

Supplementary Materials for

A novel proangiogenic B cell subset is increased in cancer and chronic inflammation

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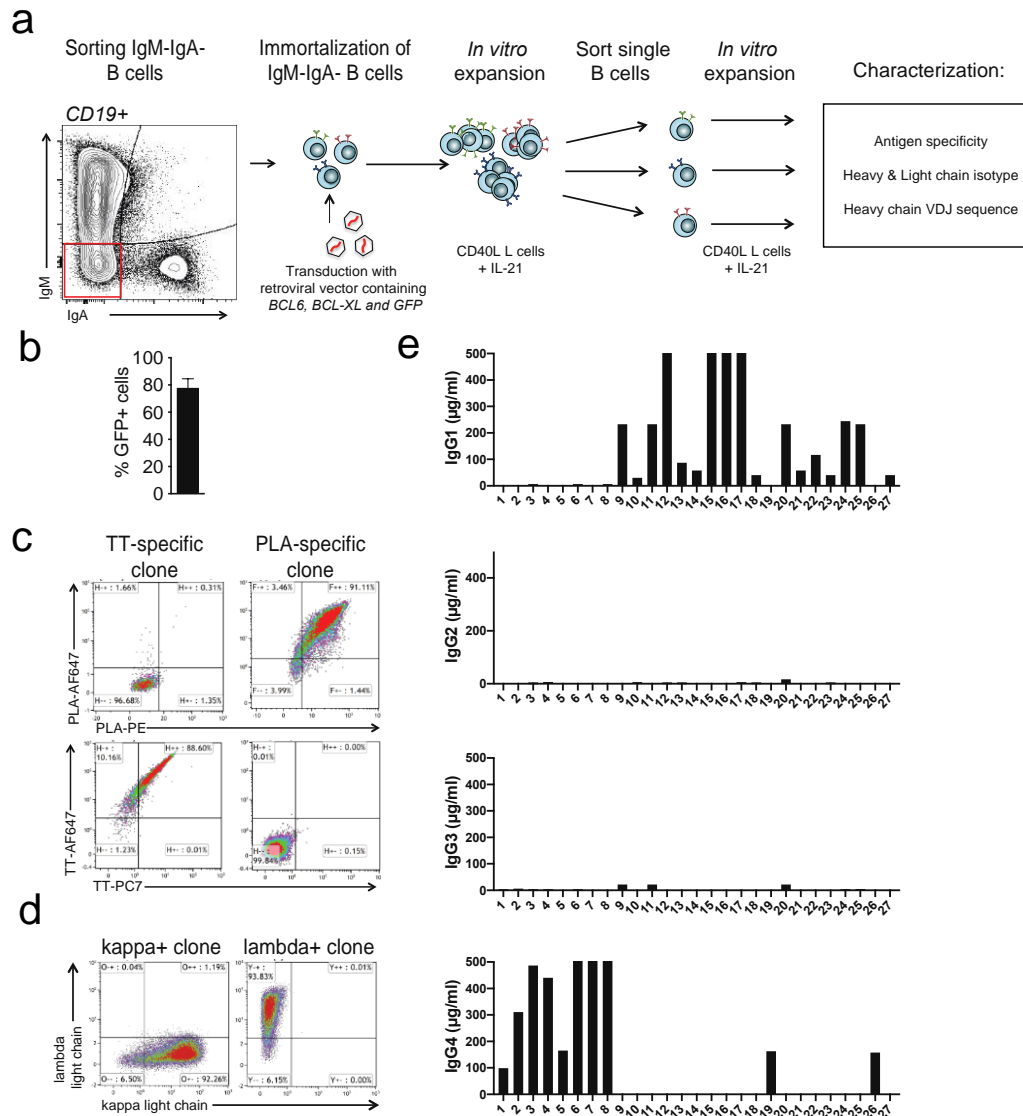


Fig. S1. Generation and characterization of B cell clones. (a) CD19+IgM-IgA- B cell pools were sorted and immortalized. After 3 days *in vitro* expansion the transduction efficiency was measured by analysis of GFP expression (b). Single cells specific for PLA or TT were sorted and expanded for 6 weeks in the presence of CD40L and IL-21 (a). Antigen specificity of purified clones was assessed by staining with fluorescently labeled TT and PLA (c). Immunoglobulin light chain isotypes were determined by flow cytometry (d) and immunoglobulin heavy chain isotypes were determined using bead suspension array (e).

