# Biomimetic tissue models reveal the role of hyaluronan in melanoma proliferation and invasion

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## Supplementary Table 1: Primer list

| Gene | Gene Accession number | Start | Forward | End | Start | Reverse | End |
|---|---|---|---|---|---|---|---|
| *Rpl-p0* | NM_007475 | 512 | GGACCCGAGAAGACCTCCTT | 531 | 596 | GCACATCACTCAGAATTTCAATGG | 573 |
| *Has1* | NM_008215 | 1107 | CTATGCTACCAAGTATACCTCG | 1128 | 1214 | TCTCGGAAGTAAGATTTGGAC | 1194 |
| *Has2* | NM_008216.3 | 696 | TGTACGGTGCCTTTTTAGCC | 715 | 873 | TTCACAGATTGCAAACATTTCC | 852 |
| *Has3* | NM_008217 | 1250 | CAAGTCTTACTTTCGGGAATGG | 1271 | 1449 | TAGCCTTGATAATGCCCACC | 1430 |
| *Ki67* | NM_001081117.2 | 9709 | GATGCAAAAACTCTGAAGGAGG | 9730 | 9875 | GGAGGTGAAAACCACACTGG | 9856 |
| *Hyal1* | NM_008317.5 | 1162 | CCAAGGAATCATGCCAGG | 1179 | 1339 | GAGAAACTGGCAGGGTTGAG | 1320 |
| *Hyal2* | NM_010489.2 | 1180 | ATAGTCAACGTGTCCTGGGC | 1199 | 1318 | TATGGCCAGGCACTAGGC | 1301 |

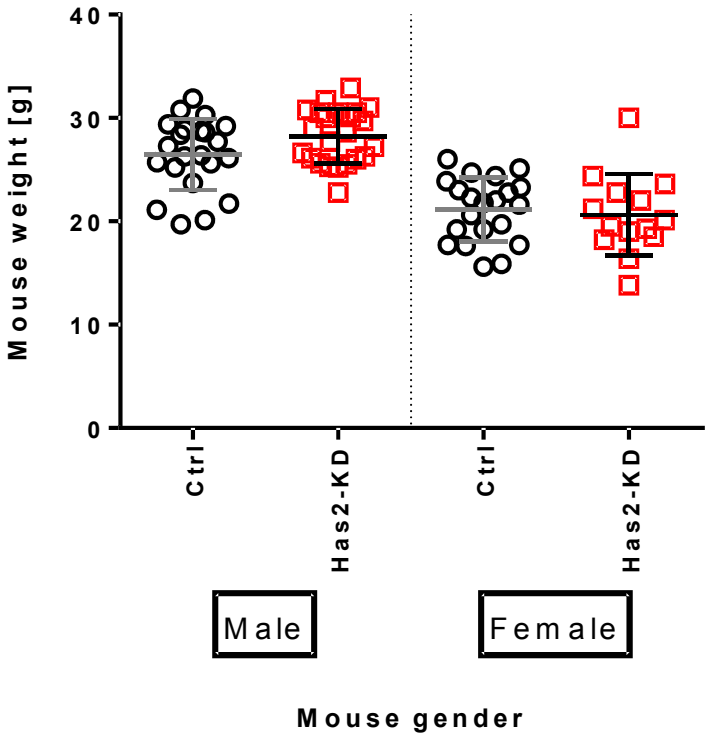


**Figure S1: Weight of Ctrl and Has2-KD mice.** The standard starting age for the 4OHT treatment was 8 weeks after birth and treatment lasted for 12 days. Weight of 4OHT-treated mice with ages between 7 and 35 weeks is shown. Both genders showed no weight specific effect due to *Has2*-KD. Different starting age of treatment did not result in any differences in body weight. Each dot represents an individual mouse.

