Supplementary materials

Flow cytometry

Cells were fixed with 4% (v/v) paraformaldehyde (Junsei, Tokyo, Japan) for 10 min and washed twice with Dulbecco's phosphate-buffered saline (DPBS; Welgene Inc., Daegu, Korea). The fixed cells were stained with fluorescence-unconjugated glial cell line-derived neurotrophic factor (GDNF) family receptor alpha 1 (GFRα1; a SSC-specific marker) primary antibody diluted in DPBS, or florescence-unconjugated alpha smooth muscle actin (αSMA; a peritubular myoid cell-specific marker), GATA binding protein 4 (GATA4; a sertoli cell-specific marker), or luteinizing hormone receptor (LHR; a leydig cell-specific marker) primary antibody diluted in Hank's balanced salt solution (HBSS, Invitrogen) containing 0.1% (w/v) saponin (Sigma-Aldrich, St. Louis, MO) for 16 h at 4℃. Subsequently, the detection of GFRα1 primary antibody was conducted by incubating Alexa Fluor 488-conjugated secondary antibody diluted in DPBS for 2 h at 4℃, and the detection of αSMA, GATA4, and LHR primary antibodies was conducted by incubating Alexa Fluor 488-conjugated secondary antibody diluted in HBSS containing 0.1% (w/v) saponin for 1 h at 4℃. Supplementary Table S2 describes the detailed information and dilution rate of the used antibodies. Then, the stained cells were rinsed with DPBS and sorted using a FACSCalibur (Becton Dickinson, Franklin Lakes, NJ), and data were analyzed using the BD CellQuest Pro Software™ (Becton Dickinson).

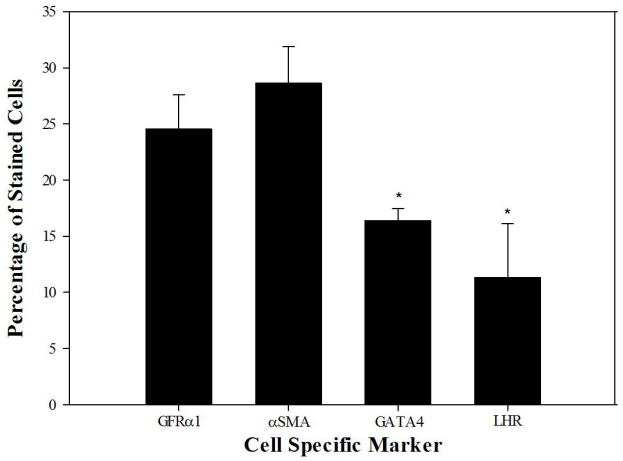

Fig. S1. The percentage of undifferentiated SSCs and peritubular myoid, sertoli and leydig cells coexisted in SSC population sorted from outbred ICR mouse testis-derived testicular cells using a MACS technique. Testicular cells were retrieved enzymatically from testis derived from ICR mice, and SSC population was prepared by sorting these testicular cells using a MACS technique based on anti-Thy1 antibody. Subsequently, the SSC population was stained with SSC-specific GFR α1, peritubular myoid cell-specific αSMA, sertoli cell-specific GATA4 and leydig cell-specific LHR antibodies and the percentage of each cell type was determined by flow cytometry. In the SSC population, cells showing positivity against GFR α1, αSMA, GATA4 and LHR were detected, and the percentage of GFR α1-positive SSCs and αSMA-positive peritubular myoid cells was significantly higher than those of GATA4-positive sertoli and LHR-positive leydig cells. All data shown are means $\pm$ standard deviation of three independent experiments. *$p < 0.05$.

