Appendix A. Supplementary data for:

Mechanism of irradiation-induced mammary cancer metastasis: A role for SAP-dependent Mkl1 signaling

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Content of the supplementary data:

Table S1. Differential probesets in 4T1-derived tumors grown in preirradiated *vs.* nonirradiated stroma. **(Table S1 is provided as a separate .xlsx file.)**

Figure S1. Irradiation induces collagen accumulation in 4T1 mammary tumors.

Figure S2. Irradiation induces an elevated nuclear localization of Mkl1 protein in 4T1-FL- and 4T1-ΔSAP-derived tumors.

Figure S3. Irradiation induces SAP-dependent tenascin-C, Fgfbp1 and Car12 protein expression.

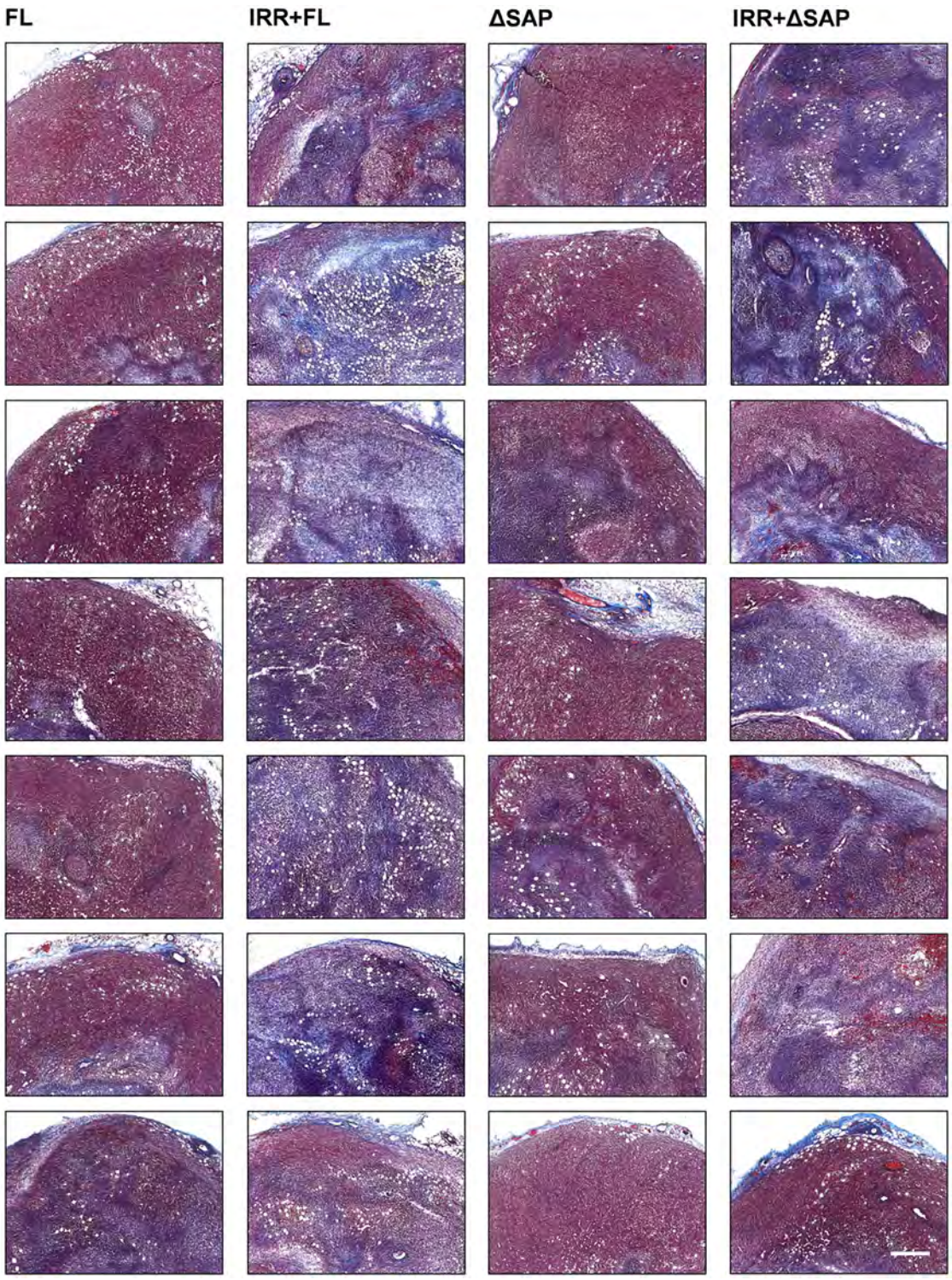


Figure S1. Irradiation induces collagen accumulation in 4T1 mammary tumors. Masson's trichrome stain of cryosections for visualization of collagens (blue) in 4T1-FL- and 4T1-ΔSAP-derived tumors grown in either nonirradiated or 20 Gy preirradiated stroma, designated as FL, IRR+FL, ΔSAP and IRR+ΔSAP tumors. Images for all 7 mice per group are shown. Scale bar, 500 μm.

