

Figure S1. CD70 is upregulated in EGFR TKI resistant cells. Related to Figure 1. (A) *CD70* RNA expression in HCC827 and HCC4006 parental and ER cells as determined by RNAseq analysis (n = 3). Box plots depict the median (line) as well as the 25th and 75th percentile, with whiskers showing 1.5x the interquartile range (IQR). *p < 0.0001 vs parental cells; one-way ANOVA. (B) EMT marker expression in OR cells as determined by Western blotting. (C – E) Expression of *CD70* in EGFR wild-type (WT) and mutant NSCLC cell lines (C) and clinical specimens from the PROSPECT (D) and TCGA (E) datasets. Boxes depict median \pm 1 quartile, with whiskers extending to the most extreme datapoint within 1.5 IQR from the box boundaries. (F) *CD70* protein by Western blotting in H1975 parental cells and osimertinib resistant (OR) variants that had undergone EMT and PC9 parental cells and erlotinib resistant (ER) variants that developed resistance through the acquisition of T790M secondary mutations. (G) *CD27* RNA expression in parental and ER variants as determined by RNAseq. Dots represent individual data points with the midline depicting the mean and error bars showing SD. (H) Cell surface *CD27* expression as determined by flow cytometry. Raji cells served as a positive control.

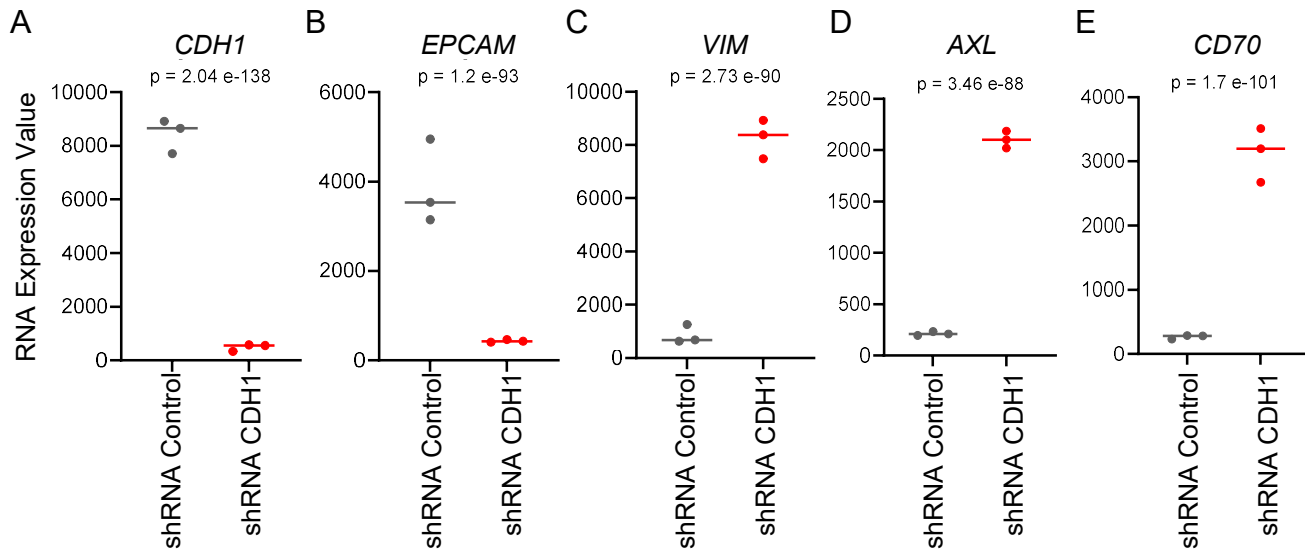


Figure S2. *CD70* RNA is increased in cells that have undergone EMT. Related to Figure 3. (A-D) Gene expression of epithelial markers *CDH1* (A) and *EPCAM* (B) and mesenchymal markers *VIM* (C) and *AXL* (D) in HCC827 cells after knockdown of *CDH1*. (E) Induction of EMT by *CDH1* knockdown was associated with increased expression of *CD70*. Dots represent individual data points and horizontal lines represent the mean.

