Supplemental Figure 1: Controlling for growth rate, identically sized ID tumors are more likely to respond to RT + IC in situ vaccination. Female, C57BL/6 mice were injected on the same day intentionally aiming for either Intradermal (ID) or Subcutaneous (SC) placement of the tumor. Mice were monitored weekly, and whenever tumors reached treatment size (190-230mm^3), they were collected into a treatment group and treated with either PBS control, or our previously published in situ vaccine (12Gy external beam radiation on treatment day 1 followed by intratumoral hu14.18-IL2 immunocytokine on treatment days 6-10). **A)** Frequency histogram of the number of mice reaching treatment size for each of the 'treatment waves' reached during the course of the experiment. Blue-green bars correspond to the SC waves and red-purple-brown bars correspond to ID waves. **B)** Overlap in time to treatment size between ID and SC implanted tumors occurred for multiple SC and ID mice only on post-implant day 27, in which n=3 mice of both ID-implanted (pink) and SC-implanted (green) groups reached the 190-230mm^3 treatment window. Shown is the average +/- SEM tumor volume following ISV treatment starting on post-implant day 27, to enable comparison of treatment outcome for ID and SC tumors when all treated tumors have the same tumor size and growth rate at the time treatment is initiated.

Supplemental Figure 2: Controlling for starting size, ID tumors are more likely to respond to RT + IC in situ vaccination. Female, C57BL/6 mice were injected on the same day intentionally aiming for either Intradermal (ID) or Subcutaneous (SC) placement of the tumor. Mice were monitored weekly, and whenever tumors reached treatment size (190-230mm^3), they were collected into a treatment group and treated with either PBS control or our previously published in situ vaccine (12Gy external beam radiation on treatment day 1 followed by intratumoral hu14.18-IL2 immunocytokine on treatment days 6-10). For **A** and **B**, the Average +/- SEM tumor volume is plotted for each individual wave, for the SC tumors (in **A,** shades of blue) and the ID tumors (in **B,** shades of red) to enable comparison of treatment

outcome for 190-230mm^3 tumors. Although numbers are small, there are no significant differences in overall survival between the 4 waves shown in **A**, and there are no significant differences between any of the 8 waves plotted in **B** as determined by Cox proportional hazards modeling, for both ID (p=0.877) and SC (p=0.340) implanted tumors. This indicates that in this setting, the initial growth rate of the tumor is not influencing response to treatment. **C)** Tumor volumes for each individual mouse in the 4 waves of treatment for SC mice. **D)** Tumor volumes for each individual mouse in the 8 waves of treatment for ID mice.

Supplemental Figure 3: Depth of implantation impacts growth rate and degree of response to multiple immunotherapies in the CT26 cancer model. A) Treatment Schema. Female, 6 week old Balb/c mice were implanted intentionally either ID or SC with 1x10^6 CT26 colon adenocarcinoma cells [20] as described in the methods section. Mice were then randomized to receive either no treatment, a single IT injection of 50µg CpG1826 (TriLink Biotechnologies) on day 5 post-implantation (orange arrow), or a single IP injection of 200µg anti-CTLA4 (IgG2c, Bristol-Myers Squibb) antibody on day 7 post-implantation (green arrow) [21]. **B)** For all mice, tumors were measured in a blinded fashion, and the mobile or fixed physical status of the tumor was evaluated and recorded. The table in B documents the physical exam status for every mouse in the experiment on each measurement day. Mobile tumors were coded red, fixed tumors were coded blue, and tumors with both properties or intermediate findings were coded purple. Tumors that were not yet palpable were coded grey. Mice that had a tumor and were rendered disease free have a 'DF' in the cell in this graph starting on the measurement day tumor was no longer palpable. Mice that were euthanized due to tumor burden or found dead from metastases have an 'X' in the cell in this graph starting on the measurement day they were euthanized or found dead. Results demonstrate that, like the B78 melanoma tumor model in C57Bl/6 mice, the CT26 colon adenocarcinoma in Balb/c mice can develop in both the ID and SC space, and that the vast majority of intentionally ID-implanted

