Supplementary Methods

Culture of human embryonic kidney 293 (HEK293) cells. HEK293 cells were cultured in Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Thermo Fisher Scientific) and incubated in 5% CO_2 at 37 °C.

Western blotting. HEK293 cells were transfected using FuGENE HD (Promega) with 500 ng/mL of plasmid pcDNA3.1-HA-CCND1 (Addgene, 172649) and 500 ng/mL of *DYRK1B* plasmid (empty vector [EV], wild-type [WT] or with a P/LP-null variant) and they seeded in a poly-lysine coated 6-well plate at a concentration of 0.5×10^6 cells/mL. 48 hours after transfection, the cells were harvested and the proteins were extracted using Pierce RIPA buffer (Thermo Fischer Scientific), supplemented with 1 mM dithiothreitol (Thermo Fischer Scientific) and protease and phosphatase inhibitors (Roche). The proteins were quantified using Pierce Rapid Gold BCA protein assay kit (Thermo Fischer Scientific). 20 µg of proteins were denatured at 95 °C for 5 minutes with Laemmli buffer 4× (Thermo Fischer Scientific) and loaded on a 10% precast gel (Bio-Rad) for electrophoresis. After migration, the proteins were transferred on a nitrocellulose membrane (GE Healthcare) and the non-specific sites on the membranes were blocked for 1 hour in the blocking buffer (Tris buffered saline [TBS], 0.1% Tween 20, 5% bovine serine albumin). The membranes were then incubated overnight at 4°C with primary antibodies: DYRK1B (#5672 Cell signaling Technology; diluted at 1/1000 in the blocking buffer), phospho-CCND1 (at p.T286 amino acid) (#3300 Cell signaling Technology; diluted at 1/1000 in the blocking buffer) and CCND1 (Origene; diluted at 1/2000 in the blocking buffer), following by fluorescent anti-rabbit secondary antibody (SA5-35571 Thermo Fischer Scientific; diluted at 1/5000 in the blocking buffer) and fluorescent anti-mouse secondary antibody (#35518 Thermo Fischer Scientific; diluted at 1/5000 in the blocking buffer). Nitrocellulose membranes were revealed using the Odyssey CLx imaging system (LI-COR

Bioscience). β-actin was used as a loading control to normalize data (#3700 Cell Signaling Technology; diluted at 1/4000 in the blocking buffer).

Table S1. Clinical data of participants included in the RaDiO study.

	Adults				Children/adolescents	
Adiposity	Obesity	Overweight (with no obesity)	Normal-weight	NA	Obesity	Normal-weight
N	1,526	2,859	2,875	8	1,043	1,042
Sex	M:514 / F:1,012	M:1,823 / F:1,036	M:1,252 / F:1,623	M:3 / F:5	M:486 / F:557	M:540 / F:502
Age at investigation (years)	51 ± 13	54 ± 12	48 ± 12	45 ± 8.4	13 ± 2.1	18 ± 3.3
BMI (kg/m^2)	37 ± 7.3	27 ± 1.5	22 ± 1.9	NA	31 ± 5.4	20 ± 2.3
Type 2 Diabetes	537	1,144	497	1	NA	NA
Fasting glucose (mmol/L)	6.4 ± 2.4	6.5 ± 2.4	8.3 ± 3	5.2 ± 0.3	NA	NA

Data are the mean ± SD or numbers (%)

BMI**,** body mass index; ***F***, female**;** ***M***, male; ***NA***, not available.

Table S2. Rare *DYRK1B* variants identified in the RaDiO study.

