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Supplemental Information

Do Pluripotent Stem Cells Exist in Adult Mice as Very Small Embryonic Stem Cells?

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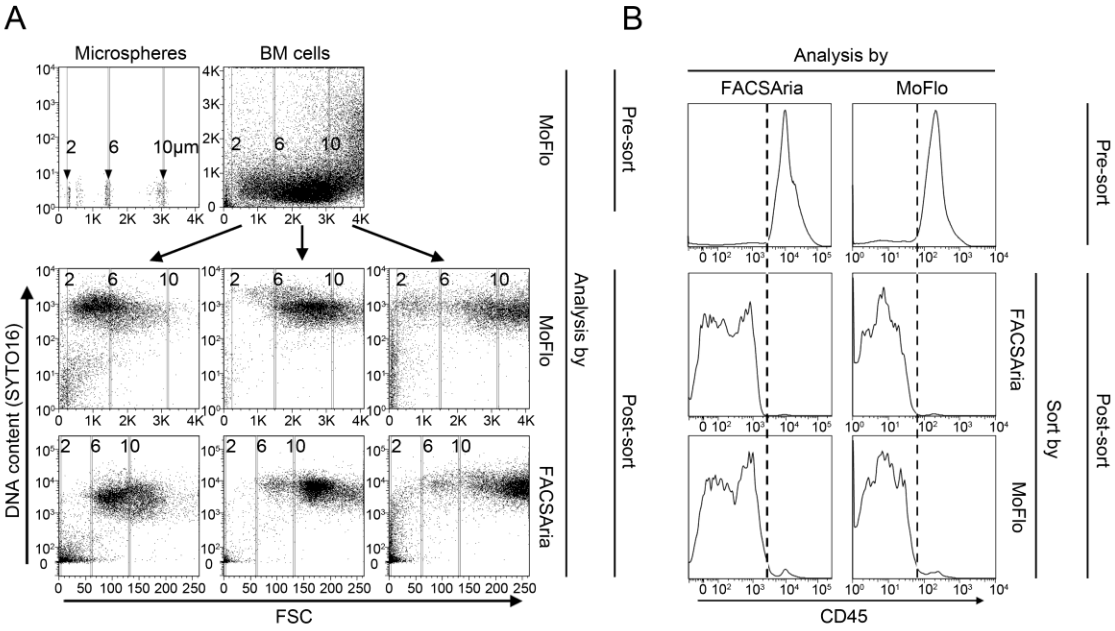
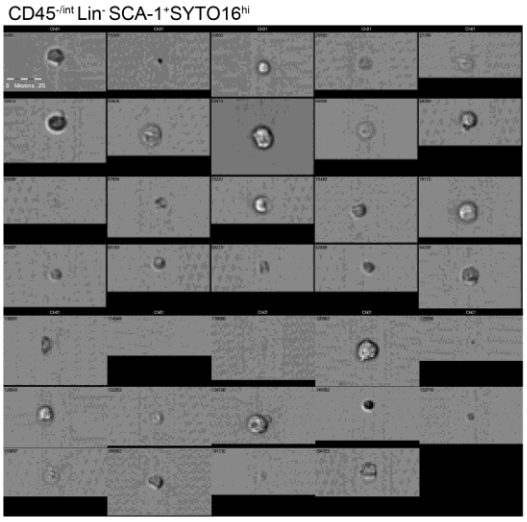
Figure S1


A
Microspheres
BM cells
2
6
10μm
10
MoFlo
FACSAria
Analysis by
DNA content (SYTO16)
FSC
B
Analysis by
FACSAria
MoFlo
Pre-sort
Post-sort
Sort by
CD45

C


CD45-/int Lin- SCA-1+SYTO16hi

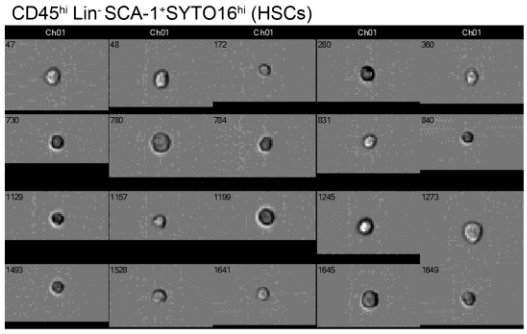

CD45hi Lin- SCA-1+SYTO16hi (HSCs)

