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Figure S1

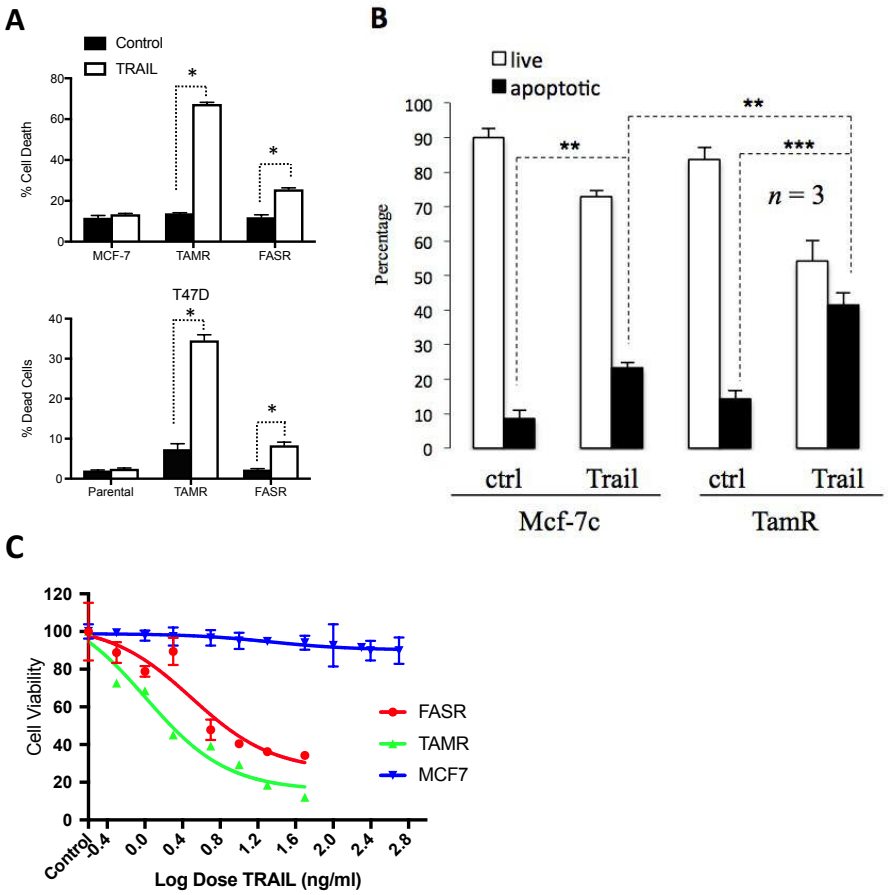


Figure S1: Endocrine-resistant MCF-7 cells demonstrate sensitivity to TRAIL. A) MCF-7 and T47D parental and endocrine-resistant cells were treated with 20ng/ml TRAIL for 18 hours and % cell death assessed by fixable live/dead assay *p <0.01 n=3. B) MCF-7 and TamR cells (Vivanco et al- derived) were treated with TRAIL as in (A) and apoptotic cells assessed by Annexin-V assay. (*p<0.03). C) IC50 curves - MCF-7, TAMR and FASR cells treated at increasing doses of TRAIL and viability measured by cell titer blue assay.

