(Takara). All primer pairs did not show cross-reactivity to rat genes as described previously [2]. hBNP primer sequence (146 bp): sense GCA AAA TGG TCC TCT ACA CC; antisense CAG GAC TTC CTC TTA ATG CC; hcTn-I primer sequence (218 bp): sense AAT TGC AGC TGA AGA CTC TG; antisense GAC TTT TGC CTC TAT GTC GT; hMHC-β primer sequence (214 bp): sense GCT AAA GGT CAA GGC CTA CA; antisense GCA GAT CAA GAT CTG GCA AA; hNkx 2.5 primer sequence (205 bp): sense GAG AGT TTG TGG CGG CGA TT; antisense CGA CGG CGA GAT AGC AAA GG; hSMA primer sequence (485 bp): sense TCT GGA CGC ACA ACT GGC ATC GT; antisense TAC ATA GTG GTG CCC CCT GAT AG; hsm22-α primer sequence (468 bp): sense CGG CTG GTG GAG TGG ATC ATA; antisense CCC TCT GTT GCT GCC CAT CTG A; hCD31 primer sequence (469 bp): sense AGG TCA AGC AGC ATC GTG GTC AAC AT; antisense TTG TCT TTG AAT ACC GCA G; hCD34 primer sequence (380 bp): sense AAT GAG GCC ACA ACA AAC ATC ACA; antisense CTG TCC TTC TTA AAC TCC GCA CAG C; hKDR primer sequence (468 bp): sense CAA ATG TGA AGC GGT CAA CAA AGT T; antisense ATG CTT TCC CCA ATA CTT GTC GTC T; heNOS primer sequence (456 bp): sense AAC CAC ATC AAG TAT GCC ACC AAC C; antisense CGT GCC GAT CTC AGT GCT CA; hGAPDH primer sequence (596 bp): sense CTG ATG CCC CCA TGT TCG TC; antisense CAC CCT GTT GCT GTA GCC AAA TTC G; Total GAPDH primer sequence (320 bp): sense GTG CCA GCC TCA TGT TCG TC; antisense CGC CAG TGT ACT CCA CGA CAT TTC G

Real-time PCR for analyzing the human-specific gene expression of angiogenic growth factors and the cardiomyogenic and vasculogenic lineage markers in rat ischemic myocardium following USSC transplantation

To quantify the mRNA expression of human-specific genes of angiogenic factors and the cardiomyogenic and vasculogenic markers in rat ischemic myocardium following human cell transplantation, real-time PCR was performed on an ABI PRISM 7000 sequence detector (Applied Biosystems) as described previously [11]. Total RNA was prepared as described above and the expression of the related genes was quantified using the SYBR green reagent (2x SYBR Green Supermix; Bio-Rad, Hercules, CA) following the instructions of the manufacturer on a Bio-Rad Cycler. PCR of anigiogenic factors was performed in multiplicate in optimized conditions: 95°C denatured for 3 minutes, followed by 40 cycles of 45 seconds at 94°C, 45 seconds at 55°C, and 45 seconds at 72°C. PCR of human-specific cardiomyogenic and vasculogenic genes was also performed in multiplicate in optimized conditions: 95°C denatured for 10 minutes, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Primer for human-specific angiogenic factors (VEGF, bFGF, HGF and SDF-1) and human-specific CMC, SMC and EC markers (hcTn-I, hSM22α, and hKDR) were confirmed not to cross-react with rat genes by using rat heart samples. (data not shown).

Primers: human VEGF (hVEGF), forward, 5-TCTCCCTGATCGGTGACAGT-3,

reverse, 5-GGCAGAGCTGAGTGTTAGCA-3; human bFGF (hbFGF), forward, 5-TTTCTAGCTTCCATCCTTTCTCC-3, reverse, 5–AGTTACCAGCTCCCCCAAAA-3; human HGF (hHGF), forward, 5-GCACCCACCAATACAACTGTC-3, reverse, 5-TGACTCTCCAGTAGTTGTCTTAGGATT-3; human SDF-1 (hSDF-1), forward, 5-CCTCCCCCAACCTTAGATGT-3, reverse, 5-CAGACGATCACACCATGGAA; human GAPDH (hGAPDH), forward, 5-CACTGAATCTCCCCTCCTCA-3, reverse, 5-TCCCCTCTTCAAGGGGTCTA-3; hcTn-I, forward: 5-CGGAGAGTGAGGATCTCTGC-3, reverse: 5-TCGGTGTCCTCCTTCTTCAC-3; hSM22α, forward: 5-AAGAAAGCGCAGGAGCATAA-3, reverse: 5-AAGGCCAATGACATGCTTTC-3; and hKDR, forward: 5-TTTTTGCCCTTGTTCTGTCC-3, reverse: 5-TCATTGTTCCCAGCATTTCA-3.

No other products were amplified because melting curves showed only one peak in each primer pair. Fluorescence signals were measured over 40 PCR cycles. The cycle number at which the signals crossed a threshold set within the logarithmic phase was recorded. For quantification, we evaluated the difference in cycle threshold between the AP-treated group and vehicle control of each gene. The efficiency of amplification of each pair of primers was determined by serial dilutions of templates and all were 0.9. Each sample was normalized with the loading references of hGAPDH. *Ct* values used were the means of the triplicate replicates. Experiments were repeated at least three times.

