Reprogramming normal cells into tumor precursors requires ECM stiffness and oncogene-mediated changes of cell mechanical properties

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Supplementary Information

Supplementary Figure 1
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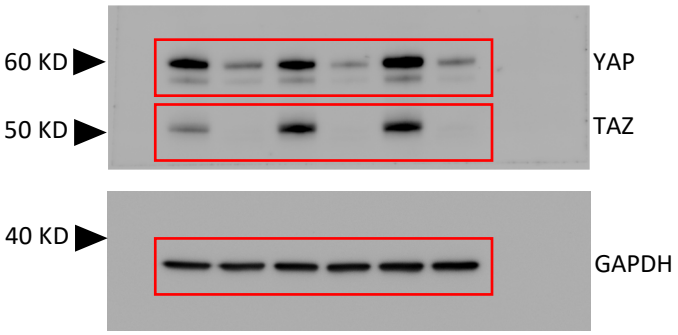
Supplementary Discussion

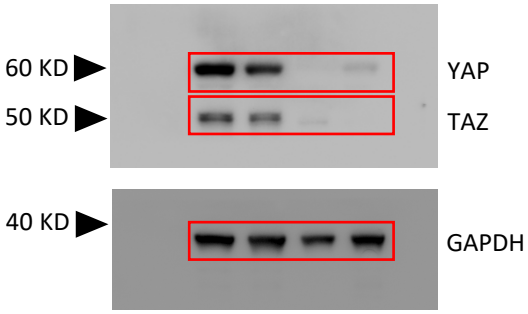
Supplementary Figure 1.

Uncropped blots with molecular weight markers.

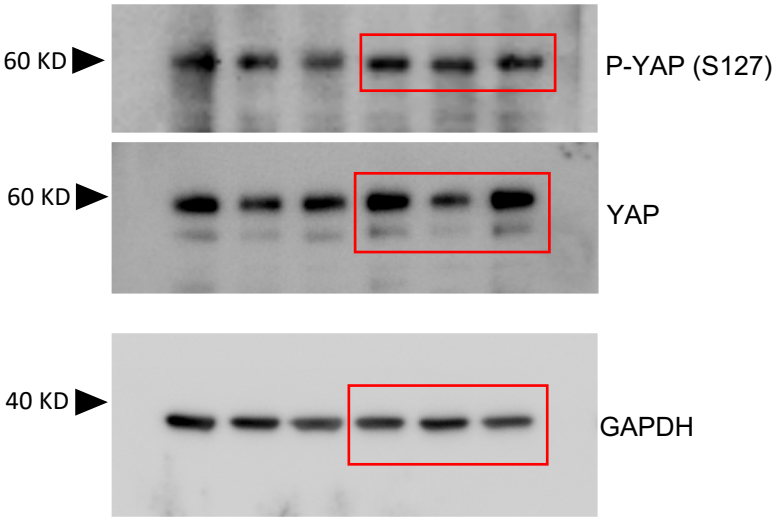
Extended Data Fig. 2h



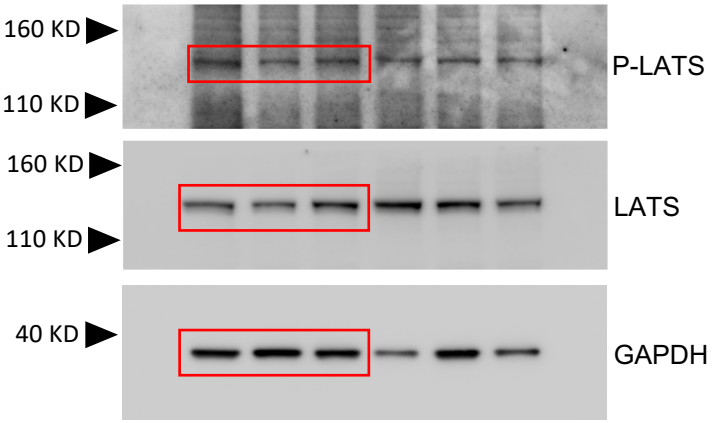
Extended Data Fig. 4b



Extended Data Fig. 5a



Extended Data Fig. 5b



Supplementary Figure 1.

Supplementary Table 1.

Supplementary Data File containing the List of GO terms significantly enriched (p-value ≤ 0.05) in HER2-induced genes whose expression is dependent on YAP/TAZ and substrate stiffness (referred to Extended Data Fig. 4i).

Supplementary Table 2.

siRNA sequences used

siControl (siAS)	AllStars Negative Control siRNA 1027280 (Qiagen)
siYAP#1	CUGGUCAGAGAUACUUCUU
siYAP#2	GACAUCUUCUGGUCAGAGA
siYAP#3	GCTTTGAGTTCTGACATCC
siTAZ#1	AGGUACUUCCUCAAUCACA
siTAZ#2	ACGUUGACUUAGGAACUUU
siTAZ#3	AGGUACUUCCUCAAUCACA

siRNA mixes were composed as following: mix siYAP/TAZ#1 contains siYAP#1 and siTAZ#1, mix siYAP/TAZ#2 contains siYAP#2 and siTAZ#2, mix siYAP/TAZ#3 contains siYAP#3 and siTAZ#3.

