SUPPLEMENTARY FIGURES

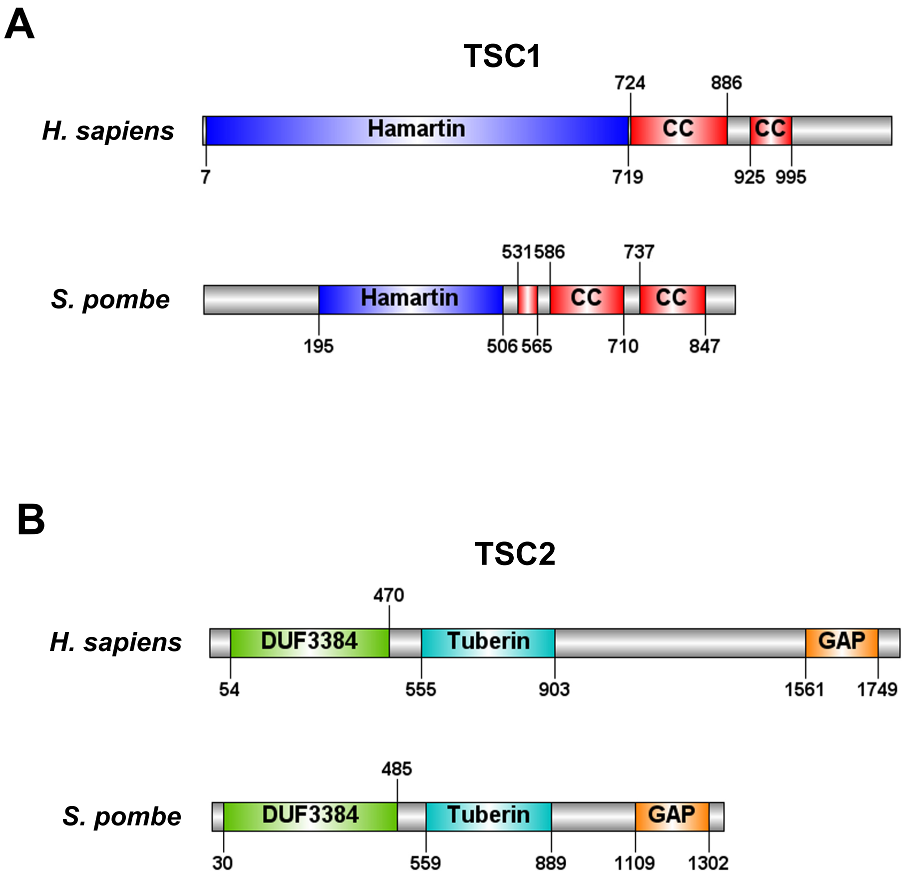


Figure S1 - Domain structure of human *TSC1* and *TSC2* in comparison to their respective fission yeast orthologs. CC, Coiled Coil; GAP, GTPase-activating protein; DUF, Domain of unknown function.

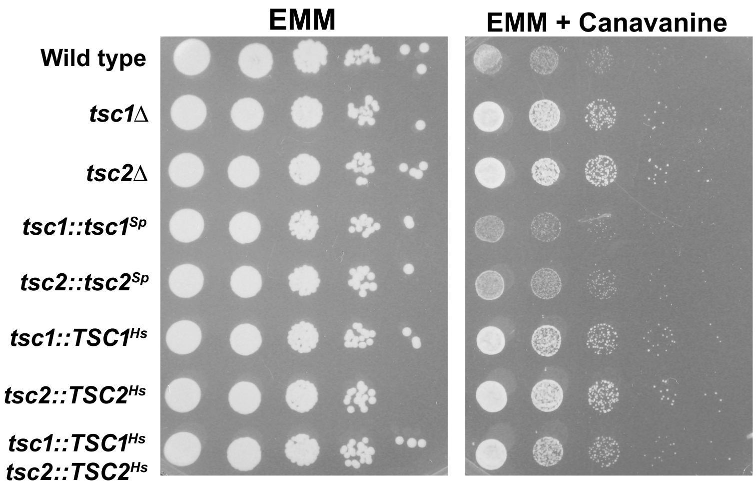


Figure S2 – The human *TSC1* or *TSC2* genes are unable to complement the loss of fission yeast *tsc1* or *tsc2*, respectively. Ten-fold serial dilutions of the indicated yeast cultures were plated on YES media, or YES media containing 60 µg/ml canavanine, and incubated for three days at 30°C.

