

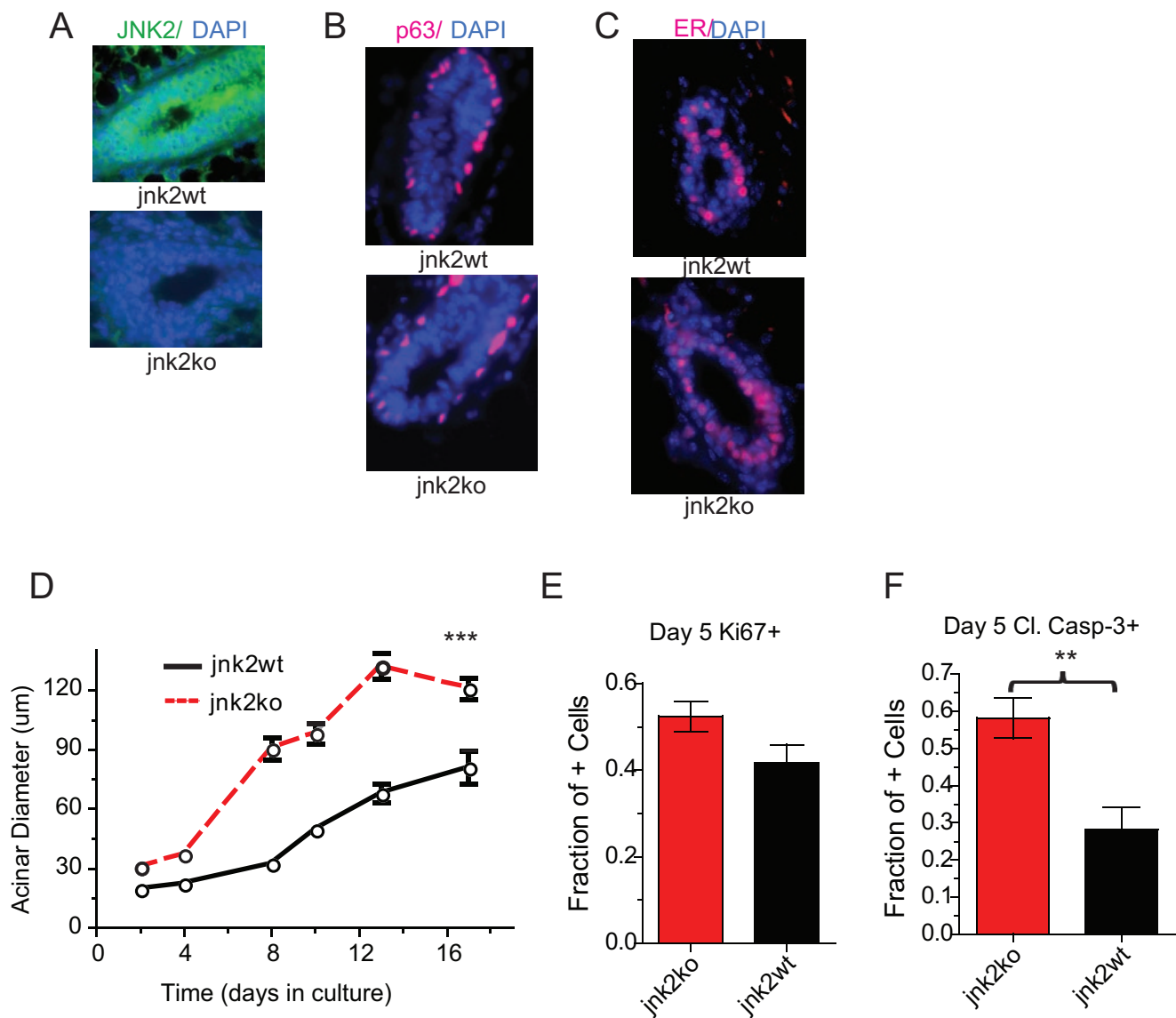
SUPPLEMENTARY DATA

Supplemental Experimental Procedures:

