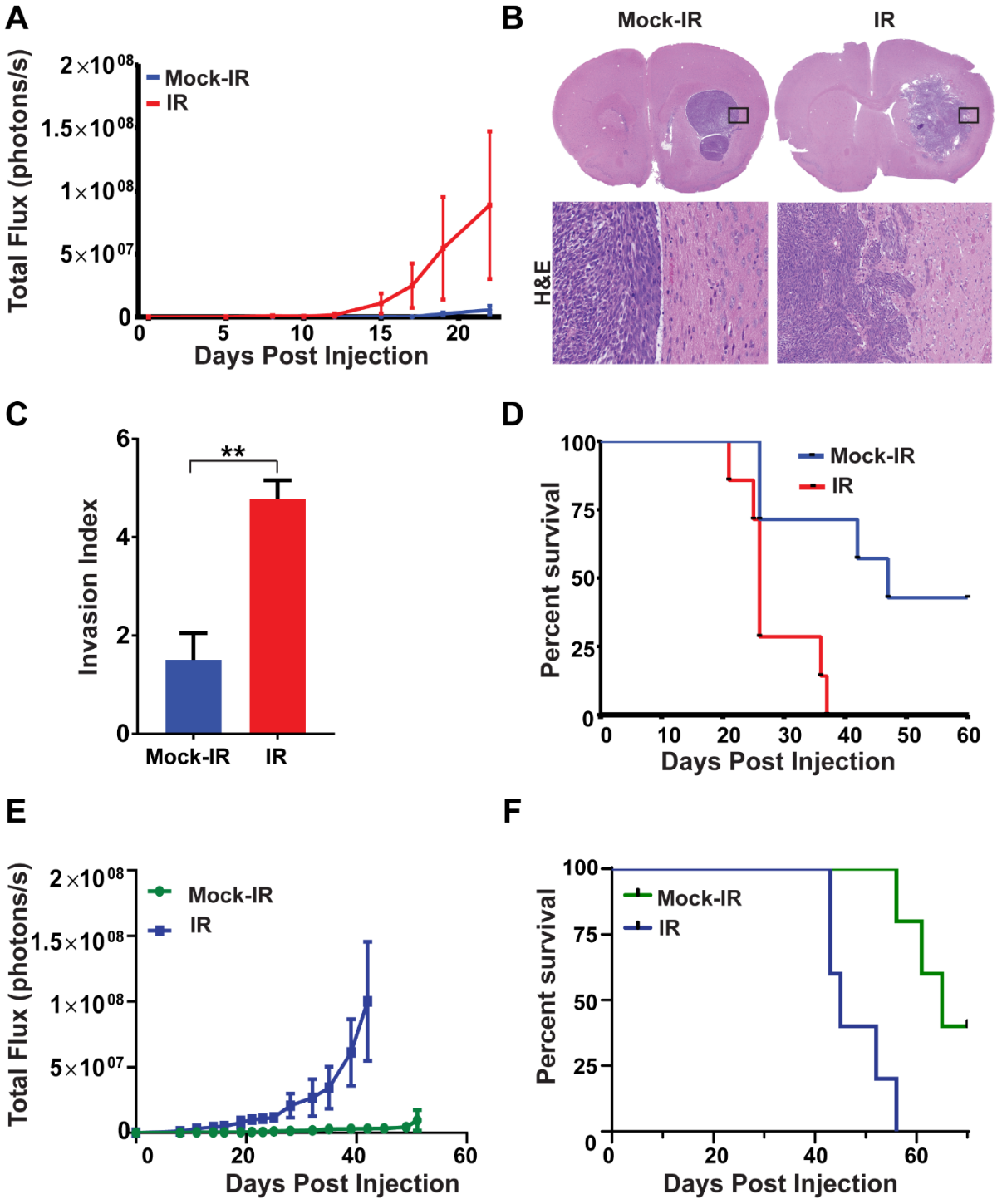
Elimination of radiation-induced senescence in the brain tumor microenvironment attenuates glioblastoma recurrence

