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Imaging reveals the connection between spontaneous coronary plaque ruptures, atherothrombosis and myocardial infarctions in HypoE/SRBI$^{-/-}$ mice

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Disclosures: None.

Short running title: **Coronary plaque ruptures in HypoE/SRBI$^{-/-}$**

ABSTRACT

Background: The hyperlipidemic mouse model HypoE/SRBI$^{-/-}$ has been shown to develop occlusive coronary atherosclerosis followed by myocardial infarctions and premature deaths in response to high-fat, high-cholesterol diet (HFC). However, the causal connection between myocardial infarctions and atherosclerotic plaque rupture events in the coronary arteries has not been investigated so far. **Objective**: To assess whether diet-induced coronary plaque ruptures trigger atherothrombotic occlusions resulting in myocardial infarctions in HFC-fed HypoE/SRBI$^{-/-}$ mice. **Methods:** HypoE/SRBI$^{-/-}$ mice were characterized with respect to the individual dynamics of myocardial infarction(s) and features of infarct-related coronary atherosclerosis by serial non-invasive molecular and functional imaging, histopathology, and a pharmaceutical intervention. Detailed histological analysis of whole mouse hearts was performed when spontaneously occurring acute myocardial infarctions were diagnosed by imaging. **Results:** Using the imaging-triggered approach we discovered thrombi in 32 (10.8%) of all 296 atherosclerotic coronary plaques in 14 HFC-fed HypoE/SRBI$^{-/-}$ mice. These thrombi typically were found in arteries presenting with inflammatory plaque phenotypes. Acetylsalicylic acid treatment did not attenuate the development of atherosclerotic coronary plaques, but profoundly reduced the incidence of premature deaths, the number of thrombi (7 in 249 plaques) and also the degree of inflammation in the culprit lesions. **Conclusions:** HFC-induced ruptures of coronary plaques trigger atherothrombosis, vessel occlusions, myocardial infarctions and sudden death in these mice. Thus, the HypoE/SRBI$^{-/-}$ mouse model mimics major features of human coronary heart disease and might therefore be a valuable model for the investigation of molecular and cellular parameters driving plaque rupture-related events and the development of new interventional approaches.

Key Words: hyperlipidemic mouse model; atherosclerosis; thrombosis; plaque vulnerability; imaging; aspirin

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INTRODUCTION

Cardiovascular diseases are the leading cause of deaths worldwide [1]. Atherosclerosis underlies these pathologies in the majority of cases and may trigger sudden life-threatening events such as myocardial infarction or stroke. Currently, ruptures of so-called 'vulnerable' atherosclerotic plaques are considered a primary cause of these events. Plaque ruptures occur acutely, trigger thrombus formation and occlusion of the respective artery lumen finally resulting in acute ischemic events.

Vulnerable plaques are advanced atherosclerotic lesions consisting of a necrotic core, an abundant accumulation of lipids and extracellular matrix, a variety of inflammatory cells and a fibrous cap. The individual composition of plaques is widely accepted to determine the individual vulnerability and outcome rather than their size. It seems likely that multiple plaque ruptures can occur, followed by healing of the vessel wall associated with increase in lumen narrowing, whereas plaque ruptures seem to result in clinical events only when certain factors coincide (recently reviewed in [2]).

For studying the pathophysiology of atherosclerosis and its clinical sequelae a broad spectrum of animal models has been developed. As an example advanced lesions resembling features of vulnerable plaques were found in brachiocephalic arteries of the widely used Apolipoprotein E-deficient mouse ($ApoE^{-/-}$) [3-5]. Recently, atherothrombotic events have been reported in $ApoE^{-/-}$ mice upon surgical carotid constriction and stress [6]. However, clinical events such as myocardial infarctions or stroke were not observed in these models questioning their clinical relevance.

In 2002, the hypomorphic apolipoprotein E (HypoE) mouse was introduced [7]. This mouse was combined with a knock-out in scavenger receptor class B type I ($HypoE/SRBI^{-/-}$ [8, 9]) or the recently

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published ApoE$^{-/-}$/Fbn1(C1039G$^{+/-}$ [10]) carrying a mutation (C1039G$^{+/-}$) in the fibrillin-1 (Fbn1) gene, presenting with advanced plaque phenotypes, spontaneous myocardial infarction and sudden deaths.

However, the connection between spontaneous myocardial infarctions, plaque events and atherothrombosis in the coronary arteries has not yet been shown. We therefore study here whether ruptures of vulnerable coronary plaques trigger occlusive thrombus formation and cause the observed myocardial infarctions in HFC-fed HypoE/SRBI$^{-/-}$ mice employing serial non-invasive imaging combined with whole-heart thin-slice histopathology and a pharmaceutical intervention.

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METHODS

All animal experiments performed in the study were approved by the local authorizing agency of North Rhine-Westphalia. Homozygous double-transgenic HypoE/SRBI$^{-/-}$ mice [8] were enrolled in this study. Mice were fed a standard chow diet (4.5% fat, 0.022% cholesterol; Altromin, Germany) before the start of the experiment and were assigned to the following study groups:

a) Follow up until spontaneous death: 37 mice (19 male, 18 female; median age: 18 weeks) were put on HFC diet (7.5% cocoa butter, 15.8% fat, 1.25% cholesterol, 0.5% sodium cholate; Altromin, Germany) [8], 12 mice (3 male, 9 female) continued the chow diet. Plasma cholesterol levels were assessed at day 21 after onset of diet (chow: 253±44 mg/dl, HFC: 1300±276 mg/dl). During an observation period of up to 60 days, mice were studied by serial high-resolution positron emission tomography (PET) using ^{18}F-FDG and echocardiography three times a week (see below).

b) Follow up until first defect in ^{18}F-FDG-PET: 36 mice (11 male, 25 female; median age: 13 weeks) were put on HFC diet (n=21) or left on chow (n=15) and subjected to the identical serial imaging protocol as described above. In this cohort ^{18}F-FDG-PET was used to immediately trigger the excision of the respective heart when the first ^{18}F-FDG-PET defect occurred. For the chow group, PET scans were performed for 8 weeks. After whole body perfusion with paraformaldehyde (4%) in phosphate-buffered saline, hearts were excised, weighed and embedded in paraffin for detailed histology. Hearts of animals which died spontaneously before the defined end point (n=7) were excluded.

c) Anti-thrombotic intervention: 11 HypoE/SRBI$^{-/-}$ mice (8 male, 3 female; median age: 15 weeks) were put on a modified HFC diet where acetylsalicylic acid (HFC-ASA) at a dose of 500 mg/kg diet was added (Altromin, Germany). Mice were studied by ^{18}F-FDG-PET 14, 21, and 28

days on HFC-ASA diet. Afterwards mice were sacrificed, hearts were excised and processed as detailed above.

