
Supplementary information

Synthetic super-enhancers enable precision viral immunotherapy

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Supplementary Information Guide

Title: **Synthetic super-enhancers enable precision viral immunotherapy**

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Please find below the following Supplementary Information for Koeber et al.

1. **Supplementary Table S1** | DNA sequences of the top 32 enhancer fragment hits (Related to Fig. 1)
2. **Supplementary Table S2** | Antibodies used in this study for immune profiling.
3. **Flow cytometry gating strategy for single cell QC analysis and sample processing** (Related to Fig. 3h and Extended Data Fig. 5 and 6a). E28 is shown.
4. **Unprocessed EMSA gel images** (Related to Fig. 2)
5. **Image analysis, segmentation and quantitative analysis** (Related to Fig. 4 and Extended Data Fig. 9)
6. **Harmony software image analysis pipeline for human tissue (Related to Fig. 4 and Extended Data Fig. 9)**
7. **Western immunoblots uncropped gels** (Related to Extended Data Fig. 2g and Extended Data Fig. 10d)
8. **Typical example of flow gating** for all tumours assessed for GFP and mCherry costaining (GFP tumours cells and mCherry transgene, respectively). Related to Extended Data Fig. 13j

ID1101	chr11	82553161	82553319
GTGAGTGTGACTCACATGCTGGCATCAGAGCCCCTCTTAGAGCTTAGAACACTGGGCCTCTGTCAGGCCAAAGAAAAGGAGTCCCTATGAA TAGCCATCTCCACAAATAGCAGTTACTGTCACCTTCTTAAAGACACAAGCATGGCTGGAGACCGGCACTG			
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ID3836	chr7	33027949	33028108
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ID2082	chrX	48968923	48969082
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>ID4035	chr19	2695387	2695546
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ID1667	chr7	43347035	43347194
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ID2816	chr19	8134074	8134233
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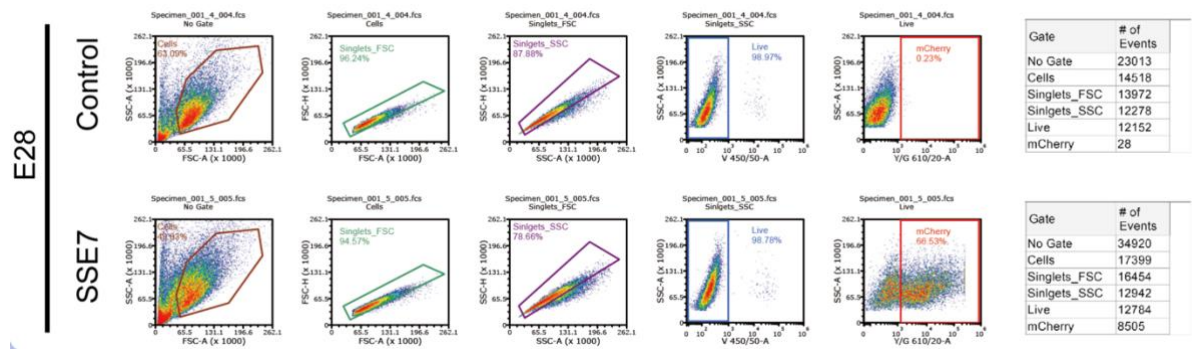
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ID4421	chr16	62699342	62699446
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1. Supplementary Table S1 | Sequences of the top 32 fragments.

Mouse Flow cytometry antibodies	Supplier	Cat number.	Antibody dilution
NK1.1	BD Biosciences	PK136/Cat #564144	1/300
CD4	BD Biosciences	GK1.5/ Cat# 564667	1/300
CD45	BD Biosciences	30-F11/ Cat# 565710	1/200
CD11B	BD Biosciences	M1/70/ Cat# 565080	1/200
CD3	BD Biosciences	17A2/Cat# 564380	1/300
CD8a	BD Biosciences	53-67/ Cat# 612898	1/300
FOXP3	eBioscience	FJK-16S/ Cat#48577382	1/100
CD62L	BD Biosciences	MEL-14/ Cat#563117	1/400
TIM-3	Biolegend	RMT-23/Cat#119721	1/100
CD27	Biolegend	LG.3A10/Cat# 119721	1/200
GITR	BD Biosciences	DTA-1/Cat# 563390	1/200
CD44	Biolegend	IM7/ Cat#103059	1/200
CD25	Biolegend	PC61/Cat#102017	1/200
LAG3	eBioscience	ebioC9B7W/Cat#46-2231-82	1/100
TCF7	Cell Signalling Technology	C63D9/ Cat#14456S	1/100
PD-1	BD Biosciences	J43/ Cat#562523	1/200
Ki67	eBioscience	SolA15/ Cat# 25-5698-82	1/200
TOX	Miltenyi	REA473/ Cat#130-118-335	1/100
Granzyme b	BD Biosciences	GB11/ Cat#560213	1/200
CD24	BD Biosciences	M1/69/ Cat# 564664	1/400
LY6G	BD Biosciences	IA8/Cat# 565707	1/100
CD45	BD Biosciences	30F11/Cat# 624287	1/400
CD68	Biolegend	FA-11/Cat#137017	1/200

