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Supplemental Material to:

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A high throughput screen to identify enhancers of ADAR-mediated RNA-editing

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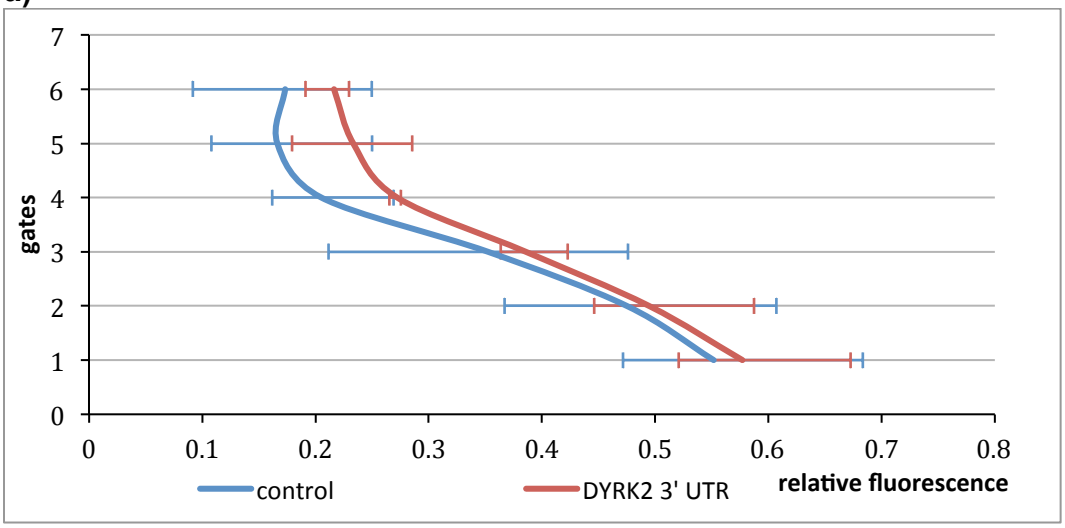
Supplementary data

Table S1: Impact of isolated candidates on editing levels determined by direct sequencing of a reporter construct. The hit # is indicated and the predicted gene. Increase in editing relative to a MOCK control is indicated for two independent experiments. Sometimes only the 3' UTR of a gene was contained. The proposed molecular function is indicated where known

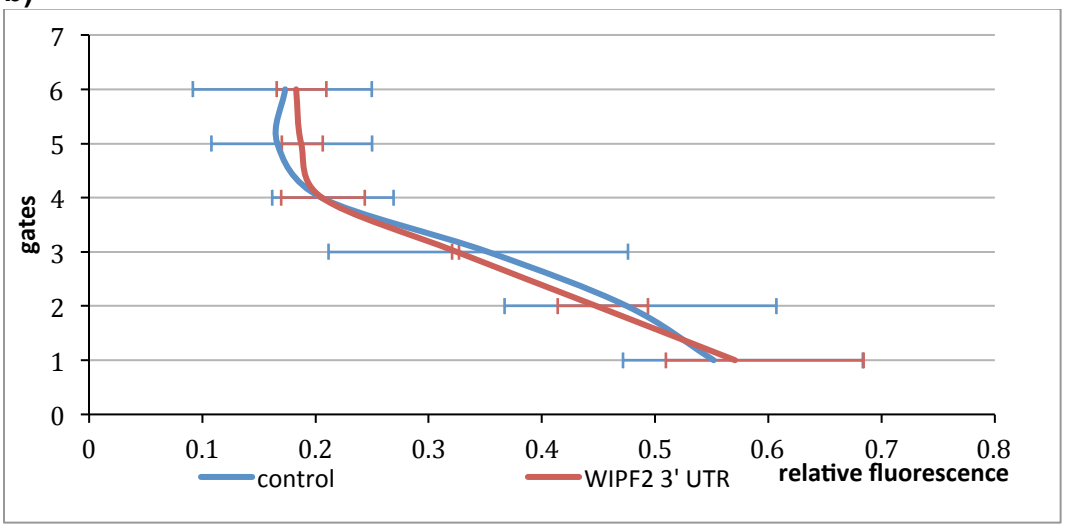
#	protein	Increase in editing level (vs. control) [in %] (1^{st}/2^{nd} run)	sequence	function
1	**chromosome 5 contig**	18 / 42	?	unknown
2	**FABP7**	18 / 27	coding	fatty acid binding
10	**STK38**	14.5 / 6.5	coding	kinase
11	**SSRP1**	17 / 23.5	coding	DNA-binding
21	**KAT5/HTATIP**	16.5 / 28	coding	acetyl transferase
22	**DYRK2**	50 / n.d.	3'UTR	RNA
23	**WIPF2**	19 / 18	3'UTR	RNA
32	**SOX1**	15 / 18	3'UTR	RNA
35	**GPX4**	16.5 / 24.5	coding	glutathione peroxidase
36	**DSS1/SHFM1**	35 / 22.5	coding	proteasome subunit
40	**hnRNP A2B1**	12 / 10	Coding	RNA-binding
42	**ZBTB4**	27 / 40	3'UTR	RNA
44	**chromosome 17 contig**	14.5 / 33.5	?	unknown
48	**DNAJB6**	38.5 / 29.5	coding	chaperone
52	**Q5TGE2**	30.5 / 2.5	3'UTR	RNA
60	**TCTP**	47 / 20.5	coding	calcium binding/ microtubule stabilization

