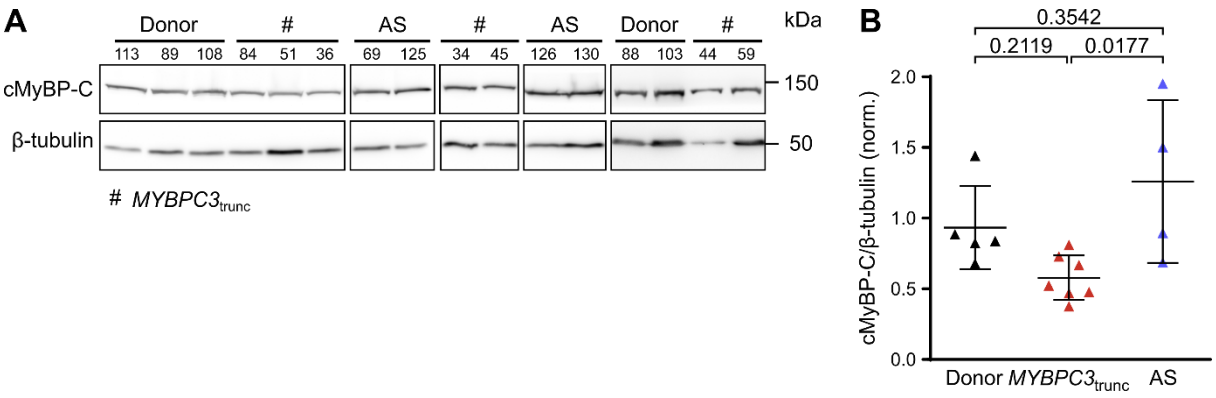
Supplementary Information

This file includes:

