## **Supplemental Items**

**Supplemental Information Inventory:** 

- 1. Figure S1. High-level study metrics. Related to Figure 1.
- 2. Figure S2. Discriminant transcript and metabolomic pathways between IR and IS. Related to Figure 2.
- 3. Figure S3. Changes in plasma in response to weight gain and loss. Related to Figure 3.
- 4. Figure S4. Patterns observed in response to weight gain and loss. Related to Figure 4.
- 5. Figure S5. Analysis of personal and other sources of omic variation. Related to Figure 6.
- 6. Figure S6. Distribution of plasma metabolite concentrations across the dataset following normalization. Related to Figures 2, 3 and 4.
- 7. Table S1. An overview of clinical parameters measured in the study. Related to Figure 1.
- 8. Table S2. Significant differences in transcripts, proteins, metabolites, microbes, clinical assays and pathways between IR and IS individuals. Related to Figure 2. See separate Excel table.
- 9. Table S3. Significant differences in transcripts, proteins, metabolites, metabolites and clinical assays across the weight gain/loss perturbation. Related to Figures 3, 4 and 5.
- 10. Table S4. Validation of metabolomic changes in a validation cohort. Related to Figure 3.
- 11. Table S5. Read depth and mapping fraction for RNA-seq data. Related to Figures 2, 3, 4 and 6.
- 12. Table S6. Read and assembly stats for microbiome samples. Related to Figures 2, 3, 4, 5 and 6.









**Figure S2. Discriminant transcript and metabolomic pathways between IR and IS. Related to Figure 2.** A) Key pathways that are discriminant between IR and IS transcriptomes at baseline. Gene expression differences at a FDR < 0.2 significance cutoff were used as input into the DAVID pathway analysis suite, and pathways from either KEGG or Gene Ontology biological process categories were assessed. As multiple redundant pathways were identified as significant, key unique pathways are highlighted here (see Table S2 for the full list). B) Changes in the number of significant metabolites using different SSPG cutoffs for defining IR and IS. C) Significant metabolites between IR and IS for three different families of biomolecules: amino acids, acylcarnitines and plasmalogens. Intensities are plotted after normalization to IS. D) Top panel: performance measures of Random Forest and AdaBoost models in terms of Precision, Recall and F1 Score. Bottom left: Confusion matrices for 8 held-out samples

for Random Forest and AdaBoost. Bottom right:  $\Delta_{SSPG}$  computation at time  $(t^n)$  using regression: Predicted vs. real delta SSPG values using LASSO and Elastic Net regression.



Pathway enrichment

Pathway	Database	FDR
Glycerophospholipid metabolism	HMDB	0.013
Arginine and proline metabolism	HMDB	0.016
Manine, aspartate and glutamate metabolism	HMDB	0.025



Figure S3. Changes in plasma in response to weight gain and loss. Related to Figure 3. A) Metabolites associated with changes in Body Mass Index (BMI). Pathway analysis using HMDB pathways is performed for metabolites associated with BMI. Top pathways along with the associated corrected p-values are shown. B) Differences in individual metabolites at peak weight and after weight loss for individual metabolites comprising top pathways. Intensities are plotted after normalization to baseline. C) Plasma leptin levels across the weight gain and loss perturbations. Plasma leptin was measured by two separate immunoassay methods (Olink Proseek and Luminex). Intensities were normalized to baseline values for each participant, and boxplots for log2 intensities at peak weight, weight loss and followup timepoints are shown for both measurement methods.



**Figure S4. Patterns observed in response to weight gain and loss. Related to Figure 4.** A) Longitudinal multi-omic pathway analysis reveals long-term changes. Pathway analysis of biomolecules comprising longitudinal Cluster 02, which fails to return to baseline following weight loss. B) Weighted gene coexpression network analysis and correlation with clinical variables. Weighted gene-coexpression network analysis (WGCNA) was applied to transcriptomic data for all participants and all timepoints, and a heatmap and dendrogram detailing the clustering results is shown (note only the top 5000 genes are plotted). The identified co-expressed gene modules were tested for biological pathway enrichment (Gene Ontology terms) as well as association with clinical variables (see Fig. 4 C and D).



