

A

		Expression Intensity				
		negative	weak	intermediate	strong	
IKK α	Normal	0/64; 0%	6/64; 9%	23/64; 36%	35/64; 55%	
	PDAC	8/79; 10%	21/79; 27%	42/79; 53%	8/79; 10%	*
p62	Normal	19/82; 23%	19/82; 23%	37/82; 45%	7/82; 9%	*
	PDAC	5/87; 6%	12/87; 14%	29/87; 33%	41/87; 47%	*
NQO1	Normal	19/47; 41%	8/47; 17%	18/47; 38%	2/47; 4%	*
	PDAC	1/64; 2%	4/64; 6%	34/64; 53%	25/64; 39%	*
NRF2	Normal	12/67; 18%	30/67; 45%	21/67; 31%	4/67; 6%	*
	PDAC	0/86; 0%	15/86; 17%	29/86; 34%	42/86; 49%	*
MDM2	Normal	10/59; 17%	20/59; 33%	28/59; 47%	2/59; 3%	*
	PDAC	1/65; 2%	10/65; 15%	25/65; 38%	29/65; 45%	*

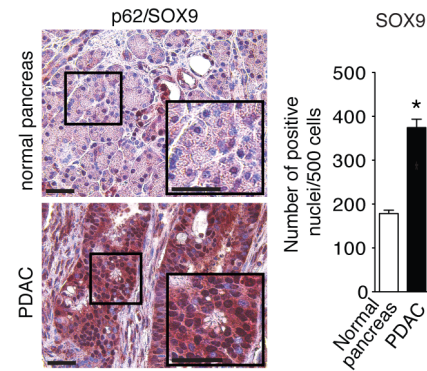
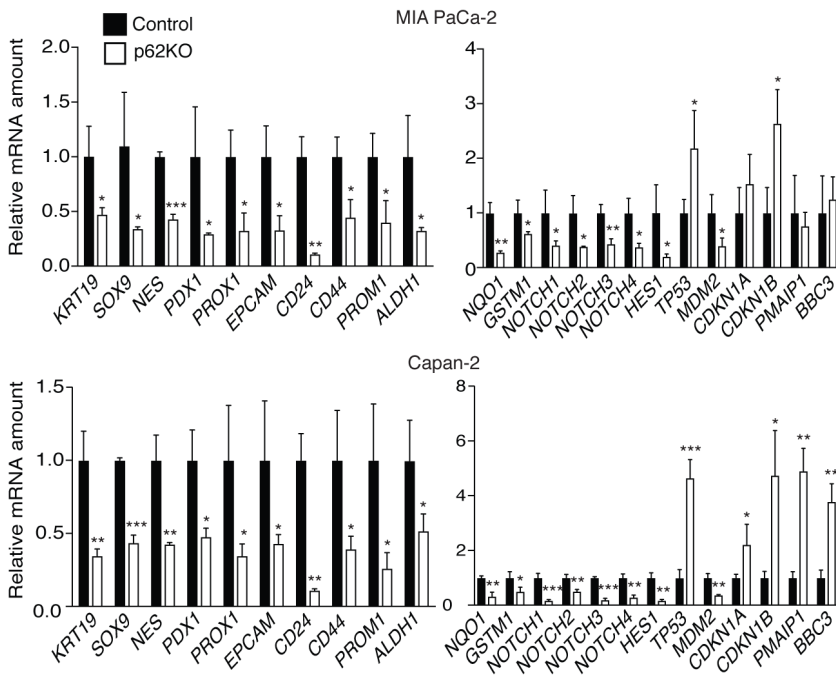
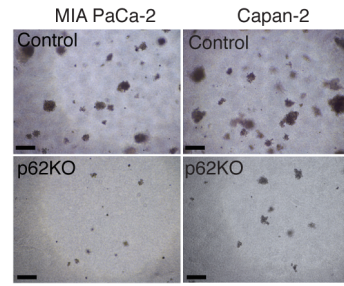
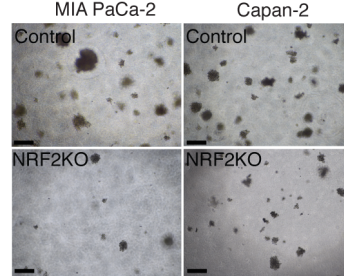
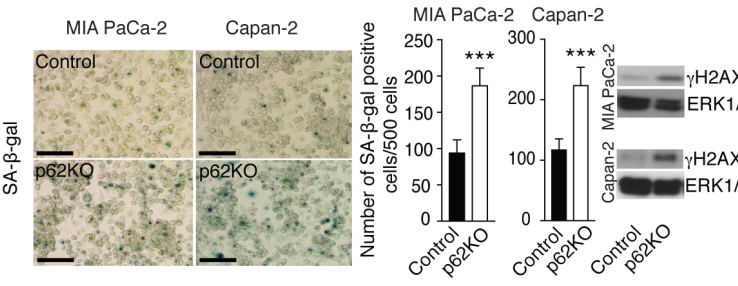
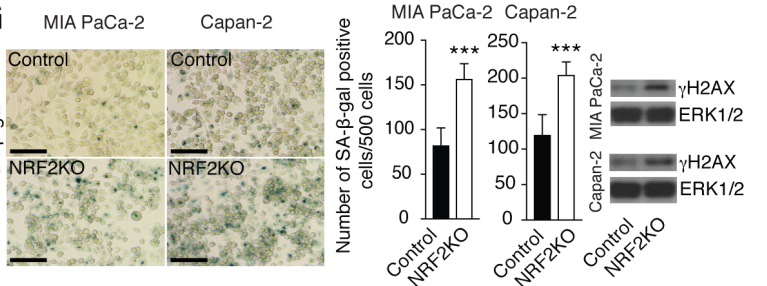
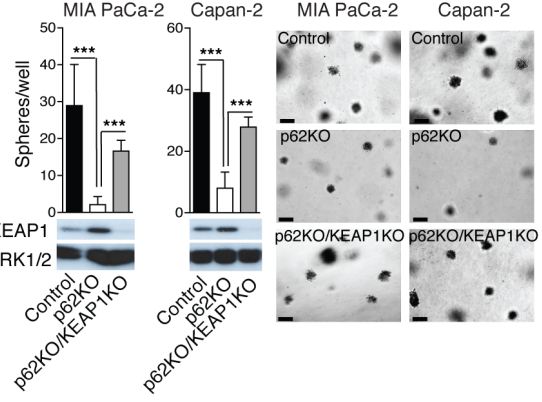
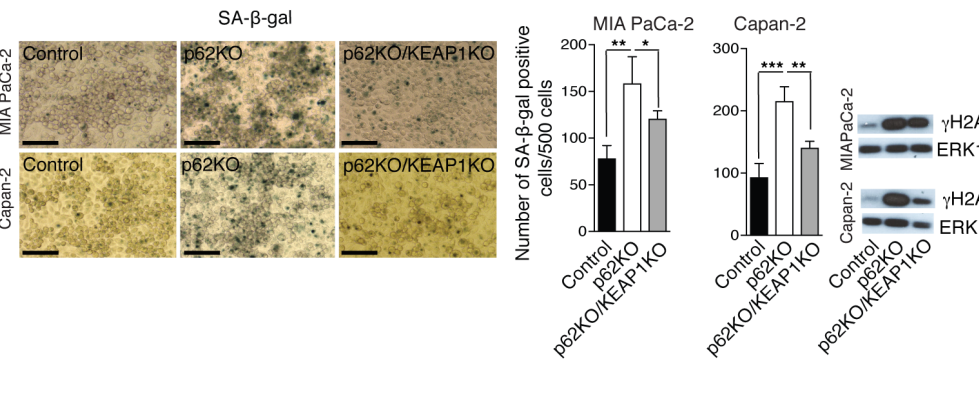
B**C****D****E****F****G****H****I**

