Fig S1. SOX9 is a regulator of CRC cell growth and proliferation.
A, western blots showing no change in β-catenin levels upon SOX9 RNAi and no change in SOX9 levels upon inhibition of Wnt signalling by dnTCF overexpression in DLD-1 cells. Cells were transfected with either the empty pcDNA3.1 vector (EV), or indicated plasmids and harvested for western blotting 48h later. *B,* correlation between CTNNB1 expression and essentiality in 53 Cancer Cell Line Encyclopedia (CCLE) CRC lines. *C,* correlation between SOX9 expression and essentiality in 53 CCLE CRC lines. *D,* correlation between essentiality scores of SOX9 and CTNNB1 (β-catenin) in 56 CCLE CRC lines. *E,* correlation between SOX17 gene effect and CTNNB1 gene effect in 56 CCEL CRC lines. In (B-E), essentiality (gene effect) is measured using Chronos scores. Lower scores indicate a stronger growth reduction upon CRISPR KO. Each data point represents a single cell line. Cell lines passing threshold Chronos score values of -0.2 and expression values of 2 TPM are coloured orange, while others are coloured grey. All data were sourced from the 22Q1 public DepMap release. *F,* top 10 genes with essentiality scores most correlated with SOX9 across all CCLE cancer cell line datasets. β-catenin shows the 7th best correlation.

A c-Myc-335 enhancer sequence: (Chr8:127400835–127401814)

```
CGCTCCATAGAGCCTGCAGAGGGCACTAGACTGGGAATTAGAAAACCTGATTTCCCTTCCAGCTCCACCTCTGACCAATTGCCTGACCC
TGGTCAAATTGCTTAACCTCTTCCTATCTCAGCTCCCTATCCATAAAACAGAGGGACGAATAAACTCTCCTCCTACCACTAAGAGGTGT
AGCCAGAGTTAATACCCTCATCGTCCTTTGAGCTCAGCAGATGAAAGGCACTGAGAAAAGTACAAAGAATTTTTATGTGCTATTGACTT
TATTTTATTTTATGTGGGGGAGGGAGCCGGGCAACTGCTTTCTCTGAATCAAAGGGCAGGAACCCAGCAAGTTTCTCAG
GATTGGGGCTTAGACTGGGCTGTGTATACAGACAGTGCCAGCCAACCCCACAGTTCAGTTTCCTTTAACCTGGTGCTCCAGGCAATAA
CTGTGCAACTCTGCAATTTAACAATGTGTTCTTTGTCCCACAACTGTTCTCGTTTCTCAACTGCCCAGGTAATATGTTTGGGCCTGTAG
GAAGAGTCAAATAGTTAATAAGGGAAGGGTTTGGCATGCCCTACGTAAGTTCTACCAGCAAGTCCCAACAAGAAGGCATTCTGTGTCTC
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AATGCATTGGTGAGCTGTGTTTTGTTTATGGTTTTATGCCATCTCATTTTCCCATGGATATTGTTTTCTAAAGAGCCCTTAAGTTTAC
GTCAGCTTTTAAAGCTACCAGCAGCACCATTTCAGTTCATATTAAGCCCTTAATATGGTATGAATAGGAGAGCTATTAGACTAAAGAGC
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T
```

Legend: **TCF sites** CDX sites CAG sites SOX sites

B

Probe	Sequence
WT	CAATTTAACAATGTGTTCTTTGTCCCACAA
5' mut	CAATTTAA**ACC**TGTGTTCTTTGTCCCACAA
3' mut	CAATTTAACAATGTGTTCT**GGT**TCCCACAA
5'+3' mut	CAATTTAA**ACC**TGTGTTCT**GGT**TCCCACAA

C

Mouse homologue of c-Myc-335 (chr15:61490400-61491920)

```
CATGGCAGAGGTCTTGAAAGCATGTATAAACAAACACACACACAAAGATCACAAACAAAATTCTAGACCCTATTCTGTAGCTAACTACA
TGTTTGACTTTGGAAATTGCAAACATTTTCCCTATCTTCATTGTTCTCTACTGACATCAACAAAATTACTTCAAATTTCTTTGTCAGTT
CATTTAGCCAGGACTCCAGCAAATGTATCAATCCATTTATCCTGCAGGGGGAGCTAGACCGGGTTCTGAAAACCCCGGTTTCTCTTCCC
AGCCCCATACTGGGTGATTGTGTGACCCTGGGCAAACTGTTTAACCTCCCCCATATCTTTAGGTATCGTCCATAAAACAGAGGGATGAAT
AAACTCTCCTCCTACCACTAAGGGGTGTAGCCAGGTTAATACCCTCACCATCCTTTGAGCTCCGCAGATGAAAGGCACTGAGAAAAGT
ACAAAGTATTTTTATGTGCTATTGACTTTATTTTATTTTATGTGGGGGAGGAAGTTTGCCCCAGCTGGAAAGTTGCTTTCTCTGAATCA
AAGGGCAGGAACCCAGCAAGTTTCTCAGGATCAGGACCTTAGGCTGGGCTGTGTGTACAGAGTGCCAACCAACCCCACAGTTCAGTTTC
CTTTAACCTGGTGCTCCAGGCAATAACTGTGCAACTCTGCAATTTAACAATGTGTTCGTTGTCCCACAACTGTTCTTGTTTCGCAATTG
CCCAGGTAATATGTTTGGGCCTGTAGGAAGAGTCAAATAGTTAATGAGGGAAGGGCTTGGCATGCCCTACATAGGTTCTTCCAGCAAGT
CTCAGCACGAATACATTCTCTTGCTCCTCTCTCCTCTGCTTCTTGACCTACCCTCCAAATCTTGCCAGTGTCACAACCAGGAATGAGTC
CTCTTTCCCGGCAGGCTGTCATACCAGTTTGGCTGTTATCAATGCCTGGGTGAGGTGTATTTTGGTTATAGTTTTACACCATTTATTTT
CCCAGGGATATTATGATTTCTAAGGGCCCTTGGGTTTGCATTGGCTTTTCACACTACCAGCAACATCATTTCGATGCTTATTAAGCCTT
TAGTATGGTATGAATAGAAGAGCTCTTGGACTAAAGTACCATAATCATCCCTAAGAAAAGCGTCCATCCCCAACCTTGACGTGGTCCCT
GAACCTCTAAGAAGTGTCATCCCTCTGAGGTTTGTCTGATGAACGCTTTCTCTGGGAGATCCCAAGAAGTCACTGTATCAGACACAGCT
TAGAAGGGCACTTTCTAGCCATTACAAGTCCCCTAGTTGAGCATCTCCATTTTGTCTACGTGATGAGCCTTAATTAAATTTGCCAGGGT
TTGATGCTTTACTCCCAGATGTGGGGCTGACAGAGATTGCTGACATAAATAAAGTGTGCTCCAAGTGTTTGCTCAGCTCTGGGCTCAGC
CAGGACAGATGCAGCTTCTTGAATGTCATTCCCAAGATATCCTGGAGGTATTTAGCTTCCCTCCCTCTACTCTGGAAAAGATACCATGG
GCAACATC
```