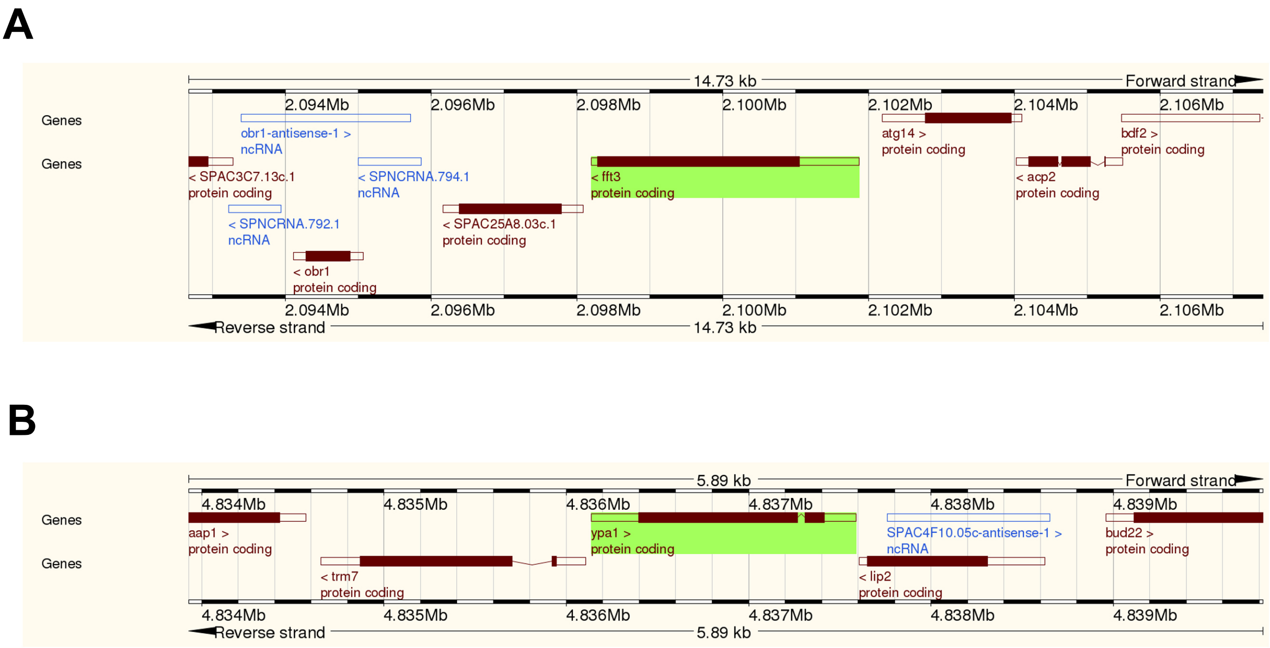
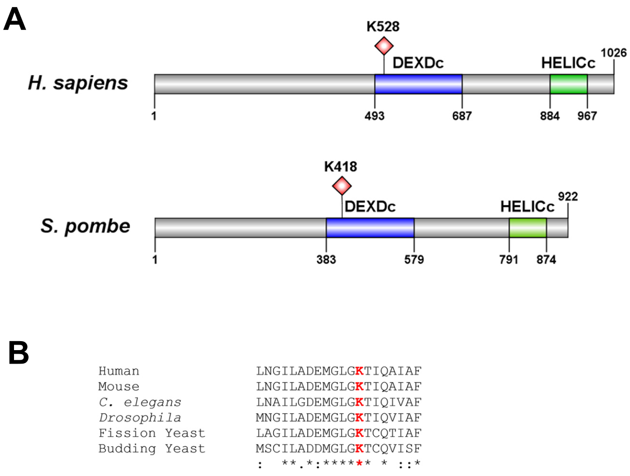
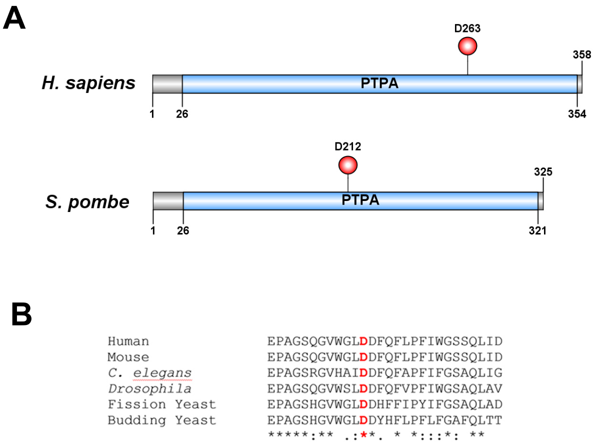


