

Endothelial-Specific Cre Mouse Models: Is Your Cre CREdible?

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ABSTRACT

The field of vascular biology has gained enormous insight from the use of Cre and Cre/ERT2 mouse models to temporally and spatially manipulate gene expression within the endothelium. Models are available to constitutively or inducibly modulate gene expression in all, or a specified subset of endothelial cells (ECs). However, caution should be applied to both the selection of allele and the analysis of resultant phenotype: many similarly-named Cre models have divergent activity patterns, while ectopic or inconsistent Cre or Cre/ERT2 expression can dramatically affect results. In an effort to disambiguate previous data and to provide a resource to aid appropriate experimental design, here we summarise what is known about Cre recombinase activity in the most widely used endothelial-specific Cre and Cre/ERT2 mouse models.

ABBREVIATIONS

EC	endothelial cell
GFP	green fluorescent protein
YFP	yellow fluorescent protein
tpA	transcriptional termination
β-gal	beta-D-galactosidase
MLPA	multiplex ligation-dependent probe amplification
MGI	mouse genome informatics
TG	transgene
tm	targeted mutation
bp	base pair
kb	kilo base pair
PAC	P1-derived artificial chromosome
BAC	bacterial artificial chromosome
E	embryonic day
P	postnatal day
DA	dorsal aorta
CV	cardinal vein
END	endocardial lining of heart

INTRODUCTION

The Cre-loxP system: The Cre-loxP system is a powerful genetic tool that allows mice with tissue-specific genetic loss or gain of function to be generated with relative ease. Cre recombinase, a protein from the bacteriophage P1, binds a 34bp *loxP* recognition sequence. Upon binding to two *loxP* sites in the same orientation, Cre recombinase causes any intervening DNA to be excised from the genome leaving only a single *loxP* site¹⁻⁴. Coding regions of interest can therefore be flanked with *loxP* sites such that they are removed from the genome upon Cre binding.

Restricting the expression of Cre recombinase with specific enhancer/promoter elements therefore allows for spatial control of *loxP*-mediated excisions. Temporal control of gene excision beyond that which can be regulated by promoter/enhancer activity can be provided by using the modified Cre/ERT2, in which Cre recombinase activity must be actively induced by tamoxifen administration.

Determining expression patterns of Cre and Cre/ERT2 mouse alleles:

Successful gene targeting with Cre and Cre/ERT2-*loxP* is critically determined by the

precise expression pattern of Cre recombinase. A single Cre-mediated excision event is passed on during cell division to all subsequent daughter cells. In this way, Cre recombination is able to mark cell lineages. It follows, therefore, that brief expression of Cre in an unreported cell type early in development can have profound consequences on any later phenotype or fate-mapping experiment. So too can lack of Cre expression in a small number of cells within a theoretically Cre-positive population ⁵. Given the dynamic nature of spatio-temporal gene expression, it can sometimes be challenging to predict or detect these events. Further, copy number variation and site of integration effects can strongly influence the expression patterns of transgenes, such that independently-derived transgenic mouse alleles driving Cre or Cre/ERT2 with identical regulatory sequences may have significantly different Cre expression patterns ⁶. Consequently, many endothelial-specific Cre and Cre/ERT2 alleles have some degree of unreported activity, inconsistent recombination efficiency and/or parent of origin effects. Generational drift can also strongly influence outcomes, as Cre expression may be lost or altered in mice many generations downstream of those original characterized. All these variables can lead to results that are difficult to interpret and in some cases seemingly contradictory. A thorough characterisation of Cre expression in each different mouse model is therefore essential in order to generate meaningful data.

Spatial information about Cre recombinase activity can be tracked using Cre reporter mice, in which a reporter gene (e.g. *GFP*, *YFP*, *lacZ*) is inserted at a ubiquitously expressed locus but preceded by a *loxP*-flanked transcriptional termination (tpA) sequence. Therefore, the reporter gene is transcribed only when Cre mediated recombination has excised the termination sequence. The most common of these reporter mice is the R26R-*LacZ*, where *loxP*-tpA-*loxP* precedes the β -*gal* gene at the ubiquitously expressed ROSA26 locus ^{7,8}. Because unanticipated Cre activity can vary between mice, it is imperative that such analyses extend to testing multiple different mice expressing the Cre transgene, preferably from different parents ⁹. However, although Cre reporter mice provide excellent spatial information on Cre activity, their sensitivity is limited by nature of the reporter protein: it can be challenging to assess small differences in Cre levels, while length of reporter half-life can also be an issue. Precise temporal information can also be obtained using qPCR to measure mRNA expression of a floxed gene directly, although this can only reliably detect a difference of 2-fold or more over a control. Improvements on qPCR exist, such as the extension MLPA (eMLPA) assay which also detects the presence of circular excised DNA ¹⁰. However, as such assays require tissue homogenization, they do not provide detailed spatial information afforded by Cre reporter animal, and are thus best utilized as additional assays rather than as a replacement.

Side-effects of Cre-induced recombination: There have been reports linking Cre-recombinase activity in mammalian cells with growth inhibition, DNA damage and ectopic activation of an anti-viral immune response ¹¹⁻¹³. These effects are thought to be independent of legitimate *loxP* recombination, instead associated with high levels of Cre recombinase, secondary recombination at pseudo-*loxP* sites and associated DNA damage. While so far poorly studied in mouse models, these potentially confounding responses may be an important consideration for some applications in vascular biology.

ENDOTHELIAL-SPECIFIC CRE AND CRE/ERT2 MOUSE MODELS

This review provides a summary of the most commonly used endothelial-specific constitutive and inducible Cre models and their Cre recombinase activity, both as a resource to aid experimental design and to facilitate interpretation of previously reported data. Whilst not intended to be an exhaustive list, those discussed here constitute the vast majority of all Cre mouse alleles currently in use to study gene function in the endothelium. To aid reference we have used the official MGI nomenclature throughout: transgenes randomly inserted into the genome are denoted as *Tg(Gene locus-Cre)^{serialnumber labcode}*, while knock-in models are instead denoted as *Gene^{tm#(Cre)labcode}*, tm# indicating designated targeted mutation number.

Pan-endothelial models

Constitutively active pan-endothelial Cre mouse models

In constitutively active pan-endothelial Cre transgenic mice, Cre recombinase activity is driven by enhancer and/or promoter regions that are generally considered to be expressed in all endothelial cell (EC) populations from early in development. Mouse models using these Cre alleles are therefore frequently referred to simply as endothelial-specific knock out animals. However, important differences exist between different constitutively active pan-endothelial alleles, including wide variations in expression in different vascular beds and onset of enhancer/promoter activity. A detailed understanding of the precise expression pattern of Cre recombinase activity is therefore important when interpreting any phenotypes generated with these models. Here we examine what is known about Cre expression in the most frequently cited pan-endothelial constitutive Cre alleles.

