

The transcription factor MAFF regulates an atherosclerosis relevant network connecting inflammation and cholesterol metabolism

von Scheidt – Identification of MAFF as a regulator of the LDLR

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1 **Abstract**

2 **Background** – Coronary artery disease (CAD) is a multifactorial condition with both genetic
3 and exogenous causes. The contribution of tissue specific functional networks to the
4 development of atherosclerosis remains largely unclear. The aim of this study was to identify
5 and characterise central regulators and networks leading to atherosclerosis.

6 **Methods** – Based on several hundred genes known to affect atherosclerosis risk in mouse (as
7 demonstrated in knock-out models) and human (as shown by genome-wide association studies
8 (GWAS)) liver gene regulatory networks were modeled. The hierarchical order and regulatory
9 directions of genes within the network were based on Bayesian prediction models as well as
10 experimental studies including chromatin immunoprecipitation DNA-Sequencing (ChIP-Seq),
11 ChIP mass spectrometry (ChIP-MS), overexpression, siRNA knockdown in mouse and human
12 liver cells, and knockout mouse experiments. Bioinformatics and correlation analyses were
13 used to clarify associations between central genes and CAD phenotypes in both human and
14 mouse.

15 **Results** – The transcription factor *MAFF* interacted as a key driver of a liver network with three
16 human genes at CAD GWAS loci and eleven atherosclerotic murine genes. Most importantly,
17 expression levels of the low-density lipoprotein receptor (*LDLR*) gene correlated with *MAFF*
18 in 600 CAD patients undergoing bypass surgery (STARNET) and a hybrid mouse diversity
19 panel involving 105 different inbred mouse strains. Molecular mechanisms of *MAFF* were
20 tested under non-inflammatory conditions showing a positive correlation between *MAFF* and
21 *LDLR in vitro* and *in vivo*. Interestingly, after LPS stimulation (inflammatory conditions) an
22 inverse correlation between *MAFF* and *LDLR in vitro* and *in vivo* was observed. ChIP-MS
23 revealed that the human CAD GWAS candidate *BACH1* assists *MAFF* in the presence of LPS

1 stimulation with respective heterodimers binding at the MAF recognition element (MARE) of
2 the *LDLR* promoter to transcriptionally downregulate *LDLR* expression.

3 **Conclusion** – The transcription factor *MAFF* was identified as a novel central regulator of an
4 atherosclerosis/CAD relevant liver network. *MAFF* triggered context specific expression of
5 *LDLR* and other genes known to affect CAD risk. Our results suggest that *MAFF* is a missing
6 link between inflammation, lipid and lipoprotein metabolism and a possible treatment target.

7 **Keywords**

8 Atherosclerosis, BACH1, coronary artery disease, inflammation, key driver analysis, LDLR,
9 lipid metabolism, MAFF, network modeling

1 **Translational Perspective**

2 **What is new?**

- 3 • Our study identified the transcription factor *MAFF* as key driver gene in a liver-
4 specific network involving several genes with established, genome-wide significant
5 association to coronary artery disease (CAD).
- 6 • *MAFF* regulated context-specifically expression of *LDLR* in experimental model
7 systems and human individuals.
- 8 • *MAFF* induced *LDLR* expression under non-inflammatory conditions. After LPS
9 stimulation *MAFF* downregulated *LDLR* expression via heterodimerisation with
10 *BACH1* and binding at the maf-recognition element (MARE) – also known as stress-
11 responsive element – in the promoter of *LDLR*.

12 **What are the clinical implications?**

- 13 • Cholesterol metabolism and inflammation represent two major causes of CAD. Here
14 we identified a transcriptional regulator (*MAFF*) to differentially affect the major
15 determinant of cholesterol levels (*LDLR*) dependent on the inflammatory state.
- 16 • Further studies of the transcription factor *MAFF*, its interaction partners and
17 downstream cascades might generate new therapeutic targets to treat
18 hypercholesterolemia, inflammation and reduce CAD risk.

1 **Introduction**

2 Coronary artery disease (CAD), a globally leading cause of death,^{1, 2} is brought about by
3 atherosclerosis of the epicardial arteries, which is prompted by a multifactorial interplay of
4 genetic and lifestyle factors.³ From a functional point of view, the mechanisms which result in
5 CAD can be grouped into different pathways or networks.⁴⁻⁷ A systematic analysis of genes
6 identified by genome-wide association studies (GWAS) of CAD patients and genetic mouse
7 models of atherosclerosis revealed a strong concordance of relevant networks and pathways for
8 atherosclerosis between the two species.⁸ However, characterization and regulation of these
9 functional networks is far from being complete.⁹ Central mechanisms for atherosclerosis are
10 the disturbance of cholesterol metabolism and inflammation, which – like CAD – have
11 multifactorial etiologies.¹⁰⁻¹² Clinical as well as epidemiological trials and Mendelian
12 randomization studies confirmed that elevation of plasma cholesterol and increased
13 inflammation promote the progression of atherosclerosis and CAD¹³⁻¹⁶, whereas reduction of
14 plasma cholesterol levels and inflammatory processes lowered significantly the subsequent risk
15 of cardiovascular events.^{10, 17-19} Both, cholesterol metabolism and inflammatory responses are
16 largely orchestrated in the liver. Therefore, the analysis focused on hepatic tissue to further
17 elucidate regulatory gene networks involved in atherosclerosis.

18

19 **Methods**

20 Data used in this study are available in persistent repositories. Human data from STARNET are
21 accessible through the Database of Genotypes and Phenotypes (dbGAP). Mouse data from
22 HMDP are accessible through the Mouse Phenome Database (MPD). All experimental data
23 supporting the findings of this study can be requested from qualified researchers at the German
24 Heart Center Munich from the corresponding author. An expanded and detailed materials and

1 methods section is provided in the supplement section (**Expanded Methods**). Human and
2 mouse candidate genes were retrieved from the literature.^{8, 20-30} Gene-gene relations of
3 atherosclerotic genes were retrieved utilising Bayesian gene regulatory networks derived from
4 previous expression analyses on human and mouse tissues as described.³¹⁻³⁶ The key driver
5 analysis (KDA) was based on an established algorithm to identify central regulators of
6 atherosclerosis relevant networks.^{31, 37-40} ChIP-Seq experiments of *MAFF* on human HepG2
7 cells were performed as described.⁴¹⁻⁴⁴ Binding capacities of *MAFF* and heterodimerisation
8 partners were confirmed using ChIP-Seq data. siRNA experiments targeting liver genes were
9 performed in cultured AML12 murine and Hep3b human liver cells. *Maff* overexpression
10 experiments were performed in AML12 cells. ChIP-MS was performed to identify *MAFF*
11 binding partners. Molecular docking of heterodimerisation partners was assessed based on
12 lowest free energy.⁴⁵⁻⁴⁸ The STARNET study was conducted in accordance with the provisions
13 of the Declaration of Helsinki and the International Conference on Harmonization Good
14 Clinical Practice guidelines. The protocol was approved by an independent ethics committee
15 and all patients provided written informed consent. All animal studies in mice followed the
16 guidelines of the Animal Care and Use Committees of the University of California Los Angeles.
17 The approach is graphically summarised in **Figure 1**.

18

19 **Results**

20 **Bioinformatics identification of the MAFF network**

21 The bioinformatics approach was based on a comprehensive search for mouse genes that have
22 been previously found to affect the manifestation of atherosclerosis in genetically engineered
23 mouse models as well as human chromosomal loci significantly associated with CAD in GWAS
24 and the respective annotation of responsible genes at these loci.²⁰⁻³⁰ Specifically, 244 human

1 CAD GWAS candidate genes (**Supplemental Table 1**) and 827 mouse atherosclerosis genes
2 (**Supplemental Table 2**) were used to model gene regulatory networks using a key driver
3 analysis.⁴⁹ Liver Bayesian network models composed from multiple published genetic and gene
4 expression datasets were constructed to retrieve gene-gene regulatory relationships in each
5 dataset,³¹⁻³⁶ followed by summarising the individual liver networks into a union liver Bayesian
6 network (**Expanded Methods**). The mouse atherosclerosis genes and human CAD GWAS
7 genes were then mapped separately to the liver Bayesian network model to retrieve subnetworks
8 (specific parts of the global Bayesian liver network) of disease genes and to predict key
9 regulatory genes in these subnetworks. Several subnetworks enriched for known atherosclerosis
10 or CAD associated genes were identified. **Figure 2** displays the interconnected top 10 liver
11 subnetworks containing mouse atherosclerosis or human CAD genes. **Table 1** lists these
12 subnetworks in mouse and human ranked by fold enrichment of disease genes in each
13 subnetwork. Based on mouse atherosclerosis genes, the top five key driver genes of the
14 regulatory networks were *Maff*, *Illb*, *Ccl7*, *Atf3* and *Cxcl10*. With the exception of *Maff* these
15 genes have already been shown to have significant effects on atherosclerosis in mouse models,
16 which may serve as positive control for this approach.⁵⁰⁻⁵³ Regarding the human CAD genes,
17 the top ranked key driver gene *ALDH2* is known to reside at a CAD GWAS locus. The key
18 driver *SERPINE1*, which is also part of the *MAFF* network, shares several atherosclerosis
19 relevant genes with *MAFF*.

20 The top ranked liver subnetwork, over-represented with both mouse atherosclerosis and human
21 CAD candidate genes, was predicted to be orchestrated by *MAFF/Maff*, which interacts with
22 24 atherosclerosis related genes. A number of these genes are known to be associated with lipid
23 metabolism and others with inflammation, and eleven genes (*Atf3*, *Epha2*, *Gdf15*, *Ldlr*, *Nr4a3*,
24 *Phlda1*, *Serpine1*, *Tnfaip3*, *Tnfrsf12a*, *Trib1* and *Zfp36*) were found to affect atherosclerosis in

1 genetically engineered mouse models.⁵³⁻⁶³ On the human side, the *MAFF* interacting genes
2 *LDLR*, *MCL1* and *TRIB1* reside at genome-wide significant CAD GWAS loci.

3 *MAFF* is a member of the MAF family, which consists of large and small MAF proteins. Large
4 MAFs possess a transactivation domain and modulate regulatory processes. Small MAFs are
5 lacking a transactivation domain and are therefore classified as transcriptional repressors.⁶⁴
6 *MAFF*, a small MAF, is a basic region leucine zipper (bZIP)-type transcription factor composed
7 of a DNA binding domain and a leucine zipper domain necessary for dimerisation. *MAFF* can
8 mediate both transcriptional activation or repression by forming heterodimers with other bZIP
9 transcription factors. But the precise mechanism by which *MAFF* forms specific dimers, and
10 therefore induces or represses specific target genes is currently not well established.

11

12 **Prediction of regulatory directionality in the MAFF/Maff network**

13 Individual liver Bayesian network models were constructed using multiple genetic and gene
14 expression datasets from mouse and human studies and combined the networks into one union
15 liver network (**Expanded Methods**). As the directionality between two genes in a network
16 might differ across studies due to different environmental perturbations and physiological states,
17 the dominant direction that is supported by more datasets was taken as the directionality
18 between two genes (**Figure 2**).

19 Based on this data *ATF3*, *TRIB1*, *SERPINE1*, *FOSL2*, and *ZFP36* were predicted to be upstream
20 of *MAFF*, i.e. these genes appear to affect regulation of the transcription factor. All other genes
21 of interest in the context of atherosclerosis (*ARID5B*, *CLCF1*, *CREM*, *CXCL8*, *DUSP5*, *EPHA2*,
22 *FOXP1*, *GDF15*, *LDLR*, *MCL1*, *NAV2*, *NR4A3*, *PHLDA1*, *PPPLR15A*, *SLC20A1*, *TGFBI*,

1 *TNFAIP3*, *TNFRSF12A* and *TSC22D1*) were predicted to be downstream of *MAFF* and
2 therefore likely to be regulated by the transcription factor *MAFF*.

3

4 **Confirmation of *Maff* coexpression with lipid metabolism and inflammation processes in** 5 **mouse atherosclerosis models**

6 The Hybrid Mouse Diversity Panel (HMDP) is a set of 105 different inbred mouse strains,
7 which were studied under different dietary conditions and different genetic backgrounds.^{65, 66}
8 Ldl and Vldl cholesterol levels increased from chow diet over high-fat diet to the atherogenic
9 transgenic mice on high-fat diet (26mg/dl vs. 42mg/dl, vs. 92mg/dl; $p < 0.001$). In transgenic
10 mice inflammation associated factors like *Il1b*, *Il6* and *Tnfa* were also upregulated.

11 On regular chow (Pearson's $r = 0.30$, $p = 9.88e-07$) and high-fat diet (Pearson's $r = 0.35$, $p = 1.78e-$
12 07) positive correlations between *Maff* and *Ldlr* were detected. By contrast, in mice on high-fat
13 diet with transgenic expression of human APOE-Leiden and cholesteryl ester transfer protein
14 (*CETP*), causing increased hyperlipidemia and inflammation, significant inverse correlations
15 between *Maff* and *Ldlr* were observed (Pearson's $r = -0.27$, $p = 4.65e-05$). Correlations between
16 *Maff* and its network interaction partners are summarised in **Supplemental Table 3**. Also,
17 associations between *Maff* and various molecular and biochemical phenotypes were studied,
18 showing significant correlations with atherosclerosis related traits under non-inflammatory
19 conditions (mice on chow and high-fat diet): total cholesterol (Pearson's $r = 0.31$, $p = 7.90e-4$),
20 unesterified cholesterol (Pearson's $r = 0.38$, $p = 2.22e-3$), body weight (Pearson's $r = 0.32$,
21 $p = 1.54e-3$); and inflammatory conditions (atherogenic): aortic lesion area (as a measure of
22 atherosclerotic lesions) (Pearson's $r = -0.37$, $p = 1.34e-3$), *Il6*-levels (element of the
23 inflammasome axis; Pearson's $r = 0.45$, $p = 5.24e-6$), *Tnfa*-levels (as a measure of inflammation;
24 Pearson's $r = 0.33$, $p = 1.23e-3$) and *Mcp1*-levels (recruiting monocytes, memory T cells and

1 dendritic cells to the sites of inflammation; Pearson's $r=0.33$, $p=1.09e-3$). Furthermore, the
2 density of absolute values of Pearson correlation coefficient r was assessed between gene pairs of
3 the *Maff* network as a parameter of network gene coexpression or connection activity and the
4 network coexpression activity between HMDP panels (chow vs. high fat vs. atherogenic) was
5 compared. Overall, significantly increased gene-gene coexpression from chow diet over high-
6 fat diet to the transgenic group was identified ($p<0.01$) (**Supplemental Figure 1**). The gradual
7 elevation of coexpression activity of the *Maff* network along with the accompanying increases
8 in cholesterol levels from low-atherogenic to high-atherogenic conditions suggests a context-
9 specific role of *Maff* in the regulation of the liver gene network and LDL cholesterol.

10

11 **Confirmation of MAFF coexpression with CAD and related processes in human liver**

12 To study the effects of *MAFF* in humans with CAD phenotype data from the Stockholm-Tartu
13 Atherosclerosis Reverse Network Engineering Task (**STARNET**) were used. STARNET
14 provides RNA-sequencing data from different tissues of 600 CAD patients undergoing
15 coronary artery bypass graft (CABG) surgery. All patients gave written informed consent to
16 donate tissue samples prior to CABG surgery.⁹ Based on liver samples in STARNET, a strong
17 positive correlation between expression levels of *MAFF* and *LDLR* (Pearson's $r=0.57$, $p=4.7e-$
18 49) was detected (**Figure 3a**). Studying the *MAFF* network expression values, 22 out of the 24
19 predicted neighbouring genes were found to be significantly correlated to *MAFF* expression
20 (**Supplemental Table 1**).