Figure S1

a)



b)



c)

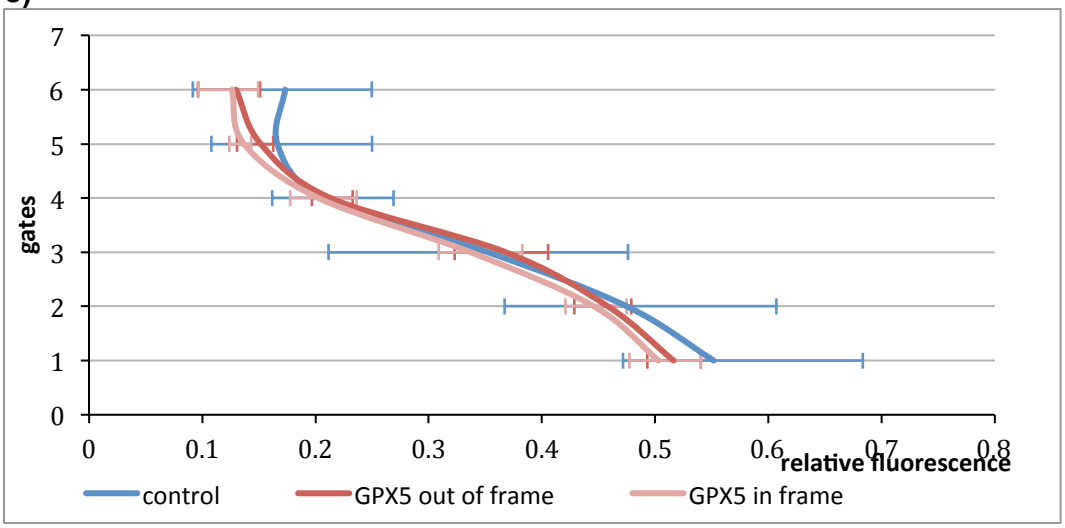
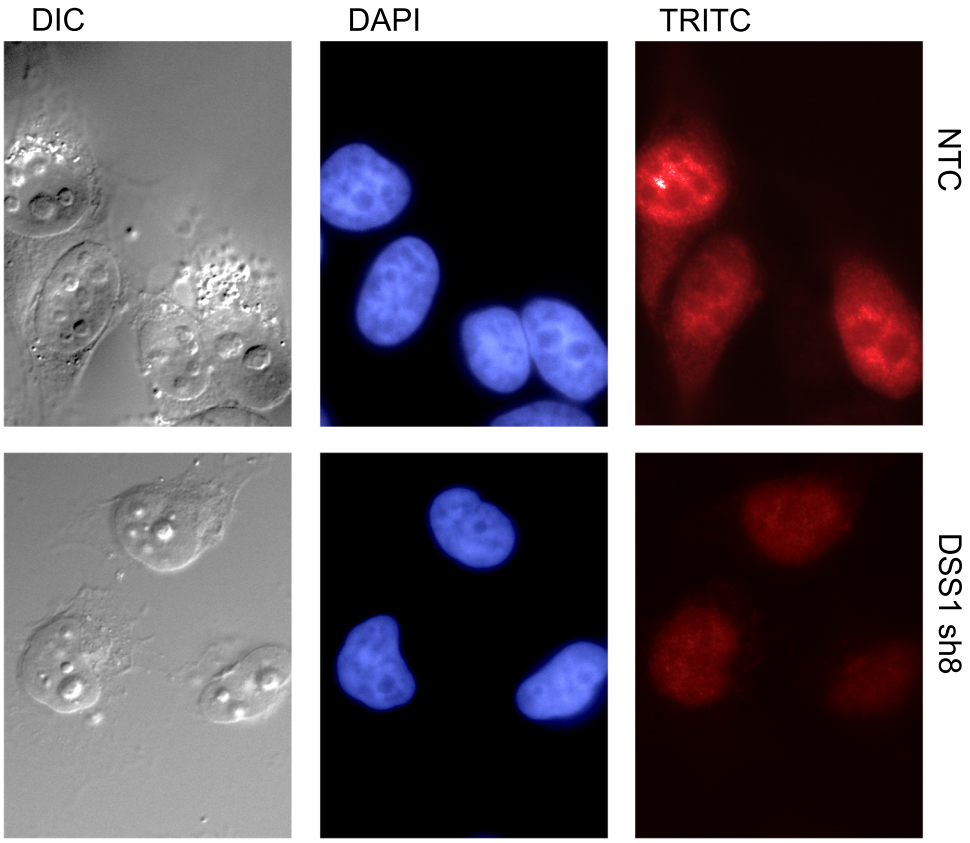
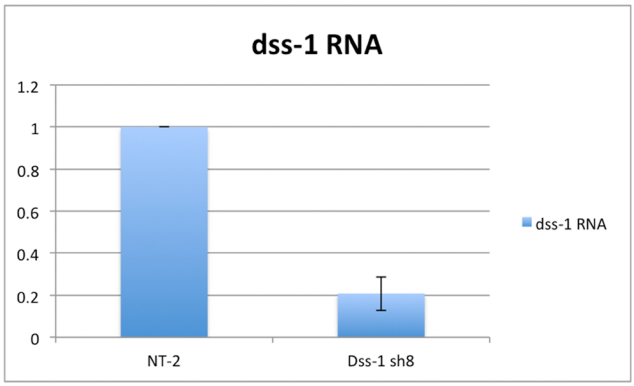


Figure S1: Verification of enhancers of editing in tissue culture cells by FACS analysis of a RFP-editable-stem-loop-GFP construct. HeLa cells were transfected with an editable RFP-GFP construct and an empty vector (control) or the candidates identified in the yeast screen cloned in-frame or out-of-frame, as indicated. In the absence of editing, only RFP fluorescence can be seen, while editing leads to a simultaneous expression of GFP. Stimulation of editing leads to a shift of fluorescence intensities along the x-axis to the right. **a)** the 3' UTR of the DYRK2 kinase had a stimulatory effect on ADAR2 activity in HeLa cells, as in the primary screen in yeast cells. **b)** In contrast, the 3' UTR of WIPF2 but also the coding region of GPX5 **(c)** did not stimulate editing, indicating that some, but not all candidate clones identified in the yeast screen could act in tissue culture cells.

