Supplemental Figure Legends.

Figure S1. GST-MDA-7 toxicity in GBM cells is dependent on cathepsin proteases, and weakly dependent on caspase 8. GBM6 and GBM12 cells were treated 24 h after plating with vehicle (DMSO), IETD (50 μM) or cathepsin B inhibitor (CB inhib.) (1 μM) followed by treatment with GST or GST-MDA-7 (50 nM). Cells were isolated for viability analyses 72 h after GST-MDA-7 treatment as judged in triplicate by trypan blue dye exclusion assay (± SEM, n = 3, # $p < 0.05$ value less than vehicle treated cells).

Figure S2. GST-MDA-7 causes vesicularization of LC3-GFP in UOK121LN cells that is blocked by 3-methyl adenine. UOK121LN cells in 4-well glass chamber slides in triplicate were transfected with a plasmid to express LC3-GFP and 24 h after transfection treated with vehicle (PBS) or 3-methyl adenine (3MA, 5 mM) followed 30 min later by GST or GST-MDA-7 (50 nM). Six h after GST-MDA-7 treatment, cells were examined under visible light or under fluorescent light with appropriate filters at 40X magnification. The data are representative images from the triplicate plating (n = 2).

Figure S3. Knock down of ATG5 and Beclin1 suppresses GST-MDA-7–induced autophagy and cell killing in human GBM cells. U251 cells were plated in triplicate, and 12 h after plating transfected with either a vector control plasmid to express a non-specific scrambled siRNA (siSCR) or with plasmids to knock-down expression of Beclin 1 (siBeclin 1) or of ATG5 (siATG5). Twelve h after transfection, cells were treated with GST or GST-MDA-7 (30 nM). Forty eight h after GST-MDA-7 exposure cell viability was determined by trypan blue exclusion assay on isolated cells (± SEM, n = 3). In parallel, U251 cells were plated in 4-well chamber glass slides in triplicate, and 12 h after plating were transfected with a plasmid to express LC3-GFP, and in parallel co-transfected with either a vector control scrambled siRNA plasmid (siSCR) or with plasmids to knock-down the expression of either Beclin 1 (siBeclin 1) or ATG5 (siATG5). Twelve h after transfection, cells were treated with GST or GST-MDA-7 (100 nM). Twenty four h after GST-MDA-7 exposure the U251 cells were examined under visual light or under fluorescent light at 40X magnification. The mean number of intense