Rat homologue of c-Myc-335 (chr7:93157287-93158520)

```
TGACCCTGGACAAACTGTCTAACCTCCCCATATCTTTAGGTCTCATCCATAAAACAGAGGGATGAACAAACTCTCCTCCTACCAGTAAG
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TGACTTTATTTTATTTTATGTGGGGGAGGGAGGTTTGCCCCAGCTGGAAAGTTGCTTTCTCTGAATCAAAGGGCAGGAACCCAGCAAGTT
TCTCAGGATCAGGACCTTAGGCTGGGCTGTGTGTACAGAATGCCAACCAACCCCAGAGTTCAGTTTCCTTTAACCTGGTGCTCCAGGCA
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GTAGGAAGAGTCAAATAGTTAATGAGGGAAGGGCTTGGCATGCCCTACATAGGTTCTTCCAGCAAGTCTCAGCAAGAACACATTCTCTT
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TAAGAGACCTTGGGTTTGTATTGGCTTTTCAAGCTACCAGCAATGTCATTTCAATGTTTATTAAGCCCTTAATATGGTATGAATAGGAG
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CTCTCTGAGGTGTGTCTGATGAGAGCTTTCTCTGGGAGATGCCCAAGAAGTCAGTGCATTTGACACAACTCAGAAGGGCACTTTCTAGCC
ATTACAAGTCCCCTAGTCGAGCATCTCCATTTCATCTACGTGATGAGCCTTGATTAAATTTGCCAGGGTTTGATTCTTTACTCCCAGAT
GGGGGGCTGACAGAGATTGCTGACATAAATAAAGTGTGCCCCGAGTGTTTGCTCAGCTCTGGGCTCAGCCAAGACAGATGTGGCTTCTT
GAATGTCATTCCCAAGATATCCTGGAGGTATTTAGCTTCCCTCCCTCTATTCTGGAAAAGATACCATGGGCAACATC
```

Fig S2. The c-Myc-335 WRE is bound and regulated by SOX9.
A, sequence of the c-Myc-335 WRE with regulatory motifs annotated. Positions shown are based on the GRCh38.p13 primary assembly of the *Homo sapiens* genome. *B,* sequences (5'-3') of the SOX site probes used for EMSA. The SOX sites in the WT and corresponding locations in the mutant probes have been underlined. Mutated bases are emboldened. *C,* sequences of the mouse and rat homologues of the c-Myc-335 WRE with TCF and SOX sites annotated. Positions are based on the GRCm39 (mouse) and mRatBN7.2 (rat) genome assemblies. TCF and SOX sites conserved in at least 2 organisms are underlined.

Human Myc+8 enhancer sequence:
Chr8:127742878-127744069

```
GGTCTTCAGCTCCCTGTACTTTGGGATTTTAATCTACCACCACCCATAAATCAATAAATAATTACTTTCTTTGACTCTGACTCCTAGAA
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GCGAATTTCAAAGGGAGATGCAATCCACAGAAGTATAGTAGTTCAAAGGGTTACAAAAGCAAGGGCCTCTTAAACAGCTCAGTCTTTGC
CCCTTTGTGGCCTAGGGCTGGAGTGCAGCTCTGGGGTGACTCACTTGGGAATCGGGAAGGTGTTAGTCTGAATCACTAAGTCCAGGCAA
GCCCTCAGAATAGGAGAGAGTGTTCCTAGCAAGGAAAACAACTCTCCATTCCAAATAATCAGGAAAGAACTTTAGGGATGTGGAGCTTG
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GCCATTGTGCAACCTATTTTGTAGATAATAATCAAGAATCGGACGTGAAGGGGAGGAGTTTGCAACTTGGTCAGGAATGTATAAGAAGG
AATAAGCTAATTCTGACTATGCCCTTTATCCATGCACTATCCAGGAATTAATGACTCTCCCAGAGGATTCCTGGAATGATTTTGTTGA
GGGATGGAATGTATAAAGAGGAAGGAAGTGTTATTTTATGCTGCCATTTGGAAGCAACAAAGGAGATCAACAGTATGAAAACAATCAAT
CAAATTTGAAAATGAACAAAGTTTTCACAATCCCAGCCTAATACTTAGAGAGCTCACAGCTTGGATGCATAAGTAAAGAGTTCTCTGCT
GGTCTTTAAGACAAACTCTCACACAAAACTTGGGAAAAAGGACAAAATGTTGCATTAGGGGGTTTTCTGTGGTTTGTTTGCAATAACT
ATAATTGGCTCAATCAATAATTATTTTTTAGTATACACACTAAGGGCCCCTGTAGCATTTTTTCCCATCGATAAATAATCCTTAGTCTA
GAAAATGCCGAGGGATGTTCTCCACCCTTGTCT
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Mouse
Chr15: 61863502-61864717

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GGTCTCTAGCATCCTGGAGTGTCTTTGGGACTTTAATTCACCATCATTCATAAACCAATAAGTAATTACTATCTTTGACACCCCCCCCC
CATGATCTATTGAAGCGTTTAACATTTAGTTTTTGATCTTATTTTTGGCTCTTTTAGACTCACCCTCAGCTTCAAACTGTTACACCCTC
TATCCTACATTCAAAAGAAAACACAAACCCCAGTAGTAGTTTGAAATACTTTGAGTAGTTCAAAGGATTAGCAAAAGGGGGGAGCAGGG
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GTTAGGTTGAATCACTCAGTCCAGGCAAGCCCAAAGAATAGAGGAAAGCATTCCTCATTAGGAAAACAACTCCTGTTCCAAATGATCAG
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GTGGTTTGTTTACAATAAGAGATGAGTCACAATAATGATTTTTTTTTTTTTTTTTAGCATGACTGCTAAGGAACCCTGAAGCATTTCTT
CCCAACAAAAACTAATCTTTAGGTTATAACATGCTGGAAGATGAGCTTCAAGCCTGTCTA
```

Rat
Chr7:93599915-93601095

```
GGTCTCTAACATCCTGGACTGTCTTTGGGATTTCACCATCATTCATAAACCAATAAGTAATTACTGTCTTTGATACACACACCCCCTAA
GATAATCTCCTGAACCATTTAACACTTAGATTTTGATCTTTTTGGCTCCTTTAGACCCACACTCAGCTTCAAACTATCCCACCCTCTAT
CCTAAATTCAAAGGAAAAGACAAACCCCAAACACTGAGTAATTCAAAGGGTAACAAAAGGGGGCGGGGGGGCGGGGGTGGTGTGCTCGT
AATAAGTTCCCCCCACTTTGCCCTTTGGTGAGCCTAGGCTGGAGTGGTGCCCTAGGGTGACTCACTTGAGACCCGGGAAGGTGTTAGATT
GAATCACTCAGTCCAGGCAAGCCCAAGGACTAGGAGAGCATTCCTCATTAGGAAAACAACTTGTTCCAAATGATCAGGAAACAAGTTTA
AGGATTCAGAGTTTGGCTGTGGGAATGGAAGGGAAGTATCACAGTCCCCTGTCAGGGCACTTCTCAGCTTTACCAAGCCAAGGAATGGT
CTGAAAACAGGACATCAGCCAGCTTCCTTCCAGAAAGTCAGTCTGAGCTTGAACATAACACAGGAAGGCTCTTGGTAGCCAATCTGGA
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GAATATACAAGCATTGCCTGGTCTCTGTCGTTCACCCATGACGGTGGAATTGAAAGCTGCCCCAAAGGATTCCTGGGATGGTGTTCTC
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GTCACAATGATGACTTGTTAGTATCGACTGCTTAAGGGACCCTGAAGCATTTCTTCCCAACAAAAAGTAATCATTAGGTTATAACAAGC
TGGAAGACAGGCTTCAAGACTGTTT
```

Legend: TCF sites SOX sites

Fig S3. Sequences of the human Myc+8 WRE and homologues in mice and rats.
Positions shown are based on the GRCh38.p13 (human), GRCm39 (mouse) and mRatBN7.2 (rat) genome assemblies. Putative TCF and SOX9 binding sites are annotated and sites conserved in at least 2 species are underlined.

