Electronic Supplementary Material (ESI) for Analytical Methods.
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SUPPLEMENTARY INFORMATION

for the article “An integrated homogeneous SPARCL™ immunoassay for the rapid biomarker detection on a chip” (by Natalia Sandetskaya, Nicole Isserstedt-John, Andreas Kölsch, Sebastian Schattschneider and Dirk Kuhlmeier)

1. Custom-made luminometer for the SPARCL™ measurements on a chip

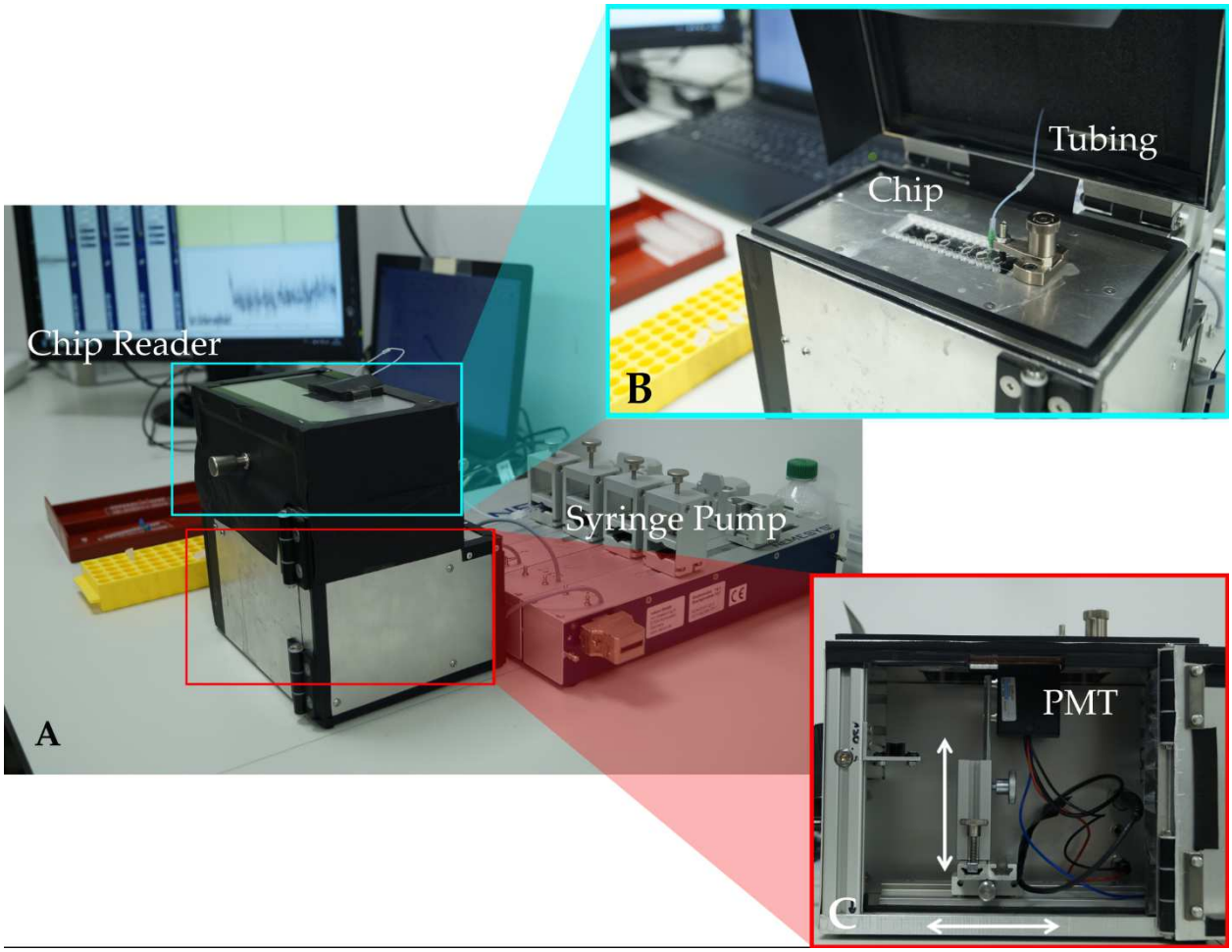

Fig. S1 Custom-made luminometer for the SPARCL™ measurements on the chip. A: Overview of the instrument with the closed lid. B: Chip positioning on the top panel of the instrument and its connection to the syringe pump. C: Luminometer with the side panel opened.

