Supplemental Material to:

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Dual functions of autophagy in the response of breast tumor cells to radiation; cytoprotective autophagy with radiation alone and cytotoxic autophagy in radiosensitization by vitamin D_3

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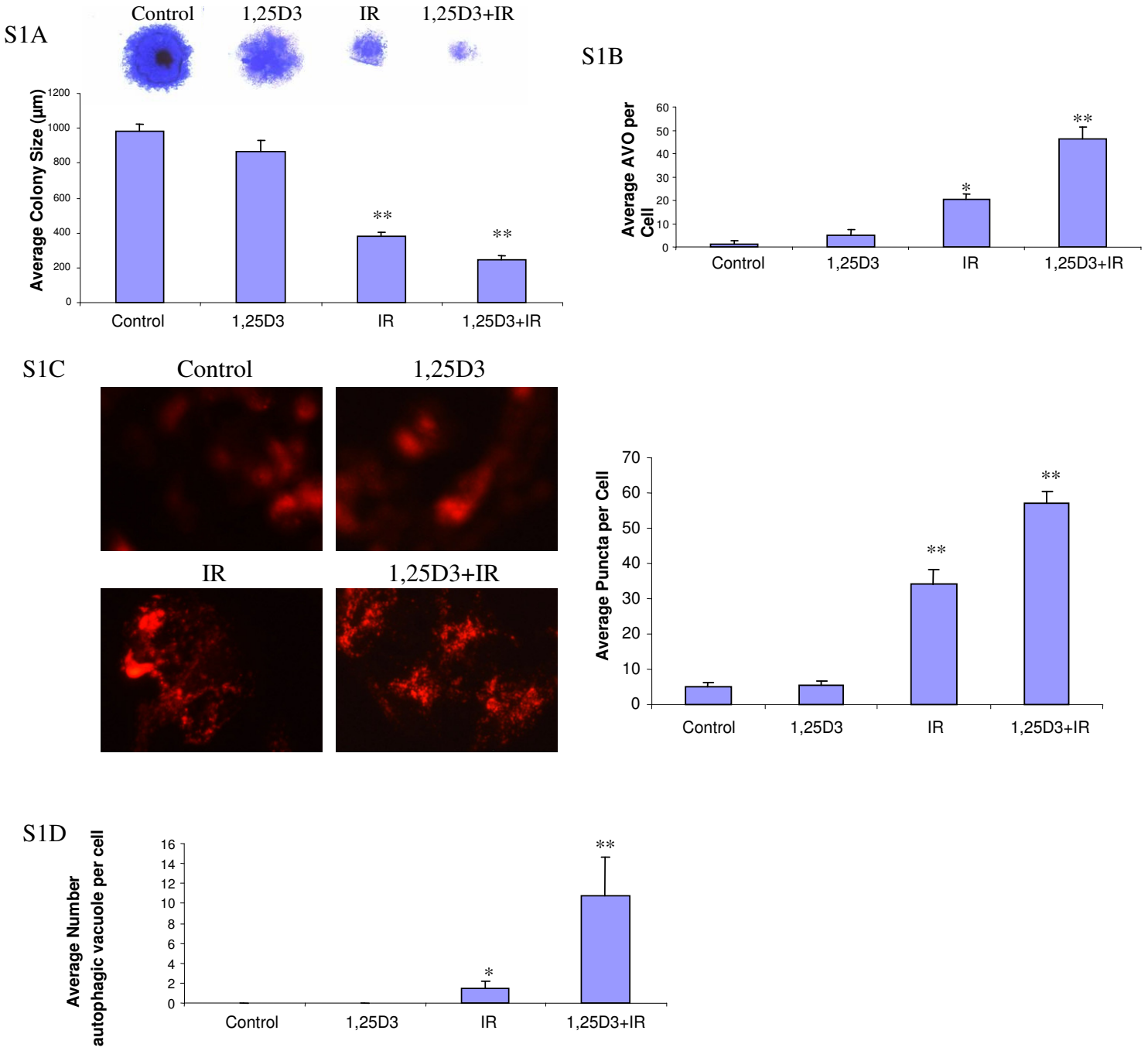
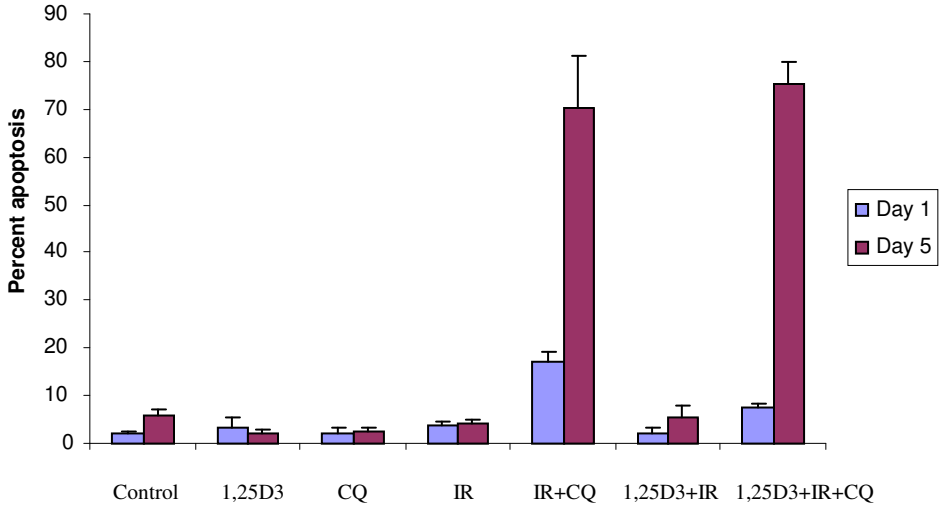