Figure S2

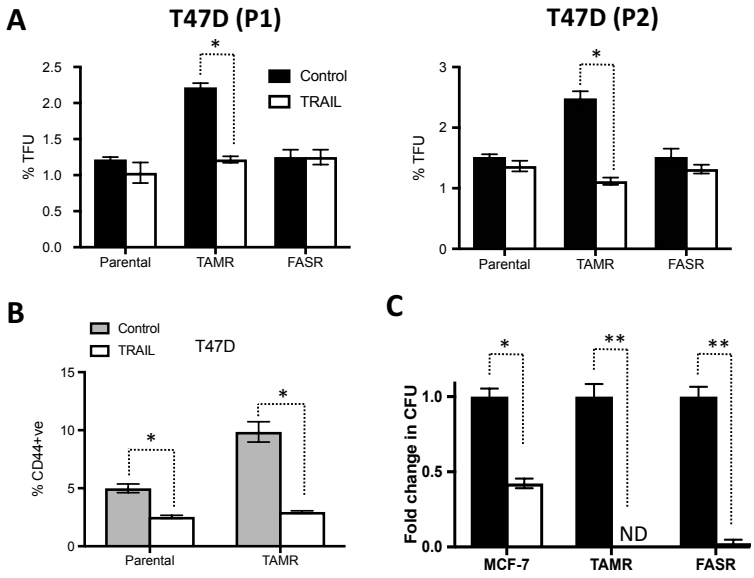


Figure S2: Endocrine-resistant CSCs demonstrate sensitivity to TRIAL. A) Tumoursphere forming capability and B) % CD44+ve cells of parental T47D and T47D-derived endocrine-resistant cell lines after 18 hours treatment with 20ng/ml TRAIL compared to their respective untreated controls *p<0.03. C) Fold change in colony forming units (CFU) of parental MCF-7 and endocrine-resistant cell lines following 20ng/ml TRAIL treatment for 18 hours *p<0.01, **p<0.001.

Figure S3

A

Sample No.	Chemo Tx	Endocrine Tx
90	Epirubicin Cyclophosphamide, Capecitabine	-
149	-	-
94	Epirubicin	-
61	-	-
202	-	-
46	EOX	Tamoxifen, Letrozole, Fulvestrant
123	-	-
69	Epirubicin, Cisplatin, 5FU	Tamoxifen, Letrozole, anastrazole
180	-	-
165	-	-
72	-	-
196	-	-
59	EOX, Paclitaxel, Capecitabine	Tamoxifen, Letrozole, Fulvestrant, exemestane, Diethylstilbestrone
68	FEC, Capecitabine	Tamoxifen, Anastrazole, Fulvestrant
164	-	-
62	-	Fulvestrant
127	-	Tamoxifen
188	-	Tamoxifen
125	-	-
29	FEC, epirubicin	Anastrazole, Exemestane, Fulvestrant, Letrozole
44	EOX	Tamoxifen, Letrozole
77	-	-
61/66	EOX, Paclitaxel, Capecitabine	Tamoxifen, Letrozole, Fulvestrant, Diethylstilbestrone
81	FEC, Paclitaxel, Capecitabine	Tamoxifen, anastrazole
52	CMF, Vinorelbine, Docetaxel, Capecitabine, Taxotere, EC	Tamoxifen, Letrozole
*81A	FEC, Paclitaxel, Capecitabine	Tamoxifen, anastrazole

FEC – Fluorouracil (5FU), Epirubicin, cyclophosphamide. **EOX** – Epirubicin, oxaliplatin, capecitabine

* Sample 81A is the same patient as sample 81 but sample taken at later timepoint following re-presentation

B

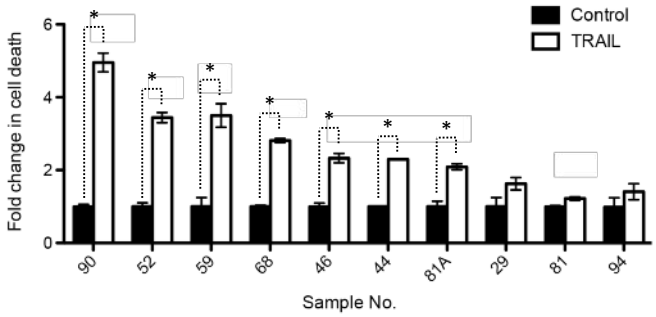


Figure S3: Primary-derived patient samples. A) Previous lines of therapy received by patient samples prior to sample acquisition. B) Primary human patient samples were treated with 100ng/ml TRAIL for 18 hours and fold change in cell death assessed by fixable live/dead assay (*$p<0.05$).

Figure S4

A

"Report as per RCPathguidelines:

CUbbs 151: Cores of breast tissue shows poorly differentiated adenocarcinoma compatible with grade 3 invasive ductal carcinoma NST (gland/acinous formation, 3; nuclear atypia/pleomorphism 3; mitotic count 3) (actual mitotic count 13 per 10 high-power fields). No in situ elements seen. There is no vascular invasion. B5b

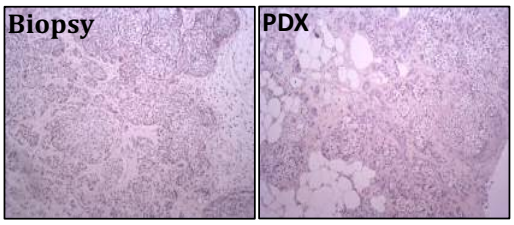
PDX 151: Mouse mammary gland shows a deposit of poorly differentiated adenocarcinoma compatible with invasive ductal carcinoma NST grade 3 (gland/acinous formation, 3; nuclear atypia/pleomorphism 3; mitotic count 3) (actual mitotic count 12per 10 high-power fields). There is marked lympho-vascular invasion. There is also cancerisation of the mouse mammary ducts.