Human Myc-29 enhancer sequence:
Chr8:127706818-127707901

```
AAAAGGTTGGGCTGGTTTCAAATGTTCTCAGAACCAAAGCAACACACGCCTTTCAGAGCGCTGGAGAAAATGCTGTTGAACAAGCTCTC
ATGGCTCAACAGTGGAGATCTGAACAATGTATCTGACTATTAACATAATTTGAGAAGAGAGGCTCCCTCTTAACCTGTATTTTTGGCTC
ACTCAAAAAAGTGCTCCTGTCAGCCTTCAATTTCTCTACCTGGGTCTCTAGTCTGAGGACAGCATCCTTTTGGAAGACTTCAGCAATT
TCTGTGGAGCCATGTTTAAATTGGGCAAGTGGAAAACTAACTCCCTTTAAGAAAAGTGGCATTGTTTTGCAGTCAGGTAATTCCACGTG
GGCTCACTGGTTACTTTCCATGGGAATAGTCTTTGAGGAAGAAAGGGCCTTTGTTGAGATTTGTTTTGTTTTGAGAACAGTCAATTGGT
TCTTTCTACTCTTCCTTCTAGGGCGCCAATCTTTCCAAGGATAACCACAAAGTGCAAGCAAAAATAATGAAAAAGGAAAGAAAGAAAGA
AAATCTGTATGCAAACCCAGGTTGTCATTATCTGGCGTTGTTTTCGGCCATTCTTGTCTTCTGACTCCTACCTGGCACACAGATCACTC
CTGCCCACTCCTGCCCATTACTCTCTATCTTGTACACGCCTTGCTGGTTACATATGGAGGACCATGGAAGAAAAGCATTGCTGTAAATTT
CCTCCTTCTCAAGGAATCCTGCTCCCTGAGTTTCTGCTTAAGTGACTTCTCACCTTCAAACTCACTGAGGTTATTCTTGTTTGGCTTCA
GATAAGTGGCTGAGATTTTAATTGTTCTCAATTGGTCTGTTTATCAGGGTAACTGTTTCAGTTTTCTTCAAGACAAATGATTAGATCCT
TCGGTCTTTATCAGTATATTAAGTATGTTTTTCAGGTGTGGACTTAACTTTGCCCTCCACTCCCAGCAACCGTTTCCTTTCATAACTTG
GGTGTAGTTTAAACACACACACACACACACACACACACACACACACTTGCTCAGAGTTATTTTGGAAAGTGTATTCTTTACCAGTGA
CACCAACCTCAAAAGA
```

Mouse
Chr15:61831213-61832304

```
CAGATACTAGGCCCGCTTAAAAAAAAAATCACAGAACCAAAGCAACTACCCATTGCTGAAAGAGCCAAGAGAAGATATCAGACAAGGAC
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TTCACTCAAAAGGAAAACATTCCTTTTAGCTTTAAATTTCTCTGCCTTGGTCTCTCATCTGAAGACCACATCCTCTTTGAGGACTTGGA
CAGTTTCTGTGGAGCCATGTTTAAATTGAGTGAGTGAAAAGCTAACTGTCTTTTATAAAAGTAGCATTGTTTTGCAGTCAGGTAATTTC
GGGCCGGTTCAGCTTTTACTTTCCATGGGAATAGTCTTTGAGGAAAAAAGGGGCCTTTGTTGAATTTTGTTTTGTTTTGAAACTAGTCA
GTTAGTTCTTTTTACCTTTCTTTTGGGGGGCGCCAGTCTTTCTAAAAACAACCACAAAATTCTGGAAGAAGTAATGAAAAGAGGAAGAA
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ACTGAGATCCAGAGTGCTCTCAGTAGGTCTGTTTATCATCACAAACTGCTTCAGCTTTCCTCAGGACACAGGGTAGGTAGTTTATCTGT
GTATTGGGGTGTGTTCTTCAGGTGTGGAGTTAACTTTGTCTTCCGCTTCCGGCTGCAGTTCCTCTTCCTTAATTAGATATACATTATTT
GAGCATGCGCACATGCTCGCTCTCGCACATACACAGAGCGAGAAACAGAGAGACAGAAACACAAAGGCAGAGAGATAGAGGCAGAGAGA
CAGAGACAAAGAGAGCCCAAAAGA
```