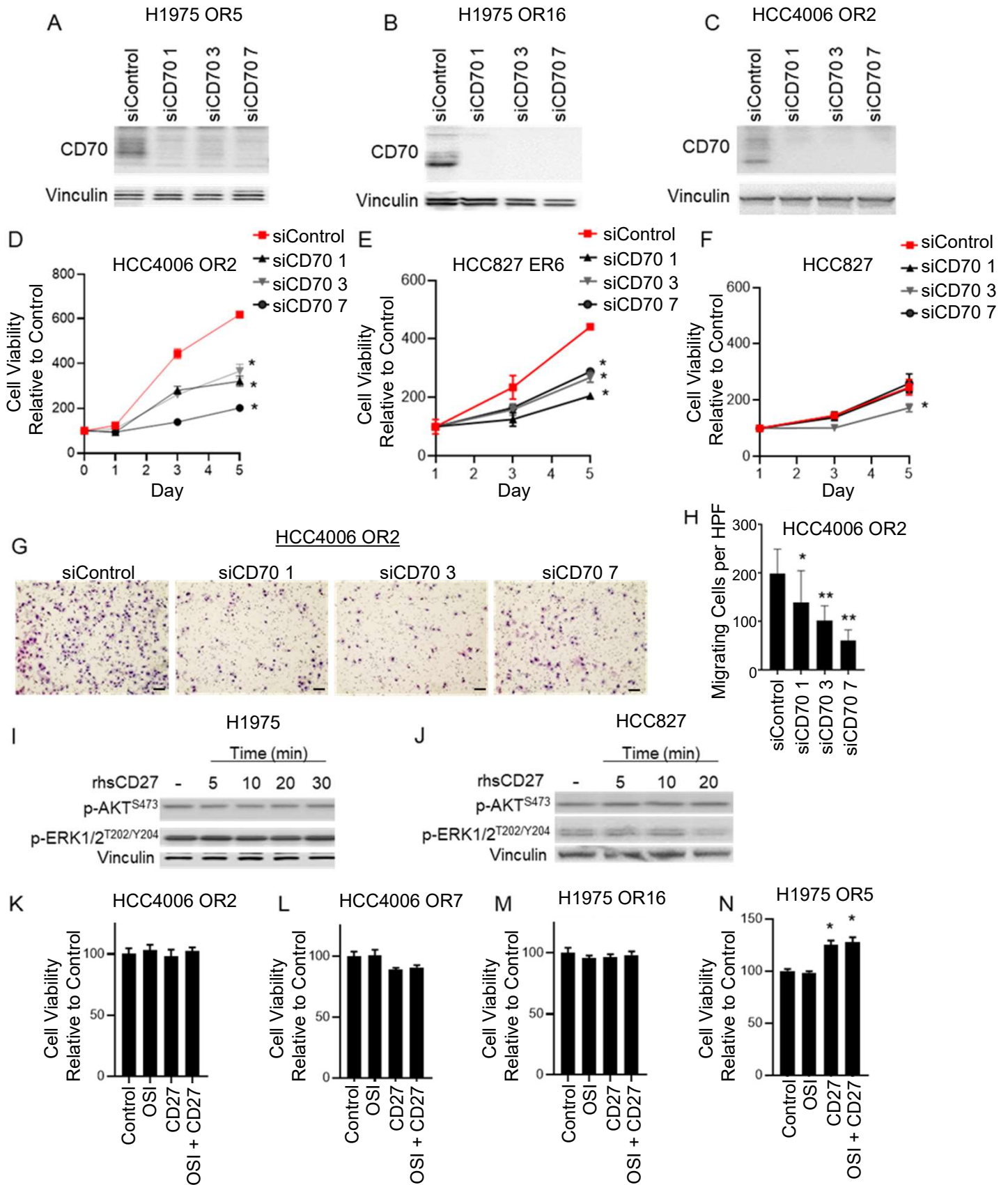


Figure S3. CD70 promotes tumor cell growth and invasiveness. Related to Figure 4. (A - C) siRNA mediated knockdown of CD70 as determined by Western blotting. (D - F) Cell growth (mean viability \pm SD) of HCC4006 OR2, HCC827 ER6, and HCC827 cells treated with siRNA targeting CD70 (n = 3). **p <

