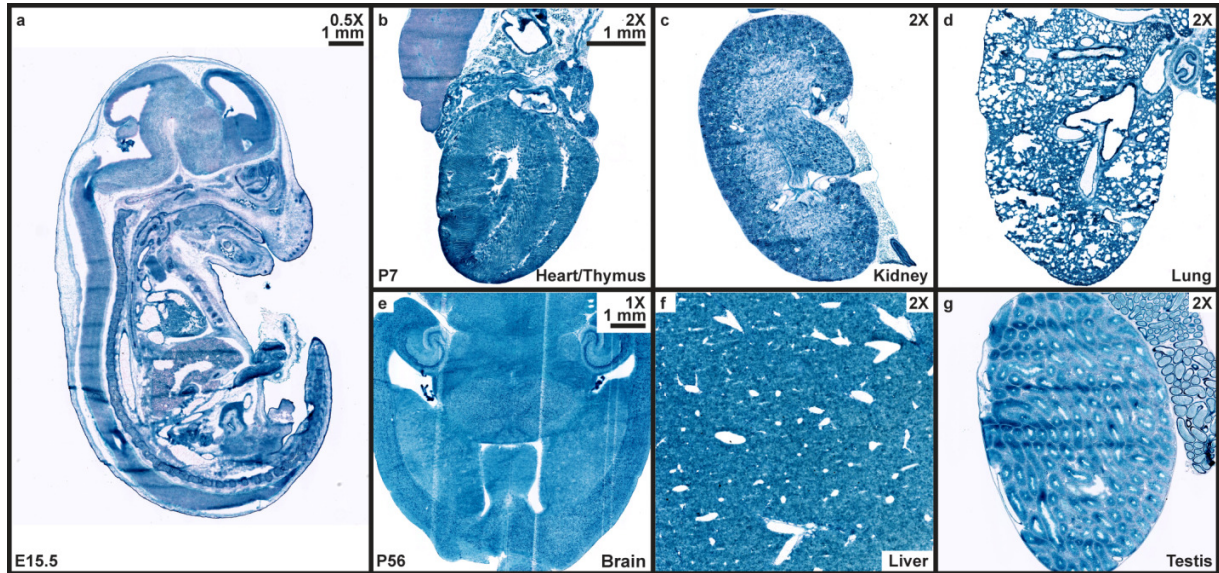
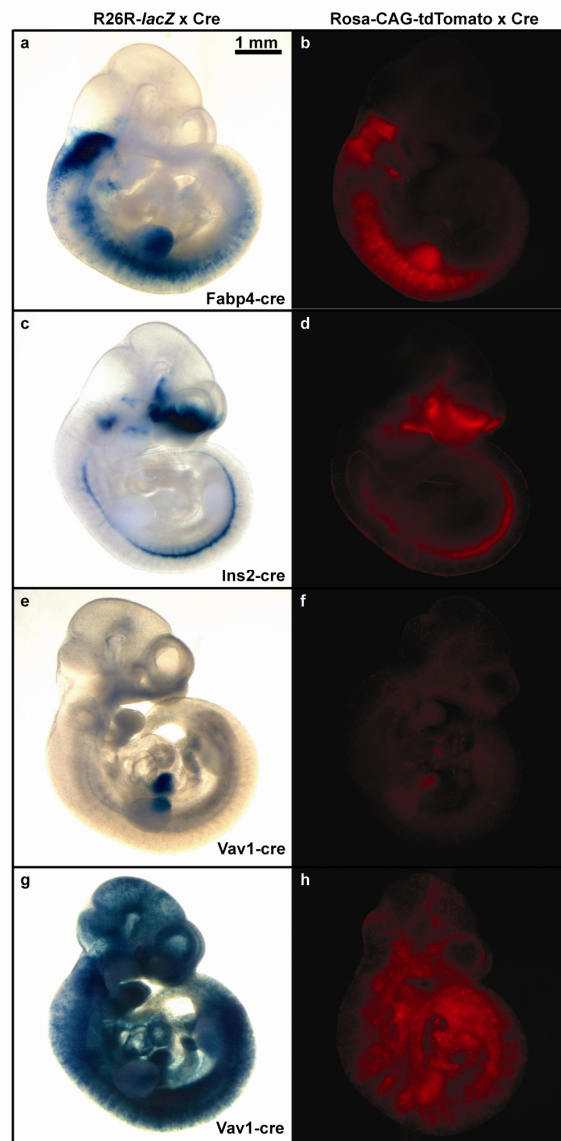


