Supplementary Table S1: Primer sequences used for cloning antigen into pET-21b bacterial expression vector

Primer	Sequence	Antigen cloned and sequenced
T7 Clon (for)	5'CGCCGCCGCGGATCCGCGACGCGTCGACCATCATCATCATCATCATATG GCTAGCATGACTGGTGGACAGCAAATG 3'	4B7, 5H6, T7 1-2a (cloned)
T7 NdeI Clon For	5'ATAGAATCACATTAAACAGGAATTCCATATGGAATTCCATCATCATCATCA TCATGGTGTTATGGCTAGCATGACTGGTGGACAGCAAATG 3'	4H4 (cloned)
T7 reverse primer	5'CCTCCTTTCAGCAAAAAACCCC 3'	4B7, 4H4, 5H6 (cloned)
T7Select seq primer (for)	5' TGCTAAGGACAACGTTATCGG3′	4B7, 4H4, 5H6, T7 1-2a (sequenced)
New pET T7 Prom Seq	5′ AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGG 3′	4B7, 4H4, 5H6, Ro52, CDR2, T7 1-2a (sequenced)
CDR2-NdeI-For	5′ATCACATTAAACAGGAATTCCATATGGAATTCCATCATCATCATCATCATG GTGTTATGGCTAGCATGACTGGTGGACAGCAAATGGGTGGGATGCTGGCG GAAAACCTGGTAGAGGAGT 3′	CDR2 (cloned)
CDR2-Xho I Rev:	5′CGCGGATCCCGCTCGAGCGGTAGAGCTAGAGGTTCAATTAAGAATGAGA GGAGAGTGATC 3′	CDR2 (cloned)
CDR2-Seq	5′ AGGAATATGGGCTCGTGTTAAAGGAGAACAGTGAAC 3′	CDR2 (sequenced)
Ro52-SalI For	5′CGCCGCCGCGGATCCGCGACGCGTCGACCATCATCATCATCATCATATG GCTTCAGCAGCACGCTTGACAATGATGTGGGAGG 3′	Ro52 (cloned)
Ro52-XhoI Rev	5′CGCGGATCCCGCTCGAGCGGCCATCAATAGTCAGTGGATCCTTGTGATC CAATA 3′	Ro52 (cloned)
Ro52-Seq	5′ TCCATGCCAAGTTGGGATTTTCCTGGACTATGAGGCTGGCAT 3′	Ro52 (sequenced)

Supplementary Table S2: Demographics of 21 ovarian cancer patients used in the test set

StudyID	Age at Dx	BSID	DxStageCd	TumorClass	TumorGrade	DFI	OS	TTR1	RecurInterval	Chemo	CA125Recode	DxInterval	EvidenceofDiseaseCd
P283	51	4680	3C	Serous	Grade 3 (high)	11.63	82	18.27	-18.27	pre chemo	2393	0.00	EOD
		5110			Poorly differentiated				-13.60	in chemo	22	4.67	Not Specified
		12322							1.67	recur post chemo	95	19.93	Not Specified
P295	71	5042	4	Serous		5.83	32	10.67	-10.67	pre chemo	388	0.00	EOD
		7183							-5.77	post chemo	18	4.90	NED
		12269							3.97	recur in chemo	64	14.63	Not Specified
P336	60	12563	3C	Serous	Grade 3 (high)	15.10	85	20.30	-20.23	pre chemo	1185	0.07	EOD
		12912			Poorly differentiated				-10.00	post chemo	20	10.30	NED
		15164							23.67	recur pre chemo	185	43.97	EOD
P341	40	12671	3C	Serous	Grade 3 (high)	0.57	38	14.60	-14.50	pre chemo	1246	0.10	EOD
		12897			Poorly differentiated				-7.10	in chemo	37	7.50	Not Specified
		13555							3.17	in chemo	87	17.77	Not Specified
P300	69	7174	3C	Serous	Grade 3 (high)	4.90	65	10.17	-10.17	pre chemo	1226	0.00	EOD
		11499	3C		Poorly differentiated				-3.73	post chemo	12	6.43	NED
		12359	3C						4.50	recur in chemo	246	14.67	Not Specified
P326	77	12380	3C	Serous	Moderately to	10.13	36	16.27	-16.23	pre chemo	1012	0.03	EOD
		12898	3C		poorly differentiated				-4.30	in chemo	41	11.97	Stable Disease
		13541	3C						3.43	recur in chemo	115	19.70	Tolerating treatment
P342	60	12675	3C	Serous	Grade 3 (high)	3.20	19	7.67	-7.63	pre chemo	182	0.03	EOD
		12863	3C		Poorly differentiated				-3.30	in chemo	5	4.37	NED
		12908	3C						0.90	in chemo	34	8.57	Not Specified
		13507	3C						6.03	in chemo	228	13.70	EOD

