

Supplementary Figures

Figure S1

A

#	Visible?	Stained?	MS/MS View: 342 Proteins in 302 Clusters With 4 Decoys	Probability Legend:		Accession Number	Molecular Weight	Protein Grouping Ambiguity						
				over 95%	80% to 94%				50% to 79%	20% to 49%	0% to 19%			
1				sp P02766 TTHY_HUMAN Transthyretin OS=Homo sapiens GN=TTR PE...	sp P02766 TTHY_HUMAN	16 kDa		44	38	53				
2				sp P02743 SAMP_HUMAN Serum amyloid P-component OS=Homo sapi...	sp P02743 SAMP_HUMAN	25 kDa		35	23	38				
3				sp P01857 IGHG1_HUMAN Ig gamma-1 chain C region OS=Homo sapie...	sp P01857 IGHG1_HUMAN	36 kDa		9						2
4				sp P02649 APOE_HUMAN Apolipoprotein E OS=Homo sapiens GN=APO...	sp P02649 APOE_HUMAN	36 kDa		6		8				
5				Cluster of sp P12883 MYH7_HUMAN Myosin-7 OS=Homo sapiens GN=...	sp P12883 MYH7_HUMAN	223 k...	*	299	332	332	286	442	357	290
6				Cluster of sp P68032 ACTC_HUMAN Actin, alpha cardiac muscle 1 OS=...	sp P68032 ACTC_HUMA...	42 kDa	*	96	99	102	84	128	107	91
7				Cluster of sp P17661 DESM_HUMAN Desmin OS=Homo sapiens GN=D...	sp P17661 DESM_HUMAN	54 kDa	*	18	74	73	54	61	69	47
8				Cluster of sp P35609 ACTN2_HUMAN Alpha-actinin-2 OS=Homo sapie...	sp P35609 ACTN2_HUM...	104 k...	*	20	56	56	43	78	54	32
9				sp P25705 ATPA_HUMAN ATP synthase subunit alpha, mitochondrial O...	sp P25705 ATPA_HUMAN	60 kDa	*	4	24	41	34	41	37	26
1				sp P06576 ATPB_HUMAN ATP synthase subunit beta, mitochondrial OS...	sp P06576 ATPB_HUMAN	57 kDa	*		41	48	42	61	34	30
1				sp P08590 MYL3_HUMAN Myosin light chain 3 OS=Homo sapiens GN=...	sp P08590 MYL3_HUMAN	22 kDa	*	12	34	39	30	58	48	35
1				Cluster of sp P68871 HBB_HUMAN Hemoglobin subunit beta OS=Hom...	sp P68871 HBB_HUMAN	16 kDa	*	2	39	21	34	58	13	41
1				sp P00560 PGK_YEAST Phosphoglycerate kinase OS=Saccharomyces ce...	sp P00560 PGK_YEAST	45 kDa								

