The original uploaded version contained characterization of the zebrafish phenotype from kmt2d and kdm6a/al mutants. These lines were lost during the covid shutdown, and upon revision for publication we were unable to validate these mutants or obtain another allele. Therefore we removed these data from this revised version of the manuscript.

The role of Kabuki Syndrome genes *KMT2D* and *KDM6A* in development: Analysis in Human sequencing data and compared to mice and zebrafish

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Abstract

KMT2D and *KDM6A* are epigenetic regulators that have been implicated in Kabuki Syndrome, a rare congenital birth defect with multiple tissue and organ abnormalities, including craniofacial and heart defects. Our previous study identified human families with mutations in the epigenetic modifiers KMT2D and KDM6A, which is implicated in 32% and 10% of Kabuki Syndrome patients respectively. To understand the connection to Kabuki syndrome patients, and the transcriptional targets of KMT2D and KDM6A in humans, we performed RNA sequencing (seq) of lymphoblastoid cells from Kabuki Syndrome patients carrying mutations in *KMT2D* and *KDM6A*. We identified 1995 significant changes in transcriptional targets for *KMT2D* and 1917 for *KDM6A*, as compared to control. When compared with RNA-seq datasets obtained from other mouse and zebrafish studies, our analysis revealed *KMT2D* mutations affect the expression of 76 orthologous genes across all three datasets. Similarliy, *KDM6A* afftects the expprssion of 7 orthologous genes across three datasets. Despite the differences in cell types, stages, and species in the comparison between the transcriptomic datasets, there are common gene expression changes associated with KMT2D and KDM6A mutations. qPCR on novel zebrafish mutants confirmed the differentially expression in KMT2D or KDM6A mutant backgrounds. Taken together, our results show that *KMT2D* and *KDM6A* regulate common and unique genes across humans, mice, and zebrafish for early craniofacial and cardiac development and this information contributes to the understanding of epigenetic dysregulation during development of Kabuki syndrome.

Running Title:

Kabuki gene expression across zebrafish, mice, and humans

Key words:

Cardiac development, Kabuki Syndrome, epigenetics, cardiac neural crest cells, RNA sequencing, zebrafish, mice, human

Introduction

Kabuki syndrome is a rare congenital abnormality with characteristic facial features superficially similar to the makeup used by actors in traditional Japanese theatre where the name arises. The craniofacial malformation results from defects in the eyelid tears, arched and broad eyebrows, depressed nasal tip, and prominent or cupped ears (Adam et al., 2019). These patients also present with cardiac issues as well as growth defects such as short stature and microcephy. Approximately 28-80 % of Kabuki Syndrome patients are afflicted with congenital heart defects (Digilio et al., 2017). Common heart defects include atrial and ventricular septal defects, bicuspid aortic valves, aortic coarctation, double outlet right ventricle, transposition of great arteries, infundibular pulmonary stenosis, dysplastic mitral valve, Tetralogy of Fallot, etc. (Cocciadiferro et al., 2018; Digilio et al., 2017; Shangguan et al., 2019; Yap et al., 2020).

The combination of defects observed in Kabuki Syndrome are similar to those observed in embryos with deficiencies in neural crest cells (NCCs), a multipotent population of cells that give rise to craniofacial cartilage and bone, cardiac outflow track as well as multiple other derivatives. NCCs form early in development as part of the central nervous system, and then migrate long distances to begin a differentiation program. Because they undergo multiple morphogenetic and cellular processes, NCCs rely on a precise gene and epigenetic regulatory network for their development. Several epigenetic processes are important for development including DNA methylation (Serra-Juhe et al., 2015), histone modifications (Zaidi et al., 2013; Q. J. Zhang & Liu, 2015), chromatin remodeling (Bruneau, 2010; Delgado-Olguin, Takeuchi, & Bruneau, 2006), and microRNA (Toni, Hailu, & Sucharov, 2020) and contribute to the etiology of developmental disorders. Because of the ability to transcriptionally regulate many genes at the same time, defects in epigenetic regulators help to explain why patients with the same genotype and syndrome often have large variations in phenotype, penetrance, and severity. Indeed, mutations in chromatin

remodeler Chd7 and histone acetyltransferase Kat6a/b are associated with birth defects leading to Charge and Ohdo syndromes, respectively (Vissers et al., 2004) (Campeau et al., 2012).

