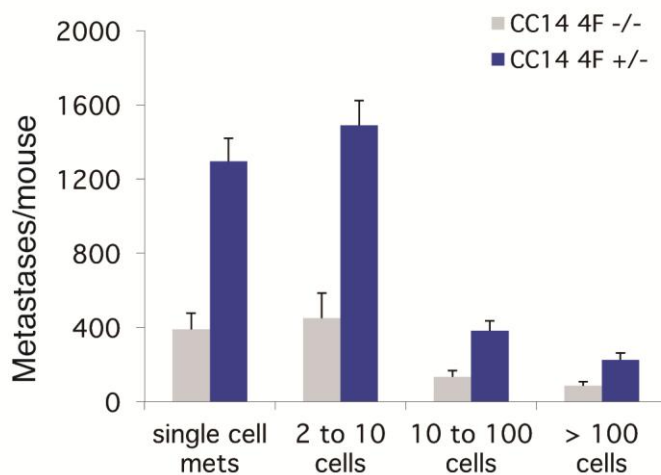
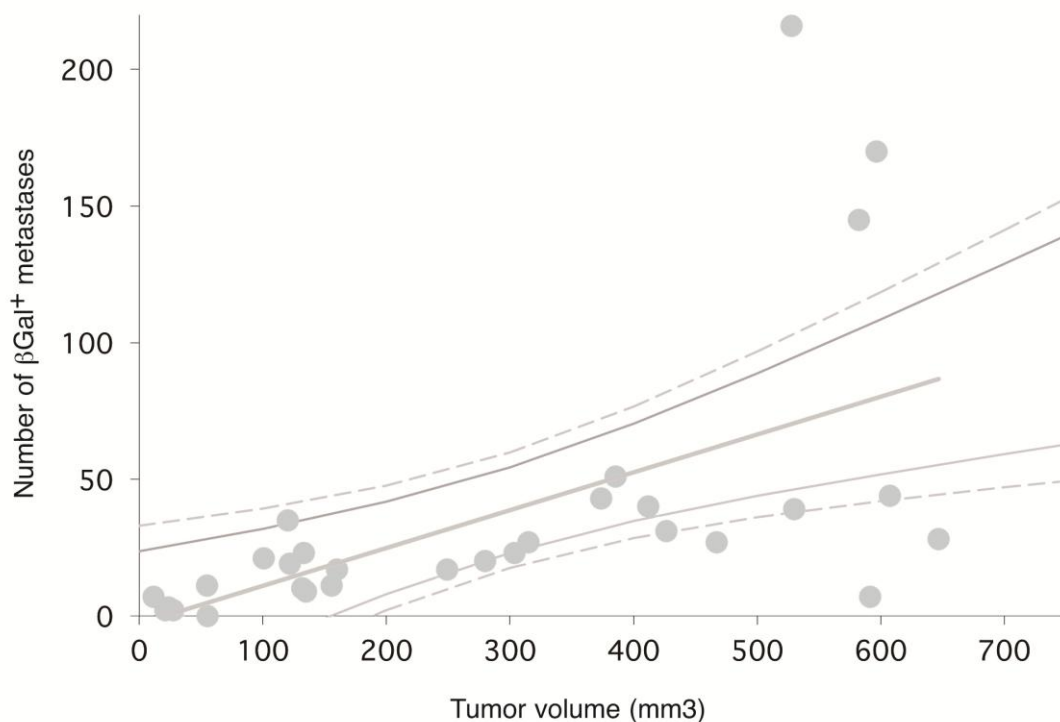