**Figure S5.** Analysis of personal and other sources of omic variation. Related to **Figure 6.** A) Variance decomposition analysis of selected 'omes. The variance across all timepoints was deconvolved into experiment-dependent variation (i.e. due to the perturbation), personal variation (within an individual), or other types of variation (technical or unknown sources). This is plotted as a scatterplot for all 'omes (top panel) as well as individual 'omics types (see Fig. 6A for others). B) Variation in 'omics analyte abundance within participants versus across participants. The coefficients of variation for proteins, metabolites and transcripts are plotted across steady-state timepoints (T1 and T4) within an individual (red) and across individuals (blue).



**Figure S6. Distribution of plasma metabolite concentrations across the dataset following normalization. Related to Figures 2, 3 and 4.** Heatmap showing the abundance of each metabolite in each sample of the study; each color in the top column header represents a different individual in the study (clustering distance = Pearson correlation, clustering method = Complete).

Metric	IR_average	IR_sd	IS_average	IS_sd	FDR
SSPG	206.35	37.8	77.2	27.15	2.35E-07
ETHNICITY(C/B/A)	8/0/5	-	6/3/1	-	0.1
AGE	58	5	56	6	0.612
SEX(M/F)	6/7	-	3/7	-	0.667
Waist Circumference	104	8	97	7	0.048
Systolic BP	123	7	121	14	0.6
Diastolic BP	78	6	76	4	0.32
BMI	30.51	2.9	28.57	2.81	0.612
A1C	5.55	0.26	5.49	0.41	1
AG	8.25	2.43	9.33	1.97	0.612
ALB	3.9	0.17	3.92	0.17	1
ALKP	81	17.82	105	26.77	0.612
ALT	35.9	11.75	38.67	9.48	0.887
AST	21.88	4.26	21.67	3.33	1
BASO	0.73	0.56	0.76	0.4	1
BASOAB	0.04	0.03	0.04	0.02	1
BUN	12.38	2.5	14.67	1.97	0.612
CA	9.08	0.32	9.02	0.24	1
CHOI	188.56	30.7	188.6	27.05	1
CHOLHDI	4 14	1 27	3 1	1 01	0.612
CI	104 63	2.26	103 17	2 64	0.736
CO2	27.5	1.85	28.17	1 17	0.871
CR	0.78	0.13	0.95	0.22	0.612
FOS	1 78	0.15	2 73	1 73	0.612
EOSAB	0.11	0.06	0.15	0.12	0.887
GLOB	3 19	0.00	3	0.12	0.612
GLUB	91.88	15 59	89.17	12 56	1
нст	40.18	53	A1 1A	3.81	1
ны	40.10	9.01	67.4	29.04	0.667
	12 51	2.09	12 92	1 22	1
hcCPD	1 16	2.08	13.85	1.52	0.612
IGM	121 25	58.04	72 75 92	20.92	0.012
I GIVI	121.25	0.27	45.85	20.92	1
	4.01	21.22	102	22.09	0.832
	2 50	1.09	105	0.97	0.633
	2.39	1.08 E 24	20.46	0.87 E 46	0.012
	29.70	0.25	29.40	0.66	0.617
	20.74	2.70	20.64	1.05	0.017
MCHC	22.74	1	22.62	0.42	1
MCV	99.51	0.71	01.2	2.91	1
MONO	6 99	9.71	7 10	2.04	1
MONOAR	0.88	0.08	7.19	0.16	1
IVIONOAD	140.29	0.08	140.67	2.66	1
	140.56	2.39	140.07	2.00	1
	2.64	5.37	23.87	1.07	0.824
NEUTAD	5.04	0.08	3.20	1.05	0.824
NHUL	141	30.92	121.2	29.17	1
	239.13	90.09	220.57	59.32	1
KBC	4.54	0.38	4.52	0.49	
KDW	14.1	1.74	13.74	0.72	1
IBIL	0.69	0.29	0.53	0.13	0.612
IGL	129.67	40.64	90	59.27	0.612
I'P	7.09	0.21	6.92	0.29	0.667
WBC	5.98	0.76	5.44	1.73	0.676
Weight	86.95	12.23	78.89	10.31	0.612
Weight Gain	2	1.1	3.6	1.7	0.029

**Table S1. An overview of clinical parameters measured in the study. Related to Figure 1.** Both participant statistics as well as clinical blood panel results are shown for IR and IS subgroups in the study.