KMT2D, also known as MLL2 methylates histone 3 at lysine 4 (H3K4) which is associated with transcriptional repression, and *KDM6A*, demethylates histone 3 at lysine 27 (H3K27), mostly associated with transcriptional actiation (Ali, Hom, Blakeslee, Ikenouye, & Kutateladze, 2014; Hong et al., 2007; Koutsioumpa et al., 2019; Lan et al., 2007), both genes are frequently mutated in patients with Kabuki Syndrome (Cocciadiferro et al., 2018; Gazova, Lengeling, & Summers, 2019; Luperchio, Applegate, Bodamer, & Bjornsson, 2019; Shangguan et al., 2019; Tekendo-Ngongang, Kruszka, Martinez, & Muenke, 2019; Yap et al., 2020) (Ang et al., 2016; Lee, Lee, & Lee, 2012; Schwenty-Lara, Nurnberger, & Borchers, 2019; Serrano, Demarest, Tone-Pah-Hote, Tristani-Firouzi, & Yost, 2019). *KMT2D* or lysine-specific methyltransferase 2D, is also known as *MLL2* or myeloid/lymphoid or mixed-lineage leukemia 2. It belongs to a family of 7 SET1-like histone methyltransferases (Ali et al., 2014). KMT2D contains a SET domain for histone methylation, PHD and coiled-coil domains, and zinc finger domains (Ali et al., 2014). The PHD domain of zebrafish *kmt2d*, which shares 86.1% identity with human *KMT2D (***Figure 1A***)*.

Recent studies have significantly contributed to our understanding of cardiac development and function as regulated by *KMT2D* (Ang et al., 2016; Schwenty-Lara, Nurnberger, et al., 2019; Serrano et al., 2019), and *KDM6A* (Lee et al., 2012). The PHD domain of zebrafish *kmt2d*, which shares 86.1% identity with human *KMT2D*, was targeted by CRISPR-mediated gene disruption in a recent study on *kmt2d* in heart development (Serrano et al., 2019). The study generated 3 alleles with premature stop codons that cause a truncation of the PHD domain (Serrano et al., 2019) and categorized phenotypes, including heart edema and microcephaly, and a shorter body axis (Serrano et al., 2019). They report a robust Notch signaling hyperactivation in endocardial and endothelial cells that affect vasculogenesis and angiogenesis, and a rescue of cardiovascular phenotype by pharmacological inhibition of Notch signaling (Serrano et al., 2019). Another recent study on *kmt2d* showed morpholino-based knockdown of *kmt2d* in *Xenopus* causes heart defects (Schwenty-Lara, Nurnberger, et al., 2019), and impairs NCC development (Schwenty-Lara, Nehl, & Borchers, 2019). In mouse studies, development of limbs, palate, central nervous system, and heart are impaired upon mutating *Kmt2d*, with hypoplasia in frontonasal bone, fully penetrant cleft palate, mandible hypoplasia, deficits in palatal shelf elevation and cranial base ossification

111 (Fahrner et al., 2019; Shpargel, Mangini, Xie, Ge, & Magnuson, 2020). Further, disruption of
112 *Kmt2d* show disorganized interventricular septum, hypoplasia of compact myocardium indicating
113 decreased cardiomyocyte proliferation and failure of outflow tract septation, suggesting a role of
114 *Kmt2d* in cardiac development (Ang et al., 2016).

115 *KDM6A* (and *kdm6l* in zebrafish) or Lysine Demethylase 6A, is also known as *UTX* or
116 Ubiquitously transcribed tetratricopeptide repeat, X chromosome. UTX, UTY and JMJD3
117 comprise a subfamily of proteins containing JmjC-domain (Jumonji C) and zinc fingers, and they
118 are evolutionarily conserved from *Caenorhabditis elegans* to human (Klose, Kallin, & Zhang,
119 2006; Lan et al., 2007; Shi & Whetstine, 2007). The JmjC-domain is associated with histone
120 demethylation (Accari & Fisher, 2015), while the tetratricopeptide repeats at N-terminal regions
121 of UTX and UTY are predicted protein interaction motifs (Lan et al., 2007). It is important to note
122 that *KDM6A* or *UTX* is an X-linked gene, with 2 copies in females (XX) and 1 copy in males (XY),
123 where the Y chromosome has the homolog, *UTY* (Itoh et al., 2019). Studies indicate that *UTY* and
124 *UTX* diverged from a common ancestor, yet the functional role of *UTY* in histone demethylation
125 needs further investigation, because some studies show that it has a lower histone demethylation
126 activity than *UTX* (Faralli et al., 2016; Hong et al., 2007; Itoh et al., 2019; Lan et al., 2007;
127 Shpargel, Starmer, Wang, Ge, & Magnuson, 2017; Shpargel, Starmer, Yee, Pohlers, & Magnuson,
128 2014; Walport et al., 2014).

129 To determine the effect of patient mutations on the transcriptome, we performed RNA-seq
130 analysis on human patient lymphoblastoid cells (LBCs). We determined that 1995 genes were
131 differentially regulated for *KMT2D*, and 1917 for *KDM6A*, as compared to control LBCs. We then
132 compared common gene targets of *KMT2D* and *KDM6A* from published zebrafish, mice, with our
133 human RNA-seq datasets in order to prioritize downstream genes for further study. Our results
134 reveal that some of the genes which are involved in cardiogenesis, cardiac function, vasculature,
135 neurogenesis, and overall development, are regulated by *KMT2D* and *KDM6A* across zebrafish,
136 mice, and humans. Our *in vivo* analysis in CRISPR-mutated zebrafish indicate that *kmt2d* and
137 *kdm6a* regulate genes involved in early embryonic, cardiac, and NCC development. Our results
138 support the idea that mutations in zebrafish KMT2D and KDM6A have defects similar to Kabuki
139 Syndrome patients, supporting previous studies in the field, identifies targets in human patients
140 LBCs, and presents a pan-species perspective of the roles of *KMT2D* and *KDM6A* in development.

