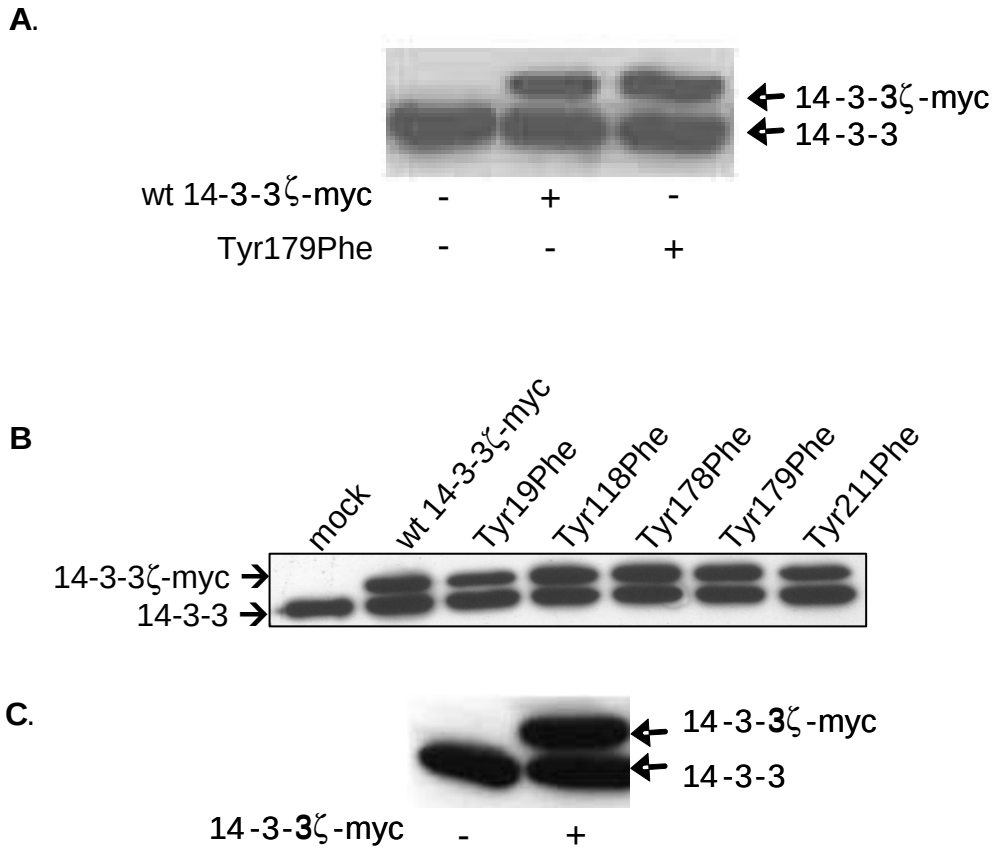
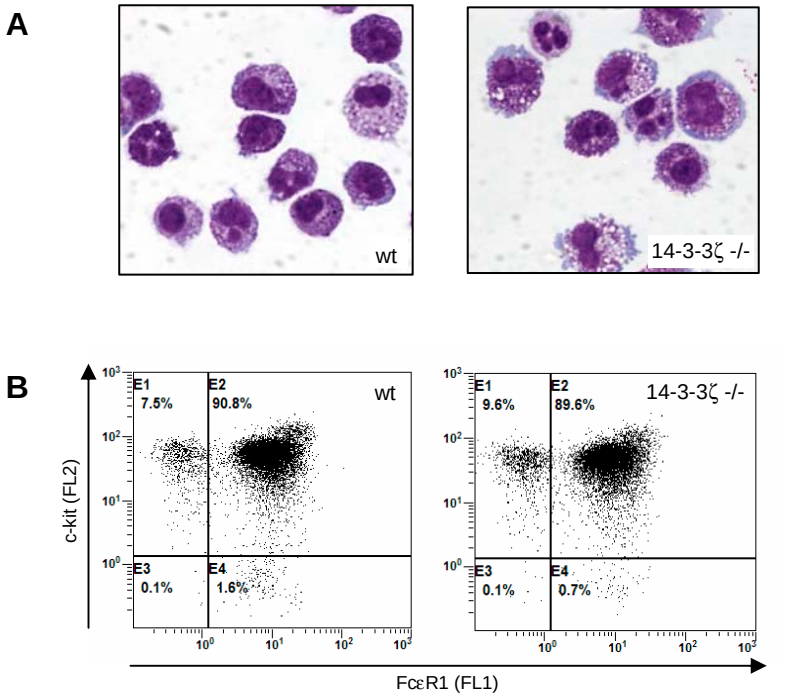