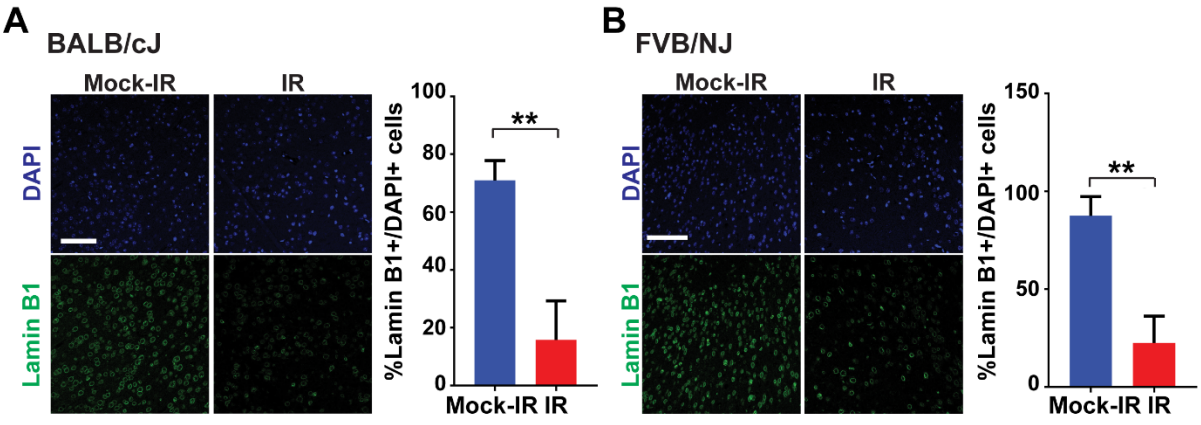
Eliot Fletcher-Sananikone et. al; Supplemental Data

Supplemental Figure 1



Supplemental Figure 1. A, C57BL/6J mice were mock irradiated (Mock-IR) or intra-cranially irradiated (IR) with 10 Gy of X-rays (6 mice per cohort). After 30 days, mice were intra-cranially implanted with 2,500 CT2A cells expressing firefly luciferase. Tumor growth was monitored by BLI imaging over a 30-day period. Plot represents average signal intensity (photons per second) for each cohort versus time post-injection. Note marked increase in the rate of tumor growth (*red line*) in pre-irradiated mouse brains (P=0.0003, error bars S.E.M). **B,** H&E-stained sections of mock-irradiated or irradiated mouse brains bearing CT2A tumors. High magnifications show representative areas spanning the tumor borders. Note marked increase in tumor size and infiltration in the pre-irradiated mouse brain. **C,** Plot depicts average Invasion Index for mock-irradiated or irradiated cohorts (P= 0.0016, error bars S.D). **D,** Kaplan-Meier curves show survival of mock-irradiated or pre-irradiated mice implanted with CT2A cells and then monitored over a 60-day period (n=6 per cohort, P= 0.0176). **E,** FVB/NJ-C57BL/6J mice were mock irradiated (Mock-IR) or intra-cranially irradiated (IR) with 10 Gy of X-rays (5 mice per cohort). After 30 days, mice were intra-cranially implanted with 50,000 NS2262 cells expressing firefly luciferase, and tumor growth monitored by BLI imaging. Plot represents average signal intensity (photons per second) for each cohort versus time post-injection. (P<0.0001, error bars S.E.M). **F,** Kaplan-Meier curves show survival of mock-irradiated or pre-irradiated mice implanted with NS2262 cells (n=5 per cohort, P=0.0042).

