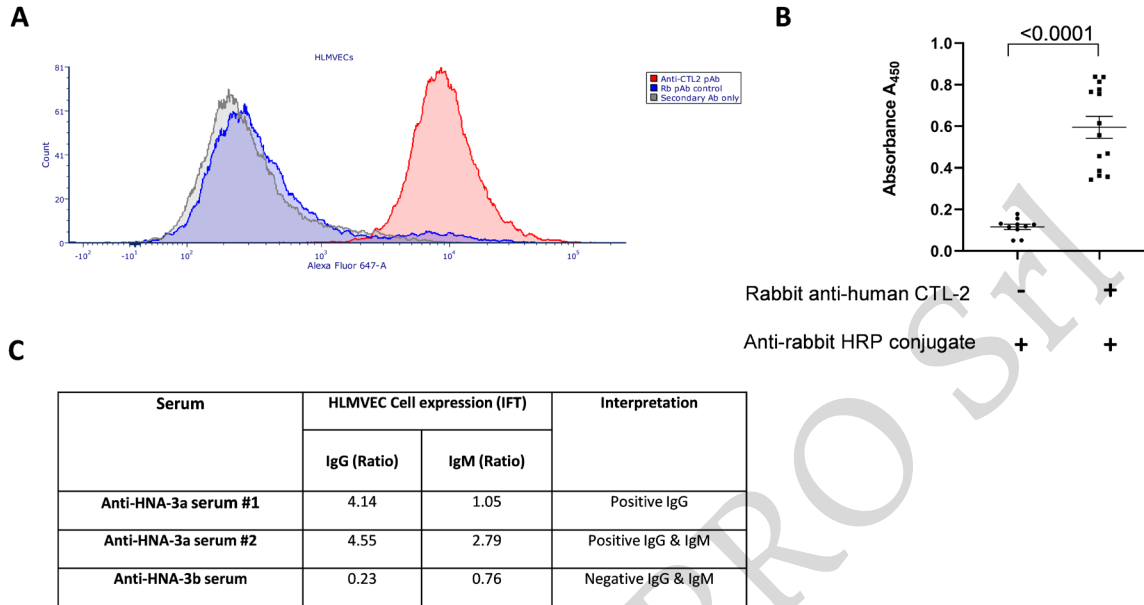


**ONLINE SUPPLEMENTARY CONTENT**

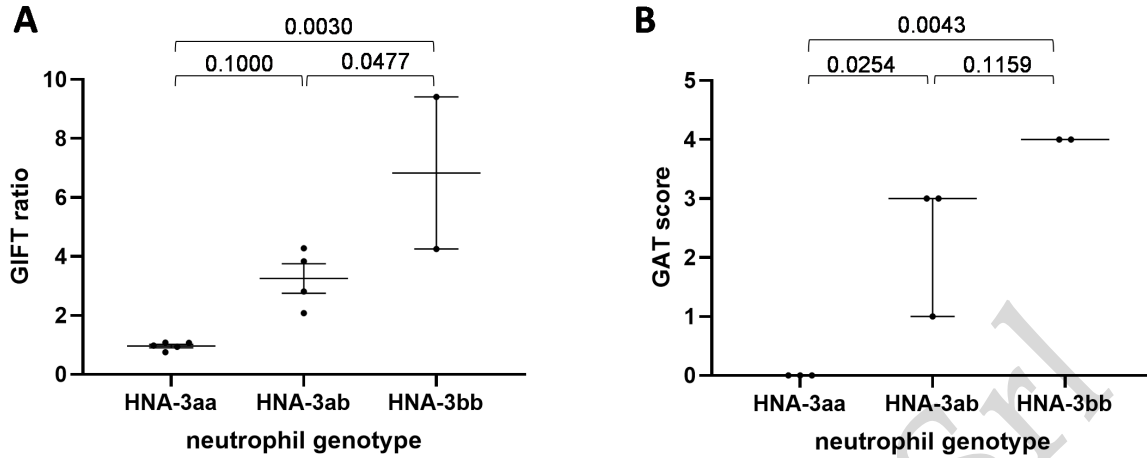


**Figure S1 - HLMVEC reactivity to anti-HNA-3a sera, anti-HNA-3b serum, and anti-CTL-2 polyclonal antibody**

**A:** CTL-2 protein total expression level was evaluated on the surface of HLMVECs. Confluent HLMVECs were trypsinized (trypsin-EDTA 0.25%, Gibco, ThermoFisher Scientific, Waltham, MA, USA) to remove them from the plate, washed in PBS and incubated with either rabbit polyclonal anti-human CTL-2 antibody (0.2 mg/ml, Abcam), rabbit polyclonal IgG control (0.2 mg/ml, Cat#AB-105-C, R&D Systems, Bio-Techne, Minneapolis, MN, USA) or left untreated for 60 min. After 2 washes, cells were incubated with a goat anti-rabbit Cy5-conjugated antibody (4µg/mL, Cat#A10523, Invitrogen, Waltham, MA, USA) for 30 min in the dark. Samples were then analysed using flow cytometry (BD FACS Canto II) and MFI was recorded. One representative experiment of four replicates is shown.

**B:** CTL-2 protein total expression level was evaluated on surface of HLMVECs by in-house ELISA. 2.5x10<sup>4</sup> HLMVECs were seeded in 96 well plate (tissue culture-treated, Corning, Sommerville, MA, USA). After 24 hrs, HLMVECs were fixed with 4% paraformaldehyde (Cat#28906, Pierce, ThermoFisher Scientific, Waltham, MA, USA) in PBS for 10 min at room temperature. After 3 washes in PBS, HLMVECs were incubated with rabbit polyclonal anti-human CTL-2 antibody (0.2 mg/ml, Abcam) for 60 min. HLMVECs were then washed 6 x in PBS-Tween 0.1% (Cat#P1379, Sigma-Aldrich, St. Louis, MO, USA) and incubated with HRP-conjugated anti-rabbit IgG antibody (0.3µg/mL, Cat#81-6120, Zymax Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) 1 hr at room temperature. 