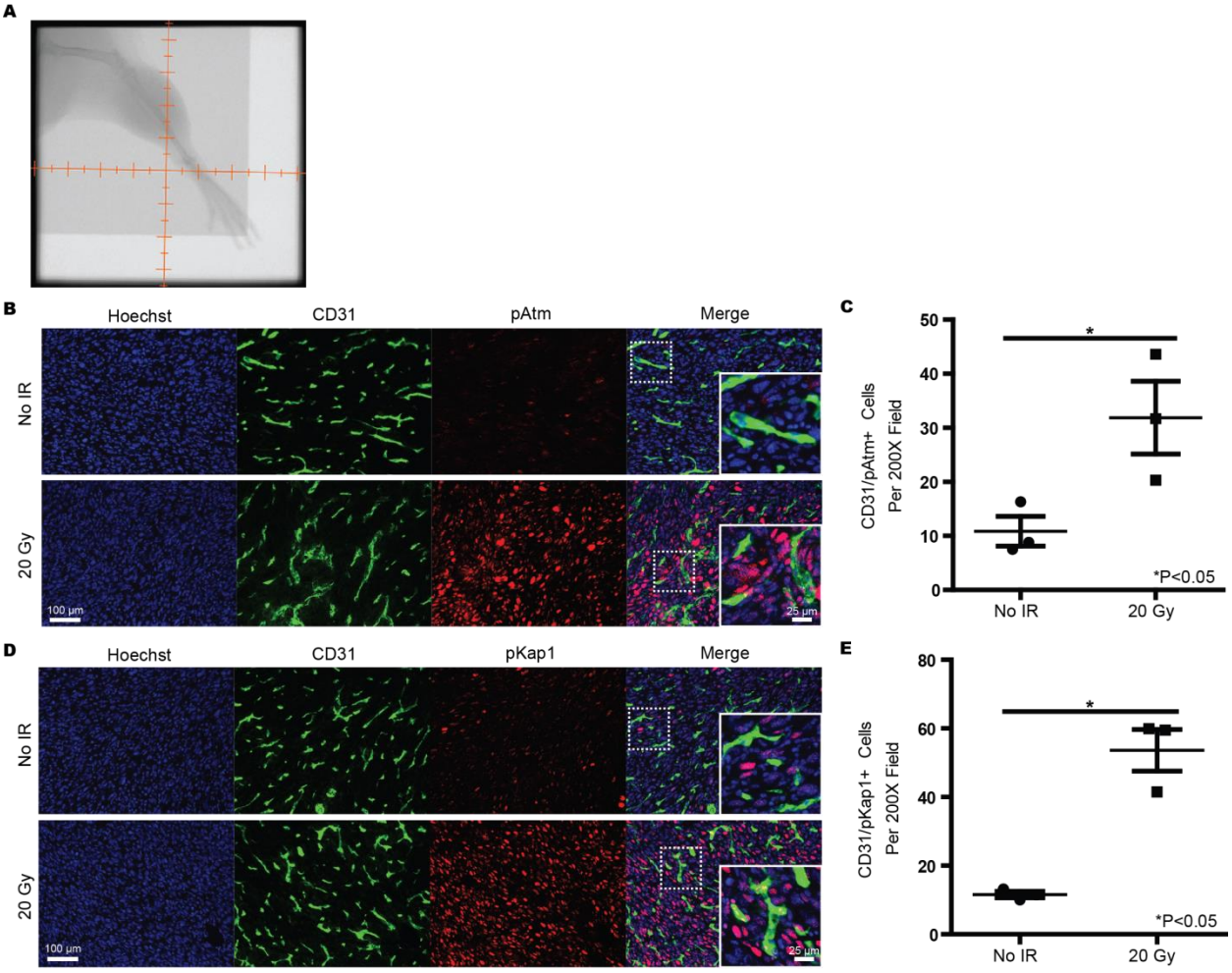
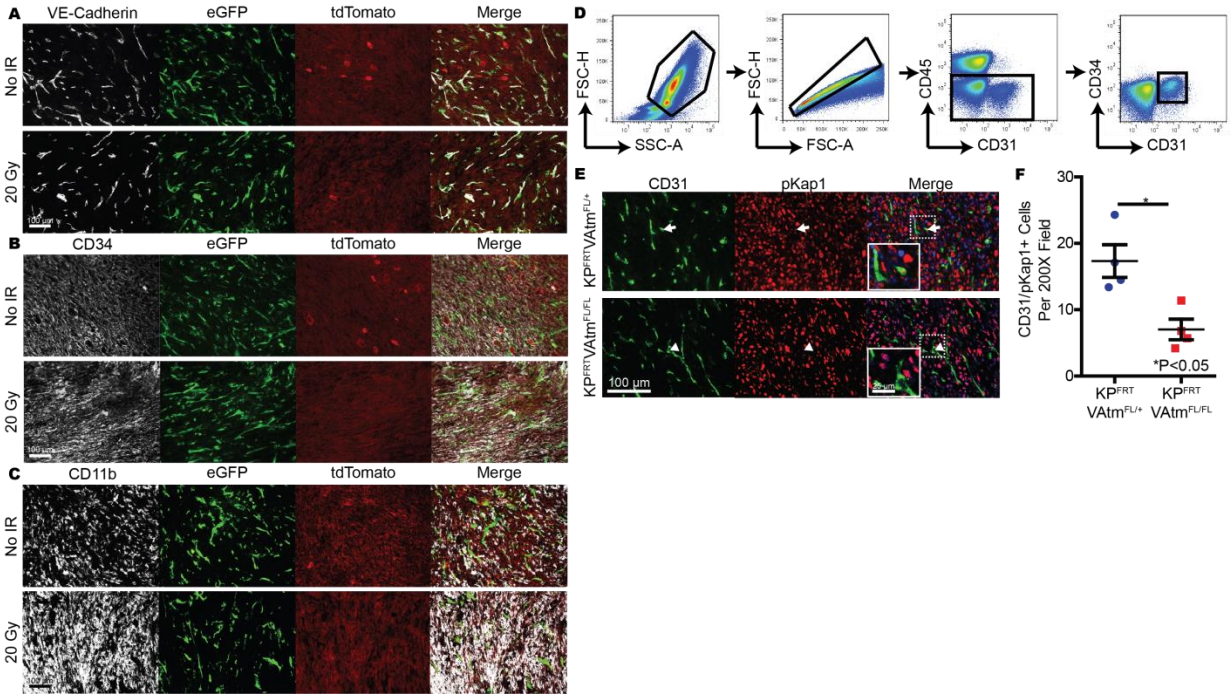
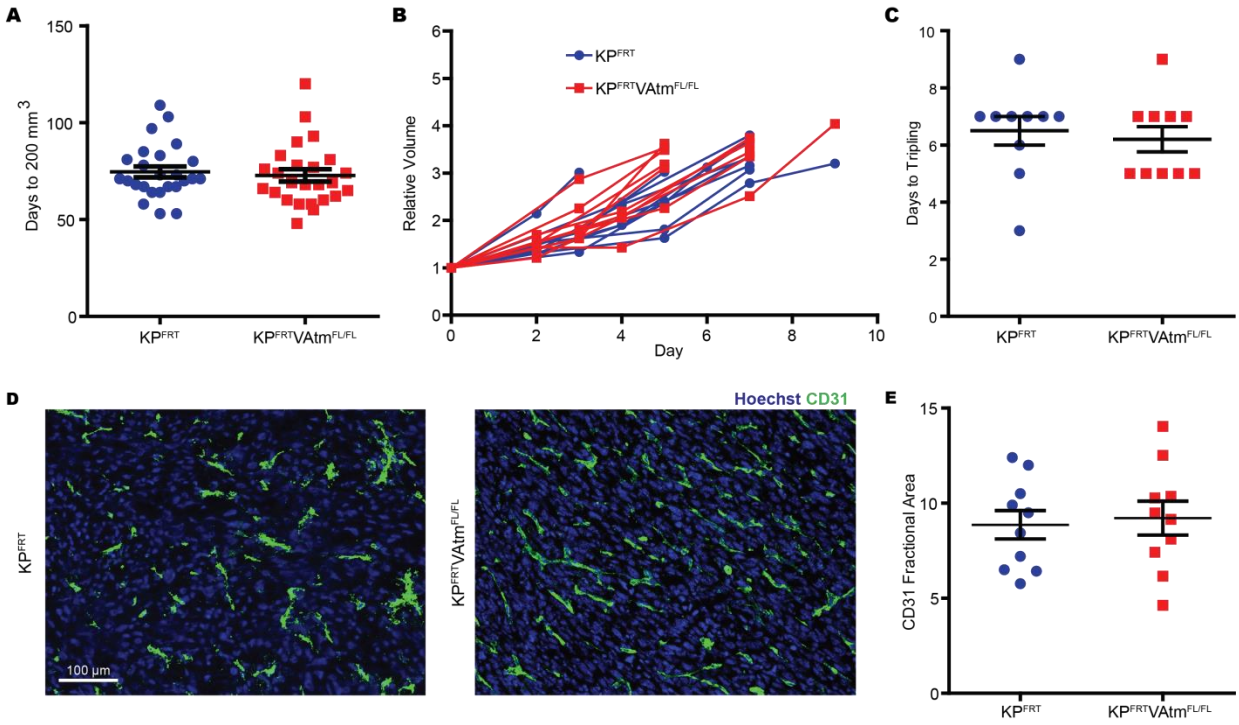
SUPPLEMENTARY MATERIAL



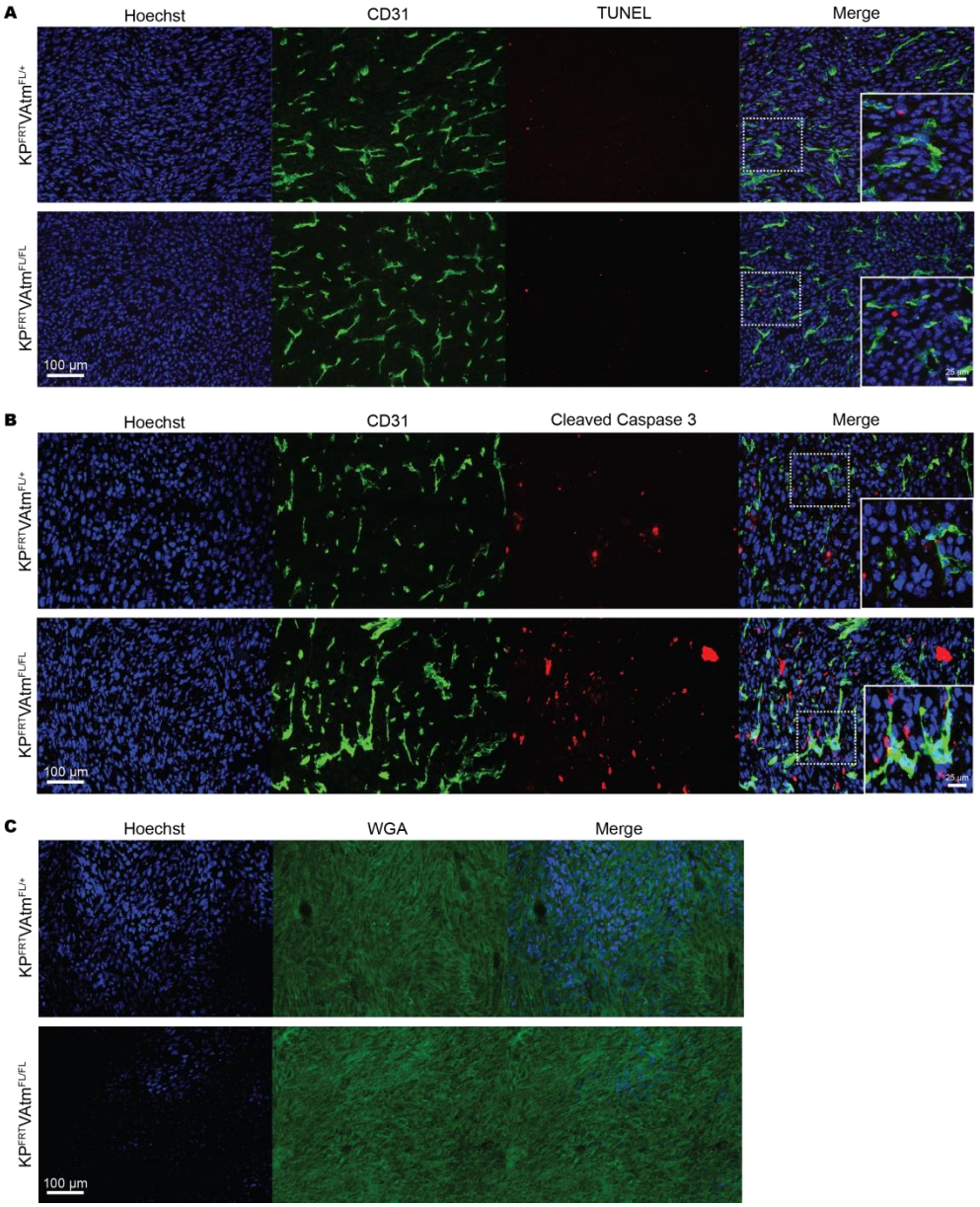
Supplementary Figure 1. Atm is activated in endothelial cells of primary sarcomas following radiation. (**A**) Representative fluoroscopy image of the radiation field for a sarcoma treated with ionizing radiation using a 40 mm x 40 mm square collimator. (**B**-**E**) Immunofluorescence and quantification of CD31 and pAtm double positive cells (**B** and **C**) or CD31 and pKap1 double positive cells (**D** and **E**) in unirradiated primary soft tissue sarcomas from KP^{loxP} mice (No IR) and sarcomas 4 hours after irradiation