

Supplementary Table S1: SOX HMG-box domain constructs

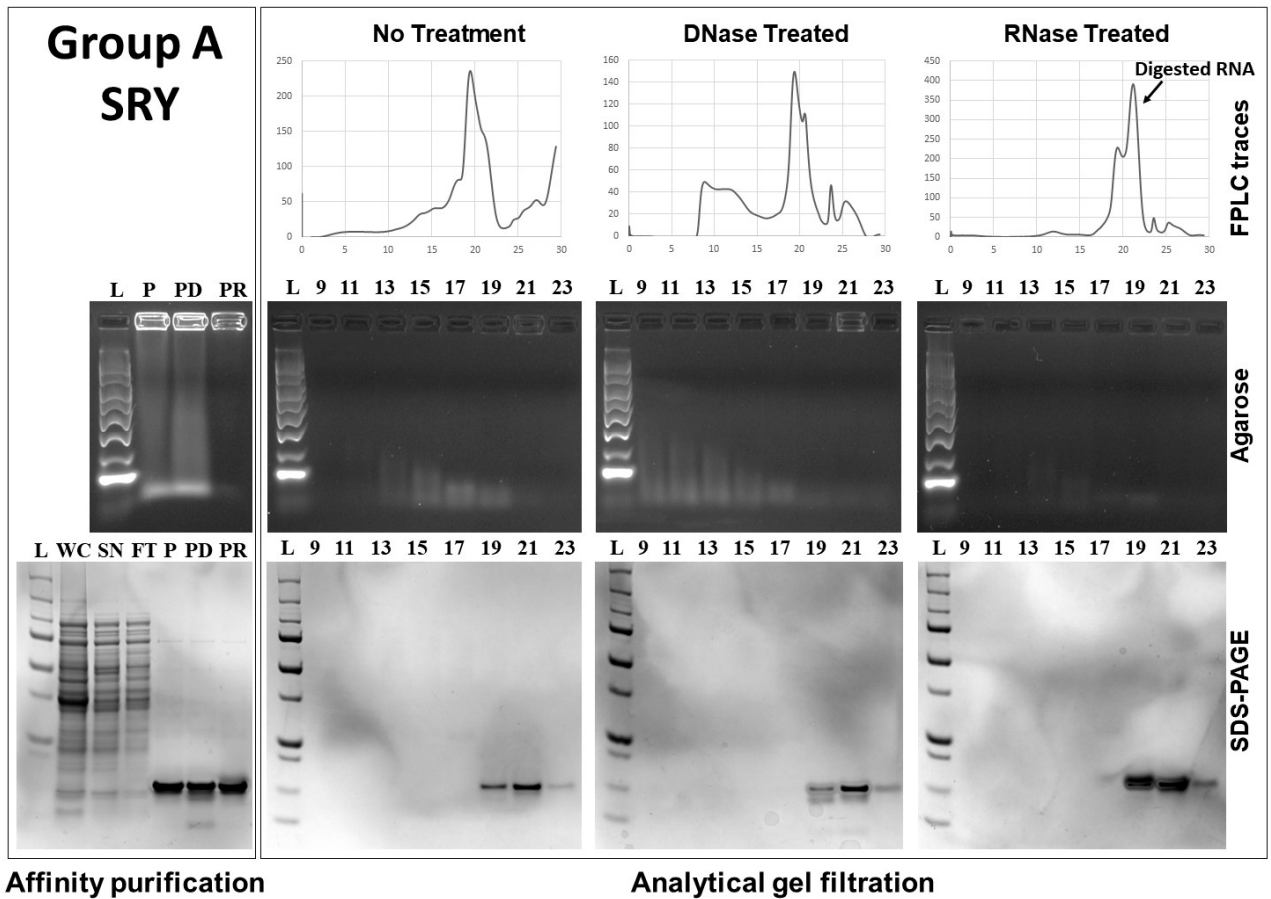
| Protein | Uniprot ID | Residues | Plasmid | Resistance | Affinity tag | Description |
|---------|------------|----------|------------|---------------|--------------|----------------|
| SRY | Q05066 | 58-140 | pMCSG21 | Spectinomycin | X6 His | HMG-box domain |
| SOX2 | P48431 | 39-117 | pMCSG21 | Spectinomycin | X6 His | HMG-box domain |
| SOX6 | P35712 | 613-700 | pMCSG21 | Spectinomycin | X6 His | HMG-box domain |
| SOX9 | P48436 | 103-183 | pMCSG21 | Spectinomycin | X6 His | HMG-box domain |
| SOX11 | P35716 | 47-135 | pET-30a(+) | Kanamycin | X6 His | HMG-box domain |
| SOX15 | O60248 | 47-135 | pET-30a(+) | Kanamycin | X6 His | HMG-box domain |
| SOX17 | Q9H6I2 | 66-145 | pMCSG21 | Spectinomycin | X6 His | HMG-box domain |
| SOX21 | Q9Y651 | 6-94 | pET-30a(+) | Kanamycin | X6 His | HMG-box domain |
| SOX30 | O94993 | 335-423 | pET-30a(+) | Kanamycin | X6 His | HMG-box domain |

