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Version: Supplemental Material

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**Article:**

Blick, C, Ramachandran, A, Wigfield, S et al. (8 more authors) (2013) Hypoxia regulates FGFR3 expression via HIF-1 $\alpha$  and miR-100 and contributes to cell survival in non-muscle invasive bladder cancer. *British Journal of Cancer*, 109 (1). pp. 50-59. ISSN 0007-0920

<https://doi.org/10.1038/bjc.2013.240>

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## **Parallel RNA interference screens identify EGFR activation as an escape mechanism in FGFR3 mutant cancer**

Herrera-Abreu et al

Supplementary tables and figure legends

### **Supplementary table 1. Cell lines included in screen**

Cell lines included in screen, along with EC50 of PD173074, transfection conditions, and cellular phenotype.

### **Supplementary table 2. siRNA screen results for effect of siRNA on cell survival.**

### **Supplementary table 3. siRNA screen results for effect of siRNA on sensitivity to PD173074.**

### **Supplementary Figure legends**

#### **Supplementary Figure 1. siRNA screen results**

A and B) Effect of siRNA on cell survival and sensitivity to PD170734 across the panel of cell lines for siRNA targeting FGFR1, FGFR2 and FGFR3.

B. Effect of siRNA on sensitivity PD173074 for all siRNA in FGFR3 mutant cell lines, with siEGFR indicated in red. EGFR siRNA is not clearly delineated in MGHU3 and RT112M.

#### **Supplementary Figure 2. Combined targeting of EGFR and FGFR3**

A. Lapatinib and gefitinib increase sensitivity to PD170374, and decreased growth in the combination, in *FGFR3* mutant bladder cancer cell lines (*Top*) with no effect in other cell lines (*Bottom*).

B. 94-10 cell lines treated with indicated doses of PD173073, with or without gefitinib at 250nM or 500nM, for 72 hours. (*Left*) absolute effect on growth indicating the sensitivity of the cell line to gefitinib (*Right*) growth relative to cells treated with gefitinib alone. Gefitinib restores sensitivity to FGFR inhibition.

C. Clonogenic assay of combined PD173074 500nM and cetuximab (100ug/ml) treatment.

### **Supplementary Figure 3. Combined targeting of FGFR3 and EGFR**

A. Western blot of MGHU3 cell lysates treated for the indicated times with 500nM PD173074, 250nM Gefitinib, combination, or vehicle, blotted for phosphorylated and total EGFR. EGFR phosphorylation is upregulated at 6 hours by PD173074.

B. 97.7, RT112M and MGHU3 cells were treated with PD173074 500nM, gefitinib 250nM or the combination and subjected to PI flow cytometric analysis (FACS).

C. RT112M cell line treated for the indicated times with 500nM PD173074, 250nM Gefitinib, or the combination, before lysis. Lysates were immunoprecipitated with FGFR3 antibody, or normal IgG control, before blotting with FGFR3 or EGFR antibodies.

### **Supplementary Figure 4. CDC25C is an EGFR phosphatase but is likely not relevant to the EGFR feedback in this context.**

A. Sensitivity to PD173074 expressed as Z score for siRNAs against CDC25C in three *FGFR3* mutant bladder cancer cell lines.

B. RT112M cells were transfected with siCON or individual siRNA targeting CDC25C, lysates made 72 hours post transfection, and blotted with indicated antibodies.

C. Analysis of CDC25C-Thr48 phosphorylation following PD173074 in RT112M cells

**Supplementary Figure 5. Mouse weights from xenograft experiments**

A. RT112M xenografts were established in nude mice, and divided randomly into 4 groups treated with vehicle, PD173074 20mg/mg IP, gefitinib 110mg/kg PO, or combination of both inhibitors for 3 days. Mean body weight of mice per group.

