# Supplementary Materials

## Pharmacodynamic studies

### Methods

Optional, paired radiologic guided fine needle aspiration core tumor and/or skin punch biopsies were obtained in eligible patients with accessible tumors at baseline and on day 15 prior to treatment and were immediately placed into RNA*later* (for RT-PCR) or formalin (for immunohistochemistry)**.**

RT-PCR: Total RNA was extracted from tumor samples preserved in RNA*later* using the RNeasy Mini kit (Qiagen). cDNA was synthesized using the Applied Biosystems high capacity cDNA reverse transcription kit, following the manufacturer's instructions. An EGF/PDGF pathway array (Qiagen # PAHS-040) was used to examine differences in gene expression between baseline and day 15 pre-dose tumor biopsies, following the manufacturer's instructions. This $RT^2$ human pathway array evaluates 84 genes involved in the EGF/PDGF pathways. Samples were amplified using the ABI Step One Plus RT-PCR system. Relative expression of the mRNA analyzed was estimated using the formula: $2^{-\Delta CT}$, where $\#C_T = C_T$ (mRNA) – $C_T$ (Housekeeper).

Immunohistochemistry: Core tumor and skin biopsies (baseline and prior to dosing on day 15) were fixed in formalin immediately after the procedure and processed in to paraffin wax blocks. Sections were deparaffinized using standard histologic procedures, and an antigen retrieval method (pressure cooker and high EDTA buffer) was used to ensure optimal antigen integrity and expression. Differences in expression of EGFR, AKT, p-AKT, ERK1/2, p-ERK1/2 were determined by IHC using commercially available antibodies against these proteins. IHC staining was scored by eye (from a minimum of 1000 cells for the per cent markers) by a physician trained in gastrointestinal pathology who was blinded to the results.

## Results

### Pharmacodynamic Analysis: EGFR, pAkt /t-Akt ratio and pERK/t-ERK ratio

The effect of treatment on changes in EGFR expression, pERK/t-ERK and pAkt /t-Akt ratios was determined by immunohistochemistry in paired skin biopsies obtained at baseline and on day 15 of cycle 1 from 6, 4 and 7 patients, respectively**.** There was no significant change in expression from baseline with treatment in any of these proteins as measured by paired t-tests **(Supplementary figure 1A-C).** However, a trend towards decreased pERK/t-ERK ratio (p=0.14) **(Supplementary Figure 1C)** was noted with treatment.

### EGF/PDGF pathway gene array

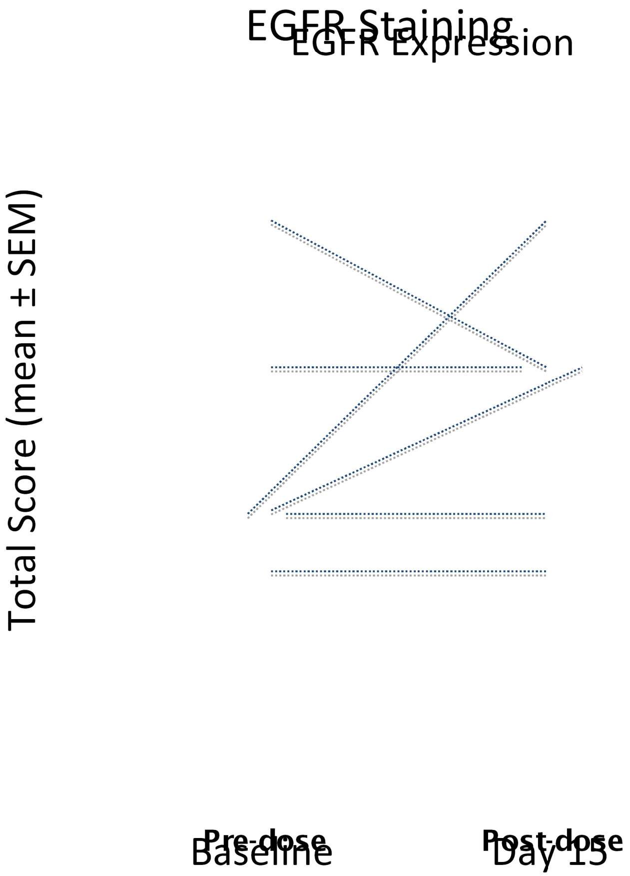
The effect of treatment changes in expression of EGF and PDGF pathways genes was determined by gene array in paired tumor biopsies obtained at baseline and on day 15 of cycle 1 from the 3 patients with satisfactory amounts of available tissue. Several pro-survival genes including those involved in the PI3K pathway (PIK3CA and the mTOR effector protein, RPS6KB1) and the PDGF pathway (PDGFA, PDGFB, and PDGFRA) were upregulated with treatment, while expression of EGF was downregulated **(Supplementary Figure 1D).** Individual gene array data from the three patients are shown in **Supplemental Figure 2.**

