

Supplementary Information

Title:

TWIST1 drives cisplatin resistance and cell survival in an ovarian cancer model, via upregulation of GAS6, L1CAM, and Akt signalling

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Figure S1

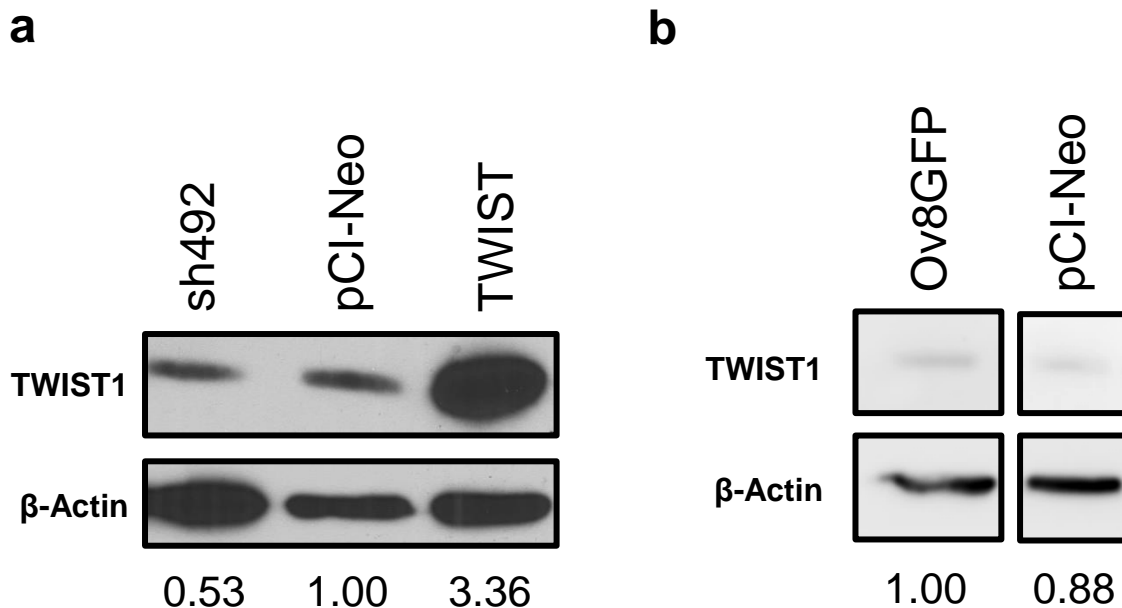


Figure S1. Cells transfected with empty pCI-Neo vector exhibit intermediate phenotype. **a.** Quantification of representative western blot data demonstrating that TWIST1 expression is reduced 2-fold by sh492 and increased approximately 3.5-fold by expression of a second copy of the gene. **b.** Transfection of cells with empty pCI-Neo vector does not substantially affect TWIST1 expression levels. Representative western data shown. Blots cropped for clarity; full blots are shown in Supplementary Figure S5.

