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Supplemental information

A SARS-CoV-2 antibody broadly neutralizes SARS-related coronaviruses and variants by coordinated recognition of a virus-vulnerable site

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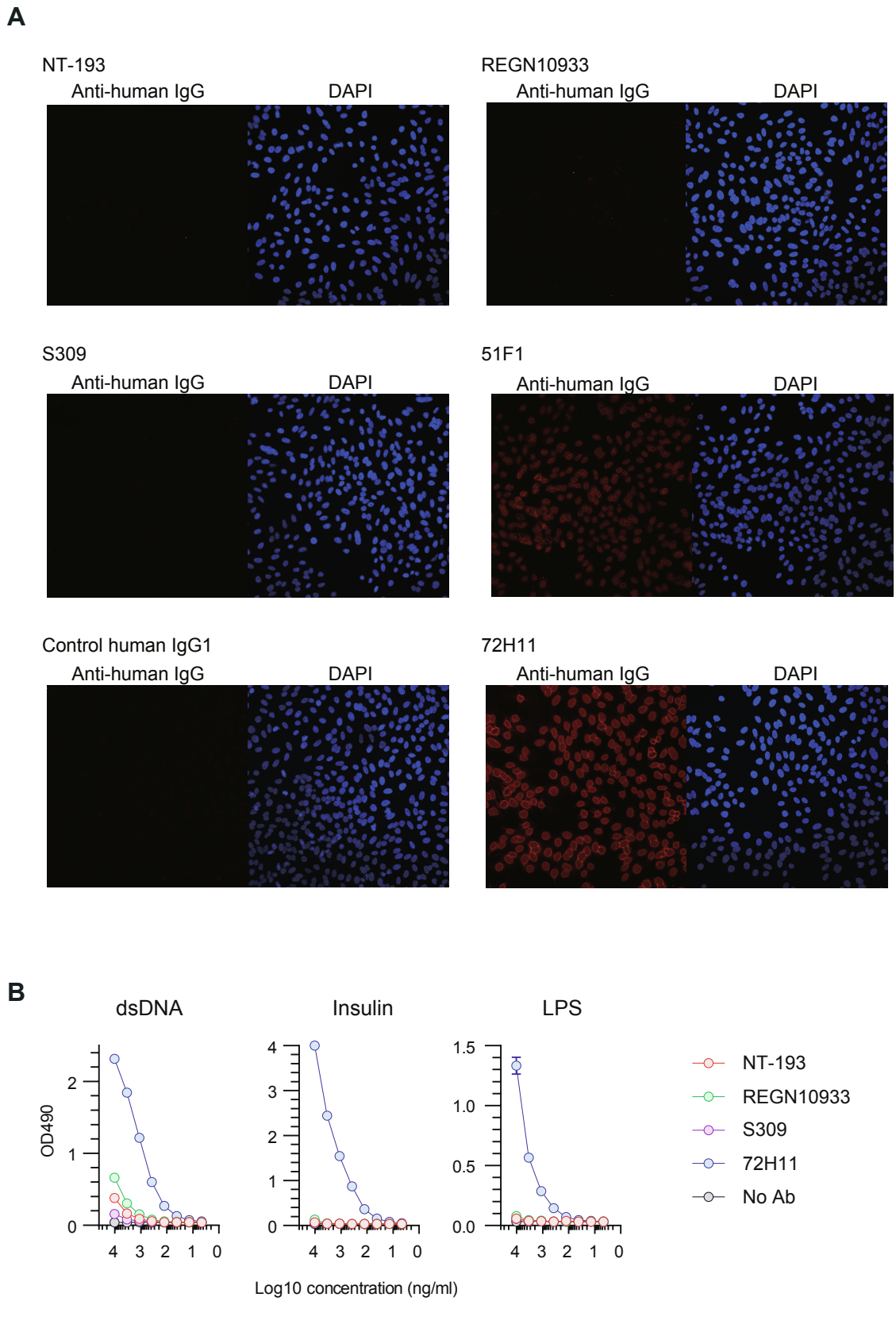

Figure S1. NT-193 is neither autoreactive or polyreactive, Related to Figure 1.
(A) Autoreactivity of NT-193 was assessed under immunofluorescence assay using HEp-2 cells. The representative staining of human IgG1 antibodies is shown from NT-193, REGN10933, and S309 along with reference antibodies (51F1, 72H11, and polyclonal human IgG1). (B) Polyreactivity of NT-193 was assessed under ELISA using dsDNA, insulin, and LPS as coated antigens. Polyreactive human IgG1 (72H11) was loaded as positive control. Values represent mean ± SD. The representative data from three independent experiments are shown.

