

*Supporting Information for:*

Development of a Mix-and-Read Assay for Human  
Asprosin using Antibody-Oligonucleotide Probes and  
Thermofluorimetric Analysis

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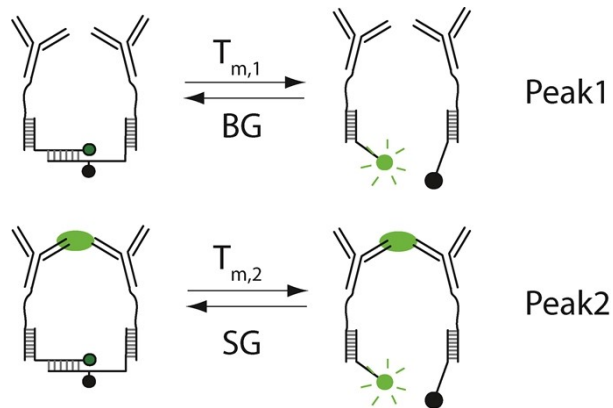
**Supporting Information (SI) Contents:**

Page S-2: The design of asprosin TFA assay, **Figure S-1**.

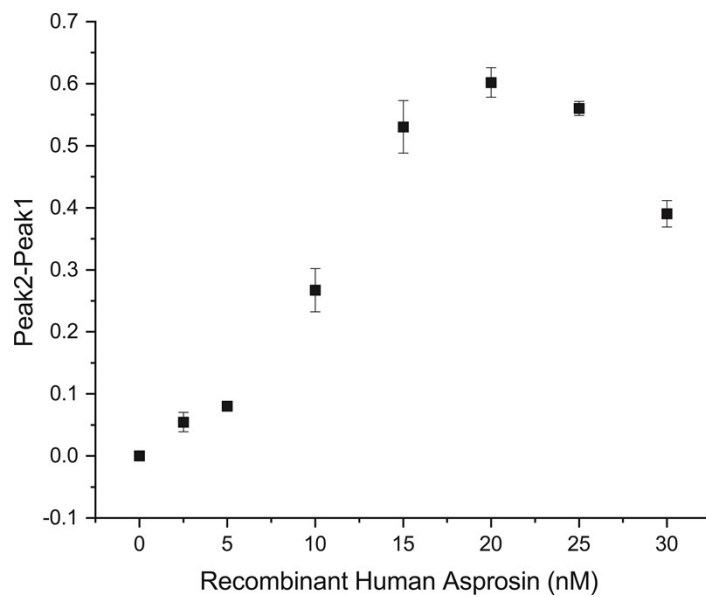
Page S-2: Calibration curve of asprosin TFA assay in DMEM, **Figure S-2**.

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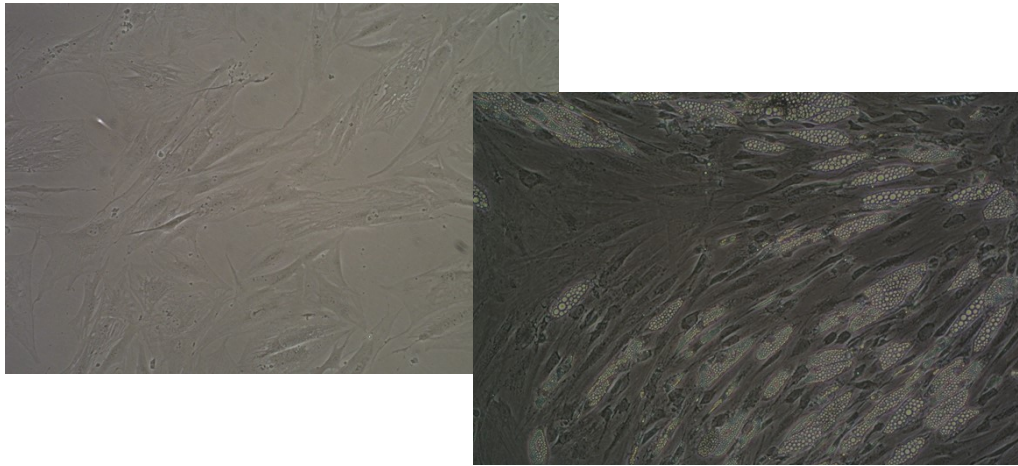
Page S-3: Asprosin secretion from human adipocytes, **Figure S-4**.



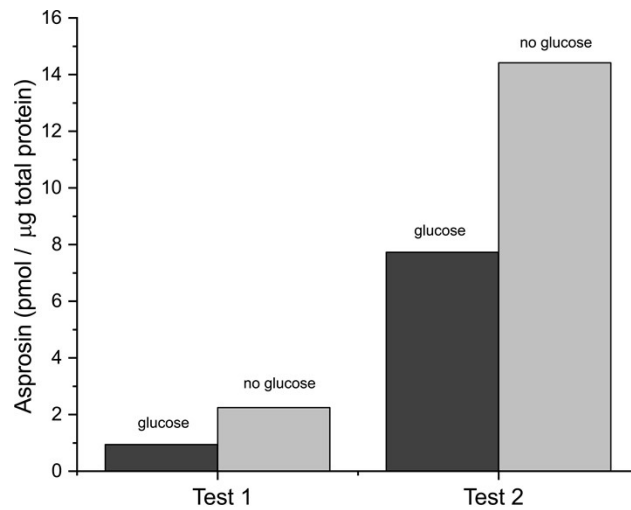
**Figure S-1.** The design of homogeneous asprosin assay using thermofluorimetric analysis (TFA). BG: background of fluorescence changing from complexes without targets, which is shown as Peak 1 in the derivative curve; SG: signal of fluorescence changing from complexes with target binding, providing the Peak 2 in the derivative curve.



**Figure S-2.** Calibration curve of asprosin TFA assay in DMEM with 15 nM probes, which exhibits an LOD of 0.6 nM.



**Figure S-3.** Poietics™ Human Visceral Preadipocytes were purchased from Lonza, which were cultured (left) and differentiated according to the manufacturer’s manual (right).



**Figure S-4.** Asprosin secretion from human adipocytes. Differentiated adipocytes were used for asprosin secretion sampling in DMEM media with  $4.5 \text{ g L}^{-1}$  glucose or without glucose in different dates. Two tests have been done in different batches of cells, and both showed around a two-fold increase of asprosin secretion in media without glucose compared to the media with glucose.