Figure S1 p62 Accumulates in Human PDAC and Its Depletion Decreases Progenitor and Cancer Stem Cell Markers, Related to Figure 1

(A) The table depicts the numbers and percentages of human cancerous (n = 64-87) or healthy (n = 47-82) pancreatic tissues with relative staining intensities (arbitrarily indicated as negative, weak, intermediate or strong) of the indicated markers. *, p < 0.001, normal vs. PDAC. (B) Sox9 and p62 double IHC staining of normal human pancreas and PDAC. Sox9 positive nuclei were visualized with DAB in brown and cytoplasmic p62 was stained with AEC (reddish). Quantitation of Sox9 staining is shown to the right (n = 52). Scale bars: 25 μ m. (C) RNA was extracted from MIA PaCa-2 and Capan-2 cells in which p62 was ablated or not (n = 3). Expression of the indicated genes was analyzed by Q-RT-PCR. (D) Representative images of spheres formed by WT or p62-ablated MIA PaCa-2 and Capan-2 cells. Scale bars: 100 μ m (E) Representative images of spheres formed by WT or NRF2-ablated MIA PaCa-2 and Capan-2 cells. Scale bars: 100 μ m. (F) SA- β -gal staining of control and p62-ablated MIA PaCa-2 and Capan-2 cells (n = 6) and IB analysis of γ -H2AX. Scale bars: 100 μ m. (G) SA- β -gal staining of control and NRF2-ablated MIA PaCa-2 and Capan-2 cells (n = 6) and IB analysis of γ -H2AX. Scale bars: 100 μ m. (H) Quantification and representative images of spheres formed by WT and p62-ablated MIA PaCa-2 and Capan-2 cells with or without ablated KEAP1 and IB analysis of KEAP1. Scale bars: 100 μ m. (I) Quantification and images of SA- β -gal staining of WT and p62-ablated MIA PaCa-2 and Capan-2 cells with or without ablated KEAP1 and γ -H2AX IB. Scale bars: 100 μ m. (I). Results in B, C and F-I are mean \pm SEM; *, p < 0.05; **, p < 0.01; ***, p < 0.001. Statistical significance was calculated using Student's t test.

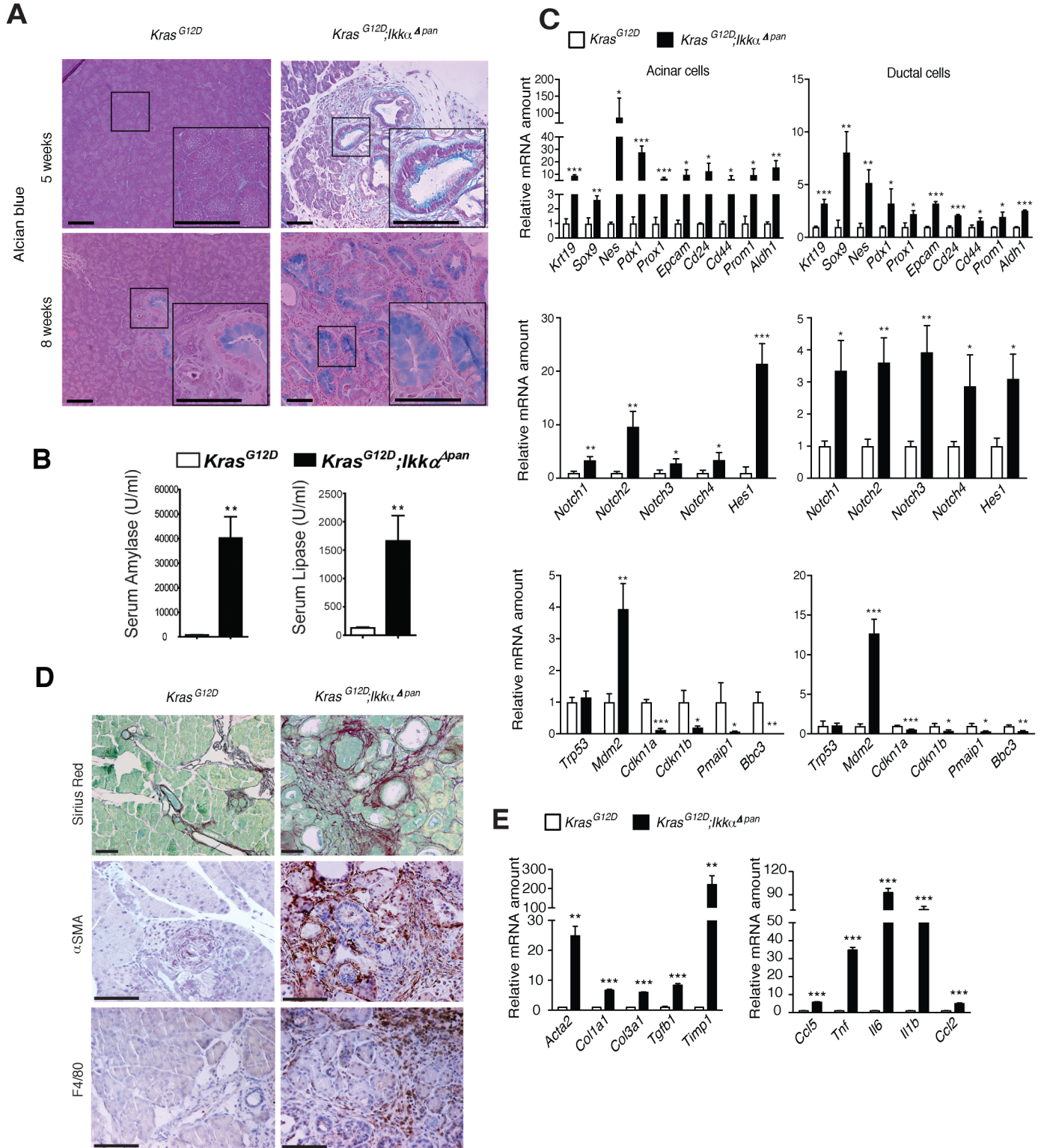


Figure S2 Loss of Pancreatic IKK α in *Kras*^{G12D} Mice Leads to Acinar Cell Damage, Fibrosis, Inflammation and Up-regulation of Progenitor and Stem Cell Markers, Related to Figure 2