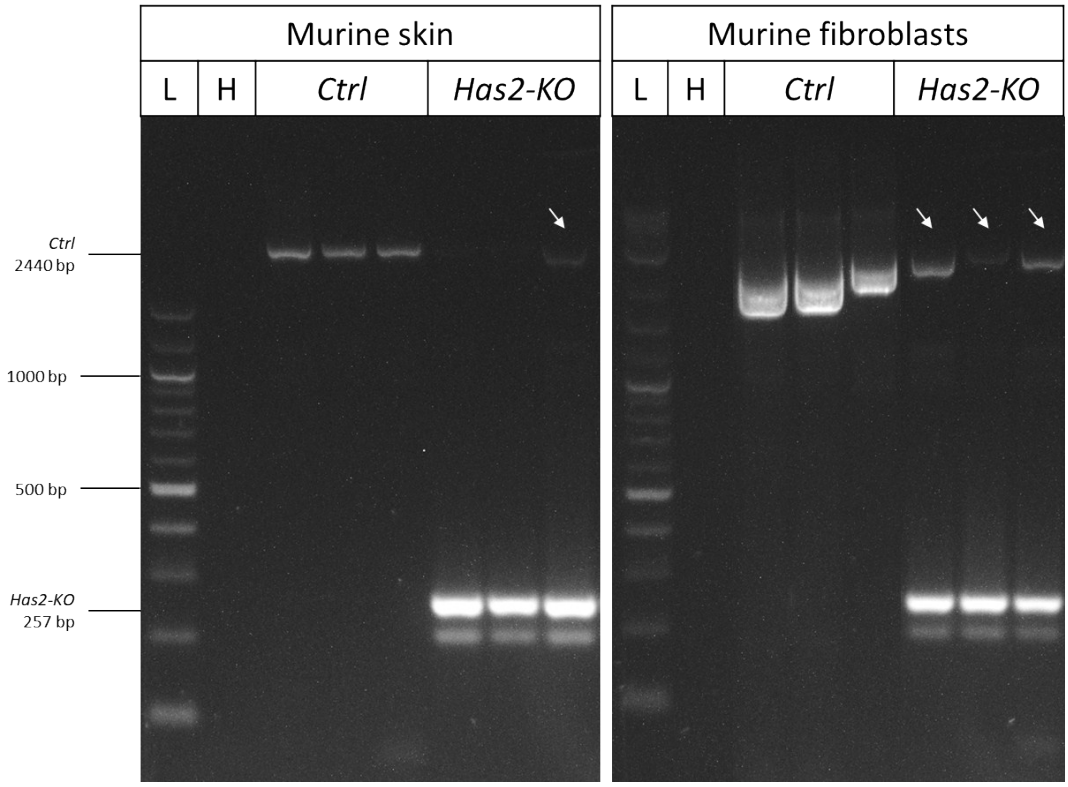


**Figure S2: Incomplete deletion of *Has2*-Exon2.** PCR amplification with flanking primers around *Has2*-Exon2 revealed successful but incomplete deletion in both full thickness skin samples and explanted fibroblasts. White arrows indicate undeleted alleles at 2440 bp in samples, where Cre-dependent excision clearly occurred (257 bp product). L=ladder, H=water.

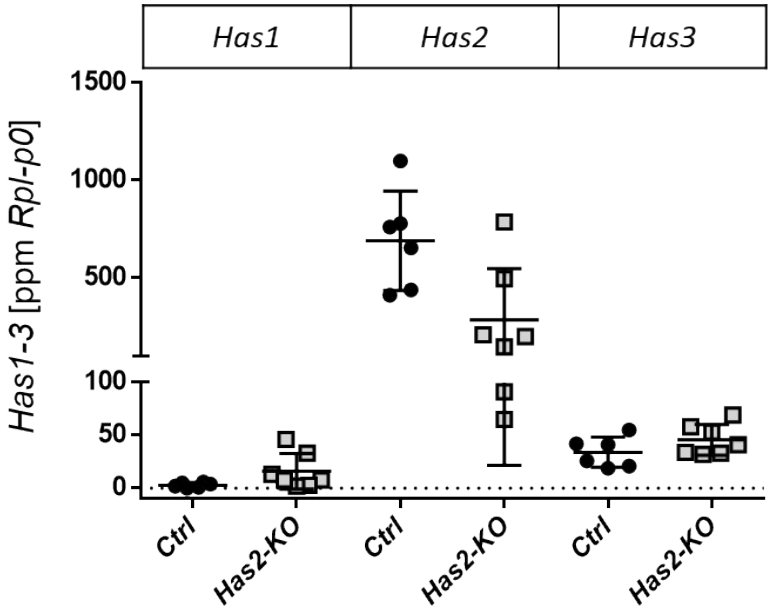


**Figure S3: No compensation by Has isoenzymes.** Full thickness skin samples were analyzed for all *Has* genes expression in RT-qPCR. Results were given as n(*Has*X) parts per million n(*Rpl-p0*). Compared to *Has2* gene expression, *Has1* and *Has3* expression were ~100-fold lower. While *Has2* expression was decreased by knockout induction, *Has1* and *Has3* expression kept their level. No compensatory upregulation was observed in these matched samples.

