

Inventory of Supplemental Data:

Supplemental Figure 1: Molecular characterization of SCC tumors and cell lines

Supplemental Figure 2: Excision of *p63* in murine SCC

Supplemental Figure 3: Microarray identification of FGFR2 in SCC

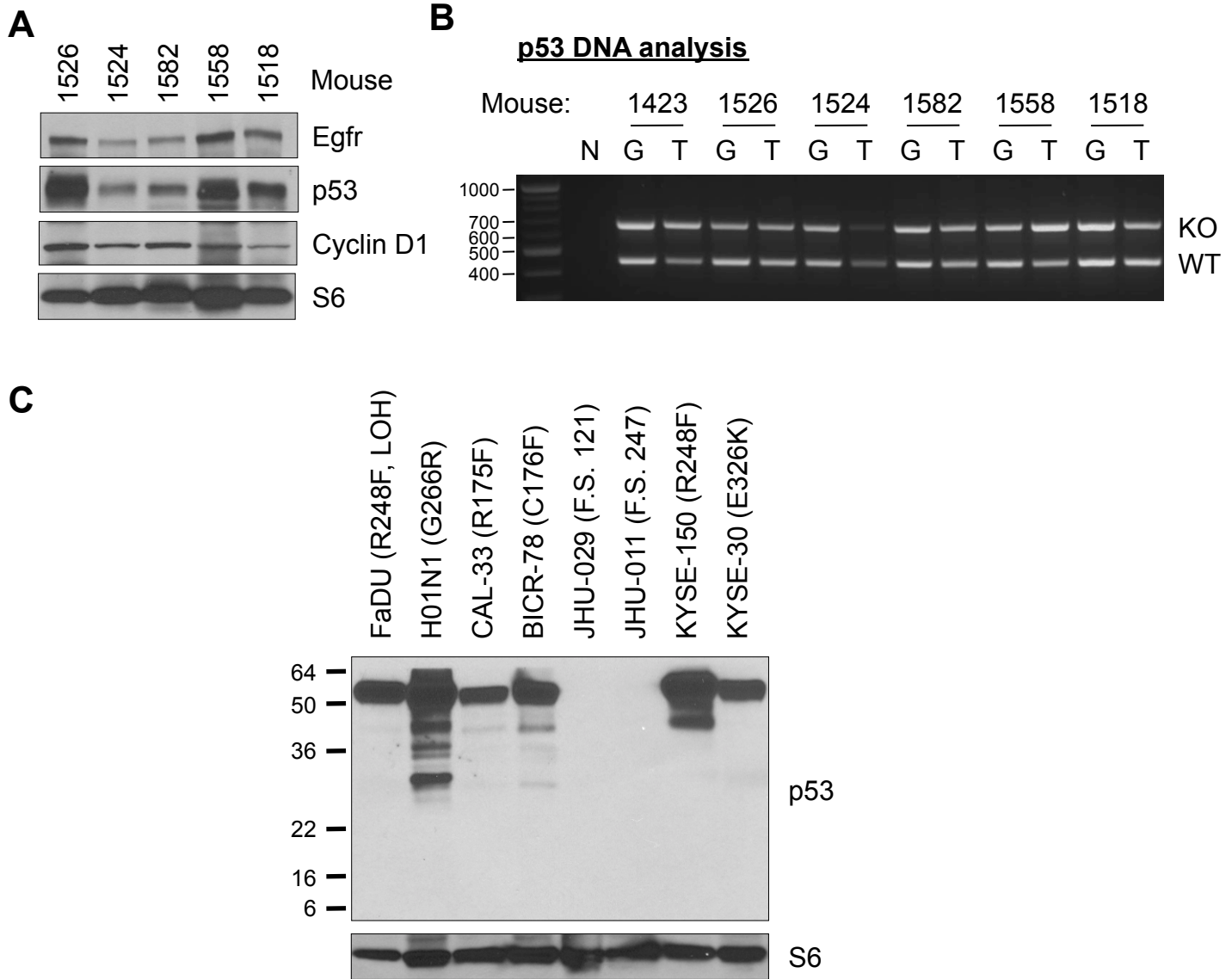
Supplemental Figure 4: Direct functional regulation of *Fgfr2* by p63

Supplemental Figure 5: Activation of p63-Fgfr2-Fgf7 axis in SCC and hair follicles

