BRG1-SWI/SNF-dependent regulation of the Wt1 transcriptional landscape mediates epicardial activity during heart development and disease

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Epicardium-derived cells (EPDCs) contribute cardiovascular cell types during development and in adulthood respond to Thymosin β4 (Tβ4) and myocardial infarction (MI) by reactivating a fetal gene programme to promote neovascularization and cardiomyogenesis. The mechanism for epicardial gene (re-)activation remains elusive. Here we reveal that BRG1, the essential ATPase subunit of the SWI/SNF chromatin-remodelling complex, is required for expression of Wilms’ tumour 1 (Wt1), fetal EPDC activation and subsequent differentiation into coronary smooth muscle, and restores Wt1 activity upon MI. BRG1 physically interacts with Tβ4 and is recruited by CCAAT/enhancer-binding protein β (C/EBPβ) to discrete regulatory elements in the Wt1 locus. BRG1-Tβ4 co-operative binding promotes optimal transcription of Wt1 as the master regulator of embryonic EPDCs. Moreover, chromatin immunoprecipitation-sequencing reveals BRG1 binding at further key loci suggesting SWI/SNF activity across the fetal epicardial gene programme. These findings reveal essential functions for chromatin-remodelling in the activation of EPDCs during cardiovascular development and repair.
SWI/SNF catalytic subunits are expressed in the epicardium. We found that BRG1 and BRM were localized throughout the developing heart (Fig. 1a,b; Supplementary Fig. 1a–d) and notably were expressed in the epicardium overlapping with the expression domain of Wt1 (Fig. 1c; Supplementary Fig. 1b–e). In the adult heart, BRG1 expression was comparatively reduced in the myocardium and absent from the quiescent epicardium (compare Fig. 1a with Fig. 1d; Supplementary Fig. 1a,b). Moreover, BRM localization was excluded from the epicardium (compare Fig. 1b with Fig. 1e), which also lacked Wt1 expression, consistent with the reported down-regulation of the embryonic epicardial gene program24 (Fig. 1f; Supplementary Fig. 1b). However, in response to MI, BRG1 and BRM expression in the epicardium increased (Supplementary Fig. 1f,g), most notably in the expanded subepicardial region (ses; Fig. 1g,h) and co-localized with Wt1 re-expression. Wt1 expression was further enhanced by stimulation with Tβ4 (priming) before MI24 (compare

![Figure 1](image_url)

**Figure 1** | BRG1 and BRM are expressed in the developing and adult epicardium. (a–i) Immunostaining for BRG1, BRM and Wt1 in mouse E11.5 (a–c), adult (d–f) and adult primed- (g–i) injured (day 4 post-MI) hearts (g–i). Arrows mark myocardium, arrowheads mark epicardium and double-arrow indicates the expansion of the subepicardial space post-MI. ep, epicardium; my, myocardium; ses, subepicardial space. All scale bars 100 μm.
Fig. 1i with Supplementary Fig. 1(h). Taken together, the spatiotemporal expression of BRG1 and BRM and colocalization with Wt1, suggests that SWI/SNF complexes may regulate epicardial Wt1 expression and, subsequently, EPDC activation. In support of this, EPDCs expressing enhanced GFP from the Wt1 locus of Wt1\textsuperscript{GFP/Cre} + knock-in mice\textsuperscript{17} were positive for BRG1 and BRM, in both the developing and post-MI adult heart (Supplementary Fig. 1c–h).

**SWI/SNF complex binds regulatory elements in the Wt1 locus.**

To determine whether SWI/SNF directly regulates Wt1 expression, we examined BRG1 and BRM binding to the Wt1 promoter in vivo. Using sequence alignment software (www.dcode.org), we compared mouse and human genomes to identify evolutionarily conserved regions that may act as sites for SWI/SNF association\textsuperscript{7,8} and found seven ECRs in the Wt1 locus, which were named according to their location in relation to the TSS: two distal, located upstream of the TSS (starting at \(\approx -4.4\) kb and \(-3.4\) kb and extending for 393 bp and 126 bp, respectively), three proximal, located immediately upstream of the TSS (starting at \(\approx -0.3\) kb, \(-0.2\) kb and \(-4.2\) bp and extending for 101 bp, 129 bp and 439 bp, respectively) and two located within intron 1 (starting at \(\approx +4.0\) kb and \(+5.8\) kb and extending for 106 bp and 188 bp, respectively; Fig. 2a; Supplementary Table 1). ChIP on embryonic hearts with an anti-BRG1 antibody revealed that, of the seven ECRs, BRG1 was strongly associated with the proximal promoter (\(-42\) bp) and intronic (+4.0 kb and +5.8 kb) ECRs of Wt1, but not with a non-ECR element (ECR) localized upstream of the TSS (starting at \(\approx -3.8\) kb and extending for 100 bp) confirming specificity of the ChIP signal (Fig. 2b). BRM, in contrast, only weakly associated with the +5.8 kb ECR (Fig. 2c), whereas BAF180 revealed a similar binding pattern to BRG1 (Fig. 2d). The co-occupancy patterns observed on Wt1 ECRs could result from transient binding by separate SWI/SNF components (BRG1 or BAF180) in different subsets of Wt1-expressing cells, or could be due to a direct interaction between these proteins. We, therefore, examined potential complex formation at Wt1 ECRs by sequential ChIP (re-ChIP). Strong re-ChIP signals were detected with an anti-BAF180 antibody on BRG1-enriched Wt1 ECRs (Fig. 2b) in hearts at embryonic day (E) 11.5 (Fig. 2e), confirming SWI/SNF complex formation. In combination, the ChIP experiments revealed enrichment of SWI/SNF containing BRG1 and BAF180 in regulatory regions of murine Wt1 in the embryonic heart, which directly correlated with epicardial-specific Wt1 expression (Fig. 1c). Moreover, even though BRM is expressed in the developing epicardium (Fig. 1b), BRG1 is the preferred ATPase subunit of the SWI/SNF complex binding at the Wt1 locus.

