1   **Supplemental Data**

2   **Supplemental Methods**

3   **Kinetic/affinity analysis and fitting procedure for SPR**

4   Kinetic/affinity analysis of FK binding to immobilized CD46 was performed using

5   three models in combination with the Biacore T100 evaluation software version 2.0.3.

6   A first included a 1:1 fitting model provided by the Biacore T100 evaluation software

7   itself, which failed to describe the obtained binding data satisfyingly. Second, two

8   trivalent models were tested and included the equations described by Lortat-Jacob *et*

9   *al*. (2) and a variation of the equations described by Fournel *et al*. (1) (Johan

10   Hoebeke, personal communication). Both trivalent models resulted in reasonable

11   good fitting for Ad11- and 35-FK to immobilized CD46 judged by χ^2 values, but they

12   showed relatively large errors for Ad3- and Ad7-FK binding kinetics. Third, a two-

13   stage reaction model was applied to globally fit the binding data, which provided the

14   lowest χ^2 values for all four FK binding reactions. In this model, analyte (A) binds to

15   ligand (B) to form the complex AB, then complex AB changes to ABx, which cannot

16   dissociate directly to A+B. The net reactions are described by equations 1–6, where

17   σ_{kd1}, σ_{kd2}, σ_{ka1}, and σ_{ka2} are standard deviation for individual k_{d1}, k_{d2}, k_{a1}, and k_{a2},

18   respectively. σKD is the standard deviation for overall K_D.

$$A + B \underset{kd_1}{\overset{ka_1}{\Leftrightarrow}} AB \underset{kd_2}{\overset{ka_2}{\Leftrightarrow}} ABx \text{ (Eq.1)}$$
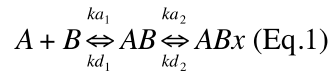
$$A = conc$$

$$B[0] = R\max, \frac{dB}{dt} = -(ka_1 * A * B - kd_1 * AB) \text{ (Eq.2)}$$

$$AB[0] = 0, \frac{dAB}{dt} = (ka_1 * A * B - kd_1 * AB) - (ka_2 * AB - kd_2 * ABx) \text{ (Eq.3)}$$

19

$$ABx[0] = 0, \frac{dABx}{dt} = (ka_2 * AB - kd_2 * ABx) \text{ (Eq.4)}$$

$$\text{Total response}: AB + ABx + RI$$

$$KD = \frac{kd_1}{ka_1} \frac{kd_2}{kd_2 + ka_2} \text{ (Eq.5)}$$

$$\sigma KD = \sqrt{(\frac{kd_2}{ka_1(kd_2 + ka_2)}\partial_{kd_1})^2 + (\frac{kd_1(kd_2 + ka_2) - kd_1kd_2}{ka_1(kd_2 + ka_2)^2}\sigma_{kd_2})^2}$$
$$+ (\frac{kd_1kd_2}{ka_1^2(kd_2 + ka_2)}\sigma_{ka_1})^2 + (\frac{kd_1kd_2}{ka_1(kd_2 + ka_2)}\sigma_{ka_2})^2 \text{ (Eq.6)}$$

20

Legends to supplemental figures

SFig. 1. Flow cytometry profiles of CD46 and DSG-2 expression. CD46 was analyzed in A549 human lung cells, parental rodent CHO and three different stable and clonal CHO-CD46 transfectants with increasing CD46 levels (A), in human 16HBE14o bronchial epithelial cells (C), or in parental M010119 and stable transfected M010119-eGFP-CD46#8 cells (E). Numbers after slash indicate MFI values resulting from utilizing the MCI20.6 anti-CD46 antibody (A, C), or GB24 anti-CD46 (stains both, endogenous and eGFP-tagged CD46) (E). Controls using isotype antibody were in the range from 0.8 to 1 (not shown). DSG-2 was analyzed in A549 cells and in parental M010119 cells (B), in 16HBE14o cells (D) and stable transfected M010119-eGFP-CD46#8 cells (F) with 6D8 anti-DSG-2 antibody.

SFig. 2. Control for cross-linking experiment shown in Fig. 3 with CARex-huFc replacing CD46ex-huFc. (A) CHO-CD46#2 cells or (B) A549 cells were pre-incubated for 1 h in the cold using the indicated concentrations of adapter CARex-huFc alone or in combination with a 2-fold increase series of goat-anti human Fc antibody. Following addition of the different eGFP-expressing vectors for another 1 h, cells were washed and analyzed 48 h p.i.

SFig. 3. Analysis of recombinant Ad-FK and CD46ex-huFc proteins. (A, B) Individual FK proteins were produced using the Baculovirus expression system. Purified FK proteins from Ad3, Ad5, Ad7, Ad11, and Ad35 were analyzed by either 12.5% reducing PAGE and sypro ruby red staining (A), or by 10% native PAGE and Coomassie Blue staining (B). (C) Analysis of recombinant CD46ex-huFc. Three μg of purified CD46ex-huFc were analyzed by 10% native PAGE and Coomassie Blue staining.

