Dear Editor,

Takotsubo syndrome (TS) is a stress-induced non-ischaemic cardiomyopathy that is more common in women, but is associated with higher morbidity and mortality in males. Also known as broken-heart syndrome, TS is characterised by transient left ventricular (LV) dysfunction independent of obstructive coronary artery disease. TS is a polygenic condition and nowhere is this more evident than the use of positive inotropes, such as isoprenaline (ISO) in pre-clinical models. There is no standard therapy for broken-heart syndrome because the mechanisms underlying the condition remain unknown. Furthermore, there is no consensus on predisposition for Takotsubo and our goal was to better understand the regulatory mechanism as a first step towards improved treatment plans. Suberanilohydroxamic acid or SAHA, a drug approved for cancer treatment by the US Food and Drug Administration has previously been shown to improve cardiopulmonary function. We tested the hypothesis that the cardioprotective benefit of SAHA in a pre-clinical model of Takotsubo is conferred by an epigenetic acetylation/deacetylation (Ac/Dc) axis.

Eight-week-old 129/Sv mice were divided into four groups of five animals. Group 1 received intraperitoneal (IP) saline and served as healthy controls. Mice in group 2 served as a SAHA only control receiving IP injections every third day (100 mg/kg, illustrated by red arrows). The remaining groups (3 and 4) received subcutaneous isoprenaline (ISO, 25 mg/kg) once daily for the first 5 days to induce cardiomyopathy (illustrated by blue arrows). The remaining ISO-injury mice were therapeutically administered SAHA (ISO/SAHA). Group 4 commenced SAHA treatment on the day after ISO administration was concluded (ISO/SAHA, referred to as Reversal or “REV”) (Fig. 1a).

ISO significantly increased picrosirius-stained collagen content in the infarcted LV (Fig. 1b, group 3). SAHA treatment attenuated this ISO-induced collagen content (Fig. 1b, group 4). As protein accumulation is characteristic of TS, we assessed collagen deposition. SAHA treatment attenuated ISO-induced injury by decreasing collagen. Quantitative histological analysis confirmed that LV collagen content was ~3% for untreated and SAHA only groups when compared to 8% in the ISO-induced injury group. Collagen deposition in the REV group was reduced to 4%. These findings indicated that treatment with SAHA reduced ISO-induced LV collagen deposition.

Since the area of ISO-induced injury was vastly improved following SAHA treatment, we assessed protein accumulation using chemical mapping. For each tissue section, an FPA-FTIR image of 6 × 8 grids was collected on the area of infarction of 1.04 × 1.38 mm² (Fig. 1c). The absorbance spectra showed a decrease in the intensity of the amide I band (α-helix secondary protein structure) and that was correlated with an increase in the amide II band (Fig. 1d, i). The amide I/II intensity ratios obtained from the inverted second derivative spectra confirmed these changes, and showed a decrease in all treatment groups (ISO = 1.8, SAHA = 1.8, ISO/SAHA = 1.6) when compared to the control group (2.0) (Fig. 1d, ii). The SAHA group had an increased intensity at 1515 cm⁻¹ that was associated with α-helical protein structures. Given that collagen is largely comprised of α-helical structures, the FPA-FTIR imaging results were consistent with histological observations. Taken together, this data suggested that treatment with SAHA influenced the composition of secondary protein structures in ISO-induced injury.