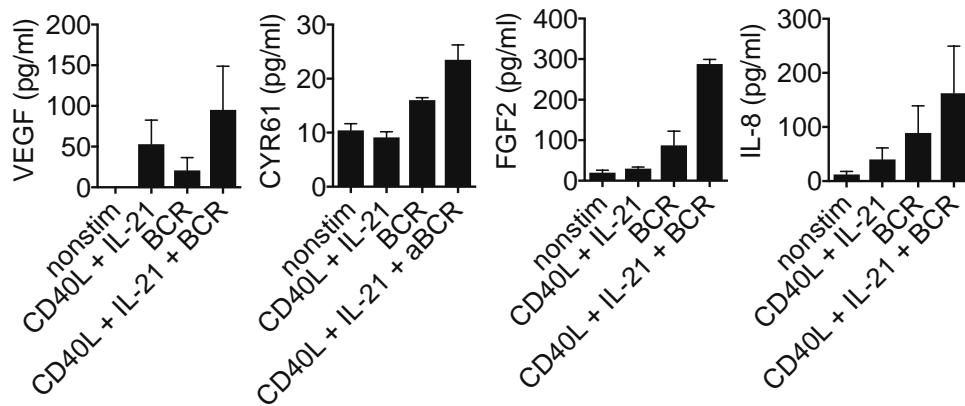


Fig. S3 Primary B cells can produce pro-angiogenic cytokines. Measurement of secreted pro-angiogenic cytokines in supernatants of purified primary peripheral B cells after 3 days stimulation.

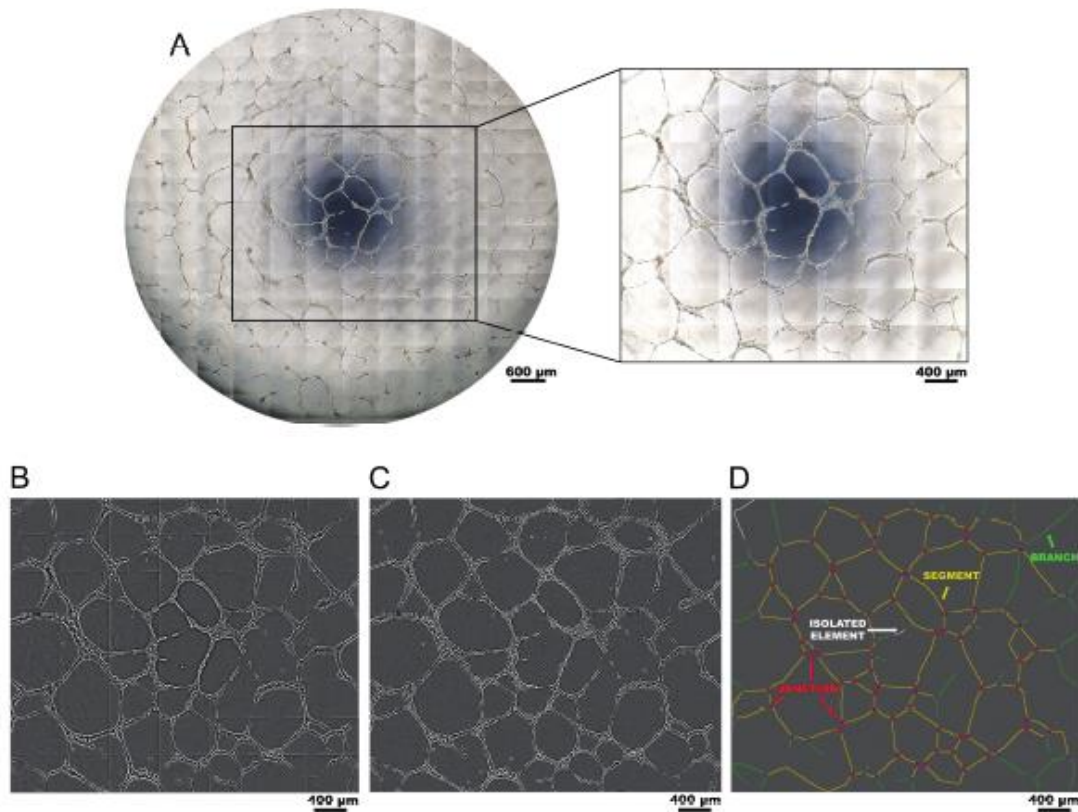


Fig. S4 Data processing and quantification of HUVEC tube formation assay. **(a)** Original image of the whole well and representative area of the well. **(b)** Adobe Photoshop processing step 1 - background reduction: Filter → High Pass (Radius: 10 px) and step 2 - **(c)** stitching lines removal: Tools → Spot Healing Brush Tool (Size: 15 px). **(d)** Final quantification performed with the use of ImageJ and default settings of Angiogenesis Analyzer. The number of junctions and total length [px] were used as parameters for quantification of the rate of tube formation.

