

Fig. S1. Expression of mutant Arf6 alter Fc ε **RI internalization but not IgE binding.** RBL cells transiently transfected with WT Arf6^{CFP} (**A,C**) or Arf6-Q67L^{CFP} (**B,D**) were primed with IgE^{AF555} and crosslinked (**C-D**), or not (**A-B**), with DNP²⁴ for 10' at 37°C before fixation and imaging. Scale bars = 10 μ m.

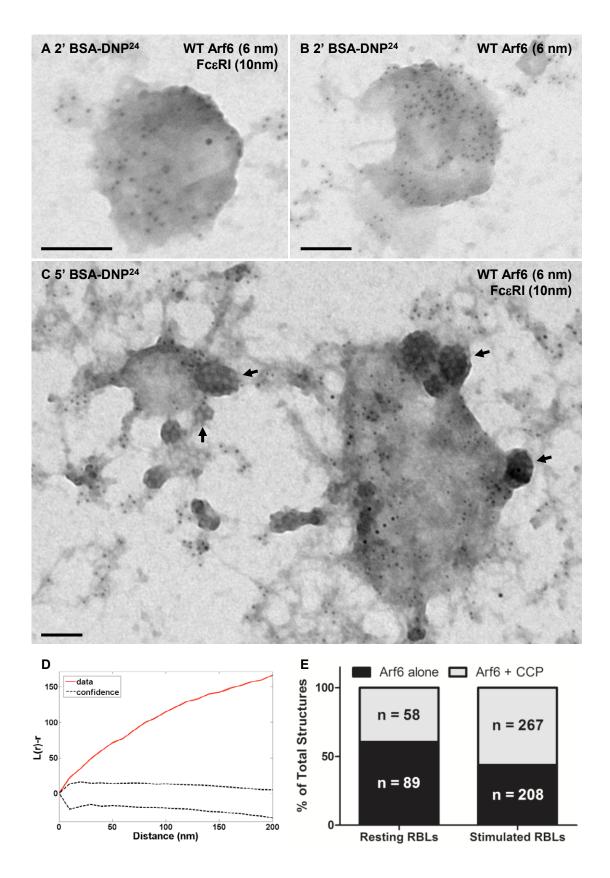


Fig. S2. Arf6 positive structures are often with/connected to CCP. (A-C) TEM image of membrane sheets prepared from cells expressing WT Arf6^{CFP} (6 nm gold). Samples were primed with IgE and stimulated for 2' (A-B) or 5' (C) with DNP-gold (10 nm gold). (D) Ripley's bivariate statistical co-clustering test corresponding to images (C). (E) Quantification of Arf6 structures alone or connected to a CCP in resting or stimulated RBLs. Scale bars = 100 nm (A-C).

