

# AFM13 Is the Most Advanced Bispecific NK-Cell Engaging Antibody in Clinical Development Substantially Enhancing NK-Cell Effector Function and Proliferation

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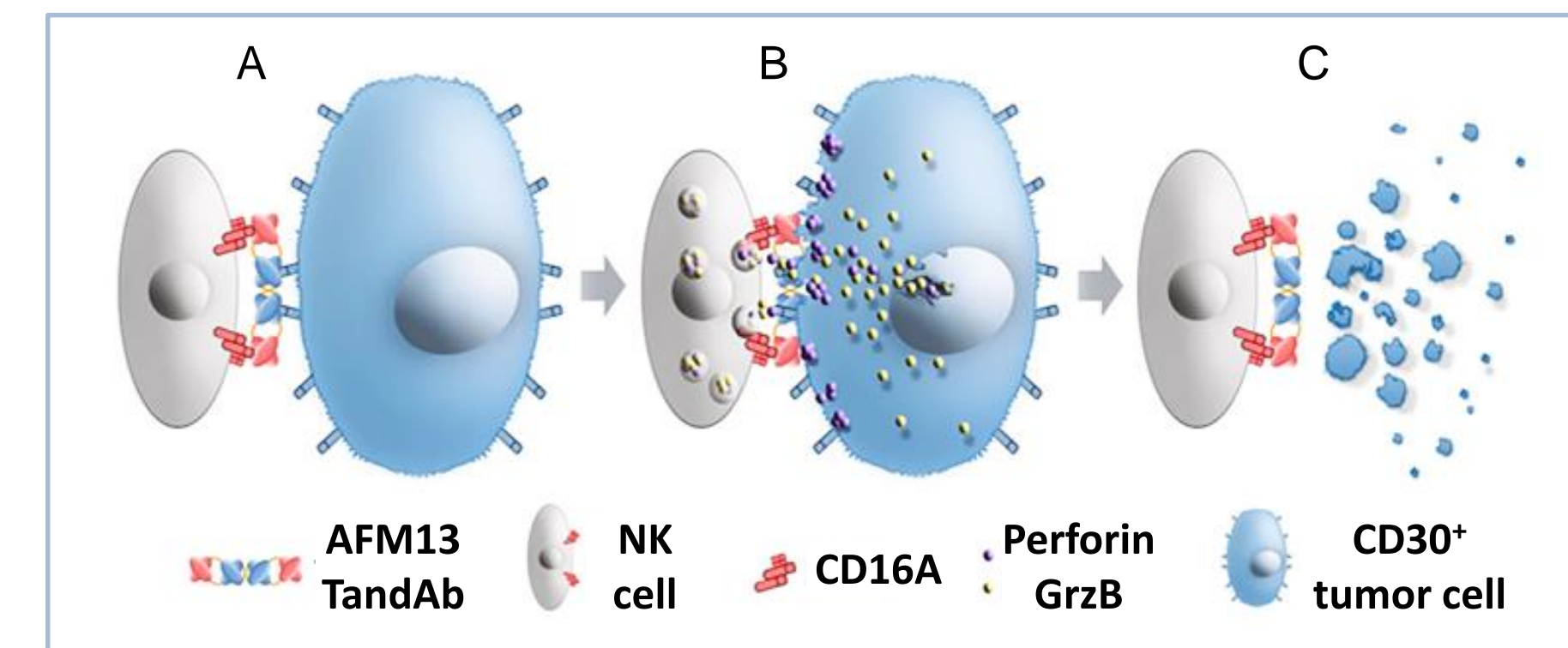
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## Introduction

AFM13 is an NK-cell engaging CD30/CD16A bispecific tetravalent TandAb antibody currently in Phase 2 clinical development in Hodgkin lymphoma (HL) and other CD30<sup>+</sup> malignancies. It engages NK-cells through CD16A with high affinity and specificity and confers significantly stronger NK-cell activation when compared to other therapeutic antibodies.

We have previously shown synergistic efficacy when NK-cell activation by AFM13 is combined with check-point modulation such as anti-PD-1 treatment, which is known to unleash T-cell and NK-cell activity. The goal of this study was to identify further strategies for combination treatments and biomarkers that potentially indicate NK-cell responses to AFM13 treatment.