The transcriptome of Takotsubo and how SAHA influences gene behaviour is poorly understood. Apical LV tissues were assessed for Takotsubo-like response to SAHA treatment using Li-COR protein quantification, transcriptional expression index (TEI) by RNA-seq (sequencing) and H3K9/14 acetylation/deacetylation by ChIP-seq (Fig. 1e). Gene set enrichment analysis (GSEA) identified pathways implicated in ISO-induced cardiomyopathy were vastly improved by SAHA (Fig. 1f). As these results suggested that treatment with the hydroxamic acid could influence cardiac remodelling, we assessed actively transcribed genes that belong to these highly connected pathways. Histone H3 acetylation (ac) and methylation (me) are powerful determinants that regulate cardiac transcription. We assessed H3K9/14ac, H3K+4me3, H3K9me1, H3K9me2, H3K9me3, H3K27me3 and H3K36me3 (Fig. 1g). Li-COR analyses showed a dramatic change in H3K9/14ac following ISO-induced injury and SAHA treatment (Fig. 1h). H3K4me3 another permissive mark of gene transcription was also elevated in SAHA-treated animals (REV). Since the acetyltransferases CBP (KAT3A) and EP300 (KAT3B) are known to regulate cardiac transcription and are subject to the Ac/Dc axis by SAHA, we assessed their protein levels. We also examined co-repressive deacetyltransferases RYBP, HDAC1, HDAC2 and HDAC3. We showed that CBP (KAT3A) protein levels were reduced in ISO-induced cardiomyopathy but reversed by SAHA treatment when compared to control animals (no injury) (Fig. 1i). Histone acetyltransferase EP300 (KAT3B) was significantly reduced in the REV group with RYBP reduced in ISO-induced cardiomyopathy animals. These results suggested the Ac/Dc modifiers that influence ISO-induced cardiomyopathy were attenuated by SAHA.

Pathways directly regulated by ISO-induced cardiomyopathy and reversed by SAHA included cardiac development, heart failure, congestion heart failure, cardiomyopathy, cardiac scarring, collagen biosynthesis, extracellular matrix organisation and cardiac metabolism (Fig. 1j). We also assessed genetic candidates associated with predisposition to Takotsubo derived recently from the Swedish Coronary Angiography and Angioplasty Registry (SCAAR). RNA-seq shows SAHA treatment influenced the TEI (transcriptional expression index) of genes with a shift towards cardiac benefit (Fig. 1k). These results suggested that the core pathways identified could be regulated by SAHA treatment. The recent SCAAR screen found no genetic link with Takotsubo for polymorphisms in the Adrb1, Bag3 and Grk5 genes. However, we observed a dramatic shift in the TEI
following SAHA treatment, suggesting the expression of stress-
induced genes could also be under epigenetic control.

While total histone acetylation is often assessed by protein blotting it is not informative of genomic location. To determine whether the cardiac benefit conferred by SAHA treatment was regulated by an Ac/Dc axis, we performed chromatin immunoprecipitation assays from LV tissues followed by sequencing (ChIP-seq). We assessed H3K9/14ac signals in all groups. SAHA treatment influenced acetylation and deacetylation of genes implicated with Takotsubo (Fig. 1). Surprisingly, this included prominent lysine deacetylation of genes that are neither random nor limited to any one gene, but rather, co-