3,3',5,5'-Tetramethylbenzidine (TMB) (Cat#34022, Thermo Fisher Scientific, Waltham, MA, USA) substrate solution was prepared as per manufacturer's instructions and added to the cells for 10 min at room temperature. Sulphuric acid (1M) was added to stop the reaction and absorbance at 450 nm was measured on microplate reader (PowerWave XS2, BioTek, Millennium Science, Mulgrave, VIC, AU).

**C:** Phenotyping of HLMVECs against anti-HNA-3a serum #1 and anti-HNA-3a serum #2 as well as an anti-HNA-3b serum as per GIFT protocol: 25x10<sup>5</sup> HLMVECs were mixed 1:1 volume (25 µL) with control or test sera in a 96-well plate (Nunc). The plate was incubated (30°C, 30 min), centrifuged to pellet HLMVECs, inverted to drain, and HLMVECs were washed with PBS/EDTA/BSA 0.2%. Goat anti-human F(ab')<sub>2</sub> fragment IgG-PE and IgM-AF647 (both Jackson ImmunoResearch, West Grove, PA, USA) were diluted n/100 and n/400 respectively in PBS/EDTA/BSA 0.2% buffer and added to the plate which was then incubated at room temperature in the dark for 30 min. HLMVECs were then washed and analysed by flow cytometry (BD FACS Canto II). GIFT ratio was determined as the ratio between the serum MFI value and the average MFI value from three independent wells incubated with negative control serum. Positive result: Ratio >2.0.



### Figure S2 - Neutrophil reactivity to anti-HNA-3b serum

Neutrophils were isolated from 10 healthy volunteers and GIFT (A) and GAT (B) were performed against an anti-HNA-3b serum. GIFT data are presented as mean  $\pm$  SEM and GAT data are presented as median and IQR.  $P < 0.05$  was considered statistically significant.