

CXCL12 drives natural variation in coronary artery anatomy across diverse populations

SUPPLEMENTARY FIGURES AND TEXT

Pamela E. Rios Coronado^{1†}, Daniela Zanetti^{2,3,4†}, Jiayan Zhou^{3,2†}, Jeffrey A. Naftaly¹, Pratima Prabala¹, Azalia M. Martínez Jaimes^{5,1}, Elie N. Farah⁶, Xiaochen Fan¹, Soumya Kundu^{7,8}, Salil S. Deshpande⁹, Ivy Evergreen⁷, Pik Fang Kho^{2,3}, Austin T. Hilliard³, Sarah Abramowitz^{10,11,12}, Saiju Pyarajan¹³, Daniel Dochtermann¹³, Million Veteran Program¹⁴, Scott M. Damrauer^{15,16,17}, Kyong-Mi Chang^{15,18}, Michael G. Levin^{10,15}, Virginia D. Winn¹⁹, Anca M. Pașca²⁰, Mary E. Plomondon^{21,22}, Stephen W. Waldo^{21,22,23}, Philip S. Tsao^{3,24,25}, Anshul Kundaje^{7,8}, Neil C. Chi⁶, Shoa L. Clarke^{2,3,26‡}, Kristy Red-Horse^{1,27,28*‡}, Themistocles L. Assimes^{2,3,25,29*‡}

¹Department of Biology, Stanford University; Stanford, CA, USA

²Department of Medicine, Division of Cardiovascular Medicine, Stanford University School of Medicine; Stanford, CA, USA

³VA Palo Alto Health Care System; Palo Alto, CA, USA

⁴Institute of Genetic and Biomedical Research, National Research Council; Cagliari, Sardinia, Italy

⁵Department of Developmental Biology, Stanford University School of Medicine; Stanford, CA, USA

⁶Department of Medicine, Division of Cardiology, University of California San Diego; La Jolla, CA, USA

⁷Department of Genetics, Stanford University School of Medicine; Stanford, CA, USA

⁸Department of Computer Science, Stanford University; Stanford, CA, USA

⁹Institute for Computational and Mathematical Engineering, Stanford University School of Medicine; Stanford, CA, USA

¹⁰Department of Medicine, Division of Cardiovascular Medicine, University of Pennsylvania Perelman School of Medicine; Philadelphia, PA, USA

¹¹Sarnoff Cardiovascular Research Foundation; McLean, VA, USA

¹²Donald and Barbara Zucker School of Medicine at Hofstra/Northwell; Hempstead, NY, USA

¹³Center for Data and Computational Sciences, VA Boston Healthcare System; Boston, MA, USA

¹⁴A list of consortium members can be found in the Supplementary File

¹⁵Corporal Michael J. Crescenz VA Medical Center; Philadelphia, PA, USA

¹⁶Department of Surgery, University of Pennsylvania Perelman School of Medicine; Philadelphia, PA, USA

¹⁷Department of Genetics, University of Pennsylvania Perelman School of Medicine; Philadelphia, PA, USA

¹⁸Department of Medicine, Division of Gastroenterology and Hepatology, University of Pennsylvania Perelman School of Medicine; Philadelphia, PA, USA

¹⁹Department of Obstetrics and Gynecology, Stanford University School of Medicine; Stanford, CA, USA

²⁰Department of Pediatrics, Neonatology, Stanford University School of Medicine; Stanford, CA, USA

²¹Department of Medicine, Rocky Mountain Regional VA Medical Center; Aurora, CO, USA

²²CART Program, VHA Office of Quality and Patient Safety; Washington, DC, USA

²³Division of Cardiology, University of Colorado School of Medicine; Aurora, CO, USA

²⁴Department of Medicine, Stanford University School of Medicine; Stanford, CA, USA

²⁵Cardiovascular Institute, Stanford University School of Medicine; Stanford, CA, USA

²⁶Department of Medicine, Stanford Prevention Research Center, Stanford University School of Medicine; Stanford, CA, USA

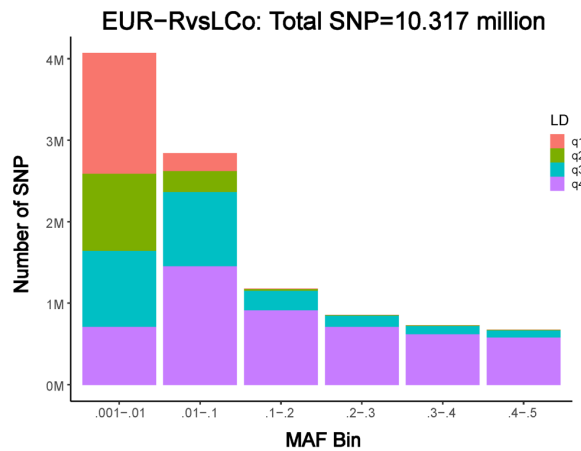
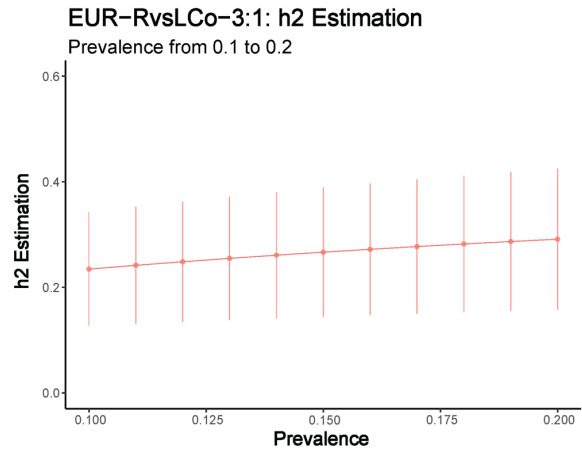
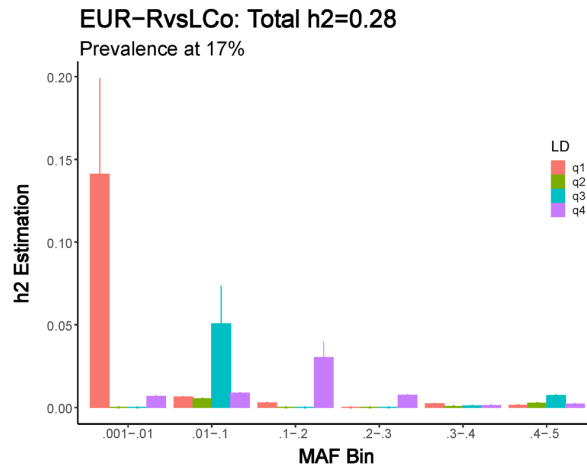
²⁷Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine; Stanford, CA, USA

²⁸Howard Hughes Medical Institute; Chevy Chase, MD, USA

²⁹Department of Epidemiology and Population Health, Stanford University School of Medicine; Stanford, CA, USA

†Pamela E. Rios Coronado, Daniela Zanetti, and Jiayan Zhou contributed as co-lead junior authors.

‡Shoa L. Clarke, Kristy Red-Horse, and Themistocles L. Assimes contributed as co-supervising senior authors.

