Developmental Cell *16*

Supplemental Data

Scara5 Is a Ferritin Receptor Mediating Non-Transferrin Iron Delivery

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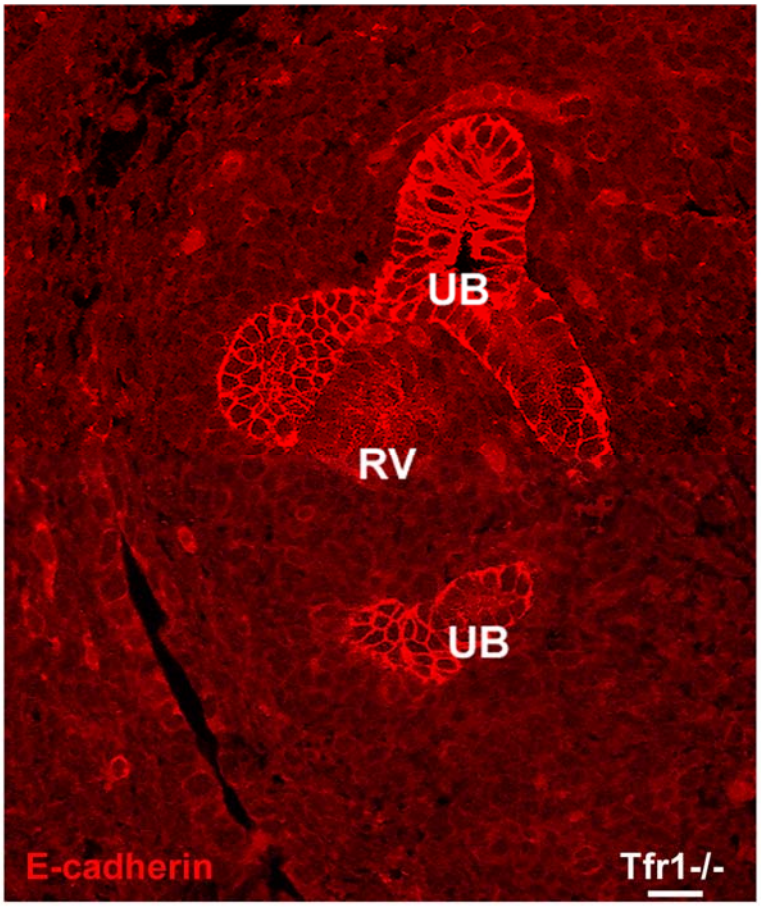

Figure S1. Initial Stages of Renal Development Were Preserved in TfR1$^{-/-}$ Mice
A surviving E11.5 embryo demonstrated the first stage of ureteric bud (UB) elongation and branching and mesenchymal to epithelial transition (E-cadherin^{+} renal vesicle, RV). Bar = 10μm.