Figure S1. Bone Marrow Cell Analysis with Different Flow Cytometers, Related to Figure 2. (A) Representative re-analysis data of sorted BM cells. A MoFlo machine was used to sort events by FSC into 2-6, 6-10, and >10 μm (expected-size) subgroups (upper). Each subgroup was re-analyzed with the MoFlo (middle) and FACSAria (lower). Vertical lines indicate the positions of 2-, 6-, and 10-μm-microspheres. (B) Representative re-analysis data of sorted $CD45^{-/int}$ cells. These cells were sorted in parallel by FACSAria and MoFlo, and then re-analyzed with both of these sorters. Dotted lines indicate the threshold between $CD45^{-/int}$ and $CD45^{hi}$. (C) Images of $CD45^{-/int}Lin^{-}SCA\text{-}1^{+}SYTO16^{hi}$ cells (candidate VSELs) and $CD45^{hi}Lin^{-}SCA\text{-}1^{+}SYTO16^{hi}$ cells (HSCs) captured by flow cytometry imaging. Shown here are 34 candidate VSELs and 20 HSCs, randomly chosen. The dotted bar in the top-left image indicates 20 μm.

Figure S2

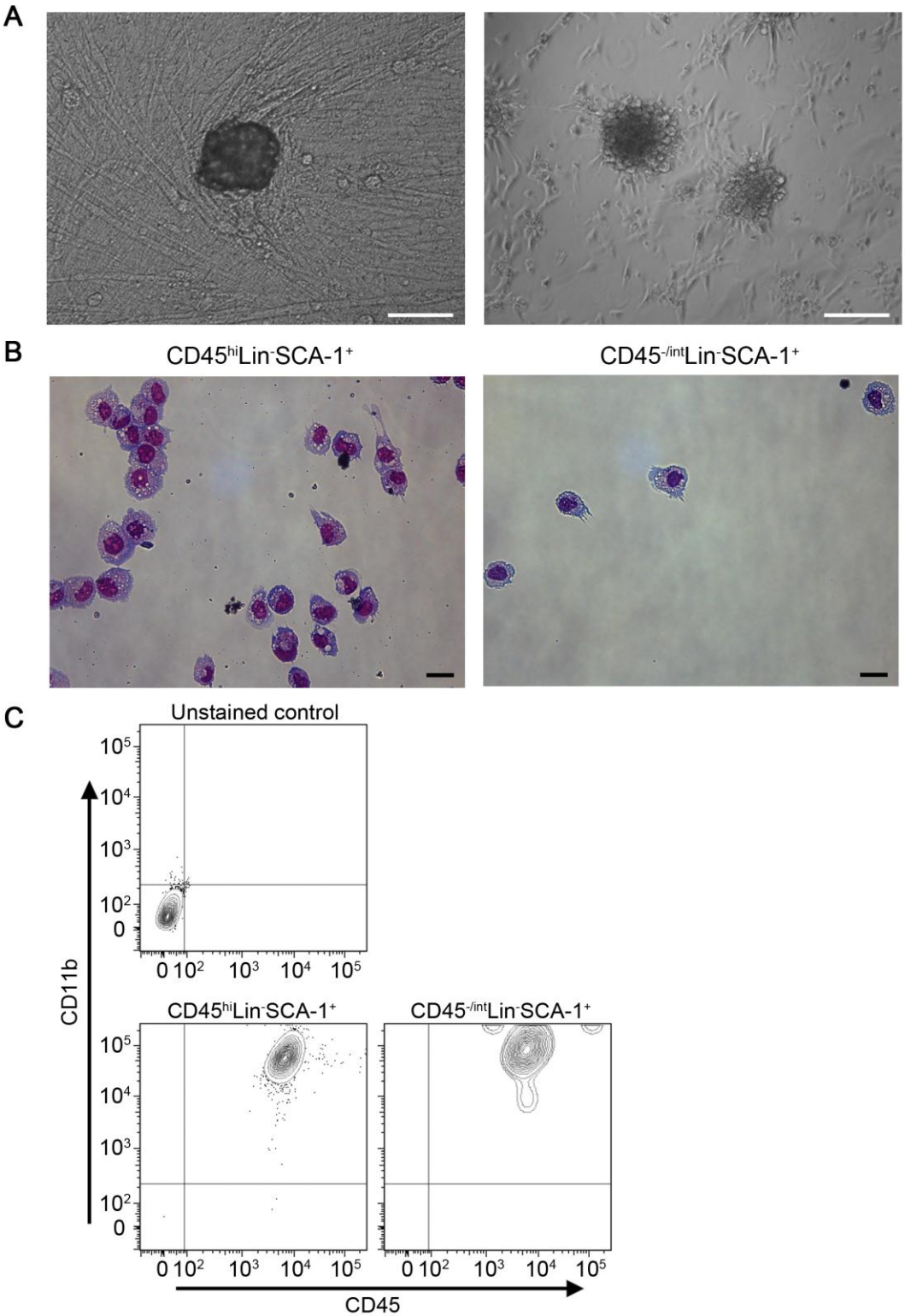

A
B
CD45hiLin-SCA-1+
CD45-/intLin-SCA-1+
C
Unstained control
CD11b
CD45
0 10² 10³ 10⁴ 10⁵