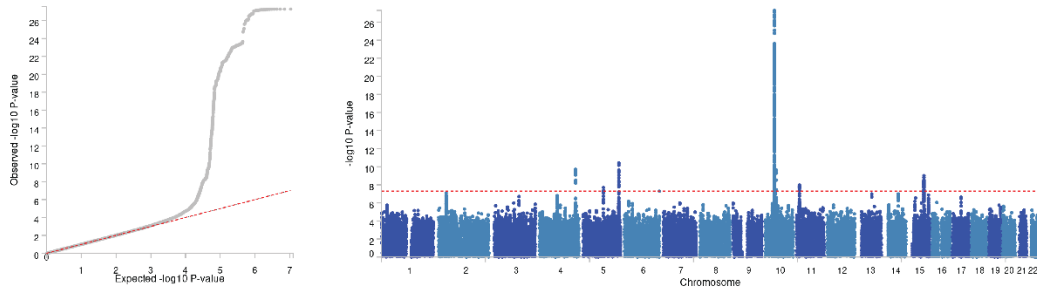
A**B****C**

Supplementary Figure 1. Estimating heritability for coronary artery dominance. Analyses performed using GREML-LDMS-I as implemented in GCTA for right versus left/co-dominance in European cohort. (A) Number of genetic variants by minor allele frequency (MAF) and linkage disequilibrium (LD) score bins (B) h2 estimation on a liability scale assuming prevalence of left and co-dominance from 10% to 20% (C) Stratified h2 contribution to total h2 by MAD and LD score bins.

