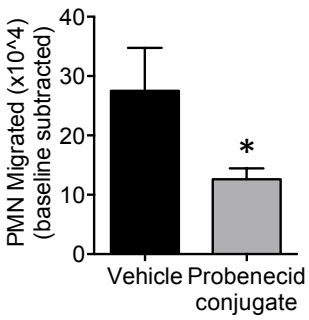
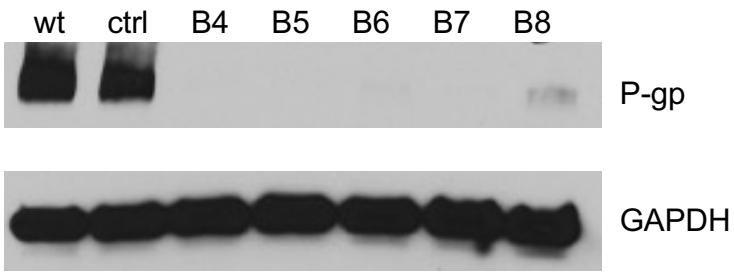


Supplementary Figure 1. Probenecid conjugate effectively inhibits HXA_3-induced neutrophil migration. HCT-8 epithelial monolayers were grown on inverted transwell inserts, and pretreated for 1 hour with 100 μM probenecid conjugate. Monolayers were then infected apically with *S*. typhimurium strain SL1344 for 1 hr, then washed and inverted. Neutrophils were added to the top of the well (basolateral side) and allowed to migrate for two hours, followed by quantitation of mpo as described. Data shown are mean +/- S.D. of a representative of 2 independent experiments; N = 6 per each experiment. *=$p<0.05$ by Student *t* test..

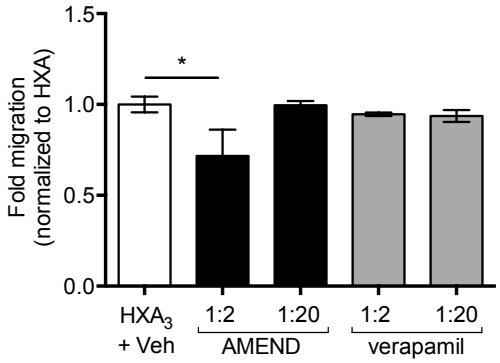


Supplementary Figure 2. sHRNA knockdown of P-glycoprotein in T84 cells. T84 cells were infected with lentiviral particles carrying shRNA constructs targeting *mdr1a*. Cell lines B4-B8 contain independent targeting constructs and are compared to non-transfected cells (lane 1) and cells transfected with control shRNA (lane 2). Lysates were separated by gel electrophoreseis, transferred to nitrocellulose and probed for P-gp (a) or GAPDH loading control (b). As all constructs induced significant knockdown of P-gp expression by western blot, clones B4 and B5 were chosen for further analysis.

Supplementary Table 1. Bakerbond® SepPak extraction columns used to screen AMEND activity

SepPak [1]SPE™ column (functional surface)	Retention of AMEND activity
Octadecyl (C_{18})	++++
Octyl (C_8)	++++
Phenyl (C_6H_5)	+
Amino (NH_2)	-
Cyano (CN)	+
Silica Gel (SiOH)	+
Florisil® (Mg_2SiO_3)	+
Carboxylic Acid (COO^-)	+
Aromatic Sulfonic Acid ($C_6H_5SO_3H$)	++
H_2O-phobic SC-DVB (SO_3)	-
Narc™-2	++++
Quaternary Amine (NH_4^+)	-
Diol (COHCOH)	+

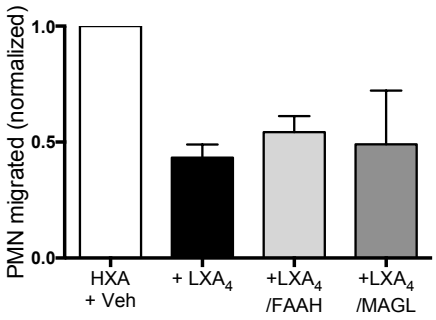
[1]SPE™ = 10 μm solid-phase extraction system from J.T.Baker.