Supplementary Results

FISH Analysis to Assess the Mechanism of Cardiac Regeneration Following USSC Transplantation

To ensure whether cardiac repair occurred though cell fusion or not, we performed FISH with human genome and rat genome probes using tissue samples 28 days post MI and USSC transplantation. The specificity of the probes was tested in tissues of normal rat heart and rat heart immediately after human cell transplantation. We confirmed that these two probes did not cross-react with the other species cells (data not shown). The FISH analysis revealed that most of the USSC-derived cells expressing human genome were not paired with rat genome, indicating very few incidence of cell fusion between transplanted USSCs and recipient cells (cell fusion ratio, 1.5±0.8%) **(Supplementary Figure VII)**.

These findings indicate that multi-lineage differentiation without cell fusion may be a major contributing factor to the process of cardiac repair following transplantation of USSCs.

Human-Specific Gene expression of Cardiomyogenic, Smooth Muscle and Endothelial Lineage Markers in Rat Ischemic Myocardium Following USSC Transplantation

To further confirm the immunohistochemical results regarding cardiomyogenesis

and vasculogenesis using a molecular approach, we performed RT-PCR with rat ischemic myocardium from each of the different transplant groups using human-specific primers for BNP, cTn-I, MHC-β and Nkx 2.5 as human CMC lineage markers, human-specific primers for sm22α and SMA as human SMC markers and human-specific primers for CD31, eNOS and KDR as human EC markers. The RT-PCR analysis revealed the expression of human-specific cardiomyogenic, arteriogenic and vasculogenic genes in rat ischemic myocardium 28 days following USSC transplantation. Notably, the gene expression profile of all markers, except hSMA and sm22α, were not detected in USSCs before transplantation, but were significantly augmented in ischemic myocardium after USSC transplantation. In addition, to quantify the expression of cariomyogenic and vasculogenic genes, we performed real-time PCR with rat ischemic myocardium, using human-specific primer cTn-I, human-specific primer sm22α and human-specific primer KDR. The gene expression profile of all markers was significantly augmented in ischemic myocardium after USSC transplantation compared with Fbr or PBS treatment **(Supplementary Figure VIII)**.

Transplanted USSCs highly express the angiogenic growth factors

We analyzed by real-time RT-PCR whether transplanted USSCs may express the angiogenic cytokines such as human VEGF (hVEGF), human bFGF (hbFGF), human HGF (hHGF) and human SDF-1 (hSDF-1) at day 5. Expression of hVEGF, hbFGF, hSDF-1 and

hHGF in the infarct and peri-infarct borders was confirmed at higher levels in the USSC-transplanted animals than the Fbr or PBS-treated groups ($P<0.05$) **(Supplementary Figure IX)**. Thus, transplanted USSCs may enhance myocardial angiogenesis by overexpressing hVEGF, hbFGF, hHGF and hSDF-1.

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Supplementary Figure Legends

Supplementary Figure I

Histological evaluation of myocardial neovascularization after myocardial infarction (MI).

a: Representative histochemical staining for isolectin B4 in each group at day 28 (x200).

b: Capillary density in rats receiving USSCs, Fbr or PBS at day 28. *, P<0.05; **, P<0.01

(n=10 in all groups).

Supplementary Figure II

Left ventricular (LV) functional evaluation by echocardiography and a micromanometer-tipped conductance catheter.

a: Representative recording of M-mode echocardiography 28 days after cell or PBS administration. Lateral wall motion was well preserved following USSC transplantation (arrow: endocardium in the lateral wall).

b: Change in echocardiographic parameters during 23 days (between day 5 and day 28 after cell transplantation). FS, fractional shortening; RWMS, regional wall motion score. *, $P<0.05$; **, $P<0.01$ (n=10 in all groups).

c: Invasive hemodynamic parameters following USSC, Fbr or PBS administration at day 28. + dP/dt and -dP/dt, Maximum and minimum derivative of LV pressure; EF, ejection fraction; LVEDP, LV end-diastolic pressure. *, $P<0.05$; **, $P<0.01$. (n=10 in each group).

Supplementary Figure III

Representative immunofluorescent staining for immature cardiac markers and HMA in HD group at day 5 or 28.

a-d: Representative double immunofluorescent staining for for Nkx2.5 and HMA in HD

group at day 5 (x 400). *a,* Nkx2.5; *b*, HMA; *c*, DAPI; *d*, merge.

e-h: Representative double immunofluorescent staining for for GATA4 and HMA in HD group at day 5 (x 400). *e,* GATA4; *f*, HMA; *g*, DAPI; *h*, merge.

i-l: Representative double immunofluorescent staining for for cTn-I and HMA in HD group at day 28 (x 400). *i*, HMA; *j*, cTn-I; *k*, DAPI; *l*, merge.

Supplementary Figure IV

Histological evaluation of human CMC, EC and SMC development in rat ischemic myocardium at day 28.

a-e: Representative double immunofluorescent staining for cTn-I and human nuclear antigen (HNA) in HD USSC group at day 28. Human CMCs were identified as double-positive cells for cTn-I (green) and HNA (red). *a*, merge, x100; *b*, cTn-I, x400; *c*, HNA, x400; *d*, DAPI, x400; *e*, merge x400. White arrows show nuclei of human CMCs.

f-j: Representative double immunofluorescent staining for vWF (red) and HNA (green) in HD USSC group at day 28. Human ECs were identified as double positive cells for vWF and HNA. *f*, merge, x100; *g*, vWF, x400; *h*, HNA, x400; *i*, DAPI, x400; *j*, merge, x400.

k-o: Representative double immunofluorescent staining for smooth muscle actin (green) and HLA-ABC (red) in HD group at day 28. Human SMCs were identified as double

positive cells for SMA and HLA-ABC. *k*, merge, x100; *l*, SMA, x400; *m*, HLA-ABC, x400; *n*, DAPI, x400; *o*, merge, x400.

