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SUPPLEMENTARY INFORMATION

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Age-related remodelling of oesophageal epithelia by mutated cancer drivers

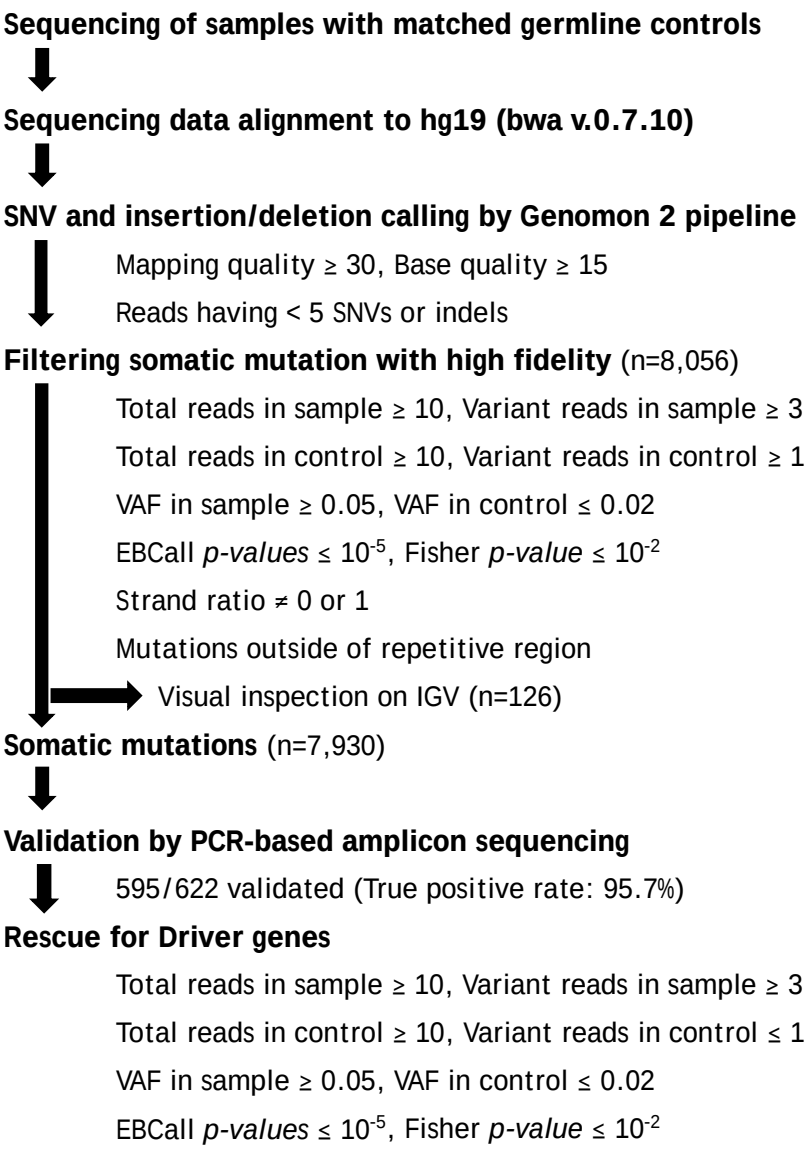
Akira Yokoyama[1,2,14], Nobuyuki Kakiuchi[1,3,14], Tetsuichi Yoshizato[1,14], Yasuhito Nannya[1], Hiromichi Suzuki[1], Yasuhide Takeuchi[1,4], Yusuke Shiozawa[1], Yusuke Sato[1], Kosuke Aoki[1], Soo Ki Kim[1], Yoichi Fujii[1], Kenichi Yoshida[1], Keisuke Kataoka[1], Masahiro M. Nakagawa[1], Yoshikage Inoue[1,5], Tomonori Hirano[1,3], Yuichi Shiraishi[6], Kenichi Chiba[6], Hiroko Tanaka[7], Masashi Sanada[8], Yoshitaka Nishikawa[2], Yusuke Amanuma[2], Shinya Ohashi[2], Ikuo Aoyama[2], Takahiro Horimatsu[2], Shin'ichi Miyamoto[3], Shigeru Tsunoda[5], Yoshiharu Sakai[5], Maiko Narahara[9], J. B. Brown[10], Yoshitaka Sato[11], Genta Sawada[12], Koshi Mimori[12], Sachiko Minamiguchi[4], Hironori Haga[4], Hiroshi Seno[3], Satoru Miyano[6,7], Hideki Makishima[1], Manabu Muto[2,15] & Seishi Ogawa[1,13,15]*