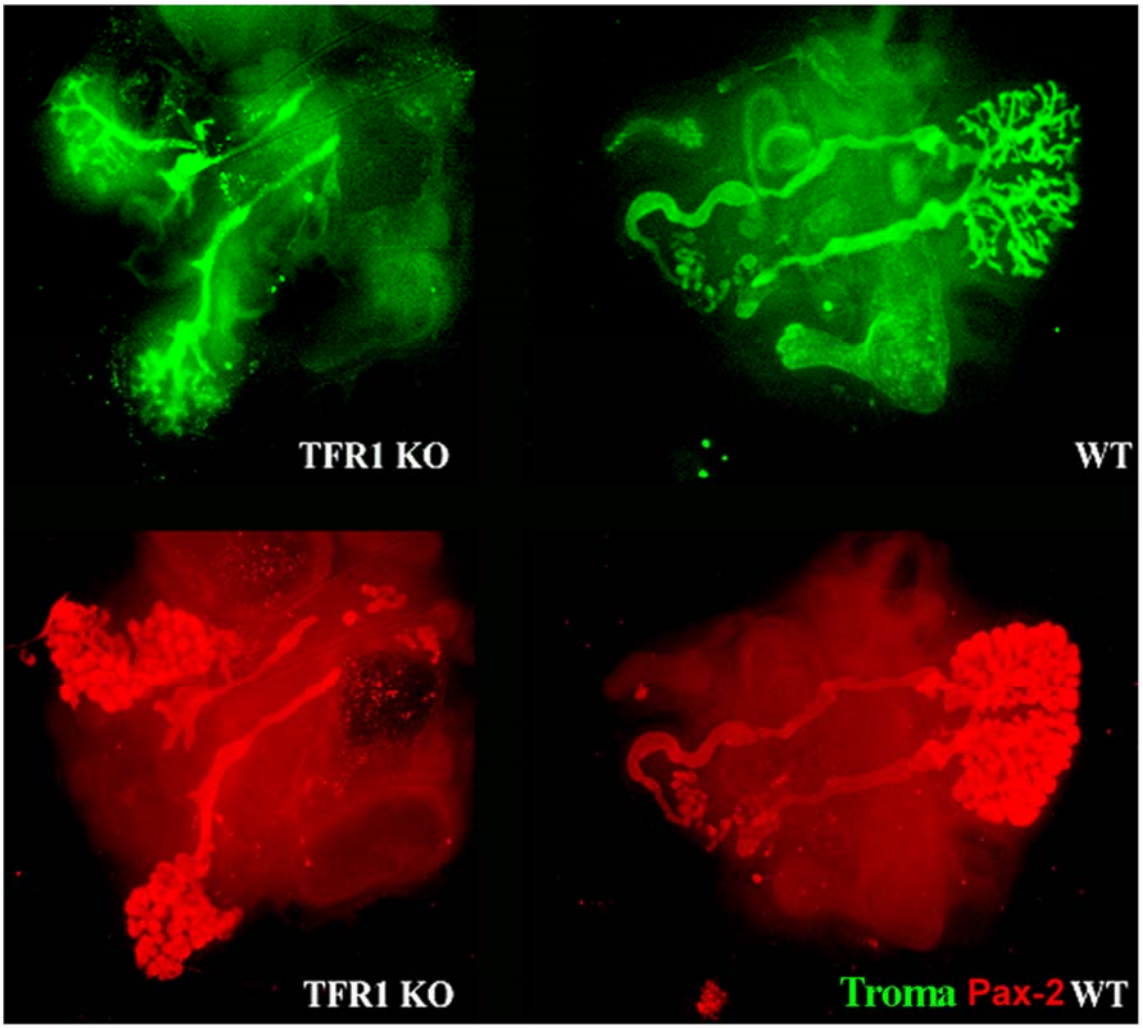

Figure S2. Development of the Ureteric Bud and Metanephric Mesenchyme in Explanted Urogenital Sheets (E11), Cultured with Ferric Ammonium Citrate for 5 Days

Both $TfR1^{-/-}$ and $TfR1^{+/+}$ sheets developed branched ureteric buds ($Troma^{+}Pax2^{+}$) and cap mesenchyme ($Pax2^{+}$) demonstrating the competence of $TfR1^{-/-}$ progenitors to form renal structures. Bars=10μm.

