

(A) Validation of a subset of ARHGEF2 interactors. Tetracycline-inducible Flag-tagged PP6 regulatory subunits were immunoprecipitated with an antibody specific for Flag covalently attached to agarose beads and protein complexes were probed for the presence of endogenous ARHGEF2. Specific antibodies for ARHGEF2 and Flag were used to detect protein abundance in cell lysates. α -tubulin was used as a loading control. Representative of two independent experiments. (B) Gene ontology (GO) enrichment analysis of the ARHGEF2 interaction network (http://www.geneontology.org/), based on biological process. See Table S2 for more details.



| | 10 | 20 | 30 | 40 | 50 |
|------------------------------------|--------------------------|----------------------------------|---|----------------------------------|----------------------------------|
| Q92974 Homo sap | | | | <mark>msri</mark> -e | SLTRARIDRS |
| Q60875 Mus musc | | | | <mark>msri</mark> -e | SLTRARIDRS |
| Q865S3 Canis lu | | | | <mark>msri</mark> -e | SLTRARTERS |
| Q5FVC2 Rattus n | | | | <mark>msri</mark> -e | SLTRARIDRS |
| F1N1F7 Bos taur | | | | <mark>msri</mark> -e | SLTRARIDRS |
| Q6AX47 Xenopus | | | | <mark>MSWK</mark> -G | RRPSERVPAG |
| Q6NY14 Xenopus | - | | | <mark>MSRI-E</mark> | SFGKAPAERT |
| H3AF10 Latimeri | | RSFSSLFLSL | RREEKKYSKE | RGEKMSRV-E | VLAKARNERI |
| B2DCZ9 Sus scro | | | | | |
| W5UTH7 Ictaluru | | | | <mark>msrv</mark> - t | DVSKVRQERM |
| E7F4U1 Danio re | | | | <mark>MSRAS</mark> E | PLPKARQERM |
| K7FEF2 Pelodisc | | | | | |
| Consistency | 0000000000 | 0000000000 | 0000000000 | 0 0 0 0 <mark>6 6 5 4</mark> 0 4 | 3 4 3 4 <mark>5 5 2 4 5</mark> 3 |
| | | | | | |
| | 60 | 70 | 80 | 90 | 100 |
| Q92974 Homo sap | | EKMKE | -AKDARYTNG | HLFTTISVSG | MTMCYACNKS |
| Q60875 Mus musc | | ЕКМКЕ | -AKDARYING | HLFTTISVSG | MTMCYACNKS |
| Q865S3 Canis lu | | EKMKE | - AKDARYING | HLFTTISVSG | MTMCYACNKS |
| Q5FVC2 Rattus n | | EKMKE | -AKDARYTNG | HLFTTISVSG | MTMCYACNKS |
| F1N1F7 Bos taur | | EKMKE | - AKDARYING | HLFTTISVSG | MIMCIACNES |
| Q6AX47 Xenopus | | MKDSK | - EKDPRYING | HLFTTITVSG | TTMCFVCNKS |
| Q6NY14 Xenopus | | MKD | - SKDPRYING | HLFTTITVSG | TIMCFVCNKS |
| H3AF10 Latimeri | | EKMKEREKEA | KEKEARYING | HLFNSITVSG | LTQCYACNKS |
| B2DCZ9 Sus scro | | MKE | - AKDARYING | HLFTTISVSG | MTMCYACNKS |
| W5UTH7 Ictaluru | | ERMRERERA | REREARYTNG | HLFTSLTVSG | TTLCSACNKS |
| E7F4U1 Danio re | | ERMKAREKET | KERESRYSNG | HLFTSLSVSA | TTLCSACNKS |
| K7FEF2 Pelodisc | | | | | |
| Consistency | , 5 5 2 2 2 3 3 5 6 6 | 4 6 5 5 5 0 0 0 0 0 | 0 5 7 6 6 8 8 7 8 8 | 8887676887 | 5868 <mark>56</mark> 8888 |
| Consistency | <u>55222555</u> 00 | 4 033300000 | | 000/0/000/ | 3000300000 |
| | 110 | 120 | 130 | 14(|) 150 |
| Q92974 Homo sap | | TCNVTIHNRC | KDTLANCTKV | KOKOOKAALL | KNNTA-LQSV |
| Q60875 Mus musc | | TCNVTIHNRC | KDTLANCTKV | KOKOOKAALL | RNNTA-LOSV |
| Q865S3 Canis lu | | TCNVTIHNRC | KDTLANCTKV | KOKOOKAALL | KNSTA-LQSV |
| Q5FVC2 Rattus n | | TCNVTIHNRC | KDTLANCTKV | KQKQQKAALL | RNNTA-LQSV |
| F1N1F7 Bos taur | | TCNVTIHNRC | KDTLANCTKV | KQKQQKAALL | KNNTA-LQSV |
| Q6AX47 Xenopus | | TCSVSIHNRC | KDALPSCTKV | KQKQQKTAFL | KNNSA-LQNV |
| Q6NY14 Xenopus | | TCNVTIHNRC | KDALPSCTKV | KQKQQKAAFL | KNNSA-LQNV |
| H3AF10 Latimeri | | TCNVTIHNRC | KDTLANCTKV | KQKQQKAALV | KNSSA-LQTV |
| B2DCZ9 Sus scro | | TCNVTIHNRC | KDTLANCTKV | KOKOOKAALL | KNNTA-LQSV |
| W5UTH7 Ictaluru | | TCNVTIHNRC | RDSLPNCAKM | KQRQQKLALM | RNSSSYSGTV |
| E7F4U1 Danio re | | TCNVTIHNRC | RDTLANCAKM | KQKQQRLALG | RNTAT - LHNV |
| K7FEF2 Pelodisc | | ACNVTIHNRC | KDTLPNCTKV | KOKOOKAALL | KNNSA-LQSV |
| _ | 888888868* | 9*9*9***** | 8 * 7 * 6 8 * 8 * 8 | **9**97*87 | 8 * 7 7 8 <mark>0</mark> 8 7 7 * |
| Consistency | 000000000 | 9 9 9 9 9 8 8 8 8 8 | 0 1 1 0 0 0 0 0 0 | ********* | 0 * / / 0 <mark>0</mark> 0 / / * |
| | 160 | 170 | | 19(|) |
| Q92974 Homo sap | | ERPSSAIYPS | DSFRQSLLGS | RRGRSSLSLA | KSVSTTNIAG |
| Q60875 Mus musc | | ERPTSALYPS | DSFROSLLGS | RRGLSSLSLA | KSVSTINIAG |
| Q865S3 Canis lu | | ERPSSAIYPS | DSFROSLLGS | RRGRSSLSLA | KSVSTINIAG |
| Q5FVC2 Rattus n | | ERPTSAIYPS | DSFRQSLLGS | RRGLSSLSLA | KSVSTINIAG |
| F1N1F7 Bos taur | | ERPSSAIYPS | DI-ROSLLGS | RRGRSSLSLA | KSVSTINIAG |
| Q6AX47 Xenopus | | ERPNSAIYPS | DI-ROSLLGS DSLRHSILGS | RRGRSSLSLA | KSVSTINIAG |
| Q6NY14 Xenopus | | ERPNSAIYPS | DSLRHSILGS | RRGRASLSLS | KSVSTINIAG |
| H3AF10 Latimeri | | DRPSSAVYLS | DSERHSTEGS DSFRQSLLGS | RRGRASISIS | KSVSTINIAG |
| B2DCZ9 Sus scro | | ERPSSAVILS | DSFRQSLLGS DSFRQSLLGS | RRGRITITLS | KSVSTINIAG |
| W5UTH7_Ictaluru | | ERPSSALIPS | DSFRQSLLGS DS <mark>L</mark> RQSLLGS | RERSPISIA | |
| E7F4U1 Danio re | | | ETLRQSFLGS | | KSVSTNNIAG |
| K7FEF2_Pelodisc | | ERPSSAIYPS ERPNSAIYPS | | RKGRSSLSLN RRGRPTLSLS | KSVSTNNIAG |
| | 8 * * 7 * 0 8 6 5 8 | | ESFRQTLLGS | | KSVSTTNIAG |
| Consistency | 8 6 7 7 7 8 6 5 8 | 9 * * <mark>7</mark> * * 9 * 8 * | 88 <mark>5</mark> *898*** | * 9 * <mark>7 7 7 9 8 * 7</mark> | * * * * * 8 * * * * |
| | 210 | 220 | 220 | |) 250 |
| 000074 | | | | | |
| Q92974_Homo_sap | | RRILSQSTDS | LNMRNRTLSV | ESLIDEA-EV | IYSELMSDFE |
| Q60875_Mus_musc | | RQILSQSTDS | LNMRNRTLSV LNMRNRALSV | ESLIDEG <mark>V</mark> EV | FYNELMSDFE |
| | | | NMDNDATCV | ESLIDEGAEV | I <mark>Y</mark> SELMSDFE |
| Q865S3_Canis_lu | | RRILSQSTDS | | | |
| Q865S3_Canis_lu Q5FVC2_Rattus_n | | RQILSQSTDS | LNMRNRTLSV | ESLIDEG <mark>V</mark> EV | FYNELMSDFE |