with 20 Gy (20 Gy) (n=3 mice per group). High magnification images are shown in the insets. All data are represented as mean ± SEM.



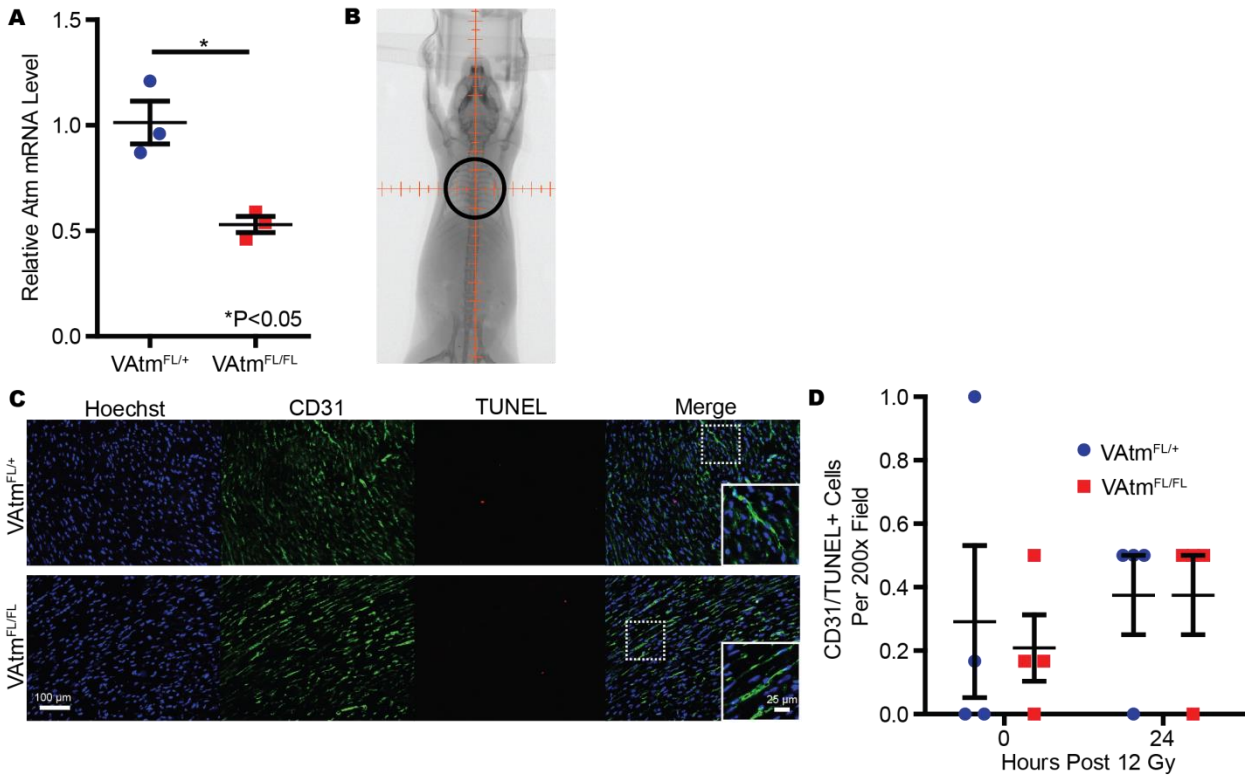
Supplementary Figure 2. Dual recombinase technology enables Cre to recombine alleles in the endothelial cells of primary sarcomas. (**A**-**C**) Representative fluorescence images of soft tissue sarcomas initiated with adeno-FlpO in *KP^{FRT};VE-Cadherin-Cre; mTmG* mice in the absence of radiation (No IR) and two weeks following irradiation with 20 Gy (20 Gy) stained for VE-Cadherin (**A**), CD34 (**B**), and CD11b (**C**). Images represent three mice per group. (**D**) Representative flow cytometry plots and gates used for isolation