

**Supplementary Table 1:** Histological analysis of biopsies from stem cell transplant recipients that were HuNoV positive (Table 1A) or HuNoV negative (Table 1B). Alternating white and grey columns are used to separate biopsies by patients. Immunosuppression in patients and controls ; TCD- T cell depleted allograft, Tac - tacrolimus, Sir - sirolimus, Steroids, CsA-Cyclosporine, MMF- Mycophenylate mofetil. The duodenal biopsies are identified by prefix Du, ileal biopsy by prefix IL, Colon biopsies are identified by prefix Co. RtCo – Right colon, Lt Co- left colon, SigCo- Sigmoid colon. Ctrl indicates biopsies from HuNoV-negative patients. Multiple biopsies from the same patient are labeled alphabetically.(-) indicates absence of a particular histological feature; (+, ++) indicates prevalence of the histological feature was observed. N- Nausea, V- vomiting, D- Diarrhea. A – Anorexia, NA – Not applicable, ND – Not detected CMV - Cytomegalovirus Virus and C diff – Clostridium difficile. Standard GVH grade is used to indicate the severity. § - Patient is 4 years out of transplant had chronic GVH of skin and liver. # - Patient >1 year post transplant. The minus sign for biopsy collection indicates that biopsy was obtained prior to the stool that was tested to confirm the HuNoV infection. All the patients had GI symptoms as indicated in the table when they were biopsied.

**Supplementary Table 1A. HuNoV-positive stem cell transplant recipients**

Intestinal segment		Duodenum														Ileum	Colon				
Patient	N1	N3			N4		N5	N6		N7		N8	N9		N10	N11	N11	N1			N12
Immunosuppression	TCD	Tac,Sir, Steroids	TCD		TCD	TCD	CsA, MMF	CsA, MMF		Tac, Sir	TCD	TCD	CsA, MMF	TCD	TCD	TCD	TCD	TCD			Tac, Sir
Biopsy	Du1	Du3A	Du3B1	Du4A	Du4B	Du5	Du6	Du7A	Du7B	Du8	Du9A	Du9B	Du10	Du11	IL11	RtCo1	LtCo1	SigCo1	RtCo12		
Biopsy Collection from the first HuNoV-positive stool (Days)	119	-45	7	47	148	0	-140	-74	-10	19	-155	-33	unknown	-7	83	130	130	130	-21		
Histological Observation	Detection of VP1 by IF	ND	+	++	ND	ND	ND	+	ND	ND	ND	ND	+	ND	ND	++	ND	ND	ND	ND	
	Gastric metaplasia	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	+	ND	ND	ND	ND	
	Basolateral vacuoles in epithelium	++	++	++	++	++	++	++	++	++	++	++	ND	++	++	ND	ND	ND	ND	ND	
	Crypt elongation	ND	ND	ND	ND	ND	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	Edema	+	+	+	+	ND	+	+	ND	ND	+	ND	+	+	+	+	ND	ND	ND	ND	
	Hemorrhage	+	+	+	+	+	+	+	+	+	ND	+	+	+	+	+	ND	ND	ND	ND	
	Flattened and expanded capillaries	ND	+	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	ND	ND	ND	ND	
	Cell debris	ND	+	+	ND	ND	ND	ND	ND	ND	ND	+	+	ND	ND	+	ND	ND	ND	ND	
	Heterochromatic nuclei in crypt	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	
GI presentation	V, D, & wasting	D, cramps	PI	D, partial SB obstruction	D	D, A	D, N	N, V & poor intake	D & A	N, D, weakness & abdominal cramping	V, D, dehydration	Blood per rectum	D	N, V & D	D	V, D, & wasting	V, D, & wasting	V, D, & wasting	D, cramps, gas and bloating		
Other complications	GVH	Grade 0	Grade 0	Grade 0	Grade 1	Grade 0	Grade 0	Grade 1	Grade 1	Grade 0	§	#	#	Grade 0	Grade 1	Grade 1	Grade 0	Grade 0	Grade 0	Grade 0	
	Co-Infections	NA	NA	NA	C diff	NA	NA	NA	NA	NA	NA	NA	NA	C diff	NA	NA	NA	NA	NA	NA	