Figure S2

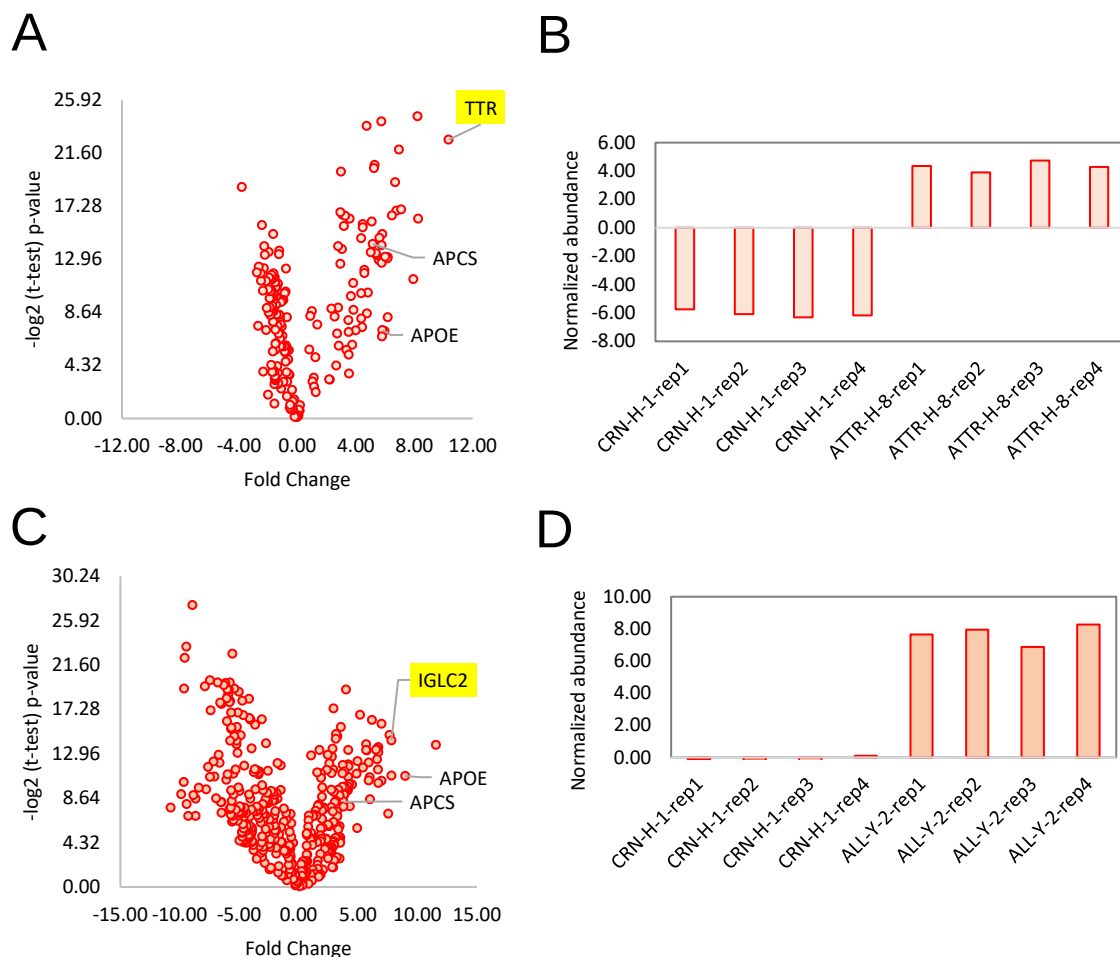
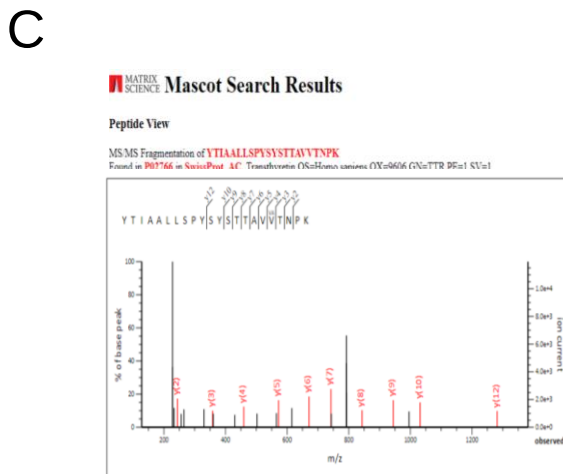
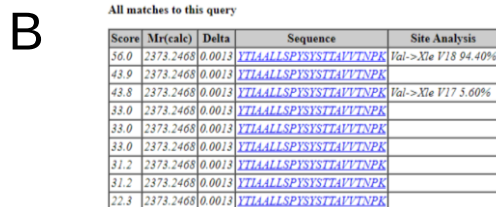


Figure S2. **A.** Volcano plot of Congo red negative heart autopsy specimen CRN-H-1 versus Congo red positive heart autopsy specimen for transthyretin amyloidosis (ATTR-H-8); **B.** Transthyretin normalized abundance in CRN-H-1 versus ATTR-H-8; **C.** Volcano plot of Congo red negative heart CRN-H-1 versus Congo red positive lymph node for AL amyloidosis (ALL-Y-2); **D.** Immunoglobulin lambda light chain in CRN-H-1 “H-A-009” vs Congo red positive lymph node (ALL-Y-2).



