S.c. 1 MSGLPPPPPGFEEDSDLALPPPPPPPPGYEIEELDNPMVPSSVNEDTFLPPPPPPPSNFE

H.s. 1 --------MAGVFPYRGPGNPVPGPLAPLP-DYMSEEKLQEKARKWQQL----QAKRYAE
S.c. 61 INAEEIVDFTLPPPPPPPGLDELETKAEKKVELHGKRKLDIGKDTFVTRKSRKRAKKMTK

H.s. 48 KRKFGFVDAQKEDMPPEHVREIIRDHGDMTNRKFRHDKRVYLGALKYMPHAVLKLLENMP
S.c. 121 KAKRSNLYTPKAEMPPEHLRKIINTHSDMASKMYNTDKKAFLGALKYLPHAILKLLENMP

H.s. 108 MPWEQIRDVPVLYHITGAISFVNEIPWVIEPVYISQWGSMWIMMRREKRDRRHFKRMRFP
S.c. 181 HPWEQAKEVKVLYHTSGAITFVNETPRVIEPVYTAQWSATWIAMRREKRDRTHFKRMRFP

 **R G Q G**

 **V S**

H.s. 168 PFDDEEPPLDYADNILNVEPLEAIQLELDPEEDAPVLDWFYDHQPLRDSRKYVNGSTYQR
S.c. 241 PFDDDEPPLSYEQHIENIEPLDPINLPLDSQDDEYVKDWLYDSRPLEEDSKKVNGTSYKK

 **P LR P S G**  **GPC**  **N P**

H.s. 228 WQFTLPMMSTLYRLANQLLTDLVDDNYFYLFDLKAFFTSKALNMAIPGGPKFEPLVRDIN
S.c. 301 WSFDLPEMSNLYRLSTPLRDEVTDKNYYYLFDKKSFFNGKALNNAIPGGPKFEPLYP--R

  **S**

H.s. 288 LQDEDWNEFNDINKIIIRQPIRTEYKIAFPYLYNNLPHHVHLTWYHTPNVVFIK-TEDPD
S.c. 359 EEEEDYNEFNSIDRVIFRVPIRSEYKVAFPHLYNSRPRSVRIPWYNNPVSCIIQNDEEYD

 ****

H.s. 348 LPAFYFDPLINPISHRHS-VKSQEPLPDDDE-EFELPEFVEPFLK-DTPLYTDNTANGIA
S.c. 419 TPALFFDPSLNPIPHFIDNNSSLNVSNTKENGDFTLPEDFAPLLAEEEELILPNTKDAMS

H.s. 404 LLWAPRPFNLRSGRTRRALDIPLVKNWYREHCPAGQPVKVRVSYQKLLKYYVLNALKHRP
S.c. 479 LYHSPFPFNRTKGKMVRAQDVALAKKWFLQHPDEEYPVKVKVSYQKLLKNYVLNELHPTL

H.s. 464 PKAQKKRYLFRSFKATKFFQSTKLDWVEGWLQVCRQGYNMLNLLIHRKNLNYLHLDYNFN
S.c. 539 PTNHNKTKLLKSLKNTKYFQQTTIDWVEAGLQLCRQGHNMLNLLIHRKGLTYLHLDYNFN

H.s. 524 LKPVKTLTTKERKKSRFGNAFHLCREVLRLTKLVVDSHVQYRLGNVDAFQLADGLQYIFA
S.c. 599 LKPTKTLTTKERKKSRLGNSFHLMRELLKMMKLIVDTHVQFRLGNVDAFQLADGIHYILN

 **R G**  **SA** **G**

 **N**

H.s. 584 HVGQLTGMYRYKYKLMRQIRVCKDLKHLIYYRFNTGPVGKGPGCGFWAAGWRVWLFFMRG
S.c. 659 HIGQLTGIYRYKYKVMHQIRACKDLKHIIYYKFNK-NLGKGPGCGFWQPAWRVWLNFLRG

 **P**  **E**

H.s. 644 ITPLLERWLGNLLARQFEGRHSKGVAKTVTKQRVESHFDLELRAAVMHDILDMMPEGIKQ
S.c. 718 TIPLLERYIGNLITRQFEGR-SNEIVKTTTKQRLDAYYDLELRNSVMDDILEMMPESIRQ

H.s. 704 NKARTILQHLSEAWRCWKANIPWKVPGLPTPIENMILRYVKAKADWWTNTAHYNRERIRR
S.c. 777 KKARTILQHLSEAWRCWKANIPWDVPGMPAPIKKIIERYIKSKADAWVSAAHYNRERIKR

