# Supplementary Material

# αSMA⁺ fibroblasts suppress Lgr5⁺ cancer stem cells and restrain colorectal cancer progression

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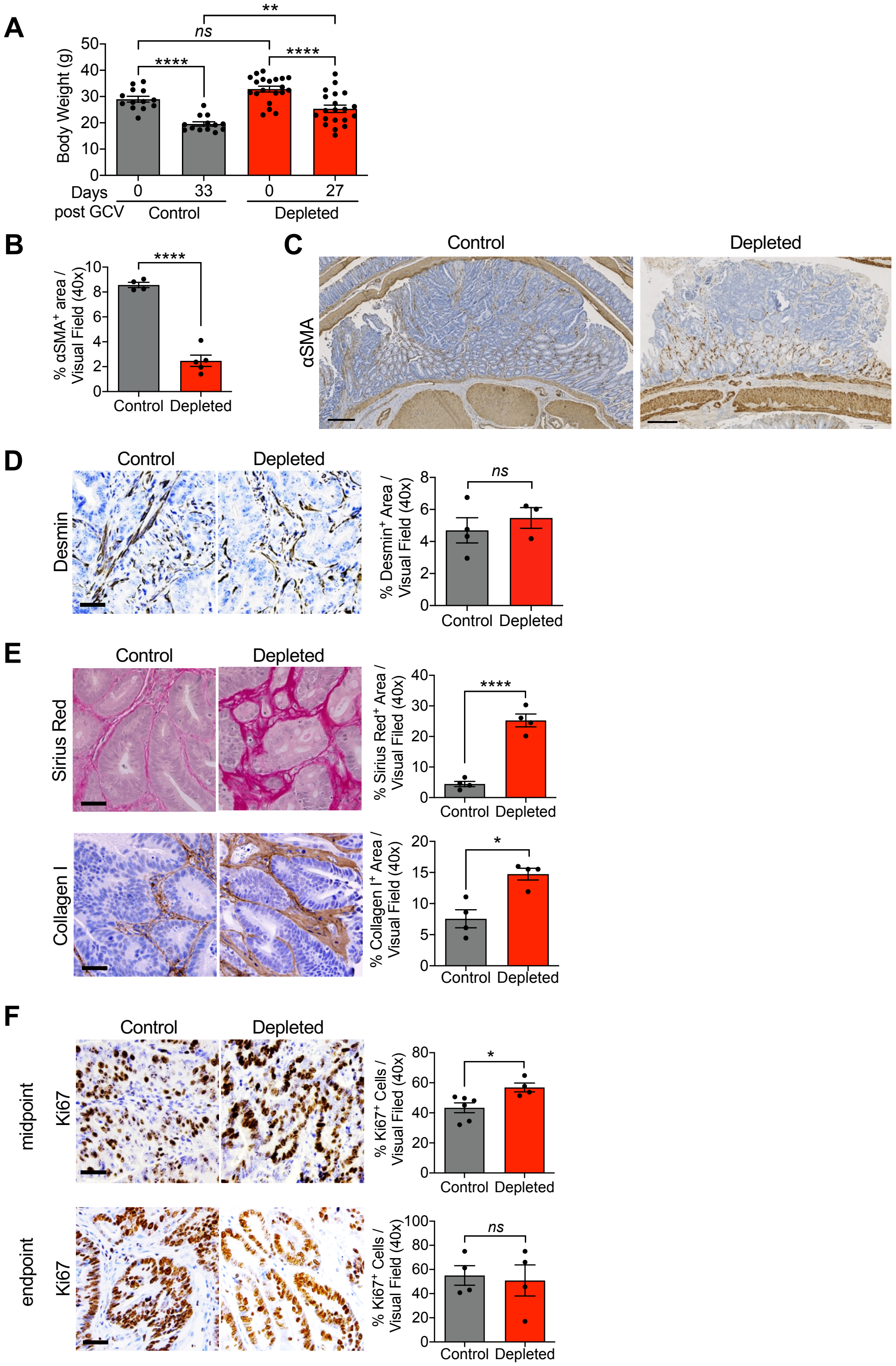
## Supplementary Figure Legends

