SUPPLEMENTARY MATERIALS

Table 1S. List of unique peptide sequences confirmed by MS-MS analysis. The selections were based on high Mascot scores. The overall coverage of all amino acid sequences (70 aa) of unique peptides was calculated on primary sequences of gamma isoform (247 aa) to be 28.3%. The data was based on 5 independent experiments, and each individual MS/MS data was collected from 15 samples.

MS-MS Spectra	Unique Peptides	Mascot Score	Coverage %	Peptide Sequences
4	3	199	17	**NVTELNEPLSNEER** **ELEAVCQDVLSLLDNYLIK** **YLAEVATGEK**
3	3	132	16	**NVTELNEPLSNEER** **ELEAVCQDVLSLLDNYLIK** **LGLALNYSVFYYEIQNAPEQACHLAK**
3	2	130	10	**NVTELNEPLSNEER** **YLAEVATGEK**
3	2	118	16	**NVTELNEPLSNEER** **ELEAVCQDVLSLLDNYLIK**
Total coverage of peptides			**28.3 %**	**(70 aa of 247 aa)**

Supplementary figure legend

Figure S1.

Novel interaction of Btk with 14-3-3 in primary mouse B cells. Whole cell lysate (WCL) was co-immunoprecipitated with 14-3-3, using pan-14-3-3 antibody. Samples were analyzed by western blot, using anti-Btk to confirm the association of Btk with 14-3-3. Mouse Btk-/- (KO-Btk) cells served as negative control. Quantitative analysis shows the increased association of Btk with 14-3-3 upon activation with anti-IgM (70% increase in wt-Btk and as expected a low

increase 12% in Xid mice).

Figure S2.

Inhibition of Btk interaction with 14-3-3. Namalwa and K562 cells were treated with the non-peptide 14-3-3 inhibitor BV02 (10 μM) and incubated for 24 hours in the cell culture medium. Control samples were incubated with DMSO.

Figure S3.

Btk homologs from different species share a consensus 14-3-3 binding motif. High degree conservation of the consensus 14-3-3 binding motif in Btk from different species. Sequence alignment of the 14-3-3 binding region for Btk.

Figure S4.

Kinetics/dynamics of 14-3-3 binding capacity to Btk in RBL-2H3 cells. Time course of the increase in 14-3-3ζ interaction with phosphorylated Btk during pervanadate stimulation. Phosphorylation of Btk in RBL-2H3 cells increased after serum and pervanadate exposure, and the affinity of Btk for 14-3-3ζ increased as this phosphorylation increased. The cells were lysed after 0, 0.5, 1, 2.5, 5, 10, 15, 30 and 45 min of pervanadate treatment. Lysates were subjected to immunoprecipitation using anti-pan-14-3-3 antibody and blotted with anti-Btk antibody.

Figure S1.

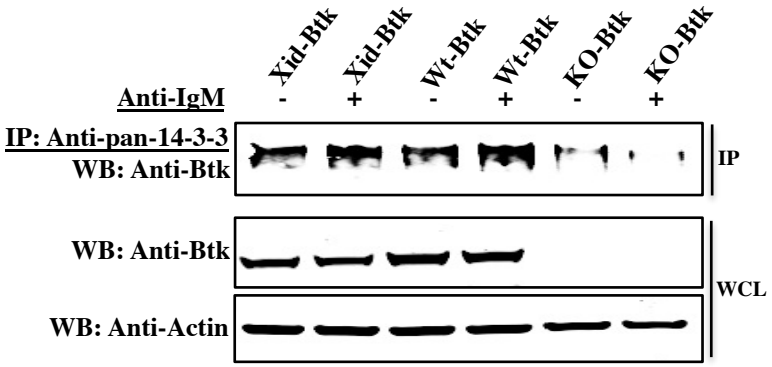


Figure S2.

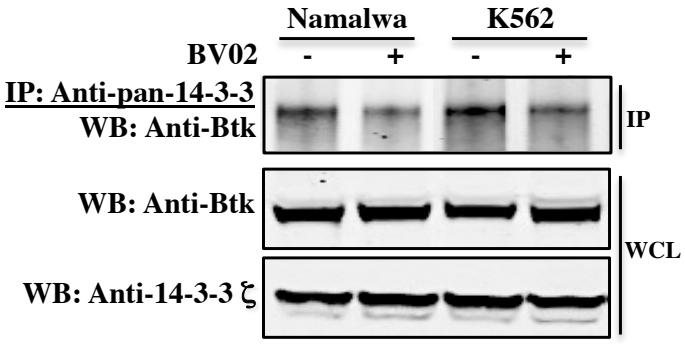


Figure S3.

Serine 51(S51)

14-3-3 binding motif RXRXXS/T (phospho)

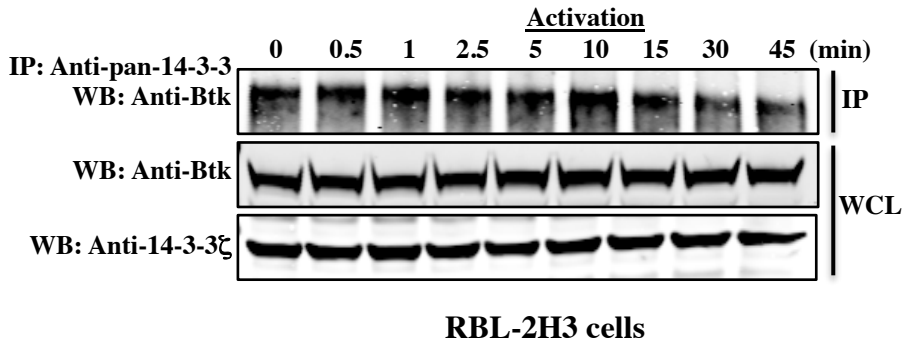
Human	LSYYEYDFE-----**RGRRGS**-----KKGSIDVEK
Mouse	LSYYEYDFE-----**RGRRGS**-----KKGSIDVEK
Cow	LSYYEYDFE-----**RGRRGS**-----KKGSIDVEK
Elephant	LSYYEYDFE-----**RGRRGS**-----KKGSIDVEK
Platypus	LSYYEYDFE-----**RGRRGS**-----KKGSVDIEK
Frog	IIYYEYDFD------**RGRRGS**-----KKGSVDLDK
Chicken	LSYYEYDFE-----**RGRRGS**-----KKGSVDIEK
Fish	IAYYDYDLE-----KGKKKG-----LKGTVGIDK

Threonine 495 (T495)

14-3-3 binding motif RXRXXS/T (phospho)

Human	CLLNYLREM-----**RHRFQT**-----QQLLEMCKD
Mouse	CLLNYLREM-----**RHRFQT**-----QQLLEMCKD
Cow	CLLNYLREM-----**RHRFQT**-----QQLLEMCKD
Elephant	CLLNYLREM-----**RHRFQT**-----QQLLEMCKD
Platypus	CLLSYLRET------**RHRFQS**-----LQLLEMCKD
Frog	CLLNYLKDL-----RGRVSQ-----GDLLSMCSD
Chicken	NGCLLNFLR-----QRRFQP-----AELLEMCKD
Fish	LLSYLREGL-----KQHPSP-----IQLLEMCKD

Figure S4.



RBL-2H3 cells