Supplementary Material for Ponicsan, et. al.

Contains:

Supplementary Table 1. Pol II peptides crosslinked to B2 RNA(81-130)

Peptide[1]	Sequence	Amino acid start-end	Missed cleav-ages	Modifi-cations[2]	Charge	M/Z calculated	M/Z Error, ppm[3]	Elution time from HPLC (min)	Mascot score sample 1[4]	Mascot score sample 2[4]	Crosslink intensity sample 1[5]	Crosslink intensity sample 2[5]	Average purified Pol II intensity[5]
RPB1-1a	K.RVQFGVLSPDELKR.M	20-33	2		2	744.4136		64.031	*	*	4.61x10^5	2.49x10^5	1.73x10^8
RPB1-1b	K.RVQFGVLSPDELKR.M	20-33	2		3	548.6458	1.5183	61.133	48	*	7.41x10^5	2.96x10^5	3.95x10^7
RPB1-2	K.RMSVTEGGIK.Y	33-42	1		2	539.2897	0.2944	34.466	*	22	4.47x10^5	3.87x10^5	1.10x10^5
RPB1-3a	K.RMSVTEGGIKYPETTEGGRPK.L	33-53	2		4	574.0456	0.2008	42.347	23	*	1.70x10^5	4.42x10^4	4.32x10^6
RPB1-3b	K.RMSVTEGGIKYPETTEGGRPK.L	33-53	2	O (M)	4	578.0443					NP	NP	1.25x10^6
RPB1-4a	K.FGVEQPEGDEDLTKEK.G	164-179	1		2	910.9340		47.539	*	*	2.15x10^4	2.62x10^4	2.71x10^7
RPB1-4b	K.FGVEQPEGDEDLTKEK.G	164-179	1		3	607.6232	1.8589	47.208	26	23	2.94x10^5	3.72x10^5	1.29x10^6
RPB1-5	K.KILLSPER.V	213-220	1		2	478.3004	3.0263	39.658	25	26	1.03x10^6	7.87x10^5	2.77x10^6
RPB1-6	R.RNEQNGAAAHVIAEDVK.L	292-308	1	D (NQ)	3	608.3063	5.8652	41.202	31	28	1.23x10^5	5.19x10^4	3.61x10^5
RPB1-7a	R.AEIQELAMVPR.M	513-523	0		2	628.8374					NP	NP	2.45x10^8
RPB1-7b	R.AEIQELAMVPR.M	513-523	0	O (M)	2	636.8345	2.8359	59.685	44	59	9.00x10^5	1.57x10^6	5.31x10^7
RPB1-8	K.KSLGTSAGSLVH.I	643-654	1		2	578.8197	1.3424	38.700	26	*	1.91x10^5	3.90x10^5	NP
RPB1-9	H.TIGIGDSIADSK.T	686-697	0		2	588.8090	0.7821	51.669	*	25	3.38x10^5	3.30x10^5	8.31x10^7
RPB1-10	R.QTFENQVNR.I	735-743	0		2	568.2782	0.9375	31.297	*	36	3.08x10^5	3.67x10^5	5.46x10^6
RPB1-11	K.SLSEYNNFK.S	759-767	0		2	551.2642	1.5133	46.116	*	23	1.31x10^6	8.60x10^5	1.09x10^7
RPB1-12a	R.TLPHFIKDDYGPESR.G	806-820	1		2	887.9416	1.1684	53.894	31	31	3.61x10^5	8.09x10^4	1.43x10^8
RPB1-12b	R.TLPHFIKDDYGPESR.G	806-820	1		3	592.2968	2.0027	53.887	28	*	4.83x10^6	1.53x10^6	1.10x10^7