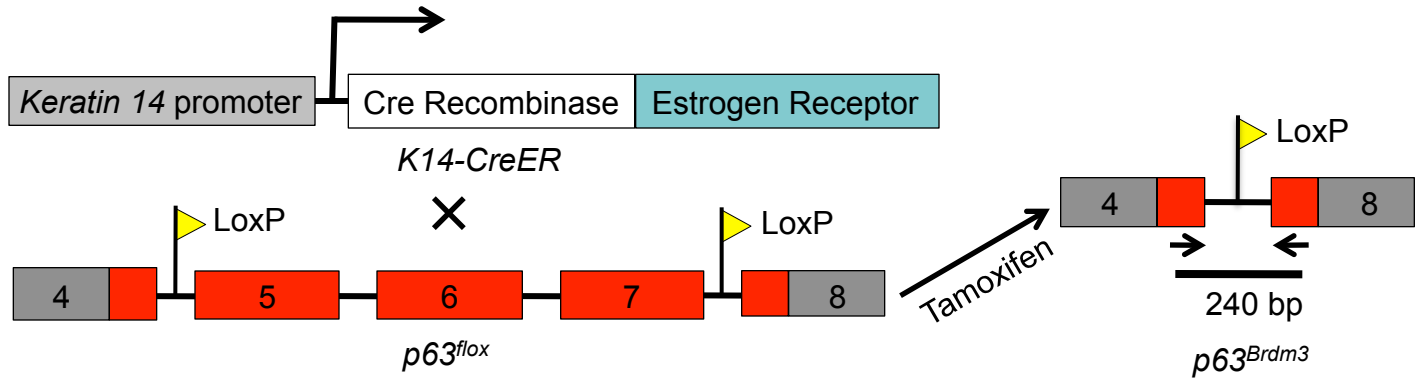
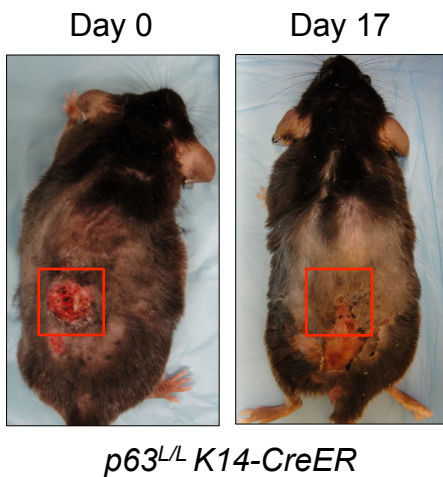
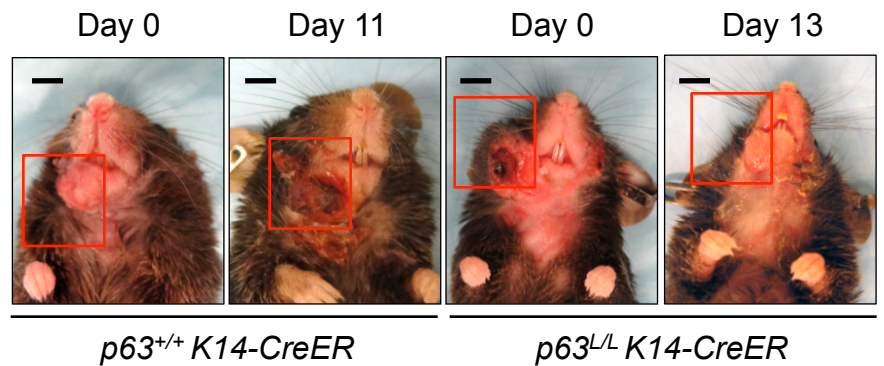
Supplemental Figure 6: AZD4547 treatment of SCC cells and tumors

Supplemental Table 1: Sequences for primers used in this study

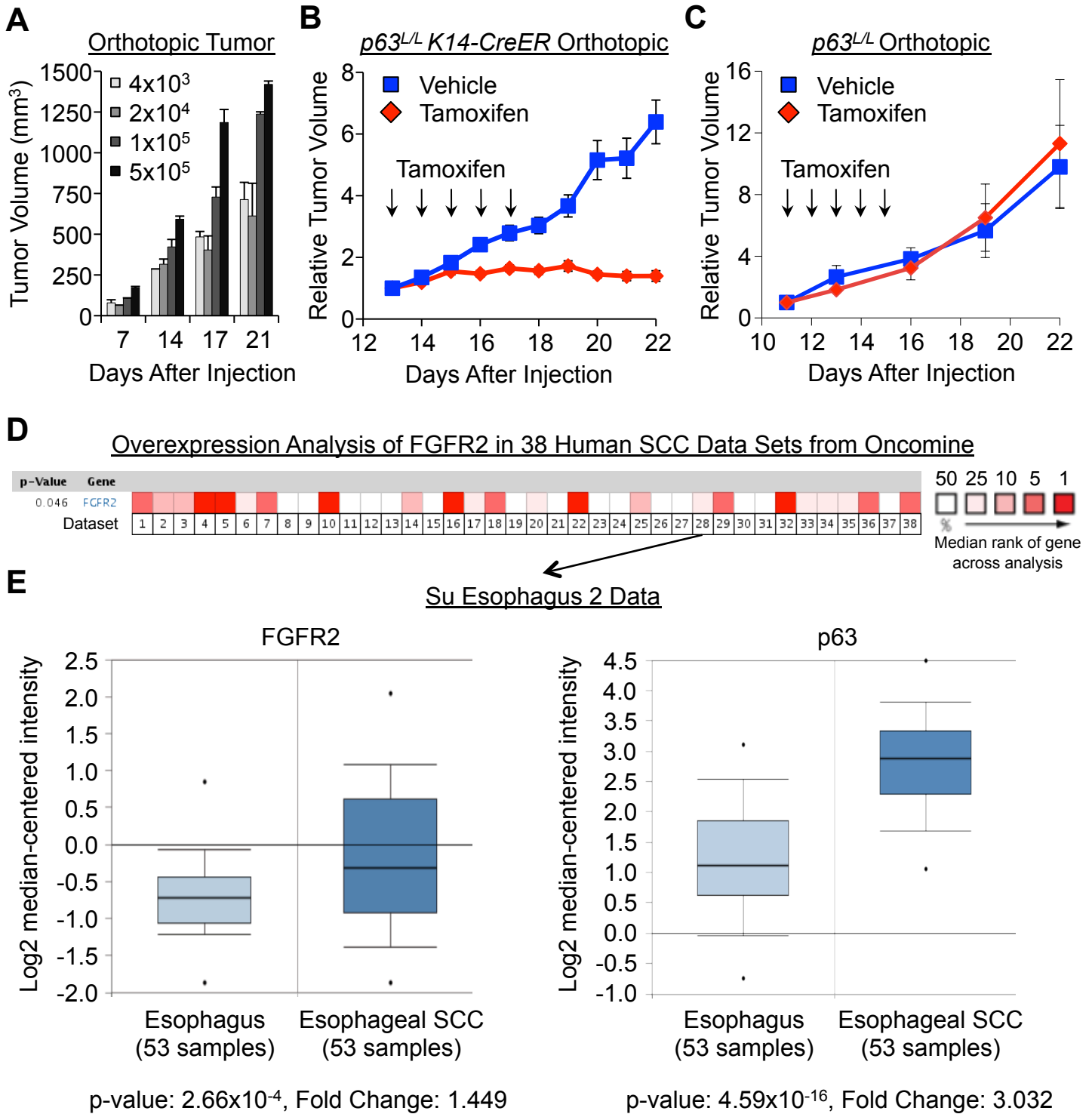
Supplemental References for Supplemental Figure 3



