

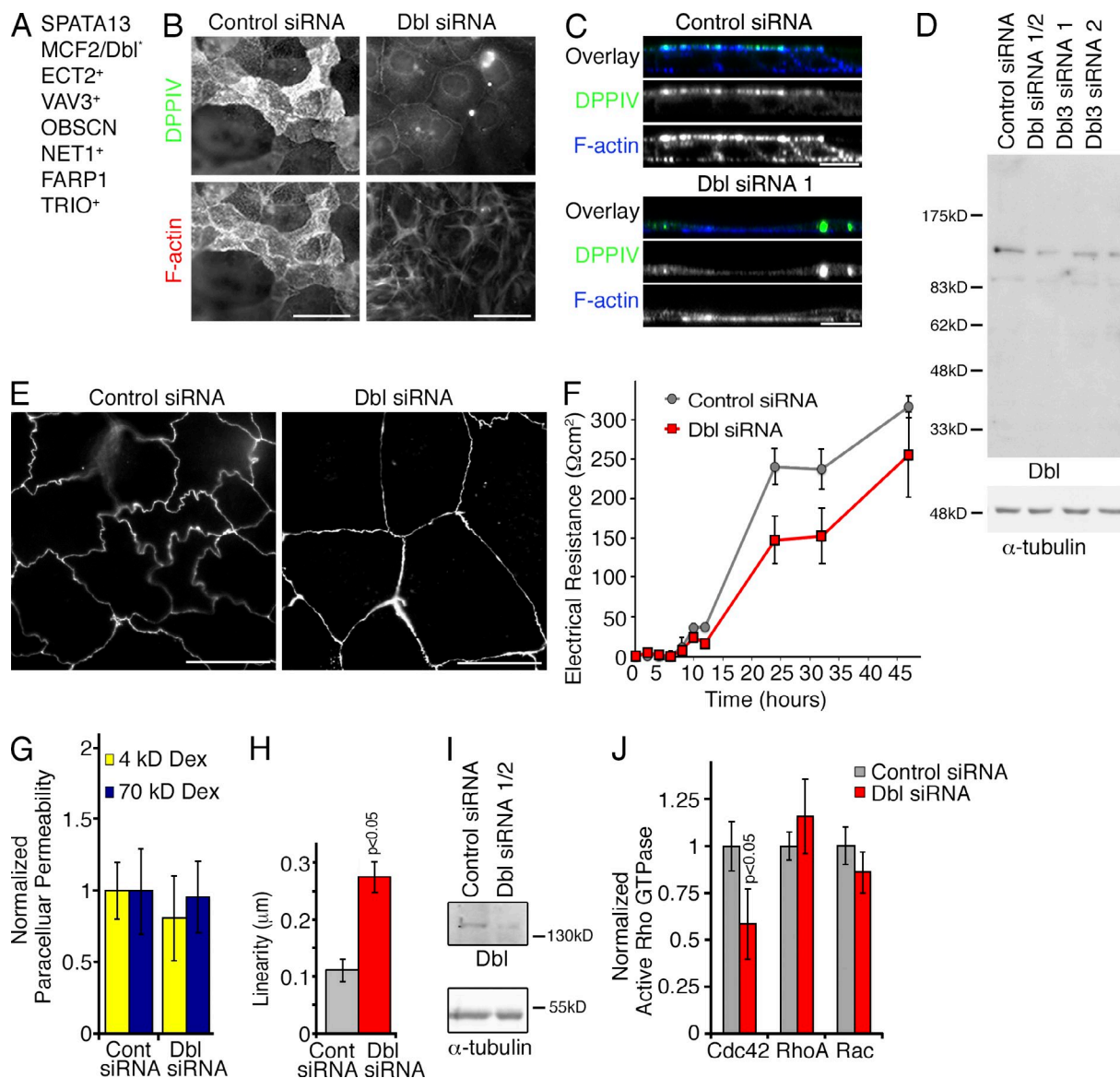
Zihni et al., <http://www.jcb.org/cgi/content/full/jcb.201304064/DC1>

Figure S1. Dbl regulates apical differentiation but not epithelial barrier formation. (A) Caco-2 cells were screened for GEFs regulating junction formation and apical differentiation using a functional siRNA screen. The table shows positive hits of the screen that were confirmed in two independent rounds of screening. (B and C) Caco-2 cells transfected with control or Dbl-specific (targeting all isoforms) siRNAs were processed for confocal microscopy as indicated. Shown are confocal zy sections from the apical membrane (A) and z sections to illustrate the observed cell flattening and reduced accumulation of apical F-actin and DPPIV. (D) Caco-2 cells were transfected with siRNAs as indicated and analyzed by immunoblotting total cell extracts with anti-Dbl antibodies. (E) Caco-2 cells transfected with control and Dbl-specific siRNAs were fixed and stained to the tight junction protein ZO-1. Shown is an epifluorescence image. (F and G) Caco-2 cells transfected with siRNAs were subjected to a calcium switch protocol. Tight junction formation was then assayed by measuring transepithelial electrical resistance and fluorescent dextran permeability. Note that the effect of Dbl on junction formation was measured at the same time as the effect of SH3BP1; hence, the control values are the same as those previously published in Elbediwy et al. (2012). (H) The linearity of tight junctions was determined by measuring the length of linear junctional fragments in images such as those in E. Shown are means \pm SD ($n = 10$). (I and J) Levels of Dbl depletion and active Rho GTPases in Caco-2 cells were measured after transfection of siRNAs (J shows means \pm SD, $n = 3$). Bars: (B) 30 μm ; (C) 10 μm ; (E) 20 μm .

