Supplementary Information

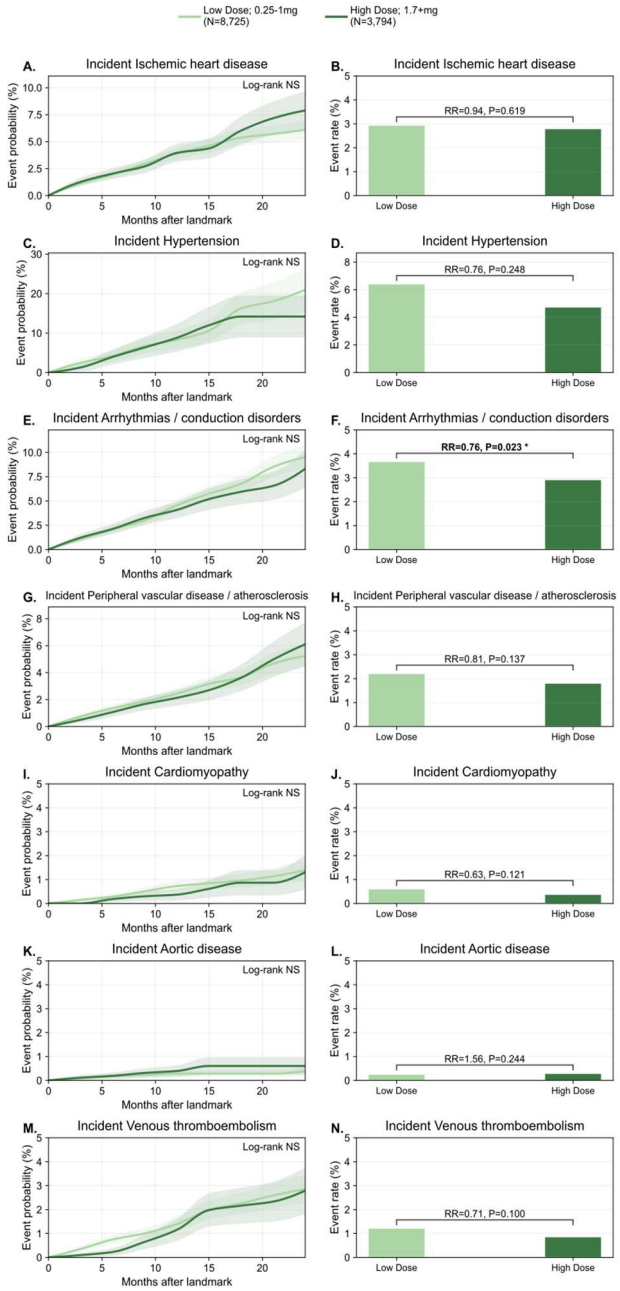

Fig. S1. Higher maximum semaglutide dose was associated with lower post-landmark incidence of select cardiovascular conditions. Patients were grouped by the maximum semaglutide dose reached by the 2-year landmark as low dose (0.25–1.0 mg; n = 8,725) or high dose (≥1.7 mg; n = 3,794), and incident cardiovascular outcomes were assessed only after the landmark among patients event-free through that timepoint. Left-column panels show cumulative post-landmark event probability over follow-up for incident ischemic heart disease (A), incident hypertension (C), incident arrhythmias/conduction disorders (E), incident peripheral vascular disease/atherosclerosis (G), incident cardiomyopathy (I), incident aortic disease (K), and incident venous thromboembolism (M). Right-column panels show the corresponding post-landmark event rates with relative risks (RR) and nominal P values for incident ischemic heart disease (B), incident hypertension (D), incident arrhythmias/conduction disorders (F), incident peripheral vascular disease/atherosclerosis (H), incident cardiomyopathy (J), incident aortic disease (L), and incident venous thromboembolism (N).

