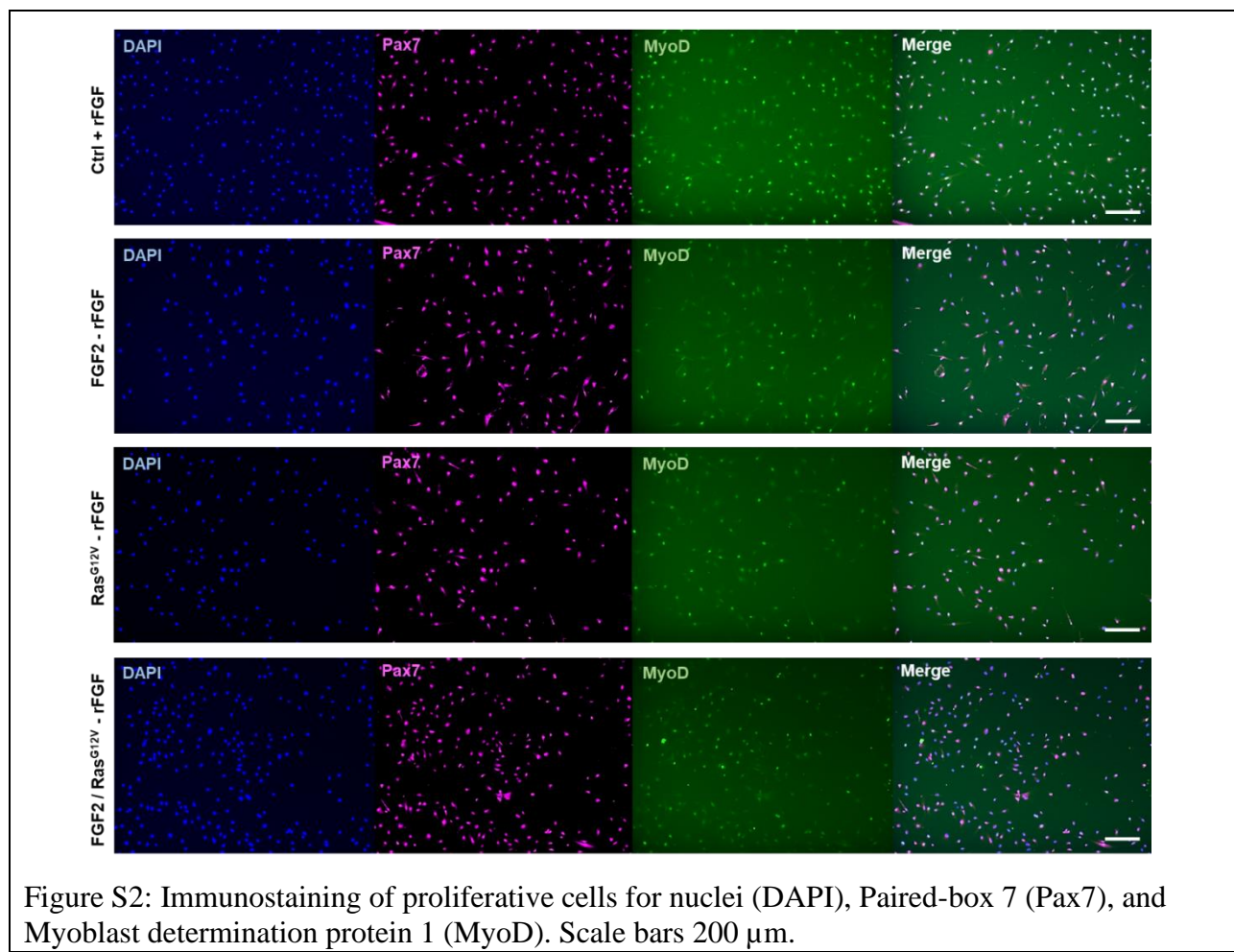
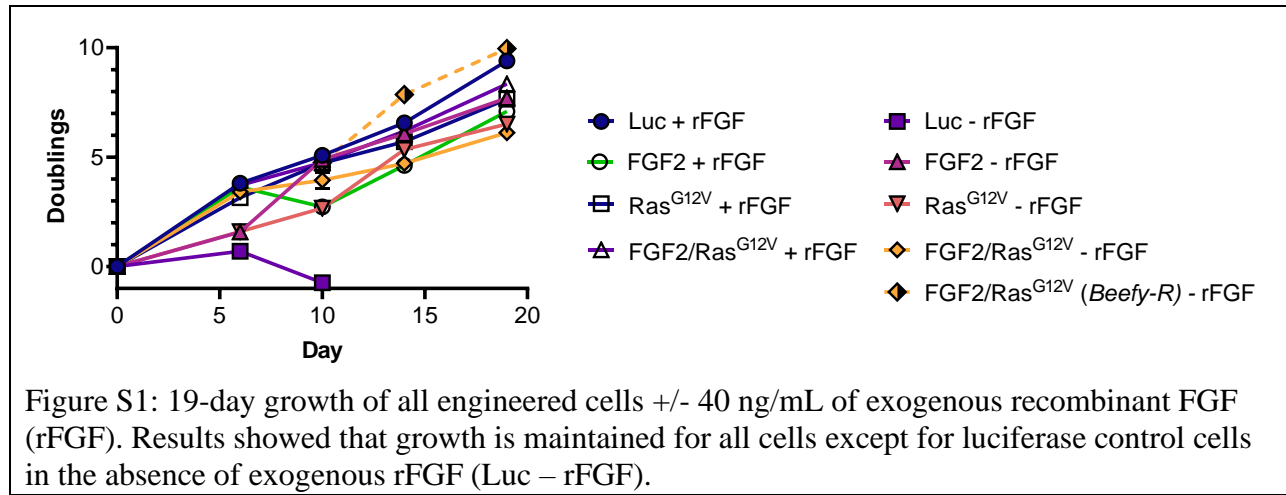