RPB1-13	K.TAETGYIQR.R	854-862	0		2	519.7644	0.7042	31.650	29	34	9.58x10^5	6.92x10^5	5.46x10^6
RPB1-14	R.NSINQVVQLR.Y	881-890	0		2	585.8331	1.9054	56.422	*	59	1.09x10^6	9.78x10^5	1.95x10^8
RPB1-15a	K.KLVIVNGDDPLSR.Q	1019-1031	1	D (NQ)	2	713.8987	0.7550	60.745	65	44	2.83x10^5	2.21x10^5	1.70x10^8
RPB1-15b	K.KLVIVNGDDPLSR.Q	1019-1031	1	D (NQ)	3	476.2682	1.3753	60.860		21	NP	3.00x10^5	9.93x10^6
RPB1-16	R.LKELINISK.K	1124-1132	1		2	529.3344	1.8349	53.301	*	29	8.41x10^5	6.01x10^5	7.17x10^7
RPB2-1a	K.FEQIYLSKPTHWER.D	88-101	0		2	917.4678		58.509	*		3.22x10^5	NP	8.11x10^7
RPB2-1b	K.FEQIYLSKPTHWER.D	88-101	0		3	611.9809	3.9846	58.881	38	40	1.82x10^6	2.79x10^5	4.37x10^6
RPB2-2	K.TVIKEGEEQLQTQHQK.T	131-146	1		3	632.6656	0.6749	36.939	19	24	7.21x10^5	4.87x10^5	1.56x10^8
RPB2-3a	K.MATNTVYVFAK.K	200-210	0		2	622.8208	4.5398	61.180	41	26	2.15x10^6	2.16x10^6	2.02x10^8
RPB2-3b	K.MATNTVYVFAK.K	200-210	0	O (M)	2	630.8183	5.4382	56.045	46	53	1.66x10^6	1.78x10^6	5.67x10^7
RPB2-4a	K.MATNTVYVFAKK.D	200-211	1		2	686.8683	2.3294	53.740	36	56	6.59x10^5	3.25x10^5	6.81x10^3
RPB2-4b	K.MATNTVYVFAKK.D	200-211	1	O (M)	2	694.8658	1.5449	47.328	29	56	4.33x10^5	3.77x10^5	6.72x10^4
RPB2-5	K.TRIISDGLK.Y	437-445	1		2	501.8008	1.3885	38.977	22	20	2.12x10^5	3.04x10^5	6.91x10^4
RPB2-6	K.YSLATGNWGDQK.K	446-457	0		2	670.3175	4.4807	55.842	66	64	2.65x10^6	2.12x10^6	1.04x10^7
RPB2-7a	K.YSLATGNWGDQKK.A	446-458	1		2	734.3650	1.5864	48.946	63	43	1.09x10^6	5.75x10^5	8.77x10^7
RPB2-7b	K.YSLATGNWGDQKK.A	446-458	1		3	489.9124		48.946	*	*	3.32x10^6	2.02x10^6	1.91x10^7
RPB2-8a	K.QAMGVYITNFHVR.M	731-743	0		3	512.5978					NP	NP	2.73x10^7
RPB2-8b	K.QAMGVYITNFHVR.M	731-743	0	O (M)	2	776.3905	1.7298	60.231	34	24	3.85x10^5	3.63x10^5	4.00x10^7
RPB2-8c	K.QAMGVYITNFHVR.M	731-743	0	O (M)	3	517.9294	4.4636	60.147	22	25	1.60x10^6	1.98x10^6	4.54x10^6
RPB2-9a	H.VLYYPQKPLVTTR.S	750-762	0		2	789.4562	4.3074	55.156	25	35	4.33x10^5	2.57x10^6	1.07x10^5
