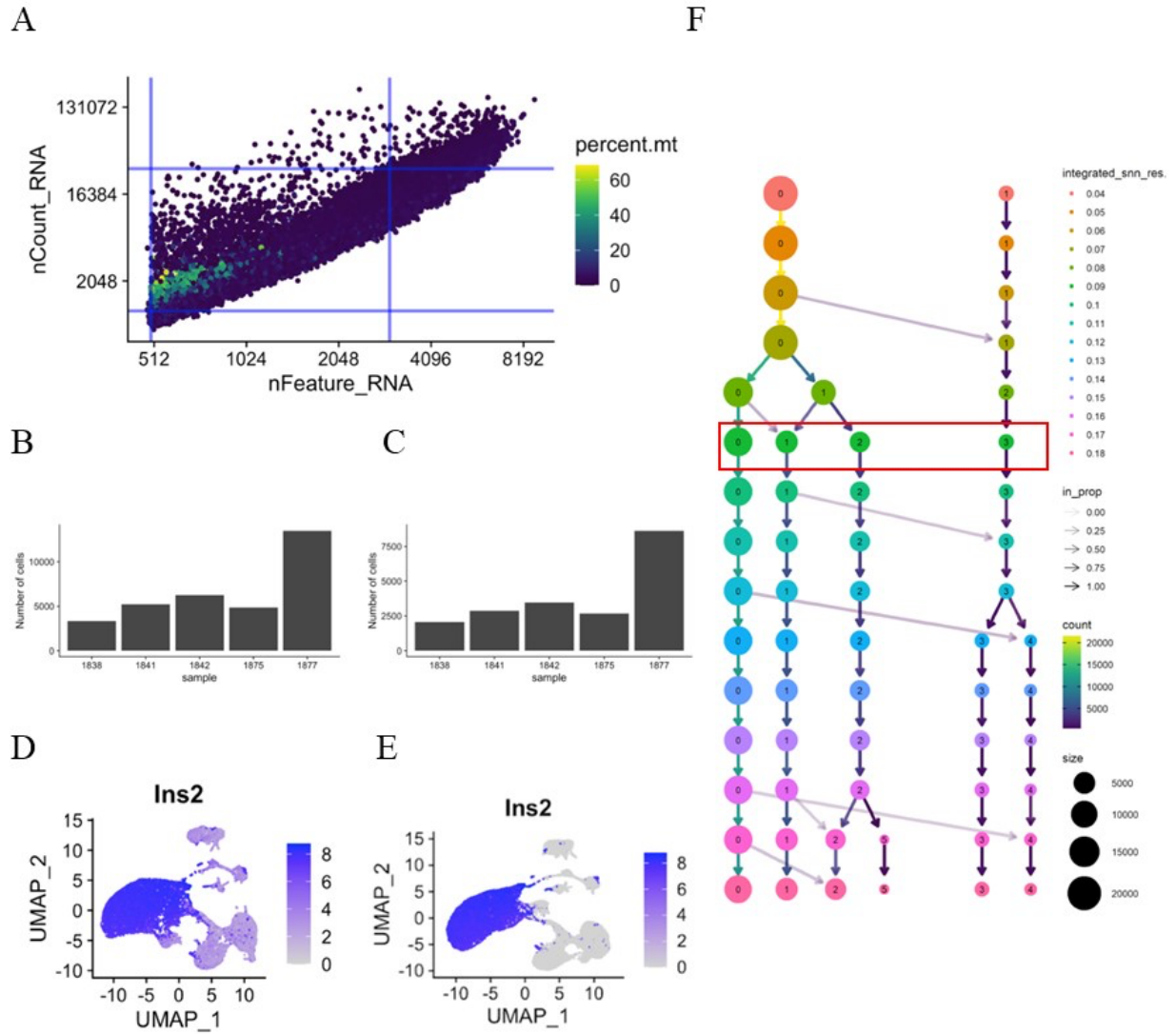
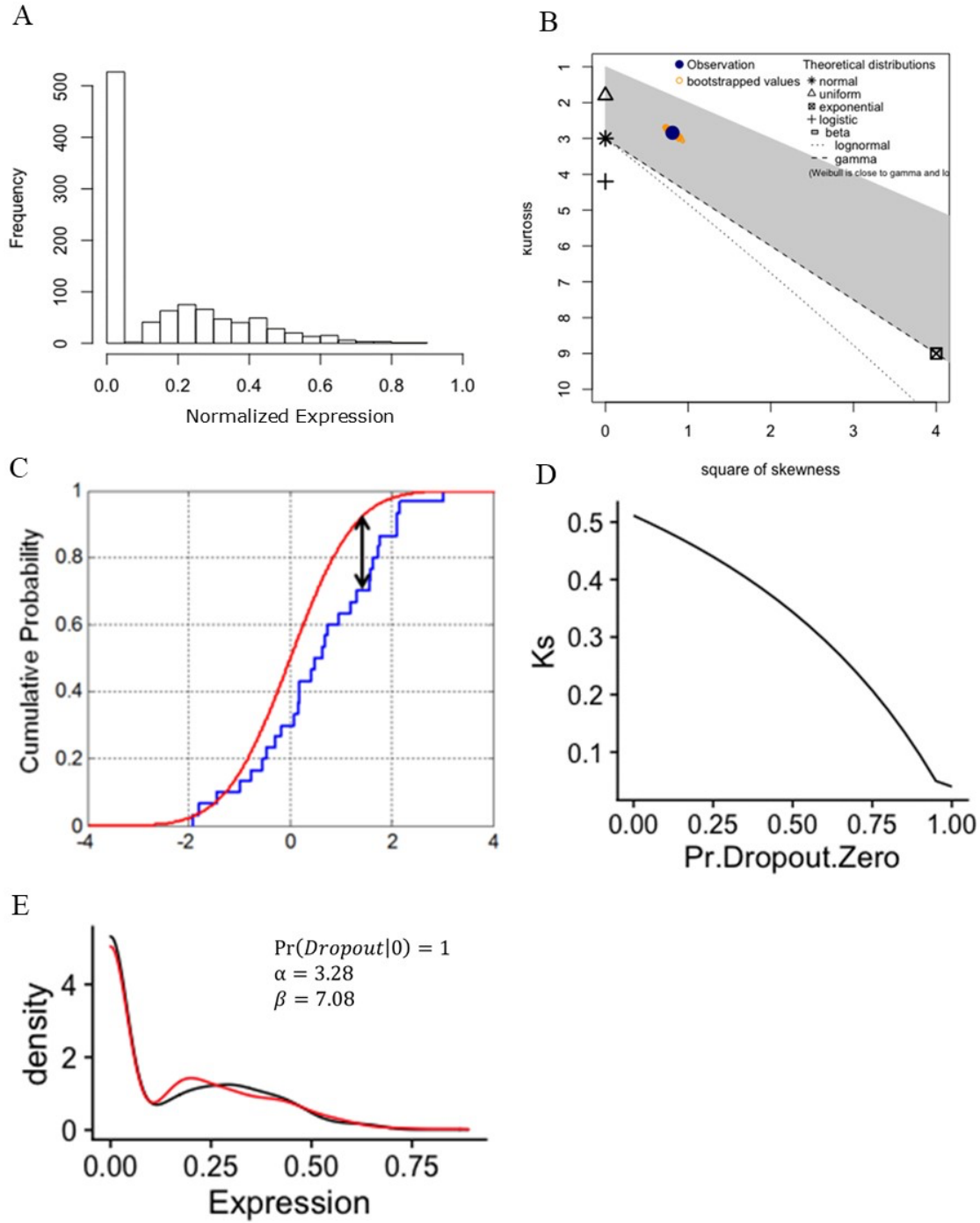