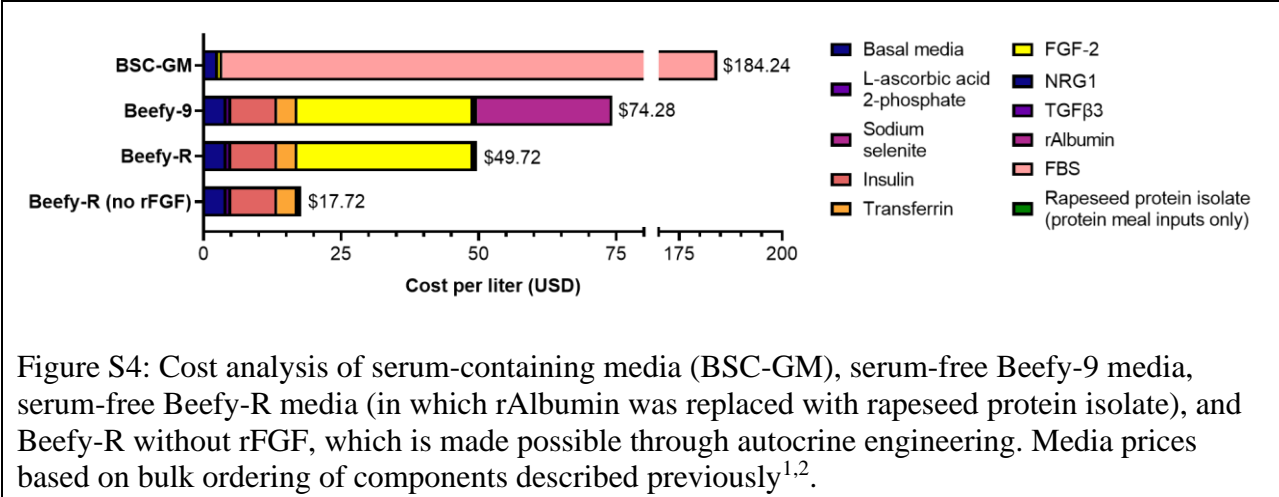
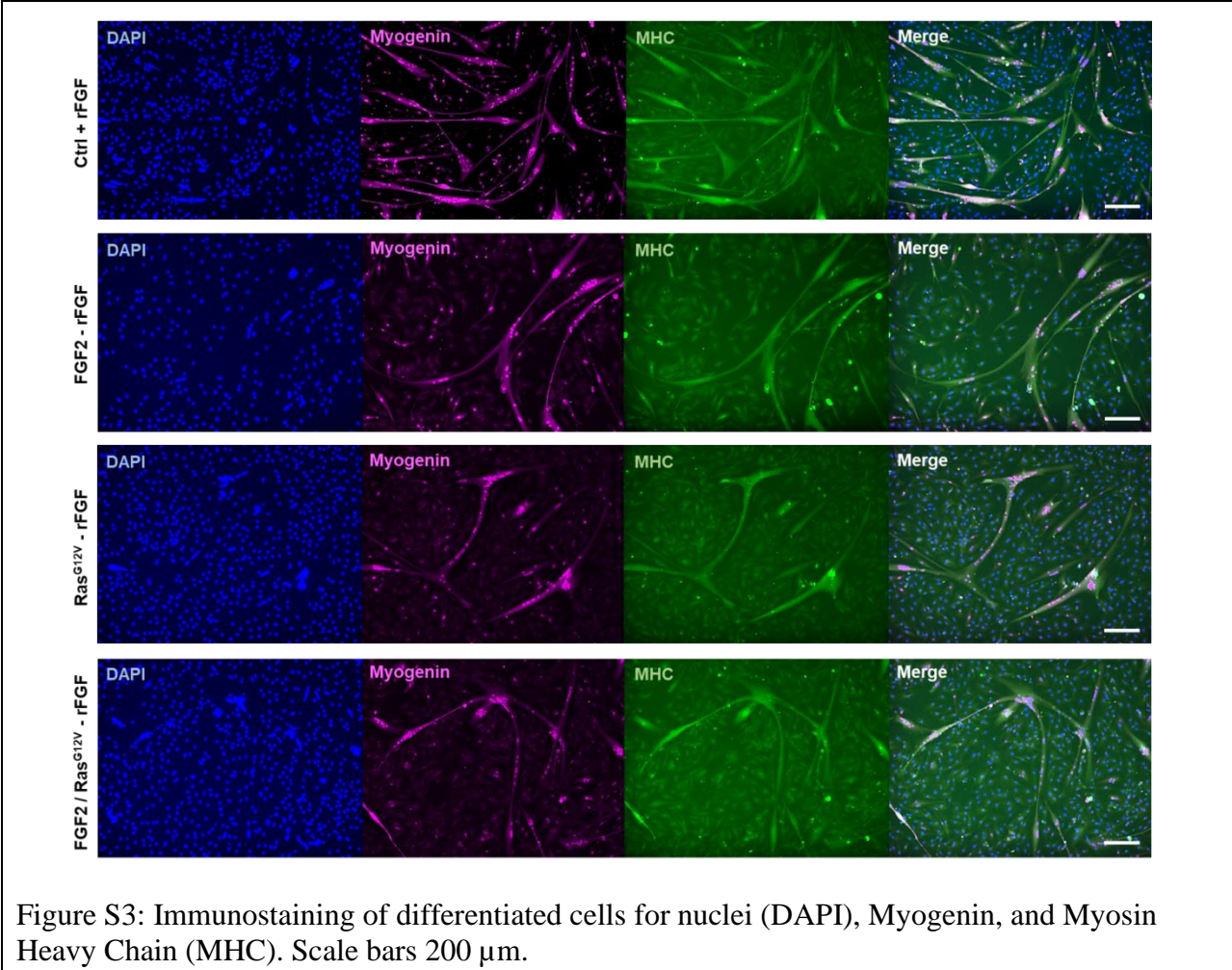


