**Figure S1: qPCR of FGF2 mRNA expression in H1581 and L6 cells**

FGF2 mRNA.epsFor end-point qPCR, RNA was isolated from L6-WT, L6-V51M, H1581-WT and H1581-V561M cells using the Qiagen RNeasy mini kit. Contaminating genomic DNA was removed with recombinant DNase I, which was then inhibited using EDTA and heat. cDNA was generated using the BioRad iScript cDNA synthesis kit. qPCR was performed on the BioRad CFX96 Touch Real-Time PCR system using the BioRad iTaq SYBR Green mix. Primers were as follows:

L6 GAPDH F: 5’-GATGGTGAAGGTCGGTGTGA-3’;

L6 GAPDH R: 5’-TTGAGGTCAATGAAGGGGTC-3’;

H1581 GAPDH F: 5’-GAAGGTGAAGGTCGGAGTCA-3’;

H1581 GAPDH R: 5’-TTGAGGTCAATGAAGGGGTC-3’;

L6 FGF2 F: 5’-GTGTGTGCGAACCGGTACCT-3’;

L6 FGF2 R: 5’-GCTCTTAGCAGACATTGGAAG-3’;

H1581 FGF2 F: 5’-GTGTGTGCTAACCGTTACCT-3’;

H1581 FGF2 R: 5’-GCTCTTAGCAGACATTGGAAG-3’

**Table S1 and S2 Proliferation after various timepoints**

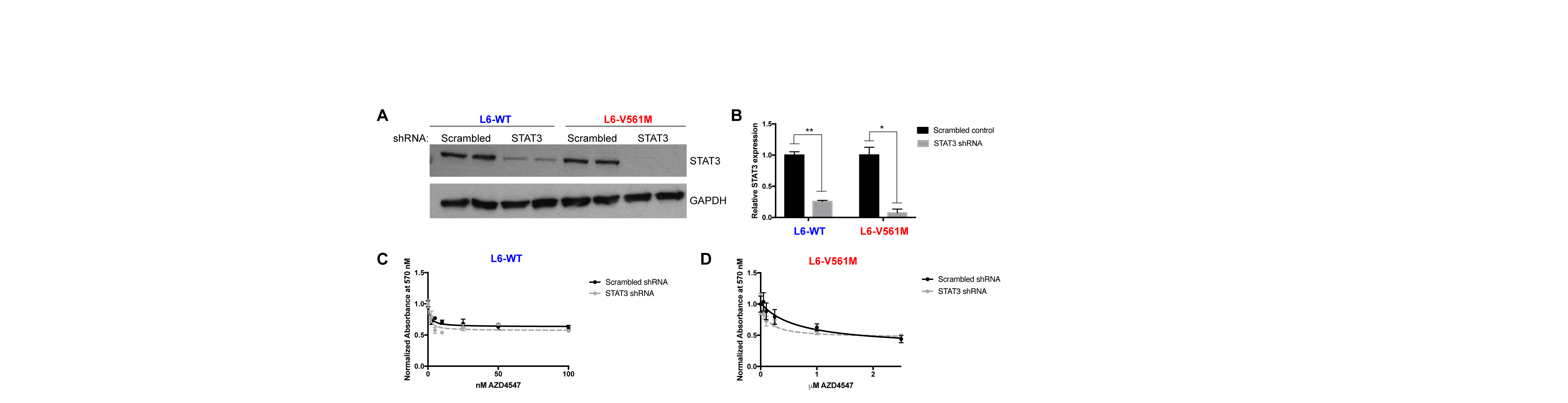
|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **L6 WT** | | | **L6 V561M** | | |  |  |
|  | Initial cell count | Final cell count | % Proliferation | Initial Cell Count | Final Cell Count | % Proliferation | Fold-increase in V561M cells | P-value |
| 72 h | 5000 | 7000 | 140 | 5000 | 15000 | 300 | 2 | 0.03 |
| 9000 | 18000 | 200 | 6000 | 26000 | 433.33 |
| 5000 | 8000 | 160 | 6000 | 17000 | 283.33 |
| 96 h | 8000 | 26000 | 325 | 4000 | 43000 | 1075 | 4.4 | 0.00004 |
| 4000 | 23000 | 575 | 6000 | 61000 | 1016.67 |
| 5000 | 18000 | 360 | 5000 | 50000 | 1000 |
| 120 h | 5000 | 47000 | 940 | 5000 | 89000 | 1780 | 2.5 | 0.03 |
| 4000 | 30000 | 750 | 7000 | 130000 | 1857.14 |
| 5000 | 46000 | 920 | 4000 | 110000 | 2750 |
| 168 h | 5000 | 350000 | 7000 | 3000 | 660000 | 22000 | 2.7 | 0.03 |
| 5000 | 200000 | 4000 | 5000 | 790000 | 15800 |
| 4000 | 340000 | 8500 | 4000 | 630000 | 15750 |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **H1581-WT** | | | **H1581-V561M** | | |  |  |
|  | Initial cell count | Final cell count | % Proliferation | Initial Cell Count | Final Cell Count | % Proliferation | Fold-increase in V561M cells | P-value |
| 72 h | 5000 | 13000 | 260 | 6000 | 44000 | 733.33 | 3 | 0.003 |
| 7000 | 12000 | 171.43 | 5000 | 34000 | 680 |
| 4000 | 13000 | 325 | 4000 | 32000 | 800 |
| 96 h | 5000 | 24000 | 480 | 5000 | 46000 | 920 | 2 | 0.0003 |
| 8000 | 35000 | 437.5 | 4000 | 40000 | 1000 |
| 6000 | 30000 | 500 | 7000 | 67000 | 957.14 |
| 120 h | 9000 | 85000 | 944.44 | 6000 | 120000 | 2000 | 2 | 0.04 |
| 6000 | 110000 | 1833.33 | 5000 | 150000 | 3000 |
| 6000 | 77000 | 1283.33 | 5000 | 140000 | 2800 |
| 168 h | 6000 | 330000 | 5500 | 5000 | 570000 | 11400 | 2.75 | 0.01 |
| 7000 | 240000 | 3428.57 | 6000 | 1000000 | 16666.67 |
| 4000 | 260000 | 6500 | 5000 | 720000 | 14400 |

