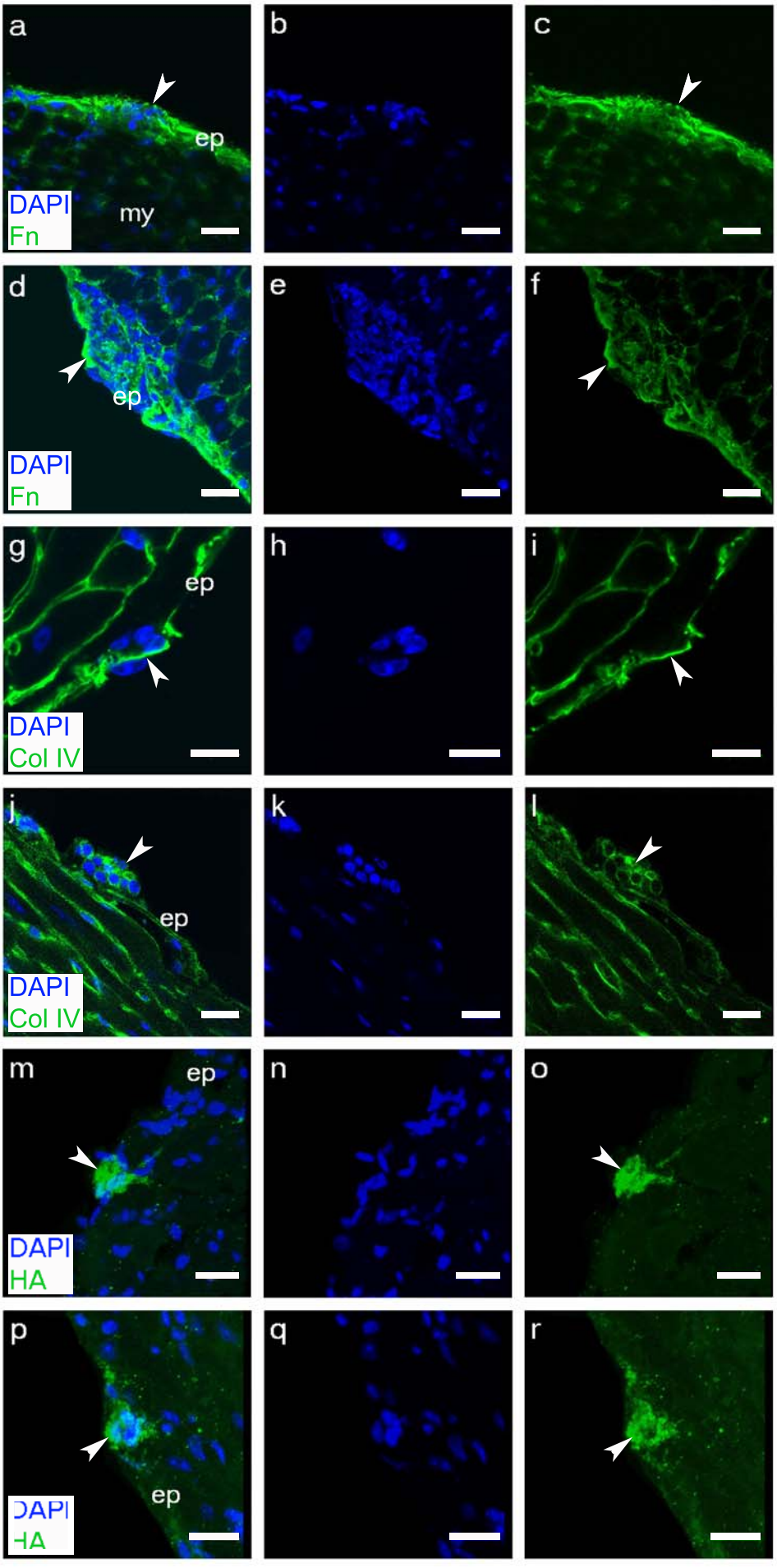
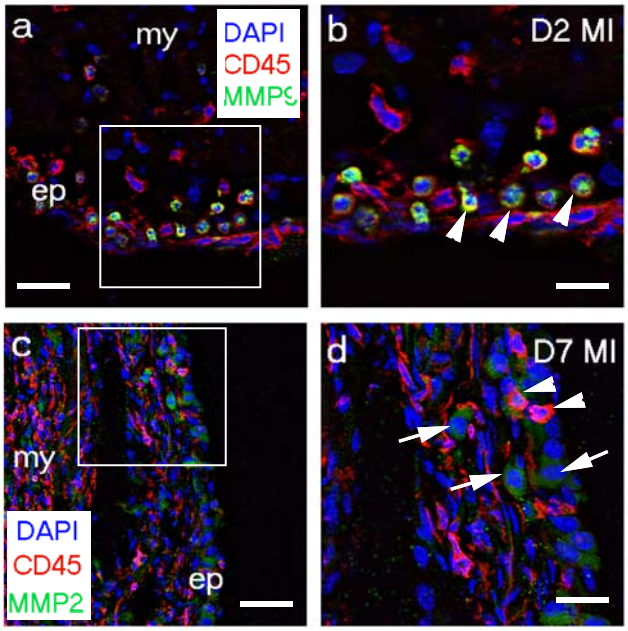
Balmer et al_Supp. Fig. 1



