

Supplementary information

IgA transcytosis and antigen recognition govern ovarian cancer immunity

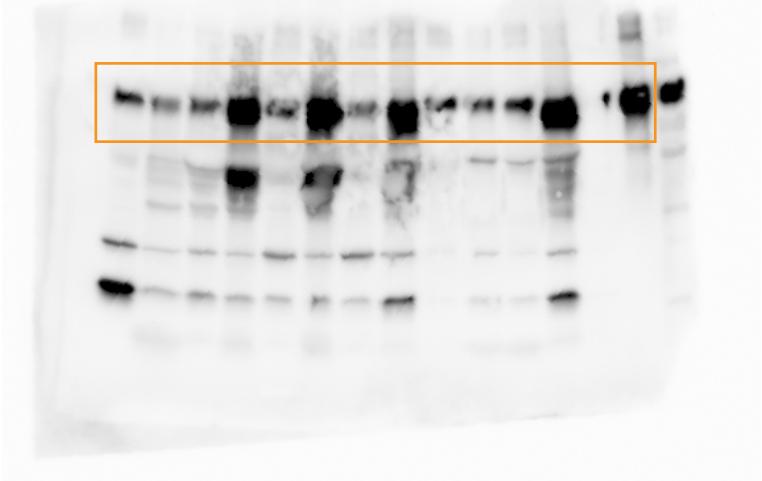
In the format provided by the
authors and unedited

Supplementary Figure 1

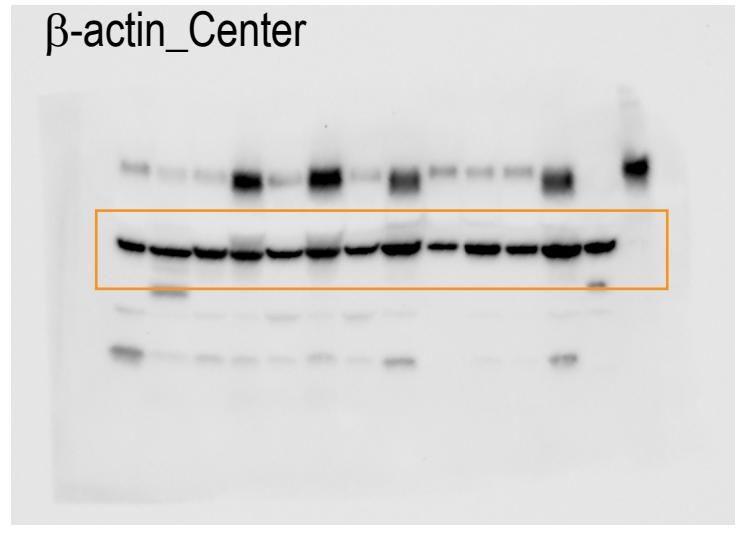
Uncropped western blots

Fig1c_Center and Right

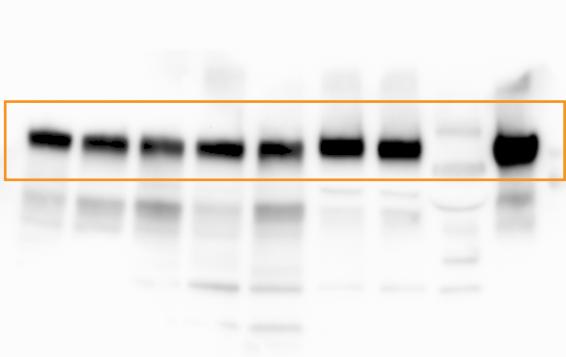
PIGR_Center



β-actin_Center



PIGR_Right



β-actin_Right

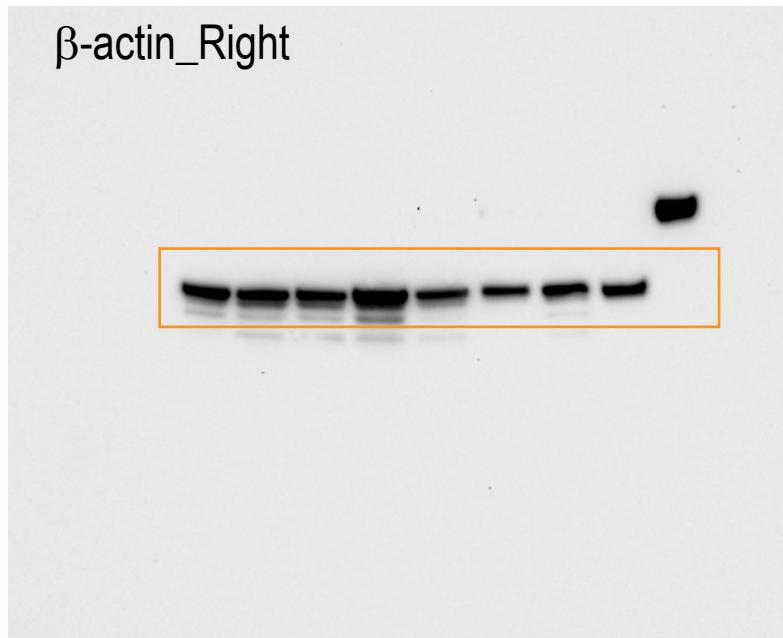
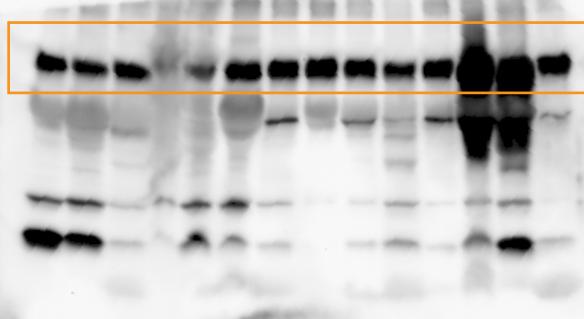
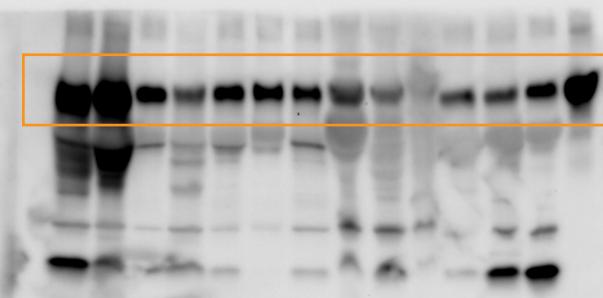


