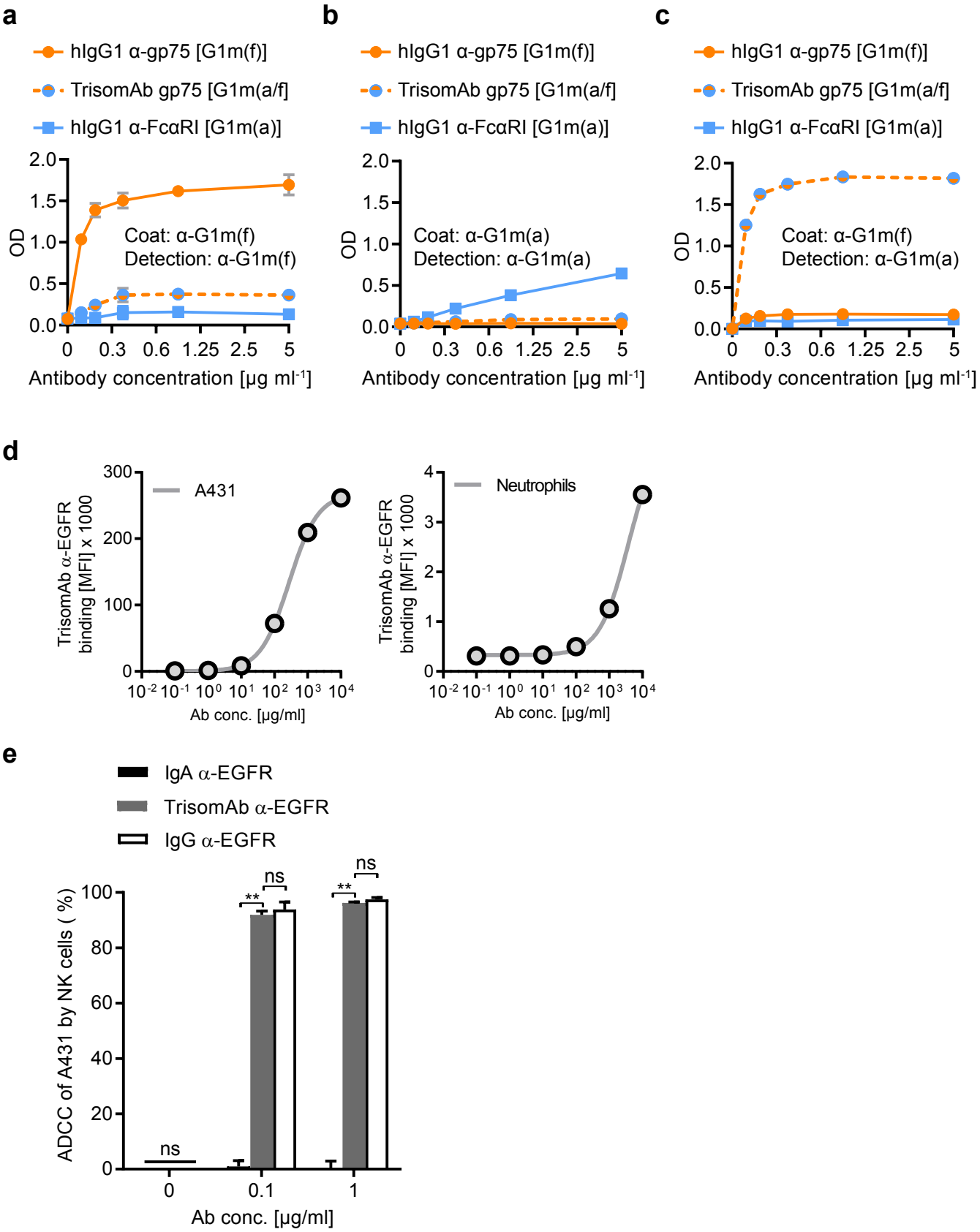
Table 1. Antibody binding affinity to FcαRI, FcγRIIIa and EGFR determined by surface plasmon resonance