Rat
Chr7: 93553494- 93554583

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CTGATACTAGGCCTGTTTGAAAAAATTTTTTTCACAGAACCAAAGCAGCTACCCATTGCTGGAAGAGTAGAGAGAAGATATCAGACAAG
AAGTCTGCTCAATGGCCAATATCTGAACAGGGCATCTCACTGTGTGCATTGGCATAGTCCAAGTAGGGATAGAGAGCTACTGATTTTTT
TTTTGGTTCCCTCTAAAGGAAAACATTCCTTTTAGCTTTAAATTTCTCTGCCTTGGTCTCTAGTCTGAAGACCACATCCTCTTTGAGGA
TCTGAACAGTTTCTATGGAGCCATGTTTAAATTGAGTGAGTGAACAGCTAACTCCCTTTTATAAAAGTAGCATTGTTTTTGCAGCCAGG
TAATTTCAGGCGGGTTCAGCTTTTACTTTCCATGGGAATAGTCTTTGAGGAAGAAAGGGCCTTTGTTGGATTTTGTTTTGTTTTGAGA
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TGGAATCCTGCCCAAGATTTCTTATTTCATACCTCTCCAGTGTCTGCGTGCAGAGGAGCACTGAAAGAGCTATGGTAAAACTGTCCTCT
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GTAGGTAGGTTCTCCAATGTTTATCTGTGTGATGGAGTGTGTTCTTCAGGTGTGGAGTTAACTTTGCCCTGAGCTTCCGGCAGCCGTTT
TCCTTCCTTAATTAGGGTGTACATTGTTTAAGCATGAGCAGAGAGACAAAGAGACAGAGAGACAGAGGCAGAGAGAGAGACAGAGAGAC
AGAGACAATGAAAACTGAGAGA
```

Legend: `TCF sites` `SOX sites`

Fig S4. Sequences of the human Myc-29 WRE and homologues in mice and rats.
Positions shown are based on the GRCh38.p13 (human), GRCm39 (mouse) and mRatBN7.2 (rat) genome assemblies. Putative TCF and SOX9 binding sites are annotated and sites conserved in at least 2 species are underlined.

Human Myc-521 enhancer sequence:
Chr8:127214627-127216810

ACAGTGTTAGACAGTACAAAGGGCATTTTTACCTCCAACACATCTTTCATGAAGAGTTTTTTTATCTAGGTTTCATGGAGCCCTACGGTAAATAAATTTGCCCAAGTT
CTAACAAGATTCTGGAATCTCATATGTGGGTATGTTCATGTTGGGGTGTGTGTGTGTGTGTATGTGGGGTGTATGGGTGTCTGTGTTTTTCCTCTGAAAAGCACC
ATAGTCTCTGCCACATTTTTAAAAAGGTGCATAACCCTTTAAACAGGAACCATTTACTTTAAATGGAATCAGAAATCTCACTATAAAAAAGCAATAATATTAATTTC
TACTGTGTGTGTCTCTTGCCTTTTATTCCATGTTCACAATTCATCTTATTTTCATTTAAGACAACCAACTGGGCTAGTTAGAGCCGAGATTTGAGCCCAGTTTTTCT
CACTGACTCTAAAACCTATCCATGCTCCTAAACCTCCTCACCATTGGAGGGCATTGCTGTTTACCCTTTCAGTTTCAGCTGTACTATCAAAAGCAGCAAATCAAGGG
CAGGGACCACAGCTTGACCTTAAACTATTCAACTGCACTTAATTACCCATTGTAGTGGAAGCACATAAATACAGGATGTCCCCTCACTCTGCCATTCTGCTTTGCAT
CATTATCAAAAAGACAAAGGTAATTCATGTCTAATTACTCACTCACCGCAAGGTCAGAATATTTATTTTTATCATACTTGTGATTTTTAGGATTAGTGATATCAGTA
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TGGGATTAATTTACTCAGAGATCTGGAGTAGCAATAACTTGTGTCTTTCCAAAACCCCCGCAGTCAATTAATGAGATCAATTTAAAGTGGCAGTATTGAAAGGGAAA
ACCCAAACACTTAGATATAGCAGTATGCAACTGACCTATTCCTTTTTCCATTCAGATTCAGTCAGTCAAATATATCTTATTGACTTTGACTACTGTCATCATAACTG
TGTTAGCTAATTGTCTGAAGTTTTCATTCATTCTCTGTCTTTGCAGATGTCACTGCTATTCAATCTGTATTTCCTTGCTCTCGCTGTCTA*TCTGCACTAACA
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GGGGGAGATGTGAACAAAGTCTTAGAGTCTAGTTGATAGTAAGAATATGAAATGAATATGACTCACTGATGTGATATAGTCTTCAAAAAACTAAATTTGGTGTTTGA
ATGCGTAGAAATATATTAGGTTTTACAACATAAAATTTCCATTTTGGTAGATGGAAATGTTTGAATAAAGGCAATTTCATAAGGTTCATAGTCTGTAGAGTGAAATG
GAAGTGGAAAAACCTCACTTTGCTCTGTTCTGCTCTACTTAGCTCATACTTGAAGTAATCATTCAGTTCTTGATGCCATACAGACAAAGAAGGTAGTGGACAAAAAG
AGACACACATATTCAGTAGATAGCAACCAAGCTGGGAGGGGAAC

Mouse
Chr15: 61317631- 61319835

AGCCATTTATTTATTTGTTTGTTTATTTATTTACACCAAAACACCTTGAATCAATAAATTGTGATCTAGATCTCATGCAGCCCTAAAAATCTGTTTGCTTAAGTTCT
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CTTACTATTTATGACATGCCCATAATTCATCTCACTTGAGCTCAAGACAGGCAGCTAAACTAGGCATAGCATAGCCCAGAGTAACAACTAGCACTGTGCTCCTGGCC
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GCTATATAGTTATTGTCACAGGTATGCTGAGTCACAGAGCAAATAAGGAAGTTTATGAGTTGACAGAGAAAAGGCTACAAAGAGGTTAAGATAATGTTCCCTGTTAAT
CACAGGTGTCTTGTGAAATAGATGCATTGTTTATTTATATTGTCCATTTATTGAGCAGTCGCTGCTTGGGAAACCGAAACTAGCCCAAATAGTGAGGATTAAATAAA
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GCTCCCCTGTCAATATGATAGGCCTAAGTTAGGGGCAAAGTGAATACTGTTTTAAGAATCTTTAGTTGGGAGATGAAATAAGAATACTTGATGCAGCATAGTAGCCA
AAAGACTAAATTTAGGGATTGAATGCATAATATTACATCAGGTTTTAGAATGTGAAACCTCTACTGTCAAAAACCAAAAGTCAGCAAGGAGCTAGGGAAATGGTTCT
GAGGATAATGTGCTTGGTATACAAGCATGAGGACGTGAGCTTGGGTATCCTGCACTTACAGGAAGCTGGTCACAGAAGCATGCCTCTCTAAACTGGCTATGGGGCTT
GTGAGAGATGGAGACAAGAGAATTCTAAGGGCTTACTGGCCAACCAGTCTAAGCAAAATAGATTC

Rat
Chr7:92991347-92993522

GTTTGTTTGTTTGTTTGTTTGTTCGTTTGTTCACACACATGAAAATACTTTGAATCAAGAGATTGTGATCTAGGCCACACGGGCCCTGAAAATCTGTTTGCTCAAG
TTCTAAGAACCTTCTGGAATTTAATAAAGACAGCATTCTATGTATGTGTGTGTTTAGGGCTGTATTCCTCTTCTGACAAAAGACCCTGTAGCATTTATTGTTAAGAAGT
TCATCACTGTACACAAGGGTTAAGAGCCATTGGCTTGAAATGGGGTTAGGAACATAAATTATTTATAGAGAAGCAATAATATTAACGTATATATTACAAGTCTCTTA
CTATTTGTGACTTGCCCATAATTCACCTCATTTGAGCTCAAGCTAAGCAGCTACACTAGTCAGAGCACAGCCCAGTGTAGTAACTAACACTGTATTCCTGGCCCTTA
CTATTAGAGGGCATTGATGTTTACCACCTTGACTTCAACTGCACAAAGCAAGGGCAAGGACGCACAGTATGACCTTAAACTATTCAACTCCACTTAATTACCCATTGT
AGTAGAAGCACATAAATACAGGATGTCCCTTTGCTCCCCCATTCTGCTTTGCATCATTATCAGAAAGACAAATGTAATTCATGTCCAATTACTCACTCACCGCAAGG
TCAGAATATTTATTTTTATCGTACTGGTGATTTTTAGGATTAGGGTATCAGTATTTATTCATGGCTAACCCAAACCAAACATCCTGTTGATATCTATGAATTGCTA
TATAGTTATTGTCACAGGTATGCTGAGTCACAGAGCAAATAAGGAAGTTTATGAGTTGACAGGGAAAAGGCTGCAAGAGGGAAAGATAAGGTCCCCTGGGTAATCACT
GGTGTCTTGCGAAATAGAGGCATTGTTTACATTGTTCATCTATTGAGGACTGGGTGCTCGAGAAACTGGGAAACTGGGAGGGCTTAAACAAAATTCAAT
CATTACTTTTAAAATGTGATGCTGGGACATTTTGATTTAATTTATGAACTGCTCATTTTAGCAATCAGTTTTTTTTCCTCTGCCTCGTAGAGAAGAGCTTTGTCTA
GTTCTCAGATGTAGACAGAAGTAAGAGAGGTAGAAAACTAAAGGAAACTCTTCTGGGTAGAGTTCATTTGCTCAGAGATCTGGAGTAGAATTAACTGACGTCCTACC
GGGACCTTGGCAGTCAGTTCCTGAGATGAGAGTGACAGTCTTAAAAAGGAGAAGCCAAGCCCTCAACTGTATCAATGTGTATGGTTCATTTCTTTCCTTATTCACAT
TCAGCCAAGCAAATACACTTTATCTTCCGTCATTGTCATCAAGACAGAACTAATTGCCTGAAGATTTCACTAATTCTGGGTCTTTACAGATGGCCTTGTTATAAGTT
GTGCCTGAGATTCAAATCATTTCCTTTCTCTAGATACCTGTTATAACAGTTAACAGGTGTGTGAGTGTGTATGTGTGTGTCTGTGTGTCTGTGTGTCTGTGTGTCTGTG
TCTGTGTGTCTGTGTGTGGCTGTGTGTTTCTTAAGATGTGTCCTTATTCCTCCTCATATTTTTGTACATACTAGTAGTCTGGAGAACAGGCTTCTAATTGTGGTTCC
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GGGGTAATGTGAACAAAGTTTTAAGACTCTTTAGTGGAGAGAAGATGAAATTATAATCCTTGATGCAATGTAGTTTCCGAAGGACTAAAGTTGGGGTTTGAATGCAT
AATATTACATCAGTTTATAGAAATGTGAAGCCTTTATTCTCATGAATCAAACGTCATTGTGGAGCTAGCAAAATAGTTCTGAGGATAATGTACTTGATACACAAGGA
TGAGGACATGAGATTGGATACCCTGCACCCACAGGAAGCTGGTCATAGAAGCATGCATCTCTAAACTGGCTAAGGGGCTTGGGAGAGATGGAGAGAAGAGGCTTCAA
GGGCTTACTGGCCAGCCAGTCTAAGCAAAACAGGTC

Legend: TCF sites   SOX sites

Fig S5. Sequences of the human Myc-521 WRE and homologues in mice and rats.
Positions shown are based on the GRCh38.p13 (human), GRCm39 (mouse) and mRatBN7.2 (rat) genome assemblies. Putative TCF and SOX9 binding sites are annotated and sites conserved in at least 2 species are underlined.

Fig S6. ChIP-seq tracks of SOX9-bound Wnt targets in CRC cells.
A, ChIP-seq tracks showing the binding of SOX9 to the Myc+8, Myc-29, and Myc-521 WREs. *B,* SOX9 ChIP-seq tracks of top 5 Wnt target genes in HT29 cells. SOX9 ChIP-seq dataset generated by Shi et al 2015.

A

Defa5 promoter sequence:
Chr8:705735