Supplementary Figure V

a-d: Representative double immunohistochemistry for HNA (red) and cTn-I (green) 4 weeks after MI in each group.

e: Percent ratio of human CMCs/ total HNA^+ cells and that of human CMCs/ total CMCs in each group. *, P<0.05; **, P<0.01.

Supplementary Figure VI

Histological evaluation of regenerative and resident CMC proliferation in rat ischemic myocardium at day 7

a-d: Representative double immunofluorescent staining for cTn-I and Ki67 at day 7 in PBS (*a*), Fbr (*b*), LD (*c*) and HD (*d*) groups (x100). White arrows show nuclei of the double-positive cells indicating the proliferative CMCs in the ischemic myocardium.

e: Density of the proliferative CMCs at day 7 was dose-dependently increased in rat ischemic myocardium following USSC transplantation. **, P<0.01. (n=10 in all groups).

Supplementary Figure VII

Quantification of cell fusion vs differentiation by FISH analysis

a- f: Representative double FISH for rat genome (red) and human genome (green) in HD group at day 28. Fused cells were identified as double positive cells for rat genome and human genome (x400). *a*, rat genome; *b*, DAPI; *c*, human genome; *d*, merge of *a*, *b* and *c*, (x400); *e*, merge of *a* and *c* , x400; *f*, Higher magnification (x 800?) of the square in the image *d*. Arrrow, fused nucleus; arrowhead, human cell-derived nucleus without cell fusion.

Supplementary Figure VIII

Molecular analyses of human-specific gene expression in USSCs pre transplantation and in LV tissue samples 4 weeks after the infusion of cells or PBS.

a: RT-PCR analysis for human-specific genes of CMC, SMC and EC lineage markers in Fbrs and USSCs before transplantation. lane 1, human heart (positive control); lane 2, Fbrs; lane 3, USSCs.

b: RT-PCR analysis to evaluate expression of human-specific genes of CMC (hBNP, hcTn-I, hMHC-β and hNkx 2.5), SMC (hsm22α and hSMA) and EC (hKDR, heNOS and hCD31) lineages in rat ischemic myocardium at day 28. Human specific genes of CMC, SMC and EC markers were dose-dependently expressed in USSC-treated animals. lane 1, human heart (positive control); lane 2, PBS group; lane 3, Fbr group; lane 4, LD group; lane 5, HD group.

c: Real-time PCR for quantification of the expression of human-specific genes of CMC

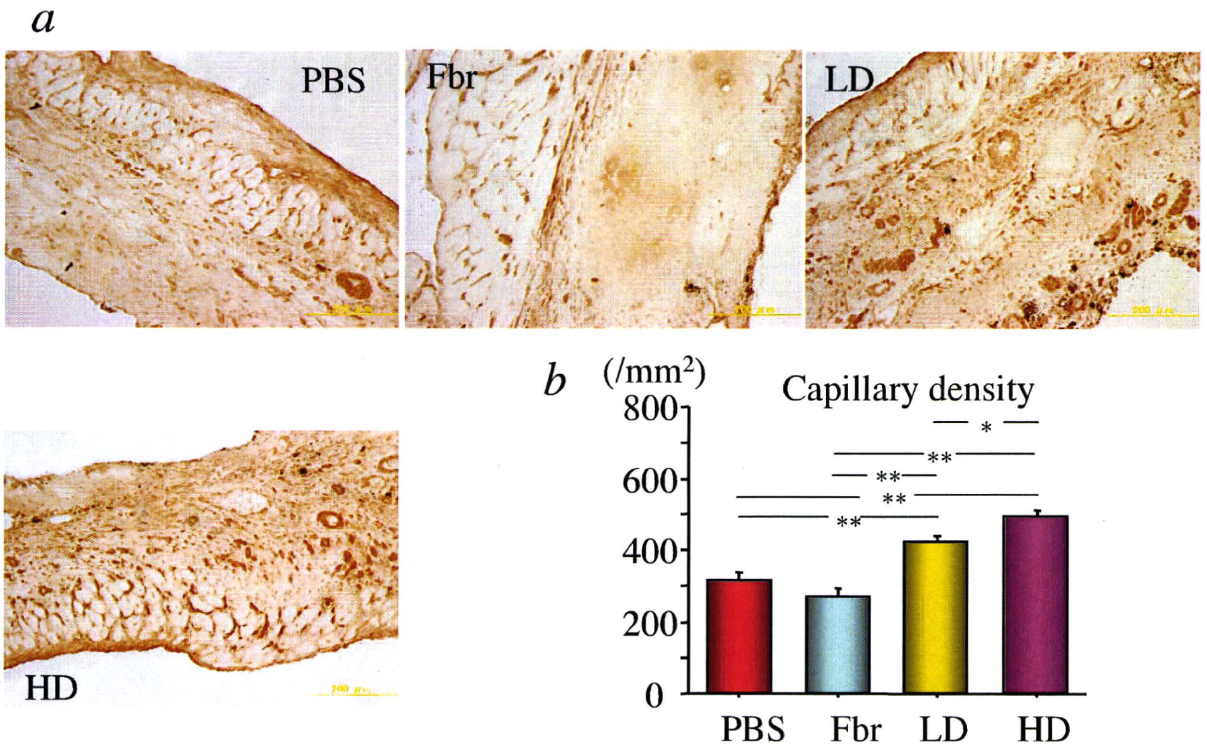
(cTn-I), SMC (sm22α) and EC (KDR) lineage markers in the rat ischemic myocardium at day 28. The human-specific cardiomyogenic and vasculogenic gene expression was significantly enhanced in USSC-transplanted groups compared with Fbr and PBS-treated groups. **, P<0.01.

