Akinyi et al (bioRxiv 2025/09/07). *QKI ensures splicing fidelity during cardiogenesis by engaging the U6 tri-snRNP to activate splicing at weak 5' splice sites*.

Mitch Murphy

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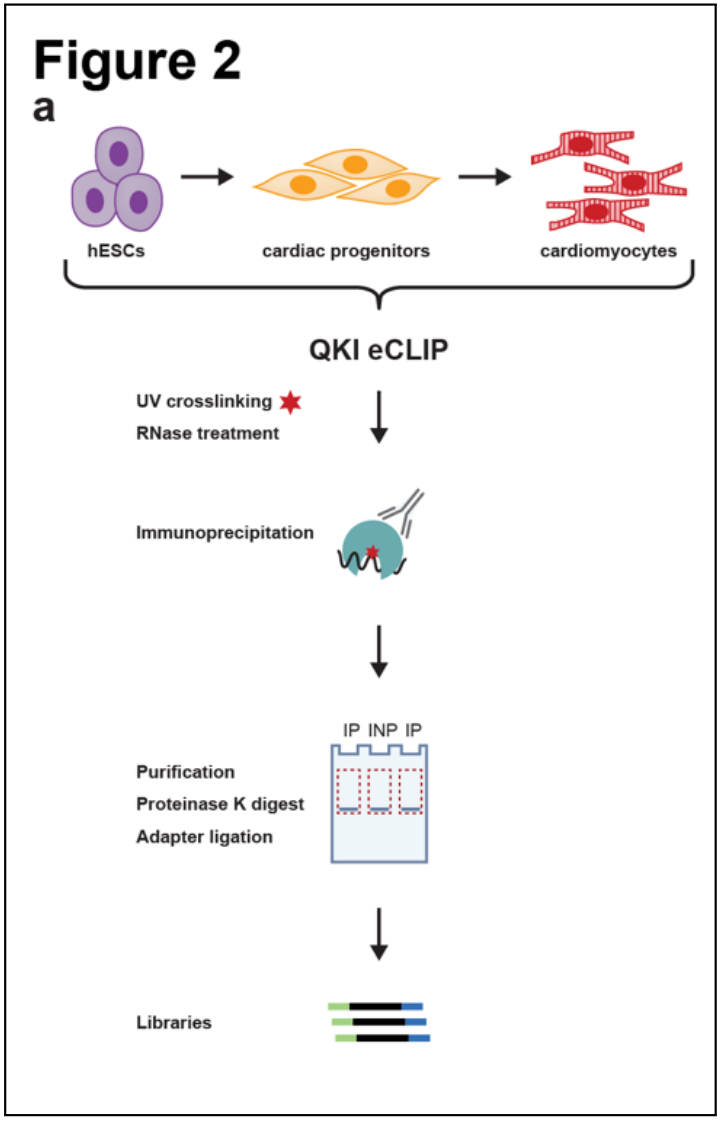
2025/09/24

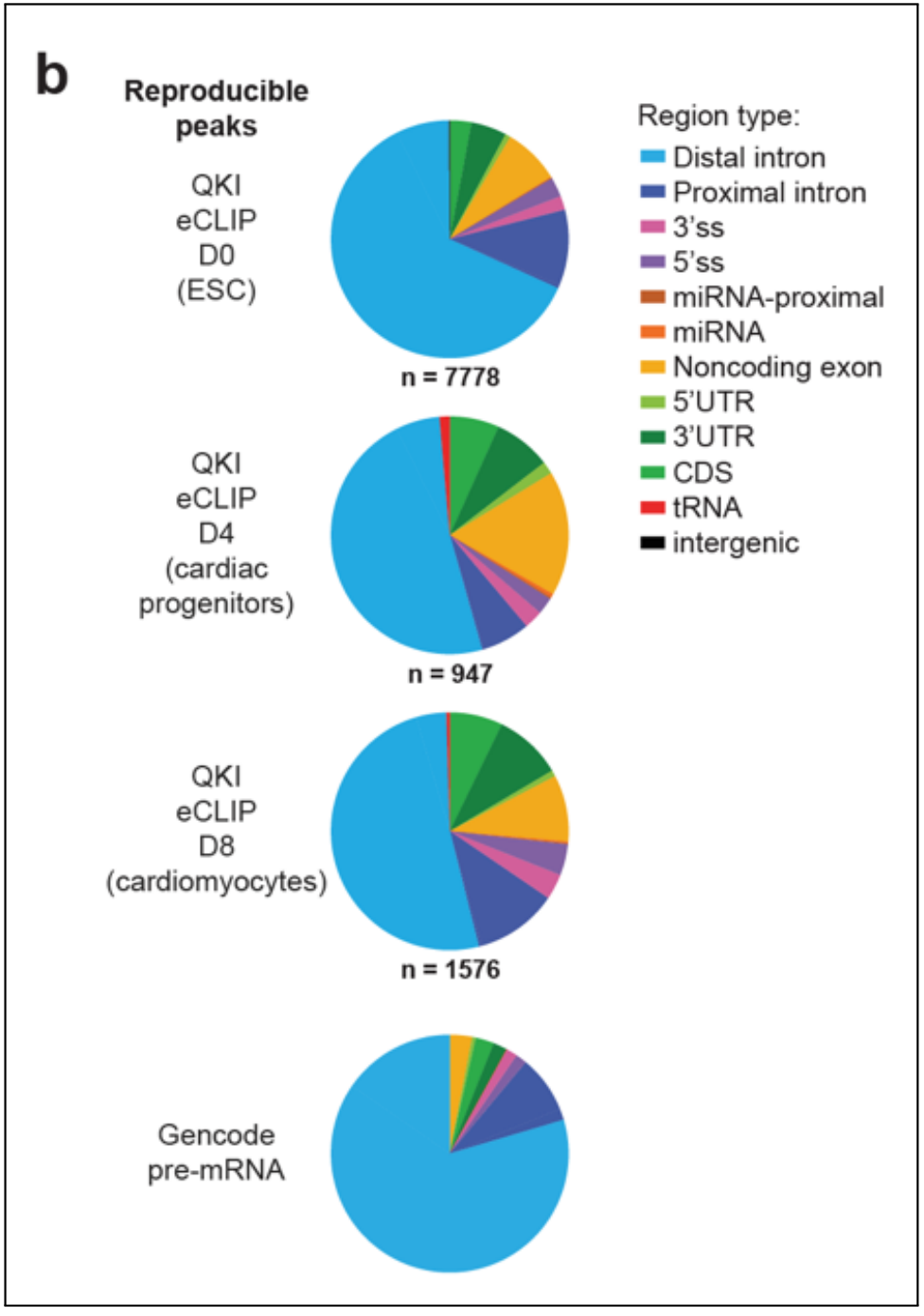
Stage-resolved eCLIP maps transcriptome-wide QKI binding landscapes during human cardiac specification

While prior efforts have explored QKI-dependent events based on the presence of QKI motifs, the fact that QKI is expressed throughout cardiac differentiation (Extended Data Fig. 1a-c) suggests that the definition of its mechanistic role in heart development requires experimental stage-resolved mapping of QKI-RNA interactions.