Supplementary Table 3.

qRT-PCR primer sequences used

Target	Forward primer sequence	Reverse primer sequence
Human *CTGF*	AGGAGTGGGTGTGTGACGA	CCAGGCAGTTGGCTCTAATC
Human *CYR61*	CCTTGTGGACAGCCAGTGTA	CCTTGTGGACAGCCAGTGTA
Human *AXL*	CACCAGCAAGAGCGATGTGT	CGGTCCTGGGGATTTAGCTC
Human *SNAI2*	GGGGAGAAGCCTTTTTCTTG	TCCTCATGTTTGTGCAGGAG
Human *CD10*	TGGATCTTGTAAGCAGCCTCA	GCACAACGTCTCCAAGTTGC
Human *aSMA*	TGCCGACCGAATGCAGAAGG	TGGAGCCACCGATCCAGACA
Human *K14*	CACCTCTCCTCCTCCCAGTT	GACACCACCTTGCCATCGT
Human *K8*	TCCTCAGGCAGCTATATGAAGAG	GGTTGGCAATATCCTCGTACTGT
Human *K18*	AATCTTGGTGATGCCTTGGA	ACCCTGCTTCTGCTGGCTTA
Human *K19*	ACACTGGCAGAAACGGAGGC	TGAGCCGCTGGTACTCCTGA
Human *MUC1*	TTGGCTGTCTGTCAGTGCCG	ACATAGCGCCCATGGGTGTG
Human *E-CAD*	CCCACCACGTACAAGGGTC	CTGGGGTATTGGGGGCATC
Human *CLDN4*	GACTGTGCCTTGCTCACCGA	CCACCACTGCCCAAACCTGT
Human *GAPDH*	CTCCTGCACCACCAACTGCT	GGGCCATCCACAGTCTTCTG
Murine *DN p63*	CCTGGAAAACAATGCCCAGAC	GAGGAGCCGTTCTGAATCTGC
Murine *Slug*	CTCACCTCGGGAGCATACAG	GACTTACACGCCCCAAGGATG
Murine *k5*	TCTCTTCTGGCTACGGAGGA	GAAGCTCATGCCTCCTTGAC
Murine *aSma*	TGCTGTCCCTCTATGCCTCT	GAAGGAATAGCCACGCTCAG
Murine *Procr*	GGAGAAAGGGCTGGACTGGT	CCCCTCCCACACACACACTT
Murine *k14*	AGGACCTGAAGAGCAAGATC	TCCTTGAGGCTCTCAATCTG
Murine *Axin2*	GAGATGACGCCTGTGGAACC	CCTGCTCAGACCCCTCCTTT
Murine *Mhy11*	GGTGAACGCCCTCAAGAGCA	TCTGAGTCCCGAGCGTCCAT
Murine *Areg*	TGGTGAACGGTGTGGAGAAA	TGTGATAACGATGCCGATGC
Murine *Esr*	GGATGCTGAACCGCCCATGA	CAGCCAGGCACACTCGAGAA
Murine *k19*	AGGAGCTGAACACCCAGGTC	GGGCTTCAAAACCGCTGAT
Murine *Pgr*	TCCGGAACTTACACATTGATGACCA	CCACATGGTAAGGCACAGCGA
Murine *Sox9*	AGGCCACGGAACAGACTCAC	CCCCTCTCGCTTCAGATCAA
Murine *Amy*	GGTTGCCTTCAGGAATGTGG	GTGAGCTTTGCCATCACTGC
Murine *Yap*	AAGGAGAGACTGCGGTTGAA	CCTGAGACATCCCAGGAGAA
Murine *Taz*	ACCCCAGGAAGGTGATGAAT	CTGCTGAGTGGTCAGTGCAT
Murine Yap^{S127A}	ACAGCATGTTCGAGCTCATG	TGTGACGTTCATCTGGGACA
Murine *Rac1*	CCTGAAGTGCGACACCACTG	CCTCGCTGTGTGAGAGCTGA
Murine *Ctgf*	CTGCCTACCGACTGGAAGAC	CATTGGTAACTCGGGTGGAG
Murine *Gapdh*	ATCCTGCACCACCAACTGCT	GGGCCATCCACAGTCTTCTG
Murine *18s-rRNA*	TGTCTCAAAGATTAAGCCATGC	GCGACCAAAGGAACCATAAC

Supplementary Discussion

Another point of discussion relates to the material nature of the ECM and its effect on oncogene-expressing cells. Our studies employed defined, covalently crosslinked ECM allowing tuning of stiffness. These hydrogels are typically elastic (Extended Data Fig. 7), while natural ECMs are complex viscoelastic entities, with weak (e.g., ionic, hydrogen) bonds also allowing stress relaxation. In addition, cells dynamically remodel their ECM[30]. Time-dependent stress relaxation (i.e., the viscous effect) has been shown to contribute, on top of ECM stiffness, to stem cell fate and to YAP mechanosignaling[30,31], highlighting the need of designing more complex biomaterials recapitulating both elastic and viscous characteristics, as well as changes in the ECM composition, in future studies of oncogene-mediated mechanotransduction.