RPB2-9b	H.VLYYPQKPLVTTR.S	750-762	0		3	526.6399		55.156	*	*	4.38x10^6	2.98x10^5	8.90x10^4
RPB2-10a	K.KGFDQEEVFEKPTR.E	821-834	1		2	855.4283		47.381	*	*	3.49x10^4	1.89x10^4	1.32x10^8
RPB2-10b	K.KGFDQEEVFEKPTR.E	821-834	1		3	570.6213	2.5361	46.786	45	42	1.46x10^6	1.06x10^6	6.44x10^6
RPB2-11a	K.TVTLPENEDELESTNR.R	870-885	0		2	923.9369	2.3919	53.773	21	40	3.47x10^5	2.33x10^5	3.54x10^7
RPB2-11b	K.TVTLPENEDELESTNR.R	870-885	0		3	616.2937					NP	NP	1.39x10^7
RPB2-12a	R.GNEVLYNGFTGR.K	1024-1035	0		2	663.8255	4.2029	59.237	44	51	3.22x10^5	2.41x10^5	3.61x10^7
RPB2-12b	R.GNEVLYNGFTGR.K	1024-1035	0	D (NQ)	2	664.3175	1.2727	56.675	*	43	1.70x10^5	8.42x10^5	1.05x10^7
RPB2-13a	R.GPIQILNRQPMEGR.S	1065-1078	1		3	536.9595		58.315	*	*	3.98x10^5	1.87x10^5	NP
RPB2-13b	R.GPIQILNRQPMEGR.S	1065-1078	1	O (M)	3	542.2911	4.3854	52.060	24	30	4.58x10^5	6.52x10^5	2.58x10^6
RPB2-14	R.NKTQISLVR.M	1142-1150	1		2	529.8195	0.6955	39.019	27	*	9.21x10^5	5.71x10^5	2.52x10^5
RPB3-1a	K.SEYSELDEDESQAPYDPNGKPER.F	206-228	0		2	1328.0701					NP	NP	6.36x10^7
RPB3-1b	K.SEYSELDEDESQAPYDPNGKPER.F	206-228	0		3	885.7158	0.9992	48.323	78	74	5.84x10^5	4.64x10^5	2.19x10^5
RPB3-1c	K.SEYSELDEDESQAPYDPNGKPER.F	206-228	0	D (NQ)	4	664.7847					NP	NP	9.31x10^6
RPB5-1a	R.ALIVVQQGMTPSAK.Q	102-115	0		2	721.9054	3.6639	58.713	55	72	3.97x10^6	3.11x10^6	2.41x10^8
RPB5-1b	R.ALIVVQQGMTPSAK.Q	102-115	0	O (M)	2	729.9029	1.7324	49.651	60	61	3.30x10^6	3.50x10^6	5.07x10^7
RPB5-2	R.ALIVVQQGMTPSAKQSLVDMAPK.Y	102-124	1		3	815.4345	3.1331	58.760	*	24	1.37x10^5	1.73x10^5	NP
RPB5-3a	K.QSLVDMAPK.Y	116-124	0		2	494.7603	2.7503	51.855	*	53	3.37x10^6	3.86x10^6	1.16x10^8
RPB5-3b	K.QSLVDMAPK.Y	116-124	0	O (M)	2	502.7577	1.1785	37.239	18	23	1.11x10^6	1.80x10^6	1.14x10^7
RPB8-1a	R.ADQFEYVMYGK.V	85-95	0		2	675.8026					NP	NP	5.89x10^7
RPB8-1b	R.ADQFEYVMYGK.V	85-95	0	O (M)	2	683.8028	2.3618	54.423	49	44	6.76x10^5	1.64x10^6	4.94x10^6
RPB8-2	K.VYRIEGDETSTEAATR.L	96-111	1		3	599.9587	1.0242	38.358	*	33	2.45x10^5	3.09x10^5	5.34x10^7