Figure S2. Co-culture with the Myoblast C2C12 Cell Line, Related to Figure 3. (A) Representative photographs of spontaneously aggregated C2C12 cells on day 10 of culture in 2% FCS. White scale bars, 100 μm. (B) Representative photographs of cells derived from $CD45^{hi}Lin^{-}SCA\text{-}1^{+}$ and $CD45^{-/int}Lin^{-}SCA\text{-}1^{+}$ fractions (May-Giemsa staining; scale bars, 15 μm). (C) FACS analysis of the expression of CD45 (hematopoietic lineage marker) and CD11b (macrophage marker) in proliferating cells derived from $CD45^{hi}Lin^{-}SCA\text{-}1^{+}$ (lower left) and $CD45^{-/int}Lin^{-}SCA\text{-}1^{+}$ (lower right) cells.

Figure S3

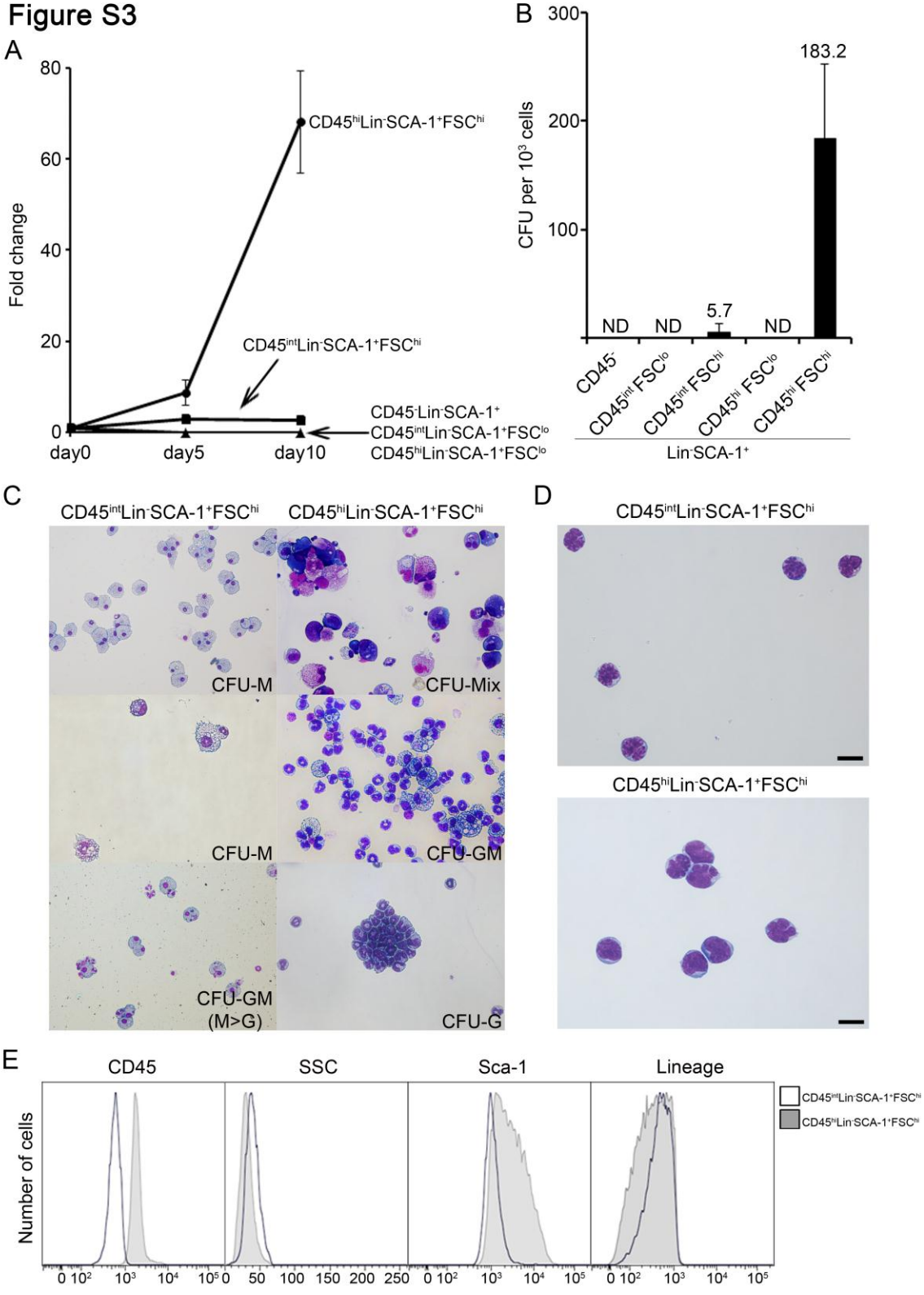

A
Fold change
day0
day5
day10
CD45hiLin-SCA-1+FSChi
CD45intLin-SCA-1+FSChi
CD45-Lin-SCA-1+
CD45intLin-SCA-1+FSClo
CD45hiLin-SCA-1+FSClo
B
CFU per 10^3 cells
183.2
5.7
ND
CD45-
CD45int FSClo
CD45int FSChi
CD45hi FSClo
CD45hi FSChi
Lin-SCA-1+
C
CD45intLin-SCA-1+FSChi
CD45hiLin-SCA-1+FSChi
CFU-M
CFU-Mix
CFU-M
CFU-GM
CFU-GM (M>G)
CFU-G
D
CD45intLin-SCA-1+FSChi
CD45hiLin-SCA-1+FSChi
E
CD45
SSC
Sca-1
Lineage
Number of cells