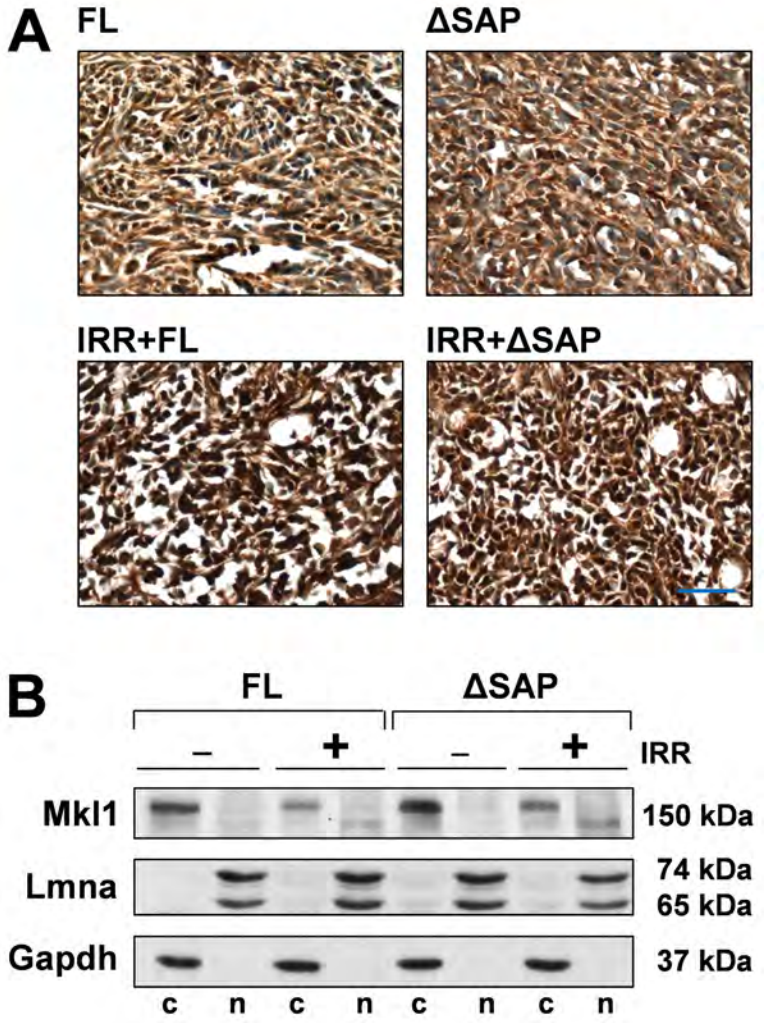
