

figure S1

Sequence alignment of chicken, mouse, and human proteins. The alignment shows conserved regions and specific mutations across the protein sequence.

	10	20	30	40	50	60	70	80	
chicken						MERPVKDGIIYVQHCKFGKRT			
mouse						MESVEPPVKDGILYQOHVKFGKRC			
human						MTRGARLRSARAQLNQLSLDGGTGSGQKGKCEFPSSLSSVSPGLEAAALLAVTMDPLETPIKDGILYQOHVKFGKRC			
	90	100	110	120	130	140	150	160	
chicken	WRKIRAQI	FAASPF	GVARMEKF	DARD--HGT	VSDISL--QRC	AARRVIRLSDC	CVSVPFMGT	ESCPKATAAFYLT	TTEKNY
mouse	WRKVWALLY	AGGP	SGVARLES	WDVRDGLGPAGDR	STGPSRGER	RVIRLADCVSVLPAD	GESCPRDTG	AFLIT	TERSH
human	WRKVWALLY	AGGP	SGVARLES	WEVRDGLGAAGDRS	AGPGRGER	RRVIRLADCVSVLPAD	GESCPRDTG	AFLIT	TERSH
	170	180	190	200	210	220	230	240	
chicken	VLAEEQRDE	WIEQLCQLAF	QGKKEAEQSS	STGLQP---IPMEE	ENCLYSSWQDLTE	EFFPVLVRL	RTTEAAARCE	LHGHYVLA	
mouse	LLAAQHRQS	WVDPICQLAF	PGTGECSSGS	GQAESPKRGFV	PMEEENSYSSWQEV	TEFPVIVQRTEAT	SRCQLKGPYLLV	L	
human	LLAOHQHRAWMGPICQLAF	PCTGEASSG	TDQSPKRGLV	PMEEENSYSSWQEV	GEGFVQFPVV	QRTEAATRCQLKGPA	L	LL	
	250	260	270	280	290	300	310	320	
chicken	LPHSLTLKDAQSQQPLLT	WPYPFLRK	FQDQNIFSFEA	GRRSDSGEFTFT	SPRAELCR	AVAAAIA	CQQQ---	GQE	
mouse	GQDDIQLRET	SKPQACFS	WPyRFLRK	YSGDKGVFSFEA	RRCDSGEGLF	AFSSPRAD	ICGVAAA	AIARQ	
human	GPDAIQLREAKGT	ALYSWPyHFLRK	FSDKGVS	FEAGRRCRCHSGEGLF	FAFSTPCAPDLCRA	VAGAIARQ	ERRLPEL	AM	
	330	340	350	360	370	380	390	400	
chicken	SPQPSAQGLSNQPWGAE	EDPQCSPTLGRAHSGHS	SAYP---SLNLLRF	PVEPEAPAPIV	YAS	IARGQQPHFRPCP	GQ		
mouse	PPCPPLPRALSLPSLEPP	GELREVAPG	FELPTPRKLPLTD	PGPQSLPLLLSP	--TQEGPASGL	YASVCK	---QTSKHTGT		
human	QPCPPLPRATSLPSLDTP	GELREMPPGPEPPT	TSRKMH	PGQSLPLLLGP	--EPNDLASGL	YASVCK	---RASGPPG		
	410	420	430	440	450	460	470	480	
chicken	PLPEHLYENIFTAQPRP	-LAEEEAE	EEEGR-WEL	GCRAPEGHSS-EAAV	PYPARSAPQ	PHTQRWAPGGSRG	GAEEEPSRP		
mouse	--AEHLYENIVCMLEA	SPGLTNGGPEAQ	ECPGGGRSPLGSP	IYHNTEDLSWFGSAQ	DSNLEAQYR	RLL	LELEDEAGSAGRS		
human	--NEHLYENLCVLEA	SPTLHGGEPEPHEGP	-GSRSPTTSP	IYHNGQDLSWFGP	ANDSTLEAQYR	RLL	LELDQVEG	--TGRP	
	490	500							
chicken	KPQRTLRAKLVRL	LSRD--GPGARDWS							
mouse	GAQAGIKAKLVT	LIITRERKKGPAPCDR							
human	DPQAGFKAKLVT	LLSRERRKGPAPCDR							