A detailed description of imaging methodology, immunohistochemistry, blood and tissue analyses can be found in the supplement.

Statistics

Statistical analysis was performed using Systat Sigmaplot 11. Depending on the existence of equal variances and normal distribution of data, data sets were compared using an unpaired or paired group comparison test, or the equivalent non-parametric rank sum test, respectively. For the comparison of frequencies, Pearson's chi-square test was applied. P values < 0.05 were considered statistically significant.

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RESULTS

Molecular and functional imaging discovers multiple spontaneously occurring myocardial infarctions. As expected, all HFC-fed mice prematurely died within 28 days, whereas no death was observed in the chow group (Fig. 1A).

Serial ^{18}F-FDG-PET found the first myocardial infarctions already four days after onset of the HFC diet with an increase in defect size starting after 2 weeks of HFC (Figs. 1B&1C. In clear contrast, no myocardial infarction occurred in the 12 chow-fed animals (Fig. 1B, left panel), of which five were even followed up until day 60.

In recognition of the broad temporal variability of the occurrence of myocardial infarctions and the time point of individual death, we defined three distinct time points for all mice on HFC diet: t0 = baseline, t1 = first ^{18}F-FDG defect and t2 = final ^{18}F-FDG-PET scan before spontaneous death (Supplemental Fig. 1). The right panel of Fig. 1B illustrates that ^{18}F-FDG defect sizes increase towards the time of the last PET scan in HFC-fed mice. Echocardiography showed a significant decrease of ejection fractions by the time of the first ^{18}F-FDG defect with progressive deterioration in HFC-fed animals, accompanied by an increase of endsystolic volumes. Left ventricular myocardial mass increased over time. No changes in ventricular function or myocardial mass were observed in the chow group (Supplemental Fig. 2). In summary, myocardial infarctions occurring in HFC-fed HypoE/SRBI$^{-/-}$ mice gradually lead to an impairment of cardiac function.

To study whether coronary plaque ruptures and atherothrombosis underlie myocardial infarctions, a separate group of HypoE/SRBI$^{-/-}$ mice was sacrificed as soon as the first myocardial infarction occurred. Here, characteristic signs of subacute infarctions were found: HFC-hearts were bigger and showed inhomogeneous white-colored patches in the left ventricular myocardium especially at the apex as compared to chow (Supplemental Fig. 3A). In line with the increase in

myocardial mass relative heart weights of HFC-fed mice were higher compared to those of chow-fed mice (Supplemental Fig. 3B). However, mean cardiomyocyte diameters were similar between the HFC and chow group (Supplemental Fig. 3C) suggesting structural changes rather than myocardial hypertrophy causing the increased heart weights in HFC-fed mice. Moreover, areas of myocardial infarction on histological slices matched the respective ^{18}F-FDG defects in co-registered slices (Fig. 2). Distinct regions of tissue demise, inflammation and remodeling characterizing post-ischemic myocardial tissue were identified *in situ* by immunohistochemistry (Supplemental Fig. 4).

Imaging-triggered histology reveals vulnerable plaque phenotypes associated with coronary atherothrombosis. Analysis of hearts from HFC-fed mice revealed not only regions of subacute infarctions, but a clear correspondence of these regions with atherothrombosis in the supplying coronary artery (Fig. 2). We found a striking difference in the number and phenotype of coronary plaques between the HFC-fed and the chow-fed HypoE/SRBI$^{-/-}$ group: in 14 HFC-fed mice enrolled, 296 coronary atherosclerotic plaques were observed (Fig. 3A). This is ~10 times more plaques per mouse than in the chow group (34 plaques in 15 hearts, Supplemental Fig. 5).

The most striking finding was the occurrence of intraluminal thrombi in 32 (10.8%) of the 296 atherosclerotic plaques in 12 out of 14 HFC-fed mice (Fig. 3B). Detailed analysis of the atherosclerotic lesions associated with local atherothrombosis demonstrated a high inflammatory activity, as indicated by local accumulation of macrophages, MPO-expressing cells (e.g. activated neutrophils) and strong expression of the phagocyte activity marker S100A9 (Fig. 4A). Most interestingly, inflammatory activity in atherosclerotic lesions was higher in those associated with thrombus formation as compared to non-thrombotic lesions as indicated by S100A9 (Fig. 4B).

Intervention with acetylsalicylic acid (ASA) reduces the incidence of atherothrombosis and premature deaths. To further study the causal relationship between coronary atherothrombosis

and myocardial infarctions we fed HypoE/SRBI$^{-/-}$ mice with HFC & ASA (n=13, HFC-ASA) as published before [11]. Remarkably, all HFC-ASA mice survived the observation period of 28 days (Fig. 5A). Although ^{18}F-FDG-PET proved the occurrence of myocardial infarctions, the defects were significantly smaller than in HFC-fed mice and typically occurred later (Fig. 5B).

Histological analysis of 10 HFC-ASA mice sacrificed after 29 days on diet showed 249 coronary atherosclerotic plaques, which corresponds well with HFC-fed animals (Fig. 5B). In addition, the localization (Supplemental Fig. 6) and degree of stenosis of plaques (Fig. 5C) in both groups were similar. However, the incidence of plaque-associated intraluminal thrombosis was significantly reduced from 32 in 296 plaques (10.8%) in HFC-fed mice to only 7 in 249 plaques (2.8%) in HFC-ASA mice (Fig. 5B; Supplemental Figs. 6&7).