Figure S3 – Ensembl genome browser generated view of the genomic regions surrounding (A) *fft3* or (B) *ypa1*.



```
Human           LNGILADEMGLGKTIQAIAF
Mouse           LNGILADEMGLGKTIQAIAF
C. elegans      LNAILGDEMGLGKTIQIVAF
Drosophila      MNGILADEMGLGKTIQVIAF
Fission Yeast   LAGILADEMGLGKTCQTIAF
Budding Yeast   MSCILADDMGLGKTCQVISF
                :  **.*:****** * ::*
```

Figure S4 – The ATPase domain of human SMARCAD1 is conserved in fission yeast. (A) Domain structure of human SMARCAD1 compared to its fission yeast ortholog, Fft3p. The conserved helicase domain is shown in green. The conserved lysine residue in the ATPase domain is indicated with a red diamond. (B) A clustalW alignment of the relevant portion of the ATPase domain of Fft3p.



```
Human               EPAGSQGVWGLDDFQFLPFIWGSSQLID
Mouse               EPAGSQGVWGLDDFQFLPFIWGSSQLID
C. elegans          EPAGSRGVHAIDDFQFAPFIFGSAQLIG
Drosophila          EPAGSQGVWSLDDFQFVPFIWGSAQLAV
Fission Yeast       EPAGSHGVWGLDDHFFIPYIFGSAQLAD
Budding Yeast       EPAGSHGVWGLDDYHFLPFLFGAFQLTT
                    *****:** .:**. * *:::*: **
```

Figure S5 – The PPIase domain of human PTPA is conserved in fission yeast. (A) Domain structure of human PTPA compared to its fission yeast ortholog, Ypa1p. The conserved aspartate residue in the catalytic domain is indicated with a red diamond. (B) A clustalW alignment of the relevant portion of the catalytic domain of Ypa1p.

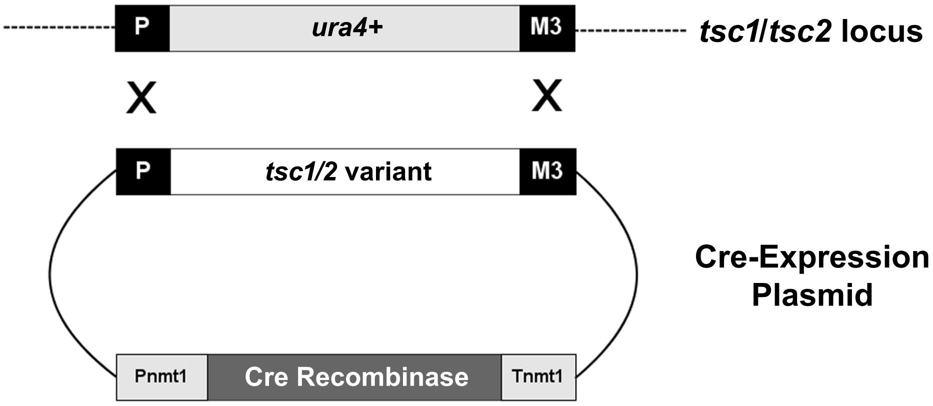


Figure S6 – Schematic describing the recombinase mediated cassette exchange of *tsc1* and *tsc2* variants at the *tsc1/tsc2* loci. P, *loxP* site; M3, *loxM3* site; P_{nmt1}, *nmt1* promoter; T_{nmt1}, *nmt1* terminator.

