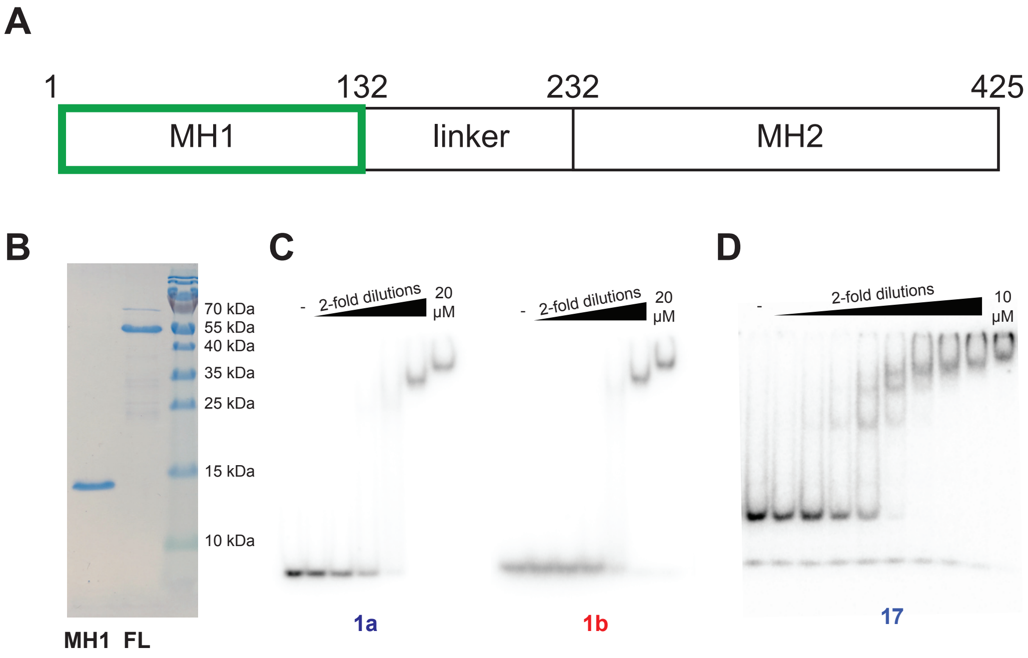
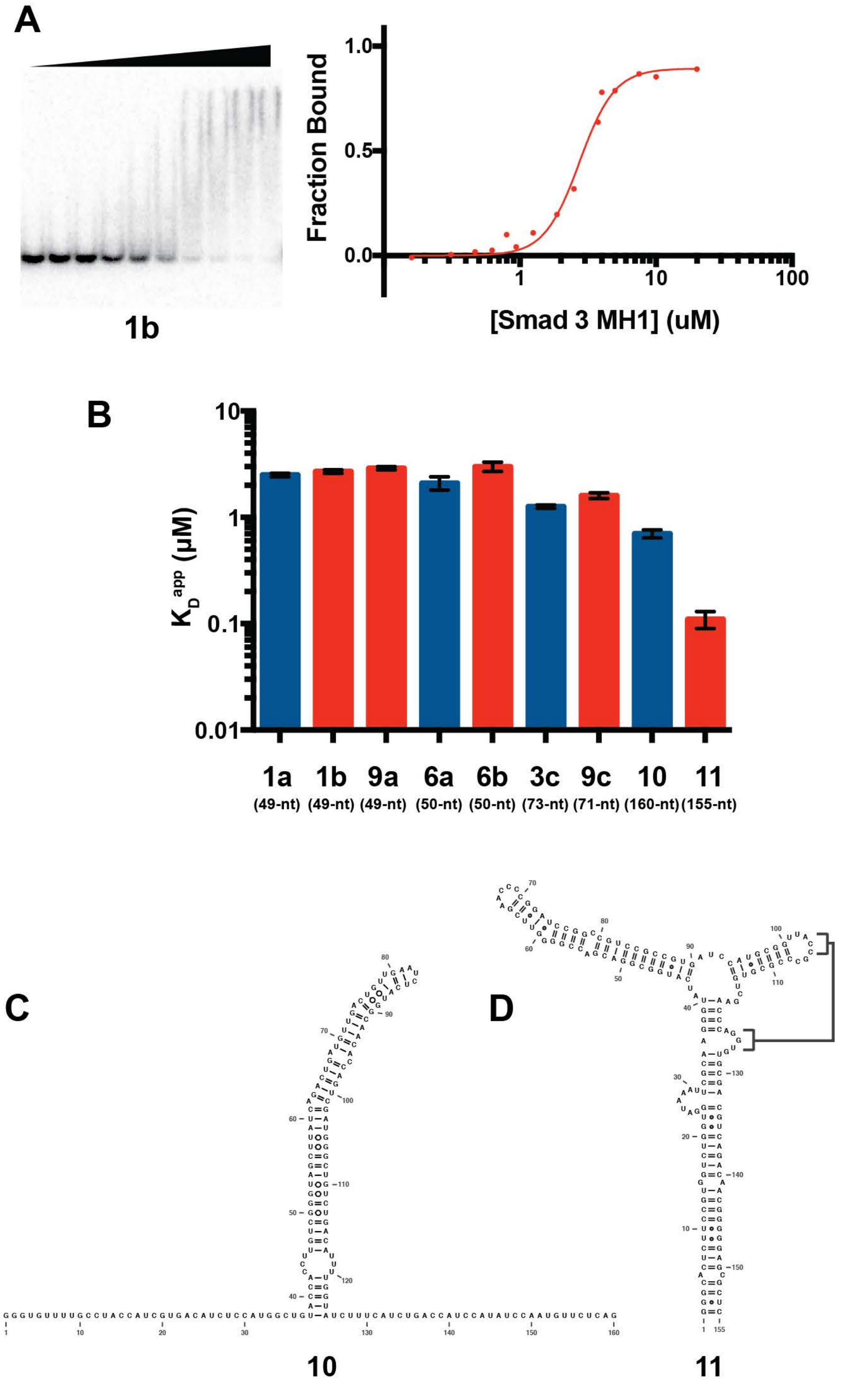