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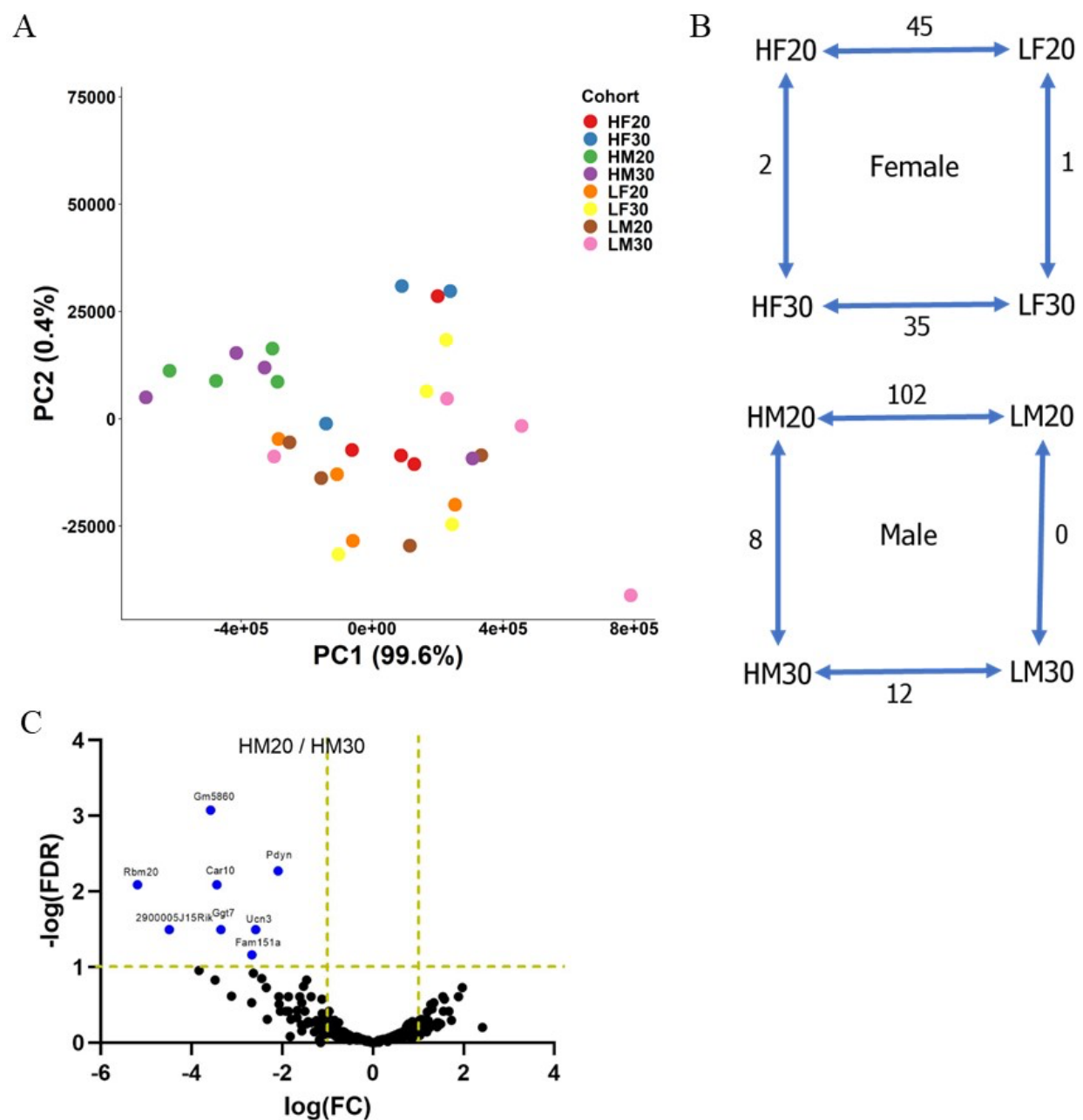
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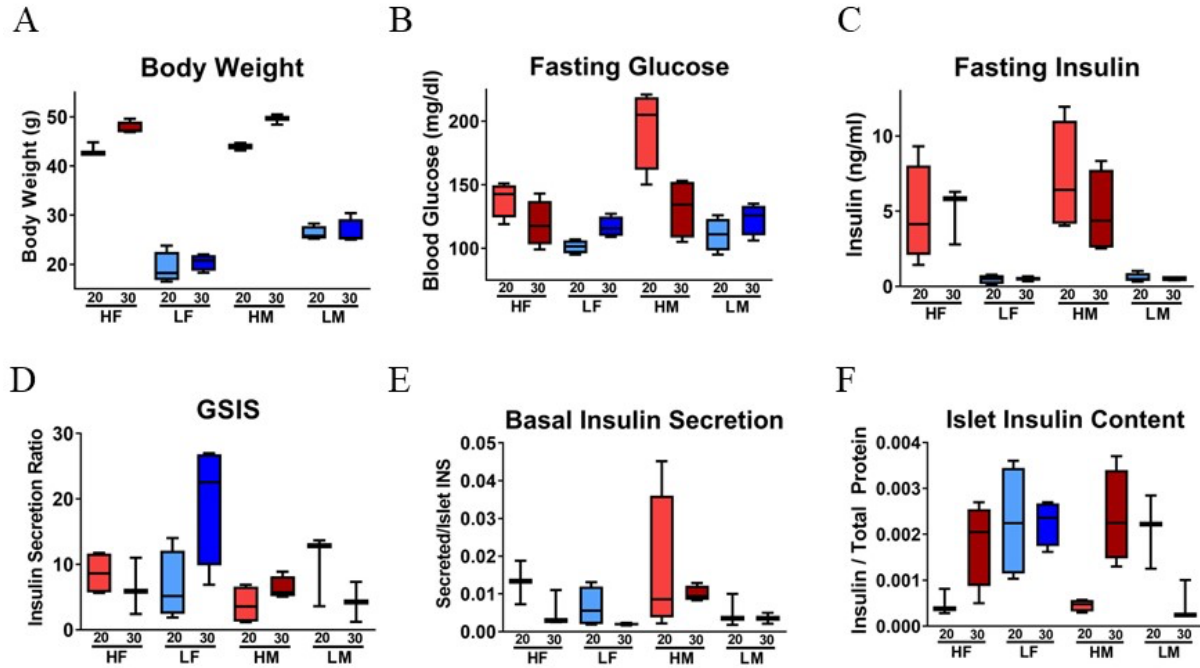
Supplemental Figure 1. Single cell RNA sequencing quality control. **(A)** Total counts, features, and mitochondrial RNA expression plotted for each cell. Cells within blue box and without mitochondrial gene expression were included in analysis. **(B)** Number of cells identified in individual mice prior to quality control, **(C)** and after quality control. **(D)** Expression of *Ins2* across all cells prior to ambient RNA removing using SoupX, **(E)** and after ambient RNA removal. **(F)** Resolution analysis to determine the number of β -cell subpopulations. Selected resolution highlighted with red box.