Fig1c_Left

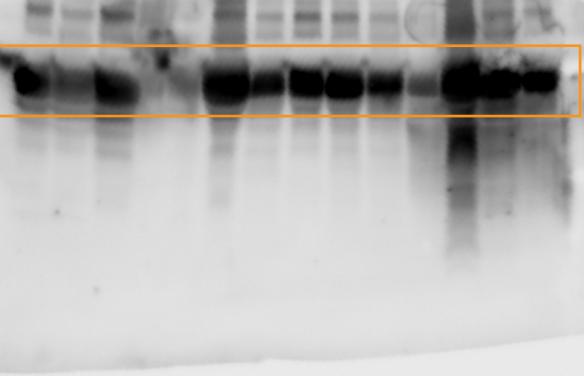
PIGR_Left_Blot1



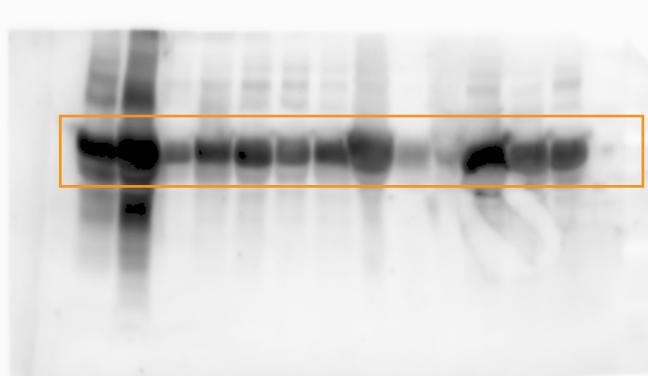
PIGR_Left_Blot2



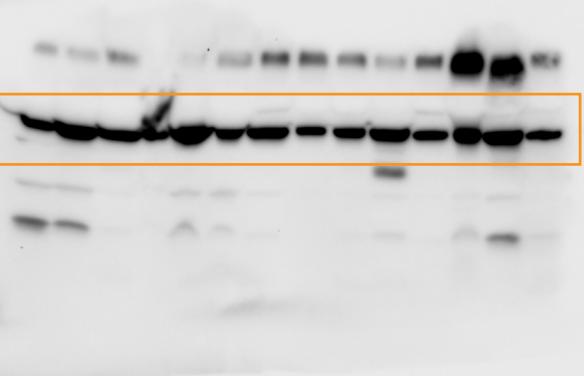
IgA_Left_Blot1



IgA_Left_Blot2



β -actin_Left_Blot1



β -actin_Left_Blot2

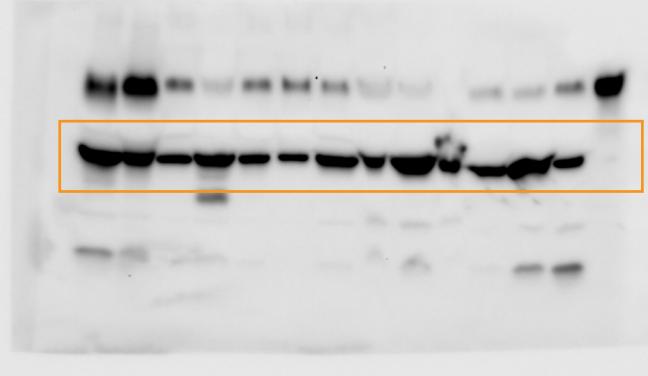
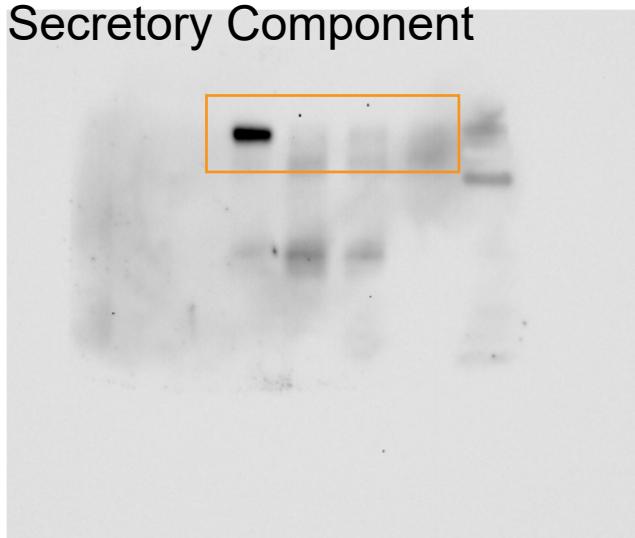
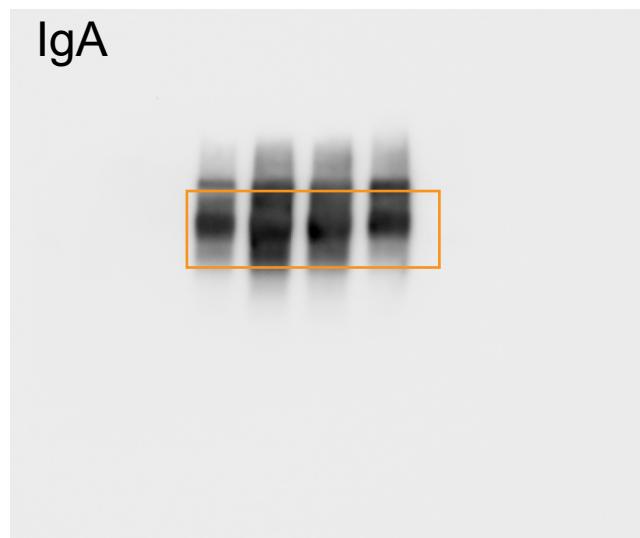