| F1N1F7_Bos_taur | HFNDESPLGL | RRILSQSTDS | L N M R N R T L S V | E S L I D E G <mark>A</mark> E V | IYNELMSDFE |
|--|---|--|--|--|--|
| Q6AX47_Xenopus_ | | RRILSQSTDS | LNMRNRTLSV | ESLIDEG <mark>A</mark> EV | V Y SQLMSDFA |
| Q6NY14_Xenopus_ | | RRILSQSTDS | L N M R N R T L S V | ESLIDEG <mark>A</mark> EV | VYSQLMSDFA |
| H3AF10_Latimeri | | RRILSQSTDS | LN I KNRTLSV | ESLIDEG <mark>A</mark> EV | I Y NQLMGNLE |
| B2DCZ9_Sus_scro | HFNDESPLGL | RRILSQSTDS | LN <mark>MRNRTLSV</mark> | E S L I D E G <mark>A</mark> E V | I Y NELMSDFE |
| W5UTH7_Ictaluru | TLSDDSPLGL | RRILSQSTDS | LN FRSRTMSM | ESLNDEG-EV | Y Y A S V L E E L E |
| E7F4U1_Danio_re | | RRILSHSTDS | LN FRNRAMSM | ESLNDEG – ET | Y Y T S MME E L E |
| K7FEF2_Pelodisc | | RRILSQSTDS | LNMRNRTLSV | ESLIDEG <mark>A</mark> EF | VYSQLMSDFE |
| Consistency | <mark>5</mark> 6788 <mark>**</mark> 9*9 | * 8 * * * 8 * * * * | * * <mark>7 9 9 *</mark> 8 9 * 8 | * * * <mark>7 * * 9 </mark> 4 * 8 | 6 * <mark>6 7 8 9</mark> 7 8 7 8 |
| | | | | | |
| | | | | | |
| Q92974_Homo_sap | | WSLAVDSSFL | QQHKKEVMKQ | QDVIYELIQT | ELHHVRTLKI |
| Q60875_Mus_musc | | WSLAVDSSFL | QQHK <mark>KEVMK</mark> K | QDVIYELIQT | ELHHVRTLKI |
| Q865S3_Canis_lu | | WSLAVDSSFL | <mark>Q</mark> QQK <mark>K</mark> EVMKQ | QDVIYELIQT | ELHHVRTLKI |
| Q5FVC2_Rattus_n | | WSLAVDSSFL | QQHKKEVMKK | QDVIYELIQT | ELHHVRTLKI |
| F1N1F7_Bos_taur | | WSLAVDSSFL | QQHKKEVMKQ | QDVIYELIQT | ELHHVRTLKI |
| Q6AX47_Xenopus_ | | WSLAVDNNYL | QQHKKDVMKR | QDVIYELIQT | EVHHVRTLKI |
| Q6NY14_Xenopus_ | | WSLAVDNNYL | QQHKKDVMKR | QDVIYELIQT | EVHHVRTLKI |
| H3AF10_Latimeri | | WSLAVDTNFL | QQHRKDIIKR | QDVIYELIQT | ELHHVRTLKI |
| B2DCZ9_Sus_scro | | WSLAVDSSFL | Q QHKKEVMKQ | QDVIYELIQT | ELHHVRTLKI |
| W5UTH7_Ictaluru | | WSMVVDSAYL | QTHRKDIIKR | QDVIYELIQT | ELHHVRTLRI |
| E7F4U1_Danio_re | | WSLAVDSSYL | QSQRKDVIKR | QDVIYELIQT | EFNHVRTLRI |
| K7FEF2_Pelodisc | | WSLAVDNNYL | QQHKKEVMKR | QDVIYELIQT | ELHHVRTLKI |
| Consistency | <mark>5</mark> 7788 <mark>*</mark> 6*** | **99** <mark>778</mark> * | * 888 <mark>*</mark> 898 * 7 | * * * * * * * * * | * 8 9 * * * * 8 * |
| | 210 | 320 | | 340 | 350 |
| Q92974 Homo sap | | EELHLEPGVV | QGLFPCVDEL | SDIHTRFLSQ | LLERRRQALC |
| Q60875 Mus musc | | EELQMEPEVV | QGLFPCVDEL | SDIHTRFLSQ | LLERRRQALC |
| Q865S3 Canis lu | | EELQLEPGVV | QGLFPCVDEL | SDIHTRFLNQ | LLERRRQALC |
| Q5FVC2 Rattus n | | EELQMEPEVV | QGLFPCVDEL | SDIHTRFLSQ | LLERRRQALC |
| F1N1F7 Bos taur | | EELQLEPGVV | OGLFPCVDEL | TDIHTRFLSO | LLDRRRQALC |
| Q6AX47 Xenopus | | EDLOMDPALV | NKMFPCVDEL | SDIHIRFLIQ | LLERRKDSLA |
| Q6NY14 Xenopus | | EDLQMDPALV | NKMFPCVDEL | SDIHIRFLIO | LLERRKDSLA |
| H3AF10 Latimeri | | EDLSMDQTLV | HSMFPVLDEL | SDLHVRFLSQ | LLERRKDSLV |
| B2DCZ9 Sus scro | | EELQLEPGVV | QGLFPCVDEL | SDIHTRFLSO | LLERRRQALC |
| W5UTH7 Ictaluru | | DEVQLEPGVV | HALFPCLEKL | LVLHTRFLTO | LLNRRLHCLQ |
| E7F4U1 Danio re | | E E VMME MG V V | HAIFPCLDOL | LLIHTNFLSO | LLQRRSNSLA |
| K7FEF2 Pelodisc | | | | | |
| | MTNMFRKGML | EDLQMDPALV | QSMFPCVDEL | SDVHDRFLTQ | LLVGSKESLA |
| — | | EDLQMDPALV 9887887 <mark>5</mark> 8* | QSMFPCVDEL 658**8898* | SDVHDRFLTQ 779*69**6* | LLVGSKESLA **688667*5 |
| Consistency | MTNMFRKGML *757*9 <mark>5</mark> *** | | | | LLVGSKESLA **688667* <mark>5</mark> |
| Consistency | * 7 <mark>5 7 * 9</mark> <mark>5</mark> * * * | | <mark>65</mark> 8**8898* | 779* <mark>6</mark> 9** <mark>6</mark> * | |
| Consistency | * 7 <mark>5 7 * 9 5</mark> * * * | 9 8 8 7 8 8 7 <mark>5</mark> 8 * | <mark>65</mark> 8**8898* | 779* <mark>6</mark> 9** <mark>6</mark> * | * * <mark>6 8 8 6 6 7 * </mark> 5 |
| Consistency | * 7 5 7 * 9 <mark>5</mark> * * * | 9887887 <mark>5</mark> 8* | 658**8898* | 779* <mark>69**6</mark> * | * * <mark>6 8 8 6 6 7 *</mark> 5 |
| Consistency Q92974_Homo_sap | * 7 5 7 * 9 5 * * * | 9887887 <mark>5</mark> 8* | 658**8898* | 779*69**6* | * * 6 8 8 6 6 7 * 5 |
| Consistency Q92974_Homo_sap Q60875_Mus_musc | * 757 * 95 * * * | 988788758*370 RLGDLLI RLGDLLI | 658**8898* | 779*69**6 | * * 688667 * 5 |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu | * 757*95*** | 988788758 * | 658**8898* | 779*69**6 | * * 688667 * 5 400 RHSKALKLYK RHTKALKLYK RHTKALKLYK |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n | * 757*95*** PGSTRNFVIH PGSTRNFVIH PGSTRNFVIH PGSTRNFVIH PGSTRNFVIH | 988788758* | 658**8898* | 7 7 9 * 6 9 * * 6 * | **688667*5 400 RHSKALKLYK RHTKALKLYK RHTKALKLYK RHTKALKLYK |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur | * 757*95*** | 988788758*370 RLGDLLI RLGDLLI RLADLLI RLADLLI RLGDLLI | 658**8898* | 7 7 9 * 6 9 * * 6 * | ** 688667 *5 400 RHSKALKLYK RHTKALKLYK RHTKALKLYK RHTKALKLYK |
| Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ | * 757*95*** | 988788758* | 658**8898* 380 SQFSGPSAEQ SQFSGSNAEQ SQFSGSNAEQ SQFSGPSAEQ NQFSGTNGER | 7 7 9 * 6 9 * * 6 * | **688667*5 400 RHSKALKLYK RHTKALKLYK RHTKALKLYK RHTKALKLYK QHHKAMKLYK |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ | 7 7 7 5 * * * | 988788758* | 6 5 8 * * 8 8 9 8 * | 7 7 9 * 6 9 * * 6 * | * * 688667 * 5 400 RH SKALKLYK RH TKALKLYK RH TKALKLYK RH TKALKLYK QH HKAMKLYK QH HKAMKLYK RH TKALKLYK RH TKALKLYK |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru | A 757*95*** PGSTRNFVIH PGSTRNFVIH PGSTRNFVIH PGSTRNFVIH SDSNKNFVIN SNSNKNFVIN SNSNKNFVIN PGSPRNFVIH PGSPRNFVIH PGSTHNFTIS | 9 8 8 7 8 8 7 5 8 * RLGD L L I RLGD I L I | 6 5 8 * * 8 8 9 8 * | 779*69**6* | ** 688667 *5 400 RH5KALKLYK RHTKALKLYK RHTKALKLYK RHTKALKLYK QHHKAMKLYK QHHKAMKLYK RHTKAVKLYK RHTKALKLYK GHLKAVKLYK |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ H3AF10_Latimeri B2DCZ9_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re | A 757*95*** PGSTRNFVIH PGSTRNFVIH PGSTRNFVIH PGSTRNFVIH SDSNKNFVIN SNSNKNFVIN SNSNKNFVIN PGSPRNFVIH PDSTHNFTIS SNSNRNFTIQ | 9 8 8 7 8 8 7 5 8 * | 6 5 8 * * 8 8 9 8 * | 7 7 9 * 6 9 * * 6 * | ** 688667 *5 |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6AX14_Xenopus_ H3AF10_Latimeri B2DCZ9_Sus_scro W5UTH7_Ictaluru E7F401_Danio_re K7FEF2_Pelodisc | A 757*95*** | 9 8 8 7 8 8 7 5 8 * | 658**8898* 380 SQFSG PSAEQ SQFSG PSAEQ SQFSG PSAEQ SQFSG PSAEQ NQFSG TNAER NQFSG TNAER TQFSG TNAER TQFSG TNAEQ QQFSG QCADE QQFSG QCADE AQFSG HSAEQ | 7 7 9 * 6 9 * * 6 * 390 M C K T Y S E F C S M R K T Y S E F C S M R K T Y S E F C S M R K T Y S E F C S L K K T Y T E F A S M K K A Y T E F A S M K K A Y T E F A S M K K T Y A E F C S M K K T Y A E F C S M K K A Y A E F C S | ** 688667 *5 |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6AX14_Xenopus_ H3AF10_Latimeri B2DCZ9_Sus_scro W5UTH7_Ictaluru E7F401_Danio_re K7FEF2_Pelodisc | A 757*95*** PGSTRNFVIH PGSTRNFVIH PGSTRNFVIH PGSTRNFVIH SDSNKNFVIN SNSNKNFVIN SNSNKNFVIN PGSPRNFVIH PDSTHNFTIS SNSNRNFTIQ | 9 8 8 7 8 8 7 5 8 * | 6 5 8 * * 8 8 9 8 * | 7 7 9 * 6 9 * * 6 * | ** 688667 *5 |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6AX14_Xenopus_ H3AF10_Latimeri B2DCZ9_Sus_scro W5UTH7_Ictaluru E7F401_Danio_re K7FEF2_Pelodisc | 2 7 5 7 * 9 5 * * * PGSTRNFVIH PGSTRNFVIH PGSTRNFVIH SDSNKNFVIN SDSNKNFVIN SDSNKNFVIN SDSNKNFVIN SDSNKNFVIN PGSPRNFVIH PDSTHNFTIS SNSNRNFIQ NGRNKNIVRH 56867 * 9886 | 9 8 8 7 8 8 7 5 8 * | 6 5 8 * * 8 8 9 8 * | 7 7 9 * 6 9 * * 6 * 390 M C K T Y S E F C S M R K T Y S E F C S M R K T Y S E F C S M R K T Y S E F C S I K K A Y K E F C S M K K A Y K E F C S M K K A Y K E F C S M K K C Y V E F C S M K K C Y V E F C S M K K C Y V E F C S M K K A Y A E F C S M K K A Y A E F C S M K K A Y A E F C S M K K A Y A E F C S M K K A Y A E F C S | * * 688667 * 5 400 RH SKALKLYK RHTKALKLYK RHTKALKLYK RHTKALKLYK QHHKAMKLYK QHHKAMKLYK QHHKAMKLYK GHLKAVKLYK RHTKALKLYK RHTKAVKLYK RHTKAVKLYK RHTKAVKLYK RHTKAVKLYK |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6NY14_Xenopus_ Q6NY14_Xenopus_ H3AF10_Latimeri B2DCZ9_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency | 7 5 7 * 9 5 * * * 9 G 5 TRN FVIH 9 G 5 TRN FVIN 5 N S NKN FVIN 9 G 5 PRN FVIH 9 D 5 THN FTIS 5 N S N RN FTIQ N G RNKN IVRH 5 6 8 6 7 * 9 8 8 6 | 9 8 8 7 8 8 7 5 8 * | 6 5 8 * * 8 8 9 8 * | 7 7 9 * 6 9 * * 6 * M C K T Y S E F C S M R K T Y S E F C S M R K T Y S E F C S M R K T Y S E F C S M K K T Y T E F A S M K K A Y K E F C S M K K A Y K E F C S M K K A Y K E F C S M K K A Y A E F C S M K K A Y K E F C S M K K K Y K E F C S M K K K Y K E F C S K K K K K | ** 688667 * 5 |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ U6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap | 7 7 7 9 5 * * * * * * * * * * * * * * * * * * * | 9 8 8 7 8 8 7 5 8 * | 6 5 8 * * 8 8 9 8 * | 7 7 9 * 6 9 * * 6 * | ** 688667 * 5 |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ U6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc | x 7 5 7 * 9 5 * * * PG S TRN FVIH S D S NKN FVIN PG S TRN FVIH S D S NKN FVIN PG S PRN FVIH PG S RNN FVIN PG S RN FVIN PG R R FVIN PG R R FVIN PG R R FVIN PG R R FVIN PG R | 9 8 8 7 8 8 7 5 8 4 | 658**8898* | 7 7 9 * 6 9 * * 6 * | ** 688667 * 5 |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ U6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu | X 7 5 7 * 9 5 * * * | 9 8 8 7 8 8 7 5 8 4 | 6 5 8 * * 8 8 9 8 * | 7 7 9 * 6 9 * * 6 * | ** 688667 * 5 |
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| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6NX14_Xenopus_ Q6NY14_Xenopus_ U3PF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru | A 7 5 7 * 9 5 * * * PG STRNFVIH PG STRNFVIH PG STRNFVIH PG STRNFVIH PG STRNFVIH SD SNKNFVIN SD SNKNFVIN SD SNKNFVIN SD SNKNFVIN PG S PRNFVIH PG S PRNFVIH PG S PRNFVIH PJ STHNFTIS SN SNKNFVIN SN SNKNFVIN PG S PRNFVIH PJ STHNFTIS SN SNKNFVIN SN SN SN SN SN SN SNKNFVIN SN SN SN SN SN SN SN SN SN S | 9 8 8 7 8 8 7 5 8 7 R L G D L L I R L G D I L I R L G D I L I R L G D I L V R L G D I L V | 6 5 8 * * 8 8 9 8 * 5 0 F 5 0 P 5 A E Q 5 0 F 5 0 P 5 A E Q 5 0 F 5 0 P 5 A E Q 5 0 F 5 0 P 5 A E Q 5 0 F 5 0 P 5 A E Q 5 0 F 5 0 P 5 A E Q N 0 F 5 0 T N A E R N 0 F 5 0 T N A E R 1 0 F 5 0 T N A E Q 1 0 F 5 0 P 5 A D Q 2 0 F 5 0 | 7 7 9 * 6 9 * * 6 * MCK TY SEFCS MRK TY SEFCS MRK TY SEFCS MRK TY SEFCS MRK TY SEFCS MKK TY SE | ** 688667 *5 |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6NY14_Xenopus_ Q6NY14_Xenopus_ U3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F401_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ U35FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F401_Danio_re | x 7 5 7 * 9 5 * * * | 9 8 8 7 8 8 7 5 8 7 R L G D L L I R L G D I L I | 6 5 8 * * 8 8 9 8 * S 0 F S 0 F S A E Q S 0 F S 0 F S A E Q S 0 F S 0 F S A E Q S 0 F S 0 F S A E Q S 0 F S 0 F S A E Q S 0 F S 0 F S A E Q N 0 F S 0 T N A E Q T 0 F S 0 | 779*69**6* MCKTYSEFCS MRKTYSEFCS MRKTYSEFCS MRKTYSEFCS MKKTYSEFCS MKKTYSEFCS MKKTYEFAS MKKAYKEFCS MKKTYAEFCS MKKTYAEFCS MKKTYAEFCS MKKTYAEFCS MKKTYAEFCS MKKTYAEFCS MKKTYAEFCS MKKTYAEFCS MKKTYAEFCS MKKTYEFAS MKKTYEFAS MKKTYEFAS MKKTYEFAS MKKTYEFAS MKKTYEFAS MKKTYEFAS MKKTYEFAS MKKTYEFAS MKKTYEFCS MKTYEFCS MKKTYE | ** 688667 *5 |
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| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ U3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency | A 7 5 7 * 9 5 * * * PG S TRN FVIH PG S TRN FVIH S D S NKN FVIN S D S NKN FVIN PG S PRN FVIH PD S THN FTIS S N S NKN FVIN S S S R S S S S S S S S S S S S S S S S | 9 8 8 7 8 8 7 5 8 4 | 6 5 8 * * 8 8 9 8 * | 7 7 9 * 6 9 * * 6 * MCK TY SEFCS MRK TY SEFCS MRK TY SEFCS MRK TY SEFCS MKK TY SE | ** 688667 * 5 |
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| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6NY14_Xenopus_ Q6NY14_Xenopus_ Q6NY14_Xenopus_ B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap | A 7 5 7 * 9 5 * * * P 6 S TRN FVIH P 6 S P 8 FVIH P 6 S P 7 | 9 8 8 7 8 8 7 5 8 7 R L G D L L I R L G L I R L G L I G I R K T R S A Q F I R K T R S A R K R R R S A S A S A S A S A S A S A S A S | 6 5 8 * * 8 8 9 8 * S 0 F S 0 F S A E Q S 0 F S 0 F S A E Q S 0 F S 0 F S A E Q S 0 F S 0 F S A E Q S 0 F S 0 F S A E Q S 0 F S 0 F S A E Q N 0 F S 0 T N A E Q T 0 F S 0 | 7 7 9 * 6 9 * * 6 * M C K T Y S E F C S M R K T Y S E F C S M R K T Y S E F C S M R K T Y S E F C S M R K T Y S E F C S M K K T Y S E F C S M K K T Y S E F C S M K K Y K K K Y K E F C S M K K Y K K K Y K E F C S M K K Y K K K Y K K K K K K K K K K K K | ** 688667*5 |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ U3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FE72_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q6AX47_Xenopus_ Q6AX47_Xenopus_ Q6AX47_Xenopus_ U3FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ U3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc | x 7 5 7 * 9 5 * * * | 9 8 8 7 8 8 7 5 8 4 | 6 5 8 * * 8 8 9 8 * | 7 7 9 * 6 9 * * 6 * MCK TY SEFCS MRK TY SEFCS MRK TY SEFCS MRK TY SEFCS MKK TY SEFCS MK TY SE | ** 688667 *5 |

