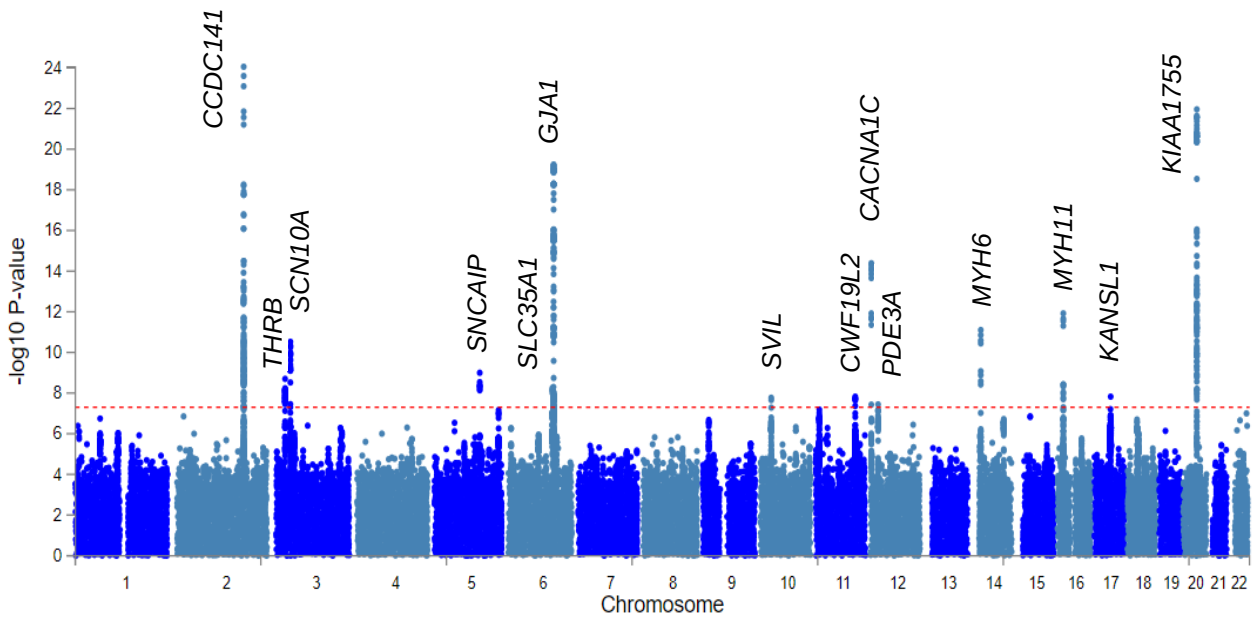
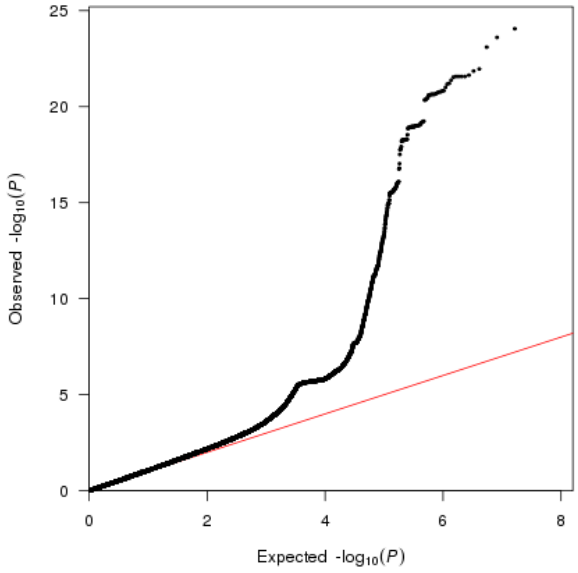
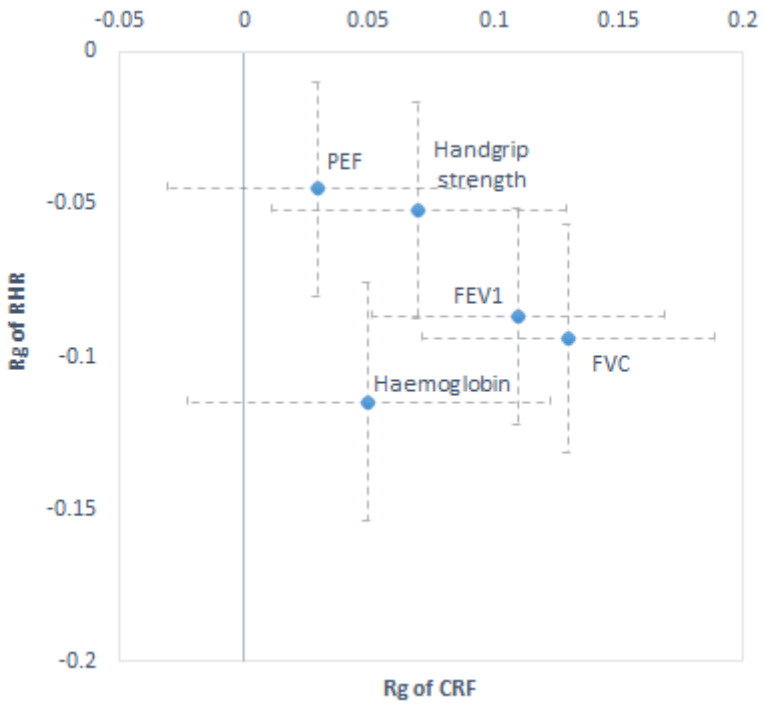


(a)

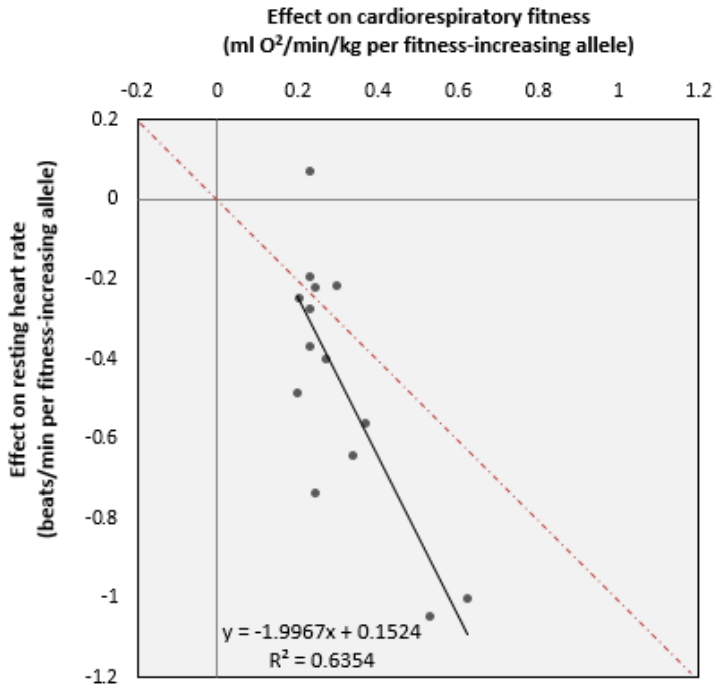


(b)

