Supplementary Information

Matlab Code

```
Matlab script for manuscript "[18F]-fluoroethyltyrosine-induced Cerenkov
luminescence improves image-guided surgical resection of glioma"

Written By Alan Wright and David Lewis

clear all


ImportN=importdata('XXX.tif');%Enter files here. Both should be a 3D matrix
ImportT=importdata('XXX.tif');%with the same rowsxcols and a value of 2 for
dimension 3
min_intensity=0; %enter here the min pixel intensity
max_intensity=255; %enter here the maximum pixel intensity

ImportN(:,:,1)=255-ImportN(:,:,1);
ImportT(:,:,1)=255-ImportT(:,:,1);


Normal_data=double(ImportN);
Tumour_data=double(ImportT);


Normal_ops_list=0;
Tumour_ops_list=0;


[ii,jj,kk]=size(Normal_data);
[i2,j2,k2]=size(Tumour_data);
Tumour_mask=zeros(ii,jj);
Normal_mask=zeros(ii,jj);

Tumour_ops=zeros(i2,j2);
Normal_ops=zeros(i2,j2);

tlist=0;
nlist=0;

if (ii==i2)&&(jj==j2)&&(kk==k2)&&(kk==2)
    for i=1:ii
        for j=1:jj
            tp=Tumour_data(i,j,2);
            if tp==255
                Tumour_mask(i,j)=1;
                Tumour_ops(i,j)=Tumour_data(i,j,1);
                Tumour_ops_list=cat(1,Tumour_ops_list,Tumour_data(i,j,1));
            end
            np=Normal_data(i,j,2);
            if np==255
                Normal_mask(i,j)=1;
                Normal_ops(i,j)=Normal_data(i,j,1);
                Normal_ops_list=cat(1,Normal_ops_list,Normal_data(i,j,1));
            end
            clear tp
            clear np
        end
```

```
end
    Normal_ops_list(1,:)=[];
    Tumour_ops_list(1,:)=[];

targets=cat(1,zeros(length(Normal_ops_list),1),ones(length(Tumour_ops_list)
,1));
    ops=cat(1,Normal_ops_list,Tumour_ops_list);
    [X,Y,T,AUC,OPTROCPT] = perfcurve(targets',ops',1);
    subplot(1,2,1)
    plot(X,Y)
    AUC
    BLAH=sqrt(X.*X+(1-Y).*(1-Y));
    [C,I]=min(BLAH);
    OPTROC2=cat(2,X(I),Y(I));
    OPTROC2T=T(I);


    %section for creating boxplots of imaging data
   Scaling_factor=(max_intensity-min_intensity)/255;

corrected_Tumour_ops_list=(Tumour_ops_list*Scaling_factor)+min_intensity;

corrected_Normal_ops_list=(Normal_ops_list*Scaling_factor)+min_intensity;
   A= corrected_Normal_ops_list.';
   B= corrected_Tumour_ops_list.';
   C = [A B];
   A1=size(A);
   B1=size(B);
   grp = [zeros(1,(A1(1,2))),ones(1,(B1(1,2)))];
   Ylim2=(max_intensity+10);
   subplot(1,2,2)
   boxplot(C,grp,'Labels',{'Normal','Tumour'},'OutlierSize',1)
   ylim([min_intensity Ylim2]);
   ylabel('Radiance (p/sec/cm2/sr)');

   mean_normal=mean(corrected_Normal_ops_list);
   std_normal=std(corrected_Normal_ops_list);
   mean_tumour=mean(corrected_Tumour_ops_list);
   std_tumour=std(corrected_Tumour_ops_list);
   corr_OPTROC2T=((OPTROC2T*Scaling_factor)+min_intensity);


   Export1=[OPTROCPT OPTROC2 I OPTROC2T corr_OPTROC2T AUC mean_normal
std_normal mean_tumour std_tumour];
   Export2=Export1.';
   Export3=horzcat(X,Y,T);



   [l1,m1] =
ndgrid(1:size(corrected_Normal_ops_list,1),1:size(corrected_Normal_ops_list
,2));
   [l2,m2] =
ndgrid(1:size(corrected_Tumour_ops_list,1),(1:size(corrected_Tumour_ops_lis
t,2))+size(corrected_Normal_ops_list,2));
   z =
accumarray([l1(:),m1(:);l2(:),m2(:)],[corrected_Normal_ops_list(:);correcte
d_Tumour_ops_list(:)]);
   z(z == 0) = NaN;
```

```
    save('XXX_FMa.csv','Export2','Export3','-ascii','-double','-tabs');
    save('XXX_FMb.csv','z','-ascii','-double','-tabs')
    saveas(gcf,'XXX_FM.png')



else
    error='wrong format'
end
```

