figure S1

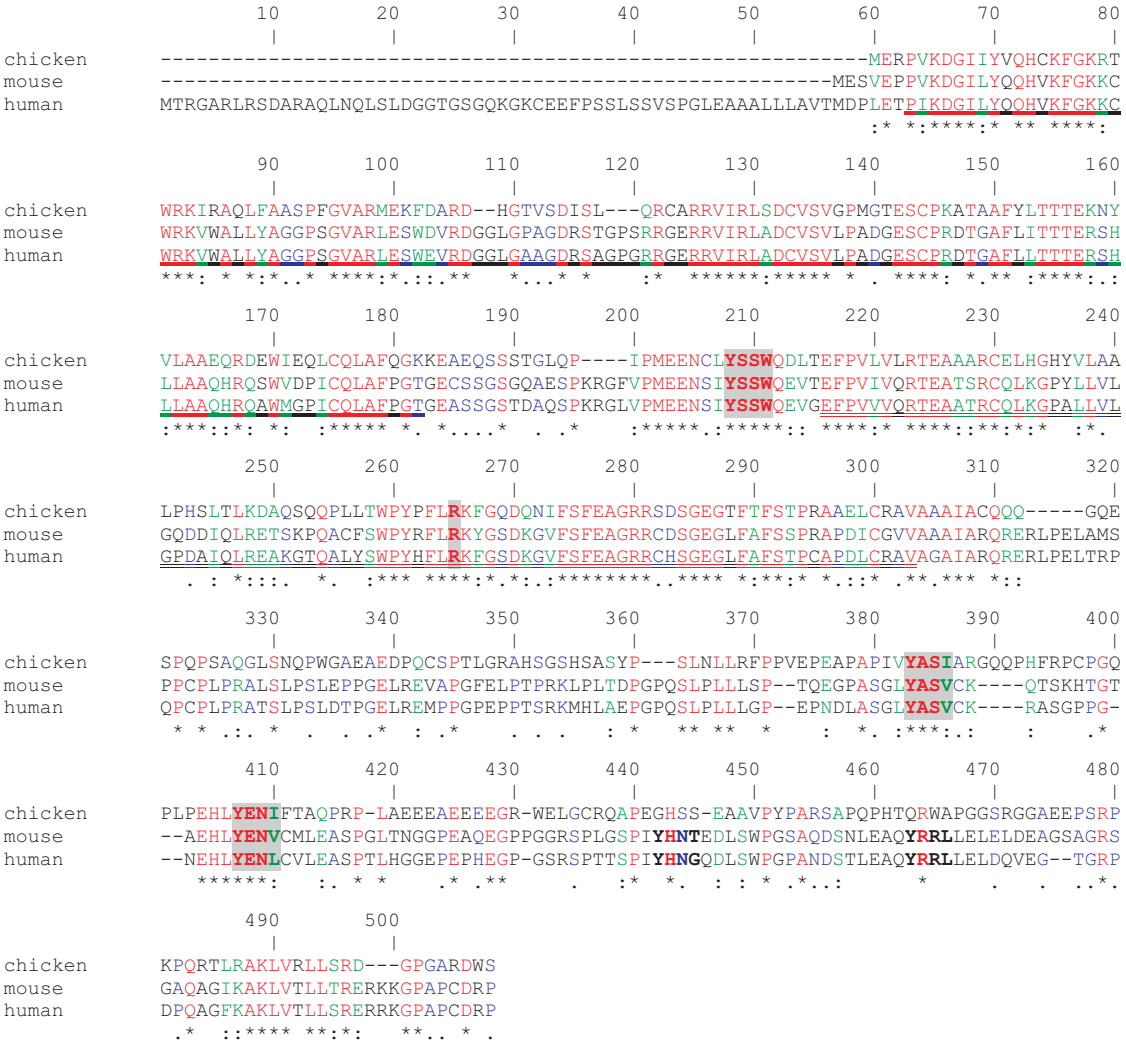
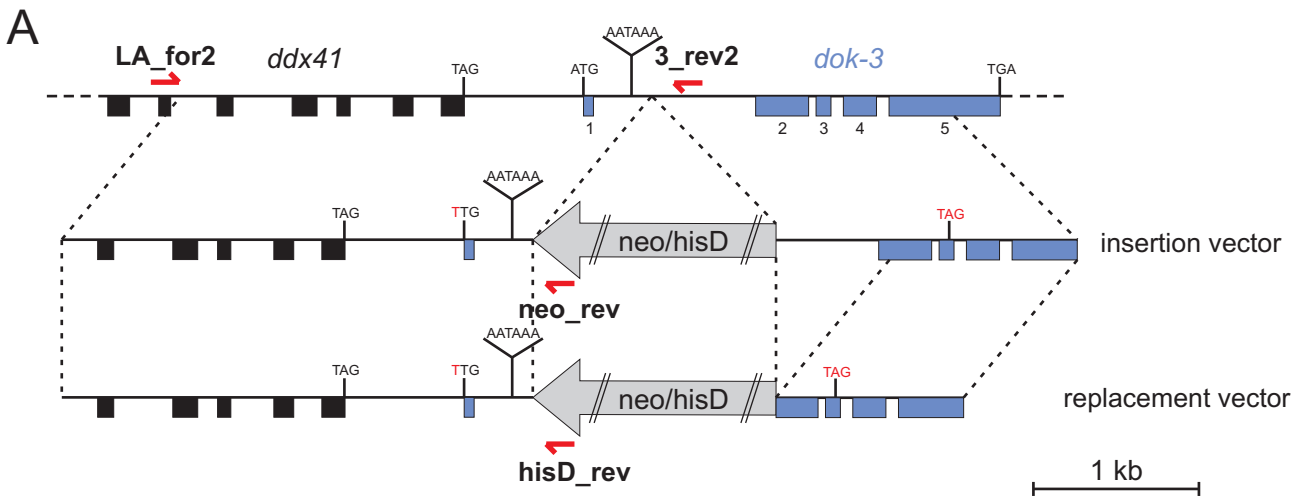
