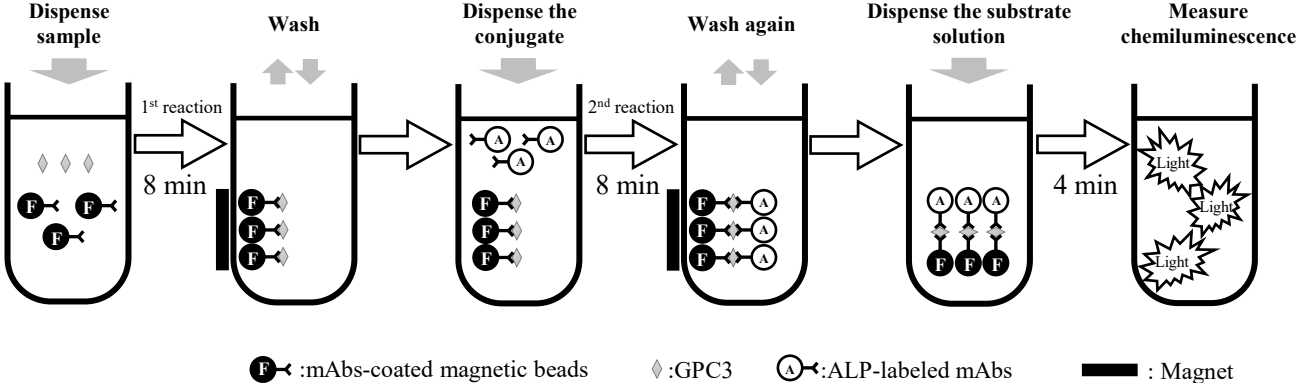
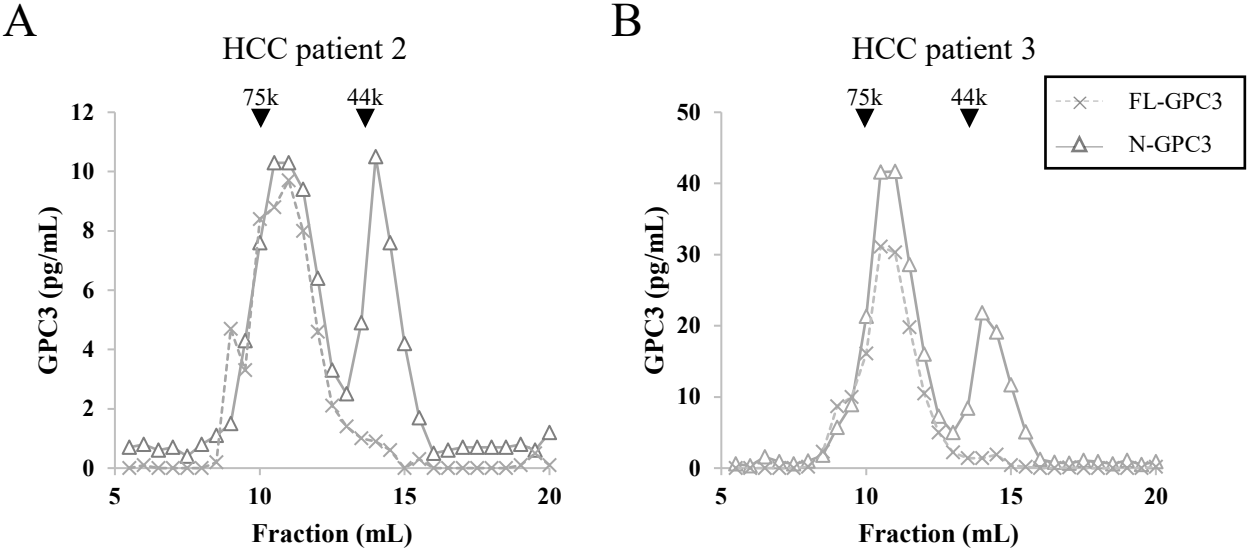


# Supplementary Figure 1: Assay procedure on the LUMIPULSE.



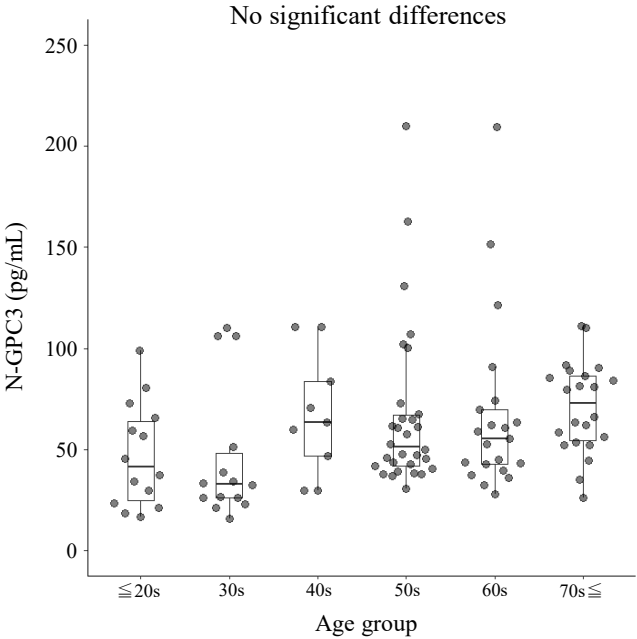
Condition	Volume
mAbs-coated magnetic beads solution	50 $\mu$ L
Sample volume	20 $\mu$ L
ALP-labeled mAbs solution	50 $\mu$ L