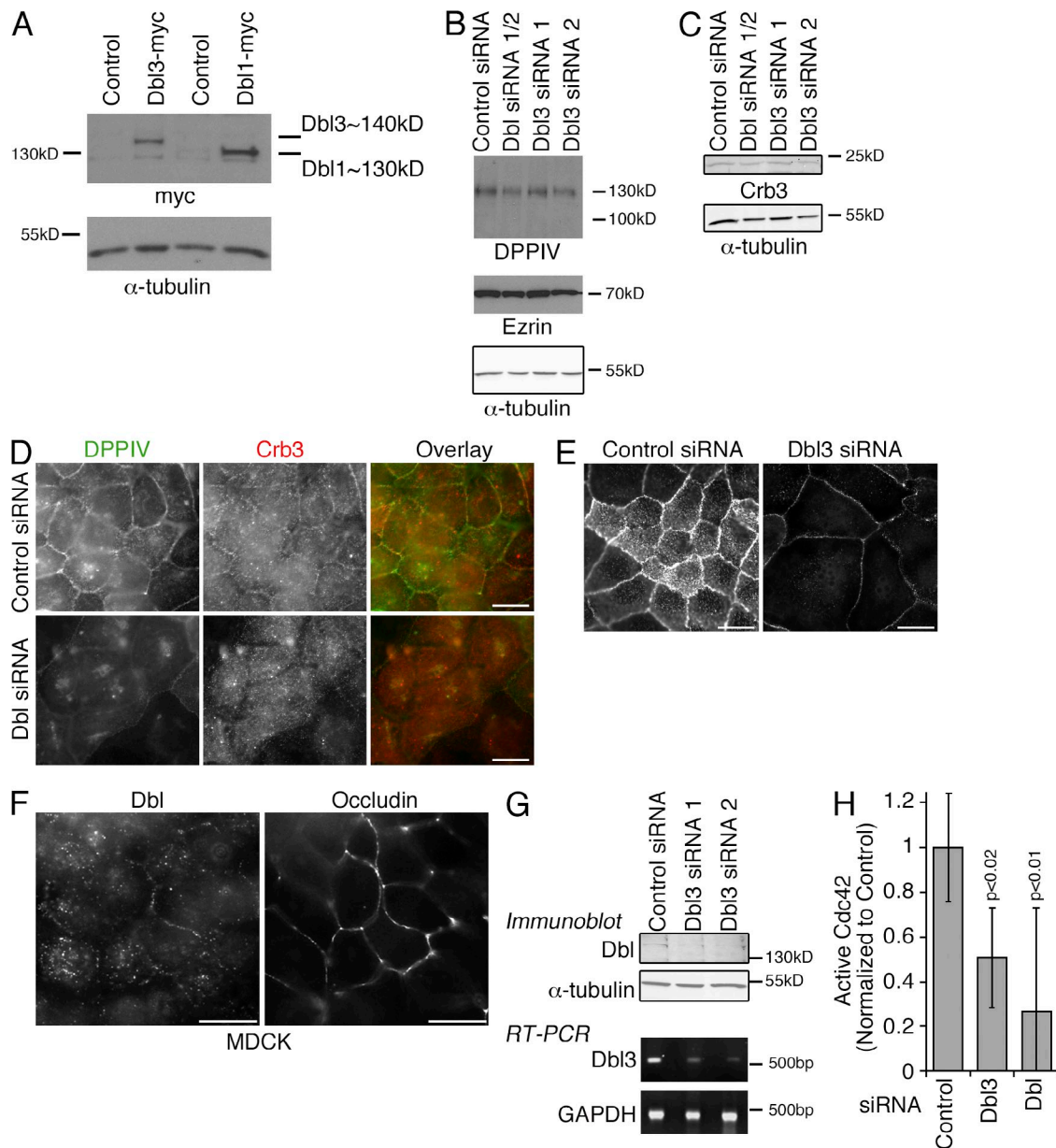


Figure S2. **Analysis of Dbl expression and depletion.** (A) MDCK cell lines stably expressing Dbl-Myc isoforms were processed for immunoblotting using anti-myc antibodies. (B and C) Total cell extracts of Caco-2 cells that had been transfected with control siRNAs and siRNAs targeting all Dbl isoforms or Dbl3 only were immunoblotted with antibodies against DPPIV, ezrin, Crb3, and α -tubulin. (D and E) Caco-2 cells transfected with siRNAs were processed for immunofluorescence using antibodies against the apical marker DPPIV and the apical polarity regulator Crb3 (D) or ezrin (E). Shown are epifluorescence images. (F) Expression of endogenous Dbl in MDCK cells was analyzed by epifluorescence using antibodies against Dbl and occludin. (G and H) MDCK cells were transfected with siRNAs as indicated and expression of Dbl3 was analyzed by immunoblotting and RT-PCR (G). (H) The effect of Dbl depletion on total levels of active Cdc42 (shown are means \pm SD, $n = 4$). Bars, 20 μ m.

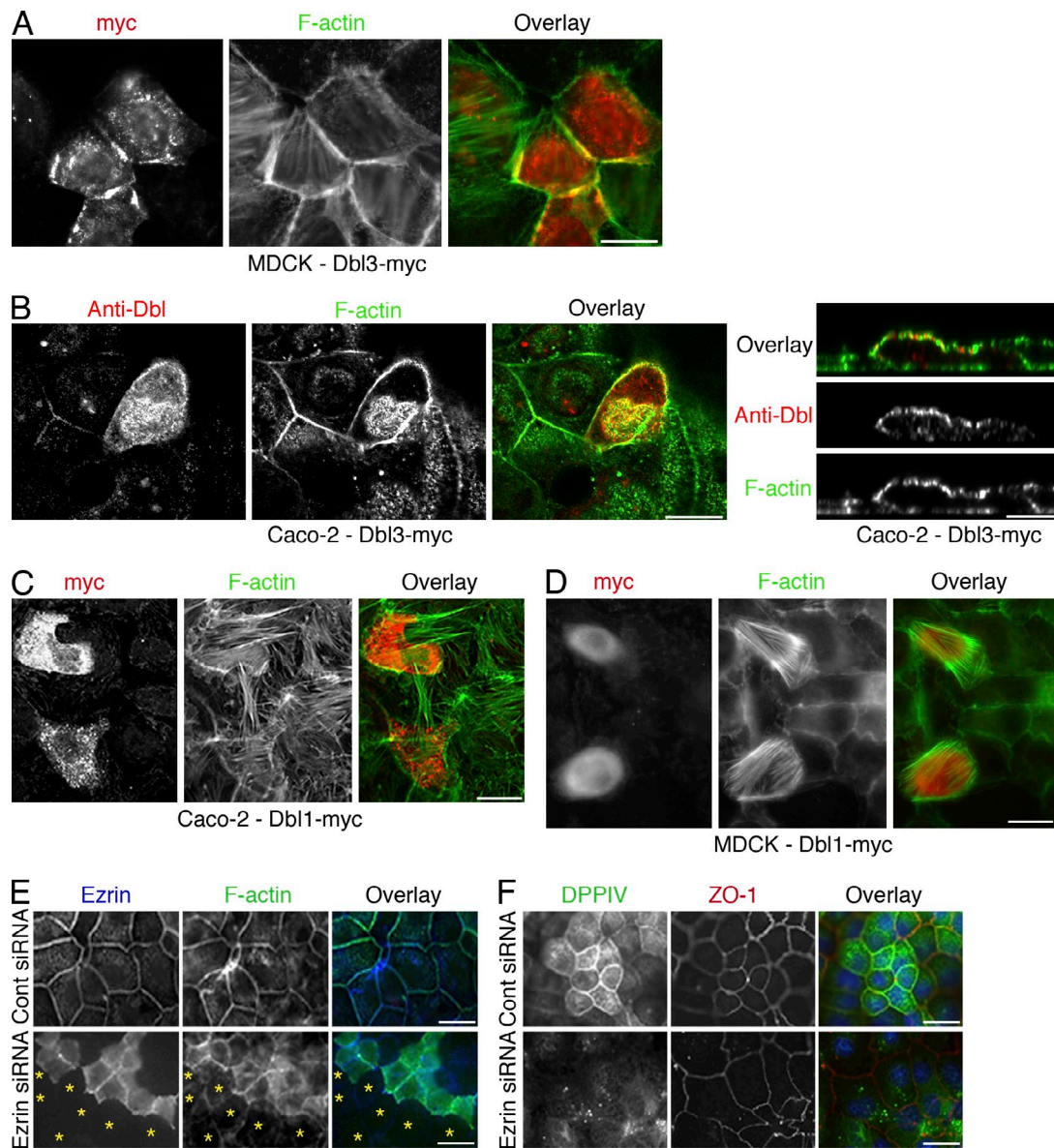


Figure S3. **Expression of Dbl isoforms and ezrin analysis.** (A–D) Myc-tagged Dbl isoforms were transiently expressed in MDCK and Caco-2 cells and localized by immunofluorescence using antibodies against the myc tag (A, C, and D) or Dbl (B). (E and F) Caco-2 cells were transfected with siRNAs and processed for immunofluorescence as indicated. Bars: (A, C, and D) 5 μ m; (B) 10 μ m; (E and F) 20 μ m.