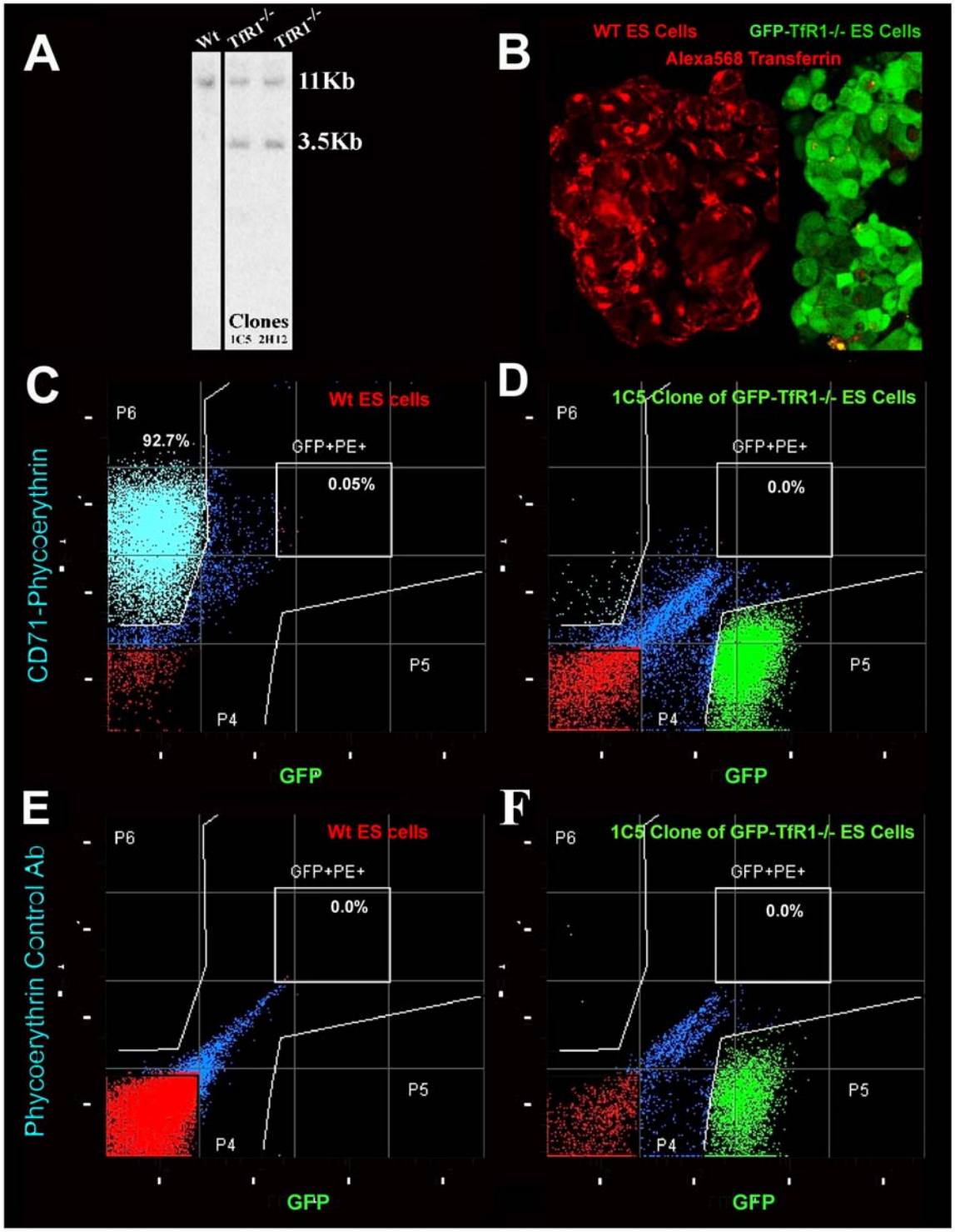

Figure S3. Authentication of GFP-TfR1$^{-/-}$ ES Cells

(A) Southern blotting verified the knockin of GFP in the Rosa26 locus of TfR1$^{-/-}$ ES clones. The Southern blot demonstrated the wild type (11kb) and the knockin allele (3.5kb) using EcoRV digestion of genomic DNA and a cDNA probe described in Srinivas et al, 2001.

(B) WT but not GFP-TfR1$^{-/-}$ ES clones captured Alexa568-transferrin (red).

(C and D) WT ES clones expressed cell surface TfR1-CD71 (92.7% of the clone) whereas GFP^{+}TfR1$^{-/-}$ ES cells did not express this epitope (0% of GFP-TfR1$^{-/-}$ ES cells).

(E and F) Isotype matched phycoerythrin control antibodies did not recognize WT or GFP-TfR1$^{-/-}$ ES cells.