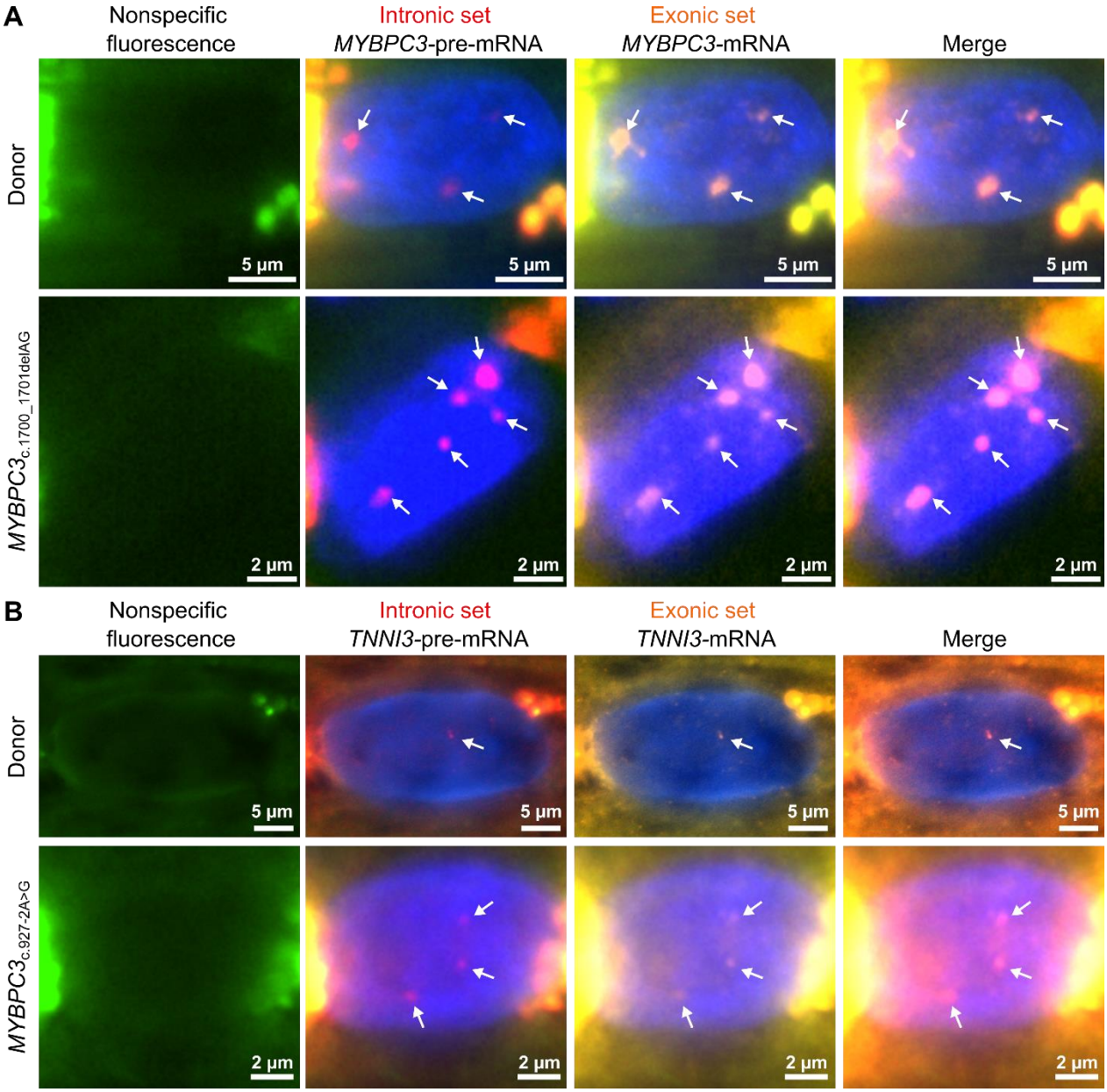
Supplement Fig. 1-3

Supplement Tables 1-4



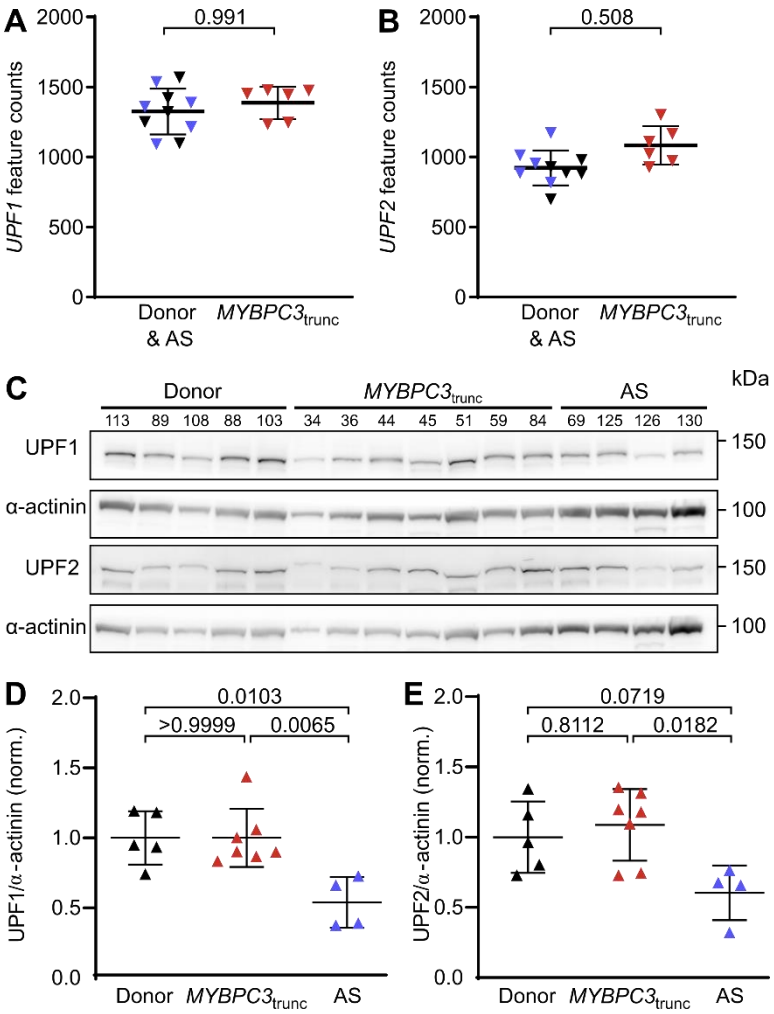
Supplement Fig. 1. cMyBP-C expression in *MYBPC3*$_{trunc}$ mutations relative to β-tubulin

Protein extracts from frozen tissue samples were analyzed on western blots after SDS-PAGE. **A** Exemplary western blot for quantification of cMyBP-C relative to β-tubulin. Each membrane was cut into pieces according to the expected size of the protein and incubated with the respective antibody. **B** Relative quantification of cMyBP-C to β-tubulin from six individual western blot membranes. Each triangle represents the mean of the six experiments for each individual. Analyzed individuals were H88, H89, H103, H108, and H113 in the donor group, H34, H36, H44, H45, H51, H59, and H84 in the *MYBPC3*$_{trunc}$ patient group and H69, H125, H126, and H130 in the AS-patient group. For comparison of HCM-patient results with donors or AS-patients, one-way analysis of variance (ANOVA) and Tukey's *post-hoc* test were performed. ANOVA yielded significant variation among groups ($F(2,13)=5.30$, $p=0.0207$). Mean ± SD and *p*-values from Tukey's test are indicated in the figure.



