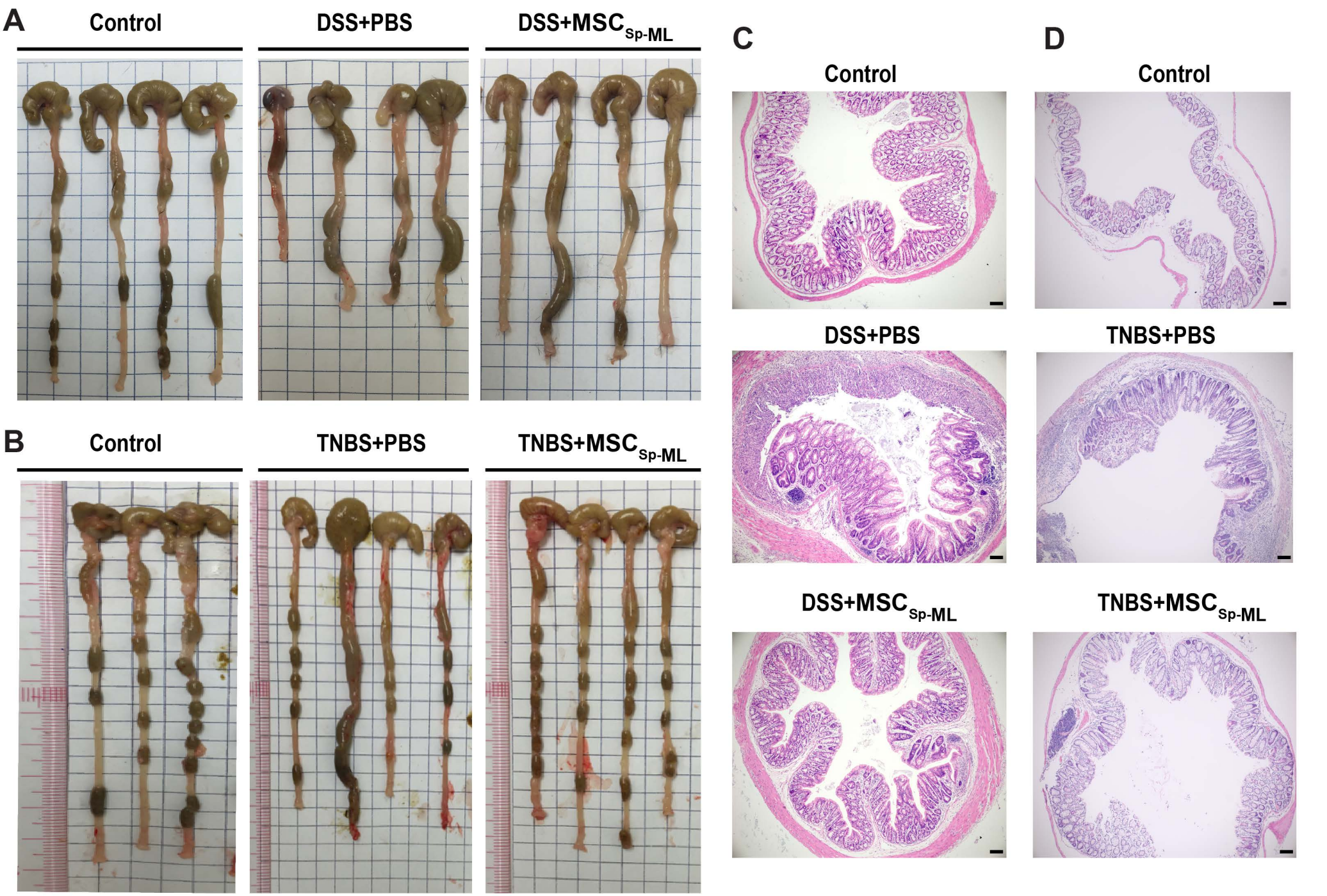
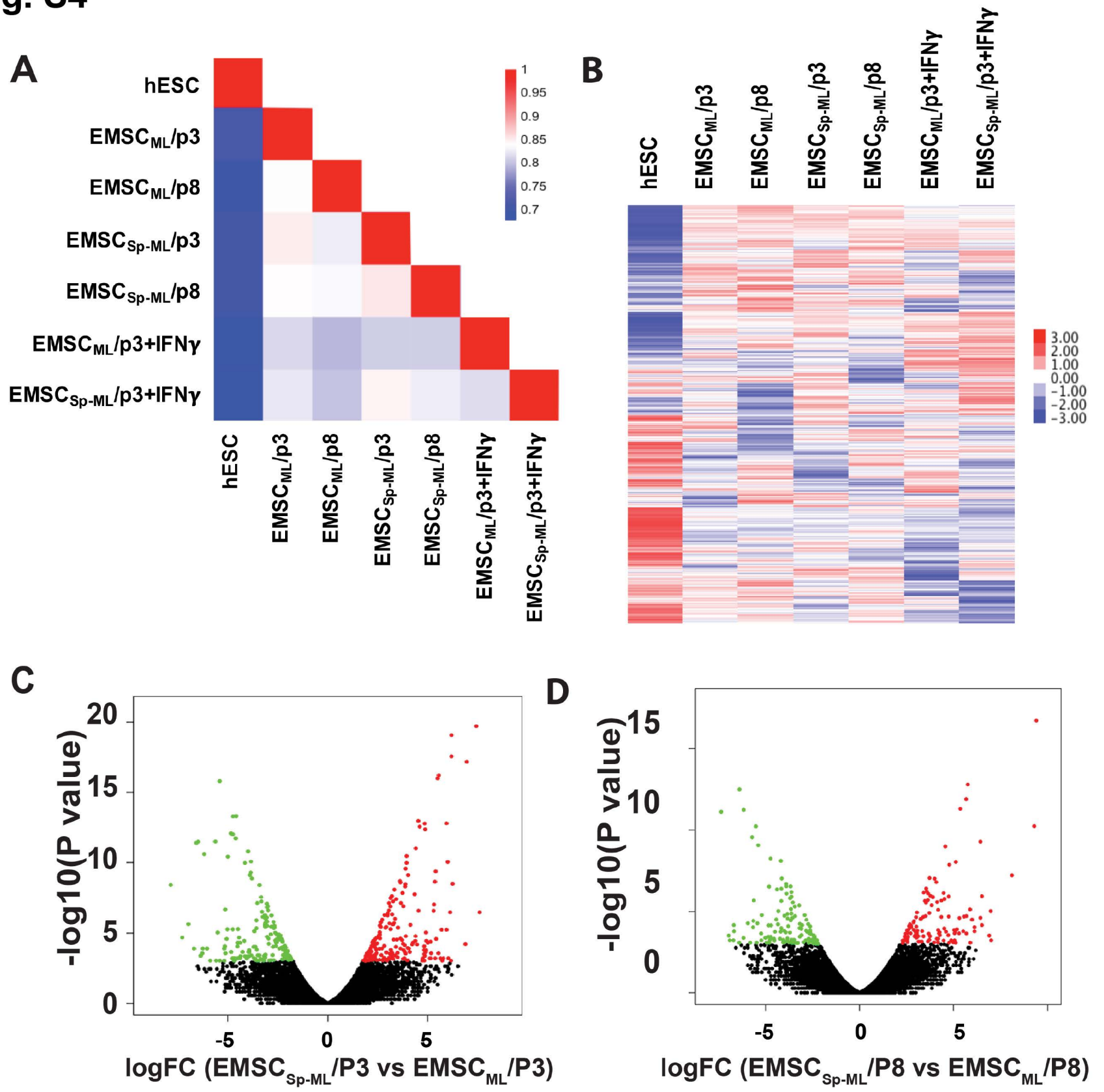

A
Control
DSS+PBS
DSS+MSC Sp-ML
B
Control
TNBS+PBS
TNBS+MSC Sp-ML
C
Control
DSS+PBS
DSS+MSC Sp-ML
D
Control
TNBS+PBS
TNBS+MSC Sp-ML

Fig. S4


A
hESC
EMSC$_{ML}$/p3
EMSC$_{ML}$/p8
EMSC$_{Sp-ML}$/p3
EMSC$_{Sp-ML}$/p8
EMSC$_{ML}$/p3+IFNγ
EMSC$_{Sp-ML}$/p3+IFNγ
1
0.95
0.9
0.85
0.8
0.75
0.7
B
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
C
-log10(P value)
20
15
10
5
0
-5
0
5
logFC (EMSC$_{Sp-ML}$/P3 vs EMSC$_{ML}$/P3)
D
-log10(P value)
15
10
5
0
-5
0
5
logFC (EMSC$_{Sp-ML}$/P8 vs EMSC$_{ML}$/P8)