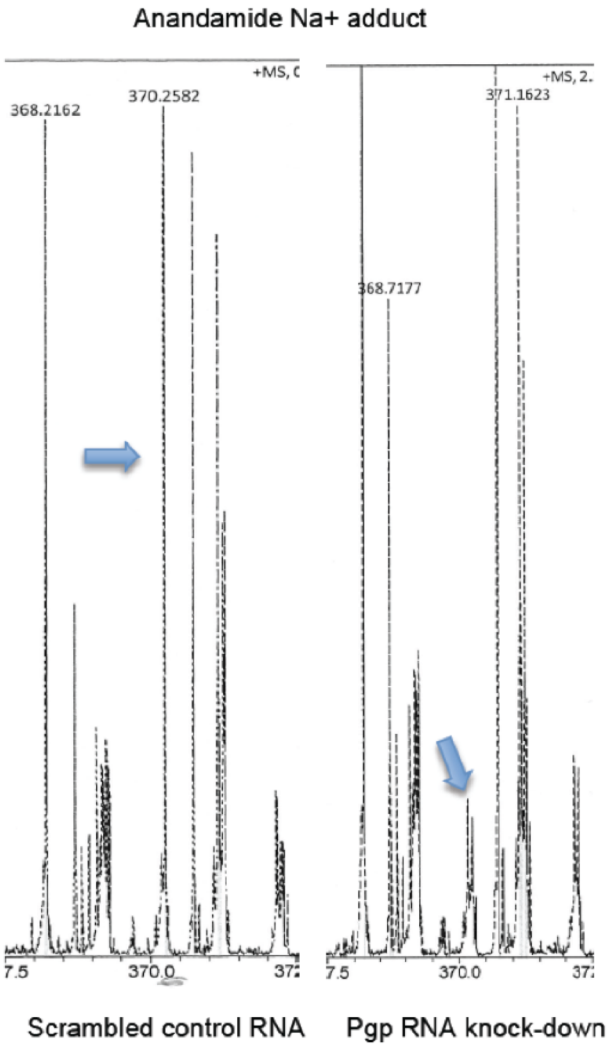
Supplementary Figure 3. AMEND secretion is P-gp dependent. T84 epithelial cells were treated with vehicle or with 40 μM verapamil hydrochloride, a P-gp inhibitor. Enriched supernatant fractions were prepared and evaluated for AMEND inhibitory activity in the cell-free migration assay. Pre-treatment with verapamil inhibited secretion of the AMEND inhibitory compounds. Data shown are mean +/- SD of a representative of two independent experiments. *=$p<0.05$ by one-way ANOVA.