## Tumor-targeting of NK-cells by AFM13

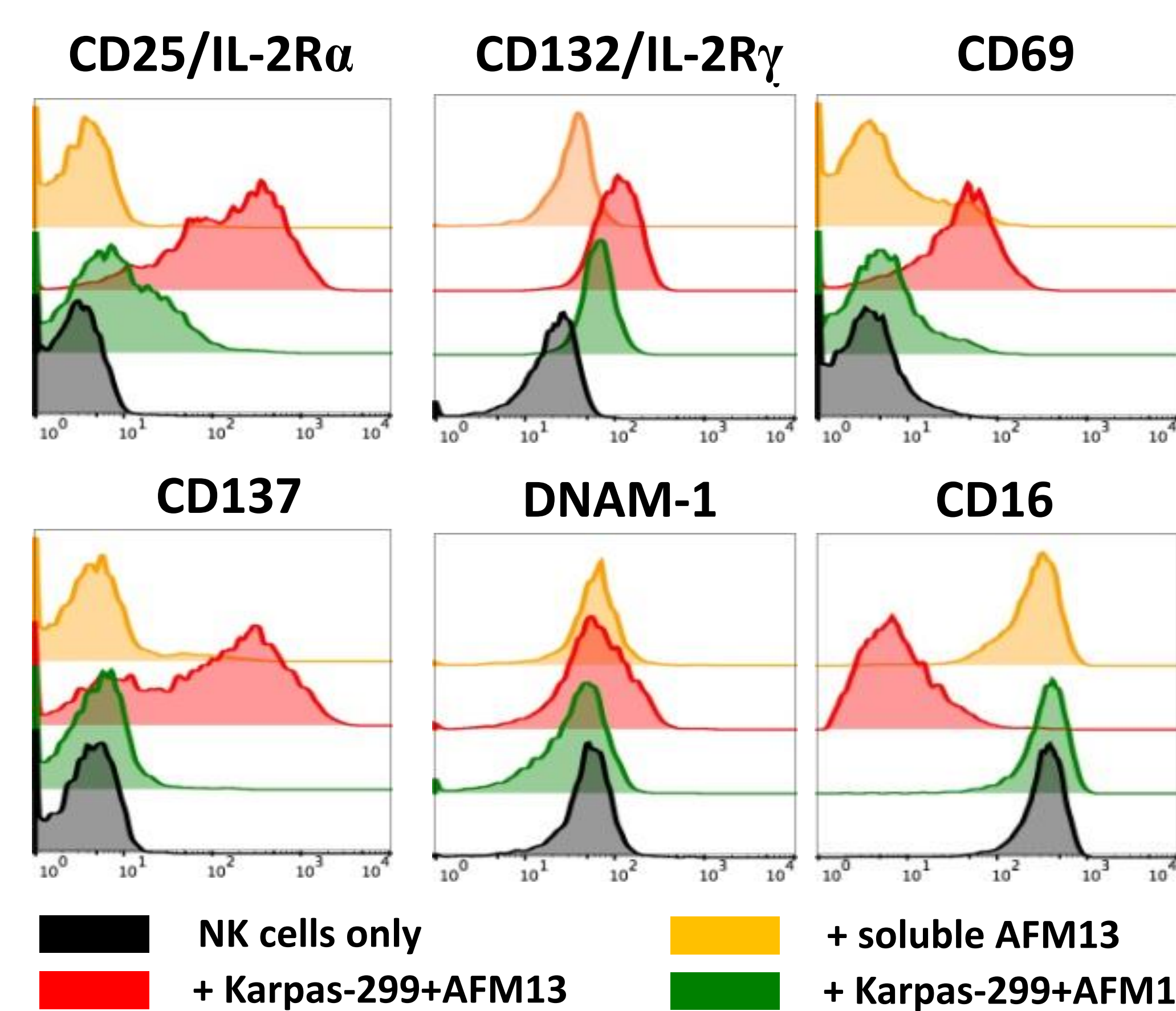


A. AFM13-mediated engagement of CD16A<sup>+</sup> NK-cells and CD30<sup>+</sup> tumor cells. B. NK-cell activation and release of perforin and granzyme B (GrzB). C. Induction of tumor cell death