Supplementary Figures



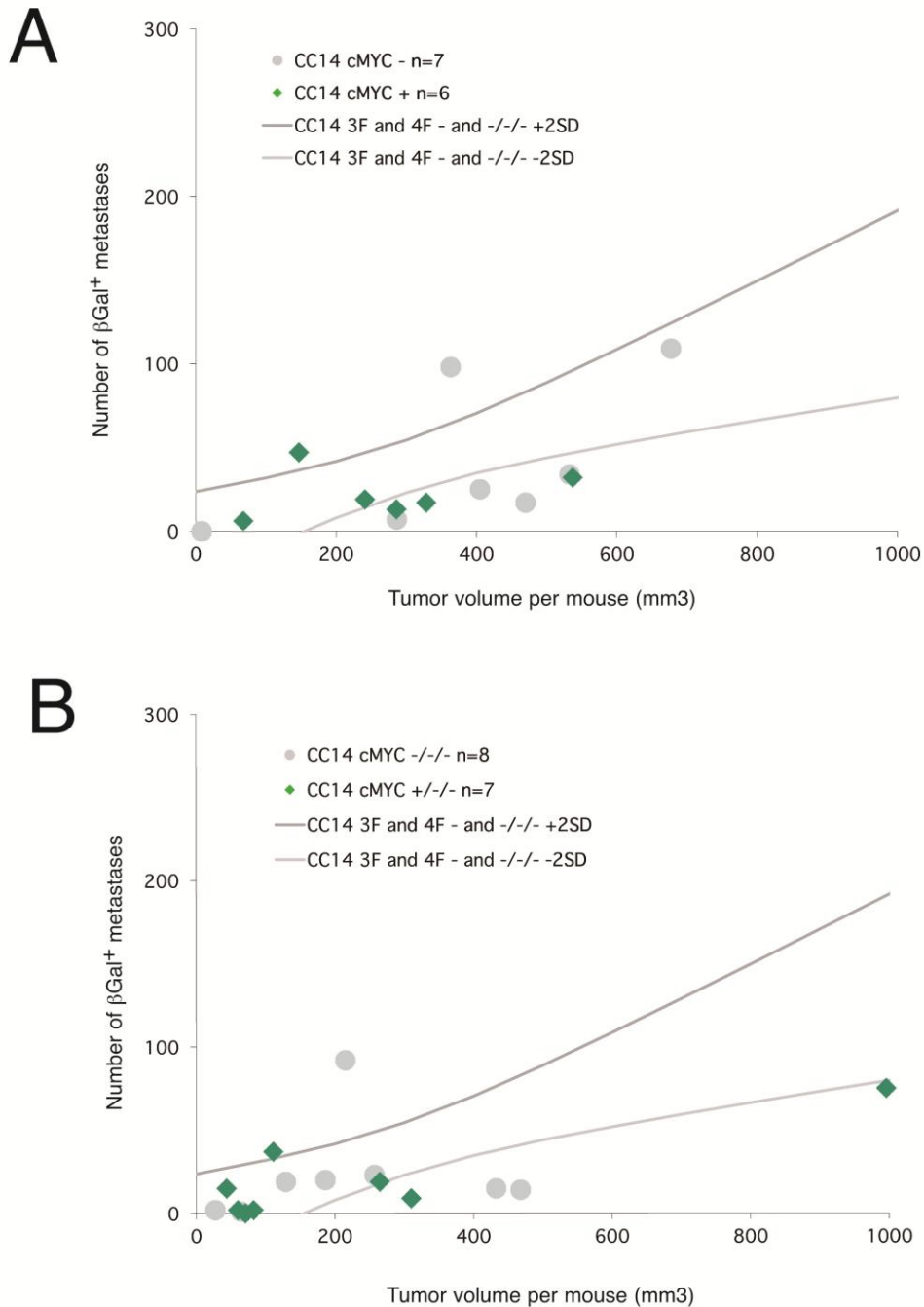
Supplementary Figure S1. Quantification of 4F-induced lung metastases by size in CC14 cells.

Lung metastases were scored after injection of human tumor cells into the circulation. In all cases $p < 0.05$. See main text for details.