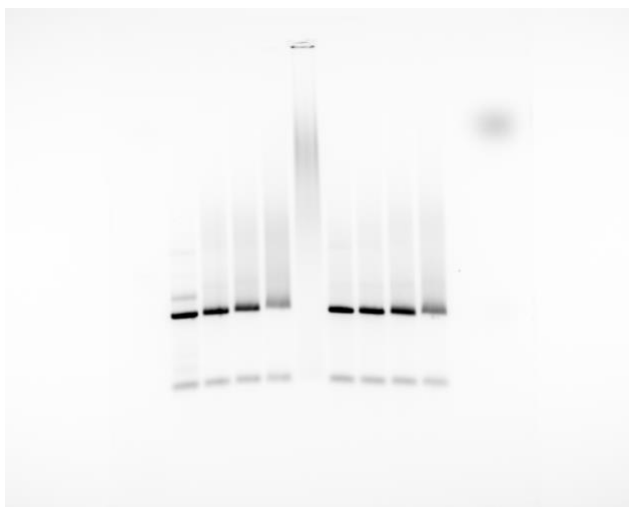
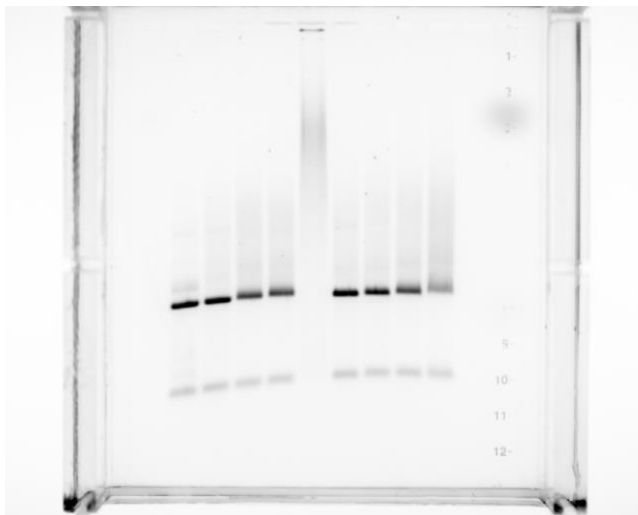
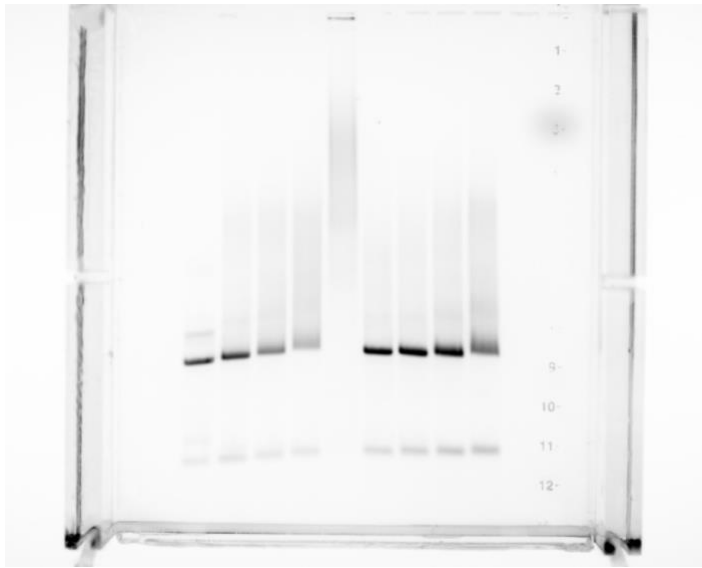
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LY6C	BD Biosciences	AL-21/Cat# 563011	1/200
CD206	Biolegend	C068C2/Cat#141723	1/400
MHCII	Biolegend	M5-114-15-2/Cat#107643	1/200
CD11c	Biolegend	N418/Cat#117336	1/100
CD49D	Biolegend	R1-2/Cat#103611	1/200
XCR1	Biolegend	ZET/Cat#148208	1/200
CD16	Biolegend	S17014E/Cat#158003	1/200
PDL1	Biolegend	10F.9G2/Cat# 124324	1/200
CD64	Biolegend	X54-5/7.1/Cat#139314	1/200
CD32b	Thermo Scientific	AT130-2/Cat# 17-0321-82	1/200
Ki67	Invitrogen	SolA15/Cat# 56-5698-82	1/200
IFN- γ	Biolegend	XMG1.2/Cat#505813	1/100
IL-2	eBiosciences	JES6-5H4/Cat#17-7021-82	1/100
TNF- α	Invitrogen	MP6-XT22/Cat#25-7321-82	1/100