21 *MAFF* expression was also associated with several cardiometabolic traits in the STARNET
22 database. Importantly, *MAFF* expression was inversely associated with the SYNTAX-Score I
23 – a measure of CAD severity (Pearson's $r=-0.1$; $p<0.01$). Weight (Pearson's $r=-0.19$; $p=4.4e-6$)
24 and BMI (Pearson's $r=-0.15$; $p=2.90e-4$) were found to be significantly inversely correlated to

1 *MAFF* expression, whereas *MAFF* expression was positively correlated with hsCRP (Pearson's
2 $r=0.1$; $p<0.01$). Notably, *MAFF* expression values were higher in women ($p=5.5e-3$) (**Figure**
3 **3b**), such that it might be of interest that 9 *MAFF* interacting genes in **Supplemental Table 3**
4 (*ATF3*, *EPHA2*, *FOXP1*, *GDF15*, *SERPINE1*, *SLC20A1*, *TGFBI*, *TNFRSF12A* and *ZFP36*)
5 have been recognised as sexually dimorphic in mice.³⁴ We note that the phenotypic correlations
6 between *MAFF* and phenotypes other than CAD were weak in this human CAD cohort
7 compared to the genetically defined mouse populations. This could be explained by low
8 phenotypic variability or medication in the CAD cohort.

9 Summarising the results from the STARNET cohort, lower levels of *MAFF* expression were
10 correlated with I) lower levels of *LDLR* expression (less capacity to lower circulating LDL
11 cholesterol), II) higher risk for complex and severe CAD, and III) male gender.

12 Coexpedia⁶⁷, an open tool for exploring biomedical hypotheses via coexpression analyses,
13 revealed in 467 different studies by gene set analysis the most relevant biological correlates of
14 *MAFF* to be *LDLR* mediated cholesterol biosynthetic process ($p=1.40e-23$), negative regulation
15 of apoptotic process ($p=8.47e-7$), and inflammatory response ($p=3.28e-5$). Further, gene set
16 analysis of disease ontology highlighted several CAD relevant traits and risk factors. *MAFF*
17 expression was associated with arthritis ($p=1.86e-20$), ischemia ($p=7.76e-12$), myocardial
18 infarction ($p=1.37e-11$), atherosclerosis ($p=4.14e-10$) and coronary heart disease ($p=1.74e-8$).
19 On risk factor level hypertension ($p=3.15e-12$), obesity ($p=9.38e-12$), diabetes mellitus
20 ($p=1.44e-9$), kidney disease ($p=9.49e-9$) and hypercholesterolemia ($p=7.32e-5$) showed
21 significant association with *MAFF*.

22

23 **In vitro validation of MAFF/Maff regulatory capacities**

1 To confirm the role of *MAFF* orchestrating the predicted regulatory liver network of CAD genes
2 Hep3b and AML12 were studied representing human and mouse hepatocyte cell lines,
3 respectively. siRNA-knockdown (KD) of *MAFF* in human cells and *Maff* in mouse cells
4 showed consistent, significant reductions of the *LDLR/Ldlr* expression ($p < 0.001$) (**Figure 4a+b**)
5 (**Expanded Methods**), as well as consistent and significant perturbations of other neighbouring
6 genes. *MAFF/Maff*-KD caused significant upregulation of *EPHA2/Epha2*, *GDF15/Gdf15* and
7 *TNFAIP3/Tnfaip3*, in contrast to *LDLR/Ldlr* downregulation in both species. These results are
8 in line with the predictions of the Bayesian networks in that these genes are downstream of
9 *MAFF/Maff* (**Supplemental Figure 2**).

10 Knockdown of *MAFF/Maff* did not perturb expression levels of *Trib1* in AML12 cells and only
11 slightly decreased expression values in human Hep3b cells. By contrast, siRNA knockdown of
12 *TRIB1/Trib1* led to decreased levels of *MAFF/Maff* in both cell lines ($p < 0.05$), suggesting that
13 the known CAD risk gene *TRIB1* might be upstream acting as a regulator of *MAFF/Maff*
14 expression levels, which was consistent with the Bayesian modeling.

15 Further, the effect of *Maff* overexpression was investigated using plasmid DNA transfection in
16 mouse AML12 cells and revealed a significant upregulation of the *Ldlr* expression ($p = 0.002$)
17 (**Figure 4c**). Based on these silencing and overexpression experiments in the absence of
18 inflammatory stimuli, *Maff* and *Ldlr* expression was found to be positively correlated *in vitro*.

19

20 **Ldlr reduction in Maff^{-/-} mouse models**

21 To explore *in vivo* the effects of *MAFF* on the expression levels of *Ldlr*, an inbred *Maff^{-/-}* mouse
22 model on C57BL/6-background was employed. Homozygous null mice are viable and fertile
23 and show no obvious functional deficiencies. Liver samples from *Maff^{-/-}*, *Maff^{+/-}* and wildtype

1 (WT) mice, all fed with chow diet, were collected. Expectedly, circulating cholesterol levels
2 were low in mice lacking a pro-atherosclerotic background (e.g. *Apoe*^{-/-}, *Ldlr*^{-/-}). *Maff*^{-/-} mice
3 showed no significant differences on serum cholesterol levels compared to WT mice.
4 Significant decrease of *Ldlr* expression levels in *Maff*^{-/-} mice was observed compared to WT
5 mice (p=0.028) (**Figure 4d**) and lower amounts of *Ldlr* protein were confirmed by Western blot
6 analysis in *Maff*^{-/-} compared to WT mice (p=0.010). This is in line with the *in vitro* findings.
7 There was no significant difference between the groups and heterozygous *Maff* mice.

8

9 **Context specific influence on MAFF and LDLR in the presence of LPS stimulation**

10 *Maff* has been described to be a context specific transcription factor.⁶⁴ Pro-inflammatory
11 lipopolysaccharide (LPS) was used intraperitoneally as a strong inductor of acute systemic
12 inflammation in male *Maff* WT and knockout (KO) mice. A significant induction of *Maff*
13 expression levels (p<0.001) was detected in WT animals six hours after LPS treatment. *Maff*
14 mRNA was not detectable in *Maff*^{-/-} mice. In addition to changes in *Maff* levels, there was a
15 significant decrease of *Ldlr* expression levels in the WT group (p<0.001), but no significant
16 change in *Maff*^{-/-} mice, suggesting that *Maff* and its network are sensitive to inflammatory
17 processes and that *Maff* is involved in inflammation induced suppression of *Ldlr* (**Figure 4e-f**).
18 Circulating Ldl/Vldl cholesterol levels were non-significantly elevated in *Maff* WT mice
19 compared to LPS treated *Maff* WT mice (66.1mg/dl vs. 61.4mg/dl, p=0.24) six hours after LPS
20 injection. Further, expression of the pro-inflammatory cytokine *Tnfa* was assessed in *Maff* WT
21 and *Maff*^{-/-} mice with and without LPS treatment. *Tnfa* expression was significantly
22 upregulated in *Maff* WT and *Maff*^{-/-} mice after LPS stimulation (p<0.001). However,
23 comparing *Tnfa* expression between both groups 6 hours after LPS stimulation, upregulation of
24 *Tnfa* expression was found to be significantly increased in *Maff* WT mice (p=0.048) (**Figure**

1 **4g**). Under inflammatory conditions – using LPS stimulation – *Maff* and *Ldlr* expression was
2 found to be inversely correlated in *Maff* WT mice.

3 **Identification of the regulatory MAFF binding site**

4 Next, the regulatory role of *MAFF* as a transcription factor was investigated in the network.
5 Potential binding sites of *MAFF* were assessed using Chromatin-Immuno-Precipitation-DNA-
6 Sequencing (ChIP-Seq) data on human HepG2 cells as studied in the ENCODE project.⁶⁸
7 Computational analysis revealed a Leucine-Zipper binding motif to be enriched in binding
8 elements of gene members of the *MAFF* network (20 of 24 genes, Fisher's exact test, fold
9 change=4.03, p=3.2e-34). Moreover, multiple *MAFF* binding sites were identified in the *LDLR*
10 gene, including the promoter region (**Figure 5**). These results suggest that MAFF has the
11 potential to bind to the promoter regions of the network member genes and regulate their
12 expression.

13

14 **MAFF binding partners in homeostasis and inflammation**

15 To further elucidate the role of *MAFF/Maff* and its binding partners in homeostasis and
16 inflammation Chromatin Immunoprecipitation followed by mass spectrometry (ChIP-MS) was
17 performed in Hep3b and AML12 liver cells. Both cell lines were treated with either vehicle
18 PBS or LPS (10ng/ml) for 48h with and without *MAFF/Maff* siRNA knockdown.

19 *MAFF/Maff* siRNA knockdown and LPS stimulation identified *BACH1/Bach1* as relevant
20 transcriptional interaction partner of *MAFF/Maff* (**Figure 6**). Specifically, LPS induction
21 determined *BACH1/Bach1* as a robust *MAFF/Maff* interactor in both cell lines. On expression
22 level, LPS stimulation led to a significant increase of *BACH1/Bach1* expression in human
23 Hep3b (p=0.006) and mouse AML12 cells (p=0.001) compared to controls (vehicle) (**Figure**

1 **7a+b**). *BACH1/Bach1* is a known repressor of the MARE – also known as stress-responsive
2 element – and downregulates transcription (**Figure 8**).⁶⁹ Of note, *BACH1* is a human CAD
3 GWAS candidate gene and the underlying mechanism of how *BACH1* contributes to CAD is
4 unclear.⁷⁰

5 Also the heterochromatin markers *Trim28 (Kap1)* and *Cbx3*, as well as the RNA related factors
6 *Dhx40*, *Srrm1* and *Srsf2/5* were enriched in *Maff* ChIP-MS of LPS stimulated AML12 mouse
7 liver cells. A clear reduction of the enrichment signal in response to *Maff* knockdown indicated
8 that these proteins are specific *Maff* interactors (**Figure 6c+d**). *Maff* interactors and their
9 individual enrichment patterns under different conditions are summarised after normalisation
10 in **Figure 7c**.

11 Further, it was studied if activating transcription factors bind with *MAFF/Maff* under basal
12 conditions and in the presence of LPS stimulation in both cell lines. No enrichment of activating
13 transcription factors was identified comparing both conditions.

14

15 **Verification of MAFF-BACH1 heterodimers binding at the LDLR promotor**

16 Based on the findings of the ChIP-MS approach in human Hep3b cells, human ChIP-Seq data
17 was used to validate the binding capacities of the *MAFF* heterodimerisation partners at the
18 *LDLR* promoter. Three dimensional structures of heterodimers were modeled (**Expanded**
19 **Methods**) and confirmed the preferred binding of the *MAFF-BACH1* complex at the *LDLR*
20 promoter as measured by the lowest free energy ($p=9.5e-05$) (**Supplemental Figure 3**).

1 **Discussion**

2 Gene regulatory network modelling based on hundreds of genes known to affect atherosclerosis
3 retrieved a dense liver network highly enriched for mouse atherosclerosis and human CAD
4 GWAS candidate genes. Notably, the network – centered at the transcription factor *MAFF* –
5 contains *LDLR* and inflammatory genes, which are all implicated to play causal roles in
6 coronary artery disease. Prediction by Bayesian models and experimental studies clarified the
7 hierarchical order and regulatory direction of the genes within the network as well as the central
8 role of *MAFF* as a regulating element.

9 Under non-inflammatory conditions a positive correlation between *MAFF* and *LDLR*
10 expression *in vitro* and *in vivo* was identified, whereas inflammatory conditions led to inverse
11 correlation between *MAFF* and *LDLR* expression. Most importantly, after acute induction of
12 systemic inflammation via LPS stimulation, we observed that binding of *MAFF-BACH1*
13 heterodimers at the MARE in the *LDLR* promoter region significantly downregulated *LDLR*
14 expression (**Figure 8**).

15 *BACH1* resides at a genome-wide significant CAD GWAS locus and the underlying mechanism
16 how *BACH1* contributes to CAD progression is hitherto unknown.⁷⁰ Based on pathway analyses
17 *BACH1* has been described to be involved in vascular remodeling, proliferation and
18 transcriptional regulation.⁷¹ We speculate that *BACH1* promotes atherosclerosis based on the
19 downregulation of the *LDLR* via *MAFF*.

20 The transcriptome studies in mouse models and human samples as well as *in vitro* and *in vivo*
21 *MAFF* perturbation experiments provided evidence supporting a context-specific role of *MAFF*
22 in the regulation of *LDLR* as well as other genes. *In vivo* data from STARNET, including liver
23 tissue samples from a large set of CAD patients undergoing bypass surgery showed a significant
24 positive correlation between expression of the transcription factor *MAFF* and the *LDLR*. Lower

1 expression values of *MAFF* were found in men and were significantly correlated with more
2 complex and severe coronary artery lesions as measured by the Syntax Score I. Likewise, a
3 positive correlation between *Maff* and *Ldlr* was found in mice on chow and high-fat diets in
4 HMDP. In mice with transgenic expression of human *APOE-Leiden* and *CETP*, which display
5 a significant increase of inflammatory cytokines, showed in contrast a significant inverse
6 correlation between *Maff* and the *Ldlr*. These results support a context-specific relationship
7 between the expression of *Maff* and *Ldlr*.

8 Substantial convergence of tissue-specific regulatory mechanisms was observed in the
9 *MAFF/Maff* liver specific network between human and mouse. The finding that knockdown of
10 *Maff* *in vitro* in a mouse hepatocyte cell line led to reduced expression of the *Ldlr* was validated
11 in a human *in vitro* model, highlighting similar interaction patterns between *MAFF* and *LDLR*
12 in human and mouse. Besides *LDLR*, the other experimentally examined neighbouring genes
13 within the *MAFF* network (*EPHA2*, *GDF15*, *TNFAIP3*, and *TRIB1*) also showed convergent
14 regulation in human and mouse liver cell lines. The *in vivo* data in *Maff* knockout mice strongly
15 supported this assumption. Additionally, the presence of *MAFF* binding motifs in the *LDLR*
16 gene region and 19 out of the overall 24 additional predicted neighbouring genes using human
17 ChIP-Seq data were identified. However, under baseline conditions it was not achievable to
18 identify the molecular partners effecting *LDLR/Ldlr* downregulation following *MAFF/Maff*
19 silencing, in contrast to inflammatory conditions. Nevertheless, *Maff* overexpression in AML12
20 cells led to significant upregulation of the *Ldlr* under non-inflammatory conditions.

21 *MAFF* was also found to be sensitive to environmental stimuli (e.g. high-fat diet, inflammation).
22 Treatment with lipopolysaccharides markedly increased inflammatory cytokine levels in the
23 liver of wildtype mice, as measured by *Il1b*, *Il6* and *Tnfa* levels and led to an excessive
24 upregulation of *Maff* that was accompanied by reduced *Ldlr* expression. The same pattern of

1 elevated inflammatory mediators and inverse correlation of *Maff* and *Ldlr* expression levels has
2 been identified in the HMDP in mice with transgenic implementation of human *APOE-Leiden*
3 and *CETP*. This is in line with findings in the literature, that cholesterol accumulation in cells
4 of genetically modified mouse models triggers the inflammasome and results in elevated release
5 of inflammatory mediators such as *Il1b*, *Il6* and *Tnfa*.⁷²

6 Indeed, inflammation and perturbation of cholesterol levels coincide in several human
7 conditions, including rheumatoid arthritis (RA), which goes along with an increased risk of
8 cardiovascular events.⁷³⁻⁷⁷ Recently, Fernández-Ortiz and colleagues studied lipid metabolism
9 in different stages of RA disease activity and observed that total cholesterol, LDL cholesterol
10 and oxidized LDL cholesterol were slightly elevated in the low disease activity group, whereas
11 high disease activity individuals showed distinct reduction of total cholesterol, HDL cholesterol
12 and LDL cholesterol along a drastic increase of pro-atherosclerotic oxidized LDL cholesterol.⁷⁸
13 These alterations are mirrored by a gene set analysis of disease ontology⁶⁷ focused on *MAFF*
14 expression revealing its association with arthritis, LDLR expression, atherosclerosis and
15 myocardial infarction risk. Thus, our offer an explanation on that *MAFF* could be a link between
16 inflammation, lipid and lipoprotein metabolism and cardiovascular disease.