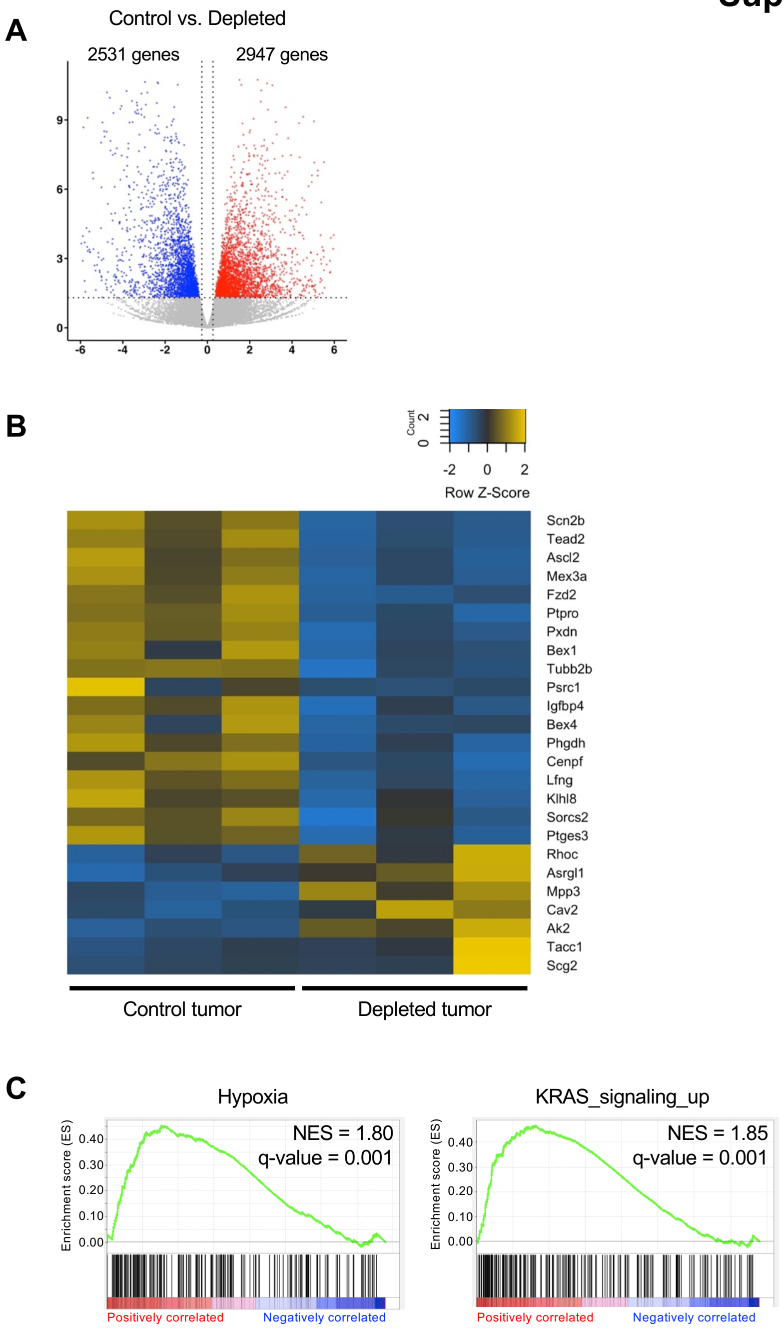
## Supplementary Figure 1



**Supplementary Figure 1. Phenotypic characterization of αSMA$^+$ CAF-depleted tumors.**

**A.** Body weight of control and depleted mice following GCV treatment to deplete αSMA$^{+}$ CAFs. Control, n=13; depleted, n=20. **B.** Quantitation of αSMA staining in CAF-depleted (n = 5) and control (n = 4) tumors (representative images in Figure 1H). **C.** Representative immunohistochemistry for αSMA$^{+}$ CAF-depleted and control tumors, Scale bar: 200 μm. **D.** Representative immunohistochemistry and quantification for desmin in αSMA$^{+}$ CAF-depleted (n = 3) and control (n = 4) tumors, Scale bar: 50 μm. **E.** Representative picrosirius red staining and immunohistochemistry for type I collagen in control (n = 4) and (n = 4) depleted tumors, and quantification of staining. Scale bar: 50 μm. **F.** Midpoint: Representative immunohistochemistry and quantification for Ki67 in αSMA$^{+}$ CAF-depleted (n = 4) and control (n = 6) tumors at 20 days post GCV. Endpoint: Representative immunohistochemistry and quantification for Ki67 in αSMA$^{+}$ CAF-depleted (n = 4) and control (n = 4) tumors at endpoint. Scale bar: 50 μm. GCV: ganciclovir. The data are presented as mean ± SEM. **A**, one-way ANOVA with Sidak's multiple comparison test performed; **B**, **D**, **E top panel**, **F**, unpaired t-test performed; **E bottom panel**, Mann-Whitney test performed. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, *ns*: not significant.