| F1N1F7_Bos_taur | h s h g <mark>m</mark> e e e <mark>r</mark> Q | D <mark>ltkal</mark> glvk | ELLSNVDQDV | HELEK <mark>GA</mark> RLQ | EIYN <mark>RMD</mark> PRA |
|--|---|---|--|---|--|
| Q6AX47 Xenopus | N S K G <mark>D</mark> E E E <mark>H Q</mark> | D <mark>L A E</mark> S <mark>L R</mark> L L K | DLISTIDQDV | HNLEK <mark>NL</mark> RLQ | EIY <mark>Q</mark> RIDSKS |
| Q6NY14 Xenopus | N S K G <mark>D</mark> E E E <mark>Y</mark> L | D L AE S L R M V K | ELITTIDQDV | HNLEK <mark>SL</mark> RLQ | EIYQRIDSKS |
| H3AF10 Latimeri | | DL <mark>AQAL</mark> SLIK | DLIFTVDQEV | FECEKSLRLQ | EIYNRVDSKA |
| B2DCZ9 Sus scro | | DLTTALGLVK | ELLSNVDQDV | HELEKGARLQ | EIYNRMDPRA |
| W5UTH7 Ictaluru | | GLKQALTLLR | DLLNGVEQQV | LELERTORLO | EIRSRLDPRS |
| | | | | | |
| E7F4U1_Danio_re | | SL <mark>AQ</mark> SL <mark>T</mark> LIR | ELLC <mark>S</mark> VDQQV | QELER <mark>AQ</mark> RLQ | E I Q <mark>S R L D</mark> P R A |
| K7FEF2_Pelodisc | | DL <mark>GMAL</mark> TLVK | DLISAIDLEV | HEQEK <mark>SA</mark> RLQ | E I Y <mark>G</mark> RVDGRV |
| Consistency | 7 8 6 8 <mark>4</mark> 8 8 8 <mark>4</mark> 6 | 8 <mark>* 5 4 8 * 4</mark> 9 8 8 | 8 <mark>*</mark> 8 6 <mark>5</mark> 9 9 8 6 9 | 6 7 7 * 8 <mark>4 5</mark> * * * | * * 7 <mark>5</mark> * 7 * <mark>6</mark> 8 7 |
| | | | | | |
| | | 520 | 530 | | 550 |
| Q92974_Homo_sap | | GP <mark>FGREELLR</mark> | RKLIHDG <mark>C</mark> LL | WKTATG-RFK | DV <mark>L</mark> VLLMTDV |
| Q60875 Mus musc | QTP <mark>V</mark> <mark>PG</mark> K | GP <mark>FGRDELLR</mark> | RKLIHEG <mark>C</mark> LL | WKTAT <mark>G-RFK</mark> | DV <mark>L</mark> LLMTDV |
| Q865S3 Canis lu | QAP <mark>V</mark> PSK | GPFGREELLR | RKLIHDGCLL | WKTATG-RFK | DV <mark>LMLLMTD</mark> V |
| Q5FVC2 Rattus n | | GPFGRDELLR | RKLIHDGCLL | WKTATG-RFK | DVLLLLMTDV |
| F1N1F7 Bos taur | | GPFGREELLR | RKLIHDGCLL | WKTATG-RFK | DVLMLLMTDV |
| Q6AX47 Xenopus | | DPFSKEELLR | RKLIHEGSLL | WKTAAG-RFK | DVIMLLMTDT |
| Q6NY14 Xenopus | | HSFSKEELLR | RKLIHEGSLL | WKTAAG-RFK | DVIMLLMTDT |
| H3AF10 Latimeri | | RLFRKEEFQR | RKLIHDGFML | WKNSSG-RFK | DVQVLLMTDV |
| _ | | | | | |
| B2DCZ9_Sus_scro | | G P F G R E L L R | RKLIHDGCLL | WKTAAG-RFK | D V <mark>L M L L M T D V</mark> |
| W5UTH7_Ictaluru | | AMFRPAELLR | RQLIHEG <mark>T</mark> LL | WKTPSS-RLK | D V <mark>Q V L L M T D V</mark> |
| E7F4U1_Danio_re | | GVFHGAELLR | RGLIHEGALL | WKTAQG <mark>S</mark> RLK | DV <mark>HVLLMTD</mark> V |
| K7FEF2_Pelodisc | | GF <mark>FSKDELLR</mark> | RKLVHDG <mark>C</mark> LL | WKTATG-RFK | DILVLLMTDV |
| Consistency | 5 5 4 <mark>8 0 0 0</mark> 4 <mark>7</mark> 5 | 54 * 566 * 98 * | * 8 * 9 * 8 * <mark>5</mark> 9 * | * * 9 8 6 9 <mark>0</mark> * 8 * | * 9 <mark>5 7 * * * * *</mark> 8 |
| | | | | | |
| | | | | <u></u> 590 | 600 |
| Q92974 Homo sap | | Y I F P T L D K P S | V V S L Q N L I V R | D I A N Q <mark>E K G M F</mark> | LIS <mark>AA</mark> PPE |
| Q60875 Mus musc | | YIFTSLDKPS | VVSLQNLIVR | DIANQAKGMF | LIS <mark>SG</mark> PPE |
| Q865S3 Canis lu | | YIFPALDKPS | VVSLQNLIVR | DIANQEKGMF | LIS <mark>AA</mark> PPE |
| Q5FVC2 Rattus n | | YIFTSLDKPS | VVSLQNLIVR | DIANQAKGMF | LIS <mark>SG</mark> PPE |
| F1N1F7 Bos taur | | YIFPALDKPS | VVSLQNLIVR | DIANQEKGMF | LISAAPPE |
| Q6AX47 Xenopus | | YYFPTLDKSP | VISLQNLIVR | DIANQEKGMF | LISAK PPE |
| | | | | | |
| Q6NY14_Xenopus_ | | YYFPTLDRSP | VISLQNLIVR | DIANQEKGMF | LIS <mark>AK</mark> PPE |
| H3AF10_Latimeri | | YTFPSMDKPS | VISLQNLIVR | DIANQERGIF | LISAA – <mark>S</mark> PPE |
| B2DCZ9_Sus_scro | | YIFPALDKPS | VVSLQNLIVR | D I A N Q E K G M F | LIS <mark>AA</mark> PPE |
| W5UTH7_Ictaluru | | YI FASLDKSA | VVSLQNLLVR | DIANQERGLF | LIS <mark>SE<mark>FS</mark>PPE</mark> |
| E7F4U1_Danio_re | LVFMQEKDQK | YVFPTLDKPS | VLCLQNLIVR | DIANQQRGMF | LIS <mark>HS</mark> – <mark>T</mark> PPE |
| K7FEF2_Pelodisc | TTELOEKDOK | FTFPALDKPA | | | |
| | HIF HØERDØR | FIFFALDAFA | VISLQNLIVR | D I A N Q E K G M F | LLS <mark>GT</mark> PPE |
| Consistency | 9989**** | 96*769*977 | VISLQNLIVR *88***9** | DIANQEKGMF * * * * * 78 * 8 * | LLSGT PPE * 9 * <mark>6 5 <mark>0 0</mark> * * *</mark> |
| Consistency | | | | | |
| · | 9 9 8 9 <mark>* * * * *</mark> 9 | 9 <mark>6 * 7 6</mark> 9 * 9 7 7 | * 8 8 <mark>* * * *</mark> 9 * * | * * * * <mark>* 7 8</mark> * 8 * | |
| ¥ | 9 9 8 9 * * * * * 9 610 | 9 6 * 7 6 9 * 9 7 7 620 | * 8 8 * * * * 9 * * 630 | * * * * * 7 8 * 8 * 640 | * 9 * <mark>6 5 0 0</mark> * * * 650 |
| Q92974_Homo_sap | 9989*****9 610 MYE <mark>VHT<mark>AS</mark>RD</mark> | 9 6 * 7 6 9 * 9 7 7 620 D <mark>R S TW I R V I</mark> Q | * 8 8 * * * * 9 * * 630 QS <mark>VRTCPSRE</mark> | * * * * * <mark>7 8 * 8</mark> * 640 DFPLIETEDE | * 9 * 6 5 0 0 * * * 650 A ^Y LRRIK <mark>ME</mark> L |
| Q92974_Homo_sap Q60875_Mus_musc | 9 9 8 9 * * * * * * 9 610 MYE VHT AS RD MYE VHA <mark>AS</mark> RD | 96*769*977 620 DRSTWIRVIQ DRTTWIRVIQ | * 8 8 * * * * 9 * * 630 QSVRTCPSRE QSVRLCPSRE | * * * * * 78 * 8 * 640 DFPLIETEDE DFPLIETEDK | * 9 * 6 5 0 0 * * * 650 AYLRRIKMEL AYLRRIKTKL |
| Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu | 9 9 8 9 * * * * * * 9 610 MYE VHT A S RD MYE VHA A S RD MYE VHA A S RD | 96*769*977 620 DRSTWIRVIQ DRTTWIRVIQ DRSTWVRVIQ | * 8 8 * * * * 9 * * 630 QSVRTCPSRE QSVRLCPSRE QSVRLCPSRE | * * * * * 7 8 * 8 * 640 DFPLIETEDE DFPLIETEDK DFPLIETEDE | * 9 * 6 5 0 0 * * * |
| Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n | 9989*****9 610 MYEVHTASRD MYEVHAASRD MYEVHTASRD MYEVHAASRD | 96*769*977 620 DRSTWIRVIQ DRTTWIRVIQ DRSTWVRVIQ DRTTWIRVIQ DRTTWIRVIQ | * 8 8 * * * * 9 * * 630 QSVRTCPSRE QSVRLCPSRE QSVRVCPSRE QSVRLCPSRE | *****78*8* 640 DFPLIETEDE DFPLIETEDK DFPLIETEDE DFPLIETEDE DFPLIETEDK | * 9 * 6 5 0 0 * * * AYLRRIKMEL AYLRRIKTKL AYLRRIKTKL AYLRRIKTKL |
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| Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6AX47_Xenopus_ Q6AX47_Xenopus_ Q6AX14_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc | 9 9 8 9 * * * * * 9 | 9 6 7 6 9 7 6 9 7 7 7 | * 8 8 * * * * 9 * * * 630 Q S V R T C P S R E Q S V R L C P S R E Q S V R V C P S R E Q S | * * * * * 7 8 * 8 * D FPLIETEDE D FPLIETEDE D FPLIETEDE D FPLIETEDE D FPLIETEDE E FPLIETEDE E FPLIETEDE E FPLIETEDE B FPLIETEDE G FQLIETEDE B FPLIETEDE S GVENE B FPLIETEDE S GVENE | * 9 * 6 5 0 0 * * * AYLRRIKMEL AYLRRIKMEL AYLRRIKMEL AYLRRIKMEL AYLRRIKMEL AYLRRIKMEL AYLRRIKMEL AKLRKLREVI VLRNLKDEI AKLRKLREVI VLRNLKDEI AYLRRIKMEL ALRRIKADI ALRRIKADI ALRRIKADI ALRRIKADI ASLRKIKDQM 9 4 * 7 8 8 4 5 8 700 RGLFRSESLE RGLFRSESLE RGLFRLESFE RGLFRTDSMD RCLFRSSLE NLFRADTPV RTLFRAESLE * 49 * 5 8 8 4 6 |