(A) Alcian Blue staining of pancreatic sections from indicated mouse strains. Scale bars: 50 μ m. (B) Analysis of serum amylase and lipase in 5-week-old *Kras*^{G12D} and *Kras*^{G12D};*Ikk α* ^{Apan} mice (n = 4). (C) Q-RT-PCR analysis of ductal, progenitor, stem cell and senescence markers and Notch and p53 pathway genes in acinar and ductal cell fractions obtained from 5-week-old *Kras*^{G12D} and *Kras*^{G12D};*Ikk α* ^{Apan} mice (n = 6). (D) Sirius Red staining and α SMA and F4/80 IHC of pancreatic tissue sections from 5-week-old *Kras*^{G12D} and *Kras*^{G12D};*Ikk α* ^{Apan} mice. Scale bars: 50 μ m. (E) Q-RT-PCR analysis of cytokine, chemokine and fibrosis-related genes in pancreata of 5-week-old mice (n = 6). Results in B, C and E are mean \pm SEM; *, p < 0.05; **, p < 0.01; ***, p < 0.001. Statistical significance was calculated using Student's t test.

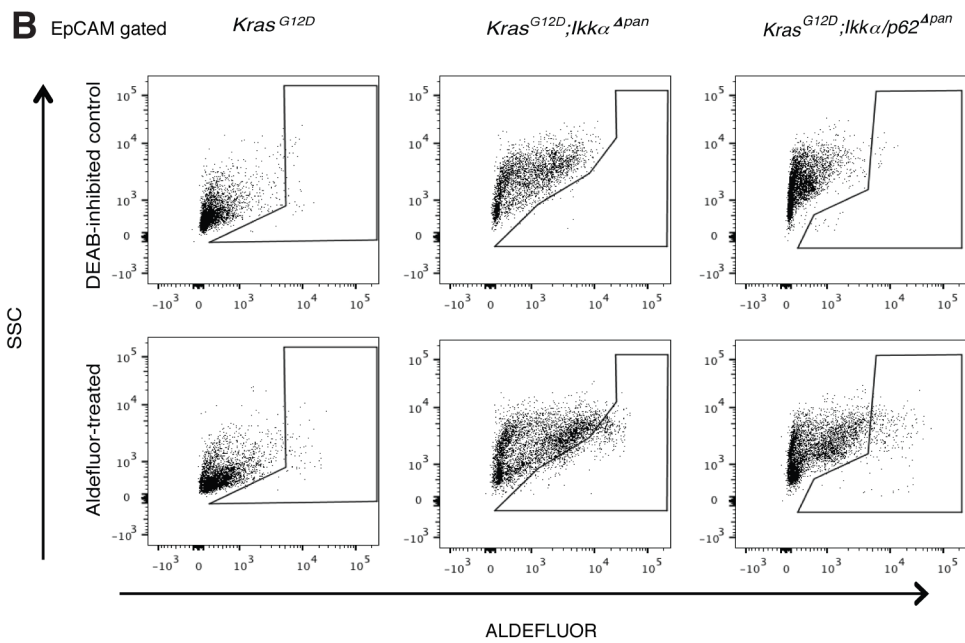
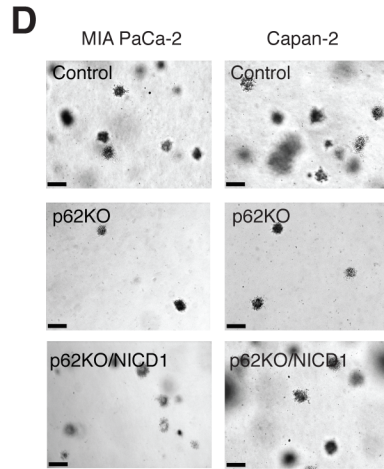
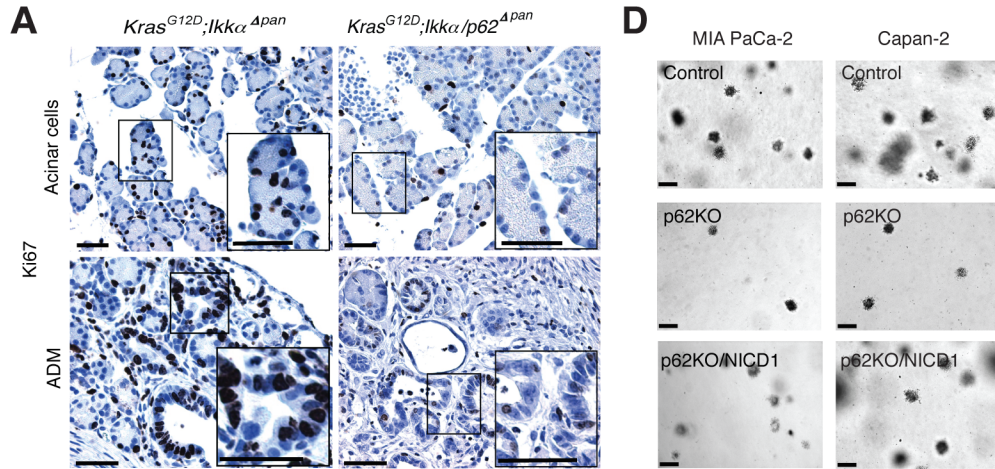
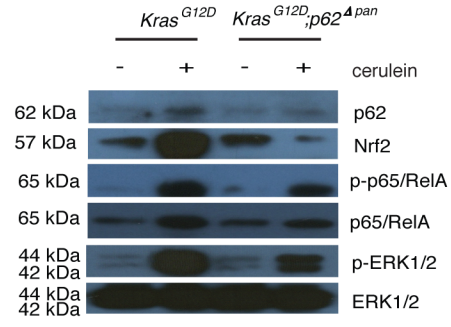


Figure S3 Pathologies Caused by IKK α Loss are Rescued by p62 Ablation, Related to Figure 3

(A) Ki67 IHC of acinar cells and ADM of *Kras*^{G12D} and *Kras*^{G12D};*p62*^{Apan} mice. Scale bars: 25 μ m. (B) Representative FACS plots showing frequency of ALDH expression in EpCAM⁺ cells from 8-week-old *Kras*^{G12D} (n = 3), *Kras*^{G12D};*Ikk α* ^{Apan} (n = 7), and *Kras*^{G12D};*Ikk α* /*p62*^{Apan} (n = 4) mice. (C) Representative light microscopy images of spheres formed by sorted ALDH⁺ cell fractions from B. Scale bars: 100 μ m. (D) Representative images of control and p62-ablated MIA PaCa-2 and Capan-2 cells with or without NICD1 overexpression. Scale bars: 100 μ m.