Supplementary Figure 1. Characterisation of extracellular matrix protein expression in adult epicardial cell clusters.

Immunofluorescent staining for ECM markers in adult epicardium highlighted the presence of cell clusters surrounded by matrix as demarcated by antibodies against Fibronectin (Fn; **a**-**f**), Collagen IV (Col IV; **g**-**l**) and Hyaluronic acid (HA; **m**-**r**). White arrowheads highlight the containment of cells within clusters in the epicardium of adult, wild type mouse hearts; ep, epicardium. Scale bars 20μm (**a**-**f**, **j**-**l**); 10μm; (**g**-**i**).

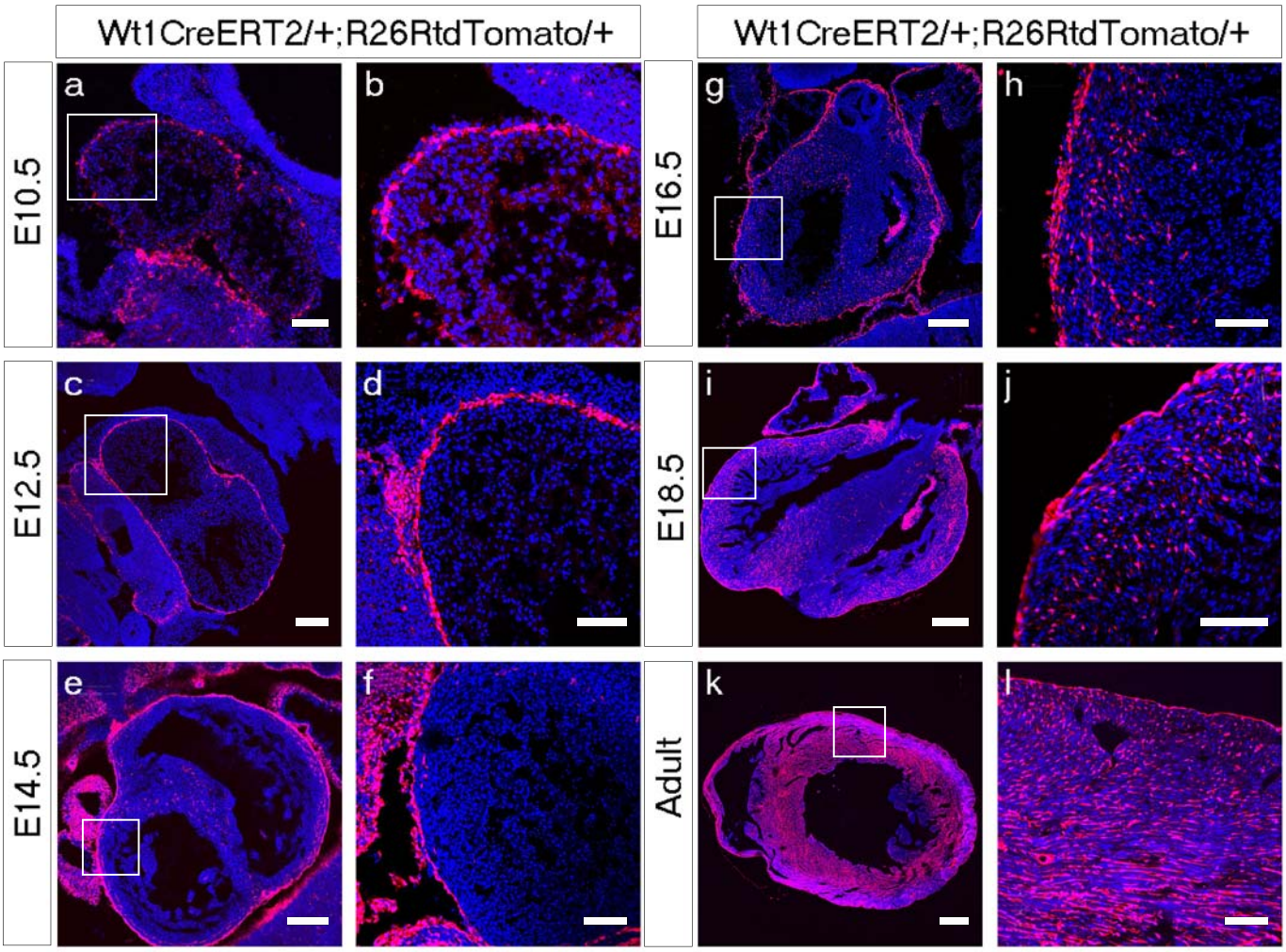
Balmer et al_Supplementary Fig. 2



Supplementary Figure 2. Localisation of MMP2 and MMP9 to regions of CD45-cell cluster associated ECM breakdown post-MI.