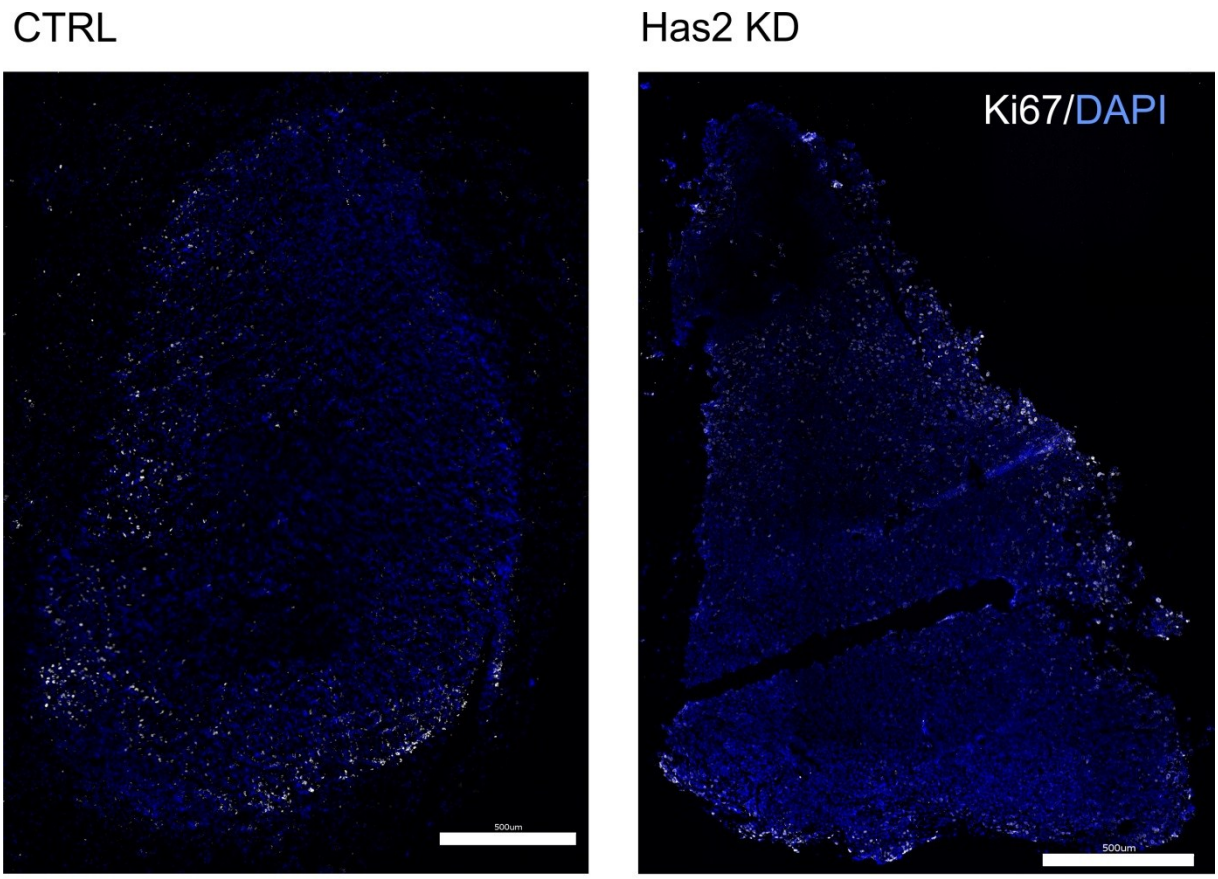


**Figure S4: Overview of Ki67 expression in experimental tumors.** Cryosections from tumors were stained with anti-Ki67 antibody (white) and DAPI (blue) as described in the methods section. Quantitation of the fluorescence signal was performed with Image J, see Figure 2. (scale bar: 500 µm).