**Supplementary Table 1B. HuNoV-negative stem cell transplant recipients**

Intestinal segment		Duodenum										
Patient	1	2	3	4	5	6	7	8	9	10	11	
Immunosuppression	TCD	TCD	TCD	Tac	CsA	Tac, Sir	Tac, MMF	Tac	Tac, Sir	TCD	TCD	
Biopsy	Ctrl Du1	Ctrl Du2	Ctrl Du3	Ctrl Du4	Ctrl Du5	Ctrl Du6	Ctrl Du7	Ctrl Du8	Ctrl Du9	Ctrl Du10	Ctrl Du11	
Histological Observation	Detection of VP1 by IF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	Gastric metaplasia	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	Basolateral vacuoles in	+	+	+	+	+	+	+	+	+	+	
	Crypt elongation	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	Edema	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	Hemorrhage	+	ND	+	+	ND	ND	+	+	ND	+	
	Flattened and expanded	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	Cell debris	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Heterochromatic nuclei in crypt	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
GI presentation	D & N	None	N	None	None	N	D & N	N	D	D	N	
Other complications	Upper GI GVH	Grade 1	Grade 0	Grade 1	Grade 0	Grade 1	Grade 1	Grade 1	Grade 1	Grade 0	Grade 0	
	Co-Infections	ND	ND	ND	ND	ND	ND	ND	ND	CMV	CMV	

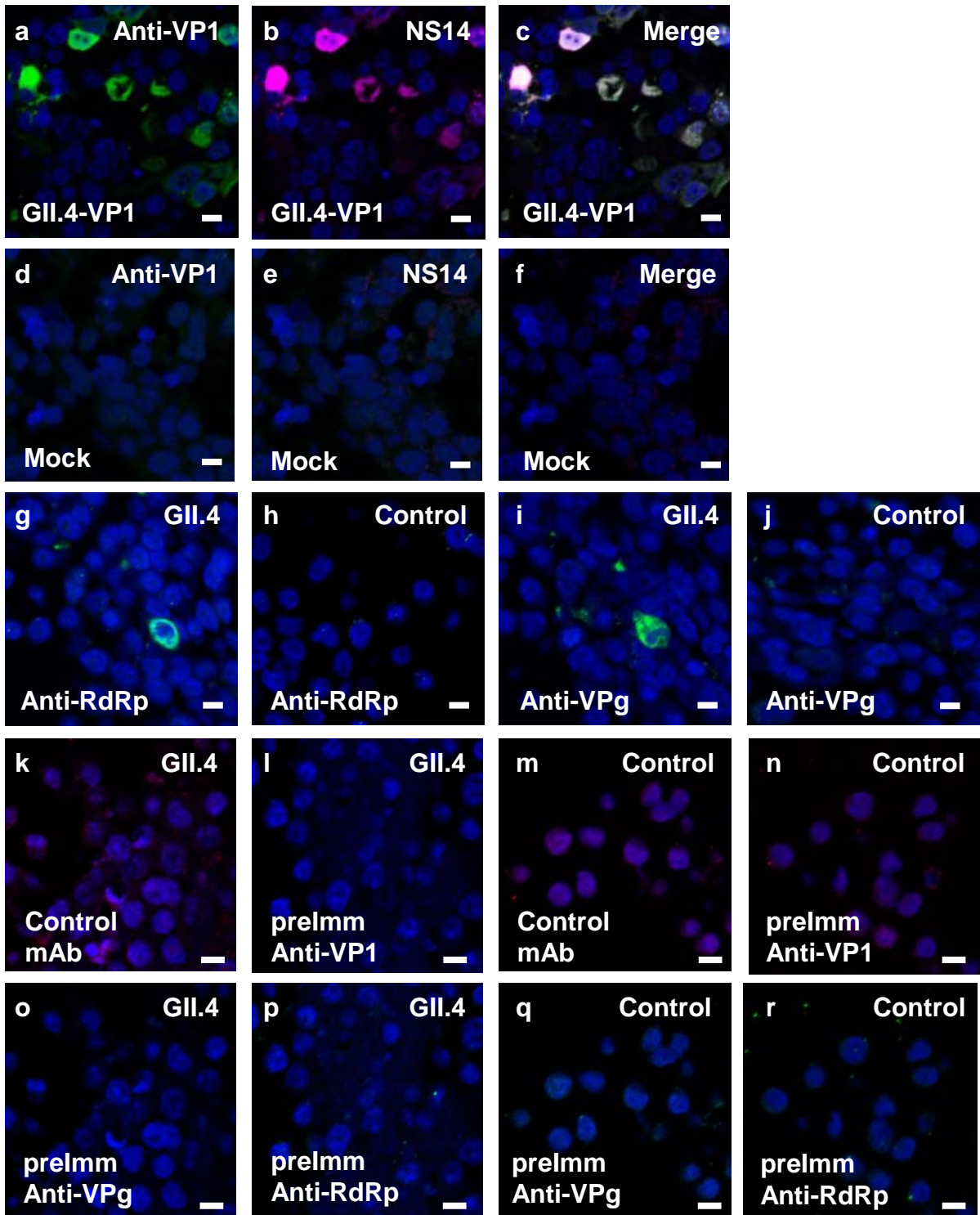
**Supplementary Table 2. Cell types positive for HuNoV VP1 by immunofluorescence**

