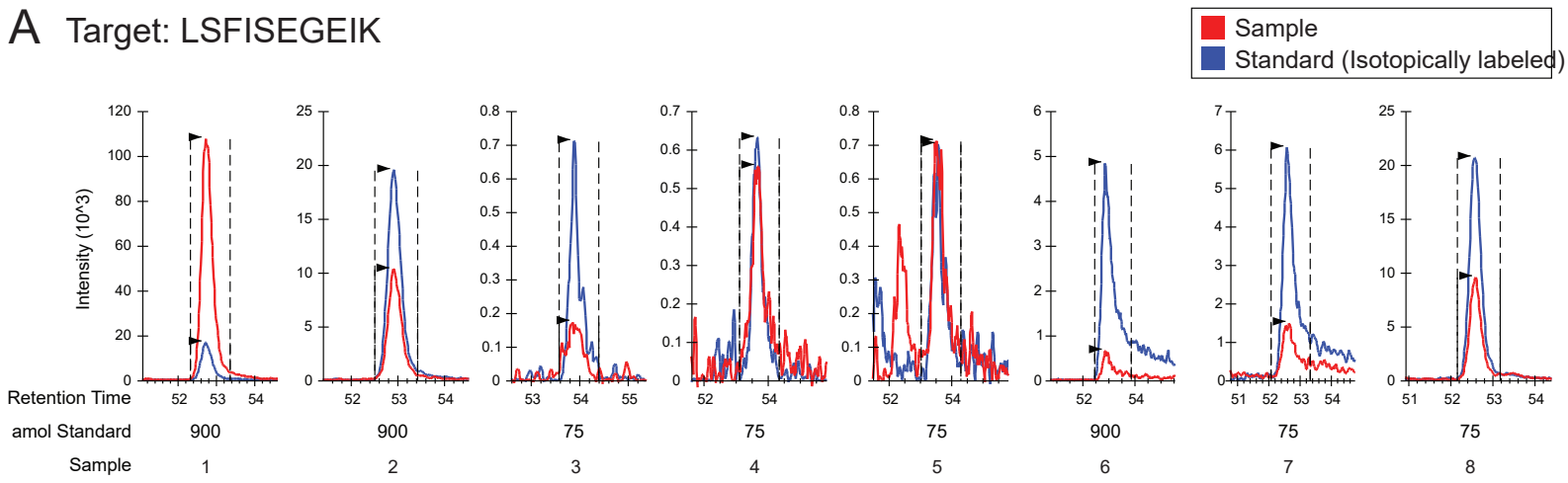
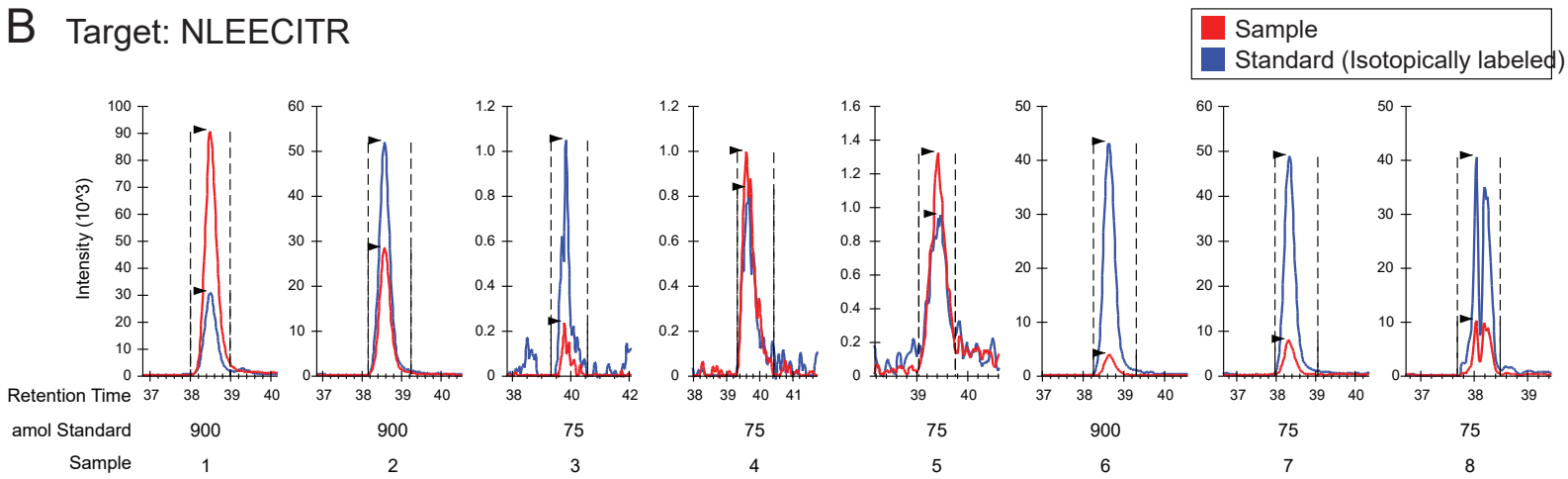