Supplementary Table S2: Nucleic acid binding substrate sequences

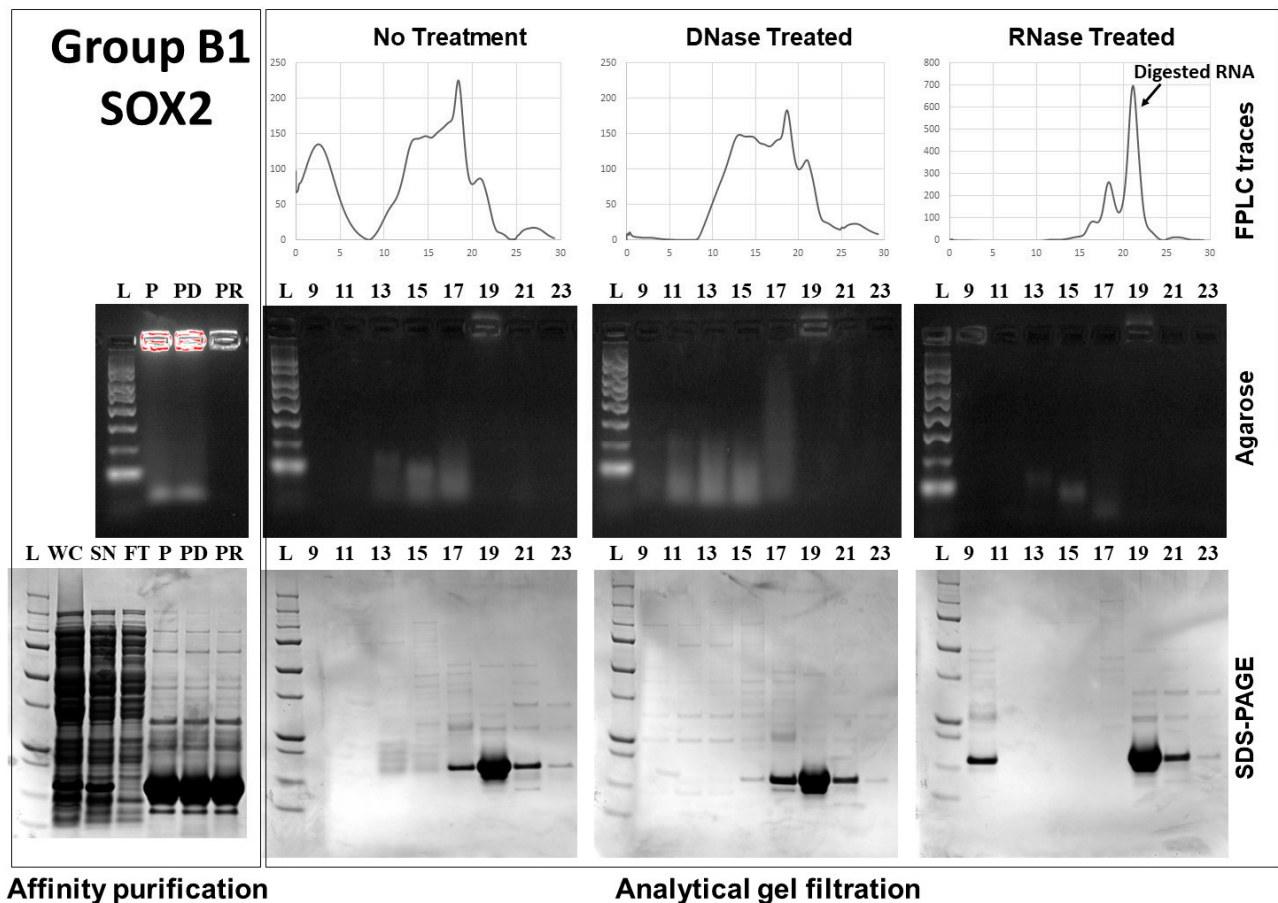
| Name | Sequence | Source/description |
|--------------|---|---|
| 60-mer ssDNA | 5'-GTTAGAGGTGCCCCACAGGCGGCGGTTAGTATTCCCCGCCGCCTGGGGCACCGGGGCAC-3' | Beak and feather disease virus loop construct |
| 22-mer ssDNA | 5'-CCCATCTTAGTATATTAGTTA-3' | Random primer |
| 12-mer ssDNA | 5'-ATCGATCGATCG-3' | Short repeating sequence containing all nucleotides |
| 40-mer RNA | 5'-GGUCGCCGUGGCCACUUCGAAAGGGGUGGAAAGGGCGACC-FAM-3' | ES2 lncRNA hairpin "Loop A" [39] |

