Naarmann-de Vries *et al.*, Supplementary Material

siRNAs

siRNA	Sequence	Reference
HNRNPK #1	AGACUACAAUGCCAGUGUUdTdT	(1)
HNRNPK #2	CUGUGGAAUGCUUAAAUUAdTdT	(1)
HNRNPK #3	GGAACAAGCAUUUAAAAGdTdT	(1)
HNRNPE1 #1	CUACUCGAUUCAAGGACAAdTdT	(1)
HNRNPE1 #2	UGAACCAGGUGGCAAGACAdTdT	(1)
HNRNPE1 #3	UCACCAUUCCAAAUAACUUdTdT	(1)
PRMT1	GGACAUGACAUCCAAAGAUdTdT	
ctrl.	AGGUAGUGUAAUCGCCUUGdTdT	(1)

Antibodies for Western blot (WB) and immunofluorescence staining (IF)

Name	Company/ Reference	Catalogue number	Dilution (WB)	Dilution (IF)
PRMT1	Merck, Darmstadt, Germany	07-404	1:2000	1:200
HNRNPK	Santa Cruz, Dallas, TX, USA	sc-28380	1:500	1:200
HNRNPK	Abcam, Cambridge, UK	ab52600		1:200
Non-R^{met} K	(1)		1:10	
admR	(2)		1:50	
HNRNPE1	Santa Cruz, Dallas, TX, USA	sc-16504	1:500	1:200
ALOX15	(3)			1:200
VINCULIN	Merck, Darmstadt, Germany	V9131	1:3000	
ELAVL1	Santa Cruz, Dallas, TX, USA	Sc-5261	1:200	
DDX6	Novus Biologicals, Centennial, CO, USA	NB200-191	1:5000	1:800
DCP1A	Abnova, Taipei, Taiwan	H00055802-M06		1:400
HNRNPL	Santa Cruz, Dallas, TX, USA	sc-32317	1:500	1:100
RPS3	NEB, Ipswich, MA, USA	#2579	1:1000	
RPS19*	Abcam, Cambridge, UK	ab181365	1:1000	1:200
RPS19**	Abcam, Cambridge, UK	ab40833	1:500	1:200
RPS19***	Abnova, Taipeh, Taiwan	H00006223-M01	1:500	
RPL19	Santa Cruz, Dallas, TX, USA	K-12/sc-100830	1:200	
GAPDH	Abcam, Cambridge, UK	8245-100	1:5000	
ACTB	Merck, Darmstadt, Germany	A1978	1:2000	
UBIQUITIN	Cell Signaling, Danvers, MA, USA	#3936	1:1000	
Puromycin	Merck, Darmstadt, Germany	MABE343	1:25000	

RPS19* used for Western blot analyses if not otherwise stated
RPS19** applied for Figure 6B
RPS19*** applied for Figure 2H

Cloning primers

Construct	Primer	Sequence
pET16b-RPS19 wt	fw	ttagttggatccgatgcctggagttactg
	rv	cgaggcggatcctactaatgcttcttg
pET16b-RPS19 W52R	fw	gatgagaaccggttctacacgcgagc
	rv	gctcgcgtgtagaaccggttctcatc
pET16b-RPS19 F21A	fw	agagctctggcagccgccctcaaaaagtccggg
	rv	cccggacttttgagggcggctgccagagctct
pSUPERIOR-Scr	fw	gatccccaggtagtgtaatcgccttgttcaagagacaaggcgattaca ctacctttttta
	rv	agcttaaaaaaggtagtgtaatcgccttgtctcttgaacaaggcgatta cactacctggg
pSUPERIOR-RPS19(495)	fw	gatccccgcacaaagagcttgctcccttcaagagagggagcaagct ctttgtgctttta
	rv	agcttaaaaagcacaaagagcttgctccctctcttgaagggagcaag ctctttgtgcggg
pSUPERIOR-RPS19(738)	fw	gatccccactgacacctcagggacaattcaagagattgtccctgaggt gtcagttttta
	rv	agcttaaaaaactgacacctcagggacaatctcttgaattgtccctgag gtgtcagtggg

qPCR primer

target	Primer	Requence	Reference
RPS19	fw	acacgcgagctgcttccaca	
	rv	agtttgcggccgccatcttg	
ACTB	rv	tccctggagaagagctacg	(4)
	rv	gtagtttcgtggatgccaca	(4)
18S	fw	ctggataccgcagctaggaata	(5)
	rv	ccggtccaagaatttcacctct	(5)
28S	fw	cccagtgctctgaatgtcaa	(6)
	rv	agtgggaatctcgttcatcc	(6)
ALOX15	fw	aggtcaggttcccttgttac	
	rv	gaattaacccgtccttccag	
HBG1	fw	aaaccctgggaaggctcct	(1)
	rv	tcccaggagcttgaagttc	(1)
blaR	fw	gaaatgagaacaggggcatc	
	rv	ttagccctcccacacataac	

