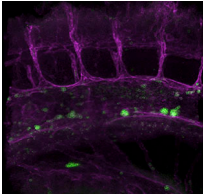
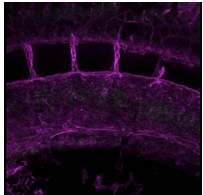


SUPPLEMENTAL MATERIAL

Gao et al., <http://www.jem.org/cgi/content/full/jem.20130733/DC1>

Video 1. The +9.5 element is required for HSC genesis in the AGM. Whole-mount three-dimensional confocal imaging of +9.5^{+/+} embryos at E10.5 with c-Kit (green) and CD31 (magenta) antibodies. Three-dimensional reconstructions were generated from z-stacks (100–120 optional sections) and the video is displayed at 5 frames per second. Related to Fig. 2.



Video 2. The +9.5 element is required for HSC genesis in the AGM. Whole-mount three-dimensional confocal imaging of +9.5^{-/-} embryos at E10.5 with c-Kit (green) and CD31 (magenta) antibodies. Three-dimensional reconstructions were generated from z-stacks (100–120 optional sections) and the video is displayed at 5 frames per second. Related to Fig. 2.

Table S1. +9.5 *cis*-regulatory element deletion does not alter expression of components of Notch, Wnt and BMP4 pathways

Notch Pathway	Gene Name	log₂ fold change
Receptor	<i>Notch1</i>	0.06
	<i>Notch2</i>	-0.19
	<i>Notch3</i>	0.35
	<i>Notch4</i>	0.84
Ligand	<i>Dll1</i>	0.31
	<i>Dll3</i>	0.40
	<i>Dll4</i>	0.59
	<i>Jag1</i>	0.51
	<i>Jag2</i>	-0.27
	S2 cleavage	<i>Adam10</i>
	<i>Adam17</i>	-0.23
S3 cleavage	<i>Psen1</i>	-0.25
	<i>Psen2</i>	-0.78
	<i>Ncstn</i>	-0.02
	<i>Aph1a</i>	-0.20
	<i>Aph1b</i>	0.42
	<i>Aph1c</i>	-0.37
	<i>Psene</i>	-0.03
DNA binding complex	<i>Rbpj</i>	-0.37
	<i>Maml1</i>	-0.07
	<i>Maml2</i>	0.09
	<i>Maml3</i>	-0.16
BMP4 Pathway		
Ligand	<i>Bmp4</i>	0.50
Receptor	<i>Bmpr1a</i>	0.63
	<i>Bmpr1b</i>	0.50
	<i>Bmpr2</i>	0.24
Complex	<i>Smad1</i>	0.45
	<i>Smad2</i>	0.02
	<i>Smad3</i>	0.03
	<i>Smad4</i>	0.13
	<i>Smad5</i>	0.33
	<i>Smad6</i>	0.29
	<i>Smad7</i>	0.21
	<i>Smad9</i>	0.26
	Wnt Pathway	
Ligand	<i>Wnt1</i>	0.21
	<i>Wnt2</i>	0.21
	<i>Wnt2b</i>	0.50
	<i>Wnt3</i>	1.04
	<i>Wnt3a</i>	2.43
	<i>Wnt4</i>	0.46
	<i>Wnt5a</i>	0.41
	<i>Wnt5b</i>	0.55
	<i>Wnt6</i>	0.34
	<i>Wnt7a</i>	0.72
	<i>Wnt7b</i>	0.11
	<i>Wnt8a</i>	2.43
	<i>Wnt8b</i>	1.35
	<i>Wnt9a</i>	0.24
	<i>Wnt9b</i>	0.51
	<i>Wnt10a</i>	0.56
<i>Wnt10b</i>	0.60	
<i>Wnt11</i>	0.58	
<i>Wnt16</i>	0.66	
Receptor	<i>Fzd1</i>	0.19
	<i>Fzd2</i>	0.44

	Gene Name	log ₂ fold change
	<i>Fzd3</i>	0.70
	<i>Fzd4</i>	0.49
	<i>Fzd5</i>	0.54
	<i>Fzd6</i>	0.80
	<i>Fzd7</i>	0.22
	<i>Fzd8</i>	0.26
	<i>Fzd9</i>	-0.54
	<i>Fzd10</i>	0.89
	<i>Lrp5</i>	-0.14
	<i>Lrp6</i>	0.14
Complex	<i>Dvl1</i>	0.21
	<i>Dvl2</i>	0.03
	<i>Dvl3</i>	0.11
	<i>Axin1</i>	-0.10
	<i>Axin2</i>	0.28
	<i>Csnk1a1</i>	-0.06
	<i>Csnk1d</i>	0.01
	<i>Csnk1e</i>	0.23
	<i>Csnk1g1</i>	0.08
	<i>Csnk1g2</i>	0.04
	<i>Csnk1g3</i>	0.32
	<i>Gsk3a</i>	-0.11
	<i>Gsk3b</i>	0.14
	<i>Apc</i>	0.18
	<i>Ctnnb1</i>	0.36
DNA-binding complex	<i>Tcf3</i>	0.00
	<i>Tcf4</i>	0.30
	<i>Tcf7</i>	0.46
	<i>Tcf12</i>	0.31
	<i>Tcf15</i>	0.84
	<i>Tcf19</i>	0.33
	<i>Tcf20</i>	-0.06
	<i>Tcf21</i>	0.64
	<i>Tcf23</i>	1.00
	<i>Tcf25</i>	-0.05
	<i>Tcf15</i>	0.81
	<i>Tcf711</i>	0.48
	<i>Tcf712</i>	0.07
	<i>Tle1</i>	0.28
	<i>Tle2</i>	0.25
	<i>Tle3</i>	-0.16
	<i>Tle4</i>	0.15
	<i>Tle6</i>	-0.24