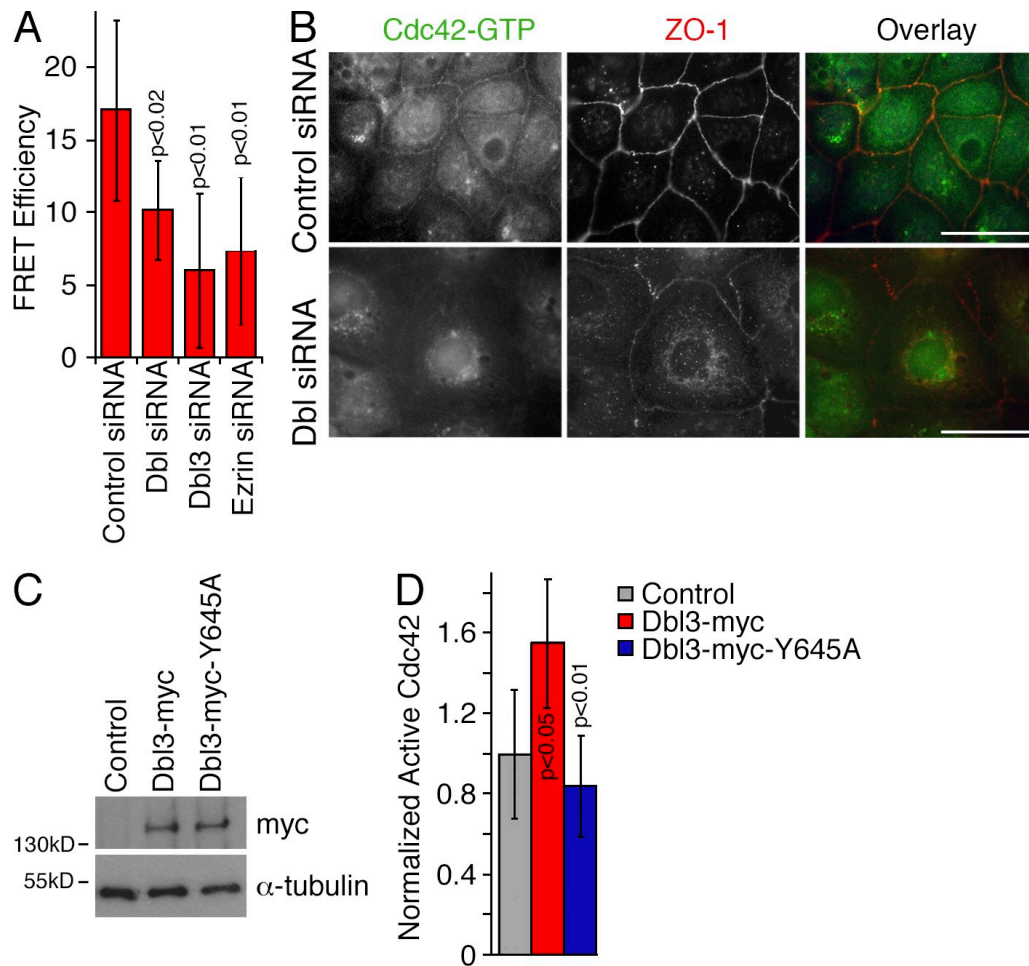


Figure S4. **Db1 is required for enrichment of active Cdc42 at apical cell-cell contacts.** (A) Total levels of apical FRET activity after transfection of siRNAs and FRET Cdc42 biosensor (shown are means \pm SD, $n \geq 12$ imaged fields). (B) Caco-2 cells were transfected with siRNAs and processed for immunofluorescence using anti-Cdc42-GTP and ZO-1 antibodies. (C and D) Caco-2 cells were transiently transfected with plasmids encoding the indicated constructs. The cells were then extracted for immunoblotting (C) and determination of total levels of active Cdc42 by G-LISA assay (D; shown are means \pm SD, $n = 5$). Bars, 20 μ m.

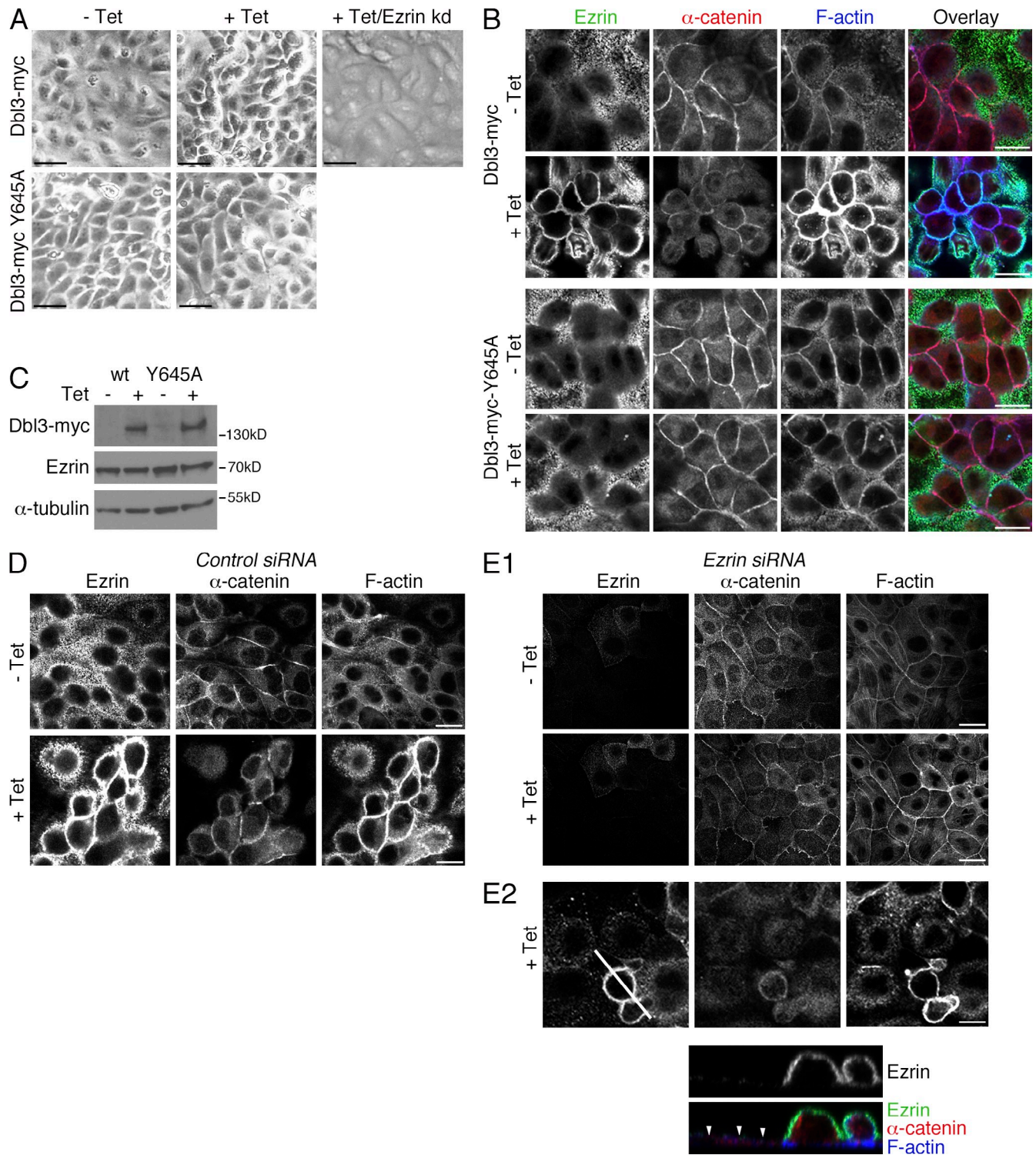


Figure S5. **Dbl3 activates the Par3–Par6–aPKC pathway.** (A–C) Conditional MDCK cell lines stably expressing active or mutant Dbl3-myc were analyzed by phase-contrast microscopy (A), immunofluorescence followed by confocal microscopy (B; shown are confocal xy sections taken at the apical–lateral border), or immunoblotting (C). (D and E) Dbl3-myc MDCK cells were transfected with siRNAs and incubated with tetracycline as indicated before analysis by immunoblotting and immunofluorescence combined with confocal microscopy (xy sections were taken at the apical–lateral border). (E2) A z scan and the corresponding xy section of an area that contains ezrin-expressing and -depleted cells. Bars: (A) 30 μ m; (B, D, and E) 10 μ m.

