Supplemental figure legends

Fig S1. Hantavirus RNA synthesis.

For mRNA synthesis (left side), a capped primer 10-20 nt in length is cleaved from a host mRNA after a G residue, and this G is aligned opposite the template C at position +3. After its extension to **….G**UAG$_{OH}$, the primer can be extended without realignment to produce a mRNA in which exactly one 5' UAG repeat has been deleted (2nd level on left side). The same primer can also be realigned before extension; to produce a "wt" mRNA with all three UAG repeats (3rd level on left side). If the same primer is cleaved a 2nd time (after realignment) by the cap-dependent, G-specific endonuclease, the remaining fragment (5' **p**UAG) can be used to prime antigenome synthesis (left side red arrow). Alternatively, antigenome synthesis can be initiated by GTP opposite the C at position +3 at the 3' end of the genome template (right side). After its extension to **pppG**UAG$_{OH}$ and realignment on the genome template, the pppG can be cleaved by the G-specific endonuclease acting in a cap-independent fashion, to generate the pUAG 5' ends (right side). When the complementary ends of the genome anneal, the dsRNA so formed will have flush ends without the 5' tri-phosphate.

Fig S2. Non-denaturing PAGE analysis of gel-purified pppRNA1.

^{32}P-CTP-labelled, gel-purified pppRNA1 (T7-3G) synthesized in the presence of 5-biotin-UTP (Biotyn.), or its absence, was electrophoresed on 10% non-denaturing polyacrylamide gel, which was stained with ethidium bromide (below) and autoradiographed (above). Note that EtBr staining strongly over-estimates the amount of dsRNA present.

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Fig S3. Interaction of differentially purified 5' ppp-RNA1s with RIG-I.

500ng of minimally-purified (RNA1 biot.), PAGE purified (RNA1* biot.), and PAGE + RNase III-treated (RNA1** biot.) biotinylated RNA1s were bound to streptavidin beads and used to "pull down" either purified (his-tagged) RIG-I, or a vaccinia virus E3L-GST fusion protein, in the same pull-down assay; the E3L protein serving as a positive control (43). This comparison shows that the binding of RIG-I to the beads was due to the dsRNA by-products of the T7 reaction, rather than the 5' ppp-ssRNA.