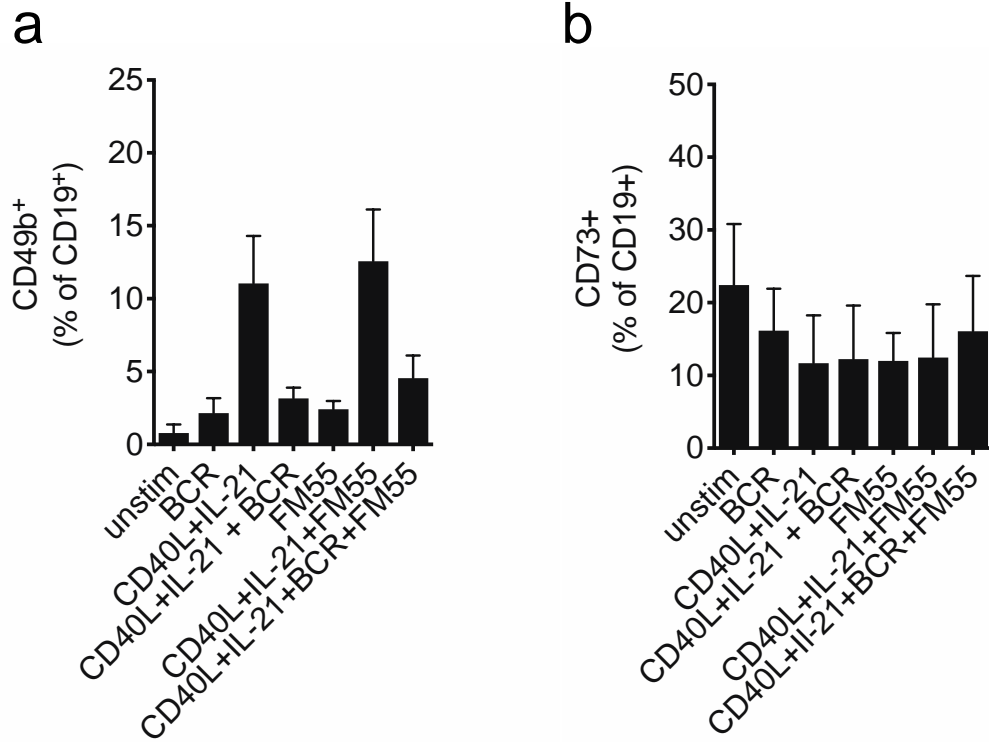


Fig. S5 Effect of co-culturing melanoma cells with primary B cells on the expression of CD49b (a) and CD73 (b). B cells were cultured for three days and expression of CD49b and CD73 was analyzed by flow cytometry.