GWAS: Right vs Left Dominance

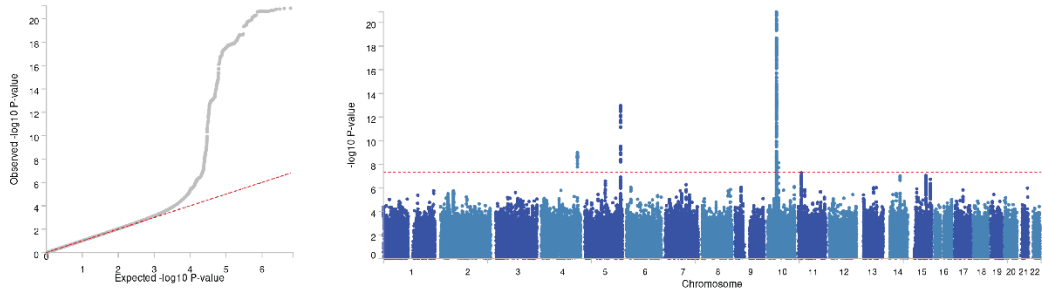
All ancestry MVP participants

A



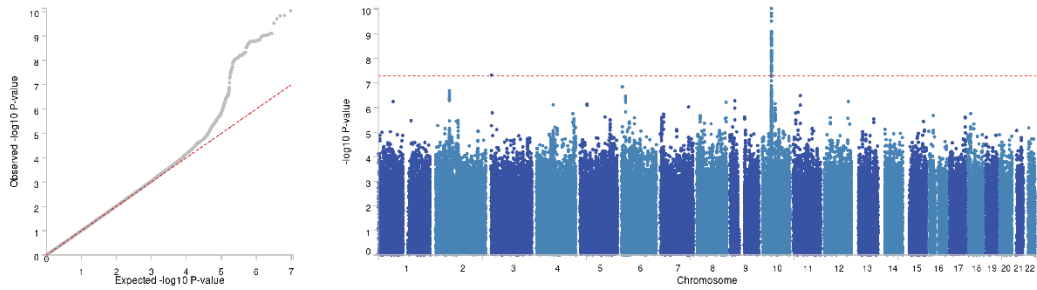
European ancestry MVP participants

B



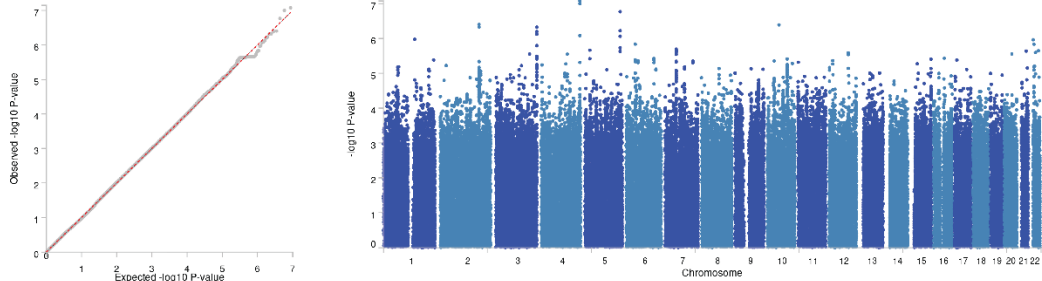
African ancestry MVP participants

C

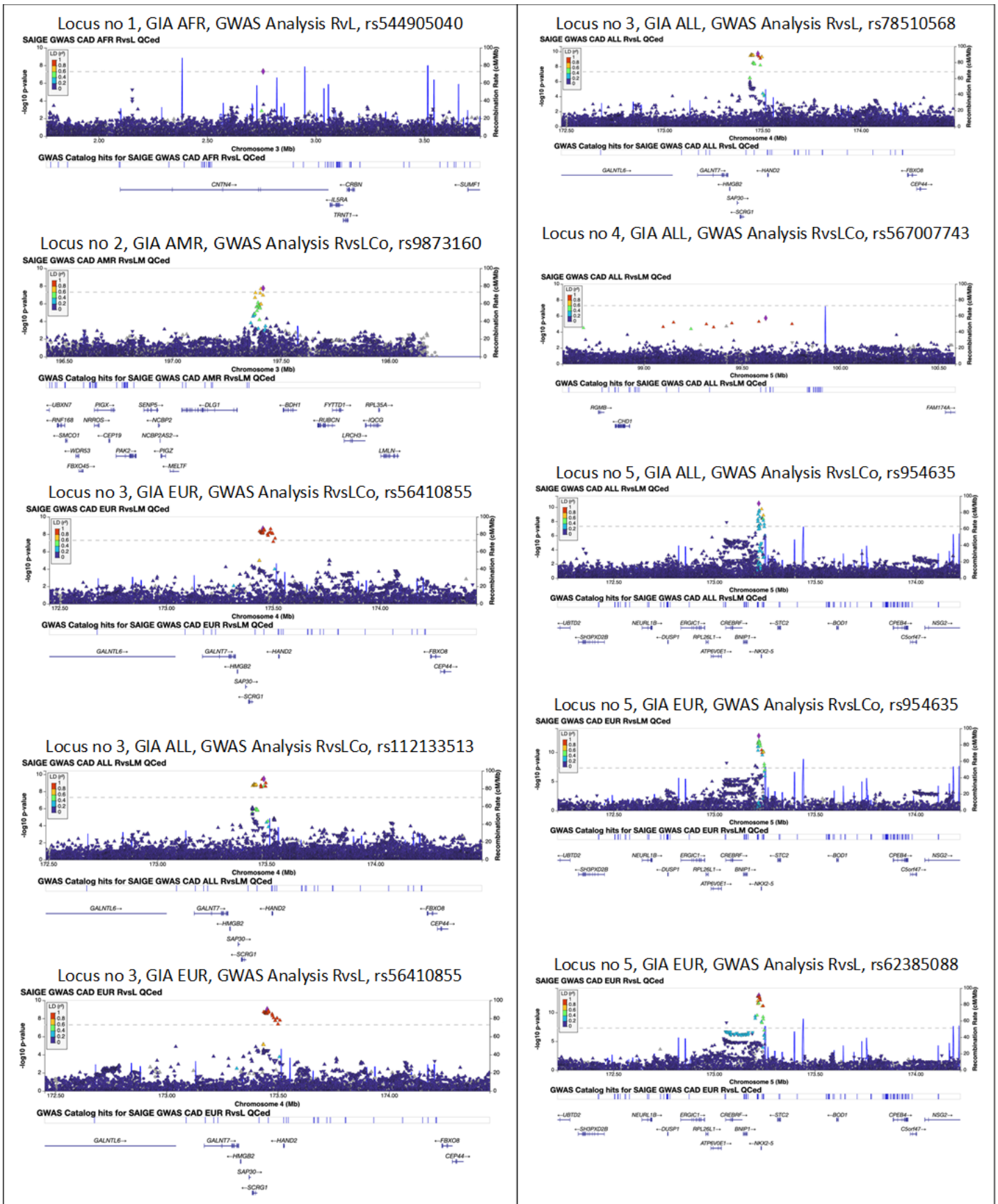


Admixed American ancestry MVP participants

D

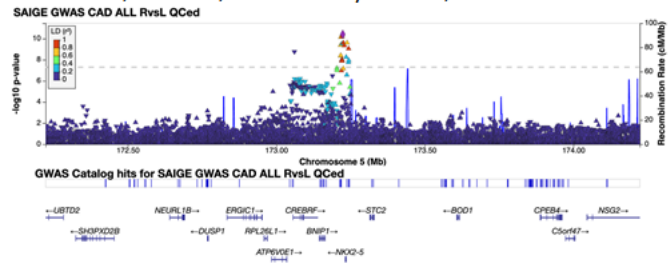


Supplementary Figure 2. Genome-wide association study (GWAS) of right versus left dominance in the Million Veteran Program (MVP). Q-Q and Manhattan plots for All ancestry (A), European (B), African (C) and Admixed American (D) cohorts.

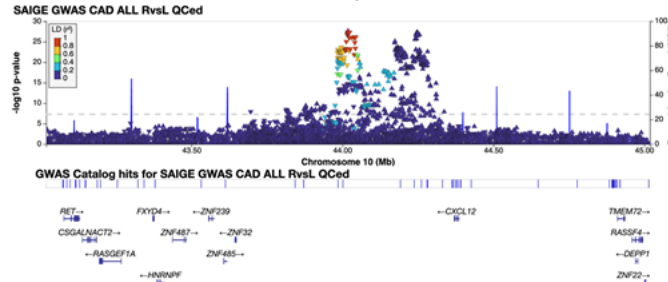


Supplementary Figure 3. LocusZoom plots around lead genetic variants for analyses listed in Table 1.

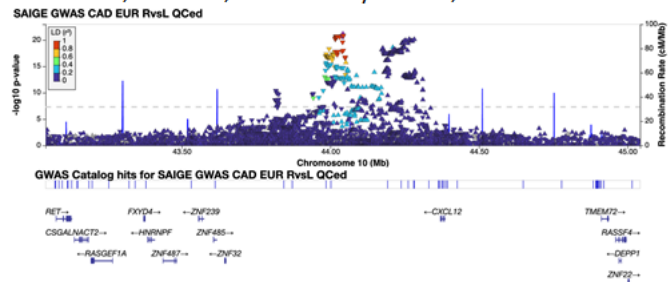
Locus no 5, GIA ALL, GWAS Analysis RvsL, rs5589355



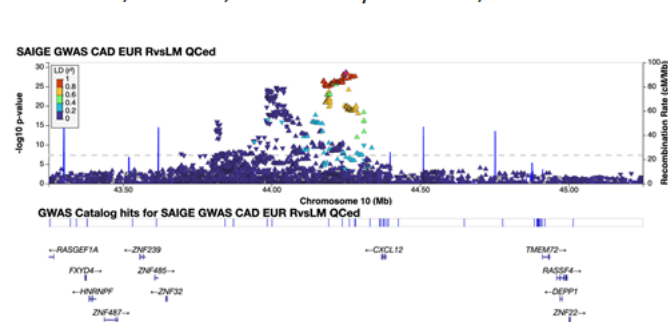
Locus no 6, GIA ALL, GWAS Analysis RvsL, rs2902339



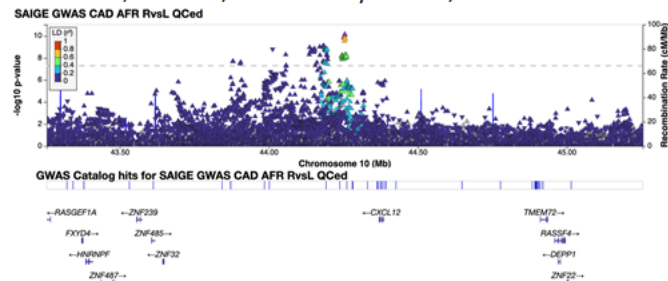
Locus no 6, GIA EUR, GWAS Analysis RvsL, rs7917534



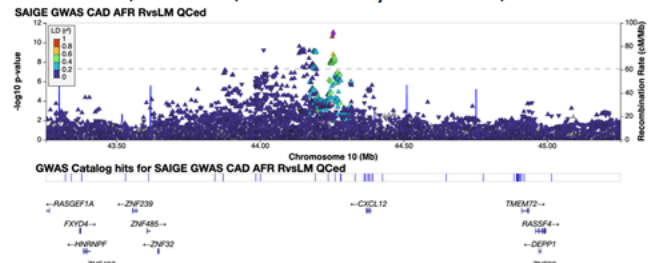
Locus no 6, GIA EUR, GWAS Analysis RvsLCo, rs589655



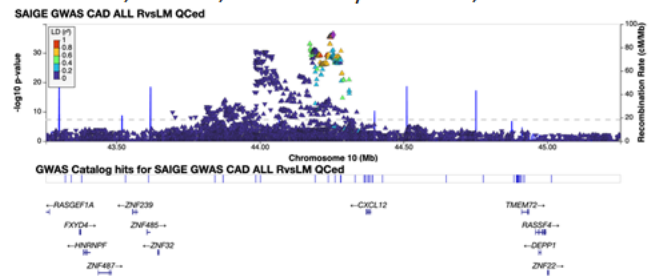
Locus no 6, GIA AFR, GWAS Analysis RvsL, rs2576354



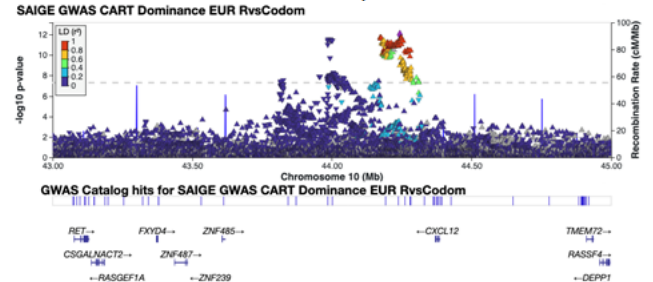
Locus no 6, GIA AFR, GWAS Analysis RvsLCo, rs2576354