Immunofluorescent staining for matrix metalloproteinase (MMP) markers, revealed MMP9 co-localised to CD45+ cells (**a**; white arrowheads in **b**) within the epicardium 2 days post-MI and cells that stained for both CD45+ and MMP2 at 7 days post-MI (**c**; white arrowheads in **d**) alongside neighbouring cells that were CD45- and MMP2+ at 7 days post-MI (white arrows in **d**) suggesting MMP-activity may contribute to the breakdown of ECM associated with the dispersal of CD45+ cell clusters (Figure 1, 3 and Supp. Figure 1) following injury. White box inserts in **a** and **c** depicted at higher magnification in **b** and **d** respectively; ep, epicardium; my, myocardium. Scale bars 50μm (**a**, **c**); 10mm (**b**, **d**).

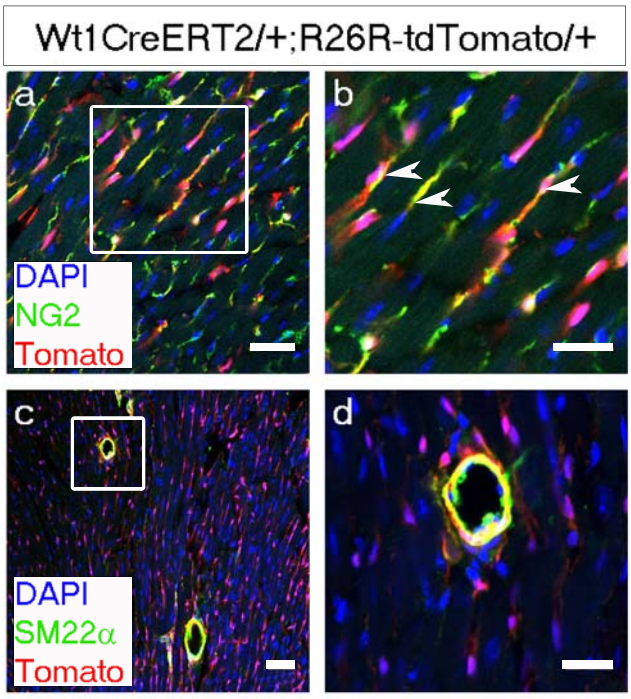
Balmer et al_Supplementary Fig. 3



Supplementary Figure 3. Wt1CreERT2;R26R-tdTomato efficiently labels the epicardium and epicardial cell derivatives throughout development.