2. Supplementary Table S2 | Antibodies used for immune cell characterization of tumours.

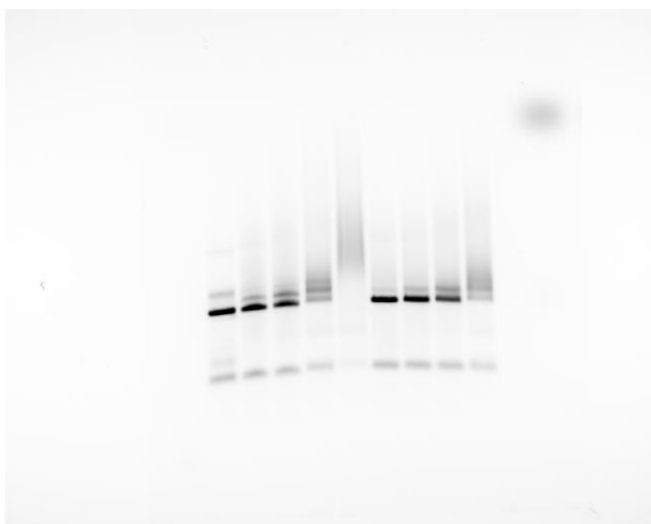
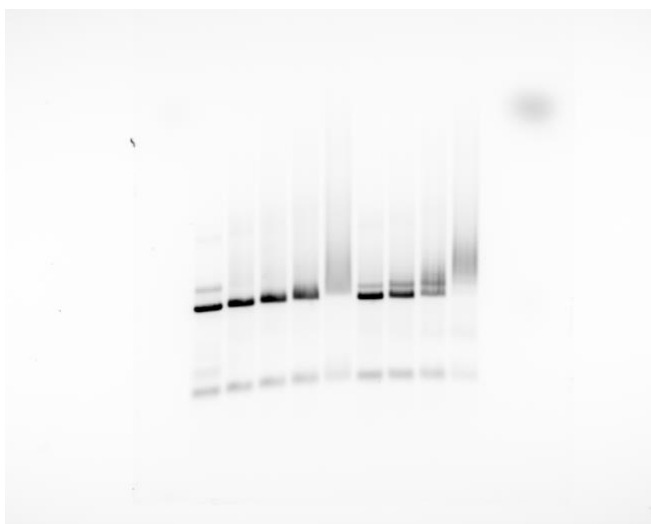
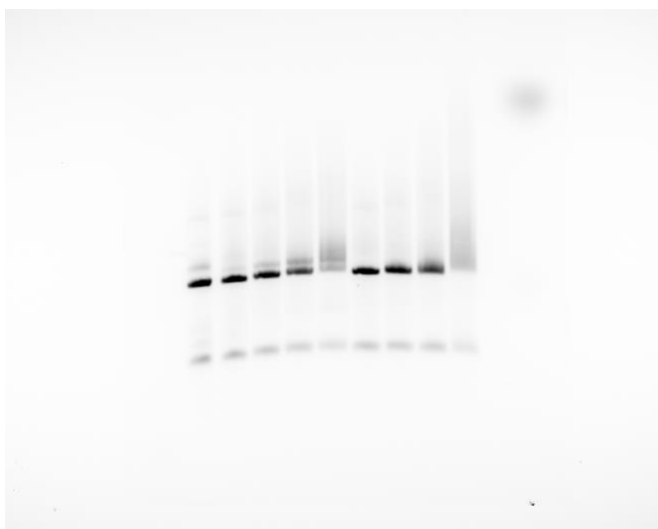


3. Flow cytometry gating strategy for single cell QC analysis and sample processing
 (Related to Fig. 3h and Extended Data Fig. 5 and 6a). E28 is shown.