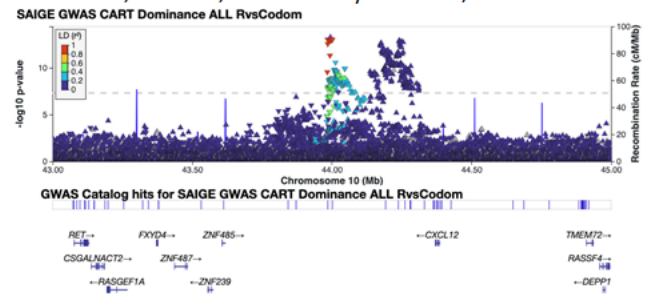
Locus no 6, GIA ALL, GWAS Analysis RvsLCo, rs606314



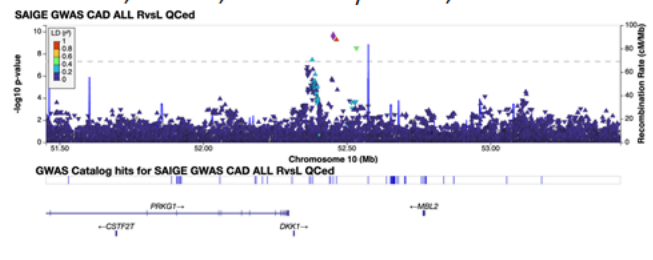
Locus no 6, GIA EUR, GWAS Analysis RvsCo, rs583489



Locus no 6, GIA ALL, GWAS Analysis RvsCo, rs918864065

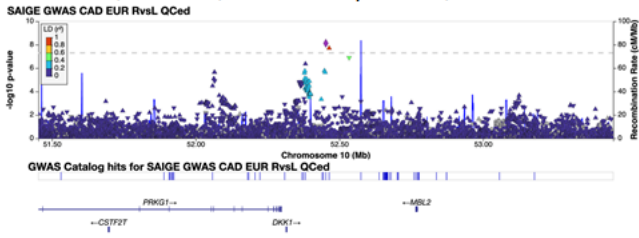


Locus no 7, GIA ALL, GWAS Analysis RvsL, rs1194743

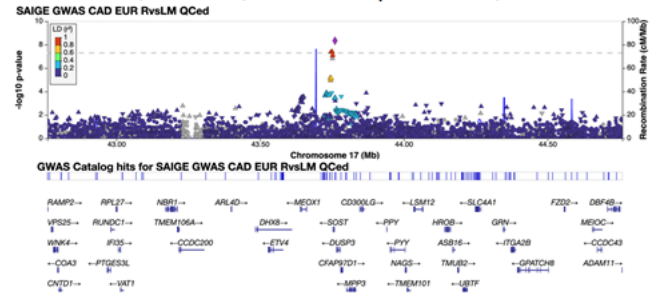


Supplementary Figure 3 (con't). LocusZoom plots around lead genetic variants for analyses listed in Table 1.

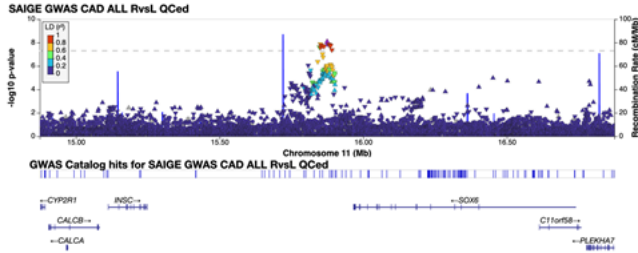
Locus no 7, GIA EUR, GWAS Analysis RvsL, rs1194743



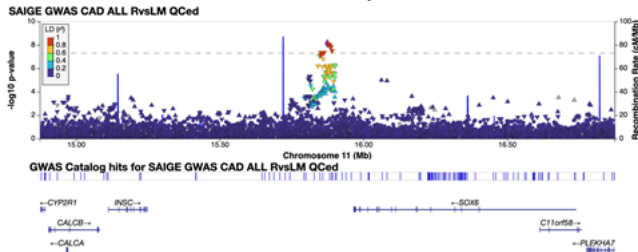
Locus no 10, GIA EUR, GWAS Analysis RvsLCo, rs851058



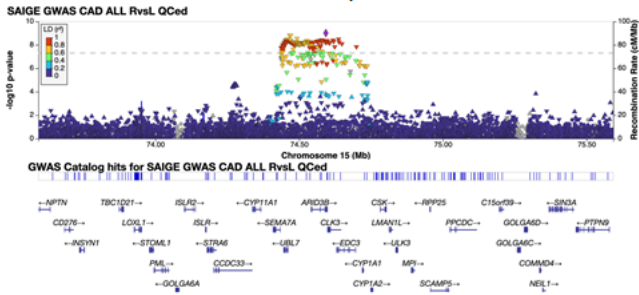
Locus no 8, GIA ALL, GWAS Analysis RvsL, rs35342212



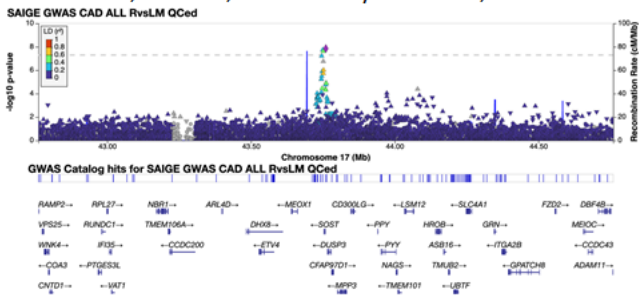
Locus no 8, GIA ALL, GWAS Analysis RvsLCo, rs35342212