Fig. 2c

Secretory Component



IgA



Left panel (IgA treated OVCAR3)

Total Erk1/2



DUSP5

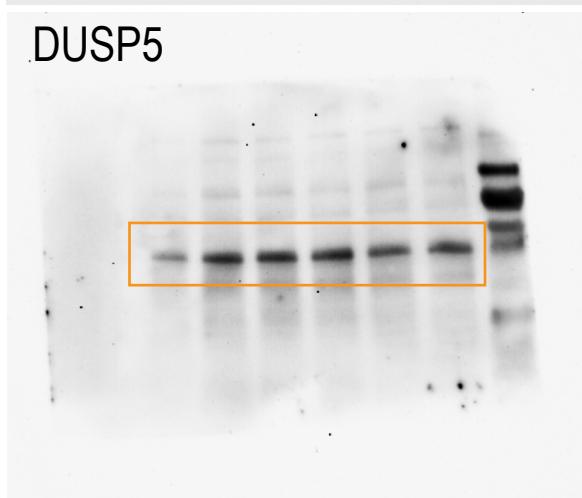
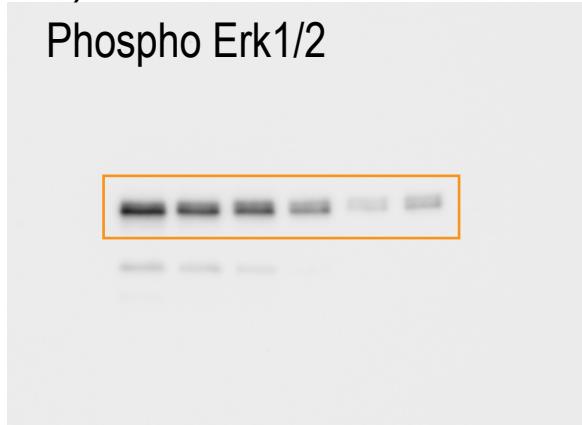


Fig.2e

Phospho Erk1/2



β -actin

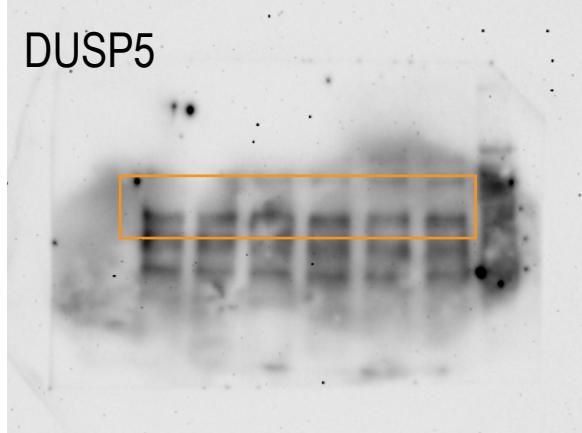


Right panel (IgG treated OVCAR3)

