Supplemental Materials

Molecular Biology of the Cell

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Subcellular optogenetic inhibition of G proteins generates signaling gradients and cell migration

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Supplemental figure legends

Fig. S1. CRY2-mCh lacking the RGS4Δ domain is not capable of inducing reverse βγ translocation. Images show a HeLa cell expressing CRY2-mCh, CIBN-CaaX, and YFP-γ9. The corresponding plot shows the mean intensity values of CRY2-mCh and YFP-γ9 at the plasma membrane within the region of OA. Endogenous CXCR4 receptors were activated with 50 ng/ml SDF-1α (2:05), resulting in YFP-γ9 translocation to intracellular membranes. Unlike CRY2-mCh-RGS4Δ (Fig.2), localized optical recruitment of CRY2-mCh to the plasma membrane did not lead to reverse βγ translocation. Receptor deactivation by 20 μM AMD-3100 did however lead to reverse βγ translocation. These results show that the ability of CRY2-mCh-RGS4Δ to induce reverse βγ translocation (Fig.2) is due to its GAP activity rather than a peculiar effect caused by OA or the use of CRY2/CIBN constructs.

Fig. S2. CRY2-mCh-RGS4Δ can initiate migratory responses in opposite directions in two nearby cells. Localized OA was applied to two RAW 264.7 cells expressing CRY2-mCh-RGS4Δ, CIBN-CaaX, PH(Akt)-Venus, and CXCR4, followed by uniform receptor activation with 50 ng/ml SDF-1α. OA was applied on opposite sides for the two cells. The DIC image sequence shows that both cells generated lamellipodia oriented in the direction opposite to the side of OA. After the last frame (12:40), images were captured in the mCh and Venus channels to verify subcellular localization of the CRY-RGS construct and PIP3 sensor.

Fig. S3. Reversing the side of OA reverses the directional responses in a RAW cell expressing CRY2-mCh-RGS4Δ, CIBN-CaaX, PH(Akt)-Venus, and CXCR4. Receptors were activated with 50 ng/ml SDF-1α (2:05). Upon localized OA at one side of the cell, the cell generated a PIP3

gradient and initiated migration in the direction opposite to the side of OA. This directional response was reversed upon switching the side of OA with respect to the cell.

Fig. S4. Light-triggered localization of CRY2-mCh does not generate directional PIP3 or migratory responses. Images show a RAW cell expressing CRY2-mCh, CIBN-CaaX, PH(Akt)-Venus, and CXCR4. Similar to CRY2-mCh-PGK1 (Fig. 3D), localized optical recruitment of CRY2-mCh to the plasma membrane does not influence the direction of PIP3 responses or lamellipodia formation. These negative controls show that the directional effects generated by the CRY2-RGS and CRY2-GRKct constructs are due to their ability to inhibit G protein subunit activity.

Fig. S5. Directional migration initiation in a RAW cell with uniform activation of endogenous C5a receptors and localized G protein inhibition by CRY2-mCh-RGS4Δ. Images show a RAW cell expressing CRY2-mCh-RGS4Δ, CIBN-CaaX, and PH(Akt)-Venus. C5a receptors were activated with 10 μM FKP-(D-Cha)-Cha-r, resulting in a uniform PIP3 response and cell protrusions. Upon adaptation of these transient responses, subsequent localized OA resulted in the formation of a PIP3 gradient and migration initiation oriented in the direction opposite to the side of OA.

Fig. S6. A CRY-GRK3ct construct can generate directionally responsive PIP3 and lamellipodia responses similar to the CRY-GRK2ct construct. (A) Images show a RAW cell expressing CRY2-mCh-GRK3ct, CIBN-CaaX, PH(Akt)-Venus, and CXCR4. (B) Time-stacks corresponding to the images in (A). Optical recruitment of the CRY-GRK3ct construct to one side of the cell, together with uniform receptor activation by 50 ng/ml SDF-1α resulted in a PIP3 gradient and lamellipodia oriented in the direction opposite to the side of OA. Switching the side of OA reversed these directional responses.