Biology Open (2018): doi:10.1242/bio.031302: Supplementary information

SUPPLEMENTARY FILES

```
Sequence 1:NP_000359.1  Gene:TSC1  HGNCID:12362          Length:1164 Species: H. sapiens
Sequence 2:NP_593028.1  Gene:tsc1  PomBaseID:SPAC22F3.13  Length:899  Species: S. pombe

                    Alignment Length: 1033    Identity: 187/1033 (18%)
                    Similarity: 302/1033 (29%)     Gaps: 480/1033 (46%)

Human    41 LVNTLVDYYLETSSQPALHILTTLQEPHDKHLLDRINEYVGKAATRLSILSLLGHVIRLQPSWKH 105
            |||.||.|              .:|.|  |.|......:.....||:.|||::..|||.|....:
pombe   175 LVNVLVHY--------------GIQRP--KELSSCFCHHFLNPPTRIPILSVMVEVIRRQGPRLY 223

Human   106 KLSQAPLLPSLLKCLKMDTDVVVLTTGVLVLITMLPMIPQSGKQHLLDFFDIFGRLS-------- 162
            ::.|......:|||.:.||..::|:..:..::.:|..|..|....|...|.|:.|.|
pombe   224 EIPQTGFYDLVLKCAEFDTSPILLSYALSFILMILSHICNSLDDSLYRLFCIYLRFSMIDPTSGF 288

Human   163 -------SWCLKKPGHVAEVYLVHLHASVYA---------------------------------L 187
                   :|         ||:  |...|.||                                 |
pombe   289 PSSTASGNW---------EVF--HDFMSTYASTTTSQTDSSYNDVHDIVGSSQPDYLESLDYSQL 342

Human   188 FHRLYGMYPCNFVSFLRSH--YSMKENLE---TF-EEVVKPMME-----HVRIHPELVTGSKDHE 241
            |..||.:||.||:.|||..  |:.|.|.:   :| :|::....:     |: .|...:..:.:.|
pombe   343 FSILYALYPINFLEFLRDPKLYASKHNFQIRYSFNQELLSTKSDGLLGRHL-AHSNFLKYTAETE 406

Human   242 L-DPRRWKRLETHDVVIECAKISLDPTEASYEDGYSVSHQISARFPHRSADVTTSPYADTQNSYG 305
            | |..||.||::..||..|                                       ::.|:.|
pombe   407 LTDKSRWTRLDSIAVVALC---------------------------------------NSLNAVG 432

Human   306 CATST--PYSTSRLMLLNMPGQLPQTL--SSPSTRLITEPPQATLWSPSMVCGMTTPPTSPGNVP 366
            .|.|.  |:.          |:||.|.  :|.:|.|:..|                      |..
pombe   433 IAESVMDPFG----------GKLPTTYEETSSATGLLAYP----------------------NES 465

Human   367 PDL-SHPYSKVFGTTAGGKGTPLGTPATSPPPAPLCHSDDYVHISLPQATVTPPRKEERMDSARP 430
            .|: |.|:|                                  ||.||
pombe   466 HDIASEPFS----------------------------------ISWPQ----------------- 479

Human   431 CLHRQHHLLNDRGSEEPPGSKGSVTLSDLPGFLGDLASEEDSIEKDKEEAAISRELSEITTAEAE 495
                            .|...|||
pombe   480 ----------------NPSISGSV----------------------------------------- 487

Human   496 PVVPRGGFDSPFYRDSLPGSQRKTHSAASSSQGASVNPEPLHSSLDKLGPDTPKQAFTPIDLPCG 560
                                    |||.:..:....|.|
pombe   488 ------------------------HSATTFDKAQLSNTE-------------------------- 502

Human   561 SADESPAGDRECQTSLETSIFTPSPCKIPPPTRVGFGSGQPPPYDHLFEVALPKTAHHFVIRKTE 625
                                                     ..||::
pombe   503 -----------------------------------------DSYDNI------------------ 508

Human   626 ELLKKAKGNTEEDGVPSTSPMEVLDRLIQQGADAHSKELNKLPLPSKSVDWTHFGGSPPSDEIRT 690
                 :.|.:..:||.|...::.     ::|:       |.|.|.|:|:       |..:|.||.
pombe   509 -----SHGTSYSEGVSSIHMVKG-----ERGS-------NNLELTSESL-------SSTNDTIRR 549

Human   691 LRDQLLLLHNQLLYERFKRQQHALRNRRLLRKVIKAAALEEH-------NAAMKDQLKLQEKDIQ 748
            |:..||.|.|:|.:|:|.||||.....:|.|:.|...|:|..       |...|.|::|...:|.
pombe   550 LQRDLLFLQNELRFEKFVRQQHLQNIGKLHREHILDMAVESERQKLLLTNKRYKAQIELLNSEID 614

Human   749 MWKVSLQKEQARYNQLQEQRDTMVTKLHSQIRQLQHDREEFYNQSQELQTKLEDCRNMIAELR-- 811
            ..:   .:.||..|    :|.......:::|:.|:.:::.:.::..||::.:|...:.:..:|
pombe   615 KHR---SESQAALN----RRVKWENDFNNKIKALREEKKAWKSEESELKSSIESLISQLESIRNS 672

Human   812 -IELKKANNKVCHTELLLSQVSQKLSNSESVQQQMEFLNRQLLVLGEVNELYLEQLQNKHSDTTK 875
             |::..:.|::   ||.|.....||...|.....:....:|:               :..|||:.
pombe   673 QIDIAFSKNQL---ELKLQLYETKLKEYEQHLSCVNISKKQV---------------SSSSDTSF 719

Human   876 EVEMMKAAYRKELEKNRSHVLQQTQRLDTSQKR-ILELESHLAKKDHLLLEQKKYLEDVKLQARG 939
            ....|          :.|.:|..::.:...|:| ::|.|.|..|           ||...|..:.
pombe   720 GNTKM----------DSSMILSNSEAVSDEQERELIESEKHRMK-----------LESENLHLQA 763

Human   940 QLQAAESRYEAQKRITQVFELEILDLYGRLEK---------------DGLLKKLEEEK 982
            .::..:...||   |..|:|.:|.||..||..               |..|.|:.|.|
pombe   764 NIELLKKDLEA---INVVYEAKIFDLEKRLSSEANAPELHNPVNLNYDAQLSKISEIK 818
```

