Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

SUPPORTING INFORMATION

Design of Peptide-based Lateral Flow Assay for the Detection of the Biomarker Mdm2

Bryan Gosselin, [†,‡] Maurice Retout, [†] Ivan Jabin [‡] and Gilles Bruylants[†,*]*

[†] Engineering of Molecular NanoSystems, Ecole Polytechnique de Bruxelles, Université libre de Bruxelles (ULB), avenue F. D. Roosevelt 50, CP165/64, B-1050 Brussels, Belgium

[‡] Laboratoire de Chimie Organique, Université libre de Bruxelles (ULB), Avenue F. D. Roosevelt 50, CP160/06, B-1050 Brussels, Belgium

Ivan.Jabin@ulb.be; Gilles.Bruylants@ulb.be

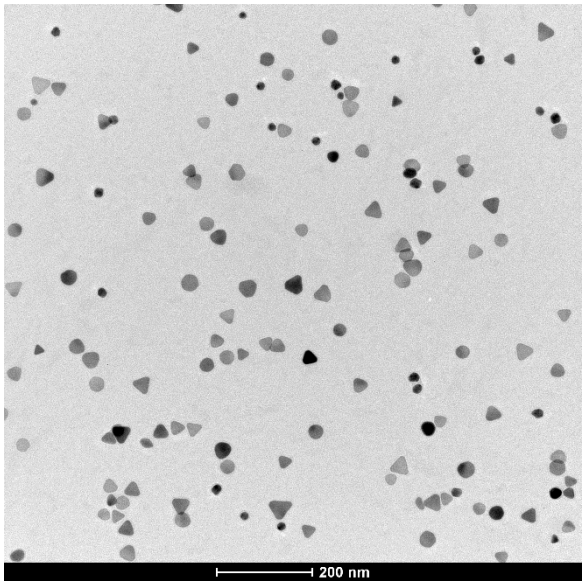

Figure S1. Additional TEM image of AgNPls-X_4

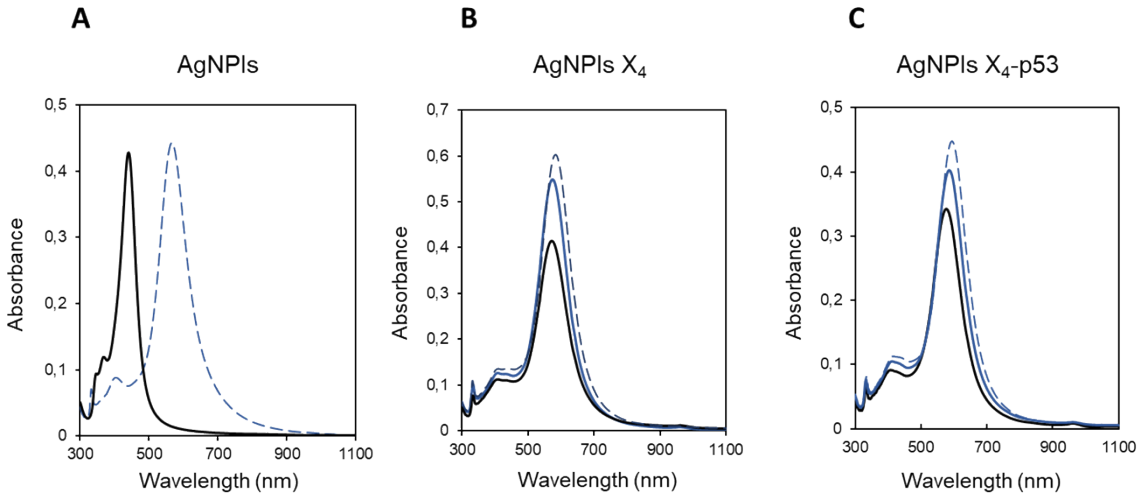

Figure S2.UV-Vis spectra of (a) AgNPls after 5 minutes of suspension in either water (blue dashed line) or 0.1X (black full line), (b) AgNPls-X_4 and (c) AgNPls-X_4-p53 after 2 hours of suspension in either water (blue dashed line), 0.5X (blue full line) or 1X PBS (black full line).