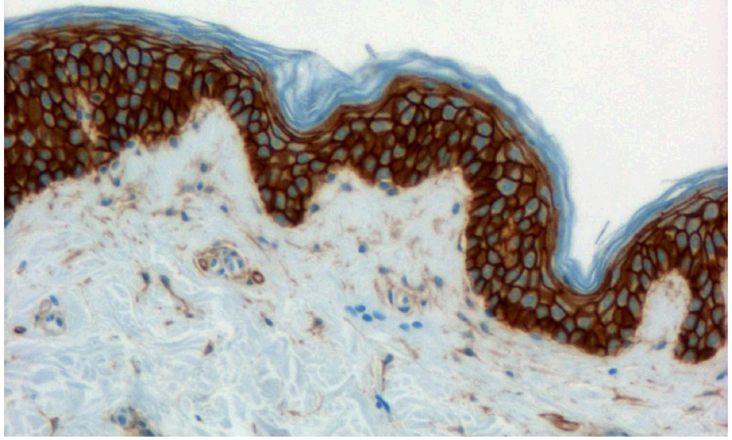
## FIGURE LEGENDS

**Supplementary Figure 1:** Immunohistochemical pharmacodynamic analyses of paired pre-treatment and day 15 skin biopsies for EGFR (1A), p-Akt/t-Akt (1B) and p-ERK/t-ERK (1C). EGF/PDGF pathways gene array on paired pre-treatment and day 15 tumor biopsies are shown in figure 1D.

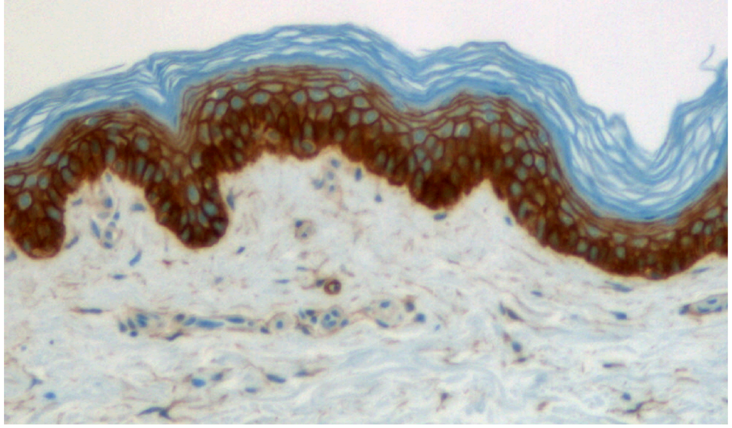
**Supplemental Figure 2:** EGF/PDGF pathways gene array on 3 individual patients' paired pre-treatment and day 15 tumor biopsies (figures 2A, 2B, 2C)