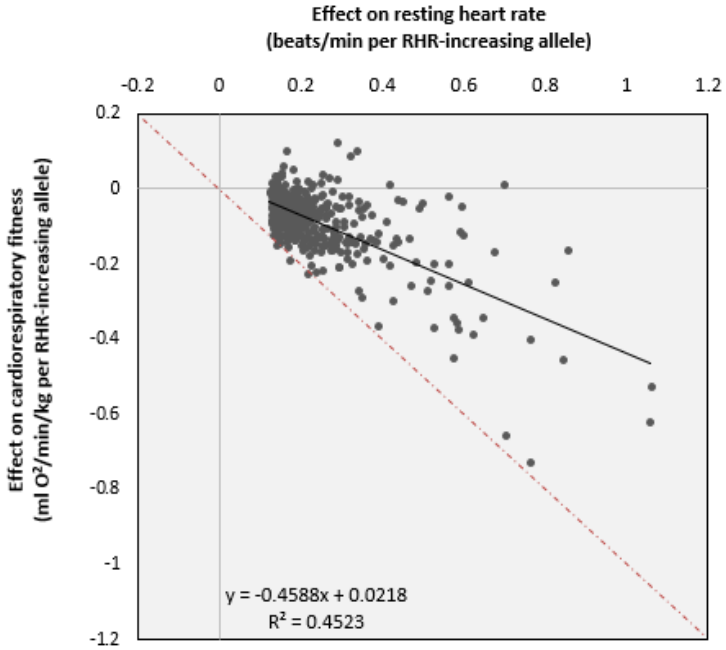
Supplementary Figure 1. **Manhattan plot (a) and Q-Q plot (b) of genome-wide association analyses on cardiorespiratory fitness among up to 69,416 participants in UKBB.** (a) 14 independent genome-wide significant loci are annotated. On the Manhattan plot, red horizontal line indicates genome-wide significance threshold (p < 5 x 10-8); (b) On the Q-Q plot, the red diagonal line indicates null association estimate. LD score regression estimated λ_{GC}=1.15, LD score regression intercept = 1.02 (95% CI 1.00 -1.04), suggesting that apparent inflation evident in the QQ-plot can be attributed to polygenicity of cardiorespiratory fitness.



Supplementary Figure 2. Comparison of genetic correlations between cardiorespiratory fitness (CRF), resting heart rate (RHR) and various physiologically relevant traits, including forced expiratory volume 1 (FEV1), forced vital capacity (FVC), peak expiratory flow (PEF), handgrip strength, and haemoglobin.

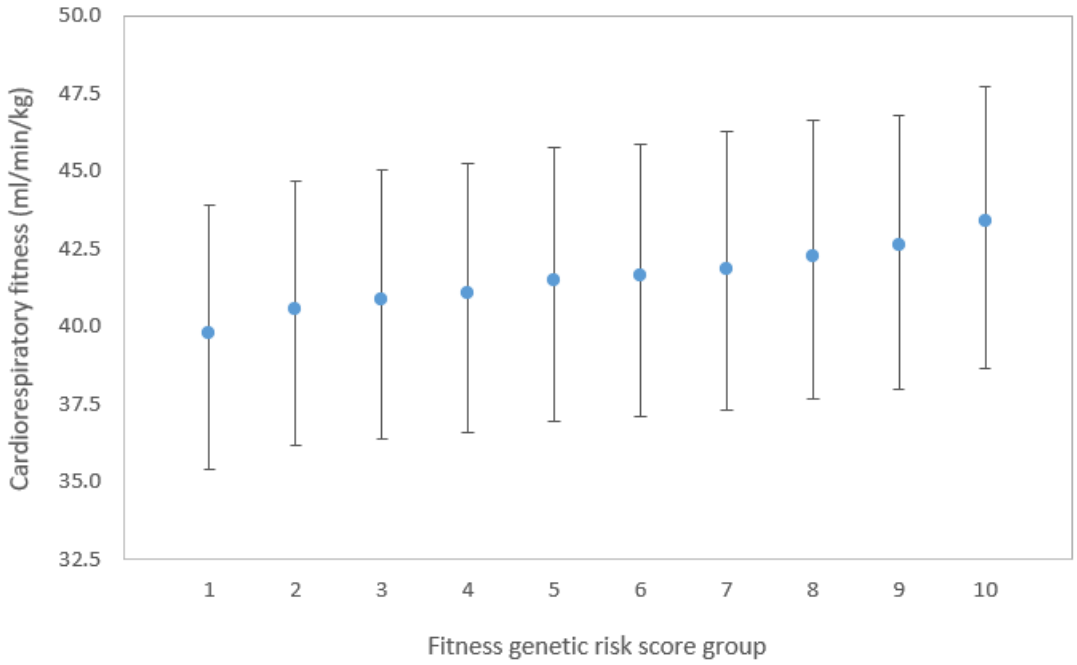


(a)

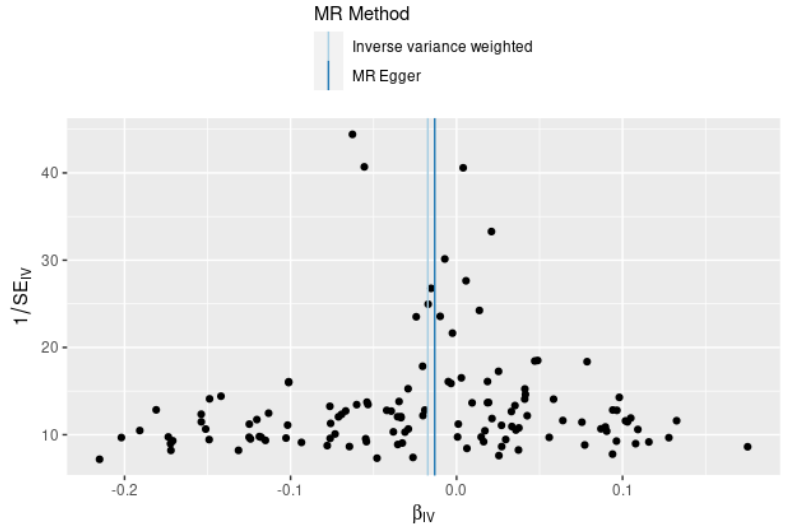


(b)

