Supplementary Information: Development of a fluorescent lateral flow test for the detection of the obesity biomarker leptin in human serum

Margarita Ortiz-Martinez,[a,b] Katerina Kourentzi,[a] Richard C. Willson,[a,b,c] Marco Rito-Palomares [b,c] and Mirna González-González* [b,c]

a. Department of Chemical and Biomolecular Engineering, University of Houston, Houston, TX, USA.

b. Tecnologico de Monterrey, Institute for Obesity Research, Monterrey, Nuevo León, México.

c. Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Monterrey, Nuevo León, México.

*Corresponding author E-mail: mirnagonzalez@tec.mx

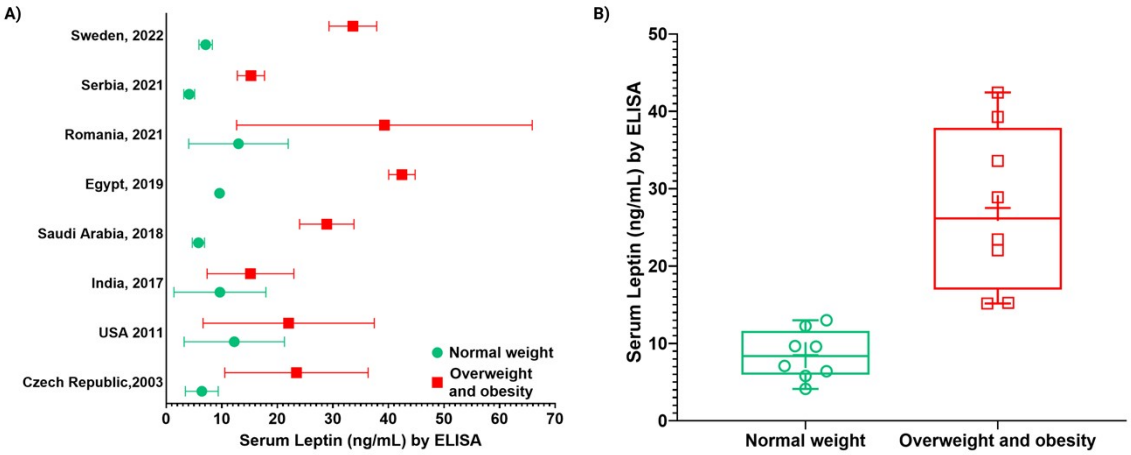

Fig. S1 Serum leptin levels in adults reported in previous studies. Eight studies were selected from literature research (conducted in 2023) that met the following criteria: published in the last 20 years, leptin levels measured in human serum from adults by ELISA, results reported for normal weight (NW) compared to overweigh/obesity (OW/Ob) and the number of samples analyzed stated explicitly. A) Mean leptin levels (standard deviation as error bars) are presented for each study: Czech Republic (NW n=8 and OW/Ob n=8)[1], United States of America (NW n=69 and OW/Ob n=41) [2], India (NW n=150 and OW/Ob n=180)[3], Saudi Arabia (NW n=40 and OW/Ob n=96) [4], Egypt (NW n=40 and OW/Ob n=50)[5], Romania (NW n=12 and OW/Ob n=17)[6], Serbia (NW n=9 and OW/Ob n=23)[7] and Sweden (NW n=25 and OW/Ob n=19)[8]. B) A boxplot summarizes the leptin levels across all studies, showing the minimum values, 25th percentile, median, 75th percentile and maximum values with the "+" symbol indicating the mean. Both graphs indicate higher leptin levels in the overweight and obesity group across all geographical regions, consistent with existing literature. These findings support the potential use of leptin measurements at point of care as a tool for assessing metabolic state.

