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Supplemental Material to:

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A novel tetravalent bispecific TandAb (CD30/CD16A) efficiently recruits NK cells for the lysis of CD30+ tumor cells

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Figure 1S: Amino acid sequence of the V_H and V_L of the anti-CD16A clone LSIV21.

Figure 2S: SDS-PAGE and SE-HPLC of CD30/CD16A TandAb. (A) Reducing and non-reducing SDS-PAGE. Determination of the apparent molecular weight of CD30/CD16A TandAb (2 µg per lane) was performed under reducing and non-reducing conditions in 10% Tricine SDS-PAGE and subsequent staining with the NuPAGE Colloidal Blue Staining Kit (Invitrogen, catalog number LC6025). Molecular weight marker Mark12 unstained standard (Invitrogen, catalog number LC5677) was used to estimate TandAb mass. The main protein species, with an apparent molecular weight of approximately 50 kDa, represents the monomeric polypeptide of the CD30/CD16A TandAb. **(B)** Analysis of CD30/CD16A TandAb homodimer, aggregates and fragment by SE-HPLC. CD30/CD16A TandAb preparations were analyzed on a Superdex 200 column employing 40 mM Na_2HPO_4, pH 7.0 buffer as the mobile phase at a flow rate of 0.5 mL/min with a HPLC system (Agilent HP1100/HP1200). The results from the SE-HPLC revealed a purity of the CD30/CD16A TandAb of 96.2% homodimer. The content of aggregates was reported as 3.8%.

Figure 3S: Kinetics of target cell lysis mediated by CD30/CD16A TandAb, diabody, anti-CD30 IgG and Fc-enhanced anti-CD30 IgG in cytotoxicity assays. $1x10^4$ calcein-labeled KARPAS-299 target cells were incubated for the indicated time periods with increasing concentrations of CD30/CD16A TandAb, CD30/CD16A diabody, anti-CD30 IgG and Fc-enhanced anti-CD30 IgG together with freshly isolated human NK cells at an E:T ratio of 5:1. Percent specific target cell lysis was calculated from the fluorescent calcein released into the cell culture supernatant from apoptotic target cells and plotted as mean and

SD of duplicates in the diagram together with the sigmoidal-dose response curves determined by non-linear regression.

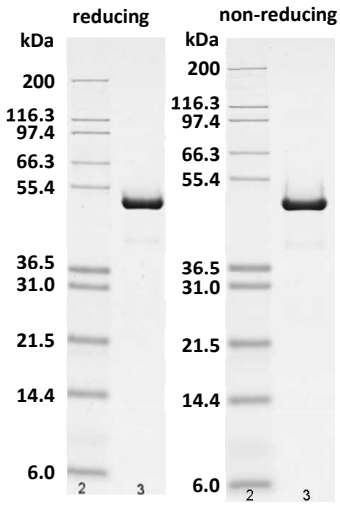
Figure 1S

V_H:
QVQLVQSGAEVKKPGESLKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGG
STSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGSAYYYDFADYWGQ
GTLVTVSS

V_L:
SYVLTQPSSVSVAPGQTATISCGGHNIGSKNVHWYQQRPGQSPVLVIYQDNKRPSGIP
ERFSGSNSGNTATLTISGTQAMDEADYYCQVWDNYSVLFGGGTKLTVL

Figure 2S

A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non-reducing
kDa
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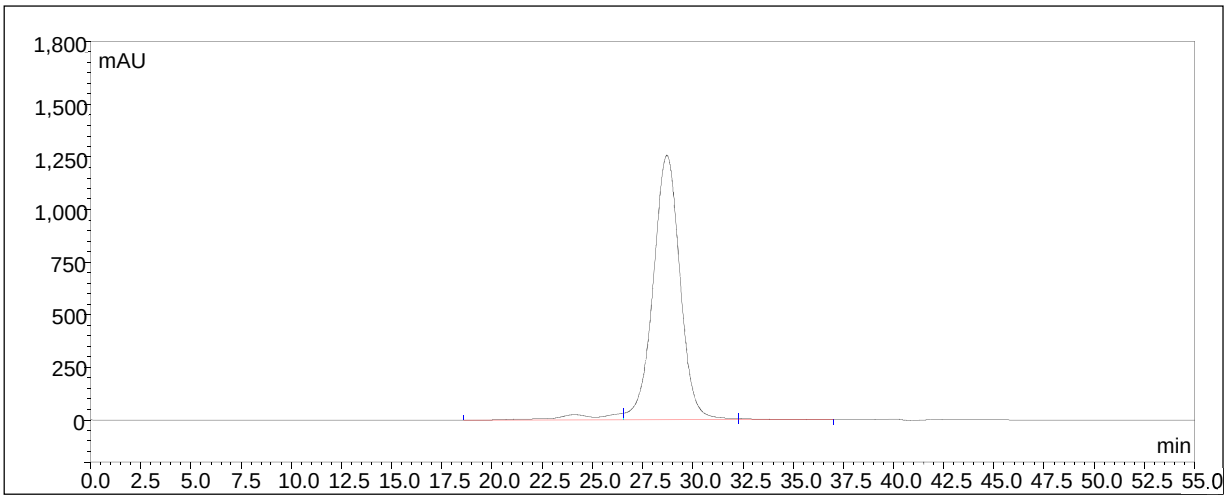
66.3
55.4
36.5
31.0
21.5
14.4
6.0
2
3