Fig. S7. Localized βγ inhibition directs the formation of a PIP3 gradient and cell protrusions in a RAW cell with uniform activation of endogenous C5a receptors. Images show a RAW cell expressing CRY2-mCh-GRK2ct, CIBN-CaaX, and PH(Akt)-Venus. Following optical recruitment of the CRY2-GRKct construct to one side of the cell, C5a receptors were activated with 10 μM FKP-(D-Cha)-Cha-r. This resulted in the formation of a PIP3 gradient and cell protrusions oriented in the direction opposite to the side of OA.

Supplemental movie legends

Movie 1. Local OA of CRY2-mCh-RGS4Δ followed by uniform activation of CXCR4. The movie shows a RAW cell expressing CRY2-mCh-RGS4Δ (red), CIBN-CaaX, PH(Akt)-Venus (green), and CXCR4. Localized OA (white box) was applied to recruit the CRY2-RGS construct to one side of the cell. Subsequent addition of spatially uniform SDF-1α (50ng/ml) initiated cell migration in the opposite direction relative to the side of OA. The movie corresponds to the data presented in Fig. 3A.

Movie 2. Uniform activation of CXCR4 followed by local OA of CRY2-mCh-RGS4Δ. The movie shows a RAW cell expressing CRY2-mCh-RGS4Δ (red), CIBN-CaaX, PH(Akt)-Venus (green), and CXCR4. SDF-1α (50ng/ml) was added prior to OA, resulting in a PIP3 increase and cell protrusions. Localized OA was then applied to one side of the cell, resulting in the initiation of cell migration in the opposite direction.

Movie 3. Local OA of CRY2-mCh-GRK2ct followed by uniform activation of CXCR4. The movie shows a RAW cell expressing CRY2-mCh-GRK2ct (red), CIBN-CaaX, PH(Akt)-Venus (green), and CXCR4. The CRY-GRKct construct was optically recruited to one side of the cell. Subsequent receptor activation by 50 ng/ml SDF-1α resulted in the generation of a PIP3 gradient and lamellipodia oriented in the opposite direction relative to the side of OA. Switching the side of OA caused these responses to reverse direction.

Movie 4. Adaptation to uniform CXCR4 activation followed by local OA of CRY2-mCh-RGS4Δ. The movie shows a RAW cell expressing CRY2-mCh-RGS4Δ (red), CIBN-CaaX, PH(Akt)-Venus (green), and CXCR4. Receptors were activated with SDF-1α (50ng/ml) leading to the generation of PIP3 and cell protrusions. After these responses adapted, localized OA of the CRY-RGS construct to one end of the cell resulted in the generation of a PIP3 gradient and migration initiation in the direction opposite to the side of OA. The movie corresponds to the data presented in Fig. 5.

Figure S1.

