Fig. S1.
Fig. S2.

A

Blot: pTyr

-  +  -  +  -  +

TCL  IgG  EPS8  dasatinib

B

Blot: EPS8

-  +  -  +  -  +

TCL  IgG  EPS8  dasatinib
Fig. S2.
Fig. S3

| Cell Line | G1 (%) | S (%) | G2/M (%) |
|-----------|--------|-------|----------|
| HN4       | 63.7   | 16.2  | 15.6     |
| 602F      | 61.3   | 17.4  | 18.4     |
| FFFF      | 58.2   | 14.7  | 23       |
Fig. S4.
Fig. S4.
Fig. S4.
Legends to Supplementary Figures:

**Figure S1: EPS8 is constitutively tyrosine-phosphorylated in HN4 cells.** HN4 cells were deprived of serum for 16 h, and then lysed with or without addition of 10 ng/ml EGF for 10 min. EPS8 was immunoprecipitated as described in Methods and proteins resolved by SDS-PAGE and western blotted with antibodies that recognize phosphotyrosine (A). Antibody was then removed and the blot incubated with EPS8 antibody (B). TCL, 50 μg total cell lysate (5% input).

**Figure S2: Dasatinib reduces tyrosine phosphorylation of EPS8. A. & B.** HN4 cells were treated with 400 nM dasatinib, or an equivalent volume of vehicle, for 16h and then lysed. EPS8 was immunoprecipitated as described in Methods and proteins resolved by SDS-PAGE and western blotted with anti-phosphotyrosine antibody (A). Subsequently, antibody was removed and the blot incubated with EPS8 antibody (B). TCL, 50 μg total cell lysate (5% input). **C. & D.** HN4/FFFF cells, cultured in the presence or absence of dasatinib, were lysed, EPS8 immunoprecipitated, and western blotted with anti-phosphotyrosine (C) followed by antibody removal and reprobing with anti-EPS8 (D).

**Figure S3: EPS8 Src phosphorylation site mutants show reduced exit from G2/M.** The indicated cells were trypsinized, counted, fixed, stained and analyzed by flow cytometry, as described in Methods.

**Figure S4: Impact of Src phosphorylation site mutants of EPS8 on keratin expression.** HN4 parental cells, or cells overexpressing wild-type EPS8, FFFF-, FFF-, or 602F-EPS8, or HN12 cells as
control, were grown in triplicate on coverslips, fixed with cold methanol, and immunostained with keratin antibody (A) or with mouse IgG as a negative control (B). Samples were stained with AlexaFluor488-conjugated secondary antibodies and counterstained with DAPI. Original magnification, x400. (C). Cultures of the indicated cell lines were photographed at 100x under phase contrast. Bar = 50 μm.

**Figure S5: Increased kinase activity in FFFF- and 602F-EPS8-expressing cells.** Total protein lysates were prepared from cell lines, resolved by SDS-PAGE, and western blotted sequentially with the indicated antibodies.

**Figure S6: Expression of PTEN in HN4 and derivative cell lines.** Total protein lysates were prepared from the indicated cell lines, resolved by SDS-PAGE, and western blotted sequentially with PTEN and GAPDH antibodies.