Table S1. **Alignment of CRAL-TRIO domains.** The CRAL-TRIO domains of DOME and the indicated proteins were aligned using the Eukaryotic Linear Motif (<http://elm.eu.org>). The leucine residue mutated to inactivate the CRAL-TRIO domain of Db13 is labelled with an asterisk.

Db13	---QDIAFLS	GGR-----G	KDNAWIIITFP	-----E	NCNFRCIPEE	VI*AKVITYLT	SIARQ	-----	NGSDS	
AAD25756/1-0	EDLATAAAYMN	G-----V	RESHPVCYNV	HSEELY...	Q	TIGSEKNREK	FLRWRFLQME	KGIQK	-----LNLKPGGVT	
A49678/1-0	YYDIARHQIV	EVA----GD	KYRKILVFS	ACRMP	---	SHOLDHSK	LLGYLKHITLD	QYVES	-----DYTLT	
00939/1-0	EAETGKIYRS	SC-----V	KLRLPVLIMR	PSV-----	---	ENSKSVKG	QIRYLVCME	NAVQN	-----LPPGEE	
035239/1-0	EILSGKFTIL	NVR-----	PTASTALFT	ARLHH	---	PHKSAQHV	VLQALFYLLD	RAVDS	-----FETQRN	
CAA15638/1-0	KRTTFDYADIL	PLP-----GLT	PDKCKVSLYC	FRFE	---	ASKMHTE	DTRAFFMVSD	CRFVT	---PD DLAKPDVLS	
AAF25952/1-0	LQQRVVFLLT	GGR-----	RRGPLLFCP	ATP-----	---	RRDRLKPE	DLRRLSYLI	SIPSD	-----AAKNL	
NIP2_HUMAN/1-0	YKVIISHGGY	YGD-----G	LNALIVFAVC	FMPES	---	S	QPNRYRLMDN	LFKYVIGTL	LLVAE	-----NYMIV
CAB55301/1-0	-----AFLS	GGR-----G	KDNAWIIITFP	-----E	NCNFRCIPEE	VI*AKVITYLT	SIARQ	-----	NGSDS	
YUQP_CAEEL/1-0	RRKHAPIDII	GPQ----RKE	DGDRLVVDR	AGRIDV...	S	GLMKSVPQTE	YLHEMFRSFP	EIQRR	---LM KMEAETGVQC	
Q9ZWD6/1-0	ELREVLQYYP	QGYH---GV	KEKRPVYIER	LGKAHP...	S	KLMRITIDR	YLKYHVOEFP	RALQE	KPPA CSIAAKRRIC	
Q91870/1-0	EILSGKFTVL	PGR-----	ENAAALALFT	ARLHR	---	PDVTHKA	VLQAIYQLD	KAIER	-----VEQRN	
043304/1-0	PPALLEEYFA	GGWH---YQ	IDRPLYLIR	LQMDT...	K	GLMKAVGEEA	LLRHVSVN	EGQKR	---CEG STROLGRPIS	
190854/1-0	TIEAGYPSVL	SSR-----	KYRVVLMFN	LENWQ	---	SQEIFDE	ILQAYCFIL	KLEN	-----EETQT	
AAF02821/1-0	DLDLLQFFTL	QGL-----	RSNRIFRIV	GKYPF	---	ARVVSAR	LKKYISQKIS	NOCPE	---GPLCLVY	
CAB51563/1-0	ENATGKQVLL	G-----YD	KDRPCLLYL	PAR	---	QNTKTSPL	QIRHLVFKS	CAIDL	-----MPPGVE	
KARI_RAT/1-0	ILKEKVAFVS	GGR-----	KRGPILTFP	ART	---	NHDRIRQE	DLRKLVTNLA	SVPSE	-----DVCKR	
048940/1-0	ELAQQKVFMG	G-----HD	KIRPILMVF	GGRHF	---	QNDGGLDE	FKRFVYVLD	KVCAS	-----MPPGQE	
023217/1-0	EGETGKASRA	SF-----HD	RQKRVVLMR	PAM	---	QNSTSQEG	NIRHLVYLL	NAIN	-----LPGQK	
A53057/1-0	PPDVLQKFMF	GGDV---GH	KDSVLRIEP	WGYLDM...	K	GIMYSCKKSD	LEKSKLQCS	KHLKD	---LEA QSEKVGKPT	
4437/1-0	EKPLIAKFYP	QYH---KT	KDRPVYFEE	LGAVNL...	H	EMNKVTEER	MLKNLWVEY	SVVQY	RLPA CSRAAGHLE	
Q23130/1-0	FNDISAHETI	QVIA---DC	RVRPVIVVY	AYRLEP...	---	SSKIDHAR	LLQYLVOIID	KIVDQ	-----DNYTIV	
048939/1-0	PADLRRAVDR	SQILGLSGYS	RELPVFAIG	VGLST	---	FDKASVHY	YVQSHIQIN	YRERI	ILPS ASKKQGRPIT	
SC14_SCHPO/1-0	EKEAVSKYYP	QFYH---KT	IDRPPVYEQ	IGNIDL...	K	KLYQITPER	MMQNLYVEY	MLALK	RFFA CSRKAGGLIE	
CAB63153/1-0	DLEGVAVYMR	G-----Y	KEHPVCYNA	YGVFKE(5)	R	VFGDEEKLNK	FLRWRVQLR	RGVKM	---LHFKPGGVN	
Q22824/1-0	NMSAVAEYFP	GGIM---GKS	KRDVVMQA	MAKAHP...	K	TLVKAGPTSQ	LFQLCISET	MSFKI	---IR QTEBERTERM	
Q17910/1-0	NPIFQKRLMP	RGEI-LEKT	NQNRLWYIE	YATITV...	E	SIAHSIRSE	ACKFQFLQF	YMLRK	---VM EQEBERTGCLS	
DBS_HUMAN/1-0	QKKRFAFLS	GGR-----G	QDSPVITFP	DYP	---	AFSEIPDK	EFQNVMTYLT	SIPSL	-----QDAGI-	
CAB62036/1-0	ETWSLLFWH	G-----Y	KNRPPCLIVR	LGLAFL	---	KLPSHERPR	FAQAIISQV	HGVLH	-----LLTPENS	
AAF18525/1-0	GSEFKLVFT	H-----GV	KQHVVIYSS	YGEFQN...	KE	IFSDKEKLSK	FLKWRVQFQ	KCVRS	-----LDFSPKAS	
024659/1-0	DLDDDKVYMG	G-----A	RTRPILLGF	PVKNF	---	SAKRDMPK	FKSYCVYLLD	SICAR	-----IPRGQE	
TTPA_RAT/1-0	LLKAGYHGV	RSR-----	PTSRVLIVR	ISYWD	---	PKVETAYD	VFRVSLITS	LIVQE	-----VEQRN	
NF1_DROME	EFKTKLSMNI	FYQA---GTS	KSYVVFYFI	ARRYK	---	IGETNGD	LIYHVILTL	KPFCH	---SPFEVVID	
AAD23650/1-0	EIDVELKYYP	QGYH---GV	KDRPVYIER	LGQVDA...	T	KLMQVTTIDR	YVKYHVFREF	KTFNI	KLPA CSIAAKKHD	
Q93546/1-0	DHPVHHHWKT	GLTG---ESGI	IPNTIVNIEQ	TGSNDY...	W	GMLHSYPTNE	ILRARVHDL	SMLKA	---VM DLEKKTQOC	
Q22467/1-0	DCSVPLRYLP	GSLI---GL	HENNVSLSQM	IGLHA...	A	GLMPATRNSD	LYRMRJAES	GVMQI	---IR KMEKEQKPL	
Q06705/1-0	NLELQKATIQ	G-----Y	NDMRPVILVR	PRLHH	---	SDQTEQE	LEKFSLLVIE	QSKLF	-----PKNYPA	
081806/1-0	LIEKLEIFKI	HGR-----	KRKIKILRI	GKFFP	---	ARFLSLDV	LKKYLEEKIF	PRLGR	-----KPPAVLY	
AAF19259/1-0	PPVIQLYDS	GGLC---GY	YECPVYFNI	IGSLDP...	K	GLLLSASKQD	MRKRKVKC	LLLHE	---CEL QTQKLGRRKIE	
Q921J8/1-0	PPVIQKYMP	GGLC---GY	RDCPWYDI	IGPLDP...	K	GLLFSVTKQD	LLKTKMRDC	RILHE	---CDL QTERLGRKIE	
076636/1-0	DHPILKQYFP	LGLVG---ETG	KDNQLLVIEC	AGRIDL...	M	GILKSVHLS	FLIQRFKFS	KMLAA	---MN EMERKYGTQC	
015915/1-0	IREIGSAGCV	YVN---KR	KKRPILFAV	PRNDT	---	LKNVPSL	KFKNLVYWL	QGFSR	-----MDEPKGIE	
YNX1_YEAST/1-0	ENESGKQVIL	G-----YE	NDARPILYLK	PGR	---	QNTKTSR	QVQHLVFMLE	RVDF	-----MPAGQD	
001324/1-0	EEDELNKYVP	IDVIG-QNHQ	DDNKVLMFER	TGKIDI...	S	GLVDNVLMHK	FMQIKLMMK	GVHQK	---VV AERKTRGQS	
BAA90344/1-0	EAETGKIYRA	DY-----K	KHRTVLVLR	PGL	---	ENTTSKGG	QIKYLVYCL	KAIMS	-----LTDQE	
Q9ZW01/1-0	ELAEGLAYVA	G-----L	DECRPVLFVR	IKQDY	---	QKLHTQKQ	LTRLVFTLE	VAIST	-----MSRNV	
BAA90976/1-0	DIASLKALYQ	T-----GV	NCRTVMVVV	GRNIP	---	VTLIDMDK	ALLYFHVMD	HAIVK	-----EYVLV	
061528	VLRDGIAVLP	GGRC-----	RAQAVIVCP	SREQP	---	VNQDNLRN	VFLYLFVETS	KMARE	-----K	
YN02_CAEEL/1-0	RPTVIKQYFP	GCNH---NS	KARPMYILR	FQGLD...	K	GMLRSCGVEN	LVKLTISIC	DGLQR	---AAE ATRKLGTPIS	
YJ05_YEAST/1-0	NTELQNVGIL	TFDA---NG	ANKKATWNL	YGQLV...	K	KKELFQNVDK	FVRYRGLM	KGLSL	-----LDFTSSDNN	
YQF6_CAEEL/1-0	SIAPFLQFIA	SSRL(4)WS	EHNALFVFR	AWSQP...	K	EFIKTFKTS	YLLHCFGYS	MLQQLLRRE	KKQSADKGPV	
017907/1-0	PPPECLEKYCG	YGLL---G	TERPILMSL	LGNVDV...	E	GLLRSVASLD	YIKFSAAE	KGMKL	---CEE KAKESGRPFE	
5824562/1-0	NHPIKHKWKY	GITG---PSGH	MDNVIVNIEQ	CGKTDY...	T	GMMETYSILE	VMRARMVDL	QMLHH	---VM ELEAKTGQA	
P78783/1-0	NFVKASMYFI	WGQ-----	KKRAIVFLN	LHNFIP...	---	PKNTKDMEE	LKALILYAM	NARLF	-----LDSEQNAK	
YN04_YEAST/1-0	ENETGKQVIL	GF-----	NAKRPLYMK	NGR	---	QNTSSFR	QVQELVYMM	TATTV	-----APQGV	
YAS2_SCHPO/1-0	DDDFVRQLRI	GKCFI-FGE	KHNRPVCIYR	ARLHK	---	VGDVSPES	VERLTWVME	TARLI	-----LKPPIE	
NF1_HUMAN	EFKAKTSLI	FYQA---GTS	KANPIFYV	ARRFK	---	TGQINGD	LIYHVLLTL	KPYVA	-----KPYEII-	
Q9XVU9/1-0	GKDCERFHI	SQLN--YEVT	SKNLHVFOK	MEGTDI...	K	EILKVMPLS	VLSYFMLQ	NFSRA	---MA HTERKTGKTS	
Consensus/60%	pchhnpbbb.	sh.....D	+pGpslbbb.	hspH.....	...	pshs.pc	b1+bb1bbbE	phbpp.....s.bp	