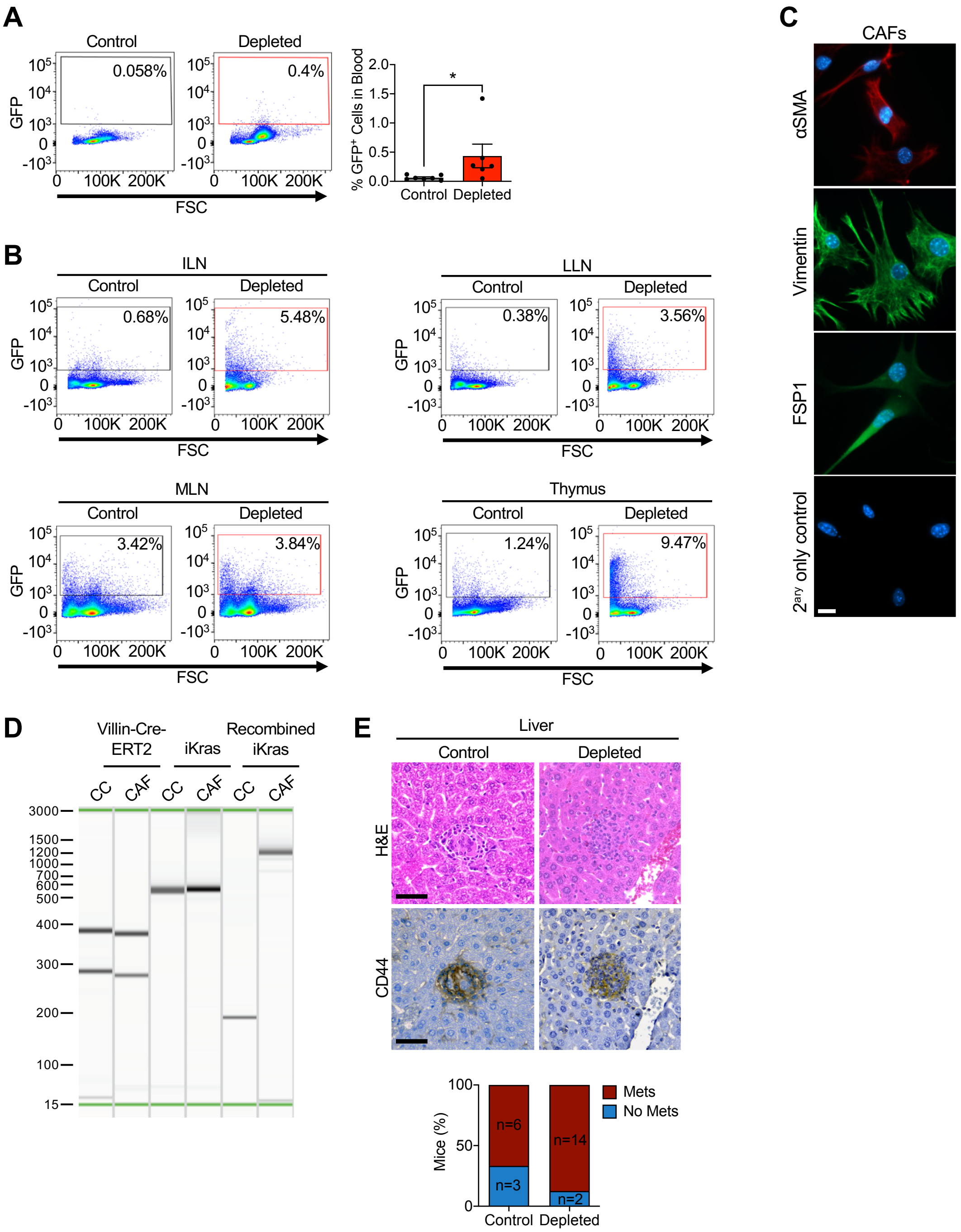
## Supplementary Figure 2



**Supplementary Figure 2. Depleted tumors are associated with malignancy-related gene signatures.**

**A.** Volcano plots showing downregulated and upregulated genes identified by RNA sequencing in control vs. depleted tumors. **B.** Heatmap of genes associated with CRC recurrence in control compared to depleted tumors. **C.** Selected GSEA enrichment plots associated with malignancy from the RNA analysis comparing control and depleted tumors. FDR: false discovery rate; NES: normalized enrichment score.

