Novel SCRG1/BST1 axis regulates self-renewal, migration, and osteogenic differentiation potential in mesenchymal stem cells

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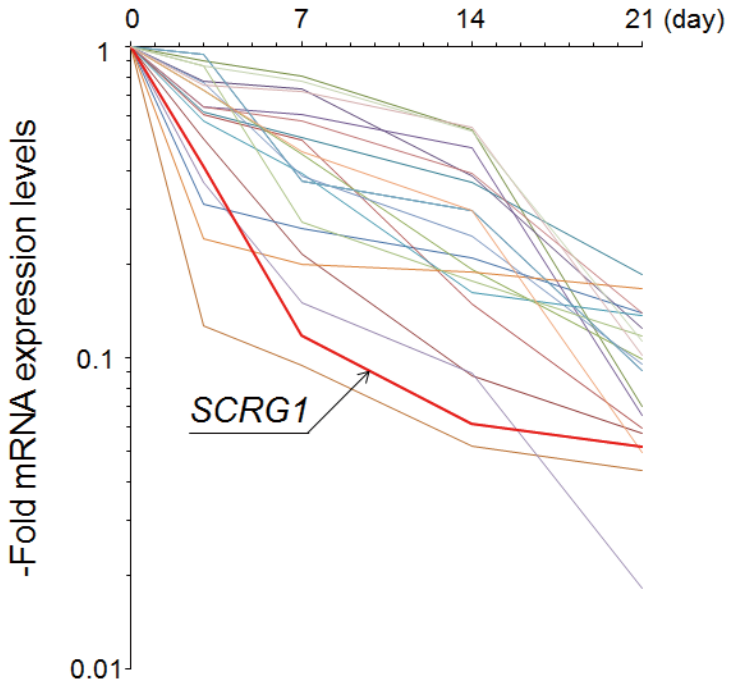
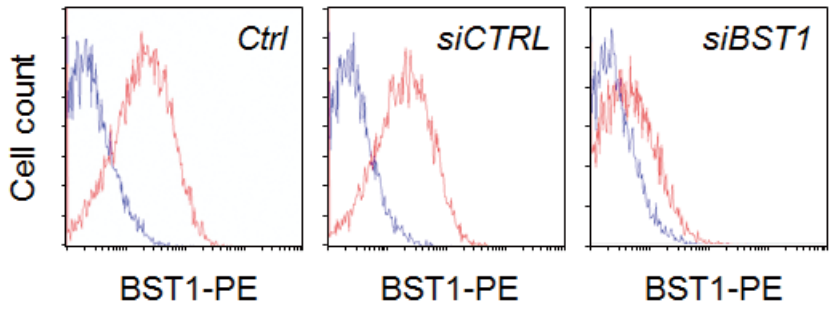
Supplemental Table S1. Genes for which expression was more than 5-fold downregulated 21 days after osteogenic induction.

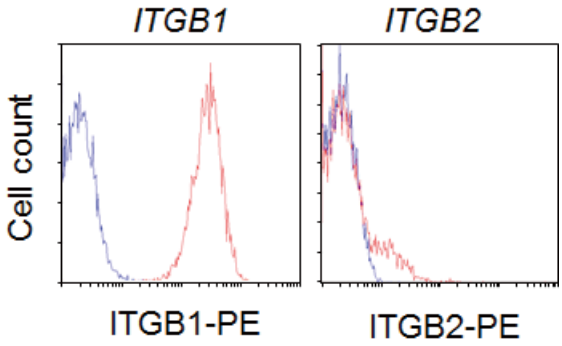
Symbol	Gene name	Relative expression levels compared with day 0				Fold chenge in day 21 (-)
		Day 3	Day 7	Day 14	Day 21	
IL6	interleukin 6 (interferon, beta 2)	0.619	0.513	0.368	0.185	5.4
LDB2	LIM domain binding 2	0.241	0.201	0.189	0.167	6.0
CRIP2	cysteine-rich protein 2	0.639	0.580	0.393	0.140	7.1
HAPLN1	hyaluronan and proteoglycan link protein 1	0.314	0.262	0.210	0.139	7.2
HAS2	hyaluronan synthase 2	0.580	0.392	0.163	0.137	7.3
RDX	radixin	0.774	0.732	0.384	0.124	8.0
KRT7	keratin 7	0.869	0.273	0.176	0.118	8.5
EML4	echinoderm microtubule associated protein like 4	0.872	0.777	0.534	0.113	8.9
LIN7C	lin-7 homolog C (*C. elegans*)	0.753	0.722	0.549	0.103	9.8
MEOX2	mesenchyme homeobox 2	0.727	0.454	0.192	0.099	10.1
PRPS1	phosphoribosyl pyrophosphate synthetase 1	0.770	0.386	0.247	0.096	10.4
CKB	creatine kinase, brain	0.949	0.370	0.299	0.091	11.0
SEC23A	Sec23 homolog A (*S. cerevisiae*)	0.900	0.803	0.543	0.070	14.4
RASA3	RAS p21 protein activator 3	0.643	0.608	0.476	0.065	15.3
BST1	bone marrow stromal cell antigen 1	0.607	0.502	0.150	0.060	16.8
MEST	mesoderm specific transcript homolog (mouse)	0.506	0.216	0.088	0.057	17.4
PENK	proenkephalin	0.408	0.118	0.062	0.052	19.3
AP4S1	adaptor-related protein complex 4, sigma 1 subunit	0.727	0.460	0.298	0.050	20.1
SCRG1	scrapie responsive gene 1	0.127	0.095	0.052	0.043	23.0
KRT14	keratin 14	0.365	0.151	0.089	0.018	54.7
KRT16	keratin 16	0.324	0.122	0.115	0.015	64.6