Dbl3	RFTIILDRRL	DT----	---	WSSLKIS	LQKISA.SFP	GNLHLVLR	PTSF--	LQRT	FTDIGPWSQ	EDFMLLIPVV
AAD25756/1-0	SLLOIHLKLN	APGVSR...	---	TEIIVGIKRV	IETQD.NYP	EFVSRNIFN	VEFW---	FYA	MRAVLSPELT	QRTKSRVVA
A49678/1-0	YLHGLTSDN	KPS----	---	LSWLRLDA	YREFDR.KYK	KNIKALYTVH	PTM---	FIKT	LLILFKPLIS	PKFGQKIFYV
T00939/1-0	QMVMMIDPHG	YSLAN----	---	VSLRRTTKET	AHVLOE.HYF	ERLAFAVLYN	PEK---	FFEP	FWKVARPELE	PKTRKVKFV
O35239/1-0	GLVFFIYDCMG	SNYAN----	---	FELDGLKKV	LNLKGA.AFP	ARKKKVLIVR	APW---	FRV	PYSIISLLAK	DKVREIPIL
CAA15638/1-0	GEVQIFDMKG	TTMRHI...	...	RLTISTLRAY	IKFLQL.AFP	VRLRAIHMIN	CPT---	YLDR	IVSVVVKPIS	DEVFKLIRFH
AAF25952/1-0	GFTVIIDMRG	NG-----	---	NCSTNAKTI	LKVLOE.HFS	ANHNVVLIK	PDN-----	F	WQQRASISS	HKYKFEITTV
NIP2_HUMAN/1-0	YLNAGTTRRK	MPS-----	---	LGWLKRC	YQQLDR.RLR	KNLKSIIIVH	PSW---	FIRT	LLAVTRFELS	SKFSQKIRYV
CAB55301/1-0	RFTIILDRRL	DT----	---	WSSLKIS	LQKISA.SFP	GNLHLVLR	PTSF--	LQRT	FTDIGPWSQ	EDFMLLIPVV
YUQP_CAEEL/1-0	YMHYIFDLEA	LNFDPD...	...	GVVNGPFRVS	WQLVGO.HYR	EFIDKFIIVN	SFS---	YINV	LWSALSFP	EQSKQRIVFA
Q9ZWD6/1-0	STTTIILVQG	LGIKNF...	...	PTAANLVAAM	SKIDNS.YYF	ETLHRMYIVN	AGTG--	FKKM	LWPAAQKPLD	AKTIAKHVL
Q91870/1-0	GLIFIVDMIN	SSYGN----	---	FDYELCVKI	LNLKGA.AFP	ARKKCVFIVS	SELW---	FRA	PFAVLRIVR	EKLRERVTV
O43304/1-0	SWTCLLDLEG	LNMRLH...	...	RPGVKALLRM	IEVDED.NYP	ETLGRLLIVR	APR---	VFPV	LWTLISPEIN	ENTRRFLIY
190854/1-0	GFCIIEFKG	FTMQQA...	...	SLRTSDLRKM	VDMLQD.SFP	ARFKAIHFH	QPW---	YFTT	TYNVVKPEL	SKLLERFVH
AAF02821/1-0	MHSTVQKDDN	SPG-----	---	ITILRWI	YEDLPS.DIK	DRLQLVYFTH	PGLR--	SRLV	IATLGRLLS	GGLYWKIKYV
CAB51563/1-0	TLALLINKFS	SSNRS----	---	NPSVGQKQV	LNIQLT.HYC	ERLGRALVIN	IEW---	AVWG	EFKLSPEID	PITREKLFN
KAR1_RAT/1-0	GFTVIIDMRG	SK-----	---	WDLIKPL	LKTLQE.AFP	AEIHVALIHK	PDN-----	F	WQKQKINFGS	SKFIFETSMV
O48940/1-0	KFVGAELKGC	WGYSN----	---	SDVRGYLSA	LSIQLD.YYP	ERLGLFIVN	APY---	IFMK	VWQIVVPSID	NKTKKIVFV
O23217/1-0	QMSWLDFTG	WSMAV----	---	NPPMKTTRT	IHIQON.YYP	ERLGIAPLYN	PPR---	LFQA	VYRAAKYELD	PRTAKVKFV
A53057/1-0	GLTVVDFMEN	VGSKHM...	...	KPGLDMYLIL	LQVLED.NYP	EMMKRLFVIN	APT---	LFVP	LYKIVKPLIS	EDMKKIVFL
4437/1-0	TSCTIMDLKG	ISISSA...	...	YSVMSVYREA	SYISON.YYF	ERMGFYIIN	APFG--	FST	AFRLKPELID	PVTYSKIFIL
Q23130/1-0	YFHYGLRSHN	K-----	---	PPVRWLFQA	YKQLDR.RFK	KNLKALYVH	PTR----	FIRI	IFSLFKGHS	SKFENFHYV
O48939/1-0	TCIKVLDMTG	LKLSAL...	...	NQIKLLTII	SSIDDL.NYP	EKTNTYIVN	APY---	IFSA	CWKVVKPLQ	EDTRRKHQVL
SC14_SCHPO/1-0	TSCTIMDLKG	VGITSI...	...	HSVYSYIRQA	SSISQD.YYP	ERMGFYIIN	APWG--	FSS	APNLKGFED	BATVKKHIL
CAB63153/1-0	SIIQVTDLKD	MPK-----	---	RELRVASNQI	LSLQD.NYP	ELVATKIFIN	VEW---	YFSV	IYSMFSPELT	QRTKSKIVMS
Q22824/1-0	GVIIMDLGC	FSDMLL...	...	TPTLKVYMSL	LTMQLN.IFP	DFARRIYIIN	CDA---	MMSA	VYAMVSPVLS	SQTRKVV--
Q17910/1-0	SLRRIVNMDG	YEINPFT.MV	...	FVTSGLTAYY	SQLFHFENYF	ELVTPVDMVN	IAKW---	IHV	PYKIAKAMP	TGFSEKFRLH
DBS_HUMAN/1-0	PILVIDRRR	DK-----	---	WTSVKAS	VLRTAA.SFP	ANLQLVLRV	PTGF--	FQRT	LSDIAPKRN	DDFKMVPV1
CAB62036/1-0	ELTVVDCVCG	LSPLR----	---	IPMQMRRSC	SSIQD.HFP	NRLGCLFIR	LFP---	VVRV	ISQTFIQIR	PITRKSIVRIE
AAF18525/1-0	SFVVFSDFRN	APGLGQ...	...	RALWQIFIKR	VKQFED.NYP	EFVAKELFN	VEW---	YIPY	YKTPGSIIS	PRTKSKMVS