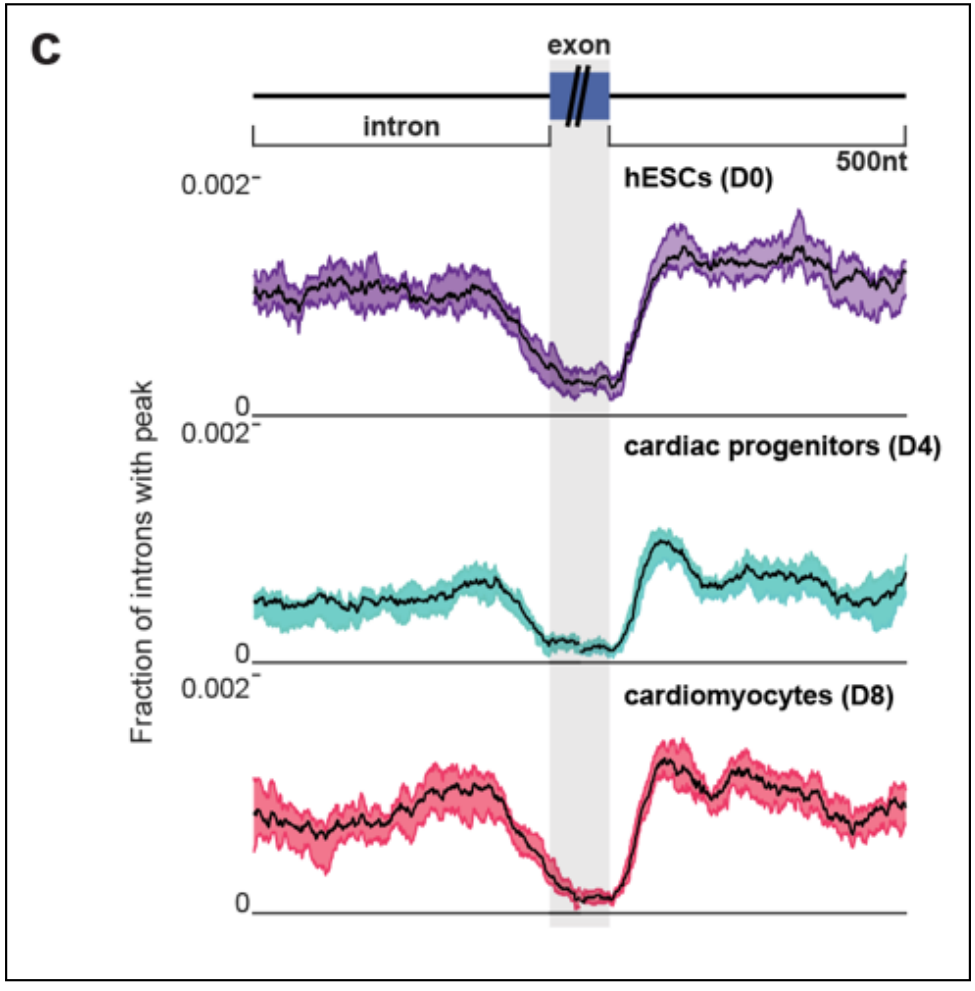
We therefore performed **enhanced crosslinking immunoprecipitation followed by sequencing (eCLIP-seq) across key stages of human cardiac commitment, namely pluripotency, cardiac progenitors, and cardiomyocytes, to chart the time-resolved RNA interaction landscape of QKI** (Fig. 2a, Extended Data Fig. 4a).



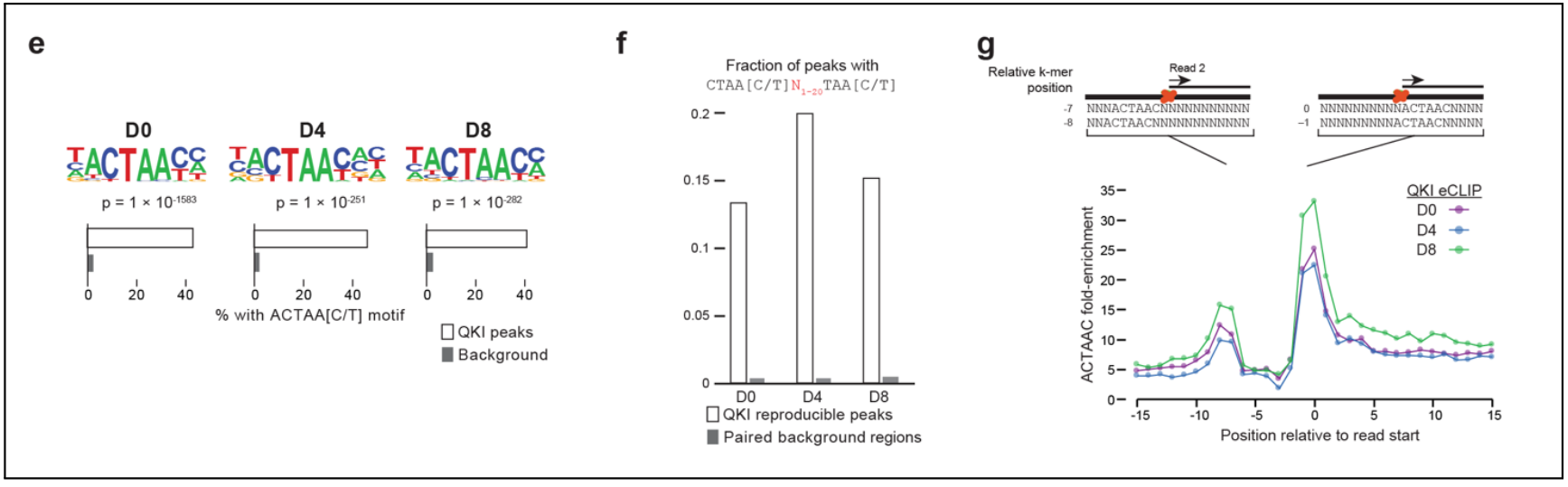
Using a custom adaptation of the **Irreproducible Discovery Rate framework to incorporate three replicates**, we identified 7,778 reproducible QKI peaks at D0 (pluripotency), 947 at D4 (cardiac progenitors), and 1,576 at D8 (cardiomyocytes) (Fig. 2b).



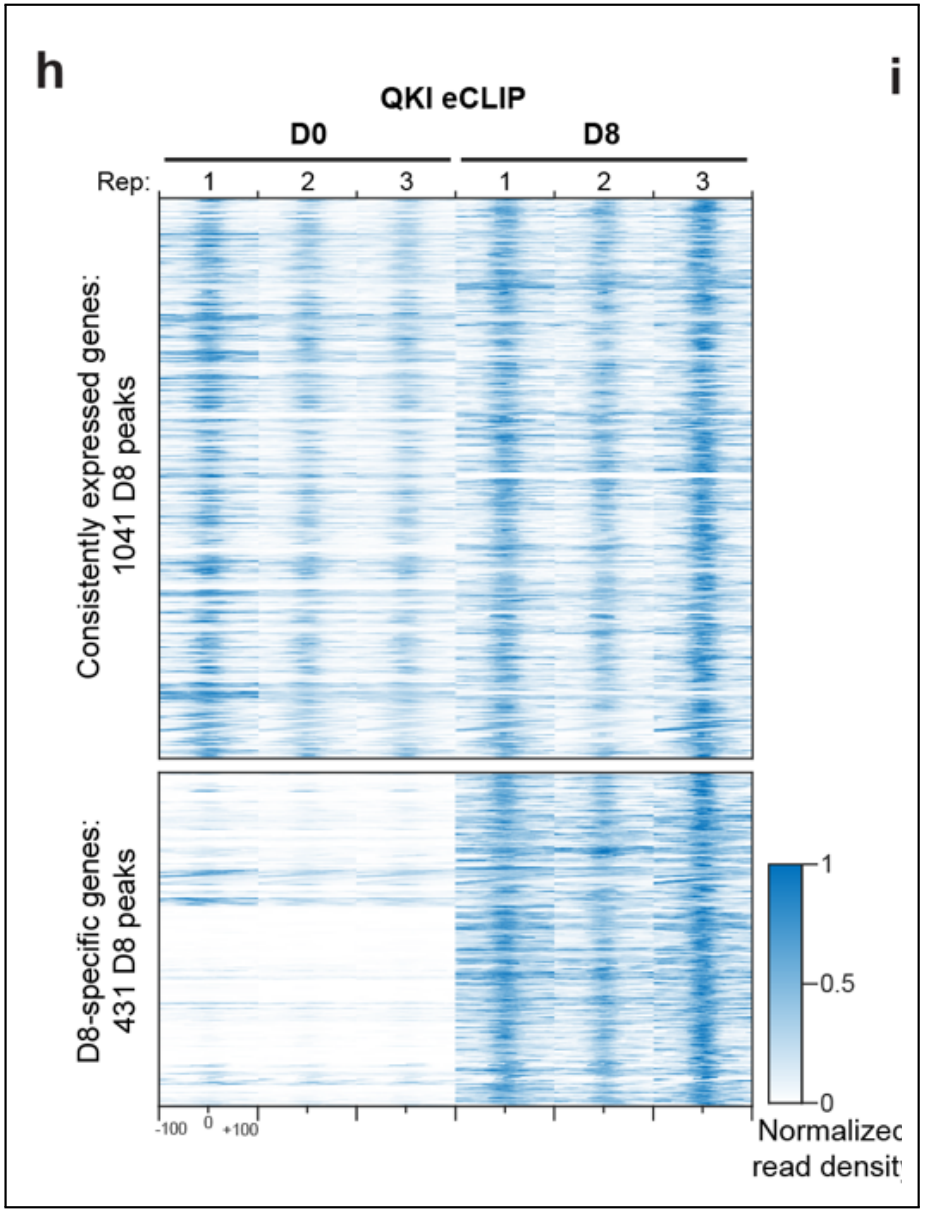
Across all timepoints, 74% of QKI binding events localized to introns (Fig. 2b, Extended Data Fig. 4b), with a **particular enrichment in 5' and 3' splice sites as well as adjacent proximal intronic regions, consistent with roles in splicing regulation** (Fig. 2c).