of tumor and heart endothelial cells by FACS and flow cytometry analysis of endothelial cell cycle progression. (**E**) Immunofluorescence and (**F**) quantification of CD31 and pKap1 double positive cells in sarcomas from *KP^{FRT}VAtm$^{FL/+}$* and *KP^{FRT}VAtm$^{FL/FL}$* mice 4 hours following irradiation with 20 Gy (n=4 mice per group). An endothelial cell positive for pKap1 in the sarcoma from a *KP^{FRT}VAtm$^{FL/+}$* mouse (arrows) and an endothelial cell negative for pKap1 in the sarcoma from a *KP^{FRT}VAtm$^{FL/FL}$* mouse (arrowheads) are indicated and shown at higher magnification in the insets. Data are represented as mean ± SEM.



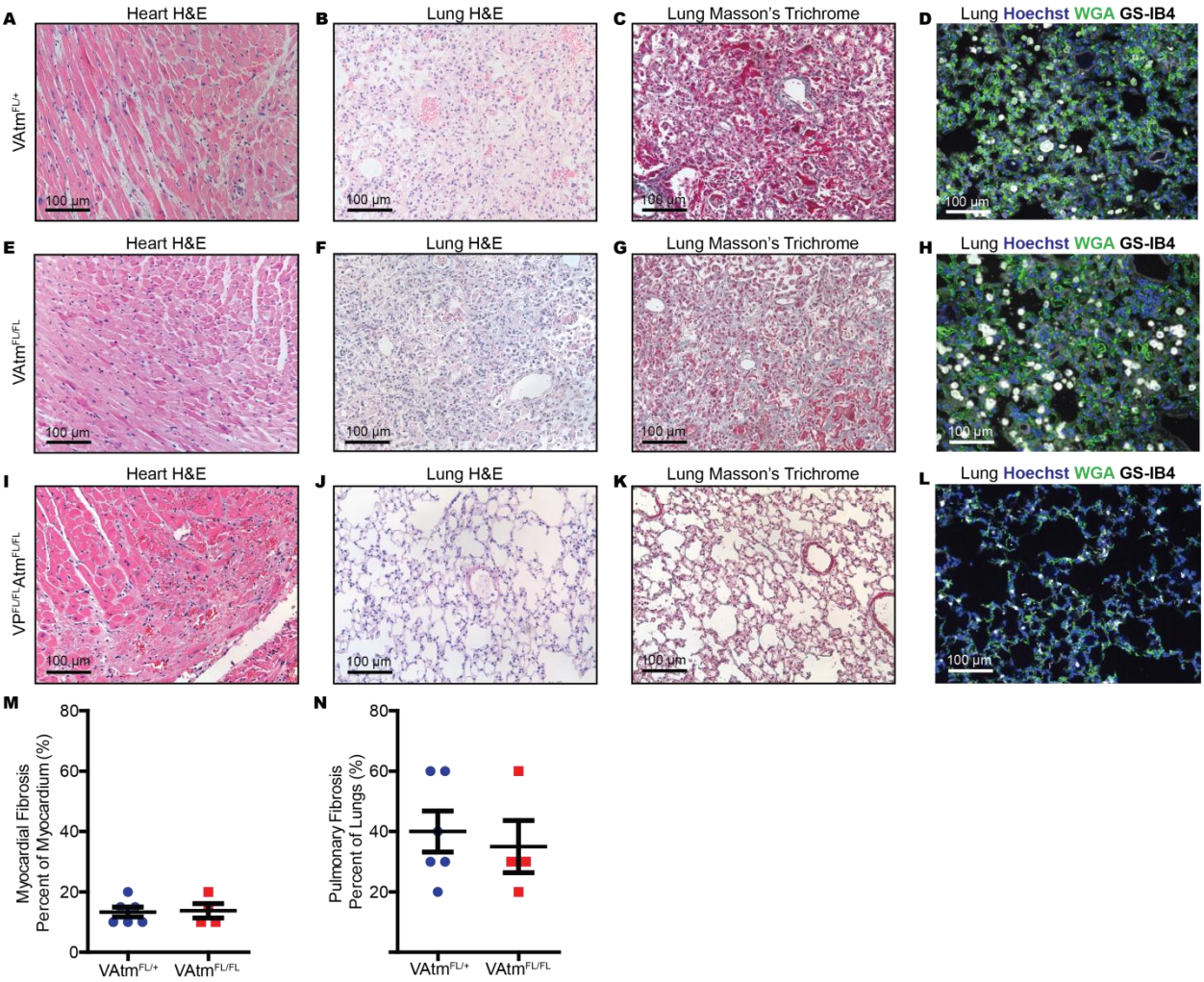
Supplementary Figure 3. Deletion of *Atm* in endothelial cells does not affect primary sarcomagenesis. (**A**) Time for sarcomas in KP^{FRT} and $KP^{FRT}VAtm^{FL/FL}$ mice to reach 200 mm^3 following intramuscular injection of adeno-FlpO (n=25 mice per group). (**B**) Tumor growth curves and (**C**) time to tripling for unirradiated sarcomas in KP^{FRT} and $KP^{FRT}VAtm^{FL/FL}$ mice (n=10 mice per group). (**D**) Immunofluorescence images and (**E**) quantification of endothelial cell marker CD31 in sarcomas from KP^{FRT} and $KP^{FRT}VAtm^{FL/FL}$ mice (n=10 mice per group). $KP^{FRT}VAtm^{FL/FL}$ mice are reproduced from Figure 2 for comparison with an independent cohort of KP^{FRT} mice. All data are represented as mean ± SEM.