Table S2. Significant differences in transcripts, proteins, metabolites, microbes, clinical assays and pathways between IR and IS individuals. Related to Figure 2. Individual analyte types are separated into unique tabs in the attached excel table. For RNA-seq, differentially expressed transcripts (Ensembl) at FDR < 0.01 are listed along with associated fold changes and p and q values for the test. For transcriptomic pathways, differential transcripts between IR and IS at FDR < 0.2 were used as input to the DAVID pathway analysis suite. Significant pathways from either KEGG or Gene Ontology biological process categories are listed. For proteomic pathways, proteomic data ranked by differential expression between IR and IS was used as input into GORILLA. Significant pathways are listed. For the microbiome, average abundance values from both 16S sequencing (in percentage) and whole genome shotgun sequencing (mWGS, in %) are listed for IR and IS individuals for bacteria that are significantly different (p < 0.05) between the group. Taxonomic units that are detected by both methods are bolded, with their prevalence indicated by columns labeled as "nonzeros". For targeted Proseek multiplex assays, immunoassays were performed on plasma and the groupwise differences between IR and IS across all timepoints is shown. For Luminex cytokine/chemokine/adipokine assays, immunoassays were performed on plasma and the groupwise differences between IR and IS across all timepoints is shown. For clinical parameters, clinical blood panels were performed by the Stanford core lab, and significant analytes between IR and IS across all timepoints are shown. For metabolomics, LC-MS metabolomics differences between IR and IS (across all timepoints) are shown.

Table S3. Significant differences in transcripts, proteins, metabolites, metabolites and clinical assays across the weight gain/loss perturbation. Related to Figures 3, 4 and 5. Individual analyte types are separated into unique tabs in the attached excel table. For RNA-seq, differentially expressed transcripts (Ensembl) at FDR < 0.01 are listed along with associated fold changes and p and q values for the test for both the differences between baseline and weight gain and weight gain vs weight loss. For the microbiome, average changes in abundance (T2 minus T1) from both 16S sequencing (in percentage) and whole genome shotgun sequencing (mWGS, in %) are listed for IR and IS individuals for bacteria that are significantly different (p < 0.05) between the group. Taxonomic units that are detected by both methods are bolded, with their prevalence indicated by columns labeled as "nonzeros". For metabolomics data, differences across all timepoints are shown, as well as BMI-associated changes. For Proseek multiplex protein assays, all immunoassays were performed on plasma and associations with changes in BMI in the study are shown. For Luminex cytokine/chemokine/adipokine assays, the immunoassays were performed on plasma and associations with changes in BMI in the study are shown. Clinical blood panels were performed by the Stanford core lab, and significant associations with changes in BMI in the study are shown.

