

Supplementary Figures for the article:

p53 Forms Redox-Dependent Protein–Protein Interactions through Cysteine 277

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Legends to figures

Supplementary Figure S1. reversible redox-dependent protein complexes of p53 are observed upon continuous H₂O₂ production or under low glucose conditions.

(A) HEK293T cells transiently expressing Flag-p53 (WT) were treated with diamide for 15 min, followed by refreshing the medium. Protein samples were collected at the indicated recovery times (*) after diamide removal.

(B) p53 does not form detectable redox-dependent protein-protein interactions upon prolonged H₂O₂ treatment in HEK293T cells cultured in high glucose media (4.5 g/l).

(C) p53 does not form detectable redox-dependent protein-protein interactions upon prolonged H₂O₂ treatment in H1299 cells cultured in high glucose media (4.5 g/L).

(D) Continuous exogenous H₂O₂ production upon addition of Glucose Oxidase (GOX) induces formation of several p53 redox-dependent protein complexes (indicated by asterisks) in cells cultured in high-glucose (4.5g/L) media. HEK293T cells transiently expressing Flag-p53 (WT) were cultured in high-glucose media or refreshed to PBS 1h prior to treatment with diamide (200 μM, 15min) or H₂O₂ (200 μM, 15min). 100 mU/mL or 1000 mU/mL of GOX was added to cells for the indicated times.

(E) H₂O₂ treatment induces similar p53 redox-dependent complexes as diamide does in cells cultured in low-glucose (1g/L) media. HEK293T cells transiently expressing Flag-p53 (WT) were cultured in low-glucose media or refreshed to PBS 1h prior to the treatment of diamide (200 μM, 15min) or H₂O₂ (200 μM, 15min). 100 mU/mL or 1000 mU/mL of GOX was added to cells for the indicated times. Note that in high glucose media GOX seems to induce more p53 redox-dependent complexes, probably because the enzyme produces more H₂O₂ at this higher substrate concentration.

Supplementary Figure S2. p53 cysteine conservation in vertebrate species.

Alignment of human p53 protein sequences from 5 groups of vertebrate species including fish, amphibians, birds, reptiles and mammals. All protein sequences were downloaded from the ENSEMBL database and the sequence alignment was performed in Jalview (version 15.0) software. The alignments were colored based on the extent of conservation (Threshold 10). Cysteines were further colored in orange in Adobe Illustrator.

Supplementary Figure S3. C277 is required for p53 disulfide-dependent protein interactions.

- (A) Evaluation of transcriptional activity of p53 cysteine mutants (to Alanine or Serine) in H1299 cells.
- (A) Evaluation of disulfide-dependent protein complexes of p53 cysteine mutants (to Alanine or Serine) in H1299 cells.
- (B) Evaluation of disulfide-dependent protein complexes of p53 WT, C182S, C277S and C176S mutants in HEK293T cells.

Supplementary Figure S4. p53 does not form disulfide-dependent homodimers upon diamide treatment.

- (A) Flag-p53 and HA-p53 were expressed in HEK293T cells either separately or co-expressed. Immunoprecipitation using anti-Flag or anti-HA was conducted after diamide treatment and Western blots were probed with different fluorescence-conjugated secondary antibodies against Flag (Green) or HA(Red). Flag-p53 (green) and HA-p53(red) were both oxidized to form a complex (*, around 100kDa) with another protein, but not as a homodimer or homo-oligomer as no overlapping signal of green and red was observed. p53 is known to function as a (non-disulfide dependent) tetramer, and Flag-p53 can indeed pull-down HA-p53. The high-molecular weight band containing HA-p53, which is independent of homodimerization of p53 as indicated in the model in (B).
- (B) HA-p53 and Flag-p53 form non-covalent tetramers of different composition (other combinations are possible). Diamide treatment induces the formation of disulfide-dependent complexes with an unknown protein. Although Flag-p53 binds HA-p53 non-covalently, HA-p53 can be found to migrate as a redox-sensitive complex in the Flag-directed IP.

Supplementary Figure S5. Reproducible results among 4 replicates in each condition.

- (A) WB detection of p53 disulfide-dependent dimerization for the four replicates prepared for MS/MS analysis for each condition.
- (B) Bar graph showing the amounts of protein identified by MS in each replicate (R1, R2, R3, R4) and condition.
- (C) Box plot showing the distribution of Label-Free quantification (LFQ) intensities of proteins in each replicate and condition. Flag-p53 is indicated as the top outlier with the highest and roughly equal LFQ value in all samples.
- (D) Principle component analysis (PCA) of LFQ value in all samples.

Supplementary Figure S6. PANTHER Gene Ontology (GO) analysis of redox dependent-p53 binding partners.

GO enrichment analysis for 167 diamide-induced p53 interactors was performed using the PANTHER Classification System online (<http://pantherdb.org/>). The top significant terms (p-value < 0.001) in biological process, molecular function and cellular component were presented in the bar plots.

- (B) GO analysis of molecular function.
- (C) GO analysis of cellular component.

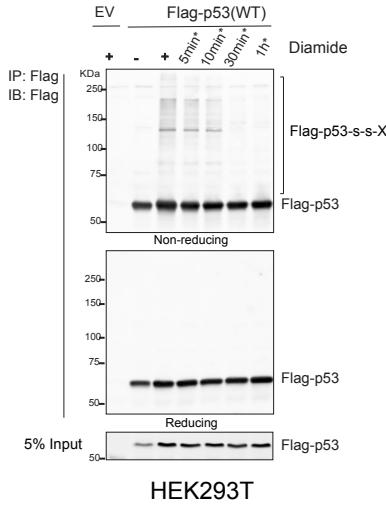
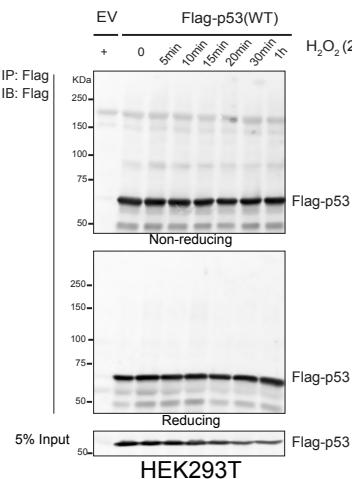
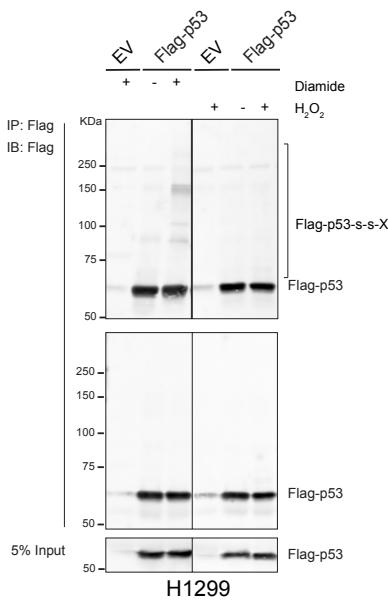
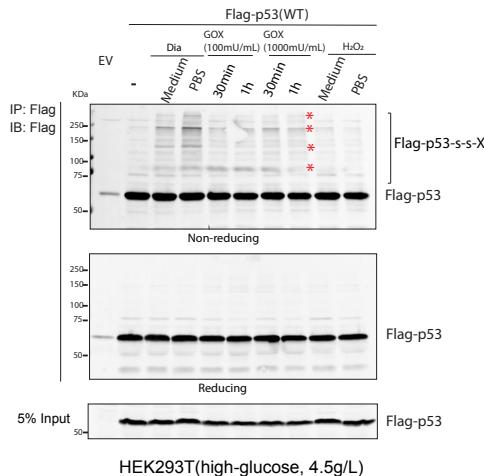
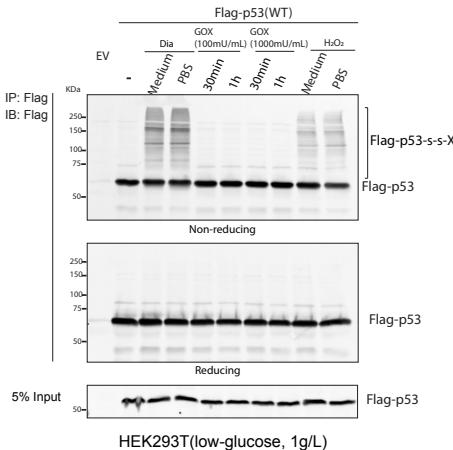
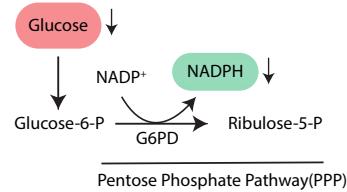
Supplementary Figure S7. Validation of the disulfide-dependent interaction between p53 and 14-3-3 θ .