 **G S
 V**

 **A**

H.s. 764 GATVDKTVCKKNLGRLTRLYLKAEQERQHNYLKDGPYITAEETVAVYTTTVHWLESRRFS
S.c. 837 GAHVEKTMVKKNLGRLTRLWIKNEQERQRQIQKNGPEITPEEATTIFSVMVEWLESRSFS

 **R KR**
 **P**

H.s. 824 PIPFPPLSYKHDTKLLILALERLKEAYSVKSRLNQSQREELGLIEQAYDNLHEALSRIKR
S.c. 897 PIPFPPLTYKNDTKILVLALEDLKDVYASKVRLNASEREELALIEEAYDNPHDTLNRIKK

H.s. 884 HLLTQRAFKEVGIEFMDLYSHLVPVYDVEPLEKITDAYLDQYLWYEADKRRLFPPWIKPA
S.c. 957 YLLTQRVFKPVDITMMENYQNISPVYSVDPLEKITDAYLDQYLWYEADQRKLFPNWIKPS

H.s. 944 DTEPPPLLVYKWCQGINNLQDVWETSEGECNVMLESRFEKMYEKIDLTLLNRLVRLIVDH
S.c. 1017 DSEIPPLLVYKWTQGINNLSEIWDVSRGQSAVLLETTLGEMAEKIDFTLLNRLLRLIVDP

  **PK**

H.s. 1004 NIADYMTAKNNVVINYKDMNHTNSYGIIRGLQFASFIVQYYGLVMDLLVLGLHRASEMAG
S.c. 1077 NIADYITAKNNVVINFKDMSHVNKYGLIRGLKFASFIFQYYGLVIDLLLLGQERATDLAG
 **H A DK T**

 **N D**

 **V I**

H.s. 1064 PPQMPNDFLSFQDIATEAAHPIRLFCRYIDRIHIFFRFTADEARDLIQRYLTEHPDPNNE
S.c. 1137 PANNPNEFMQFKSKEVEKAHPIRLYTRYLDRIYMLFHFEEDEGEELTDEYLAENPDPNFE
 **LY D**

 **S**

 **T**

 **A**

H.s. 1124 NIVGYNNKKCWPRDARMRLMKHDVNLGRAVFWDIKNRLPRSVTTVQWENSFVSVYSKDNP
S.c. 1197 NSIGYNNRKCWPKDSRMRLIRQDVNLGRAVFWEIQSRVPTSLTSIKWENAFVSVYSKNNP

H.s. 1184 NLLFNMCGFECRILPKCRTSYEEFTHKDGVWNLQNEVTKERTAQCFLRVDDESMQRFHNR
S.c. 1257 NLLFSMCGFEVRILPRQRME-EVVSNDEGVWDLVDERTKQRTAKAYLKVSEEEIKKFDSR

H.s. 1244 VRQILMASGSTTFTKIVNKWNTALIGLMTYFREAVVNTQELLDLLVKCEHKIQTRIKIGL
S.c. 1316 IRGILMASGSTTFTKVAAKWNTSLISLFTYFREAIVATEPLLDILVKGETRIQNRVKLGL

H.s. 1304 NSKMPSRFPPVVFYTPKELGGLGMLSMGHVLIPQSDLRWSKQTDVGITHFRSGMSHEEDQ
S.c. 1376 NSKMPTRFPPAVFYTPKELGGLGMISASHILIPASDLSWSKQTDTGITHFRAGMTHEDEK

H.s. 1364 LIPNLYRYIQPWESEFIDSQRVWAEYSLKRQEAIAQNRRLTLEDLEDSWDRGIPRINTLF
S.c. 1336 LIPTIFRYITTWENEFLDSQRVWAEYATKRQEAIQQNRRLAFEELEGSWDRGIPRISTLF

H.s. 1424 QKDRHTLAYDKGWRVRTDFKQYQVLKQNPFWWTHQRHDGKLWNLNNYRTDMIQALGGVEG
S.c. 1496 QRDRHTLAYDRGHRIRREFKQYSLERNSPFWWTNSHHDGKLWNLNAYRTDVIQALGGIET

H.s. 1484 ILEHTLFKGTYFPTWEGLFWEKASGFEESMKWKKLTNAQRSGLNQIPNRRFTLWWSPTIN
S.c. 1556 ILEHTLFKGTGFNSWEGLFWEKASGFEDSMQFKKLTHAQRTGLSQIPNRRFTLWWSPTIN

H.s. 1544 RANVYVGFQVQLDLTGIFMHGKIPTLKISLIQIFRAHLWQKIHESIVMDLCQVFDQELDA
S.c. 1616 RANVYVGFLVQLDLTGIFLHGKIPTLKISLIQIFRAHLWQKIHESIVFDICQILDGELDV
 **M F KF**