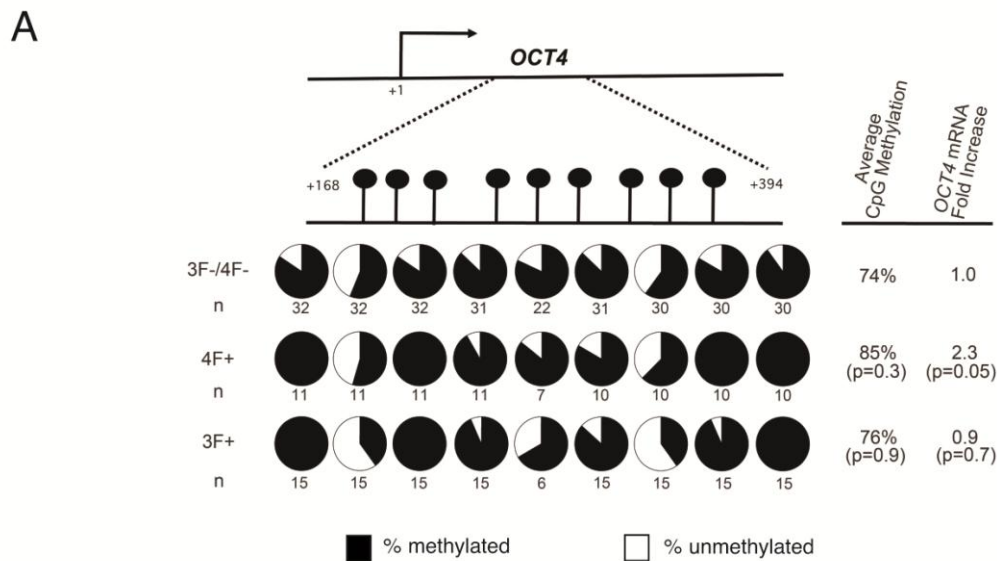
Supplementary Figure S2. Plot of the number of β Gal⁺ lung metastases versus total tumor volume per mouse for all CC14 controls.

The individual tumor values and metastases for 4F and 3F uninduced (-, -/-) samples is denoted with round markers. The trend line is shown bisecting the control space, flanked on either side by the average ± 2 standard deviation (SD) space limits (with $p < 0.05$; thin solid lines) or the ± 3 SD space limits (with $p < 0.01$; broken thin lines). Note the presence of rare outliers.



Supplementary Figure S3. Plots of the number of βGal^+ lung metastases versus total tumor volume per mouse for cMYC tumors.

Plots for cMYC+ vs cMYC- (**A**) and for cMYC+/-/- and cMYC-/-/- (**B**) tumors, all compared with the +/- 2SD space of controls (see Fig S2). P values were determined by the unpaired two-tailed Student's t test comparing all samples within a given class (e.g. cMYC+) versus the entire control (all uninduced samples) pool and were as follows: Top) Tumor volume cMYC+ vs controls p=0.3. Number of metastases cMYC+ vs. controls p=0.3. Bottom) Tumor volume cMYC+/-/- vs controls p=0.9, and 3F+/-/- vs. controls p=0.8. Number of metastases cMYC+/-/- vs. controls p=0.8.