## Key results

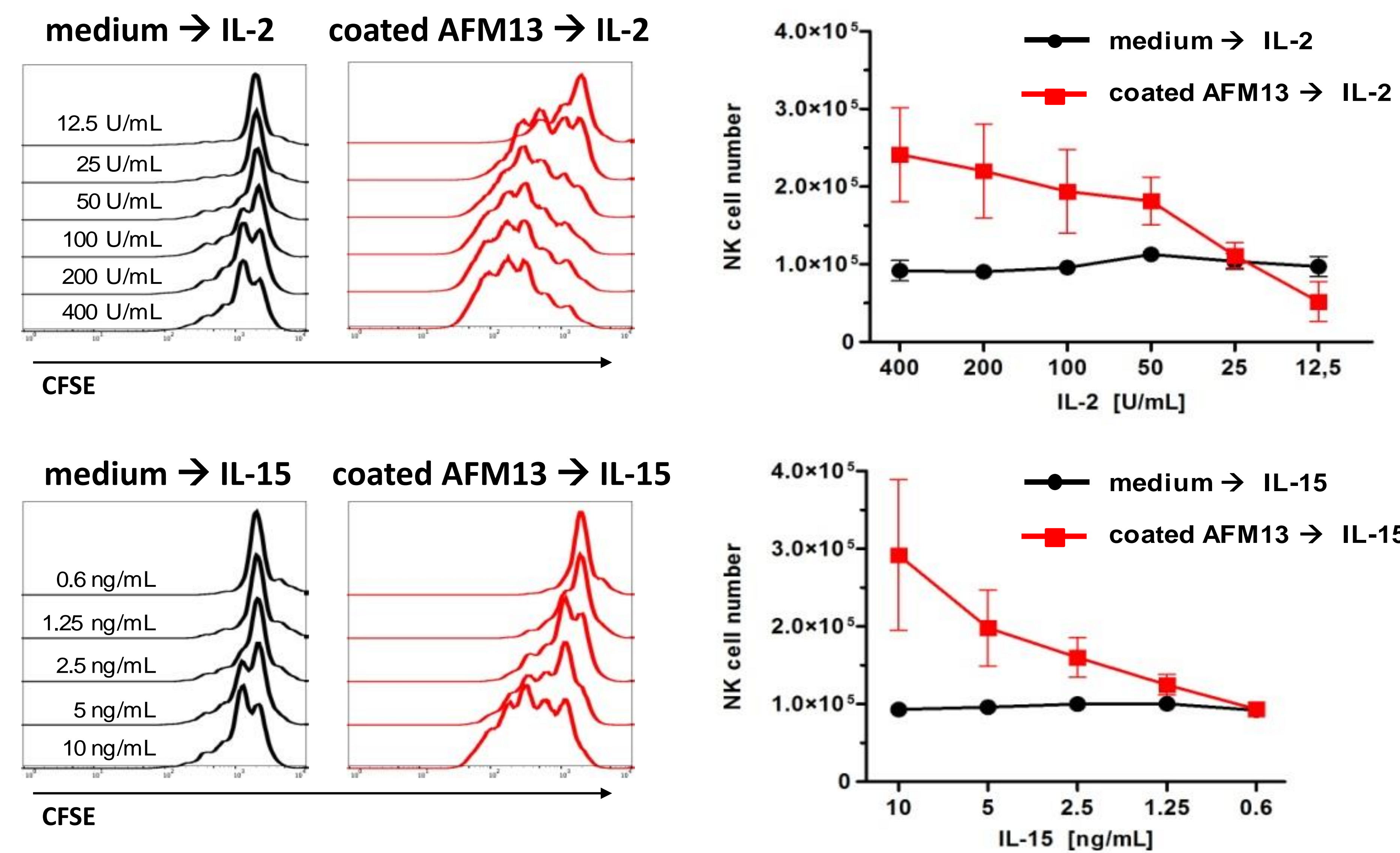
1. AFM13 amplifies IL-2 and IL-15-mediated NK-cell proliferation
2. AFM13 is more potent than anti-CD30 IgG
3. AFM13 enhances NK-cell cytotoxicity to CD30<sup>+</sup> tumor cells
4. AFM13-activated NK-cells can recover from transient exhaustion upon culture in IL-2