Biopsy	Number of VP1 positive cells per section*			
	Epithelial cells	Lamina Propria		
		Macrophages	T cell	Dendritic cell**
Duodenum (4/14)	5-20	>20	>20	4
Jejunum (16/19)	0-10	7-10	ND	2
Ileum (1/1)	3	>20	>20	0

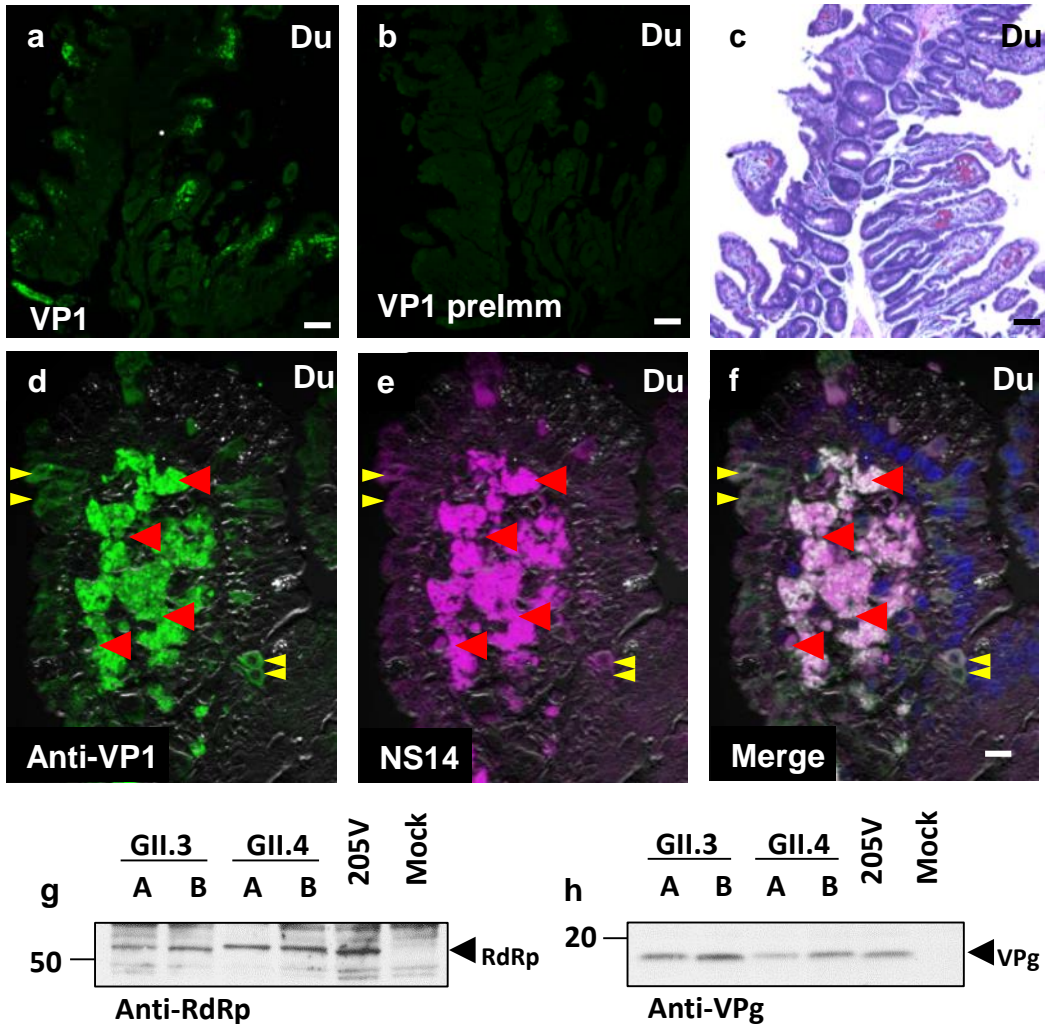
ND-not done. \*\* Not all dendritic cells were positive for the presence of VP1. The numbers in parentheses indicate the number of biopsies positive for VP1 out of the total number of biopsies analyzed. 2-3 biopsy sections were tested for VP1 expression in duodenal and jejunum biopsies. 5 sections were analyzed for the ileal biopsy. For the immune cells, a single section was analyzed per biopsy.