Figure S2

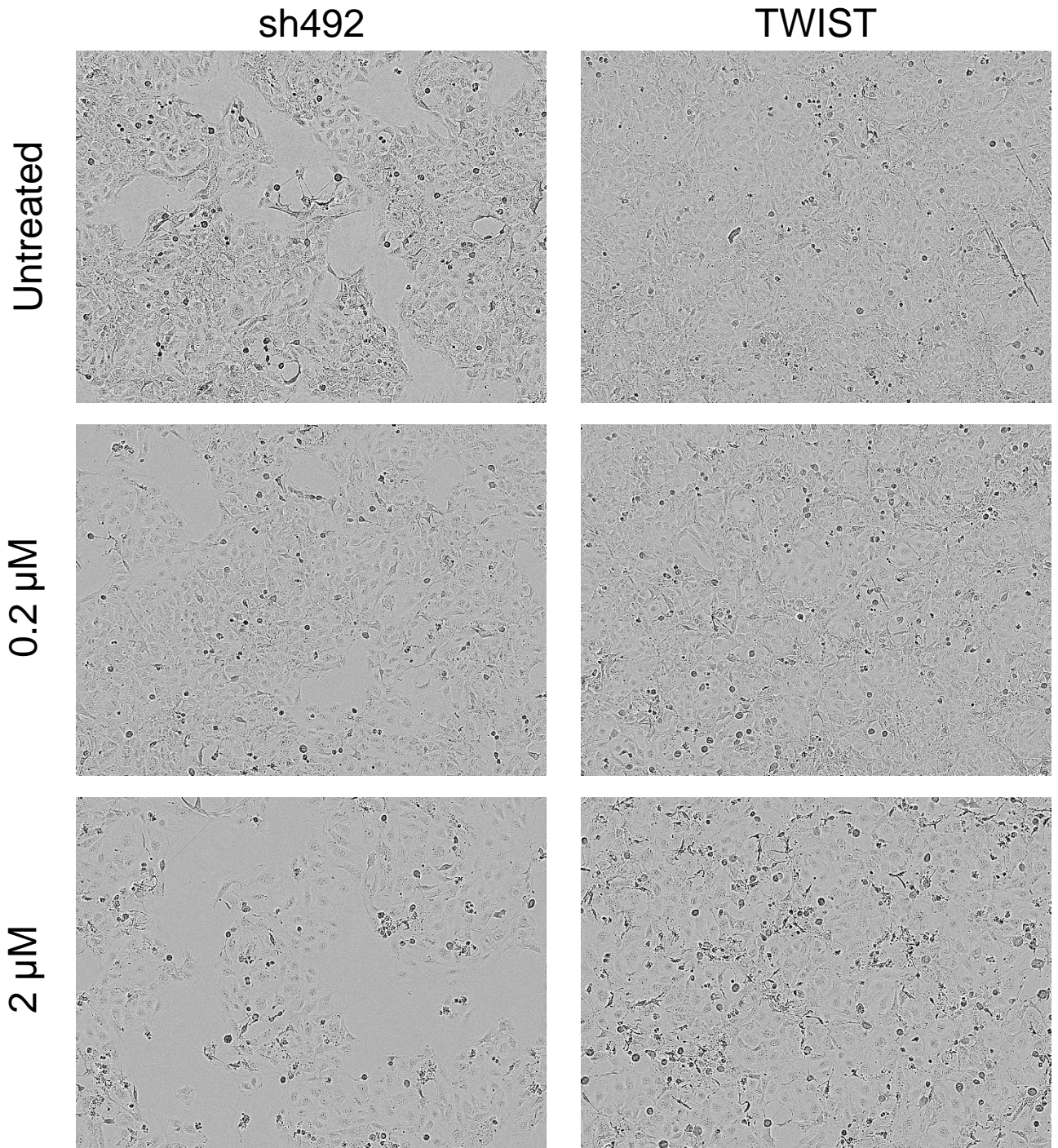


Figure S2. Representative images of Ov8GFP-TWIST and Ov8GFP-sh492 cells acquired three days post treatment with the indicated doses of cisplatin. Images such as these were analyzed to determine confluence, which is graphed in Figure 1c.