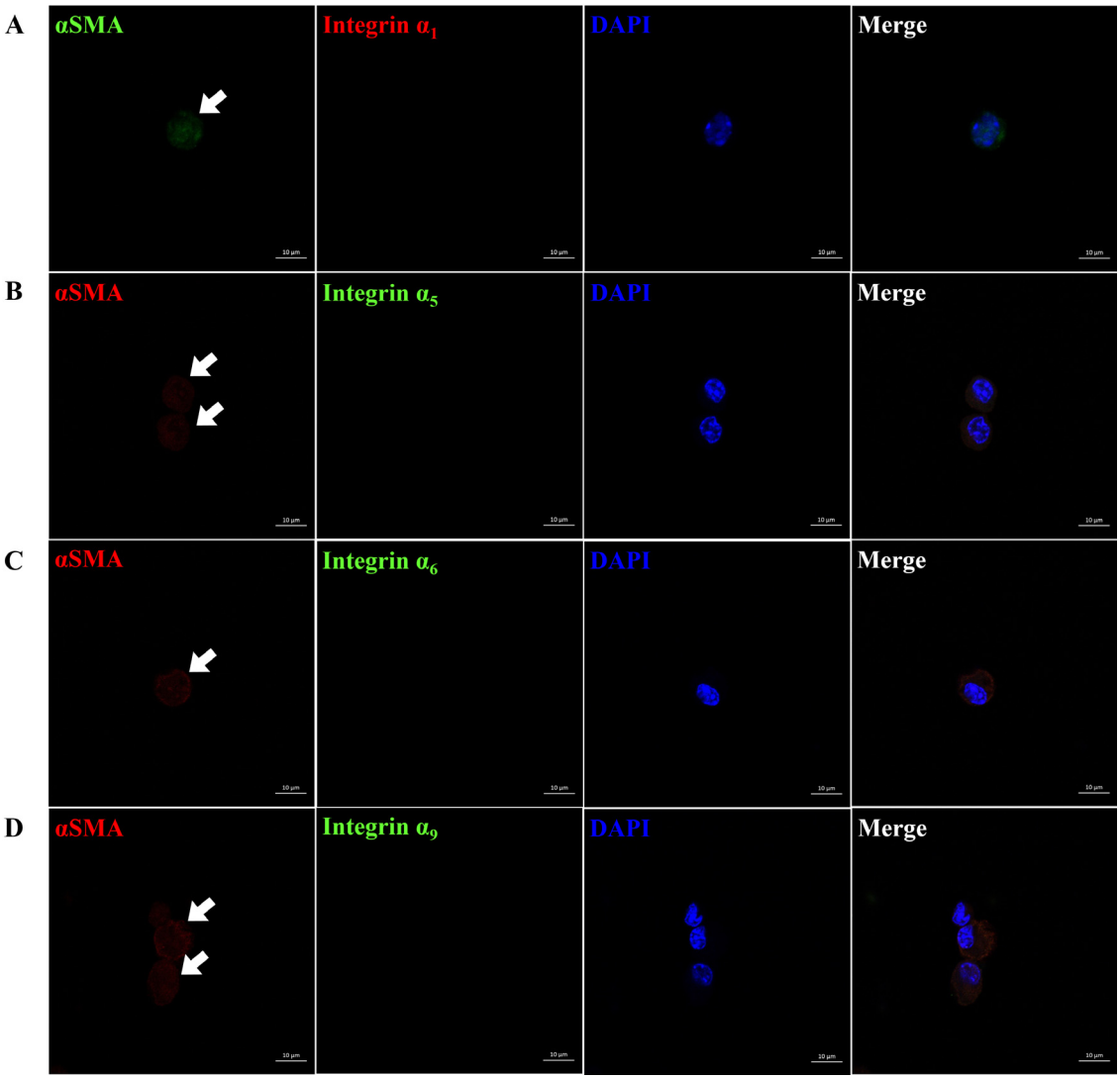

Fig. S2. Identification of integrin α and β subunit proteins expressed on the surface of peritubular myoid cells derived from outbred ICR mouse testes. In order to prepare SSC population which the proportion of SSCs in testicular cells was increased, testicular cells retrieved by treating enzymatically testis derived from ICR mice were sorted using a MACS technique based on anti-Thy1 antibody. Subsequently, the protein expression of integrin α and β subunits in the peritubular myoid cells included in the sorted SSC population was identified by immunocytochemistry. As the result, any localization of integrin α_1, α_5, α_6, α_9, α_V, α_E, β_1 and β_5 subunit proteins subunit proteins (A~H) were not detected on the surface of peritubular myoid cells expressing αSMA (arrow; a peritubular myoid cell-specific marker). All figures are representative immunocytochemistry images of integrin subunit proteins expressed on the surface of peritubular myoid cells. Nuclear counterstaining was conducted using DAPI. n=3. Scale bars represent 10 μm.

