Supplemental material

Supplementary Figure S1

A Monoclonal antibody to chCAR (mAb12-36) recognizes D1, while polyclonal antibodies (Rb25 or Rb40) bind to D1 and D2 of chCAR. Extracts of E. coli BL21 containing GST-fusion proteins were run on SDS PAGE followed by Western blotting using antibodies to chCAR.

B Silver or coomassie stained SDS PAGE (with or without the reducing agent β-mercaptoethanol as indicated) of recombinant CAR polypeptides used in binding studies or adhesion/neurite outgrowth assays. CAR polypeptides are shown schematically. Ig domains are shown as loops linked by disulphide bonds. N-linked sugars are indicated by a Y. The extracellular CAR polypeptides chCAR-D1D2-Fc, mCAR-D1D2-w/oFc, or chCAR-D1D2-w/oFc were derived from supernatants of COS-7 cells. Secreted Fc fusion proteins were harvested by protein A sepharose 4B, and the Fc portion was proteolytically cleaved. chCAR-D1D2-w/oFc-decl was generated by deglycosylation by PNGaseF.

mCAR-D1D2, mCAR-D1 and mCAR-D2 were produced in E. coli BL21 as GST tagged proteins. After purification, the GST part was proteolytically cleaved, and CAR domains were further purified by ion exchange and/or gel filtration chromatography.

C Amino acid sequence of chCAR obtained by cDNA cloning. Peptide sequences obtained by Edmann degradation of the 36 kDa CAR component or of tryptic digests of the 36 kDa band of immunoaffinity purified chCAR using mAb12-36 are printed in bold.

Supplementary Figure S2

Chick tectal cells were cultivated on immobilized TN-C or TN-R in the presence of 0.48 mg/ml of the fiber knob Ad2 C428N for 24 h. Total neurite length was measured per view

field. Application of the fiber knob resulted in an increase of the total neurite length. Error bars indicate SEM. **p < 0.005, ***p < 0.0005; Mann-Whitney U Test.

Supplementary Figure S3

Characterization of FN fragments FN30 and FN40.

A The commercially available product FN40 (Calbiochem) is heterogeneous showing three major bands in SDS PAGE. These components were further separated by size exclusion chromatography and analyzed in SDS PAGE by coomassie staining. Fraction 14 and fraction 19 show major bands at 40 or 30 kDa, respectively. Both are able to interact with CAR. Fraction 14 (FN40) and fraction 19 (FN30) were used in binding assays. The molecular masses of standard proteins are indicated at the left of the panel.

B For further characterization of FN30 its exact molecular mass was determined by mass spectrometry (30,926.00).

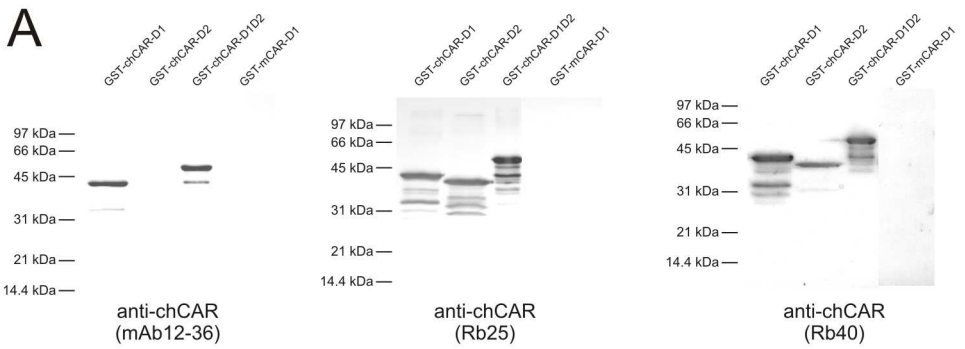
C The N-terminus of FN30 (fraction 19) was determined by Edman degradation and is printed in bold within the corresponding hFN sequence (isoform 9). The calculated mass is 30,924.3.