File S1 – Protein sequence alignment of human *TSC1* and *S. pombe tsc1* generated using the DRSC integrative ortholog prediction tool (http://www.flyrnai.org/cgi-bin/DRSC_orthologs.pl). Residues highlighted in red correspond to amino acids altered in clinical cases of TSC.

```
Sequence 1:XP_011520940.2  Gene:TSC2  HGNCID:12363            Length:1879     Species: H. sapiens
Sequence 2:NP_592909.1     Gene:tsc2  PomBaseID:SPAC630.13c   Length:1339     Species: S. pombe

                  Alignment Length: 1773        Identity:  341/1773 (19%)
                  Similarity: 562/1773 (32%)    Gaps:      684/1773 (39%)


Human     174 RALFFKVIKDYPSNE--DLHERLEVFKALTDNGRHITYLEEELADFV-------LQWMD-----V 224
              |..||:.|:..|..:  :|..||:....|||.|..|:.:|..:...:       |||..     .
pombe      97 RHTFFQTIEKDPFQDIAELQLRLKSLSVLTDEGHKISGIENRVGPLLSAWFNQYLQWQSQATELQ 161

Human     225 GLSSEFLLV-----------LVNLVKFNSCYLDE-YIARMVQMICLLCVRTASSVDIEVSLQVLD 277
              |..::..||           ..|||||....:.| .:.::|..:..:|........:...|...|
pombe     162 GKDADSKLVHLLFFKSLFKFSTNLVKFQWFLVPEPQMLQLVNSVVQICNHARLEDVVTEVLMFFD 226

Human     278 AVVCYNCLPAESLPLFIVTLCRT-INVKELCEPCWKLMRNLLGTHLGHSAIYNMCHLMEDRAYME 341
              :::.|:.:|..||...::.||.| |:.....:....::.||:.:.:.:.|..|:.::::......
pombe     227 SMIRYSVIPKASLYDTVLILCSTYISTYSYSKLAQSVIFNLISSPVSNLAFENVFNILQYNRSNV 291

Human     342 DAPLLRGAVFFVGMALWGAHRLYSLRNSPTS--VLPSFYQAMACP---NEVVSYEIVLSITRLIK 401
              :|  :||||..:...:     |..::|...:  .|.|..:....|   ||.|.:||:.::...::
pombe     292 NA--VRGAVRLMRFLM-----LQEVKNDAIASITLSSSIEFTEFPLGFNENVDFEILGTVYLFLR 349

Human     402 --KYRKELQVVAWDILLNIIERLLQ--QLQTLDSPELRTIVHDLLTTVEELCDQNEFHG----SQ 458
                .....|..:.|..:|||:....|  .|:...|.|..:.........:.:.|..:|..    .|
pombe     350 TPSILNRLNFLDWHRILNILMYCSQYLPLKASTSKEAFSKTAAFANIYDRVLDFLDFEALIPLLQ 414

Human     459 ERYFELVERCADQRP------ESSLLNLI-SYRAQSIHPAKDGWIQNLQALMERFFRSESRGAVR 516
              :...:||....|..|      ...||.|. :|..  |.|....|:.||:.|:         |..:
pombe     415 QFQVKLVFFLKDVLPVLKPKIRKKLLRLFETYNL--IFPCNQYWVFNLEFLL---------GIYQ 468

Human     517 IKVLDV----LSFVLLINRQFYEEE-----LINSVVISQLSHIPEDKDHQVRKLATQLLVDLA-- 570
              .|..|:    |.|.|:.:.....:|     |.:..:...:....::.|..|......:|..|:
pombe     469 CKTFDLEDRALLFKLVEDACSVADENSAPILCSKFLFPVIESFSKESDDCVVSPVYNMLFFLSVN 533

Human     571 ------EGCHTHHFNSLLDIIEKVMARSLSPPPELEERDVAAYSASLED---VKTAVLGLLVILQ 626