Rare variants in *DYRK1B* (NM_004714.3)	Position (hg19)	MAC in RaDiO	MAC in GnomAD	REVEL score	ACMG criteria	Category
c.7G>A, p.V3I	19:40322501	1	7	0.03	-	Neutral
c.14C>T, p.P5L	19:40322494	1	1	0.14	PS3, PM2	P/LP
c.80G>A, p.R27Q	19:40321407	2	6	0.07	-	Neutral
c.92G>C, p.R31P	19:40321395	1	0	0.24	PM2	Neutral
c.118G>T, p.A40S	19:40321369	1	0	0.10	PM2	Neutral
c.170A>G, p.K57R	19:40321317	1	3	0.19	PM2	Neutral
c.202A>C, p.K68Q	19:40321185	1	5	0.23	PS3, PP5	Neutral
c.209G>A, p.R70Q	19:40321178	1	17	0.17	PS3	Neutral
c.236C>T, p.S79L	19:40321151	1	2	0.14	PM2	Neutral
c.256_258del, p.K86del	19:40321129	1	0	-	PS3, PM2, PM4	P/LP
c.305G>A, p.R102H	19:40321082	2	8	0.06	PS3, PM5	P/LP
c.359G>T, p.G120V	19:40321028	1	0	0.97	PS3-null, PM2, PP3	P/LP-null
c.391C>T, p.H131Y	19:40320649	1	0	0.39	PS3, PM2	P/LP
c.470G>A, p.R157Q	19:40320570	1	1	0.31	PS3, PM2	P/LP
c.500C>T, p.T167M	19:40320540	1	10	0.36	PS3	Neutral
c.506T>G, p.M169R	19:40320534	2	0	0.39	PM2	Neutral
c.515A>G, p.Y172C	19:40320525	1	3	0.40	PM2	Neutral
c.526C>A, p.L176M	19:40319218	1	16	0.18	PS3	Neutral
c.536A>T, p.H179L	19:40319208	1	0	0.65	PS3-null, PM2, PP3	P/LP-null
c.668C>T, p.T223M	19:40319076	3	30	0.40	PS3	Neutral
c.746A>G, p.N249S	19:40318998	2	1	0.29	PM2	Neutral
c.775G>A, p.D259N	19:40318969	1	0	0.90	PS3-null, PM2, PP3	P/LP-null
c.845C>T, p.P282L	19:40318259	1	0	0.86	PS3-null, PM2, PP3	P/LP-null
c.967A>G, p.N323D	19:40318053	1	0	0.23	PS3, PM2	P/LP
c.971G>T, p.R324L	19:40318049	1	5	0.21	PS3	Neutral
c.1003G>A, p.A335T	19:40318017	4	3	0.09	PM2	Neutral
c.1030C>T, p.R344C	19:40317990	2	8	0.42	-	Neutral
c.1031G>A, p.R344H	19:40317989	2	14	0.26	-	Neutral
c.1045C>T, p.R349W	19:40317975	1	1	0.23	PS3-null, PM2	P/LP-null
c.1046G>A, p.R349Q	19:40317974	1	2	0.09	PM2	Neutral
c.1054G>A, p.G352R	19:40317966	1	0	0.15	PS3, PM2	P/LP
c.1055G>C, p.G352A	19:40317965	5	155	0.06	-	Neutral
c.1057G>T, p.G353C	19:40317963	1	1	0.25	PS3, PM2	P/LP
c.1072C>T, p.R358*	19:40317948	1	0	-	PVS1, PS3-null, PM2	P/LP-null
c.1073G>A, p.R358Q	19:40317947	1	4	0.06	PS3, PM2	P/LP
c.1079C>A, p.T360K	19:40317941	1	0	0.07	PS3, PM2	P/LP
c.1111G>A, p.G371R	19:40317612	3	2	0.19	PS3, PM2	P/LP
c.1111G>C, p.G371R	19:40317612	1	1	0.20	PS3, PM2	P/LP
c.1196C>A, p.A399D	19:40317527	1	0	0.08	PM2	Neutral
c.1208G>A, p.R403H	19:40317515	1	1	0.16	PM2	Neutral
c.1229G>A, p.R410H	19:40317494	1	0	0.48	PS3, PM2	P/LP
c.1252G>A, p.A418T	19:40317471	9	22	0.03	-	Neutral
c.1285G>A, p.G429S	19:40317438	3	103	0.06	-	Neutral
c.1285G>C, p.G429R	19:40317438	3	5	0.09	-	Neutral
c.1295G>A, p.R432H	19:40317428	1	1	0.10	PM2	Neutral
c.1328C>T, p.P443L	19:40317395	1	0	0.06	PM2	Neutral
c.1336A>G, p.S446G	19:40317387	3	11	0.07	-	Neutral
c.1341T>A, p.S447R	19:40317382	1	0	0.28	PS3, PM2	P/LP
c.1349C>T, p.T450I	19:40317374	1	0	0.19	PM2	Neutral
c.1358C>T, p.A453V	19:40317365	1	5	0.09	-	Neutral
c.1386C>G, p.S462R	19:40317337	5	101	0.15	-	Neutral
c.1414G>A, p.G472S	19:40316924	2	0	0.25	PM2	Neutral
c.1441C>T, p.R481W	19:40316897	1	0	0.18	PM2	Neutral
c.1450C>T, p.R484C	19:40316888	1	7	0.24	-	Neutral
c.1463G>A, p.R488Q	19:40316875	1	14	0.13	-	Neutral
c.1469G>T, p.C490F	19:40316869	1	0	0.06	PM2	Neutral
c.1470T>G, p.C490W	19:40316868	3	8	0.21	-	Neutral
c.1481G>A, p.G494E	19:40316857	1	11	0.11	-	Neutral
c.1675C>T, p.P559S	19:40316570	1	2	0.09	PM2	Neutral