0.0001; *p = 0.0027; one-way ANOVA. (G) Representative images of migrating HCC4006 OR2 cells following siRNA-mediated knockdown of CD70. Scale bars = 1000 μ m. (H) Mean number of migrating cells \pm SD per high powered field (HPF) following siRNA-mediated knockdown of CD70 (n = 3). *p = 0.002; **p < 0.0001; one-way ANOVA. (I & J) Western blotting of p-AKT and p-ERK1/2 in H1975 and HCC827 cells following treatment with 500 ng/ml rhCD70. (K – N) Relative cell viability (mean \pm SD) of osimertinib resistant cells following 5-day treatment with 500 ng/ml rhCD27, osimertinib (OSI; 100 nM), or the combination (n = 6). *p < 0.0001; one-way ANOVA.

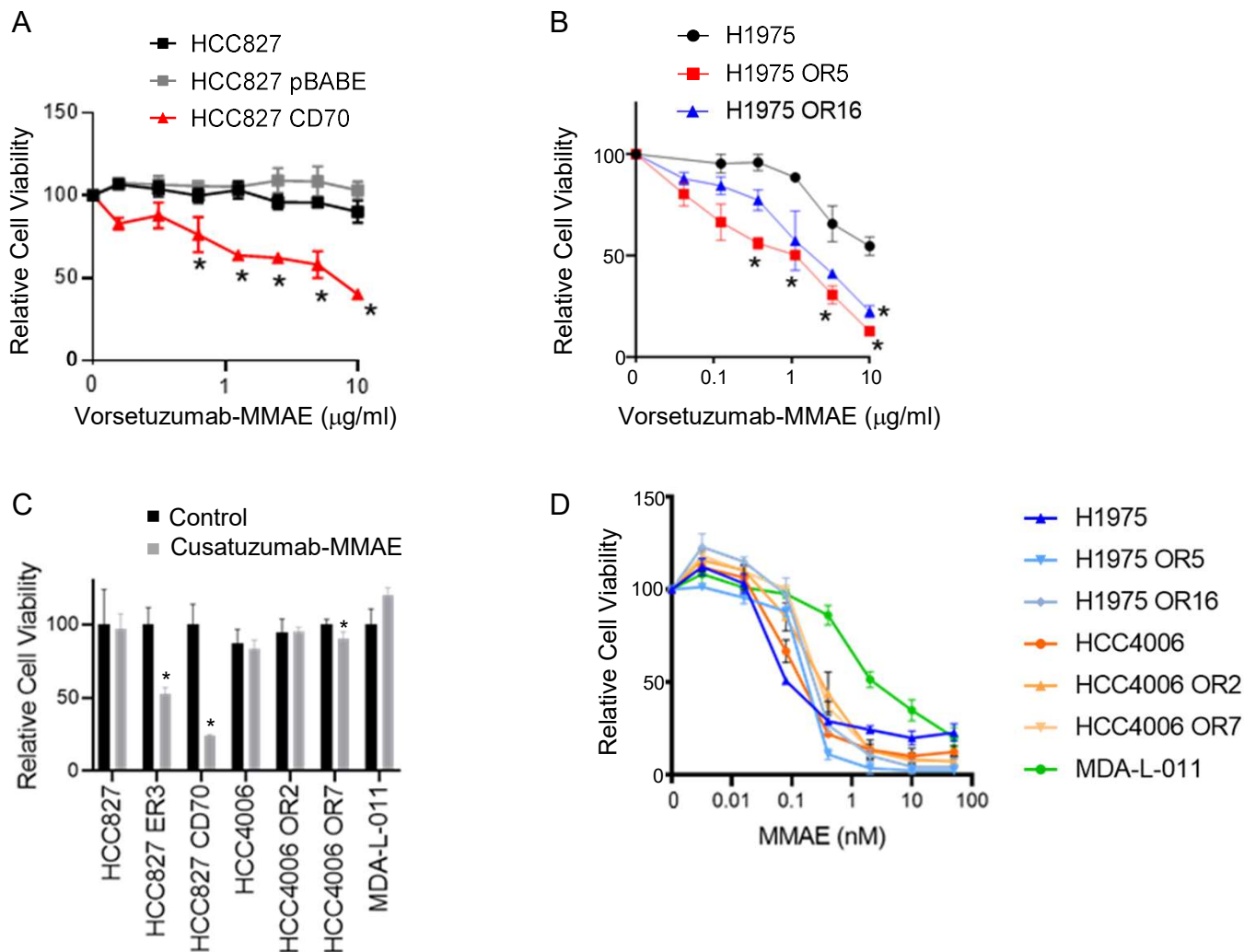


Figure S4. CD70 ADCs have activity against EGFR TKI resistant cells. Related to Figure 6. (A) Dose response curve of HCC827 cells with or without CD70 expression treated with vorsetuzumab-MMAE ($n = 3$). $*p \leq 0.01$; multiple t-tests. (B) Dose response curve of H1975 parental and OR cells with treated with vorsetuzumab-MMAE ($n = 3$). $*p \leq 0.01$; multiple t-tests. (C) Cell viability of parental and EGFR TKI resistant cells following cusatuzumab-MMAE (3 mg/ml) treatment ($n = 3$). $*p < 0.005$; multiple t-test. (D) Dose response curve of parental and EGFR TKI resistant cells treated with MMAE ($n = 3$). All graphs show mean viability relative to control \pm SD.