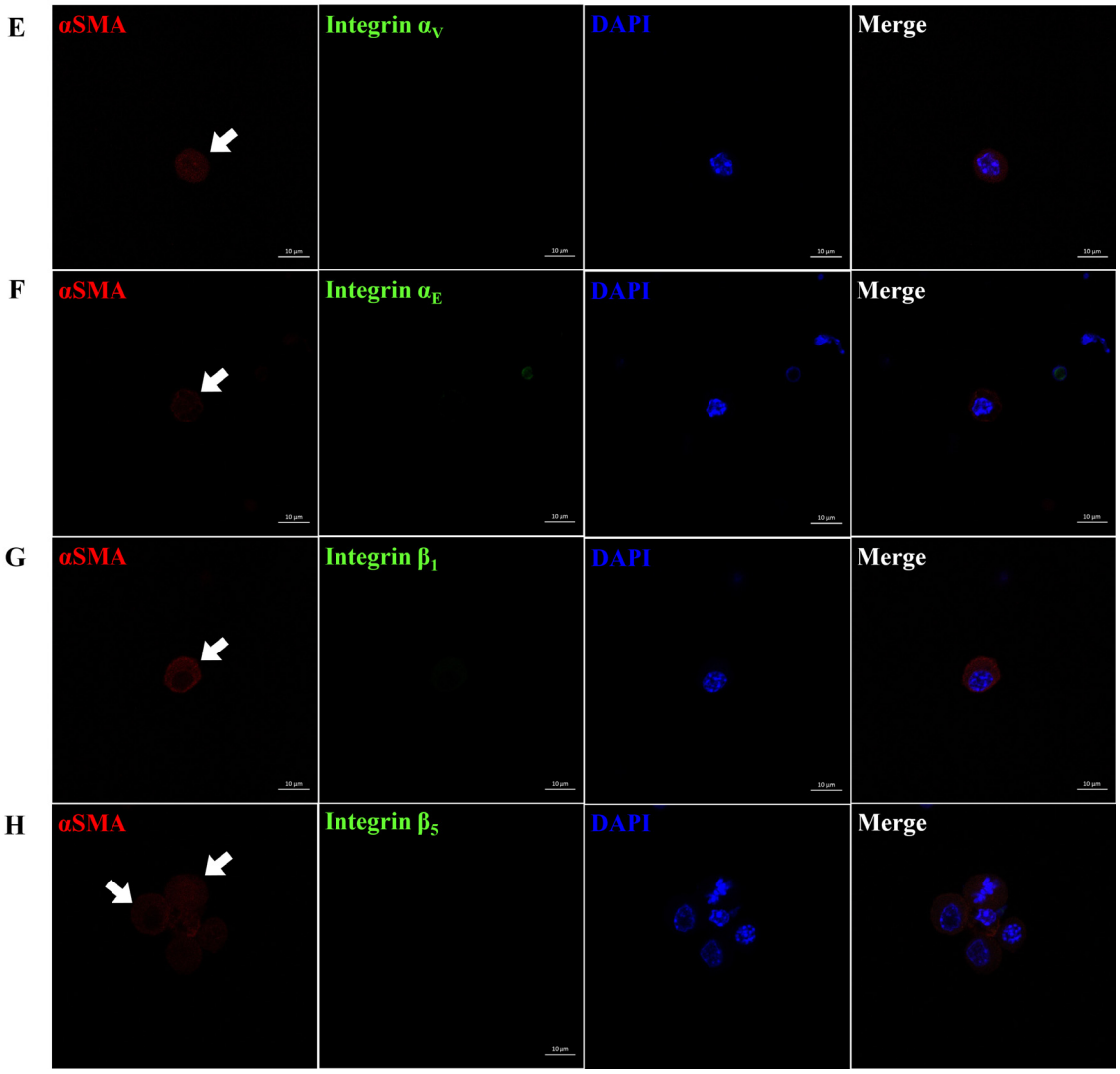

Fig. S2. Continued.