Supplementary Figure IX

Quantification of the human-specific angiogenic gene expression in the rat ischemic myocardium.

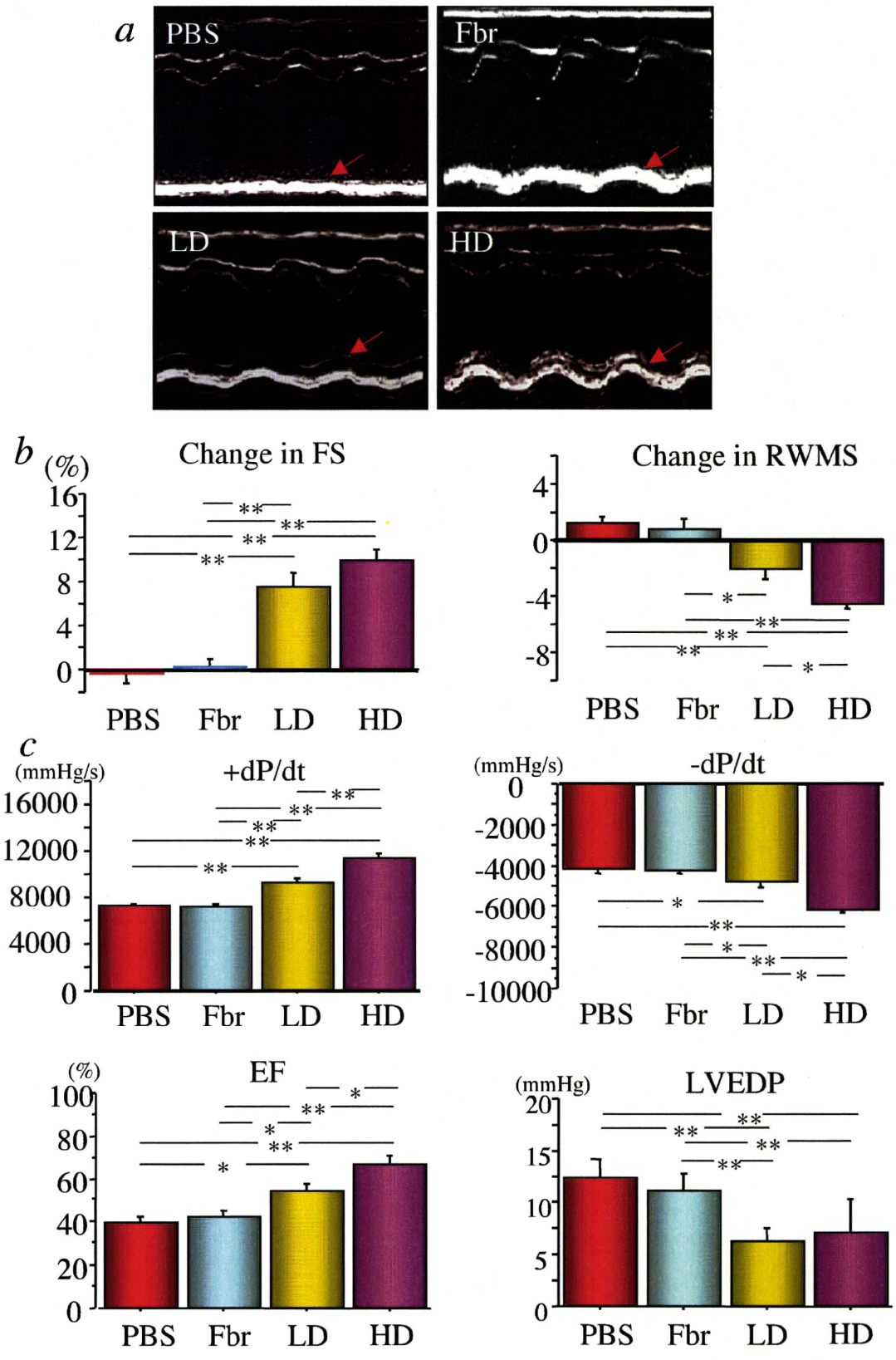
a-d: Real-time PCR for hVEGF (*a*), hbFGF (*b*), hHGF (*c*) and hSDF-1 (*d*) in infarct and peri-infarct areas. The human-specific angiogenic gene expression was significantly enhanced in USSC-transplanted groups compared with Fbr and PBS groups. *, P<0.05; **, P<0.01.

