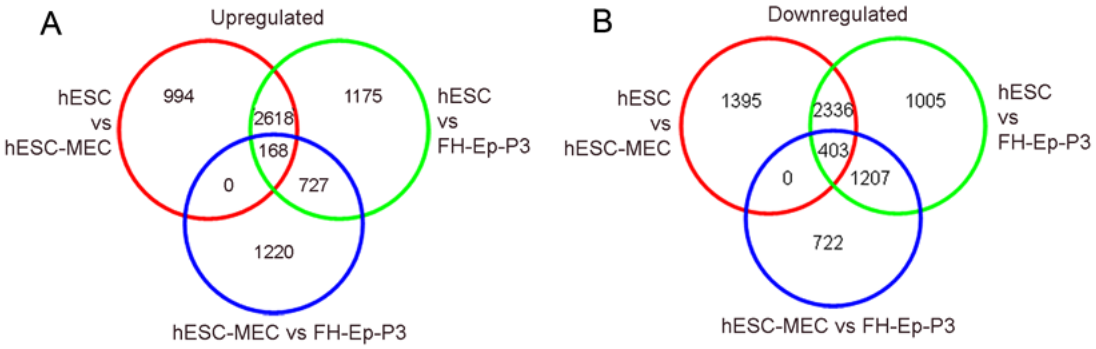


C

Gene Expression Change	hESC vs hESC-MEC	hESC-MEC vs FH-Ep-P3	hESC vs FH-Ep-P3
Expressed More (Upregulated)	**3780 (100%)**	**2115 (100%)**	**4688 (100%)**
GO Term - Biological Process	68%	62%	65%
GO Term - Molecular Function	73%	68%	70%
GO Term - Cellular Process	71%	67%	70%
KEGG Pathways Represented	588	372	710
Expressed Less (Downregulated)	**4134 (100%)**	**2332 (100%)**	**4951 (100%)**
GO Term - Biological Process	65%	67%	67%
GO Term - Molecular Function	71%	71%	72%
GO Term - Cellular Process	72%	68%	71%
KEGG Pathways Represented	644	425	763

