SUPPLEMENTARY MATERIAL

Development and characterization of phospho-ubiquitin antibodies to monitor PINK1-PRKN signaling in cells and tissue

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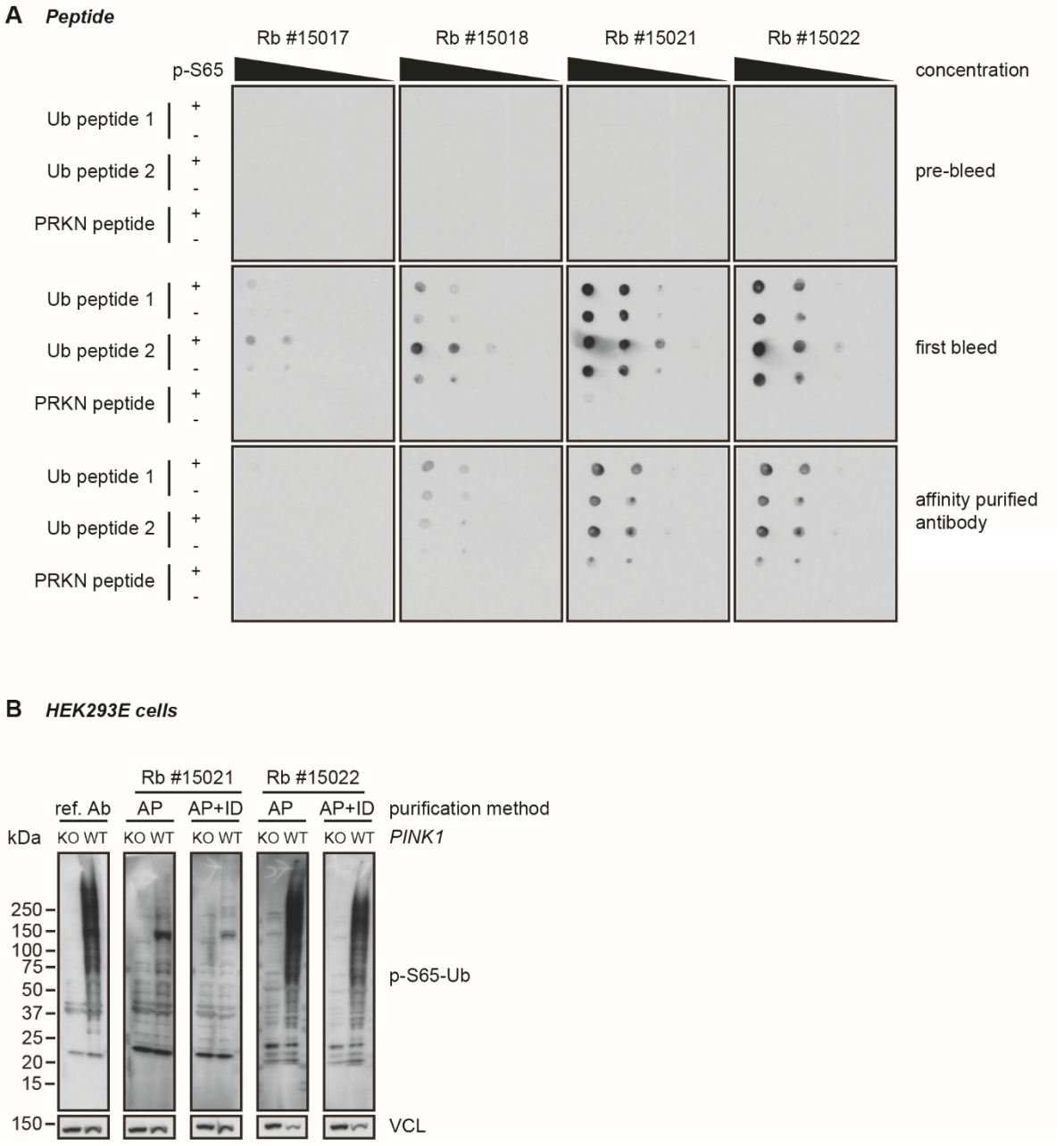
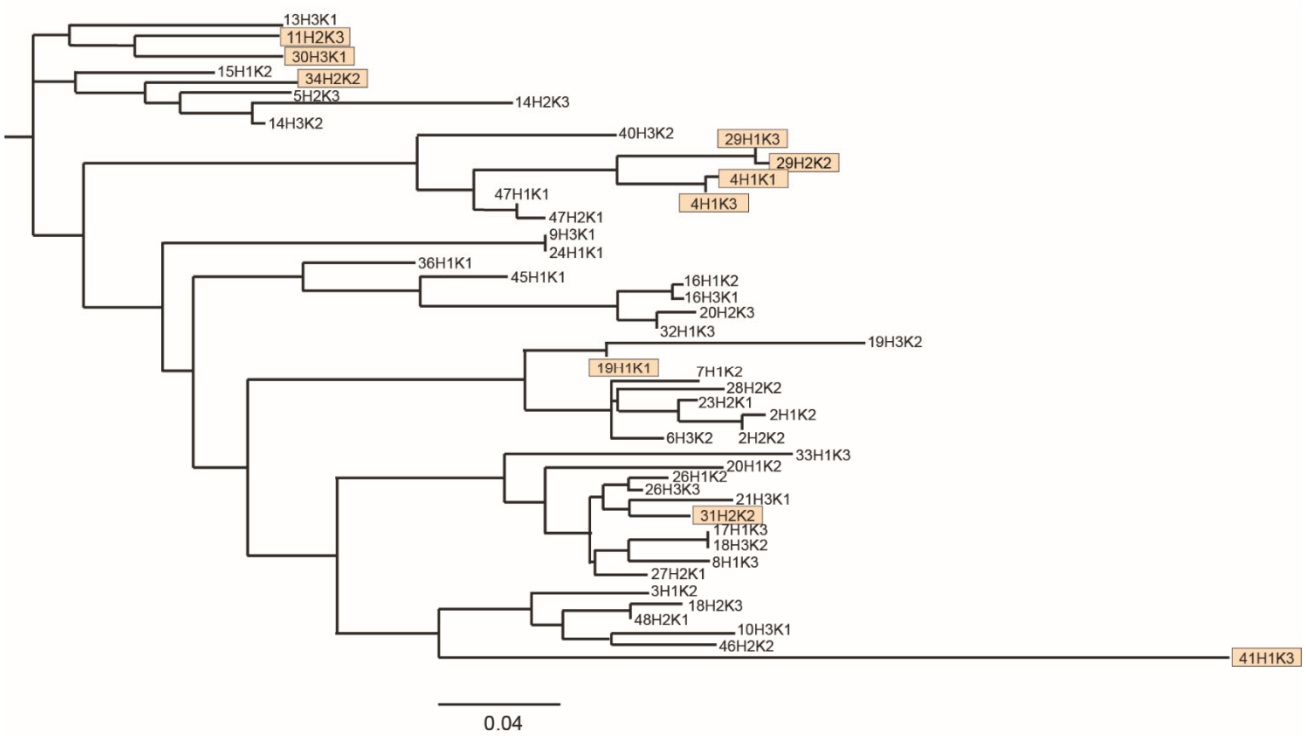
Running title: Development of recombinant phospho-ubiquitin antibodies