Supplement Fig. 2. Representative images of *MYBPC3* and *TNNI3* RNA-FISH in donor and *MYBPC3*$_{trunc}$ patient cardiac tissue cryosections

To visualize active transcription in cardiomyocyte nuclei, cryosections (10-16 µm thick) of left ventricular heart tissue were hybridized with fluorescently labeled DNA probes against exonic and intronic RNA from *MYBPC3* **A** or *TNNI3* **B**. One representative nucleus is shown for donor (upper rows) and *MYBPC3*$_{trunc}$ patient (mutation *MYBPC3*$_{c.1700\_1701delAG}$ H51) (lower rows). First column shows non-specific fluorescence (green); second column shows intronic transcript signals (red); third column shows exonic transcript signals (orange); fourth column shows a merge of intronic, exonic and DAPI (blue) fluorescence. Co-localization of intronic and exonic signals in the nucleus indicate active transcription sites (aTS), marked by white arrows.



Supplement Fig. 3. Relative expression of UPF1 and UPF2 in *MYBPC3*$_{trunc}$- and AS-patients

Relative expression of UPF1 and UPF2 were examined in *MYBPC3*$_{trunc}$- and AS-patients and donors by RNA-sequencing and western blotting. **A and B** Feature counts for UPF1 **(A)** and UPF2 **(B)** were derived from DeSeq2 analysis and compared as groups *MYBPC3*$_{trunc}$ vs. AS-patients (blue triangles) and donors (black triangles). *MYBPC3*$_{trunc}$-patients (red triangles) showed comparable feature counts as the control group. **C** Representative western blots of UPF1 and UPF2 and α-actinin as loading control. For each UPF, the membrane was cut into two pieces according to the molecular weight of the target protein and incubated with the respective antibody. **D and E** Quantification of relative protein expression for UPF1 **(D)** and UPF2 **(E)** from four western blot membranes. Each triangle represents the mean of the four experiments. Both UPF1 and UPF2 did not show a significant alteration in HCM-patients compared to donors. Interestingly, both proteins were downregulated in AS-patients at protein level, due to unknown reasons. For comparison of HCM-patient results with donors or AS-patients, one-way analysis of variance (ANOVA) and Tukey's *post-hoc* test were performed. ANOVA yielded significant variation among groups ((D) $F(2,13)=8.24$, $p=0.0049$ (E) $F(2,13)=5.31$, $p=0.0206$). Mean ± SD and *p*-values from Tukey's test are indicated in the figure.

Supplement Table 1 Available clinical data from all patient and donor samples used throughout the manuscript

Name	Mutation (pathogenic)	Age, [year]	Sex	NYHA	LVOT[a] [mm Hg]	ST[b] [mm]	PWT[c]	FS[d] [%]	Sample type	Sydney heart bank code
H34	*MYBPC3*$_{c.3288delG}$	38	m	III	30	28	12	44	myectomy	
H36	*MYBPC3*$_{c.2864\_2865delCT}$	16	f	I	80	20	12	70	myectomy, IVS[e]	
H44	*MYBPC3*$_{c.3697C>T}$	29	f	III	100	27	13	65	myectomy,	
H45	*MYBPC3*$_{c.1458-6G>A}$	63	f	III	13	20	10	49	myectomy, IVS	
H51	*MYBPC3*$_{c.1700\_1701delAG}$	26	f	I	110	24	15	74	myectomy	
H59	*MYBPC3*$_{c.3490+1G>T}$	64	m	III	n.d.[f]	19	12	34	myectomy	
H66	None, AS	41	f	II	50	12	11	38	myectomy	
H69	None, AS	32	m	II	n.d.	22	17	n.d.	myectomy	
H84	*MYBPC3*$_{c.927-2A>G}$	45	m	NYHAII-III and ICD[g]; later NYHAIV and heart transplantation	n.d.	n.d.	n.d.	n.d.	explant, LV[h]	
H88	None, donor	56	m	n.d.	n.d.	n.d.	n.d.	n.d.	explant, LV	3.149
H89	None, donor	41	f	n.d.	n.d.	n.d.	n.d.	n.d.	explant, LV	3.073
H103	None, donor	48	f	n.d.	n.d.	n.d.	n.d.	n.d.	explant, LV	5.089
H108	None, donor	48	m	n.d.	n.d.	n.d.	n.d.	n.d.	explant, IVS	6.004
H113	None, donor	48	m	n.d.	n.d.	n.d.	n.d.	n.d.	explant, LV	6.052
H125	None, AS	57	m	II	80	14	12	normal	myectomy	
H126	None, AS	60	f	III	50	13	13	normal	myectomy	
H130	None, AS	28	f	III	120	26	24	n.d.	myectomy	