Supplementary Table S3: SOX HMG-box domain mutant constructs

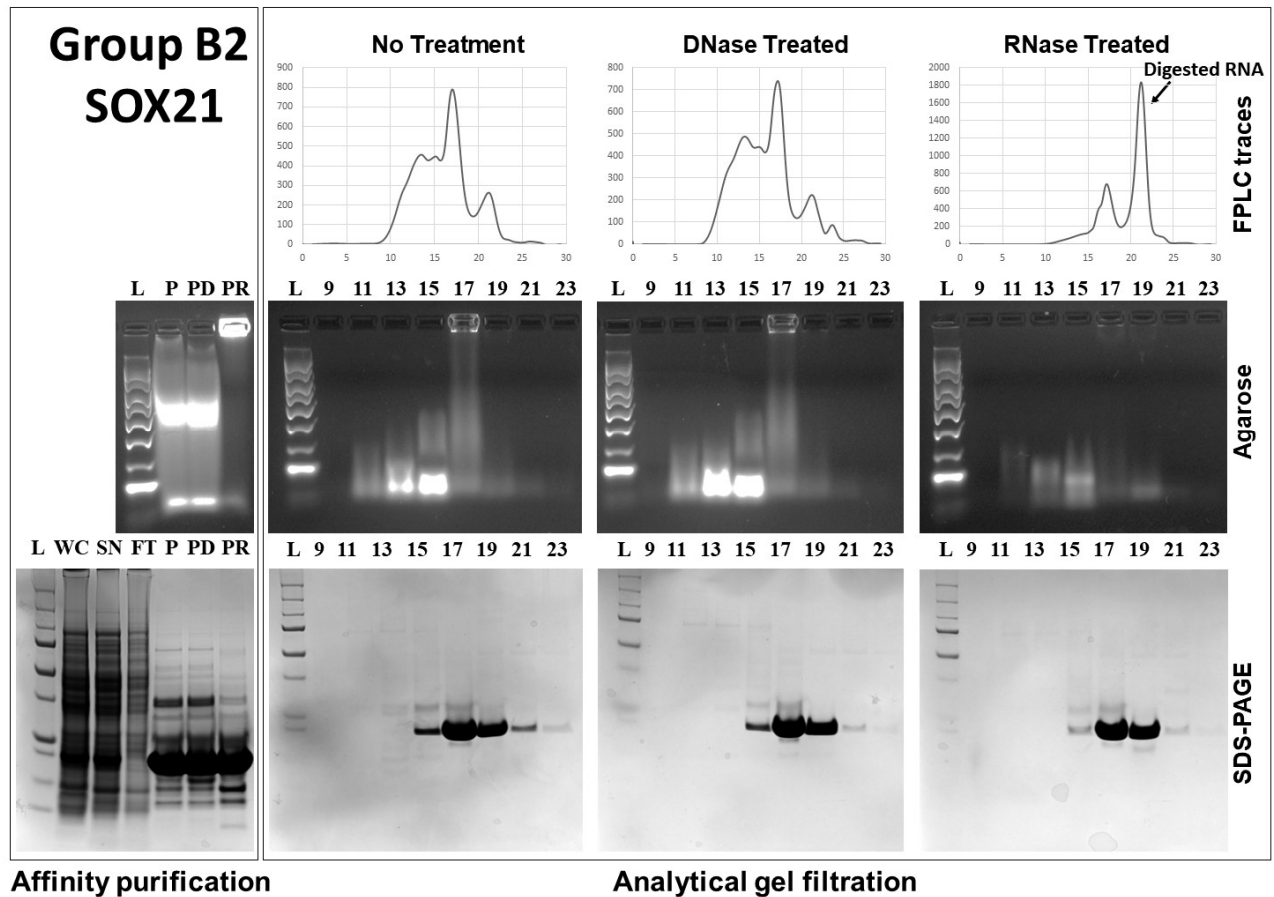
| Protein | Uniprot ID | Residues | Plasmid | Resistance | Affinity tag | Description |
|-------------------|------------|----------|------------|---------------|--------------|----------------------------|
| SOX17 WT | Q9H6I2 | 66-145 | pMCSG21 | Spectinomycin | X6 His | HMG-box domain |
| SOX17 Δ N | Q9H6I2 | 74-145 | pET-30a(+) | Kanamycin | X6 His | Δ N HMG-box domain |
| SOX17 Δ C | Q9H6I2 | 66-137 | pET-30a(+) | Kanamycin | X6 His | Δ C HMG-box domain |
| SOX17 Δ CN | Q9H6I2 | 74-137 | pET-30a(+) | Kanamycin | X6 His | Δ CN HMG-box domain |
| SRY Δ C | Q05066 | 58-129 | pET-30a(+) | Kanamycin | X6 His | Δ C HMG-box domain |
| SOX2 Δ C | P48431 | 39-110 | pET-30a(+) | Kanamycin | X6 His | Δ C HMG-box domain |
| SOX11 Δ C | P35716 | 47-118 | pET-30a(+) | Kanamycin | X6 His | Δ C HMG-box domain |