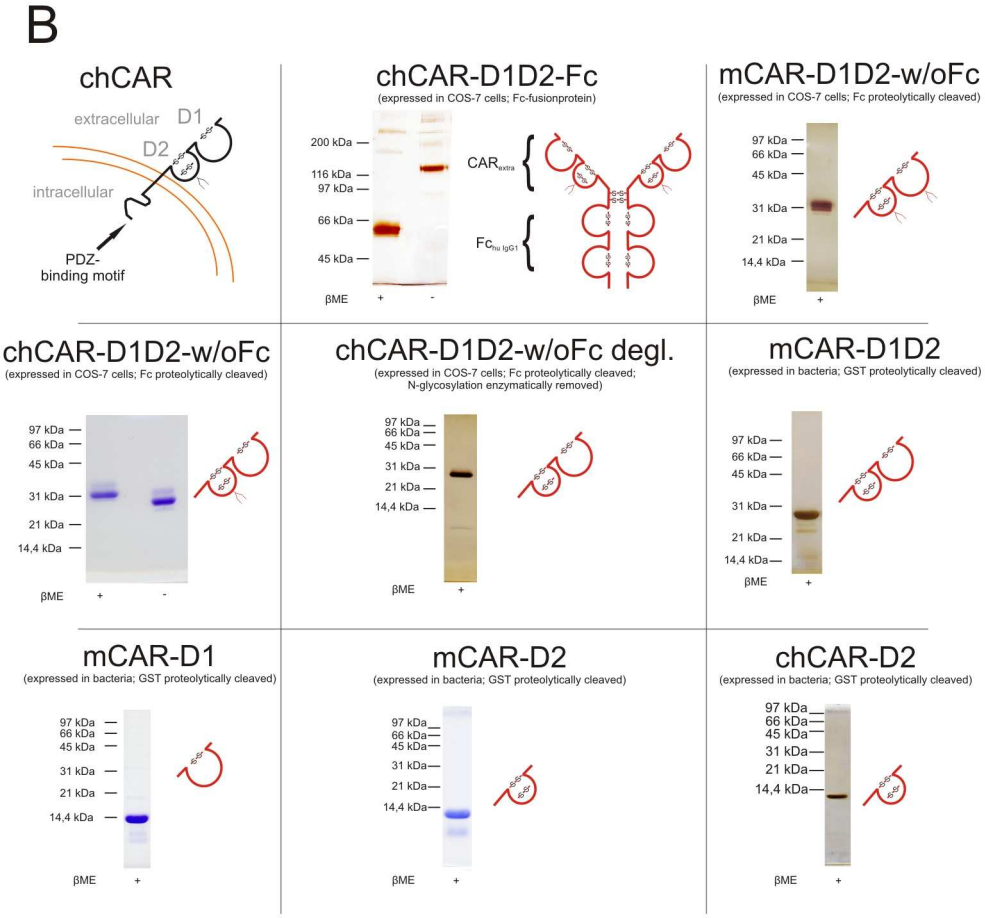
Supplementary Figure S4

Homotypic aggregation of CAR-expressing CHO or NIH 3T3 cells.

A-B Stable chCAR transfected CHO or NIH 3T3 cells reveal stronger aggregation in comparison to parental cells. Cells were incubated in DMEM without FCS at 90 rpm at 37°C. Samples were taken at different time periods of incubation and analyzed by a cell counter (Coulter Beckmann). Aggregation is considered as the percentage decrease in particles. Error bars indicate SEM. Bar, 200 µm.

Figure S1





C

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  1 MEPPPLVLLV GLALLGAAGL TRSLSITSAE SAFEKAQGER VTLPCTFELS
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101 DPKSGDGSVD ILNLKSADTG TYQCKVKKAP GVESLKIQLN VLVKPASTKC
151 SIEGSQEIGK DIVLKCASQE GTPLLYYDWR RVVTGTQGLP ATSVLNKNTG
201 ELLLKNASKD YSGTYSCVAS NRVGTDECSV ELNVTPPINT AGVIAGAILG
251 TLLGLALLAF LVICCCKKHR EKKYEKEVHH EIREDVLPPK SRSSTARSYI
301 GSNRSSLGSM SPSNMEGYSK TPYSQVPSED FERTSGQNQT IASSKVAAPN
351 LSRMGAVPVM IPAQSKDGSI V
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Figure S2