tumors exhibit the “mobile” physical exam finding, and the vast majority of intentionally SC-implanted tumors exhibit the “fixed” physical exam finding. For all experiments, only ID-implanted mice with ‘mobile’ tumors and SC-implanted mice with ‘fixed’ tumors for greater than half of all measurement days were included in the analysis of tumor growth or survival graphs (C-J, below); (the number of mice included in analyses of tumor growth or survival per group, n, out of the 10 initial mice implanted in each group, is indicated below each graph). **C and D)** average tumor volume for ID (red) and SC (blue) tumors in response to no treatment (dotted lines), CpG immunotherapy (solid lines in **C**), or anti-CTLA4 immunotherapy (solid lines in **D**). Note that when one mouse in the group dies, the average can no longer be graphed. **E)** Individual and mean +/- SEM tumor volumes for each of the treatment groups represented in C and D on day 19 post implantation, the last day for which all mice were alive. Statistical analysis was conducted using a one-way ANOVA. No corrections were made for multiple comparisons. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$. **F, G, and H)** Tumor volumes for each individual mouse in each treatment group are plotted over the course of the experiment to demonstrate the range and variability seen. ID tumors are depicted in red, and SC tumors are depicted in blue. **F** shows all mice treated with IT CpG, **G** shows all mice treated with anti-CTLA4, and **H** shows all untreated tumors (dotted lines). **I)** Survival analysis of ID (red) and SC (blue) tumor-bearing untreated mice (dotted lines), and mice treated with CpG (solid lines). **J)** Survival analysis of ID (red) and SC (blue) tumor-bearing untreated mice (dotted lines), and mice treated with anti-CTLA4 (solid lines). Survival analysis in **I** and **J** was conducted using the Kaplan-Meier method, with comparisons between groups conducted using log-rank tests. Results demonstrate that on day 19 post implantation, untreated SC tumors were significantly larger than untreated ID tumors ($p<0.0001$), and untreated ID-implanted mice had a significantly longer overall survival ($p < 0.01$) compared to untreated SC-implanted mice. Untreated SC-implanted mice also had significantly larger tumors than SC-implanted mice treated with CpG ($p<0.0001$) or anti-CTLA4 ($p<0.0001$) on day 19 post implantation. SC-implanted mice treated

with anti-CTLA4 also had significantly larger tumors than ID-implanted mice treated with anti-CTLA4 by day 19 ($p<0.05$). Due to a mouse in the SC-implanted untreated group dying on day 19 post implantation, group comparisons beyond this point for mice with SC-implanted tumors were not technically possible. Nevertheless, comparison of remaining groups on day 26 post implantation by one-way ANOVA demonstrated that ID-implanted untreated mice had significantly larger tumors than ID-implanted mice treated with CpG ($p<0.05$) as well as ID-implanted mice treated with anti-CTLA4 ($p<0.01$). Compared to SC-implanted untreated mice, a greater overall survival was detected in SC-implanted mice treated with anti-CTLA4 ($p<0.001$), but not for mice treated with CpG. Compared to ID-implanted untreated mice, a trend toward greater overall survival was detected in ID-implanted mice treated with anti-CTLA4 ($p=0.064$). No difference in survival was detected among ID-implanted mice treated with CpG vs. untreated ID implanted mice. However ID-implanted mice treated with CpG did have a greater overall survival compared to SC-implanted mice treated with CpG ($p<0.01$). Together, these data suggest that in the CT26 tumor model implanted in balb/c mice, ID- and SC-implanted tumors may behave differently in both the untreated and immunotherapy-treated settings. These CT26 tumors display the same fixed/mobile physical properties associated with SC vs. ID implantation that would allow for more homogeneous selection of study populations when selected for either fixed or mobile features. See Supplemental Table 1 for a summary of all p values referenced for the combinations of tumor depths and immunotherapies studied in this CT26 model.

