

Supplemental table 1

Reference	Entry	Gene_name	# peptides	average ratio BirA*Rad18 (Biotin + IR) over BirA*GFP (Biotin + IR)	average +IR/ IR for BirA*Rad18	p-value	z-score from p-value	Ranked by +IR/- IR	known properties	references
splP05090 APOD_HUMAN	P05090	APOD_HUMAN	2	3.614306649	2.80730729	0.34212458	2.11445083	Apolipoprotein D		
splQ14676 MDC1_HUMAN	Q14676	MDC1_HUMAN	18	2.343450223	2.59422339	0.00478479	13.9346784	Mediator of DNA damage checkpoint protein 1		
splQ8NCN4 RN169_HUMAN	Q8NCN4	RN169_HUMAN	3	4.209486458	2.52264283	0.15389746	4.32607435	E3 ubiquitin-protein ligase RNF169		
splO00469 PLOD2_HUMAN	O00469	PLOD2_HUMAN	2	2.273552105	2.0637377	0.13712442	4.64553899	Procollagen-lysine,2- oxoglutarate 5- dioxygenase 2		
splQ96RL1 UIMC1_HUMAN	Q96RL1	UIMC1_HUMAN	4	4.124663651	2.00170986	0.13985809	4.59089242	BRCA1-A complex subunit RAP80		
splQ8ND82 Z280C_HUMAN	Q8ND82	Z280C_HUMAN	4	2.884018089	1.83803559	0.10228989	5.45688885	Zinc finger protein 280C		
splQ12888 TP53B_HUMAN	Q12888	TP53B_HUMAN	9	2.460705668	1.47525806	0.27813614	2.68768254	Tumor suppressor p53-binding protein 1		
splO76064 RNFB_HUMAN	O76064	RNFB_HUMAN	3	3.525593049	1.43792037	0.21736264	3.37020797	E3 ubiquitin-protein ligase RNF8		
splQ9Y253 POLH_HUMAN	Q9Y253	POLH_HUMAN	4	2.981581688	1.43776166	0.2735299	2.73391379	DNA polymerase eta		
splQ92547 TOPB1_HUMAN	Q92547	TOPB1_HUMAN	2	1.799041616	1.43481979	0.18412957	3.82955742	DNA topoisomerase 2- binding protein 1		
splQ6IQ32 ADNP2_HUMAN	Q6IQ32	ADNP2_HUMAN	3	1.531802691	1.4313704	0.25458588	2.93260766	ADNP homeobox protein 2	loss of ADNP2 sensitizes cells to oxidative damage	Kushnir et al. 2008
splQ14865 ARI5B_HUMAN	Q14865	ARI5B_HUMAN	7	2.028032118	1.41108765	0.12088891	5.45688885	AT-rich interactive domain-containing protein 5B	recruited to laser stripes	Leung et al. 2017
splQ7Z3K3 POGZ_HUMAN	Q7Z3K3	POGZ_HUMAN	4	2.200100193	1.40345814	0.04368266	7.81238853	Pogo transposable element with ZNF domain	regulates RPA recruitment	Baude et al. 2016
splQ96JM2 ZN462_HUMAN	Q96JM2	ZN462_HUMAN	2	1.977431057	1.36364133	0.14902904	4.41506485	Zinc finger protein 462	a possible ATM/ATR substrate	Matsuoka et al. 2007
splP38398 BRCA1_HUMAN	P38398	BRCA1_HUMAN	5	3.767225405	1.34237	0.3535389	2.02359671	Breast cancer type 1 susceptibility protein		
splP00387 NB5R3_HUMAN	P00387	NB5R3_HUMAN	3	1.377093544	1.30749984	0.18199308	3.86186729	NADH-cytochrome b5 reductase 3		
splQ96JM3 CHAP1_HUMAN	Q96JM3	CHAP1_HUMAN	9	1.790946216	1.26485029	0.16834868	4.07761049	Chromosome alignment-maintaining phosphoprotein 1	Interacts with MDC1, Rev7	<a href="https://thebiogrid.org/interaction/809571">https://thebiogrid.org/interaction/809571</a>
splP53634 CATC_HUMAN	P53634	CATC_HUMAN	2	1.493040003	1.2359247	0.36276237	1.95229862	Dipeptidyl peptidase 1		
splQ5TGY3 AHDC1_HUMAN	Q5TGY3	AHDC1_HUMAN	3	1.661335963	1.18417252	0.20662094	3.51051255	AT-hook DNA-binding motif-containing protein 1	PARylated after damage	Zhen and Yu 2018
splQ9H582 ZN644_HUMAN	Q9H582	ZN644_HUMAN	2	1.370318072	1.16824298	0.17367926	3.99131214	Zinc finger protein 644		Dungrawala et al. 2015
sp P10244 MYBB_HUMAN	P10244	MYBB_HUMAN	7	1.325345254	1.15757628	0.37618351	1.85172606	Myb-related protein B		
splQ2NL82 TSR1_HUMAN	Q2NL82	TSR1_HUMAN	2	1.705754251	1.15661838	0.33220811	2.19587801	Pre-rRNA-processing protein TSR1 homolog		
splQ14839 CHD4_HUMAN	Q14839	CHD4_HUMAN	11	2.249456748	1.10753189	0.13931482	4.60166696	Chromodomain- helicase-DNA-binding protein 4		Pan et al. 2012