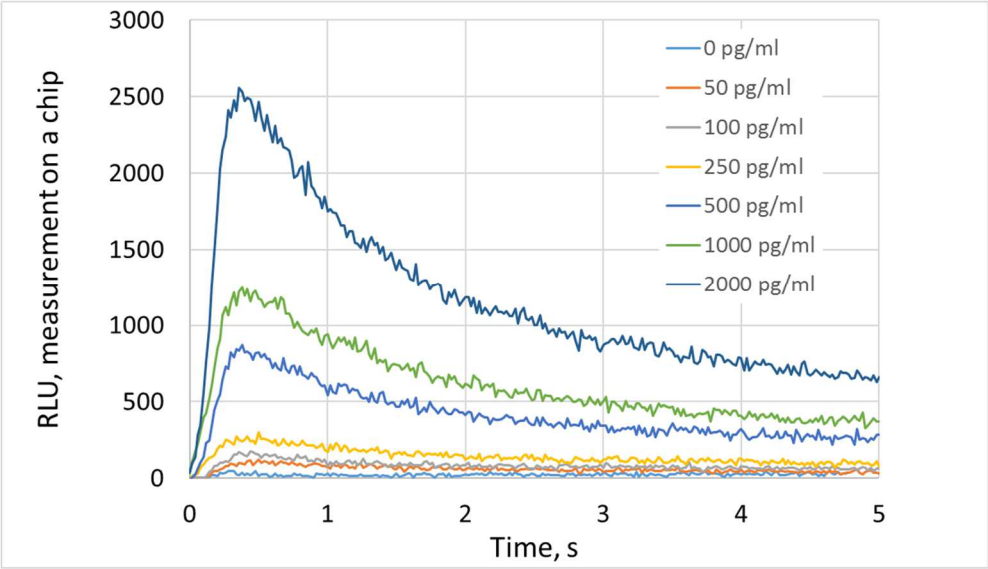

Fig. S2 Exemplary signal development for various concentration of TIMP1 after injection of the trigger at 0 s.

2. Determination of the limits of detection and quantification for SPARCL™ on a chip

Ten replicates of blank samples were measured on the chip (Supplementary table 1, A). Additionally, TIMP1 dilutions in the range 50-2000 pg/ml were prepared in PBS and measured in five replicates of each concentration (Supplementary table 1, B).

Supplementary table 1. Calibration of SPARCL™ on a chip. A: Estimation of the noise luminescence S_0. B: calibration for the analytical range of TIMP1 concentration.

A

S0	**S0, RLU**
0_1	47,5
0_2	49,2
0_3	45,4
0_4	33,9
0_5	44,9
0_6	91,4
0_7	35,7
0_8	65,0
0_9	47,0
0_10	50,2
Average S/S0	**51,0**
SD	16,5

B

TIMP1, pg/ml	**Average RLU**	**SD (RLU)**	**S/S0**	**SD (S/S0)**
0	51,0	16,5	**1,0**	0,3
50	119,4	34,0	**2,3**	0,7
100	204,4	68,7	**4,0**	1,3
250	236,6	74,2	**4,6**	1,5
500	819,8	126,0	**16,1**	2,5
1000	1185,1	71,2	**23,2**	4,8
2000	2017,0	247,1	**39,5**	14,1

The average S/S_0 (Supplementary table 1, B) were used for the calibration curve (Fig. S2). Using the acquired data, the limit of detection LOD and limit of quantification LOQ were determined as follows:

LOD = Average S_0 + 3*SD

LOQ = Average S_0 + 10*SD,

where SD is a standard deviation for serial measurements of the signal for the blank sample S_0.

LOD (RLU) = 51 + 3*16.5 = 100.5 RLU

LOD (S/S_0) = 100.5/51 ≈ 2

LOD (TIMP1, pg/ml) = (2-1)/0.0203 = 49,3 pg/ml

LOD (RLU) = 51 + 10*16.5 = 216 RLU

LOD (S/S_0) = 216/51 ≈ 4,2

LOD (TIMP1, pg/ml) = (4.2-1)/0.0203 = 157.6 pg/ml

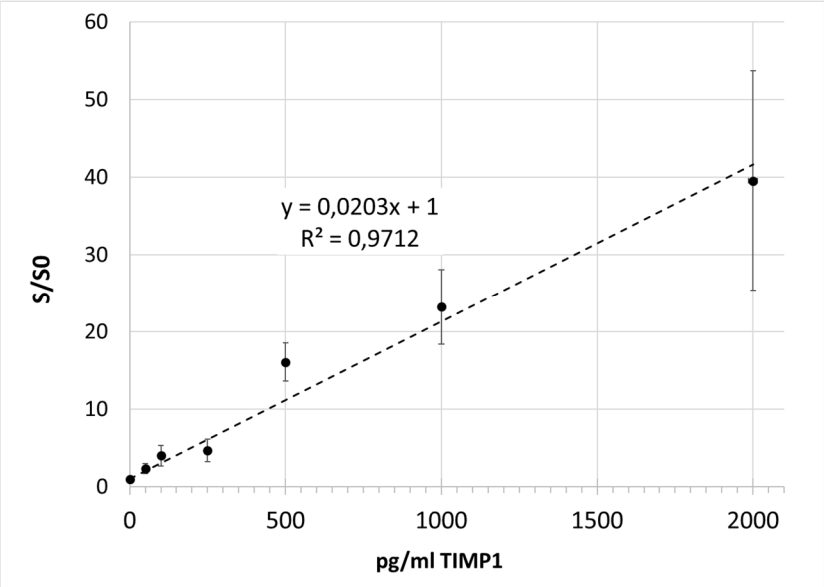

Fig. S3 Calibration curve for the homogeneous SPARCL™ for the quantitative detection of TIMP1 on a chip. The error bars designate the standard deviation, number of replicates n=5.