Figure S3

Ov8GFP

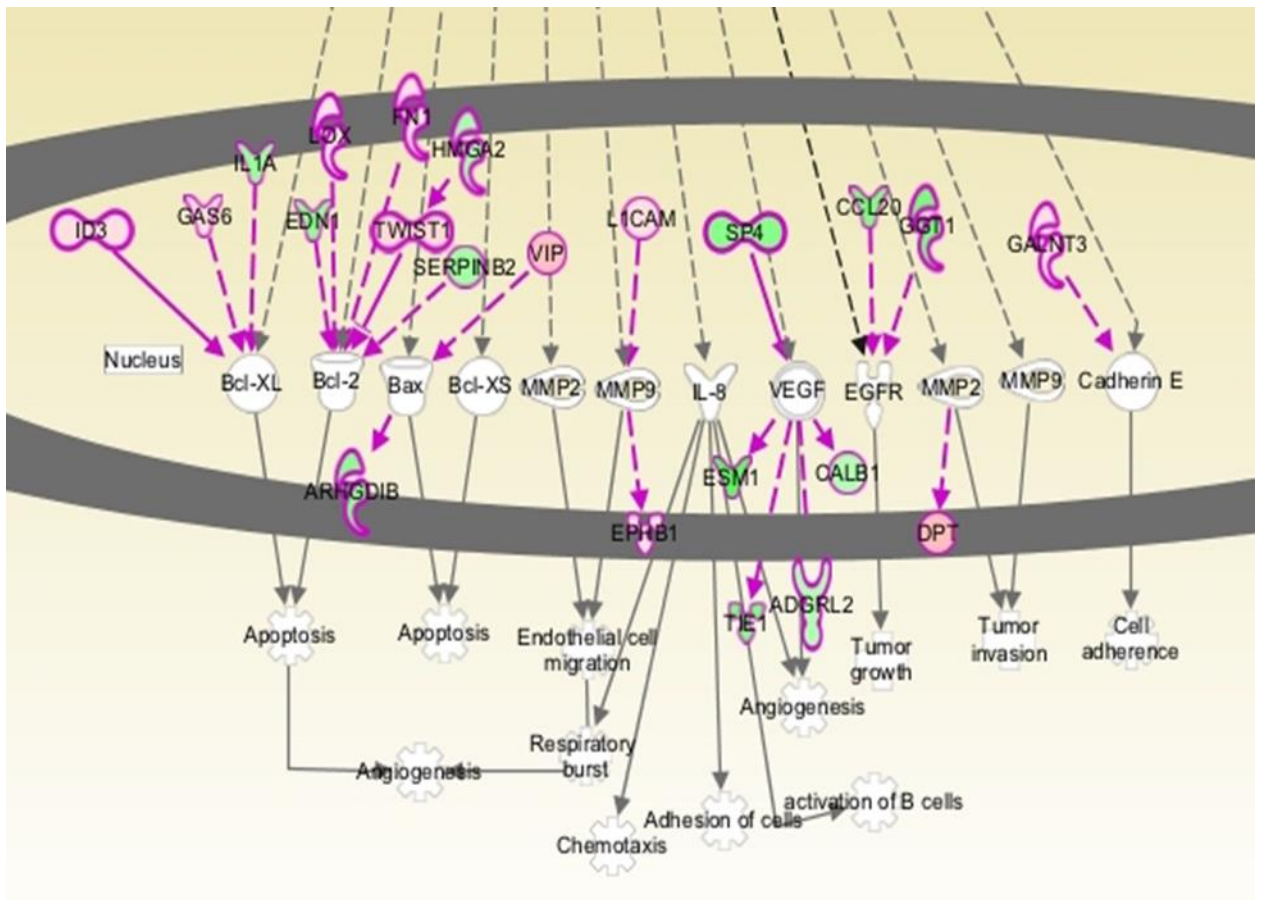


Figure S3. Ingenuity Pathway Analysis revealed that TWIST1 and known TWIST1 associated proteins such as IL-8, MMP2, MMP9, and E-cadherin lie in common pathways intersecting apoptosis and tumour growth and invasion. L1CAM and HMGGA2, two TWIST1 associated genes identified by our RNA seq analysis, also appear.