GPCR	Assay Mode	% Activity	Assay Mode	Agonist used	% Inhibition
CB2 (CNR2)	**Agonist**	18%	Antagonist	CP55940	-15%
ADORA3	**Agonist**	10%	Antagonist	2-Cl-IB-MECA	-8%
CXCR4	**Agonist**	16%	Antagonist	CXCL12	-2%
P2RY1	**Agonist**	11%	Antagonist	2-methylthio-ADP	-1%
CXCR3	**Agonist**	6%	Antagonist	CXCL11	-18%
MTNR1B	**Agonist**	6%	Antagonist	2-Iodomelatonin	-14%
GPR120	**Agonist**	9%	Antagonist	GW9508	-13%
ADCYAP1R1	**Agonist**	4%	Antagonist	PACAP-27	-17%
AGTRL1	**Agonist**	4%	Antagonist	Apelin-13	-12%
AVPR2	**Agonist**	5%	Antagonist	Vasopressin	-10%
C5L2	**Agonist**	3%	Antagonist	Complement C5a	-17%
CHRM1	**Agonist**	-1%	Antagonist	Acetylcholine	-16%
CRHR2	**Agonist**	3%	Antagonist	Sauvagine	-15%
DRD3	**Agonist**	0%	Antagonist	Dopamine	-15%
EDNRA	**Agonist**	3%	Antagonist	Endothelin I	-10%
OXTR	**Agonist**	3%	Antagonist	Oxytocin	-10%
PPYR1	**Agonist**	1%	Antagonist	Pancreatic Polypeptide	-11%
CALCR-RAMP3	**Agonist**	10%	Antagonist	Calcitonin	31%
EDG8	**Agonist**	14%	Antagonist	S-1-P	18%
CALCR-RAMP2	**Agonist**	6%	Antagonist	Calcitonin	17%
MTNR1A	**Agonist**	11%	Antagonist	2-Iodomelatonin	14%
CCKBR	**Agonist**	0%	Antagonist	CCK-8	22%
CXCR5	**Agonist**	4%	Antagonist	CXCL13	19%
PTGER2	**Agonist**	2%	Antagonist	Prostaglandin E2	19%
HTR1B	**Agonist**	-1%	Antagonist	Serotonin / 5-HT	17%
OPRD1	**Agonist**	-1%	Antagonist	DADLE	15%
GPR92	**Agonist**	2%	Antagonist	Oleoyl LPA	15%
HTR1A	**Agonist**	4%	Antagonist	Serotonin / 5-HT	15%
PTGIR	**Agonist**	-1%	Antagonist	Beraprost	14%
NPBWR1	**Agonist**	0%	Antagonist	Neuropeptide W23	14%
TBXA2R	**Agonist**	-2%	Antagonist	I-BOP	14%
OPRL1	**Agonist**	2%	Antagonist	Orphanin FQ	12%
HTR1E	**Agonist**	1%	Antagonist	Serotonin / 5-HT	12%
DRD4	**Agonist**	1%	Antagonist	Dopamine	12%
HTR1F	**Agonist**	3%	Antagonist	Serotonin / 5-HT	12%
P2RY2	**Agonist**	-2%	Antagonist	UTP	12%
EDG7	**Agonist**	0%	Antagonist	Oleoyl LPA	12%
PTGER3	**Agonist**	0%	Antagonist	Prostaglandin E2	12%
FPR1	**Agonist**	3%	Antagonist	WKYMVm-NH2	11%
CXCR6	**Agonist**	7%	Antagonist	CXCL16	11%
AVPR1B	**Agonist**	2%	Antagonist	Vasopressin	11%
GPR119	**Agonist**	6%	Antagonist	Oleoyl Ethanolamide	10%
CRTH2	**Agonist**	2%	Antagonist	PGD2	10%
NPY1R	**Agonist**	1%	Antagonist	Peptide YY	10%
HRH3	**Agonist**	2%	Antagonist	R-a methylhistamine	10%
UTR2	**Agonist**	0%	Antagonist	Urotensin II	10%

