Supporting Information

Recognition of the glycan headgroup of stage-specific embryonic antigen-4 by a cancer-targeting antibody

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Running title: *Antibody recognition of SSEA-4*

Material Included

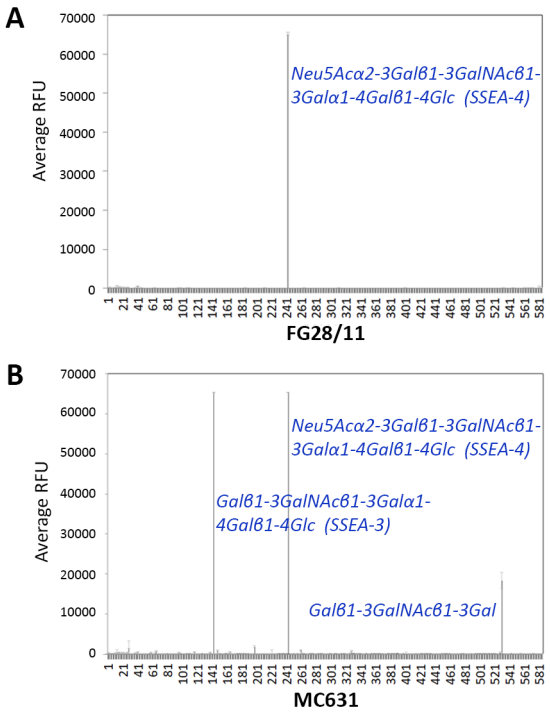

Figure S1. Binding of FG28/11 by glycan array. Antibodies **A)** FG28/11 and **B)** MC631 binding to the Consortium for Functional Glycomics glycan array (CFG, core H, version 5.1), composed of 585 glycans. FG28/11 only bound to SSEA-4 (Neu5Acα2-3Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1), whereas MC631 bound to SSEA-4, SSEA-3 (Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1) and the trisaccharide Galβ1-3GalNAcβ1-3Gal.

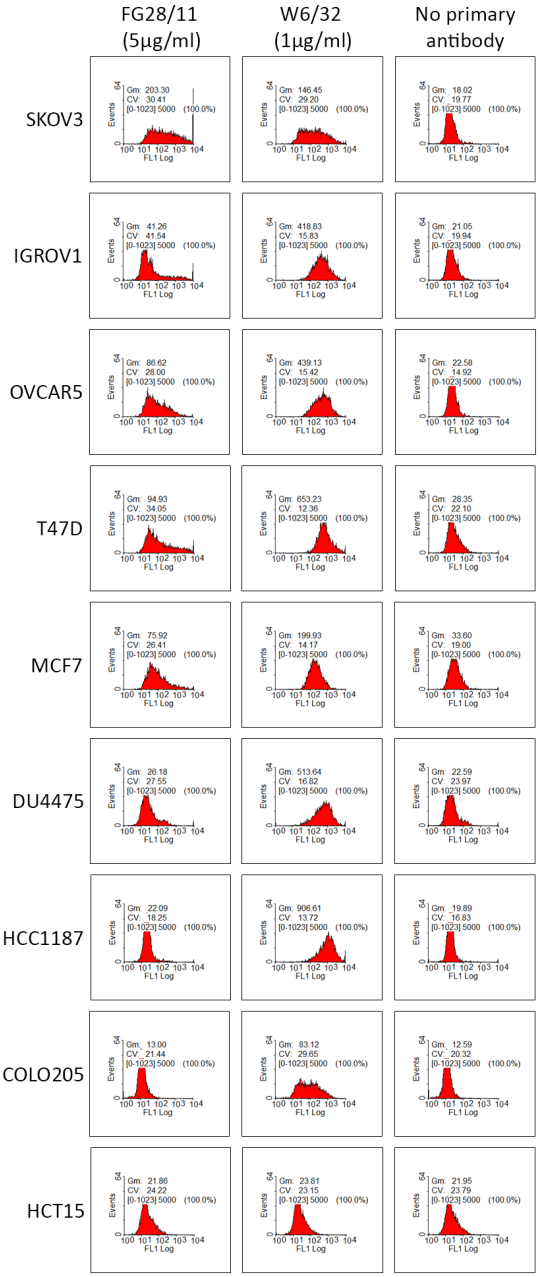

Figure S2. Tumor cell binding of the FG28/11 mAb. A panel of cancer cell lines were stained with FG28/11 mAb (5µg/ml) and analyzed by flow cytometry. The anti-HLA-A,B,C mAb W6/32 (1µg/ml) and no primary mAb were included as positive and negative controls, respectively. Results were expressed as Geometric mean (Gm) with % CV. Ovarian cancer cell lines are SKOV3, IGROV1 and OVCAR5. Breast cancer cell lines are T47D, MCF7, DU4475 and HCC1187. Colorectal cancer cell lines are COLO205 and HCT15.