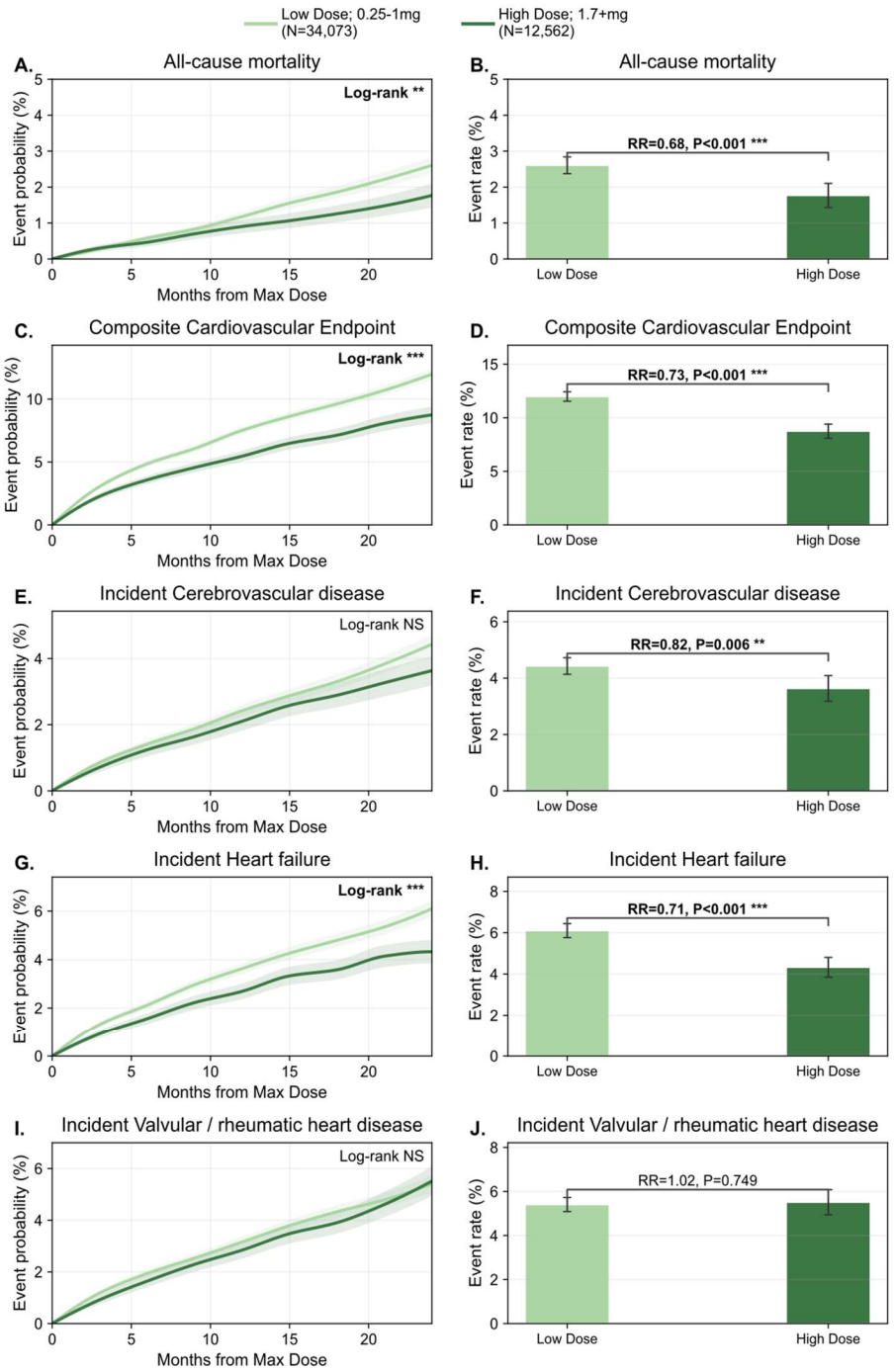

Fig. S2. Higher maximum semaglutide dose was associated with lower cardiovascular risk. Patients with at least one cardiovascular condition were grouped by the maximum semaglutide dose reached within a 2-year observation window as low dose (0.25–1.0 mg; n=34,073) or high dose (≥1.7 mg; n=12,562). For each patient, outcomes were tracked from the date they first achieved their maximum dose, with follow-up extending up to 24 months thereafter. Left-column panels show cumulative event probability and right-column panels show the corresponding 24-month event rates for all-cause mortality (A, B), composite cardiovascular events (C, D), incident cerebrovascular disease (E, F), incident heart failure (G, H), and incident valvular/rheumatic heart disease (I, J). Error bars represent 95% confidence intervals derived from Greenwood's formula; relative risks and nominal P values comparing high versus low dose are shown on the bar plots. Higher-dose semaglutide was associated with lower risk across all outcomes examined.

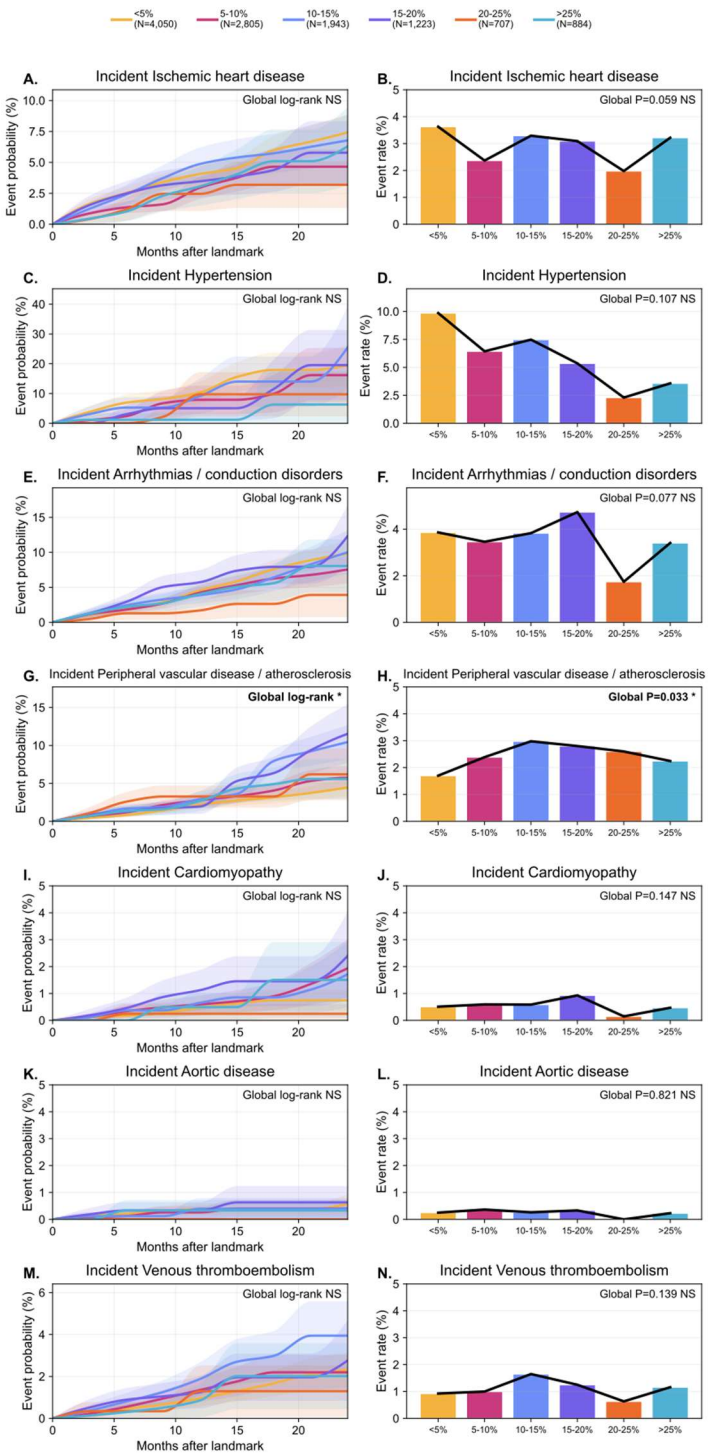

Fig. S3. Semaglutide 2-year maximum weight-loss landmark analysis: incident cardiovascular conditions by weight-loss category. Patients were categorized according to the maximum percentage reduction in body weight achieved before the 2-year landmark as <5% (n = 4,050), 5–10% (n = 2,805), 10–15% (n = 1,943), 15–20% (n = 1,223), 20–25% (n = 707), or >25% (n = 884), and incident cardiovascular outcomes were assessed only after the landmark among patients event-free through that timepoint. Left-column panels show cumulative post-landmark event probability over follow-up for incident ischemic heart disease (A), incident hypertension (C), incident arrhythmias/conduction disorders (E), incident peripheral vascular disease/atherosclerosis (G), incident cardiomyopathy (I), incident aortic disease (K), and incident venous thromboembolism (M). Right-column panels show the corresponding post-landmark event rates across weight-loss categories for incident ischemic heart disease (B), incident hypertension (D), incident arrhythmias/conduction disorders (F), incident peripheral vascular disease/atherosclerosis (H), incident cardiomyopathy (J), incident aortic disease (L), and incident venous thromboembolism (N), with global P values indicated.