Motif enrichment analysis using HOMER revealed a dominant ACTAAY motif enriched >15-fold above background and present in >40% of peaks (Fig. 2e-g), matching the known in vitro QKI recognition sequence. Furthermore, a bipartite consensus motif ($ACTAA[C/T]N_{1-20}TAA[C/T]$) previously shown to mediate high-affinity QKI binding was found in 13-20% of peaks (>33-fold enrichment) (Fig. 2f), reinforcing the specificity and quality of our datasets. Reverse transcription stop-site analysis relative to QKI motifs revealed stereotypical pileups at position 0 and -1, with secondary enrichment at -7/-8 (Fig. 2g), consistent with reverse transcription termination at crosslinked direct QKI-RNA contacts.



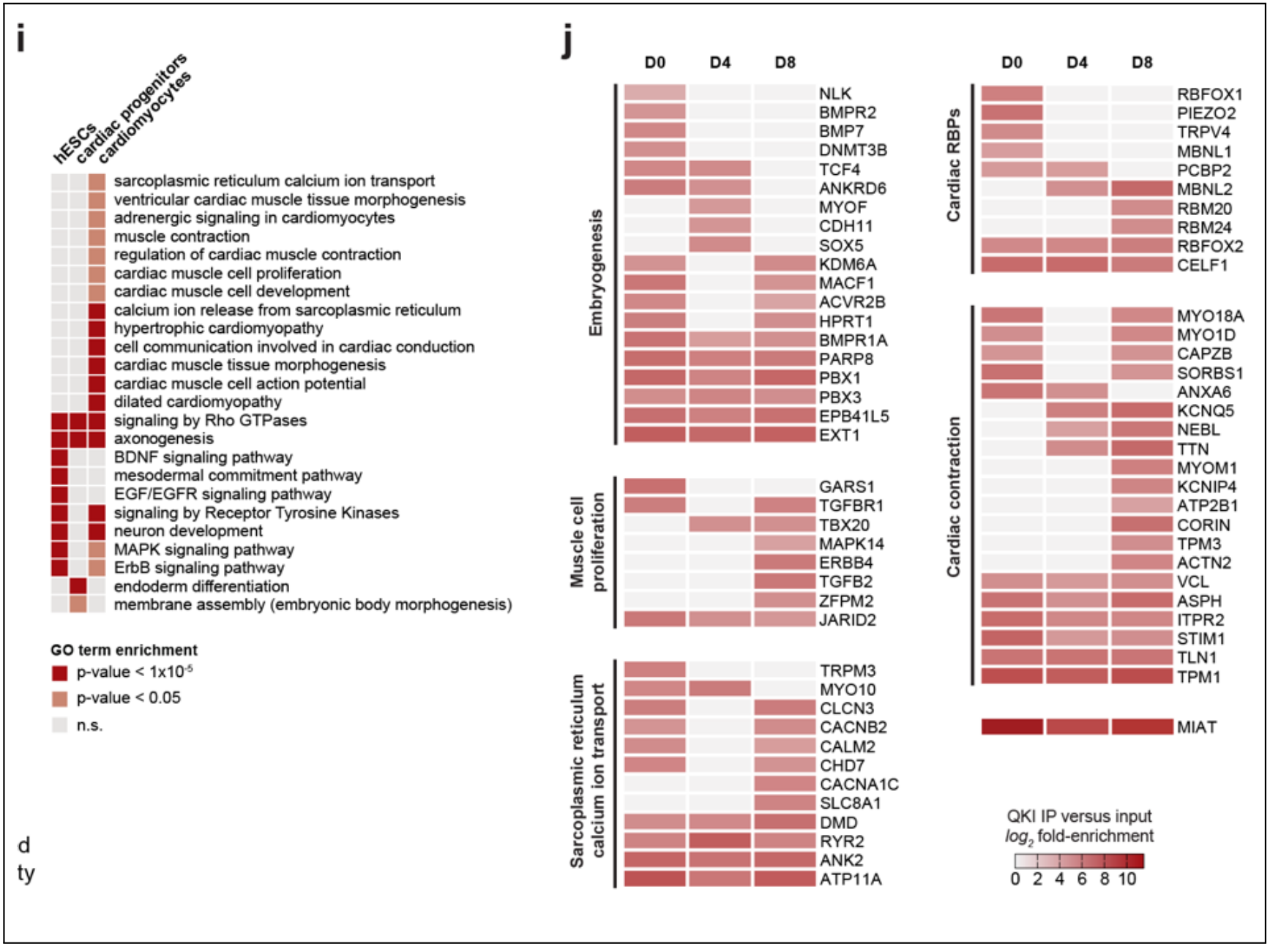
Considering the cardiomyocyte interactome, we observed two distinct sets of targets: 72% of peaks were found in genes consistently expressed between stem cells and cardiomyocytes, and those were consistently QKI-bound as early as stem cells (Fig. 2h, Extended Data Fig. 4d). In contrast, the 28% of peaks found in cardiomyocyte-specific genes (>5-fold induced in cardiomyocytes) were largely only QKI-bound in cardiomyocytes (Fig. 2h, Extended Data Fig. 4d). Thus, underlying gene expression change enables a dramatic retuning of the QKI regulatory landscape as cardiogenesis proceeds, maintaining a broad network present in pluripotent cells while newly including a more focused cardiac-specific program post-specification.



Although QKI binds broadly in pluripotent hESCs (D0) and cardiac progenitors (D4), these targets comprised a heterogeneous set of developmental regulators without a unifying lineage identity (Fig. 2b,i,j). Notably, functional annotation using gene ontology (GO term) analysis at early stages revealed an unexpected enrichment for neuronal genes, but not cardiac-associated transcripts (Fig. 2i,j).

QKI binding in cardiomyocytes (D8) showed a pronounced and functionally coherent enrichment for mRNAs encoding key components of the contractile apparatus and calcium signaling pathways (Fig. 2i,j). These included core sarcomeric genes such as TTN, NEBL, ACTN2, TPM3, TLN1, and STIM1, cell cycle regulators such as NIN, as well as central regulators of calcium handling and excitation-contraction coupling such as RYR2, ATP11A, SLC8A1, CAMK2D, and CACNA1C (Fig. 2j).

GO enrichment analysis of D8-bound transcripts underscored this transition, with top categories including sarcomere organization, muscle contraction, actin filament-based processes, and regulation of cytosolic calcium ion concentration (Fig. 2i).