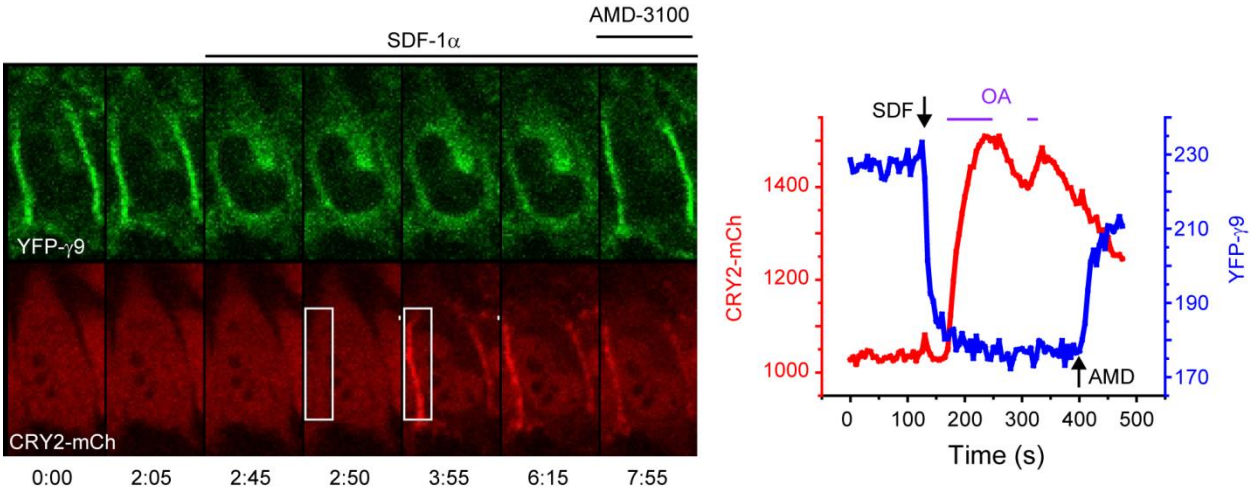

AMD-3100
SDF-1α
YFP-γ9
CRY2-mCh
0:00
2:05
2:45
2:50
3:55
6:15
7:55
SDF
OA
AMD
CRY2-mCh
YFP-γ9
1400
1200
1000
230
210
190
170
0
100
200
300
400
500
Time (s)

Figure S2.

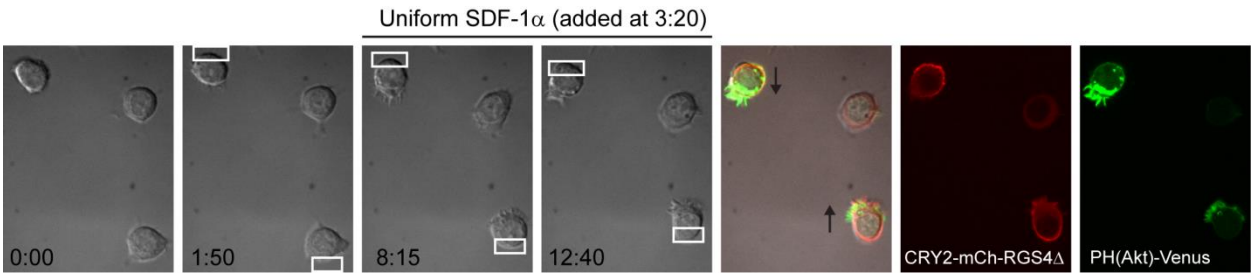

Uniform SDF-1α (added at 3:20)
0:00
1:50
8:15
12:40
CRY2-mCh-RGS4Δ
PH(Akt)-Venus

Figure S3.