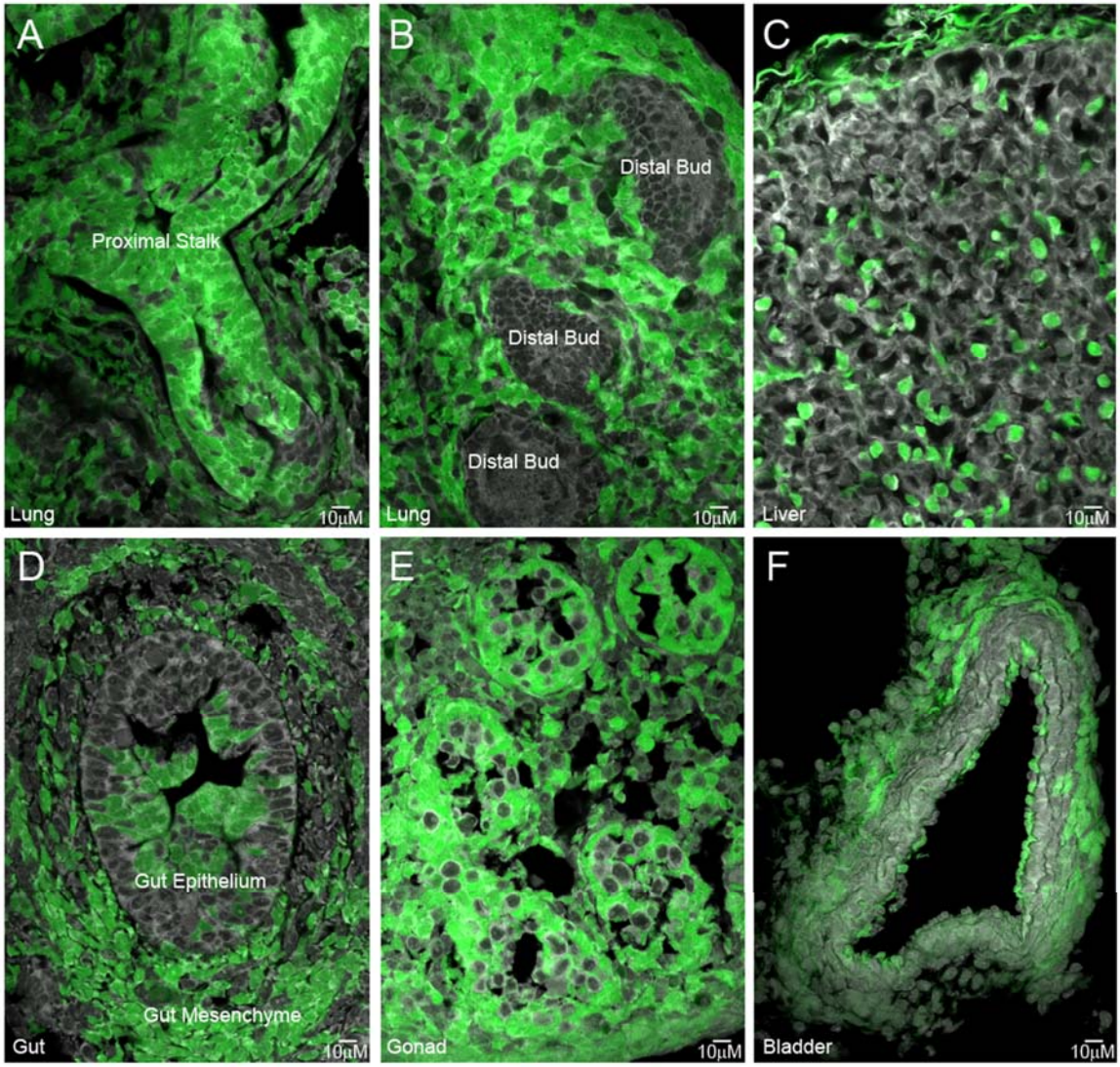

Figure S4. Extensive Integration of GFP-TfR1$^{-/-}$ (green) Cells in Epithelia and Mesenchyme of E15

(A and B) Lung
(C) Liver
(D) Midgut
(E) Gonad
(F) Bladder

GFP-TfR1$^{-/-}$ cells were present in proximal stalks (A) but not in distal tips of the lung (B).