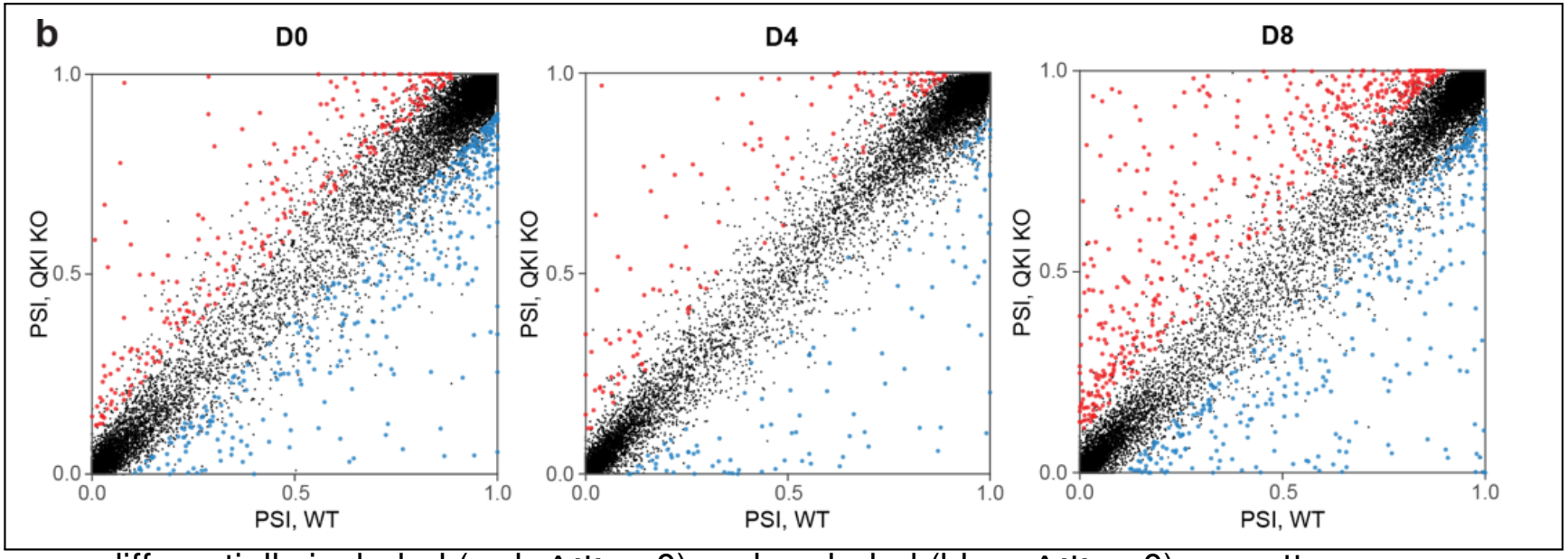

i
hESCs
cardiac progenitors
cardiomyocytes
sarcoplasmic reticulum calcium ion transport
ventricular cardiac muscle tissue morphogenesis
adrenergic signaling in cardiomyocytes
muscle contraction
regulation of cardiac muscle contraction
cardiac muscle cell proliferation
cardiac muscle cell development
calcium ion release from sarcoplasmic reticulum
hypertrophic cardiomyopathy
cell communication involved in cardiac conduction
cardiac muscle tissue morphogenesis
cardiac muscle cell action potential
dilated cardiomyopathy
signaling by Rho GTPases
axonogenesis
BDNF signaling pathway
mesodermal commitment pathway
EGF/EGFR signaling pathway
signaling by Receptor Tyrosine Kinases
neuron development
MAPK signaling pathway
ErbB signaling pathway
endoderm differentiation
membrane assembly (embryonic body morphogenesis)
GO term enrichment
p-value < 1x10-5
p-value < 0.05
n.s.
d
ty
j
D0
D4
D8
Embryogenesis
NLK
BMPR2
BMP7
DNMT3B
TCF4
ANKRD6
MYOF
CDH11
SOX5
KDM6A
MACF1
ACVR2B
HPRT1
BMPR1A
PARP8
PBX1
PBX3
EPB41L5
EXT1
Muscle cell proliferation
GARS1
TGFBR1
TBX20
MAPK14
ERBB4
TGFB2
ZFPM2
JARID2
Sarcoplasmic reticulum calcium ion transport
TRPM3
MYO10
CLCN3
CACNB2
CALM2
CHD7
CACNA1C
SLC8A1
DMD
RYR2
ANK2
ATP11A
Cardiac RBPs
RBFOX1
PIEZO2
TRPV4
MBNL1
PCBP2
MBNL2
RBM20
RBM24
RBFOX2
CELF1
Cardiac contraction
MYO18A
MYO1D
CAPZB
SORBS1
ANXA6
KCNQ5
NEBL
TTN
MYOM1
KCNIP4
ATP2B1
CORIN
TPM3
ACTN2
VCL
ASPH
ITPR2
STIM1
TLN1
TPM1
MIAT
QKI IP versus input
log2 fold-enrichment
0 2 4 6 8 10

Together [...Figure 2 reveals...] that the **QKI RNA interactome is dynamically rewired during cardiac differentiation, with a pronounced shift toward transcripts that govern the contractile machinery in terminally differentiated cardiomyocytes.**

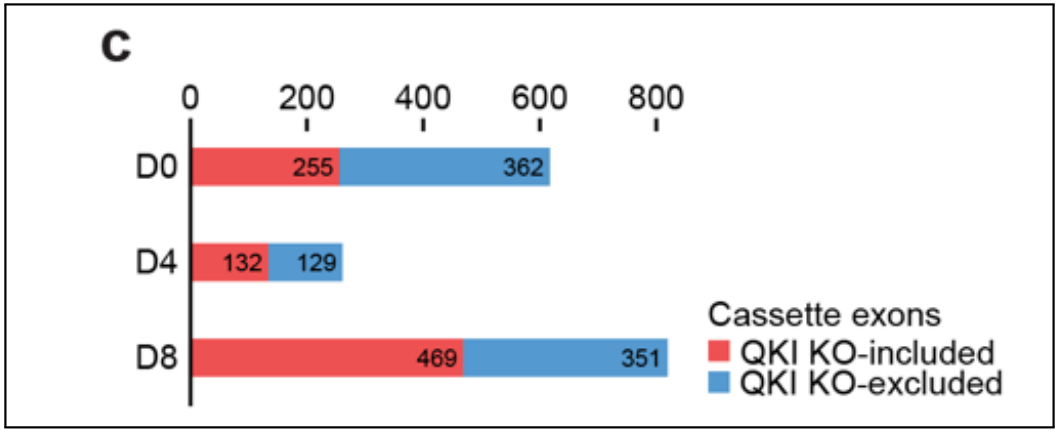
QKI ensures splicing fidelity in essential cardiac genes

To identify splicing events regulated by QKI during cardiac differentiation, paired with our direct targets identified by eCLIP, we performed differential splicing analysis using rMATS53 from our RNA-seq data profiling wild-type and QKI KO cells at matched timepoints (day 0, 4, and 8) (Fig. 3a-c). [...] **The most prominent splicing changes were in the inclusion and exclusion of cassette exons**, although other alternative splicing events were also present to a lesser extent (Fig. 3a).

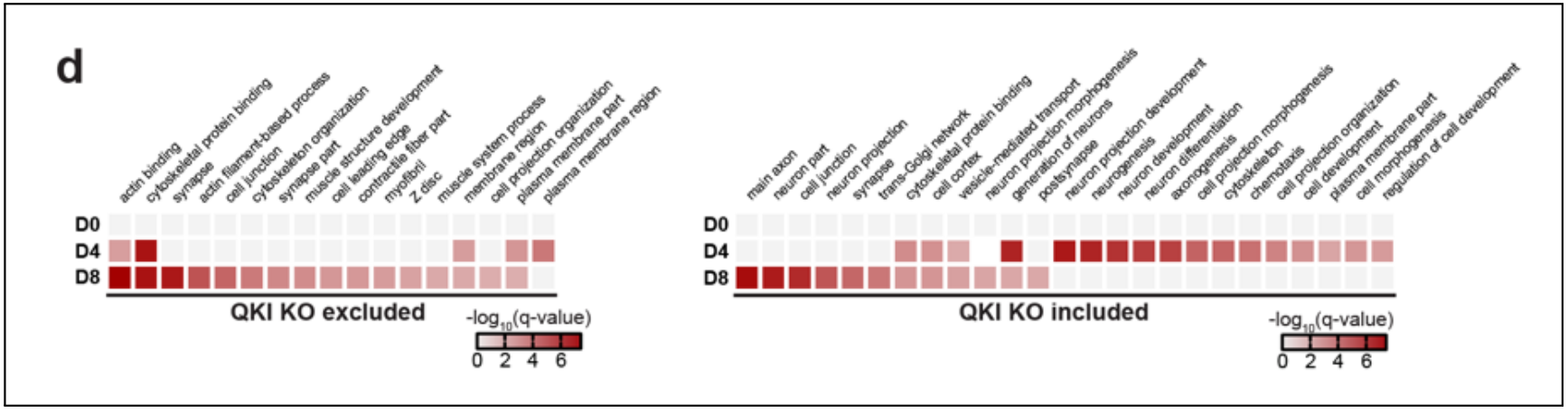
In total, we observed hundreds of significantly QKI knockout-included (255 (D0), 132 (D4), and 469 (D8)) or knockout-excluded (362 (D0), 129 (D4), and 351 (D8)) exons at each timepoint (Fig. 3b,c, Supplementary Table 2).