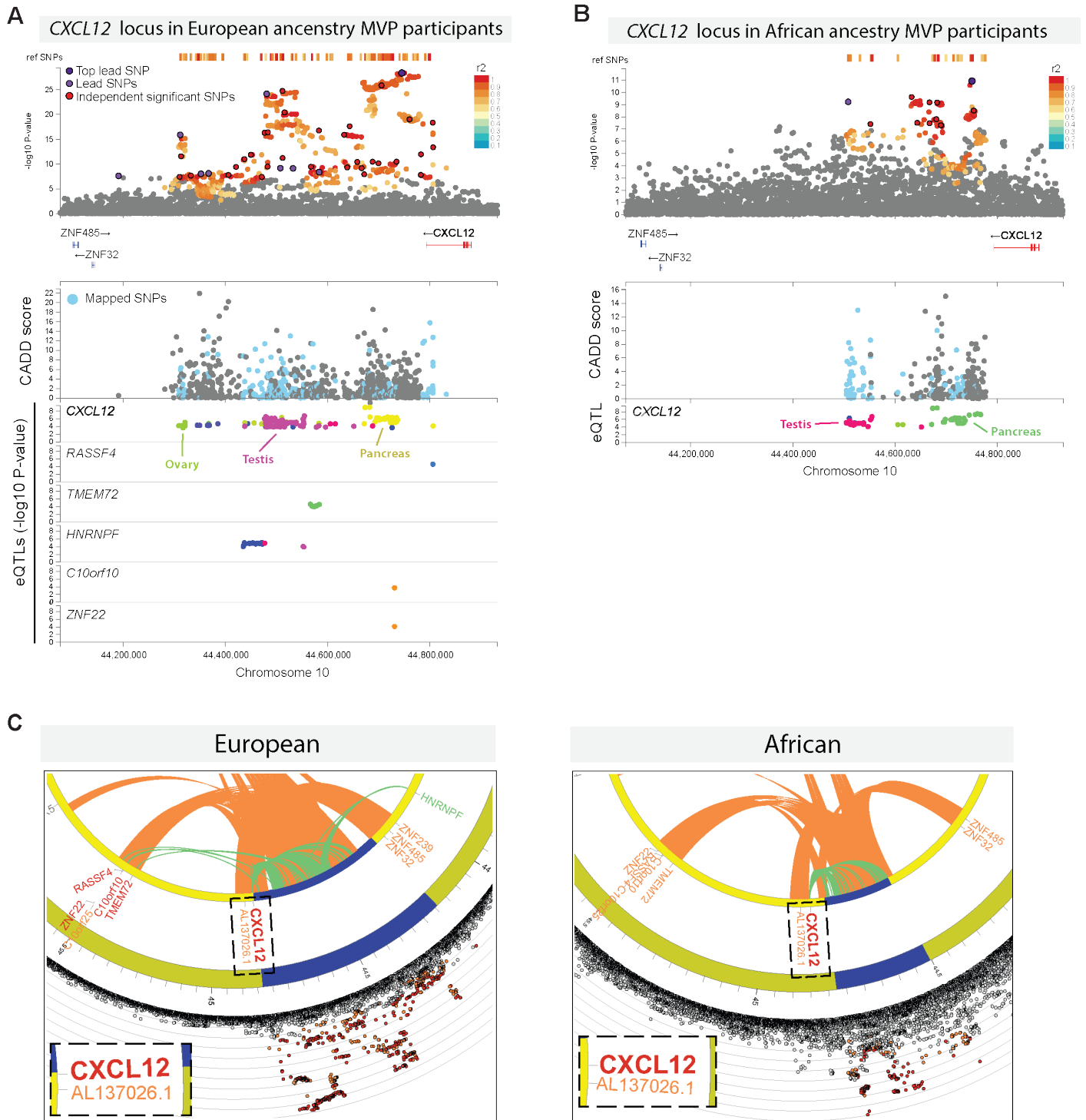
Locus no 9, GIA ALL, GWAS Analysis RvsL, rs58473469



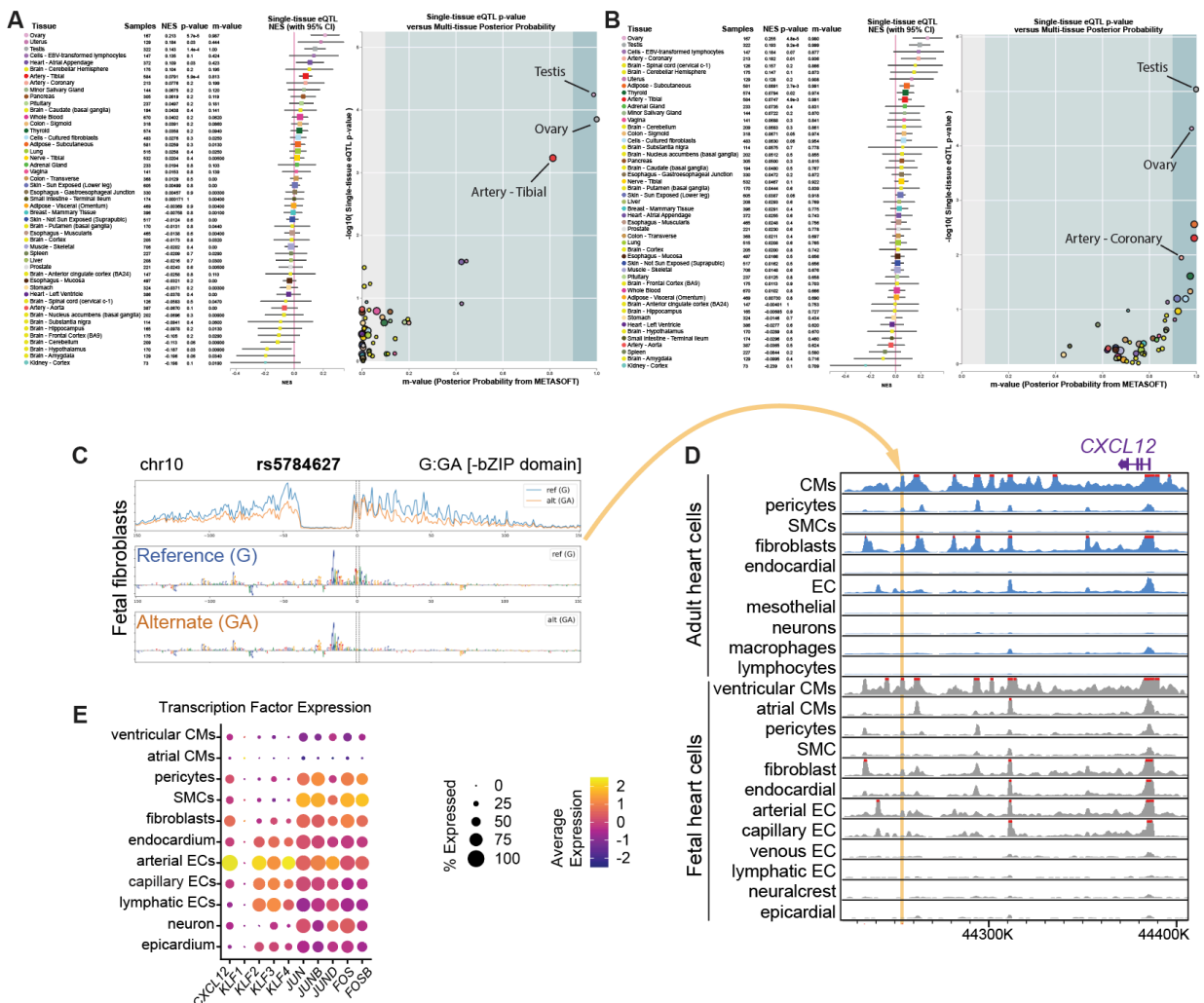
Locus no 10, GIA ALL, GWAS Analysis RvsLCo, rs851058



Supplementary Figure 3 (con't). LocusZoom plots around lead genetic variants for analyses listed in Table 1.