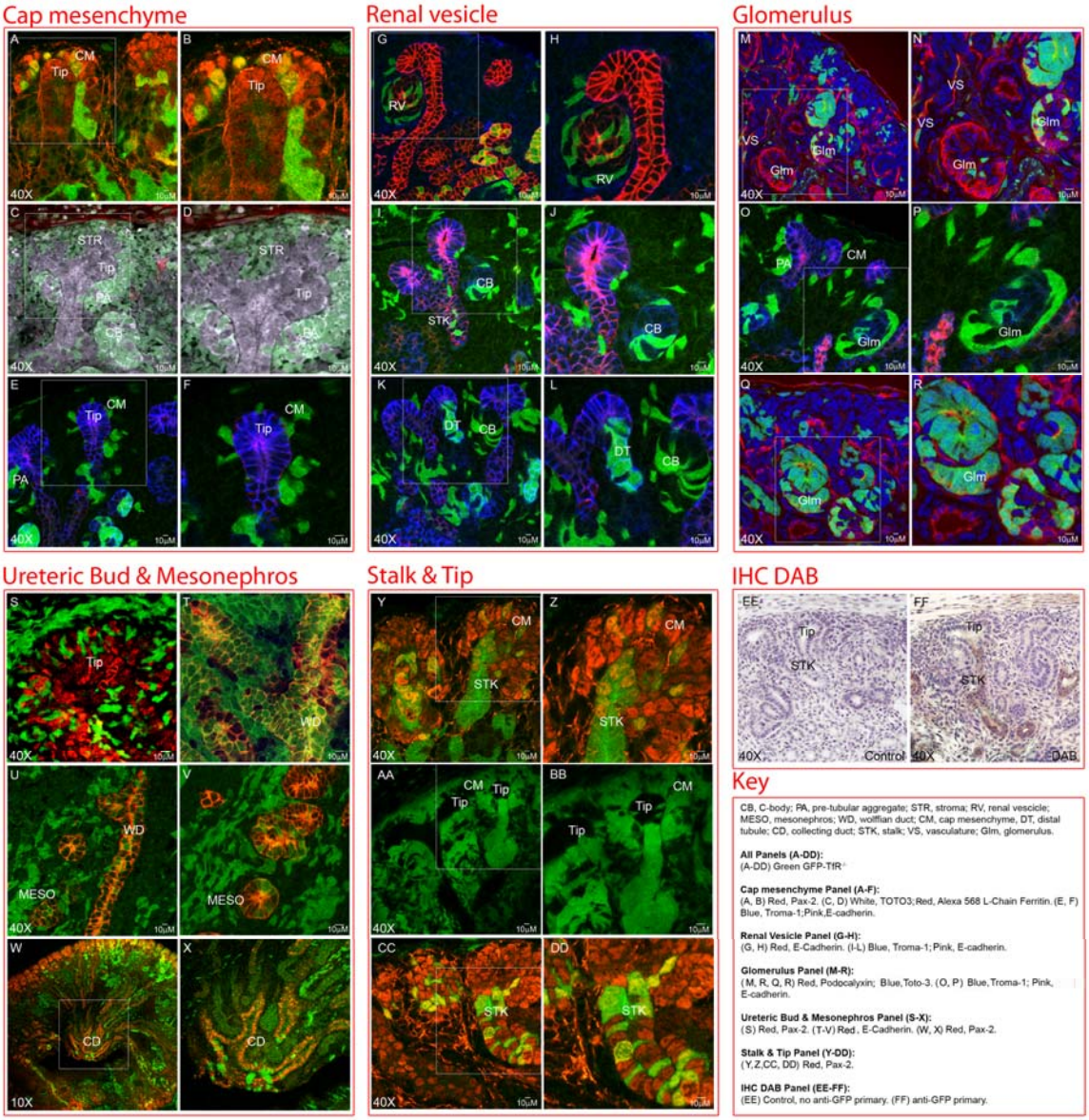

Figure S5. Extensive Integration of GFP-TfR1$^{-/-}$ (green) Cells in the E15 Kidney
GFP-TfR1$^{-/-}$ cells differentiated into cap mesenchyme (A-F,S,Y,Z,AA,BB) expressing Pax2 (A,B,S,Y,Z), as well as the epithelial derivatives of cap mesenchyme, including pretubular aggregates, renal vesicles and C-bodies (C, D, G-L) and segments of the nephron (distal tubule: K, L; glomerulus: M-R). The Wolffian duct (T-V), UB stalks (Y-DD, EE, EF) and collecting ducts (W, X) also demonstrated many examples of GFP-TfR1$^{-/-}$ integration, whereas these cells were infrequent in UB tips (C, D, Y-FF), despite extensive chimerism (C, D, AA, BB).

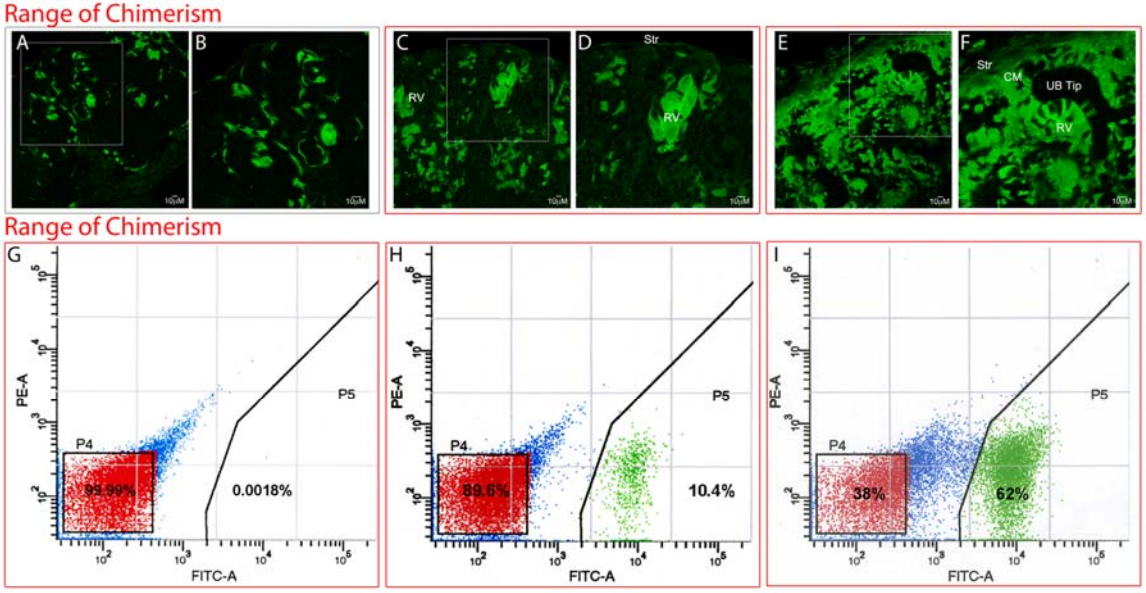

Figure S6. A Range of GFP-TfR1$^{-/-}$ Cell Chimerism Was Detected in E15 Kidneys using (A-F) Confocal Microscopy and (G-I) FACS Analysis
Six different kidneys are shown. Note extensive chimerism in stroma, cap mesenchyme and renal vesicles, but few GFP-TfR1$^{-/-}$ cells were present in the tips of the UB (F).