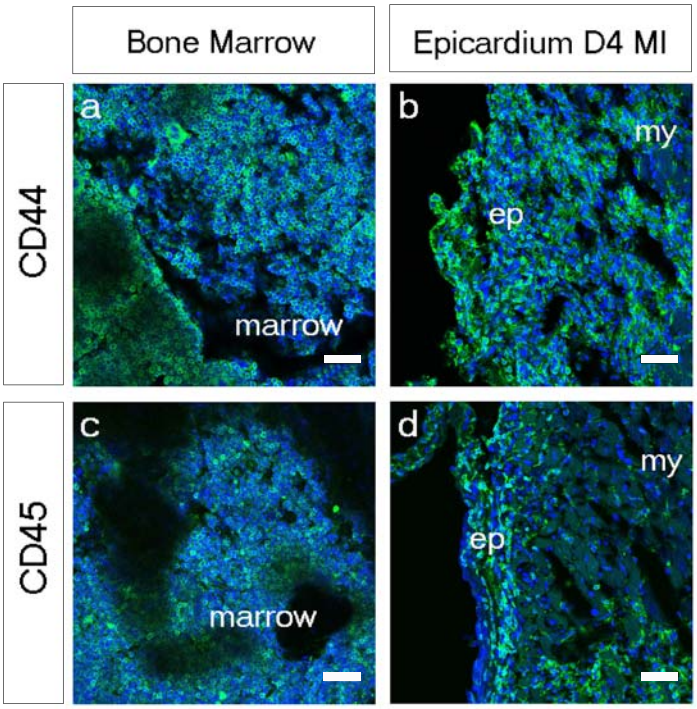
Wt1CreERT2/+; R26R-tdTomato dams were injected with tamoxifen and progesterone at E9 and again at E11, embryos were analysed for tdTomato+ expression at E10.5 (**a**, **b**), E12.5 (**c**, **d**), E14.5 (**e**, **f**), E16.5 (**g**, **h**), E18.5 (**i**, **j**) and adult stages (**k**, **l**). The efficient labelling of the epicardium at all stages of development into adulthood excluded the possibility of suboptimal recombination, due to failed tamoxifen induction, accounting for the lack of Wt1+/tomato+ cells observed in the expanded epicardium post-injury (see Fig. 3e-h). Scale bars: 100μm (**a**, **f**, **h**, **j**, **l**); 50μm (**b**); 200μm (**c**, **e**); 90μm (**d**); 300μm (**g**, **i**); 600μm (**k**).

Balmer et al_Supplementary Fig. 4



Supplementary Figure 4. Wt1+ cells can give rise to pericytes and vascular smooth muscle cells in the adult heart. Wt1CreERT2/+; R26R-tdTomato dams were injected with tamoxifen and progesterone successively at E9 and E11, pups were delivered at E18.5 and fostered. IMF revealed tomato+ cells (arising from the Wt1+ lineage) that were also positive for the pericyte marker NG2 (**a, b**) and the vascular smooth muscle cell marker SM22α, where tomato+/Sm22α+ cells were seen lining the wall of a coronary vessel (**c**, **d**); white inset boxes in **a** and **c** are shown at higher magnification in **b** and **d** respectively. Scale bars: 30μm (**a**); 20 μm (**b, d**); 50 μm (**c**).

