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Supplemental Data

Loss-of-Function Mutations in *TBC1D20* Cause Cataracts and Male Infertility in *blind sterile* Mice and Warburg Micro Syndrome in Humans

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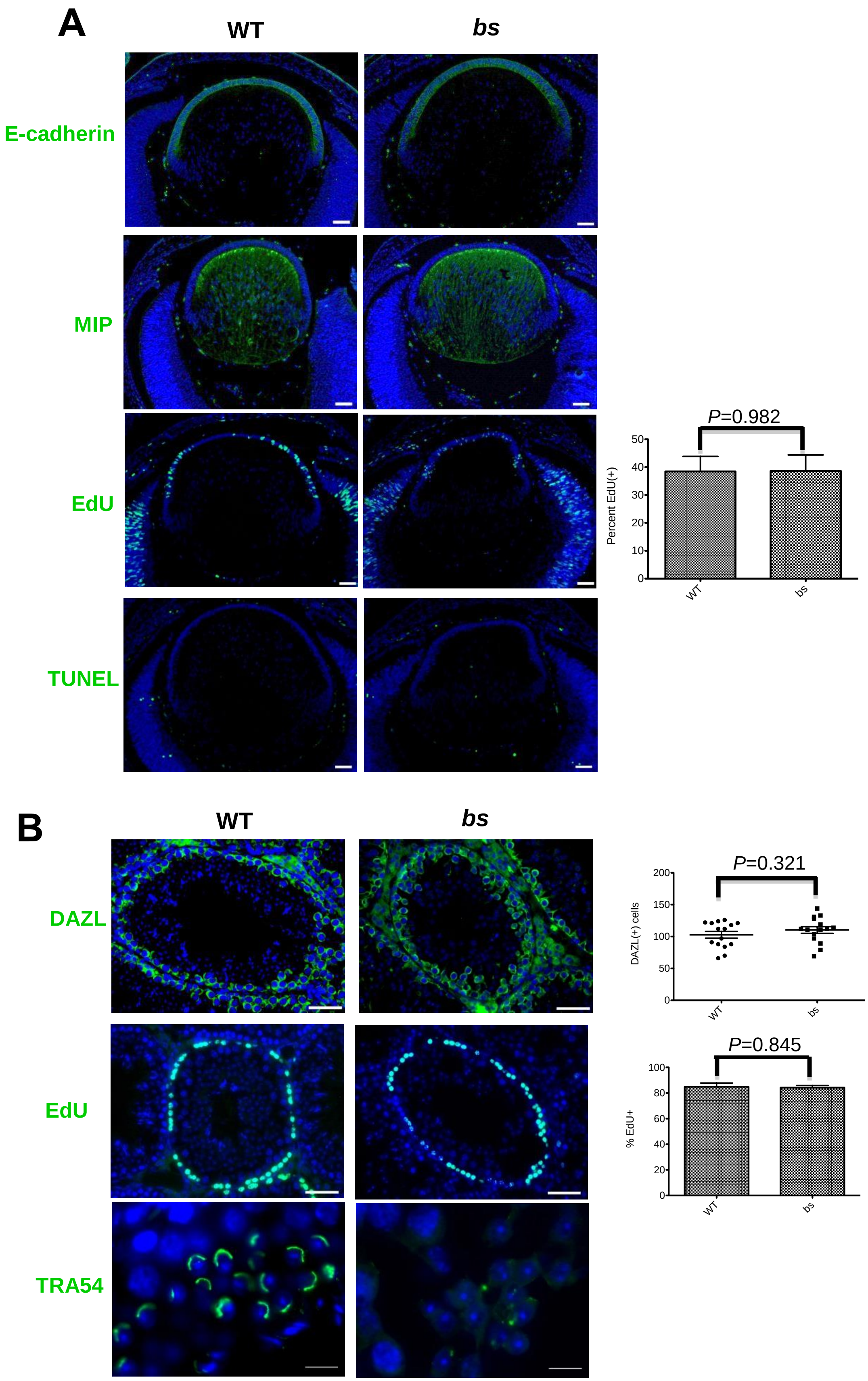
A
WT
bs
E-cadherin
MIP
EdU
TUNEL
P=0.982
Percent EdU(+)
50
40
30
20
10
0
WT
bs
B
WT
bs
DAZL
EdU
TRA54
P=0.321
DAZL(+) cells
200
150
100
50
0
WT
bs
P=0.845
% EdU+
100
80
60
40
20
0
WT
bs

Figure S1: *bs* lens and testicular phenotypes. (**A**) Immunostaining with E-cadherin (top panel) and MIP (second panel) did not reveal any abnormalities in E15.5 *bs* lens epithelial and fiber cells respectively; the number of EdU(+) cells (n=3, third panel) and the number of TUNEL(+) cells (n=3, fourth panel) did not significantly differ between E15.5 WT or *bs* lenses. (**B**) The number of spermatogonia and Sertoli cells following DAZL immunostaining (top panel) did not significantly different between WT and *bs* tubules (n=15); the number of EdU-positive cells (middle panel) did not differ between WT and *bs* tubules (n=15). Scale bars = 25 µm. Crescent-shaped TRA54 immunostaining (bottom panel) of spermatids was evident in WT, whereas in *bs* TRA54 positive immunostaining revealed presence of small punctae. Scale bars = 5 µm. *P* values were determined by Student's *t* test and are shown on top of each graph and error bars represent SEM.