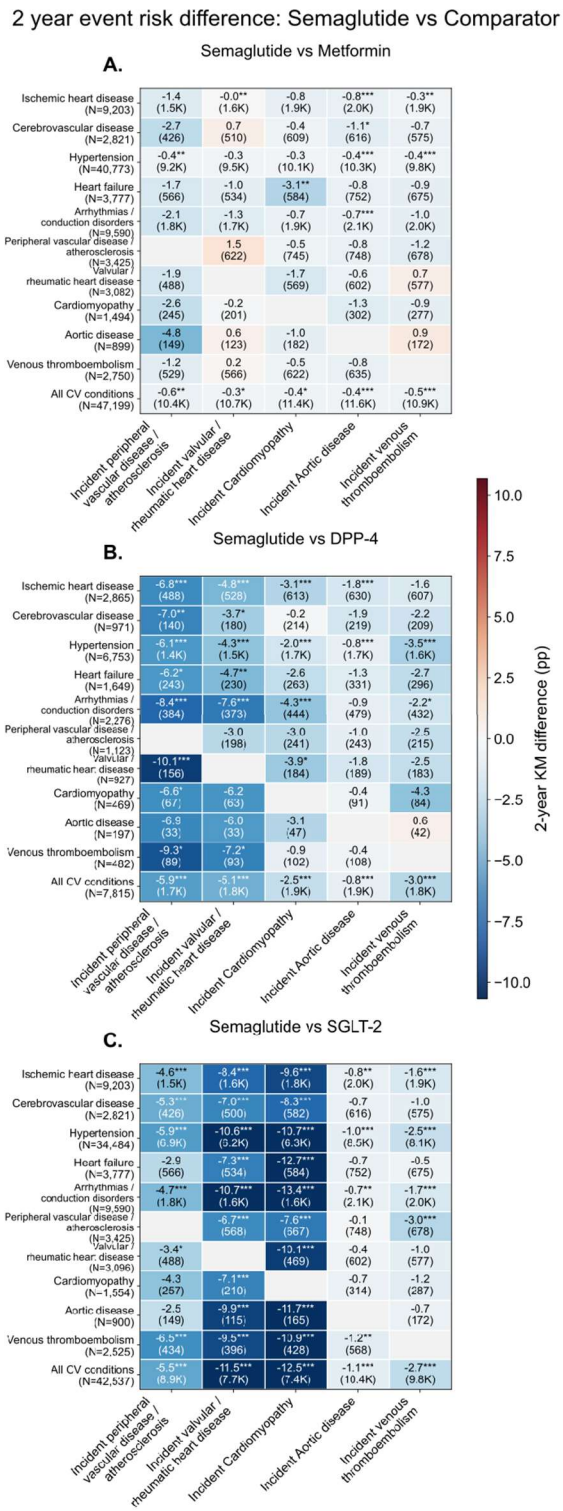

Fig. S4. Semaglutide vs comparator anti-diabetic drugs' absolute 2-year event risk differences across baseline cardiovascular burden subgroups for additional cardiovascular outcomes. (A) Semaglutide vs Metformin; (B) Semaglutide vs DPP-4 inhibitors; (C) Semaglutide vs SGLT-2 inhibitors. Heatmaps show absolute 2-year event risk differences across baseline cardiovascular burden subgroups. Rows indicate baseline cardiovascular burden subgroups, whereas columns indicate incident peripheral vascular disease/atherosclerosis, valvular/rheumatic heart disease, cardiomyopathy, aortic disease, and venous thromboembolism. Cell values represent the difference in approximate 2-year event risk between semaglutide and the respective comparator, expressed in percentage points (pp). Asterisks denote statistical significance (* p<0.05, ** p<0.01, *** p<0.001).