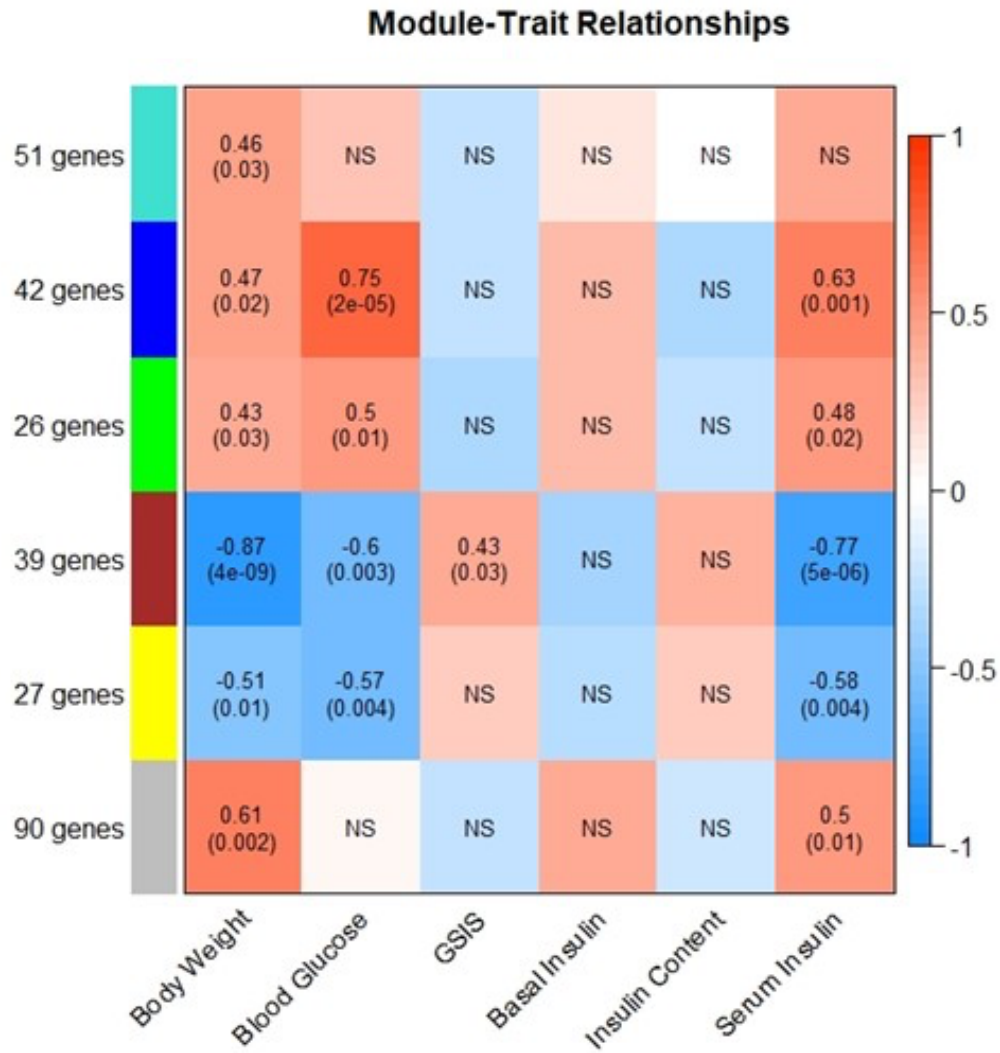
Supplemental Figure 2. Quantifying expected expression for β -cell expression of *Dcd*. **(A)** Distribution of expression across all β -cells. **(B)** Cullen and Frey analysis identifies *Dcd* expression to be beta distributed. **(C)** Kolmogorov-Smirnov test identifies alpha and beta parameters that minimize Ks between real (blue line) and simulated (red line) distribution of cells expressing *Dcd*. **(D)** Estimating percent of cells not expressing *Dcd* due to gene drop out by iterating alpha and beta parameters that minimize Ks between real and simulated data. **(E)** Density plot visualizing estimated parameters for distribution of *Dcd* expression. Red line shows distribution of actual data, black line shows distribution of simulated data based on optimal alpha and beta parameters. From these parameters, expected expression is calculated.