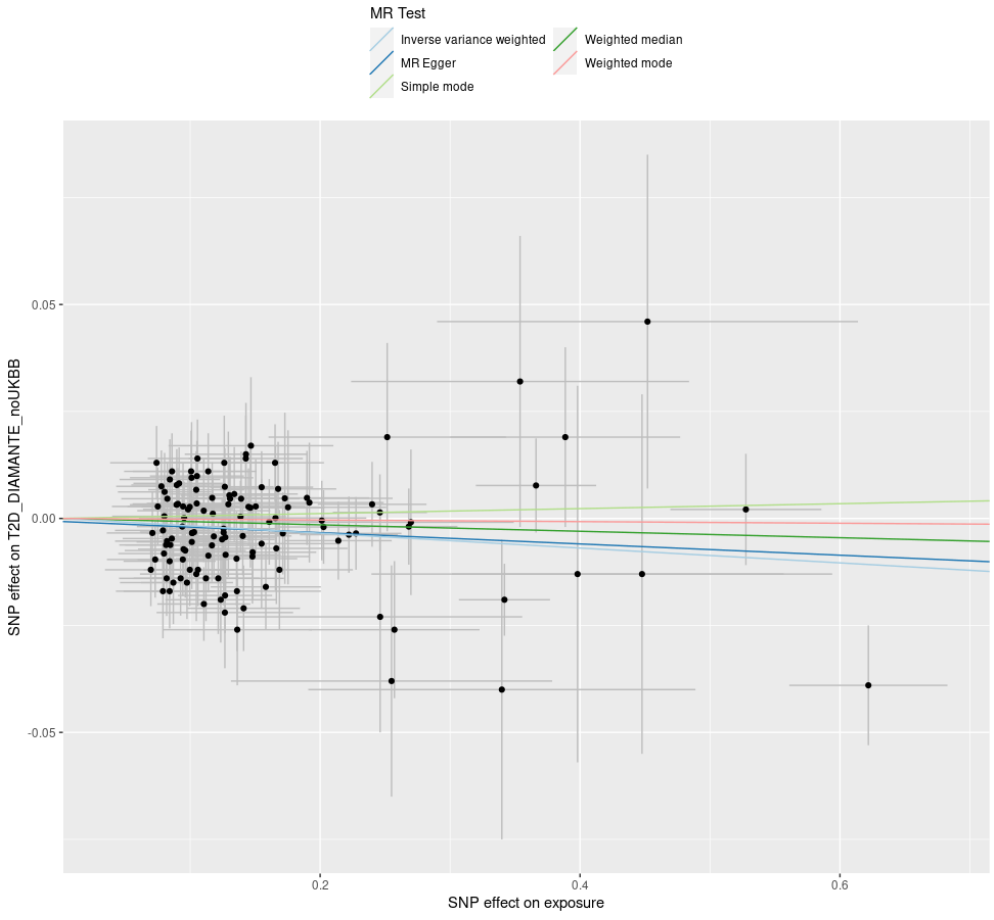
Supplementary Figure 3. Comparison of effect of cardiorespiratory fitness (CRF) and resting heart rate (RHR)-associated independent variants (p <5 x 10^{-8}) in UK Biobank. (a) effect of 14 distinct genome-wide significant CRF associated variants (effect alleles aligned to CRF-increasing) on RHR in UK Biobank (n=69,416); (b) effect of 427 distinct genome-wide significant RHR associated variants (effect alleles aligned to RHR-increasing) on CRF in UK Biobank (n= 452,941).



Supplementary Figure 4. Mean (and 95% confidence interval) cardiorespiratory fitness levels by decile groups of genetic risk score constructed using 160 independent genome-wide significant variants in the UK Biobank study (n=69,416). The mean difference between the highest GRS group and the lowest is 3.6 ml O_2 min^{-1} kg^{-1}

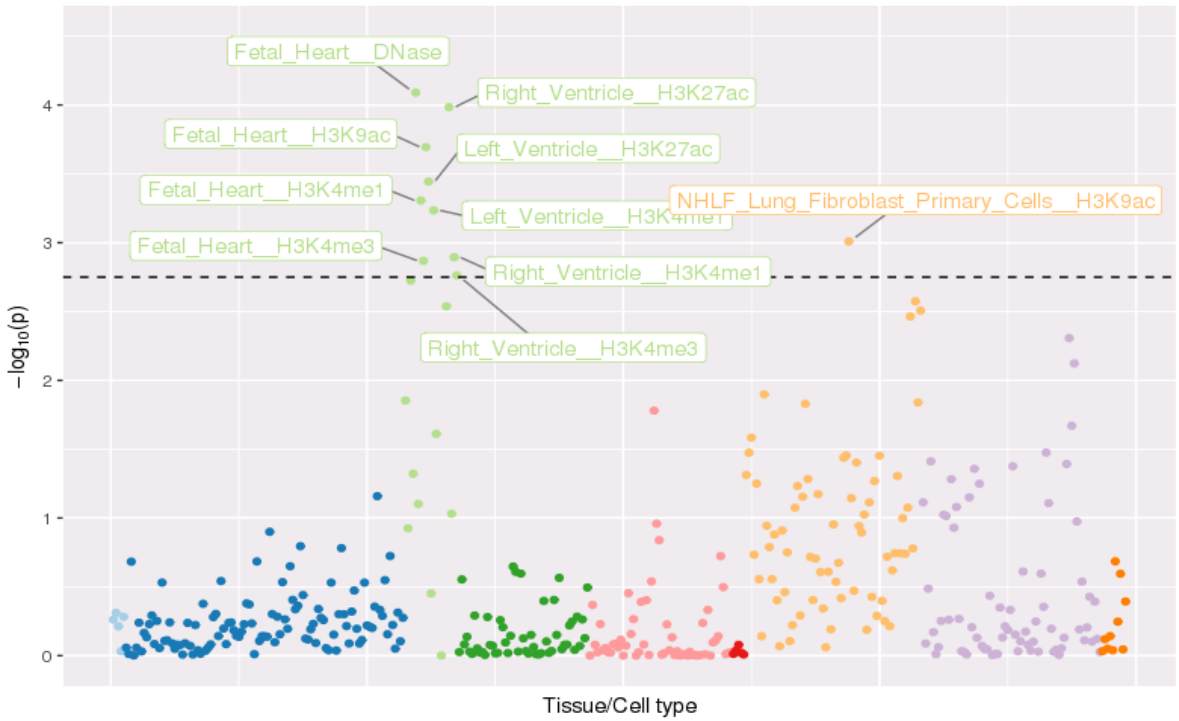


(a)

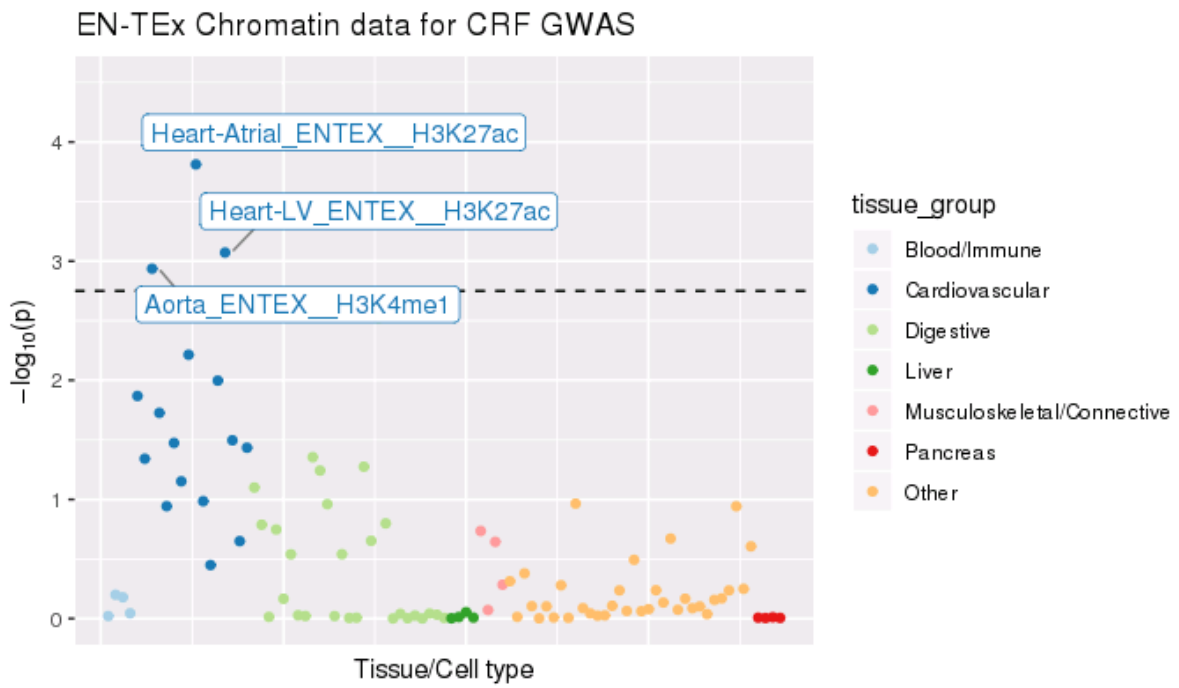


(b)