A


DIC
DAPI
TRITC
NTC
DSS1 sh8

B


dss-1 RNA
1.2
1
0.8
0.6
0.4
0.2
0
NT-2
Dss-1 sh8
dss-1 RNA

C

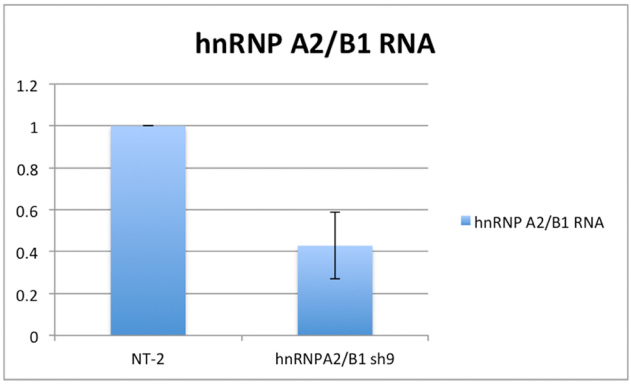

hnRNP A2/B1 RNA
1.2
1
0.8
0.6
0.4
0.2
0
NT-2
hnRNPA2/B1 sh9
hnRNP A2/B1 RNA

Figure S2: delivery of shRNAs against DSS1 and hnRNP A2B1 reduce the corresponding protein and RNA levels. shRNAs directed against hnRNP DSS1 or hnRNP A2/B1 were delivered using lentiviral mediated transfection. Stable knock-down clones were selected and tested for knockdown efficiency. A) Repression of DSS-1 protein expression was tested on a cell line stably expressing myc-DSS1, using an anti-myc antibody.

(B) DSS1 RNA levels were measured by qPCR after knockdown with either a non-target control (NT-2) or an anti *DSS-1* shRNA (sh8) which leads to about 5 fold reduction in RNA levels.

(C) hnRNP A2/B1 RNA levels were measured by qPCR after knockdown of a non-target control (NT-2) or a hnRNP A2/B1 –specific shRNA (sh9). Knockdown leads to a 50% reduction in RNA levels.