Fig. S5. Bulk RNA expression profiling of GLP1R with Heart and Cardiovascular Samples in this study. https://nfer-workspaces.com/rna_lab/results?token=glp+1r&ranges=%5B0%2C0.013%2C1.004%2C6.651%5D&cell_line_data_source=not_cell_line&single_cell_data_source=not_single_cell&diff_data_type=gtex_tcga&input_type=gene&collections=%5Broot-cells-and-tissues%2Croot-diseases%5D&enriched_study=high&include_cohen_d_pos=true&include_cohen_d_neg=true&single_cell_details_sort=adj_score&tissue_spec_dataset=gtex&include_cohen_d_pos_tiss_spec=true&include_cohen_d_neg_tiss_spec=true&cluster_name=all&cluster_category=all&study_exp_range=%5B0%2C14%5D&includeSamples=all&ensg_symbol=ENSG00000112164&enst_symbol=none&extraction_mode=ees_tagged&corr_data_type=whole_group&data_type=cohort_info&groupA=_%24eyIxIjp7InNhbXBsZUNvdW50Ijo2Mzk5MCwiZXhwUmFuZ2UiOiswLDE0XSwiY2F0ZWdvcnlKb2luIjp7IndpdGhpbl9jYXRlZ29yeV9qb2luIjp7Im5mZXItc3R1ZGlcyI6InVuaW9uIiwibmZlci1waGxzLWFuZC10aXNzdWVzIjoiaW50ZXJzZWN0aW9uIiwibmZlci1kcnVncyI6ImludGVyc2VjdGlvbiIsImNlbGwtbGluZS1jZWN0aW9uIiwibmZlci1hZ2UiOiJpbnRlcnNlY3Rpb24iLCJuZmVyLWdlbmRlciI6ImludGVyc2VjdGlvbiIsInJhdy1lZXRhZGF0YSI6ImludGVyc2VjdGlvbiJ9fSwiYWxsU2FtcGxlcyI6ImFsbCIsImF0bGFzIjoiaW50ZXJzZWN0aW9uIiwiY2VsbFR5cGUiOiJhbGwiLCJwb3NpdGlvbiI6ImFsbCIsInRpdGxlIjoiMTcgMSBjaGFyYWN0ZXJpc3RpYyB8IDE0NzkgU3R1ZGllcyJ9fQ%3D%3D&prune_outliers=false
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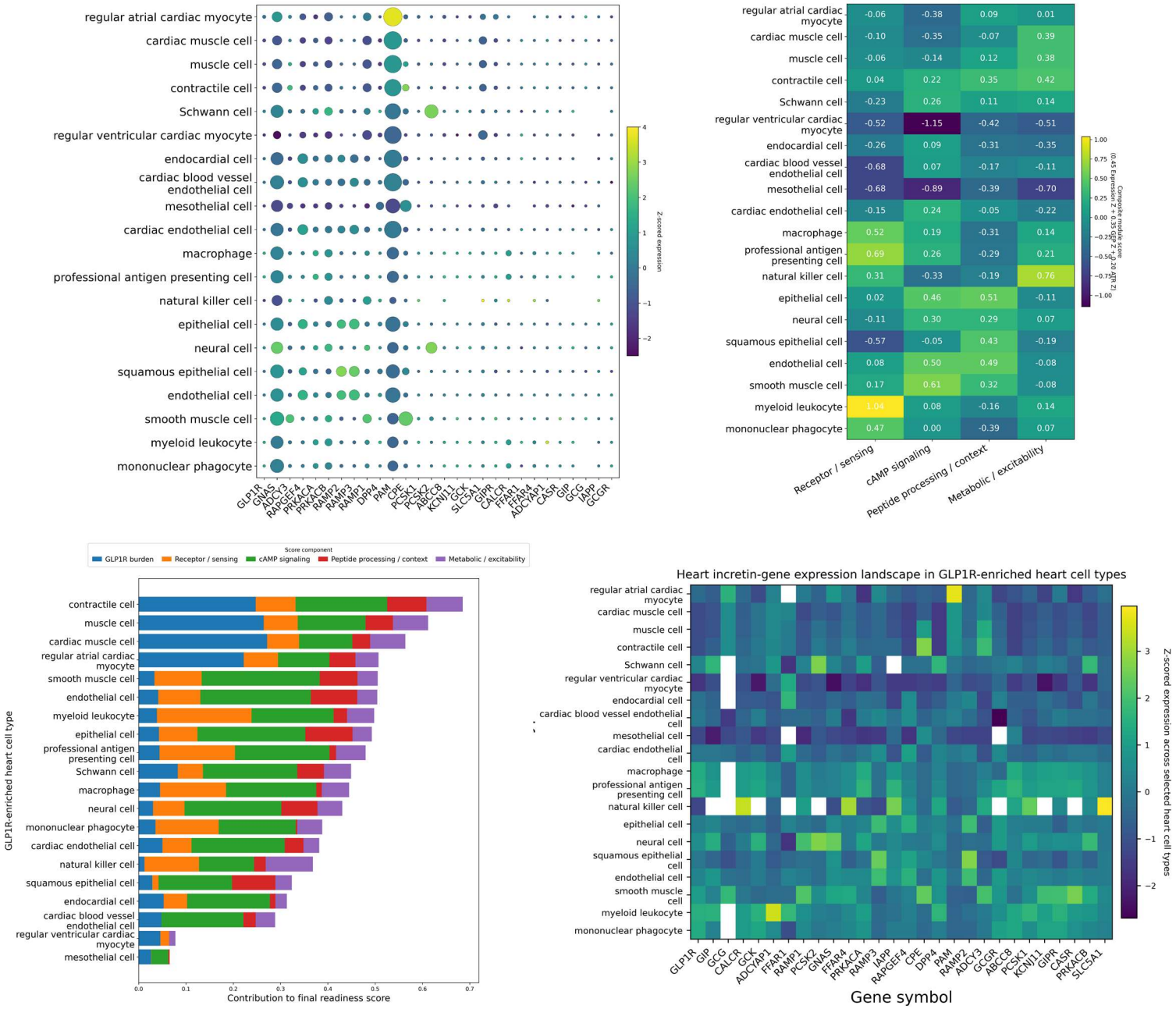

Fig. S6. System of incretin genes and their expression patterns in single-cell RNA-sequencing data from heart tissue, with emphasis on GLP1R-enriched cardiovascular cell types. (Top left), Dot plot of semaglutide-relevant genes across GLP1R-enriched heart cell types. Dot size denotes the fraction of cells within each cell type expressing the indicated gene, and dot color denotes gene-wise z-scored expression across the selected heart cell types. **(Top Right),** Functional module heatmap summarizing semaglutide-response machinery across the same GLP1R-enriched heart cell types. Module scores represent composite z-scored signals derived from receptor and sensing genes, cAMP signaling genes, peptide-processing and contextual-modulation genes, and metabolic or excitability-associated genes. **(Bottom Left),** Ranked stacked-bar plot of a composite semaglutide functional-readiness score across GLP1R-enriched heart cell types. Total bar length represents the final readiness score, and colored segments denote the relative contribution from GLP1R burden, receptor and sensing context, cAMP signaling, peptide-processing context, and metabolic or excitability-associated programs. **(Bottom Right),** Heatmap of gene-wise z-scored expression across GLP1R-enriched heart cell types, highlighting coordinated enrichment patterns across cardiomyocyte-contractile, endothelial-epithelial, neuroglial and immune compartments. Together, these panels move beyond receptor detection alone to identify heart cell types that are transcriptionally positioned for potential semaglutide responsiveness.

Table S1. Clinical evidence of cardiovascular benefits for Semaglutide and Tizepatide.