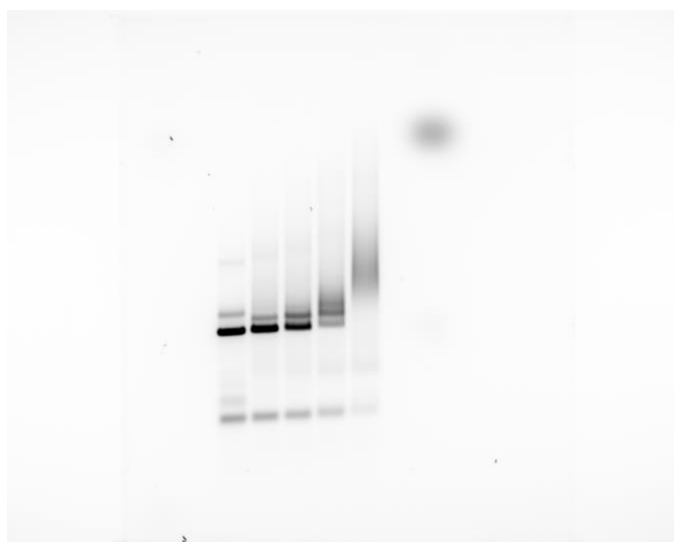
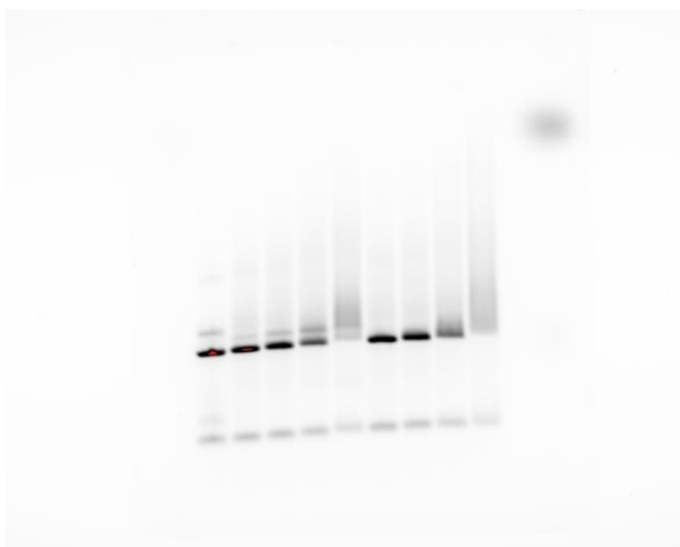
G7 lysates on FL SSE7 (Fig. 3d) n=3



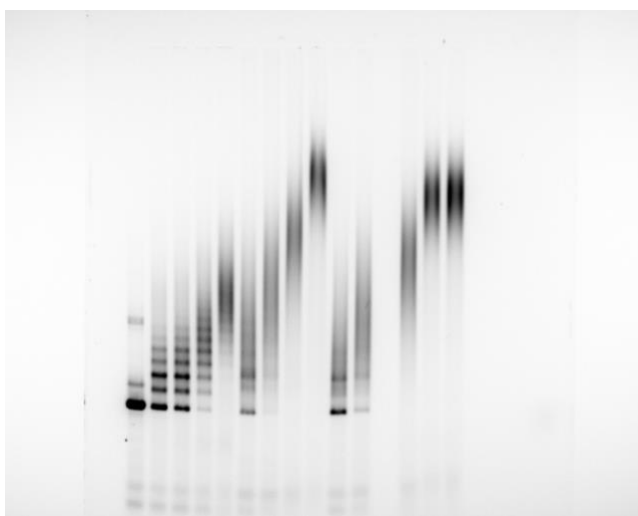
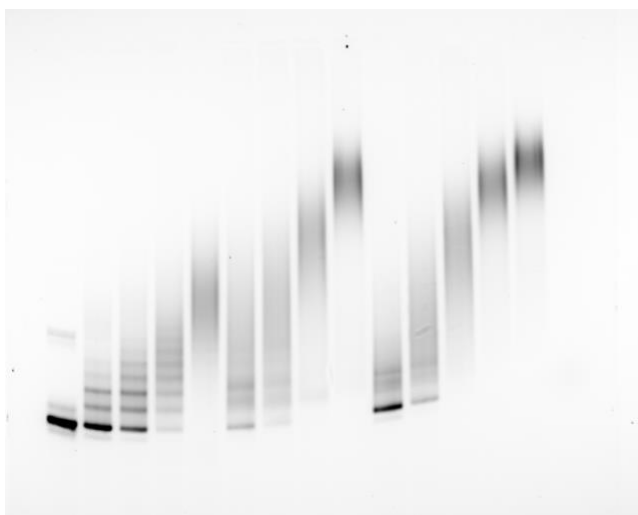
hFib OE lysates on FL SSE7 (figure 2f) n=3