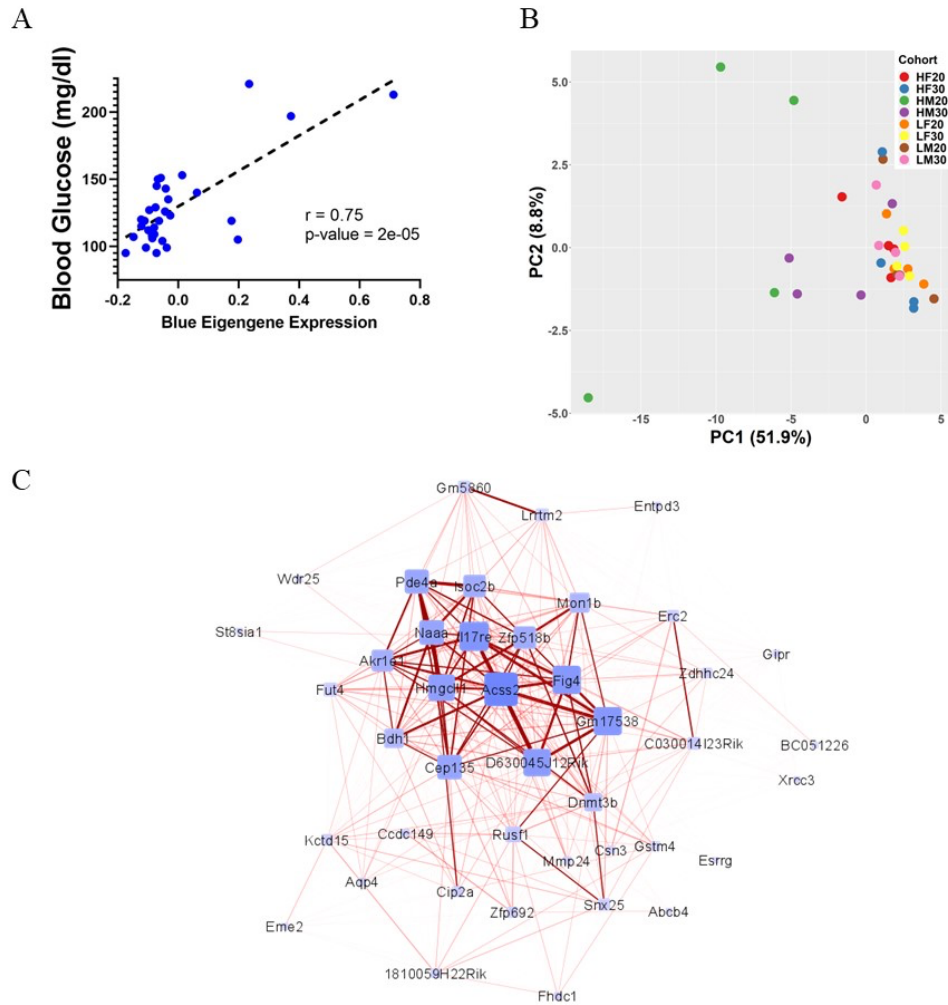
Supplemental Figure 3. β -cell-specific gene *Pdyn* is differentially expressed in obese males. **(A)** Principal component analysis of bulk RNA sequencing data normalized with only β -cell-specific genes. **(B)** Number of differentially expressed genes in females and males, across diets and age. **(C)** Differentially expressed genes between 20wk high-fat males and 30wk high-fat males. Vertical golden lines indicate threshold for significance based on average log fold change, horizontal line indicates threshold for significance based on FDR corrected p-value. Blue genes are significantly under-expressed in comparison. HF20 – 20wk high-fat female, HF30 – 30wk high-fat female, HM20 – 20wk high-fat male, HM30 – 30wk high-fat male, LF20 – 20wk low-fat female, LM20 – 20wk low-fat male, LF30 – 30wk low-fat female, LM30 – 30wk low-fat male.