Comparator A	Comparator B	Parameter	P-value
SC-untreated	ID-untreated	Day 19 tumor volume	<0.0001
SC-untreated	SC-CpG	Day 19 tumor volume	<0.0001
SC-untreated	SC-anti-CTLA4	Day 19 tumor volume	<0.0001
SC-anti-CTLA4	ID-anti-CTLA4	Day 19 tumor volume	0.0359
SC-CpG	ID-CpG	Day 19 tumor volume	0.1290
ID-untreated	ID-CpG	Day 26 tumor volume	0.0358
ID-untreated	ID-anti-CTLA4	Day 26 tumor volume	0.0055
ID-CpG	SC-CpG	Day 26 tumor volume	0.0018
ID-untreated	SC-untreated	Overall survival	0.0014
ID-CpG	ID-untreated	Overall survival	0.3589
SC-CpG	SC-untreated	Overall survival	0.2685
ID-CpG	SC-CpG	Overall survival	0.0010
ID-CTLA4	ID-untreated	Overall survival	0.0675
SC-CTLA4	SC-untreated	Overall survival	0.0005
ID-CTLA4	SC-CTLA4	Overall survival	0.1464

Supplemental Table 1: Summary p-values for the analyses conducted for the CT26 colon adenocarcinoma model, corresponding with Supplemental Figure 3. Day 19 and Day 26 analyses were conducted using a one-way ANOVA, with multiple comparisons conducted using the Fisher's Least Square Difference test. Survival comparisons were conducted using the log-rank test of Kaplan-Meier estimates. P-values were not corrected for multiple comparisons.

Supplemental Figure 4: Depth of implantation impacts growth rate and degree of response to multiple immunotherapies in the Panc02 cancer model. A) Treatment Schema. Female, 6 week old C57Bl/6 mice were implanted either intentionally ID or SC with $1x10^6$ Panc02 pancreatic adenocarcinoma cells [22] as described in the methods section. Mice were then randomized to receive either no treatment, a combination of 250 µg/dose agonist CD40 antibody (blue arrow, derived from nude mice ascites following FGK 45.5 hybridoma cell injection and ammonium sulfate precipitation as described previously [23]) intraperitoneally on days 5 and 12 post implantation plus intratumoral injections of 50 µg/dose CpG (orange arrow, TriLink Biotechnologies) on days 8 and 15 post implantation, or a combination of intraperitoneal injections of 200 µg/dose anti-CTLA4 antibody (green arrow, IgG2c, Bristol-Myers Squibb) on

days 2, 5, and 8 post implantation plus intratumoral injections of 75,000 biological units of IL2 (red arrow, BRB Preclinical Biologics Repository) on days 5-9 post implantation. **B)** For all mice, tumors were measured in a blinded fashion, and the mobile or fixed physical status of the tumor was evaluated and recorded. The table in **B** documents the physical exam status for every mouse in the experiment on each measurement day. Mobile tumors were coded red, fixed tumors were coded blue, and tumors with both properties or intermediate findings were coded purple. Tumors that were not yet palpable were coded grey. Mice that had a tumor and were rendered disease free have a 'DF' in the cell in this graph starting on the measurement day tumor was no longer palpable. Mice that were euthanized due to tumor burden or found dead from metastases have an 'X' in the cell in this graph starting on the measurement day they were euthanized or found dead. Results demonstrate that, like the B78 melanoma tumor model in C57Bl/6 mice, Panc02 pancreatic adenocarcinoma in C57Bl/6 mice can develop in both the ID and SC space, and that the vast majority of intentionally ID-implanted tumors exhibit the "mobile" physical exam finding, and the vast majority of intentionally SC-implanted tumors exhibit the "fixed" physical exam finding. For all experiments, only ID-implanted mice with 'mobile' tumors and SC-implanted mice with 'fixed tumors for greater than half of all measurement days were included in the analysis\ of tumor growth graphs (C-J, below); (the number of mice included in analyses of tumor growth per group, n, out of the 9 or 10 initial mice implanted in each group, is indicated below each graph). **C and D)** average tumor volume for ID (red) and SC (blue) tumors in response to no treatment (dotted lines), CD40 + CpG immunotherapy (solid lines in **C**), or anti-CTLA4 + IL2 immunotherapy (solid lines in **D**). Note that when one mouse in the group dies, the average can no longer be graphed. **E)** Individual and mean +/- SEM tumor volumes for each of the treatment groups represented in **C** and **D** on day 23 post implantation, the last day for which all mice were alive. Statistical analysis was conducted using a one-way ANOVA with multiple comparisons performed using uncorrected Fisher's Least Square Difference. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$. **F,**