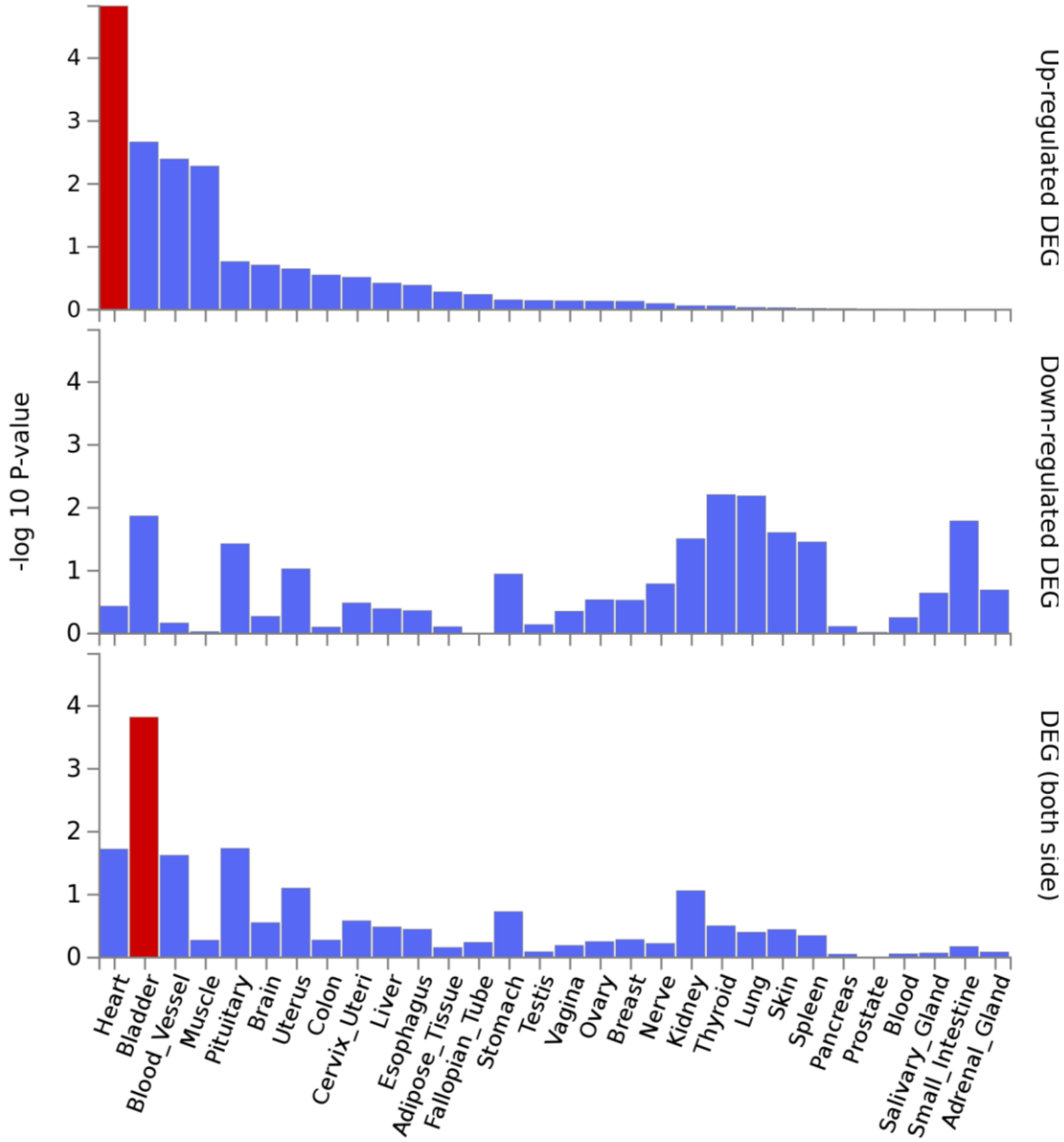
Supplementary Figure 5 (a) Funnel plot for MR analyses of Radial filtered fitness score on T2D. (b) Scatter plot for various MR analyses of Radial filtered fitness score on T2D with slope indicates effect estimate of causal associations. After removing outliers using Radial method, I^2 =0.0%, p-value for Cochran's Q = 0.686.



Supplementary Figure 6. Tissue-specific enrichment for cardiorespiratory fitness GWAS using LD score regression applied to specifically expressed genes (LDSC-SEG). Each dot represents a tissue or cell type further categorised into 9 general tissue groups indicated by different colours. Expression enrichment in 396 ROADMAP Epigenetics chromatin data. Horizontal dash line represents the significance threshold of false discovery rate = 0.05, –log10 (P) = 2.85 (chromatin). Any tissue and cell type above the line is considered enriched.



Supplementary Figure 7. LD score regression applied to specifically expressed genes (LDSC-SEG) EN-TEx chromatin enrichment results for cardiorespiratory fitness GWAS. A total of 93 labels of tissue and cell types are included in the analyses. Each dot represents a tissue or cell type categorised into 8 tissue groups marked in different colours. Horizontal dash line represents the significance threshold of false discovery rate = 0.05, (a) –log10(P) = 2.75 (gene expression). (b) –log10 (P) = 2.85 (chromatin). Any tissue and cell type above the line is considered enriched.



Supplementary Figure 8. Tissues enriched with differentially expressed genes sets identified using prioritised genes associated with cardiorespiratory fitness. Output from FUMA using GTEx v8 30 general tissue types.

Supplementary Table 1. UK Biobank participant characteristics of those with cardiorespiratory fitness estimates from exercise testing, stratified by sex and incident type 2 diabetes status. Participants with prevalent type 2 diabetes at baseline were excluded from this analysis.

Sex	**Women**			**Men**		
T2D status	Pooled	Non-T2D	T2D	Pooled	Non-T2D	T2D
N	39,395	38,663	732	34,179	33,059	1,120
Age (y)	57 ± 8	57 ± 8	60 ± 8	58 ± 8	58 ± 8	60 ± 8
Height (cm)	163 ± 6	163 ± 6	161 ± 6	176 ± 7	176 ± 7	174 ± 7
Weight (kg)	70.1 ± 13.0	69.9 ± 12.9	80.7 ± 16.7	84.9 ± 13.3	84.6 ± 13.1	92.6 ± 15.9
BMI (kg·m^2)	26.4 ± 4.7	26.4 ± 4.6	30.9 ± 5.8	27.4 ± 3.8	27.3 ± 3.8	30.4 ± 4.7
FFM (kg)	44.1 ± 4.8	44.1 ± 4.7	46.6 ± 6.2	63.2 ± 7.5	63.1 ± 7.4	65.3 ± 8.5
Waist circumference (cm)	83.1 ± 11.6	82.9 ± 11.5	94.5 ± 12.8	95.5 ± 10.6	95.2 ± 10.4	103.7 ± 12.1
Resting heart rate (bpm)	67 ± 10	67 ± 10	70 ± 11	65 ± 11	65 ± 11	68 ± 12
Systolic blood pressure (mmHg)	130 ± 17	130 ± 17	137 ± 17	135 ± 16	135 ± 16	139 ± 16
Diastolic blood pressure (mmHg)	78 ± 10	78 ± 10	81 ± 10	81 ± 9	81 ± 9	82 ± 10
VO_2max (ml O_2 min^{-1} kg^{-1} FFM)	**39.8 ± 7.1**	**39.8 ± 7.0**	**36.6 ± 6.8**	**43.1 ± 6.4**	**43.2 ± 6.3**	**40.0 ± 5.9**
VO_2max (ml O_2 min^{-1} kg^{-1} body mass)	25.5 ± 5.7	25.6 ± 5.7	21.6 ± 4.8	32.4 ± 5.8	32.5 ± 5.8	28.6 ± 5.1

Values are means ± standard deviations. T2D: Type 2 diabetes. BMI: Body mass index. FFM: Fat-free mass. VO_2max: Maximal oxygen consumption.

