

Figure 6-Supplement 1. Mattijssen et al.

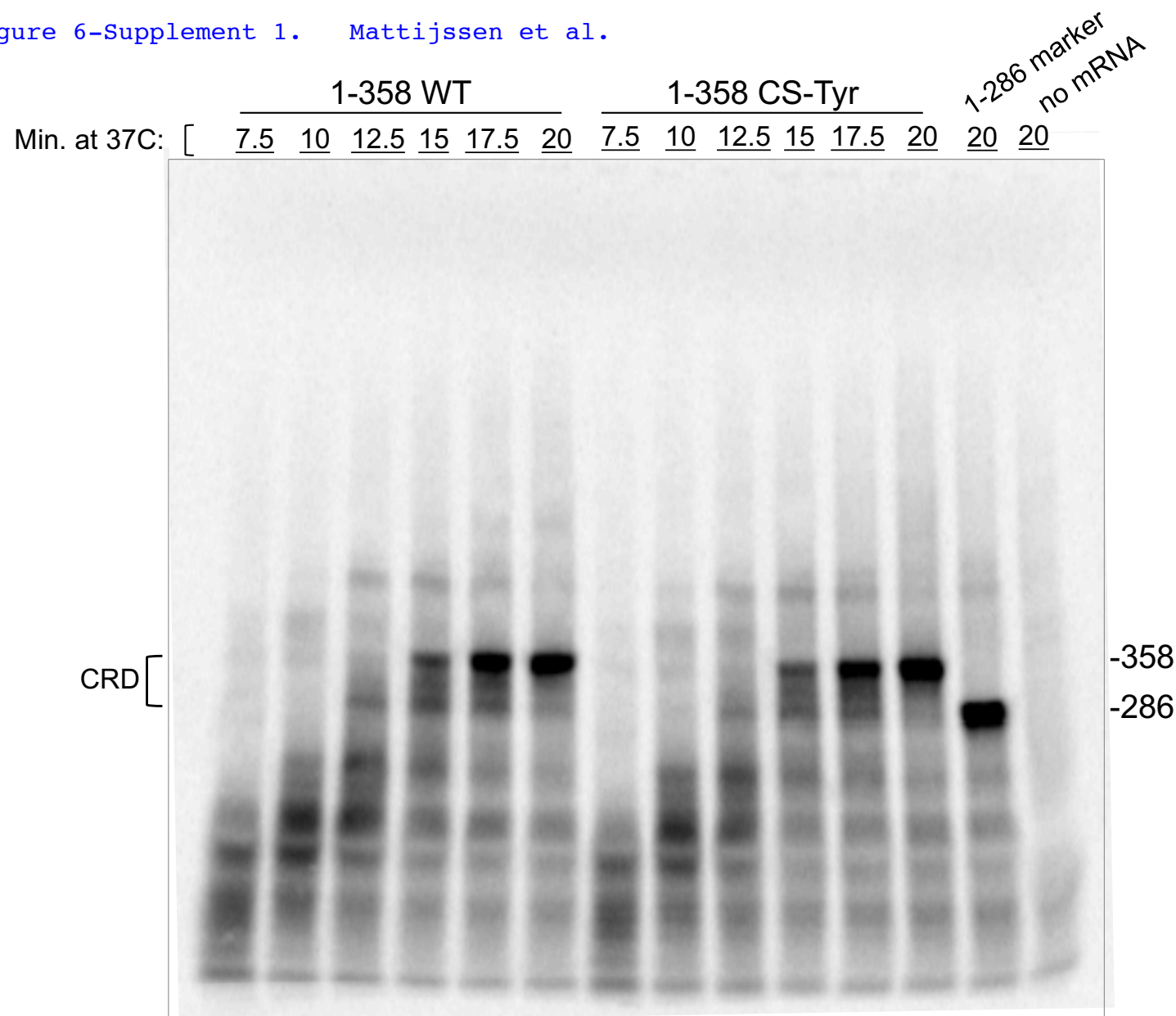


Figure legend: Time course in vitro translation of T7-synthesized mRNAs. -358 and -286 at right indicate positions of N- and C- terminal boundaries of the CRD, as indicated by bracket on the left. The 358 size bands resulted from in vitro translation of the T7-synthesized mRNAs (see methods below and text).
Methods: HEK293 cell lysate was prepared from cells at 70% confluency by adding an equal volume of lysis buffer (10 mM HEPES pH 7.3, 10 mM KAc, 0.5 mM MgAc, 5 mM DTT and protease inhibitors (Roche) to a PBS-washed cell pellet and incubating for 45 mins on ice. The cells were then passed 10 times through a 30.5 G needle and checked under a microscope for a lysis of >60%. The lysate was spun at 14,000 g for 1 minute to remove debris and nuclei. The supernatant was aliquoted and immediately frozen at -80C. DNA templates for T7 RNA polymerase-mediated transcription, were generated by PCR to obtain the following fragments: Flag-LARP4-1-286 (to mark the start of the CRD) and 2 versions of Flag-LARP4- 358 (end of the CRD), a WT version and CS-Tyr. Using the *mMESSAGE mMACHINE*® T7 Ultra Kit (Thermofisher), 7^mG 5' capped and 3' polyadenylated mRNAs were generated. Polyadenylation after addition of PolyA polymerase was confirmed by denaturing gel electrophoresis (not shown). The in vitro translation reaction contained the following in 10 ul: 40% cell extract, 50 mM KAc, 2.5 mM MgAc, 20 U Supersasin, 200 ng mRNA template, 1.6 mM HEPES pH 7.3, 2 mM creatine phosphate, 0.01 ug/ul creatine kinase, 10 uM spermidine, 10 uM amino acid mix (no methionine), 10.2 uCi 35S-Methionine (Perkin Elmer)). Reactions were placed at 37C and after indicated times placed on ice and quenched by addition of 10 ul EDTA (25 mM final). The reactions were then subjected to immunoprecipitation using *Anti-FLAG*® M2 Magnetic Beads (Sigma) according to manufacturer's protocol. Immunoprecipitated material was eluted from the beads using SDS buffer containing b-mercaptoethanol and heated for 5 mins at 80C. Samples were loaded on an SDS-PAGE gel, then blotted to nitrocellulose and imaged on a phosphorimage screen.