- D**
- Error tolerant search + manual verification of MS/MS spectra
 - Transthyretin mutation ¹²²V to I/L detected from heart tissue

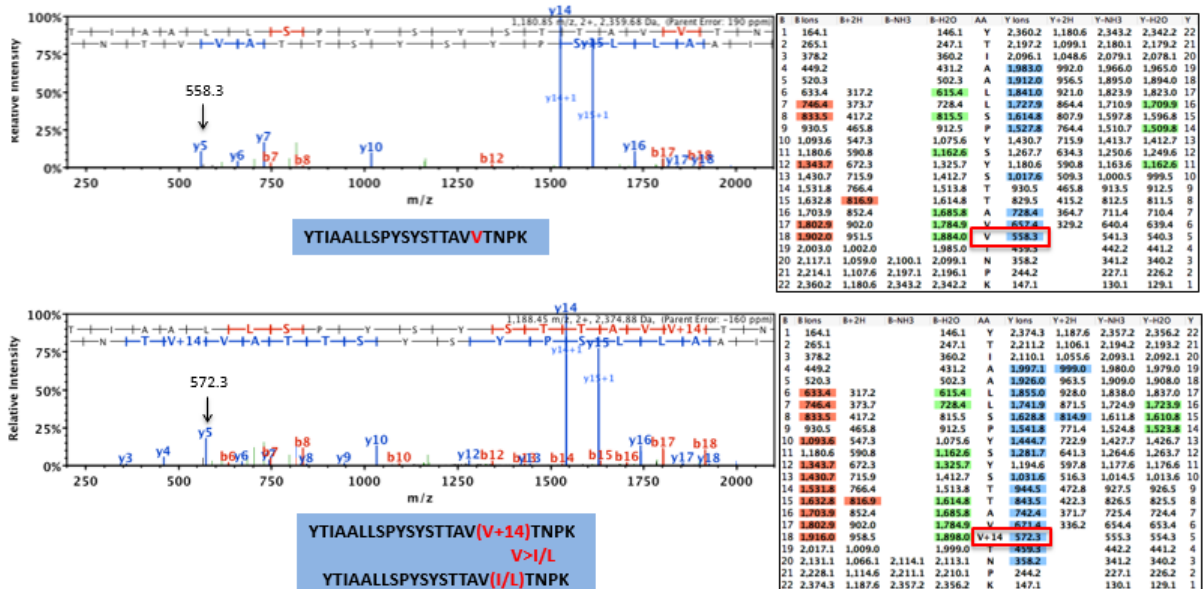


Figure S4

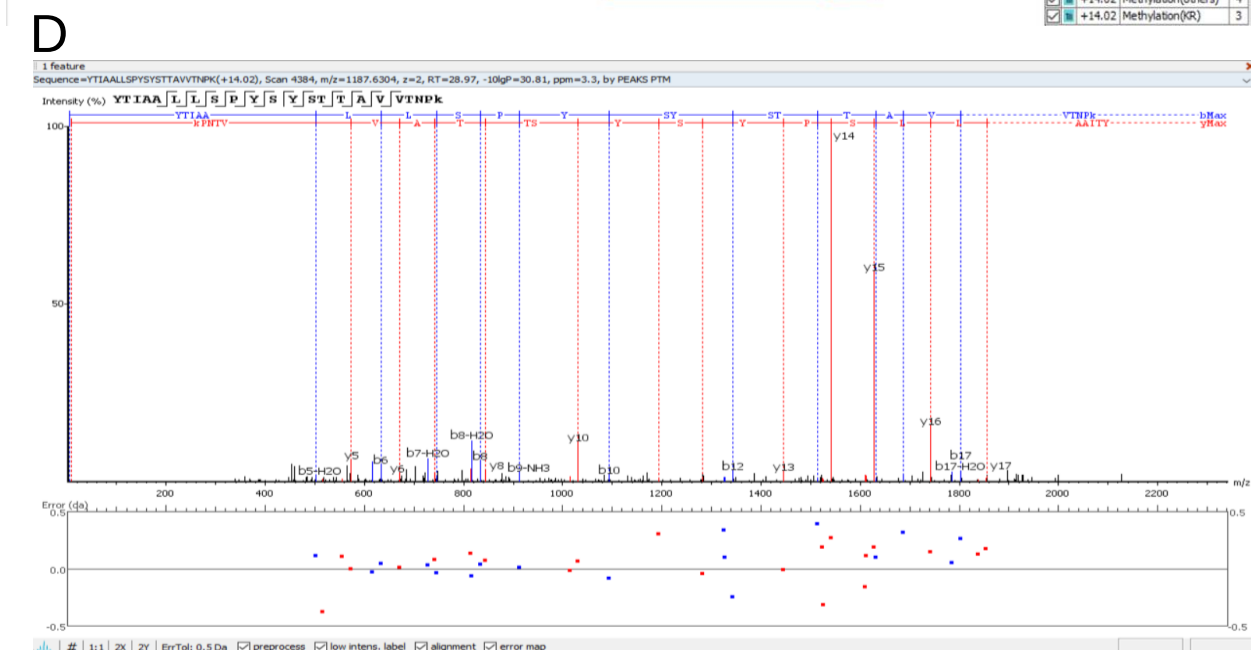
