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Supplemental information

Differential translocation of bacteriophages across the intestinal barrier in health and Crohn's disease

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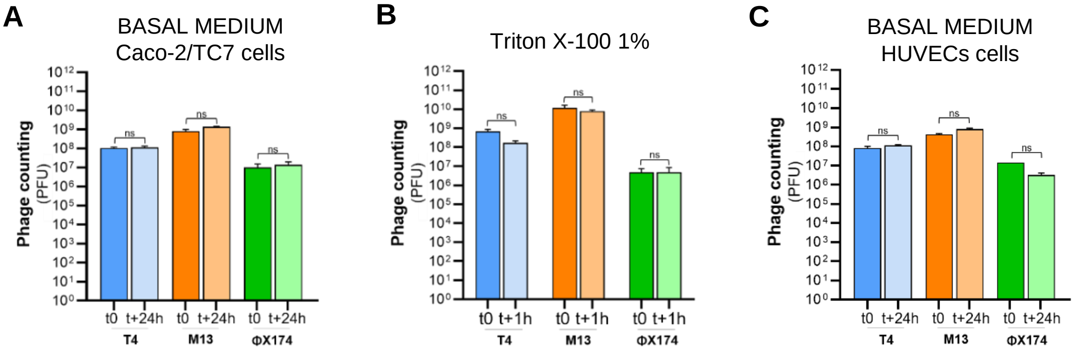
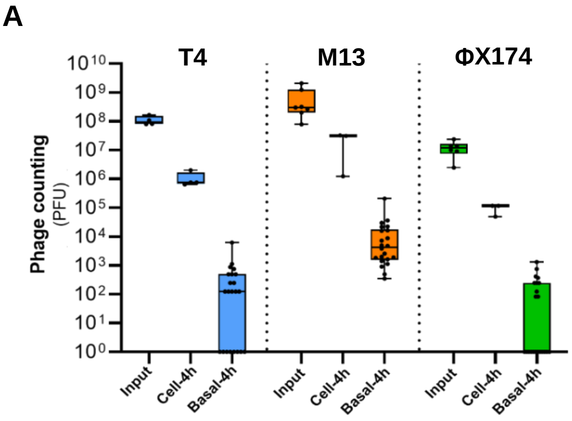
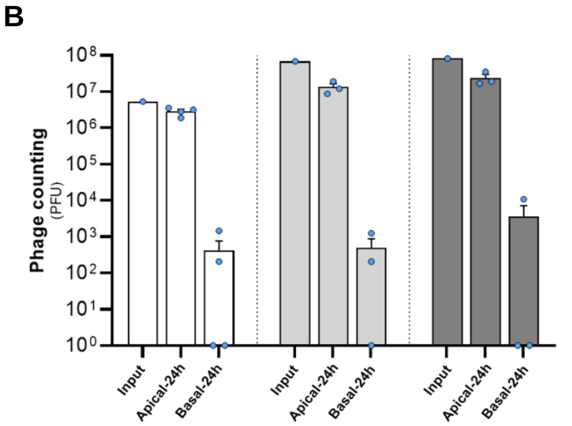


Figure S1 : Cell culture medium and Triton X-100 do not impact phage viability

(A) Titration of T4, M13, and ΦX174 phages immediately after suspension (t0) and 24 h after incubation in Caco-2/TC7 culture medium. **(B)** Titration of T4, M13, and ΦX174 phages immediately after suspension (t0) and following a 1 h incubation with 1% Triton X-100 (t+1h), used in this study to lyse eukaryotic cells. **(C)** Titration of T4, M13, and ΦX174 phages immediately after suspension (t0) and 24 h after incubation in HUVEC culture medium (t+24h). All experiments were performed in triplicates [n = 3]. Data are represented as mean ± SEM.



Phage	**(Cell / Input)** Ratio in %	**(Basal / Input)** Ratio in %
T4	1.063 (± 0.5334)	0.0005231 (± 0.001228)
M13	1.547 (± 0.04163)	0.005495 (± 0.01393)
ΦX174	0.93 (± 0.5048)	0.003295 (± 0.007477)
p-value	ns	M13 *vs.* T4 : p=0.0011**



Input (PFU)	**Apical after 24h** (PFU)	**Basal after 24h** (PFU)	**(Basal / Input)** Ratio in %
5.38 x 10^6	2.86 x 10^6 (±7.22 x 10^5)	4.18 x 10^2 (±7.02 x 10^2)	0.007752 (±0.01305)
6.88 x 10^7	1,34 x 10^7 (±5.35 x 10^6)	4.86 x 10^2 (±6.69 x 10^2)	0.0007071 (±0.0009741)
8.25x 10^7	2.4 x 10^7 (±1.01x 10^7)	3.6 x 10^3 (±6.24 x 10^3)	0.004377 (±0.00758)