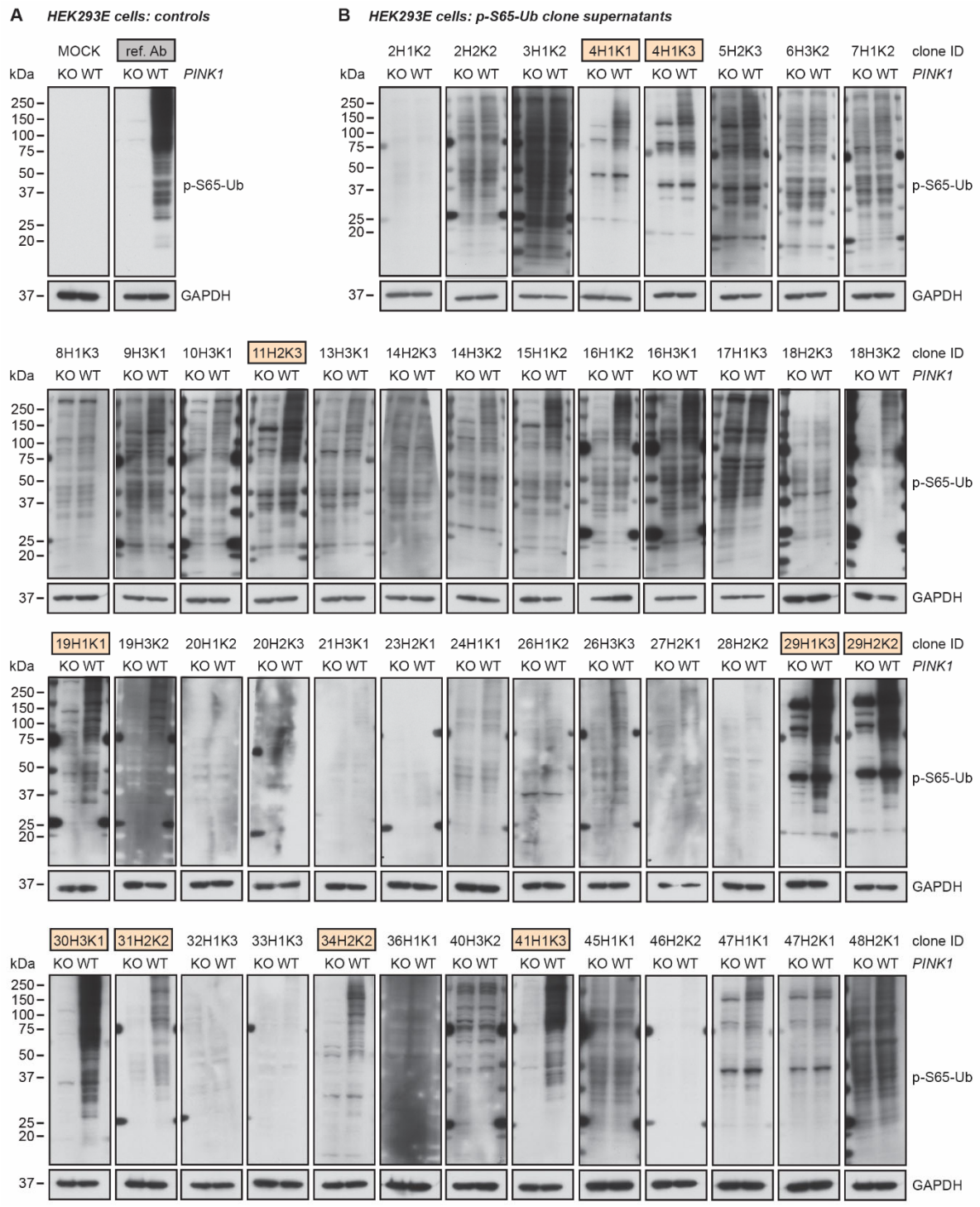
Supplementary Figure 1. Identification of p-S65-Ub immunopositive bleeds in four rabbits. (**A**) Dot blot analyses to identify p-S65-Ub positive bleeds from immunized rabbits. Two sets of 13-mer p-S65-Ub peptides containing the p-S65 in its center position that were used for rabbit immunizations as well as negative controls from their non-phosphorylated counterparts and 12-mer non-/phosphorylated PRKN peptides containing the S65 in its center position were spotted