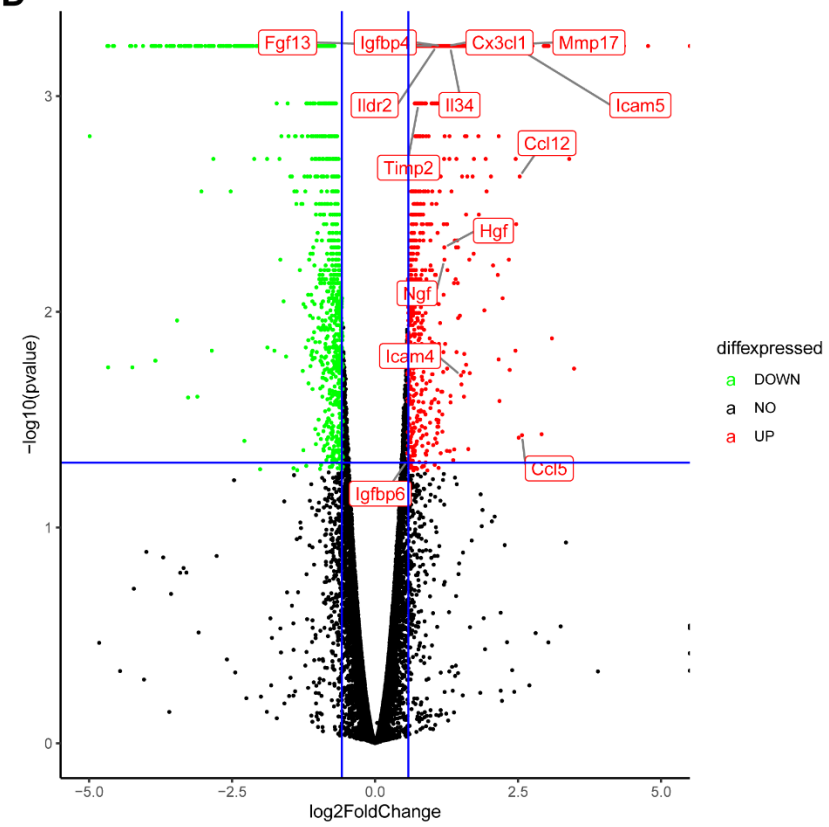
Supplemental Figure 2

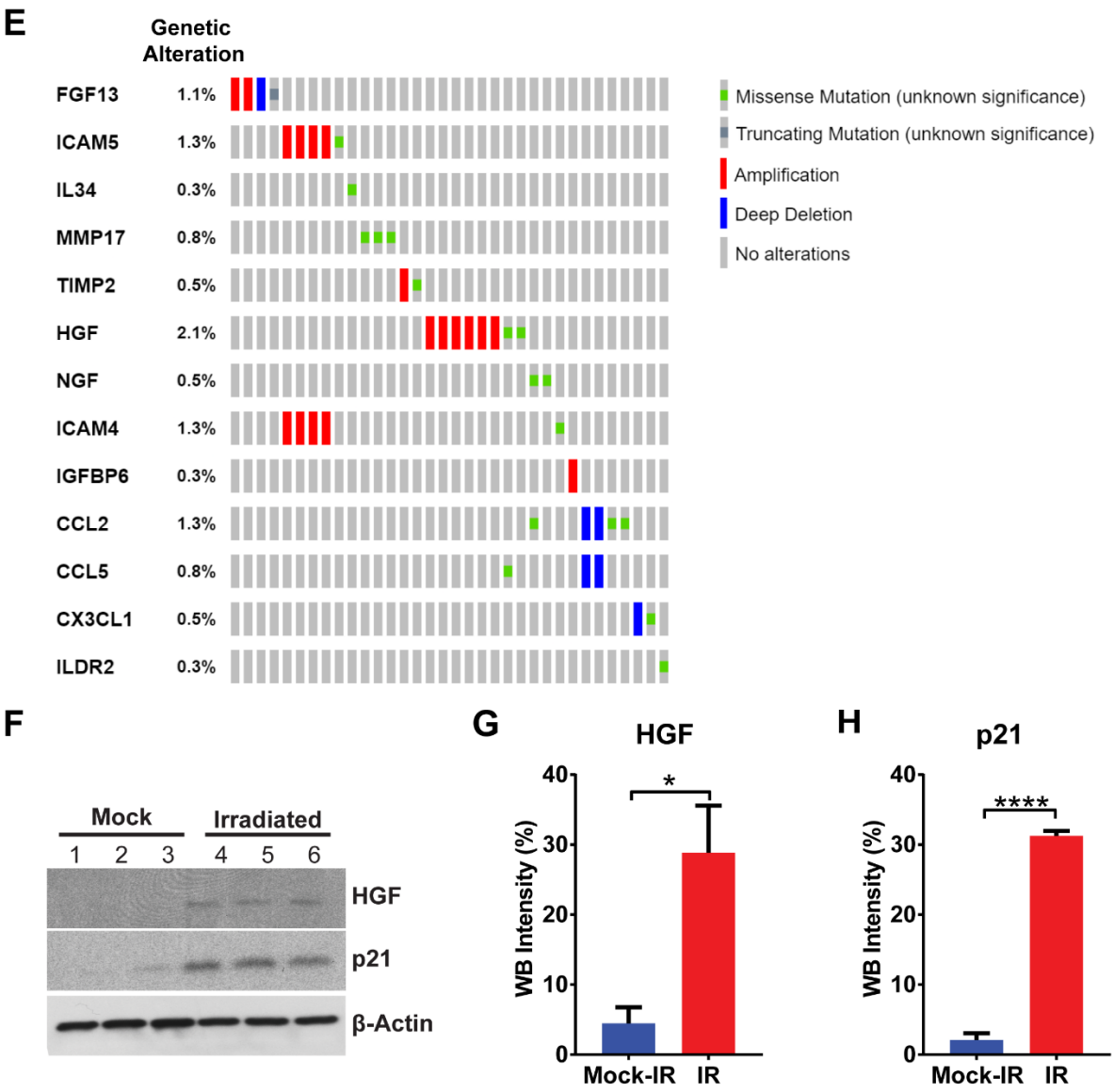


C

Gene	log2 FC
Ccl5	2.567
Ccl12	2.527
Icam5	2.432
Cxcl10	2.265
Mmp17	1.883
Icam4	1.500
Il34	1.308
Cx3cl1	1.261
Ngf	1.211
Hgf	1.209
Igfbp4	1.165
Ildr2	1.076
Fgf13	1.039
Il12a	0.805
Timp2	0.760
Mmp2	0.623
Icam1	0.621
Igfbp6	0.567
Il1f9	0.549
Il18	0.450
Ifngr2	0.322
Cxcl14	0.310
Fgf10	0.288
Timp1	0.274