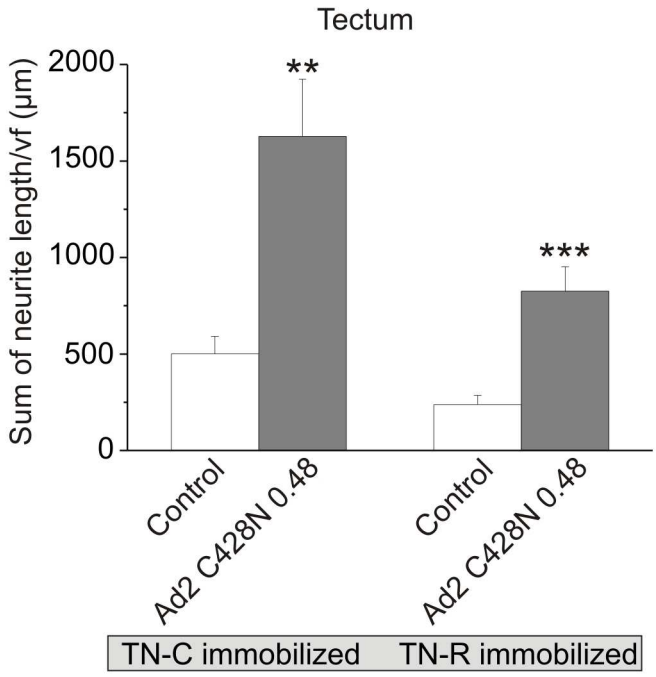

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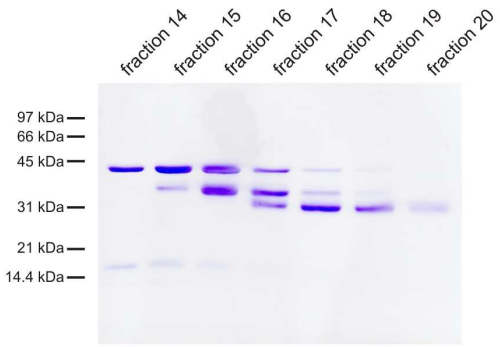
Figure S3

A



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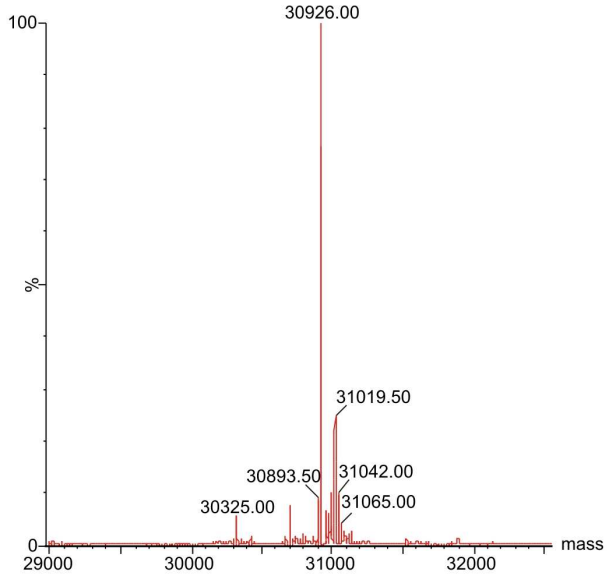