**Supplementary Fig. 1. Validation of antibodies to detect HuNoV antigens.** The ability of a polyclonal anti-VP1 (green, a) and the monoclonal antibody NS14 (magenta, b) to detect HuNoV VP1 was analyzed using HEK cells expressing GII.4 VP1 (a-c) or mock-transfected cells (d-f). HuNoV VP1 was detected by both the anti-VP1 and NS14 antibodies in an overlapping pattern in GII.4 VP1-expressing HEK cells (white, c) but not in the mock-transfected cells (d-f). Monoclonal antibody 9E3, an isotype control for NS14 (k and m) and pre-immune VP1 serum (l and n) did not detect VP1 in the GII.4 VP1-expressing HEK cells (k and l) or the mock-transfected cells (m and n). HEK cells expressing full-length HuNoV genome were used to detect RdRp and VPg. Anti-RdRp detected RdRp in cells expressing the HuNoV genome (g) but not the control (h). Similarly, anti-VPg detected VPg in cells expressing the HuNoV genome (i) but not the control (j). The pre-immune sera for anti-VPg or anti-RdRp did not detect VPg or RdRp in cells expressing the HuNoV genome (o and p) or the control (q and r). Scale bars 10 $\mu$ m.

Supplementary Figure 1.



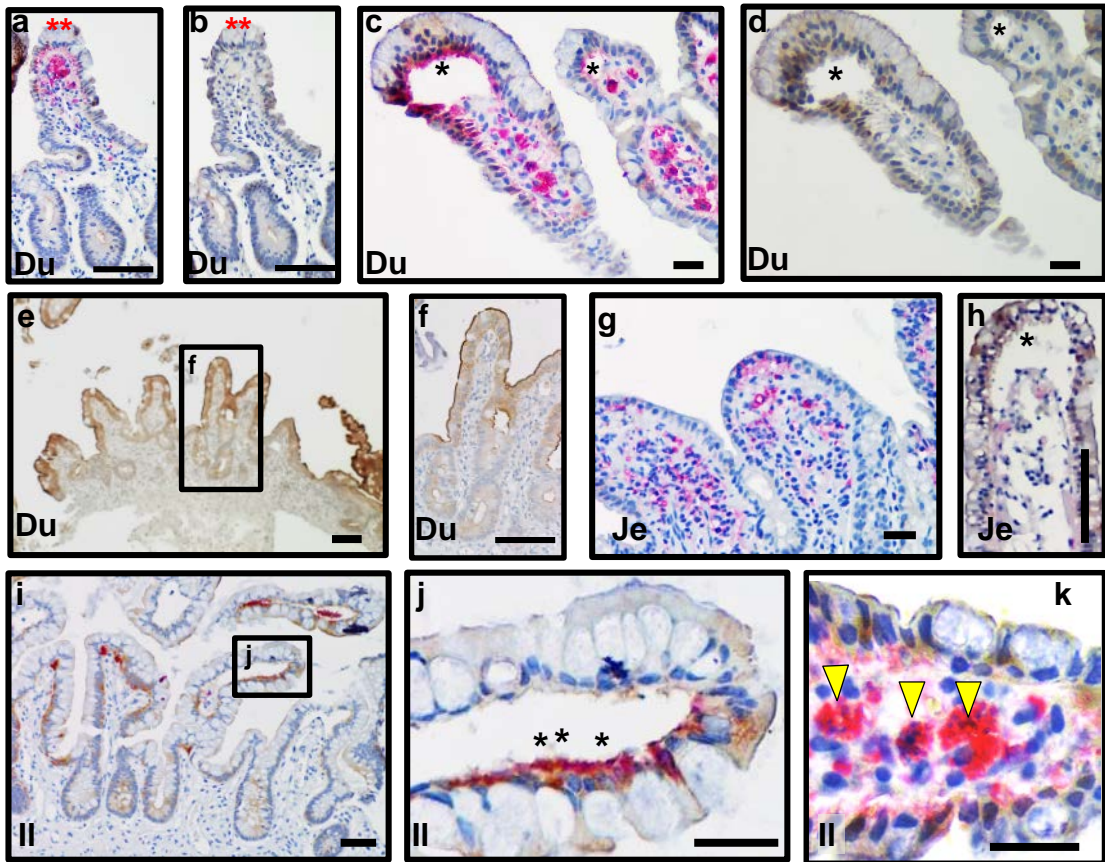
## Supplementary Figure 2.