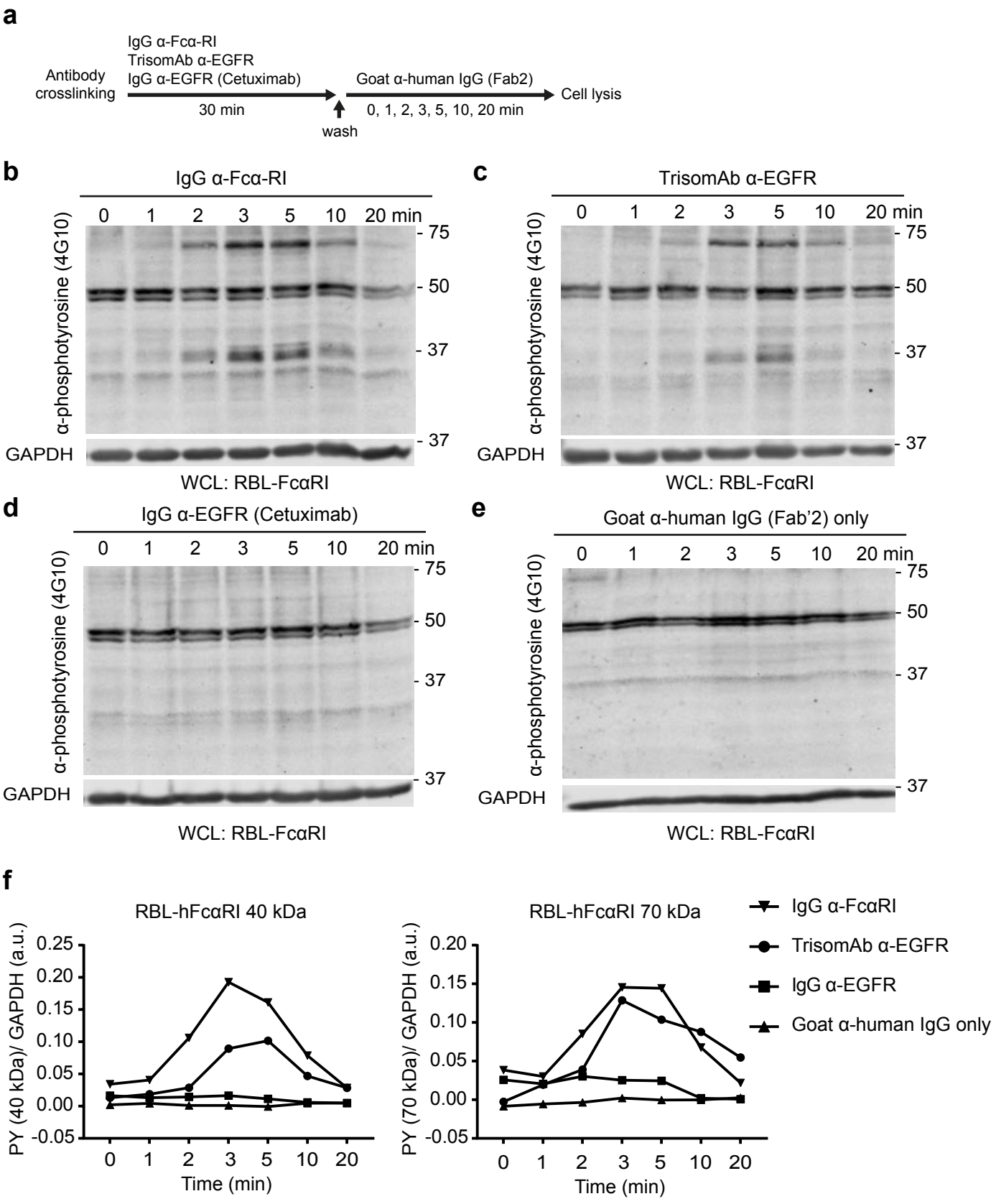
	1/kD value		
	TrisomAb α-EGFR (bsAb)	hIgG1 α-FcαRI (mAb)	hIgG1 α-EGFR (mAb)
FcαRI (Protein)	1,38E+08	2,74E+08	ND
FcγRIIIa (Protein)	1,23E+07	4,11E+06	5,06E+06
α-hIgG1 (mAb)	3,78E+08	4,06E+08	5,43E+08
EGFR (Protein)	ND	ND	2,56E+09

Supplementary Figure 1



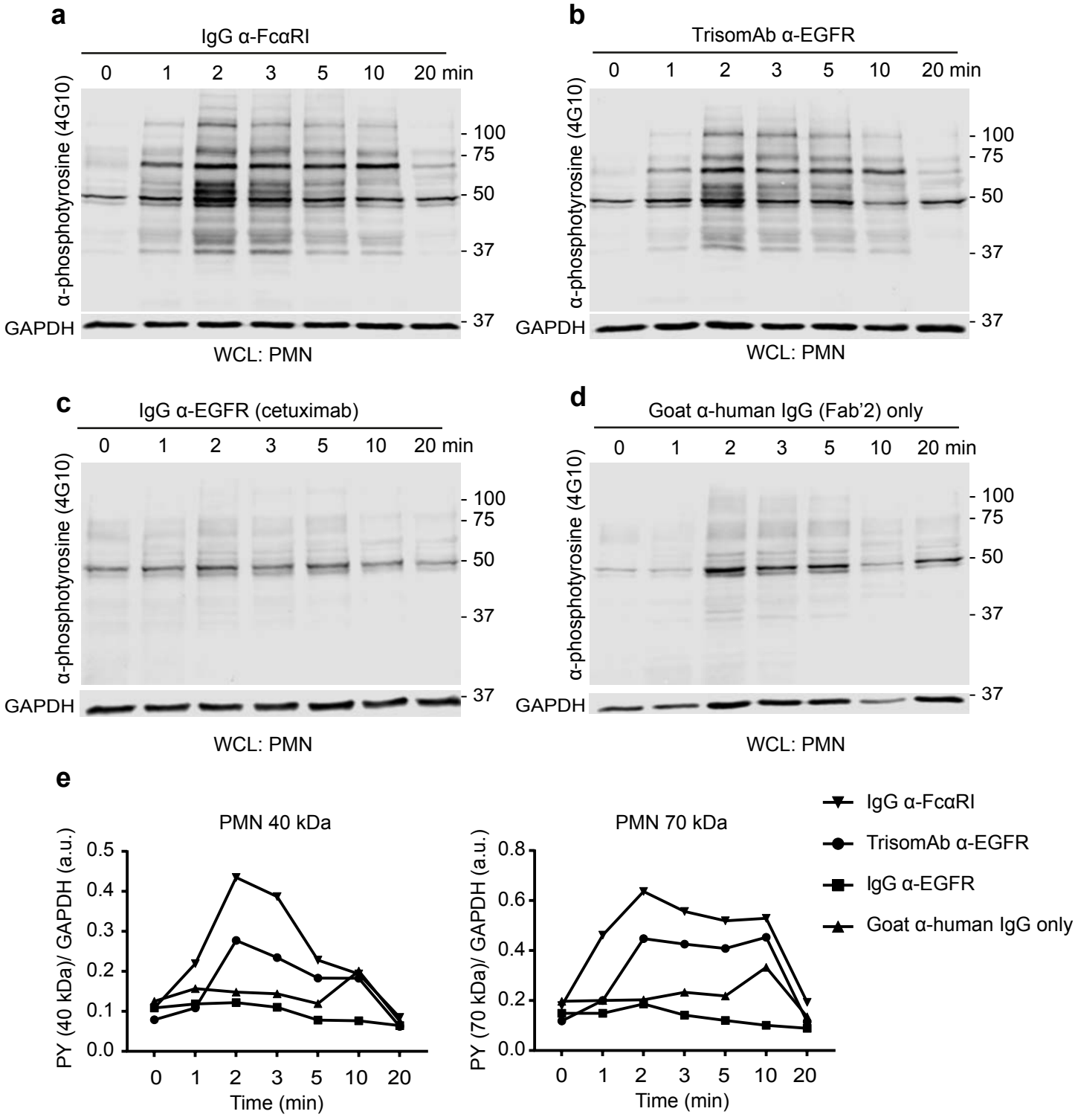
Supplementary Figure 1. TrisomAb α-gp75 Fab-arm exchange and ADCC of A431 cells by NK cells. (a-c) Sandwich ELISA showing successful Fab-arm exchange utilizing the allotypic differences between TrisomAb and its parental forms. (d) Dose-response curve of TrisomAb α-EGFR binding to A431 cells and neutrophils assessed by flow cytometry. (e) ADCC of A431 cells by NK cells (E:T 5:1) in the presence of 0, 0.1 and 1 µg ml-1 TrisomAb, IgA and IgG α-EGFR antibodies. Cell Titer-Blue Cell Viability Assay was performed after 24 hours. Data shows mean ± SD of one representative example of > nine independent experiments a-c. OD, optical density. Data shows one representative example of three independent experiments d, e; error bars showing S.E.M. *P < 0.05 ** P < 0.01 analysis of variance (Two-way ANOVA) with Tukey multiple comparisons correction.