**Supplementary Figure S1: The R26R-lacZ reporter allele is expressed throughout all tissues and cell types**



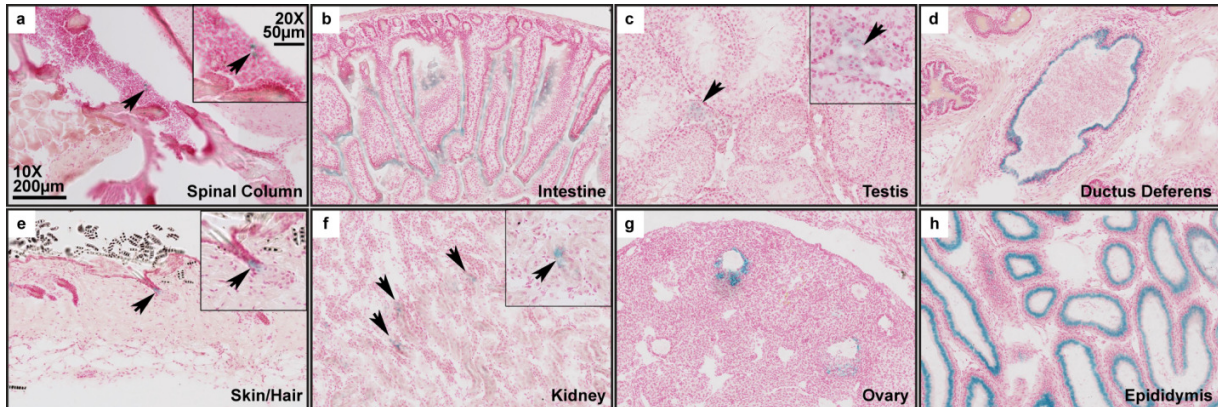
**Supplementary Figure S1** (a-g) Select datae from reporter validation experiments utilizing germline deleted R26R-lacZ mice crossed to C57Bl/6J animals to analyze reporter gene expression in the absence of cre. (a) E15.5 embryo showing  $\beta$ -galactosidase expression throughout all developing tissues. (b-d) Ubiquitous  $\beta$ -galactosidase expression in postnatal day 7 Heart/Thymus (b), Kidney (c), and Lung. (e-g) Ubiquitous  $\beta$ -galactosidase expression in adult (P56) Brain (e), Liver (f), and Testis/Epididymis (g).

**Supplementary Figure S2: Cre-mediated R26R-lacZ reporter gene expression is recapitulated using an independent fluorescent reporter strain**



**Supplementary Figure S2** Comparison of reporter expression patterns in E10.5 embryos harvested from timed matings between R26R-lacZ and Rosa-CAG-tdTomato female mice crossed to male mice harboring the cre allele indicated. (a and b) Fabp4-cre/R26R-lacZ embryonic reporter expression is reproduced in Fabp4-cre/Rosa-CAG-tdTomato embryos. (c and d) Ins2-cre/R26R-lacZ embryonic reporter expression is reproduced in Ins2-cre/Rosa-CAG-tdTomato embryos. (e and f, g and h) Inconsistent Vav1/R26R-lacZ embryonic reporter expression highlighted in Figure 5 is recapitulated in Vav1-cre/Rosa-CAG-tdTomato embryos.

**Supplementary Figure S3: Endogenous  $\beta$ -galactosidase activity in B6.129S4-*Gt(ROSA)26Sor<sup>tm1Sor</sup>/J* mice**



**Supplementary Figure S3** (a-h) Select tissue sections at 10x magnification from R26R-lacZ mice expressing endogenous  $\beta$ -galactosidase. (a) Isolated cells express  $\beta$ -gal in vertebral bone marrow (arrowhead). (b) Intestinal lumen displays endogenous activity in R26R-lacZ mice. (c) Interstitial cells (arrowhead) of adult testis display isolated areas of expression. (d) Endothelial cells throughout the ductus deferens express homogenously. (e) Endogenous expression in cells located apically in the sebaceous glands of hair follicles (arrowhead). (f) Isolated renal tubules (arrowheads) exhibit diffuse  $\beta$ -gal activity. (g) Select follicular granulosa cells have endogenous  $\beta$ -gal expression. (h) Background expression observed in epithelial cells of the ductuli efferentes and ductus epididymis. Insets represent 20x magnified images of the select tissue to highlight areas of endogenous  $\beta$ -gal expression.

