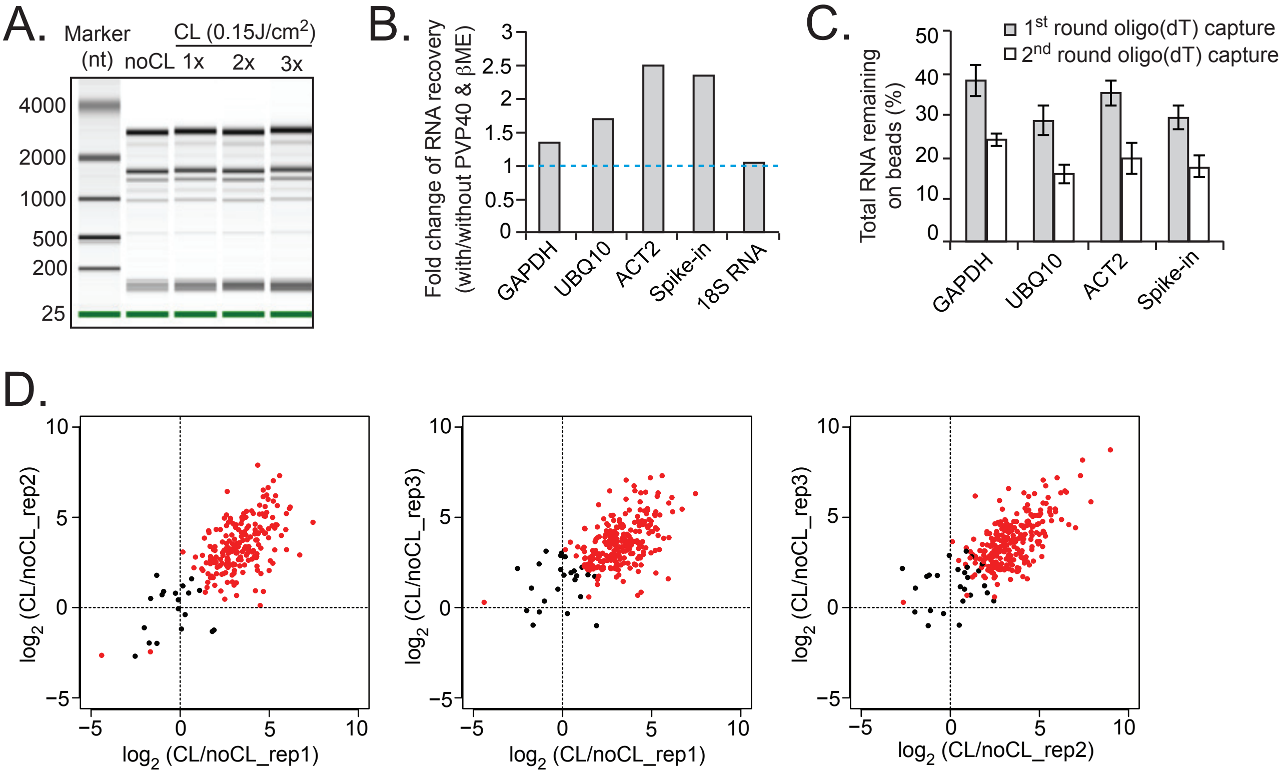
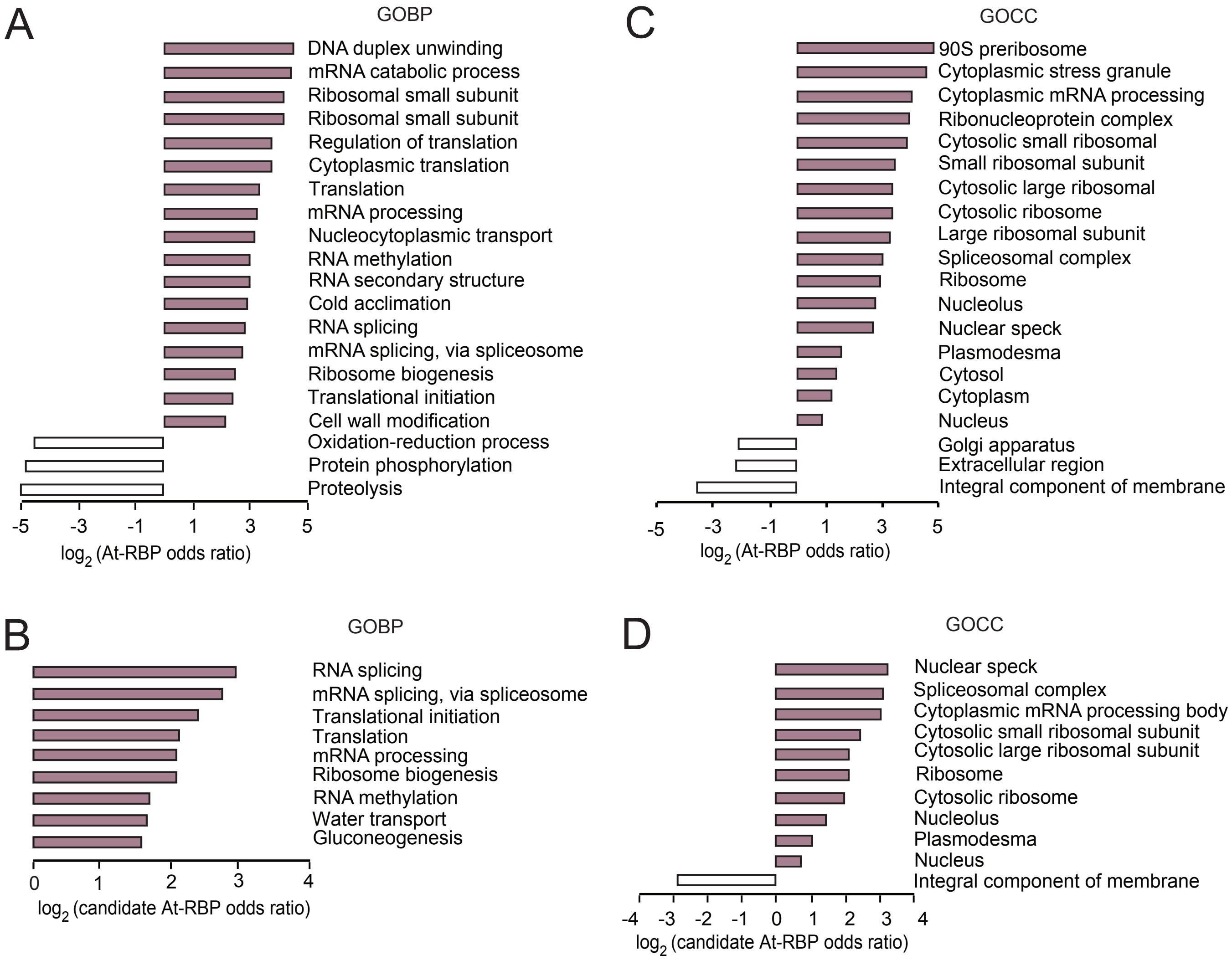