Thrombocyte aggregation assays did not reveal a clear effect of the ASA treatment, although this might be partly due to a low platelet count (median: 550.4x10^3/µl and 381.0x10^3/µl in HFC and HFC-ASA fed animals, respectively; Fig. 6A) potentially impairing the assay. In contrast, ASA treatment significantly attenuated the HFC-mediated rise in WBCs (Fig. 6A) suggesting inhibition of diet-induced systemic inflammatory activity. In addition, S100A9 was significantly reduced locally at sites of atherosclerotic lesions in HFC-ASA mice as compared to HFC mice (Fig. 6B) but not in the post-ischemic myocardium as assessed by S100A9 and Mac-3 stainings (Supplemental Fig. 8).

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DISCUSSION

Emerging experimental models of atherosclerosis such as the HypoE/SRBI$^{-/-}$ mouse present with diet induced hyperlipidemia leading to advanced coronary plaque phenotypes, spontaneous myocardial infarction and sudden death, thus potentially closely mimicking human coronary artery disease [8]. We studied here whether ruptures of vulnerable coronary plaques trigger occlusive thrombus formation and cause the observed myocardial infarctions in HFC-fed HypoE/SRBI$^{-/-}$ mice.

In a first step, we employed non-invasive imaging to study the temporal and spatial dynamics of spontaneous myocardial infarctions previously described in HypoE/SRBI$^{-/-}$. Serial ^{18}F-FDG-PET demonstrated for the first time *in vivo* that consecutive myocardial infarctions occur in individual HFC-fed HypoE/SRBI$^{-/-}$ mice with large variations in time point, extent and fatality. In correlative histological studies, the typical patterns of recent ischemia with necrosis and immune cell infiltration could be observed side-by-side with myocardial fibrosis, supporting the notion of myocardial infarctions occurring sequentially in HypoE/SRBI$^{-/-}$ mice. In connection with the functional impairment assessed by echocardiography and the temporal dynamics of mortality, multiple myocardial infarctions are the most likely cause of the spontaneous premature deaths in HFC-fed HypoE/SRBI$^{-/-}$ mice.

However, we not only used the serial ^{18}F-FDG-PET imaging approach to assess myocardial infarctions but also to trigger an immediate histological evaluation of the coronary arteries upon the first occurrence of myocardial infarcts. This analysis revealed a connection of myocardial infarctions and atherothrombosis in the supplying coronary arteries. In particular, we found the majority of thrombi in medium and large coronary arteries in the basal left ventricular myocardium. This may also explain the predominantly septal location of the thrombotic coronary arteries since the septal arteries are rather direct proximal branches of the right or left coronary artery or the

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aortic sinus itself [12]. To further study the connection between coronary atherothrombosis, myocardial infarctions and spontaneous deaths in HFC-fed HypoE/SRBI$^{-/-}$ mice we treated a subset of animals with ASA, which significantly reduced coronary atherothrombosis (78% compared to non-treated) and premature deaths in the 4-week treatment interval.

Intra-plaque and intraluminal thrombi have been described in larger arteries of ApoE$^{-/-}$ mice on high-cholesterol diets before [3, 6]. However, in none of these models thrombotic plaques in coronary arteries were reported. In addition, no clinical events such as stroke or myocardial infarction arising from the thrombotic plaque events were observed.

Based on these striking differences between the HypoE/SRBI$^{-/-}$ mouse studied here and classical mouse models of atherosclerosis we thoroughly investigated the plaque phenotype of culprit lesions of myocardial infarctions occurring under HFC diet. In particular, we looked for vulnerable plaque phenotypes, which are considered a primary cause of plaque rupture events. Hallmarks of vulnerable plaques have been derived mainly from human autoptic studies of sudden death cases. There, typically only one culprit plaque has been identified as the primary cause of the fatal atherothrombosis despite multiple other coronary artery plaques [13, 14]. In our study, HFC-fed HypoE/SRBI$^{-/-}$ mice exhibit several key features of human vulnerable plaques as recently defined [15]: They are (a) found in vessels of larger size and proximal segments of the coronary tree, (b) are lipid- and cholesterol-rich, (c) present with perivascular inflammation, (d) exhibit thrombi communicating with the necrotic core, and most importantly (e) are associated with clinical events – myocardial infarctions and/or spontaneous deaths.

ASA treatment did not significantly interfere with plaque development in HFC-fed HypoE/SRBI$^{-/-}$: no difference in the number and extent of coronary plaques between treated and non-treated mice was observed suggesting no substantial anti-atherosclerotic effects of ASA in this mouse model. In agreement with this finding, it was shown that ASA had no effect on the

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development of atherosclerotic lesions in ApoE$^{-/-}$ mice [6, 16]. In these studies, in which plaque rupture of carotid artery plaques was either mechanically induced or spontaneously occurring, it was additionally demonstrated that ASA directly reduced atherothrombosis by its anti-platelet action. This finding could not be confirmed in our study, most probably due to technical issues.

Other effects exerted by ASA might contribute to the reduction of thrombotic events in HypoE/SRBI$^{-/-}$. Cyrus et al. reported that low dose ASA treatment in low-density lipoprotein receptor-deficient under high fat diet suppressed vascular inflammation [11]. This observation is in line with our finding of reduced systemic and local inflammatory activity under ASA treatment. Taken into account that inflammation is amongst the main drivers for plaque vulnerability, suppression of inflammatory activity in HypoE/SRBI$^{-/-}$ mice by ASA might induce plaque stabilization and therefore might result in fewer plaque ruptures and thrombotic events.