Legends to Supplementary Figures

Figure S1: Immunofluorescence staining of K562 cells with antibodies for PLA analysis.

K562 cells were either left untreated (0d) or induced for erythroid maturation with 1.5 mM sodium butyrate for eight days (8d). The localization of DDX6 and the P-body marker DCP1A, HNRNPK and RPS19, as well as HNRNPL, was detected with specific antibodies as indicated, which were recognized by fluorophore-coupled secondary antibodies (anti-rabbit-Cy3, anti-mouse-FITC). Staining of nuclei with DAPI. **B)** Quantification of the PLA assay shown in (Figure 3A). Red signals per cell were determined in 10 images of three independent experiments each. Student's t-test for significance (***$p<0.001$).

Figure S2: Analysis of melting curves of His-RPS19 variants

Analysis of melting curves of His-RPS19 wild type or the related variants, as indicated (mean±s.d., n=3).

Figure S3: Analysis of the HNRNPK – RPS19 interaction with the TSA.

A-F) Raw data (T_m) of the TSA analysis for RPS19 wt and variants W52R and non-related F21A as control, with the non-methylated HNRNPK and HNRNPK^{5Rmet} derived peptides P1, P2 and P1m, P2m, respectively, which were used to extract ΔT_m values in Figure 4D and E as well as Figure S3 D-F. **D-F)** TSA analysis of the interaction between RPS19 wt, -W52R and -F21A and peptide P1 (D), peptide P2 (E) and peptide P2m (F). The ΔT_m relative to the reaction without peptide addition is shown.

Figure S4:

Specificity control of immunofluorescence staining.

K562 wt cells and RPS19-depleted K562 clone A were subjected to immunofluorescence analysis as in Figure 7D, except that primary ALOX15 and RPS19 antibodies were omitted.