```
GATGCATTC........................ACTCCTTGAACTAAATCCGAATTTTTATTTTAATCTGATAAACTTGGCCTA
CTATTTTACTG..................TCCCCTATAGCCTGATAAGGTCATTGACCTCTCCATACTGGCACCAGCGGGA
GACTACTCA................CAAAAGCCTCCTACATGAGGTTAGTAATATCCCTGAATCCTGCAATGAATT
AACTCTCTAC..............CCCAGGTCTGCCCCCAGAGAGTCATCCAGAGAGTACCAGGGACCATCTTCA
GAAAACAAG.............CCCCCAAACTTCTTGAATGAAAGCGCTGTTGTTTTTCTTTTTTGAATATATA
AAAGTAAAT...........TGGGAAACAGAACAGGATAGTAATACCCTTATCATCATTAACACCTTGGAT
CAAGAAGAGG.........ACAGACTCACGCTTTGATGAAAGCTGGGAGAAAGAGGAGCATCAAAGGGAT
CTTGAGAAC..........TTCCCCTCCCAATCACATGCCCACCTCCTCTCACTGCAGCTTTCTGTCTCAG
GTCTTCTCC...........TAAATCCAGGCTGACTCCTCACTCCCCACATATCCACTCCTGCTCTCCCTC
CTGCAGGTGACCCCAGCCAGAGGACCATCGCCATCCT
```

Legend: **TCF sites** (red) CDX sites (yellow) CAG sites (cyan) **SOX sites** (purple) Exon (blue)

B

Defa6 promoter sequence:
Chr8:6926680–6926025