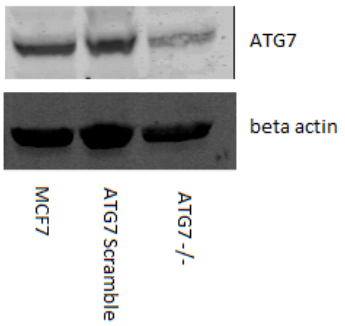
Supplemental Figure 1: S1A. Clonogenic survival was assessed after 14 days post-treatment. Colonies were imaged and average colony width was measured using image analysis software, QCapturePro. Values shown are from a representative experiment with triplicate samples for each condition. **S1B.** Average number of AVOs were counted per cell in three fields for each condition and are represented in the graph. **S1C.** Average number of AVO RFP puncta were counted per cell in three fields for each condition and are represented in the graph. **S1D.** Average numbers of AVOs were counted per cell (images presented in Figure 2D), from at least 10 representative cells, for each condition and are represented in the graph.

S2.

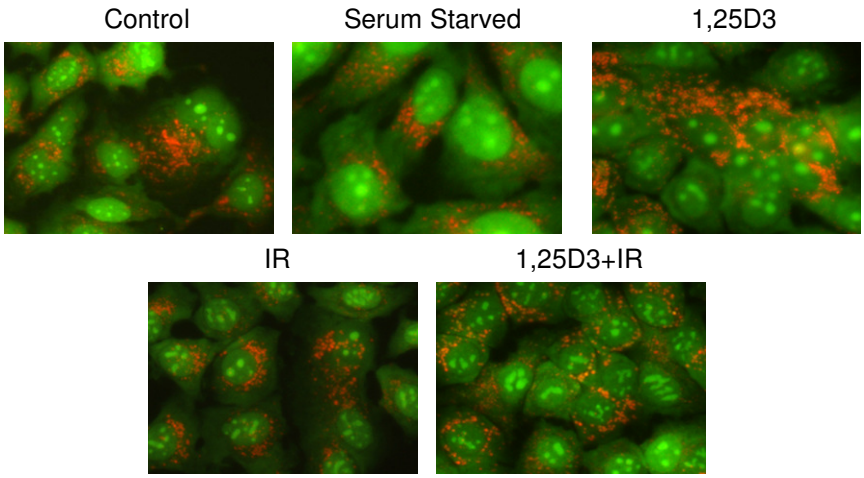


Supplemental Figure 2: Average number of TUNEL positive cells were counted per cell in three fields for each condition on the days indicated and is represented in the graph. All TUNEL positive cells were confirmed with DAPI via altered nuclear morphology.

