Supporting information

Label-Free Imaging of Histamine Mediated G Protein-Coupled Receptors Activation in Live Cells

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S1. SPR profile demonstrating the histamine desensitization of endogenous GPCR activation of HeLa cells.

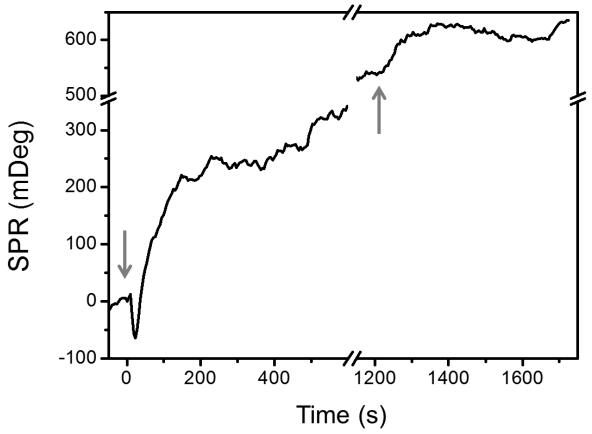

Figure S1. SPR profile of endogenous GPCR stimulation of HeLa cells by two successive 6 μM histamine injections (indicated by arrows). The HeLa cells were first exposed to 6 μM histamine (t = 0 s) for 600 s, washed with buffer, followed by another 6 μM histamine injection (t = 1200 s). The profile is the average of all cells in the imaging field. The attenuate SPR response (~250 mDeg for 1st injection, ~100 mDeg for 2nd injection) indicates the desensitization of histamine GPCR receptors. [1-2]

S2. SPR profiles of histamine triggered endogenous GPCR activation after antagonist treatments.

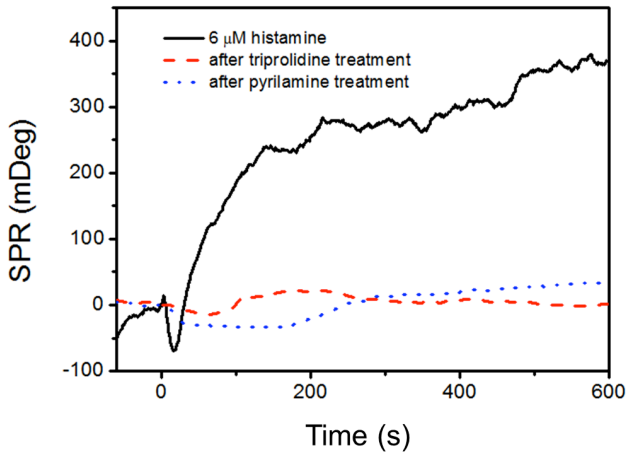

Figure S2. SPR profiles of endogenous GPCR stimulation by 6 μM histamine in HeLa cells after 30 min treatment of H1 receptor antagonists: 10 μM triprolidine (red dashed line), 10 μM pyrilamine (blue dotted line), and without H1 receptor antagonist treatment (black solid line) for a comparison.

S3. P1 and P2 peaks of triphasic P-EIM response of individual cells.