Given the human-like nature of spontaneous plaque rupture and myocardial infarctions the HypoE/SRBI$^{-/-}$ mouse should also provide an excellent basis for studying pharmaceutical interventions. Recently, immunosuppression by the sphingosine-1 phosphate analogue FTY720 has been shown to improve left ventricular function in HypoE/SRBI$^{-/-}$ put on HFC diet for only 3 weeks [17]. In a recent publication, the same group employed HypoE/SRBI$^{-/-}$ with an inducible Cre-mediated gene repair of the HypoE allele together with switching mice to a normal chow diet. A subgroup of mice surviving the HFC diet was subsequently given orally FTY720 in drinking water and compared to non-treated mice with respect to left ventricular function up to 15 weeks. As expected, untreated mice showed a progressive impairment of LV function over time. In FTY720 treated mice initially LV function also dropped but was almost completely restored by 15 weeks. As in our ASA-treatment intervention, FTY720 did not reduce atherosclerosis but resulted in systemic immunosuppression and reduced cardiac inflammation [18]. Further studies should investigate the immunosuppressive effect of ASA accordingly. Other clinically approved pharmaceutical interventions such as treatment with statins, which is known to reduce

cardiovascular events in humans [19], are warranted to further evaluate the human-like nature of the HypoE/SRBI$^{-/-}$ model. If these are successful, the HypoE/SRBI$^{-/-}$ model could be used for testing emerging pharmaceutical interventions in a preclinical setting.

Ruptures of inflammatory plaques might not be solely responsible for the formation of intravascular thrombi in HypoE/SRBI$^{-/-}$. Liu et al. reported that the incidence of atherothrombosis in their ApoE$^{-/-}$-based model of vulnerable plaque formation was highly dependent on the concomitant increase in blood thrombogenicity introduced by adenovirus-mediated prothrombin overexpression [6]. SR-BI-deficiency has been previously shown to alter platelet function resulting in a higher susceptibility for thrombosis and therefore further contribute to atherothrombotic events in HFC-fed HypoE/SRBI$^{-/-}$ [20]. The cholate fraction of the HFC diet, which was reported to be hepatotoxic and alter lipid metabolism [21], is rather unlikely to substantially promote atherothrombosis in HypoE/SRBI$^{-/-}$. Nakagawa-Toyama and colleagues recently reported that myocardial infarctions, cardiomegaly and premature death can be induced in HypoE/SRBI$^{-/-}$ mice using the HFC diet even without cholate [9].

Although the concept of plaque ruptures of vulnerable plaques underlying clinical events such as myocardial infarctions in humans is well accepted, the hypothesis of a straightforward single and first plaque rupture in a patient leading to the clinical event has been recently debated. More likely and in accordance with the notion, that atherosclerosis is a systemic vascular disease, multiple plaque ruptures could occur at various vessel sites in individuals, followed by healing of the vessel wall. These might be associated with subclinical, maybe unspecific symptoms while only under certain circumstances, which still have to be finally studied, a plaque rupture would result in a clinical event [2]. Interestingly, the HypoE/SRBI$^{-/-}$ mouse would support the multiple plaque rupture hypothesis: Using serial ^{18}F-FDG-PET imaging, we detected multiple infarctions in individual HFC-fed HypoE/SRBI$^{-/-}$ mice which were well tolerated in the majority of cases, therefore

maybe resembling subclinical events in humans. Only a few myocardial infarctions lead to the 'clinical event' of sudden death.

LIMITATIONS

Studies in humans suggest that atherosclerotic plaque development and rupture may be gender-related [22]. The investigation of gender effects in this study was severely hampered by the tedious and inefficient breeding of HypoE/SRBI$^{-/-}$ mice that finally results in only small groups of animals available for the various experiments. However, mortality in the largest group of HFC-fed HypoE/SRBI$^{-/-}$ in our study which had comparable numbers of male and female mice did not show a difference in the "clinical endpoint" mortality, which suggests no differences in plaque vulnerability. Future studies in larger cohorts of HypoE/SRBI$^{-/-}$ are warranted to finally assess if gender effects on plaque phenotypes and vulnerability exist.

CONCLUSION

The HypoE/SRBI$^{-/-}$ mouse model mimics a broad range of characteristic features of human coronary artery disease encompassing the development of advanced/vulnerable plaques, plaque ruptures, coronary atherothrombosis and myocardial infarction. In contrast to other models and previous studies, we discover here that there is a causal connection between plaque ruptures and myocardial infarctions and, most interestingly, multiple subclinical events may occur before it comes to sudden death. Thus, the HypoE/SRBI$^{-/-}$ mouse might have a great potential as a preclinical animal model for developing new diagnostic and therapeutic strategies towards the prevention of plaque rupture and myocardial infarctions.

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DISCLOSURE

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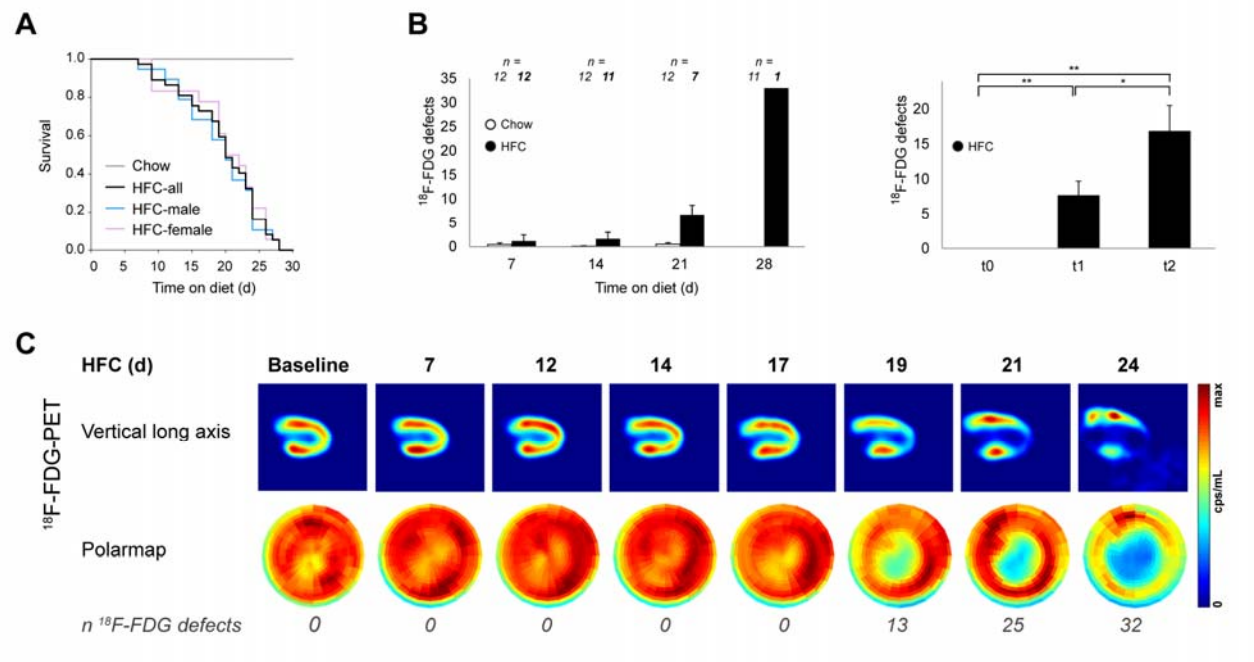
Figure 1