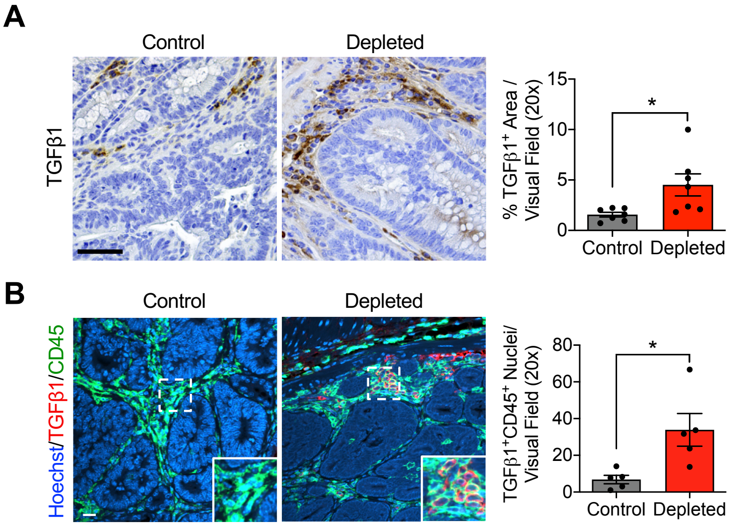
# Supplementary Figure 3



**Supplementary Figure 3. Depletion of αSMA⁺ CAFs increases liver metastasis.**

**A.** Representative FACS dot plots and quantification of the percentage GFP⁺ CTCs in the blood of control (n = 7) and depleted (n = 6) mice at endpoint. **B.** Representative FACS dot plots for data presented in Figure 2B. **C.** Immunostaining of iKAP CAFs for αSMA (red), vimentin (green), and FSP-1 (green). Blue nuclei stained with Hoechst. Scale bar: 20 µm. $2^{ary}$ only control: no primary antibody, Alexa Fluor 488 secondary antibody only. **D.** Electrogram of PCR products from cancer cells (CC) and CAFs for the indicated alleles. PCR was performed for the Villin-Cre-ERT2 allele (300 and 400 bp), iKras allele (600 bp), and Cre-mediated recombined iKras allele (unrecombined allele: 1000 bp, recombined allele: 180 bp). **E.** Representative images of H&E and CD44 immunohistochemistry in liver sections and quantification of mice with liver metastasis (identified by CD44 staining). Control, n = 9 mice; depleted, n = 16 mice. Scale bars: 100 µm. The data are presented as mean ± SEM. **A**, Mann-Whitney test performed. * $P < 0.05$, *ns*: not significant.

# Supplementary Figure 4



**Supplementary Figure 4. αSMA$^+$ CAF-depleted tumors show increased TGFβ1 in immune cells.**

**A.** Representative immunohistochemistry for TGFβ1 in control (n = 7) and depleted (n = 7) tumors and quantification of staining. Scale bar: 50 µm. **B.** Costaining for CD45 (green) and TGFβ1 (red) in control (n = 5) and depleted (n = 5) tumors. Scale bar, 20 µm. **A**, **B**, unpaired t-test with Welch's correction performed. * $P < 0.05$, ** $P < 0.01$, *ns*: not significant.