G, and H) Individual tumor volumes for each individual mouse in each treatment group are plotted over the course of the experiment to demonstrate the range and variability seen. Results show that untreated ID-implanted and SC-implanted tumors grew at approximately the same rate with no statistical difference detected in day 23 tumor volume. However, due to invasion of the leg musculature and innervation, SC-implanted mice were more likely to meet criteria for euthanasia per our animal protocol, which did translate to a statistically significantly greater overall survival for ID-implanted mice compared to SC-implanted mice (log rank analysis, p =0.01); these data are evident by the indication of dead or euthanized mice in the untreated groups in **B**. ID-implanted mice treated with both CD40 + CpG and anti-CTLA4 + IL2 immunotherapies had a trending lower tumor volume at day 23 post implantation compared to ID-implanted untreated mice (p=0.07 and 0.054, respectively) (**E**). On day 23 post implantation, ID-implanted mice treated with CD40 + CpG had a lower tumor volume than SC-implanted mice ($p<0.05$, **E**). Similarly, ID-implanted mice treated with anti-CTLA4 + IL2 had a lower tumor volume than SC-implanted mice ($p<0.01$, **E**). Due to a mouse in the SC-implanted untreated group dying on day 23 post implantation, group comparisons beyond this point were not technically possible. Nevertheless, comparison of tumor size in the remaining groups on day 30 post implantation (in **C** and **D**) by one-way ANOVA demonstrated that ID-implanted untreated mice had significantly larger tumors than ID-implanted mice treated with either CD40 + CpG ($p < 0.001$) or with anti-CTLA4 + IL2 ($p < 0.001$). It should be noted, however, that a difference between ID-implanted and SC-implanted tumors treated with CD40+CpG could no longer be detected at day 30 post implantation. Together, these data suggest that the Panc02 tumor line in C57Bl/6 mice displays the same mobile/fixed physical exam findings associated with either ID or SC localization of the tumor, and may exhibit a difference in response to either CD40+CpG or anti-CTLA4 + IL2 immunotherapy based on implanted tumor depth. See Supplemental Table 2 for a summary of all p values referenced for the combinations of tumor depths and immunotherapies studied in this Panc02 model.

Comparator A	Comparator B	Parameter	P-value
SC-untreated	ID-untreated	Day 23 tumor volume	0.1197
ID-untreated	ID-CD40+CpG	Day 23 tumor volume	0.0758
ID-untreated	ID-CTLA4 + IL2	Day 23 tumor volume	0.0543
ID-CD40+CpG	SC-CD40+CpG	Day 23 tumor volume	0.0265
ID-CTLA4 + IL2	SC-CTLA4 + IL2	Day 23 tumor volume	0.0042
ID-untreated	ID-CD40+CpG	Day 30 tumor volume	<0.0001
ID-untreated	ID-CTLA4 + IL2	Day 30 tumor volume	<0.0001
ID-CD40+CpG	SC-CD40+CpG	Day 30 tumor volume	0.5925
ID-CTLA4 + IL2	SC-CTLA4 + IL2	Day 30 tumor volume	0.00091

Supplemental Table 2: Summary p-values for the analyses conducted for the pancreatic adenocarcinoma model, corresponding with Supplemental Figure 4. Day 23 and Day 30 analyses were conducted using a one-way ANOVA, with multiple comparisons conducted using the Fisher's Least Square Difference test. P-values were not corrected for multiple comparisons.