on membranes in different concentrations (0.2-25 µM; 5-fold serial dilution). Blots were probed with rabbit sera from before immunization (pre-bleed), after immunization (first bleed), or after affinity-purification. (**B**) WT and *PINK1* KO HEK293E cells were treated for 8 h with 20 µM CCCP and cell lysates were used for western blot analyses. Blots were probed with the reference antibody or bleeds from two rabbits (Rb #15021 and #15022) with either affinity purification (AP) alone or also immuno-depletion (AP+ID). VCL was used as loading control. Ref. Ab - reference antibody, AP - affinity purification, ID - immuno-depletion, KO - knockout, WT - wild-type.



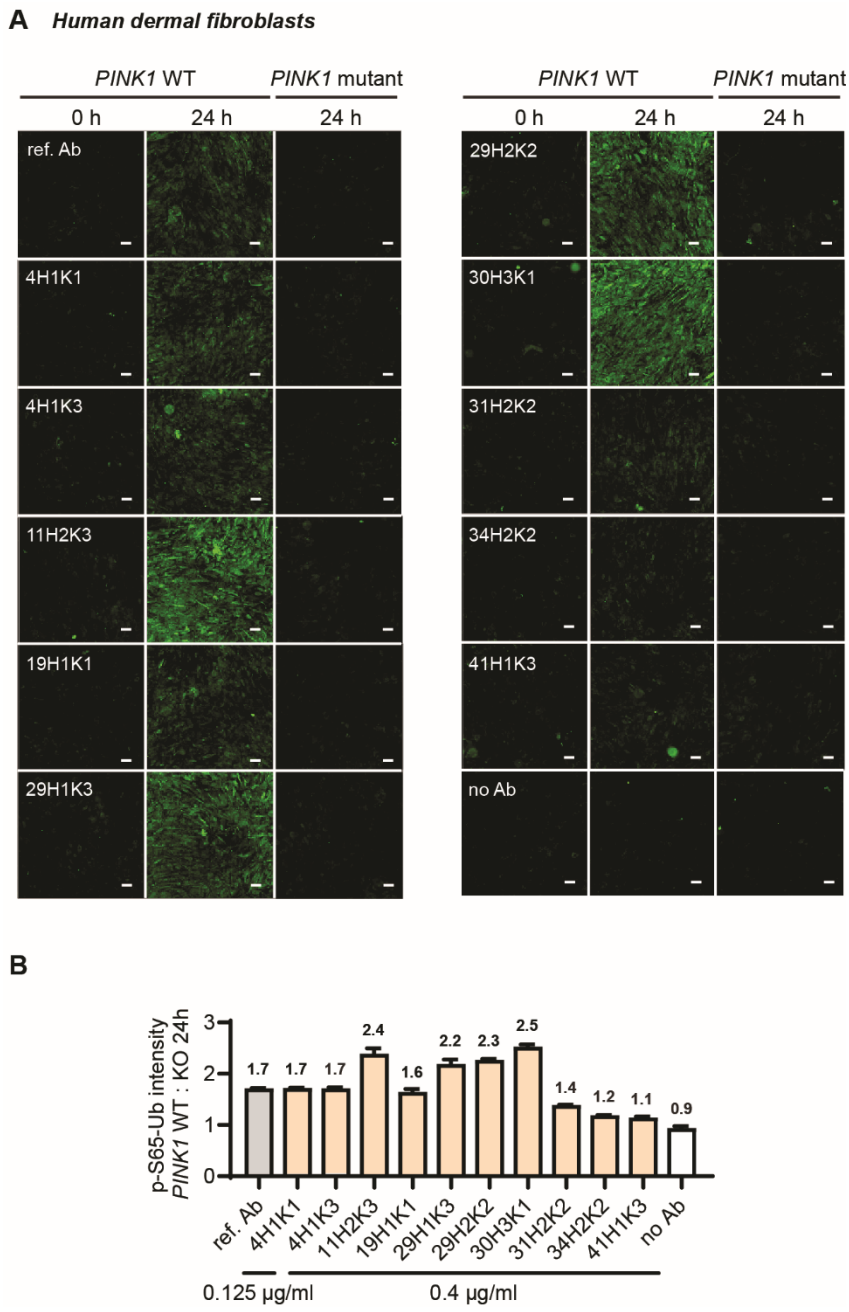
Supplementary Figure 2. Phylogenetic sequence tree of p-S65-Ub clone variable region.

A phylogenetic sequence tree was generated based on the p-S65-Ub antibody clone amino acid sequence for combined heavy and kappa variable regions. The top ten promising p-S65-Ub clone supernatants are highlighted in light orange. Scale bar: 0.04 nucleotide substitutions per site.



Supplementary Figure 3. Western blot screening of top recombinant p-S65-Ub clone supernatants. WT and *PINK1* KO HEK293E cells were treated for 24 h with 20 μM CCCP and cell lysates were used for western blot analyses. (**A**) Representative western blot images from

supernatant produced by HEK293 cells transfected with a media control containing all media and transfection reagent except the plasmids (MOCK) vector that served as negative control or from the reference antibody that served as positive control. (**B**) Representative western blot images from 47 p-S65-Ub recombinant antibody supernatants. GAPDH was used as loading control. The top ten promising p-S65-Ub antibody clones are highlighted in light orange. ref. Ab - reference antibody, KO - knockout, WT - wild-type.



Supplementary Figure 4. Characterization of the top recombinant p-S65-Ub clone supernatants for immunocytochemistry staining in human dermal fibroblasts. All ten p-S65-Ub clone supernatants (0.4 µg/ml) and the reference antibody (0.125 µg/ml) were evaluated by immunocytochemistry in fibroblasts treated with 2 µM valinomycin for 0 or 24 hours. (**A**) Representative images of p-S65-Ub immunoreactive signals (green) in human primary skin

fibroblasts carrying WT or homozygous *PINK1*^{Q456X} mutation. (**B**) Fluorescence intensities of each clone were quantified by high content imaging and compared relative to the treated *PINK1* mutant fibroblasts. Fold changes are labeled at the top of each bar. N = 2. Samples stained without primary antibody (no Ab) was used a negative control. Scale bar: 50 μm. ref. Ab - reference antibody, WT - wild-type.