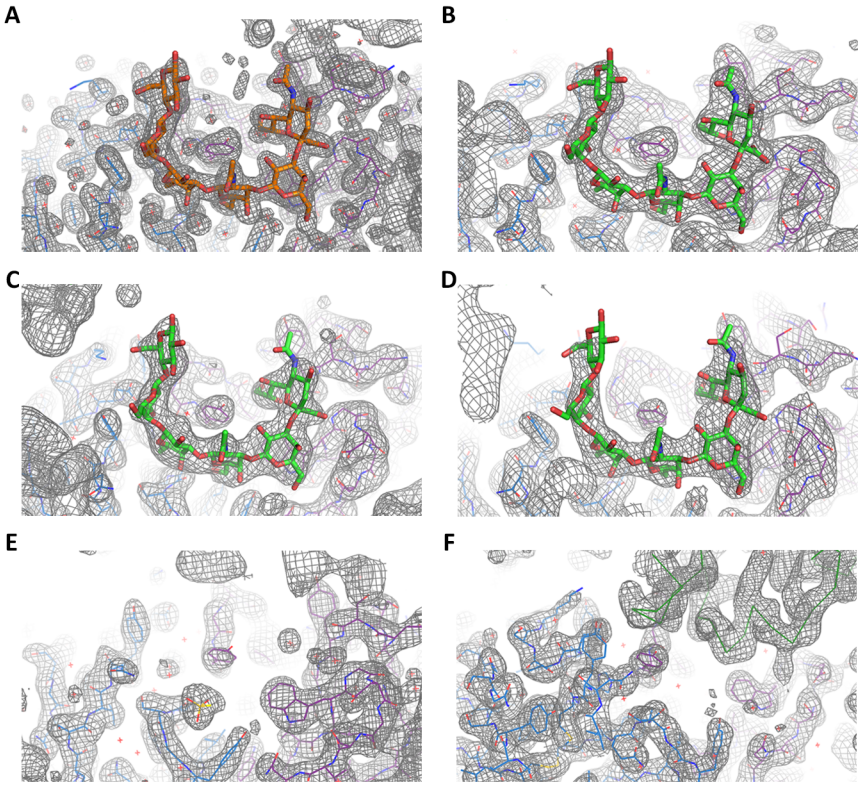

Figure S3. Electron density maps of the ch28/11 Fab binding sites in the presence and absence of the SSEA-4 hexasaccharide. **A)** Fab:SSEA-4 $P2_1$ crystal form. Hexagonal $P6_2$ crystals had three ch28/11 Fab:SSEA-4 complexes in the asymmetric unit: **B)** complex 1, **C)** complex 2 and **D)** complex 3. Unliganded ch28/11 crystals had 2 Fab molecules in the asymmetric unit: **E)** Fab 1 and **F)** Fab 2. Composite omit $2F_o - Fc$ electron density maps (displayed at 1.5σ level) are shown for each structure as grey mesh. Residues from each Fab are depicted as lines with L chain in blue and H chain in purple. Ligands are depicted as sticks with carbon color coded for the $P2_1$ crystal form (orange) and the $P6_2$ crystal form (green).