Supplemental Figure 5: Depth of implantation impacts growth rate, overall survival, and degree of response to combination anti-CTLA4 and immunocytokine immunotherapy in the B16-GD2 melanoma model. A) Treatment Schema. Female, 6 week old C57Bl/6 mice were implanted either intentionally ID or SC with $5x10^5$ B16-GD2 murine melanoma cells (B16-F10 melanoma cells transfected to express GD2 as described previously [23]) as described in the methods section. Mice were then randomized to receive either no treatment or a combination of 200μg intraperitoneal anti-CTLA4 antibody (green arrow, IgG2c, Bristol-Myers Squibb) on day 5 post implantation plus intratumoral injections of 50 g/dose hu14.18-IL2 immunocytokine (IC) as described in the Methods section (black arrows) on days 2-6 post implantation. **B)** For all mice, tumors were measured in a blinded fashion, and the mobile or fixed physical status of the tumor was evaluated and recorded. The table in B documents the physical exam status for every mouse in the experiment on each measurement day. Mobile tumors were coded red, fixed tumors were coded blue, and tumors with both properties or

intermediate findings were coded purple. Tumors that were not yet palpable were coded grey. Tumor-bearing mice that were rendered disease free have a 'DF' in the cell in this graph starting on the measurement day tumor was no longer palpable. Mice that were euthanized due to tumor burden or found dead from metastases have an 'X' in the cell in this graph starting on the measurement day they were euthanized or found dead. Results demonstrate that, like the B78 melanoma tumor model in C57Bl/6 mice, the B16-GD2 model in C57Bl/6 mice can develop in both the ID and SC space, and that the majority of intentionally ID-implanted tumors exhibit the "mobile" physical exam finding, and the majority of intentionally SC-implanted tumors exhibit the "fixed" physical exam finding. Of note, two ID-implanted tumors in the untreated group failed to develop, but almost all of the mice with ID-implanted tumors that were treated with immunotherapy (which began before tumors were palpable) did not develop a tumor. For all experiments, ID-implanted mice with 'fixed' tumors and SC-implanted mice with 'mobile' tumors for greater than half of the measurement days they could be measured were excluded from the analysis (number of mice per group, n, indicated below each graph, of 9 or 10 mice initially implanted in each group). **C)** Average tumor volume for ID (red) and SC (blue) tumors in response to no treatment (dotted lines) or anti-CTLA4 + IC immunotherapy (solid lines). Note that when one mouse in the group dies, the average can no longer be graphed. **D)** Survival curves of ID (red) and SC (blue) B16-GD2 tumor-bearing untreated mice (dotted lines), and mice treated with anti-CTLA4+IC immunotherapy (solid lines). Survival analysis was conducted using the Kaplan-Meier method, with comparisons between groups conducted using log-rank tests. Statistical comparison of these survival times are shown in Supplemental Table 3. **E)** Individual and mean +/- SEM tumor volumes for each of the treatment groups on day 17 post implantation, the last day for which all mice were alive. Statistical analysis was conducted using a one-way ANOVA with multiple comparisons performed uncorrected Fisher's Least Square Difference. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$. **F and G)** Individual tumor volumes are plotted over the course of the experiment for all untreated mice (F) and

immunotherapy treated mice (G). Results demonstrate that untreated SC-implanted mice exhibited larger tumors on day 17 post implantation compared to untreated ID-implanted mice ($p < 0.01$) and had a significantly shorter overall survival ($p < 0.05$). No difference (in tumor volume or overall survival) could be detected between SC-implanted mice treated with anti-CTLA4 + IC immunotherapy and untreated SC-implanted mice. By contrast, ID-implanted tumors treated with anti-CTLA4+IC had a smaller average tumor volume on day 17 post implantation compared to SC-implanted treated tumors ($p<0.05$) treated similarly and also had a significantly longer overall survival ($p<0.0001$) than mice with SC-implanted tumors treated similarly. No statistically significant difference could be detected between ID-implanted tumors treated with immunotherapy compared to untreated ID-implanted tumors due to the substantial variance in the experimental group attributable to the SC-implanted untreated group using the one-way ANOVA. However, a student's t test comparing ID-implanted treated and untreated tumors shows they are substantially different ($p = 0.018$). Further, 0 of 8 ID-implanted mice treated with anti-CTLA4 + IC developed a palpable tumor (after excluding the single ID-implanted mouse with a 'fixed' phenotype as indicated in B), whereas all 9 of 9 SC-implanted mice developed tumors ($p < 0.0001$ by fisher's exact test). This does not appear to be a byproduct of only implantation depth, as 5 out of 7 ID-implanted untreated mice developed tumors ($p < 0.001$ by fisher's exact test, when compared to the 0 of 8 mice with ID-implanted tumors that received anti-CTLA4 + IC). Together, these data show that implantation depth may have a profound influence on baseline growth rate, as well as degree of response to anti-CTLA4 + IL2 immunotherapy. Indeed, the B16-GD2 model under these conditions may represent the extreme case where SC-implanted tumors appear to have no response to immunotherapy (no difference from SC-implanted untreated tumors), and ID-implanted tumors have a 'maximum' response to immunotherapy (almost no tumors develop during the experimental period). See Supplemental Table 3 for a summary of all p values referenced for the combinations of tumor depths and immunotherapies studied in this B16-GD2 model.