Supplemental Figure 1. Molecular characterization of SCC tumors and cell lines. (A) Western blot analysis of indicated proteins in murine SCC tumors. Ribosomal S6 serves as a loading control. (B) PCR analysis for loss of *Wild-type* p53 in murine SCC tumors. N= no DNA, G= genomic DNA, T= Tumor DNA. *Wild-type* allele PCR product is 450 base pairs; *null* allele PCR product is 650 base pairs. Note reduced WT band in tumor 1423, suggesting LOH. (C) Western blot analysis of p53 protein in Human SCC cell lines using DO-1 antibody recognizing an epitope between amino acids 11 and 25. Ribosomal S6 serves as a loading control. Known p53 mutations are indicated. LOH = loss of heterozygosity. F.S. = frameshift mutation.

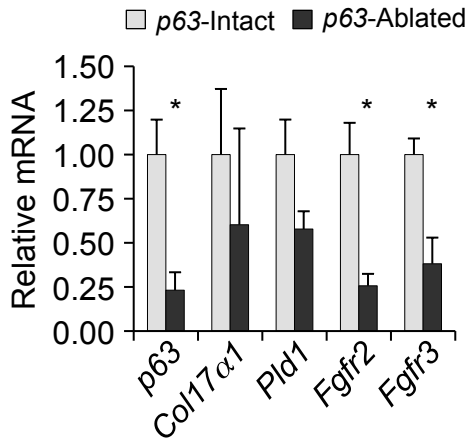
A**B****C**

Supplemental Figure 2. Excision of *p63* in murine SCC. (A) Schematic of generation of the $p63^{Brdm3}$ allele. Cre-mediated recombination results in excision of exons 5, 6, and 7, which encode the majority of the DNA-binding domain (red). PCR of genomic DNA with primers in exons 4 and 8 result in a 240bp product only in the recombined allele, as seen in Figure 2B. (B) Regression of cutaneous $p63^{L/L} K14-CreER$ tumor following tamoxifen treatment as described in Figure 2D. (C) Progression of $p63^{+/+} K14-CreER$ oral SCC tumor (left) versus regression of $p63^{L/L} K14-CreER$ tumor (right) following tamoxifen treatment as in Figure 2D. Scale bar = 0.5cm