Reference	Entry	Gene_name	# peptides	average ratio BirA*Rad18 (Biotin + IR) over BirA*GFP (Biotin + IR)	average +IR/ IR for BirA*Rad18
splQ9NS91 RAD18_HUMAN	Q9NS91	RAD18_HUMAN	148	74.53554124	1.04482982

## Supplemental table 1

**Supplemental table 1. Multiplexed mass spectrometry reveals that BirA<sup>R118G</sup>-Rad18 preferentially biotinylates DDR proteins following irradiation.** The biotinylation assay was carried out as described in the methods section followed by MS (technical duplicate). Biotinylated proteins with less than two peptides were excluded. The dataset for BirA<sup>R118G</sup>-RAD18 was normalized to the dataset of BirA<sup>R118G</sup>-GFP. The ratio of BirA<sup>R118G</sup>-RAD18 (Biotin + IR) over BirA<sup>R118G</sup>-GFP (Biotin + IR) was calculated and only the biotinylated proteins with a ratio of  $\geq 1.3$  were considered. Proteins were then ranked based on descending order of the ratio of BirA<sup>R118G</sup>-RAD18 (Biotin + IR) over BirA<sup>R118G</sup>-RAD18 (Biotin - IR). The p-values were determined for each protein followed by a z-score analysis. Only proteins with a z-score  $\geq 2$  were considered. Proteins highlighted in red, yellow and blue are known DDR proteins, proteins indirectly linked to DDR, or an uncharacterized protein, respectively.

## Supplemental table 1

### References

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Kushnir M, Dresner E, Mandel S, Gozes I. 2008. Silencing of the ADNP-family member, ADNP2, results in changes in cellular viability under oxidative stress. *J Neurochem* **105**: 537-545.

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## Supplemental table 2

Entry	Protein_name	# peptides	Average ratio (BirA*GFP Biotin + IR)/(BirA*Rad18 Biotin + IR) <b>ratio above 1.3</b>	P value
P22670	MHC class II regulatory factor RFX1	2	1.430833674	0.073724535
Q8IVM0	Coiled-coil domain-containing protein 50	5	1.773655911	0.080727133
Q8IU81	Interferon regulatory factor 2-binding protein 1	2	1.632965357	0.088136168
P50479	PDZ and LIM domain protein 4	9	1.930271679	0.102811944
Q8IZ21	Phosphatase and actin regulator 4	2	2.126480984	0.10322152
Q16630	Cleavage and polyadenylation specificity factor subunit 6	3	1.351906761	0.115516788
Q9ULU4-11	Isoform 11 of Protein kinase C-binding protein 1	8	1.550534173	0.144797445
Q96KR1	Zinc finger RNA-binding protein	14	2.382830666	0.152050352
Q96PV6	Leukocyte receptor cluster member 8	5	1.661165384	0.161373721
Q15942	Zyxin	11	3.686219925	0.187217857
Q15637-5	Isoform 5 of Splicing factor 1	9	1.736531068	0.187379903
P05412	Transcription factor AP-1	4	1.659432134	0.196817003
P54259	Atrophin-1	4	1.855896859	0.198955126
Q9H0W8	Protein SMG9	2	2.770152429	0.238132297
Q13371	Phosducin-like protein	2	1.353596924	0.257799919
P23588	Eukaryotic translation initiation factor 4B	6	2.991465836	0.259043263
Q14156	Protein EFR3 homolog A	2	1.694267035	0.271922006
P49757	Protein numb homolog	12	2.87602877	0.273134178
Q43303	Centriolar coiled-coil protein of 110 kDa	3	1.581254127	0.27888867
Q9UNF1	Melanoma-associated antigen D2	9	1.733862072	0.296969196
Q969G3	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1	5	1.84583864	0.310089533

## Supplemental table 2

**Supplemental table 2. Multiplexed mass spectrometry reveals that BirA<sup>R118G</sup>-GFP does not preferentially biotinylate DDR proteins following irradiation.** The biotinylation assay was carried out as described in the methods section followed by MS. Biotinylated proteins with less than two peptides were excluded. The ratio of BirA<sup>R118G</sup>-GFP (Biotin + IR) over BirA<sup>R118G</sup>-RAD18 (Biotin + IR) was calculated and only the biotinylated proteins with a ratio of  $\geq 1.3$  were considered. The p-values were determined for biotinylated proteins and ranked in descending order.