**Table S1 and S2 Proliferation after various timepoints**

Cells were starved for 12 h, then counted using the Countess automated cell counter. Approximately 5000 cells were plated in each well of a 6-well plate. Actual cell counts for each well were recorded, and cells were incubated for 72h-168h before trypsinization and counting using the Countess automated cell counter. % cell proliferation was calculated for each well based on initial and final cell counts.

**Figure S2: STAT3 knockdown sensitizes H1581-V561M cells to AZD4547 treatment.**



**Figure S2: STAT3 knockdown sensitizes H1581-V561M cells to AZD4547 treatment.**

**A** L6-WT and L6-V561M cells were infected with virus containing shRNA targeting STAT3 or a non-targeting scrambled control. Lysates were harvested and immunoblots of two replicate lysates were performed as described in 2A. **B** STAT3 expression levels were quantified using ImageJ and normalized to a GAPDH loading control and plotted using GraphPad Prism. **C, D** IC50 curves for AZD4547 treatment of L6-WT and L6-V561M cells treated with scrambled control or STAT3 targeting shRNA were generated as described for Figure 1. Significance values: \*<0.05; \*\*<0.01, \*\*\*<0.001.

**Table S3: AZD4547 sensitivity of L6 shRNA-treated cells**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **IC50 [scrambled] (nM)** | **IC50 [shRNA *STAT3*] (nM)** | **Fold-change** |
| **L6-WT** | 1.4 ± 0.6 | 1.2 ± 0.7 | Within error |
| **L6-V561M** | 700 ± 400 (0.7 µM) | 140 ± 50 | ↓5 |
| **Fold-change** | 500 | 117 | -- |