Table S1. Buffer composition for the running buffer screening

	Buffer A	Buffer B	Buffer C	Buffer D
Bovine serum albumin (%)	0.5	0.5	1.0	1.0
Tween-20 (%)	0.5	0.5	0.5	0.5
PEG 3350 (%)	0.0	0.5	0.0	0.5

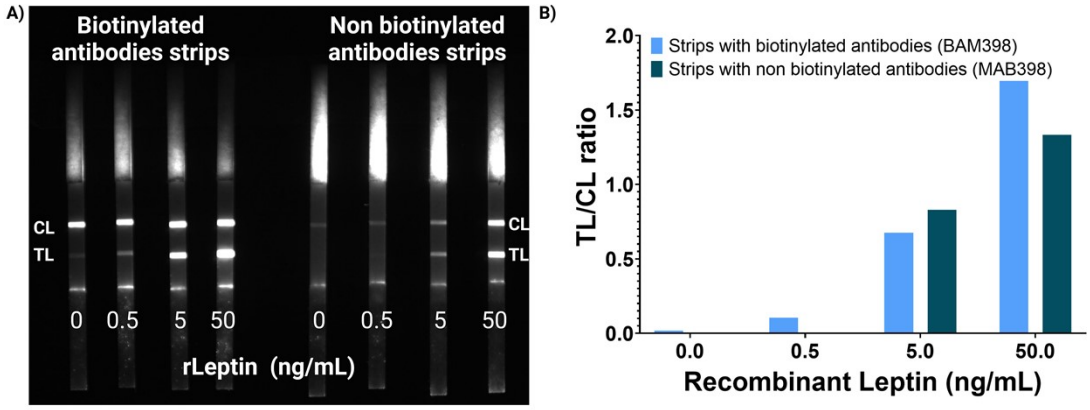

Fig. S2 The orientation of the detection antibodies (conjugated to the reporter particles) and capture antibodies (immobilized on the test line of the strip) was selected based on the performance of the systems, tested using a serial curve of recombinant leptin. A) Grayscale images of the strips obtained using a FluorChem gel documentation system, are shown for both tested orientations. B) The graph presents the ratio of fluorescence intensity of the test and the control line at varying recombinant leptin concentrations (0-50 ng/mL). The configuration using biotinylated antibodies as capture on the strip and nonbiotinylated detection antibodies conjugated to carboxylate-modified Europium (III) chelate polystyrene particles demonstrated superior performance, with detection at lower concentrations, minimal nonspecific binding and a clear signal to concentration response. This system was chosen for the following experiments. CL: Control line; rLeptin: Recombinant leptin; TL: Test line.

Table S2. Final buffer compositions used to dilute serum samples in the screening assay.

	Buffer E	Buffer F	Buffer G	Buffer H
Bovine serum albumin (%)	0.50	0.25	0.25	0.00
Tween-20 (%)	0.50	0.50	0.50	0.50
PEG 3350 (%)	0.00	0.50	0.00	0.50

All buffers were prepared as 2× stocks and mixed with serum to obtain the final concentrations listed.