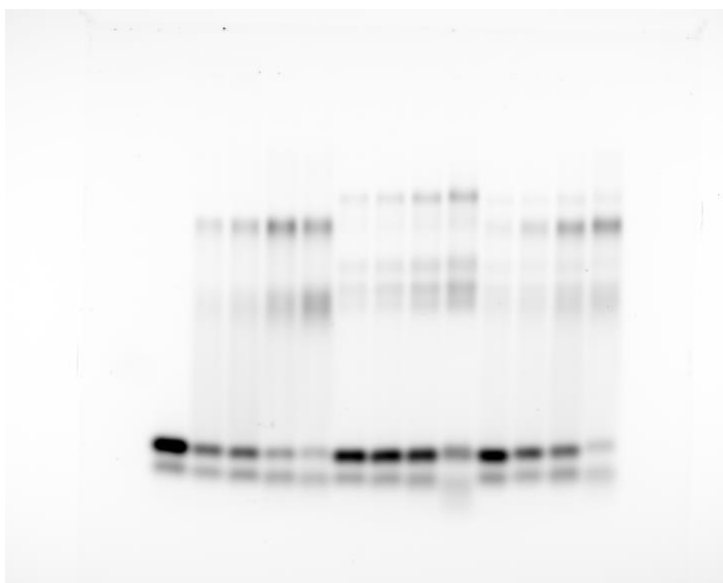
HFib lysates figure 2f



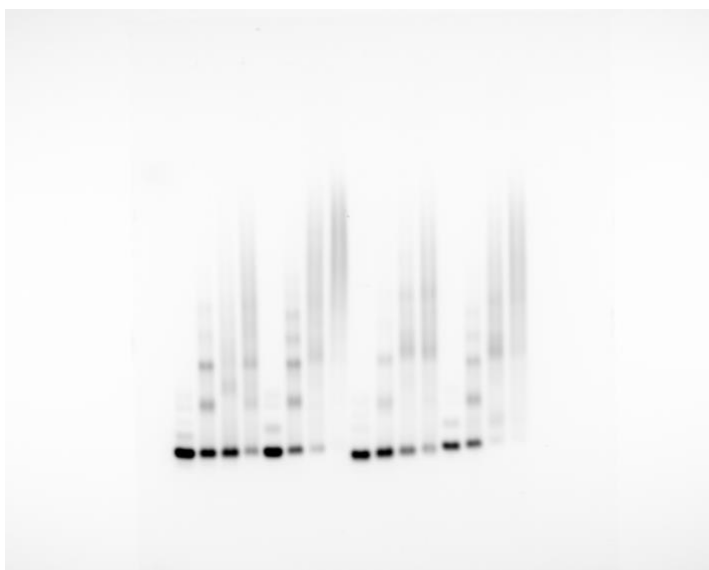
Recombinant protein on FL SSE7 figure 3f (n=3)



Recombinant proteins on FGF4 extended figure 4f (n=1)