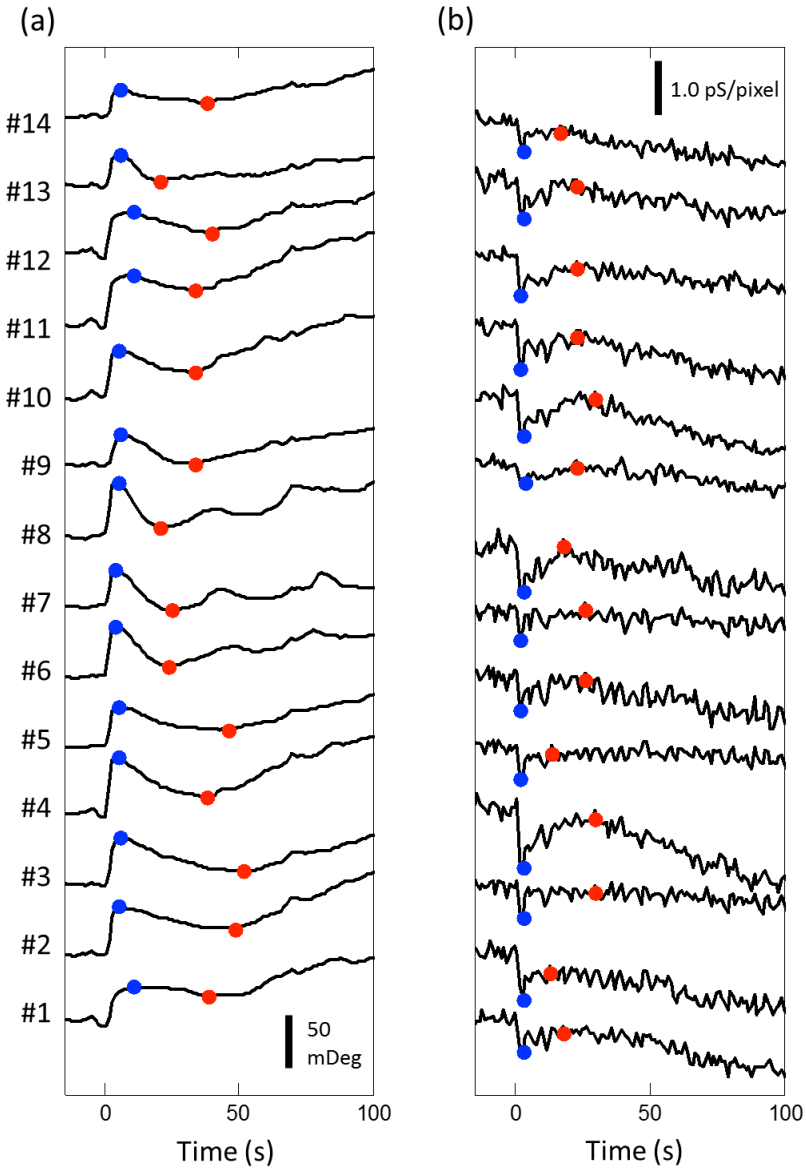

Figure S3. The SPR (a) and EIM (b) profiles of individual cells from Fig. 2 in the main text. The peaks of P1 and P2 phases were identified and marked by the blue (P1) and red (P2) dots. The corresponding peak positions are summarized in Table S1.

Table S1. The P1 and P2 peak positions of all 14 cells identified from the SPR and EIM profiles in Fig. S3.

#cell	Time (s)			
	SPR – P1	**EIM – P1**	**SPR – P2**	**EIM – P2**
1	11	3	39	18
2	5	3	49	13
3	6	3	52	30
4	5	3	38	30
5	5	2	46	14
6	4	2	24	26
7	4	2	25	26
8	5	3	21	18
9	6	4	34	23
10	5	3	34	30
11	11	2	34	23
12	11	2	40	23
13	6	3	21	23
14	6	5	38	17
Average	6.4	2.9	35.4	22.4
STDV	2.6	0.9	9.9	5.7

S4. Simulation of cell-matrix distance change of the P2 phase cellular response.

The observed negative P2 phase of SPR profile corresponds to the cell-matrix adhesion alteration caused by histamine activation.

First, we use Winspall program (http://www.res-tec.de/downloads.html) to simulate the SPR angle of the Au region (71.110 Deg). And based on the SPR angle scan profiles of the Au region and cell region, we calculate the average SPR angle of the cell region (73.473 Deg), and the average cell-matrix distance (103 nm; in good agreement with the typical cell/substrate distance[3]) from Winspall simulation. The simulation parameters are listed in Table S2. Next, by changing the cell-matrix distance (thickness of buffer solution between Au film and cell bottom membrane), the simulated values of SPR angle at different cell-matrix distances are plotted in Fig. S4 (black square). Although the SPR signal (or SPR angle) follows an exponential decay with increasing cell-matrix distance in the full range (e.g. 0 to 400 nm),[4] for a short range of cell-matrix distance from 100 nm to 121 nm, a linear fitting was applied (Fig. S4, red line). The slope of the linear fitting indicates that 1 mDeg SPR angle difference represents ~0.048 nm cell-matrix distance change.

Table S2. The refractive indices of different layers for Winspall simulation.

Layer	Thickness (nm)	Refractive index
Prism	-	1.51361
Chromium (Cr)	2	3.06769+3.36054*i*
Gold (Au)	47	0.16146+3.64199*i*
Buffer solution	variable	1.334
Cell membrane	5	1.470
Cell cytoplasm	-	1.3745

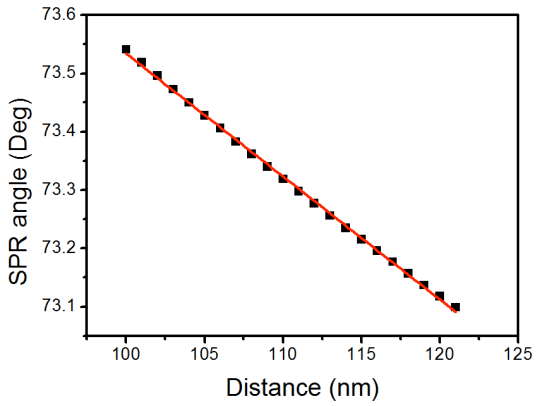

Figure S4. Relationship between the SPR angle and cell-matrix distance under the typical P-EIM experiment conditions from Winspall simulation (black square), and the corresponding linear fit (red line).

References

(1) Gainetdinov, R. R.; Premont, R. T.; Bohn, L. M.; Lefkowitz, R. J.; Caron, M. G. *Annu. Rev. Neurosci.* **2004,** *27*, 107-144.
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(3) Giebel, K.; Bechinger, C.; Herminghaus, S.; Riedel, M.; Leiderer, P.; Weiland, U.; Bastmeyer, M. *Biophys. J.* **1999,** *76*, 509-516.
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