[1] Pol II subunit and peptide number (e.g. RPB2-10 is subunit 2, peptide 10). Letters represent different charge and moficiation states of the same peptide.

[2] The modifications column indicates whether the peptide was determined to be oxidized (O) on methionine (M) or deamidated (D) on asparigine (N) or glutamine (Q).

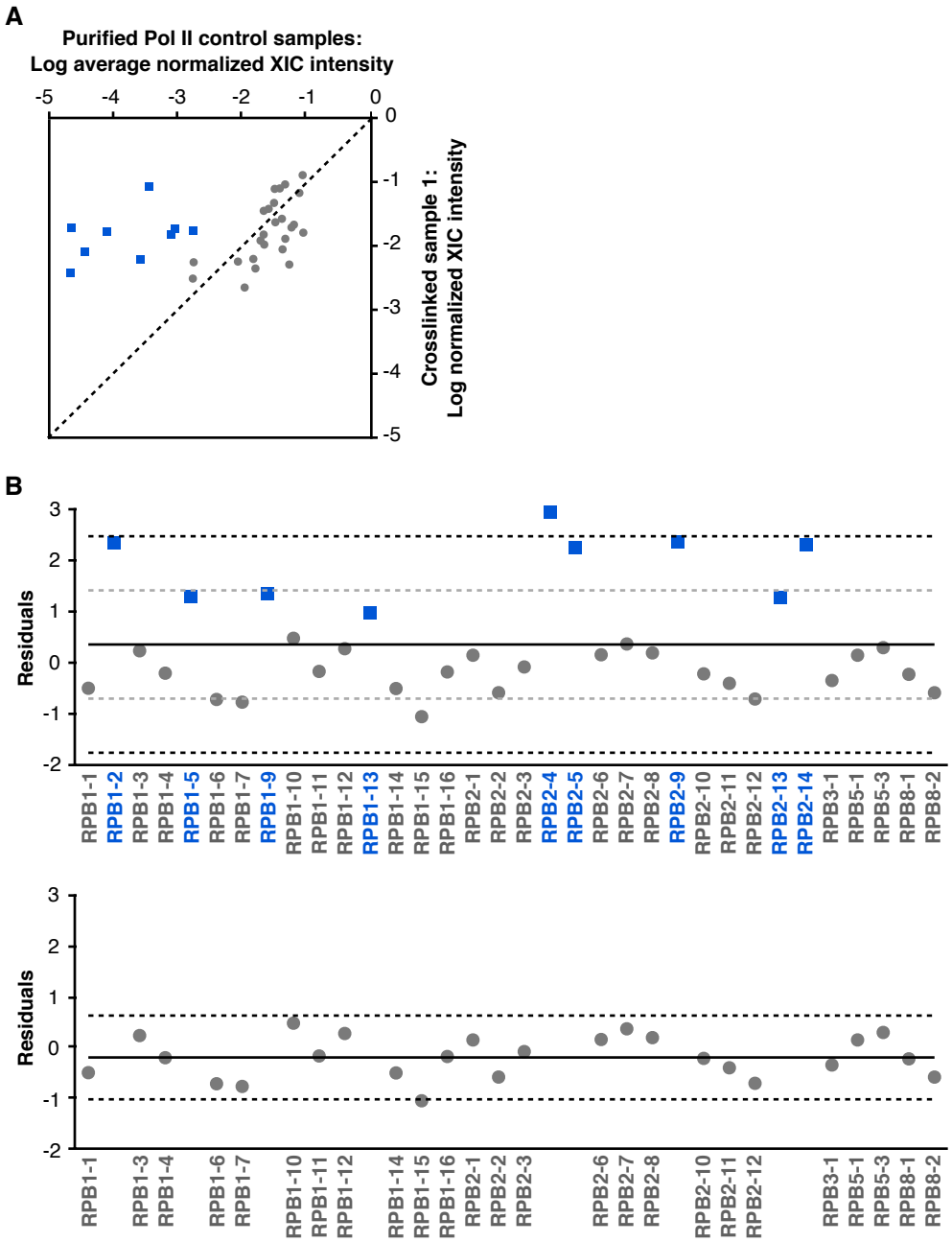
[3] The average ppm M/Z error [I(M/Z Obs - M/Z Calc)/ M/Z Calc * 1,000,000I] for both crosslinked samples (ppm is 1/1000 Da).

[4] Asterisk indicates the peptide was manually identified by the XIC rather than by Mascot. Blank space indicates a peptide form was not identified (but was present in the non-crosslinked sample).