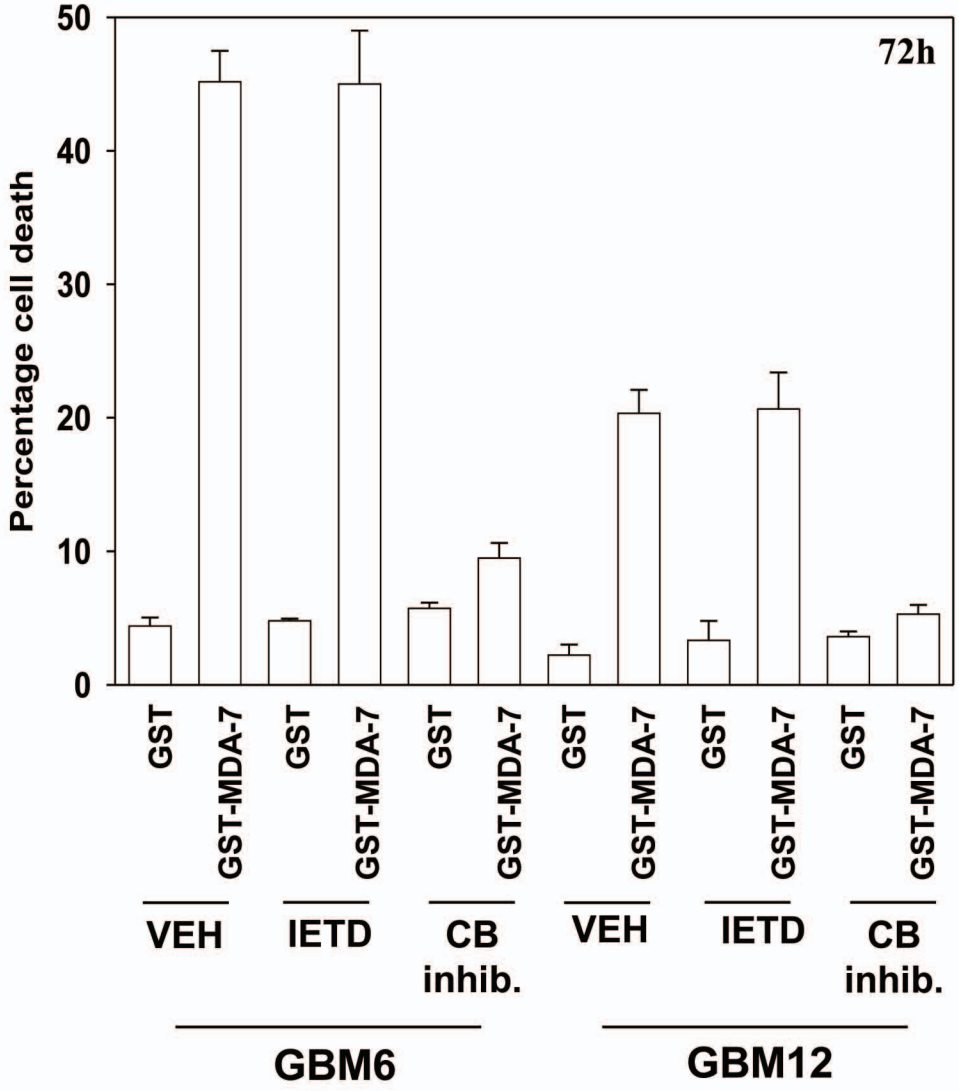
LC3-GFP staining vesicles per cell was determined from multiple fields (from 40 cells) from multiple treatment conditions (± SEM, n = 3 independent studies).

Figure S4. Loss of PERK expression reduces GST-MDA-7 toxicity; expression of activated mutant K-RAS promotes GST-MDA-7 toxicity. Transformed MEFs: wild type transfected with vector (WT) or to express K-RAS D13 (WT K-RAS D13); PERK -/- transfected with vector (PERK -/-) or to express K-RAS D13 (PERK -/- K-RAS D13) were cultured for 36 h and then treated with GST or GST-MDA-7 (0-100 nM, as indicated). Cells were isolated for viability analyses 72 h after GST-MDA-7 treatment as judged in triplicate by trypan blue dye exclusion assay (± SEM, n = 3). Inset Panel. Transformed mouse embryonic fibroblasts (wild type, WT; deleted for PERK, PERK -/-), 24 h after plating, treated with GST or GST-MDA-7 (100 nM). Twenty four h after GST-MDA-7 treatment, cells were isolated and subjected to SDS PAGE to determine the expression of Beclin 1, ATG5, the cleavage status of LC3 and GAPDH (n = 2).