Supplementary Figures

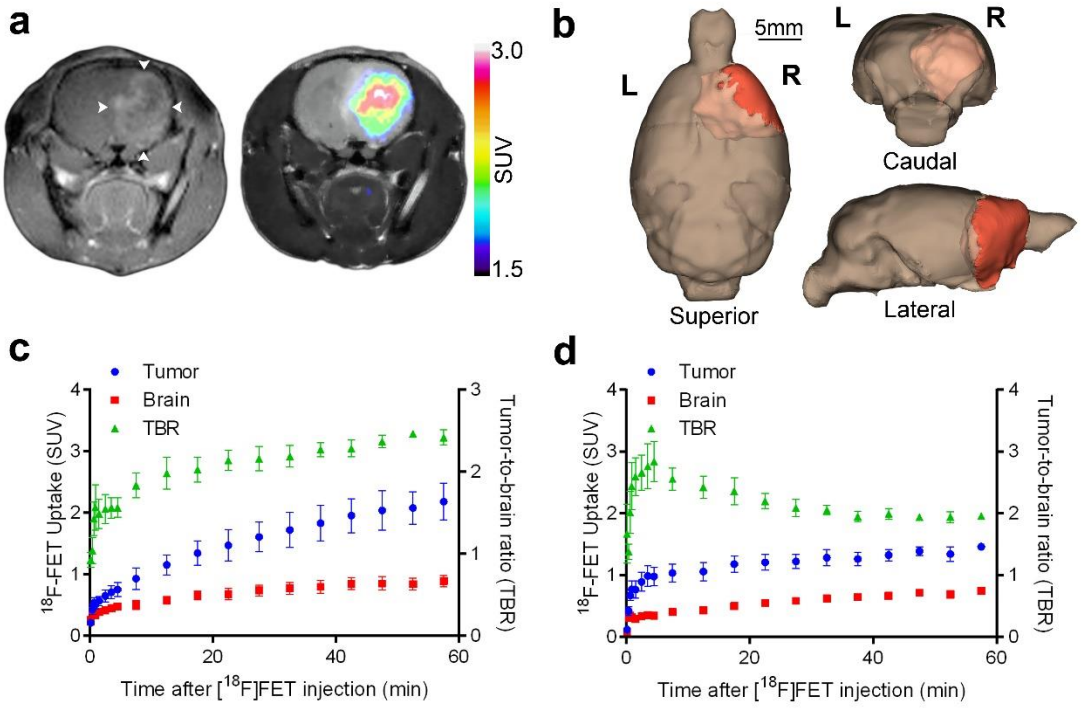

Figure S1. Dynamic measurements of tumor uptake of [^{18}F]-fluoroethyltyrosine (FET). (a) Contrast agent-enhanced T_1-weighted MR image of an enhancing F98 human glioblastoma (left) and a FET PET image, rendered in false color, overlaid on a T_2-weighted MR image (right). Tumor location is arrowed. (b) 3D segmentation and visualisation of the F98 tumor using FET PET and normal brain tissue using MRI. Dynamic measurements of FET uptake in tumor and contralateral brain in (c) F98 (n=4) and (d) U87 (n=3) glioblastomas.