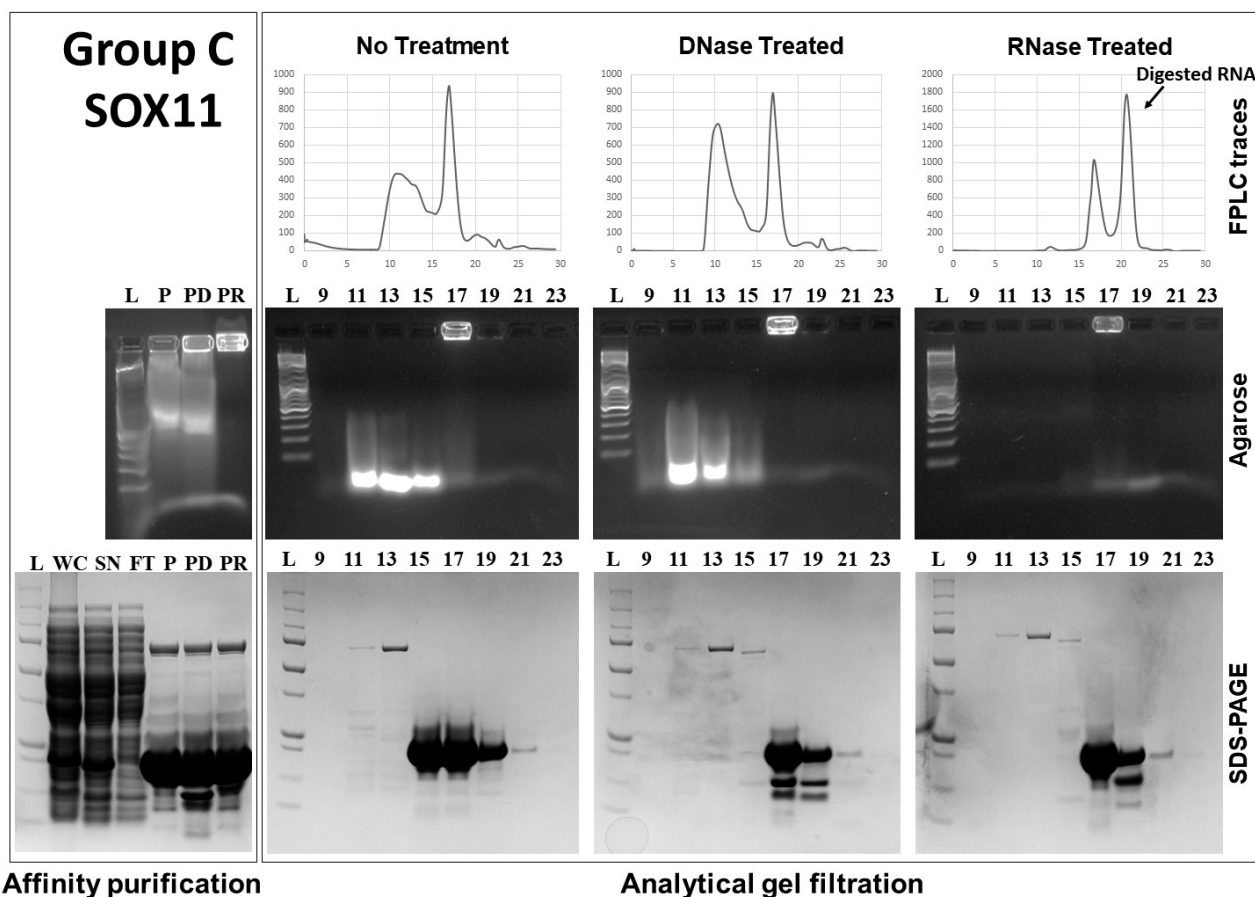
Supplementary Figure S1: The SRY HMG-box domain co-purifies with RNA during affinity and size exclusion chromatography. Left panel shows different stages during affinity chromatography. The FPLC trace, agarose gel, and SDS-PAGE analysis of the samples are indicated on the right. During purification, samples were taken for whole cell (WC), supernatant (SN), flowthrough (FT), purified eluant (P), purified eluant treated with DNase (PD), and purified eluant treated with RNase (PR). The right panel shows the subsequent analytical gel filtration of no treatment, DNase treated, and RNase treated SRY. In no treatment and DNase treated samples, SRY HMG-box elutes around 19 to 21 mL, and RNA can be detected in fractions 13 to 19. In RNase treated samples, the RNA-related peak shifts dramatically to fraction 22.