# Supplementary Figure 1A



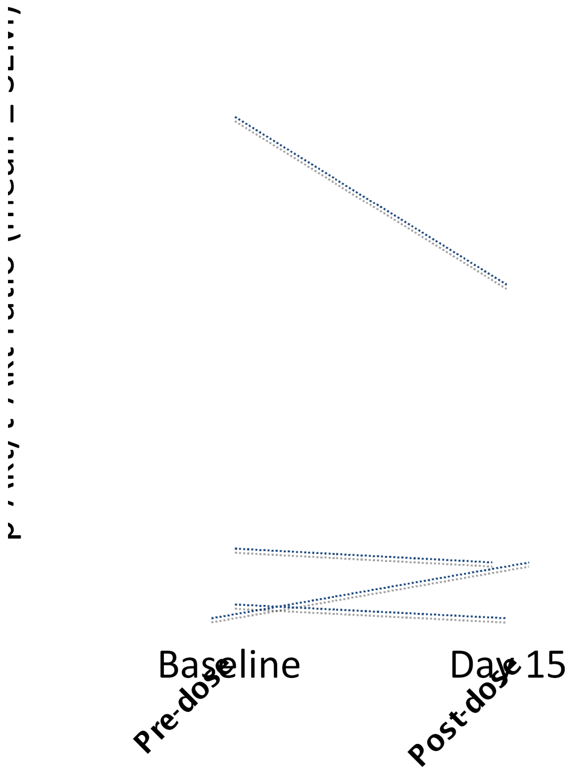


Baseline

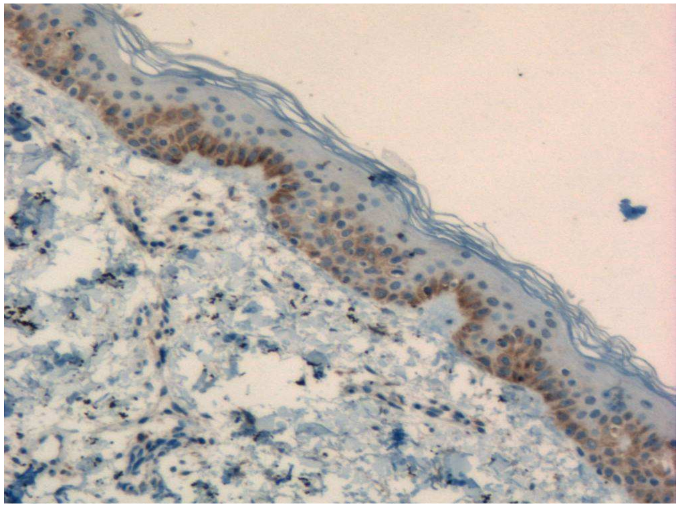


Day 15

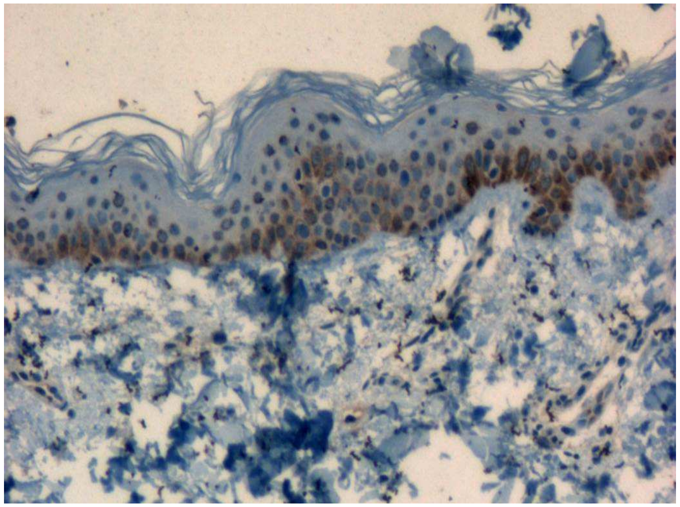