## Supplementary Tables

### Supplementary Table 1: Mouse genotypes.

| | | | | | | Villin-Cre$^{ERT2}$; LSL-KrasG12D/+; Apc F/F; Trp53 F/F; rtTA | | |
|---|---|---|---|---|---|---|---|---|
| ID | Tamoxifen start date | Doxycycline start date | GCV start date | DOD | GCV treatment (days) | αSMA-TK/0 | αSMA-RFP/0 | Luc/0 |
| G894 | 9/4/15 | 9/4/15 | 10/26/15 | 11/17/15 | 22 | 0 | RFP | 0 |
| M26 | 9/4/15 | 9/4/15 | 10/26/15 | 11/12/15 | 17 | 0 | RFP | 0 |
| M129 | 10/28/15 | 10/28/15 | 1/9/16 | 1/28/16 | 19 | 0 | 0 | L |
| M144 | 10/28/15 | 10/28/15 | 1/9/16 | 1/24/16 | 15 | 0 | 0 | L |
| M148 | 10/28/15 | 10/28/15 | 1/9/16 | 1/16/16 | 7 | 0 | 0 | L |
| V226 | 12/8/15 | 12/8/15 | 2/2/16 | 2/21/16 | 19 | 0 | RFP | n.d. |
| V202 | 12/8/15 | 12/8/15 | 2/2/16 | 3/2/16 | 29 | 0 | RFP | 0 |
| M196 | 12/8/15 | 12/8/15 | 2/2/16 | 3/30/16 | 57 | 0 | 0 | 0 |
| V218 | 12/8/15 | 12/8/15 | 2/2/16 | 3/26/16 | 53 | 0 | 0 | n.d. |
| M200 | 12/8/15 | 12/8/15 | 2/2/16 | 3/10/16 | 37 | 0 | 0 | n.d. |
| V227 | 12/8/15 | 12/8/15 | 2/2/16 | 6/6/16 | 125 | 0 | RFP | n.d. |
| 159F | 12/8/15 | 12/8/15 | 2/2/16 | 3/23/16 | 50 | 0 | RFP | n.d. |
| V212 | 12/8/15 | 12/8/15 | 2/2/16 | 3/13/16 | 40 | 0 | 0 | n.d. |
| V215 | 12/8/15 | 12/8/15 | 2/2/16 | 3/15/16 | 42 | 0 | 0 | 0 |
| V232 | 12/8/15 | 12/8/15 | 2/2/16 | 5/5/16 | 93 | 0 | RFP | n.d. |
| V264 | 1/3/16 | 1/3/16 | 2/19/16 | 4/10/16 | 51 | 0 | 0 | L |
| V271 | 1/3/16 | 1/3/16 | 2/19/16 | 4/29/16 | 70 | 0 | 0 | L |
| V269 | 1/3/16 | 1/3/16 | 2/19/16 | 3/23/16 | 33 | 0 | 0 | L |
| V282 | 1/3/16 | 1/3/16 | 2/19/16 | 3/17/16 | 27 | 0 | 0 | L |
| V275 | 1/3/16 | 1/3/16 | 2/19/16 | 6/25/16 | 127 | 0 | RFP | L |
| V276 | 1/3/16 | 1/3/16 | 2/19/16 | 6/11/16 | 113 | 0 | 0 | L |
| V325 | 1/3/16 | 1/3/16 | 2/19/16 | 4/9/16 | 50 | 0 | RFP | L |
| V299 | 1/3/16 | 1/3/16 | 2/19/16 | 7/25/16 | 157 | 0 | 0 | L |
| V302 | 1/3/16 | 1/3/16 | 2/19/16 | 3/21/16 | 31 | 0 | RFP | L |