Drug	Population / condition	Cardiovascular findings established or supported in humans	Evidence tier	Key reference(s)
Semaglutide	Overweight/obesity with established ASCVD, without diabetes	Reduced 3-point Major Adverse Cardiovascular Events (MACE), defined as cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke	Randomized trial; label-supporting	SELECT / NEJM (New England Journal of Medicine)
Semaglutide	Type 2 diabetes with high cardiovascular risk	Reduced 3-point MACE versus placebo; strongest individual component signal was for nonfatal stroke; cardiovascular death alone was not significantly reduced	Randomized trial	SUSTAIN-6 / NEJM (New England Journal of Medicine)
Semaglutide	Obesity-related HFpEF	Improved heart-failure symptoms, physical limitations, exercise function, body weight, inflammatory measures and NT-proBNP; pooled analyses also showed reduction in cardiovascular death or worsening heart-failure events, driven mainly by fewer worsening heart-failure events	Randomized trials / pooled randomized analyses	STEP-HFpEF / NEJM, Circulation, JACC (New England Journal of Medicine)
Semaglutide	Obesity/overweight with prevalent heart failure in SELECT	Improved MACE and a heart-failure composite endpoint; favorable signals for cardiovascular death and all-cause death in the prevalent-heart-failure subgroup	Prespecified randomized subgroup analysis	Deanfield et al. / Lancet (The Lancet)
Semaglutide	ASCVD plus overweight/obesity, without diabetes	Associated with lower revised MACE-3, revised MACE-5, all-cause mortality, cardiovascular-related mortality and hospitalization for heart failure	Real-world observational	Smolderen et al. / Diabetes Obes Metab, JAHA/JACC-type reports (PMC)
Semaglutide	Broad clinical-practice comparator analyses	Associated with lower myocardial infarction or stroke composite risk versus sitagliptin-like comparator strategies in routine care	Real-world observational	Clinical-practice comparative analyses (PMC)
Tirzepatide	Obesity-related HFpEF	Reduced the composite of cardiovascular death or worsening heart-failure events, largely through fewer worsening heart-failure events; improved health status/KCCQ and exercise tolerance; cardiovascular death alone was not significantly reduced	Randomized trial	SUMMIT / NEJM, ACC, Circulation (New England Journal of Medicine)
Tirzepatide	Type 2 diabetes with established ASCVD	Demonstrated cardiovascular noninferiority versus dulaglutide for the composite of cardiovascular death, myocardial infarction, or stroke; superiority for MACE was not established	Randomized CV outcomes trial	SURPASS-CVOT / NEJM, ACC (American College of Cardiology)
Tirzepatide	Type 2 diabetes, BMI ≥25, pre-existing ischemic heart disease	Associated with lower risk of a composite of acute myocardial infarction, ischemic stroke and all-cause mortality; acute myocardial infarction and all-cause mortality were individually lower	Real-world observational	Dani et al. / JACC Advances-like observational study (JACC)
Tirzepatide	Broader real-world comparative analyses	Cardiovascular benefit in practice appears broadly comparable to semaglutide overall, with favorable but not definitive signals relative to active comparators	Real-world observational / comparative effectiveness	Observational comparative analyses and reviews (Taylor & Francis Online)

Table S2. Baseline Demographic and Clinical Characteristics of Semaglutide-Treated Patients by Maximum Dose Reached During the 2-Year Observation Period

Dosage	Number of unique patients	Age, Mean (SD)	Female, %	T2DM Prevalence at Baseline, %	Baseline HBA1C, Mean (SD)
0.25 mg	2,192	57.1 (12.6)	71.3	31.2	6.0 (1.3)
0.5 mg	3,383	60.1 (12.3)	65.3	33.4	6.6 (1.7)
1.0 mg	2,386	59.9 (12.0)	63.1	40.7	6.9 (1.7)
1.7 mg	353	53.4 (11.5)	75.8	46.2	5.7 (0.5)
2.0 mg	1,095	59.4 (11.7)	64.4	46.2	6.9 (1.7)
2.4 mg	1,108	53.6 (10.9)	67.5	44.9	5.6 (0.6)

Table S3. Baseline Demographic and Clinical Characteristics of Low-Dose and High-Dose Semaglutide-Treated Patients During the 2-Year Observation Period.

Dose group	Number of unique patients	Age, Mean (SD)	Female, %	Baseline T2DM Prevalence,%	Baseline HBA1C, Mean (SD)	Number of Semaglutide prescriptions, Median [IQR]		Change in Semaglutide prescriptions (Post - Pre landmark), Median [IQR]
						0 to 2 years (Landmark period)	2-4 years (Post-landmark)	
Low Dose (0.25mg - 1mg)	8,725	59.3 (12.3)	65.9	30.2	6.6 (1.7)	1 [1-2]	0 [0-2]	-1 [-2, -1]
High Dose (1.7mg+)	3,794	57.8 (12.1)	66.6	27.1	6.4 (1.5)	5 [1-9]	1 [0-2]	-4 [-9, -1]

Table S4. Semaglutide prescription counts by weight-loss category before and after key treatment landmarks, median [IQR].

Weight loss Category	Number of unique patients	Number of Semaglutide prescriptions, Median [IQR]			Change in Semaglutide prescriptions (Post - Pre landmark), Median [IQR]
		0 to 2 years (Landmark period)	2-4 years (Post landmark)	(2 years post max weight loss attainment)	
<5%	4,050	1 [1, 2]	0 [0, 2]	0 [0, 2]	-1 [-3, -1]
5-10%	2,805	2 [1, 5]	0 [0, 2]	0 [0, 2]	-2 [-5, -1]
10-15%	1,943	2 [1, 7]	0 [0, 2]	1 [0, 2]	-3 [-7, -1]
15-20%	1,223	4 [1, 9]	1 [0, 2]	1 [0, 2]	-4 [-8, -1]
20-25%	707	5 [1, 10]	1 [0, 2]	1 [0, 2]	-5 [-9, -1]
25%+	884	5 [2, 11]	0 [0, 1]	0 [0, 1]	-4 [-8, -1]

Table S5. Single Cell RNA profile of GLP1R in the human heart.