COHORT 1 METABOLITE	FORMULA	COHORT 1 DELTA CHANGE	COHORT 2 METABOLITE	FORMULA	COHORT 2 DELTA CHANGE
L-Tyrosine	C9H11NO3	UP	tyrosine	C9H11NO3	UP
Benzyl sulfate   p-Cresol sulfate	C7H8O4S	DOWN	p-cresol sulfate	C7H8O4S	DOWN
L-Carnitine	C7H15NO3	DOWN	carnitine	C7H15NO3	DOWN
Citric acid   Isocitric acid	C6H8O7	DOWN	citrate	C6H8O7	DOWN
N-Acetylputrescine	C6H14N2O	UP	N-acetylputrescine	C6H14N2O	UP
C6H12O7	C6H12O7	UP	galactonate	C6H12O7	UP
C6H12O6	C6H12O6	UP	chiro-inositol	C6H12O6	DOWN
FA(6:0, OH)	C6H12O3	UP	alpha-hydroxy isoca proate	C6H12O3	DOWN
L-Glutamic Acid	C5H9NO4	UP	glutamate	C5H9NO4	UP
C5H9NO4	C5H9NO4	UP	N-acetylserine	C5H9NO4	DOWN
Ribitol	C5H12O5	DOWN	ribitol	C5H12O5	UP
C5H10O6	C5H10O6	UP	ribonate	C5H10O6	UP
C5H10O5	C5H10O5	UP	xylose	C5H10O5	DOWN
L-Glutamine	C5H10N2O3	DOWN	glutamine	C5H10N2O3	DOWN
C4H9NO2	C4H9NO2	DOWN	2-aminobutyrate	C4H9NO2	DOWN
C4H8O3	C4H8O3	DOWN	3-hydroxybutyrate (BHBA)	C4H8O3	DOWN
L-Aspartic acid	C4H7NO4	UP	aspartate	C4H7NO4	UP
C4H7NO4	C4H7NO4	DOWN	iminodiacetate (IDA)	C4H7NO4	DOWN
L-Serine	C3H7NO3	DOWN	serine	C3H7NO3	DOWN
L-Lactic acid	C3H6O3	UP	lactate	C3H6O3	DOWN
FA(22:6)	C22H32O2	DOWN	docosahexaenoate (DHA; 22:6n3)	C22H32O2	DOWN
AC(12:0)	C19H37NO4	DOWN	laurylcarnitine	C19H37NO4	DOWN
FA(18:1, 2OH)	C18H34O4	UP	octadecanedioate	C18H34O4	DOWN
AC(10:0)	C17H33NO4	DOWN	decanoylcarnitine	C17H33NO4	DOWN
AC(9:1)	C15H29NO4	DOWN	octanoylcarnitine	C15H29NO4	DOWN
Hypaphorine	C14H18N2O2	UP	tryptophan betaine	C14H18N2O2	UP
FA(12:0, OH)	C12H24O3	DOWN	3-hydroxylaurate	C12H24O3	DOWN
FA(12:1)	C12H22O2	DOWN	5-dodecenoate (12:1n7)	C12H22O2	DOWN
FA(10:0, OH)	C10H20O3	DOWN	3-hydroxydecanoate	C10H20O3	DOWN
Gamma-Glutamylglutamine	C10H17N3O6	DOWN	gamma-glutamylglutamine	C10H17N3O6	DOWN
C10H16N2O7	C10H16N2O7	DOWN	gamma-glutamylglutamate	C10H16N2O7	UP

## Table S4. Validation of metabolomic changes in a validation cohort. Related to

**Figure 3.** Significant weight-responsive metabolites (Cohort 1) were compared to data from a separate weight-change cohort conducted in Sweden (Cohort 2) (see STAR Methods). The subset of metabolites that were interrogated in both studies were compared (based on elemental composition). The list of overlapping metabolites that were significant in both cohorts is listed above, and the subset that changed in the same direction in response to weight change is indicated in green.