# Supplementary Figure 2: Size exclusion chromatography analysis of GPC3 proteins.

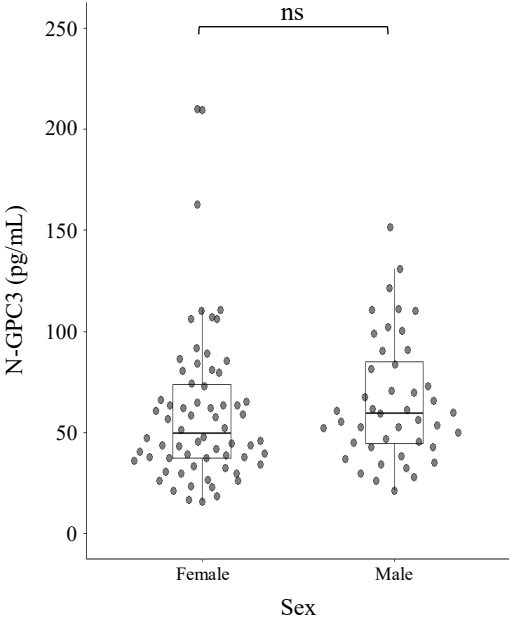


# Supplementary Figure 3: Effects of age and sex on serum GPC3.

A



B



**Supplementary Table 1: Intra-assay reproducibility analysis of the GPC3 assays.**

	FL-GPC3			N-GPC3		
	Specimen1	Specimen2	Specimen3	Specimen1	Specimen2	Specimen3
Measurement Value (pg/mL)	36.7	375.9	4070.8	48.2	386.0	3982.6
	38.9	395.1	3942.1	46.2	403.9	3883.4
	38.2	395.8	4020.1	46.5	403.6	3802.0
	37.6	396.1	4016.6	45.3	396.5	3926.3
	36.9	405.3	4073.9	46.0	412.4	3981.3
	39.4	397.1	4003.6	45.8	407.0	4008.4
Average (pg/mL)	38.0	394.2	4021.2	46.3	401.6	3930.7
CV %	2.8	2.5	1.2	2.2	2.3	2.0

## Supplementary Table 2: Inter-assay reproducibility analysis of the GPC3 assays.

FL-GPC3		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		
Measurement Value (pg/mL)	Specimen1	38.5	35.8	38.1	36.9	29.9	37.2	36.2		
		37.7	35.1	37.3	35.8	30.3	35.8	35.6		
	Specimen2	401.8	348.0	390.2	373.4	327.1	379.5	379.5		
		404.9	355.2	390.6	379.1	331.8	372.8	377.4	7 days	
	Specimen3	3975.6	3890.7	3719.8	3723.2	3307.0	3878.3	3724.1	Average	CV
		3962.1	3868.1	3741.6	3714.8	3503.9	3914.9	3720.4	pg/mL	%
Average (pg/mL)	Specimen1	38.1	35.5	37.7	36.4	30.1	36.5	35.9	35.7	7.3
	Specimen2	403.4	351.6	390.4	376.3	329.5	376.2	378.5	372.2	6.4
	Specimen3	3968.9	3879.4	3730.7	3719.0	3405.5	3896.6	3722.3	3760.3	4.9
N-GPC3		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		
Measurement Value (pg/mL)	Specimen1	48.5	43.1	46.4	41.3	37.1	47.4	43.5		
		47.9	43.1	47.3	43.7	37.8	46.1	45.5		
	Specimen2	405.8	348.0	395.5	388.0	344.6	398.4	372.8		
		405.9	348.3	395.4	383.7	356.6	396.3	386.3	7 days	
	Specimen3	3939.7	3911.1	3704.3	3733.0	3388.2	3901.2	3781.2	Average	CV
		3922.1	3896.3	3709.6	3811.2	3392.3	3947.7	3791.4	pg/mL	%
Average (pg/mL)	Specimen1	48.2	43.1	46.9	42.5	37.5	46.8	44.5	44.2	8.1
	Specimen2	405.9	348.2	395.5	385.9	350.6	397.4	379.6	380.4	5.8
	Specimen3	3930.9	3903.7	3707.0	3772.1	3390.3	3924.5	3786.3	3773.5	4.9

**Supplementary Table 3: Dilution linearity analysis of the GPC3 assays.**

	Sample	r	Slope	Intercept
FL-GPC3	1	1.000	0.99 (0.97-1.01)	1.90 (0.26-3.55)
	2	1.000	0.99 (0.98-1.00)	1.38 (0.49-2.27)
	3	1.000	1.00 (0.98-1.02)	0.94 (-0.70-2.58)
N-GPC3	1	1.000	0.99 (0.98-1.01)	3.16 (0.97-5.35)
	2	1.000	1.00 (0.98-1.01)	2.79 (0.60-4.98)
	3	1.000	0.99 (0.96-1.03)	3.92 (-1.09-8.94)

**Supplementary Table 4: The number of outliers.**

	NC	NASH	HCC
AFP	2 [3.3%]	0 [0.0%]	12 [17.1%]
N-GPC3	2 [3.3%]	3 [6.0%]	10 [14.3%]
PIVKA-II	2 [3.3%]	10 [20.0%]	10 [14.3%]