Suppl. Fig. 1. Expression levels of transfected 14-3-3ζ-myc and endogenous 14-3-3. HEK 293T cells were transfected with constructs expressing either wild type (wt) or mutant forms of 14-3-3ζ-myc (A,B). 48 hours after transfection the cells were lysed and lysates were subjected to immunoblot analysis using the EB1 anti-14-3-3 pAb. CTL-EN cells were electroporated with 14-3-3ζ-myc (C). 48 hours after electroporation the cells were lysed and lysates were subjected to immunoblot analysis using the EB1 anti-14-3-3 pAb. Quantification of several experiments indicated that wt 14-3-3ζ-myc and the Tyr179Phe mutant were expressed at an average of 81% (std. dev. -/+25%) of the total endogenous 14-3-3 proteins.



Suppl. Fig. 2. Bone marrow-derived mast cells derived from 14-3-3ζ -/- mice are not defective in differentiation. Isolated cells were cultured in 20% WEHI-3 conditioned medium for 4-12 weeks, at which time >89% of the cells were identified as mast cells by May Grunwald-Giemsa staining (A) and by flow cytometric analysis for cell surface expression of c-*kit* (clone: 2B8, PE-conjugated, eBioscience) and FcεRI (clone: MAR-1, FITC-conjugated)(B).

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                                Y179
1433beta_human                  NFSVFYYEIL
1433beta_bovine                 NFSVFYYEIL
1433beta_mus                    NFSVFYYEIL
1433beta_rat                    NFSVFYYEIL
1433beta_xenopus                NFSVFYYEIL

1433epsil_human                 NFSVFYYEIL
1433epsil_bovine                NFSVFYYEIL
1433epsil_mus                   NFSVFYYEIL
1433epsil_rat                   NFSVFYYEIL
1433epsil_xenopus               NFSVFYYEIL

1433sigma_human                 NFSVFHYEIA
1433sigma_bovine                NFSVFHYEIA
1433sigma_mus                   NFSVFHYEIA
1433sigma_rat                   NFSVFHYEIA

1433eta_human                   NFSVFYYEIQ
1433eta_bovine                  NFSVFYYEIQ
1433eta_mus                     NFSVFYYEIQ
1433eta_rat                     NFSVFYYEIQ
1433eta_xenopus                 NFSVFYYEIQ

1433gamma_human                 NYSVFYYEIQ
1433gamma_bovine                NYSVFYYEIQ
1433gamma_mus                   NYSVFYYEIQ
1433gamma_rat                   NYSVFYYEIQ
1433gamma_xenopus               NYSVFYYEIQ

1433theta/tau_human             NFSVFYYEIL
1433theta/tau_bovine            NFSVFYYEIL
1433theta/tau_mus               NFSVFYYEIL
1433theta/tau_rat               NFSVFYYEIL
1433theta/tau_xenopus           NFSVFYYEIL