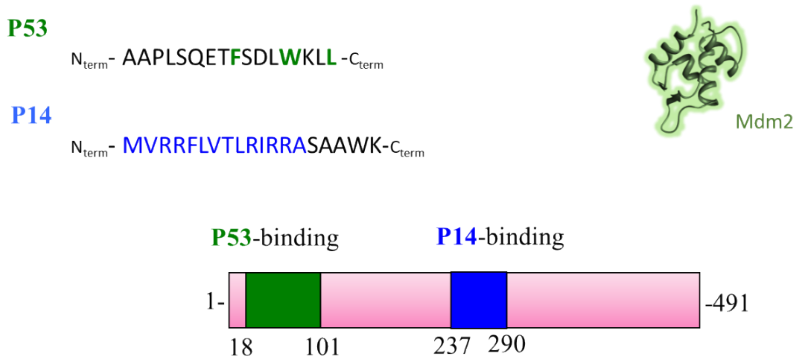

Figure S3. Amino acids sequence of peptide aptamers

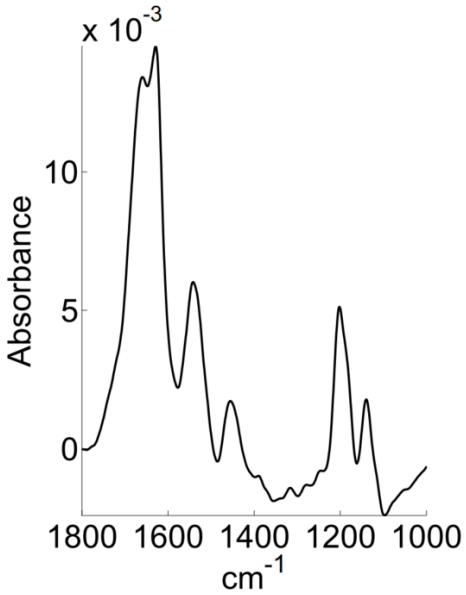

Figure S4. ATR-FTIR spectrum of p53 peptide. Characteristic peaks at 1650 cm^{-1} and 1530 cm^{-1} related to amide I and II band, respectively.

	Hydrodynamic diameter	Zeta Potential
AgNPls X_4	34 ± 1 nm	- 52 ± 2 mV
AgNPls X_4-p53	39 ± 1 nm	- 52 ± 4 mV

Table S1. DLS measurement and Zeta potential of AgNPls-X_4 and AgNPls-X_4-p53.