HEK293T cells were transfected with Flag-p53 WT in combination with siRNA for 14-3-3 θ or a scrambled Ctrl siRNA. After 48h of transfection, IP and WB using a Flag antibody were performed to examine whether the shifted band recognized by the 14-3-3 θ antibody is indeed the presumed p53-S-S-14-3-3 θ interaction. In the upper left panel, the disulfide-dependent, covalent p53-S-S-14-3-3 θ complex (*) is clearly present in the control condition using both Flag (-p53) and 14-3-3 θ staining, whereas it is largely gone upon 14-3-3 θ depletion (si14-3-3 θ) (*). The pattern of other intermolecular disulfide-dependent complexes containing Flag-p53 seems not to be affected by 14-3-3 θ knockdown. In the lower right panel, consistently, reduced 14-3-3 θ (#) is only observed in

the control condition. A lower level of 14-3-3 θ is detected upon siRNA-mediated knock-down in the cleared lysate as well (input).

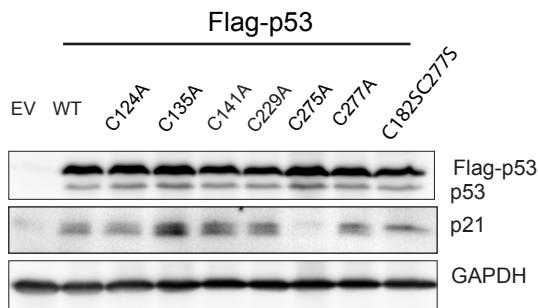
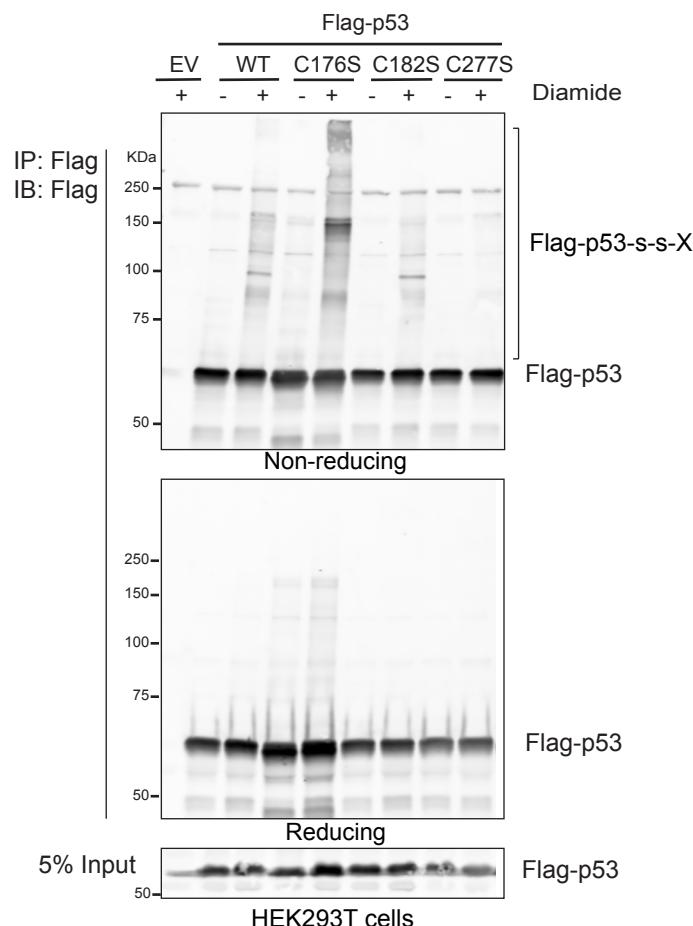
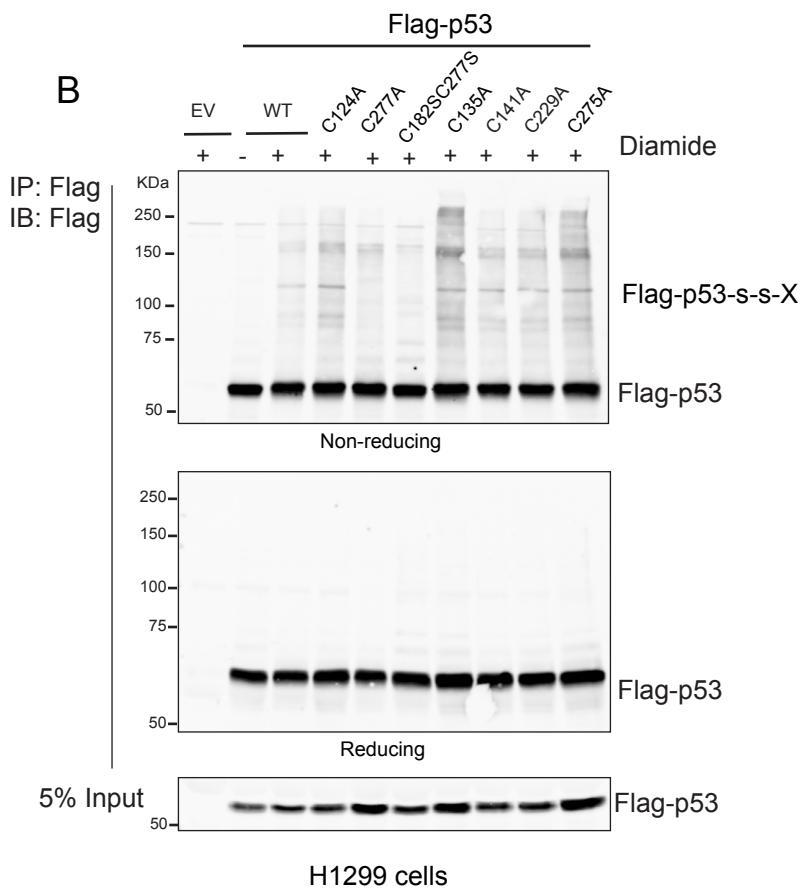
Supplementary Figure S8. Further Validation of the disulfide-dependent interaction between p53 and 53BP1.

HEK293T cells were transfected with HA-p53 WT or C277S alone or along with GFP-53BP. 48 h after transfection and following 15-min diamide treatment, the p53-53BP1 complex was precipitated using HA-antibody coated beads. The beads were washed with a wash buffer containing low salt (L, 150 mM NaCl), high salt (H, 1M NaCl) or high salt plus 10 mM DTT (D). The wash buffer was otherwise identical to the lysis buffer (see Methods section), minus the alkylating agent. The p53 - 53BP1 interaction was examined by WB using HA and GFP antibodies.