D

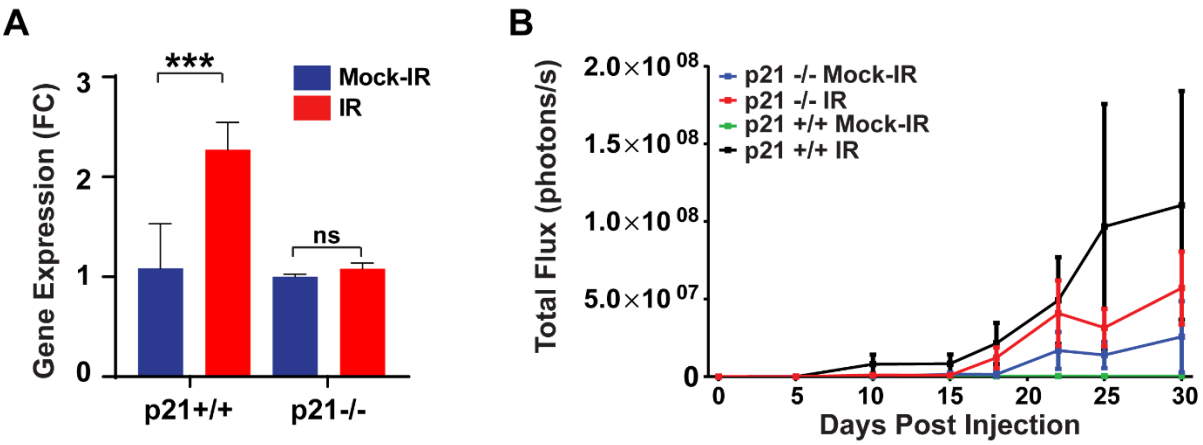




Supplemental Figure 2. A, Plot shows the percentage of Lamin B1-positive cells in the cortex of mock-irradiated or irradiated brains of BALBcJ mice (n=3 per cohort) with associated images showing immunofluorescence staining of the cortex of mock-irradiated and irradiated brains for Lamin B1 and DAPI (P=0.0082, error bars S.D). Scale bar=100 μm. **B,** Plot shows the percentage of Lamin B1-positive cells in the cortex of mock-irradiated or irradiated brains of FVB/NJ mice (n=3 per cohort) with associated images showing immunofluorescence staining of the cortex of mock-irradiated and irradiated brains for Lamin B1 and DAPI (P=0.0037, error bars S.D). Scale bar=100 μm. **C,** List of SASP genes up-regulated (Log Fold Change) in mock-irradiated