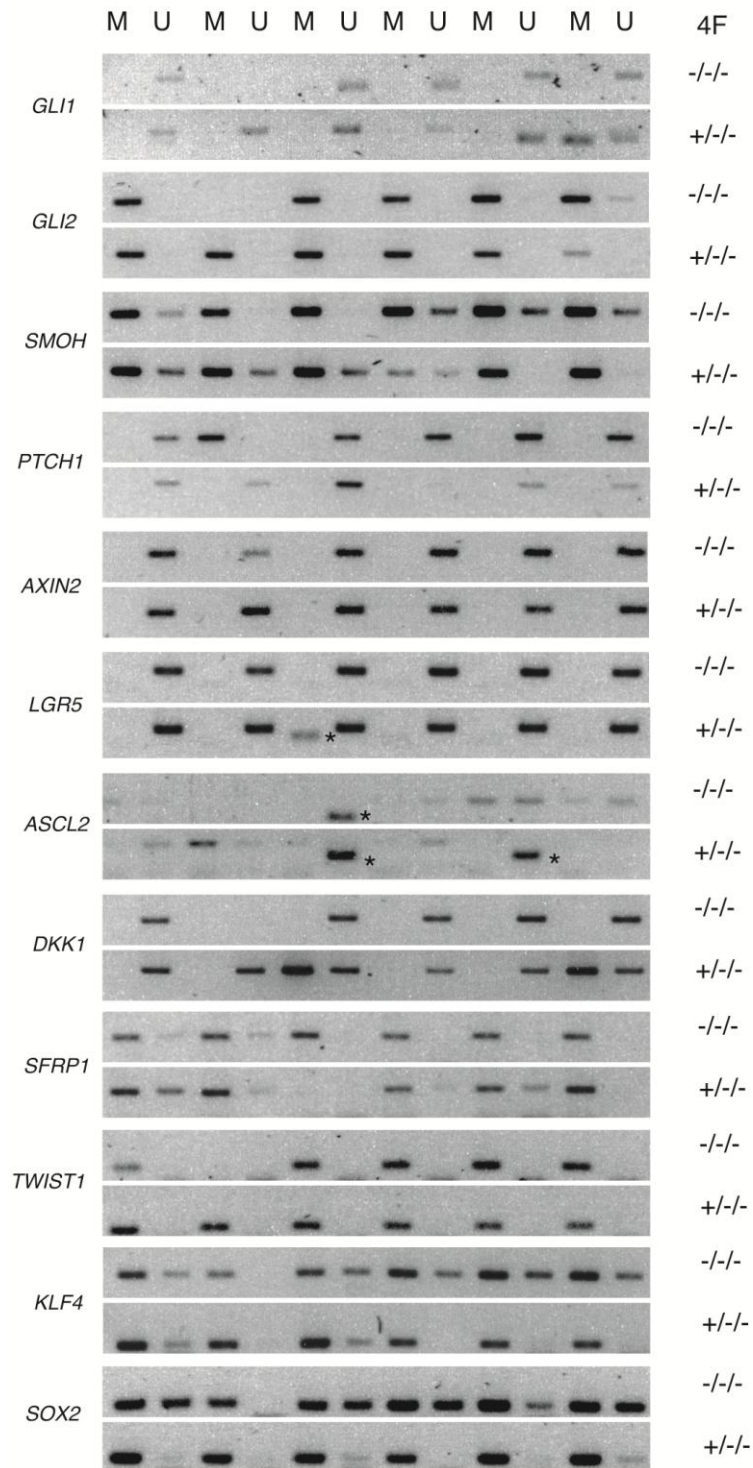
B

	4F -/-		4F +/-		n	3F -/-		3F +/-		n	MSP primer region
	M	U	M	U		M	U	M	U		
<i>PTCH1</i>	17	83	17	92	12	50	100	100	100	4	-9490 to -9164
<i>SMOH</i>	100	100	100	92	12	100	100	100	100	4	-683 to -477
<i>GLI1</i>	0	100	0	100	6	0	100	0	100	4	-3535 to -3375
<i>GLI2</i>	83	33	100	17	12	100	55	100	55	11	-6173 to -5847
<i>SFRP1</i>	100	33	100	70	10	100	55	100	91	11	-259 to +11
<i>DKK1</i>	20	100	33	100	12	9	100	9	100	11	-92 to +360
<i>LGR5</i>	0	100	0	100	12	0	100	0	100	4	-617 to -459
<i>AXIN2</i>	0	100	25	100	12	27	100	36	100	11	-3292 to -3173

Supplementary Figure S4. Reprogramming-driven DNA methylation changes.