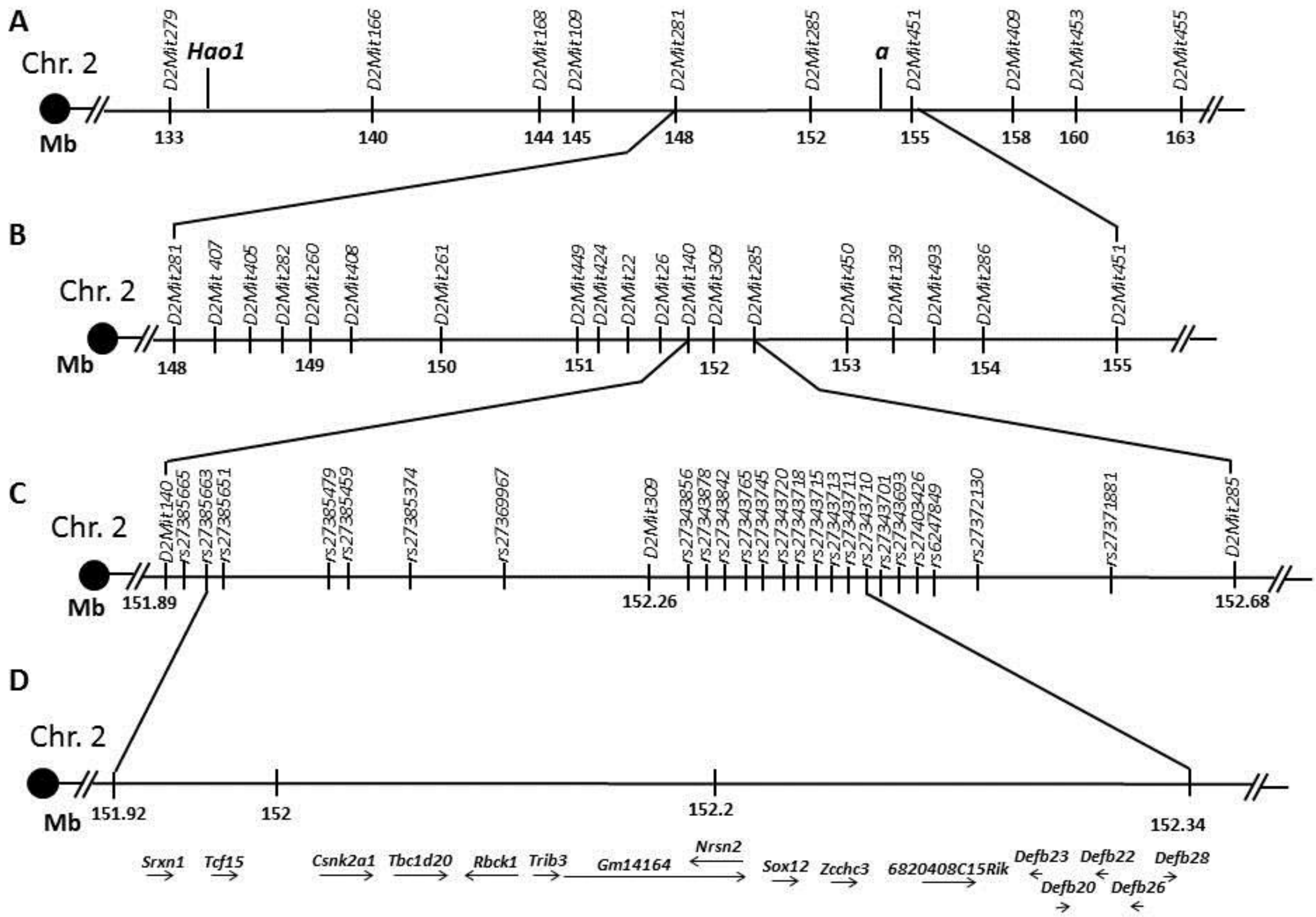

Figure S2: Positional cloning of the *bs* locus. (**A**) Initial microsatellite mapping assigned the *bs* mutation to the region between *D2Mit281* and *D2Mit451*. (**B**) Subsequent fine mapping identified additional recombinants which further narrowed the *bs* region to between *D2Mit140* and *D2Mit285*. (**C**) SNP markers refined the *bs* critical region to between rs27385663 and rs27343710. (**D**) The 416kb *bs* critical region contained 14 known genes and 2 hypothetical genes.

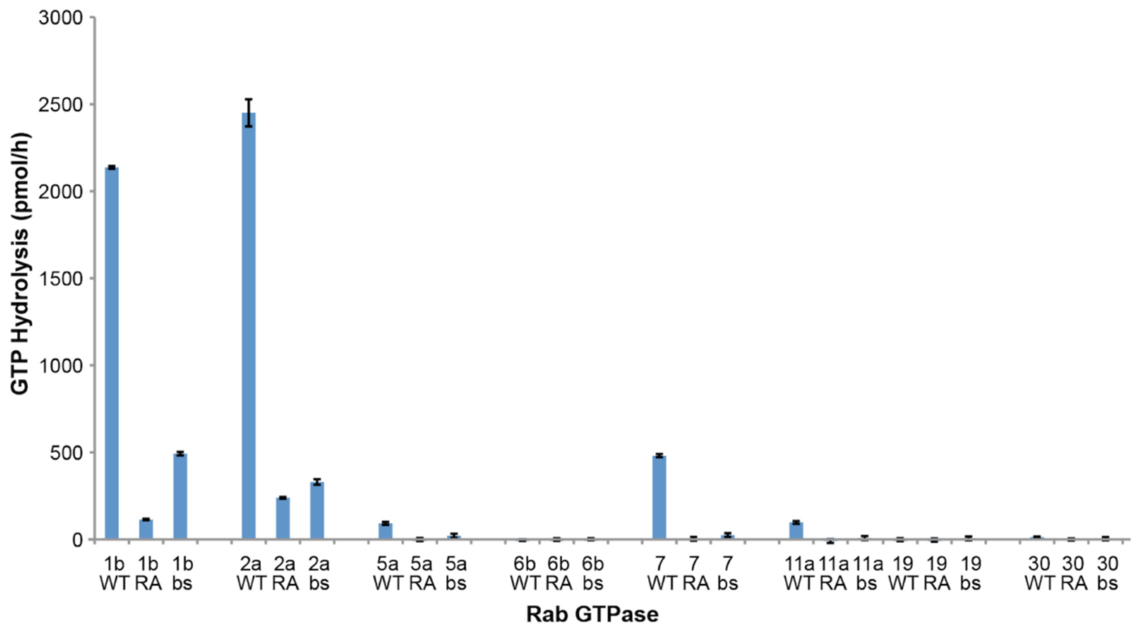

Figure S3: TBC1D20 mediated GTP hydrolysis screen. The biochemical analysis revealed that WT mouse TBC1D20 has a high activity towards RAB1 and RAB2 and only a residual activity towards several other RABs . The mutant TBC1D20-bs shows residual activity towards RAB1 and RAB2 similarly to the previously characterized RA mutant. Each point on the graph represents the mean values from three independent experiments and error bars indicate SD.

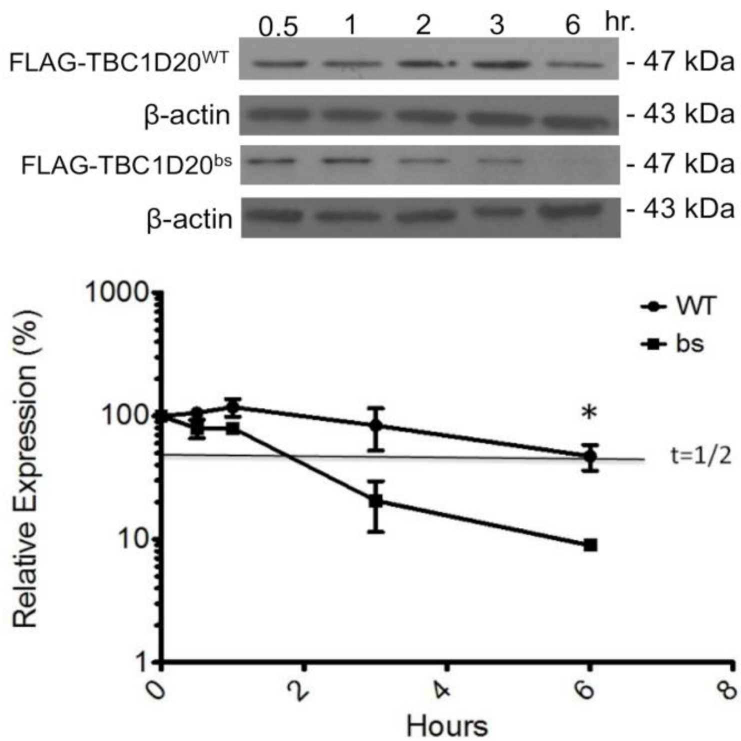

Figure S4: Stability of TBC1D20^{WT} and TBC1D20^{bs} proteins. Western blots of HEK 293 cell lysates transiently transfected with FLAG-tagged *Tbc1d20*^{WT} or *Tbc1d20*^{bs} clones treated with cycloheximide. 6 hr following the treatment there was significantly (P <0.05) less FLAG-TBC1D20^{bs} protein than FLAG-TBC1D20^{WT} protein relative to β-actin. Each point on the graph represents the mean values from three independent western blots done in triplicates and error bars represent SEM.