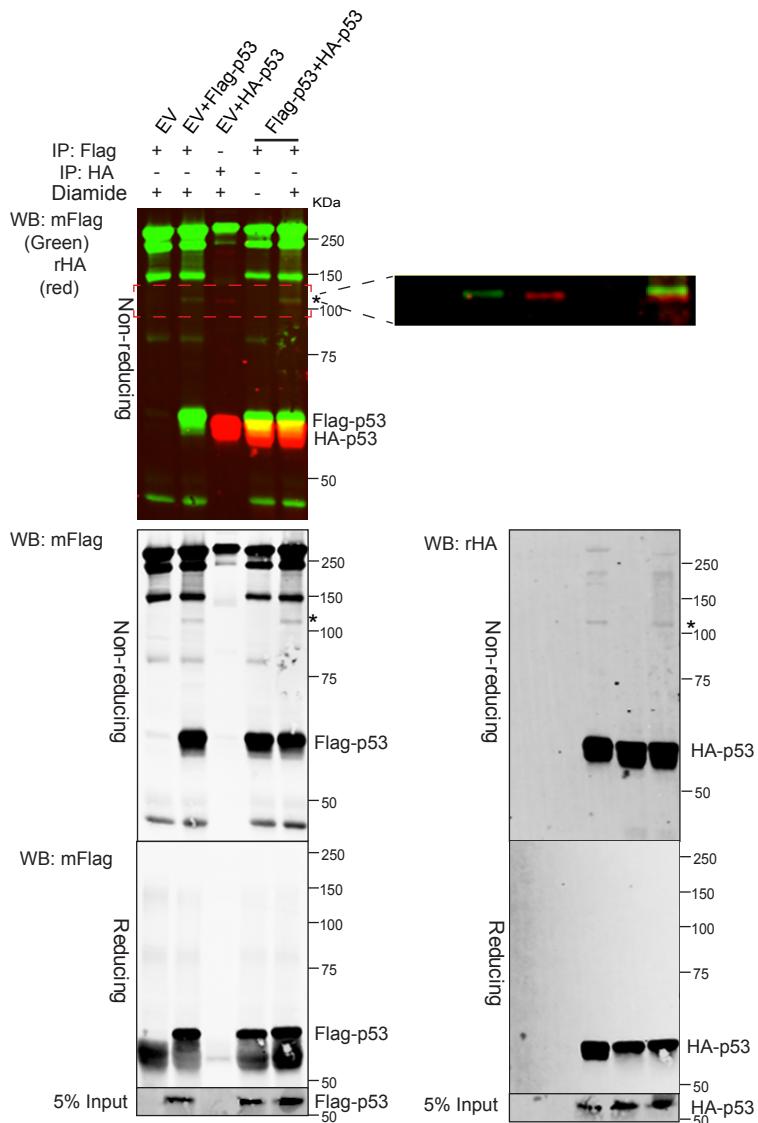
A**B****C****D****E****Figure S1.**

A

| | | | |
|------------|---|-----|--|
| Fish | ENSSANP00000016801[<i>Sinocyclocheilus_anshuiensis</i>] | 90 | QSGTAKSVCTYSSDLNKLFCQLAKTCPVQMVEAPPQGSVIRATAIYKKSEHVAEVRRCPHHERTPDG..DGLAPAAHLIRVEGNSRALYREDDVNSRHSVVVPEAPQLGAETTVLYNFMCNSSCMGMNRRPILTTITLETHDQOLLGRRSFEVRVACPGDRKTEESNFRKDQ 268 |
| | ENSCGOP00000014430[<i>Cottoperca_gobio</i>] | 103 | KSGTAKSVTSTYSELLNKLCQLAKTCPVSPVELVSKELPPGTVLRTAVYKKSEHVAEVRRCPHHQNEDA...AEHRSHLIRVEGSQRAQYFEDQHTKRQSVTPYEPPLQGSEITTLLSFMCNSSCMGMNRRPILTTITLETHDQOLLGRRSFEVRVACPGDRKTEEENSTKLQ 278 |
| | ENSTSP000005027925[<i>Oncorhynchus_tshawytscha</i>] | 161 | OSSTAKSVTCTYSPDLNKLFCQLAKTCPVQIVVDHPPPGAVVRALAIYKKLSDVADVRRCPHHQSTSEN..NEGPAPRGHLVRVEGNQRSEYMEDGNTLROSVLVPYEPPOVGSECTTLYNFMCNSSCMGMNRRPILTTITLETHDQOLLGRRSFEVRVACPGDRKTEEEINLKKQO 340 |
| | ENSCCRP000010073273[<i>Cyprinus_carpio</i>] | 115 | QSGTAKSVCTYSSDLNKLFCQLAKTCPVQMLVDAPPQGSVIRATAIYKKSEHVAEVRRCPHHERTLDG..DGLAPAAHLIRVEGNSRALYREDDMIYRHSVVVPEAPQLGAETTVLYNFMCNSSCMGMNRRPILTTITLETHDQOLLGRRSFEVRVACPGDRKTEESNFRKDQ 271 |
| | ENSSGRP000000059520[<i>Sinocyclocheilus_grahami</i>] | 90 | QSGTAKSVCTYSPDLNKLFCQLAKTCPVQMVEAPPQGSVIRATAIYKKSEHVAEVRRCPHHERTPDG..DGLAPAAHLIRVEGNSRALYREDDVNSRHSVVVPEAPQLGAETTVLYNFMCNSSCMGMNRRPILTTITLETHDQOLLGRRSFEVRVACPGDRKTEESNFRKDQ 268 |
| | ENSRHP000000066963[<i>Sinocyclocheilus_rhinoferos</i>] | 90 | QSGTAKSVCTYSSDLNKLFCQLAKTCPVQMVEAPPQGSVIRATAIYKKSEHVAEVRRCPHHERTPDG..DGLAPAAHLIRVEGNSRALYREDDVNSRHSVVVPEAPQLGAETTVLYNFMCNSSCMGMNRRPILTTITLETHDQOLLGRRSFEVRVACPGDRKTEESNFRKDQ 268 |
| | ENSNBRP000000026855[<i>Neolamprologus_bricharius</i>] | 114 | KSGTAKSVTSTYSEVLNKLYCQLAKTPIEVLVSKEPPQAVLRTATVYKKTEHADVVRCPHHQNEDS...AEHRSHLIRVEGNQRACYFEDLHTKRQSVTPYEPPLQGSEMTTILLSFMCNSSCMGMNRRPILTTITLETHDQOLLGRRSFEVRVACPGDRKTEESNFRKDQ 289 |
| | ENSKMAP000000018969[<i>Kryptolebias_marmoratus</i>] | 114 | NGSMAKSVTSTYSEKLKKLFCQLAKTPIEVLVSEELPOGAVLRTATVYKKSDHVAEVVKRCPHHQTEDS...SDNRSHLIRLEQLGSQVQYFEDPNTLRQSVTPYEPPLQGSKHTTILLSFMCNSSCMGMNRRPILTTITLETHDQOLLGRRSFEVRVACPGDRKTEESNFRKDQ 289 |
| | ENSXCOPO00000002276[<i>Iphophorus_couchianus</i>] | 71 | DSGTAKSVTSTYSVKGKLFCQLAKTTPIGVLVKEEPPQAVIRATAVYKKTEHGEVVKRCPHHQSEDL...SDNKSHLIRVEGSQLAOYFEDPNTRRHSSVTVPYERPQLGSEMTTILLSFMCNSSCMGMNRRPILTTITLETHDQOLLGRRSFEVRVACPGDRKTEEGNLEK..244 |
| | ENXETP0000053759[<i>Xenopus_tropicalis</i>] | 138 | QSGTAKSVTCTYSTDLNKLFCQLAKTCPPLLVRVERPPLGLSILRQNSPMMLRVEGNSKAYMEDVGTGRHSSVCPVGPQVGETCTVLYNFMCNSSCMGMNRRPILTTITLETHDQOLLGRRSFEVRVACPGDRRTEEDNCTKKR 268 |
| Amphibians | ENSCABP00000018274[<i>Chelonoidis_abingdonii</i>] | 89 | QSGTAKSVTCTYSPQLNKLYCQLAKTCPVQIRTAQSOPPAGSIIIRATAVYKKSEHVAEVRRCPHHHERCEEY..RDGVAPARHLIRLEGNOQAHYYDDENTKRSQSVTPYETPLQGSDCTTLYNFMCNSSCMGMNRRPILTTITLEGRHROLLGRRCFEVRVACPGDRRTEEEFNCKKL 296 |
| | ENSGAGP00000006237[<i>Gopherus_agassizii</i>] | 117 | QSGTAKSVTCTYSPQLNKLYCQLARTCPVQIRTAQSOPPAGSIIIRATAVYKKSEHVAEVRRCPHHHERCEEY..RDGVAPARHLIRLEGNOQAHYYDDENTKRSQSVTPYEMPLQGSDCTTLYNFMCNSSCMGMNRRPILTTITLEGRHROLLGRRCFEVRVACPGDRRTEEEFNCKKL 296 |
| | ENNSMRP0000000503879[<i>Meristogenys_merianae</i>] | 98 | QSGTAKSVTCTYSPVNLNKLCQIAKTCVCIKVSSLPPAGTIRATAVYKKSEHVAEVVKRCPHHERSQD..NTSLAPAPQHLIRVEGNOHAQYFSDRNTKRSVTPYEPQLGSXTTLYNFMCNSSCMGMNRRPILTTITLEGRHROLLGRRCFEVRVACPGDRRTEEEFNCKKL 276 |
| | ENSPVIP00000017506[<i>Pogona_vitticeps</i>] | 115 | TSGMAKSVTCTYSPDLNKLFCQLAKTCPVPHIKVSASPPADAVIRTMAYKKSEHIAVDVKRCPHHERSPD..FDKDTAPAEHLIRVEANPAQKYISDTSKRSVTPVYEQPLGTDSTTLYNFMCNSSCMGMNRRPILTTITLEPLQGMLIGRRSFEVRCACPGDRRTEEEFNCKKL 294 |
| | ENSPSIP00000014905[<i>Pelodiscus_sinensis</i>] | 102 | QSGTAKSVTCTYSPDLNKLFCQLAKTCPVQIKVSSOPPPGSVIRATAVYKKSEHVAEVVRCPHHERSADY..SDGVAPAQHLIRLEGNOQAYYRDDENTKRSQSVTPYETPLQGSDCTTLYNFMCNSSCMGMNRRPILTTITLESKNGOLLGRRCFEVRVACPGDRRTEEEFNHRKKL 281 |
