# Supplementary tables

Supplementary table 1. Comparison of FcyRI-binding profiles by BGB-A317 and BGB-

A317/IgG4<sub>S228P</sub>

	$k_a(1/Ms)$	$k_d(1/s)$	$K_A$ (1/M)	$K_{D}\left( \mathrm{M} ight)$
BGB-A317/IgG4 <sub>S228P</sub>	7.12 x 10 <sup>5</sup>	1.99 x 10 <sup>-3</sup>	$3.57 \ge 10^8$	2.80 x 10 <sup>-9</sup>
BGB-A317	ND	ND	ND	ND

 $K_A$ : affinity.  $K_D$ : dissociation constant. ND: not detectable.

Supplementary table 2. Comparison of PK parameters between BGB-A317 and BGB-

A317/IgG4<sub>S228P</sub>

	Dose (mg/kg, QW)	C <sub>max</sub> (µg/ml)	AUC <sub>0-168h</sub> (µg/ml-h)
BGB-A317	1	10.6	1087
BGB-A317	10	112.8	11396
BGB-A317/IgG4 <sub>S228P</sub>	10	73.0	6000

	1	11	21	31	41	51	61	71	81
IgG4 S228P	ASTKGPSV	FPLAPCSRST	SESTAALGCL	VKDYFPEPVI	TVSUNSGALTS	GVHTFPAVL(	SSGLYSLSSV	VTVPSSSLGT	<b>KTYTCNVDHKPS</b>
IgG4 ₩t	ASTKGPSV	FPLAPCSRST	SESTAALGCL	VKDYFPEPVI	TVSUNSGALTS	GVHTFPAVL(	SSGLYSLSSV	VTVPSSSLGT	<b>KTYTCNVDHKPS</b>
BGB-A317	ASTKGPSV	FPLAPCSRST	SESTAALGCL	VKDYFPEPVI	TVSUNSGALTS	GVHTFPAVL(	SSGLYSLSSV	VTVPSSSLGT	<b>KTYTCNVDHKPS</b>
Consensus	astkgpsv	fplapcsrst	sestaalgcl	vkdyfpepvt	tvswnsgalts	gvhtfpavlo	(ssglyslssv	vtvpssslgt)	tytcnvdhkps
	91	101	111	121	131	141	151	161	171
IgG4 S228P	NTKVDKRVI	ESKYGPPCP <mark>P</mark>	CPAP <mark>EFL</mark> GGP	SVFLFPPKPF	<b>WTLMISRTPE</b>	VTCVVV <mark>D</mark> VS(	EDPEVQFNWY	VDGVEVHNAK.	TKPREEQFNSTY
IgG4 Wt	NTKVDKRVI	ESKYGPPCP <mark>S</mark>	CPAP <mark>EFL</mark> GGP	SVFLFPPKPP	<b>WTLMISRTPE</b>	vtcvvv <mark>d</mark> vs(	EDPEVQFNWY	VDGVEVHNAK	TKPREEQFNSTY
BGB-A317	NTKVDKRVI	ESKYGPPCP <mark>P</mark>	CPAP <mark>PVA</mark> GGP	SVFLFPPKPF	<b>WTLMISRTPE</b>	VTCVVV <mark>A</mark> VS	EDPEVQFNWY	VDGVEVHNAK.	TKPREEQFNSTY
Consensus	ntkvdkrv	eskygppcpp	cpapeflggp	svflfppkpk	dtlmisrtpe	vtcvvvdvs	ledberdtum	vdgvevhnakt	tkpreeqfnsty
	181	191	201	211	221	231	241	251	261
IgG4 S228P	RVVSVLTV	<mark>L</mark> HQDWLNGKE	YKCKVSNKGL	PSSIEKTISH	KAKGQPREPQV	YTLPPSQEEN	TKNQVSLTCL	VKGFYPSDIA	VEWESNGQPENN
IgG4 Wt	RVVSVLTV	lhqdwlngke	YKCKVSNKGL	PSSIEKTISH	KAKGQPREPQV	YTLPPSQEED	TKNQVSLTCL	VKGFYPSDIAV	VEWESNGQPENN
BGB-A317	RVVSVLTV	VHQDWLNGKE	YKCKVSNKGL	PSSIEKTISF	KAKGQPREPQV	YTLPPSQEED	TKNQVSLTCL	VKGFYPSDIAV	VEWESNGQPENN
Consensus	rvvsvltv.	lhqdwlngke	ykckvsnkgl	pssiektisk	akgqprepqv	ytlppsqeer	atknqvsltclv	vkgfypsdiav	vewesngapenn
	271	281	291	301	311	321	331	341	351
IgG4 S228P	YKTTPPVLI	DSDGSFFLYS	<mark>R</mark> LTVDKSRWQ	EGNVFSCSVN	HEALHNHYT(	KSLSLSLGK			
IgG4 Wt	YKTTPPVLI	DSDGSFFLYS	<mark>r</mark> ltvdksrwq	EGNVFSCSVN	IHEALHNHYT(	KSLSLSLGK			
BGB-A317	YKTTPPVLI	DSDGSFFLYS	KLTVDKSRWQ	EGNVFSCSVN	THE ALHNHYT (	KSLSLSLGK			
Consensus	vkttppvl	dsdasfflvs	rltvdksrwa	eanvfscsvi	healhnhyto	kslslslak			

Amino acid sequence alignment of the Fc-hinge regions of IgG4<sub>S228P</sub>, IgG4wt and BGB-A317 (or BGB-A317/IgG4variant). Differences in the sequences are highlighted in blue.