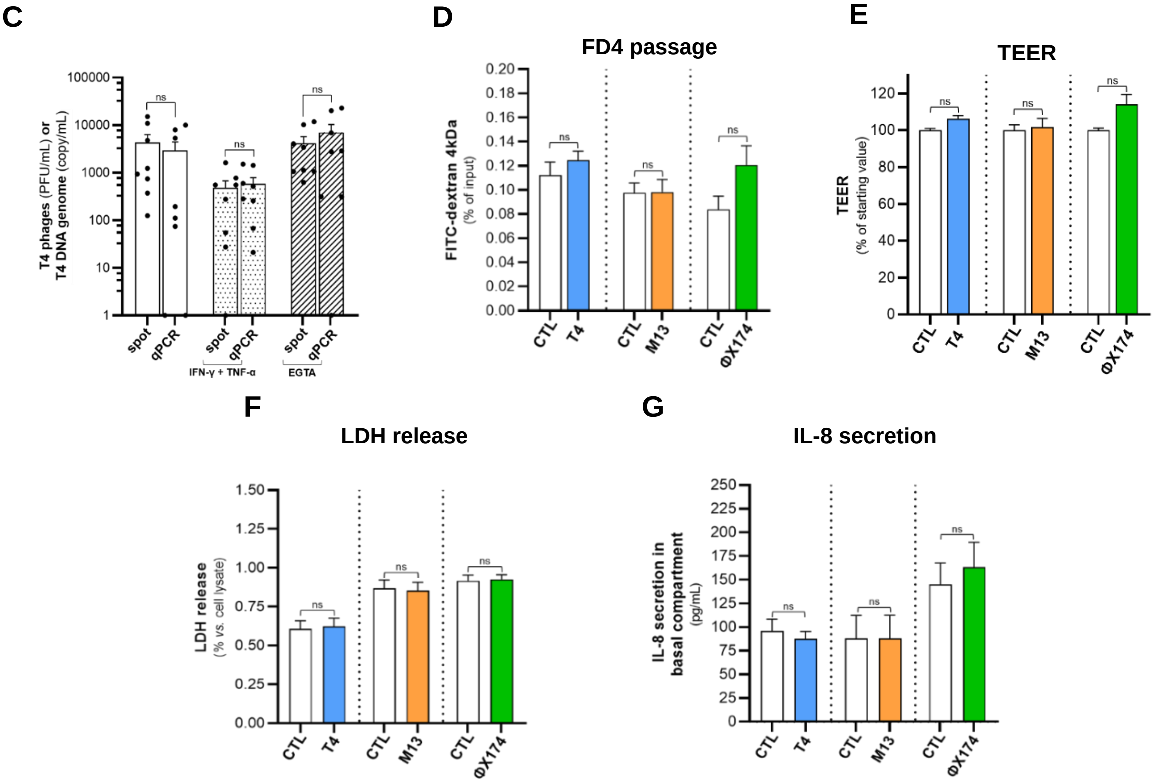


Figure S2 : Internalization, translocation and functionality of phages crossing Caco-2/TC7 intestinal epithelial cells and absence of impact on permeability, cytotoxicity, or inflammation (legend on following page)

Figure S2 : Internalization, translocation and functionality of phages crossing Caco-2/TC7 intestinal epithelial cells and absence of impact on permeability, cytotoxicity, or inflammation

(A) Titration of T4, M13, and ΦX174 phages associated to Caco-2/TC7 cells (cell) and translocated phages (basal) after 4 h of incubation in the apical compartment. The input refers to the phages initially added to the apical compartment. The table displays the percentage of phages associated to and translocated across Caco-2/TC7, calculated relative to the input. A minimum of four independent experiments [n ≥ 4] were performed. **(B)** Titration of T4 phages in the apical and basal compartments after 24 h of incubation following their addition to the apical compartment. The input refers to the number of phages initially added to the apical compartment. The table shows the initial input of T4 phages, the number of phages recovered from both the apical and basal compartments after 24 h, and the percentage of phages translocating across the Caco-2/TC7 monolayer, calculated relative to the input. One independent experiment was conducted in triplicate or quadruplicate. **(C)** Titration of T4 phages in the basal compartment after 24 h of apical incubation, measured by plaque assays (spot) and qPCR (a calibration curve using T4 DNA was used to determine the genome copy/mL) under both physiological, or compromised barrier conditions (IFN-γ + TNF-α, and EGTA). Two independent experiments were conducted in quadruplicate. **(D)** FITC-Dextran 4 kDa (FD4) passage from the apical to the basal compartment during 24 h in the presence of the indicated phage compared to control (CTL) condition without phage. **(E)** Measure of transepithelial electrical resistance (TEER) after 24 h of incubation with medium containing no phages (CTL) or the indicated phages. Values are expressed as a percentage relative to the starting value for each well and then normalized to the control condition. **(F)** Release of lactate dehydrogenase (LDH) by the cells in the apical compartment, after 24 h of incubation with medium containing no phages (CTL) or the indicated phages. Values are expressed as the percentage of LDH measured in the whole cell lysate. **(G)** Secretion of pro-inflammatory cytokine IL-8 by the cells within the basal compartment after 24 h of incubation with medium containing no phages (CTL) or the indicated phages. For all the experiments, a minimum of four independent experiments [n ≥ 4] were performed. All data are represented as mean ± SEM.