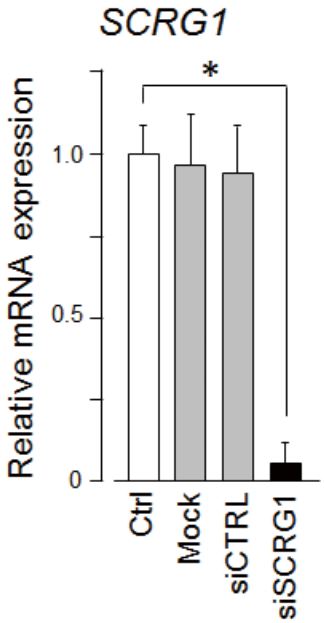
Supplemental Figure S1: Synthesis and secretion of SCRG1 are downregulated in hMSCs after osteogenic commitment. Whole genome expression was analyzed after 0, 3, 7, 14, and 21 days culture of primary hMSCs in osteogenic differentiation medium. Genes for which expression was more than 5-fold downregulated 21 days after osteogenic induction are represented and listed in supplementary Table S1.



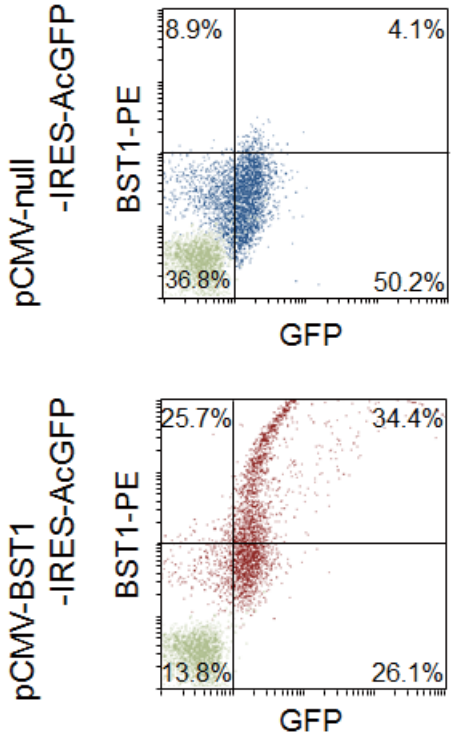
Supplemental Figure S2: siBST1 transfection reduces expression of cell surface BST1. Expression of cell surface BST1 was analyzed by flow cytometry of UE7T-13 cells transfected with siBST1 and treated with PE-conjugated anti-BST1 antibody. Specific antibody (red) and isotype control IgG (blue) are shown.



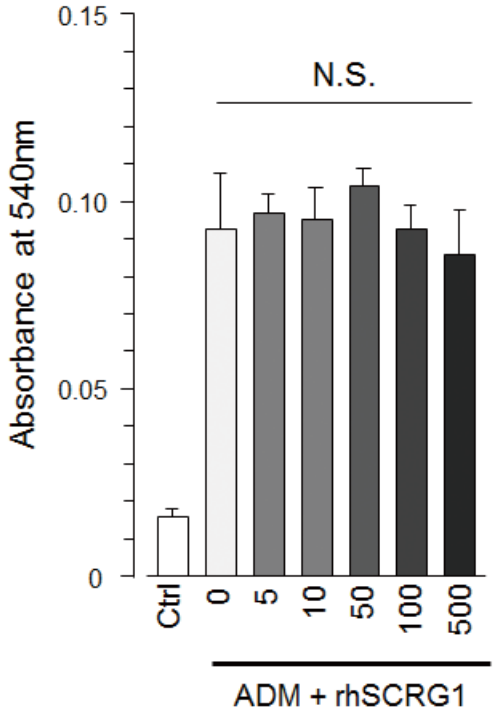
Supplemental Figure S3: UE7T-13 cells do not express β2 integrin. UE7T-13 cells were incubated with anti-β1 integrin and anti-β2 integrin antibodies for 1 h at 4°C. The cells were then incubated with PE-conjugated secondary antibody for 1 h at 4°C in the dark. Acquisition was performed using an EPICS XL ADC System. Specific antibody (red) and isotype control IgG (blue) are shown.