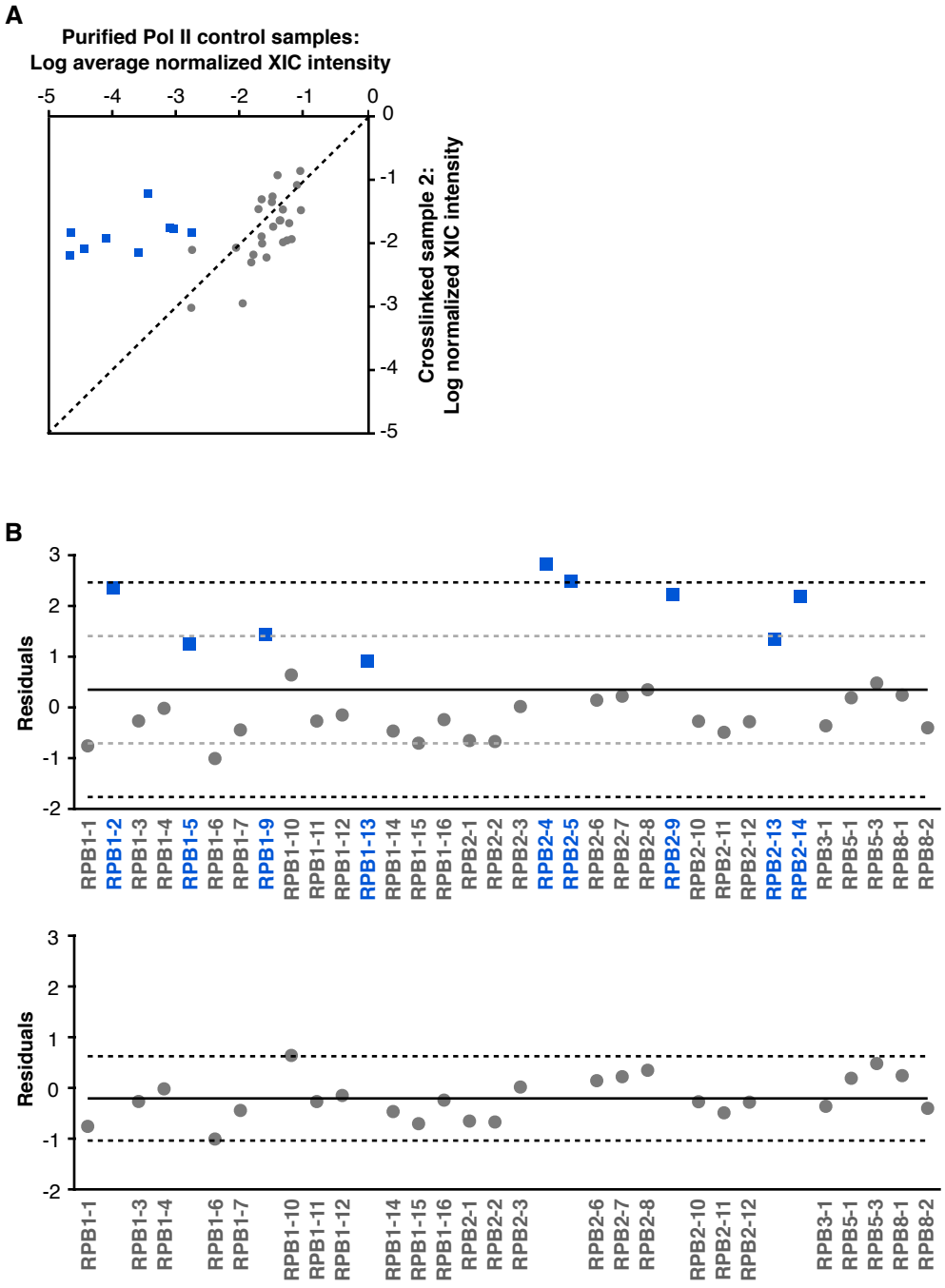
[5] NP, peptide form was not present.