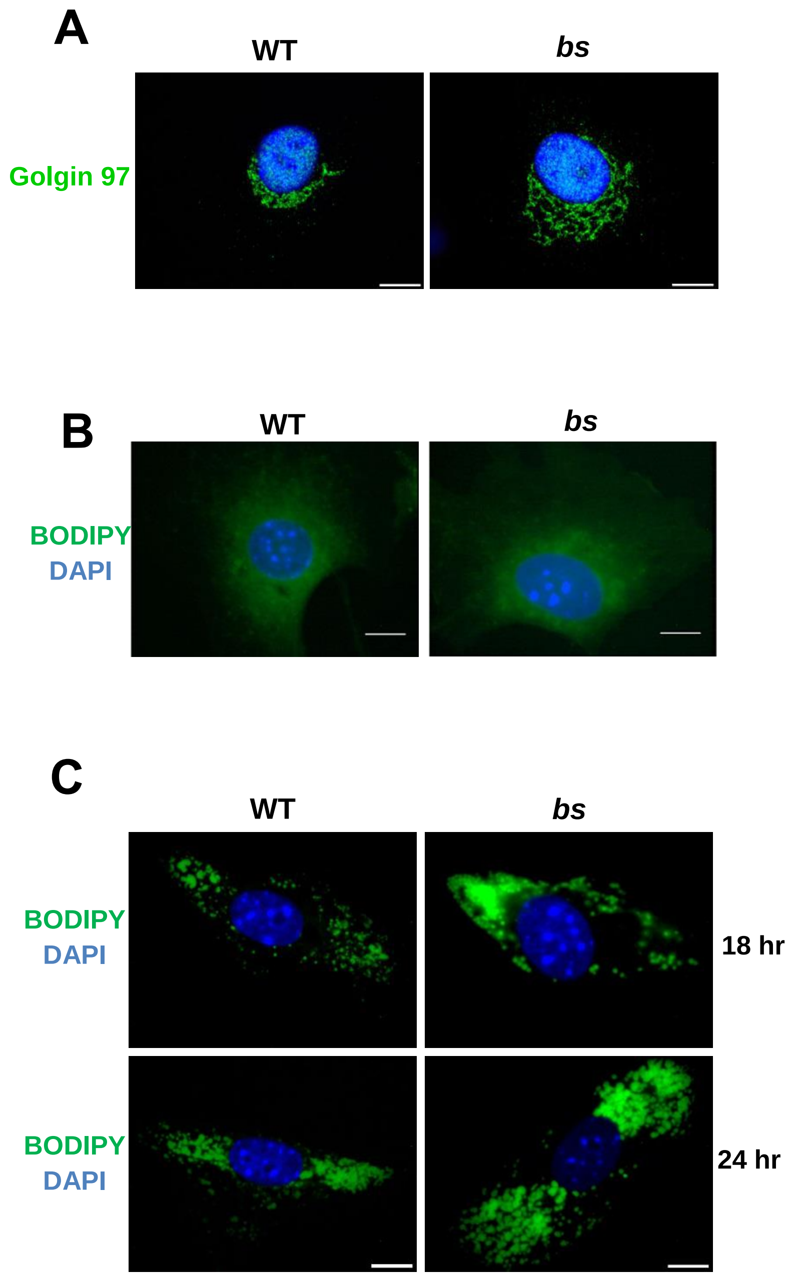

Figure S5: Cellular phenotypes of WT and *bs* mEFs. Enlarged *trans*-Golgi (**A**) were observed following immunostaining with golgin-97 (green) in *bs* when compared to WT mEFs. (**B**) Staining of WT and *bs* mEFs with BODIPY 493/503 did not identify LDs in either cell line. However, oleic acid treatment (**C**) for 18 hr (top panel) and 24 hr (bottom panel) following staining with the neutral lipid dye BODIPY 493/503 (green) revealed expanded LD structures in *bs* when compared to WT mEFs,. DNA was stained with DAPI (blue). Scale bars = 5 µm.

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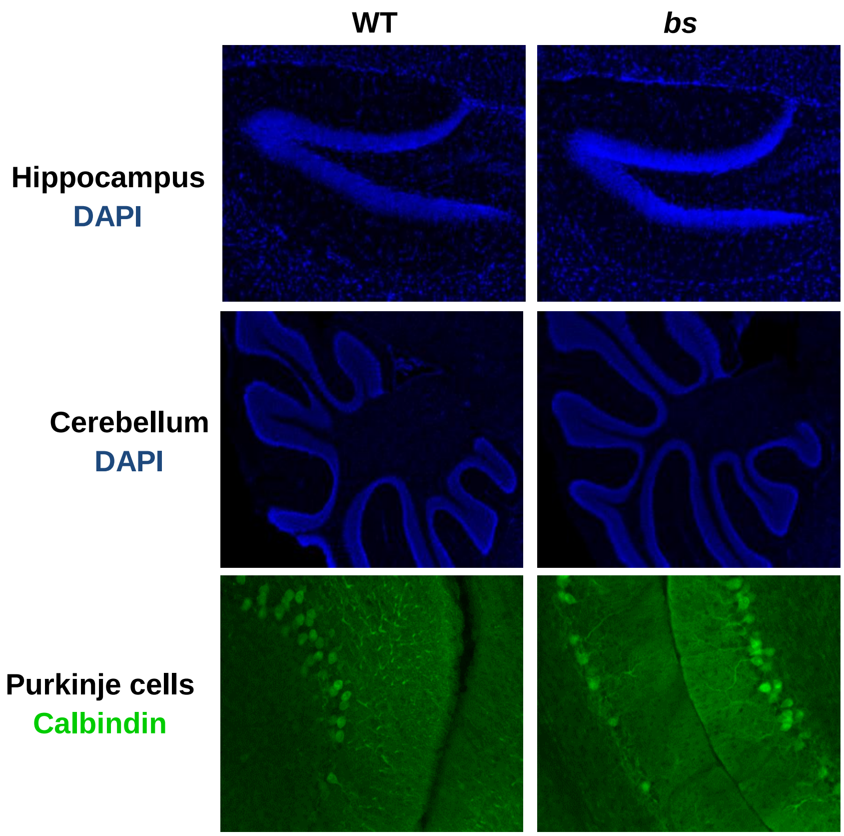

Figure S6: *bs* brain phenotypes. No obvious morphological abnormalities were noted in adult *bs* brains following DAPI staining of hippocampus (top panel) and cerebellum (middle panel). No difference in Purkinje cell morphology was noted between WT and *bs* following calbindin staining (bottom panel).

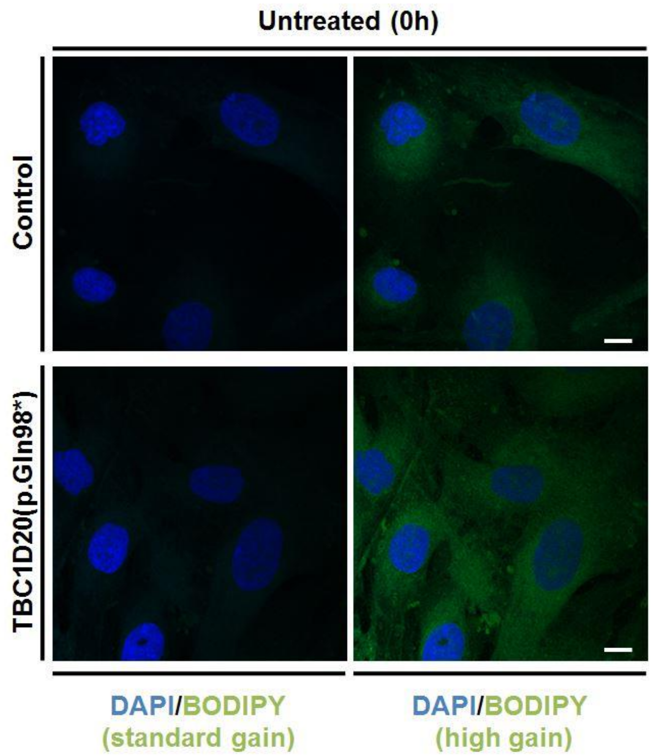

Figure S7: BODIPY 493/503 staining of untreated control and TBC1D20 (p.Gln98*) human fibroblasts. Cells were stained using the neutral lipid dye BODIPY 493/503 (green) and DNA was stained with DAPI (blue). Using standard gain to measure BODIPY fluorescence (as when cells were treated with oleic acid), very little fluorescent signal was recorded (left panels). Using higher gain (right panels), fluorescent signal was recorded but did not identify LDs in these cells. Scale bars = 10 µm.