Supplementary Figure 2



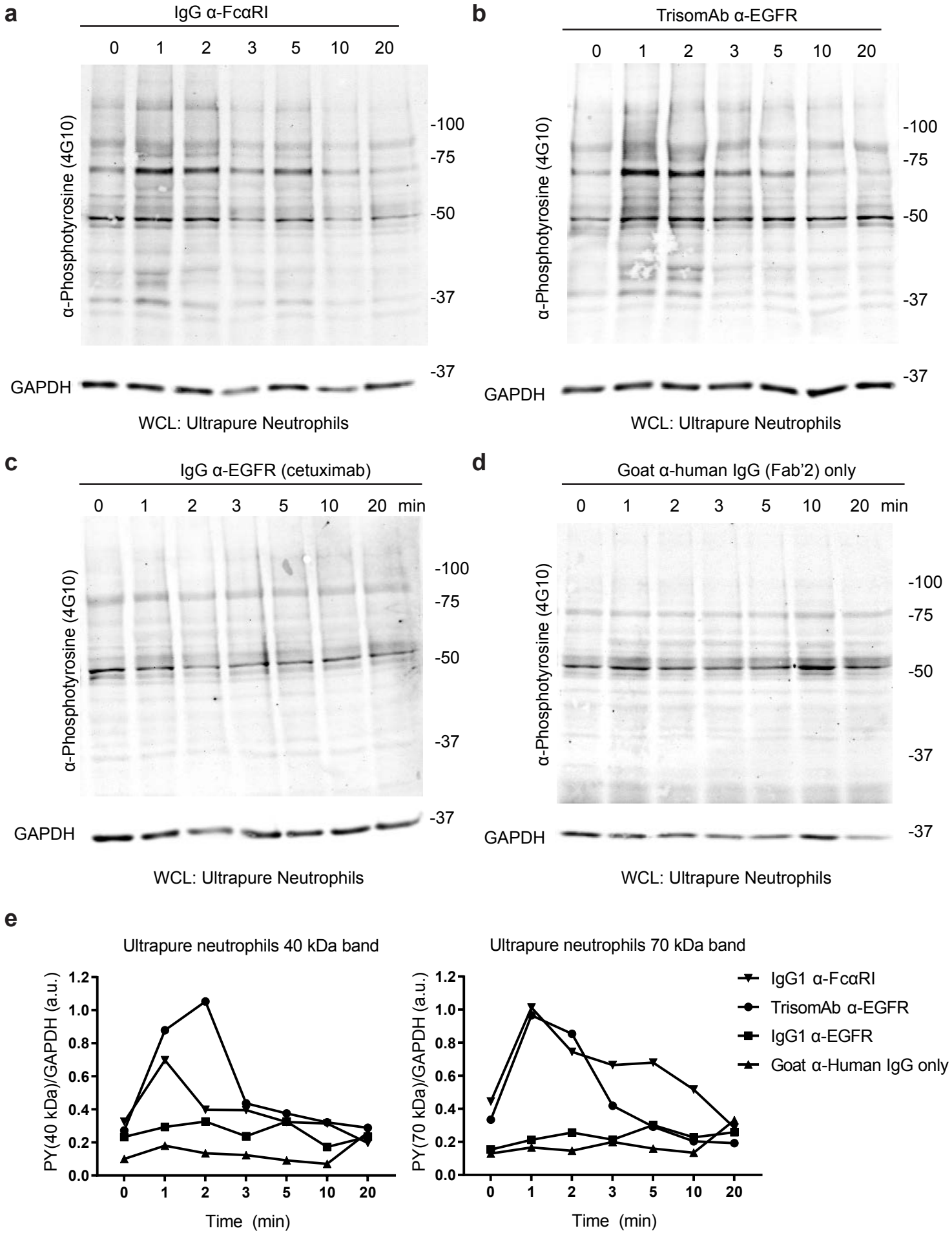
Supplementary Figure 2. TrisomAb-induced clustering of FcαRI elicits tyrosine signaling in RBL-FcαRI cells. (a) Schematic representation of FcαRI crosslinking prior to RBL lysis. hFcαRI at RBL surface was clustered by incubation with TrisomAb α-EGFR and the parental IgG α-FcαRI and IgG α-EGFR mAbs for 30 minutes followed by incubation with a secondary goat α-human F(ab')2 for 0, 1, 2, 3, 5, 10 and 20 minutes. IgG α-EGFR and secondary antibody only are used as negative controls. (b-e) Immunoblot analysis of protein extracts prepared from RBL-FcαRI incubated with the parental IgG α-FcαRI (b), TrisomAb α-EGFR (c), IgG α-EGFR (d) or secondary antibody only (e). Extracts were probed with Abs against phosphorylated tyrosine (4G10) and GAPDH. (f) Quantification of tyrosine phosphorylated proteins after crosslinking FcαRI on RBL-FcαRI cells with indicated antibodies. Ratio of phosphotyrosine (PY) band at 40 kDa (left panel) or 70 kDa (right panel) over GAPDH in arbitrary units (A.U.) is depicted. Data shows one representative example and quantification of three independent experiments b-f. WCL, whole cell lysate.