Supplementary Table 1. ELISA screening of B cell supernatants

Ranking	B Cell ID	ELISA detection for proteins or peptides						Generated recombinant antibody clones
		p-S65-Ub protein	Ub protein (non-phospho)	p-S65-Ub peptide	Ub peptide (non-phospho)	p-S65-PRKN peptide	PRKN peptide (non-phospho)	
1	19G7[1]	2.269	0.054	1.846	0.05	0.05	0.049	-
2	10F4[1]	2.262	0.051	1.976	0.046	0.047	0.048	2H1K2, 2H2K2
3	22A9[1]	2.214	0.051	1.979	0.048	0.047	0.049	3H1K2
4	22C1[1]	2.192	0.056	1.821	0.048	0.048	0.048	4H1K1, 4H1K3
5	40C7[1]	2.114	0.047	1.794	0.05	0.05	0.051	5H2K3
6	4B3[1]	2.107	0.046	1.472	0.048	0.048	0.053	6H3K2
7	44B5[1]	2.104	0.053	1.909	0.161	0.049	0.056	7H1K2
8	11G6[1]	2.085	0.051	1.931	0.05	0.052	0.049	8H1K3
9	14D10[1]	2.049	0.053	1.62	0.049	0.049	0.049	9H3K1
10	2E1[1]	2.045	0.049	1.577	0.052	0.047	0.06	10H3K1
11	22H5[1]	2.016	0.05	1.92	0.051	0.047	0.055	11H2K3
12	44D8[1]	2.003	0.048	2.005	0.132	0.051	0.052	-
13	5A6[1]	1.988	0.049	1.591	0.047	0.048	0.05	13H3K1
14	24H10[1]	1.986	0.051	1.51	0.052	0.05	0.051	14H2K3, 14H3K2
15	44D11[1]	1.975	0.05	1.866	0.165	0.05	0.052	15H1K2
16	15F8[1]	1.974	0.05	1.594	0.049	0.048	0.049	16H1K2, 16H3K1
17	5D6[1]	1.963	0.049	1.661	0.051	0.048	0.055	17H1K3
18	41E10[1]	1.948	0.047	1.811	0.048	0.048	0.049	18H2K3, 18H3K2
19	1F4[1]	1.933	0.049	1.451	0.049	0.049	0.051	19H1K1, 19H3K2
20	4E10[1]	1.92	0.049	1.507	0.049	0.047	0.054	20H1K2, 20H2K3
21	14C2[1]	1.9	0.049	1.781	0.05	0.048	0.056	21H3K1
22	23C11[1]	1.875	0.05	1.539	0.049	0.049	0.049	-
23	44E1[1]	1.87	0.052	1.689	0.077	0.071	0.05	23H2K1
24	8B3[1]	1.865	0.047	1.635	0.049	0.052	0.05	24H1K1
25	9F8[1]	1.854	0.048	1.4	0.046	0.048	0.049	-
26	24A7[1]	1.823	0.052	1.608	0.048	0.052	0.052	26H3K3, 26H3K3
27	1D1[1]	1.818	0.048	1.556	0.047	0.048	0.05	27H2K1
28	7D6[1]	1.817	0.051	1.311	0.052	0.049	0.058	28H2K2
29	3C11[1]	1.803	0.051	1.584	0.048	0.049	0.05	29H1K3, 29H2K2
30	34E2[1]	1.788	0.049	1.819	0.054	0.053	0.051	30H3K1
31	15E3[1]	1.772	0.055	1.296	0.05	0.049	0.053	31H2K2
32	28D5[1]	1.771	0.052	1.009	0.048	0.049	0.049	32H1K3
33	15E9[1]	1.771	0.049	0.728	0.053	0.048	0.056	33H1K3
34	27E3[1]	1.762	0.05	1.486	0.048	0.05	0.049	34H2K2
35	6H7[1]	1.758	0.069	1.229	0.182	0.049	0.053	-
36	28H[1]	1.73	0.047	1.651	0.054	0.049	0.07	36H1K1
37	2G5[2]	0.635	0.105	0.084	0.048	0.047	0.049	-
38	5C10[2]	0.443	0.046	0.149	0.049	0.048	0.05	-
39	37G1[2]	0.433	0.049	0.157	0.048	0.05	0.053	-
40	2B1[2]	0.423	0.048	0.052	0.048	0.048	0.047	40H3K2
41	3F8[3]	1.754	0.047	1.367	0.067	1.831	0.062	41H1K3
42	17G4[3]	1.449	0.05	0.602	0.072	1.25	0.071	-
43	6B8[3]	1.072	0.065	0.45	0.053	0.711	0.053	-
44	21E11[3]	0.715	0.052	0.221	0.05	0.335	0.053	-
45	30G3[4]	0.727	0.047	0.497	0.047	0.046	0.048	45H1K1
46	25B9[4]	1.023	0.049	0.68	0.046	0.047	0.047	46H2K2
47	31A7[4]	1.019	0.049	0.856	0.048	0.049	0.051	47H1K1, 47H2K1
48	37F1[4]	0.95	0.048	0.903	0.048	0.051	0.051	48H2K1
	44A3	2.387	0.062	2.243	0.453	0.048	0.053	
	44B6	2.322	0.055	2.091	0.476	0.058	0.06	
	44B3	2.292	0.057	2.217	1.157	0.059	0.077	
	44B7	2.288	0.051	2.031	0.896	0.051	0.058	
	5D2	2.207	0.051	1.955	0.49	0.049	0.052	
	44C6	2.202	0.051	1.956	0.35	0.05	0.055	
	44C4	2.199	0.052	1.881	0.253	0.05	0.056	
	5C6	2.169	0.568	1.912	0.566	0.049	0.049	
	44D2	2.117	0.065	1.929	1.472	0.061	0.07	
	44D5	2.093	0.25	2.09	1.409	0.348	0.283	
	44C5	2.09	0.055	1.637	0.215	0.048	0.058	