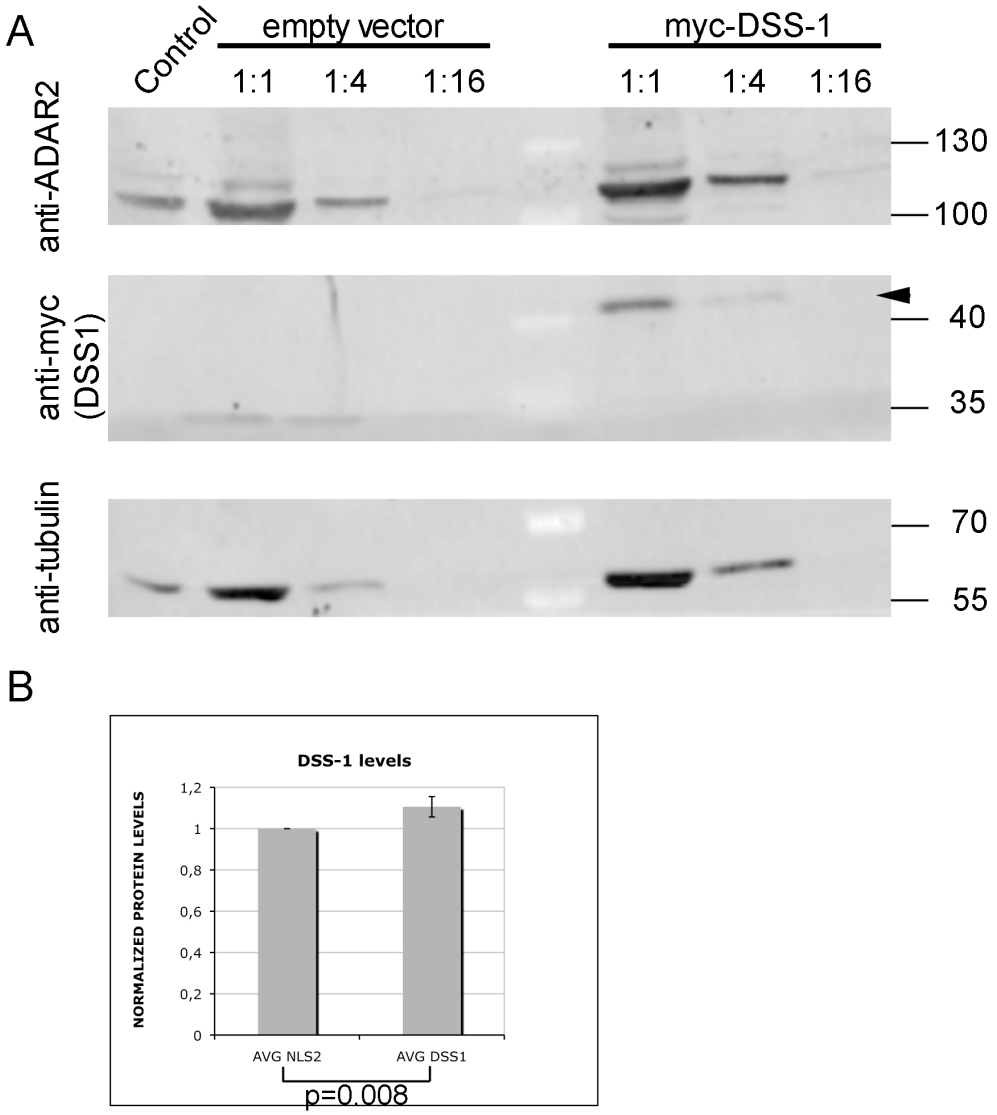


Figure S3: DSS1 increases cellular ADAR2 levels. Quantitative western blots for cells transfected with myc-DSS1 or an empty vector control. **A)** A dilution series of HeLa cells transfected with myc-DSS1 or an empty vector was loaded on an SDS gel, blotted and detected with antibodies as indicated. ADAR2 levels were detected with an anti ADAR2 antibody (top row). DSS1 (arrowhead) or the tag of the empty vector were detected with an anti-myc antibody (middle row). For quantification, alpha-tubulin was quantified with a suitable antibody (bottom row). Bands were

directly quantified using fluorimetry of fluorochrome labeled antibodies on an FX-Pro scanner (BioRad).

B) Quantification of three independent experiments shows a significant increase of ADAR2 in the presence of DSS1.