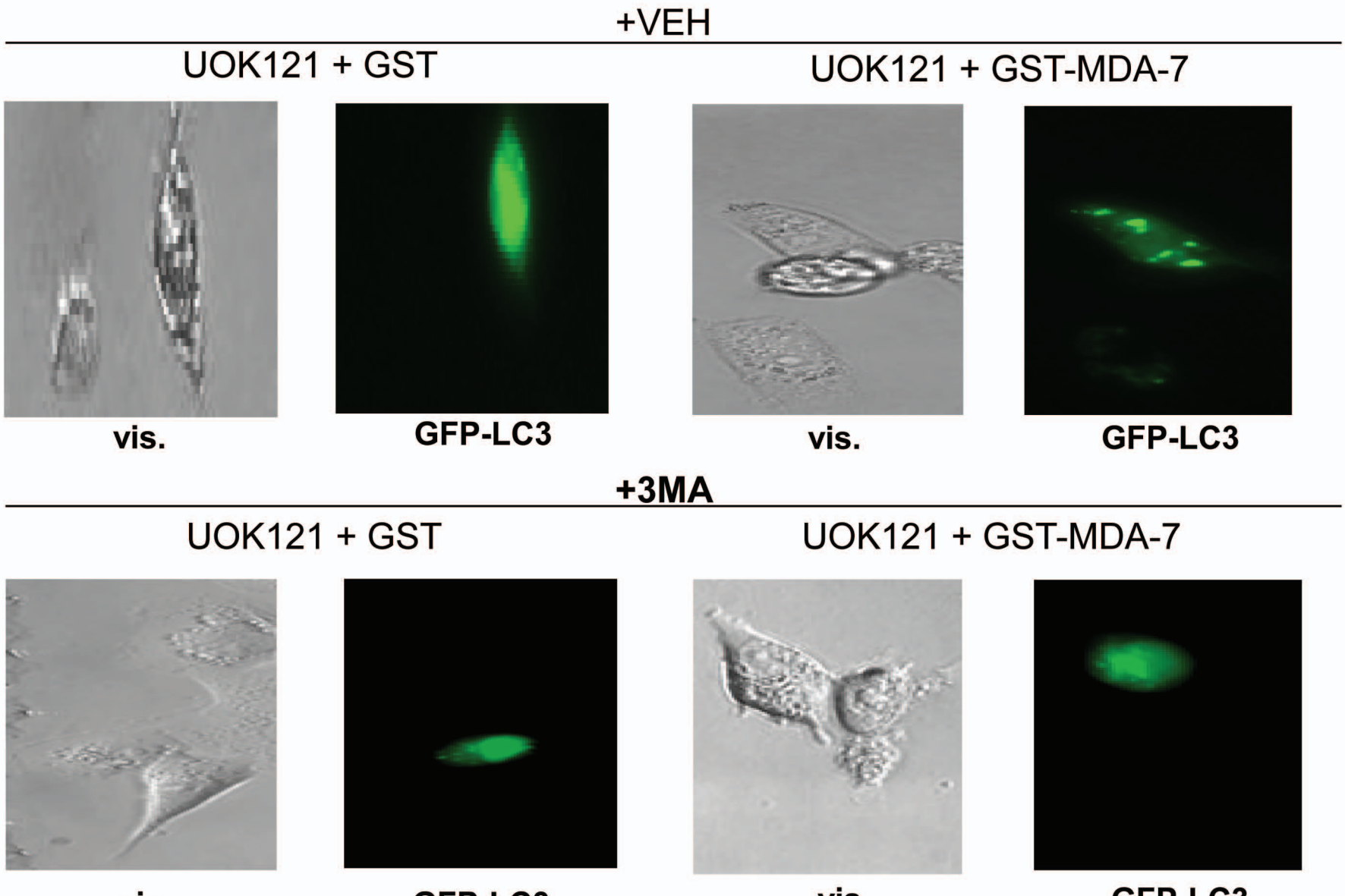
Figure S5. Knock-down of CD95 levels or expression of dominant negative PERK blunts GST-MDA-7–induced activation of p38 MAPK and JNK1/2. *Upper panel*: UOK121LN cells in triplicate were transfected with a plasmid to express LC3-GFP in parallel with siRNA molecules: an siSCR control; to knock down CD95 expression. Thirty six h after transfection treated with GST or GST-MDA-7 (50 nM). Six h after GST-MDA-7 treatment the cells were treated with GST or GST-MDA-7 (50 nM). Six h after GST-MDA-7 treatment the cells were isolated and the phosphorylation of JNK1/2 and p38 MAPK determined by immunoblotting (n = 2). *Lower panel*: UOK121LN cells were transfected with either a vector control plasmid (CMV) or a plasmid to express dominant negative PERK, and 24 h after transfection treated with GST or GST-MDA-7 (50 nM). Six h after GST-MDA-7 treatment the cells were isolated and the phosphorylation of ERK1/2, JNK1/2 and p38 MAPK determined, and the expression of MCL-1, by immunoblotting (n = 2).