49   STable 1. Overview kinetics/affinity analysis of Ad-FKs binding to immobilized CD46ex-huFc

Annalyte / RU chip / binding experiment	Flow rate (ul / min)	k_{a1} ($M^{-1}s^{-1}$) [a]	k_{d1} (s^{-1})	k_{a2} (s^{-1})	k_{d2} (s^{-1})	% χ^2/R_{max}	K_D (M)
Ad3-FK / 1121 / 1	55	2.04×10^5 (0.0014)	7.11×10^{-4} (0.019)	1.97×10^{-3} (0.0037)	1.64×10^{-4} (0.0022)	0.64 - 1.31	2.68×10^{-10} (0.0093)
Ad3-FK / 1121 / 2	30	1.92×10^5 (0.0013)	7.18×10^{-4} (0.020)	2.03×10^{-3} (0.0037)	1.65×10^{-4} (0.0022)	0.66 - 1.28	2.81×10^{-10} (0.0101)
Ad3-FK / 2630 / 3	30	2.93×10^5 (0.0035)	6.36×10^{-4} (0.019)	2.02×10^{-3} (0.0046)	1.98×10^{-4} (0.0029)	1.36 - 2.78	1.94×10^{-10} (0.0079)
Ad7-FK / 1121 / 4	30	4.20×10^4 (0.012)	3.39×10^{-4} (0.059)	3.77×10^{-3} (0.036)	5.08×10^{-5} (0.061)	0.03 - 0.50	1.07×10^{-10} (0.0249)
Ad7-FK / 2630 / 5	30	2.44×10^5 (0.0047)	5.88×10^{-4} (0.015)	1.18×10^{-3} (0.0035)	2.58×10^{-4} (0.0064)	0.44 - 0.93	4.32×10^{-10} (0.0195)
Ad7-FK / 2630 / 6	30	1.44×10^5 (0.0046)	3.30×10^{-4} (0.011)	8.67×10^{-4} (0.048)	2.90×10^{-4} (0.015)	0.25 - 0.71	5.72×10^{-10} (0.0419)
Ad11-FK / 1121 / 7	30	9.04×10^5 (0.021)	9.86×10^{-5} (0.091)	6.96×10^{-4} (0.11)	7.57×10^{-5} (0.39)	2.86 - 5.05	1.07×10^{-11} (0.0531)
Ad11-FK / 2630 / 8	30	7.96×10^5 (0.012)	3.93×10^{-4} (0.018)	1.03×10^{-3} (0.0054)	1.28×10^{-4} (0.01)	0.27 - 3.72	5.47×10^{-11} (0.0527)
Ad11-FK / 2630 / 9	30	1.08×10^6 (0.00086)	4.90×10^{-4} (0.032)	1.45×10^{-2} (0.023)	2.77×10^{-4} (0.046)	3.52 - 4.19	8.48×10^{-12} (0.199)
Ad35-FK / 1121 / 10	30	3.54×10^6 (0.0088)	2.16×10^{-4} (0.015)	1.12×10^{-3} (0.0081)	1.06×10^{-4} (0.0037)	0.12 - 3.03	5.23×10^{-12} (0.0751)
Ad35-FK / 2630 / 11	30	2.12×10^6 (0.0034)	7.96×10^{-4} (0.030)	1.33×10^{-3} (0.0042)	1.51×10^{-4} (0.0049)	0.27 - 3.08	3.85×10^{-11} (0.0222)
Ad35-FK / 2630 / 12	30	3.60×10^6 (0.0057)	3.85×10^{-4} (0.019)	1.30×10^{-3} (0.0061)	1.31×10^{-4} (0.0076)	0.16 - 4.76	9.76×10^{-12} (0.0833)

50

51   [a] values in parentheses are errors of the fitting procedure

52

References

1. **Fournel, S., S. Wieckowski, W. Sun, N. Trouche, H. Dumortier, A. Bianco, O. Chaloin, M. Habib, J. C. Peter, P. Schneider, B. Vray, R. E. Toes, R. Offringa, C. J. Melief, J. Hoebeke, and G. Guichard.** 2005. C3-symmetric peptide scaffolds are functional mimetics of trimeric CD40L. Nat Chem Biol **1:**377-82.

2. **Lortat-Jacob, H., E. Chouin, S. Cusack, and M. J. van Raaij.** 2001. Kinetic analysis of adenovirus fiber binding to its receptor reveals an avidity mechanism for trimeric receptor-ligand interactions. J Biol Chem **276:**9009-15.