1433zeta_human                  NFSVFYYEIL
1433zeta_bovine                 NFSVFYYEIL
1433zeta_mus                    NFSVFYYEIL
1433zeta_rat                    NFSVFYYEIL
1433zeta_xenopus                NFSVFYYEIL
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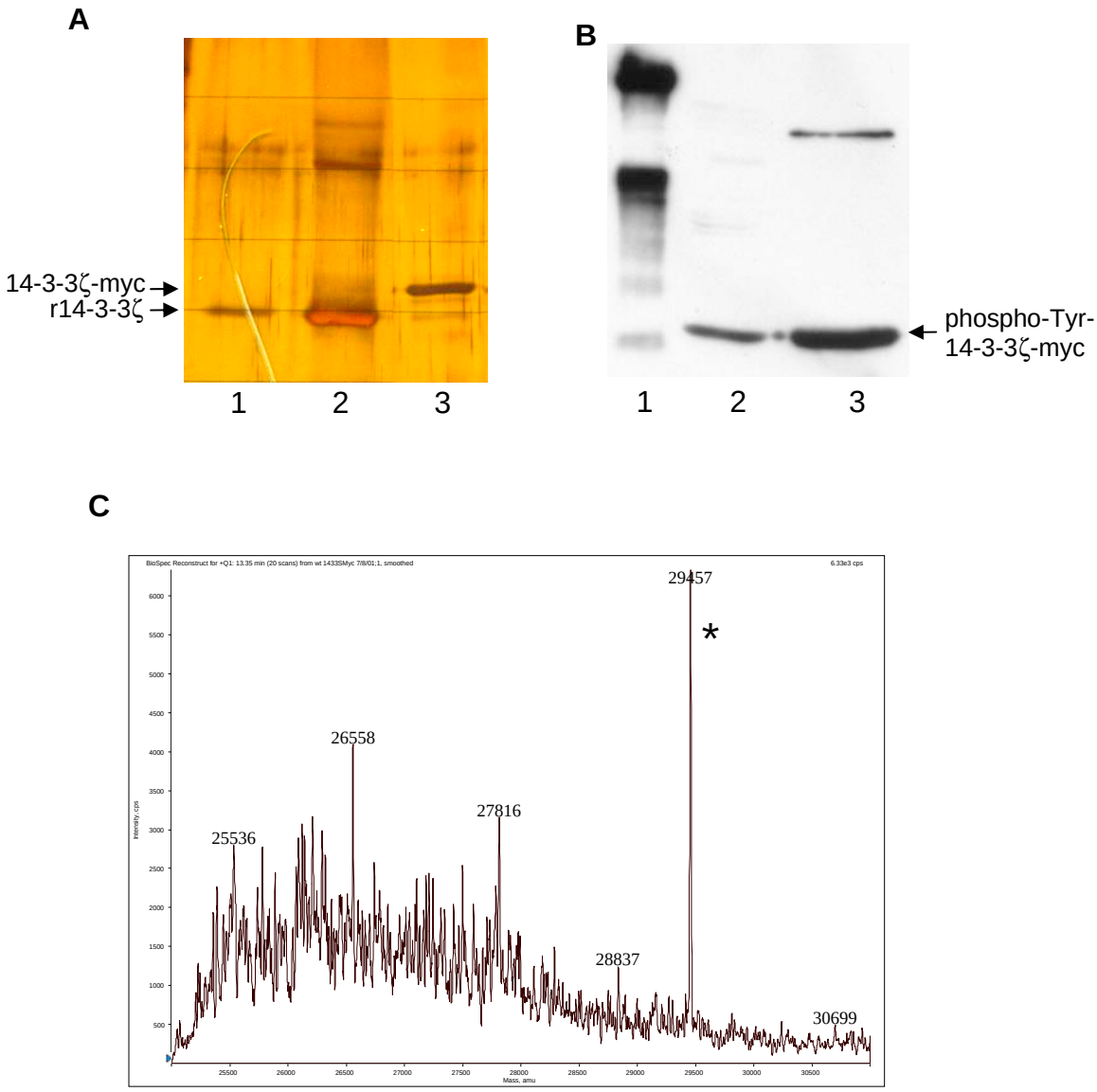
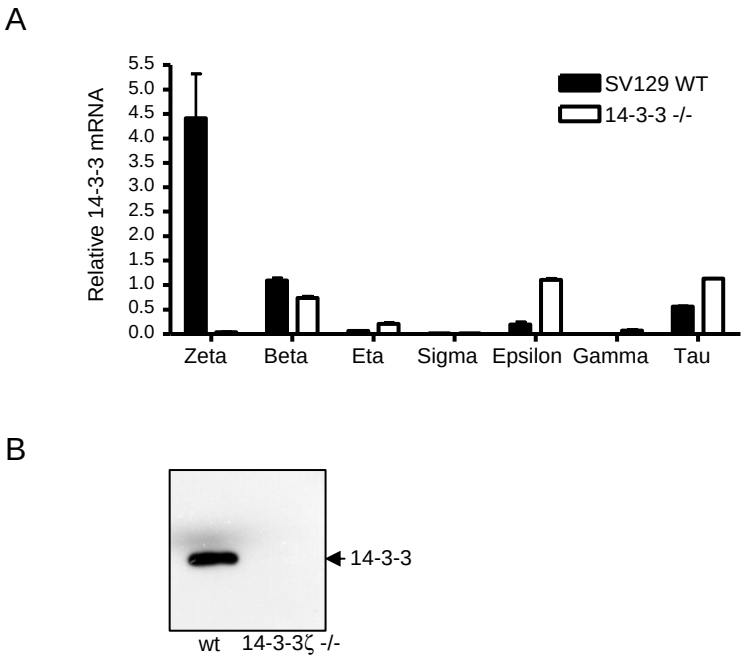
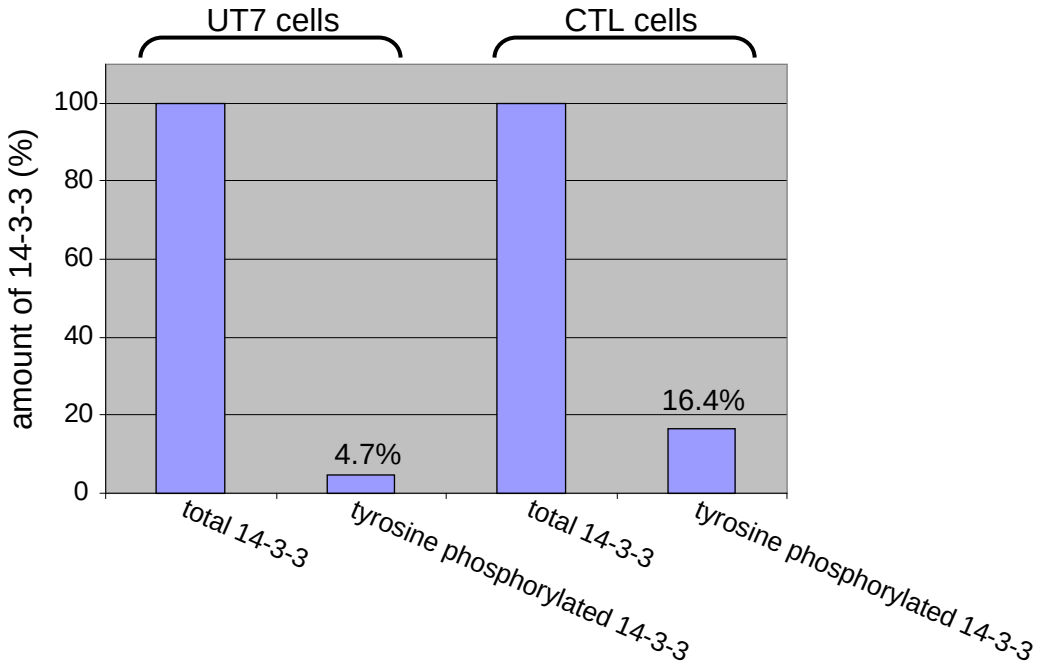
The 14-3-3 family proteins are highly conserved across metazoan species. As shown below Tyr179 is conserved in 1) all species from *Xenopus* to *Homo sapiens* and 2) in all 7 isoforms.