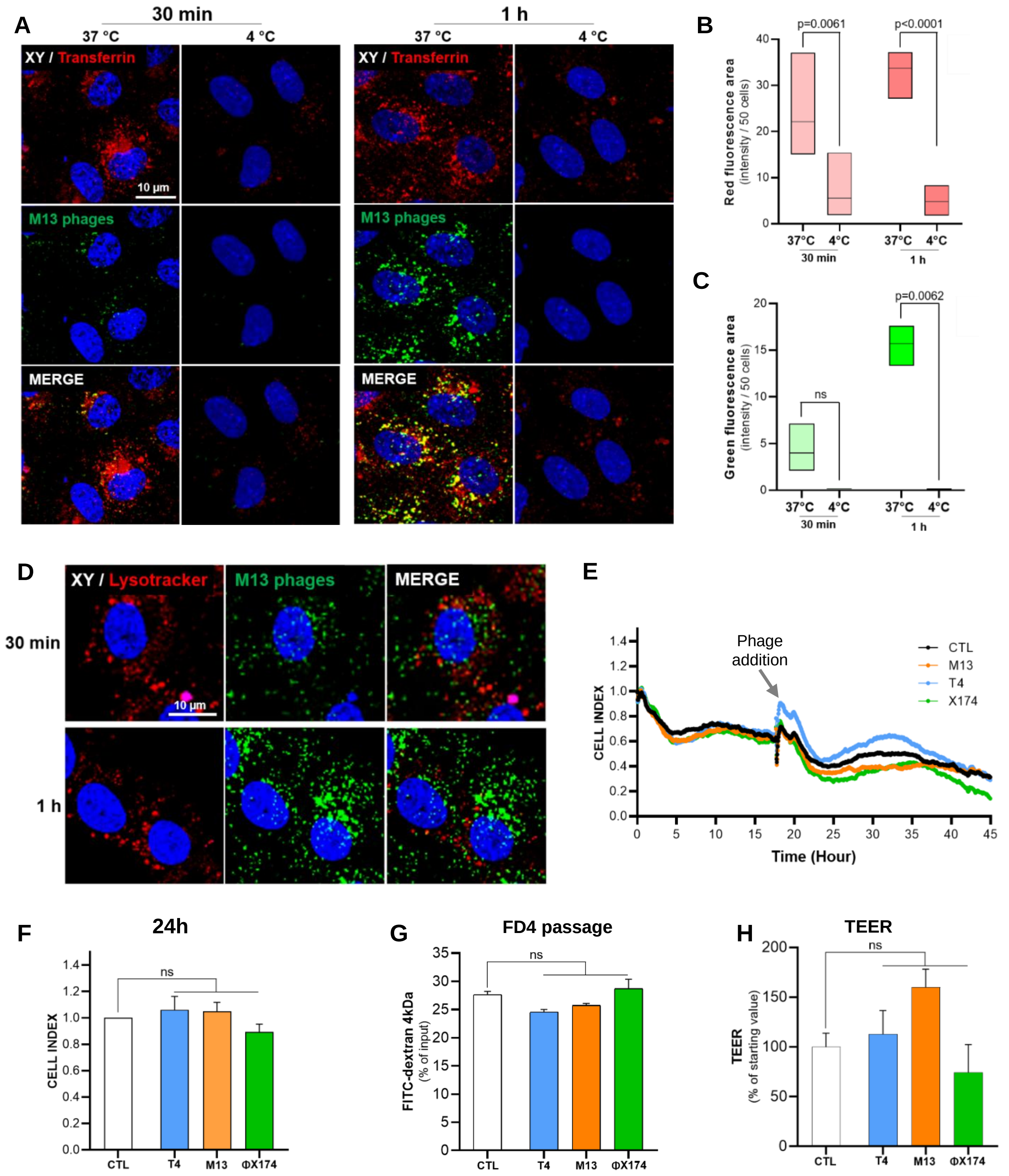


Figure S3 : Visualization of phages in the endocytic pathway in HUVECs cells following short incubation periods and phage impact on cellular permeability
(A) Images of CF488-labeled M13 phages and Cy3-labeled transferrin in HUVECs after 30 min and 1 h, at 37°C, and 4°C. Quantification of the **(B)** red fluorescence area (transferrin), and **(C)** green fluorescence area (M13 phages) in HUVECs following 30 min and 1 h, at 37°C, and 4°C, as shown in panel (A). Data represent mean values obtained from analysis of 5 randomly selected images. **(D)** Images of CF488-labeled M13 phages and Lysotracker Deep Red in HUVECs after 30 min and 1 h. For A and D: scale bar, 10 µm. **(E)** Representative plot of real-time permeability (cell index) of HUVECs in presence of the indicated phage relative to control (CTL) condition using xCELLigence system. **(F)** Cell index values obtained after 24 h in presence of the indicated phage relative to control (CTL) condition without phage after real time measure of HUVECs permeability, shown in panel (E). **(G)** FITC-Dextran 4 kDa (FD4) passage from the apical to the basal compartment after 24 h in presence of the indicated phage relative to control (CTL) condition. **(H)** Measure of transendothelial electrical resistance (TEER) after 24 h of incubation with medium containing no phages (CTL) or the indicated phages. Values are expressed as the percentage relative to the starting value for each well and then normalized to the control condition. For (E) to (H), three independent experiments [n=3] were performed. Data are represented as mean ± SEM.

