Supporting Information

Besson et al. 10.1073/pnas.1103873108

SI Materials and Methods

Histological Analyses. Primary antibodies: β-galactosidase (β-gal; Promega), laminin and b-IIItubulin (Sigma), Pax7, MF20, and nestin (DSHB), Vimentin, CCAAT displacement protein (CDP), Keratin14 (K14), and Doublecortin (DCX) (Santa Cruz Biotechnology), Keratin 15 (K15) and MASH1 (BD Biosciences), Keratin 5 (K5), Bmi1, GFP, AE15, and, Runx2 (Abcam), Keratin 6 (K6; Progen), BrdU (AbD Serotec), Glial GFAP (DakoCytomation), Ki67 (BD Pharmingen), phospho-histone H3 (Upstate Biotechnology), P-Cadherin (P-Cadh; Zymed Laboratories), glial cell line-derived neurotrophic factor receptor-α1 (GFRA1; Neuromics), Sox9 (R&D), and PW1 (1). Antibody binding was revealed using species-specific secondary antibodies coupled to Alexa Fluor 488 (Molecular Probes), FITC (DakoCytomation), Cy3, Cy5, or peroxidase (Jackson ImmunoReserach). Nuclei were counterstained with DNA (DAPI) or nuclear fast red (Sigma).

FACS Analyses. Antibodies: rat anti-mouse CD49f-PE, rat anti-mouse hematopoietic lineage flow mixture-Pacific blue (Lin: CD3, CD45R:B220, CD11b, TER-119, Ly-6G), rat anti-mouse CD34-biotin (Ram34), rat anti-mouse Sca1-PE, rat anti-mouse cKit-APC, streptavidin-PE-Cy7 (all from BD Biosciences), and rat anti-mouse CD34-Pacific blue (Clinisciences).

1. Relaix F, et al. (1996) Pw1, a novel zinc finger gene implicated in the myogenic and neuronal lineages. *Dev Biol* 177:383–396.

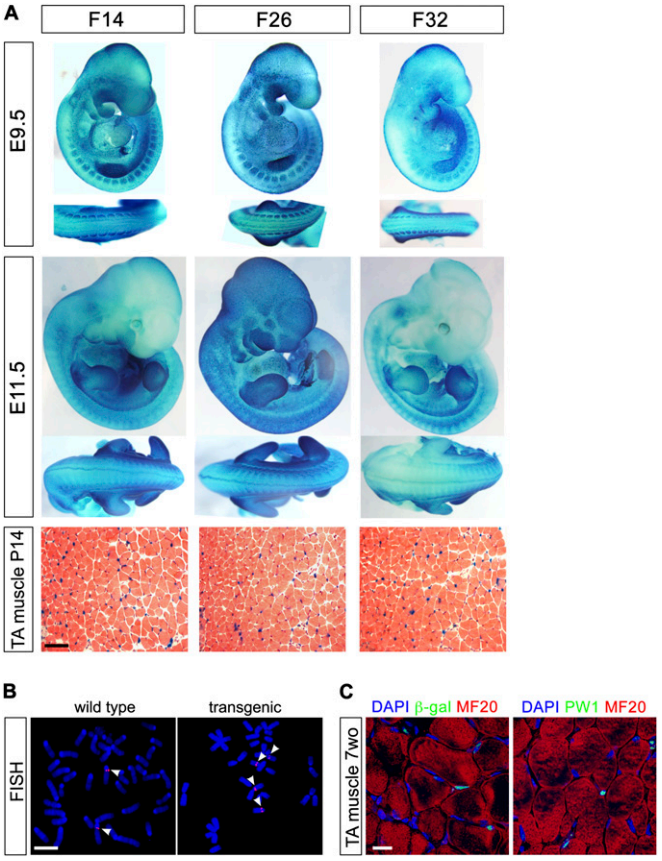

Fig. S1. (*A*) Three *Pw1*^{IRESnLacZ} founders displayed similar reporter expression profiles. Sagittal and dorsal views of 9.5 and 11.5 whole embryos from three Tg (*Pw1*^{IRESnLacZ}) founders (F14, F26, and F32) stained for X-Gal (*Top* and *Middle*). X-Gal staining of 14-d postnatal *Tibialis Anterior* (TA) muscle from the three transgenic founders. (*B*) FISH metaphase preparations showing single chromosomal insertion. (*C*) Transverse section of TA muscle from 7-wk-old transgenic mice were immunostained for β-gal and PW1 and did not reveal expression in the myonuclei [as defined by being in myofibers expressing sarcomeric myosin expression (MF20)]. [Scale bars, 100 μm (*B*), 75 μm (*C*), 10 μm (*A*).]

