Microbial iCLIP2: Enhanced mapping of RNA-Protein interaction by promoting protein and RNA stability

Nina Kim Stoffel[1], Srimeenakshi Sankaranarayanan[1], Kira Müntjes[1], Nadine Körtel[2], Anke Busch[2], Kathi Zarnack[3], Julian König[2], Michael Feldbrügge[1]

[1] Institute of Microbiology, Heinrich Heine University Düsseldorf, Cluster of Excellence on Plant Sciences, 40204 Düsseldorf, Germany

[2] Institute of Molecular Biology gGmbH, Ackermannweg 4, 55128 Mainz, Germany

[3] Buchmann Institute for Molecular Life Sciences (BMLS), Goethe University Frankfurt, Max-von-Laue-Straße 15, 60438 Frankfurt am Main, Germany

Table of contents:

Supplemental Figure S1 2

Supplemental Figure S2 3

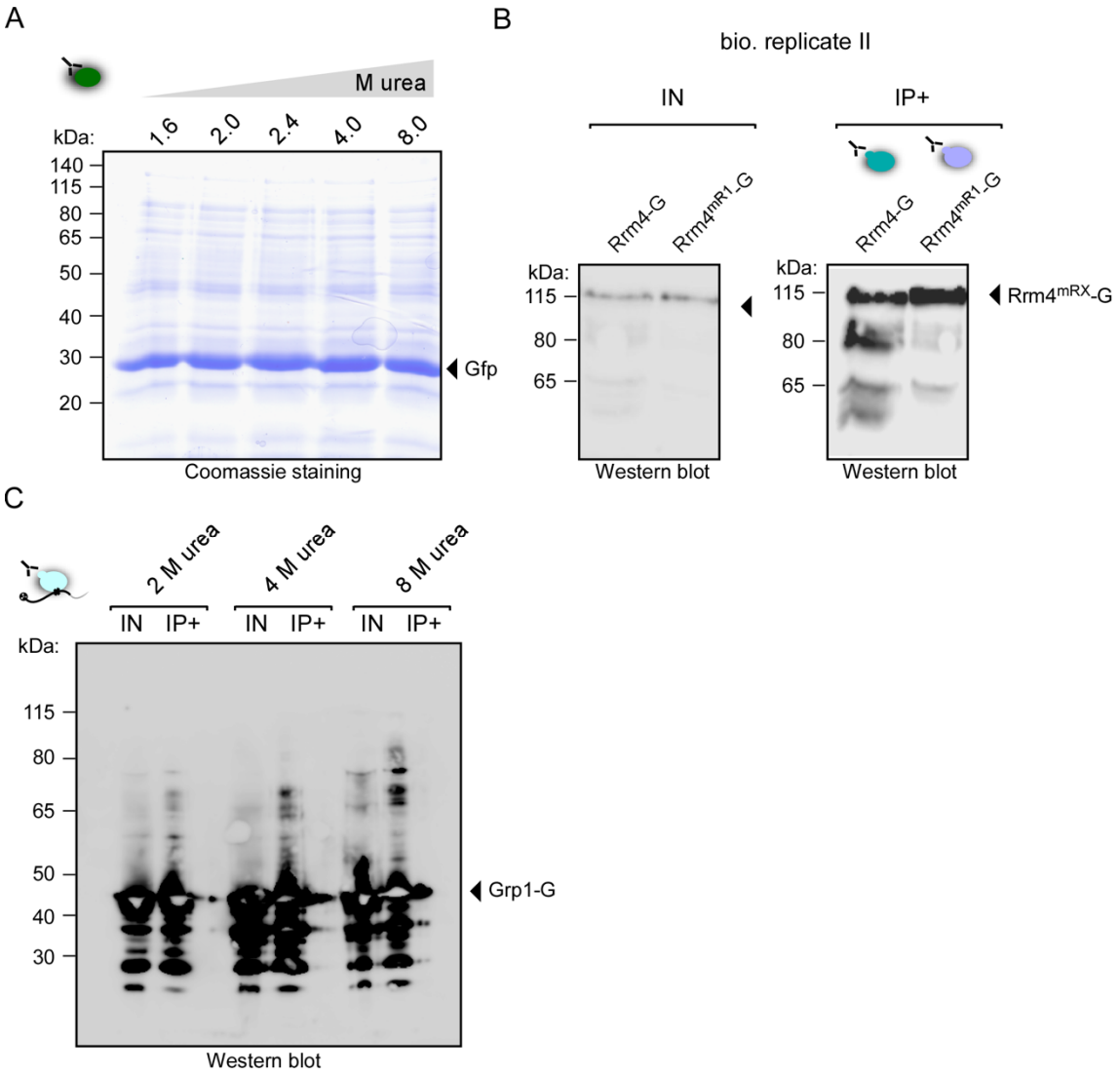
Supplemental Figure S3 4

Supplemental Figure S4 5

Table S1. Summary of different cell lysis conditions 6

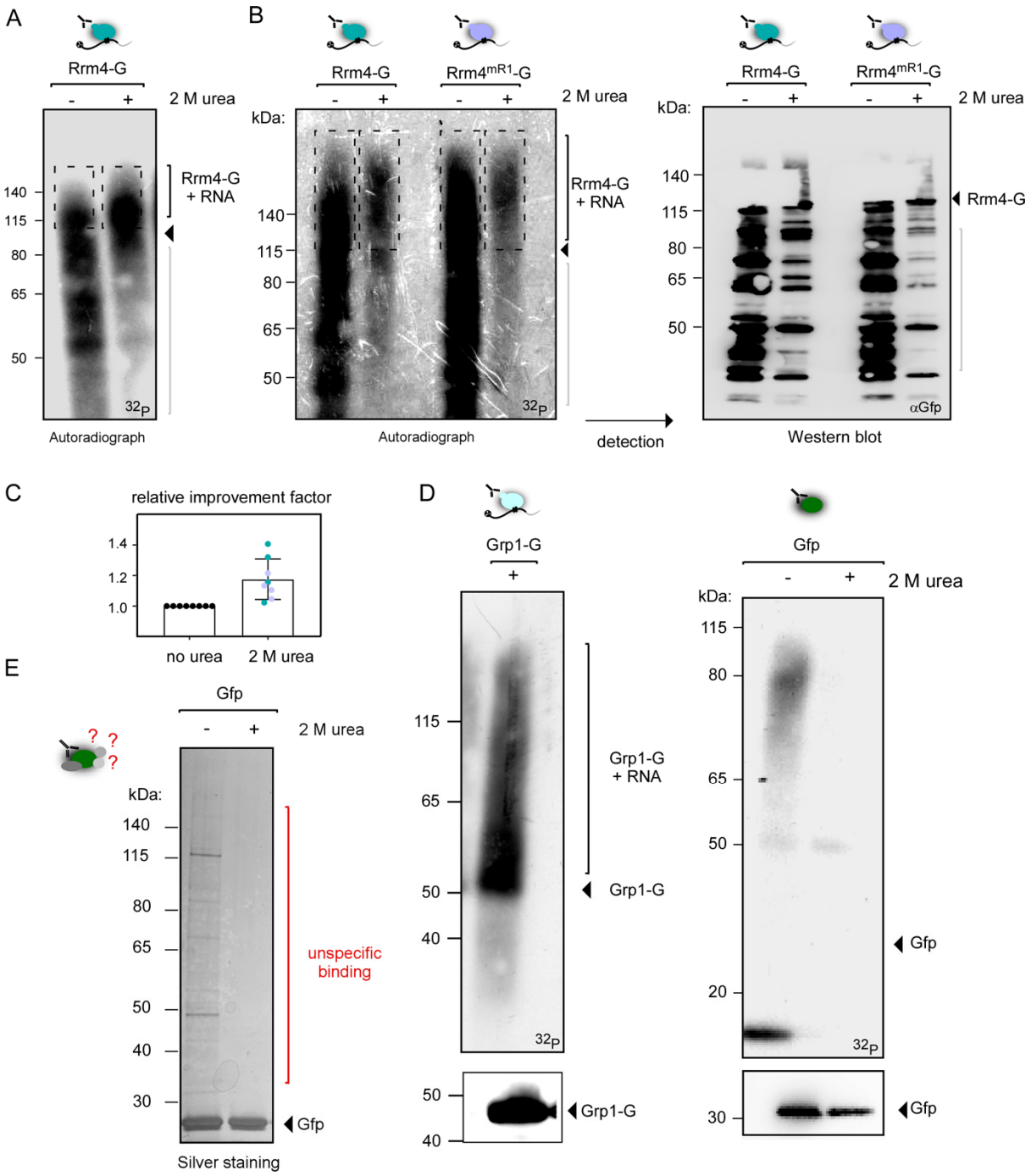
Table S2. Mitochondrial respiratory chain complex I and III 7