O24659/1-0	KFVCIVDLKG	WGYSN----	---	CDIRAYIAA	IEIMQN.YYP	ERLGRALMH	VPY---	MPMK	AWKMIYFID	NVTRDFVVFV
TPPA_RAT/1-0	GVKAIIDLEG	WQISHA...	...	QITPSVAKKI	AAVITD.SFP	LKVRGIHLIN	EPV---	IFHA	VFSMIKPELT	EKIKGRHILH
NF1_DROME	FHTCSNRFR	R-----	---	TEFLQW	FVLPET.VAY	ENVHAVIYN	CNS---	WVR	EYTKFDRIL	APLKGKRLK
AAD23650/1-0	QSTTILVQG	VGLKSF...	...	KAARDLLQRI	QKIDSQ.NYP	ETLNRMFLIN	AGSG--	FRL	LWSIVKSELD	PKTTAKHVL
Q93546/1-0	SVIYIMDLTG	IKFDKR...	...	TLLTGGLSAI	SAPMAE.HYV	ELVHSFVLVN	VPA---	FISA	IWTIAKPLP	ERTRNKCNIL
Q22467/1-0	GTSVIFDLG	LSMVQI...	...	LAALKVVTTM	LSQLQE.MFP	DVIRKIFIVN	TPT---	FIQV	LWSMISPLCA	KQTQQVKIL
Q06705/1-0	STTIIDFQNG	FMSMN----	---	MDYAPVKFL	ITCFEA.HYF	ESLGHLLHK	AEW---	IFNP	IWNLIKWLD	PVVASKIVFT
O81806/1-0	VHTGQRSEN	EPG-----	---	ISALRAI	YDAPV.NVR	DNLQEVYFTH	PGLQ--	SRLF	LATCGRFLS	GGLYGLRYI
AAF19259/1-0	MALMVFDMEG	LSLKHL...	...	KPAVEVYQOF	FSILEA.NYP	ETLKNLIVR	APK---	LFPV	AFNLVKSMS	EETRRKIVIL
Q921J8/1-0	TIVMIFDCG	LGLKHF...	...	KPLVEVYQEF	PGLLEE.NYP	ETLKFMLIVK	ATK---	LFPV	CYNLMKPELS	EDTRKIVVL
O76636/1-0	SVIYIIDLGG	LKFDDA...	...	SIVTGPYRIL	WASVYT.AYP	EWINTLFLIN	APS---	FMTL	LWKAIGPLP	ERTRNKVRIC
O15915/1-0	QPCFIVDYKD	FGSGN----	---	MDMKTNLEA	MHFLLD.HCP	ERMGQSLFD	PEA---	LWF	AKKISPEIN	EVTLSKVRFI
YNX1_YEAST/1-0	SLALLIDFKD	YDPVPK(5)K	...	IPPIGVGKEV	LHILQT.HYP	ERLGRALTN	IPW---	LAWT	FLKLIHPID	PLTRKIVFD
O01324/1-0	GGLFIMDLG	ISFSPK...	...	SVLTGPYRIM	WGTLFD.HYP	QLLQKIIIVN	APS---	FVNV	LHQACSPLE	EDYKKEIVIT
BAA90344/1-0	KMVVLDQFS	WTLGS----	---	TPLKVIRET	VNVLOD.CYP	ERLGLAILYN	PPR---	IFES	FWKIVKPELD	HETYKVKFV
Q9ZM01/1-0	QFVILFDIA	A-----	---	AVLLVTLIV	LFSFSL.NVE	LLILCNSTAH	LFP---	FFFF	FIYKTNPKLL	QISISF----
BAA90976/1-0	YFHTLTSEYN	H-----	---	LDSDFLKKL	YDVVDV.KYK	RNLKAVYVH	PTF---	RSKV	STWFFITVSV	SGLKDIHHV
O61528	GFLVVIDMRG	K-----	---	QWTNVRRH	LKALSS.IES	SSTVQVFIK	PER-----	F	WEKQAKQMSL	GTWDFEVEI
YN02_CAEEL/1-0	SWSLVVDLGG	LSMRHL...	...	RPGVQCLLKI	IEIVEA.NYP	ETMGQVLVVR	APR---	VFPV	LWTLISPEID	EKTRKFMVS
YJ05_YEAST/1-0	YMTQVHDYKG	VSVWRM...	...	SDIKNCSKTV	IGIFQK.YYP	ELLYAKYFVN	VPT---	VFGW	VYDLIKKQVD	ETTRKIVVVL
YQF6_CAEEL/1-0	QFIVIFDLNT	VNIIDVYVNM	...	SGYMKLWQIR	SELWQD.WFP	EMVQRIYITN	PPR---	LLGL	LWKVARVFS	EENLKRTEI
O17907/1-0	QMTLVFDLEN	ITSAHF...	...	KQFASSFTTL	VSLFQD.HYP	LFIRKILIR	APEM--	ARIA	YASITAILQD	PITRLVEMPS
5824562/1-0	WILYVMDITG	LQYNKK...	...	DLVTGSMKSL	ADFMAD.HYV	EMIKVGVVKN	AQTVN--	VPYK	KIRVVVKFK	AGRLRWWRV
P78783/1-0	GVLGVLDITY	FSRKN----	---	IDLDFARVF	AETPON.YYF	EILGQALIVG	SGFRMALFEG	VMSIGYELD	PEVRSWFC	
YN04_YEAST/1-0	KITVIVDFKS	YKEPGIITDK	...	APPISIARMC	LNVMQD.HYP	ERLAKCVLIN	IPW---	FAWA	FLKMMYFELD	PATKAKIVFL
YAS2_SCHPO/1-0	TATVVFDMTD	FMSMN----	---	MDYGPLKFM	IKCFEA.HYF	ECLGECIVHK	APW---	LFQG	VWSIKSWLD	PVVVSVKFT
NF1_HUMAN	---VVDLTH	TGPSN----	---	RFKTDPLSKW	FVVPFG.FAY	DNVSAVIYN	CNS---	WVR	EYTKYHERLL	TGLKSKRLV
Q9XVU9/1-0	SVVCLLDLKG	LNLMDFMNPL	...	SGPAQLARIV	VQVWAE.YES	EHLCKLLIN	PEG---	IISV	MWQTKRLVD	PNTVEKLAFL
Consensus/60%	.bshlbdBct	bsh.....sbssb+ph	bpblps.pap	-plpphblp	sPB...	bb.s	baplhpsFls	ppscpKl.bl

