Fc receptor	WT52	WT52-E	WT52-D
V158 hFcγRIIIa	> 100	1.7 ± 0.03	-
F158 hFcγRIIIa	> 100	2.2 ± 0.03	-
mFcγRIV	11.8 ± 0.04	0.19 ± 0.08	-
hFcγRI	0.65 ± 0.07	0.34 ± 0.04	18.0 ± 0.03
mFcγRI	11.7 ± 0.10	0.52 ± 0.02	-

Suppl. Tab. S1. Binding of WT52, WT52-E and WT52-D to human and mouse Fc gamma receptors. The binding of WT52, WT52-E and WT52-D to human and mouse Fc gamma receptors was determined by ELISA. WT52 is the parental llama-human chimeric antibody. WT52-E contains the S239D/I332E amino acid substitutions in the CH2 domain. These substitutions have been shown to be associated with increased ADCC in mice. WT52-D contains the E233P/L234V/L235A amino acid substitutions in the CH2 domain. These amino acid substitutions have been shown to strongly impair ADCC both *in vitro* and *in vivo*. Binding of antibodies to Fc receptors is expressed as EC_{50} (nM). Values represent the mean ± SD of 2 experiments performed in triplicate.

		EC_{50} (pM)			E_{MAX} (% lysis)		
Cell line	**Donor**	**WT52**	**WT52-E**	**WT52-D**	**WT52**	**WT52-E**	**WT52-D**
MKN-45 (23653)	A	10 ± 2.3	4.8 ± 1.2	-	16 ± 3.1	46 ± 3.3	-
	B	11 ± 3.9	7.4 ± 3.0	-	18 ± 2.0	40 ± 7.3	-
	C	25 ± 5.7	3.4 ± 0.3	-	21 ± 4.7	43 ± 14	-
NCI-H441 (9429)	A	-	87 ± 46	-	-	32 ± 1.1	-
	B	-	99 ± 58	-	-	14 ± 1.6	-
	C	-	210 ± 69	-	-	28 ± 2.4	-

Suppl. Tab. S2. ADCC activity of engineered antibodies. The ADCC activity of WT52, WT52-E and WT52-D were compared in classical ^{51}Cr-release assays using MKN-45 human gastric carcinoma cells, NCI-H441 human lung carcinoma cells and NK cells from 3 different donors. The numbers in parenthesis correspond to the MFI values observed by flow cytometry analysis of MET expression (see Suppl. Tab. S5). ADCC activity is expressed as both EC_{50} (nM) and E_{MAX} (% cell lysis). Values represent the mean of 3 independent measurements.

Assay	Method	Unit	G52 (germlined)	WT52 (parental)
HGF competition	ELISA	IC_{50} (nM)	1.02 ± 1.19	1.24 ± 1.23
		E_{MAX} (%)	94.04 ± 4.05	97.14 ± 5.44
Native MET binding (A549)	Flow cytometry	EC_{50} (nM)	0.44 ± 0.16	0.32 ± 0.01
		E_{MAX} (MFI)	12564 ± 785	11872 ± 1207
Native MET binding (MKN-45)	Flow cytometry	EC_{50} (nM)	0.62 ± 0.11	0.67 ± 0.09
		E_{MAX} (MFI)	22397 ± 6040	22207 ± 5797
Inhibition of HGF-induced MET auto-phosphorylation (A549)	ELISA	IC_{50} (nM)	5.73 ± 0.89	5.45 ± 1.36
		E_{MAX} (%)	75.40 ± 2.60	76.35 ± 1.30

Suppl. Tab. S3. *In vitro* characterization of the germlined G52 antibody. Germlined G52 was compared to parental WT52 in various *in vitro* assays. HGF competition was analyzed by ELISA using a MET-Fc chimera in solid phase and biotinylated HGF plus increasing antibody concentrations in solution. Binding was revealed using HRP-conjugated streptavidin. Binding to native MET was assessed by flow cytometry using A549 human lung carcinoma cells or MKN-45 human gastric carcinoma cells and fluorescein isothiocyanate-conjugated secondary anti-human antibodies. Antagonistic activity was measured by stimulating A549 cells with a fixed concentration of HGF plus increasing concentrations of antibodies. MET phosphorylation was determined by ELISA.

Receptor	G52	ARGX-111
V158 hFcγRIIIa	> 100	2.8 ± 0.05
F158 hFcγRIIIa	> 100	4.2 ± 0.03
mFcγRIV	12.0 ± 0.02	0.89 ± 0.04
hFcγRI	0.62 ± 0.03	0.35 ± 0.03
mFcγRI	11.0 ± 0.01	13.0 ± 0.01
cFcγRIIIa	40.0 ± 14.10	0.43 ± 0.11
hMET	0.11 ± 0.08	0.10 ± 0.10
cMET	0.10 ± 0.11	0.11 ± 0.11
mMET	> 100	> 100

Suppl. Tab. S4. Binding of G52 and ARGX-111 to human, mouse and simian Fc receptors. Binding of G52 and ARGX-111 to the indicated Fc receptors was determined by ELISA. G52 is the germlined version of WT52. ARGX-111 is the afucosylated version of G52 engineered to contain the H433K/N434F substitutions in the CH3 region. These substitutions are known to increase binding to human FcRn at acidic pH while not affecting its affinity at neutral pH, thus enhancing antibody recycling from the sorting endosome. Afucosylation is known to increase affinity for human FcγRIIIa but not for its mouse orthologue FcγRIV. Furthermore, neither G52 nor ARGX-111 cross-reacts with mouse MET (mMET). However, ARGX-111 binds with high affinity to the cynomologous monkey (*Macaca fascicularis*) receptors FcγRIIIa (cFcγRIIIa) and MET (cMET), suggesting that the monkey is a suitable species for testing the toxicological properties of this antibody (see Discussion). Antibody binding is expressed as EC_{50} (nM). Values represent the mean ± SD of 2 experiments performed in triplicate.