Supplemental Material



Supplementary Figure I

Supplemental Material



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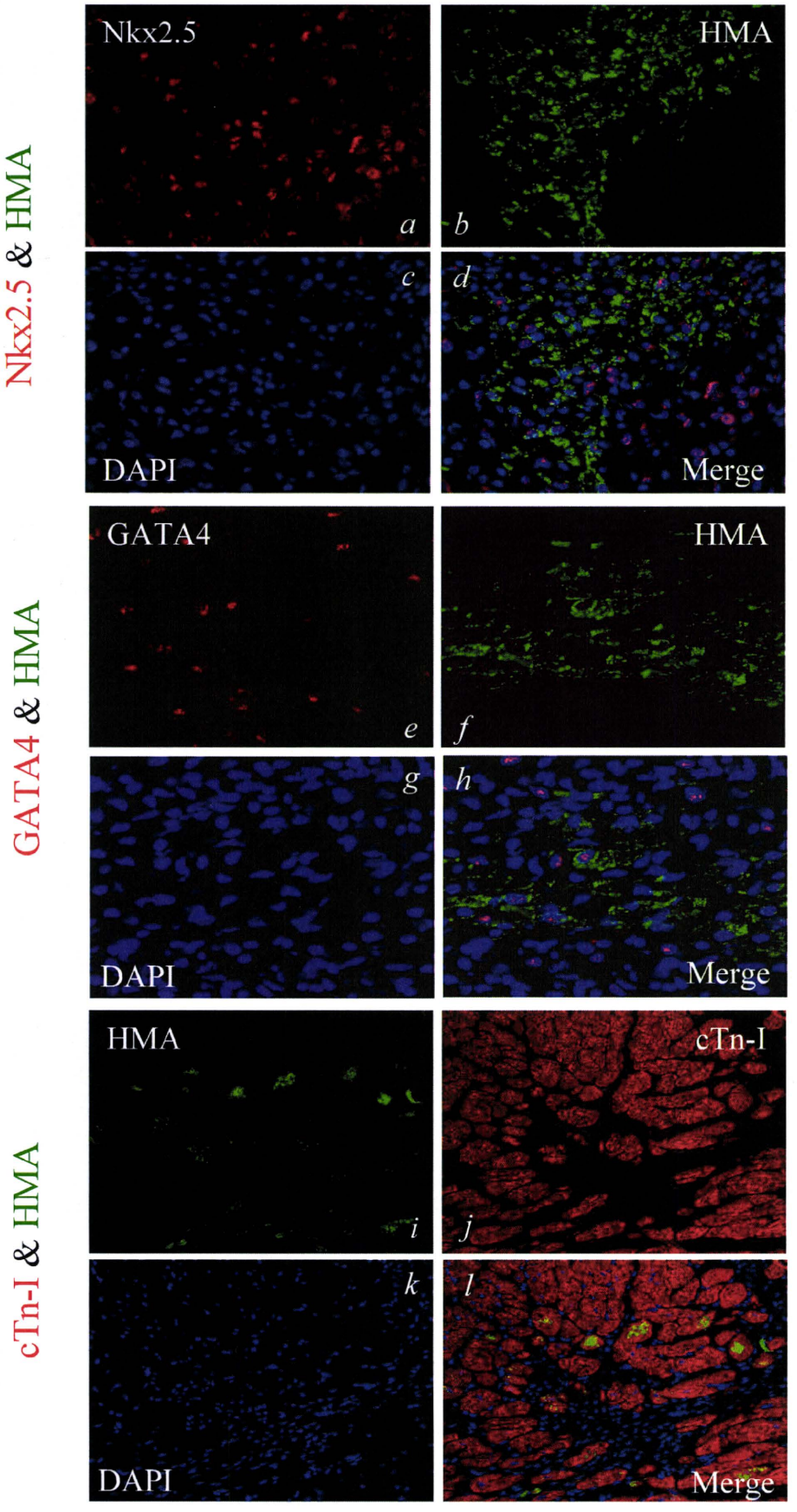

Supplementary Figure III

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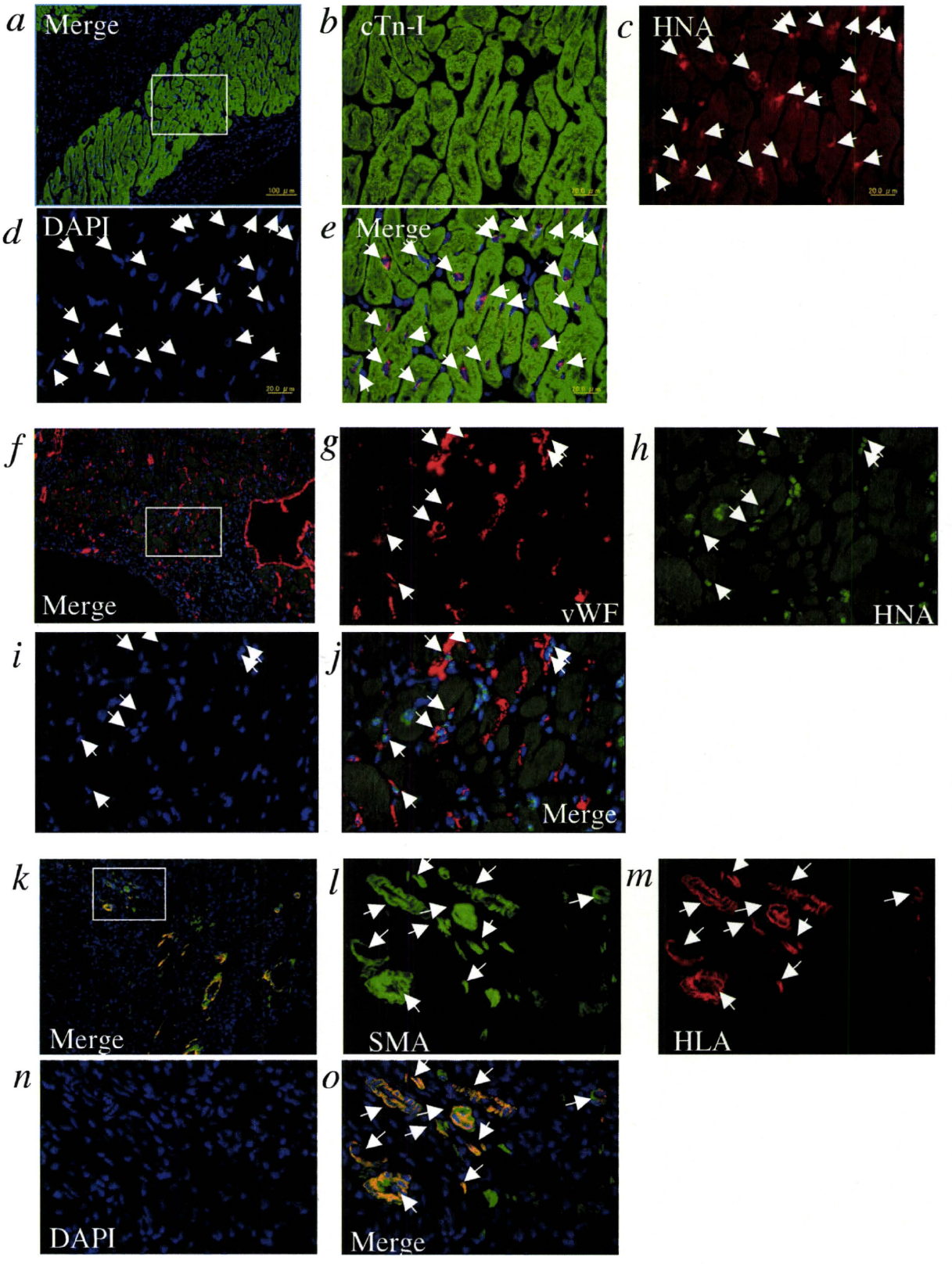