A



B

■ *Kras^{G12D}* □ *Kras^{G12D},p62^{4pan}*

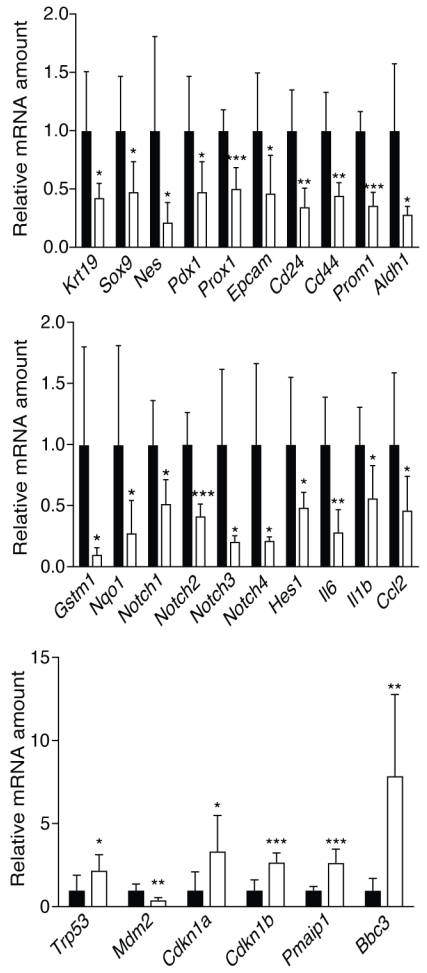


Figure S4 p62 Ablation Inhibits NRF2 Activation, Transcription of Ductal, Progenitor and Stemness Markers and *Mdm2* in Cerulein-Induced Acinar-to-Ductal Metaplasia, Related to Figure 4 (A) IB analysis of pancreatic lysates from *Kras^{G12D}* and *Kras^{G12D};p62^{Apan}* mice prepared 3 days after treatment with PBS or cerulein. (B) Q-RT-PCR analysis of pancreatic RNA from cerulein-treated *Kras^{G12D}* and *Kras^{G12D};p62^{Apan}* mice (n = 3). Results in B are mean \pm SEM; *, p < 0.05; **, p < 0.01; ***, p < 0.001. Statistical significance was calculated using Student's t test.