| F1N1F7_Bos_taur | S P R G E R L L Q D | AIR <mark>EV</mark> E <mark>G</mark> LKD | LLVG <mark>PGV</mark> EL <mark>L</mark> | LTPREPPLPM | E - PDSGGNTS |
|---|---|--|---|--|--|
| Q6AX47 Xenopus | S Y R G E K L I S E | AIKEVEALKD | VIVG <mark>SASEL</mark> P | LLRTEQNQTS | D-TSHSR |
| Q6NY14 Xenopus | T Y R G E K L I G D | TIKEVE <mark>A</mark> LKD | VIIGAGSEL P | LLRTEQNHPL | D-TSNSQ |
| H3AF10 Latimeri | | AIKEVE <mark>K</mark> LKD | LLVGGSVELS | VSVVDGDRNE | CLSSEGTROH |
| B2DCZ9 Sus scro | | AIREVEGLKD | LLVGPGVELL | LTSREPALPV | E-TDSGGNTS |
| W5UTH7 Ictaluru | | AITEVDRLTE | LLLGSGSGIP | LACTINGHHN | CT |
| _ | | | | | |
| E7F4U1_Danio_re | | AIVEVDRLSD | VLLSSCIER P | QSCRLNGEAT | E <mark>VQ</mark> L |
| K7FEF2_Pelodisc | | AIREVECLKD | LFMGAGRDWD | QNHPADPDGT | GS |
| Consistency | 6 <mark>4</mark> 8 9 * 7 * 8 <mark>4</mark> 7 | 99 <mark>6**84</mark> *89 | 8 8 7 <mark>9</mark> 5 5 5 7 7 <mark>4</mark> | <mark>6 5 3 5 5</mark> 4 4 4 4 4 | 4 <mark>0</mark> 3 3 3 <mark>4 1 0 1</mark> 4 |
| | | | | | |
| | 760 | | <u></u> 780 | | 800 |
| Q92974 Homo sap | PG <mark>VTANGE</mark> AR | TFNGSIELCR | ADSDSS-QR- | D R <mark>N G N Q L R S P</mark> | QEEALQRLVN |
| Q60875 Mus musc | PG <mark>VTANGEAR</mark> | TFNGSIELCR | ADSDSS-QK- | D R <mark>N G N Q L R S P</mark> | QEEVLQPLIN |
| Q865S3 Canis lu | | NFNGSIELCR | TDSDSS-QK- | DR <mark>NGNQL</mark> RAP | QEEALQRLVN |
| Q5FVC2 Rattus n | | TFNGSIELCR | ADSDSS-QK- | DRNGNQLRSP | QEEALQPLVN |
| F1N1F7 Bos taur | | TFNGSIELCR | ADSDSS-QK- | DRNGNQLRSP | QEEALQRLVN |
| | | | | | |
| Q6AX47_Xenopus_ | | TINGSCDLGK | DDDPKD R - | NG <mark>NQVQN</mark> KSP | HEEALQRIVN |
| Q6NY14_Xenopus_ | | AVNGSCDVAK | EDDPKD R - | NG <mark>NQLQN</mark> KSP | REDALQRIVN |
| H3AF10_Latimeri | S S D <mark>A T N G E N G</mark> | G V N G Q P D F R R | T <mark>SISVQK</mark> DR- | NG <mark>NQLQQ</mark> RSP | QEEVYQSLVN |
| B2DCZ9_Sus_scro | PG <mark>VTANGE</mark> AR | TF <mark>NG</mark> S <mark>IEL</mark> CR | ADSDSS-QK- | D R <mark>N G N Q L</mark> R S P | Q E E A L Q R L V N |
| W5UTH7 Ictaluru | GAL <mark>LINGQEV</mark> | LVNGSQETAA | SQDG- | N G <mark>N Q L E D K T P</mark> | SEEVSQRLVN |
| | TSKTADKERI | SVNGTYDTNG | PSNKDR- | NGNQLLDKLL | NEEVCQRLVN |
| K7FEF2 Pelodisc | | SLNGASELCL | ADSDSGORDG | NGNQLQQKVP | REELIDRLAT |
| _ | 5547657754 | 56**748645 | 5765540360 | 75*5484868 | 6*96696989 |
| Consistency | | | | | 0 9 0 0 9 0 9 0 9 |
| | 010 | 030 | 020 | 0.40 | 050 |
| | | | | | |
| Q92974_Homo_sap | | AVAQQDTLME | ARFPEGPERR | EKLCRANSRD | GEAGRAGAAP |
| Q60875_Mus_musc | | VVVQQERL <mark>M</mark> E | ALFPEGPERW | EKLSRANSRD | GEAGRAAVAS |
| Q865S3_Canis_lu | | A <mark>V</mark> AQQDTL <mark>M</mark> E | A R F P E G P E R R | EKLARANSRD | G <mark>E</mark> AGRVGP <mark>AP</mark> |
| Q5FVC2_Rattus_n | LYG <mark>L</mark> LQGL <mark>Q</mark> A | VVVQQERL <mark>M</mark> E | ALFPEGPERW | EKL <mark>SRANSR</mark> D | G <mark>E</mark> AGRAAV <mark>AS</mark> |
| F1N1F7 Bos taur | LYG <mark>L</mark> LHGLQA | AVAQQDTLME | ARFPEGPERR | EKLTRANSRD | GEAGRAGG <mark>VP</mark> |
| Q6AX47 Xenopus | | LVIQQDTLQD | TPMQDVSDKK | ERLCRSNSR- | -DPILVEPLS |
| Q6NY14 Xenopus | | MVIQQDTLQD | APLQDVGDKK | ERLCRTNSR- | -DPILIEPKS |
| H3AF10 Latimeri | | VVVQQDTLLE | LCIQEVVDKK | EKLTRSNSR- | -DGPLGDPGS |
| _ | | | | | |
| B2DCZ9_Sus_scro | | AVAQQDTLME | ARFPEGPERR | EKLTRANSRD | GEAGRAGAAP |
| W5UTH7_Ictaluru | | A VIRQDSIVE | F CARE GG TSR | SRP | - GRSLSREGT |
| E7F4U1_Danio_re | LST <mark>Q</mark> LHSIQA | AVIRQDSFLE | VYMQKDTSP- | | <mark>TT</mark> |
| | | | | | |
| K7FEF2_Pelodisc | LYG <mark>L</mark> HGL <mark>Q</mark> A | A <mark>VGQQXRGG</mark> G | RWEK <mark>V</mark> SRANS | <mark>R</mark> | - KGVLGQLAP |
| _ | LYGL <mark>L</mark> HGLQA 8765*879*9 | | | | |
| _ | | A <mark>V</mark> GQQXRG <mark>G</mark> G | RWEKVSRANS | <mark>R</mark> | - <mark>KGVLGQL</mark> AP |
| _ | 8 7 6 <mark>5</mark> * 8 7 9 * 9 | A <mark>VGQQXRGG</mark> G 6*68*86757 | RWEKVSRANS 5255754664 | R | - <mark>KGVLGQL</mark> AP 2 5 4 3 4 4 3 3 5 5 |
| Consistency | 8 7 6 <mark>5</mark> * 8 7 9 * 9 | AVGQQXRGGG 6*68*86757 | RWEKVSRANS 5255754664 | R | -KGVLGQLAP 2543443355 900 |
| Consistency Q92974_Homo_sap | 8765*879*9 860 VAPEKQ <mark>ATEL</mark> | AVGQQXRGG 6*68*86757 870 ALLQRQHALL | RWEKVSRANS 5255754664 880 QEELR <mark>RCRRL</mark> | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc | 8765*879*9 860 VAPEKQATEL VTPEKQATEL | AVGQQXRGGG 6*68*86757 870 ALLQRQHALL ALLQRQHTLL | RWEKVSRANS 5255754664 880 QEELRRCRL QEELRCQRL | R 6552545552 890 GEERATEAGS GEERATEAGS | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu | 8765*879*9 860 VAPEKQATEL VTPEKQATEL VAPDKQATEL | AVGQQXRGGG 6*68*86757 | RWEKVSRANS 5255754664 880 QEELRRCRRL QEELRRCQRL QEELRRCRL | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n | 8765*879*9 860 VAPEKQATEL VTPEKQATEL VAPDKQATEL VTPEKQATEL | A V G Q V X K G G 6 * 68 * 8 6 7 5 7 | RWEKVSRANS 5255754664 880 QEELRCCRL QEELRCCRL QEELRCCRL | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur | 8765 8879 9 | A V G Q V X K G G 6 * 68 * 8 6 7 5 7 ALLQ RQHALL ALLQ RQHTLL ALLQ RQHALL ALLQ RQH SLL ALLQ RQH SLL | RWEKVSRANS 5255754664 880 QEELRRCRRL QEELRCQRL QEELRRCRL QEELRRCRL | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n FIN1F7_Bos_taur Q6AX47_Xenopus_ | 8765 8879 9 APEKQATEL VAPEKQATEL VAPDKQATEL VTPEKQATEL AVPEKQATEL KTVEKQ - TDL | A V G Q Q XRG G G 6 * 68 * 8 6 7 5 7 ALLQ RQHALL ALLQ RQH TLL ALLQ RQH SLL ALLQ RQH SLL ALLQ RQH SLL ALLQ RQH SLL | RWEKVSRANS 5255754664 880 QEELRRCQRL QEELRCQRL QEELRRCQRL QEELRRCQRL QEELRRCRL QEELRRCRL QEELRRCRL | R 6552545552 GEERATEAGS GEERATEAGS GEERATEAGS GEERATEAGS GEERATEAGS CEERAQEAGL | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ | 8765 8879 89 | A V G Q V X K G G 6 * 68 * 8 6 7 5 7 ALLQ RQHALL ALLQ RQHTLL ALLQ RQHALL ALLQ RQH SLL ALLQ RQH SLL | RWEKVSRANS 5255754664 880 QEELRRCRRL QEELRCQRL QEELRRCRL QEELRRCRL | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n FIN1F7_Bos_taur Q6AX47_Xenopus_ | 8765 8879 89 | A V G Q Q XRG G G 6 * 68 * 8 6 7 5 7 ALLQ RQHALL ALLQ RQH TLL ALLQ RQH SLL ALLQ RQH SLL ALLQ RQH SLL ALLQ RQH SLL | RWEKVSRANS 5255754664 880 QEELRRCQRL QEELRCQRL QEELRRCQRL QEELRRCQRL QEELRRCRL QEELRRCRL QEELRRCRL | R 6552545552 GEERATEAGS GEERATEAGS GEERATEAGS GEERATEAGS GEERATEAGS CEERAQEAGL | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ | 8765 8879 89 | A V G Q Q XRG G G 6 * 68 * 8 6 7 5 7 ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL ALLQ RQHSLL ALLQ RQHSLL TLLQ RQHTLL TLLQ RQHTLL | RWEKVSRANS 5255754664 | R 6552545552 GEERATEAGS GEERATEAGS GEERATEAGS GEERATEAGS GEERATEAGS GEERATEAGS CEERAQEAGL | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LETRLRESEQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n FIN1F7_Bos_taur Q6AY14_Xenopus_ Q6NY14_Xenopus_ H3AF10_Latimeri B2DCZ9_Sus_scro | 8765 8879 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 | A V G Q X R G G 6 * 68 * 8 67 57 ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL TILQ RQHTLL TILQ RQHTLL ALLQ RQHTLL ALLQ RQHTLL ALLQ RQHALL | RWEKVSRANS 5255754664 880 QEELRRCQRL QEELRRCQRL QEELRCRRL QEELRCRRL QEELRCRRQ QEELRCRRQ QEELRCRQ QEELRRCRRQ QEELRRCRRA | R 6552545552 GEERATEAGS GEERATEAGS GEERATEAGS GEERATEAGS GEERATEAGS CEERAQEAGL CDDRAQEAGL CEEKAQEARE | - KGVLGQLAP 2543443355 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LETRLRESEQ LQARLQGSEA LEARLRESEQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru | 8765 8879 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 | A V G Q V X K G G 6 * 68 * 8 6 7 5 7 | RWEKVSRANS 5255754664 QEELRCCRL QEELRCCRL QEELRCCRL QEELRCCRL QEELRCRQ QEELRCRQ QEELRCRQ QEELRCKRQ QEELSRCRKA QEELRCRRL QEELRCRR | R 6552545552 GEERATEAGS GEERATEAGS GEERATEAGS GEERATEAGS GEERATEAGS CEERAQEAGL CDDRAQEAGL CEERAQEARE GEERATEAGS | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LETRLRESEQ LQARLQGSEA LEARLRESEQ AEGRLKDSEK |
| Consistency Q92974_Homo_sap Q608755_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re | 8765 8879 9 8765 8879 9 860 VAPEKQATEL VTPEKQATEL VTPEKQATEL KTVEKQ - TDL - ADKQ - TEL KTTEKTSNEL VAPEKQATEL GEVGTA - GEL GTPAGSVGDF | A V G Q V XRGGG 6 8 8 8 6 7 5 7 ALLQRQHALL ALLQRQHALL ALLQRQHALL ALLQRQHALL ALLQRQHALL TLLQRQHALL NLLQRQHILL ALLQRQHILL ALLQRQHSLL ALLQRQHSLL ALLQRQHSL | RWEKVSRANS 5255754664 880 QEELRCQRL QEELRCQRL QEELRCQRL QEELRCRL QEELRCRL QEELRCRQ QEELRCRQ QEELRCRQ QEELRCRA QEELRCRA QEELRCRA | R 6 5 5 2 5 4 5 5 5 2 G EERATEAGS G EERATEAGS G EERATEAGS G EERATEAGS C EERAQEAGL C D D RAQEAGL C EERAQEARE G EERATEAGS C EERAQEARE C D RAQEAGL C EERAQEARE G EERATEAGS C EERAQEARE G EERATEAGS C EERAQEARE C D RAQEAGL C E C C C C C C C C C C C C C C C C C C | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LETRLRESEQ LQARLQGSEA LEARLRESEQ AEGRLKDSEK |
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| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86583_Canis_lu Q857VC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc | 8765 8879 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 | A V G Q V XRGGG 6 8 8 8 6 7 5 7 ALLQRQHALL ALLQRQHALL ALLQRQHALL ALLQRQHALL ALLQRQHALL TLLQRQHALL NLLQRQHILL ALLQRQHILL ALLQRQHSLL ALLQRQHSLL ALLQRQHSL | RWEKVSRANS 5255754664 880 QEELRCQRL QEELRCQRL QEELRCQRL QEELRCRL QEELRCRL QEELRCRQ QEELRCRQ QEELRCRQ QEELRCRA QEELRCRA QEELRCRA | R 6 5 5 2 5 4 5 5 5 2 G EERATEAGS G EERATEAGS G EERATEAGS G EERATEAGS C EERAQEAGL C D D RAQEAGL C EERAQEARE G EERATEAGS C EERAQEARE C D RAQEAGL C EERAQEARE G EERATEAGS C EERAQEARE G EERATEAGS C EERAQEARE C D RAQEAGL C E C C C C C C C C C C C C C C C C C C | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LETRLRESEQ LQARLQGSEA LEARLRESEQ AEGRLKDSEK |
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| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n FIN1F7_Bos_taur Q6AY14_Xenopus_ Q6NY14_Xenopus_ H3AF10_Latimeri B3DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency | 8765 8879 9 8765 8879 89 VAPEKQATEL VTPEKQATEL VTPEKQATEL VTPEKQATEL KTVEKQ TEL KTVEKQ TEL KTVEKQ TEL GEVGTA GEL GTPAGSVGDF KAQEKQATEL 3556774689 | A V G Q V X R G G 6 * 68 * 8 67 57 | RWEKVSRANS 5255754664 S80 QEELRRCRL QEELRRCQRL QEELRRCRL QEELRRCRL QEELRRCRL QEELRRCRL QEELRRCRL QEELRRCRL QEELRRCRL QEELRRCRL QEELRRCRL QEELRCRL QEELRRCRL QEELRRCRL QEELRRCRL QEELRRCRL QEELSRCRL QEELSRCRQL * 8 * 9 5 * 7764 | R 890 G EERATEAGS G EERATEAGS G EERATEAGS G EERATEAGS G EERATEAGS C EERAQEAGL C DDRAQEAGL C EERAQEAG G EERATEAGS | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LEARLRESEQ AEGRLKDSEK VQDPAKGQDK LEARLRESEQ 886887997 |
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| Consistency Q92974_Homo_sap Q60875_Mus_musc Q66553_Canis_lu Q5FVC2_Rattus_n FIN1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ H3AF10_Latimerii B2DC29_Sus_scro W5UTH7_Ictaluru E7F401_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q6FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ H3AF10_Latimerii B2DC29_Sus_scro W5UTH7_Ictaluru | 8765 8879 9 VAPEKQATEL VTPEKQATEL VTPEKQATEL VTPEKQATEL VTPEKQATEL KTVEKQ - TDL - ADKQ - TEL KTVEKQ - TDL QEVGTA - GEL GEVGTA - GEL GEVGTA - GEL GEVGTA - GEL GEVGTA - GEL GEVGTA - GEL 910 ARALLEREAE | A V G Q X RG G G 6 0 8 0 8 0 7 5 7 | RWEKVSRANS 5255754664 5255754664 GEELRCRL GEELRCR GEELRCRL | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LETRLRESEQ LETRLRESEQ AGRLKDSEK VQDPAKGQK LEARLRESEQ 886887997 950 RRSLPAGDAL |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q66875_Mus_musc Q66553_Canis_lu Q5FVC2_Rattus_n FINIF7_Bos_taur Q6NY14_Xenopus_ Q6NY14_Xenopus_ Q6NY14_Xenopus_ U57F401_Datimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n FINIF7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re | 8765 8879 9 VAPEKQATEL VTPEKQATEL VTPEKQATEL VTPEKQATEL VTPEKQATEL KTVEKQ - TDL - ADKQ - TEL KTVEKQ - TDL QEVGTA - GEL GEVGTA - GEL GEVGTA - GEL GEVGTA - GEL GEVGTA - GEL GEVGTA - GEL 910 ARALLEREAE | A V G Q Q XRG G G 6 * 6 8 * 8 6 7 5 7 | RWEKVSRANS 5255754664 5255754664 SESS754664 SESS75666 SESS756766 SESS76767676 SESS76767676 <td>R </td> <td>- KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ BAGRLKDSEK VQDPAKGQDK LEARLRESEQ B86887997 950 RRSLPAGDAL</td> | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ BAGRLKDSEK VQDPAKGQDK LEARLRESEQ B86887997 950 RRSLPAGDAL |
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| Consistency Q92974_Homo_sap Q60875_Mus_musc Q66875_Mus_musc Q6553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q60875_Mus_musc Q66853_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ Q6NY14_Xenopus_ Q6NY14_Xenopus_ Q6NY14_Xenopus_ Q6NY14_Xenopus_ Q5FVC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc | B 7 6 5 8 8 7 9 8 9 VAPEKQATEL VAPEKQATEL VAPEKQATEL VAPEKQATEL KTVEKQ TEL KTVEKQ TEL KTVEKQ TEL KTVEKQ TEL KTVEKQ TEL GEVGTA GEL GEVGTA GEL GEVGTA GEL GEVGTA GEL GEVGTA GEL GEVGTA GEL GEVGTA GEL GEVGTA GEL GEVGTA GEL GEVGTA GEL GEL GEVGTA GEL GEL GEL GEL GEL GEL GEL GEL | A V G Q Q XRG G G 6 0 8 0 8 0 7 5 7 ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL ILQ RQHALL ILQ RQHILL ALLQ RQHILL ALLQ RQHILL ALLQ RQHILL ALLQ RQHILL ALLQ RQHILL ALLQ RQHALL ALLQ RQHAL ALLQ RQHAL ALLQ RQHALL ALLQ RQHAL ALLQ RQHAL ALLQ RQHAL ALLQ RQHALL ALLQ RQHAL ALLQ RQHAL AL | RWEKVSRANS 5255754664 5255754664 GEELRCRL GEELRRCRL GEELRCRL GEELRCRL < | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LETRLRESEQ LEARLRESEQ AEGRLKDSEK VQDPAKGQDK LEARLRESEQ 86887997 950 RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL |
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| Consistency Q92974_Homo_sap Q60875_Mus_musc Q66875_Mus_musc Q66553_Canis_lu Q5FVC2_Rattus_n FIN1F7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q92974_Homo_sap | 8 7 6 5 8 8 7 9 8 9 VA PEKQATEL VA PEKQATEL VT PEKQATEL VT PEKQATEL KTVEKQ - TDL - ADKQ - TEL KTVEKQ - TDL - ADKQ - TEL KTVEKQATEL GEVGTA - GEL GEVGTA - GEL GEVGTA - GEL GEVGTA - GEL 9 00 10 10 10 10 10 10 10 10 10 | A V G Q Q XRG G G 6 * 6 8 * 8 6 7 5 7 ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL ALLQ RQHALL TLQ RQHTLL TLQ RQHTLL TLQ RQHTLL ALLQ RQHTLL ALQ RQHALL ALQ RQHALL ALQ RQHAL ALQ RQHALC ALQ RQHALC ALQ RQHALG C ARRQLALG E ARRQLAALG E ARRQLAALG B A CON AN | RWEKVSRANS 525575464 525575464 S25575464 S25575464 S25575464 SEELRCRL SEELSRCR SEESER SEESER SEESER SEGE | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LETRLRESEQ LETRLRESEQ AEGRLKDSEK VQDPAKGQDK LEARLRESEQ 868887997 950 RRSLPAGDAL CSLPSPDLL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL CSLPSPDLL RRSLPAGDAL |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n FINIF7_Bos_taur Q6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1NIF7_Bos_taur Q6AX47_Xenopus_ Q6NY14_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc | 8 7 6 5 8 7 9 * 9 VAPEKQATEL VAPEKQATEL VAPEKQATEL VAPEKQATEL VAPEKQATEL VAPEKQATEL KTVKQ TEL KTVKQ TEL KTVKQ TEL KTVKQ TEL GEVGATEL GEVGTA GEL GEVGTA GEL GEVGTA GEL GEVGCATEL GEVGCATEL GEVGTA GEL GEVGTA GEL GEVGTA GEL GEVGTA GEL GEVGTA GEL RALLEREAE RALLEREAE ARALLEREAE RALLEREAE RALLEREAE RALLEREAE RALLEREAE RALLEREVE ARALLEREVE ARALLEREVE ARALLEREVE ARALLEREVE SEGSEERTEREVE SEGSEERTEREVE SEGSEERTEREVE YUSFNPQ YLSFNPQ | A V G Q Q XRG G G 6 * 6 8 * 8 6 7 5 7 | RWEKVSRANS 5255754664 5255754664 GEELRCRL GEELRCR GEELRCRL | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LETRLRESEQ LETRLRESEQ LETRLRESEQ AEGRIKDSEK VQDPAKGQDK LEARLRESEQ 866887997 950 RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL CSLPSPDLL RSLPAGDAL 1000 LGSP-EERLQ LGSP-EERLQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86583_Canis_lu Q5FVC2_Rattus_n FINIF7_Bos_taur Q6AX14_Xenopus_ Q6AX14_Xenopus_ Q6NX14_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q86583_Canis_lu Q5FVC2_Rattus_n FINIF7_Bos_taur Q6AX14_Xenopus_ Q6NY14_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_re K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q92974_Homo_sap Q60875_Mus_musc Q92974_Homo_sap Q60875_Mus_musc Q86583_Canis_lu | B 7 6 5 B 7 9 * 9 VA PEKQATEL VA PEKQATEL VA PEKQATEL VA PEKQATEL VA PEKQATEL AVPEKQATEL AVPEKQATEL AVPEKQATEL GEVGTA-GEL ARALLEREAE REKLERE ARALLEREAE REKLEREVE ARALLEREVE ARALLEREVE ARALLEREVE ARALLEREVE ARALLEREVE ARALLEREVE ARALLEREVE GERAFLEREVE BES 8 8 8 7 8 5 6 | A V G Q Q XRGGG 6 * 68 * 8 6757 ALLQRQHALL ALLQRQHALL ALLQRQHALL ALLQRQHALL ALLQRQHALL TLLQRQHTLL TLQRQHTLL TLQRQHTLL ALLQRQHTLL ALLQRQHALL ALLQRQHALL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHSL ALLQRQHS ALLQRQHSL ALLQRQHS ALLQRQ ALLQR ALLQR ALLQR ALLQR ALLQR ALLQR ALLQR | RWEKVSRANS 525575464 525575464 SEELRCRL GEELRCRL GEELSCRCL GEES | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LETRLRESEQ LEARLRESEQ AEGRLKDSEK VQDPAKGQDK LEARLRESEQ 86887997 950 RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL CSLPSPDLL RSLPAGDAL CSLPSPDLL RSLPAGDAL CSLPSPDLL RSLPAGDAL CSLPSPDLL CSP-EERLQ LGSP-EERLQ LGSP-EERLQ |
| Consistency Q92974_Homo_sap Q60875_Mus_musc Q86553_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_rek X7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc Q865S3_Canis_lu Q5FVC2_Rattus_n F1N1F7_Bos_taur Q6AX47_Xenopus_ Q6NX14_Xenopus_ H3AF10_Latimeri B2DC29_Sus_scro W5UTH7_Ictaluru E7F4U1_Danio_rek K7FEF2_Pelodisc Consistency Q92974_Homo_sap Q60875_Mus_musc | B 7 6 5 B 7 9 * 9 VA PEKQATEL VA PEKQATEL VA PEKQATEL VA PEKQATEL VA PEKQATEL AVPEKQATEL AVPEKQATEL AVPEKQATEL GEVGTA-GEL ARALLEREAE REKLERE ARALLEREAE REKLEREVE ARALLEREVE ARALLEREVE ARALLEREVE ARALLEREVE ARALLEREVE ARALLEREVE ARALLEREVE GERAFLEREVE BES 8 8 8 7 8 5 6 | A V G Q Q XRG G G 6 * 6 8 * 8 6 7 5 7 | RWEKVSRANS 5255754664 5255754664 GEELRCRL GEELRCR GEELRCRL | R | - KGVLGQLAP 2543443355 900 LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LEARLRESEQ LETRLRESEQ LETRLRESEQ LETRLRESEQ LETRLRESEQ AEGRIKDSEK VQDPAKGQDK LEARLRESEQ 866887997 950 RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RRSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL RSLPAGDAL CSLPSPDLL RSLPAGDAL 1000 LGSP-EERLQ LGSP-EERLQ |