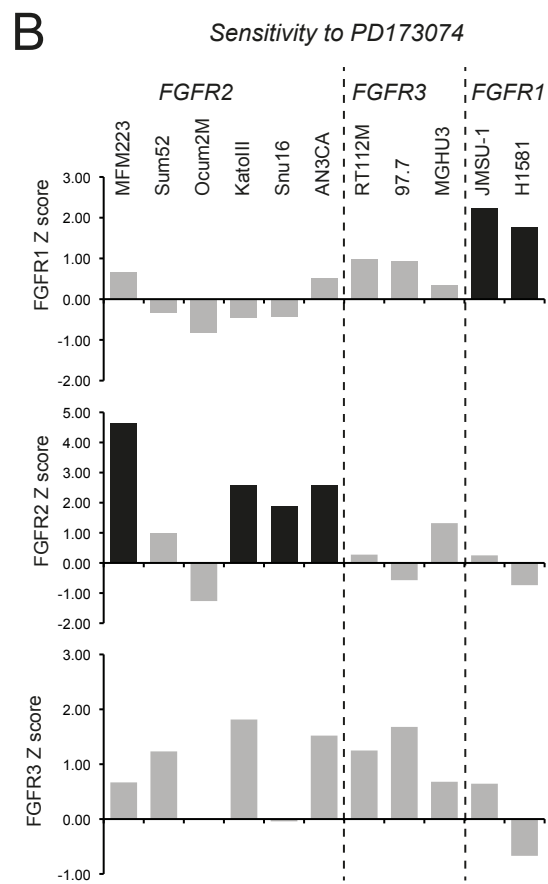
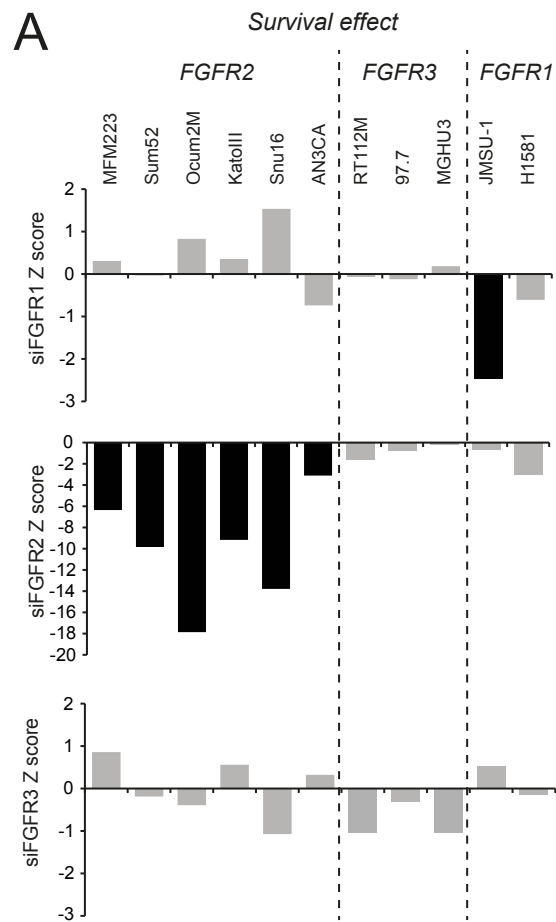
B. RT112M xenografts were established in nude mice, and divided randomly into 4 groups treated with vehicle, PD173074 15mg/mg IP (days 0-3 and days 7-10), cetuximab 40mg/kg IP (days 0,3,7,10) or combination of both inhibitors. Mean body weight of mice per group.

**Supplementary Figure 6. Expression of EGFR and HGF from a publically available bladder cancer gene expression data set.**

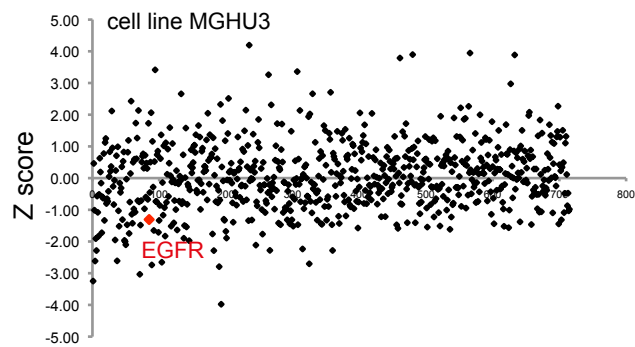
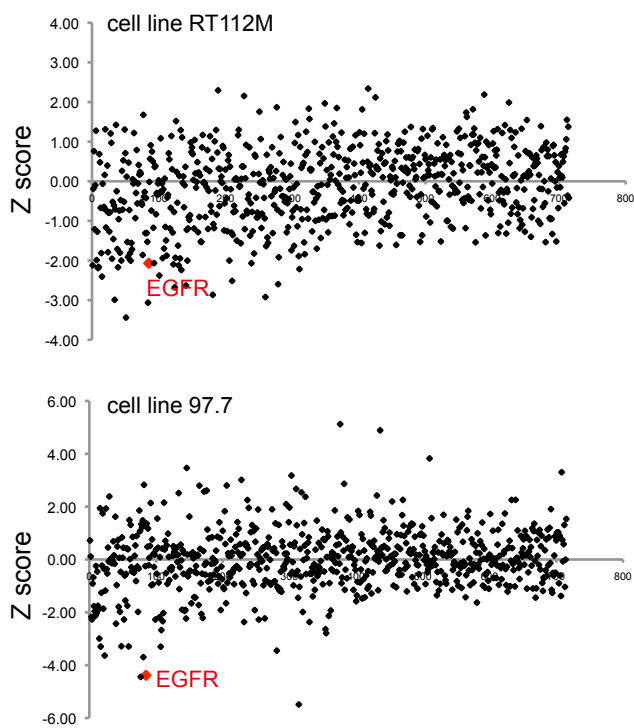
A. Expression of EGFR mRNA (201983\_s\_at) in a publically available gene expression data set of normal bladder, invasive bladder cancers, and superficial type bladder cancers (36). Comparison between groups with Students T test.