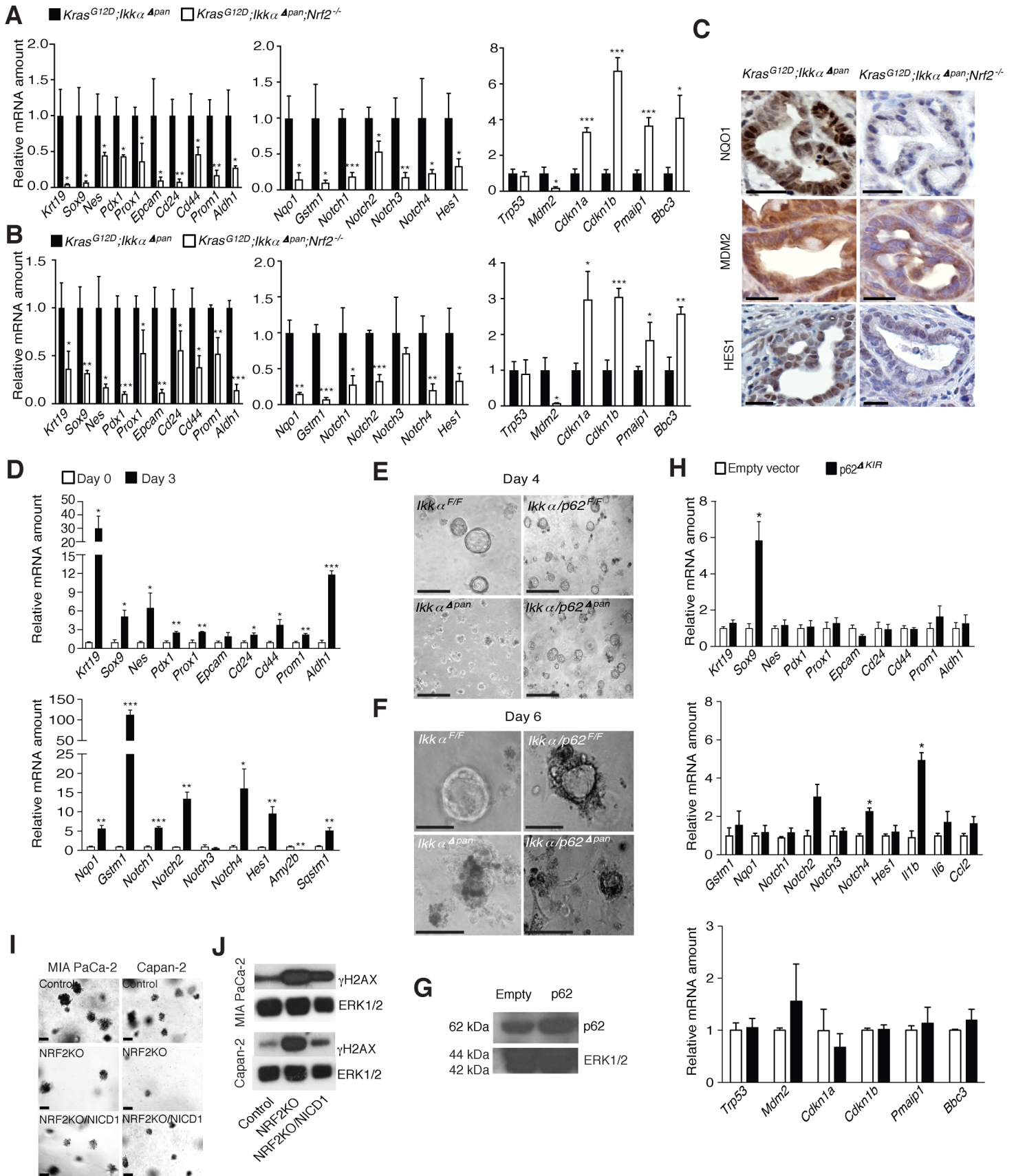


Figure S5 Effects of NRF2, p62 and Matrigel Culture on Gene Expression and Ductal Structure Formation, Related to Figure 5 (A, B) Q-RT-PCR analysis of indicated mRNAs in acinar (A) and ductal (B) pancreatic cells isolated from 5-week-old *Kras*^{G12D};*Ikkα*^{Apan} and *Kras*^{G12D};*Ikkα*^{Apan};*Nrf2*^{-/-} mice (n = 5). (C) NQO1, MDM2 and HES1 IHC of pancreatic tissue sections from 5-week-old mice (ductal lesions are shown). Scale bars: 25 μm. (D) Q-RT-PCR analysis indicated mRNAs in freshly isolated acinar cells (d0) and ductal structures formed after 3 days in Matrigel (d3) by the same cells (n = 5). (E, F) Images of ductal structures formed by the indicated acinar cell genotypes after 4 (E) and 6 (F) days in Matrigel. Scale bar: 50 μm (E), 20 μm (F). (G) p62 IB analysis of WT acinar cells transfected with either empty or p62 expression vectors. (H) Q-RT-PCR analysis of acinar cells transfected with p62 KIR⁻ (*p62*^{AKIR}) or empty expression vectors (n = 3). (I) Representative images of spheres formed by WT or NRF2-ablated MIA PaCa-2 and Capan-2 cells with or without NICD1 overexpression. Scale bars: 100 μm. (J) IB analysis of indicated proteins in whole cell lysates. All results depicted by bar graphs are mean ± SEM *, p < 0.05; **, p < 0.01; ***, p < 0.001. Statistical significance was calculated using Student's t test.

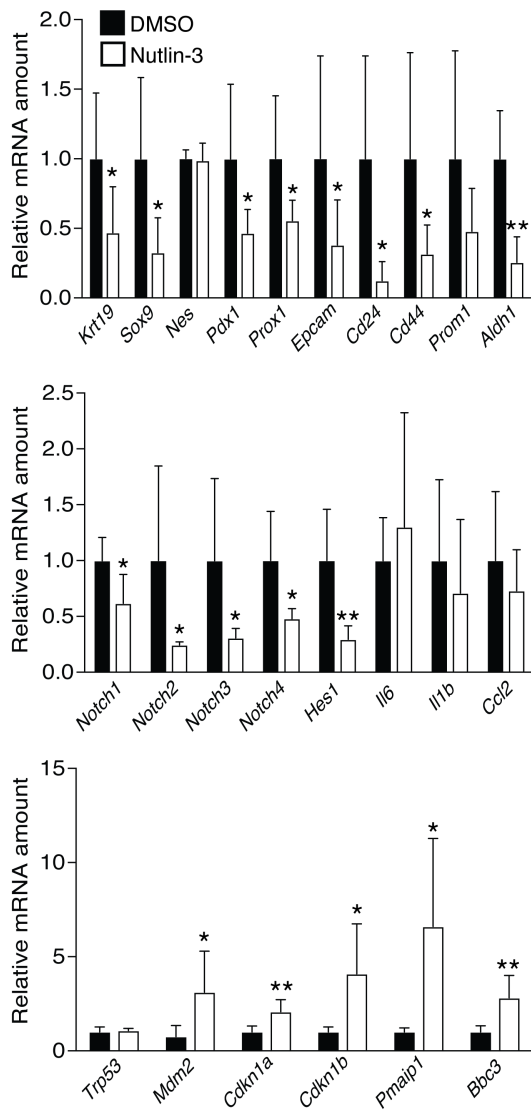
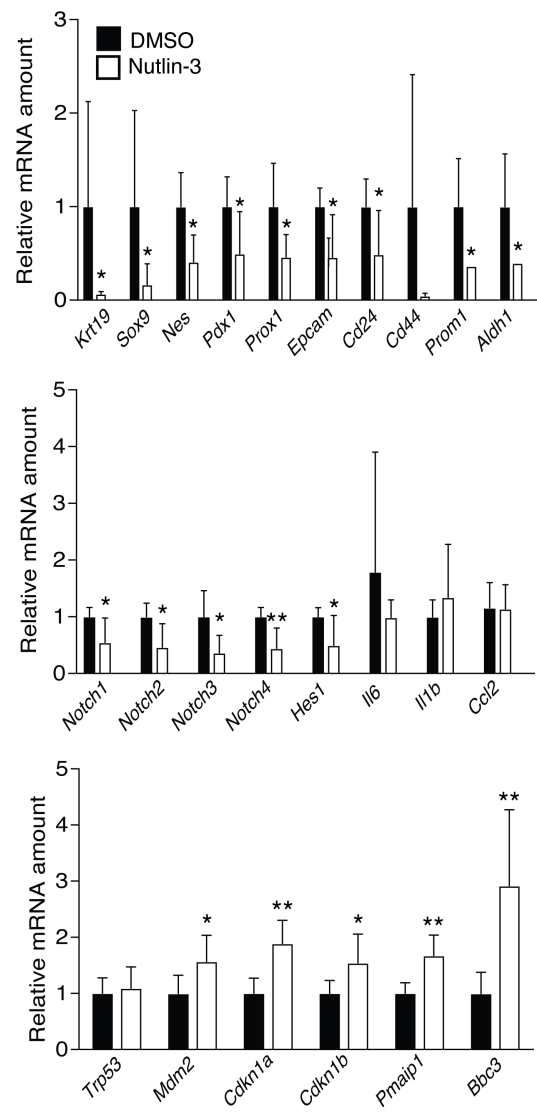
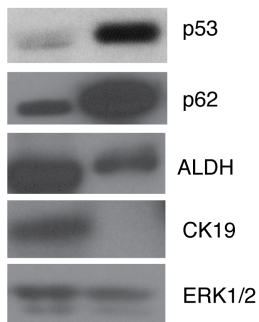
A**B****C***Ikkα^{4pan} Ikkα/Atg7^{Δpan}*

Figure S6 In Vivo Nutlin-3 Treatment Impairs Expression of Ductal, Progenitor and Stemness Markers, Related to Figure 6 (A, B) Q-RT-PCR analysis of mRNAs isolated from pancreata of *Kras*^{G12D} (A) and *Kras*^{G12D};*Ikkα*^{Apan} (B) mice (n = 7 each group) treated as indicated. (C) IB analysis of the indicated proteins in pancreatic lysates from *Ikkα*^{Apan} and *Ikkα/Atg7*^{Apan} mice (n = 4 each group). Results in A and B are mean ± SEM *, p < 0.05; **, p < 0.01; ***, p < 0.001 by Student's t test.