versus irradiated cohorts (n=3 per cohort). **D,** Volcano plot showing the differentially expressed genes from mock-irradiated versus irradiated cohorts. SASP genes that show significant differential expression are highlighted in the plot. **E,** The TCGA database was used to analyze alterations in SASP genes in GBM. **F,** Western blot of whole brain lysates from mock-

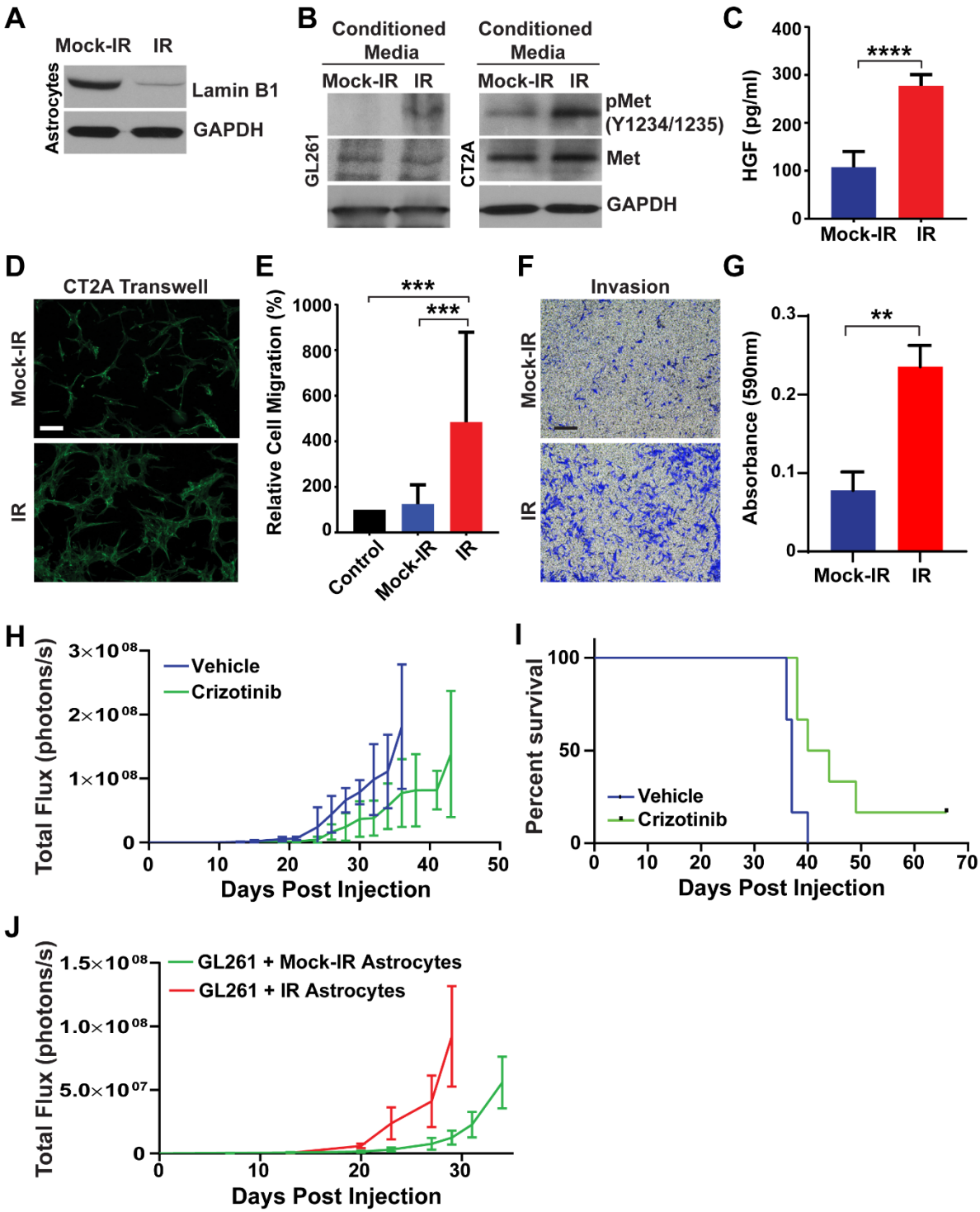
irradiated or irradiated cohorts (n=3 per cohort) for HGF, p21 and β-Actin (loading control). **G,** Plot represents quantification of HGF western blot signal intensity normalized to loading control (n=3, P= 0.0162, error bars S.D). **H,** Plot represents quantification of p21 western blot signal intensity normalized to loading control (n=3, P <0.0001, error bars S.D).