Tek-Cre (Tie2-Cre): The first endothelial-specific Cre mouse models used the regulatory sequences of the *Tek* gene (also known as *Tie2*), and remain the most commonly used. *Tek/Tie2* encodes an angiopoietin receptor, a member of the receptor tyrosine kinase family, that is expressed in all ECs from very early in development and into adulthood¹⁴⁻¹⁸. In 1997 Schlaeger *et al.* demonstrated that a combination of the *Tek/Tie2* promoter region with an intron 1 enhancer sequence is capable of directing strong EC-specific reporter gene expression¹⁹. Subsequently, by placing *Cre* cDNA between a 2.1kb promoter region and a 10kb fragment from intron 1, two groups simultaneously created *Tek-Cre* transgenic mouse alleles: *Tg(Tek-cre)^{1Ywa}*²⁰ and *Tg(Tek-cre)^{12Flv}*²¹. Other *Tek-Cre* alleles made with the same strategy are *Tg(Tek-cre)^{5326Sato}*, *Tg(Tek-cre)^{1Rwng}* and *Tg(Tek-cre)^{1Xyfu}*²²⁻²⁴. However, these alleles have limited characterisation and are far less widely used than the *Tg(Tek-cre)^{1Ywa}* and *Tg(Tek-cre)^{12Flv}* alleles. Despite being made using the same transgenic strategy, important differences in the activity of Cre recombinase exist between the *Tg(Tek-cre)^{1Ywa}* and *Tg(Tek-cre)^{12Flv}* mouse alleles, presumably due to copy number variation or position effects from transgene insertion sites.

In both *Tg(Tek-cre)^{1Ywa}* and *Tg(Tek-cre)^{12Flv}*, Cre activity begins at E7.5 in a subset of cells in the extra embryonic mesodermal yolk sac. At this stage there is no Cre activity observed in the embryo proper. By E8.5, *Tg(Tek-cre)^{1Ywa}* shows some Cre reporter gene expression within ECs of the embryo, with patchy activity observed in the dorsal aorta and in a small subset of ECs from the common atrial chamber²⁰. In contrast, *Tg(Tek-cre)^{12Flv}* is reported to drive strong pan-endothelial Cre recombinase activity in all small blood vessels as well as the dorsal aorta and umbilical vessels at E8.5²⁵. With both alleles, Cre activity is reported to be pan-EC by E9.5 and remains such throughout development (Fig. 1A,B). In the heart, both alleles show Cre recombinase activity in the endocardium, endocardial cushions and all valves by

E13.5, in addition to the coronary vasculature. In the adult, reporter gene activity is observed in all vascular beds ²¹ and in ECs of the lymphatic vasculature, as is to be expected from their venous endothelial origin ²⁶.

All *Tek-Cre* mouse models show some degree of Cre recombinase activity in the haematopoietic lineage. With the *Tg(Tek-cre)^{1Ywa}* allele, a small number of circulating cells are reported to be Cre positive in the adult mouse ²⁰. However, using the *Tg(Tek-cre)^{12Flv}* allele, Tang *et al.*, report that 82% of blood cells from the embryonic yolk sac and 85% of circulating adult blood cells show Cre reporter gene activity ²⁷. These data suggest that *Tie2* is transiently expressed in a precursor cell that gives rise to both blood and endothelial lineages.

The regulatory sequences that drive expression of the *Tek-Cre* transgene also drive expression in the female germ line. To avoid the generation of a null allele, all *Tek-Cre* alleles must be transmitted to offspring via the male ^{21,28}. However, even when this is carefully adhered to, null alleles are known to appear within *Tie2-Cre* colonies ⁵. This is likely due to variable expression of the *Tie2* regulatory sequences very early in embryogenesis and/or their germ cell activation.

Tek-Cre mouse alleles are prone to variable and non-specific Cre recombinase activity ⁵. Theis *et al.*, report non-specific recombination in 13% of the second generation using the *Tg(Tek-cre)^{5326Sato}* allele ²². Despite this not being reported in their original characterisations, it is now apparent that other *Tek-Cre* alleles also suffer from a similar variability. Whilst using the *Tg(Tek-cre)^{12Flv}* allele, we and others have observed Cre recombinase activity can range from EC-specific to near ubiquitous within a single litter ⁵.

***Cdh5-Cre (VE-Cadherin-Cre)*:** Cadherin 5 (*Cdh5*, also known as vascular endothelial (VE) cadherin), is localised at the EC junction, where it mediates cell adhesion and plays a crucial role in vessel assembly. *Cdh5* null mice die at mid gestation due to severe vascular defects, and abnormal somite and heart development (reviewed ²⁹⁻³¹). A region extending 2.2kb from the *Cdh5* transcriptional start site is reported to contain both an enhancer element able to independently drive EC expression and a negative regulatory element inhibiting *Cdh5* expression in other cell types ³². This 2.2kb regulatory region drives Cre recombinase expression in the *Tg(Cdh5-cre)^{7Mlia}* mouse, with Cre activity detected in the yolk sac at E7.5, and in the embryo by E8.5. However, *Tg(Cdh5-cre)^{7Mlia}* is expressed only sporadically within the ECs of the dorsal aorta at E10.5, and Cre activity is also variable between littermates at early stages ³³. Cre recombinase reporter gene activity increases during development with expression in all observed blood and lymphatic vasculature by E14.5 and into adulthood ³³.

Other constitutive *Cdh5-Cre* mouse alleles have been independently generated, including *Tg(Cdh5-cre)^{1Nmoc}* and *Tg(Cdh5-cre)^{1Spe}* ^{34,35}. These alleles both use the same 2.2kb upstream fragment previously described, but *Tg(Cdh5-cre)^{1Spe}* has the addition of flanking 2kb insulator sequences from the chicken γ -globin gene. *Tg(Cdh5-cre)^{1Spe}* shows patchy expression at E9.5, with more widespread activity observed at E10.5 but a curious absence in the head vasculature at this time point. The *Tg(Cdh5-cre)^{1Nmoc}* is not well characterised, but it has been shown that Cre recombinase reporter gene expression can be observed in the dorsal aorta by E10.5 and is pan-EC in the adult ³⁴.

Similar to *Tie2-Cre*, the *Cdh5-Cre* alleles show Cre recombinase reporter gene expression in haematopoietic cells. For the *Tg(Cdh5-cre)^{1Spe}* allele, it has been demonstrated that 85% of foetal blood cells and 96% of CD45+ adult bone marrow-derived cells were positive for Cre recombinase reporter gene activity³⁵. Away from the haematopoietic system, little non-specific or ubiquitous Cre activity has been noted for these alleles, although they are far less widely used than *Tie2/Tek-Cre* and therefore variations may not have been reported.

