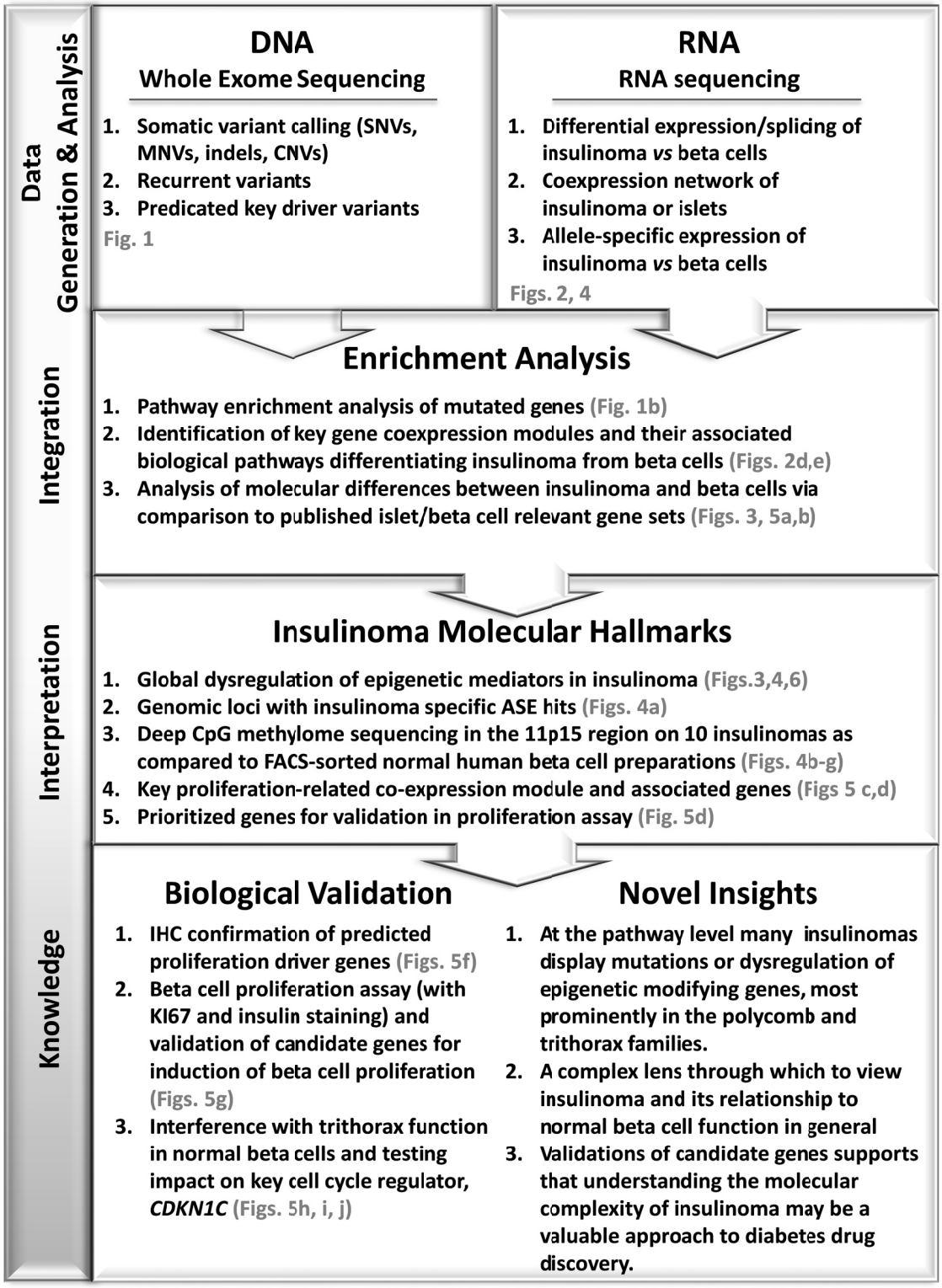
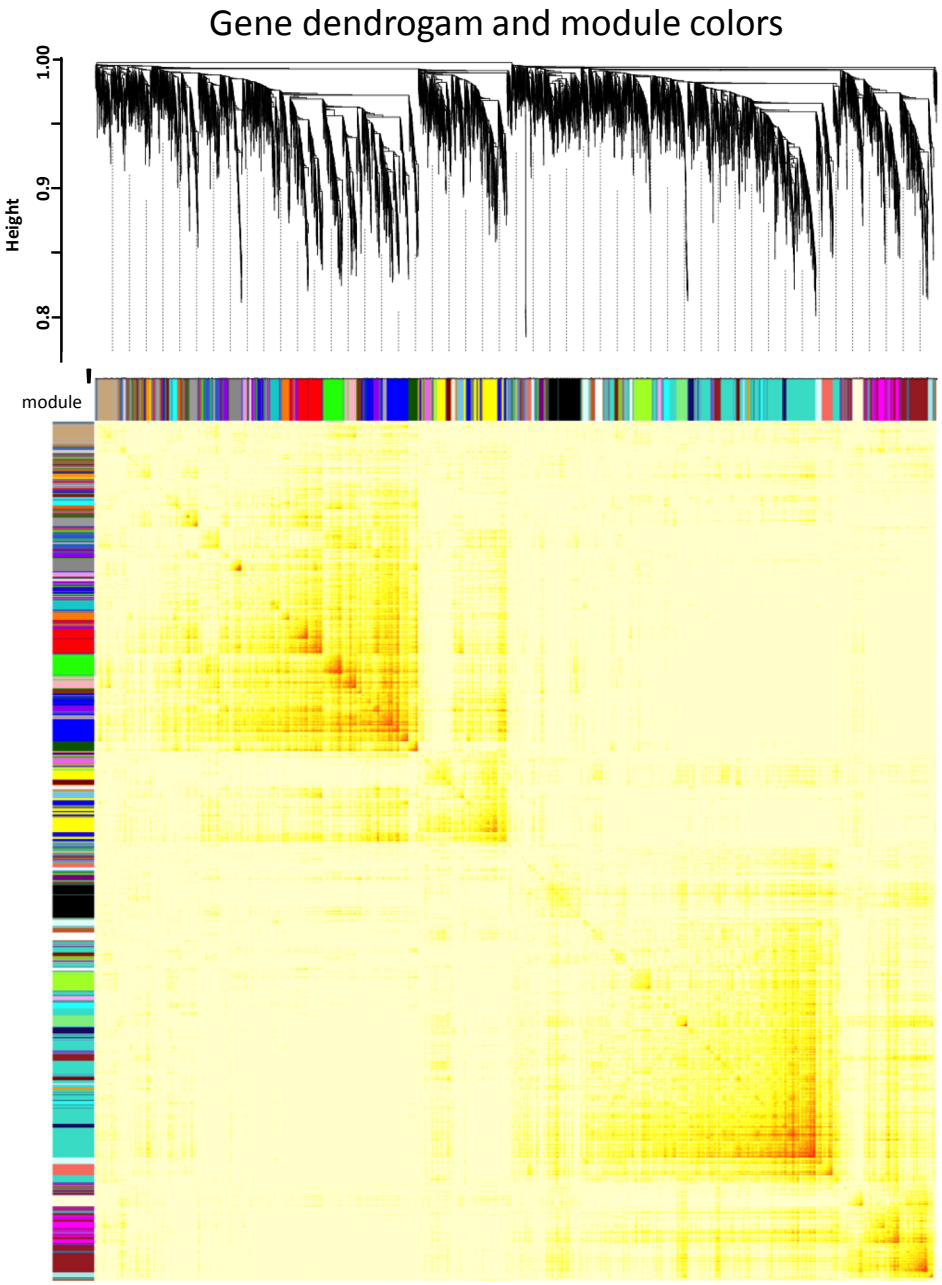
## Supplementary Figure 1