Table S1. 16 RefSeq candidate genes identified in the *bs* critical region

Gene symbol	Gene name	RefSeq accession no.
Srxn1	sulfiredoxin 1 homolog (S. cerevisiae)	NM_029688.5
Tcf15	transcription factor 15	NM_009328.2
Csnk2a1	casein kinase 2, alpha 1 polypeptide	NM_007788.3
Tbc1d20	TBC1 domain family, member 20	NM_024196.3
Rbck1	RanBP-type and C3HC4-type zinc finger containing 1	NM_001083921.1
Trib3	tribbles 3 homolog (Drosophila)	NM_175093.2
Gm14164*	predicted gene 14164	NR_033505.1
Nrsn2	neurensin 2	NM_001009948.1
Sox12	SRY-box containing gene 12	NM_011438.2
Zcchc3	zinc finger, CCHC domain containing 3	NM_175126.4
6820408C15Rik*	RIKEN cDNA 6820408C15 gene	NM_177656.3
Defb23	defensin beta 23	NM_001037933.2
Defb20	defensin beta 20	NM_176950.3
Defb22	defensin beta 22	NM_001002791.2
Defb26	defensin beta 26	NM_001039120.2
Defb28	defensin beta 28	NM_001037502.2

Table S2. Mouse primer sequences

Primer target/name	Sequence 5’->3’:
6820408C15Rik cDNA 1065F	GTTCAGATCGCCAGCCACATAC
6820408C15Rik cDNA 1236R	GGGGTTTCTGAGTATTCACGACCA
6820408C15Rik cDNA 1556R	TCTTGTAAGTCACCAGCCGGAAC
6820408C15Rik cDNA 371F	CACCGGTATCTGGAGACAGCCTA
6820408C15Rik cDNA 741F	TACAGCCACGAAGGCTACCTCAG
6820408C15Rik cDNA 860R	CCCGAGGATACTCTGCTGCTGTA
Ccna1 cDNA F	TTCTGGAAGCTGACCCATTC
Ccna1 cDNA R	GGCAAGGCACAATCTCATTT
Clgn cDNA F	AGAATGGGAGGCACCACATA
Clgn cDNA R	TCTGGGTTGGGAATCTTCTG
Crem cDNA F	GCGACAACCGCATCAGAG
Crem cDNA R	TCCTTCCCTGTTTTCCTTATTT
Csnk2a1 cDNA 1066F	TTCAACGATATCTTGGGCAGACA
Csnk2a1 cDNA 1081R	CCAAGATATCGTTGAAACGTGGA
Csnk2a1 cDNA 1267R	AACTCATTCGAGCCTGGTCCTTC
Csnk2a1 cDNA 1551R	TCAGACACGGTGCTTCTGAAGTG
Csnk2a1 cDNA 582F	AACCCCTGCCTTGGTTTTTGAAC
Csnk2a1 cDNA 899F	TGGATATGTGGAGCTTGGGTTGT
D2Mit109 F	CCTCTCCAATCAGTCACATGG
D2Mit109 R	TTGTCTTACCTTCATCAGAGATGC
D2Mit136 F	CCCCACATAGGAAACACAGC
D2Mit136 R	ATCTTCACATATGCATGTATACTTGTG
D2Mit139 F	AGATGTAAAGGTCATCTCTCAGCC
D2Mit139 R	AGAAGCAAGGGCAACTTCAA
D2Mit140 F	CTGCCTCCTGTTTTGAAAGC
D2Mit140 R	GACATGTATACACGTGTGCGC
D2Mit166 F	ATCTGCCTCAAGATACTAAGTAGATGC
D2Mit166 R	GTTGTATATGTATATGTGCAACACACA
D2Mit168 F	CTCACAGACACTGCACTATTACACA
D2Mit168 R	TGTTCCTGCTATTGTTTTGGG
D2Mit194 F	CTCACAGACACTGCACTATTACACA
D2Mit194 R	TGTTCCTGCTATTGTTTTGGG
D2Mit22 F	GCTCCCTTTCCTCTTGAACC
D2Mit22 R	GGGCCCTTATTCTATCTCCC
D2Mit26 F	TGTTCTTTGCTCATCCACCA
D2Mit26 R	AGGCTGATGGTAACAGTGGG
D2Mit260 F	ACATAGAAACAAGCATACATGCA
D2Mit260 R	CTGTGGTAAACTTTAAATAATGGTGG

D2Mit261 F	TGAACCCTGGGCTTAATCAC
D2Mit261 R	AAACCCTGTCTCAAAAAAAAAGG
D2Mit281	TTAGCATGACATGATGGATACTCC
D2Mit281	TCACATCTCAGAGGGGCTG
D2Mit282 F	GCAACCTCAAACATACTCCATG
D2Mit282 R	CTCTTCACAGATTCCCCCTG
D2Mit285 F	TCAATCCCTGTCTGTGGTAGG
D2Mit285 R	TATGACACTTACAAGGTTTTTGGTG
D2Mit286 F	GGCCATGCTCTTTTTTTTACC
D2Mit286 R	CCCTGTGCTCTTGCTTTTTC
D2Mit309 F	ACAAATGCCACTCTCACATCC
D2Mit309 R	TATTTCTCAGAGTCACTAGGAGTGATG
D2Mit405 F	TGATTATATCTTGGAATACACGTGTG
D2Mit405 R	CTGTGTAGCAAAACAGTTTATGGC
D2Mit407 F	AAAGAGAGAACTAACTCTCAAAAGTCG
D2Mit407 R	GAAGGTCTGCAGGTTTGGTC
D2Mit408 F	TGCTCACATCATGCCCTTAA
D2Mit408 R	CATTTTAGGCATGTGCATGG
D2Mit409 F	CAACGTGTTTTCAGTCTAAGAGATG
D2Mit409 R	AAGAGAAGGTAATCACAAACCCC
D2Mit424 F	CACCCTAAGTTGTCCTCACATG
D2Mit424 R	GCCTCATCTATAGATTGTGTGCA
D2Mit449 F	GTATCCAAAGTCTGCCACTTCC
D2Mit449 R	CTCCTCAGAACCCCACACAT
D2Mit450 F	AGGAATTTCTGCTATTCCTCATATAA
D2Mit450 R	AAGAGTTTTAAGTAAGGGTTGTAGACC
D2Mit451 F	CATTAGATAGACTGGGCAAGGG
D2Mit451 R	TCCTCCCTCCAAACCCTC
D2Mit453 F	CCTGAAATTTCCCTTCATAGTAGG
D2Mit453 R	GAAGACACCCACAAGACTAATGC
D2Mit493 F	GTCTCTACCTGAGTTTCCATCACA
D2Mit493 R	TCCCGAGTTGTCCCTCTATG
D2Mit494 F	TGAGTCGAGGCAGATGGG
D2Mit494 R	TTCTTCTCCTGGGATTGTGG
D2Mit495	AGACCCTGTCTCACACCACC
D2Mit495	ATGTGGTCCTGATTTTTGGG
D2Mit57	CCTCACAACTATGTCAGGTAATGG
D2Mit57	GGATGAGGGATTAAAAATAGATGC
Defb20 Exon 1F (398bp)	GCTCCCTGTCTGTCCATGTGAGT
Defb20 Exon 1R	TGAGATAGCCGGAGCTGAAAATG
Defb20 Exon 2F (471bp)	TGTCTGCTGTCTCTCCCTGTGAA

Defb20 Exon 2R	ACAAGGAGCGGATACAGGCAAAA
Defb22 cDNA 2F	GAACCTACTGCCTACTGACAGG
Defb22 cDNA 601R	GGATATTAGGTCAGGAGAAAAGAGG
Defb23 cDNA 1F	AGACCCCGGGCTTTTCTAC
Defb23 cDNA 594R	CCCAGGAGAAAGACCTGCTA
Defb26 cDNA 152F	CTGCAGAGTCCGATGTCAAG
Defb26 cDNA 646R	GGGAGGTGTTCATATCCCAGA
Defb26 Exon 1F	ACAGCAGGCTCACTTCTCCCATT
Defb26 Exon 2F	TCTAGAGGGCAGAGGTTCGGTTT
Defb26 Exon 2R	GGGTGTTTTAGAGTCATTTGCATCC
Defb26 Exon1R	TGAAATGATGCTTCTCACCACCA
Defb28 EXON1 F	CCTTGGCCTTCCTCAGATTT
Defb28 EXON1 R	GATCATACCCAGAGCCTCACA
Defb28 EXON2 F	GTCTCCCTGAACCCACCATT
Defb28 EXON2 R	ACCCATGTCAAGACCCCTTT
Defb28 EXON3 F	AAACCCCAGTCCTCCAGAAT
Defb28 EXON3 R	TTTCAGATTTGAGTTTCAGGACA
Gm14164 EXON 5.1 F	TGTGCATGCTGGGAGTTTAATACA
Gm14164 EXON 5.1 R	CCAGTGTGGAGGAAAGTACATGG
Gm14164 EXON 5.2 F	TGCCATCCCTTTGTCCTTACCTT
Gm14164 EXON 5.2 R	CCCTATTTCCCAGACAAGGTCGT
Gm14164 EXON 5.3 F	TGTCCGCCTGAGTGTTTTCTCTG
Gm14164 EXON 5.3 R	TGAGGGGGAACATAAGGATCTCTC
Gm14164 EXON 5.4 F	TCAGAGTCACCTCTCCCCTTTGA
Gm14164 EXON 5.4 R	GGATTCTGGTGTCTCGCTGATTG
Gm14164 EXON1 F	CCAGGGCAGTGGGATTGTTATTT
Gm14164 EXON1 R	TGGCTTTGAACTTAGAGATCTGATGG
Gm14164 EXON2 F	TAGCCATGATTGGCTCCAAACTC
Gm14164 EXON2 R	GTTCTGCCAACAGGACAATGGAC
Gm14164 EXON3 F	CAACTTTCCTATGCCGCAGTGAC
Gm14164 EXON3 R	GTCGGATCCTGTCTCTGGCAAC
Gm14164 EXON4 F	CCAGCTCTGTTTGGCACTCCTC
Gm14164 EXON4 R	CCCCAACCTCCCACTTTAAGAAC
Nrsn2 EXON2 F	AAAACACTCAGGCGGACAGACAG
Nrsn2 EXON2 R	CTGGCCACCCTAGCATCTCTCTT
Nrsn2 EXON3 F	GATGTTCTGTGAGGCCCATTTCA
Nrsn2 EXON3 R	AAGGTGATGAAAGCAAGGAGTGC
Oct4 cDNA F	AGACCACCATCTGTCGCTTC
Oct4 cDNA R	GGTCTCCAGACTCCACCTCA
Odf1 cDNA F	AGATCCTCTGGGCGATTTCT
Odf1 cDNA R	TGATGTTCGGGTGTGAGAGA