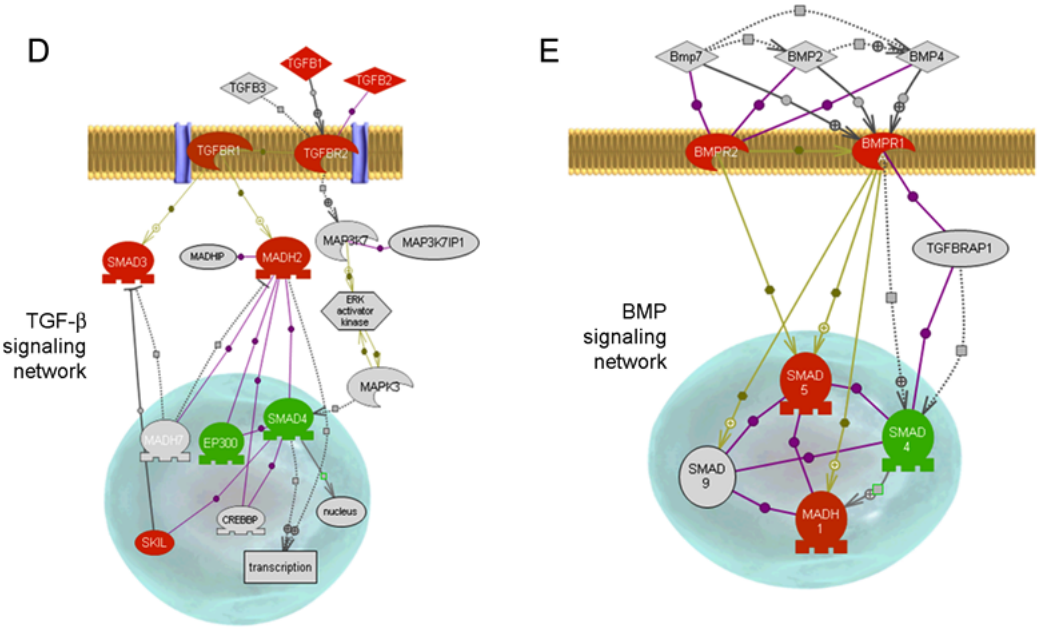


Fig. S1. Microarray analysis of gene expression. Shown are differences in gene expression profiles in hESC, hESC-MEC and FH-Ep-P3 cells. Venn diagrams in A and B compare upregulated genes and downregulated genes in cells. The analyses were based on 47,700 transcripts. Despite similarities in hESC-MEC and FH-Ep-P3, 1220 genes were uniquely upregulated and 722 genes were downregulated in cells, respectively. Table in (C) provides numbers of upregulated and downregulated genes. Also, gene ontology groupings of differentially expressed genes and cellular pathways represented in differentially expressed genes, are shown, according to KEGG. Multiple biological and cellular processes, molecular functions and pathways were included in differentially expressed lists. To examine changes in discrete pathways, we focused on TGF-β (D) and BMP (E) signaling networks in hESC-MEC and primary fetal human liver cells, i.e., FH-Ep-PP. This was to probe whether networks were regulated similar to FH-Ep-P3 cells, as previously described (Inada et al., 2008). Genes expressed in hESC-MEC at levels higher than FH-Ep-PP cells are in red compartments, genes expressed at lower level in hESC-MEC are in green compartments and genes not represented in the analysis are in grey compartments. Data in D showed that in hESC-MEC, TGF-β and TGF-βR1 and TGF-βR2 receptors were expressed, along with SMAD2 (MADH2) and SMAD3, although SMAD4 was expressed less well. Data in E indicate that BMP pathway was active with higher expression in hESC-MEC of BMPR1 and BMPR2 receptors, as well as of the intracellular transducers, SMAD5 and SMAD1 (MADH1), whereas SMAD4 was expressed less well. This should be useful because TGF-β and BMP signaling pathways participate in mesenchymal differentiation.

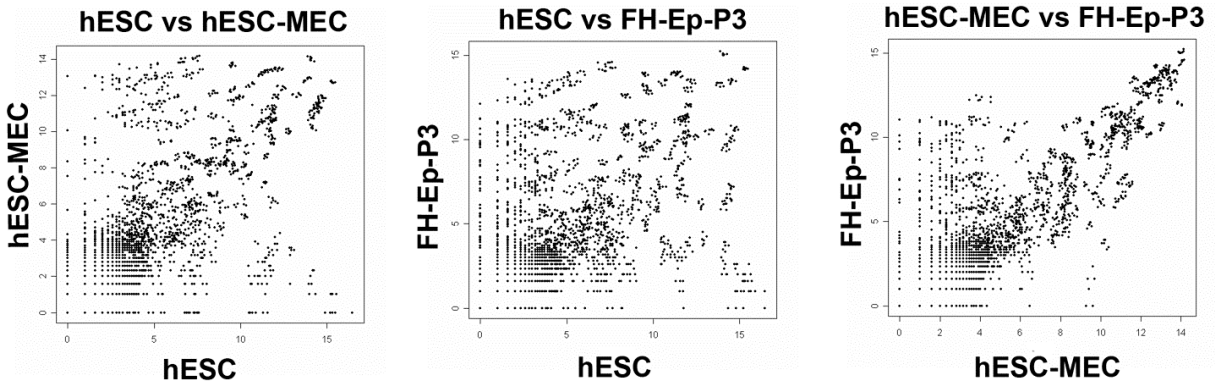


Fig. S2. Comparison of global microRNA expression profiles in various cell types. The data indicated that microRNA expression in hESC was markedly different from hESC-MEC (panel on left) and FH-Ep-P3 (middle panel), whereas the profiles of microRNA expression assumed greater similarities in hESC-MEC and FH-Ep-P3 cells (panel on right).