To investigate whether Wt1 is a direct BRG1–SWI/SNF target in the adult epicardium, we performed ChIP experiments using intact (Fig. 2fg) and injured-adult hearts (Fig. 2h–l). The adult heart does not ordinarily express Wt1 (Fig. 1f; Supplementary Fig. 1b) and neither BRG1, nor BRM associated with Wt1 ECRs in the intact adult heart (Fig. 2fg), as expected given the lack of expression of BRG1 and BRM in adult epicardium (Fig. 1d,e). However, in primed- (+T\(\beta\)4) and injured-adult hearts with evident Wt1 re-expression at day 4 post-MI (arrowheads in Fig. 1i), BRG1 had a similar binding pattern to that in the embryonic heart (compare Fig. 2bh). BRM was also found to bind to \(-42\) bp, \(+4.0\) kb and \(+5.8\) kb ECRs (Fig. 2i), in contrast to the lack of binding in epicardial hearts and suggesting that in the reactivated adult epicardium BRM may act redundantly with BRG1 to regulate Wt1 expression. By contrast, in non-primed-, injured-adult hearts that exhibited low levels of Wt1 at day 4 post-MI (arrowheads in Fig. 2i) there was minimal association of BRG1 and BRM to Wt1 ECRs (Fig. 2jk), even though BRG1 and BRM were expressed (Supplementary Fig. 1bg). Overall, these data demonstrate the existence of ECRs in the Wt1 locus that are bound by SWI/SNF complexes containing BRG1 or BRM (adult epicardium only) as the catalytic subunit. Occupancy at these ECRs dynamically alters during embryogenesis into adulthood and responds to exogenous T\(\beta\)4 and injury, suggesting that chromatin–remodelling of the Wt1 locus by SWI/SNF is amplified by T\(\beta\)4 and directly associates with enhanced Wt1 and epicardial activity\textsuperscript{23,24}.

To further characterize the remodelling of the Wt1 locus in the developing and adult heart, we investigated the association of \(-42\) bp, \(+4.0\) kb and \(+5.8\) kb ECRs, with RNApol II and canonical epigenetic modifications in histone H3 tails, previously implicated in gene activation and repression: K4me1, K4me3, K27ac and K27me3 (ref. 25). When Wt1 was ‘off’ (that is, intact adult heart) the repressive H3K27me3, catalysed by the Polycomb complex 2 (PRC2)\textsuperscript{26}, was the only epigenetic mark found to significantly associate to \(-42\) bp, \(+4.0\) kb and \(+5.8\) kb ECRs (compare Supplementary Fig. 2e with Supplementary Fig. 2a–d; \(p < 0.05\) for the three Wt1 ECRs; the two-tailed Student’s t-test was used to determine the statistical significance). In contrast, when Wt1 was ‘on’ (that is, embryonic and primed-, injured-adult hearts) RNApol II phosphorylated at serine five (confined to promoter regions and required for the initiation of transcription\textsuperscript{27}) was enriched in \(-42\) bp and \(+4.0\) kb ECRs (Supplementary Fig. 2f), suggesting that these elements act as bona fide regulatory elements of gene expression. Moreover, H3K4me1 (associated with putative enhancers) was enriched in \(+4.0\) kb and \(+5.8\) kb ECRs (Supplementary Fig. 2g), whereas H3K4me3 (associated with active promoters) was enriched in all three Wt1 ECRs (Supplementary Fig. 2h). Finally, while the activating histone mark H3K27ac (associated with TSS and active enhancers) was strongly enriched in \(-42\) bp and \(+4.0\) kb ECRs (Supplementary Fig. 2i), the counteracting, mutually exclusive modification H3K27me3 (associated with inactive promoters) was also detected in the three ECRs (Supplementary Fig. 2j), suggesting differences in the epigenetic remodelling of the Wt1 locus, which likely reflects the dynamic changes in spatiotemporal expression. Of note, when Wt1 activation is ‘low’ (that is, non-primed, injured-adult hearts) a similar epigenetic profile was observed on Wt1 ECRs (Supplementary Fig. 2k–o), suggesting that cardiac injury alone, which leads to minimal association of BRG1 and BRM to Wt1 ECRs (Fig. 2jk), is sufficient to induce remodelling of the Wt1 locus and subsequent low level Wt1 re-expression (Fig. 2l), however, this is suboptimal when compared with ectopic T\(\beta\)4 treatment (Fig. 1i).

**C/EBP\(\beta\) recruits the SWI/SNF complex into the Wt1 locus.**

Targeting of the SWI/SNF complex to the Wt1 locus within the epicardium is likely mediated by sequence-specific transcription factors, which access silent chromatin and initiate cell specific gene activation through recruitment of chromatin–remodelling complexes and co-activator partners\textsuperscript{5}. To this end, the pioneering C/EBPs have been shown to mediate epicardial gene activation during heart development and injury\textsuperscript{28}. In addition, the C/EBP\(\beta\) isoform has been shown to recruit the SWI/SNF complex to specific genomic loci, via direct interaction with BRG1 (ref. 29). In silico analysis of Wt1 \(-42\) bp, \(+4.0\) kb and \(+5.8\) kb ECR sequences predicted the existence of C/EBP\(\beta\) binding sites (Supplementary Table 2), raising the possibility that C/EBP\(\beta\) may recruit SWI/SNF complexes containing BRG1 to the Wt1 ECRs. To explore this further, we performed ChIP with an anti-C/EBP\(\beta\) antibody and observed enrichment of C/EBP\(\beta\) in all three ECRs in fetal and primed-, injured-adult hearts.
(Supplementary Fig. 3a–d). Subsequent re-ChIP analyses with an anti-BRG1 antibody confirmed complex formation between C/EBPβ and BRG1 on Wt1 ECRs (Supplementary Fig. 3b,d). Furthermore, siRNA-targeting of C/EBPβ transcripts resulted in decreased expression of Wt1 and the Wt1-downstream target gene Raldh2 (ref. 21) in mouse primary epicardial cells (Supplementary Fig. 3e,f), supporting a role for a C/EBPβ-SWI/SNF remodelling complex in the regulation of Wt1 ECRs.

**Wt1 ECRs drive epicardial gene expression in vivo.** To obtain in vivo evidence of Wt1 ECR activity, we employed an established transient transgenic enhancer reporter assay20 to engineer Wt1 ECR-\( \beta \)-galactosidase (\( \beta \)-gal) activity. The reporting of individual Wt1 ECR activity lacks cooperative interaction with cis-acting elements (enhancers or negative regulatory regions) as would be associated with the intact gene locus and consequently \( \beta \)-gal-reporter expression would not

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**Figure 2 | ATP-dependent SWI/SNF complexes bind ECRs in the Wt1 locus.** (a) Sequence alignment of the Wt1 locus from mouse and human. Peak heights indicate degree of sequence homology; pink bars above the peaks denote evolutionary conserved regions (ECRs); yellow represents the 5’ untranslated region (UTR); blue indicates exon 1. Boxed regions were selected for further analysis by ChIP. Numbers above boxed regions denote ECR approximate distances upstream (–) or downstream (+) of the transcription start site (TSS). (b–d) ChIP-qPCR data using chromatin derived from embryonic hearts at E11.5 and anti-BRG1, anti-BRM or anti-BAF180 antibodies. Note that no enrichment was detected in non-ECR elements (–ECR). (e) Sequential ChIP (re-ChIP) with an anti-BAF180 antibody using embryonic heart-derived chromatin pulled-down with anti-BRG1. (f) ChIP-qPCR data using chromatin derived from adult intact (no MI) hearts and anti-BRG1 or anti-BRM antibodies. (g) ChIP-qPCR data using chromatin derived from adult non-primed, injured-hearts at day 4 post-MI and anti-BRG1 or anti-BRM antibodies. Note that no enrichment was detected in non-ECR elements (–ECR). (h) ChIP-qPCR data using chromatin derived from adult primed- (+TJ4), injured-hearts at day 4 post-MI and anti-BRG1 or anti-BRM antibodies. (i) ChIP-qPCR data using chromatin derived from adult non-primed, injured-hearts at day 4 post-MI and anti-BRG1 or anti-BRM antibodies. (j) Wt1 immunostaining in post-MI adult myocardium; ses, subepicardial space. Scale bar 100 μm.