Comparator A	Comparator B	Parameter	P-value
SC-untreated	ID-untreated	Day 17 tumor volume	0.0004
SC-untreated	SC-anti-CTLA4 + IC	Day 17 tumor volume	0.1706
SC-anti-CTLA4 + IC	ID-anti-CTLA4+IC	Day 17 tumor volume	0.0026
ID-untreated	ID-anti-CTLA4+IC	Day 17 tumor volume (LSD)	0.5985
ID-untreated	ID-anti-CTLA4+IC	Day 17 tumor volume (Students t test)	0.0182
SC-untreated	ID-untreated	Overall Survival	0.0124
SC-untreated	SC-anti-CTLA4 + IC	Overall Survival	0.9096
SC-anti-CTLA4 + IC	ID-anti-CTLA4+IC	Overall Survival	<0.0001
ID-untreated	ID-anti-CTLA4+IC	Overall Survival	0.0152

Supplemental Table 3: Summary p-values for the analyses conducted for the B16-GD2 melanoma model, corresponding with Supplemental Figure 5. Day 17 analyses were conducted using a one-way ANOVA, with multiple comparisons conducted using the Fisher's Least Square Difference test except where otherwise noted. Survival comparisons were conducted using the log-rank test of Kaplan-Meier estimates. P-values were not corrected for multiple comparisons.

Supplemental Figure 6: Depth of implantation affects tumor growth rate in untreated mice, but not response to potent anti-CTLA4 plus IL2 immunotherapy, in the MOC2 tumor model. A) Treatment Schema. Female, 6 week old C57Bl/6 mice were implanted either intentionally ID or SC with $1x10^6$ MOC2 murine squamous cell head and neck cancer cells [24] as described in the methods section. Mice were then randomized to receive either no treatment, or a combination of intraperitoneal injections of anti-CTLA4 antibody (green arrow, IgG2c, Bristol-Myers Squibb) on days 2, 5, and 8 post implantation plus intratumoral injections of 75,000 biological units of IL2 (red arrow, BRB Preclinical Biologics Repository) on days 5-9 post implantation. **B)** For all mice, tumors were measured in a blinded fashion, and the mobile or fixed physical status of the tumor was evaluated and recorded. The table in B documents the physical exam status for every mouse in the experiment on each measurement day. Mobile