B. Expression of HGF mRNA (209960\_at) in a publically available gene expression data set of normal bladder, invasive bladder cancers, and superficial type bladder cancers (36). Comparison between groups with Students T test.

# Supplementary Figure 1

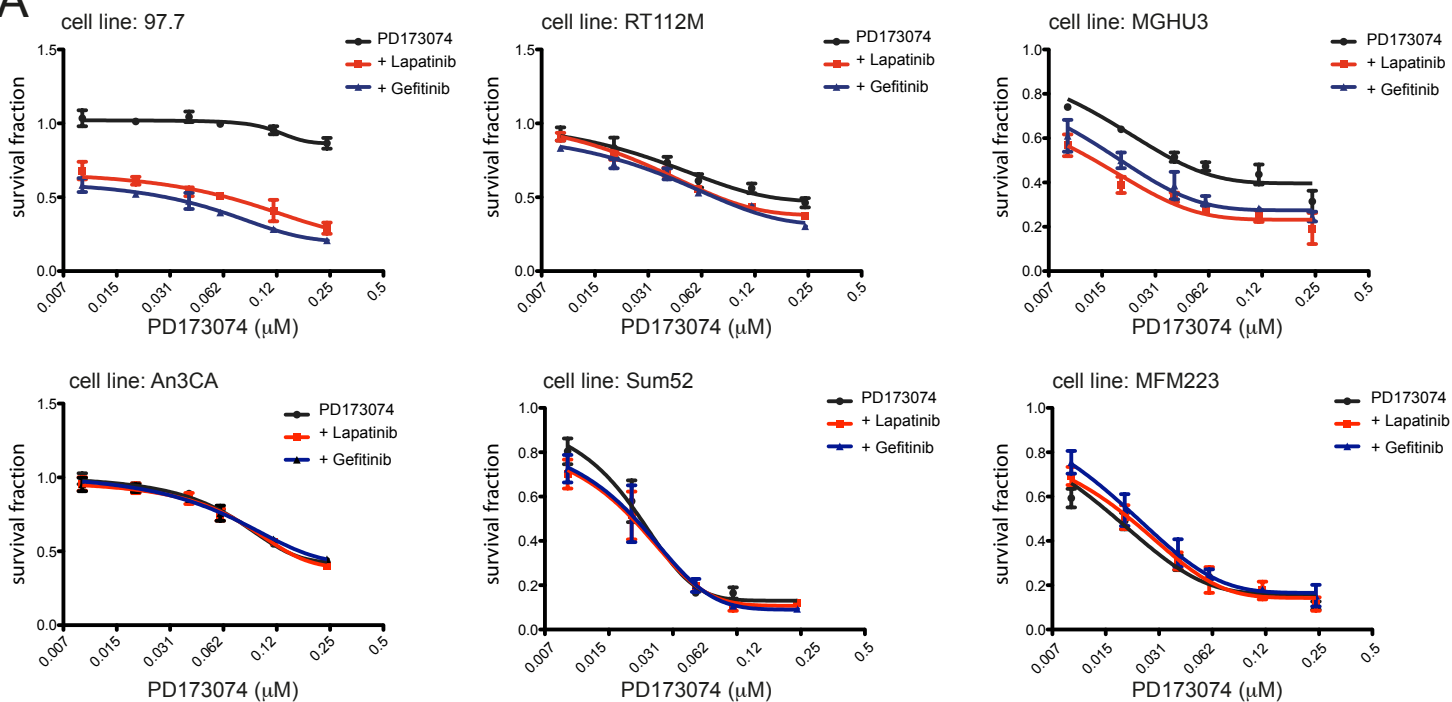