| RQE LGSP-DERLQ DFSDL-TELEK DFS DLTE-LEKLP TKE IEDDEVARLH RQE LGSP-DERLQ DGQQ DGSDIDVD |
|--|
| DFS DLTE-LEKLP FKE IEDDEVARLH RQE LGSP-DERLQ GQQ DGSDIDVD |
| rke ie <mark>ddevarl</mark> h Roe lgsp-derlo Goo dgsdidvd |
| RQ <mark>E LGSP-DERLQ</mark> GQQ DGSDIDVD |
| GQQ DG <mark>S</mark> D IDVD |
| |
| |
| PG YCDGPERLOE |
| |
| 3 4 5 3 3 <mark>5 3 0</mark> 3 4 4 5 4 |
| |
| . 1040 1050 |
| |
| ESRDGEAVA |
| ESRDGEPTA |
| <mark>ES</mark> <mark>RDGEPMV</mark> |
| ESRDGEPTA |
| ESRDGEPVA |
| ES <mark>V QELKGVNRGS</mark> |
| ESV QELRDAEQGS |
| EGS QEVREEGSNP |
| SRDGEPVA |
| |
| |
| ES <mark>Saeareh</mark> a |
| ES <mark>Saeareha</mark> |
| ES <mark>Saeareh</mark> a |
| |