141

142

143 **Materials and Methods**

144

145 **Human Cell Samples**

146 Patient-derived lymphoblastoid cell lines (LCLs) used in this study were previously generated
147 from KS patients with mutations in *KMT2D*/*MLL2* and *KDM6A* (Van Laarhoven et al 2015).
148 Control LCLs from age and demography-matched subjects, were obtained from the Coriell
149 Institute for Medical Research https://www.coriell.org/

150

151 **RNAseq of human Kabuki Syndrome patients and comparison across taxa**

152 Human LCLs from *KMT2D* and *KDM6A* patients and controls, as mentioned above, were grown
153 to a count of 10^6 and pelleted. RNA was extracted by Zymo Research Direct-zol™ RNA
154 MiniPrep kit (Catalog #R2050). Two cDNA libraries for each genotype (e.g. wildtype, *KMT2D*
155 mutant, *KDM6A* mutant) were prepared from separate RNA MiniPreps using the Nugen mRNA
156 kit for Illumina sequencing. Sequencing was performed on an Illumina NovaSEQ6000 (150bp
157 paired-end reads) at the Genomics and Microarray Shared Resource Core Facility at University
158 Of Colorado Denver Cancer Center. Libraries were sequenced to an average depth of 42 million
159 reads. Reads were aligned to the human genome (hg38) using STAR Aligner (v2.7.3a) (Dobin et
160 al., 2013), and gene counts were computed from STAR using quant mode (Dobin et al., 2013).
161 Differential expression was performed in R using Deseq2 (Love, Huber, & Anders, 2014).

162 We compared our human RNAseq data to previously published datasets of KMT2D and
163 KDM6A mutants from mice and zebrafish (Ang et al., 2016; Fahrner et al., 2019; Itoh et al.,
164 2019; Lei & Jiao, 2018; Serrano et al., 2019; Shpargel et al., 2017). RNAseq datasets were
165 downloaded from the NCBI Gene Expression Omnibus. Datasets included GSE129365 (Kmt2d

166 mouse chondrocytes, (Fahrner et al., 2019)), GSE103849 (Kdm6a mouse neural crest cells,
167 (Shpargel et al., 2017), GSE121703 and GSE128615 (mouse Cd4 cells, (Itoh et al., 2019)),
168 GSE110392 (mouse neural stem cells, (Lei & Jiao, 2018)). In addition an RNAseq dataset of
169 whole embryo zebrafish Kmt2d mutants was provided by Serrano et al. (Serrano et al., 2019).
170 Where necessary, downloaded RNAseq datasets were re-analyzed using DESeq2 for differential
171 expression.

172 To identify potential core genes regulated by Kmt2d and Kdm6a we found the
173 intersection set of genes differentially expressed at a nominal p-value of 0.05 in each dataset for
174 all Kmt2d and Kdm6a RNAseq datasets respectively. To do this, we used BioMart as
175 implemented in R to convert non-human gene symbols into orthologous human ensembl
176 identifiers, while allowing multiple mappings to account for gene duplications. Intersection sets
177 were computed from these human ensemble identifiers.

178

179 **Statistical Analysis**

180 Data shown are the means ± SEM from the number of samples or experiments indicated in the
181 figure legends. All assays were repeated at least three times with independent samples. *P* values
182 were determined with Student's *t* tests using GraphPad Prism.

183

184 **Results**

185 **Comparative analyses of the transcriptome of KS patients reveal common targets of *KMT2D***
186 **and *KDM6A***

187 To translate our results into the context of human birth defects, we investigated the
188 transcriptome of 2 human Kabuki Syndrome patients carrying mutations in *KMT2D* and *KDM6A*,
189 by RNA sequencing of their lymphoblastoid cells (LBCs). The patient with *KMT2D* mutation has
190 a transition c.4288T>C (p.C1430R) (Figure 1A) and the patient with *KDM6A* mutation has a
191 microdeletion in chromosome X resulting in a deletion of *KDM6A* (Figure 1A). Both mutations

192 are predicted to result in a loss of function mutation. As controls, we used LBCs from age and
193 ethnicity matched individuals. To ascertain differential gene expression, generated reads from
194 RNA-seq were mapped to the human genome using STAR aligner (Dobin et al., 2013) and
195 differential expression was performed in R with DESeq2. Differentially expressed genes (nominal
196 p value <= 0.05) from *KMT2D* and KDM6A showed 1995 and 1917 differentially expressed genes
197 as compared to control LBCs. Gene Ontology analysis suggests that many of these genes regulate
198 pathways as presented in Table , consistent with both source and timing of cells that were
199 sequenced.