Tissue	Cell Type	Expression ln(CP10K)	% Cells Expressing Genes
heart	regular atrial cardiac myocyte	1.83	1.36
heart	cardiac muscle cell	1.77	1.11
heart	muscle cell	1.77	1.05
heart	contractile cell	1.77	0.92
heart	dermis microvascular lymphatic vessel endothelial cell	2.20	0.53
heart	Schwann cell	1.88	0.52
heart	aggregated	1.80	0.41
heart	cell	1.80	0.41
heart	regular ventricular cardiac myocyte	1.72	0.38
heart	fetal cardiomyocyte	2.06	0.34
heart	endocardial cell	1.84	0.33
heart	cardiac blood vessel endothelial cell	1.85	0.32
heart	mesothelial cell	1.76	0.29
heart	neuronal receptor cell	1.69	0.28
heart	cardiac endothelial cell	1.85	0.28
heart	mature NK T cell	2.58	0.22
heart	natural killer cell	1.76	0.21
heart	macrophage	1.97	0.21
heart	professional antigen presenting cell	1.97	0.20
heart	epithelial cell	1.86	0.18
heart	squamous epithelial cell	1.85	0.18
heart	neural cell	1.90	0.18
heart	endothelial cell	1.88	0.18
heart	smooth muscle cell	1.93	0.17
heart	cardiac muscle myoblast	1.67	0.16
heart	myeloid leukocyte	1.97	0.16
heart	myoblast	1.67	0.16
heart	lymphoid lineage restricted progenitor cell	2.02	0.16
heart	hematopoietic precursor cell	2.02	0.16
heart	endothelial cell of lymphatic vessel	1.83	0.15
heart	fibroblast	1.90	0.15
heart	mononuclear phagocyte	1.96	0.15
heart	blood vessel endothelial cell	1.91	0.15
heart	endothelial cell of vascular tree	1.91	0.15
heart	precursor cell	1.70	0.15
heart	connective tissue cell	1.90	0.14
heart	activated CD8-positive, alpha-beta T cell	1.91	0.12
heart	adipocyte	1.74	0.12
heart	ventricular cardiac muscle cell	2.03	0.12
heart	CD4-positive, alpha-beta cytotoxic T cell	2.83	0.11
heart	mononuclear cell	1.97	0.11
heart	pericyte	1.97	0.11
heart	innate lymphoid cell	1.92	0.10
heart	leukocyte	1.96	0.10
heart	immature innate lymphoid cell	1.99	0.09
heart	mural cell	1.96	0.09
heart	perivascular cell	1.96	0.09
heart	vein endothelial cell	2.16	0.09
heart	myeloid cell	1.93	0.08
heart	lymphocyte	1.97	0.08
heart	hematopoietic cell	1.95	0.08
heart	fibroblast of cardiac tissue	1.74	0.08
heart	progenitor cell	2.05	0.08
heart	neuron	1.92	0.08
heart	mature alpha-beta T cell	2.39	0.08
heart	CD4-positive, alpha-beta T cell	2.83	0.06
heart	stromal cell	2.59	0.06
heart	CD8-positive, alpha-beta T cell	1.91	0.05
heart	capillary endothelial cell	2.20	0.04
heart	smooth muscle myoblast	1.55	0.03
heart	T cell	2.43	0.03
heart	vascular associated smooth muscle cell	2.25	0.02
heart	mesothelial cell of epicardium	2.11	0.02
heart	monocyte	2.31	0.01
heart	cardiac neuron	1.99	0.01
heart	endothelial cell of artery	2.32	0.01

Table S6. Constituent medications within the DPP-4 and SGLT2 comparator groups.

Comparator Group	Constituent Drugs
DPP-4 inhibitors	sitagliptin, saxagliptin, linagliptin, alogliptin
SGLT2 inhibitors	empagliflozin, dapagliflozin, canagliflozin

Table S7. ICD-9 and ICD-10 code definitions for baseline cardiovascular burden phenotypes used in subgroup assignment.

Phenotype	Disease Name	ICD10 Code Prefixes	ICD9 Code Prefixes
Ischemic heart disease	Ischemic heart disease	I20, I21, I22, I23, I24, I25	410, 411, 412, 413, 414
Cerebrovascular disease	Cerebrovascular disease	I60, I61, I62, I63, I64, I65, I66, I67, I68, I69	430, 431, 432, 433, 434, 435, 436, 437, 438
Hypertension	Hypertension	I10, I11, I12, I13, I14, I15, I16	401, 402, 403, 404, 405
Heart failure	Heart failure	I50	428
Arrhythmia / Conduction disorders	Arrhythmias / conduction disorders	I44, I45, I46, I47, I48, I49	426, 427
Peripheral / Vascular disease atherosclerosis	Peripheral vascular disease / atherosclerosis	I70, I73, I74	440, 443, 444
Valvular / rheumatic heart disease	Valvular / rheumatic heart disease	I00, I01, I02, I05, I06, I07, I08, I09, I34, I35, I36, I37, I38, I39	390, 391, 392, 393, 394, 395, 396, 397, 398, 424
Cardiomyopathy	Cardiomyopathy	I42, I43	425
Aortic disease	Aortic disease	I71	441
Venous thromboembolism	Venous thromboembolism	I26, I80, I81, I82	415.1, 451, 452, 453

Table S8. Interval between index date and most recent qualifying preindex CV diagnosis.

Cohort	Cardiovascular subgroup	Number of unique patients	Gap to most recent qualifying preindex ICD, median [IQR], d
semaglutide	all cv conditions	53114	108 [26, 340]
semaglutide	aortic disease	1013	416 [165, 740]
semaglutide	arrhythmia conduction disorder	10611	214 [49, 639]
semaglutide	cardiomyopathy	1735	197 [50, 672]
semaglutide	cerebrovascular disease	3147	359 [111, 987]
semaglutide	heart failure	4221	137 [36, 431]
semaglutide	hypertension	45704	118 [30, 344]
semaglutide	ischemic heart disease	10206	162 [43, 443]
semaglutide	peripheral vascular disease atherosclerosis	3745	238 [77, 679]
semaglutide	valvular rheumatic heart disease	3441	315 [92, 831]
semaglutide	venous thromboembolism	3122	608 [159, 1,535]
metformin	all cv conditions	132970	55 [6, 264]
metformin	aortic disease	2911	225 [44, 666]
metformin	arrhythmia conduction disorder	25457	139 [13, 590]
metformin	cardiomyopathy	3864	145 [22, 579]
metformin	cerebrovascular disease	10332	239 [38, 831]
metformin	heart failure	9175	81 [9, 374]
metformin	hypertension	109617	63 [7, 260]
metformin	ischemic heart disease	30177	128 [15, 496]
metformin	peripheral vascular disease atherosclerosis	9057	163 [25, 555]
metformin	valvular rheumatic heart disease	8439	263 [40, 824]
metformin	venous thromboembolism	6261	346 [41, 1,170]
DPP-4 inhibitors	all cv conditions	11875	88 [11, 398]
DPP-4 inhibitors	aortic disease	298	282 [80, 764]
DPP-4 inhibitors	arrhythmia conduction disorder	3105	132 [17, 476]
DPP-4 inhibitors	cardiomyopathy	632	181 [32, 588]
DPP-4 inhibitors	cerebrovascular disease	1321	233 [54, 808]
DPP-4 inhibitors	heart failure	2217	75 [11, 345]
DPP-4 inhibitors	hypertension	9558	113 [19, 437]
DPP-4 inhibitors	ischemic heart disease	3838	151 [26, 568]
DPP-4 inhibitors	peripheral vascular disease atherosclerosis	1471	165 [39, 533]
DPP-4 inhibitors	valvular rheumatic heart disease	1221	224 [39, 655]
DPP-4 inhibitors	venous thromboembolism	665	298 [36, 1,006]
SGLT2 inhibitors	all cv conditions	74169	24 [4, 128]
SGLT2 inhibitors	aortic disease	3577	308 [84, 621]
SGLT2 inhibitors	arrhythmia conduction disorder	33798	42 [7, 220]
SGLT2 inhibitors	cardiomyopathy	14410	55 [9, 259]
SGLT2 inhibitors	cerebrovascular disease	8543	252 [55, 812]
SGLT2 inhibitors	heart failure	34279	21 [4, 105]
SGLT2 inhibitors	hypertension	56957	65 [11, 249]
SGLT2 inhibitors	ischemic heart disease	31601	59 [10, 251]
SGLT2 inhibitors	peripheral vascular disease atherosclerosis	10912	154 [35, 468]
SGLT2 inhibitors	valvular rheumatic heart disease	16479	101 [15, 394]
SGLT2 inhibitors	venous thromboembolism	4748	410 [71, 1,323]

