Supplementary Figure Legends

Supplementary Figure 1

(**a-d**) Chemotherapeutic drugs do not induce DRAM-3 in Hep-G2 (**a**), A549 (**b**), LNCaP (**c**) or H1299 (**d**) cells. RT-qPCR analysis was performed on cells treated for 24h with Actinomycin D (2 nM), Etoposide (10 μM) and Cisplatin (5 μM). Each condition was measured in triplicate, and data displayed are means of DRAM-3/18S RNA levels from 3 experiments with the untreated condition (Co) normalised to 1. Error bars are SD.

Supplementary Figure 2

(**a**) DRAM-3 and WIPI2 do not co-localize. Saos-2 pWZL DRAM-3 cells were with α-Myc-Tag and α-WIPI2 antibody for IF analysis. Images were taken with a Zeiss LSM710 microscope with 63x objective. (**b**) Quantification of Immunofluorescence analysis of DRAM-3 and LC3B overlap. Images of Saos-2 pWZL-DRAM-3 cells were taken with a Zeiss LSM710 microscope with 63x objective. Cells with medium overexpression (estimated 25% of the overall population; ~70% show low expression and ~5% high expression) were used for the experiment. Two cells per image in 17 images were analysed using the Zeiss Zen software co-localzation tool, after setting appropriate cut-offs. The results are represented as means; 'overall' is the average of all cells analysed, while 'high' denotes the mean of cells with apparently high costaining and 'low' the mean of cells with apparently low co-staining (1 each picked from each image). Error bars are SD. (**c**) A high proportion of DRAM-3 positive LC3 puncta are localized to autolysosomes/lysosomes. Saos-2 pWZL DRAM-3 cells retrovirally overexpressing EGFP-LC3

were generated and stained with α-Myc-Tag and α-LAMP2 antibodies for IF analysis. In control conditions, few LC3 puncta could be observed (data not shown) and so therefore treated cells with 100nM Bafilomycin A to cause autophagosome accumulation. Images were taken with a Zeiss LSM710 microscope using a 63x objective.

Supplementary Figure 3

(**a-b**) DRAM-3 induction does not induce substantial levels of apoptosis. Saos-2 DRAM-3 tet-on cells were subjected to doxycycline treatment for 48h and then analysed for caspase 3 cleavage by western blotting (**a**). Treatment of cells with TNF and cycloheximide was used as a positive control. (**b**) Cells were also stained with Annexin V and propidium iodide prior to analysis by flow cytometry. Early apoptotic death (PI-/Annexin V+) is shown in the lower right quadrant. Late apoptotic death or necrotic death (PI+/Annexin V+) is shown in the upper right quadrant. (**c**) DRAM-3 expression is attenuated in DRAM-3 Tet-On cells following DRAM-3 activation and 10 days recovery without doxycycline. DRAM-3 inducible cells were subjected to 7 days doxycycline treatment, followed by a 10 day doxycycline-free recovery growth and re-subjection to doxycycline. DRAM-3 induction is markedly attenuated in the second round of doxycycline treatment.

Supplementary Figure 4

(a-c) DRAM-3 overexpression affects autophagy in control conditions. Saos-2 cells were subjected to 1,2 and 4h of EBSS starvation (3 experiments). LC3B conversion and p62 were determined by Western blot analysis (**a-c**) and LC3BII/Actin levels were quantified using ImageJ (right panels). (**d-f**). Quantification of LC3B-II/Actin levels in wild-type (GFP Crispr) and

DRAM-3 Crispr knockout cells following treatement with chloroquine. Quantification of the blots is shown below each blot.

Supplementary Figure 5

(**a**) DRAM-3 disruption does not change early endosome and lysosome appearance. EEA1 antibody was used to visualise early endosomes, and LAMP2 antibody to visualise lysosomes in Saos-2 CRISPR Control and DRAM-3 cells. Immunofluorescence analysis was performed on a Zeiss 710 confocal microscope with 63x objective. (**b**) DRAM-3 overexpression does not affect endocytic uptake of BSA. Saos-2 pWZL and pWZL DRAM-3 were serum- and amino acid-starved (EBSS) for 3h 30min, then BSA (1 mg/ml) was added back for different time points. Western blot analysis was performed to determine the endocytic uptake of BSA. (**c**) Uptake kinetics of (**b**). The uptake of each time point in the 2 cell lines was quantified from 3 experiments. Values displayed are means, error bars are SD.

Supplementary Figure 6

(**a**) DRAM-3 overexpression protects from Glucose starvation-induced cell death. MDA-MB-231 cells containing pWZL Control and pWZL DRAM-3 contructs were subjected to 24h Glucose starvation (DMEM glucose free + 2% dialized FBS). The starvation media was exchanged after 24h and the surviving cells were grown in normal growth media for 3-4 days before staining them for colony formation with Giemsa modified stain. (**b**) Glucose-starvation induced cell death is mainly non-apoptotic. Saos-2 pWZL and pWZL DRAM-3 cells were subjected to 24h Glucose starvation with and without addition of apoptosis inhibitor 50µM z-

Vad-FMK (b). 4 µg/ml Cycloheximide (CHX) + 5ng/ml TNF-α (TNF) was used as an apoptosis-inducing positive control. (**c**) DRAM-3 expressing and vector control cells (pWZL) were treated as indicated and were then stained with Annexin V and propidium iodide prior to analysis by flow cytometry. Early apoptotic death (PI-/Annexin V+) is shown in the lower right quadrant. Late apoptotic death or necrotic death (PI+/Annexin V+) is shown in the upper right quadrant. (**d**) Crispr knockout of DRAM-3 does not decrease survival following glucose deprivation. Saos-2 control (GFP Crispr) and DRAM-3 Crispr knockout cells were subjected to 24h Glucose starvation (DMEM glucose free + 2% dialized FBS). The starvation media was exchanged after 24h and the surviving cells were grown in normal growth media for 3-4 days before staining for colony formation with Giemsa-modified stain. (**e**) Inhibition of autophagy by treatment with chloroquine does not affect cell death induced by glucose deprivation in either DRAM-3 expressing or vector control cells (pWZL). Cells were stained with Annexin V and propidium iodide prior to analysis by flow cytometry. Early apoptotic death (PI-/Annexin V+) is shown in the lower right quadrant. Late apoptotic death or necrotic death (PI+/Annexin V+) is shown in the upper right quadrant. FACS profiles are representative for 3 experiments.

Supplementary Figure 7

DRAM-3 overexpressing cells show greater LC3B-II levels in control and glucose-starvation conditions. Saos-2 pWZL and pWZL DRAM-3 cells were subjected to 24h Glucose starvation (DMEM glucose free + 2% dialized FBS) and then analysed for LC3B conversion by western blotting. 3 experiments were quantified using ImageJ and the results were plotted as means of LC3BII/Actin levels, error bars are SEM.

Supplementary Figure 8

DRAM-3 is amplified in a number of tumour types. Using publically available datasets in cBioPortal (www.cBioPortal.org), *dram-3* genetic alterations were mapped in a range of cancer types.