| | ENSPMP00000017811[<i>Podarcis_murais</i>] | 84 | PSGTAKSVTCTYSPDLNKLFCQLAKTCPVQIKVSSOPPPGSVIRATAVYKKSEHVAEVVRCPHHERSGGRRGJIAPEAHLIRVEGNLQAOYLSQONTKRSVTPYEAQPLGTESSK..LYNFMCNSSCMGMNRRPILTTITLEGHOLLGRRCFEVRVACPGDRRTEEEFNHRKKM 264 |
| | ENSPCEVP000005020111[<i>Gopherus_evgoodei</i>] | 117 | QSGTAKSVTCTYSPQLNKLYCQLARTCPVQIRTAQSOPPAGSIIIRATAVYKKSEHVAEVRRCPHHHERCEEY..RDGVAPARHLIRLEGNOQAHYYDDENTKRSQSVTPYEMPLQGSDCTTLYNFMCNSSCMGMNRRPILTTITLEGRHROLLGRRCFEVRVACPGDRRTEEEFNCKKL 296 |
| | ENSLTP00000024105[<i>Pelodus_castaneus</i>] | 120 | QSGTAKSVTCTYSPDLNKLFCQLAKTCPVQIKVSSOPPPGSVIRATAVYKKSEHVAEVVRCPHHERCTEY..SDGVAPARHLIRLEGNOQAYYHDDNHTKRSQSVTPYEPPLQGSDCTTLYNFMCNSSCMGMNRRPILTTITLEGHOLLGRRCFEVRVACPGDRRMEETTQRKKS 299 |
| | ENSLTP00000024160[<i>Laticephala_laticaudata</i>] | 138 | PSGTAKSVTYTFSPLNKLFCQMGKTCVVLKLAVGVPPPGSVVRAVAYKRSEHMAEVVKRCPHHERPEHN..DEAPIADHLIRVEGNLQAYYTHPTTNRHSVLPYQKPVQGMACTI..QTLTLETAQEVLGRRCFDVRCACPGDRRMEETTQRKAK 317 |
| | ENSPTXP00000020660[<i>Peudonaja_textilis</i>] | 1 |MAVYKRMSEHMAEVVRCPHHERPEHN..DEAPIADHLIRVEGNLQAYYTHPTTNRHSVLPYQKPVQGMASTI..QTLTLETAQEVLGRRCFDVRCACPGDRRTEEE..128 |
| Reptiles | ENSLSDP00000017092[<i>Lonchura_striata_domestica</i>] | 16 | VTCTYSPSLNKLFCRLAKTCPVQVVRGVPPPGALLRAVAYKKSEHVAEVVRCPHHERCG..PDGNAPAQHLIRVEGNGQARYHDDETTTRHSSVAVPYEPPEVGSECTTLYNFMCNSSCMGMNRRPILTTITLEGRHROLLGRRCFEVRVACPGDRRHEEEITORKG 186 |
| | ENSACCP0000002024610[<i>Aquila_chrysaetos_chrysaetos</i>] | 1 |VYKRMSEHMAEVVRCPHHERCGGP..GDGLAPAPQHLIRVEGNGP0..RHSVAVPYEPPE..VYKRMSEHMAEVVRCPHHERCGGG..SDGLAPAPQHLIRVEGNGQARYHDDETTTRHSSVTPVYEPPEVGSDCTTLYNFMCNSSCMGMNRRPILTTITLEGRHROLLGRRCFEVRVACPGDRRHEEEFNCKKL 125 |
| | ENSSHBP000005007544[<i>Strigops_habroptila</i>] | 55 | AMGLRCPOQFSVVLHKLFCRLAKTCPVQVWGVPPPGALLRAVAYKKSEHVAEVVRCPHHERAGGD..SEGNAPAQHLIRVEGNGQARYHDDETTTRHSSVTPVYEPPEVGSDCTTLYNFMCNSSCMGMNRRPILTTITLEGRHROLLGRRCFEVRVACPGDRRHEEEFNCKKL 231 |
| | ENSCPFP00000017149[<i>Camariaynchus_parvulus</i>] | 72 | VTCTYSPALNKLFCRLAKTCPVQVVRGVPPPGALLRAVAYKKSEHVAEVVRCPHHERCG..TDGNAAPAQHLIRVEGNGQARYQDDETTTRHSSVAVPYEPPEVGSECTTLYNFMCNSSCMGMNRRPILTTITLEPLQGMLIGRRSFEVRCACPGDRRHEEEFNCKKL 244 |
| | ENSTGUP000000326551[<i>Taeniopygia_guttata</i>] | 96 | FLLIMYFFPQYSPSLNKLFCRLAKTCPVQVVRGVPPPGALLRAVAYKKSEHVAEVVRCPHHERCG..GLVHTVLYNFMCNSSCMGMNRRPILTTITLEPLQGMLIGRRSFEVRCACPGDRRHEEEFNCKKL 259 |
| | ENSOANP00000037616[<i>Ornithorynchus_anatinus</i>] | 188 | QSGTAKSVTCTYSPPLNKLFCQLAKTCPVQLWDSPPPPAGARVARAMAYKKTDHRAEVVKRCPHHERSSD..DGDAAPAQHLIRVEGNGQATYLNDKETTRQSVVVPYEPQVGCEYTTVFYNYMCNSSCMGMNRRPILTTITLEASQGLGRQSFEVRCACPGDRKSDSEENHQKKG 366 |
| | ENSPCIP00000039513[<i>Phascolarctos_cinerarius</i>] | 223 | HSGTAKSVTCTYSPELNKLFCQLAKTCPVQLWTSPPAGARVARAMAYKKSEHMTEVVKRCPHHERCTEH..DALAPPQHLIRVEGNLRAEYLNDDATTMRQSVSIPIYESPEVGCDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSQOLLGRRSFEVRVACPGDRRTEEEFNRKKG 402 |
| | ENSVURP00001004263[<i>Vombatus_urinus</i>] | 91 | HSGTAKSVTCTYSPELNKLFCQLAKTCPVQLWTSPPAGSIRVARAMAYKKSEHMTEVVKRCPHHERCTEH..DALAPPQHLIRVEGNLRAEYLNDDATTMRQSVSIPIYESPEVGCDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSQOLLGRRSFEVRVACPGDRRTEEEFNRKKG 270 |
| | ENSDNOP00000004075[<i>Dasyurus_novemcinctus</i>] | 107 | RSGTAKSVTCTYSPILNKLFCQLAKTCPVQLWNNSPPPPGARVARAMAYKKSEHMTEVVRCPHHERCSEH..GDGLAPPOQHLIRVEGNLHAEYLDINTFRHSVVPYEMPEVGADCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSGNLLGRRSFEVRVACPGDRRNEEDNFRKKG 286 |
| | ENSLAFTP00000006292[<i>Loxodonta_africana</i>] | 111 | HSGTAKSVTCTYSPDLNKLFCQLAKTCPVQLWASPPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLEDSTS..TLEDSGNLLGRNSFEVRVACPGDRRNEEDNFRKKG 290 |