Figure S4

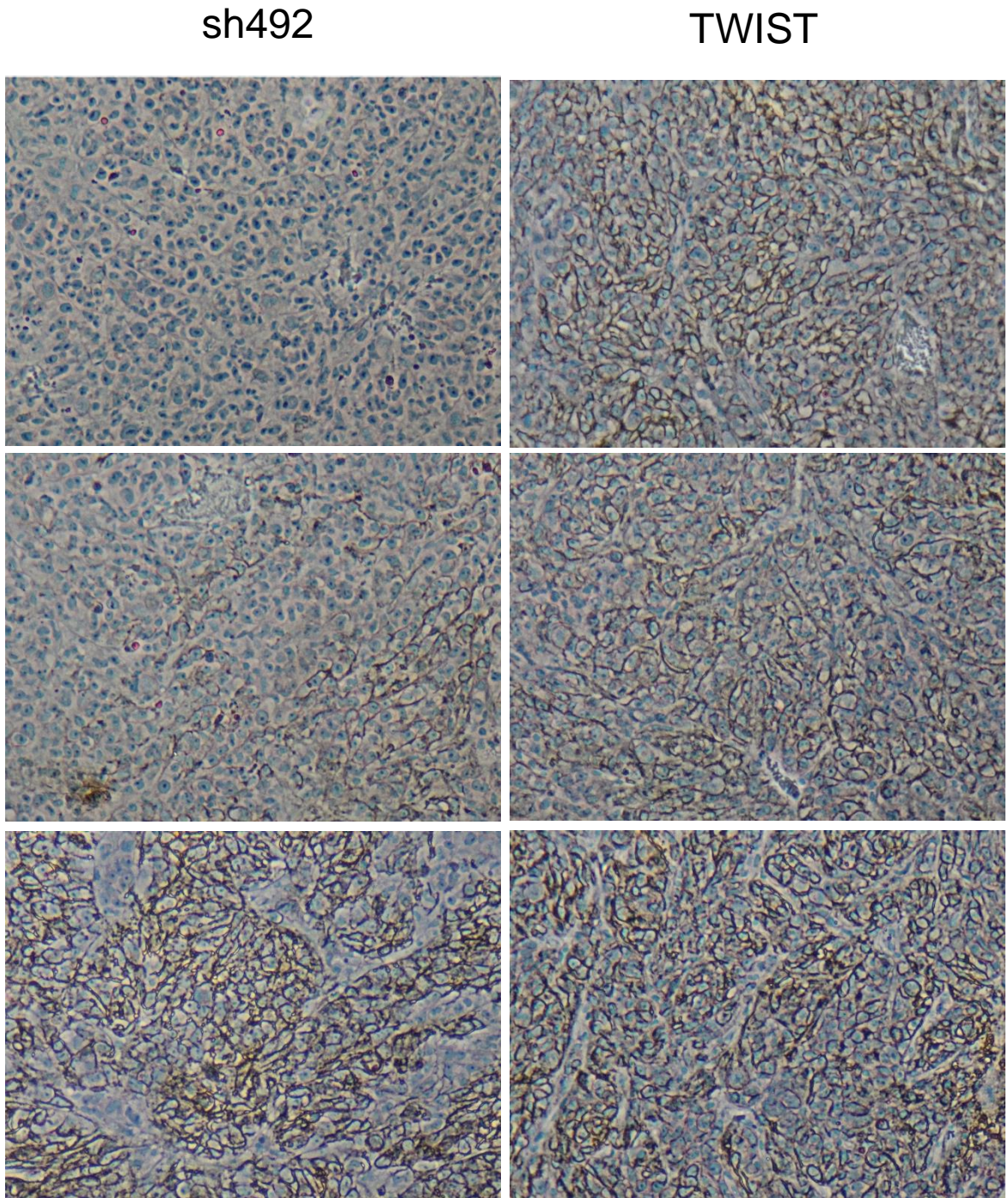


Figure S4. Representative images of Ov8GFP-sh492 and Ov8GFP-TWIST1 tumours from mice, immunostained for cell surface L1CAM. Ov8GFP-sh492 tumours were heterogeneous, with different tumour masses displaying no staining (top), light staining (middle) or dark staining. TWIST1 tumours were stained more uniformly and were typically dark with some lighter areas.

