S1 | Pharmacological inhibitors or dominant negative proteins used to distinguish clathrin-independent mechanisms of endocytosis

	Clathrin-independent mechanism of endocytosis			
Inhibitors or DN proteins (see Refs 1–10)	**Caveolar**	**RhoA-regulated**	**CDC42-regulated**	**ARF6-regulated**
Dynamin-2 DN	Inhibits	Inhibits	No effect / enhances	No effect
ARF6 DN	ND	ND	No effect	Inhibits recycling[d, 11]
Cholesterol depletion	Inhibits	ND	Inhibits[a]	Inhibits[12]
Actin polymerization modifiers (LatA, CytoD, Jas)	Inhibits[13]	Inhibits[7]	Inhibits[14]	Inhibits recycling [15]
Genistein (general tyrosine kinase inhibitor)	Inhibits	No effect	Inhibits	ND
PP2 (Src family kinase inhibitor)	Inhibits	No effect	No effect	ND
DN CAV1[b]; CAV1 KD	Inhibits	No effect	No effect	ND
Clostridium toxin B (Rho GTPase inhibitor)	No effect	Inhibits	Inhibits	ND
DN RhoA	No effect	Inhibits	No effect	ND
DN CDC42	No effect	No effect	Inhibits	ND
AP-180 DN[c]	No effect	No effect	No effect	ND
Chlorpromazine[c]	No effect	No effect	No effect	ND

AP-180, adaptor protein-180; ARF6, ADP-ribosylation factor 6; CAV1, caveolin-1; CDC42, cell-division cycle-42; CytoD, cytochalasin D; DN, dominant negative; Jas, jasplakinolide; KD, knock-down; LatA, latrunculin A; ND, not determined; PP2, 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine.

[a] Inhibition varies with cell type and level of cholesterol depletion[1,3,16,17].
[b] CAV1 tagged with green fluorescent protein at N-terminus originally reported to be a DN[5].
[c] Inhibits clathrin-dependent endocytosis (see Refs 1,9).
[d] Also shown to inhibit uptake of carboxypeptidase E (see Ref. 18).

References

1. Cheng, Z.J. *et al*. Distinct mechanisms of clathrin-independent endocytosis have unique sphingolipid requirements. *Mol. Biol. Cell* **17**, 3197–3210 (2006).
2. Henley, J.R., Krueger, E.W., Oswald, B.J. & McNiven, M.A. Dynamin-mediated internalization of caveolae. *J. Cell Biol.* **141**, 85–99 (1998).
3. Kirkham, M. *et al*. Ultrastructural identification of uncoated caveolin-independent early endocytic vehicles. *J. Cell Biol.* **168**, 465–476 (2005).
4. Orlandi, P.A. & Fishman, P.H. Filipin-dependent inhibition of cholera toxin: evidence for toxin internalization and activation through caveolae-like domains. *J. Cell Biol.* **141**, 905–915 (1998).
5. Pelkmans, L., Kartenbeck, J. & Helenius, A. Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER. *Nature Cell Biol.* **3**, 473–483 (2001).
6. Sabharanjak, S., Sharma, P., Parton, R.G. & Mayor, S. GPI-anchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-independent pinocytic pathway. *Dev. Cell* **2**, 411–423 (2002).
7. Sauvonnet, N., Dujeancourt, A. & Dautry-Varsat, A. Cortactin and dynamin are required for the clathrin-independent endocytosis of γc cytokine receptor. *J. Cell Biol.* **168**, 155–163 (2005).
8. Sharma, D.K. *et al*. Selective stimulation of caveolar endocytosis by glycosphingolipids and cholesterol. *Mol. Biol. Cell* **15**, 3114–3122 (2004).
9. Singh, R.D. *et al*. Selective caveolin-1-dependent endocytosis of glycosphingolipids. *Mol. Biol. Cell* **14**, 3254–3265 (2003).
10. Naslavsky, N., Weigert, R. & Donaldson, J.G. Convergence of non-clathrin- and clathrin-derived endosomes involves Arf6 inactivation and changes in phosphoinositides. *Mol. Biol. Cell* **14**, 417–431 (2003).
11. Brown, F.D., Rozelle, A.L., Yin, H.L., Balla, T. & Donaldson, J.G. Phosphatidylinositol 4,5-bisphosphate and Arf6-regulated membrane traffic. *J. Cell Biol.* **154**, 1007–1017 (2001).
12. Naslavsky, N., Weigert, R. & Donaldson, J.G. Characterization of a nonclathrin endocytic pathway: membrane cargo and lipid requirements. *Mol. Biol. Cell* **15**, 3542–3552 (2004).
13. Pelkmans, L., Püntener, D. & Helenius, A. Local actin polymerization and dynamin recruitment in SV40-induced internalization of caveolae. *Science* **296**, 535–539 (2002).

14. Gauthier, N.C. et al. Glycosylphosphatidylinositol-anchored proteins and actin cytoskeleton modulate chloride transport by channels formed by the *Helicobacter pylori* vacuolating cytotoxin VacA in HeLa cells. *J. Biol. Chem* **279**, 9481–9489 (2004).
15. Radhakrishna, H. & Donaldson, J.G. ADP-Ribosylation factor 6 regulates a novel plasma membrane recycling pathway. *J. Cell Biol.* **139**, 49–61 (1997).
16. Gauthier, N.C. *et al*. *Helicobacter pylori* VacA cytotoxin: a probe for a clathrin-independent and Cdc42-dependent pinocytic pathway routed to late endosomes. *Mol. Biol. Cell* **16**, 4852–4866 (2005).
17. Patel, H.K. *et al*. Plasma membrane cholesterol modulates cellular vacuolation induced by the *Helicobacter pylori* vacuolating cytotoxin. *Infect. Immun.* **70**, 4112–4123 (2002).
18. Arnaoutova, I., Jackson, C.L., Al-Awar, O.S., Donaldson, J.G. & Loh, Y.P. Recycling of raft-associated prohormone sorting receptor carboxypeptidase E requires interaction with ARF6. *Mol. Biol. Cell* **14**, 4448–4457 (2003).