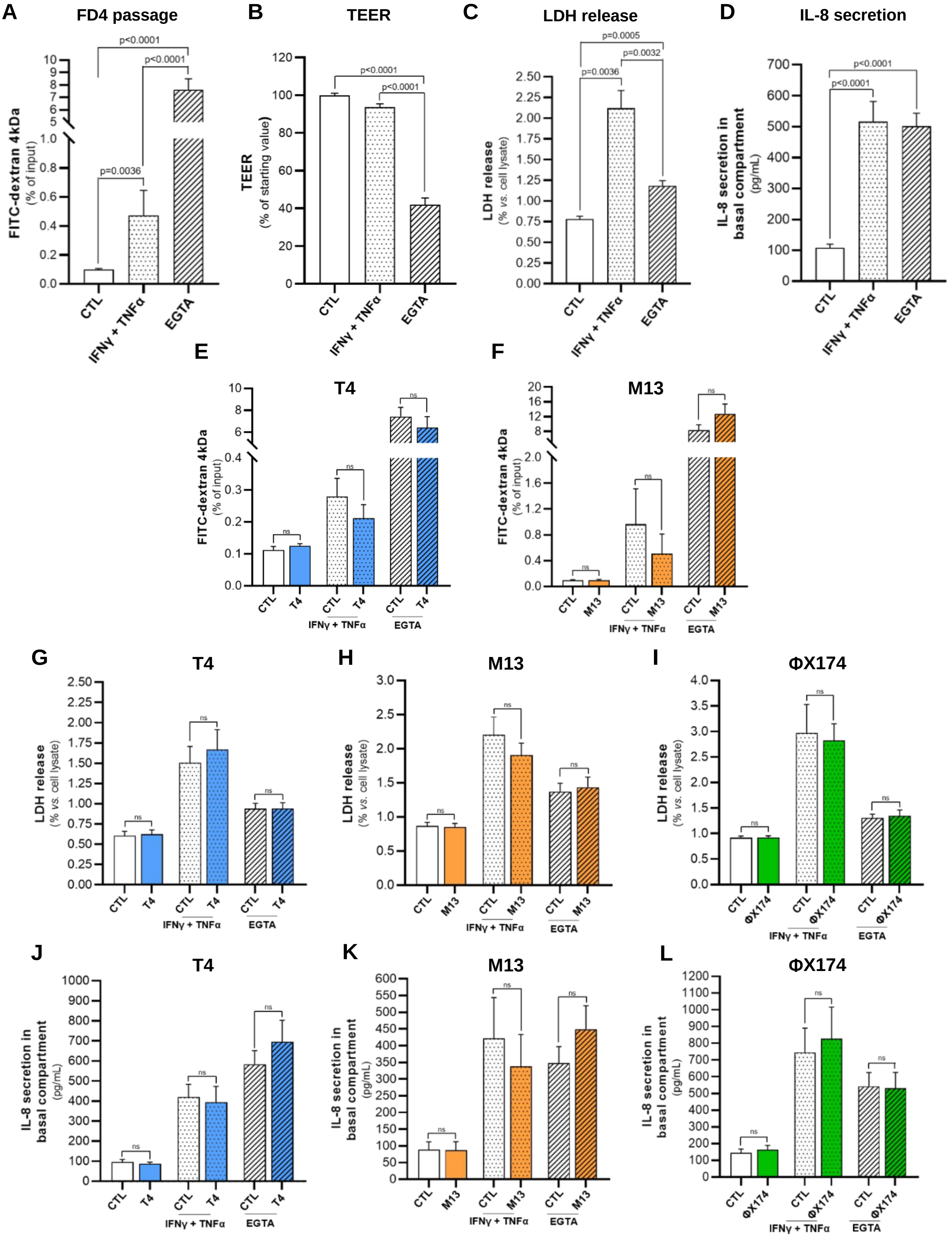


Figure S4 : Phages do not induce hyperpermeability, cytotoxicity, or inflammation in Caco-2/TC7 cells under compromised barrier conditions (legend on following page)

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Caco-2/TC7 were treated with IFNγ + TNFα in the basal compartment during 48 h, or with EGTA in the apical compartment during 26 h. **(A)** FITC-Dextran 4 kDa (FD4) passage from the apical to the basal compartment (24 h of FD4 flux). **(B)** Measure of transepithelial electrical resistance (TEER). Values are expressed as a percentage relative to the starting value for each well and then normalized to the control condition. **(C)** Release of lactate dehydrogenase (LDH) from cells (expressed as the percentage of LDH measured in the whole cell lysate). **(D)** Secretion of pro-inflammatory cytokine IL-8 by the cells into the basal compartment. For (A) to (D), these data were pooled from control groups used in independent experiments involving T4, M13, and ΦX174 phages, with at least 3 independent experiments conducted for each [n ≥ 9]. Data are represented as mean ± SEM. **(E-F)** FITC-Dextran 4 kDa (FD4) passage from the apical to the basal compartment during 24 h in presence of **(E)** T4, or **(F)** M13 phages relative to control (CTL) condition, under healthy, and compromised barrier conditions (IFNγ + TNFα, or EGTA). (**G-I**) Release of lactate dehydrogenase (LDH) from cells after 24 h in presence of **(G)** T4, **(H)** M13, or **(I)** ΦX174 phages relative to control (CTL) condition, under healthy, and compromised barrier conditions (IFNγ + TNFα, or EGTA). (**J-L**) Secretion of pro-inflammatory cytokine IL-8 by the cells into the basal compartment after 24 h in presence of **(J)** T4, **(K)** M13, or **(L)** ΦX174 phages relative to control (CTL) condition, under healthy, and compromised barrier conditions (IFNγ + TNFα, or EGTA). For (E) to (L), a minimum of three independent experiments [n ≥ 3] were performed. Data are represented as mean ± SEM.