Supplementary Table 2. Sequentially adjusted logistic regression models of the association of sex- and age-residualised cardiorespiratory fitness with incident type 2 diabetes in UK Biobank. Participants with prevalent type 2 diabetes at baseline were excluded from analyses.

	Per ml $O_2 \cdot min^{-1} \cdot kg^{-1}$ fat-free mass		
	Pooled	**Women**	**Men**
Model 1	0.94 (0.93 to 0.94) [$4x10^{-4}$]	0.95 (0.94 to 0.96) [$9x10^{-3}$]	0.93 (0.92 to 0.94) [$5x10^{-3}$]
Model 2	0.96 (0.95 to 0.97) 5[$x10^{-13}$]	0.97 (0.96 to 0.98) [$3x10^{-7}$]	0.95 (0.94 to 0.96) [$1x10^{-10}$]
Model 3	0.97 (0.96 to 0.98) [$5x10^{-9}$]	0.97 (0.96 to 0.98) [$1x10^{-5}$]	0.97 (0.96 to 0.98) [$8x10^{-6}$]
	Per ml $O_2 \cdot min^{-1} \cdot kg^{-1}$ body mass		
	Pooled	**Women**	**Men**
Model 1	0.88 (0.87 to 0.89) [$1x10^{-4}$]	0.88 (0.87 to 0.89) [$3x10^{-3}$]	0.88 (0.87 to 0.89) [$2x10^{-3}$]
Model 2	0.91 (0.90 to 0.92) [$7x10^{-20}$]	0.91 (0.90 to 0.92) [$7x10^{-14}$]	0.91 (0.90 to 0.92) [$4x10^{-16}$]
Model 3	0.96 (0.95 to 0.97) [$9x10^{-8}$]	0.96 (0.94 to 0.98) [$7x10^{-5}$]	0.96 (0.95 to 0.98) [$7x10^{-5}$]

Values are odds ratios (95% confidence intervals). Figures in square brackets are exact p values. CRF: Cardiorespiratory fitness. COPD: Chronic obstructive pulmonary disease.

Model 1 is adjusted for age and sex.

Model 2 is additionally adjusted for ethnicity (White, other), hypertension (yes/no), history of stroke (yes/no), history of heart failure (yes/no), history of heart disease (yes/no), history of atrial fibrillation (yes/no), history of COPD (yes/no), history of cancer (yes/no), betablocker use (yes/no), calcium channel blocker use (yes/no), ACE inhibitor use (yes/no), Diuretic use (use (yes/no), bronchodilator use (yes/no), lipid-lowering agent (yes/no), iron deficiency medication (yes/no), smoking (never, former, current), alcohol consumption (never, former, current), meat intake, oily fish intake, fruit and vegetable intake, salt intake, employment (yes/no), and Area Deprivation Index.

Model 3 is additionally adjusted for adiposity (fat mass for fat-free mass scaled fitness, BMI for body mass scaled fitness).

Supplementary Table 3. Results for the 14 independent genome-wide significant loci associated with cardiorespiratory fitness in European ancestry individuals in the UK Biobank study (N = 69,416).

Lead SNP	chr	pos	EA	OA	EAF	beta (s.e.)	p	Function	Nearest Gene*
rs142556838	2	179747068	C	T	0.91	0.62 (0.06)	**1.6E-24**	intron	CCDC141
rs4811602	20	36849088	G	A	0.53	-0.34 (0.04)	**1.7E-22**	intron	KIAA1755
rs1012020	6	122110346	A	G	0.90	0.52 (0.06)	**1.1E-19**	intergenic	GJA1
rs11062107	12	2187041	A	G	0.83	-0.37 (0.05)	**2.5E-15**	intron	CACNA1C
rs3915425	16	15912544	T	C	0.68	0.27 (0.04)	**8.2E-13**	intron	MYH11
rs422068	14	23864804	T	C	0.64	0.25 (0.04)	**1.1E-11**	intron	MYH6
rs6599251	3	38785809	G	T	0.45	0.23 (0.03)	**7.4E-11**	intron	SCN10A
rs111299422	5	121868475	D	I	0.70	-0.23 (0.04)	**1.4E-09**	intergenic	SNCAIP
rs1561076	3	24497993	A	G	0.68	-0.23 (0.04)	**2.0E-09**	intron	THRB
rs4754197	11	107096785	G	A	0.78	-0.24 (0.04)	**9.5E-09**	intergenic	CWF19L2
rs17695155	10	29820758	T	C	0.60	0.20 (0.04)	**1.1E-08**	intron	SVIL
rs1572226	6	118635079	C	G	0.49	-0.20 (0.03)	**1.4E-08**	intron	SLC35F1
rs62070949	17	44308053	A	G	0.84	-0.30 (0.05)	**1.7E-08**	intergenic	KANSL1
rs67622553	12	20454434	G	T	0.77	-0.23 (0.04)	**4.7E-08**	intergenic	PDE3A

* Nearest genes for the intergenic variants were selected as the genes located closest to the variants that were also mapped by either eQTL or CI by FUMA. Chr: chromosome; pos: position; EA: effect allele; OA: other allele; EAF: effect allele frequency

Supplementary Table 4. Genetic correlation between cardiorespiratory fitness, resting heart rate and several physiologically relevant traits using LD score regression.

Traits	CRF (UK Biobank)			RHR (UK Biobank)		
	Rg	s.e.	p-value	Rg	s.e.	p-value
CRF (Fenland)	0.78	0.17	3.1E-06	-0.43	0.12	4.2E-04
RHR (UK Biobank)	-0.68	0.03	5.4E-120	--	--	--
Handgrip strength	0.07	0.03	0.016	-0.01	0.02	0.442
FEV1	0.11	0.03	3.7E-04	-0.09	0.02	2.5E-06
FVC	0.13	0.03	1.9E-05	-0.09	0.02	6.8E-07
FEV1/FVC	-0.03	0.03	0.290	0.004	0.02	0.814
PEF	0.03	0.03	0.420	-0.05	0.02	0.012
Haemoglobin	0.05	0.04	0.148	-0.12	0.02	3.7E-09

CRF: cardiorespiratory fitness; RHR: resting heart rate; FEV1: forced expiratory volume1; FVC: forced vital capacity; PEF: peak expiratory flow rate

Supplementary Table 5. Validity of the four constructed genetic instruments for cardiorespiratory fitness in the Fenland study.