### 1. AFM13 induces an activated NK-cell phenotype



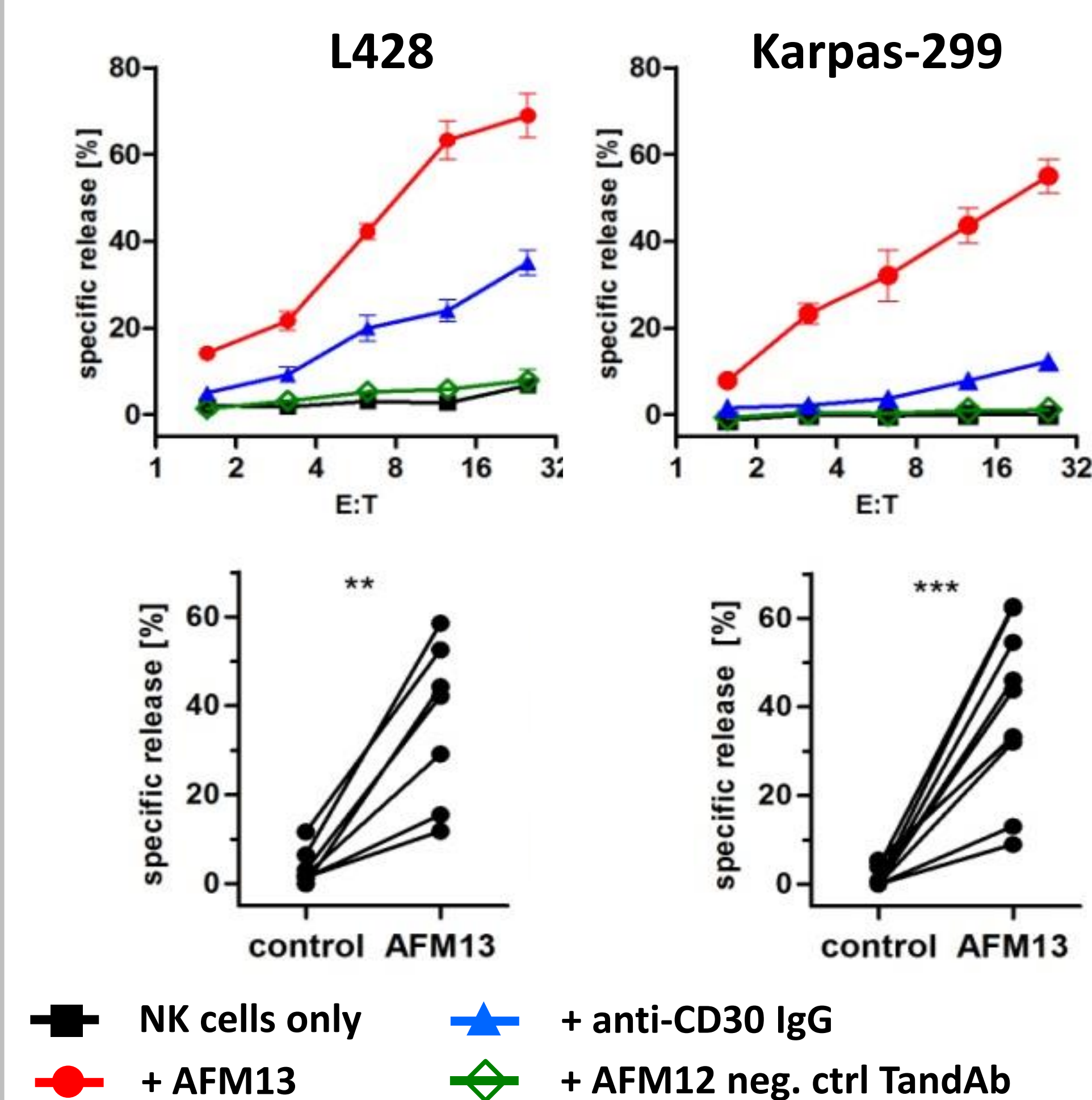
Purified human naïve NK-cells were co-cultured for 48 h with CD30<sup>+</sup> Karpas-299 cells (anaplastic large cell lymphoma) in the presence of the AFM13 (0.1 µg/mL). Controls: NK-cells only; NK-cells treated with soluble AFM13; NK-cells co-cultured with Karpas-299 cells with CD16AxCD19 AFM12 TandAb as a negative control. After the co-culture, the expression of NK-cell receptors was analyzed by FACS. Comparable NK-cell activation was achieved after co-culture with CD30<sup>+</sup> L428 cells (classical Hodgkin lymphoma) in the presence of AFM13.