Supplementary Figure 4. Mass spectrometry of 14-3-3ζ-myc. HEK 293 cells were transfected with a construct for the expression of 14-3-3ζ-myc and after 48h, the cells were lysed and 14-3-3ζ-myc was immunoprecipitated with the 9E10 mAb covalently coupled to cyanogen bromide Sepharose beads. 14-3-3ζ-myc was eluted using 0.1M Glycine pH 2.4 and neutralized in 1M Tris pH 8. (A) Purity of the 14-3-3ζ-myc (lane 3) was determined by silver stain relative to 155ng (lane 1) and 1.5µg (lane 2) recombinant 14-3-3ζ (r14-3-3ζ). The difference in molecular weight between the r14-3-3ζ and 14-3-3ζ-myc is due to the epitope tag. (B) Tyrosine phosphorylation of the eluted material was confirmed by 4G10 Western blot with an A431 cell lysate positive (lane 1) and two independent preparations of 14-3-3ζ-myc (lanes 2 and 3). Eluted material was then subjected to ion-spray mass spectrometry (C). The primary mass spectrum was transformed mathematically (using an output mass range of 25,000 to 35,000 mass units) to give the mass profile shown. The observed mass of 29,457 (*) was compared with the calculated mass of 29380 for 14-3-3ζ-myc with *N*-terminal acetylation. The observed mass difference of +77 is consistent with the covalent addition of a single phosphate group (theoretical = +80amu).