Total Erk1/2



DUSP5



Phospho Erk1/2



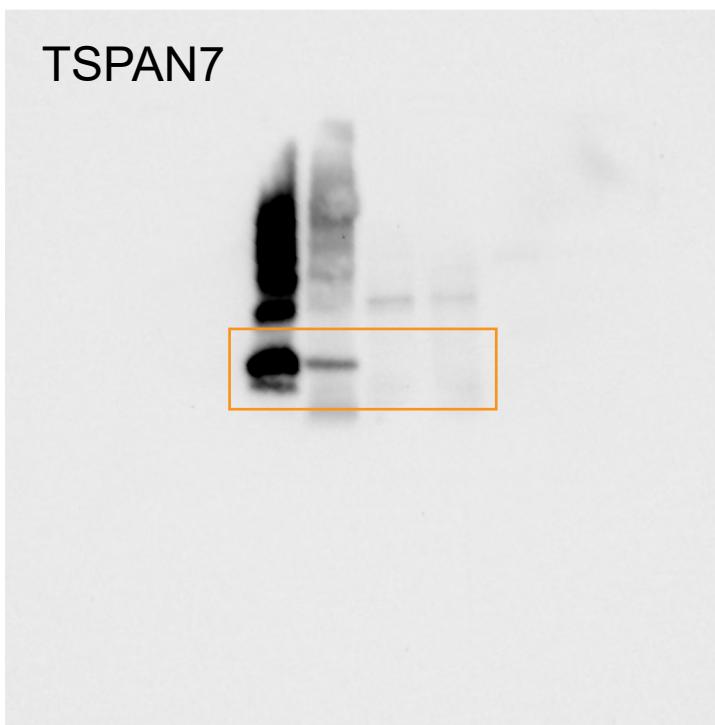
β -actin



Fig.3c

Left panel

TSPAN7

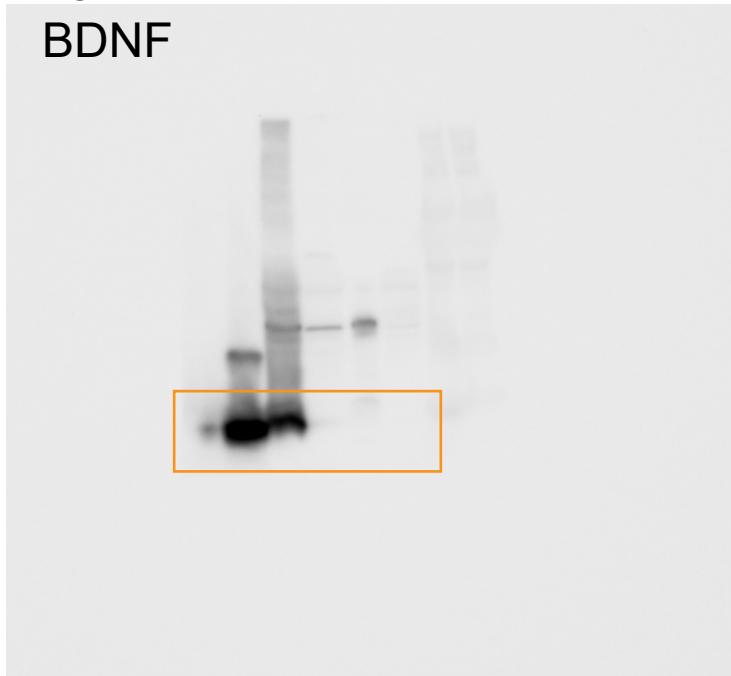


β -actin for TSPAN7



Right panel

BDNF



β -actin for BDNF

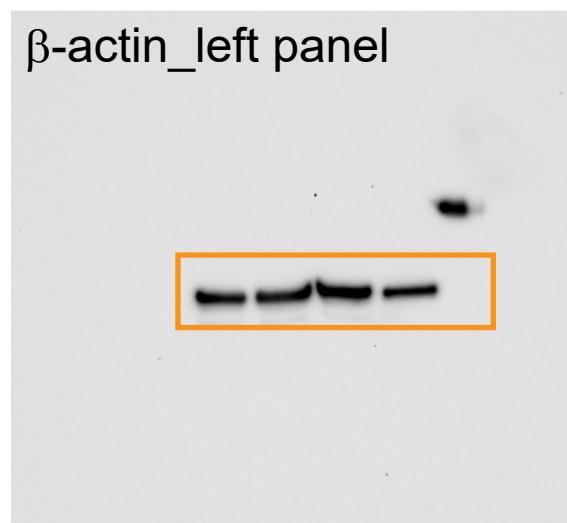


Extended Data Figure 3c

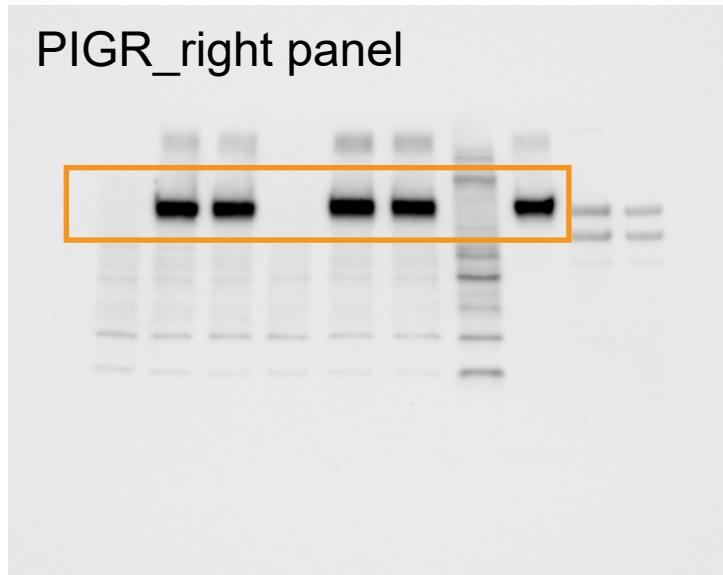
PIGR_left panel



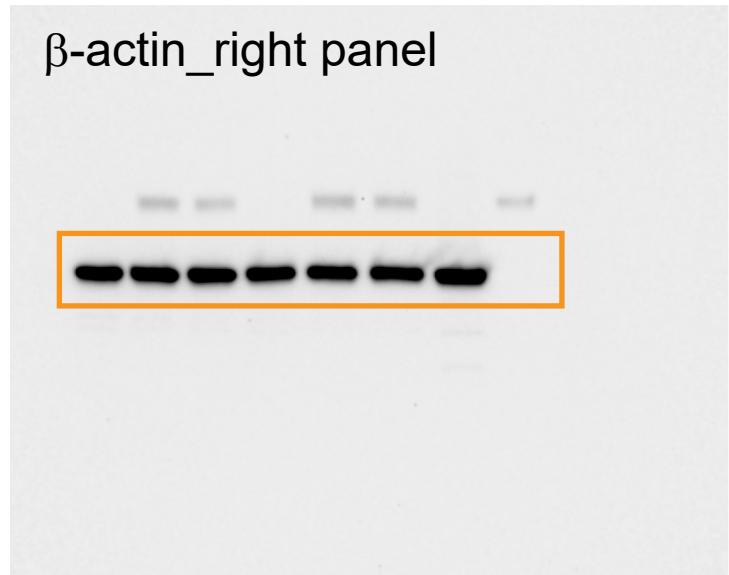
β -actin_left panel



PIGR_right panel



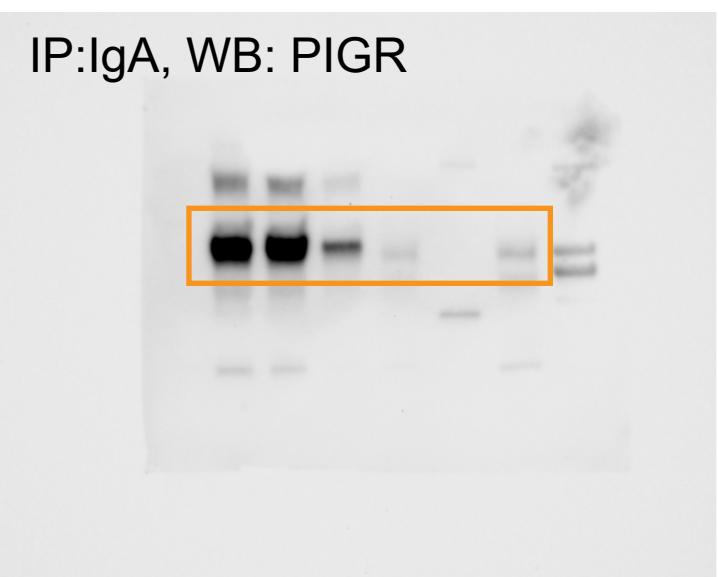
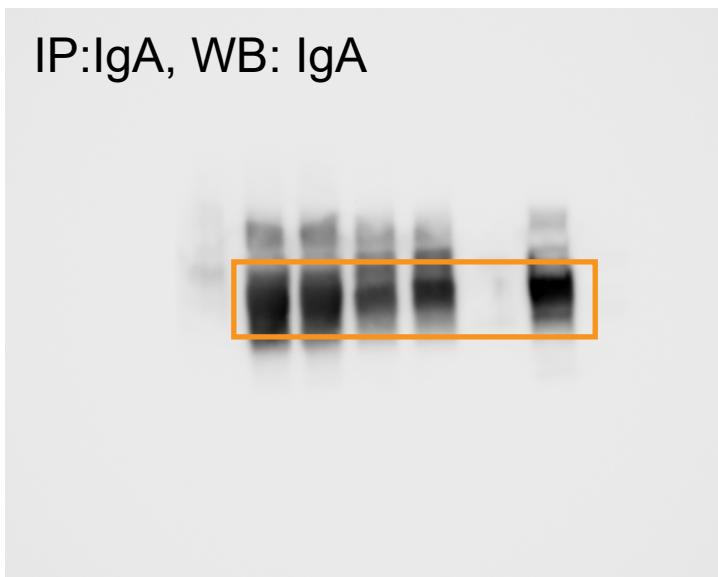
β -actin_right panel



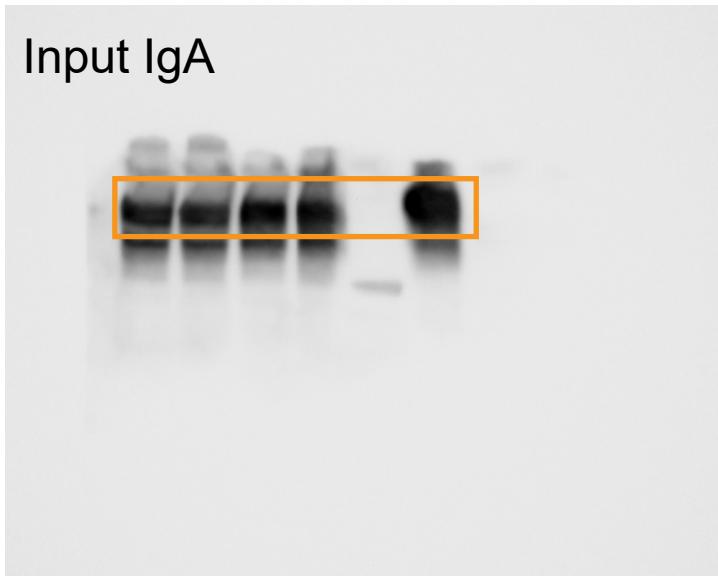
Extended Data Figure 4a

IP:IgA, WB: IgA

IP:IgA, WB: PIGR



Input IgA



Input PIGR



Extended Data Figure 4h

t-ERK1/2_Left panel



t-ERK1/2_Right panel



p-ERK1/2_Left panel



p-ERK1/2_Right panel



DUSP5_Left panel



DUSP5_Right panel



β -actin_Leftt panel



β -actin_Right panel



Supplementary Table 1

Statistical information associated with Figure 1a							
Ig -class	Population	Min.	25th Percentile	Median	75th Percentile	Max.	IQR
IgA	B cells	0.214285714	0.585060351	0.691489362	0.80126183	1	0.216201479
IgA	Plasma cells	0.333333333	0.402777778	0.493519442	0.644444444	0.909090909	0.241666667
IgA	Plasmablasts	0.289473684	0.42503639	0.537878788	0.666666667	1	0.241630277
IgG	B cells	0	0.011288805	0.031914894	0.075709779	0.333333333	0.064420974
IgG	Plasma cells	0.037220844	0.218181818	0.35413643	0.414755733	0.584541063	0.196573915
IgG	Plasmablasts	0	0.257575758	0.331491713	0.446969697	0.537425854	0.189393939
IgM	B cells	0	0.134241245	0.210526316	0.333333333	0.785714286	0.199092088
IgM	Plasma cells	0	0.076719577	0.130434783	0.2	0.397022333	0.123280423
IgM	Plasmablasts	0	0.051313436	0.102040816	0.166666667	0.243093923	0.115353231

Horizontal black lines with each box present median values; boxes extend from 25th to 75th percentile of values; whiskers extend to a maximum of 1.5 X IQR (interquartile range=75th percentile-25th percentile) beyond the boxes; dots denote the value of all observations for each box; the lowest dots are the minimum values and highest dots are the maximum values for each box.