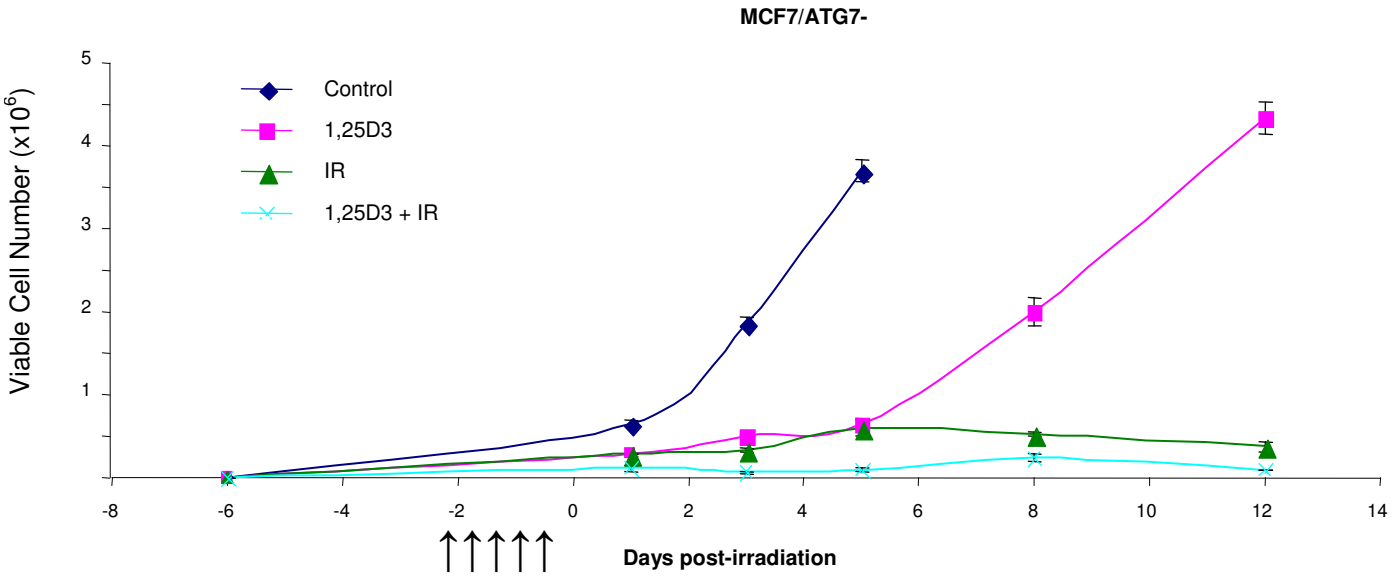
S3A



S3B

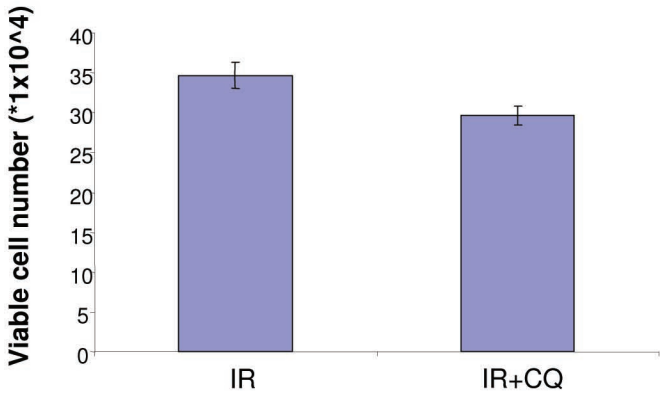


S3C

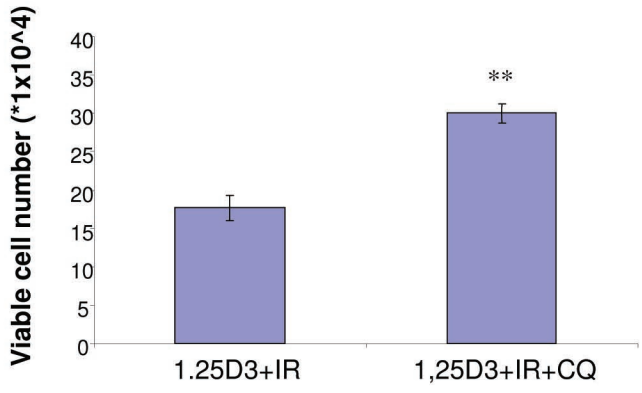


Supplemental Figure 3: Response to radiation or 1,25D$_3$ + radiation in MCF-7 cells with silencing of ATG7. S3A MCF7 cells that were stably transfected with either an empty vector or with a plasmid for the silencing of ATG7 were obtained from Dr. Kelekar. ATG7 levels were confirmed by Western blotting comparing parental