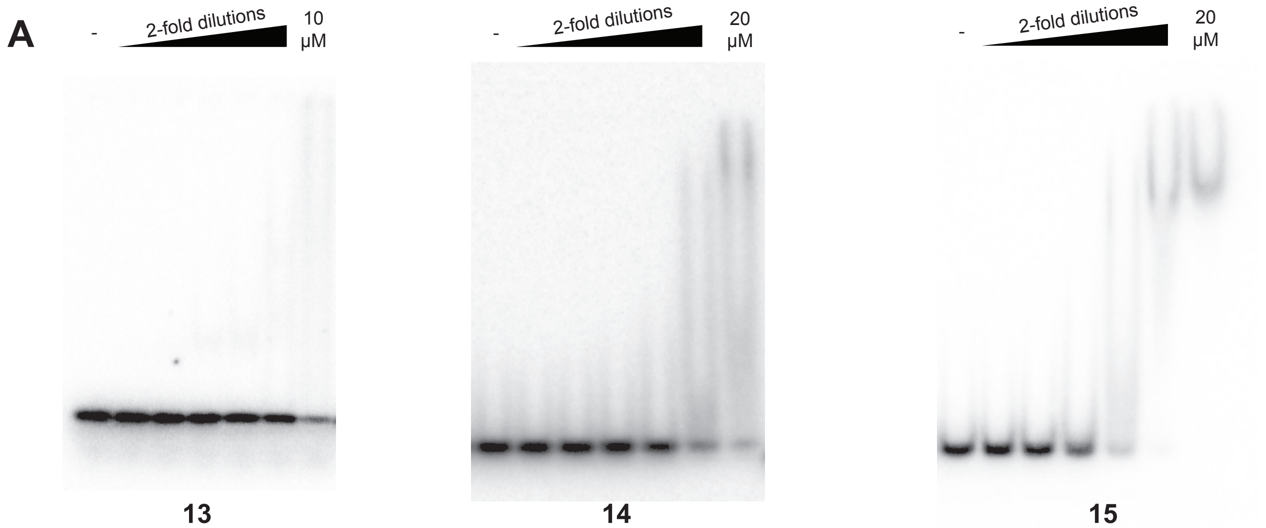
Figure S1