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Db13
AAD25756/1-0 ML.---SSVS D LTYID..D KQTPELNT LQ...
A49678/1-0 RP.--AKVRE T LKYIP..A DDEEVQY F KT...
T00939/1-0 NY.-----LS E S EHV KLEQ LGI ERQV LKY DDFLK
YS.DDPNTKV IMEENFD..M EKMELEAF N DD...
O35239/1-0 T.-----TS E V T Q H I P . R E C L E K N L A Y V K ...
CAA15638/1-0 T.-----QSIN T L Y E F V P . R E M L E E E Y A G A G ...
AAF25952/1-0 S.-----IE S N K I V E . . S H Q L T G D F E Q Q L ...
NIP2_HUMAN/1-0 F.---NLAEI A E L V P M E . . Y V G I E E C I K Q V D Q E L N
CAB55301/1-0 ML.---SSVS D LTYID..D KQTPELNT LQ...
YUQP_CAEEL/1-0 G.---SNWKE E L D I V D . . K E C L E F E R Y A M I P ...
Q9ZWD6/1-0 EP.---KSLF K E H V I D . . S S Q L E E F L A S C S ...
Q91870/1-0 K.-----AH E V N H I P . K E S L E E H L A T S K ...
O43304/1-0 SG.SNYQGP G V D Y I D . R E V I E D F L A E S V ...
190854/1-0 G.-----DDL S G F Y Q E I D . E N I L E S D F A T L P ...
AAF02821/1-0 SR.-----LQ Y W E D I K . . K G E V E I P D F V K N H ...
CAB51563/1-0 EP.-----D R Y V P . K D Q L D S N E F S L H ...
KARI_RAT/1-0 S.-----VE G T K L V D . P S Q L T E E F D S L D ...
O48940/1-0 EK.--NKVKS T L E E M E . . E S Q V E E I F P S L P ...
O23217/1-0 YP.KDKASDE L M T T H F D . V E N L E K E F A E A T ...
A53057/1-0 G.---GDYKD T L E Y I D . A E E L E A Y L A T K S ...
4437/1-0 G.---SSYQK E L L K Q I P . A E N L E V K F A K S E ...
Q23130/1-0 MC.---IDEL E N A L S V A . R L N L E S P I R D H D K S F S
O48939/1-0 P.---GCCGRD E L T I M D . . Y S S L E H F C R R E G S ...
SC14_SCHPO/1-0 G.---SNYKS A L L E Q I P . A D N L E A K L A N C Q ...
CAB63153/1-0 KE.---GNAAE T L Y K F I R . P E D I E V Q Y A L S R ...
Q22824/1-0 --.-----FA I S Q Y A D . S H K L E G D R T K I A V ...
Q17910/1-0 DR.---HFIE T L T E D I N . I D D I E V S L A N D A ...
DBS_HUMAN/1-0 ML.---SSVP D H G Y I D . K S Q L T E D L A T L D ...
CAB62036/1-0 G.---ETFHR V I S E Y I Q . - - T L E S Y L A S N C N ...
AAF18525/1-0 GP.---SKSAE T I F K Y V A . P E V V E V K Y A L S K ...
O24659/1-0 DD.---KSLQE V H Q E I D . D S Q I E D T L A K L A ...
TTPA_RAT/1-0 G.---NNYKS S L L Q H F P . - D I L E L E Y A N E S ...
NF1_DROME FL.---ESPN K L T D F I D A E Q Q K L E G A T L S L D E ...
AAD23650/1-0 G.---NKYQS K L L E I I D . S N E L E E F L A N C T ...
Q93546/1-0 N.---SEWRV E V L K M A E . G S C L E S Y W N D E E D ...
Q22467/1-0 G.---NDWKQ H K E N I G . E E V E F E R W A T R K ...
Q06705/1-0 KN.-----I D E H K F I Q . P Q Y I E R Y L A E N D ...
O81806/1-0 SR.-----V D Y W E H V R . R N E I E M P E F V Y D H ...
AAF19259/1-0 GD.---NWKQ E L T K F I S . P D Q L E V E F A T M T ...
Q9Z1J8/1-0 G.---SNWKE G L K L I S . P E E L E A H F A T L T ...
O76636/1-0 SGN--SDWKT S V Q K H A H . I D N I E K H W A T L V ...
O15915/1-0 NS(5)KRTEA E L L E Y I D . I E N L E Q N L A N L D ...
YNX1_YEAST/1-0 EP.-----F V K Y V P . K N E L D S L Y A D L K ...
O01324/1-0 S.---EPAIG A I Q K H A D . K C F L E S D L A D L E ...
BAA90344/1-0 YS.SDKESQK I M A D V F D . L D K L D S A F A R N P ...
Q9ZW01/1-0 --.---EHTC D Y T Q N S R . R G I R T F V D L S T A T ...
BAA90976/1-0 D.---SLHQL F S A I S P E . Q I D F E P F V L E Y D A ...
O61528 S.-----F E S L K I I D . S S H L E K T V A S Y P ...
YN02_CAEEL/1-0 GG.SGGDLKE E R K H I E . E K F I E D F L A S C L ...
YJ05_YEAST/1-0 TD.-----G S K C Q Y L K . - D C P Y E G Y A K D K ...
YQP6_CAEEL/1-0 S.-----D K S D L A G . S S C R E G E F V N T V P ...
O17907/1-0 E.---SDWKW S E A Q I V N . L D A W E M Y W A N L V ...
5824562/1-0 G.---NRNFG L G V F H S D . E E Q V A D F I A K Q V ...
P78783/1-0 K.-----P A Q V S G Y I D . S K Y I E L S M H I Q F D ...
YN04_YEAST/1-0 G-(6)SDWKE E L L K Y I D . P E E L E E E L A T L K ...
YAS2_SCHPO/1-0 RN.-----Y R D Q Q Y I N . P D N I L K E F A P N P ...
NF1_HUMAN FI.---DCPG K A E H I E H E Q Q K L E A A T L A L E E ...
Q9XVU9/1-0 SN.-----V P D K K Y L E . P E A I E V E Y A T Y R ...
Consensus/60% s.....p.bp pLhcb1c... ppLP.pbGGp .s...