Figure S5

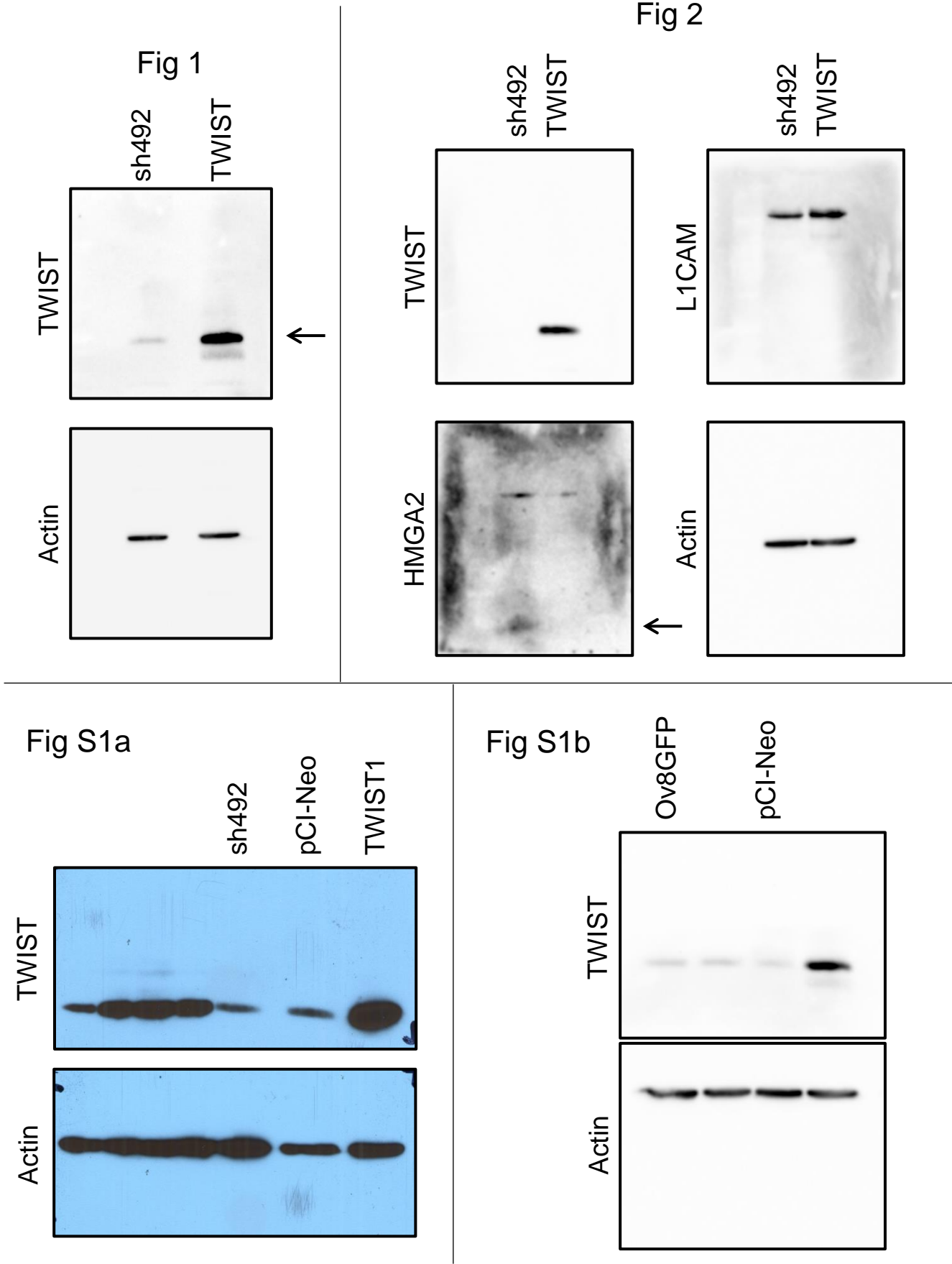


Figure S5. Full-length blots for the indicated figures from the main text, plus Figure S1.