Supplementary Figure 3



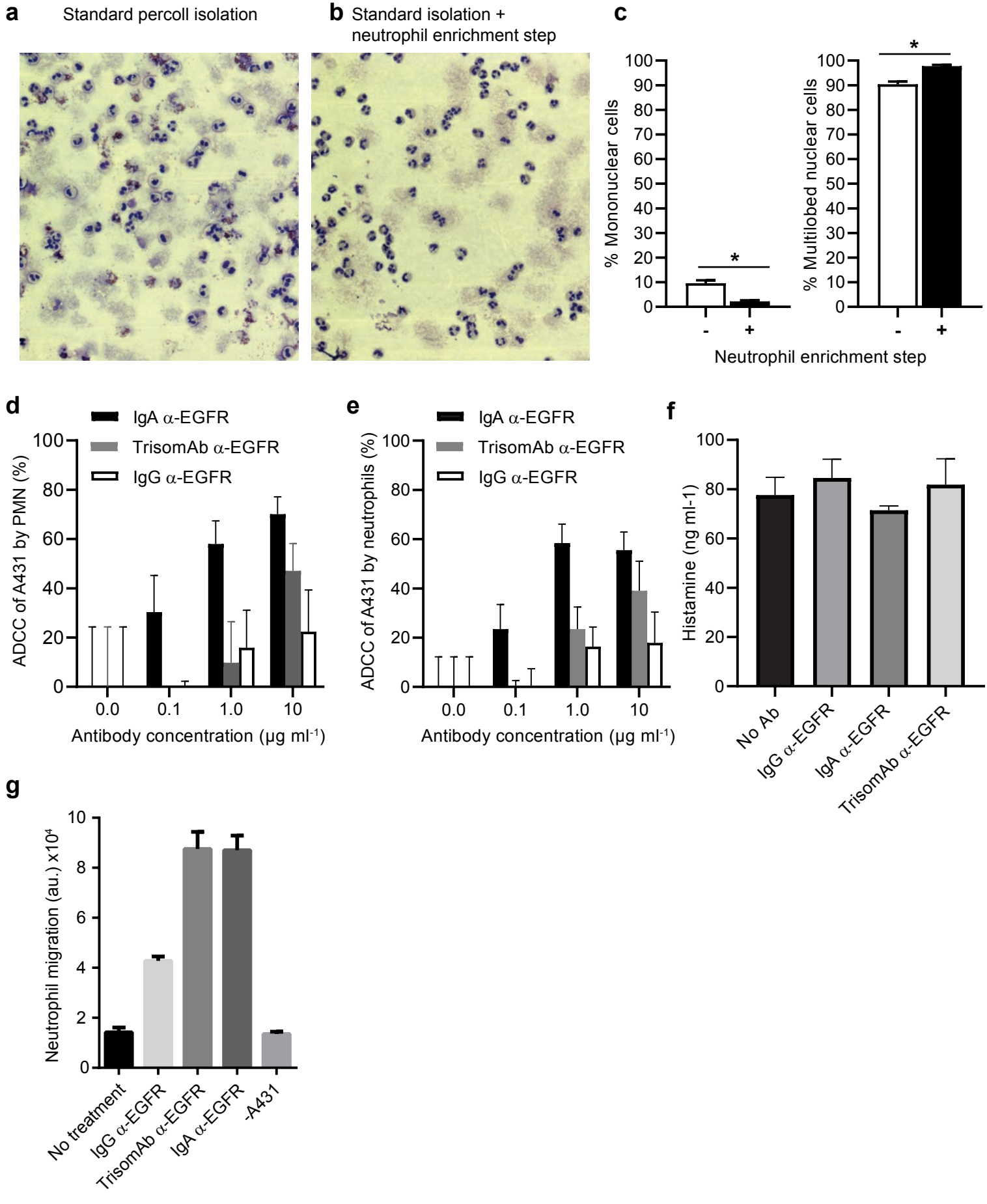
Supplementary Figure 3. TrisomAb-induced clustering of FcαRI elicits tyrosine signaling in PMN. (a-d) Immunoblot analysis of protein extracts prepared from PMN incubated with the parental IgG α-FcαRI (a), TrisomAb α-EGFR (b), IgG α-EGFR (c) and secondary antibody only (d). Extracts were probed with Abs against phosphorylated tyrosine (4G10) and GAPDH. (e) Quantification of tyrosine phosphorylated proteins after crosslinking FcαRI on PMN with indicated antibodies. Ratio of phosphotyrosine (PY) band at 40 kDa (left panel) or 70 kDa (right panel) over GAPDH in arbitrary units (A.U.) is depicted. Data shows one representative example and quantification of three independent experiments a-e. WCL, whole cell lysate. PMN, polymorphonuclear cell.