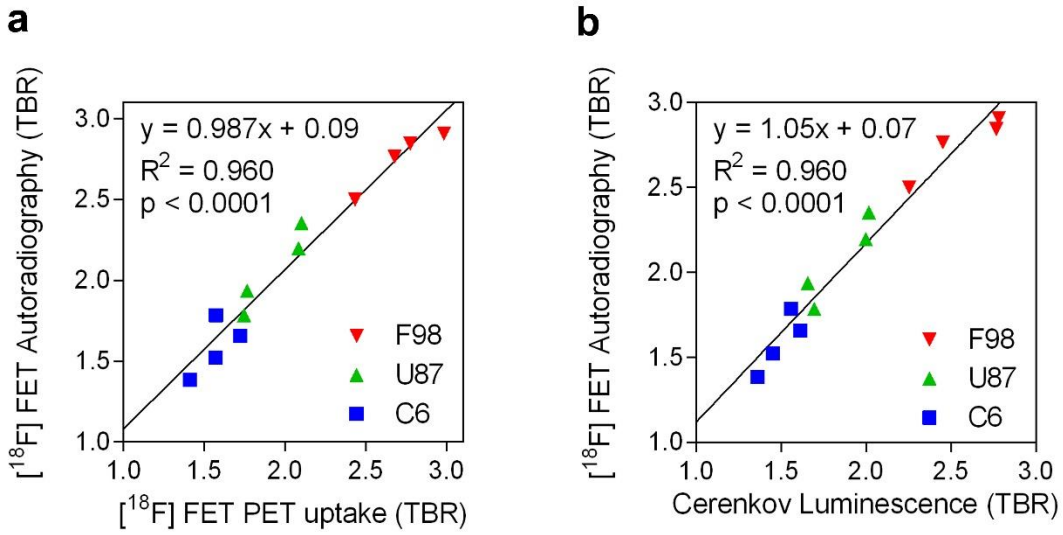

Figure S2. Correlation between tumor FET PET signal in vivo and tumor FET signal on autoradiograms of excised brains (a) and between the tumor FET signal on autoradiograms and FET-induced tumor Cerenkov luminescence (b) in the three different glioblastoma models.

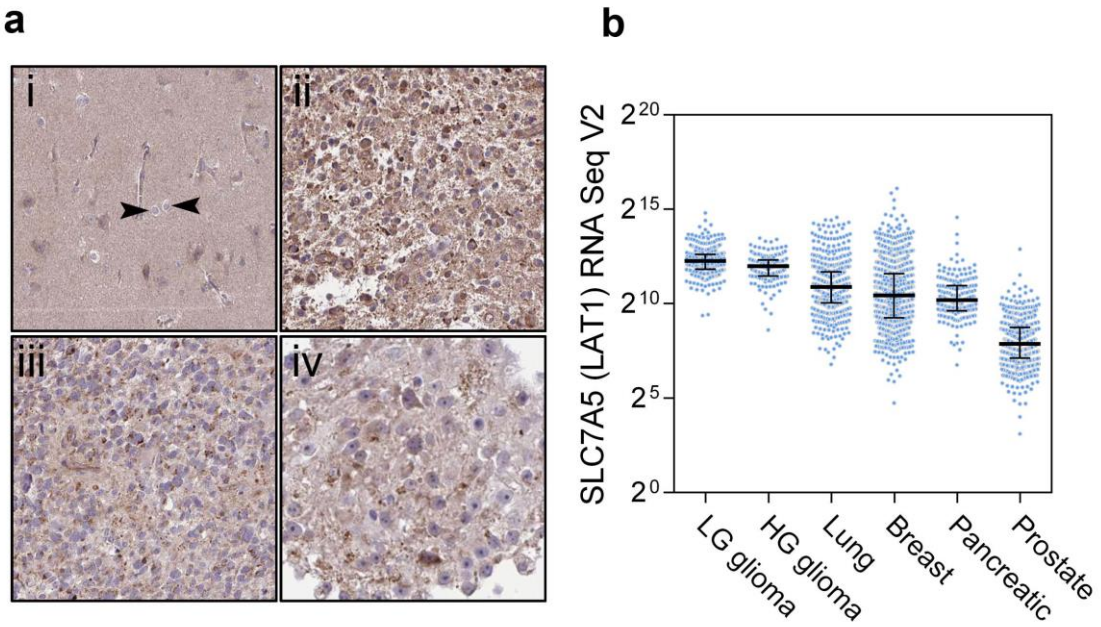

Figure S3. LAT1 (SLC7A5) is highly expressed in clinical low and high-grade gliomas (a) SLC7A5 immunohistochemistry (HPA052673) of human glial tissue showing moderate plasma membrane and cytoplasmic staining of LAT1 in cancer compared to normal glial cells. (i) Normal cerebral cortex with negative glial staining (arrowed), (ii) low grade glioma patient (ID#2909) showing moderate intensity staining. (iii) high-grade glioma (ID#2802) with moderate intensity staining. (iv) U87 glioblastoma with moderate intensity staining. Images were taken from the Human Protein Atlas (www.proteinatlas.org). (b) SLC7A5 RNA levels, showing high expression in low and high-grade (GBM) glioma. Glioma expression is higher than a number of other common malignancies. Reanalysis of data from the TCGA Research Network (http://cancergenome.nih.gov/).