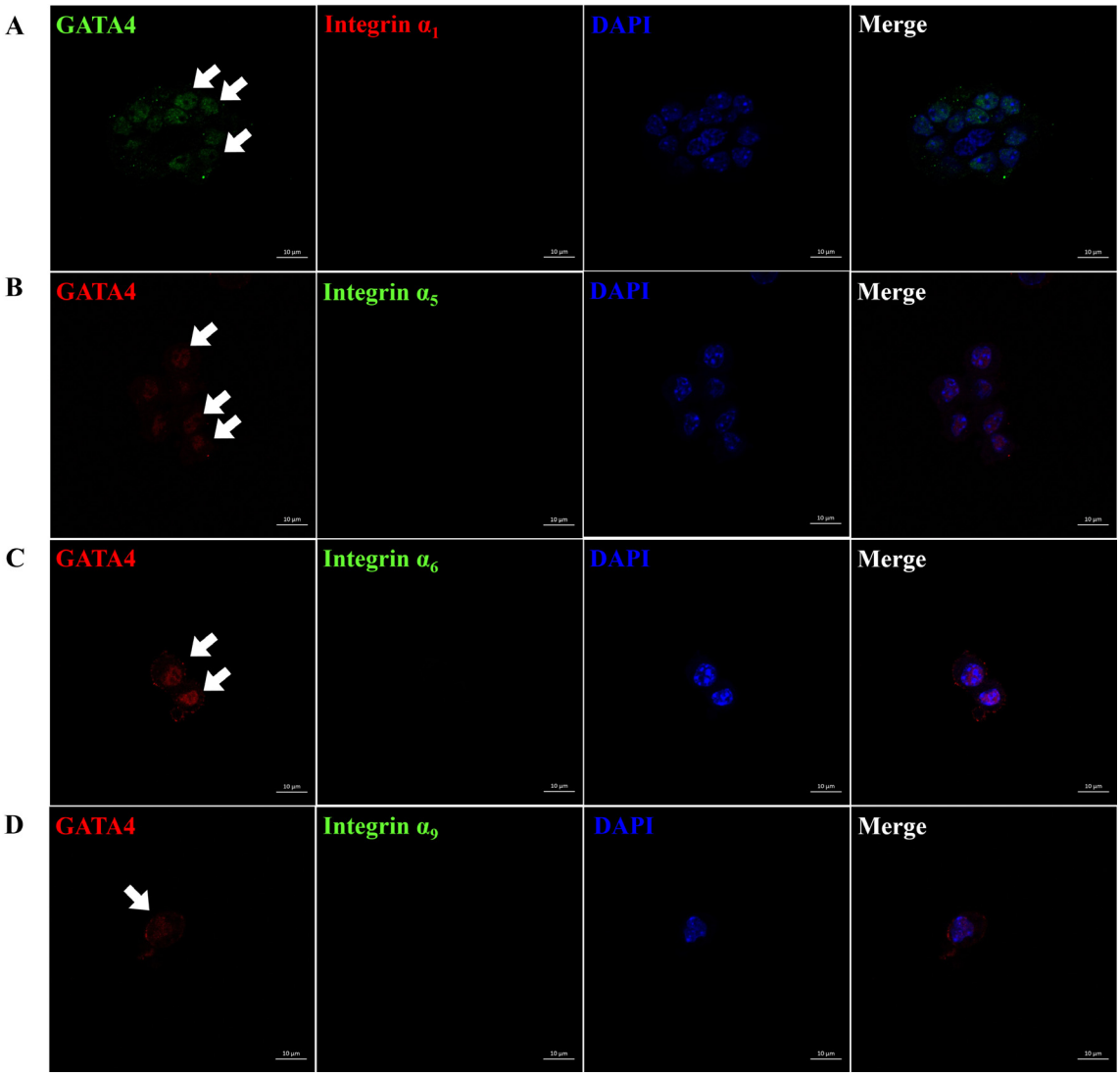

Fig. S3. Identification of integrin α and β subunit proteins expressed on the surface of sertoli cells derived from outbred ICR mouse testes. In order to prepare SSC population which the proportion of SSCs in testicular cells was increased, testicular cells retrieved by treating enzymatically testis derived from ICR mice were sorted using a MACS technique based on anti-Thy1 antibody. Subsequently, the protein expression of integrin α and β subunits in the sertoli cells included in the sorted SSC population was identified by immunocytochemistry. As the results, any localization of integrin α_1, α_5, α_6, α_9, α_V, α_E, β_1 and β_5 subunit proteins (A~H) were not detected on the surface of sertoli cells stained positively against GATA4 (arrow; a sertoli cell-specific marker). All figures are representative immunocytochemistry images of integrin subunit proteins expressed on the surface of sertoli cells. Nuclear counterstaining was conducted using DAPI. n=3. Scale bars represent 10 μm.