```
TCACATGGGTTTCTTGAAATAAATCTTTTGGCTTTAGTTTTACTAACTCTTTACC..GT..CC..TGAGT
TCTTTTCCCTTATAGGCTGATAAGGTCATTATCTTCTCCACACTGTGCCCCACAAGAGCTATTCACCCCA
TAATCTCAAAGCCTCCTTCATGAGGGCAGAAATGCCCCCTGAATCCTGCAATGAATTAACTCTCTACTCTA
GCGGGATCCAGCTCTGGCCTCAAGGTCTAGACCTCCAGAGAGTGGCCAGCCCCACCTTCAGAAAATAAGAG
GCATTTGATTCCTGAAATTATTCATTGAAAGCACTGTTCTTTTCTTTTTTGAATATTAACAAGTAAATATT
CCAGCAGATG................................CATCACTATCAGCTGGGACCAGAACAGACAC
TCAATAAACA.....................AAGGAGCATCAAAGGGACATGGAGGGCAAGG
GTAGCTCTTCTGCTCCCCAATCACATGCACTCCCCGTCTCACCGCAACATCTGTCCCTGAGCCTTCTCCCA
GCAGACCTATAAATCCAGGCTGGCTCCTCACTCCCCACACATCTGCTCCTGCTCTCTCTCCTCCAGCGACC
CTAGCCAGtAGAACCCT
```

C

Defa5-luc, LS174T cells

Relative activity

100 — WT (~100), ~99
TCF ****
5' SOX ****
3' SOX ****
CDX ****
CAG ****

D

Defa6-luc, LS174T cells

Relative activity

WT (~100)
TCF ****
3' SOX ****
5' SOX ****
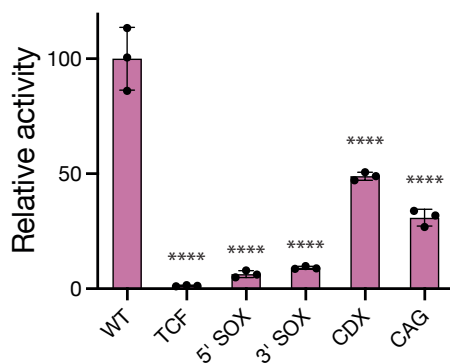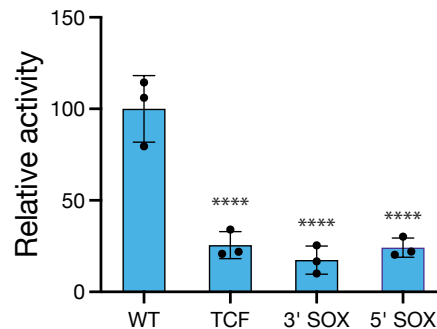
Fig S7. Synergistic upregulation of Paneth cell defensins by Wnt signalling and SOX9.
A, sequence of the *Defa5* WRE with regulatory motifs annotated. The region between nucleotides -617 to +60 with respect to the transcription start site (TSS) were cloned to make the reporter. The ATG at +40 was changed to AGT in the reporter. *B,* sequence of the *Defa6* WRE with regulatory motifs annotated. Positions shown are based on the GRCh38.p13 primary assembly of the *Homo sapiens* genome. The region between nucleotides -604 to +52 with respect to the TSS were cloned into the promoter. ATG at +41 was changed to AGT in the reporter. *C,* luciferase assay data showing a reduction in Defa5-luc activity in LS174T cells upon the mutation of TCF, SOX, CDX, or CAG sites. *D,* luciferase assay showing a reduction in Defa6-luc activity in LS174T cells upon mutation of TCF or SOX sites. Data in (C) and (D) are shown as mean ± s.d. of biological triplicates. ns P > 0.05, *P < 0.05 ,** P < 0.01, *** P < 0.001. **** P < 0.0001. P-values were calculated using one-way ANOVA followed by Dunnett's test.

A

TOPflash

GGTACCTGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCCCCGG**GAGATCAAAGG**GGG
T**AAGATCAAAGG**GGGT**AAGATCAAAGG**GGTCGG**GAGATCAAAGG**GGGT**AAGATCAAAGG**GGGT**AAGAT
CAAAGG**GGTCGACCTCGAGGATATCAAGATCTGGCCTCGGCGGCCAAGCTT

B

SOXflash

AAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTGGCCTAACTGGCCGGTACCTGA
GCTCGCTAGCCTCGAGATTT**AACAATGT**GT**TCTTTGTC**CCACATTT**AACAATGT**GT**TCTTTGTC**CCAC
ATTT**AACAATGT**GT**TCTTTGTC**CCACAGATCTGGCCTCGGCGGCCAAGCTT

C

TOP/SOX

ACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTG
GCCTAACTGGCCGGTACCTGAGCTCGCTAGCCTCGAGTCGG**AACAATGT**G**TTCTTTGT**GTAC**GAGATC
AAAGG**GGGT**AACAATGT**G**TTCTTTGT**GTAC**AAGATCAAAGG**GGTCAAGCTT

Legend: `TCF sites` `SOX sites`

Fig S8. Sequence determinants of synergistic activation by Wnt signalling and SOX9.
Sequences of the (A) TOPflash, (B) SOXflash, and (C) TOP/SOX synthetic reporters with TCF and SOX sites annotated.

A
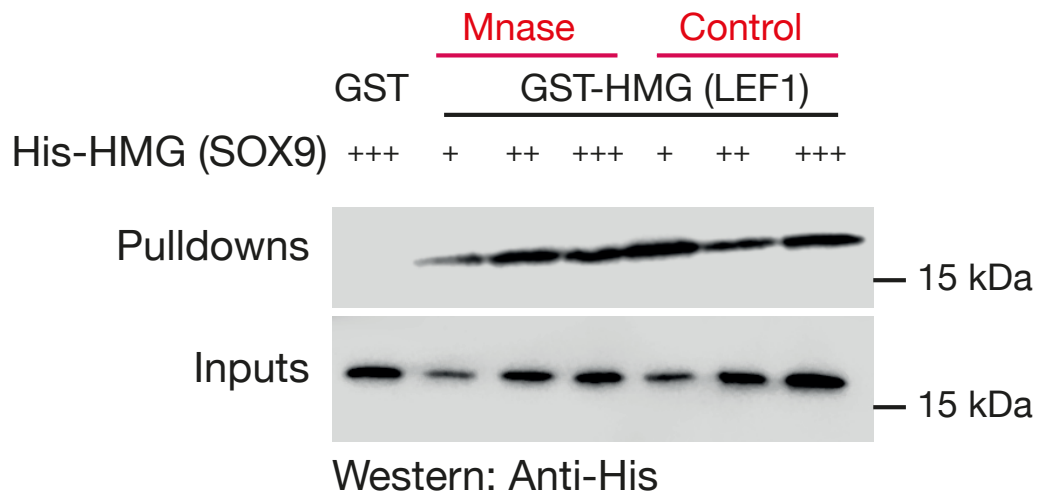
B

Uniprot ID, species, protein	Sequence	Positions
Q04887 Mouse SOX9	KLA**D**QYPHLHNAELSKTLGKLWRLLNESEKRPFVEEAERLRVQHKK**D**HP**D**Y	122–172
P48436 Human SOX9	KLA**D**QYPHLHNAELSKTLGKLWRLLNESEKRPFVEEAERLRVQHKK**D**HP**D**Y	122–172
A0A337SCT0 Cat SOX9	KLA**D**QYPHLHNAELSKTLGKLWRLLNESEKRPFVEEAERLRVQHKK**D**HP**D**Y	122–172
P48434 Chick SOX9	KLA**D**QYPHLHNAELSKTLGKLWRLLNESEKRPFVEEAERLRVQHKK**D**HP**D**Y	122–172
Q6F2E7 X. tropicalis SOX9	KLA**D**QYPHLHNAELSKTLGKLWRLLNEGEKRPFVEEAERLRIQHKK**D**HP**D**Y	122–172
Q9DFH1 D. rerio SOX9b	KLA**D**QYPHLHNAELSKTLGKLWRLLNEGEKRPFVEEAERLRVQHKK**D**HP**D**Y	108–158
