Supplementary Table 1 - Characteristics of coronary artery disease cases and controls in UK Biobank

	Cases	Controls
N Individuals	4831	115,455
Age ± SD, years	62.1 ± 5.9	56.7 ± 7.9
Male, n (%)	3908 (80%)	53,028 (45.9%)
Lipid Lowering Therapy, n (%)	3998 (82.8%)	18,482 (16.0%)
Ever Smoker, n (%)	2528 (52.3%)	52,629 (45.6%)
Hypertension, n (%)	3373 (69.8%)	22,809 (19.6%)
Diabetes Mellitus, n (%)	880 (18.2%)	5524 (4.8%)
Body Mass Index ± SD, kg/m^2	29.3 ± 4.8	27.5 ± 4.8

Supplementary Table 2 - Findings from Stage 1 UK Biobank CAD GWAS

UK Biobank Stage 1 Analysis - Genome Wide Significant Loci								
SNP	**Chr**	**Gene**	**Description**	**EA**	**EAF**	**OR**	**95%CI**	**P**
rs646776	1	*(*1p13*/SORT1)*	downstream	T	0.78	1.17	1.11-1.23	1.3×10^{-8}
rs9349379	6	*PHACTR1*	intronic	G	0.41	1.15	1.11-1.20	3.4×10^{-11}
rs140570886	6	*LPA*	intronic	C	0.02	1.92	1.68-2.20	2.2×10^{-21}
rs4977575	9	*(*9p21*/CDKN2B-AS1)*	intergenic	G	0.49	1.24	1.19-1.29	5.4×10^{-23}
rs28451064	21	*(KCNE2)* Gene Desert	intergenic	A	0.13	1.18	1.11-1.25	2.1×10^{-8}

* Genes for SNPs that are outside the transcript boundary of the protein-coding gene are shown in parentheses [eg, (*KCNE2*)].
Chr = Chromosome, EA = Effect Allele, EAF = Effect Allele Frequency, OR = Odds Ratio, CI = Confidence Interval

Supplementary Table 7 - Definitions of diseases/traits for PheWAS in 112,338 individuals of European ancestry from UK Biobank

Phenotype	Definition	Sample Size	Covariates
Waist Hip Ratio Adj BMI	Waist-to-hip ratio measurement at enrollment was quantile-normalized separately in males and females, and then combined	112,159	Age, Body Mass Index, Sex, Principal Components, Genotyping Chip
Body Fat Percentage	Body fat percentage as measured by an impedance device for body composition at enrollment was quantile-normalized separately in males and females, and then combined	110,365	Age, Body Mass Index, Sex, Principal Components, Genotyping Chip
Systolic BP	Automated systolic BP measurement at enrollment	104,611	Age, Age^2, Body Mass Index, Sex, Principal Components, Genotyping Chip
Diastolic BP	Automated diastolic BP measurement at enrollment	104,610	Age, Age^2, Body Mass Index, Sex, Principal Components, Genotyping Chip
Peripheral Vascular Disease	History of peripheral vascular disease or intermittent claudication during verbal interview or hospitalization for ICD code I731, I738, I739, I743, I744, I745	692	Age, Sex, Principal Components, Genotyping Chip
Gout	History of gout during verbal interview	1612	Age, Sex, Principal Components, Genotyping Chip
Migraine	History of migraine during verbal interview	3161	Age, Sex, Principal Components, Genotyping Chip
COPD	History of chronic obstructive airway disease, emphysema/chronic bronchitis or emphysema during verbal interview	2363	Age, Sex, Principal Components, Genotyping Chip
Lung Cancer	History of lung cancer, small cell lung cancer or non-small cell lung cancer during verbal interview	115	Age, Sex, Principal Components, Genotyping Chip
Breast Cancer	History of breast cancer during verbal interview	2382	Age, Sex, Principal Components, Genotyping Chip
Colorectal Cancer	History of large bowel cancer/colorectal cancer, colon cancer/sigmoid cancer or rectal cancer during verbal interview	616	Age, Sex, Principal Components, Genotyping Chip
Any Cancer	History of any cancer during verbal interview	9530	Age, Sex, Principal Components, Genotyping Chip

Abbreviations: Adj, adjusted; COPD, chronic obstructive pulmonary disease; ICD, international classification of disease; BP, blood pressure

Supplementary Table 8 - Characteristics of publicly available GWAS included in phenome-wide association study