Supplementary Materials: Engineered autocrine signaling eliminates muscle cell FGF2 requirements for cultured meat production





FGF2	ATGGCGGCAGGTTCAATAACTACCCTGCCTGCTCTGCCTGAGGATGGCGGATCTGGTGCTTTTCCACCTGG CCACTTCAAGGACCCCAAGAGGCTGTACTGCAAGAACGGCGGATTCTTCCTGAGGATTCACCCCGACGGA AGAGTGGATGGCGTGC GCGAAAAGAGCGACCCTCACATCAAGCTGCAACTGCAGGCCGAAGAGAGAGGC GTCGTACAGCATCAAAGGCGTGTGCGCCAATAGATACCTGGCCATGAAGGAAGATGGCAGGCTGCTGGCCT CCAAATGCGTGACCGACGAGTGCTTCTTCTTCGAACGCCTGGAAAGCAACAACCTACAACACCTACAGAAG CCGCAAGTACTCCTCTTGGTACGTGGCCCTGAAGAGGACCGGCCAGTACAAGCTGGGACCTAAGACAGGC CCTGGACAGAAGGCCATCCTGTTCCCTTCCAATGTCCGCCAAAAGTggatctggcgaagcagagctctctgcatgtggcga cgtggaagagaacctggacct
Ras ^{G12V}	ATGACGGAATATAAGCTGGTGGTGGTGGGCGCCGTCGGTGTGGGCAAGAGTGC GCTGACCATCCAGCTGA TCCAGAACCATTTGTGGACGAATACGACCCCACTATAGAGGATTCTACCGGAAGCAGGTGGTCATTGA TGGGGAGACGTGCCTGTTGGACATCCTGGATACCGCCGGCCAGGAGGAGTACAGCGCCATGCGGGACCA GTACATGCGCACCGGGGAGGGCTTCTGTGTGTGTTTGCATCAACAACACCAAGTCTTTTGAGGACATCC ACCAAGTACAGGGAGCAGATCAAACGGGTGAAGGACTCGGATGACGTGCCCATGGTGCTGGTGGGGAACA AGTGTGACCTGGCTGCACGCACTGTGGAATCTCGGCAGGCTCAGGACCTCGCCGAAGCTACGGCATCCC CTACATCGAGACCTCGGCCAAGACCCGGCAGGGAGTGGAGGATGCCTTCTACACGTTGGTGC GTGAGATC CGGCAGCACAAGCTGCGGAAGCTGAACCCTCCTGATGAGAGTGGCCCCGGCTGCATGAGCTGCAAGTGTG TGCTCTCTAA