Comparison between	P value	Test performed
IgA vs. IgG	1e-10	Two-way ANOVA followed by Dunnett's ad hoc tests for multiple comparison was performed on arcsin-transformed percentage data
IgA vs. IgM	1e-10	

Supplementary Table 2

Statistical information associated with Figure 2a

Cell type	Treatment and time	Number of cells (n) quantified
WT OVCAR3	IgG, 0 min	319
WT OVCAR3	IgG, 15 min	290
WT OVCAR3	IgG, 1 hr	342
WT OVCAR3	IgG, 8 hr	366
WT OVCAR3	IgA, 0 min	348
WT OVCAR3	IgA, 15 min	287
WT OVCAR3	IgA, 1 hr	342
WT OVCAR3	IgA, 8 hr	278
WT OVCAR3	Pep-IgA, 0 min	212
WT OVCAR3	Pep-IgA, 15 min	307
WT OVCAR3	Pep-IgA, 1 hr	321
WT OVCAR3	Pep-IgA, 8 hr	278
PIGR ^{CRSIPR} OVCAR3	IgA, 0 min	282
PIGR ^{CRSIPR} OVCAR3	IgA, 15 min	260
PIGR ^{CRSIPR} OVCAR3	IgA, 1 hr	313
PIGR ^{CRSIPR} OVCAR3	IgA, 8 hr	304

Between treatment groups	P value	Test performed
WT OVCAR3 IgA_0 min vs. WT_OVACR3_IgA_15 min	<0.0001	Unpaired two-tailed t-test
WT_OVACR3_IgA_15 min vs. WT_OVACR3_IgA_1 hr	<0.0001	Unpaired two-tailed t-test
WT_OVACR3_IgA_1 hr vs. WT_OVACR3_IgA_8 hr	<0.0001	Unpaired two-tailed t-test
WT_OVCAR3_IgG_8 hr vs. WT_OVACR3_IgA_8 hr	<0.0001	Unpaired two-tailed t-test
WT_OVCAR3_IgA_8 hr vs. WT_OVACR3_Pep-IgA_8 hr	<0.0001	Unpaired two-tailed t-test
WT_OVCAR3_IgA_8 hr vs. PIGR ^{CRSIPR} _OVACR3_IgA_8 hr	<0.0001	Unpaired two-tailed t-test

Statistical information associated with Figure 2f

Effector cell type	Target cell type	Target-effector ratio	P value	Test performed
NY-ESO-1-TCR-transduced T-cells	OVCAR3 ^{Luci} -NY-ESO-1	1:1	0.0127	
NY-ESO-1-TCR-transduced T-cells	OVCAR3 ^{Luci} -NY-ESO-1	1:10	0.0255	
NY-ESO-1-TCR-transduced T-cells	OVCAR3 ^{Luci} -NY-ESO-1	1:20	0.0063	ordinary one-way ANOVA
FSH-CER T-cells	OVCAR3 ^{Luci}	1:1	0.0248	
FSH-CER T-cells	OVCAR3 ^{Luci}	1:10	0.0006	
FSH-CER T-cells	OVCAR3 ^{Luci}	1:20	<0.0001	

Supplementary Table 3

Statistical information associated with Figure 3b

Growth curve statistics

Between treatment groups	P value	Test performed
Tumour#1: PBS vs. iIgA	0.0155	Paired two-tailed t-test
Tumour#1: iIgA vs. Ab1	0.0144	Paired two-tailed t-test
Tumour#2: PBS vs. iIgA	0.0175	Paired two-tailed t-test
Tumour#2: iIgA vs. Ab2	0.0044	Paired two-tailed t-test
Tumour#2: iIgA vs. Ab3	0.0061	Paired two-tailed t-test

Tumour weight statistics

Between treatment groups	P value	Test performed
Tumour#1: PBS vs. iIgA	<0.0001	Unpaired two-tailed t-test
Tumour#1: iIgA vs. Ab1	<0.0001	Unpaired two-tailed t-test
Tumour#2: PBS vs. iIgA	<0.0001	Unpaired two-tailed t-test
Tumour#2: iIgA vs. Ab2	<0.0001	Unpaired two-tailed t-test
Tumour#2: iIgA vs. Ab3	<0.0001	Unpaired two-tailed t-test

Supplementary Table 4

Statistical information associated with Figure 3d

Growth curve statistics

Between treatment groups	P value	Test performed
iIgA vs. iIgG	0.0292	Paired two-tailed t-test
iIgA vs. α TSPAN7	0.0177	Paired two-tailed t-test
α TSPAN7 vs. α BDNF	0.0064	Paired two-tailed t-test

Tumour weight statistics

Between treatment groups	P value	Test performed
iIgA vs. iIgG	0.0006	Unpaired two-tailed t-test
iIgA vs. α TSPAN7	0.0308	Unpaired two-tailed t-test
iIgA vs. α BDNF	0.0465	Unpaired two-tailed t-test

Supplementary Table 5

Statistical information associated with Figure 3f

Growth curve statistics

Between treatment groups	P value	Test performed
F(ab') ₂ -αTSPAN7 vs. F(ab') ₂ -αBDNF	0.0255	Paired two-tailed t-test
F(ab') ₂ -iIgA vs. F(ab') ₂ -iIgG	0.0563	Paired two-tailed t-test