B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1,250
1,000
750
500
250
0
min
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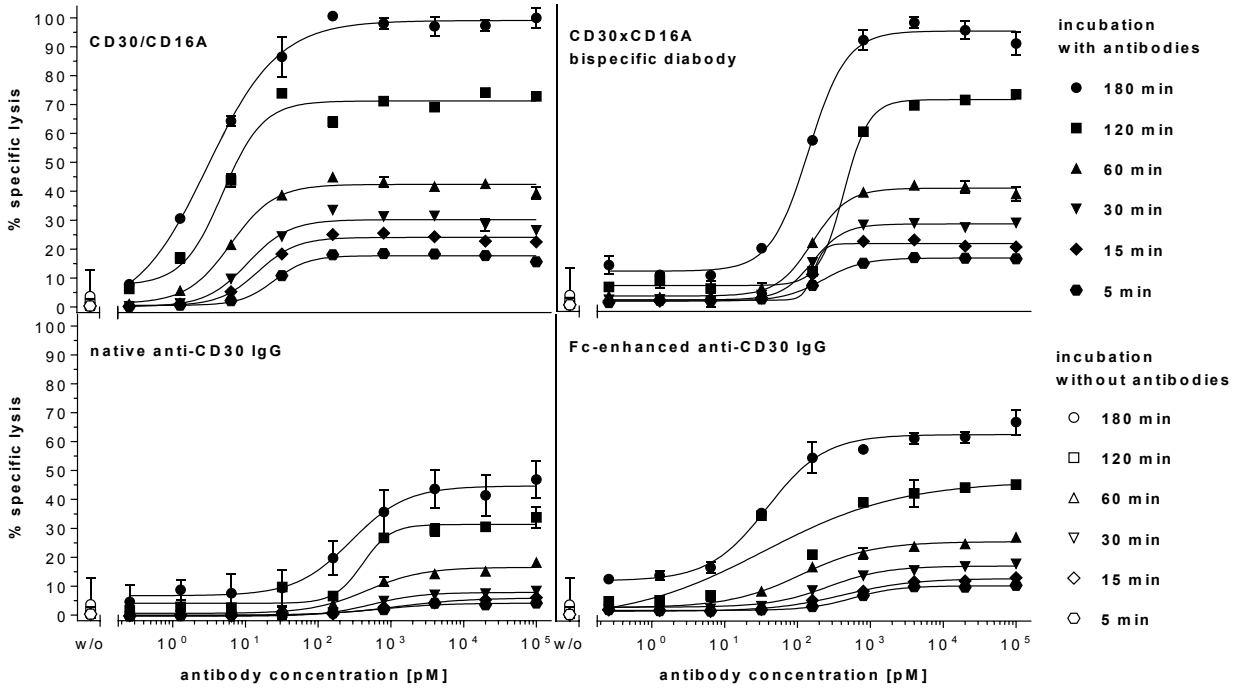
Figure 3S


CD30/CD16A
CD30xCD16A
bispecific diabody
native anti-CD30 IgG
Fc-enhanced anti-CD30 IgG
% specific lysis
antibody concentration [pM]
w/o
incubation
with antibodies
180 min
120 min
60 min
30 min
15 min
5 min
incubation
without antibodies
180 min
120 min
60 min
30 min
15 min
5 min

SUPPLEMENTARY Table 1S: Affinities of parental and affinity-matured anti-CD16A scFv

anti-CD16A scFv clone		K_D [nM]
parental	50NI	14423
affinity matured	LSIII49	651
	LBIII3	2406
	LBIII5	913
	LBIII6	501
	LSIV14	594
	LSIV21	393