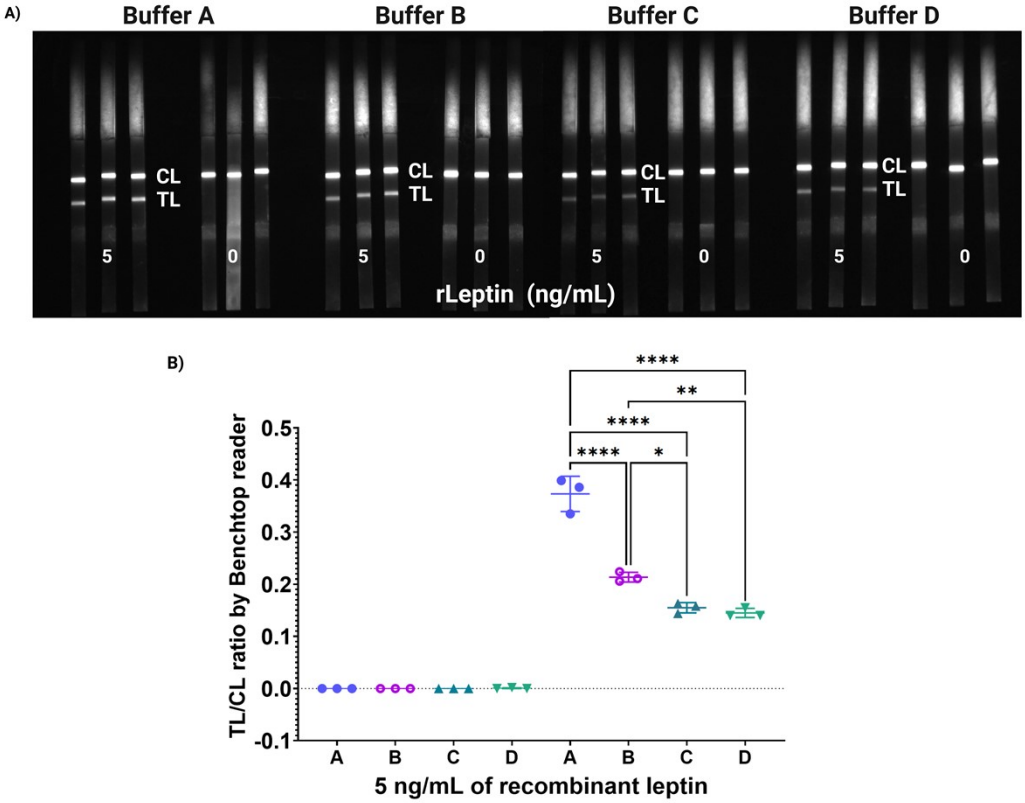

Fig. S3 Running buffer screening was performed to select the optimal buffer for leptin detection in the developed LFA. Four buffer compositions with either no recombinant leptin (0 ng/mL) or a low concentration (5 ng/mL), in triplicate. A) Grayscale images of the strips, captured with the FluorChem gel documentation system, show minimal to no signal for the blanks and strong signals on the test line for the low positive samples across all buffer formulations. The control lines exhibited similar intensity across tested conditions. B) The test line/control lines (TL/CL) ratio, measured using a Benchtop reader, is presented in the graph. Consistent with the images, the fluorescence intensity for the blank was zero or near zero and the low positive samples were clearly distinguishable from blanks in all formulations. Group mean differences (0 and 5 ng/mL) were analyzed by one-way ANOVA with Tukey's post-hoc test for multiple comparisons (α= 0.05). While no significative difference was observed among blank TL/CL ratios, Buffer A demonstrated a significantly higher signal for the positive samples and was therefore selected for the subsequent experiments in buffer. CL: Control line; LFA: Lateral flow assay; LOD: Limit of detection; rLeptin: Recombinant leptin; TL: Test line. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