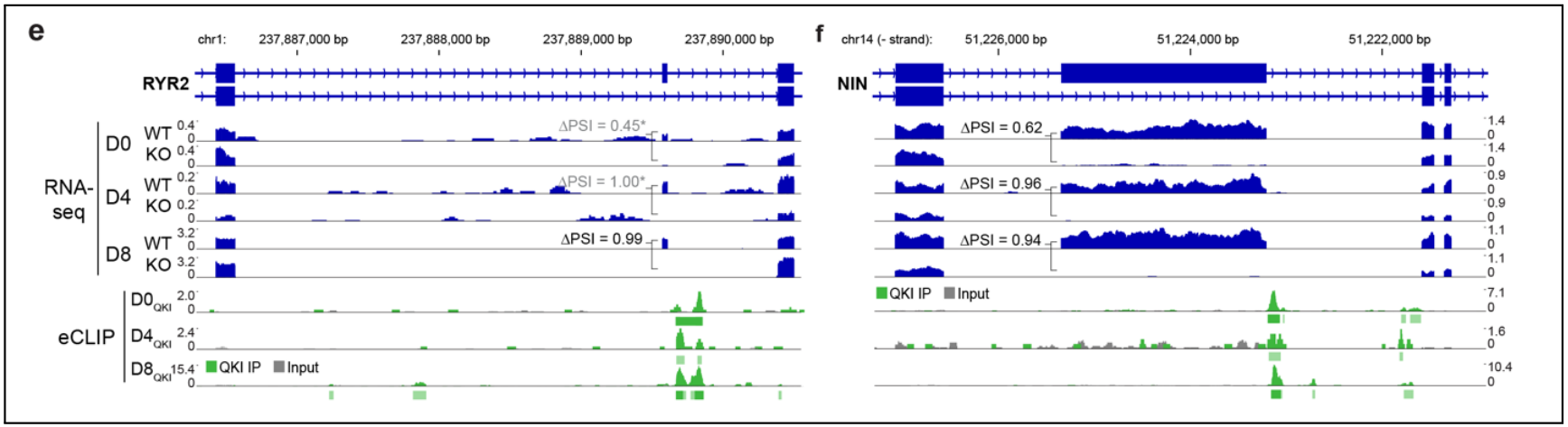
differentially included (red, $\Delta\Psi > 0$) and excluded (blue, $\Delta\Psi < 0$) cassette exons



Although QKI binds broadly in pluripotent hESCs (D0) with significant splicing changes upon its loss, affected transcripts generally lacked coherent functional enrichment (Fig. 3d). In cardiac progenitors (D4), QKI-KO-induced exon inclusions were unexpectedly enriched in neuronal genes, suggesting a role for QKI in repressing non-cardiac exons (Fig. 3d). In contrast, **QKI-dependent exon inclusion (i.e., KO-excluded exons) at the cardiomyocyte stage (D8) predominantly affected transcripts essential for cardiac structure and contractility (Fig. 3d), highlighting a central role of QKI in ensuring proper exon inclusion in genes encoding the cardiac contractility apparatus.**

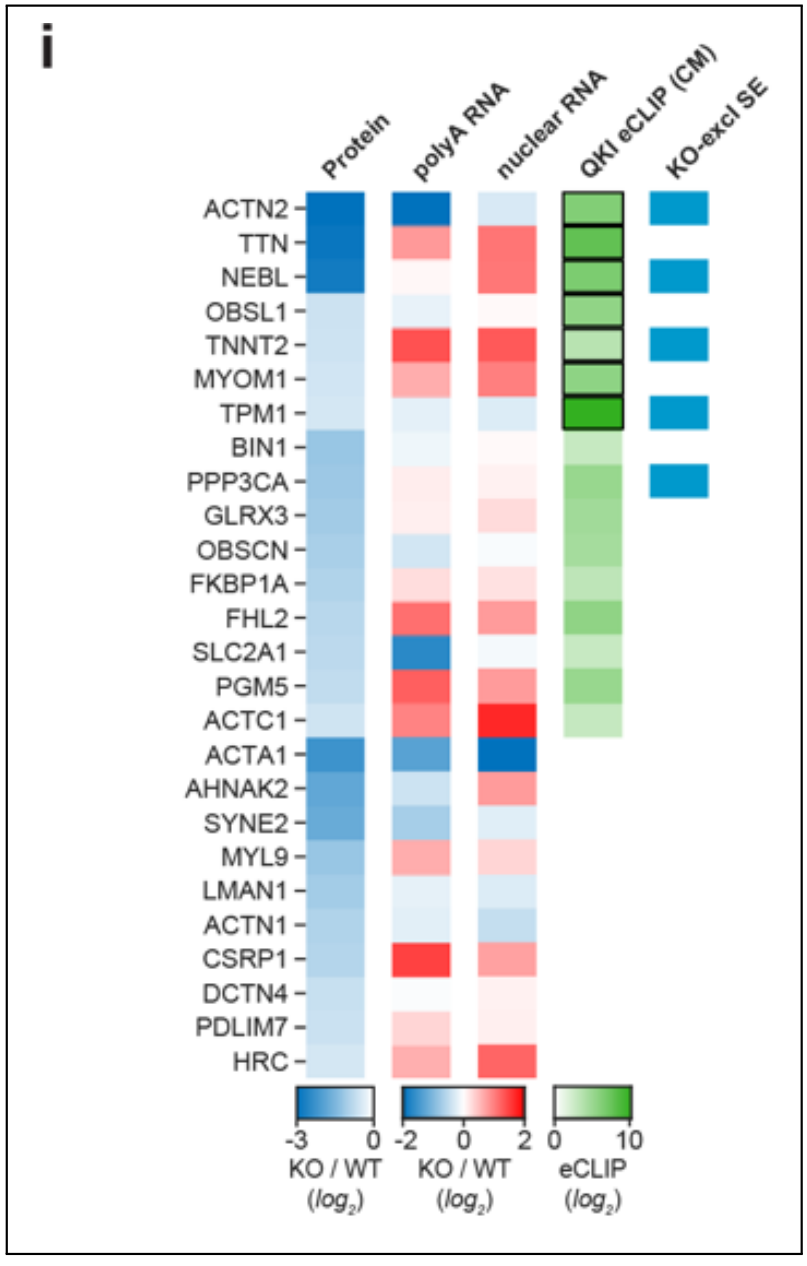


Our QKI eCLIP data enabled us to identify directly regulated QKI-dependent splicing events based on concurrent QKI binding. Several events observed in our dataset provide empirical transcriptome-wide validation of direct QKI binding at QKI-dependent splicing events previously observed in mouse cardiomyocytes or whole heart tissue, such as ACTN2, BIN1, RYR2, NEBL, AKAP9, RBFOX2, and TTN34, 36 or in neuronal cells during development, including NIN (a key regulator of the cell cycle) (Fig. 3e,f, Extended Data Fig. 5a-c). [...] **These results underscore that the role of QKI in ensuring exon inclusion at cardiac-relevant exons reflects a more universal regulatory principle.**