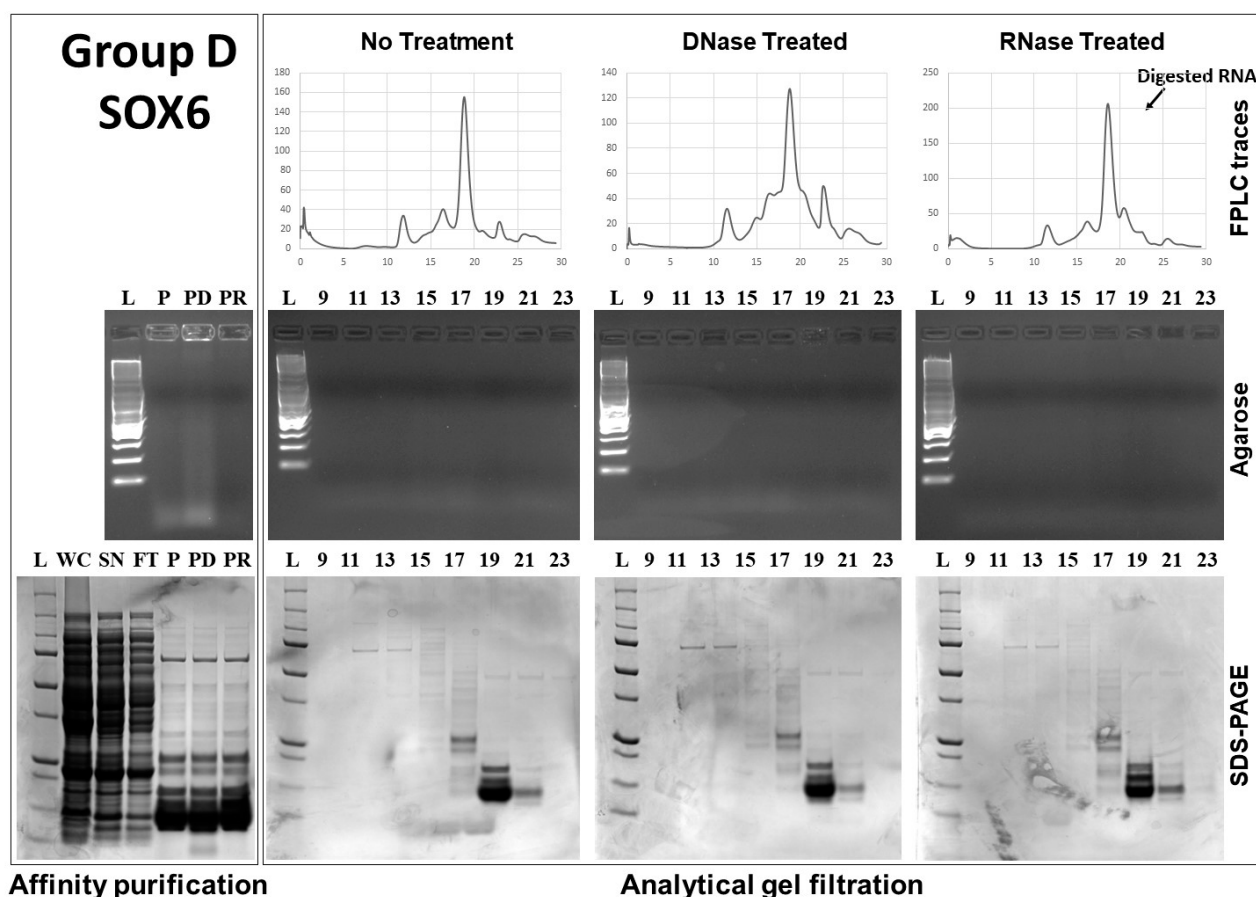
Supplementary Figure S2: The SOX2 HMG-box domain co-purifies with RNA during affinity and size exclusion chromatography. Left panel shows different stages during affinity chromatography. The FPLC trace, agarose gel, and SDS-PAGE analysis of the samples are indicated on the right. During purification, samples were taken for whole cell (WC), supernatant (SN), flowthrough (FT), purified eluant (P), purified eluant treated with DNase (PD), and purified eluant treated with RNase (PR). The right panel shows the subsequent analytical gel filtration of no treatment, DNase treated, and RNase treated SOX2. In no treatment and DNase treated samples, SOX2 HMG-box elutes around 19 to 21 mL, and RNA can be detected in fractions 10 to 19. In RNase treated samples, the RNA-related peak shifts to fraction 22.