# Supplementary Figure 1B



pAkt Staining

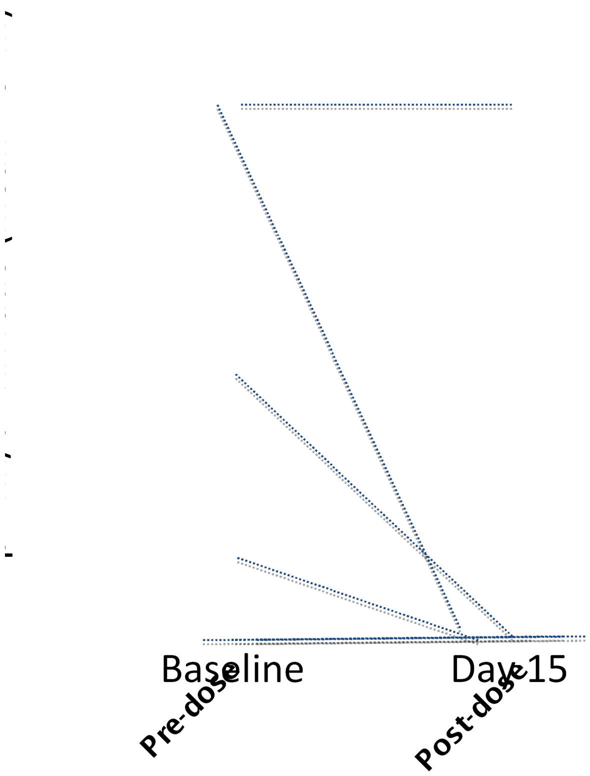


Baseline

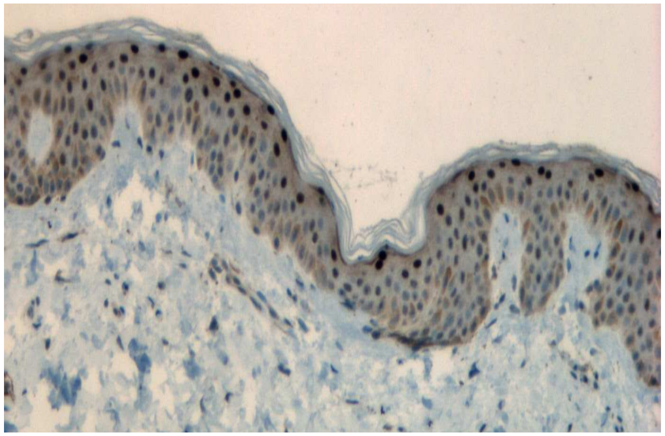


Day 15

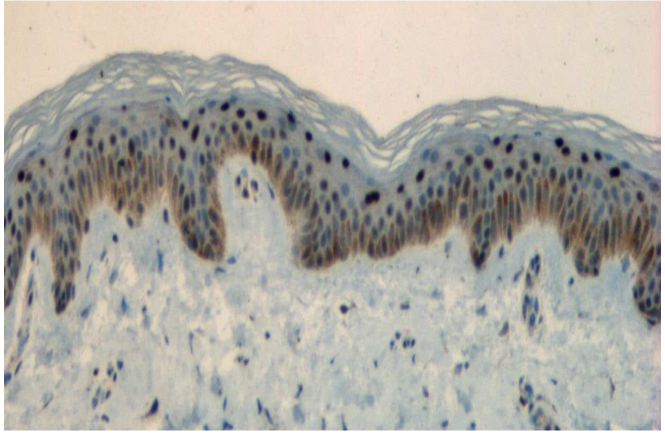
# Supplementary Figure 1C



pERK1/2 Staining