# Strategy for Integrating Molecular Landscapes in Human Insulinomas

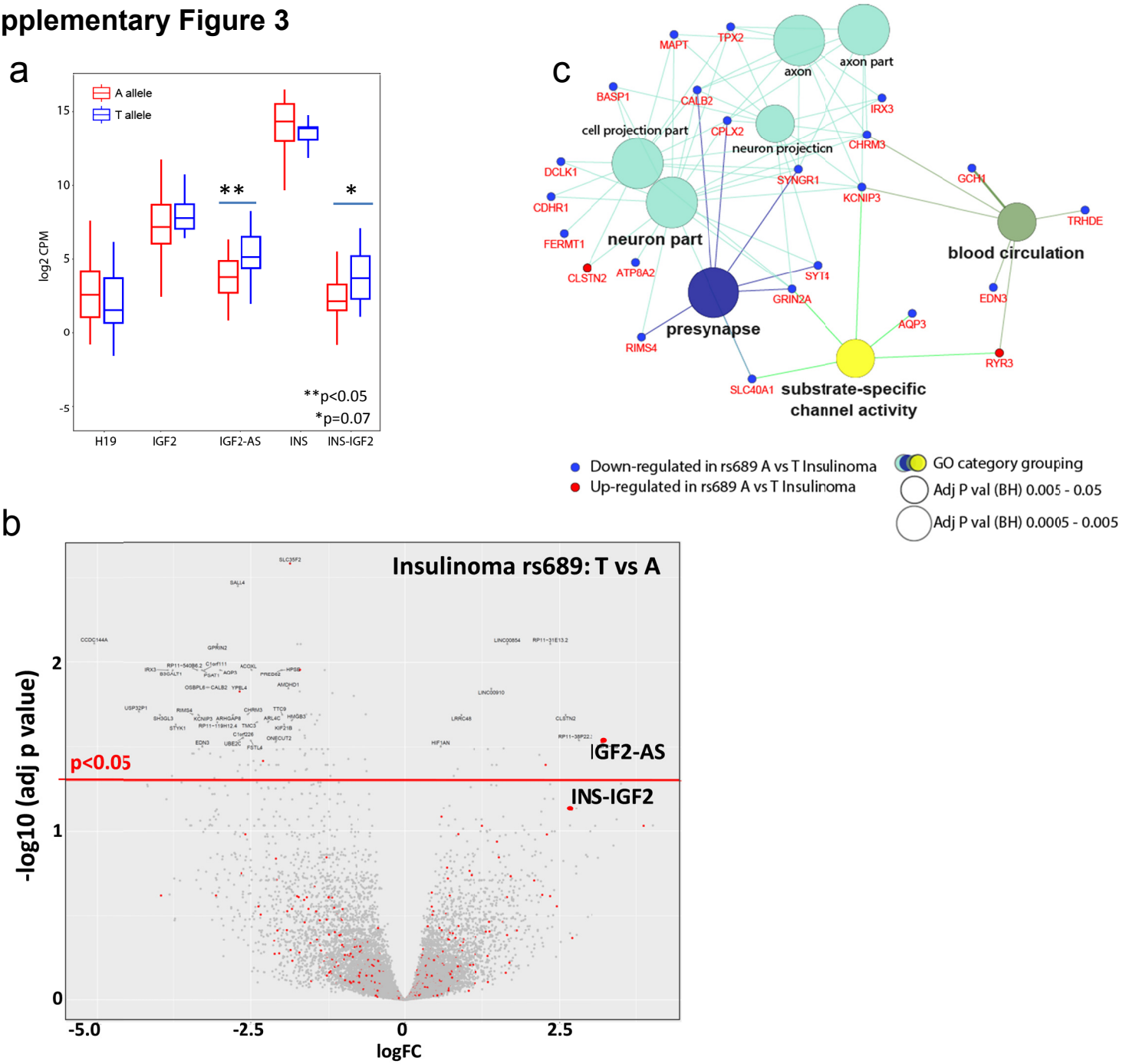


## Supplementary Figure 2.



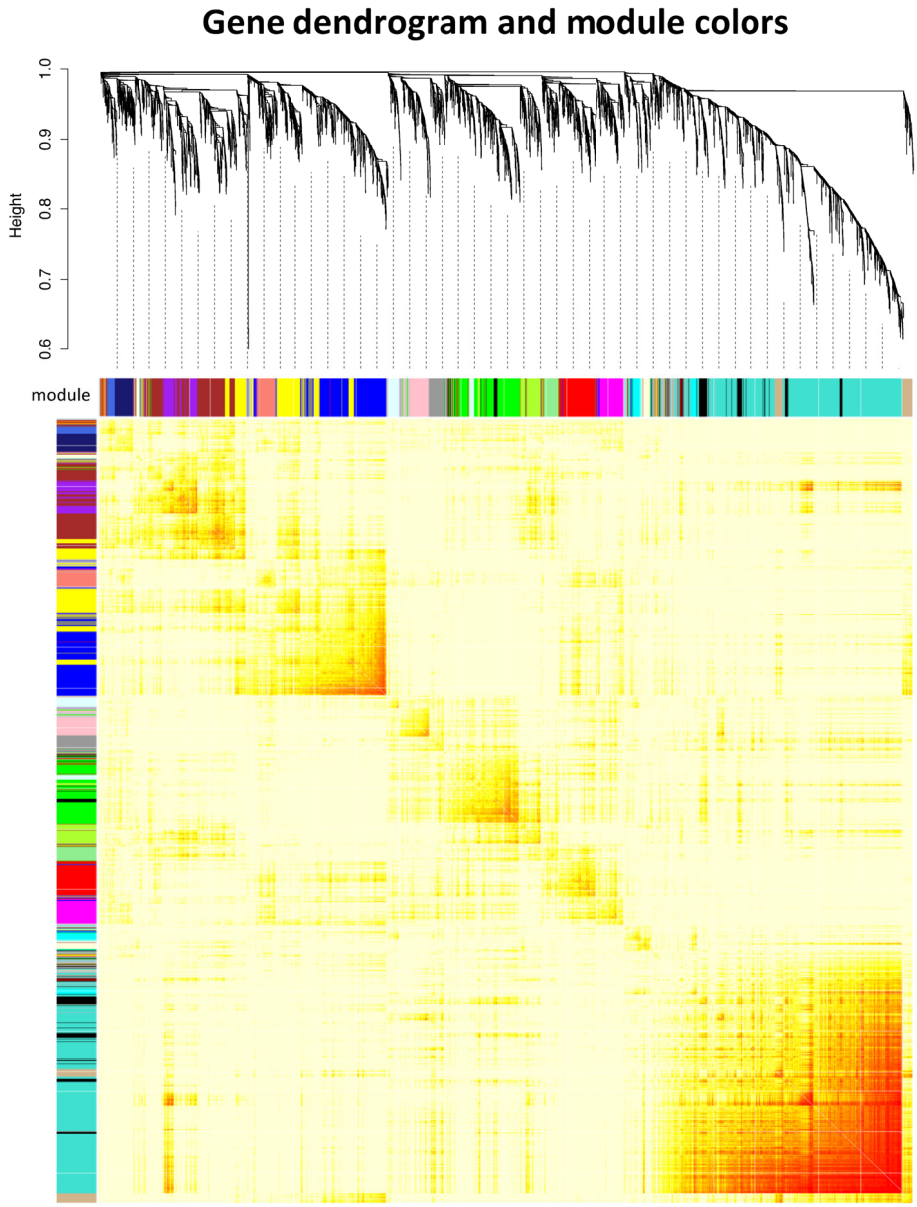

**The Complete Insulinoma Co-Expression Network.**
Clustering of the Topological Overlap Matrix (TOM) for expressed protein coding genes in insulinomas based on RNAseq data. In the symmetric heatmap, all the genes are represented in rows and columns in the same order. The color intensity reflects the connection strength between two genes, with red representing the strongest signal and white representing the weakest signal. Gene modules were denoted in both x any y axes in different colors.

## Supplementary Figure 3




**Effects of the rs689 SNP site on chromosome 11 gene expression.**
**a.** Insulinoma samples were separated according to whether they expressed the A (reference allele) (n=17) or the T allele (n=8) at rs689, a SNP within the insulin gene. Differential expression analysis of select genes physically located near insulin revealed higher expression of IGF2-AS and near significant elevation of INS-IGF2 read-through transcript. **b.** Expanded genome-wide differential expression analysis between these two groups revealed 109 differentially expressed genes at adjusted p value >0.05 (Supplementary Data 17). **c.** Pathway enrichment analysis of these 109 differentially expressed genes, performed by ClueGO using GO annotation databases, revealed several significantly enriched pathways. The node and edge plot shows the enriched pathways and the differentially expressed genes found associated with them. Small nodes which are red and blue indicate whether that gene was significantly up- or down- regulated, respectively, in the comparison of T vs A allele-expressing insulinomas. The size of the nodes associated with pathway annotations relate to their respective adjusted p values as indicated while the coloring of the nodes relates to the GO annotation assigned grouping.