| Birds | ENSBBEP00000018891[<i>Bison_bison_bison</i>] | 107 | HSGTAKSVTCTYSPSLNKLFCQLAKTCPVQLWDSPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLEDNTFRHSVVPYEPSEIDSECTTLYHYNMCMNSSCMGMNRRPILTTITLEDCSCGNLLGRNSFEVRVACPGDRRTEEEFNLRKKG 286 |
| | ENSNVPI00000005141[<i>Neovison_vison</i>] | 100 | HSGTAKSVTCTYSPSLNKLFCQLAKTCPVQLWSSPPPADTVCRAMA..SDGLAPPOQHLIRVEGNLRAKYLDDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSGNVNLGRSSFEVRVACPGDRRTEEEFNFRKKG 279 |
| | ENSCCDRP0000013165[<i>Camelus_dromedarius</i>] | 102 | HSGTAKSVTCTYSPPLNKLFCQLAKTCPVQLWSSLPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDASGNLLGRNSFEVRVACPGDRRTEEEFNFRKKG 281 |
| | ENSCFAP00000045749[<i>Felis_catus</i>] | 210 | QSGTAKSVTCTYSPPLNKLFCQLAKTCPVQLWRSPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLHAKYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDASGNLLGRNSFEVRVACPGDRRTEEEFNFRKKG 389 |
| | ENSBTAP00000001420[<i>Bos_taurus</i>] | 107 | QSGTAKSVTCTYSPSLNKLFCQLAKTCPVQLWDSPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPSEIDSECTTLYHYNMCMNSSCMGMNRRPILTTITLEDCSCGNLLGRNSFEVRVACPGDRRTEEEFNLRKKG 286 |
| | ENSCAFP00000064590[<i>Canis_lupus_familiaris</i>] | 166 | HSGTAKSVWTYSPPLNKLFCQLAKTCPVQLWSSPPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSGNVNLGRNSFEVRVACPGDRRTEEEFNFRKKG 345 |
| | ENSTTRP00000009721[<i>Turtiosus_truncatus</i>] | 108 | HSGTAKSVTCTYSPPLNKLFCQLAKTCPVQLVSSPPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSGNVNLGRNSFEVRVACPGDRRTEEEFNFRKKG 287 |
| | ENSESEAP000005023399[<i>Equus_asinus_asinus</i>] | 102 | NGSTAKSVTCTYSPTLNKLFCQLAKTCPVQLVSSPPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSGNVNLGRNSFEVRVACPGDRRTEEEFNFRKK 281 |
| | ENSSCHIP0000030141[<i>Capra_hircus</i>] | 103 | HSGTAKSVTCTYSPSLNKLFCQLAKTCPVQLWSSPPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSGNVNLGRNSFEVRVACPGDRRTEEEFNFRKKG 282 |
| | ENSSSCP00000050664[<i>Sus_scrofa</i>] | 163 | HSGTAKSVTCTYSPALNKLFCQLAKTCPVQLWSSPPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDASGNLLGRNSFEVRVACPGDRRTEEEFNFLKKG 342 |
| Mammals | ENSMMP000000104299[<i>Mus_musculus</i>] | 114 | HSGTAKSVTCTYSPSLNKLFCQLAKTCPVQLWSTPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSGNVNLGRNSFEVRVACPGDRRTEEEFNFRKG 292 |
| | ENSRNOP00000074031[<i>Rattus_norvegicus</i>] | 113 | HSGTAKSVTCTYSPSLNKLFCQLAKTCPVQLWSTPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSGNVNLGRNSFEVRVACPGDRRTEEEFNFRKG 291 |
| | ENSMAPU00000025852[<i>Mesocricetus_auratus</i>] | 117 | HSGTAKSVTCTYSPSLNKLFCQLAKTCPVQLWSTPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNGMAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDPSGNLLGRNSFEVRVACPGDRRTEEEFNFRKG 295 |
| | ENSPTRP00000014836[<i>Pan_trichotes</i>] | 115 | HSGTAKSVTCTYSPDLNKFQQLAKTCPVQLWSTPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNSQHMTEVVRCPHHERCSD..TLEDSGNLLGRNSFEVRVACPGDRRTEEEFNLRKKG 293 |
| | ENSGGOP00000030954[<i>Gorilla_gorilla_gorilla</i>] | 76 | HSGTAKSVTCTYSPTLNKFQQLAKTCPVQLWDSTPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSGNVNLGRNSFEVRVACPGDRRTEEEFNLRKKG 254 |
| | ENSMMPU00000011334[<i>Macaca_mulatta</i>] | 155 | HSGTAKSVTCTYSPDLNMFQQLAKTCPVQLWDSTPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSGNVNLGRNSFEVRVACPGDRRTEEEFNFRKKG 333 |
| | ENSPAP00000010404[<i>Pan_paniscus</i>] | 115 | HSGTAKSVTCTYSPALNMFQQLAKTCPVQLWDSTPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSGNVNLGRNSFEVRVACPGDRRTEEEFNFRKK 293 |
| | ENSBOP00000008181[<i>Saimiri_boliviensis_boliviensis</i>] | 65 | HSGTAKSVTCTYSPALNMFQQLAKTCPVQLWDSTPPPGTRVTRAMA..SDGLAPPOQHLIRVEGNLRAEYLDRTNTFRHSVVPYEPPEVGSDCTTLYHYNMCMNSSCMGMNRRPILTTITLEDSSGNVNLGRNSFEVRVACPGDRRTEEEFNFRKK 25 |