Plzf cDNA F	GAGCACACTCAAGAGCCACA
Plzf cDNA R	GTGGCAGAGTTTGCACTCAA
Prm2 cDNA F	GAAGGCGGAGGAGACACTC
Prm2 cDNA R	GGGAGGCTTAGTGATGGTG
Pxmp4 cDNA 1187R	GGCTCCAGAACTTCTCAGCAGAAT
Pxmp4 cDNA 13F	AGCCAGGACCCTTGACCGAGTG
Pxmp4 cDNA 514F	CCTTGCACACTGCGGTGATTTG
Pxmp4 cDNA 612R	AGAGGTAGGTCATGGAGGACTGT
Rbck1 cDNA 1193F	ACCGAACCTACTGAGTGCCCTGT
Rbck1 cDNA 1362R	GGGCATGAGTAGGTGCTGTCAAT
Rbck1 cDNA 1585F	GCACATGAATTGCAGGGAGTACC
Rbck1 cDNA 1789R	GACCCAGCAGATCTCAGTGTGG
Rbck1 cDNA 2065R	GCTCAGCTGGGTCCTTGTAACAC
Rbck1 cDNA 268F	GGGCAGAGTTGCTTCTACCTTCC
Rbck1 cDNA 772F	CAACACGTCACTCAACCCACAAG
Rbck1 cDNA 845R	CCTTGAAGCCCAAATCTTCCAAC
Rhox1 cDNA F	AATGTGGCCTCAGCAACAG
Rhox1 cDNA R	TCTGCACTTTGGCTTCACAC
Rhox8 cDNA F	AGGAACTGGAGCGCATTTT
Rhox8 cDNA R	TCAGGCATTTCCTGTATTTGG
rs13476843 F (436bp)	CCCTTGTTTTGAGCCTCTGATCC
rs13476843 R	GCGTTCATCATATCCCCATCAAC
rs27343693 156F	TGCATGGAACCATCTTTTCAAAC
rs27343693 627R	TGCTAACTCAGCATTGGGTGATG
rs27343701 F (497bp)	TTGGAAGTAATCATTTGCCATCAA
rs27343701 R	GTTGGTTAGGTGCCAACCACCTT
rs27343710 F (461bp)	TGTCCACAAAGCAGCAGGCTTAC
rs27343710 R	TCCCCAAACAACTCTACCACCAA
rs27343711 F (499bp)	GCCTGTTCAGCCAATGTTTTCAA
rs27343711 R	CGTTCCAGTGTTTGGGTCAGC
rs27343713 F (486bp)	CCATGACTACACACACATCAGACACC
rs27343713 R	TTTGAAAACATTGGCTGAACAGG
rs27343715 F (383bp)	CCATGACTACACACACATCAGACACC
rs27343715 R	GAAGGTAGGTGAAGGCAGGAGGA
rs27343718 F (500bp)	TCCTCCCAAAGTACACACTCAAACA
rs27343718 R	AAAGAAACACGCAAGGTGATTGC
rs27343720 F (456bp)	GCACATCCATAAACACCGCATTT
rs27343720 R	TGGCAGGGAAGTTTGTAAGGTGA
rs27343745 F (458bp)	CAAGGAAAAACATGTCATGGACAGA
rs27343745 R	GGCTTCTTCCACACTCAAAGATACA
rs27343764 F (483bp)	CTTAGTGATGCCTGGGCCTCTG

rs27343764 R	TCAGTCATTGTGAGAAATGCAAGG
rs27343765 209F	CCTCCCAAGTGCTGGGATTAAAG
rs27343765 663R	AAAAATGGGAGGGGAGGAGGAG
rs27343795 F (466bp)	CACATCAGGCAGCTCCAAGGTAT
rs27343795 R	CCTAAGGGGTAAGCCAGAGTTGC
rs27343842 F (495bp)	GGCCACTAGGTGGTACTTGAAATCC
rs27343842 R	TGTTCATTTGATGTCTCAGAGCAGTG
rs27343856 F	CATTATCTCTGCCTCCCCCAAAT
rs27343856 R	CCCTGAGGGATGATGAAACACAC
rs27343878 F (447bp)	AGAAGCTTGGCCTTCGTCTTACG
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rs27372130 F (484bp)	AGCTACCCAACTACAGCCAGGTG
rs27372130 R	CAACAACAAACCCCACCCTACAA
rs27385374 F	GCCCAGGCTGTCAATAAGCTTTC
rs27385374 R	TCCCCTCTTTGACCACATCCTAA
rs27385459 F (407bp)	AACTTCTGATCCTTCCCAAGTGC
rs27385459 R	GTCCTGGATCTCAAGGGAGAAGC
rs27385480 F (463bp)	TGAGGATCAGAGAAAGGTAGAAGGACA
rs27385480 R	GAGTTGGTGTCTGTGGCCTTGAG
rs27385624 F (459bp)	CTGGCCTCACCAACACTTGTACC
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rs27385629 F (466bp)	TATCCCTGTAAGCCCTGCACTGA
rs27385629 R	GACCATGGAGGCCAGAAGAAGAC
rs27385631 F (475bp)	TACGATCCCAGCACCTCAGAGAC
rs27385631 R	GCAGCCACACAGGATTACTGTCA
rs27385649 F (465bp)	ACTCTTGGGGCTGATTTTCAAGC
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rs27385651 F (492bp)	GCTCTGGAGACAAGGACCCATCT
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rs27385663 F (456bp)	GAGCATCTTATGTCGCCACTGCT
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rs27385665 F (494bp)	TCCAGAGGAGTGTTTGTTGGACTG
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rs27403426 147F	GGGCAGTTATCATAGGGCCTCAC
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rs6247849 F (487bp)	GTGGAGGGTCTGGAGAGATGTT
rs6247849 R	GCTGCCAAAATCAAAAACACTGC
Scp3 cDNA F	CAACAACAAAAGATTTTTCAGCA
Scp3 cDNA R	TTTGCAACATAGCCATTTCTTTT
Scrt2 Exon 1F	CTGGGAGCTGTCCAGTGCTGAAG
Scrt2 Exon 1R	ACCCTGCCACTTTCCTTTGTACG
Scrt2 Exon 2.1F	CATGGACAGAACTGAGCCAACCT
Scrt2 Exon 2.1R	CGTACGTCTTGCCGCACTCTGC
Scrt2 Exon 2.2F	TGGACGCCTTCTTCATCTCTGAC
Scrt2 Exon 2.2R	GCAGTGCTTGTGGAGGTAGGACT
Scrt2 Exon 2.3F	ATGCACGTGCTCACGCACAACCT
Scrt2 Exon 2.3R	GACAAAGTGGGTGGGTGCGAAG
Sox12 cDNA 1017R	GTCCTCGGACAGGGTCGGAGAGG
Sox12 cDNA 1210F	GACTATTGCACCCCCGAGGTTA
Sox12 cDNA 1368R	CCGAGGGGCCCCAATACCTGAT
Sox12 cDNA 1454R	GGGAGGGGTATGTCTGGATTTGT
Sox12 cDNA 1605R	AGTGAGGGGGAGGGGTTAAGGTA
Sox12 cDNA 172F	ACTGACCTCCCCCTCGCACAGAC
Sox12 cDNA 448F	AAGACCCCGAGCGGCCACATTA
Sox12 cDNA 519F	AATCATGGACCAGTGGCCCGACA
Sox12 cDNA 536R	GGCCACTGGTCCATGATTTTTC
Sox12 cDNA 615R	GAACGGGATCTTCTCCGAGTCCT
Sox12 cDNA 651F	GGCGGACTACCCGGACTACAAGT
Sox12 cDNA 679R	GCCGGTACTTGTAGTCCGGGTAG
Sox12 cDNA 81F	TCCTTGGCCCTGCAAAACAA
Sox12 cDNA 870F	GGAAGTACGCCTGCTGGAGACC
Srxn1 cDNA 1F	AAGGAAGAGGTATGGGGCTACGC
Srxn1 cDNA 382R	AGGTCTGAAAGGGTGGACCTCAC
Srxn1 Exon 1.3F	CTCGGGGACAGGTAGGATCACAG
Srxn1 Exon 1.3R	CAGTCCGTCAAGGTCACACAGG
Stra8 cDNA F	GCTTTTGACGTGGCAAGTTT
Stra8 cDNA R	AACACAGCCAAGGCTTTTGA
Tbc1d20 cDNA 1080F	CTTTGAGCTGGCATCAACTCAGC
Tbc1d20 cDNA 1174R	GGACATCTTTTGTTCGAGCCTCA
Tbc1d20 cDNA 1573R	GGCCCAACTTTTTAGGCAGTGAC
Tbc1d20 cDNA 16F	GCCGAACTGGGGTTAGGT
Tbc1d20 cDNA 1F	GAACTGGGGTTAGGTCCGTGGT
Tbc1d20 cDNA 335R	CTGGTGTTCACGTTGAGGAGCTT
Tbc1d20 cDNA 374F	TGAGCAAGGACTACCAGCAA
Tbc1d20 cDNA 505R	GATCCAGGACGAGGAGGAT
Tbc1d20 cDNA 683F	TCATTGACCAAGTGAGCCCAGAG