**Legends to Supplementary Tables:**

**Table S1.** Genes showing significant downregulation in 602F-EPS8-expressing HN4 cells.

**Table S2.** Genes showing significant downregulation in dasatinib-treated HN4 cells.

**Table S3.** Genes showing significant upregulation in 602F-EPS8-expressing HN4 cells.

**Table S4.** Genes showing significant upregulation in dasatinib-treated HN4 cells.
**Table 1: KEGG Pathways**

| Category | ID | Description | GeneRatio | BgRatio | pvalue | padj | geneID |
|----------|----|-------------|-----------|---------|--------|-------|--------|
| Parkinson's disease | KEGG | hsa04110 | Cell cycle | 65/1070 | 124/5712 | 1.51e-17 | 3.25e-15 |
| Parkinson's disease | KEGG | hsa03503 | DNA replication | 26/1070 | 36/5712 | 3.47e-12 | 1.49e-10 |
| Parkinson's disease | KEGG | hsa03030 | Ribosome biogenesis in eukaryotes | 40/72/5712 | 72/1070 | 2.44e-12 | 1.31e-10 |
| Parkinson's disease | KEGG | hsa03160 | Huntington's disease | 49/172/5712 | 182/5712 | 5.09e-06 | 1.3e-06 |
| Parkinson's disease | KEGG | hsa03430 | Mismatch repair | 5/172/5712 | 21/5712 | 9.10e-06 | 2.2e-06 |
| Parkinson's disease | KEGG | hsa05122 | Parkinson's disease | 45/1070 | 130/5712 | 1.04e-05 | 1e-05 |
| Parkinson's disease | KEGG | hsa03190 | Oxidative phosphorylation | 54/1070 | 171/5712 | 1.27e-05 | 1e-05 |
| Parkinson's disease | KEGG | hsa03410 | Pyrimidine metabolism | 34/1070 | 99/5712 | 1.00e-17 | 1e-17 |
| Parkinson's disease | KEGG | hsa03411 | Ubiquitin mediated proteolysis | 42/1070 | 134/5712 | 1.63e-05 | 1e-05 |
| Parkinson's disease | KEGG | hsa03450 | Non-homologous end-joining | 8/170/5712 | 43/5712 | 1.00e-06 | 1e-06 |
| Parkinson's disease | KEGG | hsa03420 | Nucleotide excision repair | 34/1070 | 171/5712 | 1.00e-17 | 1e-17 |
| Alzheimer's disease | KEGG | hsa04110 | Cell cycle | 47/1070 | 166/5712 | 1.00e-17 | 6.2e-17 |
| Alzheimer's disease | KEGG | hsa03411 | Base excision repair | 42/1070 | 134/5712 | 1.00e-17 | 6.2e-17 |
| Alzheimer's disease | KEGG | hsa03414 | Oocyte meiosis | 34/1070 | 171/5712 | 1.00e-17 | 6.2e-17 |
| Alzheimer's disease | KEGG | hsa03412 | Prostate cancer repair | 27/1070 | 87/5712 | 1.00e-17 | 6.2e-17 |

**Additional Pathways**

| Category | ID | Description | GeneRatio | BgRatio | pvalue | padj | geneID |
|----------|----|-------------|-----------|---------|--------|-------|--------|
| Parkinson's disease | KEGG | hsa05120 | RNA transport | 65/1070 | 147/5712 | 5.78e-13 | 1e-13 |
| Parkinson's disease | KEGG | hsa05130 | Ribosome biogenesis in eukaryotes | 40/72/5712 | 72/1070 | 2.44e-12 | 1.31e-10 |
| Parkinson's disease | KEGG | hsa03030 | DNA replication | 26/1070 | 36/5712 | 3.47e-12 | 1.49e-10 |
| Parkinson's disease | KEGG | hsa03430 | Mismatch repair | 5/172/5712 | 21/5712 | 9.10e-06 | 2.2e-06 |
| Parkinson's disease | KEGG | hsa05122 | Parkinson's disease | 45/1070 | 130/5712 | 1.04e-05 | 1e-05 |
| Parkinson's disease | KEGG | hsa03410 | Pyrimidine metabolism | 34/1070 | 99/5712 | 1.00e-17 | 1e-17 |
| Parkinson's disease | KEGG | hsa03411 | Ubiquitin mediated proteolysis | 42/1070 | 134/5712 | 1.63e-05 | 1e-05 |
| Parkinson's disease | KEGG | hsa03450 | Non-homologous end-joining | 8/170/5712 | 43/5712 | 1.00e-06 | 1e-06 |
| Parkinson's disease | KEGG | hsa03420 | Nucleotide excision repair | 34/1070 | 171/5712 | 1.00e-17 | 1e-17 |
| Alzheimer's disease | KEGG | hsa04110 | Cell cycle | 47/1070 | 166/5712 | 1.00e-17 | 6.2e-17 |
| Alzheimer's disease | KEGG | hsa03411 | Base excision repair | 42/1070 | 134/5712 | 1.00e-17 | 6.2e-17 |
| Alzheimer's disease | KEGG | hsa03414 | Oocyte meiosis | 34/1070 | 171/5712 | 1.00e-17 | 6.2e-17 |
| Alzheimer's disease | KEGG | hsa03412 | Prostate cancer repair | 27/1070 | 87/5712 | 1.00e-17 | 6.2e-17 |