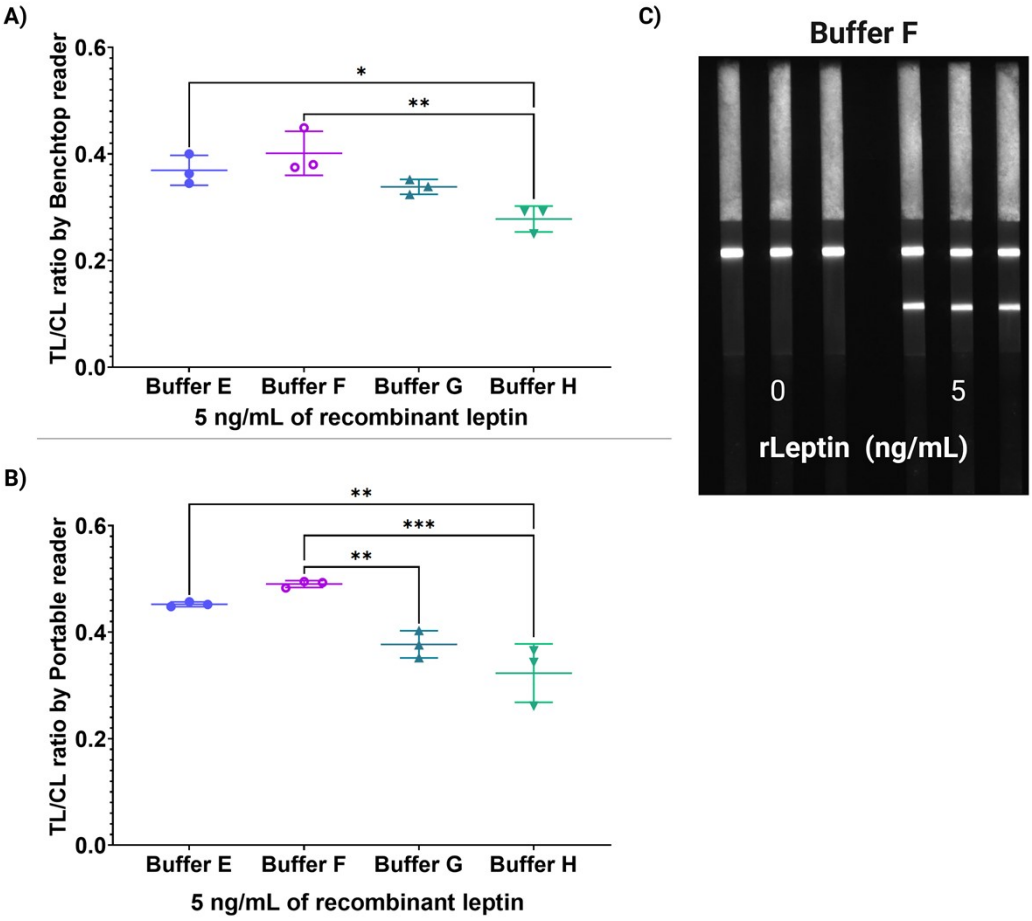

Fig. S4 A second running buffer screening was conducted to select the optimal buffer for diluting serum samples for leptin detection in the developed LFA. Calf serum (diluted 50% in each of the four buffers) was tested to identify the buffer that achieved lower non-specific binding while maintaining strong signal intensity for the positive sample (5 ng/mL). The graphs show the TL/CL ratio for the four buffers with 5 ng/mL of recombinant leptin added, measured using a A) Benchtop reader and B) Portable reader. Group mean differences (0 and 5 ng/mL) were analyzed by one-way ANOVA with Tukey's post-hoc test for multiple comparisons ($\alpha = 0.05$). In both readers, Buffer F demonstrated the higher TL/CL ratio for the positive samples and was therefore selected for the following experiment with calf and human serum. For the four buffers measured in both readers the blank intensity was zero or near zero with no significant difference among them (data provided in the Supplementary Data File). C) Grayscale images of the selected buffer F strips, captured with the FluorChem gel documentation system, show no signal for the blanks and strong signals on the test line for the low positive samples. CL: Control line; LFA: Lateral flow assay; LOD: Limit of detection; rLeptin: Recombinant leptin; TL: Test line. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table S3. Summary of literature studies for leptin detection with potential for point-of-care

Year	Description	Measurement method	Linear range	Limit of detection	Biological samples tested	Main results in biological samples	Time to result	Reference
2015	Chemiluminescence immunosensor: hemin/G-quadruplex DNAzymes and Fe_3O_4 /PD/Au nanocomposites	Chemiluminescence Analyzer	1-800 pg/mL	0.3 pg/mL	20 human serum samples	The concentration by ELISA and the concentration obtained by the proposed method showed no significant difference between the conventional method and the proposed method by the Student t-test	More than 3h	[9]
2017	Inkjet-printed point-of-care immunoassay	Microarray scanner and mobile phone-based fluorescence microscope	Scanner 0.01-1000 ng/mL Mobile phone 0.1-1000 ng/mL	Scanner 0.037 ng/mL Mobile phone 0.71 ng/mL	Pediatric patients with obesity and lean n=13	Concordance with ELISA assessed by graphical comparison only	More than 90 minutes	[10]
2018	Single-walled carbon nanotubes–chitosan nanocomposites glassy carbon electrode	Differential pulse voltammetry	0-1000 ng/mL	5 pg/mL	One human serum sample spiked with leptin (dilution not reported)	The linear regression coefficient for the ELISA and biosensor was 0.9949	Not reported	[11]
2019	Porous graphene functionalized black phosphorus (PG-BP) composite electrode	Square wave voltammetry	0.150-2500 pg/mL	0.036 pg/mL	One human serum sample diluted 100-fold, spiked with leptin	The recovery in serum was in the range of 98 ± 2.6%–100.8 ± 0.84%	More than 2 h	[12]
2020	Molecularly imprinted polymers onto gold working electrodes	Electrochemical Impedance Spectroscopy	1-32 ng/mL	0.110 ng/mL	Serum from adults n=70	The conductance signal and leptin levels measured by ELISA showed a strong correlation, r=0.97791 (Pearson correlation)	40 min	[13]
2021	Graphite paper-based impedimetric biosensor	Electrochemical Impedance Spectroscopy	0.2-20 pg/mL	0.00813 pg/mL	Healthy children's serum (dilution not reported) spiked with leptin n=5	The recovery was in the range of 97.3-101.7%	90 min	[14]

Table S3. Summary of literature studies for leptin detection with potential for point of care (continued)