Participant Identifier	Timepoint	Trimmed reads	Fraction Mapped
ZJTKAE3	1	41217787	0.994
ZJTKAE3	2	44563611	0.992
ZJTKAE3	3	42854136	0.993
ZJTKAE3	4	46698867	0.993
ZK112BX	1	32354021	0.965
ZK112BX	2	34366551	0.963
ZK112BX	3	32701809	0.961
ZK112BX	4	18401108	0.959
ZK4CK8Y	1	13281979	0.977
ZK4CK8Y	2	46366564	0.987
ZK4CK8Y	3	20661317	0.987
ZK4CK8Y	4	44485215	0.992
ZKFV71L	1	9949488	0.981
ZKFV/1L	2	26920406	0.988
ZKFV/IL	3	22390173	0.980
ZKEV/IL	4	36961237	0.993
ZKVR420	2	30574718	0.990
7KV/P426	2	23059678	0.994
ZKVR420	3	23035078	0.982
71 6318R	1	42392503	0.997
71 6318R	2	45403170	0.997
71 6318R	3	39346873	0.990
ZL6318R	4	47343881	0.994
ZL9BTWF	1	26132750	0.995
ZL9BTWF	2	38092899	0.998
ZL9BTWF	3	26412111	0.992
ZL9BTWF	4	32837330	0.996
ZLGD9M0	1	24180646	0.988
ZLGD9M0	3	42798325	0.998
ZLPRB8E	1	48759209	0.992
ZLPRB8E	2	13486634	0.987
ZLPRB8E	3	13703986	0.985
ZLPRB8E	4	21426079	0.980
ZLPZSOH	1	19885777	0.989
ZLPZSOH	2	10500452	0.954
ZLPZSOH	3	17565278	0.987
ZLPZSOH	4	17847264	0.989
ZLTUJTN	2	36530224	0.992
ZLTUJTN	3	35747043	0.988
ZLTUJTN	4	35394019	0.990
ZM7JY3G	1	41579948	0.993
ZM/JY3G	2	40127702	0.995
ZM7JY3G	3	29403577	0.993
ZM/JY3G	4	40165302	0.993
ZIVIBH10Z	1	63476524	0.962
	2	53113730	0.900
	3	42051005	0.962
ZMBVNEM	2	45300712	0.992
	2	51/7879/	0.992
ZMBVNEM	4	38594024	0.992
ZVGW5EI	1	14977108	0.991
ZVGW5EI	2	25467371	0.991
ZVGW5FI	3	24558474	0.992
ZVM4N7A	1	12883989	0.994
ZVM4N7A	2	30106252	0.990
ZVM4N7A	3	34049640	0.991
ZVTCAK9	1	19295425	0.990
ZVTCAK9	2	23708120	0.993
ZVTCAK9	3	56448999	0.993
ZW61YGW	1	29488957	0.991
ZW61YGW	2	21080657	0.992
ZW61YGW	3	27035844	0.992
ZWCZHHY	1	19691104	0.991
ZWCZHHY	2	24531478	0.992
ZWCZHHY	3	23007507	0.990
ZWFDEY0	1	28307124	0.993
ZWFDEY0	2	18462648	0.992
ZWFDEY0	3	36625835	0.992
ZWHMV5E	1	34453080	0.992
ZWHMV5E	2	38872637	0.992
ZWHMV5E	3	29782389	0.992
	1	34924914	0.993
	2	37565095	0.992
2112KJY 7V17KJV	3	34723083	0.992
2112NJ1 7V7I\\\/45	4	36983654	0.991
Z1/10043		23125720	0.993
Z171W45	2	24499140	0.991
21/10045	5	22339060	0.992