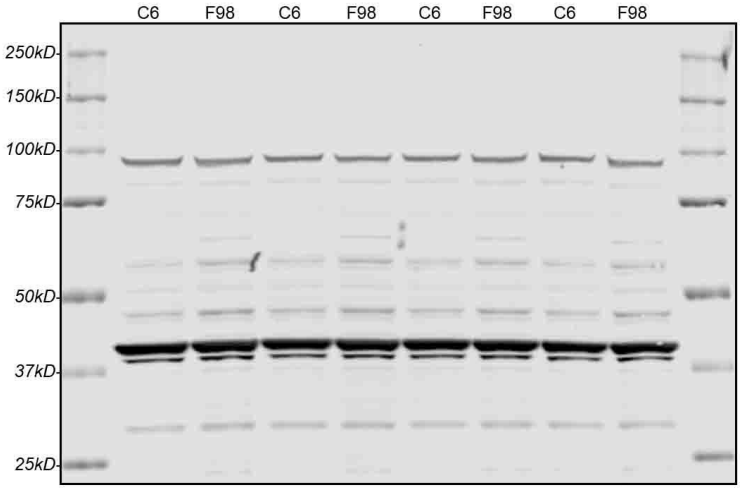

Figure S4. Western blot of LAT1 (SLC7A5) protein expression in C6 and F98 glioblastoma cell lines. LAT1 is at 55 kD and β-actin at 42 kD.