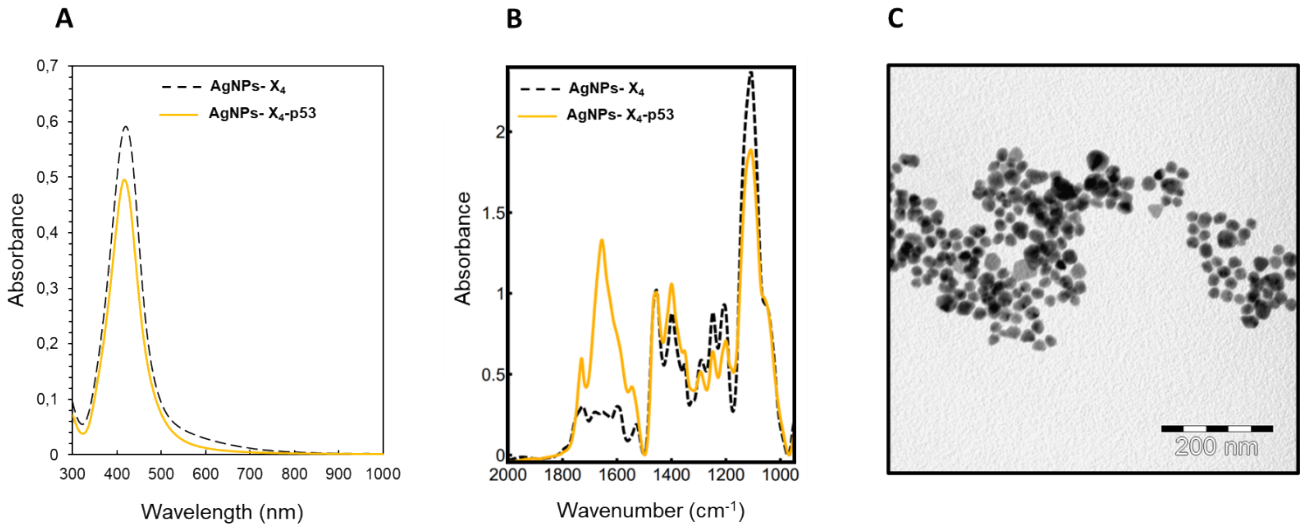

Figure S5. (A) UV-Vis spectra recorded in water at pH 7 for AgNPs-X_4, before and after the coupling of p53 (AgNPs-X_4-p53). (B) ATR-FTIR spectra of AgNPs-X_4 (black dashed line) and AgNPs-X_4-p53 (yellow plain line). (C) TEM image of AgNPs-X_4

Table S2. Composition of the different buffers attempted.

Code Name	Buffer	Proteins	Polymers	Surfactants	Salts	Others	Results
B1	20 mM Tris.HCl pH 8	2% BSA	/	0.1% T20	50 mM KCl	/	FP
B2	0.5X PBS	2% BSA	/	0.1% T20	/	/	FP
B3	1X PBS	5% BSA	1% PEG	0.2% T20	/	/	FP (-)
B4	0.5X PBS	5% BSA	1% PEG	0.5% T20	/	/	FP (+)
B5	1X PBS	5% BSA	1% PEG	0.05% T20	/	/	FP (-)
B6	/	0.2X SuperBlock	1% PEG	0.2% T20	/	/	FP (+)
B7	/	0.2X Casein	1% PEG	0,2% T20	/	/	FP (-)
B8	10 mM Borate pH 8.5	5% BSA	1% PEG	0.05% T20	150 mM NaCl	/	FP
B9	10 mM TRIS.HCl pH 8	5% BSA	1% PEG	0.05% T20	150 mM NaCl	/	FP
B10	0.1X PBS	5% BSA	1% PEG	0.05% T20	/	5% trehalose	FP (+)
B11	0.1X PBS	5% BSA	1% PEG	0.05% T20	/	5% glycerol	FP (+)
B12	1X PBS	10% BSA	1% PEG	0.05% T20	/	/	FP
B13	1X PBS	5% BSA + 0.2X Casein	1% PEG	0.05% T20	/	/	FP(-)
B14	1X PBS	5% BSA	0.5% PEG	0.05% T20	/	/	FP
B15	1X PBS	5% BSA	2% PEG	0.05% T20	/	/	FP
B16	1X PBS	5% BSA + 0.2X Casein	1% PEG	0.05% T20	/	/	FP (-)
B17	1X PBS	10% BSA + 0.4X Casein	1% PEG	0.05% T20	/	/	No FP but no TP
B18	1X PBS	5% BSA	1% PVP	0.05% T20	/	/	FP (+)
B19	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.1% T20	200mM KCl	1 mM NaEDTA	FP (-)
B20	25 mM Tris.HCl pH 8	/	1% PEG	0.1% T20	200mM KCl	1 mM NaEDTA	FP (+)
B21	25 mM Tris.HCl pH 8	5% BSA	/	0.1% T20	200mM KCl	1 mM NaEDTA	FP (+)
B22	25 mM Tris.HCl pH 8	/	/	0.1% T20	200mM KCl	1 mM NaEDTA	FP (+)
B23	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.2% T20	200mM KCl	1 mM NaEDTA	FP
B24	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.1% T20	200mM KCl	2 mM NaEDTA	FP
B25	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.1% T20	200mM KCl	1 mM NaEDTA + 2 mM DTT	FP
B26	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.1% T20	100mM KCl	1 mM NaEDTA	FP
B27	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.4% T20	100mM KCl	1 mM NaEDTA	FP (+)
B28	25 mM Tris.HCl pH 8	5% BSA	1% PEG	2% NaDeOx	100mM KCl	1 mM NaEDTA	No FP but NPs remains on the bottom
B29	25 mM Tris.HCl pH 8	5% BSA	1% PEG	2% NaDeOx	100mM KCl	/	No FP but NPs remains on the bottom
B30	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.5% NaDeOx	100mM KCl	1 mM NaEDTA	No FP but no TP