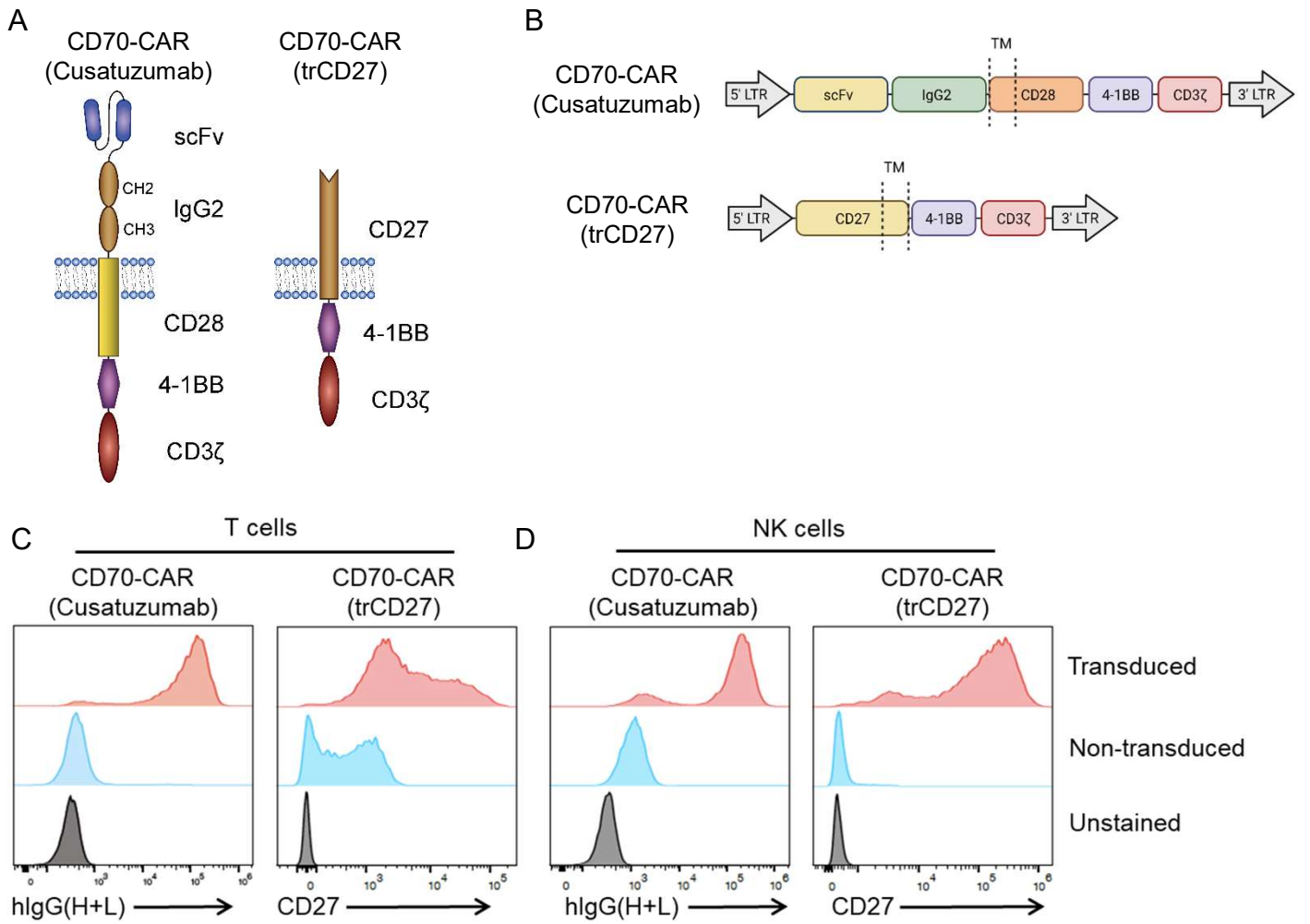


Figure S5. CD70 CAR constructs. Related to Figure 6. (A & B) Diagram of CD70 CAR constructs. (C & D) CD70 CAR expression in T cells (C) and NK cells (D) as evaluated by flow cytometry.

Table S1. Cell surface genes which displayed a log2fold change of 3 or greater in expression in EGFR TKI resistant cells as compared to parental cells. Related to Figure 1.