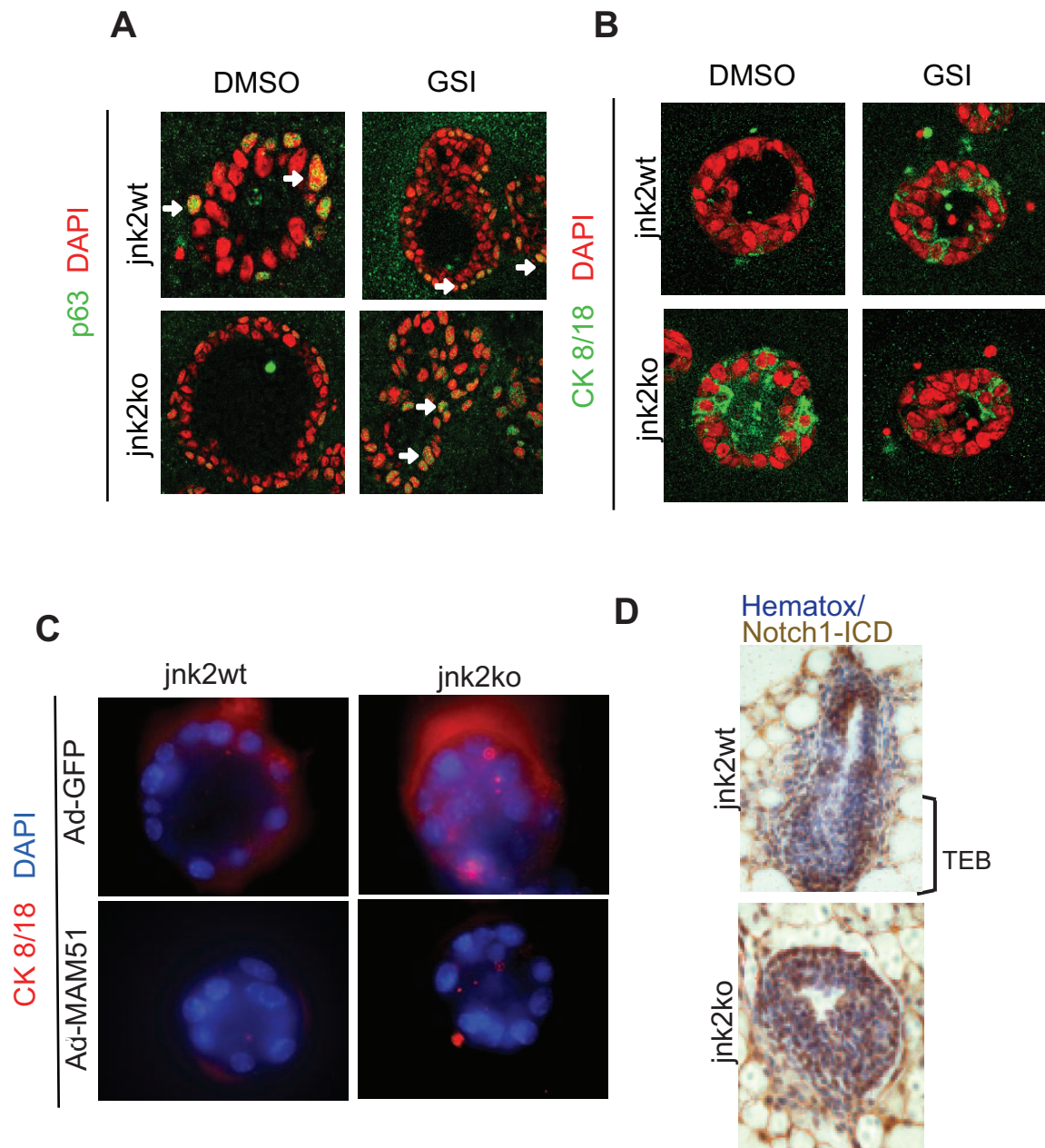
Primers used (all are against mouse, unless otherwise indicated):

qPCR primers:

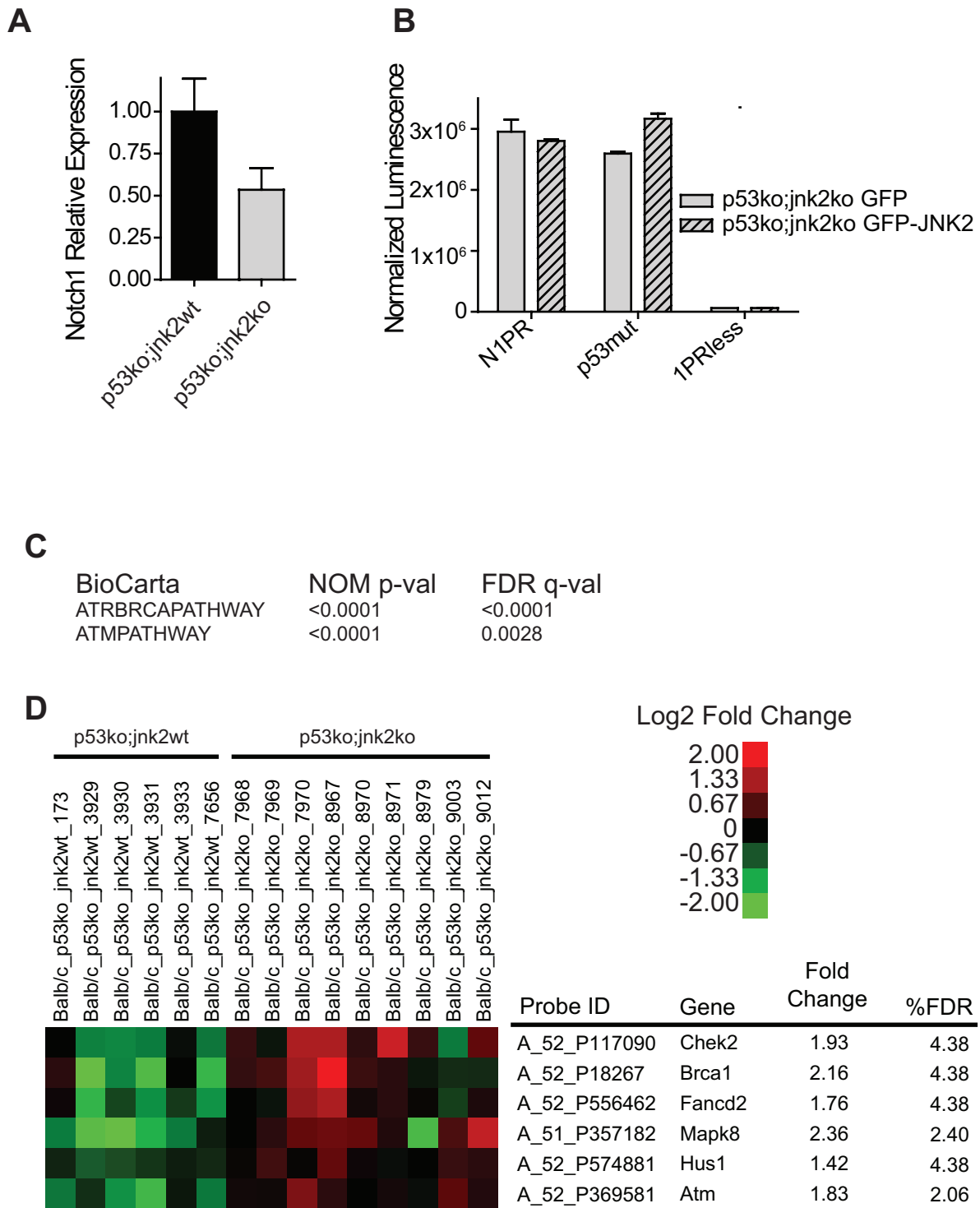
hes1 NM_008235.2 (5'-CACAGAAAGTCATCAAAGCC and 5'-TGCTTGACAGTCATTTCCAG),
notch1 NM_008714.3 (5'-CTACAGAAGGTTACACAG and 5'-CAGAGGTAGGAGTTGTCACG),
p53 NM_001127233.1 (5'-TGAACCGCCGACCTATCCTTA and GGCACAAACACGAACCTCAAA),
twist1 NM_011658.2 (5'-AATTCACAAGAATCAGGGCGTGTTGGG and 5'-TCTATCAGAATGCAGAGGTTGTTGGG),
snai1 NM_011427.2 (5'-TCCAAACCCACTCGGATGTGAAGA and 5'-TTGGTGCTTGTGGAGCAAGGACAT),
snai2 NM_011415.2 (5'-CACATTTCGAACCCACACATTGGCT and 5'-TGTGCCCTCAGGTTTGATCTGTCT),
zeb1 NM_011546.3 (5'-CAGTGTTCATGTTTAAGAGCA and 5'-GTCTTTCATCCTGGTTTCCG),
klf4 NM_010637.3 (5'-CATTATCAAGAGTCTATGCCA and 5'-CACAGTGGTAAGGTTTCTCG),
cdh1 NM_009864.2 (5'-GCCAAGTACATCCTCTATTCTC and 5'-GCAACGAATCCCTCAAAGAC),
gapdh NM_008084.2 (5'-CGTGGAGTCTACTGGCGTCTTTCAC and 5'-CGGGGATGATGAGCCTTTTGGC),
brca1 NM_009764.3 (5'-CCAAAGAAGTAATGACCGTG and 5'-GCTAACTATCCACTTTCCTCC),
gata-3 NM_008091.3 (5'-ACGAATCCAGCACAGAAGG and 5'-ATGTCCTGCTCTCCTTG),
ck14 NM_016958.1 (5'-TCTTCAGCAAGACAGAGGAG and 5'-CTCCAGGTTATTCTCCAGGG),
p63 NM_001127259.1 (5'-GTTCAATGAGGGACAGATTGC and 5'-GAATTCAGTGCCAACCTGTG),
Human *brca1* NM_007294.3 (5'-CCCAGAAGAATTTATGCTCGT and 5'-CATTGACCACATCTCCTCTG),
Human *gapdh* BC096440.1 (5'-AAGGTGAAGGTTCGGAGTCAA and 5'-AATGAAGGGGTCATTGATGG).
ChIP primers:
p21 NC_000006.11 (5'-GATTCCTTTCTATCAGCCC and GTCACAAGATACATACCACCT),
gapdh NC_000012.11 (5'-GCCAAAGACAGAAGCCAGGA and 5'-CAGGATAGGACTCAGGGAATACAG),
notch1 NC_000068.7 (5'-GTGACCGTGGAACGTCTA 5'-CTGTCCTAGGGCTCCAC)