[1]Department of Pathology and Tumor Biology, Kyoto University, Kyoto, Japan. [2]Department of Clinical Oncology, Kyoto University Hospital, Kyoto, Japan. [3]Department of Gastroenterology and Hepatology, Kyoto University, Kyoto, Japan. [4]Department of Diagnostic Pathology, Kyoto University, Kyoto, Japan. [5]Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan. [6]Laboratory of DNA Information Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [7]Laboratory of Sequence Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [8]Department of Advanced Diagnosis, Clinical Research Center, Nagoya Medical Center, Nagoya, Japan. [9]Department of Human Genetics, McGill University, Montreal, Quebec, Canada. [10]Laboratory of Molecular Biosciences, Life Science Informatics Research Unit, Kyoto University, Kyoto, Japan. [11]Sato Clinic, Okayama, Japan. [12]Department of Surgery, Kyushu University Beppu Hospital, Beppu, Japan. [13]Department of Medicine, Center for Hematology and Regenerative Medicine, Karolinska Institute, Stockholm, Sweden. [14]These authors contributed equally: Akira Yokoyama, Nobuyuki Kakiuchi, Tetsuichi Yoshizato. [15]These authors jointly supervised this work: Manabu Muto, Seishi Ogawa. *e-mail: sogawa-tky@umin.ac.jp