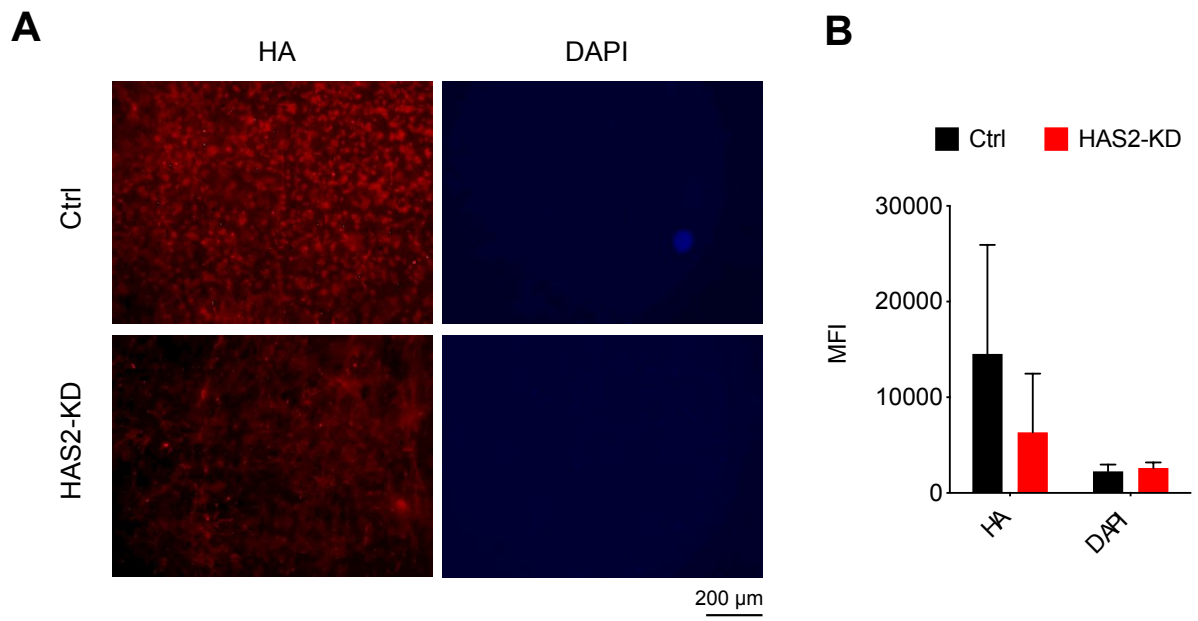


**Figure S5: Efficiency of decellularization method.** (A) FbECMs were stained with HAPB and DAPI, and visualized using fluorescence microscopy. (Scale bars:100 µm) (B) Quantification of mean (±SD) fluorescence signal intensity of HABP and DAPI using ImageJ (n = 3). Difference in HABP signal intensity could be observed in *Ctrl* and *Has2-KD* FbECM. Cells were fully removed as shown by absence of DAPI signal in (A).