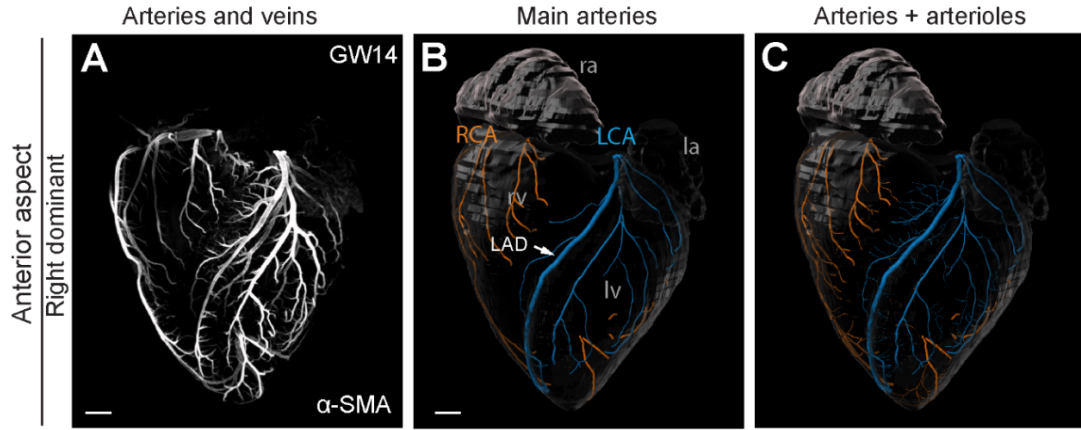
Supplementary Figure 4. Genomic analyses of *CXCL12* dominance variants. (A and B) LocusZoom plots derived by FUMA-GWAS in European (A) and African (B) populations show substantial signal overlap between ancestral groups. SNPs not in LD with any lead or independent significant SNPs are grey. (A, B bottom panels) CADD scores of SNPs in LD with independent significant SNPs in the locus zoom plots, including SNPs mapped (blue) and not mapped (grey) to genes by eQTLs and/or chromatin interactions. Scores >10 are predicted to affect the expression of nearby genes. Expression quantitative trait loci (eQTL) from the Genotype-Tissue Expression (GTEx) project database are shown for the same variants. eQTLs implicate *CXCL12* and five other genes in European populations, but only *CXCL12* in African populations. (C) Circos plots mapping genes by chromatin interactions (orange arcs), eQTLs (green arcs), or both (red gene name). Only *CXCL12* is mapped by both in African populations.



Supplementary Figure 5. Expression QTLs (eQTLs) and machine learning methods suggest regulatory regions for *CXCL12*. (A and B) eQTL multi-tissue plots from the GTExPortal for two genetic variants, rs1870634 and rs7074248, found to be independently significant for dominance in EUR (A) and AFR (B) GWAS, respectively. While the strongest effects of tissue expression for these SNPs are in ovary, testis, and the uterus, rs1870634 is also an eQTL for the tibial artery (A) and rs7074248 is an eQTL for both tibial and coronary arteries (B). (C) Predicted per-base accessibility for reference and alternate alleles of rs5784627 suggest a disrupted bZIP domain motif. (D) Sequencing tracks of chromatin accessibility near *CXCL12*. Yellow highlights rs5784627 region where open chromatin is coincident with *CXCL12* promoter accessibility in select cardiac cell types, including coronary artery cells. (E) scRNAseq from fetal hearts reveals co-expression of *CXCL12* with bZIP domain transcription factors *JUN* and *FOS*.