Consortium	Outcome/Trait (Units)	Sample Size	Genotyping
GLGC[1]	LDL cholesterol (SD) HDL cholesterol (SD) Total cholesterol (SD) Triglycerides (SD)	Up to 188,587 individuals	37 studies using metabochip, 23 studies using various arrays
MAGIC[2]	Fasting Insulin Adjusted for BMI (SD)	Up to 96,496 individuals	Various arrays, imputation to 2.5 million SNPs using HapMap reference panel
MAGIC[3]	Insulin Secretion (SD)	Up to 5,318 individuals	Various Arrays imputation to 2.4 million SNPs using HapMap reference panel
GIANT[4]	Height (SD)	Up to 253,288 individuals	Various arrays, imputation to 2.5 million SNPs using HapMap reference panel
GIANT[5]	Body Mass Index (OR)	Up to 263,407 individuals total, focusing on the upper 5th percentile (cases) and lower 5th percentile (controls) of BMI the distribution	Various arrays, imputation to 2.8 million SNPs
CKDGen[6]	Cystatin C/Creatinine Serum estimated Glomerular Filtration Rate (mL/min/1.73m2)	Up to 133,413 individuals	Various arrays, imputation to 2.5 million SNPs using HapMap reference panel
IIBDGC[7]	Inflammatory Bowel Disease (OR)	Up to 38,155 cases and 48,485 controls of European Ancestry	Various arrays, imputation to 9 million SNPs using 1000 Genomes reference panel
ADIPOGen[8]	Adiponectin (SD)	Up to 39,883 individuals of European Ancestry	Various arrays, imputation to 2.7 million SNPs using HapMap reference panel
DIAGRAM[9]	Type 2 Diabetes (OR)	Meta-analysis of up to 34,840 cases and 114,981 controls in individuals of primarily European Ancestry	Various arrays, imputation to 2.5 million SNPs using HapMap reference panel

DIAGRAM, DIAbetes Genetics Replication And Meta-analysis; GIANT, Genetic Investigation of ANthropometric Traits; GLGC, Global Lipids Genetics Consortium; MAGIC, Meta-Analyses of Glucose and Insulin-related traits Consortium; CKDGen, Chronic Kidney Disease Genetics Consortium; IIBDGC, International Inflammatory Bowel Disease Genetics Consortium; SNPs, single nucleotide polymorphism; LDL cholesterol, low-density lipoprotein cholesterol; HDL cholesterol, high-density lipoprotein cholesterol; SD, standard deviation; BMI, body mass index; OR, odds ratio

Supplementary Note

Functional characterization of ARHGEF26(Val29Leu) missense variant

Cell culture

Human Aortic Endothelial Cells (HAEC), Human Umbilical Vein Endothelial Cells (HUVEC), and Human Coronary Artery Smooth Muscle Cells (HCASMC) were purchased from Lifeline Cell Technology and maintained in VascuLife EnGS Endothelial Medium and SMC Medium (Lifeline Cell Technology) free of antibiotics at 37°C and 5% CO_2. HAEC, HUVEC, and HCASMC at passages 2-6 were used for experiments. HL60 cell line was purchased from Sigma-Aldrich. HEK293 and THP-1 cell lines were purchased from ATCC. HEK293 was maintained in high-glucose Dulbecco's Modified Eagle Medium with GlutaMA Supplement and 10% fetal bovine serum (Thermo Fisher Scientific). HL60 and THP-1 cells were maintained in RPMI 1640 Medium supplemented with 10% non-heated-inactivated fetal bovine serum (Thermo Fisher Scientific). HL60 cells were differentiated for 5 days in medium containing 1.3% DMSO for leukocyte TEM assays. Cell line specificity was confirmed with tissue-specific markers: HAEC were von Willebrand Factor positive and smooth muscle a-actin negative, HCASMC were von Willebrand Factor negative and smooth muscle a-actin positive. Both cell types were confirmed to be mycoplasma negative.

siRNA and *ARHGEF26* constructs

Silencer Select siRNA against 3'UTR of human *ARHGEF26* was customized from Thermo Fisher Scientific. Targeting efficiency of siRNA was confirmed by western blot of transfected cells. Non-targeting siRNA control was purchased from Thermo Fisher Scientific. The cDNA containing the complete open-reading frame of human *ARHGEF26* (NM_015595.3) was obtained from the Mammalian Gene Collection (MGC) and cloned with an N-terminal FLAG-GGGS sequence onto a pcDNA3.4 mammalian expression vector (Thermo Fisher Scientific) using NEBuilder HiFi DNA Assembly Master Mix (NEB). Wild-type ARHGEF26 and 29Leu mutant

was generated by site-directed mutagenesis (Q5 kit, NEB) and sanger-sequenced. Vector without FLAG-GGGS-ARHGEF26 insert is used as control vector.

Transfection

HAEC and HCASMC were transfected in 6-well format using Lipofectamine 2000 Transfection Reagent (Invitrogen) following manufacture's protocol. Briefly, cells were plated at 90% confluency the day prior to transfection. Then cells were washed and replenished with Opti-MEM I Reduced Serum Medium. Per well, cells were co-transfected with 50nM siRNA with 1ug/mL *ARHGEF26* vector (final concentration). Medium was replaced at 4 hours post-transfection. Cells were trypsinized and re-plated one-day after transfection (HAEC), or re-plated and starved in serum-free medium (HCASMC).