Supplemental Figure 3



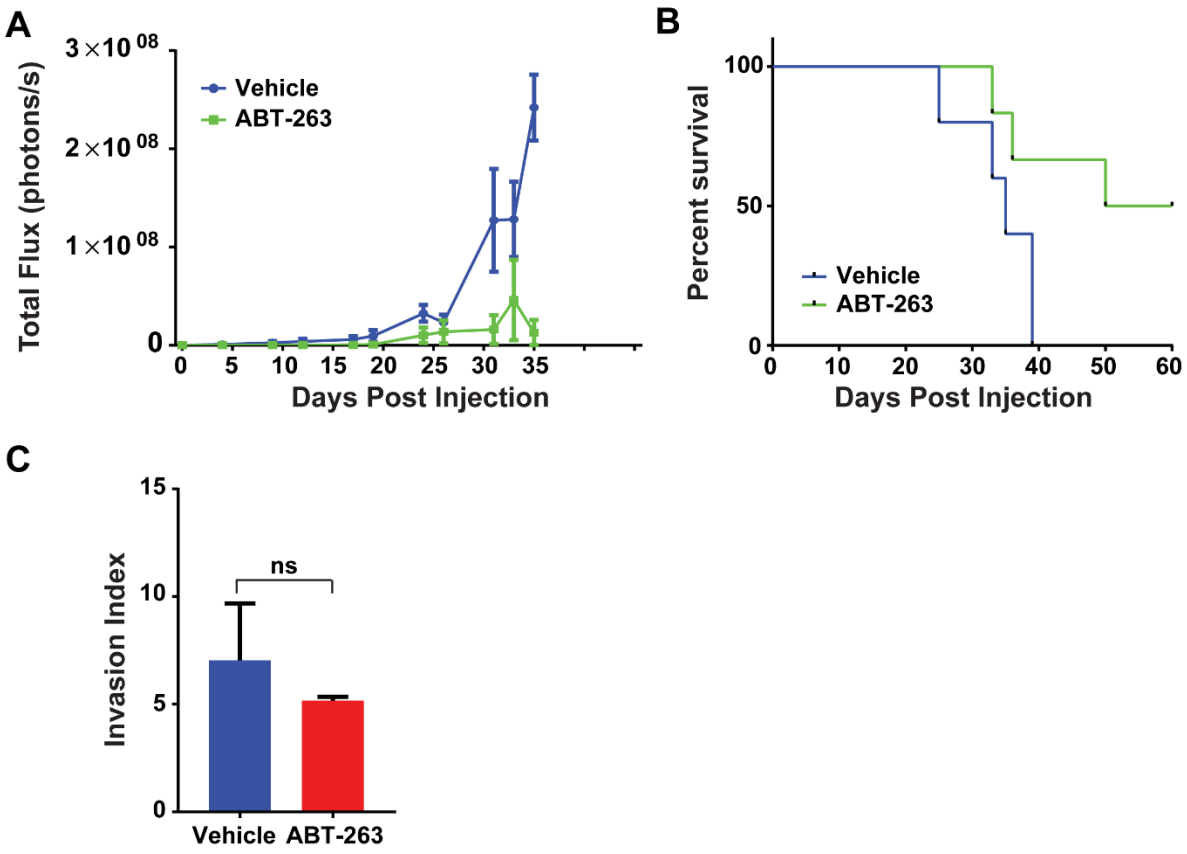
Supplemental Figure 3. A, Plot shows relative expression of *HGF* gene in mock-irradiated versus irradiated astrocytes as quantified by qRT-PCR. (n=3, P=0.001, error bars S.D). **B,** p21+/+ or p21-/- mice were mock irradiated or cranially irradiated with 10 Gy of X-rays (6 mice per cohort). After 30 days, mice were intra-cranially implanted with 2,500 CT2A cells expressing firefly luciferase. Tumor growth was monitored by BLI imaging. Plot represents average signal intensity (photons per second) for each cohort versus time post-injection. Note promotion of tumor growth in irradiated p21+/+ mice but not in p21-/- mice (P= 0.2043, error bars S.E.M).