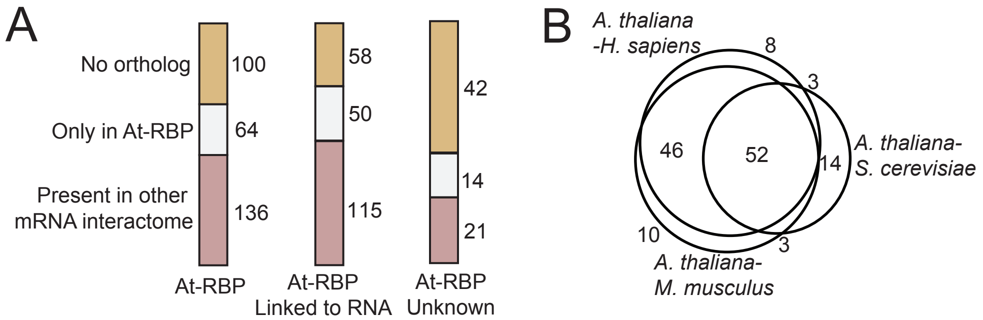
Supplemental Figure 1. Optimization of the Arabidopsis mRNA interatome capture protocol.
(A). RNA extracted from four-day-old etiolated seedlings was cross-linked (CL) either once, twice or three times at UV 150 mJ/cm2. Non-cross-linked (noCL) seedlings were used as control. RNA integrity was analysed on a Bioanalyzer 2100 using an RNA pico chip, and is depicted as the gel representation of in-chip electrophoresis.
(B). Lysates were prepared from noCL four-day-old etiolated seedlings using a lysis buffer with or without polyvinylpyrrolidone 40 (PVP40) and β-mercaptoethanol (β-ME). RNA captured on oligo(dT) beads was extracted and used for qRT-PCR. Measurements are the average of three technical replicates and are shown as fold change in RNA recovery upon addition of PVP40 and β-ME to lysis buffer.
(C). Two rounds of oligo(dT) capture were performed using a lysate from noCL four-day-old etiolated seedlings. Aliquots of the lysate were taken after the first and second round of oligo(dT) capture, respectively, followed by RNA extraction and qRT-PCR analysis. Measurements are the average of three technical replicates with error bars representing the SD. Measurements are shown relative to the input.
(D). Scatter plots comparing the protein enrichment in CL over noCL based on LC-MS/MS intensities of two biological replicates. Proteins significantly enriched (FDR < 1%) in CL or noCL are depicted in red. Proteins that lack enrichment are depicted in black.