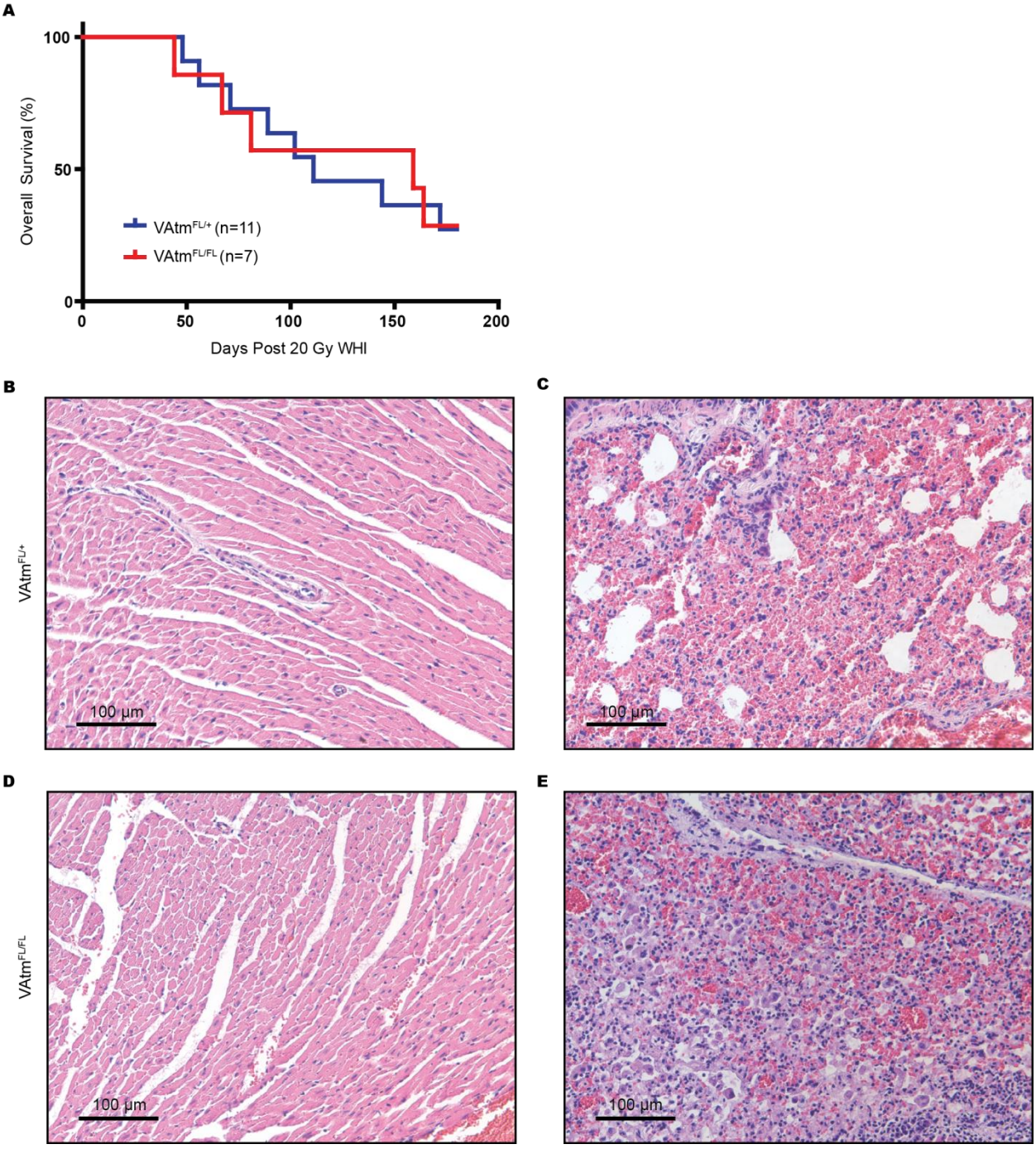
Supplementary Figure 4. Representative immunofluorescence images from *KP^{FRT}VAtm$^{FL/+}$* and *KP^{FRT}VAtm$^{FL/FL}$* mice. (**A**) Representative immunofluorescence for CD31 and TUNEL in sarcomas from *KP^{FRT}VAtm$^{FL/+}$* and *KP^{FRT}VAtm$^{FL/FL}$* mice 4 hours following irradiation with 20 Gy. High magnification images are shown in the insets. Quantification is shown in **Figure 3B**. (**B**) Representative immunofluorescence for CD31 and cleaved caspase 3 in sarcomas from *KP^{FRT}VAtm$^{FL/+}$* and *KP^{FRT}VAtm$^{FL/FL}$* mice 24 hours following irradiation with 20 Gy. High magnification images are shown in the insets. Quantification is shown in **Figure 3D**. (**C**) Representative immunofluorescence of Hoechst 33342 perfusion in sarcomas from *KP^{FRT}VAtm$^{FL/+}$* and *KP^{FRT}VAtm$^{FL/FL}$* mice 24 hours following irradiation with 20 Gy. Cell membranes were counterstained with wheat germ agglutinin (WGA). Quantification is shown in **Figure 3G**.