Table S1. Clone Characteristics

Clone ID	Gender	Donor ID	Heavy chain isotype	Light chain isotype	V gene and allele	J gene and allele	D gene and allele
1	Female	1	IgG4	lambda	V1-18*04	J5*02	D5-12*01
2	Male	2	IgG4	lambda	V3-53*01	J4*02	D6-13*01
3	Male	2	IgG4	kappa	V1-46*01	J3*02	D3-10*01
4	Male	2	IgG4	kappa	V1-2*02	ND	ND
5	Male	2	IgG4	kappa	V5-51*01	J3*02	D3-22*01
6	Male	2	IgG4	kappa	V3-30*03	J1*01	D4-23*01
7	Male	2	IgG4	kappa	V3-33**01	J3*01	D1-20*01
8	Male	3	IgG4	kappa	ND	ND	ND
9	Female	4	IgG1	ND	ND	ND	ND
10	Male	5	IgG1	lambda	V1-69*09	J6*02	D6-13*01
11	Male	5	IgG1	kappa	V3-30*03	J1*01	D5-18*01
12	Male	5	IgG1	kappa	V3-74*01	J2*01	D3-10*01
13	Male	5	IgG1	kappa	V3-74*01	J6*02	D4-17*01
14	Male	5	IgG1	kappa	V3-30*03	J4*02	D3-22*01
15	Male	6	IgG1	kappa	V3-20*01	J3*01	ND
16	Male	6	IgG1	kappa	V1-18*01	J4*02	D1-26*01
17	Male	6	IgG1	kappa	V3-23*04	J4*02	D2-2*01
18	Male	6	IgG1	kappa	V3-9*01	J4*02	D5-12*01
19	Male	6	IgG4	lambda	V3-30*03	J1*01	D4-23*01
20	Male	6	IgG1	kappa	ND	ND	ND
21	Male	7	IgG1	kappa	V3-20*01	J6-03	D2-15*01
22	Male	7	IgG1	kappa	V3-53*04	J5*03	D5-13*01
23	Male	7	IgG1	kappa	V3-9*01	J4*02	D4-17*01
24	Male	7	IgG1	ND	V1-58*01	J4*02	D1-26*01
25	Male	7	IgG1	kappa	V5-51*01	J2*02	D3-16*01
26	Male	7	IgG4	kappa	V1-69*01	J4*02	D3-9*01
27	Male	8	IgG1	lambda	V4-59*01	J6*03	D3-9*01

ND Not determined

Table S2. Melanoma patient characteristics

Donor ID	Age at sampling	Gender	Diagnosis and stage	Material analyzed	Analysis
M1	45	female	melanoma stage IIIc	Resected tissue	Confocal microscopy
M2	93	male	melanoma stage IV	Resected tissue	Confocal microscopy
M3	73	female	melanoma stage IV	Resected tissue	Confocal microscopy
M4	75	male	melanoma stage IV	Resected tissue	Confocal microscopy
M5	65	male	melanoma stage IV	Resected tissue	Confocal microscopy
M6	33	female	melanoma stage IV	Resected tissue	Confocal microscopy
M7	52	female	melanoma stage IIIa	Resected tissue	Confocal microscopy
M8	56	female	melanoma stage IIIc	Resected tissue	Confocal microscopy
M9	65	male	melanoma stage IV	Resected tissue	Confocal microscopy
M10	36	female	melanoma stage IV	PBMC	Flow cytometry
M11	74	female	melanoma stage IV	PBMC	Flow cytometry
M12	56	female	melanoma stage IIIb	PBMC	Flow cytometry
M13	73	female	melanoma stage IV	PBMC	Flow cytometry
M14	53	male	melanoma stage IIIc	PBMC	Flow cytometry
M15	67	male	melanoma stage IV	PBMC	Flow cytometry
M16	33	male	melanoma stage IIIb	PBMC	Flow cytometry
M17	69	male	melanoma stage IV	PBMC	Flow cytometry
M18	45	female	melanoma stage IIIc	PBMC and resected tissue	Flow cytometry
M19	93	male	melanoma stage IV	PBMC and resected tissue	Flow cytometry
M20	53	male	melanoma stage IIIc	PBMC	Flow cytometry
M21	73	female	melanoma stage IV	PBMC	Flow cytometry
M22	50	male	melanoma stage IV	PBMC and resected tissue	Flow cytometry
M23	93	male	melanoma stage IIIb	PBMC	Flow cytometry
M24	69	female	melanoma stage IV	PBMC	Flow cytometry
M25	77	female	melanoma stage IV	PBMC	Flow cytometry
M26	61	male	melanoma stage IV	PBMC	Flow cytometry
M27	86	female	melanoma stage IV	PBMC	Flow cytometry
M28	65	male	melanoma stage IV	PBMC	Flow cytometry
M29	85	male	melanoma stage IV	PBMC	Flow cytometry
M30	77	male	melanoma stage IV	PBMC	Flow cytometry
M31	65	female	melanoma stage IV	PBMC	Flow cytometry
M32	60	female	melanoma stage IV	PBMC	Flow cytometry
M33	70	female	melanoma stage IV	PBMC	Flow cytometry