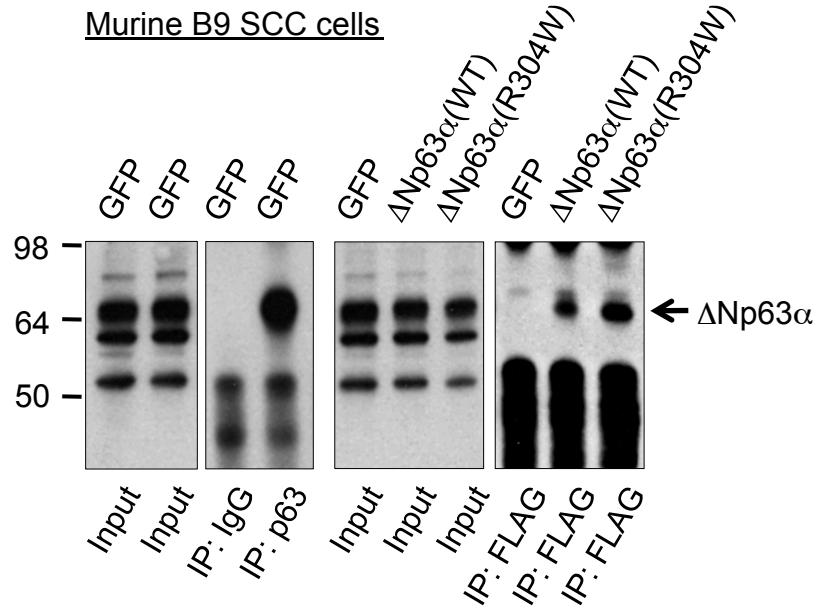


Supplemental Figure 3. Microarray identification of FGFR2 in SCC. (A) Growth of orthotopic tumors with varying numbers of viable CD31^{neg} CD45^{neg} cells (n=2 per set). Tumor and stromal cells were not separated during FACS sorting, and cell number represents total number of tumor and stromal cells injected. (B) Tumor growth of *p63^{L/L} K14-CreER* orthotopic tumors following tamoxifen (n=12) or vehicle (n=14) treatment. Tumor volume is normalized to size prior to first treatment. $p < 0.0001$ as assessed by multiple measures ANOVA. Error bars +/- SEM. (C) Tumor growth of *p63^{L/L}* orthotopic tumors following tamoxifen (n=6) or vehicle (n=6) treatment. Tumor volume is normalized to size prior to first treatment. p-value is not significant as assessed by multiple measures ANOVA. Error bars +/- SEM. (D) Gene rank analysis of FGFR2 overexpression in Oncomine microarray datasets. Numbers refer to citations for primary data found in Supplemental References. (E) Representative FGFR2 and p63 levels in Esophagus compared to Esophageal SCC from the "Su Esophagus 2" dataset (Ref. 28). Whiskers indicate 95th percentile of data. p-values calculated by Student's t-test.