B31	25 mM Tris.HCl pH 8	5% BSA	1% PEG	5% NaDeOx	100mM KCl	1 mM NaEDTA	No FP but NPs remains on the bottom
B32	25 mM Tris.HCl pH 8	5% BSA	1% PEG	2% NaDeOx + 0.05% T20	100mM KCl	1 mM NaEDTA	No FP but NPs remains on the bottom
B33	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.1% NaDeOx	100mM KCl	1 mM NaEDTA	FP
B34	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.05% NaDeOx	100mM KCl	1 mM NaEDTA	FP
B35	25 mM Tris.HCl pH 8	5% BSA	1% PEG	1% T20	100mM KCl	1 mM NaEDTA	FP (+)
B36	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.25% NaDeOx + 0.1% T20	100mM KCl	1 mM NaEDTA	No FP but no TP
B37	25 mM Tris.HCl pH 8	1% BSA	1% PEG	0.25% NaDeOx + 0.2% T20	100mM KCl	1 mM NaEDTA	No FP and TP
B38	25 mM Tris.HCl pH 8	1% BSA	1% PEG	0.25% NaDeOx + 0.4% T20	100mM KCl	1 mM NaEDTA	No FP and TP (+)
B39	25 mM Tris.HCl pH 8	1% BSA	1% PEG	0.25% NaDeOx + 0.1% T20	100mM KCl	1 mM NaEDTA	No FP but no TP
B40	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.25% NaDeOx + 0.4% T20	100mM KCl	1 mM NaEDTA	No FP and TP (++)
B41	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.3% NaDeOx + 0.4% T20	100mM KCl	1 mM NaEDTA	Similar as B40
B42	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.25% NaDeOx + 0.2% T20	100mM KCl	1 mM NaEDTA	Similar as B40
B43	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.3% NaDeOx + 0.4% T20	200mM KCl	1 mM NaEDTA	Similar as B40
B44	25 mM Tris.HCl pH 8	5% BSA	1% PEG	0.25% NaDeOx + 0.2% T20	200mM KCl	1 mM NaEDTA	Similar as B40

Abbreviations:

T20= Tween 20 **NaDeOX**= Sodium deoxycholate

PVP= PVP K15 **PBS**= Phosphate Buffer Saline

PEG= PEG6000 **BSA**= Bovine Serume Albumin

Results:

Optimization was an iterative process. buffer list separated by thick black line correspond to the different optimization phase. Yellow colored buffers are the buffer composition reference for the following phase. Green colored buffer is the most optimal buffer composition.

FP= False Positive **TP**= True Positive

Intensity of signal (FP or TP) are expressed as **(+)** *for an increase and* **(-)** *for a decrease compared to reference buffer of the phase*