|  |  |  |  |  |  | Villin-Cre$^{ERT2}$; LSL-KrasG12D/+; Apc F/F; Trp53 F/F; rtTA |  |  |
|---|---|---|---|---|---|---|---|---|
| ID | Tamoxifen start date | Doxycycline start date | GCV start date | DOD | GCV treatment (days) | α-SMA-TK/0 | α-SMA-RFP/0 | Luc/0 |
| G826 | 9/4/15 | 9/4/15 | 10/26/15 | 11/2/15 | 7 | TK | 0 | n.d. |
| G846 | 9/4/15 | 9/4/15 | 10/26/15 | 11/27/15 | 32 | TK | 0 | L |
| G862 | 9/4/15 | 9/4/15 | 10/26/15 | 12/1/15 | 36 | TK | 0 | L |
| G876 | 9/4/15 | 9/4/15 | 10/26/15 | 10/30/15 | 4 | TK | RFP | L |
| G877 | 9/4/15 | 9/4/15 | 10/26/15 | 12/3/15 | 38 | TK | RFP | L |
| G893 | 9/4/15 | 9/4/15 | 10/26/15 | 11/24/15 | 29 | TK | 0 | L |
| M2 | 9/4/15 | 9/4/15 | 10/26/15 | 11/27/15 | 32 | TK | 0 | L |
| M9 | 9/4/15 | 9/4/15 | 10/26/15 | 11/6/15 | 11 | TK | 0 | 0 |
| M11 | 9/4/15 | 9/4/15 | 10/26/15 | 12/3/15 | 38 | TK | 0 | L |
| M80 | 10/28/15 | 10/28/15 | 1/9/16 | 1/26/16 | 17 | TK | 0 | 0 |
| M126 | 10/28/15 | 10/28/15 | 1/9/16 | 1/27/16 | 18 | TK | 0 | 0 |
| M169 | 12/8/15 | 12/8/15 | 2/2/16 | 3/4/16 | 31 | TK | RFP | L |
| V204 | 12/8/15 | 12/8/15 | 2/2/16 | 2/15/16 | 13 | TK | RFP | n.d. |
| V225 | 12/8/15 | 12/8/15 | 2/2/16 | 2/28/16 | 26 | TK | 0 | n.d. |
| V228 | 12/8/15 | 12/8/15 | 2/2/16 | 3/4/16 | 31 | TK | RFP | 0 |
| V234 | 12/8/15 | 12/8/15 | 2/2/16 | 3/4/16 | 31 | TK | 0 | 0 |
| 160F | 12/8/15 | 12/8/15 | 2/2/16 | 3/4/16 | 31 | TK | RFP | 0 |
| V213 | 12/8/15 | 12/8/15 | 2/2/16 | 3/8/16 | 35 | TK | 0 | 0 |
| V265 | 1/3/16 | 1/3/16 | 2/19/16 | 3/25/16 | 35 | TK | RFP | L |
| V277 | 1/3/16 | 1/3/16 | 2/19/16 | 3/25/16 | 35 | TK | 0 | L |
| V297 | 1/3/16 | 1/3/16 | 2/19/16 | 3/17/16 | 27 | TK | RFP | L |
| V284 | 1/3/16 | 1/3/16 | 2/19/16 | 3/13/16 | 23 | TK | 0 | L |
| V324 | 1/3/16 | 1/3/16 | 2/19/16 | 3/20/16 | 30 | TK | RFP | L |
| V295 | 1/3/16 | 1/3/16 | 2/19/16 | 3/17/16 | 27 | TK | 0 | 0 |
| V305 | 1/3/16 | 1/3/16 | 2/19/16 | 3/20/16 | 30 | TK | 0 | L |
| V304 | 1/3/16 | 1/3/16 | 2/19/16 | 3/25/16 | 35 | TK | 0 | L |
| V319 | 1/3/16 | 1/3/16 | 2/19/16 | 3/17/16 | 27 | TK | 0 | L |
| V327 | 1/21/16 | 1/21/16 | 3/9/16 | 3/26/16 | 17 | TK | 0 | L |
| V336 | 1/21/16 | 1/21/16 | 3/9/16 | 3/22/16 | 13 | TK | 0 | 0 |
| V350 | 1/21/16 | 1/21/16 | 3/9/16 | 3/25/16 | 16 | TK | 0 | L |
| V356 | 1/21/16 | 1/21/16 | 3/9/16 | 4/5/16 | 27 | TK | 0 | L |
| V382 | 1/21/16 | 1/21/16 | 3/9/16 | 3/29/16 | 20 | TK | RFP | 0 |