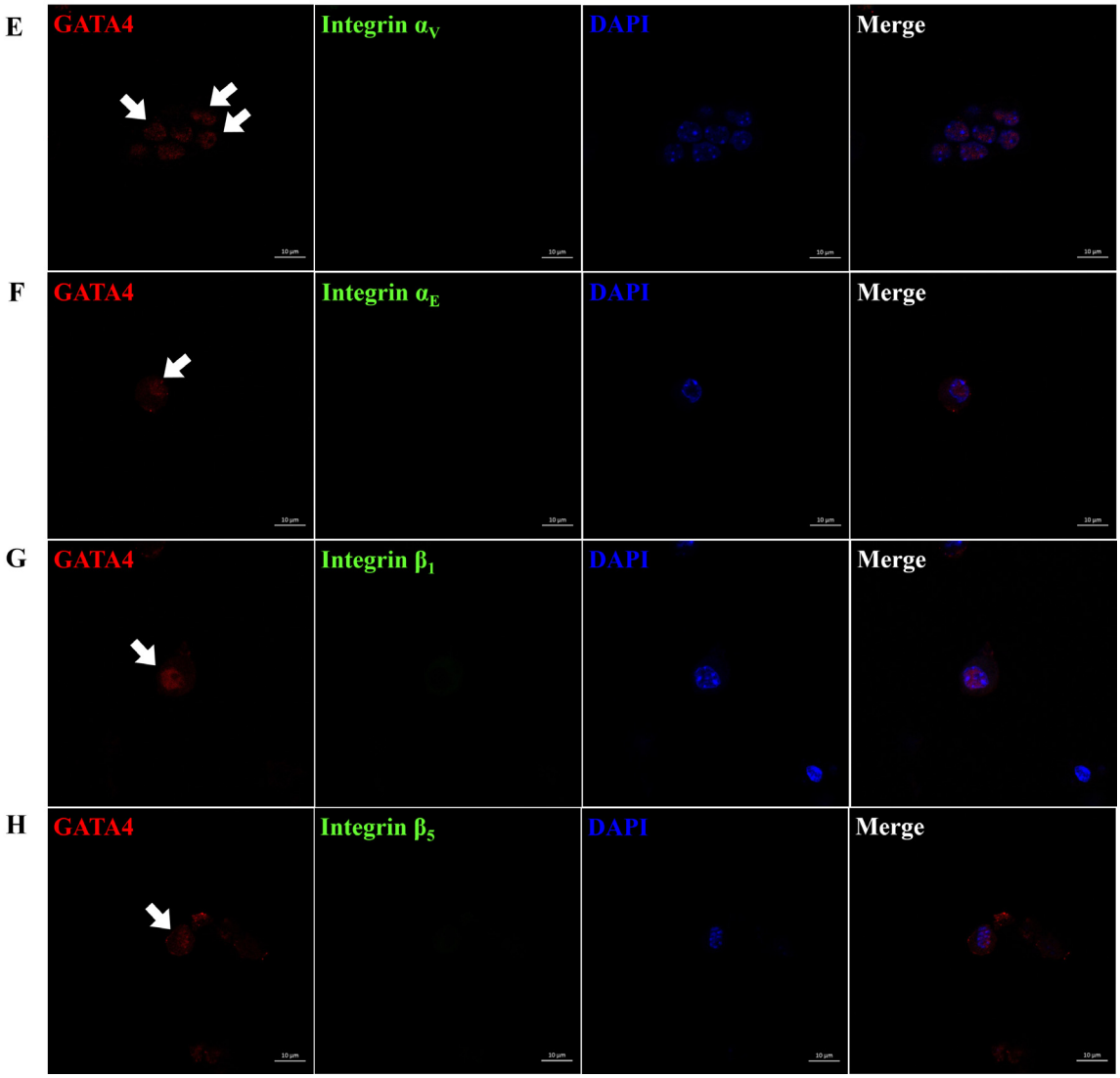

Fig. S3. Continued.