VL (106)
1 10 20 30 40 50
1 10 20 30 40 50 60
ENVLTQSPAIMSASPGEKVTMTCSAS**SSV**-------**NY**M**H**WYQQKSSTSPKLWIY**D****T**-------
SKLAS

60 70 80 90 100 (Seq)
70 80 90 100 110 (IMGT)
GVP-GRFSGSG--SGNSYSLTIRTMEAEDVATYFC**FQ**A**SGY**P**LT**FGGGTKLELK

VH (117)
1 10 20 30 40 50
60
1 10 20 30 40 50 60
QVQLKESGP-GLVAPSQSLSITCTVS**GFSL**----**NSYG**VSWVRQPPGKGLEWLGV**IWGD**---
GS**T**NYHS

70 80 90 100 110 (Seq)
70 80 90 100 110 (IMGT)
ALM-SRLRISKDNSKRQVFLKLNSLQTDDTATYYC**TKPGSGYA**FAYWGQGTLVTVSS

Figure S4. Variable domain sequences of ch28/11 Fab. The light (VL) and heavy (VH) variable domains are shown with the CDR sequences show in bold (red). The sequential numbering used in the PDB files (black) and IMGT numbering scheme (orange) are shown for comparison. Residues with a double underline are contact residues for all structures, while residues with a single underline are additional contact residues found in one or multiple, but not all five Fab:SSEA-4 complexes.

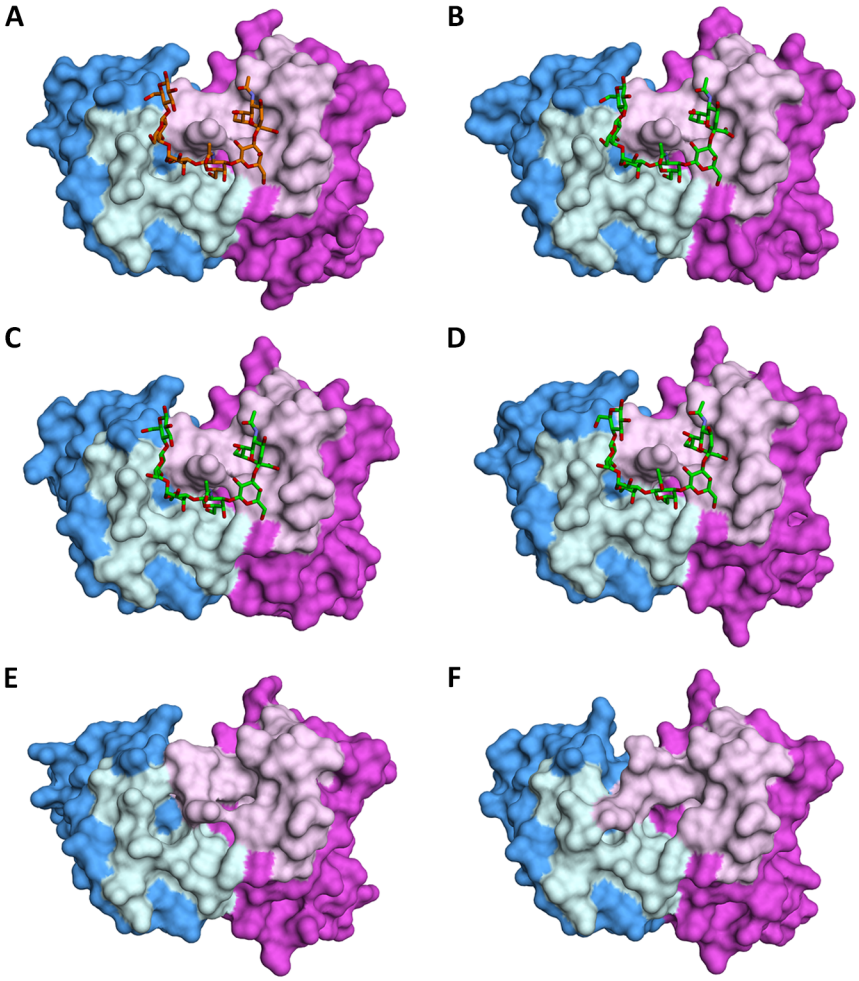

Figure S5. Crystal structures of ch28/11 Fab with and without bound SSEA-4 displayed as solvent-accessible end-on views. **A)** $P2_1$ crystal form. Hexagonal $P6_2$ crystal structure showing Fab:SSEA-4: **B)** complex 1 **C)** complex 2, and **D)** complex 3. Unliganded ch28/11 crystal: **E)** Fab 1, and **F)** Fab 2. Solvent-accessible surfaces are shown for the VL (blue) and VH (magenta) with L chain CDRs in pale blue and H chain CDRs in pale pink. Each SSEA-4 is depicted as sticks with carbon atoms color coded for the $P2_1$ crystal form (orange) and the $P6_2$ crystal form (green).

