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).