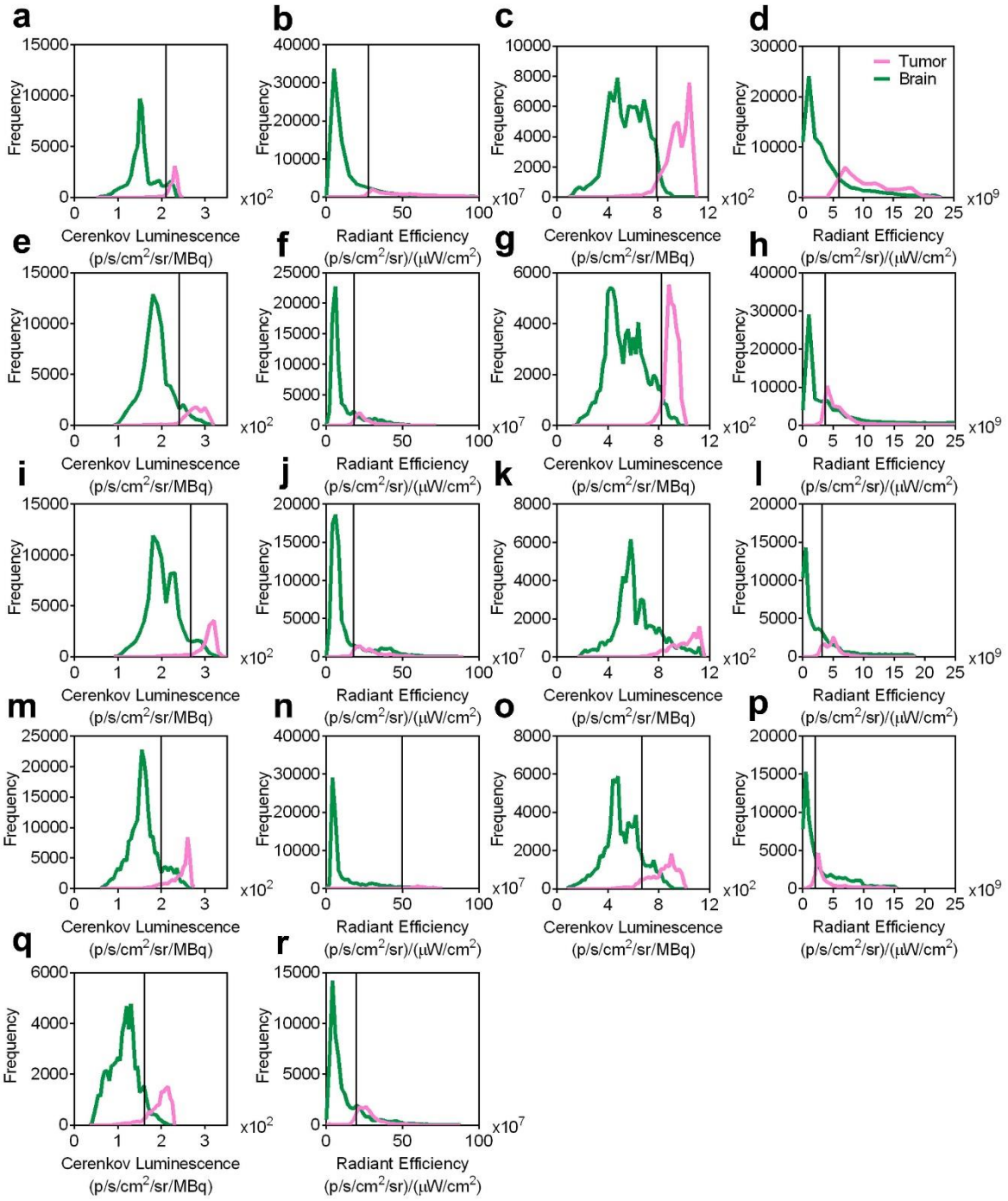

Figure S5. Histograms of FET Cerenkov Luminescence (a, e, I, m, q, c, g, k, o) and 5-ALA radiant efficiency (b, f, j, n, r, d, h, l, p) in tumor (magenta) and normal brain regions (green) in U87 (a, b, e, f, I, j, m, n, q, r) and F98 (c, d, g, h, k, l, o, p) glioblastoma tumors.