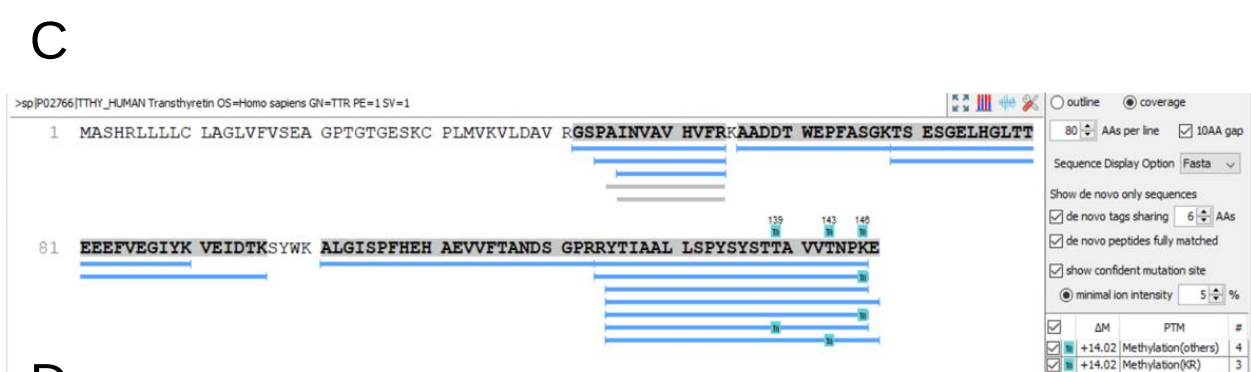
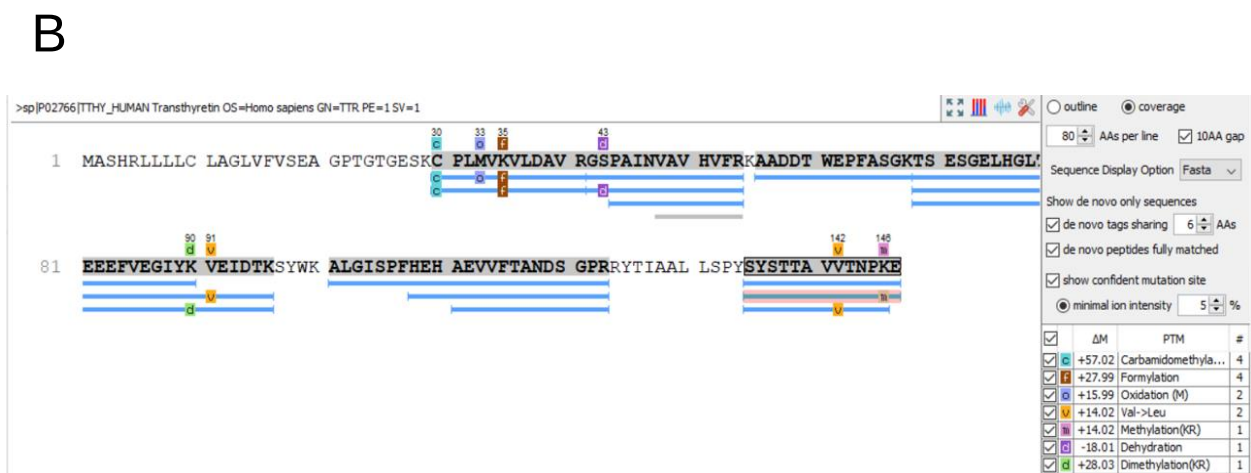
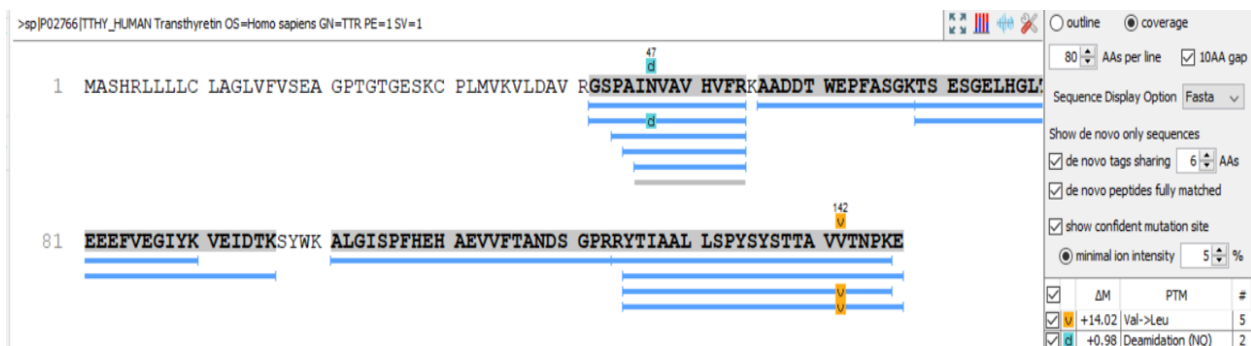