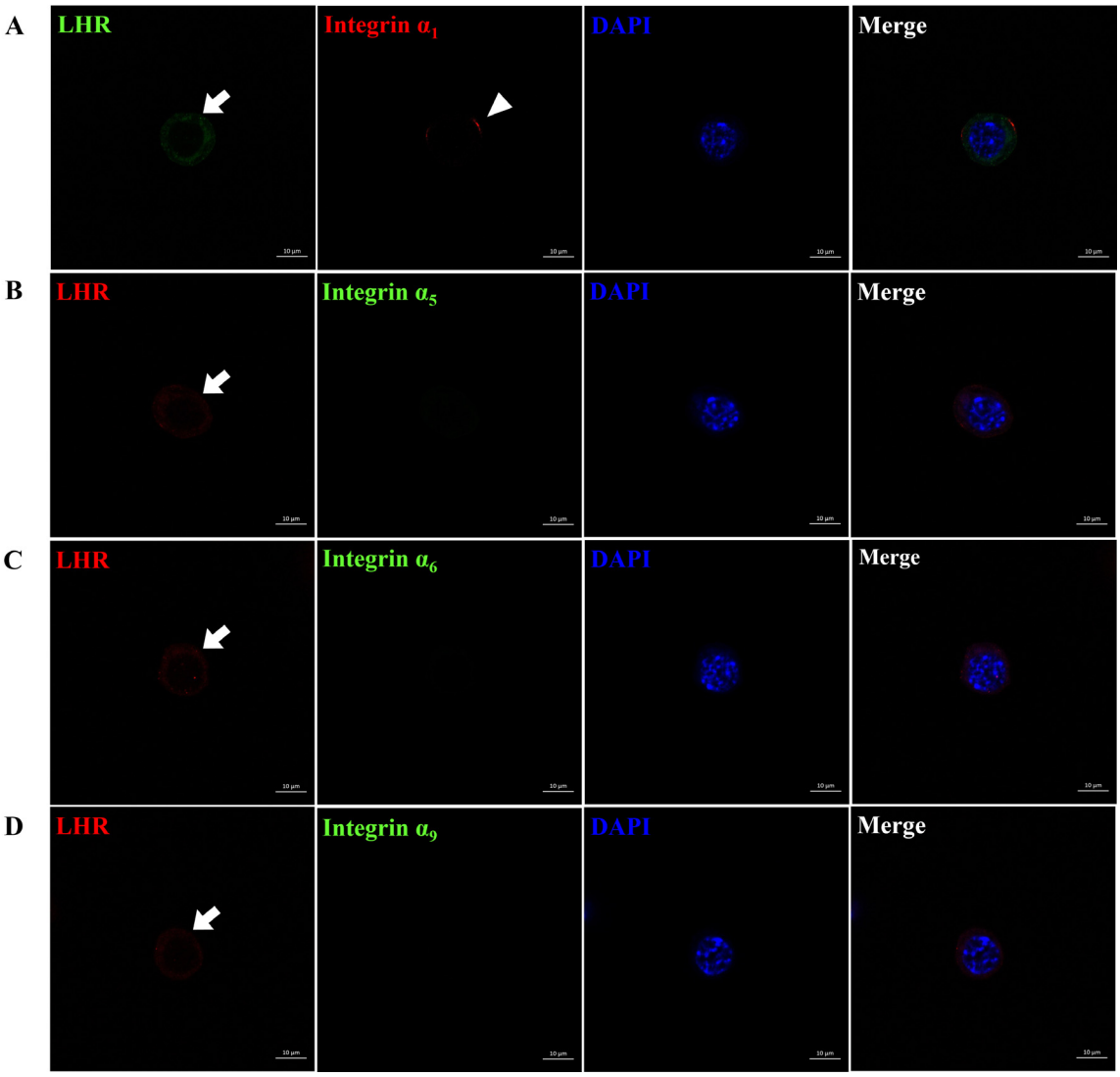

Fig. S4. Identification of integrin α and β subunit proteins expressed on the surface of leydig cells derived from outbred ICR mouse testes. In order to prepare SSC population which the proportion of SSCs in testicular cells was increased, testicular cells retrieved by treating enzymatically testis derived from ICR mice were sorted using a MACS technique based on anti-Thy1 antibody. Subsequently, the protein expression of integrin α and β subunits in the leydig cells included in the sorted SSC population was identified by immunocytochemistry. As the results, integrin α_1 subunit protein (arrow head; A) was localized on the surface of leydig cells stained positively against LHR (arrow; a leydig cell-specific marker). However, integrin α_5, α_6, α_9, α_V, α_E, β_1 and β_5 subunit proteins (B~H) were not detected on the surface of leydig cells expressing LHR. All figures are representative immunocytochemistry images of integrin subunit proteins expressed on the surface of leydig cells. Nuclear counterstaining was conducted using DAPI. n=3. Scale bars represent 10 μm.