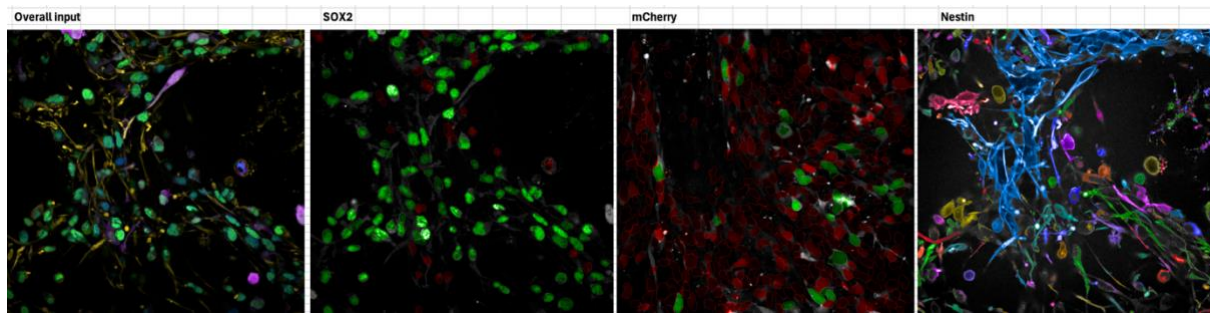


Recombinant proteins on the constituent fragments of SSE7 Figure 2g (n=1)



4. Uncropped full gel images for EMSA (Related to Fig. 3d-f)

Example of cell scoring/segmentation



5. Image analysis, segmentation and quantitation for human tissue slices | Example of cell segmentation/scoring.

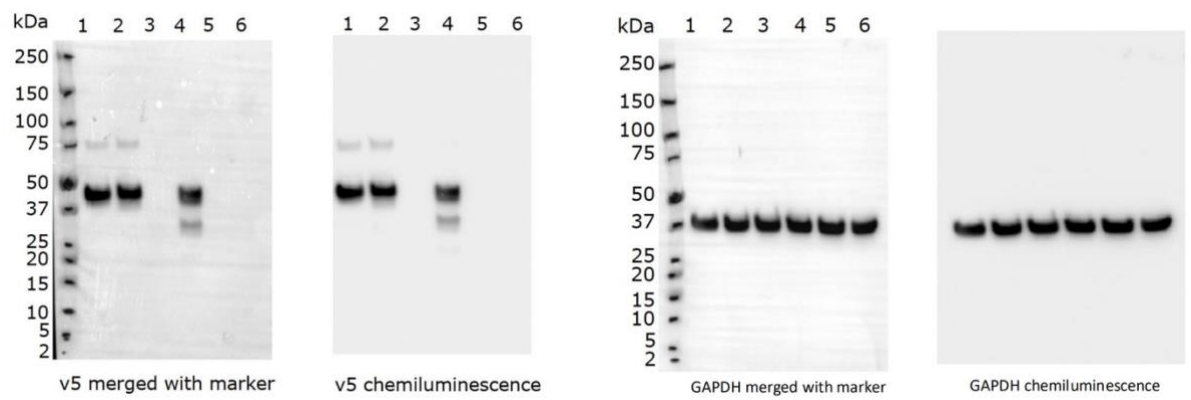
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Find Nuclei	Input	Method	Output
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Calculate Morphology Properties	Input	Method	Output
	Population : Nuclei Region : Nucleus	Method : Standard Area Roundness	Property Prefix : Nucleus
Calculate Intensity Properties	Input	Method	Output
	Channel : DAPI Population : Nuclei Region : Nucleus	Method : Standard Mean	Property Prefix : Intensity Nucleus DAPI
Select Population	Input	Method	Output
	Population : Nuclei	Method : Filter by Property Nucleus Area [μm^2] : > 26 Intensity Nucleus DAPI Mean : > 0 Boolean Operations : F1 and F2	Output Population : Nuclei Selected
Select Population (6)	Input	Method	Output
	Population : Nuclei Selected	Method : Common Filters Remove Border Objects Region : Nucleus	Output Population : whole_nuclei

Find Image Region	Input	Method	Output
	Channel : Alexa 647 ROI : None	Method : Common Threshold Threshold : <u>0.58</u> Split into Objects Area : > 0 px ² Fill Holes	Output Population : Image Region nestin Output Region : Image Region
Calculate Intensity Properties (2)	Input	Method	Output
	Channel : Alexa 488 Population : whole_nuclei Region : Nucleus	Method : Standard Mean	Property Prefix : Intensity Nucleus Alexa 488
Calculate Intensity Properties (3)	Input	Method	Output
	Channel : Alexa 555 Population : whole_nuclei Region : Nucleus	Method : Standard Mean	Property Prefix : Intensity Nucleus Alexa 555
Select Population (2)	Input	Method	Output
	Population : whole_nuclei	Method : Filter by Property Intensity Nucleus Alexa 488 Mean : > <u>1900</u>	Output Population : Sox2 +
Select Population (3)	Input	Method	Output
	Population : whole_nuclei	Method : Filter by Property Intensity Nucleus Alexa 555 Mean : > <u>1700</u>	Output Population : mcherry +
Select Population (4)	Input	Method	Output
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Calculate Intensity Properties (4)	Input	Method	Output