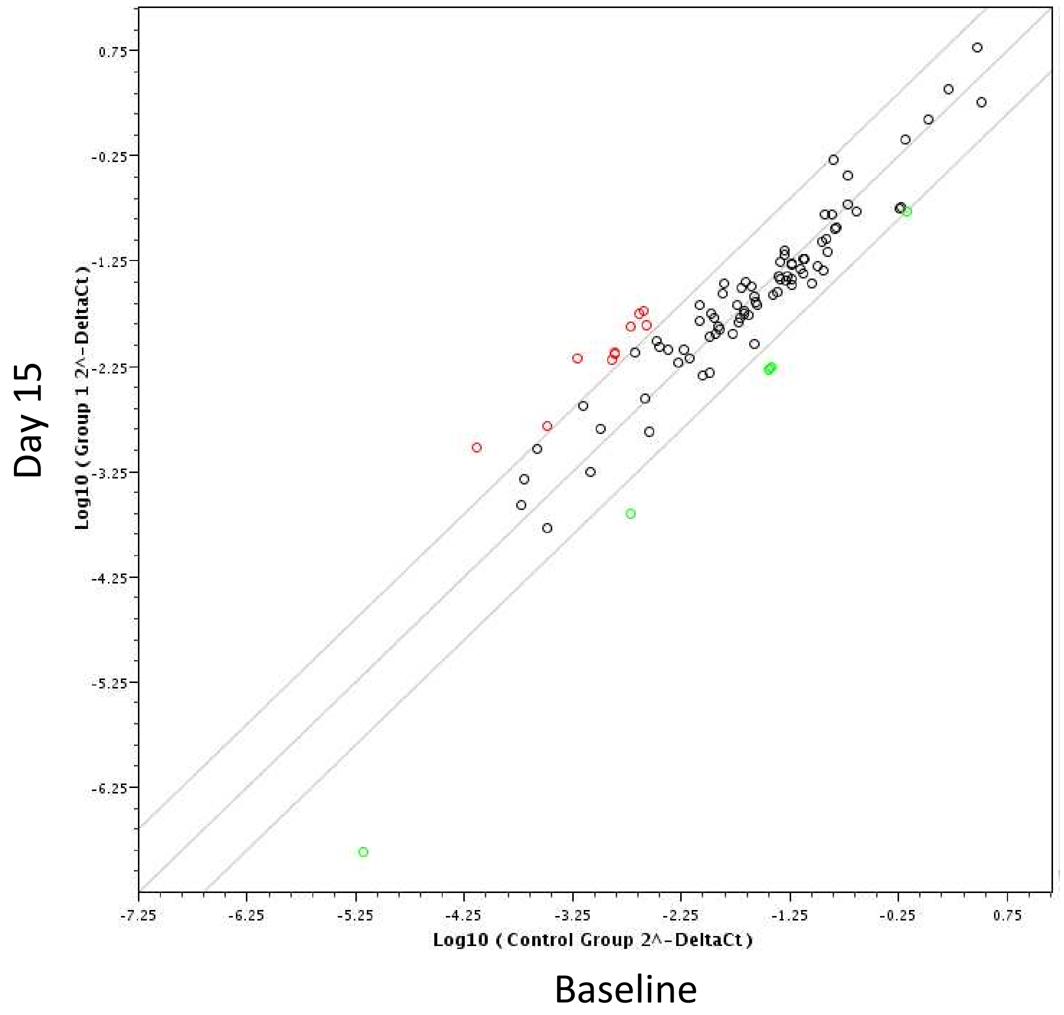


Baseline



Day 15

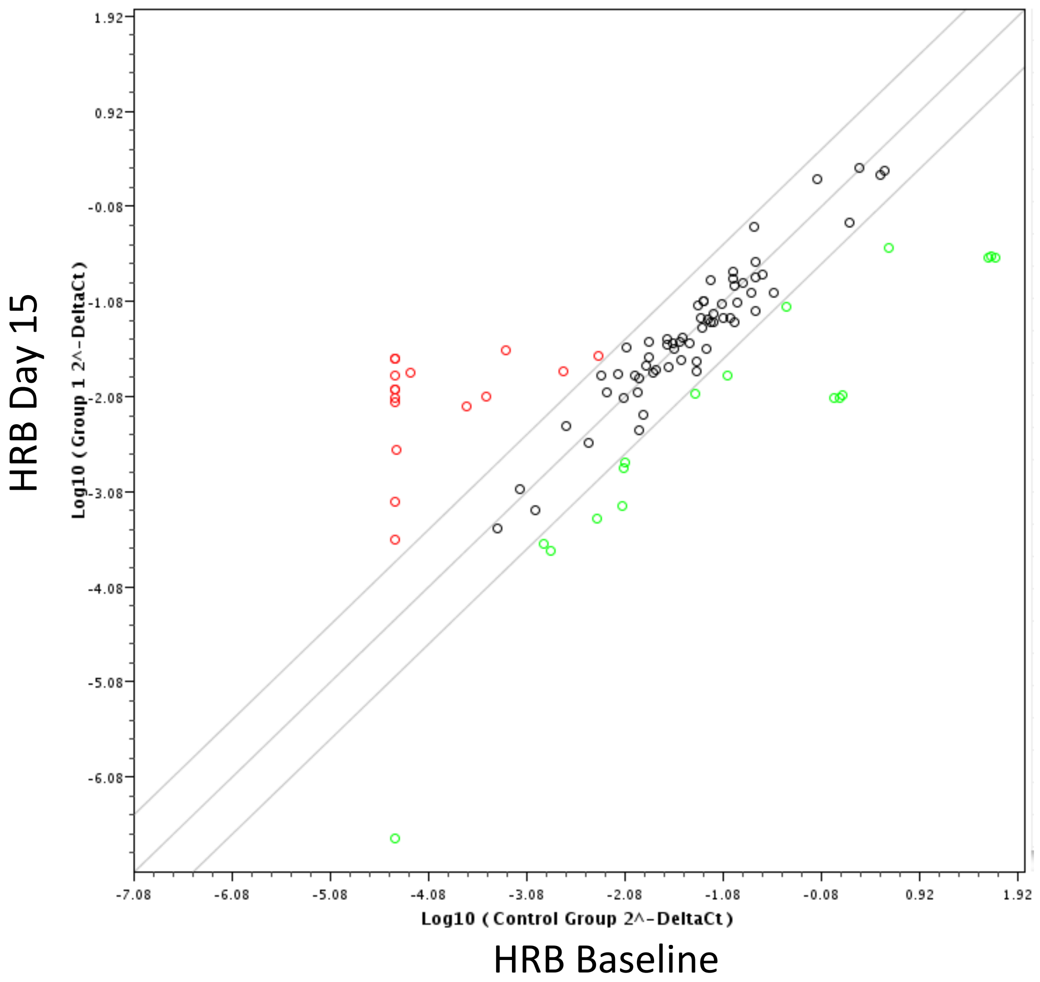
# Supplementary Figure 1D



| Gene Symbol | Fold Regulation |
|---|---|
| EIF4E | 7.4334 |
| GAB1 | 5.1088 |
| GSK3A | 5.3814 |
| HBEGF | 6.9981 |
| LTA | 13.2289 |
| PDGFA | 5.0388 |
| PDGFB | 10.8222 |
| PDGFRA | 4.6157 |
| PIK3CA | 7.8412 |
| RPS6KB1 | 5.6274 |