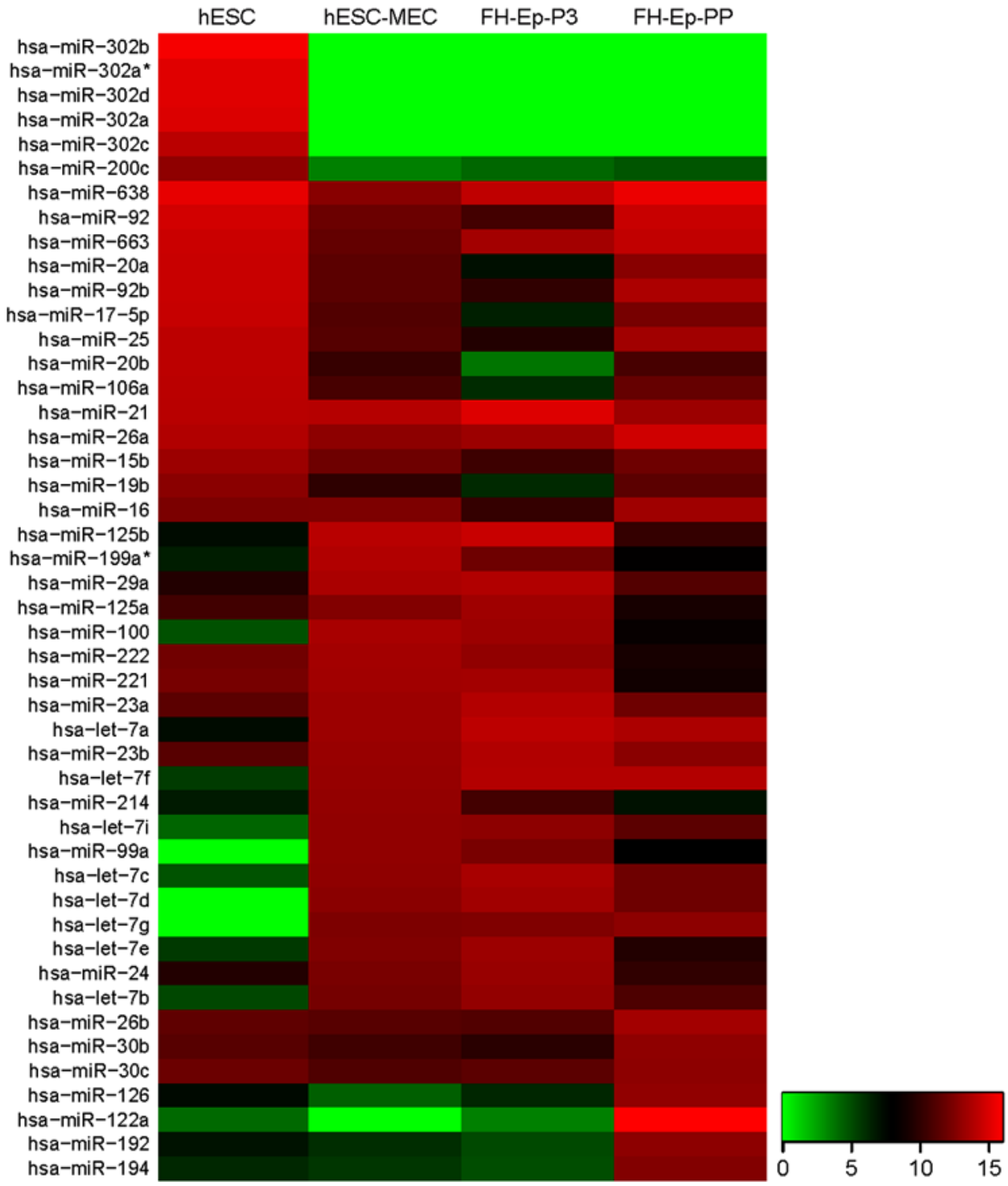


Fig. S3. Heat map analysis of differentially expressed microRNAs in various cell types. These data indicated that hESC diverged from hESC-MEC and other cell types, whereas hESC-MEC became more closely related to cells from fetal human liver**.**