200 We asked whether gene expression changes observed in our human RNA-seq datasets of
201 KMT2D and KDM6A mutations are similar to those observed in datasets from the experimental
202 model systems mouse and zebrafish. To do this, we compared RNA sequencing datasets for
203 *Kmt2d*-mutant mice and zebrafish generated by other researchers (see methods) to our human
204 dataset. For mice, the RNA sequencing dataset is derived from embryonic hearts (Ang et al., 2016),
205 and the zebrafish data are derived from whole embryos (Serrano et al., 2019). Likewise, our
206 *KDM6A* human RNA seq datasets were compared to three distinct RNA seq datasets of mice that
207 are mutant for *Kdm6a*. The mice datasets are derived from NCCs (Shpargel et al., 2017), neural
208 stem cells (Lei & Jiao, 2018), and CD4+ cells (Itoh et al., 2019). For *KDM6A*, the comparison is
209 only between humans and mice, since zebrafish RNA sequencing datasets for *kdm6a* mutation are
210 not available.

211 For each, we asked what genes are differentially expressed in all three datasets. A
212 comparison between KMT2D datasets from 3 species revealed 76 orthologous genes differentially
213 expressed in all three datasets (Figure 1B). Heat map analysis shows that 30 of the 76 common
214 targets are either upregulated or downregulated in a similar pattern across the 3 species, while 46
215 do not share a similarity in expression patterns among the 3 species (Figure 1C). The dissimilarity
216 is likely due to differences in cell type and age. For *KDM6A*, 7 common targets were found
217 between human and the 3 mice datasets (Figure 1A). The variation in the number of common
218 targets between *KMT2D* and *KDM6A* datasets could partly be due to the differences in the roles
219 and targets of *KMT2D* and *KDM6A*, as well as functional redundancy of other family members
220 across species (Akerberg, Henner, Stewart, & Stankunas, 2017; Crump & Milne, 2019; Fellous,
221 Earley, & Silvestre, 2019; Nottke, Colaiacovo, & Shi, 2009).

222 Despite the dissimilarity among datasets, genes identified in these analyses may represent
223 a set of genes commonly regulated by KMT2D and KDM6A across tissue types and taxa. We
224 identified several genes based on known roles in regulating development with special focus on
225 genes that affect development of craniofacial, cardiac, or NCC development including *KMT2D*
226 datasets are *SOX12*, *SLC22A23*, *FOXP4*, *NR2F6*, and *SIX3*. The genes for *KDM6A* datasets are
227 *ID3*, *PRDM1*, and *ANXA4*.

Genes common to *KMT2D* datasets	**Table 1: Characteristics relevant to cardiac and overall development**
SOX12	Expressed mice outflow tract 12.5-16.5 dpc (days post coitum), *Sox4*$^{+/-}$*11*$^{+/-}$*12*$^{-/-}$ mice have common trunk or truncus arteriosus, a CHD with single outflow tract (Bhattaram et al., 2010; Hoser et al., 2008; Paul, Harvey, Wegner, & Sock, 2014), expressed broadly throughout neural plate and migrating NCCs (Uy, Simoes-Costa, Koo, Sauka-Spengler, & Bronner, 2015)
SLC22A23	Expressed in heart and integral to mouse heart development expressed in heart, integral in mouse heart development (Aberg et al., 2012; Christoforou et al., 2008; Ekizoglu, Seven, Ulutin, Guliyev, & Buyru, 2018)
FOXP4	Mutants develop two hearts having proper chamber formation, and normal trabecular and compact myocardial development, with the same heart rate but asynchronous, lethal at E12.5 (Li, Zhou, Lu, & Morrisey, 2004; Y. Zhang et al., 2010)
NR2F6	Important for normal cardiac morphogenesis, including the development of coronary vasculature, left ventricular compact zone, and heart valves PMID: (Crispino et al., 2001; Hermann-Kleiter & Baier, 2014; Huggins, Bacani, Boltax, Aikawa, & Leiden, 2001)
SIX3	Early brain development (Inbal, Kim, Shin, & Solnica-Krezel, 2007), mutation implicated in holoprosencephaly, coloboma, cleft lip/palate (Lacbawan et al., 2009)

228

Genes common to *KDM6A* datasets	**Table 2: Characteristics relevant to cardiac and overall development**
ID3	Knockout in various combinations with *ID1/2/4* causes ventricular septation defects, outflow tract atresia, missing heart tube-forming region, decreased heart size and function (Y. Chen et al., 2004; Cunningham et al., 2017; Fraidenraich et al., 2004; Hu, Xin, Hu, Sun, & Zhao, 2019)
PRDM1	Functions in mesoderm of second heart field, where it interacts with Tbx1, during outflow tract morphogenesis in mouse embryo (Vincent et al., 2014))
ANXA4	Expressed in cardiac myocytes and upregulated in human failing hearts (Lewin et al., 2009; Matteo & Moravec, 2000)

Comparison of the *KMT2D* and *KDM6A* datasets between human patients generated by us, with those of mice and zebrafish generated by other groups, show variations in the expression patterns of the some genes that are common across different species and cell types. The observation indicates there may be a tight spatial and temporal orchestration of the roles of *KMT2D* and *KDM6A* in gene regulation.