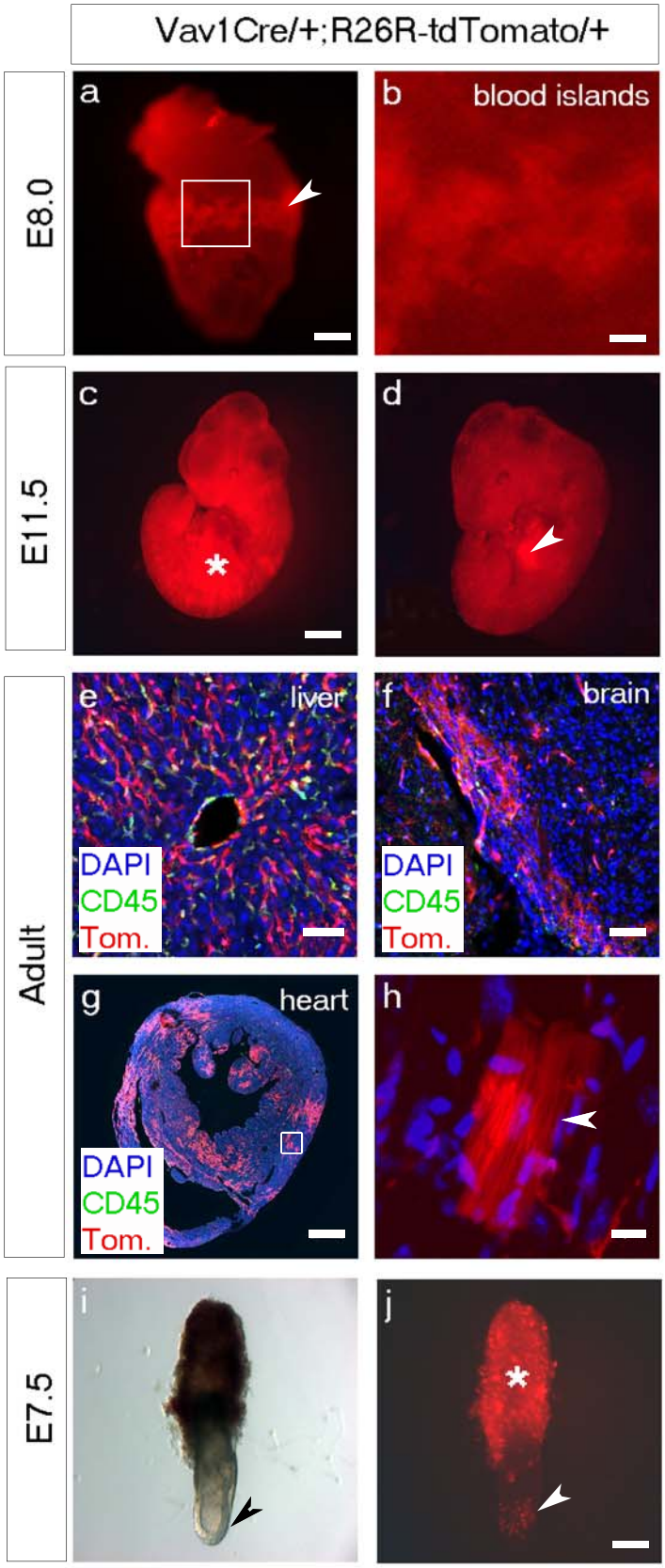
Balmer et al_Supplementary Fig. 5



Supplementary Figure 5. CD44+ and CD45+ bone marrow cells reside in the expanded region of the epicardium post-MI.

Immunofluorescent staining detected CD44+ and CD45+ cells in adult bone marrow (**a**, **b**). Equivalent CD44+ and CD45+ sub-populations were located in the adult epicardium 4 days post-MI (**c**, **d**). ep, epicardium. Scale bars: 50μm (**a-d**).

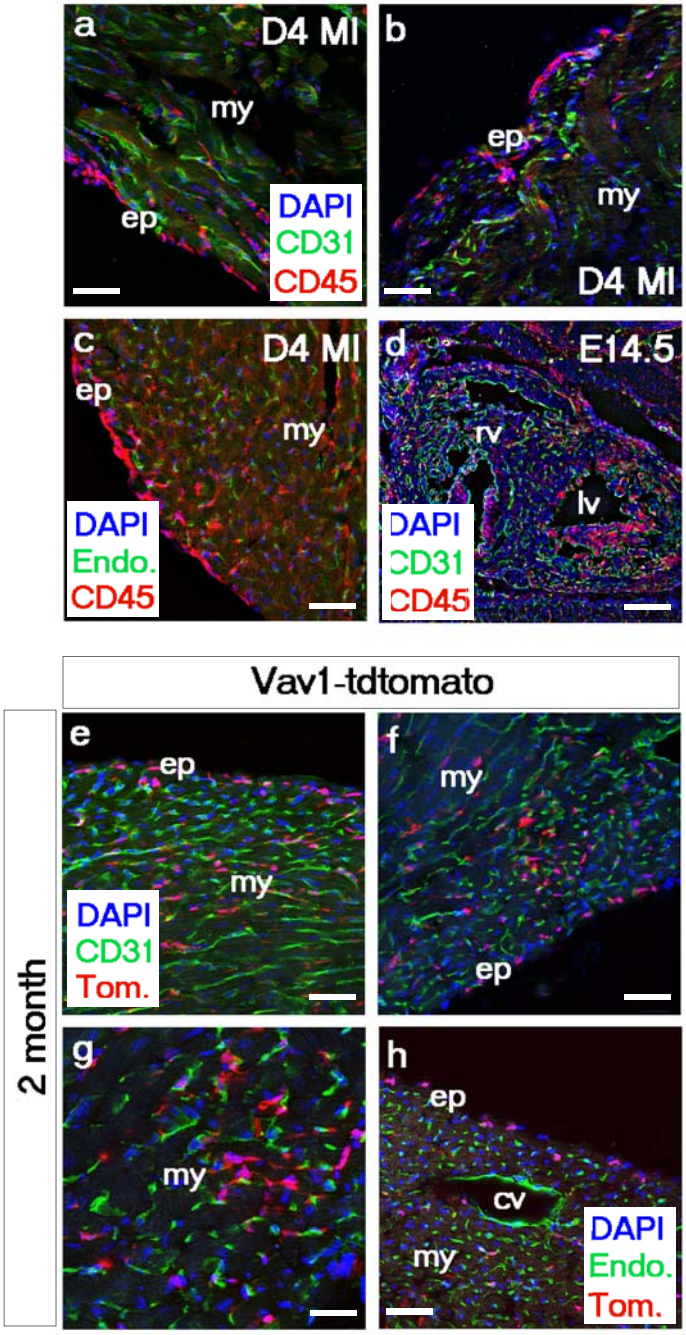
Balmer et al_Supplementary Fig. 6



Supplementary Figure 6. Vav1-Cre;R26R-tdTomato lineage traces haemagenic endothelium of the yolk sac blood islands, AGM and liver in addition to derivatives in heart and brain.