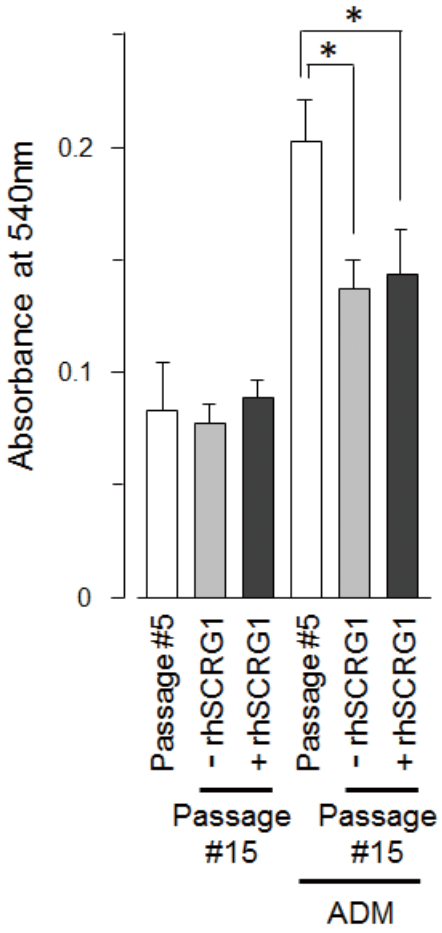
Supplemental Figure S4: siRNA against SCRG1 clearly knocked down the mRNA expression level. UE7T-13 cells were transfected with siRNA for SCRG1 (siSCRG1). mRNA expression of SCRG1 was measured by qRT-PCR and normalized to GAPDH; results are expressed as fold increase or decrease relative to the control (Ctrl). Data are presented as mean ± SD. *$p < 0.05$ was considered significant.



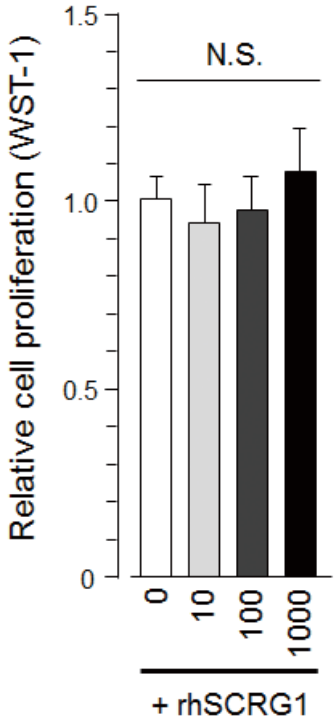
Supplemental Figure S5: The transfection efficiencies of pCMV-BST1-IRES-AcGFP and pCMV-null-IRES-AcGFP into UE7T-13 cells. UE7T-13 cells were transfected with pCMV-null-IRES-AcGFP (upper panels) or pCMV-BST1-IRES-AcGFP (lower panels). BST1 and GFP expression was investigated by flow cytometry. Bicistronic vector pCMV-BST1-IRES-AcGFP simultaneously express the same mRNA transcript of BST1 and AcGFP genes. Therefore, BST1 overexpressing cells transfected with pCMV-BST1-IRES-AcGFP were detected as GFP-positive cells.