tumors were coded red, fixed tumors were coded blue, and tumors with both properties or intermediate findings were coded purple. Tumors that were not yet palpable were coded grey. Tumor-bearing mice that were rendered disease free have a 'DF' in the cell in this graph starting on the measurement day tumor was no longer palpable. Mice that were euthanized due to tumor burden or found dead from metastases have an 'X' in the cell in this graph starting on the measurement day they were euthanized or found dead. Results demonstrate that, like the B78 melanoma tumor model, the MOC2 model in C57Bl/6 mice can develop in both the ID and SC space, and that the majority of intentionally ID-implanted tumors exhibit the "mobile" physical exam finding, and the majority of intentionally SC-implanted tumors exhibit the "fixed" physical exam finding. For all experiments, only ID-implanted mice with 'mobile' tumors and SC-implanted mice with 'fixed tumors for greater than half of all measurement days were included in the analysis (number of mice per group, n, indicated below each graph, out of the 9 or 10 mice initially injected in each group). **C)** Average tumor volume for ID (red) and SC (blue) tumors in response to no treatment (dotted lines) or anti-CTLA4 + IL2 immunotherapy (solid lines). **D)** Individual and mean +/- SEM tumor volumes for each of the treatment groups represented in **C** on day 32 post implantation, the last day of the experiment. Statistical analysis was conducted using a one-way ANOVA with multiple comparisons performed using uncorrected Fisher's Least Square Difference. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$. **E and F)** Individual tumor volumes are plotted over the course of the experiment for all untreated mice (**E**) and for all anti-CTLA4 + IL2 immunotherapy treated mice (**F**). Results demonstrate that untreated SC-implanted mice had larger untreated tumors at day 32 post implantation compared to ID-implanted mice ($p < 0.0001$). Untreated SC-implanted mice had significantly larger tumors than anti-CTLA4 + IL2 treated SC-implanted mice ($p<0.0001$). Similarly, untreated ID-implanted mice had tumors that showed a trend toward being larger than immunotherapy treated mice with ID-implanted tumors ($p = 0.054$). However an effect of implantation depth was not detected between SC-implanted and ID-implanted mice treated with anti-CTLA4 + IL2 immunotherapy;

this is because the vast majority of tumors implanted at both depths were rendered disease-free by treatment by day 32. However, although not evaluated as a predetermined study endpoint, the data do appear to suggest that the SC-implanted tumors treated with immunotherapy are larger than the ID-implanted tumors treated with immunotherapy on day 14, before all immunotherapy-treated tumors begin to completely respond. This suggests that the effect of immunotherapy on ID vs. SC implanted tumors may vary depending on the tumor model under investigation. It is certainly conceivable that in the MOC2 model, a less potent immunotherapy (anti-CTLA4 alone or IT IL2 alone, for example) may have elicited a difference in response between ID- and SC-implanted mice. These data suggest that in the MOC2 model, tumors grow at different rates based on tumor implantation depth, though both ID and SC implanted tumors are similarly responsive to potent anti-CTLA4 + IL2 immunotherapy when this regimen is applied under these conditions. See Supplemental Table 4 for a summary of all p values referenced for the combinations of tumor depths and immunotherapies studied in this MOC2 model.

Comparator A	Comparator B	Parameter	P-value
SC-untreated	ID-untreated	Day 32 tumor volume	<0.0001
SC-untreated	SC-anti-CTLA4 + IL2	Day 32 tumor volume	<0.0001
ID-untreated	ID-anti-CTLA4 + IL2	Day 32 tumor volume	0.0524
SC-anti-CTLA4 + IC	ID-anti-CTLA4 + IL2	Day 32 tumor volume	>0.9999

Supplemental Table 4: Summary p-values for the analyses conducted for the MOC2 head and neck cancer model, corresponding with Supplemental Figure 6. Day 32 analyses were conducted using a one-way ANOVA, with multiple comparisons conducted using the Fisher's Least Square Difference test. P-values were not corrected for multiple comparisons.

Supplemental Video 1: Demonstration of “Fixed” phenotype of implanted B78 tumor. The C57BL/6 mouse in the video was implanted SC with B78 syngeneic melanoma cells and allowed to grow for 4 weeks. The tumor exhibited a “fixed” phenotype on physical exam following lateral displacement of the skin. Motion of the finger in the video causes the skin to displace with the finger, sliding over the deeper musculature and tumor. The tumor remains “fixed” to the underlying structures and does not appear to be physically connected to the skin compartment.

Supplemental Video 2: Demonstration of “Mobile” phenotype of implanted B78 tumor. The C57BL/6 mouse in the video was implanted ID with B78 syngeneic melanoma cells and allowed to grow for 4 weeks. The tumor exhibited a “mobile” phenotype on physical exam following lateral displacement of the skin. As skin is displaced by the finger in the video, the tumor displaces as well, moving with the skin. This tumor phenotype is almost fully associated with the true skin compartment and does not appear attached or anchored to any underlying tissue.