Supplementary Table 2. Pol II peptides crosslinked to B2 RNA(81-115)

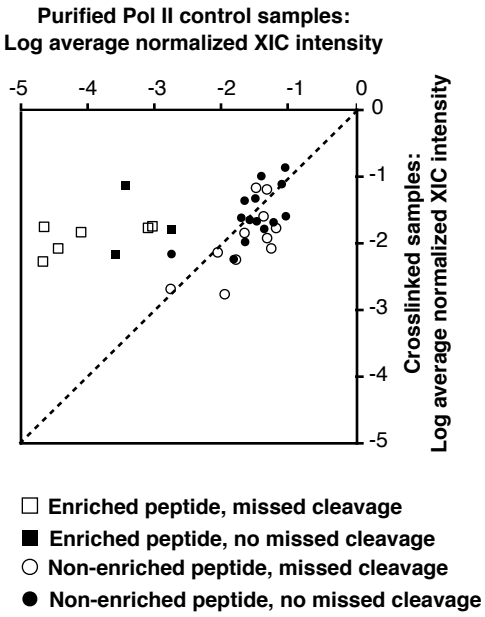
Subunit	Peptide	Amino acid start-end	M/Z calculated	M/Z error ppm	Missed cleavages	Mascot score
RPB1	K.LLVDSNNPK.I	117-125	500.2766	1.10	0	26
RPB1	N.TFHYAGVSAK.N	1106-1115	540.7770	0.55	0	34
RPB1	G.GAMSPSYSPTSPAYEPR.S	1587-1603	899.4090	0.27	0	61
RPB1	M.SPSYSPTSPAYEPR.S	1590-1603	769.8605	1.00	0	69
RPB1	K.YSPTSPTYSPTTPK.Y	1874-1887	763.8730	0.98	0	28
RPB1	K.YSPTSPTYSPTSPVYTPTSPK.Y	1888-1908	1129.5530	5.47	0	36
RPB1	K.YSPTSPTYSPTSPK.Y	1909-1922	756.8626	2.46	0	44
RPB2	K.GEIGDATPFNDAVNVQK.I	994-1010	887.9346	0.77	0	31
RPB3	R.ITELTDENVK.F	11-20	581.3052	2.98	0	28
RPB8	R.IEGDETSTEAATR.L	99-111	690.3187	1.14	0	50



Supplementary Figure 1. Nine peptides in crosslinked sample 1 are significantly enriched compared to the control samples. The figure and the statistical approach are the same as Figure 5, with the exception that total XIC values for peptides from crosslinked sample 1 were used instead of the averages of XIC values from both crosslinked samples. See the legend of Figure 5 and the text for a more detailed description. **(A)** Comparison of the XIC values of peptides crosslinked to B2 RNA(81-130) in sample 1 to the average XIC values from the control samples. **(B)** Statistical analysis to determine which peptides were significantly enriched in crosslinked sample 1.



Supplementary Figure 2. Nine peptides in crosslinked sample 2 are significantly enriched compared to the control samples. The figure and the statistical approach are the same as Figure 5, with the exception that total XIC values for peptides from crosslinked sample 2 were used instead of the averages of XIC values from both crosslinked samples. See the legend of Figure 5 and the text for a more detailed description. **(A)** Comparison of the XIC values of peptides crosslinked to B2 RNA(81-130) in sample 2 to the average XIC values from the control samples. **(B)** Statistical analysis to determine which peptides were significantly enriched in crosslinked sample 2.



Supplementary Figure 3. Plot depicting trypsin missed cleavage in peptides crosslinked to B2 RNA(81-130). Trypsin missed cleavages are found in both crosslinked enriched and non-enriched peptides. The squares represent peptides statistically enriched in the crosslinked samples, whereas the circles represent peptides that were neither over- nor under-represented in the crosslinked samples.