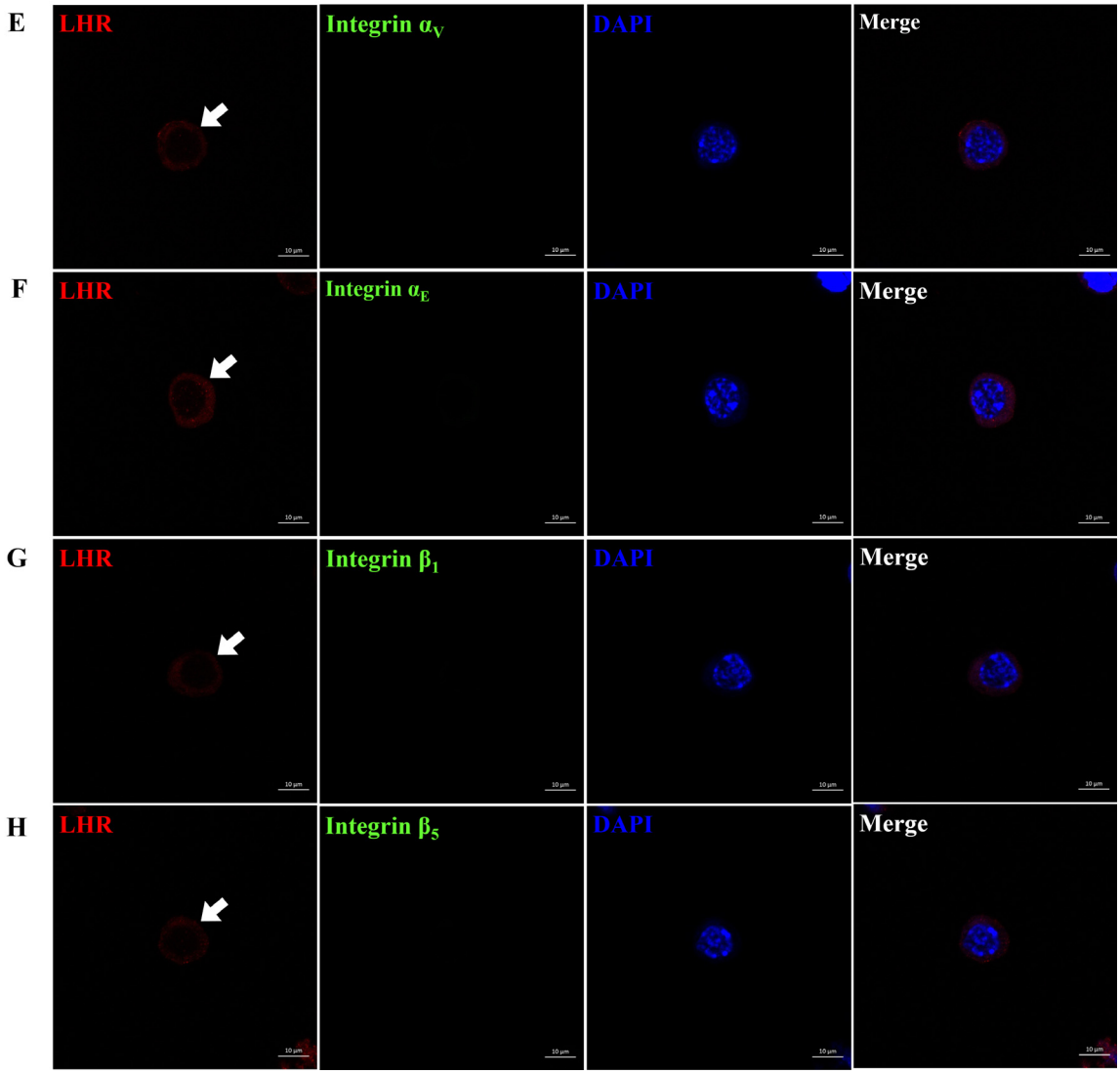

Fig. S4. Continued.

Table S1. Oligonucleotide primers and PCR cycling conditions

Genes	GeneBank number	Primer sequence		Size (bp)	Temp[a]
		Sense (5'>3')	Anti-sense (5'>3')		
Actb	NM_007393.4	TCTTTGATGTCACGCACGAT	TACCACAGGCATTGTGATGG	201	60
Itga1	NM_001033228	TGGCCAACCCAAAGCAAGAA	AGGGCCCACATGCCAGAAAT	200	60
Itga2	NM_008396	TGTGCACCCCCAGAGCACTT	TGTTCACTTGAAGGCCCGGA	181	60
Itga3	NM_013565	AGCAACCTGCAGATGCGAGC	CTCATGCGCATCTTCCCCAG	158	60
Itga4	NM_010576	AGCAAAAAGGCATAGCGGGG	AACGCTGGCTTCCTTCCCAC	160	60
Itga5	NM_010577	AGGCTGCGCTGTGAGTTTGG	TGCCGAGGCAGGATCTGGTA	178	60
Itga6	X63251	AGGTTCGAGTGACGGTGTTT	GTATCGGGGAATGCTGTCAT	185	60
Itga7	NM_008398	GCTTCCCAGACATTGCCGTG	TCCATCCACATCCAGGCCAC	182	60
Itga8	NM_001001309	GCATTCTTGACGTGGGCTGG	ATCCTCTGGGGAGGCAGCAG	154	60
Itga9	NM_133721	GGGGCAGGTCACCGTCTACC	AGCCACATCTGGGAACCCGT	156	60
Itga10	BC115770	GCTGTCTCCATGCCACAGGC	GTGGGGAGGCATCACATCCA	186	60
Itga11	NM_176922	TCCGGTAACCCAGGGCAACT	GCTTCCACACTCGTGCGACC	172	60
Itgav	NM_008402	AAGGCGCAGAATCAAGGGGA	CCAGCCTTCATCGGGTTTCC	194	60
Itgal	NM_008400	GGAAGCCTGGTGGGCTCAGT	AGCTCAGCACAACCACCCGA	180	60
Itgam	NM_008401	CTTTGCAATTGAGGGCACGC	GAAGGCTCCACCTGCCCAGT	150	60
Itgad	NM_001029872	TGTGGAGAAGCCCGTCGTGT	AGTGGCAGGCGCACAGTCAT	157	60
Itgax	NM_021334	GCTAGGGGACGTGAATGGGG	GGAGGGGATCTGGGATGCTG	165	60
Itgae	NM_008399	ACACAAGCCAAAGCCCTTCT	CAGGCTCTTGACTCTGGGTG	186	60
Itgb1	NM_010578	CTGGTCCCGACATCATCCCA	CCGTGTCCCACTTGGCATTC	167	60
Itgb2	NM_008404	GGTGGCTCAGATCGGGGTTC	TGCACCTGTTGCATTGGCAG	165	60
Itgb3	NM_016780	CCCCACCACAGGCAATCAAA	CCCTCTGGGGCATCTCGATT	166	60
Itgb4	BC080751	GGCCAGTGGCTCTCTCAGCA	GTGGTCAGCAAGCTCGTGGG	151	60
Itgb5	NM_010580	AGGGCGTCCTATGCTCAGGC	AGACACAACGGCCTCGGTCA	161	60
Itgb6	NM_021359	GTCCAAGGTGGCTGTGCCTG	TGCGGGAGACAGGGTTTTCA	199	60
Itgb7	NM_013566	AAGGAGGGCTCTGCAGTGGG	TACAGTTGGCTGCCAGGGGA	182	60
Itgb8	BC125343	GCCTCAAGGTGCGCTCTCAA	AGGCTGCCCCAAGAACCAAG	181	60