**KEGG Pathways**

- **Parkinson's disease**
  - hsa05120: Parkinson's disease (p = 3.12e-05)
  - hsa03030: DNA replication (p = 4.70e-01)
  - hsa03410: Pyrimidine metabolism (p = 9.99e-09)
  - hsa03420: Nucleotide excision repair (p = 1.61e-09)

- **Alzheimer's disease**
  - hsa04110: Cell cycle (p = 1.62e-09)
  - hsa03411: Base excision repair (p = 1.62e-09)
  - hsa03414: Oocyte meiosis (p = 1.62e-09)
  - hsa03412: Prostate cancer repair (p = 1.62e-09)
| Category | ID | Description | GeneRatio | pvalue | padj | geneID | Count |
|----------|----|-------------|-----------|--------|-------|--------|-------|
| KEGG     | hsa03016 | DNA replication | 30/387 | 36/372 | 4.87E-20 | 1.02E-17 | 36/5712 |
|          | hsa04110 | Cell cycle | 60/387 | 124/372 | 1.77E-19 | 1.86E-17 | 212/5712 |
|          | hsa04140 | Spliceosome | 56/387 | 125/372 | 2.48E-16 | 1.47E-14 | 193/5712 |
|          | hsa04140 | Spliceosome | 56/387 | 125/372 | 2.48E-16 | 1.47E-14 | 193/5712 |
|          | hsa04140 | Spliceosome | 56/387 | 125/372 | 2.48E-16 | 1.47E-14 | 193/5712 |
|          | hsa04140 | Spliceosome | 56/387 | 125/372 | 2.48E-16 | 1.47E-14 | 193/5712 |
|          | hsa10100 | Metabolic pathways | 207/387 | 117/372 | 7.11E-05 | 0.000169 | 9/5712 |
|          | hsa05164 | Huntington's disease | 49/387 | 182/372 | 9.46E-05 | 0.001728 | 9/5712 |
|          | hsa05170 | Amyotrophic lateral sclerosis | 63/387 | 16/372 | 0.000190 | 0.004287 | 2/5712 |
|          | hsa05188 | Ribosome biogenesis in eukaryotes | 38/387 | 132/372 | 1.77E-05 | 0.000816 | 1/5712 |
|          | hsa05188 | Ribosome biogenesis in eukaryotes | 38/387 | 132/372 | 1.77E-05 | 0.000816 | 1/5712 |
|          | hsa05188 | Ribosome biogenesis in eukaryotes | 38/387 | 132/372 | 1.77E-05 | 0.000816 | 1/5712 |
|          | hsa05188 | Ribosome biogenesis in eukaryotes | 38/387 | 132/372 | 1.77E-05 | 0.000816 | 1/5712 |
|          | hsa05190 | Carbohydrate metabolism | 49/387 | 121/372 | 2.10E-05 | 0.000301 | 1/5712 |
|          | hsa05190 | Carbohydrate metabolism | 49/387 | 121/372 | 2.10E-05 | 0.000301 | 1/5712 |
|          | hsa05190 | Carbohydrate metabolism | 49/387 | 121/372 | 2.10E-05 | 0.000301 | 1/5712 |
|          | hsa05190 | Carbohydrate metabolism | 49/387 | 121/372 | 2.10E-05 | 0.000301 | 1/5712 |
|          | hsa05190 | Carbohydrate metabolism | 49/387 | 121/372 | 2.10E-05 | 0.000301 | 1/5712 |