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**Table 1 | Fold enrichment of BRG1, BRM and BAF180 at ECRs.**

| Antibody | Embryonic | Adult | Adult, MI |
|----------|-----------|-------|-----------|
| BRG1     | Fold enrichment ±s.d. | | |
| BRM      | Fold enrichment ±s.d. | | |
| BAF180   | Fold enrichment ±s.d. | | |

**Fold enrichment**

| Antibody | Embryonic | Adult | Adult, MI |
|----------|-----------|-------|-----------|
| BRG1     | Fold enrichment ±s.d. | | |
| BRM      | Fold enrichment ±s.d. | | |
| BAF180   | Fold enrichment ±s.d. | | |
be expected to precisely mimic endogenous gene expression and instead overlap in-part with Wt1 in the developing embryo11,32 (Fig. 3a–i; Supplementary Figs 4,5). The –42 bp ECR, which based on its genomic location (Fig. 2a) is predicted to act as a Wt1 core promoter, recapitulated at least some of the regions of Wt1 expression (Supplementary Fig. 5), and importantly drove β-gal activity strongly throughout the epicardium covering the developing ventricles, atria and outflow tract (arrowheads and arrows, respectively in Fig. 3a,b; Supplementary Figs 4,5). The observed expression in the outflow tract was considerably elevated and regionally expanded relative to the corresponding Wt1 expression (Fig. 3a, Supplementary Fig. 5), likely due to the

Figure 3 | Wt1 ECRs drive gene expression and BRG1 is required for EPDC activity. (a–i) Wt1 ECRs are sufficient to drive LacZ expression in the epicardium at E11.5. Whole-mount (a,c,e,f,h) and transverse-sectioned hearts (b,d,g,i) are shown. Arrowheads mark epicardium covering the ventricles; arrows mark epicardium covering the atria (a,b) and outflow tract (a). Two (−42 bp), five (+4.0 kb) and six (+5.8 kb) transgenic mice were examined to assess the reproducibility of reporter activity patterns. (j–s) BRG1 and Wt1 immunostaining of E11.5 hearts documenting BRg1 deletion (asterisks in j,q) and Wt1 reduction (asterisks in m,r) in the epicardium of Brg1 cKOs compared to littermate controls (arrowheads in j,k,l,p). Note reduced BRG1 expression also in the myocardium (arrows; compared j with l versus o with q) of Gata5Cre;Brg1flox/flox but not Wt1CreERT2;Brg1flox/flox hearts. (n,s) BRG1 ChIP–qPCR data. Three independent ChIP experiments were performed per group (n = 10 hearts) and are presented as fold enrichment over IgG. (t,u) Wt1 immunostaining of E16.5 hearts documenting fewer Wt1-positive cells in the epicardium (arrowheads) and EPDCs invading the subepicardial and myocardial layers (arrows) in Brg1 cKOs. Note magnified views of (red) inset boxes in t and u. (v) Quantification of Wt1-expressing cells in E16.5 hearts as % = (total number of Wt1-positive cells per section/total number of DAPI nuclei per section) × 100; co, n = 3 hearts; mut, n = 4 hearts. (w,x) Endomucin (EMCN) and smooth-muscle myosin heavy chain (SM-MHC) immunostaining of E16.5 hearts documenting a reduction of patent coronary vessels, containing endothelium (arrow) surrounded by smooth muscle (arrowheads), in Brg1 cKOs hearts. Note magnified views of (red) inset boxes in panels w and x. (y) Quantification of patent vessels per field in E16.5 hearts; 6 fields analysed per section per genotype; co, n = 3 hearts; mut, n = 4 hearts. All error bars are data ± s.d. Significant differences (P values) were calculated using two-tailed Student’s t-test (**P ≤ 0.01; ***P ≤ 0.001). co, control; ep, epicardium; la, left atrium; lv, left ventricle; mut, mutant; my, myocardium; ra, right atrium; rv, right ventricle; v, ventricle. All scale bars 200 μm, except j,o,t,u,w,x 100 μm and magnified views of inset boxes in t,u,w,x 20 μm.
absence of cis-acting repressive elements present in the Wt1 locus. Elsewhere in the developing embryo, the −42 bp ECR drove β-gal activity strongly in domains of the spinal cord and brain (arrows in Supplementary Fig. 4a and Supplementary Fig. 5), where lateral motor neurons expressing Wt1 are being formed, and the mesothelium of the body wall and gut, which represent further sites of known Wt1 expression (arrow in Supplementary Fig. 4b; Supplementary Fig. 5). The activity of the more distal, intronic +4.0 kb (Fig. 3c,d; Supplementary Fig. 4d−f) and +5.8 kb (Fig. 3e–g; Supplementary Fig. 4g–i) ECR transgenes was relatively weaker, when compared to the core promoter element (−42 bp ECR), and restricted to epicardial cell clusters. Of note, the +5.8 kb ECR transgene also drove β-gal activity in the urogenital region (Supplementary Fig. 4j–l), where Wt1 plays a pivotal role in development. In the absence of ECR-LacZ transgene integration, no β-gal activity was observed confirming signal specificity (Fig. 3h,i).