Premature deaths and serial PET imaging in HypoE/SRBI$^{-/-}$ mice under HFC diet

(A) HFC-fed mice (n=37, 19 male, 18 female) died spontaneously within 28 days while all chow-fed mice (n=12) survived (Mantel-Cox test, *P*<0.001). Mortality curves are similar for both genders. (B) Animals were imaged by ^{18}F-FDG-PET three times a week until spontaneous death (HFC, n=12) or until day 30 (chow, n=12). Left: The HFC-fed group showed an exponential increase in ^{18}F-FDG defects over time in contrast to the chow group. Right: At baseline, no ^{18}F-FDG defects were detected (t0). First ^{18}F-FDG defects (t1) considerably vary in time (days on HFC-diet: median 19, range 7-26) and significantly grew towards the last ^{18}F-FDG-PET (t2) before death (days on diet: median 21, range 7-28). Data are means + s.e.m., * *P*<0.05, ** *P*<0.01 by paired *t*-test and Wilcoxon signed-rank test. (C) Sample case showing physiological uptake of ^{18}F-FDG throughout the left ventricle at baseline. PET reveals suddenly occurring ^{18}F-FDG defects indicative of myocardial infarctions in the apex and inferoseptal wall. The first defect was observed at day 19 of HFC diet with a considerable increase in defect size over time.

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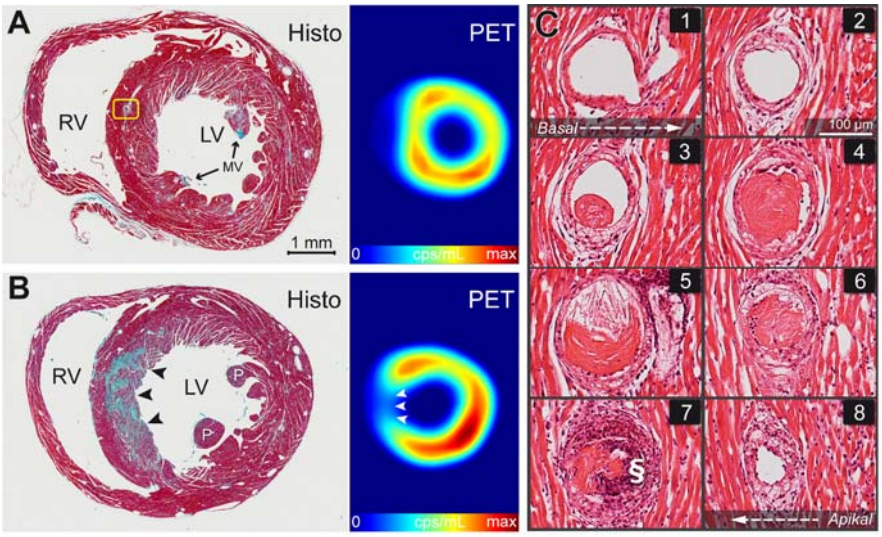
Figure 2



Atherothrombosis induces myocardial infarctions in HFC-fed HypoE/SRBI$^{-/-}$

HFC-fed mouse heart explanted after 14 days on diet upon occurrence of the first ^{18}F-FDG defect. (A) Masson Goldner-stained cross section taken from the basal region of the heart reveals atherothrombosis in the septal coronary artery (yellow box) but no sign of myocardial infarction. The corresponding PET-image shows normal ^{18}F-FDG uptake. (B) Downstream of the intraluminal thrombus a subacute myocardial infarction was identified in the septum characterized by massive post-ischemic myocardial fibrosis (black arrows) corresponding to a septal ^{18}F-FDG-PET defect (white arrows). (C) HE-stained serial cross sections of the septal coronary artery (SA) reveal an occluding thrombus associated with perivascular inflammation and inflammatory infiltrates (§). LV = left ventricle, MV = mitral valve, P = papillary muscle, RV = right ventricle.