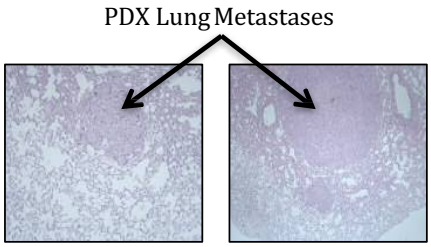
Additional comments

CUbbs 151: The tumour grows in a predominantly solid sheets, and at the periphery of the core, one third of the tumour showed nested appearance within desmoplastic stroma. There is no gland formation, 0%. There is marked nuclear pleomorphism with large pleomorphic nuclei, some in bizarre shapes. The tumour has a well defined edge (pushing edge) into the fat. The tumour shows a fine capillary network dividing the tumour into small compartments. No significant inflammatory response isseen.

PDX 151: The tumour grows predominantly in solid sheets, and at the periphery one 10th of the tumour shows nested appearance is with minimal desmoplastic stromal response. There is no gland formation (0%). There is marked nuclear pleomorphism with large pleomorphic nuclei, some in bizarre shapes. The tumour has a well defined edge (pushing edge) into the mouse fat pad. The tumour shows a fine capillary network dividing the tumour into small compartments. No significant inflammatory response is seen.

In conclusion, both tumours show similar histologic features. On assessment of the Nottingham criteria of grading of breast cancer the tumour is only identical. Addition tumour characteristics of growth pattern, tumour stroma, tumour infiltrating lymphocytes/inflammation, tumour vessels, specific nuclear characteristics, both tumours also show near exact morphology on further interrogation of the resemblance of the tumours. Additionally both CUbbs 151 nd PDX 151 demonstrate the same receptor staining patterns for both ER (-ve, 0/8), PR (-ve) and HER2 (+2, FISH –ve). "





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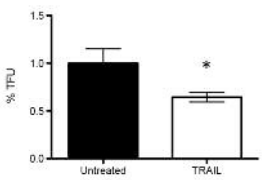

C

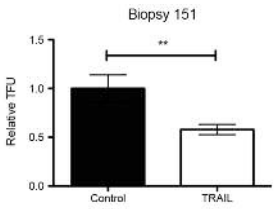

Figure S4: Endocrine-resistant primary and PDX tumour cells are sensitive to TRAIL. A) Histological assessment of Cubbs 151 core tissue biopsy and PDX 151 tumour in mouse. B) MaCa 3366 TAMR tumours treated with 16mg/kg TRAIL *in vivo* were excised, dissociated and treated with 100ng/ml TRAIL for 18 hours *ex vivo* under tumoursphere culture conditions *p<0.05. C) Original tumour biopsy cells (from which PDX 151 was established) were treated with 100ng/ml TRAIL for 18 hours under tumoursphere culture conditions **p<0.01.