Kdr-Cre (Flk1-Cre, VEGFR2-Cre): *Kdr (Flk1/VEGFR2)* encodes a receptor tyrosine kinase absolutely required for VEGF-induced EC proliferation, survival and migration³⁶. Widely expressed in early ECs, *Kdr* is also robustly expressed in the precursor cells that give rise to endothelial, haematopoietic and muscle cell lineages³⁷. The *Kdr-Cre* mouse allele *Tg(Kdr-cre)^{15962Brei}* was generated using a 969-bp fragment of the *Kdr* promoter and a previously characterised 510-bp EC-specific enhancer from intron 1 of the *Kdr* gene³⁷⁻³⁹. While the *Kdr*-intron1 enhancer is active in ECs by E7.8³⁸, the earliest Cre recombination activity demonstrated in the *Tg(Kdr-cre)^{15962Brei}* allele is reported at E9.5 exclusively in the head and vessels of the upper trunk, although activity is pan-endothelial by E11.5³⁹. *Tg(Kdr-cre)^{15962Brei}* driven reporter gene activity is seen in coronary vessels and the endocardium but is absent from the myocardium³⁹. Similar to *Tek-Cre*, *Tg(Kdr-cre)^{15962Brei}* also shows Cre mediated recombination in most haematopoietic cells and in the bone marrow.

Another *Kdr-Cre* allele, *Kdr^{tm1(cre)Sato}*, was generated by knock in of Cre recombinase into the first exon of the *Kdr* locus, thus creating a null allele. *Kdr^{tm1(cre)Sato}* is active slightly earlier than *Tg(Kdr-cre)^{15962Brei}* with Cre recombination observed at E8.5⁴⁰. However, the *Kdr^{tm1(cre)Sato}* allele is also active in haematopoietic cells, skeletal and cardiac muscle, limiting its usefulness as an EC-specific Cre.

Summary: Differences in expression patterns between superficially similar Cre models can have profound consequences for the interpretation of phenotypes driven by these transgenes. Therefore, when using any constitutively active pan-EC Cre model, the origins of the specific Cre allele in use must be considered. Further, the extensive potential for non-specific recombination events, and the inherent variability of many of these Cre alleles, should be taken into account during experimental design. Where possible, Cre activity or target gene expression should be assessed in every animal studied, either by the inclusion of a Cre-reporter allele or by direct analysis of gene expression. Further information on the controls required are also described in detail elsewhere⁹.

Inducible pan-endothelial Cre/ERT2 mouse models

Cre/ERT2, an inducible version of Cre recombinase, can be combined with EC-specific enhancer/promoter regions to enable both temporal and spatially controlled activity of Cre recombinase. Cre/ERT2 was created by fusing Cre recombinase cDNA with a mutated version of the estrogen receptor ligand binding domain (ERT2), thus creating a specific receptor for the tamoxifen metabolite OHT. In Cre-ERT2 mouse models, Cre recombinase is still expressed according to its driving regulatory sequences, but remains sequestered in the cytoplasm in complex with Hsp90. After tamoxifen administration, OHT binds to ERT2, preventing interaction with Hsp90 and allowing the Cre recombinase to move to the nucleus where it can recombine *loxP* sites^{41,42}. Tamoxifen can therefore be administered to induce Cre recombinase activity at any required developmental stage, with minimal toxicity (reviewed by⁴³).

Cre/ERT2 mouse models are particularly useful in vascular biology, where constitutive gene deletion often causes lethality early in development. Here we detail the most cited pan-endothelial inducible Cre mouse alleles, with an emphasis on recombination specificity, timing and recombination efficiency.

Cdh5-Cre/ERT2 (VE-cadherin-Cre/ERT2): *Cdh5-Cre/ERT2* alleles are the most commonly used inducible EC-specific Cre mice. There are three different alleles that use regions of the *Cdh5* locus to drive Cre/ERT2 expression: *Tg(Cdh5-cre/ERT2)^{CIVE23Mlia}*⁴⁴, *Tg(Cdh5-cre/ERT2)^{#Ykub}*⁴⁵ and the most widely used *Tg(Cdh5-cre/ERT2)^{1Rha}*⁴⁶. The *Tg(Cdh5-cre/ERT2)^{CIVE23Mlia}* allele (or *VE-cadherin-Cre/ERT2*) was developed using the same 2.2kb *Cdh5* enhancer/promoter element previously used to drive the constitutively-active VE-cadherin-Cre^{32,44}. By contrast, the *Tg(Cdh5-cre/ERT2)^{#Ykub}* was created using a BAC clone containing 200.3kb upstream region of *Cdh5*⁴⁵, while the *Tg(Cdh5-cre/ERT2)^{1Rha}* allele (commonly referred to as *Cdh5-PAC-Cre/ERT2*) were created by inserting the Cre/ERT2 cDNA at the start codon of the *Cdh5* gene in a *Cdh5*-carrying PAC, which was then randomly integrated into the genome^{46,47}.

The *Tg(Cdh5-cre/ERT2)^{1Rha}* allele has been used extensively to study the post-natal vasculature, with tamoxifen administration between P1 and P6 able to induce pan-EC Cre-mediated recombination in both retina and brain endothelium^{46,48}. Tamoxifen injection at two weeks after birth has also been shown to produce efficient recombination in the endocardium⁴⁹. However, as with many inducible Cre alleles, there have been some reports of experimental variability in recombination efficiency, both in relative levels of gene knock-down in different mice given identical tamoxifen doses, and between different floxed allele genes within the same mouse⁵⁰. Relatively little information is available about the Cre expression patterns in the post-natal retina of the *Tg(Cdh5-cre/ERT2)^{CIVE23Mlia}* and *Tg(Cdh5-cre/ERT2)^{#Ykub}* alleles.

Despite their popularity, the activity of *Cdh5-Cre/ERT2* alleles have not been consistently characterised during embryonic development. The *Tg(Cdh5-cre/ERT2)^{CIVE23Mlia}* allele is best described, with fairly robust Cre activity at most embryonic stages two days after tamoxifen administration, and greater recombination efficiency reported after multiple days of tamoxifen dosing⁴⁴. Although less characterised, the *Tg(Cdh5-cre/ERT2)^{1Rha}* allele is also active in embryonic stages, with intraperitoneal (I.P) injection for three consecutive days from E11.5 known to induce reporter gene expression in ECs of the valves, coronary vessels and the endocardium by E15.5⁵¹. When directly compared with *Tg(Tek-cre)^{12Flv}* for activity during embryonic development, the *Tg(Cdh5-cre/ERT2)^{1Rha}* allele drove similarly patterned but weaker *Rosa26R:lacZ* Cre-reporter gene activity at both E9.5 and E10.5 (when induced three and two days earlier), suggesting that genetic deletions using the two different Cre alleles will result in different levels of recombination and therefore potentially affect phenotypes (Fig. 1A,B). Further, section analysis found evidence of mosaic activity in the dorsal aorta similar to that seen with the constitutively active *Tg(Cdh5-cre)^{7Mlia}* (Fig. 1B,C). As with post-natal mice, considerable intra-litter variability was also detected at E13.5 (Fig. 1C).