c.1732C>T, p.P578S	19:40316513	31	294	0.05	-	Neutral
c.1742C>T, p.A581V	19:40316503	1	2	0.16	PM2	Neutral
c.1799G>A, p.R600H	19:40316446	1	5	0.06	-	Neutral
c.1823A>T, p.D608V	19:40316422	1	0	0.14	PM2	Neutral
c.1840C>A, p.P614T	19:40316405	2	38	0.05	-	Neutral
c.1855C>T, p.R619C	19:40316390	1	6	0.04	PS3-null	Neutral

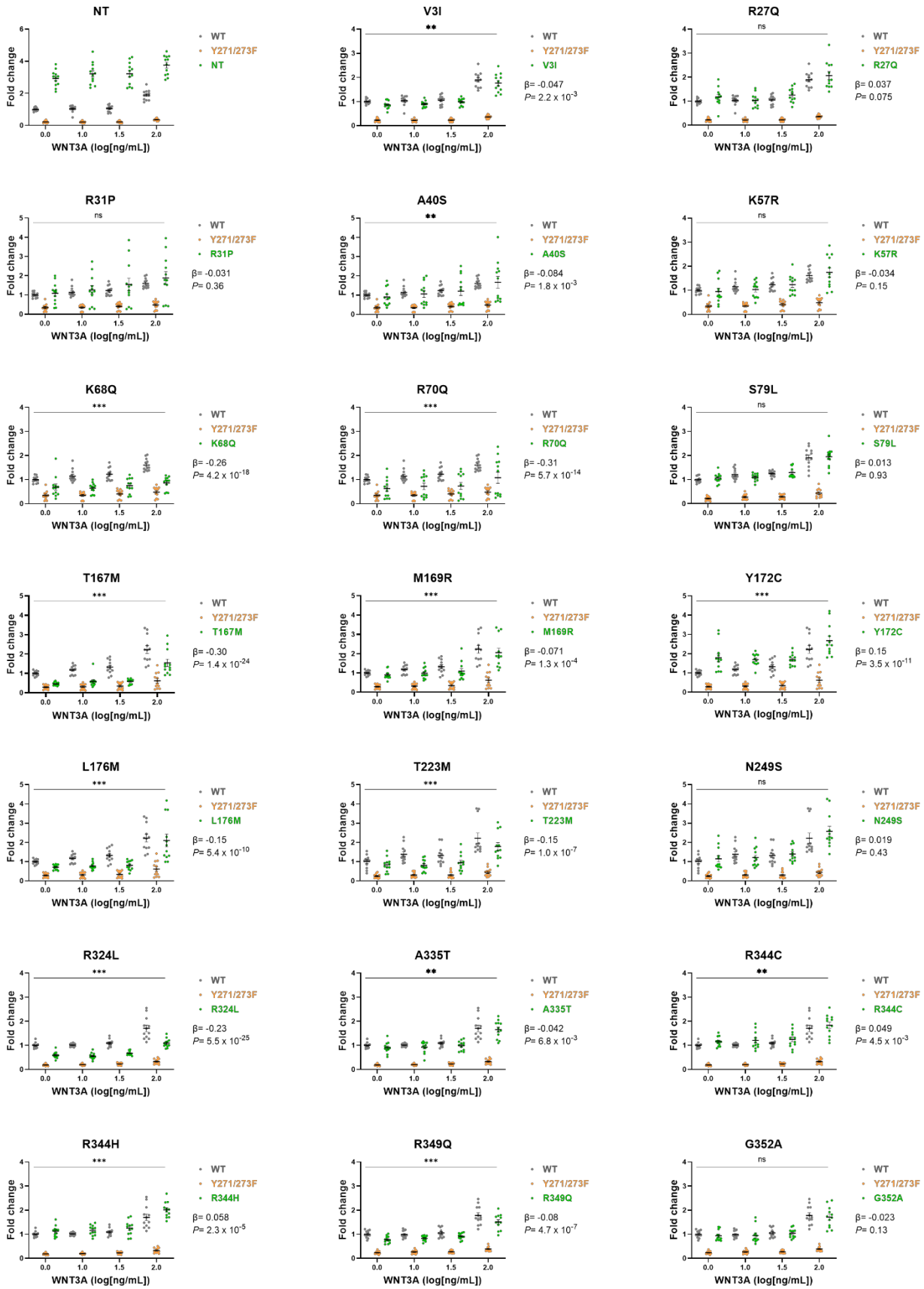
ACMG, American College of Medical Genetics and Genomics; ***GnomAD*****,** genome aggregation database (version 2.1.1); ***MAC*****,** minor allele count; ***P/LP*****,** pathogenic or likely pathogenic variant; ***P/LP-null*****,** fully inhibitory (*i.e.* null) P/LP variant; ***PM-*****,** moderate pathogenicity ACMG criterion; ***PP-*****,** supporting pathogenicity criterion; ***PS-*****,** strong pathogenicity ACMG criterion; ***PVS-*****,** very strong pathogenicity ACMG criterion; ***REVEL***, rare exome variant ensemble learner.