A**C****B****Figure S3.**

A



B

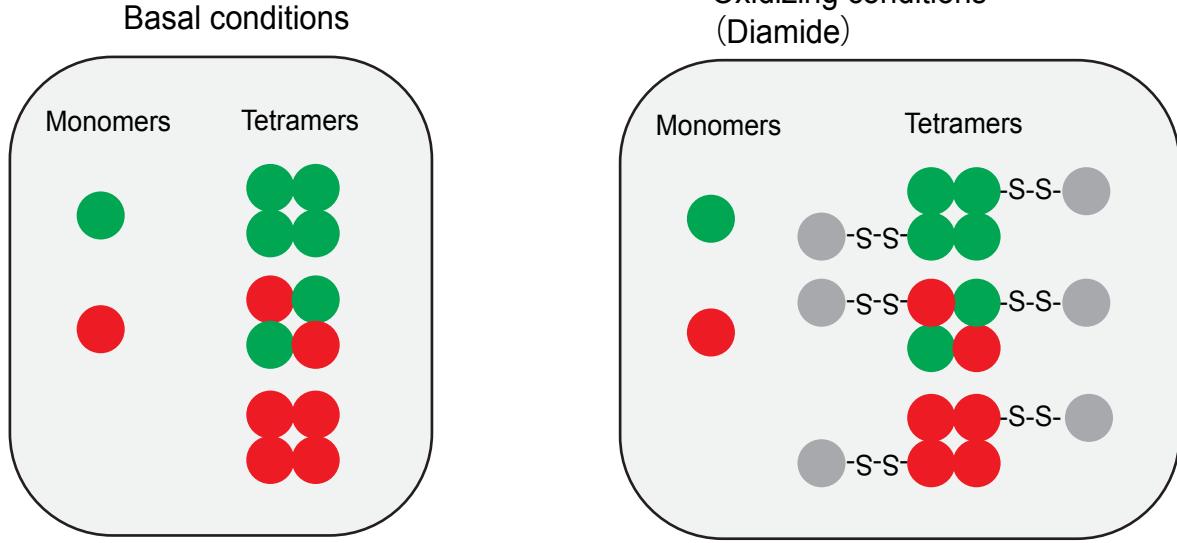
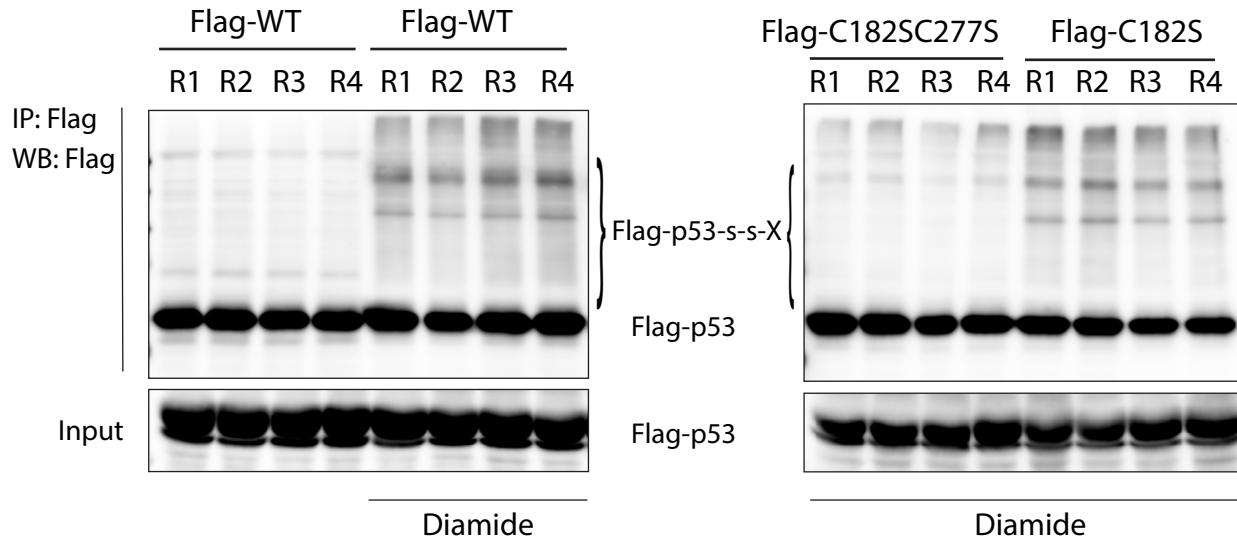
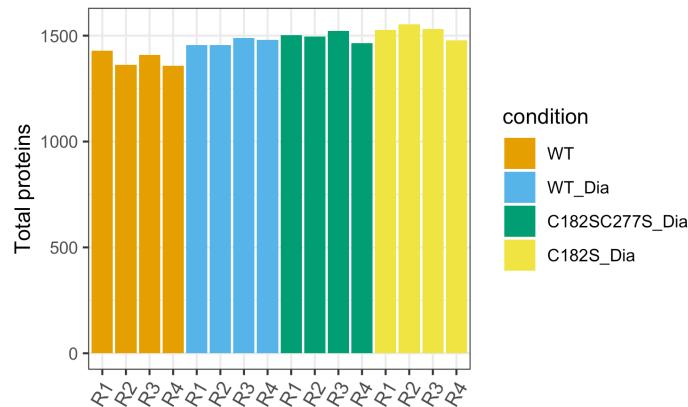


Figure S4.

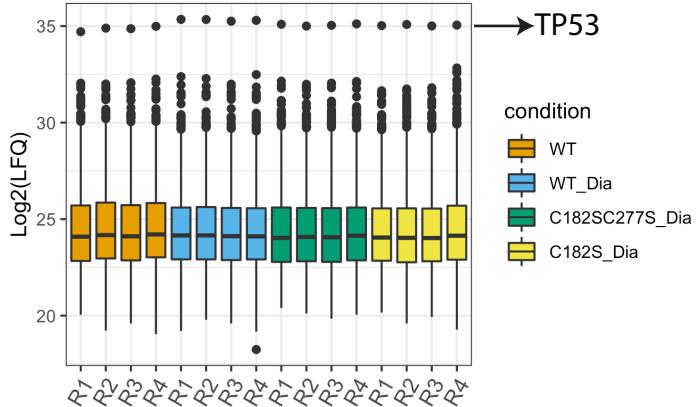
A



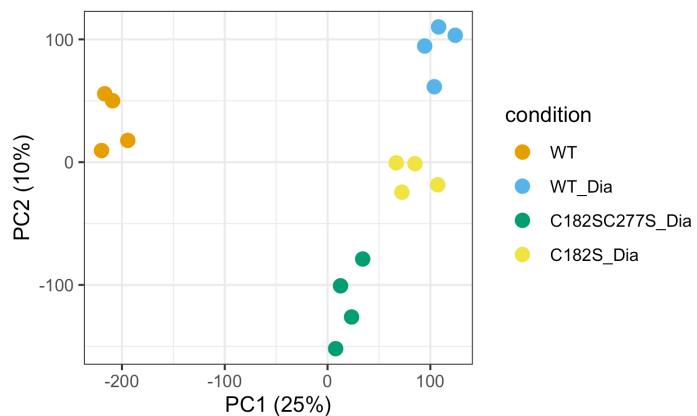
B



C

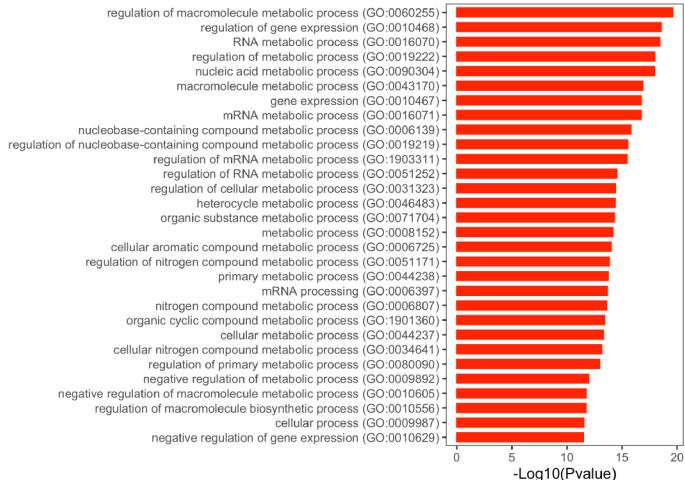


D

**Figure S5.**

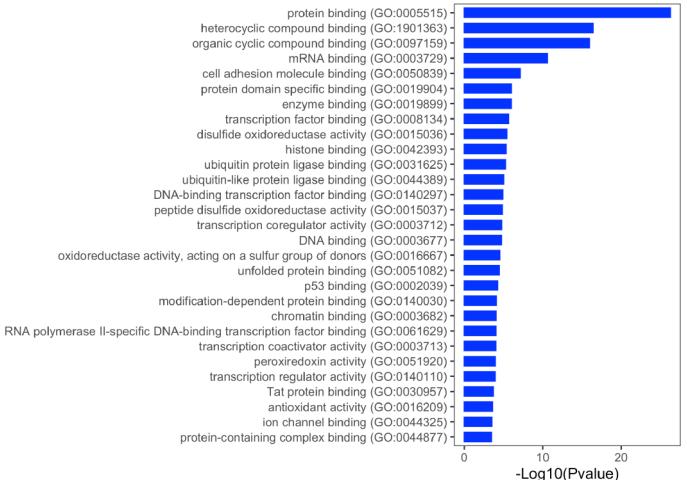
A

GO biological process



B

GO molecular function



C

GO cellular component

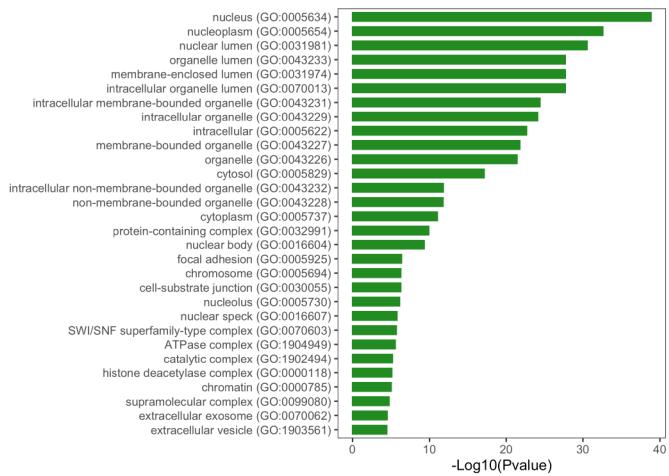


Figure S6.