Supplemental Figure S1



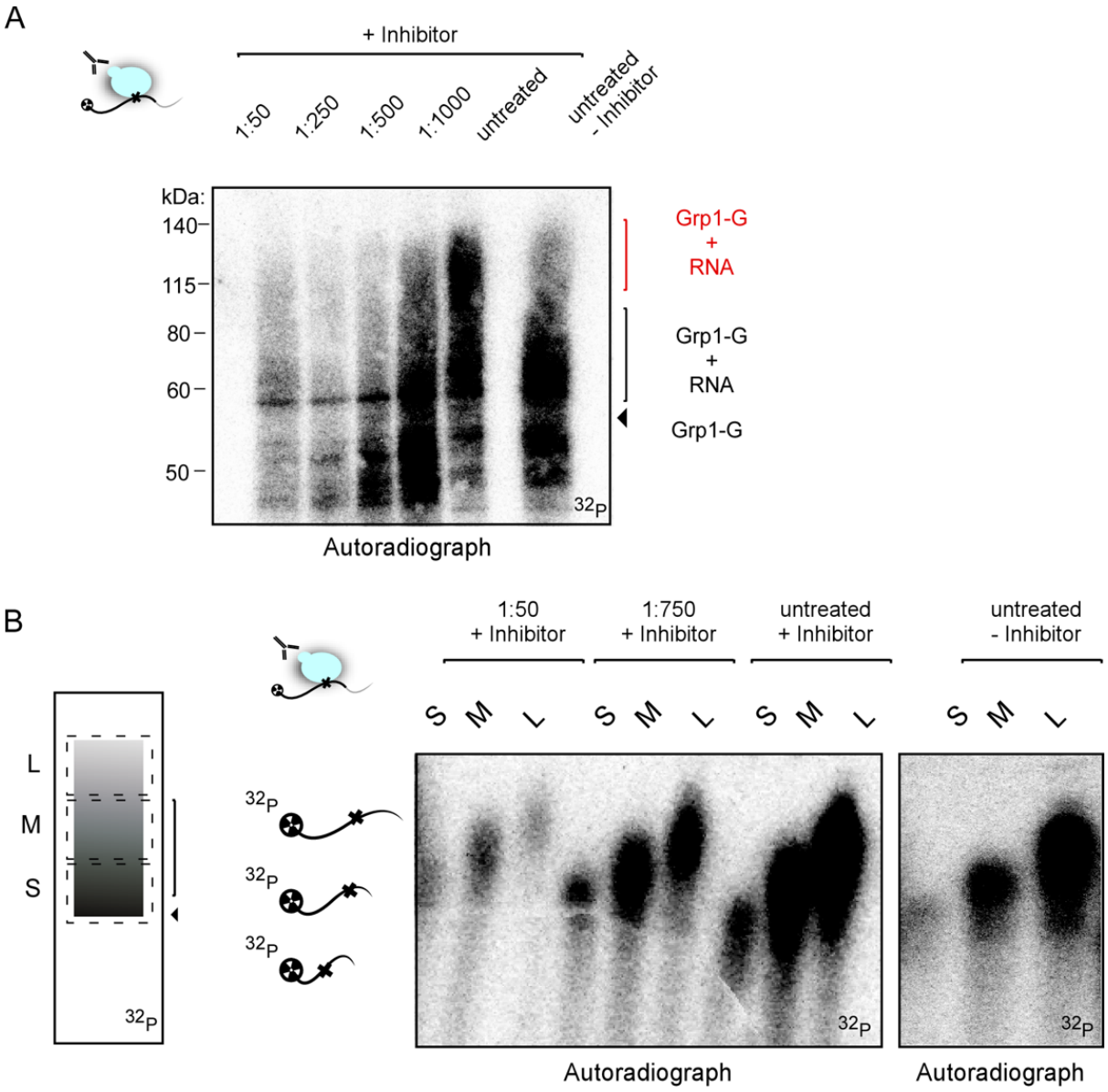
Supplemental Fig. S1. Increased protein stability by improved iCLIP2 protocol. (A) Coomassie-stained SDS-gel of Gfp IP, purified under different urea molarities conditions. Gfp was expressed in *E.coli* and the respective cell lysate was used as starting material for IP. **(B)** Western Blot analysis Rrm4-Gfp IP (IP+) by the usage of lysing buffer containing 8 M urea. Cell lysate (Input = IN) **(C)** Western blot of Grp1-Gfp iCLIP2 (IP+) experiments and respective cell lysate (IN) were produced under different urea conditions (2-8 M urea) - the respective autoradiograph is depicted in Fig. 3B. cell lysate (IN)