Instrument*	n_snp†	Association with measured fitness in Fenland					2-sample MR‡			
		beta	s.e.	t	p	AdjR^2	beta	s.e.	p	CochQp
1	14	1.42	0.19	7.36	2.0E-13	0.54%	1.40	0.19	5.7E-06	0.06
2	370	1.08	0.13	8.36	<2.6E-16	0.70%	1.09	0.13	6.5E-16	0.05
3	148	1.04	0.14	7.23	5.4E-13	0.52%	1.04	0.14	2.1E-11	0.02
4	160	1.23	0.12	10.40	<2.6E-16	1.08%	1.15	0.11	7.6E-19	0.01

* All instrument effects were aligned as fitness-increasing.

Instrument 1: 14 independent, genome-wide significant variants associated with exercise-based fitness in UK Biobank ($p < 5 \times 10^{-8}$);

Instrument 2: 370 genome-wide significant RHR-associated variants that passed the Radial test and available in the Fenland genotyping data;

Instrument 3: 148 Radial-filtered RHR-associated variants that were also nominally significant in exercise-based fitness GWAS results in UK Biobank ($p < 0.05$);

Instrument 4: the combined fitness score: 160 independent variants combining the list of variants from (1) and (3); variant pairs in LD ($r^2 > 0.01$) were identified and the variants selected as independent top fitness variants (among (1)) were preferred.

† Only SNPs available in the Fenland study were included in the analyses, which is the reason why the number of SNPs listed here do not match the number of SNPs included in the instruments constructed in the UK Biobank.

‡ 2-sample MR approach: the genetic instruments of fitness in UK Biobank were treated as the exposure, fitness GWAS in the Fenland study was the outcome.

Supplementary table 6. Results of Mendelian randomisation analyses between cardiorespiratory fitness and type 2 diabetes.

exposure*	nsnp	method	beta	s.e.	p	CochQp
Combined fitness score	157	Inverse variance weighted	-0.028	0.016	0.086	<0.001
	157	MR Egger	0.003	0.036	0.937	
	157	Egger Intercept	-0.005	0.005	0.330	
	157	Weighted median	-0.007	0.010	0.442	
	157	Penalised weighted median	-0.008	0.009	0.400	
	157	MR-PRESSO Raw	-0.029	0.017	0.085	
	148	MR-PRESSO Outlier-corrected	-0.019	0.008	**0.016**	
	160	One-sample MR†	-0.037	0.007	**8.8E-08**	
Radial filtered fitness score	126	Inverse variance weighted	-0.017	0.006	**0.005**	0.686
	126	MR Egger	-0.014	0.013	0.274	
	126	Egger Intercept	-0.001	0.002	0.780	
	126	Weighted median	-0.009	0.010	0.373	
	126	Penalised weighted median	-0.008	0.010	0.412	
	126	One-sample MR†	-0.022	0.008	**0.005**	

GWAS summary statistics of the outcome type 2 diabetes were obtained from Mahajan *et al*.[96] with UK Biobank cohort depleted

Effect sizes are log Odds of diabetes per unit higher fitness (ml $O2 \cdot min^{-1} \cdot kg^{-1}$ FFM)

* The combined fitness score used here contains 157 variants instead of 160 because only 121 variants were directly available in the type 2 diabetes GWAS, 36 proxies (LD > 0.8) were found using UK Biobank Random 25K unrelated White British cohort as the reference panel; The Radial filtered fitness score was obtained by conducting Radial-filtering from fitness to type 2 diabetes first, removing 34 variants as outliers, hence, 126 variants remained for further analyses.

† Logistic regression model was used to assess the association between 160-variant or Radial-filtered 126-variant fitness GRS and prevalent type 2 diabetes in the UK Biobank; the model was adjusted for age, sex, genotyping array, first 10 principal components; a total of 18,994 cases and 330,636 non-cases were included in the analyses.

Supplementary Table 7. Mendelian Randomisation results for cardiorespiratory fitness on intermediate traits.

Outcome	Method	Radial-filtered	n_SNPs	betaIVW*	sebetaIVW	pIVW	CochQp
Fasting Insulin	Inverse variance weighted	No	156	-0.0073	0.0052	0.1602	0.00
	MR Egger	No	156	-0.0135	0.0113	0.2363	
	Egger Intercept	No	156	0.0010	0.0017	0.5430	
	Weighted median	No	156	-0.0129	0.0052	0.0144	
	Inverse variance weighted	Yes	134	-0.0112	0.0032	**0.0006**	0.79
	MR Egger	Yes	134	-0.0145	0.0068	0.0339	
	Egger Intercept	Yes	134	0.0006	0.0010	0.5624	
	Weighted median	Yes	134	-0.0132	0.0051	**0.0110**	
Fasting glucose	Inverse variance weighted	No	156	0.0011	0.0057	0.8544	0.00
	MR Egger	No	156	-0.0032	0.0125	0.7982	
	Egger Intercept	No	156	0.0007	0.0018	0.7019	
	Weighted median	No	156	0.0000	0.0043	1.0000	
	Inverse variance weighted	Yes	140	-0.0002	0.0027	0.9260	0.98
	MR Egger	Yes	140	-0.0038	0.0057	0.5059	
	Egger Intercept	Yes	140	0.0006	0.0008	0.4233	
	Weighted median	Yes	140	0.0000	0.0042	0.9980	
2-hour glucose	Inverse variance weighted	No	156	-0.0157	0.0072	0.0293	0.00
	MR Egger	No	156	0.0042	0.0156	0.7877	
	Egger Intercept	No	156	-0.0033	0.0023	0.1524	
	Weighted median	No	156	-0.0120	0.0084	0.1537	
	Inverse variance weighted	Yes	141	-0.0099	0.0051	0.0524	0.86
	MR Egger	Yes	141	-0.0044	0.0122	0.7161	
	Egger Intercept	Yes	141	-0.0009	0.0016	0.5947	
	Weighted median	Yes	141	-0.0115	0.0078	0.1441	
HbA1c	Inverse variance weighted	No	155	-0.0011	0.0048	0.8205	0.00
	MR Egger	No	155	0.0063	0.0104	0.5467	
	Egger Intercept	No	155	-0.0012	0.0015	0.4257	
	Weighted median	No	155	0.0004	0.0049	0.9330	
	Inverse variance weighted	Yes	137	-0.0009	0.0031	0.7753	0.74
	MR Egger	Yes	137	0.0046	0.0065	0.4840	
	Egger Intercept	Yes	137	-0.0009	0.0009	0.3253	
	Weighted median	Yes	137	0.0005	0.0048	0.9248	
BMI	Inverse variance weighted	No	157	-0.0033	0.0038	0.3883	0.00
	MR Egger	No	157	-0.0012	0.0085	0.8925	
	Egger Intercept	No	157	-0.0003	0.0012	0.7782	
	Weighted median	No	157	-0.0002	0.0022	0.9411	
	Inverse variance weighted	Yes	108	-0.0024	0.0015	0.1028	0.11
	MR Egger	Yes	108	-0.0044	0.0031	0.1568	
	Egger Intercept	Yes	108	0.0003	0.0005	0.4698	
	Weighted median	Yes	108	-0.0003	0.0022	0.8883	
SHBG	Inverse variance weighted	No	160	0.0092	0.0036	0.0106	0.00
	MR Egger	No	160	-0.0012	0.0078	0.8772	
	Egger Intercept	No	160	0.0017	0.0011	0.1345	
	Weighted median	No	160	0.0027	0.0011	0.0173	
	Inverse variance weighted	Yes	99	0.0059	0.0008	**9.6E-11**	0.11
	MR Egger	Yes	99	0.0042	0.0019	0.0284	
	Egger Intercept	Yes	99	0.0003	0.0003	0.3090	
	Weighted median	Yes	99	0.0056	0.0013	**2.4E-05**	

*effect size are per unit higher of fitness (ml $O2 \cdot min^{-1} \cdot kg^{-1}$ FFM).