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		p-S65-Ub protein	Ub protein (non-phospho)	p-S65-Ub peptide	Ub peptide (non-phospho)	p-S65-PRKN peptide	PRKN peptide (non-phospho)	
	44B4	2.083	0.05	1.952	1.48	0.048	0.053	
	10G11	2.073	0.276	1.816	0.691	0.22	0.415	
	44G7	2.06	0.063	2.079	0.843	0.051	0.055	
	44F9	2.048	0.052	1.765	1.547	0.049	0.051	
	44A4	2.039	0.057	2.203	2.06	0.053	0.052	
	44F6	2.03	0.052	1.849	1.551	0.049	0.055	
	44A1	1.942	0.081	1.97	0.459	0.051	0.054	
	31B7	1.926	0.051	1.872	0.387	0.059	0.072	
	44C8	1.925	0.049	2.083	1.998	0.05	0.055	
	44F8	1.906	0.05	1.809	1.433	0.05	0.052	
	44F10	1.798	0.05	1.593	0.372	0.05	0.051	
	31D10	1.739	0.048	1.146	0.048	0.048	0.048	
	12B6	1.728	0.048	1.253	0.048	0.049	0.049	
	34H4	1.719	0.048	1.445	0.048	0.054	0.049	
	18D5	1.664	0.049	1.387	0.051	0.048	0.053	
	44E9	1.661	0.05	1.945	0.197	0.055	0.063	
	3H8	1.658	0.046	1.25	0.047	0.047	0.049	
	44F4	1.645	0.053	1.854	1.031	1.401	1.023	
	27A6	1.621	0.064	1.413	0.05	0.049	0.048	
	22A8	1.602	0.057	0.922	0.064	0.051	0.06	
	39D5	1.571	0.048	1.135	0.048	0.049	0.049	
	3D1	1.55	0.047	1.103	0.048	0.05	0.049	
	11H6	1.535	0.048	0.94	0.047	0.047	0.048	
	6B6	1.529	0.048	1.676	0.102	0.049	0.052	
	31C2	1.528	0.047	0.961	0.049	0.049	0.051	
	44H8	1.518	0.051	0.833	0.094	0.05	0.053	
	44C7	1.508	0.056	1.922	2.093	0.059	0.057	
	28C4	1.507	0.051	1.023	0.053	0.048	0.052	
	44F3	1.481	0.085	1.664	1.298	0.361	0.055	
	44B9	1.469	0.053	1.445	0.97	0.051	0.056	
	44C11	1.466	0.052	1.585	0.775	0.054	0.055	
	1B11	1.458	0.047	0.837	0.05	0.048	0.054	
	25B11	1.448	0.05	0.912	0.048	0.049	0.049	
	15G10	1.438	0.049	0.981	0.049	0.05	0.05	
	21B9	1.434	0.048	1.429	1.254	0.049	0.06	
	13D4	1.428	0.051	0.815	0.061	0.051	0.057	
	39E5	1.415	0.047	0.549	0.048	0.048	0.05	
	44A11	1.41	0.059	1.768	1.005	0.051	0.054	
	44G10	1.407	0.05	1.175	0.056	0.048	0.049	
	35D10	1.395	0.048	1.419	0.05	0.05	0.047	
	37G3	1.395	0.048	0.715	0.056	0.048	0.05	
	32B2	1.393	0.046	1.448	0.046	0.049	0.047	
	19G9	1.373	0.05	1.046	0.049	0.049	0.049	
	30H4	1.356	0.047	1.14	0.048	0.048	0.05	
	1H3	1.347	0.048	0.763	0.05	0.049	0.048	
	25D10	1.342	0.051	0.904	0.049	0.052	0.051	
	29C11	1.331	0.049	1	0.049	0.048	0.049	
	37D10	1.326	0.047	1.389	0.05	0.054	0.049	
	28D7	1.322	0.058	0.937	0.048	0.048	0.05	
	2B11	1.322	0.048	0.675	0.047	0.047	0.054	
	44C1	1.31	0.052	2.059	0.053	0.05	0.049	
	24A10	1.304	0.05	0.654	0.048	0.048	0.048	
	13A8	1.302	0.049	0.652	0.05	0.051	0.055	
	28F11	1.3	0.047	1.21	0.05	0.047	0.055	
	11B3	1.292	0.048	0.782	0.05	0.047	0.051	
	33A5	1.268	0.049	0.764	0.05	0.051	0.05	
	20G5	1.245	0.049	0.631	0.047	0.049	0.053	
	35D5	1.241	0.047	1.073	0.07	0.061	0.05	
	22E9	1.233	0.052	0.758	0.048	0.047	0.047	
	44A2	1.163	0.055	1.709	1.69	0.054	0.133	
	44E4	1.153	0.078	2.08	1.758	0.101	0.134	
	3G1	1.146	0.047	0.681	0.134	0.048	0.053	
	10F10	1.141	0.049	0.771	0.049	0.077	0.05	
	11C1	1.141	0.047	0.512	0.049	0.048	0.048	
	32D1	1.109	0.047	0.954	0.051	0.047	0.05	
	24F4	1.106	0.047	0.687	0.048	0.049	0.048	
	2H6	1.097	0.048	0.671	0.048	0.05	0.049	
	22B6	1.096	0.051	0.433	0.048	0.05	0.057	