**C** *Sensitivity to PD173074*

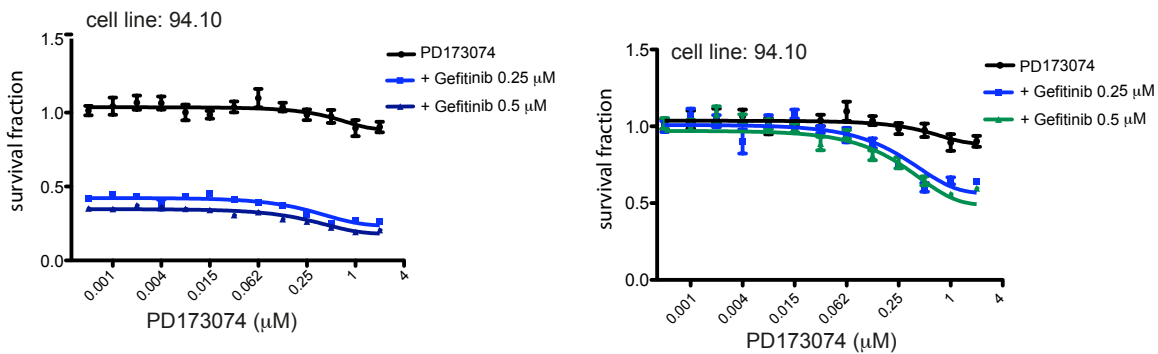


# Supplementary Figure 2

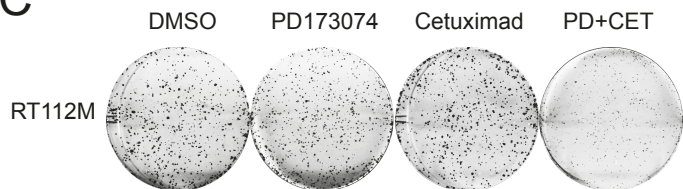
**A**



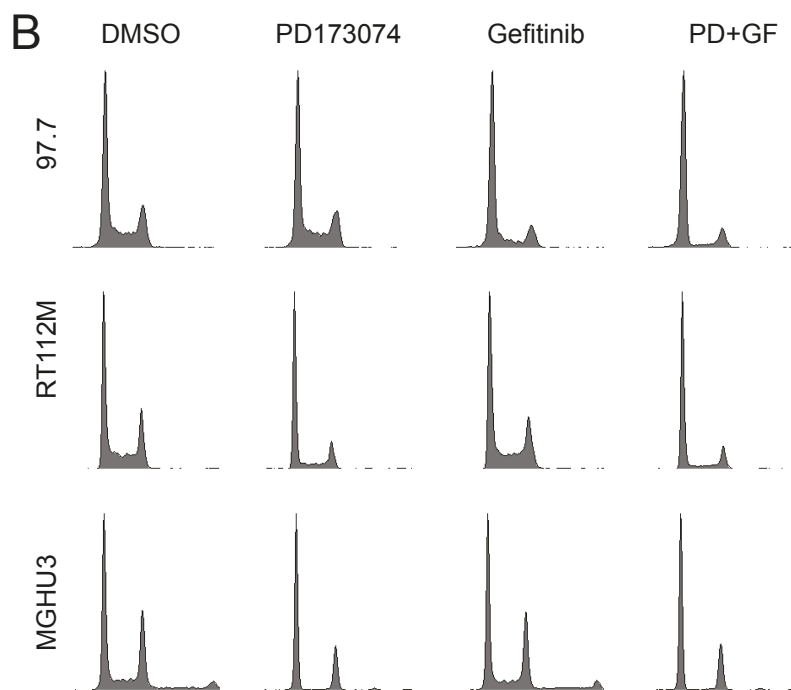
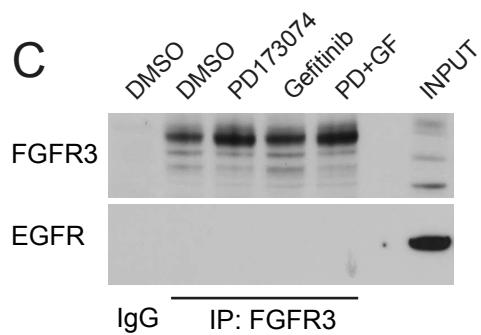
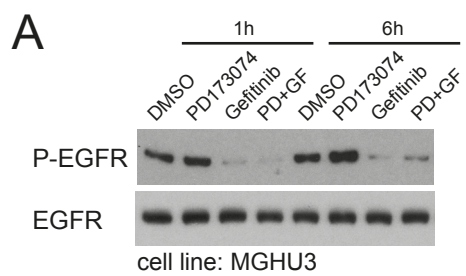
**B**