### 2. AFM13 amplifies NK-cell proliferation to IL-15 and low IL-2



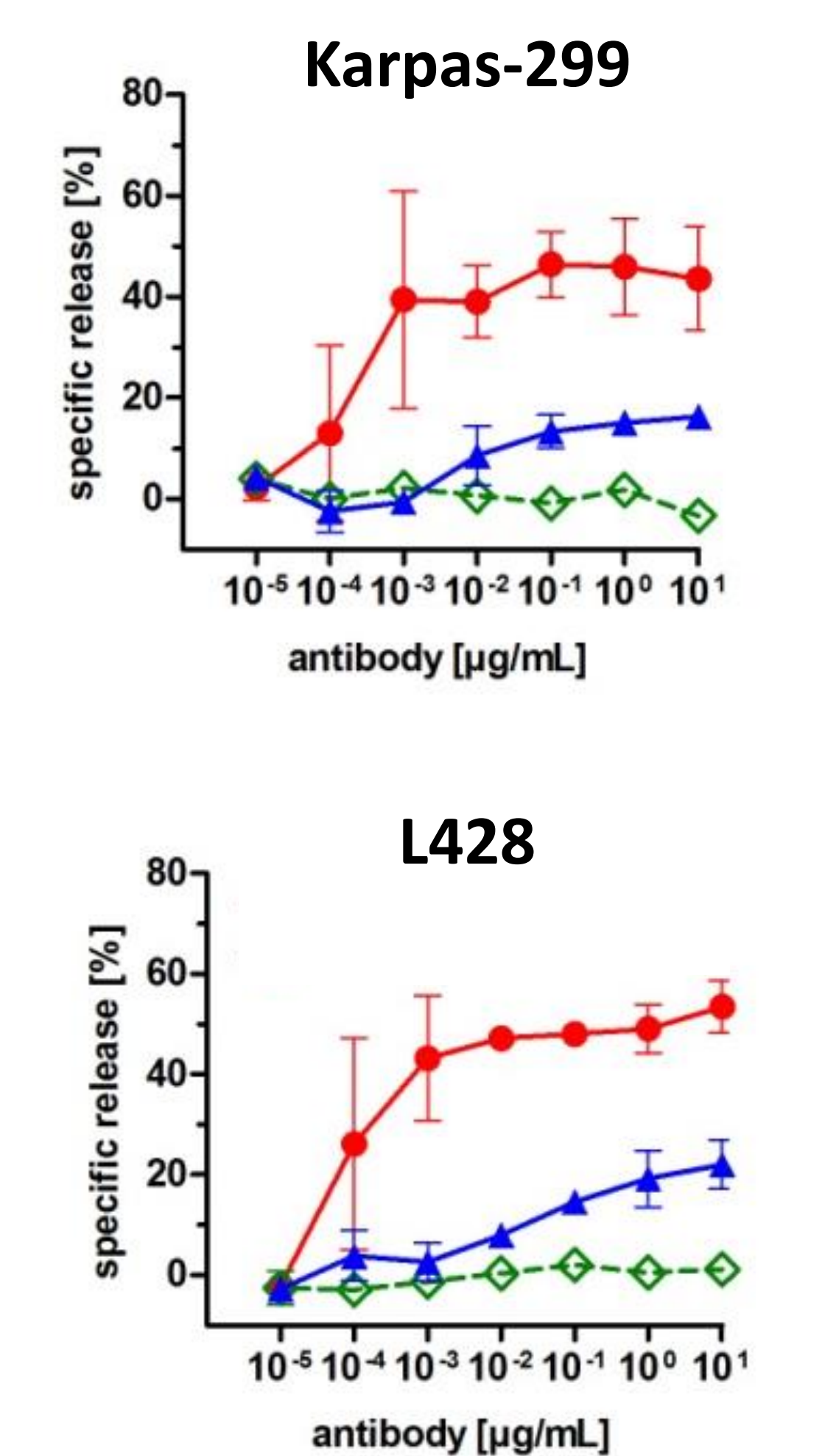
The sensitivity of NK-cells to IL-2 and IL-15 was examined in CFSE-labeled NK-cells that were activated by plastic-coated AFM13 or medium as a negative control for 18 h and afterwards replated and incubated for 5 days with IL-2 (12–400 U/mL) or IL-15 (0.6–10 ng/mL). CFSE dilution was compared to only IL-2-treated NK-cells. Absolute NK-cell numbers were monitored by FACS.

### 3. AFM13 improves killing of CD30<sup>+</sup> tumor cells by NK cells



In short-term exposure experiments (4 h), NK-cell cytotoxicity was measured in <sup>51</sup>Cr release assays against L428 or Karpas-299 cells in the presence of AFM13 at escalating effector:target (E:T) ratios. Controls: no antibody; AFM12 negative control TandAb. Below, cumulative cytotoxicity data (E:T 6:1) with/without AFM13 are shown.