Figure S5

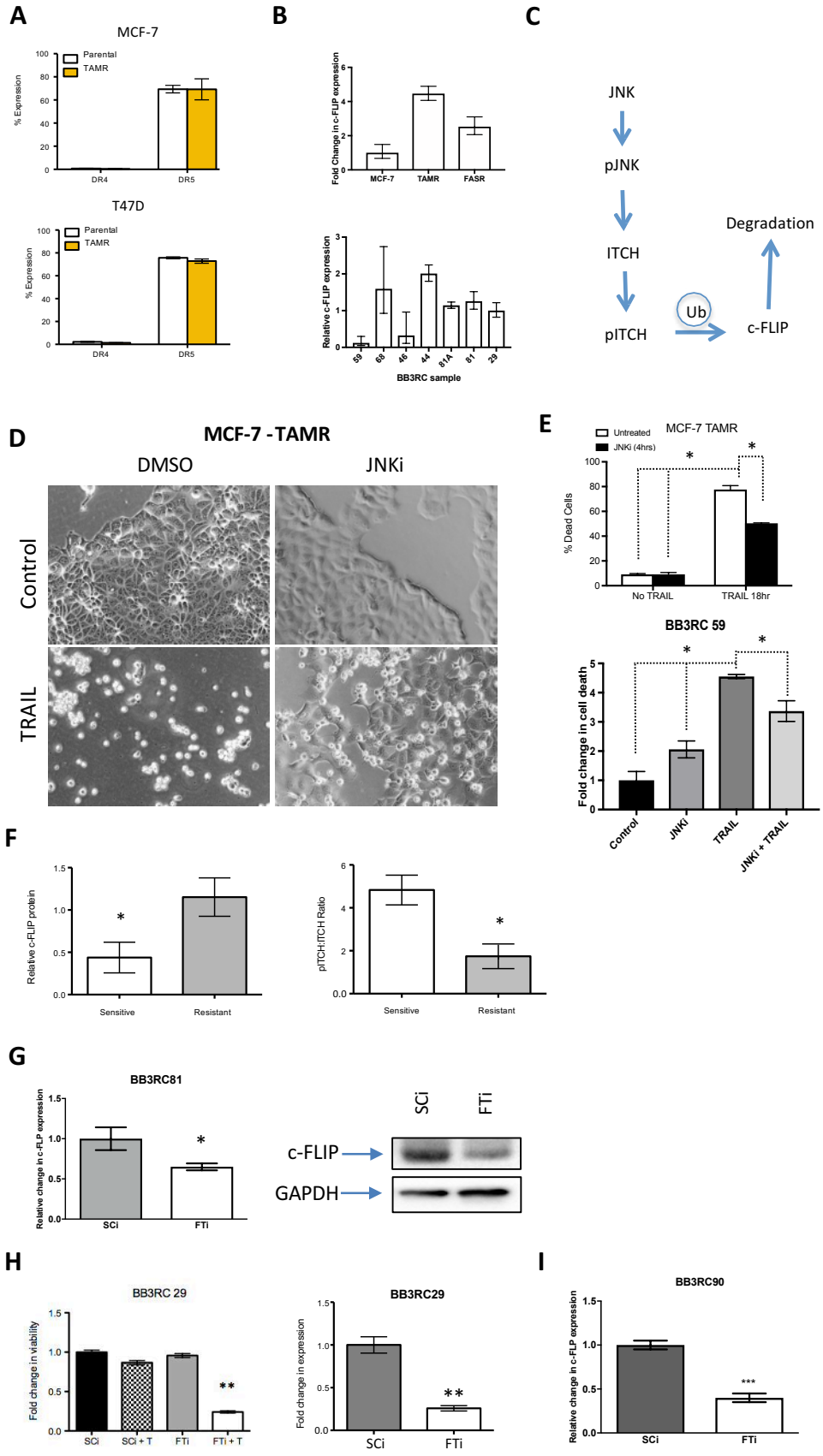

Figure S5: Mechanism of endocrine-resistant tumour cell sensitivity to TRAIL. A) Percentage expression of DR4 and DR5 on cell surface of parental and TAMR MCF-7 and T47D cells. B) c-FLIP expression as determined by QPCR in MCF-7, TAMR and FASR samples and primary endocrine-resistant samples. C) Hypothesised mechanism of c-FLIP degredation and subsequent sensitivity to TRAIL in endocrine-resistant tumour cells. D) Light microscopy images of MCF-7 TAMR cells with or without pre-treatment with Jnk inhibitor for 4hrs and treated with or without TRAIL. E) MCF-7 TAMR and endocrine-resistant, TRAIL-resistant primary metastatic sample BB3RC59 were pre-treated with or without Jnk inhibitor for 4hrs and treated with or without 100ng/ml TRAIL for 18 hours. F) Average relative c-FLIP levels and ITCH activation in endocrine-naive and endocrine-resistant primary tumour samples. G) Fold change in c-FLIP expression, as determined by QPCR, in BB3RC81 primary tumour cells following 48 hour incubation with c-FLIP targeted (FTi) and scrambled control (SCi) siRNA (*p<0.05). C-FLIP protein levels following 48 hours incubation with SCi or FTi siRNA. H) Endocrine-resistant, TRAIL-resistant primary metastatic sample BB3RC29 was incubated with c-FLIP targeted (FTi) and scrambled control (SCi) siRNA for 48hrs and treated with or without 100ng/ml TRAIL for 18 hours (*p<0.05). Fold change in c-FLIP expression, as determined by QPCR, in BB3RC29 primary tumour cells following 48 hour incubation with c-FLIP targeted (FTi) and scrambled control (SCi) siRNA (**p<0.03, ***p<0.01).