17 **Limitations**

18 Bioinformatics analyses on key drivers of liver gene expression may be further enriched in the
19 future as the numbers of loci showing significant signals in human CAD GWAS and mouse
20 candidate genes will increase within the next years. However, our previous network modeling
21 of CAD GWAS loci demonstrated that network predictions are relatively insensitive to changes
22 in the number of loci included, supporting the robustness of the overall patterns of disease
23 pathways and networks.⁷⁹ In this study, a *MAFF* centered regulatory network was revealed
24 involving multiple atherosclerosis related genes in mouse and human, with profound effects of

1 *MAFF* on downstream targets, and its regulation by inflammatory mediators. The molecular
2 mechanisms affecting *MAFF* expression as well as *MAFF* downstream targets remain
3 inadequately understood. Specifically, the molecular partners of *MAFF* executing
4 transcriptional activation of *LDLR* under non-inflammatory conditions remain to be elusive. By
5 contrast, *BACH1* was identified to physically interact with *MAFF* in the presence of LPS
6 stimulation. While we provided multiple layers of evidence placing *MAFF* in the center of a
7 top ranked liver-specific key-driver network linking lipid metabolism and inflammation, further
8 investigations should aim to identify potential mechanisms relevant for cardiovascular risk via
9 regulation of *MAFF*, *LDLR* and downstream pathways. Of specific interest would be the
10 elucidation of the molecular model explaining *MAFF*-related *LDLR* induction and subsequent
11 atheroprotective effects. In this respect our findings may be a starting point for a better
12 elucidation of interactions between inflammatory processes, hypercholesterolemia and CAD
13 risk.

14 **In conclusion**

15 The transcription factor *MAFF* is the key driver of a liver specific network which includes a
16 large number of genes known to affect atherosclerosis in mouse and human. Depending on the
17 underlying context (non-inflammatory vs. inflammatory) *MAFF* is able to mediate activation
18 or repression of gene expression.^{80, 81} Under non-inflammatory conditions *MAFF* appears to
19 induce *LDLR* expression. In the presence of LPS stimulation, heterodimerisation of *MAFF* with
20 the CAD GWAS candidate and transcriptional regulator *BACH1*⁸² binding at the MARE in the
21 promoter region of the *LDLR* led to downregulation of *LDLR* expression. It appears that both,
22 the degree of inflammation (none vs. excessive) and expression values of *MAFF* modulate these
23 processes. Our experiments in different model systems and human samples demonstrate a direct

- 1 connection between *MAFF* and the *LDLR* and also revealed significant changes in this
- 2 relationship under inflammatory conditions (**Figure 8**).

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1 **Author contributions**

2 MVS drafted the manuscript and performed the literature search of mouse genes. YZ performed
3 the bioinformatics modeling. *In vitro* experiments have been performed by MVS, NC and TV.
4 TV contributed with KO mouse models. MY and PAE supported the Maff^{-/-} mouse model.
5 MW and MM supported the ChIP-MS approach. AJL provided *in vivo* data from HMDP. OF,
6 AR, JK and JB contributed with human data from STARNET. SP performed the MAFF binding
7 approach. XY supervised the bioinformatics analysis. All authors participated in the analyses
8 of the data and critically reviewed the manuscript, written by MVS, YZ, AJL, XY and HS.

9

10 **Disclosures**

11 None.

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1 References

- 2 1. Writing Group M, Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das
3 SR, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jimenez MC, Judd SE,
4 Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER, 3rd, Moy
5 CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ,
6 Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW,
7 Turner MB, American Heart Association Statistics C and Stroke Statistics S. Executive Summary: Heart
8 Disease and Stroke Statistics--2016 Update: A Report From the American Heart Association.
9 *Circulation*. 2016;133:447-54.
- 10 2. Nichols M, Townsend N, Scarborough P and Rayner M. Cardiovascular disease in Europe
11 2014: epidemiological update. *Eur Heart J*. 2014;35:2929.
- 12 3. Khera AV, Emdin CA, Drake I, Natarajan P, Bick AG, Cook NR, Chasman DI, Baber U, Mehran R,
13 Rader DJ, Fuster V, Boerwinkle E, Melander O, Orho-Melander M, Ridker PM and Kathiresan S.
14 Genetic Risk, Adherence to a Healthy Lifestyle, and Coronary Disease. *N Engl J Med*. 2016;375:2349-
15 2358.
- 16 4. Kessler T, Vilne B and Schunkert H. The impact of genome-wide association studies on the
17 pathophysiology and therapy of cardiovascular disease. *EMBO Mol Med*. 2016;8:688-701.
- 18 5. Makinen VP, Civelek M, Meng QY, Zhang B, Zhu J, Levian C, Huan TX, Segre AV, Ghosh S, Vivar
19 J, Nikpay M, Stewart AFR, Nelson CP, Willenborg C, Erdmann J, Blakenberg S, O'Donnell CJ, Marz W,
20 Laaksonen R, Epstein SE, Kathiresan S, Shah SH, Hazen SL, Reilly MP, Lusic AJ, Samani NJ, Schunkert H,
21 Quertermous T, McPherson R, Yang X, Assimes TL and Genome-Wide CAD. Integrative Genomics
22 Reveals Novel Molecular Pathways and Gene Networks for Coronary Artery Disease. *Plos Genet*.
23 2014;10.
- 24 6. Shu L, Chan KHK, Zhang GL, Huan TX, Kurt Z, Zhao YQ, Codoni V, Tregouet DA, Consortium C,
25 Yang J, Wilson JG, Luo X, Levy D, Lusic AJ, Liu SM and Yang X. Shared genetic regulatory networks for
26 cardiovascular disease and type 2 diabetes in multiple populations of diverse ethnicities in the United
27 States. *Plos Genet*. 2017;13.
- 28 7. Ghosh S, Vivar J, Nelson CP, Willenborg C, Segre AV, Maekinen VP, Nikpay M, Erdmann J,
29 Blankenberg S, O'Donnell C, Maerz W, Laaksonen R, Stewart AFR, Epstein SE, Shah SH, Granger CB,
30 Hazen SL, Kathiresan S, Reilly MP, Yang X, Quertermous T, Samani NJ, Schunkert H, Assimes TL,
31 McPherson R and Consortium C. Systems Genetics Analysis of Genome-Wide Association Study
32 Reveals Novel Associations Between Key Biological Processes and Coronary Artery Disease. *Arterioscl*
33 *Throm Vas*. 2015;35:1712-1722.
- 34 8. von Scheidt M, Zhao Y, Kurt Z, Pan C, Zeng L, Yang X, Schunkert H and Lusic AJ. Applications
35 and Limitations of Mouse Models for Understanding Human Atherosclerosis. *Cell Metab*.
36 2017;25:248-261.
- 37 9. Franzen O, Ermel R, Cohain A, Akers NK, Di Narzo A, Talukdar HA, Foroughi-Asl H,
38 Giambartolomei C, Fullard JF, Sukhvasi K, Koks S, Gan LM, Giannarelli C, Kovacic JC, Betsholtz C,
39 Losic B, Michoel T, Hao K, Roussos P, Skogsberg J, Ruusalepp A, Schadt EE and Bjorkegren JL.
40 Cardiometabolic risk loci share downstream cis- and trans-gene regulation across tissues and
41 diseases. *Science*. 2016;353:827-30.
- 42 10. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, Hegele RA, Krauss RM,
43 Raal FJ, Schunkert H, Watts GF, Boren J, Fazio S, Horton JD, Masana L, Nicholls SJ, Nordestgaard BG,
44 van de Sluis B, Taskinen MR, Tokgozoglu L, Landmesser U, Laufs U, Wiklund O, Stock JK, Chapman MJ
45 and Catapano AL. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence
46 from genetic, epidemiologic, and clinical studies. A consensus statement from the European
47 Atherosclerosis Society Consensus Panel. *Eur Heart J*. 2017;38:2459-2472.
- 48 11. Weber C and Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat*
49 *Med*. 2011;17:1410-22.

- 1 12. Geovanini GR and Libby P. Atherosclerosis and inflammation: overview and updates. *Clin Sci*
2 (*Lond*). 2018;132:1243-1252.
- 3 13. Cohen JC, Boerwinkle E, Mosley TH, Jr. and Hobbs HH. Sequence variations in PCSK9, low LDL,
4 and protection against coronary heart disease. *N Engl J Med*. 2006;354:1264-72.
- 5 14. Ference BA, Yoo W, Alesh I, Mahajan N, Mirowska KK, Mewada A, Kahn J, Afonso L, Williams
6 KA, Sr. and Flack JM. Effect of long-term exposure to lower low-density lipoprotein cholesterol
7 beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis. *J*
8 *Am Coll Cardiol*. 2012;60:2631-9.
- 9 15. Holmes MV, Asselbergs FW, Palmer TM, Drenos F, Lanktree MB, Nelson CP, Dale CE,
10 Padmanabhan S, Finan C, Swerdlow DI, Tragante V, van Iperen EP, Sivapalaratnam S, Shah S, Elbers
11 CC, Shah T, Engmann J, Giambartolomei C, White J, Zabaneh D, Sofat R, McLachlan S, consortium U,
12 Doevendans PA, Balmforth AJ, Hall AS, North KE, Almqvister B, Hoogeveen RC, Cushman M, Fornage
13 M, Patel SR, Redline S, Siscovick DS, Tsai MY, Karczewski KJ, Hofker MH, Verschuren WM, Bots ML,
14 van der Schouw YT, Melander O, Dominiczak AF, Morris R, Ben-Shlomo Y, Price J, Kumari M, Baumert
15 J, Peters A, Thorand B, Koenig W, Gaunt TR, Humphries SE, Clarke R, Watkins H, Farrall M, Wilson JG,
16 Rich SS, de Bakker PI, Lange LA, Davey Smith G, Reiner AP, Talmud PJ, Kivimaki M, Lawlor DA,
17 Dudbridge F, Samani NJ, Keating BJ, Hingorani AD and Casas JP. Mendelian randomization of blood
18 lipids for coronary heart disease. *Eur Heart J*. 2015;36:539-50.
- 19 16. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. 1999;340:115-26.
- 20 17. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R,
21 Collins R, Simes R and Cholesterol Treatment Trialists C. Efficacy and safety of cholesterol-lowering
22 treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of
23 statins. *Lancet*. 2005;366:1267-78.
- 24 18. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau
25 J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A,
26 Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF,
27 Troquay RPT, Libby P, Glynn RJ and Group CT. Antiinflammatory Therapy with Canakinumab for
28 Atherosclerotic Disease. *N Engl J Med*. 2017;377:1119-1131.
- 29 19. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, Koenig W, Libby P,
30 Lorenzatti AJ, Macfadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ and Group JTS.
31 Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of
32 rosuvastatin: a prospective study of the JUPITER trial. *Lancet*. 2009;373:1175-82.
- 33 20. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, Dixon RJ, Meitinger T,
34 Braund P, Wichmann HE, Barrett JH, Konig IR, Stevens SE, Szymczak S, Tregouet DA, Iles MM, Pahlke
35 F, Pollard H, Lieb W, Cambien F, Fischer M, Ouwehand W, Blankenberg S, Balmforth AJ, Baessler A,
36 Ball SG, Strom TM, Braenne I, Gieger C, Deloukas P, Tobin MD, Ziegler A, Thompson JR, Schunkert H,
37 Wtccc and the Cardiogenics C. Genomewide association analysis of coronary artery disease. *N Engl J*
38 *Med*. 2007;357:443-53.
- 39 21. Schunkert H, Konig IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, Preuss M, Stewart AF,
40 Barbalic M, Gieger C, Absher D, Aherrahrou Z, Allayee H, Altshuler D, Anand SS, Andersen K,
41 Anderson JL, Ardissino D, Ball SG, Balmforth AJ, Barnes TA, Becker DM, Becker LC, Berger K, Bis JC,
42 Boehnke SM, Boerwinkle E, Braund PS, Brown MJ, Burnett MS, Buysschaert I, Cardiogenics,
43 Carlquist JF, Chen L, Cichon S, Codd V, Davies RW, Dedoussis G, Dehghan A, Demissie S, Devaney JM,
44 Diemert P, Do R, Doering A, Eifert S, Mokhtari NE, Ellis SG, Elosua R, Engert JC, Epstein SE, de Faire U,
45 Fischer M, Folsom AR, Freyer J, Gigante B, Girelli D, Gretarsdottir S, Gudnason V, Gulcher JR, Halperin
46 E, Hammond N, Hazen SL, Hofman A, Horne BD, Illig T, Iribarren C, Jones GT, Jukema JW, Kaiser MA,
47 Kaplan LM, Kastelein JJ, Khaw KT, Knowles JW, Kolovou G, Kong A, Laaksonen R, Lambrechts D,
48 Leander K, Lettre G, Li M, Lieb W, Loley C, Lotery AJ, Mannucci PM, Maouche S, Martinelli N,
49 McKeown PP, Meisinger C, Meitinger T, Melander O, Merlini PA, Mooser V, Morgan T, Muhleisen TW,
50 Muhlestein JB, Munzel T, Musunuru K, Nahrstaedt J, Nelson CP, Nothen MM, Olivieri O, Patel RS,
51 Patterson CC, Peters A, Peyvandi F, Qu L, Quyyumi AA, Rader DJ, Rallidis LS, Rice C, Rosendaal FR,

1 Rubin D, Salomaa V, Sampietro ML, Sandhu MS, Schadt E, Schafer A, Schillert A, Schreiber S,
2 Schrezenmeir J, Schwartz SM, Siscovick DS, Sivananthan M, Sivapalaratnam S, Smith A, Smith TB,
3 Snoep JD, Soranzo N, Spertus JA, Stark K, Stirrups K, Stoll M, Tang WH, Tennstedt S, Thorgeirsson G,
4 Thorleifsson G, Tomaszewski M, Uitterlinden AG, van Rij AM, Voight BF, Wareham NJ, Wells GA,
5 Wichmann HE, Wild PS, Willenborg C, Witteman JC, Wright BJ, Ye S, Zeller T, Ziegler A, Cambien F,
6 Goodall AH, Cupples LA, Quertermous T, Marz W, Hengstenberg C, Blankenberg S, Ouwehand WH,
7 Hall AS, Deloukas P, Thompson JR, Stefansson K, Roberts R, Thorsteinsdottir U, O'Donnell CJ,
8 McPherson R, Erdmann J, Consortium CA and Samani NJ. Large-scale association analysis identifies 13
9 new susceptibility loci for coronary artery disease. *Nat Genet.* 2011;43:333-8.

10 22. Coronary Artery Disease Genetics C. A genome-wide association study in Europeans and
11 South Asians identifies five new loci for coronary artery disease. *Nat Genet.* 2011;43:339-44.