Tbc1d20 cDNA 873R	CACGATCACAGCTGCAAAGT
Tbc1d20 del1659-1670F	TTGGGCACGTCCTGATGGACTTGAGGTTATACG
Tbc1d20 del1659-1670R	CGTATAACCTCAAGTCCATCAGGACGTGCCCAA
Tbc1d20 E6/cDNA F (109bp)	CAGCTGGCTTATCACCTGGTTTG
Tbc1d20 E6/cDNA R (109bp)	TGCAAAGTAAATGGGCATAAGT
Tbc1d20 Exon 6 F	CCTGGCTTCGTGGTACATTTTTG
Tbc1d20 Exon 6 R	AGATCACGGATCAAGTTGGATGG
Tbc1d20 ORF 1128R	CACCGTCAGCCCCATCACT
Tbc1d20 ORF 353F	GAGAAGGGCTCCAGGAAGAGCTA
Tbc1d20 ORF 468R	CACCACCAGCAGAAATGTGACC
Tbc1d20 ORF 731F	GCCACCCACTTATGCCCATTTAC
Tbc1d20 ORF 849R	CTGAGGGATCTGGGACAACAGGT
Tbc1d20 ORF Fwd (no ATG)	GCCCTCCGGCCCTCAAAG
Tbc1d20 ORF Fwd (w/ATG)	ATGGCCCTCCGGCCCTC
Tbc1d20 ORF Rev (21nt)	TTAGGGGAACAGCTGCAGCTG
Tbc1d20 ORF Rev (22nt)	TTAGGGGAACAGCTGCAGCTGG
Tbc1d20 t691a	GGCACGTCCTGATGGACATGAGGTTATACGACTTC
Tbc1d20 t691a antisense	GAAGTCGTATAACCTCATGTCCATCAGGACGTGCC
Tbc1d20 w/STOP; + 5' XbaI R	GGATCCTCTAGATTAGGGGAACAGCTGCAGCTGG
Tbc1d20: no ATG; + 5' NotI F	AAGCTTGCGGCCGCGGCCCTCCGGCCCTCAAAG
Tbc1d20: w/STOP; + 5' XbaI R	GGATCCTCTAGATTAGGGGAACAGCTGCAGCTG
Tcf15 cDNA 257F	AGACCGCACGCAGAGCGTGAAC
Tcf15 cDNA 324F	GACCGAAAGCTGTCTAAGATCGAG
Tcf15 cDNA 433R (Exon 1)	AAACACGGCTGCCCGTCGTC
Tcf15 cDNA 54F (Exon 1)	GTGCTGTACCCGGACGTGAG
Tcf15 cDNA 623R	AAAACCAGGGATCCAGGTTCATC
Tcf15 cDNA 656R	TCATGGCCTCTTTCTCTGGAGTC
Tcf15 Exon 1.4F	CTGAGCGAGGACGAGGAGAACC
Tcf15 Exon 1.4R	CTTAGCTTCCATGGGGGTACCTG
Tcf15 EXON2 F	GCCTTTTCAGCAGACTCACC
Tcf15 EXON2 R	GCAAGTCTCATTGTCCACTGC
Tnp1 cDNA F	ACAAGGGCGTCAAGAGAGGT
Tnp1 cDNA R	CATCACAAGTGGGATCGGTA
Trib3 cDNA 1091R	AGGAGACAGCGGATCAGACAGC
Trib3 cDNA 1349R	CTTGGCCCAAAAAGTCAGGAGA
Trib3 cDNA 370F	TCAGACTTGTCACCTGCTGTGG
Trib3 cDNA 440R	CCTTGCTCTCGTTCCAAAAGGAT
Trib3 cDNA 58F	CAGCACTTTAGCAGCGGAAGAGG
Trib3 cDNA 595F	CGGCTCCTTTACATCTTTTTCACG
Trib3 cDNA 769R	CAAAGCGACGCAGCTTGAGGTC
Trib3 cDNA 872F	CTGCCTACGTGGGACCAGAGATA

Zcchc3 cDNA 1003F	AGGTCCAGCCTGAAGACCCTCT
Zcchc3 cDNA 1025R	AAGAGGGTCTTCAGGCTGGACCT
Zcchc3 cDNA 130F	CCAGAAGCGTCGGGTTTCAGAG
Zcchc3 cDNA 1335R	CTCTTCCCCGCACCTGAAACAC
Zcchc3 cDNA 1381R	AGAGGTTGCACACGATGACCTTC
Zcchc3 cDNA 1574R	TCTCCATTCAAAACGATTAAAAA
Zcchc3 cDNA 563F	ACCCTACGGGTGAGGCGTCAGA
Zcchc3 cDNA 610R	CGCTCGCGCTCTTTTTCCTGTA
Zcchc3 cDNA 890F	AGTTCGACGTGAGTTTCCGATCC
Zcchc3 cDNA 941F	TCTACGAGGAGAAGCGAGAGCTG