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		p-S65-Ub protein	Ub protein (non-phospho)	p-S65-Ub peptide	Ub peptide (non-phospho)	p-S65-PRKN peptide	PRKN peptide (non-phospho)	
	27C9	1.069	0.048	0.88	0.047	0.048	0.049	
	25H5	1.067	0.051	0.65	0.051	0.049	0.049	
	40D5	1.054	0.062	0.352	0.049	0.049	0.075	
	42H10	1.041	0.046	0.516	0.052	0.051	0.05	
	8H4	1.012	0.047	0.313	0.048	0.048	0.051	
	44E2	0.929	0.061	1.819	1.53	0.05	0.066	
	29G9	0.91	0.047	0.347	0.05	0.047	0.048	
	44H1	0.899	0.049	1.518	1.054	0.047	0.051	
	12F1	0.877	0.049	0.372	0.046	0.049	0.052	
	44B10	0.861	0.054	0.571	0.056	0.05	0.049	
	38A11	0.861	0.048	0.479	0.047	0.051	0.047	
	9E6	0.858	0.771	1.027	1.033	0.663	1.155	
	18D8	0.854	0.05	0.215	0.048	0.049	0.048	
	44F7	0.846	0.051	0.597	0.272	0.05	0.06	
	21G2	0.837	0.054	0.259	0.047	0.051	0.053	
	4B4	0.823	0.05	0.334	0.047	0.048	0.048	
	44H6	0.759	0.054	1.533	0.398	0.058	0.061	
	44D6	0.751	0.053	1.351	0.784	1.1	0.432	
	30A10	0.712	0.047	0.37	0.048	0.048	0.048	
	28H8	0.666	0.048	0.34	0.048	0.048	0.055	
	44B8	0.655	0.052	1.553	0.376	0.048	0.058	
	25H10	0.629	0.048	0.244	0.046	0.049	0.047	
	34G11	0.607	0.047	0.223	0.051	0.049	0.069	
	35A7	0.595	0.046	0.202	0.049	0.047	0.047	
	44G8	0.576	0.05	1.236	0.89	0.54	0.068	
	44E6	0.568	0.05	0.392	0.061	0.048	0.054	
	44G9	0.523	0.049	1.757	1.494	0.049	0.051	
	44E5	0.507	0.053	2.122	0.446	0.05	0.052	
	28C5	0.464	0.049	0.276	0.048	0.05	0.057	
	30E6	0.444	0.046	0.628	0.401	0.05	0.05	
	44G3	0.437	0.061	0.177	0.077	0.055	0.054	
	44H7	0.415	0.054	1.48	0.713	0.051	0.053	
	25A6	0.414	0.051	0.168	0.05	0.048	0.05	
	10G5	0.391	0.051	0.17	0.049	0.13	0.053	
	44G6	0.381	0.055	1.474	0.753	0.05	0.053	
	44H9	0.37	0.054	0.187	0.051	0.214	0.05	
	4A11	0.355	0.047	0.101	0.046	0.049	0.048	
	39B1	0.335	0.047	0.116	0.056	0.051	0.049	
	31E4	0.325	0.047	0.098	0.048	0.048	0.047	
	29D6	0.296	0.047	0.15	0.049	0.047	0.047	
	44C3	0.25	0.054	1.48	0.973	0.067	0.059	
	5C8	0.242	0.046	0.096	0.048	0.049	0.048	
	27E4	0.233	0.051	0.133	0.051	0.048	0.049	
	44E7	0.173	0.063	1.279	1.279	0.049	0.064	
	25F2	0.165	0.051	0.092	0.051	0.049	0.048	
	27A8	0.153	0.051	0.083	0.046	0.047	0.05	
	44G1	0.149	0.215	1.189	0.352	0.09	0.145	
	44H5	0.147	0.056	1.647	0.915	0.049	0.058	
	2F6	0.143	0.047	0.696	0.812	0.065	0.194	
	32F5	0.135	0.048	0.06	0.049	0.048	0.049	
	44C2	0.124	0.05	0.787	0.162	0.05	0.053	
	44H10	0.12	0.05	0.083	0.049	0.048	0.048	
	44F5	0.104	0.064	1.678	1.269	0.076	0.136	
	44B2	0.097	0.062	0.749	0.491	0.049	0.055	
	44D7	0.081	0.056	0.766	0.334	0.051	0.052	
	44G11	0.069	0.083	0.823	0.277	0.104	0.122	
	44H2	0.061	0.048	1.194	0.42	0.047	0.05	
	44A8	0.06	0.055	1.718	1.216	0.077	0.079	
	44A7	0.06	0.095	0.494	0.164	0.053	0.053	
	44E3	0.059	0.05	0.425	0.05	0.049	0.05	
	44E10	0.059	0.059	0.898	0.237	0.053	0.055	
	44B11	0.056	0.054	1.695	1.533	0.057	0.073	
	44D1	0.054	0.05	1.109	1.09	0.05	0.051	
	44H4	0.054	0.052	0.237	0.113	0.048	0.051	
	44D3	0.053	0.05	1.757	1.686	0.051	0.056	
	44F11	0.052	0.051	2.096	0.544	0.052	0.058	
	44D10	0.052	0.051	1.615	0.258	0.05	0.052	
	44B1	0.052	0.049	1.422	1.055	0.073	0.115	