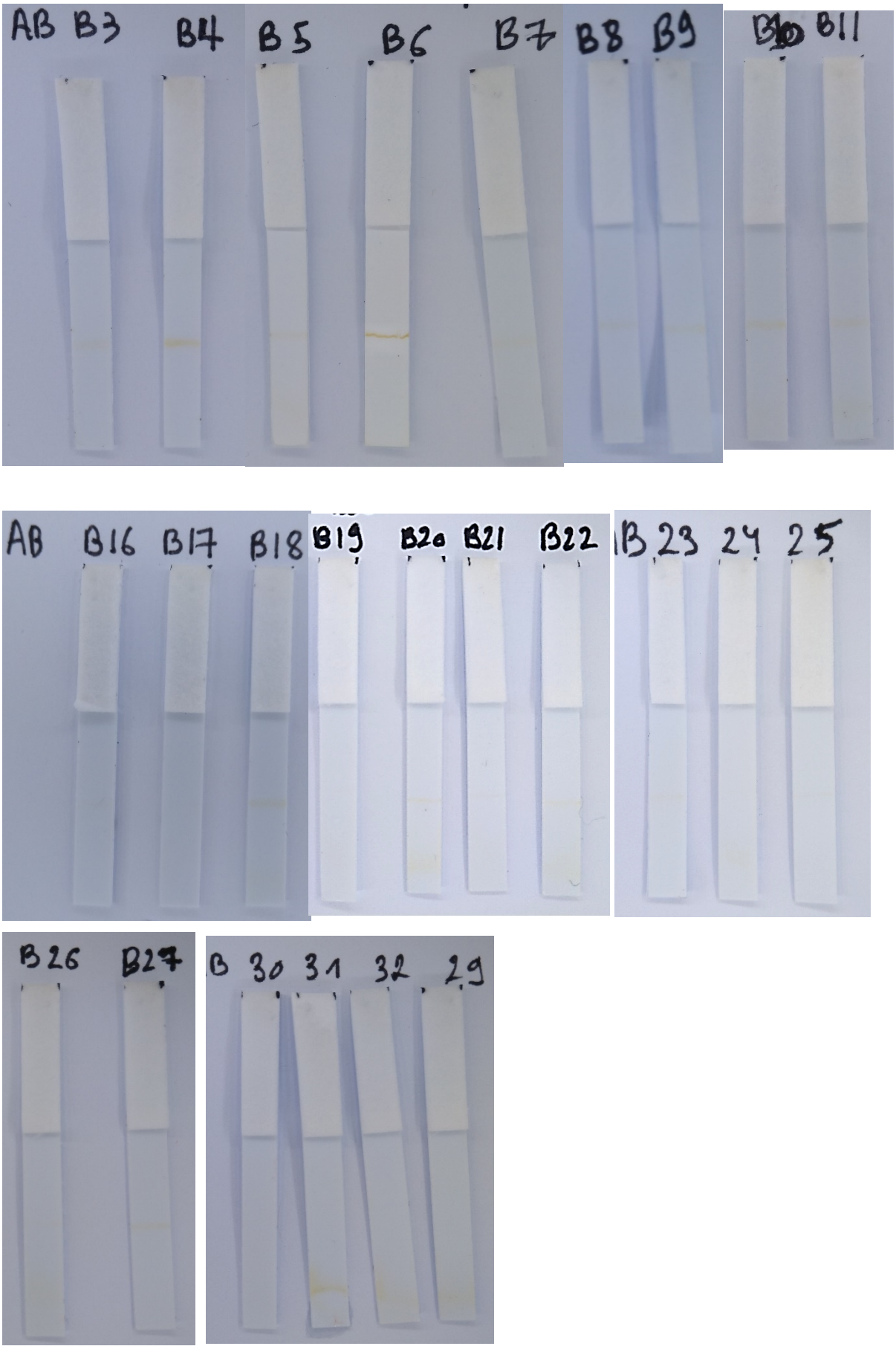

Figure S6. Picture of half strip without Mdm2 with different buffer composition

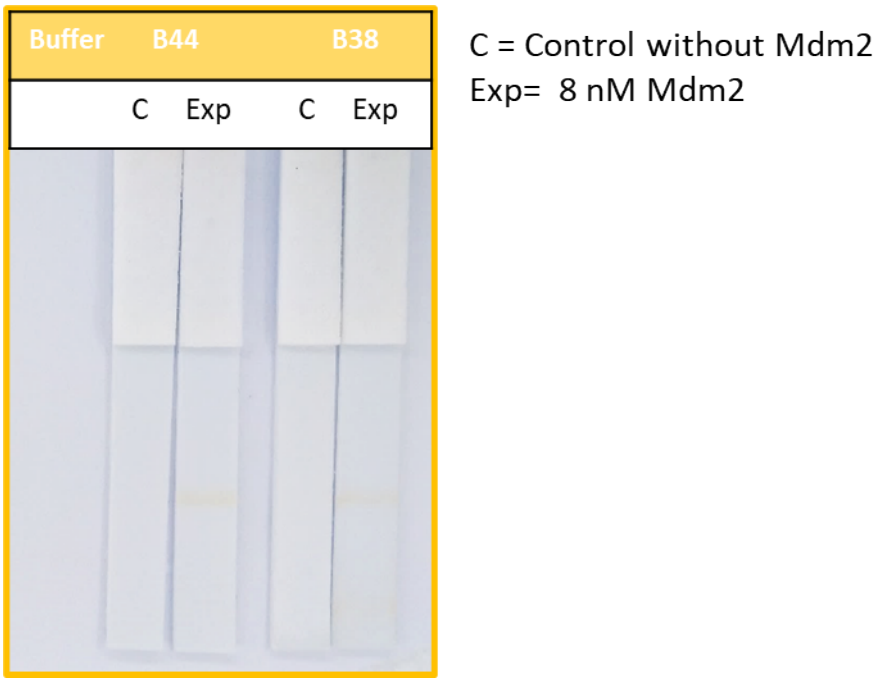

Figure S7. Picture of p14-BSA with and without Mdm2 for Buffer B44 and B38.