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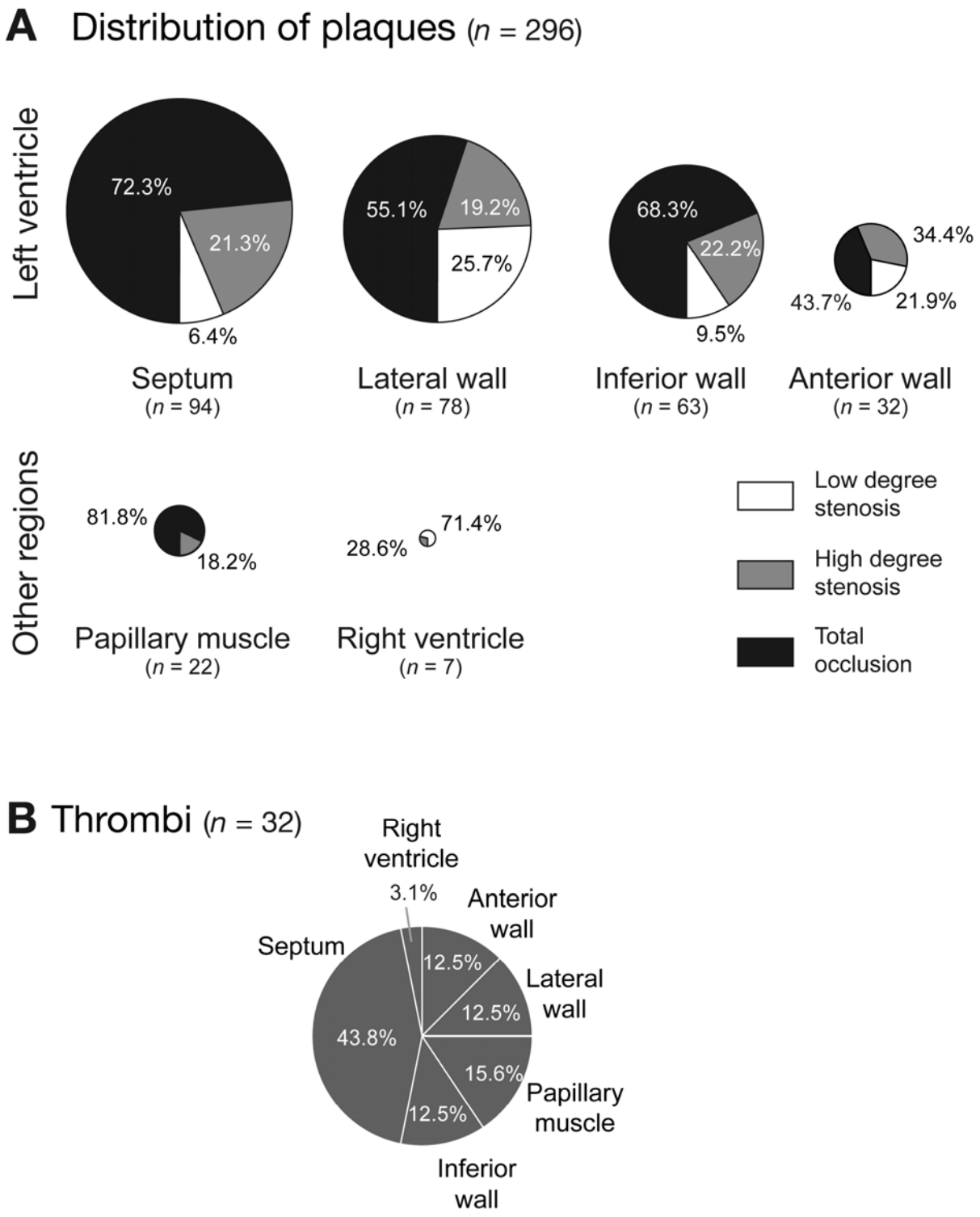
Figure 3


A Distribution of plaques (n = 296)
Left ventricle
72.3%
21.3%
6.4%
Septum
(n = 94)
55.1%
19.2%
25.7%
Lateral wall
(n = 78)
68.3%
22.2%
9.5%
Inferior wall
(n = 63)
34.4%
43.7%
21.9%
Anterior wall
(n = 32)
Other regions
81.8%
18.2%
Papillary muscle
(n = 22)
28.6%
71.4%
Right ventricle
(n = 7)
Low degree stenosis
High degree stenosis
Total occlusion
B Thrombi (n = 32)
Right ventricle
3.1%
Anterior wall
12.5%
Lateral wall
12.5%
Papillary muscle
15.6%
Inferior wall
12.5%
Septum
43.8%

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Histological evaluation of coronary plaques and thrombi in HFC-fed HypoE/SRBI$^{-/-}$

(A) Degree of stenosis and location of coronary atherosclerotic plaques (low degree stenosis equals <50% luminal narrowing, high degree stenosis equals 50%-99% luminal narrowing, and total occlusion). In HFC-fed mice (n=14) a total of 296 plaques were found, predominantly in the left ventricle and with a high rate of occluded arteries. (B) 32 of 296 plaques (10.8%) showed thrombus formation with a higher incidence in arteries supplying the septum.