Discussion

In animal models, mutations or knockdowns in Kabuki Syndrome genes show similar NCC related phenotypes. *kmt2d* mutations in zebrafish cause defects in cardiovascular development, angiogenesis and aortic arch formation, as well as cardiac hypoplasticity (Serrano et al., 2019) while Kmt2d associates with genes in cardiomyocytes, and myocardial deletion of *Kmt2d* reduces cardiac gene expression during murine heart development (Ang et al., 2016). In *Xenopus*, morpholino-based knockdown of *kmt2d* causes cardiac hypoplasticity, reduced expression of early cardiac developmental genes *tbx20*, *isl1*, *nkx2.5* (Schwenty-Lara, Nurnberger, et al., 2019), and impaired formation and migration of NCCs by reducing the expression of early NCC-specific

246 markers like *pax3*, *foxd3*, *twist*, and *slug* (Schwenty-Lara, Nehl, et al., 2019). Interestingly we did
247 not observe these same defects in single zebrafish Kabuki Syndrome mutants. It is likely that
248 genetic compensation between closely related paralogs are able to compensate for the loss of
249 function of a single gene. Future studies will test this by the creation of double and triple KS mutant
250 zebrafish.

251 Less is known about *kdm6a* and its zebrafish paralog *kdm6al*. In mouse embryonic stem cells,
252 *KDM6A* or *Utx* promotes the differentiation (ESCs) into a cardiac lineage, and serves as a co-
253 activator of core cardiac transcription factors like *Srf, Nkx2.5*, *Tbx5*, and *Gata4* (Lee et al., 2012).
254 *KDM6A* or *Utx* associates with cardiac-specific genes like *Anf* and *Baf60c*, to help recruit Brg1,
255 an ATP-dependent chromatin remodeler that activates transcription of core cardiac transcription
256 factors (Hang et al., 2010; Lee et al., 2012; Lickert et al., 2004). A neural crest-specific conditional
257 deletion of *KDM6A* or *Utx* allele with a *Wnt1-Cre* transgene causes CHDs like patent ductus
258 arteriosus and craniofacial defects including frontonasal hypoplasia, facial depression, and eyelid
259 tears (Shpargel et al., 2017). In a percentage of the embryos a cleft palate is observed. Interestingly
260 the phenotype is more severe in females likely due to the remaining copy of UTY on the Y
261 chromosome. Because UTY has a non-functional methyltransferase, it is also possible that the
262 ability of UTY to rescue UTX in males is methyltransferase independent (Shpargel et al., 2017).
263 Consistent with the mouse data, zebrafish kdm6a and kdm6al mutants have minor reductions in
264 gene expression, craniofacial cartilage and cardiac morphology as well as a reduction in histone
265 modifications. Whether this function is methyltransferase dependent in zebrafish remains to be
266 determined.

267 We present a comparison between the transcriptome of zebrafish, mice, and humans with
268 mutations in *KMT2D,* and a comparison between transcriptomes of humans and three distinct cell
269 populations of mice with mutations in *KDM6A*. Although our analyses involves transcriptomic
270 datasets from different species, cell types, mutations, and developmental stages, we identify some
271 common targets of KMT2D and KDM6A that transcend these biological differences. A subset of
272 genes involved in cardiac development and physiology, vasculature, and overall development,
273 appeared as common targets across all datasets for each mutation. Specifically, our analysis
274 revealed 76 genes as common targets of *KMT2D* upon comparing our human datasets with the
275 zebrafish and mouse datasets. 30 out of 76 genes show a similar expression pattern across the 3

species, while 46 genes do not. The dissimilar expression patterns of the 46 genes can partly be explained by the fact that the transcriptomes of the 3 species are derived from different cell populations derived during different stages. Hence, epigenetic regulation by KMT2D is tightly orchestrated spatially and temporally. On the other hand, a similar expression pattern in 3 species for the 30 genes by KMT2D indicates that its role is partly conserved. For *KDM6A*, we identified 7 common targets upon comparing human RNA-seq datasets with 3 mice datasets obtained from neural crest, neural stem, and CD4+ cells. Our *in vivo* analysis of *kmt2d* and *kdm6a* mutations in zebrafish corroborate the findings of previous studies investigating these two genes in cardiac development and disease (Ang et al., 2016; Lee et al., 2012; Schwenty-Lara, Nurnberger, et al., 2019; Serrano et al., 2019). The caveat with this type of transcriptomic analyses comparison is that the RNA-seq datasets are obtained from different sources and cell types across three species of humans, mice, and zebrafish, harboring different mutations in *KMT2D* and *KDM6A*. Despite the non-uniformity, there are some shared gene regulatory networks, and thus can serve as a resource to identify core or shared biological signatures of *KMT2D* and *KDM6A*. Although, a more direct comparison between similar mutations and cell-types are warranted in future studies, this analyses identifies the common targets of *KMT2D* and *KDM6A* across different species, cell types, and mutations.

