Supplementary Figures

Oligonucleotide primer table

1. 5' TAGGGATATCTTGGCTGCAGCAGCAAATGGAACTGGCAAG
2. 5' CAGATTCTTCTTTACGCAGCCGCATTTCCCCTAAGTGTTC
3. 5' ATTAACCTGATGGAATAAGCTAGCCTGAAAGGAGTAACG
4. 5' TAGGGATATCTTAGCTGCAGCAGCAAATGGAACAGGCAAGA
5. 5' CCAGATGATAGTATTGGATCAGGCAGATAAGTTGCTGTC
6. 5' TATCTCCATCGTATTGGACAATCAGGTCGCTTTGGTCAT

Figure Legends

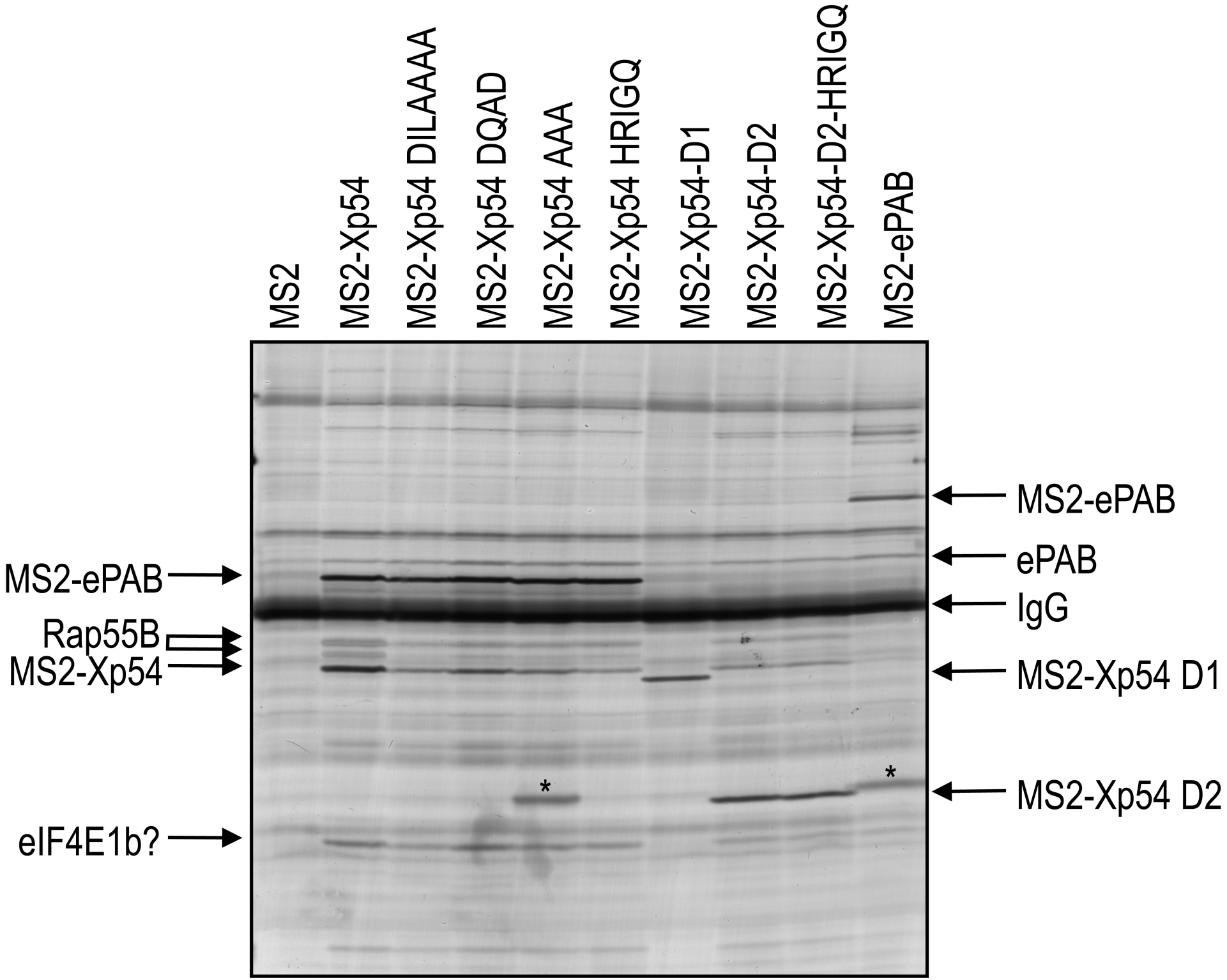
S1. The levels of MS2 fusion proteins expressed in oocytes are approximately constant.
Lysates were prepared from oocytes injected with mRNAs encoding the indicated proteins, and immunoprecipitated with MS2 monoclonal antibodies prior to SDS-PAGE gel electrophoresis and silver staining (see Minshall *et al.*, 2007) for further detail). Identities of abundant p54 co-immunoprecipitating proteins are indicated. * Protein not consistently found to co-immunoprecipitate.