Table S1

| Gene | Expression | | Log2 Fold Change | p Value | Function |
|-----------------|------------|----------|------------------|----------|--|
| | sh492 | TWIST | | | |
| HCLS1 | 1.9192 | 0.3462 | -2.4710 | 5.00E-05 | Antigen signaling |
| ESM1 | 19.8290 | 5.3762 | -1.8829 | 5.00E-05 | Proangiogenic |
| GGT1 | 7.5759 | 2.6660 | -1.5068 | 5.00E-05 | Glutathione metabolism |
| TIE1 | 4.2598 | 1.5563 | -1.4526 | 5.00E-05 | Endothelial adhesion molecule |
| CCL20 | 21.4373 | 7.9192 | -1.4367 | 5.00E-05 | Downstream of IL6/NFkB/STAT3 - immune attractant |
| ABCA1 | 1.7954 | 0.7054 | -1.3478 | 5.00E-05 | Cholesterol homeostasis |
| SP4 | 10.2972 | 4.1698 | -1.3042 | 5.00E-05 | Primarily neural transcription factor |
| ARHGDI3 | 8.3662 | 3.4472 | -1.2792 | 1.50E-04 | Microenvironmental communication to inhibit metastasis |
| LSAMP | 0.6617 | 0.2736 | -1.2739 | 2.50E-04 | Neuron growth |
| MIR484,NDE1 | 36.8636 | 15.6657 | -1.2346 | 1.50E-04 | MicroRNA |
| EDN1 | 11.9078 | 5.0866 | -1.2271 | 5.00E-05 | Endothelin |
| IL1A | 6.1556 | 2.7691 | -1.1525 | 2.00E-04 | Proinflammatory cytokine |
| CTPS2 | 10.0673 | 4.8606 | -1.0505 | 1.50E-04 | CTP synthase |
| SERPIN2 | 57.3030 | 28.1986 | -1.0230 | 5.00E-05 | Protease inhibitor, antiapoptotic |
| HMGA2 | 17.6553 | 8.9826 | -0.9749 | 1.00E-04 | DNA binding, chromosome condensation, DNA repair regulator |
| LOC643201 | 18.1145 | 9.4289 | -0.9420 | 1.50E-04 | Pseudogene |
| LPHN2 | 34.4611 | 18.9748 | -0.8609 | 1.50E-04 | Adhesion linked GPCR |
| CALB1 | 68.4107 | 38.3483 | -0.8351 | 1.00E-04 | Cytosol calcium buffering |
| FAM129A | 9.9252 | 17.6015 | 0.8265 | 2.50E-04 | ER stress response, regulates translation |
| DPYSL3 | 11.3916 | 20.8860 | 0.8746 | 5.00E-05 | L1CAM pathway member, putative migration function |
| GAS6 | 21.2476 | 39.7292 | 0.9029 | 1.00E-04 | Growth/adhesion/migration/survival. Interacts with Axl |
| CHN1 | 11.6973 | 22.1486 | 0.9210 | 5.00E-05 | Lipid stimulated GTPase |
| GREM1 | 24.7823 | 47.6102 | 0.9420 | 5.00E-05 | BMP inhib, proFGF signaling |
| SHISA9 | 7.0954 | 13.6759 | 0.9467 | 2.00E-04 | Synaptic protein |
| EPHB1 | 8.8684 | 17.2622 | 0.9609 | 1.00E-04 | Adhesion/migration signaling. ERK/JNK, possibly angiogenesis |
| RHOBTB1 | 1.7298 | 3.4910 | 1.0130 | 5.00E-05 | GTPase involved with actin skeleton |
| L1CAM | 17.3307 | 37.0653 | 1.0967 | 5.00E-05 | Cell adhesion, migration in drug resistant cancers |
| PCSK9 | 4.3111 | 9.2437 | 1.1004 | 5.00E-05 | Cholesterol homeostasis |
| OAS3 | 1.3659 | 2.9771 | 1.1240 | 1.50E-04 | RNA synthesis inhibitor |
| LOX | 4.5291 | 10.5536 | 1.2204 | 5.00E-05 | Crosslinks ECM |
| DOK7 | 8.9338 | 21.3466 | 1.2567 | 5.00E-05 | Neuromuscular interface |
| ID3 | 17.8285 | 43.0758 | 1.2727 | 5.00E-05 | Inhibitor of bHLH binding |
| LEPREL1 | 8.2163 | 21.0238 | 1.3555 | 5.00E-05 | Collagen assembly and linkage |
| CAPN6 | 2.0987 | 5.5978 | 1.4153 | 5.00E-05 | May inhibit apoptosis, promote angiogenesis |
| FN1 | 225.0440 | 620.2980 | 1.4628 | 1.50E-04 | Fibronectin |
| COL12A1 | 22.0742 | 61.1653 | 1.4704 | 5.00E-05 | Collagen 12 alpha 1 |
| AMIGO2 | 1.3070 | 3.9771 | 1.6055 | 5.00E-05 | Cell-cell communication, neg regulator of apoptosis |
| GALNT3 | 0.3538 | 1.1292 | 1.6743 | 1.50E-04 | Oligosaccharide biosynthesis |
| COL4A4 | 1.2217 | 4.2168 | 1.7873 | 5.00E-05 | Collagen alpha 4 |
| HOXA3 | 0.3283 | 1.1875 | 1.8548 | 5.00E-05 | Developmental transcription factor - angiogenesis and patterning |
| ATOH8 | 0.2612 | 0.9560 | 1.8718 | 5.00E-05 | bHLH developmental TF |
| GDF6 | 0.4460 | 1.8793 | 2.0753 | 5.00E-05 | Bone development - BMP signal responsive |
| PXDNL | 0.5750 | 2.4422 | 2.0866 | 5.00E-05 | Oxidative stress responsive endonuclease |
| MIR1909,REXO1 | 12.5434 | 53.5919 | 2.0951 | 5.00E-05 | MicroRNA |
| MIR5193,UBA7 | 0.7722 | 5.1067 | 2.7254 | 5.00E-05 | MicroRNA |
| BDKRB1 | 0.4423 | 3.1086 | 2.8133 | 5.00E-05 | Receptor for bradykinin, an inflammatory vasodilator |
| LINC00452 | 0.2309 | 2.0640 | 3.1600 | 5.00E-05 | Noncoding RNA |
| TWIST1 | 20.2035 | 209.0290 | 3.3710 | 5.00E-05 | EMT, angiogenesis, metastasis, stem cell phenotype |
| MIR4324,SLC6A16 | 0.7451 | 21.5666 | 4.8552 | 5.00E-05 | MicroRNA |
| VIP | 0.0000 | 0.4792 | N/A | 5.00E-05 | Vasodilator, also involved in survival |
| DPT | 0.0000 | 1.2281 | N/A | 5.00E-05 | ECM protein involved with TGFb |
| KCNA10 | 0.0000 | 0.2646 | N/A | 5.00E-05 | Voltage gated potassium channel |

Table S1. Complete list of differentially expressed genes output by RNA-seq analysis. Genes are ordered according to \log_2 fold change; genes downregulated when TWIST1 is overexpressed appear in green, and those upregulated appear in red. TWIST1 itself and genes selected for further study are shown in darker shades.