**Supplementary Fig. 2a-f. Anti-VP1 specifically detects HuNoV VP1 in duodenal biopsy.** Serial sections of a duodenal biopsy from a HuNoV-positive patient was stained, with guinea pig anti-VP1 (a) and pre-immune VP1 (b). The histology of the same biopsy was analyzed by H&E staining (c). A small portion of the same biopsy is shown at a higher magnification (d-f) stained with polyclonal serum guinea pig anti-VP1 (green, d) and mAb NS14 (magenta, e). The two antibodies show overlapping staining (white, f). VP1 was detected in enterocytes (yellow arrowheads, d-f) as well as in the cells of the lamina propria (red arrowheads, d-f). Nuclei are stained blue by DAPI (f). Scale bars a-c 100 $\mu$ m; d-f 10 $\mu$ m.

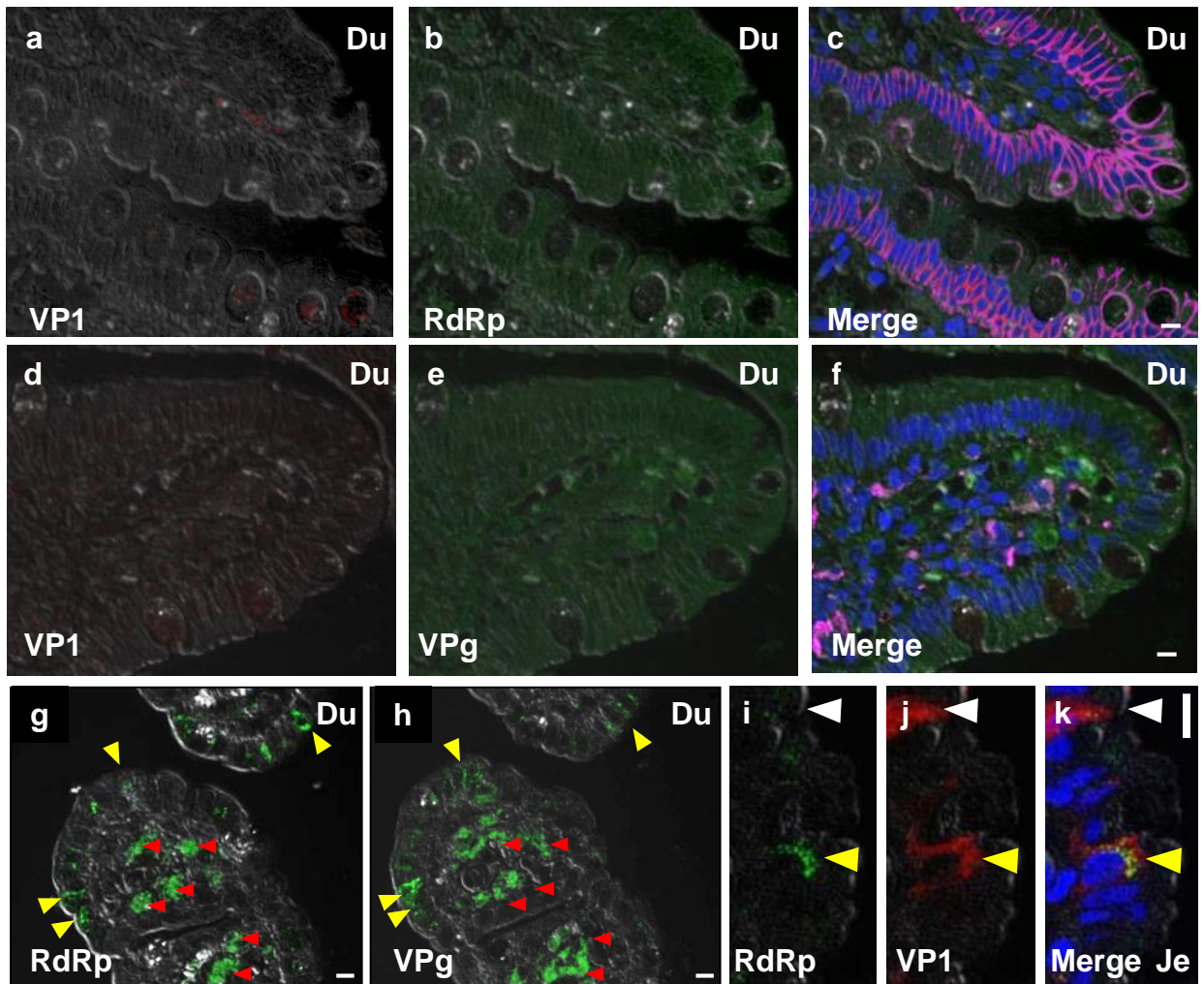
**Supplementary Fig. 2g and h. Validation of antibodies raised against GII.3 RdRp and VPg to detect GII.4 RdRp and VPg by western.** Full-length constructs that expressed the HuNoV genome were transfected into HEK293FT cells. The cells were harvested after 24hrs and non-structural proteins RdRp (g) and VPg (h) were detected by western blot. HEK293FT cells were transfected with the constructs expressing full length HuNoV genomes of - **GII.3** (U201 clones A and B served as positive control), **HuNoV GII.4** construct (SAGA clones A and B served as the test), **205V** (HuNoV GII.3-GFP) was an additional positive control and **mock** (empty vector shows background and served as a negative control). Primary antibodies for panel g: rabbit anti-GII.3 RdRp, and for panel h: rabbit anti-GII.3 VPg. goat anti-Rb HRP was used to detect the primary antibodies.