Supplementary Figure 4



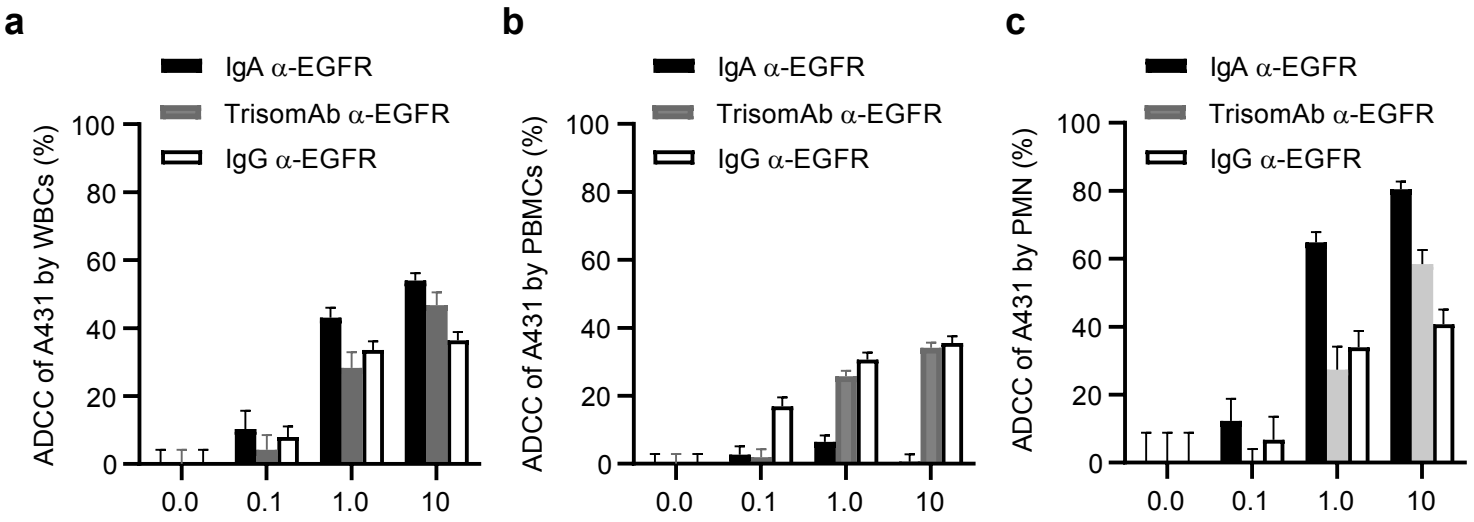
Supplementary Figure 4. TrisomAb-induced clustering of FcαRI elicits tyrosine signaling in neutrophils. (a-d) Immunoblot analysis of protein extracts prepared from enriched neutrophils incubated with the parental IgG α-FcαRI (a), TrisomAb α-EGFR (b), IgG α-EGFR (c) and secondary antibody only (d). Extracts were probed with Abs against phosphorylated tyrosine (4G10) and GAPDH. (e) Quantification of tyrosine phosphorylated proteins after crosslinking FcαRI on neutrophils with indicated antibodies. Ratio of phosphotyrosine (PY) band at 40 kDa (left panel) or 70 kDa (right panel) over GAPDH in arbitrary units (A.U.) is depicted. Data shows one representative example and quantification of two independent experiments a-e. WCL, whole cell lysate.