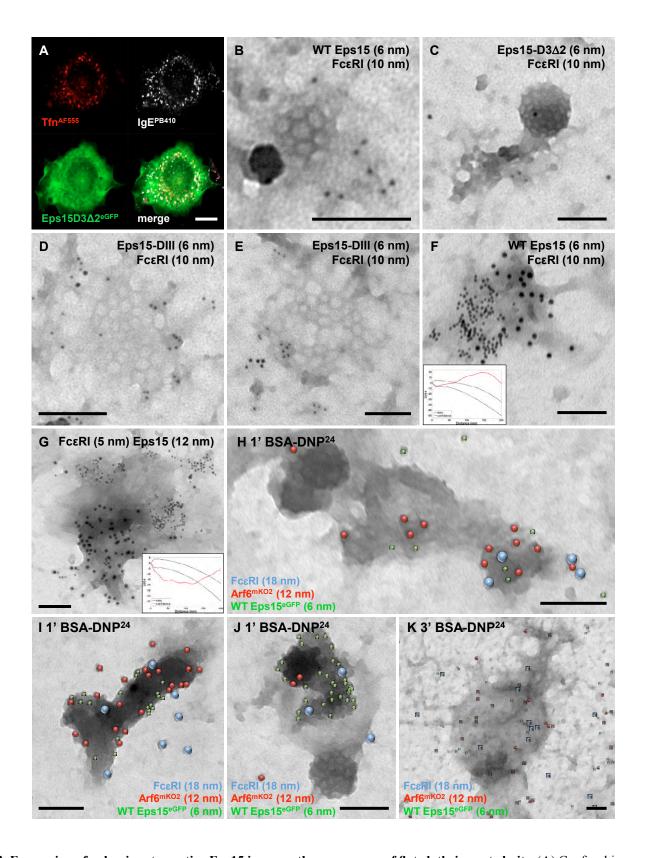


Fig. S3. Expression of a dominant negative Eps15 increase the occurrence of flat clathrin coated pits. (A) Confocal image of RBL cells transiently transfected with Eps15 D3 Δ 2^{eGFP} primed with IgE^{PB410} and stimulated with DNP²⁴ and tfn^{AF555} (Invitrogen) for 10' at 37°C. (**B-G**) Electron micrograph of RBL-2H3 cells expressing WT Eps15^{eGFP} (**B,F-G**), Eps15 D3 Δ 2^{eGFP} (**C**) or Eps15 DIII^{eGFP} (**D-E**) (6 nm gold); primed with IgE and stimulated with DNP-gold before imaging. Insert graph on images **F** and **G** are Ripley's bivariate statistical co-clustering test for Eps15 and Fc ε RI. (**H-K**) Electron micrographs of membrane sheets triply labelled for WT Eps15^{eGFP} (6 nm gold, green dots), Arf6^{mKO2} (12 nm gold, red dots) and Fc ε RIβ (18 nm gold, blue dots) after 1' (**H-J**) or 3' (**K**) stimulation at 37°C. Scale bars = 10 μ m (**A**) or 100 nm (**B-K**).



Movie 1. Z-slices throughout a RBL cells (showed in Fig. **1G**) primed with IgE^{AF488} and stimulated with DNP²⁴ and tfn^{AF555} (Invitrogen) for 10' at 37°C, showing the complete internalization of both transferrin and IgE receptors. Scale bar = 5 μ m.



Movie 2. Z-slices throughout a RBL cells (showed in Fig. 1H) primed with IgE^{AF488} and stimulated with DNP²⁴ and tfn^{AF555} (Invitrogen) for 10' at 37°C, beforehand treated with siRNA anti-clathrin HC, showing the complete internalization of the IgE receptors while transferrin stays on the cell surface. Scale bar = 5 μ m.





Movie 3 and 4. 3D EM reconstructions of the Arf6 structures observed in Figure 3D and G.



Movie 5. 3D EM reconstruction of a clathrin coated pit with WT Dynamin gold labels present at the neck of the vesicle.



Movie 6. Z-stack maximum intensity projection of a RBL cell stably expressing WT Dynamin^{eGFP} and primed with IgE^{AF555} showing a diffuse and homogeneous distribution of WT Dynamin^{eGFP}. Scale bar = 5 μ m.



Movie 7. Z-stack maximum intensity projection of a RBL cell stably expressing Dynamin-K44A^{eGFP} and primed with IgE^{AF555}, showing the presence of Dynamin-K44A^{eGFP} in punctate structures near the cell surface. Scale bar = 5 μ m.



Movie 8. Z-stack maximum intensity projection of a RBL cell transiently expressing WT Eps15^{eGFP} showing the accumulation of bright Eps15 structures connected, or next, to the plasma membrane. Scale bar = 10μ m.





Movie 9 and 10. 3D EM reconstructions of the Eps15 structures observed in Figure 7A and D.



Movie 11. Z-stack maximum intensity projection of the RBL cell presented in Figure **8G**, showing a high degree of Fc ε RI internalization after crosslinking with DNP²⁴-BSA Scale bar = 5 μ m.



Movie 12 Z-stack maximum intensity projection of the RBL cell presented in Figure **8I**, showing the accumulation of aggregated Fc ϵ RI on the cell surface and the absence of internalization upon crosslinking with DNP²⁴-BSA after a 30' treatment with Pitstop2 at 60 μ M. Scale bar = 5 μ m.