**Fig. S1.** *In vitro* acetylation of TBX5. Bacterially expressed MBP-TBX5 is acetylated by KAT2B (lane 1-0.5 µg, lane 2-1.0µg and lane 3-2.0µg). KAT2B do not acetylate MBP (lane 4-0.5µg, lane 5-1.0µg and lane 6-2.0µg).

**Fig. S2.** Prediction of acetyl lysine residue in TBX5. Web-based tools PredMod (A), PHOSIDA (B) and PAIL (C) are used and Lys234 is predicted in all three sites. (D) Western blot analysis showing acetylation of both TBX5 and mutant TBX5 K234R suggesting Lys234 is not the target for acetylation.

**Fig. S3.** Mapping the acetylation residue in TBX5. (A) Peptide sequences used from acetylated proteins to derive the motif. (B) Sequence logo plot for non-acetylated proteins indicating no enrichment of specific amino acids around the key lysine residue. The stretches of peptides were randomly selected from non-acetylated proteins (Uniprot database). (C) Western blot analysis showing acetylation status of both wildtype TBX5 and mutant TBX5K339R in H9c2 cells. (D) TBX5K339R mutant protein retains KAT2B interaction. Pulldown assay showing the association of KAT2B with wild type TBX5 (lane-2) and mutant TBX5K339R (lane-3). (E) Reporter assays showing the effect of TBX5 lysine mutants on MYH6 promoter. All the mutants altered the MYH6 promoter activity but a significant reduction of promoter activity is only observed with the mutant K339R.

**Fig. S4.** (A) Cellular localization of TBX5-WT-FLAG or TBX5-K339R-FLAG in untreated and leptomycin B-treated cells. Data are shown for Cos7 cells (left) and C2C12 cells (right). (B) Representative images and cellular distribution of TBX5-WT in Garcinol-treated cells. (C) Representative images of the co-localization between TBX5-K339R and endogenous CRM1 outside the nucleus. Results are mean ± SD from 3 individual experiments, n=150-200 cells per condition and per experiment.

**Fig. S5.** (A) Aminoacid alignments of human, murine and zebrafish kat proteins were generated to study their percentage of similarity using ClustalW2. (B) RT-PCR expression analysis of kat2b and kat2a in zebrafish. The analyses were carried out on 36, 48, 60 and 72h post fertilized embryos. H and T denote heart and tail regions and + and – denote +RT (retrotranscriptase) and –RT samples respectively. β-actin1 is used as a control. The agarose gels show the ubiquitous expression pattern of both kat2b and kat2a at different developmental stages. (C) *In situ* hybridization using kat2a and kat2b probes was performed on 48 hpf zebrafish embryos. Expression in the cardiac region is indicated with a black arrow (H=heart), whereas expression in the pectoral fins (PF) is highlighted by dashed circles.

**Fig. S6.** (A) Schematic representation of the CRISPR-Cas target sites for kat2a (exon 1, + strand), kat2b (exon 6, - strand) and tbx5a (exon 2, - strand). The PAM sequence is red bolded. (B) T7 assay confirmed the presence of indels in kat2a, kat2b and tbx5a injected embryos. For kat2a, 6/7 analysed embryos gave a positive result (presence of extra bands of expected size), whereas 7/7 gave a positive result for both kat2b and tbx5a injected embryos (C = control). A diagram of the PCR products used for the assay is shown (TS = target site). Bands of unexpected sizes are also found due to the presence of polymorphic intronic regions in the PCR products used for the assay. (C) Table indicating the number of embryos
harbouring mutations in *kat2a*, *kat2b* and *tbx5a* Cas9+sgRNA injected embryos. Rate of mutagenesis is defined as the average percentage of mutant alleles in all alleles analysed for each individual embryo.