Fig S4. Transfection controls

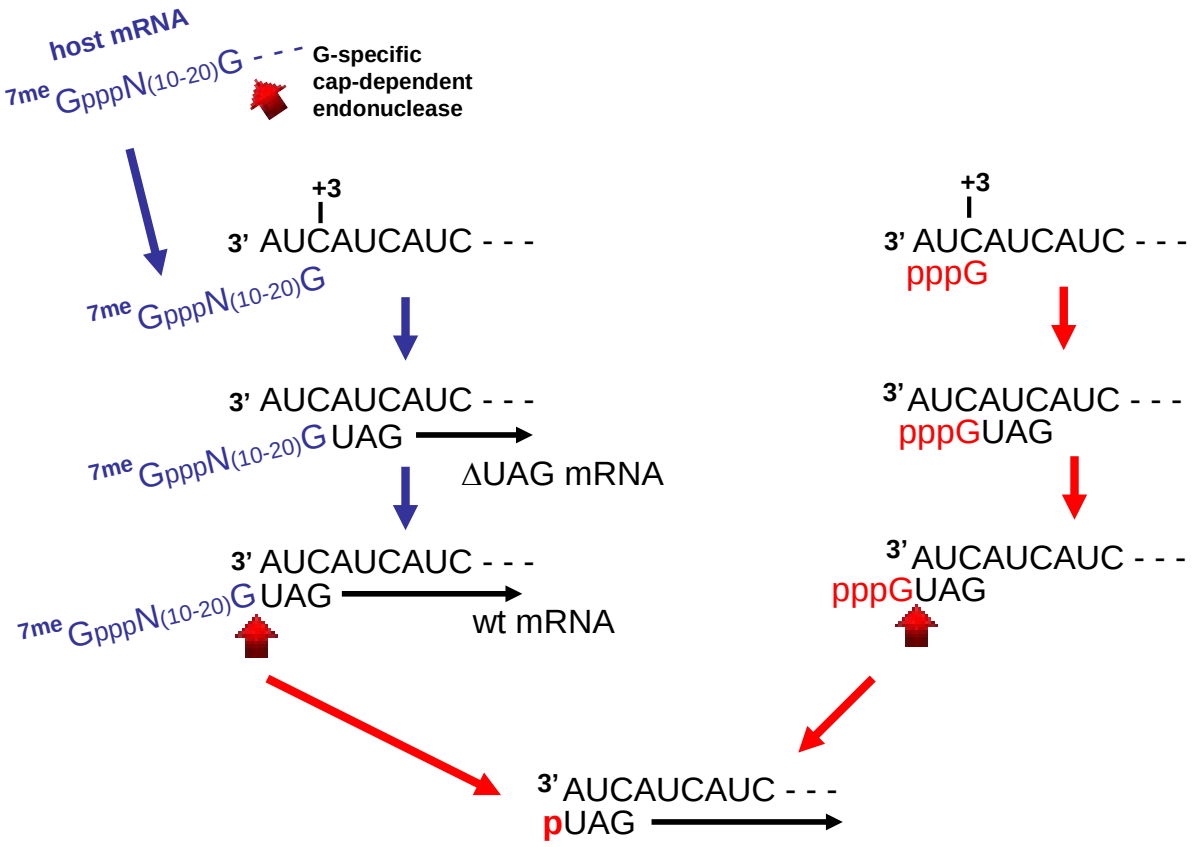
Various synthetic ssRNAs (1 ug) and tRNA (3 ug) (as indicated on the left) were tested for their ability to activate IFNβ, when transfected into A549 cells by themselves. A 20'mer RNA annealed to 1 ug of 5' ppp-RNA1** served as a positive control (lane 2).

Fig S5A. RNase III resistance of Jun -1/60^{mod} RNA.