Vavl1-Cre;R26R-tdTomato embryos were dissected at E7.5, E8.0 and E11.5 and the expression of tdTomato assessed by direct fluorescence. At E8.0 tdTomato+ cells were observed in the trophoblast (**a**; white asterice) and in presumptive blood islands (**b**; white arrowheads). At E11.5, embryos with the expected staining in haematopoietic regions were observed, including the aorta-gonad-mesonephros (AGM) region (**c**), foetal liver (**d**) and adult liver (**e**; 3 out of 6 embryos analysed). However, other embryos showed a variable degree of colonisation of tdTomato+ cells throughout the developing embryo including the brain (**f**) and adult heart (**g**)**,** containing tdTomato+ cardiomyocytes (**h**). This suggested that non-specific Cre recombination could occur in the Vav1-Cre mouse line and indeed, sporadic tdTomato+ cells were detected in the epiblast at E7.5 (black arrowhead in **i**, and white arrowhead in **j**; white asterice in **j** highlights maternal tdTomato+ cell contribution to the ectoplacental cone). For the analyses described, only those embryos with none or less than 5% non-specific colonisation were selected. Scale bars: 30 μm (**a**, **b**, **i**, **j**); 50 μm (**c, d**); 800μm (**g**); 10 μm (**h**).

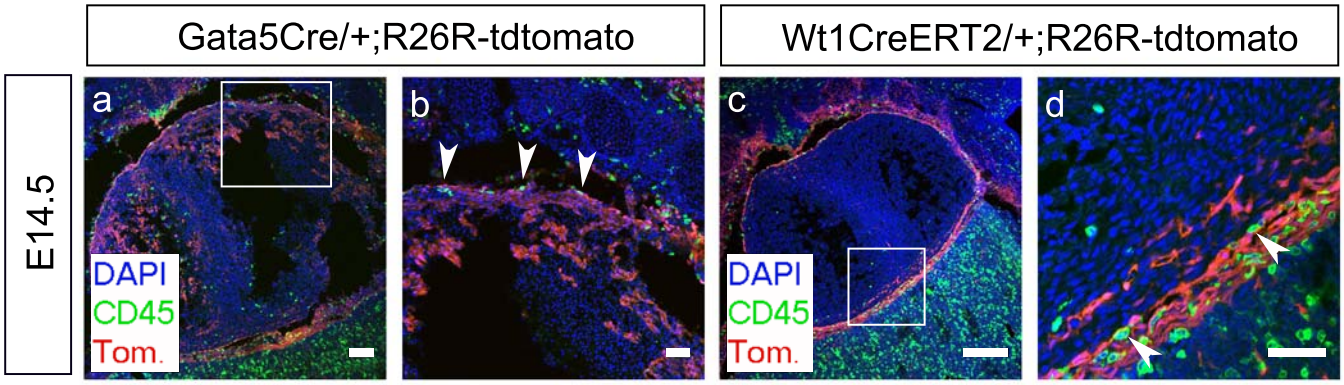
Balmer et al_Supplementary Fig. 7



Supplementary Figure 7. CD45+/Vav1-tdTomato+ cells are negative for endothelial markers during development and following BM transplantation or injury.

Immunostaining for CD31 (**a**, **b**) and endomucin (**c**) at 4 days post-MI revealed CD45+ cells did not have an endothelial cell phenotype. CD31 co-staining also revealed CD45+ cells within the heart during development at E14.5 were not endothelial cells (**d**). Transplantation of Vav1-tdTomato cells into irradiated hosts were examined at 2 months for either CD31 (**e-g**) or endomucin staining (**h**) to reveal that donor Vav1-tdTomato+ cells did not adopt an endothelial cell fate. cv, coronary vessel; ep, epicardium; my, myocardium. Scale bars. 50 μm (**a**, **c**, **e**, **f**, **h**) 30 μm (**b**, **g**); 200μm (**d**).