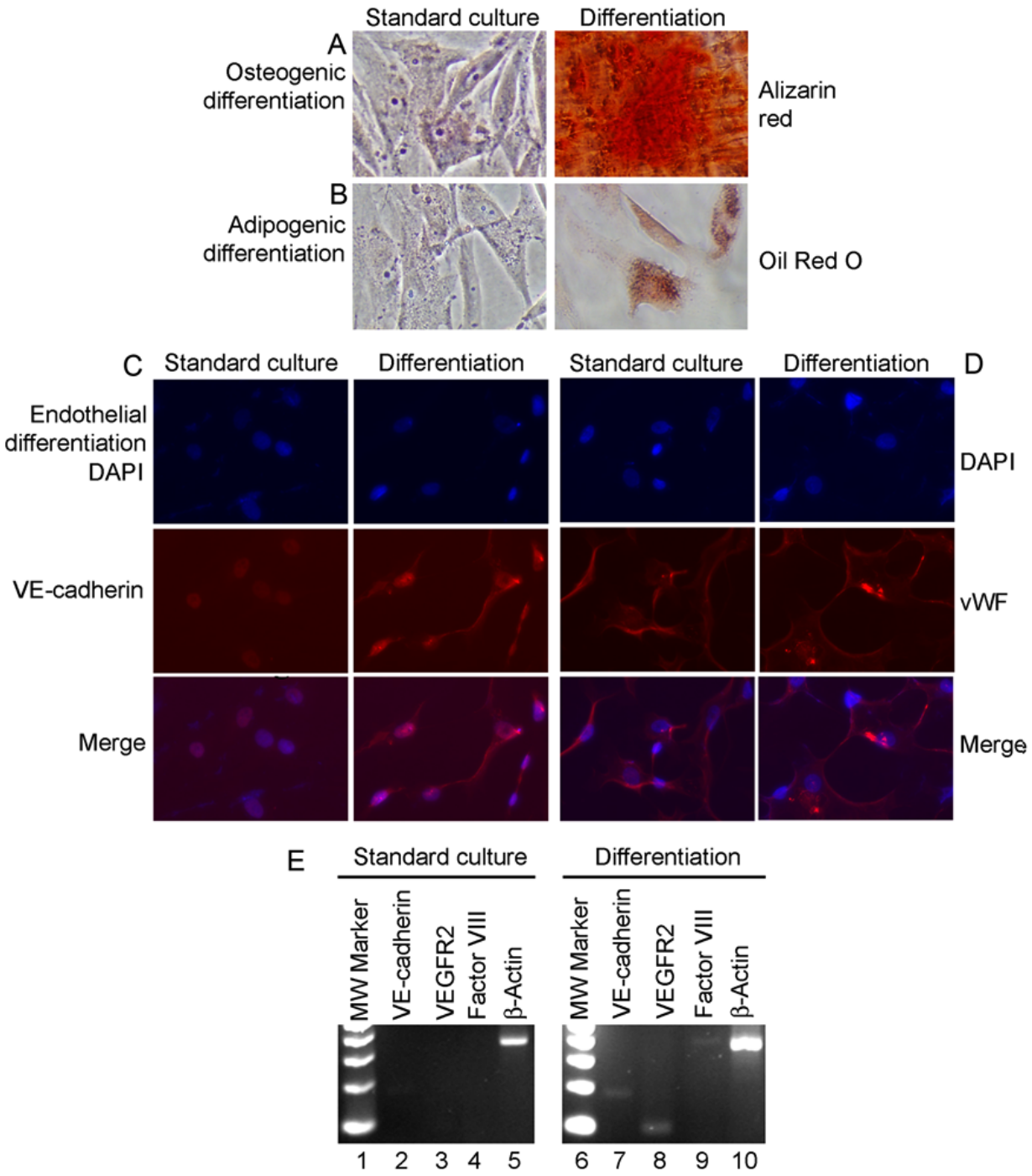
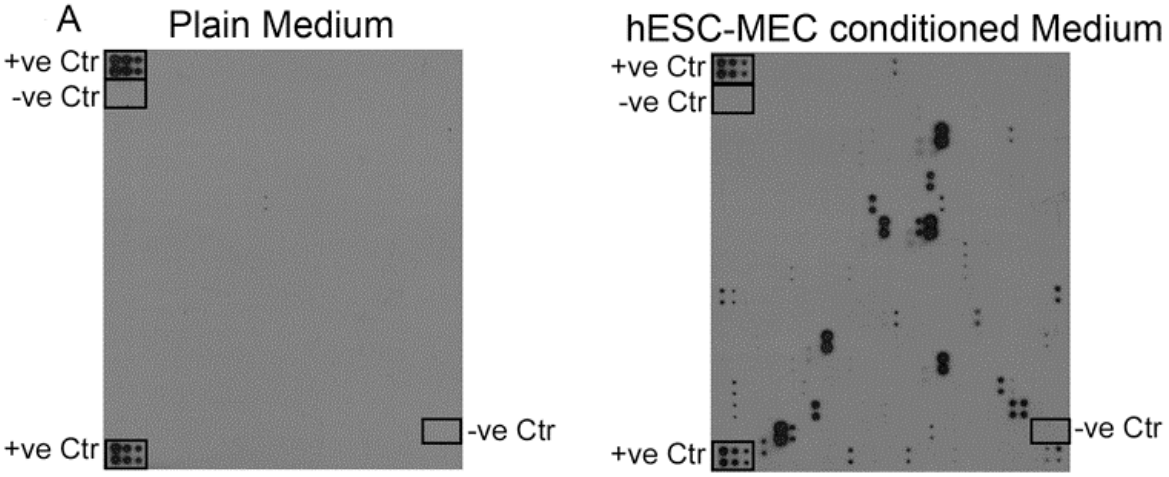