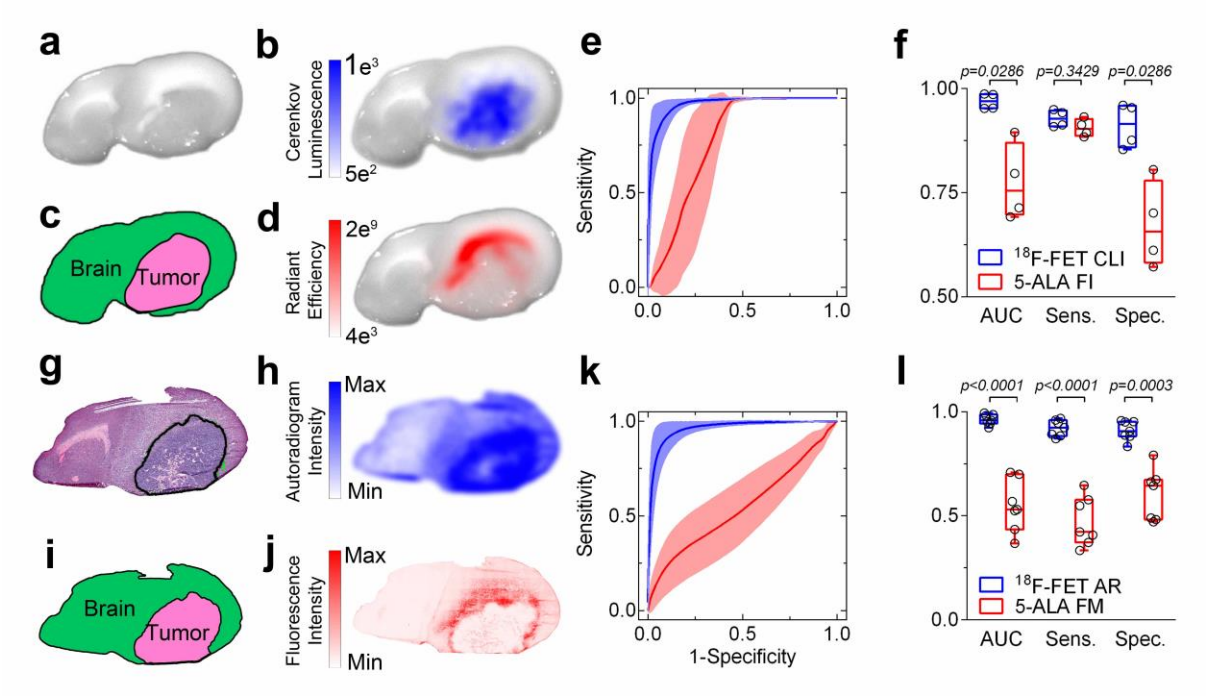

Figure S6. Cerenkov luminescence imaging (CLI) with [^{18}F]-fluoroethyltyrosine (FET) more accurately delineates rat F98 glioblastoma and surrounding brain than 5-aminolevulinic acid (5-ALA) fluorescence imaging. Representative white light image of a rat brain slice containing a F98 human glioblastoma (a), overlaid with the corresponding FET CL (b) and 5-ALA-induced fluorescence images (d). Annotated segmentation (c) obtained by co-registration of the white light image (a) and corresponding H&E stained section. The tumor margin is delineated by a black line (g). FET autoradiography (h), 5-ALA confocal microscopy (j) and annotated segmentation (i) obtained from an H&E stained section (g). (e) Receiver operating characteristic (ROC) curves for FET CLI (blue) and 5-ALA fluorescence (red), data are the mean ± s.d. (n=4). (f) Areas under the ROC curves (AUC), sensitivity and specificity for detection of tumor using FET CLI and 5-ALA-induced fluorescence (n=4) at CLI_{OptROC} and $5\text{-}ALA_{OptROC}$. (k) ROC curves obtained from FET autoradiography (blue) and confocal microscopy of 5-ALA-induced fluorescence (red). Data are the mean ± s.d. (n=7). (l) Areas under the ROC curves (AUC), sensitivity and specificity for detection of tumor using FET autoradiography and confocal microscopy of 5-ALA-induced fluorescence (n=7). Box and whisker plots represent the range, interquartile range and the median,. Data were compared using 2-tailed Mann-Whitney and unpaired t tests as appropriate.