**Supplementary Table S1: Tissues annotated for  $\beta$ -galactosidase expression in P7 and P56 reporter mice**

| System                   | Organs           | Structures                       | Substructures/Cell Types    |
|--------------------------|------------------|----------------------------------|-----------------------------|
| Reproductive System      | Testis           | Seminiferous Tubules             | Leydig/Interstitial Cells   |
|                          |                  |                                  | Sertoli Cells               |
|                          | Epididymis       | Head, Body and Tail Region       | Spermatogonia/Spermatocytes |
|                          |                  |                                  | Interstitial Tissues        |
|                          | Ovary            | Corpus Luteum                    | Epithelium                  |
| Oocyte/Ovum              |                  |                                  |                             |
| Uterus                   | Endometrium      |                                  |                             |
|                          |                  | Endometrium Glandular Epithelium |                             |
|                          |                  | Myometrium                       |                             |
| Renal/Urinary System     | Kidney           | Medulla and Cortex Regions       | Renal Tubules               |
|                          |                  | Renal Pelvis/Papilla             | Renal Corpuscles            |
|                          | Urinary Bladder  | Urothelium                       |                             |
|                          |                  | Smooth Muscle                    |                             |
| Endocrine System         | Adrenal gland    | Cortex                           |                             |
|                          |                  | Medulla                          |                             |
|                          | Pancreas         | Pancreatic Ducts                 | Acinar Cells                |
|                          |                  |                                  | Islets of Langerhans        |
| Haemolymphoid System     | Spleen           | Red Pulp                         | Megakaryocytes              |
|                          |                  | White Pulp                       |                             |
|                          |                  | Trabeculae                       |                             |
|                          | Thymus           | Cortex                           |                             |
|                          |                  | Medulla                          |                             |
|                          | Lymph Node       |                                  |                             |
| Gastrointestinal System  | Large Intestine  | Epithelium                       |                             |
|                          |                  | Smooth Muscle                    |                             |
|                          | Small Intestine  | Epithelium                       |                             |
|                          |                  | Smooth Muscle                    |                             |
| Stomach                  | Epithelium       |                                  |                             |
|                          | Smooth Muscle    |                                  |                             |
| Liver and biliary system | Liver            | Bile ducts                       | Hepatocytes                 |
| Respiratory System       | Lung             | Bronchiole                       |                             |
|                          |                  | Alveolus                         |                             |
| Cardiovascular System    | Heart            | Ventricles                       | Myocardium                  |
|                          |                  |                                  | Endocardium                 |
|                          |                  | Atria                            | Myocardium                  |
|                          |                  | Valves                           | Endocardium                 |
|                          | Arterial System  | Aorta                            |                             |
|                          | Pulmonary Artery |                                  |                             |
|                          | Venous system    | Vena Cava                        |                             |
| Musculoskeletal System   | Skeletal Muscle  |                                  |                             |
|                          |                  |                                  |                             |
| Integumentary System     | Bone             | Trabecular/Compact               |                             |
|                          |                  | Marrow                           |                             |
|                          |                  | Dermis                           |                             |
|                          | Skin             | Epidermis                        |                             |
|                          |                  | Hair Follicle                    |                             |
| Adipose tissue           | White Fat        |                                  |                             |
|                          | Brown Fat        |                                  |                             |
|                          | Mammary gland    |                                  |                             |
| Nervous System           | Brain            | Cerebral Cortex                  |                             |
|                          |                  | Cerebellum                       |                             |
|                          |                  | Hippocampus                      |                             |
|                          |                  | Thalamus                         |                             |
|                          |                  | Midbrain                         |                             |
|                          |                  | Hypothalamus                     |                             |
|                          |                  | Medulla                          |                             |
|                          |                  | Pons                             |                             |
|                          |                  | Olfactory Lobe                   |                             |
|                          | Choroid Plexus   |                                  |                             |
| Spinal Cord              | Cervical         | White/Gray Matter                |                             |
|                          | Thoracic         | White/Gray Matter                |                             |
|                          | Lumbar/Sacral    | White/Gray Matter                |                             |
| Peripheral Nerves        |                  |                                  |                             |
| Sensory System           | Eye              | Retina                           |                             |
|                          |                  | Optic Nerve                      |                             |
|                          |                  | Cornea                           |                             |
|                          |                  | Lens                             |                             |