Table S3. EoE patient characteristics

Donor ID	Age (years)	Gender	Food allergy	Symptoms	ENDOSCOPIC FINDINGS						HISTOLOGY				SYMPTOMS (current)		TREATMENT (current)						
					severe (oesophageal blockage)	Edema	Furrows	Exudates	Rings	Strictures	Others	Peak epithelial Eos/hpf (size of hpf 0.3072mm ²)	Peak epithelial Eos 0 (0), 1 (0-40), 3 > 40	Peak stromal/lamina propria Eos/hpf (size of hpf 0.3072mm ²)	Peak stromal/lp Eos	Spongiosis 1 = absent, 2 = mild, 3 = moderate, 4 = severe	Fibrosis 1 = absent, 2 = mild, 3 = moderate, 4 = severe	EoE-Drug Budesonide 1 = Y (mg/day), 2 = N	EoE-Drug Fluticasone 1 = Y (mg/day), 2 = N	EoE-Drug 3 1 = Y (mg/day), 2 = N	PPI 1 = Y (which), 2 = N	Systemic Steroids 1 = Y (mg/day), 2 = N	Dietary Treatment 1 = N, 2 = Elemental Diet, 3 = Targeted Diet, 4 = Empirical Diet, 5 = Other Diets (describe)
EoE1	37	m	No	severe (oesophageal blockage)	Yes	No	No	Yes	No	No	0	0	0	0	1	6	1; 2mg/day	2	2	1 (esomep)	2	1	2
EoE2	18	f	No	moderate (frequent difficulty swallowing)	Yes	Yes	No	Yes	No	No	35	1	50	2	2	3	2	1; 2	2	2	2	2	2
EoE3	24	m	No	mild (occasional difficulty swallowing)	Yes	No	No	Yes	No	No	17	1	40	2	2	2	2	1; 0.5	2	2	2	2	2
EoE4	56	m	No	absent	No	No	No	Yes	No	No	0	0	0	0	1	2	2	1; 0.5	2	2	2	2	2
EoE5	45	f	No	absent	Yes	Yes	No	Yes	No	No	58	2	5	1	2	2	2	2	2	Pantoprazol	2	2	2
EoE6	35	f	No	moderate (frequent difficulty swallowing)	Yes	Yes	No	Yes	Yes	No	35	1	40	2	2	3	2	1; 1.0	2	2	2	2	2
EoE7	44	m	No	moderate (frequent difficulty swallowing)	Yes	Yes	Yes	Yes	No	No	63	2	15	1	4	3	2	2	2	2	2	4 (milk 3 months)	2
EoE8	86	m	No	mild (occasional difficulty swallowing)	No	No	No	No	No	Nodules	0	0	0	0	1	1	2	1; 0.5	2	2	2	2	2
EoE9	27	m	No	mild (occasional difficulty swallowing)	Yes	Yes	Yes	Yes	No	No	170	2	30	1	3	3	2	1; 2	2	2	2	2	2
EoE10	66	m	No	absent	No	No	No	Yes	Yes	No	0	0	10	1	1	3	2	2	2	2	2	4 (milk 3 months)	2
EoE11	41	m	No	severe (oesophageal blockage)	Yes	Yes	No	Yes	No	No	29	1	10	1	2	2	2	1; 0.5	2	2	2	2	2
EoE12	74	m	No	moderate (frequent difficulty swallowing)	No	No	No	No	No	Nodules	0	0	4	1	1	2	2	1; 0.5	2	2	2	2	2
EoE13	17	f	Yes (oral allergy syndrome)	mild (occasional difficulty swallowing)	Yes	Yes	No	Yes	No	No	80	2	20	1	3	2	2	2	2	2	2	4 (milk 3 months)	2
EoE14	23	m	No	moderate (frequent difficulty swallowing)	Yes	Yes	Yes	Yes	No	No	42	2	not measured		4	3	2	2	2	2	2	2	2
EoE15	22	m	No	moderate (frequent difficulty swallowing)	Yes	Yes	No	Yes	No	No	50	2	5	1	3	2	2	2	2	2	2	4 (milk 3 months)	2
EoE16	51	m	No	mild (occasional difficulty swallowing)	Yes	Yes	No	Yes	No	No	74	2	150	2	3	2	2	1; 0.5	2	2	2	2	2
EoE17	71	m	No	mild (occasional difficulty swallowing)	No	No	No	Yes	No	Nodules	0	0	10	1	2	3	2	1; 0.5	2	2	2	2	2
EoE18	62	m	Yes (oral allergy syndrome)	moderate (frequent difficulty swallowing)	Yes	No	No	Yes	No	No	7	1	40	2	2	3	2	1; 0.5	2	2	2	2	2