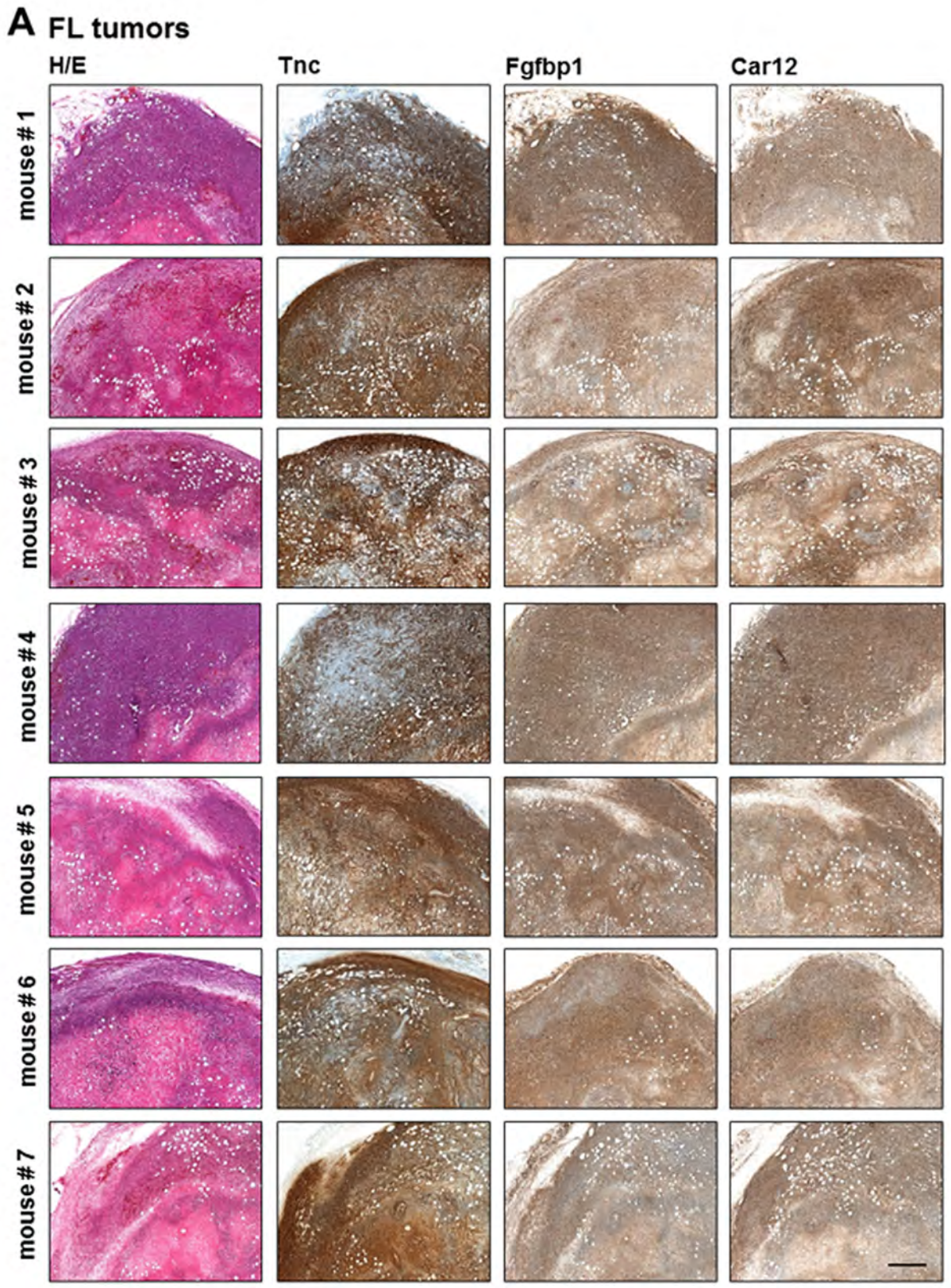
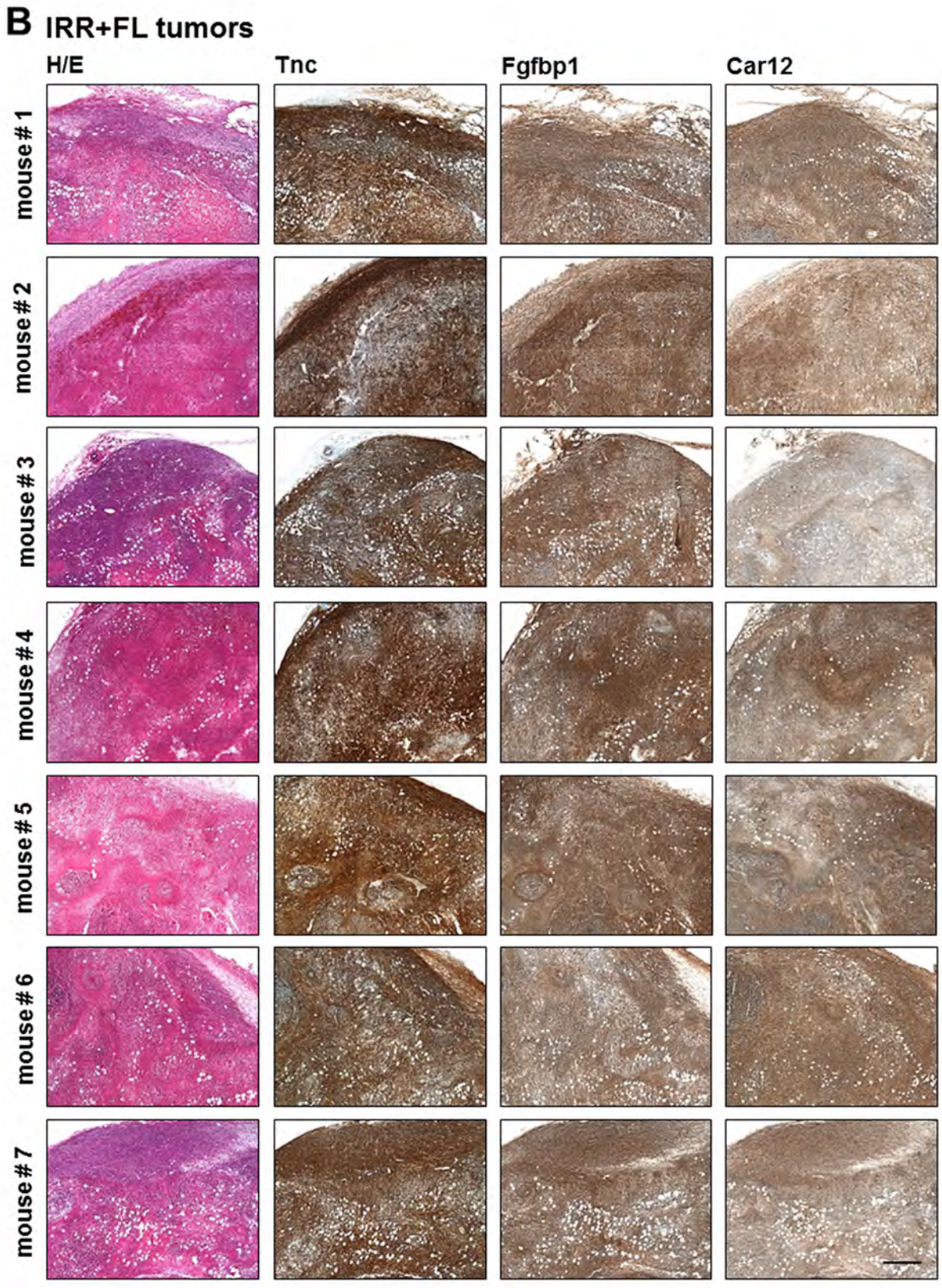