MCF7 cells with those transfected with scrambled ATG7 and to those with ATG7 knockdown. **S3B** Autophagy was monitored by acridine orange staining. **S3C** Cells were exposed to radiation alone (5x2 Gy), or 1,25D$_3$ prior to irradiation and viable cell number was determined by exclusion of trypan blue at the indicated days following the initiation of radiation exposure. ↑ indicates dose of 2 Gy radiation received.

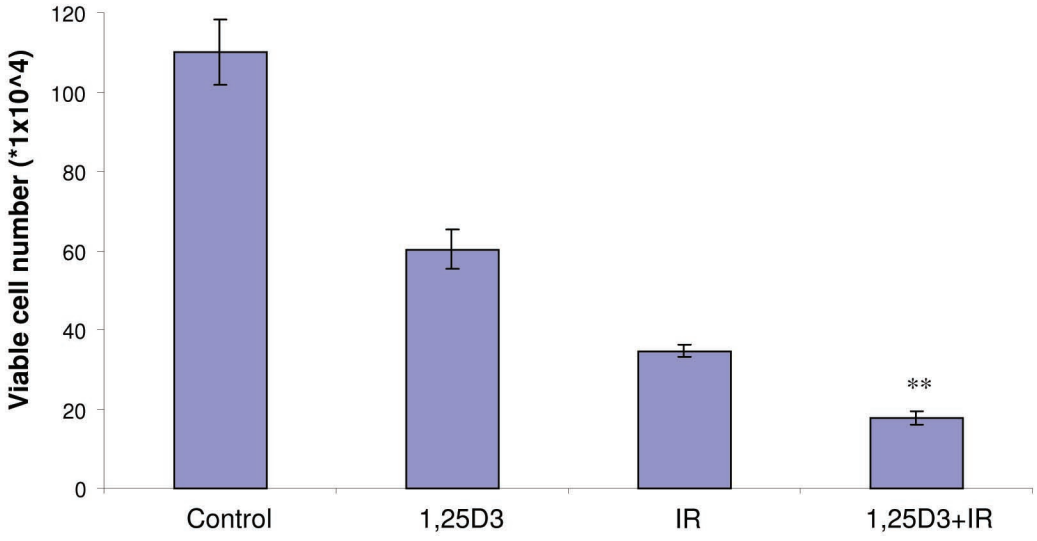
S4A.