12 23. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, Ingelsson E,
13 Saleheen D, Erdmann J, Goldstein BA, Stirrups K, Konig IR, Cazier JB, Johansson A, Hall AS, Lee JY,
14 Willer CJ, Chambers JC, Esko T, Folkersen L, Goel A, Grundberg E, Havulinna AS, Ho WK, Hopewell JC,
15 Eriksson N, Kleber ME, Kristiansson K, Lundmark P, Lyytikainen LP, Rafelt S, Shungin D, Strawbridge
16 RJ, Thorleifsson G, Tikkanen E, Van Zuydam N, Voight BF, Waite LL, Zhang W, Ziegler A, Absher D,
17 Altshuler D, Balmforth AJ, Barroso I, Braund PS, Burgdorf C, Claudi-Boehm S, Cox D, Dimitriou M, Do
18 R, Consortium D, Consortium C, Consortium CD, Doney AS, El Mokhtari N, Eriksson P, Fischer K,
19 Fontanillas P, Franco-Cereceda A, Gigante B, Groop L, Gustafsson S, Hager J, Hallmans G, Han BG,
20 Hunt SE, Kang HM, Illig T, Kessler T, Knowles JW, Kolovou G, Kuusisto J, Langenberg C, Langford C,
21 Leander K, Lokki ML, Lundmark A, McCarthy MI, Meisinger C, Melander O, Mihailov E, Maouche S,
22 Morris AD, Muller-Nurasyid M, Mu TC, Nikus K, Peden JF, Rayner NW, Rasheed A, Rosinger S, Rubin
23 D, Rumpf MP, Schafer A, Sivananthan M, Song C, Stewart AF, Tan ST, Thorgeirsson G, van der Schoot
24 CE, Wagner PJ, Wellcome Trust Case Control C, Wells GA, Wild PS, Yang TP, Amouyel P, Arveiler D,
25 Basart H, Boehnke M, Boerwinkle E, Brambilla P, Cambien F, Cupples AL, de Faire U, Dehghan A,
26 Diemert P, Epstein SE, Evans A, Ferrario MM, Ferrieres J, Gauguier D, Go AS, Goodall AH, Gudnason V,
27 Hazen SL, Holm H, Iribarren C, Jang Y, Kahonen M, Kee F, Kim HS, Klopp N, Koenig W, Kratzer W,
28 Kuulasmaa K, Laakso M, Laaksonen R, Lee JY, Lind L, Ouwehand WH, Parish S, Park JE, Pedersen NL,
29 Peters A, Quertermous T, Rader DJ, Salomaa V, Schadt E, Shah SH, Sinisalo J, Stark K, Stefansson K,
30 Tregouet DA, Virtamo J, Wallentin L, Wareham N, Zimmermann ME, Nieminen MS, Hengstenberg C,
31 Sandhu MS, Pastinen T, Syvanen AC, Hovingh GK, Dedoussis G, Franks PW, Lehtimaki T, Metspalu A,
32 Zalloua PA, Siegbahn A, Schreiber S, Ripatti S, Blankenberg SS, Perola M, Clarke R, Boehm BO,
33 O'Donnell C, Reilly MP, Marz W, Collins R, Kathiresan S, Hamsten A, Kooner JS, Thorsteinsdottir U,
34 Danesh J, Palmer CN, Roberts R, Watkins H, Schunkert H and Samani NJ. Large-scale association
35 analysis identifies new risk loci for coronary artery disease. *Nat Genet.* 2013;45:25-33.

36 24. Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, Saleheen D, Kyriakou T, Nelson
37 CP, Hopewell JC, Webb TR, Zeng L, Dehghan A, Alver M, Armasu SM, Auro K, Bjornnes A, Chasman DI,
38 Chen S, Ford I, Franceschini N, Gieger C, Grace C, Gustafsson S, Huang J, Hwang SJ, Kim YK, Kleber
39 ME, Lau KW, Lu X, Lu Y, Lyytikainen LP, Mihailov E, Morrison AC, Pervjakova N, Qu L, Rose LM, Salfati
40 E, Saxena R, Scholz M, Smith AV, Tikkanen E, Uitterlinden A, Yang X, Zhang W, Zhao W, de Andrade
41 M, de Vries PS, van Zuydam NR, Anand SS, Bertram L, Beutner F, Dedoussis G, Frossard P, Gauguier D,
42 Goodall AH, Gottesman O, Haber M, Han BG, Huang J, Jalilzadeh S, Kessler T, Konig IR, Lannfelt L, Lieb
43 W, Lind L, Lindgren CM, Lokki ML, Magnusson PK, Mallick NH, Mehra N, Meitinger T, Memon FU,
44 Morris AP, Nieminen MS, Pedersen NL, Peters A, Rallidis LS, Rasheed A, Samuel M, Shah SH, Sinisalo
45 J, Stirrups KE, Trompet S, Wang L, Zaman KS, Ardissino D, Boerwinkle E, Borecki IB, Bottinger EP,
46 Buring JE, Chambers JC, Collins R, Cupples LA, Danesh J, Demuth I, Elosua R, Epstein SE, Esko T,
47 Feitosa MF, Franco OH, Franzosi MG, Granger CB, Gu D, Gudnason V, Hall AS, Hamsten A, Harris TB,
48 Hazen SL, Hengstenberg C, Hofman A, Ingelsson E, Iribarren C, Jukema JW, Karhunen PJ, Kim BJ,
49 Kooner JS, Kullo IJ, Lehtimaki T, Loos RJ, Melander O, Metspalu A, Marz W, Palmer CN, Perola M,
50 Quertermous T, Rader DJ, Ridker PM, Ripatti S, Roberts R, Salomaa V, Sanghera DK, Schwartz SM,
51 Seedorf U, Stewart AF, Stott DJ, Thiery J, Zalloua PA, O'Donnell CJ, Reilly MP, Assimes TL, Thompson

1 JR, Erdmann J, Clarke R, Watkins H, Kathiresan S, McPherson R, Deloukas P, Schunkert H, Samani NJ,
2 Farrall M and Consortium CAD. A comprehensive 1,000 Genomes-based genome-wide association
3 meta-analysis of coronary artery disease. *Nat Genet.* 2015;47:1121-30.

4 25. McPherson R and Tybjaerg-Hansen A. Genetics of Coronary Artery Disease. *Circ Res.*
5 2016;118:564-78.

6 26. Howson JMM, Zhao W, Barnes DR, Ho WK, Young R, Paul DS, Waite LL, Freitag DF, Fauman
7 EB, Salfati EL, Sun BB, Eicher JD, Johnson AD, Sheu WHH, Nielsen SF, Lin WY, Surendran P, Malarstig
8 A, Wilk JB, Tybjaerg-Hansen A, Rasmussen KL, Kamstrup PR, Deloukas P, Erdmann J, Kathiresan S,
9 Samani NJ, Schunkert H, Watkins H, CardioGramplusC4D, Do R, Rader DJ, Johnson JA, Hazen SL,
10 Quyyumi AA, Spertus JA, Pepine CJ, Franceschini N, Justice A, Reiner AP, Buyske S, Hindorff LA, Carty
11 CL, North KE, Kooperberg C, Boerwinkle E, Young K, Graff M, Peters U, Absher D, Hsiung CA, Lee WJ,
12 Taylor KD, Chen YH, Lee IT, Guo X, Chung RH, Hung YJ, Rotter JI, Juang JJ, Quertermous T, Wang TD,
13 Rasheed A, Frossard P, Alam DS, Majumder AAS, Di Angelantonio E, Chowdhury R, Epic CVD, Chen YI,
14 Nordestgaard BG, Assimes TL, Danesh J, Butterworth AS and Saleheen D. Fifteen new risk loci for
15 coronary artery disease highlight arterial-wall-specific mechanisms. *Nat Genet.* 2017;49:1113-1119.

16 27. Nelson CP, Goel A, Butterworth AS, Kanoni S, Webb TR, Marouli E, Zeng L, Ntalla I, Lai FY,
17 Hopewell JC, Giannakopoulou O, Jiang T, Hamby SE, Di Angelantonio E, Assimes TL, Bottinger EP,
18 Chambers JC, Clarke R, Palmer CNA, Cubbon RM, Ellinor P, Ermel R, Evangelou E, Franks PW, Grace C,
19 Gu D, Hingorani AD, Howson JMM, Ingelsson E, Kastrati A, Kessler T, Kyriakou T, Lehtimaki T, Lu X, Lu
20 Y, Marz W, McPherson R, Metspalu A, Pujades-Rodriguez M, Ruusalepp A, Schadt EE, Schmidt AF,
21 Sweeting MJ, Zalloua PA, AlGhalayini K, Keavney BD, Kooner JS, Loos RJF, Patel RS, Rutter MK,
22 Tomaszewski M, Tzoulaki I, Zeggini E, Erdmann J, Dedoussis G, Bjorkegren JLM, Consortium E-C,
23 CardioGramplusC4D, group UKBCCCw, Schunkert H, Farrall M, Danesh J, Samani NJ, Watkins H and
24 Deloukas P. Association analyses based on false discovery rate implicate new loci for coronary artery
25 disease. *Nat Genet.* 2017;49:1385-1391.

26 28. Webb TR, Erdmann J, Stirrups KE, Stitzel NO, Masca NG, Jansen H, Kanoni S, Nelson CP,
27 Ferrario PG, Konig IR, Eicher JD, Johnson AD, Hamby SE, Betsholtz C, Ruusalepp A, Franzen O, Schadt
28 EE, Bjorkegren JL, Weeke PE, Auer PL, Schick UM, Lu Y, Zhang H, Dube MP, Goel A, Farrall M, Peloso
29 GM, Won HH, Do R, van Iperen E, Kruppa J, Mahajan A, Scott RA, Willenborg C, Braund PS, van
30 Capelleveen JC, Doney AS, Donnelly LA, Asselta R, Merlini PA, Duga S, Marziliano N, Denny JC, Shaffer
31 C, El-Mokhtari NE, Franke A, Heilmann S, Hengstenberg C, Hoffmann P, Holmen OL, Hveem K, Jansson
32 JH, Jockel KH, Kessler T, Kriebel J, Laugwitz KL, Marouli E, Martinelli N, McCarthy MI, Van Zuydam NR,
33 Meisinger C, Esko T, Mihailov E, Escher SA, Alver M, Moebus S, Morris AD, Virtamo J, Nikpay M,
34 Olivieri O, Provost S, AlQarawi A, Robertson NR, Akinsansya KO, Reilly DF, Vogt TF, Yin W, Asselbergs
35 FW, Kooperberg C, Jackson RD, Stahl E, Muller-Nurasyid M, Strauch K, Varga TV, Waldenberger M,
36 Wellcome Trust Case Control C, Zeng L, Chowdhury R, Salomaa V, Ford I, Jukema JW, Amouyel P,
37 Kontto J, Investigators M, Nordestgaard BG, Ferrieres J, Saleheen D, Sattar N, Surendran P, Wagner A,
38 Young R, Howson JM, Butterworth AS, Danesh J, Ardissino D, Bottinger EP, Erbel R, Franks PW, Girelli
39 D, Hall AS, Hovingh GK, Kastrati A, Lieb W, Meitinger T, Kraus WE, Shah SH, McPherson R, Orho-
40 Melander M, Melander O, Metspalu A, Palmer CN, Peters A, Rader DJ, Reilly MP, Loos RJ, Reiner AP,
41 Roden DM, Tardif JC, Thompson JR, Wareham NJ, Watkins H, Willer CJ, Samani NJ, Schunkert H,
42 Deloukas P, Kathiresan S, Myocardial Infarction G and Investigators CAEC. Systematic Evaluation of
43 Pleiotropy Identifies 6 Further Loci Associated With Coronary Artery Disease. *J Am Coll Cardiol.*
44 2017;69:823-836.

45 29. Braenne I, Civelek M, Vilne B, Di Narzo A, Johnson AD, Zhao Y, Reiz B, Codoni V, Webb TR,
46 Foroughi Asl H, Hamby SE, Zeng L, Tregouet DA, Hao K, Topol EJ, Schadt EE, Yang X, Samani NJ,
47 Bjorkegren JL, Erdmann J, Schunkert H, Lusi AJ and Leducq Consortium CADGd. Prediction of Causal
48 Candidate Genes in Coronary Artery Disease Loci. *Arterioscler Thromb Vasc Biol.* 2015;35:2207-17.

49 30. Schunkert H, Gotz A, Braund P, McGinnis R, Tregouet DA, Mangino M, Linsel-Nitschke P,
50 Cambien F, Hengstenberg C, Stark K, Blankenberg S, Tiret L, Ducimetiere P, Keniry A, Ghorri MJ,
51 Schreiber S, El Mokhtari NE, Hall AS, Dixon RJ, Goodall AH, Liptau H, Pollard H, Schwarz DF, Hothorn

1 LA, Wichmann HE, Konig IR, Fischer M, Meisinger C, Ouwehand W, Deloukas P, Thompson JR,
2 Erdmann J, Ziegler A, Samani NJ and Cardiogenics C. Repeated replication and a prospective meta-
3 analysis of the association between chromosome 9p21.3 and coronary artery disease. *Circulation*.
4 2008;117:1675-84.

5 31. Zhu J, Zhang B, Smith EN, Drees B, Brem RB, Kruglyak L, Bumgarner RE and Schadt EE.
6 Integrating large-scale functional genomic data to dissect the complexity of yeast regulatory
7 networks. *Nat Genet*. 2008;40:854-61.

8 32. Derry JM, Zhong H, Molony C, MacNeil D, Guhathakurta D, Zhang B, Mudgett J, Small K, El
9 Fertak L, Guimond A, Selloum M, Zhao W, Champy MF, Monassier L, Vogt T, Cully D, Kasarskis A and
10 Schadt EE. Identification of genes and networks driving cardiovascular and metabolic phenotypes in a
11 mouse F2 intercross. *PLoS One*. 2010;5:e14319.

12 33. Wang SS, Schadt EE, Wang H, Wang X, Ingram-Drake L, Shi W, Drake TA and Lusk AJ.
13 Identification of pathways for atherosclerosis in mice: integration of quantitative trait locus analysis
14 and global gene expression data. *Circ Res*. 2007;101:e11-30.

15 34. Yang X, Schadt EE, Wang S, Wang H, Arnold AP, Ingram-Drake L, Drake TA and Lusk AJ.
16 Tissue-specific expression and regulation of sexually dimorphic genes in mice. *Genome Res*.
17 2006;16:995-1004.

18 35. Schadt EE, Molony C, Chudin E, Hao K, Yang X, Lum PY, Kasarskis A, Zhang B, Wang S, Suver C,
19 Zhu J, Millstein J, Sieberts S, Lamb J, GuhaThakurta D, Derry J, Storey JD, Avila-Campillo I, Kruger MJ,
20 Johnson JM, Rohl CA, van Nas A, Mehrabian M, Drake TA, Lusk AJ, Smith RC, Guengerich FP, Strom
21 SC, Schuetz E, Rushmore TH and Ulrich R. Mapping the genetic architecture of gene expression in
22 human liver. *PLoS Biol*. 2008;6:e107.

23 36. Tu Z, Keller MP, Zhang C, Rabaglia ME, Greenawalt DM, Yang X, Wang IM, Dai H, Bruss MD,
24 Lum PY, Zhou YP, Kemp DM, Kendziora C, Yandell BS, Attie AD, Schadt EE and Zhu J. Integrative
25 analysis of a cross-loci regulation network identifies App as a gene regulating insulin secretion from
26 pancreatic islets. *PLoS Genet*. 2012;8:e1003107.

27 37. Makinen VP, Civelek M, Meng Q, Zhang B, Zhu J, Levian C, Huan T, Segre AV, Ghosh S, Vivar J,
28 Nikpay M, Stewart AF, Nelson CP, Willenborg C, Erdmann J, Blakenberg S, O'Donnell CJ, Marz W,
29 Laaksonen R, Epstein SE, Kathiresan S, Shah SH, Hazen SL, Reilly MP, Coronary ADG-WR, Meta-
30 Analysis C, Lusk AJ, Samani NJ, Schunkert H, Quertermous T, McPherson R, Yang X and Assimes TL.
31 Integrative genomics reveals novel molecular pathways and gene networks for coronary artery
32 disease. *PLoS Genet*. 2014;10:e1004502.