Supplemental Figure 1



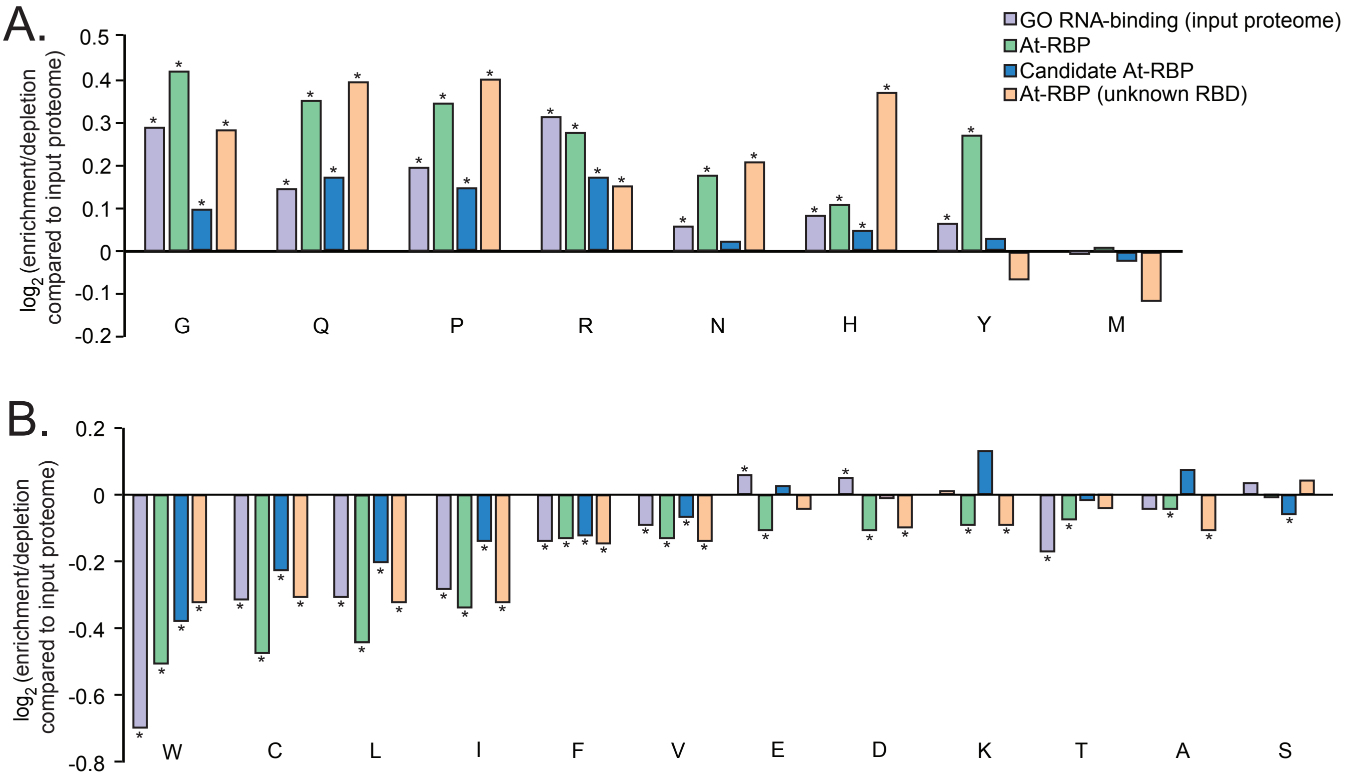
Supplemental Figure 2. GO enrichment analysis of At-RBPs and candidate At-RBPs.
(A-B). The most significantly over- and under- represented Gene Ontology (GO) terms for biological process in At-RBPs (A) and candidate At-RBPs (B).
(C-D). The most significantly over- and underrepresented GO terms for cellular compartment in At-RBPs (C) and candidate At-RBPs (D).