Supplementary Figure IV

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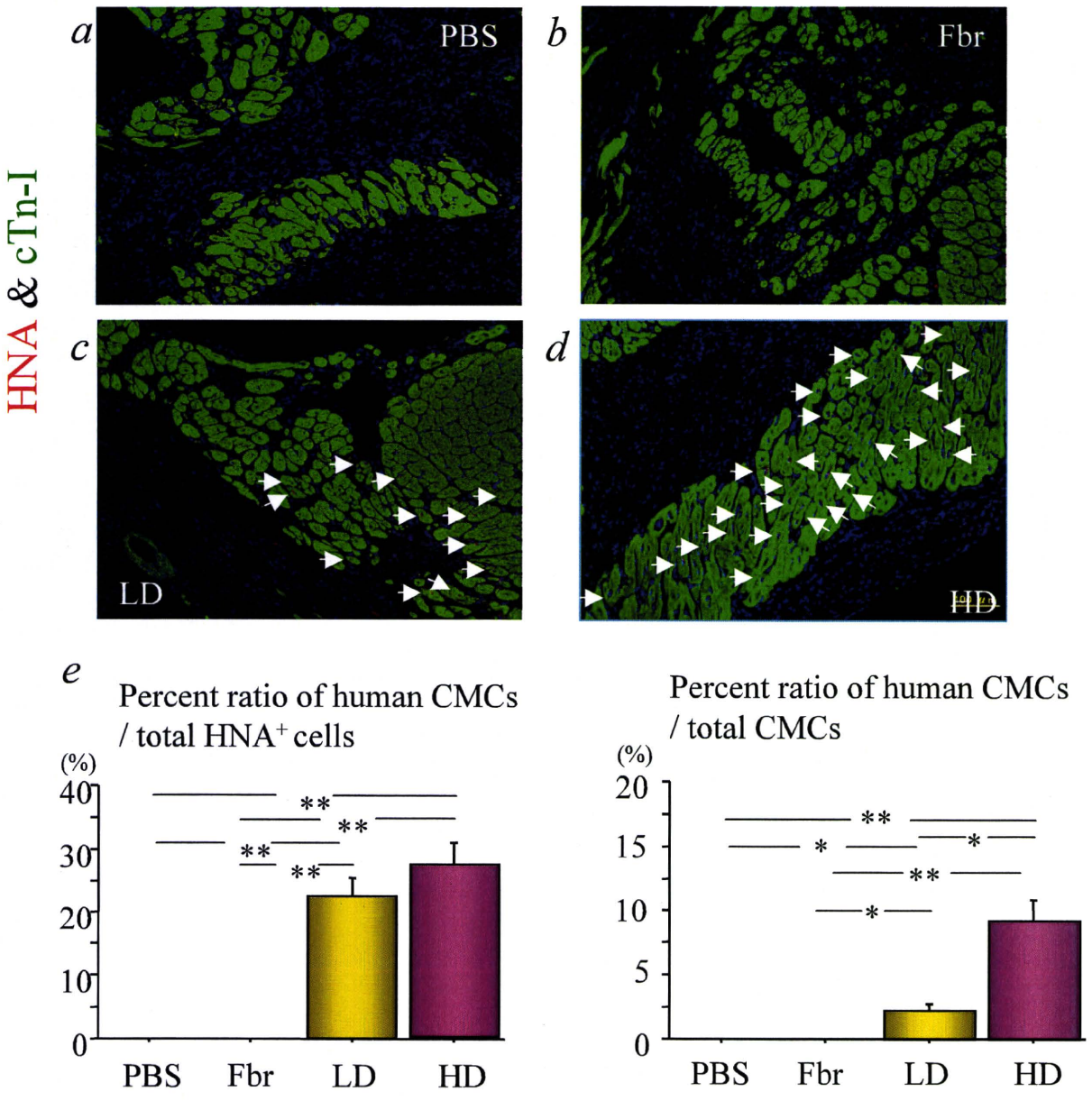

