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

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Supplementary Information

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Supplementary Figure 1.

Uncropped blots with molecular weight markers.
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  | AGGAGTGCGGTTGGTGGAGCA  | CCAGGCAGTTGGCTCCTAACT   |
| Human CYR61 | CAGTCGGGACACCGAGGCTGA  | CAGTCGGGACACCGAGGCTGA  |
| Human AXL   | CAGCAAAAGAGCTAGGAAGGT  | CAGCAAAAGAGCTAGGAAGGT  |
| Human SNAI2 | GGGAGAGGACACGAGGCTGA  | CAGTCGGGACACCGAGGCTGA  |
| Human CD10  | TGGAGGGTGTGGCTGAGCGA  | CAGTCGGGACACCGAGGCTGA  |
| Human aSMA  | TGAGCCACGGAATGCGAGGAG | CAGTCGGGACACCGAGGCTGA  |
| Human K14   | CACCTCTCGCTCCTCCAGTT  | GACGACATCGCCATCTGCTC   |
| Human K8    | TCTCCAGCGAGCTATATGGAAG | GGTGCGGAAAATATCCGCTACTG |
| Human K18   | AATCTTGATGCGCTCCGTTGA | CAGTCGGGACACCGAGGCTGA  |
| Human K19   | ACACCTGGCAGAAACCGAGGAC | TGGAGGGTGTGGCTGAGCGA  |
| Human MUC1  | TGGGCTTGTCCTGAGGCTCG  | ACATAGCGCCATGGGTGGTG   |
| Human E-CAD | CACCCAGCTTACAGGGGTG   | CAGTCGGGACACCGAGGCTGA  |
| Human CLDN4 | GACCTCGGCTTGGCTCAAGGA | CAGTCGGGACACCGAGGCTGA  |
| Human GAPDH | TTCCTGCCGCACCAACTCT    | CAGTCGGGACACCGAGGCTGA  |
| Murine DN | p63 | CAGTCGGGACACCGAGGCTGA  | CAGTCGGGACACCGAGGCTGA  |
| Murine Slug | CTACCTCGGAGACCATCAG  | CAGTCGGGACACCGAGGCTGA  |
| Murine k5   | TCTCTTCTGCGTACGAGGAGA | GGTGCGGAAAATATCCGCTACTG |
| Murine aSMA | TGCTGGCACTGAGAACGAGCTA | CAGTCGGGACACCGAGGCTGA  |
| Murine Procr | GGAGAGACGCGGCTGAGGCTT | CAGTCGGGACACCGAGGCTGA  |
| Murine k14  | AGGAGGGTGTGGCTGAGCGA  | CAGTCGGGACACCGAGGCTGA  |
| Murine Axin2 | GACAATGGAGCAGCTGTGGAAC | CAGTCGGGACACCGAGGCTGA  |
| Murine Mhy11 | GGGCCACAGCTGCTCCATGCT  | CAGTCGGGACACCGAGGCTGA  |
| Murine Areg | TTGGAGGGTGTGGCTGAGCGA | CAGTCGGGACACCGAGGCTGA  |
| Murine Esr | GGGAGAGGACGGAAGCAAGT   | CAGTCGGGACACCGAGGCTGA  |
| Murine k19  | AGGAGGGTGTGGCTGAGCGA  | CAGTCGGGACACCGAGGCTGA  |
| Murine Pgr  | TCGGAGAACTTGACATACGGCAAC | CAGTCGGGACACCGAGGCTGA  |
| Murine Sox9 | AGGCACCAGAACAGCTCAC  | CAGTCGGGACACCGAGGCTGA  |
| Murine Amy  | GGTGCGGCTCAGATGGTG   | CAGTCGGGACACCGAGGCTGA  |
| Murine Yap  | AAGGAGGACAGCTGCGGTGAA | CAGTCGGGACACCGAGGCTGA  |
| Murine Taz  | ACCCACAGGGAAGTGAATGT  | CAGTCGGGACACCGAGGCTGA  |
| Murine YapS127A | ACAGAATGGAGGAGCAGGAGA | CAGTCGGGACACCGAGGCTGA  |
| Murine Rac1 | CCTGGAGGGTGTGAGAGCTGCA | CAGTCGGGACACCGAGGCTGA  |
| Murine Ctgf | CTGCTGGCACTGAGGAGCAGAC | CAGTCGGGACACCGAGGCTGA  |
| Murine Gapdh | ATCTGGGACACCGAGGCTGA  | CAGTCGGGACACCGAGGCTGA  |
| Murine 18s-rRNA | TGTCTCAGGTAAGGACCACCTAACCAGA | CAGTCGGGACACCGAGGCTGA  |
**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. 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, 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.