Gene Name	Log2 Fold Change	Adjusted p-value
<i>EMP3</i>	3.456120	2.21E-53
<i>CD70</i>	5.115689	2.65E-39
<i>FSCN1</i>	3.340315	6.16E-39
<i>ADAMTS1</i>	5.027088	1.10E-26
<i>FHL1</i>	4.875225	1.61E-26
<i>CDH2</i>	5.403338	3.30E-24
<i>MDGA1</i>	6.358142	3.31E-22
<i>AKAP12</i>	5.501246	1.46E-21
<i>ILDR2</i>	6.225538	4.53E-21
<i>MYLK</i>	4.424685	1.46E-20
<i>CAP2</i>	3.301247	2.68E-20
<i>RRAD</i>	3.236440	6.70E-19
<i>S100A3</i>	3.658456	1.24E-16
<i>DSC3</i>	3.655640	1.01E-15
<i>DAB2</i>	4.604081	6.55E-15
<i>ADAMTSL4</i>	3.018830	2.63E-14
<i>ANO2</i>	7.380809	4.67E-14
<i>NTNG2</i>	4.818666	9.72E-14
<i>LZTS1</i>	7.599672	1.40E-13
<i>SCN9A</i>	3.884876	3.08E-13
<i>ACKR3</i>	7.524005	6.08E-12
<i>AXL</i>	3.720214	7.86E-12
<i>ADAMTS2</i>	7.583414	1.03E-11
<i>APBB1IP</i>	7.720331	5.28E-11
<i>PTX3</i>	5.211632	6.97E-11
<i>PIEZO2</i>	6.431404	3.42E-10
<i>DYSF</i>	3.168602	1.55E-09
<i>NCKAP5</i>	3.869194	2.36E-09
<i>COL6A2</i>	4.244008	2.43E-09
<i>GPR1</i>	6.179602	4.20E-09
<i>CAMK2A</i>	5.248377	4.39E-09
<i>LRRC7</i>	7.072014	5.99E-09
<i>GAS7</i>	4.720206	1.36E-08
<i>CDH13</i>	6.871683	2.17E-08
<i>FILIP1L</i>	3.411498	2.29E-08
<i>ADAM12</i>	5.244629	3.09E-08
<i>SYNC</i>	4.683973	4.10E-08
<i>SSTR1</i>	6.799371	5.55E-08
<i>L1CAM</i>	4.628323	1.01E-07
<i>ITGB8</i>	3.127380	1.03E-07
<i>TLR4</i>	5.853496	1.27E-07
<i>RELN</i>	3.805221	1.57E-07
<i>CORO2B</i>	3.041626	1.77E-07
<i>KIF26A</i>	3.598145	1.93E-07
<i>ROBO4</i>	4.940439	2.43E-07
<i>DUSP15</i>	4.346493	3.76E-07
<i>LPAR4</i>	4.883461	3.79E-07
<i>SEMA3D</i>	6.307769	4.07E-07
<i>C14orf37</i>	3.720417	4.49E-07
<i>ADAM33</i>	6.172884	5.99E-07
<i>DAW1</i>	3.638512	6.27E-07
<i>CDH4</i>	3.989199	6.33E-07
<i>NCAM1</i>	5.835802	6.35E-07