Characterization of human and mouse CD64<sup>+</sup> cells within A431 allogeneic xenograft model. (a) Representative images of mCD64 and hCD64 IHC staining. (b) Comparison of mCD64<sup>+</sup> and hCD64<sup>+</sup> cells within tumor tissues by FACS. (Note: CD64 =  $Fc\gamma RI\alpha$ )



Cytokine production by M2 macrophages. To determine whether anti-PD-1 antibodies with Fc $\gamma$ R-binding ability could activate primary M2 macrophages to increase cytokine production in response to the cross-linking with PD-1-positive HuT78/PD-1 cells, the in vitro-differentiated M2 cells (as described in the Methods and Materials) were co-cultured with HuT78/PD-1 cells in the presence of anti-PD-1 Abs at the final concentration of 10 µg/ml in 96-well v-bottomed plates. After overnight co-culture, cell-free supernatants were assayed for cytokines using a multiplex kit (Millipore, HCYTOMAG-60K) and Luminex 200 (Merck). The results of triplicate data points with M2 macrophages from two individuals (donor 136, **a** and donor 173, **b**) were presented as mean + SD (error bar).



The effect of anti-PD-1 antibody treatment on tumor-infiltrating T cells. (a) Representative images of hCD8 and hPD-1 IHC staining on tumor tissues from BGB-A317- or BGB-A317/IgG4<sub>S228P</sub> treated mice. (b-c) Quantified result of hCD8 and hPD-1 IHC staining intensity in indicated groups. The IHC staining intensity was scored by 2 pathologists independently. (d-e) The result of FACS analysis of tumor infiltrated hCD8 and hPD-1 positive cells in indicated groups.



Multiplex immunofluorescence analysis of immune biomarkers in A431 tumor samples. (a) Digitized images of immunofluorescent staining of BGB-A317/IgG4<sub>S228P</sub>treated tumors. The same tumor section sample was stained with anti-mCD64, anti-F4/80, anti-murine CD11b, and neutrophil antibodies. Blue color presents staining of nuclei. Other immune cell biomarkers are coded by colored words on the top of the images. The immune staining photos were analyzed by inForm, and double positive cells are indicated in yellow. (b) Co-localization of BGB-A317/IgG4<sub>S228P</sub> with CD8<sup>+</sup> T cells and mCD64<sup>+</sup> immune cells. Arrows indicate the pockets of cells co-stained positively at the junction sites by the three biomarkers: hCD8, BGB-A317/IgG4<sub>S228P</sub> and mCD64. BGB-A317/IgG4<sub>S228P</sub> was biotinylated and dosed at 10 mg/kg to NOD/SCID mice cotransplanted with A431 cells and human PBMCs twice a week for 3 weeks. One day after the last dose, the mice were sacrificed and the tumor tissues were processed for immunofluorescence staining by anti-hCD8, anti-mCD64 and streptavidin Alexa Fluor® 647 conjugate (Life Technologies). The images were captured by Vectra. (Note: CD64 = FcγRIα)







**Immunofluorescence analysis of cytotoxic T cell (CTL) markers in A431 tumor samples.** The tumor tissue samples of A431 xenograft mice treated with BGB-317 or BGB-A317/IgG4S228P were stained with antibodies against perforin (ab180773, Abcam), CD107a (Lysosomal-associated membrane protein 1, LAMP1, 11215-H08H, Sinobiological), or granzyme B (10345-H08H, Sinobiological) in together with CD8 (SP57, Ventana), respectively. (**a-c**) The representative images of the colocalization of perforin, CD107a or granzyme B with CD8<sup>+</sup> T cells. The immune cell biomarkers are

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color-coded by the words on the right of the images. Blue color presents staining of nuclei. The staining of perforin and granzyme B was only observed on non-tumor cells, while CD107a was also detected on tumor cells. (**d-f**) Quantification of the percentage of perforin<sup>+</sup>/CD8<sup>+</sup>, CD107a<sup>+</sup>/CD8<sup>+</sup> and granzyme B<sup>+</sup>/CD8<sup>+</sup> T cells within total cells in tumor samples treated with BGB-A317 or BGB-A317/IgG4S228P. The horizontal bars and P values indicates the statistical significances between the treatment groups. The images were captured by Vectra and analyzed by inForm.