Supplemental Figure S2



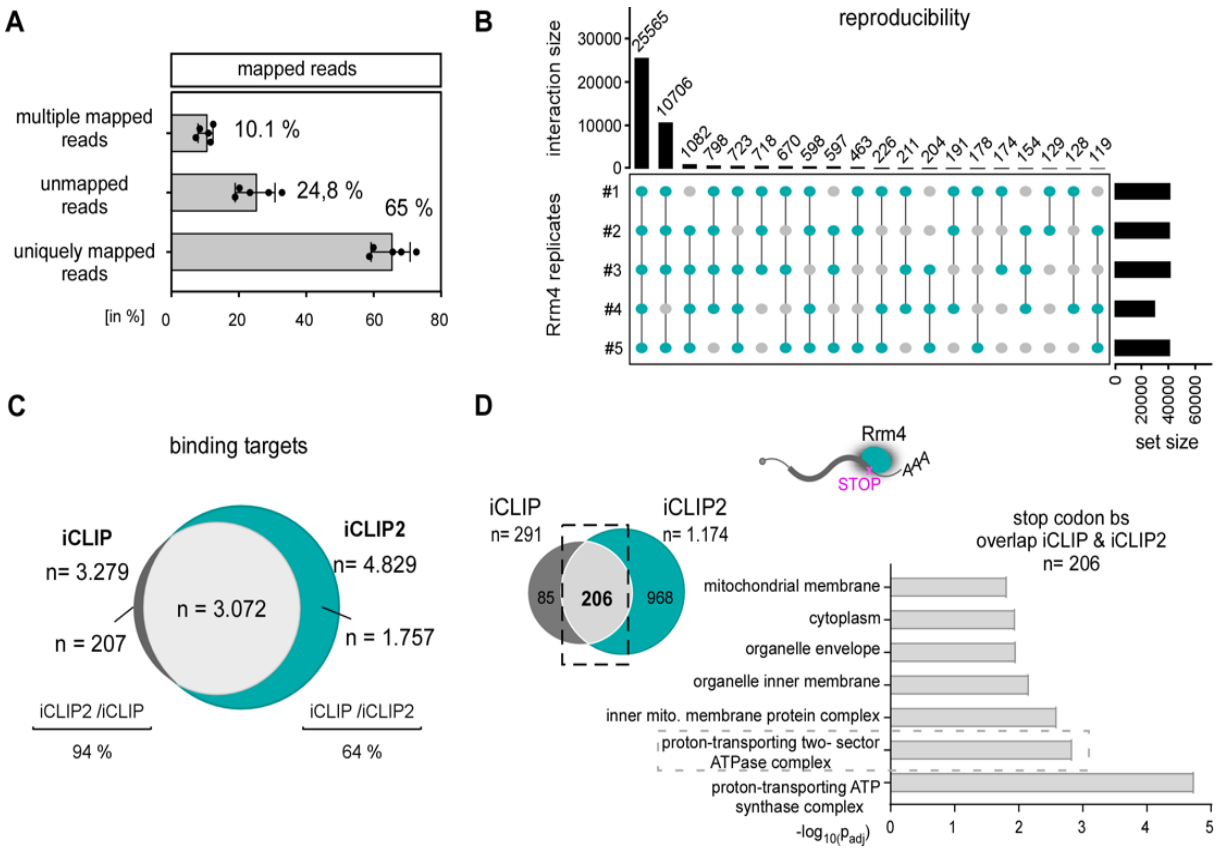
Supplemental Fig. S2. 2 M urea increases protein stability. (A -B) Autoradiograph of Rrm4-Gfp-RNA complexes by the usage of IPs +/-2 M urea containing lysis buffer (biological replicate). In B the respective Western blot is depicted. **(C)** The relative improvement factor was calculated by dividing the results of relative area quantification (refer to Figure 3D) with the untreated buffer (+ 2M urea/ - urea). **(D)** Autoradiograph of Grp1-Gfp-RNA complexes and free cytoplasmatic Gfp-RNA complexes, purified +/- 2 M urea conditions. Respective Western blots are below. **(E)** Silver-stained SDS-gel from IP Gfp samples prepared -/+ 2 M urea containing lysis buffer. Unspecific co-purified proteins are indicated in red.