<i>CRYAB</i>	3.110369	2.24E-06
<i>AMPH</i>	5.685837	2.32E-06
<i>OLR1</i>	5.738856	1.15E-05
<i>STAC2</i>	4.015361	1.26E-05
<i>SLC14A1</i>	4.916806	2.56E-05
<i>COL16A1</i>	3.979897	4.50E-05
<i>HTR7</i>	3.454280	6.49E-05
<i>GYPC</i>	5.463758	6.76E-05
<i>SLC13A5</i>	4.521131	9.75E-05
<i>C10orf90</i>	4.505605	0.0001771
<i>CSPG4</i>	4.533254	0.000188749
<i>PHOSPHO1</i>	4.120589	0.000251998
<i>SNAP25</i>	4.085223	0.000270866
<i>IL21R</i>	3.679359	0.000335016
<i>FMN2</i>	4.582655	0.000387031
<i>XIRP1</i>	4.780643	0.000423883
<i>PALM2-AKAP2</i>	3.682301	0.000876154
<i>CD207</i>	3.845447	0.0016433
<i>OLIG2</i>	4.029482	0.002328891
<i>SCN5A</i>	3.755273	0.002423911
<i>ALPP</i>	3.059086	0.002658332
<i>TNFRSF8</i>	3.280728	0.00305236
<i>FABP3</i>	3.780470	0.005923231
<i>PDGFRA</i>	3.038692	0.006700453
<i>MAMDC2</i>	3.362973	0.00892553
<i>MKRN3</i>	3.822993	0.015264194

Table S2. Cellular localization of the 10 most significant cell surface genes upregulated in EGFR TKI resistant cells as compared to parental cells. Related to Figure 1.

Gene Name	Log2 Fold Change	Adjusted p-value	Localization
<i>EMP3</i>	3.46	2.21E-53	myelin sheaths, organ tissue
<i>CD70</i>	5.12	2.65E-39	transiently expressed on activated immune cells
<i>FSCN1</i>	3.34	6.16E-39	highly expressed throughout brain
<i>ADAMTS1</i>	5.03	1.10E-26	liver, gallbladder, ovary
<i>FHL1</i>	4.88	1.61E-26	thyroid, testis, heart & skeletal muscle
<i>CDH2</i>	5.40	3.30E-24	highly expressed in brain, liver, kidney, heart
<i>MDGA1</i>	6.36	3.31E-22	highly expressed in cerebellum
<i>AKAP12</i>	5.50	1.46E-21	kidney, cerebellum, reproductive organs
<i>ILDR2</i>	6.23	4.53E-21	brain, kidney
<i>MYLK</i>	4.42	1.46E-20	smooth muscle, colon