C

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 51 MKEINLAPDS SSVVVSGLMV ATKYEVSVYA LKDTLTSRPA QGVVTTLENV
101 SPPRRARVTD ATETTITISW RTKTETITGF QVDAVPANGQ TPIQRTIKPD
151 VRSYTITGLQ PGTDYKIYLY TLNDNARSSP VVIDASTAID APSNLRFLAT
201 TPNSLLVSWQ PPRARITGYI IKYEKPGSPP REVVPRPRPG VTEATITGLE
251 PGTEYTIYVI ALKNNQKSEP LIGRKKTVQK TPF
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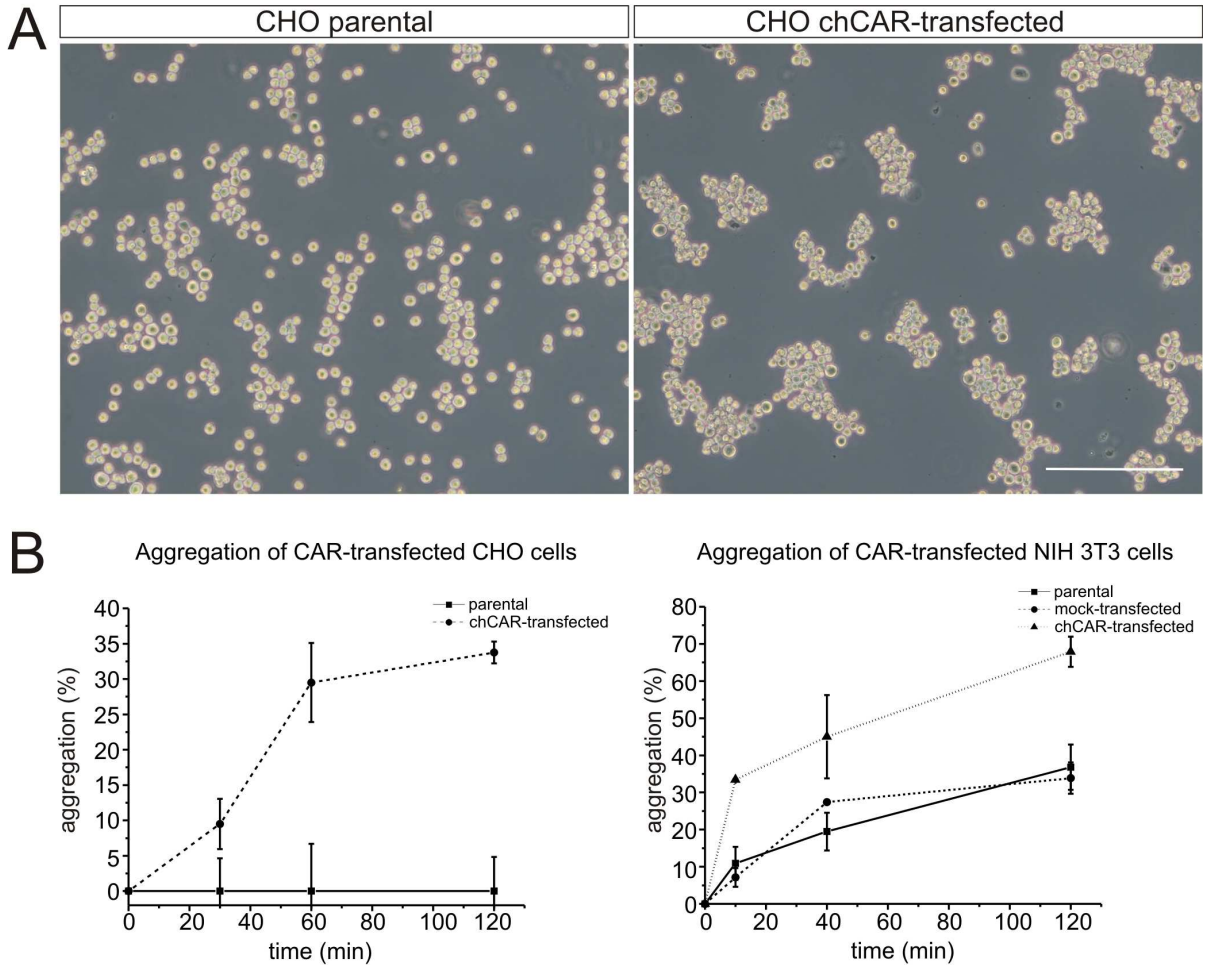
Figure S4



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