                    :.|..|.|..|:.....|..|.|:....:.   :..|...|.|   ::..:..:|.||:
pombe     534 FQNPGLKDCIDHIFQQLISDTSSVTVRRLATSTLIR---LFYYYYDLRDAVPIQETLAKMLEILE 595

Human     627 TKLYTLPASHATRVYEMLVSHIQLHYKHSYT-LPIASSIRL-QAFDFLLLLRADSLHRLGLPNKD 689
              |..:..       |..||.....|.::.:.| :.|..:|.| :.|..|              |.|
pombe     596 TPSFPF-------VSRMLCLQFFLRFRANGTSIYICENIDLNEPFKVL--------------NVD 639

Human     690 GVVRFSPYCVCDYMEPERGSEK-----------KTSGPLSPPTGPPGPAPAGPAVRLG----SVP 739
              ..:..:.|.:.|........||           |.|               ..:...|    :.|
pombe     640 SELIPAVYPISDSFVNSATVEKHIWERKENDLIKIS---------------NHSTEYGKDFVTFP 689

Human     740 YSLLFRVLLQCLKQESDWKVLKLVLGRLPESLRYKVLIFTSPCSVDQLCSALCSMLSGPKTLERL 804
              .|.|.|...:.:..||:|.:|..::..|.:.:..:.:...:...:..|...:|.::     .||:
pombe     690 TSSLLRFYRKSMATESNWTILMFMITHLADQISNRSMFIGALEEIYNLLDFMCDIV-----FERV 749

Human     805 ---RGAPEGFSRTDLHLAVVPVLTALISYHNYLDKTKQREMVYCLEQGLIHRCASQCVV---ALS 863
                 ...|....:.::.:.::..:..|..||:...:.::.|:|.....|| .:....|.|   :|.
pombe     750 SISAEIPSNIRKANIMIPILQNVQMLFVYHDQFSRAQEDELVSVFFAGL-QKWNEACHVSIHSLM 813

Human     864 ICSVEMPDIIIKALPVLVVKLTHISATASMAVPLLEFLSTLARLPHLYRNFAAEQYASVFAISLP 928
              :|..|:|..|.|.||.::|.|:.:.....::|.:||||.:|||||.|..||....|..:|||:|.
pombe     814 LCCYELPVSIRKQLPAILVTLSRLITKPDLSVHILEFLCSLARLPDLIANFTDADYRQIFAIALK 878

Human     929 Y------TNPSK------------FNQYIVCLAHHVIAMWFIRCRLPFRKDFVPFITKGLRSNVL 975
              |      |..||            ::.|::.||:.|:.:||:..||..||.|||:|.:||:
pombe     879 YIQHRDFTKESKDSNDTESILKNSYSSYVLALAYSVLQIWFLSLRLTERKKFVPWILRGLK---- 939

Human     976 LSFDDTPEKDSFRARSTSLNERPKSLRIARPPKQGLNNSPPVKEFKESSAAEAFRCRSISVSEHV 1040
              |:.:..|
pombe     940 LASEGKP---------------------------------------------------------- 946

Human    1041 VRSRIQTSLTSASLGSADENSVAQADDSLKNLHLELTETCL---DMMARYVFSNFTAVPKRSPVG 1102
                                                 |.:.||   |||.::.:||...   .:...
pombe     947 -----------------------------------LEDLCLVQYDMMQQFCYSNSDI---NNQTS 973
```

```
Human  1103 EFLLAGGRTKTWLVGNKLVTVTTSVGTGTRSLLGLDSGELQSGPESSSSPGVHVRQTKEAPAKLE 1167
            .|:.:...::||:.||.|.|:..||.:|
pombe   974 TFVDSDVESETWIRGNSLFTINVSVNSG------------------------------------- 1001

Human  1168 SQAGQQVSRGARDRVRSMSGGHGLRVGALDVPASQFLGSATSPGPRTAPAAKPEKASAGTRVPVQ 1232
                                               ||