Supplemental Figure 4. Metabolic and islet phenotypes from bulk RNA sequencing mice. **(A)** body weight, **(B)** blood glucose, **(C)** serum insulin levels collected after 4-hour fast. **(D)** Glucose-stimulated insulin secretion (GSIS), **(E)** Basal insulin secretion, **(F)** Islet insulin content collected after isolated islets were rested overnight. N = 4 mice per age X sex X diet cohort. N= 10 islets per individual for islet phenotypes. Middle bar represents mean, box represents 25th and 75th quartile, whiskers represent minimum and maximum values. HF20 – 20wk high-fat female, HF30 – 30wk high-fat female, HM20 – 20wk high-fat male, HM30 – 30wk high-fat male, LF20 – 20wk low-fat female, LM20 – 20wk low-fat male, LF30 – 30wk low-fat female, LM30 – 30wk low-fat male.



Supplemental Figure 5. Correlation between phenotype and module eigengene expression. Number of genes within each module reported on y-axis, phenotypic trait reported on x-axis. For each module-trait relationship, the Pearson correlation between eigengene expression and phenotype value is reported (top) along with an FDR-corrected p-value for the correlation (bottom). Color of box indicates strength of correlation. NS - non-significant association based on FDR-corrected p-value.



Supplemental Figure 6. Overview of blue module. **(A)** Correlation between blue module eigengene expression and blood glucose levels across all cohorts. **(B)** Principal component analysis of gene expression within the blue module, segregated by cohort. **(C)** Blue module network structure in across all cohorts. Size and color of node indicates overall connectivity within the network, thickness of edges indicates strength of correlation between gene pairs.

Supplemental Table 1. Differential gene expression between β -cell subtypes. Average log fold change (Avg_logFC) is calculated by dividing expression within subtype by expression in all other β -cells. Pct.1 is percent of cells in subtype expressing the gene, pct.2 is percent of all other β -cells expressing the gene. P_Val_adj is Bonferroni corrected p-value. Genes with Avg_logFC > 0.1 and p_val_adj <0.01 are considered significantly enriched in the subpopulation.

gene	cluster	avg_logFC	pct.1	pct.2	p_val	p_val_adj
Acly	Beta 1	0.669781	0.995	0.93	0	0
Rgs4	Beta 1	0.635721	0.986	0.906	0	0
Peg3	Beta 1	0.580347	0.971	0.841	0	0
Glul	Beta 1	0.549264	0.908	0.742	0	0
Gpx3	Beta 1	0.536209	0.949	0.859	0	0
Syt13	Beta 1	0.483343	0.972	0.868	0	0
Camk2n1	Beta 1	0.482152	0.962	0.86	0	0
Wnt4	Beta 1	0.480665	0.625	0.454	0	0
Ddc	Beta 1	0.470963	0.747	0.539	0	0
Ucp2	Beta 1	0.469822	0.899	0.749	0	0
Spock2	Beta 1	0.441173	0.892	0.753	0	0
Abcc8	Beta 1	0.431929	0.925	0.786	0	0
Hepacam2	Beta 1	0.427329	0.797	0.625	0	0
Syt7	Beta 1	0.425812	0.925	0.8	0	0
Sorbs2	Beta 1	0.425438	0.818	0.63	0	0
Sphkap	Beta 1	0.422549	0.829	0.662	0	0
Gatsl2	Beta 1	0.421583	0.814	0.651	0	0
Gnas	Beta 1	0.408791	0.999	0.985	0	0
Prnp	Beta 1	0.408473	0.943	0.816	0	0
Cplx2	Beta 1	0.40633	0.836	0.677	0	0
Rnase4	Beta 1	0.381295	0.978	0.895	0	0
Lgals3bp	Beta 1	0.37729	0.983	0.89	0	0
Atp2a2	Beta 1	0.377279	0.957	0.841	0	0
Ptprn	Beta 1	0.374313	0.981	0.902	0	0
Sez6l	Beta 1	0.356734	0.873	0.737	0	0

Supplemental Table 2. Differential gene expression between all β -cells in 20wk high-fat fed males and 30wk high-fat fed males. Average log fold change (Avg_logFC) is calculated by dividing expression within 20wk high-fat fed males by expression in 30wk high-fat fed males. Pct.1 is percent of 20wk high-fat fed male β -cells expressing the gene, pct.2 is percent of 30wk high-fat fed male β -cells expressing the gene. P_Val_adj is Bonferroni corrected p-value. Genes with Avg_logFC > 0.1 and p_val_adj <0.01 are considered significantly differentially expressed.

p_val	avg_logFC	pct.1	pct.2	p_val_adj	Gene
0	-1.89523	0.123	0.97	0	RP23-81C12.3
7.27E-290	1.220785	0.905	0.51	1.02E-285	Gm10076
1.92E-198	1.198659	0.844	0.562	2.70E-194	Uba52
4.28E-166	-0.89875	0.4	0.694	6.02E-162	Rpl10-ps3
1.82E-133	0.686969	0.99	0.945	2.55E-129	Rpl38
1.85E-128	-0.67703	0.75	0.888	2.60E-124	Rpl39-ps
1.20E-119	0.752163	0.937	0.797	1.69E-115	Rpl35
1.87E-114	0.722633	0.62	0.242	2.63E-110	Atp5l
1.65E-110	0.628877	0.992	0.959	2.31E-106	Rps29
7.13E-108	0.606599	0.987	0.952	1.00E-103	Rpl37a
1.71E-105	-0.63204	0.506	0.705	2.39E-101	Gm13841
1.00E-97	0.611405	0.985	0.937	1.41E-93	Rpl37
1.92E-97	0.561776	0.985	0.954	2.69E-93	Rps21
1.77E-96	-0.07931	0.007	0.151	2.48E-92	Gcg
1.52E-95	0.442955	0.393	0.066	2.13E-91	Gm4617
1.35E-89	0.452039	0.502	0.15	1.90E-85	Dbi
1.80E-87	0.473683	0.504	0.159	2.52E-83	Usp50
2.76E-83	0.582446	0.82	0.582	3.88E-79	Serf2
2.21E-82	-0.69301	0.721	0.868	3.10E-78	Mif
1.44E-73	0.593773	0.806	0.601	2.02E-69	Sec61g
1.72E-73	-0.4969	0.963	0.774	2.42E-69	mt-Atp6
9.13E-71	0.427517	0.9	0.67	1.28E-66	mt-Nd3
1.86E-68	-0.52397	0.964	0.783	2.61E-64	mt-Co3
2.83E-68	0.326981	0.246	0.024	3.97E-64	Hist1h2ap
2.80E-63	-0.16625	0.931	0.702	3.94E-59	Malat1