Q9DFH2 D. rerio SOX9a	KLA**D**QYPHLHNAELSKTLGKLWRLLNEVEKRPFVEEAERLRVQHKK**D**HP**D**Y	124–174
Q9VA17 D. melanogaster SOX100B	VMSKQYPHLQNSELSKSLGKLWKNLKDSDKKPFMEFAEKLRMTHKQ**E**HP**D**Y	94–144
Q23045 C. elegans egl-13	KILKAYPDMHNSNISKILGSRWKGMSNSEKQPYYEEQSRLSKLHMEQHP**D**Y	346–396

Fig S9. Highly conserved residues mediate a DNA-independent TCF/SOX9 interaction.
A, western blot analysis of a GST pulldown assay showing that the interaction of the HMG domain of LEF1 (GST-tagged) with the HMG domain of SOX9 (His tagged) is unaffected by treatment with Mnase, suggesting that it does not require DNA. *B,* sequence comparison of SOX9 proteins across species showing conservation of residues mediating the TCF/SOX9 interaction.

| Protein	HMG sequence	Positions
SOX9	RRKLA<mark>D</mark>QYPHLHNAELSKTLGKLWRLLNESEKRPFVEEAERLRVQHKK<mark>D</mark>HP<mark>D</mark>YK	120–173
SOX8	RRKLA<mark>D</mark>QYPHLHNAELSKTLGKLWRLLSESEKRPFVEEAERLRVQHKK<mark>D</mark>HP<mark>D</mark>YK	117–170
SOX10	RRKLA<mark>D</mark>QYPHLHNAELSKTLGKLWRLLNESDKRPFIEEAERLRMQHKK<mark>D</mark>HP<mark>D</mark>YK	119–172
SOX7	RKRLAVQNPDLHNAELSKMLGKSWKALTLSQKRPYVDEAERLRLQHMQ<mark>D</mark>YPNYK	60–113
SOX17	RKRLAQQNPDLHNAELSKMLGKSWKALTLAEKRPFVEEAERLRVQHMQ<mark>D</mark>HPNYK	83–136
SOX18	RKRLAQQNPDLHNAVLSKMLGKAWKELNAAEKRPFVEEAERLRVQHLR<mark>D</mark>HPNYK	100–153
SOX4	RRKIM<mark>E</mark>QSPDMHNAEISKRLGKRWKLLKDSDKIPFIREAERLRLKHMA<mark>D</mark>YP<mark>D</mark>YK	74–127
SOX11	RRKIM<mark>E</mark>QSPDMHNAEISKRLGKRWKMLKDSEKIPFIREAERLRLKHMA<mark>D</mark>YP<mark>D</mark>YK	64–117
SOX12	RRKIM<mark>D</mark>QWPDMHNAEISKRLGRRWQLLQDSEKIPFVREAERLRLKHMA<mark>D</mark>YP<mark>D</mark>YK	55–108
SOX5	RRKILQAFPDMHNSNISKILGSRWKAMTNLEKQPYYEEQARLSKQHLEKYP<mark>D</mark>YK	571–624
SOX6	RRKILQAFPDMHNSNISKILGSRWKSMSNQEKQPYYEEQARLSKIHLEKYPNYK	636–689
SOX13	RRKILQAFPDMHNSSISKILGSRWKSMTNQEKQPYYEEQARLSRQHLEKYP<mark>D</mark>YK	439–492
SOX1	RRKMAQENPKMHNSEISKRLGAEWKVMSEAEKRPFIDEAKRLRALHMK<mark>E</mark>HP<mark>D</mark>YK	66–119
SOX2	RRKMAQENPKMHNSEISKRLGAEWKLLSETEKRPFIDEAKRLRALHMK<mark>E</mark>HP<mark>D</mark>YK	56–109
SOX3	RRKMALENPKMHNSEISKRLGADWKLLTDAEKRPFIDEAKRLRAVHMK<mark>E</mark>YP<mark>D</mark>YK	154–207
SOX14	RRKMAQENPKMHNSEISKRLGAEWKLLSEAEKRPYIDEAKRLRAQHMK<mark>E</mark>HP<mark>D</mark>YK	23–76
SOX21	RRKMAQENPKMHNSEISKRLGAEWKLLTESEKRPFIDEAKRLRAMHMK<mark>E</mark>HP<mark>D</mark>YK	23–76
SOX15	RRQMAQQNPKMHNSEISKRLGAQWKLLDEDEKRPFVEEAKRLRARHLR<mark>D</mark>YP<mark>D</mark>YK	64–117
SRY	RRKMALENPRMRNSEISKQLGYQWKMLTEAEKWPFFQEAQKLQAMHREKYPNYK	75–128
SOX30	RPALAKANPAANNAEISVQLGLEWNKLSEEQKKPYYDEAQKIKEKHRE<mark>E</mark>FPGWV	352–405

Fig S10. Conservation of TCF-interacting residues across human SOX9 proteins.
When mutated in SOX9, D125,168,171 prevent it from binding to the HMG domain of TCFs. Amino acid residues in other human SOX family members that have acidic (D/E) residues at the corresponding positions are highlighted.
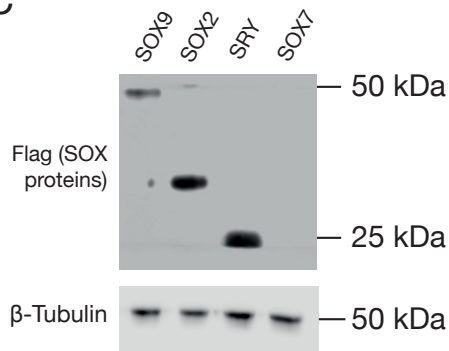
A

	D125	D168	D171
SOX9	D	D	D
SOX2	Q	E	D
SRY	L	K	N
SOX7	V	D	N

B

TOPflash, HEK293T cells

C

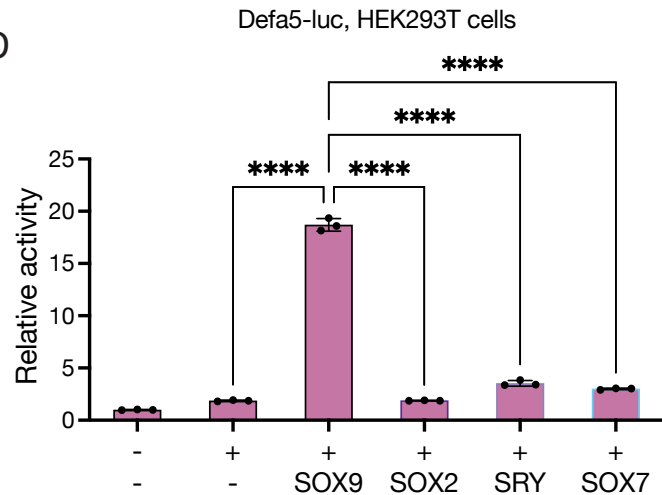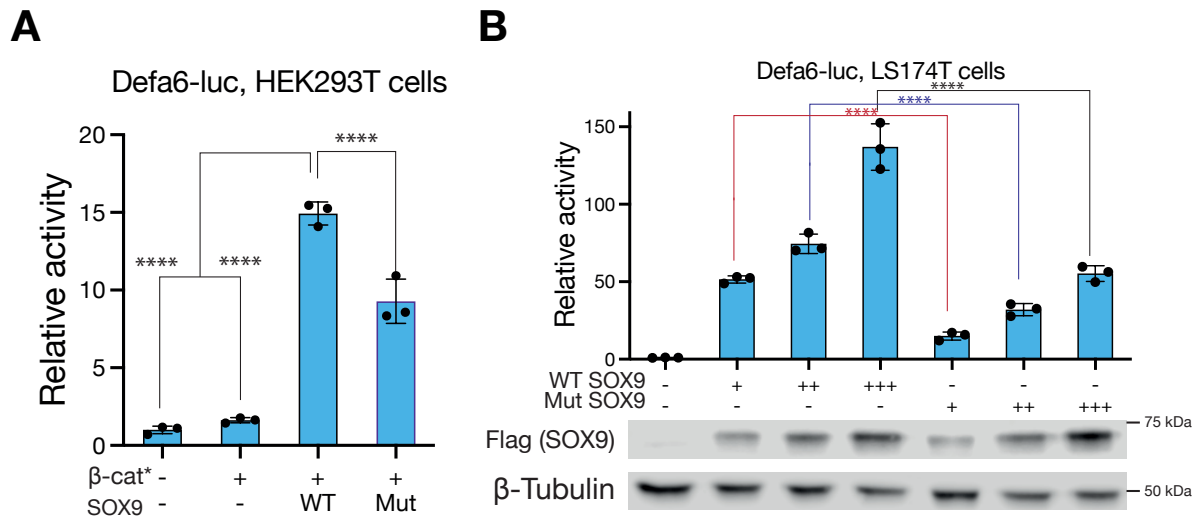Flag (SOX proteins)

β-Tubulin

D

Defa5-luc, HEK293T cells
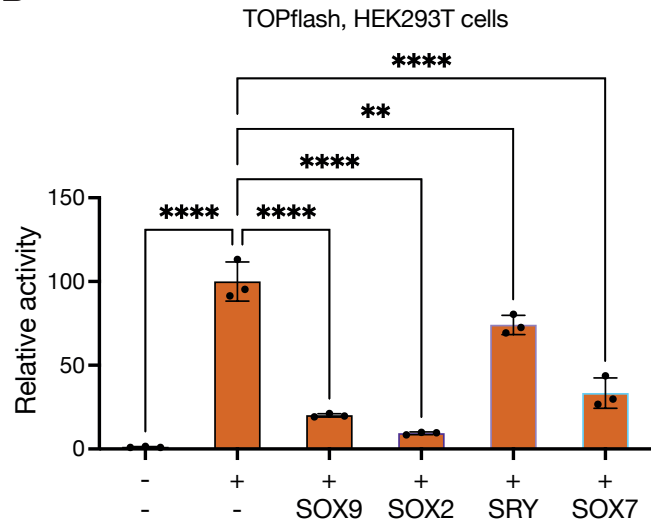
Fig S11. SOX9 is unique in its ability to activate the *Defa5* promoter.