P356	44	12867	3C	Serous	Grade 3 (high)	9.07	39	13.77	-13.70	pre chemo	542	0.07	EOD
		12920	3C		Poorly differentiated				-9.07	in chemo	11	4.70	Tolerating treatment
		13549	3C						-1.63	post chemo	89	12.13	EOD
P367	49	13606	4	Serous	nd	4.33	19	10.90	-10.90	pre chemo	788	0.00	EOD
		14698	4						-4.33	in chemo	29	6.57	Tolerating treatment
		14893	4						2.20	recur in chemo	3084	13.10	EOD
P370	59	13615	3C	Serous	Grade 3 (high)	2.63	32	8.03	-8.03	pre chemo	1200	0.00	EOD
		14697	3C		Poorly differentiated				-2.63	in chemo	13	5.40	
		14832	3C						2.50	recur in chemo	300	10.53	EOD
P178	35	887	3C	Serous	Grade 3 (high)	3.50	34	9.83	-9.80	pre chemo	16906	0.03	EOD
		1667	3C		Poorly differentiated				-4.20	in chemo	203	5.63	NED
		3817	3C						1.57	recur in chemo	506	11.40	EOD
P378	49	14828	4A	Serous	Grade 3 (high)	11.67	61	28.27	-28.27	pre chemo	1725	0.00	EOD
		15180	4A		Poorly differentiated				-12.37	in chemo	9	15.90	Not Specified
		15281	4A						-1.87	post chemo	60	26.40	Not Specified
P392	47	15175	3C	Serous	Grade 3 (high)	8.53	28	13.67	-13.67	pre chemo	13154	0.00	EOD
		15256	3C		Poorly differentiated				-5.93	post chemo	9	7.73	NED
		15292	3C						0.37	recur in chemo	60	14.03	EOD
P393	67	15190	3C	Serous	Grade 3 (high)	4.10	29	12.07	-11.10	pre chemo	4040	0.97	
		15259	3C		Poorly differentiated				-5.03	in chemo	26	7.03	EOD
		15291	3C						0.23	recur pre chemo	3500	12.30	EOD
P265	49	4347	3C	Serous	Grade 3 (high)	4.87	24	9.50	-9.53	pre chemo	2693	-0.03	EOD
		4694	3C		Poorly differentiated				-4.87	in chemo	54	4.63	Not Specified
		5109	3C						-0.43	recur post chemo	204	9.07	Not Specified
P386	56	15155	3C	Serous	Grade 3 (high)	16.80	52	22.73	-22.73	pre chemo	584	0.00	

		15178	3C		Poorly differentiated				-17.27	in chemo	16	5.47	Not Specified
		15251	3C						-10.50	post chemo	14	12.23	NED
		15769	3C						1.17	recur in chemo	166	23.90	EOD
P410	62	15260	3C	Serous	Grade 3 (high)	8.13	45	14.77	-14.77	pre chemo	3643	0.00	EOD
		15776	3C		Poorly differentiated				-3.23	post chemo	24	11.53	NED
		15796	3C						1.20	recur in chemo	568	15.97	Not Specified
P413	55	15272	3C	Serous	Grade 3 (high)	7.83	48	24.53	-24.50	pre chemo	3217	0.03	EOD
		15770	3C		Poorly differentiated				-15.53	in chemo	8	9.00	Not Specified
		15792	3C						-10.87	in chemo	7	13.67	NED
P376	57	14822	3C	Serous	Grade 3 (high)	17.27	61	34.33	-34.47	pre chemo	1619	-0.13	EOD
		15264	3C		Poorly differentiated				-9.93	post chemo	7	24.40	NED
		15740	3C						-0.73	post chemo	10	33.60	EOD
P398	72	15226	3C	Serous	Grade 3 (high)	8.67	25	15.20	-15.20	pre chemo	120	0.00	EOD
		15266	3C		Poorly differentiated				-10.30	in chemo	24	4.90	EOD
		15781	3C						1.87	recur in chemo	38	17.07	EOD
P400	47	15229	3C	Serous	Grade 3 (high)	4.27	19	9.63	-9.63	pre chemo	8658	0.00	EOD
		15274	3C		Poorly differentiated				-4.50	in chemo	6	5.13	Not Specified
		15659	3C						0.17	recur pre chemo	106	9.80	EOD

Note: Average age of onset = 56 years, range 35-79

Average TTR = 15.48 months, range 7.7-34.3

Average DFI (interval between date of last chemo and recurrence) = 7.76 months (range 0.57-17.27)

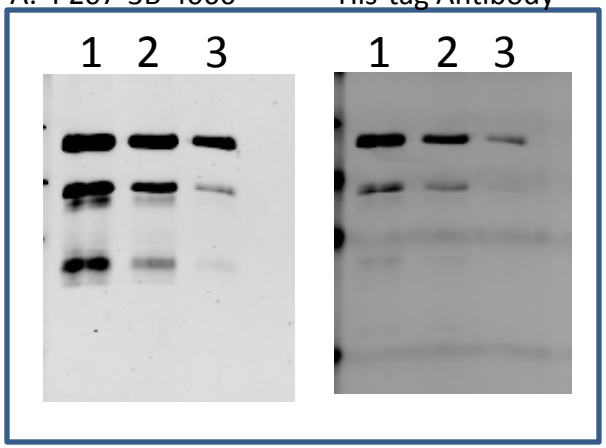
antigen that is shown on the western blot image at time T2 shows that the normalized signal intensity for that particular antigen is above its cutoff. Underneath each western blot images in a panel, the normalized signal intensity value of each protein band is shown.