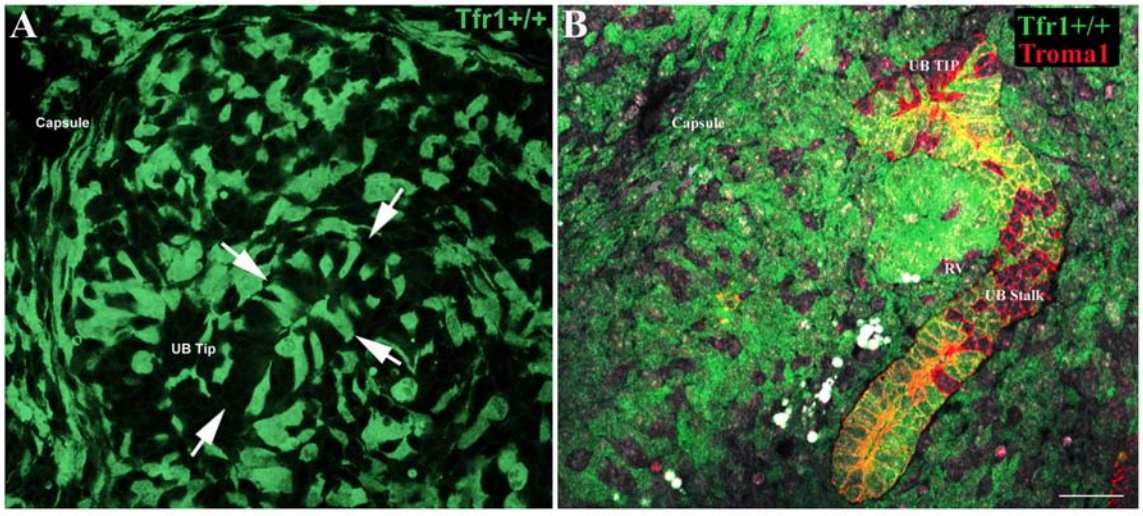

Figure S7. GFP-TfR1$^{+/+}$ ES Cells (green) Populate All Compartments of the Kidney
(A) Arrows depict GFP-TfR1$^{+/+}$ cells in the UB tip.
(B) Troma1 depicts GFP-TfR1$^{+/+}$ cells in UB tip and stalk. RV = renal vesicle. Bar = 30μm.