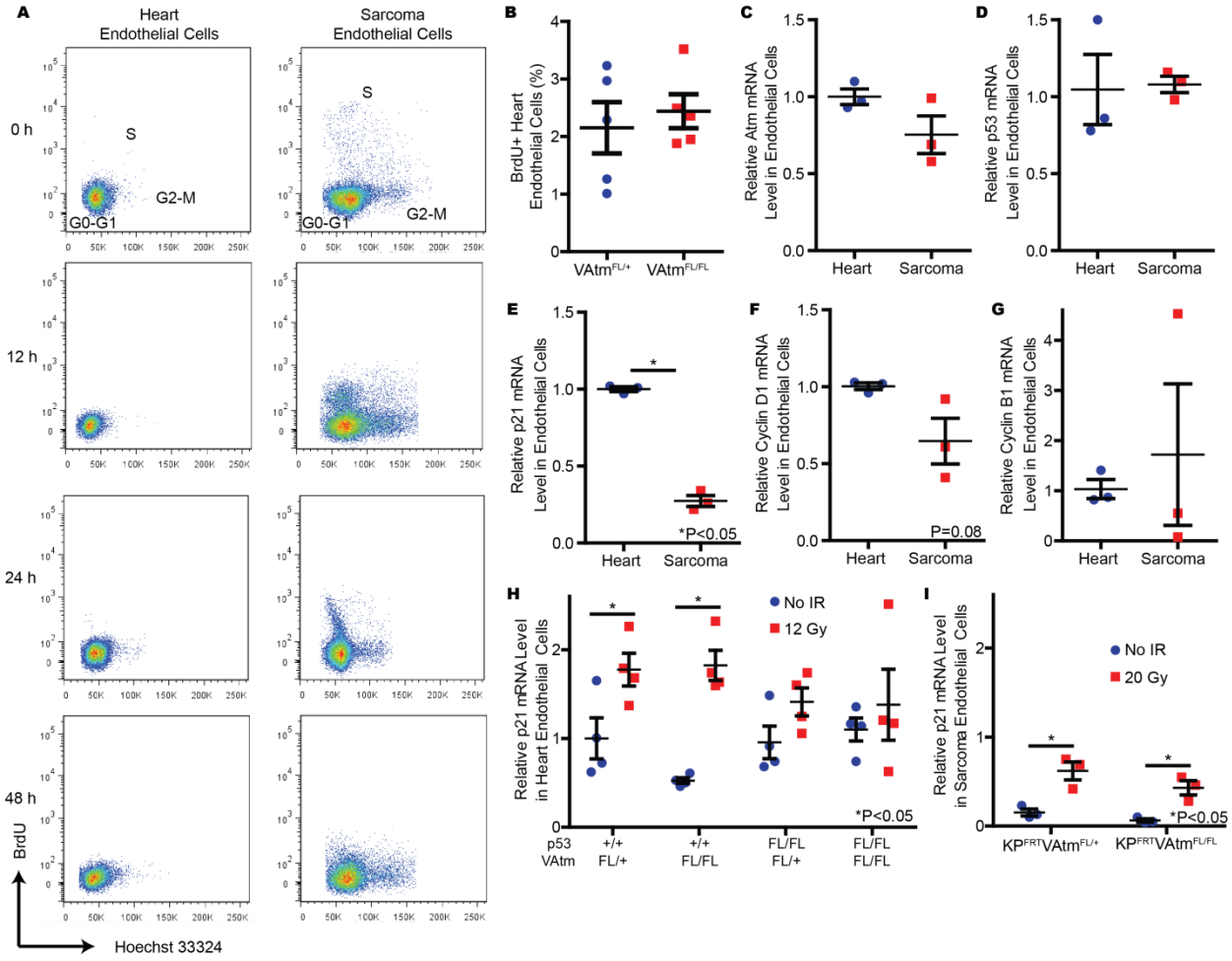
Supplementary Figure 5. Deletion of *Atm* does not radiosensitize heart endothelial cells. (**A**) Expression of *Atm* mRNA measured by qRT-PCR in FACS isolated cardiac endothelial cells ($CD45^-$ $CD34^+$ $CD31^+$) from mice with the indicated genotype (n=3 mice per group). Data are represented as mean ± SEM. (**B**) Representative fluoroscopy image of the radiation field for a mouse treated with whole-heart irradiation using a 15 mm circular collimator. (**C**) Immunofluorescence for CD31 and TUNEL in hearts from $VAtm^{FL/+}$ and $VAtm^{FL/FL}$ mice 24 hours following irradiation with 12 Gy. High magnification images are shown in the insets. (**D**) Quantification of CD31 and TUNEL double positive cells in hearts from $VAtm^{FL/+}$ and $VAtm^{FL/FL}$ mice 24 hours following irradiation with 12 Gy and in unirradiated controls (n=5 mice per group). All data are represented as mean ± SEM.