n.d.: not determined

## Supplementary Table 2: GSEA of differentially regulated pathways in depleted compared to control tumors.

**Positively regulated pathways**

| Pathway name | Size | ES | NES | Nom p-val | FDR q-val | FWER p-val | Rank at max | Leading edge |
|---|---|---|---|---|---|---|---|---|
| HALLMARK_TNFA_SIGNALING_ VIA_NFKB | 191 | 0.5698 | 2.2740 | 0.0000 | 0.0000 | 0.0000 | 3083 | tags=47%, list=20%, signal=58% |
| HALLMARK_INFLAMMATORY_RESPONSE | 191 | 0.5229 | 2.0725 | 0.0000 | 0.0000 | 0.0000 | 2203 | tags=32%, list=14%, signal=37% |
| HALLMARK_KRAS_SIGNALING_ UP | 192 | 0.4662 | 1.8504 | 0.0000 | 0.0014 | 0.0040 | 3262 | tags=40%, list=21%, signal=49% |
| HALLMARK_PANCREAS_BETA_CELLS | 39 | 0.5808 | 1.8070 | 0.0000 | 0.0013 | 0.0050 | 1629 | tags=33%, list=10%, signal=37% |
| HALLMARK_HYPOXIA | 185 | 0.4520 | 1.7988 | 0.0000 | 0.0012 | 0.0060 | 3062 | tags=36%, list=20%, signal=44% |
| HALLMARK_INTERFERON_ GAMMA_RESPONSE | 181 | 0.4500 | 1.7595 | 0.0000 | 0.0020 | 0.0120 | 4257 | tags=42%, list=27%, signal=57% |
| HALLMARK_IL6_JAK_STAT3_SIGNALING | 83 | 0.4830 | 1.7322 | 0.0000 | 0.0024 | 0.0160 | 4377 | tags=46%, list=28%, signal=63% |
| HALLMARK_APOPTOSIS | 152 | 0.3810 | 1.4768 | 0.0066 | 0.0490 | 0.3360 | 3243 | tags=32%, list=21%, signal=39% |
| HALLMARK_KRAS_SIGNALING_DN | 175 | 0.3619 | 1.4254 | 0.0090 | 0.0751 | 0.4930 | 2313 | tags=33%, list=15%, signal=38% |
| HALLMARK_SPERMATOGENESIS | 119 | 0.3644 | 1.3698 | 0.0234 | 0.1175 | 0.7050 | 1911 | tags=24%, list=12%, signal=27% |
| HALLMARK_P53_PATHWAY | 181 | 0.3377 | 1.3437 | 0.0240 | 0.1367 | 0.7870 | 4082 | tags=36%, list=26%, signal=48% |
| HALLMARK_IL2_STAT5_SIGNALING | 188 | 0.3298 | 1.3095 | 0.0395 | 0.1725 | 0.8810 | 4031 | tags=36%, list=26%, signal=48% |
| HALLMARK_COMPLEMENT | 172 | 0.3302 | 1.3021 | 0.0452 | 0.1698 | 0.8950 | 3865 | tags=37%, list=25%, signal=49% |
| HALLMARK_BILE_ACID_METABOLISM | 107 | 0.3486 | 1.2952 | 0.0749 | 0.1682 | 0.9110 | 4249 | tags=35%, list=27%, signal=47% |
| HALLMARK_MYOGENESIS | 190 | 0.3246 | 1.2839 | 0.0529 | 0.1729 | 0.9410 | 3167 | tags=28%, list=20%, signal=35% |
| HALLMARK_ESTROGEN_RESPONSE_ EARLY | 189 | 0.3220 | 1.2723 | 0.0651 | 0.1779 | 0.9540 | 3720 | tags=34%, list=24%, signal=44% |
| HALLMARK_OXIDATIVE_PHOSPHORYLATION | 188 | 0.3194 | 1.2667 | 0.0661 | 0.1753 | 0.9580 | 5781 | tags=48%, list=37%, signal=76% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| HALLMARK_UV_RESPONSE_UP | 147 | 0.3237 | 1.2483 | 0.0950 | 0.1910 | 0.9770 | 3667 | tags=31%, list=24%, signal=40% |
| HALLMARK_HEME_METABOLISM | 181 | 0.3180 | 1.2378 | 0.0846 | 0.1971 | 0.9840 | 4902 | tags=42%, list=31%, signal=61% |
| HALLMARK_ALLOGRAFT_REJECTION | 180 | 0.3104 | 1.2270 | 0.0904 | 0.2046 | 0.9890 | 2729 | tags=25%, list=18%, signal=30% |
| HALLMARK_XENOBIOTIC_METABOLISM | 180 | 0.3020 | 1.1947 | 0.1089 | 0.2516 | 0.9940 | 4393 | tags=43%, list=28%, signal=60% |
| HALLMARK_ADIPOGENESIS | 188 | 0.2933 | 1.1585 | 0.1680 | 0.3073 | 0.9990 | 5339 | tags=44%, list=34%, signal=66% |
| HALLMARK_COAGULATION | 121 | 0.3051 | 1.1490 | 0.2025 | 0.3153 | 0.9990 | 3806 | tags=40%, list=24%, signal=52% |
| HALLMARK_ANDROGEN_RESPONSE | 93 | 0.3146 | 1.1391 | 0.2122 | 0.3216 | 0.9990 | 4471 | tags=37%, list=29%, signal=51% |
| HALLMARK_HEDGEHOG_SIGNALING | 36 | 0.3694 | 1.1254 | 0.2825 | 0.3384 | 0.9990 | 3567 | tags=36%, list=23%, signal=47% |
| HALLMARK_PROTEIN_SECRETION | 93 | 0.2960 | 1.0696 | 0.3314 | 0.4584 | 1.0000 | 6099 | tags=48%, list=39%, signal=79% |
| HALLMARK_ESTROGEN_RESPONSE_LATE | 189 | 0.2607 | 1.0437 | 0.3718 | 0.5086 | 1.0000 | 3286 | tags=26%, list=21%, signal=33% |
| HALLMARK_UV_RESPONSE_DN | 135 | 0.2702 | 1.0384 | 0.3808 | 0.5038 | 1.0000 | 4642 | tags=37%, list=30%, signal=52% |
| HALLMARK_CHOLESTEROL_HOMEOSTASIS | 71 | 0.2855 | 0.9909 | 0.4703 | 0.6101 | 1.0000 | 4582 | tags=38%, list=29%, signal=54% |
| HALLMARK_INTERFERON_ALPHA_RESPONSE | 86 | 0.2671 | 0.9339 | 0.5741 | 0.7486 | 1.0000 | 4257 | tags=36%, list=27%, signal=49% |
| HALLMARK_APICAL_JUNCTION | 193 | 0.2301 | 0.9176 | 0.6845 | 0.7691 | 1.0000 | 4332 | tags=29%, list=28%, signal=40% |
| HALLMARK_REACTIVE_OXYGEN_SPECIES_ PATHWAY | 45 | 0.2732 | 0.8774 | 0.6769 | 0.8487 | 1.0000 | 5686 | tags=44%, list=36%, signal=70% |
| HALLMARK_GLYCOLYSIS | 193 | 0.2187 | 0.8625 | 0.8048 | 0.8588 | 1.0000 | 4090 | tags=27%, list=26%, signal=37% |
| HALLMARK_TGF_BETA_ SIGNALING | 52 | 0.2534 | 0.8450 | 0.7504 | 0.8726 | 1.0000 | 3706 | tags=23%, list=24%, signal=30% |
| HALLMARK_FATTY_ACID_METABOLISM | 148 | 0.2178 | 0.8364 | 0.8302 | 0.8656 | 1.0000 | 5167 | tags=35%, list=33%, signal=52% |
| HALLMARK_PEROXISOME | 100 | 0.2221 | 0.8060 | 0.8818 | 0.8962 | 1.0000 | 5401 | tags=43%, list=35%, signal=65% |
| HALLMARK_PI3K_AKT_MTOR_SIGNALING | 99 | 0.2140 | 0.7784 | 0.9032 | 0.9130 | 1.0000 | 5465 | tags=36%, list=35%, signal=56% |