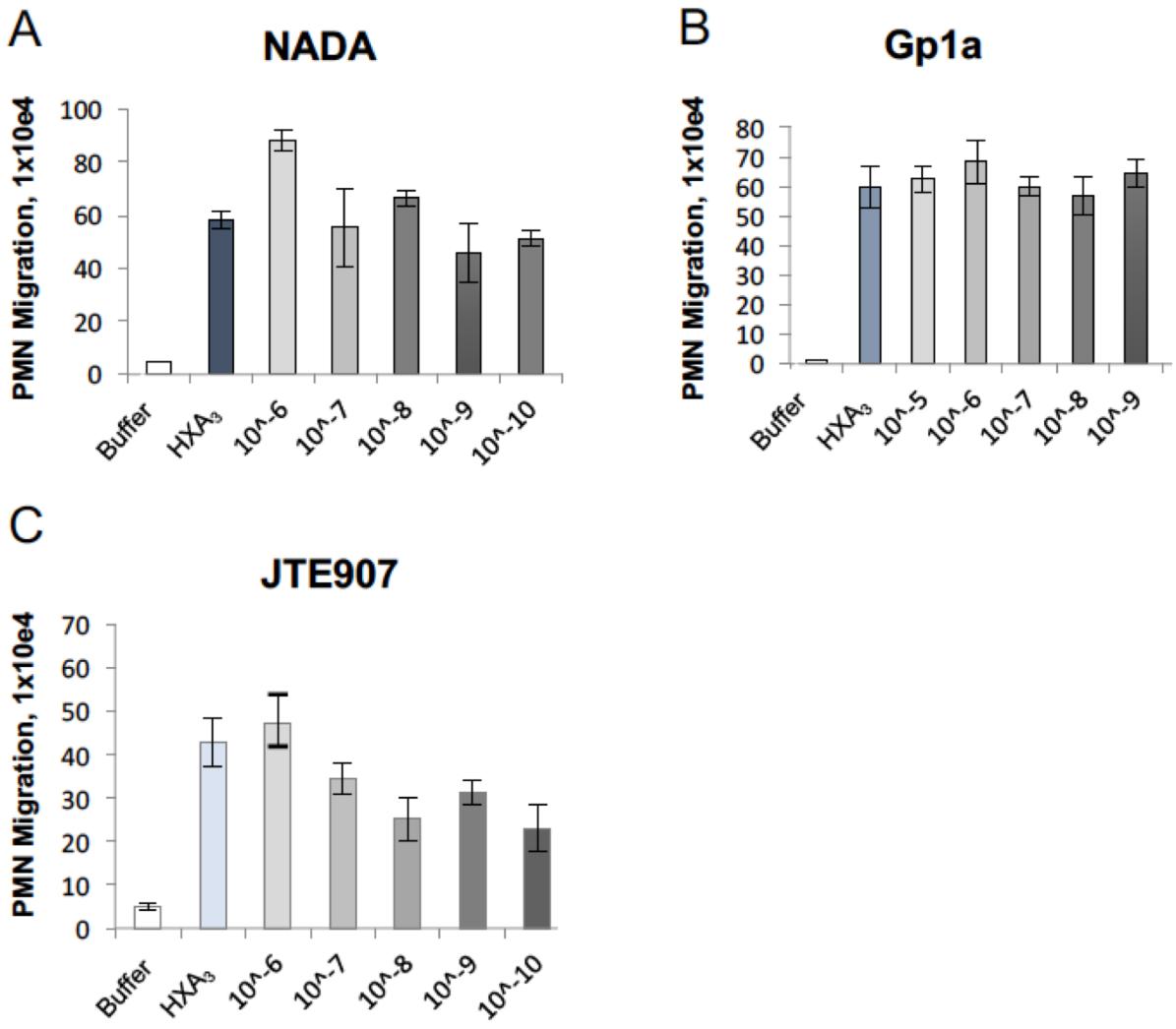
Supplementary Table 2. Results of GPCR activity screen with enriched AMEND. Large scale preparations of AMEND from T84 cells were prepared and screened in the DiscoveRx GPCR Beta-arrestin activation assay in both agonist and antagonist mode. Percent activity is shown for both modes; for antagonist activity, percent inhibition of GPCR activation by the listed agonist is shown. Rows highlighted in dark orange and purple show GPCRs for which AMEND displayed agonist activity above an arbitrary threshold of 10%. GPCRs highlighted in light orange and blue had negative inhibition percentages but no corresponding activity in the agonist assay. Rows highlighted in yellow contain GPCRs against which AMEND displayed both agonist and antagonist activity, which may reflect independent components of this mixture. Rows shown in green contain GPCRs against which AMEND displayed antagonist activity above the arbitrary cutoff of 10%.



Supplementary Figure 4. FAAH and MAGL assays are endocannabinoid specific and do not affect the inhibitory activity of Lipoxin A4. Experiments were performed and normalized as in Figure 2C. Lipoxin was used at 10 nM. Data are mean +/- S.E.M. combined from two independent experiments.



Supplementary Figure 5. P-gp dependent anandamide Na^+ adduct secretion from T84 cells. Enriched T84 cell supernantants from control or B4-mdr1 knockdown cell lines were subjected to electrospray ionization mass spectrometry. Arrow indicates peak of anandamide Na^+ adduct.



Supplementary Figure 6. Assessment of CB2 activity to imposed gradients of HXA_3 preparations. Dose responses to NADA (A; 10^{-6}-10^{-10}M), GP1a (B; 10^{-6}-10^{-9}M), and JTE 907 (C; 10^{-6}-10^{-10}M) were evaluated for HXA_3 inhibitory activity in the cell-free migration assay. Data shown are mean +/- SD of a representative of two independent experiments. Dose response values of PMN transepithelial migration were not statistically different when compared to the PMN transepithelial migration induced by imposed gradients of HXA_3 itself as determined by one way ANOVA.