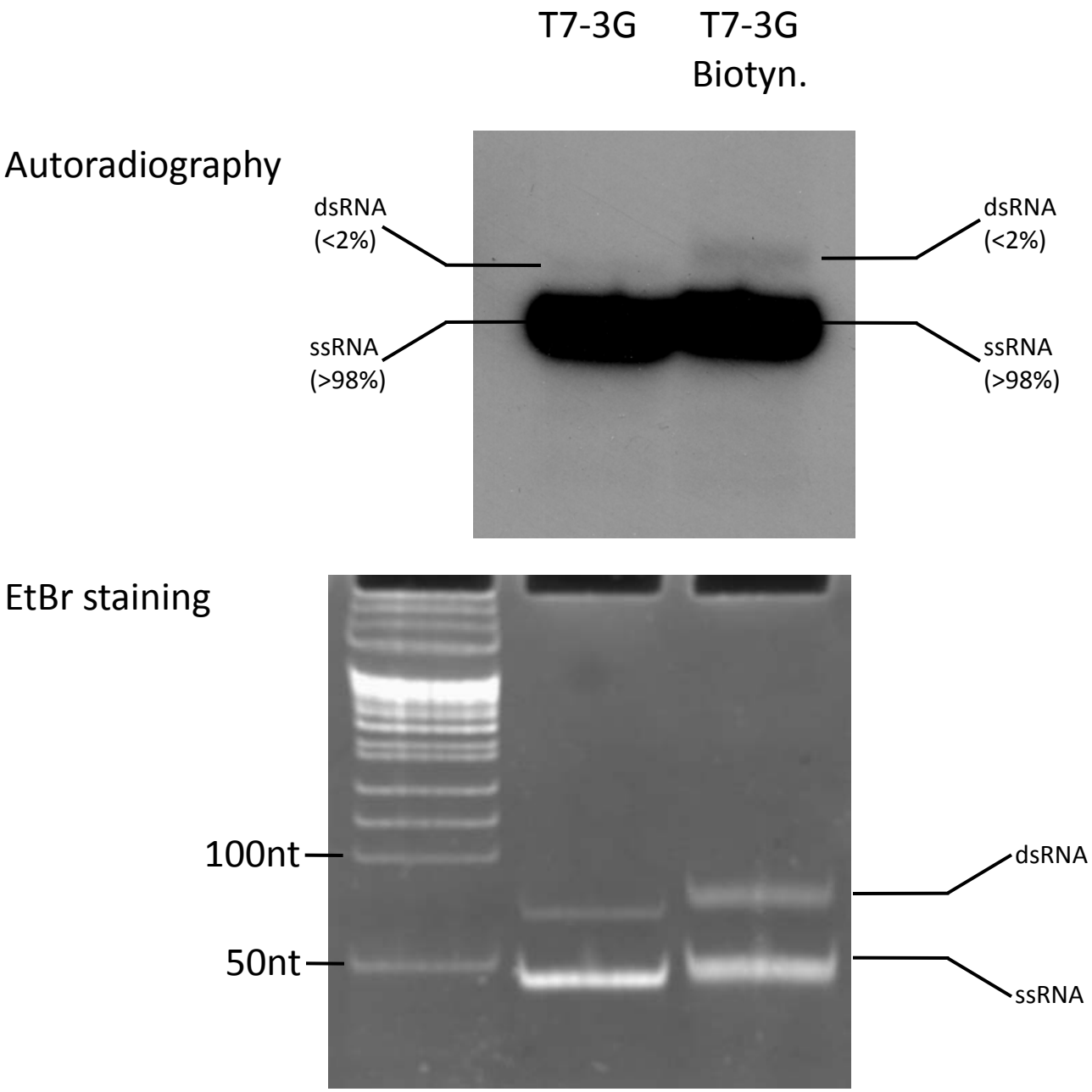
Equal amounts of Jun -1/60^{mod} RNA were either untreated, mock-treated, or RNase III-treated, and then analyzed by non-denaturing (10%) PAGE, and stained with EtBr. Poly-I/C served as a positive control.

Fig S5B. Formation of Junin dsRNA panhandles.

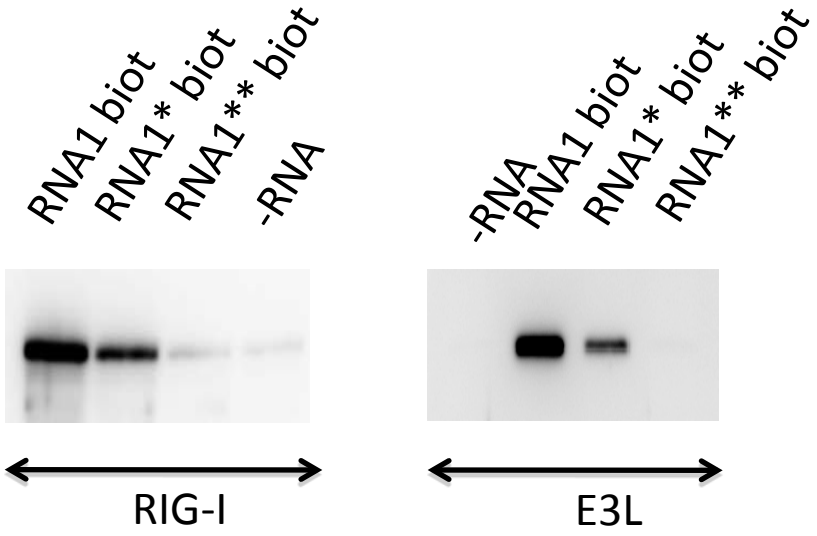
600 ng of 5' ppp Jun -1/60^{mod} RNA were annealed (or not) with the same amount of synthetic complementary oligonucleotides as indicated on the left. The RNA samples then analyzed by non-denaturing (10%) PAGE, and stained with EtBr.