2021	Surface Plasmon Resonance imaging inmunosensor	Imaging and imageJ	0.23 to 5 ng/mL	0.07 ng/mL	Serum from normally nourished children and children with malnutrition n=18	The immunosensor showed a strong positive correlation with ELISA values by Pearson correlation r= 0.991	More than 1 hour	[15]
2022	Cyanogen bromide electrochemical biosensor	Electrochemical impedance spectroscopy	0.05-100 pg/mL	0.0086 pg/mL	Human serum samples diluted 1000-fold, spiked with leptin n=5	The recovery in serum obtained was in the range of 95.76 to 105.02%	More than 1 hour	[16]
2022	Reduced graphene oxide-gold electrochemical biosensor	Differential pulse voltammetry	0.001-1000 pg/mL	0.00087 pg/mL	Human serum samples with varying BMI diluted 10-fold n=6	The serum leptin content of obese people is significantly higher	More than 2 hours	[17]
2022	Aptasensor based on Au and TiO_2 nanoparticles	Electrochemical impedance spectroscopy	1-100 pg/mL and 100 pg/mL-1ng/mL	0.312 pg/mL	One human serum sample diluted 1:10 and spiked with leptin	An agreement was found between the added leptin and the aptasensor's performance, with recovery values ranging from 96.40 to 107.79%	Not reported	[18]
2023	Screen-printed electrode surface coated with platinum nanospheres and gold nanoparticles	Differential pulse voltammetry	1.0 fg/mL- 10 pg/mL	0.31 fg/mL	One commercial serum sample diluted 1:10 and spiked with leptin	The calculated recovery values using the biosensor were between 106.2% and 109.0%	More than 90 min	[19]
2025	Fluorescent based	Fluorescence	0.25-100 ng/mL	0.25 ng/mL	Human serum samples from adults n=30	The LFA detected endogenous leptin in 25% diluted human serum (0.21-15.62 ng/mL), with a strong correlation with ELISA-measured leptin levels (r=0.96).	Less than 1 hour (5 samples by triplicate)	Present study