**Negatively regulated pathways**

| Pathway name | Size | ES | NES | Nom p-val | FDR q-val | FWER p-val | RANK AT MAX | LEADING EDGE |
|---|---|---|---|---|---|---|---|---|
| HALLMARK_E2F_TARGETS | 186 | -0.6553 | -2.9231 | 0.0000 | 0.0000 | 0.0000 | 3513 | tags=69%, list=23%, signal=88% |
| HALLMARK_G2M_CHECKPOINT | 185 | -0.5678 | -2.5512 | 0.0000 | 0.0000 | 0.0000 | 3138 | tags=50%, list=20%, signal=62% |
| HALLMARK_MYC_TARGETS_V1 | 183 | -0.5155 | -2.3179 | 0.0000 | 0.0000 | 0.0000 | 4648 | tags=57%, list=30%, signal=80% |
| HALLMARK_MITOTIC_SPINDLE | 196 | -0.4308 | -1.9542 | 0.0000 | 0.0000 | 0.0000 | 4419 | tags=41%, list=28%, signal=56% |
| HALLMARK_MYC_TARGETS_V2 | 54 | -0.5144 | -1.9091 | 0.0000 | 0.0000 | 0.0000 | 4044 | tags=65%, list=26%, signal=87% |
| HALLMARK_WNT_BETA_CATENIN_SIGNALING | 39 | -0.4885 | -1.6423 | 0.0118 | 0.0032 | 0.0190 | 1550 | tags=28%, list=10%, signal=31% |
| HALLMARK_DNA_REPAIR | 138 | -0.3401 | -1.4742 | 0.0000 | 0.0178 | 0.1190 | 4204 | tags=39%, list=27%, signal=53% |
| HALLMARK_ANGIOGENESIS | 34 | -0.3474 | -1.1690 | 0.2282 | 0.2272 | 0.8400 | 1968 | tags=24%, list=13%, signal=27% |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION | 189 | -0.2557 | -1.1540 | 0.1159 | 0.2261 | 0.8650 | 2086 | tags=23%, list=13%, signal=26% |
| HALLMARK_APICAL_SURFACE | 41 | -0.2813 | -1.0056 | 0.4548 | 0.5691 | 0.9990 | 3360 | tags=39%, list=22%, signal=50% |
| HALLMARK_NOTCH_SIGNALING | 32 | -0.2834 | -0.9359 | 0.5924 | 0.7451 | 1.0000 | 1550 | tags=19%, list=10%, signal=21% |
| HALLMARK_MTORC1_SIGNALING | 188 | -0.1998 | -0.9095 | 0.7416 | 0.7652 | 1.0000 | 3855 | tags=28%, list=25%, signal=36% |
| HALLMARK_UNFOLDED_PROTEIN_RESPONSE | 107 | -0.1958 | -0.8088 | 0.9018 | 0.9054 | 1.0000 | 3872 | tags=29%, list=25%, signal=38% |