Supplementary Figure 5



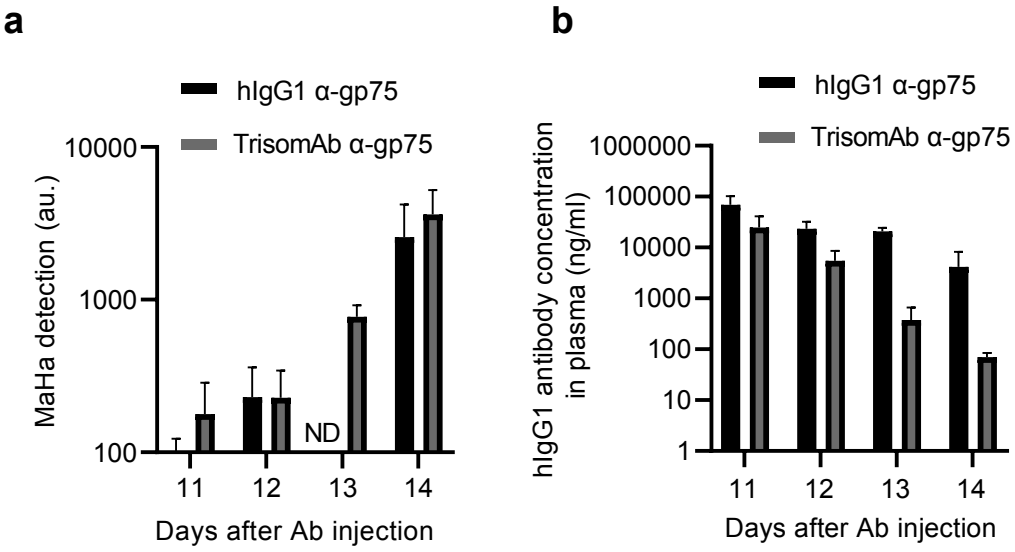
Supplementary Figure 5. ADCC of A431 cells by enriched neutrophils, histamine detection and neutrophil chemotaxis. (a, b) Images of neutrophils isolated with standard percoll isolation method (a) or isolated with an additional neutrophil enrichment step (b). (c) Quantification of mononuclear and multilobed nuclear cells with or without neutrophil enrichment step. (d, e) ADCC of A431 cells by PMN (E:T 50:1) (d) and enriched neutrophils (E:T 50:1) (e) in the presence of 0, 0.1, 1 and 10 µg ml-1 TrisomAb, IgA and IgG α-EGFR antibodies. Cell Titer-Blue Cell Viability Assay was performed after four hours. (f) Histamine detection in supernatant from ADCC assay shown in d measured by ELISA. (g) Quantification of neutrophil migration towards supernatant derived from PMN-mediated tumor killing experiments. Data shows one representative example of two independent experiments d-f and three independent experiments g; error bars showing S.E.M. *P < 0.05 analysis of variance (One-way ANOVA) with Tukey multiple comparisons correction in c.

Supplementary Figure 6



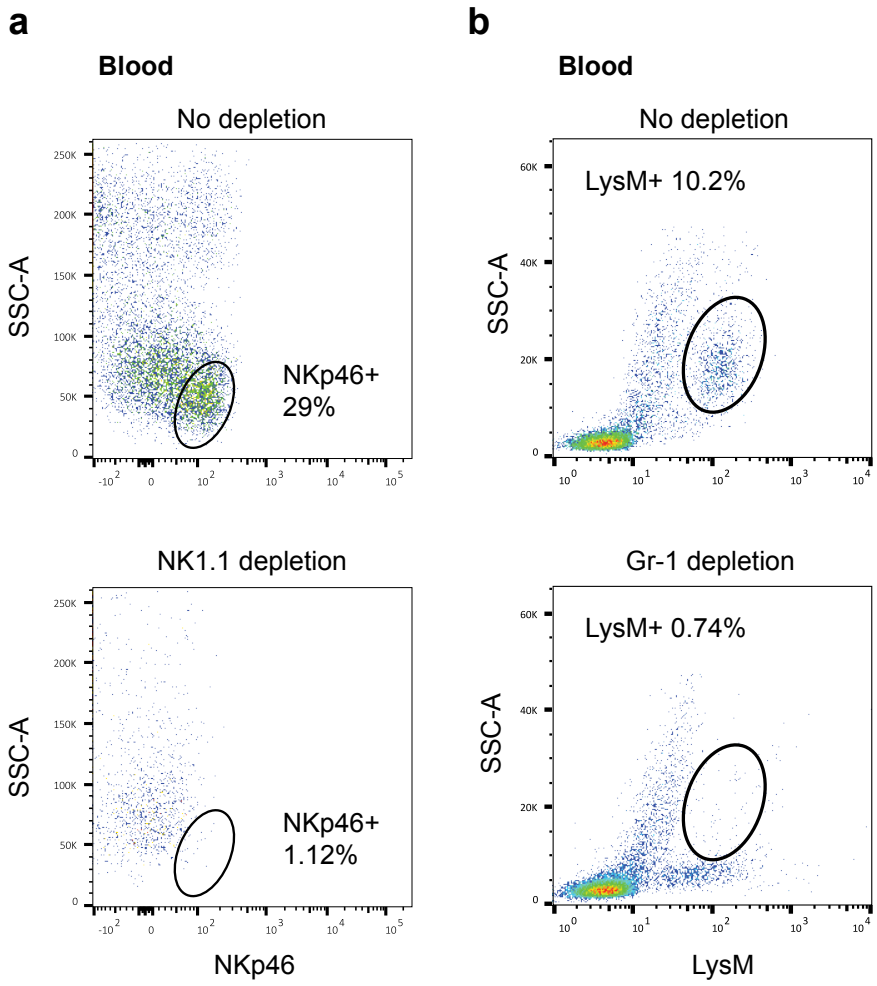
Supplementary Figure 6. ADCC by WBC. (a-c) ADCC of A431 cells by WBC (a), PBMC (b) and PMN (c) in the presence of 0, 0.1, 1 and 10 µg ml-1 TrisomAb, IgA and IgG α-EGFR antibodies. Cell Titer-Blue Cell Viability Assay was performed after four hours. (d) WBC, White blood cells. PBMC, peripheral blood mononuclear cells. PMN, polymorphonuclear cells. Data shows one representative example of two independent experiments d-f; error bars showing S.E.M.