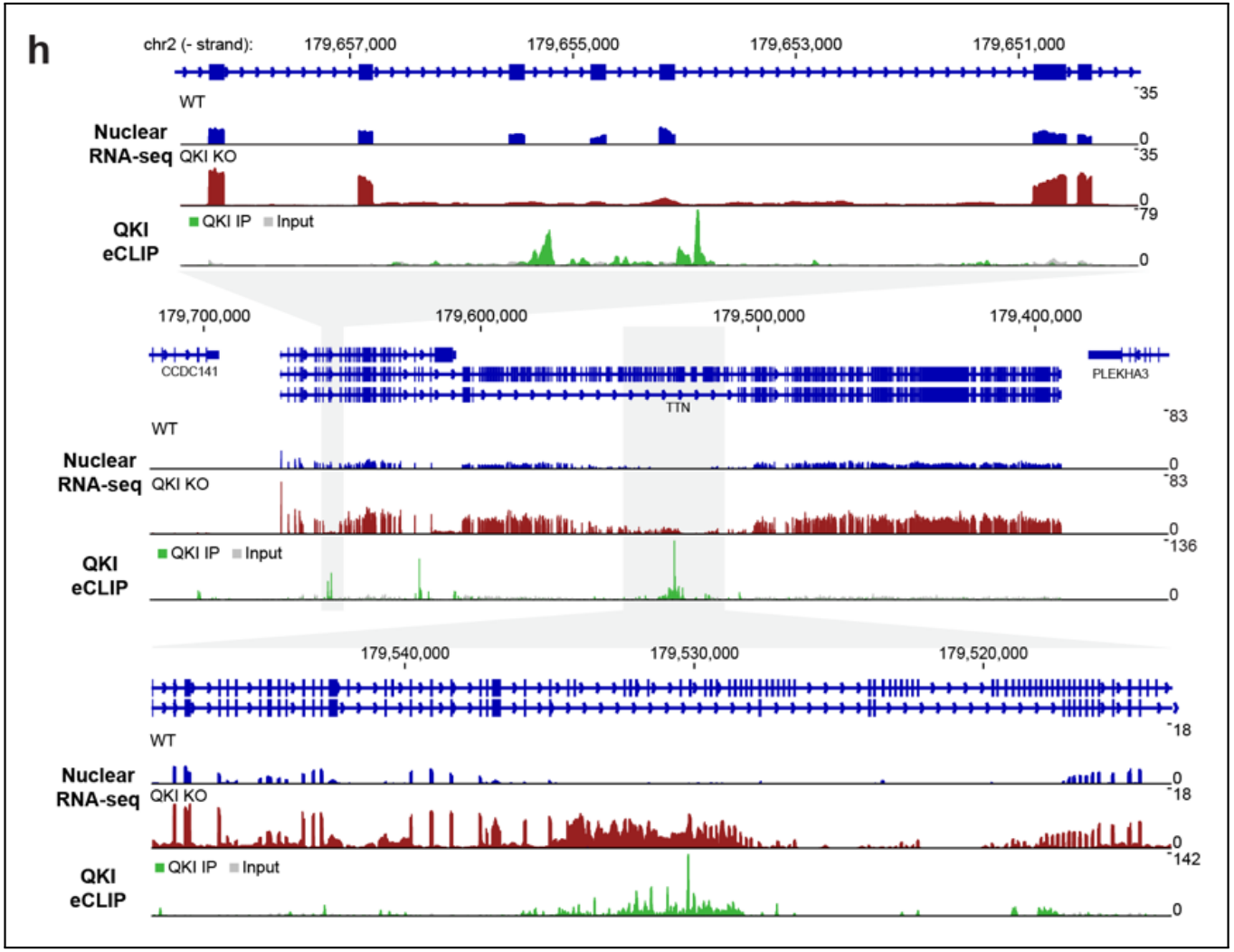


QKI loss leads nuclear sequestration of mis-spliced sarcomeric transcripts, resulting in depletion of essential sarcomeric proteins

Aligning with the inability to assemble sarcomeres, we observed that **among the top 10 most depleted proteins were three core proteins essential for cardiomyocyte structure and contractile function: TTN (Titin), NEBL (Nebulin), and ACTN2 (Alpha-actinin-2)** (Fig. 3g-i). [...] Although ACTN2 RNA was decreased (4.5-fold) in QKI knockout, NEBL RNA was 1.7-fold increased (Fig. 3i, Extended Data Fig. 6a,b), indicating a dichotomy between RNA and protein expression.

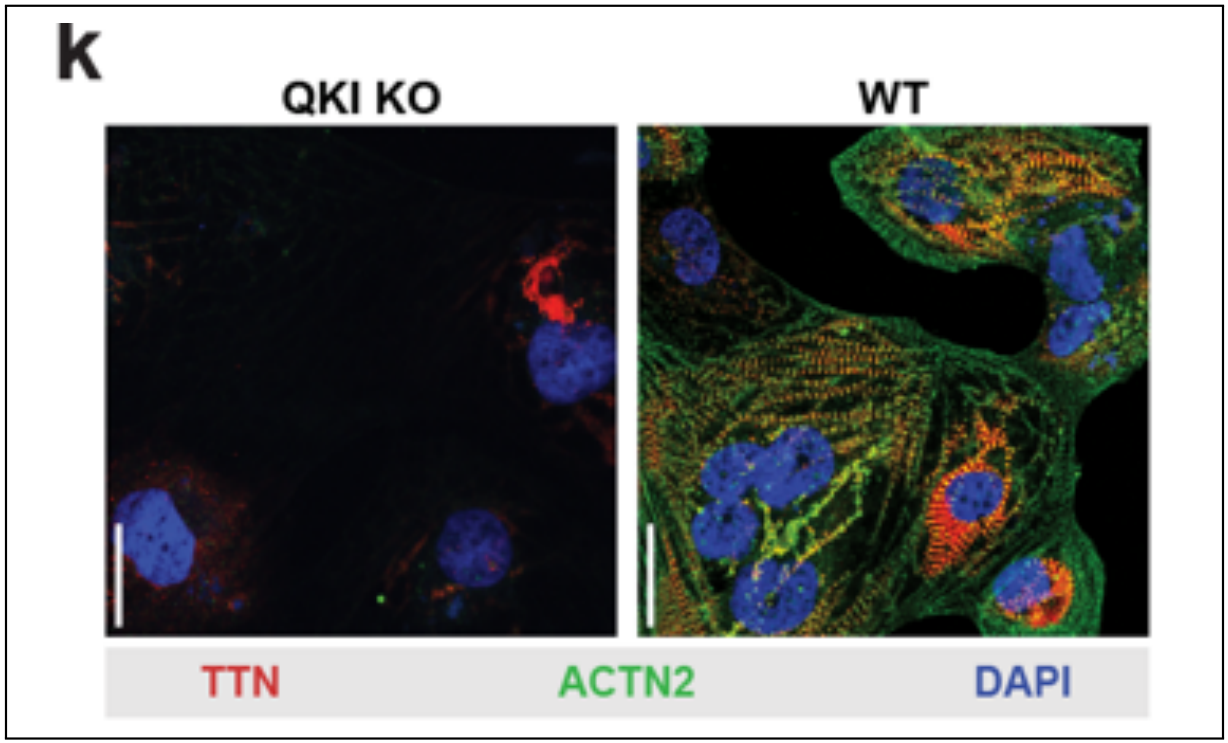


The altered RNA splicing and increased nuclear retention of TTN, [...] a central architectural scaffold of the cardiac sarcomere, was particularly notable. TTN encodes a [...] protein that spans half the sarcomere, coordinating sarcomere assembly, stability, and passive elasticity, and mutations in TTN are a leading cause of familial cardiomyopathies [...].



The extensive QKI binding and multi-exon splicing changes observed in our nuclear RNA-seq of QKI KO (Fig. 3h) extend prior findings **indicating QKI control of TTN splicing and suggest that TTN is dramatically altered upon QKI loss and nuclear-retained.**