Table S1: Genes used. Gene sequences used in constructs (upper case), followed by 2A linker sequences used after FGF-2 for bi-cistronic expression with Ras^{G12V} (lower case).

Component	Concentration	Supplier	Catalog #
DMEM/F12 basal media	N/A	ThermoFisher	11320033
2-Phospho-L-ascorbic acid trisodium salt	200 µg/mL	Sigma	49752-10G
Insulin (human, recombinant)	20 µg/mL	Sigma	91077C-250MG
Transferrin (human, recombinant)	20 µg/mL	InVitria	777TRF029
Sodium selenite	20 ng/mL	Sigma	S5261-10G
Fibroblast growth factor (FGF-2)	40 ng/mL	PeptoTech	100-18B
Neuregulin (NRG1)	0.1 ng/mL	PeptoTech	100-03
Transforming growth factor (TGFβ3)	0.1 ng/mL	R&D Systems	8420-B3-005/CF
UltraPure Water	5.8% (v/v)	ThermoFisher	10977015
Antibiotic/Antimycotic	1% (v/v)	ThermoFisher	1540062
Recombinant albumin	0.8 mg/mL	Sigma	A9731-1G

Table S2: Components of the various media used throughout this study. “Beefy-9 + FGF” had all components, “Beefy-9 – FGF” had all components except FGF-2, “B8 + FGF” had all components except recombinant albumin, and “B8 – FGF” had all components except FGF-2 and recombinant albumin.

References

1. Stout, A. J. *et al.* Simple and effective serum-free medium for sustained expansion of bovine satellite cells for cell cultured meat. *Commun Biol* **5**, 1–13 (2022).
2. Stout, A. J. *et al.* A Beefy-R culture medium: replacing albumin with rapeseed protein isolates. 2022.09.02.506409 Preprint at <https://doi.org/10.1101/2022.09.02.506409> (2022).