33 38. Wang IM, Zhang B, Yang X, Zhu J, Stepaniants S, Zhang C, Meng Q, Peters M, He Y, Ni C,
34 Slipetz D, Crackower MA, Houshyar H, Tan CM, Asante-Appiah E, O'Neill G, Luo MJ, Thieringer R, Yuan
35 J, Chiu CS, Lum PY, Lamb J, Boie Y, Wilkinson HA, Schadt EE, Dai H and Roberts C. Systems analysis of
36 eleven rodent disease models reveals an inflammatoric signature and key drivers. *Mol Syst Biol*.
37 2012;8:594.

38 39. Yang X, Zhang B, Molony C, Chudin E, Hao K, Zhu J, Gaedigk A, Suver C, Zhong H, Leeder JS,
39 Guengerich FP, Strom SC, Schuetz E, Rushmore TH, Ulrich RG, Slatter JG, Schadt EE, Kasarskis A and
40 Lum PY. Systematic genetic and genomic analysis of cytochrome P450 enzyme activities in human
41 liver. *Genome Res*. 2010;20:1020-36.

42 40. Shu L, Zhao Y, Kurt Z, Byars SG, Tukiainen T, Kettunen J, Orozco LD, Pellegrini M, Lusk AJ,
43 Ripatti S, Zhang B, Inouye M, Makinen VP and Yang X. Mergeomics: multidimensional data
44 integration to identify pathogenic perturbations to biological systems. *BMC Genomics*. 2016;17:874.

45 41. Zhang Y, Liu T, Meyer CA, Eeckhoutte J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM,
46 Brown M, Li W and Liu XS. Model-based analysis of ChIP-Seq (MACS). *Genome Biol*. 2008;9:R137.

47 42. Landt SG, Marinov GK, Kundaje A, Kheradpour P, Pauli F, Batzoglou S, Bernstein BE, Bickel P,
48 Brown JB, Cayting P, Chen Y, DeSalvo G, Epstein C, Fisher-Aylor KI, Euskirchen G, Gerstein M, Gertz J,
49 Hartemink AJ, Hoffman MM, Iyer VR, Jung YL, Karmakar S, Kellis M, Kharchenko PV, Li Q, Liu T, Liu XS,
50 Ma L, Milosavljevic A, Myers RM, Park PJ, Pazin MJ, Perry MD, Raha D, Reddy TE, Rozowsky J, Shores
51 N, Sidow A, Slattery M, Stamatoyannopoulos JA, Tolstorukov MY, White KP, Xi S, Farnham PJ, Lieb JD,

1 Wold BJ and Snyder M. CHIP-seq guidelines and practices of the ENCODE and modENCODE consortia.
2 *Genome Res.* 2012;22:1813-31.

3 43. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, Cheng JX, Murre C, Singh H and Glass
4 CK. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements
5 required for macrophage and B cell identities. *Mol Cell.* 2010;38:576-89.

6 44. Machanick P and Bailey TL. MEME-CHIP: motif analysis of large DNA datasets. *Bioinformatics.*
7 2011;27:1696-7.

8 45. Lu X, Guanga GP, Wan C and Rose RB. A novel DNA binding mechanism for maf basic region-
9 leucine zipper factors inferred from a MafA-DNA complex structure and binding specificities.
10 *Biochemistry.* 2012;51:9706-17.

11 46. Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC,
12 Gonzales NR, Gwadz M, Hurwitz DI, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA,
13 Zhang D, Zheng C, Geer LY and Bryant SH. CDD/SPARCLE: functional classification of proteins via
14 subfamily domain architectures. *Nucleic Acids Res.* 2017;45:D200-D203.

15 47. Kozakov D, Hall DR, Xia B, Porter KA, Padhorney D, Yueh C, Beglov D and Vajda S. The ClusPro
16 web server for protein-protein docking. *Nat Protoc.* 2017;12:255-278.

17 48. Vajda S, Yueh C, Beglov D, Bohnuud T, Mottarella SE, Xia B, Hall DR and Kozakov D. New
18 additions to the ClusPro server motivated by CAPRI. *Proteins.* 2017;85:435-444.

19 49. Erdmann J, Kessler T, Munoz Venegas L and Schunkert H. A decade of genome-wide
20 association studies for coronary artery disease: the challenges ahead. *Cardiovasc Res.*
21 2018;114:1241-1257.

22 50. An SJ, Jung UJ, Choi MS, Chae CK, Oh GT and Park YB. Functions of monocyte chemotactic
23 protein-3 in transgenic mice fed a high-fat, high-cholesterol diet. *J Microbiol Biotechnol.* 2013;23:405-
24 13.

25 51. Kirii H, Niwa T, Yamada Y, Wada H, Saito K, Iwakura Y, Asano M, Moriwaki H and Seishima M.
26 Lack of interleukin-1beta decreases the severity of atherosclerosis in ApoE-deficient mice.
27 *Arterioscler Thromb Vasc Biol.* 2003;23:656-60.

28 52. Heller EA, Liu E, Tager AM, Yuan Q, Lin AY, Ahluwalia N, Jones K, Koehn SL, Lok VM, Aikawa E,
29 Moore KJ, Luster AD and Gerszten RE. Chemokine CXCL10 promotes atherogenesis by modulating the
30 local balance of effector and regulatory T cells. *Circulation.* 2006;113:2301-12.

31 53. Gold ES, Ramsey SA, Sartain MJ, Selinummi J, Podolsky I, Rodriguez DJ, Moritz RL and Aderem
32 A. ATF3 protects against atherosclerosis by suppressing 25-hydroxycholesterol-induced lipid body
33 formation. *J Exp Med.* 2012;209:807-17.

34 54. Jiang H, Li X, Zhang X, Liu Y, Huang S and Wang X. EphA2 knockdown attenuates
35 atherosclerotic lesion development in ApoE(-/-) mice. *Cardiovasc Pathol.* 2014;23:169-74.

36 55. Bonaterra GA, Zugel S, Thogersen J, Walter SA, Haberkorn U, Strelau J and Kinscherf R.
37 Growth differentiation factor-15 deficiency inhibits atherosclerosis progression by regulating
38 interleukin-6-dependent inflammatory response to vascular injury. *J Am Heart Assoc.*
39 2012;1:e002550.

40 56. Ishibashi S, Goldstein JL, Brown MS, Herz J and Burns DK. Massive xanthomatosis and
41 atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice. *J Clin Invest.*
42 1994;93:1885-93.

43 57. Qing H, Liu Y, Zhao Y, Aono J, Jones KL, Heywood EB, Howatt D, Binkley CM, Daugherty A,
44 Liang Y and Bruemmer D. Deficiency of the NR4A orphan nuclear receptor NOR1 in hematopoietic
45 stem cells accelerates atherosclerosis. *Stem Cells.* 2014;32:2419-29.

46 58. Hossain GS, Lynn EG, Maclean KN, Zhou J, Dickhout JG, Lhoták S, Trigatti B, Capone J, Rho J,
47 Tang D, McCulloch CA, Al-Bondokji I, Malloy MJ, Pullinger CR, Kane JP, Li Y, Shiffman D and Austin RC.
48 Deficiency of TDAG51 protects against atherosclerosis by modulating apoptosis, cholesterol efflux,
49 and peroxiredoxin-1 expression. *J Am Heart Assoc.* 2013;2:e000134.

50 59. Kremen M, Krishnan R, Emery I, Hu JH, Slezicki KI, Wu A, Qian K, Du L, Plawman A, Stempien-
51 Otero A and Dichek DA. Plasminogen mediates the atherogenic effects of macrophage-expressed

1 urokinase and accelerates atherosclerosis in apoE-knockout mice. *Proc Natl Acad Sci U S A*.
2 2008;105:17109-14.

3 60. Wolfrum S, Teupser D, Tan M, Chen KY and Breslow JL. The protective effect of A20 on
4 atherosclerosis in apolipoprotein E-deficient mice is associated with reduced expression of NF-
5 kappaB target genes. *Proc Natl Acad Sci U S A*. 2007;104:18601-6.

6 61. Muñoz-García B, Madrigal-Matute J, Moreno JA, Martin-Ventura JL, López-Franco O, Sastre C,
7 Ortega L, Burkly LC, Egido J and Blanco-Colio LM. TWEAK-Fn14 interaction enhances plasminogen
8 activator inhibitor 1 and tissue factor expression in atherosclerotic plaques and in cultured vascular
9 smooth muscle cells. *Cardiovasc Res*. 2011;89:225-33.

10 62. Arndt LD, J; Jeromin, F; Thiery, J; Burkhardt; R. Heterozygous deficiency of Tribbles homolog-
11 1 gene (Trib1) increases atherosclerotic lesions in ApoE-knockout mice. *12 Jahrestagung Deutsche*
12 *Vereinte Gesellschaft für Klinische Medizin und Laboratoriumsmedizin eV (DGKL)*. 2015;P068.

13 63. Yin K, Tang SL, Yu XH, Tu GH, He RF, Li JF, Xie D, Gui QJ, Fu YC, Jiang ZS, Tu J and Tang CK.
14 Apolipoprotein A-I inhibits LPS-induced atherosclerosis in ApoE(-/-) mice possibly via activated
15 STAT3-mediated upregulation of tristetraprolin. *Acta Pharmacol Sin*. 2013;34:837-46.

16 64. Blank V. Small Maf proteins in mammalian gene control: mere dimerization partners or
17 dynamic transcriptional regulators? *J Mol Biol*. 2008;376:913-25.

18 65. Ghazalpour A, Rau CD, Farber CR, Bennett BJ, Orozco LD, van Nas A, Pan C, Allayee H, Beaven
19 SW, Civelek M, Davis RC, Drake TA, Friedman RA, Furlotte N, Hui ST, Jentsch JD, Kostem E, Kang HM,
20 Kang EY, Joo JW, Korshunov VA, Laughlin RE, Martin LJ, Ohmen JD, Parks BW, Pellegrini M, Reue K,
21 Smith DJ, Tetradis S, Wang J, Wang Y, Weiss JN, Kirchgessner T, Gargalovic PS, Eskin E, Lusk AJ and
22 LeBoeuf RC. Hybrid mouse diversity panel: a panel of inbred mouse strains suitable for analysis of
23 complex genetic traits. *Mamm Genome*. 2012;23:680-92.

24 66. Bennett BJ, Davis RC, Civelek M, Orozco L, Wu J, Qi H, Pan C, Packard RR, Eskin E, Yan M,
25 Kirchgessner T, Wang Z, Li X, Gregory JC, Hazen SL, Gargalovic PS and Lusk AJ. Genetic Architecture of
26 Atherosclerosis in Mice: A Systems Genetics Analysis of Common Inbred Strains. *PLoS Genet*.
27 2015;11:e1005711.

28 67. Yang S, Kim CY, Hwang S, Kim E, Kim H, Shim H and Lee I. COEXPEDIA: exploring biomedical
29 hypotheses via co-expressions associated with medical subject headings (MeSH). *Nucleic Acids Res*.
30 2017;45:D389-D396.

31 68. Cheng Y, Ma Z, Kim BH, Wu W, Cayting P, Boyle AP, Sundaram V, Xing X, Dogan N, Li J,
32 Euskirchen G, Lin S, Lin Y, Visel A, Kawli T, Yang X, Patacsil D, Keller CA, Giardine B, Mouse EC,
33 Kundaje A, Wang T, Pennacchio LA, Weng Z, Hardison RC and Snyder MP. Principles of regulatory
34 information conservation between mouse and human. *Nature*. 2014;515:371-5.

35 69. Zhang X, Guo J, Wei X, Niu C, Jia M, Li Q and Meng D. Bach1: Function, Regulation, and
36 Involvement in Disease. *Oxid Med Cell Longev*. 2018;2018:1347969.

37 70. van der Harst P and Verweij N. Identification of 64 Novel Genetic Loci Provides an Expanded
38 View on the Genetic Architecture of Coronary Artery Disease. *Circ Res*. 2018;122:433-443.

39 71. Schunkert H, von Scheidt M, Kessler T, Stiller B, Zeng L and Vilne B. Genetics of coronary
40 artery disease in the light of genome-wide association studies. *Clin Res Cardiol*. 2018;107:2-9.

41 72. Emini Veseli B, Perrotta P, De Meyer GRA, Roth L, Van der Donck C, Martinet W and De
42 Meyer GRY. Animal models of atherosclerosis. *Eur J Pharmacol*. 2017;816:3-13.

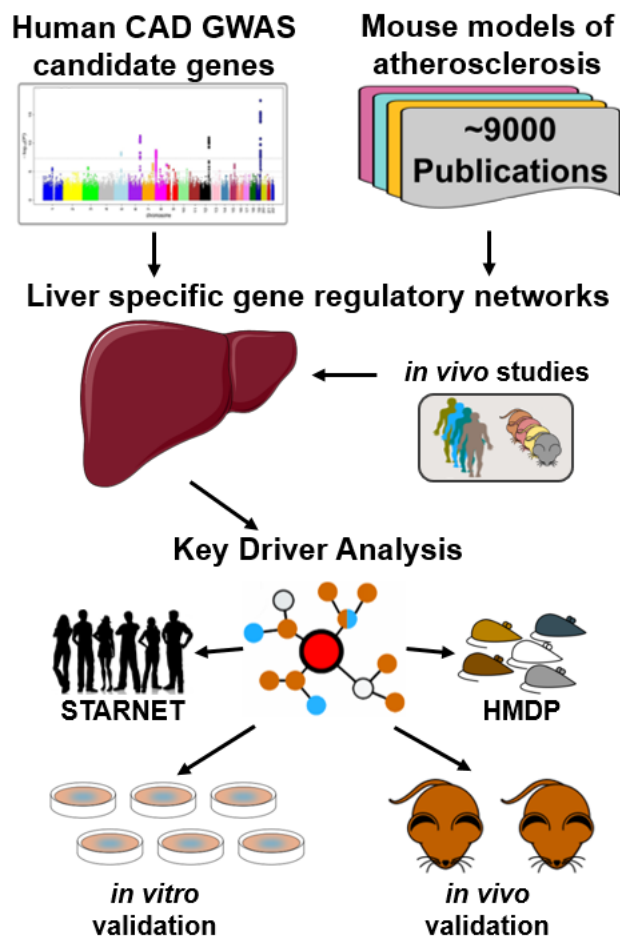
43 73. Castaneda S, Nurmohamed MT and Gonzalez-Gay MA. Cardiovascular disease in
44 inflammatory rheumatic diseases. *Best Pract Res Clin Rheumatol*. 2016;30:851-869.

45 74. Gonzalez-Gay MA, Gonzalez-Juanatey C and Martin J. Rheumatoid arthritis: a disease
46 associated with accelerated atherogenesis. *Semin Arthritis Rheum*. 2005;35:8-17.

47 75. Lopez-Mejias R, Castaneda S, Gonzalez-Juanatey C, Corrales A, Ferraz-Amaro I, Genre F,
48 Remuzgo-Martinez S, Rodriguez-Rodriguez L, Blanco R, Llorca J, Martin J and Gonzalez-Gay MA.
49 Cardiovascular risk assessment in patients with rheumatoid arthritis: The relevance of clinical,
50 genetic and serological markers. *Autoimmun Rev*. 2016;15:1013-1030.