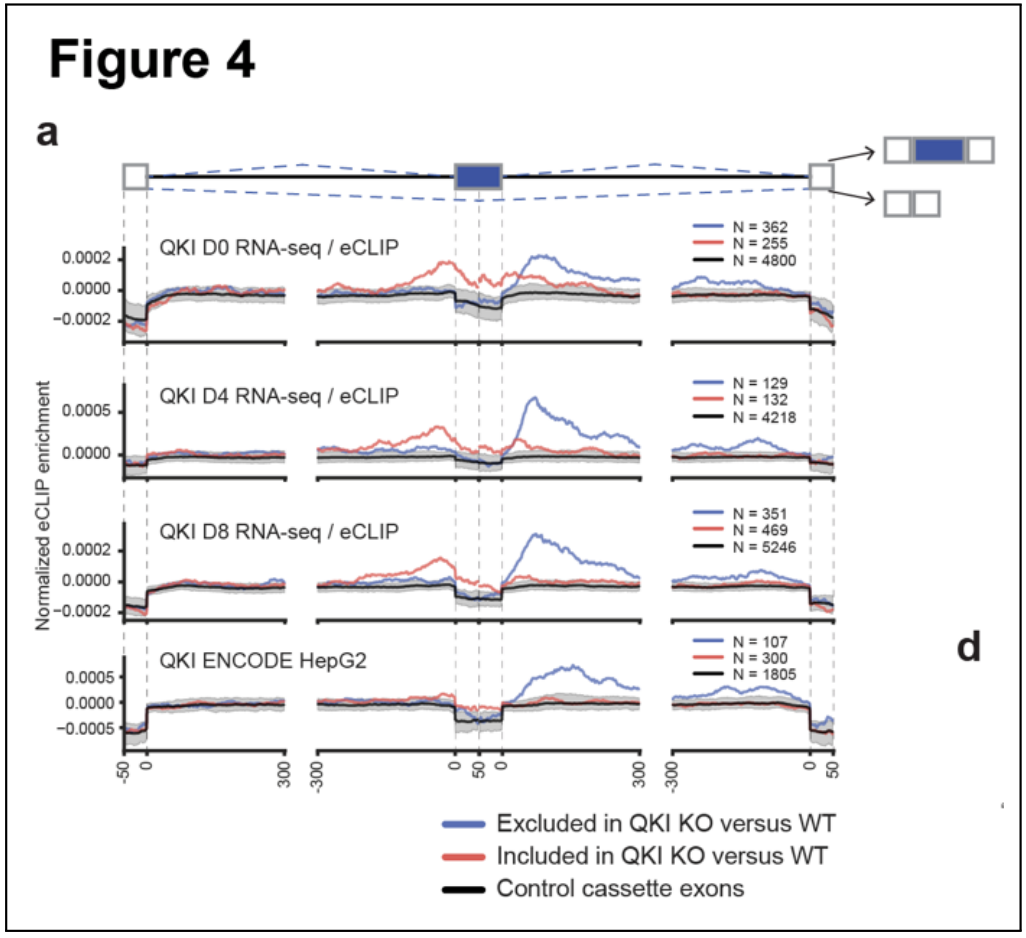
Immunofluorescence staining also revealed near-complete loss of TTN protein along with ACTN2 in QKI-deficient cells (Fig. 3k), supporting a model in which defective splicing and nuclear sequestration of TTN transcripts underlie the failure to assemble sarcomeres in the absence of QKI.



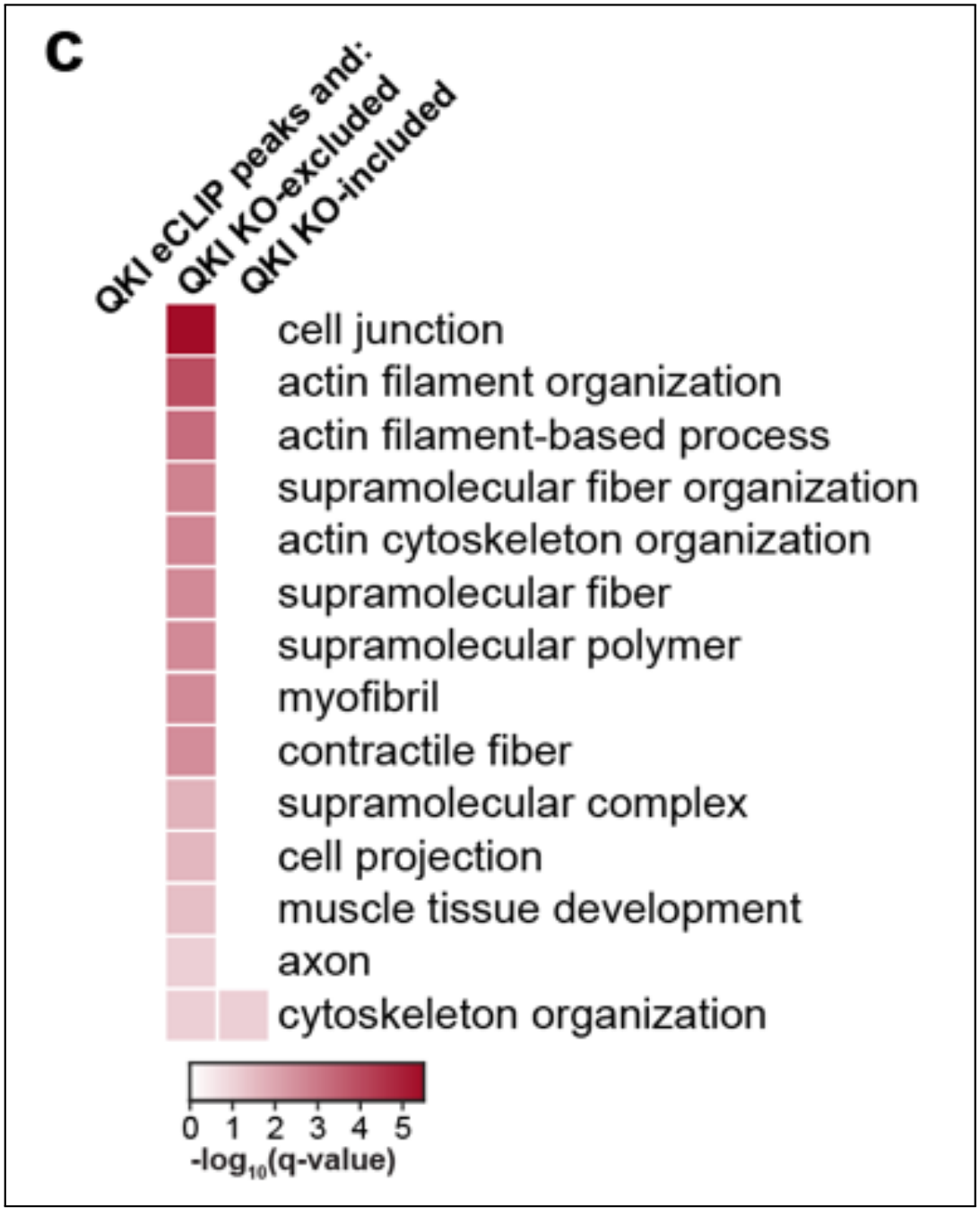
Together, these results highlight that the exclusion of exons upon QKI loss not only drives altered isoform expression or decreased RNA abundance likely via nonsense-mediated decay; rather, QKI loss leads to a broad disruption of pre-mRNA processing that often results in nuclear retention. The subsequent failure to produce proteins necessary for sarcomere architecture and cardiac function links QKI-mediated exon inclusion to the sarcomere defects observed in QKI KO cardiomyocytes.

