Fig. S1. *In vitro* acetylation of TBX5. Bacterially expressed MBP-TBX5 is acetylated by KAT2B (lane1-0.5 µg, lane2-1.0µg 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.