Supplementary Figure V

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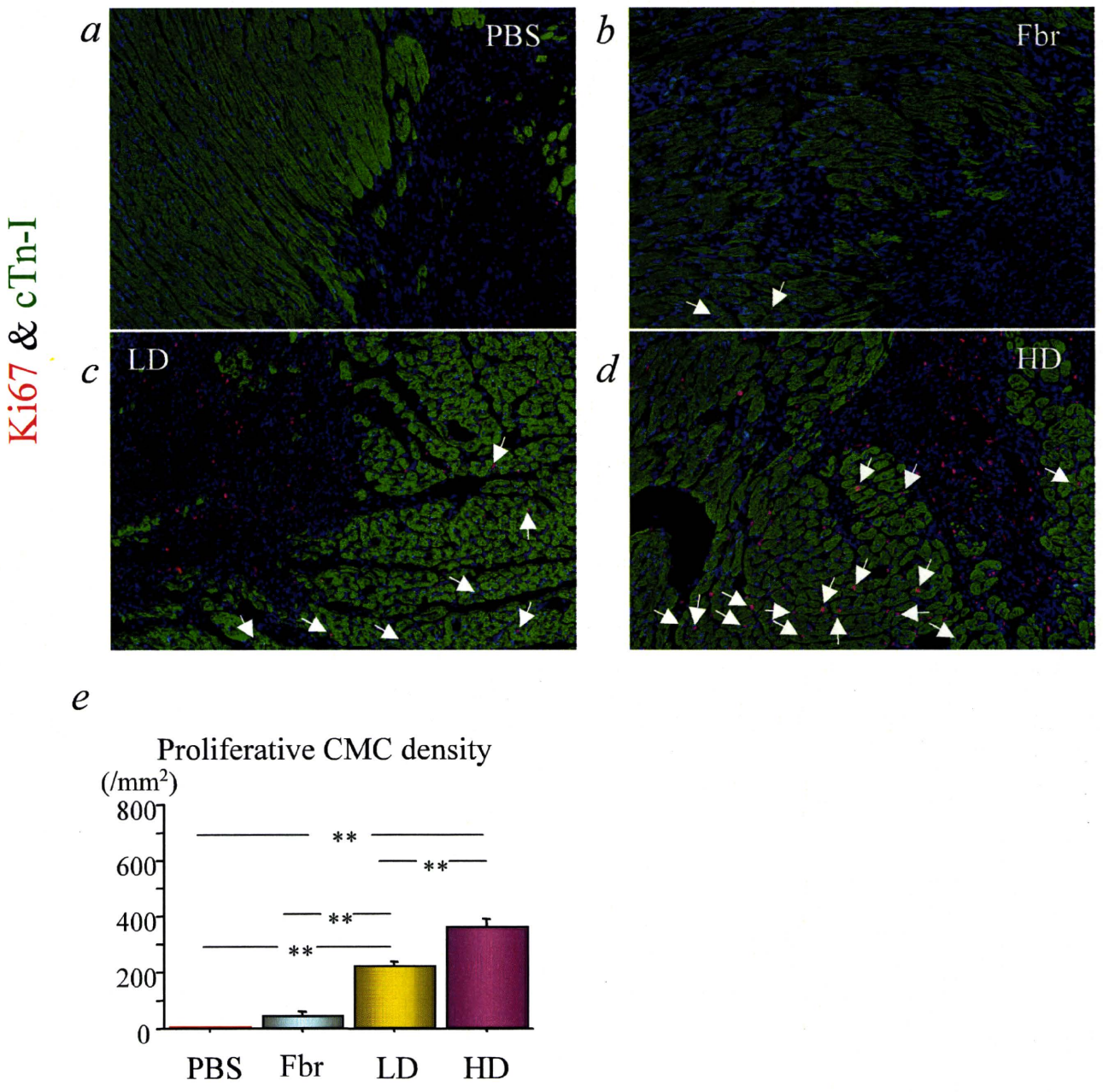
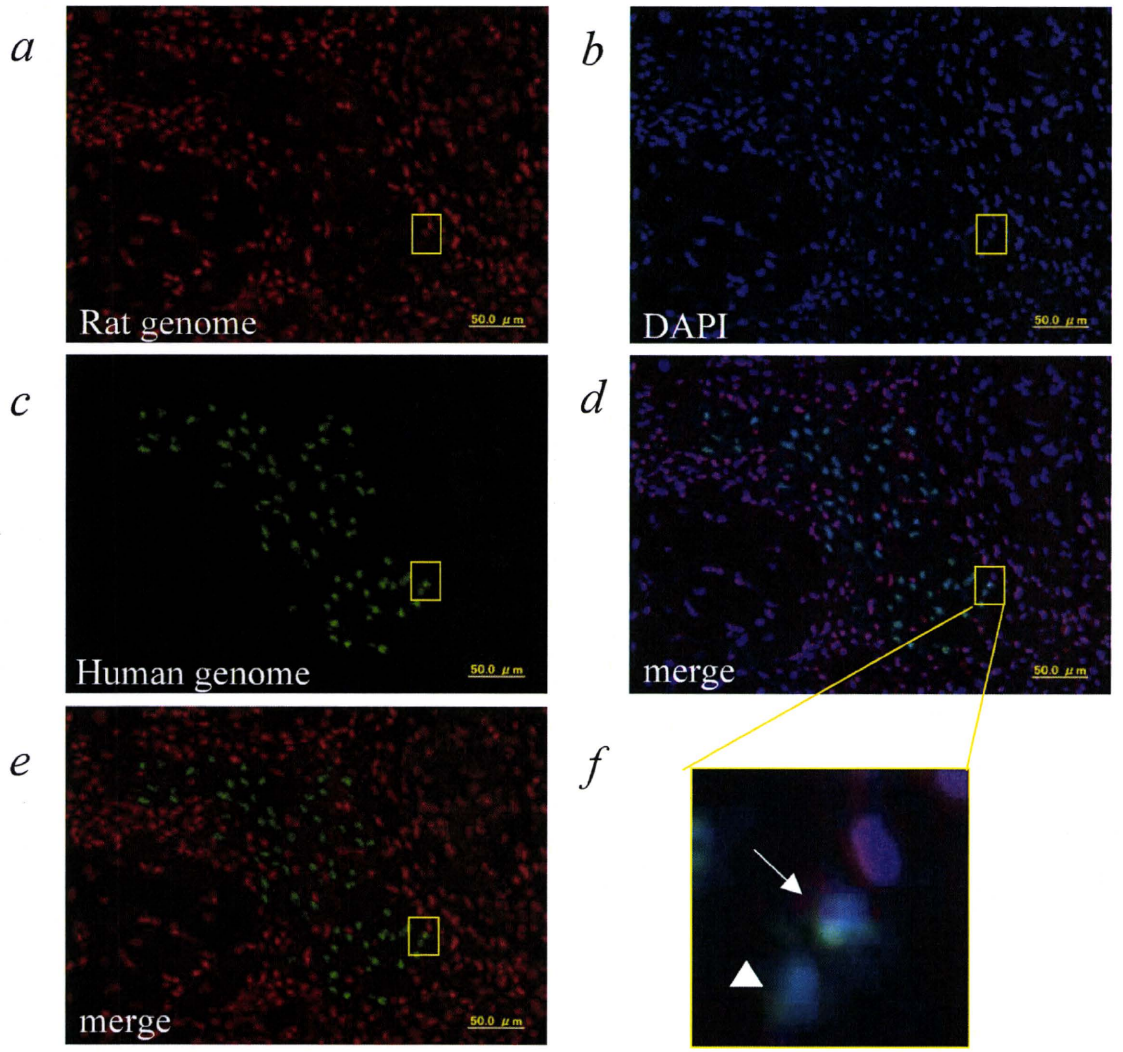