Table S4. Antibodies used for flow cytometry

Target	Label	Clone	Isotype	Company
CD73	FITC	AD2	mIgG1,k	Biologend
CD49b	APC	P1E6-C5	mIgG1,k	Biologend
CD112	PE	TX31	mIgG1,k	Biologend
CD276	PE	DCN.70	mIgG1,k	Biologend
CD325	PE	8C11	mIgG1,k	Biologend
CD53	PE	HI29	mIgG1,k	Biologend
CD19	APC/Fire750	HIB19	mIgG1,k	Biologend
Viability dye	Zombie yellow	viability dye	-	Biologend
CD39	BV711	A1	mIgG1,k	Biologend
FLT1 (EIC)	PE*	sc-57137	mouse	Santa Cruz Biotech
IgA	AF488	goat polyclonal	goat polyclonal	Jackson laboratories
IgM	Perp/Cy5.5	MHM-88	mIgG1,k	Biologend
lambda light chain	Pacific blue	MHL-38	mIgG2a,k	Biologend
kappa light chain	AF647	MHK-49	mIgG1,k	Biologend

Table S5. Primers used for real-time PCR

Target gene	Forward 5' --> 3'	Reverse 5' --> 3'
ADM	TTG GCA GAT CAC TCT CTT AG	TTC CAC TTC TTT CGA AAC TC
CCL22	GCG TGG TGT TGC TAA CCT TC	CCA CGG TCA TCA GAG TAG GC
CYR61	TTG ATT GCA GTT GGA AAA GG	GCC TTG TAA AGG GTT GTA TAG
EF1A	CTG AAC CAT CCA GGC CAA AT	GCC GTG TGG CAA TCC AAT
FGF2	GCT GTA CTG CAA AAA CGG GG	TAG CTT GAT GTG AGG GTC GC
IL10	GTG ATG CCC CAA GCT GAG A	CAC GGC CTT GCT CTT GTT TT
IL16	CAG TGT TAA TCC CTA TTG CAC	ATT GTT GAG AGA GGG ACT TC
MDK	AAG ATA AGG TGA AGA AGG GC	TCA AAC TTG TAC TTG CAG TC
PDGFA	AAG ACC AGG ACT GTC ATT TAC G	TTG ACG CTG CTC GTG TTG C
TGFB2	ATC CTG AGC CCG AGG AAG TC	GAT GGC ATT TTC GGA GGG GA
TNFSF10	CTT ACG TGT ACT TTA CCA ACG	CAT TCT TGG AGT CTT TCT AAC G
SEMA4A	ATC ATT AAA GAA GTC CTG GC	ATT GTA GAC AGT GGA AGA GG
VEGFA	AAT GTG AAT GCA GAC CAA AG	GAC TTA TAC CGG GAT TTC TTG

Table S6. Antibodies used for confocal microscopy staining

Antibody	Label	Clone	Isotype	Company	Order number	Concentration used
Primary						
CYR61	-	3H3	Mouse IgG1	Novus Biologicals	NBD1-04285	10 ug/ml
VEGF	Dylight 633*	Rabbit polyclonal	Rabbit polyclonal	GeneTex	GTX102643	5.8 ug/ml
CD20	-	EP459Y	Rabbit IgG	Abcam	AB78237	0.148 ug/ml
CD73	-	2B6	Mouse IgG2b	Antibodies online	ABN238367	10 ug/ml
CD49b	AF546**	HAS-3	Mouse IgG2a	Abcam	Ab10800	13 ug/ml
CD138		MI15	Mouse IgG1	Novus Biologicals	NBP1-54523	0.27 ug/ml
IgG4	Dy650*	EP4420	Rabbit	Abcam	Ab186921	10 ug/ml
Secondary						

Mouse IgG	AF405	goat polyclonal	goat polyclonal	Invitrogen	A-31553	10 ug/ml
Rabbit	AF488	goat polyclonal	goat polyclonal	Invitrogen	A-11008	1 ug/ml
Mouse IgG2b	AF594	goat polyclonal	goat polyclonal	Invitrogen	A-21145	4 ug/ml
Isotype controls						
Mouse IgG1				Agilent Dako	X0931	10 ug/ml
Mouse IgG2a				Agilent Dako	X0943	13 ug/ml
Mouse IgG2b				Agilent Dako	X0944	10 ug/ml
Rabbit				Agilent Dako	X0936	10 ug/ml