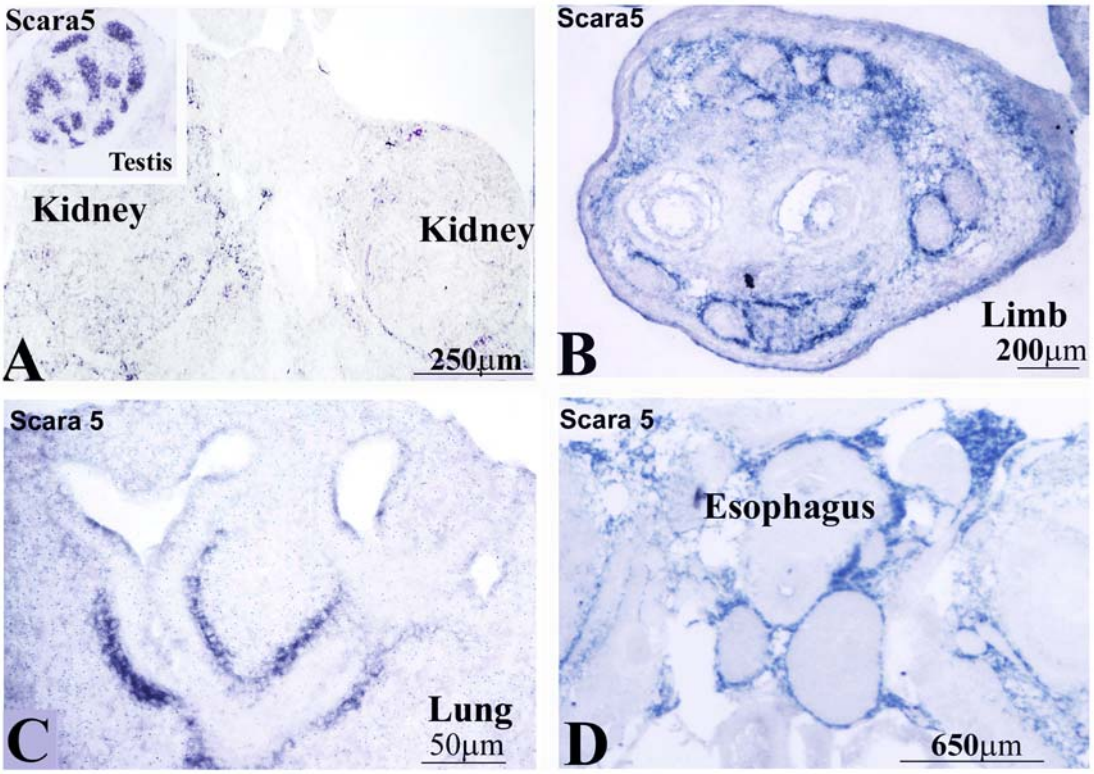

Figure S8. In Situ Hybridization of Scara5 in Embryonic Organs
Scara5 was expressed by (A) epithelia of the testis and kidney stroma, (B) by perimyseal limb mesenchyme, (C) by airway mesenchyme, and (D) by stroma of the upper gastrointestinal tract and great vessels.