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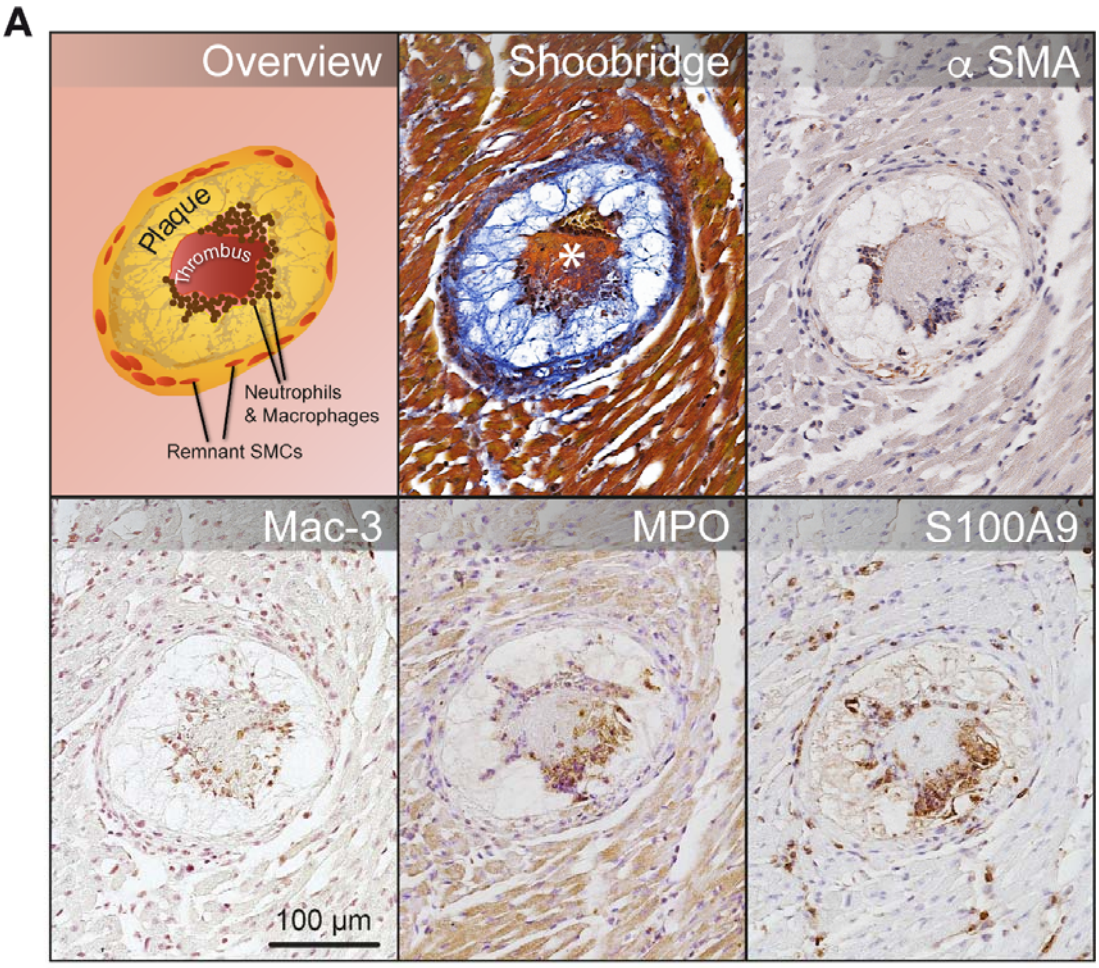
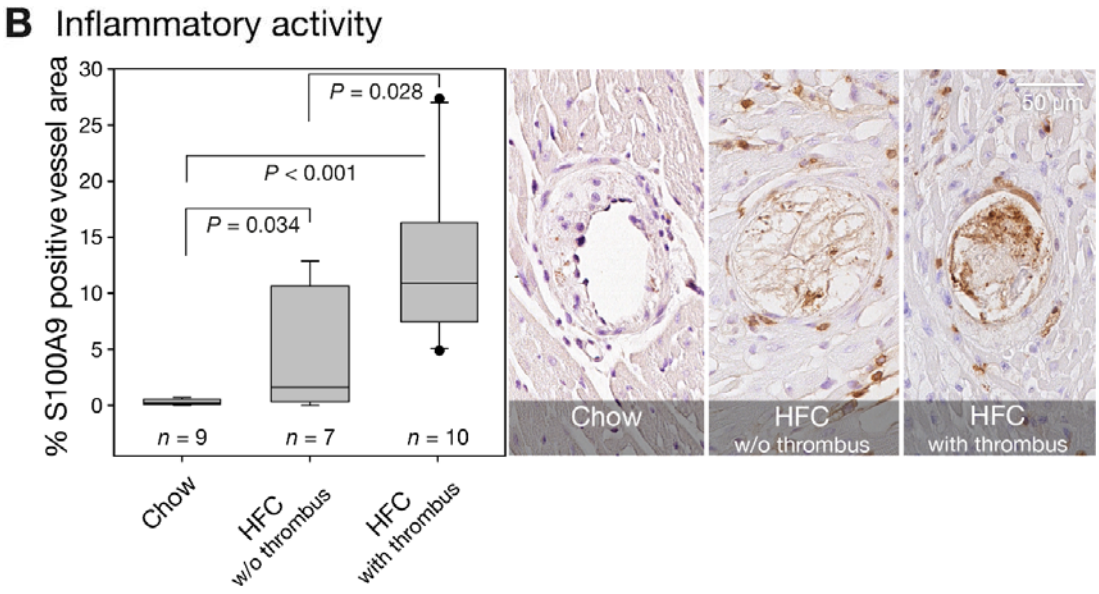
Figure 4


A
Overview
Shoobridge
α SMA
Plaque
Thrombus
Neutrophils
& Macrophages
Remnant SMCs
Mac-3
MPO
S100A9
100 μm


B Inflammatory activity
% S100A9 positive vessel area
30
25
20
15
10
5
0
P = 0.028
P < 0.001
P = 0.034
n = 9
n = 7
n = 10
Chow
HFC w/o thrombus
HFC with thrombus
50 μm
Chow
HFC w/o thrombus
HFC with thrombus

#

Atherothrombosis in HFC-fed HypoE/SRBI$^{-/-}$ is related to local inflammatory activity

(A) Cross sections of an atherosclerotic plaque with thrombotic vessel occlusion. Shoobridge's polychrome staining allows for discriminating between cardiomyocytes (red-orange) and connective tissue (blue) but also serves to identify fibrin clots (*). Smooth muscle cells (α-SMA^{+}) in the arterial vessel wall demonstrating media degradation. Immunohistochemistry shows macrophages (Mac-3), neutrophils (MPO), and phagocyte/neutrophil activity (S100A9) reflecting inflammatory activity in the lesion. (B) Inflammatory activity assessed by S100A9 staining was significantly higher in HFC-fed versus chow-fed mice and further increased in thrombus-associated plaques (Mann-Whitney rank sum test).

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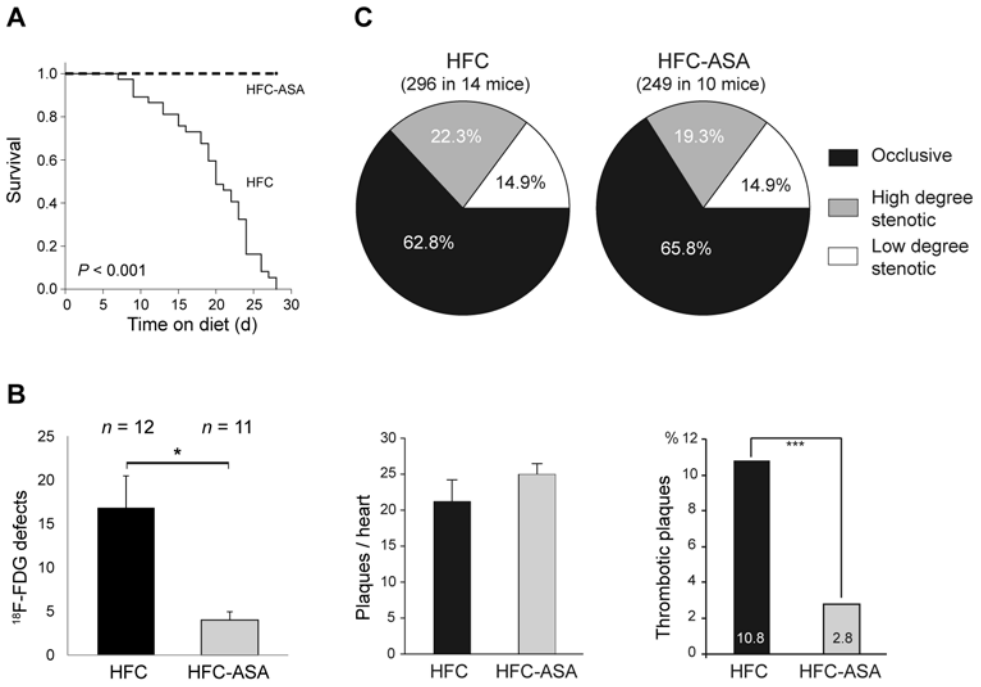
Figure 5