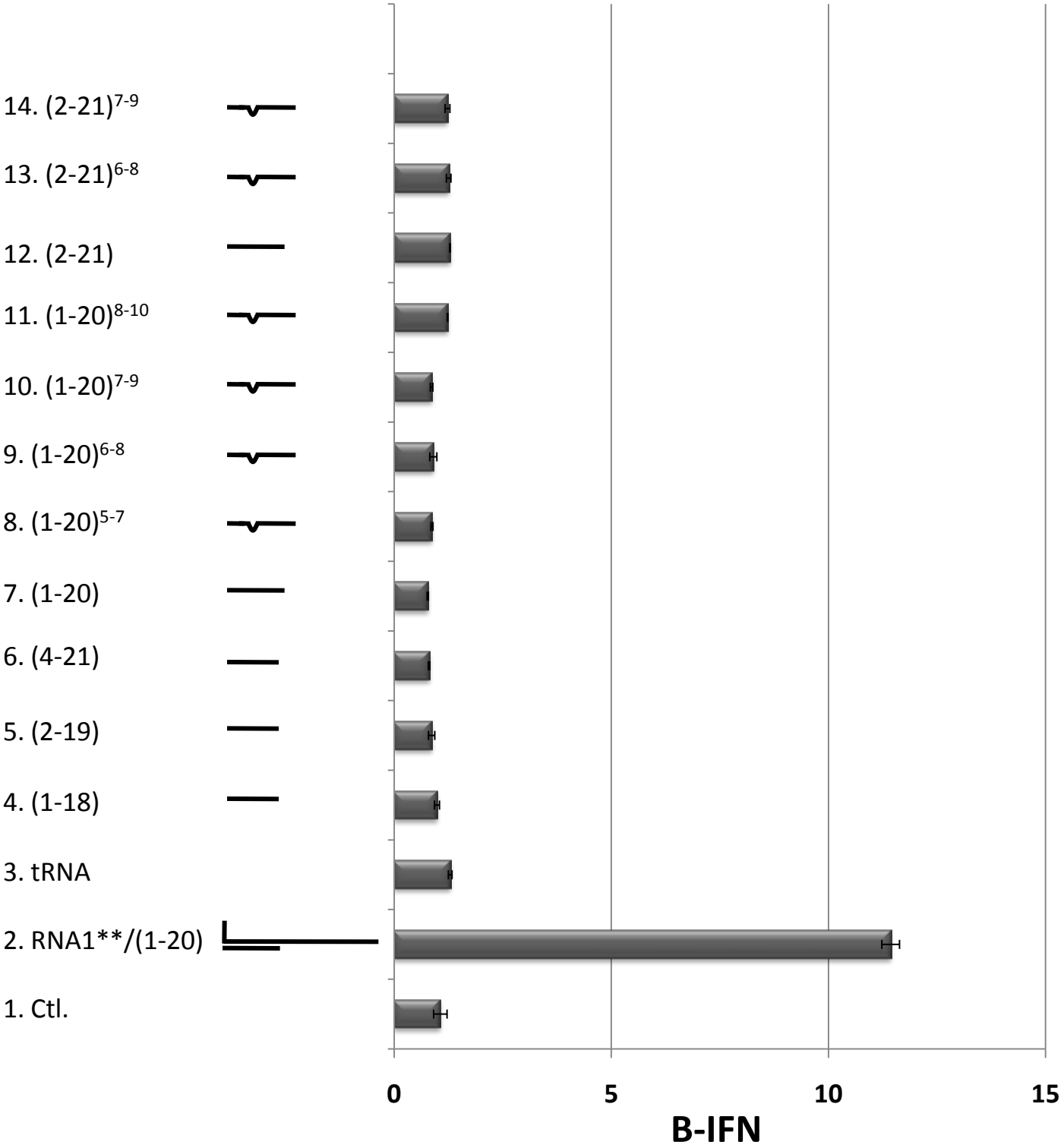
Marq et al. Fig. S1



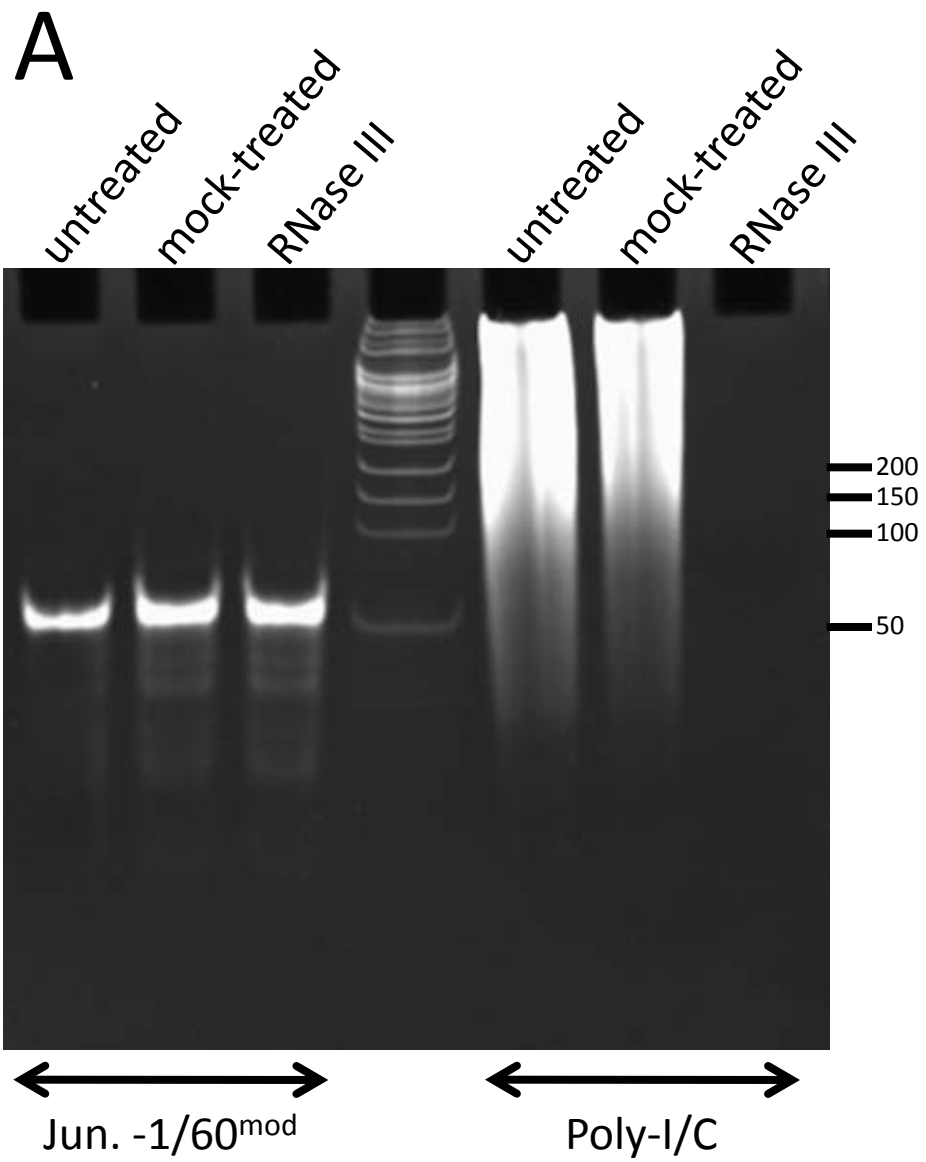