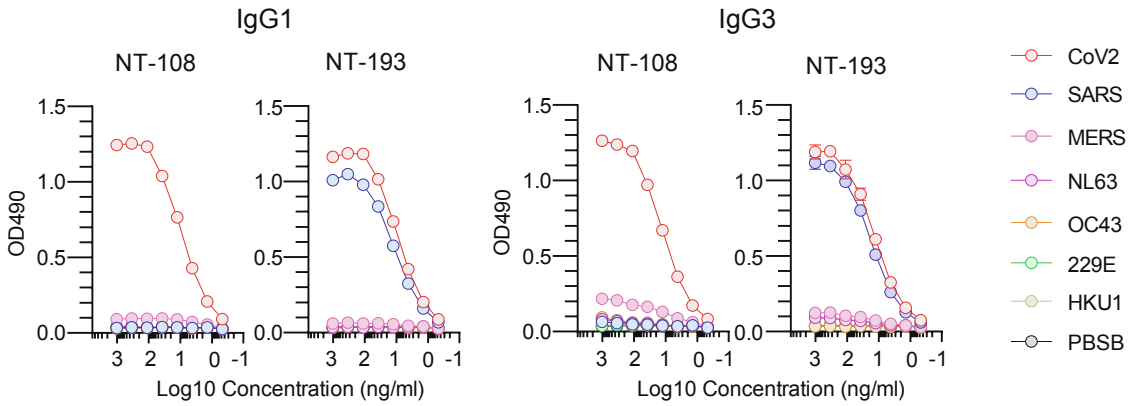

Figure S2. NT-108 and NT-193 fail to cross-react coronavirus S-trimer antigens, Related to Figure 2.

S-trimer antigens from the indicated coronaviruses were coated on ELISA plate and the binding of serially diluted NT-108 and NT-193 were assessed. IgG1 subclass is shown in left and IgG3 subclass in right panel. Values represent mean ± SD. The representative data from three independent experiments are shown.