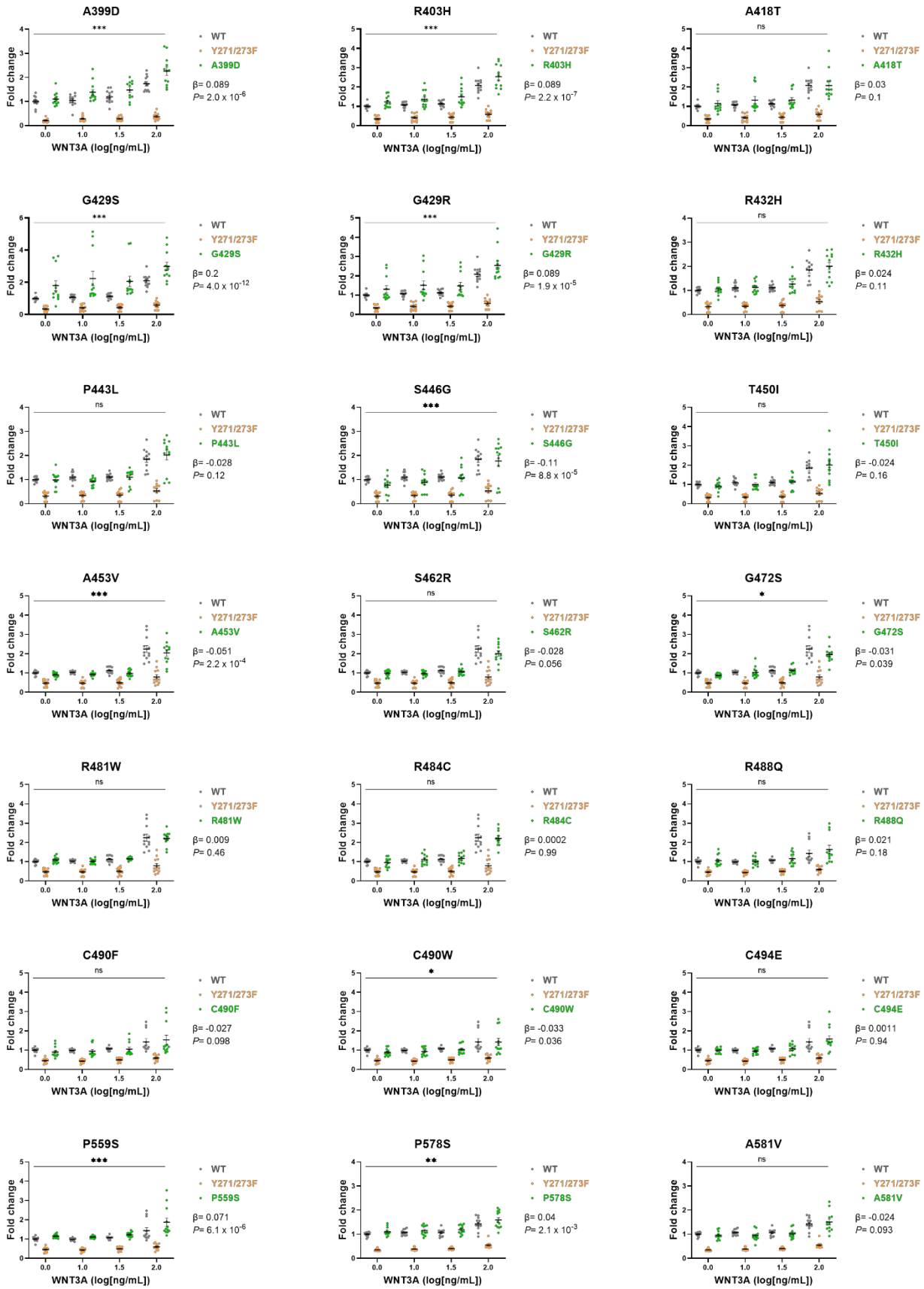
Table S3. Null mutations of *DYRK1B* (NM_004714.3) detected in 52K and TOPMed studies.

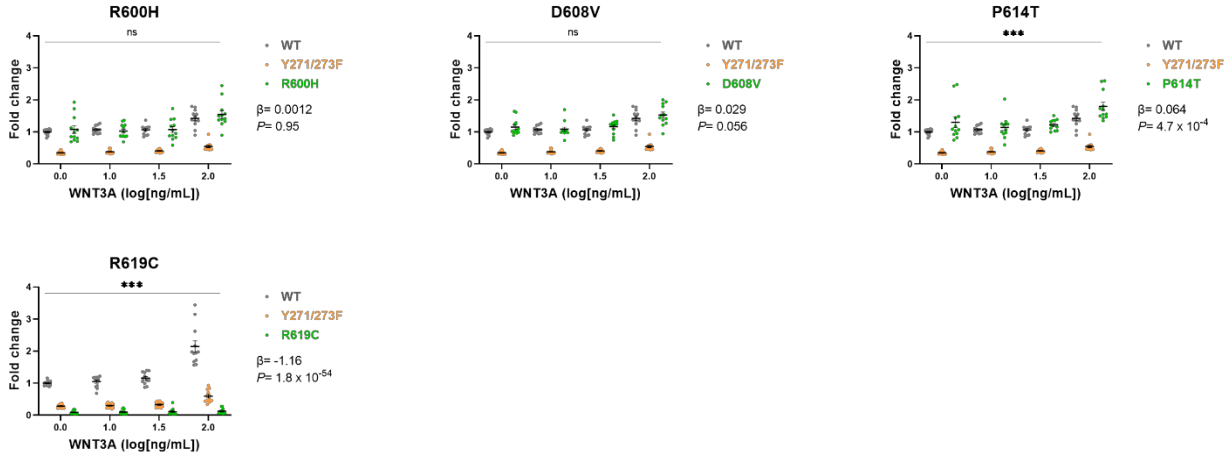
Chr	Position (Hg38)	Mutation	52K	TOPMed
19	40321205	c.184-3_184-2insGGGC		X
19	40318298	c.808-2A>C		X
19	40318281	c.823C>T, p.Gln275Ter	X	X
19	40316889	c.1449C>G, p.Tyr483Ter	X	X
19	40316876	c.1462C>T, p.Arg488Ter		X
19	40316713	c.1528_1531del, p.Gln511ArgfsTer52		X
19	40316611	c.1633del, p.Gln545SerfsTer19		X
19	40316611	c.1633_1634insC, p.Gln545ProfsTer30		X
19	40316491	c.1753_1754insC, p.Gln585ProfsTer23		X