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Table S2. siRNA sequences of confirmed hits of the library screening

Gene	First round siRNAs		Second round siRNAs	
	siGenome		ON-TARGETplus	
SPATA13	5'-GAAGAAACUUGCCAUGUUA-3'		5'-CCACACAGGAACACGGUGA-3'	
SPATA13	5'-GGAGAGCAUCGACAAGUA-3'		5'-GUACCAUGCUCUAAAAGCGA-3'	
SPATA13	5'-GGACCAACGUCAUCCGGGA-3'		5'-CUGCAACCUCAGCGUGAA-3'	
SPATA13	5'-GCGCAGCUAGCCACUAAUU-3'		5'-GGACCAACGUCAUCCGGGA-3'	
VAV3	5'-UAAUAGAUCUUCAGCAGUA-3'		5'-GAAACAUUAUCACGACUUU-3'	
VAV3	5'-GCAGAGACCGAACUUAAUA-3'		5'-GCAAUUAGCAUUAAGUACA-3'	
VAV3	5'-GCAAAGCACAUCAAGAUUU-3'		5'-AGUACAAGAUAGCCAAUA-3'	
VAV3	5'-AGACCGAACUUUAAUUAAG-3'		5'-AGACCGAACUUUAAUUAAG-3'	
TRIO	5'-GAUAAGAGGUACAGAGAUU-3'		5'-GUAAGAAGUGAAAGAUUC-3'	
TRIO	5'-GGAAGUCGCUCCUUGACAA-3'		5'-CGACCUAUCCGUAGCAUUA-3'	
TRIO	5'-CAACGGAGAGUCCAUGUUA-3'		5'-GGAAUACAACCACGAAGAA-3'	
TRIO	5'-GAACACCAACUUCAGAUAA-3'		5'-AGAACAGGGUAAUUGCAUUA-3'	
OBSCN	5'-GCAGACAGCGACACCUAUA-3'		5'-GAUGUGAGCUGCAGAUUCA-3'	
OBSCN	5'-AGACAUACCGCGAAGAU-3'		5'-CCAAGGUGGUGUUUUCUA-3'	
OBSCN	5'-GGAAGGACAUACACUCUCA-3'		5'-GCAGACAGCGACACCUAUA-3'	
OBSCN	5'-CAGGAGAGAUCCAUUUGU-3'		5'-GAUGGAGAGUUCGUGAUU-3'	
MCF2	5'-GGUGUAACCGCAAGUUUG-3'		5'-GAACUGGGCAAGAUUAA-3'	
MCF2	5'-GGUAUCAUCUGUUGAAGAA-3'		5'-UACAGAACGGGAUUAAGUU-3'	
MCF2	5'-GGAAGAAGUUUAAUUGUC-3'		5'-GAAGAAGGCACUCGAUGC-3'	
MCF2	5'-GCAACAGGAUCAAUUACA-3'		5'-GAGCUCAGUUAGUGAUUUG-3'	
ECT2	5'-GAUAAAGGAUGAUCUUGAA-3'		5'-GCACUCACCUUGUAGUUGA-3'	
ECT2	5'-GCACUCACCUUGUAGUUGA-3'		5'-CAGAGGAGAUUAAGACUAU-3'	
ECT2	5'-GAAGGGCUCUUAUGACAUC-3'		5'-UACAGCAAUGCCAAACGA-3'	
ECT2	5'-CAGAGGAGAUUAAGACUAU-3'		5'-GUAUAGAGUCGUCUUUCUA-3'	
FARP1	5'-CAGGAAAACUCGUGUCCA-3'		5'-CAGGAAAACUCGUGUCCA-3'	
FARP1	5'-AAGAAGAACUCACAAGGUA-3'		5'-CAGGAGGCAUUUGAAGUUC-3'	
FARP1	5'-GCACGUUGUUGUUAAGUUU-3'		5'-CCGGAAAACUCGGGGAUCA-3'	
FARP1	5'-CAGCGCAGCUCUCUUGAUU-3'		5'-CUUGGAACGUGGACAGAAG-3'	
NET1	5'-GGCAAUAUAUGAAAUGUCC-3'		5'-GGAGGAUGCUAUUUGAUUA-3'	
NET1	5'-GAAGUCGCCUAGUCAAAUA-3'		5'-GAGUCUCCUUCAGUCGAA-3'	
NET1	5'-GGAGGAUGCUAUUUGAUUA-3'		5'-UCACGUCCUUGGCAAUUU-3'	
NET1	5'-UCACGUCCUUGGCAAUUU-3'		5'-UUGGUGAUCUGGACUCUUA-3'	

Reference

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