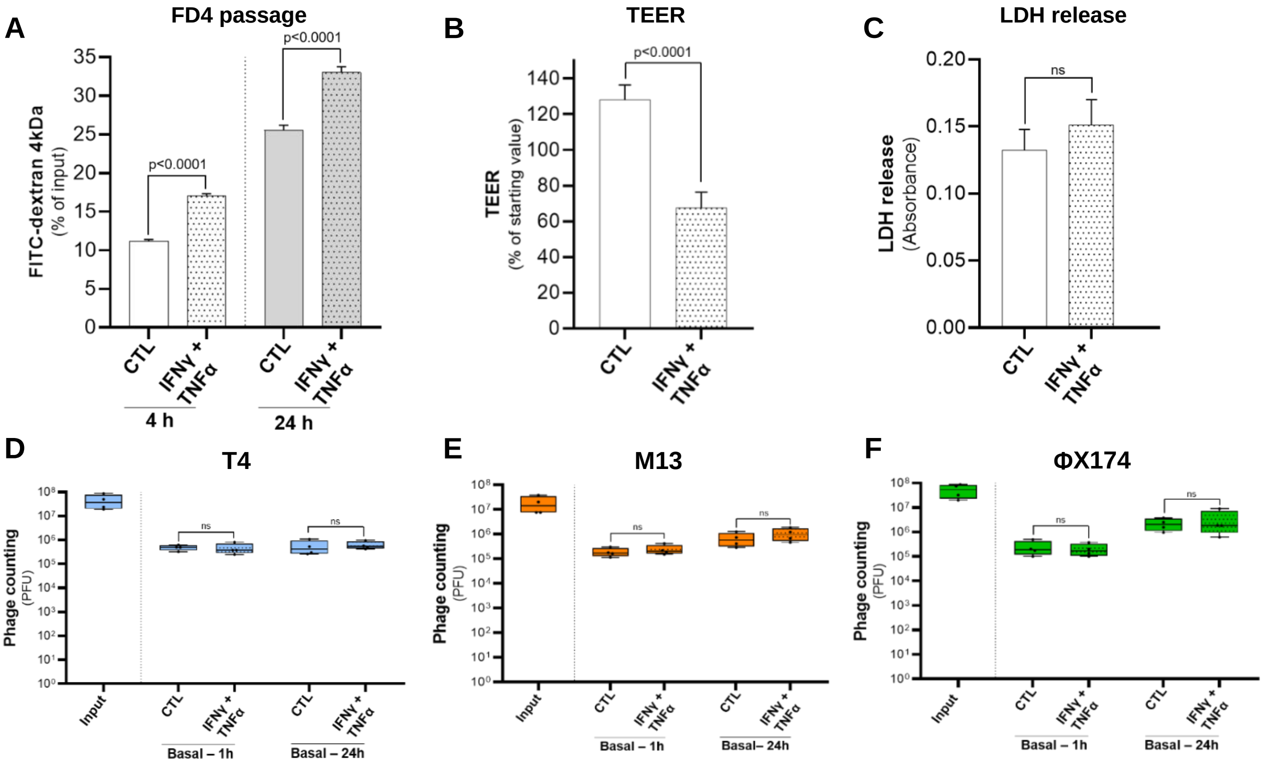

Figure S5 : Phage translocation is not impacted by inflammatory condition in HUVECs cells
(A) FITC-Dextran 4 kDa (FD4) passage from the apical to the basal compartment after 22 h (4 h of FD4 flux), and 42 h (4 h of FD4 flux) of IFNγ + TNFα treatment. **(B)** Measure of transendothelial electrical resistance (TEER) after 18 h of IFNγ + TNFα treatment. Values are expressed as a percentage relative to the starting value for each well and then normalized to the control condition. **(C)** Release of lactate dehydrogenase (LDH) from cells after 42 h of IFNγ + TNFα treatment. For (A), (B), (C), experiments were pooled from control groups used in independent experiments involving T4, M13, and ΦX174 phages [n = 9]. **(D-F)** Titration of **(D)** T4, **(E)** M13, and **(F)** ΦX174 phages in the basal compartment (basal) after 24 h of incubation. The input represents the phages added in the apical compartment at the beginning of the experiment. Conditions include healthy barrier condition (CTL) relative to compromised barriers conditions induced by IFNγ and TNFα. For (D), (E), (F), three independent experiments [n = 3] were performed. Data are represented as mean ± SEM.