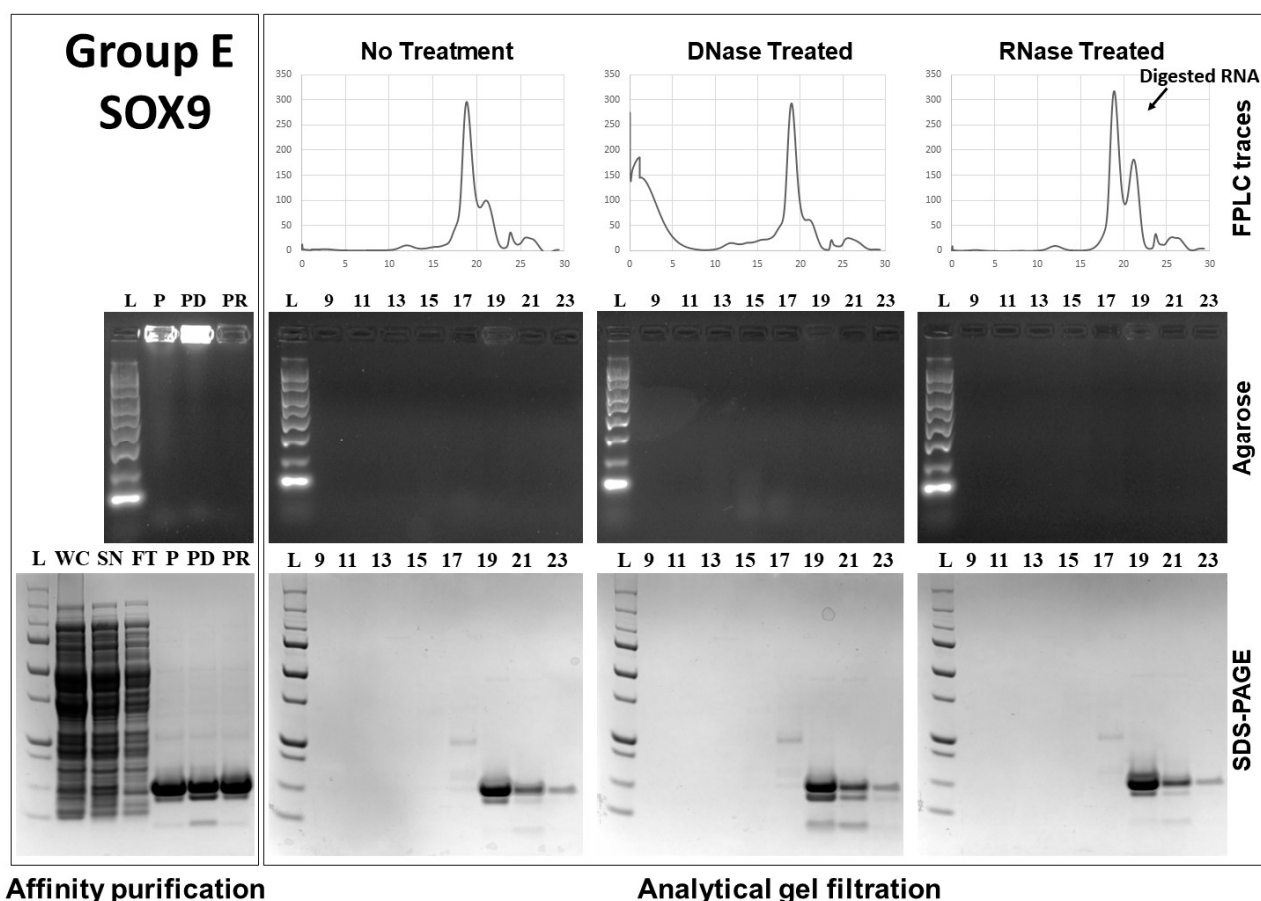
Supplementary Figure S3: The SOX21 HMG-box domain co-purifies with RNA during affinity and size exclusion chromatography. Left panel shows different stages during affinity chromatography. The FPLC trace, agarose gel, and SDS-PAGE analysis of the samples are indicated on the right. During purification, samples were taken for whole cell (WC), supernatant (SN), flowthrough (FT), purified eluant (P), purified eluant treated with DNase (PD), and purified eluant treated with RNase (PR). The right panel shows the subsequent analytical gel filtration of no treatment, DNase treated, and RNase treated SOX21. In no treatment and DNase treated samples, SOX21 HMG-box elutes around 17 to 19 mL, and RNA can be detected in fractions 10 to 17. In RNase treated samples, the RNA-related peak shifts to fraction 22.