S2. Tethered p54 proteins do not differentially affect reporter mRNA levels. The stability of ^{33}P-labelled Firefly luciferase mRNAs was assessed 12 h after injection by agarose gel electrophoresis and autoradiography (see Minshall *et al.*, 2007). rRNA was stained with ethidium bromide as a loading control. While reporter mRNA was partially degraded between injection (0 h), and harvesting 12 hours later, this was observed in oocytes co-injected with all MS2-fusion protein mRNAs. We did not observe any direct correlation between reporter mRNA levels, and expression (Fig.1C).

S3. CPEB and Xp54 interact with ePAB

Oocyte lysates (untreated or treated with RNAse) were immunoprecipitated with monoclonal CPEB antibody (panel A), or analysed by Sepharose 6 HR 10/30 FPLC gel filtration (panel B; see Minshall *et al.*, 2007). Using western blotting it is shown that ePAB both co-immunoprecipitates and co-fractionates with CPEB. The interaction between CPEB and ePAB is RNA-independent, unlike that between CPEB and the IMP3 RNA-binding protein Vg1RBP, which requires RNA (panels A and B). (C). Mass spectrometry evidence that the binding of ePAB to MS2-Xp54-DQAD is enhanced and the binding of Rap55B to p54-DQAD is diminished, relative to wild-type MS2-Xp54. Lysates were prepared from oocytes injected with MS2-Xp54 mRNAs, immunoprecipitated with MS2 antibodies and

separated by SDS-polyacrylamide gel electrophoresis followed by silver staining. The band at around 70 kDa, whose binding to MS2-Xp54-DQAD relative to wild type MS2-Xp54 was enhanced, was submitted to mass spectrometry. The peptides (YQGVNLYVK, ALDTMNFEVIK) revealed this band to correspond to ePAB, the maternal (embryonic) form of the poly(A)-binding protein (Voeltz *et al.*, 2001). The interaction between MS2-Xp54 and ePAB was verified by western blotting (Fig.1C). The protein doublet at around 55 kDa, whose binding to MS2-Xp54-DQAD relative to wild type p54 was reduced, was also sequenced. The peptides of both proteins (LNTETFGVSGR, SFFDNISSEMK, EEVYEIIFR, FEGDFDFESANAQFNR, YEGILYTIDTENSTVALAK, QSGPQSQPAPLNVPPPAAPVLGTVNDENR, TDSGVETQNSDGNPEEDPLGPNTYYDR) revealed them to correspond to Rap55B, presumably spliced isoforms of Rap55B. The interaction between MS2-Xp54 and Rap55 was verified by western blotting (Fig.1C).


MS2
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MS2-Xp54 DQAD
MS2-Xp54 AAA
MS2-Xp54 HRIGQ
MS2-Xp54-D1
MS2-Xp54-D2
MS2-Xp54-D2-HRIGQ
MS2-ePAB
MS2-ePAB
Rap55B
MS2-Xp54
eIF4E1b?
MS2-ePAB
ePAB
IgG
MS2-Xp54 D1
MS2-Xp54 D2
*
*