 Q92974_Homo_sap
 SES

 Q60875_Mus_musc
 SES

 Q65533_Canis_lu
 SES

 Q5FVC2_Rattus_n
 SES

 Q5FVC2_Rattus_n
 SES

 Q5FVC2_Rattus_n
 SES

 Q5FVC2_Rattus_n
 SES

 Q6AX47_Xenopus_SES
 G6AX47_Xenopus_SES

 Q6NY14_Xenopus_SES
 H3AF10_Latimeri

 H3AF10_Latimeri
 TTD

 B2DC23_Sus_scros
 SES

 W5UTH7_Ictaluru
 SHC

 E7F401_Danio_re
 SHC

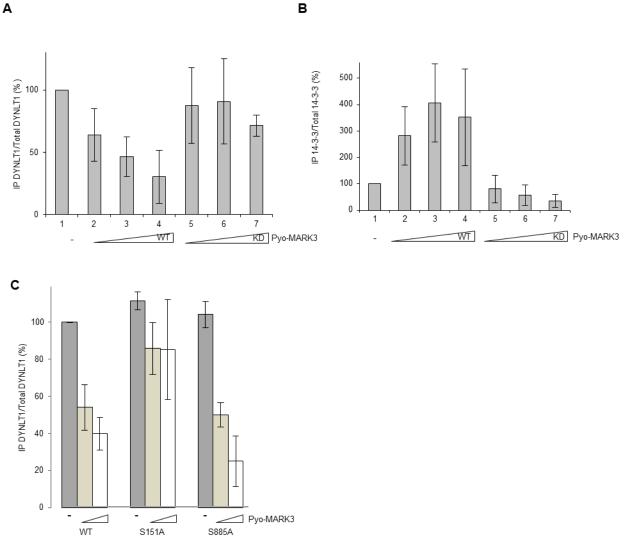
 K7FEF2_Pelodisc
 LDS

 Consistency
 6560

Alignment score = 903772.00 Alignment score per aligned residue pair = 14.73 Sequence identities = 41201 Percent sequence identity = 0.67 Number of sequences = 12 Alignment length = 1054 Number of residues = 11577 Number of gaps = 1071 Date of the analysis: 11/30/2016

Figure S2. Full alignment of ARHGEF2 orthologs in vertebrates.

The color code is based on the conserved residues with red or 10 being the most conserved. The conservation score is performed by PRALINE.





(A, B) Quantification of the DYNLT1 and 14-3-3 interactions with ARHGEF2 in the presence of increasing wildtype MARK3 (WT) or kinase-defficient MARK3 (KD) as observed in Figure 3C. Data in (A) and (B) are means ± SD from four independent experiments. C) Quantification of DYNLT1 interaction with wild-type ARHGEF2 (WT) and its mutants S151A and S885A in the presence of increasing amounts of MARK3 as observed in Figure 3D. Data in (C) are means \pm SD from three independent experiments.

в

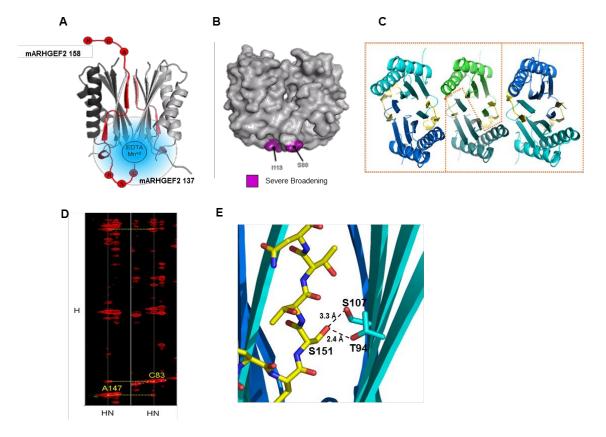


Figure S4. Structural characterization of the DYNLT1-ARHGEF2 interaction.

(A) Schematic representation of the PRE-tag conjugated to the N-terminus of the ARHGEF2 peptide (137-158). (B) Residues exhibiting substantial peak broadening induced by the addition of the PRE-tagged peptide. (C) Crystallographic contact sites of the DYNLT1-ARHGEF2 (peptides 136-164) chimera. Three molecules of DYNLT1/ARHGEF2 (136-164) chimera are present in the asymmetric unit (outlined in orange), two of which form homodimers, while the third forms a dimer with a molecule in another crystallographic symmetry unit. ARHGEF2 residues are highlighted in yellow. The asymmetric units are related to each other by the space group P3121. (D) Nuclear Overhauser Effect Spectroscopy (NOESY). Strips of the NOESY spectrum of the DYNLT1-ARHGEF2 chimera highlighting NOE connections between residues A147 and C83, consistent with the crystal structure. (E) ARHGEF2 Ser¹⁵¹ side chain hydrogen bonds with DYNLT1. Hydrogen bonds formed by the side chain hydroxyl group of ARHGEF2 Ser¹⁵¹ with the side chain hydroxyls of DYNLT1 T94 and S107 are shown as dashed lines with distances between oxygen atoms indicated.

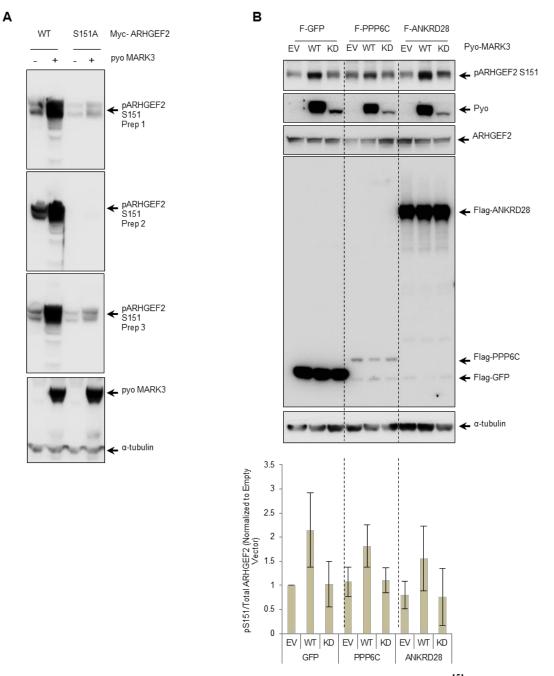
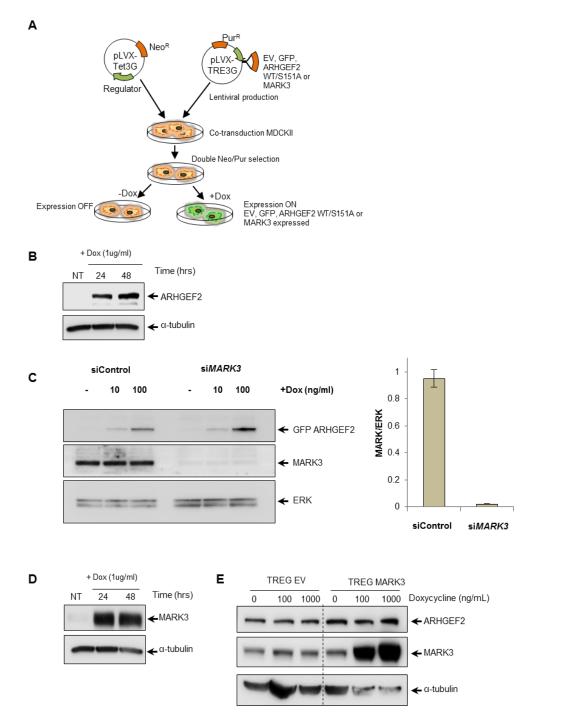
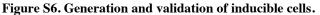


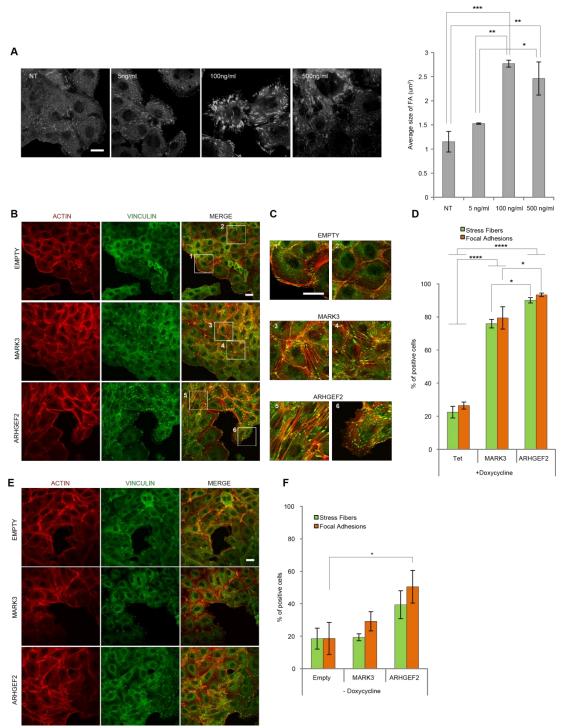
Figure S5. MARK3 but not PP6 regulates the phosphorylation of ARHGEF2 Ser¹⁵¹.

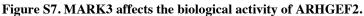
(A) Validation of the specificity of the ARHGEF2 phospho-S151 antibody. Western blot of HEK293T overexpressing Myc-tagged wild-type ARHGEF2 (WT) or the mutant (S151A) alone or in the presence of pyo-tagged MARK3 probed with three different preparations of phospho-S151-specific antibodies (designed by Cell Signaling Technology). We retained prep 2 for our analysis. (B) Western blot of tetracycline-inducible HEK293 cell lines carrying inducible expression of Flag-tagged GFP, catalytic subunit PPP6C and regulatory subunit ANKRD28. The cells were induced overnight with tetracycline (500ng/ml) and transfected with empty vector (EV), wild type MARK3 (WT) or kinase deficient MARK3 (KD). Endogenous ARHGEF2 phosphorylation was evaluated using the phospho-S151 specific antibodies from panel A. Data are means \pm SD of four independent experiments.





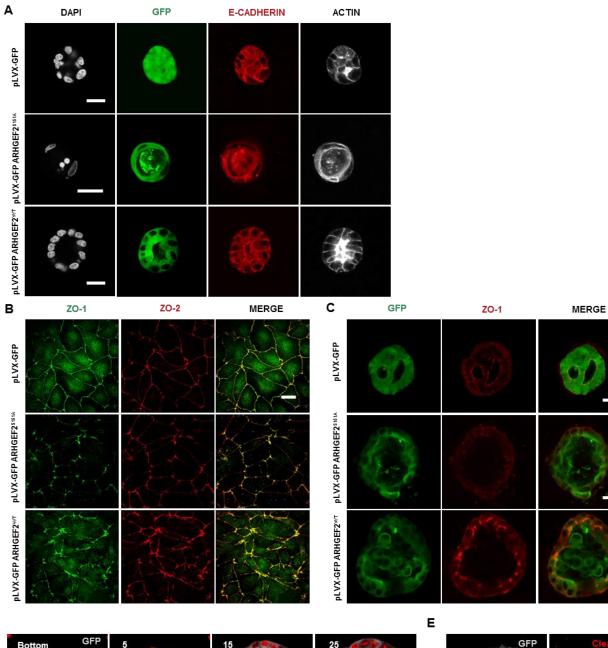
(A) Schematic of the generation process of MDCKII cells stably expressing MARK3, ARHGEF2^{WT} or ARHGEF2^{S151A} under control of a tetracycline-inducible (TETi) promoter. (B) WB of the selected colonies expressing inducible ARHGEF2. (C) WB of inducible MDCKII cells expressing pLVX-GFP ARHGEF2^{WT} with increasing doses of doxycycline treated with a control or a of specific pool of *MARK3* siRNA. Right panel: quantification of MARK3 protein is shown. Data are means \pm SD of three independent experiments. (D) Validation of inducible MARK3. (E) Evaluation of the effect of EV and MARK3 induction on ARHGEF2 expression. (B, D and E. Representative of three independent experiments.