Fig. 2. **Determination of sensitivity of different combination of antigens for predicting ovarian cancer recurrence using venn diagram.** Venn diagrams represented the immunoreactivity of each antigen (above its cutoff) with ovarian cancer patient's serum sample obtained at time T2 (the blood sample collected approximately 3-15 months before the clinical recurrence, ideally with normal CA125 and no evidence of disease). Panels (A-B) represent venn diagram of different combination of antigens, Ro52, CDR2 and 5H6 (A), and Ro52, HARS, and CDR2 (B) used for determining sensitivity for predicting ovarian cancer recurrence.

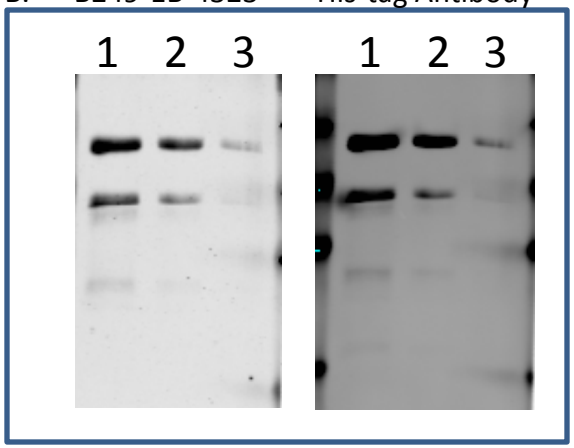
Supplementary Fig. 1. **Western blot and graphical representation of difference in immunoreactivity of Ro52 protein with serum IgGs using varying amounts of Ro52 protein.** Varying amounts of Ro52, such as 0.3 µg, 0.15 µg and 0.06 µg were loaded and SDS-PAGE followed by immunoscreening using serum samples obtained from 1 ovarian cancer patient (A) and a women with benign disease (B) was performed using western blot as described in figure legend 1 (Fig. 1). Supplementary Fig. 1C is a graphical representation of difference in immunoreactivity of Ro52 antigen with ovarian cancer patient P203-3B-4066 and women with benign disease B249-1B-4323 using varying amounts of Ro52 protein. This is utilized to choose the optimum amount of Ro52 protein in

micrograms for which the signal intensity of the Ro52 protein band remained within the detectable range when measured with Odyssey software.

A. P207-3B-4066 His-tag Antibody

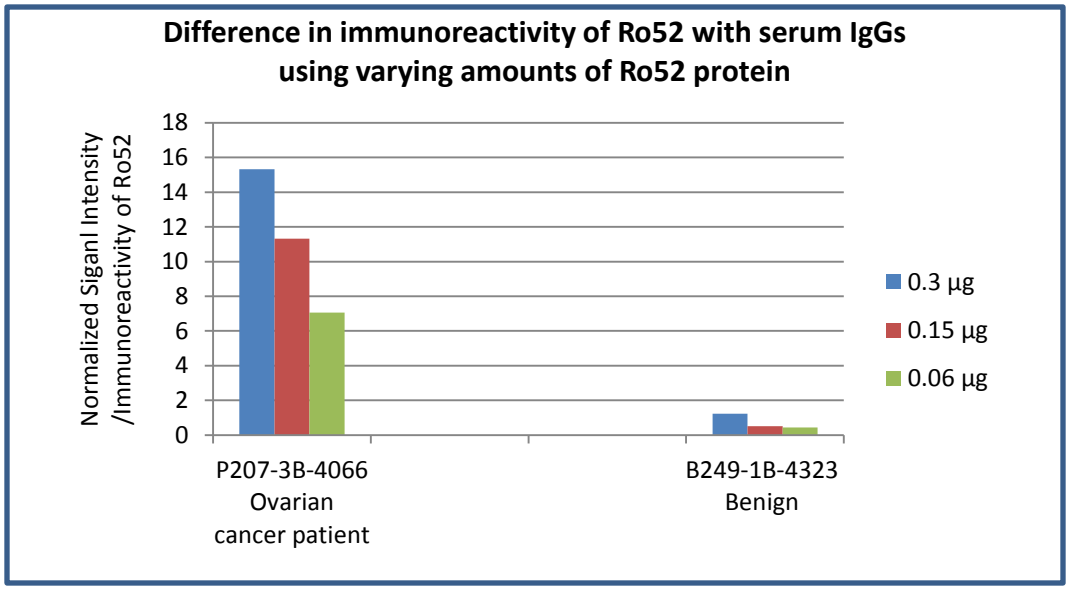


B. B249-1B-4323 His-tag Antibody



Lane number	1	2	3
Ro52 protein (57.06 kDa)	0.3 µg	0.15 µg	0.06 µg

C.



Supplementary Figure 1(A-C)