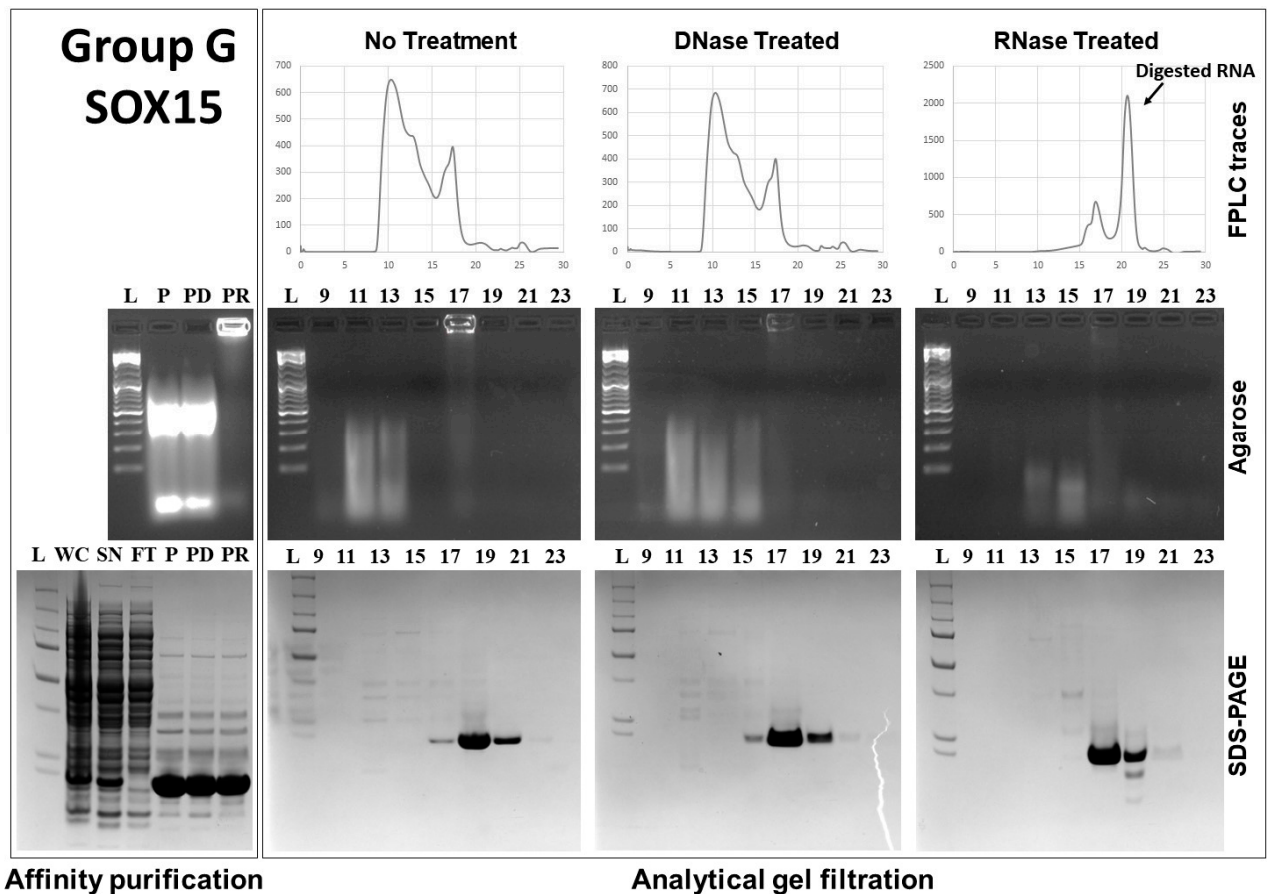
Supplementary Figure S4: The SOX11 HMG-box domain co-purifies with RNA during affinity and size exclusion chromatography. Left panel shows different stages during affinity chromatography. The FPLC trace, agarose gel, and SDS-PAGE analysis of the samples are indicated on the right. During purification, samples were taken for whole cell (WC), supernatant (SN), flowthrough (FT), purified eluant (P), purified eluant treated with DNase (PD), and purified eluant treated with RNase (PR). The right panel shows the subsequent analytical gel filtration of no treatment, DNase treated, and RNase treated SOX11. In no treatment and DNase treated samples, SOX11 HMG-box elutes around 17 to 19 mL, and RNA can be detected in fractions 9 to 16. In RNase treated samples, the RNA-related peak shifts to fraction 21.