Figure S3. *In vitro* Assessment of Hematopoietic Lineage Potential among Candidate VSELs, Related to Figure 4. (A) Results of cell proliferation in a liquid culture. $Lin^-SCA\text{-}1^+$ cells were cultured in IMDM plus 10% FCS supplemented with 10 ng/ml of SCF, IL-3, and Flt3 ligand. Shown are the mean ± SD from three independent experiments. (B) Representative methylcellulose colony assays of FACS-purified populations. Hematopoietic colonies developed only from $CD45^{int}Lin^-SCA\text{-}1^+FSC^{hi}$ and $CD45^{hi}Lin^-SCA\text{-}1^+FSC^{hi}$ cells. Data shown here are mean ± SD in five independent experiments. CFU, colony-forming unit; ND, not detected. (C) Representative photographs of cell components of colonies derived from each $Lin^-SCA\text{-}1^+$ fraction indicated. (May-Giemsa staining). CFU-M, CFU-macrophage; CFU-Mix, CFU-erythroid and myeloid cells; CFU-GM, CFU-granulocytes/macrophages; CFU-G, CFU-granulocytes. Scale Bar, 20 μm. (D) Morphological and (E) immunophenotypical comparisons of freshly-isolated $CD45^{int}Lin^-SCA\text{-}1^+FSC^{hi}$ and $CD45^{hi}Lin^-SCA\text{-}1^+FSC^{hi}$ cells. Scale bars, 10 μm. These data were similar in three independent experiments.