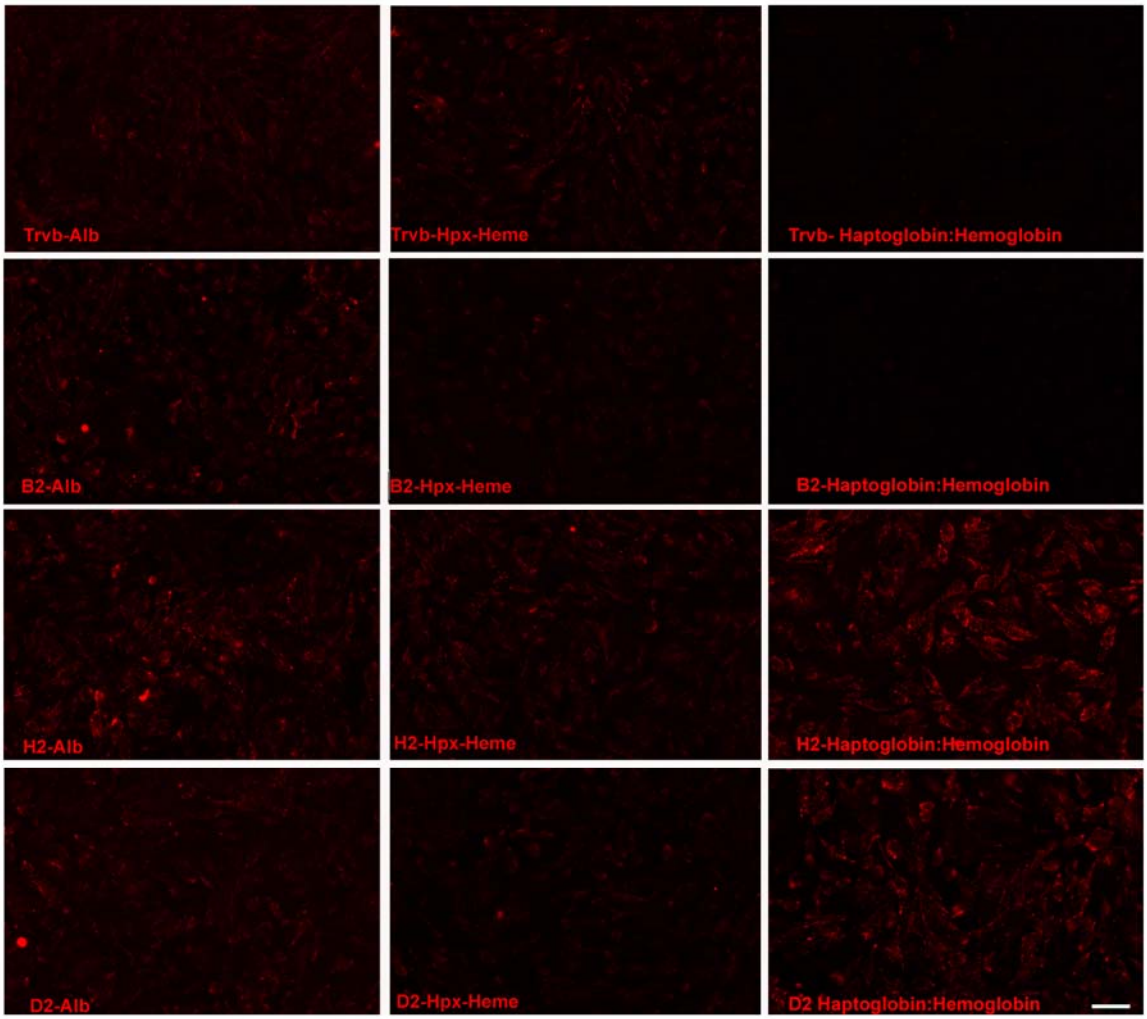

Figure S9. Endocytosis of Fluorescent Albumin (Alb), Heme-Hemopexin (hpx-

heme), and Haptoglobin-Hemoglobin Was Analyzed in Scara5$^-$ (Trvb, B2) and Scara5$^+$ (D2 and H2) Clones
Haptoglobin-hemoglobin alone was recognized by Scara5$^+$ clones. Bar = 10μm.

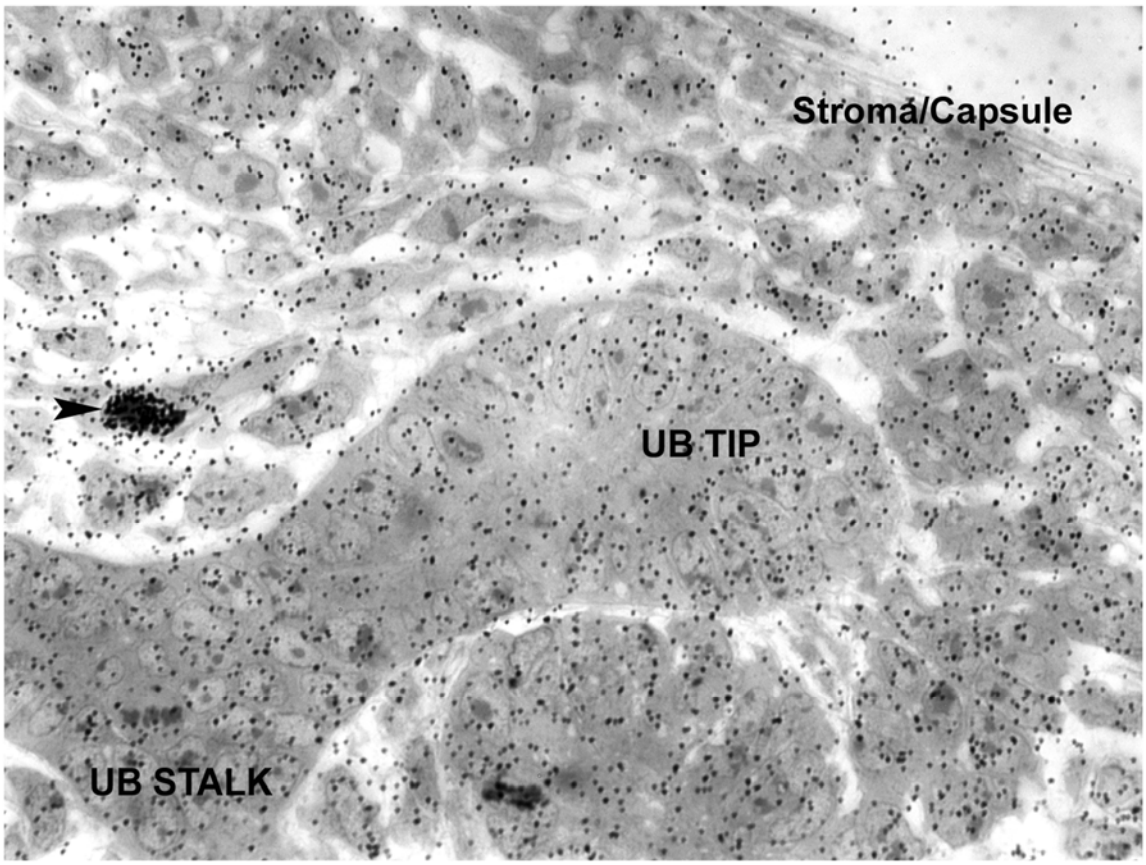

Figure S10. ^{55}Fe-Radioautography
Embryonic kidney was harvested 12-24 hours after ^{55}Fe administration (0.8mCi, Orlic et al., 1974) and ^{55}Fe was detected in Epon sections (1μm) coated with K5D emulsion (Ilford) and developed with Microdol after 3 mo. ^{55}Fe was distributed uniformly in the kidney except for red cells in nascent capillaries (arrowhead).

SUPPLEMENTAL REFERENCE

Orlic, D., Lev, R., and Rosenthal, W.S. (1974). Fetal rat utilization of 55Fe absorbed by fetal intestine from swallowed amniotic fluid. Blood *43*, 429–436.