Supplementary Information

Contents

Supplementary Method 1: **Flow chart of mutation calling**



hg19; Homo sapiens (human) genome assembly GRCh37

bwa; Burrows-Wheeler Aligner

SNV; single nucleotide variant

VAF; variant allele fraction

EBCall; Empirical Bayesian mutation Calling

IGV; Integrative Genomics Viewer

Supplementary Method 2; a sample script for a random effects model meta-analysis

```
#############################################################################
# load library
library(metafor)
#############################################################################
# function for calculating the coordinate of intersection point of perpendicular line from (x,y) to line with "a" slope
and "b" intercept
calc_dis <- function(a, b, x1, y1){
  x <- (x1+a*y1-b*a)/(a^2+1)
  return(c(x=x, y=a*x+b))
}
#############################################################################
# data read
res <- read.table("Path to data_for_script.txt")

res_nd <- subset(res, Path=="nondysplasia")
res_nd_4 <- subset(res_nd, Size==4)
res_nd_0.8 <- subset(res_nd, Size==0.8)
res_nd_0.2 <- subset(res_nd, Size==0.2)
res_nd_4_l <- subset(res_nd_4, ESCC_risk==0)
res_nd_4_h <- subset(res_nd_4, ESCC_risk==1)
res_nd_0.8_l <- subset(res_nd_0.8, ESCC_risk==0)
res_nd_0.8_h <- subset(res_nd_0.8, ESCC_risk==1)
res_nd_0.2_l <- subset(res_nd_0.2, ESCC_risk==0)
res_nd_0.2_h <- subset(res_nd_0.2, ESCC_risk==1)
#############################################################################
# This is an example of the code to calculate effect of ESCC risks on "mutation number".
# Modify this code for other metrices.
#############################################################################
# For 4mm2 sample
# Set up the regression line of samples without risk
out_4_l <- lm(res_nd_4_l$mut_num~res_nd_4_l$age-1);out_4_l
out2_4_l <- summary(out_4_l)

# Calculate the distance from the regression line to each risk (-) sample
```

```
dis_info_4_l <- NULL
for(i in 1:nrow(res_nd_4_l)){
  tmp_age <- res_nd_4_l[i,"age"]
  tmp_num <- res_nd_4_l[i,"mut_num"]
  tmp_res <- calc_dis(out_4_l$coefficients[1], 0,tmp_age, tmp_num)
  dis <- sqrt((tmp_age- tmp_res[1])^2+(tmp_num- tmp_res[2])^2)
  if(out_4_l$coefficients[1]*tmp_age -tmp_num<0){
    judge <- 1 #above
    dis <- dis
  }else{
    judge <- 0 # below
    dis <- -dis
  }
  tmp_res2 <- c(dis,judge)
  dis_info_4_l <- rbind(dis_info_4_l,tmp_res2)
}
colnames(dis_info_4_l) <- c("dis","judge")
rownames(dis_info_4_l) <- 1:nrow(dis_info_4_l)
dis_info_4_l <- as.data.frame(dis_info_4_l)

# Set up the regression line of samples with risk
out_4_h <- lm(res_nd_4_h$mut_num~res_nd_4_h$age-1);out_4_h
out2_4_h <- summary(out_4_h)

# Calculate the distance from the regression line to each risk (+) sample
dis_info_4_h <- NULL
for(i in 1:nrow(res_nd_4_h)){
  tmp_age <- res_nd_4_h[i,"age"]
  tmp_num <- res_nd_4_h[i,"mut_num"]
  tmp_res <- calc_dis(out_4_l$coefficients[1], 0,tmp_age, tmp_num)
  dis <- sqrt((tmp_age- tmp_res[1])^2+(tmp_num- tmp_res[2])^2)
  if(out_4_l$coefficients[1]*tmp_age -tmp_num<0){
    judge <- 1 #above
    dis <- dis
  }else{
    judge <- 0 # below
```

```
    dis <- -dis
  }
  tmp_res2 <- c(dis,judge)
  dis_info_4_h <- rbind(dis_info_4_h,tmp_res2)
}
colnames(dis_info_4_h) <- c("dis","judge")