Supplementary References

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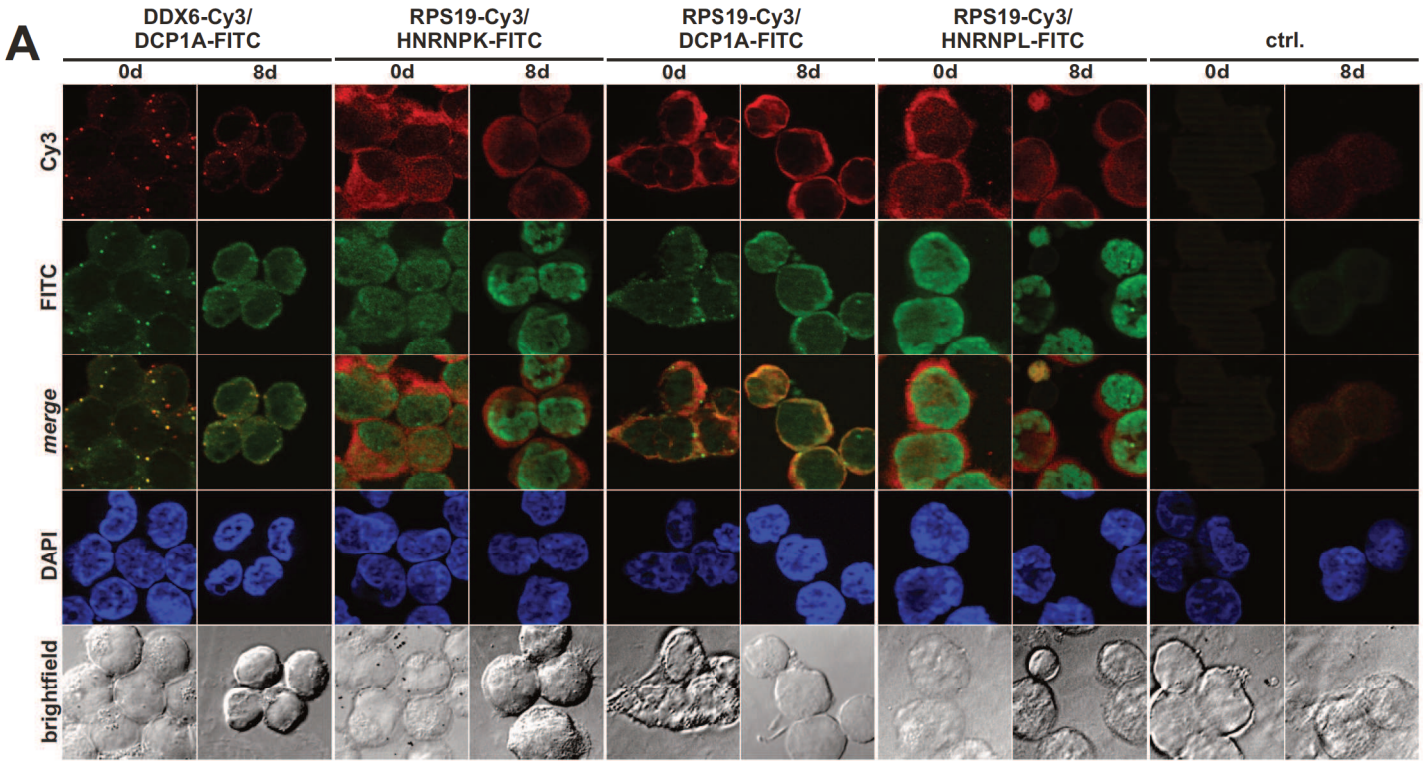
2. Gross, H., Hennard, C., Masouris, I., Cassel, C., Barth, S., Stober-Grasser, U., Mamiani, A., Moritz, B., Ostareck, D., Ostareck-Lederer, A. *et al.* (2012) Binding of the heterogeneous ribonucleoprotein K (hnRNP K) to the Epstein-Barr virus nuclear antigen 2 (EBNA2) enhances viral LMP2A expression. *PLoS One*, **7**, e42106.
3. Rapoport, S.M., Schewe, T., Wiesner, R., Halangk, W., Ludwig, P., Janicke-Hohne, M., Tannert, C., Hiebsch, C. and Klatt, D. (1979) The lipoxygenase of reticulocytes. Purification, characterization and biological dynamics of the lipoxygenase; its identity with the respiratory inhibitors of the reticulocyte. *Eur J Biochem*, **96**, 545-561.
4. Stohr, N., Lederer, M., Reinke, C., Meyer, S., Hatzfeld, M., Singer, R.H. and Huttelmaier, S. (2006) ZBP1 regulates mRNA stability during cellular stress. *J Cell Biol*, **175**, 527-534.
5. Naarmann-de Vries, I.S., Brendle, A., Bahr-Ivacevic, T., Benes, V., Ostareck, D.H. and Ostareck-Lederer, A. (2016) Translational control mediated by hnRNP K links NMHC IIA to erythroid enucleation. *J Cell Sci*, **129**, 1141-1154.
6. Weidensdorfer, D., Stohr, N., Baude, A., Lederer, M., Kohn, M., Schierhorn, A., Buchmeier, S., Wahle, E. and Huttelmaier, S. (2009) Control of c-myc mRNA stability by IGF2BP1-associated cytoplasmic RNPs. *RNA*, **15**, 104-115.

Figure S1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DDX6-Cy3/
DCP1A-FITC
RPS19-Cy3/
HNRNPK-FITC
RPS19-Cy3/
DCP1A-FITC
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HNRNPL-FITC
ctrl.
0d
8d
Cy3
FITC
merge
DAPI
brightfield

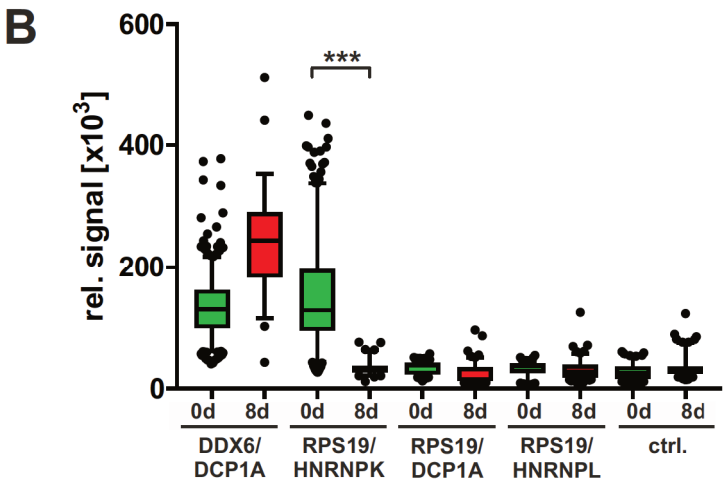
B
rel. signal [x10^3]
600
400
200
0
***
0d
8d
DDX6/
DCP1A
RPS19/
HNRNPK
RPS19/
DCP1A
RPS19/
HNRNPL
ctrl.