figure S2

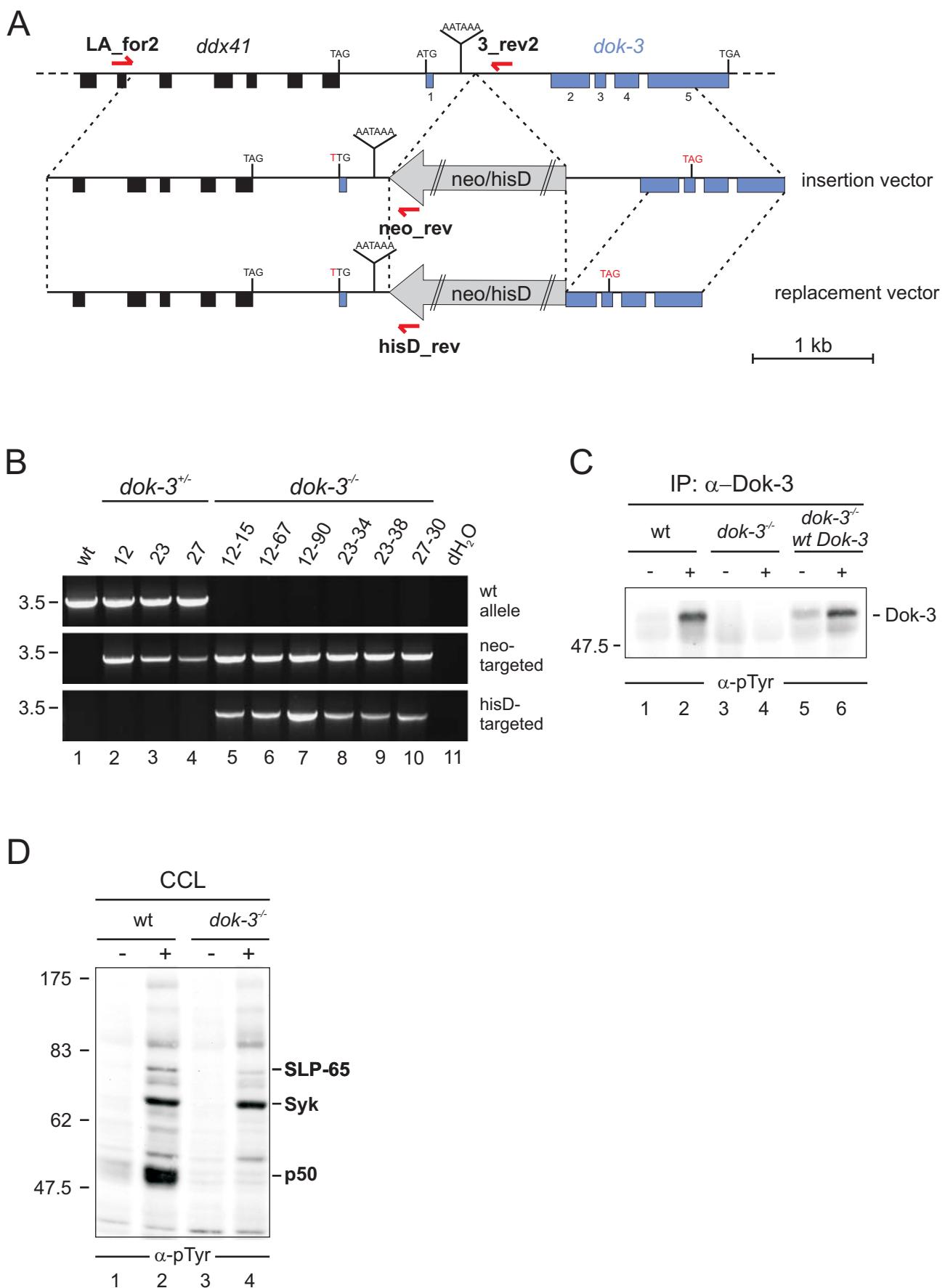