A
1
132
232
425
MH1
linker
MH2
B
70 kDa
55 kDa
40 kDa
35 kDa
25 kDa
15 kDa
10 kDa
MH1
FL
C
-
2-fold dilutions
20 μM
1a
-
2-fold dilutions
20 μM
1b
D
-
2-fold dilutions
10 μM
17

# Figure S2


A
1b
Fraction Bound
1.0
0.5
0.0
1
10
100
[Smad 3 MH1] (uM)
B
$K_D^{app}$ (µM)
10
1
0.1
0.01
1a (49-nt)
1b (49-nt)
9a (49-nt)
6a (50-nt)
6b (50-nt)
3c (73-nt)
9c (71-nt)
10 (160-nt)
11 (155-nt)
C
D
GGGUGUUUUGCCUACCAUCGUGACAUCUCCAUGGCUGU–AUCUUUCAUCUGACCAUCCAUAUCCAAUGUUCUCAG
10
11

## Figure S3



**13**

5′-r(GUAU**GUCUAGAC**UGAA)-3′
3′-d(CATA**CAGATCTG**ACTT)-5′

**14**

5′-r(GUAU**GUCUAGAC**UGAA)-3′
3′-r(CAUA**CAGAUCUG**ACUU)-5′

**15**

5′-r(UCAACAUCA**GUCUG**AUAAGCUA)-3′
3′-r(AGUUGUAGU**CAGAC**UAUUCGAU)-5′

**Supplementary Table 1.** Complete list of RNA sequences used in this study

| Construct number | Template pri-miRNA | Sequence |
|---|---|---|
| **1a** | hsa-miR-21 central stem | GGACGCUUAUCAGACUGAUGUUGUUCGCAACACCAGUCGAUGGGCGUCC |
| **1b** | hsa-miR-21 central stem ΔSBE | GGACGCUUAUAAAACUGAUGUUGUUCGCAACACCAGUUUAUGGGCGUCC |
| **2a** | hsa-miR-105 central stem | GGACGAUGCUCAGACUCCUGUGUUCGCACGGAUGUUUGAGCAUCGUCC |
| **3a** | hsa-miR-199a1 central stem | GGACAACCAGUGUUCAGACUACCUGUUUCGACAGUAGUCUGCACACUGGUUGUCC |
| **3b** | hsa-miR-199a1 minimal SBE | GGACAAUCAGACUACCUGUUUCGACAGGUAGUCUGCUUGUCC |
| **3c** | hsa-miR-199a1 stem loop | GGCCAACCCAGUGUUCAGACUACCUGUUCAGGAGGCUCUCAAUGUGUACAGUAGUCUGCACAUUGGUUAGGCC |
| **4a** | hsa-miR-215 central stem | GGACGAUUGACAGACAAUAUGCGUUCGCGCAGUUUGUCUGUCAUUCGUCC |
| **4b** | hsa-miR-215 natural loop | GGACGAUUGACAGACAAUAUAGCUGAGUUUGUCUGUCAUUCGUCC |
| **5a** | hsa-miR-421 central stem | GGACGUUAAAUGUUUGUUGAGCGUUCGCGCUCAACAGACAUUAAUCGUCC |
| **6a** | hsa-miR-509 central stem | GGACGUACUGCAGACAGUGGGCGUUCGCGCGUACGUCUGUGGGUACGUCC |
| **6b** | hsa-miR-509 central stem ΔSBE | GGACGUACUGCCAGAAGUGGGCGUUCGCGCGUACUCUGGUGGGUACGUCC |
| **7a** | hsa-miR-600 natural loop | GGACGCUCUUGUCUGUCAGGCAGUGGAGUUACUUACAGACAAGAGCGUCC |
| **8a** | hsa-miR-631 central stem | GGACGUGGCCCAGACCUCAGCUUCGGCUGAUGGACUGAGUCACGUCC |
| **9a** | cel-miR-84 central stem | GGACGUGAGGUAGUAUGUAAGCGUUCGCGCUUUCAACUAACUCGCGUCC |
| **9b** | cel-miR-84 short | GGUGAGGUAGUAUGUAAGCUUCGGCUUUCAACUAACUCGCC |
| **9c** | cel-miR-84 stem loop | GGCAUCUGAGGUAGUAUGUAAUAUUGUAGACUGUCUAUAAUGUCCACAAUGUUUCAACUAACUCGGCUGCC |
| **10** | hsa-miR-21 extended | GGGUGUUUUGCCUACCAUCGUGACAUCUCCAUGGCUGUACCACCUUGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACCAGUCGAUGGGCUGUCUGACAUUUUGGUAUCUUUCAUCUGACCAUCCAUAUCCAAUGUUCUCAG |
| **11** | VAI | GGGCACUCUUCCGUGGUCUGGUGGAUAAAUUCGCAAGGGUAUCAUGGCGGACGACCGGGGUUCGAACCCCGGAUCCGGCCGUCCGCCGUGAUCCAUGCGGUUACCGCCCGCGUGUCGAACCCAGGUGUGCGACGUCAGACAACGGGGGAGCGCUC |