A, Table showing the identity of amino acid residues in human SOX2, SRY, and SOX7 proteins at positions corresponding to human SOX9 residues D125, D168, and D171. *B,* Luciferase assay showing repression of TOPflash activity by SOX9, SOX2, SRY, and SOX7. *C,* western blots against the Flag tag (to detect Flag-tagged SOX proteins) and β-Tubulin in HEK293T cells transfected for the experiment in (A). Although SOX7 potently suppressed TOPflash activity, it could not be detected on a western. *D,* Luciferase assay showing only SOX9 cooperates with β-catenin to activate the *Defa5-luc* reporter in HEK293T cells. For (B) and (D), HEK293T cells were transfected with indicated reporters and plasmids expressing β-catenin* and indicated SOX proteins. Luciferase activity was measured 24h later. Bars represent mean ± s.d. of biological triplicates. ns $P > 0.05$, *$P < 0.05$,** $P < 0.01$, *** $P < 0.001$. **** $P < 0.0001$. P-values were calculated using one-way ANOVA followed by Dunnett's test.

A

Defa6-luc, HEK293T cells

Relative activity

| β-cat* | - | + | + | + |
| SOX9 | - | - | WT | Mut |

B

Defa6-luc, LS174T cells

Relative activity

| WT SOX9 | - | + | ++ | +++ | - | - | - |
| Mut SOX9 | - | - | - | - | + | ++ | +++ |

Flag (SOX9) — 75 kDa

β-Tubulin — 50 kDa

Fig S12. Non-DNA contacting TCF-interacting residues play a role in the activation of Wnt-SOX9 activated enhancers.

Luciferase assay data showing the activation of Defa6-luc by β-catenin* and WT or Mut SOX9 in HEK293T cells (A) and by WT or Mut SOX9 in LS174T cells (B). Western blots in (B) show relative expression levels of WT and Mut SOX9 with Tubulin as loading control. Each bar represents mean ± SD from three replicates (N = 3). ns P > 0.05, *P < 0.05 ,** P < 0.01, *** P < 0.001. **** P < 0.0001. P-values were calculated using one-way ANOVA followed by Dunnett's test.

Construct	Target gene	pGIPZ clone ID	Target sequence
Scr	Scrambled negative control	RHS4346	ATCTCGCTTGGGCGAGAGTAAG
SOX9-1	SOX9	V2LHS_92504	TAAGACTGCAGTGAACAAG
SOX9-2	SOX9	V2LHS_11387	ATGTCTTGAAGGTTAACTG

Table S1. Targeting sequences and clone IDs of the pGPIZ RNAi clones used to create LS174T cell lines expressing scrambled (Scr) or SOX9-targeting shRNAs.
"Target sequence" denotes the sequence of the mature antisense siRNA.

Construct	Target sequence
SOX9 RNAi / shRNA 1	AAGCTCTGGAGACTTCTGA
shRNA 2	ACCACCCGGATTACAAG
shRNA 3	TTGTTCACTGCAGTCTTAA

Table S2. Targeting sequences of SOX9 targeting shRNA sequences cloned into pSUPER for transient transfection RNAi experiments

Construct	Site	Original site	Mutated site
5' SOX	SOX9	AACAATGT	AAaccTGT
3' SOX	SOX9	TCTTTGTC	gaTgTGTC
TCF+SOX	SOX9 (3' SOX)	TCTTTGTC	gaTgTGTC
	TCF	CCTTTGAGC	CCTgTtcGC
		GATGAAAGG	GAgtAcAGG
		GTACAAAGA	GTACAcAtc
		AATCAAAGG	AAgaAcAGG

Table S3. Site-directed mutagenesis of the c-Myc-335 reporter

Reporter	Site	Original	Mutated
Defa5	**TCF**	GCTTTGATG	GCTGTGCGG
		CATCAAAGG	CCGCACAGG
		GAACAAAGG	GCCCACAGG
	5' SOX	ATGAAAGC	ATTCCAGC
	3' SOX	CTGTTGTT	CTGCGTTT
	CAG	TACTGA	TTCACA
		TACTGG	TTCACG
		CACTGG	CTCACG
		ACAGAC	AGACTC
		GGCTGA	GCCACA
		CCAGCC	CGTGGC
Defa6	**TCF**	TCTCAAAGC	TCGTACAGC
		ATTGAAAGC	AGGGACAGC
		GAACAAAGG	GCCCACAGG
		CATCAAAGG	CCGCACAGG
	5' SOX	CTATTCAC	CGATGCCC
		TCCTTCAT	TACTGCCT
		GCTTGGAG	GATTTGCG
		ACATGGAG	AAAATTGCG
	3' SOX	CACAAGAG	CAACCGAG
		CTCAAAGC	CTACCAGC
		TACAATGA	TAACCTGA

Table S4. Site-directed mutagenesis of the *Defa5/6* reporters

Target	Primer
Defa5	ACCTCAGGTTCTCAGGCAAG
	CTGATTTCACACACCCCGGA
G6PD (control)	GAGGCCGTGTACACCAAGAT
	TCAGGGAGCTTCACGTTCTT
Myc	TACAACACCCGAGCAAGGAC
	AGCTAACGTTGAGGGGCATC

Table S5. Primers used for RT-qPCR

Target	Primer
c-Myc-335 (Peak)	TCCTATCTCAGCTCCCTATCCA
	CTGCTGAGCTCAAAGGACGA
c-Myc-335 flank 1	GGCTCTCACCCTTCAACCAA
	GCTTGTGACTTAGCCTGGGT
c-Myc-335 flank 2	GGCTCTGGTTGGGGGTTTTA
	TGAGGCGGAAGTCAACACAG
Control	CGATTCAGTGCCGCATTAGC
	TAATCGGCTGGATCTCCCCA

Table S6. Primers used for ChIP-qPCR