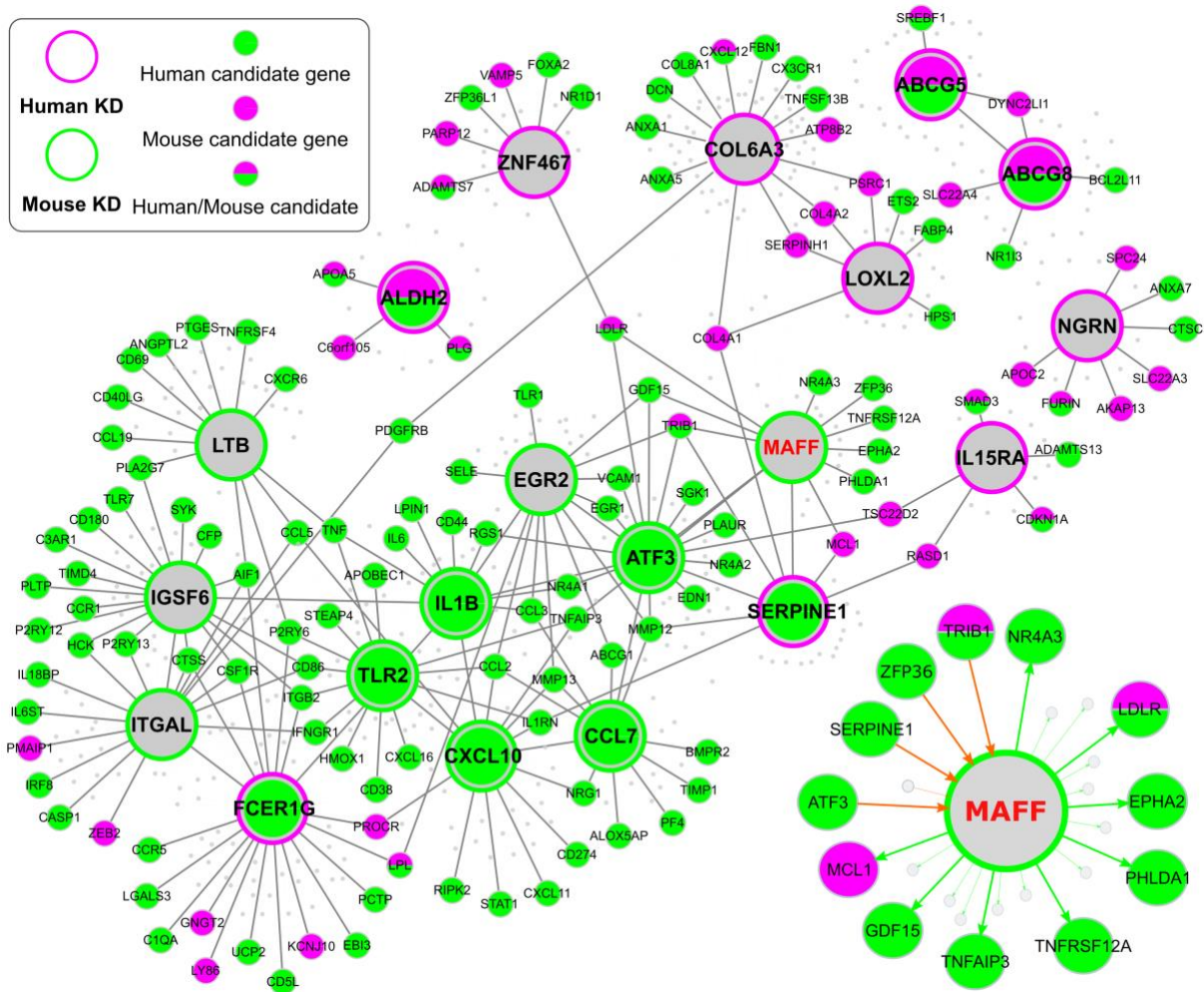
- 1 76. Schieir O, Tosevski C, Glazier RH, Hogg-Johnson S and Badley EM. Incident myocardial
2 infarction associated with major types of arthritis in the general population: a systematic review and
3 meta-analysis. *Ann Rheum Dis*. 2017;76:1396-1404.
- 4 77. Skeoch S and Bruce IN. Atherosclerosis in rheumatoid arthritis: is it all about inflammation?
5 *Nat Rev Rheumatol*. 2015;11:390-400.
- 6 78. Fernandez-Ortiz AM, Ortiz AM, Perez S, Toledano E, Abasolo L, Gonzalez-Gay MA, Castaneda
7 S and Gonzalez-Alvaro I. Effects of disease activity on lipoprotein levels in patients with early arthritis:
8 can oxidized LDL cholesterol explain the lipid paradox theory? *Arthritis Res Ther*. 2020;22:213.
- 9 79. Zhao Y, Chen J, Freudenberg JM, Meng Q, Rajpal DK and Yang X. Network-Based
10 Identification and Prioritization of Key Regulators of Coronary Artery Disease Loci. *Arterioscler*
11 *Thromb Vasc Biol*. 2016;36:928-41.
- 12 80. Katsuoka F and Yamamoto M. Small Maf proteins (MafF, MafG, MafK): History, structure and
13 function. *Gene*. 2016;586:197-205.
- 14 81. Massrieh W, Derjuga A, Doualla-Bell F, Ku CY, Sanborn BM and Blank V. Regulation of the
15 MAFF transcription factor by proinflammatory cytokines in myometrial cells. *Biol Reprod*.
16 2006;74:699-705.
- 17 82. Tan MK, Lim HJ, Bennett EJ, Shi Y and Harper JW. Parallel SCF adaptor capture proteomics
18 reveals a role for SCFFBXL17 in NRF2 activation via BACH1 repressor turnover. *Mol Cell*. 2013;52:9-
19 24.
- 20

1 **Figures**



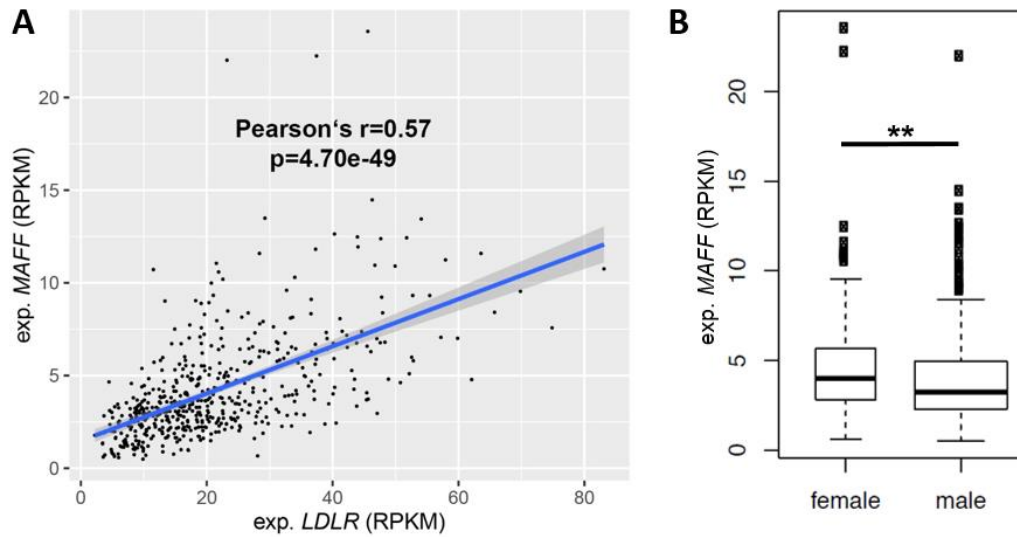
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3 **Figure 1.** Study workflow: Human and mouse atherosclerosis candidate genes were used to first model
4 liver specific regulatory networks and second decipher key driver genes of gene regulatory networks in
5 both species. Prediction of bioinformatics modeling was validated in human and mouse genetic studies
6 as well as in in vitro and in vivo experiments. CAD: Coronary artery disease; GWAS: Genome wide
7 association study.



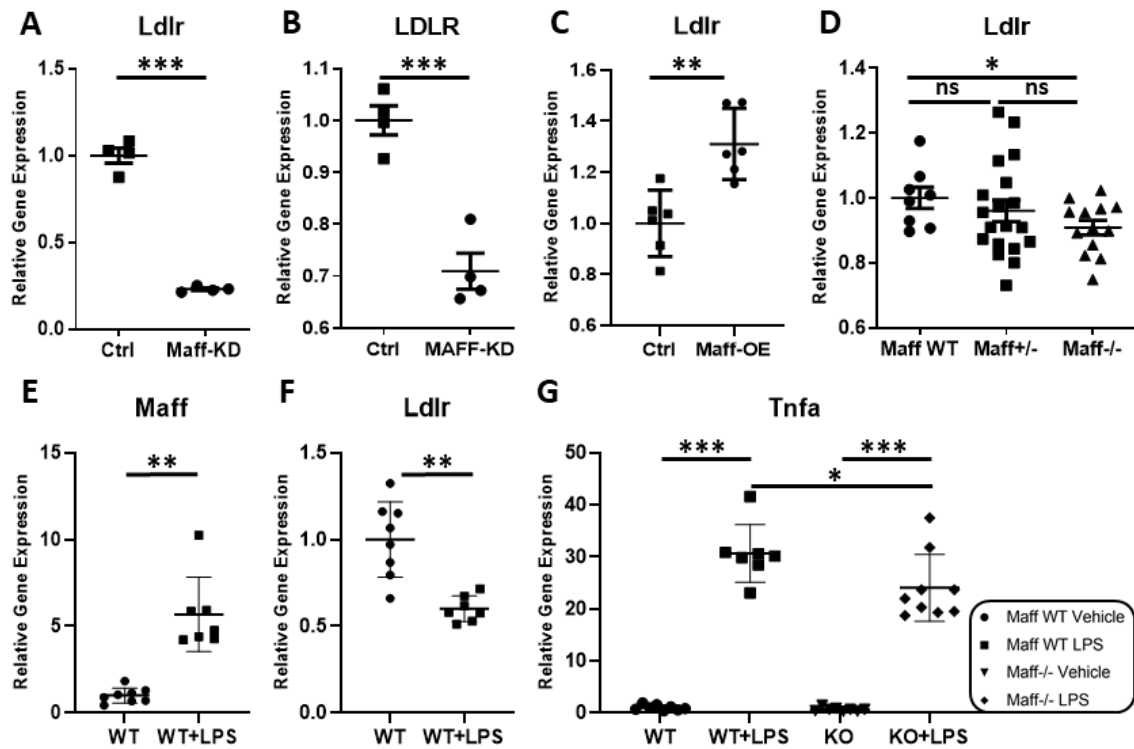
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2 **Figure 2.** Liver specific regulatory subnetworks and their key driver genes. The key driver analysis was
 3 performed on human and mouse networks respectively and the architecture of the illustrated network is
 4 based on both, mouse and human data. Key drivers are depicted as the largest nodes in the networks.
 5 All genes highlighted in solid green have already been studied to have a significant effect on
 6 atherosclerosis in genetically engineered mouse models. Human CAD GWAS candidate genes are
 7 highlighted in magenta. Key driver genes in grey need to be validated. Genes with both colors have an
 8 effect on atherosclerosis/CAD in human and mouse. Lower right: The MAFF network is the top ranked
 9 key driver gene network based on mouse data and closely connected to other human key driver
 10 subnetworks. Directionality between genes was based on the consensus of directional predictions from
 11 Bayesian networks constructed from different datasets, with the directionality predicted by the majority
 12 of studies shown. Red arrows indicate genes that are predicted to regulate MAFF, whereas green arrows
 13 indicate genes that are predicted to be regulated by the transcription factor MAFF. CAD: coronary artery
 14 disease; GWAS: Genome wide association study; Human KD: Human key driver gene; Mouse KD:
 15 Mouse key driver gene; MAFF: v-Maf avian musculoaponeurotic fibrosarcoma oncogene homolog F.



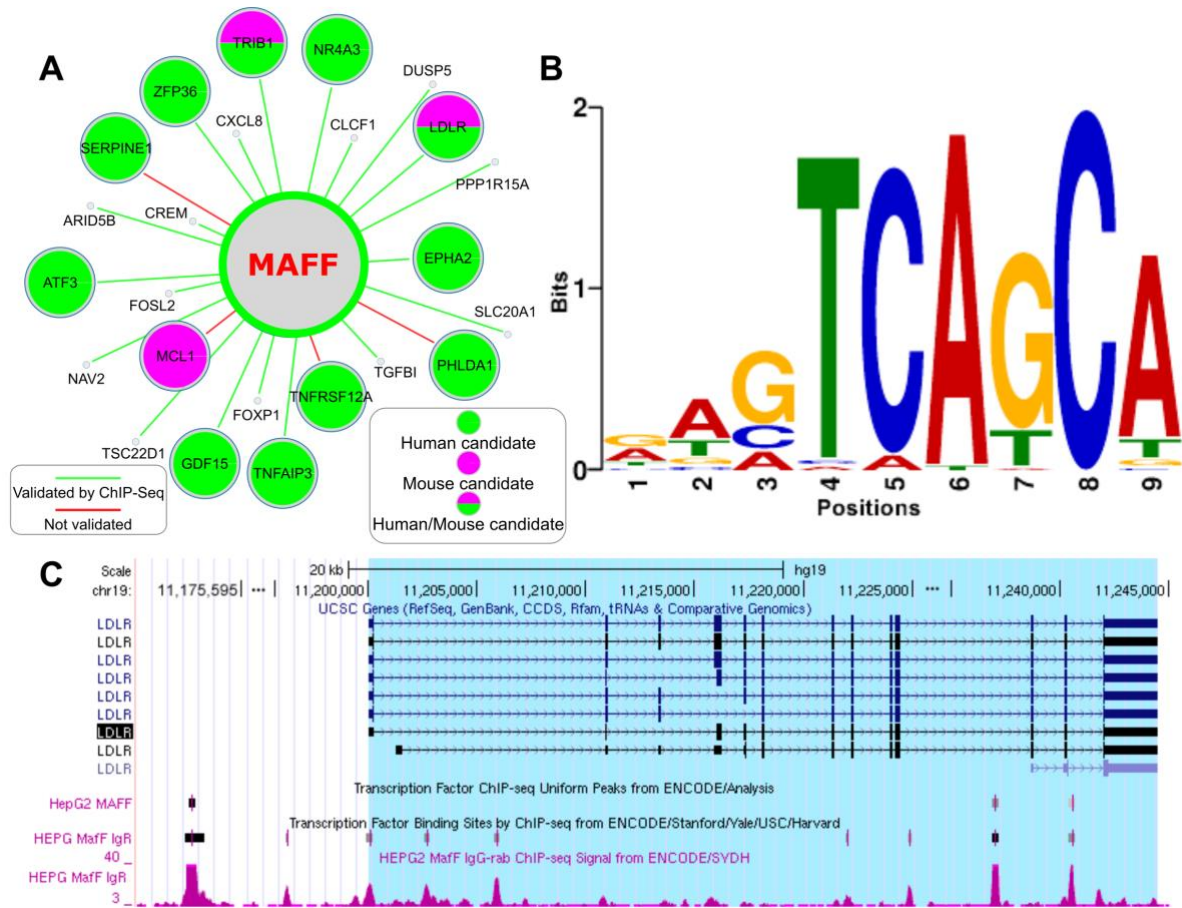
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2 **Figure 3.** Correlation of expression levels of MAFF in human liver samples from the Stockholm-Tartu
 3 Atherosclerosis Reverse Network Engineering Task (STARNET) with A: LDLR and B: Sex. **
 4 indicates $p<0.01$. LDLR: low-density lipoprotein receptor; MAFF: v-Maf avian musculoaponeurotic
 5 fibrosarcoma oncogene homolog F; RPKM: Reads per kilobase million.