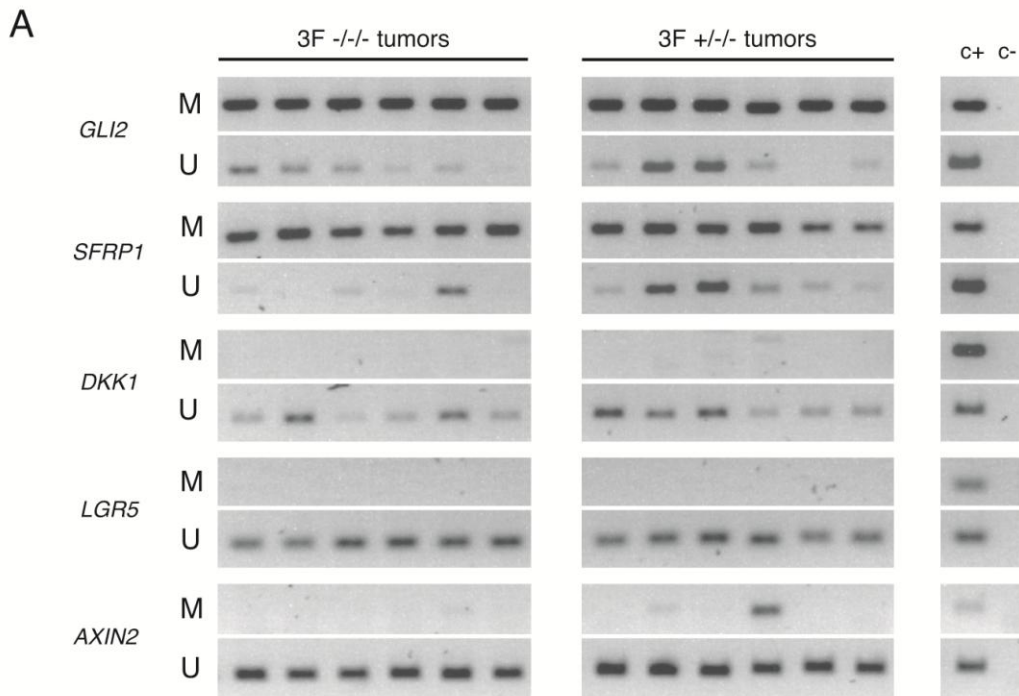
A) Reprogramming does not change the methylation of OCT4. Analysis was performed as for NANOG in Fig. 5; The upper diagram shows the position of analyzed CpG dinucleotides in the 5' promoter region of *OCT4*. The methylation status (represented in pie charts in the lower part of the diagram) was determined by bisulfite sequencing. Uninduced controls were pooled as there were no differences between 4F- vs. 3F- samples.

B) Quantification of methylation specific PCR (MSP) results for 3F+/- vs 3F-/- tumors (Figs. S4, S5) testing for both methylated and unmethylated status of selected CpG islands for different genes as indicated. The numbers of tumors analyzed (n) and the position of the islands (MSP primer region Fig. S5B) in relation to the initiator ATG are indicated.



Supplementary Figure S5. MSP analyses of the methylation status of CpG islands of selected genes.

PCR reaction products for methylated (M) and unmethylated (U) states were loaded on agarose gels and photographed. The images show representative examples for the genes indicated in 4F+/- and 4F-/- tumors. Bands with asterisks denote primer dimers. (see Fig. 5)



