



## Supplementary Materials for

### **PI3K $\gamma$ Adaptor Subunits Define Coupling to Degranulation and Cell Motility by Distinct PtdIns(3,4,5)P<sub>3</sub> Pools in Mast Cells**

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#### **This PDF file includes:**

Descriptions of Movies m1 to m9.

## Movie descriptions

### Movie m1 (m1.p84\_z\_stack)

A stack of deconvoluted images of a KO BMMC reconstituted with WT p110 $\gamma$  and HA-p84 was taken 2 minutes after stimulation with 2  $\mu$ M adenosine, and runs through adjacent focal planes to visualize the distribution of PH<sub>Btk</sub>-GFP (green) and subcortical F-actin stained with rhodamine-phalloidin (red).

### Movie m2 (m2.p101\_z\_stack)

KO BMMCs were complemented with WT p110 $\gamma$  and HA-p101. Subsequently experiments were performed exactly as for movie m1.

### Movie m3 (m3.p84\_ade.mov)

Time-lapse video microscopy was used to visualize PtdIns(3,4,5)P<sub>3</sub> in KO BMMCs reconstituted with p84 and p110 $\gamma$ . Images were acquired immediately after stimulation with adenosine over 360 frames (1 frame / 2 s). The movie runs at 15 frames / s.

### Movie m4 (m4.p101\_ade.mov)

Live-cell video microscopy for visualization of PtdIns(3,4,5)P<sub>3</sub> with PH<sub>Btk</sub>-GFP in KO BMMCs reconstituted with p101 and p110 $\gamma$ . Images were acquired immediately after stimulation with adenosine over 360 frames (1 frame/ 2 s). The movie runs at 15 frames / s.

### Movie m5 (m5.p84\_DNP\_ade.mov)

Live-cell video microscopy for visualization of PtdIns(3,4,5)P<sub>3</sub> with PH<sub>Btk</sub>-GFP in KO BMMC reconstituted with p84 and p110 $\gamma$ . Cells were sensitized overnight with 100 ng/ml anti-DNP IgE. Images were acquired immediately after stimulation with DNP-HSA + adenosine over 400 frames (1 frame/ 2 s). The movie runs at 15 frames / s.

### Movie m6 (m6.p101\_DNP\_ade.mov)

Live-cell video microscopy for visualization of PtdIns(3,4,5)P<sub>3</sub> with PH<sub>Btk</sub>-GFP in KO BMMC complemented with p101 and p110 $\gamma$ . Cells were sensitized overnight with 100 ng/ml anti-DNP IgE. Images were acquired immediately after DNP-HSA and adenosine were added (1 frame/ 2 s; 400 frames). The movie runs at 15 frames / s.

### Movie m7 (m7.p101\_ade\_SIP.mov)

Surface intensity plots (SIPs) of the cells monitored in movie m4. Intensity of GFP-PH<sub>Btk</sub> is shown. The intensity scale matches the one in fig. S5.

### Movie m8 (m8.p101\_ade\_NOC.mov)

Live-cell video microscopy for visualization of PtdIns(3,4,5)P<sub>3</sub> with PH<sub>Btk</sub>-GFP in KO BMMC reconstituted with p101 and p110 $\gamma$ . Cells were treated with the microtubule disruptor nocodazole (1  $\mu$ M for 3h) prior to stimulation with adenosine. Images were acquired immediately after adenosine stimulation over 360 frames (1 frame/ 2 s). The movie runs at 15 frames / s.

### Movie m9 (m9.p101\_ade\_NOC\_SIP.mov)

Surface intensity plots of the cells monitored in movie m8. Intensity of GFP-PH<sub>Btk</sub> is shown. Intensity scale used is the same as that in fig. S5.