Ranking	B Cell ID	ELISA detection for proteins or peptides						Generated recombinant antibody clones
		p-S65-Ub protein	Ub protein (non-phospho)	p-S65-Ub peptide	Ub peptide (non-phospho)	p-S65-PRKN peptide	PRKN peptide (non-phospho)	
	44D4	0.052	0.052	1.09	0.967	0.05	0.056	
	44F1	0.052	0.051	0.874	0.545	0.051	0.063	
	44F2	0.052	0.052	0.941	0.398	0.047	0.055	
	44H3	0.052	0.049	0.196	0.092	0.048	0.113	
	14E7	0.052	0.052	0.047	0.047	0.052	0.048	
	44G4	0.051	0.052	1.726	0.976	0.05	0.052	
	44A6	0.051	0.053	1.227	0.373	0.056	0.065	
	44H11	0.051	0.05	1.136	0.489	0.049	0.051	
	44G5	0.05	0.053	1.877	0.775	0.049	0.056	
	39G9	0.05	0.051	0.048	0.046	0.048	0.048	
	44E8	0.049	0.057	1.777	0.979	0.069	0.085	
	44E11	0.049	0.051	1.683	1.547	0.049	0.056	
	44G2	0.049	0.072	1.195	0.121	0.049	0.05	
	44D9	0.049	0.049	0.116	0.059	0.05	0.052	
	44C9	0.049	0.05	0.091	0.065	0.049	0.082	
	44C10	0.048	0.05	0.272	0.049	0.048	0.049	
	44A10	0.048	0.049	0.13	0.047	0.05	0.049	
	44A5	0.048	0.049	0.158	0.072	0.049	0.051	
	39G11	0.048	0.063	0.047	0.048	0.049	0.058	
	44A9	0.047	0.05	1.48	0.269	0.053	0.051	

Ub - ubiquitin, "-" - clone not available.

[1] B cells that detect both p-S65-Ub protein and peptide.

[2] B cells that only detect p-S65-Ub protein but not peptide.

[3] B cells that detect both p-S65-Ub protein and peptide but additionally also recognize p-S65-PRKN peptide.

[4] B cells that weakly detect p-S65-Ub protein and peptide.