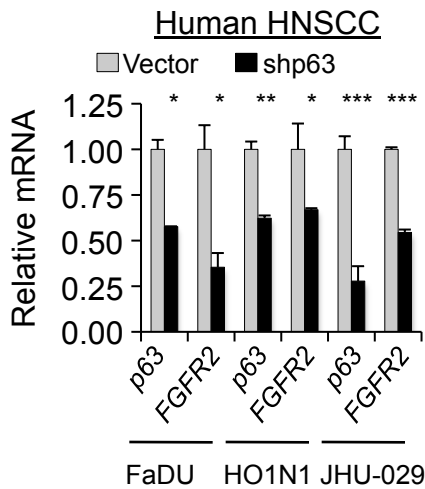
A Autochthonous Murine SCC



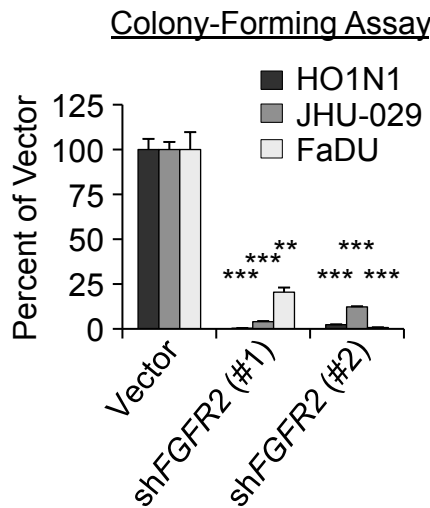
B Murine B9 SCC cells



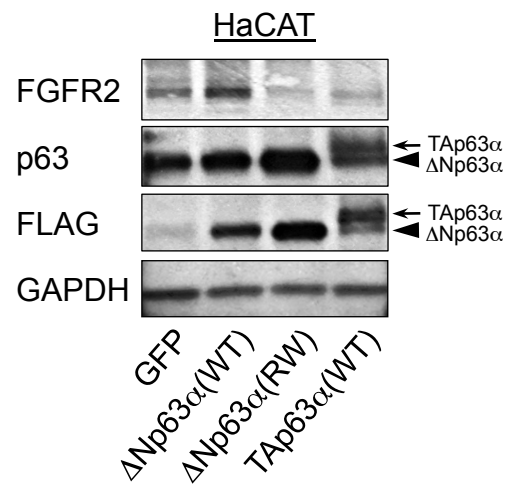
C Human HNSCC



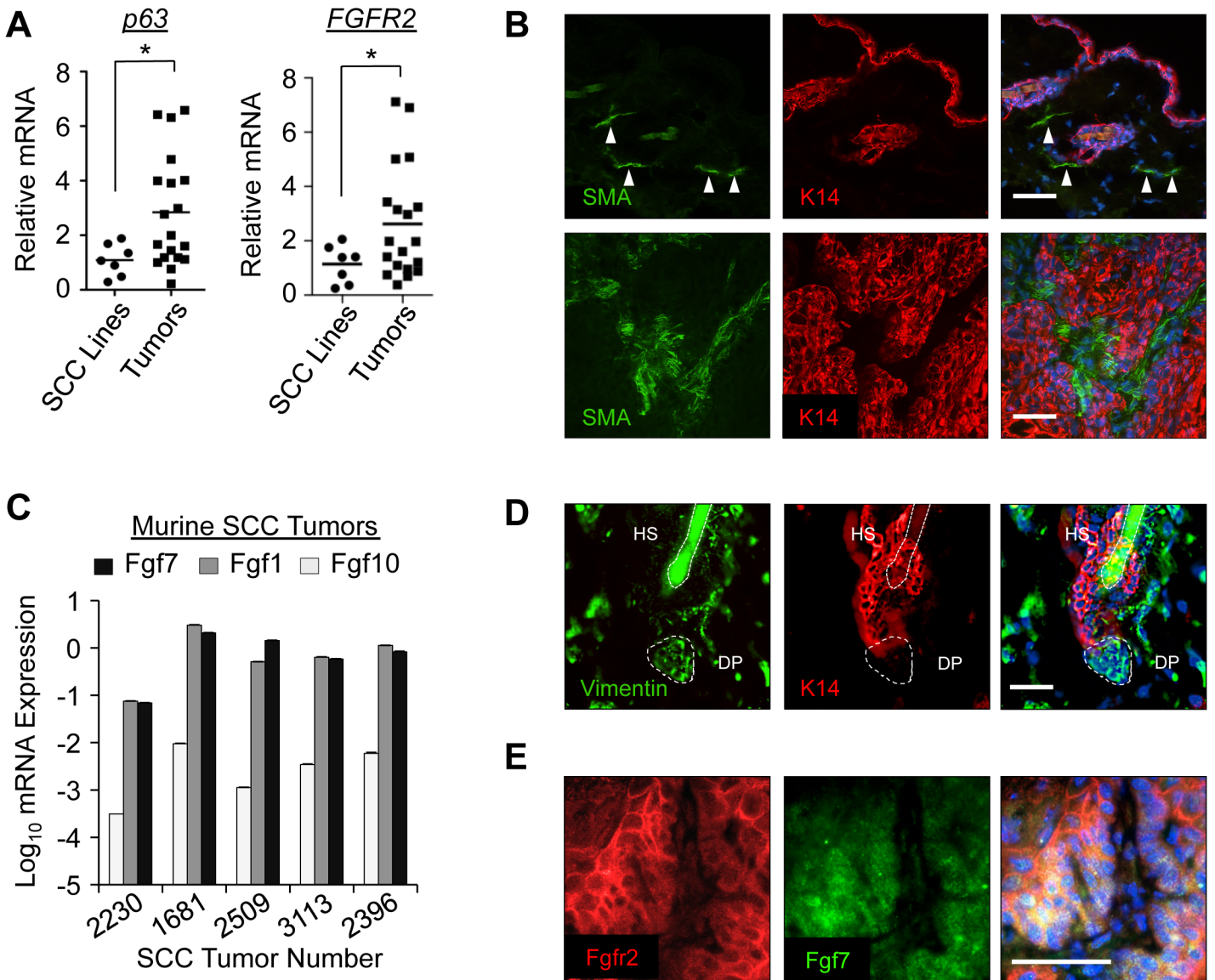
D Colony-Forming Assay



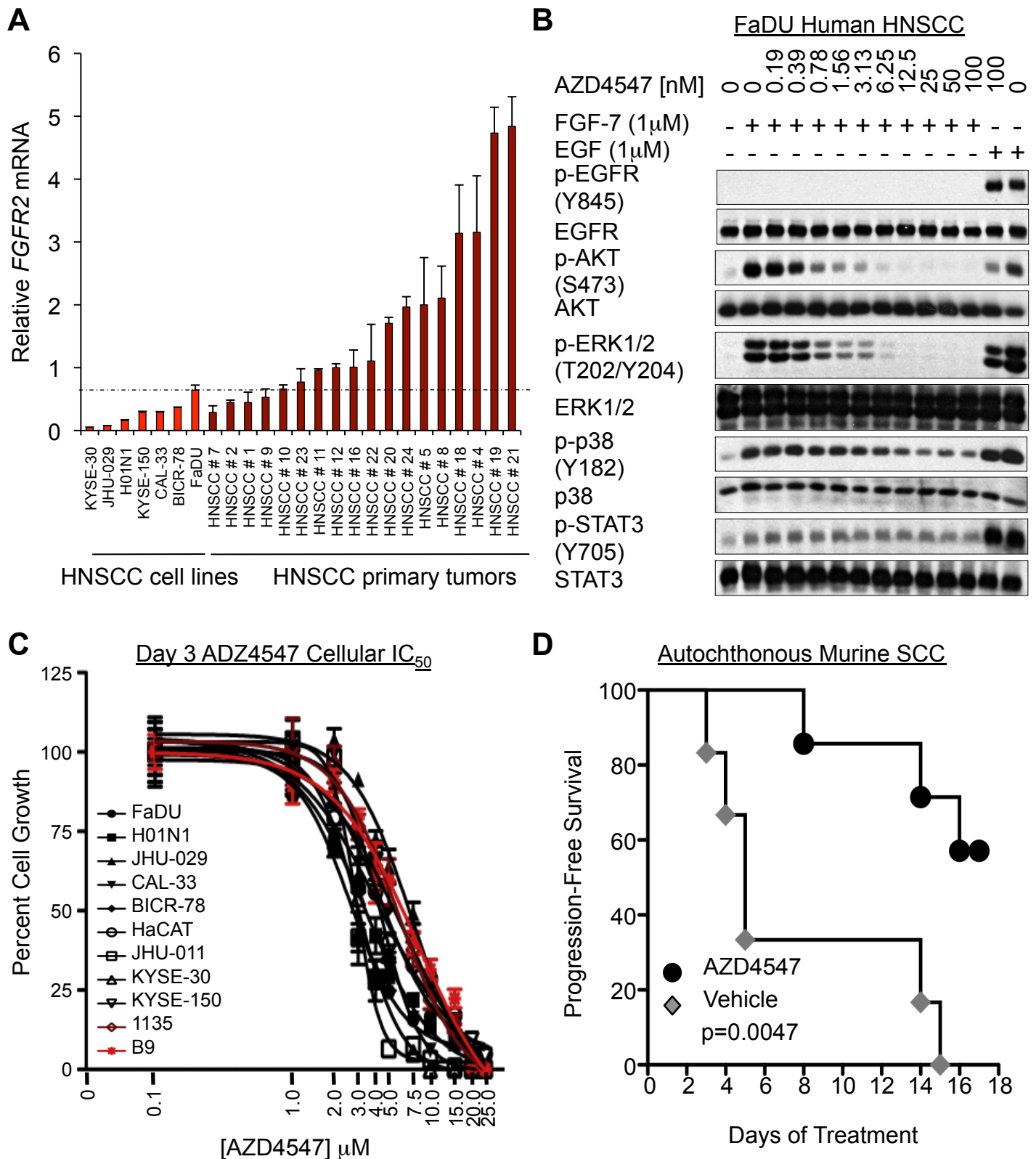
E HaCAT



Supplemental Figure 4. Direct functional regulation of *Fgfr2* by p63. (A) QRT-PCR of mRNA levels of indicated genes in p63-intact (n=3) or p63-ablated (n=3) autochthonous tumors on day 6 after tamoxifen or vehicle treatment, respectively. *p<0.05. Error bars represent +/- SEM. (B) Western blot analysis of murine B9 SCC cells following immunoprecipitation with the α-p63 H129 antibody or control IgG antibody (left) or FLAG-epitope tagged wild-type ΔNp63α (WT) or DNA-binding deficient ΔNp63α (R304W) following immunoprecipitation with α-FLAG M2 antibody (right). Blots probed with α-p63 4A4 antibody. (C) Quantitative Real-Time PCR of mRNA levels of p63, and FGFR2 mRNA after p63 knock down in human HNSCC cells 72 hours following lentiviral-mediated knock-down of p63 (shp63) or infection with control shRNA (Vector). *p<0.05, **p<0.01, ***p<0.001 as assessed by student's unpaired t-test. Error bars indicate +/- SEM. (D) FGFR2 is required for colony formation of HNSCC cells. Quantification Human HNSCC cells plated on plastic 72 hours after infection with control lentivirus, or lentivirus expressing p63 or FGFR2-directed shRNAs. **p<0.01, ***p<0.001 as assessed by students unpaired t-test. Error bars represent +/- SEM of 3 biological replicates. (E) Selective induction of FGFR2 by ΔNp63α requires DNA binding. Expression of FGFR2 in human HaCAT cells following stable expression of GFP, or FLAG-tagged wild type (WT) or R304W mutant (RW) ΔNp63α or TAp63α. Arrow indicates TAp63α; arrowhead indicates ΔNp63α. GAPDH serves as a loading control.