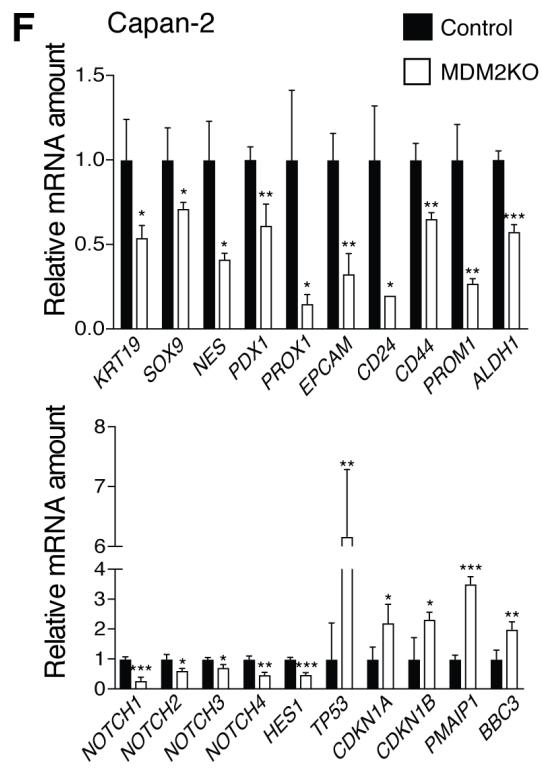
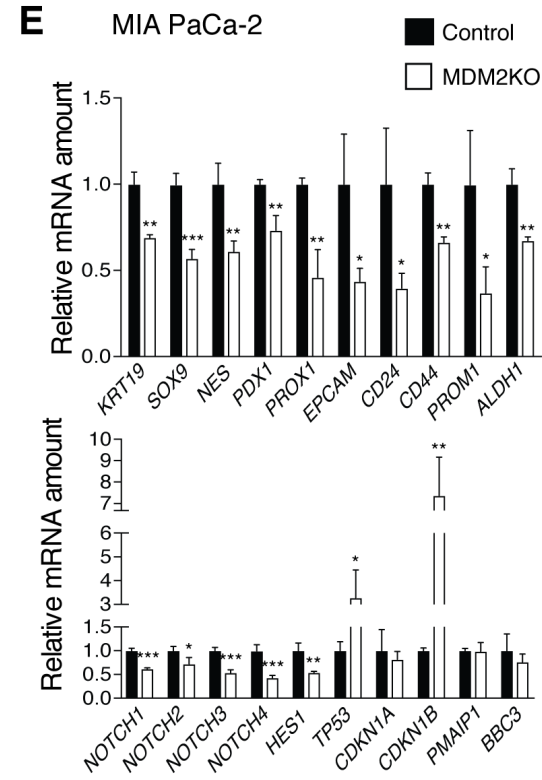
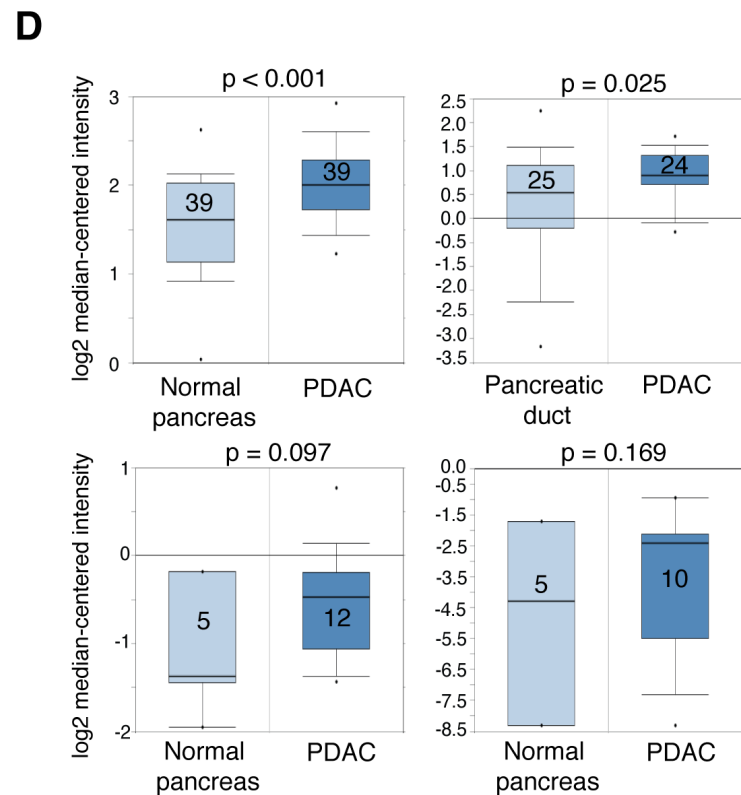
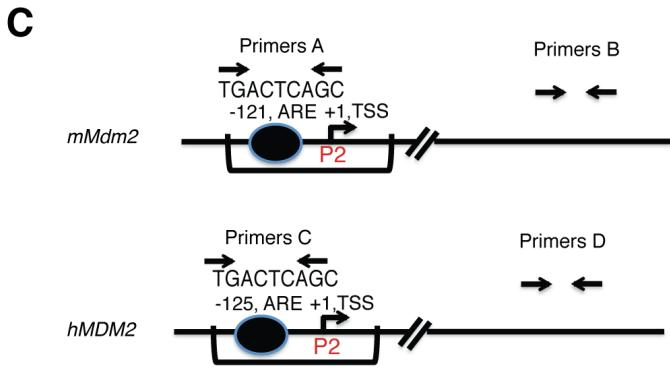
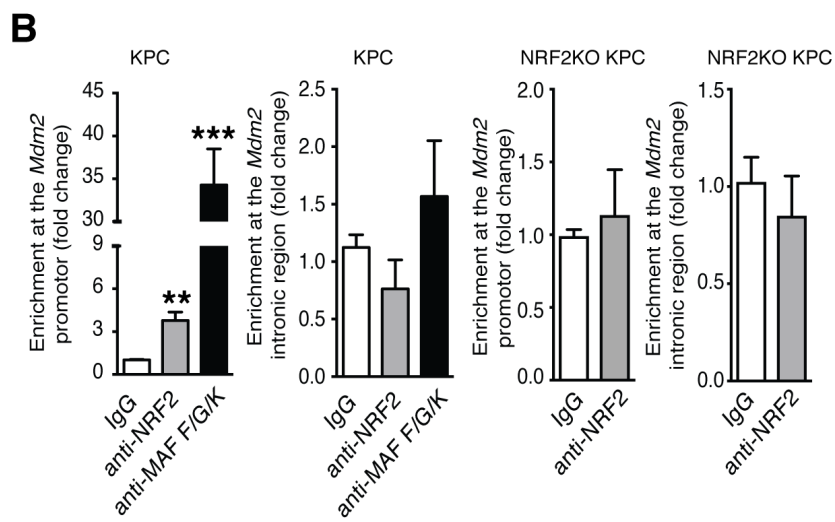
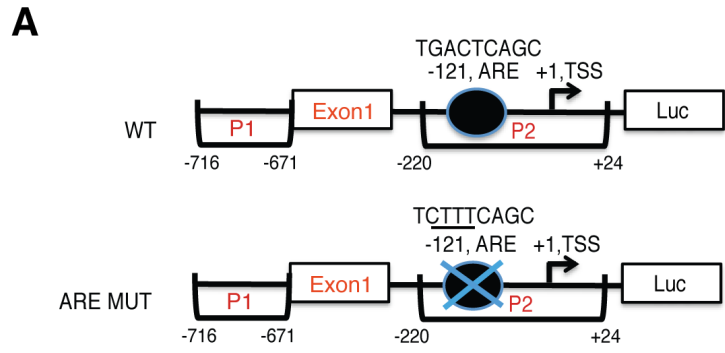