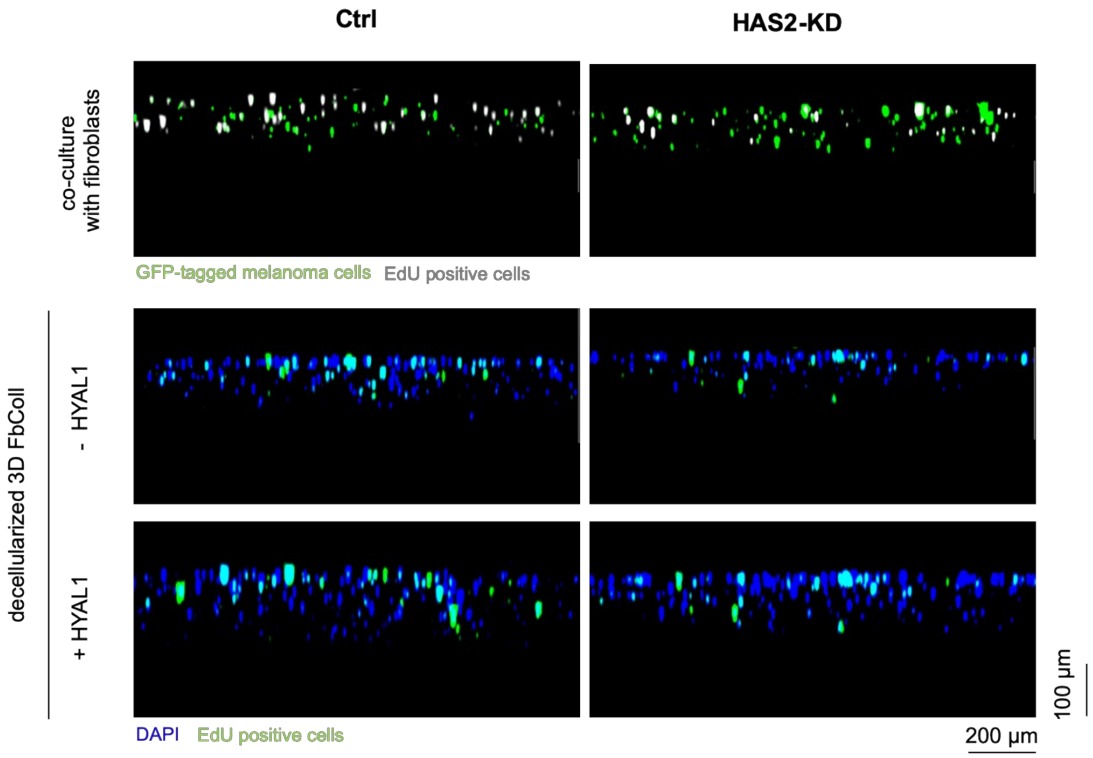


**Figure S6: Representative fluorescence images of EdU assay.** GFP-tagged melanoma cells stained with EdU and DAPI. For the analysis, GFP signal were used to distinguish fibroblasts and melanoma cells in the co-culture system. EdU positive cells were analyzed throughout the matrix thickness by counting cells using home-built MATLAB script.

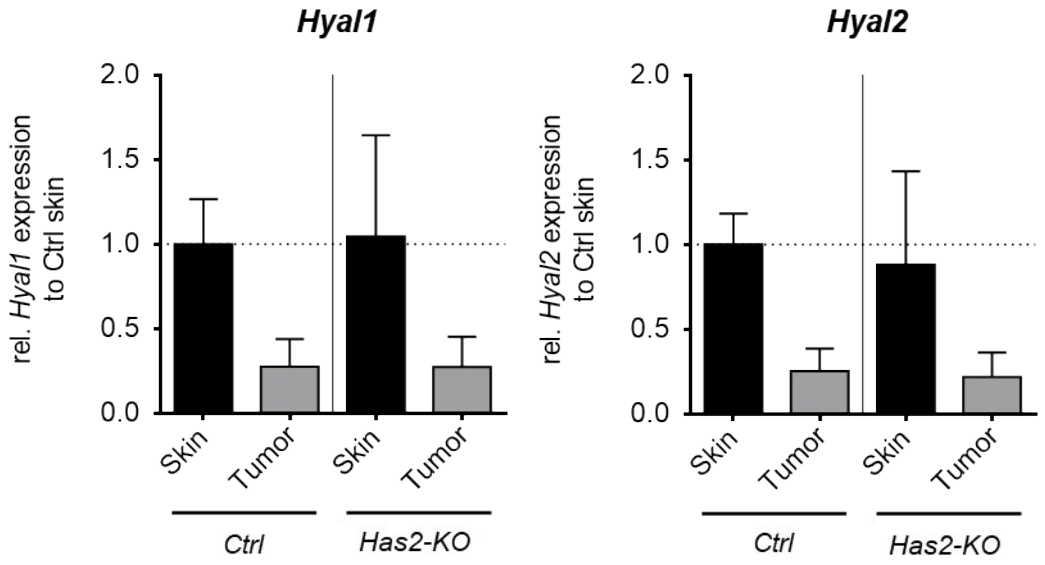


**Figure S7: Hyaluronidase-1 and -2 gene expression in skin and tumor.** Full thickness skin and tumor mass samples were analyzed for *Hyal1* and *-2* gene expression with RT-qPCR. Results were first calculated as n(*Hyal*X) parts per million n(*Rpl-p0*) and then normalized to mean skin *Ctrl* values. Knockdown of *Has2* showed no effects on *Hyal1* or *Hyal2* expression, neither in skin nor in tumor sample. Tumor tissue gene expressions per cell were at ~30% compared to skin samples, but readily detectable.