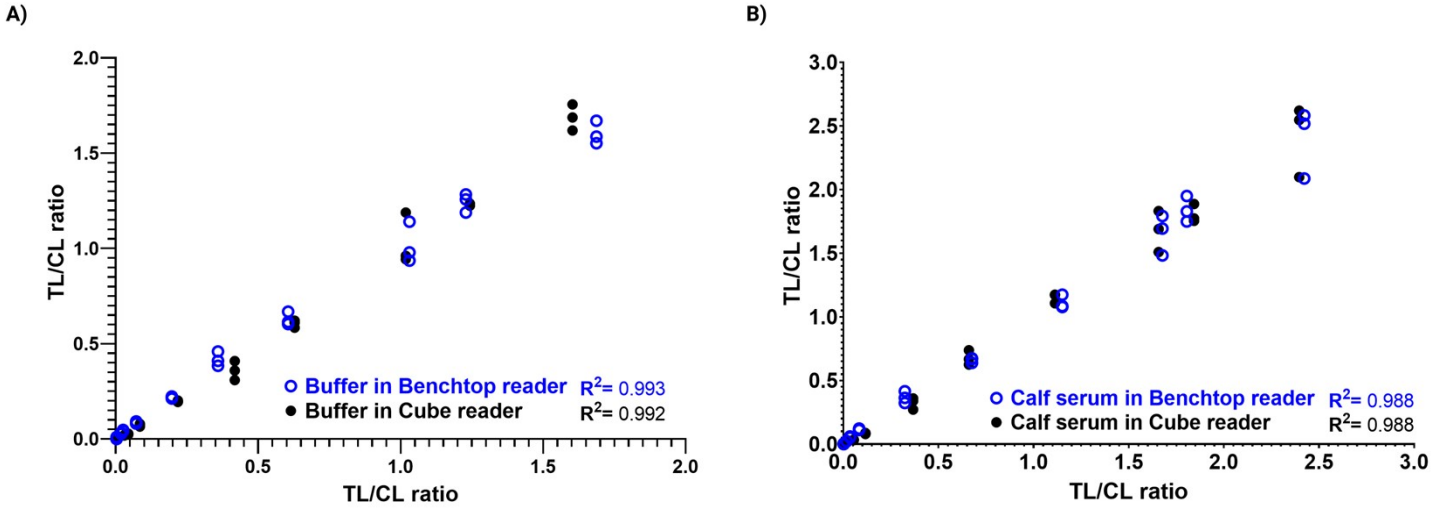

Fig. S5 Comparison of benchtop and portable fluorescence readers. Two linear regression analyses were performed for each serial dilution curve: one with hollow circles, where the X-axis represents the triplicate readings from the benchtop and the y-axis the mean of the data from the portable reader; and another with filled circles, where the X-axis corresponds to the triplicate readings from the portable reader and the Y-axis the mean of the data from the benchtop reader. Data represent serial dilution curves of rLeptin spiked in A) Buffer containing 0.5% BSA and 0.5% Tween-20, and B) 50% Calf serum with final concentrations of 0.25% BSA, 0.5% Tween-20 and 0.5% PEG3350. In both matrices, the TL/CL ratio obtained from both readers follows a linear relationship, with coefficients of determination (R2) greater than 0.98. R^2: Coefficient of determination; CL: Control line; LFA: Lateral flow assay; PEG: Polyethylene Glycol; PBS: Phosphate buffered saline; rLeptin: Recombinant leptin; TL: Test line.

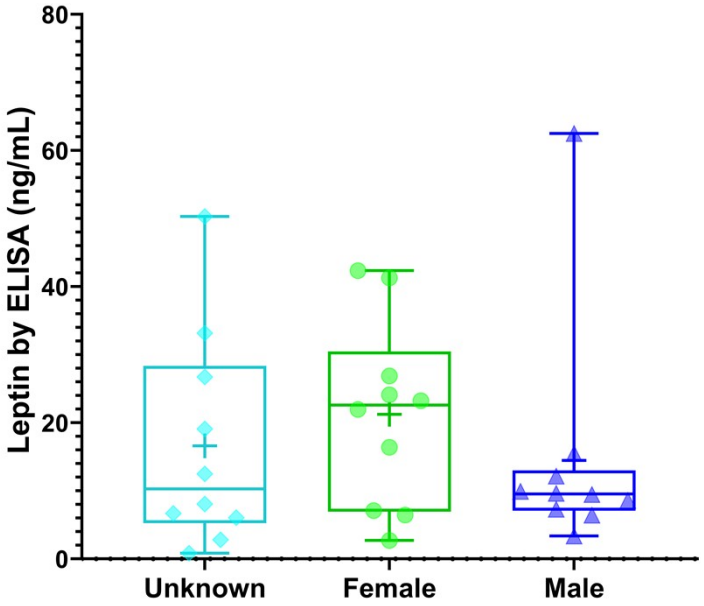

Fig. S6 Leptin levels in human serum samples measured in triplicate using the Human Leptin Quantikine QuicKit ELISA (QK398, R&D Systems). Three groups (n= 10 each) were evaluated: unknown sex, female, and male. The box plots represent, from bottom to top the minimum values, 25th percentile, median, 75th percentile and maximum values. The + symbol indicates the mean.