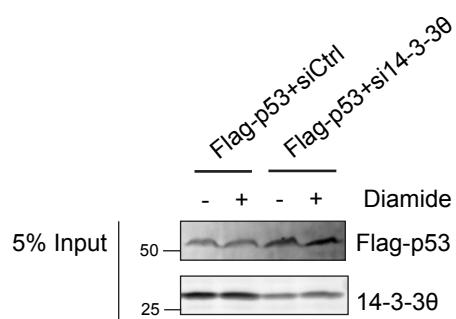
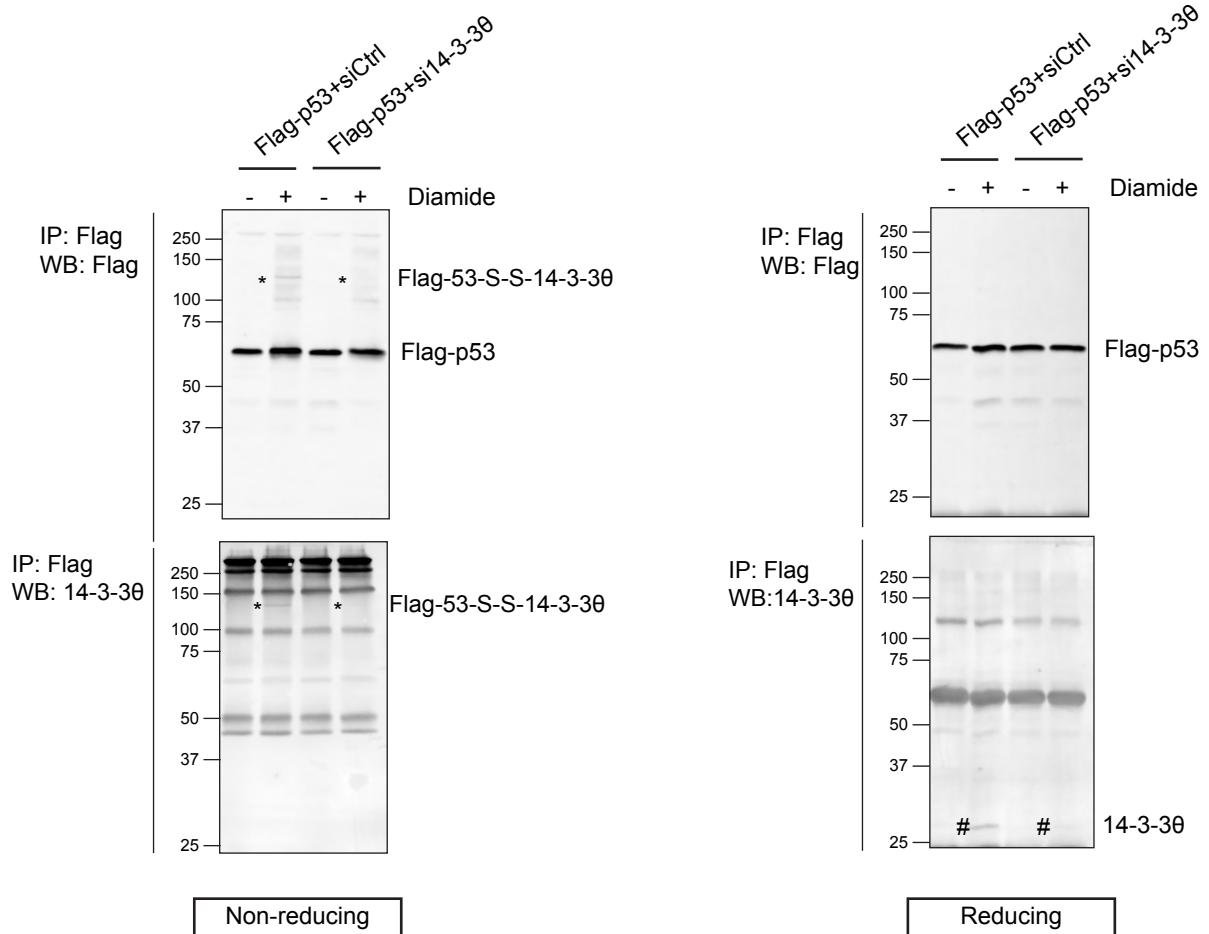


Figure S7.

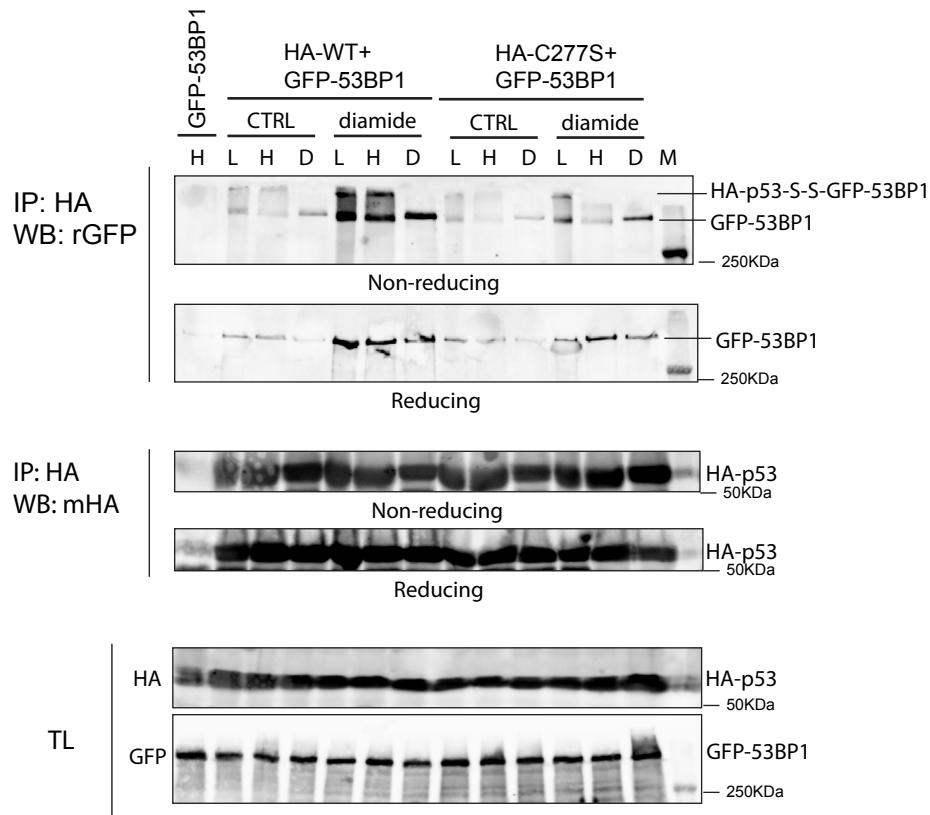


Figure S8.

Table S1. Primers for mutagenesis PCR.