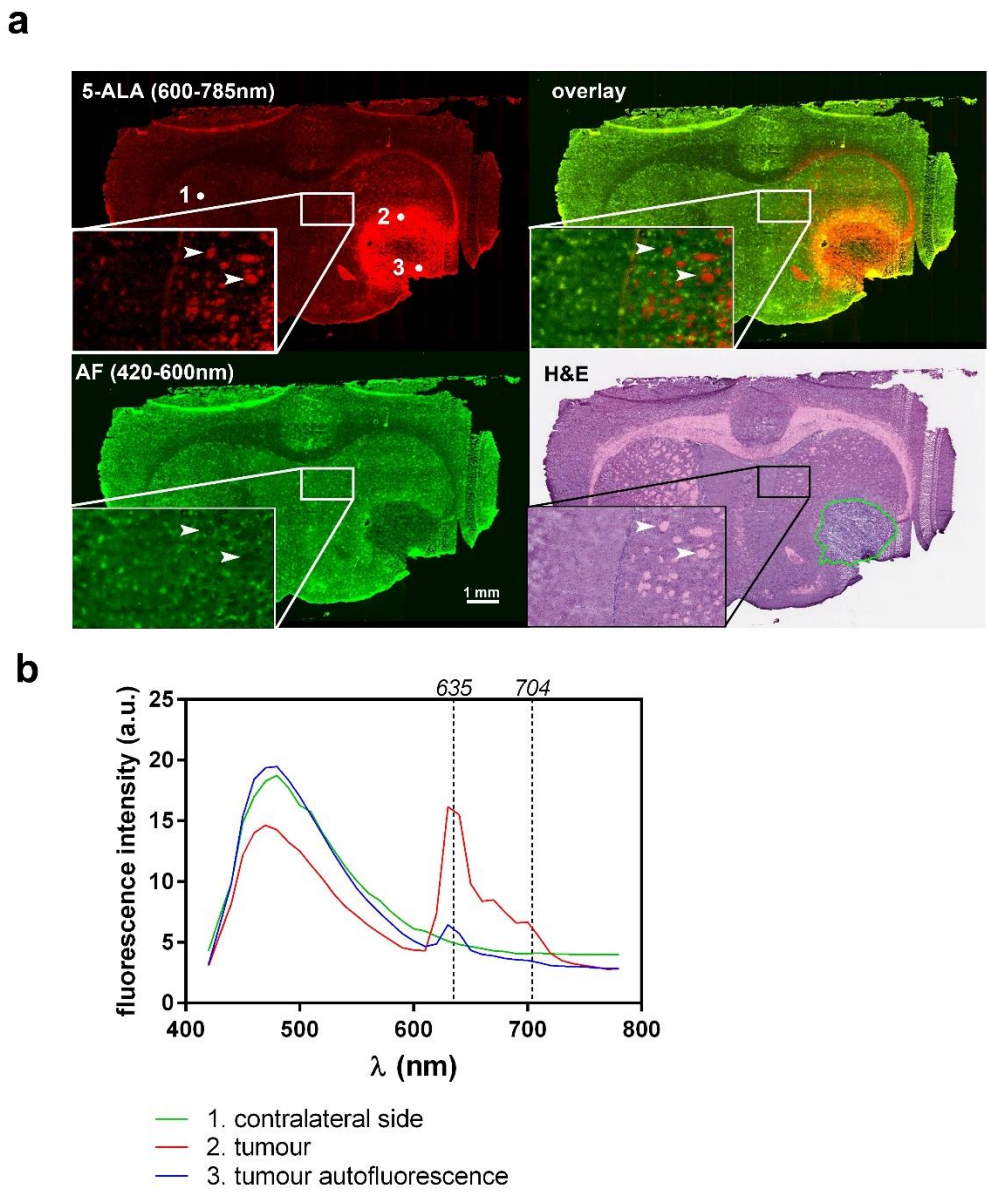

Figure S7. Localization of 5-ALA fluorescence in U87 glioblastoma. (a) 5-ALA-induced fluorescence (top left); tissue autofluorescence (AF) (bottom left); overlay of images in a and b (top right). The same section stained by H&E is shown bottom right. 5-ALA-induced fluorescence in white matter tracts on the ipsilateral side is arrowed on the higher magnification insets. (b) Fluorescence emission spectra with an autofluorescence peak at about 480 nm and characteristic PPIX peaks at 635 and 704 nm. Spectra were acquired from the three points shown in the top left image in (a).

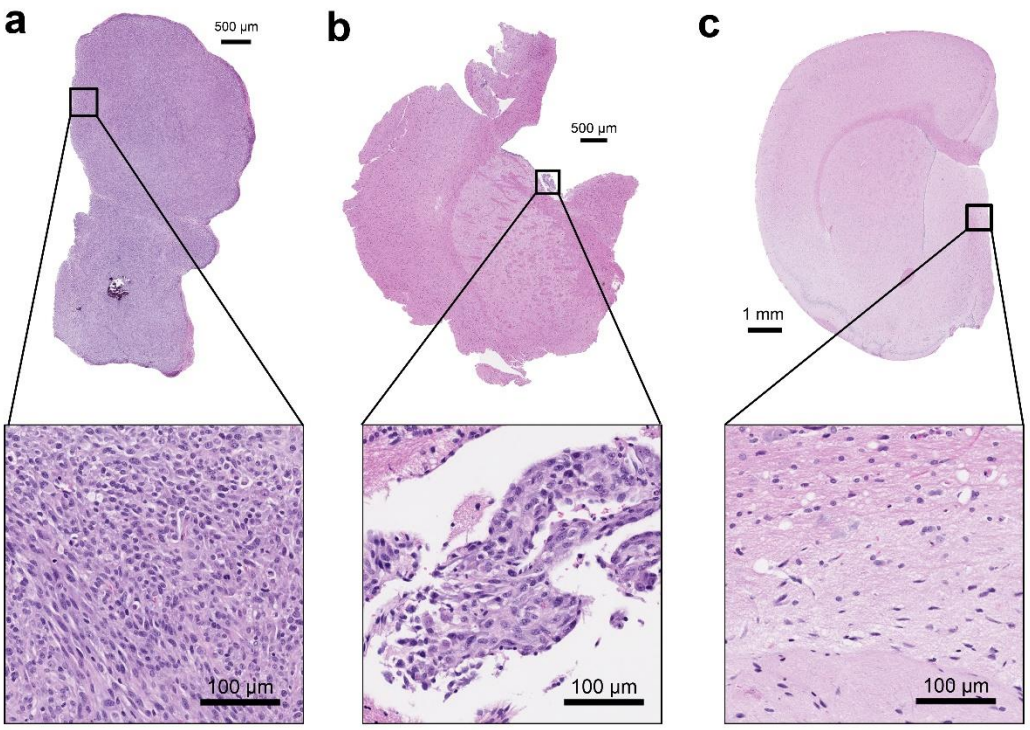

Figure S8. H&E staining of resected U87 tumor specimens removed under FET CLI guidance. FET CLI positive fragments (a) and (b) showed the presence tumor cells. No tumor cells were found in the remaining FET CLI negative brain (c).