rownames(dis_info_4_h) <- 1:nrow(dis_info_4_h)
dis_info_4_h <- as.data.frame(dis_info_4_h)

# Mann-Whitney U test
w.out <- wilcox.test(dis_info_4_h$dis,dis_info_4_l$dis, alternative="greater")

#############################################################################
# For 0.8mm2 sample
# Set up the regression line of samples without risk
out_0.8_l <- lm(res_nd_0.8_l$mut_num~res_nd_0.8_l$age-1);out_0.8_l
out2_0.8_l <- summary(out_0.8_l)

# Calculate the distance from the regression line to each risk (-) sample
dis_info_0.8_l <- NULL
for(i in 1:nrow(res_nd_0.8_l)){
  tmp_age <- res_nd_0.8_l[i,"age"]
  tmp_num <- res_nd_0.8_l[i,"mut_num"]
  tmp_res <- calc_dis(out_0.8_l$coefficients[1], 0,tmp_age, tmp_num)
  dis <- sqrt((tmp_age- tmp_res[1])^2+(tmp_num- tmp_res[2])^2)
  if(out_0.8_l$coefficients[1]*tmp_age -tmp_num<0){
    judge <- 1 #above
    dis <- dis
  }else{
    judge <- 0 # below
    dis <- -dis
  }
  tmp_res2 <- c(dis,judge)
  dis_info_0.8_l <- rbind(dis_info_0.8_l,tmp_res2)
}
colnames(dis_info_0.8_l) <- c("dis","judge")
```

```
rownames(dis_info_0.8_l) <- 1:nrow(dis_info_0.8_l)
dis_info_0.8_l <- as.data.frame(dis_info_0.8_l)

# Set up the regression line of samples with risk
out_0.8_h <- lm(res_nd_0.8_h$mut_num~res_nd_0.8_h$age-1);out_0.8_h
out2_0.8_h <- summary(out_0.8_h)

# Calculate the distance from the regression line to each risk (+) sample
dis_info_0.8_h <- NULL
for(i in 1:nrow(res_nd_0.8_h)){
  tmp_age <- res_nd_0.8_h[i,"age"]
  tmp_num <- res_nd_0.8_h[i,"mut_num"]
  tmp_res <- calc_dis(out_0.8_l$coefficients[1], 0,tmp_age, tmp_num)
  dis <- sqrt((tmp_age- tmp_res[1])^2+(tmp_num- tmp_res[2])^2)
  if(out_0.8_l$coefficients[1]*tmp_age -tmp_num<0){
    judge <- 1 #above
    dis <- dis
  }else{
    judge <- 0 # below
    dis <- -dis
  }
  tmp_res2 <- c(dis,judge)
  dis_info_0.8_h <- rbind(dis_info_0.8_h,tmp_res2)
}
colnames(dis_info_0.8_h) <- c("dis","judge")
rownames(dis_info_0.8_h) <- 1:nrow(dis_info_0.8_h)
dis_info_0.8_h <- as.data.frame(dis_info_0.8_h)

# Mann-Whitney U test
w.out <- wilcox.test(dis_info_0.8_h$dis,dis_info_0.8_l$dis, alternative="greater")

#############################################################################
# For 0.2mm2 sample
# Set up the regression line of samples without risk
out_0.2_l <- lm(res_nd_0.2_l$mut_num~res_nd_0.2_l$age-1);out_0.2_l
out2_0.2_l <- summary(out_0.2_l)
```

```
# Calculate the distance from the regression line to each risk (-) sample
dis_info_0.2_l <- NULL
for(i in 1:nrow(res_nd_0.2_l)){
  tmp_age <- res_nd_0.2_l[i,"age"]
  tmp_num <- res_nd_0.2_l[i,"mut_num"]
  tmp_res <- calc_dis(out_0.2_l$coefficients[1], 0,tmp_age, tmp_num)
  dis <- sqrt((tmp_age- tmp_res[1])^2+(tmp_num- tmp_res[2])^2)
  if(out_0.2_l$coefficients[1]*tmp_age -tmp_num<0){
    judge <- 1 #above
    dis <- dis
  }else{
    judge <- 0 # below
    dis <- -dis
  }
  tmp_res2 <- c(dis,judge)
  dis_info_0.2_l <- rbind(dis_info_0.2_l,tmp_res2)
}
colnames(dis_info_0.2_l) <- c("dis","judge")
rownames(dis_info_0.2_l) <- 1:nrow(dis_info_0.2_l)
dis_info_0.2_l <- as.data.frame(dis_info_0.2_l)

# Set up the regression line of samples with risk
out_0.2_h <- lm(res_nd_0.2_h$mut_num~res_nd_0.2_h$age-1);out_0.2_h
out2_0.2_h <- summary(out_0.2_h)

# Calculate the distance from the regression line to each risk (+) sample
dis_info_0.2_h <- NULL