Marq et al. Fig.S2



Marq et al. Fig.S3

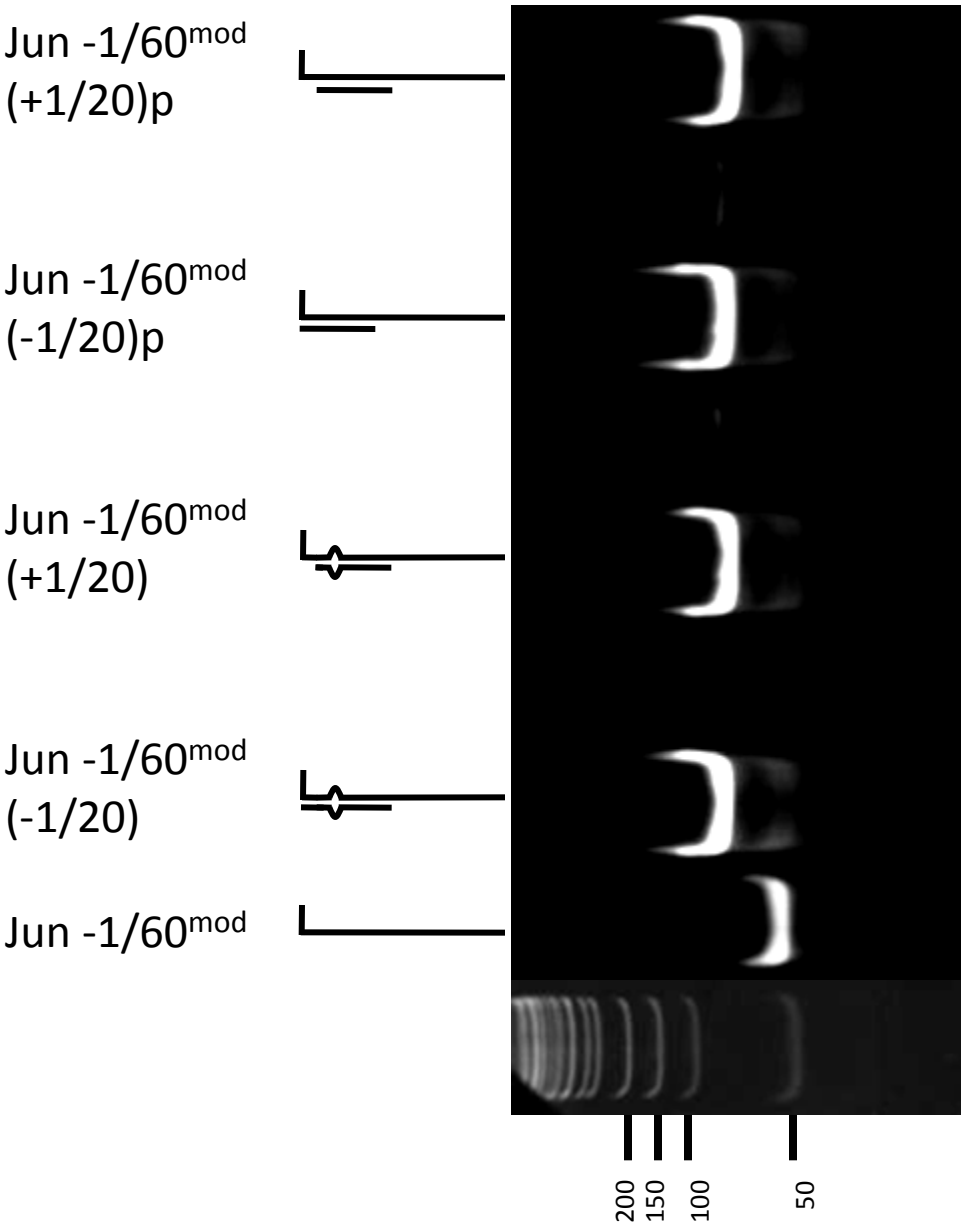


Marq et al. Fig. S4



Marq et al. Fig. S5A

B



Marq et al. Fig. S5B