### Supplementary Figure 3.



**Supplementary Fig. 3. Detection of HuNoV VP1 in duodenum, jejunum and ileum by immunohistochemistry.** VP1 (red) and villin (brown) in duodenal (a-f), jejunal (g and h) and ileal (i-k) biopsies from HuNoV-positive (a-d, g-k) and HuNoV-negative (e and f) patients. Area highlighted by black rectangles and labeled in e and i are magnified in f and j, respectively. Loss of villin expression from the apical surface of the villus epithelium was observed in the biopsies from HuNoV-positive patients (a-d and g-k). VP1 was analyzed in serial sections of a duodenal biopsy stained with anti-VP1 (red, a and c) but not stained with pre-immune anti-VP1 (b and d). Panel (a) shows a larger area of the biopsy shown in Figure 1h, j & k. In the HuNoV-negative patient, VP1 was not detected; however, apical villin was detected in the villous epithelial cells (e and f). The jejunal villi (g and h) show the presence of VP1, but it was absent in the crypts (g). In the ileum, VP1 antigen is present in the lamina propria cells (yellow arrowheads, k). Scale bars 50 $\mu$ m.

### Supplementary Figure 4.



**Supplementary Fig. 4. Immunofluorescence-based detection of HuNoV non-structural proteins in biopsies.** HuNoV-negative biopsies (a-c and d-f) did not show staining for VP1 (red, a, c, d and f), RdRp (green, b), and VPg (green, e). Epithelial cells were identified by detection of E-cadherin expression (magenta, c) and macrophages by CD68 (magenta, f). Nuclei are stained with DAPI (blue, c and f).

Non-structural proteins RdRp (green, g) and VPg (green, h) were detected in serial sections of a HuNoV-positive duodenal biopsy. The matched areas between the two serial sections that are positive for both RdRp and VPg staining are indicated by arrowheads. The yellow arrowheads indicate the epithelial cells while the red arrowheads indicate the cells of the lamina propria positive for RdRp and VPg.

Detection of HuNoV proteins in a jejunal biopsy (i-k), RdRp (green, i and k) and VP1 (red, j and k) were detected in epithelial cells. Some cells show the presence of VP1 alone (white arrowhead, i-k), while some epithelial cells show the presence of both RdRp and VP1 (yellow arrowhead, i-k) in a perinuclear pattern. Scale Bars – 20  $\mu$ m.