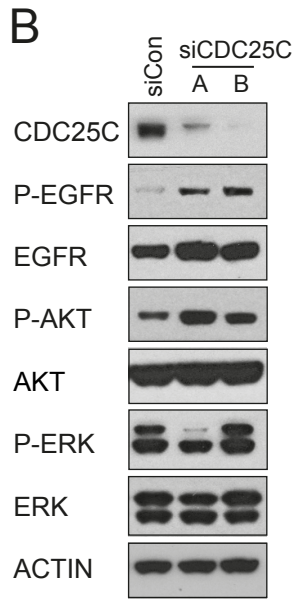
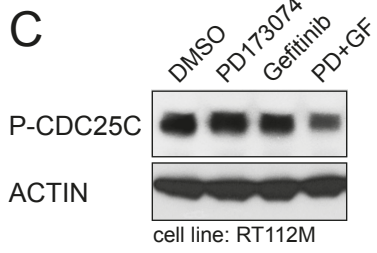
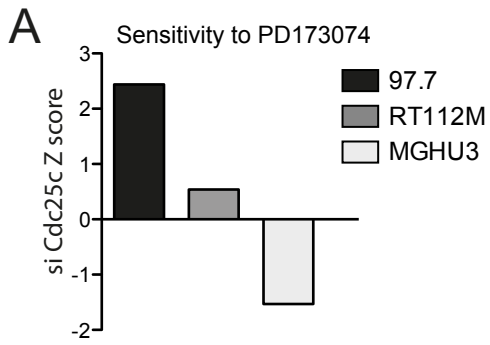
**C**



# Supplementary Figure 3



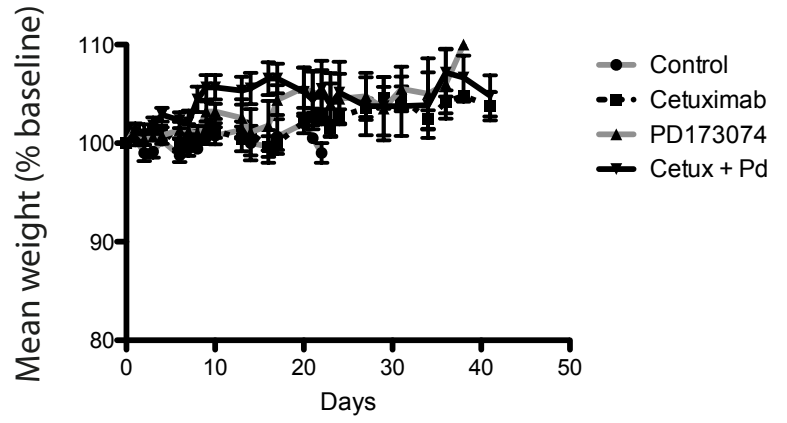
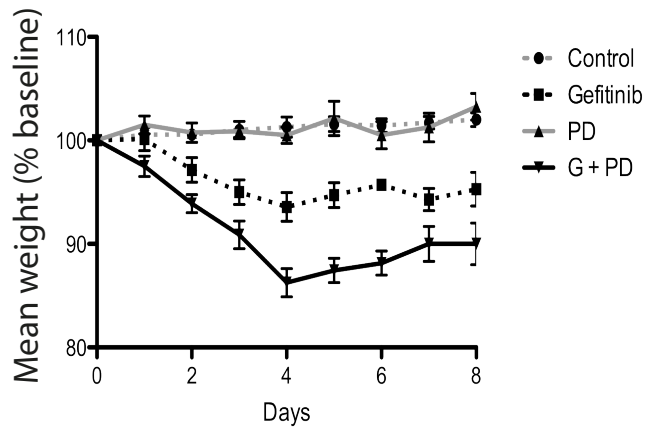
# Supplementary Figure 4





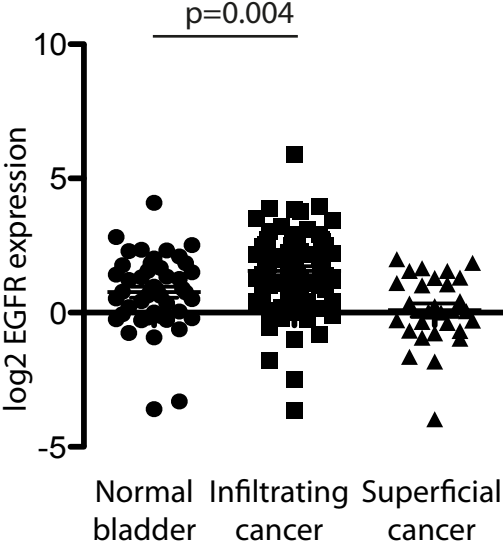
# Supplementary Figure 5

## A



Supplementary Figure 6

A



B