Supplemental Table 3. Differential gene expression between beta 1 and beta 2 subtypes. Average log fold change (Avg_logFC) is calculated by dividing expression within Beta 1 by expression in Beta 2. Pct.1 is percent of Beta 1 cells expressing the gene, pct.2 is percent of Beta 2 cells expressing the gene. P_Val_adj is Bonferroni corrected p-value. Genes with Avg_logFC > 0.1 and p_val_adj <0.01 are considered significantly differentially expressed.

p_val	avg_logFC	pct.1	pct.2	p_val_adj	Gene
0	0.484005	0.546	0.263	0	Ddc
0	0.459297	0.838	0.711	0	Ucp2
0	0.454491	0.627	0.356	0	Hepacam2
0	0.443471	0.99	0.976	0	mt-Nd1
0	0.438423	0.987	0.958	0	Acly
0	-0.18834	0.023	0.215	0	Ak4
0	-0.23468	0.029	0.228	0	Mgarp
0	-0.33205	0.069	0.291	0	Hilpda
0	-0.33307	0.096	0.384	0	Gys1
0	-0.37517	0.984	0.996	0	Eif1
0	-0.38668	0.202	0.513	0	P4ha2
0	-0.4045	0.966	0.986	0	Fau
0	-0.43226	0.212	0.51	0	Cdkn1a
0	-0.45035	0.081	0.437	0	Slc2a1
0	-0.48068	0.363	0.711	0	P4ha1
0	-0.48095	0.472	0.771	0	Pfkl
0	-0.51603	0.283	0.626	0	Egln1
0	-0.52548	0.094	0.38	0	Ldha
0	-0.53734	0.938	0.972	0	Pkm
0	-0.54822	0.323	0.708	0	Pfkl
0	-0.56701	0.946	0.982	0	Bsg
0	-0.57694	0.659	0.884	0	Gpi1
0	-0.58394	0.402	0.689	0	Cebpd
0	-0.59889	0.632	0.799	0	Sod2
0	-0.66421	0.924	0.98	0	Aldoa

Supplemental Table 4. Differential gene expression between Beta 1 cells in 20wk high-fat fed males and 30wk high-fat fed males. Average log fold change (Avg_logFC) is calculated by dividing expression within beta 1 cells of 20wk high-fat fed males by expression in beta 1 cells 30wk high-fat fed males. Pct.1 is percent of Beta 1 cells in 20wk high-fat fed males expressing the gene, pct.2 is percent of Beta 1 cells in 30wk high-fat fed males expressing the gene. P_Val_adj is Bonferroni corrected p-value. Genes with Avg_logFC > 0.1 and p_val_adj < 0.01 are considered significantly differentially expressed.

p_val	avg_logFC	pct.1	pct.2	p_val_adj	Gene
1.56E-287	-2.13328	0.115	0.984	2.18E-283	RP23-81C12.3
2.75E-165	1.228821	0.945	0.544	3.86E-161	Gm10076
7.05E-106	1.175078	0.863	0.571	9.90E-102	Uba52
5.40E-98	-0.88653	0.414	0.707	7.58E-94	Rpl10-ps3
2.00E-79	0.72301	0.995	0.944	2.82E-75	Rpl38
1.13E-72	0.635999	0.997	0.941	1.59E-68	Rpl37a
1.74E-71	0.691517	0.995	0.962	2.45E-67	Rps29
1.43E-69	0.676092	0.994	0.935	2.01E-65	Rpl37
3.86E-69	0.776445	0.949	0.792	5.42E-65	Rpl35
5.36E-69	0.752005	0.643	0.219	7.52E-65	Atp5l
5.08E-65	0.62566	0.991	0.957	7.14E-61	Rps21
8.01E-63	-0.65683	0.498	0.713	1.12E-58	Gm13841
1.26E-60	-0.62399	0.762	0.876	1.76E-56	Rpl39-ps
6.39E-51	0.447893	0.451	0.086	8.97E-47	Gm4617
4.39E-48	0.478908	0.54	0.167	6.17E-44	Usp50
4.46E-47	-0.85928	0.997	0.921	6.27E-43	mt-Co3
5.60E-45	-0.81853	0.997	0.921	7.86E-41	mt-Atp6
1.43E-44	0.57623	0.849	0.6	2.01E-40	Serf2
1.01E-43	0.383597	0.302	0.025	1.42E-39	Hist1h2ap
1.68E-43	0.444955	0.54	0.183	2.36E-39	Dbi
3.66E-43	0.570022	0.961	0.853	5.13E-39	Rpl35a
1.78E-41	0.63003	0.831	0.6	2.51E-37	Sec61g
4.56E-41	0.429884	0.992	0.944	6.40E-37	Rps8
2.99E-37	0.401338	0.93	0.738	4.20E-33	Ndufa2
1.28E-36	-0.32228	0.087	0.336	1.80E-32	Wdr89

Supplemental Table 5. β -cell-specific genes identified in single cell RNA sequencing analysis.