**BRG1 is required for activation of the Wt1 locus.** We next tested whether BRG1 plays an essential role in Wt1 regulation. We first used siRNA to specifically knock-down Brg1 in mouse primary epicardial cells and observed reduction in the expression of Wt1 and Raldh2 (Supplementary Fig. 6a,b). Subsequently, we used Gata5Cre (Fig. 4d–f) and Wt1CreERT2 Cre-drivers crossed with a Brg1lox/lox mouse line to conditionally delete Brg1 in the mouse epicardium by E11.5 and examined Wt1 expression (Fig. 3j−r; Supplementary Fig. 6c,d). Both Cre strains induced reduction of Brg1 levels in the developing heart. Gata5Cre was relatively more efficient (Supplementary Fig. 6c), however, this was explained by Gata5Cre targeting Cre activity additionally in non-epicardial cells (Supplementary Fig. 6e,f), such that BRG1 was reduced in the developing endocardium/myocardium and epicardium as compared to Wt1CreERT2 targeting, which was epicardial-specific, albeit with variable efficiency (compare Fig. 3j,l with Fig. 3o,q). Notably, targeting of Brg1 led to decreased Wt1 and Raldh2 expression in mutant hearts, compared to control littermates (Fig. 3k,p,r; Supplementary Fig. 6d). Moreover, reduced Wt1 expression was strongly associated with reduced binding of BRG1 to Wt1 ECRs in Brg1-deficient hearts (Fig. 3n,s), further supporting that BRG1 occupancy is required for Wt1 activity during murine epicardial development.

To determine the functional consequences of deleting Brg1 in the developing epicardium, we extended the phenotypic analysis of Brg1-deficient embryos to E16.5 and assessed EPDC mobilization and accompanying coronary smooth muscle differentiation (Fig. 3t–v; Supplementary Fig. 6g–p). No live embryos positive for the Gata5Cre allele and bearing two copies of the Brg1 floxed alleles (Gata5Cre; Brg1F/F Mut) were collected at E16.5; indeed harvesting of embryos at earlier developmental stages revealed that Brg1 mutants died between E12.5 and E16.5 (Supplementary Fig. 6g). At E12.5, mutant hearts exhibited lower levels of coronary smooth muscle markers compared to littermate controls (Co) (Supplementary Fig. 6h), including SM22a (smooth muscle protein 22-alpha) and SMAx (smooth muscle actin, alpha). In addition, endothelial/endoocardial markers (Supplementary Fig. 6i) such as platelet and endothelial cell adhesion molecule 1 (PECAM) and the tyrosine-protein kinase receptor Tie2, and myocardial markers (Supplementary Fig. 6j), such as the myosin heavy chain polypeptides α and β (αMHC and βMHC, respectively), were also reduced in mutants, reflecting Cre activity and the requirement for Brg1 in both epicardial and non-epicardial compartments (Supplementary Fig. 6e). Moreover, histological characterization of transverse sections from mutant hearts revealed under-developed and disorganized chamber walls, with no clear ventricular septation and dilated atria (compare Supplementary Fig. 6k with 6l). The severity of these cardiac malformations is likely to induce embryonic lethality, preventing the examination of patent coronary vasculature at E16.5. Rare surviving mutants, collected at E14.5 (n = 8 out of 73 embryos) and E15.5 (n = 1 out of 16 embryos), revealed small, under-developed hearts (Supplementary Fig. 6m,n), a lack of Wt1-positive epicardial cells and a disorganized endomucin (EMCN)-positive endocardium/endothelium (Supplementary Fig. 6o,p), respectively. Consequently, in order to address the functional consequences of epicardial deletion of Brg1 on EPDC activity at later stages of embryogenesis, we focused our studies on the characterization of mutant embryos generated using the epicardial-specific Wt1CreERT2 Cre-driver. Wt1-derived Cre activity was induced by tamoxifen delivery to delete Brg1 from E10 and while mutant embryos collected at E16.5 appeared grossly normal, immunostaining revealed a reduction in the number of Wt1-positive cells located in the epicardium or invading the subepicardial space and adjacent myocardial layer (arrowheads and arrows, respectively in Fig. 3t,u), which was statistically significant compared to littermate control hearts (Fig. 3v; P < 0.001; the two-tailed Student’s t-test was used to determine the statistical significance). Additional immunostaining studies using antibodies against the coronary smooth muscle marker SM-MHC (smooth muscle-myosin heavy chain polypeptide 11) and endothelial marker EMCN, showed a dramatic decrease in the number of patent coronary blood vessels (an endothelial layer surrounded by supporting smooth cells) in embryos positive for the Wt1CreERT2 allele and bearing two copies of the Brg1 floxed alleles (Wt1CreERT2; Brg1F/F Mut; Fig. 3w–y), further confirming the requirement for Brg1-mediated regulation of EPDC activity in the developing heart.

**Tβ4 interacts with BRG1 augmenting Wt1 activation post-MI.** Collectively our data revealed that the SWI/SNF complex promotes Wt1 activity in the developing and adult epicardium, via binding of its catalytic subunit at three discrete cis-regulatory elements in the Wt1 locus. Moreover, SWI/SNF-mediated function is enhanced by stimulation with exogenous Tβ4 before MI. Insight into how Tβ4 amplifies SWI/SNF chromatin-remodelling emerged with the identification of BRG1 as a potential binding partner of Tβ4 via a yeast two-hybrid (Y2H) screen using a mouse embryonic complementary DNA (cDNA) library as prey and Tβ4 as bait to identify molecular interactors. To validate the Y2H finding, we performed co-immunoprecipitation experiments, between EGFP-tagged Tβ4 and endogenous BRG1 (Fig. 4a,b). Tβ4-EGFP co-immunoprecipitated with BRG1, but not with the control IgG indicating specificity of binding (Fig. 4a). Moreover, subsequent immunoblotting for BRG1 confirmed successful immunoprecipitation of the ATPase subunit by the anti-BRG1 antibody (Fig. 4a). Reciprocal co-immunoprecipitation experiments, using an anti-Tβ4 antibody for pull-down with anti-BRG1 and anti-EGFP antibodies for immunoblotting, further confirmed physical interaction between BRG1 and Tβ4 (Fig. 4b). Tβ4 is expressed in the developing and adult injured heart, including the epicardium (Supplementary Fig. 1e; Supplementary Fig. 7a–c), in an equivalent spatiotemporal pattern to BRG1 supporting a potential protein–protein interaction. To probe a physical association between BRG1 and Tβ4 in vivo, we performed Duolink II in situ proximity ligation assay (PLA) with specific PLUS and MINUS PLA probes against BRG1 and Tβ4 antibodies in embryonic and postnatal hearts (Fig. 4c–e). Nuclear PLA positive signals (red ‘spuckles’) were present in myocardial and epicardial cells in the developing heart (arrow and arrowheads, respectively in Fig. 4c) and in the
Figure 4 | Tβ4 interacts with SWI/SNF enhancing activation of the adult epicardium. (a,b) Immunoprecipitation experiments with Tβ4-EGFP-transfected cells using anti-BRG1 (a) or anti-Tβ4 (b) antibodies. The original uncropped images of gels are shown in Supplementary Fig. 11. (c–e) Duolink in situ analysis using anti-rabbit PLUS and anti-mouse MINUS PLA probes specific for Tβ4 and BRG1 antibodies respectively, in embryonic (c), intact adult (d) and post-MI hearts (e). (f–h) Duolink in situ analysis using anti-rabbit PLUS and anti-mouse MINUS PLA probes against BRG1 and BAF155. (i–k) re-ChIP with anti-Tβ4, anti-BAF180 or anti-BRG1 antibodies using chromatin pulled-down with anti-BRG1 (i) or anti-BAF155 (j) to examine Tβ4-SWI/SNF complex formation in E11.5 (i) and adult post-MI, primed hearts (j,k). (l) ChIP-qPCR data from chromatin derived from post-MI, Tβ4 knockout (KO) adult hearts showing minimal binding of BRG1 in Wt1 ECRs. (m) Wt1 and Raldh2 expression analysis by qRT-PCR compared to sham-operated animals. (n) ChIP-qPCR data from chromatin generated from primed- ( + Tβ4), and injured- (MI) adult Tβ4 knockout hearts revealing BRG1 strong enrichment in Wt1 ECRs. (o) Wt1 and Raldh2 expression analysis by qRT-PCR compared to sham-operated animals. At least three independent ChIP experiments per antibody per sample group (n = 10 fetal hearts; n = 3 adult hearts) were performed. ChIP results are presented as fold enrichment over input, whereas re-ChIP results are present in fold enrichment over the level of ChIP with negative control IgG antibody. (p) List of representative BRG1-enriched epicardial genes shared between PBS- and Tβ4-primed, -injured (MI) adult hearts and representative UCSC browser snapshots of the Aldh1a2 and Tbx18 loci (highlighted in blue in the list of genes) derived from the Brg1 ChIP-seq experiments. All error bars are data ± s.d. Significant differences (P values) were calculated using two-tailed Student’s t-test (*P ≤ 0.05; **P ≤ 0.01). ep, epicardium; my, myocardium; ses, subepicardial space. All scale bars 50 μm.