Table S3. Human primer sequences

Primer target:	Primer sequence 5'->3':
TBC1D20 coding exon 1	GTAGCGCGACGGCCAGTCCCTCAGCCTGTTCCTCTC
	CAGGGCGCAGCGATGACGAGCGGGTGCTACGTCC
TBC1D20 coding exon 2	GTAGCGCGACGGCCAGTCCCTTGGTTTGAAAGGACC
	CAGGGCGCAGCGATGACGCTAAGAGGAGATACTTACCTGTACC
TBC1D20 coding exon 3	GTAGCGCGACGGCCAGTAGGAGAGCTCTGAAGGTCCC
	CAGGGCGCAGCGATGACGCCCTCTAGGCCTTTTGTG
TBC1D20 coding exon 4	GTAGCGCGACGGCCAGTTTAGATCAGGGCTCAGGGTG
	CAGGGCGCAGCGATGACTGGATCTTGATATGCGTTGC
TBC1D20 coding exon 5	GTAGCGCGACGGCCAGTACAATGAGGAAACTGCGCTC
	CAGGGCGCAGCGATGACAACTCACTGGCCTGTTTTCTG
TBC1D20 coding exon 6	GTAGCGCGACGGCCAGTTGCATCTTTCCATAATCCCC
	CAGGGCGCAGCGATGACTCCTAAATGCTTCCTTCTCACC
TBC1D20 coding exon 7	GTAGCGCGACGGCCAGTCCAACCCTATCCCACCAG
	CAGGGCGCAGCGATGACAACATTTGGGCTGAGTCCTG
TBC1D20 coding exon 8	GTAGCGCGACGGCCAGTTAAAGGCAAACACAAACGGG
	CAGGGCGCAGCGATGACGAGCTTAGGCCAGTGGAATC
RAB1A coding exon 1	GTAGCGCGACGGCCAGTCTCGACCCCTTTAAGATCCC
	CAGGGCGCAGCGATGACGTTTCTTTCGATTACCCGTGG
RAB1A coding exon 2	GTAGCGCGACGGCCAGTCCAAGATACCACTGGGGATG
	CAGGGCGCAGCGATGACGTGTGGCTGTTTGCAGTTTTG
RAB1A coding exon 3	GTAGCGCGACGGCCAGTAAAGATGAGGACCAAGTTTGAC
	CAGGGCGCAGCGATGACGAAAGTGGATGGAGTGGCTG
RAB1A coding exon 4	GTAGCGCGACGGCCAGTGACATTAATCAAATAATGGCTTCCTAC
	CAGGGCGCAGCGATGACGATTTCCCCTCCCTCCTACAC
RAB1A coding exon 5	GTAGCGCGACGGCCAGTCATAATGGAGCGTGAGGAAC
	CAGGGCGCAGCGATGACGTCTGTTAGGTCAGGAACGATG
RAB1A coding exon 6	GTAGCGCGACGGCCAGTTTTCACTTGGGTTCAGATTGC
	CAGGGCGCAGCGATGACGCCATTATGGATGGTAGAAATGC
RAB1B coding exon 1	GTAGCGCGACGGCCAGTAGTGATGGGAGTGGGCG
	CAGGGCGCAGCGATGACAAGAGGGACCCGGATGTG
RAB1B coding exon 2	GTAGCGCGACGGCCAGTTGACAGAGTGAGACCTTATTGC
	CAGGGCGCAGCGATGACTACAGCTTCACCCCTGGTTC
RAB1B coding exon 3	GTAGCGCGACGGCCAGTCAGCTGAGTGCTGGGAATTG
	CAGGGCGCAGCGATGACCGCTGTGTCCCACTAGGAG
RAB1B coding exon 4	GTAGCGCGACGGCCAGTGTGTCGCTCTTCCCAAAATC
	CAGGGCGCAGCGATGACTCCAGGAAGCATCAGGAAAG
RAB1B coding exon 5	GTAGCGCGACGGCCAGTCTGAGCCCAGGACTTAAGGG
	CAGGGCGCAGCGATGACGCAAACTCCTGACAAGGGAG
RAB1B coding exon 6	GTAGCGCGACGGCCAGTAACACCACAGCCAAGGTAGC
	CAGGGCGCAGCGATGACACCCCAAAGCCACAGACTC
RAB2A coding exon 1	GTAGCGCGACGGCCAGTGTTTCGAGGCTGAGCGG
	CAGGGCGCAGCGATGACACTGCGGAAATGGAGCG
RAB2A coding exon 2	GTAGCGCGACGGCCAGTCCCTGGCTGCCTCTTTTC
	CAGGGCGCAGCGATGACAACAGCGTAACAACAAATCCAG
RAB2A coding exon 3	GTAGCGCGACGGCCAGTCATGTCTTGGAAGTAAACATTCTG
	CAGGGCGCAGCGATGACATGGCTGCAGGAAGTCAGTG

RAB2A coding exon 4	GTAGCGCGACGGCCAGTTTTATGCACTCCTTCCCTACC CAGGGCGCAGCGATGACCGGGGCAGAAAGGCTAAG
RAB2A coding exon 5	GTAGCGCGACGGCCAGTTGGATATGGCTAAAAGTGGTTC
	CAGGGCGCAGCGATGACTTTTATAATTTAAGAGGCTGGTAACTC
RAB2A coding exon 6	GTAGCGCGACGGCCAGTTGGTACACTTTCCACAAATGC
	CAGGGCGCAGCGATGACTGATGTTCAAAGACCATCCG
RAB2A coding exon 7	GTAGCGCGACGGCCAGTAATATGTATTACGTCGATTGACTTTC
	CAGGGCGCAGCGATGACTTGGATGACTGTAAACTAATGGTG
RAB2A coding exon 8	GTAGCGCGACGGCCAGTATTGTTGCTAAAATCTACTTGCTG
	CAGGGCGCAGCGATGACTGACGGATTAAAGTCTTCTGTGAC
CSNK2A1 3'UTR	CCCATCTTGGCTTTAGTAACTTTATC
	TTAGCGTCCTCTAAAGTTTACCATTAT
TBC1D20 exon 2	CACCAGGCTCTGAACAGTGAT
	CTCCTTCACTGATAGCCATGC
TBC1D20 exon 4	ATGCCAGAGGAACAGAGAGAA
	TAGTGCAGCTGAGGGTTGC
TBC1D20 exon 6	TGTCTGACTTCAGGCACGTC
	AAAGTAAATCGGCATCAGTGG
TBC1D20 exon 7	CCTCAGGACTTGCCCTATGA
	TGGGGGAAACTGAACAAAAA
TBC1D20 exon 8	AGTGATGGGGCTGACAGTG
	CCATTCCAGGGCACTTTTC
RBCK1 exon 5	GGGTGCACCTTCATCAACA
	TCGTCGGGCTGGTATGAG
RBCK1 exon 3	CTTGCAGCAGTGGGTGATT
	GTAGGCACTGTCCCCATTCT

Table S4. Clinical details of WARBM individuals with *TBC1D20* mutations.

Individual	1.1	1.2	2	3.1	3.2	4	5
TBC1D20 Mutation	c.199C>T p.Arg67*	c.199C>T p.Arg67*	c.292C>T p.Gln98*	c.352_353delCA p.Gln118Glufs*9	c.352_353delCA p.Gln118Glufs*9	c.672G>A p.Trp224*	Deletion exons 2-8
Sex	Male	Female	Female	Female	Female	Male	Female
Ethnic origin	Polish	Polish	Dutch	Pakistani	Pakistani	Egyptian	Pakistani
Consanguinity	Non-consanguineous (but families originate from a common small village)	Non-consanguineous (but families originate from a common small village)	Distant consanguinity	Non consanguineous (but families originate from a common small village)	Non consanguineous (but families originate from a common small village)	First cousin	First cousins
Current age (at assessment)	21 years	16 years	6 years	13 years	11 years	14 years	15 years
Head circumference	50cm (-4.11SD)	51cm (-3.15SD)	47 cm (-4.54SD).	50,5cm (-3.11SD)	50cm (-3.17SD)	Age 14: 47.5 (-5.03SD) Age 9: 46.2 cm (-5.02SD)	49cm (-4.50SD)
Height	Not recorded as difficult to measure accurately due to severe contractures	Not recorded as difficult to measure accurately due to severe contractures	100cm (-3.81SD)	132cm (-3.29SD)	122cm (-3.21SD)	Age 14: 120 cm (-5.06SD) Age 9: 100cm (-5.71SD)	10 months: 66cm (-2.34SD) Otherwise not recorded
Weight	Age 15: 28 kg (-9.92SD)	Age 6: 13 kg (-3.75SD)	Not recorded	20 kg (-5.17SD)	18 kg (-4.53SD)	Age 14: 18kg (-6.52SD) Age 9: 16kg (-5.08SD)	24.5kg (-6.04SD)
Pregnancy (any problems)	No	No	No	Not recorded	Not recorded	Pre-eclampsia	Placenta previa
Gestation	40 weeks	40 weeks	37 weeks	40 weeks	40 weeks	36 weeks	37 weeks
Birth weight	2.2kg (-2.93SD)	2.2kg (-2.82SD)	2.6kg (-0.47)	2.2kg (-2.82SD)	2.57kg (-1.91SD)	2kg (-1.79SD)	2.4kg (-0.99SD)
Birth length	49cm (-1.02SD)	48cm (-1.19SD)	45cm (-1.53SD)	Not recorded	Not recorded	Not recorded	Not recorded
Birth head circumference	32cm (-2.52SD)	33cm (-1.25SD)	Not recorded	Not recorded	Not recorded	Not recorded	31.5cm (-1.03SD)
Karyotype (please note any cytogenetic abnormalities detected on array)	46, XY	46, XX	46,XX Normal Array CGH (105K)	46, XX	46, XX	46,XY	46 XX
Severe Postnatal growth retardation	+	+	+	+	+	+	+
Postnatal Microcephaly	+	+	+	+	+	+	+
Developmental delay/ mental retardation	Profound mental retardation	Profound mental retardation	Severe mental retardation	Profound mental retardation with autistic features	Profound mental retardation	Profound mental retardation with autistic features (thumb sucking , head banging and fear from	Severe mental retardation