Gene.stable.ID	Gene.name
ENSMUSG000000086714	0610009E02Rik
ENSMUSG000000070532	1700084C01Rik
ENSMUSG000000086390	1810019D21Rik
ENSMUSG000000085455	1810059H22Rik
ENSMUSG000000036552	2210404J11Rik
ENSMUSG000000043833	2900005J15Rik
ENSMUSG000000091636	A330050F15Rik
ENSMUSG000000071567	A830039N20Rik
ENSMUSG000000089809	A930011G23Rik
ENSMUSG000000042476	Abcb4
ENSMUSG000000026198	Abcb6
ENSMUSG00000000686	Abhd15
ENSMUSG000000097145	AC102739.1
ENSMUSG000000097383	AC130216.1
ENSMUSG000000097312	AC131780.1
ENSMUSG000000034853	Acot11
ENSMUSG000000043587	Acpl2
ENSMUSG000000020333	Acsl6
ENSMUSG000000027605	Acss2
ENSMUSG000000074207	Adh1
ENSMUSG000000042429	Adora1
ENSMUSG000000033717	Adra2a
ENSMUSG000000033715	Akr1c14
ENSMUSG000000045410	Akr1e1
ENSMUSG000000020256	Aldh1l2

Supplemental Table 6. Bulk RNA sequencing differential expression analysis for β -cell-specific genes. For each analysis, log fold change, counts per million, p-value, and fdr corrected p-value are reported. hf - 20wk high-fat females/30wk high-fat females, hm - 20wk high-fat males/30wk high-fat males, lf - 20wk low-fat females/30wk low-fat females, lm - 20wk low-fat males/30wk low-fat males, f20 - 20wk high-fat females/20wk low-fat females, m20 - 20wk high-fat males/20wk low-fat males, f30 - 30wk high-fat females/30wk low-fat females, m30 - 30wk high-fat males/30wk low-fat males, age - all 20wk mice/all 30wk mice, diet - all high-fat mice/ all low-fat mice, sex - all females/all males.

Gene.name	Gene	hf_logFC	hf_logCPM	hf_PValue	hf_FDR
Gm5860	ENSMUSG00000085022	-0.082107426	7.513984219	0.85063417	0.989357442
Pdyn	ENSMUSG00000027400	-0.327040319	12.10666437	0.412914101	0.892219835
Car10	ENSMUSG00000056158	-0.093792668	4.704952766	0.815374712	0.989357442
Rbm20	ENSMUSG00000043639	-0.469861092	3.801321284	0.320459044	0.892219835
Ucn3	ENSMUSG00000044988	-0.619304611	9.401425117	0.157996714	0.875911606
2900005J15Rik	ENSMUSG00000043833	0.624795158	3.063310301	0.202824547	0.892219835
Ggt7	ENSMUSG00000027603	-0.583585178	5.148752455	0.154807516	0.873556699
Fam151a	ENSMUSG00000034871	-0.551653616	7.769859022	0.267992949	0.892219835
Brsk2	ENSMUSG00000053046	0.054638887	2.669524981	0.918081295	0.989357442
Mns1	ENSMUSG00000032221	-0.050080636	5.072095688	0.895851016	0.989357442
Tat	ENSMUSG00000001670	-0.008736211	6.019045931	0.985201609	1
Ffar3	ENSMUSG00000019429	0.281173065	3.548861351	0.545593143	0.945441292
Ttyh1	ENSMUSG00000030428	0.755295873	10.64911071	0.081747825	0.659285338
Tmem215	ENSMUSG00000046593	0.107359097	8.485376282	0.770481377	0.989357442
Nyap2	ENSMUSG00000054976	0.089339266	4.331287876	0.87447964	0.989357442
Lrrtm2	ENSMUSG00000071862	-0.200946622	5.596960593	0.619575241	0.945441292
Cdh8	ENSMUSG00000036510	0.503616009	3.83311436	0.346791763	0.892219835
Acss2	ENSMUSG00000027605	0.739560945	3.19627282	0.115580751	0.750727862
Th	ENSMUSG00000000214	0.308079733	8.08242172	0.427657188	0.892219835
Utp14b	ENSMUSG00000079470	0.526911706	2.179213433	0.497762916	0.930728293
Zfp324	ENSMUSG00000004500	0.265970775	3.295170825	0.580080308	0.945441292
Gucy2c	ENSMUSG00000042638	0.679410571	7.492405834	0.206955805	0.892219835
Cyp7b1	ENSMUSG00000039519	0.15367837	4.53476516	0.689630334	0.97905534
D630045J12Rik	ENSMUSG00000063455	0.062653056	3.393415684	0.927592316	0.989357442
Entpd3	ENSMUSG00000041608	-0.09652747	10.43136193	0.808111191	0.989357442

Supplemental Table 7. Gene significance and module membership for weighted gene co-expression analysis. For each gene, module membership is reported. Gene significance (GS) for each trait is reported as a Pearsons correlation, along with p-value (p.GS) associated with correlation. Module membership (MM) for each module reported as Pearsons coefficient along with p-value (p.MM) associated with correlation. BG - Blood glucose, GSIS - Glucose-stimulated insulin secretion, Basal.INS - Basal insulin secretion, INS.content - islet insulin content.