Supplementary Table 8. Multivariable Mendelian randomisation analysis results of cardiorespiratory fitness on type 2 diabetes adjusted for intermediate traits.

Covariate	n_snp	beta†	s.e.	p	p_heterogeneity
None	126	-0.017	0.006	0.005	0.686
Fasting Insulin (adj.BMI)	124	-0.015	0.006	0.018	0.764
Fasting Glucose (adj.BMI)	124	-0.018	0.006	0.003	0.907
2-hr Glucose (adj.BMI)	124	-0.015	0.006	0.013	0.708
HbA1c	124	-0.015	0.006	0.012	0.914
BMI	126	-0.015	0.006	0.017	0.757
All 5 above intermediate traits	124	-0.013	0.006	0.043	0.968
Sex hormone binding globulin	126	-0.016	0.006	0.011	

*adj.BMI: adjusted for body mass index in the original genome-wide association analysis model for the intermediate trait

† beta values are log Odds of diabetes per ml O2·min^{-1}·kg^{-1} FFM.

Supplementary Table 9. Associations between the enhanced 160-SNP genetic risk score for cardiorespiratory fitness and Protein Targets assessed by the aptamer-based technology (SomaScan©) in 10,707 individuals from the Fenland study.

Target	TargetFullName	UniProtId	GeneName	beta	se	p
N-terminal pro-BNP	N-terminal pro B-type natriuretic peptide	P16860	*NPPB*	0.058	0.008	9.45E-13
MSP	Hepatocyte growth factor-like protein	P26927	*MST1*	-0.042	0.009	2.59E-06
MYL6B	Myosin light chain 6B	P14649	*MYL6B*	0.036	0.009	4.66E-05
MXRA7	Matrix-remodeling-associated protein 7	P84157	*MXRA7*	0.033	0.009	1.26E-04
CD248	Endosialin	Q9HCU0	*CD248*	0.033	0.009	1.93E-04
MYPC1	Myosin-binding protein C, slow-type	Q00872	*MYBPC1*	0.032	0.009	2.29E-04
WISP-2	WNT1-inducible-signaling pathway protein 2	O76076	*WISP2*	0.031	0.009	4.24E-04
HS3S4	Heparan sulfate glucosamine 3-O-sulfotransferase 4	Q9Y661	*HS3ST4*	0.030	0.009	5.06E-04
MMP-2	72 kDa type IV collagenase	P08253	*MMP2*	0.031	0.009	5.21E-04
Apo B	Apolipoprotein B	P04114	*APOB*	0.030	0.009	5.81E-04
MYOC	Myocilin	Q99972	*MYOC*	0.028	0.008	6.33E-04
VPS29	Vacuolar protein sorting-associated protein 29	Q9UBQ0	*VPS29*	-0.030	0.009	6.60E-04
SHBG	Sex hormone-binding globulin	P04278	*SHBG*	0.026	0.008	9.86E-04
PAFAH	Platelet-activating factor acetylhydrolase	Q13093	*PLA2G7*	0.029	0.009	9.91E-04

Supplementary Table 10. DEPICT gene-set enrichment results for significant independent variants associated with cardiorespiratory fitness ($p<10^{-5}$).

Original gene set description	Nominal P value	FDR	First 10 genes in the reconstituted gene set (Z score)
sequence-specific DNA binding RNA polymerase II transcription factor activity	2.25E-06	**<0.05**	NR2F6 (3.0)|C5orf41 (2.9)|ACVR2B (2.5)|ZFPM2 (2.4)|LMX1B (2.4)|ZNF219 (2.4)|KIAA1267 (2.3)|TNRC6B (2.2)|WT1-AS (2.2)|PVRL1 (2.2)
RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in positive regulation of transcription	2.11E-05	<0.20	WT1-AS (3.8)|ZFPM2 (2.9)|LMX1B (2.6)|ACVR2B (2.4)|SGIP1 (2.2)|MAPT (2.2)|SMAD1 (2.1)|BNC2 (2.1)|ENSG00000224376 (2.0)|THRB (1.7)
muscle cell differentiation	2.84E-05	<0.20	C1orf105 (5.1)|CCDC141 (3.9)|SCN5A (3.8)|SVIL (3.2)|NKX2-5 (3.0)|PLN (2.9)|PLCE1 (2.9)|MYH7 (2.6)|TTN (2.6)|KRT83 (2.5)
muscle structure development	3.64E-05	<0.20	C1orf105 (6.3)|CCDC141 (3.8)|SCN5A (3.6)|MYH7 (3.4)|TTN (3.4)|SVIL (2.9)|NKX2-5 (2.9)|PLN (2.8)|MYH6 (2.6)|PLCE1 (2.3)
muscle tissue development	3.82E-05	<0.20	C1orf105 (5.8)|SCN5A (4.4)|CCDC141 (3.8)|MYH7 (3.3)|SVIL (3.2)|NKX2-5 (3.1)|MYH6 (2.9)|TTN (2.9)|PLCE1 (2.6)|PLN (2.6)
RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity	4.58E-05	<0.20	WT1-AS (2.7)|CDH11 (2.7)|LMX1B (2.6)|ACVR2B (2.3)|SMAD1 (2.1)|MAPT (2.0)|TSLP (1.8)|KIAA1267 (1.8)|SOCS6 (1.8)|DSP (1.8)
RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity	6.54E-05	<0.20	ENSG00000253744 (2.5)|ZFPM2 (2.3)|NKX2-5 (2.3)|ARHGEF40 (2.2)|KIAA1755 (2.1)|HABP2 (2.0)|DSP (1.9)|BNC2 (1.9)|PVRL1 (1.9)|NR2F6 (1.9)
striated muscle tissue development	7.50E-05	<0.20	C1orf105 (6.0)|SCN5A (4.5)|CCDC141 (4.1)|NKX2-5 (3.4)|MYH7 (3.4)|MYH6 (3.0)|TTN (3.0)|SVIL (2.9)|PLCE1 (2.7)|OSBPL6 (2.6)
negative regulation of cell migration	1.25E-04	<0.20	USHBP1 (2.8)|ADAMTSL3 (2.7)|ENSG00000224376 (2.0)|CACNA1C (2.0)|C3orf54 (1.9)|MUC5B (1.9)|PLEKHM1 (1.8)|KIAA1755 (1.8)|ADM (1.7)|RASGRF2 (1.7)
cell-matrix adhesion	1.26E-04	<0.20	CCDC141 (2.8)|AMT (2.8)|DAG1 (2.3)|PLEKHA3 (2.3)|FKBP7 (2.3)|ADAMTSL3 (2.2)|THRB-IT1 (2.2)|USHBP1 (2.1)|ENSG00000224376 (1.9)|PLEC (1.9)
striated muscle cell differentiation	1.34E-04	<0.20	C1orf105 (6.0)|SCN5A (5.1)|CCDC141 (4.5)|NKX2-5 (3.7)|PLN (3.5)|KRT83 (3.3)|SVIL (3.3)|MYH7 (3.2)|MYH6 (3.0)|PLCE1 (2.8)
muscle organ development	1.61E-04	<0.20	C1orf105 (6.9)|SCN5A (3.7)|CCDC141 (3.5)|MYH7 (3.5)|TTN (3.4)|OSBPL6 (2.8)|NKX2-5 (2.8)|SVIL (2.8)|MYH6 (2.5)|PLN (2.3)
increased cardiac muscle contractility	2.09E-04	<0.20	MYH7 (8.0)|MYH6 (7.9)|PLN (6.7)|TTN (6.2)|NKX2-5 (5.4)|SCN5A (4.7)|C1orf105 (4.1)|CACNA1C (3.6)|ENSG00000237975 (2.8)|DSP (2.4)

Supplementary Table 11. MAGMA gene-set analysis using cardiorespiratory fitness GWAS summary statistics as implemented in FUMA.