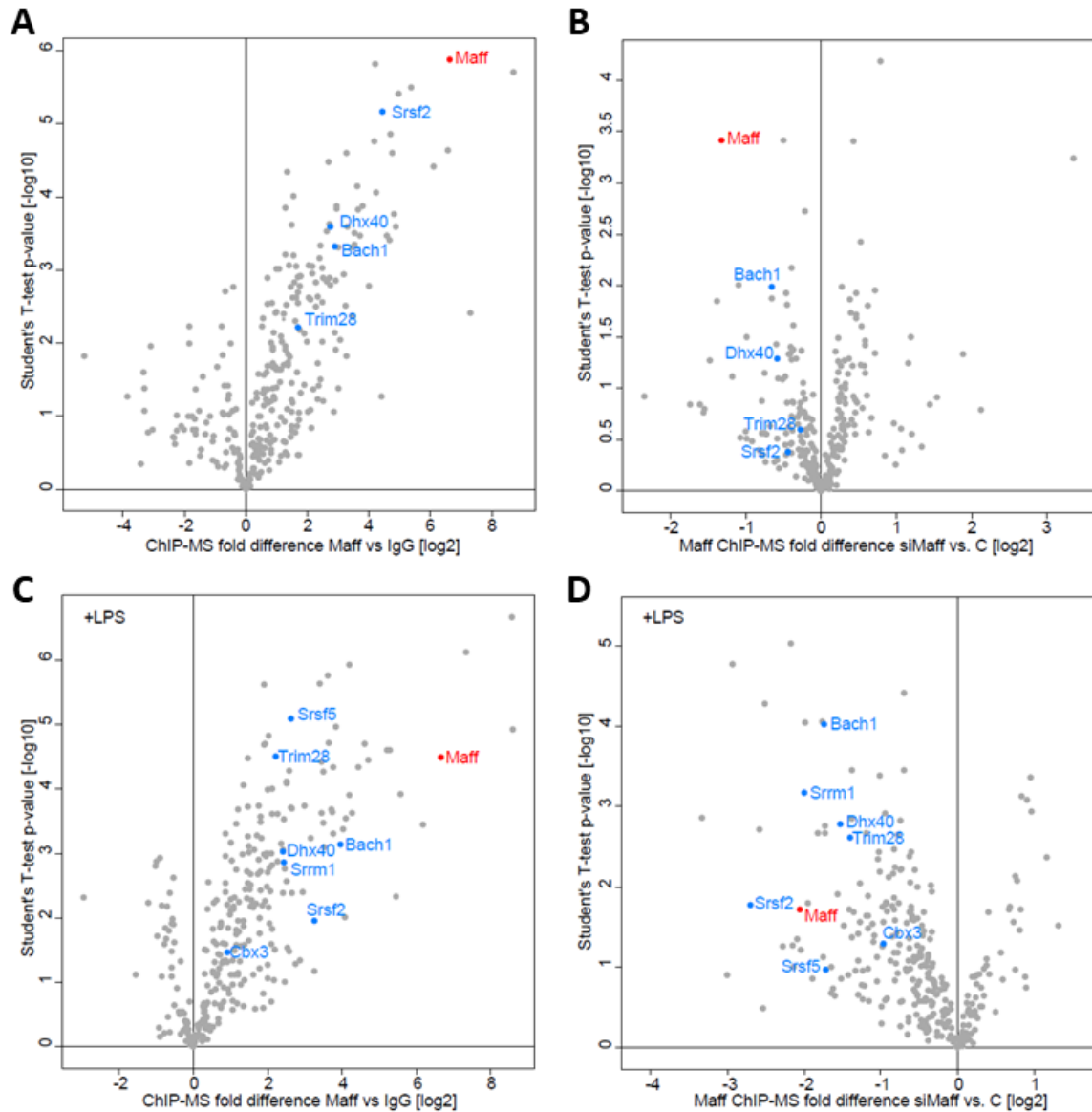


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2 **Figure 4.** A: In vitro results of *Ldlr* expression after siRNA-knockdown of *Maff* compared to controls
3 (vehicle) in mouse AML12 liver cells. B: In vitro results of *LDLR* expression after siRNA-knockdown
4 of *MAFF* compared to controls (vehicle) in human Hep3b liver cells. C: In vitro results of *Ldlr*
5 expression cells after *Maff* overexpression compared to controls (vehicle) in mouse AML12. D: In vivo
6 results of *Ldlr* expression in *Maff*^{-/-} mice compared to *Maff*^{+/-} and WT mice. E: In vivo results of *Maff*
7 expression in *Maff* WT mice 6 hours after LPS stimulation compared to controls (vehicle). F: In vivo
8 results of *Ldlr* expression in *Maff* WT mice 6 hours after LPS stimulation compared to controls (vehicle).
9 G: In vivo results of *Tnfa* expression in *Maff* WT and *Maff*^{-/-} mice 6 hours after LPS stimulation
10 compared to controls (vehicle). *** indicates $p < 0.001$, ** indicates $p < 0.01$, * indicates $p < 0.05$, ns
11 indicates non-significant. Bonferroni correction was applied for multiple comparison. Ctrl: control
12 group; KD: knockdown; LDLR: low-density lipoprotein receptor; LPS: lipopolysaccharide; MAFF: v-
13 Maf avian musculoaponeurotic fibrosarcoma oncogene homolog F; OE: overexpression; WT: wildtype.

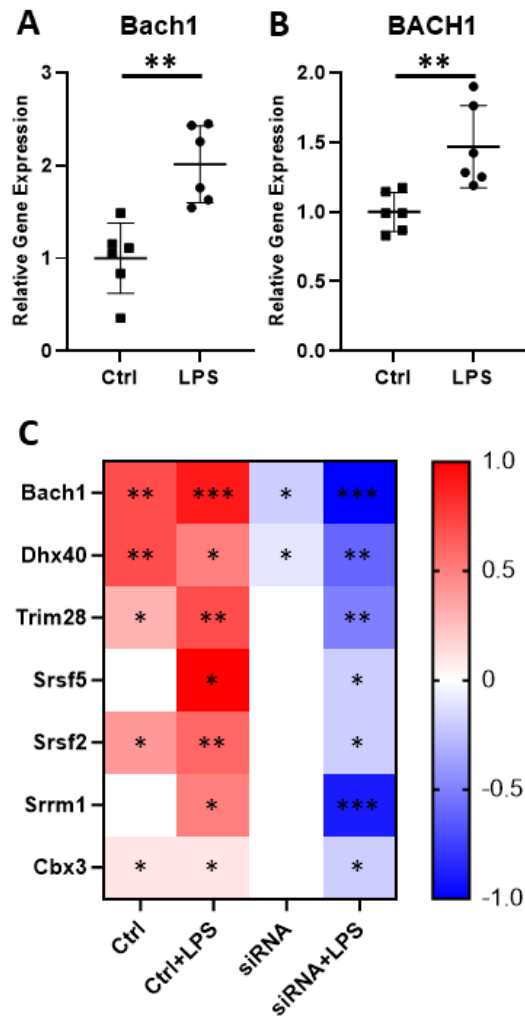


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 2 **Figure 5.** A: ChIP-seq data of human HepG2 cells supports potential binding of MAFF to genes in the
 3 MAFF subnetwork. Green edges indicate a binding motif was shared in the selected network genes,
 4 whereas red edges indicate that no known shared binding motif was found in the particular network
 5 genes. A matching binding motif was found in 20 out of 24 predicted interaction partners of MAFF. B:
 6 The matching motif among the MAFF network genes, which agrees with the previously known MAFF
 7 binding motif. The matching motif was identified using publically available ChIP-Seq data of the MAFF
 8 gene in human HepG2 cells from ENCODE. The height of the letter represents the frequency of the
 9 observed nucleotide in that position. C: Presence of the MAFF binding motif (small boxes below)
 10 upstream and within the LDLR gene (highlighted in blue).



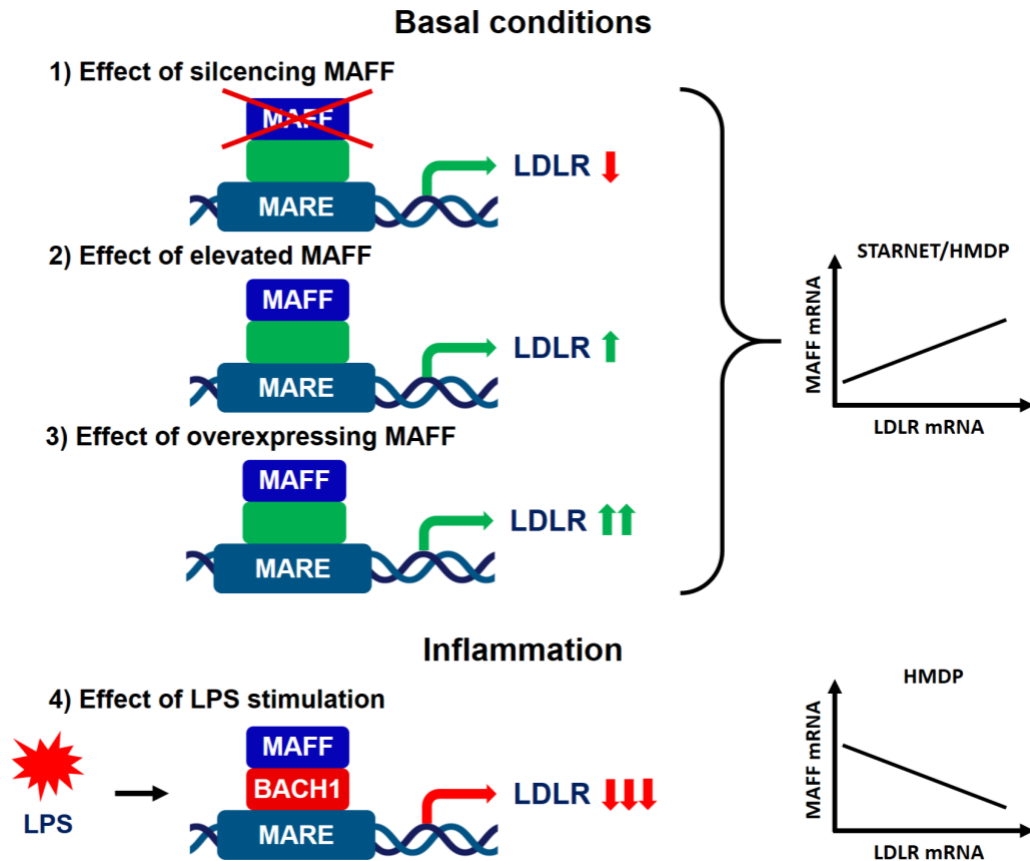
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2 **Figure 6.** Volcano plot of the p-values (y-axis) vs. the log₂ protein abundance differences (x-axis) of
 3 Maff binding partners in AML12 cells identified by ChIP-MS under (A) homeostatic conditions, (B)
 4 after Maff siRNA knockdown (which led to a 91% decrease on protein level), (C) LPS stimulation and
 5 (D) Maff siRNA knockdown in combination with LPS stimulation. Significant Maff interaction partners
 6 were highlighted in blue. Enrichment of binding partners is provided as fold difference compared to
 7 negative control (IgG) in panel A and C and compared to control (Maff WT) after siRNA knockdown
 8 in panel B and D. C: control (Maff WT); IgG: nonspecific IgG served as negative control.



1

2 **Figure 7.** LPS stimulation led to a significant increase of BACH1/Bach1 expression in mouse AML12
 3 cells (A) and human Hep3b cells (B) compared to controls (vehicle). (C) The heat map of z-scored Maff
 4 ChIP-MS visualises LFQ intensities of selected Maff interactors in extracts from AML12 cells under
 5 homeostatic conditions (Ctrl), LPS stimulation (Ctrl+LPS), after Maff siRNA knockdown (siRNA) and
 6 after Maff siRNA knockdown in combination with LPS stimulation (siRNA+LPS). Provided are
 7 adjusted p-values. *** indicates $p < 0.001$, ** indicates $p < 0.01$, * indicates $p < 0.05$.



1

2 **Figure 8.** The role of the transcription factor MAFF in activation or repression of the LDLR is based on
3 heterodimerisation partners and environmental conditions. MAFF heterodimers bind at the MAF
4 recognition element (MARE) of the LDLR promoter and execute regulation of the LDLR. Under basal
5 conditions 1) MAFF knockdown/knockout led to reduced LDLR expression, 2) elevated MAFF
6 expression was correlated with higher expression of LDLR in a human CAD cohort (STARNET) and
7 in wildtype mice of the hybrid mouse diversity panel (HMDP), 3) Overexpression of Maff using plasmid
8 DNA transfection led to increased Ldlr expression in vitro. In the presence of LPS stimulation MAFF-
9 BACH1 heterodimers result in downregulation of the LDLR in vivo. HMDP mice on atherogenic
10 background (transgenic expression of human APOE-Leiden and cholesteryl ester transfer protein
11 (CETP)) showed increased inflammation and revealed that elevated Maff expression correlates with
12 lower Ldlr expression. BACH1: BTB domain and CNC Homolog 1; MAFF: v-Maf avian
13 musculoaponeurotic fibrosarcoma oncogene homolog F; MARE: Maf recognition element; LDLR: low-
14 density lipoprotein receptor; LPS: lipopolysaccharide.

1 **Table 1.** Listed are the top 10 key driver genes detected in human and mouse liver networks based on
 2 the bioinformatics approach. Several genes have already been studied and confirmed with regard to
 3 atherosclerosis/CAD. FDR: False discovery rate.

4

Species	Key Driver Gene	Studied effect on atherosclerosis	Network size (Genes)	Atherosclerosis associated genes	FDR	Fold Enrichment
Mouse	<i>Maff</i>	no	25	11	1.50E-05	19.08
	<i>Il1b</i>	yes	27	11	1.50E-05	19.08
	<i>Ccl7</i>	yes	34	12	1.17E-05	16.53
	<i>Atf3</i>	yes	50	15	3.89E-06	14.05
	<i>Cxcl10</i>	yes	46	13	1.26E-05	13.23
	<i>Egr2</i>	no	50	14	7.79E-06	13.11
	<i>Igsf6</i>	no	66	18	1.49E-06	12.77
	<i>Ltb</i>	no	44	12	2.45E-05	12.77
	<i>Itgal</i>	no	52	14	8.81E-06	12.61
	<i>Tlr2</i>	yes	57	15	5.90E-06	12.32
Human	<i>ALDH2</i>	yes	19	4	1.10E-04	31.29
	<i>IL15RA</i>	no	20	4	1.50E-04	29.72
	<i>ZNF467</i>	no	22	4	2.70E-04	27.02
	<i>NGRN</i>	no	32	5	1.87E-07	23.22
	<i>LOXL2</i>	no	28	4	1.15E-03	21.23
	<i>ABCG8</i>	yes	30	4	1.73E-03	19.82
	<i>ABCG5</i>	yes	33	4	3.00E-03	18.01
	<i>SERPINE1</i>	no	34	4	3.55E-03	17.48
	<i>COL6A3</i>	no	69	6	1.22E-07	12.92
	<i>FCER1G</i>	no	81	5	3.16E-03	9.17

1 **Supplemental Materials Appendix**

2 **Supplemental Table 1.** List of 244 human CAD candidate genes based on annotations of 169 known
3 significant and suggestive human CAD GWAS loci.

4 **Supplemental Table 2.** List of 827 mouse atherosclerosis genes as defined by a significant effect on
5 atherosclerotic lesion size or composition when perturbed in a mouse model.

6 **Supplemental Table 3.** Correlation analysis of network genes in HMDP and STARNET based on
7 Pearson correlation coefficient.