Figure S6. GST-MDA-7 toxicity in RCCs is enhanced by inhibition of MEK1 and AKT signaling. UOK121LN cells were infected (400 moi) with control empty vector virus (CMV), dominant negative (dn) AKT and/or dnMEK1. Twenty four h after infection cells were treated with GST or GST-MDA-7 (50 nM). Cells were isolated, and cell viability was determined by trypan blue exclusion assays in triplicate using a hemacytometer. (± SEM, n = 3, * $p < 0.05$ value greater than the amount of cell killing in than CMV infected cells).

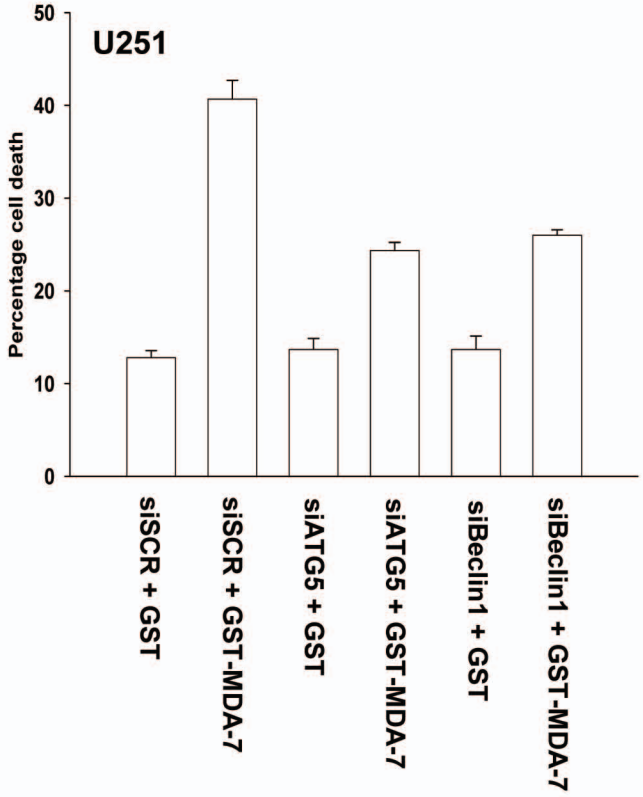
Park et al. Figure S1

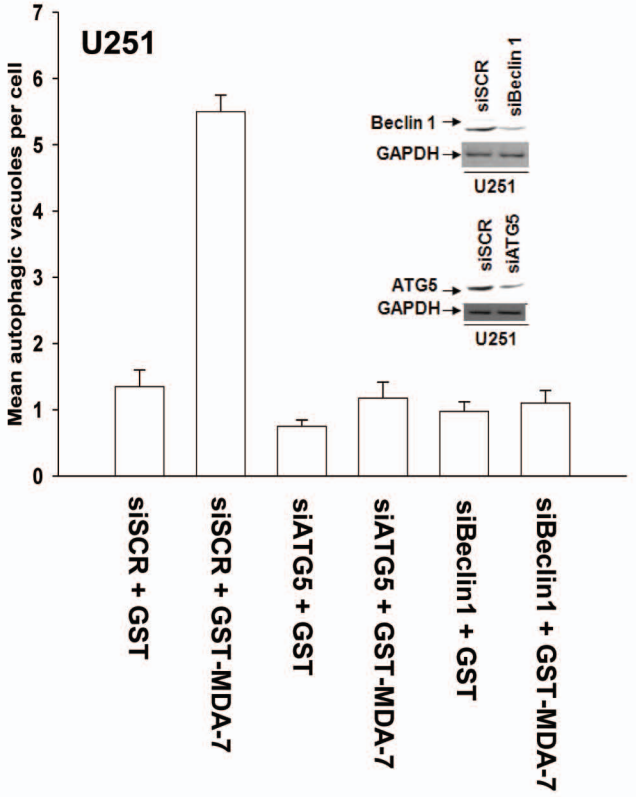


Park et al. Figure S2



Park et al. Figure S3





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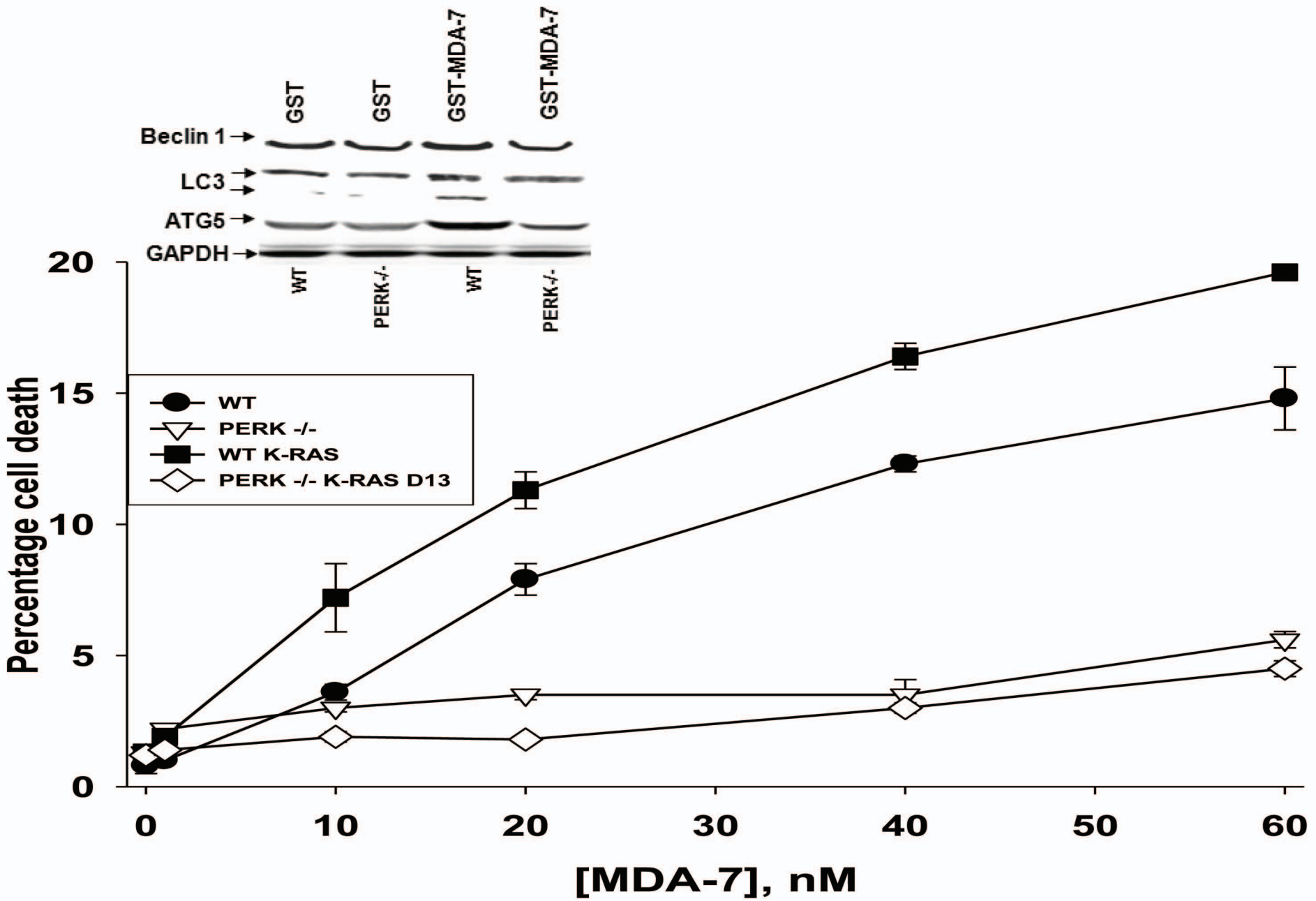

