Supplemental Table 1. Compounds and mouse models available to examine the relative importance of endosomal and cell surface signaling by GPCRs in the ENS. These include new and established tools to probe the functional significance of GPCR endocytosis, and novel approaches to define the importance of βArrs in the control of GPCR signaling.

Target	Tool	Effect and mechanism of action	GPCR examined in ENS	Endpoint examined	Ref
Dynamin	Dynasore Dyngo4a Dynole 34-2 Dyngo4a inactive	Inhibition; blocks dynamin GTPase and clathrin coated vesicle formation	NK_1R, DOR, MOR, SSTR2A	Microscopy, Ca^{2+} signaling, MAPK signaling	(3, 14-17, 20)
	Ryngo 1-23	Activation; increases dynamin GTPase activity	-	-	(6)
Clathrin	PitStop2 PitStop2 inactive	Inhibition of clathrin-dependent processes	MOR, NK_1R	Microscopy	(9, 16)
General Endocytosis	Hypertonic sucrose	Formation of clathrin microcages, Non-selective	NK_1R, MOR, DOR	Microscopy	(9, 12, 17)
	Phenylarsine oxide	Crosslinks sulfur groups	NK_1R, MOR	Microscopy	(4, 13)
	K^+ depletion	Blocks clathrin coated pit formation	MOR	Microscopy	(13)
	Concanavalin A	Non-selective	NK_1R	Microscopy	(4)
Beta Arrestins	βArr2 global knockout mouse	Genetic Deletion	MOR, NK_1R	Tension Recordings, fecal output, Microscopy	(8, 11, 16, 19)
	βArr2 conditional knockout mouse	Genetic Deletion	-	-	(21)

	Barbadin	Inhibits βArr-AP2 interaction	-	-	(1)
GRK	Global knockout (GRK6) mouse	GRK deletion	MOR	Fecal output	(18)
	Cmpd101	Inhibition of GRK2/3 activity	-	-	(10)
	Knockin mice	GRK phosphosite-deficient receptors	-	-	(2, 5)
Endosomal Trafficking (endosomal pH)	Bafilomycin A1	Vacuolar-type H^+-ATPase inhibitor	NK_1R, SSTR2A, MOR	Microscopy	(4, 15, 20)
	Monensin	Carboxylic ionophore	NK_1R, NK_3R	Microscopy	(4, 7, 12)
	NH_4Cl	Lysosomotropic weak base	NK_1R	Microscopy	(4)
	Chloroquine	Lysosomotropic weak base	NK_1R	Microscopy	(4)
Golgi transport	Brefeldin A	Disassembly of Golgi complex	SSTR2A	Microscopy	(20)
***De novo* receptor synthesis**	Cycloheximide	Blocks protein synthesis	MOR, DOR, NK_1R	Microscopy	(4, 13, 17)