Leukocyte TEM assay

Leukocyte TEM assay was modified from previously described[10]. HAEC was plated on a HTS Transwell 96-well permeable insert with 5.0 μm pore size (Corning) in 40 μL/well medium and allowed to settle for 8 hours. Then the transwell was replaced with complete medium contain 10 ng/mL TNF-α (PeproTech) and cultured overnight. The next day, 235 μL/well serum-free endothelial cell medium containing 0.25% BSA with vehicle or 50ng/mL SDF-1 (PeproTech) was placed on a 96-well white receiver plate. The medium in the transwell insert was removed and replaced with 75 μL/well serum-free endothelial cell medium containing 0.25% BSA and 200,000 differentiated HL60 cells. The insert was then gently placed in the receiver plate and incubated at 37°C for 5 hours with lid on. The insert was removed and HL60 migrated into the receiver plate was quantified with a luminescent assay (CellTiter-Glo, Promega). Standard curve of HL60 cells was prepared by serial dilutions on an identical white receiver plate, with total HL60 cell input set as 100%. Differences in means of percentage of migrated cells per well were assessed by two-way ANOVA with uncorrected Fisher's LSD test within vehicle and SDF-1 subgroups, respectively, and significance threshold set as $P < 0.05$.

Leukocyte adhesion assay

HAEC were transfected and re-plated on a black-wall, clear-bottom 96-well plate and cultured until 100% confluence (48-72-hour post-transfection). Prior to the assay, HAEC were treated with 10 ng/mL TNF-α overnight. THP-1 cells were labeled with Calcein-AM cell-permeant dye (Thermo Fisher Scientific), washed, and added to wells containing HAEC at 200,000 /well in serum-free medium containing 0.25% BSA, and incubated at 37°C for 1 hour. The wells were washed four times in 37°C PBS. After the final wash, the plate was drained thoroughly and 100 μL TBS buffer containing 1% NP-40 was added to each well. The plate was agitated for 10 min protected from light, and the fluorescence was measured on a plate reader. Standard curve was generated on an identical, separate plate. Differences in means of fluorescent intensity were assessed by one-way ANOVA with Dunnett's multiple comparisons test, and a multiplicity adjusted P value set as 0.05 for statistical significance.

VSMC proliferation

HCASMC were transfected and re-plated on a 96-well plate in serum-free medium and starved. After 48 hours, the plate was replaced with medium containing serum and cells are allowed to proliferate for 72 hours. To measure cell proliferation, the medium was removed and cell numbers in each well were counted with a luminescent assay (CellTiter-Glo, Promega). Differences in means of luminescence were assessed by one-way ANOVA with Dunnett's multiple comparisons test, and a multiplicity adjusted P value set as 0.05 for statistical significance.

Western blot

Cells were harvested with lysis buffer (150mM NaCl, 50mM Tris HCl, 0.5 % NP-40 and 0.1% sodium deoxycholate, pH 7.5) supplemented with fresh protease inhibitors (Pierce Protease Inhibitor Mini Tablet, EDTA free). Cell lysate was incubated for 15min in rotation and centrifuged at 20,000 g for 15min at 4°C to remove insoluble materials. The protein concentration in the supernatant was measured by a bicinchoninic acid (BCA) assay kit (Thermo Fisher

Scientific) and normalized with Laemmli sample buffer. Equal amount of protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 4-20% Mini-PROTEAN TGX precast gels (Bio-Rad Laboratories), transferred to nitrocellulose membrane, and blocked with 5% non-fat milk in Tris-buffered saline supplemented with 0.05% Tween-20 (TBST) at room temperature for 1 hour. The membrane was then probed with primary antibodies to ARHGEF26 (Sigma-Aldrich), FLAG (M2 HRP-conjugated, Sigma-Aldrich), or actin (HRP-conjugated, Santa Cruz Biotechnology), respectively, in 1% non-fat milk in TBST. The HRP-conjugated anti-rabbit secondary antibody was then incubated at room temperature for 1 hour for ARHGEF26 blots. After extensive washing, the membranes were imaged by an enhanced chemiluminescence substrate (EMD Millipore) and imaged on Amersham Imager 600 (GE Healthcare).

Cycloheximide chase assay

FLAG-tagged WT or 29Leu FLAG-ARHGEF26 was overexpressed in HEK293 cells for 48 hours. One day prior to the cycloheximide chase, WT and 29Leu ARHGEF26-transfected cells (12 wells each) were plated on the same 24-well plate at 150,000 cells per well in 500µL medium. For the cycloheximide chase, 500µL medium containing 100 µg/mL or 200 µg/mL cycloheximide (Enzo Life Sciences) was added to each well to achieve 50 µg/mL or 100 µg/mL final concentration. Cells were harvested in lysis buffer at indicated time points post chase, and BCA-normalized lysate (20 µg/time points) were probed for FLAG by Western blot. For each cycloheximide dose, 2 blot sections (WT and 29Leu) from the same treated plate were blotted on same membrane and simultaneously imaged.

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