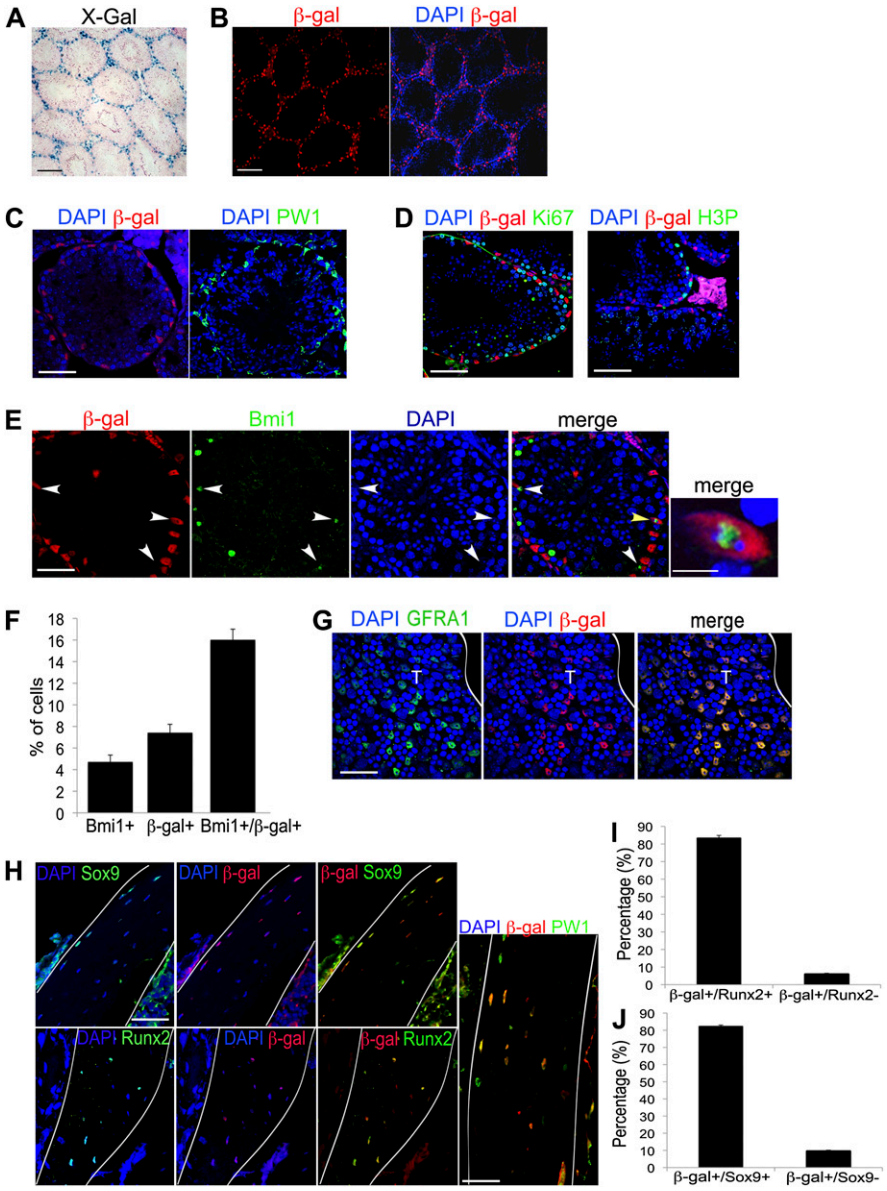

Fig. S2. (*A–G*) Reporter expression identifies undifferentiated spermatogonia. (*A–C*) Cross sections of 7-wk-old transgenic seminiferous tubules stained for X-Gal (*A*) or immunostained for β-gal (red, *B and C*) and PW1 (green, *C*). Reporter activity and endogenous PW1 protein were detected in a small percentage of cells located near the basement membrane. (*D*) Representative cross sections of 7-wk-old transgenic seminiferous tubules immunostained for β-gal (red) and either Ki67 (green) or phospho-histone H3 (H3P). Reporter expression does not overlap with Ki67 nor phospho-histone H3. (*E*) Colocalization of β-gal (red) and Bmi1 (green) in cross section of 7-wk-old transgenic seminiferous tubules. We note β-gal$^+$ cells next to Bmi1$^+$ cells (white arrowheads), as well as a small percentage of cells coexpressing the reporter and Bmi1 (yellow arrowhead and higher magnification on the left). (*F*) Schematic representation of the percentage (%) of cells positive for Bmi1, β-gal, and Bmi1/β-gal. More than 100 tubules from three independent experiments were analyzed. Values represent mean % ± SEM. (*G*) Representative