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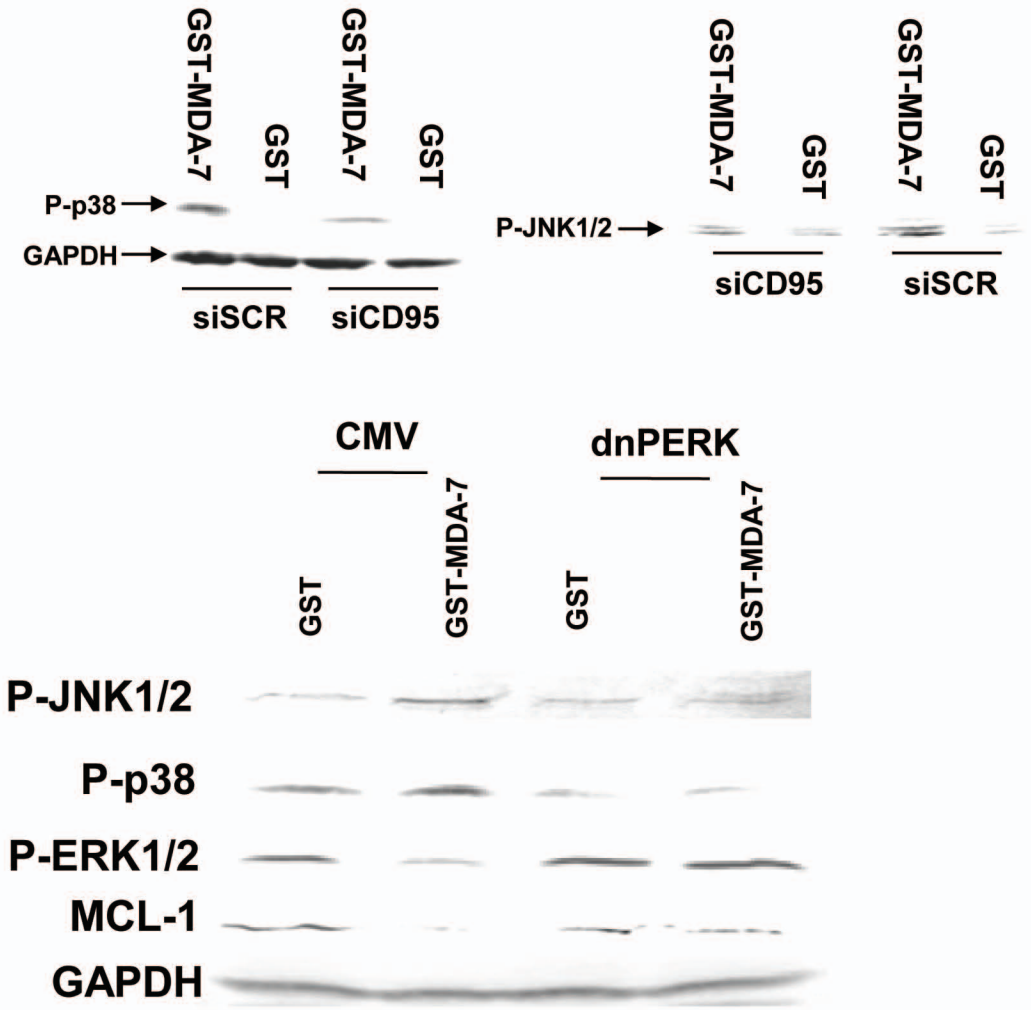

Park et al. Figure S6