expanding subepicardial space upon injury (arrowheads in Fig. 4e), but not in the intact adult heart (Fig. 4d). This result was in agreement with the expression pattern of BRG1 (Fig. 1a,d,g).

To test the specificity of the nuclear PLA signals both a positive control with BRG1 and BAF155 (another subunit of the SWI/SNF complex, gene alias SMARCC1, with a similar expression pattern;
Supplementary Fig. 7d–f) antibodies (Fig. 4f–h) and negative controls with Tβ4 and BRG1 antibodies on their own (Supplementary Fig. 7g,h) or BRG1 and Tβ4 antibodies on Tβ4 knockout samples (Supplementary Fig. 7i) were included. Similar to BRG1 and Tβ4, nuclear BRG1 and BAF155 PLA signals were detected exclusively in the myocardium and epicardium of both embryonic and post-MI hearts, confirming the presence of functional SWI/SNF chromatin–remodelling complexes (arrow and arrowheads in Fig. 4f,h). Further confirmation of an interaction between Tβ4 and the SWI/SNF catalytic subunit was obtained in vivo by re-ChIP analyses (Fig. 4i,j). Robust re-ChIP signals were detected with an anti-Tβ4 antibody on BRG1-enriched Wt1 ECRs in embryonic (Fig. 4i) and adult primed- (+Tβ4), injured- hearts (Fig. 4j). Of note, the latter BRG1-enriched elements were also enriched for BAF180 (Figs 2e and 4j), confirming the formation of SWI/SNF complexes containing Tβ4, BRG1 and BAF180 in epicardial cells. Likewise, re-ChIP experiments using BRM-enriched Wt1 ECRs revealed that BRM interacts with Tβ4, but not BRG1 in primed-, injured-adult hearts (Fig. 4k), resulting in the formation of Tβ4-BRM/SWI–SWI/SNF complexes in the adult reactivated epicardium. Re-ChIP experiments using BRM-enriched Wt1 ECRs at E11.5 were not possible due to the low binding of BRM in the Wt1 locus (Fig. 2c).

Following our observations that Tβ4 priming enhanced SWI/SNF-mediated activation of Wt1 in the adult epicardium (Fig. 1i versus Fig. 2i; 2h,i versus Fig. 2j,k; Supplementary Fig. 1b), we tested whether endogenous levels of Tβ4 (Supplementary Fig. 7b,c) were sufficient to promote SWI/SNF complex activity. Tβ4 knockout mice, followed by MI, revealed a modest increase in SWI/SNF-binding Wt1 ECRs and associated increase in Wt1 expression during heart injury (Fig. 4l,m; also in development, Supplementary Fig. 8), however, priming with ECRs augmenting in significantly enhanced binding of the SWI/SNF complex to Wt1 ECRs resulting in Wt1 reactivation (Fig. 4n,o). This suggested that endogenous Tβ4 is dispensable for optimal SWI/SNF binding and reinforced a functional requirement for ectopic (endogenous) Tβ4 priming in nuclear targeting of SWI/SNF-mediated regulation of Wt1. To further investigate the role of Tβ4 in SWI/SNF transcriptional activation of Wt1, we cloned the Wt1 ECRs into a chromatinized episomal reporter, pREP4 (ref. 36), and transfected the constructs into the human cell line SW13 that lacks BRG1 and BRM, but expresses all other SWI/SNF components. SW13 cells were transfected with luciferase reporters (−0.2 kb-luc, −42 bp-luc, +4.0 kb-luc or +5.8 kb-luc) and either BRG1/BRM/Tβ4 alone or Tβ4 in combination with BRG1 or BRM expression constructs. A myc-tagged Tβ4 construct, carrying a nuclear localization signal (NLS-Tβ4-myc), was used to drive Tβ4 into the nucleus. Restoring BRG1 expression to SW13 cells resulted in a significant increase in activity of all Wt1 ECR reporters, with the only exception being the −0.2 kb ECR (Supplementary Fig. 9; −0.2 kb-luc: 1.085 ± 0.13 versus −42 bp-luc: 1.211 ± 0.14; +4.0 kb-luc: 1.359 ± 0.11; and +5.8 kb-luc: 1.430 ± 0.20; P ≤ 0.001 (−42 bp-luc), P ≤ 0.0001 (+4.0 kb-luc) and P ≤ 0.01 (+5.8 kb-luc)); one-way analysis of variance (ANOVA) followed up by Tukey’s multiple comparison test was used for comparing three or more groups). A lack of activity at the −0.2 kb ECR was anticipated given that no BRG1 binding was detected for this element, suggesting that it does not convey any regulatory function. Restoring BRM expression also resulted in a significant increase in +4.0 kb-luc and +5.8 kb-luc reporter activity (Supplementary Fig. 9; 1.241 ± 0.07 and 1.376 ± 0.06, respectively, P ≤ 0.01 (+4.0 kb-luc) and P ≤ 0.05 (+5.8 kb-luc)). Tβ4 expression alone did not induce Wt1 ECR reporter activity (Supplementary Fig. 9), however, co-expression of BRG1 or BRM with Tβ4 significantly increased −42 bp, +4.0 kb and +5.8 kb-luc luciferase activity over BRG1, BRM and Tβ4 alone (Supplementary Fig. 9; −42 bp-luc: 1.406 ± 0.12 (BRG1 + Tβ4) and 1.175 ± 0.19 (BRM + Tβ4) versus 1.211 ± 0.14 (BRG1) and 1.055 ± 0.06 (BRM), P ≤ 0.001 for both ECR-luc; +4.0 kb-luc: 1.646 ± 0.238 (BRG1 + Tβ4) and 1.578 ± 0.11 (BRM + Tβ4) versus 1.359 ± 0.11 (BRG1) and 1.241 ± 0.07 (BRM), P ≤ 0.001 and P ≤ 0.0001 respectively; +5.8 kb-luc: 1.887 ± 0.414 (BRG1 + Tβ4) and 1.684 ± 0.215 (BRM + Tβ4) versus 1.430 ± 0.20 (BRG1) and 1.376 ± 0.06 (BRM), P ≤ 0.01 and P ≤ 0.05 respectively; one-way ANOVA followed up by Tukey’s multiple comparison test was used for comparing three or more groups). These results are in line with our in vivo observations (Fig. 4i–o) and support direct activation of Wt1 ECRs by SWI/SNF complexes containing Tβ4.