Supplementary Figure S5: The SOX6 HMG-box domain co-purifies with RNA during affinity and size exclusion chromatography. Left panel shows different stages during affinity chromatography. The FPLC trace, agarose gel, and SDS-PAGE analysis of the samples are indicated on the right. During purification, samples were taken for whole cell (WC), supernatant (SN), flowthrough (FT), purified eluant (P), purified eluant treated with DNase (PD), and purified eluant treated with RNase (PR). The right panel shows the subsequent analytical gel filtration of no treatment, DNase treated, and RNase treated SOX6. While co-purified RNA can be seen in affinity purification samples, the RNA bands are very faint in the gel filtration samples.



Supplementary Figure S6: The SOX9 HMG-box domain co-purifies with RNA during affinity and size exclusion chromatography. Left panel shows different stages during affinity chromatography. The FPLC trace, agarose gel, and SDS-PAGE analysis of the samples are indicated on the right. During purification, samples were taken for whole cell (WC), supernatant (SN), flowthrough (FT), purified eluant (P), purified eluant treated with DNase (PD), and purified eluant treated with RNase (PR). The right panel shows the subsequent analytical gel filtration of no treatment, DNase treated, and RNase treated SOX9. While co-purified RNA can be seen in affinity purification samples, the RNA bands are very faint in the gel filtration samples.



Supplementary Figure S7: The SOX15 HMG-box domain co-purifies with RNA during affinity and size exclusion chromatography. Left panel shows different stages during affinity chromatography. The FPLC trace, agarose gel, and SDS-PAGE analysis of the samples are indicated on the right. During purification, samples were taken for whole cell (WC), supernatant (SN), flowthrough (FT), purified eluant (P), purified eluant treated with DNase (PD), and purified eluant treated with RNase (PR). The right panel shows the subsequent analytical gel filtration of no treatment, DNase treated, and RNase treated SOX15. In no treatment and DNase treated samples, SOX15 HMG-box elutes around 17 to 19 mL, and RNA can be detected in fractions 9 to 16. In RNase treated samples, the RNA-related peak shifts to fraction 22.

References:

39. Holmes, Z.E.; Hamilton, D.J.; Hwang, T.; Parsonnet, N.V.; Rinn, J.L.; Wuttke, D.S.; Batey, R.T. The Sox2 transcription factor binds RNA. *Nat. Commun.* **2020**, *11*, 1-12.