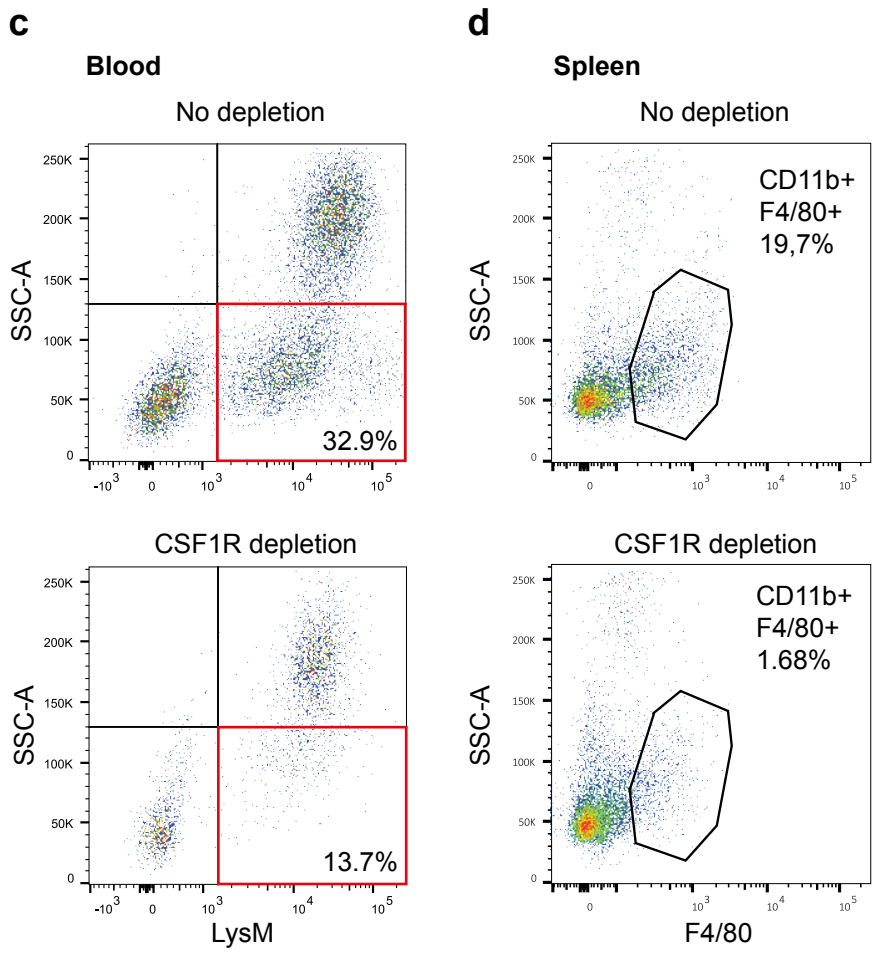
Supplementary Figure 7



Supplementary Figure 7: Detection of MAHA and therapeutic antibodies over time. (a) Mouse anti-human antibody (MAHA) detection over time in serum derived from mice treated with hIgG1 α-gp75 or TrisomAb α-gp75. (b) hIgG1 antibody concentration over time in serum derived from mice treated with hIgG1 α-gp75 or TrisomAb α-gp75. ND, not detectable.