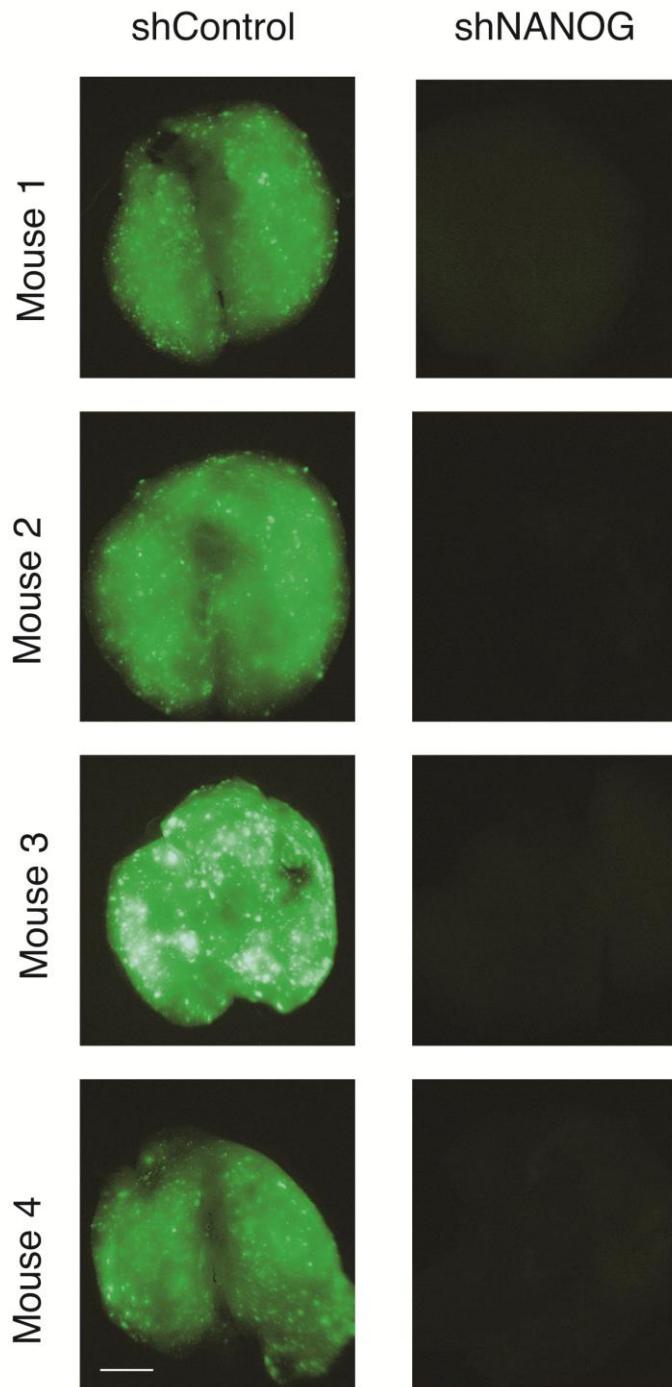
B

		Forward (5' → 3')	Reverse (5' → 3')	Position in relation to initiator ATG
<i>PTCH1</i>	M	TGTGTAAATATCGTTTATTAGCGGC	TATACGAATTCGTATCGATCCCTCG	-9490 to -9164
	UM	TGTGAAATATTGTTTATTAGTGGTGG	TACAAATTCATATCAATCCCTCAA	-9488 to -9166
<i>SMOH</i>	M	CGGGGTATTAGATTTTTTAGTTC	CCGACTCCTTTTACTCTAACTCG	-683 to -477
	UM	TGGGGTATTAGATTTTTTAGTTC	CAACTCCTTTTACTCTAACTCACT	-680 to -478
<i>GLI1</i>	M	TTTAGTTTTGGATCGCGTATTTTC	CCCTATTTTACCTCTATATCCCGAA	-3535 to -3375
	UM	TTTAGTTTTGGATTGTGATTTTTGA	CCCTATTTTACCTCTATATCCCAA	-3535 to -3375
<i>GLI2</i>	M	TTGGGGAATTTTTACGTTTTTTAC	AAAAAAAACAACCGACTCACG	-6173 to -5847
	UM	GGGAATTTTTATGTTTTTATGT	AAAAAAAACAACCACTCACAA	-6125 to -5848
<i>SFRP1</i>	M	TGTTTCGTCGATTTTCGGGAGTC	CGATACCCATACCGACTCTACGC	-254 to +10
	UM	TTTTTTTTGTTGTGATTTTTGGGAGTT	CCAATACCCATACCACTCTACACC	-259 to +11
<i>DKK1</i>	M	GTAGTCGAATCGGTACGGTTTC	GAATACAAAATCCCGACGC	-92 to +360
	UM	TTTGTAGTTGAATTGGTATGGTTTT	CCAAATACAAAATCCCAACAC	-90 to +208
<i>LGR5</i>	M	TTTTTTCGAAGTAGTTTTTTTCGA	GCGATACTAACGAATTCGACG	-617 to -459
	UM	TTTTTTTGAAGTAGTTTTTTTGA	CACAATACTAACAAATTCCAACACT	-617 to -460
<i>AXIN2</i>	M	AGAAATGTTGTATTTTAGAGTCGG	GAAAAACATTCACACCGAA	-3292 to -3175
	UM	TGTATTAGAAATGTTGATTTAGAGTT	CCAAAAACATTCACACCGAA	-3292 to -3173
<i>SOX2</i>	M	AAATTAGACGAGGTTTTGTTGATTTTC	ACCCAAAAAATAATTTAACCGCT	-1942 to -1688
	UM	GAAATTTAGATGAGGTTTTGTTGATTTTC	ACCCAAAAAATAATTTAACCACT	-1943 to -1688
<i>KLF4</i>	M	CGTATTTTTGTTCCGGTGTC	GAAATATTTACTTTAAATTCGACGTT	-2617 to -2455
	UM	GAGTGTATTTTTGTTGGTGTGTTG	CAAAATATTTACTTTAAATTCCAACTT	-2619 to -2424
<i>SNAIL1</i>	M	TTCGTTAATGTTACGTTTTTAGGC	CGATAATCTAACCGTTCTAACG	-564 to -416
	UM	TTTTTTTGTAAATGTTATTTTTAGGT	CAATATCTAACACTTCTAACACC	-567 to -416
<i>ASCL2</i>	M	AAGGTGTCGTTTGTAGTTTTTAGTTC	GAAATAAAAAATCAAATTCGGA	-1196 to -1080
	UM	GGTGTGTTTGTAGTTTTTAGTTC	AAAAATAAAAAATCAAATTCGAAA	-1198 to -1080
<i>TWIST1</i>	M	TTTTTCGAGTATTTTCGAGGC	GTTTCTAAAAACGTAACCGCG	-1103 to -952
	UM	TGTTTTTTTTGAGTATTTTTGAGGT	AACATTTCTAAAAACATAACCAACACC	-1106 to -948