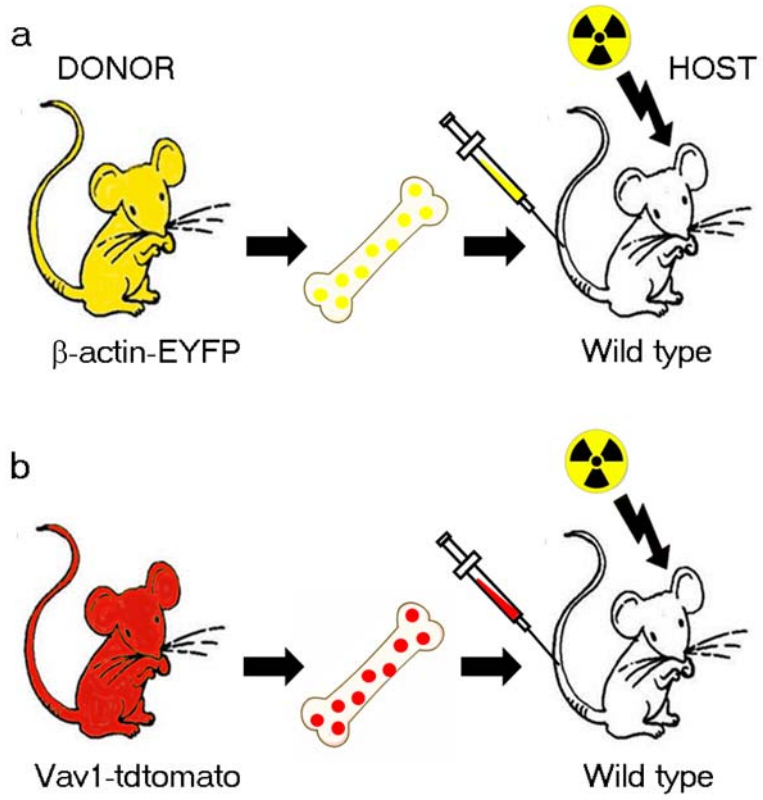
Balmer et al_Supplementary Fig. 8



Supplementary Figure 8. Gata5 and Wt1 lineages do not give rise to CD44+ or CD45+ cells during development.

Gata5Cre/+;R26RtdTomato (**a**, **b**) and Wt1CreERT2/+;R26RtdTomato (**c, d**) lineage traces were used to determine the origin of cells residing in the epicardial clusters. Immunofluorescent staining at E14.5 revealed CD45+ cells (highlighted by white arrowheads) were not tomato+ and, therefore, did not arise from Gata5+ or Wt1+ lineages. Scale bars: 100μm (**a**); 50 μm (**b, d**); 200 μm, (**c**).

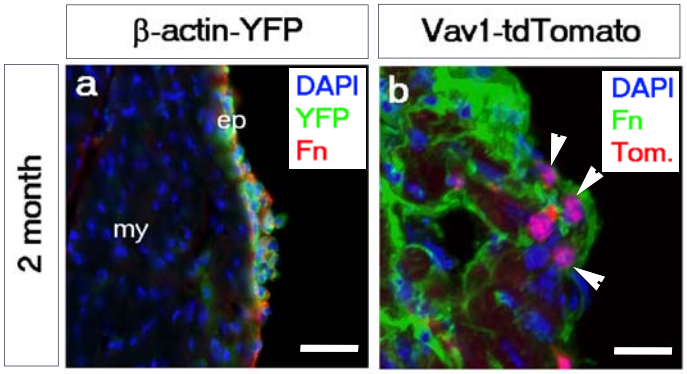
Balmer et al_Supplementary. Fig. 9



Supplementary Figure 9. Overview of transplantation experiments with whole bone marrow and Vav1-tdTomato haematopoietic stem cells.

Whole bone marrow cells were isolated from the femurs of β-actinCre;R26R-EYFP mice (**a**), or lin-negative cells were sorted from femur-extracted bone marrow from Vav1-Cre;R26RtdTomato mice (**b**) and injected into the tail vein of unlabelled wild type host mice that had been sub-lethally (6Gy) or lethally irradiated (successive 4Gy plus 5 Gy). Reconstitution of the host bone marrow was predicted to occur between 7 and 14 days according to previous studies[46]. Hearts were analysed at 2 and 6 months post transplantation.