Serial imaging of ^{18}F-FDG defects and plaque analysis in HypoE/SRBI$^{-/-}$ under ASA treatment

HypoE/SRBI$^{-/-}$ mice (n=11) were put on a combined HFC and acetyl-salicylic acid diet (HFC-ASA) and imaged by ^{18}F-FDG-PET at day 14, 21 and 28 after onset of diet. (A) All mice with ASA treatment survived the observation period in contrast to mice under HFC only diet (n=37; Mantel-Cox test, *P*<0.001). (B) Final scan ^{18}F-FDG defect sizes were significantly larger in HFC-fed HypoE/SRBI$^{-/-}$ (median days on diet: 21 days) as compared to age-matched ASA-treated HypoE/SRBI$^{-/-}$. Histological evaluation of coronary plaques in HFC-ASA-fed mice revealed a comparable number of coronary plaques per heart but a considerable lower incidence of thrombotic plaques as compared to the HFC group. Data are means + s.e.m, * *P*<0.05, *** *P*<0.001 by Mann-Whitney rank sum test. (C) ASA treatment does not change the coronary plaque burden in HFC-fed mice.

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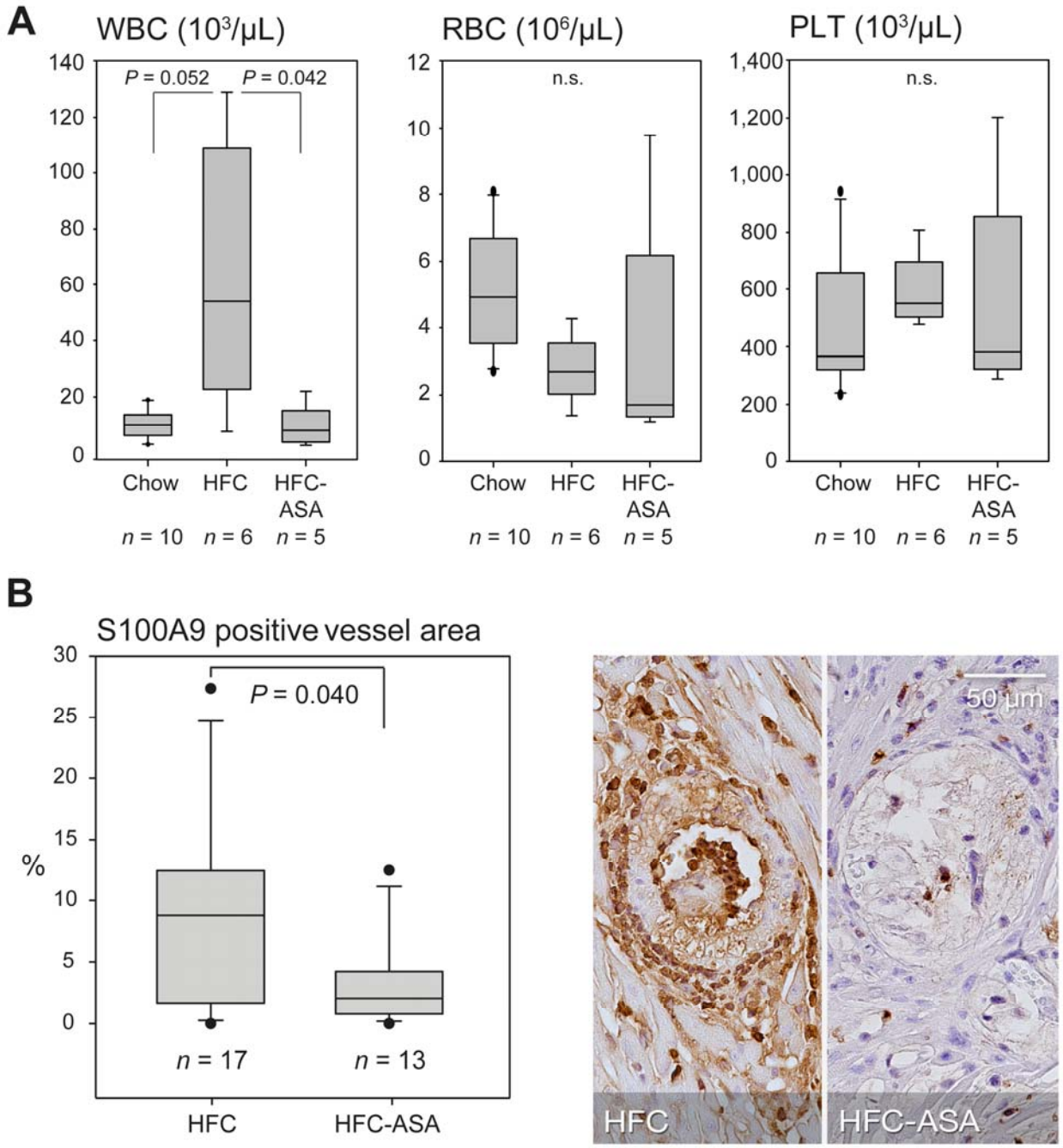
Figure 6



Plaque-associated and systemic inflammatory activity in HFC-fed HypoE/SRBI$^{-/-}$ with and without ASA treatment

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(A) HFC induced rise in circulating white blood cells (WBC) was attenuated by ASA, suggesting a systemic anti-inflammatory effect while the concentration of erythrocytes (RBC) and platelets (PLT) remained unaffected. (B) S100A9 immunolocalisation at the site of maximal plaque extend revealed significantly decreased S100A9 expression in septal arteries of ASA-treated animals. *P*-values calculated by Mann-Whitney rank sum test.