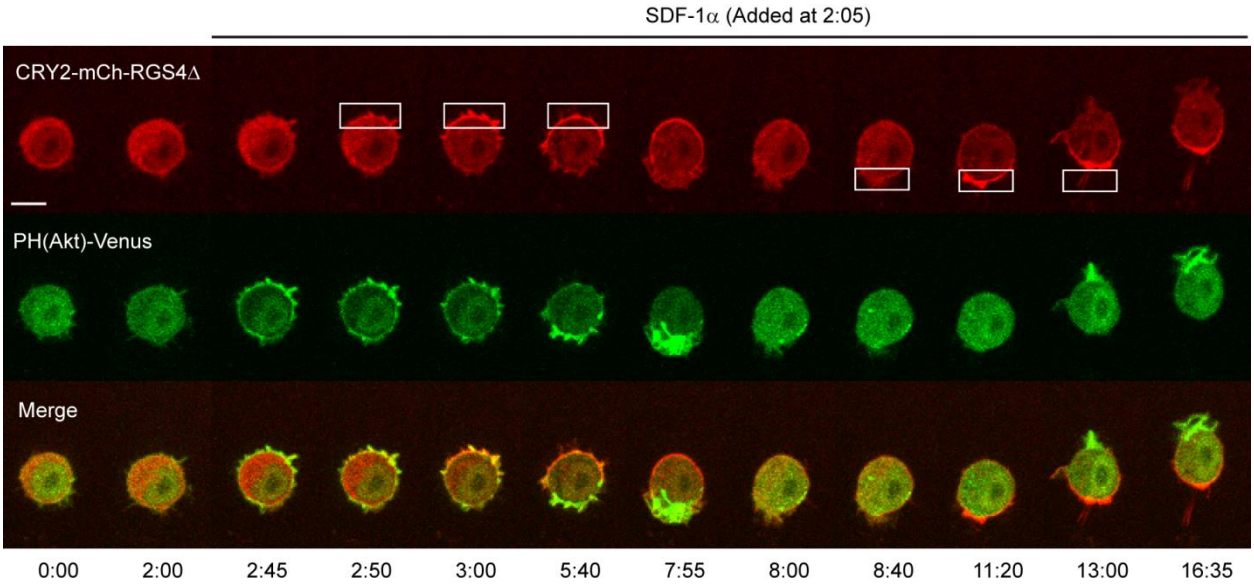

SDF-1α (Added at 2:05)
CRY2-mCh-RGS4Δ
PH(Akt)-Venus
Merge
0:00
2:00
2:45
2:50
3:00
5:40
7:55
8:00
8:40
11:20
13:00
16:35

Figure S4.

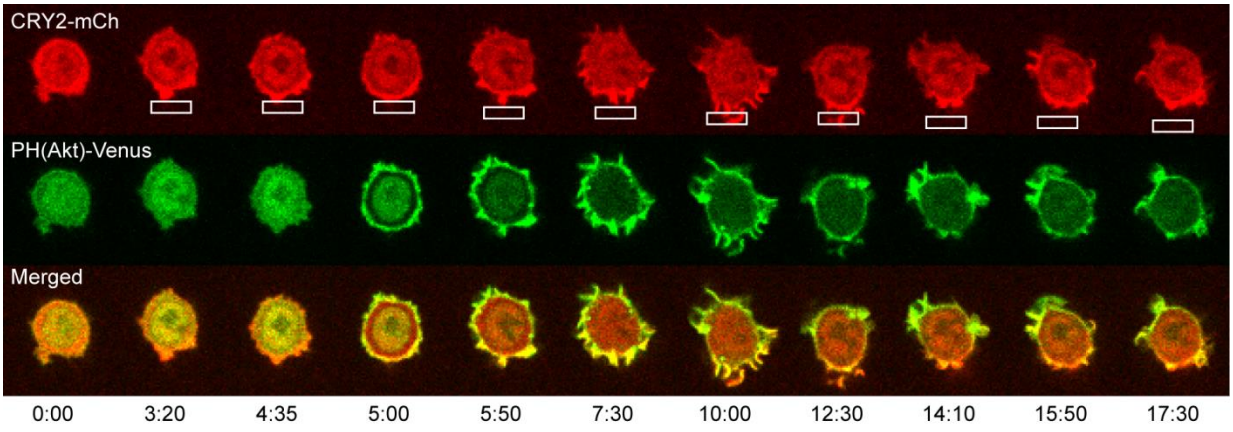

CRY2-mCh
PH(Akt)-Venus
Merged
0:00
3:20
4:35
5:00
5:50
7:30
10:00
12:30
14:10
15:50
17:30

Figure S5.