| Gene Symbol | Fold Regulation |
|---|---|
| EGF | -8.6306 |

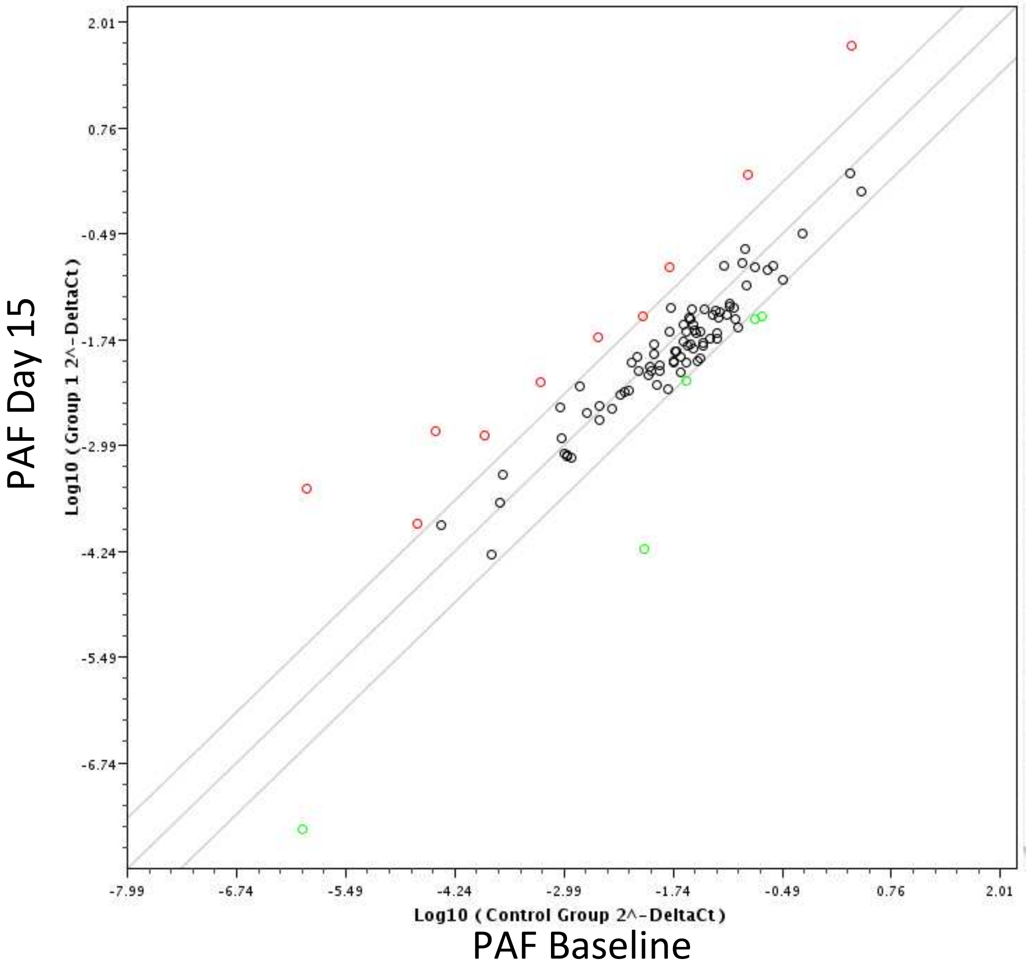
# Supplementary Figure 2A



| Gene Symbol | Fold Regulation |
|---|---:|
| AKT2 | 16.8659 |
| CASP3 | 49.2885 |
| CHUK | 5.0968 |
| EIF4E | 541.3265 |
| ELK1 | 192.9434 |
| GAB1 | 360.8404 |
| GSK3A | 210.5031 |
| HBEGF | 271.1823 |
| IKBKB | 7.7123 |
| LTA | 6.8402 |
| MAP2K7 | 59.055 |
| NFATC3 | 32.5836 |
| NUP62 | 25.9504 |
| PDGFB | 255.0754 |
| PIK3CA | 537.0225 |
| RPS6KB1 | 262.7201 |

| Gene Symbol | Fold Regulation |
|---|---:|
| BCL2 | -4.8672 |
| BRAF | -5.335 |
| FASLG | -13.4312 |
| FN1 | -12.9583 |
| IL2 | -5.3709 |
| PDGFRA | -9.7587 |
| PLAT | -7.3537 |
| PLCG1 | -4.848 |
| RPS6KA5 | -6.5592 |
| STAT1 | -4.9692 |