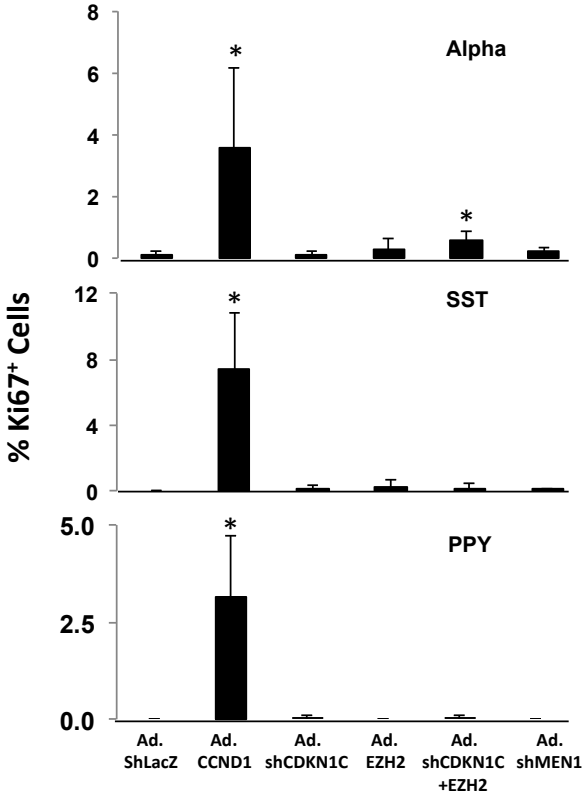
## Supplementary Figure 4




**The Complete Human Islet Co-Expression Network.**

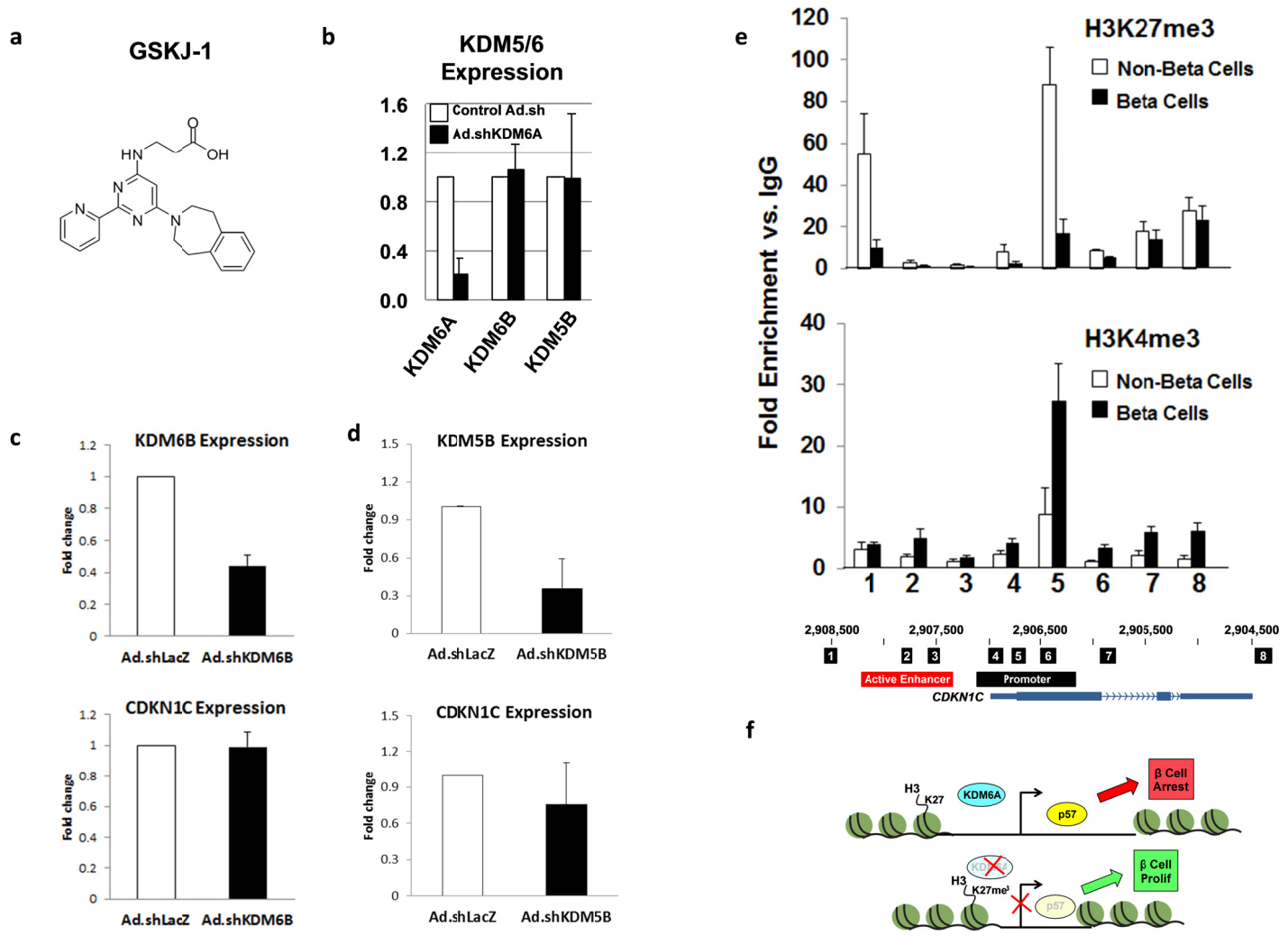
The same as **Supplementary Figure 2**, but for the human islet RNAseq samples from Fadista et al[30].