8 **Supplemental Figure 1.** Comparison of pair-wise correlations among all *Maff* network genes according
9 to their expression values in three HMDP populations. Large and bright circles represent strong
10 correlations. A: Mice on chow diet. B: Mice on high-fat diet. C: Mice on high-fat diet with transgenic
11 implementation of human *APOE-Leiden* and *CETP*.

12 **Supplemental Figure 2.** A: In vitro results of MAFF neighbour gene expression after siRNA
13 knockdown of *MAFF* in human Hep3b liver cells and B: In vitro results of *Maff* neighbour gene
14 expression after siRNA knockdown of *Maff* in mouse AML12 liver cells. * indicates $p < 0.05$, **
15 indicates $p < 0.01$, *** indicates $p < 0.001$. Ctrl: control group; KD: knockdown; LDLR: low-density
16 lipoprotein receptor; MAFF: v-Maf avian musculoaponeurotic fibrosarcoma oncogene homolog F.

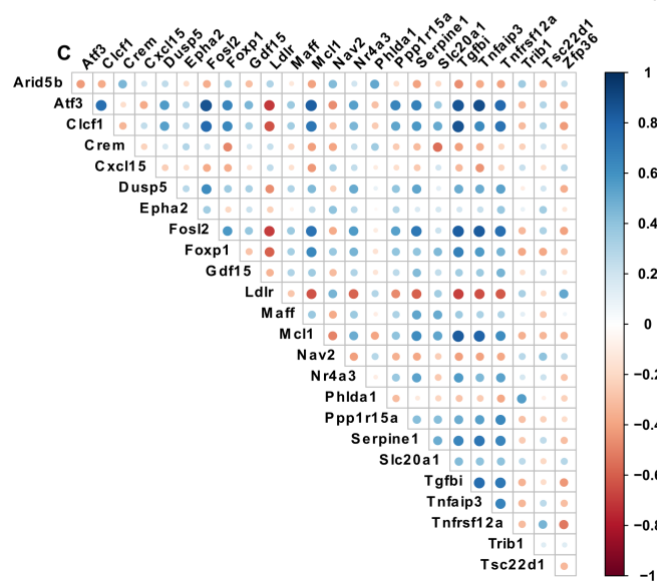
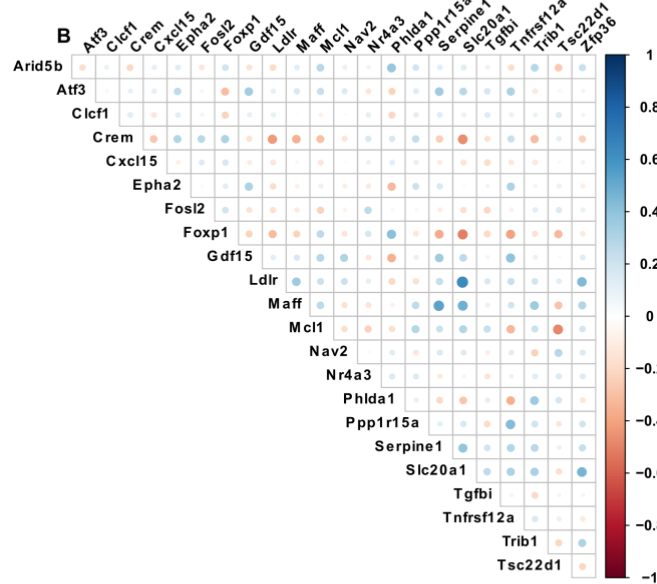
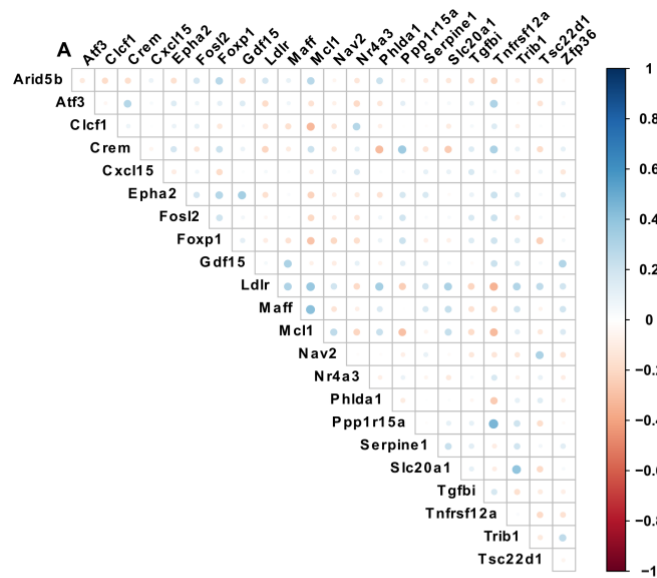
17 **Supplemental Figure 3.** Three-dimensional binding of the heterodimerised transcription factor *MAFF*
18 (blue) and the transcriptional repressor *BACH1* (red) at the MARE of the *LDLR* promoter in the presence
19 of LPS stimulation. BACH1: BTB domain and CNC Homolog 1; MAFF: v-Maf avian
20 musculoaponeurotic fibrosarcoma oncogene homolog F; MARE: Maf recognition element; LDLR: low-
21 density lipoprotein receptor; LPS: lipopolysaccharide.

1 **Supplemental Table 3.** Correlation between *Maff/MAFF* and its network genes under different conditions. HMDP mice on chow diet, high-fat diet and with
 2 transgenic implementation of human *APOE-Leiden* and *CETP* (left). On the right, human data from the STARNET cohort.

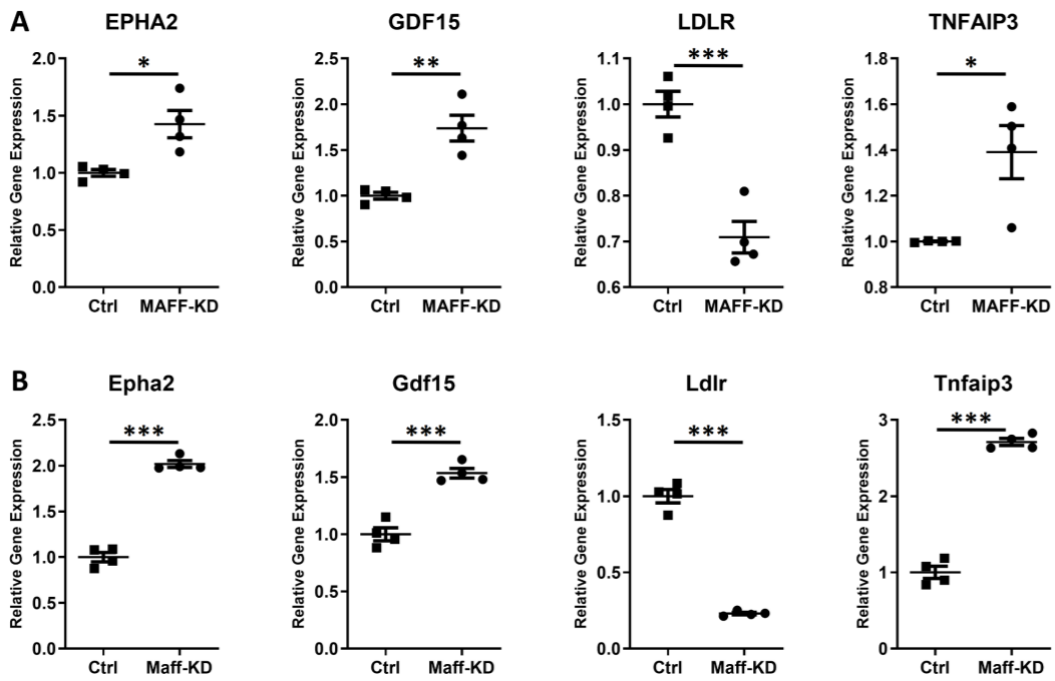
Gene	Mouse data (HMDP)										Human data		Gene
	Chow - both sexes		High-fat - female		High-fat - male		Transgenic - female		Transgenic - male		STARNET - both sexes		
	Pearson's R	p-value	Pearson's R	p-value	Pearson's R	p-value	Pearson's R	p-value	Pearson's R	p-value	Pearson's R	p-value	
<i>Arid5b</i>	0.10	1.30E-01	0.08	2.31E-01	0.13	5.87E-02	-0.12	7.33E-02	0.09	3.79E-01	0.04	3.05E-01	<i>ARID5B</i>
<i>Atf3</i>	0.06	3.24E-01	0.17	1.70E-02	0.09	1.78E-01	0.26	7.31E-05	0.37	1.58E-04	0.66	2.26E-68	<i>ATF3</i>
<i>Clefl</i>	-0.17	6.67E-03	0.01	8.50E-01	-0.01	8.39E-01	0.26	7.66E-05	0.34	4.35E-04	0.43	1.54E-26	<i>CLCF1</i>
<i>Crem</i>	-0.10	1.01E-01	-0.27	1.07E-04	-0.35	4.89E-08	-0.22	8.67E-04	-0.21	3.29E-02	0.35	2.97E-17	<i>CREM</i>
<i>Dusp5</i>	n/a	n/a	n/a	n/a	n/a	n/a	0.09	2.00E-01	0.31	1.57E-03	0.62	5.74E-59	<i>DUSP5</i>
<i>Epha2</i>	0.03	6.44E-01	-0.08	2.33E-01	-0.08	2.23E-01	-0.06	3.89E-01	-0.07	4.92E-01	0.62	2.99E-59	<i>EPHA2</i>
<i>Fosl2</i>	0.01	8.68E-01	0.00	9.51E-01	-0.11	8.88E-02	0.30	5.72E-06	0.37	1.36E-04	0.54	3.54E-42	<i>FOSL2</i>
<i>Foxp1</i>	-0.15	1.56E-02	-0.07	3.05E-01	-0.23	5.95E-04	0.26	7.38E-05	0.33	6.36E-04	-0.03	4.43E-01	<i>FOXP1</i>
<i>Gdf15</i>	0.31	4.14E-07	0.17	1.74E-02	0.16	1.46E-02	0.09	1.91E-01	0.31	1.54E-03	0.57	2.88E-48	<i>GDF15</i>
<i>Ldlr</i>	0.30	9.88E-07	0.35	1.78E-07	0.30	2.90E-06	-0.27	4.65E-05	-0.19	4.98E-02	0.57	4.70E-49	<i>LDLR</i>
<i>Mcl1</i>	0.42	5.30E-12	0.27	9.79E-05	0.25	1.62E-04	0.37	1.35E-08	0.35	3.82E-04	0.47	4.86E-31	<i>MCL1</i>
<i>Nav2</i>	-0.14	2.50E-02	-0.16	2.16E-02	0.03	7.06E-01	-0.38	9.23E-09	-0.26	7.24E-03	0.31	2.03E-13	<i>NAV2</i>
<i>Nr4a3</i>	-0.08	2.00E-01	-0.06	3.61E-01	-0.14	3.38E-02	0.22	1.10E-03	0.37	1.44E-04	0.35	5.81E-17	<i>NR4A3</i>
<i>Phlda1</i>	0.16	1.21E-02	-0.07	3.07E-01	0.00	9.53E-01	-0.09	1.83E-01	-0.03	7.83E-01	0.48	8.69E-33	<i>PHLDA1</i>
<i>Ppp1r15a</i>	0.00	9.70E-01	0.10	1.56E-01	0.26	7.86E-05	0.32	1.91E-06	0.28	4.25E-03	0.67	2.05E-72	<i>PPP1R15A</i>
<i>Serpine1</i>	0.17	7.92E-03	0.42	2.01E-10	0.54	1.40E-18	0.53	4.63E-17	0.37	1.57E-04	0.43	1.54E-26	<i>SERPINE1</i>
<i>Slc20a1</i>	0.23	1.73E-04	0.40	3.28E-09	0.49	5.78E-15	0.50	1.44E-15	0.24	1.59E-02	0.41	2.66E-23	<i>SLC20A1</i>
<i>Tgfb1</i>	-0.16	1.27E-02	0.02	7.54E-01	0.09	1.55E-01	0.31	3.97E-06	0.35	2.54E-04	0.31	1.22E-13	<i>TGFBI</i>
<i>Tnfaip3</i>	n/a	n/a	n/a	n/a	n/a	n/a	0.32	1.81E-06	0.27	6.00E-03	0.46	6.02E-30	<i>TNFAIP3</i>
<i>Tnfrsf12a</i>	-0.18	3.11E-03	0.13	5.55E-02	0.19	3.79E-03	0.30	4.52E-06	0.27	6.26E-03	0.62	5.74E-59	<i>TNFRSF12A</i>
<i>Trib1</i>	0.20	1.50E-03	0.31	5.93E-06	0.37	1.03E-08	0.11	8.98E-02	0.08	4.13E-01	0.4	3.49E-22	<i>TRIB1</i>
<i>Tsc22d1</i>	-0.06	3.35E-01	-0.29	2.90E-05	-0.11	9.25E-02	-0.18	6.88E-03	-0.26	7.57E-03	0.48	8.35E-33	<i>TSC22D1</i>
<i>Zfp36</i>	0.20	1.75E-03	0.30	1.40E-05	0.18	5.70E-03	0.05	4.19E-01	0.07	4.85E-01	0.6	3.50E-54	<i>ZFP36</i>

3

1 Supplemental Figure 1.

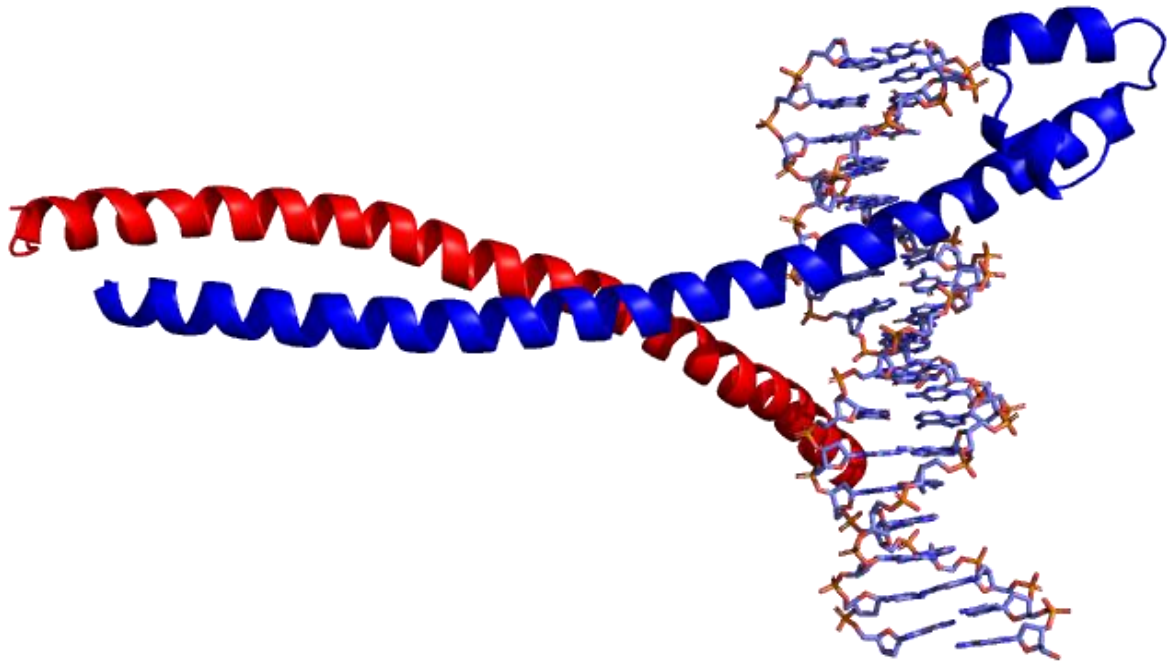


1 Supplemental Figure 2.



2

1 Supplemental Figure 3.



2