[a]LVOT, left ventricular outflow tract pressure gradient; [b]ST, septum thickness; [c]PWT, posterior wall thickness; [d]FS, fractional shortening; [e]IVS, intraventricular septum; [f]n.d., no data; [g]ICD, implantable cardioverter-defibrillator; [h]LV, left ventricle

Supplement Table 2 Primer *UPF3B* and *MYBPC3*-mRNA quantification

Primer	Sequence 5'->3'	Modifications
TNNI3 reverse transcription primer	CAGCTCAGAGAGAAGCTTTA	
TNNI3 real-time F	CTCCAACTACCGCGCTTATG	
TNNI3 real-time R1	GCAGAGTCTTCAGCTGCAATTT	
TNNI3 probe	TTTTCTTGGCGTGCGGCTCC	5'-Atto 550, 3'-BMN-Q590
MYBPC3 real-time F2	AGGACCAGGTCAACCTCACA	
MYBPC3 real-time R2	CTCTCCCACGTTGCTGATCTT	
MYBPC3 probe	AAGGTCATCGACGTGCCA	5'-FAM, 3'-BMN-535
UPF3B reverse transcription primer	TGGCTAATACCACTTTCCTGCT	
UPF3B real-time F	AAGAGCCAGTGGGCAAAGTT	
UPF3B real-time R	CGCTCTCATCTTCAGGTCTCT	
UPF3B probe	ATTGCCCAAGCGTTCTGATAGCGA	5'-FAM, 3'-BMN-535
GAPDH reverse transcription primer	CCGTTCAGCTCAGGGATGAC	
GAPDH real-time F	CACCAGGGCTGCTTTTAAC	
GAPDH real-time R	ATGGGTGGAATCATATTGGAAC	
GAPDH probe	CCCTTCATTGACCTCAACTACATGGTTTACA	5'-VIC/HEX, 3'-BMN-Q535

Supplement Table 3: Rank metric score results from the NMD gene set derived from GSEA

NMD-specific genes from the NMD gene set	Rank metric score of *MYBPC3*$_{trunc}$ patients vs. donors	Rank metric score of AS-patients vs. donors
PABPC1	0.07	-0.19
EIF4G1	-0.91	-0.83
ETF1	-0.04	-0.10
GSPT1	-0.45	-0.32
GSPT2	-0.23	-0.19
NCBP2	0.23	0.10
NCBP1	-0.06	-0.20
EIF4A3	0.14	0.25
CASC3	-0.03	-0.12
MAGOHB	0.31	0.25
MAGOH	0.36	0.46
UPF2	0.53	0.26
RNPS1	0.12	0.19
RBM8A	0.21	0.20
UPF3B	1.25	1.07
UPF3A	0.20	0.06
PPP2CA	0.47	0.61
SMG1	0.13	-0.16
SMG7	-0.25	-0.26
PPP2R1A	0.12	0.17
PPP2R2A	-0.48	-0.27
UPF1	0.11	-0.01
DCP1A	0.04	-0.13
PNRC2	0.78	0.67
SMG5	0.43	0.22
SMG8	-0.24	0.13
SMG6	0.26	0.04
SMG9	0.16	0.13

Supplement Table 4: Consequences of cMyBP-C/*MYBPC3* mutations

Patient	Mutation (*MYBPC3*, cMyBP-C)	Protein consequences at cMyBP-C domain	Position of the resulting PTC upstream of next exon-exon junction [bp]
H34	Deletion (c.3288delG, p.Glu1096Aspfs*93)	Frameshift, truncation in C9 (exon 31)	62
H36	Deletion (c.2864_2865delCT, p.Pro955Argfs*95)	Frameshift, truncation in C8 (exon 29)	41 and 104
H44	Termination (c.3697C>T, p.Gln1233*)	PTC[a], truncation in C9 (exon 32)	115
H45	Splice mutant (c.1458-6G>A, p.Leu487fs*)	Intron containing PTC between exon 16 and 17 is not spliced, truncation in C3	248
H51	Deletion (c.1700_1701delAG, p.Glu567Glyfs*4)	Frameshift, truncation in C3 (exon 18)	78
H59	Splice mutant (c.3490+1G>T, p.Glu1111Alafs*25)	Frameshift due to skipping of exon 31, truncation in C9	62
H84	Splice mutant (c.927-2A>G, p.Asp310fs*)	Intron containing PTC between exon 11 and 12 is not spliced, truncation between C1 and C2	252

[a]PTC, premature termination codon