## Supplementary Figure 5



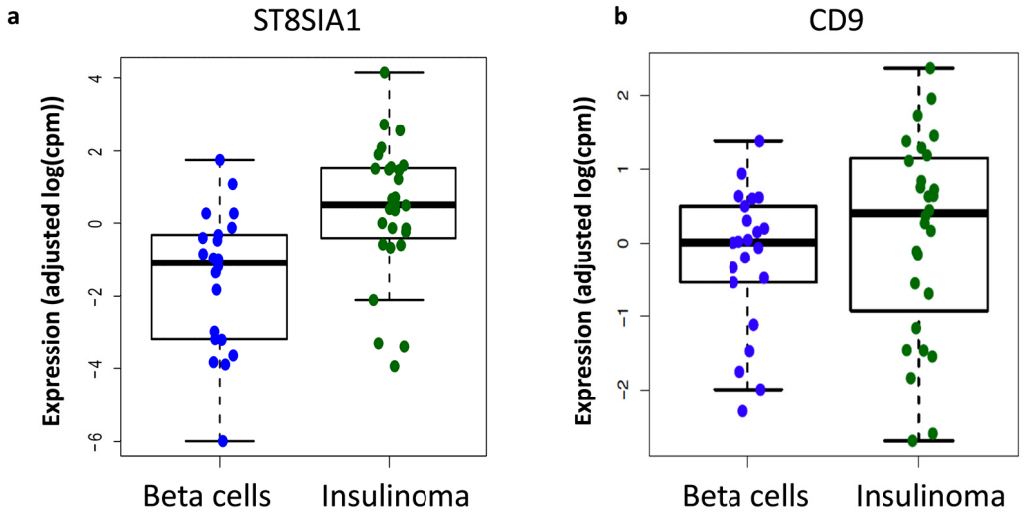

**The effect of adenoviral delivery of epigenetic modifiers and cell cycle genes on proliferation in non-beta cells.** Ki67 was used as a marker for proliferation, in dispersed human islets, co-immunolabeled with glucagon (alpha cells), somatostatin (delta cells) or PP (PP cells). As expected, *CCND1* induces proliferation in all cells, whereas overexpressing *EZH2*, or silencing *MEN1, CDKN1C* or a combination of *EZH2* overexpression plus *CDKN1C* silencing had no effect. Results are expressed as the percent of the three specific endocrine cell types in which Ki67 labeling was present. A minimum of 1000 cells were counted for each islet cell type. Error bars indicate SEM, and * indicates $p < 0.05$ by Student's unpaired T-test.

## Supplementary Figure 6




**Direct regulation of the cell cycle inhibitor gene, CDKN1C encoding p57$^{KIP2}$ by the trithorax member, KDM6A.** **a.** The structure of the pharmacologic *KDM6A* inhibitor, GSKJ-1. **b.** Efficacy of adenoviral silencing *KDM6A* in human islets on expression of *KDM6A*, but not on the related genes *KDM6B* and *KDM5B*, demonstrating the specificity of the Ad.shKDM6A virus on *KDM6A* expression. Error bars indicate SEM. **c.** Lack of effect of adenoviral silencing of *KDM6B* on *CDKN1* expression. Error bars indicate SEM. **d.** Lack of effect of adenoviral silencing of *KDM5B* on *CDKN1C* expression. Error bars indicate SEM. **e.** The upper panel shows ChIP of FACS-sorted beta and non-beta cells for the repressive, polycomb-dependent H3K27me3 mark on the *CDKN1C* region. H3K27me3 is present in upstream and promoter regions of *CDKN1C* in non-beta cells, consistent with the absence of *CDKN1C* expression in non-beta cells. In contrast, this mark is reduced or absent on *CDKN1C* in beta cells, consistent with expression of *CDKN1C* in beta cells. The lower panel shows similar experiments for the open trithorax-dependent chromatin mark, H3K4me3, showing that this region is predicted to be accessible in beta cells, but not non-beta cells, consistent again with *CDKN1C* expression in beta cells, but not other islet cell types. **f.** A cartoon illustrating these changes in beta cells under basal conditions, and (top) and beta cells in which KDM6A has been inhibited with GSKJ-1 or Ad.shKDM6A (bottom), and highlighting the direct interaction of the trithorax member, KDM6A, with the *CDKN1C* locus.

## Supplementary Figure 7



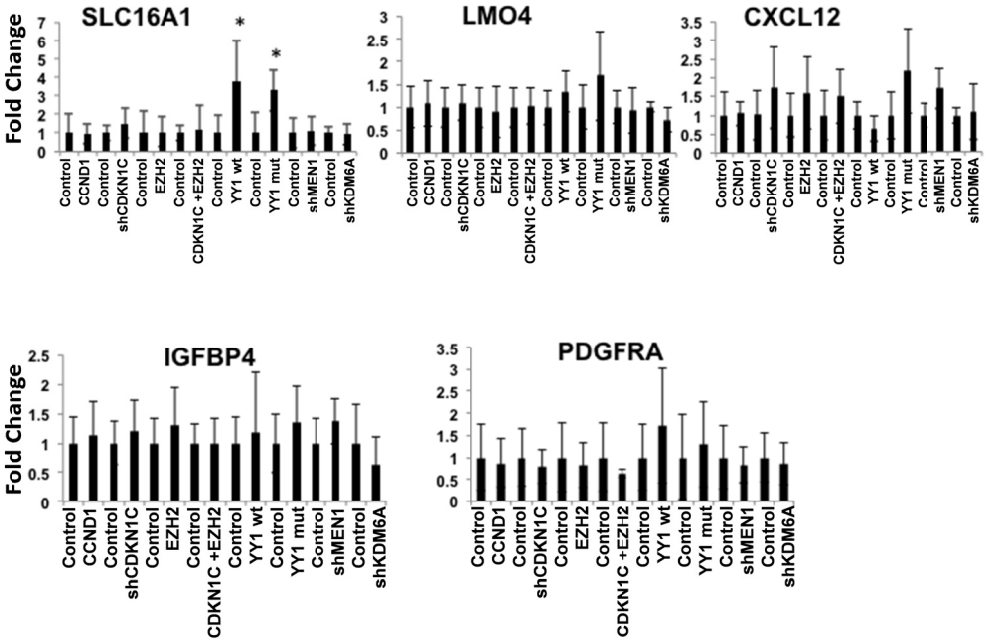

**Heterogeneous expression of ST8SIA1 and CD9 in human beta cells and insulinomas.** Box and whisker plot of RNAseq or ST8SIA1 and CD9, canonically associated with beta cell heterogeneity[49], expressed as FPKM for each of the two genes among 22 sets of FACS-sorted beta cells and the 25 insulinomas. The data do not allow for assessment of heterogeneity within beta cells in a single islet or insulinoma, but indicate that both beta cells and insulinomas display marked inter-specimen heterogeneity, likely due at least in part, to heterogeneity of cells within a given sample.