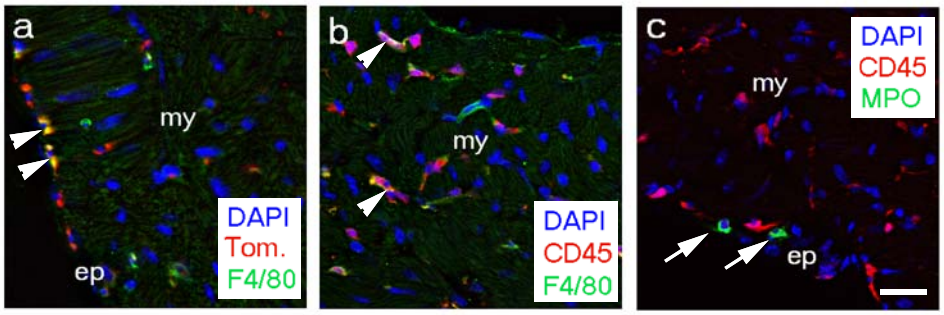
Balmer et al_Supplementary Fig. 10



Supplementary Figure 10. Transplanted whole bone marrow and Vav1-tdTomato haematopoietic stem cells traffic into adult epicardial clusters.

2 months after transplantation (Supplementary Fig. 9) both YFP+ whole BM cells and Vav1-tdTomato HCs were observed in host adult epicardial cell clusters (**a**, **b**; arrowheads in **b** highlight individual tdTomato+ cells encapsulated by fibronectin (Fn)+ ECM). ep, epicardium; my, myocardium. Scale bars: 100μm (**a**); 30 μm **(b).**

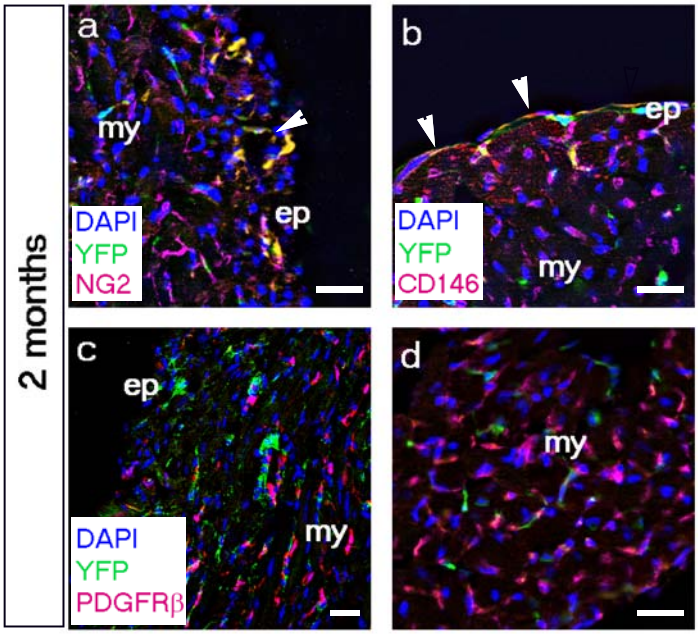
Balmer et al_Supplementary Fig. 11



Supplementary Figure 11. A minor sub-population of transplanted CD45+/Vav1-tdTomato+ cells were positive for the macrophage marker F4/80, but none were positive for the neutrophil marker MPO.

Immunostaining for F4/80 revealed some (less than 10%) of the transplanted Vav1-tdTomato (**a**) or CD45+ cells (**b**) were indicative of activated macrophages; consistent with the majority of transplanted cells staining negative for the myeloid lineage marker Ly6c (Figure 6t). MPO immunostaining revealed that none of the donor cells were neutrophils (**c**); ep, epicardium; my, myocardium. Scale bar 50μm (**a-c**)

Balmer et al_Supplementary Fig. 12



Supplementary Figure 12. Donor BM (YFP+) cells arising from within the adult epicardium differentiated, in part, into NG2+/CD146+ pericytes but not PDGFRβ+ fibroblasts.

Immunostaining for NG2 (**a**) and CD146 (**b**; positive cells highlighted by white arowheads) confirmed that a proportion of donor BM (YFP+) cells differentiated into pericytes (NG2+ cells adjacent to a coronary vessel highlighted by white arrowhead in **a**). Immunostaining for PDGFRβ excluded a contribution to the mesenchymal/fibroblast lineage in either epicardium (**c**) or the underlying myocardium (**d**). ep.epicardium; my, myocardium. Scale bars 50 μm (**a-d**).