Among these variants, two null variants from 52K and seven null variants from TOPMed were kept for further association analysis with type 2 diabetes risk.

Fig. S1. Effect of neutral *DYRK1B* variants on Wnt signaling, according to luciferase assays

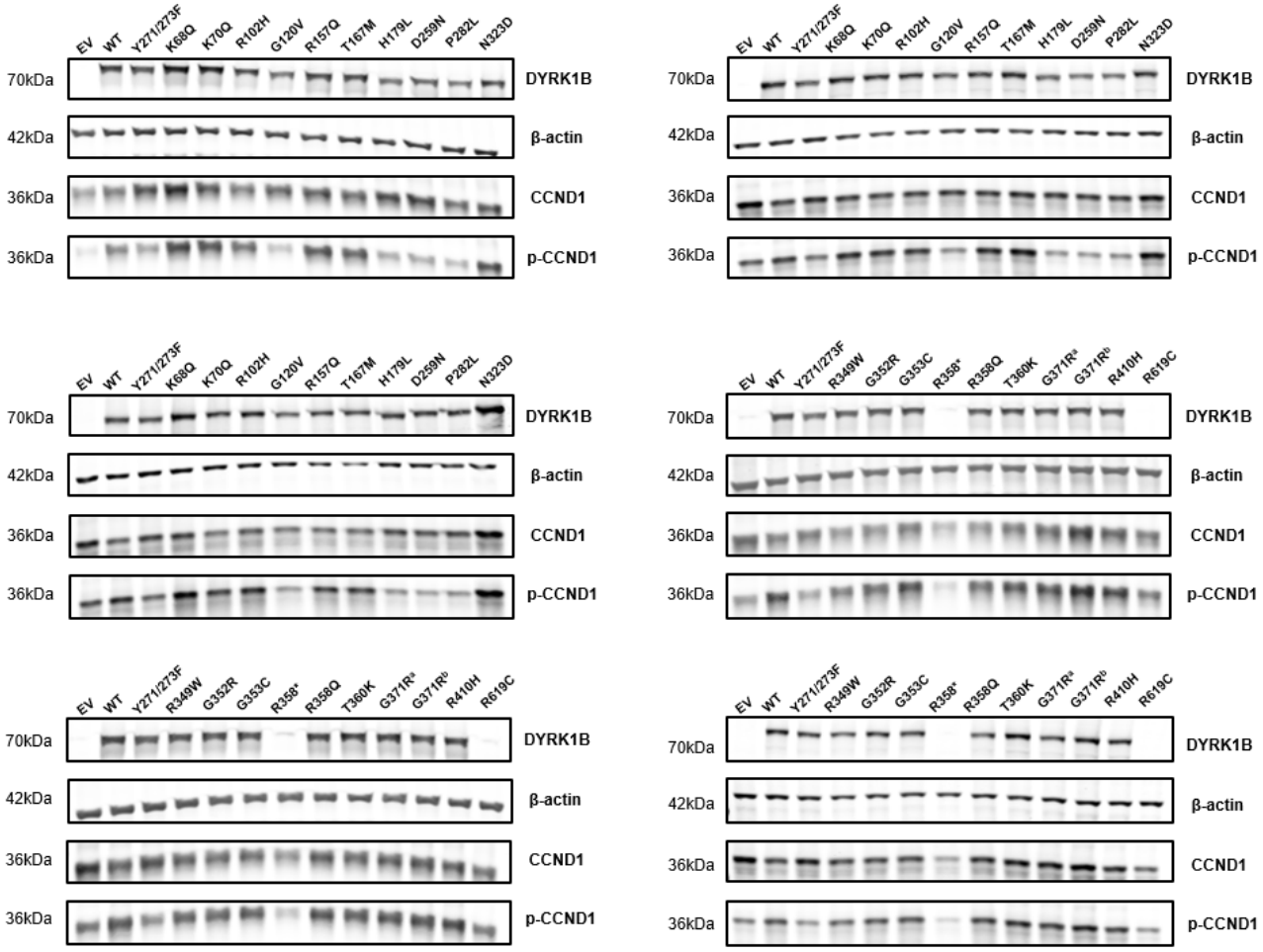



A399D
***
WT
Y271/273F
A399D
β= 0.089
P= 2.0 x 10-6
R403H
***
WT
Y271/273F
R403H
β= 0.089
P= 2.2 x 10-7
A418T
ns
WT
Y271/273F
A418T
β= 0.03
P= 0.1
G429S
***
WT
Y271/273F
G429S
β= 0.2
P= 4.0 x 10-12
G429R
***
WT
Y271/273F
G429R
β= 0.089
P= 1.9 x 10-5
R432H
ns
WT
Y271/273F
R432H
β= 0.024
P= 0.11
P443L
ns
WT
Y271/273F
P443L
β= -0.028
P= 0.12
S446G
***
WT
Y271/273F
S446G