Pdgfb-Cre/ERT2: Platelet derived growth factor B (PDGFB) is a high affinity ligand for PDGF receptors PDGFRA and PDGFRB. *Pdgfb* is expressed in ECs, where it is crucial for the proper arrangements of pericytes and smooth muscle cells in the vessel wall⁵². However, *Pdgfb* is neither exclusively nor ubiquitously expressed in

the vasculature: expression is observed in neurons, pericytes, smooth muscle and megakaryocytes⁵³⁻⁵⁵. In E11.5 embryos, *Pdgfb* is expressed in capillary and arterial ECs but not venous ECs⁵⁶, and is down-regulated in the mature vasculature, although expression remains in capillaries into the adult stages⁵⁶.

The *Tg(Pdgfb-icre/ERT2)^{1Frut}* allele was created by insertion of Cre/ERT2 coding cDNA at the start codon of the *Pdgfb* gene in a PAC, which was then randomly integrated into the genome⁵⁷. Like *Tg(Cdh5-cre/ERT2)^{1Rha}*, this allele has been best characterized in post-natal mice, where it has been used extensively to study angiogenesis in the neonatal retina. When induced at P1, Cre recombination can be observed in all ECs, although *Pdgfb* itself is only highly expressed at the growing edge of the developing vascular network⁵⁷. Mice injected with tamoxifen at P1 to P4 have been shown to have efficient pan-EC recombination at P5⁵⁸. In the adult, tamoxifen administration by gavage has been shown to induce Cre recombination in most capillary beds except the liver or the central nervous system after 48 hours, although no Cre expression was seen in larger arteries and veins at this stage⁵⁷. Tamoxifen administration in the adult mouse can also result in pan-EC recombination in coronary vessels, with recombination reported to be at ~99% of all coronary vascular ECs but entirely absent in the endocardium⁵⁹.

The *Tg(Pdgfb-icre/ERT2)^{1Frut}* allele is not well characterised in the embryo and may be of limited use for developmental studies due to the highly heterogeneous nature of *Pdgfb* expression between vascular beds, particularly its absence from venous ECs^{56,60}. When directly compared to the inducible *Tg(Cdh5-cre/ERT2)^{1Rha}* and *Tg(Tek-cre)^{12Flv}*, *R26RlacZ* reporter gene driven by *Tg(Pdgfb-icre/ERT2)^{1Frut}* was notably absent in venous ECs at both E10.5 and E13.5 (Fig. 1B-C).

Tek-Cre/ERT2: The *Tg(Tek-cre/ERT2)^{1Amd}* allele utilizes a similar approach to the constitutively active Tek-Cre models, placing the Cre/ERT2 cDNA between the promoter and intron 1 enhancer sequences^{19,61}. The *Tg(Tek-cre/ERT2)^{1Soff}* allele places Cre/ERT2 cDNA at the coding ATG of the *Tie2* gene within a BAC, which was then randomly inserted into the genome⁶². Although fairly widely used, *Tg(Tek-cre/ERT2)^{1Amd}* requires up to five weeks of tamoxifen induction to induce pan-EC gene recombination in the adult, limiting its applications, while the efficiency and expression profile of the *Tg(Tek-cre/ERT2)^{1Soff}* allele has not been fully reported.

Summary: Although a powerful tool for vascular researchers, EC Cre/ERT2 mouse models do not come without their problems. In addition to the expression variability and characterization limitations described for standard Cre models, susceptibility of floxed alleles to recombination can differ⁶³, whilst experimental variations in the method and dose of tamoxifen administration can also affect the efficiency of Cre/ERT2-driven gene deletion⁶⁴. Conversely, Cre/ERT2 alleles also have the potential for Cre activity without tamoxifen induction ('leakiness'), which must be controlled for in experimental design⁶⁵. Lastly, there is a time lag between tamoxifen induction and effective excision of *loxP* sites by Cre recombination that ranges from 6-24 hours⁶⁶. Therefore, when using these models, it is essential to accurately determine recombination efficiency in target tissues for each floxed allele and each tamoxifen administration regime, when possible in every experimental animal.

Subtype-specific endothelial models

Endothelial heterogeneity is an essential feature of the vasculature. Differential gene expression in specific EC sub-populations is essential for the creation of a hierarchically branched vascular system, for angiogenesis and lymphangiogenesis, and for organ specialization in response to local signals ⁶⁷ (and reviewed ⁶⁸⁻⁷⁰). While some “pan-EC” Cre models may inadvertently omit some EC subtypes, a number of Cre alleles have also been generated to deliberately target specific types of ECs. These include arterial and lymphatic ECs, sprouting angiogenic ECs and the endocardium. At the time of writing, there are no reported venous or capillary EC-specific Cre alleles, although both would clearly be valuable additional resources.

Arterial-specific Cre and Cre/ERT2 mouse models

Sox17-Cre: The SOX17 transcription factor is expressed in arterial but not venous ECs from early in development, in addition to the definitive endoderm and multiple haematopoietic lineages. An arterial EC-specific Cre allele, *Sox17^{tm1(iCre)Heli}*, was generated by knock-in of the codon-improved Cre recombinase ⁷¹ into exon 1 of the *Sox17* locus ⁷². This allele should not be confused with *Sox17^{tm2.1(iCre)Heli}*, which is constitutively active in all *Sox17*-expressing tissues ⁷³, or *Sox17^{tm4.1(iCre/ERT2)Heli}*, which is expressed in arterial and venous ECs, pancreas and haematopoietic cells ⁷⁴. Although *Sox17* is first expressed in the early definitive endoderm from E6.0 ⁷⁵, the earliest activity of the constitutive *Sox17^{tm1(iCre)Heli}* allele is at E8.5 and exclusive to the developing dorsal aorta. This specificity was attributed to a second promoter sequence between exons 3 and 4 of the *Sox17* locus, which directs expression of a shorter *Sox17* mRNA transcript that functions earlier in the endoderm but does not influence the Cre transcript. At E9.5 and E10.5, *Sox17^{tm1(iCre)Heli}*-mediated recombination was detected in the dorsal aorta and was absent from the cardinal vein, although it was seen in the umbilical vein. From E10.5 all labelled vessels could be traced directly to the dorsal aorta, and later in development Cre activity was seen in arteries throughout all visceral organs examined. However, reporter activity was also seen in non-ECs within the thymus, spleen, pancreas and liver at E16.5-E18.5. Further, although embryos homozygous for the *Sox17^{tm1(iCre)Heli}* allele were indistinguishable from wild-type littermates, *Sox17* plays a crucial role in acquisition and maintenance of arterial identity ⁷⁶. Disruption to the *Sox17* locus could therefore have a synergistic effect with other genes deleted using this allele, confounding interpretation of any resulting phenotype.

Bmx-Cre/ERT2: *Bmx*, a member of the Tec tyrosine kinase family, is expressed in arterial ECs of both fetal and adult tissues ⁷⁷. The *Tg(Bmx-cre/ERT2)^{1Rha}* allele is an inducible transgenic arterial EC-specific Cre mouse originally developed to investigate the role of Notch signalling in postnatal arteries ⁷⁸. cDNA encoding Cre/ERT2 was introduced downstream of the promoter for the *Bmx* gene in a PAC4 vector, which was then randomly inserted in the mouse genome ⁷⁹. Daily I.P injections of tamoxifen from P10 to P13 induced Cre recombinase activity specifically within arterial ECs in the P28 retina and adult intestine, ovary and uterus. Cre recombination was also observed specifically in arteries of various organs at E18.5, although details of the tamoxifen induction strategy are unclear. In a later study, Kidoya et al induced *Tg(Bmx-cre/ERT2)^{1Rha}* activity from E10.5 onward, with reporter expression seen in SMA-positive arteries at E14.5 ⁸⁰.