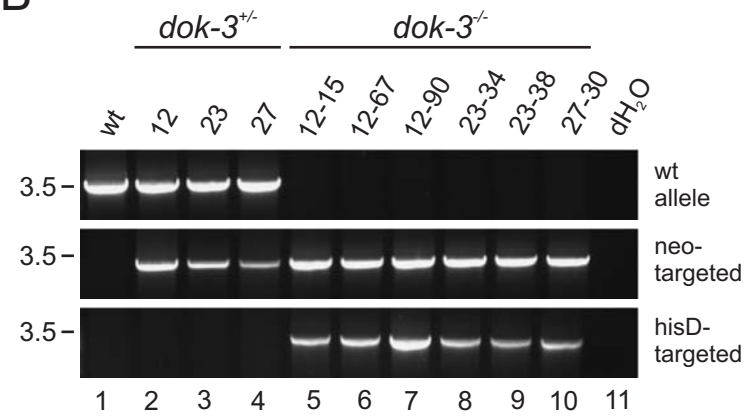
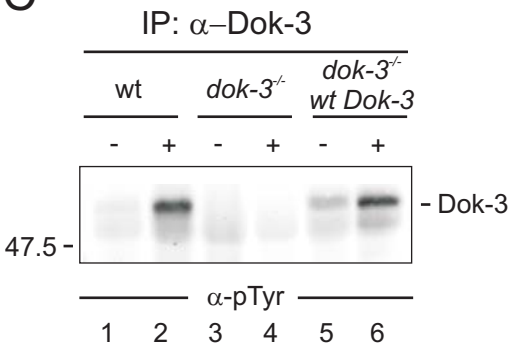