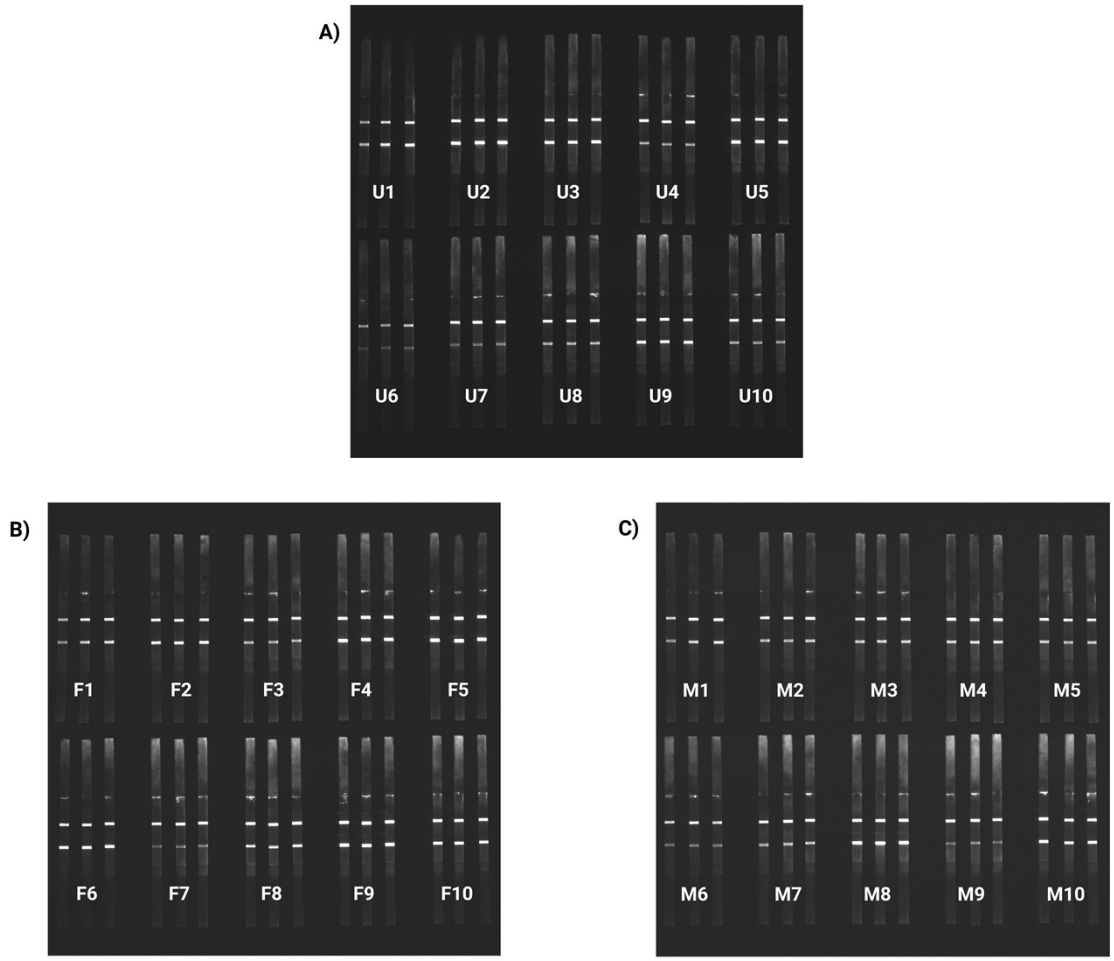

Fig. S7 Images of the 30 human serum samples tested in triplicate at 25% dilution in Running Buffer (0.25% BSA, 0.5% Tween-20, and 0.5% PEG 3350 in 1x PBS) acquired using the FluorChem gel documentation system. The developed LFA successfully detected endogenous leptin in all the samples tested, demonstrating minimal variability. A) Unknown sex samples B) Female samples C) Male samples. BSA: Bovine serum albumin; PEG: Polyethylene Glycol; PBS: Phosphate buffered saline.

References

1 M. Rosická, M. Krsek, M. Matoulek, Z. Jarkovská, J. Marek, V. Justová and Z. Lacinová, Serum ghrelin levels in obese patients: the relationship to serum leptin levels and soluble leptin receptors levels, *Physiol. Res.*, 2003, **52**, 61–6.

2 S. Wang, D. B. Reed, S. Goli and D. Goswami, Blood leptin and C-reactive protein provide more sensitive assessment than blood lipids and other inflammatory biomarkers in overweight university students, *Nutr. Res.*, 2011, **31**, 586–593.

3 S. Mishra, V. Gupta, S. Mishra, R. Sachan and A. Asthana, Serum level of orexin-A, leptin, adiponectin and insulin in north Indian obese women, *Diabetes Metab. Syndr. Clin. Res. Rev.*, 2017, **11**, S1041–S1043.

4 H. S. Al-Amodi, N. A. Abdelbasit, S. H. Fatani, A. T. Babakr and M. M. Mukhtar, The effect of obesity and components of metabolic syndrome on leptin levels in Saudi women, *Diabetes Metab. Syndr. Clin. Res. Rev.*, 2018, **12**, 357–364.

5 N. M. Rashad, S. E. Sayed, M. H. Sherif and M. Z. Sitohy, Effect of a 24-week weight management program on serum leptin level in correlation to anthropometric measures in obese female: A randomized controlled clinical trial, *Diabetes Metab. Syndr. Clin. Res. Rev.*, 2019, **13**, 2230–2235.