| | | |
|---|---|---|
| **12** | U55 | UUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUU |
| **13** | RNA/DNA hybrid duplex with 2 SBEs | 5’ – r(GUAUGUCUAGACUGAA) – 3’<br>3’ – d(CATACAGATCTGACTT) – 5’ |
| **14** | dsRNA duplex with 2 SBEs | 5’ – d(GTATGTCTAGACTGAA) – 3’<br>3’ – d(CATACAGATCTGACTT) – 5’ |
| **15** | miR-21 RNA duplex | 5’ – r(UCAACAUCAGUCUGAUAAGCUA) – 3’<br>3’ – r(AGUUGUAGUCAGACUAUUCGAU) – 5’ |
| **16** | 30-bp hairpin | GGAUCGAUCGAUCGAUCGGCAUCGAUCGGCUUCGGCCGAUCGAUGCCGAUCGAUCGAUCGAUCC |
| **17** | Group II intron D4A | GGGGUCUCGUAUUGCAGAAAUGACAACAUCUGCCGUAACCAAUCGGGUAAAAGGUGGUCAAAUCAAGCGAGAC |
| **17a** | D4a truncation 1 | GGGUCUCGUAUUGCAGAAAUGACAACAUCUUUCGAGGUGGUCAAAUCAAGCGAGAC |
| **17b** | D4a truncation 2 | GGGUCUCGUAUUGCAGAAAUGACUUCGGUCAAAUCAAGAGAGAC |
| **17c** | D4a truncation 3 | GGGUCUCGUAUUGCAGAAAUAAAUCAAGCGAGAC |
| **17d** | D4a isolated terminal loop | GGGUCUCGUAUUGCAGAAAUGACACAUCUUGCCCGUAACCAAUCGGGUAAGGUGUGUCAAAUCAAUGCGAGAC |
| **17e** | D4a isolated apical loop | GGGUCUCGUAUUGCAGAUGACACAUCUGCCGUAACCAAUCGGGUAAAAGGUGUGUCAUCUGCAAUGCGAGAC |
| **18** | ai5-gamma D3 | GGGUAUAAAAAGCUAAUGCCAUAUUGUAAUGAUAUGGAUAAGAAUUAUUAUUCUAAAGAUGAAAAUCUGCUAACUUAUACUG |
| **19** | dsDNA with 1 SBE | 5’ – GTATGTCTCAGATGAA – 3’<br>3’ – CATACAGAGTCTACTT – 5’ |
| **20** | NF-KB aptamer | GAUACUUGAAACUGUAAGGUUGGCGUAUC |
| **21** | RRE | GGUCUGGGCGCAGCGCAAGCUGACGGUACAGGCC |

## Supplementary Figure 1

**SMAD3 RNA binding is conferred by the MH1 domain. A)** SMAD3 domain map with the nucleic acid-binding MH1 domain highlighted in green. **B)** SDS-PAGE gel of the purified protein constructs used for all experiments. **C)** Representative EMSAs of full-length SMAD3 binding to pri-miRNA constructs with and without the SBE. This binding recapitulates the affinity and the lack of sequence specificity conferred by SMAD3 MH1. **D)** Representative EMSA of full-length SMAD3 binding to **17**. This binding recapitulates the high affinity for complex RNA structures conferred by SMAD3 MH1.

## Supplementary Figure 2

**SMAD3 does not preferentially bind pri-miRNA stem loops. A)** Representative EMSAs and curve fits for replicate experiments performed on pri-miRNA constructs **B)** $K_D^{app}$ values calculated from replicate experiments on pri-miRNA constructs. pri-miRNA constructs containing putative SBEs are shown in blue and negative controls are shown in red. **C)** Secondary structure of the miRNA construct **10** containing the central stem loop and flanking single-stranded regions. RNA folding algorithms predict a stable central stem and many unstable conformations for the flanking regions. **D)** Secondary structure of the length matched control RNA construct **11.** The secondary structure is based on experimental probing data (51).

## Supplementary Figure 3

**SMAD3 does not bind dsRNA or RNA/DNA hybrid duplexes.** Representative EMSAs of poor SMAD3 binding to RNA-containing duplexes.

## Supplementary Table 1

**Complete list of RNA sequences used in this study**