Gene Set	N genes	Beta	Beta STD	SE	P	Pbon
GO_bp:go_regulation_of_cardiac_muscle_contraction	70	0.65676	0.039576	0.11696	9.97E-09	**1.54E-04**
GO_bp:go_cardiac_muscle_contraction	125	0.40524	0.032585	0.08102	2.87E-07	**4.45E-03**
GO_bp:go_regulation_of_actin_filament_based_movement	40	0.68116	0.031053	0.14684	1.77E-06	**2.74E-02**
GO_bp:go_cardiac_muscle_cell_action_potential	63	0.51723	0.029574	0.11169	1.84E-06	**2.84E-02**
GO_bp:go_regulation_of_striated_muscle_contraction	84	0.46534	0.030706	0.10093	2.03E-06	**3.14E-02**
GO_bp:go_striated_muscle_contraction	158	0.31838	0.028757	0.071131	3.83E-06	5.93E-02
GO_bp:go_regulation_of_heart_rate	92	0.41899	0.028929	0.095165	5.38E-06	8.33E-02
GO_bp:go_ventricular_cardiac_muscle_cell_action_potential	32	0.72529	0.02958	0.16736	7.38E-06	1.14E-01
GO_bp:go_cardiac_muscle_cell_contraction	64	0.48413	0.0279	0.11321	9.55E-06	1.48E-01

* Bold gene sets were enriched based on Bonferroni corrected p-value

Supplementary Table 12. Enriched gene-sets from various MSigDB resourses (FDR ≤ 0.05) using FUMA with 160 prioritised genes associated with cardiorespiratory fitness.

Database	GeneSet	N	n	P-value	adjusted P	Genes
KEGG	dilated cardiomyopathy	89	6	7.1E-05	0.0133	CACNA1C, MYH6, MYH7, TTN, ITGA4, PLN
KEGG	hypertrophic cardiomyopathy	82	5	4.6E-04	0.0432	CACNA1C, MYH6, MYH7, TTN, ITGA4
REACTOME	muscle contraction	206	11	6.9E-07	0.0010	GUCY1A2, SLN, CACNA1C, MYH6, MYH11, TTN, SCN5A, SCN10A, SCN11A, NKX2-5, PLN
GO BiologicalProcesses	cardiac muscle contraction	132	9	9.8E-07	0.0072	CACNA1C, MYH6, MYH7, TTN, SCN5A, SCN10A, NKX2-5, PLN, GJA1
GO BiologicalProcesses	striated muscle contraction	166	9	6.5E-06	0.0239	CACNA1C, MYH6, MYH7, TTN, SCN5A, SCN10A, NKX2-5, PLN, GJA1
GO BiologicalProcesses	heart process	278	11	1.2E-05	0.0305	CACNA1C, MYH6, MYH7, TTN, SRC, THRB, SCN5A, SCN10A, NKX2-5, PLN, GJA1
Cancer gene modules	module201 (B lymphoma)	48	5	3.6E-05	0.0070	MYH7, MYH11, SCN5A, PLN, GJA1
Cancer gene modules	module387 (B lymphoma, breast cancer; liver cancer)	49	5	4.0E-05	0.0070	MYH7, MYH11, SCN5A, PLN, GJA1
Cancer gene modules	module329 (B lymphoma; liver cancer)	51	5	4.9E-05	0.0070	MYH7, MYH11, SCN5A, PLN, GJA1

Supplementary Table 13. DEPICT results for tissue and cell-type specific enrichment using significantly independent variants from cardiorespiratory fitness GWAS ($p < 10^{-5}$).

MeSH term	Name	MeSH 1st level term	MeSH 2nd level term	Nominal P value	FDR	Top 10 tissue-specific expressed genes (Z score)
A07.541	Heart	Cardiovascular System	Heart	1.42E-04	<0.05	NR2F6 (3.0)|C5orf41 (2.9)|ACVR2B (2.5)|ZFPM2 (2.4)|LMX1B (2.4)|ZNF219 (2.4)|KIAA1267 (2.3)|TNRC6B (2.2)|WT1-AS (2.2)|PVRL1 (2.2)
A07.541.358	Heart Atria	Cardiovascular System	Heart	1.62E-04	<0.05	WT1-AS (3.8)|ZFPM2 (2.9)|LMX1B (2.6)|ACVR2B (2.4)|SGIP1 (2.2)|MAPT (2.2)|SMAD1 (2.1)|BNC2 (2.1)|ENSG00000224376 (2.0)|THRB (1.7)
A07.541.358.100	Atrial Appendage	Cardiovascular System	Heart	1.90E-04	<0.05	C1orf105 (5.1)|CCDC141 (3.9)|SCN5A (3.8)|SVIL (3.2)|NKX2-5 (3.0)|PLN (2.9)|PLCE1 (2.9)|MYH7 (2.6)|TTN (2.6)|KRT83 (2.5)
A07.541.560	Heart Ventricles	Cardiovascular System	Heart	4.65E-04	<0.05	C1orf105 (6.3)|CCDC141 (3.8)|SCN5A (3.6)|MYH7 (3.4)|TTN (3.4)|SVIL (2.9)|NKX2-5 (2.9)|PLN (2.8)|MYH6 (2.6)|PLCE1 (2.3)
A07.541.510.110	Aortic Valve	Cardiovascular System	Heart	3.53E-03	<0.20	C1orf105 (5.8)|SCN5A (4.4)|CCDC141 (3.8)|MYH7 (3.3)|SVIL (3.2)|NKX2-5 (3.1)|MYH6 (2.9)|TTN (2.9)|PLCE1 (2.6)|PLN (2.6)
A07.541.510	Heart Valves	Cardiovascular System	Heart	3.53E-03	<0.20	WT1-AS (2.7)|CDH11 (2.7)|LMX1B (2.6)|ACVR2B (2.3)|SMAD1 (2.1)|MAPT (2.0)|TSLP (1.8)|KIAA1267 (1.8)|SOCS6 (1.8)|DSP (1.8)
A05.360.319.679.690	Myometrium	Urogenital System	Genitalia	5.86E-03	<0.20	ENSG00000253744 (2.5)|ZFPM2 (2.3)|NKX2-5 (2.3)|ARHGEF40 (2.2)|KIAA1755 (2.1)|HABP2 (2.0)|DSP (1.9)|BNC2 (1.9)|PVRL1 (1.9)|NR2F6 (1.9)