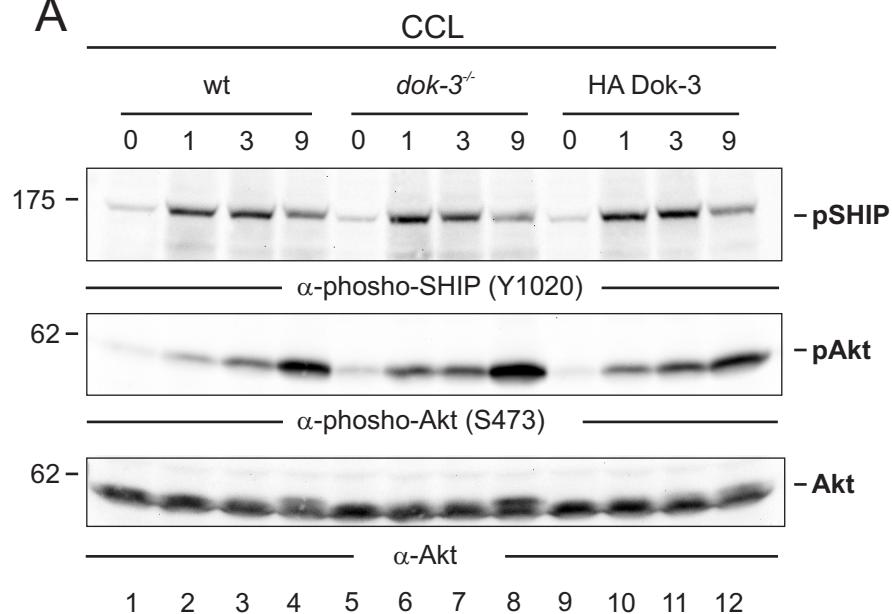
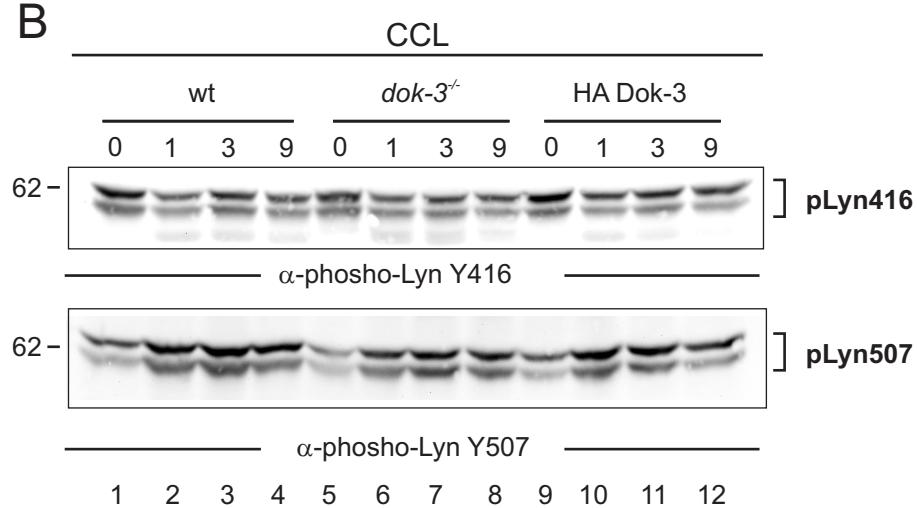