It is unlikely that the *Tg(Bmx-cre/ERT2)^{1Rha}* allele can be used for studying the earliest events in arterio-venous specification in the embryo, as formation of the dorsal aorta and cardinal vein has occurred before *Bmx* is first detected at E10.5

^{81,82}. Additionally, *Bmx^{lacZ}* expression was not observed in smaller arterioles, potentially indicating incomplete *Tg(Bmx-cre/ERT2)^{1Rha}* activity in these beds. Variable *Tg(Bmx-cre/ERT2)^{1Rha}* activity at the distal ends of arterioles has also been noted ⁸³. It is not reported whether the *Tg(Bmx-cre/ERT2)^{1Rha}* allele is active in any non-EC tissues following tamoxifen induction, although *Bmx* expression has been detected in the embryonic endocardium, thymus and tongue of the adult mouse ⁸¹.

Summary: The limited characterization of the above arterial specific Cre alleles indicates that testing specificity and activity of Cre at the time point and tissue of interest prior to breeding compound mouse lines would be highly advisable.

Angiogenic-specific Cre mouse models

Sprouting angiogenesis is the best studied form of angiogenesis, in which new blood vessels form from existing vessels. During sprouting angiogenesis, environmental cues induce quiescent ECs to develop motile behaviour and migrate towards a pro-angiogenic signal. These leading ECs, termed ‘tip cells’, are followed by proliferative ‘stalk cells’ that maintain the connection to the pre-existing vasculature ⁸⁴.

Esm1-CreERT2: The inducible *Tg(Esm1-cre/ERT2)^{1Rha}* allele (also denoted as *Esm1(BAC)-iCreERT2*) targets tip cells in the postnatal retina, a commonly used model for studying angiogenesis ⁸⁵. It was made using a BAC transgenic strategy, in which an *iCreERT2*-containing cassette was inserted immediately downstream of the *Esm1* promoter, deleting exon 1 ⁶⁷. Cre activity of *Tg(Esm1-cre/ERT2)^{1Rha}* mice was mostly restricted to sprouting ECs in the P5 retina following tamoxifen induction at P4, consistent with the tip cell-specific expression patterns of both endogenous *Esm1* mRNA ^{86,87} and *Esm1* protein ⁶⁷. Retinas analysed at P6, following single tamoxifen pulses between 6-96 hours prior to analysis, demonstrated consistent Cre activity at the angiogenic front. Induction 24-96 hours before analysis also labelled an increasing number of cells in the arteries, but not the veins, a pattern attributed to the migration of angiogenic tip cells into the forming arteries ^{88,89}. The only other tissue in which the expression of this Cre allele is reported is in Lewis Lung carcinoma tumours, where both *Esm1* protein and *Tg(Esm1-cre/ERT2)^{1Rha}* activity is seen not just in tip cells, but throughout the tumour vasculature. Embryonic activity of *Tg(Esm1-cre/ERT2)^{1Rha}* has not yet been described.

Apelin-Cre/ERT2: Apelin (*Apln*) is a pro-angiogenic ligand for the *Apln* receptor APLNR/APJ. The knock-in *Apln^{tm1.1(cre/ERT2)Bz}* allele was generated by inserting *CreERT2* cDNA at the *Apln* locus, deleting exon 1 ⁹⁰. This inducible allele is able to distinguish sprouting vessels from mature, quiescent vessels: adult mice show significantly fewer ECs labelled one week after tamoxifen induction, compared with induction in the embryo. Further, *Apln^{tm1.1(cre/ERT2)Bz}* activity is reactivated in various tissue injury models that stimulate sprouting angiogenesis, including myocardial infarction (MI), hindlimb ischaemia-reperfusion, and numerous tumour models. In all cases, robust EC-specific *Apln^{tm1.1(cre/ERT2)Bz}* activity is seen in newly-formed vessels, a subset of which are actively proliferating, whilst remaining at low levels in regions/tissue remote from the injury ⁹¹. The *Apln^{tm1.1(cre/ERT2)Bz}* allele also targets angiogenic ECs in the postnatal retina: *Apln^{tm1.1(cre/ERT2)Bz}*-driven reporter activity is more localised to the peripheral angiogenic front of the P6 retina compared with *Tg(Cdh5-cre/ERT2)^{1Rha}*, and labels more ECs when induced at P2-P4 compared with P5-P7, consistent with decreasing angiogenic sprouting in the more developed

retinas⁹². Tamoxifen administration in adult mice resulted in no *Apln*^{tm1.1(cre/ERT2)Bz} activity in the retina, again demonstrating lack of activity in mature, quiescent ECs.

In the embryo, the *Apln*^{tm1.1(cre/ERT2)Bz} allele drives recombination in the heart specifically within the coronary endothelium, while no recombination is seen in the endocardium, smooth muscle cells or lymphatic ECs after tamoxifen administration at E10.5^{90,91}. *Apln*^{tm1.1(cre/ERT2)Bz} is also active in ECs throughout other developing organs in which angiogenesis is occurring, and analysis of Cre-expressing APLN-positive ECs at E10.5 demonstrates they are proliferative⁹¹. Interestingly, *Apln*^{tm1.1(cre/ERT2)Bz} Cre activity is absent from the dorsal aorta.

There have been some reports of 'leakiness' with the *Apln*^{tm1.1(cre/ERT2)Bz} allele, resulting in Cre/ERT2 recombinase activity in the absence of tamoxifen induction⁹¹. This was observed at levels significantly lower than those seen following tamoxifen treatment. Nevertheless, for accurate interpretation of experimental data it is important to include an un-induced negative control.

Summary: Although both these Cre/ERT2 alleles are described as labelling sprouting angiogenic ECs, there are considerable differences in reporter gene expression driven by *Apln*-Cre/ERT2 compared to *Esm1*-Cre/ERT2, indicating that they label different subsets of angiogenic ECs. This should be considered when selecting a model.

Cre models specific to different compartments of the coronary vasculature

In addition to the arterial, venous, lymphatic and microvascular heterogeneity seen in the systemic vasculature, coronary ECs are also developmentally heterogeneous, differentially originating from the sinus venosus (SV), endocardium and proepicardium⁹³. There has been much debate over the relative contributions of these different sources of ECs, in which the interpretation of various constitutive or inducible Cre alleles has underpinned much of the discrepancies between different fate-mapping studies⁹⁴. This final section will give a brief overview of the EC-specific Cre alleles used to address the origins of the coronary endothelium.