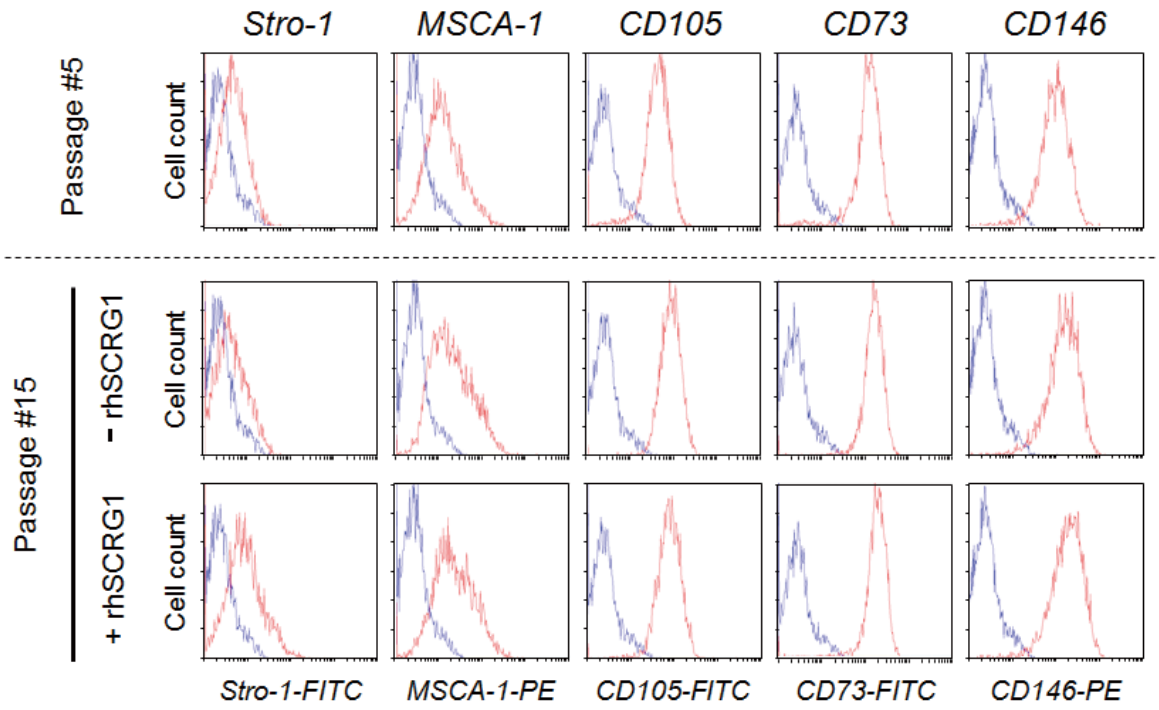
Supplemental Figure S6: Adipogenic differentiation of UE7T-13 cells was not suppressed by SCRG1. UE7T-13 cells were cultured in adipogenic differentiation medium (ADM) containing various concentrations of rhSCRG1 (5-500 ng/mL). After 2 weeks, lipid droplets were stained with Oil Red O, extracted with DMSO, and absorbance was measured at 540 nm.



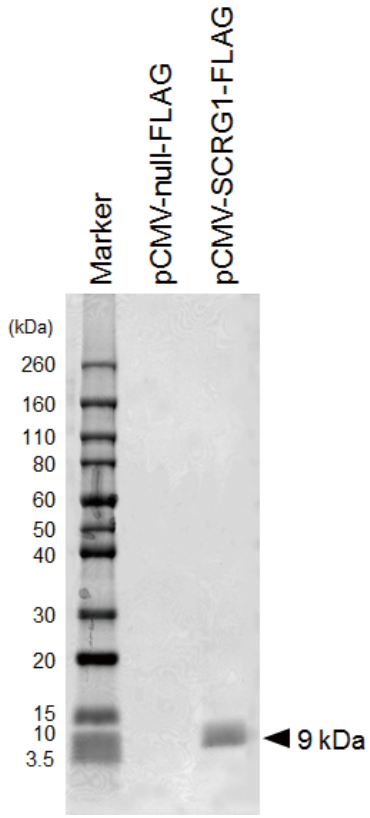
Supplemental Figure S7: Adipogenic differentiation of hMSCs was not preserved by SCRG1 after *ex vivo* expansion. Primary cultured hMSCs (passage #5) were subcultured ten times in the presence (passage #15 +rhSCRG1) or absence (passage #15 -rhSCRG1) of 500 ng/mL rhSCRG1. The cells were cultured in adipogenic differentiation medium (ADM). After 2 weeks, lipid droplets were stained with Oil Red O staining, extracted with DMSO, and absorbance was measured at 540 nm.



Supplemental Figure S8: Cell proliferation activity was not affected by the addition of rhSCRG1 in UE7T-13 cells. UE7T-13 cells were cultured in growth medium containing various concentrations of rhSCRG1 (0–1000 ng/mL) on 96-well culture plates for 5 days. After incubation periods, proliferation was evaluated by WST-1 assay.



Supplemental Figure S9: Expression of hMSC markers Stro-1, MSCA-1, CD73, CD105, and CD146 were unchanged by long-term culture. Primary cultured hMSCs (passage #5) were subcultured ten times in the presence (passage #15, +rhSCRG1) or absence (passage #15, -rhSCRG1) of 500 ng/mL rhSCRG1. Expression of Stro-1, MSCA-1, CD73, CD105, and CD146 was examined by flow cytometry with FITC- or PE-conjugated specific antibodies. Specific antibody (red) and isotype control IgG (blue) are shown.



Supplemental Figure S10: Purified rhSCRG1 was detected as a single 9-kDa band in SDS-PAGE. C-terminal FLAG-tagged recombinant human SCRG1 (rhSCRG1) was produced by a pSCRG1-FLAG vector and FreeStyle MAX 293 Expression System. rhSCRG1 secreted into the culture medium from HEK293F cells was purified by anti-FLAG M2 Agarose Affinity Gel. The purified rhSCRG1 was separated and analyzed by SDS-PAGE and CBB staining.