Ensembl	moduleCo	GS.datTraits.Body.Weight	p.GS.datTraits.Body.Weight	GS.datTraits.BG	p.GS.datTraits.BG
ENSMUSG00000000214	black	-0.387992956	0.028216289	-0.23515641	0.195120973
ENSMUSG00000000215	red	-0.309477385	0.084776654	-0.302141111	0.092819842
ENSMUSG00000000276	turquoise	0.064094056	0.72746158	0.232612645	0.200136582
ENSMUSG00000000301	grey	0.029904252	0.870935943	0.129581654	0.479660091
ENSMUSG00000000305	turquoise	0.041785284	0.820370928	-0.371521376	0.036293511
ENSMUSG00000000686	turquoise	-0.42817346	0.014493631	-0.50392194	0.00327653
ENSMUSG00000001566	brown	-0.624583403	0.000132982	-0.29371165	0.102764746
ENSMUSG00000001622	blue	0.540872835	0.001392444	0.546872544	0.001200561
ENSMUSG00000001670	brown	-0.40936357	0.019987791	-0.593071929	0.000347258
ENSMUSG00000001901	grey	0.023437458	0.898683446	-0.155419059	0.39566846
ENSMUSG00000002324	yellow	-0.379070406	0.032386271	-0.347145643	0.051577533
ENSMUSG00000002799	grey	-0.013889375	0.939858524	-0.216466327	0.23405717
ENSMUSG00000004500	grey	-0.185246593	0.310086547	-0.415549288	0.01801725
ENSMUSG00000005232	yellow	-0.434483292	0.012961012	-0.506161316	0.003119249
ENSMUSG00000005268	brown	-0.785512708	1.01E-07	-0.506473638	0.003097841
ENSMUSG00000005373	grey	-0.231611791	0.20213426	-0.110208346	0.548198081
ENSMUSG00000005640	brown	-0.714201877	4.42E-06	-0.463128446	0.007600276
ENSMUSG00000005682	turquoise	0.363108836	0.041087765	0.273308203	0.130143212
ENSMUSG00000006463	blue	0.268343806	0.137549569	0.344493334	0.053509939
ENSMUSG00000006641	red	0.105057934	0.567159291	-0.047508882	0.79624947
ENSMUSG00000007594	turquoise	-0.109718162	0.549989813	-0.250249194	0.167156975
ENSMUSG00000007908	blue	0.61171477	0.000199239	0.575492537	0.000568915
ENSMUSG00000008461	turquoise	0.604336805	0.00024926	0.496766039	0.003825919
ENSMUSG00000009246	brown	-0.547401038	0.001184826	-0.061098002	0.739751736
ENSMUSG00000009739	turquoise	0.454182593	0.009021879	0.458518641	0.008306997

Supplemental Table 8. Network connectivity for all β -cell genes. Strength of connectivity for each gene within its assigned module reported as k-within value.

Gene	moduleColor	All_kWithin	hf20_kWithin	hm20_kWithin	lf20_kWithin	lm20_kWithin	hf30_kWithin
ENSMUSG00000000214	black	3.061009444	4.216736063	0.715978632	0.959009053	4.515787698	0.710101472
ENSMUSG00000000215	red	2.680056484	6.10788962	5.808888359	3.695878561	4.181470523	7.384521496
ENSMUSG00000000276	turquoise	0.372522114	7.432389626	4.585141312	12.68478687	9.21723427	8.178172693
ENSMUSG00000000301	grey	0.285150275	11.54473485	14.13396877	12.60693335	9.28650462	15.55721377
ENSMUSG00000000305	turquoise	0.791828722	9.410496738	4.850622595	13.57004274	9.690189964	10.92924283
ENSMUSG00000000686	turquoise	1.537749999	4.270789321	3.911499917	8.624234995	6.025118295	11.49768394
ENSMUSG00000001566	brown	0.382724588	8.141217542	4.270162913	7.202536054	2.613167434	4.613872846
ENSMUSG00000001622	blue	0.709826254	10.38650173	2.592431943	5.536986515	4.261273285	3.248942462
ENSMUSG00000001670	brown	1.900481103	8.474266263	4.025060145	9.028137433	7.77874078	7.045516776
ENSMUSG00000001901	grey	0.052604157	19.50900488	8.386575984	15.37058172	11.78941683	10.47787439
ENSMUSG00000002324	yellow	0.161223547	3.401832672	5.248428873	0.852245905	3.361569186	3.243890919
ENSMUSG00000002799	grey	0.030332378	7.19502349	13.28209487	8.727800487	18.51703303	14.22393678
ENSMUSG00000004500	grey	0.066910094	14.44259317	10.67127991	16.54096184	12.97447512	19.79910121
ENSMUSG00000005232	yellow	1.584764699	3.198698278	2.149865892	2.787889679	7.76155106	3.715523454
ENSMUSG00000005268	brown	3.221853608	9.045878743	5.746257291	3.466950659	4.802587313	6.100967891
ENSMUSG00000005373	grey	0.069666893	16.46022747	12.0053316	10.84508861	9.593634653	19.60815624
ENSMUSG00000005640	brown	1.537854104	8.722337182	5.822238008	4.060335187	13.5552928	6.107506813
ENSMUSG00000005682	turquoise	8.924142198	8.641116697	12.21529901	15.0477548	6.992438159	11.72693347
ENSMUSG00000006463	blue	1.172718057	4.882319158	2.590179084	8.574723785	10.99031821	8.771941302
ENSMUSG00000006641	red	1.026610144	6.749799716	6.16937584	4.36084843	4.679082731	3.978082492
ENSMUSG00000007594	turquoise	0.842756222	6.263414112	5.123389463	2.607824003	9.974447615	12.81048387
ENSMUSG00000007908	blue	4.590049479	5.164482483	10.92930213	6.984019547	5.100055148	12.57665928
ENSMUSG00000008461	turquoise	2.065857425	7.674505909	12.26005426	13.93931118	6.779764532	10.16485118
ENSMUSG00000009246	brown	0.306474124	8.910776765	7.73802338	9.706351064	13.07996639	4.583305512
ENSMUSG00000009739	turquoise	4.100165482	4.661999158	5.821822903	10.49353729	9.751222861	6.385883693