**Supplementary Table S2: Tissues annotated for  $\beta$ -galactosidase expression at E15.5**

| System                   | Organs                    | Structures                         | Substructures/Cell Types   |
|--------------------------|---------------------------|------------------------------------|--|
| Limb                     | Forelimb                  | Forearm                            | Mesenchyme<br>Radius<br>Ulna<br>Skin   |
|                          |                           | Upper Arm                          | Mesenchyme<br>Humerus<br>Skeletal Muscle<br>Skin                             |
|                          | Handplate                 | Digit 1                            |  |
|                          |                           | Digit 2                            |  |
|                          |                           | Digit 3                            |  |
| Mesenchyme               | Head Mesenchyme           |                                    |  |
|                          | Trunk Mesenchyme          | Body-wall Mesenchyme               |  |
|                          | Tail Mesenchyme           |                                    |  |
| Cardiovascular System    | Arterial System           | Aorta                              |  |
|                          | Heart                     | Atrium                             | Interatrial Septum<br>Cardiac Muscle   |
|                          |                           | Endocardial Cushion                |  |
|                          |                           | Pericardium                        |  |
|                          |                           | Valve                              |  |
|                          |                           | Ventricle                          | Interventricular Septum<br>Cardiac Muscle                                    |
|                          | Pericardial Cavity        |                                    |  |
| Venous System            | Portal Vein<br>Vena Cava  |                                    |  |
| Glands                   | Adrenal Gland             |                                    |  |
| Hemolymphoid System      | Spleen                    |                                    |  |
|                          | Primordium                |                                    |  |
|                          | Thymus                    |                                    |  |
|                          | Primordium                |                                    |  |
| Integumental System      | Muscle                    |                                    |  |
|                          | Skin                      | Epidermis<br>Dermis                |  |
| Nervous System           | Central Nervous System    | Forebrain                          | Diencephalon<br>Hypothalamus<br>Thalamus<br>Telencephalon<br>Cerebral Cortex |
|                          |                           | Midbrain                           |  |
|                          |                           | Hindbrain                          | Medulla  |
|                          |                           | Ganglion                           | Cranial<br>Cranial   |
|                          | Peripheral Nervous System | Nerve                              | Spinal Cord  |
|                          |                           | Spinal                             | Dorsal Root Ganglion   |
|                          | Sensory Organ             | Ear                                | Inner Ear<br>Cornea  |
| Eye                      |                           | Eye Lid<br>Lens<br>Retina          |  |
| Nose                     |                           |                                    |  |
| Alimentary System        | Gut                       | Foregut                            | Esophagus<br>Pharynx<br>Tongue<br>Stomach                                    |
|                          |                           | Hindgut                            |  |
|                          |                           | Midgut Gland                       | Salivary Gland<br>mesenchyme   |
|                          | Oral region               | Upper Jaw                          | Palatal Shelf<br>Skeleton<br>Maxilla   |
|                          |                           | Lower Jaw                          | Meckel's Cartilage<br>mesenchyme<br>Skeleton<br>Tooth                        |
|                          |                           | Oral Epithelium                    |  |
|                          |                           | Tooth                              |  |
| Liver and Biliary System | Gall Bladder<br>Liver     |                                    |  |
| Renal and Urinary System | Bladder<br>Metanephros    |                                    |  |
| Reproductive System      | Ovary<br>Testis           |                                    |  |
| Respiratory System       | Lung                      |                                    |  |
|                          | Respiratory Tract         | Larynx<br>Trachea<br>Main Bronchus |  |
|                          | Diaphragm                 |                                    |  |
| Skeleton                 | Axial Skeleton            | Cervical region                    | Vertebra   |
|                          |                           | Lumbar Region                      | Vertebra   |
|                          |                           | Thoracic region                    | Rib<br>Vertebra<br>Vertebra  |
|                          | Tail Region               | Vertebra                           |  |
| Cranium                  | Chondrocranium            |                                    |  |
| Extraembryonic Component | Arterial System           | Umbilical Artery                   |  |
|                          | Venous System             | Umbilical Vein                     |  |