A role for BRG1-SWI/SNF in global gene activation. To investigate whether the role for BRG1-SWI/SNF complexes is restricted to the Wt1 locus, or whether they might regulate other epicardial loci, we employed ChIP-seq to perform a comparative analysis of the binding of BRG1 in PBS- versus Tβ4-primed, injured- (MI) adult hearts (Fig. 4p; Supplementary Fig. 10). Among the shared genes, between PBS- and Tβ4-primed, injured-adult hearts, we identified binding at Wt1, confirming our targeted ChIP-qPCR data, and at other fetal epicardial loci such as Tbx18, Aldh1a2 and Tcf21 (Fig. 4p). BRG1 binding was also evident at adult epicardial genes (Fig. 4p) previously identified in a transcriptomic study of the adult murine epicardium, including the membrane proteins uroplakin 3b (Upk3b), uroplakin 1b (Upk1b), glycoprotein m6a (Gpm6a) and the transcription factor basoonulin-1 (Bcn1). In addition, key myocardial genes Myh6 (also known as αMHC) and Myh7 (also known as βMHC), encoding for myosin heavy chains alpha and beta, respectively, and other cardiac-related genes, such as Colla2 (collagen 1 alpha-2, a fibroblast marker) and Acta2 (also known as SM actin, alpha 2, a smooth muscle marker), were also represented in the list of shared BRG1-enriched genomic regions (Supplementary Fig. 10), suggesting novel gene-regulating functions for the SWI/SNF chromatin–remodelling complex in the (adult) injured heart. Of significance, the expression of some of these genes (for example, αMHC and βMHC) was found to be decreased upon Gata5Cre-remediated deletion of Brg1 in the developing non-epicardial compartment (Supplementary Fig. 6h,i), in agreement with the hypothesis that the function of the SWI/SNF chromatin–remodelling complex in the developing heart is recapitated in the adult injured heart. To validate the genomic binding of BRG1, we analysed the ChIP-seq profile in already described sites of SWI/SNF occupancy, such as our intrinsic +4.0 kb ECR within the Wt1 locus (Fig. 2) and the 5 kb region upstream of the Myh7 TSS. While BRg1-binding was detected in these regions in both PBS- and Tβ4-primed, -injured samples, considerably more peaks were observed in the Tβ4-primed sample set (Supplementary Fig. 10). Likewise, BRG1 peak density was comparatively higher in epicardial loci such as Tcf21, Upk3b and Tbx18 and cardiac-related loci such as Acta2 in the Tβ4-primed, -injured sample set versus the non-primed (PBS) group (Fig. 4p; Supplementary Fig. 10). As such, we speculate that Tβ4 may play a role in amplifying BRG1-targeting of downstream embryonic epicardial loci, as important for the reactivation of EPDCs following adult heart injury. Taken together, our ChIP-seq data supports BRG1–SWI/SNF binding of Wt1 and suggests a role for chromatin–remodelling in activation of further key epicardial genes, such as Tbx18 and Tcf21.
Discussion

Here we reveal that epicardial activity is epigenetically regulated during development and adult heart injury and that Wt1, the master regulator of epicardial EMT, is dynamically controlled by SWI/SNF chromatin-remodelling complexes containing BRG1 and Tb4 (Figs 1–4). Tb4 lacks a DNA-binding domain, but physically interacts with SWI/SNF (Fig. 4) and serves to augment BRG1 and BAF/BRG1 binding at specific enhancer sites within the Wt1 locus (Figs 2 and 4). BRG1, as the preferred SWI/SNF ATPase subunit, was essential to maintain Wt1 expression and associated EPDC activity (Fig. 3). Tb4–SWI/SNF contained the epicardial-specific pioneer transcription factor C/EBPb (Supplementary Fig. 3), which likely functions to target the epicardial-specific pioneer transcription factor C/EBPb along with the BAF53 subunit, tightly binds via nuclear actin as an essential component of the SWI/SNF

The reactivation of embryonic genes is a hallmark of attempts by the adult heart to compensate for cardiovascular stress and disease. This includes pathologic hypertrophy which is associated with the up-regulation of a signature fetal gene program.43 BRG1 is reactivated in the adult myocardium in response to cardiac stress and forms a complex with its embryonic partners, HDACs (histone deacetylases) and PARP (poly ADP ribose polymerase), to induce z-mycosin heavy chain (MHC) to β-MHC switch.44 Fetal gene re-expression, in this setting, is insufficient to counteract the pathology and suppress adverse remodelling and is unable to instigate a regenerative response. There is a requirement, therefore, for amplification of the epigenetic effector signals and to elicit targeting to key loci, upstream of multiple developmental pathways. In this regard, the relative levels of Tb4 appeared to be critical for efficient SWI/SNF complex activity and Wt1 expression (Figs 1, 2 and 4), which likely sits upstream of EPDC activation and cell fate. This was particularly relevant in the context of reactivation of the adult epicardium upon myocardial injury (Figs 2 and 4), whereby ectopic Tb4 priming resulted in optimal activation of SWI/SNF-enhanced reactivation of EPDCs (Figs 2 and 4) and subsequent differentiation into coronaries and muscle.23,24; while injury alone (despite inducing an up-regulation of endogenous Tb4 expression) resulted in minimal SWI/SNF activation (Fig. 2) of EPDCs,44 which subsequently adopts a default fibroblast differentiation program.22 ChIP-seq revealed an extensive range for Tb4–BRG1–SWI/SNF activity following adult cardiac injury at additional epicardial and cardiac gene loci (Fig. 4). Further studies will be required to dissect the functional implications of primoscuous BRG1–Tb4 activity in the context of promoting adult cell activation, in light of our previous findings that Tb4 treatment prior to MI facilitates improved heart repair.4 Epigenetic induction, with co-factor amplification, of fetal genes to activate adult progenitor populations may represent a general paradigm in cell-based tissue repair. More specifically, targeting dynamic chromatin signatures at key embryonic genes that underlie epicardial cell activation could represent a therapeutic strategy to regenerate the injured-adult mammalian heart.