Table S2. Primers for genotyping and RT-PCR

Primer	Sequence
+9.5 flanking Forward	5'-ATGTCCTTTCGGATCTCCTGCC-3'
+9.5 flanking Reverse	5'-GGTAAACAGAGCGCTACTCCTGTGTGT-3'
18S rRNA Forward	5'-CGCCGCTAGAGGTGAAATTCT-3'
18S rRNA Reverse	5'-CGAACCTCCGACTTTCGTTCT-3'
Gata2 Forward	5'-GCAGAGAAGCAAGGCTCGC-3'
Gata2 Reverse	5'-CAGTTGACACACTCCCGGC-3'
c-Kit Forward	5'-AGCAATGGCCTCACGAGTTCTA-3'
c-Kit Reverse	5'-CCAGGAAAAGTTTGGCAGGAT-3'
Myb Forward	5'-CGAAGACCCTGAGAAGGAAA-3'
Myb Reverse	5'-GCTGCAAGTGTGGTTCTGTG-3'
Mpl Forward	5'-ACTTTGATCCAGCGGGTGCT-3'
Mpl Reverse	5'-CAGGAAGTCACTGATTCAG-3'
Gfi1 Forward	5'-TCCGAGTTCGAGGACTTTTG-3'
Gfi1 Reverse	5'-CATGCATAGGGCTTGAAAGG-3'
Gfi1b Forward	5'-AGGAGCGGGAGATGTTGCT-3'
Gfi1b Reverse	5'-GGGTGACTCAGAGAGTGGTGATT-3'
Runx1 Forward	5'-TCACTGGCGCTGCAACAA-3'
Runx1 Reverse	5'-TCTGCCGAGTAGTTTTTCATCGTT-3'
Lyl1 Forward	5'-AAGCGCAGACCAAGCCATAG-3'
Lyl1 Reverse	5'-AGCGCTCACGGCTGTTG-3'
Ikzf1 Forward	5'-CGGGCATGTACCCAGTCATTA-3'
Ikzf1 Reverse	5'-GCTCCTATCTTGACAGGTCTTC-3'
Sfpi1 Forward	5'-GGCAGCGATGGAGAAAGC-3'
Sfpi1 Reverse	5'-GGACATGGTGTGCGGAGAA-3'
TAL1 Forward	5'-GAGGCCCTCCCATATGAGA-3'
TAL1 Reverse	5'-GCGCCGCACTACTTTGGT-3'
Bex6 Forward	5'-CAGGAGACAGAAGCCAAGAGAAA-3'
Bex6 Reverse	5'-TGAGGAACCACTTGAAAACCT-3'
Gpr56 Forward	5'-CTCTGCTCCAGGAGAAAGTCA-3'
Gpr56 Reverse	5'-GCGGACAGCAGGTTTATGT-3'
Mfsd2b Forward	5'-CCCAGATGTAGCTCGCCTCTA-3'
Mfsd2b Reverse	5'-GCAGACTTCCACACACAGGATAAGT-3'
Slc35d3 Forward	5'-GGACCCGGCCATGGT-3'
Slc35d3 Reverse	5'-TCATGGCACAGCCGATCA-3'
Fcho1 Forward	5'-TGAGGTCTCTACCATTTGTGA-3'
Fcho1 Reverse	5'-TTGGCCCTTTCCTAATGAA-3'
Lin28b Forward	5'-CCTGCAAAAGAGAAAGCCAAA-3'
Lin28b Reverse	5'-CACAGTCCACATCTGATCCATCA-3'
Gpr65 Forward	5'-CTTACCCCTTCCACGTGAT-3'
Gpr65 Reverse	5'-ACGTTTATGTCGCGCTCTAA-3'
Hhex Forward	5'-TCGCCGAGCTAAATGGAGAA-3'
Hhex Reverse	5'-CAACGCATCCTTTTTGTTGCT-3'
Mmrn1 Forward	5'-TTTACCAAGTGGAAAGAGTTCTTGT-3'
Mmrn1 Reverse	5'-GGATGTCTTCTGAGATCTTCTGGAA-3'
Cdk6 Forward	5'-TGCTCAACCCATCGAGAAGTT-3'
Cdk6 Reverse	5'-TCAGGCATTTCAGAAGTAGGTCITT-3'