The importance of histone modifications in cardiac and craniofacial development and function has been the focus of many recent studies (Stein et al., 2011). In mice, H3K27me3 is important for postnatal cardiac homeostasis (Delgado-Olguin et al., 2012), and the Polycomb complex regulates heart development (He et al., 2012) (Lee et al., 2012; Monroe et al., 2019). In zebrafish, H3K27me3 has recently been shown to silence structural genes in proliferating cardiomyocytes during wound invasion and regeneration of injured hearts (Ben-Yair et al., 2019). ChIP-sequencing studies on primary neonatal murine cardiomyocytes revealed that H3K4me3 is enriched, while H3K27me3 is reduced, at promoters of cardiac-specific genes like *Mef2c*, *Gata4*, and *Tbx5* (Z. Liu et al., 2016). This study further showed that early re-patterning of H3K4me3 and H3K27me3 occurs during reprogramming of induced cardiomyocytes (iCMs), which are used for modeling cardiac diseases and regeneration (Z. Liu et al., 2016). Cardiac reprogramming and epigenetics have been focused in other publications (Engel & Ardehali, 2018; Ieda et al., 2010; L. Liu, I. Lei, H. Karatas, et al., 2016; Liu, Lei, & Wang, 2016; Monroe et al., 2019). In craniofacial

306 development, we have identified Kat2a and Kat2b as being important in regulating cartilage and
307 bone development in both zebrafish and mice.

308 To conclude, several features of the roles of *KMT2D* and *KDM6A* remain to be deciphered,
309 especially other factors that cross-talk with H3K4me3/K27me3 which are linked to craniofacial
310 defects (Shpargel et al., 2017; Vallianatos & Iwase, 2015; Wilderman, VanOudenhove, Kron,
311 Noonan, & Cotney, 2018) and CHDs such as *KDM1A*/*LSD1*, *KDM4A*, *KDM4C*, *EZH2*(Agger et
312 al., 2007; Ahmed & Streit, 2018; L. Chen et al., 2012; Lee et al., 2012; Nicholson et al., 2013;
313 Rosales, Carulla, Garcia, Vargas, & Lizcano, 2016; Willaredt, Gorgas, Gardner, & Tucker, 2012;
314 Wu et al., 2015; Yang et al., 2019; Q. J. Zhang et al., 2011). We take a small step towards
315 informing future studies which will be using various species to study the regulation of development
316 and disease by *KMT2D* and *KDM6A*. Taken together, a comprehensive understanding of
317 epigenetic regulation of heart and craniofacial development and disease, in combination with large
318 next-generation sequencing datasets from patients that are continuously being generated and
319 animal models will contribute to the emerging field of personalized medicine to translate
320 epigenetics-based research from the bench to the bedside.

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328

329 **Conflicts of Interest**

330 The authors declare no conflicts of interest

331

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609 **Figure Legends**

610

611 **Figure 1**: *KMT2D* shares common targets across humans, mice and zebrafish.

612 Table: Mutation information of patients.

613 A. Venn diagrams showing overlap between gene lists derived from RNA-sequencing (seq)
614 analysis of *KMT2D* mutant datasets from humans, mice, and zebrafish as labeled.

615 B. Heat maps showing 76 common targets among human, mice, and zebrafish. Genes selected
616 for RT-qPCR in Figure 1 C. are highlighted in green boxes.

617

618 **Figure 2**: *KDM6A* shares common targets across humans and various cell types of mice, which
619 are similarly regulated in zebrafish.

620 A. Venn diagrams showing overlap between gene lists derived from RNA-seq analysis of
621 *KDM6A* mutant datasets from humans and three distinct cell types of mice, as labeled.

622 B. Heat maps showing 7 common targets among human and 3 cell types of mice. Abbreviations:
623 Ms – mouse, NCC – neural crest cells, NSC – neural stem cells, Cd4 – Cd4 T lymphocytes.
624 Genes selected for RT-qPCR in Figure 2 C. are highlighted in green boxes.

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Table Gene Affected	Sex	Status	Effect at cDNA Level	Effect at Protein Level	Chromosomal Location (hg19)
KMT2D	♂	Proband	c.4288T>C	p.C1430R	chr12:47726789(hg18)
KDM6A	♀	Proband	Microdeletion, Gene deleted	Gene deleted	chrX:43620636-46881568