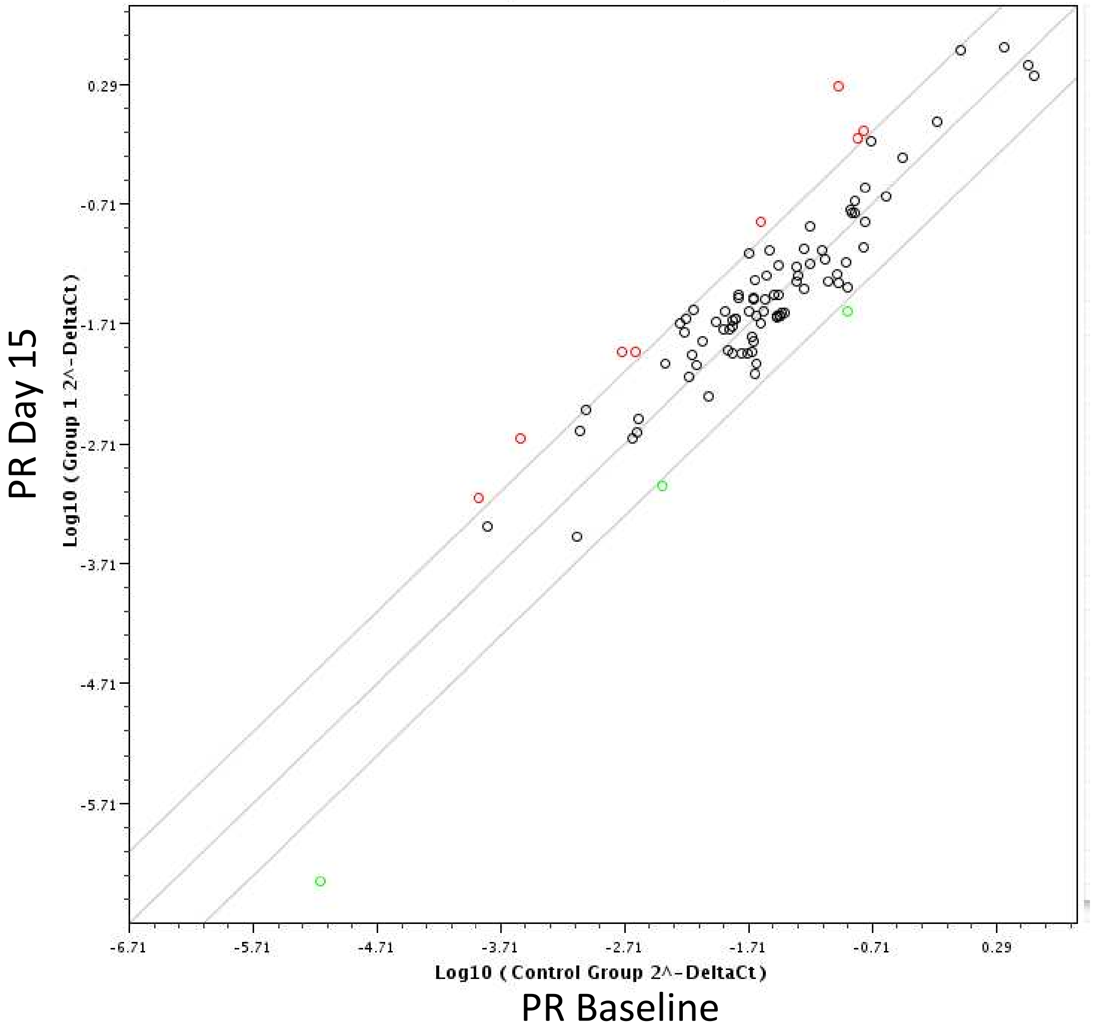
# Supplementary Figure 2B



| Gene Symbol | Fold Regulation |
|---|---|
| AKT3 | 7.5456 |
| COL1A1 | 8.0664 |
| FASLG | 10.411 |
| FN1 | 12.3685 |
| LTA | 43.0704 |
| MAPK10 | 5.765 |
| PDGFA | 10.5181 |
| PDGFRA | 268.1517 |
| STAT5A | 4.1905 |
| B2M | 26.9093 |

| Gene Symbol | Fold Regulation |
|---|---|
| EGF | -135.5841 |
| EPS8 | -4.8472 |
| RPS6KA5 | -4.4136 |

# Supplementary Figure 2C



| Gene Symbol | Fold Regulation |
|---|---|
| AKT3 | 5.6515 |
| BCL2 | 4.8775 |
| COL1A1 | 18.2718 |
| IL2 | 5.3406 |
| LTA | 7.8581 |
| PDGFA | 6.1265 |

| Gene Symbol | Fold Regulation |
|---|---|
| EGFR | -4.8474 |
| EGR1 | -4.4853 |