[a]Temp: temperature.

Table S2. Primary and secondary antibodies

Antibody name	Catalog number	Company	Application	Dilution rate
Biotin-conjugated rat anti-mouse Thy1	NPBI-28033	Novus	MACS	1 : 250
APC-conjugated American hamster anti-mouse integrin α_1	142606	BioLegend	ICC	1 : 50
FITC-conjugated rabbit anti-human integrin α_5	orb222105	Biorbyt	ICC, FI	1 : 50, 1 : 100
Alexa Fluor 488-conjugated rat anti-human/mouse α_6	313607	BioLegend	ICC, FI	1 : 50, 1 : 100
FITC-conjugated rabbit anti-mouse integrin α_9	orb188618	Biorbyt	ICC, FI	1 : 50, 1 : 100
FITC-conjugated rabbit anti-human integrin α_V	orb7231	Biorbyt	ICC, FI	1 : 50, 1 : 100
FITC-conjugated Armenian hamster anti-mouse integrin α_E	121420	BioLegend	ICC	1 : 50
FITC-conjugated mouse anti-mouse integrin β_1	ab21845	Abcam	ICC, FI	1 : 50, 1 : 100
FITC-conjugated mouse anti-mouse integrin β_5	11-0497	eBioscience	ICC	1 : 50
Rabbit anti-human GFRα1	sc-10716	Santa Cruz	ICC, FC	1 : 50
Rabbit anti-mouse αSMA	ab5694	Abcam	ICC, FC	1 : 50
Goat anti-mouse GATA4	sc-1237	Santa Cruz	ICC, FC	1 : 50
Goat anti-mouse LHR	sc-26341	Santa Cruz	ICC, FC	1 : 50
Alexa Fluor 488-conjugated chicken anti-rabbit IgG	A21441	Invitrogen	ICC, FC	1 : 50, 1 : 100
Alexa Fluor 546-conjugated donkey anti-rabbit IgG	A10040	Invitrogen	ICC	1 : 50
Alexa Fluor 488-conjugated donkey anti-goat IgG	A11055	Invitrogen	ICC, FC	1 : 50, 1 : 100
Alexa Fluor 568-conjugated donkey anti-goat IgG	A11057	Invitrogen	ICC	1 : 50
LEAF^{TM} Purified rat anti-mouse integrin α_5	103807	BioLegend	AIA	1 : 100
Rat anti-mouse integrin α_6	MAB1378	Chemicon International	AIA	1 : 50
Mouse anti-human integrin $\alpha_9\beta_1$	MAB2078Z	Chemicon International	AIA	1 : 10
LEAF^{TM} Purified rat anti-mouse integrin α_V	104108	BioLegend	AIA	1 : 10

MACS: magnetic activated cell sorting, ICC: immunocytochemistry, FI: fluorescence immunoassay, FC: flow cytometry, AIA: antibody inhibition assay.