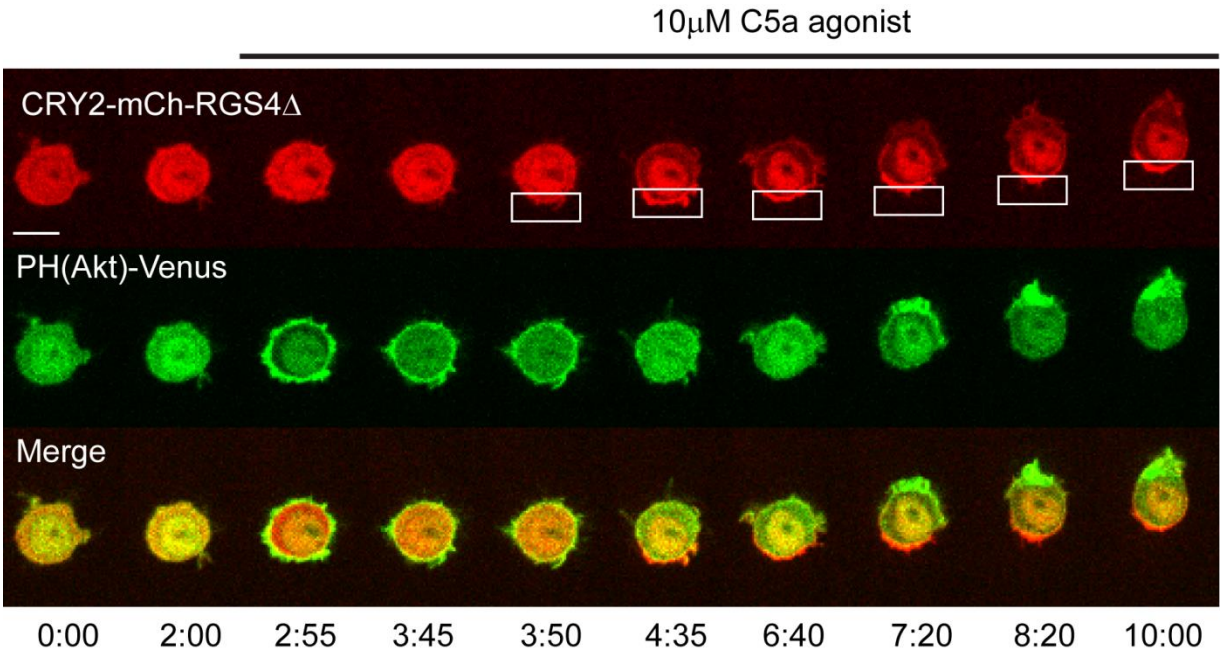

10μM C5a agonist
CRY2-mCh-RGS4Δ
PH(Akt)-Venus
Merge
0:00
2:00
2:55
3:45
3:50
4:35
6:40
7:20
8:20
10:00

Figure S6.