Methods

Animal models. All animal experiments were carried out according to UK Home Office project Licence PPL30/2987 compliant with the UK animals (Scientific Procedures) Act 1986 and approved by the local Biological Sciences Ethical Review Process. Mice were housed and maintained in a controlled environment. Gata5−/−, Tbx1+10GFP; Wt1Cdr2ERT2, R26RtdTomato; Brg1flox/flox were kept in a C57Bl/6J inbred background. For timed mating experiments, 8–12 week old mice were set-up overnight and females checked for vaginal plugs the following morning; the date of a vaginal plug was set as embryonic day (E) 0.5. For tamoxifen-dependent tissue-specific gene inactivation using the Wt1CreERT2 driver, 2 mg of 4-hydroxytamoxifen (Sigma) were administered to pregnant mice by intraperitoneal (i.p.) injection at both E9.5 and E10.5 to maximize Cre-ERT2 activity in the developing epicardium.

Thymosin β4 administration. The injection regimen of Tb4 for priming of wild-type C57BL/6J, Tb4−/− and Wt1GFP;Cre + mice have been previously described.43 Briefly, adult mice received i.p. injection of clinical grade Tb4 (12 mg/kg; RegeneRex Biopharmaceuticals Inc.) or vehicle (PBS) daily for 7 days. On the eighth day, MI was performed and further injections of Tb4/vehicle were given on day 0 and day 2 post surgery.

Myocardial infarction. All surgical and pharmacological procedures were performed in accordance with the Animals (Scientific Procedures) Act 1986 (Home Office, UK). For MI experiments, adult mice (12–14 week old; 25–30 g body weight) were primed with Tb4 or vehicle, as described above, before surgical procedures. MI was induced in isoflurane-anaesthetized mice by permanent ligation of the left anterior descending coronary artery (LAD). For sham controls, a suture was passed under the LAD but not ligated. Upon recovery (day 0), animals received i.p. injection of Tb4 (12 mg kg−1) or vehicle (PBS). Further injections were given every second day. Hearts were harvested at 4 days following ligation.

Immunodetection methods. Cryosections were processed for indirect immunofluorescence using standard methods. AlexaFlour secondary antibodies (Invitrogen, 1:200) were used in all cases. Where mouse primary antibodies were used, 1 h incubation with AffiniPure Fab Fragment Goat Anti-Mouse IgG (Jackson ImmunoResearch, 1:50) before primary antibody incubation was performed to reduce non-specific signal. Imaging was performed using a Zeiss Axio Imager Z1 microscope with an AxioCam MRm camera attachment running AxioVision software release 4.8 (Carl Zeiss).

Duolink in situ analysis. Cryosections were processed as for immunofluorescence, up to and including Fab fragment treatment and incubation with primary antibodies. Duolink was performed as per manufacturer’s instructions (Olink Bioscience) using anti-rabbit PLUS and anti-mouse MINUS PLA probes and Orange detection reagents. Imaging was performed using a Zeiss Axio Imager Z1 microscope with an AxioCam MRm camera attachment running AxioVision software release 4.8.

Whole-embryo in situ hybridization. RNA in situ hybridization on whole-embryo was performed on E11.5 embryos fixed with 4% formaldehyde solution. In brief, embryos were treated with proteinase K for 30 min and post-fixed with 4% embryo was performed on E11.5 embryos fixed with 4% formaldehyde solution. In this regard, the relative levels of Tb4 appeared to be critical for efficient SWI/SNF complex activity and Wt1 expression (Figs 1, 2 and 4), which likely sits upstream of EPDC activation and cell fate. This was particularly relevant in the context of reactivation of the adult epicardium upon myocardial injury (Figs 2 and 4), whereby ectopic Tb4 priming resulted in optimal activation of SWI/SNF-enhanced reactivation of EPDCs (Figs 2 and 4) and subsequent differentiation into coronaries and muscle.23,24; while injury alone (despite inducing an up-regulation of endogenous Tb4 expression) resulted in minimal SWI/SNF activation (Fig. 2) of EPDCs,44 which subsequently adopts a default fibroblast differentiation program.22 ChIP-seq revealed an extensive range for Tb4–BRG1–SWI/SNF activity following adult cardiac injury at additional epicardial and cardiac gene loci (Fig. 4). Further studies will be required to dissect the functional implications of primoscuous BRG1–Tb4 activity in the context of promoting adult cell activation, in light of our previous findings that Tb4 treatment prior to MI facilitates improved heart repair.4 Epigenetic induction, with co-factor amplification, of fetal genes to activate adult progenitor populations may represent a general paradigm in cell-based tissue repair. More specifically, targeting dynamic chromatin signatures at key embryonic genes that underlie epicardial cell activation could represent a therapeutic strategy to regenerate the injured-adult mammalian heart.