Supplementary Figure S6. Gene regions analyzed by MSP.

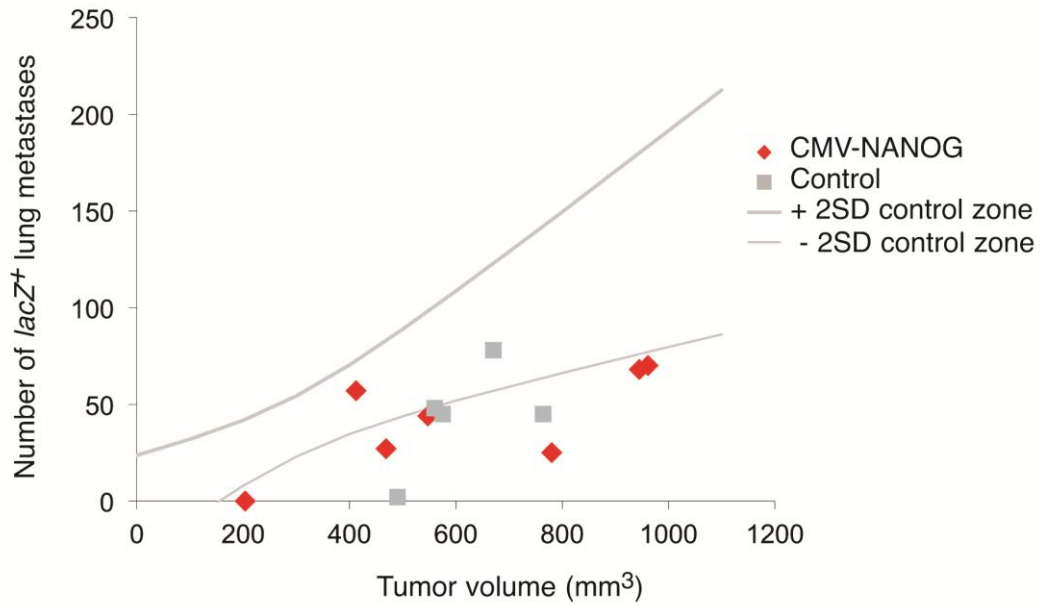
A) Examples of MSP reactions, amplified from 3F+/- and 3F-/- tumor DNA and run in agarose gels with positive fully methylated (c+) and negative fully unmethylated (c-) controls for individual genes as noted (see text). See Fig. S4 for of 4F+/- and 4F-/- MSP examples.