Supplemental Figure S3



Supplemental Fig. S3. Optimal RNase I conditions for microbial iCLIP2 protocol. (**A**) Autoradiography of RNase I titration series for Grp1-Gfp. In addition, intrinsic RNase digestion was tested (untreated - Inhibitor) The non-adequate-separated Grp1-Gfp-RNA complexes are marked in red. (**B**) Autoradiograph of RNase I titration for Grp1-Gfp-RNA complexes (1:50, 1:1750), untreated +/- Inhibitor. The respective autoradiograph was separated into short (S), medium (M) and long (L) RNAs. The respective size-selected Grp1-RNA complexes were isolated and separated by a 6 % TBE-Urea-gel as described by Huppertz et al., 2014.

Supplemental Figure S4



Supplemental Fig. S4. Improvements achieved in iCLIP2 data set by optimizing the protocol. (**A**) Bar chat of mapped reads out of the 5 biological Rrm4 replicates of iCLIP2. The majority of reads are uniquely mapped, as expected. The high number of unmapped reads is due to the low-quality genome of *U. maydis*. Reads within the „unmapped“ group for example could be manually determined as rRNAs of *U. maydis*. (**B**) UpSet plot of 5 biological replicates of iCLIP2 Rrm4. Indicating high reproducibility of the biological replicates (**C**) Ven diagram of iCLIP vs. iCLIP2 Rrm4 overall binding targets (**D**) Bar chart of gProfile analysis of stop codon binding targets determined within both iCLIP and iCLIP2 datasets (overlap n=206)

Table S1. Summary of different cell lysis conditions

Technique:	1 Bead mill jar	2 Bead mill tubes	3 Homogenizer	4 Vibrax
Cryogenic conditions	+	++	++	
Time efficiency		++	++	+
High throughput/ Volumina		++	++	++
Mechanical separation/ chemical lysis		++		

[a] For details of the four different techniques see Materials and methods.

Table S2. Mitochondrial respiratory chain complex I and III

Respiratory chain complex I	
N	
NDUFV1	UMAG_11170
NDUFV2	UMAG_00634
NDUFS1	UMAG_10695
NDUFA2	UMAG_10820
Q	
NDUFA5	UMAG_11517
NDUFS2	UMAG_11162
NDUFS3	UMAG_11896
NDUFS7	UMAG_11038
NDUFS8	UMAG_11448
Pp-a	
ND1	mito-DNA
NDUFA3	UMAG_11731
NDUFA8	UMAG_05598
NDUFA13	UMAG_01311
Pp-b	
ND2	mito-DNA
NDUFC1	unkown
NDUFC2	unkown
ND3	mito-DNA
ND4L	mito-DNA
ND6	mito-DNA
PD-a	
NDUFB5	unkown
NDUFB10	UMAG_12001
NDUFB11	UMAG_00153
NDUFB6	unkown
ND4	mito-DNA
PD-B	
NDUFAB1	UMAG_00778
NDUFB7	UMAG_11217
NDUFB3	UMAG_05102
NDUFB8	UMAG_03956
ND5	mito-DNA
NDUFB9	UMAG_05625
NDUFB2	unkown
added at later stage	
NDUFA1	unkown
NDUFA10	unkown
NDUFS5	UMAG_06051
NDUFB1	unkown
NDUFB4	unkown
NDUFA9	UMAG_00381
NDUFA7	unkown
NDUFA11	UMAG_11495
NDUFAB1	UMAG_00778
NDUFA6	UMAG_02437
NDUFS4	UMAG_00512
NDUFV3	Unknown
NDUFA12	UMAG_10847
NDUFS6	UMAG_11583
Respiratory chain complex III	
ISP	UMAG_10507
QCR7	UMAG_04237
QCR10	UMAG_11097
QCR9	UMAG_11212
Cyt1	UMAG_11534
QCR6	UMAG_00940
QCR8	UMAG_11288
QCR2	UMAG_01478
COR1	unkown
Cytb	unkown