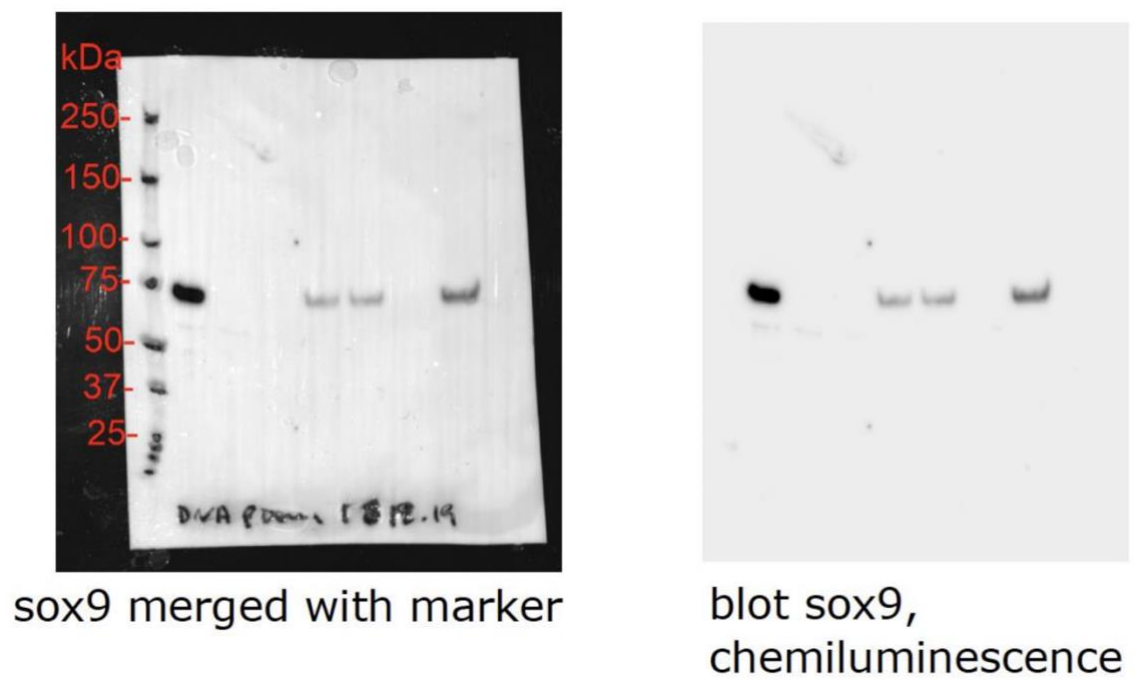
	Channel : Alexa 647 Population : nestin + Region : Nucleus	Method : Standard Mean	Property Prefix : Intensity Nucleus Alexa 647
Select Population (5)	Input Population : whole_nuclei	Method Method : Filter by Property Intensity Nucleus Alexa 488 Mean : > <u>1900</u> Intensity Nucleus Alexa 555 Mean : > <u>1700</u> Boolean Operations : F1 and F2	Output Output Population : Sox2 mcherry +
Select Population (7)	Input Population : whole_nuclei	Method Method : Filter by Property nestin + : > 0 Intensity Nucleus Alexa 555 Mean : > <u>1700</u> Boolean Operations : F1 and F2	Output Output Population : Nestin + mCherry+
Define Results	Results Method : List of Outputs Population : Image Region nestin Number of Objects Population : whole_nuclei Number of Objects Sox2 + : Mean+StdDev mcherry + : Mean+StdDev nestin + : Mean+StdDev Sox2 mcherry + : Mean+StdDev Population : Nuclei Selected whole_nuclei : Mean Population : Nuclei Number of Objects Population : Sox2 + Number of Objects Intensity Nucleus Alexa 488 Mean : Mean Population : mcherry + Number of Objects Intensity Nucleus Alexa 555 Mean : Mean Population : nestin + Number of Objects Intensity Nucleus Alexa 647 Mean : Mean Population : Sox2 mcherry + Number of Objects Population : Nestin + mCherry+ Number of Objects		
	Object Results Population : Image Region nestin : None Population : whole_nuclei : Use Selected Well Results Population : Nuclei Selected : None Population : Nuclei : None Population : Sox2 + : None Population : mcherry + : None Population : nestin + : None Population : Sox2 mcherry + : None Population : Nestin + mCherry+ : None		