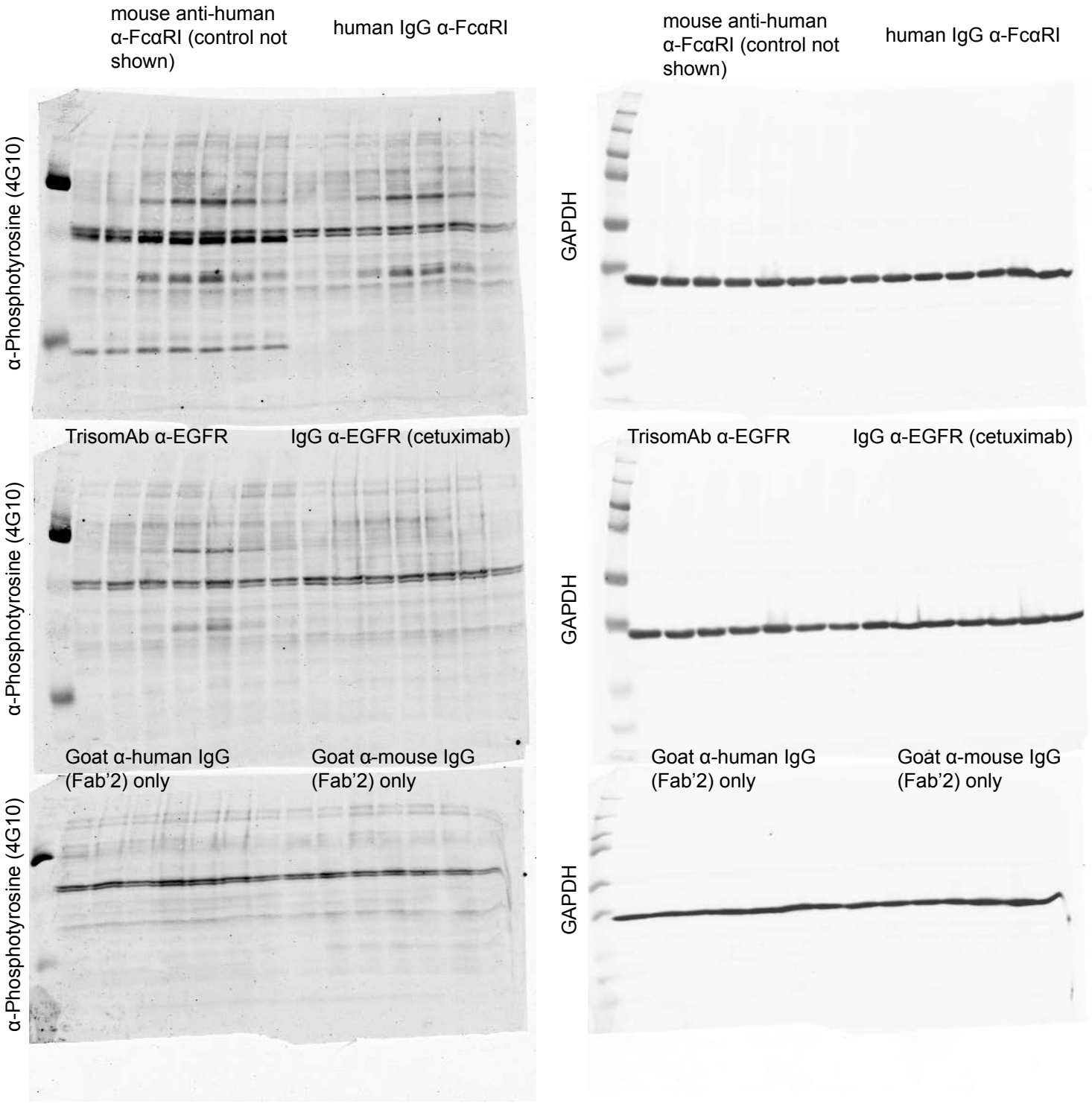
Supplementary Figure 8



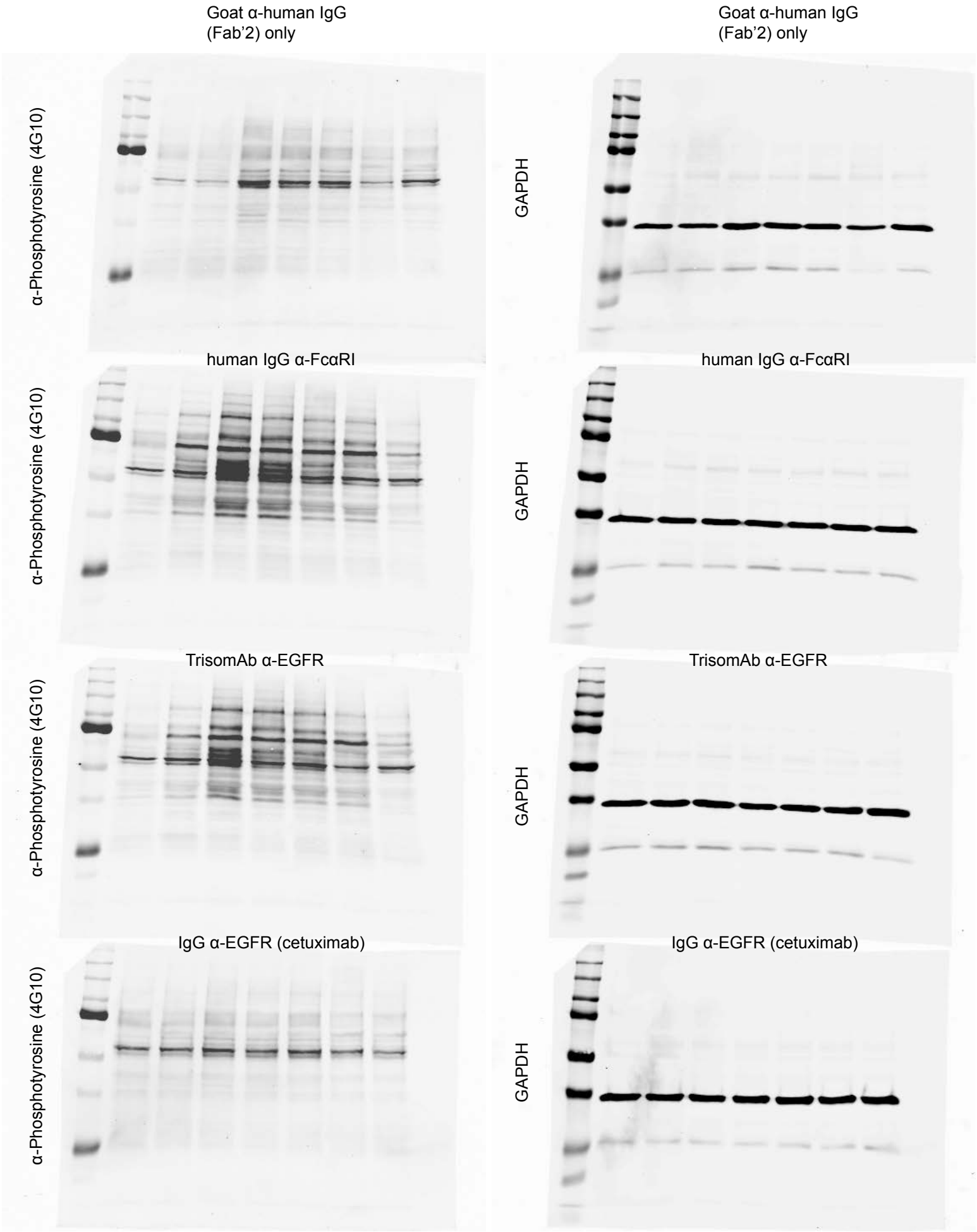


Supplementary Figure 8. Depletion of NK cells, neutrophils and macrophages. Cellular subsets were depleted as indicated in the method section. (a) Confirmation of NK1.1 depletion in blood derived 3 days after initiation of cell depletion. (b) Confirmation of Gr-1 depletion in blood derived 3 days after initiation of cell depletion. (c, d) Confirmation of CSF1R depletion in blood (c) and spleen (d) derived 5 days after initiation of cell depletion.

Supplementary Figure 9: Full unedited gel for Supplementary Figure 2



Supplementary Figure 10: Full unedited gel for Supplementary Figure 3



Supplementary Figure 11: Full unedited gel for Supplementary Figure 4