Supplemental Figure 5. Activation of p63-Fgfr2-Fgf7 axis in SCC and hair follicles. (A) Up-regulation of *p63* and *Fgfr2* in Human SCC tumors versus cell lines. Relative mRNA expression was determined by QRT-PCR in human HNSCC cell lines (n=7) and tumors (n=18). Bar indicates mean value. *p<0.05 as assessed by students unpaired t-test. (B) Immunofluorescent staining of murine skin (top) or SCC tumors (bottom) with indicated antibodies. K14= Keratin 14, SMA = Smooth Muscle Actin. Hoechst dye (blue) identifies nuclei. Arrowheads indicate blood vessels. Scale bar = 25µm. (C) Relative mRNA expression *Fgf2* IIIb ligands in murine SCC tumors. Error bars +/- SEM. (D) Immunofluorescent staining of murine hair follicles in telogen with indicated antibodies. DP = Dermal papilla. HS = Hair Shaft. Scale bar = 25µm (E) Immunofluorescent staining of murine SCC tumors with indicated antibodies. Scale bar = 25µm.



Supplemental Figure 6. AZD4547 treatment of SCC cells and tumors. (A) Quantitative Real-Time PCR of mRNA levels of *FGFR2* mRNA in human HNSCC cell lines and HNSCC primary tumors. mRNA levels were normalized to β -actin. Error Bars +/- SEM. (B) AZD4547 does not inhibit EGF-mediated signaling. Cells were serum-starved and pre-treated for 1 hour with indicated dose of AZD4547, followed by a 15-minute stimulation with indicated ligand. Protein levels were assessed by western blotting with indicated antibodies. (C) Growth of HNSCC cells following AZD4547 treatment. Cell growth at indicated doses of AZD4547 was assessed using Cell-TiterGlo after 3 days of treatment. Note 1135 and B9 are murine SCC cell lines. Error Bars +/- SEM. (D) Kaplan-Meier curve of mice bearing DMBA-induced SCC tumors treated daily oral AZD4547 (12.5mg/kg, n=7) or vehicle control (n=6). Tumors were considered to have progressed when they reached 150% original volume. P-value calculated using log-rank test.