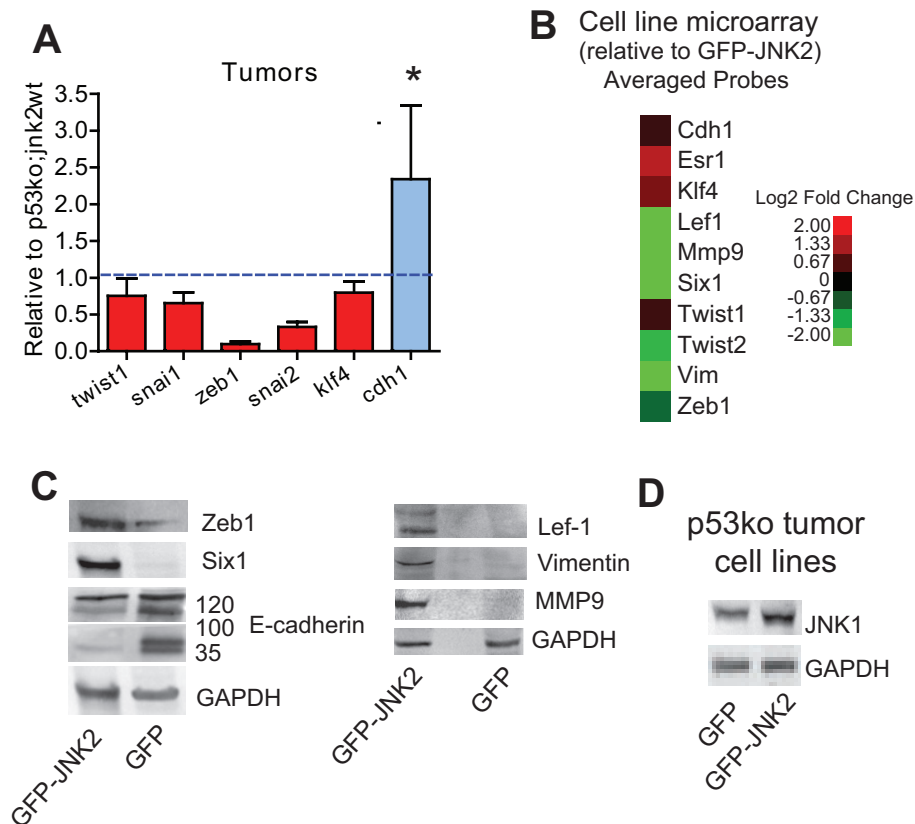
Supplementary Figure S1: Effect of JNK2 on mammary epithelial cells. **A.** Representative images of adult (8 wk-old) ducts probed for JNK2 expression; **B-C.** Images of p63⁺ basal cells and ER⁺ luminal cells in adult ducts ($n = 3$); **D.** *Jnk2wt* and *jnk2ko* mammary epithelial cells were isolated and grown in 3D culture. Acinar diameter was measured at indicated time points. Linear regression analysis was performed, $***p < 0.0001$; **E-F.** Quantification of Ki67⁺ and cleaved caspase 3⁺ cells grown in 3D culture. Nonparametric *t*-test was performed, $**p < 0.001$.