| Mutant | Primers(5' to 3') |
|----------|--|
| p53C124S | Forward: GGGACAGCCAAGTCTGTGACTAGCACGTACTCCCTGCCCTCA Reverse: TGAGGGCAGGGGAGTACGTGCTAGTCACAGACTGGCTGTCCC |
| p53C124A | Forward: GGGACAGCCAAGTCTGTGACTGCCACGTACTCCCTGCCCTCA Reverse: TGAGGGCAGGGGAGTACGTGGCAGTCACAGACTGGCTGTCCC |
| p53C135S | Forward: CCTGCCCTCAACAAGATGTTAGCCAACACTGCCAAGACCTGC Reverse: GCAGGTCTGGCCAGTTGGCTAAACATCTTGTGAGGGCAGG |
| p53C135A | Forward: CCTGCCCTCAACAAGATGTTGCCAACACTGCCAAGACCTGC Reverse: GCAGGTCTGGCCAGTTGGCAACATCTTGTGAGGGCAGG |
| p53C141S | Forward: GCCAACTGGCCAAGACCAGGCCCTGTGCAGCTGTGGGT Reverse: ACCCACAGCTGCACAGGGCTGGCTTGCCAGTTGGCA |
| p53C141A | Forward: TGCCAACCTGGCCAAGACCGCCCTGTGCAGCTGTGGGT Reverse: ACCCACAGCTGCACAGGGGCGGTCTTGGCCAGTTGGCA |
| p53C176S | Forward: ACGGAGGTTGTGAGGCGCAGCCCCCACCATGAGCGCTG Reverse: CAGCGCTCATGGTGGGGCTGCCCTCACAACTCCGT |
| p53C182S | Forward: TGCCCCCACCATGAGCGCAGCTCAGATAGCGATGGTCT Reverse: AGACCATCGCTATCTGAGCTGCCTCATGGTGGGGCA |
| p53C182A | Forward: TGCCCCCACCATGAGCGCGCCTCAGATAGCGATGGTCT Reverse: AGACCATCGCTATCTGAGGCGCGCTCATGGTGGGGCA |
| p53C229S | Forward: CCTGAGGTTGGCTTGACAGTACCAACATCCACTACAAC Reverse: AGTTGTAGTGGATGGTGGTACTGTCAGAGCCAACCTCAGG |
| p53C229A | Forward: CCTGAGGTTGGCTTGACGCTACCAACATCCACTACAAC Reverse: AGTTGTAGTGGATGGTGGTAGCGTCAGAGCCAACCTCAGG |
| p53C275S | Forward: ACAGCTTGAGGTGCGTGTAGTCCTGTCTGGGAGAGAC Reverse: GTCTCTCCAGGACAGGCACAAACAGCACCTCAAAGCTGT |
| p53C275A | Forward: ACAGCTTGAGGTGCGTGTAGTCCTGTCTGGGAGAGAC Reverse: GTCTCTCCAGGACAGGCACAAACAGCACCTCAAAGCTGT |
| p53C277S | Forward: TTGAGGTGCGTGTGGCCAGTCCTGGGAGAGACCGGG Reverse: GCCGGTCTCTCCAGGACTGGCACAAACAGCACCTCAA |
| p53C277A | Forward: TGAGGTGCGTGTGGCCGCTCTGGGAGAGACCGGGCGCA Reverse: TGCGCCGGCTCTCCAGGAGCGGCACAAACAGCACCTCA |

Table S2. Diamide-dependent interactors of p53.

| | Diamide-dependent interactors | |
|----|-------------------------------|--|
| 1 | TLK2 | The interaction with the proteins indicated in Red is significantly enriched in all three comparisons p53 WT vs p53 WT +Diamide, p53 WT +Diamide vs p53 C182SC277S +Diamide and p53 C182S + Diamide vs p53 C182SC277S +Diamide, but not in the comparison p53 WT +Diamide vs p53 C182S +Diamide. The binding of these proteins therefore likely depends on p53 C277. |
| 2 | ARNT | |
| 3 | C18orf25 | |
| 4 | PPP1R2;PPP1R2P3 | |
| 5 | UBXN7 | |
| 6 | RANBP3 | |
| 7 | COPS2 | |
| 8 | DCTPP1 | |
| 9 | TP53BP1 | |
| 10 | HDGFRP2 | |
| 11 | PCYT1A | |
| 12 | YWHAE | |
| 13 | SAFB | |
| 14 | MDM2;MDM2 isoform KB9;mdm2 | |
| 15 | SLC4A1AP | |
| 16 | YWHAZ | |
| 17 | PSME3 | |
| 18 | DFFA | |
| 19 | YWHAQ | |
| 20 | RPRD1A | |
| 21 | ZNF428 | |
| 22 | TTC1 | |
| 23 | CNN2 | |
| 24 | ARID3B | |
| 25 | PUS7 | |
| 26 | CTDP1 | |
| 27 | NCL | |
| 28 | RAD18 | |
| 29 | SRCAP | |
| 30 | GLMN | |
| 31 | SMAP;C11orf58 | |
| 32 | ELL | |
| 33 | RBM17 | |
| 34 | NCOR1 | |
| 35 | RCC2 | |
| 36 | TRIM33 | |
| 37 | FTO | |
| 38 | BCLAF1 | |
| 39 | TUBA1A;TUBA3E | |
| 40 | PPAN-P2RY11;PPAN | |
| 41 | GSTP1 | |
| 42 | PSMD9 | |
| 43 | CPSF7 | |
| 44 | RNF126 | |
| 45 | GRB2 | |
| 46 | NR2C2AP | |
| 47 | SMARCC1 | |
| 48 | POGK | |
| 49 | CARHSP1 | |
| 50 | PTBP1 | |
| 51 | CSTF2 | |

| | |
|-----|----------------------------|
| 52 | CDKN2A |
| 53 | RNGTT |
| 54 | TOX4 |
| 55 | JUN |
| 56 | YWHAB |
| 57 | ZFP36L2;ZFP36L1 |
| 58 | SUPT4H1 |
| 59 | BPTF |
| 60 | SRSF5 |
| 61 | SAFB2 |
| 62 | CTTN |
| 63 | ZXDC;ZXDA;ZXDB |
| 64 | CIAPIN1 |
| 65 | DUS1L |
| 66 | DNAJC9 |
| 67 | PHAX |
| 68 | UBTF |
| 69 | CD2BP2 |
| 70 | HMGB1;HMGB1P1 |
| 71 | MNAT1 |
| 72 | FOXK2 |
| 73 | RING1 |
| 74 | SRSF6 |
| 75 | EP400 |
| 76 | GINS3 |
| 77 | XPO5 |
| 78 | NELFA |
| 79 | PDCD11 |
| 80 | VBP1 |
| 81 | NME1;NME2;NME1-NME2;NME2P1 |
| 82 | EIF4B |
| 83 | ZPR1 |
| 84 | CHERP |
| 85 | GLRX3 |
| 86 | TXN |
| 87 | PRDX2 |
| 88 | YWHAG |
| 89 | CNBP |
| 90 | CNN3 |
| 91 | SERBP1 |
| 92 | SH3GL1 |
| 93 | POLR1E |
| 94 | CACYBP |
| 95 | AASDHPPPT |
| 96 | PHF8 |
| 97 | IMPDH2 |
| 98 | TRIM24 |
| 99 | PRDX6 |
| 100 | TXLNA |
| 101 | FOXK1 |
| 102 | ANP32E |
| 103 | PNN |

| | |
|-----|----------------------|
| 104 | BOLA2 |
| 105 | ACIN1 |
| 106 | CHD8 |
| 107 | PDLIM1 |
| 108 | TXNL1 |
| 109 | FLNB |
| 110 | TTC4 |
| 111 | SRSF3 |
| 112 | PFDN2 |
| 113 | NUDC |
| 114 | SNW1 |
| 115 | YARS |
| 116 | KHDRBS1 |
| 117 | TCOF1 |
| 118 | ARMC6 |
| 119 | POGZ |
| 120 | USP48 |
| 121 | NUCD2 |
| 122 | HIRIP3 |
| 123 | MAP4 |
| 124 | EIF5A;EIF5A2;EIF5AL1 |
| 125 | MBD3 |
| 126 | RBM25 |
| 127 | IRF2BPL |
| 128 | TPM3;DKFZp686J1372 |
| 129 | PPP5C |
| 130 | KIF4A |
| 131 | NASP |
| 132 | UBXN1 |
| 133 | MCMBP |
| 134 | CHD1 |
| 135 | UBA1 |
| 136 | CBX3 |
| 137 | RPS12 |
| 138 | DNAJA1 |
| 139 | TCEA1 |
| 140 | HCFC1 |
| 141 | AIP |
| 142 | TMPO |
| 143 | STIP1 |
| 144 | U2SURP |
| 145 | ZRANB2 |
| 146 | USP5 |
| 147 | RBM26 |
| 148 | IRF2BP1 |
| 149 | HDLBP |
| 150 | NPM1 |

| | |
|-----|---------|
| 151 | FLNA |
| 152 | PRDX1 |
| 153 | DNAJC7 |
| 154 | HNRNPA3 |
| 155 | TLN1 |
| 156 | RNH1 |
| 157 | FHL1 |
| 158 | KHSRP |
| 159 | SRRM2 |
| 160 | HSPH1 |
| 161 | ZC3H14 |
| 162 | CCT2 |