Figure S7. NRF2 Directly Controls *MDM2* Transcription in PDAC, Related to Figure 7 (A) Reporter constructs used to assess the functionality of the NRF2 binding site/ARE upstream to the *Mdm2* promoter 2 (P2). (B) Chromatin immunoprecipitation assays probing NRF2 and small MAF protein recruitment to the *Mdm2/MDM2* promoters in WT and NRF2 ablated KPC (n = 3) cells. (C) Positions of the ChIP primer sets used to assess NRF2 binding to the *Mdm2/MDM2* control region, including the P2 promoter with the adjacent NRF2 binding site. (D) Relative *MDM2* mRNA amounts in normal and cancerous pancreatic tissue were obtained from www.oncomine.org and expressed as log₂ median-centered intensity. Numbers within the bars represent study participants. p values were obtained from public datasets. (E, F) Q-RT-PCR mRNA analysis of MIA PaCa-2 cells (E) and Capan-2 cells (F), transfected with either MDM2 KO CRISPR/Cas9 or control vector (n = 3). Results in B, E and F are mean ± SEM; *, p < 0.05; **, p < 0.01; ***, p < 0.001 by Student's t test.

Table S1, related to the STAR Methods. Primers used for analyzing changes in gene expression levels.

Gene name (Forward-F; Reverse-R;)	Primer sequences
<i>mAldh1(Aldh1a1) F</i>	CTGTGAAGGCTGCAAGACAGG
<i>mAldh1(Aldh1a1) R</i>	GTCAGCCAGCTTGTTTCAGCAG
<i>mAmy2b F</i>	TGGTGACAAGGTGCAACAATG
<i>mAmy2b R</i>	GATTGCCTGAGCCACACATG
<i>mαSma (Acta2) F</i>	GTTTCAGTGGTGCCTCTGTCA
<i>mαSma (Acta2) R</i>	ACTGGGACGACATGGAAAAG
<i>mCcl2 F</i>	GCCAGCTCTCTTCCCTCCA
<i>mCcl2 R</i>	CCCAGAAGCATGACAGGGAC
<i>mCcl5 F</i>	AATCCCCTACTCCCCTCGG
<i>mCcl5 R</i>	TTCTTGGGTTTGCTGTGCAG
<i>mCd3(Cd3e) F</i>	GAGAGACATCGCCTTCTGTGG
<i>mCd3(Cd3e) R</i>	GCTGAAGAGCAAGCTGTGGAG
<i>mCd19 F</i>	TATGCAGCTCCTCAGCTCCAC
<i>mCd19 R</i>	CCATGCTGGTTCTAGGTCGTC
<i>mCd24(Cd24a) F</i>	TGACCGATAAGGCCATAGTGC
<i>mCd24(Cd24a) R</i>	CGCCTGGTAGTTCCTTCCAAC
<i>mCd44 F</i>	CGGAATCTGCAGAGTGTGGAC
<i>mCd44 R</i>	CAGGAATGACGTCTCCAATCG
<i>mCd133(Prom1) F</i>	AATTCGCTCAGCAGCAGTGAC
<i>mCd133(Prom1) R</i>	TGCTTAGGCTTGGTCTGATGC
<i>Ck19 (Krt19) F</i>	GGGGGTTTCAGTACGCATTGG
<i>Ck19 (Krt19) R</i>	GAGGACGAGGTCACGAAGC
<i>mCol1a1 F</i>	TAGGCCATTGTGTATGCAGC
<i>mCol1a1 R</i>	ACATGTTTCAGCTTTGTGGACC
<i>mCol13 F</i>	TAGGACTGACCAAGGTGGCT
<i>mCol13 R</i>	GGAACCTGGTTTCTTCTCACC
<i>mEmr1(Adgre1): F</i>	GACTGACAACCAGACGGCTTG
<i>mEmr1(Adgre1): R</i>	TCACTGCCTCCACTAGCATCC
<i>mEpcam F</i>	TTAATGCCTAGCCGTGCTGAG
<i>mEpcam R</i>	TCTGCAGTCCGAGCTCTTCTG
<i>mGclc F</i>	TTTCATGATCGAAGGACACCA
<i>mGclc R</i>	CTGCACATCTACCACGCAGT
<i>mGclm F</i>	GGCTGATTTGGGAACCTCCAT
<i>mGclm R</i>	CGGGAACCTGCTCAACTG
<i>mGgt1 F</i>	GGTGGCGTAGAACTCAGAGC
<i>mGgt1 R</i>	TTTGCCTATGCCAAGAGGAC
<i>mGstal F</i>	CTGGACTGTGAGCTGAGTGG
<i>mGstal R</i>	CATTGAAGTGGTGAAGCAGC
<i>mGstm1 F</i>	CTACCTTGCCCGAAAGCAC
<i>mGstm1 R</i>	ATGTCTGCACGGATCCTCTC
<i>mHes1 F</i>	TCTACACCAGCAACAGTG
<i>mHes1 R</i>	TCAAACATCTTTGGCATCAC
<i>mHey1 F</i>	GCGGACGAGAATGGAAACTTG
<i>mHey1 R</i>	GCTCAGATAACGGGCAACTTC
<i>mHmox1 F</i>	CCTTCAAGGCCTCAGACAAA
<i>mHmox1 R</i>	GAGCCTGAATCGAGCAGAAC
<i>mI11-β (I11b) F</i>	CAACCAACAAGTGATATTCTCCATG
<i>mI11-β (I11b) R</i>	GATCCACACTCTCCAGCTGCA
<i>mI16 F</i>	GAGGATAACCACTCCCAACAGACC
<i>mI16 R</i>	AAGTGCATCATCGTTGTTTCATACA