Supplemental Figure 4



Supplemental Figure 4. A, Western Blot of mock-irradiated or irradiated astrocytes (10 Gy, 10 days) for Lamin B1 and GAPDH (loading control). Note decrease in Lamin B1 in irradiated astrocytes indicating induction of senescence. **B,** Western blot of serum-starved GL261 or CT2A cells (treated for 2 hours with conditioned media from mock-irradiated or irradiated astrocytes) for pY1234/1235 Met, Met, and GAPDH (loading control). Note increase in pY1234/1235 Met signal in cells treated with conditioned media from irradiated astrocytes. **C,** Plot shows levels of HGF in conditioned media from mock-irradiated or irradiated astrocytes quantified by ELISA (n=6, P<0.0001, error bars S.D). **D,** Results of Boyden Chamber Assay with CT2A cells in the top chamber and mock-irradiated or irradiated (10 Gy, 10 days) primary mouse astrocytes in the bottom chamber. Representative images show CT2A cells on the bottom surface of the trans-well membrane stained with Alexa Fluor 488 Phalloidin (green). Scale bar=100 μm. **E,** Plot shows percentage of cells per 40X microscopic field migrating towards the bottom chamber relative to migration towards media alone (n=3 per cohort, P=0.0035, error bars S.D). **F,** Primary astrocytes were isolated, seeded into a 24 well plate and treated with 10 Gy IR for 10 days. 2.5 X 10^5 GL261 cells were seeded onto inserts coated with matrigel. Inserts were fixed with 0.1% crystal violet in methanol after 24 hours. Cells invading through the matrigel were quantified by eluting crystal violet from the cells with methanol and reading absorbance at 570 nm. Scale bar=100 μm. **G,** Plot shows invasion of GL261 cells in the presence of mock-irradiated or irradiated astrocytes, quantified as absorbance of crystal violet eluted from the invading cells (n=3, P=0.0016, error bars, S.D.). **H,** C57BL/6J mice were cranially irradiated with 10 Gy of X-rays (6 mice per cohort). After 30 days, mice were intra-cranially implanted with 2,500 GL261 cells expressing firefly luciferase. Seven days after tumor cell implantation, mice were treated with 10 doses of Crizotinib (50 mg/kg) given every other day or with vehicle alone as control. Tumor growth was monitored by BLI imaging. Plot represents average signal intensity (photons per second) for each cohort versus time post-injection (P=0.1294, error bars S.E.M). **I,** Kaplan-Meier curves show survival of pre-irradiated mice implanted with GL261 cells and then treated with Crizotinib or vehicle alone (n=6 per cohort, P=0.0099). **J,** 2,500 luciferase-tagged GL261 cells were mixed with 12,500 mock-irradiated or irradiated (10 Gy, 10 days) primary mouse astrocytes, and implanted intra-cranially in C57BL/6J mice (6 mice per cohort). Tumor growth was monitored by BLI imaging. Plot represents average signal intensity (photons per second) for each cohort versus time post-injection (P=0.0025, error bars S.E.M).