Intronic QKI binding near 5’ splice sites ensures accurate exon inclusion

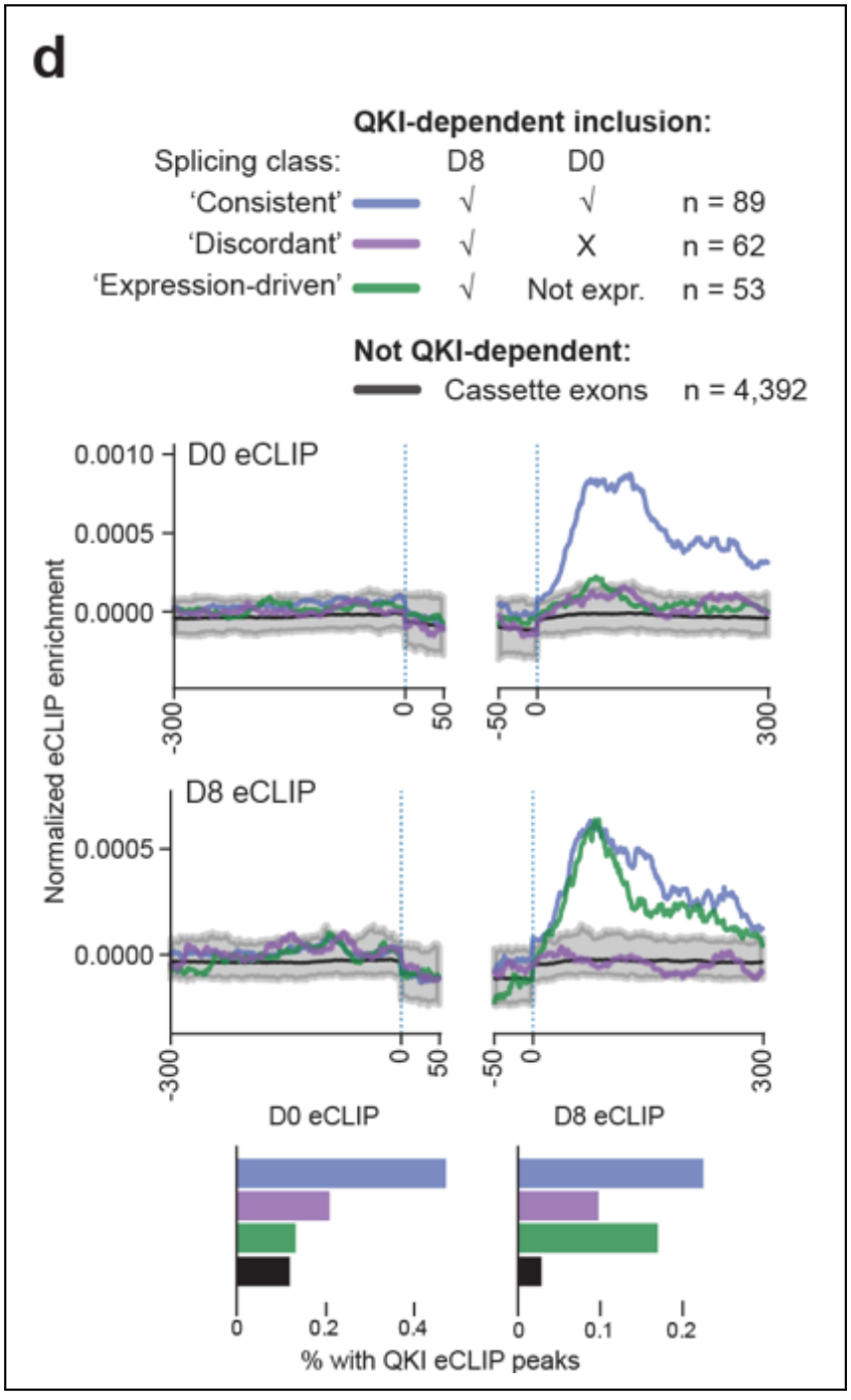
Splicing outcomes were tightly related to QKI binding position: exon inclusion upon QKI KO was characterized by QKI binding near the 5’SS (indicating **QKI binding to the 5’SS induces inclusion**), whereas QKI KO-dependent exclusion showed enriched QKI binding near the 3’SS (indicating **QKI binding to the 3’SS induces exon exclusion**) (Fig. 4a,b). This positional logic was consistent across all stages of cardiac differentiation and aligned with previously reported motif-predicted patterns [...] (Fig. 4a)



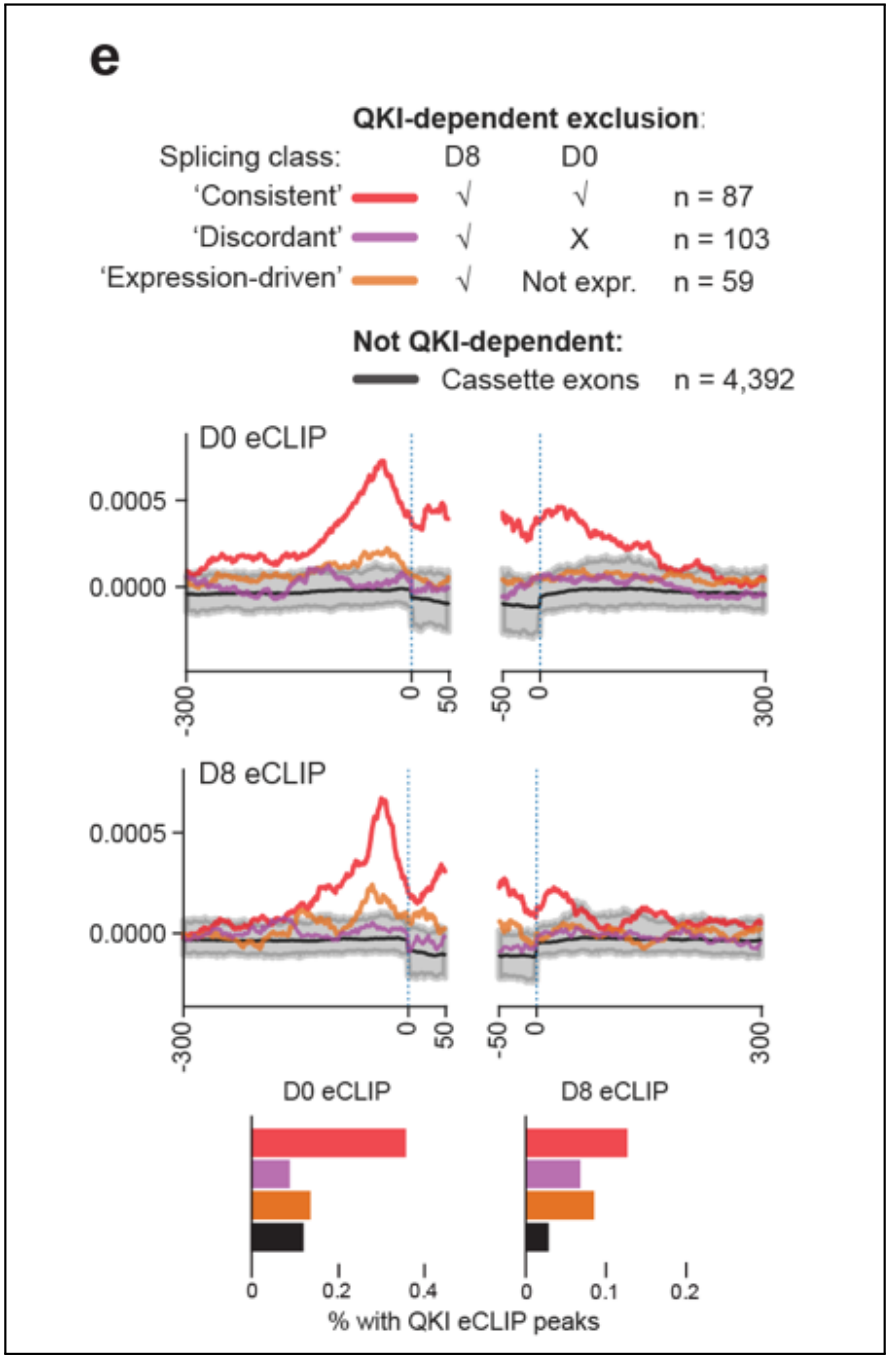
Matching our results from analysis of all differential splicing events (Fig. 3d), **genes containing exons excluded upon QKI knockout that had flanking intronic QKI peaks from eCLIP were enriched for cytoskeletal and sarcomeric genes essential for cardiac function** (Fig. 4c).



For QKI-dependent exon inclusion events, both classes showed enriched QKI binding in cardiomyocytes (Fig. 4d). The expression-driven group contained cardiomyocyte-specific transcripts encoding core sarcomeric components and regulators of contractility, including ACTN2, CACNA1C, TNNT2, and SVIL (Fig. 4d). Thus, there is a unique set of transcripts that are both selectively expressed in cardiomyocytes and rely on QKI for accurate exon inclusion, providing an explanation for the sarcomere disorganization observed upon QKI loss in cardiomyocytes. In contrast, “discordant” exons that were QKI-dependent in D8 but not D0 did not show enriched QKI binding (Fig. 4d).



Consistent with QKI being expressed in both D0 and D8, this suggests that for the discordant exons, the D8-specific dependence on QKI may occur indirectly through QKI regulation of a different cardiac-specific RBP. QKI-dependent exon exclusion events showed a similar pattern - QKI binding was enriched (proximal to the 3'SS) at consistent events but not discordant ones (Fig. 4e).



Together, these results reveal a spatial and context-dependent logic for QKI-mediated splicing [...]. QKI exerts highly selective control over exon inclusion through proximal intronic engagement at a restricted set of key cardiac transcripts required for cardiac function.

This remarkable functional specificity, emerging from widespread binding, points to an unrecognized mechanistic logic in exon recognition-one that requires QKI to convert proximal intronic binding into precise exon inclusion at loci essential for tissue-specific function.