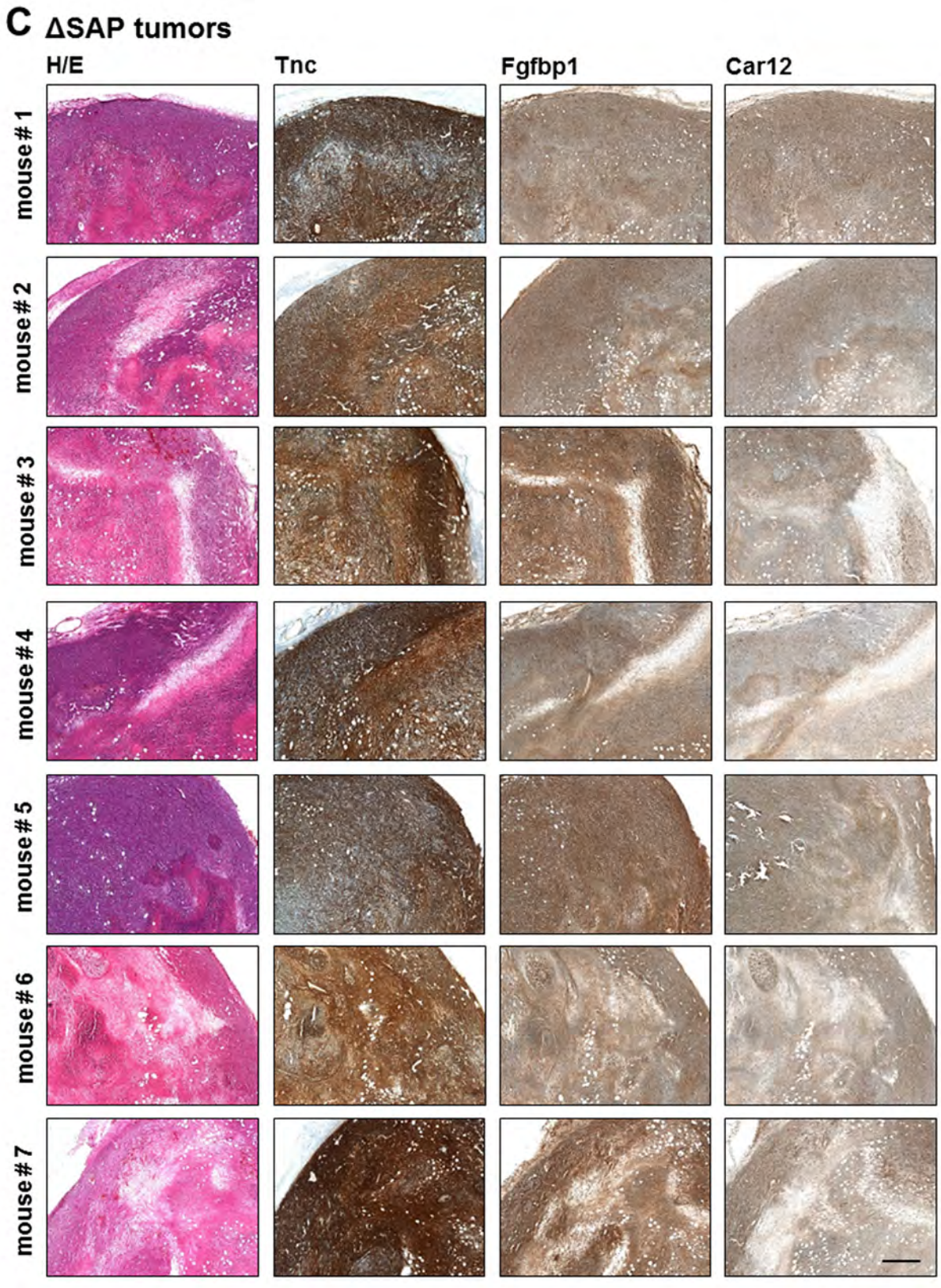