**Supplementary Table S2**
| Category            | ID       | Description                                | GenRatio | Bgratio | pvalue | padj | geneID | Pathways in cancer                                                                 |
|---------------------|----------|--------------------------------------------|----------|---------|--------|------|--------|-------------------------------------------------------------------------------------|
| KEGG                | has03010 | Ribosome                                   | 44/994   | 88/5712 | 1.71E-12 | 2.00E-10 | RPL18/RPL31/RPS5/PGK/RPL3/RPS9/RPL18/RPL28/RPL34/RPS13/RPS2/RPL24/RPL52/RPL21/RPL36/RPL5/PGK/PGK11/RPL13/RPL11/PGK/PGK | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04512 | EOM-receptor interaction                   | 43/994   | 85/5712 | 1.84E-12 | 2.00E-10 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04510 | Focal adhesion                              | 71/994   | 200/5712 | 3.05E-10 | 2.21E-08 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04514 | Cell adhesion molecules (CAMs)              | 45/994   | 130/5712 | 1.34E-06 | 7.26E-05 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04142 | Lyssosome                                   | 41/994   | 121/5712 | 7.26E-05 | 6.00E-05 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has05410 | Hypertrophic cardiomyopathy (HCM)           | 30/994   | 83/5712 | 3.04E-05 | 0.0010999 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04411 | Protein processing in endoplasmic reticulum | 49/994   | 165/5712 | 0.00011655 | 0.0039312 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04010 | MAPK signaling pathway                      | 70/994   | 266/5712 | 0.00012502 | 0.0039312 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04514 | Dilated cardiomyopathy                      | 30/994   | 90/5712 | 0.00016992 | 0.00488251 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has05222 | Small cell lung cancer                      | 28/994   | 84/5712 | 0.00027266 | 0.00060361 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04522 | Pathways in cancer                          | 80/994   | 323/5712 | 0.00036345 | 0.00713089 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04521 | Arrhythmogenic right ventricular cardiomyopathy (ARVC) | 25/994   | 74/5712 | 0.00046048 | 0.00826979 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04670 | Leukocyte transendothelial migration         | 34/994   | 114/5712 | 0.00068711 | 0.01149647 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has05111 | Other glycan degradation                     | 9/994    | 17/5712 | 0.000902 | 0.01398084 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has05146 | Ameboliases                                 | 31/994   | 104/5712 | 0.00017767 | 0.00713089 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04444 | Endoctyosis                                 | 51/994   | 201/5712 | 0.00024502 | 0.00361222 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| KEGG                | has04513 | Type I diabetes mellitus                    | 15/994   | 41/5712 | 0.00025762 | 0.03277063 | TFGA/ITG24/COX2/TCN/NGC/BB8/LAMB1/LAM/LAM/COMP/COA1A/ALAMA/HBF4/ITG8/FGF/HSCA/COA1A/ITG84/COA2D8/ITG2A/1A  |
| Category | ID | Description | GeneRatio | BgRatio | pvalue | padj | geneID |
|----------|----|-------------|-----------|---------|---------|------|--------|
| KEGG | hsa04512 | ECM-receptor interaction | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa04142 | Lysosome | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa04510 | Focal adhesion | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa04670 | Leukocyte transendothelial migration | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa05146 | Amoebiasis | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa04810 | Regulation of actin cytoskeleton | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa04145 | Phagosome | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa04380 | Pathways in cancer | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa05412 | Arhythogenic right ventricular cardiomyopathy (ARVC) | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa00245 | Other genetic degradation | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa04514 | Cell adhesion molecules (CAMs) | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa00531 | Glycosaminoglycan degradation | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa04510 | Hypertrophic cardiomyopathy (HCM) | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa04514 | Dilated cardiomyopathy | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa04974 | Protein digestion and absorption | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa04380 | Osteoclast differentiation | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |
| KEGG | hsa05500 | Bacterial invasion of epithelial cells | 10.36E-06 | 9.83E-06 | 0.00452457 | 0.00854884 |