Figure S4A. PEAKS PTM and SPIDER assignment of V122I/L by V->Leu (Xle) with +14.02 mass shift on peptide RYTI AALLSPYSTTAV**V**TNPKE; **SB.** both V->Leu (Xle) and methylation on RYTI AALLSPYSTTAV**V**TNP**K**E. **S4C.** Methylation on peptide RYTI AALLSPYSTTAV**V**TNP**K**E; **S4D.** MS/MS assignment for RYTI AALLSPYSTTAV**V**TNP**K**(+14)E (methyl on K).

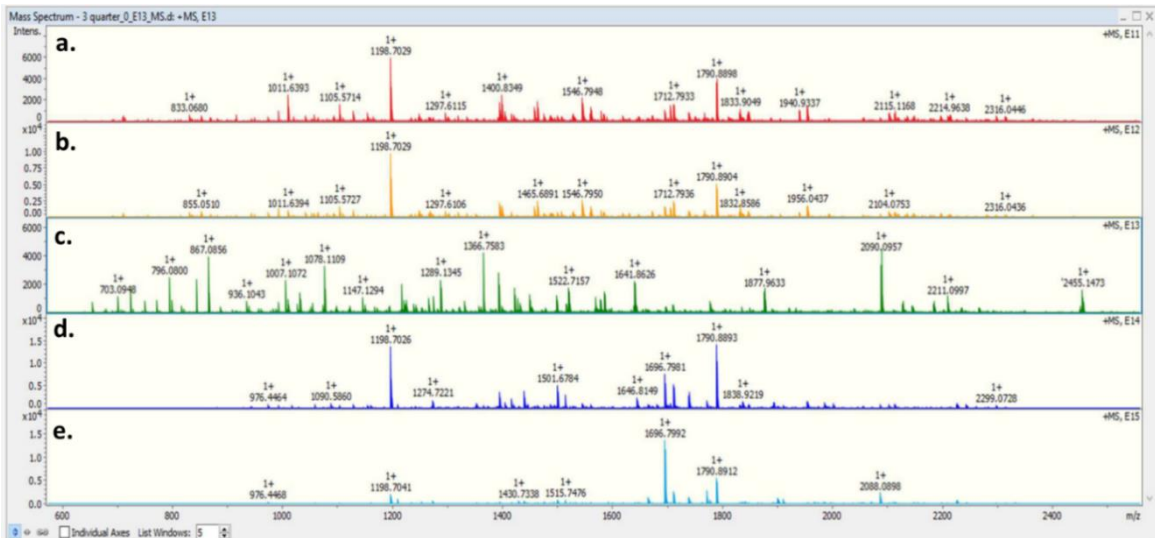
Figure S5

Figure S5. On-tissue trypsin digestion of FFPE heart tissue from **A.** Congo red positive (CR+) and **B.** Congo red negative (CR-) biopsy specimen; **C.** in-solution trypsin digestion of a transthyretin protein standard; **D.** Congo red positive (CR+) and **E.** Congo red negative (CR-) autopsy specimen.

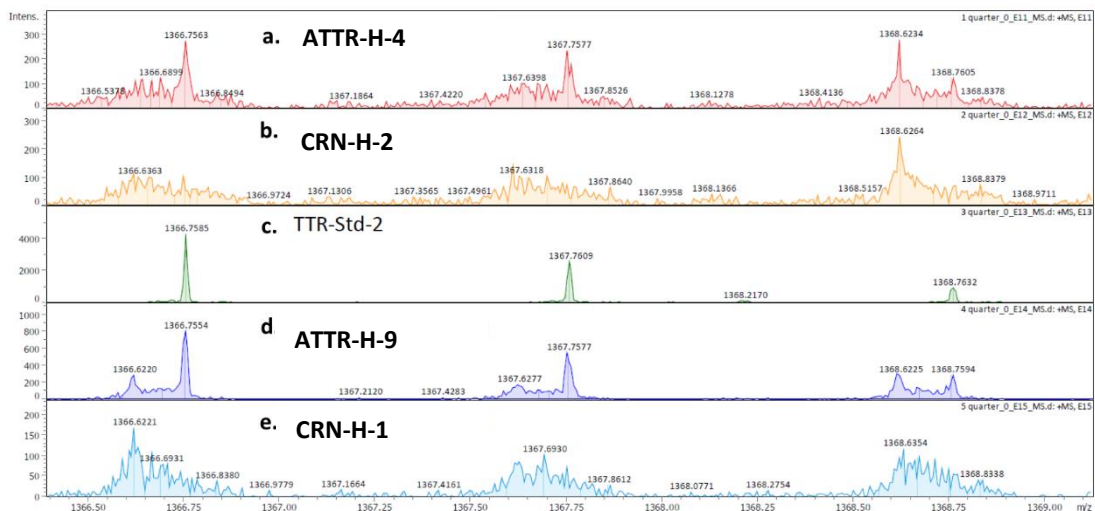
Figure S6

Figure S6. Peptide GSPAINVAVHVFR (m/z 1366.7) as potential biomarker for transthyretin observed only in **A.** Congo red positive heart biopsy (ATTR-H-4), **C.** transthyretin protein standard (TTR-Std-2) and **D.** Congo red positive heart autopsy (ATTR-H-9) but not in the **B.** Congo red negative heart biopsy (CRN-H-2) and **E.** Congo red negative heart autopsy specimen (CRN-H-1).

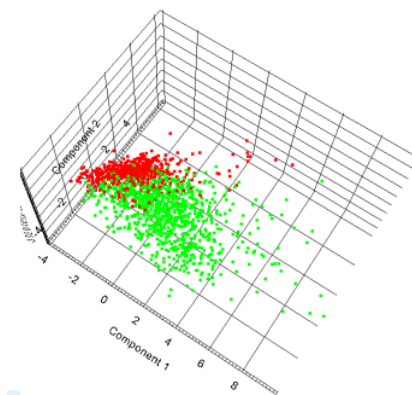
Figure S7

Fig. S7. Principal component analysis of Congo red negative biopsy specimen (CRN-H-2) versus Congo red positive specimen (ATTR-H-4).