Supplemental Figure 2



Supplemental Figure 3. Conservation of RBPs across kingdoms.
(A). Categorization of At-RBPs based on conservation between Arabidopsis, yeast, mouse, and human.
(B). Overlap of At-RBPs with proteins identified in mRNA interactomes of yeast (Beckmann et al. 2015), mouse (mESC (Kwon et al., 2013, HL-1 (Liao et al. 2016)) and human (HEK293 (Baltz et al., 2012), HuH7 (Beckmann et al. 2015)).

Supplemental Figure 3



Supplemental Figure 4. Analysis of amino acid enrichment/depletion in the Arabidopsis mRNA interactome.
Log2 enrichment (A) and depletion (B) of amino acids of At-RBPs (green), proteins from the input proteome annotated as ‘RNA-binding’ (light purple), At-RBPs with unknown RBD (light orange) and candidate At-RBP (blue) compared to the input proteome. Amino acids that are significantly ($p < 0.001$) enriched/ depleted compared to the input proteome are marked with asterisks. Number of proteins in each RBP set: input proteome N=8246, GO RNA-binding (input proteome) N= 339, At-RBPs N=300, candidate At-RBPs N= 437, At-RBPs (unknown RBD) N= 61.

Supplemental Figure 4

Supplemental Table 1: Zinc-finger proteins not associated with RNA-binding identified by mRNA interactome capture.

Gene ID	Protein name	Protein domain	FDR
AT3G06410	Zinc finger CCCH domain-containing protein 34	zf-CCCH	4.36E-15
AT5G18550	Zinc finger CCCH domain-containing protein 58	zf-CCCH	6.85E-15
AT2G47850	Zinc finger CCCH domain-containing protein 32	zf-CCCH	7.18E-11
AT3G15680	Ran BP2/NZF zinc finger-like superfamily protein	zf-RanBP	2.53E-08
AT1G67325	RanBP2-type zinc finger protein	zf-RanBP	9.1E-08
AT5G63260	Zinc finger CCCH domain-containing protein 67	zf-CCCH	9.84E-07
AT3G62240	RING/U-box superfamily protein	zf-C2H2, zf-RING	2.98E-06
AT1G10170	NF-X1-type zinc finger protein NFXL1	zf-NF-X1	1.81E-05
AT2G02160	Zinc finger CCCH domain-containing protein 17	zf-CCCH	2.9E-05
AT1G75560	zinc knuckle (CCHC-type) family protein	zf-CCHC	1.66E-03

Supplemental Table 2: Examples of potential non-canonical RBPs identified by mRNA interactome capture.