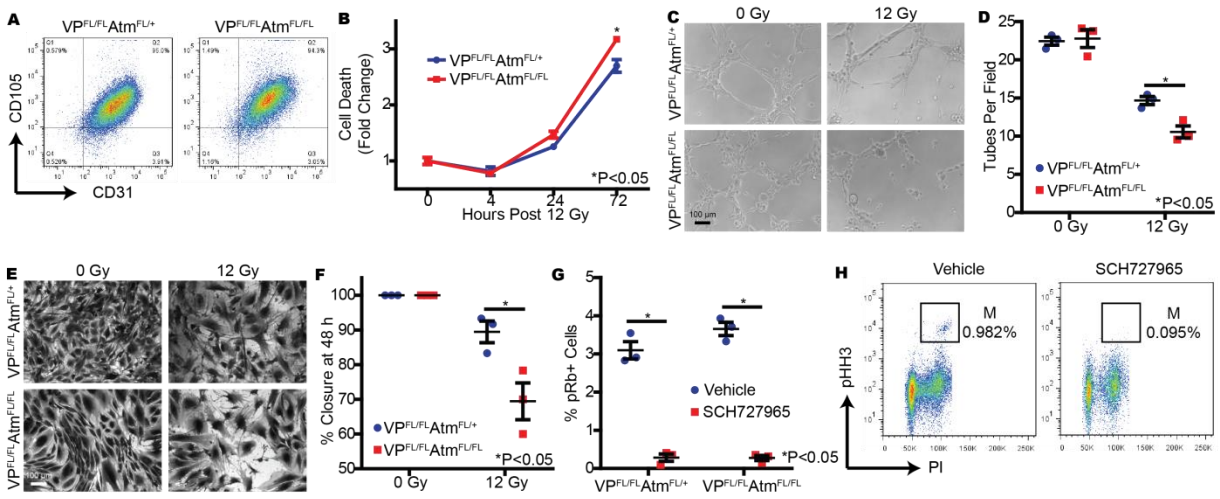
Supplementary Figure 6. *VAtm*$^{FL/+}$ and *VAtm*$^{FL/FL}$ mice develop lung fibrosis following whole-heart irradiation with 12 Gy. (**A**-**D**) Representative sections from *VAtm*$^{FL/+}$ mice after whole-heart irradiation with 12 Gy. Hematoxylin and eosin (H&E) staining of the myocardium (**A**) and lungs (**B**). Lungs stained with Masson's trichrome (**C**) or immunofluorescence for wheat germ agglutinin (WGA) and *Griffonia simplicifolia*-IB$_4$ lectin (GS-IB$_4$) (**D**). (**E**-**H**) Representative sections from *VAtm*$^{FL/FL}$ mice after whole-heart irradiation with 12 Gy. H&E staining of the myocardium (**E**) and lungs (**F**). Lungs stained with Masson's trichrome (**G**) or immunofluorescence for WGA and GS-IB$_4$ (**H**). (**I**-**L**) Representative sections from *VP*$^{FL/FL}$*Atm*$^{FL/FL}$ mice after whole-heart irradiation with 12 Gy. H&E staining of the myocardium (**I**) and lungs (**J**). Lungs stained with Masson's trichrome (**K**) or immunofluorescence for WGA and GS-IB$_4$ (**L**). (**M** and **N**) Quantification of myocardial fibrosis (**M**) and pulmonary fibrosis (**N**) in *VAtm*$^{FL/+}$ and *VAtm*$^{FL/FL}$ mice at the time of death following whole-heart irradiation with 12 Gy (n=6 and 4 mice respectively). All data are represented as mean ± SEM. Panel (**E**) is reproduced here from **Figure 4D** for comparison.



Supplementary Figure 7. *VAtm*$^{FL/+}$ and *VAtm*$^{FL/FL}$ mice develop pulmonary hemorrhage and inflammation following whole-heart irradiation with 20 Gy. (**A**) Kaplan-Meier plots of overall survival for *VAtm*$^{FL/+}$ and *VAtm*$^{FL/FL}$ mice following 20 Gy whole-heart irradiation. (**B** and **C**) Hematoxylin and eosin (H&E) stained sections of the myocardium (**B**) and lungs (**C**) from a *VAtm*$^{FL/+}$ mouse 89 days after whole-heart irradiation with 20 Gy. (**D** and **E**) H&E stained sections of the myocardium (**D**) and lungs (**E**) from a *VAtm*$^{FL/FL}$ mouse 67 days after whole-heart irradiation with 20 Gy.