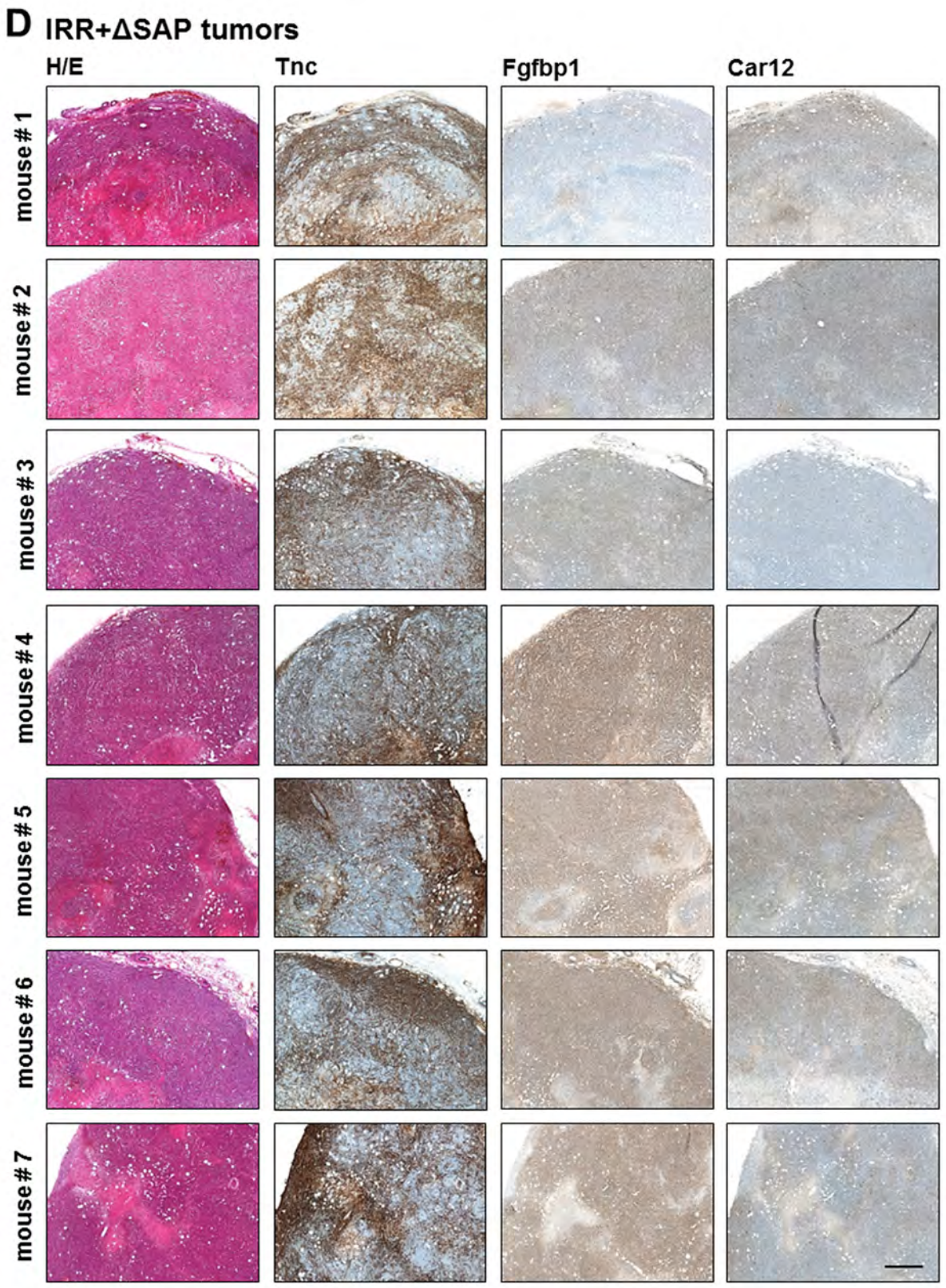