β= -0.11
P= 8.8 x 10-5
T450I
ns
WT
Y271/273F
T450I
β= -0.024
P= 0.16
A453V
***
WT
Y271/273F
A453V
β= -0.051
P= 2.2 x 10-4
S462R
ns
WT
Y271/273F
S462R
β= -0.028
P= 0.056
G472S
*
WT
Y271/273F
G472S
β= -0.031
P= 0.039
R481W
ns
WT
Y271/273F
R481W
β= 0.009
P= 0.46
R484C
ns
WT
Y271/273F
R484C
β= 0.0002
P= 0.99
R488Q
ns
WT
Y271/273F
R488Q
β= 0.021
P= 0.18
C490F
ns
WT
Y271/273F
C490F
β= -0.027
P= 0.098
C490W
*
WT
Y271/273F
C490W
β= -0.033
P= 0.036
C494E
ns
WT
Y271/273F
C494E
β= 0.0011
P= 0.94
P559S
***
WT
Y271/273F
P559S
β= 0.071
P= 6.1 x 10-6
P578S
**
WT
Y271/273F
P578S
β= 0.04
P= 2.1 x 10-3
A581V
ns
WT
Y271/273F
A581V
β= -0.024
P= 0.093
Fold change
WNT3A (log[ng/mL])