		Average*	Std Error	% of Control
Anandamide	control	3.34	0.56	
(AEA)	B4-mdr1a	1.96	0.06	59
	B5-mdr1a	2.04	0.13	61
Palmitoyl ethanolamide	control	27.20	5.14	
(PEA)	B4-mdr1a	10.87	1.50	40
	B5-mdr1a	2.20	0.26	8
Oleoyl ethanolamide	control	5.97	0.98	
(OEA)	B4-mdr1a	2.53	0.12	42
	B5-mdr1a	2.57	0.21	43
2-Arachidonoyl Glycerol	control	35.53	6.35	
(2-AG)	B4-mdr1a	32.17	1.37	91
	B5-mdr1a	29.47	1.19	83
Noladin ether	control	33.43	13.32	
	B4-mdr1a	154.03	3.47	461
	B5-mdr1a	56.23	1.32	168
N-Arachidonoyl Dopamine	control	0.00	0.00	
	B4-mdr1a	1.97	0.06	N/A
	B5-mdr1a	1.90	0.00	N/A
*(normalized to anandamide-d8 standard, which was at 1µg/µL)				

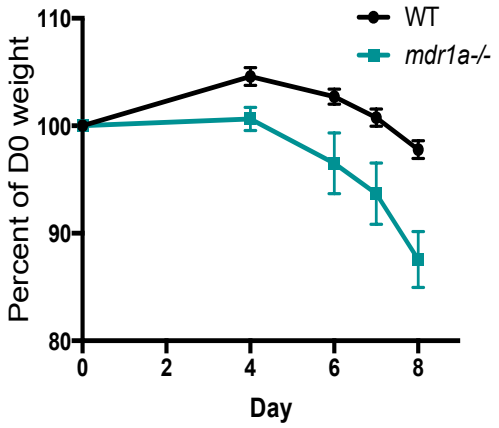
Supplementary Table 3. Endocannabinoids are secreted from epithelial cells via P-glycoprotein. Semi-quantitative MS analysis was performed to compare enriched AMEND preparations from control T84 cells to those with shRNA-mediated P-gp knockdown (*B4-mdr1a* and *B5-mdr1a)*. Relative abundance of each compound was calculated by comparison with measured intensity of anandamide-d8 standard; while this is only accurately quantitative for anandamide itself, it allowed for comparison of relative units between samples for the remaining compounds.

Compound Name	Assay Format	Assay Target	Result Type	RC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response
CP55940	Agonist	CNR2	EC50	0.003	1.83	-4.71	98.2	99.3
2-arachidonoyl glycerol (2AG)	Agonist	CNR2	EC50	0.281	0.72	-6.62	139.3	142.3
AEA + aLEA	Agonist	CNR2	EC50	>1				5.63
AEA + aLEA + OEA	Agonist	CNR2	EC50	>1				4.51
AEA + OEA	Agonist	CNR2	EC50	>1				0
aLEA + OEA	Agonist	CNR2	EC50	>1				1.53
alpha-linolenoyl ethanolamide (aLEA)	Agonist	CNR2	EC50	>15.5				22.4
AMEND	Agonist	CNR2	EC50	>1				4.8
Anandamide (AEA)	Agonist	CNR2	EC50	2.22	0.88	-1.03	70	64.1
oleoyl ethanolamide (OEA)	Agonist	CNR2	EC50	>3.08				10.2

Supplementary Table 4. CB2 (CNR2) agonist effects of synthetic AEA, OEA, and α-LEA. The synthetic compounds AEA, OEA, and α-LEA were prepared individually, and in combination, and examined in the DiscoveRx GPCR Beta-arrestin activation assay in the agonist mode targeted to CNR2. Full dose curves for EC50 analysis were carried out across a dose range of 10μM-1fM. Compound CP55940 is a potent, non-selective cannabinoid receptor agonist, and is used as standard positive control.

		Average EC content*:	
	genotype	wt	mdr1a-/-
Anandamide (AEA)		76.0	44.5
Palmitoyl ethanolamine (PEA)		25.5	30.8
Oleoyl ethanolamide (OEA)		104.0	68.6
2-Arachidonoyl Glycerol (2-AG)		76.6	122.0
Noladin ether (NE)		68.3	104.8
N-Arachidonoyl Dopamine		8.4	21.3
*normalized to anandamide-d8, which was at 1µg/1µL			

Supplementary Table 5. Endocannabinoids are differentially present in wild-type vs. P-gp deficient mice. Semi-quantitative MS analysis was performed as described in Fig. 3 legend, and data analysis was performed as in Supplementary table 3. Data shown are, for each genotype, from six groups of three mice each pooled prior to MS.



Supplementary Figure 7. DSS colitis in P-gp deficient mice.

FVB wild-type and *mdr1a*-/- mice were treated with DSS as in Fig. 1. n=5 mice per group.