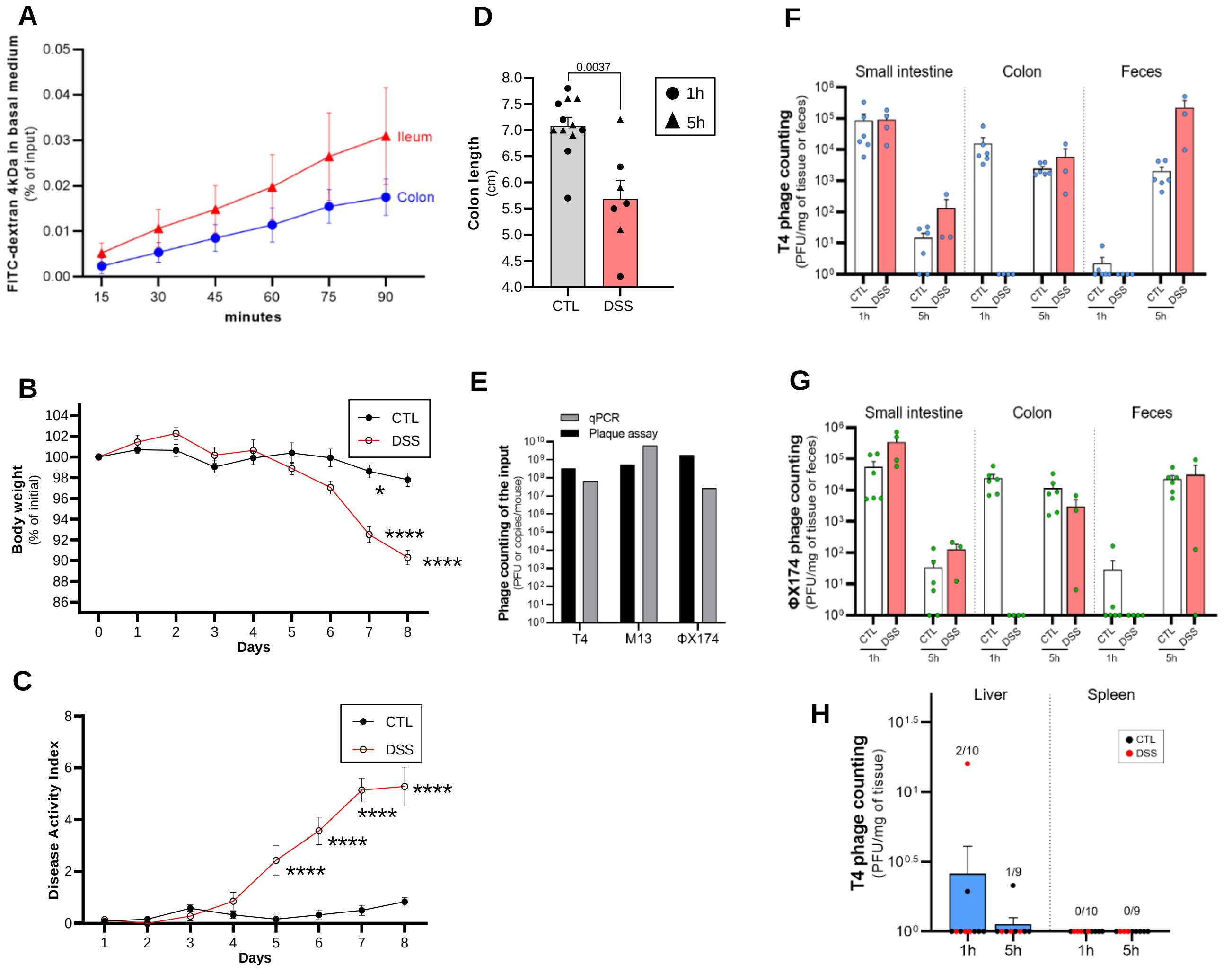

Figure S6 : Phage translocation in mice models
(A) Level of FITC-Dextran 4kDa (FD4) in basal medium over 90 min for ileum (red curve) and colon (blue curve) tissues from healthy mice in the *ex vivo* Ussing chamber model. For each intestinal segment, six replicates were performed. Data are represented as the percentage of fluorescent tracer added in the apical chamber over time and as mean ± SEM. **(B)** Bodyweight changes in control and DSS-treated mice throughout the *in vivo* experiment. **(C)** Disease Activity Index (DAI) scores in control and DSS-treated mice. **(D)** Colon length measured at day 9 in both groups. Symbols indicate the mice sacrified either 1 h (circles) or 5 h (triangles) after phage cocktail oral gavage. **(E)** Titration of T4, M13 and ΦX174 phages in the input sample by plaque assays and qPCR. **(F-G)** Titration of T4 **(F)** or ΦX174 **(G)** phages in the small intestine, colon and feces of control and DSS-treated mice at 1h and 5 h post-oral administration. **(H)** Titration of T4 phages in the liver and spleen of control and DSS-treated mice, 1h and 5 h after oral administration. Numbers indicate mice positive for phage detection out of the total tested. Phage quantification in (F), (G) and (H) was performed by plaque assays. Data are represented as mean ± SEM ; n = 6 mice per control group (1h and 5h post-gavage) and n = 3 or 4 mice per DSS-treated group (1h and 5h post-gavage).

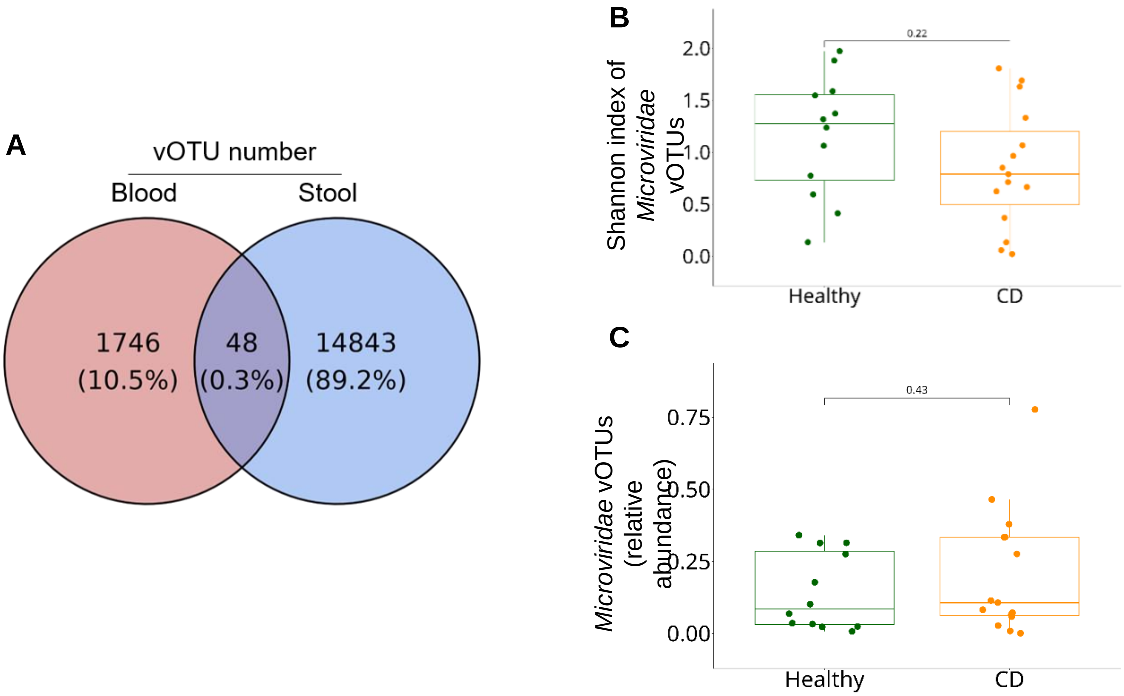

Figure S7 : vOTUs identified in blood and fecal samples from all participants and analysis of fecal *Microviridae.*
(A) Venn diagram of the vOTUs identified in blood and fecal samples from all participants. The final vOTU dataset was obtained by excluding all sequences detected in the negative controls and retaining only those present in at least one biological sample across the 29 participants in the cohort. **(B)** Alpha diversity of *Microviridae* in fecal samples, quantified using Shannon index, and compared between patients with Crohn’s disease (CD) and healthy subjects. (**C)** Overall relative abundance of *Microviridae* vOTUs in fecal samples, separated by disease status.