Supplementary Figure 8. Sarcoma endothelial cells proliferate more rapidly than heart endothelial cells. (**A**) Representative flow cytometry analysis of BrdU incorporation and DNA content (Hoechst 33324) of heart and sarcoma endothelial cells ($CD45^-$ $CD34^+$ $CD31^+$) at the indicated time points following BrdU injection. (**B**) Flow cytometry quantification of BrdU incorporation into heart endothelial cells from *VAtm*$^{FL/+}$ and *VAtm*$^{FL/FL}$ mice following 7 daily injections of BrdU (n=5 mice per group). (**C**-**G**) Expression of *Atm* (**C**), *p53* (**D**), *p21* (**E**), *Cyclin D1* (**F**), and *Cyclin B1* (**G**) mRNA measured by qRT-PCR in FACS isolated heart and primary sarcoma endothelial cells ($CD45^-$ $CD34^+$ $CD31^+$) (n=3 mice per group). (**H**) qRT-PCR measurement of *p21* mRNA expression in FACS isolated cardiac endothelial cells from *VAtm*$^{FL/+}$, *VAtm*$^{FL/FL}$, *VP*$^{FL/FL}$*Atm*$^{FL/+}$, and *VP*$^{FL/FL}$*Atm*$^{FL/FL}$ mice 4 hours after whole-heart irradiation with 12 Gy (12 Gy) or in unirradiated controls (No IR) (n=4 mice per group). (**I**) qRT-PCR measurement of *p21* mRNA expression in FACS isolated sarcoma endothelial cells from *KP*^{FRT}*VAtm*$^{FL/+}$ and *KP*^{FRT}*VAtm*$^{FL/FL}$ mice 4 hours after sarcoma irradiation with 20 Gy (20 Gy) or in unirradiated controls (No IR) (n=3 mice per group). Values normalized to heart endothelial cells from unirradiated *VAtm*$^{FL/+}$ mice. All data are represented as mean ± SEM.



Supplementary Figure 9. Deletion of *Atm* radiosensitizes cardiac endothelial cells in vitro. (**A**) Flow cytometry analysis of CD31 and CD105 expression to assess the purity of primary cardiac endothelial cells isolated from $VP^{FL/FL}Atm^{FL/+}$ and $VP^{FL/FL}Atm^{FL/FL}$ mice using antibody-coated magnetic beads and grown in vitro. (**B**) Relative cell death of primary cardiac endothelial cells from $VP^{FL/FL}Atm^{FL/+}$ and $VP^{FL/FL}Atm^{FL/FL}$ mice assessed by annexin V staining at various time points after irradiation with 12 Gy (n=3 independent experiments). (**C** and **D**) Representative images (**C**) and quantification (**D**) of tube formation for primary cardiac endothelial cells from $VP^{FL/FL}Atm^{FL/+}$ and $VP^{FL/FL}Atm^{FL/FL}$ mice following no irradiation (0 Gy) or irradiation with 12 Gy (12 Gy) (n=3 independent experiments). (**E** and **F**) Representative images (**E**) and quantification (**F**) of cell migration for primary cardiac endothelial cells from $VP^{FL/FL}Atm^{FL/+}$ and $VP^{FL/FL}Atm^{FL/FL}$ mice following no irradiation (0 Gy) or irradiation with 12 Gy (12 Gy) (n=3 independent experiments). (**G**) Flow cytometry quantification of Rb phosphorylation in primary cardiac endothelial cells from $VP^{FL/FL}Atm^{FL/+}$ and $VP^{FL/FL}Atm^{FL/FL}$ mice treated with DMSO (Vehicle) or 500 nM SCH727965 for 24 hours. (**H**) Flow cytometry analysis of mitosis marker phosphorylated histone H3 (pHH3) for primary cardiac endothelial cells treated with DMSO (Vehicle) or 500 nM SCH727965, a cyclin-dependent kinase inhibitor, for 24 hours. All data are represented as mean ± SEM.

Supplementary Table 1. Echocardiographic measurements in *VAtm*$^{FL/+}$ and *VAtm*$^{FL/FL}$ mice.

Genotype	Time after 12 Gy WHI	LVDd (mm)	LVDs (mm)	IVS (mm)	PW (mm)	AET (ms)	HR (BPM)	FS (%)	LVm (mg)	mVcfc (circ/s)
VAtm$^{FL/+}$ (n=6)	No IR	2.93±0.16	1.33±0.16	0.95±0.07	0.96±0.06	40.07±1.14	610.7±16.88	55±3.9	80.83±6.06	432.71±33.56
	6 Weeks	3.16±0.06	1.39±0.07	1±0.06	1.14±0.14	41.41±2.1	585.79±45.11	55.81±2.17	110.62±14.79	438.8±23.02
	1 Year	3.45±0.14	1.74±0.18	1.06±0.06	1.1±0.06	39.46±2.21	632.42±42.68	50.29±3.3	130.53±7.19	400.45±31.43
VAtm$^{FL/FL}$ (n=6)	No IR	2.96±0.12	1.24±0.12	0.92±0.02	1.15±0.07	41.41±1.57	583.45±35.36	58.43±2.48	94.37±7.58	456.17±19.48
	6 Weeks	3.16±0.07	1.37±0.06	0.98±0.02	0.98±0.1	40.86±0.95	596.73±21.28	56.7±1.41	97.29±2.7	442.41±17.87
	1 Year	3.87±0.23	1.89±0.2	0.98±0.07	1.07±0.07	**55.27±2.09***	**467.54±16.31***	51.64±2.61	127.33±12.41	337.62±21.56

Left ventricular end diastolic dimension (LVDd), left ventricular end systolic dimension (LVDs), intraventricular septum (IVS), left ventricular posterior wall (PW), aortic ejection time (AET), fractional shortening (FS), heart rate (HR), left ventricular mass (LVm), heart rate corrected mean velocity of circumferential fiber shortening (mVcfc). Data are represented as mean ± SEM. *P<0.05 by two-way ANOVA with Bonferroni correction to compare genotypes at each time point.