## Table S5. Read depth and mapping fraction for RNA-seq data. Related to Figures 2, 3, 4 and 6.

Subject ID and Timepoint	Total reads QC_raw	Total reads QC_trim	Assembled 16S Amplicons QC_combined	% Total Reads Utilized	16S Amplicons QC_nonchimera	% Chimeric Amplicons	16S Amplicons QC_nonhost	% Human Host Amplicons	Final % Total Reads Utilized
ZMBVNFM-01	212838	3 212838	68062	63.96%	55842	17.95%	55842	0.0000%	52.47%
ZMBVNFM-02	187860	187860	59641	63.50%	50377	15.53%	50377	0.0000%	53.63%
ZMBVNFM-03	173742	173742	58023	66.79%	45472	21.63%	45472	0.0000%	52.34%
ZMBVNFM-04	217376	217376	67521	62.12%	52157	22.75%	52157	0.0000%	47.99%
ZMBH10Z-01	208022	208022	55557	53.41%	44222	20.40%	44222	0.0000%	42.52%
ZMBH10Z-02	135330	135330	35019	51.75%	27953	20.18%	27953	0.0000%	41.31%
ZMBH10Z-03	183918	183918	52781	57.40%	42990	18.55%	42990	0.0000%	46.75%
ZMBH10Z-04	385794	385566	82727	42.91%	68807	16.83%	68806	0.0015%	35.67%
ZM7JY3G-01	188920	188920	59789	63.30%	46114	22.87%	46114	0.0000%	48.82%
ZM7JY3G-02	213760	213760	65047	60.86%	51521	20.79%	51521	0.0000%	48.20%
ZM7JY3G-04	104192	104192	33937	65.14%	26807	21.01%	26807	0.0000%	51.46%
ZY7IW45-01	282522	282522	37598	26.62%	29379	21.86%	29379	0.0000%	20.80%
ZY7IW45-02	284600	284600	33526	23.56%	25253	24.68%	25253	0.0000%	17.75%
ZY7IW45-03	255716	5 255716	34555	27.03%	26155	24.31%	26155	0.0000%	20.46%
ZY7IW45-04	286612	286612	45051	31.44%	36161	19.73%	36161	0.0000%	25.23%
ZY1ZKJY-02	126058	126058	23412	37.14%	17575	24.93%	17575	0.0000%	27.88%
ZY1ZKJY-03	153340	153340	32928	42.95%	25492	22.58%	25492	0.0000%	33.25%
ZY1ZKJY-04	159038	159038	32138	40.42%	25476	20.73%	25476	0.0000%	32.04%
ZWFDEY0-01	288392	288392	53102	36.83%	42010	20.89%	42010	0.0000%	29.13%
ZWFDEY0-02	278026	278026	50320	36.20%	38813	22.87%	38813	0.0000%	27.92%
ZWCZHHY-01	315574	315574	45969	29.13%	34864	24.16%	34864	0.0000%	22.10%
ZWCZHHY-02	297806	297806	42404	28.48%	34224	19.29%	34224	0.0000%	22.98%
ZWCZHHY-03	303286	303286	37432	24.68%	26008	30.52%	26008	0.0000%	17.15%
ZWCZHHY-04	403014	403014	56908	28.24%	34202	39.90%	34201	0.0029%	16.97%
ZW61YGW-01	249072	249072	34173	27.44%	24138	29.37%	24138	0.0000%	19.38%
ZW61YGW-02	260356	260356	35549	27.31%	23929	32.69%	23929	0.0000%	18.38%
ZW61YGW-03	245966	245966	36651	29.80%	24213	33.94%	24213	0.0000%	19.69%
ZVM4N7A-01	294366	294366	50306	34.18%	36150	28.14%	36150	0.0000%	24.56%
ZVM4N7A-02	340620	340620	54607	32.06%	41586	23.84%	41586	0.0000%	24.42%
ZVGW5FI-01	265576	265576	54886	41.33%	46680	14.95%	46680	0.0000%	35.15%
ZVGW5FI-02	219608	219608	17367	15.82%	13565	21.89%	13565	0.0000%	12.35%
ZVGW5FI-03	477886	477886	40152	16.80%	29671	26.10%	29671	0.0000%	12.42%
ZKVR426-01	161084	161084	52407	65.07%	40687	22.36%	40687	0.0000%	50.52%
ZKVR426-02	215364	215364	68380	63.50%	55217	19.25%	55217	0.0000%	51.28%
ZKVR426-03	210612	210612	64734	61.47%	53638	17.14%	53638	0.0000%	50.94%
ZKVR426-04	142794	142794	47505	66.54%	38246	19.49%	38246	0.0000%	53.57%
ZKFV71L-01	145478	145478	46775	64.31%	39793	14.93%	39793	0.0000%	54.71%
ZKFV71L-02	450904	450822	85366	37.87%	71860	15.82%	71860	0.0000%	31.87%
ZKFV71L-04	168898	168898	53904	63.83%	43174	19.91%	43174	0.0000%	51.12%
ZK4CK8Y-01	394636	394578	76636	38.84%	64809	15.43%	64807	0.0031%	32.84%
7K4CK8Y-02	188732	188732	57539	60.97%	48467	15.77%	48467	0.0000%	51.36%
ZK4CK8Y-03	250860	250860	73642	58.71%	63292	14.05%	63292	0.0000%	50.46%
7K4CK8Y-04	205860	205860	63108	61.31%	55843	11.51%	55843	0.0000%	54.25%
ZK112BX-01	202666	202666	60336	59.54%	48051	20.36%	48051	0.0000%	47.42%
ZK112BX-02	178968	178968	53497	59.78%	47936	10.39%	47936	0.0000%	53.57%
7K112BX-03	212606	212606	66887	62.92%	51399	23.16%	51399	0.0000%	48.35%
ZJTKAE3-01	193464	193464	54088	55.92%	41351	23.55%	41351	0.0000%	42.75%
ZJTKAE3-02	164388	164388	43381	52.78%	33910	21.83%	33910	0.0000%	41.26%
ZJTKAE3-04	110246	110246	30474	55.28%	23393	23.24%	23393	0.0000%	42.44%
Table S6	Poad	and as	sombly s	tate f	or microbi	omo sar	nnlos Po	lated to E	liguros 2

Table S6. Read and assembly stats for microbiome samples. Related to Figures 2,3, 4, 5 and 6.