Fig. 1  Therapeutic benefit of SAHA targeting the Ac/Dc axis. a Illustration of ISO-induced Takotsubo-like cardiomyopathy. In the pre-clinical model, mice were divided into four groups. Group 1 received IP saline and served as healthy controls. Group 2 served as SAHA only control receiving IP injections every third day. The remaining groups (3 and 4) received subcutaneous ISO once daily for the first 5 days to induce TS-like cardiomyopathy. Group 4 commenced SAHA treatment on the day after ISO administration was concluded (Reversal). Mice were sacrificed 9 days following administration of the fifth ISO dose, on day 14. b Quantitative assessment of LV collagen content. Representative picrosirius red-staining images are shown. Data presented as the mean ± SEM. Control vs. ISO, exact P-value 0.001 (**), ISO vs. Reversal, exact P-value 0.001 (**). Calculated using paired Student’s t-test. Picrosirius red staining of normal myocardium (n = 9), SAHA (n = 5), Reversal (ISO/SAHA n = 5). c FPA-FTIR chemical imaging showing lipid and protein distribution using a Bruker Hyperion 2000 FTIR microscope equipped with a 64 × 64 element FPA detector and a 15× objective lens and acquired in transmission mode. d Comparison of FPA-FTIR (i) absorbance spectra and (ii) inverted second derivative spectra within the amide region (1800–1200 cm⁻¹). e Apical LV tissues were assessed for TS-like response to SAHA treatment using Li-COR protein quantification, transcriptional expression index (TEI) determined by RNA-seq and chromatin modification by ChIP-seq. f Heatmap of cardiomyopathy pathways from multi-contrast GSEA derived from the Human Phenotype Ontology (HPO). Pathway significance is calculated by FDR P < 0.05 using multi-contrast analysis for ISO vs. healthy controls, SAHA vs. healthy controls, ISO/SAHA vs. healthy controls (Reversal), SAHA vs. ISO and ISO/SAHA vs. ISO (n = 3 each group). g Histone lysine map for the major sites assessed of H3 acetylation and methylation, these include the transcriptionally permissive marks H3K4me3, H3K9/14ac, and H3K36me3. The suppressed expression is also shown in the bar graph. h Heatmap of cardiomyopathy pathways from multi-contrast GSEA derived from the Human Phenotype Ontology (HPO). Pathway significance is calculated by FDR P < 0.05 using multi-contrast analysis for ISO vs. healthy controls, SAHA vs. healthy controls, ISO/SAHA vs. healthy controls (Reversal), SAHA vs. ISO and ISO/SAHA vs. ISO (n = 3 each group). i Heatmap of histone modification in apical LV tissue from ISO and SAHA-administered mice. Bar plots represent mean values of Li-COR Odyssey quantification of protein arrested against total α-tubulin. Data are represented as the mean ± SEM. * P < 0.05, ** P < 0.01 vs. control, #P < 0.05. Calculated using one-way ANOVA (n = 3 each group). j Protein levels of histone-modifying enzymes (lysine acetyltransferases EP300 and CBP including the deacetyltransferases RYBP, HDAC1, HDAC2 and HDAC3) in apical LV tissue from ISO and SAHA-administered mice. Bar plots represent mean values of Li-COR Odyssey quantification of protein arrested against total α-tubulin. Data are represented as the mean ± SEM. * P < 0.05, ** P < 0.01 vs. control, #P < 0.05. Calculated using one-way ANOVA (n = 3 each group). k Relationship of histone modification to transcriptional expression index (TEI) determined by RNA-seq and chromatin modification by ChIP-seq. l Heatmap of cardiomyopathy pathways from multi-contrast GSEA derived from the Human Phenotype Ontology (HPO). Pathway significance is calculated by FDR P < 0.05 using multi-contrast analysis for ISO vs. healthy controls, SAHA vs. healthy controls, ISO/SAHA vs. healthy controls (Reversal), SAHA vs. ISO and ISO/SAHA vs. ISO (n = 3 each group). m Heatmap of cardiomyopathy pathways from multi-contrast GSEA derived from the Human Phenotype Ontology (HPO). Pathway significance is calculated by FDR P < 0.05 using multi-contrast analysis for ISO vs. healthy controls, SAHA vs. healthy controls, ISO/SAHA vs. healthy controls (Reversal), SAHA vs. ISO and ISO/SAHA vs. ISO (n = 3 each group). n Heatmap of cardiomyopathy pathways from multi-contrast GSEA derived from the Human Phenotype Ontology (HPO). Pathway significance is calculated by FDR P < 0.05 using multi-contrast analysis for ISO vs. healthy controls, SAHA vs. healthy controls, ISO/SAHA vs. healthy controls (Reversal), SAHA vs. ISO and ISO/SAHA vs. ISO (n = 3 each group).

AUTHOR CONTRIBUTIONS
S.R., P.M., C.S. performed ISO-induced cardiomyopathy. N.M., J.V., T.C. performed FPA-FTIR chemical imaging. I.K., S.M., J.O., H.K.N., K.A.H. performed, analysed and interpreted the data from Li-COR, CHIP-Seq and RNA-Seq including bioinformatic analyses. A.E.O. interpreted the data, prepared the figures and wrote the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY
The datasets analysed in the current study are available from the corresponding author on reasonable request.

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ADDITIONAL INFORMATION

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