Supplemental Methods

Mouse Leg Irradiation:

Specially designed custom Lucite jigs were constructed to immobilize mice without the use of anesthetics as shown in Supplemental Figure 1. These jigs were constructed to fit 4 mice on a Lucite base plate, which was centered underneath the x-ray unit at a 50 cm target to skin distance. A Lucite fixture including a lead shielding with cut-outs to reveal the animal's leg was constructed to fit over the mouse holders aligned by Lucite plugs on the corners that fit precisely into the base plate (see Supplemental Figure 1). The animal's right leg could be gently pulled through a hole in the mouse holder for exposure to radiation (foot gently held in place by tape). Using the lighted collimator field locator on the x-ray unit the radiation field size was set to encompass the attached piece of lead. Notice that most of the mouse is outside the radiation field. The lead was added to ensure that the radiation field did not expose the animal's side (abdomen). As can be seen the animal's leg was open to the radiation field and verification was made using the light field prior to radiation. A special "Lucite" leg was constructed (approximately 1 cm in diameter) and cut in half to enable the placement of a TLD mid-plane. These jigs were used to conduct TLD dosimetry to establish dose and dose/rate (TLD dosimetry was conducted by the Physics Section, Radiation Oncology Branch). The thickness of lead used was 9 mm, which reduced the dose underneath the lead to <1% of the primary dose. Likewise, the dose outside the collimator was <1% of the dose to the leg. The x-ray unit ran at 300 kV peak, with 2 mm aluminum filtration at a dose rate of 1.8 Gy/min. A dose of 35 Gy was delivered to the leg of mice. We have used this model for over 10 years with impressive reproducibility with respect to leg contraction over the time span.

Preparation of tissue samples:

Tissues including skin, subcutaneous tissue and muscle were processed for either RNA, protein analysis or staining. For RNA and protein extraction, samples were snap-frozen and stored at -80ºC. For RNA, Trizol (Invitrogen, Carlsbad, CA), was coupled with a standard phenol-choloroform extraction and quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware). Protein lysates were made with RIPA buffer (Pierce Scientific, Rockford, IL) containing protease inhibitor cocktail (Roche, San Francisco, CA) and quantified using a detergent compatible (DC) protein assay (Biorad, Hercules, CA). Additional leg tissue was decalcified, formalin-fixed, paraffin embedded and cut into 5µm sections.

In vitro Irradiation:

1522 and HCT116 cells were chosen for *in vitro* experimentation including transfection and exposure to radiation. The irradiator used is the same Therapax DXT300 x-ray irradiator as previously mentioned in the *in vivo* work. The x-ray unit ran at 300 kV peak, with 2 mm aluminum filtration at a dose rate of 1.8 Gy/min. Total dose delivered to 1522 cells was 10Gy and total dose delivered to HCT116 cells was 2Gy.

ELISA:

To evaluate the expression of c-Met and TGF-ß, protein samples from cell lines and fibrotic and control tissue were used to run single analyte ELISA run according to manufacturer's instructions (R&D Systems, Minneapolis, MN) and absorbance was read using a Biotek µQuant plate reader equipped with KCjunior Software (Biotek, Winooski, VT).

Western Blotting:

To evaluate the response of 1522 cell line to transfection with c-Met siRNA, western blotting was conducted for c-Met, TGF-β, CTGF (all from Cell Signaling Technology, Danvers, MA) and PDGF-R (Millipore, Billerica, MA). Cells were transfected with c-Met siRNA or scramble

siRNA and protein was extracted 48 hours later. 20µg of protein was run on a 4-12% NuPAGE Bis-Tris pre-cast gel (Invitrogen, Grand Island, NY), transferred to nitrocellulose, blocked in 5% milk, and incubated with primary antibodies overnight. After incubating with goat anti-rabbit IgG secondary antibody they were quantified with ImageJ software [1].

1. Schneider CA, Rasband WS, Eliceiri KW. Nih image to imagej: 25 years of image analysis. *Nature methods* 2012;9:671-675.