## Supplementary Figure 8

**a Insulinomas vs beta cells**

| Gene | log2FC | Avg FPKM | FDR |
|---|---|---|---|
| PDGFRA | 3.72233 | 1.93358 | 3.46E-08 |
| CXCL12 | 3.61433 | 3.1339 | 1.47E-07 |
| LMO4 | 1.59807 | 2.16362 | 0.0000412 |
| SLC16A1 | 1.45058 | 1.17362 | 0.0008609 |
| IGFBP4 | 1.48303 | 5.69898 | 0.0011412 |
| FCGRT | 0.861763 | 4.57001 | 0.020955 |
| CD302 | 0.625648 | 3.09759 | 0.0699045 |
| TNS1 | -0.727501 | 7.00011 | 0.161207 |
| C1QBP | 0.280112 | 5.62119 | 0.29666 |
| LDHA | 0.252584 | 4.9272 | 0.64934 |
| OAT | 0.0802741 | 5.84896 | 0.728528 |
| SMAD3 | -0.081958 | 4.56325 | 0.891364 |

**b Transduced Human Islets**



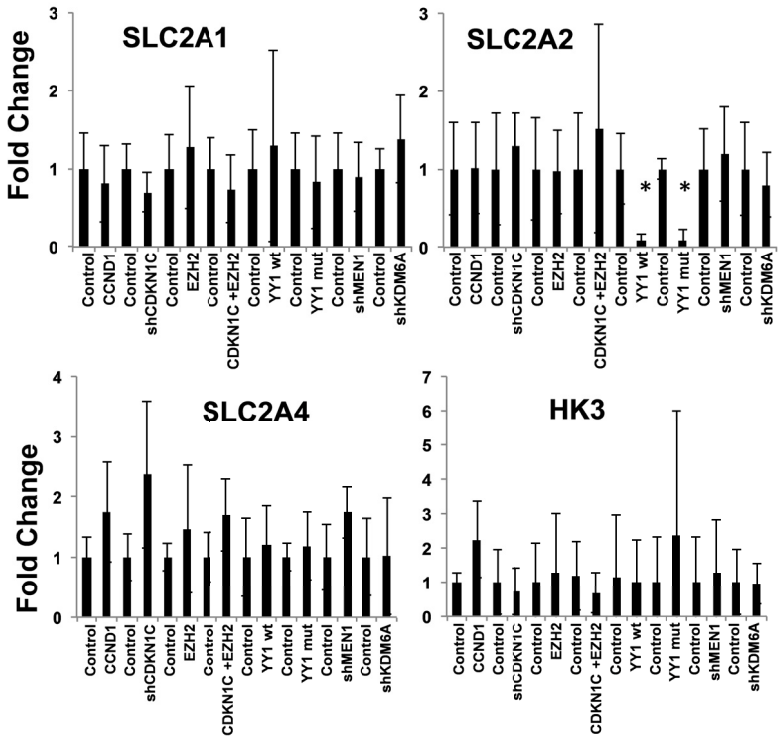

**Differential expression of "disallowed genes" in insulinomas (left) and effect of cell cycle and epigenetic manipulation on these genes in normal islets (right).** **a.** Differential expression of candidate disallowed genes in insulinomas vs. beta cells, extracted from **Supplementary Data 8**. The yellow-shaded are statistically significantly increased in insulinomas as compared to FACS-sorted beta cells, and may in theory contribute to the abnormal secretion of insulin in insulinomas. **b.** Effects of adenoviral treatment of human islets with a control adenovirus, or adenoviruses expressing *CCND1, EZH2, YY1,* the Thr372Arg mutant of *YY1*, or encoding shRNAs for *CDKN1C, KDM6A*, or *MEN1* as shown. Among these, only mutant and wild-type *YY1* altered expression of only one "disallowed gene", *SLC16A*, the pyruvate-lactate transporter. Error bars indicate SEM, and * indicates p <0.05 by Student's unpaired T-test.

# Supplementary Figure 9

**a Insulinomas vs beta cells**

| Gene | log2FC | Avg FPKM | FDR |
|---|---|---|---|
| SLC2A1 | -1.46509 | 4.3283 | 9.42E-09 |
| HK3 | 3.15643 | -0.455474 | 3.95E-08 |
| SLC2A4 | 3.07589 | -0.942312 | 2.01E-07 |
| SLC2A2 | -4.78802 | 2.62279 | 5.77E-07 |
| HK1 | 1.54803 | 3.16401 | 0.0016013 |
| SLC2A3 | 1.22031 | 4.38292 | 0.0118842 |
| GCK | -0.723758 | 6.01771 | 0.147475 |
| HK2 | 0.880302 | 0.594225 | 0.22354 |
| SLC2A6 | -0.1446 | 4.33046 | 0.705597 |
| SLC2A5 | N/A | <1 | N/A |