<i>mJag1 F</i>	GCCACCTGTGTGGATGAGATC
<i>mJag1 R</i>	GGCACTTGGCACCACCTATGTC
<i>mMdm2 F</i>	TTCGTGAGAACTGGCTTCCAG
<i>mMdm2 R</i>	AGGCACATCCAAGCCTTCTTC
<i>mMmp7 F</i>	CCCGGTACTGTGATGTACCC
<i>mMmp7 R</i>	AATGGAGGACCCAGTGAGTG
<i>mMuc5ac F</i>	CAGGACTCTCTGAAATCGTACCA
<i>mMuc5ac R</i>	AAGGCTCGTACCACAGGGA
<i>mMuc6 F</i>	CGGCTGCGTCTGTCCTAAG
<i>mMuc6 R</i>	GCATAGTCACATGGGCATTCTC
<i>mNes F</i>	CCCTGAAGTCGAGGAGCTG
<i>mNes R</i>	CTGCTGCACCTCTAAGCGA
<i>mNotch1 F</i>	GATGGCCTCAATGGGTACAAG
<i>mNotch1 R</i>	TCGTTGTTGTTGATGTCACAGT
<i>mNotch2 F</i>	AACGAGAAGGTCCAGCTGTCC
<i>mNotch2 R</i>	ATGTGGCATCGGAGACATACG
<i>mNotch3 F</i>	AGTCAATGGCTTCAGCTGCAC
<i>mNotch3 R</i>	GGAGTGCTTGCACACTCATCC
<i>mNotch4 F</i>	CTGGACCAGAATGCGAGACAG
<i>mNotch4 R</i>	GTTGTAGCCAGATGGCTGTGG
<i>mNoxa (Pmaip1) F</i>	TACCACCTGAGTTCGCAGCTC
<i>mNoxa (Pmaip1) R</i>	CAGTTATGTCCGGTGCCTCC
<i>mNqo1 F</i>	AGCGTTCGGTATTACGATCC
<i>mNqo1 R</i>	AGTACAATCAGGGCTCTTCTCG
<i>mPdx1 F</i>	TTAACCTAGGCGTCGCACAAG
<i>mPdx1 R</i>	TTCCAGAAGTCTGCCAGCATC
<i>mProx1 F</i>	GGAGATGGCTGAGAACAAGC
<i>mProx1 R</i>	AGACTTTGACCACCGTGTCC
<i>mPuma (Bbc3) F</i>	CGAAGACTCCAGAAGCAGCAG
<i>mPuma (Bbc3) R</i>	TGCTATTGAGGCACCTTGCTG
<i>mp21 (Cdkn1a) F</i>	TGAGGAGGAGCATGAATGGAG
<i>mp21 (Cdkn1a) R</i>	CATCACCAGGATTGGACATGG
<i>mp27 (Cdkn1b) F</i>	GCCTGACTCGTCAGACAATCC
<i>mp27 (Cdkn1b) R</i>	CTTCTGCAGCAGGTCGCTTC
<i>mp53 (Trp53) F</i>	GACCATCCTGGCTGTAGGTAGC
<i>mp53 (Trp53) R</i>	CAGTCTTCGGAGAAGCGTGAC
<i>mp62 (Sqstm1) F</i>	GCTGCCCTATACCCACATCT
<i>mp62 (Sqstm1) R</i>	CGCCTTCATCCGAGAAAC
<i>mSox9 F</i>	CGGAACAGACTCACATCTCTCC
<i>mSox9 R</i>	GCTTGCACGTCGGTTTTTG
<i>mTgfb1 F</i>	TTGCTTCAGCTCCACAGAGA
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<i>mTimp1 R</i>	GTAAGGCCTGTAGCTGTGCC
<i>mTnf F</i>	GCACAGAAAGCATGACCCG
<i>mTnf R</i>	GCCCCCATCTTTTGGG
<i>m18s F</i>	AGCCCCTGCCCTTTGTACACA
<i>m18s R</i>	CGATCCGAGGGCCTCACTA
<i>hALDH1 (ALDH1a1) F</i>	TTAGCTGATGCCGACTTGGAC
<i>hALDH1 (ALDH1a1) R</i>	TCCTGGATGCGGCTATAACAAC
<i>hCD24 F</i>	AGTGCAGTGGTGCATCTCAG
<i>hCD24 R</i>	GTGGCAGGTGCCTGTAATCC
<i>hCD44 F</i>	TTGCATTGCAGTCAACAGTCG

<i>hCD44 R</i>	CTGTCCTCCACAGCTCCATTG
<i>hCD133 (PROM1) F</i>	GCGTTGGAGAACATGAACAGC
<i>hCD133 (PROM1) R</i>	TGAGAGATGACCGCAGGCTAG
<i>hCK19 (KRT19) F</i>	ATCCTGAGTGACATGCGAAGC
<i>hCK19 (KRT19) R</i>	AACCAGGCTTCAGCATCCTTC
<i>hEPCAM F</i>	GATCCTGACTGCGATGAGAGC
<i>hEPCAM R</i>	GCAGTGTTACACACCAGCAC
<i>hGSTM1 F</i>	AGCAACGCCATCTTGTGCTAC
<i>hGSTM1 R</i>	TGGTTGTCCATGGTCTGGTTC
<i>hHES1 F</i>	CCGGAGCTGGTGCTGATAAC
<i>hHES1 R</i>	TCAGTAGCGCTGTTCCAGGAC
<i>hNES F</i>	AGGACCAAGAAGCTGGCTCAGG
<i>hNES R</i>	CCAGTGAAGCCATCCTGCTC
<i>hNFE2L2 F</i>	GCCTGTAAGTCCTGGTCATCG
<i>hNFE2L2 R</i>	TTGTGAGATGAGCCTCCAAGC
<i>hNOTCH1 F</i>	GATGCCAACATCCAGGACAAC
<i>hNOTCH1 R</i>	CCGGATCAGGATCTGGAAGAC
<i>hNOTCH2 F</i>	CACTGTGGCCAACCAGTTCTC
<i>hNOTCH2 R</i>	CTGGCAGTGTCTGGAATGTC
<i>hNOTCH3 F</i>	ATCTGGTTGCTGCTGACATCC
<i>hNOTCH3 R</i>	ATTGACATCCATGCCATCAGC
<i>hNOTCH4 F</i>	CACTAGGCGAGGACAGCATTG
<i>hNOTCH4 R</i>	GCCTGAGCACATCACAACCTCC
<i>hNQO1 F</i>	CTGGAGTGCAGTGGTGTGATC
<i>hNQO1 R</i>	AGGCAGGAGAATTGCTGGAAC
<i>hPDX1 F</i>	TCCTACAGCACTCCACCTTGG
<i>hPDX1 R</i>	GGAGCCTTCCAATGTGTATGG
<i>hPROX1 F</i>	CGAAGCGAGAAGGCAACAAC
<i>hPROX1 R</i>	CGACATGGCAGTGTTTCAGTTC
<i>hP27 (CDKN1B) F</i>	TCCGGCTAACTCTGAGGACAC
<i>hP27 (CDKN1B) R</i>	TGCAGGTCGCTTCCTTATTCC
<i>hP53 (TP53) F</i>	CCTTGCTTGCAATAGGTGTGC
<i>hP53 (TP53) R</i>	AGTGCAGGCCAACTTGTTTCAG
<i>hP62 (SQSTM1) F</i>	GAAGAGCAGCTCACAGCCAAG
<i>hP62 (SQSTM1) R</i>	AAGGCGATCTTCCTCATCTGC
<i>hSOX9 F</i>	CATCAAGACGGAGCAGCTGAG
<i>hSOX9 R</i>	GGCTGTAGGCGATCTGTTGG
<i>h18S F</i>	GGACACGGACAGGATTGACAG
<i>h18S R</i>	CAACTAAGAACGGCCATGCAC