### 4. AFM13 is more potent than anti-CD30 IgG

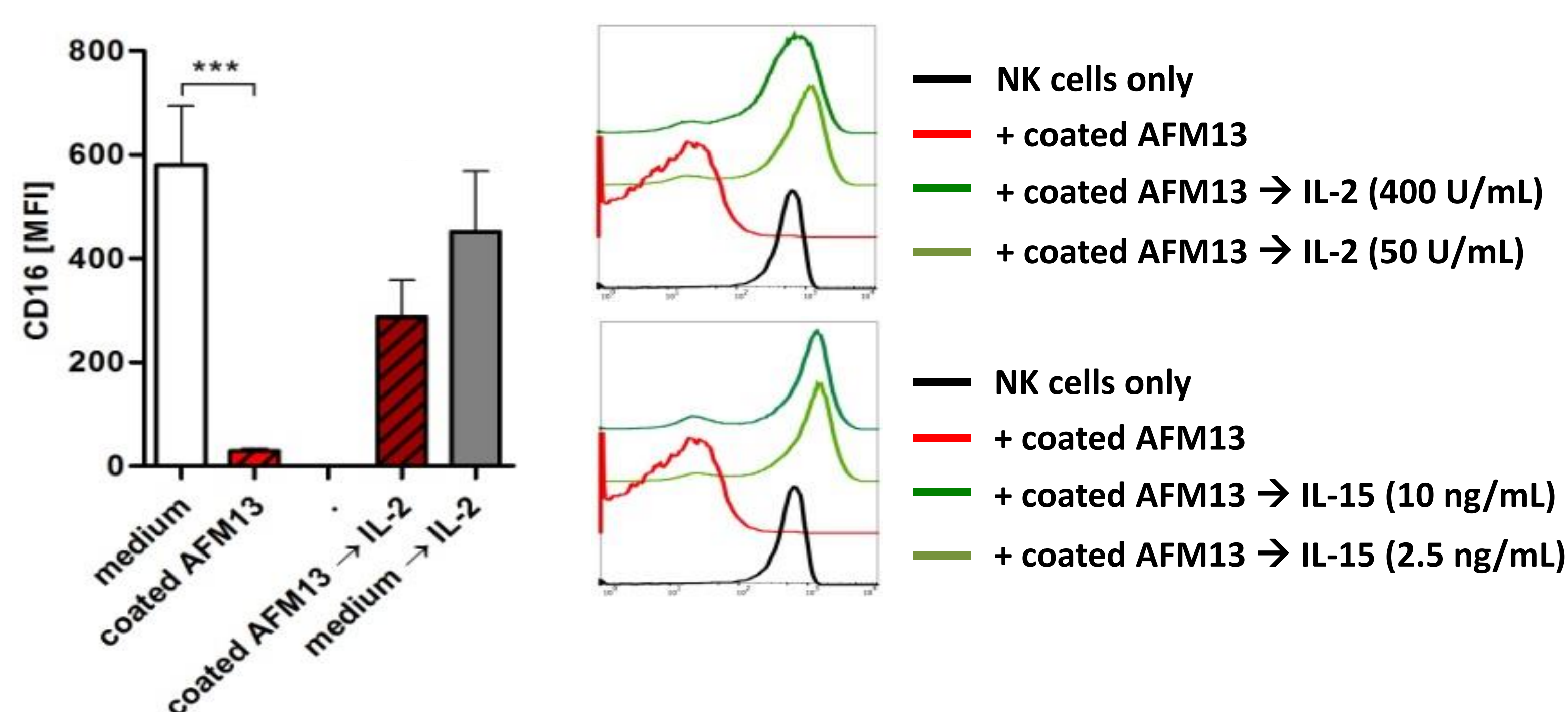


Legend for cytotoxicity graphs:  
● + AFM13    ▲ + anti-CD30 IgG  
◇ + AFM12 neg. ctrl TandAb

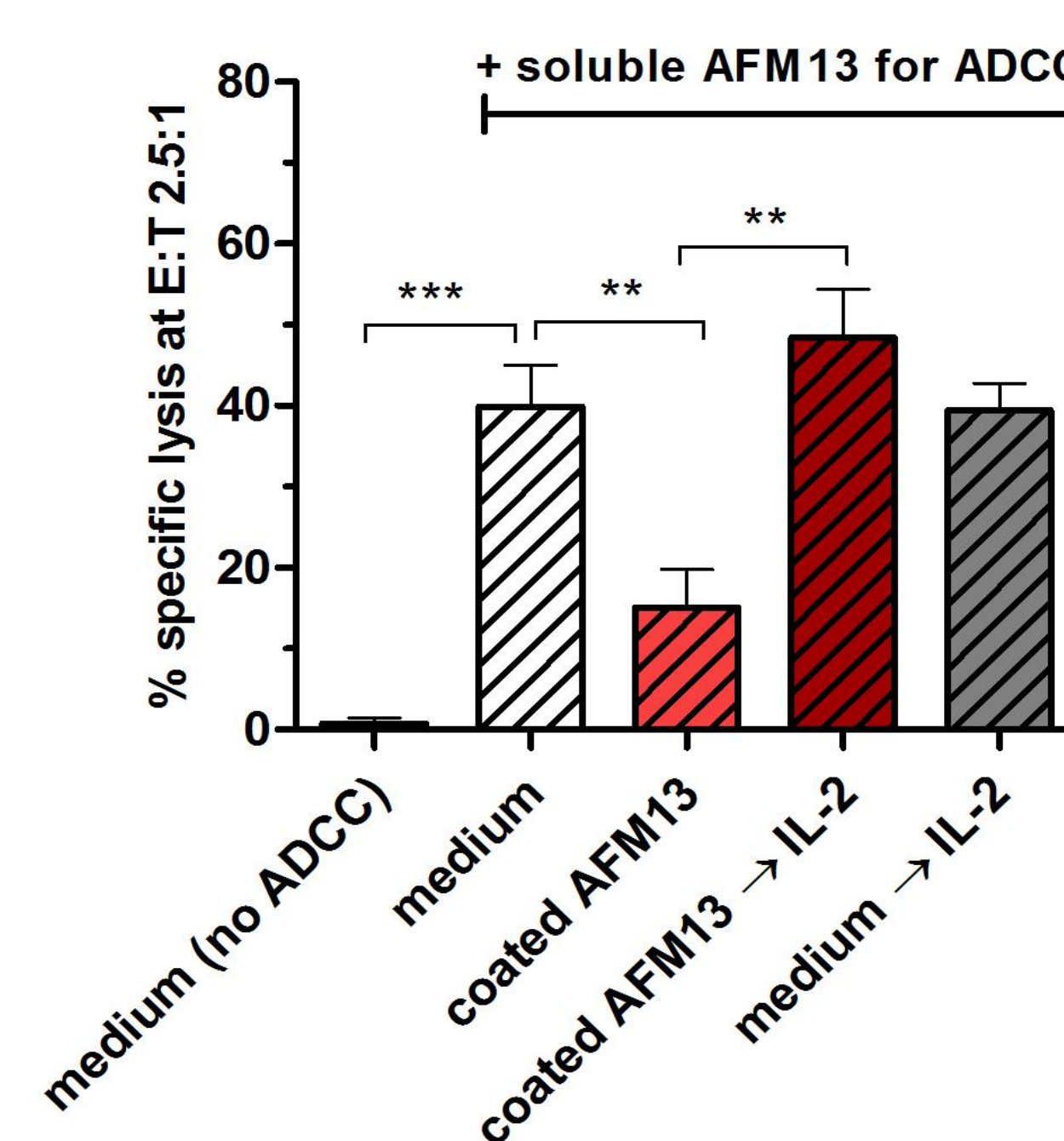
In a <sup>51</sup>Cr release assay, the potency of AFM13 was compared to the parental anti-CD30 mAb against CD30<sup>+</sup> Karpas-299 and L428 cells. Data are depicted for a range of 10<sup>-6</sup>–10 µg/mL (E:T 25:1). Negative control AFM12 TandAb.