Gene ID	Protein name	Protein domain	Function	FDR
Cytoskeleton				
AT1G49240	ACTIN 8	Actin	structural constituent of cytoskeleton	2.49E-04
AT1G04820 AT4G20890	Tubulin alpha-4 chain Tubulin beta-3 chain	Tubulin	structural constituent of cytoskeleton	6.63E-06 9.47E-06
Enzymes/metabolism				
AT1G20620	Catalase-3 (CAT3)	Catalase	catalyzes the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen	1.17E-03
AT5G64100	Peroxidase 69	Peroxidase	uses H_2O_2 to catalyse oxidative reactions	1.05E-04
AT5G20250	RAFFINOSE SYNTHASE 6 (RS6)	Raffinose_syn	involved in carbohydrate metabolism	2.88E-04
AT3G17390	S-adenosylmethionine synthase 4 (METK4)	S-adoMet_synt	biosynthesis of S-adenosylmethionine	1.97E-05
AT5G63650	Serine/threonine-protein kinase (SRK2G)	Pkinase	SNF1-related protein kinase	3.96E-05
AT2G32000	DNA topoisomerase type IA	Toprim	DNA unwinding, involved in DNA replication	4E-05
AT4G14990	Topoisomerase II-associated protein (PAT1)	PAT1	isomerase activity	3.32E-03
Membrane proteins/transport				
AT5G03280	Ethylene-insensitive protein 2 (EIN2)	Nramp	Involved in ethylene signal transduction	3.58E-09
AT4G27500	Proton pump-interactor 1 (PPI1)	No annotated domains	interacts with H+-ATPase and regulates its activity	6.85E-15
AT1G20110	FYVE DOMAIN PROTEIN REQUIRED FOR ENDOSOMAL SORTING 1 (FREE1)	FYVE	regulates multivesicular/ prevacuolar compartment protein sorting	2.41E-04
AT3G53420	Aquaporin PIP2-1	MIP	Transport of water and small solutes	2.09E-05
AT2G37170	Aquaporin PIP2-2	MIP	Transport of water and small solutes	8.79E-03
AT4G35100	Aquaporin PIP2-7	MIP	Transport of water and small solutes	5.44E-05
AT2G38750	ANNEXIN 4 (ANN4)	Annexin	Ca^{2+} dependent membrane binding protein	4.91E-07
Photoreceptors				
AT1G09570	Phytochrome A (PHYA)	GAF;HATPase_C; HisKA;PAS;PHY	red/far-red light photoreceptor	1.49E-03
AT3G45780	Phototropin-1 (PHOT1)	Pkinase	Blue-light photoreceptor	3.48E-03
Miscellaneous				
AT5G56030	Heat shock protein 90.2 (HSP90.2)	HSP90	Molecular chaperone assisting in the synthesis and folding of proteins	5.07E-03
AT2G34040	Apoptosis inhibitory protein 5 (API5)	API5	unknown	4.81E-13
AT1G10170	NF-X1-type zinc finger protein (NFXL1)	Zf-NF-X1	negative regulator of the trichothecene phytotoxin-induced defense response	1.81E-05
AT2G42680	Multiprotein-bridging factor 1A (MBF1A)	HTH_3;MBF1	Transcriptional co-activator	2.7E-03
AT2G32080	Purine-rich alpha 1 (PURA1)	PurA	Transcription factor	2.52E-09