Supplemental Table 1: Sequences for primers used in this study

Real-Time PCR		
<i>Mouse</i>	<i>Forward Primer (5'-3')</i>	<i>Reverse Primer (5'-3')</i>
Total p63	CTGTAAGTCCAGATTGCGAA	CTCATTGAACTCACGGCTCA
Δ Np63	GGAAAACAATGCCCAGACTC	GTGGAATACGTCCAGGTGGC
TAp63	TTACAGATCTGCCATGTTCGC	CCCAGATATGCTGGAAGACC
Fgfr1	CAACCGTGTGACCAAGTGG	TCCGACAGGTCCTTCTCCG
Fgfr2 IIIb	GCTGGCTCTGTTCAATGTGACGG	CTCACAGGCGCTTGCTGTTTGG
Fgfr2 IIIc	GTGGTTGCCCCGGGGAATCG	GGTGTGGTGACCGTTCAACGACA
Fgfr3	CCACCGACAAGGAGCTAGAGG	CGGTGACAGGCTTGGCAGTA
Fgfr4	CATGCAGTGCCTGCCGGGAA	TGTTGGTGGCGCAGCCGAAT
Egfr	GTTGAGGCAACGACCGCCA	ACTGCCATCAGCGGGGACCT
β 4 Integrin	TGTGTTCCAGGTGTTTGAGC	CAATGGTGTAGTCGCTGGTG
α 6 Integrin	AGCCCCAGGGACTTACAAC	GGGCACGAGACTTTCATCAT
Coll7 α 1	GGAAGTCCGGTTGGAGAAGCA	CCTCGGATGCTTCCACTTGA
Fgf1	CGGCTCGCAGACACCAATGAGG	GGCCTGAGGGTTAGCGCAGC
Fgf7	CAGAACAAGTCAAGGAGCAACCG	GTCGCTCGGGGCTGGAACAG
Fgf10	CTGTCCGTACAGTGTCTGGAGATA	CCCAGCCCCACCACAACAT
β -actin	ATGAGCTGCCTGACGGCCAGTTCATC	TGGTACCACCAGACAGCACTGTGTTG
Etv4	GCTGCGCCCCGAAAACAAGC	GCGGAGGCAGAGACCTGAGGT
Egr1	ACCTGACCACAGAGTCTTTTC	GTCGGAGGATTGGTCATGCT
Pld1	AGAGTAAAATGGAGCACCGCT	GAATGGGATGCACCCTTCT
<i>Human</i>	<i>Forward Primer (5'-3')</i>	<i>Reverse Primer (5'-3')</i>
Δ Np63	GGAAAACAATGCCCAGACTCA	TGTTGAGGAGCCCCAGGTT
Δ Np63 probe	TTAGTGAGCCACAGTACAC	
<i>Human</i>	<i>Purchased from Applied Biosystems</i>	
β -actin	Hs99999903_m1	
FGFR2	Hs00256382_m1	
Chromatin Immunoprecipitation		
<i>Mouse</i>	<i>Forward Primer (5'-3')</i>	<i>Reverse Primer (5'-3')</i>
Fgfr2 -2.4 Kb	CCACCCTCCACCCCCTACGG	CGTCATGAGGCCTTCTGGGGA
Fgfr2 -12.5 Kb	CCCCCTCAGCCACAGTGGGT	GCCTCTTGTGGCGCTCCCAG
Pld1 +15.3 Kb	ACCTCGGTAAGCAACCCTTG	GGAAGCTGGGCTAAACGACT
Coll7 α 1 -6.5 Kb	GCGAGCCTCAAACGGAAATC	GAAGTGGTTCCCAGCTCCTC
Genomic DNA PCR primers		
<i>Mouse</i>	<i>Forward Primer</i>	<i>Reverse Primer</i>
p63 ^{Brdm3} loading	CAGAGGAGGCAACACAGGATAGA	CCGGGGGATCCGAATTCATCGA
	CAATGGTAGGCTCACTCTGGGAGATGATA	AACACACACTGGCAGGACTGGCTAGG