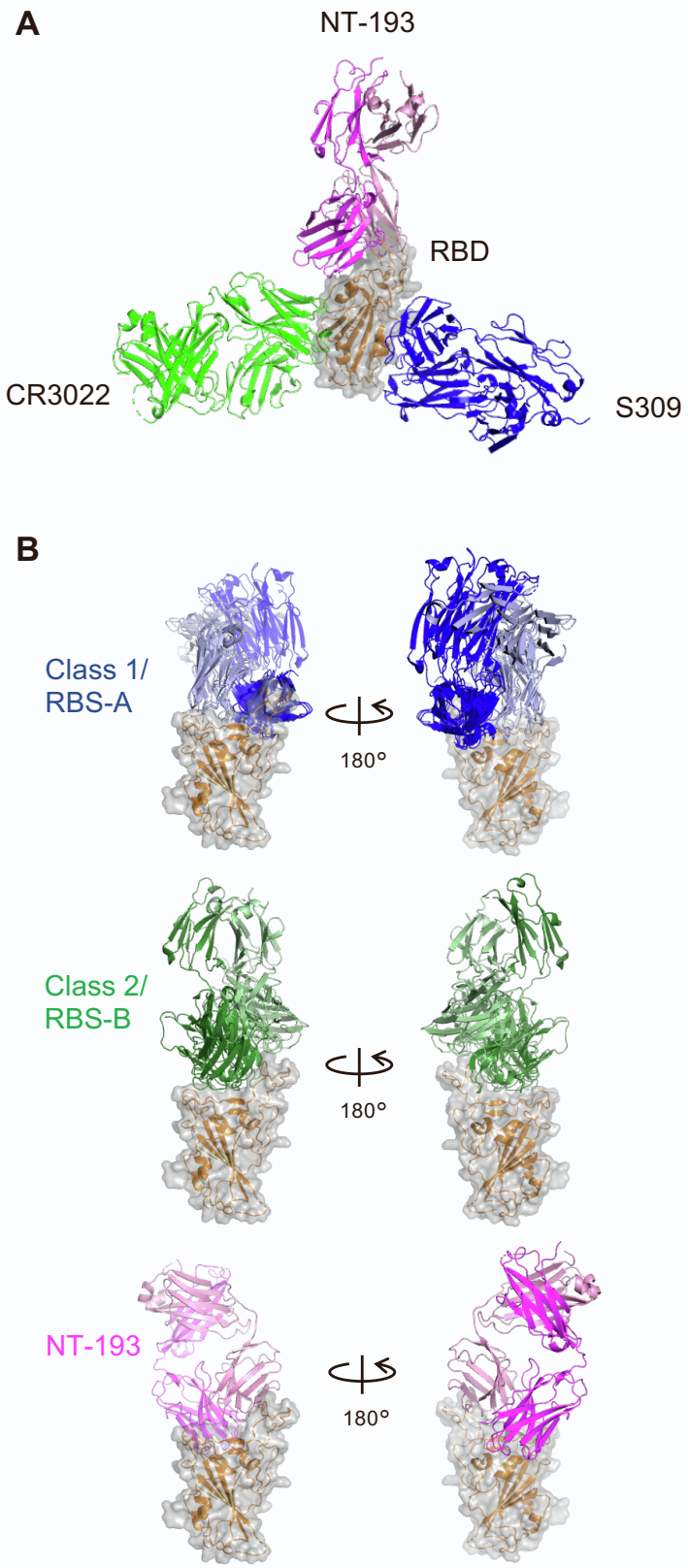

Figure S3. The binding mode of NT-193 is distinct from previously isolated antibodies, Related to Figure 3.

(A) The comparison of the NT-193 (magenta and pink) complex with CR3022 (green) and S309 (blue) complexes. The cartoon models are shown (magenta: NT-193 heavy chain, pink: NT-193 light chain, orange: RBD). (B) The comparison of the NT-193 complex (magenta and pink) with class 1/ RBS-A antibody complexes (dark and light blue) and class 2/RBS-B ones (dark and light green). The cartoon models are shown (magenta: NT-193 heavy chain, pink: NT-193 light chain, orange: RBD). The left models are shown at the same angle as the right one of NT-193 complex in Figure 3A. Left and right angles are almost 180 degree rotated.