Supplemental Figure 5



Supplemental Figure 5. A, C57BL/6J mice were mock irradiated or intra-cranially irradiated with 10 Gy of X-rays (6 mice per cohort). After 5 days, mice were treated with either vehicle or ABT-263 (50 mg/kg) daily for 25 days. At 30 days post-IR, mice were intra-cranially implanted with 2,500 CT2A cells expressing firefly luciferase. Tumor growth was monitored by BLI imaging over a 30-day period. Plot represents average signal intensity (photons per second) for each cohort versus time post-injection ($P < 0.0001$, error bars S.E.M). Note marked delay in tumor growth (green line) in mouse brains pre-treated with ABT-263. **B,** Kaplan-Meier curves show survival of pre-irradiated mice, treated with vehicle or ABT-263, implanted with CT2A cells and then monitored over a 60-day period. (n=6 per cohort, $P=0.0460$). **C,** Plot depicts average Invasion Index for irradiated and vehicle- or ABT-263-treated cohorts (n=3, $P=0.344$, error bars S.D).

Supplemental Table: Primer sequences for qRT-PCR

1	mGapdh	forward	TCTCCCTCACAATTTCCATCC
		Reverse	GGGTGCAGCGAACTTTATTG
2	mCcl5	forward	AAAGACGAGGACAGCACTTAC
		Reverse	CTCGCTGTTCATTGCCAAATAC
3	mCcl12	forward	CAGTGAGGTGCATAGCGTAAT
		Reverse	CTCCTTCTGGGACATTGCTATC
4	mIgfbp6	forward	AGACTACAAAGGAGAGCAAACC
		Reverse	GAACAGGATTGGGCCGTATAG
5	mHgf	forward	AGACTACAAAGGAGAGCAAACC
		Reverse	GAACAGGATTGGGCCGTATAG
6	mTimp2	forward	CAGTGAGGTGCATAGCGTAAT
		Reverse	CTCCTTCTGGGACATTGCTATC
7	mIl34	forward	CTGTCTTGGGATCCTACTTGAC
		Reverse	CCTGTAAGGTCACACTCCTTATC
8	mIldr2	forward	CCAGCAAGAGTGGATGCAAA
		Reverse	TAAGACGCACCCAGAAAGGT
9	mCx3cl1	forward	CCAGCAAGAGTGGATGCAAA
		Reverse	TAAGACGCACCCAGAAAGGT
10	mIcam5	forward	AGGCTGCCCAGAACATATTAC
		Reverse	CCTTCCATGACTTCCTCCTTTC
11	mIcam4	forward	CCAGCAAGAGTGGATGCAAA
		Reverse	TAAGACGCACCCAGAAAGGT
12	mNgf	forward	CAGTGAGGTGCATAGCGTAAT
		Reverse	CTCCTTCTGGGACATTGCTATC
13	mFgf13	forward	AAAGACGAGGACAGCACTTAC
		Reverse	CTCGCTGTTCATTGCCAAATAC
14	mMmp17	forward	GTCTTGGAAGCCACCCTACT
		Reverse	TGAGTGTGACACCAGCATCT
15	mIgfbp4	forward	AGAAGCCCCTGCGTACATTG
		Reverse	TGTCCCCACGATCTTCATCTT