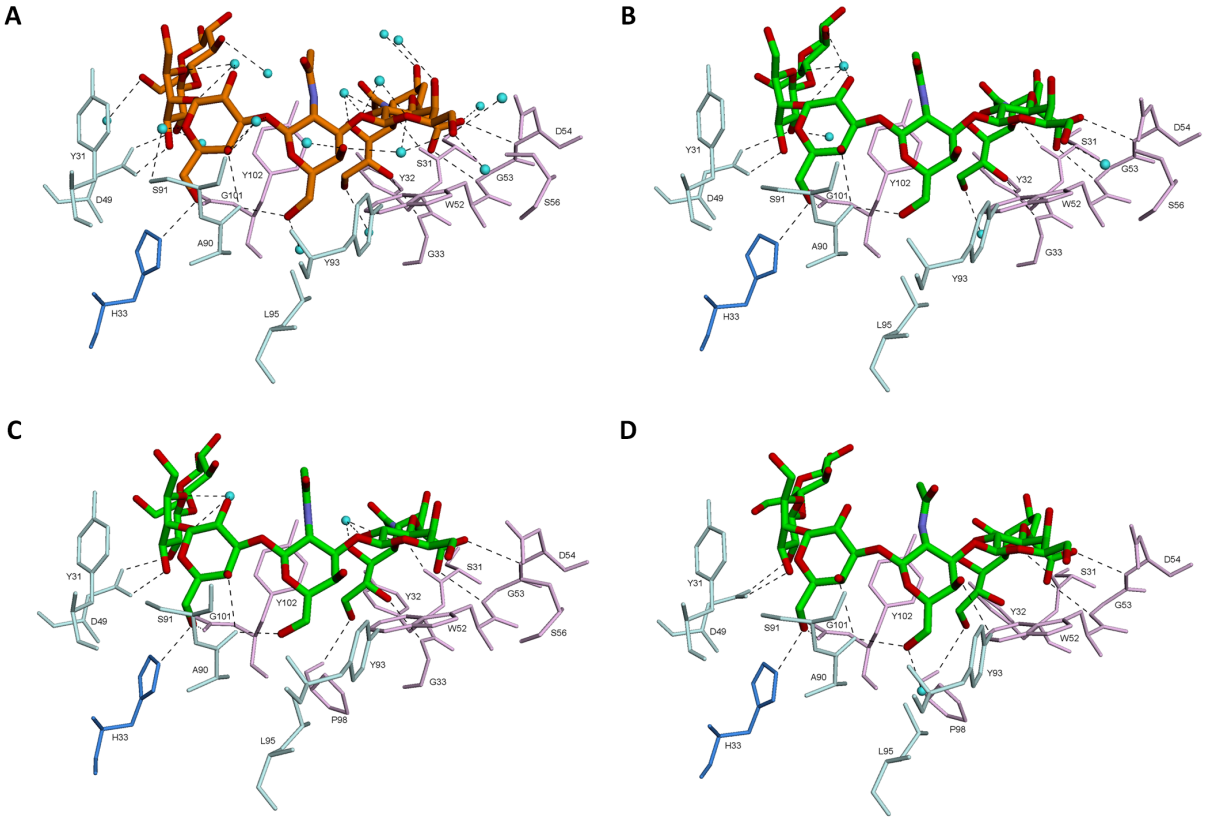

Figure S6. Details of the interactions between ch28/11 Fab and SSEA-4. **A)** $P2_1$ crystal form. Hexagonal $P6_2$ crystal structure showing Fab:SSEA-4: **B)** complex 1 **C)** complex 2, and **D)** complex 3. Fab residues are depicted as sticks with L chain CDR residues in pale blue, H chain CDR residues in pale pink, and a L chain framework residue in blue. Bound glycans are depicted as sticks with carbon color coded for the $P2_1$ crystal form (orange) and the $P6_2$ crystal form (green). Water molecules are depicted as light blue spheres. Each residue has been labeled with a residue number (amino acids in 1 letter code). Hydrogen bonding interactions with the bound glycan are shown as black dashed-lines.