A Target: LSFISEGEIK



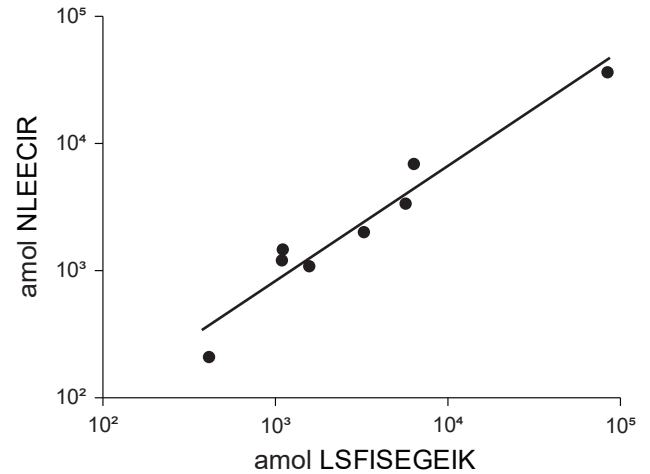
B Target: NLEECITR



C

Sample #	Source	Sample Type	ORF1p Simoa (pg/ml)	Plasma Volume (ml)	Total amol Simoa	Total amol LSFISEGEIK	Total amol NLEECIR
1	MGH	GE Cancer	1231	3.5	107444	84023	36415
2	MGH	GE Cancer	284	3	21262	6345	6938
3	BioIVT	Healthy	0.06	6	9	413	210
4	BioIVT	Pool (2)	0.24	4	24	1089	1210
5	BioIVT	Pool (2)	0.13	4	13	1105	1472
6	MGH	Healthy	3.0	5	374	1568	1085
7	MGH	Healthy	4.7	5	586	3261	2015
8	MGH	Healthy	232	5	28928	5683	3370

D



Supplementary Figure 23. Targeted proteomics analysis of patient plasma. Samples selected for analysis included two GE cancer patient plasmas with high ORF1p, healthy patients (BioIVT) with very low or undetectable ORF1p, and three healthy patient samples with high ORF1p in the 62H12:Ab6 assay. ORF1p was immunoprecipitated on 62H12-conjugated magnetic beads, digested and prepared for analysis as described above, and assayed for target peptides (A) LSFISEGEIK and (B) NLEECITR, with quantification against spiked-in internal isotopically-labeled standard; results are listed in (C). Traces are from injection of 15% of sample. (C) Assay data: measured and total ORF1p in the samples by Simoa and MS and starting plasma volumes analyzed. Simoa values were from 62H12:Ab6 for samples from MGH (Massachusetts General Hospital) and Nb5:Ab6 for samples from BioIVT; Pool (2): for samples 4 and 5, samples from two healthy patients each were pooled. For healthy samples that were not measurable, the assay LOD was used. (D) Comparison of the two peptides assays. Sample values strongly correlate ($R^2 = 0.989$, linear regression, 99.4% correlation (t test), $p < 0.0001$ for both).