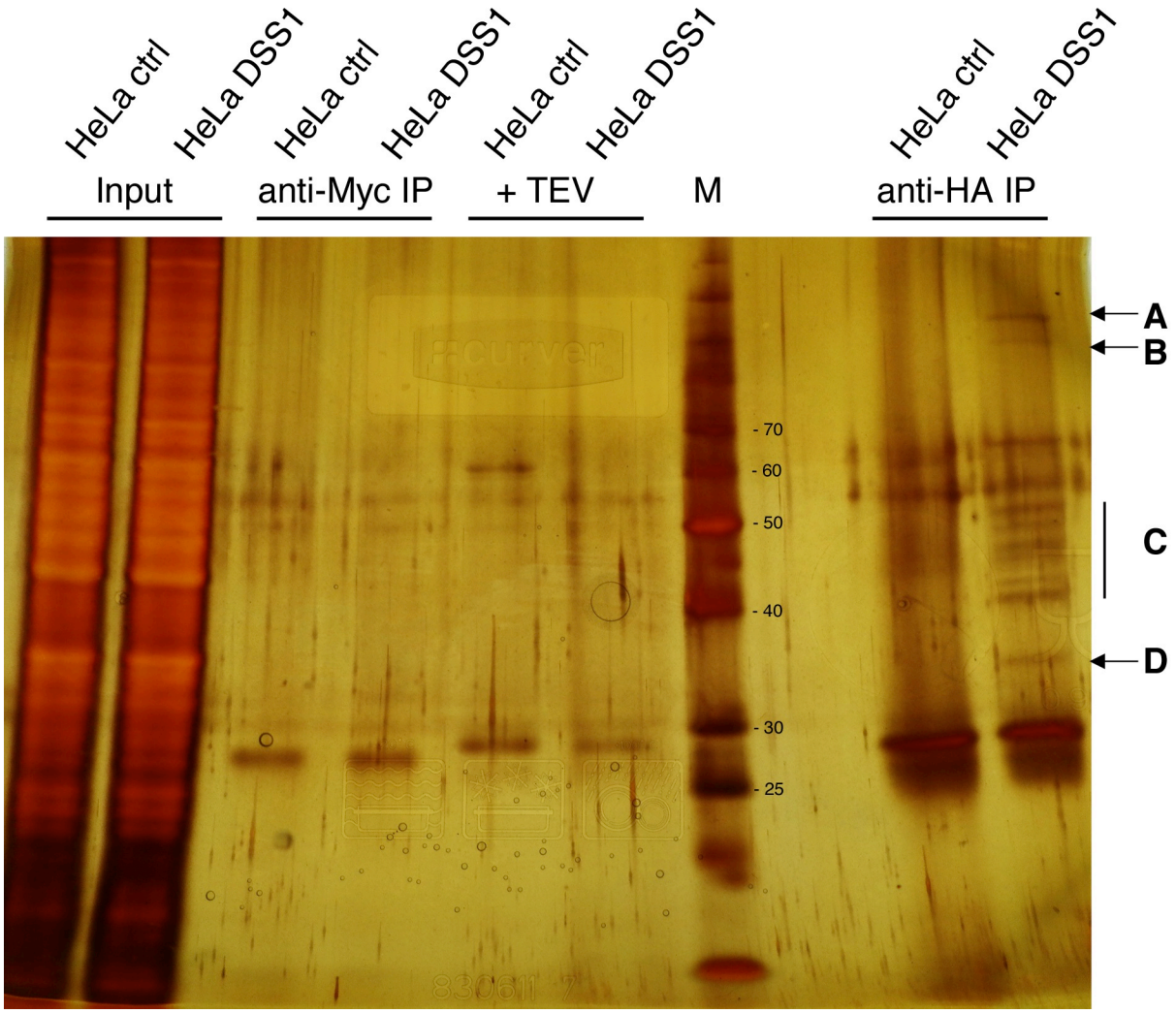


Figure S4: Representative gel image of a purification of TAP-tagged DSS1. Aliquots from each purification step as well as the final purified proteins were loaded on a 7-17% SDS gradient gel and silver stained. The marked bands were cut and subjected to mass spectrometric analysis. Protein identities are indicated in Supplementary table 2.

Supplementary Table 2: Protein bands purified by TAP tagging and cut out from an SDS PAGE as depicted in supplementary figure 4. The identified proteins, the number of assigned spectra and the % coverage are indicated. hnRNP proteins that were copurified are highlighted in yellow.

	assigned spectra	**% coverage**
Band A, 116 kDa		
26S proteasome non-ATPase regulatory SU1	39	30.00%
Band B, 106 kDa		
26S proteasome subunit p97	38	24.00%
Bands C 59, 56, 52, 5, 49, 47, 45 kDa		
26S protease regulatory SU 8	16	30.00%
26S proteasome SU 9	14	25.00%
Proteasome 26S SU, ATPase, 1]	5	13.00%
26S protease regulatory SU 6B	8	15.00%
proteasome SU p42	3	8,0%
heterogeneous nuclear ribonucleoprotein G	**18**	**39%**
heterogeneous nuclear ribonucleoprotein D	**2**	**8.50%**
heterogeneous nuclear ribonucleoprotein C	**2**	**7.30%**
Band D 32 kDa		
26S proteasomeregulatory SU 14	9	15.00%
26S proteasome regulatory SU 13	2	2,4%
heterogeneous nuclear ribonucleoprotein A2/B1	**11**	**27.00%**
heterogeneous nuclear ribonucleoprotein A1 isoform b	**3**	**8,3%**