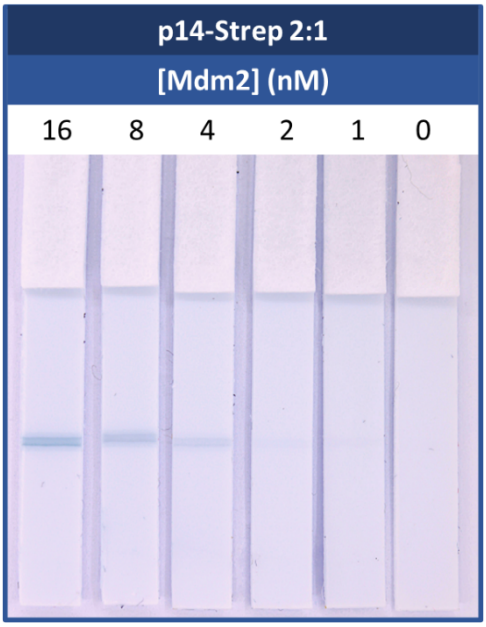

Figure S8. Pictures of the p14-Strep 2:1 strip used to detect different concentrations of Mdm2 ranging from 16 nM to 0 nM with AgNPls-X_4-p53.

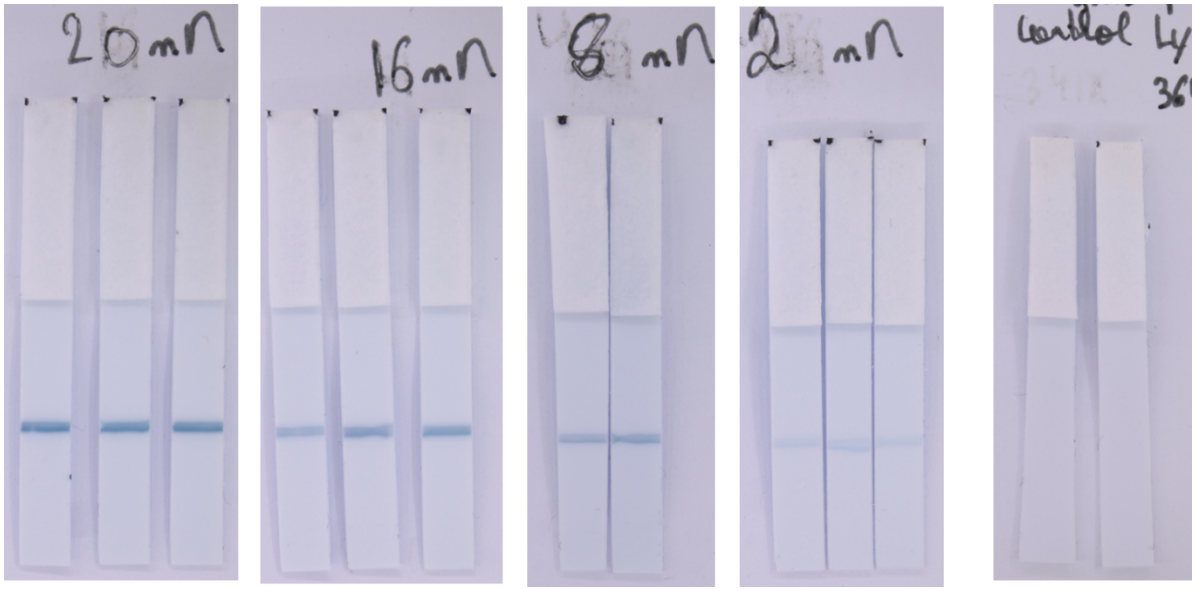

Figure S9. Picture of the replicates dipstick assays as a function of different concentrations of Mdm2 spiked in HEK293 cell lysate.