Table S9. ICD-9 and ICD-10 code definitions for the composite cardiovascular endpoint, including fatal and nonfatal components.

Component	ICD10 Code Prefixes	ICD9 Code Prefixes
All-cause death	N/A	N/A
Myocardial infarction/Acute coronary syndrome	I21, I22, I23, I24	410, 411
Stroke/cerebrovascular event	I60, I61, I62, I63, I64, I65, I66, I67, I68, I69	430, 431, 432, 433, 434, 435, 436, 437, 438

Supplementary Text

Cardiac GLP1R geography provides biologic plausibility

The expression data provide a plausible biological framework for this clinical pattern. Prior work has shown that GLP1R is detectable in human cardiac tissue and that cardiac or endocardial/endothelial GLP-1R signaling can mediate cardioprotective effects in experimental systems[7,19]. Our atlas-based analyses extend that rationale in two ways. First, they show that the human heart is not transcriptionally silent for GLP1R at the tissue level. Second, they resolve that signal into distinct cell populations.

Following is a potential dose-stratified interpretation of the cardiac GLP1R atlas. At lower semaglutide exposure, pharmacologic activity may be concentrated in the most prevalent GLP1R-expressing cardiac populations, particularly atrial and broader cardiomyocyte, or contractile-cell compartments, in which GLP1R-positive cells are relatively more frequent (**Table S5**, green-highlighted rows). At moderate to high exposure, engagement may extend to a broader set of less prevalent but still recurrent endothelial, endocardial, vascular-associated, and stromal populations (**Table S5**, yellow-highlighted rows), potentially broadening favorable effects on vascular tone, myocardial loading conditions, endothelial biology and inflammatory signaling. At very high exposure, semaglutide may additionally engage rare GLP1R-expressing immune-cell subsets, including CD4-positive alpha-beta T-cell and related lymphoid compartments (**Table S5**, red-highlighted rows).

Whole-body receptor geography and implications for organ-directed pharmacology

The whole-body single-cell atlas further refines this interpretation by showing that GLP1R tissue geography depends on how receptor burden is summarized. Prevalence-weighted GLP1R Engagement Potential (GEP) prioritizes tissues in which GLP1R-positive cells are comparatively frequent, whereas Absolute Target Load (ATL) prioritizes tissues containing the largest aggregate reservoir of GLP1R-positive cells. In this framework, systemic metabolic effects and organ-specific cardiovascular effects need not arise from the same receptor geography. Rather, semaglutide exposure may simultaneously engage pancreatic and other whole-body compartments that shape weight loss while also interacting with a substantial cardiac target reservoir capable of supporting direct heart-specific biology.

Distributed GLP1R signaling programs across myocardial, vascular, cardio-immune cells

Examination of GLP1R signal in the heart shows distribution across several biologically meaningful compartments rather than being restricted to a single rare niche (**Fig. S6 - top left panel**). Cardiomyocyte-contractile states, especially regular atrial cardiac myocytes, cardiac muscle cells, muscle cells and contractile cells, show the strongest overall GLP1R burden, consistent with the idea that the myocardium itself is a major transcript-level reservoir of semaglutide responsiveness. At the same time, the data shows that these cardiomyocyte-like compartments also co-express canonical downstream signaling genes of GLP1R such as GNAS, ADCY3, RAPGEF4, PRKACA and PRKACB, suggesting that receptor detection is embedded

within a productive signaling framework rather than occurring in isolation (**Fig. S6 - top left panel**). Vascular-interface states, including endothelial, cardiac endothelial, endocardial and cardiac blood vessel endothelial cells, show recurrent enrichment of RAMP2, RAMP3, RAPGEF4, DPP4 and related contextual genes, a pattern that is concordant with literature implicating GLP-1 receptor agonists in endothelial protection, vascular anti-inflammatory signaling and attenuation of atherosclerotic biology[4–7]. Finally, myeloid, macrophage and antigen-presenting populations show distinct enrichment of CALCR, ADCYAP1, FFAR1 and related signaling genes, raising the possibility that part of semaglutide's cardiovascular benefit may be mediated through immunometabolic remodeling rather than through cardiomyocytes alone, a concept that is broadly compatible with the known anti-inflammatory and cardiometabolic effects of GLP-1 receptor agonism[4,6,7].

Computing potential for functional semaglutide-response modules

We computed a heatmap to distill this gene-level complexity into a functional architecture (**Fig. S6 - top right panel**). Cardiomyocyte-contractile compartments retain comparatively strong receptor burden and appreciable cAMP signaling competence, supporting the interpretation that semaglutide-responsive biology in heart may include direct myocardial interfaces. In contrast, endothelial and epithelial compartments are especially notable for stronger peptide-processing or contextual-modulation signatures, including RAMP2, RAMP3, DPP4, CPE and RAPGEF4, suggesting that these cell types may be particularly important for vascular and tissue-interface responses to GLP-1 receptor agonism (**Fig. S6 - top right panel**). Myeloid leukocytes, macrophages and professional antigen-presenting cells show comparatively prominent receptor and sensing module scores, which is particularly interesting because semaglutide has reduced major adverse cardiovascular events in people with overweight or obesity and established cardiovascular disease in the SELECT trial, while related work increasingly points to inflammatory and vascular mechanisms as important mediators of benefit, beyond weight loss alone[1,4,6,7]. The relatively distinct Schwann-cell and neural-cell module patterns suggest a peripheral neuroglial niche that may participate in incretin biology, but this remains more hypothesis-generating than the vascular-inflammatory axis, for which the clinical and experimental literature is already stronger[4,6,7].