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12 h
MS2
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12 h
MS2-Xp54
0 h
12 h
MS2-Xp54 DILAAAA
0 h
12 h
MS2-Xp54 DQAD
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12 h
MS2-Xp54 AAA
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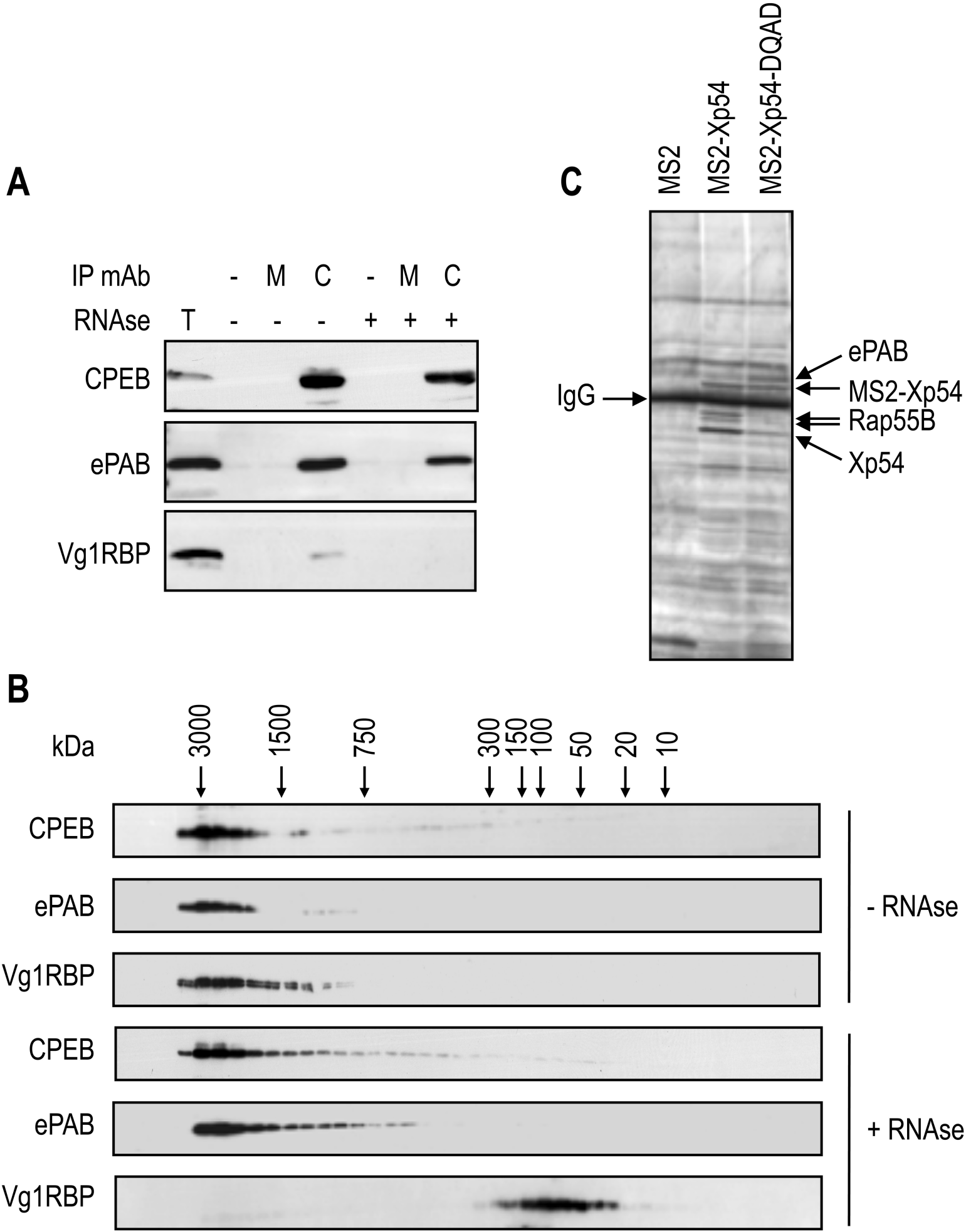
rRNA
32P Luc-MS2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RNAse T - - - + + +
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Vg1RBP
C
MS2
MS2-Xp54
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IgG
ePAB
MS2-Xp54
Rap55B
Xp54
B
kDa
3000
1500
750
300
150
100
50
20
10
CPEB
ePAB
Vg1RBP
- RNAse
CPEB
ePAB
Vg1RBP
+ RNAse