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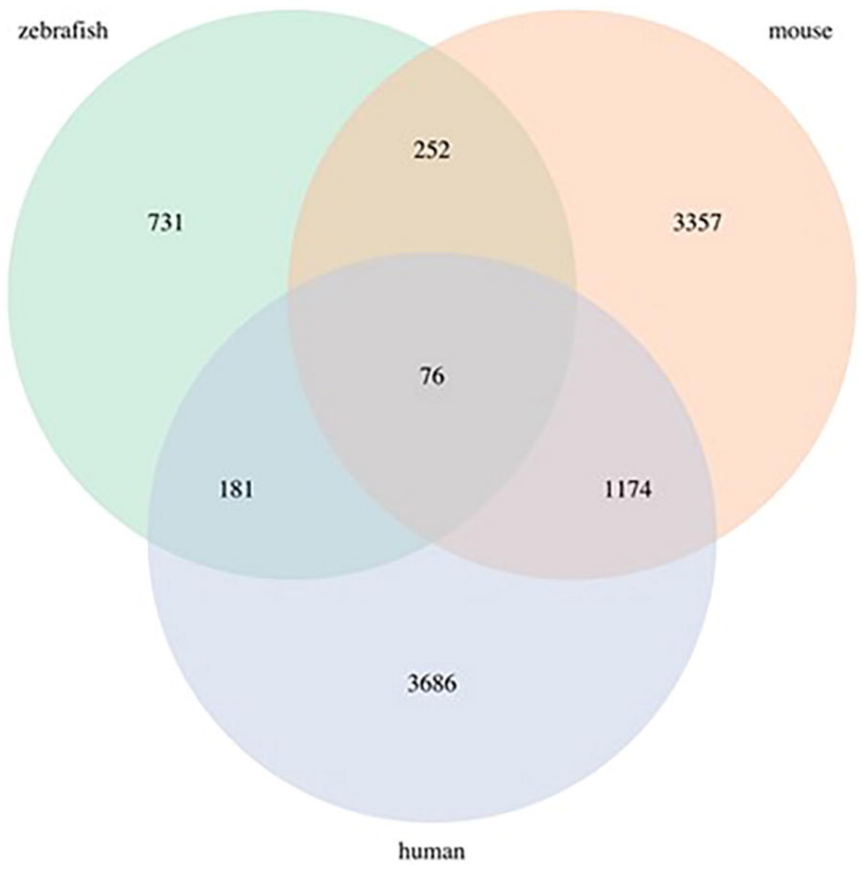
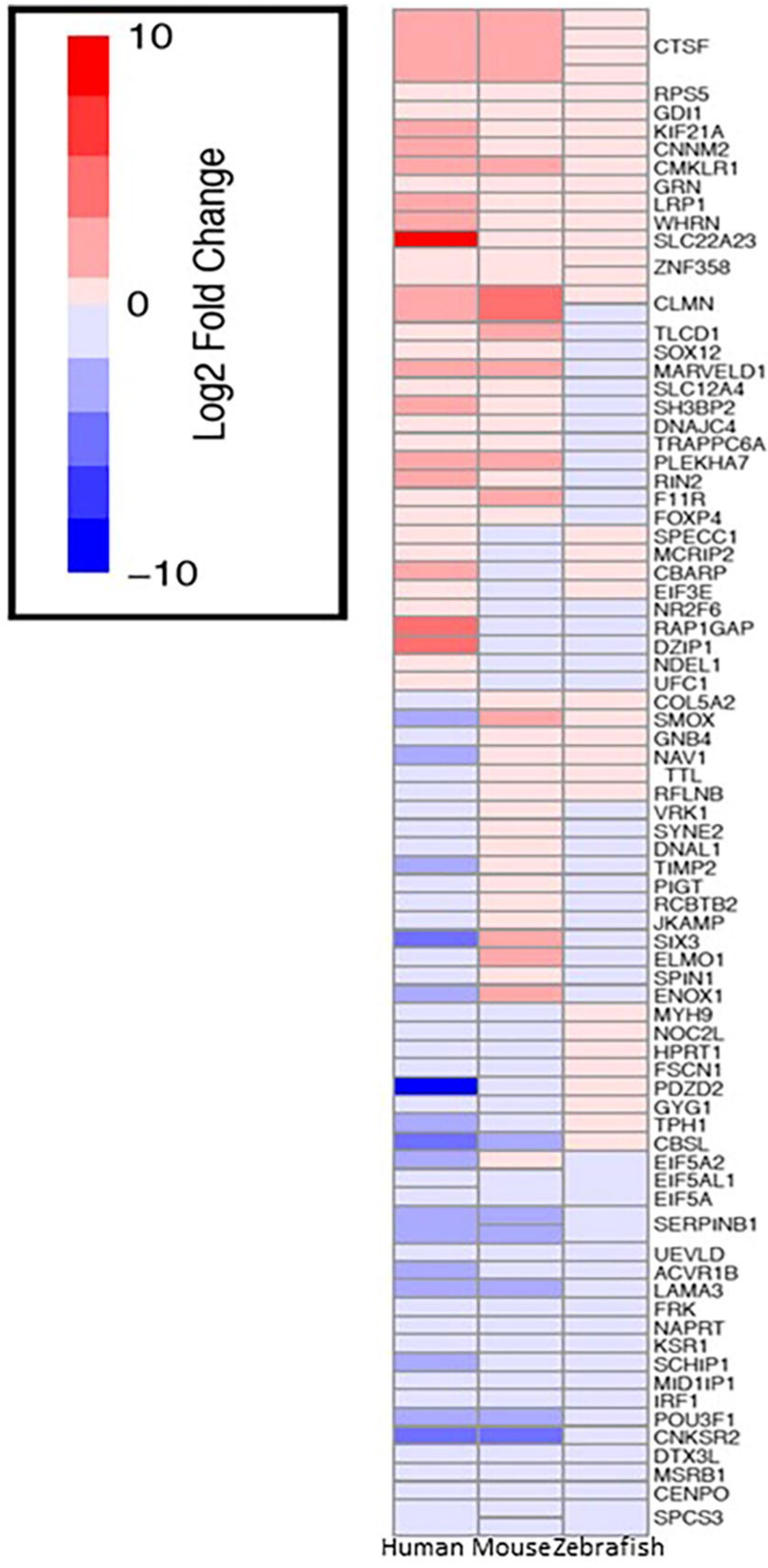
Figure 4: Table Gene Affected	Sex	Status	Effect at cDNA Level	Effect at Protein Level	Chromosomal Location (hg19)
KMT2D	♂	Proband	c.4288T>C	p.C1430R	chr12:47726789(hg18)
KDM6A	♀	Proband	Microdeletion, Gene deleted	Gene deleted	chrX:43620636-46881568