Table S1 : Phages do not induce hyperpermeability of Caco-2/TC7 cells in compromised barrier conditions. Measure of transepithelial electrical resistance (TEER) after 24 h of incubation with medium containing no phages (CTL), or the indicated phages, under healthy, and compromised barrier conditions (IFNγ + TNFα or EGTA treatment). A minimum of three independent experiments [n ≥ 3] were performed. Values are expressed as a percentage relative to the starting value for each well, and then compared to the control condition.

TEER (% of starting value)						
	CTL	**T4**	**CTL**	**M13**	**CTL**	**ΦX174**
	100 (± 3.643)	106.2 (± 6.758)	100 (± 9.452)	101.7 (± 14.86)	100 (± 3.649)	114.3 (± 14.49)
p-value	p>0.99		p>0.99		p>0.99	
IFNγ + TNFα	97.27 (± 7.581)	105.8 (± 16.78)	90.77 (± 10.82)	89.99 (± 7.991)	91.35 (± 10.91)	92.82 (± 7.656)
p-value	p>0.99		p>0.99		p>0.99	
EGTA	48.04 (± 13.02)	58.29 (± 20.81)	31.06 (± 16.16)	22.72 (± 11.42)	45.9 (± 28.30)	46.80 (± 30.25)
p-value	p>0.99		p>0.99		p>0.99	

Table S2 : Percentage of translocated phages across intestinal tissue in healthy mice.
Percentage of phages that translocated after 90 min of addition in the apical compartment using the Ussing chamber system, calculated relative to the input. For each phage, three ileum and colon tissue samples taken from two or three individual mice were analyzed.

Phage	**Ileum (Basal / Input)** Ratio in %	**Colon (Basal / Input)** Ratio in %
T4	0.0008730 (± 0.001496)	0.0004333 (± 0.0005859)
M13	9.348e-005 (± 0.0001615)	1.207e-005 (± 0.00001987)
ΦX174	0.0004167 (± 0.0007217)	0
p-value	ns	ns

Table S3 : Detailed information about the participants included in the study.
“CD” indicates patient with Crohn’s disease and “H” indicates healthy subject

Participant	Sampling date	Sex	Age	Age at diagnosis	Localisation (Montreal)	Flare/ Remission	C-reactive protein (mg/L)	Harvey-Bradshaw Index	Smoking	Surgery	Antibiotic (last month)	Undergoing treatment
CD1	2020	Female	53	37	L2	Remission	1	2	Past	No	No	Yes
CD2	2020	Female	40	NA	E3	Remission	1	NA	Past	No	No	No
CD3	2020	Female	38	NA	L3L4	Flare	1	4	Never	No	No	No
CD4	2020	Male	44	NA	L3L4	Remission	0.6	1	Never	No	No	No
CD5	2020	Female	40	20	L3	Remission	5	2	Past	No	No	Yes
CD6	2020	Male	60	55	L3	Flare	5	7	Never	No	No	Yes
CD7	2019	Female	26	16	L3	Flare	89.9	NA	Past	No	No	No
CD8	2020	Male	42	13	L3	Flare	6.3	3	Never	No	No	Yes
CD9	2020	Male	47	12	L2	Remission	7.8	0	Past	No	No	No
CD10	2018	Female	50	47	L1	Remission	1	NA	Current	No	No	Yes
CD11	2020	Female	33	19	L3	Flare	5.3	NA	Past	No	No	No
CD12	2020	Male	22	20	L3	Remission	5	3	Never	No	No	Yes
CD13	2020	Male	56	52	L1	Flare	8.7	7	Past	No	No	Yes
CD14	2020	Female	56	20	L3	Remission	1	2	Never	No	No	Yes
CD15	2020	Female	37	27	L3	Flare	1	7	Past	No	No	No
H1	2020	Female	26	NA	NA	NA	NA	NA	Never	No	No	No
H2	2020	Female	28	NA	NA	NA	NA	NA	Never	No	No	No
H3	2020	Male	31	NA	NA	NA	NA	NA	Never	No	No	No
H4	2020	Male	29	NA	NA	NA	NA	NA	Never	No	No	No
H5	2020	Male	36	NA	NA	NA	NA	NA	Never	No	No	No
H6	2021	Female	25	NA	NA	NA	NA	NA	Current	No	No	No
H7	2021	Male	32	NA	NA	NA	NA	NA	Never	No	No	No
H8	2021	Female	38	NA	NA	NA	NA	NA	Current	No	No	No
H9	2021	Female	27	NA	NA	NA	NA	NA	Never	No	No	No
H10	2021	Female	29	NA	NA	NA	NA	NA	Never	No	No	No
H11	2021	Female	56	NA	NA	NA	NA	NA	Never	No	No	No
H12	2021	Female	41	NA	NA	NA	NA	NA	Never	No	No	No
H13	2021	Female	28	NA	NA	NA	NA	NA	Never	No	No	No
H14	2021	Female	55	NA	NA	NA	NA	NA	Never	No	No	No

Table S4. Characteristics of the 16 vOTUs detected both in fecal and blood samples retrieved from the same subject (shared blood/fecal vOTUs).
"CD" indicates patient with Crohn's disease and "HS" indicates healthy subject.
"NA" indicates values that could not be assigned. Phages encoding an integrase in their genome were classified as temperate, while those lacking an integrase were considered virulent, excepted for *Microviridae* that were excluded from this analysis.