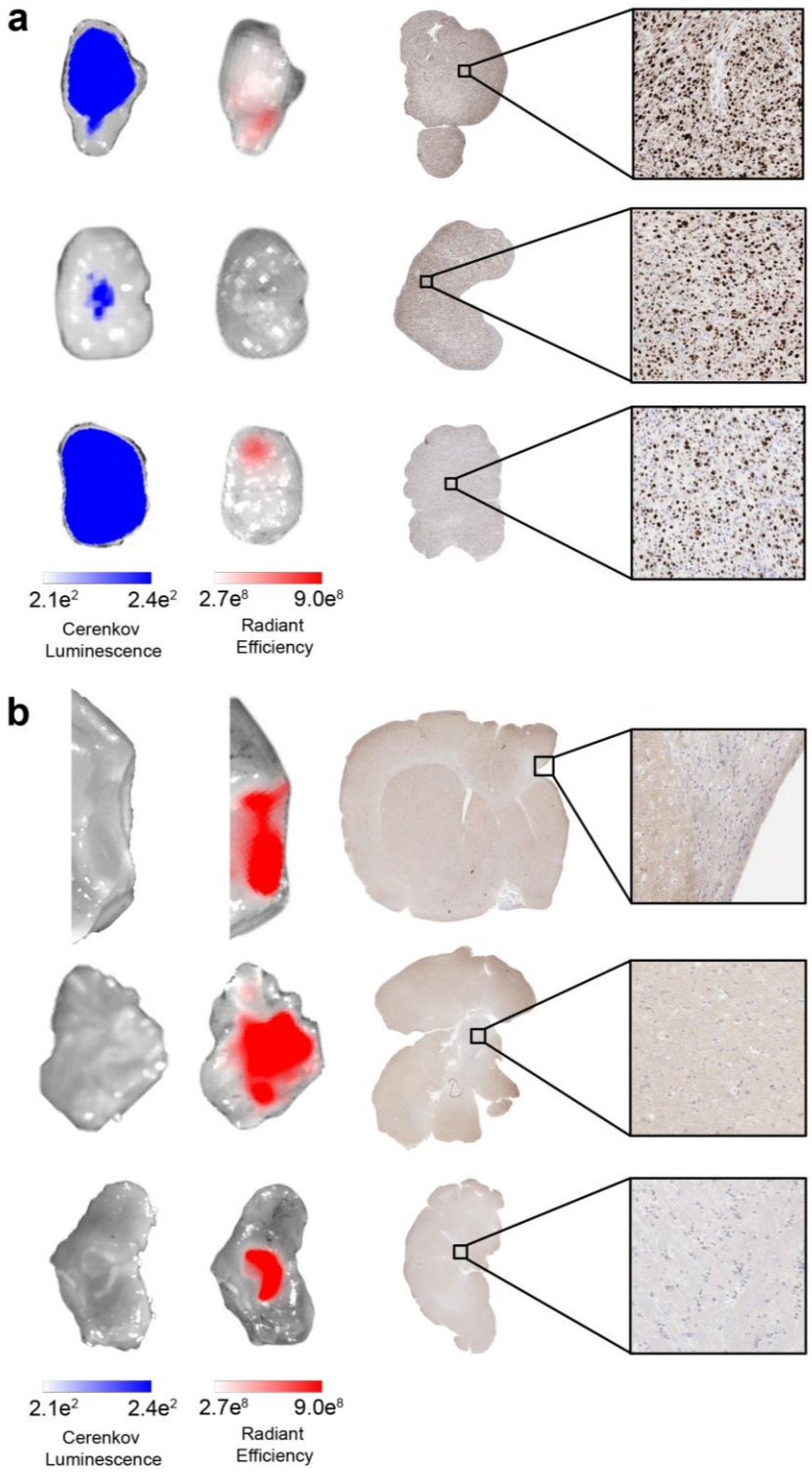

Figure S9. Tumour resections with positive FET CLI signal (a) and remaining brain or tumour resections positive for 5-ALA derived florescence. (a) FET CLI positive resections contained ki67 positive tumor cells; (b) 5-ALA derived florescence tissue was negative for tumor cells determined by serial sectioning and Ki67 staining.