for(i in 1:nrow(res_nd_0.2_h)){
  tmp_age <- res_nd_0.2_h[i,"age"]
  tmp_num <- res_nd_0.2_h[i,"mut_num"]
  tmp_res <- calc_dis(out_0.2_l$coefficients[1], 0,tmp_age, tmp_num)
  dis <- sqrt((tmp_age- tmp_res[1])^2+(tmp_num- tmp_res[2])^2)
  if(out_0.2_l$coefficients[1]*tmp_age -tmp_num<0){
    judge <- 1 #above
    dis <- dis
```

```
  }else{
    judge <- 0 # below
    dis <- -dis
  }
  tmp_res2 <- c(dis,judge)
  dis_info_0.2_h <- rbind(dis_info_0.2_h,tmp_res2)
}
colnames(dis_info_0.2_h) <- c("dis","judge")
rownames(dis_info_0.2_h) <- 1:nrow(dis_info_0.2_h)
dis_info_0.2_h <- as.data.frame(dis_info_0.2_h)

# Mann-Whitney U test
w.out <- wilcox.test(dis_info_0.2_h$dis,dis_info_0.2_l$dis, alternative="greater")

# Apply the same calculation to 0.8mm2 and 0.2 mm2 samples

# Meta analysis for ESCC risk
out.m <- rbind(c(mean(dis_info_0.2_h$dis),sd(dis_info_0.2_h$dis),length(dis_info_0.2_h$dis),
                 mean(dis_info_0.2_l$dis),sd(dis_info_0.2_l$dis),length(dis_info_0.2_l$dis)),
               c(mean(dis_info_0.8_h$dis),sd(dis_info_0.8_h$dis),length(dis_info_0.8_h$dis),
                 mean(dis_info_0.8_l$dis),sd(dis_info_0.8_l$dis),length(dis_info_0.8_l$dis)),
               c(mean(dis_info_4_h$dis),sd(dis_info_4_h$dis),length(dis_info_4_h$dis),
                 mean(dis_info_4_l$dis),sd(dis_info_4_l$dis),length(dis_info_4_l$dis)))
colnames(out.m) <- c("mean_h","sd_h","num_h","mean_l","sd_l","num_l")
out.m <- as.data.frame(out.m)

# Calculate effect sizes and outcome measures
# "SMDH" for the standardized mean difference with heteroscedastic population variances in the two groups (Bonett,
2008, 2009).
out.m2 <- escalc(measure="SMDH", m1i=mean_h,m2i=mean_l,
                 sd1i=sd_h,sd2i=sd_l,n1i=num_h,n2i=num_l, data=out.m)

# Random-effects model
out.m3=rma(yi, vi, data=out.m2)

# Plot forest plot
```

```
forest(out.m3,showweights=T,alim=c(-4,4),at=(-4:4),
       slab=c("0.2 mm2","0.8 mm2", "4 mm2"),cex=1.2,cex.lab=1,cex.axis=1,
       main="Number of mutations and ESCC risk")
text(1,5,paste("P=",signif(out.m3$pval,3)))

#########################################################################
# Meta analysis for age
out.m <- rbind(c(sqrt(out2_0.2_h$r.squared),length(dis_info_0.2_h$dis),
                 sqrt(out2_0.2_l$r.squared),length(dis_info_0.2_l$dis)),
               c(sqrt(out2_0.8_h$r.squared),length(dis_info_0.8_h$dis),
                 sqrt(out2_0.8_l$r.squared),length(dis_info_0.8_l$dis)),
               c(sqrt(out2_4_h$r.squared),length(dis_info_4_h$dis),
                 sqrt(out2_4_l$r.squared),length(dis_info_4_l$dis)))
colnames(out.m) <- c("coef_h","num_h","coef_l","num_l")
out.m <- as.data.frame(out.m)

# Calculate effect sizes and outcome measures
# "ZCOR" for the Fisher's r-to-z transformed correlation coefficient (Fisher, 1921).
out.m2 <- escalc(measure="ZCOR", ri=coef_l,ni=num_l,data=out.m)

# Random-effects model
out.m3=rma(yi, vi, data=out.m2)

# Plot forest plot
forest(out.m3,showweights=T,alim=c(-2,2),at=(0:20-10)/10,
       slab=c("0.2 mm2","0.8 mm2", "4 mm2"),cex=1.2,cex.lab=1,cex.axis=1,
       main="Number of mutations and age in risk (-)")
text(1,5,paste("P=",signif(out.m3$pval,3)))
```

Supplementary Note 1. Acknowledgements

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