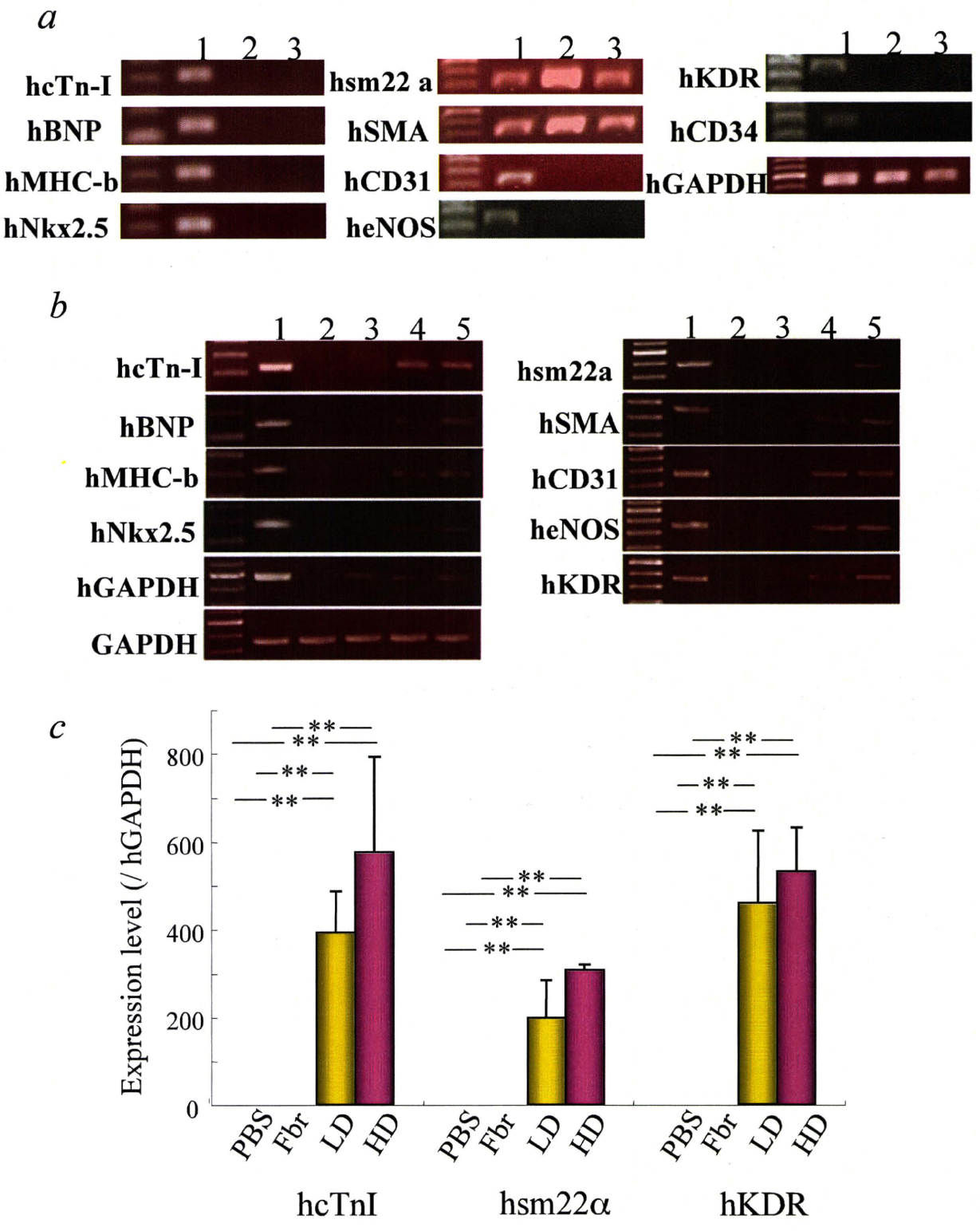
Supplementary Figure VI

Supplemental Material



Supplementary Figure VII

Supplemental Material



Supplementary Figure VIII