Suppl. Fig. 5. 14-3-3ζ represents the major isoform in bone marrow-derived mast cells. (A) $5x10^6$ bone marrow-derived mast cells were lysed in 1 mL of TRIzol reagent (Invitrogen) and RNA was prepared. 2μg of RNA was reverse transcribed using the Omniscript Reverse Transcription kit (Qiagen) and oligo (dT)12-18 (Invitrogen). Real-time RT-PCR was performed in triplicate with the QuantiTect SYBR Green PCR system (Qiagen) on a Rotorgene 6000 series PCR machine (Corbett Research). Relative expression levels of 14-3-3 isoforms were determined using the comparative quantification feature of the Rotorgene software and β-Actin as an internal control. Real-time RT-PCR assays were performed for 43 cycles (95° C for 20 s, 50° C for 30 s and 72° C for 30 s). Supplementary Table 1 lists the primer sequences used. (B) bone marrow derived mast cells obtained from wt and 14-3-3ζ -/- SV129 mice were lysed and subjected to Western blot analysis using the anti-14-3-3 pAb as in Figure 1.



Suppl. Fig. 6. The stoichiometry of Tyr179 phosphorylation. We have examined the fraction of endogenous 14-3-3 in both UT7 and CTL-EN cells that is phosphorylated on Tyr179 following cytokine stimulation. For these experiments, we precipitated either total endogenous 14-3-3 proteins using a phospho-serine peptide (Biotin-NHS-KGGFDFNGPYLGPPHSR(pS)LPDGG) or endogenous phospho-Tyr179-14-3-3 using GST-SH2^{Shc} as described for Figure 1B and quantified the 14-3-3 signals in each pulldown following immunoblotting with anti-14-3-3 antibodies. Quantification of the results from two experiments indicates that GST-SH2^{Shc} precipitates 4.7% of the total 14-3-3 from UT7 cells and 16.4% of the total 14-3-3 from CTL-EN cells following cytokine stimulation. These results are also in line with the amount of PI 3-kinase activity we observe to be recruited to Tyr179 in Figure 4. Because essentially all the PI 3-kinase activity recruited to 14-3-3ζ-myc interacts via Tyr179 (Fig. 4), comparing the PI 3-kinase associated with wt 14-3-3ζ-myc to the total PI 3-kinase activity in the cell following GM-CSF stimulation provides a measure of the PI 3-kinase activity recruited to 14-3-3ζ via Tyr179 and an indication of the stoichiometry of Tyr179 phosphorylation. The results from two experiments indicates that the PI 3-kinase associated with 14-3-3ζ-myc in a Tyr179-dependent manner constitutes 5.25% (standard deviation +/- 0.6) of the total PI 3-kinase activity in CTL-EN cells.