**Supplementary Table S3: Tissues annotated for  $\beta$ -galactosidase expression at E10.5**

| System                   | Organs                              | Structures  | Substructures/Cell Types             |
|--------------------------|-------------------------------------|---|--------------------------------------|
| Branchial Arches         | 1st Arch                            | Mandibular Component                                  | Ectoderm<br>Endoderm<br>Mesenchyme   |
|                          |                                     | Maxillary Component                                   | Ectoderm<br>Endoderm<br>Mesenchyme   |
|                          | 2nd Arch                            |   |                                      |
|                          | 3rd Arch                            |   |                                      |
|                          | 4th Arch                            |   |                                      |
| Limb                     | Forelimb Bud                        | Ectoderm<br>Mesenchyme                                |                                      |
|                          | Hindlimb Bud                        | Ectoderm<br>Mesenchyme                                |                                      |
| Mesenchyme               | Head Mesenchyme                     |   |                                      |
|                          | Trunk Mesenchyme                    | Body-wall Mesenchyme<br>Paraxial Mesenchyme<br>Somite |                                      |
| Cardiovascular System    | Arterial System                     | Dorsal Aorta  |                                      |
|                          | Heart                               | Atrio-ventricular Canal                               |                                      |
|                          |                                     | Atrium  |                                      |
|                          |                                     | Bulbous Cordis  |                                      |
|                          |                                     | Endocardial Cushion                                   |                                      |
|                          |                                     | Outflow Tract   |                                      |
|                          |                                     | Primitive Ventricle                                   | Cardiac Muscle<br>Endocardial Lining |
| Venous System            | Sinus Venosus<br>Cardinal Vein      |   |                                      |
| Nervous System           | Central Nervous System              | Forebrain   | Diencephalon<br>Telenchephalon       |
|                          |                                     | Hindbrain   |                                      |
|                          |                                     | Midbrain  |                                      |
|                          |                                     | Presumptive Spinal Cord                               |                                      |
|                          |                                     | Neural Tube   |                                      |
|                          |                                     | Ganglion  |                                      |
|                          | Periphereal Nervous System          | Cranial   |                                      |
|                          |                                     | Spinal  | Dorsal Root Ganglion                 |
| Sensory Organs           | Ear                                 | External Ear  |                                      |
|                          |                                     | Inner Ear   | Otocyst                              |
|                          | Eye                                 | Lens Pit  |                                      |
|                          |                                     | Mesenchyme  |                                      |
|                          |                                     | Optic Cup   |                                      |
| Nose                     | Optic Stalk                         |   |                                      |
|                          | Fronto-nasal Process                |   |                                      |
| Alimentary System        | Gut                                 | Foregut   | Oesophagus<br>Stomach                |
|                          |                                     | Hindgut   |                                      |
|                          |                                     | Midgut  |                                      |
|                          | Oral Region                         | Oral Epithelium                                       |                                      |
| Liver and Biliary System | Liver                               | Hepatic Primordium                                    |                                      |
| Respiratory System       | Lung Rudiments<br>Respiratory Tract | Main Bronchus   |                                      |
| Urogenital System        | Mesonephros                         |   |                                      |
| Extraembryonic Component | Umbilical Vein                      |   |                                      |
|                          | Umbilical Artery                    |   |                                      |

## **Supplementary Methods**

### **Intraperitoneal injection of tamoxifen for inducible cre driver lines**

A number of the lines characterized on cre.jax.org express tamoxifen-inducible creER alleles. The following procedure was utilized to induce recombination in creER strains analyzed by The Jackson Laboratory Cre Repository.

Tamoxifen (Sigma-Aldrich) was dissolved in corn oil at a concentration of 20 mg/ml by shaking overnight at 37°C protected from light. For the duration of injections, tamoxifen solution was stored at 4°C. Injection dose was determined by weight, using approximately 75 mg tamoxifen/kg body weight.

For the duration of injections, mice were housed in a separate animal room to reduce potential hazards to animal care personnel. For adult mice, a standard dose of 100µl tamoxifen/corn oil solution (above) was administered via intraperitoneal injection (using an ACUC approved injection procedure) once every 24 hours for a total of 5 consecutive days. Following the final injection, mice were quarantined for 24 hours before returning them to their normal animal room. For cre characterization work at the Jackson Laboratory, there was a 7-day waiting period between the final injection and necropsy/histological analysis. Throughout the course of tamoxifen injections and the post-injection wait period, mice were closely monitored for any adverse reactions to the treatment.