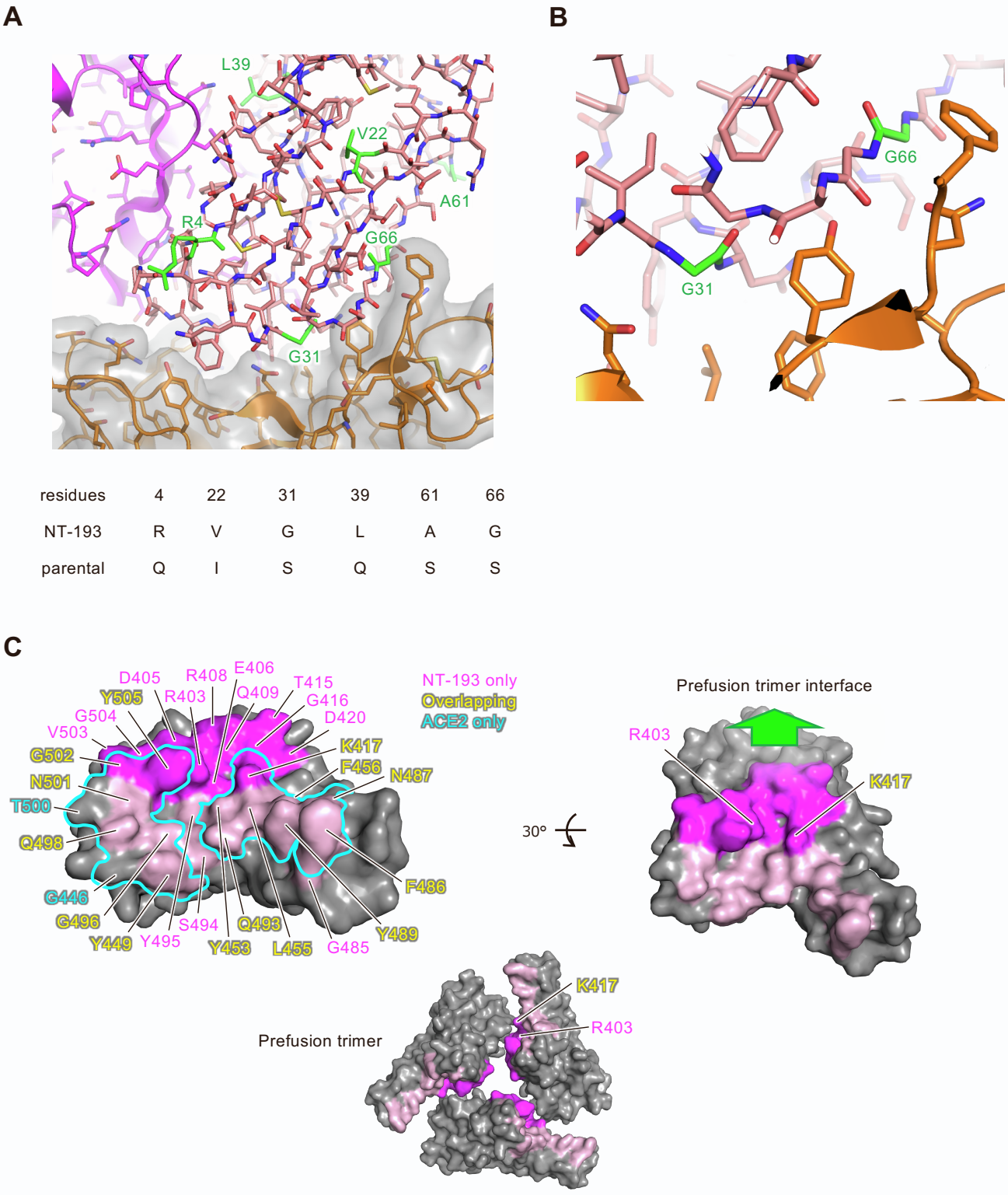

Figure S4. NT-193 recognizes open RBD conformation via germline-encoded residues, Related to Figures 3 and 4.

(A, B) Mapping of somatically mutated sites onto the complex structure. (A) The NT-193 complex (magenta and pink stick models) with RBD (orange with transparent surface) is shown (top panel). The somatically mutated residues in NT-193 light chain are colored in green. The amino acid substitutions by these somatic hypermutations are summarized in the lower panel. (B) The closed-up view of the binding interfaces around G31 and G66 is shown. (C) Binding sites of NT-193 Fab-RBD complex. ACE2 and NT-193 binding residues on RBS. The binding areas for ACE2, NT-193 HC and LC are shown in cyan frame, magenta, and light pink, respectively. Top left, the surface models of RBS are shown. The angle is the same as Figure 3C. Top right, the model is rotated by about 30 degree compared with one of top left. Bottom, the model of RBD in closed prefusion trimer is shown.

Figure S5. Footprints of NT-193 differ from other antibodies, Related to Figures 3 and 4. The CoV2 RBD residues are colored based on conservation with CoV1 similarly to Figure 3. The binding residues of RBD for ACE2 and each antibody are colored: ACE2 (cyan), NT-193 (magenta), class 1/RBS-A (green), class 2/RBS-B (orange), class 3 (blue), and class 4 (brown). The residues which contact ACE2, antibody, or RBD within a distance of 4 Å are defined as binding residues. The reported mutations (red) are shown in the column between CoV1 and CoV-2 sequences.