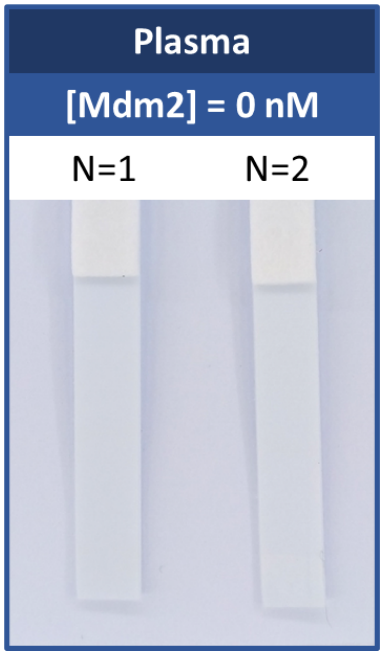

Figure S10. LFA Picture of p14-BSA strip with AgNPls- AgNPls-X_4-p53 in absence of Mdm2 run in human plasma.

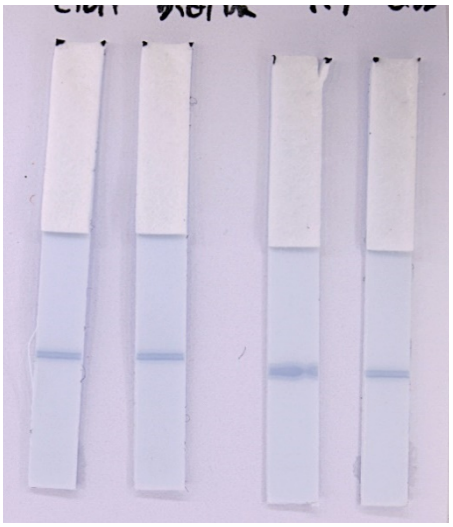

Figure S11. Pictures of different dipstick assays with a test line composed of p14-Strep 2:1 with negative cell lysate. Different optimizations were tested: (1) addition of EtOH (2) addition of biotin excess to block remaining available binding site (3) use strip with a p14: Strep ratio of 4:1 to avoid remaining available binding site (4) classical conditions. As observed, no significant improvement of the false positive were obtained.

Bioconjugation Protocol:

In a 1.5 mL glass vial, 1000 µL AgNPls-X_4 was added. Then, 100 µL of MES buffer (100mM, pH 5.8), 60 µL EDC.HCl (6 mM) and 60 µL Sulfo-NHS (10 mM) were successively added. The mixture is stirred during 1 hours. Then, 20 µL anti-Mdm2 pAB (2 mg/mL) was added. Mixture was stirred 4h at room temperature. The mixture was transferred in a 1.5 mL Protein Lobind Eppendorf. the Eppendorf was completed to 1.5 mL with BSA 1% to block surface and centrifugated at 18000 g during 20' at room temperature. The supernatant was discarded and the pellet was redispersed in BSA 1%. Centrifugation and redispersion cycle were repeated twice as described. Note that for the last cycle, the resulting nanoparticles were resuspended in 5 mM phosphate buffer (pH 7).