### 5. Recovery of AFM13-mediated NK-cell cytotoxicity after pre-activation by AFM13

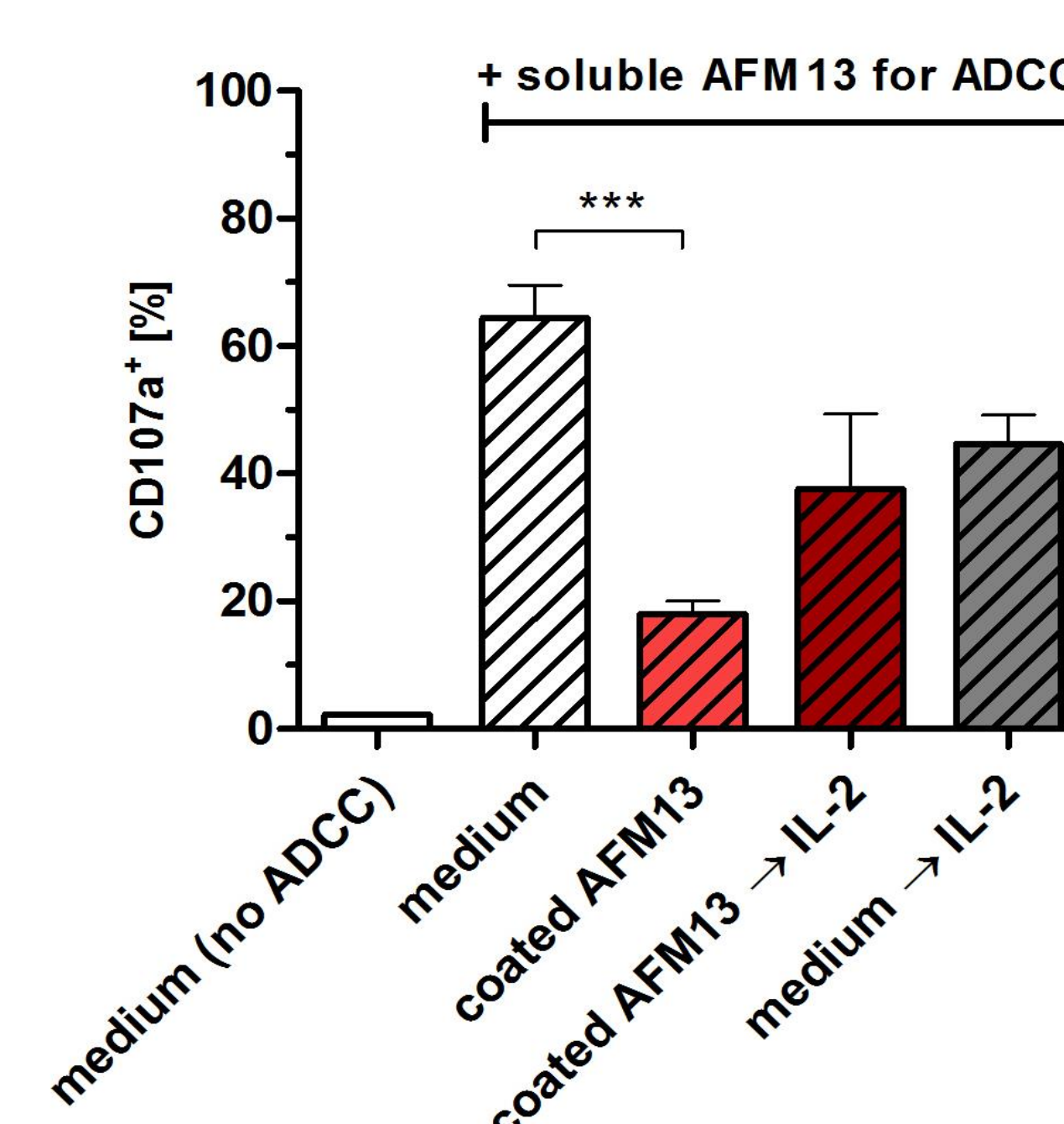
#### Recovery of CD16 expression



#### Recovery of AFM13-mediated Karpas-299 lysis

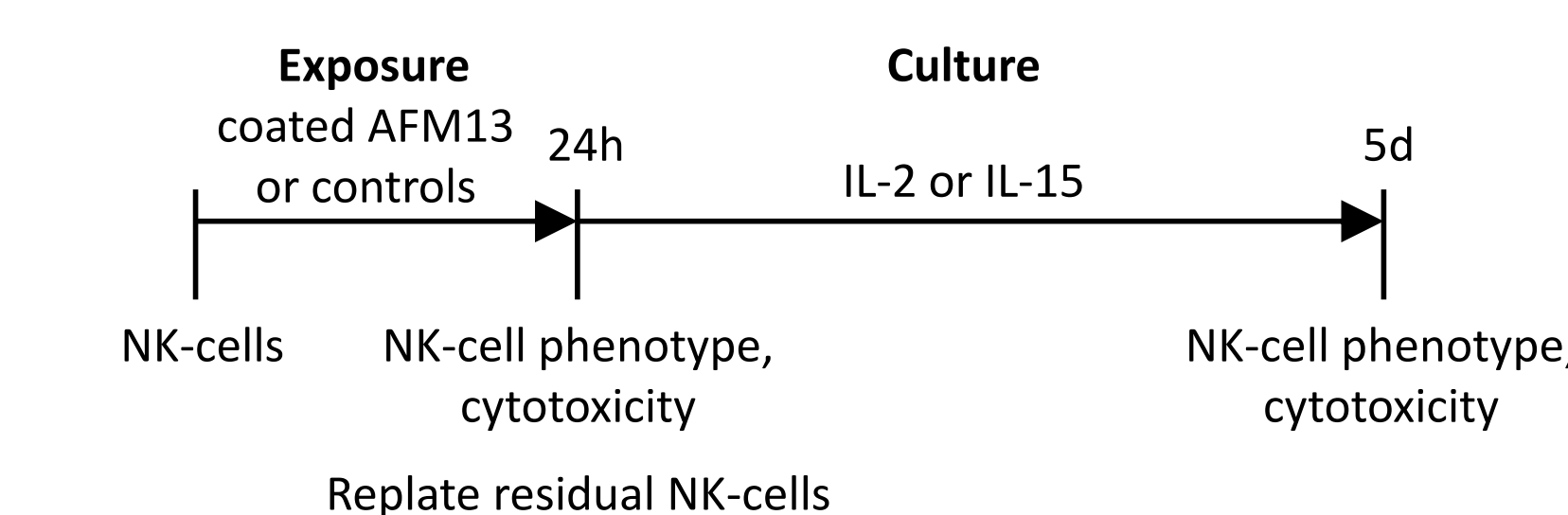


#### Recovery of AFM13-mediated degranulation



NK-cells were activated on plastic-coated AFM13 (or medium as a negative control). After 24 h, NK-cells were harvested and tested as follows: CD16 expression was analyzed by FACS. NK-cell cytotoxicity and CD107a expression (a marker for NK-cell degranulation) was assessed after 4 h co-culture with Karpas-299 cells in the presence/absence of AFM13 by FACS and <sup>51</sup>Cr release assays, respectively.

Residual NK cells were replated and incubated with IL-2 (400 U/mL) or IL-15 (10 ng/mL), or as indicated. After 5 days, NK-cell CD16 expression as well as cytotoxicity and CD107a expression was assessed in response to Karpas-299 cells in the presence/absence of AFM13.



## Disclosures

- U.W., T.G., A.K., J.K. and M.T. are employees of Affimed GmbH.