**b Transduced Human Islets**



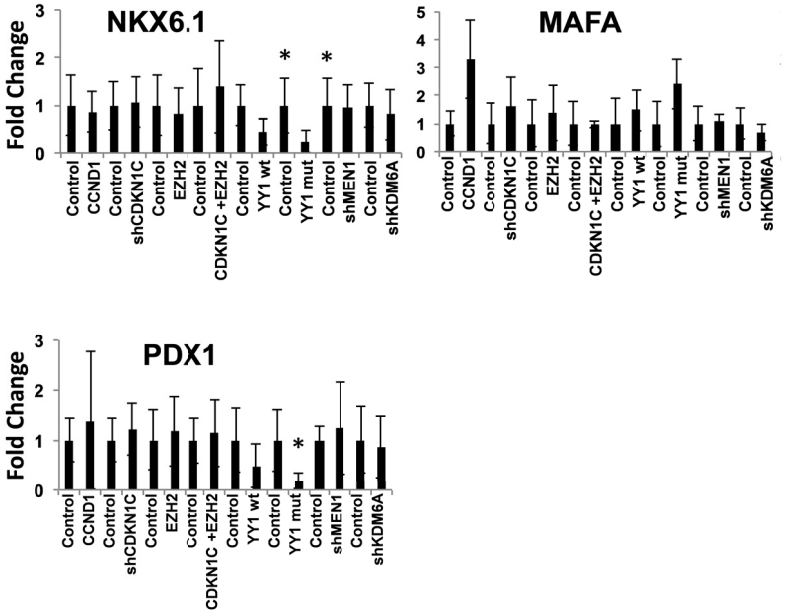

**Differential expression of glucose transporters and hexokinases in insulinomas (left) and effect of epigenetic manipulation and effect of cell cycle and epigenetic manipulation on these genes in normal islets (right).** **a.** Differential expression of glucose transporter and hexokinase genes in insulinomas vs. beta cells, extracted from **Supplementary Data 8**. The yellow-shaded are statistically significantly different in insulinomas as compared to FACS-sorted beta cells, and may in theory contribute to the abnormal secretion of insulin in insulinomas. **b.** Effects of adenoviral treatment of human islets with a control adenovirus, or adenoviruses expressing *CCND1, EZH2, YY1,* the Thr372Arg mutant of *YY1*, or encoding shRNAs for *CDKN1C, KDM6A*, or *MEN1*, as shown. Among these, only mutant and wild-type *YY1* altered expression of only one of the genes in this group, *SLC2A2*, encoding the Glut2 transporter. Error bars indicate SEM, and * indicates p <0.05 by Student's unpaired T-test.

## Supplementary Figure 10

**a Insulinomas vs beta cells**

| Gene | log2FC | Avg FPKM | FDR |
|---|---|---|---|
| PDX1 | -1.15702 | 6.32986 | 0.0138055 |
| NKX6-1 | -1.11 | 6.58 | 0.0708053 |
| NEUROD1 | -0.897058 | 6.53137 | 0.104482 |
| ISL1 | -0.545179 | 6.36173 | 0.119793 |
| NKX2-2 | -0.68 | 5.28 | 0.308324 |
| MAF | 0.0552567 | 4.60599 | 0.89735 |
| MAFB | -0.0838149 | 7.77628 | 0.901517 |
| MAFA | -0.0906068 | 6.84971 | 0.9272 |
| NEUROG3 | 0 | <1 | N/A |
| ARX | N/A | 0-1 | N/A |

**b Transduced Human Islets**




**Differential expression of key beta cell transcription factors in insulinomas (left) and effect of epigenetic manipulation and effect of cell cycle and epigenetic manipulation on these genes in normal islets (right). a.** Differential expression of transcription factor genes in insulinomas vs. beta cells, extracted from **Supplementary Data 8**. None are strongly different in insulinomas as compared to FACS-sorted beta cells. The minor decreases may reflect contamination of insulinomas with non-beta cell types such as endothelial cells and fibroblasts. **b.** Effects of adenoviral treatment of human islets with a control adenovirus, or adenoviruses expressing *CCND1, EZH2, YY1,* the Thr372Arg mutant of *YY1*, or encoding shRNAs for *CDKN1C, KDM6A*, or *MEN1*, as shown. Among these, only mutant and wild-type *YY1* altered expression of only two of the genes in this group, *NKX6.1* and *PDX1*. Error bars indicate SEM, and * indicates $p < 0.05$ by Student's unpaired T-test.