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Co-Immunoprecipitation and immunoblotting. For the co-IP experiments (Fig. 4a,b), HEK293 cells were transfected using Polyfect Transfection Reagent (Qagen). Approximately 2.4 × 10^6 cells were seeded into 60 mm tissue culture dishes and transfected 24 h later with 8 μg of T4EGFP-N3 and 80 μg Polyfect. After 48 h of post-transfection, cells were lysed as detailed above and 750 μl of lysate was used for immunoprecipitation (IP) using Dynabeads Protein G (Invitrogen), as per manufacturer’s instructions but with amendments to the incubation times. Antibodies used for IP were: Brst1-H188 (Santa Cruz); TATA box binding protein (TBP)-N1 (Santa Cruz) and control IgG (Abcam). Brg1 antibody was diluted in 1% PBS plus 0.02% Tween-20 were added to 50 μl Dynabeads and incubated with rotation for 2 h at room temperature. As a positive control for IP, breast cancer cell line MCF-7 (ATCC) was lysed and used as a control for transient transfected cells. For the co-IP experiments, primers that added a BamHI site to the 5′ end and an XhoI site to the 3′ end of the TATA box-EGFP-N3 template were used to amplify the 4.0 kb luciferase—was then excised from pGL3-promoter using NotI and XhoI and ligated into pTRE4-promoter plasmid. TBP was ligated with Brst1-H188 and TBP into pGL3-Promoter, also for ChIP-qPCR (Supplementary Table 3), but relevant restriction sites were inserted in the forward and reverse sequences to allow cloning of ECR). PCR was performed using primers that added NotI and XhoI and ligated into pGEM3-promoter, also for ChIP-qPCR (Supplementary Table 3), but relevant restriction sites were inserted in the forward and reverse sequences to allow cloning of ECR). PCR was performed using primers that added NotI and XhoI and ligated into pTRE4 promotor, ChIP-qPCR (Supplementary Table 3), but relevant restriction sites were inserted in the forward and reverse sequences to allow cloning of ECR). PCR was performed using primers that added NotI and XhoI and ligated into pTRE4-promoter plasmid. For the data presented in Fig. 3, however, fold enrichment values are presented as the fold-change over the level of ChIP with negative control IgG antibody (ChIP signal/IgG signal) to allow comparison between control (co) and mutant (mut) samples.

ChIP-sequencing. Brst1 ChIP-seq was performed on PBS- and TATA-primed, injured-adult hearts (biological replicates) collected four days after MI. Briefly, tissues were cross-linked with 1% formaldehyde at room temperature for 10 min, incubated in nuclei extraction buffer first, then in lysis buffer and sonicated. 

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Solubilized chromatin was incubated overnight at 4 °C with a specific rabbit polyclonal anti-Brg1 antibody (Millipore; Supplementary Table 4) coupled to anti-rabbit IgG Dynabeads (Invitrogen). Immunoprecipitated chromatin was then purified by standard phenol-chloroform extraction and DNA libraries were prepared with the NEBNext Ultra DNA Library Prep Kit for Illumina according to the manufacturer's protocol. Libraries were quantified with Qubit dsDNA HS Assay Kit (Invitrogen) and KAPA Library Quantification Kit for Illumina, and then sequenced on an Illumina NextSeq 500 instrument. Sequence reads were mapped with Bowtie (version 1.0.0), using in 2 - X 2000 parameter, to the mouse reference genome (mm9), obtained from the UCSC genome browser. Peaks were called using MACS2 (version 2.0.10) using bgdcomp function. Genes closest to peaks were determined using Bedtools (version 2.17.0). Bigwig files were generated using an enhanced perl script (courtesy of Dr Jim Hughes, Weatherhall Institute of Molecular Medicine, University of Oxford, UK). Plots were made using ChiPSeeker and Bioconductor packag**

Sequential Chip.** After primary ChIP, specific immunoprecipitates were washed, eluted, incubated with 10 mM dithiothreitol at 37 °C for 30 min, diluted 10 times in ChIP buffer (10 mM EDTA, 50 mM Tris–HCl (pH 8.0), 1% SDS, protease inhibitors) and re-immunoprecipitated with the secondary antibodies. Isolation of re-immunoprecipitated chromatin was done according to manufacturer's protocol (Millipore). Extracted DNA was used for qPCR (see above). Fold enrichment was determined as the fold-change in per cent IgG (ChIP signal/IgG signal) at the target regions.**

**siRNA experiments.** siRNA sequences targeting Brg1 and C/EbetaR transcripts (Silencer Select Pre-designed siRNA from Ambion) were transduced into mouse primary epicardial cells using Lipofectamine 2000 (Life Technologies) according to the manufacturer's instructions.

**Statistical analysis.** Animal numbers and sample sizes reflected the minimal number needed for statistical significance based on previous experience. For MI studies, hearts showing no morphological signs of infarct (for example, tissue blanching around the ligating suture) at the stage of sample collection were excluded from further analysis. No randomization or blinding was used. Statistics were calculated using Microsoft Excel and Prism Graphpad software. The statistical significance between two groups was determined using two-tailed Student’s t-test (Microsoft Excel), and the data were reported as mean ± s.d. Among three or more groups (for example, data shown in Supplementary Fig. 9), one-way analysis of variance (ANOVA) followed up by Tukey’s multiple comparison test was used for comparisons (Prism Graphpad software). A value of P < 0.05 was considered statistical significant.

**Data availability.** The ChiP-seq data that support the findings of this study have been deposited in Gene Expression Omnibus (GEO) with the accession number GSE76239. All other relevant data are available from the corresponding authors on reasonable request.

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Acknowledgements
This work was funded by the British Heart Foundation (programme grant: RG/08/003/25264) and supported by the BHF Oxbridge Regenerative Medicine Centre (RM/13/3/30159). We are grateful to W.T. Pu (Boston, USA) for generously providing the Wt1CreERT2/+ and Wt1CreERT2/+ mouse strains; to J.V. Barnett (Nashville, USA), S. De Val (Oxford, UK), C. Englert (Jena, Germany), P. Hohenstein (Edinburgh, UK), A. Imbalzano (Worcester, USA), M. Mommersteeg (Oxford, UK), S. Naidu (Indiana-Police, USA), H. Scholz (Berlin, Germany), B. Wilm (Liverpool, UK) and K. Zhao (Bethesda, USA) for providing reagents including cell lines, expression constructs and plasmids; to J. K. Takeuchi (Tokyo, Japan), B. G. Bruneau (San Francisco, USA) and A. Chappell (London, UK) for Cre protocols and technical assistance. We thank Regenerex Biopharmaceuticals Inc. (http://www.regenerex.com) for provision of clinical grade Thymosin Tβ4.

Author contributions
J.M.V., S.H. and C.V.C. carried out experiments (except MI surgeries), analysed the data and contributed figures for the manuscript; S.B., K.N.D. and M.R. performed all MI surgeries. D.N.B. processed samples and performed immunofluorescence. X.S. and L.E.H. performed whole-embryo in situ hybridization. R.W. and T.S.S. provided technical assistance with the BRG1 ChIP-seq studies, and D.G. analysed the data set generated. D.M. and P.C. provided the Brgl1-floxed mice. B.D. generated Wt1 ECR-Hsp68-LacZ transgenic mice. J.M.V. and P.R.R. established the hypotheses, supervised the studies and co-wrote the manuscript.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing interests: The authors declare no competing financial interests.

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How to cite this article: Vieira, J. M. et al. BRG1-SWI/SNF-dependent regulation of the Wt1 transcriptional landscape mediates epicardial activity during heart development and disease. Nat. Commun. 8, 16034 doi: 10.1038/ncomms16034 (2017).

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