H.s. 1604 LEIETVQKETIHPRKSYKMNSSCADILLFASYKWNVSRPSLLADSKDVMDSTTTQKYWID
S.c. 1676 LQIESVTKETVHPRKSYKMNSSAADITMESVHEWEVSKPSLLHETNDSFKGLITNKMWFD

 **I L**

 **R**

H.s. 1664 IQLRWGDYDSHDIERYARAKFLDYTTDNMSIYPSPTGVLIAIDLAYNLHSAYGNWFPGSK
S.c. 1736 VQLRYGDYDSHDISRYVRAKFLDYTTDNVSMYPSPTGVMIGIDLAYNMYDAYGNWFNGLK

 **V**

H.s. 1724 PLIQQAMAKIMKANPALYVLRERIRKGLQLYSSEPTEPYLSSQNYGELFSNQIIWFVDDT
S.c. 1796 PLIQNSMRTIMKANPALYVLRERIRKGLQIYQSSVQEPFLNSSNYAELFNNDIKLFVDDT
 **D L**

H.s. 1784 NVYRVTIHKTFEGNLTTKPINGAIFIFNPRTGQLFLKIIHTSVWAGQKRLGQLAKWKTAE
S.c. 1856 NVYRVTVHKTFEGNVATKAINGCIFTLNPKTGHLFLKIIHTSVWAGQKRLSQLAKWKTAE
 **DPA R T**

 **N D K**

 **Y**

H.s. 1844 EVAALIRSLPVEEQPKQIIVTRKDMLDPLEVHLLDFPNIVIKGSELQLPFQACLKVEKFG
S.c. 1916 EVSALVRSLPKEEQPKQIIVTRKAMLDPLEVHMLDFPNIAIRPTELRLPFSAAMSIDKLS

H.s. 1904 DLILKATEPQMVLFNLYDDWLKTISSYTAFSRLILILRALHVNNDRAKVILKPDKTTITE
S.c. 1976 DVVMKATEPQMVLFNIYDDWLDRISSYTAFSRLTLLLRALKTNEESAKMILLSDPTITIK

H.s. 1964 PHHIWPTLTDEEWIKVEVQLKDLILADYGKKNNVNVASLTQSEIRDIILGMEISAPSQQR
S.c. 2036 SYHLWPSFTDEQWITIESQMRDLILTEYGRKYNVNISALTQTEIKDIILGQNIKAPSVKR

H.s. 2024 QQIAEIE-----KQTKEQSQLTAT--QTRTVNKHGDEIITSTTSNYETQTFSSKTEWRVR
S.c. 2096 QKMAELEAARSEKQNDEEAAGASTVMKTKTINAQGEEIVVVASADYESQTFSSKNEWRKS

H.s. 2077 AISAANLHLRTNHIYVSSDDIKETGYTYILPKNVLKKFICISDLRAQIAGYLYGVSPPDN
S.c. 2156 AIANTLLYLRLKNIYVSADDFVEEQNVYVLPKNLLKKFIEISDVKIQVAAFIYGMSAKDH

H.s. 2137 PQVKEIRCIVMVPQWGTHQTVHLPGQLPQHEYLKE---MEPLGWIHTQPNESPQLSPQDV
S.c. 2216 PKVKEIKTVVLVPQLGHVGSVQIS-NIPDIGDLPDTEGLELLGWIHTQTEELKFMAASEV

H.s. 2194 TTHAKIMADNPSWDGEKTIIITCSFTPGSCTLTAYKLTPSGYEWGRQNTDKGNN-PKGYL
S.c. 2275 ATHSKLFADK----KRDCIDISIFSTPGSVSLSAYNLTDEGYQWGEENKDIMNVLSEGFE

H.s. 2253 PSHYERVQMLLSDRFLGFFMVPAQSSWNYNFMGVRHDPNMKYELQLANPKEFYHEVHRPS
S.c. 2331 PTFSTHAQLLLSDRITGNFIIPSGNVWNYTFMGTAFNQEGDYNFKYGIPLEFYNEMHRPV

H.s. 2313 HFLNFALLQEGEVYSADREDLYA
S.c. 2391 HFLQFSELAGDEELEAEQIDVFS

**Figure S2. Alignment of *Saccharomyces cerevisiae* (S.c.) and human (H.s.) Prp8.** Suppressor substitutions are listed below the yeast sequence in black for U4-cs1 (from Kuhn *et al.* 1999, Kuhn and Brow 2000, this study (underlined), or double underlined if from both), green for *prp28-1* (Price et al. 2014), and red for *brr2-1* (Kuhn et al. 2002).