6. Image analysis pipeline in Harmony

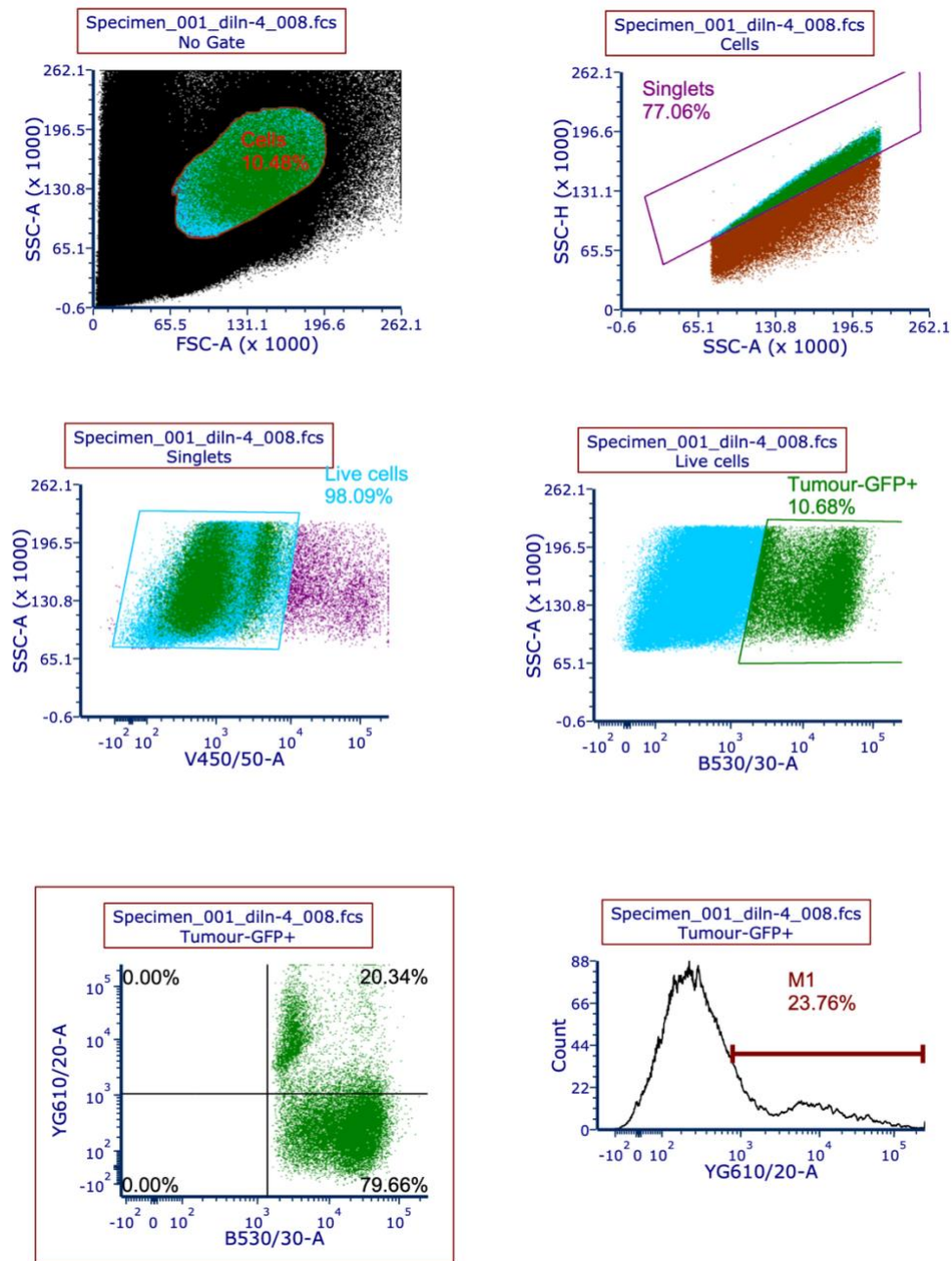
Related to ED Fig. 2g



Related to ED Fig. 10d



7. Western blots uncropped gels



8. Typical example of flow gating for all tumours processed in Extended Data 13j