						noise or children)	
	No progress in developmental milestones (speech absent, no eye contact, reaction to sounds)	No progress in developmental milestones (speech absent, eye contact present, reaction to sounds)	1 year 9 months: rolling. 2 years and 6 months: Recognized her parents and grandparents, Babbling, Hand wringing movements Sitting for a short time but falls easily. Age 5: Unable to sit on own, dress or feed herself. At age 7 : Some words (mama, eat), and recognizes some words. Remembers things and shows it when she likes something. Stands with support			8 months: Head control 5 years: Sat with support Never learnt to walk or talk	Smiled at 3 months Rolled at 13 months. 15 months- claps, waves. Pulled herself along the floor at 3 years. At 15 years: Cannot sit without support. Reaches out and takes objects. Reasonable understanding , follows instructions, responds to questions. Can feed herself if handed food. Some single words, but unclear.
Axial Hypotonia	Severe generalized hypotonia	Severe generalized hypotonia,	Yes	Moderate- severe generalized hypotonia in the in first years of life; can sit alone and can reach sitting position or roll alone	Moderate- severe generalized hypotonia in the in first years of life; can sit alone but cannot reach the sitting position or roll alone	Yes	Profound central hypotonia in the first years of life
Limb spasticity and reflexes	Decreased reflexes from upper and lower limbs. Flexion contractures of the knee and toes	Elevated, symmetrical patellar reflexes, decreased other reflexes from upper and lower limbs. Babinski sign (+). Flexion contractures of the knee and toes	Hypertonia of legs and arms with hyperreflexia and pathological plantar responses. Limited flexion of hips at age 5; Subluxation of hips age 7	Increased muscular tone, more severe at the lower limbs with fixed flexed posture of the knees (more severe than K4.2)	Increased muscular tone, more severe especially the lower limbs with fixed flexed posture of the knees Surgery for pes equino-varus	At age 8 tendon release operation was done for spasticity At age 14 upper and lower limb spasticity	Hypertonia in all four limbs, more marked in lower limbs, brisk reflexes, up going plantars, very limited flexion at ankles and hips.
Nerve conduction studies	Not performed	Not performed	Not performed	At 3 and 5 years: Slow motor nerve conduction of the lower limbs	At 3 years: Slow motor nerve conduction lower limbs	Not performed	Not performed
Speech	Absent	Absent	Age 7: Very limited says some words (mama, eat), and recognizes some words	Absent	4-5 words	No	Some single words, but unclear speech, understood by parents.
Walking	No	No	No	No	No	No	No
Seizures	Yes	Yes	No	Febrile seizures at 2 ys of age	At 10 months status epilepticus	No	No

					with fever, then some episodes of febrile seizures, resolved by 3 years, no therapy		
EEG findings	Abnormal organization of the electric activity	Abnormal organization of the electric activity, generalized changes with spike, waves and spike-and-wave discharges	Not performed	Age 2: Abnormal activity, multifocal paroxysmal activity at age	Abnormal organization of the electric activity, no paroxysmal activity	Age 5 normal: No focal sharp waves or spikes, Background 3-7 c/sec mixture of theta and delta waves	Not performed
Brain imaging	Brain MRI scan at age 13ys: cortical atrophy, hypoplastic corpus callosum wide lateral ventricles	Brain MRI scan at age 3ys: cortical atrophy, hypoplastic corpus callosum	Brain MRI at 2 ½ years: Bilateral frontoparietal PMG, bilateral occipital loss of white matter around posterior horns of the lateral ventricles, corpus callosum hypogenesis especially of the splenium, mild cerebellar hypoplasia	Brain MRI at 2 years: Bilateral frontoparietal PMG, Megacisterna magna Hypoplastic cerebellar vermis At 4 years 11 months: Progressive cerebellar atrophy of the vermis and hemispheres	Brain MRI at 6 months and 2 years 8 months: Bilateral frontoparietal PMG, megacisterna magna, progressive cerebellar atrophy	Brain MRI scan at the age of 7 months: Bilateral frontoparietal PMG, cortical and central atrophy and hypogenesis of corpus callosum	Brain MRI at 19 months: Very minor degree of superior cerebellar vermian atrophy, otherwise normal.
Microopthalmia	Yes	Yes	Yes , short axial length (13.44 mm in the right eye and 12.72 in the left eye)	Not recorded	Not recorded	No	
Microcornea	Yes	Yes	Yes	Not recorded	Not recorded	No	
Biltateral Congenital Cataracts	+	+	+	+	+	+	+
Pinpoint Pupils	No	Yes	Not recorded	No	No	Age 4: Pinpoint pupils age 4 Age 14: Pupils were reactive to light with sluggish dilatation on mydriatic	Not pinpoint at 15 years, although small pupils noted on review at 2 years of age.
Glaucoma	Yes	Yes	Yes	Yes		Yes	No
Progressive optic nerve atrophy	Yes	Yes	Pale, oblique implanted optic nerves	Pale papilla	Pale papilla	Normal optic nerves age 5	Bilateral pale discs.
ERG	Not performed	Not performed	Not performed	Normal at 5 years	Moderate delay in the major components	Not done	No recordable responses at 6 months
VEP	Not performed	Not performed	Not performed	Irregular morphology of the evoked cortical response; delay	Irregular morphology of the evoked cortical response; delay	Age 5: VEP studies showed intact visual	No recordable responses at 6 months

				in the major components	in the major components		
Vision	Severely impaired	Severely impaired		Severely impaired	Severely impaired		Severely impaired
Hypogonadism (state which abnormalities noted)	Small penis, bilateral cryptorchidism	Not apparent	Not apparent	Not apparent	Not apparent	Small penis and, hypoplastic scrotum with right undescend ed testis and small left testis	Not apparent
Feeding difficulties	Yes	Yes	No	Yes	Reduced appetite		Gastroesop hageal reflux as a baby, now eats well
Sleep abnormalities	Yes	Yes	Yes	Yes	Yes Frequent nocturnal awakenings		No, sleeps well.
Facial features			Nevus of unna on the forehead, deep set eyes, scarce peri-orbital fat, normal ears, normal palate	Brachycephaly, deep set eyes, ptosis, prominent nasal bridge, low anterior hairline and hirsutism	Brachycephaly, deepset eyes, ptosis, prominent nasal bridge, low anterior hairline	Prominent nose with anteverted nostrils and long philtrum.	High nasal bridge, deep set eyes, prominent chin.
Other			Thin teeth enamel, with brown looking dentin Left convex scoliosis	Right renal agenesis	Normal abdominal and heart scan	Hyperextesi bility in the wrist joints and elbows	Nystagmus. Dental crowding.