***Aplnr*-Cre/ERT2:** The SV is a transient developmental structure at the venous pole of the heart. The apelin receptor APLNR (also known as APJ) is highly expressed in the SV endothelium, but not at appreciable levels in the endocardium or epicardium at E10.5-12.5. The *Tg(Aplnr-cre/ERT2)^{#Krh}* allele was generated using BAC recombineering technology: Cre/ERT2 cDNA was inserted at the *Aplnr/Apj* start site, which was then randomly integrated into the genome⁹⁵. When induced by tamoxifen at E9.5, robust reporter expression was detected by E10.5 in the SV and in many, but not all, coronary vessels in the developing heart, predominantly on the dorsal and lateral sides. In the embryo, *Tg(Aplnr-cre/ERT2)^{#Krh}* was reported to mimic that of *Aplnr/Apj*, with activity seen throughout the microvasculature, in the cardinal vein and intersomitic vessels. In the adult mouse, the *Tg(Aplnr-cre/ERT2)^{#Krh}* allele has also been used to label EC-specific *Aplnr/Apj* expression in non-muscularised vessels of <~50µm diameter in skeletal muscle, white adipose tissue and brown adipose tissue⁹⁶.

***Fabp4*-Cre and *Fabp4*-Cre/ERT2:** Fatty acid binding protein 4 (FABP4), often referred to as an adipocyte and macrophage-specific protein AP2, is also expressed in ECs. This includes coronary vessel ECs, although no expression is detected in endocardial ECs^{97,98}. As expected, the *Tg(Fabp4-cre)^{1Rev}* allele, generated by

driving Cre cDNA expression with a 5.4kb *Fabp4*/Ap2 promoter/enhancer fragment and then randomly inserting into the genome⁹⁹, directs Cre activity in coronary but not endocardial ECs⁹⁹. The inducible *Tg(Fabp4-cre/ERT2)^{1pc}*¹⁰⁰ allele was created using the same transgenic promoter/enhancer approach as *Tg(Fabp4-cre)^{1Rev}*, and was also able to selectively label coronary, but not endocardial, ECs⁹⁸.

Nfatc1-Cre and Nfatc1-Cre/ERT2: The endocardium is a specialised EC layer that lines the inner surface of the heart, from which cells can delaminate and migrate to the developing cardiac cushions, as well as contributing to coronary vessel endothelium and mural cell populations¹⁰¹. *Nfatc1*, a calcium/calciueurin-dependent transcription factor, is highly expressed in the endocardium whilst relatively absent in most other blood ECs. The *Nfatc1* locus has therefore been used to develop a number of Cre alleles primarily utilised to study the endocardium.

A constitutively active *Nfatc1^{tm1.1(cre)Bz}* allele (generated by Bin Zhou at Albert Einstein College of Medicine and sometimes referred to as *Nfatc1-Cre*) was created by knock-in of *Cre-IRES* cDNA downstream of the translational stop codon of the mouse *Nfatc1* locus¹⁰². The resulting allele was reported to drive *Cre* mRNA expression and Cre-driven recombination specifically within endocardial ECs at both E9.5 and E10.5, with no expression reported in SV ECs, proepicardium, epicardium or myocardium. This *Nfatc1^{tm1.1(cre)Bz}* allele was used to propose a model by which endocardial cells form some coronary vessels via angiogenesis¹⁰². However, other independently generated *Nfatc1-Cre* alleles have given contradictory results. Confusingly, these were generated by a group led by a different scientist also named Bin Zhou, based at the Shanghai Institutes for Biological Sciences of the Chinese Academy of Sciences. Until recently, mice generated by these two scientists were both labelled using a single lab code (*Bz*) on MGI. Although this has now been corrected, researchers should be aware of possible confusion in papers published prior to 2018, and check references carefully to ensure they are using their desired mouse model. Bin Zhou (Shanghai, now denoted *Bzsh*) generated a series of *Nfatc1-Cre* and *Dre* alleles with slightly different expression patterns compared to *Nfatc1^{tm1.1(cre)Bz}*, the crucial difference being activity in SV ECs. For example, their constitutively active *Nfatc1^{tm2(cre)Bzsh}* allele, which was generated using the identical targeting strategy detailed for *Nfatc1^{tm1.1(cre)Bz}*, showed Cre recombinase activity in SV ECs at E9.5 and E11.5, in addition to endocardial activity⁴⁹. Together, these results suggest that constitutive *Nfatc1-Cre* activity may not be entirely restricted to the endocardium in the developing heart, and so alone may be unsuitable for definitive studies of coronary vessel origins.

To overcome specificity issues, inducible *Nfatc1-Cre/ERT2* models have also been developed. The *Nfatc1^{tm1.1(cre/ERT2)Bzsh}* allele was generated by homologous recombination to replace exon 1 of the *Nfatc1* locus with *Cre/ERT2* cDNA¹⁰³. Immunostaining for estrogen receptor (ESR) showed at E9.5 *Nfatc1^{tm1.1(cre/ERT2)Bzsh}* is expressed in atrial and ventricular endocardial ECs, but is absent in the SV. Tamoxifen injection at E8.0-E8.5 could therefore activate Cre activity specifically in the endocardium before the coronary vasculature forms, but presumably after the early transient expression of *Nfatc1* in the SV. Consistent with this, no reporter labelling was observed in the SV at E11.5¹⁰³, indicating that *Nfatc1-CreER* is a more reliable tool for labelling endocardial cells when induced in the specified developmental window. A second inducible knock-in allele, *Nfatc1^{em1(cre/ERT2)Bzsh}*, also

showed similar endocardial-specific immunostaining of early *Cre/ERT2* expression¹⁰⁴.

***Npr3-Cre/ERT2*:** *Npr3* encodes natriuretic peptide receptor 3 and was found to be specifically expressed in endocardial ECs^{49,104}. The inducible *Npr3^{tm1.1(cre/ERT2)Bzsh}* allele was generated using homologous recombination to insert *CreERT2* cDNA in frame with the translational start codon at the *Npr3* locus¹⁰⁴. Immunostaining for ESR showed *Npr3-Cre/ERT2* expression in the atrial and ventricular endocardium, but not SV, between E8.5 to E11.5. Following tamoxifen induction at E8.5, Cre recombination was seen in approximately 73% of ventricular endocardial cells at E10.5, 78% at E11.5 and 80% at E12.5, whilst the SV remained unlabelled. Although *Npr3^{tm1.1(cre/ERT2)Bzsh}* is not exclusive to the endocardium (expression is also seen in dorsal aorta EC, a subset of non-EC mesenchymal cells, and a subset of TBX18-positive epicardial cells⁴⁹), it is active in the adult¹⁰⁵. This is different to *Nfatc1^{tm2(cre)Bzsh}*, which cannot be activated by tamoxifen injection after embryonic stages. Tamoxifen induction of *Npr3^{tm1.1(cre/ERT2)Bzsh}* in the adult heart labels over 90% of endocardial cells after 48 hours. As in the embryonic heart, labelling was also seen in a subset of epicardial cells, but not vascular ECs.