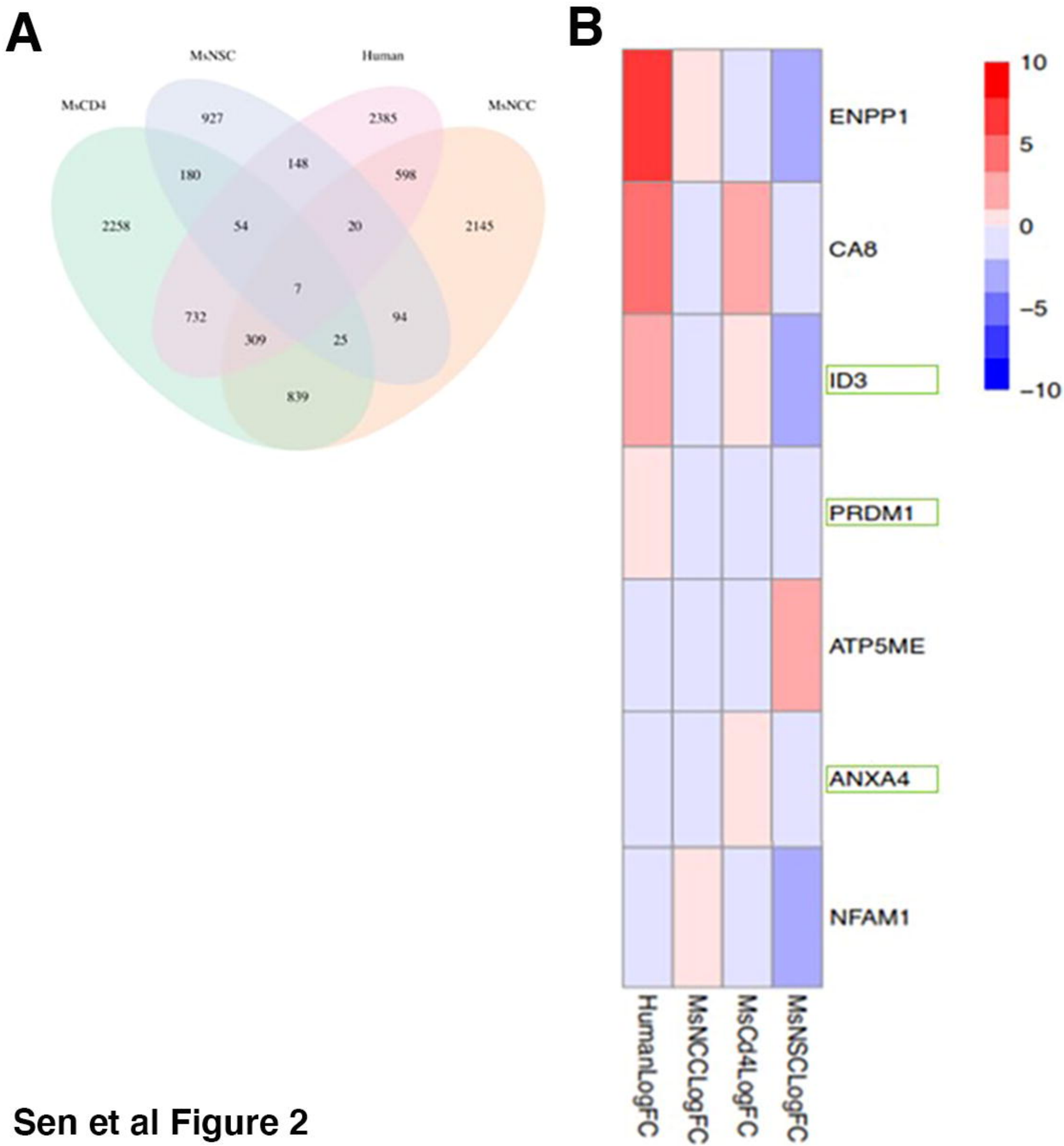
B



C



Sen Figure 1



Sen et al Figure 2