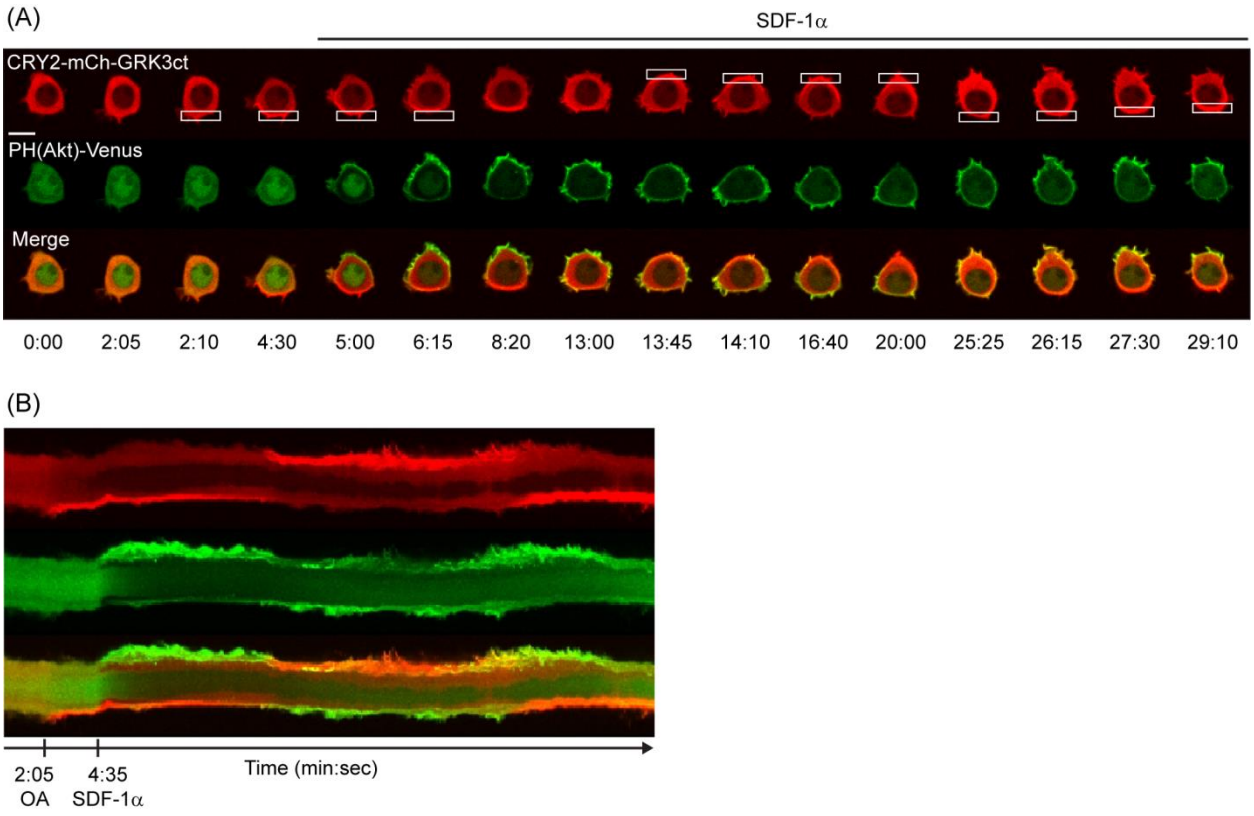
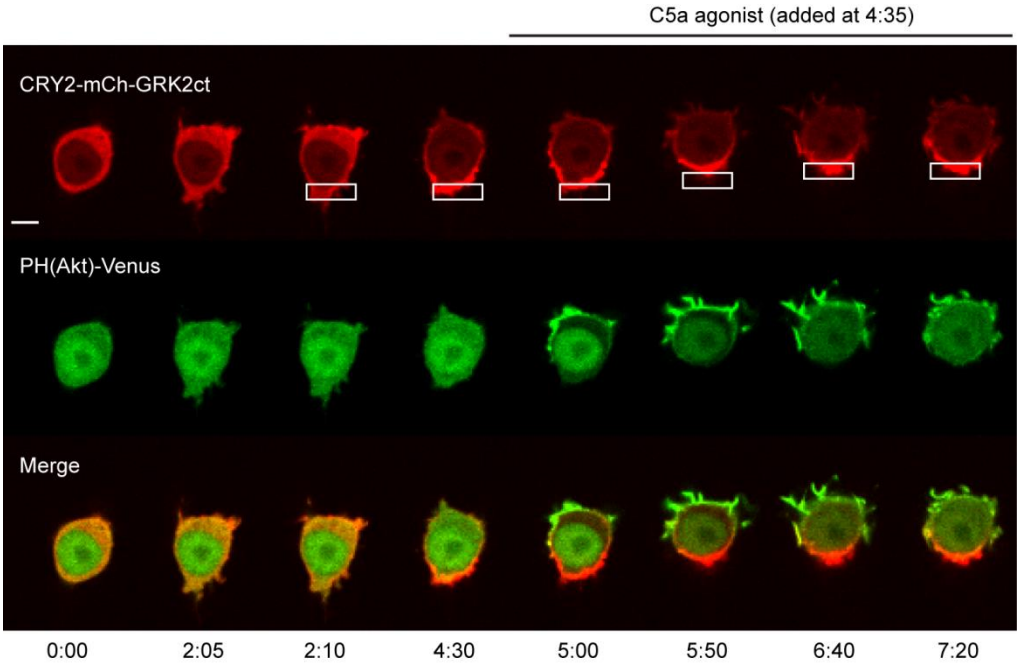

(A)
SDF-1α
CRY2-mCh-GRK3ct
PH(Akt)-Venus
Merge
0:00
2:05
2:10
4:30
5:00
6:15
8:20
13:00
13:45
14:10
16:40
20:00
25:25
26:15
27:30
29:10
(B)
2:05
OA
4:35
SDF-1α
Time (min:sec)

Figure S7.


C5a agonist (added at 4:35)
CRY2-mCh-GRK2ct
PH(Akt)-Venus
Merge
0:00
2:05
2:10
4:30
5:00
5:50
6:40
7:20