Figure S2. Irradiation induces an elevated nuclear localization of Mkl1 protein in 4T1-FL- and 4T1-ΔSAP-derived tumors. (A) Immunohistochemical detection of Mkl1 in cryosections of 4T1-FL- and 4T1-ΔSAP-derived tumors grown in either nonirradiated or 20 Gy preirradiated stroma, designated as FL, IRR+FL, ΔSAP and IRR+ΔSAP tumors. Scale bar, 50 μm. **(B)** Immunoblot detection of Mkl1 in cytoplasmic (c) and nuclear (n) extracts of the primary tumors described in (A). (-) and (+) indicate tumors grown in nonirradiated and preirradiated stroma, respectively. Successful separation of the nuclear from the cytoplasmic components and equal loading were verified by the detection of lamin A/C (Lmna) and Gapdh as a nuclear and cytoplasmic marker, respectively.


A FL tumors
H/E
Tnc
Fgfbp1
Car12
mouse # 1
mouse # 2
mouse # 3
mouse # 4
mouse # 5
mouse # 6
mouse # 7


B IRR+FL tumors
H/E
Tnc
Fgfbp1
Car12
mouse # 1
mouse # 2
mouse # 3
mouse # 4
mouse # 5
mouse # 6
mouse # 7


C ΔSAP tumors
H/E
Tnc
Fgfbp1
Car12
mouse # 1
mouse # 2
mouse # 3
mouse # 4
mouse # 5
mouse # 6
mouse # 7


D IRR+ΔSAP tumors
H/E
Tnc
Fgfbp1
Car12
mouse # 1
mouse # 2
mouse # 3
mouse # 4
mouse # 5
mouse # 6
mouse # 7

E

FL tumors

Mouse #	Tnc	Fgfbp1	Car12
1	++	+++	++
2	+++	++	+++
3	++	++	++
4	++	+++	+++
5	+++	+++	++
6	++	++	++
7	+++	++	++

IRR+FL tumors

Mouse #	Tnc	Fgfbp1	Car12
1	+++	+++	+++
2	+++	+++	+++
3	+++	+++	+
4	+++	+++	+++
5	+++	+++	+++
6	+++	++	+++
7	+++	+++	++

ΔSAP tumors

Mouse #	Tnc	Fgfbp1	Car12
1	+++	++	+++
2	+++	+++	++
3	+++	+++	++
4	+++	+++	++
5	++	+++	+++
6	+++	++	++
7	+++	+++	++

IRR+ΔSAP tumors

Mouse #	Tnc	Fgfbp1	Car12
1	+	-	+
2	+	+	+
3	+	+	-
4	+	++	+
5	+	+	+
6	+	+	+
7	+	+	+

Figure S3. Irradiation induces SAP-dependent tenascin-C, Fgfbp1 and Car12 protein expression. H&E stain and immunohistochemical detection of tenascin-C, Fgfbp1 and Car12 in sequential sections of 4T1-FL- and 4T1-ΔSAP-derived tumors grown in either nonirradiated or 20 Gy preirradiated stroma, designated as FL **(A)**, IRR+FL **(B)**, ΔSAP **(C)** and IRR+ΔSAP **(D)** tumors. Images for all 7 mice per group are shown. Scale bar, 500 μm. **(E)** Semiquantitative summary of the data shown in (A, B, C and D). Tnc, Fgfbp1 and Car12 protein expression is classified in four categories: +++, high; ++, moderate; +, low; -, absent.