B) List of the position and sequences of MSP primers for selected genes as indicated.



Supplementary Figure S7. Loss of lung metastases driven by NANOG knock-down.

Panels show fluorescent images of dissected lungs from mice injected into the tail vein with control (*shControl*) and *shNANOG/P8* conditions. The left lung lobes were dissected and stained with XGAL to reveal βGal^+ metastases in great detail. Here the images show the overall GFP fluorescence from the *GFP⁺/LacZ⁺* human colon cancer cells forming metastases. Note that cells with compromised NANOG fail to form GFP⁺ metastases. Scale bar = 3.2 mm for all panels.



Supplementary Figure S8. Overexpression of NANOG does not increase the number of metastases in the lungs from subcutaneous xenografts.

The graph shows the number of metastases and total tumor weight per animal for control CC14 and CC14-CMV-NANOG cells. Expression of NANOG over 100 fold was verified in the experimental xenografts at the end of the experiments by RT-qPCR (not shown).

Supplementary Table S1. RT-qPCR primers used.

Gene name	Direction	Sequence
<i>NANOG</i>	FW	AAATTGGTGATGAAGATGTATTCCG
	RV	GCAAAACAGAGCCAAAAACG
<i>NANOGP8</i>	FW	GCTGCCTTCAAGCATCTGTT
	RV	TTAAGAGGTGGACTGGAAAAA
<i>OCT4 (5'UTR)</i>	FW	GGGTTGAGTAGTCCCTTCGC
	RV	TAGCCAGGTCCGAGGATCAA
<i>SOX2 (5'UTR)</i>	FW	GAGAGTGTGGCAAAAGGGGG
	RV	CGCCGCCGATGATTGTTATT
<i>KLF4 (5'UTR)</i>	FW	ATCTCGGCCAATTTGGGGTT
	RV	TTGACGCAGTGTCTTCTCCC
<i>GLI1</i>	FW	AGCGTGAGCCTGAATCTGTG
	RV	CAGCATGTACTGGGCTTTGAA
<i>GLI2</i>	FW	AGCAGCAGCAACTGTCTGAG
	RV	CACATGAGCCGTGTCCAG
<i>PTCH1</i>	FW	GGCAGCGGTAGTAGTGGTGTTCC
	RV	TGTAGCGGGTATTGTCGTGTGTG
<i>AXIN2</i>	FW	AGTGTGAGGTCCACGGAA
	RV	ACTGCCACACGATAAGGAG
<i>LGR5</i>	FW	GGAGCATTCACTGGCCTTTA
	RV	CTGGACGGGGATTTCTGTTA
<i>cMYC</i>	FW	TGGTCTTCCCCTACCCTCTCAAC
	RV	GATCCAGACTCTGACCTTTTGCC
<i>DKK1</i>	FW	TCCGAGGAGAAATTGAGGAA
	RV	CCTGAGGCACAGTCTGATGA
<i>SNAIL1</i>	FW	AAGAGGCCTTCCCAGTGGCCATT
	RV	GGGAGCTTCCCAGTGAGTCT
<i>SNAIL2</i>	FW	TTAGAACTCACACGGGGGAGAA
	RV	GCTACACAGCAGCCAGATTC
<i>ZEB2</i>	FW	TCCTAATATTCCGCCTGTCTG
	RV	GGCATGAAAATGGAGTGGAT
<i>FOXC2</i>	FW	AGTTCATCATGGACCGCTTC
	RV	GCTCCTCCTTCTCCTTGGAC
<i>TWIST1</i>	FW	GCCGGAGACCTAGATGTCATT
	RV	CCCACGCCCTGTTTCTTTGA
<i>HPRT</i>	FW	AAGATGGTCAAGGTCGCAAG
	RV	CTCCAGATGTTTCCAAACTCAAC
<i>βACTIN</i>	FW	ACAGAGCCTCGCCTTTGC
	RV	GGAATCCTTCTGACCCATGC