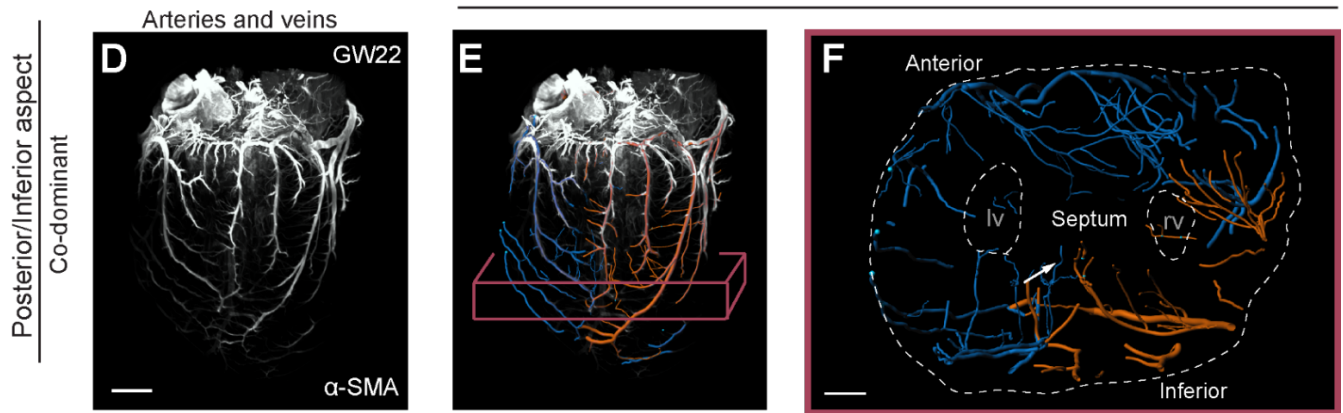
3D Reconstruction

3D Tracing and Rendering



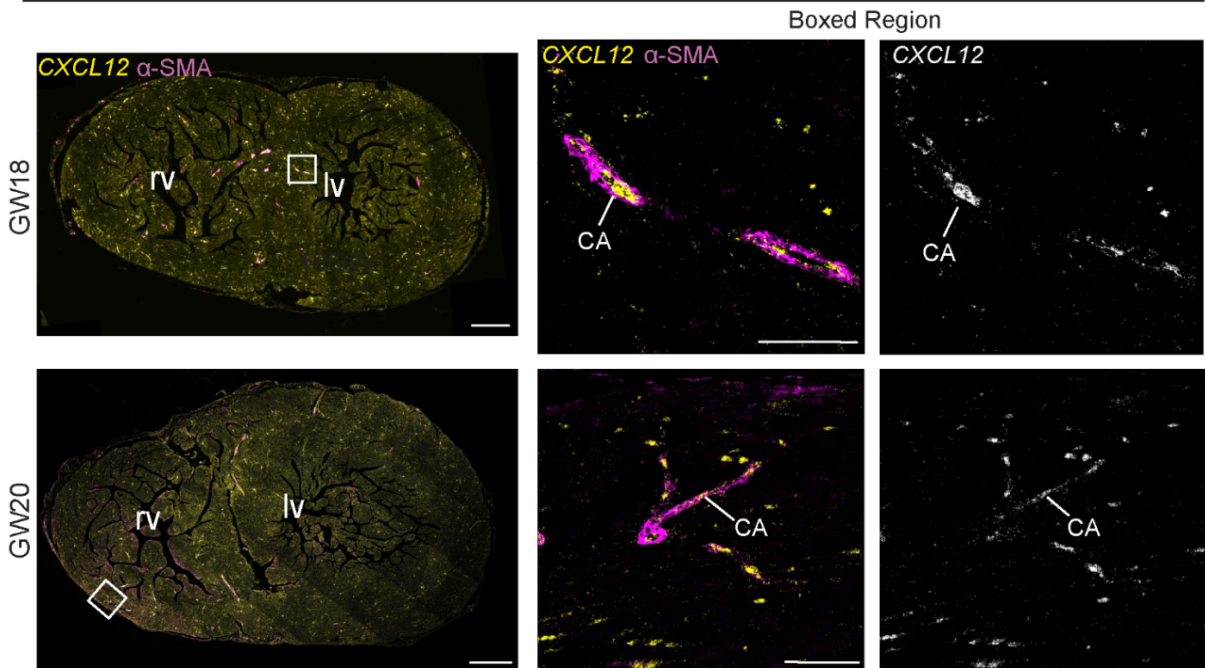
3D Reconstruction

3D Tracing and Rendering

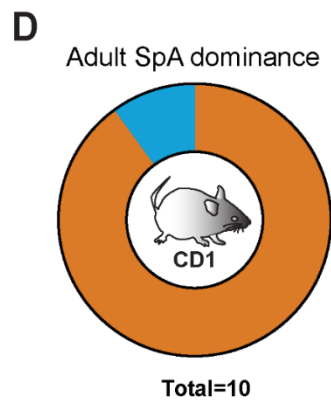
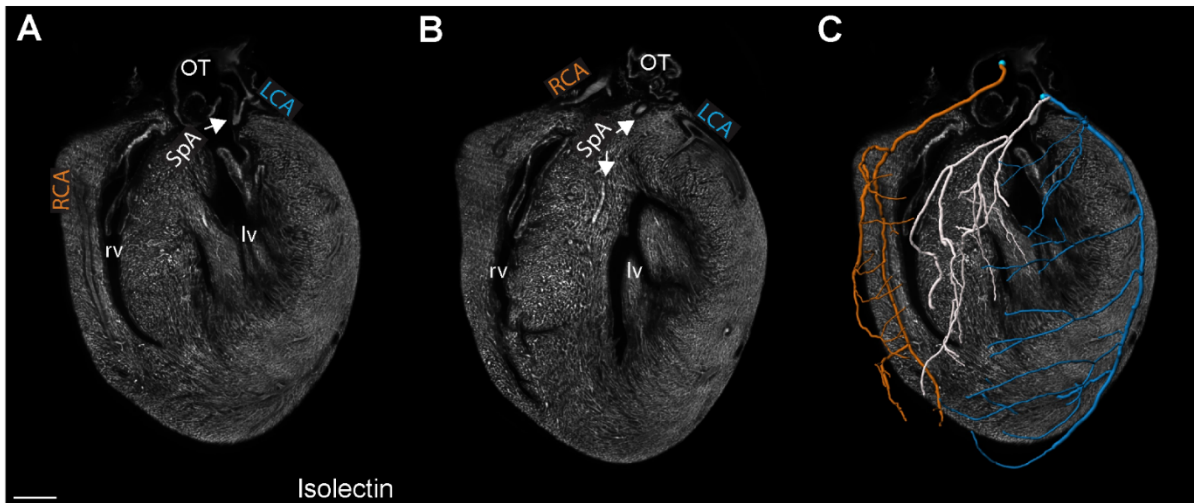


G

FISH

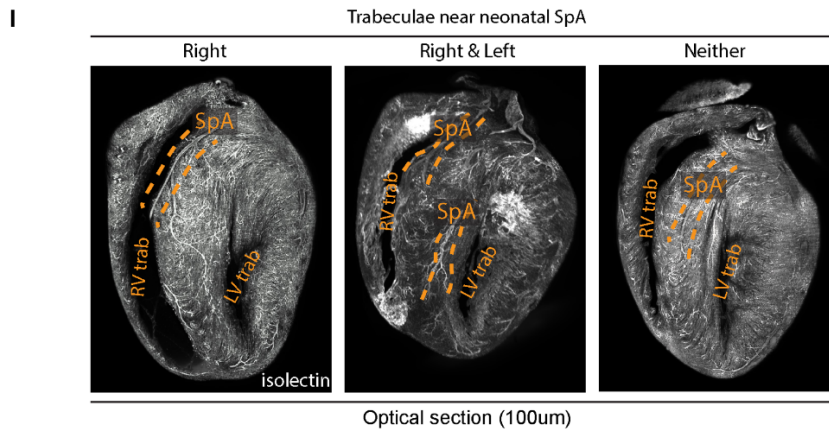
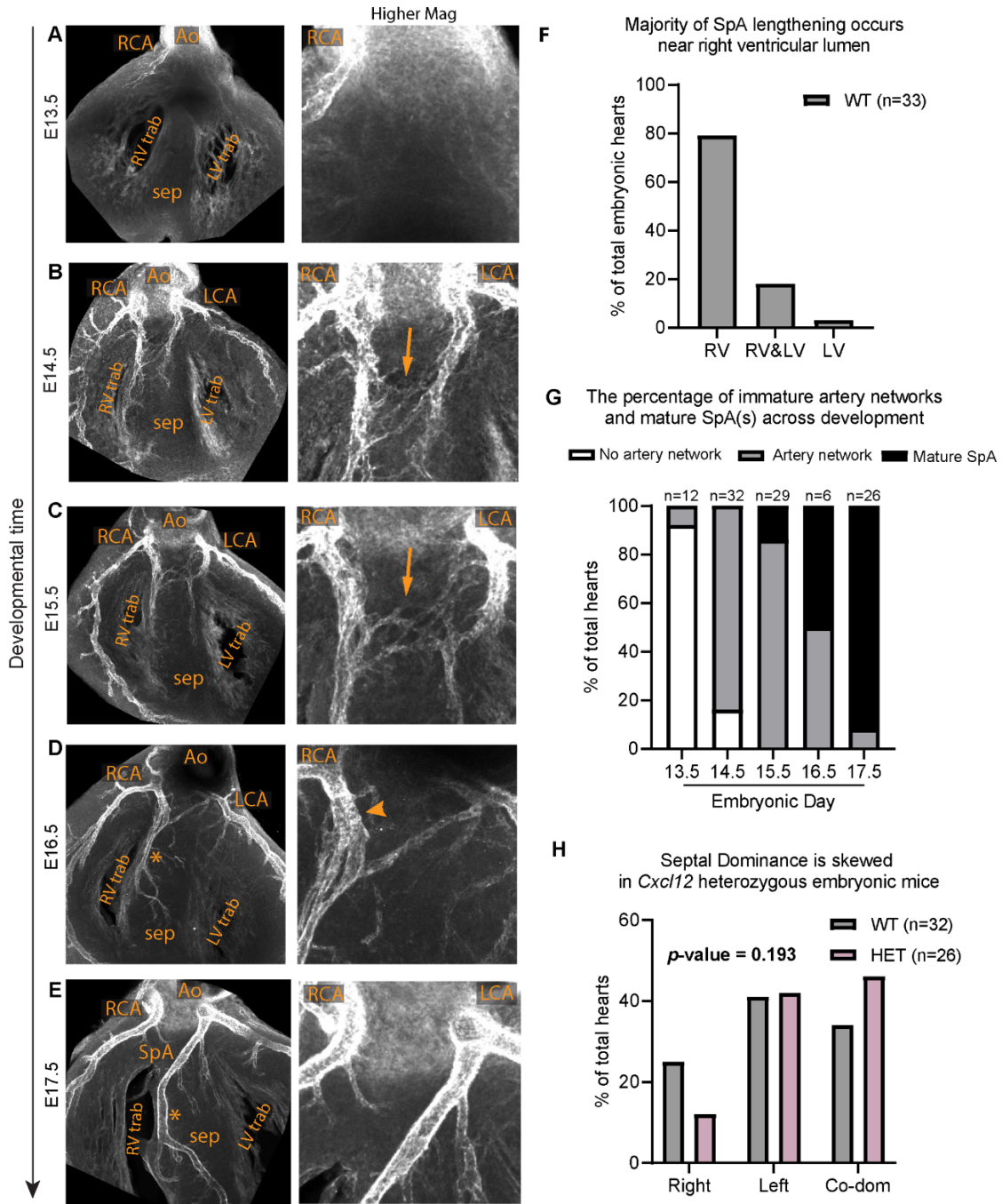


Supplemental Figure 6. Artery imaging and *CXCL12* expression in fetal hearts. (A) Anterior view of a three-dimensional (3D) reconstruction of a gestational week (GW) 14 heart subjected to whole-organ immunolabeling for alpha-smooth muscle actin (α -SMA). (B and C) 3D reconstruction of representative tracings of α -SMA⁺ arteries. Traces highlight branches of the main arteries (B) or main with lower order arteries (C) that originated from the right (RCA, orange) or left (LCA, blue) coronary ostia where they meet the aorta. (D-F) Example of a co-dominant fetal heart as indicated by the presence of both RCA and LCA branches within the IVG. (E) and inferior septum (arrow in F). (G) *CXCL12* fluorescence *in situ* hybridization (FISH) and α SMA immunolabeling on transverse sections through hearts at the indicated ages showed expression in coronary arteries (CA) and other unlabeled cells within the myocardium. LAD, left anterior descending; LCA, left coronary artery; lv, left ventricle; RCA, right coronary artery; rv, right ventricle. Scale bars: A and B 500 μ m; D, 2mm; F, 1mm; G, left panels, 1mm; middle panels 100 μ m.