# Supplementary Table 1.

# Insulinoma and Patient Characteristics.

| Insulinoma # | Age/ Gender | Mt. Sinai ID # | Histology | Diameter | Benign vs. Malig* | Insulinoma DNA Seq | WBC DNA Seq | RNAseq (RIN) | Lowest Blood Glucose/Insulin/ C-Peptide/Prolinsulin | Ki67 | Hypoglycemia Resolved Postop | FH MEN1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 53F | 4418 | Insulinoma | 1.4 cm | B | WES | WES | good | 31/10.8/?/? | <1% | Yes | No |
| 2 | 48M | 4950 | Insulinoma | 3.6 cm | B | WES | WES | 8.3 | 34,53/11.5/2.7/? | 3-5% | Yes | No |
| 3 | 73F | 4973 | Insulinoma | 1.9 cm | B | WES | WES | 6.7 | 43/5,13/2.1,2.6/? | <1% | Yes | No |
| 4 | 82M | 4974 | Insulinoma | 2.2 cm | B | WES | WES | 4.7 | 38/11.9,17.5/7.2,5.4/? | <1% | Yes | No |
| 5 | 43M | 5318 | Insulinoma | 1.5 cm | B | WES | WES | 5.8 | 47/23/6.63/199.5 | 2-3% | Yes | No |
| 6 | 18M | 5320 | Insulinoma | 3.0 cm | B | WES | WES | 5.6 | 33/48/6.55/250.4 | 7% | Yes | No |
| 7 | 83M | 5322 | Insulinoma | 1.0 cm | B | WES | WES | N/A | 40/3.7/2.47/38 | N/A | Yes | No |
| 8 | 58M | 5326 | Insulinoma | 1.5 cm | B | WES | WES | N/A | <50/35/2867/320 | 3% | Yes | No |
| 9 | 59M | 5329 | Insulinoma | 1.0 cm | B | WES | WES | 7.4 | 50/7/7.33/? | <1% | Yes | No |
| 10 | 57F | 5331 | Insulinoma | 2.2 cm | B | WES | WES | 6.9 | 42/13/2.29/41.1 | N/A | Yes | No |
| 11 | 46M | 5333 | Insulinoma | 2.4 cm | B | WES | WES | 7.9 | 38/<2/1.36/14.2 | <2% | Yes | No |
| 12 | 83M | 5404 | Insulinoma | 1.8 cm | B | WES | WES | N/A | 35/37/500/0.48 | N/A | N/A | N/A |
| 13 | 59M | 5405 | Insulinoma | 1.5 cm | B | WES | WES | N/A | N/A | N/A | Yes | N/A |
| 14 | 45M | 5406 | Insulinoma | 2.7 cm | B | WES | WES | N/A | N/A | 0.2 mito/hpf | N/A | N/A |
| 15 | 65F | 5407 | Insulinoma | N/A | B | WES | WES | N/A | N/A | N/A | N/A | N/A |
| 16 | 63F | 5408 | Insulinoma | N/A | B | WES | WES | N/A | N/A | N/A | N/A | N/A |
| 17 | 33F | 5863 | Insulinoma | 2.5 cm | M | WES | WES | 8.1 | 36/?/2.2/? | 15% | No | No |
| 18 | 35M | 5967 | Insulinoma | 1.6 cm | B | WES | WES | 7.2 | 49/13.8/11.9/38 | 2% | Yes | Yes |
| 19 | 61M | 6066 | Insulinoma | 2.8 cm | B | WES | WES | 8.4 | 42/188/18.7/NA | 3-20% | Yes | No |
| 20 | 35M | 6107 | Insulinoma | 1.5 cm | B | WES | WES | 7.7 | 23/57.7/19.9/38 | 2% | Yes | No |
| 21 | N/A | 5564 | Insulinoma | N/A | B | no pair | N/A | 7.8 | no info per IRB | N/A | N/A | N/A |
| 22 | N/A | 5565 | Insulinoma | N/A | B | no pair | N/A | 8.6 | no info per IRB | N/A | N/A | N/A |
| 23 | 52F | 5594 | Insulinoma | N/A | B | no pair | N/A | 7.5 | 39/7.7/2.1/53 | N/A | Yes | No |
| 24 | 48F | 5595 | Insulinoma | N/A | M | no pair | N/A | 7.5 | 15/85/10.8/1300 | N/A | Yes | No |
| 25 | 29F | 5596 | Insulinoma | N/A | B | no pair | N/A | 5.2 | 24/19.9/4.5/91 | N/A | No | No |
| 26 | 48F | 5597 | Insulinoma | N/A | B | no pair | N/A | 7.3 | 36/12.5/6.2/420 | N/A | Yes | No |
| 27 | 32M | 5599 | Insulinoma | N/A | B | no pair | N/A | 7.0 | 32/92.3/5.6/130 | N/A | Yes | No |
| 28 | 76F | 5600 | Insulinoma | N/A | B | no pair | N/A | 5.9 | 49/16.8/4.9/210 | N/A | Yes | No |
| 29 | 30M | 5601 | Insulinoma | N/A | B | no pair | N/A | 7.9 | 42/21.9/5.5/45 | N/A | Yes | No |
| 30 | 30M | 5602 | Insulinoma | N/A | B | no pair | N/A | 8.0 | 42/21.9/5.5/46 | N/A | Yes | No |
| 31 | 60M | 5603 | Insulinoma | N/A | B | no pair | N/A | 7.0 | 43/26.2/3.5/480 | N/A | Yes | No |
| 32 | 58F | 6957 | Insulinoma | 1.1 cm | B | no pair | N/A | 7.6 | 46/5/3.4/86.2 | <2% | Yes | No |
| 33 | 60F | 8003 | Insulinoma | 0.9 cm | B | WES | WES | N/A | 33/6.5/1.7/4.2 | 3% | Yes | No |
| 34 | 71M | 9398 | Insulinoma | 1.0 cm | B | WES | WES | N/A | 39/?/?/239 | 1% | Yes | No |
| 35 | 65F | BGI.INS15 | Insulinoma | N/A | B | WES | WES | N/A | N/A | N/A | N/A | N/A |
| 36 | 48F | BGI.INS20 | Insulinoma | N/A | B | WES | WES | N/A | N/A | N/A | N/A | N/A |
| 37 | 53M | BGI.INS38 | Insulinoma | N/A | B | WES | WES | N/A | N/A | N/A | N/A | N/A |
| 38 | 53F | BGI.INS40 | Insulinoma | N/A | B | WES | WES | N/A | N/A | N/A | N/A | N/A |

**Notes: NA** = Not available;
**Normal blood glucose** = 70-110 mg/dl;
**Benign vs Malignant** diagnosis is based on pathologist report, although in several cases the Ki67 labeling index exceeds 3%;
**No Pair** indicates that no WBC DNA was available, so WES was not performed.

## Supplementary Table 2.

## Predicted Insulinoma Key Driver Variants.

| | | | |
|---|---|---|---|
| AASS | FLNC | MCC | PVRL1 |
| ADAMTS9 | FUT8 | MEN1 | PZP |
| ADNP2 | GAK | MICAL1 | RGS4 |
| AOX1 | GLB1 | MLH3 | RNF111 |
| ARHGAP35 | GPS1 | MLLT4 | SLC27A4 |
| ASB1 | GRN | MYOCD | SLK |
| ATR | H3F3A | NCAM2 | SMARCC1 |
| BMP5 | HSPA5 | NDNL2 | SMURF1 |
| BRINP3 | IL7R | NEK5 | SNX18 |
| CAD | IMPDH1 | NLRC3 | STAG2 |
| CCDC146 | IRAK2 | NLRP5 | STARD13 |
| CDX2 | ITPKB | OTOF | SUFU |
| CNTNAP4 | KATNAL1 | P2RY4 | TBC1D9B |
| COL4A5 | KATNB1 | PAX6 | TG |
| CPNE3 | KDM5B | PCDHA9 | TMCO1 |
| CREBBP | KDM6A | PER2 | TMTC1 |
| CYP4F12 | KLHL1 | PITPNM2 | TPM1 |
| DRD2 | KLHL24 | PITPNM3 | VCP |
| DSCAML1 | KMT2C | PIWIL2 | VIM |
| DUOX2 | LIPE | PLCE1 | VPS13C |
| DUSP1 | LMO2 | PLXNA4 | WDR48 |
| ERBB4 | MAP3K15 | PNPLA3 | YY1 |
| ESF1 | MAST4 | PSIP1 | ZBTB44 |