Summary: Due to the ongoing controversy regarding the exact origins of different types of vessels within the heart, a detailed knowledge of the expression pattern of a chosen Cre and/or Cre/ERT2 model is crucial for any research in this area. A careful literature check and a detailed genealogy for any chosen mouse model would also be recommended, as many of these alleles (and the labs of origin) have similar or identical names.

CONCLUSION AND FUTURE OUTLOOK

EC-specific Cre mouse models have greatly expanded our knowledge of vascular biology. However many are not thoroughly characterised and have recombination in cell populations other than the intended cell type, confounding the interpretation of resulting data. Constitutively active pan-EC Cre alleles can display variable Cre recombinase activity even between littermates, and all show recombination in cells of the haematopoietic lineage. Inducible Cre mouse alleles will also have inherent problems with Cre leakiness and variation in recombination efficiency. Therefore, to generate reliable and reproducible data using EC-specific Cre mouse models it is clear that Cre allele activity must be adequately monitored for each specific mouse model, target tissue and time point of interest.

As our understanding of the genome expands, so do the options for the manipulation of tissue-specific gene expression. For example, it is now possible to combine *Cre-lox* with the *Dre-rox* system, in which Dre recombinase catalyzes recombination between *rox* sites¹⁰⁶, thus enabling knockout specificity to be controlled by two regulatory sequences¹⁰⁷. To this end, a Dre-switchable CrexER allele has been generated that has two *rox* sites flanking the ESR, so the Cre-ESR fusion protein is restricted to the cytoplasm until Dre-driven excision of the ESR coding sequence occurs¹⁰⁷. For EC-specific Dre expression, Pu *et al.* have already generated a novel *Tek-Dre* allele (provisionally named *Tek-Dre^{tm1(dre)Bzsh}*) by knocking in a Dre cassette in-frame with the ATG of endogenous *Tek*¹⁰⁷. By combining this Tek-Dre allele with organ- or EC subtype-specific expression of CrexER, recombination of a floxed target gene can be restricted to increasingly precise cell populations.

With our increased understanding of EC heterogeneity, the combination of EC-subtype-specific enhancers with new recombinase technology looks set to provide a powerful next-generation toolbox with which to interrogate the complex systems governing EC development and behaviour.

FIGURE LEGENDS

Figure 1. A direct comparison of the embryonic expression pattern and intensity of the Cre reporter R26R:lacZ when crossed with different EC-specific Cre alleles on the C57/Bl6 background. Embryonic age was established from date of plug in mother, all embryos were fixed, stained and sectioned using identical conditions across each age group as previously described ¹⁰⁸. For tamoxifen administration, 100µl tamoxifen (10mg/ml in a 10:1 solution of peanut oil and 100% ethanol) was administered orally via gavage on two consecutive days as indicated, and embryos taken 48 hours after last dose. When two embryos for the same allele are shown, they are littermates. E=embryonic day, da=dorsal aorta, cv=cardinal vein, end=endocardial lining of heart

Table 1. Summary of the most widely-used EC-specific Cre and Cre/ERT2 alleles.

CapEC= capillary endothelial cells, AEC= Arterial endothelial cells, SVEC=sinus venosus endothelial cells, CEC= coronary endothelial cells, VEC=vein endothelial cells, EndEC=endocardial endothelial cells. MGI ID number can be used at <http://www.informatics.jax.org/> to obtain further information about origin, recombinase activity, mouse availability and a complete list of papers referencing each allele. Note that ID numbers must include "MGI:" when added into search box.

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Disclosures,

Nothing to disclose

Highlights

Summary of vascular specific Cre mouse models and what is known about their Cre expression patterns from embryo to adulthood.

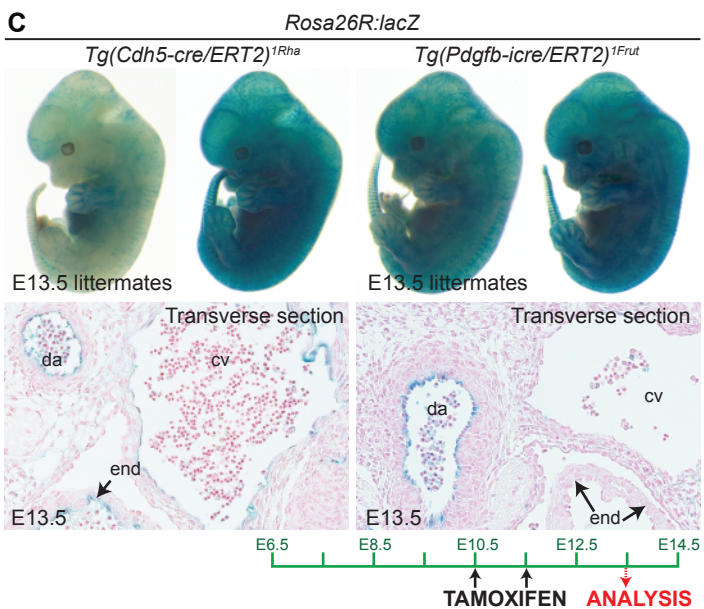
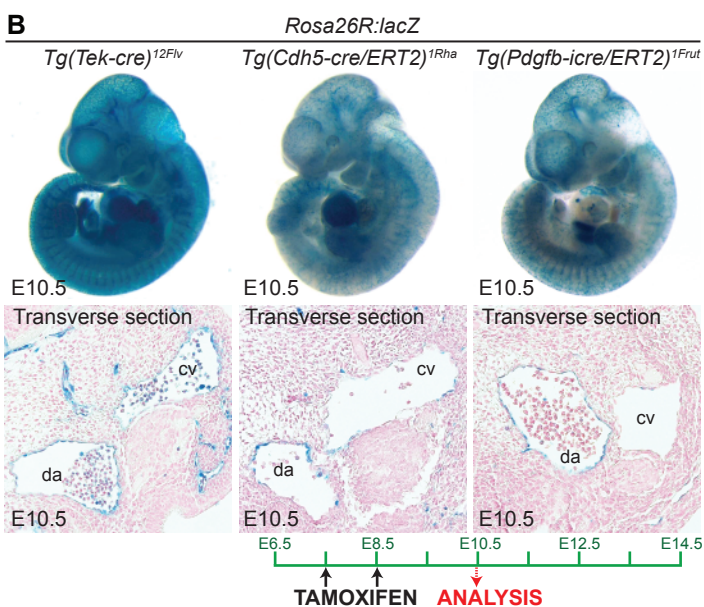
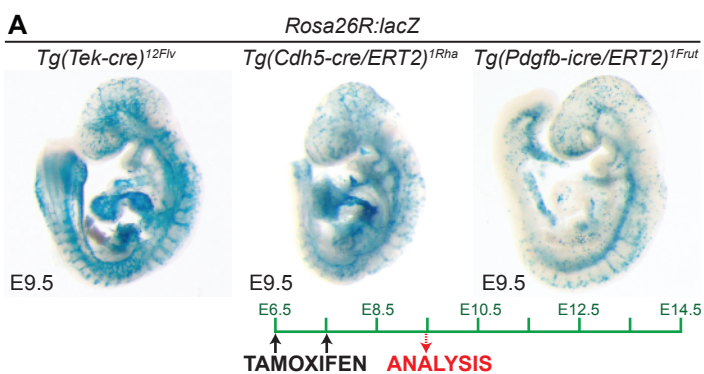