(A) Immunofluoresnce of inducible MDCKII cells expressing pLVX-GFP ARHGEF2^{WT} in the presence of increasing doses of doxycycline fixed and stained for VINCULIN. Four high magnification fields per experiment were quantified. Data are means of two independent experiments . Scale bar, 20 μm. Statistical significance was determined by a one-way ANOVA test with a Bonferroni post-test correction for multiple comparisons. *P=0.0165; **P=0.023 (5 ng VS 100 ng) and **P=0.0017 (NT VS 500 ng); ***P=0.0003. (B) MDCKII cells expressing inducible pLVX-Empty Vector, MARK3 or wild-type ARHGEF2. Confocal images of subconfluent cells induced

with 100 ng/mL of doxycycline for 18 hours, fixed and stained for ACTIN and VINCULIN. (C) Detail of the boxed areas shown in A. (D) Quantification of stress fibers and focal adhesions of images shown in A, expressed as percent of positive cells. (E) Uninduced MDCKII as in (B), treated with DMSO 18 hours. In (F) quantification of images shown in (E). Scale bars, 20 μ m. Data in (D) and (F) are means \pm SD of three independent experiments, a total of n=100-200 cells per condition were counted. Statistical significance was determined by a two-way ANOVA test with a Bonferroni post-test correction for multiple comparisons. In (D), *P=0.0136 for SF and *P=0.0143 for FA; ****P<0.0001., In (F) *P=0.0182.





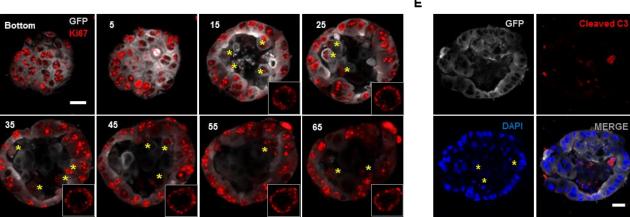


Figure S8. Phosphorylation of ARHGEF2 Ser¹⁵¹ is required for normal cell polarity

(A) 3D culture at four days of MDCKII cells expressing inducible pLVX-GFP, pLVX-GFP ARHGEF2^{WT} or mutant S151A. GFP fluorescence was visualized and cysts were stained for E-CADHERIN, ACTIN and DAPI. Representative of two independent experiments. Scale bar 20 μm. (B,C) Tight junction staining in MDCKII expressing pLVX-GFP, pLVX-GFP ARHGEF2^{WT} or mutant S151A. In (B), 2D staining of ZO-1 and ZO-2. In (C), 3D staining of ZO-1. Scale bar 20 um. Representative of three independent experiments. (D, E) Ki67 and cleaved caspase 3 staining in MDCKII spheroids expressing inducible pLVX-GFP ARHGEF2^{WT} at day seven. In (D), Ki67 staining through different Z stacks (the number represent the Z stack step in μm), the yellow asterisks mark the luminal cells proliferating. Representative of two independent experiments. Scale bar 20 μm. In (E), Cleaved Caspase 3 (C3) staining; the yellow asterisks mark the groups of apoptotic luminal cells that coincide with luminal nuclear fragmentation as observed in the DAPI staining. Scale bar 20 μm. Representative of two independent experiments.

| Summary | | FlagBirA*-ARH | | | GEF2 | | | | |
|-----------|------------------------|---|----|----|-------|------|------|-------|-------|
| Gene ID P | Protein ID Gene Nam | m Full name | | | trols | А | В | Total | SAINT |
| 9181 | 15011974 ARHGEF2 | Rho/Rac guanine nucleotide exchange factor 2 | | | | 2388 | 2739 | 5127 | - |
| 9859 | 109255228 CEP170 | centrosomal protein 170kDa | 38 | 35 | 31 | 236 | 140 | 376 | 1.00 |
| 26586 | 148664244 CKAP2 | cytoskeleton associated protein 2 | 1 | | | 106 | 51 | 157 | 1.00 |
| 55291 | 255918192 PPP6R3 | protein phosphatase 6 regulatory subunit 3 | 10 | 4 | 4 | 82 | 47 | 129 | 1.00 |
| 23243 | 68131557 ANKRD28 | ankyrin repeat domain 28 | 3 | 1 | | 86 | 42 | 128 | 1.00 |
| 79649 | 147903302 MAP7D3 | MAP7 domain containing 3 | 7 | 7 | 5 | 68 | 26 | 94 | 1.00 |
| 22870 | 151101459 PPP6R1 | protein phosphatase 6 regulatory subunit 1 | 4 | 2 | 1 | 62 | 28 | 90 | 1.00 |
| 54477 | 19923493 PLEKHA5 | pleckstrin homology domain containing A5 | | | | 66 | 21 | 87 | 1.00 |
| 283373 | 157743284 ANKRD52 | ankyrin repeat domain 52 | | | | 67 | 15 | 82 | 1.00 |
| 9701 | 37537701 PPP6R2 | protein phosphatase 6 regulatory subunit 2 | | | | 41 | 17 | 58 | 1.00 |
| 2011 | 254028234 MARK2 | microtubule affinity regulating kinase 2 | 2 | | | 35 | 14 | 49 | 1.00 |
| 5358 | 209862851 PLS3 | plastin 3 | 8 | 8 | 7 | 22 | 26 | 48 | 0.90 |
| 5537 | 183603929 PPP6C | protein phosphatase 6 catalytic subunit | 5 | 3 | 2 | 32 | 14 | 46 | 1.00 |
| 157922 | 186659512 CAMSAP1 | calmodulin regulated spectrin associated protein 1 | 2 | 2 | 1 | 38 | 6 | 44 | 0.94 |
| 22919 | 6912494 MAPRE1 | microtubule associated protein RP/EB family member 1 | 6 | 4 | 4 | 24 | 19 | 43 | 1.00 |
| 220134 | 21450832 SKA1 | spindle and kinetochore associated complex subunit 1 | | | | 26 | 13 | 39 | 1.00 |
| 4137 | 8400715 MAPT | microtubule associated protein tau | 1 | | | 22 | 16 | 38 | 1.00 |
| 4140 | 193083125 MARK3 | microtubule affinity regulating kinase 3 | 4 | 3 | | 25 | 9 | 34 | 1.00 |
| 55201 | 50428935 MAP1S | microtubule associated protein 1S | | | | 24 | 9 | 33 | 1.00 |
| 9053 | 310750366 MAP7 | microtubule associated protein 7 | | | | 23 | 10 | 33 | 1.00 |
| 57509 | 50348611 MTUS1 | microtubule associated tumor suppressor 1 | | | | 21 | 12 | 33 | 1.00 |
| 4690 | 5453754 NCK1 | NCK adaptor protein 1 | 4 | 4 | 3 | 20 | 12 | 32 | 0.91 |
| 51512 | 253970412 GTSE1 | G2 and S-phase expressed 1 | | | | 24 | 4 | 28 | 1.00 |
| 5528 | 31083280 PPP2R5D | protein phosphatase 2 regulatory subunit B', delta | 3 | 2 | | 17 | 8 | 25 | 0.94 |
| 23271 | 44955929 CAMSAP2 | calmodulin regulated spectrin associated protein family member 2 | | | | 20 | 4 | 24 | 1.00 |
| 256714 | 270483740 MAP7D2 | MAP7 domain containing 2 | | | | 17 | 4 | 21 | 1.00 |
| 10298 | 5031975 PAK4 | p21 protein (Cdc42/Rac)-activated kinase 4 | 4 | 2 | 1 | 10 | 8 | 18 | 0.79 |
| 9201 | 4758128 DCLK1 | doublecortin like kinase 1 | 2 | | | 9 | 7 | 16 | 1.00 |
| 51248 | 7706025 PDZD11 | PDZ domain containing 11 | | | | 9 | 6 | 15 | 1.00 |
| 9928 | 7661878 KIF14 | kinesin family member 14 | 1 | | | 11 | 3 | 14 | 0.99 |
| 283638 | 163644261 KIAA0284 | centrosomal protein 170B | | | | 8 | 4 | 12 | 1.00 |
| 79884 | 88759339 MAP9 | microtubule associated protein 9 | | | | 8 | 4 | 12 | 1.00 |
| 23332 | 214010173 CLASP1 | cytoplasmic linker associated protein 1 | | | | 9 | 3 | 12 | 0.99 |
| 1855 | 32479521 DVL1 | dishevelled segment polarity protein 1 | | | | 5 | 4 | 9 | 1.00 |
| 121441 | 206597465 NEDD1 | neural precursor cell expressed, developmentally down-regulated 1 | 1 | | | 5 | 4 | 9 | 1.00 |
| 6729 | 226371618 SRP54 | signal recognition particle 54kDa | 1 | | | 4 | 3 | 7 | 0.99 |

Table S1. ARHGEF2 interactors reported in this study –Summary.

Summary of the BioID analysis for ARHGEF2. The spectral counts of the top 3 controls out of 14 are shown. "A" and "B" denote two independent biological replicates. "Total" is the sum of the spectral counts for the prey. Bait Spectral Counts are highlighted in yellow. SAINT or significant analysis interactome score >0.79 was used to identified *bona fide* interactors.

| GO biological process complete | Accession number | Homo sapiens - REFLIST (20972) | ; upload_1 (35) | upload_1 (expected) | upload_1 (over/under) | upload_1 (fold Enrichment) | upload_1 (P- value) | -Log ₁₀ P-value |
|--|------------------|--------------------------------------|--------------------|------------------------|--------------------------|----------------------------------|------------------------|----------------------------|
| microtubule cytoskeleton organization | GO:0000226 | 397 | 12 | 0.66 | + | 18.11 | 9.90E-09 | 8.004365 |
| cytoskeleton organization | GO:0007010 | 960 | 16 | 1.6 | + | 9.99 | 5.50E-09 | 8.259637 |
| single-organism organelle organization | GO:1902589 | 1547 | 16 | 2.58 | + | 6.2 | 6.70E-06 | 5.173925 |
| microtubule-based process | GO:0007017 | 574 | 13 | 0.96 | + | 13.57 | 3.41E-08 | 7.467246 |
| regulation of microtubule cytoskeleton organization | GO:0070507 | 129 | 5 | 0.22 | + | 23.22 | 2.06E-02 | 1.686133 |
| regulation of microtubule polymerization or depolymerization | GO:0031110 | 56 | 5 | 0.09 | + | 53.5 | 3.46E-04 | 3.460924 |
| regulation of microtubule-based process | GO:0032886 | 153 | 5 | 0.26 | + | 19.58 | 4.70E-02 | 1.327902 |
| negative regulation of microtubule binding | GO:1904527 | 2 | 2 | 0 | + | > 100 | 4.54E-02 | 1.342944 |
| COPII vesicle coating | GO:0048208 | 61 | 4 | 0.1 | + | 39.29 | 2.93E-02 | 1.533132 |
| COPII-coated vesicle budding | GO:0090114 | 64 | 4 | 0.11 | + | 37.45 | 3.54E-02 | 1.450997 |
| cellular localization | GO:0051641 | 2131 | 15 | 3.56 | + | 4.22 | 4.73E-03 | 2.325139 |
| organelle organization | GO:0006996 | 2952 | 23 | 4.93 | + | 4.67 | 3.21E-08 | 7.493495 |
| cellular component organization | GO:0016043 | 5382 | 26 | 8.98 | + | 2.89 | 2.03E-05 | 4.692504 |
| cellular component organization or biogenesis | GO:0071840 | 5548 | 26 | 9.26 | + | 2.81 | 4.09E-05 | 4.388277 |
| vesicle coating | GO:0006901 | 63 | 4 | 0.11 | + | 38.04 | 3.33E-02 | 1.477556 |
| protein complex subunit organization | GO:0071822 | 1205 | 11 | 2.01 | + | 5.47 | 2.17E-02 | 1.663540 |
| vesicle targeting, rough ER to cis-Golgi | GO:0048207 | 61 | 4 | 0.1 | + | 39.29 | 2.93E-02 | 1.533132 |
| vesicle targeting, to, from or within Golgi | GO:0048199 | 65 | 4 | 0.11 | + | 36.87 | 3.76E-02 | 1.424812 |
| vesicle targeting | GO:0006903 | 77 | 5 | 0.13 | + | 38.91 | 1.66E-03 | 2.779892 |
| organelle localization | GO:0051640 | 462 | 9 | 0.77 | + | 11.67 | 4.31E-04 | 3.365523 |
| establishment of organelle localization | GO:0051656 | 395 | 8 | 0.66 | + | 12.14 | 1.99E-03 | 2.701147 |
| mitotic cell cycle process | GO:1903047 | 761 | 9 | 1.27 | + | 7.09 | 2.74E-02 | 1.562249 |
| mitotic cell cycle | GO:000278 | 789 | 10 | 1.32 | + | 7.59 | 3.68E-03 | 2.434152 |
| establishment or maintenance of cell polarity | GO:0007163 | 154 | 5 | 0.26 | + | 19.45 | 4.85E-02 | 1.314258 |
| Unclassified | UNCLASSIFIED | 3875 | 5 | 6.47 | - | 0.77 | 0.00E+00 | |
| Analysis Type: | PANTHER Overr | epresentatio | on Test (relea | se 20160715 | 5) | | | |
| Annotation Version and Release Date: | GO Ontology da | tabase Rele | ased 2016-12 | -28 | | | | |
| Analyzed List: | upload_1 (Hom | o sapiens) | | | | | | |
| Reference List: | Homo sapiens (| all genes in o | database) | | | | | |
| Bonferroni correction: | TRUE | 1 | | | | | | |
| Bonferroni count: | 8404 | | | | | | | |

Table S2. GO enrichment analysis of the ARHGEF2 network.