A

Antibody		NT-193						
Ab conc. for resistance check (μg/mL)		8	4	2	1	0.5	0.25	No Ab
Ab conc. for passaging (μg/mL)	50							
	16.7							
	5.5							
	1.9							
	0.6							
	No Ab							

Antibody		NT-108 + NT-193						
Ab conc. for resistance check (μg/mL)		8	4	2	1	0.5	0.25	No Ab
Ab conc. for passaging (μg/mL)	50							
	16.7							
	5.5							
	1.9							
	0.6							
	No Ab							

No CPE
Parental
G504V

B

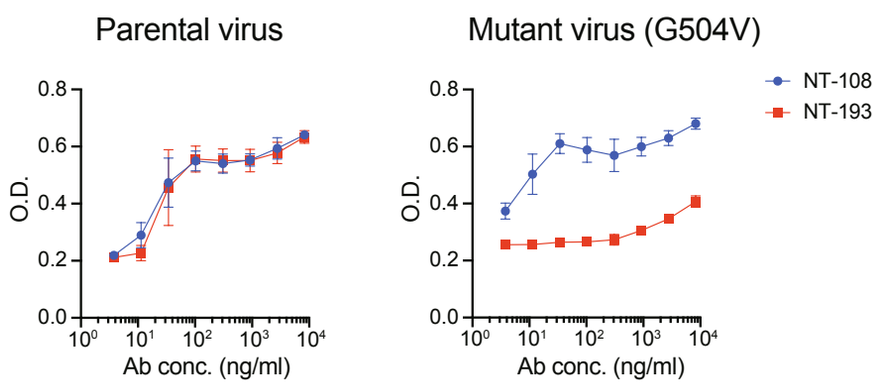

Figure S6. Different screening approach selects the same escape mutation, Related to Figure 5.

(A) CoV2 virus was passaged three times in the presence of NT-193 IgG3 or NT-108/NT-193 IgG3 antibodies at several concentrations (0.6, 1.9, 5.5, 16.7, and 50 μg/mL). After third passages, the viral RNAs were collected from CPE^+ wells (red or orange) and subjected to RBD sequencing (red; G504V mutation, orange; no mutation). In parallel, the viruses from CPE^+ wells were further cultured in the presence of NT-193 or NT-108/NT-193 (0.25, 0.5, 1, 2, 4, and 8 μg/mL) for resistance check. (B) Neutralizing activity of NT-108 and NT-193 IgG3 against G504V mutant and parental viruses was assessed. The CPEs were expressed as O.D. of MTT assay. Value represents mean ± SD. The representative data from two independent experiments are shown.

Table S1. Data collection statistics, Related to Figures 3 and 4.

RBD_NT-193	
Wavelength	0.98
Resolution range	49.3 - 2.80 (2.94 - 2.80)
Space group	P 2_1 2_1 2_1
Unit cell	84.07 106.89 148.07 90 90 90
Total reflections	227819 (30744)
Unique reflections	33594 (4373)
Multiplicity	6.8 (7.0)
Completeness (%)	100.0 (100.0)
Mean I/sigma(I)	10.3 (1.5)
Wilson B-factor (Å^2)	47
R-merge	0.153 (1.33)
R-meas	0.166 (1.43)
R-pim	0.063 (0.54)
CC1/2	0.99 (0.71)
Resolution range in refinement	45.1 - 2.80 (2.90 - 2.80)
Reflections used in refinement	33522 (3301)
Reflections used for R-free	1661 (169)
R-work	0.226 (0.351)
R-free	0.256 (0.379)
CC(work)	0.919 (0.756)
CC(free)	0.904 (0.812)
Number of non-hydrogen atoms	4833
macromolecules	4788
ligands	28
solvent	17
Protein residues	621
RMS(bonds)	0.008
RMS(angles)	1.22
Ramachandran favored (%)	95.91
Ramachandran allowed (%)	3.76
Ramachandran outliers (%)	0.33
Rotamer outliers (%)	1.32
Clashscore	4.01
Average B-factor (Å^2)	64
macromolecules	64
ligands	93
solvent	51
Number of TLS groups	18