Table 1. Summary of the most widely-used EC-specific Cre and Cre/ERT2 alleles

Gene	Mouse Line	Regulation of Cre Activity	Expression	Non-EC Expression	Earliest Reported Activity	MGI ID	Ref
<i>Tek</i> (<i>Tie2</i>)	<i>Tg(Tek-cre)</i> ^{1Ywa}	10kb enhancer and 2.1kb promoter	Pan-EC	Heart valves, HCs	E7.5	MGI:2450311	20
	<i>Tg(Tek-cre)</i> ^{12Flv}	10kb enhancer and 2.1kb promoter	Pan-EC	Heart valves, HCs	E7.5	MGI:2136412	21
	<i>Tg(Tek-cre)</i> ^{5326Sato}	10kb enhancer and 2.1kb promoter	Pan-EC	HCs, some ectopic expression	E9.5	MGI:2445474	22
	<i>Tg(Tek-cre)</i> ^{1Rwng}	10kb enhancer and 2.1kb promoter	Pan-EC	HCs	E7.5	MGI:3608912	23
	<i>Tg(Tek-cre)</i> ^{1Xyfu}	10kb enhancer and 2.1kb promoter	Pan-EC	HCs	Unknown	MGI:2683222	24
	<i>Tg(Tek-cre/ERT2)</i> ^{1Soff}	Endogenous locus, Cre/ERT2 inserted at start codon of gene within a BAC	Pan-EC	None reported	Unknown	MGI:3837451	62
	<i>Tg(Tek-cre/ERT2)</i> ^{1Arnd}	10kb enhancer and 2.1kb promoter	Pan-EC	None reported	Unknown	MGI:2450312	61
<i>Cdh5</i> (<i>VE-cad</i>)	<i>Tg(Cdh5-cre)</i> ^{7Mlia}	2.2kb promoter	Pan-EC	HCs	E7.5	MGI:3620560	33
	<i>Tg(Cdh5-cre)</i> ^{1Nmoc}	2.2kb promoter	Pan-EC	HCs	E10.5	MGI:3842516	34
	<i>Tg(Cdh5-cre)</i> ^{1Spe}	2.2kb promoter flanked by 2kb insulator	Pan-EC	HCs	E9.5	MGI:3836418	35
	<i>Tg(Cdh5-cre/ERT2)</i> ^{CIVE23Mlia}	2.2kb promoter	Pan-EC	HCs	E9.5	MGI:3700149	44
	<i>Tg(Cdh5-cre/ERT2)</i> ^{#Ykub}	Endogenous locus, Cre/ERT2 inserted at start codon within BAC	Pan-EC	None reported	Not reported	MGI:5705396	45
	<i>Tg(Cdh5-cre/ERT2)</i> ^{1Rha}	Endogenous locus, Cre/ERT2 inserted at start codon within PAC	Pan-EC	None reported	E9.5	MGI:3848982	46
<i>Kdr</i> (<i>Flk1</i>)	<i>Tg(Kdr-cre)</i> ^{15962Brei}	2.3kb enhancer and 939bp promoter	Pan-EC	HCs	E9.5	MGI:3038968	39
	<i>Kdr</i> ^{tm1(cre)Sato}	Endogenous locus, targeted knock-in of CreERT2 cDNA into exon 1	Pan-EC	HCs skeletal and cardiac muscle	E8.5	MGI:2655253	40
<i>Pdgfb</i>	<i>Tg(Pdgfb-icre/ERT2)</i> ^{1Fru}	Endogenous locus, Cre/ERT2 inserted at start codon in PAC,	CapEC, AEC	Keratinocytes, megakaryocytes	E9.5	MGI:3793852	57
<i>Sox17</i>	<i>Sox17</i> ^{tm1(iCre)Heli}	Endogenous locus, targeted knock-in of CreERT2 cDNA deleting exon 1	Some AECs	Thymus, spleen, pancreas and liver	E8.5	MGI:3852645	72
<i>Bmx</i>	<i>Tg(Bmx-cre/ERT2)</i> ^{1Rha}	Endogenous locus, Cre/ERT2 inserted at start codon within PAC	Some AECs	None reported	E10.5	MGI:5513853	78
<i>Esm1</i>	<i>Tg(Esm1-cre/ERT2)</i> ^{1Rha}	Endogenous locus, Cre/ERT2 inserted at start codon within BAC	Tip ECs	None reported	P4	-	67
<i>Apln</i>	<i>Apln</i> ^{tm1.1(cre/ERT2)Bz}	Endogenous locus, targeted knock-in of CreERT2 cDNA into gene deleting exon 1	Sprouting ECs	None reported	E10.5	MGI:5637737	90
<i>Aplnr</i> (<i>Apj</i>)	<i>Tg(Aplnr-cre/ERT2)</i> ^{#Krh}	Endogenous locus, Cre/ERT2 inserted at start codon of BAC	SV and derived CECs, capECs, VECs	None reported	E10.5	MGI:5637737	95
<i>Fabp4</i> (<i>Ap2</i>)	<i>Tg(Fabp4-cre)</i> ^{1Rev}	5.4kb promoter fragment	CEC	Adipocytes and macrophages	E16.5	MGI:2386686	99
	<i>Tg(Fabp4-cre/ERT2)</i> ^{1lpc}	5.4kb promoter fragment	CEC	Adipocytes and macrophages	E16.5	MGI:2387425	100
<i>Nfatc1</i>	<i>Nfatc1</i> ^{tm1.1(cre)Bz}	Endogenous locus, targeted knock-in of Cre cDNA into translational stop codon	EndEC	Hair follicle stem cells, osteoblasts, macrophages	E9.0	MGI:5471107	102
	<i>Nfatc1</i> ^{tm2(cre)Bzsh}	Endogenous locus, targeted knock-in of Cre cDNA into translational stop codon	EndEC, SVEC	None reported	E9.5	MGI:6163733	49
	<i>Nfatc1</i> ^{tm1.1(cre/ERT2)Bzsh}	Endogenous locus, targeted knock-in of CreERT2 cDNA into gene deleting exon 1	EndEC, SVEC	None reported	E8.5	MGI:5637438	103
	<i>Nfatc1</i> ^{em1(cre/ERT2)Bzsh}	Endogenous locus, targeted knock-in of Cre/ERT2 cDNA into 3'UTR	EndEC, SVEC	None reported	E7.75	MGI:5804178	104
<i>Npr3</i>	<i>Npr3</i> ^{tm1.1(cre/ERT2)Bzsh}	Endogenous locus, targeted knock-in of Cre/ERT2 cDNA into translational start codon	EndEC	Some epicardial cells	E8.5	MGI:5804184	104