Figure S4

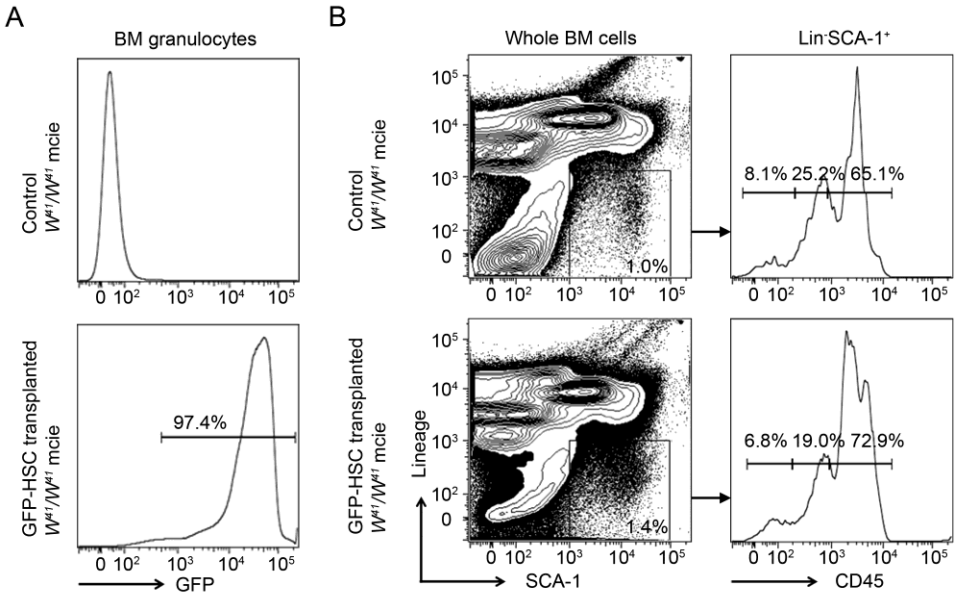


Figure S4. Analysis of Bone Marrow from Mice with EGFP$^+$-HSC Transplants, Related to Figure 4. (A) FACS analysis of GFP$^+$ cells in BM granulocytes from control mice (upper) and mice, 3 months after they were irradiated and received intravenous transplants of 10^2 long-term HSCs (lower). (B) A representative histogram of CD45 expression within the BM Lin$^-$SCA-1$^+$ fraction from control mice (right upper) and transplant-recipient mice (right lower) 3 months after transplantation. Data were similar in three independent experiments.

Table S1. Hierarchy of FACS gating and frequency of populations, Related to Figure 1.

Population	Frequency (% of lysis-buffer-treated BM, mean ± SD, n=22)
FSC & SSC gated	37.28 ± 2.79
SYTOX-Blue^{-}	32.94 ± 2.68
Lin^{-}SCA-1^{+}	0.489 ± 0.095
CD45^{-}	0.027 ± 0.013
FSC^{lo}	0.024 ± 0.009
FSC^{hi}	0.001 ± 0.001
CD45^{int}	0.070 ± 0.027
FSC^{lo}	0.041 ± 0.017
FSC^{hi}	0.019 ± 0.010
CD45^{hi}	0.385 ± 0.011
FSC^{lo}	0.023 ± 0.010
FSC^{hi}	0.331 ± 0.058

FSC^{lo}, <10 μm; FSC^{hi}, >10 μm.

Table S2. Age of Mice and Purification strategy for mouse VSEL used by Ratajczak group, Related to Figure 1.

Year	Authors	PMID	Age of mice used	Purification Strategy
2006	Kucia M et al.	16498386	3–4 wk & 1 yr	$CD45^-Lin^-SCA\text{-}1^+$
2008	Zuba-Surma EK et al.	18031297	4–8 wk	Size-beads & $CD45^-Lin^-SCA\text{-}1^+7\text{-}AAD^-$
2008	Dawn B et al.	18420834	4–6 wk	Size-beads & $CD45^-Lin^-SCA\text{-}1^+$
2008	Zuba-Surma EK et al.	18430437	6 wk & 15 wk	Size-beads & $CD45^-Lin^-SCA\text{-}1^+$
2008	Kucia M et al.	18511604	4-6 wk & 1 yr	$CD45^-Lin^-SCA\text{-}1^+$
2008	Zuba-Surma EK et al.	18951465	4–8 wk	Size-beads & $CD45^-Lin^-SCA\text{-}1^+7\text{-}AAD^-$
2009	Shin DM et al.	19641521	4–5 wk	Size by lymphoid cells & $CD45^-Lin^-SCA\text{-}1^+$
2010	Shin DM et al.	20508611	4–5 wk	$CD45^-Lin^-SCA\text{-}1^+$
2010	Wojakowski W et al.	20596650	4–8 wk	Size-beads & $CD45^-Lin^-SCA\text{-}1^+$
2011	Zuba-Surma EK et al.	20629987	4–6 wk	Size by lymphoid cells & $CD45^-Lin^-SCA\text{-}1^+$
2011	Ratajczak J et al.	21034791	4-8 wk	Size by lymphoid cells & $CD45^-Lin^-SCA\text{-}1^+7\text{-}AAD^-$
2012	Shin DM et al.	22023227	4–5 wk	$CD45^-Lin^-SCA\text{-}1^+$

Table S3. Primer sequences for *Oct4*, Related to Figure 3.

Pair		Sequence	Amplicon size	Reference
1	Sense	5'-CACGAGTGGAAAGCAACTCA	246bp	Toyooka et at., 2008
	Antisense	5'-AGATGGTGGTCTGGCTGAAC		
2	Sense	5'-ACCTTCAGGAGATATGCAAATCG	70bp	Kucia et al. 2006a
	Antisense	5'-TTCTCAATGCTAGTTCGCTTTCTCT		
3	Sense	5'-AGTTGGCGTGGAGACTTTGC	160bp	Liu et at. 2009
	Antisense	5'-CAGGGCTTTCATGTCCTGG		
4	Sense	5'-TACAGCAGATCACTCACATCG	133bp	Mm.PT.51.7439100.g Integrated DNA Technologies
	Antisense	5'-GTAGCCTCATACTCTTCTCGTTG		
	Probe	5'-ACCACATCCTTCTCTAGCCCAAGC		