Towards a composite functional-readiness framework for semaglutide cardiac activity

The functional-readiness ranking is useful because it prioritizes cell types not simply by GLP1R abundance but by the combination of receptor burden, receptor-context genes, downstream cAMP machinery, peptide-processing capacity and metabolic or excitability-associated effectors (**Fig. S6 - bottom left panel**). In this framework, contractile and muscle-like states rank highly because they combine substantial GLP1R burden with signaling genes such as GNAS, ADCY3 and RAPGEF4 and with downstream contextual genes including CPE and PAM. Smooth-muscle and endothelial-interface states also emerge as highly ranked, which is biologically plausible given the substantial literature on GLP-1 receptor agonist effects on endothelial function, vascular inflammation and atherosclerotic remodeling[6,7]. Myeloid leukocytes, macrophages and antigen-presenting cells rank prominently because they combine receptor-adjacent sensing programs with inflammatory and peptide-context genes, supporting an immunometabolic interpretation of

semaglutide action that is consistent with the cardiovascular and HFpEF clinical data (**Fig. S6 - bottom left panel**). In SELECT, semaglutide lowered cardiovascular event risk in overweight or obesity without diabetes, and in STEP-HFpEF and STEP-HFpEF DM it improved symptoms, physical limitations and exercise-related outcomes in obesity-related HFpEF, indicating that the net cardiovascular phenotype of semaglutide likely reflects integrated myocardial, vascular and inflammatory effects rather than a single-cell-type mechanism[1–4].

Single cell RNA patterns hint at broad model of possible semaglutide cardiac activity

The expression heatmap provides the gene-level resolution underlying the higher-order summaries (**Fig. S6 - bottom right panel**). Several striking patterns stand out. First, cardiomyocyte-contractile states show coordinated expression of GLP1R with GNAS, CPE, PAM, RAPGEF4 and ADCY3, supporting a model in which receptor-positive myocardial compartments may be capable of signal transduction rather than merely harboring sparse receptor transcripts. Second, endothelial, epithelial and squamous epithelial states show recurrent RAMP2, RAMP3, RAPGEF4 and DPP4 signals, consistent with an interface biology centered on peptide sensing and vascular or barrier regulation (**Fig. S6 - bottom right panel**). Third, immune and myeloid compartments show notable enrichment of CALCR, ADCYAP1 and FFAR1, suggesting that incretin-adjacent and nutrient-sensing programs may intersect in inflammatory cell states relevant to cardiovascular remodeling. Finally, neural and Schwann compartments show PCSK2-rich signatures, implying specialized neuropeptide-processing capacity. The overall picture is that semaglutide activity potential in heart is most plausibly distributed across three major axes: a myocardial-contractile axis, a vascular-interface axis and an inflammatory-immunometabolic axis, with a smaller neuroglial niche that deserves follow-up. This distributed model is more congruent with current clinical and mechanistic literature than a narrow interpretation based on cardiomyocytes or endothelial cells alone[4–6].

Implications for endpoint selection, dose optimization and high-dose extrapolation

The most defensible interpretation of our cumulative single cell RNA analysis is that semaglutide's cardiovascular benefit is unlikely to be explained solely by receptor presence in one dominant heart cell type. Instead, the data support a distributed-response model in which GLP1R-positive cardiomyocyte-contractile cells provide one plausible interface, endothelial and endocardial cells provide a strong vascular-context interface, and macrophage-myeloid populations provide an inflammatory-immunometabolic interface. This interpretation is aligned with the clinical observation that semaglutide reduces cardiovascular events in obesity without diabetes and improves obesity-related HFpEF outcomes, and with mechanistic literature emphasizing vascular protection, reduced endothelial dysfunction and attenuation of inflammatory remodeling as important components of GLP-1 receptor agonist biology[20–24].

Although weight reduction likely contributes to benefit for selected outcomes, the absence of a consistent monotonic association between achieved weight loss and cardiovascular risk suggests additional organ-directed mechanisms. Semaglutide's cardiovascular effects may therefore reflect not only systemic changes in adiposity, but also direct or indirect actions on cardiac, vascular, and immune biology, including effects on glycemic physiology, blood pressure, vascular

inflammation, endothelial function, myocardial loading conditions, neurohormonal signaling and arrhythmic susceptibility.

These findings have immediate translational implications. Current incretin development and clinical use are often benchmarked primarily against the magnitude of total-body weight reduction. Our results argue that this strategy is too narrow for cardiovascular medicine. In the present framework, weight loss is a whole-body pharmacodynamic readout, but cardiovascular protection may also reflect organ-directed biology. That distinction matters for dose optimization, endpoint selection and cross-drug comparison. More broadly, these data support incorporating dedicated cardiovascular endpoints, and not weight loss alone, into therapeutic optimization.

Extrapolation of this empirical real-world relationship to semaglutide 7.2 mg yields a hypothetical maximum weight change of approximately −29.8% over the 2-year landmark period (**Fig. 2**). While our analysis is based on maximum dose reached and the lowest observed pre-landmark weight over 2 years in routine care, the STEP UP trial evaluated a protocolized once-weekly 7.2 mg semaglutide high-dose regimen over 72 weeks. These clinical trial outcomes appear directionally concordant, with STEP UP showing approximately 20.7% mean loss under the efficacy estimate and 31.2% of participants achieving at least 25% weight loss[21]. Our landmark-derived, real-world model based on maximum attained dose and lowest observed pre-landmark weight is closer to an upper-envelope estimate of achievable pharmacologic effect than to a trial-average endpoint measured at a fixed week under protocolized titration, retention, and adherence conditions. In that context, the extrapolated value of ~29.8% understandably sits above the trial-average response but within the broader range suggested by the high-responder tail of STEP UP. The implication is that real-world pharmacological imputation can be useful for estimating directionality and attainable response range at higher exposures but should not be treated as a one-to-one surrogate for randomized-trial mean efficacy when dose-escalation schedules, treatment persistence, patient selection and endpoint definitions differ.