Tumour weight statistics

Between treatment groups	P value	Test performed
F(ab') ₂ -αTSPAN7 vs. F(ab') ₂ -αBDNF	0.0030	Unpaired two-tailed t-test
F(ab') ₂ -iIgA vs. F(ab') ₂ -αBDNF	0.0006	Unpaired two-tailed t-test
F(ab') ₂ -iIgA vs. F(ab') ₂ -iIgG	0.6145	Unpaired two-tailed t-test
F(ab') ₂ -iIgA vs. F(ab') ₂ -αTSPAN7	0.3231	Unpaired two-tailed t-test

Supplementary Table 6

Statistical information associated with Figure 4a

Growth curve statistics

Between treatment groups	P value	Test performed
iIgA vs. PBS	0.0067	Paired two-tailed t-test
iIgA vs. α TSPAN7	0.1504	Paired two-tailed t-test

Tumour weight statistics

Between treatment groups	P value	Test performed
iIgA vs. PBS	<0.0001	Unpaired two-tailed t-test
iIgA vs. α TSPAN7	0.4336	Unpaired two-tailed t-test

Supplementary Table 7

Statistical information associated with Figure 4b

Growth curve statistics

Between treatment groups	P value	Test performed
iIgA vs. PBS	0.0197	Paired two-tailed t-test
iIgA vs. α TSPAN7	0.0140	Paired two-tailed t-test
iIgA vs. α TSPAN7+ α NK1.1	0.0175	Paired two-tailed t-test

Tumour weight statistics

Between treatment groups	P value	Test performed
iIgA vs. PBS	<0.0001	Unpaired two-tailed t-test
iIgA vs. α TSPAN7	0.0004	Unpaired two-tailed t-test
α TSPAN7 vs. α TSPAN7+ α NK1.1	0.9119	Unpaired two-tailed t-test

Supplementary Table 8

Statistical information associated with Figure 4d

Between treatment groups	P value	Test performed
Uncoated:with- α CD351 ^{Neut} vs. Uncoated:no- α CD351 ^{Neut}	0.8115	Unpaired two-tailed t-test
α TSPAN7 ^{coated} :with- α CD351 ^{Neut} vs. α TSPAN7 ^{coated} :no- α CD351 ^{Neut}	0.0244	Unpaired two-tailed t-test

Statistical information associated with Figure 4e

Between treatment groups	P value	Test performed
PBS vs. iIgA	0.0036	Unpaired two-tailed t-test
iIgA vs. α TSPAN7	0.0132	Unpaired two-tailed t-test
α TSPAN7 vs. α TSPAN7+ α NK1.1	0.6264	Unpaired two-tailed t-test

Supplementary Table 9

Statistical information associated with Figure 4f

Growth curve statistics

Between treatment groups	P value	Test performed
WT:iIgA vs. WT:PBS	0.0068	Paired two-tailed t-test
WT:iIgA vs. CR:IgA	0.0015	Paired two-tailed t-test
WT: α BDNF vs. CR: α BDNF	0.0107	Paired two-tailed t-test
WT: α TSPAN7 vs. CR: α TSPAN7	0.0080	Paired two-tailed t-test
WT:PBS vs. CR:PBS	0.1143	Paired two-tailed t-test
WT:PBS vs. CR:iIgA	0.9062	Paired two-tailed t-test

Tumour weight statistics

Between treatment groups	P value	Test performed
WT:iIgA vs. CR:iIgA	0.0266	Unpaired two-tailed t-test
WT: α BDNF vs. CR: α BDNF	0.7354	Unpaired two-tailed t-test
WT: α TSPAN7 vs. CR: α TSPAN7	0.0009	Unpaired two-tailed t-test
WT:PBS vs. CR:PBS	0.8425	Unpaired two-tailed t-test
CR:PBS vs. CR:iIgA	0.8637	Unpaired two-tailed t-test

Supplementary Table 10

Patient Derived specimens			
Specimen type	ID	Experiment	Description
Solid tumours	S1	FACS, B cell immortalization	High grade Serous Ovarian Cancer
Solid tumours	S2	FACS, B cell immortalization	High grade Serous Ovarian Cancer
Solid tumours	S3	FACS	High grade Serous Ovarian Cancer
Solid tumours	S4	FACS	High grade Serous Ovarian Cancer
Solid tumours	S5	FACS, B cell immortalization	High grade Serous Ovarian Cancer
Solid tumours	S6	FACS	High grade Serous Ovarian Cancer
Solid tumours	S7	FACS, B cell immortalization	High grade Serous Ovarian Cancer
Solid tumours	S8	FACS	High grade Serous Ovarian Cancer
Solid tumours	S9	FACS	High grade Serous Ovarian Cancer
Solid tumours	S10	FACS, BCR sequencing	High grade Serous Ovarian Cancer
Solid tumours	S11	FACS, B cell immortalization	High grade Serous Ovarian Cancer
Solid tumours	S12	FACS	High grade Serous Ovarian Cancer
Solid tumours	S13	FACS	High grade Serous Ovarian Cancer
Solid tumours	S14	FACS, B cell immortalization	High grade Serous Ovarian Cancer
Solid tumours	S15	FACS, B cell immortalization	High grade Serous Ovarian Cancer
Solid tumours	S16	FACS	High grade Serous Ovarian Cancer
Solid tumours	S17	FACS	High grade Serous Ovarian Cancer
Solid tumours	S18	FACS, B cell immortalization	High grade Serous Ovarian Cancer
Solid tumours	S19	FACS	High grade Serous Ovarian Cancer
Solid tumours	S20	FACS	High grade Serous Ovarian Cancer
Solid tumours	S21	FACS, B cell immortalization	High grade Serous Ovarian Cancer
Solid tumours	S22	FACS, B cell immortalization	High grade Serous Ovarian Cancer
Solid tumours	S23	FACS	High grade Serous Ovarian Cancer
Solid tumours	S24	FACS	High grade Serous Ovarian Cancer
Solid tumours	S25	FACS	High grade Serous Ovarian Cancer
Solid tumours	S26	FACS	High grade Serous Ovarian Cancer
Solid tumours	S27	FACS	High grade Serous Ovarian Cancer
Solid tumours	S28	FACS	High grade Serous Ovarian Cancer
Solid tumours	S29	FACS, BCR sequencing	High grade Serous Ovarian Cancer
Ascites	A1	Co-Immunoprecipitation	High grade Serous Ovarian Cancer
Ascites	A2	Co-Immunoprecipitation	High grade Serous Ovarian Cancer
FFPE-TMA	MCC1	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC2	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC3	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC4	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC5	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC6	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC7	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC8	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC9	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC10	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC11	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC12	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC13	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC14	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer
FFPE-TMA	MCC15	Multiplex Immunofluorescence	High grade Serous Ovarian Cancer