References

1. **Beautrait A, Paradis JS, Zimmerman B, Giubilaro J, Nikolajev L, Armando S, Kobayashi H, Yamani L, Namkung Y, Heydenreich FM, Khoury E, Audet M, Roux PP, Veprintsev DB, Laporte SA, and Bouvier M.** A new inhibitor of the beta-arrestin/AP2 endocytic complex reveals interplay between GPCR internalization and signalling. *Nat Commun* 8: 15054, 2017.
2. **Bradley SJ, Wiegman CH, Iglesias MM, Kong KC, Butcher AJ, Plouffe B, Goupil E, Bourgognon JM, Macedo-Hatch T, LeGouill C, Russell K, Laporte SA, Konig GM, Kostenis E, Bouvier M, Chung KF, Amrani Y, and Tobin AB.** Mapping physiological G protein-coupled receptor signaling pathways reveals a role for receptor phosphorylation in airway contraction. *Proc Natl Acad Sci U S A* 113: 4524-4529, 2016.
3. **Duraffourd C, Kumala E, Anselmi L, Brecha NC, and Sternini C.** Opioid-induced mitogen-activated protein kinase signaling in rat enteric neurons following chronic morphine treatment. *PLoS One* 9: e110230, 2014.
4. **Grady EF, Gamp PD, Jones E, Baluk P, McDonald DM, Payan DG, and Bunnett NW.** Endocytosis and recycling of neurokinin 1 receptors in enteric neurons. *Neuroscience* 75: 1239-1254, 1996.
5. **Grecksch G, Just S, Pierstorff C, Imhof AK, Gluck L, Doll C, Lupp A, Becker A, Koch T, Stumm R, Hollt V, and Schulz S.** Analgesic tolerance to high-efficacy agonists but not to morphine is diminished in phosphorylation-deficient S375A mu-opioid receptor knock-in mice. *J Neurosci* 31: 13890-13896, 2011.
6. **Jackson J, Papadopulos A, Meunier FA, McCluskey A, Robinson PJ, and Keating DJ.** Small molecules demonstrate the role of dynamin as a bi-directional regulator of the exocytosis fusion pore and vesicle release. *Mol Psychiatry* 20: 810-819, 2015.
7. **Jenkinson KM, Mann PT, Southwell BR, and Furness JB.** Independent endocytosis of the NK(1) and NK(3) tachykinin receptors in neurons of the rat myenteric plexus. *Neuroscience* 100: 191-199, 2000.
8. **Kang M, Maguma HT, Smith TH, Ross GR, Dewey WL, and Akbarali HI.** The role of beta-arrestin2 in the mechanism of morphine tolerance in the mouse and guinea pig gastrointestinal tract. *J Pharmacol Exp Ther* 340: 567-576, 2012.
9. **Lay J, Carbone SE, DiCello JJ, Bunnett NW, Canals M, and Poole DP.** Distribution and trafficking of the mu-opioid receptor in enteric neurons of the guinea pig. *Am J Physiol Gastrointest Liver Physiol* 311: G252-266, 2016.
10. **Lowe JD, Sanderson HS, Cooke AE, Ostovar M, Tsisanova E, Withey SL, Chavkin C, Husbands SM, Kelly E, Henderson G, and Bailey CP.** Role of G Protein-Coupled Receptor Kinases 2 and 3 in mu-Opioid Receptor Desensitization and Internalization. *Mol Pharmacol* 88: 347-356, 2015.
11. **Maguma HT, Dewey WL, and Akbarali HI.** Differences in the characteristics of tolerance to mu-opioid receptor agonists in the colon from wild type and beta-arrestin2 knockout mice. *Eur J Pharmacol* 685: 133-140, 2012.
12. **Mann PT, Southwell BR, and Furness JB.** Internalization of the neurokinin 1 receptor in rat myenteric neurons. *Neuroscience* 91: 353-362, 1999.
13. **Minnis JG, Patierno S, Kohlmeier SE, Brecha NC, Tonini M, and Sternini C.** Ligand-induced mu opioid receptor endocytosis and recycling in enteric neurons. *Neuroscience* 119: 33-42, 2003.
14. **Patierno S, Anselmi L, Jaramillo I, Scott D, Garcia R, and Sternini C.** Morphine induces mu opioid receptor endocytosis in guinea pig enteric neurons following prolonged receptor activation. *Gastroenterology* 140: 618-626, 2011.
15. **Pelayo JC, Poole DP, Steinhoff M, Cottrell GS, and Bunnett NW.** Endothelin-converting enzyme-1 regulates trafficking and signalling of the neurokinin 1 receptor in endosomes of myenteric neurones. *J Physiol* 589: 5213-5230, 2011.

16. **Poole DP, Lieu T, Pelayo JC, Eriksson EM, Veldhuis NA, and Bunnett NW.** Inflammation-induced abnormalities in the subcellular localization and trafficking of the neurokinin 1 receptor in the enteric nervous system. *Am J Physiol Gastrointest Liver Physiol* 309: G248-259, 2015.
17. **Poole DP, Pelayo JC, Scherrer G, Evans CJ, Kieffer BL, and Bunnett NW.** Localization and regulation of fluorescently labeled delta opioid receptor, expressed in enteric neurons of mice. *Gastroenterology* 141: 982-991 e918, 2011.
18. **Raehal KM, Schmid CL, Medvedev IO, Gainetdinov RR, Premont RT, and Bohn LM.** Morphine-induced physiological and behavioral responses in mice lacking G protein-coupled receptor kinase 6. *Drug Alcohol Depend* 104: 187-196, 2009.
19. **Raehal KM, Walker JK, and Bohn LM.** Morphine side effects in beta-arrestin 2 knockout mice. *J Pharmacol Exp Ther* 314: 1195-1201, 2005.
20. **Zhao P, Canals M, Murphy JE, Klingler D, Eriksson EM, Pelayo JC, Hardt M, Bunnett NW, and Poole DP.** Agonist-biased trafficking of somatostatin receptor 2A in enteric neurons. *J Biol Chem* 288: 25689-25700, 2013.
21. **Zhu L, Almaca J, Dadi PK, Hong H, Sakamoto W, Rossi M, Lee RJ, Vierra NC, Lu H, Cui Y, McMillin SM, Perry NA, Gurevich VV, Lee A, Kuo B, Leapman RD, Matschinsky FM, Doliba NM, Urs NM, Caron MG, Jacobson DA, Caicedo A, and Wess J.** beta-arrestin-2 is an essential regulator of pancreatic beta-cell function under physiological and pathophysiological conditions. *Nat Commun* 8: 14295, 2017.