6 A.-V. Sitar-Tăut, A. Cozma, A. Fodor, S.-C. Coste, O. H. Orasan, V. Negrean, D. Pop and D.-A. Sitar-Tăut, New Insights on the Relationship between Leptin, Ghrelin, and Leptin/Ghrelin Ratio Enforced by Body Mass Index in Obesity and Diabetes, *Biomedicines*, 2021, **9**, 1657.

7 A. Korac, B. Srdic-Galic, A. Kalezic, A. Stancic, V. Otasevic, B. Korac and A. Jankovic, Adipokine signatures of subcutaneous and visceral abdominal fat in normal-weight and obese women with different metabolic profiles, *Arch. Med. Sci.*, 2021, **17**, 323–336.

8 L. T. van Egmond, E. M. S. Meth, J. Engström, M. Ilemosoglou, J. A. Keller, H. Vogel and C. Benedict, Effects of acute sleep loss on leptin, ghrelin, and adiponectin in adults with healthy weight and obesity: A laboratory study, *Obesity*, 2023, **31**, 635–641.

9 Y. He, J. Sun, X. Wang and L. Wang, Detection of human leptin in serum using chemiluminescence immunosensor: Signal amplification by hemin/G-quadruplex DNAzymes and protein carriers by Fe3O4/polydopamine/Au nanocomposites, *Sensors Actuators B Chem.*, 2015, **221**, 792–798.

10 D. Y. Joh, A. M. Hucknall, Q. Wei, K. A. Mason, M. L. Lund, C. M. Fontes, R. T. Hill, R. Blair, Z. Zimmers, R. K. Achar, D. Tseng, R. Gordan, M. Freemark, A. Ozcan and A. Chilkoti, Inkjet-printed point-of-care immunoassay on a nanoscale polymer brush enables subpicomolar detection of analytes in blood, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, E7054–E7062.

11 Q. Zhang, Y. Qing, X. Huang, C. Li and J. Xue, Synthesis of single-walled carbon nanotubes–chitosan nanocomposites for the development of an electrochemical biosensor for serum leptin detection, *Mater. Lett.*, 2018, **211**, 348–351.

12 J. Cai, X. Gou, B. Sun, W. Li, D. Li, J. Liu, F. Hu and Y. Li, Porous graphene-black phosphorus nanocomposite modified electrode for detection of leptin, *Biosens. Bioelectron.*, 2019, **137**, 88–95.

13 C. M. Mihailescu, D. Stan, M. Savin, C. A. Moldovan, S. Dinulescu, C. H. Radulescu, B. Firtat, G. Muscalu, C. Brasoveanu, M. Ion, D. Dragomir, D. Stan and A. C. Ion, Platform with biomimetic electrochemical sensors for adiponectin and leptin detection in human serum, *Talanta*, 2020, **210**, 120643.

14 B. Özcan and M. K. Sezgintürk, A novel and disposable GP- based impedimetric biosensor using electropolymerization process with PGA for highly sensitive determination of leptin: Early diagnosis of childhood obesity, *Talanta*, 2021, **225**, 121985.

15 A. Sankiewicz, A. Hermanowicz, A. Grycz, Z. Łukaszewski and E. Gorodkiewicz, An SPR imaging immunosensor for leptin determination in blood plasma, *Anal. Methods*, 2021, **13**, 642–646.

16 İ. Uludağ and M. K. Sezgintürk, A direct and simple immobilization route for immunosensors by CNBr activation for covalent attachment of anti-leptin: obesity diagnosis point of view, *3 Biotech*, 2022, **12**, 33.

17 C. Zhang, L. Liu, H. Li, J. Hu, J. Zhang, H. Zhou, Z. Zhang and X. Du, An oriented antibody immobilization based electrochemical platform for detection of leptin in human with different body mass index, *Sensors Actuators B Chem.*, 2022, **353**, 131074.

18 C. Erkmen, G. A. Tiğ and B. Uslu, First label-free impedimetric aptasensor based on Au NPs/TiO2 NPs for the determination of leptin, *Sensors Actuators B Chem.*, 2022, **358**, 131420.

19 C. Erkmen, G. Aydoğdu Tig and B. Uslu, Evaluation of aptamer and molecularly imprinted polymers as a first hybrid sensor for leptin detection at femtogram levels, *Talanta*, 2023, **265**, 124809.