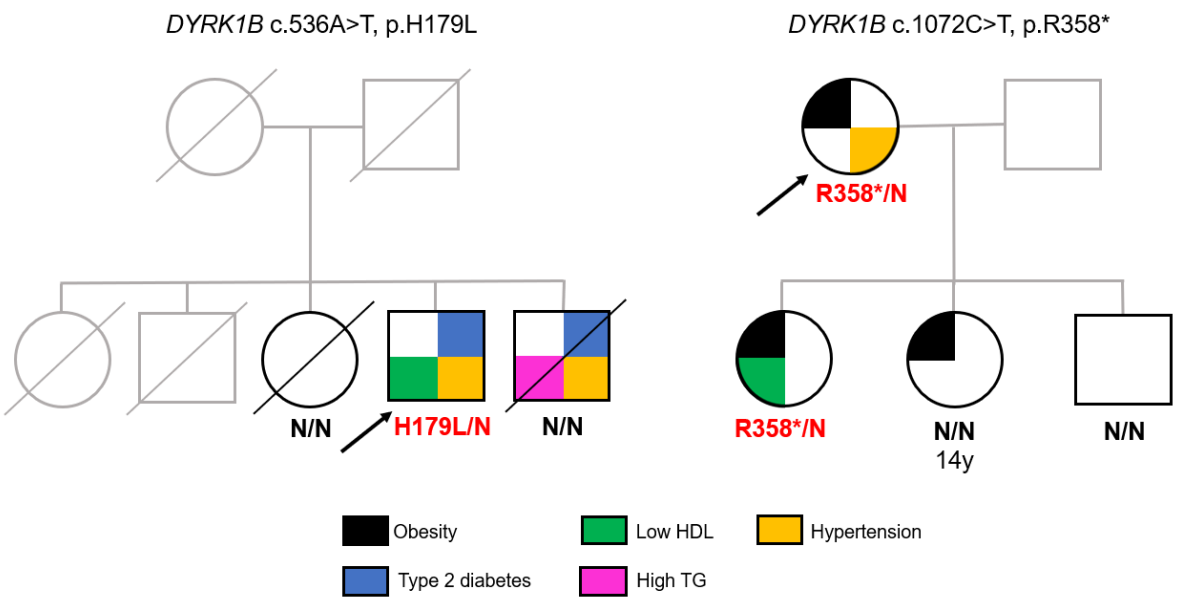
The figures illustrate fold changes in luciferase activity, normalized to β-galactosidase, within HEK293 cells that were either transfected or left non-transfected (designated as the non-transfected [NT] condition). This transfection involved the use of wild-type or mutated *DYRK1B* plasmids, along with the TOPflash (*i.e.* TCF reporter) plasmid. The response was measured across varying concentrations of WNT3A (0, 10, 30, and 100 ng/mL), relative to the baseline activity observed with the wild-type DYRK1B. Positive and negative control conditions, *i.e.* WT and Y271/273F, were respectively represented in grey and orange. Data are the mean ± SEM of the fold changes from four independent experiments performed in technical triplicates. The effect of each *DYRK1B* variant was analysed using linear regression model (with estimates and p-values on the right) and confirmed with ANOVA model (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ *versus* wild-type). ***NT***, not transfected; ***WT***, wild-type.

Fig S2. Effect of P/LP-null *DYRK1B* variants on CCND1 by phosphorylation by Western blotting



The figures illustrate the protein expression of DYRK1B, CCND1 and p-CCND1 within transfected HEK293 cells. This transfection involved the use of empty vector or wild-type or P/LP-null *DYRK1B* plasmids, along with CCND1 plasmid. Four independent experiments were performed for each P/LP-null variant. ***EV***, empty vector; ***WT***, wild-type; ***CCND1***, cyclin D1; ***p-CCND1***, CCND1 phosphorylated.

Fig S3. Co-segregation of p.H179L and p.R358* with metabolic traits in two families



The figure shows the co-segregation of two P/LP-null variants (p.H179L and p.R358*) with metabolic traits in two families. The arrows indicate the individual sequenced in the RaDiO study. Family members were sequenced by Sanger sequencing. Obesity was defined as BMI ≥30 kg/m^2, Type 2 diabetes as fasting glucose ≥7.0 mmol/l and/or used treatment of hyperglycemia, low HDL levels as ≤1.04 mmol/l in men and ≤1.30 mmol/l in women, high TG levels as ≥1.70 mmol/l and hypertension by systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg. ***NN***, wild-type; ***HDL***, high-density lipoprotein; ***TG***, triglyceride.