Name of the vOTUs	Family	Lifestyle	Host Phylum	Homology with known gut viruses	Participant
SampleCD8_ Contig922	NA	Virulent	Bacillota	Yes	CD
SampleH14_ Contig702	NA	Temperate	Bacteroidota	Yes	CD
SampleCD11_ Contig2059	*Anelloviridae*	Virulent	NA	Yes	CD
SampleCD11_ Contig727	*Microviridae*	NA	NA	Yes	CD
CrossAssembly_ Contig15411	*Microviridae*	NA	Bacillota	Yes	CD
SampleH2_ Contig566	NA	Virulent	Bacteroidota	Yes	CD
SampleH14_ Contig1913	NA	Temperate	Bacillota	Yes	HS
SampleH1_ Contig1177	NA	Virulent	NA	Yes	HS
SampleH13_ Contig1617	NA	Virulent	Bacillota	No	HS
SampleCD2_ Contig30	*Suoliviridae*	Virulent	NA	Yes	CD
SampleCD2_ Contig5485	NA	Virulent	NA	Yes	CD
CrossAssembly_ Contig3237	*Autographiviridae*	Virulent	Pseudomonadota	Yes	CD
CrossAssembly_ Contig8120	*Autographiviridae*	Virulent	Pseudomonadota	Yes	CD
SampleH1_ Contig422	*Microviridae*	NA	Bacteroidota	Yes	CD
CrossAssembly_ Contig1712	Unclassified	Temperate	Bacillota	No	CD
SampleCD10_ Contig6328	NA	Virulent	Bacillota	No	CD

Table S5 : Number of pairs of reads per sample.
The number of pairs obtained after sequencing is indicated in the "Raw" column. The number of pairs remaining after quality-filtering, and removal of non-human reads is indicated in the "Clean and non-human" column. The samples with less than 100,000 pairs of clean, and non-human reads were excluded from the analysis. “CD” indicates patient with Crohn’s disease and “H” indicates healthy subject

SampleName	Status	Sample Type	Raw	Clean and non-human
H1_F	Healthy	Stool	4 557 777	3 302 598
H2_F	Healthy	Stool	5 141 556	3 538 237
H3_F	Healthy	Stool	4 575 463	3 314 041
H4_F	Healthy	Stool	5 006 463	3 702 841
H5_F	Healthy	Stool	6 630 408	4 868 696
H6_F	Healthy	Stool	6 043 597	4 533 278
H7_F	Healthy	Stool	149	113
H8_F	Healthy	Stool	12 607 586	10 702 899
H9_F	Healthy	Stool	4 957 375	4 008 417
H10_F	Healthy	Stool	161	122
H11_F	Healthy	Stool	47 423 047	36 124 946
H12_F	Healthy	Stool	3 848 503	3 021 191
H13_F	Healthy	Stool	3 944 094	3 101 396
H14_F	Healthy	Stool	10 220 371	8 099 646
CD1_F	Crohn	Stool	3 398 455	2 610 231
CD2_F	Crohn	Stool	11 438 302	8 205 616
CD3_F	Crohn	Stool	27 092 512	19 524 928
CD4_F	Crohn	Stool	18 270 751	12 128 024
CD5_F	Crohn	Stool	8 453 588	5 729 131
CD6_F	Crohn	Stool	4 522 582	3 789 808
CD7_F	Crohn	Stool	2 985 683	2 478 116
CD8_F	Crohn	Stool	10 219 909	8 279 831
CD9_F	Crohn	Stool	7 091 001	5 360 552
CD10_F	Crohn	Stool	9 143 203	7 378 428
CD11_F	Crohn	Stool	9 290 609	7 084 801
CD12_F	Crohn	Stool	6 350 692	5 222 735
CD13_F	Crohn	Stool	7 983 876	6 487 386
CD14_F	Crohn	Stool	636 447	498 993
CD15_F	Crohn	Stool	3 500 715	2 724 997
H1_B	Healthy	Blood	25 145 743	6 790 645
H2_B	Healthy	Blood	13 872 445	5 336 467
H3_B	Healthy	Blood	17 469 863	6 122 420
H4_B	Healthy	Blood	16 038 090	3 433 116
H5_B	Healthy	Blood	19 342 807	5 840 315
H6_B	Healthy	Blood	1 916 528	185 563
H7_B	Healthy	Blood	2 347 924	837 299
H8_B	Healthy	Blood	2 733 222	788 996
H9_B	Healthy	Blood	2 689 347	723 438
H10_B	Healthy	Blood	2 486 126	573 092
H11_B	Healthy	Blood	1 397 921	160 240
H12_B	Healthy	Blood	3 711 044	691 480
H13_B	Healthy	Blood	2 312 559	68 548
H14_B	Healthy	Blood	5 506 340	2 713 132
CD1_B	Crohn	Blood	25 941 062	8 696 199
CD2_B	Crohn	Blood	14 183 825	8 720 837
CD3_B	Crohn	Blood	29 669 303	14 833 794
CD4_B	Crohn	Blood	20 129 214	11 955 938
CD5_B	Crohn	Blood	16 967 073	10 236 927
CD6_B	Crohn	Blood	4 631 975	1 917 021
CD7_B	Crohn	Blood	2 048 220	12 333
CD8_B	Crohn	Blood	7 197 457	3 434 820
CD9_B	Crohn	Blood	6 813 381	3 293 988
CD10_B	Crohn	Blood	6 542 891	3 338 429
CD11_B	Crohn	Blood	7 374 010	2 778 364
CD12_B	Crohn	Blood	9 614 014	4 117 958
CD13_B	Crohn	Blood	5 606 138	2 207 758
CD14_B	Crohn	Blood	5 555 652	2 052 908
CD15_B	Crohn	Blood	8 436 207	4 380 873
	Microtube		5 242 956	2 930 415
	BloodCollectionTube		10 853 654	4 427 872