figure S2


A
LA_for2
ddx41
TAG
ATG
AATAAA
3_rev2
dok-3
TGA
1
2
3
4
5
TTG
neo/hisD
insertion vector
neo_rev
replacement vector
hisD_rev
1 kb


B
dok-3+/-
dok-3-/-
wt
12
23
27
12-15
12-67
12-90
23-34
23-38
27-30
dH2O
3.5
wt allele
neo-targeted
hisD-targeted
1 2 3 4 5 6 7 8 9 10 11


C
IP: α–Dok-3
wt
dok-3-/-
dok-3-/- wt Dok-3
- + - + - +
47.5
Dok-3
α-pTyr
1 2 3 4 5 6

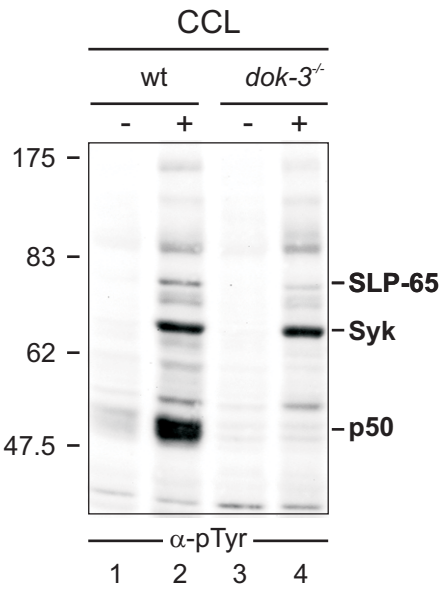
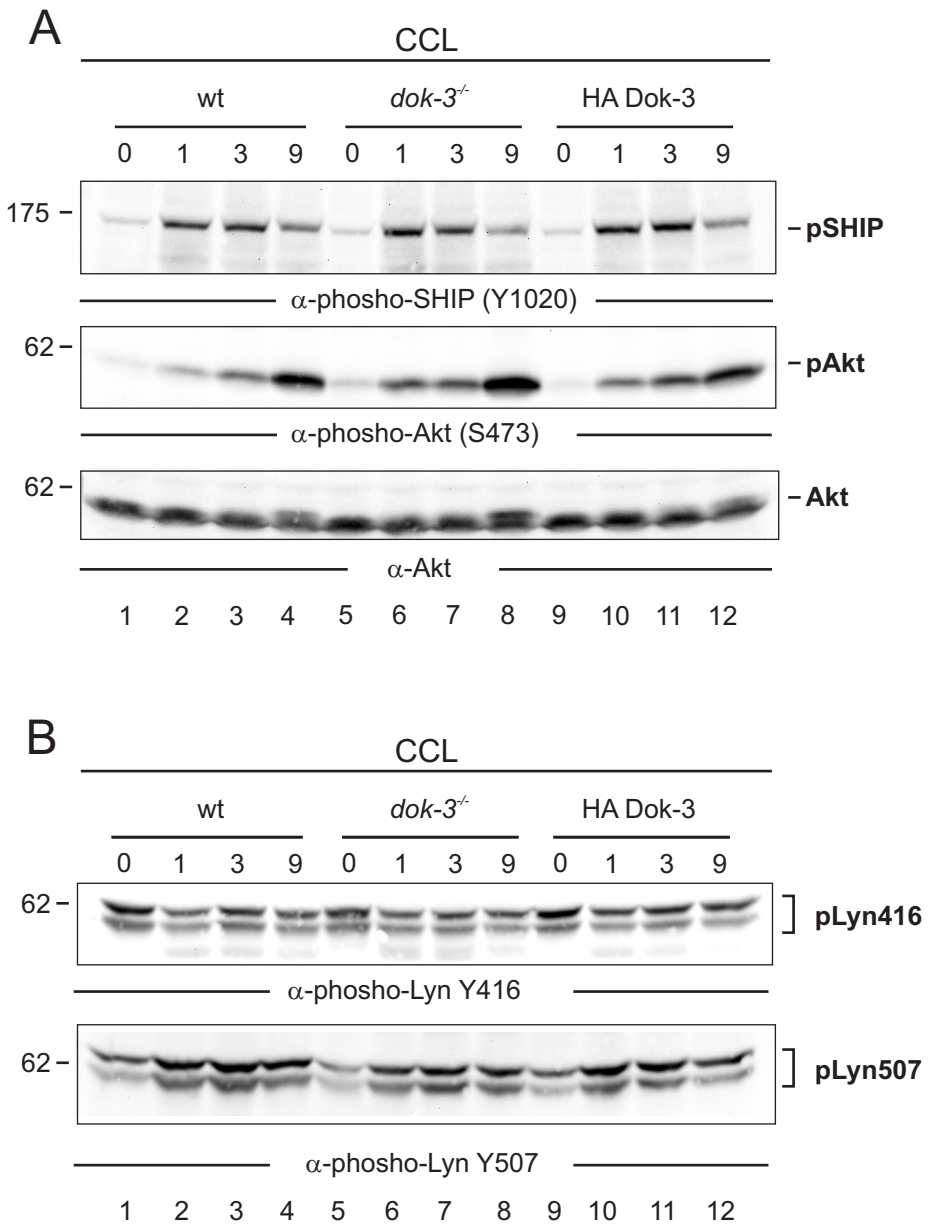

D
CCL
wt
dok-3-/-
- + - +
175
83
62
47.5
SLP-65
Syk
p50
α-pTyr
1 2 3 4

figure S3


A
CCL
wt
dok-3-/-
HA Dok-3
0 1 3 9 0 1 3 9 0 1 3 9
175
pSHIP
α-phosho-SHIP (Y1020)
62
pAkt
α-phosho-Akt (S473)
62
Akt
α-Akt
1 2 3 4 5 6 7 8 9 10 11 12
B
CCL
wt
dok-3-/-
HA Dok-3
0 1 3 9 0 1 3 9 0 1 3 9
62
pLyn416
α-phosho-Lyn Y416
62
pLyn507
α-phosho-Lyn Y507
1 2 3 4 5 6 7 8 9 10 11 12

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 neomycine (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, H_2O 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 Ca^{2+} 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 analyis 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-phophorylated SHIP (Y^{1020}), serine-phosphorylated Akt (S^{473}) and total Akt (upper, middle and lower panel, respectively) and **(B)** site-specific anti-pTyr antibodies to the activating auto-phosphorylation site of Lyn (Y^{416}) (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.