longitudinal section of 7-wk-old transgenic seminiferous tubules (T) immunostained for β-gal and GFRA1 (glial cell line-derived neurotrophic factor receptor-α1). Reporter and GFRA1 expression colocalize in the undifferentiated spermatogonia. (*H–J*) Reporter and PW1 are expressed in bone stem/progenitor cells. (*H*) Cross sections of 7-wk-old transgenic femurs immunostained for β-gal (red) and Sox9, Runx2, and PW1 (green). The white lines define the bone matrix. (*I* and *J*) Schematic representation of β-gal$^+$ cells expressing the osteo- (Runx2) and chondrogenic (Sox9) progenitor markers. In adult bone, 83% and 82% of cells positive for β-gal express Runx2 (*I*) or Sox9 (*J*), respectively. [Scale bars, 200 μm (merge in *E*), 10 μm (*A* and *B*), 50 μm (*C–E*, *G*, and *H*).]

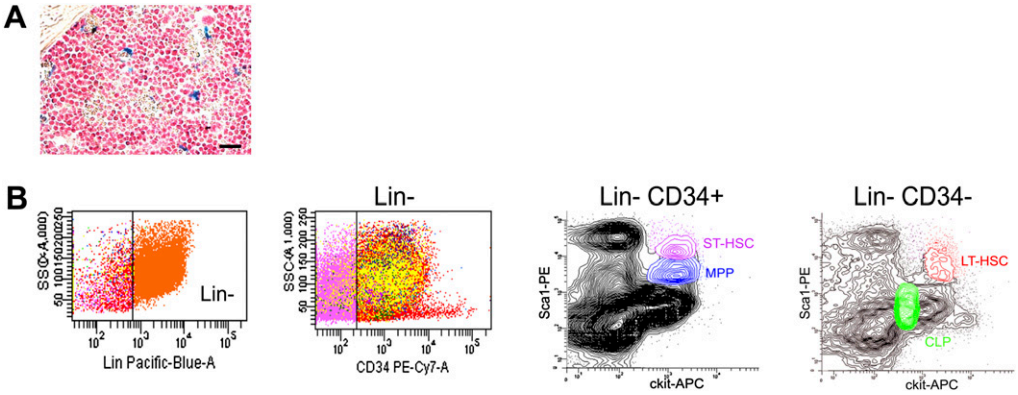

Fig. S3. (*A*) Representative section of 2-mo-old reporter mouse bone stained for β-gal. β-Gal$^+$ cells are present in the bone marrow. (*B*) FACS profiles of bone marrow cells sorted with antibodies against Lineage (Lin), CD34, Sca1, and cKit. Lin$^-$ cells were separated by CD34, Sca1, and cKit expression. (Scale bar, 20 μm.)