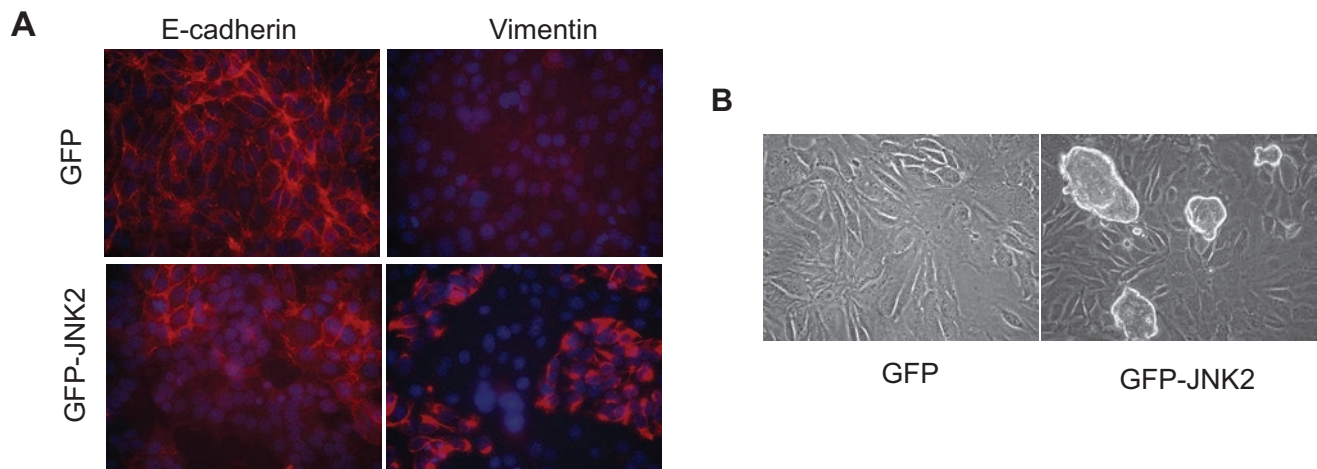
Supplementary Figure S2: Representative images of JNK2 effects on mammary cell differentiation and Notch activity. **A.** Representative staining where arrows indicate p63⁺ cells in 3D culture; **B-C.** Representative staining of ER⁺ mammary cells in 3D culture; **D.** Representative staining of Notch1^{ICD} in TEBS.



Supplementary Figure S3: JNK2 effects on gene expression in *p53ko* tumors or tumor cell lines. **A.** RNA from tumors was analyzed for Notch1 expression using qPCR. A nonparametric, two-tailed *t*-test was used to detect statistical differences between two groups; **B.** *p53ko;jnk2ko* GFP and GFP-JNK2 expressing cells were transfected with Notch1 promoter-luciferase constructs and analyzed for promoter activity. A 1 Way ANOVA with post-hoc *t*-test was performed; **C.** Gene Set Enrichment Analysis (GSEA) of *p53ko;jnk2ko* and *p53ko;jnk2wt* tumor microarrays shows upregulation of the ATRBRCPATHWAY and ATMPATHWAY gene sets in *jnk2ko* tumors; **D.** Genes significantly elevated with false discovery rate (FDR) of < 5% in the ATM/BRCA1 pathway.



Supplementary Figure S4: JNK2 promotes EMT in the p53ko tumor model. **A.** Expression of mesenchymal/stem (red) and epithelial (blue) markers in *p53ko* tumors ($n = 8$), **B.** Fold changes observed in microarray analysis of EMT/stem and differentiation markers in *p53ko;jnk2ko* GFP and GFP-JNK2 cell lines; **C.** Expression of EMT/stem-related proteins in *p53ko;jnk2ko* GFP and GFP-JNK2 cell lines by western blot; **D.** Expression of JNK1 in *p53ko;jnk2ko* GFP and GFP-JNK2 cell lines by western blot. A nonparametric, two-tailed *t*-test was also performed for A. * $p < 0.05$.



Supplementary Figure S5: JNK2 promotes vimentin expression and inhibits e-cadherin in the *p53ko;jnk2ko* cell line.
A. Adherent cells were stained using vimentin and e-cadherin primary antibodies; B. Adherent cells grown in culture.