Figure S2

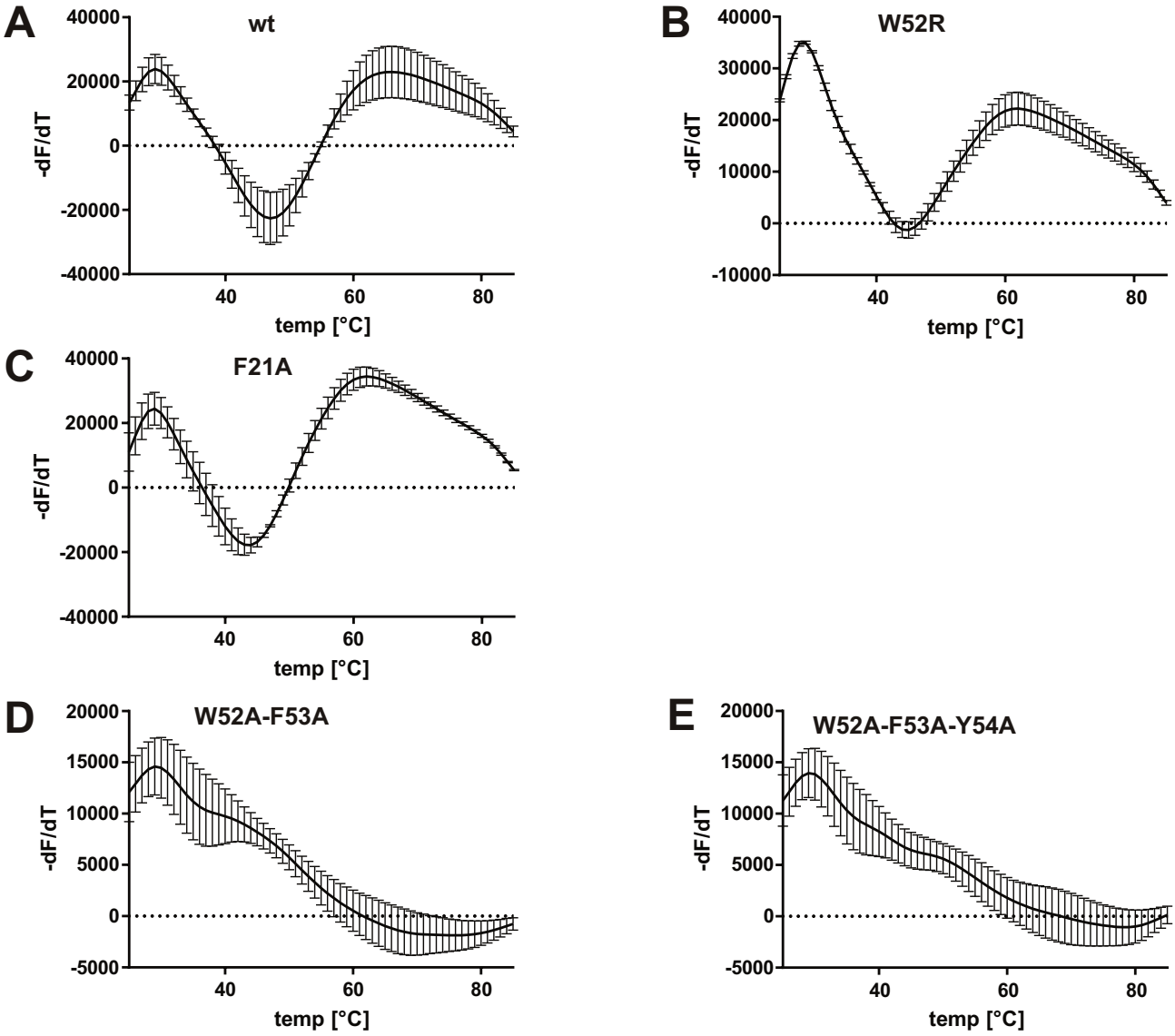

Figure S3

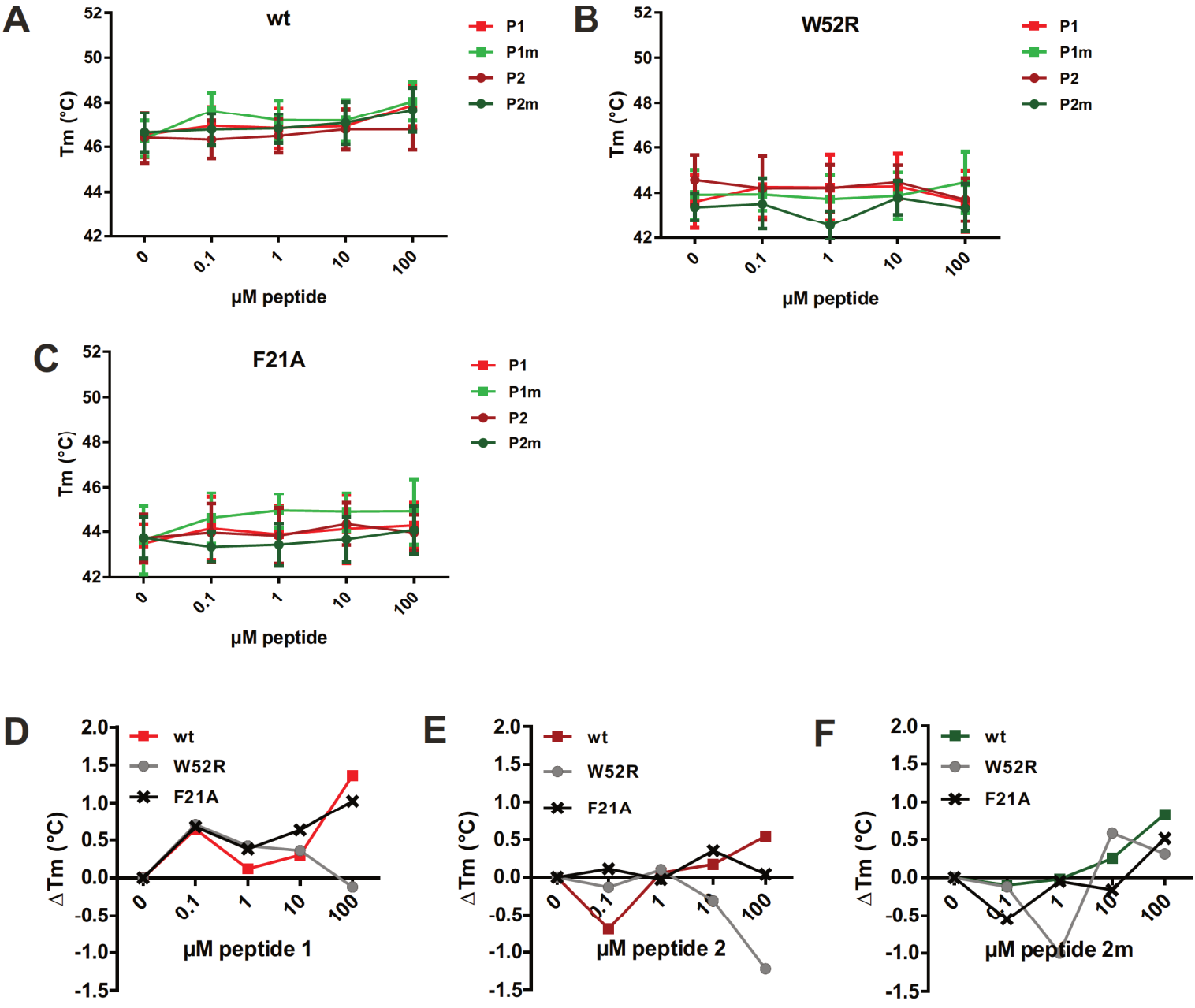
A
wt
B
W52R
C
F21A
P1
P1m
P2
P2m
Tm (°C)
52
50
48
46
44
42
0
0.1
1
10
100
µM peptide
D
E
F
wt
W52R
F21A
ΔTm (°C)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
µM peptide 1
µM peptide 2
µM peptide 2m

Figure S4