References For Supplemental Figures 3D and 3E

- 1. Cancer Type: Esophageal Cancer (*Barretina Cell Line*)**
Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A.A., Kim, S., Wilson, C.J., Lehar, J., Kryukov, G.V., Sonkin, D., et al. 2012. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* 483:603-607.
- 2. Cancer Type: Head and Neck Cancer (*Barretina Cell Line*)**
Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A.A., Kim, S., Wilson, C.J., Lehar, J., Kryukov, G.V., Sonkin, D., et al. 2012. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* 483:603-607.
- 3. Cancer Type: Squamous Cell Lung Carcinoma (*Bhattacharjee Lung*)**
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- 4. Lung Cancer Type: Squamous Cell Lung Carcinoma (*Bild Lung*)**
Bild, A.H., Yao, G., Chang, J.T., Wang, Q., Potti, A., Chasse, D., Joshi, M.B., Harpole, D., Lancaster, J.M., Berchuck, A., et al. 2006. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. *Nature* 439:353-357.
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- 6. Cancer Type: Head and Neck Cancer (*Bittner Multi-Cancer*)**
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- 7. Cancer Type: Lung Cancer (*Bittner Multi-Cancer*)**
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- 8. Head and Neck Squamous Cell Carcinoma vs Normal (*Cromer Head-Neck*)**
Cromer, A., Carles, A., Millon, R., Ganguli, G., Chalmel, F., Lemaire, F., Young, J., Dembele, D., Thibault, C., Muller, D., et al. 2004. Identification of genes associated with tumorigenesis and metastatic potential of hypopharyngeal cancer by microarray analysis. *Oncogene* 23:2484-2498.
- 9. Tongue Squamous Cell Carcinoma vs Normal (*Estilo Head-Neck*)**
Estilo, C.L., P, O.c., Talbot, S., Socci, N.D., Carlson, D.L., Ghossein, R., Williams, T., Yonekawa, Y., Ramanathan, Y., Boyle, J.O., et al. 2009. Oral tongue cancer gene expression profiling: Identification of novel potential prognosticators by oligonucleotide microarray analysis. *BMC Cancer* 9:11.
- 10. Lung Cancer Type: Squamous Cell Lung Carcinoma (*Garber Lung*)**
Garber, M.E., Troyanskaya, O.G., Schluens, K., Petersen, S., Thaesler, Z., Pacyna-Gengelbach, M., van de Rijn, M., Rosen, G.D., Perou, C.M., Whyte, R.I., et al. 2001. Diversity of gene expression in adenocarcinoma of the lung. *Proc Natl Acad Sci U S A* 98:13784-13789.
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- 12. Thyroid Gland Papillary Carcinoma vs Normal (*Giordano Thyroid*)**
Giordano, T.J., Au, A.Y., Kuick, R., Thomas, D.G., Rhodes, D.R., Wilhelm, K.G., Jr., Vinco, M., Misek, D.E., Sanders, D., Zhu, Z., et al. 2006. Delineation, functional validation, and bioinformatic evaluation of gene expression in thyroid follicular carcinomas with the PAX8-PPARG translocation. *Clin Cancer Res* 12:1983-1993.
- 13. Head and Neck Squamous Cell Carcinoma Type: Laryngeal Squamous Cell Carcinoma (*Hensen Head-Neck*)**
Hensen, E.F., De Herdt, M.J., Goeman, J.J., Oosting, J., Smit, V.T., Cornelisse, C.J., and Baatenburg de Jong, R.J. 2008. Gene-expression of metastasized versus non-metastasized primary head and neck squamous cell carcinomas: a pathway-based analysis. *BMC Cancer* 8:168.
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- 15. Head and Neck Squamous Cell Carcinoma Type: Oropharyngeal Squamous Cell Carcinoma (*Hensen Head-Neck*)**
Hensen, E.F., De Herdt, M.J., Goeman, J.J., Oosting, J., Smit, V.T., Cornelisse, C.J., and Baatenburg de Jong, R.J. 2008. Gene-expression of metastasized versus non-metastasized primary head and neck squamous cell carcinomas: a pathway-based analysis. *BMC Cancer* 8:168.
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18. **Lung Cancer Type: Squamous Cell Lung Carcinoma (Kim Lung)**
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 19. **Head and Neck Squamous Cell Carcinoma Type: Laryngeal Squamous Cell Carcinoma (Kuriakose Head-Neck)**
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Kuriakose, M.A., Chen, W.T., He, Z.M., Sikora, A.G., Zhang, P., Zhang, Z.Y., Qiu, W.L., Hsu, D.F., McMunn-Coffran, C., Brown, S.M., et al. 2004. Selection and validation of differentially expressed genes in head and neck cancer. *Cell Mol Life Sci* 61:1372-1383.
 21. **Tongue Squamous Cell Carcinoma vs Normal (Kuriakose Head-Neck)**
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Rohrbeck, A., Neukirchen, J., Roskopf, M., Pardillos, G.G., Geddert, H., Schwalen, A., Gabbert, H.E., von Haeseler, A., Pitschke, G., Schott, M., et al. 2008. Gene expression profiling for molecular distinction and characterization of laser captured primary lung cancers. *J Transl Med* 6:69.
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Su, A.I., Welsh, J.B., Sapinoso, L.M., Kern, S.G., Dimitrov, P., Lapp, H., Schultz, P.G., Powell, S.M., Moskaluk, C.A., Frierson, H.F., Jr., et al. 2001. Molecular classification of human carcinomas by use of gene expression signatures. *Cancer Res* 61:7388-7393.
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Talbot, S.G., Estilo, C., Maghami, E., Sarkaria, I.S., Pham, D.K., P, O.c., Socci, N.D., Ngai, I., Carlson, D., Ghossein, R., et al. 2005. Gene expression profiling allows distinction between primary and metastatic squamous cell carcinomas in the lung. *Cancer Res* 65:3063-3071.
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Talbot, S.G., Estilo, C., Maghami, E., Sarkaria, I.S., Pham, D.K., P, O.c., Socci, N.D., Ngai, I., Carlson, D., Ghossein, R., et al. 2005. Gene expression profiling allows distinction between primary and metastatic squamous cell carcinomas in the lung. *Cancer Res* 65:3063-3071.
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