Supplemental Figure 7. Investigating dominance in mouse hearts. (A-C) Example of the *in vivo* labeling method for identifying septal artery (SpA) dominance in mice. (D) Quantification of SpA dominance in adult CD1 mouse hearts revealed that rates are like those observed in neonatal mice. N=10 hearts. LCA, left coronary artery; lv, left ventricle; OT, outflow tract; RCA, right coronary artery; rv, right ventricle. Scale bars: 300 μ m

Jagged1+ arteries



Supplemental Figure 8. Septal artery development in embryonic mouse hearts. (A-E) Whole-organ immunolabeling of wild type mouse hearts, embryonic day (E) 13.5-17.5, with *Jagged1* to label artery endothelial cells. Higher magnifications highlight regions where septal arteries (SpA) develop. Arrows indicate interconnected, immature artery precursors in **B** and **C**. Asterisks mark where developing SpAs run along the right ventricular lumen where high *Cxcl12* expression was detected (see **Figure 5I**). Arrowheads point to arteries that have grown larger on one side in **D**, presaging dominance. (F) Quantification of SpA growth location with respect to the ventricular lumens show a bias near the right lumen with high *Cxcl12*. (G) Quantification of SpA development over developmental time. Dominance in mature SpAs is primarily established between embryonic days 16.5 and 17.5. (H) Dominance is skewed in *Cxcl12* heterozygous hearts in embryonic development, similar to postnatal stages (see **Figure 5F**). Data shown for mature SpAs at 16.5 and 17.5. (I) Representative images of neonatal SpA localized near right, right and left or neither ventricular trabeculae. Ao, aorta; LCA, left coronary artery; LV trab, left ventricle trabeculae; RCA, right coronary artery; RV trab, right ventricle trabeculae; sep, septum.

**VA Million Veteran Program:
Core Acknowledgements for Publications
May 2024**

MVP Program Office

- Sumitra Muralidhar, Ph.D., Program Director
US Department of Veterans Affairs, 810 Vermont Avenue NW, Washington, DC 20420
- Jennifer Moser, Ph.D., Associate Director, Scientific Programs
US Department of Veterans Affairs, 810 Vermont Avenue NW, Washington, DC 20420
- Jennifer E. Deen, B.S., Associate Director, Cohort & Public Relations
US Department of Veterans Affairs, 810 Vermont Avenue NW, Washington, DC 20420

MVP Executive Committee

- Co-Chair: Philip S. Tsao, Ph.D.
VA Palo Alto Health Care System, 3801 Miranda Avenue, Palo Alto, CA 94304
- Co-Chair: Sumitra Muralidhar, Ph.D.
US Department of Veterans Affairs, 810 Vermont Avenue NW, Washington, DC 20420
- J. Michael Gaziano, M.D., M.P.H.
VA Boston Healthcare System, 150 S. Huntington Avenue, Boston, MA 02130
- Elizabeth Hauser, Ph.D.
Durham VA Medical Center, 508 Fulton Street, Durham, NC 27705
- Amy Kilbourne, Ph.D., M.P.H.
VA HSR&D, 2215 Fuller Road, Ann Arbor, MI 48105
- Michael Matheny, M.D., M.S., M.P.H.
VA Tennessee Valley Healthcare System, 1310 24th Ave. South, Nashville, TN 37212
- Dave Oslin, M.D.
Philadelphia VA Medical Center, 3900 Woodland Avenue, Philadelphia, PA 19104
- Deepak Voora, MD
Durham VA Medical Center, 508 Fulton Street, Durham, NC 27705

MVP Co-Principal Investigators

- J. Michael Gaziano, M.D., M.P.H.
VA Boston Healthcare System, 150 S. Huntington Avenue, Boston, MA 02130
- Philip S. Tsao, Ph.D.
VA Palo Alto Health Care System, 3801 Miranda Avenue, Palo Alto, CA 94304

MVP Core Operations

- Jessica V. Brewer, M.P.H., Director, MVP Cohort Operations
VA Boston Healthcare System, 150 S. Huntington Avenue, Boston, MA 02130
- Mary T. Brophy M.D., M.P.H., Director, VA Central Biorepository
VA Boston Healthcare System, 150 S. Huntington Avenue, Boston, MA 02130
- Kelly Cho, M.P.H, Ph.D., Director, MVP Phenomics
VA Boston Healthcare System, 150 S. Huntington Avenue, Boston, MA 02130
- Lori Churby, B.S., Director, MVP Regulatory Affairs
VA Palo Alto Health Care System, 3801 Miranda Avenue, Palo Alto, CA 94304

- Scott L. DuVall, Ph.D., Director, VA Informatics and Computing Infrastructure (VINCI)
VA Salt Lake City Health Care System, 500 Foothill Drive, Salt Lake City, UT 84148
- Saiju Pyarajan Ph.D., Director, Data and Computational Sciences
VA Boston Healthcare System, 150 S. Huntington Avenue, Boston, MA 02130
- Robert Ringer, Pharm.D., Director, VA Albuquerque Central Biorepository
New Mexico VA Health Care System, 1501 San Pedro Drive SE, Albuquerque, NM 87108
- Luis E. Selva, Ph.D., Director, MVP Biorepository Coordination
VA Boston Healthcare System, 150 S. Huntington Avenue, Boston, MA 02130
- Shahpoor (Alex) Shayan, M.S., Director, MVP PRE Informatics
VA Boston Healthcare System, 150 S. Huntington Avenue, Boston, MA 02130
- Brady Stephens, M.S., Principal Investigator, MVP Information Center
Canandaigua VA Medical Center, 400 Fort Hill Avenue, Canandaigua, NY 14424
- Stacey B. Whitbourne, Ph.D., Director, MVP Cohort Development and Management
VA Boston Healthcare System, 150 S. Huntington Avenue, Boston, MA 02130

MVP Publications and Presentations Committee

- Co-Chair: Themistocles L. Assimes, M.D., Ph. D
VA Palo Alto Health Care System, 3801 Miranda Avenue, Palo Alto, CA 94304
- Co-Chair: Adriana Hung, M.D.; M.P.H
VA Tennessee Valley Healthcare System, 1310 24th Ave. South, Nashville, TN 37212
- Co-Chair: Henry Kranzler, M.D.
Philadelphia VA Medical Center, 3900 Woodland Avenue, Philadelphia, PA 19104