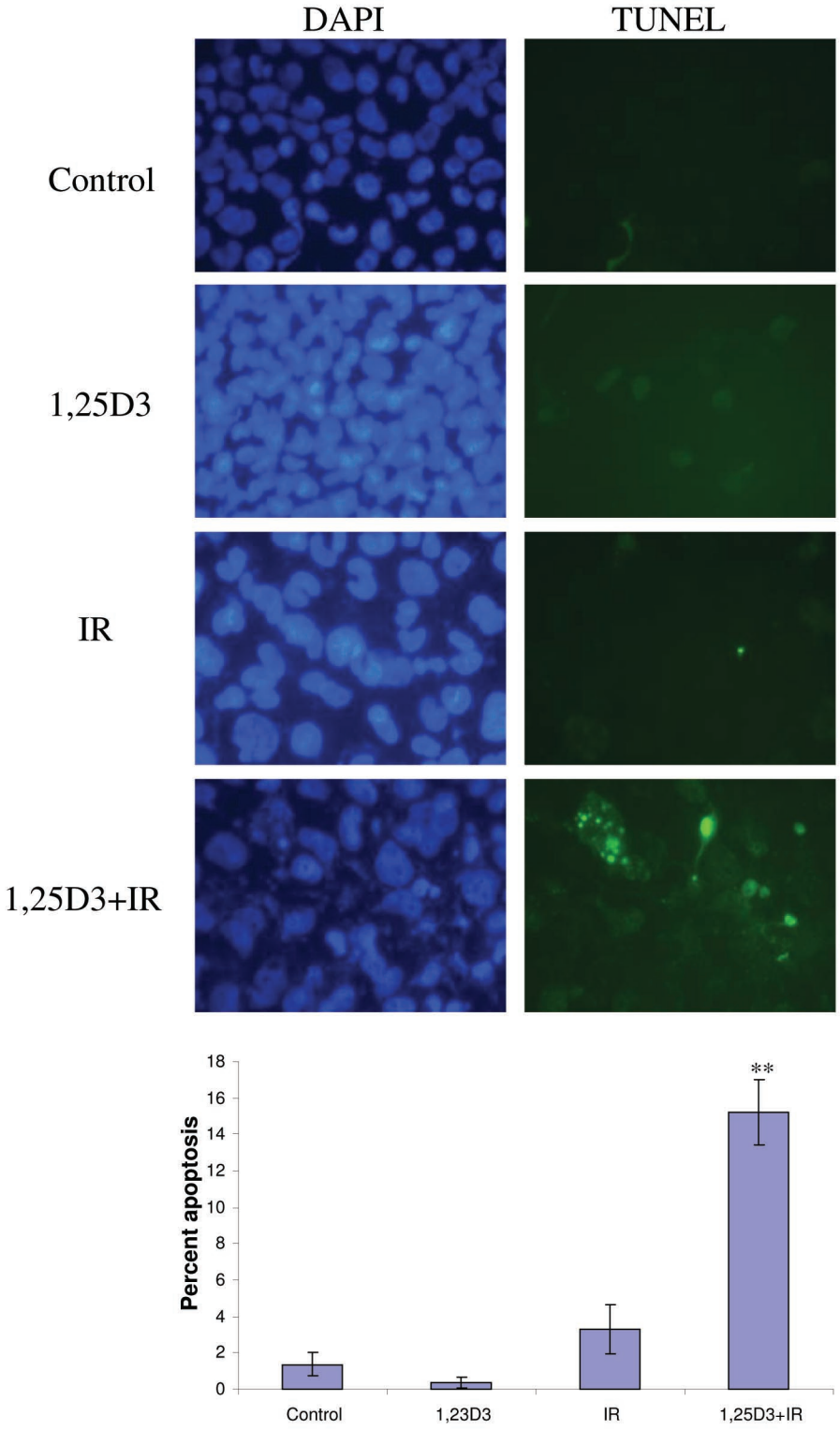
S4B.



S4C.



Supplemental Figure 4: Response to radiation or 1,25D$_3$ + radiation in MCF-7 cells in the presence or absence of Chloroquine. S4A. MCF7 cells were treated with radiation alone (4 Gy) with or without concurrent exposure to 5 μM CQ. Viable cell number was determined by trypan blue exclusion on Day 3 following treatment. **S4B.** MCF7 cells were treated with 1,25D$_3$ + radiation with or without concurrent exposure to 5 μM CQ. Viable cell number was determined by trypan blue exclusion on Day 3 following treatment. **p<0.0001 from 1,25D$_3$ +IR. **S4C.** MCF7 cells were treated with 1,25D$_3$ alone, radiation alone (4 Gy) or with 1,25D$_3$ + radiation. Viable cell number was determined by trypan blue exclusion on Day 3 following treatment. **p<0.0001 from IR.



Supplemental Figure 5. Apoptosis in Hs578t breast tumor cells. Hs578t cells were exposed to $1,25D_3$ alone, radiation alone (5x2 Gy), or $1,25D_3$ prior to irradiation and imaged with TUNEL and DAPI for the presence of apoptosis 5 days post-irradiation. Quantification is presented in the lower graph.