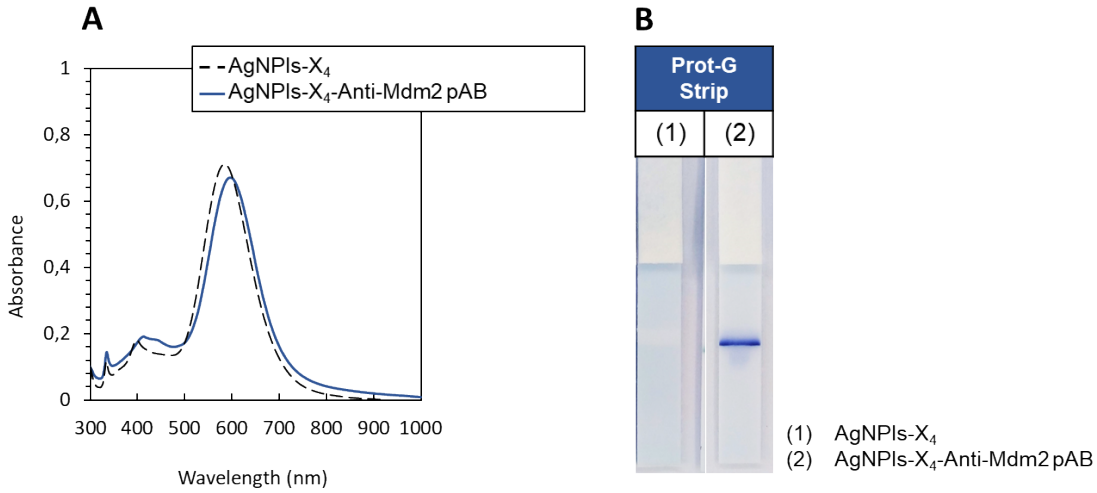

Figure S12. (A) UV-Vis spectra recorded in phosphate buffer at pH 7 for AgNPls-X_4, before and after the coupling of anti-Mdm2 pAB. (B) Pictures of different dipstick assays with a test line composed protein G (Prot-G) to confirm presence of antibodies at the surface of AgNPls-X_4. Protein G has high binding affinity for heavy chain of antibodies and, therefore, presence of antibodies at the AgNPls surface immobilizes the AgNPls a T line, leading to a positive blue line.

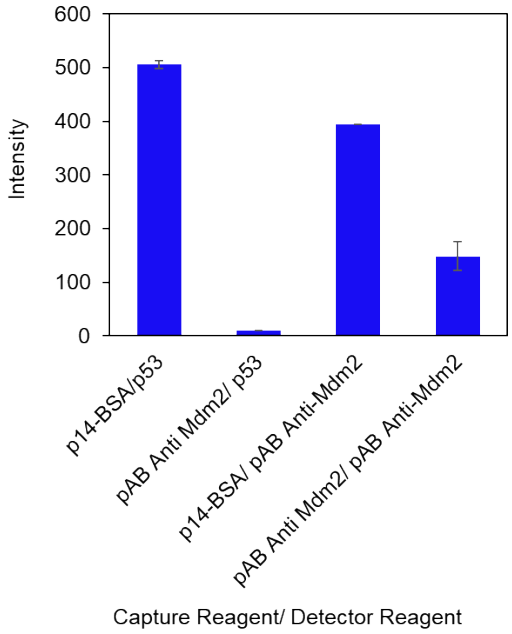

Figure S13. Signal quantification from the pictures of the dipstick assays using the ImageJ software with a test line composed of either p14-BSA or Anti-Mdm2 pAB as capture reagent and either AgNPls-X_4-p53 or AgNPls-X_4-Anti-Mdm2 pAB in the presence (final concentration of 8 nM).

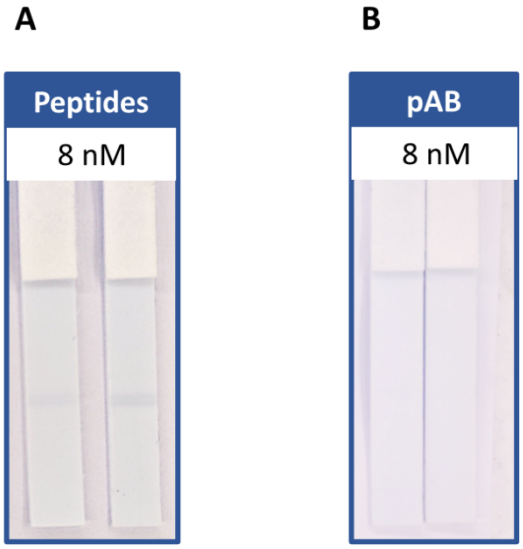

Figure S14. (A) Pictures of the 1-year old p14-BSA strip with 1 year-old AgNPls-X_4-p53 in presence of 8nM Mdm2 in duplicates (B) Pictures of the 1-week old Anti-Mdm2 pAB strip with 1 week-old AgNPls-X_4-Anti-Mdm2 pAB in presence of 8nM Mdm2 in duplicates.

Ratio	Streptavidin (19 µM)	p14-biotin (266 µM)	H_20 : Acetonitrile (3:1)
0:1	100 µL	0 µL	29 µL
1:1	100 µL	7.2 µL	21.8 µL
2:1	100 µL	14.5 µL	14.5 µL
3:1	100 µL	21.7 µL	7.3 µL
4:1	100 µL	29 µL	0 µL

Table S3. Volume of reagents to obtain different ratio of p14 Biotin:Streptavidin.