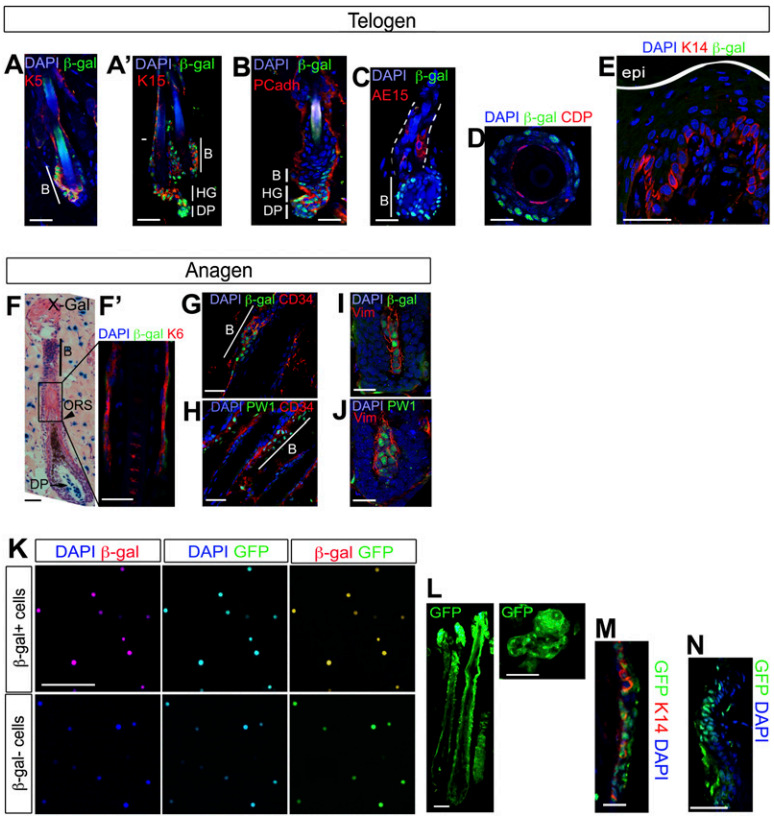

Fig. S4. (*A–J*) Representative photomicrographs of longitudinal (*A–C* and *E–J*) and transverse sections (*D*) of adult transgenic (*A–G* and *I*) and wild-type (*H* and *J*) hair follicles in telogen (7 wk old) (*A–E*) or anagen (10 wk old) (*F–J*). Hair follicles (*A–D* and *F–J*) and epidermis (*E*) are immunostained for β-gal (*A–E*, *F′*, *G*, and *I*), PW1 (*H* and *J*) and markers of the bulge (K5, *A*; K15, *A′* and CD34, *G*, and *H*), hair germ (PCadh, *B*), outer root sheath (AE15, *C*), inner root sheath, and the bulb (CDP, *D*), basal layer of the interfollicular epidermis (epi) (K14, *E*), companion layer (K6, *F′*), and dermal papilla (vimentin, *I* and *J*) or labeled after X-Gal coloration (*F*). Reporter and PW1 expression are found in the bulge (*A–C, F–H*), hair germ (*A′* and *B*), and dermal papilla (*F*, *I*, and *J*) cells but are excluded from the differentiated compartments (i.e., the bulb and the inner root sheath) of the hair follicle (*D*) and from the basal layer of the interfollicular epidermis (IFE) (*E*). (*F′*) Higher magnification of the outer and inner root sheath defined by a rectangle in (*F*) showing no reporter activity in the differentiated cells of the companion layer (K6+). B, bulge; DP, dermal papilla; epi, epidermis; HG, hair germ; ORS, outer root sheath. (*K*) Immunostaining for β-gal and GFP expression in cells sorted from the skin of 7-wk-old reporter mice as shown in Fig. 4*C*. (*L–N*) Micrographs of longitudinal sections of hair follicles derived from the β-gal$^+$ cells 3 wk after engraftment in adult nude mice revealing GFP expression in the follicles (*L*, *Left*), sebaceous gland (*L*, *Right*), and basal layer of the IFE (*M* and *N*) as defined by K14 expression (*M*). [Scale bars, 100 μm (*K, M,* and *N*), 70 μm (*E* and *F′–J*), 50 μm (*A–C* and *F*), 30 μm (*D*), 10 μm (*F* and *L*).]