pombe  1002 -----------------------------------FL---------------------------- 1003

Human  1233 EKTNLAAYVPLLTQGWAEILVRRPTGNTSWLMSLENPLSPFSSDINNMPLQELSNALMAAERFKE 1297
                             |.::|||||.|.:....|..|..|..:.|....:.|:..|:.
pombe  1004 -----------------EAVIRRPTGTTQYTFRNEASLQKFLWEENLTSSKALTRGLLC------ 1045

Human  1298 HRDTALYKSLSVPAASTAKPPPLPRSNTVASFSSLYQSSCQGQLHRSVSWAGIASQREAVGCRAP 1362
                                       |.:||.|.:
pombe  1046 ---------------------------TPSSFVSHF----------------------------- 1054

Human  1363 LCLIDSAVVMEEGSPGEVPVLVEPPGLEDVEAALGMDRRTDAYSRSSSVSSQEEKSLHAEELVGR 1427

pombe  1055 ----------------------------------------------------------------- 1054

Human  1428 GIPIERVVSSEGGRPSVDLSFQPSQPLSKSSSSPELQTLQDILGDPGDKADVGRLSPEVKARSQS 1492

pombe  1055 ----------------------------------------------------------------- 1054

Human  1493 GTLDGESAAWSASGEDSRGQPEGPLPSSSPRSPSGLRPRGYTISDSAPSRRGKRVERDALKSRAT 1557
                                               |.|.|
pombe  1055 -----------------------------------LDPHG------------------------- 1059

Human  1558 ASNAEKVPGINPSFVFLQLYHSPFFGDESNKPILLPNESQSFERSVQLLDQIPSYDTHKIAVLYV 1622
                            :.||         |:|:|||:...|..|::.:.|:||..::.|..::||
pombe  1060 ----------------ISLY---------NQPLLLPSNDDSVRRAISVFDRIPVIESLKAGLVYV 1099

Human  1623 GEGQSNSELAILSNEHGSYRYTEFLTGLGRLIELKDCQPDKVYLGGLDVCGE-DGQFTYCWHDDI 1686
            |. |...|..||:|.:.|..:..||.|||.|.|||..|  ||:.||||...: ||.|.|||.|.:
pombe  1100 GY-QQRREADILANTNPSEDFLTFLNGLGTLFELKTDQ--KVFAGGLDRENDIDGAFAYCWKDKV 1161

Human  1687 MQAVFHIATLMPTKDVDKHRCD-KKRHLGNDFVSIVYNDSGEDFKLGTIKGQFNFVHVIVTPLDY 1750
            .|.|||..|:|||.......|. ||||:||||:|::||:||.::...||..|||||::::||...
pombe  1162 TQMVFHCTTMMPTNIEHDPGCTLKKRHIGNDFVTIIFNESGLEYDFDTIPSQFNFVNIVITPESE 1226

Human  1751 ECNLVSLQCR-KDMEGL----VDTSV---AKIVSDRNLPFVARQMALHANMASQVHHSRSNPTDI 1807
            .......|.: ..::.|    :|.|:   .||||...||.:.|.:.|:|.:.|.::|..:..
pombe  1227 SIRRTGRQIKFYKVKALTKYDIDFSLFRRYKIVSSDALPAIVRDVTLNAAVFSHIYHRSAGD--- 1288

Human  1808 YPSKWIARLRHIKRLRQR 1825
            |...|..|||.:||||::
pombe  1289 YVHIWAERLRQLKRLREK 1306
```

File S2 – Protein sequence alignment of human *TSC2* and *S. pombe tsc2* generated using the DRSC integrative ortholog prediction tool (http://www.flyrnai.org/cgi-bin/DRSC_orthologs.pl). Residues highlighted in red correspond to amino acids altered in clinical cases of TSC.