Enrichment analysis of the ARHGEF2 network, based on biological process, performed using <u>http://www.geneontology.org/</u>. The analysis results are sorted hierarchically, based on their ontology. The most specific subclass first, with its parent terms indented directly below it. ARHGEF2 was excluded from the analysis. – Log₁₀ of the P value was calculated and used to generate the graph in Figure S1B.

| Gene ID | Gene Name | Saint Score | Previously Validated | Function |
|-----------------|--------------------|-------------|-------------------------|--|
| 23243 | ANKRD28 | 1 | Yes | Putative regulatory subunit of protein phosphatase 6, involved in mitosis and chromosome segregation. |
| 283373 | ANKRD52 | 1 | | Putative regulatory subunit of protein phosphatase 6, involved in mitosis and chromosome segregation. |
| 157922 | CAMSAP1 | 0.94 | | Regulation of cell morphology and cytoskeletal organization. Microtubule stabilization. |
| 22271 | CAMSAP2 | 1 | | Microtubule minus-end binding protein that may regulate the organization of non-centrosomal microtubules. |
| | | | | Microtubule stabilization. |
| | CEP170 | 1 | Yes | Centrosomal protein. Microtubule organization and cell morphology. |
| | CEP170B (KIAA0284) | 1 | | Centrosomal protein. Microtubule organization. |
| 26586 | CKAP2 | 1 | | Stabalizes microtubules and plays a role in the regulation of cell division. |
| | CLASP1 | 0.99 | | Microtubule plus-end tracking protein that promotes the stabilization of dynamic microtubules. Required for the polarization of the cytoplasmic microtubule arrays in migrating cells towards the leading edge of the cell. |
| 9201 | DCLK1 | 1 | | Involved in a calcium- signaling pathway controlling neuronal migration in the developing brain. |
| 1855 | DVL1 | 1 | | Regulates cell proliferation, acting as a transducer molecule for developmental processes, including segmentation and neuroblast specification. |
| 51512 | GTSE1 | 1 | | May be involved in p53-induced cell cycle arrest in G2/M phase by interfering with microtubule rearrangements that are required to enter mitosis. |
| 9928 | KIF14 | 0.99 | | Plays an essential role in cytokinesis. |
| | | | | Microtubule-associated protein that mediates aggregation of mitochondria resulting in cell death and genomic |
| 55201 | MAP1S | 1 | | destruction. Plays a role in anchoring the microtubule organizing center to the centrosomes. Binds to DNA. Plays a |
| | | | | role in apoptosis. Involved in the formation of microtubule bundles |
| | | | | Microtubule-stabilizing protein that may play an important role during reorganization of microtubules during |
| 9053 | MAP7 | 1 | | polarization and differentiation of epithelial cells. Associates with microtubules in a dynamic manner. May play a |
| | | | | role in the formation of intercellular contacts. |
| | MAP7D2 | 1 | | Involved in microtubule stabilization. |
| 79649 | MAP7D3 | 1 | | Promotes the assembly and stability of microtubules |
| 79884 | МАР9 | 1 | | Involved in organization of the bipolar mitotic spindle. Required for bipolar spindle assembly, mitosis |
| /3001 | | - | | progression and cytokinesis. May act by stabilizing interphase microtubules. |
| | | | | Binds to the plus end of microtubules and regulates the dynamics of the microtubule cytoskeleton. Promotes |
| 22919 | MAPRE1 | 1 | | cytoplasmic microtubule nucleation and elongation. May be involved in spindle function by stabilizing |
| | | | | microtubules and anchoring them at centrosomes. May play a role in cell migration. Promotes microtubule assembly and stability, and might be involved in the establishment and maintenance of |
| 4137 | MAPT | 1 | | neuronal polarity. |
| | | | | Involved in cell polarity and microtubule dynamics regulation. Plays a key role in cell polarity by phosphorylating |
| 2011 | MARK2 | 1 | Yes | the microtubule-associated proteins MAP2, MAP4 and MAPT/TAU at KXGS motifs, causing detachment from |
| | | | | microtubules, and their disassembly. |
| 4140 | MARK3 | 1 | | Involved in the specific phosphorylation of microtubule- associated proteins for tau, MAP2 and MAP4. |
| 57500 | MTUS1 | 1 | | Microtubule associated tumor suppressor 1. Cooperates with AGTR2 to inhibit ERK2 activation and cell |
| 57509 | WIUSI | T | | proliferation. |
| 4600 | NCK1 | 0.91 | | Adaptor protein. Plays a role in ELK1-dependent transcriptional activation in response to activated Ras signaling. |
| | - | | | Involved in actin polymerization. |
| 121441 | NEDD1 | 1 | | Required for mitosis progression. Promotes the nucleation of microtubules from the spindle. |
| 10298 | РАК4 | 0.79 | Yes | Serine-threonine kinase. Involved in cytoskeleton regulation, cell migration, growth, proliferation or cell survival and affects actin cytoskelton reorganization. |
| 51248 | PDZD11 | 1 | | Involved in biotin and ion transmembrane transport. |
| 54477 | PLEKHA5 | 1 | | Phosphatidylinositol-3-phosphate and phosphatidylinositol-3,5-bisphosphate binding. Involved in reproductive system and brain development. |
| 5358 | PLS3 | 0.9 | | Actin-bundling protein. Involved in actin and calcium ion binding. |
| 5528 | PPP2R5D | 0.94 | | Implicated in the negative control of cell growth and division. |
| 5537 | РРР6С | 1 | Yes | Catalytic subunit of protein phosphatase 6. Component of a signaling pathway regulating cell cycle progression, mitotic spindle formation and chromosome segreagation. |
| 22870 | PPP6R1 | 1 | Yes | Regulatory subunit of protein phosphatase 6. May function as a scaffolding PP6 subunit. |
| 9701 | PPP6R2 | 1 | | Regulatory subunit of protein phosphatase 6. May function as a scaffolding PP6 subunit. |
| | PPP6R3 | 1 | | Regulatory subunit of protein phosphatase 6. May function as a scaffolding PP6 subunit. |
| 55291 | | | | |
| 55291 220134 | SKA1 | 1 | | Component of the SKA1 complex, a microtubule-binding subcomplex of the outer kinetochore that is essential for proper chromosome segregation. |

Table S3. Functional Annotation of ARHGEF2 interactors.Functional annotation was made based on GO analysis and a survey of the literature. Saint score and previously validated interactors are indicated.

| | | ANA | LYSIS OF N | ARK3/C-TA | K1 COMPL | LEXES |
|--|-----------------|-----------|------------|------------|---------------------------|---|
| Protein Name | ID (Uniprot) | Gene Name | Known | MARK3* | * Control* of (*Ave. # | Comments |
| MARK3/C-TAK1 | P27448 | MARK3 | | 358 | 0 | Member of CAMK Ser/Thr protein kinase family, MARK subfamily. 1 KA1 Domain. 1 UBA Domain |
| 14-3-3 FAMILY MEMBERS | | | | | | |
| 14-3-3 protein epsilon | P62258 | YWHAE | Yes | 133 (+131) | 2 | Belongs to the 14-3-3 family. |
| 14-3-3 protein zeta/delta | P63104 | YWHAZ | Yes | 72 (+69) | 3 | Belongs to the 14-3-3 family. |
| 14-3-3 protein theta | P27348 | YWHAQ | Yes | 37(+35) | 2 | Belongs to the 14-3-3 family. |
| 14-3-3 protein eta | Q04917 | YWHAH | Yes | 33(+34) | 1 | Belongs to the 14-3-3 family. |
| 14-3-3 protein gamma | P61981 | YWHAG | Yes | 42(+40) | 2 | Belongs to the 14-3-3 family. |
| 14-3-3 protein beta | P31946 | YWHAB | Yes | 60(+51) | 9 | Belongs to the 14-3-3 family. |
| MICROTUBULE-ASSOCIATED | | | | | | |
| CLIP-associating protein 2 | 075122 | CLASP2 | | 42 | 0 | Microtubule plus-end tracking protein that promotes the stabilization of dynamic microtubules. LgRsrSdidV |
| CLIP-associating protein 1 | Q7Z460 | CLASP1 | | 32 | 0 | Microtubule plus-end tracking protein that promotes the stabilization of dynamic microtubules. LqRsrSdidV |
| GEF-H1, Rho/Rac guanine nucleotide exchange factor 2 | Q92974 | ARHGEF2 | | 28 | 0 | Activates Rho-GTPases by promoting the exchange of GDP for GTP. Localizes to the tips of cortical microtubules of the mitotic spindle during cell division, and is further released upon microtubule depolymerization. |
| Cytoskeleton-associated protein 5 | Q14008 | CKAP5 | | 27(+21) | 6 | Plays a major role in organizing spindle poles. LaReaStgvL |
| MAP4, Microtubule-associated protein 4 (MAP 4) | P27816 | MAP4 | Yes | 26(+17) | 9 | Non-neuronal microtubule-associated protein. Promotes microtubule assembly. |
| Kinesin-2 | O00139 | KIF2A | | 13(+10) | 3 | Plus end-directed microtubule-dependent motor required for normal brain development. Required for normal progression and spindle dynamics during mitosis. |
| MAP7, Microtubule-associated protein 7 | Q14244 | MAP7 | | 5 | 0 | Microtubule-stabilizing protein that may play an important role during reorganization of microtubules during polarization and differentiation of epithelial cells. |
| Microtubule-actin cross-linking factor 1, isoforms 1/2/3/5 | Q9UPN3 | MACF1 | | 2 | 0 | F-actin-binding protein which may play a role in cross-linking actin to other cytoskeletal proteins. Also bind to microtubules. VeKrrSllel, VgKafSidil |
| Microtubule-associated protein RP/EB family member 3, RP3 | Q9UPY8 | MAPRE3 | | 2 | 0 | May be involved in microtubule polymerization, and spindle function by stabilizing microtubules and anchoring them at centrosomes. May play a role in cell migration |
| SIGNALING RELATED | | | | | | |
| TBC1 domain family member 4 | O60343 | TBC1D4 | | 30(+29) | 1 | May act as a GTPase-activating protein for RAB2A, RAB8A, RAB10 and RAB14. |
| SH2 containing inositol-5-phosphatase | O00145 | INPP5D | | 10 | 0 | Phosphatidylinositol (Ptdns) phosphatase that specifically hydrolyzes the 5-phosphate of phosphatidylinositol-34,5-trisphosphate (Ptdns(3,4,5)P3) to produce Ptdins(3,4)P2, thereby negatively regulating the PISK (phosphotionsitide 3-kinase) pathways. |
| PP2A, subunit A, PR65-alpha isoform | P30153 | PPP2R1A | | 9(+8) | 1 | Serine/threonine protein phosphatase PP2A scaffolding subunit A |
| PP2A, subunit B, B-alpha isoform | P63151 | PPP2R2A | | 3 | 0 | Serine/threonine protein phosphatase PP2A regulatory subunit B family. |
| PP2C gamma | O15355 | PPM1G | | 8(+7) | 1 | Serine/threonine protein phosphatase PP2C catalytic subunit |
| PRAS40 | Q96B36 | AKT1S1 | | 4 | 0 | Subunit of TORC1 |
| Tyrosine-protein phosphatase, non-receptor type 13 | Q12923 | PTPN13 | | 3 | 0 | Tyrosine phosphatase which regulates negatively FAS-induced apoptosis and NGFR-mediated pro-apoptoti signaling, MgRaiStgsL, IIKrIScseL, IsKvpStPvhL |
| Tyrosine-protein phosphatase, non-receptor type 14 | Q15678 | PTPN14 | | 3 | 0 | Tyrosine phosphatase |

Table S4. Analysis of MARK3/C-TAK1 complexes.Summary of the different interactors found for MARK3. Previously known binding interactors are indicated. Ave.: Average.

| Data Collection Statistics: | | |
|----------------------------------|--------------------------|--|
| Space group | P 3 ₂ 2 1 | |
| Cell dimensions | | |
| a, b, c (Å) | 91.97, 91.97, 83.08 | |
| α, β, γ (°) | 90, 90, 120 | |
| Resolution (Å) | $50-2.00(2.03-2.00)^{a}$ | |
| Rmerge | 0.078 (0.348) | |
| ΙσΙ | 31.5 (6.6) | |
| Completeness (%) | 100 (100) | |
| Redundancy | 10.8 (9.2) | |
| Refinement Statistics: | | |
| Rwork | 17.4% | |
| Rfree | 21.0% | |
| No. of Reflectctions | 27423 | |
| No. of Reflections used in Rfree | 2010 | |

^a Values in parentheses are for the highest resolution shell.

Table S5: Data collection and refinement statistics for DYNLT1:ARHGEF2 chimera (PDB: 5WI4).