Cell line	Histology	EC_{50} (nM)	E_{MAX} (MFI)	MET levels
MKN-45	Gastric ca.	0.63 ± 0.14	23653 ± 12112	very high
EBC-1	NSCLC	1.7 ± 1.98	24729 ± 3076	very high
SNU-5	NSCLC	1.0 ± 0.85	21311 ± 1066	very high
786-O	Kidney	0.15 ± 0.01	26027 ± 236	very high
NCI-H596	NSCLC	0.52 ± 0.11	9930 ± 1231	high
NCI-H441	NSCLC	0.18 ± 0.03	9429 ± 38	high
A498	Kidney	0.15 ± 0.01	13930 ± 618	high
BxPC3	Pancreas ca.	0.21 ± 0.02	12393 ± 166	high
A549	NSCLC	0.55 ± 0.07	6765 ± 96	medium
NCI-H2122	NSCLC	0.48 ± 0.01	6568 ± 441	medium
Raji	Burkitt lymph.	0.17 ± 0.13	6097 ± 1076	medium
U266	Multiple myeloma	0.33 ± 0.13	6097 ± 1487	medium
NCI-H1437	NSCLC	0.38 ± 0.01	3197 ± 267	low
MDA-MB-231	Mammary ca.	0.50 ± 0.02	2609 ± 246	low
L540	Hodgkins lymph.	0.34 ± 0.01	2850 ± 118	low
U87-MG	GBM	0.40 ± 0.03	1504 ± 217	low
TOV-112D	Ovary ca.	no binding	no binding	null

Suppl. Tab. S5. ARGX-111 displays dose-dependent binding to MET-expressing human cell lines. The binding of ARGX-111 to a panel of human tumor cell lines was determined by flow cytometry. Cells were incubated with increasing concentrations of ARGX-111, and binding was revealed using phycoeritrin-conjugated secondary anti-human antibodies. EC_{50} was determined by non-linear regression of MFI values. MET expression levels were divided arbitrarily into 4 sub-groups (very high, high, medium, low) based on MFI. The MET-negative TOV-112D human ovarian carcinoma cell line was used as a negative control. NSCLC, Non-Small Cell Lung Cancer; GBM, Glioblastoma Multiforme. EC_{50} and E_{MAX} values represent the mean ± SD of 3 independent measurements.

Cell line	Donor	EC_{50} (pM)		E_{MAX} (% lysis)	
		ARGX-111	G52	ARGX-111	G52
MKN-45 (23653)	A	3.0 ± 0.3	18 ± 17	45 ± 6.2	17 ± 3.8
	B	3.7 ± 0.6	12 ± 4.2	47 ± 5.3	21 ± 0.8
	C	3.0 ± 0.4	25 ± 8.5	40 ± 7.3	18 ± 4.9
NCI-H441 (9429)	A	15 ± 12	-	40 ± 3.4	-
	B	45 ± 21	-	34 ± 1.9	-
	C	191 ± 237	-	45 ± 5.9	-
A549 (6765)	A	305 ± 270	-	23 ± 2.8	-
	B	66 ± 34	-	26 ± 6.5	-
	C	1136 ± 421	-	13 ± 9.7	-

Suppl. Tab. S6. ARGX-111 promotes ADCC of MET-expressing human tumor cell lines. The ability of ARGX-111 to promote ADCC was analyzed using a panel of human tumor cell lines expressing different MET levels (MKN-45, very high; NCI-H441, high; A549, medium). The numbers in parenthesis correspond to the MFI values observed by flow cytometry analysis of MET expression (see Suppl. Tab. S5). Cells were incubated with increasing concentrations of ARGX-111 in the presence of NK cells, and tumor cell lysis was determined by a standard ^{51}Cr-release assay. An irrelevant IgG1 (not shown) and the fucosylated G52 antibody were used as controls. Each assay was repeated using effector cells derived from three different healthy donors (A-C). Values represent the mean ± SD of 3 independent measurements. E_{MAX} (% lysis) was calculated subtracting the corresponding irrelevant IgG1 control value.

Tumor sample	$CD24^{lo}$	$CD44^{+}$	$CD24^{lo}/CD44^{+}$	$CD24^{lo}/CD44^{+}/MET^{+}$
MDA-MB-231	2.56 %	95.78 %	2.03 %	1.99 %
CRCM168	38.16 %	24.39 %	6.86 %	5.42 %
CRCM174	8.54 %	8.52 %	4.00 %	3.50 %
CRCM389	32.76 %	90.79 %	30.73 %	29.81 %

Suppl. Tab. S7. Mammary carcinoma stem cells express MET. MET expression in the $CD24^{lo}/CD44^{+}$ mammary cancer stem cell population was analyzed in MDA-MB-231 tumors and in a vital library of patient-derived primary breast cancer xenografts (see Fig. 6). Cells derived from enzymatic disgregation of MDA-MB-231, CRCM168, CRCM174 and CRCM389 experimental tumors were analyzed by flow cytometry using phycoeritrin-conjugated anti-CD24 antibodies, fluorescein isothiocyanate-conjugated anti-CD44 antibodies and allophycocyanin-conjugated anti-MET antibodies. Values are expressed as percentage over the total population and represent the mean of 3 independent measurements.