Supplementary Table 11

Statistical information associated with Extended data figure 6e

Cell type	Treatment	Number of cells (<i>n</i>) quantified
WT OVCAR3	α TSPAN7	338
PIGR ^{CRISPR} OVCAR3	α TSPAN7	319
WT OVCAR3	α BDNF	400
PIGR ^{CRISPR} OVCAR3	α BDNF	346
WT OVCAR3	iIgA	244
PIGR ^{CRISPR} OVCAR3	iIgA	311
WT OVCAR3	iIgG	420

Between treatment groups	<i>P</i> value	Test performed
WT: α TSPAN7 vs. PIGR ^{CRISPR} : α TSPAN7	<0.0001	Unpaired two-tailed t-test
WT: α BDNF vs. PIGR ^{CRISPR} : α BDNF	<0.0001	Unpaired two-tailed t-test
WT:iIgA vs. PIGR ^{CRISPR} :iIgA	<0.0001	Unpaired two-tailed t-test

Supplementary Table 12

Statistical information associated with Extended data figure 6g

Growth curve statistics

Between treatment groups	P value	Test performed
Tumour#1: PIGR ⁺ :PBS vs. PIGR ⁺ :iIgA	0.0155	Paired two-tailed t-test
Tumour#1: PIGR ⁺ :iIgA vs. PIGR ⁺ :Ab1	0.0144	Paired two-tailed t-test
Tumour#1: PIGR ⁺ :Ab1 vs. PIGR ^{CRISPR} :Ab1	0.0172	Paired two-tailed t-test
Tumour#1: PIGR ⁺ :iIgA vs. PIGR ^{CRISPR} :iIgA	0.0125	Paired two-tailed t-test
Tumour#1: PIGR ^{CRISPR} :iIgA vs. PIGR ^{CRISPR} :Ab1	0.0120	Paired two-tailed t-test
Tumour#2: PIGR ⁺ :PBS vs. PIGR ⁺ :iIgA	0.0175	Paired two-tailed t-test
Tumour#2: PIGR ⁺ :iIgA vs. PIGR ⁺ :Ab2	0.0044	Paired two-tailed t-test
Tumour#2: PIGR ⁺ :iIgA vs. PIGR ⁺ :Ab3	0.0061	Paired two-tailed t-test
Tumour#2: PIGR ⁺ :iIgA vs. PIGR ^{CRISPR} :iIgA	0.0155	Paired two-tailed t-test
Tumour#2: PIGR ⁺ :Ab2 vs. PIGR ^{CRISPR}	0.0113	Paired two-tailed t-test
Tumour#2: PIGR ⁺ :Ab3 vs. PIGR ^{CRISPR}	0.0089	Paired two-tailed t-test
Tumour#2: PIGR ^{CRISPR} :iIgA vs. PIGR ^{CRISPR}	0.0095	Paired two-tailed t-test
Tumour#2: PIGR ^{CRISPR} :iIgA vs. PIGR ^{CRISPR}	0.0107	Paired two-tailed t-test

Tumour weight statistics

Between treatment groups	P value	Test performed
Tumour#1: PIGR ⁺ :iIgA vs. PIGR ⁺ :Ab1	<0.0001	Unpaired two-tailed t-test
Tumour#1: PIGR ⁺ :iIgA vs. PIGR ^{CRISPR} :iIgA	<0.0001	Unpaired two-tailed t-test
Tumour#1: PIGR ⁺ :Ab1 vs. PIGR ^{CRISPR} :Ab1	<0.0001	Unpaired two-tailed t-test
Tumour#1: PIGR ^{CRISPR} :iIgA vs. PIGR ^{CRISPR} :Ab1	<0.0001	Unpaired two-tailed t-test
Tumour#2: PIGR ⁺ :PBS vs. PIGR ⁺ :iIgA	<0.0001	Unpaired two-tailed t-test
Tumour#2: PIGR ⁺ :iIgA vs. PIGR ⁺ :Ab2	<0.0001	Unpaired two-tailed t-test
Tumour#2: PIGR ⁺ :iIgA vs. PIGR ⁺ :Ab3	<0.0001	Unpaired two-tailed t-test
Tumour#2: PIGR ⁺ :Ab2 vs. PIGR ^{CRISPR} :Ab2	<0.0001	Unpaired two-tailed t-test
Tumour#2: PIGR ⁺ :Ab3 vs. PIGR ^{CRISPR} :Ab3	<0.0001	Unpaired two-tailed t-test
Tumour#2: PIGR ^{CRISPR} :iIgA vs. PIGR ^{CRISPR} :Ab2	<0.0001	Unpaired two-tailed t-test
Tumour#2: PIGR ^{CRISPR} :iIgA vs. PIGR ^{CRISPR} :Ab3	<0.0001	Unpaired two-tailed t-test