figure S3

A



B



Legends to Supplemental Figures

Figure S1. Dok-3 is evolutionary conserved. Amino acid sequences of chicken, mouse and human Dok-3 orthologs were aligned using the ClustalW algorithm. Conserved amino acids are depicted in red. High or weak biochemical and structural similarity of amino acid positions are indicated in green and blue, respectively. Non-conserved amino acid positions are in black. The degree of similarity is further indicated below the alignment by asterisk for fully conserved and, colon or dot for strong and weak structural similarity, respectively. The PH and PTB domains are accentuated by a single or double underscore, respectively. Avian Dok-3 shares 68% and 62% amino acid sequence homology to its murine and human orthologs, respectively.

Figure S2. Generation of *dok3*^{-/-} DT40 B cells. **(A)** Schematic representation of the genomic locus of chicken *dok-3* (upper panel). Note that exon 1 of *dok-3* is part of the 3' UTR of *ddx41* (DEAD Asp-Glu-Ala-Asp box polypeptide 41). For targeted disruption of *dok-3* alleles, an 'insertion vector' and a 'replacement vector' were constructed, which upon homologous recombination either inserted the neomycin (neo) and histidinol (hisD) resistance cassettes into intron 1 of *dok-3* (middle panel) or replaced 621 base pairs of intron 1 and exon 2 (lower panel), respectively. In both targeting vectors, the ATG start codon of *dok-3* was mutated to TTG and a stop codon was introduced 3' of the last in-frame ATG of exon 3. **(B)** Homologous recombination of the targeting constructs was confirmed by genomic PCR using the primers LA_for2 (5') and 3_rev2 (3') for wild-type alleles and neo_rev (3') or hisD_rev (3') for neo- or hisD-targeted alleles, respectively, whose position is indicated in (A) and which yielded PCR products of 3.4 kilo base pairs (kb) for wild-type (upper panel) and 3.3 kb for neo- or hisD-targeted alleles (middle and lower panel, respectively). PCR products using DNA of wild-type DT40 cells are shown in lane 1, H₂O served as negative control (lane 11). Lanes 2-4 and 5-10 represent heterozygous *dok3*^{+/+} and homozygous *dok-3*^{-/-} clones. Length of DNA marker fragments are indicated on the left in kb. Clones 12-15 (lanes 2 and 5) were generated by the replacement strategy whereas clones 23-34, 23-38 and 27-30 were generated by the insertion strategy. Clone 23-38 was used for further experiments. Note that compared to wild-type control cells, the BCR-induced Ca²⁺ response was diminished in

all *dok-3*^{-/-} clones and to the same extent. Inactivation of *dok-3* alleles had no detectable impact on the cell's morphology and their proliferative capacity (data not shown). **(C)** To confirm lack of Dok-3 protein expression, wild-type DT40 cells (lanes 1-2), *dok-3*^{-/-} mutants (lanes 3-4) and their Dok-3-reconstituted transfectants (lanes 5-6) were left untreated (-) or stimulated through their BCR for 3 min (+) and anti-Dok-3 immunopurified proteins were analyzed by anti-pTyr immunoblotting. **(D)** For analysis of global tyrosine phosphorylation patterns, untreated (-) or BCR-activated (+) wild-type and *dok-3*^{-/-} DT40 cells (lanes 1-2 and 3-4, respectively) were lysed and subjected to anti-pTyr immunoblot analysis. Relative molecular masses of marker proteins are indicated on the left in kDa.

Figure S3. The Dok-3/Grb2 module acts independently of SHIP and Csk. Wild-type DT40 cells (lanes 1-4), *dok-3*^{-/-} mutants (lanes 5-8) and their reconstituted transfectants expressing HA-tagged Dok-3 (lanes 9-12) were left untreated (0) or stimulated through their BCR for the indicated times (min). Cleared cellular lysates (CCL) were analyzed by immunoblotting with antibodies to **(A)** tyrosine-phosphorylated SHIP (Y¹⁰²⁰), serine-phosphorylated Akt (S⁴⁷³) and total Akt (upper, middle and lower panel, respectively) and **(B)** site-specific anti-pTyr antibodies to the activating auto-phosphorylation site of Lyn (Y⁴¹⁶) (upper panel) and the inhibitory pTyr residue 507 (lower panel), which is the direct substrate of Csk. Relative molecular masses of marker proteins are indicated on the left in kDa. As the presence or absence of Dok-3 expression causes little or no differences in the phosphorylation status of the investigated enzymes and/or their downstream effectors, neither SHIP nor Csk appear to be a major target of the Dok-3/Grb2 module.