Fig. S4. Multilineage differentiation potential of hESC-MEC. (A) hESC-MEC cultured under osteogenic conditions showing calcium with Alizarin red cytochemical staining (red color). (B) hESC-MEC under adipogenic conditions showing lipid by Oil Red O staining. Panels in C and D demonstrate endothelial differentiation with immunofluorescence staining for VE-cadherin (C) and von Willebrand factor (vWF) (D). hESC-MEC expressed VE-cadherin under endothelial differentiation (C), whereas vWF was present beforehand. E shows RT-PCR for VE-cadherin, vascular endothelial growth factor receptor 2 (VEGFR2), and factor VIII mRNAs as additional endothelial markers.

A



B

Proteins expressed in hESC-MEC conditioned medium

HIGHEST	Pentraxin 3
Ectodysplasin-A2	Samd4
Insulin-like growth factor binding protein 2	Urokinase plasminogen activator
Insulin-like growth factor binding protein 7	Vascular endothelial growth factor
Matrix metalloproteinase 1	Tumor necrosis factor receptor 1
Osteoprotegerin	**LOW**
Tissue inhibitor of metalloproteinase 1	Cysteine rich transmembrane BMP regulator 1
HIGH	c-src tyrosine kinase
Galectin 3	Decorin
Glypican 3	Dickkopf related protein 1
Insulin-like growth factor binding protein 6	Family with sequence similarity 3, member B
Latent transforming growth factor binding protein 1	Interleukin 7
Monocyte chemotactic protein 1	Interleukin 8
Secreted frizzled-related protein 1	Growth differentiation factor 3
Secreted protein, acidic, cyteine-rich	Interleukin 17 receptor C
Thrombospondin	Interleukin 15 receptor alpha
Thrombospondin 1	Interleukin 12 receptor beta 2
Tissue inhibitor of metalloproteinase 2	Macrophage inflammatory protein 1 alpha
MEDIUM	Nephroblastoma overexpressed protein
Angiogenin	Neuregulin 3
Endothelial monocyte activated polypeptide 2	Progranulin
Growth regulated protein alpha	Neutrophil migration protein
Low density lipoprotein receptor-related protein 6	Tissue factor pathway inhibitor
Macrophage stimulatory protein beta chain	Vascular endothelial growth factor C

Fig. S5. Cytokines in conditioned medium from hESC-MEC. Panel A shows RayBiotech cytokine membranes with plain nonconditioned medium and conditioned medium. Panel B shows list of cytokines found in conditioned medium from hESC-MEC.