## Supplementary data

## Infant antibody and B cell responses following confirmed pediatric GII.17 norovirus infections functionally distinguish GII.17 genetic clusters

Camilla A. Strother, Paul D. Brewer-Jensen, Sylvia Becker-Dreps, Omar Zepeda, Samantha May, Fredman Gonzalez, Yaoska Reyes, Benjamin D. McElvany, April M. Averill, Michael L. Mallory, Anna M. Montmayeur, Verónica P. Costantini, Jan Vinjé, Ralph S. Baric, Filemon Bucardo, Lisa C. Lindesmith, and Sean A. Diehl





(**A**). Fluorescence activated cell sorting of IgM<sup>-</sup>CD27<sup>+</sup> memory B cells from peripheral blood of an 11-month-old infant one month after a GII.17 norovirus episode. (**B**). 6XL-transduced GFP<sup>+</sup> cells were plated at 50 cells/well in 180 cultures and IgG (left) and IgA (right) were measured by ELISA and binned by optical density.

Figure S1





Figure S2. Blockade curves of polyclonal MBC culture supernatants exhibiting GII.17 VLP binding. Supernatants from the indicated polyclonal MBC cultures (well ID) were tested over a dilution series for blockade activity against binding of GII.17 (**A**) and GII.4 2012 Sydney (**B**) VLPs to PGM. ID<sub>50</sub> titers are expressed as dilution of MBC supernatant required to achieve 50% inhibition of VLP binding compared to VLP binding in the absence of supernatant pretreatment (ID<sub>50</sub>). Samples able to block 50% VLP binding at  $\geq$ 1/20 dilution are noted as positive (POS) for blockade ability.



**Figure S3: A somatically mutated GII.17 IgG mAb from subject 434 with weak binding affinity** Somatic hypermutations (SHM, silent and replacement), number of nucleotide changes involved in, and the position of each mutation in paired variable heavy and light (VH/VL) gene regions from NVG.1 are shown. CDRH/L1-3, complementarity determining region heavy (light) 1-3; FWRH/L1-3, framework region heavy (light) 1-3. Percent SHM is total number of mutated nucleotides/ total number in FWR1+CDR1+FWR2+CDR2+ FWR3. (**B**) Binding of recombinant NVG.1 (IgG) to GII.17 cluster IIIb. NHS, normal human serum (positive control).

Figure S4

NVA.1 Heavy Chain protein sequence (IGA1)

CDRH1 CDRH2 FWRH1 1 10 20 30 40 FWRH2 50 EVQLVESGGGLVKPGGSLRLSCAASGFSFSDYSMNWVRQAPGKGLEWVSSISSSSYI FWRH3 CDRH3 60 70 80 90 100 110 FWRH4 YADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARHLQLWLGFDIWGQGTMVTVS SASPTSPKVFPLSLCSTOPDGNVVIACLVQGFFPQEPLSVTWSESGQGVTARNFPPSQD IGA1 Fc region ASGDLYTTSSOLTLPATOCLAGKSVTCHVKHYTNPSODVTVPCPVPSTPPTPSPSTPPT PSPSCCHPRLSLHRPALEDLLLGSEANLTCTLTGLRDASGVTFTWTPSSGKSAV0GPPE RDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPP PSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTTFAV TSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMAEVDGTCY

NVA.1 Light Chain protein sequence (IGLC2)

CDRL2 CDRL1 10 FWRL1 20 30 40 FWRL2 50 1 QSVLTQPPSASGTPGRRVTISCSGYSSNIGYNS<u>VHWYQQ</u>FPGAAPKLLIHBNNDRPSGV **CDRL3** 100 FWRL4 110 80 90 60 70 FWRL3 IGLC2 PDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGWVFGGGGTKLTVLGQPKAAPS Fc region VTLFPPSSEELOANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKOSNNKYA

ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

**Figure S4. Amino acid sequence of native NVA.1 IGH and IGL genes**. FWR, framework region; CDR, complementarity determining region, and Fc regions are indicated.

Figure S5



**Figure S5. Expression and validation of dimeric IgA NVA.1.dA anti-GII.17 mAb.** (**A**) Nonreducing immunoblot analysis of IgA affinity purified from supernatants of HEK293A cells transfected with increasing ratios of J-chain plasmid DNA and fixed amounts (4.5  $\mu$ g) of NVA.1 heavy and light chain (IGH and IGL) plasmid DNA. Anti-human IgA-HRP was used for detection. Molecular weight marker (MW) from 55-460 kDa is included for size validation of IgA species (Ig $\alpha$ , IgA heavy chain; mIgA, monomeric IgA ~160 kDa; dIgA, dimeric IgA, ~360 kDa). (**B**) Size exclusion chromatography (SEC) of IgA affinity purified from cells transfected with 1:1:5 ratio of NVA.1 IGH:IGL:J-chain and molecular weight standards. (**C**) IgA concentrations in indicated SEC column fractions from (B). (**D**) GII.17 VLP binding by monomeric NVA.1 and dimeric NVA.1 (NVA.1dA).

Figure S6



**Figure S6. Titration curves for serum competition assays to assess NVA.1-like cluster IIIb-specific antibodies in cluster IIIb blocking sera from infected children.** Plates coated with GII.17 cluster IIIb VLP were incubated with decreasing concentrations of NVA.1 (IgA) or NVA.1G (IgG) mAb before addition of sera from cluster IIIb-infected children with blockade activity. Sera was added at the blockade ID<sub>50</sub> titer and bound serum IgA (**A**) or IgG (**B**) were detected by anti-human IgA or anti-human IgG, respectively. The lowest concentration of mAb that inhibited at least 50% of serum binding compared to no added mAb was determined.